# IDENTIFICATION OF PATHOGENS CAUSING LEAF BLIGHT IN CORN PLANTS

**THESIS**

**By**

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**FACULTY OF ANIMAL AND AGRICULTURAL SCIENCES**
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# IDENTIFICATION OF PATHOGENS CAUSING BLIGHT DISEASE IN CORN PLANTS

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One of the requirements for obtaining a
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## STATEMENT OF ORIGINALITY OF THESIS

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1. The thesis titled: **Identification of Pathogens Causing Leaf Blight Disease in Corn Plants,** and the related research are the author's own work.
2. Any ideas or quotations from others' works, whether in the form of publications or other forms, in this thesis have been acknowledged in accordance with the standard procedures of the discipline.
3. The author also acknowledges that this thesis was produced thanks to the guidance and full support of the supervisors: **Prof. Dr. Ir. Florentina Kusmiyati, M.Sc.** and **A'isyah Surya Bintang, S.P., M.Sc.**

If, at a later date, any evidence of academic misconduct is found in this thesis, the author agrees to have the bachelor's degree awarded to them revoked in accordance with the regulations of the S1 Agroecotechnology Program, Faculty of Animal Science and Agriculture, Diponegoro University.

Semarang, , August 2024

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## SUMMARY

ASIH WIDYANINGRUM. 23020220120009. 2024. Identification of Pathogens Causing Leaf Blight Disease in Corn Plants. (Advisors: **FLORENTINA KUSMIYATI** and **A'ISYAH SURYA BINTANG**)

This study aims to identify the pathogens causing corn leaf blight disease in several corn-producing regions in Central Java, namely Grobogan, Semarang, and Klaten. The research was conducted from January 2024 to June 2024 at the Laboratory of Plant Physiology and Breeding, Faculty of Animal Husbandry and Agriculture, Diponegoro University, Semarang, and on land in Pucung Village, Bancak District, Semarang Regency, Central Java.

The research was divided into several stages, namely exploration of downy mildew symptoms, identification of downy mildew morphology and morphometry, molecular identification of downy mildew, and inoculation of downy mildew pathogens. Exploration of downy mildew symptoms was carried out using a *purposive sample* method based on the intensity of attacks in three locations, namely Grobogan, Semarang, and Klaten, which are among the corn-producing centers in Central Java. Morphological and morphometric identification was performed by microscopically observing the shape and size of the spores. Molecular identification was performed using specific primers, namely PmUR/PmUF specific to the species *Peronosclerospora maydis,* PpUR/PpUF specific to the species *Pernosclerspora philippinensis,* and PsrUR/PsrUF specific to the species *Peronosclerospora sorghi.* Pathogen inoculation was carried out on Paramita F1 corn plants using a monofactor experiment and a completely randomized design (CRD). The experiment used inoculum sources from Grobogan, Semarang, and Klaten that had been molecularly identified as *Peronosclerospora sorghi.* The research environment had an average temperature of 31.9°C and an average humidity of 76%. Pathogen inoculation was carried out using the infected leaf insertion method, which involved inserting symptomatic leaf inoculum sources between test corn plants that were 1 day old after planting (DAP).

The results of research on the exploration of downy mildew symptoms show that specific symptoms of downy mildew are chlorosis parallel to the leaf veins and signs of downy mildew spores under the surface of corn leaves. Morphological and morphometric identification of the downy mildew pathogen yielded the following results: all samples had oval-shaped conidia and 3-branched conidiophores. The Grobogan sample had a conidiophore length of 376.75 µm and conidia size of 27.34 µm × 32.30 µm. The Semarang sample had a conidiophore length of 476.59 µm and a conidia size of 28.18 µm × 35.13 µm. The Klaten sample had a conidiophore length of 361.60 µm and a conidia size of 28.97 µm × 33.73 µm. Molecular identification using the three specific primers showed that the corn samples with downy mildew symptoms from the three locations were *Peronosclerospora sorghi.* Inoculation of the pathogen on Paramita F1 corn plants showed that the Grobogan sample had the fastest incubation period compared to the other samples because it

had higher spore viability than the other treatments. The incidence of downy mildew disease was 100% at 5 weeks after planting (WAP), the intensity of downy mildew disease was 24% at 5 WAP, which was categorized as resistant ( ), and the AUDPC value ranged from 70.89 to 84.35, which was also categorized as resistant. The infection rate increased with each passing week until 4 WPT, but decreased at 5 WPT. The inoculum source treatment had no significant effect on spore viability, plant height, number of leaves, chlorophyll content, stomatal opening, stomatal density, fresh crown weight, and dry crown weight because the infecting pathogen was the same, namely P. *Sorghi.*

The conclusion of this study is that the pathogen causing downy mildew from three locations is *Peronosclerospora sorghi*, based on microscopic observations and supported by molecular identification using specific primers.

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The Author

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# CHAPTER I

# INTRODUCTION

## 1.1. Background

Corn (*Zea mays* L.) is an important commodity because it is one of the food and feed crops in Indonesia. Corn can be used to make corn flour, corn syrup, corn oil, feed, and as an alternative raw material for the *biofuel* industry. Corn cob waste can be used as activated carbon to reduce pollution caused by motor vehicle exhaust gases (Gunawan *et al.*, 2020).

Corn production centers in Indonesia are spread across East Java, Central Java, West Java, North Sumatra, Lampung, East Nusa Tenggara (NTT), and Central Sulawesi. Areas in Central Java that are widely used for corn production include Grobogan, Blora, Wonogiri, and Kendal. Corn productivity in Central Java declined from 2021 to 2023, namely in 2021 by 62.56 quintals/ha, in 2022 by 59.94 quintals/ha, and in 2023 by 58.76 quintals/ha (Widyasanti, 2023).

One of the factors hindering corn production is the presence of pathogens or diseases in plants. One of the main diseases affecting corn plants is downy mildew. This disease is caused by the pathogen *Peronosclerospora* sp., which has the potential to cause crop failure with a yield loss incidence rate of up to 90% (Dewi and Paeru, 2017). *Peronosclerospora* sp. pathogens that have been identified in Indonesia are P. *maydis,* P. *philippinensis,* and P. *sorghi.* The P. *maydis* pathogen was found to attack corn plants on the islands of Kalimantan and Java, P.

*philippinensis* was found in Sulawesi, and P. *sorghi* was found in Sumatra (Hikmawati and Melina, 2011).

Corn plants infected with downy mildew exhibit specific symptoms and signs. Specific symptoms of plants affected by downy mildew include elongated chlorosis parallel to the leaf veins with visible boundaries between healthy and diseased leaves. Signs of this disease include a white color on the upper or lower surface of the leaves in the form of fine powdery hairs, which are spores of *Peronosclerospora* sp. and are usually visible in the morning (Rustiani *et al.*, 2015).

Targeted control measures are necessary given the significant economic losses caused by the disease, so information about the pathogen must be obtained. The pathogen identification process usually employs Koch's postulates based on symptoms and signs, but this method is time-consuming and lacks specificity. *Peronosclerospora* sp. is an obligate parasite that can only survive on living organisms because it cannot be cultured (Kalqutny *et al.*, 2020). Human mobility can also contribute to the increasing diversity of pathogens.

The identification process can be carried out quickly and *in real time* using molecular identification methods with PCR. The primers used can be specific primers, for example PmUF/PmUR, PpUF/PpUR, and PsrUF/PsrUR (Rustiani *et al*., 2015). The identification results can be used to study *Peronosclerospora* sp. affected by biotic and abiotic factors, resulting in variations in morphology, morphometry, and genetics ( ). Biotic factors can be in the form of plant host types, such as plant varieties. Abiotic factors in the form of the environment also play an important role in the evolution of pathogens due to climate change because

pathogens adapt to the environment (Rustiani, 2015). Therefore, identifying the pathogen that causes leaf rust in corn plants is important.

## 1.2. Research Objectives and Benefits

The purpose of this study is to identify the pathogens that cause leaf blight in corn plants in several corn-producing areas in Central Java, namely Grobogan, Semarang, and Klaten. The expected benefit of this study is to identify the pathogens that cause leaf blight in corn in several corn-producing areas in Central Java, namely Grobogan, Semarang, and Klaten.

## 1.3. Research Hypothesis

The research hypothesis tested is that the pathogen causing downy mildew disease in corn in several corn-producing areas in Central Java, namely Grobogan, Semarang, and Klaten, is *Peronosclerospora maydis*. The samples were taken from Java, and based on existing reports, the *P. maydis* pathogen has been found in Java and Kalimantan.

# CHAPTER II

# LITERATURE REVIEW

## 2.1. Corn Plant (*Zea mays* L.)

Corn is a food crop and therefore an important commodity in Indonesia. Corn is used as a food crop because it contains 75.64% carbohydrates, 10.68% protein, and 1.78% fat (Lalujan *et al*., 2017). The classification of corn according to Linnaeus (1753) is as follows:

Kingdom : Plantae

Division : Magnoliophyta

Class : Monocotyledons

Order : Poales

Family : Poaceae

Genus : *Zea*

Species : *Zea mays* L.

The morphology of corn plants consists of roots, stems, leaves, flowers, fruits, and seeds. Corn plant roots consist of seminal roots, adventitious roots, and aerial roots. There are 3-4 seminal roots located along the plant's growing point, adventitious roots emerge from the first node located 3-4 cm below the soil surface, and aerial roots are found on the first node but their roots can enter the soil to support and strengthen the corn plant (Singh, 1987). Corn has a green to purplish stem and a cylindrical . Corn plants have upright, jointed stems with a varying number of joints, ranging from 10 to 40 (Khairiyah *et al.*, 2017). Corn plants have

green leaves consisting of leaf sheaths and leaf blades. Corn leaves are perfect leaves formed from leaf sheaths that appear parallel to the stem and leaf blades that emerge from the stem nodes (Pribadi *et al.*, 2021).

Corn plants have male flowers located on the tassel at the top of the plant and female flowers located on the cob. Corn is a monoecious plant because it has both male and female flowers on one plant, with female flowers emerging from the leaf axils and male flowers developing from the apical growing point at the top of the plant (Muhadjir, 1988). Corn fruit is in the form of cobs that are milky white when forming and yellowish when mature. Corn plants usually have one or two cobs covered by husks (Pribadi *et al.*, 2021). Corn seeds are classified as single seeds because corn is a monocotyledonous plant. Corn seeds consist of a pericarp, which is a thin outer layer that prevents water loss, endosperm as a food reserve, and an embryo as a potential plant (Ernaningsih, 2021).

Sweet corn plants grow well in fertile loamy soil with good drainage. Soil that is fertile, loose, rich in organic matter, well-drained and well-aerated, and has a soil pH of around 5.5-7.0 is suitable for sweet corn cultivation (Elfarisma *et al*., 2023). Sweet corn plants can be grown in lowlands and highlands. Sweet corn plants ( ) can be grown at an altitude of 0-1800 m above sea level, but sweet corn plants in highlands have a longer harvest period (Dialista and Sugiharto, 2018). Sweet corn plants can grow optimally when supported by the right growing environment. Sweet corn plants can grow at temperatures of 21-34ºC with an optimal temperature of 23-27ºC, air humidity of 80-90%, rainfall of around 250-500 mm/year distributed evenly throughout the growing season, especially during

the flowering and grain filling phases, and sufficient direct sunlight (Dibia and Suyarto, 2017).

One of the main diseases affecting corn plants is leaf blight, which is why several corn varieties were tested for resistance to leaf blight. The results of research by Agustamia *et al.* (2016) show that the PAC 105, BS 0214, and BS 0314 varieties are resistant, while the BS 0114 and BISI 2 varieties are susceptible based on stomatal density and chlorophyll content. Stomatal density is positively correlated with disease intensity, while the higher the chlorophyll content, the lower the disease intensity because pathogens can attack plants through leaf stomata. The results of research by Pajrin *et al.* (2013) show that there are differences in resistance responses to the intensity of downy mildew (P. *maydis)* attacks, with the Bonanza variety having an intensity of 58.30% classified as susceptible, the Kumala variety having an intensity of 47.83% classified as susceptible, and the Paramita variety having an intensity of 36.56% classified as moderately resistant.

The use of commercial varieties such as Bonanza F1, for which there are no reports on resistance to downy mildew, requires the use of metalaxyl in seed treatment. Metalaxyl acts as a systemic fungicide that is absorbed by the plant and moves through the plant tissue, killing or inhibiting the growth of the fungus that causes downy mildew (Sutama *et al*., 2015). According to *the Fungicide Resistance Action Committee (*FRAC), metalaxyl belongs to the Asillalani group with the code 4. Metalaxyl works by interfering with nucleic acid synthesis and nucleic acid synthesis (RNA polymerase). Fungicides with the active ingredient metalaxyl can be used to control downy mildew, but continuous use over a long period of time has

led to resistance in the pathogens that cause downy mildew. Fungicides with the active ingredient metalaxyl are not effective in controlling downy mildew, presumably due to the occurrence of more virulent pathogen mutations, rendering resistance to fungicide use ineffective (Pakki, 2014).

## 2.2. Downy Mildew

*Downy mildew* is one of the factors that inhibit corn cultivation. Downy mildew is caused by the pathogen *Peronosclerospora* sp., which can cause crop losses of up to 90%, leading to crop failure. There are three species of *Peronosclerospora* sp. pathogens distributed in Indonesia, namely P. *maydis* in Java and Kalimantan, P. *phillipinensis* in Sulawesi, and P. *sorghi* in Sumatra (Rustiani *et al.*, 2015). The classification for *Peronosclerospora* sp. pathogens according to Muis (2018) is as follows:

Kingdom : Chromista

Division : Oomycota

Class : Oomycetes

Order : Peronosporales

Family : Peronosporaceae

Genus : *Peronosclerospora*

Species : *Peronosclerospora* sp.

The development of downy mildew in the field can be influenced by factors such as variety type, corn plant growth age, abiotic environmental factors, and the pathogen species *Peronosclerospora* sp. that causes downy mildew. The incidence

of the disease in Indonesia is dominated by abiotic environmental factors such as an average temperature of 25-30ºC, relative humidity of 80-100%, rainfall of 1000-4000 mm/year, location altitude of 0-700 m above sea level, and alluvial soil types. The species P. *maydis* is commonly found in areas with rainfall ranging from 1000-4000 mm/year, P. *sorghi* is found in the range of 1500-3000 mm/year, and P. *philippinensis* is found in the range of 1500-2000 mm/year (Wakman and Burhanuddin, 2007).

The pathogen *Peronosclerospora* sp. can spread through spores carried by wind or raindrops, which, under favorable environmental conditions, can infect plant tissues through stomata or injured parts of the plant. Once inside the plant tissue, the pathogen penetrates the cell membrane and begins to grow inside, then reproduces and spreads, causing specific symptoms and signs (Muis *et al*., 2018). Specific symptoms of plants affected by downy mildew include elongated chlorotic streaks ( ) parallel to the leaf veins, with a visible boundary between healthy and diseased leaves. A sign of this disease is the presence of white or gray powdery-like spores from *Peronosclerospora* sp., hence it is often called *downy mildew* (Agustamia *et al.*, 2016).

The host plants of *Peronosclerospora* sp. are corn, sorghum, and grasses. The *Peronosclerospora* sp. pathogen can grow on hosts such as corn, sorghum, and grass weeds, but usually does not show symptoms of downy mildew (Rustiani *et al*., 2015). The type of variety, namely susceptible and resistant varieties of corn plants, also affects the incidence of downy mildew in the field. Susceptible varieties will experience physiological disturbances in the form of metabolic and oxidative

enzyme disorders, while resistant varieties will show changes in physiological adaptation as a form of plant resistance to the pathogen that causes downy mildew (Hoerussalam and Kheri, 2013).

Plant physiological adaptation is triggered by the presence of the *Oomycetes* effector RxL96 amino acid. This amino acid is secreted into plant cells, triggering *plant cell death* and preventing further pathogen invasion of plant tissues. The physiological defense response of the host to the pathogen that causes downy mildew is triggered by the recognition of the host resistance gene (*R/Resistance)* to the effector, which then activates *effector-triggered immunity* to overcome pathogen infection (Agrios, 2005).

Plants can also adapt with the presence of AVR (*Avirulance*) genes, which are genes associated with the virulence of certain pathogens, particularly in the pathogen's that cause disease in plants. In general, AVR genes interact with plant resistance genes (R) to form a plant immune system. This interaction is known as the "AVR-R Gene System." If plants have R resistance genes that match the pathogen's AVR genes, the plants can detect effectors and trigger an immune response that can inhibit or destroy the pathogen (Agrios, 2005).

Attacks by the pathogen *Peronosclerospora* sp. usually occur during the vegetative phase, causing specific symptoms and signs. The pathogen *Peronosclerospora* sp. usually attacks corn plants 0-4 weeks after planting because the older the corn plant, the more resistant it is to attacks by the pathogen that causes downy mildew. Downy mildew disease is more likely to infect corn plants in the vegetative phase than in the generative phase because phenolic compounds that play

a role in corn plant resistance to the pathogen that causes downy mildew disease, namely *phytoalexins,* are not yet sufficiently available in young corn plants (Pakki *et al.*, 2019).

Corn plants infected with downy mildew, where the pathogen successfully invades the host tissue cells and develops well, will produce specific downy mildew symptoms and signs. The period of time required by the pathogen from the initial contact until the infection becomes apparent from the symptoms and signs visible on the plant is called the incubation period. The incubation period for downy mildew disease in corn plants is usually around 12-14 days after pathogen inoculation (Muis *et al.*, 2018).

### 2.3. Identification of Downy Mildew

Identification of downy mildew disease in corn plants is important in disease management and plant protection. The pathogen identification process usually uses the Koch's postulates method based on symptoms and signs, but this method is time-consuming and less specific (Kalqutny *et al*., 2020). Human mobility can also cause the presence of pathogens to become more diverse, so identification to the species level can only be done serologically and molecularly.

Identification of the pathogen causing leaf blight in corn plants can be done through morphological and morphometric identification and observation of specific symptoms. Morphological and morphometric identification of the pathogen causing leaf blight can be done microscopically using an electron microscope with 350 and 1000 times magnification. Sampling is assisted by attaching clear tape to the surface

of leaves that have propagules and then transferring them to a glass slide that has been stained with 2% *methylene blue* for observation. Microscopic observation is performed by observing the shape, cell wall thickness, dimensions of spores and sporangiophores, as well as the number of branches and ramifications (Adhi *et al.*, 2021).

Symptom observation was also conducted to determine whether there were similarities or differences in the effects of different pathogen species on corn plants. The results of symptom observations caused by *Peronosclerospora* sp. showed several symptoms, including narrow and stiff chlorosis, followed by stunted or inhibited plant growth, including inhibited cob formation ( ). Infection can also cause plant death when it occurs in plants less than 30 days old (Semangun, 2004).

Observations of the morphology and morphometry of three species of *Peronosclerospora* sp. found in Indonesia, namely *P. maydis*, *P. philippinensis*, and *P. sorghi*, revealed several differences. The conidia of P. *maydis* are *spherical* to *subspherical* with a diameter of 12-23 × 25-44 µm, those of P. *philippinensis* are ovoid or *elongated ovoid* with a diameter of 17-23 × 27-39 µm, *while* P. *sorghi* are oval to *spherical* with a diameter of 15-27 × 15-29 µm. P. *maydis* branches 2-4 times, P. *philippinensis* 2-4 times, and P. *sorghi* 2-3 times. The conidiophores of P. *maydis* are 150-550 µm long, those of P. *philippinensis* are 150-400 µm long, while those of P. *sorghi* are 180-300 µm long (Rustiani *et al.*, 2015).

The results of identification based on morphology and morphometry can be confirmed by molecular identification methods for the pathogen that causes leaf rust, namely using specific primers. The molecular identification method for

identifying a species utilizes the *Polymerase Chain Reaction* (PCR) method. Molecular identification is considered more accurate because it can identify down to the species level and can provide certainty regarding the relationship with pathogen species (Rustiani *et al.*, 2015).

Specific primers are used to identify pathogens down to the species level. Specific primers that can be used are PmUF/PmUR for the identification of *P. maydis*, PpUF/PpUR for the identification of *P. philippinensis*, and PsrUF/PsrUR for the identification of *P. sorghi* (Rustiani *et al.,* 2015). t identification methods are expected to produce faster, *real-time,* accurate results, more specific identification down to the species level, and can be used on large numbers of samples.

The results of downy mildew identification using specific primers proved to be able to identify the downy mildew to the species level. Downy mildew samples from Bogor and Lampung were identified as *Peronosclerospora maydis* using the PmUF/PmUR primer on 1.5% agarose gel. Downy mildew samples from Lampung, Takalar, and Bitung were identified as *Peronosclerospora philippinensis* using the PmUF/PmUR primer on 1.5% agarose gel. Downy mildew samples from Bogor, Maros, East Nusa Tenggara, and Malang were identified as *Peronosclerospora sorghi* using the PmUF/PmUR primers on a 1.5% agarose gel (Rustiani *et al.,* 2015).

The steps involved in molecular identification consist of extraction, amplification, and visualization. Extraction aims to obtain pure DNA for amplification. Extraction is carried out by isolating samples containing DNA and

separating them from other materials (Muis *et al*., 2023). Amplification aims to exponentially multiply the target DNA using the PCR method. Amplification consists of separating double-stranded DNA into single strands at a temperature of 94-98ºC (*denaturation)*, specific primer attachment to the target DNA (*annealing)*, and DNA elongation at a temperature of 72 ºC (*extension)* (Alsohaili and Bani-Hasan, 2018). Visualization is performed to check the success of the PCR reaction using *agarose gel* electrophoresis. Visualization is carried out in the form of separating PCR products based on size on *an agarose gel* using DNA dye to obtain *bands* representing the amplification products (Muis *et al.*, 2016).

The primer sequence used in the PCR reaction needs to be considered because it affects the DNA amplification results. The primer sequence affects specificity, amplification efficiency, and *annealing temperature*. Primer specificity is important because primers are designed to bind to specific target sequences in DNA, so they must be appropriate. Properly designed primers will increase amplification efficiency, which is the ability of the PCR reaction to multiply the target quickly and accurately. The primer sequence also affects *the annealing temperature*, which will impact the selectivity of the primer to bind to the target (Alsohaili and Bani-Hasan, 2018).

Identification of the pathogen that causes downy mildew needs to be carried out as a form of monitoring the spread of the pathogen. The results of the study by Rustiani *et al.* (2015) show the identification of pathogens based on the sampling areas in 13 provinces in Indonesia, namely East Java, Central Java, Special Region of Yogyakarta, West Java, Banten, Lampung, Bengkulu, West Kalimantan, East

Kalimantan, North Sulawesi, South Sulawesi, East Nusa Tenggara (NTT), and Papua. The pathogen *Peronosclerospora maydis* was identified in all survey areas. The pathogen *Peronosclerospora sorghi* was identified in West Java (Bogor), East Java (Malang), Lampung, NTT, and South Sulawesi. The pathogen *Peronosclerospora philippinensis* was identified in Lampung and Sulawesi. The results of research by Muis *et al.* (2013) show that the pathogen P. *maydis* is found in East Java, Lampung, and West Kalimantan, the pathogen P. *philippinensis* is found in South Sulawesi, while P. *sorghi* is found in North Sumatra and West Java. The spread of these three species of downy mildew pathogens across various locations in Indonesia over long distances may be due to the pathogens being carried by infected seeds from one location to another. Certain species of bulai pathogens can be transmitted through seeds, especially fresh seeds with high water content, because bulai spores can survive for a long time on the surface of seeds or even inside seed tissue, infecting new plants when the seeds are planted.

# CHAPTER III

# MATERIALS AND METHODS

The research was conducted from January 2024 to June 2024 by conducting a corn cultivation survey in the Semarang, Grobogan, and Klaten areas. Identification of pathogens causing leaf blight in corn plants was carried out at the Plant Ecology and Production Laboratory, Faculty of Animal Husbandry and Agriculture, Diponegoro University, Semarang. The research location was on land in Pucung Village, Bancak District, Semarang Regency, at an altitude of 502 meters above sea level (masl).

## 3.1. Research Materials

The materials used consisted of tools and equipment. The tools used consisted of a stereo microscope, microscope slides, Petri dishes, *cover glasses,* dropper pipettes, tweezers, *tubes,* PCR *tubes,* micropipettes, micropipette tips, *scalpels,* gloves, a PCR machine, a Gel Docs machine, an electrophoresis machine, *a cooling box, cooling gel*, a spectrophotometer, Erlenmeyer flasks, *a microwave,* writing instruments, and a camera. The materials used were corn leaf samples infected with downy mildew (*Peronosclerospora* sp.), Paramita F1 corn seeds, distilled water*, buffer,* nuclease-free water, PCR *master mix,* TBE solution, *acetone, florosafe* dye*,* and specific primers (PmUF/PmUR, PpUF/PpUR, and PsrUF/PsrUR).

### 3.2. Research Method

**Research Procedure.** This research was conducted in several stages, namely exploration of downy mildew symptoms, identification of downy mildew morphology and morphometry, molecular identification of downy mildew, and inoculation of downy mildew pathogens.

**Exploration of downy mildew symptoms.** Exploration of downy mildew symptoms was conducted by searching for corn plants with downy mildew symptoms in three locations in the Semarang, Grobogan, and Klaten areas. Samples from the Semarang area came from land in Truko Village, Bringin Subdistrict, Semarang Regency, in the form of 400 BISI 2 corn plants with an average temperature of 28.2ºC and humidity of 85%. Samples from the Grobogan area came from fields in Kuripan Village, Purwodadi Subdistrict, Grobogan Regency, consisting of 150 Baruna corn plants with an average temperature of 27.9ºC and humidity of 80%. The sample from the Klaten area came from land in Kajen Village, Ceper District, Klaten Regency, consisting of 200 BISI 2 corn plants with an average temperature of 27.2ºC and humidity of 83%.

The sampling method used was *purposive sampling* based on the presence or absence of downy mildew incidence. The downy mildew symptoms observed were systemic symptoms in the form of chlorosis parallel to the leaf veins and signs in the form of conidia below or above the leaf surface from the shoot to the fifth leaf (Adhi *et al*., 2021). Samples were stored in sealed clear plastic bags and placed in *a cooling box* containing *cooling gel.* Documentation of the exploration of downy mildew symptoms can be seen in Illustration 1.

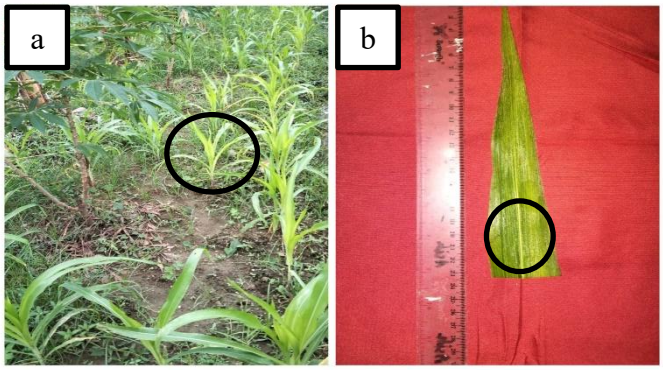



Illustration 1. Exploration of Bulai Symptoms. (a) Documentation of the land area and (b) Documentation of symptomatic plant parts.

The sampling process carried out in the field used *purposive sampling* based on the presence or absence of specific bulai symptoms in corn crops. Not all corn plants in the field showed bulai symptoms, and symptomatic samples were taken randomly from the third to fifth leaves, with 10 plant samples taken at each symptom exploration location. Sampling of corn plant groups was done randomly to avoid biased data.

**Morphological and morphometric identification of smut.** Samples of corn plants showing symptoms of smut from Grobogan, Semarang, and Klaten were observed microscopically using a microscope. The results were used for morphological identification in the form of observations of conidia shape and number of branches. The microscopic observation documentation was processed using the Image Raster application to determine the diameter of the conidia and the length of the conidiophores. Microscopic observations of powdery mildew spores were taken from the leaf surface and placed on a glass slide that had been dripped with distilled water, then covered with *a cover glass* and observed microscopically.

e leaf samples for microscopic observation were collected between 3:00 and 5:00 a.m. (Kurniawan *et al.*, 2017). Documentation of the microscopic observation activity can be seen in Illustration 2.

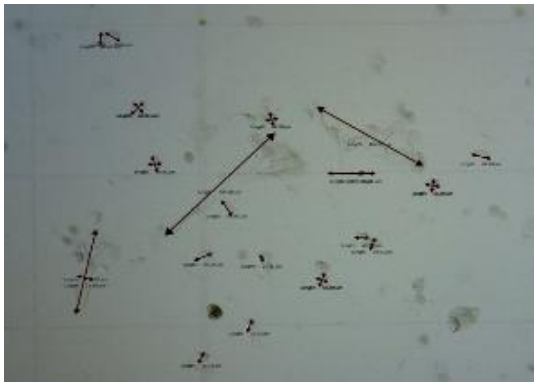


Illustration 2. Microscopic Observation of the Pathogen Causing Leaf Blight at 10×10 Magnification.

Microscopic observation was carried out by observing the morphology of the pathogen in the form of conidia and the number of branches using 10×10 magnification. The observation results were documented and processed using the *Image Raster* application to determine the morphometry of the pathogen in the form of conidia size and conidiofor length. The morphological and morphometric observation results were then compared with references to identify the pathogen.

**Molecular identification of powdery mildew.** The microscopic observation results were confirmed using molecular identification to obtain more accurate and detailed information. Molecular identification was carried out in several stages, namely sample preparation, primer preparation, PCR material preparation, DNA amplification, electrophoresis, and visualization. The stages of molecular

identification of the pathogen that causes downy mildew in corn plants can be seen in illustration 3.

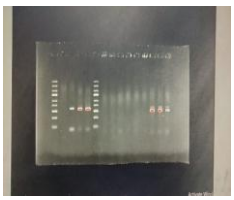
| | |
|---|---|
|  | Sample preparation involves taking 0.1 g of leaves from plants showing blight symptoms and placing them in a tube, then adding 20 µL of buffer. |
|  | Preparation of primers (*working stock*) consisting of 10 µL of 100 µM *stock primers* and 90 µL of nuclease-free water is placed in a 1.5 mL tube. |
|  | PCR material preparation consists of 9.5 µL of nuclease-free water, 12.5 µL of PCR master mix, 1 µL *of forward primer*, 1 µL of *reverse primer*, and 1 µL of extracted sample DNA mixed into a 0.2 mL PCR *tube*. |
|  | DNA amplification involves placing *a* 0.2 mL PCR *tube* into the PCR machine, then setting the PCR machine with the program and adjusting it according to the type of primer. |
|  | Electrophoresis: the sample is placed on a 1% agarose gel (with 1 µL of *florosafe* dye in 20 mL of agarose) in 400 mL of TBE solution at 50 volts for 50 minutes. |
|  | Visualization: The agarose gel from electrophoresis was visualized using Gel Docs. |

Illustration 3. Flowchart of Molecular Identification of Pathogens Causing Leaf Blight in Corn Plants.

Molecular identification of the pathogen that causes corn leaf blight begins with the preparation of samples and primers as materials for the PCR process. The prepared PCR materials are then mixed and placed in a PCR machine for DNA amplification. The PCR samples are then electrophoresed on a 1% agarose gel (stained with *fluorescein)* in TBE solution at 50 volts for 50 minutes. The samples are then visualized using Gel Docs to obtain DNA band results. The use of species-specific primers will cause DNA bands to appear only if they match the species-specific primers, making them faster and more accurate.

The primers, base sequences, and DNA product sizes used for molecular identification of the pathogen causing leaf rust disease in corn plants are shown in Table 1.

Table 1. Primers, Base Sequences, and DNA Product Sizes Used for the Identification of the Pathogen *Peronosclerospora* sp. (Rustiani *et al*., 2015)

| Primer Name | Primer Base Sequence | PCR Result | Pathogen Target |
|---|---|---|---|
| PmUF | TCG TTA TAG AAG CTA TTC ATT AG | 304 bp | *P. maydis* |
| PmUR | GCC ATC GAG TAA TCC ATT GTT | | |
| PpUF | TTT CCG TGT ATT CGG TGG AG | 117 bp | *P. philippinensis* |
| PpUR | ATG CTT TTC GAG GGA AGA GA | | |
| PsrUF | AGC AAC TCC AGT TAT GGA AGG | 500 bp | *P. sorghi* |
| PsrUR | CCT AAT GAA GGT ATG GCC CAT G | | |

Note: The last letter F stands for *forward* primer and R for *reverse* primer. If the PCR band/strip that appears matches the *base pair* (BP) size, it indicates the specific species of *the Peronosclerospora* sp. pathogen.

The amplification program for the specific primers used for molecular identification of the pathogen causing leaf blight in corn plants can be seen in Table 2.

Table 2. Amplification Program for Primer Pairs PmUF/PmUR, PpUF/PpUR, and PsrUF/PsrUR.

| Name Primer | Amplification Cycle Program | | | | | |
|---|---|---|---|---|---|---|
| | Synthesis | Denaturation | *Annealing* | Extension | Final extension | Number of cycles |
| PmUF/ PmUR | 95ºC 1 minute | 95ºC 1 minute | 57°C 1 minute | 72°C 30 seconds | 72°C 5 minutes | 35 |
| PpUF/PpUR | 95°C 1 minute | 95ºC 1 minute | 58°C 1 minute | 72°C 30 seconds | 72°C 5 minutes | 35 |
| PsrUF/ PsrUR | 95ºC 1 minute | 95ºC 1 minute | 60ºC 50 seconds | 72°C 30 seconds | 72ºC 5 minutes | 35 |

**Pathogen inoculation**. Samples obtained from symptom exploration, namely samples from Grobogan, Semarang, and Klaten, were used as inoculum sources for artificial inoculation using the infected leaf insertion method. The infected leaf insertion method was carried out by inserting the inoculum source between test corn plants that were 1 day old after sowing (HSS) (Ivayani *et al.*, 2018). Documentation of pathogen inoculation activities can be seen in Illustration 4.

Illustration 4. Pathogen Inoculation Using the Infected Leaf Insertion Method on Corn Plants.

Inoculation of the pathogen that causes downy mildew was carried out using the infected leaf insertion method in the form of leaf cuttings with specific symptoms of chlorosis parallel to the leaf veins or signs of spores on the underside of the leaf. Leaves with downy mildew symptoms as a source of inoculum were cut into pieces measuring 3-5 cm. These leaf pieces are placed around corn seeds at 1 HSS age until transplanting at 7 HSS age.

**Research Parameters.** The observation parameters studied consisted of identification parameters such as incubation period, disease incidence, disease intensity, *Area Under the Disease Progress Curve* (AUDPC) value, disease infection rate, symptom variation, spore viability, plant height, number of leaves, total chlorophyll content, stomatal opening, stomatal density, fresh weight, and dry weight.

**1. Incubation Period (days)**

The incubation period is the period between infection by a pathogen and the appearance of disease symptoms. The incubation period for leaf blight was obtained

from observations of corn plants showing symptoms of chlorosis parallel to the leaf veins after inoculation with the pathogen causing leaf blight ( ). The incubation period for corn leaf blight until the appearance of symptoms or signs of the disease ranges from 12 to 14 days (Muis *et al.*, 2018).

**2. *Disease Incidence* (%)**

*Disease incidence* is the percentage of plants infected by pathogens from the total number of plants observed (30 plants per inoculum source treatment) at 1-5 weeks after planting (WAP) at weekly intervals. Corn plants exhibiting symptoms, whether mild or severe, are counted as symptomatic plants. Disease incidence (%) according to Kurniawan *et al.* (2017) can be calculated using the formula:

$$DI = \frac{n}{N} \times 100\%$$

Explanation: DI = *Disease* incidence (%), n = number of symptomatic plants, and N = total number of plants observed.

**3. D*ise*ase *Severity* (%)**

*Disease severity* is the severity of a plant infected by a pathogen observed at 1-5 weeks after planting (WAP) at weekly intervals, where a higher disease severity score indicates a higher level of pathogen infection and a lower level of plant resistance to pathogen attack. Disease severity (%) according to Putri and Kasiamdari (2023) can be calculated using the formula:

$$DS = \frac{\Sigma(ni \times vi)}{N \times V} \times 100\%$$

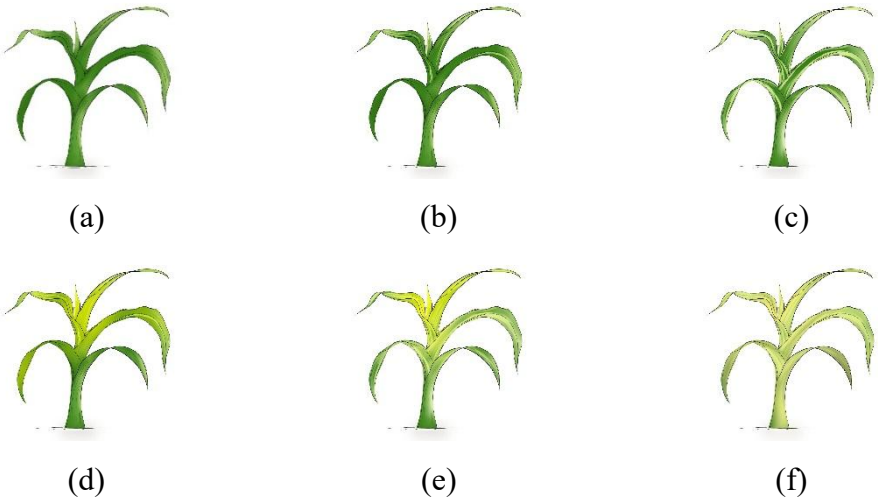
Explanation: DS = *Disease severity* or disease intensity (%), ni = number of plants observed from attack category i, vi = scale value of attack category i, N

= total number of plants observed, and V = scale value of the highest attack category.

The scoring values for the intensity level of leaf blight disease in corn plants can be seen in Table 3, and the extent of leaf blight symptoms in corn plants can be seen in Illustration 5.

Table 3. Scoring Values for Disease Intensity Levels (Agustamia *et al*., 2016)

| Damage Scale | Symptom Description |
|---|---|
| 0 | No infection |
| 1 | Light attack 0% - 20% |
| 2 | Moderate attack 20% - 40% |
| 3 | Moderate attack 40% - 50% |
| 4 | Severe attack 50% - 75% |
| 5 | Severe attack 75% - 100% |



(a) (b) (c)

(d) (e) (f)

Illustration 5. Extent of Leaf Blight Symptoms on Corn Plants. (a) No infection, (b) Mild attack 0% - 20%. (c) Moderate attack 20% - 40%. (d) Moderate attack 40% - 50%. (e) Moderately severe attack 50% - 75%. (f) Severe attack 75% - 100%.

The categories of resistance to leaf blight based on the intensity of leaf blight disease in corn plants can be seen in Table 4.

Table 4. Categories of Resistance to Downy Mildew Based on Disease Intensity
Disease (Khoiri *et al*., 2021)

| Disease Intensity | Resistance Category |
|---|---|
| 0 | Highly resistant |
| $0 < x \leq 25$ | Resistant |
| $25 < x \leq 50$ | Moderately resistant |
| $50 < x \leq 75$ | Not resistant |
| $X > 75$ | Very not resistant |

**4. *Area Under the Disease Progress Curve* (AUDPC) value**

*The Area Under the Disease Progress Curve* (AUDPC) value indicates the area under the disease progression curve. AUDPC calculations are used to identify when control is needed and to help develop effective management procedures based on disease severity. According to Putri and Kasiamdari (2023), the AUDPC value can be calculated using the following formula:

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i)$$

Explanation: AUDPC = area under the disease progression curve, $y_i$ = i-th intensity value, $t_i$ = i-th observation time.

The categories of corn plant resistance to downy mildew based on AUDPC values can be seen in Table 5.

Table 5. Categories of Resistance to Downy Mildew Based on AUDPC Values
AUDPC Values (Kalqunty and Pakki, 2020)

| AUDPC Value | Resistance Category |
|---|---|
| 0 – 100 | Resistant |
| 100 – 600 | Moderately Resilient |
| 600 – 1000 | Vulnerable |
| > 1000 | Very vulnerable |

**5. Disease Infection Rate (r) (plants/week)**

The disease infection rate is measured based on the results of disease intensity to describe the speed of disease spread in a population during a certain period of time. The disease infection rate (r) according to Pajrin *et al*. (2013) can be calculated using the formula:

$$r = \frac{2,3}{(t2-t1)} \left[ \log \frac{x2}{1-x2} - \log \frac{x1}{1-x1} \right]$$

Explanation: r = disease infection rate (plants/week), $x_1$ = proportion of disease (disease intensity) in the first observation, $x_2$ = proportion of disease (disease intensity) in the second observation, $t_1$ = time of first observation, $t_2$ = time of second observation.

**6. Symptom Variation**

Symptom variation measurements were based on systemic chlorosis, non-systemic chlorosis, stunting, and narrowed and erect leaves. These symptom variations were then entered into a table based on observation ages ranging from 1 MST to 5 MST with a 1-week interval.

**7. Spore Viability (%)**

Spore viability was calculated at 05:00 WIB using a brush and then placed in a vial containing 10 ml of sterile water. The bottle containing the spores was left in a refrigerator at 8ºC for 5 hours. Spore viability according to Kurniawan *et al*. (2017) can be calculated using the formula:

$$V = \frac{g}{g + u} \times 100\%$$

Notes: V = spore viability (%), g = number of germinated spores, and u = number of non-germinated spores.

**8. Plant Height (cm)**

Plant height was measured from the soil surface to the growing point of the plant using a measuring tape. Measurements were taken from 1 MST to 5 MST at 1-week intervals.

**9. Number of Leaves (pieces)**

Leaf count measurements were performed manually without tools from 1 MST to 5 MST at one-week intervals.

**10. Total Chlorophyll Content (mg/g)**

Total chlorophyll content in leaves was observed at 3 and 5 weeks after planting. Observations were made twice to compare chlorophyll damage at the beginning and end of planting. The leaves used for sampling were the top three leaves, which are young plant tissues. These plant tissues were collected and weighed using an analytical scale to 0.25 g, then cut into small pieces. These small

pieces were then extracted using 80% acetone by grinding them in a mortar until all the chlorophyll was dissolved. The solution was then filtered using filter paper and the filtrate was placed in a 25 mL measuring flask. The solution was placed in a cuvette and analyzed with a spectrophotometer at wavelengths of 663 nm and 645 nm. The total chlorophyll content can be calculated using Arnon's (1949) formula as follows:

$$Ca+b\ (mg/g) = [8.02 \times A663 + 20.20 \times A645] \times V/1000 \times 1/W$$

Explanation: C = total chlorophyll content (mg/g), V = volume of extract (mL), and W = wet weight of sample (g).

**11. Stomatal Opening (%)**

The stomatal density of corn leaves was observed at 3 MST. The leaves observed were the third leaves from the top. The observation was carried out using a microscope, then the number of open and closed stomata was counted. Stomatal opening according to Agustamia *et al*. (2016) can be calculated using the formula:

$$\text{Stomatal opening} = \frac{\text{Stomata membuka}}{\text{Jumlah stomata}} \times 100\%$$

**12. Stomatal Density (stomata/mm²)**

The stomatal density of corn leaves was observed at 3 MST. The leaves observed were the third leaves from the top. Stomatal density sampling began with cleaning the leaf surface of dirt. The underside of the leaf was coated with clear nail polish over an area of 1 cm, then at the tip, middle, and base. After the polish dried, clear tape was applied to cover the area of the polish layer. The tape was slowly removed until the nail polish peeled off. The sample was placed on a preparation

table and observed under a microscope, then the number of stomata and their cross-sectional area were counted. Stomatal density, according to Agustamia *et al.* (2016), can be calculated using the formula:

$$\text{Stomatal density} = \frac{\text{Jumlah stomata}}{\text{Luas penampang}}$$

**13. Fresh weight of the canopy (g/plant)**

Fresh weight measurement of plant crowns was conducted at 5 MST by weighing the plants using an analytical scale with a precision of 0.0 g.

**14. Dry weight of the canopy (g/plant)**

Measurement of dry weight of plant crowns was conducted at 5 MST by drying the plants using an oven, then weighing them using an analytical balance with an accuracy of 0.0 g.

The data obtained were processed using analysis of variance to determine the effect of the inoculum source on the parameters. Treatments that showed a significant effect on the observed parameters were further tested using a 5% level honest significant difference (HSD) test to determine the differences between inoculum sources.

# CHAPTER IV

# RESULTS AND DISCUSSION

## 4.1. Exploration of Downy Mildew Symptoms

Exploration of bulai symptoms in Baruna corn plants in the Grobogan area showed 23 plants with bulai symptoms out of a population of 150 corn plants. BISI 2 corn plants in the Semarang area had 30 plants with bulai symptoms out of a population of 400. In the Klaten area, there were 15 corn plants with bulai symptoms out of a population of 200 BISI 2 corn plants. The results of observations of bulai symptoms and signs on corn plants are presented in Illustration 6.

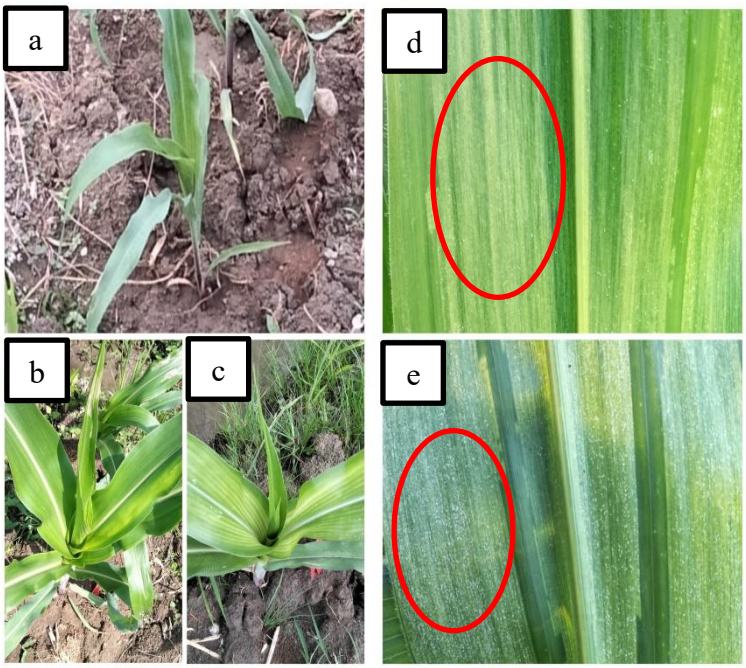



Illustration 6. Macroscopic observation of downy mildew disease. (a) Plants without downy mildew symptoms, score 0, (b) Plants with downy mildew symptoms, score 1, (c) Plants with downy mildew symptoms, score 2, (d) Mild chlorosis and few downy mildew spores, (e) Severe chlorosis and many downy mildew spores.

Variations in symptoms from samples from Grobogan, Semarang, and Klaten were not found, and only non-systemic symptoms of downy mildew were observed, namely a few leaves showing symptoms with varying disease intensity. The results of observations of downy mildew symptoms were specific, namely the presence of chlorosis parallel to the leaf veins and signs of downy mildew spores under the surface of corn leaves (Illustration 6). The specific symptoms of downy mildew are elongated chlorosis parallel to the leaf veins and white or gray powdery spores of *Peronosclerospora* sp., often referred to as *downy mildew (*Agustamia *et al.*, 2016). Chlorosis due to downy mildew is more specific than chlorosis caused by nutrient deficiency, which starts from older leaves to younger leaves, chlorosis caused by other diseases in the form of spots or dots, and chlorosis caused by environmental stress that appears evenly across all leaves (Muis *et al.*, 2018).

Healthy corn plants become symptomatic of downy mildew, beginning with the infection process by the pathogen through the leaves. Downy mildew infection occurs on leaves by damaging tissue down to the epidermis through the funnel-shaped morphology of the leaf, which can collect guttation water, allowing downy mildew spores to spread and enter the plant's stomata and begin spore germination (Putri and Kasiamdari, 2023). The pathogen causing downy mildew can infect corn plants using a special structure called haustoria to penetrate the cell walls of corn plants and then extract nutrients and water from the corn plants (Pakki *et al.*, 2019).

## 4.2. Morphological and Morphometric Identification of Downy Mildew

Morphological identification of downy mildew was performed using a 10×10 magnification microscope to observe the shape of the conidia and the number of conidiophore branches. The observation results were documented and then analyzed using the *Image Raster* application to determine the morphometry of the pathogen, namely the size of the conidia and the length of the conidiophores. The observation results of the downy mildew pathogen spores on corn plants are presented in Illustration 7.

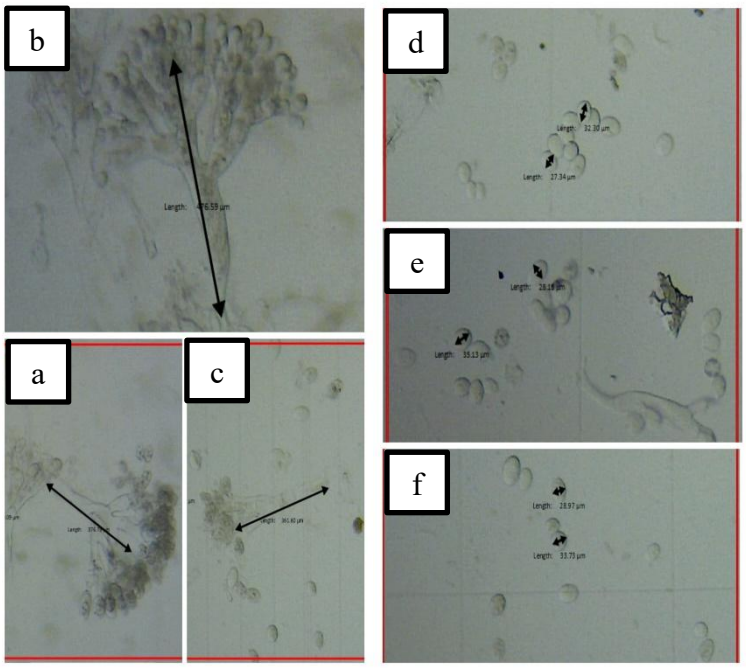



Illustration 7. Microscopic observation with 10×10 magnification. (a) Conidiophores from the Grobogan sample, (b) Conidiophores from the Semarang sample, (c) Conidiophores from the Klaten sample, (d) Conidia from the Grobogan sample, (e) Conidia from the Semarang sample, and (f) Conidia from the Klaten sample.

The Grobogan, Semarang, and Klaten samples have the same morphology, with oval conidia and 3-branched conidiophores. The morphometry of the

Grobogan sample has a conidiophore length of 376.75 µm and conidia size of 27.34 µm × 32.30 µm, the Semarang sample has a conidiophore length of 476.59 µm and a conidia size of 28.18 µm × 35.13 µm, and the Klaten sample has a conidiophore length of 361.60 µm and a conidia size of 28.97 µm × 33.73 µm. Based on the morphology and morphometry of the conidia and conidiophores, the three samples are suspected to be *Peronosclerospora sorghi (*Illustration 7). The pathogen P. *sorghi* has erect, hyaline, swollen, 2-3 branched conidiophores, 180-300 µm long, and oval, hyaline conidia, 14.4-27.3 × 15-28.9 µm in diameter (Rustiani *et al.*, 2015).

The results of the observation show that the larger morphometry is thought to be due to the evolution of the pathogen. *P. sorghi* conidia are oval-shaped, thick-walled with a thickness of 1.1-2.7 µm and a larger diameter than the diameter measured by other researchers, which is 25-31 × 36.9-42.9 µm. The increased size of *Peronosclerospora* sp. may be influenced by environmental conditions such as temperature and humidity, which affect the growth of conidia and conidiophores, as well as the type and health of the host as a source of nutrients for the growth of larger pathogens (Munkcold and White, 2016).

### 4.3. Molecular Identification of Downy Mildew

Molecular detection using specific primers can produce DNA bands from *Peronosclerospora* sp. that can be used for identification up to the species level. The results of molecular detection of downy mildew pathogens in corn plants are presented in Illustration 8.

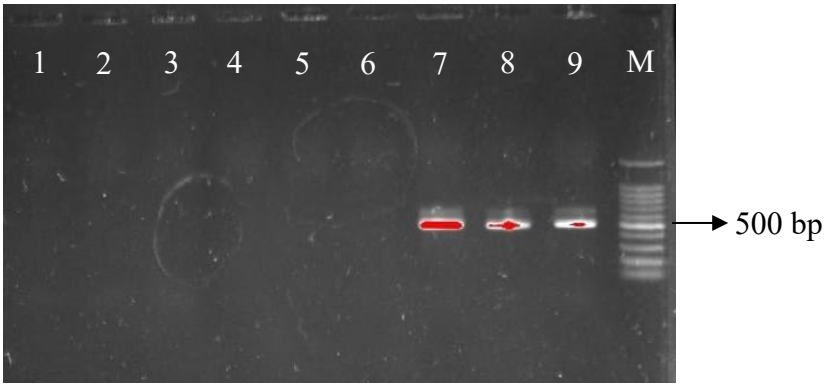



Illustration 8. Visualization of Amplified DNA Bands on 1% Agarose Gel. (M) 100bp DNA marker (ThermoSci), (1) PpUF/PpUR primer for Grobogan sample, (2) PpUF/PpUR primer for Semarang sample, (3) PpUF/PpUR primer for Klaten sample, (4) PmUF/PmUR primer for Grobogan sample, (5) PmUF/PmUR primer for Semarang sample, (6) PmUF/PmUR primer for Klaten sample, (7) PsrUF/PsrUR primer for Grobogan sample, (8) PsrUF/PsrUR primer for Semarang sample, and (9) PsrUF/PsrUR primer for Klaten sample.

The Grobogan, Semarang, and Klaten samples that had been observed based on morphology and morphometry were confirmed using specific primers. The confirmation results with specific primers for the three samples from Grobogan, Semarang, and Klaten produced DNA bands when using the specific *P. sorghi* primer, namely PsrUF/PsrUR, with a size of 500 bp (Illustration 8). Specific primers are used for pathogen identification down to the species level. For example, PsrUF/PsrUR in samples from Lampung, Makassar, East Nusa Tenggara, and Malang yielded results indicating that the pathogen causing the disease was P. *sorghi* with a size of 500 bp *(*Rustiani *et al.*, 2015).

### 4.4. Pathogen Inoculation

Pathogen inoculation yielded observations on incubation period, spore viability, disease incidence, disease intensity, AUDPC value, infection rate , plant height, number of leaves, total chlorophyll content, stomatal opening, stomatal density, fresh canopy weight, and dry canopy weight.

### 4.4.1. Incubation Period

The incubation period of downy mildew disease on Paramita F1 corn plants with inoculation treatments from different inoculum sources is presented in Table 6.

Table 6. Incubation Period of Downy Mildew Disease in Paramita F1 Corn Plants with Inoculation Treatments from Different Inoculum Sources
Different Inoculum Sources.

| Inoculum Source | Incubation Period (HST) |
|---|---|
| No Inoculum | 17.10 [a] |
| Klaten | 15.13 [ab] |
| Semarang | 14.73 [bc] |
| Grobogan | 14.40 [c] |

Different superscripts in the same column indicate significant differences (P<0.05)

The incubation period of downy mildew based on the 5% BNJ test on Paramita F1 corn plants with inoculation treatments from different inoculum sources showed no significant difference between the no inoculum treatment and the Klaten inoculum treatment, but a significantly higher difference compared to the Grobogan and Semarang inoculum treatments. The Klaten inoculum source showed an incubation period that was not significantly different from the Semarang inoculum, but was significantly higher than the Grobogan inoculum. The Semarang and Grobogan inoculum sources showed incubation periods that were not

significantly different from the fastest incubation period. Corn plants in all treatments began to show symptoms of leaf blight after 2 weeks after sowing. The incubation period or the period from pathogen infection to the onset of leaf blight symptoms and signs is approximately 12-14 days after infection (Muis *et al.*, 2018). The treatment without inoculum ( ) still showed leaf blight symptoms, presumably because leaf blight can spread from one plant to another as leaf blight spores can be carried by the wind and spread to other plants. Spores that cause downy mildew can infect corn plants up to a distance of 20-42 m, although 70-85% of infections occur within a distance of 20 m (Putri and Kasiamdari, 2023). The Grobogan and Semarang inoculum sources had the fastest incubation period, presumably because they had higher conidia counts. The percentage of downy mildew disease in several artificial inoculations on corn seedlings and sprouts was influenced by the quantity and quality of the inoculum source conidia, which affected the infection process of the downy mildew pathogen (Adhi *et al.*, 2019).

### 4.4.2. Spore Viability

The viability of spores of the downy mildew pathogen in Paramita F1 corn plants with inoculation treatments from different inoculum sources is presented in Table 7.

Table 7. Spore Viability of the Pathogen Causing Downy Mildew Disease in Paramita F1 Corn Plants with Inoculation Treatments from Different Sources of Inoculum

| Inoculum Source | Spore Viability (%) |
|---|---|
| Without Inoculum | 89.6 |
| Grobogan | 89.53 |
| Semarang | 83.0 |

| | |
|---|---|
| Klaten | 85.85 |

The viability of pathogenic spores causing downy mildew disease based on the results of variance analysis on Paramita F1 corn plants with inoculation treatments from different inoculum sources showed no significant differences. The results of no significant differences in spore viability may be due to the use of the same downy mildew pathogen species, namely *Peronosclerospora sorghi*. The results of artificial inoculation from the same species, namely P. *sorghi* from East Lampung and Pesawaran, on corn plants on farmers' land and at the University of Lampung's plant pest and disease laboratory showed significant differences in spore viability, namely 35.67% for samples from East Lampung and 32.07% for samples from Pesawaran (Kurniawan *et al.*, 2017). The research conducted showed higher spore viability, which may have occurred due to suitable temperature and humidity, namely the average temperature and humidity of the research environment, which was 30.2ºC and 85% in the morning. The pathogen *Peronosclerospora* sp. requires micro-temperature and humidity to germinate, namely humidity of 80%-90% and temperature of 18-30°C (Putri and Kasiamdari, 2023).

### 4.4.3. Incidence of Downy Mildew Disease in

The incidence of downy mildew disease on Paramita F1 corn plants with inoculation treatments from different inoculum sources is presented in Table 8.

Table 8. Incidence of Leaf Blight Disease in Paramita F1 Corn Plants with Inoculation Treatments from Different Inoculum Sources
.

| Source | Incidence of Leaf Blight |
|---|---|

| Inoculum | 1 MST | 2 MST | 3 MST | 4 MST | 5 MST |
|---|---|---|---|---|---|
| | ----------%---------- | | | | |
| Without Inoculum | 0.00 | 33.33 | 63.33 | 100.00 | 100.00 |
| Grobogan | 0 | 76.67 | 83.33 | 100.00 | 100.00 |
| Semarang | 0 | 66.67 | 73.33 | 100.00 | 100.00 |
| Klaten | 0 | 63.33 | 83.33 | 100.00 | 100.00 |

The incidence of downy mildew disease in Paramita F1 corn plants based on inoculation treatment from different inoculum sources increased each week, starting from 0% in the first week, 33.33%-76.67% in the second week, 63.33%-83.33% in the third week, and reaching 100% in the fourth and fifth weeks. The treatment without inoculum had the lowest incidence at 2 MST, but its incidence increased over time to be the same as the other treatments starting at 4 MST. The incidence value of downy mildew disease in Bonanza sweet corn increased over time, namely 10% at 8 days after inoculation (DAI), 20% at 15 DAI, 53.33% at 22 DAI, and 80% at 29 DAI (Wardani *et al.*, 2023). The increase in incidence occurred in all test plants, including those without inoculum, due to the suspected spread of spores through wind and water. The pathogen that causes downy mildew can spread through spores carried by wind or raindrops, which can then infect corn plants if the growing conditions are favorable (Muis *et al.*, 2018).

Environmental conditions greatly influence the development of downy mildew incidence caused by *P. sorghi*, namely air temperature and humidity. Environmental measurements showed that the average temperature and humidity of the research environment were 30.2°C and 85% in the morning, which was thought to be suitable for the development of the downy mildew pathogen in corn plants. The test field was also located at an altitude of 502 m above sea level, which

supported the development of the pathogen causing downy mildew in corn plants. The incidence of downy mildew disease in Indonesia is dominated by abiotic influences in the form of an average temperature of 25-30ºC, relative humidity of 80-100% ( ), and an altitude of 0-700 m above sea level (Wakman and Burhanuddin, 2007).

### 4.4.4. Intensity of Leaf Blight Disease

The intensity of downy mildew disease in Paramita F1 corn plants with inoculation treatments from different inoculum sources is presented in Table 9.

Table 9. Intensity of leaf blight disease in Paramita F1 corn plants with inoculation treatments from different inoculum sources.
.

| Source Inoculum | Disease Intensity | | | | |
|---|---|---|---|---|---|
| | 1 MST | 2 MST | 3 MST | 4 MST | 5 MST |
| | ----------%---------- | | | | |
| Without Inoculum | 0.00 | 6.67 | 12.67 | 40 | 24.67 |
| Grobogan | 0 | 15.33 | 16.67 | 40.00 | 24.67 |
| Semarang | 0.00 | 13.33 | 14.67 | 40.00 | 24.00 |
| Klaten | 0 | 12.67 | 16.67 | 40 | 24.00 |

The intensity of leaf blight disease in Paramita F1 corn plants based on inoculation treatments from different inoculum sources increased weekly until 4 weeks after sowing, starting from 0% in the first week, 6.67%-15.33% in the second week, 12.67%-16.67% in the third week, and 40% in the fourth week, but began to decline after the fifth week to around 24%. The treatment without inoculum had the lowest intensity at 2 MST, but its intensity increased over time to be the same as the other treatments starting at 4 MST. The intensity value of downy mildew disease in Asian Honey, Pioneer 21, and Pacific 339 corn developed from 18 HSI, ranging

from 10% to 20%, increasing to 39 HSI, ranging from 50% to 60%, then from 39 HSI to 60 HSI, it tended to be stable (Habibi *et al.*, 2017). The intensity value of corn rust increased in the 1-4 MST phase or vegetative phase and decreased at 5 MST, presumably because corn plants began to adapt to corn rust disease. Corn plants can have a self-defense mechanism against downy mildew in the form of a hypersensitive reaction, which is a rapid response of plants to infection that aims to limit the spread of pathogens and minimize damage to healthy corn tissue (Pakki *et al.*, 2019).

The level of corn plant resistance to downy mildew greatly affects the intensity of the disease. The intensity of downy mildew can decrease, presumably because the plants are resistant to downy mildew, thereby stopping the development of the disease. Plants physiologically stop pathogen invasion through recognition by host *resistance (R*) genes (Denance *et al*., 2013). Plants can also adapt to pathogens through AVR (*Avirulance)* genes, which interact with plant resistance genes (R) to detect effectors and trigger immune responses that inhibit or destroy pathogens (Kurata, 2020).

### 4.4.5. *Area Under the Disease Progress Curve* (AUDPC) Value

The resistance category of Paramita F1 corn varieties under inoculation treatments from different inoculum sources based on the *Area Under the Disease Progress Curve* (AUDPC) value is presented in Table 10.

Table 10. Corn Plant Resistance Categories of Paramita F1 Variety under Inoculation Treatment from Different Inoculum Sources Based on *Area Under the Disease Progress Curve* (AUDPC).

| Inoculum Source | AUDPC Value | Resistance Category |
|---|---|---|
| No Inoculum | 70.69 | Resistant |
| Grobogan | 84.35 | Resistant |
| Semarang | 80.01 | Tahan |
| Klaten | 81.69 | Resistant |

The AUDPC value without inoculum had the lowest value of 70.69, and Grobogan had the highest AUDPC value of 84.35, all of which were categorized as resistant. An AUDPC value of 0-100 indicates that corn plants are resistant to downy mildew (Putri and Kasiamdari, 2023). This AUDPC value can represent the severity of downy mildew disease over a certain period of time.

The lower the AUDPC value of the test corn plants, the higher the level of plant resistance to pathogens. The value that determines plant resistance to pathogens that cause downy mildew can be derived from the AUDPC value, where the AUDPC value is inversely proportional to plant resistance. The AUDPC value can be influenced by several factors, namely environmental factors such as humidity and temperature, which affect the intensity of downy mildew disease in corn plants (Putri and Kasiamdari, 2023).

### 4.4.6. Disease Infection Rate (r)

The disease infection rate of downy mildew on Paramita F1 corn plants with inoculation treatments from different inoculum sources is presented in Table 11.

Table 11. Disease Infection Rate (r) of Downy Mildew on Paramita F1 Corn Plants with Inoculation Treatments from Different Inoculum Sources
.

| Inoculum Source | r 1 MST | r 2 MST | r 3 MST | 4 MST | r 5 MST |
|---|---|---|---|---|---|
| Without inoculum | 0.00 | 0.28 | 0.68 | 1.49 | -0.69 |
| Grobogan | 0.00 | 0.57 | 0.15 | 1.18 | -0.69 |
| Semarang | 0.00 | 0.40 | 0.17 | 1.33 | -0.75 |
| Klaten | 0.00 | 0.40 | 0.31 | 1.18 | -0.69 |

The disease infection rate in all treatments, which was initially 0.00, increased over time as more plants became infected with downy mildew. The highest disease infection rate was observed at 4 MST and decreased at 5 MST in line with the reduction in infected plants as the plants became resistant to downy mildew. The treatment without inoculum had the lowest infection rate at 2 MST, but the infection rate increased over time until it was the same as the other treatments starting at 4 MST. A positive infection rate indicates that downy mildew on corn plants is developing and spreading, while a negative infection rate indicates that downy mildew on corn plants is beginning to decline (Wardani *et al.*, 2023).

Increased infection rates of downy mildew can be influenced by plants, pathogens, and environments that are not conducive to pathogen development. An increased infection rate is influenced by the host plants being grown, including susceptible plants, highly aggressive pathogens, and weather conditions that are very conducive to pathogen development. This causes the pathogen to quickly infect other healthy plants, meaning that in a relatively short time, the disease has spread over a wide area (Pajrin *et al.*, 2013). The infection rate of powdery mildew

( ) can also decrease due to the lack of support between plants, pathogens, and the environment. A decreased infection rate is influenced by the host plants that are resistant, non-aggressive pathogens, and weather that is less conducive to pathogen development. This causes the pathogen to take longer to infect healthy plants (Pajrin *et al.*, 2013).

### 4.4.7. Plant Height and Number of Leaves

Plant height and number of leaves on Paramita F1 corn plants with inoculation treatments from different inoculum sources are presented in Table 12.

Table 12. Plant Height and Number of Leaves in Paramita F1 Corn Plants with Inoculation Treatment from Different Inoculum Sources
Table 12. Plant Height and Number of Leaves in Paramita F

| Inoculum Source | Plant Height (cm) | Number of Leaves (pieces) |
|---|---|---|
| Without Inoculum | 123.60 | 8.53 |
| Grobogan | 120.47 | 7.73 |
| Semarang | 121.17 | 8.30 |
| Klaten | 122.03 | 8.50 |

The height of Paramita F1 corn plants treated with inoculation from different inoculum sources showed no significant difference based on the results of the analysis of variance. The insignificant difference in plant height may have occurred because the same pathogen species, *Peronosclerospora sorghi*, was used, and the disease incidence results showed that all plants were infected with downy mildew. The downy mildew pathogen inoculation treatment is also suspected to not cause differences in corn plant height compared to the generally uninoculated control ( ). Normal glutinous corn plants can reach a height of 125.85 cm at 5 MST (Lamakoma

*et al.*, 2019). Corn plants showing downy mildew symptoms do not always become stunted if the intensity is not too high and the plants can adapt to the pathogen. Plant physiology involves producing *phytoalexins* that can inhibit the release *of phytotoxins* from downy mildew, causing the pathogen that successfully invades the host plant's tissue cells to be unable to develop properly (Pakki *et al.*, 2019).

The number of leaves on Paramita F1 corn plants inoculated with different inoculum sources showed no significant difference based on the analysis of variance. The insignificant difference in the number of leaves may be due to the use of the same downy mildew pathogen species, *Peronosclerospora sorghi*, and the disease incidence results showing that all plants were infected with downy mildew. The downy mildew pathogen inoculation treatment was also suspected of not causing any difference in the number of corn leaves without downy mildew pathogen inoculation. Corn plants that grow normally at 18-35 days after planting can be categorized into the V6-V10 phase, which usually has 6-10 fully opened leaves (Aidah *et al.*, 2020).

### 4.4.8. Total Chlorophyll Content

The total chlorophyll content in Paramita F1 corn plants treated with inoculation from different inoculum sources is presented in Table 13.

Table 13. Total Chlorophyll Content in Paramita F1 Corn Plants with Inoculation Treatment from Different Inoculum Sources

| Inoculum Source | Chlorophyll Content at 3 MST | Chlorophyll Content at 5 MST |
|---|---|---|
| | ----------mg/g---------- | |

| | | |
|---|---|---|
| Without Inoculum | 0.99 | 2.25 |
| Grobogan | 0.84 | 2.02 |
| Semarang | 0.86 | 1.91 |
| Klaten | 1.02 | 2.27 |

The total chlorophyll content of Paramita F1 corn plants treated with inoculation from different inoculum sources showed no significant difference based on the results of the analysis of variance. The results of the total chlorophyll content of plants that did not differ significantly may have occurred because the pathogen species used was the same, namely *Peronosclerospora sorghi*, and the disease incidence results showed that all plants were infected with downy mildew. Inoculation of downy mildew pathogens on BS 0214, BS 0314, and BS 0114 corn feed plants showed no significant difference in total chlorophyll content, which was 2.587 mg/g, 2.512 mg/g, and 2.144 mg/g, respectively (Agustamia *et al.*, 2016).

The results of the total chlorophyll content analysis showed that as the age of the plant increased, so did its chlorophyll content, indicating that the plant was more resistant to downy mildew disease. Higher chlorophyll content indicates lower intensity of downy mildew disease in corn plants. The increase in chlorophyll content may indicate an increase in the resistance of corn plants to downy mildew disease (Agustamia *et al.*, 2016). This is in line with the results of the calculation of downy mildew disease intensity, which began to decline after 5 MST.

### 4.4.9. Stomatal Aperture and Stomatal Density

Stomatal opening and stomatal density in Paramita F1 corn plants treated with inoculation from different inoculum sources are presented in Table 14.

Table 14. Stomatal Opening and Stomatal Density in Paramita F1 Corn Plants with Inoculation Treatments from Different Sources Different Inoculants

| Source of Inoculum | Stomatal Opening (%) | Stomatal Density (stomata/mm²) |
|---|---|---|
| Without Inoculum | 78.65 | 13.96 |
| Grobogan | 84.27 | 14.39 |
| Semarang | 83.90 | 18.57 |
| Klaten | 85.43 | 15.36 |

The stomatal opening of Paramita F1 corn plants treated with inoculation from different inoculum sources showed no significant difference based on the results of the analysis of variance. The stomatal opening of plants that did not differ significantly may have occurred because the pathogen species used was the same, namely *Peronosclerospora sorghi*, and the disease incidence results showed that all plants were infected with downy mildew. The stomata of these plants are suspected to be used by downy mildew pathogens to infect corn plants. Stomata consist of guard cells and closing cells that have an automatic opening and closing mechanism, which can become an entry point for downy mildew pathogens. The more stomata that open, the greater the chance of infection by downy mildew pathogens (Agustamia *et al.*, 2016).

The stomatal density of Paramita F1 corn plants inoculated with different inoculum sources showed non- ly significant results based on the analysis of variance. The non-significant results in stomatal density may be due to the use of the same downy mildew pathogen species, *Peronosclerospora sorghi*, and the disease incidence results showing that all plants were infected with downy mildew. Inoculation of the pathogen that causes downy mildew disease in BS 0214, BS

0314, and BS 0114 corn varieties showed no significant difference in the stomatal density parameter, which was 67.093 stomata/mm², 81.778 stomata/mm², and 77.728 stomata/mm², respectively. A smaller stomatal density indicates a lower chance of infection by the pathogen causing downy mildew in corn plants, while a denser stomatal density increases the chance of infection by downy mildew (Agustamia *et al.*, 2016).

### 4.4.10. Fresh Weight and Dry Weight of the Top

The fresh weight and dry weight of the canopy in corn plants of the Paramita F1 variety with inoculation treatments from different inoculum sources are presented in Table 15.

Table 15. Fresh Weight and Dry Weight of Corn Cobs in Paramita F1 Corn Plants with Inoculation Treatments from Different Sources Different Inoculum Sources

| Inoculum Source | Fresh Weight | Dry Weight |
|---|---|---|
| Without inoculum | 47.63 | 7.70 |
| Grobogan | 43.10 | 6.29 |
| Semarang | 43.76 | 6.28 |
| Klaten | 43.42 | 6.50 |

The fresh weight and dry weight of corn plants of the Paramita F1 variety with inoculation treatments from different inoculum sources showed no significant differences based on the results of the analysis of variance. The non-significant differences in fresh weight and dry weight of corn plants may occur because the pathogen species used was the same, namely *Peronosclerospora sorghi*, and the disease incidence results showed that all plants were infected with downy mildew.

Inoculation of the pathogen causing downy mildew on sweet corn plants of the Bonanza F1 variety with control treatment, plant extract treatment (clove, noni, and garlic), and synthetic fungicide (active ingredient metalaxyl) showed no significant difference in wet weight and dry weight parameters at 5 MST (Giofanny *et al.*, 2014). This indicates that corn plants can adapt to pathogens that develop in their tissues so that damaged plant tissues can regrow, allowing the plants to continue growing even though they have been infected with downy mildew pathogens.

# CHAPTER V

# CONCLUSION AND RECOMMENDATIONS

## 5.1. Conclusion

The conclusion of this study is that the pathogen causing downy mildew disease in corn plants in the Grobogan, Semarang, and Klaten areas is *Peronosclerospora sorghi,* based on microscopic observations and supported by molecular identification using P. *sorghi-specific* primers. The Grobogan and Semarang inoculum treatments had the fastest incubation period even though they had the same species as the other treatments, presumably because they had a higher spore count. The treatment without inoculum still showed downy mildew symptoms because it was infected from other treatments as the pathogen can spread through wind or water.

## 5.2. Recommendations

The recommendation is the need for identification using multigene analysis and quarantine to control pathogen transmission, as *Peronosclerospora* sp. is *airborne*.

# REFERENCES

Aidah, S. N., and T. P. K. Indonesia. 2020. Encyclopedia of Corn: Description, Philosophy, Benefits, Cultivation, and Business Opportunities (Vol. 2). KBM Indonesia Publisher.

Adhi, S. R., F. Widiantini, and E. Yulia. 2019. Artificial inoculation method for testing infection *of Peronosclerospora maydis,* the causative agent of corn leaf blight. Jurnal Agro. 6 (1): 77-85.

Adhi, S. R., F. Widiantini, and E. Yulia. 2021. Morphometric and pathogenicity variations *of Peronosclerospora* sp., the cause of corn leaf blight disease on the island of Java, Indonesia. Indonesian Journal of Plant Pathology. 17 (5): 173-182.

Agrios, G. N. 2005. Introduction to plant pathology. Academic Press 5th edition.

Agustamia, C., A. Widiastuti, and C. Sumardiyono. 2016. The effect of stomata and chlorophyll on the resistance of several corn varieties to leaf blight. Indonesian Journal of Plant Protection. 20 (2): 89-94.

Alsohaili, S. A. and Bani-Hasan, B. M. 2018. Morphological and molecular identification of fungi isolated from different environmental sources in the northeastern desert of Jordan. Journal of Biological Sciences. 11 (3): 329-337.

Dewi, T.Q. and R.H. Paeru. 2017. Practical Guide to Corn Cultivation. Jakarta: Penebar Swadaya.

Dialista, R., and A. N. Sugiharto. 2018. The characteristics of sweet corn (*Zea mays* L. *saccharata* Sturt) at two altitudes. Journal of Agricultural Science. 2 (2): 155-163.

Dibia, N., and Suyarto, R. 2017. Corn Cultivation. Udayana University, Bali.

Elfarisna, E., E. Rahmayuni, and H. Gustia. 2023. The effect of green shell waste as an ameliorant on sweet corn plants. Indonesian Journal of Agricultural Science. 28 (4): 660-666

Ernaningsih, Z. 2021. Utilization of natural resources to improve the economy of the community in Mulusan Gunungkidul Village. Atma Inovasia Journal. 1 (4): 431-438.

Gabriel, B.P. and Riyanto. 1989. Metarhizium anisopliae (Metcha) Sor: Taxonomy, Pathology, Production, and Application. Directorate of Plant Protection, Ministry of Agriculture. Jakarta. 1-25.

Giofanny, W., Prasetyo, J., and Efri, E. 2014. The effect of several plant extracts on downy mildew disease in sweet corn (*Zea mays saccharata*). Jurnal Agrotek Tropika. 2 (3): 441-446.

Gunawan, S., H. Hasan, and R. D. W. Lubis. 2020. Utilization of corn cob adsorbents as activated carbon to reduce motor vehicle exhaust emissions. Journal of Materials, Manufacturing, and Energy Engineering. 3 (1): 38-47.

Tjasyono, Bayong. 2004. Climatology. ITB.

Harborne, J. B. 1987. Phytochemical Methods: A Guide to Modern Plant Analysis. Bandung: ITB Press. 1-78.

Hidayah, N., A. N. Istiani, and A. Septiani. 2020. Utilization of corn (*Zea mays*) as a basic ingredient for making corn chips to improve the economy of the community in Panca Tunggal Village. Journal of Community Service. 1 (1): 37-43.

Hikmawati, K. T., and P. M. Melina. 2011. Morphological characterization of *Peronosclerospora* sp., the causative agent of downy mildew in corn plants from several regions in Indonesia. Jurnal Fitomedika, 7 (3): 159-161.

Hoerussalam, A. P., and A. Khaeruni. 2013. Induction of corn (*Zea mays* L.) resistance to downy mildew through *seed treatment* and its inheritance in the S1 generation. Journal of Agricultural Science. 16 (2): 42-59.

Ivayani, I., F. Faishol, J. Prasetyo, and M. Nurdin. 2018. Effectiveness of several *Trichoderma* sp. isolates against downy mildew caused by *Peronosclerospora maydis* and corn (*Zea mays*) growth. Journal of Applied Agricultural Research. 18 (1): 39-45.

Kalqutny, S. H., S. Pakki, and A. Muis. 2020. The potential use of DNA-based molecular techniques in research on downy mildew in corn. Jurnal Agrotek Tropika. 5 (3): 163-168.

Khairiyah, K., S. Khadijah, M. Iqbal, S. Erwan, N. Norlian, and M. Mahdiannor. 2017. Growth and yield of three sweet corn varieties (*Zea mays saccharata* Sturt) with various doses of organic fertilizer on lowland swamp land. Journal of Agricultural Science. 42 (3): 230-240.

Khoiri, S., A. Abdiatun, K. Muhlisa, A. Amzeri, and D. Megasari. 2021. The incidence and severity of downy mildew disease on local Madurese maize crops in Sumenep district, East Java, Indonesia. Journal of Agrologia. 10 (1): 17-24.

Khoiri, S., K. Badami, G. Pawana, and C. S. Utami. 2021. Effectiveness of *Bacillus* isolates as a control for downy mildew disease and a growth promoter for corn plants under controlled conditions. Jurnal Rekayasa, 14 (2): 144-151.

Kurniawan, A. F., J. Prasetyo, and R. Suharjo. 2017. Identification and attack levels of downy mildew pathogens in East Lampung, Pesawaran, and South Lampung. Jurnal Agrotek Tropika. 5 (3): 163-168.

Lalujan, L. E., G. S. S. Djarkasi, T. J. N. Tuju, D. Rawung, and M. F. Sumual. 2017. Chemical and nutritional composition of local Manado yellow corn as a rice substitute. Journal of Agricultural Technology. 8 (1): 47-54.

Lamakoma, C. R., J. R. Patty, and M. Amba. 2019. The effect of liquid organic fertilizer and compound fertilizer on the growth and production of sticky corn (*Zea mays* var. *ceratina*). Journal of Agricultural Cultivation. 15 (2): 127-133.

Linnaeus, C. 1753. Species Plantarum: exhibentes plantas rite cognitas, ad genera relatas, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas. Holmiae: Impensis Laurentii Salvii.

Muhadjir, F. 1988. Characteristics of Corn Plants. Agricultural Research and Development Center, Bogor. 33-48.

Muis, A., M. J. Ryley, Y. P. Tan, R. Suharjo, N. Nonci, Y. Danaatmadja, I. Hidayat, A. Widiastuti, S. Widinugraheni, R. G. Shivas, and M. Thines. 2023. Peronosclerospora neglecta sp. nov. a widespread and overlooked threat to corn (maize) production in the tropics. Journal of Mycological Progress. 22 (2): 1-7.

Muis, A., N. Nonci, and M. B. Pabendon. 2016. Geographical distribution of *Peronosclerospora* sp., the causal organism of maize downy mildew, in Indonesia. Journal of Advances in Agriculture and Botanics. 8 (3): 143-155.

Muis, A., M. B. Pabendon, N. Nonci, and W. P. Waskito. 2013. Genetic diversity *of Peronosclerospora maydis,* the cause of downy mildew in corn, based on SSR marker analysis. Journal of Food Crop Research. 32 (3): 139-147.

Muis, A., Suriani, Kalqutny, S.H., and Nonci, N. 2018. Downy mildew disease in corn plants and control efforts. Deepublish. Sleman.

Munkvold, G., and D. White. 2016. Compendium of corn diseases (4th ed.). AACC International.

Oka, I. Nyoman. 1993. Introduction to Plant Disease Epidemiology.

Pajrin, J., J. Panggeso, I. Rosmini. 2013. Testing the resistance of several corn varieties (*Zea mays* L.) to the intensity of downy mildew disease (*Peronosclerospora maydis*) (Tadulako University).

Pakki, S. 2014. Epidemiology and control strategies for downy mildew (*Perenosclerospora* sp.) in corn crops. Journal of Agricultural Research and Development. 33 (2): 47-52.

Pakki, S., S. S. Aminah, and A. Musi. 2019. The effect of a combination of resistant varieties and metalaxyl fungicide on the incidence of *Peronosclerospora philippinensis* downy mildew disease in corn. Journal of Food Crop Agricultural Research. 3 (2): 91-99.

Prasetyo, J., C. Ginting, R. Suharjo, and H. M. Akin. 2020. Identification and testing of several Trichoderma isolates to control downy mildew (*Peronosclerospora* sp.) in corn plants. Jurnal Agrotek Tropika. 1 (1): 1-12.

Pribadi, D. U., S. Sutini, and M. Sodiq. 2021. Sweet Corn Cultivation. Graha Ilmu, Yogyakarta.

Putri, R. K., and R. S. Kasiamdari. 2023. Resistance of three corn varieties (*Zea mays* L.) to infection by the downy mildew fungus *Peronosclerospora maydis*. Scientific Journal of Biology, 14 (1): 21-31.

Ramlah, R. 2023. Molecular technology in maize (*Zea mays* L.) breeding to be applied to the agroforestry system. Journal of Agricultural Science. 3 (1): 77-82.

Rustiani, U. S., M. S. Sinaga, S. H. Hidayat, and S. Wiyono. 2015. Three species *of Peronosclerospora* causing corn downy mildew disease in Indonesia. Journal of Biology. 14 (1): 29-37.

Semangun, H. 2004. Plant Diseases in Indonesia. Gadjah Mada University Press. Yogyakarta

Setiawati, M, R., L. N. Linda, N. N. Kamaluddin, P. Suryatmana, and T. Simarmata. 2021. Application of ameliorant biofertilizer and NPK fertilizer on total N,

available P, and corn growth and yield on inceptisols. Jurnal Agro. 8 (2): 298-310.

Singh, J. 1987. Field Manual of Maize Breeding Procedures. Indian Agricultural Research Institute New Delhi. India.

Sireesha, Y., and R. Velazhahan. 2015. Assessing genetic diversity in *Peronosclerospora sorghi* causing downy mildew on maize and sorghum. Journal of Indian Phytopathology. 68 (1): 73-77.

Suharjo, R., I. G. Swibawa, J. Prasetyo, Y. Fitriana, Y. Danaatmadja, A. Budiawan, S. Roberts, N. Noorhajati, M. Amad, and M. Thines. 2020. Peronosclerospora australiensis is a synonym of P. *maydis,* which is widespread on Sumatra, and distinct from the most prevalent Java maize downy mildew pathogen. Journal of Mycological Progress. 19 (1): 1309-1315.

Sutama, K., S. Ratih, T. Maryono, and C. Ginting. 2015. The effect of *Paenibacillus polymyxa* bacteria and *Trichoderma* sp. fungi on downy mildew (*Peronosclerospora maydis* (Rac.) Shaw) in corn plants. Jurnal Agrotek Tropika. 3 (2): 199-203.

Tjasyono, Bayong. 2004. Climatology. ITB.

Wakman, W. and Burhanuddin. 2007. Management of Pre-harvest Corn Diseases. Cereal Crops Research Center. Maros. 310-313.

Wardani, N. K., M. E. Purwanto, and R. R. Brotodjojo. 2023. The effect of concentration and method of application *of Trichoderma* sp. on the incidence and severity of downy mildew (*Peronosclerospora* sp.) in sweet corn plants. Jurnal Agrivet. 29 (1): 61-73.

White, T. J., Sninsky, J. J, Galfand, D. H, and Innis, M. A. 1990. PCR Protocols: A guide to methods and applications. Academic Press, San Diego, CA. 1-437.

Widyasanti, A. A. 2023. Land area, production, and productivity of corn by province in Central Java, 2021-2023. Indonesian Central Statistics Agency.

# APPENDIX

**Appendix 1**. Description of Paramita F1 Corn Variety.

## DESCRIPTION OF PARAMITA F1 CORN VARIETY

| | |
|---|---|
| Origin | : PT. East West Seed Indonesia |
| Production code | : 679/Kpts/SR.120/1/2011 |
| Variety group | : single cross hybrid |
| Plant form | : erect |
| Plant height | : 2.20–2.35 m |
| Stem diameter | : 2.0–2.2 cm |
| Stem color | : green |
| Leaf shape | : drooping sword |
| Leaf size | : length 83–92 cm, width 7.5–8.7 cm |
| Leaf color | : green |
| *Tassel* shape | : curved and hanging |
| Tassel color | : pale yellow |
| Flowering period | : 48–53 days after planting |
| Harvest age | : 71–86 days after planting |
| Hair color | : white |
| Cob shape | : cylindrical |
| Ear size | : length 20.46–22.03 cm; diameter 4.75–4.95 cm |
| Color of cob | : yellowish white |
| Seed color | : white |
| Sugar content | : 7.6–8.5 °Brix |
| Number of seed rows | : 14–16 rows |
| Weight of 1,000 seeds | : 189–205 g |
| Weight per ear | : 285.86 – 330.6 g |
| Ear yield potential | : 21.4 – 23.59 tons/ha |
| Population per hectare | : 48,000 plants |
| Seed requirement/ha | : 9.55–10.20 kg |

**Appendix 2**. Fertilizer Requirement Calculation.

The recommended fertilizer dose for corn plants is 350 kg/ha or 350,000 g/ha of NPK fertilizer (16:16:16) (Setiawati *et al*., 2021).

The spacing between plants is 50×20 $cm^2$ .

$$\text{Population density/ha} = \frac{\text{Luas lahan}}{\text{Jarak tanam}}$$

$$= \frac{10.000}{0{,}5 \times 0{,}2}$$

$$= 100{,}000 \text{ plants/ha}$$

The required fertilizer dosage is:

$$\text{NPK} = \frac{\text{Dosis pupuk}}{\text{Populasi}}$$

$$= \frac{350.000}{100.000}$$

$$= 3.50 \text{ g/plant}$$

Fertilizer is applied twice at 1 MST (50% dose) and 3 MST (50% dose) by digging.

**Appendix 3**. Experimental *Layout* at the Pathogen Inoculation Stage.

| | | | |
|---|---|---|---|
| X1U1 | X3U1 | X2U2 | X0U1 |
| X3U2 | X2U3 | X0U2 | X1U2 |
| X1U3 | X2U1 | X3U3 | X0U3 |

Description:

$X_0$ : Without inoculum

$X_1$ : Sample from Semarang

$X_2$ : Sample from Klaten

$X_3$: Sample from Grobogan

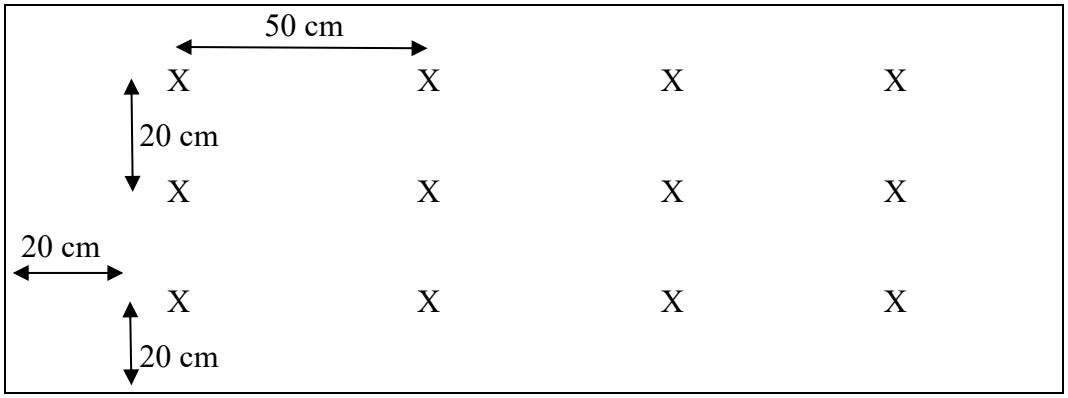
$U_1$: Replication 1

$U_2$ : Replication 2

$U_3$ : Replication 3

Each repetition consists of 10 plants

**Appendix 4**. *Layout of* the Experimental Plot at the Pathogen Inoculation Stage.




Description:

Bed height = 10-20 cm

Bed width = 80 cm

Bed length = 190 cm

Water channel width = 25-30 cm

Planting distance = 50 × 20 $cm^2$

**Appendix 5**. Measurement of Temperature, Humidity, and Sunlight Intensity from the Field.

| Age (HST) | Temperature (°C) | | | | Humidity (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | 08:00 | 12:00 | 4:00 PM | Average | 08:00 | 12:00 | 4:00 | Average |
| 1. | 30.8 | 33.7 | 32.6 | 32.0 | 83.0 | 67.0 | 79.0 | 76.3 |
| 2. | 29.7 | 34.1 | 33.4 | 31.7 | 85.0 | 52.0 | 73.0 | 70.0 |
| 3. | 30.2 | 33.6 | 32.2 | 31.6 | 82.0 | 64.0 | 77.0 | 74.3 |
| 4. | 30.4 | 34.5 | 32.8 | 32.0 | 83.0 | 58.0 | 69.0 | 70.0 |
| 5. | 30.6 | 34.9 | 33.0 | 32.3 | 85.0 | 75.0 | 69.0 | 76.3 |
| 6. | 31.0 | 35.8 | 32.9 | 32.7 | 84.0 | 75.0 | 85.0 | 81.3 |
| 7. | 30.9 | 35.7 | 32.5 | 32.5 | 83.0 | 76.0 | 84.0 | 81.0 |
| 8. | 30.3 | 35.1 | 32.3 | 32.0 | 84.0 | 75.0 | 88.0 | 82.3 |
| 9. | 30.0 | 34.9 | 31.9 | 31.7 | 96.0 | 98.0 | 63.0 | 85.7 |
| 10. | 31.4 | 35.8 | 33.2 | 33.0 | 95.0 | 96.0 | 65.0 | 85.3 |
| 11. | 31.2 | 35.9 | 32.9 | 32.8 | 96.0 | 97.0 | 63.0 | 85.3 |
| 12. | 29.6 | 34.5 | 30.7 | 31.1 | 93.0 | 95.0 | 64.0 | 84.0 |
| 13. | 28.9 | 34.4 | 30.8 | 30.8 | 85.0 | 85.0 | 75.0 | 81.7 |
| 14. | 28.8 | 34.2 | 31.0 | 30.7 | 94.0 | 96.0 | 63.0 | 84.3 |
| 15. | 28.9 | 35.3 | 31.2 | 31.1 | 96.0 | 97.0 | 98.0 | 97.0 |
| 16. | 29.9 | 34.7 | 30.6 | 31.3 | 95.0 | 97.0 | 95.0 | 95.7 |
| 17. | 29.8 | 34.9 | 30.8 | 31.3 | 98.0 | 65.0 | 79.0 | 80.7 |
| 18. | 30.1 | 34.4 | 30.3 | 31.2 | 96.0 | 98.0 | 63.0 | 85.7 |
| 19. | 30.4 | 35.5 | 31.7 | 32.0 | 90.0 | 95.0 | 60.0 | 81.7 |
| 20. | 28.8 | 34.9 | 31.0 | 30.9 | 98.0 | 69.0 | 63.0 | 76.7 |
| 21. | 31.2 | 34.6 | 32.4 | 32.4 | 85.0 | 90.0 | 60.0 | 78.3 |
| 22 | 31.1 | 35.6 | 34.5 | 33.1 | 58.0 | 52.0 | 62.0 | 57.3 |
| 23 | 31.6 | 35.5 | 32.8 | 32.9 | 85.0 | 48.0 | 64.0 | 65.7 |
| 24 | 28.4 | 33.5 | 33.1 | 30.9 | 96.0 | 63.0 | 73.0 | 77.3 |
| 25 | 30.6 | 35.6 | 34.8 | 32.9 | 39.0 | 59.0 | 55.0 | 51.0 |
| 26. | 29.5 | 34.8 | 34.2 | 32.0 | 88.0 | 61.0 | 69.0 | 72.7 |
| 27 | 29.6 | 35.7 | 33.2 | 32.0 | 75.0 | 53.0 | 68.0 | 65.3 |
| 28 | 30.3 | 35.6 | 32.9 | 32.3 | 85.0 | 46.0 | 57.0 | 62.7 |
| 29. | 32.1 | 36.1 | 33.9 | 33.6 | 63.0 | 66.0 | 67.0 | 65.3 |
| 30 | 30.9 | 36.4 | 32.0 | 32.6 | 85.0 | 53.0 | 73.0 | 70.3 |
| 31. | 31.8 | 35.9 | 31.3 | 32.7 | 73.0 | 53.0 | 65.0 | 63.7 |
| 32. | 29.3 | 33.7 | 30.3 | 30.7 | 89.0 | 65.0 | 84.0 | 79.3 |
| 33. | 30.9 | 34.6 | 29.7 | 31.5 | 80.0 | 54.0 | 82.0 | 72.0 |
| 34. | 30.1 | 33.6 | 32.8 | 31.7 | 83.0 | 54.0 | 79.0 | 72.0 |
| 35. | 28.3 | 33.3 | 32.5 | 30.6 | 88.0 | 53.0 | 70.0 | 70.3 |
| Average | 30.2 | 34.9 | 32.2 | 31.9 | 85 | 71 | 72 | 76.0 |

$$\text{Average Temperature} = \frac{((2 \times \text{suhu pagi}) + (\text{suhu siang}) + (\text{suhu sore}))}{4}$$

(Tjasyono and Bayong, 2004)

$$\text{Average Humidity} = \frac{\text{kelembaban pagi} + \text{kelembaban siang} + \text{kelembaban sore}}{4}$$

(Handoko, 1995)

**Appendix 6**. Incubation Period Data Processing.

Incubation Period Data for Bulai Disease

| Source Inoculum | Time of Onset of Symptoms | | | Total | Average |
|---|---|---|---|---|---|
| | Repeat 1 | Repeat 2 | Test 3 | | |
| | ----------hari---------- | | | | |
| Without inoculum | 17.80 | 16.50 | 17.00 | 51.30 | 17.10 |
| Grobogan | 14.30 | 15.10 | 13.80 | 43.20 | 14.40 |
| Semarang | 15.00 | 15.40 | 13.80 | 44.20 | 14.73 |
| Klaten | 16.00 | 14.80 | 14.60 | 45.40 | 15.13 |
| Total | 63.10 | 61.80 | 59.20 | 184.10 | |
| Average | 15.78 | 15.45 | 14.80 | | 15.34 |

Note: each sample contains 10 plants.

**1. Degrees of Freedom**

p (Treatment) = 4

u (Repeat) = 3

df Treatment = p – 1 = 4 – 1 = 3

db Error = p (u – 1) = 4 (3 – 1) = 8

Total degrees of freedom = db Treatment + db Error = 3 + 8 = 11

**2. Correction Factor (CF)**

$$FC = \frac{(\Sigma Yij)^2}{p.u} = \frac{(184,10)^2}{4.3} = 2824.40$$

**3. Sum of Squares (SS)**

JK Total $= [(17.80^2 + (16.50)^2 + \ldots\ldots + (14.60)^2] - FK$

$= 2841.83 - 2824.40$

$= 17.43$

SQ Treatment $= \frac{(51,30)^2 + (43,20)^2 + (44,20)^2 + (45,40)^2}{3} - FK$

$= 2837.58 - 2824.40$

$= 13.18$

JK Error = JK Total – JK Treatment

= 17.43 – 13.18

= 4.25

**4. Mean Square (MS)**

MS Treatment $= 0\frac{\text{JK Perlakuan}}{\text{db Perlakuan}} = 0\frac{13,18}{3} = 4.39$

MS Error $=\frac{\text{JK Galat}}{\text{db Galat}} =\frac{4,25}{8} = 0.53$

**5. Calculated F**

Calculated F for Treatment $=\frac{\text{KT Perlakuan}}{\text{KT Galat}} =\frac{4,39}{0,53} = 8.26$

**6. ANOVA Table**

| Source of Variation | df | SS | KT | Calculated F | F Table 5% |
|---|---|---|---|---|---|
| Treatment | 3 | 13.18 | 4.39 | 8.26** | 4.07 |
| Error | 8 | 4.25 | 0.53 | | |
| Total | 11 | 17.43 | | | |

Note: *: significant (calculated F > table F 5%) and n: not significant (calculated F < table F).

Coefficient of Variation (CV)

CV $=\frac{\sqrt{\text{KT Galat}}}{\text{rataan total}} \times 100\%$

$=\frac{\sqrt{0,53}}{15,34} \times 100\%$

= 4.75%

**7. Further Test of Significant Difference (BNJ) at the 0.05 Level**

Sd $=\sqrt{\frac{\text{KTG}}{\text{u}}} =\sqrt{\frac{0,53}{3}} = 0.42$

BNJ Table (0.05) = 4.89

Calculated BNJ = sd × BNJ Table (0.05)

= 0.42 × 4.89

= 2.06

| Treatment | Median | Without Inoculum | Klaten | Semarang | Grobogan | Notation |
|---|---|---|---|---|---|---|
| | | 17.10 | 15.13 | 14.73 | 14.40 | |
| Without inoculum | 17.10 | - | | | | a |
| Klaten | 15.13 | 1.97 | - | | | ab |
| Semarang | 14.73 | 2.37* | 1.97 | - | | bc |
| Grobogan | 14.40 | 2.70* | 2.37* | 1.97 | - | c |

Note: * = significant (>Calculated BNJ).

**Appendix 7**. Spore Viability Data Processing.

Spore Observation Data

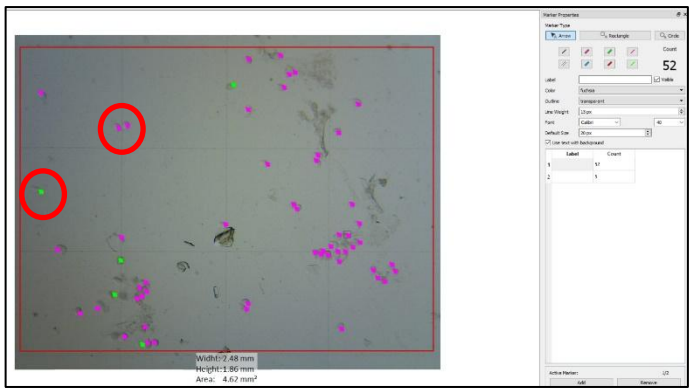
| Source Inoculum | Replication | Number of Spores (spores) | | | Spore Viability (%) |
|---|---|---|---|---|---|
| | | Viable Spores | Non-viable Spores | Number | |
| Without Inoculum | 1 | 21 | 2 | 23 | 91.30 |
| | 2 | 44 | 4 | 48 | 91.67 |
| | 3 | 55 | 9 | 64 | 85.94 |
| Grobogan | 1 | 62 | 7 | 69 | 89.86 |
| | 2 | 52 | 5 | 57 | 91.23 |
| | 3 | 42 | 6 | 48 | 87.50 |
| Semarang | 1 | 32 | 7 | 39 | 82.05 |
| | 2 | 20 | 3 | 23 | 86.96 |
| | 3 | 16 | 4 | 20 | 80.00 |
| Klaten | 1 | 62 | 11 | 73 | 84.93 |
| | 2 | 69 | 7 | 76 | 90.79 |
| | 3 | 36 | 8 | 44 | 81.82 |

Spore viability calculation:

$$V = \frac{g}{g + u} \times 100\%$$

Note: V = spore viability (%), g = number of germinated spores, and u = number of non-germinated spores.

Example of spore calculation in one unit (Grobogan 2) with magenta indicating viable spores and green indicating non-viable spores as follows:



Spore Viability Data

| Source Inoculation | Spore Viability (%) | | | Total | Average |
|---|---|---|---|---|---|
| | Repeat 1 | Repeat 2 | Repeat 3 | | |
| Control | 91.30 | 91.67 | 85.94 | 268.91 | 89.64 |
| Grobogan | 89.86 | 91.23 | 87.50 | 268.59 | 89.53 |
| Semarang | 82.05 | 86.96 | 80.00 | 249.01 | 83.00 |
| Klaten | 84.93 | 90.79 | 81.82 | 257.54 | 85.85 |
| Total | 348.14 | 360.65 | 335.26 | 1044.05 | |
| Average | 87.04 | 90.16 | 83.82 | | 87.00 |

**1. Degrees of Freedom**

p (Treatment) = 4

u (Repeat) = 3

df Treatment = p – 1 = 4 – 1 = 3

db Error = p (u – 1) = 4 (3 – 1) = 8

Total degrees of freedom = db Treatment + db Error = 3 + 8 = 11

**2. Correction Factor (CF)**

$$FC = \frac{(\Sigma Yij)^2}{p.u} = \frac{(1044{,}05)^2}{4.3} = 90836.70$$

**3. Sum of Squares (SS)**

JK Total $= [(91.30)^2 + (91.67)^2 + \ldots\ldots + (81.82)^2] - FK$

$= 91{,}023.43 - 90{,}836.70$

$= 186.73$

SQ Treatment $= \frac{(268{,}91)^2 + (268{,}59)^2 + (249{,}01)^2 + (257{,}54)^2}{3} - FK$

$= 90{,}928.67 - 90{,}836.70$

$= 91.97$

JK Error = JK Total – JK Treatment

$= 186.73 - 91.97$

$= 94.76$

**4. Mean Square (MS)**

MS Treatment $= 0\frac{\text{JK Perlakuan}}{\text{db Perlakuan}} = 0\frac{91,97}{3} = 30.66$

ME of Error $=\frac{\text{JK Galat}}{\text{db Galat}} =\frac{94,76}{8} = 11.85$

**5. Calculated F**

Calculated F for Treatment $=\frac{\text{KT Perlakuan}}{\text{KT Galat}} =\frac{30,66}{11,85} = 2.59$

**6. ANOVA Table**

| Source of Variation | df | SS | Sum of Squares | Calculated F | F Table 5% |
|---|---|---|---|---|---|
| Treatment | 3 | 91.97 | 30.66 | $2.59^{tn}$ | 4.07 |
| Error | 8 | 94.76 | 11.85 | | |
| Total | 11 | 186.73 | | | |

Note: *: significant (calculated F > table F 5%) and n: not significant (calculated F < table F).

Coefficient of Variation (CV)

CV $=\frac{\sqrt{\text{KT Galat}}}{\text{rataan total}} \times 100\%$

$=\frac{\sqrt{11,85}}{87,00} \times 100\%$

$= 3.96\%$

**Appendix 8**. Processing of Disease Incidence Data.

Incidence Data for Bulai Without Inoculum 1 MST

| Source of Inoculum | Plant Samples | Repeat 1 | Repeat 2 | Repeat 3 |
|---|---|---|---|---|
| Without Inoculum | 1 | 0 | 0 | 0 |
| | 2 | 0 | 0 | 0 |
| | 3 | 0 | 0 | 0 |
| | 4 | 0 | 0 | 0 |
| | 5 | 0 | 0 | 0 |
| | 6 | 0 | 0 | 0 |
| | 7 | 0 | 0 | 0 |
| | 8 | 0 | 0 | 0 |
| | 9 | 0 | 0 | 0 |
| | 10 | 0 | 0 | 0 |
| | Total | 0 | 0 | 0 |

Note: The number 0 indicates that the plant does not show symptoms of downy mildew, while the number 1 indicates that the plant shows symptoms of downy mildew.

Disease incidence calculation:

$$DI = \frac{n}{N} \times 100\%$$

Note: DI = *Disease* incidence (%), n = number of plants showing symptoms, and N = total number of plants observed.

Review 1 $= \frac{0}{10} \times 100\% = 0.00\%$

Test 2 $= \frac{0}{10} \times 100\% = 0.00\%$

Test 3 $= \frac{0}{10} \times 100\% = 0.00\%$

Without Inoculum 1 MST $= \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$

$= \frac{0{,}00\% + 0{,}00\% + 0{,}00\%}{3}$

$= 0.00\%$

Incidence Data Without Inoculum 2 MST

| Source of Inoculum | Plant Samples | Repeat 1 | Repeat 2 | Repeat 3 |
|---|---|---|---|---|
| Without Inoculum | 1 | 1 | 0 | 0 |
| | 2 | 1 | 0 | 1 |
| | 3 | 0 | 0 | 0 |
| | 4 | 0 | 1 | 0 |
| | 5 | 0 | 0 | 0 |
| | 6 | 1 | 0 | 1 |
| | 7 | 0 | 1 | 0 |
| | 8 | 0 | 0 | 1 |
| | 9 | 1 | 1 | 0 |
| | 10 | 0 | 0 | 0 |
| | Total | 4 | 3 | 3 |

Note: The number 0 indicates that the plant does not show symptoms of downy mildew, while the number 1 indicates that the plant shows symptoms of downy mildew.

Disease incidence calculation:

$$DI = \frac{n}{N} \times 100\%$$

Note: DI = *Disease* incidence (%), n = number of plants showing symptoms, and N = total number of plants observed.

$$\text{Repeat 1} = \frac{4}{10} \times 100\% = 40.00\%$$

$$\text{Review 2} = \frac{3}{10} \times 100\% = 30.00\%$$

$$\text{Test 3} = \frac{3}{10} \times 100\% = 30.00\%$$

$$\text{Without Inoculum 2 MST} = \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{40{,}00\% + 30{,}00\% + 30{,}00\%}{3}$$

$$= 33.33\%$$

Incidence Data Without Inoculum 3 MST

| Source of Inoculum | Plant Samples | Repeat 1 | Repeat 2 | Repeat 3 |
|---|---|---|---|---|
| Without Inoculum | 1 | 1 | 0 | 0 |
| | 2 | 1 | 0 | 1 |
| | 3 | 0 | 1 | 1 |
| | 4 | 0 | 1 | 1 |
| | 5 | 1 | 0 | 1 |
| | 6 | 1 | 1 | 1 |
| | 7 | 0 | 1 | 0 |
| | 8 | 0 | 1 | 1 |
| | 9 | 1 | 1 | 1 |
| | 10 | 0 | 1 | 0 |
| | Total | 5 | 7 | 7 |

Note: The number 0 indicates that the plant does not show symptoms of downy mildew, while the number 1 indicates that the plant shows symptoms of downy mildew.

Calculation of disease incidence:

$$DI = \frac{n}{N} \times 100\%$$

Note: DI = *Disease* incidence (%), n = number of plants showing symptoms, and N = total number of plants observed.

$$\text{Repeat 1} = \frac{5}{10} \times 100\% = 50.00\%$$

$$\text{Review 2} = \frac{7}{10} \times 100\% = 70.00\%$$

$$\text{Test 3} = \frac{7}{10} \times 100\% = 70.00\%$$

$$\text{Without Inoculum 3 MST} = \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{50{,}00\% + 70{,}00\% + 70{,}00\%}{3}$$

$$= 63.33\%$$

Incidence Data Without Inoculum 4 MST

| Source of Inoculum | Plant Samples | Repeat 1 | Repeat 2 | Repeat 3 |
|---|---|---|---|---|
| Without Inoculum | 1 | 1 | 1 | 1 |
| | 2 | 1 | 1 | 1 |
| | 3 | 1 | 1 | 1 |
| | 4 | 1 | 1 | 1 |
| | 5 | 1 | 1 | 1 |
| | 6 | 1 | 1 | 1 |
| | 7 | 1 | 1 | 1 |
| | 8 | 1 | 1 | 1 |
| | 9 | 1 | 1 | 1 |
| | 10 | 1 | 1 | 1 |
| | Total | 10 | 10 | 10 |

Note: The number 0 indicates that the plant does not show symptoms of downy mildew, while the number 1 indicates that the plant shows symptoms of downy mildew.

Disease incidence calculation:

$$DI = \frac{n}{N} \times 100\%$$

Note: DI = *Disease* incidence (%), n = number of plants showing symptoms, and N = total number of plants observed.

$$\text{Repeat 1} = \frac{10}{10} \times 100\% = 100.00\%$$

$$\text{Review 2} = \frac{10}{10} \times 100\% = 100.00\%$$

$$\text{Test 3} = \frac{10}{10} \times 100\% = 100.00\%$$

$$\text{Without Inoculum 4 MST} = \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{100{,}00\% + 100{,}00\% + 100{,}00\%}{3}$$

$$= 100.00\%$$

Incidence Data Without Inoculum 5 MST

| Source of Inoculum | Plant Samples | Repeat 1 | Repeat 2 | Repeat 3 |
|---|---|---|---|---|
| Without Inoculum | 1 | 1 | 1 | 1 |
| | 2 | 1 | 1 | 1 |
| | 3 | 1 | 1 | 1 |
| | 4 | 1 | 1 | 1 |
| | 5 | 1 | 1 | 1 |
| | 6 | 1 | 1 | 1 |
| | 7 | 1 | 1 | 1 |
| | 8 | 1 | 1 | 1 |
| | 9 | 1 | 1 | 1 |
| | 10 | 1 | 1 | 1 |
| | Total | 10 | 10 | 10 |

Note: The number 0 indicates that the plant does not show symptoms of downy mildew, while the number 1 indicates that the plant shows symptoms of downy mildew.

Disease incidence calculation:

$$DI = \frac{n}{N} \times 100\%$$

Note: DI = *Disease* incidence (%), n = number of plants showing symptoms, and N = total number of plants observed.

$$\text{Repeat 1} = \frac{10}{10} \times 100\% = 100.00\%$$

$$\text{Review 2} = \frac{10}{10} \times 100\% = 100.00\%$$

$$\text{Test 3} = \frac{10}{10} \times 100\% = 100.00\%$$

$$\text{Without Inoculum 5 MST} = \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{100{,}00\% + 100{,}00\% + 100{,}00\%}{3}$$

$$= 100.00\%$$

Incidence Data for Grobogan 1 MST

| Source of Inoculum | Plant Samples | Repeat 1 | Repeat 2 | Repeat 3 |
|---|---|---|---|---|
| Grobogan | 1 | 0 | 0 | 0 |
| | 2 | 0 | 0 | 0 |
| | 3 | 0 | 0 | 0 |
| | 4 | 0 | 0 | 0 |
| | 5 | 0 | 0 | 0 |
| | 6 | 0 | 0 | 0 |
| | 7 | 0 | 0 | 0 |
| | 8 | 0 | 0 | 0 |
| | 9 | 0 | 0 | 0 |
| | 10 | 0 | 0 | 0 |
| | Total | 0 | 0 | 0 |

Note: The number 0 indicates that the plant does not show symptoms of downy mildew, while the number 1 indicates that the plant shows symptoms of downy mildew.

Disease incidence calculation:

$$DI = \frac{n}{N} \times 100\%$$

Note: DI = *Disease* incidence (%), n = number of plants showing symptoms, and N = total number of plants observed.

Replication 1 $= \frac{0}{10} \times 100\% = 0.00\%$

Repeat 2 $= \frac{0}{10} \times 100\% = 0.00\%$

Repeat 3 $= \frac{0}{10} \times 100\% = 0.00\%$

Grobogan 1 MST $= \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$

$= \frac{0{,}00\% + 0{,}00\% + 0{,}00\%}{3}$

$= 0.00\%$

Incidence Data for Bulai Grobogan 2 MST

| Source of Inoculum | Plant Samples | Repeat 1 | Repeat 2 | Repeat 3 |
|---|---|---|---|---|
| Grobogan | 1 | 1 | 1 | 1 |
| | 2 | 1 | 1 | 0 |
| | 3 | 1 | 1 | 1 |
| | 4 | 1 | 0 | 1 |
| | 5 | 1 | 1 | 1 |
| | 6 | 1 | 1 | 1 |
| | 7 | 1 | 0 | 1 |
| | 8 | 1 | 0 | 1 |
| | 9 | 0 | 1 | 1 |
| | 10 | 0 | 0 | 1 |
| | Total | 8 | 6 | 9 |

Note: The number 0 indicates that the plant does not show symptoms of downy mildew, while the number 1 indicates that the plant shows symptoms of downy mildew.

Disease incidence calculation:

$$DI = \frac{n}{N} \times 100\%$$

Note: DI = *Disease* incidence (%), n = number of plants showing symptoms, and N = total number of plants observed.

Replication 1 $= \frac{8}{10} \times 100\% = 80.00\%$

Repeat 2 $= \frac{6}{10} \times 100\% = 60.00\%$

Repeat 3 $= \frac{9}{10} \times 100\% = 90.00\%$

Grobogan 2 MST $= \frac{\text{Ulangan 1 + Ulangan 2 + Ulangan 3}}{3}$

$= \frac{80{,}00\% + 60{,}00\% + 90{,}00\%}{3}$

$= 76.67\%$

Incidence Data for Bulai Grobogan 3 MST

| Source of Inoculum | Plant Samples | Repeat 1 | Repeat 2 | Repeat 3 |
|---|---|---|---|---|
| Grobogan | 1 | 1 | 1 | 1 |
| | 2 | 1 | 1 | 0 |
| | 3 | 1 | 1 | 1 |
| | 4 | 1 | 0 | 1 |
| | 5 | 1 | 1 | 1 |
| | 6 | 1 | 1 | 1 |
| | 7 | 1 | 1 | 1 |
| | 8 | 1 | 0 | 1 |
| | 9 | 1 | 1 | 1 |
| | 10 | 0 | 0 | 1 |
| | Total | 9 | 7 | 9 |

Note: The number 0 indicates that the plant does not show symptoms of downy mildew, while the number 1 indicates that the plant shows symptoms of downy mildew.

Calculation of disease incidence:

$$DI = \frac{n}{N} \times 100\%$$

Note: DI = *Disease* incidence (%), n = number of plants showing symptoms, and N = total number of plants observed.

$$\text{Repeat 1} = \frac{9}{10} \times 100\% = 90.00\%$$

$$\text{Replication 2} = \frac{7}{10} \times 100\% = 70.00\%$$

$$\text{Repeat 3} = \frac{9}{10} \times 100\% = 90.00\%$$

$$\text{Grobogan 3 MST} = \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{90{,}00\% + 70{,}00\% + 90{,}00\%}{3}$$

$$= 83.33\%$$

Incidence Data for Grobogan 4 MST

| Source of Inoculum | Plant Samples | Repeat 1 | Repeat 2 | Review 3 |
|---|---|---|---|---|
| Grobogan | 1 | 1 | 1 | 1 |
| | 2 | 1 | 1 | 1 |
| | 3 | 1 | 1 | 1 |
| | 4 | 1 | 1 | 1 |
| | 5 | 1 | 1 | 1 |
| | 6 | 1 | 1 | 1 |
| | 7 | 1 | 1 | 1 |
| | 8 | 1 | 1 | 1 |
| | 9 | 1 | 1 | 1 |
| | 10 | 1 | 1 | 1 |
| | Total | 10 | 10 | 10 |

Note: The number 0 indicates that the plant does not show symptoms of downy mildew, while the number 1 indicates that the plant shows symptoms of downy mildew.

Disease incidence calculation:

$$DI = \frac{n}{N} \times 100\%$$

Note: DI = *Disease* incidence (%), n = number of plants showing symptoms, and N = total number of plants observed.

$$\text{Repeat 1} = \frac{10}{10} \times 100\% = 100.00\%$$

$$\text{Replication 2} = \frac{10}{10} \times 100\% = 100.00\%$$

$$\text{Repeat 3} = \frac{10}{10} \times 100\% = 100.00\%$$

$$\text{Grobogan 4 MST} = \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{100{,}00\% + 100{,}00\% + 100{,}00\%}{3}$$

$$= 100.00\%$$

Incidence Data for Grobogan 5 MST

| Source of Inoculum | Plant Samples | Repeat 1 | Repeat 2 | Repeat 3 |
|---|---|---|---|---|
| Grobogan | 1 | 1 | 1 | 1 |
| | 2 | 1 | 1 | 1 |
| | 3 | 1 | 1 | 1 |
| | 4 | 1 | 1 | 1 |
| | 5 | 1 | 1 | 1 |
| | 6 | 1 | 1 | 1 |
| | 7 | 1 | 1 | 1 |
| | 8 | 1 | 1 | 1 |
| | 9 | 1 | 1 | 1 |
| | 10 | 1 | 1 | 1 |
| | Total | 10 | 10 | 10 |

Note: The number 0 indicates that the plant does not show symptoms of downy mildew, while the number 1 indicates that the plant shows symptoms of downy mildew.

Disease incidence calculation:

$$DI = \frac{n}{N} \times 100\%$$

Note: DI = *Disease* incidence (%), n = number of plants showing symptoms, and N = total number of plants observed.

Repeat 1 $= \frac{10}{10} \times 100\% = 100.00\%$

Replication 2 $= \frac{10}{10} \times 100\% = 100.00\%$

Repeat 3 $= \frac{10}{10} \times 100\% = 100.00\%$

Grobogan 5 MST $= \frac{\text{Ulangan 1 + Ulangan 2 + Ulangan 3}}{3}$

$= \frac{100{,}00\% + 100{,}00\% + 100{,}00\%}{3}$

$= 100.00\%$

Incidence Data for Bulai Semarang 1 MST

| Source of Inoculum | Plant Samples | Repeat 1 | Repeat 2 | Repeat 3 |
|---|---|---|---|---|
| Semarang | 1 | 0 | 0 | 0 |
| | 2 | 0 | 0 | 0 |
| | 3 | 0 | 0 | 0 |
| | 4 | 0 | 0 | 0 |
| | 5 | 0 | 0 | 0 |
| | 6 | 0 | 0 | 0 |
| | 7 | 0 | 0 | 0 |
| | 8 | 0 | 0 | 0 |
| | 9 | 0 | 0 | 0 |
| | 10 | 0 | 0 | 0 |
| | Total | 0 | 0 | 0 |

Note: The number 0 indicates that the plant does not show symptoms of downy mildew, while the number 1 indicates that the plant shows symptoms of downy mildew.

Disease incidence calculation:

$$DI = \frac{n}{N} \times 100\%$$

Note: DI = *Disease* incidence (%), n = number of plants showing symptoms, and N = total number of plants observed.

Replication 1 $= \frac{0}{10} \times 100\% = 0.00\%$

Repeat 2 $= \frac{0}{10} \times 100\% = 0.00\%$

Repeat 3 $= \frac{0}{10} \times 100\% = 0.00\%$

Semarang 1 MST $= \frac{\text{Ulangan 1 + Ulangan 2 + Ulangan 3}}{3}$

$= \frac{0{,}00\% + 0{,}00\% + 0{,}00\%}{3}$

$= 0.00\%$

Incidence Data for Bulai Semarang 2 MST

| Source of Inoculum | Plant Samples | Repeat 1 | Repeat 2 | Repeat 3 |
|---|---|---|---|---|
| Semarang | 1 | 0 | 1 | 1 |
| | 2 | 1 | 1 | 1 |
| | 3 | 1 | 1 | 1 |
| | 4 | 0 | 1 | 1 |
| | 5 | 1 | 0 | 0 |
| | 6 | 1 | 0 | 1 |
| | 7 | 1 | 1 | 0 |
| | 8 | 1 | 0 | 1 |
| | 9 | 1 | 0 | 0 |
| | 10 | 0 | 1 | 1 |
| | Total | 7 | 6 | 7 |

Note: A score of 0 indicates that the plant shows no symptoms of downy mildew, while a score of 1 indicates that the plant shows symptoms of downy mildew.

Disease incidence calculation:

$$DI = \frac{n}{N} \times 100\%$$

Note: DI = *Disease* incidence (%), n = number of plants showing symptoms, and N = total number of plants observed.

$$\text{Repeat 1} = \frac{7}{10} \times 100\% = 70.00\%$$

$$\text{Repeat 2} = \frac{6}{10} \times 100\% = 60.00\%$$

$$\text{Repeat 3} = \frac{7}{10} \times 100\% = 70.00\%$$

$$\text{Semarang 2 MST} = \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{70{,}00\% + 60{,}00\% + 70{,}00\%}{3}$$

$$= 66.67\%$$

Incidence Data for Semarang 3 MST

| Source of Inoculum | Plant Samples | Repeat 1 | Repeat 2 | Repeat 3 |
|---|---|---|---|---|
| Semarang | 1 | 0 | 1 | 1 |
| | 2 | 1 | 1 | 1 |
| | 3 | 1 | 1 | 1 |
| | 4 | 0 | 1 | 1 |
| | 5 | 1 | 0 | 0 |
| | 6 | 1 | 0 | 1 |
| | 7 | 1 | 1 | 1 |
| | 8 | 1 | 0 | 1 |
| | 9 | 1 | 0 | 1 |
| | 10 | 0 | 1 | 1 |
| | Total | 7 | 6 | 9 |

Note: The number 0 indicates that the plant does not show symptoms of downy mildew, while the number 1 indicates that the plant shows symptoms of downy mildew.

Calculation of disease incidence:

$$DI = \frac{n}{N} \times 100\%$$

Description: DI = *Disease* incidence (%), n = number of symptomatic plants, and N = total number of plants observed.

$$\text{Repeat 1} = \frac{7}{10} \times 100\% = 70.00\%$$

$$\text{Repeat 2} = \frac{6}{10} \times 100\% = 60.00\%$$

$$\text{Repeat 3} = \frac{9}{10} \times 100\% = 90.00\%$$

$$\text{Semarang 3 MST} = \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{70{,}00\% + 60{,}00\% + 90{,}00\%}{3}$$

$$= 73.33\%$$

Incidence Data for Semarang 4 MST

| Source of Inoculum | Plant Samples | Repeat 1 | Repeat 2 | Repeat 3 |
|---|---|---|---|---|
| Semarang | 1 | 1 | 1 | 1 |
| | 2 | 1 | 1 | 1 |
| | 3 | 1 | 1 | 1 |
| | 4 | 1 | 1 | 1 |
| | 5 | 1 | 1 | 1 |
| | 6 | 1 | 1 | 1 |
| | 7 | 1 | 1 | 1 |
| | 8 | 1 | 1 | 1 |
| | 9 | 1 | 1 | 1 |
| | 10 | 1 | 1 | 1 |
| | Total | 10 | 10 | 10 |

Note: The number 0 indicates that the plant does not show symptoms of downy mildew, while the number 1 indicates that the plant shows symptoms of downy mildew.

Calculation of disease incidence:

$$DI = \frac{n}{N} \times 100\%$$

Note: DI = *Disease* incidence (%), n = number of plants showing symptoms, and N = total number of plants observed.

Review 1 $= \frac{10}{10} \times 100\% = 100.00\%$

Test 2 $= \frac{10}{10} \times 100\% = 100.00\%$

Test 3 $= \frac{10}{10} \times 100\% = 100.00\%$

Semarang 4 MST $= \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$

$= \frac{100{,}00\% + 100{,}00\% + 100{,}00\%}{3}$

$= 100.00\%$

Incidence Data for Bulai Semarang 5 MST

| Source of Inoculum | Plant Samples | Repeat 1 | Repeat 2 | Repeat 3 |
|---|---|---|---|---|
| Semarang | 1 | 1 | 1 | 1 |
| | 2 | 1 | 1 | 1 |
| | 3 | 1 | 1 | 1 |
| | 4 | 1 | 1 | 1 |
| | 5 | 1 | 1 | 1 |
| | 6 | 1 | 1 | 1 |
| | 7 | 1 | 1 | 1 |
| | 8 | 1 | 1 | 1 |
| | 9 | 1 | 1 | 1 |
| | 10 | 1 | 1 | 1 |
| | Total | 10 | 10 | 10 |

Note: The number 0 indicates that the plant does not show symptoms of downy mildew, while the number 1 indicates that the plant shows symptoms of downy mildew.

Disease incidence calculation:

$$DI = \frac{n}{N} \times 100\%$$

Note: DI = *Disease* incidence (%), n = number of plants showing symptoms, and N = total number of plants observed.

Repeat 1 $= \frac{10}{10} \times 100\% = 100.00\%$

Replication 2 $= \frac{10}{10} \times 100\% = 100.00\%$

Review 3 $= \frac{10}{10} \times 100\% = 100.00\%$

Semarang 5 MST $= \frac{\text{Ulangan 1 + Ulangan 2 + Ulangan 3}}{3}$

$= \frac{100{,}00\% + 100{,}00\% + 100{,}00\%}{3}$

$= 100.00\%$

Incidence Data for Klaten 1 MST

| Source of Inoculum | Plant Samples | Repeat 1 | Repeat 2 | Repeat 3 |
|---|---|---|---|---|
| Klaten | 1 | 0 | 0 | 0 |
| | 2 | 0 | 0 | 0 |
| | 3 | 0 | 0 | 0 |
| | 4 | 0 | 0 | 0 |
| | 5 | 0 | 0 | 0 |
| | 6 | 0 | 0 | 0 |
| | 7 | 0 | 0 | 0 |
| | 8 | 0 | 0 | 0 |
| | 9 | 0 | 0 | 0 |
| | 10 | 0 | 0 | 0 |
| | Total | 0 | 0 | 0 |

Note: The number 0 indicates that the plant does not show symptoms of downy mildew, while the number 1 indicates that the plant shows symptoms of downy mildew.

Disease incidence calculation:

$$DI = \frac{n}{N} \times 100\%$$

Note: DI = *Disease* incidence (%), n = number of plants showing symptoms, and N = total number of plants observed.

Replication 1 $= \frac{0}{10} \times 100\% = 0.00\%$

Repeat 2 $= \frac{0}{10} \times 100\% = 0.00\%$

Repeat 3 $= \frac{0}{10} \times 100\% = 0.00\%$

Klaten 1 MST $= \frac{\text{Ulangan 1 + Ulangan 2 + Ulangan 3}}{3}$

$= \frac{0{,}00\% + 0{,}00\% + 0{,}00\%}{3}$

$= 0.00\%$

Data on the Incidence of Bulai Klaten 2 MST

| Source of Inoculum | Plant Samples | Repeat 1 | Repeat 2 | Repeat 3 |
|---|---|---|---|---|
| Klaten | 1 | 0 | 1 | 1 |
| | 2 | 0 | 1 | 1 |
| | 3 | 1 | 1 | 1 |
| | 4 | 0 | 1 | 0 |
| | 5 | 1 | 0 | 0 |
| | 6 | 0 | 1 | 0 |
| | 7 | 1 | 0 | 1 |
| | 8 | 1 | 1 | 1 |
| | 9 | 1 | 0 | 1 |
| | 10 | 1 | 0 | 1 |
| | Total | 6 | 6 | 7 |

Note: The number 0 indicates that the plant does not show symptoms of downy mildew, while the number 1 indicates that the plant shows symptoms of downy mildew.

Disease incidence calculation:

$$DI = \frac{n}{N} \times 100\%$$

Note: DI = *Disease* incidence (%), n = number of plants showing symptoms, and N = total number of plants observed.

$$\text{Repeat 1} = \frac{6}{10} \times 100\% = 60.00\%$$

$$\text{Repeat 2} = \frac{6}{10} \times 100\% = 60.00\%$$

$$\text{Repeat 3} = \frac{7}{10} \times 100\% = 70.00\%$$

$$\text{Klaten 2 MST} = \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{60{,}00\% + 60{,}00\% + 70{,}00\%}{3}$$

$$= 63.33\%$$

Incidence Data for Klaten 3 MST

| Source of Inoculum | Plant Samples | Repeat 1 | Repeat 2 | Repeat 3 |
|---|---|---|---|---|
| Klaten | 1 | 0 | 1 | 1 |
| | 2 | 0 | 1 | 1 |
| | 3 | 1 | 1 | 1 |
| | 4 | 0 | 1 | 1 |
| | 5 | 1 | 1 | 1 |
| | 6 | 0 | 1 | 1 |
| | 7 | 1 | 1 | 0 |
| | 8 | 1 | 1 | 1 |
| | 9 | 1 | 1 | 1 |
| | 10 | 1 | 1 | 1 |
| | Total | 6 | 10 | 9 |

Note: The number 0 indicates that the plant does not show symptoms of downy mildew, while the number 1 indicates that the plant shows symptoms of downy mildew.

Calculation of disease incidence:

$$DI = \frac{n}{N} \times 100\%$$

Note: DI = *Disease* incidence (%), n = number of plants showing symptoms, and N = total number of plants observed.

$$\text{Repeat 1} = \frac{6}{10} \times 100\% = 60.00\%$$

$$\text{Repeat 2} = \frac{10}{10} \times 100\% = 100.00\%$$

$$\text{Repeat 3} = \frac{9}{10} \times 100\% = 90.00\%$$

$$\text{Klaten 3 MST} = \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{60{,}00\% + 100{,}00\% + 90{,}00\%}{3}$$

$$= 83.33\%$$

Incidence Data for Klaten 4 MST

| Source of Inoculum | Plant Samples | Repeat 1 | Repeat 2 | Repeat 3 |
|---|---|---|---|---|
| Klaten | 1 | 1 | 1 | 1 |
| | 2 | 1 | 1 | 1 |
| | 3 | 1 | 1 | 1 |
| | 4 | 1 | 1 | 1 |
| | 5 | 1 | 1 | 1 |
| | 6 | 1 | 1 | 1 |
| | 7 | 1 | 1 | 1 |
| | 8 | 1 | 1 | 1 |
| | 9 | 1 | 1 | 1 |
| | 10 | 1 | 1 | 1 |
| | Total | 10 | 10 | 10 |

Note: The number 0 indicates that the plant does not show symptoms of downy mildew, while the number 1 indicates that the plant shows symptoms of downy mildew.

Disease incidence calculation:

$$DI = \frac{n}{N} \times 100\%$$

Note: DI = *Disease* incidence (%), n = number of plants showing symptoms, and N = total number of plants observed.

Repeat 1 $= \frac{10}{10} \times 100\% = 100.00\%$

Replication 2 $= \frac{10}{10} \times 100\% = 100.00\%$

Repeat 3 $= \frac{10}{10} \times 100\% = 100.00\%$

Klaten 4 MST $= \frac{\text{Ulangan 1 + Ulangan 2 + Ulangan 3}}{3}$

$= \frac{100{,}00\% + 100{,}00\% + 100{,}00\%}{3}$

$= 100.00\%$

Incidence Data for Klaten 5 MST

| Source of Inoculum | Plant Samples | Repeat 1 | Repeat 2 | Repeat 3 |
|---|---|---|---|---|
| Klaten | 1 | 1 | 1 | 1 |
| | 2 | 1 | 1 | 1 |
| | 3 | 1 | 1 | 1 |
| | 4 | 1 | 1 | 1 |
| | 5 | 1 | 1 | 1 |
| | 6 | 1 | 1 | 1 |
| | 7 | 1 | 1 | 1 |
| | 8 | 1 | 1 | 1 |
| | 9 | 1 | 1 | 1 |
| | 10 | 1 | 1 | 1 |
| | Total | 10 | 10 | 10 |

Note: The number 0 indicates that the plant does not show symptoms of downy mildew, while the number 1 indicates that the plant shows symptoms of downy mildew.

Disease incidence calculation:

$$DI = \frac{n}{N} \times 100\%$$

Note: DI = *Disease* incidence (%), n = number of plants showing symptoms, and N = total number of plants observed.

Repeat 1 $= \frac{10}{10} \times 100\% = 100.00\%$

Replication 2 $= \frac{10}{10} \times 100\% = 100.00\%$

Repeat 3 $= \frac{10}{10} \times 100\% = 100.00\%$

Klaten 5 MST $= \frac{\text{Ulangan 1 + Ulangan 2 + Ulangan 3}}{3}$

$= \frac{100{,}00\% + 100{,}00\% + 100{,}00\%}{3}$

$= 100.00\%$

**Appendix 9**. Processing of Disease Intensity Data.

Disease Intensity Data Without Inoculum 1 MST

| Source of Inoculum | Plant Samples | Replication 1 Scoring | Replication Score 2 | Replication Score 3 |
|---|---|---|---|---|
| Without Inoculum | 1 | 0 | 0 | 0 |
| | 2 | 0 | 0 | 0 |
| | 3 | 0 | 0 | 0 |
| | 4 | 0 | 0 | 0 |
| | 5 | 0 | 0 | 0 |
| | 6 | 0 | 0 | 0 |
| | 7 | 0 | 0 | 0 |
| | 8 | 0 | 0 | 0 |
| | 9 | 0 | 0 | 0 |
| | 10 | 0 | 0 | 0 |

Description: (0) No infection, (1) Mild attack 0% - 20%. (2) Moderate attack 20% - 40%. (3) Moderate attack 40% - 50%. (4) Moderately severe attack 50% - 75%. (5) Severe attack 75% - 100%.

Disease intensity calculation:

$$DS = \frac{\Sigma(ni \times vi)}{N \times V} \times 100\%$$

Description: DS = *Disease severity* or disease intensity (%), ni = number of plants observed from attack category i, vi = scale value of attack category i, N = total number of plants observed, and V = scale value of the highest attack category.

Replication 1

$$= \frac{(10\times0) + (0\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 0.00\%$$

Repeat 2

$$= \frac{(10\times0) + (0\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 0.00\%$$

Test 3

$$= \frac{(10\times0) + (0\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 0.00\%$$

Without Inoculum 1 MST

$$= \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{0{,}00\% + 0{,}00\% + 0{,}00\%}{3}$$

$$= 0.00\%$$

Data on Bulai Intensity Without Inoculum 2 MST

| Inoculum Source | Plant Samples | Repeat Scoring 1 | Repeat Scoring 2 | Replication Score 3 |
|---|---|---|---|---|
| Without Inoculum | 1 | 1 | 0 | 0 |
| | 2 | 1 | 0 | 1 |
| | 3 | 0 | 0 | 0 |
| | 4 | 0 | 1 | 0 |
| | 5 | 0 | 0 | 0 |
| | 6 | 1 | 0 | 1 |
| | 7 | 0 | 1 | 0 |
| | 8 | 0 | 0 | 1 |
| | 9 | 1 | 1 | 0 |
| | 10 | 0 | 0 | 0 |

Description: (0) No infection, (1) Mild attack 0% - 20%. (2) Moderate attack 20% - 40%. (3) Moderate attack 40% - 50%. (4) Moderately severe attack 50% - 75%. (5) Severe attack 75% - 100%.

Disease intensity calculation:

$$DS = \frac{\Sigma(ni \times vi)}{N \times V} \times 100\%$$

Description: DS = *Disease severity* or disease intensity (%), ni = number of plants observed from attack category i, vi = scale value of attack category i, N = total number of plants observed, and V = scale value of the highest attack category.

Replication 1

$$= \frac{(6\times0) + (4\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 8.00\%$$

Repeat 2

$$= \frac{(7\times0) + (3\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 6.00\%$$

Test 3

$$= \frac{(7\times0) + (3\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 6.00\%$$

Without Inoculum 2 MST

$$= \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{8{,}00\% + 6{,}00\% + 6{,}00\%}{3}$$

$$= 6.67\%$$

Data on Bulai Intensity Without Inoculum 3 MST

| Inoculum Source | Plant Samples | Repeat Scoring 1 | Repeat Scoring 2 | Replication Score 3 |
|---|---|---|---|---|
| Without Inoculum | 1 | 1 | 0 | 0 |
| | 2 | 1 | 0 | 1 |
| | 3 | 0 | 1 | 1 |
| | 4 | 0 | 1 | 1 |
| | 5 | 1 | 0 | 1 |
| | 6 | 1 | 1 | 1 |
| | 7 | 0 | 1 | 0 |
| | 8 | 0 | 1 | 1 |
| | 9 | 1 | 1 | 1 |
| | 10 | 0 | 1 | 0 |

Description: (0) No infection, (1) Mild attack 0% - 20%. (2) Moderate attack 20% - 40%. (3) Moderate attack 40% - 50%. (4) Moderately severe attack 50% - 75%. (5) Severe attack 75% - 100%.

Disease intensity calculation:

$$DS = \frac{\Sigma(ni \times vi)}{N \times V} \times 100\%$$

Description: DS = *Disease severity* or disease intensity (%), ni = number of plants observed from attack category i, vi = scale value of attack category i, N = total number of plants observed, and V = scale value of the highest attack category.

Replication 1

$$= \frac{(5\times0) + (5\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 10.00\%$$

Repeat 2

$$= \frac{(3\times0) + (7\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 14.00\%$$

Test 3

$$= \frac{(3\times0) + (7\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 14.00\%$$

Without Inoculum 3 MST

$$= \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{10{,}00\% + 14{,}00\% + 14{,}00\%}{3}$$

$$= 12.67\%$$

Data on Bulai Intensity Without Inoculum 4 MST

| Source of Inoculum | Plant Samples | Repeat Scoring 1 | Repeat Scoring 2 | Replication Score 3 |
|---|---|---|---|---|
| Without Inoculum | 1 | 2 | 2 | 2 |
| | 2 | 2 | 2 | 2 |
| | 3 | 2 | 2 | 2 |
| | 4 | 2 | 2 | 2 |
| | 5 | 2 | 2 | 2 |
| | 6 | 2 | 2 | 2 |
| | 7 | 2 | 2 | 2 |
| | 8 | 2 | 2 | 2 |
| | 9 | 2 | 2 | 2 |
| | 10 | 2 | 2 | 2 |

Description: (0) No infection, (1) Mild attack 0% - 20%. (2) Moderate attack 20% - 40%. (3) Moderate attack 40% - 50%. (4) Moderately severe attack 50% - 75%. (5) Severe attack 75% - 100%.

Disease intensity calculation:

$$DS = \frac{\Sigma(ni \times vi)}{N \times V} \times 100\%$$

Description: DS = *Disease severity* or disease intensity (%), ni = number of plants observed from attack category i, vi = scale value of attack category i, N = total number of plants observed, and V = scale value of the highest attack category.

Replication 1

$$= \frac{(0\times0) + (0\times1) + (10\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 40.00\%$$

Repeat 2

$$= \frac{(0\times0) + (0\times1) + (10\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 40.00\%$$

Test 3

$$= \frac{(0\times0) + (0\times1) + (10\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 40.00\%$$

Without Inoculum 4 MST

$$= \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{40{,}00\% + 40{,}00\% + 40{,}00\%}{3}$$

$$= 40.00\%$$

Data on Bulai Intensity Without Inoculum 5 MST

| Inoculum Source | Plant Samples | Repeat Scoring 1 | Repeat Scoring 2 | Replication Score 3 |
|---|---|---|---|---|
| Without Inoculum | 1 | 1 | 1 | 1 |
| | 2 | 1 | 1 | 2 |
| | 3 | 1 | 1 | 1 |
| | 4 | 1 | 2 | 1 |
| | 5 | 1 | 1 | 1 |
| | 6 | 1 | 1 | 2 |
| | 7 | 1 | 2 | 1 |
| | 8 | 1 | 1 | 2 |
| | 9 | 2 | 2 | 1 |
| | 10 | 1 | 1 | 1 |

Description: (0) No infection, (1) Mild attack 0% - 20%. (2) Moderate attack 20% - 40%. (3) Moderate attack 40% - 50%. (4) Moderately severe attack 50% - 75%. (5) Severe attack 75% - 100%.

Disease intensity calculation:

$$DS = \frac{\Sigma(ni \times vi)}{N \times V} \times 100\%$$

Description: DS = *Disease severity* or disease intensity (%), ni = number of plants observed from attack category i, vi = scale value of attack category i, N = total number of plants observed, and V = scale value of the highest attack category.

Replication 1

$$= \frac{(0\times0) + (9\times1) + (1\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 22.00\%$$

Review 2

$$= \frac{(0\times0) + (7\times1) + (3\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 26.00\%$$

Test 3

$$= \frac{(0\times0) + (7\times1) + (3\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 26.00\%$$

Without Inoculum 5 MST

$$= \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{22{,}00\% + 26{,}00\% + 26{,}00\%}{3}$$

$$= 24.67\%$$

Data on the intensity of Bulai Grobogan 1 MST

| Inoculum Source | Plant Samples | Repeat Scoring 1 | Repeat Scoring 2 | Replication Score 3 |
|---|---|---|---|---|
| Grobogan | 1 | 0 | 0 | 0 |
| | 2 | 0 | 0 | 0 |
| | 3 | 0 | 0 | 0 |
| | 4 | 0 | 0 | 0 |
| | 5 | 0 | 0 | 0 |
| | 6 | 0 | 0 | 0 |
| | 7 | 0 | 0 | 0 |
| | 8 | 0 | 0 | 0 |
| | 9 | 0 | 0 | 0 |
| | 10 | 0 | 0 | 0 |

Description: (0) No infection, (1) Mild attack 0% - 20%. (2) Moderate attack 20% - 40%. (3) Moderate attack 40% - 50%. (4) Moderately severe attack 50% - 75%. (5) Severe attack 75% - 100%.

Disease intensity calculation:

$$DS = \frac{\Sigma(ni \times vi)}{N \times V} \times 100\%$$

Description: DS = *Disease severity* or disease intensity (%), ni = number of plants observed from attack category i, vi = scale value of attack category i, N = total number of plants observed, and V = scale value of the highest attack category.

Repeat 1

$$= \frac{(10\times0) + (0\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 0.00\%$$

Repeat 2

$$= \frac{(10\times0) + (0\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 0.00\%$$

Test 3

$$= \frac{(10\times0) + (0\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 0.00\%$$

Grobogan 1 MST

$$= \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{0{,}00\% + 0{,}00\% + 0{,}00\%}{3}$$

$$= 0.00\%$$

Data on Bulai Intensity in Grobogan 2 MST

| Inoculum Source | Plant Samples | Repeat Scoring 1 | Repeat Scoring 2 | Replication 3 Scoring |
|---|---|---|---|---|
| Grobogan | 1 | 1 | 1 | 1 |
| | 2 | 1 | 1 | 0 |
| | 3 | 1 | 1 | 1 |
| | 4 | 1 | 0 | 1 |
| | 5 | 1 | 1 | 1 |
| | 6 | 1 | 1 | 1 |
| | 7 | 1 | 0 | 1 |
| | 8 | 1 | 0 | 1 |
| | 9 | 0 | 1 | 1 |
| | 10 | 0 | 0 | 1 |

Description: (0) No infection, (1) Mild attack 0% - 20%. (2) Moderate attack 20% - 40%. (3) Moderate attack 40% - 50%. (4) Moderately severe attack 50% - 75%. (5) Severe attack 75% - 100%.

Disease intensity calculation:

$$DS = \frac{\Sigma(ni \times vi)}{N \times V} \times 100\%$$

Description: DS = *Disease severity* or disease intensity (%), ni = number of plants observed from attack category i, vi = scale value of attack category i, N = total number of plants observed, and V = scale value of the highest attack category.

Repeat 1

$$= \frac{(2\times0) + (8\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 16.00\%$$

Repeat 2

$$= \frac{(4\times0) + (6\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 12.00\%$$

Test 3

$$= \frac{(1\times0) + (9\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 18.00\%$$

Grobogan 2 MST

$$= \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{16{,}00\% + 12{,}00\% + 18{,}00\%}{3}$$

$$= 15.33\%$$

Data on Bulai Intensity in Grobogan 3 MST

| Inoculum Source | Plant Samples | Repeat Scoring 1 | Repeat Scoring 2 | Replication Score 3 |
|---|---|---|---|---|
| Grobogan | 1 | 1 | 1 | 1 |
| | 2 | 1 | 1 | 0 |
| | 3 | 1 | 1 | 1 |
| | 4 | 1 | 0 | 1 |
| | 5 | 1 | 1 | 1 |
| | 6 | 1 | 1 | 1 |
| | 7 | 1 | 1 | 1 |
| | 8 | 1 | 0 | 1 |
| | 9 | 1 | 1 | 1 |
| | 10 | 0 | 0 | 1 |

Description: (0) No infection, (1) Mild attack 0% - 20%. (2) Moderate attack 20% - 40%. (3) Moderate attack 40% - 50%. (4) Moderately severe attack 50% - 75%. (5) Severe attack 75% - 100%.

Calculation of disease intensity:

$$DS = \frac{\Sigma(ni \times vi)}{N \times V} \times 100\%$$

Explanation: DS = *Disease severity* or disease intensity (%), ni = number of plants observed from attack category i, vi = scale value of attack category i, N = total number of plants observed, and V = scale value of the highest attack category.

Repeat 1

$$= \frac{(1\times0) + (9\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 18.00\%$$

Repeat 2

$$= \frac{(3\times0) + (7\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 14.00\%$$

Test 3

$$= \frac{(1\times0) + (9\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 18.00\%$$

Grobogan 3 MST

$$= \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{18{,}00\% + 14{,}00\% + 18{,}00\%}{3}$$

$$= 16.67\%$$

Data on Bulai Intensity in Grobogan 4 MST

| Source of Inoculum | Plant Samples | Repeat Scoring 1 | Repeat Scoring 2 | Replication 3 Scoring |
|---|---|---|---|---|
| Grobogan | 1 | 2 | 2 | 2 |
| | 2 | 2 | 2 | 2 |
| | 3 | 2 | 2 | 2 |
| | 4 | 2 | 2 | 2 |
| | 5 | 2 | 2 | 2 |
| | 6 | 2 | 2 | 2 |
| | 7 | 2 | 2 | 2 |
| | 8 | 2 | 2 | 2 |
| | 9 | 2 | 2 | 2 |
| | 10 | 2 | 2 | 2 |

Description: (0) No infection, (1) Mild attack 0% - 20%. (2) Moderate attack 20% - 40%. (3) Moderate attack 40% - 50%. (4) Moderately severe attack 50% - 75%. (5) Severe attack 75% - 100%.

Disease intensity calculation:

$$DS = \frac{\Sigma(ni \times vi)}{N \times V} \times 100\%$$

Description: DS = *Disease severity* or disease intensity (%), ni = number of plants observed from attack category i, vi = scale value of attack category i, N = total number of plants observed, and V = scale value of the highest attack category.

Replication 1

$$= \frac{(0\times0) + (0\times1) + (10\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 40.00\%$$

Repeat 2

$$= \frac{(0\times0) + (0\times1) + (10\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 40.00\%$$

Test 3

$$= \frac{(0\times0) + (0\times1) + (10\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 40.00\%$$

Grobogan 4 MST

$$= \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{40{,}00\% + 40{,}00\% + 40{,}00\%}{3}$$

$$= 40.00\%$$

Data on Bulai Intensity in Grobogan 5 MST

| Source of Inoculum | Plant Samples | Repeat Scoring 1 | Repeat Scoring 2 | Replication Score 3 |
|---|---|---|---|---|
| Grobogan | 1 | 1 | 1 | 1 |
| | 2 | 1 | 1 | 2 |
| | 3 | 1 | 1 | 1 |
| | 4 | 1 | 2 | 1 |
| | 5 | 1 | 1 | 1 |
| | 6 | 1 | 1 | 1 |
| | 7 | 1 | 2 | 1 |
| | 8 | 1 | 2 | 1 |
| | 9 | 2 | 1 | 1 |
| | 10 | 2 | 2 | 1 |

Description: (0) No infection, (1) Mild attack 0% - 20%. (2) Moderate attack 20% - 40%. (3) Moderate attack 40% - 50%. (4) Moderately severe attack 50% - 75%. (5) Severe attack 75% - 100%.

Disease intensity calculation:

$$DS = \frac{\Sigma(ni \times vi)}{N \times V} \times 100\%$$

Description: DS = *Disease severity* or disease intensity (%), ni = number of plants observed from attack category i, vi = scale value of attack category i, N = total number of plants observed, and V = scale value of the highest attack category.

Replication 1

$$= \frac{(0\times0) + (8\times1) + (2\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 24.00\%$$

Repeat 2

$$= \frac{(0\times0) + (6\times1) + (4\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 28.00\%$$

Test 3

$$= \frac{(0\times0) + (9\times1) + (1\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 22.00\%$$

Grobogan 5 MST

$$= \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{24{,}00\% + 28{,}00\% + 22{,}00\%}{3}$$

$$= 24.67\%$$

Semarang 1 MST Bulai Intensity Data

| Source of Inoculum | Plant Samples | Repeat Scoring 1 | Repeat Scoring 2 | Replication Score 3 |
|---|---|---|---|---|
| Semarang | 1 | 0 | 0 | 0 |
| | 2 | 0 | 0 | 0 |
| | 3 | 0 | 0 | 0 |
| | 4 | 0 | 0 | 0 |
| | 5 | 0 | 0 | 0 |
| | 6 | 0 | 0 | 0 |
| | 7 | 0 | 0 | 0 |
| | 8 | 0 | 0 | 0 |
| | 9 | 0 | 0 | 0 |
| | 10 | 0 | 0 | 0 |

Description: (0) No infection, (1) Mild attack 0% - 20%. (2) Moderate attack 20% - 40%. (3) Moderate attack 40% - 50%. (4) Moderately severe attack 50% - 75%. (5) Severe attack 75% - 100%.

Disease intensity calculation:

$$DS = \frac{\Sigma(ni \times vi)}{N \times V} \times 100\%$$

Description: DS = *Disease severity* or disease intensity (%), ni = number of plants observed from attack category i, vi = scale value of attack category i, N = total number of plants observed, and V = scale value of the highest attack category.

Replication 1

$$= \frac{(10\times0) + (0\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 0.00\%$$

Repeat 2

$$= \frac{(10\times0) + (0\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 0.00\%$$

Test 3

$$= \frac{(10\times0) + (0\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 0.00\%$$

Semarang 1 MST

$$= \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{0{,}00\% + 0{,}00\% + 0{,}00\%}{3}$$

$$= 0.00\%$$

Semarang 2 MST Bulai Intensity Data

| Inoculum Source | Plant Samples | Repeat Scoring 1 | Repeat Scoring 2 | Replication 3 Scoring |
|---|---|---|---|---|
| Semarang | 1 | 0 | 1 | 1 |
| | 2 | 1 | 1 | 1 |
| | 3 | 1 | 1 | 1 |
| | 4 | 0 | 1 | 1 |
| | 5 | 1 | 0 | 0 |
| | 6 | 1 | 0 | 1 |
| | 7 | 1 | 1 | 0 |
| | 8 | 1 | 0 | 1 |
| | 9 | 1 | 0 | 0 |
| | 10 | 0 | 1 | 1 |

Description: (0) No infection, (1) Mild attack 0% - 20%. (2) Moderate attack 20% - 40%. (3) Moderate attack 40% - 50%. (4) Moderately severe attack 50% - 75%. (5) Severe attack 75% - 100%.

Disease intensity calculation:

$$DS = \frac{\Sigma(ni \times vi)}{N \times V} \times 100\%$$

Description: DS = *Disease severity* or disease intensity (%), ni = number of plants observed from attack category i, vi = scale value of attack category i, N = total number of plants observed, and V = scale value of the highest attack category.

Replication 1

$$= \frac{(3\times0) + (7\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 14.00\%$$

Repeat 2

$$= \frac{(4\times0) + (6\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 12.00\%$$

Test 3

$$= \frac{(3\times0) + (7\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 14.00\%$$

Semarang 2 MST

$$= \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{14{,}00\% + 12{,}00\% + 14{,}00\%}{3}$$

$$= 13.33\%$$

Data on Bulai Intensity in Semarang 3 MST

| Inoculum Source | Plant Samples | Repeat Scoring 1 | Repeat Scoring 2 | Replication Score 3 |
|---|---|---|---|---|
| Semarang | 1 | 0 | 1 | 1 |
| | 2 | 1 | 1 | 1 |
| | 3 | 1 | 1 | 1 |
| | 4 | 0 | 1 | 1 |
| | 5 | 1 | 0 | 0 |
| | 6 | 1 | 0 | 1 |
| | 7 | 1 | 1 | 1 |
| | 8 | 1 | 0 | 1 |
| | 9 | 1 | 0 | 1 |
| | 10 | 0 | 1 | 1 |

Description: (0) No infection, (1) Mild attack 0% - 20%. (2) Moderate attack 20% - 40%. (3) Moderate attack 40% - 50%. (4) Moderately severe attack 50% - 75%. (5) Severe attack 75% - 100%.

Disease intensity calculation:

$$DS = \frac{\Sigma(ni \times vi)}{N \times V} \times 100\%$$

Description: DS = *Disease severity* or disease intensity (%), ni = number of plants observed from attack category i, vi = scale value of attack category i, N = total number of plants observed, and V = scale value of the highest attack category.

Replication 1

$$= \frac{(3\times0) + (7\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 14.00\%$$

Repeat 2

$$= \frac{(4\times0) + (6\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 12.00\%$$

Test 3

$$= \frac{(1\times0) + (9\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 18.00\%$$

Semarang 3 MST

$$= \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{14{,}00\% + 12{,}00\% + 18{,}00\%}{3}$$

$$= 14.67\%$$

Semarang 4 MST Bulai Intensity Data

| Inoculum Source | Plant Samples | Repeat Scoring 1 | Repeat Scoring 2 | Replication Score 3 |
|---|---|---|---|---|
| Semarang | 1 | 2 | 2 | 2 |
| | 2 | 2 | 2 | 2 |
| | 3 | 2 | 2 | 2 |
| | 4 | 2 | 2 | 2 |
| | 5 | 2 | 2 | 2 |
| | 6 | 2 | 2 | 2 |
| | 7 | 2 | 2 | 2 |
| | 8 | 2 | 2 | 2 |
| | 9 | 2 | 2 | 2 |
| | 10 | 2 | 2 | 2 |

Description: (0) No infection, (1) Mild attack 0% - 20%. (2) Moderate attack 20% - 40%. (3) Moderate attack 40% - 50%. (4) Moderately severe attack 50% - 75%. (5) Severe attack 75% - 100%.

Disease intensity calculation:

$$DS = \frac{\Sigma(ni \times vi)}{N \times V} \times 100\%$$

Description: DS = *Disease severity* or disease intensity (%), ni = number of plants observed from attack category i, vi = scale value of attack category i, N = total number of plants observed, and V = scale value of the highest attack category.

Replication 1

$$= \frac{(0\times0) + (0\times1) + (10\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 40.00\%$$

Repeat 2

$$= \frac{(0\times0) + (0\times1) + (10\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 40.00\%$$

Test 3

$$= \frac{(0\times0) + (0\times1) + (10\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 40.00\%$$

Semarang 4 MST

$$= \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{40{,}00\% + 40{,}00\% + 40{,}00\%}{3}$$

$$= 40.00\%$$

Semarang 5 MST Bulai Intensity Data

| Source of Inoculum | Plant Samples | Repeat Scoring 1 | Repeat Scoring 2 | Replication Score 3 |
|---|---|---|---|---|
| Semarang | 1 | 2 | 1 | 1 |
| | 2 | 1 | 1 | 1 |
| | 3 | 1 | 1 | 1 |
| | 4 | 2 | 1 | 1 |
| | 5 | 1 | 1 | 2 |
| | 6 | 1 | 1 | 1 |
| | 7 | 1 | 1 | 2 |
| | 8 | 1 | 1 | 1 |
| | 9 | 1 | 1 | 2 |
| | 10 | 2 | 1 | 1 |

Description: (0) No infection, (1) Mild attack 0% - 20%. (2) Moderate attack 20% - 40%. (3) Moderate attack 40% - 50%. (4) Moderately severe attack 50% - 75%. (5) Severe attack 75% - 100%.

Disease intensity calculation:

$$DS = \frac{\Sigma(ni \times vi)}{N \times V} \times 100\%$$

Description: DS = *Disease severity* or disease intensity (%), ni = number of plants observed from attack category i, vi = scale value of attack category i, N = total number of plants observed, and V = scale value of the highest attack category.

Replication 1

$$= \frac{(0\times0) + (7\times1) + (3\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 26.00\%$$

Repeat 2

$$= \frac{(0\times0) + (10\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 20.00\%$$

Test 3

$$= \frac{(0\times0) + (7\times1) + (3\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 26.00\%$$

Semarang 5 MST

$$= \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{26{,}00\% + 20{,}00\% + 26{,}00\%}{3}$$

$$= 24.00\%$$

Data on Bulai Intensity in Klaten 1 MST

| Source of Inoculum | Plant Samples | Repeat Scoring 1 | Repeat Scoring 2 | Replication Score 3 |
|---|---|---|---|---|
| Klaten | 1 | 0 | 0 | 0 |
| | 2 | 0 | 0 | 0 |
| | 3 | 0 | 0 | 0 |
| | 4 | 0 | 0 | 0 |
| | 5 | 0 | 0 | 0 |
| | 6 | 0 | 0 | 0 |
| | 7 | 0 | 0 | 0 |
| | 8 | 0 | 0 | 0 |
| | 9 | 0 | 0 | 0 |
| | 10 | 0 | 0 | 0 |

Description: (0) No infection, (1) Mild attack 0% - 20%. (2) Moderate attack 20% - 40%. (3) Moderate attack 40% - 50%. (4) Moderately severe attack 50% - 75%. (5) Severe attack 75% - 100%.

Disease intensity calculation:

$$DS = \frac{\Sigma(ni \times vi)}{N \times V} \times 100\%$$

Description: DS = *Disease severity* or disease intensity (%), ni = number of plants observed from attack category i, vi = scale value of attack category i, N = total number of plants observed, and V = scale value of the highest attack category.

Replication 1

$$= \frac{(10\times0) + (0\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 0.00\%$$

Repeat 2

$$= \frac{(10\times0) + (0\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 0.00\%$$

Test 3

$$= \frac{(10\times0) + (0\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 0.00\%$$

Klaten 1 MST

$$= \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{0{,}00\% + 0{,}00\% + 0{,}00\%}{3}$$

$$= 0.00\%$$

Data on Bulai Intensity in Klaten 2 MST

| Source of Inoculum | Plant Samples | Repeat Scoring 1 | Repeat Scoring 2 | Replication 3 Scoring |
|---|---|---|---|---|
| Klaten | 1 | 0 | 1 | 1 |
| | 2 | 0 | 1 | 1 |
| | 3 | 1 | 1 | 1 |
| | 4 | 0 | 1 | 0 |
| | 5 | 1 | 0 | 0 |
| | 6 | 0 | 1 | 0 |
| | 7 | 1 | 0 | 1 |
| | 8 | 1 | 1 | 1 |
| | 9 | 1 | 0 | 1 |
| | 10 | 1 | 0 | 1 |

Description: (0) No infection, (1) Mild attack 0% - 20%. (2) Moderate attack 20% - 40%. (3) Moderate attack 40% - 50%. (4) Moderately severe attack 50% - 75%. (5) Severe attack 75% - 100%.

Disease intensity calculation:

$$DS = \frac{\Sigma(ni \times vi)}{N \times V} \times 100\%$$

Description: DS = *Disease severity* or disease intensity (%), ni = number of plants observed from attack category i, vi = scale value of attack category i, N = total number of plants observed, and V = scale value of the highest attack category.

Replication 1

$$= \frac{(4\times0) + (6\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 12.00\%$$

Repeat 2

$$= \frac{(4\times0) + (6\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 12.00\%$$

Test 3

$$= \frac{(3\times0) + (7\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 14.00\%$$

Klaten 2 MST

$$= \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{12{,}00\% + 12{,}00\% + 14{,}00\%}{3}$$

$$= 12.67\%$$

Data on Bulai Intensity in Klaten 3 MST

| Source of Inoculum | Plant Samples | Repeat Scoring 1 | Repeat Scoring 2 | Replication 3 Scoring |
|---|---|---|---|---|
| Klaten | 1 | 0 | 1 | 1 |
| | 2 | 0 | 1 | 1 |
| | 3 | 1 | 1 | 1 |
| | 4 | 0 | 1 | 1 |
| | 5 | 1 | 1 | 1 |
| | 6 | 0 | 1 | 1 |
| | 7 | 1 | 1 | 0 |
| | 8 | 1 | 1 | 1 |
| | 9 | 1 | 1 | 1 |
| | 10 | 1 | 1 | 1 |

Description: (0) No infection, (1) Mild attack 0% - 20%. (2) Moderate attack 20% - 40%. (3) Moderate attack 40% - 50%. (4) Moderately severe attack 50% - 75%. (5) Severe attack 75% - 100%.

Disease intensity calculation:

$$DS = \frac{\Sigma(ni \times vi)}{N \times V} \times 100\%$$

Description: DS = *Disease severity* or disease intensity (%), ni = number of plants observed from attack category i, vi = scale value of attack category i, N = total number of plants observed, and V = scale value of the highest attack category.

Replication 1

$$= \frac{(4\times0) + (6\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 12.00\%$$

Repeat 2

$$= \frac{(0\times0) + (10\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 20.00\%$$

Test 3

$$= \frac{(1\times0) + (9\times1) + (0\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 18.00\%$$

Klaten 3 MST

$$= \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{12{,}00\% + 20{,}00\% + 18{,}00\%}{3}$$

$$= 16.67\%$$

Data on Bulai Intensity in Klaten 4 MST

| Source of Inoculum | Plant Samples | Repeat Scoring 1 | Repeat Scoring 2 | Replication Score 3 |
|---|---|---|---|---|
| Klaten | 1 | 2 | 2 | 2 |
| | 2 | 2 | 2 | 2 |
| | 3 | 2 | 2 | 2 |
| | 4 | 2 | 2 | 2 |
| | 5 | 2 | 2 | 2 |
| | 6 | 2 | 2 | 2 |
| | 7 | 2 | 2 | 2 |
| | 8 | 2 | 2 | 2 |
| | 9 | 2 | 2 | 2 |
| | 10 | 2 | 2 | 2 |

Description: (0) No infection, (1) Mild attack 0% - 20%. (2) Moderate attack 20% - 40%. (3) Moderate attack 40% - 50%. (4) Moderately severe attack 50% - 75%. (5) Severe attack 75% - 100%.

Disease intensity calculation:

$$DS = \frac{\Sigma(ni \times vi)}{N \times V} \times 100\%$$

Description: DS = *Disease severity* or disease intensity (%), ni = number of plants observed from attack category i, vi = scale value of attack category i, N = total number of plants observed, and V = scale value of the highest attack category.

Replication 1

$$= \frac{(0\times0) + (0\times1) + (10\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 40.00\%$$

Repeat 2

$$= \frac{(0\times0) + (0\times1) + (10\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 40.00\%$$

Test 3

$$= \frac{(0\times0) + (0\times1) + (10\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 40.00\%$$

Klaten 4 MST

$$= \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{40{,}00\% + 40{,}00\% + 40{,}00\%}{3}$$

$$= 40.00\%$$

Data on Bulai Intensity in Klaten 5 MST

| Source of Inoculum | Plant Samples | Repeat Scoring 1 | Repeat Scoring 2 | Replication Score 3 |
|---|---|---|---|---|
| Klaten | 1 | 2 | 2 | 2 |
| | 2 | 2 | 1 | 1 |
| | 3 | 1 | 1 | 1 |
| | 4 | 2 | 1 | 1 |
| | 5 | 1 | 2 | 1 |
| | 6 | 2 | 1 | 1 |
| | 7 | 1 | 2 | 2 |
| | 8 | 1 | 1 | 1 |
| | 9 | 1 | 2 | 1 |
| | 10 | 1 | 2 | 1 |

Description: (0) No infection, (1) Mild attack 0% - 20%. (2) Moderate attack 20% - 40%. (3) Moderate attack 40% - 50%. (4) Moderately severe attack 50% - 75%. (5) Severe attack 75% - 100%.

Disease intensity calculation:

$$DS = \frac{\Sigma(ni \times vi)}{N \times V} \times 100\%$$

Description: DS = *Disease severity* or disease intensity (%), ni = number of plants observed from attack category i, vi = scale value of attack category i, N = total number of plants observed, and V = scale value of the highest attack category.

Replication 1

$$= \frac{(0\times0) + (6\times1) + (4\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 24.00\%$$

Repeat 2

$$= \frac{(0\times0) + (7\times1) + (3\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 28.00\%$$

Test 3

$$= \frac{(0\times0) + (8\times1) + (2\times2) + (0\times3) + (0\times4) + (0\times5)}{10\times5} \times 100\%$$

$$= 22.00\%$$

Klaten 5 MST

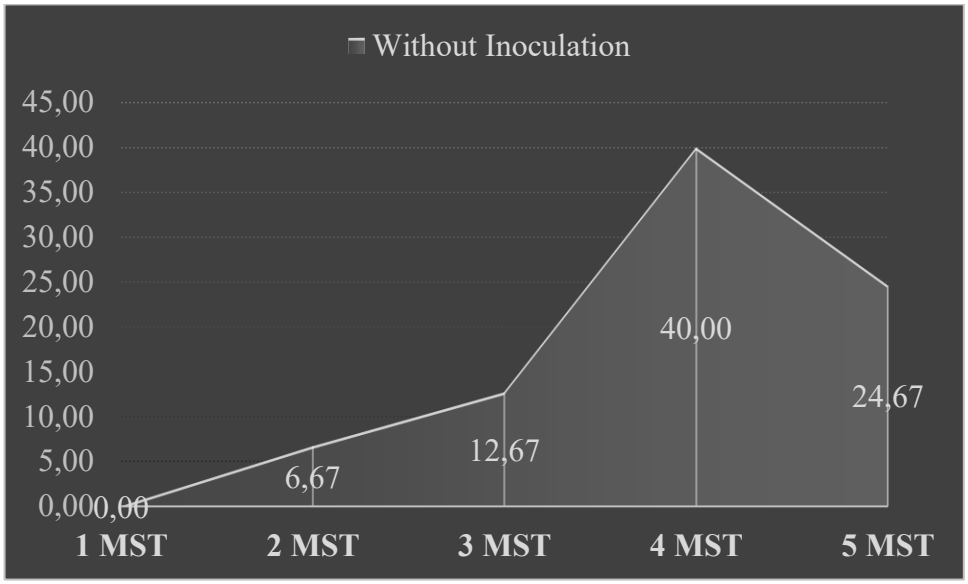
$$= \frac{\text{Ulangan 1} + \text{Ulangan 2} + \text{Ulangan 3}}{3}$$

$$= \frac{24{,}00\% + 28{,}00\% + 22{,}00\%}{3}$$

$$= 24.67\%$$

**Appendix 10**. Data Processing of *Area Under the Disease Progress Curve* (AUDPC) Values.

Intensity Curve of Bulai Samples Without Inoculum




AUDPC value calculation:

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i)$$

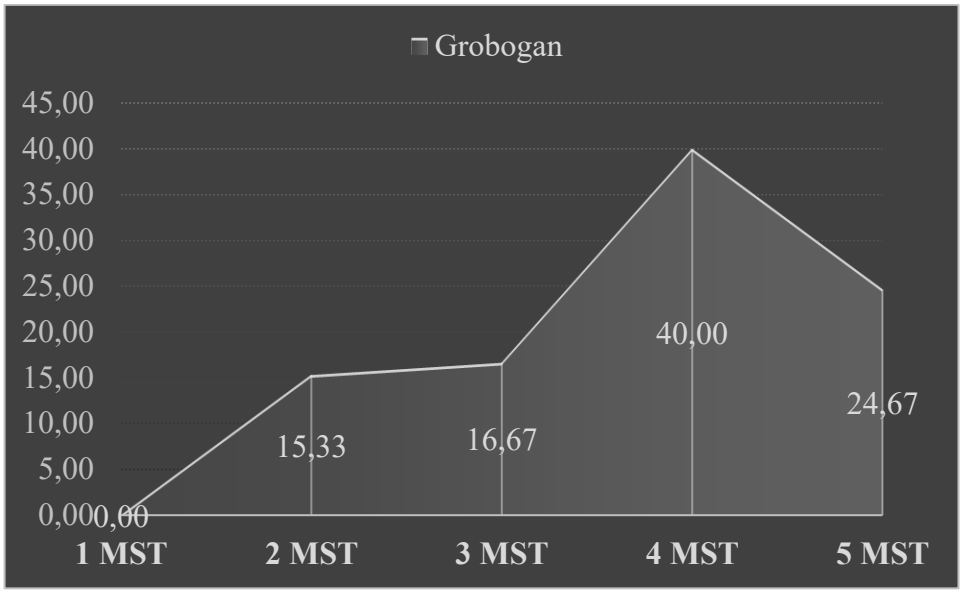
Explanation: AUDPC = area under the disease progression curve, $y_i$ = i-th intensity value, $t_i$ = i-th observation time.

$$\text{Without Inoculum} = \left(\frac{0{,}00+6{,}67}{2}\right)(2-1) + \left(\frac{6{,}67+12{,}67}{2}\right)(3-2) + \left(\frac{12{,}67+40{,}00}{2}\right)(4-3) + \left(\frac{40{,}00+24{,}67}{2}\right)(5-4)$$

$$= 3.34 + 9.67 + 26.34 + 32.34$$

$$= 70.69$$

Grobogan Sample Bulai Intensity Curve




AUDPC value calculation:

AUDPC $= \sum_{i=1}^{n-1} \left(\frac{y_i+y_{i+1}}{2}\right)(t_{i+1} - t_i)$

Explanation: AUDPC = area under the disease progression curve, $y_i$ = i-th intensity value, $t_i$ = i-th observation time.

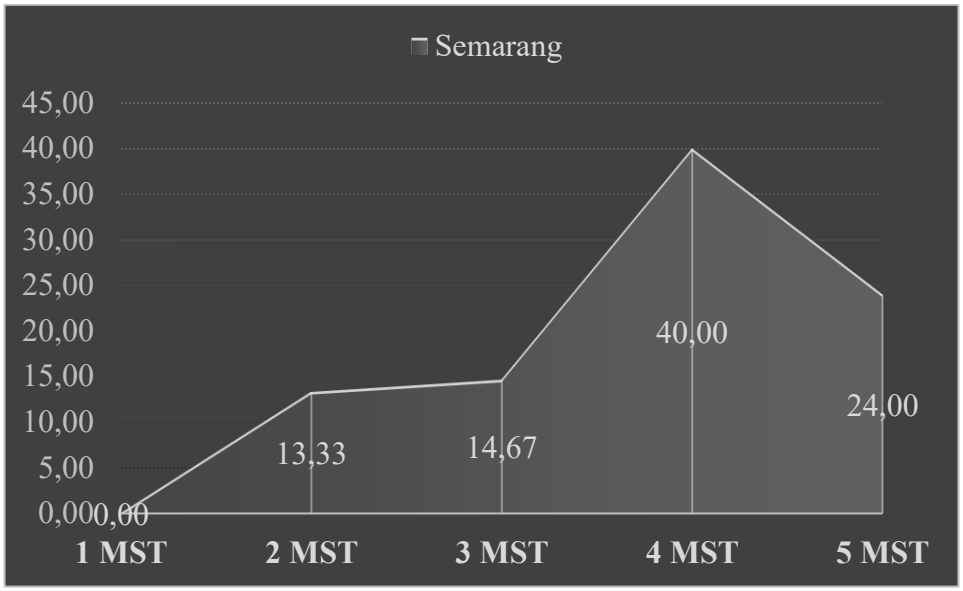
Grobogan $= \left(\frac{0,00+15,33}{2}\right)(2-1) + \left(\frac{15,33+16,67}{2}\right)(3-2) +$

$\left(\frac{16,67+40,00}{2}\right)(4-3) + 0\left(\frac{40,00+24,67}{2}\right) 0\,(5-4)$

$= 7.67 + 16.00 + 28.34 + 32.34$

$= 84.35$

Semarang Sample Bulai Intensity Curve




AUDPC value calculation:

AUDPC $= \sum_{i=1}^{n-1} \left(\frac{y_i+y_{i+1}}{2}\right)(t_{i+1} - t_i)$

Explanation: AUDPC = area under the disease progression curve, $y_i$ = i-th intensity value, $t_i$ = i-th observation time.

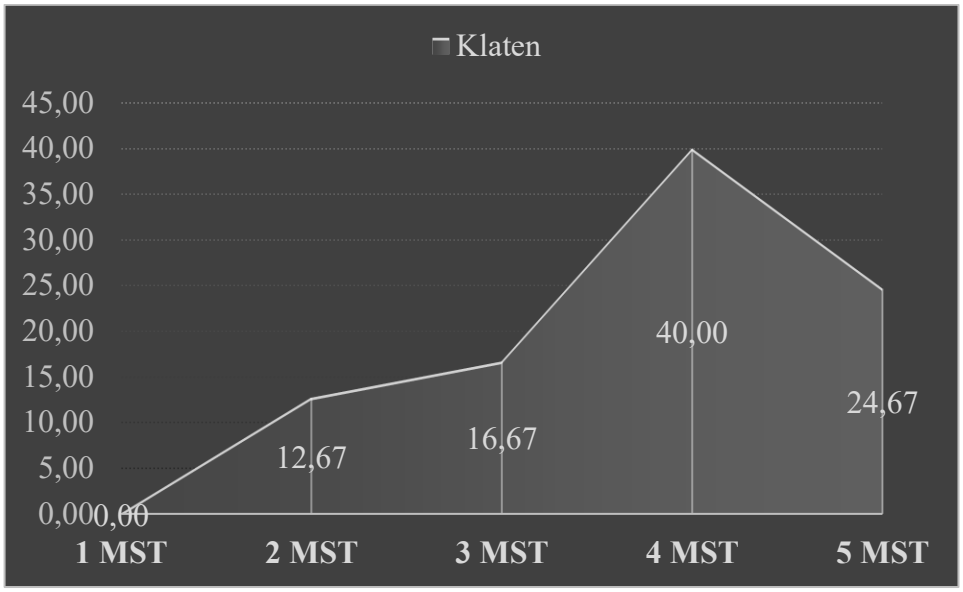
Semarang $= \left(\frac{0,00+13,33}{2}\right)(2-1) + \left(\frac{13,33+14,67}{2}\right)(3-2) +$

$\left(\frac{14,67+40,00}{2}\right)(4-3) + \left(\frac{40,00+24,00}{2}\right)(5-4)$

$= 6.67 + 14.00 + 27.34 + 32.00$

$= 80.01$

Klaten Sample Bulai Intensity Curve




AUDPC value calculation:

AUDPC $= \sum_{i=1}^{n-1} \left(\frac{y_i+y_{i+1}}{2}\right)(t_{i+1} - t_i)$

Explanation: AUDPC = area under the disease progression curve, $y_i$ = i-th intensity value, $t_i$ = i-th observation time.

Klaten $= \left(\frac{0,00+12,67}{2}\right)(2-1) + \left(\frac{16,67+12,67}{2}\right)(3-2) +$

$\left(\frac{16,67+40,00}{2}\right)(4-3) + 0\left(\frac{40,00+24,67}{2}\right) 0\,(5-4)$

$= 6.34 + 14.67 + 28.34 + 32.34$

$= 81.69$

**Appendix 11**. Processing of Disease Infection Rate Data.

Calculation of disease infection rate values:

$$r = \frac{2,3}{(t2-t1)}\left[\log\frac{x2}{1-x2} - \log\frac{x1}{1-x1}\right]$$

Explanation: r = disease infection rate, $x_1$ = proportion of disease (disease intensity) in the first observation, $x_2$ = proportion of disease (disease intensity) in the second observation, $t_1$ = time of first observation, $t_2$ = time of second observation.

Without Inoculum 1 MST $= \frac{2,3}{(1-0)}\left[\log\frac{0,00}{1-0,00} - \log\frac{0,00}{1-0,00}\right] = 0.00$ plants/week

Without Inoculum 2 MST $= \frac{2,3}{(2-1)}\left[\log\frac{0,07}{1-0,07} - \log\frac{0,00}{1-0,00}\right] = 0.28$ plants/week

Without Inoculum 3 MST $= \frac{2,3}{(3-2)}\left[\log\frac{0,13}{1-0,13} - \log\frac{0,07}{1-0,07}\right] = 0.68$ plants/week

Without Inoculum 4 MST $= \frac{2,3}{(4-3)}\left[\log\frac{0,40}{1-0,40} - \log\frac{0,13}{1-0,13}\right] = 1.49$ plants/week

Without Inoculum 5 MST $= \frac{2,3}{(5-4)}\left[\log\frac{0,25}{1-0,25} - \log\frac{0,40}{1-0,40}\right] = -0.69$ plants/week

Grobogan 1 MST $= \frac{2,3}{(1-0)}\left[\log\frac{0,00}{1-0,00} - \log\frac{0,00}{1-0,00}\right] = 0.00$ plants/week

Grobogan 2 MST $= \frac{2,3}{(2-1)}\left[\log\frac{0,15}{1-0,15} - \log\frac{0,00}{1-0,00}\right] = 0.57$ plants/week

Grobogan 3 MST $= \frac{2,3}{(3-2)}\left[\log\frac{0,17}{1-0,17} - \log\frac{0,15}{1-0,15}\right] = 0.15$ plants/week

Grobogan 4 MST $= \frac{2,3}{(4-3)}\left[\log\frac{0,40}{1-0,40} - \log\frac{0,17}{1-0,17}\right] = 1.18$ plants/week

Grobogan 5 MST $= \frac{2,3}{(5-4)}\left[\log\frac{0,25}{1-0,25} - \log\frac{0,40}{1-0,40}\right] = -0.69$ plants/week

Semarang 1 MST $= \frac{2,3}{(1-0)}\left[\log\frac{0,00}{1-0,00} - \log\frac{0,00}{1-0,00}\right] = 0.00$ plants/week

Semarang 2 MST $= \frac{2,3}{(2-1)}\left[\log\frac{0,13}{1-0,13} - \log\frac{0,00}{1-0,00}\right] = 0.40$ plants/week

Semarang 3 MST $= \frac{2,3}{(3-2)}\left[\log\frac{0,15}{1-0,15} - \log\frac{0,13}{1-0,13}\right] = 0.17$ plants/week

Semarang 4 MST $= \frac{2,3}{(4-3)}\left[\log\frac{0,40}{1-0,40} - \log\frac{0,15}{1-0,15}\right] = 1.33$ plants/week

Semarang 5 MST $= \frac{2,3}{(5-4)}\left[\log\frac{0,24}{1-0,24} - \log\frac{0,40}{1-0,40}\right] = -0.75$ plants/week

Klaten 1 MST $= \frac{2,3}{(1-0)}\left[\log\frac{0,00}{1-0,00} - \log\frac{0,00}{1-0,00}\right] = 0.00$ plants/week

Klaten 2 MST $= \frac{2,3}{(2-1)}\left[\log\frac{0,13}{1-0,13} - \log\frac{0,00}{1-0,00}\right] = 0.40$ plants/week

Klaten 3 MST $= \frac{2,3}{(3-2)}\left[\log\frac{0,17}{1-0,17} - \log\frac{0,13}{1-0,13}\right] = 0.31$ plants/week

Klaten 4 MST $= \frac{2,3}{(4-3)}\left[\log\frac{0,40}{1-0,40} - \log\frac{0,17}{1-0,17}\right] = 1.18$ plants/week

Klaten 5 MST $= \frac{2,3}{(5-4)}\left[\log\frac{0,25}{1-0,25} - \log\frac{0,40}{1-0,40}\right] = -0.69$ plants/week

**Appendix 12**. Plant Height Data Processing.

Plant Height Data at 5 MST

| Source Inoculum | Plant Height | | | Total | Average |
|---|---|---|---|---|---|
| | Repeat 1 | Repeat 2 | Repeat 3 | | |
| | ----------cm---------- | | | | |
| Without inoculum | 118.00 | 127.60 | 125.20 | 370.80 | 123.60 |
| Grobogan | 113.30 | 125.50 | 122.60 | 361.60 | 120.47 |
| Semarang | 126.30 | 121.60 | 115.60 | 363.50 | 121.17 |
| Klaten | 120.80 | 118.60 | 126.70 | 366.10 | 122.03 |
| Total | 478.40 | 493.30 | 490.10 | 1,461.80 | |
| Average | 119.60 | 123.33 | 122.53 | | 121.82 |

Note: each replicate contains 10 plants.

**1. Degrees of Freedom**

p (Treatment) = 4

u (Replication) = 3

df Treatment = p – 1 = 4 – 1 = 3

db Error = p (u – 1) = 4 (3 – 1) = 8

db Total = db Treatment + db Error = 3 + 8 = 11

**2. Correction Factor (CF)**

$$FC = \frac{(\Sigma Yij)^2}{p.u} = \frac{(1461,80)^2}{4.3} = 178071.60$$

**3. Sum of Squares (SS)**

JK Total = $[(118.00)^2 + (127.60)^2 + \ldots\ldots + (126.70)^2] - FK$

= 178,311.80 – 178,071.60

= 240.20

SQ Treatment $= \frac{(370,80)^2 + (361,60)^2 + (363,50)^2 + (366,10)^2}{3} - FK$

= 178,088.02 – 178,071.60

= 16.42

JK Error = JK Total – JK Treatment

= 240.20– 16.42

= 223.78

**4. Mean Square (MS)**

MS Treatment $= \frac{\text{JK Perlakuan}}{\text{db Perlakuan}} = \frac{16,42}{3} = 5.47$

ME of Error $= \frac{\text{JK Galat}}{\text{db Galat}} = \frac{223,78}{8} = 27.97$

**5. Calculated F**

Calculated F for Treatment $= \frac{\text{KT Perlakuan}}{\text{KT Galat}} = \frac{5,47}{27,97} = 0.20$

**6. ANOVA Table**

| Source of Variation | df | SS | KT | Calculated F | F Table 5% |
|---|---|---|---|---|---|
| Treatment | 3 | 16.42 | 5.47 | $0.20^{tn}$ | 4.07 |
| Error | 8 | 223.78 | 27.97 | | |
| Total | 11 | 240.20 | | | |

Note: *: significant (calculated F > table F 5%) and tn: not significant (calculated F < table F).

Coefficient of Variation (CV)

CV $= \frac{\sqrt{\text{KT Galat}}}{\text{rataan total}} \times 100\%$

$= \frac{\sqrt{27,97}}{121,82} \times 100\%$

= 4.34%

**Appendix 13**. Data Processing of Leaf Count.

Leaf Count Data at 5 MST

| Source Inoculum | Number of leaves | | | Total | Average |
|---|---|---|---|---|---|
| | Repeat 1 | Repeat 2 | Repeat 3 | | |
| | ----------helai---------- | | | | |
| Without inoculum | 9.0 | 8.00 | 8.6 | 25.60 | 8.53 |
| Grobogan | 7.30 | 8.00 | 7.90 | 23.20 | 7.73 |
| Semarang | 8.40 | 8.60 | 7.90 | 24.90 | 8.30 |
| Klaten | 8.70 | 8.00 | 8.80 | 25.50 | 8.5 |
| Total | 33.40 | 32.6 | 33.20 | 99.20 | |
| Average | 8.35 | 8.15 | 8.3 | | 8.27 |

Note: each replicate contains 10 plants.

**1. Degrees of Freedom**

p (Treatment) = 4

u (Replication) = 3

df Treatment = p – 1 = 4 – 1 = 3

db Error = p (u – 1) = 4 (3 – 1) = 8

Total degrees of freedom = db Treatment + db Error = 3 + 8 = 11

**2. Correction Factor (CF)**

$$FC = \frac{(\Sigma Yij)^2}{p.u} = \frac{(99,20)^2}{4.3} = 820.05$$

**3. Sum of Squares (SS)**

SS Total $= [(9.00)^2 + (8.00)^2 + \ldots\ldots + (8.80)^2] - FK$

$= 822.72 - 820.05$

$= 2.67$

SQ Treatment $= \frac{(25,60)^2 + (23,20)^2 + (24,90)^2 + (25,50)^2}{3} - FK$

$= 821.28 - 820.05$

$= 1.23$

JK Error = Total JK – Treatment JK

= 2.67– 1.23

= 1.43

**4. Mean Square (MS)**

MS of Treatment $= \frac{\text{JK Perlakuan}}{\text{db Perlakuan}} = \frac{1,23}{3} = 0.41$

ME of Error $= \frac{\text{JK Galat}}{\text{db Galat}} = \frac{1,43}{8} = 0.18$

**5. Calculated F**

Calculated F for Treatment $= \frac{\text{KT Perlakuan}}{\text{KT Galat}} = \frac{0,41}{0,18} = 2.29$

**6. ANOVA Table**

| Source of Variation | df | SS | Sum of Squares | Calculated F | F Table 5% |
|---|---|---|---|---|---|
| Treatment | 3 | 1.23 | 0.41 | $2.29^{tn}$ | 4.07 |
| Error | 8 | 1.43 | 0.18 | | |
| Total | 11 | 2.67 | | | |

Note: *: significant (calculated F > table F 5%) and n: not significant (calculated F < table F).

Coefficient of Variation (CV)

CV $= \frac{\sqrt{\text{KT Galat}}}{\text{rataan total}} \times 100\%$

$= \frac{\sqrt{0,18}}{8,27} \times 100\%$

= 5.12%

**Appendix 14**. Processing of Total Chlorophyll Content Data.

Measurement Results Using a Spectrophotometer

| Source Inoculum | Replication | Age 3 MST | | | Age 5 MST | | |
|---|---|---|---|---|---|---|---|
| | | λ 645 | λ 663 | Total Chlorophyll Content (mg/g) | λ 645 | λ 663 | Total Chlorophyll Content (mg/g) |
| Without Inoculum | 1 | 0.360 | 0.988 | 1.27 | 0.674 | 1.670 | 2.70 |
| | 2 | 0.221 | 0.604 | 0.93 | 0.523 | 1.397 | 2.18 |
| | 3 | 0.186 | 0.504 | 0.78 | 0.444 | 1.203 | 1.86 |
| Grobogan | 1 | 0.181 | 0.491 | 0.76 | 0.521 | 1.386 | 2.16 |
| | 2 | 0.219 | 0.596 | 0.92 | 0.433 | 1.181 | 1.82 |
| | 3 | 0.202 | 0.549 | 0.85 | 0.499 | 1.337 | 2.08 |
| Semarang | 1 | 0.209 | 0.563 | 0.87 | 0.377 | 1.020 | 1.58 |
| | 2 | 0.165 | 0.436 | 0.68 | 0.373 | 1.001 | 1.56 |
| | 3 | 0.244 | 0.666 | 1.03 | 0.632 | 1.628 | 2.58 |
| Klaten | 1 | 0.292 | 0.796 | 1.23 | 0.574 | 1.477 | 2.34 |
| | 2 | 0.223 | 0.613 | 0.94 | 0.583 | 1.502 | 2.38 |
| | 3 | 0.212 | 0.579 | 0.89 | 0.503 | 1.348 | 2.10 |

Total chlorophyll content calculation:

Ca+b (mg/g) = [8.02×A663 + 20.20×A645] × V/1000 × 1/W

Note: C = total chlorophyll content (mg/g), V = volume of extract (mL), and W = wet weight of sample (g).

Total Chlorophyll Content Data at 3 MST

| Source Inoculum | Total Chlorophyll Content | | | Total | Average |
|---|---|---|---|---|---|
| | Repeat 1 | Repeat 2 | Repeat 3 | | |
| | ----------mg/g---------- | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Without Inoculum | 1.27 | 0.93 | 0.78 | 2.98 | 0.99 |
| Grobogan | 0.76 | 0.92 | 0.85 | 2.53 | 0.84 |
| Semarang | 0.87 | 0.68 | 1.03 | 2.58 | 0.86 |
| Klaten | 1.23 | 0.94 | 0.89 | 3.06 | 1.02 |
| Total | 4.13 | 3.47 | 3.55 | 11.15 | |
| Average | 1.03 | 0.87 | 0.89 | | 0.93 |

**1. Degrees of Freedom**

p (Treatment) = 4

u (Repeat) = 3

df Treatment = p – 1 = 4 – 1 = 3

db Error = p (u – 1) = 4 (3 – 1) = 8

Total degrees of freedom = db Treatment + db Error = 3 + 8 = 11

**2. Correction Factor (CF)**

$$FC = \frac{(\Sigma Yij)^2}{p.u} = \frac{(11,15)^2}{4.3} = 10.36$$

**3. Sum of Squares (SS)**

SQ Total = $[(1.27)^2 + (0.93)^2 + \ldots\ldots + (0.89)^2] - FC$

= 10.70 – 10.36

= 0.34

$$\text{SQ Treatment} = \frac{(2,98)^2 + (2,53)^2 + (2,58)^2 + (3,06)^2}{3} - FK$$

= 10.43 – 10.36

= 0.07

JK Error = JK Total – JK Treatment

= 0.34– 0.07

= 0.27

**4. Mean Square (MS)**

$$\text{MS Treatment} = \frac{\text{JK Perlakuan}}{\text{db Perlakuan}} = \frac{0,07}{3} = 0.02$$

MS Error $= \frac{\text{JK Galat}}{\text{db Galat}} = \frac{0,27}{8} = 0.03$

**5. Calculated F**

Calculated F for Treatment $= \frac{\text{KT Perlakuan}}{\text{KT Galat}} = \frac{0,02}{0,03} = 0.73$

**6. ANOVA Table**

| Source of Variation | df | SS | Sum of Squares | Calculated F | F Table 5% |
|---|---|---|---|---|---|
| Treatment | 3 | 0.07 | 0.02 | $0.73^{tn}$ | 4.07 |
| Error | 8 | 0.27 | 0.03 | | |
| Total | 11 | 0.34 | | | |

Note: *: significant (calculated F > table F 5%) and n: not significant (calculated F < table F).

Coefficient of Variation (CV)

CV $= \frac{\sqrt{\text{KT Galat}}}{\text{rataan total}} \times 100\%$

$= \frac{\sqrt{0,03}}{0,93} \times 100\%$

$= 19.69\%$

Total Chlorophyll Content Data at 5 MST

| Source Inoculum | Total Chlorophyll Content | | | Total | Average |
|---|---|---|---|---|---|
| | Repeat 1 | Repeat 2 | Repeat 3 | | |
| | ----------mg/g---------- | | | | |
| Without Inoculum | 2.7 | 2.18 | 1.86 | 6.74 | 2.25 |
| Grobogan | 2.16 | 1.82 | 2.08 | 6.06 | 2.02 |
| Semarang | 1.58 | 1.56 | 2.58 | 5.72 | 1.91 |
| Klaten | 2.34 | 2.38 | 2.10 | 6.82 | 2.27 |
| Total | 8.78 | 7.94 | 8.62 | 25.34 | |
| Average | 2.20 | 1.99 | 2.16 | | 2.11 |

**1. Degrees of Freedom**

p (Treatment) = 4

u (Repeat) = 3

df Treatment = p – 1 = 4 – 1 = 3

db Error = p (u – 1) = 4 (3 – 1) = 8

Total degrees of freedom = db Treatment + db Error = 3 + 8 = 11

**2. Correction Factor (CF)**

$$FC = \frac{(\Sigma Yij)^2}{p.u} = \frac{(25,34)^2}{4.3} = 53.51$$

**3. Sum of Squares (SS)**

Total $= [(2.70)^2 + (122.18)^2 + \ldots\ldots + (2.10)^2] - FK$

$= 54.94 - 53.51$

$= 1.43$

JK Treatment $= \frac{(6,74)^2 + (6,06)^2 + (5,72)^2 + (6,82)^2}{3} - FK$

$= 53.79 - 53.51$

$= 0.28$

JK Error = JK Total – JK Treatment

$= 1.43 - 0.28$

$= 1.15$

**4. Mean Square (MS)**

$$\text{MS Treatment} = \frac{\text{JK Perlakuan}}{\text{db Perlakuan}} = \frac{0,28}{3} = 0.09$$

$$\text{MS Error} = \frac{\text{JK Galat}}{\text{db Galat}} = \frac{1,15}{8} = 0.14$$

**5. Calculated F**

$$\text{Calculated F for Treatment} = \frac{\text{KT Perlakuan}}{\text{KT Galat}} = \frac{0,09}{0,14} = 0.66$$

**6. ANOVA Table**

| Source of Variation | df | SS | KT | Calculated F | F Table 5% |
|---|---|---|---|---|---|
| Treatment | 3 | 0.28 | 0.09 | $0.66^{tn}$ | 4.07 |
| Error | 8 | 1.15 | 0.14 | | |
| Total | 11 | 1.43 | | | |

Note: *: significant (calculated F > table F 5%) and n: not significant (calculated F < table F).

Coefficient of Variation (CV)

$$CV = \frac{\sqrt{\text{KT Galat}}}{\text{rataan total}} \times 100\%$$

$$= \frac{\sqrt{0,14}}{2,11} \times 100\%$$

$$= 17.95\%$$

**Appendix 15**. Processing of Stomatal Opening Data.

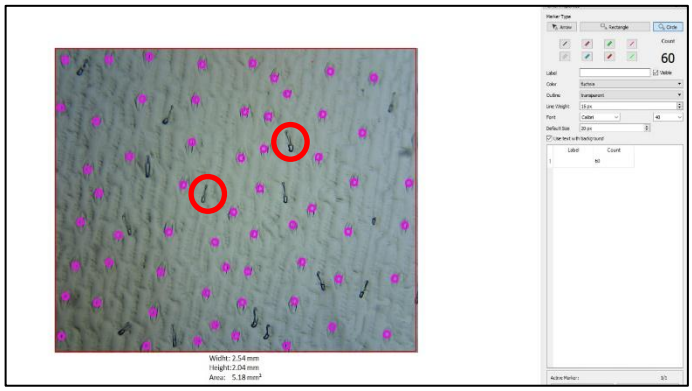
Stomatal Opening Observation Data

| Source Inoculum | Repetition | Stomatal Observation | | | Stomatal Opening (%) |
|---|---|---|---|---|---|
| | | Stomata Open | Closed Stomata | Number of Stomata | |
| Without Inoculum | 1 | 60 | 14 | 74 | 81.08 |
| | 2 | 53 | 10 | 63 | 84.13 |
| | 3 | 64 | 10 | 74 | 86.49 |
| Grobogan | 1 | 54 | 12 | 66 | 81.82 |
| | 2 | 71 | 11 | 82 | 86.59 |
| | 3 | 54 | 12 | 66 | 81.82 |
| Semarang | 1 | 86 | 25 | 111 | 77.48 |
| | 2 | 74 | 18 | 92 | 80.43 |
| | 3 | 64 | 18 | 82 | 78.05 |
| Klaten | 1 | 61 | 11 | 72 | 84.72 |
| | 2 | 62 | 13 | 75 | 82.67 |
| | 3 | 80 | 10 | 90 | 88.89 |

Stomatal opening calculation:

$$\text{Stomatal opening} = \frac{\text{Stomata membuka}}{\text{Jumlah stomata}} \times 100\%$$

Example of stomatal calculation on one unit (Without Inoculum 1) with magenta marks indicating open stomata and red circles indicating closed stomata as follows:



Stomatal Opening Data on 3-Month-Old Plants

| Source Inoculum | Stomatal Opening | | | Total | Average |
|---|---|---|---|---|---|
| | Repeat 1 | Repeat 2 | Test 3 | | |
| | ----------%---------- | | | | |
| Without inoculum | 81.08 | 84.13 | 86.49 | 251.70 | 83.90 |
| Grobogan | 81.82 | 86.59 | 81.82 | 250.23 | 83.41 |
| Semarang | 77.48 | 80.43 | 78.05 | 235.96 | 78.65 |
| Klaten | 84.72 | 82.67 | 88.89 | 256.28 | 85.43 |
| Total | 325.10 | 333.82 | 335.25 | 994.17 | |
| Average | 81.28 | 83.46 | 83.81 | | 82.85 |

**1. Degrees of Freedom**

p (Treatment) = 4

u (Repeat) = 3

df Treatment = p – 1 = 4 – 1 = 3

db Error = p (u – 1) = 4 (3 – 1) = 8

Total degrees of freedom = db Treatment + db Error = 3 + 8 = 11

**2. Correction Factor (CF)**

$$FC = \frac{(\Sigma Yij)^2}{p.u} = \frac{(994,17)^2}{4.3} = 82364.50$$

**3. Sum of Squares (SS)**

JK Total $= [(81.08)^2 + (84.13)^2 + \ldots\ldots + (88.89)^2] - FC$

$= 82,486.37 - 82,364.50$

$= 131.87$

SQ Treatment $= \frac{(251,70)^2 + (250,23)^2 + (235,96)^2 + (256,28)^2}{3} - FK$

$= 82441.50 - 82364.50$

$= 77.00$

JK Error = JK Total – JK Treatment

= 131.87– 77.00

= 54.87

**4. Mean Square (MS)**

Treatment $= \frac{\text{JK Perlakuan}}{\text{db Perlakuan}} = \frac{77{,}00}{3} = 25.67$

KT Error $= \frac{\text{JK Galat}}{\text{db Galat}} = \frac{54{,}87}{8} = 6.86$

**5. Calculated F**

Calculated F for Treatment $= \frac{\text{KT Perlakuan}}{\text{KT Galat}} = \frac{25{,}67}{6{,}86} = 3.74$

**6. ANOVA Table**

| Source of Variation | df | SS | KT | Calculated F | F Table 5% |
|---|---|---|---|---|---|
| Treatment | 3 | 77.00 | 25.67 | $3.74^{tn}$ | 4.07 |
| Error | 8 | 54.87 | 6.86 | | |
| Total | 11 | 131.87 | | | |

Note: *: significant (calculated F > table F 5%) and n: not significant (calculated F < table F).

Coefficient of Variation (CV)

CV $= \frac{\sqrt{\text{KT Galat}}}{\text{rataan total}} \times 100\%$

$= \frac{\sqrt{6{,}86}}{82{,}85} \times 100\%$

$= 3.16\%$

**Appendix 16**. Processing of Stomatal Density Data.

Stomatal Density Observation Data

| Source Inoculum | Replication | Number of Stomata (stomata) | Cross-sectional Area (mm²) | Stomatal Density (stomata/mm²) |
|---|---|---|---|---|
| Without Inoculum | 1 | 74 | 5.18 | 14.29 |
| | 2 | 63 | 5.12 | 12.30 |
| | 3 | 74 | 5.13 | 14.42 |
| Grobogan | 1 | 66 | 5.06 | 13.04 |
| | 2 | 82 | 5.10 | 16.08 |
| | 3 | 66 | 5.09 | 12.97 |
| Semarang | 1 | 111 | 5.09 | 21.81 |
| | 2 | 92 | 5.13 | 17.93 |
| | 3 | 82 | 5.13 | 15.98 |
| Klaten | 1 | 72 | 5.13 | 14.04 |
| | 2 | 75 | 5.09 | 14.73 |
| | 3 | 90 | 5.14 | 17.51 |

Stomatal density calculation:

$$\text{Stomatal density} = \frac{\text{Jumlah stomata}}{\text{Luas penampang (mm}^2\text{)}}$$

Stomatal Density Data in 3-Month-Old Plants

| Source Inoculum | Stomatal Density | | | Total | Average |
|---|---|---|---|---|---|
| | Repeat 1 | Repeat 2 | Repeat 3 | | |
| Without inoculum | 14.29 | 12.30 | 14.42 | 41.01 | 13.67 |
| Grobogan | 13.04 | 16.08 | 12.97 | 42.09 | 14.03 |
| Semarang | 21.81 | 17.93 | 15.98 | 55.72 | 18.57 |
| Klaten | 14.04 | 14.73 | 17.51 | 46.28 | 15.43 |
| Total | 63.18 | 61.04 | 60.88 | 185.10 | |
| Average | 15.80 | 15.26 | 15.22 | | 15.43 |

**1. Degrees of Freedom**

p (Treatment) = 4

u (Repeat) = 3

df Treatment = p – 1 = 4 – 1 = 3

db Error = p (u – 1) = 4 (3 – 1) = 8

Total degrees of freedom = db Treatment + db Error = 3 + 8 = 11

**2. Correction Factor (CF)**

$$FK = \frac{(\Sigma Yij)^2}{p.u} = \frac{(185{,}10)^2}{4.3} = 2855.17$$

**3. Sum of Squares (SS)**

Total SQ $= [(14.29)^2 + (12.30)^2 + \ldots\ldots + (17.51)^2] - FK$

$= 2933.48 - 2855.17$

$= 78.31$

SQ Treatment $= \frac{(41{,}01)^2 + (42{,}09)^2 + (55{,}72)^2 + (46{,}28)^2}{3} - FK$

$= 2899.98 - 2855.17$

$= 44.81$

JK Error = JK Total – JK Treatment

$= 78.31 - 44.81$

$= 33.49$

**4. Mean Square (MS)**

MS Treatment $= \frac{\text{JK Perlakuan}}{\text{db Perlakuan}} = \frac{44{,}81}{3} = 14.94$

ME of Error $= \frac{\text{JK Galat}}{\text{db Galat}} = \frac{33{,}49}{8} = 4.19$

**5. Calculated F**

Calculated F for Treatment $= \frac{\text{KT Perlakuan}}{\text{KT Galat}} = \frac{14{,}94}{4{,}19} = 3.57$

**6. ANOVA Table**

| Source of Variation | df | SS | KT | Calculated F | F Table 5% |
|---|---|---|---|---|---|
| Treatment | 3 | 44.81 | 14.94 | $3.57^{tn}$ | 4.07 |
| Error | 8 | 33.49 | 4.19 | | |
| Total | 11 | 78.31 | | | |

Note: *: significant (calculated F > table F 5%) and n: not significant (calculated F < table F).

Coefficient of Variation (CV)

$$CV = \frac{\sqrt{\text{KT Galat}}}{\text{rataan total}} \times 100\%$$

$$= \frac{\sqrt{4,19}}{15,43} \times 100\%$$

$$= 13.27\%$$

**Appendix 17**. Processing of Fresh Weight Data.

Fresh Weight Data of 5-Year-Old Crowns

| Source Inoculum | Fresh Weight of Crown | | | Total | Average |
|---|---|---|---|---|---|
| | Repeat 1 | Repeat 2 | Repeat 3 | | |
| Without inoculum | 46.79 | 48.34 | 47.75 | 142.88 | 47.63 |
| Grobogan | 43.58 | 44.55 | 41.18 | 129.31 | 43.10 |
| Semarang | 44.26 | 41.98 | 45.03 | 131.37 | 43.76 |
| Klaten | 41.32 | 48.39 | 40.56 | 130.27 | 43.42 |
| Total | 175.95 | 183.26 | 174.52 | 533.73 | |
| Average | 43.99 | 45.82 | 43.63 | | 44.49 |

**1. Degrees of Freedom**

p (Treatment) = 4

u (Repeat) = 3

df Treatment = p – 1 = 4 – 1 = 3

db Error = p (u – 1) = 4 (3 – 1) = 8

Total degrees of freedom = db Treatment + db Error = 3 + 8 = 11

**2. Correction Factor (CF)**

$$FC = \frac{(\Sigma Yij)^2}{p.u} = \frac{(533{,}73)^2}{4.3} = 23{,}738.98$$

**3. Sum of Squares (SS)**

Total SQ $= [(46.79^2 + (48.34)^2 + \ldots\ldots + (40.56)^2] - FK$

$= 23{,}828.85 - 23{,}738.98$

$= 89.87$

SQ Treatment $= \frac{(142{,}88)^2 + (129{,}31)^2 + (131{,}37)^2 + (130{,}27)^2}{3} - FK$

$= 23{,}779.29 - 23{,}738.98$

$= 40.31$

JK Error = JK Total – JK Treatment

= 89.87– 40.31

$= 49.57$

**4. Mean Square (MS)**

MS Treatment $= \frac{\text{JK Perlakuan}}{\text{db Perlakuan}} = \frac{40,31}{3} = 13.44$

EE $= \frac{\text{JK Galat}}{\text{db Galat}} = \frac{49,57}{8} = 6.20$

**5. Calculated F**

Calculated F for Treatment $= \frac{\text{KT Perlakuan}}{\text{KT Galat}} = \frac{13,44}{6,20} = 2.17$

**6. ANOVA Table**

| Source of Variation | df | SS | Sum of Squares | Calculated F | F Table 5% |
|---|---|---|---|---|---|
| Treatment | 3 | 40.31 | 18.44 | $2.17^{tn}$ | 4.07 |
| Error | 8 | 49.57 | 6.20 | | |
| Total | 11 | 89.87 | | | |

Note: *: significant (calculated F > table F 5%) and tn: not significant (calculated F < table F).

Coefficient of Variation (CV)

CV $= \frac{\sqrt{\text{KT Galat}}}{\text{rataan total}} \times 100\%$

$= \frac{\sqrt{6,20}}{44,49} \times 100\%$

$= 5.60\%$

**Appendix 18**. Processing of Dry Weight Data.

Dry Weight Data of 5-Year-Old Canopy

| Source Inoculum | Dry Weight of Crown | | | Total | Average |
|---|---|---|---|---|---|
| | Test 1 | Test 2 | Test 3 | | |
| Without inoculum | 7.32 | 7.97 | 7.80 | 23.09 | 7.70 |
| Grobogan | 6.32 | 6.73 | 5.82 | 18.87 | 6.29 |
| Semarang | 6.22 | 5.90 | 6.73 | 18.85 | 6.28 |
| Klaten | 5.81 | 7.97 | 5.73 | 19.51 | 6.50 |
| Total | 25.67 | 28.57 | 26.08 | 80.32 | |
| Average | 6.42 | 7.14 | 6.52 | | 6.69 |

**1. Degrees of Freedom**

p (Treatment) = 4

u (Repeat) = 3

df Treatment = p – 1 = 4 – 1 = 3

db Error = p (u – 1) = 4 (3 – 1) = 8

Total degrees of freedom = db Treatment + db Error = 3 + 8 = 11

**2. Correction Factor (CF)**

$$FC = \frac{(\Sigma Yij)^2}{p.u} = \frac{(80,32)^2}{4.3} = 537.61$$

**3. Sum of Squares (SS)**

SQ Total $= [(7.32^2 + (7.97)^2 + \ldots\ldots + (5.73)^2] - FC$

$= 545.95 - 537.61$

$= 8.34$

SQ Treatment $= \frac{(23,09)^2 + (18,87)^2 + (18,85)^2 + (19,51)^2}{3} - FK$

$= 541.73 - 537.61$

$= 4.12$

JK Error = Total JK – Treatment JK

= 8.34 – 4.12

= 4.22

**4. Mean Square (MS)**

Treatment $= \frac{\text{JK Perlakuan}}{\text{db Perlakuan}} = \frac{4,12}{3} = 1.37$

KT Error $= \frac{\text{JK Galat}}{\text{db Galat}} = \frac{4,22}{8} = 0.53$

**5. Calculated F**

Calculated F for Treatment $= \frac{\text{KT Perlakuan}}{\text{KT Galat}} = \frac{1,37}{0,53} = 2.60$

**6. ANOVA Table**

| Source of Variation | df | SS | KT | Calculated F | F Table 5% |
|---|---|---|---|---|---|
| Treatment | 3 | 4.12 | 1.37 | $2.60^{tn}$ | 4.07 |
| Error | 8 | 4.22 | 0.53 | | |
| Total | 11 | 8.34 | | | |

Note: *: significant (calculated F > table F 5%) and n: not significant (calculated F < table F).

Coefficient of Variation (CV)

CV $= \frac{\sqrt{\text{KT Galat}}}{\text{rataan total}} \times 100\%$

$= \frac{\sqrt{0,53}}{6,69} \times 100\%$

$= 10.85\%$