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ANALYSIS OF DISEASE SEVERITY ON CITRUS LEAVES (*CITRUS* spp.) USING *IMAGEJ* AND *PLANTIX* SOFTWARE

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Abstract

The purpose of this case study is to determine the severity of citrus leaves using Plantix software and ImageJ software. Based on the results of this case study, it was found that the severity of the disease using ImageJ software was on citrus leaves at the first location (10.641%), citrus leaves at the second location (53.278%), and citrus leaves at the third location (15.828%) which were generally the fruit plants caused by fungi and based on the results of Plantix software data, diseases in the first, second, and third locations of citrus leaves were caused by bacterial diseases. Based on the results of bacterial cell culture, isolates were found to be round white in color with a slight yellowness which is a characteristic of Xanthomonas axonopodis bacteria with a large number of ±12 colonies and a smaller number of ±35 colonies. Using software and imageJ can help farmers to find out the types of pests that attack citrus plants so that farmers can handle plants to minimize damage to citrus plants caused by pests.

Keywords: *Citrus* sp., *Disease*, *ImageJ*, *Plantix*.

INTRODUCTION

Citrus is a type of popular fruit because it has a fresh taste and contains several vitamins useful for the body. Citrus plants are often found in Indonesia, especially in the Banyuwangi and Jember areas. Citrus in Indonesia include jeruk manis, sitrun, jeruk nipis, jeruk purut, jeruk limau, and jeruk bali (Foda et al., 2021). There is a problem often faced

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by citrus farmers, namely the emergence of pests and diseases that attack, starting from the seeding period until the time of harvest arrives. Pests and diseases that exist in citrus plants are the main problems that can strike at any time and cause concern for farmers. Lack of knowledge and information related to various types of pests and diseases and the symptoms they cause causes delays in handling them and mistakes in handling. This results in citrus plants not being able to produce quality fruit and can cause plant death (Nurastuti, 2023).

In the process of growth and development of fruit plants, of course there are many factors that can inhibit growth which can usually be caused by external and internal factors. External factors usually include temperature, water, bacteria, viruses and insects. According to Wijaya et al. (2017) this effect can have an impact on fruit plants in general and citrus in particular are often attacked by bacteria.

Citrus is an annual fruit plant originating from Asia. China is believed to be the first place where oranges grow. Citrus is a plant that can grow well in tropical and subtropical regions. Types of bacteria that can infect citrus plants, for example is CVPD/HLB *(Liberobacter asiaticus*). HLB has been known to attack citrus plantations since the 1940s, almost all citrus-producing provinces in Indonesia were reported to have been seriously attacked (Murtando, 2016).

Citrus plants originate from Asia and are widely grown in the tropics. Not only are they diverse, tropical fruits that grow in Indonesia also contain vitamins, very high nutritional value, and many other health benefits (Agmalaro et al., 2013). Many Indonesian farmers cultivate citrus plants because their growth is capable of being in the highlands and lowlands (Sianturi, 2019). Citrus plants have erect, woody and hard stems with thorns that have branches that curve upwards and reach a height of ± 15 meters. Leaf shapes vary with serrate and smooth edges with slightly blunt pointed ends (Adelina, 2017).

Many cases of crop failure experienced by farmers are due to pests and diseases that attack citrus trees, and result in crop failure and losses. The lack of farmers' knowledge of diseases that can attack plants is also an obstacle that reduces the success of plant cultivation (Basri, 2020). Technological advances that are developing rapidly can help farmers solve problems easily (Fajri, 2019). Based on research by Rosadi et al. (2022) and Qurrahmah et al. (2022) there are ImageJ Software and Plantix Software which can help farmers to more easily identify diseases or pests that attack planted citrus trees. With this method, farmers can carry out proper handling of diseases so that it will increase crop productivity. Plantix Software is an application developed by PEAT GmbH in Berlin using excellent features, so that it can help farmers detect pests and diseases in plants by simply taking pictures of the plants. Plantix is an application that helps provide data to users to analyze types of diseases in fruit plants. This application is used by photographing the symptoms of disease on the leaves, then it will display the causes of the disease, symptoms, how to deal with it, and how to prevent it (Qurrahmah et al., 2022). Meanwhile, ImageJ Software is a program that can help

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process data with image objects and to determine the parameters of disease severity in leaves (Zaelani et al., 2020). ImageJ and Plantix software are applications that can detect diseased plants so that farmers can carry out proper handling and can affect crop yields (Kothari, 2018).

To find out the pathogens that cause disease in citrus plants, the plants that have been analyzed with Plantix and ImageJ software will then be subjected to bacterial culture. Bacterial culture is a way of multiplying microbes in a culture medium and by culturing in a controlled laboratory. This culture technique can be used to isolate pure cultures derived from microbes. Pure culture (axenic) is a number of populations of multicellular cells or organisms that can grow without the presence of other organisms (Mulyono, 2019). According to Nurtjahyani & Shyntya (2014) pure culture is a culture in which microbial cells originate from the division of a single cell. Pure culture has the advantage of studying or identifying microbes, including studying cultural, morphological, physiological and serological characteristics, which require a population consisting of only one type of microorganism. Slide culture is an identification technique for a colony by growing it on a piece of agar media which is then placed in a petri dish. Later on the surface of the agar will appear spots which can be identified as fungi or bacteria (Erina et al., 2018).
Based on this, a research on disease severity analysis was carried out using ImageJ and Plantix Software which aims to see the severity of the disease in citrus plants and the pathogens that cause citrus plant diseases in order to obtain good plant productivity.

METHODS

This research was conducted from September to November 2022. Sampling of citrus plant leaves was carried out in the Samarinda area, East Kalimantan. Data analysis was carried out at the Laboratory of Animal Anatomy and Microtechnics, Department of Biology, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda.

Citrus leaf samples used were taken from three different areas as representative samples in Samarinda City, where the distance between each region was quite far, in order to determine differences in disease attack on citrus plants in each region, which were taken with five repetitions. Leaves at the first location were taken in the area of the Faculty of Mathematics and Natural Sciences, leaves at the second location were taken in the area of Jl. Perjuangan 7, North Sempaja while the third leaf in the third location was taken in the Palaran area.

Identification Stage using *ImageJ* and *Plantix*

Identification was carried out in this study using ImageJ Software and Plantix Software. The entire leaf area and leaf spot area were measured using ImageJ Software, so that the percentage of severity of disease symptoms was known on lime leaves and identifying yellow spots on citrus leaves as areas with disease symptoms based on color differences (color threshold) by adjusting the saturation and brightness, while the identification of disease symptoms on lime leaves

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can be analyzed using Plantix Software. Pictures of leaves that have disease symptoms are taken so that yellow or black spots will be detected as plant symptoms based on a database of fruit or other plant diseases. Users will automatically get information about the disease, its causes and treatment.

Media Creation

NA media was prepared by weighing 28 grams of NA (Nutrient Agar) powder, then putting it into an Erlenmeyer. Homogenize the mixture using a magnetic stirrer. Covered with cotton and aluminum foil. Then sterilize in the autoclave at 121^{o}C for 15 minutes. After sterilizing the media in the Erlenmeyer, store it in the refrigerator.

Inoculation and Preparation of Pure Bacterial Cultures on NA Media

At this stage, the method is carried out by preparing the tools and materials used for inoculation in Laminar Air Flow. The NA preparation medium was heated until it melted, then poured into a petri dish (+ 15 mL) and allowed the media to cool and solidify. The next step is to pour the liquid NA medium into the petri dish and allow it to cool and solidify. Touch the surface of the colony that has been determined with a sterile loop needle then scratch it on the surface of the media. Incubate upside down at 37^{o}C for 24 hours and 96 hours. Observe the characteristics of colony growth, note the presence or absence of contamination.

The next step is count the number of colonies, the number of lesions, the diameter of the lesions and the area of the colonies. All the parameters that were searched for were carried out using ImageJ Software in a way that is, first the bacteria on NA media which had been incubated for 24 hours and 96 hours were photographed, then the existing images were input into the ImageJ Software. After that, the parameters observed for the first time were counting the number of bacterial colonies on NA media and the area. After obtaining the number of colonies and their area, then proceed with calculating the second parameter, namely the number of lesions and the diameter of the lesion. At this stage, ImageJ Software will immediately generate valid data.

RESULTS AND DISCUSSION

The sample used in this observation consisted of 15 leaves from 1 type of citrus leaf fruit plant taken from 3 different locations (Figure 1). The first location was taken from the FMIPA UNMUL campus area, the second location was taken from the struggle road, and the third location was taken from Palaran. The citrus leaves that are taken are leaves that have disease symptoms. Common disease symptoms observed on citrus leaves are yellow-brown spots.

The fruit plants from the first location were caused by the bacterium Xanthomonas axonopodis, the fruit plants from the second location were caused by sooty fungi, and the fruit plants from the third location were caused by the insect Procontarinia. Symptoms of this disease were analyzed using Plantix Software.

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Table 1. Symptoms of citrus leaf disease using plantix software

No	Sample	Symptoms of Disease
1.	Leaves of jeruk purut at FMIPA campus (location 1)	The leaves have cratered wounds. Rusty brown in color surrounded by a bright yellow halo. When ruptured, a distinct lesion is formed with a light brown or gray center and a brownish, greasy, and wet-looking margin.
2.	Leaves of jeruk purut at Jl. Perjuangan (lokasi 2)	The leaves have mildew with a gray to black color on the leaf surface. Defoliation occurs and can die.
3.	Leaves of Jeruk limau at Jl. Palaran (location 3)	The leaves have small spots like warts that cover the leaves. Leaves look deformed.

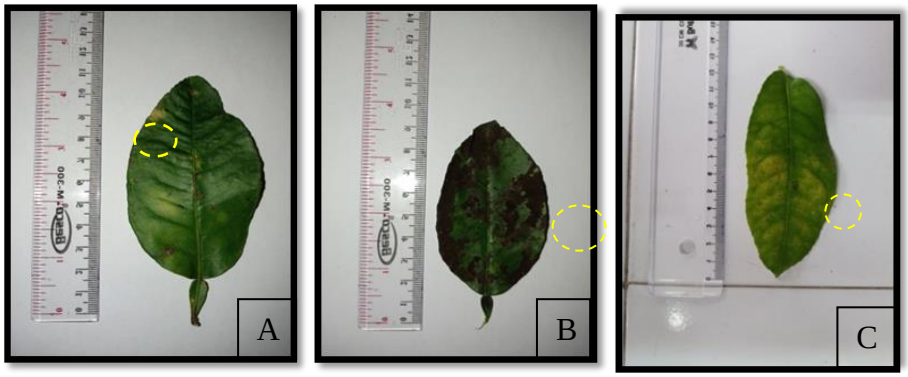

Figure 1. Leaves with disease symptoms, (A) Leaves of jeruk purut at FMIPA campus (location 1); (B) Leaves of jeruk purut at Jl. Perjuangan (location 2); and (C) Leaves of jeruk limau at Jl. Palaran (location 3). The dotted yellow circle is an example of a part of the leaf with a rusty brown crater

The results showed that the total area was the diseased area and the non-disease area on the leaves (Table 2). The leaves of citrus in the first location had a disease area of 2,098 cm^2, the second location had a disease area of 11,678 cm^2 and the third location had a disease area of 2,463 cm^2. The lowest to the highest leaf disease area was on lime leaves at locations 1, 3, and 2. Meanwhile, the area of leaves not affected by disease from highest to lowest was leaves of ctirus at location 3, namely 94,218 cm^2, leaves of citrus location 2, namely 55,883 cm^2, and leaves of citrus location 1, namely 93,213 cm^2.

The results also show the severity of the diseased leaves. The third location of the leaves of citrus has the highest leaf severity level. The percentage of severity of leaf disease symptoms from lowest to highest respectively, namely the second location of citrus leaves 8.411%, the first location of citrus leaves 10.220%, and the third location of citrus leaves 15.828%.

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Table 2. Overall area (LA), diseased area (LAP) and non-disease area (LATP) on leaves.

No.	Sample	LA (cm^2) (Average ± SD)	LAP (cm^2) (Average ± SD)	LATP (cm^2) (Average ± SD)
1.	Leaves of citrus first loc	30.915 ± 10.577	2.098 ± 2.969	93.213 ± 8.411
2.	Leaves of citrus second loc	26.471 ± 2.922	11.678 ± 2.078.	55.883 ± 10.220
3.	Leaves of citrus third loc	42.605 ± 4.820	2.463 ± 7.583	94.218 ± 15.828

Based on observations of 5 replicate samples of citrus leaves, the first location showed symptoms of the disease which could be seen from the leaves, there were cratered wounds, rusty brown in color surrounded by a bright yellow circle, when broken, a certain wound was formed with a light brown or gray center and colored edges, brownish, oily and looks wet and the average severity of disease symptoms is 10.614%, and is suspected of being affected by the bacterial disease *Xanthomonas axonopodis*. According to Iliana et al. (2020) a disease in citrus plants, namely citrus cancer caused by the presence of the bacterium *Xanthomonas axonopodis* pv. Citri with symptoms that appear as white spots under the leaves, yellow spots which will turn black brown and will form necrotic lesions. According to Kristi et al. (2022) that cancers that infect leaves have general symptoms, namely the presence of round yellow lesions measuring 1 mm, over time they will turn into brown lesions with a size of 10 mm surrounded by a circle of chlorosis and in the middle having a rough texture like cork. The general symptoms that appear on citrus leaves affected by this bacteria are brownish chlorosis lesions surrounded by a yellow halo, the lesions will rise to the surface and form cork tissue. This bacterium causes leaves to have necrotic sores on infected leaves, stems, and pods, and is considered to be one of the most serious diseases worldwide. Yield and quality of citrus infected with citrus cancer, the fruit and leaves are deformed and can fall (Wei et al., 2019).

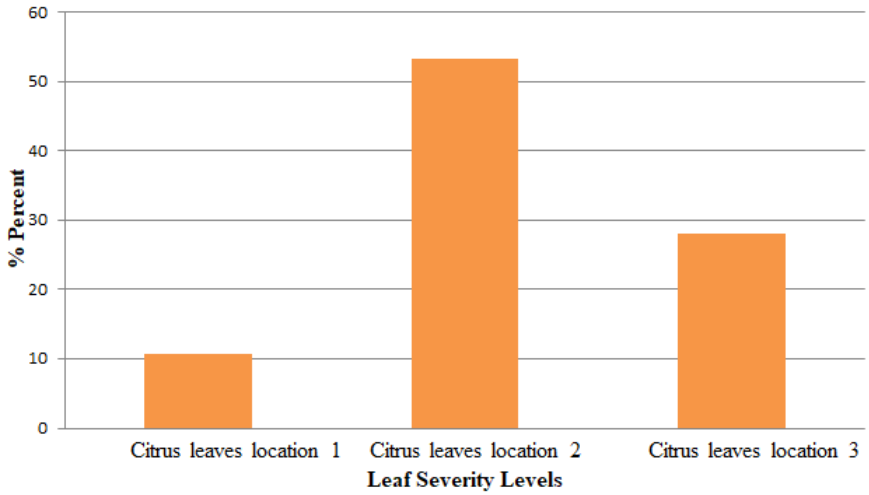

Figure 2. Leaf severity levels on fruit plants

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In the second location of the citrus leaves sample, based on observations of 5 replicate samples, the symptoms of the disease can be seen on the leaves, namely the leaves have gray to black fungus on the surface of the leaves, defoliation occurs and can die which is thought to be caused by soot fungus and the average the average severity level of lime leaves is 53.278%. According to Fiani et al. (2017) symptoms of sooty mildew disease are the presence of a thin black layer on the surface of the leaves. This black layer is part of the fungal mycelia which is spread on the leaf surface and can inhibit metabolism such as the photosynthesis process. Sooty mildew disease can cause physical damage to citrus plants. Sooty mildew disease is caused by the fungus *Capnodium citri* Berk. & Dec. which has black mycelium that is scattered and can cover the surface of citrus plant leaves (Labib et al., 2015).

In the repetition of 5 samples of citrus leaves, the third location showed symptoms of disease that could be seen on the leaves, namely the leaves had small spots like warts covering the leaves, the leaves looked deformed with an average severity level of lime leaves of 28.106%. According to Wijaya et al. (2018), insects such as flies cause spots or the appearance of black dots that are not obvious, these stains can spread widely and cause leaves to fall and die. This disease causes small spots to appear on the leaves due to insects or insect larvae which will make the leaves deformed and have holes over time (Arumingtyas et al., 2021).

The results of observations of inoculation of lime leaf samples with disease symptoms using the streak plate method aimed at culturing pathogens showed the presence of bacterial colonies. Colony results after incubation for 24 hours on the media showed colony growth with morphological characteristics such as shape (circular), margin (entire), elevation (convex), size (moderate), pigment (non-pigmented), texture (smooth), appearance (glistening) which includes the culture morphology of *Xanthomonas axonopodis* bacteria with different numbers and sizes. The number of colony sizes that look large is ± 7 colonies and the number of smaller colony sizes is ± 30 colonies. The results of the 96-hour incubation (Figure 3) showed that the large colony sizes were ± 12 colonies and the number of smaller colony sizes was ± 35 colonies.

Figure 3. Bacterial cell culture media incubation 96 hours

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Bacterial colonies for 24 hours had an overall area of 89,085 cm^2, the area of bacterial colonies was 2,131 cm2, and the area of media that did not grow colonies was 97,607 cm^2. Furthermore, the bacterial colonies for 96 hours had an overall area of 87,303 cm^2, the area of the bacterial colonies was 14,278 cm^2, and the area of the media that did not grow colonies was 83,645 cm^2. These results were derived from samples of citrus leaves which had symptoms of bacterial disease, then the bacteria were propagated using the streak plate method showing the presence of colonies of *Xanthomonas axonopodis* bacteria which grew for 24 hours incubated and experienced an increase in colony growth with incubation for 96 hours. From the results obtained in this study, it can be clearly identified the types of pests and diseases of citrus plants, can provide detailed and clear information to farmers so that they do not hesitate in diagnosing diseases and pests experienced by citrus plants. Farmers can anticipate and control diseases and pests that attack citrus plants, as well as take appropriate steps to eradicate pests and diseases by using appropriate drugs/pesticides. Citrus farmers can vary the treatment in eradicating diseases and pests (Sukri, 2016). According to Ariesdianto (2021), one of the main factors causing a decrease in the level of citrus production is disease attacks on citrus leaves. To overcome this problem, it is necessary to identify the diseased citrus leaves to determine the type of pest that infects them. Of the three locations, there were citrus plants that were most affected by pests and diseases caused by *Xanthomonas axonopodis* bacteria, where pests caused a decrease in the level of citrus production so that many farmers experienced crop failures. This is based on the Plantix application, but has not been proven in vitro.

Table 3. Total area (LA), area of bacterial colonies (LKB) and area of media that do not grow colonies in bacterial culture.

No.	Sample	LA (cm2) (Average ± SD)	LKB (cm2) (Average ± SD)	LMTTK (cm2) (Average ± SD)
1.	24 Hour Bacteria Colony	89.085 ± 10.577	2.131 ± 92.000	97.607 ± 10.577
2.	96 Hour Bacteria Colony	87.303 ± 6.434	14.278 ± 18.000	83.645 ± 3.1781

CONCLUSION

Based on the results of the study, it was found that the severity of the disease using ImageJ Software on the first location of citrus leaves (10.614%), second location of citrus leaves (53.278%), and third location of citrus leaves (28.106%) and based on data obtained from Plantix Software, the causes Diseases on citrus leaves first location, second location, third location respectively are due to the bacterial disease *Xanthomonas axonopodis*, sooty fungus, and *Procontarinia* insects.

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