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# The Pathogenic Fungi on Cempaka (Magnolia Elegans (Blume) H. Keng) Leaves at Various Altitudes in Agroforestry Systems

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***Abstract-*** This article describes the disease in the Cempaka tree that has the potential to reduce wood yield the development of Cempaka tree cultivation business Cempaka cultivation through agroforestry systems. This study aims to identify pathogenic fungi on Cempaka trees at various altitudes in the agroforestry systems. This research was carried out of on three locations of cummmunity forests that applies a agroforestry systems. The leaves sampling is obtained from three altitudes categories namely 0-400 m asl, > 400-700 m asl, and >700 m asl. The analysis of leaves to identify disease carried out in the laboratory using the steps of the Koch postulate. The results showed that Phomopsis sp. fungus is a pathogenic fungus that causes leaf spot disease in Cempaka on all three altitudes. Phomopsis sp. fungus can infect the leaf tissue through the wound or directly penetrate the leaf epidermal tissue.

***Keywords-*** *Cempaka Tree, Agroforestry, Leaf Spot, Phomopsis SP*

## I. INTRODUCTION

Cempaka trees (Magnolia elegans (Blume.) H.Keng) are plants that are in great demand by the people in North Sulawesi because they have high economic value [1]. Cempaka wood in North Sulawesi is widely used as furniture materials, house construction materials (boards, beams, floors, frames, doors and windows) [2] and the main raw materials in the construction of the Minahasa stage house or better known as the "Woloan House" [3]. Cempaka cultivation in North Sulawesi is mostly done by Minahasa people in the form of community forests with agroforestry systems [4].

The agroforestry systems proposed by Lundgren and Raintree, which are used in World Agroforestry Center publications, are a set of names for land-use systems and technologies where annual woody plants are deliberately used in the same land management units along with agricultural crops and / or animals, in various forms of space management and time management [5]. The combination of planting annual woody plants and seasonal crops can create a microclimate situation where light intensity decreases, air temperature decreases and air humidity increases [6].

A plant that is afflicted with a disease can cause losses, among others, to reduce the quantity and quality of yields and increase production costs [7]. Leaf disease needs attention because photosynthesis generally occurs in leaves where photosynthetic rate is closely related to plant diameter growth. Diameter growth takes place if photosynthesis results such as respiration, leaf replacement, root growth and height have been fulfilled [8]. Occurrence of infectious diseases that have been reported is leaf spot disease on seedlings in seedlings due to pathogenic fungi, namely Colletotrichum sp. [1].

Until now, disruption of the disease on the cempaka plants in agroforestry systems at various altitudes above sea level has not been widely reported. This study is to identify the type of pathogenic fungi that attack abruptly at three altitudes. Information obtained from identification activities can be used to determine future disease control measures which will certainly be considered in the management strategy of agroforestry systems.

## II. RESEARCH METHODS

This research was conducted for three months, namely from August to September 2017, in three different locations based on the category of place height, namely Lemoh Village, Minahasa District (0-400 masl), Rumoong Atas Village, South Minahasa District (400-700 masl), and Kinilow Village, Tomohon City (> 700 masl), North Sulawesi Province. The isolation and identification of pathogenic fungi was carried out at the Laboratory of Microbiology and Plant Disease, Department of Pests and Diseases, Faculty of Agriculture, Sam Ratulangi University, Manado. The activities of Koch's postulate were carried out at the Green House Permanent Nursery, Tondano Protected Forest Watershed Management Center (BPDASHL) in Kima Atas Village.

The steps taken in this study are as follows:

1. Observation of symptoms and signs of disease observing affected plants / showing symptoms of disease in the cempaka plants.
2. The taking of specimens is carried out by taking the leaf of cempaka which shows the symptoms and signs of disease. Each symptom of the disease is recorded and then taken to the laboratory for the next stage of research.

3. Making PDA media is based on the method used [9] with modifications to antibiotic use. The medium composition of PDA (Potatos Dextrose Agar) per liter of distilled water consisted of 39 g dissolved with distilled water then heated on a hot plate with magnetic stirrer, then sterilized by autoclaving at 121˚C, 2 atm pressure for 15 minutes.
4. Isolation of fungi from plant tissues is carried out based on the methods used [10] and [11] with modifications to the use of disinfectant materials for surface sterilization of plant tissues.
5. The leaf which is indicated as sick is sorted based on the location and symptoms of the disease and then washed in running water and placed in a container according to the symptoms and then air dried.
6. The diseased plant tissue on the cempaka leaves is then cut between the healthy and diseased parts with a size of 0.25 cm x 0.25 cm and then dipped in a disinfectant solution 2 times for 2-3 minutes then rinsed using sterile water. After that it is dried on sterile filter paper in a petri dish. The network of sick plants that have been cut and sterilized and then put into a petri dish containing PDA + AB media as much as two pieces per petri dish, then the edges of the petri dish are wrapped with parafilem and labeled and placed on a culture rack. Work is done in the LAF (Laminar Air Flow) room.
7. Identification of fungi is carried out using a microscope connected to optilab to determine the type of fungus. The identification process is done by looking at the morphological characters in a macroscopic and microscopic manner. Identification is carried out based on library references [12] and [13].
8. The implementation of the Koch Postulate was carried out at the Green House BPDASHL Permanent Nursery Tondano, Kima Atas, Manado. Inoculation is carried out with 2 treatments for each seed, which is the leaf which is injured and which is not injured. Repetition was made as many as 5 repetitions of each isolate obtained. Treatment of the opening on the seedlings is carried out with the help of sterile needles. The part of the plant to be inoculated previously is surface sterilized with 70% alcohol. Preparation of the source of the inoculum and block attachment to be carried out based on the method used [14] with modifications to the time and place of plant incubation. The source of inoculum was obtained by cutting the colonies of mushroom isolates aged 7 days with cork temporary (7 mm diameter). After that, the pieces are attached to the injured leaves. As a control, other parts of the leaves (which have also been injured) are attached with blocks to prevent colonies of fungal isolates. The block pieces that are attached to the leaves are then covered with moist cotton and aluminum foil for ± 7 days or until symptoms appear. Disease symptoms that appeared at the point of inoculation were then isolated, then isolates from the isolated isolates were identified and compared with previous isolates. If the inoculated fungal isolates produce symptoms that are identical to the symptoms of the previous cempaka leaf disease and identified as fungi that are identical to the previous isolates, the fungus is the causative agent of the disease.

## III. RESULTS AND DISCUSSION

The initial sign of leaf spot disease in the infestation in 3 agroforestry land locations is that on the leaf surface there is necrosis in the form of stains or patches with brownish color that has a clear boundary (Figure 1).

Figure 1. Leaves on cempaka leaves

This is in accordance with research which states that the initial sign of leaf spot disease on the seedlings in the nursery is the presence of stains or patches on the surface of the leaf with a clear boundary [1]. The leaves formed are generally brown, surrounded by darker boundaries. The shape of the leaf spot varies and tends to be irregular. The size of the blotch increases over time and will expand to cover all parts of the leaf. In the leaf spots that have extended the color boundary between the center and the edges will be more clear, in the middle of the spot the color is somewhat brighter than the edges of the spots.

The results of the identification of this study found one type of pathogenic fungus that causes leaf spot disease, namely Phomopsis sp. Based on the results of laboratory studies, colony morphology of the fungus Phomopsis sp. that is, having textures such as wool or cotton, white mycelium, and dark and rounded conidia. These characteristics are in accordance with the characteristics of the fungus Phomopsis sp., which is a white, grayish white to brownish colony with wool-like and round shaped texture with a dark color [15]. Conidia fungus Phomopsis sp. not bersepta, filiform, and hyaline with a size of 10.5-18 x 1 mm, whereas conidiophores have branching, filiform and hyaline [12]. Mushroom Phomopsis sp. is a fungus that has a dark picnidia, the ostiolate sinks almost round. Ostiolat is the place where fungal spores are released. Konidiophore is simple, conidia hyaline, 1 cell with two types namely ovoid to fusoid called conidial alpha and curved or bent filiform called beta conidia. This fungus is parasitic causing various black spots on the plant [16].

Figure 2. Conidia of fungi Phomopsis sp.

Mushroom Phomopsis sp. reportedly can act as plant pathogens, endophytic fungi, saprophytic fungi, and can even cause health problems in humans and animals. As a pathogen in plants, mushroom Phomopsis sp. can cause cancer, dead shoots, leaf blight, leaf blotches, root rot and fruit rot. .As for the imperfect form of the fungus Phomopsis sp. is Diaporthe sp. [17]

## IV. KOCH POSTULATE

Koch postulate is a series of activities carried out to prove the primary cause of a disease in plants. The results of the fungus inoculation were Phomopsis sp. shows that the entire inoculum causes symptoms in seedlings especially on the injured leaves.

Mushroom Phomopsis sp. cause symptoms at the point of inoculation that is injured or at the point of inokuation that is not injured. The incubation period of Phomopsis sp. to cause symptoms is 5 days. This indicates that the fungus Phomopsis sp. requires natural wounds and holes to enter the plant tissue, based on observations of fungus inoculation Phomopsis sp. cause symptoms that are identical to the natural symptoms of leaf spot on the cempaka. The fungus that has been inoculated on the seedlings can be isolated from the seedlings which show symptoms. Based on the results of the series of Koch postulate activities, it can be seen that the isolates tested were pathogenic to seedlings and fungi that were suspected to be the primary cause of symptoms of leaf spot disease in phlegm are Phomopsis sp.

(a)

(b)

Figure 3. A: Symptoms of leaf specks on the chrysanthemum, B: Chrysanthemum seeds attacked by leaf spot disease during Koch postulate activities

## V. CONCLUSION

1. The results of the identification found 1 type of pathogenic fungus causing leaf spot disease in the plague in all three altitudes, namely mushroom Phomopsis sp.
2. Fungus Phomopsis sp. can infect the leaf tissue through the wound or penetrate directly the leaf epidermis tissue.

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