Advances in Biological Sciences Research, volume 14
Proceedings of the 3rd KOBI Congress, International and National Conferences (KOBICINC 2020)

White Rot Disease on Garlic Crop: Identification of the Pathogen and Exploration of the Controlling Strategies

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ABSTRACT
Garlic is horticultural commodity which has very high economic value in Indonesia. Garlic is usually used as a cooking spice, for medicines as well as for cosmetic ingredients. However, Indonesian garlic production is very limited and only supplies at most 17.5% of domestic demand. The province of West Nusa Tenggara (WNT) is one of the largest garlic producing regions in Indonesia where it is mostly grown in the Sembalun highlands of Eastern Lombok (1200 m above sea level). Recently, garlic cultivation in WNT Province is facing an increasing problem of white rot disease. Therefore, it is beneficial to identify the symptom and sign of the disease in the field, investigate the pathogen that associated with the disease, and exploring possible strategies to control the disease. This research was conducted in July – September 2019. Samples were collected from diseased garlic crop in the Sembalun highlands and laboratory analysis was conducted at the Microbiology Laboratory, Faculty of Agriculture, Mataram University, Indonesia. The symptom of white rot disease was leaf yellowing started at the age of 75-80 days after sowing and the sign was the fungus with white mycelium on garlic bulbs and formation of sclerotia around the bulbs. Further identification in the laboratory found that the fungi has white mycelium and produced sclerotia. It is indicated that the fungus is the species of *Sclerotium cepivorum*. In addition, some controlling strategies were proposed to handle the pathogen, such as seed coating treatment, application of antagonistic fungi, and chemical control as the last option.

Keywords: *disease, garlic, Lombok, Sembalun highlands, white rot*

1. INTRODUCTION

Garlic (*Allium sativum* L.) is known as a horticultural commodity which has very high of economic value [1]. Garlic is the 14th most important vegetable crop worldwide [2]. Among the *Allium* genus, garlic is the secondly most consumed plant in the world [3]. Garlic production and acreage, over the last fifty years, have increased about 200% with more than 1.5 million hectares in 2014 [4]. In Indonesia, garlic is very important horticultural commodity because it is usually used as a cooking spice, for medicines as well as for cosmetic ingredients [5].

Garlic known as a medicinal plant because it has several bioactive compounds. It has nutritional and health benefits such as for anticancer and antibiotic as well as compounds for the treatment of metabolic, cardiovascular and respiratory system diseases [5]. Therefore, it is desirable to increase its consumption worldwide [6].

The annual demand of garlic in Indonesian is currently estimated 500,000 tons. Domestic production can fulfilled at most of 88,000 tons [7]; therefore the importation of garlic reached 412,000 tons (82.4%) yearly. This condition makes possibility of many imported garlic varieties are circulating in the traditional and modern markets in Indonesia [8].

The Sembalun highlands is located in Lombok Island, West Nusa Tenggara (WNT) Province. It is one of the garlic production regions in Indonesia. However, the garlic production in this region has declined since 1997. This decreasing was due to lot of imported garlic in the market with much cheaper price compared to the local one. Since 2017, Indonesian government was encouraging farmers to increase domestic production by increasing planting areas across the country. However, garlic cultivation

in this region recently is facing an increasing problem of the plant disease such as white rot disease of garlic in the fields. This research aimed to identify the symptom and the sign of white rot disease in the fields and investigate the pathogen that associated with the disease as well as exploring possible strategies to control the pathogen.

2. MATERIALS AND METHODS

2.1. Survey and sampling location

This research was conducted in July - September 2019. Samples of garlic diseased crop were collected from the Sembalun highlands of Eastern Lombok, WNT Province of Indonesia (Fig 1).

Laboratory analysis was conducted at the Microbiology Laboratory, Faculty of Agriculture, Mataram University, Indonesia. Equipment used were: Petri dishes, Erlenmeyer flasks, autoclave, incubator, Beaker glass, hoses, macro pipette, dropper pipette, pipette tips, Bunsen burner, test tubes, sterile cotton stick, ruler, laminar flow cabinet, hot plate, stirrer, analytical scales, test tube racks, measuring cups, scissors, tweezers, filters and evaporators. The materials used were: diseased garlic crop samples, potato dextrose agar (PDA), distilled water, cotton, tissue, 70% alcohol, paper labels, 96% ethanol, physiological NaCl solution, corn paper, wrapping paper, filter paper, chloramphenicol as an antibiotic, and aluminum foil. The research equipments were sterilized at a temperature of 121^{o}C with a pressure of 2 atm for about 15 minutes. Meanwhile, the medium used is sterilized together with the equipments.

2.2. Preparation of Potato Dextrose Agar (PDA) media

PDA media was made by dissolving 60 ml of potato dextrose extract, added 240 ml of distilled water, then added 6-grams of glucose and 6-grams of agar until the Erlenmeyer was neatly covered with sterile cotton and aluminum foil. The media was heated until homogeneous while stirring. Then it was sterilized by autoclave for 15 minutes at a pressure of 2 atm and temperature of 121°C.

2.3. Isolation of fungi from garlic diseased crop

Isolation of fungus from garlic diseased crops was conducted from root, bulb and sclerotia. PDA media containing isolated fungi were incubated for 24 hours at room temperature. Every single colony that grew from these isolates was purified. A pure culture of the fungus was maintained on PDA for identification purposes [9].

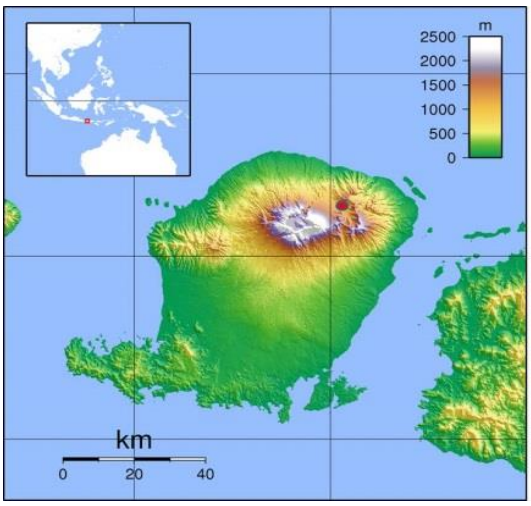

Figure 1 Survey and sampling site in the Sembalun highlands (1200 m above sea level) of North Eastern Lombok with red dot sign on the upper right part of the island.

2.4. Purification and characterization of the fungal isolates

Fungal isolates were purified by taking spores or mycelium and then spotting them on PDA media in a Petri dish. Incubation was carried out at a room temperature for 1 - 3 days. Characterization was conducted by observing the morphological characteristics of the colony growth, the colony color, insulation on the hyphae, color and shape of spores and conidiophores. The books used to identify the colonies of the fungus were: 1) The Illustrated Genera of Imperfect Fungi (Second Edition) [10], 2) The Illustrated Genera of Imperfect Fungi (Fourth Edition) [11], The Illustrated Genera of Ascomycetes (Volume I) [12], 4) Combined Keys to Illustrated Genera of Ascomycetes (Volumes I & II) [13], and 5) The Genera of Hypomycetes [14].

3. RESULT AND DISCUSSION

3.1. The disease symptom

Identification is a very precise process in an effort to suppress a pest, especially a pathogen, therefore control can be carried out more precisely because more specific pathogens are known, the life cycle and environment are not optimal and other factors that can harm the pathogen can be identified.

The symptom of the white rot disease was leaf yellowing started at the age of 75-80 days after sowing (around one month before harvesting) (Fig 2). The first symptom noted was foliar symptoms. Stunted plant were yellow followed by wilted foliage.

The older leaves dying first followed by the air leaves. The level of plant damage determinded by soil and environmental conditions. Pathogens grow in cold and humid temperatures. Therefore, in an appropriate environmental conditions, there is an increasing of pathogenic activity following the development of root systems.

The pathogen infected garlic at all growing stages, when the disease fully developed causing the plant turn to yellow and wilt. Another symptom which appearanced was the growth of mycelia on the roots, after that spreads to the bulb and causing rot. The growth of mycelia can be easily observed at the stem base when the foliage turns yellow and first appeared of leaf symptoms.

White rot pathogen can contaminated soil for many years even decades. The pathogen also known as *Sclerotium cepivorum* which occured worlwide. It is affecting alliums such as garlic and onions. The white rot disease is a big constraints for farmers because once the pathogen is infected the field then it is very difficult to remove effectively. White rot disease is usually contaminated a field through plant material or soil, therefore growing disease-free seed is very important. Once a small area of field contaminated by pathogen, then the pathogen can be easily spread to the healthy soil and plant. The machinery and flood water that was previously in contact with disease materials can also spread the pathogen [15].

Figure 2 White rot disease symptom in the field at the Sembalun highlands of Eastern Lombok, Indonesia. Leaves yellowing started at the age of 75-80 days after sowing (around one month before harvesting).

3.2. The disease signs

The disease sign was the fungus has white mycelium on garlic bulbs with sclerotia formed around the bulbs (Fig 3). Sclerotia is a survival structure of the fungus which can remain in dormant stage for years and will grow on a vulnerable host. Sclerotia can also appear on the mycelium [16].

Figure 3 Sign of the garlic white rot disease in the field at Sembalun highlands of Eastern Lombok with white mycelium and formation of sclerotia around the bulbs.

Sclerotia can infect the host plant and spreads. Sclerotia formed on rotting host tissue and then remain free in the soil. In order to control the white rot disease in the field, the number of sclerotia in the soil should be reduced to minimized fungal growing. However, multiple control strategies are needed in order to gain good results. Wind, water, tools, boots, etc., will stir up sclerotia moves from infested soil and cause spreading of the disease [16].

Sclerotium cepivorum is a soil-borne fungal pathogen. It has a monocyclic disease means that only has one reproductive cycle per season. *S. cepivorum* is a unique fungal pathogen because does not produce spores which are essential for a normal life cycle. It exists in winters as sclerotia as a survival structure. Sclerotia are tiny black globular structures which resistant to inappropriate temperature conditions. Sclerotia can survive and dormant for years when there is no host. It will germinate as a response to root exudates. Hyphal germination and growth are influence by weather as well. The mycelium grows through the soil and forms appressoria as host roots become available. The host was able to sticked and penetrated by appressoria. The disease array can be created by mycelium grows from the roots and spread to next plants. A small amount of sclerotia can cause disease and once plant infected then it will be difficult to control [16].

Environmentally, white rot pathogens are temperature dependent. Environmental conditions affect germination thereby favoring cold weather (10-20^{o}C). Germination and infection will be favoured when there is high soil moisture. However, growth of fungal and sclerotia will be stunted when the

temperature is above 20ºC. In addition, irrigation system can also a factor in spreading disease from infected to clean fields since pathogen favourable in cool weather. Therefore, this disease is of particular concern for farmers experiencing cool weather during the growing season [16].

3.3. Laboratory identification

Further identification in the laboratory found that the fungus has hyphae and produced sclerotia. It is indicated that the fungus is the species of *Sclerotium cepivorum* Berk [16]. *Sclerotium cepivorum* is a form of asexual reproduction of *Stromatinia cepivora*. It is a plant pathogen causing white rot disease in *Allium* species such garlic, leeks, and onions.

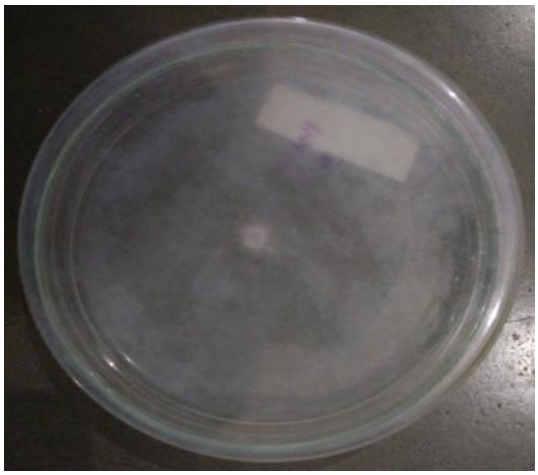

Figure 4 Mycelia of *S. cepivorum* isolated from garlic root on PDA media. It was incubated at a room temperature for three days.

Figure 5 Observation of hyphae of *S. cepivorum* isolated from garlic root under the microscope with 40x magnifications.

Globally, white rot disease is possibly the most serious constraints for production of *Allium* crop. White rot is a soil-borne disease where sclerotia can affects susceptible plants grown in infected soil. Sclerotia can spread to other areas where limited sanitation applied. Sclerotia also can survive in the soil for many years and will germinate once there are susceptible host. Therefore, it is very important to do appropriate sanitation practices. Once disease has occurred, then avoid rotation with other species of *Allium*. Infection risk can be reduced by using disease-free seed and avoiding contamination from infected land. In addition, make sure that machines, boots, and equipments should be cleaned in order to stop the spread of pathogen from infected areas. Infections usually occured in cold weather (10-20ºC) therefore it is important to plant the crops at the appropriate time to reduce the disease [16].

Importantly, white rot is a serious disease of allium family plants. The fungi can stay in the soil for many years. The white rot disease is present in all allium-producing regions and a threat to the allium production industry worldwide. The white rot disease was first found in Oregon, the United States in 1918 and the most recent in 2014 in onion fields. *S. cepivorum* is one of the most devastating pathogens of onions and garlic. Once the soil is infested, it is deemed unsuitable for onion and garlic production for up to 40 years or even more [16].

3.4. Exploration of possible controlling strategies

Some possible strategies to control the disease which are doable by farmers are: 1) Seed treatments before sowing (seed coating technique i.e. by bio-fungicide, iprodione fungicide); 2) Application of antagonistic fungi (such as *Trichoderma* sp., *Coniothyrium minitans*); and 3) Chemical control as the last option (using selective fungicides such as Rovral, Chipco green).

4. CONCLUSION

Symptom of the white rot disease is leaf yellowing started at the age of 75-80 days after sowing. Sign of the disease is the fungus has white mycelium on garlic bulbs with sclerotia formed around the bulbs. The formation of sclerotia indicated that the fungus is species of *S. cepivorum*. The isolated fungus should be identified to species level using molecular tests. Proposed controlling strategies should be tested in the glasshouse first before further application in the fields.

AUTHORS' CONTRIBUTIONS

The first author (BNH) was the main contributor of this paper, while the second to the fourth authors (NH, ARA, and MS) were the supporting contributors.

ACKNOWLEDGMENTS

These findings were part of the garlic project entitled "Low input cultivation packages on garlic in West Nusa Tenggara Province of Indonesia" through the annual budget for the West Nusa Tenggara Institute for Assessment of Agricultural Technology (WNT -IAAT), Indonesia. We thank the Indonesian Agency for Agricultural Research and Development (IAARD) for funded this research.

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