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An investigation of species associated with stem of cacao in Sulawesi under climate change

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ABSTRACT.

Phytopthora palmivora Butl. causes various diseases in cocoa, including *Phytophthora* stem canker (or cacao stem canker) and Phytophthora pod rot. However, in Sulawesi, another pathogen has been reported to infect cocoa causing stem canker and dieback. This study aimed to investigate the fungal/oomycetes species associated with stem canker lesions in Luwu Timur, Sulawesi, Indonesia and thereby identify the main putative pathogen causing stem canker of cocoa in the region. The research was carried out in the field and in the Laboratory of Plant Pests and Diseases of the Muslim University of Indonesia. The study consisted of several stages, namely sampling, preparation of various types of comparison media, isolation from tissue in stem canker lesions, culture and microscopic observation. The test plants consisted of 11 clones (9-10 year-old trees), including 5 clones showing symptoms of stem canker, namely M01, M06, Sul1, Sul2 and clone 45 (MCC02). Infected tissue excised from stem canker lesions was transferred to media plates (V8, PDA). Subcultures resulted in colonies producing hyphae and sporangia identified as *Phytophthora* spp., consistent with previous reports that *P. palmivora* causes stem canker in cacao. Furthermore, inoculation of healthy pods of four clones (both attached and detached) with infected canker tissue resulted in lesions demonstrating Phytophthora pod rot symptoms. Lesion diameter determined daily for 8 days, indicated that the largest lesions developed in M06 (101,8 cm) and the smallest in Sul1 (5.8 cm), demonstrating variation in clone resistance to *P. palmivora*. Re-isolation from the pod lesions caused by inoculations resulted in *Phytophthora* colonies and sporangia. These results are consistent with previous reports, namely that *P. palmivora* is the principle cause of cacao stem canker.

Keywords: cocoa, stem canker, *phytopthora palmivora*, climate change, plant disease

1. Introduction

Cocoa (*Theobromae cacao* L) is a plantation crop that has high economic value and is an important commodity in international trade. Several countries are trying to increase cocoa production to supply the world's cocoa needs, such as Ivory Coast which supplies 39.8% with a production of 1.5 million tons/year, Ghana supplying 19.6% and Indonesia 8.1% (ICCO : 2017). Cocoa production in Indonesia has decreased significantly in the last decade. One of the factors behind decreasing cocoa production is the presence of pests and diseases. Indonesia was once the world's third major cocoa producer, but it has now dropped to six after Brazil (Parawansa, 2020; ICCO., 2020). Previously, Ivory Coast and Ghana In 2014, the area of cocoa plantations in Indonesia was 1,774,303.97 ha with a production of 777,500 tons around 90% was cultivated by smallholders, particularly in Sulawesi, producing 500 million tons annuallyand constituting 60% of Indonesia's cocoa area. This is followed by Sumatra with a cocoa-growing area close to 300,000 ha, and a production of up to 150,000 tons (Directorate General of Plantations, 2015) Parawansa, 2014). Since then national cocoa production has decreased markedly. This can be attributed to a decrease in farm productivity. Currently, productivity of cocoa farms is low, generally under 500 kg/ha/year, while the production potential can reach more than 1 ton/ha.

The fungus *Phytophthora palmiivora* is a pathogen that can attack all parts of the plant and its symptoms can be found on the leaves, fruit, stems of cocoa (Parawansa, 2020; McMahon, 2016).

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Symptoms of the diseases caused by *palmivora* can be found on the leaves, fruit, and stems of cocoa, causing anthracnose, fruit rot and canker.

Symptoms of stem canker appear in the form of clearly demarcated wounds on the bark, surrounded by callus tissue that is often open, so that the wood looks visible from the. Other species associated with these symptoms have been isolated, raising the question of whether *P. palmivora* is the primary cause of the disease in Sulawesi cacao-growing areas.

This research was conducted to:

1. verify that the main cause of stem canker in Sulawesi is *P. palmivora* and to identify any other fungal species associated with canker symptoms
2. to compare species on different cocoa genotypes (propagated as clones)

2. Methodology

2.1 Time and place of the research

This research was conducted for 5 months from July2020 to November 2020. The research was carried out in 2 stages, namely in the field to take samples, which came from small holder gardens in East Luwu after a period of flooding and in the laboratory located at Muslim University in Makassar.

2.2 Materials and tools

The following procedures were included in implementation of the research :

1. Sampling in the field

Harvesting of cocoa stems from community plantations in Bangun Jaya Village, Kec. Tomoni Kab. East Luwu. Sampling was taken from plants that showed diseased plants, namely cocoa stems. Furthermore, the sample is analyzed in the laboratory

2. Isolation of the fungus on cocoa plants

The materials used in this study were cacao stems from4 cocoa clones that showed symptoms of stem canker The cacao clones were M01, MCC 02, Sulawesi 1 and Sulawesi 2, which had been propagated by side-grafting on root stock trees on small holder plantations in flooded locations.

Samples were taken from diseased stems by cutting into the bark a little and excising and then brought to the laboratory for isolation. In addition healthy stems were sampled in the same way. The samples (diseased and healthy) were plated onto PDA or V8 media. After isolation, they were placed in a sterile place. After obtaining pure cultures they were observed under a microscope using a reference book, Illustrated Genera of Imperfect Fungi, to identify putative species

Isolation was carried out on the stem of the cocoa plant by following the method proposed by Rodriques (1994) except that the surface sterilization procedure had been modified.. All parts of the stems of the plant were washed thoroughly in running water. Isolation was carried out in laminar airflow by cutting the stem of the cocoa plant into ± 1-2 cm. The cutting of plant parts was carried out aseptically under sterile conditions. The pieces were surface sterilized by immersion in sterile distilled water, 70% alcohol, and each for 1 minute. Then they were dried on sterile filter paper. Following this, the pieces were plated on PDA media, then incubated at room temperaturefor 4-7 days. The growth and development of cultures was observed and compared to known cultures to shed light on the possible types of fungi found on the stems of cocoa plants.

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3. Propagation of Fungal Isolates

After 4-7 days the mycelium that grew from the cut parts of the plant stem was transferred to a new medium, until a pure culture was obtained and incubated again at room temperature for one week.

The purification of fungal isolates was carried out to determine the various types of fungi that grow on the stems of cocoa plants, to facilitate observation or identification, and to facilitate the naming of the obtained fungi. Purification was carried out in the same way, namely the fungus that had been propagated previously was planted in the middle of the dish using a cork borer and stored for a week to be identified.

4. Identification of Fungal Isolates

Identification was attempted by taking a small amount of the purified fungus using tweezers, placing it on a glass slide, adding a few drops of water and then covering it with cover slip. Samples were observed under a HP microscope at X 10 and x40 magnification. The isolates were identified based on a text for the identification of fungi (Frank M. Dugan, 2005).

3. Results

Based on the results of isolation and detection on the stem of the cocoa plant (Theobroma cacao) the pathogenic fungus *Phytophtora palmivora* and other pathogenssuch as Rhizoctonia sp., and Fusarium sp. were detected. Table 1 shows the presence/absence of two of these in each of the clones sampled.

Table 1. Cacao stem clones infected with stem cancer, Phytopthora palmivora

No	Klon	Total of Isolation	Type	
			P. palmivora	*Rhizoctonia* sp
1.	M01	30	+	+
2.	MCC 02	30	+	-
3.	Sulawesi 1	30	+	-
4.	Sulawesi 2	30	+	-

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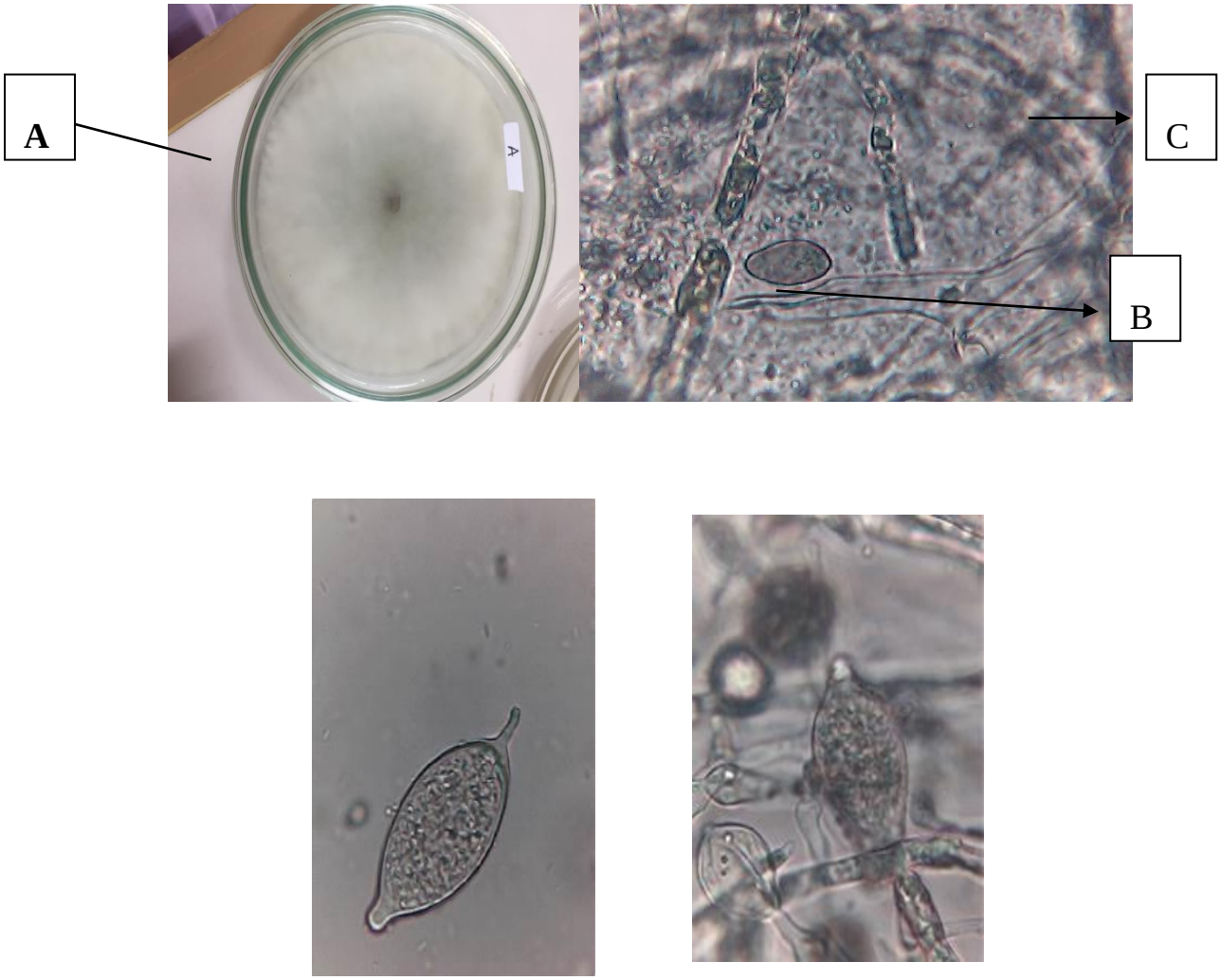

Phytophthora palmivora

Figure 1. The morphology of Phytophthora palmivora was isolated from each of the clones M01, MCC 02, Sulawesi 1 and Sulawesi 2 at the age of 8 days, (a) fungal colonies, (b) conidia, (c) hyphae (d) and (e) spora of Phytopthora palmivora (microscopic observation 100×)

Figure 1, shows structures of the fungus *Phytophthora palmivora*, including sporangia, obtained on the stems of clones M01, MCC 02, Sulawesi 1, Sulawesi 2: (a) fungal colonies on white media, one colony has merged with another (b) Sporangiumround and slightly oval shaped like a pear (and see lower photos, (c) the form of septate hyphae.

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Rhizoctonia sp.

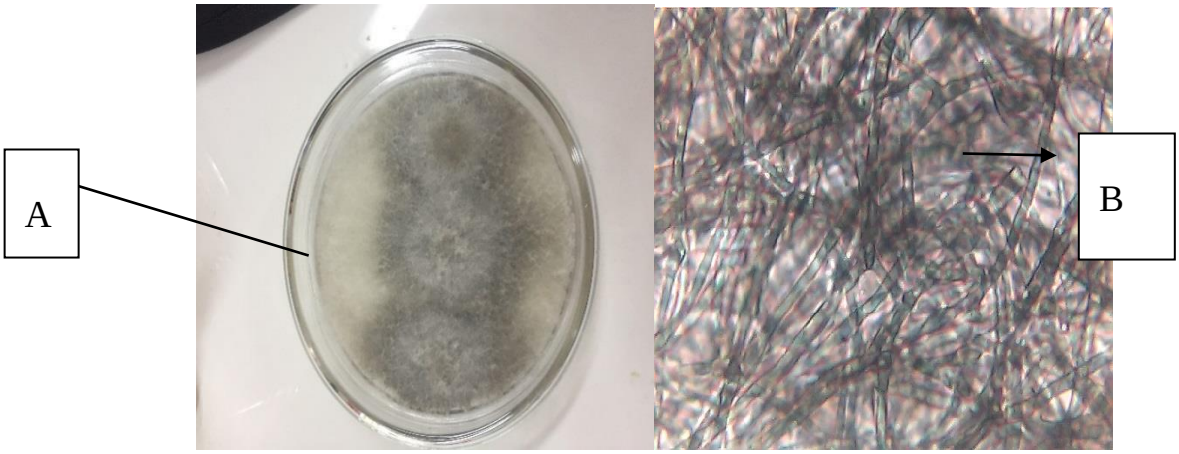

Figure 2. The morphology of Rhizoctonia in culturesof 8 days in all clones M01, MCC 02, Sulawesi 1 and Sulawesi 2 werethe same in appearance (a) fungal colonies, and (b) hyphae (microscopic observation 100×).

Figure 2, the morphological appearance of the fungus Rhizoctonia sp.showed (a) the upper fungal colonies were white like cotton, gradually changing color to blackish gray or black. Colonies with flat and thick edges and the color of the bottom lolon was black and did not form zones, (b) had no conidia, long hyphae with perpendicular branches and septate near the junction.

4. Discussion

At the identification stage, *Phytophthora palmivora* was found in all tested clones (M01, MCC02, Sulawesi 1 and Sulawesi 2) on stems that indicated the presence of stem canker symptoms . The pathogen*P.palmivora* has morphological characteristics with round and slightly oval sporangia (Mcmahon, 2016). In general, sporangia are ovoid or pear-shaped, with one clear papilla, many are globose and there are also some isolates that have obpyriform and ellipsoid shapes with sporangia size 53-61 X 32-42 m.Furthermore, the pear-shaped (ovoid) sporangia have clear papillae, are caduceus (easily separated from sporangiophores) with short stalks.

Phytophthora palmivora will release sporangia and chlamydospores when the air temperature ranges from 15-30ºC. The release and germination of zoospores occurs at a temperature of 15-30ºC while infection in the stem occurs at a temperature of 20-30ºC.. (So that the production system decreases, especially in the rainy season and dry season on land). The results showed that of the four clones tested, stem canker of *Phytopthora palmivora* was the probable causal pathogen, with the observation of typicals porangia and zoospores in isolates.

Figure 2 shows the morphology of an isolate identified as the fungus *Rhizoctonia* sp. Rhizoctonia is known to infect cacao stalks. Macroscopic characteristics were the color of the upper colony, which was white like cotton, which gradually turned white or gray, the edge of the colony was flat and thick, while the color of the bottom colony was black. Microscopic characteristics observed was the absence of conidia, with straight hyphae that have septa near the junctions (generally form a right-angle). When grown on PDA media, the mycelium will appear white at first. The fine mycelium branches to form a fine mesh and becomes septate. The distance between the two septa becomes shorter in generative structures, so the hyphae cells become short and rounded, especially as they get older. The branching of the mycelium is typical, that is, it is perpendicular.

According to Barnett and hunter (1998), among others, the mycelium is clear in some species and dark in others, the mycelium cells are usually long, the septa on the branches are formed from the

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main body, do not have conidia and other reproductive cells, have sclerotia that are light or dark in color. brown to black. Samuel and Keane (2012) added that Rhizoctonia has a hyphae branching arrangement that is perpendicular or almost perpendicular, has dolipore septa, no clamp connection, and hyphae constriction occurs near the branching point.

Rhizoctonia is a soil fungus, acts as a parasite and can also become a saprotroph. In the absence of rice plants, this fungus can infect other plants. Some *Rhizoctonia* in the asexual form (Thanatheporus), and (Ceratobasidium) areimportant pathogens of cocoa. Both forms can act as pathogens in cocoa plants. Ceratobasidium syn: Oncobasidium is known as a pathogen that causes Vascular Streak Dieback ; There are various environmental conditions that place plants at high risk of infection because these pathogens prefer wet climates to infect them.

Pathogenic fungi can infect through wounds, settle and develop in thevascular bundles. After the vascular tissue dies and the air is moist, the fungus forms purplish-white spores on infected roots. The spread of spores can be through wind, water, and agricultural tools. Fusarium sp. can grow well on a variety of agar media containing vegetable extracts. At first the mycelium was colorless, the darker the color was getting darker, finally the colony appeared to have threads. In older mycelium, thick-walled chlamydospores are formed. Fungi form numerous single-celled, colorless, oval or ovoid microclonidium, 6-15 × 2.5-4 m, less often macroconidia, coiled, colorless, mostly 2 or 3 insulated, 25-33 × 3.5 in size -5.5 .(According to Mc Mahon (2016), *Phytopthora palmivora* is the priniciple pathogenic fungus causing fruit rot and stem cancer of cocoa plants.

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