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# COMPARING THE SUSCEPTIBILITY OF TARO LEAVES WITH DIFFERENT AGES AGAINST PHYTOPHTHORA LEAF BLIGHT DISEASE

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**A B S T R A C T**

The study aims to examine Saitomo cultivar resistance to *Phytophthora colocasiae* isolate causing *leaf blight* disease following *floating leaf disc assay* method in vitro. This research was conducted in Tanralili village, Maros district. and Plant Disease Laboratory, Department of Pests and Plant Diseases, Faculty of Agriculture, Hasanuddin University, Makasssar. The research was carried out for 6 months during dry and wet seasons in 2020. Leaf ages were obtained from Satoimo cultivar of taro. Pathogen isolation was subcultured onto juice V-8 medium before putative *P. colocasiae* isolate was then tested into 2 cm square of leaf following *floating leaf disc assay* technique. Old leaf of Satoimo cultivar was the most susceptible and mature leaf was the most resistant. In this study, immature tissue did not show to have a more resistant to infection than other leaves. The causal disease-agent of leaf blight necrotic lesion on the infected leaf obtained from a wild taro had similar characters with *P. colocasiae* species.

**Keywords**: in vitro assay, leaf blight disease, putative *Phytophthora colocasiae,* Satoimo taro cultivar.

## INTRODUCTION

Taro is a potential crop as a staple food in the Tropics, particularly Indonesia, to substitute rice, sago, corn, or cassava and to strengthen food security. Taro generally grows in opened- forest areas or under the shade. Taro is a carbohydrate source, and with tuber, taro has bioactive ingredients for health due mainly to phenolic substances (Suprayatmi, M., 2017). In the future, the need for alternative carbohydrate is vital and taro can play a role. The demand of tuber export in Japan market is promisingly about 100 tonnes monthly. However, despite filling the supply and demand gap, the increase of taro production to supply food security and international market is scarce (Sulistiyono et al., 2017). One of significant causes is the threat of Phytophthora species (Drenth & Guest, 2004; Keane, 2010). Similar to other Phytophthora, they always become a primary limiting factor of the crop production system (Drenth & Guest, 2004; Guest, et al., 2004; Juan-Bachiller, 2004; Kuswinanti et al. 2019; 2020; Marelli et al., 2019).

Taro Leaf blight caused by *Phytophthora colocasiae* is the most damaging disease threatening the global taro industry (Drenth and Sendall, 2004; Erwin and Riberio, 1996; Keane, 2010; Misra et al. 2008). The pathogen is known as a close relationship with the most significant cause of famine in Ireland due to the calamitous destruction of potato crops, *P. infestans* (Keane, 2010). The pathogen infects all essential parts of the plant and every stage of taro development. The infection causes leaf lesions, and if the infection continues to develop, severe host occurs particularly vulnerable cultivar that impacts the undeveloped tuber. The agent of disease can also cause corm rot (Drenth and Sendall, 2004). The rapid spread of disease and pathogen in the environment is mainly caused by having wider-host ranges. Soon after infection, emerging sporangiospores on the infected tissue are carried by the wind, and the pathogen is equipped with a thick-walled spore (chlamydospores) that resists extreme weather (Drenth and Sendall, 2004; Erwin and Riberio, 1996). Also, the pathogen has a helping in survival for a long term in an environment (Quitugua and Trujillo, 1998).

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Furthermore, disease spread can also be helped in many ways, such as equipment contamination, insect vector, humidity, temperature, and infected tissue connection. The previous study undertaken found that ant colony contributes to spread phytophthora disease on cocoa (Junaid et al., 2020). Among other plant tissues, the corm is the most susceptible tissue infected by *P. colocasiae* (Drenth and Sendall, 2004).

The prominent sign of the disease appears in the leaves (Books, 2015). The initial symptom occurs in the upper leaf with small dark or light brown, particularly the tip and leaf margin. Water accumulation on the leaf can rapidly develop the lesion. There is no report of leaf ages' response to disease. However, understanding leaf age-pathogen interaction is necessary as it can characterize the leaf resistance of the taro cultivar. Leaf is an essential tissue to produce phytohormone against the disease (Marta, et al., 2017).

To cope with pathogen spread and yield loss due to leaf blight disease in developing taro industry in the future, seeking resistant cultivar is a very important component and one of them is by assay in vitro (Nath, 2016). Hence, this study was undertaken to examine resistance of different leaf ages of taro Satoimo cultivar against Phytophthora light blight disease that may contribute to profile resistant taro cultivar in the scale up stage.

**MATERIALS AND METHOD**

Sample collection with leaf blight and healthy leaf signs was undertaken in Sentoso Hamlet, Lekopancing Village, Kec. Tanralili, Maros Regency. For putative *P. colocasiae*, native isolate was obtained from wild taro community. Testing leaves of interest was undertaken at the Plant Disease Laboratory, Department of Pests and Plant Diseases, Faculty of Agriculture, Hasanuddin University, Makasssar. The study took place from September 2019 to March 2020.

***Subculture preparation:*** Putative *P. colocasiae* was subcultured into juice V-8 medium (Drenth and Sendall, 2004). The medium consisted of 15 g agar, 2 g $CaCo_3$, 200 mL of V-8 juice and 800 mL of distilled water, then autoclaved at 121$^{o}$C for 2 hours, then added ½ capsule chlorompenicol as antibiotic to avoid bacterium contaminant.

**Pathogen isolation:** Leaf of interest was sterilized with 70% Ethanol. The infected leaf was cut into small pieces (2 cm square) following ½ lesion and ½ healthy tissue. All activity was undertaken in the laminar airflow. The tissue (lesion and health) was submerged into 70% Ethol. Sequence events of surface sterilization consisted of leaf submersion with 1 min 70% Ethol, 1 min 2% NaOCl and 30 sec sterilized water before the leaf tissue was dried with sterile filter paper. Dried tissue was transferred into subculture juice V-8 medium. Four sheets of tissues were placed parallelly in a petri dish.

***Putative Phytophthora colocasiae isolation and detection:*** Putative isolate was collected from the infected wild taro with leaf blight sign and morphologically characterized with mycelium, sporangium shape, hyphal swelling, and papillation of sporangium. Purifying putative isolate was undertaken in the juice V-8 medium (Drenth and Sendall, 2004). Putative isolate growing into juice V-8 medium was cut with cork borer and transferred into a new subculture juice V-8 medium. Whole isolation activity was conducted with sterilized place and tools.

***Testing Satoimo cultivar using floating leaf disc assay method:*** Testing pathogenicity with floating leaf disc assay was previously demonstrated by Nath (2016). The procedure is to reach at Koch's postulate purpose. The procedure consisted of that healthy leaf was cut into a 2 cm square and subsequently inoculated putative *P. colocasiae* isolate laid into leaf center. A 2 cm square leaf with isolate was floated in the sterilized water surface on the petri dish. The leaf of interest was let to float under humid and dark condition and every day symptomatic lesion development on leaf surface was observed.

***Lesion development and disease incidence*:** Development of lesion diameter size was counted with measuring the length of necrosis on leaf trial in mm. The disease was statistically analyzed for disease incidence: the number of infected leaves divided into whole leaves observed times 100 percent. In this study, the pathogen was inoculated into three leaves in each leaf age, and for control, one leaf in every leaf age was free from pathogen inoculation.

***Pathogen isolation and identification:*** The putative *Phytophthora colocasiae* successfully obtained from symptomatic leaf blight on wild taro was confirmed with key determination of Phytophthora species (Drenth and Sendall, 2004) which the result has had similarity with the origin of the causal disease agent of leaf blight disease on taro. For more detail, the description of the pathogen was shown below (Figure 1).

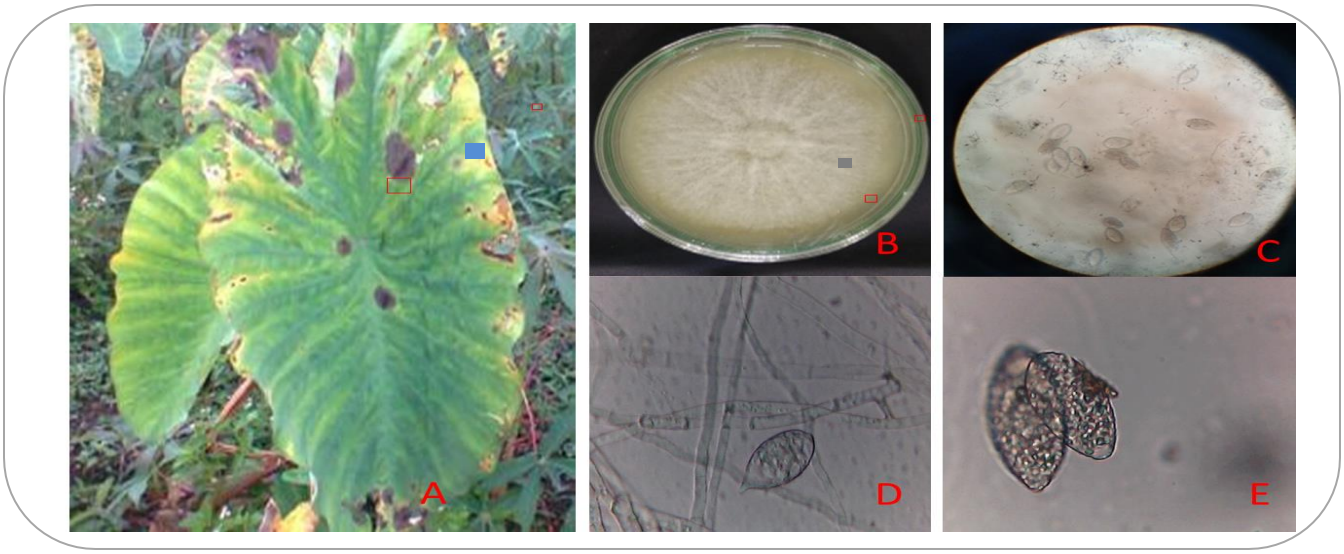


Figure 1. (A) leaf blight symptom on taro with dark brown spots and yellowing in leaf margin. Infected leaf tissue was inserted onto Juice V-8 medium (red square); B) mycelium inserted and transferred onto glass microscope (red square) shown to have cotton-like mycelium appearance; C) sporangium emerged on subculture; D) dark thick-walled hyphae (black arrow) and ovoid sporangium shape (read arrow), coducous and long-pedicle sporangium (white arrow) obtained from morphological characterization of putative Phytophthora colocasiae; E) Semi-papillate of sporangium (read arrow). Tremendous zoospores were generated in a sporangium (yellow arrow).

Sporangium shape, papillation of the sporangium and hyphal swelling are the important clues to characterize Phythophtora genus including *P. colocasiae* species (Drenth and Sendall, 2004). In the study, the observation found that sporangium obtained from putative isolate shapes like ovoid and is semi-papillate as well as long-pedicle sporangium (Figure 1 D-E). Typical hyphal swelling is absent on the isolate of interest. Whole characters shown in the study to have similarity of *P. colocasiae* causing leaf blight disease explained in the key determination of Phytophthora species (Drenth and Sendall, 2004). Overall, leaf blight lesion on taro is only caused by a single causal-disease agent, *Phytophthora colocasiae* (Drenth and Sendall, 2004; Erwin and Riberio, 1996).

**Development of symptomatic taro leaf blight:** In general, statistical analysis shown that, a significant lesion development occurred in old leaf of Satoimo cultivar after a week observation and continued to rise until the end. In contrast to old leaf on this test, the lesion development on immature and mature leaves were likely to develop insignificantly from the beginning to the end of observation compared to control. In this control, no putative *P. colocasiae* infected to immature, mature and old leaves.

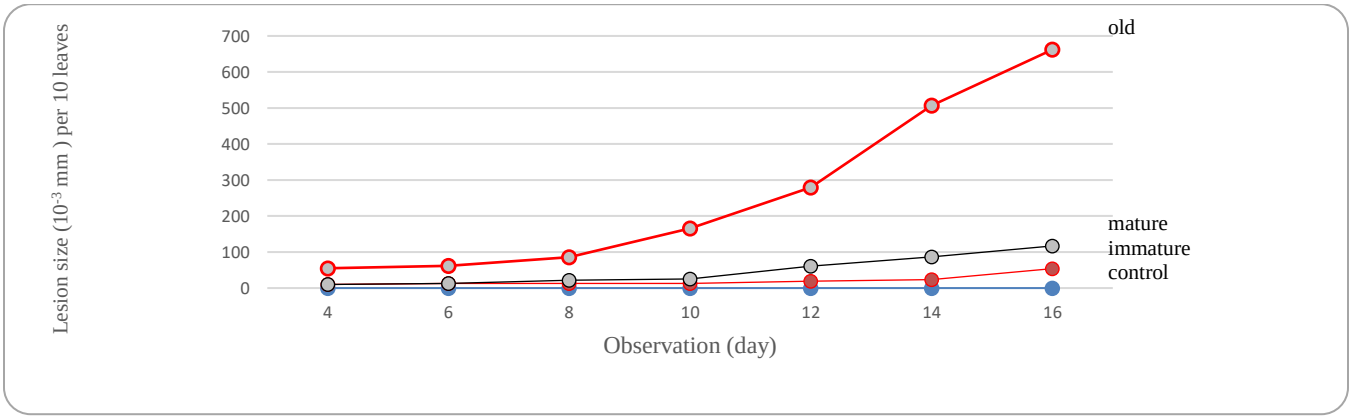


Figure 2. Necrotic lesion development in different leaf trial. Control consisted of immature, mature and old leaves with putative *P. colocasiae*


A B

Figure 3. Demonstration of floating leaf disc assay of Satoimo cultivar. A) 4 days after inoculation. The lesion absent. B) 12 days after inoculation. A significant development of leaf blight necrotic lesion. It indicates that lesion developed from the epicentral putative isolate laid in the center of leaf showing with leaf coloration.

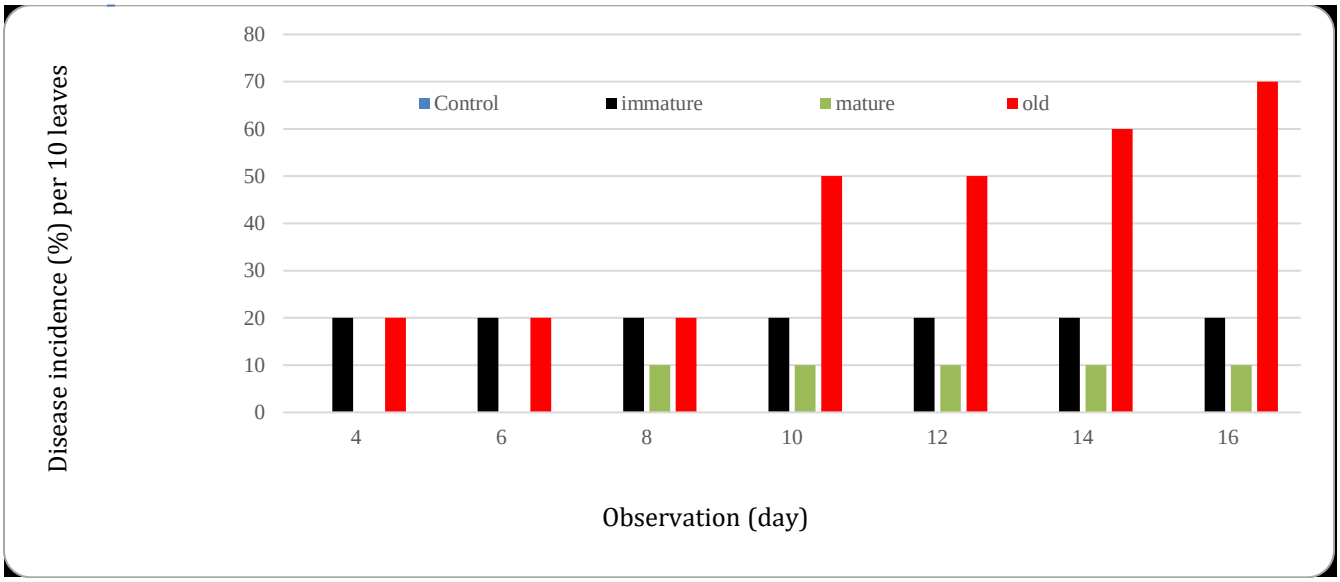


Figure 4. Disease incidence (%) signed with necrotic lesion on Satoimo cultivar leaves by the time. No disease development occurred in Control.

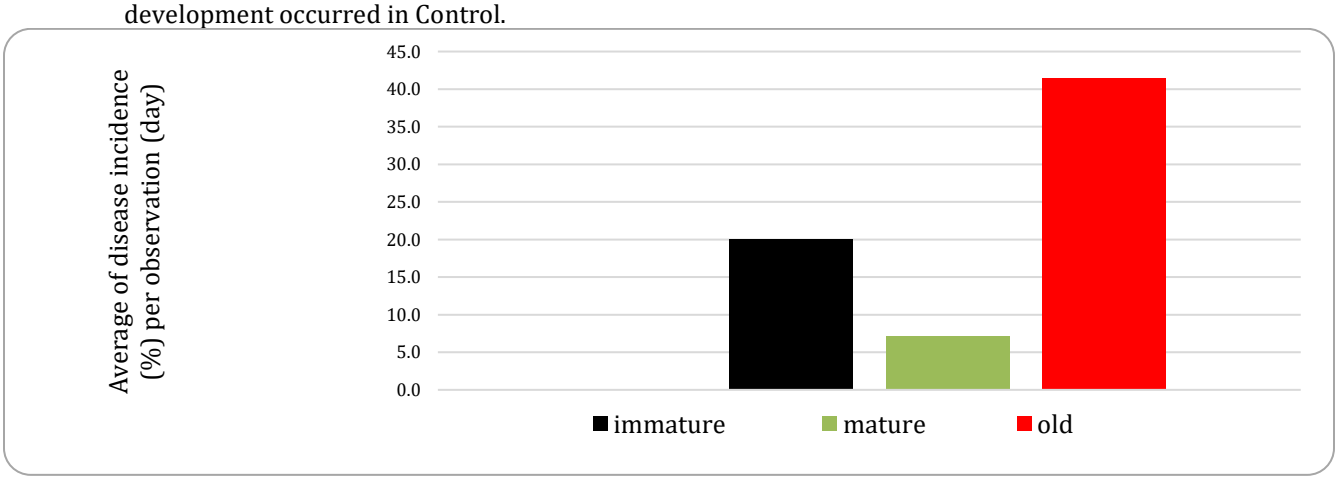


Figure 5. Average of disease incidence (%) of Satoimo cultivar leaves in entire observation (day) in different leaf development.

## DISCUSSION

Disease incidence and development of leaf blight lesion on the Satoimo taro cultivar significantly occurred in old leaves. Disease incidence in old leaf trial progressively increased by the time while disease incidence in immature and mature leaves was stagnant (Figure 4). The necrotic lesion in the leaf floating disc assay and disease development shown that necrosis rapidly developed after 10 days inoculation (Figure 2-3). Old leaves of taro experiences more significant impact of the lesion. Rapid lesion development commenced at 8 days until senescent and complete necrotic leaf (Figure 2; Figure 3B).

Generally, disease incidence and necrotic lesion significantly occur in old leaves (Figure 2,4-5). One of the main causes behind rapid necrotic lesion development on the old leaf trial of Satoimo cultivar is caused by progressive infecting to undermine the host-immune by the time and subsequently virulent putative isolate tested is still available. In contrast, mature leaf is more resistant to putative *P. colocasiae* isolate than other trials. The finding indicates that once the leaf develops in a month, old leaf immunity tends to drop, and symptomatic necrotic lesion rapidly occurs. Another is leaf canopying to underpin the disease development. Old leaf site becomes shade once the rise of new leaf is generated to cause humidity as well as adjacent rhizosphere where pathogen contacts (Quitugua and Trujillo, 1998).

## CONCLUSION

Among other leaf ages of Satoimo cultivar, the highest disease incidence progressively occurs in old leaf tissue, and the lowest is in the mature tissue. In this study, disease incidence in immature tissue is quite high. The lowest disease incidence in the leaves indicates that leaf tissue is more resistant. The causal disease-agent of leaf blight necrotic lesion on the infected leaf obtained from a wild taro has similar morfological characters with *Phytophthora colocasiae* species.

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**Contribution of Authors:**

Tutik Kuswinanti : Conduct lab acitivity and analyse data sets