ASSOCIATION OF ENDOPHYTIC FUNGI IN RICE ROOT (*Oryza sativa L*)

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Abstract

The aim of this research was to elaborate the association of endophytic fungi found in the root tissue of rice plants (Oryza sativa). The sampling location was in Sungai Kapih Village, Sambutan District, Samarinda City. This research was conducted at the Plant Disease Pest Science Laboratory, Faculty of Agriculture, Mulawarman University. The method used in this study were staining the roots of rice plants and observing endophytic fungi in the root tissue of rice plants under the microscope. The results showed that the root tissue formed hyphae and spores in the root cortex. The hyphae that were found had hyphae that were neither insulated nor insulated, forming long chains with spherical spores. In the roots of the control plants, there were no endophytic fungi that formed colonies in the root tissue.

Keywords: endophytic fungi, rice, association

PRELIMINARY

Farmers generally use pesticides excessively in controlling plant diseases without paying attention to natural enemies around the crop. The use of pesticides is carried out without taking into account the damage caused such as the occurrence of pest resistance to pesticides, pest resurgence and the death of natural enemies, damaging human health and the environment, the presence of residues in agricultural products, the emergence of new biotypes that are more resistant, and the death of biota that make up ecological habitats. not the target (Kartohardjono 2011).

Control of Plant Pest Organisms (OPT) which is commonly carried out today is control using synthetic pesticides, this happens because farmers think this control is very easy to do and produces more effective results. Control using synthetic pesticides is proven to be detrimental to humans and the agro-ecosystem environment. Environmentally friendly control is the answer to the problem of synthetic pesticides, many natural ingredients can be used as raw materials in the manufacture of biological pesticides. One of the potential raw materials for control using biological pesticides is endophytic fungi. According to (Sopialena 2020) endophytic fungi on rice plants are able to become biological agents to control pests and diseases in plants.

Endophytic fungi are fungi that live in certain tissues that are able to produce mycotoxins, enzymes and antibiotics so that their presence will be beneficial for the host plant because it increases resistance and uptake of nutrients in the soil. Compounds produced by endophytic fungi can be potential as biological controllers.

Several studies on endophytic fungi that have the potential as biological control have been carried out, but not many studies related to observe the associations of endophytic fungi on plant roots after inoculation. This study proved that endophytic fungi can be invested though the roots of tomato plant.

MATERIALS AND METHODS

The location of the research was carried out at the Laboratory of Plant Pests and Diseases, Faculty of Agriculture, Mulawarman University, Samarinda. The sampels of tomato roots were taken from the field in Sambutan District, Samarinda City.

Research procedure

a. Field Research

Penelitian dilapangan menggunakan penelitian faktorial 5 x 3 dengan 5 ulangan yang disusun dengan *Split Plot Design* (Rancangan Petak Terbagi). Adapun perlakuan pada penelitian ini adalah : Jamur endofit sebagai Petak Utama dengan 5 taraf

C1 : *Kontrol*
C2 : *Trichoderma* sp.
C3 : *Rhizopus* sp.
C4 : *Gliocladium* sp.
C5 : *Penicillium* sp.

Varietas sebagai Anak Petak dengan 3 taraf yaitu :

V1 : Ciherang
V2 : Kambang
V3 : Pandan Ungu

Inokulasi Jamur endofit dilakukan pada tanah media tanam padi sebelum transplanting. Bahan sampel akar diambil dari tanaman yang sudah panen.

b. Staining of Rice Plant Roots

Root staining was carried out to see the structure and number of fungi present in the plant root tissue so as to facilitate the observation and determination of fungal infections on rice plants. Root staining in this study used the method used by (Brundrett et al. 1996) with a dye solution that had been modified with safranin and 5% methylene blue. The rice roots are cleaned with running water and put in a container. The clean rice roots are then processed sequentially by clearing and staining. Clearing was done by soaking the roots in 10% KOH solution and heated at a temperature of 60oC to 70oC for 30 minutes, then washed with distilled water until the roots were clear. After that soak using alcohol for 1 minute and wash again with distilled water. The root staining stage was carried out in 2 stages, namely soaking using safranin for 4 hours, sterilizing with alcohol for 1 minute and washing with distilled water. Furthermore, for the second staining is done by immersion using methylene blue according to the previous staining process.

c. Observation of Endophytic Fungus on Rice Root Tissue

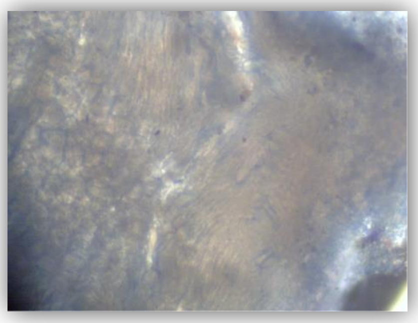
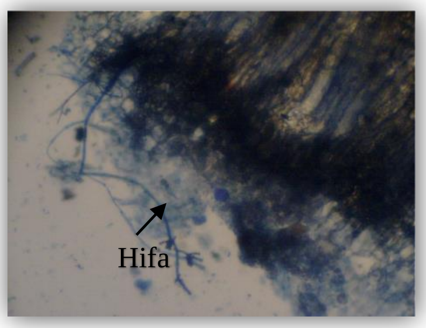
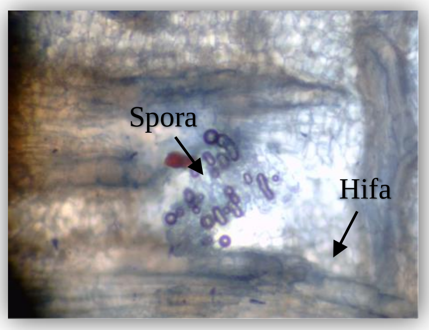
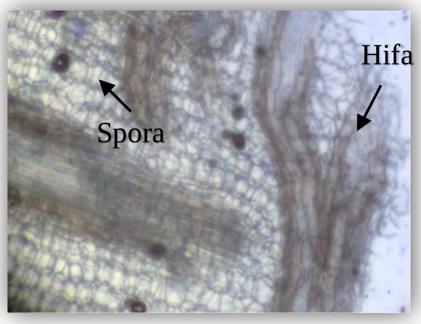
Root samples observed were treated roots after harvest without inoculation of pathogens and after inoculation of pathogens. This was done to see the effect of endophytic fungi on the ability of biocontrol agents. The stained root samples were cut 1 cm long and placed on a glass slide. Roots were observed under a microscope using optilab as a tool for taking documentation. The appearance of the structure of hyphae and spores is an indication that the root has been colonized by endophytic fungi found in the root cortex..

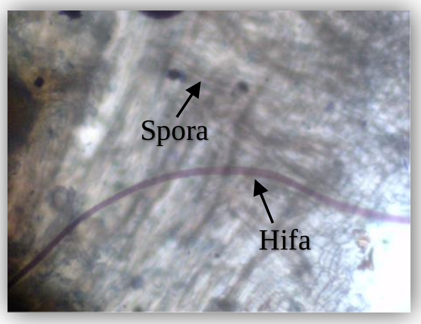
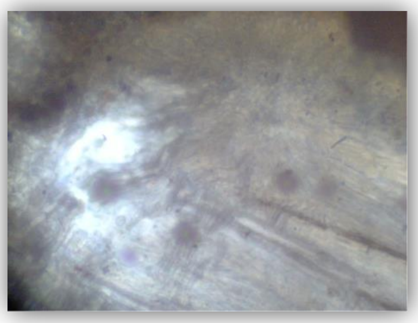
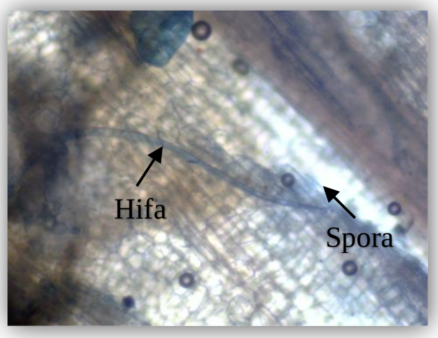
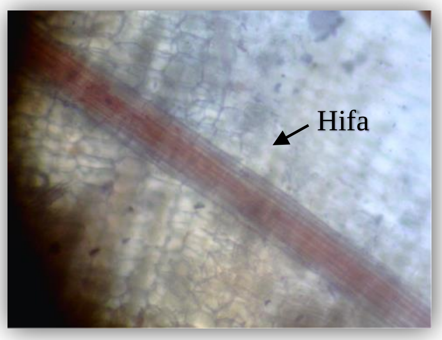
RESULTS AND DISCUSSION

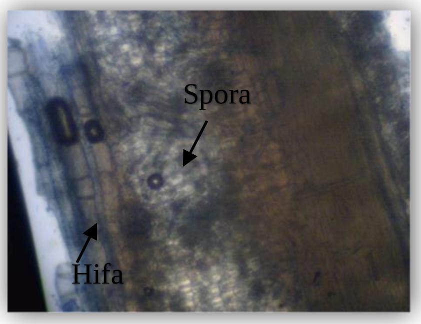
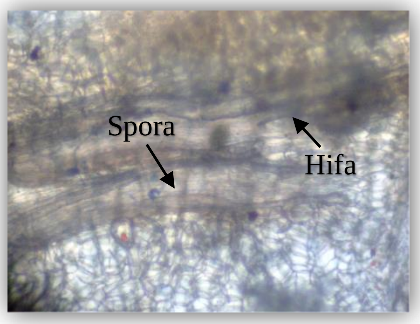
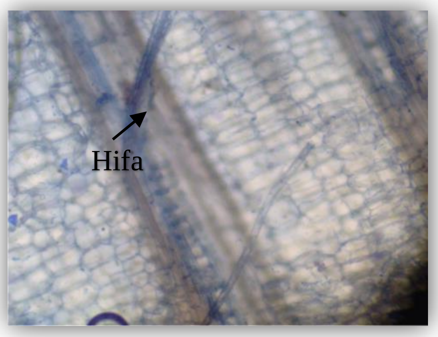
Based on microscopic observations, endophytic fungi are present in roots by forming hyphae on the root cortex and releasing spores. The hyphae were found to have neither insulated nor insulated hyphae, forming a long chain with spherical spores. No endophytic fungi were found in the roots of the control plants that formed colonies in the root tissue. The results of the observations can be seen in table 17.

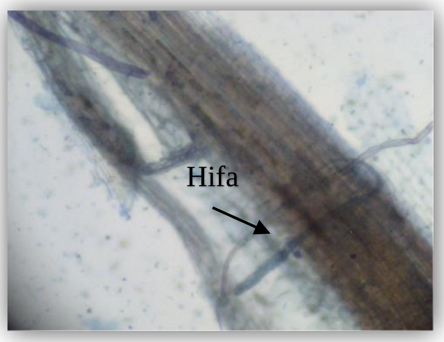
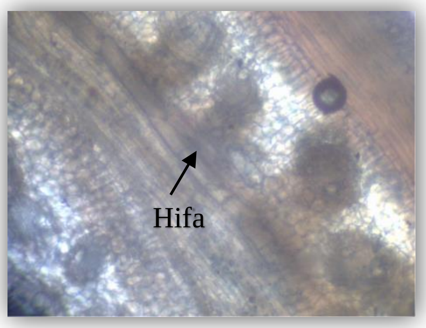
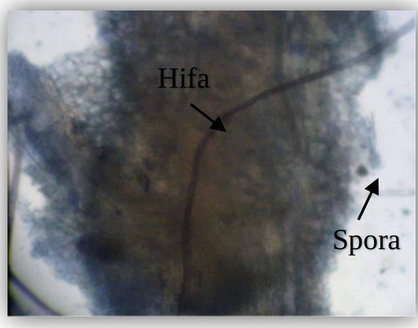
Table 17. Endophytic Fungi on Roots of Rice Plants var. Ciherang, Kambang and Purple Pandan in

every treatment

	Varieties	Treatment	Documentation
	Ciherang	Control	
		Trichoderma sp.	
		Rhizopus sp.	
		Gliocladium sp.	

		Penicillium sp.	
	Kambang	Control	
		Trichoderma sp.	
		Rhizopus sp.	

		Gliocladium sp.	
		Penicillium sp.	
	Pandan Ungu	Control	
		Trichoderma sp.	

		Rhizopus sp.	
		Gliocladium sp.	
		Penicillium sp.	

In the control treatment of the Ciherang, Kambang and Pandan Ungu varieties, it could be seen that the roots were not colonized by endophytic fungi because there were no hyphae or spore structures visible. The results of root staining in all treatments proved the presence of endophytic fungus colonization in symbiosis with rice plant roots. The hyphae seen in the Ciherang variety treated by Trichoderma sp. showed the presence of uninsulated hyphae and seen pyramids, but in the Kambang and Pandan Ungu varieties, only uninsulated hyphae were seen with round spores. This refers to the results of research conducted by (Suanda 2019) which suggests that microscopic Trichoderma sp. have uninsulated hyphae. In a study conducted by (Sugiyarto, Umniyatie, and Henuhili 2016) found that Trichoderma sp. in plant tissue to form hyphae that are not insulated with some forming a pyramid.

(Sopialena 2017) The life cycle of a pathogen starts from growing to producing reproductive organs. The entry cycle of fungi into plants includes fungal changes in the plant body and a series of changes in the host plant and the presence of pathogens (pathogenic life cycle) in it within a certain time span during the plant growth period. Important events in this cycle include: inoculation (transmission), penetration (entering the body), infection (utilization of host nutrients), invasion (expansion of attack to other tissues), spread to other sites and pathogen defense.

Fungal inoculation, the inoculum can be in the form of mycelium, spores, or sclerotium. the steps that occur in the inoculation process, starting from: the inoculum of the pathogen to the surface of the host plant's body, To perform germination an appropriate temperature and humidity are needed in the form of a layer of water on the plant surface. The wet state or form of this water film must last long enough for the inoculum to enter or penetrate into cells or tissues. Penetration of these endophytic fungi directly penetrates the surface of the plant body, through natural holes, through wounds, and through intermediaries (carriers, vectors). Fungi exploit every possible pathway to gain entry to their hosts, although individual fungal species tend to have a preferred method. Fungal pathogens often use direct penetration of plant surfaces to enter the host. This requires adhesion to the plant surface, followed by application of pressure and then enzymatic degradation of the cuticle and cell wall, to overcome the physical barriers presented by the plant surface. During cuticle and wall degradation, gene switching is switched on and off in fungi, so cutinase, followed by cellulase, then pectinase and proteases are produced, attack the cuticle, cell wall, and middle lamella in sequence. That they were encountered The pressure required for the hyphae to penetrate the cell wall was achieved by first attaching the appressorium to the plant surface with a protein adhesive. The cell wall of the apressorium is then impregnated with melanin, making it impermeable to water, and able to withstand the high turgor pressure that builds up inside the appresorium. The nucleus of the appressorium in contact with the cuticle is called the penetration pore, and its walls are thinnest at this point. The increased turgor pressure causes the pores to herniate, forming penetrating pegs, which apply great pressure to the host cuticle and cell walls. An alternative route for the entry of the fungus is through an opening that is already on the surface of the plant. This could be a natural opening or a wound.

Furthermore, the fungus goes through an infection process which is a process of starting the pathogen utilizing nutrients ('food extract') from the host. This process occurs after the pathogen makes contact with susceptible cells or tissues and obtains nutrients from these cells or tissues. During the infection process, the pathogen will grow and develop in the plant tissue. Infection that occurs in the host plant, will produce symptoms of the disease that appear from the outside such as: yellowing, changing shape (malformation), or spotting (necrotic). However, infection by endophytic fungi will not cause disease symptoms in plants, because these endophytic fungi are fungi that are very able to associate well with plants, where plants can tolerate the presence of endophytic fungi and do not cause plants to get sick. After the fungus infects the plant tissue, the fungus will then invade, which is the stage of growth and development of the fungus after infection. This process is the spread of fungi from one cell to another in the host plant tissue. Then carry out the process of colonization or the formation of more than one individual fungus.

In the treatment of Rhizopus sp., both varieties of Ciherang, Kambang, and Pandan Ungu showed uninsulated hyphae with round spores, but no sporangiophores were seen on observation. In the observed root tissue, many hyphae were colonized, so it was evident that the treatment using Rhizopus sp. effective in increasing plant resistance. This is in accordance with the statement (Izzati, Lubis, and Hasanuddin 2019) which states that Rhizopus sp. is an endophytic fungus that is able to associate with plants through roots to increase plant resistance and prevent disease attacks.

Treatment of Gliocladium sp. on Ciherang, Kambang and Pandan Ungu varieties showed that Gliocladium sp. form insulated hyphae with spherical spores. The appearance of hyphae in all treatments of this fungus proved that this fungus was able to associate well with plants and was able to colonize well on plant roots. In a study conducted (Herlina 2013) suggested that Gliocladium sp. have hyphae that are insulated by forming a pyramid as in Trichoderma sp. and this fungus is also able to associate with plants by colonizing plant parts. While in the treatment of Penicillium sp. uninsulated hyphae structure was seen in Ciherang, Kambang and Pandan Ungu varieties. Colonization of the fungus Penicillium sp. can be seen very clearly in the cross section of the observed rice plant roots. The observed results are in accordance with the microscopic morphology seen in research conducted by (Assaf et al. 2020) which also shows the morphological structure of hyphae that are not insulated and have secondary metabolites that are able to prevent disease attacks.

From all observations, the microscopic morphology of hyphae was in accordance with the identification observations of each of the characteristics of endophytic fungi that grew intracellularly in root cortical cells. Colonization that occurs in roots begins with the penetration of endophytic fungi into the epidermis and cortex tissue, then colonizes intracellularly and reproduces itself in these tissues and then spreads to other root tissues. In a study conducted by (Fitriani, Wiyono, and Sinaga 2020) endophytic fungi were found in the epidermal and cortical tissues that colonize intracellularly. This proves that the endophytic fungi that were applied were able to associate well with plants by colonizing the roots, whereas in plants that were not treated with endophytic fungi, hyphae structures were not visible in the root tissue. The endophytic fungus colonization and the reduced intensity of disease in plants treated with endophytic fungi showed that the symbiosis between endophytic fungi and plants was very good so that the suppression of blast disease in rice plants could be maximized.

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