Isolation and Evaluation of Endophytic Fungi from Paddy (Oryzasativa) for Antifungal Activity Against Fusarium sp.

Fitriani, Vivi Mardina*, Fadhliani
Biology Department, Faculty of Engineering
Universitas Samudra
Aceh, Indonesia
*vmardina@unsam.ac.id

***Abstract*—Fusariumspis one of pathogenic fungi that cause wilt disease in paddy. However, they can be control by endophytic fungi which isolated within paddy plant itself. This study aims to isolate and evaluate the type of endophytic fungi from rice plant that have the potential to inhibit the growth of pathogenic fungus particular Fusarium sp. The endophytic fungi were purified and identified on the basis of their morphological and structural characters. Pathogenic evaluation was performed in vitro and in vivo for anti-fungi and germination consisting of 5 treatments and 5 replications. The obtained data was analysed using One Way ANNOVA. The results found the two endophytic fungi associated in paddy namely Nigrospora sp and Trichoderma sp. Between those, Tricoderma sp exhibited the higher germination (80%) and inhibitory power (45.7%) compared to another. Hence, this study recommended Tricoderma sp for further anti-fungi research against Fusarium sp.**

Keywords—endophytic fungi, oryzasativa, antifungal activity

I. INTRODUCTION

Rice is one of the major commodities in Asia countries with Vietnam, India, and Indonesia as the top rice exporter [1]. The production of rice depends on the quality of seeds that must free from insect, mites, bacterial and fungal attacks [2]. Pathogenic fungi such as Alternaria sp, Rhizoctonia solani, Fusarium sp, Fusarium proliferatum and Pyricularia sp have been reported as the cause of diseases during the rice growth. These fungi can produce the mycotoxins in seeds which are harmful to the human and animal health [2,3]. Fusarium sp infection can cause disease on plants that is systemic [4]. As a consequence, it will decrease on the quality and quantity of rice production [2]. One of the promising technologies for controlling pathogenic fungi in the grains of rice (seed) is utilization of endophytic fungi [5] that found within the rice itself.

Endopyhtic fungi are fungi cluster that live and contribute to the healthy plant tissue without showing symptoms of disease [6,7]. They can be used as bio-control agents against pets and plant diseases due to their capability to produce compounds which inhibit the development of plant pathogens [7] including clavatol and pathulinpolyketides [8], salicylic acid, jasmonic acid and peroxidase [9]. Moreover, endophytic fungi could increase plant resistance from mammals, herbivores, insect, and plant diseases. The ability of endophytic fungi to produce secondary metabolites is a huge opportunity and potential to be used as pathogen eliminators in rice seed [10,11]. Therefore, this study aims to isolate the endophytic fungi associated in paddy/ rice and evaluate their activity against pathogenic fungi particularly Fusarium sp.

II. METHODS

A. Isolationand Identification of Endophytic Fungi

The endophytic fungi were isolated from the branch, stems, and roots of paddy. They were isolated using the method suggested by Sopialena et al [12] and Tumangger et al [7] with slight modification on plant surface sterilization [13]. The samples cut into ±1 cm and washed with running water. The surfaces of samples were sterilized gradually through immersion in alcohol (70%), NaOCl (5%), and alcohol (70%) for 1, 2, and 30 minutes respectively. After rinse 3 times using H_2O, they were grown in the dark condition by incubation on PDA media (10%) for 3-5 days. The growth of mycelium was recorded and transferred for further purification by separation the same isolated into the new PDA media [14]. Morphological and structural identification were carried out using microscopes [15].

B. Evaluation of Endophytic Fungi Capability for Seedling Pathogenicity

Sterilization of paddy grains was carried out using ethanol and NaOCl for 2 and 30 minutes [13]. Then, the samples were grown on the PDA media that has been cultured with the pure isolates of endophytic fungi 7 days old. A total of 10paddy seed of were planted in a petri dish and incubated for 1 week. Observations were performed on the 7 days against

germination healthy and show grains of rice's symptoms of necrosis [7,12].

C. *Pathogenic Activity of the Endophyticisolatesagainst Fusarium sp*

Antifungal activity of the isolate was tested against the *Fusarium sp*. Each pure endophytic isolate and the colony of *Fusarium sp* (diameter 5 mm) were cultured on PDA medium in a petri dish with a distance of 3 cm between them. The colony growth diameter was observed and calculated at 7 days after planting [16].

D. *Data Analysis*

The data obtained are shown descriptively to compare any data obtained from all observations.

III. Results and Discussion

A. *Identification of Endophyticfungi*

Isolation and identification of the isolate endophytic fungi were carried out macroscopically and microscopically. The results were found that there were two endophytic fungi namely *Nigrospora* sp. and *Trichoderma* sp. *Trichoderma sp* was found in the roots, stems, and leaves of rice plant, while *Nigropora* sp was only found in the stems rice. *Nigrospora* sp that was classified in the Ascomycota division of the Deuteromycetes class had brightly coloured colonies and produced the woolly colonies on PDA. The ordo Moniliales and the Dematiaceae family are plant pathogens that attack plants by infecting their host bodies. Development of their infection is influenced by humid environmental conditions. The genus Nigrospora has a variety of species, some of which act as pathogens and anti-viral for plants.

Visualization of *Nigrospora* sp using microscope exhibited the pigmented conidiophores and conidia. It also showed the septate hyaline hyphae, and hyaline. Conidiogenous cells was solitary, monoblastic, ampuliform, and bear single conidium at the apex. Macroscopically, *Nigrospora sp*with the white hyphae and radial growth type with texture of the hypha was smooth, tall, and wavy. The colonies were irregular and have flat edges. When the early growth, they were small and white with shining black conidia (Figure 1). Later on, the colonies became black. According to the morphological characteristics, the fungal isolate was identified as *Nigropsora* sp.

Another isolate found in this study was *Trichoderma sp*. It was classified into the Ascomycota division, Sordariomycetes class, Hypocreales ordo and Hypocreaceae family with the main characteristic of brightly coloured colony. The availability of *Trichoderma sp* was found in nature easily because of its ability to symbionate. They are found mainly in leaf litter and dead plant stems.

Trichoderma isolates were identified according to their characteristic such as colony colour, conidia shape, chlamydospores and conidiophores formation and phialides properties [15]. Moreover, macroscopically *Trichoderma sp* had a green colony colour with a rough and serrated texture. At the beginning of its growth, *Trichoderma sp* mycelium looks white and then turns greenish but around the mycelium there was still a white colour. The concentric growth type with a uniform height, irregular shape and serrated margins was shown in Figure 2.

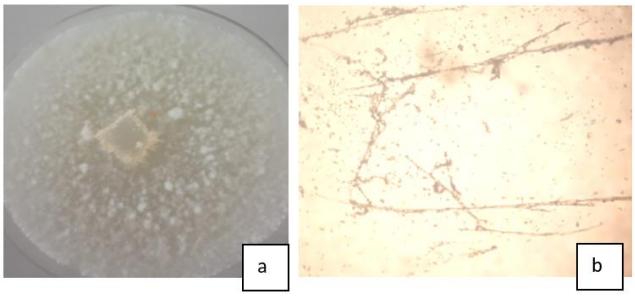

Fig. 1. *Nigrospora sp:* (a) Macroscopically and (b) Microscopically

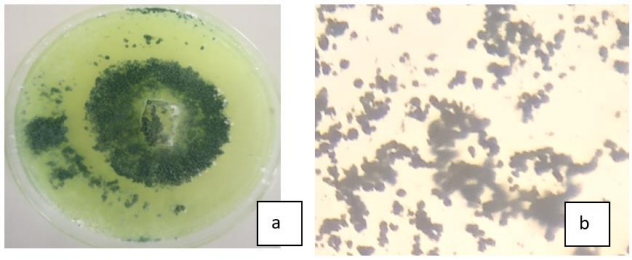

Fig. 2. *Trichoderma sp:* (a) Macroscopicallyand (b) Microscopically.

B. *Evaluation of Endophytic Fungi Capabilities for Seedling Pathogenicity*

Paddy seeds were used as an indicator to evaluate the potentially endophytic fungi towards the seed growth because they were very sensitive to inoculation treatments. Thakuria *et al* [17] reported endophytic fungi able to induce the production of auxin hormones. According to the results (Figure 3) inoculation *Trichoderma sp* and *Nogrospora sp* exhibited the positive effects on the growth of paddy seeds and the number of seeds germinated. The endophyte *Trichoderma sp* contributed the good response on the stem height, while *Nogrospora sp* demonstrated the main response on number of seeds germinated.

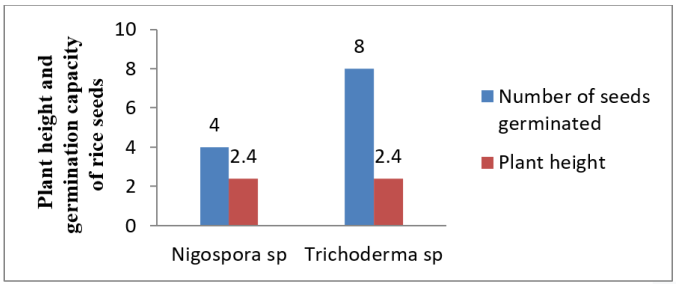

Fig. 3. Pathogenicity test of the endophytic fungi isolates on the plant height and number of seeds germinated.

Particularly, the *Tricoderma* sp increase 2.4 cm of stem height and the number of seeds germinated for 8. Besides, the *Nigrospora sp* increase stem height (2.4 cm) and the number of seeds germinated for 4 (Figure 3). This result was accordance to Tumangger et al [7] that reported the isolated fungus was able to growth the root length of 0.36 cm and the stem height of 1.98 cm and compared to the control that was1.5 cm and 0.2 cm respectively. This is because ability of endophytic fungus to improve plant growth by producing metabolites and growth promoters such as auxin, gibberellins or cytokinins. In addition, *Trichoderma sp* is an antagonistic fungus that inhibits the growth of disease-causing pathogenic fungi by producing cellulase enzymes, so they can damage the cell walls of pathogenic fungi. *T. viride* has also the ability to wrap and penetrate pathogenic hyphae and produce antibiotics that are toxic to pathogens [18]. *Nigropsora* sp plays a role in suppressing the growth of *Nilaparvata lugens* in rice [19]. Gamns *et al* [15] reported that the application of endophytic *Nigrospora sp* fungi to rice caused an increase in the number of leaf hoppers that did not hatch, apart from *Nigrospora sp* causing an extension of the old *Nilaparvata lugens* nymph (pre-maturity) stage which has an impact on the delay in reproduction of these pests because there will be a delay in laying pests on the plant. Besides, *Nigrospora sp* also functions in inhibiting the growth of the pathogenic fungus *Fusarium oxysporium* [19].

C. Capabilies of the isolated endophytic fungus against Pathogen Fusarium sp

The characteristics *Fusarium sp*, a seed-borne pathogenic fungus, are oval conidia which consist of 7 septa with hyaline colour. Their conidia are enlarged is the middle part with the two ends are tapered like a crescent moon. *Fusarium sp* can infect rice seeds with low quality of supporting storage conditions [20]. In this study, anti-fungi activity of the endophytic fungi was tested against Fusarium sp. The results revealed that the endophyte *Trichoderma sp* and *Nigrospora sp* inhibited the *Fusarium sp* growth approximately 45.7 % and 38.9% respectively (Figure 4).

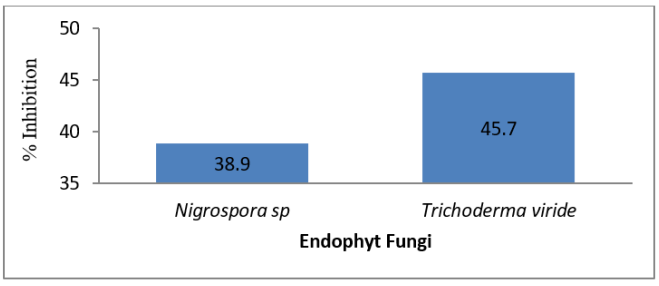

Fig. 4. The percentage of endophytic fungi inhibition against *Fusarium sp* pathogen.

Trichoderma sp was capable of producing chemical compounds (volatile and non-volatile)and enzymes that could inhibit the growth of pathogenic fungi. The antibiotic compounds were produced by *Trichoderma sp* including alamethicins, harzianic acid, peptaibols, tricholin, 6-penthyl-α-pyrone, massoilactone, gliovirin, viridine, heptelidic acid, glycoprenins, dermadindan and trichodermin [21]. In addition, *Trichoderma sp* produced cellulose enzymes that can damage the cell walls of pathogenic fungi. In this case, Septiani *et al* [22] stated that *Trichoderma sp* can produce cellulase to break down cellulose into glucose. Cellulose is the main component of the cell wall of the pathogenic fungus. On the other hand, *Nigrospora* sp was still effective in inhibiting the growth of *Fusarium* sp although their inhibition is smaller than *Trichoderma sp*. This is because the fungus *Nigospora*sp contains various components of secondary metabolite compounds which act as anti-fungal and antibacterial. *Nigrospora sp* contains phytotoxic and antibacterial nigrosporin and phomalaxton [23]. The mechanism of inhibition of endophytic fungi against pathogens is carried out through several inhibition mechanisms including antibiosis which is characterized by the formation of an inhibition zone, competition against substrates and parasitism [24].

IV. CONCLUSION

The study found two endophytic fungi associated with rice namely *Trichodrma* sp and *Nigrospora* sp. The isolated *Tricoderma* sp was able to increase stem height of 2.4 cm, the number of seeds germinated (8), and inhibit *Fusarium sp*(45.7%). On the other hand, the isolated *Nigrospora sp* was able to increase stem height of 2.4 cm, the number of seeds germinated (4) and inhibit *Fusarium* sp (38.9%).Overall, the isolated *Tricoderma* sp has the most potential to increase stem height, the number of seeds germinated and has anti-fungus activity against *Fusarium sp* as pathogenic fungus.

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