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Original Article

ENDOPHYTIC FUNGI COLONIZE AGRICULTURAL AND NON-AGRICULTURAL PLANTS IN BEDUGUL, BALI AND THEIR ANTIFUNGAL ACTIVITY AGAINST *Rhizoctonia solani* Kuhn

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ABSTRACT

Isolation of endophytic fungi was done to find alternative microorganisms as antifungal agent against *Rhizoctonia solani* Kuhn, a soil borne pathogen on many agricultural plants. The research objectives were 1) to isolate and identify endophytic fungi colonize agricultural and non-agricultural plants growing in Bedugul, Bali; and 2) to detect for their antifungal activity against *R. solani* under in-vitro conditions. The results indicated that 114 isolates of endophytic fungi were isolated from flowers, fruits, leaves, petioles, and stems of agricultural and non-agricultural plants. Ten isolates (8.8 %) were identified to species, 91 isolates (79.8 %) to genus, and 13 isolates (11,4 %) did not have spores that could not be identified morphological characters and classified as unidentified isolates. Endophytic fungi isolated including in group of *Aspergillus*, *Bipolaris*, *Cladosporium*, *Colletotrichum*, *Corynesporopsis*, *Curvularia*, *Diplodia*, *Fusarium*, *Guignardia*, *Nigrospora*, *Pestalotiopsis*, *Phomopsis* and *Xylaria*. Of the 114 fungal isolates tested, only 13 isolates (11.4 %) inhibited the growth of *R. solani* from 10.3 % to 62.2 % with a percent inhibition. The highest growth inhibition of *R. solani* was shown by *Aspergillus niger* isolated from *Solanum licopersicum* L. var *cerasiforme* (62.2 %). It could be concluded that the agricultural and non-agricultural plants growing in Bedugul, Bali were colonized by endophytic fungi. *Aspergillus niger* will be further examined on a field scale.

Key words: antifungal; endophytic fungi; *Rhizoctonia solani*

INTRODUCTION

Rhizoctonia solani Kuhn is a very common soil borne pathogen, widely distributed, and has many host plants, including crops, vegetables, fruits, and ornamental (Bohlooli et al. 2005). The fungus besides can attack any part of these crops that are in the soil, such as seeds, hypocotyl, and roots; it can also infect parts of these crops above ground, such as pods, fruits, leaves, and stems thereby reducing the production of these crops (Ogoshi and Ui 1983). Rhizoctonia foliar blight (RFB) of soybean (*Glycine max* (L.) Merrill) occurs in many tropical and subtropical regions, causing yield reductions of up to 70%; and in Brazil up to 60% (Meyera et al. 2006).

Rhizoctonia disease is difficult to eradicate because of its ecological behaviour, its extremely broad host range, and the high survival rate of sclerotia under various environmental conditions (Grosch et al. 2003). The use of fungicides is relatively expensive and risky to the safety and environmental pollution, it is also not effective in eradicating pathogen in total which resulted in the emergence of new pathogenic strains that are resistant to chemicals (Soylu et al. 2005). To reduce the use of synthetic fungicides, the use of natural antagonists, such as endophytic fungi to be a sustainable alternative for biological control of the pathogen (Clarke et al. 2006).

Endophytic fungi are fungi that spend all or part of their life cycle colonizing inter or intracellular in a healthy host plant tissues without causing disease symptoms. Endophytic fungi have been found in a wide range of host plants, including gymnosperms and angiosperms (Petrini 1986). Endophytic fungi that colonize a host plant tissues will get nutrition and protection of plants. Instead, the endophytic fungi can increase the tolerance of the host plant by producing specific functional metabolites (Redman et al. 2002). Certain endophytic fungi may promote growth and ecological adaptation to their host plants by increasing crop tolerance to environmental stress; and resistance to plant pathogens or herbivores (Waller et al. 2005). *Colletotrichum* (37-15) and *Colletotrichum* sp. isolated from plants of *Sonneratia* sp. and *Ananas comosus* respectively produced antifungal activity against the plant pathogen of *Fusarium oxysporum* f.sp. *lycopersici* (Suciatmih and Rahmansyah 2013) and *Rhizoctonia solani* (Suciatmih et al. 2011). Kim et al. (2007) report that the fermentation broth of *Fusarium oxysporum* EF119 was the most active; it suppressed the development of tomato late blight by 90% even when diluted 50-fold.

The objectives of this research are to isolate, identify, and evaluate the antifungal activity of agricultural and non-agricultural plants growing in Bedugul, Bali against *R. solani* from endophytic fungi.

METHODS

Isolation of endophytic fungi

Healthy plant organs such as stems, fruit, flowers, leaves, and petiole used to isolate the fungus, were washed with tap water until clean. The plant organs cleaned, were cut 1 cm x 1 cm and each organ of the plant placed in bottles separately.

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Pieces of the plant organs were sterilized with 70% alcohol for 1 minute and bayclin (containing 5.3% sodium hypochlorite) for 2 minutes (Nakagiri et al. 2005). Pieces of the plant organs were then rinsed with sterilized water 3 times and wrapped in a sterilized thick tissue paper for 3-4 hours (until dry). Isolation of fungi was done by direct planting techniques, by putting pieces of the dried plant organs (6 pieces) on the surface of the 2% medium of PDA that has been added chloramphenicol (200 mg / 1 liter of medium). The whole medium inoculated, was incubated at room temperature (27-28^o C). Colony morphology which has the appearance, color, and same size is considered the same isolate.

Purification of endophytic fungi

Purification of fungi producing spore was done by the isolation of a single spore (Gandjar et al. 1992). Each endophytic fungus was grown in the medium of PDA slant and incubated for 5 days. To obtain a spore suspension, endophytic fungi that were added the 4 ml of a sterilized water, is scraped using a needle, and stirred with vortex. The spore suspension was diluted with a sterilized water to get dilution 10^{-3}. The 0.1 ml of the spore suspension was spread evenly on the surface of petri dish containing PDA medium and incubated at room temperature (27-28^o C). Single colony of the fungi growing, was transferred to the PDA slant medium and ready to use.

Purification of fungi not producing spore was carried out by growing the fungi on PDA medium. Using stereo microscope, single hyphae of the fungi was then transferred to the PDA slant medium and ready to use.

Identification of endophytic fungi

Single isolate of endophytic fungi later was identified morphologically, including colony morphology of fungal culture or hyphae, spore characteristics, and reproductive structures (Nakagiri et al. 2005).

Biocontrol activity against *R. solani*

Preparation of endophytic fungal inoculum

To obtain a spore suspension, the 5 ml of a sterilized water put into test tubes each containing endophytic fungal spore that has been aged 7 days, is scraped slowly using a needle. Endophytic fungi not producing spores growing in PDA medium for 7 days, was printed with a straw of pop ice (Θ = 1 cm).

Inoculation of endophytic fungi

Two ml (10%) suspension of conidia or two prints of endophytic fungal mycelium were added to erlemeyer 100 ml each containing the 20 ml of fermentation medium potato dextrose yeast (PDY). The medium inoculated, was incubated in a shaker at room temperature (27-28^o C) and agitation speed of 90 rpm for 5 days (Suciatmih 2008).

Harvesting filtrate containing biocontrol agents

Biocontrol agents were harvested by separating the cells using centrifugation at 6000 rpm for 10 minutes (Prihatiningtian et al. 2005).

Screening test

A total of 200 ul of the fermented filtrate of endophytic fungi mixed with the 5 ml of PDA medium in a test tube, is shaken with vortex, and poured into a petri dish (Anith, 1997 modified). Once hardened medium, *R. solani* that has been rejuvenated in PDA medium, taken with a straw of pop ice (Θ = 1 cm), and grown in the middle of the medium. For control, the fermented filtrate of endophytic fungi replaced with the 200 ul of a sterilized water. The diameter of pathogen was measured on day 2 after inoculation, and the percent of growth inhibition calculated by Skidmore & Dickinson (1976):

$$P = \frac{C - T}{C} \times 100$$

Where PI = the percent of mycelial growth inhibition (%)
C = the diameter mycelium of pathogen on the controlled petri dishes (cm)
T = the diameter mycelium of pathogen on the treated petri dishes (cm)

RESULTS

One hundred and fourteen isolates of endophytic fungi were isolated from the 30 plants species (Table 1). Ten isolates (8.8%) were identified to species, 91 isolates (79.8%) to genus, and 13 isolates (11.4%) without spores so could not be identified microscopically and classified as unidentified isolates. Two species of the two genera (*Guignardia* and *Xylaria*) or 4 isolates (3.5%) including in the Ascomycota; and 11 species of 11 genera (*Aspergillus*, *Bipolaris*, *Cladosporium*, *Colletotrichum*, *Corynesporopsis*, *Curvularia*, *Diplodia*, *Fusarium*, *Nigrospora*, *Pestalotiopsis*, and *Phomopsis*) or 97 isolates (85.1%) including in the Mitosporic Fungi (Deuteromycota). No members of Basidiomycota and Zygomycota are isolated as endophytic fungi.

Fragaria vesca was a plant produced the highest diversity of endophytic fungi with 4 genera and 10 isolates was followed by *C. caudatus* with 4 genera and 7 isolates. *Widelia calendulacea* was a plant that only had two unidentified isolates.

Colletotrichum was isolated from all organs of the plant, especially the leaves (34 isolates), while *Phomopsis* and unidentified isolates were more isolated from the petioles each 8 isolates. *Xylaria* and *A. niger* were not found in petioles, fruits, and stems, but they were isolated from the leaves and flowers. *Bipolaris* was not found in the leaves, fruits, flowers, and stems, but it was only isolated from the petioles. *Curvularia* and *N. oryzae* were only found in flowers respectively.

There were four dominant endophytic fungi, namely *Colletotrichum* (49 isolates or 43.0%), *Cladosporium* (14 isolates or 12.3%), unidentified isolates (13 isolates or 11.4%), and *Phomopsis* (12 isolates or 10.5%). Of the 114 isolates of endophytic fungi tested, only 13 isolates (11.4%) inhibited the growth of *R. solani* from 10.3% - 62.2% with a percent inhibition (Figure 1). Percent of the highest growth inhibition of *R solani* (62.2%) was produced by the endophytic fungus *A. niger* isolated from the flowers of *S. licopersicum* var *cerasiforme*, while the

lowest one (10.3%) was generated by unidentified isolate (2-21) isolated from the petiole of *P. hieracioides*.

Table 1. Diversity of endophytic fungi on agricultural and non-agricultural plants

No	Host plants	Plant organs	Isolates	Fungi and isolate code	Phyla
Agricultural plants					
1.	*Abelmoschus esculentus* Moench	Leaves	4	*Colletotrichum* sp. (1-15)	Deuteromycota
				Colletotrichum sp. (1-17)	Deuteromycota
				Colletotrichum sp. (1-18)	Deuteromycota
				Colletotrichum sp. (1-46)	Deuteromycota
		Petioles	1	*Colletotrichum* sp. (1-37)	Deuteromycota
2.	*Acacia concinna* DC	Leaves	3	*Cladosporium* sp. (1-2)	Deuteromycota
				Cladosporium sp. (1-11)	Deuteromycota
				Corynesporopsis iberica (18-4)	Deuteromycota
		Petioles	1	*Cladosporium* sp. (2-10)	Deuteromycota
3.	*Annona muricata* L.	Leaves	2	*Diplodia mutila* (2-50)	Deuteromycota
				Guignardia endophyllicola (2-9)	Ascomycota
		Petioles	1	*Phomopsis* sp. (2-8)	Deuteromycota
4.	*Brassica campestris* L.	Flowers	3	*Cladosporium* sp. (1-7)	Deuteromycota
				Cladosporium sp. (2-16)	Deuteromycota
				Cladosporium sp. (2-17)	Deuteromycota
		Petioles	2	*Cladosporium* sp. (1-3)	Deuteromycota
				Cladosporium sp. (2-18)	Deuteromycota
5.	*Brassica rapa nipposinica* Mizuna	Leaves	3	*Colletotrichum* sp. (1-13)	Deuteromycota
				Colletotrichum sp. (1-24)	Deuteromycota
				Unidentified (12-1)	
6.	*Capsicum annum* L.	Flowers	1	*Fusarium* sp. (2-51)	Deuteromycota
		Leaves	5	*Colletotrichum* sp. (1-45)	Deuteromycota
				Colletotrichum sp. (2-44)	Deuteromycota
				Colletotrichum sp. (2-47)	Deuteromycota
				Colletotrichum sp. (3-12)	Deuteromycota
				Pestalotiopsis sp. (5-1)	Deuteromycota
		Petioles	1	*Pestalotiopsis* sp. (2-51)	Deuteromycota
7.	*Capsicum annuum* L. var *grossum* Sendt	Petioles	3	*C. iberica* (3-1)	Deuteromycota
				Fusarium sp. (2-28)	Deuteromycota
				Fusarium sp. (4-2)	Deuteromycota
8.	*Citrus* sp.	Leaves	2	*Phomopsis* sp. (2-20)	Deuteromycota
				Fusarium sp. (2-19)	Deuteromycota
		Petioles	2	*Colletotrichum* sp. (1-19)	Deuteromycota
				Colletotrichum sp. (1-35)	Deuteromycota
9.	*Cosmos caudatus* HBK	Flowers	5	*Curvularia* sp. (2-24)	Deuteromycota
				Fusarium sp. (2-22)	Deuteromycota
				Fusarium sp. (2-23)	Deuteromycota
				Fusarium sp. (2-33)	Deuteromycota
				Nigospora oryzae (2-32)	Deuteromycota
		Petioles	2	*Colletotrichum* sp. (1-44)	Deuteromycota
				Unidentified (7-1)	
10.	*Cucurbita moschata* Durch	Stems	1	*Colletotrichum* sp. (2-39)	Deuteromycota
		Leaves	2	*Colletotrichum* sp. (1-31)	Deuteromycota
				Colletotrichum sp. (1-32)	Deuteromycota
		Petioles	1	*Colletotrichum* sp. (3-8)	Deuteromycota
11.	*Datura fastuosa* L.	Leaves	1	Unidentified (2-34)	
		Petioles	1	*Phomopsis* sp. (2-35)	Deuteromycota
12.	*Eruca vesicaria* ssp. *sativa* (Miller) Thell.	Leaves	3	*Cladosporium* sp. (1-1)	Deuteromycota
				Cladosporium sp. (1-8)	Deuteromycota
				Colletotrichum sp. (1-25)	Deuteromycota
13.	*Fagopyrum esculentum* Moench	Leaves	1	*Phomopsis* sp. (2-53)	Deuteromycota
14.	*Fragaria vesca* L.	Fruits	2	*Colletotrichum* sp. (1-14)	Deuteromycota
				Colletotrichum sp. (2-45)	Deuteromycota
		Leaves	4	*Colletotrichum* sp. (1-12)	Deuteromycota
				Phomopsis sp. (5-2)	Deuteromycota
				Unidentified (2-12)	
				Unidentified (3-5)	
		Petioles	4	*D. mutila* (2-49)	Deuteromycota
				Phomopsis sp. (2-11)	Deuteromycota
				Unidentified (2-13)	
				Unidentified (3-6)	
15.	*Mentha piperita* L.	Leaves	3	*Colletotrichum* sp. (1-22)	Deuteromycota
				Colletotrichum sp. (2-38)	Deuteromycota
				Colletotrichum sp. (3-7)	Deuteromycota
		Petioles	2	*Cladosporium* sp. (1-9)	Deuteromycota
				Colletotrichum sp. (1-23)	Deuteromycota
16.	*Osimum basilicum* L.	Flowers	1	*Xylaria* sp. (7-3)	Ascomycota
		Petioles	1	Unidentified (2-7)	
17.	*Passiflora quadrangularis* L.	Fruits	1	*Pestalotiopsis* sp.	Deuteromycota

				(13-18)	
		Petioles	1	Unidentified (2-36)	
18.	*Pisum sativum* L.	Leaves	1	*Colletotrichum* sp. (2-40)	Deuteromycota
19.	*Plantago major* L.	Leaves	1	*Colletotrichum* sp. (1-48)	Deuteromycota
		Petioles	3	*Bipolaris* sp. (1-26)	Deuteromycota
				C. iberica (18-5)	Deuteromycota
				Unidentified (2-26)	
20.	*Plantago ovata* Husk	Leaves	1	*Colletotrichum* sp. (2-46)	Deuteromycota
21.	*Rosmarinus officinalis* L.	Leaves	1	*Xylaria* sp. (10-1)	Ascomycota
		Petioles	2	*Phomopsis* sp. (2-30)	Deuteromycota
				Phomopsis sp. (2-31)	Deuteromycota
22.	*Sechium edule* (Jack.) Sw.	Leaves	3	*Colletotrichum* sp. (1-27)	Deuteromycota
				Colletotrichum sp. (1-28)	Deuteromycota
				Colletotrichum sp. (1-30)	Deuteromycota
23.	*Solanum betaceum* Cav.	Leaves	4	*Colletotrichum* sp. (1-38)	Deuteromycota
				Colletotrichum sp. (1-40)	Deuteromycota
				Colletotrichum sp. (1-42)	Deuteromycota
				Colletotrichum sp. (1-43)	Deuteromycota
		Petioles	4	*Colletotrichum* sp. (1-29)	Deuteromycota
				Colletotrichum sp. (2-1)	Deuteromycota
				Colletotrichum sp. (2-3)	Deuteromycota
				Colletotrichum sp. (3-9)	Deuteromycota
24.	*Solanum. licopersicum* L. var *cerasiforme*	Flowers	1	*Aspergillus niger* (1-41)	Deuteromycota
		Leaves	1	*A. niger* (1-39)	Deuteromycota
25.	*Solanum tuberosum* L.	Leaves	1	*Fusarium* sp. (1-34)	Deuteromycota
		Petioles	1	*Fusarium* sp. (1-33)	Deuteromycota
26.	*Thymus vulgaris* L.	Flowers	2	*Colletotrichum* sp. (2-7)	Deuteromycota
				Phomopsis sp. (2-6)	Deuteromycota
		Petioles	2	*Phomopsis* sp. (2-5)	Deuteromycota
				Phomopsis sp. (3-3)	Deuteromycota
27.	*Widelia calendulacea* Less.	Leaves	1	Unidentified (8-2)	
		Petioles	1	Unidentified (2-48)	
Non-agricultural plants					
28.	*Picris hieracioides* L.	Leaves	6	*Cladosporium* sp. (1-5)	Deuteromycota
				Cladosporium sp. (1-10)	Deuteromycota
				Colletotrichum sp. (2-41)	Deuteromycota
				Colletotrichum sp. (2-42)	Deuteromycota
				Colletotrichum sp. (2-43)	Deuteromycota
				Colletotrichum sp. (1-16)	Deuteromycota
		Petioles	2	*Colletotrichum* sp. (2-42)	Deuteromycota
				Unidentified (2-21)	
29.	*Pueraria montana* (Lour.) Merr. var *lobata* (Willd.) Maesen & S. Almeida	Leaves	1	*Colletotrichum* sp. (1-21)	Deuteromycota
		Petioles	2	*Cladosporium* sp. (1-4)	Deuteromycota
				Phomopsis sp. (2-37)	Deuteromycota
30.	*Tectaria decurrens* (Pr.) Copel	Leaves	2	*Colletotrichum* sp. (1-20)	Deuteromycota
				Colletotrichum sp. (1-47)	Deuteromycota
		Petioles	1	*G. endophyllicola* (4-12)	Ascomycota
	Total of isolates		**114**		

DISCUSSION

Many endophytic fungi (114 isolates) colonized the 27 agricultural plants species; and the 3 non-agricultural plants species in this study was a potential source of natural bioactive for exploitation in industry, medicine, and agriculture (Guo et al. 2008).

The number of taxa (2) and the number of isolates (4 or 3.5%) of endophytic fungi from the 30 plants species included to Ascomycota was smaller than the number of taxa (11) and the number of isolates (97 or 85.1%) of endophytic fungi included to Deuteromycota. The results showed that endophytic fungi isolated from the 30 plants species was dominated by Deuteromycota. Similar results are reported by D'Souza and Bhat (2013) and Maheswari and Rajagopal (2013). Conidia morphology on the Deuteromycota have a small size so that they can spread over long distances to increase their population (Swer et al., 2011). Petrini (1986) reports that members of the Basidiomycota and Zygomycota rarely as endophytic so in this study two members of the Phyla were not isolated.

The diversity of endophytic fungi on the 30 plants species showed a difference. The composition of endophytic fungi that was different on different host plants show a preference to colonize host plants (Qadri et al. 2013). The same researchers inform that other factors such as soil conditions, climate, and the dynamics of soil microflora may also influence the colonization of endophytes in the plant tissues. Generally, different plants species have a different chemical compositions, and this may affect the microbial biomass and community composition (D'Souza and Bhat 2013).

Endophytic fungi diversity on organs of the 30 plants species showed a difference too. Similar result is reported by Ganley and Newcombe (2006). The composition of the endophytic fungi differ in different plant organs, especially between leaf and petiole could be caused by differences in the anatomical structure of the two organs. Leaf texture is softer because leaves contain mainly parenchymatous cells that are thin-walled, with chloroplasts and rich in starch (Maheswari and Rajagopal 2013),

while the petiole has a harder texture. Endophytic fungi that have affinity to the different plant tissues might be a reflection of tissue preferences of taxa dominating individually (Rodrigues and Samuels 1990) and might be reflect their capacity for utilizing or surviving within a specific substrate, such as texture and chemical content of different tissues (Photita et al. 2001).

There were four dominant endophytic fungi on the 30 plants species, namely *Colletotrichum* (49 isolates or 43.0%), *Cladosporium* (14 isolates or 12.3%), unidentified (13 isolates or 11.4%), and *Phomopsis* (12 isolates or 10.5%). Similar results are reported by Venkatesan and Suryanarayanan (2013) and Verma et al. (2013). The dominant endophytic fungi would be widely distributed on the host plants. *Colletotrichum*, *Cladosporium*, unidentified, and *Phomopsis* colonizing 18, 6, 9, and 8 plants species respectively. Endophytic fungi, such as *Aspergillus niger*, *Bipolaris*, *Curvularia*, *G. endophyllicola*, *Nigrospora oryzae*, *Pestalotiopsis*, and *Xylaria* have been widely reported as endophytic fungi (Maheswari and Rajagopal 2013; Venkatesan and Suryanarayanan 2013). In Indonesia, *Corynesporopsis iberica* and *Diplodia mutila* were a new information as endophytic fungi on *A. concinna*, *C. annuum* var *grossum*, and *P. major*; and *A. muricata* and *F. vesca* respectively.

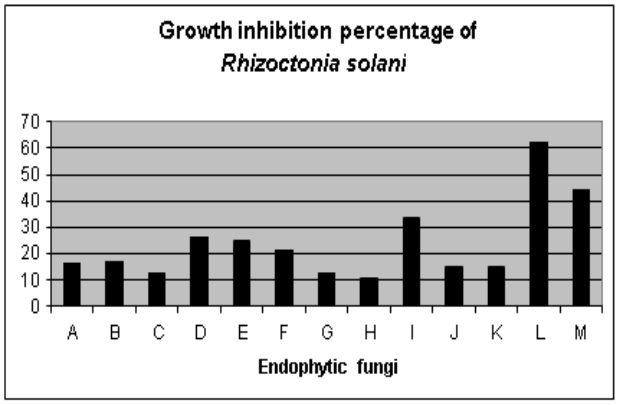

Figure 1. *Growth inhibition percentage of Rhizoctonia solani by endophytic fungi*. A = *Fusarium* sp. (2-19), B = *Phomopsis* sp. (2-35), C = *Fusarium* sp. (2-22), D = *Fusarium* sp. (2-23), E = *Fusarium* sp. (1-34), F = *Fusarium* sp. (1-33), G= *Fusarium* sp. (4-2), H= Unidentified (2-21), I= *G. endophyllicola* (4-12), J = *Phomopsis* sp. (2-8), K = Unidentified (2-12), L = *A. niger* (1-41) M = *A. niger* (1-39).

Of the 114 isolates of endophytic fungi tested, only 13 isolates (11.4%) inhibited the growth of *R. solani* from 10.3% - 62.2% with a percent inhibition (Figure 1). The 13 endophytic fungi showed antibiosis activity against *R. solani* used for toxicity tests. Fungi that inhibited the growth of *R. solani* were *A. niger* (2 isolates), *Fusarium* (6 isolates), *G. endophyllicola* (1 isolate), *Phomopsis* (2 isolates), and unidentified (2 isolates). Similar results are reported by Suciatmih et al. (2011) that *Fusarium*, *G. endophyllicola*, and *Phomopsis* inhibited the growth of *R. solani*. The same researchers inform that *Cladosporium*, *Curvularia*, *Colletotrichum*, *Nigrospora oryzae*, and *Pestalotiopsis* inhibited the growth of *R. solani*, but in this study the endophytic fungi did not inhibit *R. solani*. Possibly because endophytic fungi strains or species were different.

Percent of the highest growth inhibition of *R solani* (62.2%) was produced by the endophytic fungus *A. niger* isolated from the flowers of *S. licopersicum* var *cerasiforme* was followed by *A. niger* (44.4%) isolated from the leaves of *S. licopersicum* var *cerasiforme*. *Aspergillus niger* will be further examined on a field scale. Soenartiningsih (2010) reports that *A. niger* suppressed the growth of *R. solani* by 50%. Venkatasubbaiah and Safeeulla (2008) inform that *A. niger* isolated from the rhizosphere of coffee seedlings antagonist against *R. solani*. Interaction of *R. solani* hyphae and parasitism by *A. niger* via mycoparasite growth (*A. niger*) directly towards its host (*R. solani*) so that hyphae of the host into vacualated, collapsed, and eventually divorced disarray. Brzezinska and Jankiewicz (2012) inform that chitinase isolated from *A. niger* LOCK 62 inhibited the growth of pathogenic fungi, such as *Fusarium culmorum*, *F. solani*, and *R. solani*, but did not inhibit *Botrytis cinerea*, *Alternaria alternata*, and *F. oxysporum*.

Twenty seven species of agricultural plants and three species of non-agricultural plants growing in Bedugul, Bali were host plants of endophytic fungi. *Colletotrichum*, *Cladosporium*, unidentified isolates, and *Phomopsis* were the dominant endophytic fungi. *Corynesporopsis iberica* and *Diplodia mutila* were new endophytic fungi from plants growing in Indonesia. The highest growth inhibition of *R. solani* was shown by *A. niger* isolated from *S. licopersicum* var *cerasiforme* (62.2 %). *Aspergillus niger* will be further examined on a field scale.

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REFERENCES

Anith KN. 1997. Molecular basis of antifungal activity of a fluorescent pseudomonad (Dissertation). New Delhi: Indian Agricultural Research Institute.

Bohlooli A., Okhowat SM., and Javan-Nikkhah M., 2005. Identification of Anastomosis Group of *Rhizoctonia solani*, the Causal Agent of Seed Rot and Damping-Off of Bean in Iran. *Communication in Agricultural and Applied Biological Sciences* 70: 137-141.

Brzezinska MS., and Jankiewicz U., 2012. Production of Antifungal Chitinase by *Asprgillus niger* LOCK 62 and Its Potential Role in the Biological Control. *Current Microbiology* 65: 666–672.

Clarke BB., White JF., Hurley RH., Torres MS., Sun S., and Huff DR., 2006. Endophyte-Mediated Suppression of Dollar Spot Disease in Fine Fescues. *Plant Disease* 90: 994-998.

D'Souza MA., and Bhat DJ., 2013. Occurrence of Microfungi as Litter Colonizers and Endophytes in Varied Plant Species from the Western Ghats Forests, Goa, India. *Mycosphere* 4(3): 567-582.

Gandjar I., Koentjoro IR., Mangunwardoyo W., and Soebagya L., 1992. *Pedoman Praktikum Mikrobiologi Dasar*. Universitas Indonesia, Jakarta, 12-13.

Ganley RJ., and Newcombe G., 2006. Fungal Endophytes in Seeds and Needles of *Pinusmonticola*. *Mycological Research* 110: 318-327.

Grosch R., Scherwinski K., Lottmann J., and Berg G., 2006. Fungal Antagonists of the Plant Pathogen *Rhizoctonia solani*: Selection, Control Efficacy and Influence on the Indigenous Microbial Community. *Mycological Research* 110: 1464-1474.

Guo B., Wang Y., Sun X., and Tang K., 2008. Bioactive Natural Products from Endophytes: A Review. *Prikladnaia Biohimiia Mikrobiologiia* 44(2): 153-158.

Kim HY., Choi GJ., Lee HB., Lee SW., Lim HK., Jang KS., Son SW., Lee SO., Cho KY., Sung ND., and Kim JC., 2007. Some Fungal Endophytes from Vegetable Crops and Their Anti-Oomycete Activities Against Tomato Late Blight. *Letters in Applied Microbiology* 44: 332-337.

Maheswari S., and Rajagopal K., 2013. Biodiversity of Endophytic Fungi in *Kigelia pinnata* During Two Different Seasons. *Current Science* 104(4): 515-518.

Meyera MC., Buenob CJ., de Souzab NL., and Yorinoric JT., 2006. Effect of Doses of Fungicides and Plant Resistance Activators on the Control of Rhizoctonia Foliar Blight of Soybean, and on *Rhizoctonia solani* AG1-IA *In Vitro* Development. *Crop Protection* 25: 848-854.

Nakagiri A., Okane I., Ito T., Kramadibrata K., Suciatmih, and Retnowati A., 2005. A Guidebook to Identification of Fungi Inhabiting Mangrove and Surrounding Area in Indonesia. A Report of GTI Pilot Study on Fungal Taxonomy.

Ogoshi A., and Ui T., 1983. Diversity of Clones within An Anastomosis Group of *Rhizoctonia solani* Kuhn in A Field. *Annual Review of the Japan Phytophathological Society* 49: 239-245.

Petrini O., 1986. Taxonomy of Endophytic Fungi of Aerial Plant Tissues dalam Fokkema NJ., and Van Den Heuvel J. (eds.) *Microbiology of the Phyllosphere*. Cambridge University Press, Cambridge.
175-187.

Photita W., Lumyong S., Lumyong P., and Hyde KD., 2001. Endophytic Fungi of Wild Banana (*Musa acuminata*) at Doi Suthep Pui of National Park, Thailand. *Mycological Research* 105: 1508-1513.

Prihatiningtias W., Widyastuti SM., and Wahyuono S. 2005. Senyawa Antibakteri dari *Thievalia polygonoperda*, Fungi Endofit Tumbuhan Akar Kuning (*Fibraurea chloroleuca* Miers). *Pharmacon* 6(1): 19-22.

Qadri M., Johri S., Shah BA., Khajuria A., Sidiq T., Lattoo SK., Abdin MZ., and Riyaz-Ul-Hassan S., 2013. Identification and Bioactive Potential of Endophytic Fungi Isolated from Selected Plants of the Western Himalayas. *SpringerPlus* 2(8): 1-14.

Redman RS., Sheehan KB., Stout RG., Rodrigues RJ., and Henson JM., 2002. Thermotolerance Conferred to Plant Host and Fungal Endophyte During Mutualistic Symbiosis. *Science* 298: 1581-1581.

Rodrigues KF., and Samuels GJ., 1990. Preliminary Study of Endophytic Fungi in Tropical Palm. *Mycological Research* 94: 827-830.

Skidmore AM., and Dickinson CH., 1976. Colony Interactions and Hyphal Interference between *Septoria nodurum* and Phylloplane Fungi. *Transactions of the British Mycological Society* 66: 57-64.

Soenartiningsih, 2010. Efektivitas Beberapa Cendawan Antagonis dalam Menghambat Perkembangan Cendawan *Rhizoctonia solani* pada Jagung Secara Invitro. Prosiding Pekan Serelia Nasional: 346-352.

Soylu S., Soylu EM., Kurt S., and Ekici OK., 2005. Antagonistic Potentials of Rhizosphere-Associated Bacterial Isolates Against Soil-Borne Diseases of Tomato and Pepper Caused by *Sclerotinia sclerotiorum* and *Rhizoctonia solani*. *Pakistan Journal of Biological Sciences* 8: 43-48.

Suciatmih. 2008. Isolasi, identifikasi, skrining, dan optimasi kapang endofit penghasil antimikroorganisme dari *Dendrobium crumenatum* Sw. (anggrek merpati) (Thesis). Depok: Universitas Indonesia.

Suciatmih, and Rahmansyah M., 2013. Endophytic Fungi Isolated from Mangrove Plant and Have Antagonism Role Against Fusarium Wilt. *ARPN Journal of Agricultural and Biological Science* 8(3): 251-257.

Suciatmih, Yuliar, and Supriyati D., 2011. Isolasi, Identifikasi, dan Skrining Jamur Endofit Penghasil Agen Biokontrol dari Tanaman di Lahan Pertanian dan Hutan Penunjang Gunung Salak. *Jurnal Teknologi Lingkungan* 12(2): 171-186.

Swer H., Dkhar MS., and Kayang H., 2011. Fungal Population and Diversity in Organically Amended Agricultural Soils of Meghalaya, India. *Journal of Organic Systems* 6(2): 3-12.

Venkatasubbaiah P.., and Safeeulla KM., 2008. *Aspergillus niger* for Biological Control of *Rhizoctonia solani* on Coffee Seedlings. *Tropical Pest Management* 30: 401-406.

Venkatesan G., and Suryanarayanan S., 2013. Fungi Associated with the Leaves of Some Hydrophyte Plants. *International Journal of Current Research and Development* 1(1): 53-69.

Verma SK., Gond SK., Mishra A., Sharma VK., Kumar J., Singh DK., Kumar A., Goutam J., and Kharwar RN., 2013. Impact of Environmental Variables on the Isolation, Diversity and Antibacterial Activity of Endophytic Fungal Communities from *Madhuca indica* Gmel. at Different Locations in India. *Annals of Microbiology* 2: 721-734.

Waller F., Achatz B., Baltruschat H., Fodor J., Becker K., Fischer M., Heier T., Hu¨ ckelhoven R., Neumann C., von Wettstein D., Franken P., and Kogel KH., 2005. The Endophytic Fungus *Piriformospora indica* Reprograms Barley to Salt-Stress Tolerance, Disease Resistance, and Higher Yield. Proceedings of the National Academy Sciences, USA 102: 13386-13391.