VOL. 8, NO. 3, MARCH 2013 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science


www.arpnjournals.com

ENDOPHYTIC FUNGI ISOLATED FROM MANGROVE PLANT AND HAVE ANTAGONISM ROLE AGAINST FUSARIUM WILT

Suciatmih and Maman Rahmansyah
Microbiology Division, Research Center for Biology, Indonesian Institute of Sciences Cibinong Science Center, Jl. Jakarta - Bogor, Cibinong, West Java, Indonesia
E-Mail: suciatmih2008@yahoo.ca

ABSTRACT

Antifungal agents of endophytic fungi origin to mangrove plants were studied. Availability of those endophytic fungi competed to *Fusarium oxysporum* f.sp. *lycopersici* (FO) (Sacc.) W.C. Snyder and H.N. Hans. that has causing fusarium wilts on root tomato (*Lycopersicon esculetum* Mill.) plant cultivation had to be done. The study had dual purposes, firstly, to have isolation and turn into identification on endophytic fungi isolated from mangrove plants which is growing in Bunaken Island and Sampiran Beach, North Sulawesi; and secondly, to evaluate their antagonism as volatile and nonvolatile antifungal agents against FO under *in-vitro* conditions. Sixty nine isolates were successfully obtained as endophytic fungi gather from leaves, twigs, and roots; and these fungi are including to the genus of *Aspergillus, Colletotrichum*, *Fusarium*, *Guignardia*, *Penicillium*, *Pestalotiopsis*, *Phomopsis*, *Talaromyces*, and *Trichoderma*. Another group was hardly recognizing taxonomically because of non-sporulating endophytic fungus, so it was named as unidentified one. Among those 69 isolates tested, 22 of them (32%) showed their antagonistic character. Thirteen isolates (59%) of those 22 fungal antagonists were including as volatile and nonvolatile antifungal agents. Identified fungi such as *Aspergillus fumigatus* (34-24; it means the isolate no.34 has fermentative scheme type-24), *Penicillium* sp. (34-26), *Talaromycetes leycettanus* (37-7) and unidentified isolate (37-12) were able to inhibit FO growth by producing both volatile and nonvolatile antifungal agents. Isolates of *Aspergillus niger* (34-25), *Colletotrichum* sp. (37-15), *Fusarium* sp. (37-4), *Trichoderma harzianum* (37-14), and five isolates (codes: 37-10; 37-13; 39-2; 39-8; and 40-12) as unidentified ones showed to against FO by producing volatile antifungal agent; while five isolates of *Aspergillus niger* (codes: 35-1; 42-4; 42-5; 42-6; and 42-9), *Guignardia endophyllicola* (38-2), and three of unidentified isolates (codes: 39-6; 42-1; and 43-4) inhibited FO growth by producing nonvolatile antifungal agent.

Keywords: antagonism, endophytic fungi, *Fusarium oxysporum f.* sp. *lycopersici,* volatile, nonvolatile antifungal agents.

INTRODUCTION

Fusarium wilt is a tomato disease and has known to destroy economically the plant yield in the tropic, as due to the farming activity in Indonesia. Taufik (2008) informed that the intensity of the pathogen attack on tomato plants reached 25 to 50 percent imposition. The fungus that causes wilt disease is named *Fusarium oxysporum* f.sp. *lycopersici* (Sacc.) W.C. Snyder and H.N. Hans (then will be called FO in the next term). That fungus (FO) will attack in the nursery to cause damping off and also to the full-grown plants in the generative phase. Spore belongs to FO was able to survive in long period in the soil for several years, and so the persistence of that spore would have improved significantly inoculum potential even to destroy tomatoes cultivation in the next chance. Fusarium wilt disease was generally controlled with fungicide but it has not provided satisfactory results to the environmental sustainability (Nuryani *et al*., 2003), because the residues would kill non-target organisms, too (Untung, 1996). Therefore, a natural enemy of that FO might become an alternate effort to control effectively and has environmental friendly to realize.

Endophyte is a mutualistic symbiont that all or even the part of their life cycle occurred in healthy plant tissue, receive nutrition, and were inherent in the plant. Characteristic of their association with host plants made to favorable for increasing nutrient uptake (Chanway, 1996), potentially present resistance in plants against pathogen infection (Ting *et al*., 2007), and to promote vigorous growth effect to the plants (Ting *et al*., 2008). Endophytic fungi also produce active substance for biocontrol and antimicrobial agents, powerfully compete for the colonization of space and to gain the food, and stimulate host defense against various pathogens (Benhamau *et al*., 2000).

Following the above impetus, the research has been carried out over endophytic fungi isolated from the mangrove plant. The isolates were evaluated throughout their antagonistic character against FO. High performance capability of endophytic fungi inhibiting FO growth in the *ex-situ* exertion will become great nomination to pertain in the tomatoes cultivation in the area which has endemic in soil due to fusarium wilt problem. To have some certain fungal isolates strongly against fusarium wilt, the endophytic fungi had already been identified but some others of non-sporulating endophytic were saved for their concern as antifungal achievement.

MATERIALS AND METHODS

Sampling plant

Plant samples which are in the form of healthy leaves, twigs, and roots of mangrove were taken from Bunaken Island and Sampiran Beach, North Sulawesi, along with an expedition in the period of 15th July to 19th July 2011. Keep all the sample collections in ice box to

reside as fresh materials, and transported them to the laboratory of mycology belongs to Research Center for Biology, Indonesian Institute of Sciences, in Cibinong Science Center, West Java.

Isolation and identification of endophytic fungi

Endophytic fungi were thrived from samples by using direct planting techniques adopted from Nakagiri *et al*. (2005) work. PDA (potatoes dextrose agar) media was used to stimulate all fungal growth. The whole inoculated medium then incubated in the temperature of 27 to 28°C. The same appearance of fungal colony morphology, color, and its size were considered as the similar species. The entire representative colony of those isolates was grown separately into individual isolate. Single fungal isolate then recognized throughout the macroscopic and as well as microscopic morphology observation and all of their appearances pertain to follow Ellis (1993) and Nakagiri *et al*. (2005) guidance books for references.

All of the isolates deposited as biological resources culture belongs to the institute. Identified fungi preserved and noted to develop into collection resource of LIPI Microbial Culture Collection, Research Center for Biology, Indonesian Institute of Sciences.

Antagonism execution

Antagonism work of the endophytic fungi to FO was evaluated with dual plating technique (Figure-1a) refer to Skidmore and Dickinson (1976) method. Each of endophytic fungi and FO were grown in adjoin position at the same petridish containing PDA medium, and keep in 27 to 28°C for 5 days incubation. Zone of pathogenic discretion was quantified through the inhibition percentage on the day fifth after inoculation, based on comparable control to form on each single culture of endophytic fungi, and as well as the FO growth culture.

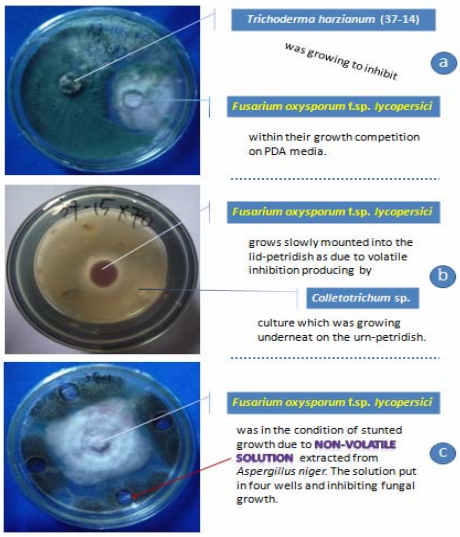

Figure-1. Representing test for life antagonism (a), volatile and nonvolatile inhibition (b and c) to FO.

Volatile antifungal test

Single endophytic fungus evaluate in the course of their antagonist effect to the FO culture which was grown together in a closed and air tight set, inside the petridish on the PDA medium. Stepping work was doing as follow: a). Five days incubation of FO culture was re-inoculated onto the center of another new (urn-) petridish containing PDA medium; b). That urn-petridish with new inoculation with FO then cupped onto the other petridish containing 5 days endophytic culture (replacing lid with that new inoculated urn-petridish); and c.) Both of them were sealed with adhesive tape to avoid air leakage.

During the 5 days incubation, put all of the cupped petridish inside a plastic bag safely under the temperature 27 to 28°C. Concerning to the control, new urn-petridish containing pathogen were placed upside down covered petri dishes containing only PDA medium, and sealed with adhesive tape, too. Sprouting growth of FO hanged and attached under the cupped petridish (Figure-1b) subsequently measured after 5 days incubation (Dennis and Webster, 1971).

Nonvolatile antifungal test

One percent dilution of endophytic-fungi-forming-spore culture was inoculated to 20 ml of Potato Dextrose Yeast (PDY) liquid medium inside the 100ml-Erlenmeyer; or even was inoculated with three single pieces of fungal mycelium belongs to endophytic-non-sporulating-fungi as well. Those cultures were incubated for 5 days in a shaker incubator at the temperature of 27 to 28°C, and keep at 90 rpm agitation speed. The filtrate was being separated from cells biomass with the centrifugation work in 3500 rpm for 10 minutes. The filtrate then used as antifungal agent for toxicity testing to FO culture.

A single piece of mycelium belongs to 5-day-old FO was cultured onto PDA medium in the new petridish, and let the fungal cultures grown at 27 to 28°C temperature for 3 days. After that period, four holes were made onto the edge of the petridish in opposite position by using a plastic straw (Figure-1c); and in that recent phase, FO was beginning to grow. Each hole then filled with 75 µl of the filtrate. Furthermore, FO were continued to keep in 27 to 28°C, and the consequences of antifungal action evaluated for the next 2 days incubation (Anith, 1997).

RESULTS AND DISCUSSIONS

Isolation and identification

Sixty-nine isolated as endophytic fungi, had been successfully segregated from plant samples of six species mangrove plants which are growing in Bunaken Island and Sampiran Beach, North Sulawesi. Nineteen isolates (28%) were identified to species, 21 isolates (30%) turn into genus, and 29 isolates (42%) do not have asexual spores which were hardly to identify microscopically and then all be classified as unidentified isolates (Table-1). Two species of two genera (*Guignardia* and *Talaromyces*) catagorized into group of Ascomycotina and 9 species of 7 genera (*Aspergillus*, *Colletotrichum*, *Fusarium*,

Penicillium, *Pestalotiopsis*, *Phomopsis*, and *Trichoderma*) affiliated as Mitosporic fungi (Deuteromycotina).

The work produced here that endophytic fungi were isolated from mangrove plants dominated by Deuteromycotina and Ascomycotina. On the other hand, endophytic fungi that were belonging to the Basidiomycotina and Zygomycotina groups could not find out in the study. It was probably due to the medium requirement in the exertion having to be specified as the fungal needed.

Isolates of endophytic fungi in this work have achieved to some species and identified as *Aspergillus fumigatus* Fres. (1 isolate), *Aspergillus niger* Van Tieghem (8 isolates), *Aspergillus* sp. (2 isolates), *Colletotrichum* sp. (7 isolates), *Fusarium* sp. (2 isolates), *Guignardia endophyllicola* Okane, Nakagiri, and Ito (5 isolates), *Penicillium* sp. (2 isolates), *Pestalotiopsis* sp. (7 isolates), *Phomopsis* sp. (1 isolate), *Talaromyces leycettanus* Evans and Stolk (3 isolates), and *Trichoderma harzianum* Rifai (2 isolates). Genera *Aspergillus* was a fungus that was found in many, the three species.

Except for *Talaromyces leycettanus*, other endophytic fungi have been revived from other plants. *Aspergillus fumigatus* can be isolated from *Juniperus communis* (Kusari *et al*., 2009) and *Aspergillus niger* from banana plants in our laboratory. Refering the last experience in handling some endophytic fungal species such as *Colletotrichum* spp., *Fusarium* spp., *Guignardia endophyllicola, Pestalotiopsis* spp., and *Phomopsis* spp. had been successful isolated from agricultural crops and plantation of riparian forest in the high altitude of foot mountain area (Suciatmih *et al*., 2011); and so do to *Penicillium* spp. were isolated from *Musa* sp. and *Brassica* sp. In the other works, Li *et al*. (2011) collected *Talaromyces flavus* from *Sonneratia apetala,* and Wu *et al*. (2011) find out *Trichoderma* spp. isolated from *Musa* spp. and *Paeonia delavayi*.

Antagonistic appearance

Among the 69 endophytic fungi isolates tested, 22 of them (32%) showed antagonistic to FO. Their antagonistic behavior affected FO in the midst of ranging from 14 to 42%. Endophytic fungi which have inhibition characteristic to the pathogen consists of *Aspergillus fumigatus* (1 isolate), *Aspergillus niger* (6 isolates), *Colletotrichum* sp. (1 isolate), *Fusarium* sp. (1 isolate), *Guignardia endophyllicola* (1 isolate), *Penicillium* sp. (1 isolate), *Talaromyces leycettanus* (1 isolate), *Trichoderma harzianum* (1 isolate), and 9 isolates achieved but were not be categorized yet. The highest pathogen growth inhibition (42%) was produced by *Trichoderma harzianum* (37-14), while the lowest (14%) one by *Guignardia endophyllicola* (39-3). *Trichoderma* spp. had been widely reported as a biocontrol agent to overcome plant pathogenic fungi. Many antibiotics and extracellular enzymes can be isolated and characterized from the fungi, and might due to the biocontrol mechanism more apparent (Zhihe *et al*., 1998). Moreover, *Trichoderma* spp. are able to compete for space and food through rhizosphere competence and induce resistance in the host plant as well (Howell, 2003).

Volatile antifungal agent

Twenty two endophytic fungi isolate that were antagonistic to FO, only 13 isolates able to produce volatile antifungal agents. Inhibition percentage of the pathogen was ranging from 12 to 43%. The fungi which have capability to produce volatile antifungal agents were *Aspergillus fumigatus* (34-24), *Aspergillus niger* (34-25), *Colletotrichum* sp. (37-15), *Fusarium* sp. (37-4), *Penicillium* sp. (34-26), *Talaromyces leycettanus* (37-7); *Trichoderma harzianum* (37-14), and 6 isolates of the unidentified (37-10, 37-12, 37-13, 39-2, 39-8, and 40-12) ones. The highest pathogen growth inhibition (43%) was formed by endophyte fungi of *Colletotrichum* sp. (37-15), while the lowest (12%) one produced by *A.spergillus niger* (34-25) (Table-2).

Table-1. Identified endophytic fungi origin to mangrove plants.

No.	Host plant	Isolated from	Acquiesce	Species names and its isolate codes	Taxa groups
Sampiran beach habitat					
1.	*Avicennia alba* Blume	leaves	1	*Aspergillus niger* (35-1)*	Deuteromycotina
			1	*Guignardia endophyllicola* (42-1)	Ascomycotina
			1	*Talaromycetes leycettanus* (35-5)	Ascomycotina
			1	Unidentified (37-13)	(unspored fungus)
		twigs	1	*Aspergillus* sp. (40-3)	Deuteromycotina
			1	*Phomopsis* sp. (37-8)	Deuteromycotina
			1	Unidentified (37-10)	(unspored fungus)
2.	*Avicennia marina* (Forssk.) Vierh.	leaves	1	*Talaromycetes leycettanus* (37-7)	Ascomycotina
			1	*Trichoderma harzianum* (37-9)	Deuteromycotina
			1	Unidentified (37-12)	(unspored fungus)
		twigs	1	*Colletotrichum* sp. (37-6)	Deuteromycotina
			1	Unidentified (37-11)	(unspored fungus)
3.	*Bruguiera* sp.	leaves	1	*Trichoderma harzianum* (37-14)	Deuteromycotina
			1	*Guignardia endophyllicola* (38-2)	Ascomycotina
			2	*Colletotrichum* sp. (38-5; 41-2)	Deuteromycotina
		twigs	6	Unidentified (43-2; 43-8; 43-9; 44-1; 44-2; 44-4)	(unspored fungi)
4.	*Ceriops* sp.	leaves	1	*Aspergillus* sp. (43-7)	Deuteromycotina
			1	*Colletotrichum* sp. (42-2)	Deuteromycotina
			1	Unidentified (42-1)	(unspored fungus)
5.	*Sonneratia* sp.	leaves	1	*Aspergillus fumigatus* (34-24)	Deuteromycotina
			2	*Colletotrichum* sp. (42-10; 42-11)	Deuteromycotina
			2	*Fusarium* sp. (37-4; 41-3)	Deuteromycotina
			1	*Guignardia endophyllicola* (39-3)	Ascomycotina
			1	*Talaromycetes leycettanus* (35-4)	Ascomycotina
		twigs	2	*Aspergillus niger* (39-11; 42-4)	Deuteromycotina
			4	*Pestalotiopsis* sp. (41-5; 41-6; 41-7; 41-8)	Deuteromycotina
			4	Unidentified (42-8; 42-13; 43-4; 43-6)	(unspored fungi)
Bunaken island habitat					
6.	*Sonneratia* sp.	leaves	1	*Colletotrichum* sp. (37-15)	Deuteromycotina
			1	*Guignardia endophyllicola* (38-1)	Ascomycotina
			1	*Pestalotiopsis* sp. (39-12)	Deuteromycotina
			1	*Aspergillus niger* (42-9)	Deuteromycotina
			1	Unidentified (40-12)	(unspored fungus)
		twigs	1	*Guignardia endophyllicola* (38-6)	Ascomycotina
			2	*Aspergillus niger* (34-25; 42-3)	Deuteromycotina
			7	Unidentified (39-6; 39-7; 39-8; 39-10; 39-13; 42-12; 43-3)	(unspored fungi)
		roots	2	*Aspergillus niger* (42-5; 42-6)	Deuteromycotina
			2	*Penicillium* sp. (34-26; 35-6)	Deuteromycotina
			2	*Pestalotiopsis* sp. (41-1; 41-4)	Deuteromycotina
			6	Unidentified (39-2; 39-4; 39-5; 39-9; 43-5; 44-3)	(unspored fungi)

*(35-1): code 35-1 means the isolate no.35 has fermentative scheme type-1

In the other occurrence, the slower growth of the pathogen indicated that volatile metabolites had been released, too. According to Perl *et al*. (2011), their investigation informed that *Aspergillus fumigatus* produces volatile metabolites, and quantified as cyclohexanone, 3-octanone, and phenethylalcohol. Ibrahim *et al*. (2011) also reported that ripe tomatoes are inoculated with *Aspergillus niger* and *Fusarium*

oxysporum produce respectively 11 and 8 volatile metabolites. Ting *et al*. (2010) introduced that *Penicillium* (BTF08) produces volatile metabolites, such as butanol, 3-methyl, β-butyrolactone, and 2-butenedinitrile. Wilkins *et al*. (2000) explained that *Trichoderma viride* isolate was able to produce volatile metabolites and consists of 2-propanol, 3-methylfuran, methyl-1-propanol, 1-pentanol, and 2-hexanone.

Table-2. Endophytic scenario against pathogenif fungi.

Fungal isolates	Percentage ability to inhibit FO growth		
	Antagonism rivalry	Volatile disturbance	Nonvolatile interruption
Aspergillus fumigatus (34-24)	28.42	23.70	10.34
Penicillium sp. (34-26)	30.40	23.70	10.30
Talaromycetes leycettanus (37-7)	15.93	17.34	10.90
Unidentified (37-12)	28.32	20.09	10.64
Aspergillus niger (34-25)	14.64	11.56	NEGATIVE
Colletotrichum sp. (37-15)	39.79	42.77	
Fusarium sp. (37-4)	39.32	37.57	
Trichoderma harzianum (37-14)	42.36	30.05	
Unidentified (37-10)	31.24	23.33	
Unidentified (37-13)	40.41	30.64	
Unidentified (39-2)	36.46	26.01	
Unidentified (39-8)	18.88	13.29	
Unidentified (40-12)	19.79	14.45	
Aspergillus niger (35-1)	35.77	NEGATIVE	41.88
Aspergillus niger (42-4)	25.64		34.62
Aspergillus niger (42-5)	24.86		17.28
Aspergillus niger (42-6)	28.74		32.54
Aspergillus niger (42-9)	34.44		15.72
Guignardia endophyllicola (38-2)	13.90		10.14
Unidentified (39-6)	24.66		10.84
Unidentified (42-1)	18.22		11.04
Unidentified (43-4)	22.48		12.60

Nonvolatile antifungal agent

Within the determination work through the 22 endophytic fungal isolates that were antagonistic to FO, only 13 isolates produced nonvolatile antifungal agent. The fungi which had growth inhibition capabilities to FO were ranging from 10 to 42%. The fungi were *Aspergillus fumigatus* (34-24), 5 isolates of *Aspergillus niger* (35-1, 42-4, 42-5, 42-6, and 42-9), *Guignardia endophyllicola* (38-2), *Penicillium* sp. (34-26), *Talaromyces leycettanus* (37-7); and 4 isolates of unidentified (37-12, 39-6, 42-1, and 43-4) ones. Fortunately, highest pathogen growth inhibition (42%) was produced by *Aspergillus niger* (35-1), while the lowest (10%) one by *Guignardia endophyllicola* (38-2).

The inhibition zone without contact with the hyphae on the evaluation of the antagonism and nonvolatile antifungal agent indicated that the secretions of inhibitors (antibiotics) were diffused from the endophytic fungi. *Aspergillus niger* produces nonvolatile antifungal agent that inhibits the growth of *Rhizoctonia solani* (Vaish and Sinha, 2006) and also inhibits the growth of *Fusarium oxysporum* f.sp. *lycopersici* (Alwathnani and Perveen, 2012). In the other study, *Guignardia endophyllicola* inhibits the growth of

Rhizoctonia solani was amount to 48.34% (Suciatmih *et al*., 2011), and *Penicillium* sp. (EU0013) reduces fusarium wilt disease in cabbage and tomato to 74 and 78%, respectively (Alam *et al*., 2010).

Effect of this study showed that *Aspergillus fumigatus* (34-24), *Penicillium* sp. (34-26), *Talaromyces leycettanus* (37-7) and unidentified (37-12) were all influencing growth of FO in the dual plating (antagonism) test, and through the production of volatile and as well as nonvolatile antifungal agents, too. Thus, those four fungi inhibited FO by producing volatile and nonvolatile antifungal agents, as well. Some isolates of *Aspergillus niger* (34-25), *Colletotrichum* sp. (37-15), *Fusarium* sp. (37-4), *Trichoderma harzianum* (37-14), and 5 isolates of unidentified (37-10, 37-13, 39-2, 39-8, and 40-12) ones were able to inhibit the pathogen through the production of volatile antifungal agent, while others 5 isolates of *Aspergillus niger* (35-1, 42-4, 42-5, 42-6, and 42-9); *Guignardia endophyllicola* (38-2), and 3 isolates of unidentified (39-6, 42-1, and 43-4) ones were able to reduce growth of the pathogen through the production of nonvolatile antifungal agent. There are various possibilities can occur to inability of endophytic fungal isolates failed to inhibit FO growth in the test media. That cause may happen as due to was not containing secondary metabolites that were antifungal (volatile and nonvolatile) or the concentration was too low for nonvolatile testing (300 µl/10 ml PDA) so increasing concentration of nonvolatile in testing is required.

On the other hand, the endophytic fungi informed containing secondary metabolites that serves as anticancer, antimalaria, antioxidant, and precursors. Endophytic fungi of some *Pestalotiopsis* spp. isolated from *Taxus wallichiana* plants produce a potential antitumor taxol compound (Mahesh *et al*., 2005). Four new norsesquiterpene peroxides [talaperoxides AD (1-4)] are separated from *Talaromyces flavus* which is isolated from mangrove plant *Sonneratia apetala* (Li *et al*., 2011); while *Phomopsis* fungi isolated from the leaves of *Taxus cuspidate*, Ginkgo biloba, and *Larix leptolepis* produce a potential anticancer taxol (Kumaran and Hur, 2009).

CONCLUSIONS

Endophytic fungi origin to mangrove plant which has produced high antifungal was *Colletotrichum* sp. (37-15) as volatile agent, while *Aspergillus niger* (35-1) recognized as nonvolatile agent. Identification toward the biochemical component release from fungal producing volatile and nonvolatile has valuable to become proponent material utilized to industrial benefit for fungal protection product, and also for medicinal purpose.

ACKNOWLEDGEMENTS

The work was made possible as funded by the Ministry of Research and Technology, Republic of Indonesia, during the Research Engineering Program in the 2011 fiscal year. We are deeply grateful to the colleagues for the critical discussion caused the data turn into comprehensible to serve.

REFERENCES

Alam S.S., K. Sakamoto, Y. Amemiya and K. Inubushi. 2010. Biocontrol of soil-borne fusarium wilts of tomato and cabbage with a root-colonizing fungus, *Penicillium* sp. EU0013. 19th World congress of soil science, soil solution for a changing world. 1-6 August, Brisbane, Australia.

Alwathnani H.A. and K. Perveen. 2012. Biological control of fusarium wilt of tomato by antagonist fungi and cyanobacteria. African Journal of Biotechnology. 11(5): 1100-1105.

Anith K.N. 1997. Molecular basis antifungal activity of a fluorescent pseudomonad. Ph.D. thesis, Indian Agricultural Research Institute, New Delhi, India.

Benhamau N., S Gagne, D. Lequere and L. Dehbi. 2000. Bacterial mediated induced resistance in cucumber: Beneficial effect of the endophytic bacterium *Serratia phymuthica* on the protection against infection by *Pythium ultimum*. Phytopathology. 90: 45-56.

Chanway C.P. 1996. Endophytes they're not just fungi. Canadian Journal of Botany. 74: 321-322.

Dennis C. and J. Webster. 1971. Antagonistic properties of species groups of *Trichoderma* I, production of non-volatile antibiotics. Transactions of the British Mycological Society. 57: 25-39.

Ellis M.B. 1993. Dematiaceous Hyphomycetes. IMI, London. p. 608.

Howell C.R. 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. Plant Disease. 87(1): 4-10.

Ibrahim A.D., H. Hussaini, A. Sani, A.A. Aliero and S.E. Yakubu. 2011. Volatile metabolites profiling to discriminate diseases of tomato fruits inoculated with three toxigenic fungal pathogens. Research in Biotevhnology. 2(3): 14-22.

Kumaran R.S. and B.K. Hur. 2009. Screening of species of the endophytic fungus *Phomopsis* for the production of the anticancer drug taxol. Biotecnology and Applied Biochemistry. 54(1): 21-30.

Kusari S., M. Lamshoft and M. Spiteller. 2009. *Aspergillus fumigatus* Fres., an endophytic fungus from *Juniperus communis* L. Horstmann as a novel source of the anticancer pro-drug deoxypodophylloxin. Journal of Applied Microbiology. 107(3): 1019-1030.

Li H., H. Huang, C. Saho, H. Huang, J. Jiang, X. Zhu, Y. Liu, L. Liu, Y. Lu, M. Li, Y. Lin and Z. She. 2011. Cytotoxic norsesquiterpene peroxides from the endophytic

fungus *Talaromyces flavus* isolated from the mangrove plant *Sonneratia apetala*. Journal of Natural Products. 74(5): 1230-1235.

Mahesh N., M.V. Tejesvi, M.S. Nalini, S. Prakash, K.R. Kini, V. Subbiah and HS Shetty. 2005. Endophytic mycoflora of inner bark of *Azadirachta indica* A. Juss. Current Science. 88(2): 218-219.

Nakagiri A., I. Okane, T. Ito, K. Kramadibrata, Suciatmih and A. Retnowati. 2005. A Guidebook to identification of fungi inhabiting mangrove and surrounding area in Indonesia. A report of GTI pilot study on fungal taxonomy.

Nuryani W., Hanudin I. Djatmika, S. Evi, dan Muhidin. 2003. Pengendalian penyakit layu fusarium pada anyelir dengan formulasi *Pseudomonas fluorescens*, *Gliocladium* sp., dan *Trichoderma harzianum*. Journal Fitopatologi Indonesia. 7(2): 71-75.

Perl T., M. junger, W. Vautz, J. Nolte, M. Kuhns, M.B.V. Zepelin and M. Quintel. 2011. Detection of characteristic metabolites of *Aspergillus fumigatus* and *Candida* species using ion mobility spectrometry - metabolic profiling by volatile organic compounds. www.aensionline.com/jasr/jasr/2009/1-8.pdf (10 November 2011).

Skidmore A.M. and C.H. Dickinson. 1976. Colony interactions and hyphal interference between *Septoria nodurum* and phylloplane fungi. Transactions of the British Mycological Society. 66: 57-64.

Suciatmih Yuliar, dan D. Supriyati. 2011. Isolasi, identifikasi, dan skrining jamur endofit penghasil agen biokontrol dari tanaman di lahan pertanian dan hutan penunjang Gunung Salak. Journal Teknologi Lingkungan. 12(2): 171-186.

Taufik M. 2008. Efektivitas agen antagonis *Trichoderma* sp. pada berbagai media tumbuh terhadap penyakit layu tanaman tomat. Prosiding seminar ilmiah dan pertemuan tahunan PEI PFI XIX Komisariat Daerah Sulawesi Selatan, 5 November.

Ting A.S.Y., S. Meon, J. Kadir, S. Radu and G. Singh. 2007. Field evaluation of non-pathogenic *Fusarium oxyaporum* isolates UPM31P1 and UPM39B3 for the control fusarium wilt in pisang berangan (*Musa*, AAA). Proceeding of the International Symposium on Recent Advances in Banana Crop Protection for Sustainable Production and Improved Livelihoods, September, ISHS Acta Horticulturae. pp. 139-144.

Ting A.S.Y., S. Meon, J. Kadir, S. Radu and G. Singh. 2008. Endophytic microorganisms as potential growth promoters of banana. Biocontrol. 53: 541-553.

Ting A.S.Y., S.W. Mah and C.S. Tee. 2010. Identification of volatile metabolites from fungal. endophytes with biocontrol potential towards *Fusarium oxysporum* f.sp. *cubense* race 4. American Journal of Agricultural and Biological Sciences. 5(2): 177-182.

Untung K. 1996. Pengantar pengelolaan hama terpadu. Gadjah Mada University Press, Yogyakarta: 25-26.

Vaish D.K. and A.P. Sinha. 2006. Evaluation of fungal antagonists against *Rhizoctonia solani* causing sheath blight of rice. Indian Journal Agricultural Research. 40(2): 79-85.

Wilkins K., K. Larsen and M. Sinkins. 2000. Volatile metabolites from mold growth on building materials and synthetic media. Chemosphere. 41: 437-44.

Wu S.H., L.X. Zhao, Y.W. Chen, R. Huang, C.P. Miao and J. Wang. 2011. Sesquiterpenoid from the endophytic fungus *Trichoderma sp.* PR-35 of *Paeonia delavayi*. Chemistry and Biodiversity. 8(9): 1717-1723.

Zhihe C., W. Qingping, X. Lihong, Z. Xiaoyan and Z. Jumei. 1998. Advance of biocontrol of *Trichoderma* and *Gliocladium*. Journal of Microbiology. 25(5): 284-286.