Plant Pathology Journal

ISSN 1812-5387

science alert

ANSInet
an open access publisher
http://ansinet.com

Plant Pathology Journal 13 (4): 257-267, 2014
ISSN 1812-5387 / DOI: 10.3923/ppj.2014.257.267

Antagonistic Activity Assessment of Fungal Endophytes from Oil Palm Tissues Against *Ganoderma boninense* Pat

[1,2]Yurnaliza, [1]I. Nyoman Pugeg Aryantha, [1]Rizkita Rachmi Esyanti and [3]Agus Susanto
[1]School of Life Sciences and Technology,
Institute Technology of Bandung (SITH ITB), Jl. Ganesha 10, Bandung, Indonesia
[2]Faculty of Mathematic and Natural Sciences,
Jl. Biotechnology No. 1. University of North Sumatera, Medan, Indonesia
[3]Indonesian Oil Palm Research Institute, IOPRI, Jl. Brigjen Katamso No. 54, Medan, Indonesia

Abstract: *Ganoderma boninense* is a major pathogen in oil palm plantations which easily infects basal stems and thus it is difficult to be eradicated. Fungal endophytes from healthy oil palm tissues can be used as biological control agents in host plants against *G. boninense*. For selecting the antagonist candidates against *G. boninense*, a simple method of dual culture assay was used. A total of 526 endophytic fungi were isolated from 531 oil palm segments in three of plantations location in Medan, North Sumatera province of Indonesia i.e. Kuala Bekala (KB), Medan Tuntungan (MT) and Medan Johor (MJ). Overall, fungal isolation rate in oil palm segments was 0.99 and colonization rate was 77.2% and the highest being found in the KB plantation. From different sample parts, we found that palm leaf midrib had the highest value of colonization and isolation rate. Based on six categories in qualitative dual culture assay, 53 isolates showed a high antagonism effect to *G. boninense*, twenty two of which were found to have a Colony Growth Inhibition (% CGI) value of more than 80%. Further studies showed that all 22 isolates exhibited a chitinolytic activity on Coloidal Chitin Bromcresol purple (CCBp) assay while only ten (crude extract of liquid culture) inhibited mycelial colony of *G. boninense*. From microscopic observation, fungal endophytes attached themselves to the hyphae of *G. boninense* at the interaction zone and causing hypae abnormalities to the pathogen. Almost all selected fungal endophytes potential to be biocontrol agents against *G. boninense* belong to the Genera of *Trichoderma*.

Key words: Antagonistic activity, fungal endophytes, oil palm, *Ganoderma boninense*

INTRODUCTION

Endophytes are microorganisms that live in plant tissues in mutualistic association with no obvious disease symptoms and a part of encountered endophytes are fungi. Fungal endophytes live naturally, systemically and asymptomatically in plant tissues. They enter plants through natural-wounds, stoma or through the pollination process (Carroll, 1988). They can colonize all parts of the host plant tissues especially dying cells. Fungal endophytes can survive in the spaces between tissues of plant cells without damaging the host, so host plant tissues appear healthy (Ghimire and Hyde, 2004). The endophytic microorganisms that occupy a plant is plentiful and has many species variations but only a few species are dominant in one host (Stone *et al*., 2000).

Symbiosis between certain endophytic fungi and their host plants give some benefits to the host. They naturally protect the host plants from insects, herbivores and pathogenic microorganism by producing metabolic compounds that inhibit pathogens from entering, growing or by inducing a systemic host defense (Rodriguez *et al*., 2009). Endophytic fungi also positively impact the host's physiology when encountering environmental stresses caused by drought (Hubbard *et al*., 2012), metal contamination (Zaurov *et al*., 2001) and salinity (Macia-Vicente *et al*., 2012). The mechanism of endophytic fungi in protecting the host plant is by mean of the production of metabolic compounds that inhibit pathogens as well as the induction of systemic host defense (Gao *et al*., 2010). Communities of endophytic fungi as symbionts affect the ecology and evolution of plant health. The number of endophytic microorganisms that occupy a plant also varies and are abundant but only a few species are dominant in one host (Stone *et al*., 2000).

Almost 400,000 types of plants that exist on earth associate with one or several types of endophytes

Corresponding Authors: Yurnaliza and I. Nyoman Pugeg Aryantha, School of Life Sciences and Technology, Institute Technology of Bandung (SITH ITB), Jl. Ganesha 10, Bandung, Indonesia

(Strobel and Daisy, 2003). However, only a few have been explored for their fungal endophytic diversity including crop plants (Zinniel *et al*., 2002), cacao (Hanada *et al*., 2010), rubber (Gazis and Chaverri, 2010), tropical palm (Azevedo *et al*., 2000), oil palm (Pinruan *et al*., 2010), medicinal plants (Khan *et al*., 2010), orchids (Bayman *et al*., 1997) and forest plants (Suryanarayanan *et al*., 2003). Structure and diversity of endophytic fungi were found to be different in each plant due to canopy cover and leaf age (Arnold and Herre, 2003), location of plant growth (Banerjee, 2011), plant health (Rodriguez *et al*., 2009) and climate (Shankar and Shashikala, 2010).

Fungal endopytes can colonize all parts of the host plant tissue especially in dying cells. Colonization of endophytic fungi on some palm species is quite high and is generally dominated by Deuteromycetes fungi and unidentified anamorf of Ascomycetes group (Carroll, 1988; Rodrigues, 1994). The colonization level of endophytic fungi in oil palm was reported in a range of 81-89% and 13 of them were identified as Basidiomycetes (Pinruan *et al*., 2010).

The potential of fungal endophytes as biocontrol agents is widely recognized. They are able to inhibit some pathogens *in vitro* and *in vivo*. Mejia *et al*. (2008) challenged fungal endophytes from cacao against three cacao pathogens: *Moniliophthora roreri* (frosty pod rot), *Phytophthora palmivora* (black pod rot) and *Moniliophthora perniciosa* (witches broom) and found that 27-65% of the morphospecies tested showed *in vitro* antagonism. Field trials showed that treatment with one fungal endophyte species, *C. gloeosporoides* significantly decreased damaging pod loss originally caused by *M. roreri* and *Phytopthora* spp. More research conducted by Arnold *et al*. (2003) showed that inoculation of leaf free endophytes with endophytic fungi *in vivo* resulted in the decrease of necrosis and mortality incidence of cacao leaf caused by *Phytopthora* sp. infection. Both results strengthen the idea of fungal endophytes as potential biological control agents to control *G. boninense*.

With the promise of fungal endophytes as biocontrol agents, a research was conducted to obtain endophytic fungi from several parts of oil palm sections and to select those having strong antagonistic effects against *G. boninense*, a pathogenic fungus in oil palm plantations that eventually kills the infected plant. This fungus has been causing major issues in oil palm and there have been no effective treatments found to control this fungus until today. Thus, isolation of endophytic fungi and selections of their antagonistic activity towards *G. boninense* is necessary to obtain potential candidates of biocontrol agents against this devastating pathogen.

MATERIALS AND METHODS

Sample collection: Fungal endophytes were isolated randomly from healthy oil palm in three locations of oil palm plantations in Medan City, North Sumatera province of Indonesia i.e. Kuala Bekala (KB), Medan Tuntungan (MT) and Medan Johor (MJ). The conditions of each sampling location are shown in Table 1. Roots, leaves, petioles and leaf midrib samples were collected in sterile plastic bags and processed within a few hours or stored at 4°C before beginning the isolation step. Basal stem rot pathogen, *G. boninense*, was obtained from the Indonesian Oil Palm Research Institute (IOPRI), Medan, Indonesia and was cultivated on Potato Dextrose Agar (PDA, Merck) medium.

Isolation of endophytic fungi: Collected samples were washed with running tap water for 20 min then roots, leaf midribs and petioles were cut into 1-2 cm long pieces whereas the main part of the leaves were cut into 2×2 cm pieces under aseptic conditions. Plant segments were sterilized in a serial process of surface sterilization with alcohol and sodium hypochlorite to kill all organisms on the plant tissue surfaces. Root segments were sterilized with 75% (v/v) ethanol for 2 min, 5.3% (v/v) sodium hypochlorite for 5 min and 75% (v/v) ethanol for 30 sec according to the method by Yurnaliza *et al*. (2008). Meanwhile, leaves, leaf midribs and petiole segments were

Table 1: Conditions of oil palm sampling locations

Area and coordinate	Temp. on Oct. 2011 (°C)	Altitude (above sea level) (m)	Palm age (years)	Soil type	Planting generation
KB					
N 03 29 07.2 E 98 38 06.4	31	79	25	Red yellow podzolic (pH±5.5)	≥1
MT					
N 03 29 12.6 E 98 34 04.5	30	91	16	Andosol (pH±6)	≥1
MJ					
N 03 31 43.0 E 98 38 06.4	30	46	20	Alluvial (pH±5,7)	1

sterilized with 95% (v/v) ethanol for 1 min, 3% (v/v) sodium hypochlorite (5 min for leaves and 10 min for petioles and palm leaf midribs), 95% (v/v) ethanol for 30 sec (Pinruan *et al*., 2010). Last, all sterilized tissues were rinsed with sterile distilled water twice and dried on sterile filter paper. This sterilization process is considered successful if there is no fungal growth when a random plant segment is rolled over an agar medium. Sterilized plant segments were then transferred into PDA medium amended with 0.1 mg mL^{-1} chloramphenicol and incubated for 3-6 days. Fungi growing from the inside of oil palm segments were observed and recorded daily. All isolated fungi were sub-cultured into a new PDA medium and were periodically ascertained for purity.

The colonization and isolation rate of all samples were calculated using the formula described by Kumar and Hyde (2004). Colonization rate was calculated using the percentage of plant segment tissues infected by one or more fungal isolates (S) from the total number of plated tissue segments (Σ S). Meanwhile, isolation rate was calculated using the total number of fungal isolated from each tissue sample (Σ E) and was then divided by the total number of plated tissue segments (Σ S):

$$\text{Colonization rate (\%)} = (S/\Sigma S)\,100$$

$$\text{Isolation rate} = \Sigma E/\Sigma S$$

Antagonistic activity: *In vitro* antagonistic assay was performed using a dual culture method on PDA medium to see which isolates were potential against *G. boninense*. This assessment was comprised of two parts. In the first part, the mycelial plugs (6 mm diameter) of each isolate and *G. boninense* were co-cultured at the same time on agar plates before it was incubated for 7 days at room temperature. Next, the antagonistic activities of endophytes against *G. boninense* were assessed qualitatively. Based on this assessment, fungal endophytes are categorized into six types (Fig. 1). In addition, fungal endophytes growth rate and inhibition rate were evaluated. Growth rate was evaluated based on the daily additional length of colony diameter and the inhibition rate was determined based on inhibition zone of *G. boninense* colony. The fungal endophyte isolates were then assigned into 5 categories based on their growth rate compared to *G. boninense*: Very slow (>8 days), slow (6-8 days), equal to *G. boninense* (5-6 days), fast (4-5 days) and very fast (≤4 days).

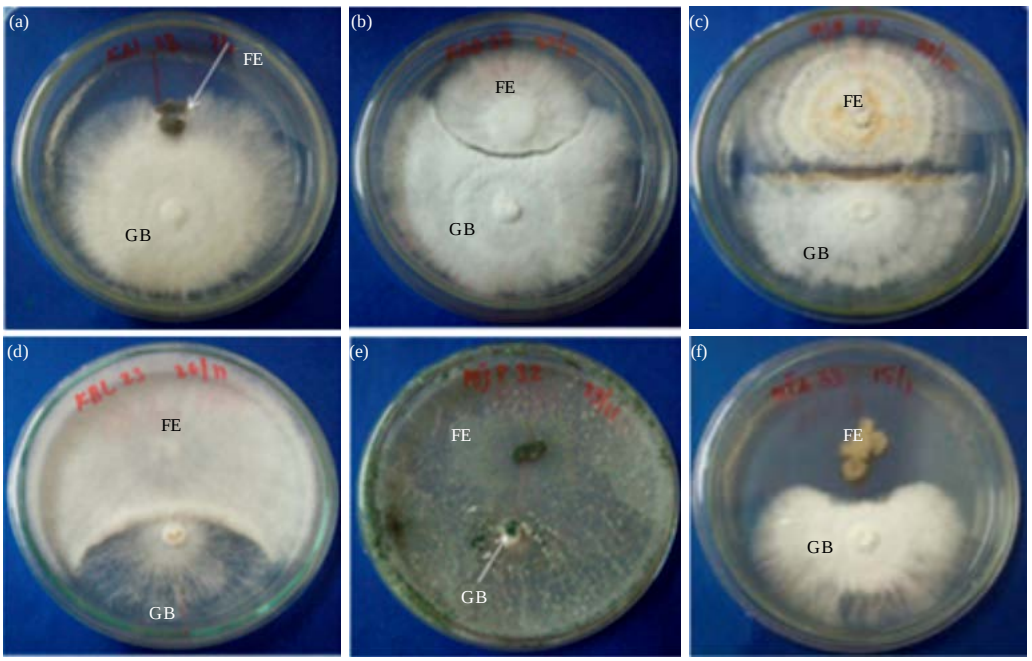

Fig. 1(a-f): Dual culture growth type of *G. boninenese* and fungal endophytes on PDA medium after 7 days incubation. There are six categories, (a) Category I: *G. boninense* inhibit the growth of fungal endophyte's, (b) Category II: *G. boninense* grows faster than fungal endophyte, (c) Category III: *G. boninense* and fungal endohytes have equal colony size, (d) Category IV: Fungal endophytes inhibit *G. boninense* without inhibition zone, (e) Category V: Fungal endophytes cover *G. boninense* colony and (f) Category VI: Fungal endophytes inhibit *G. boninense* with inhibition zone

In the second part, selected isolates (that inhibited *G. boninense*) based on the qualitative dual culture assay were again co-cultured with *G. boninense*. For fast growing isolates, *G. boninense* was planted simultaneously while for very fast growing isolates, *G. boninense* was planted three days in advance. After 7 days of incubation at room temperature, Colony Growth Inhibition (CGI) of *G. boninense* was calculated using the formula (Bivi *et al*., 2010):

$$CGI\ (\%) = \frac{(R_1 - R_2)}{R_1} \times 100$$

where, % CGI is percent of colony growth inhibition. R_1 represents the radius of *G. boninense* colony growth on the absence of fungal antagonist, R_2 represents the radius of *G. boninense* colony from antagonist direction. In addition, morphology of *G. boninense* at the interaction zone with fungal endophytes was observed using a light microscope.

Mechanism of antagonistic activity assessment: The mechanisms of antagonistic activity of selected isolates (based on the result of dual culture assay) were evaluated for the production of antifungal compound and for the activity of chitinase enzymes. Fungal isolates were cultured in Potato Dextrose Broth (PDB, Merck) medium and incubated at room temperature for 7 days in a shaker at 130 rpm. Cultures were extracted and filtered with Whatman filter paper No.1 and tested for their antifungal activity using agar well diffusion methods according to Bonjar *et al*. (2005) with modification. Eighty micro liters of the culture extract were added to a 6 mm diameter well, 0.5 cm from the margin of *G. boninense* colony and incubated for 24 h at room temperature. Uncultured PDB medium was used as a control.

Chitinolytic activity was evaluated qualitatively with Colloidal Chitin Bromocresol purple (CCBp) medium. The composition of the medium (g L^{-1}) was colloidal chitin 4.5 (prepared according to Skujins *et al*. (1965) $MgSO_4.7H_2O$ 0.3, $(NH_4)SO_4$ 3.0, KH_2PO_4 2.0 g of citric acid monohydrate 1.0, agar 15.0, bromocresol purple 0.15 and of Tween-80 200 μL, pH was adjusted to 4.7 (Agrawal and Kotasthane, 2012). The use of pH sensitive dye (bromocresol purple) is preferred because detection of clear zone around the fungal colony is easier than in assessments without. The medium was sterilized at 121°C for 15 min. Fungal endophyte plugs from the margin of colony were cut with a cork borer (diameter 6 mm) then inoculated at the center of CCBp medium and incubated at room temperature for 6-7 days. Formation of purple color in the medium was recorded.

RESULTS

Isolation of endophytic fungi from oil palm: In total, 526 endophytic fungal isolates were obtained from 531 pieces of healthy oil palm segments from three locations of oil palm plantations in Medan. Endophytes were commonly found in all sample parts. On average, colonization rate was 77.2%. Colonization rate of samples from KB (leaves and leaf midribs) and MT (roots, sticks and petioles) were higher than samples from MJ (>90%). Colonization rate in leaf midribs were higher than in roots, leaves and petioles, ranging from 82.0-92.5%. The average isolation rate of fungal endophytes from all three locations was 0.99. Isolation rate for all samples from KB and MT (except leaf samples) were higher than 1. Meanwhile, samples from MJ had isolation rates ranging from 0.7-0.9. Leaf midribs had the highest isolation rate (Table 2).

Antagonistic activities of endophytic fungi of *G. boninense*: The antagonistic activity of fungal endophytes were grouped into six categories based on dual culture growth type as described in Fig. 1. About 30.24% of fungal isolates did not inhibit *G. boninense* (category I). The rest of the isolates (14.72, 17.67, 19.69, 11.23 and 0.49%) inhibited *G. boninense* with various inhibition rate and were grouped into category II, III, IV, V and VI, respectively. Among those isolates, isolates from category IV, V and VI were considered to be more effective against *G. boninense*.

Dual culture growth type of fungal isolates from KB roots were mostly in category V. Meanwhile isolates from MT and MJ roots were mostly in category IV and I, respectively. In addition, isolates from MJ roots made up

Table 2: Colonization and isolation rate of fungal endophytes from three locations of oil palm plantations

	Colonization rate (%)				Isolation rate (ratio)			
Sample	KB	MT	MJ	Mean	KB	MT	MJ	Mean
Roots	62.5	94.4	75.0	77.78	1.13	1.22	0.85	1.06
Leaves	95.0	57.7	68.2	71.19	1.33	0.90	0.76	0.88
Leaf midrib	92.5	92.5	82.0	88.46	1.08	1.18	0.94	1.13
Petioles	89.3	96.4	55.0	73.28	1.29	1.43	0.57	0.95
Mean	85.7	82.1	68.9	77.20	1.20	1.14	0.77	0.99

KB: Kuala bekala, MT: Medan tuntungan and MJ: Medan johor

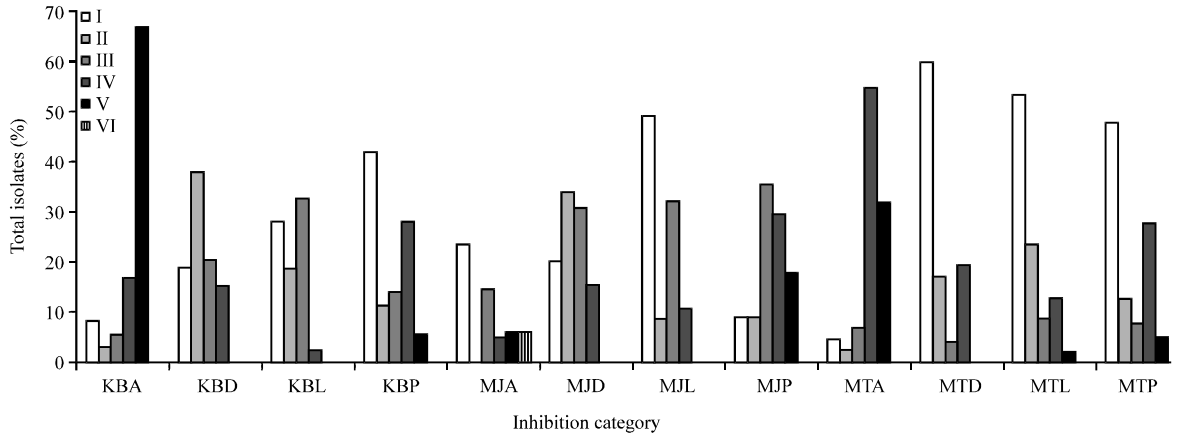

Fig. 2: Growth type of fungal endophytes towards *G. boninense* in three oil palm sample locations in Medan City. KBA: KB roots, KBD: KB leaves, KBL: KB leaf midribs, KBP: KB petioles, MJA: MJ roots, MJD: MJ leaves, MJL; MJ leaf midribs, MJP: MJ petioles, MTA: MT roots, MTD: MT leaves, MTL: MT leaf midribs, MTP: MT petioles

Table 3: Growth rate of fungal endophytes towards *G. boninense* on Ø 9 cm plate

Fungal endophyte growth (days)	Total	Inhibit *G. boninense*	Did not inhibit *G. boninense*
Very slow (>8)	14	2	12
Slow (6-8)	170	86	84
Same with GB (5-6)	146	99	47
Fast (4-5)	137	127	10
Very fast (≤4)	59	51	8
Total	526	365	161

all of category VI. Isolates from leaf samples of MT were mostly in category I while leaf samples from KB and MJ gave the most isolates in category II. Isolates from leaf midrib samples were mostly in category I for MT and MJ but not for KB. Isolates from petiole samples were mostly in category I for KB and MT and category III for MJ (Fig. 2).

A total of 365 isolates (very slow to very fast growth rate) were able to inhibit *G. boninense*, while 161 were not able to. Out of 137 fast growing isolates and 59 very fast growing isolates, 127 isolates and 51 isolates were able to inhibit *G. boninense* respectively (Table 3). Growth rates of *G. boninense* on PDA medium without fungal endophyte (represented by the time period to cover the whole medium surface on a Ø 9 mm plate) were 6-7 days (data not shown). Thus, fungal endophytes that have the ability to inhibit *G. boninense* and can grow faster than this pathogen are considered as good candidates for biological control agents. In total, 53 isolates were selected for the quantitative assessment; consisting of 50 isolates from category V with very fast growth rate plus 2 isolates from category VI and one isolate from category IV with fast growth rate.

From 53 isolates which were assessed quantitatively, 45 isolates belong to *Trichoderma* (42 from roots and 3 from petioles) and eight isolates were non *Trichoderma* (5 from petioles, 2 from leaf midribs and 1 from leaf). Not all fungal endophytes with very fast growth rate were able to inhibit *G. boninense* colony effectively when cultivated 3 days earlier. In response to fungal endophytes, *G. boninense* formed a thick biomass as a protection mechanism. Four isolates gave a CGI percent lower than 55% (two isolates from category VI and two others from category V). Possibly, these fungal endophytes have weak antagonistic activity. Only twenty two isolates had the ability to inhibit ≥80% of *G. boninense* colony, 20 isolates of which were *Trichoderma*, 1 *Lasiodiplodia* sp. and 1 unidentified Genera from Ascomycota.

Indication of mycoparasitism phenomena could be seen from the observation using a light microscope which showed some abnormalities of *G. boninense* hyphae in the dual cultures interaction zone (Fig. 3c-f). Meanwhile, Fig. 3a shows normal hyphae of *G. boninense* and Fig. 3b shows fungal endophytes that are attaching to the hyphae of *G. boninense*.

Mechanism of antagonistic activity: Inhibition activity of endophytic fungi towards *G. boninense* is by means of secondary metabolites. Antifungal compound extracted from selected endophytic fungi inhibited *G. boninense* mycelium in PDA medium (Fig. 4). From 22 isolates tested,

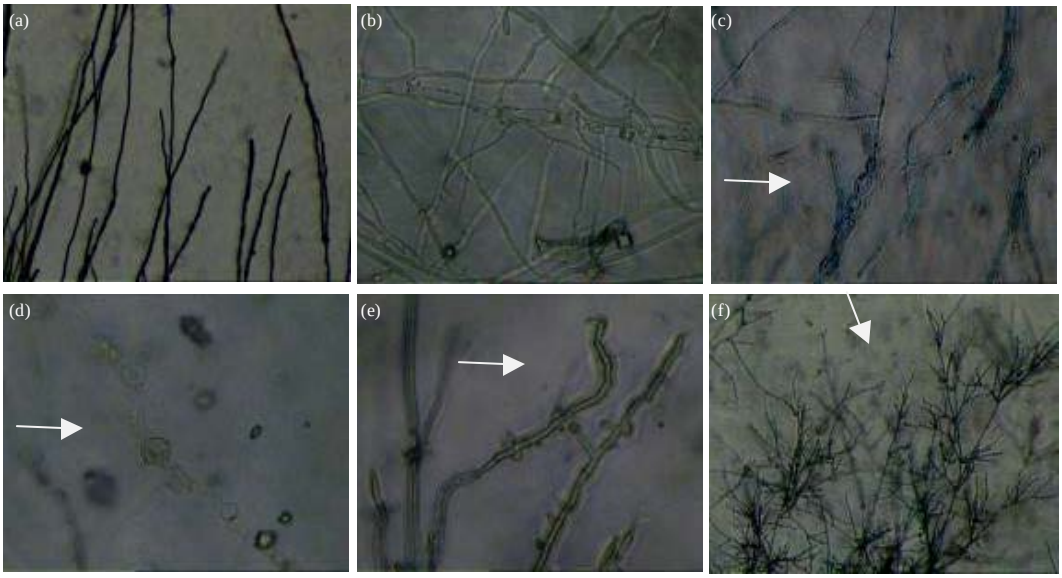

Fig. 3(a-f): Morphology of *G. boninense* hyphae in an interaction zone with some fungal endophytes. (a) Normal hyphae, (b) Fungal endophytes attaching to *G. boninense* hyphae, (c-f) Abnormalities of *G. boninense* hyphae, (c-d) Swelling and (e-f) Distortion and early branching

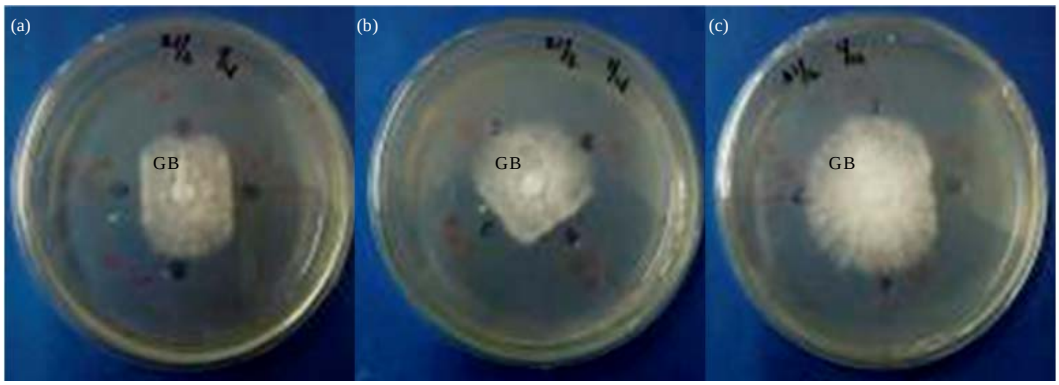

Fig. 4(a-c): Antifungal activity tests of fungal endophytes extract to *G. boninense* growth in PDA medium

antifungal activities were detected on ten isolates, nine from the genera of *Trichoderma* and 1 from non *Trichoderma*.

The chitinolytic activity assessments of fungal isolates on CCBp medium showed that almost all isolates caused color change in the medium surrounding the fungal colony from yellow to purple with various intensity. This color change was caused by activity of chitinase enzymes of fungal endophytes. This enzymes hydrolyzed chitin into monomer (N-acetylglucosamine) and causing the pH of the medium to increase from acidic to basic. Bromocresol purple in the medium which acted as pH indicator caused the medium color to change from bright-yellow to purple. Bromocresol purple has color transition range between pH 5.2-6.8. The medium become yellow if pH below than 5.2 and become purple above pH 6.8. Higher intensity of purple implied a higher chitinase activity of the fungi.

Most *Trichoderma* isolates grew fast in CCBp medium and showed high chitinase activity. One isolate (MJP 28) grew fast but gave low chitinase activity. One isolate (MTD 29) had slow growth and has a moderate chitinolytic activity. Several isolates changed CCBp medium to become completely purple in 4 days (Fig. 5).

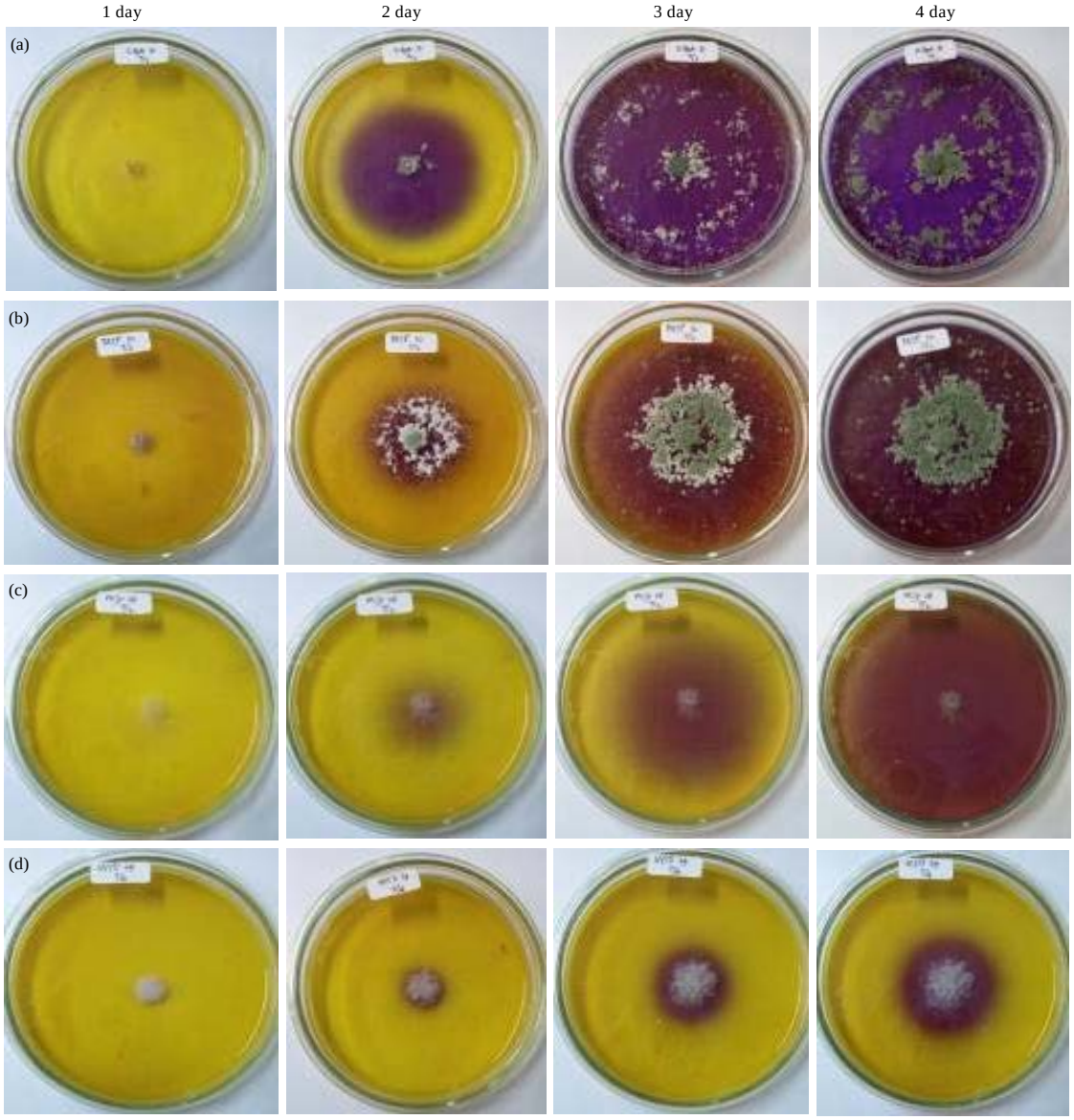

Fig. 5(a-d): Chitinolytic activity of fungal endophytes in CCBp medium supplemented with coloidal chitin and dye indicator bromocresol purple for 4 days and incubated at room temperature, (a) Fast growth and high chitinase activity, (b) Fast growth and moderate chitinase activity, (c) Fast growth and low chitinase activity and (d) Slow growth and moderate chitinase activity

After 7 days, the entire plate medium become purple but not for MTD 29 isolate. This fungus changed the color of medium first to purple then back to yellow after incubation for 2 weeks (Fig. 6) indicating the production of new compounds from N-acetylglucosamine.

DISCUSSION

The information about colonization and isolation rate is useful for estimation of presence endophyte species in host plants. Many factors in this research such as the size of plantation area, plant age, soil type, planting generation and other environmental conditions were believed to play a part for this data. Based on the average data of colonization and isolation rate, samples from KB has the highest value compared to samples from MT and MJ. This is possibly because of plantation area size is larger and the oil palm is older. Microbial endophytes were more abundant in mature palms than in middle and young palms (Zaiton *et al*., 2006). Looking specifically at the sample

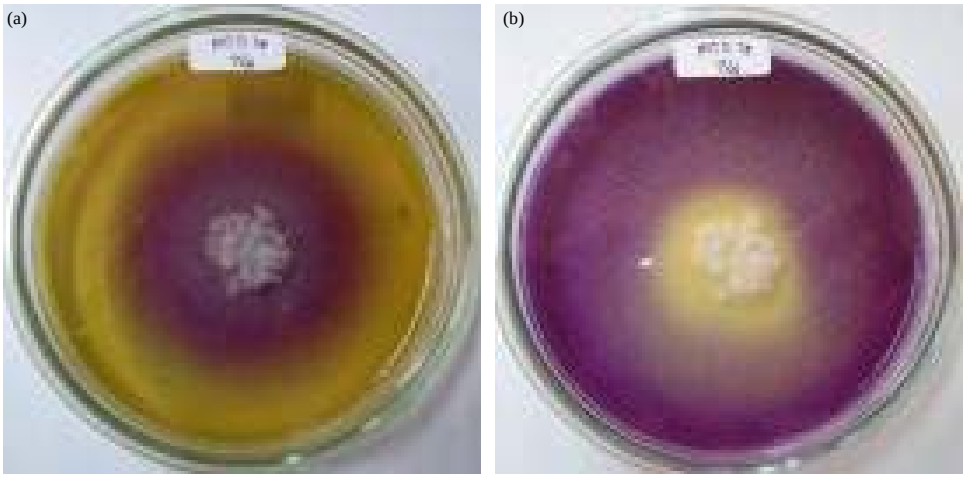

Fig. 6(a-b): Isolate of MTD 29 on CCBp medium, (a) One week incubation and (b) Two weeks incubation

parts, palm leaf midrib has the highest average value of colonization and isolation rate compared to other sample parts. Palm age and plantation location seem to have influenced the result. Meanwhile, root and petiole samples from MT have the highest colonization and isolation rate compared to root samples from KB and MJ. This might be caused by the difference in soil fertility between locations since it affects the existence of soil microorganisms. The soil type in MT is andosol soils which is formed in volcanic ash and is more fertile than podzolic soils (in KB) and alluvial soils (in MJ). Cabral *et al.* (2009) reported that incidence of *Neotypodium* endophytes in *Poa rigdifolia* was higher in high fertility soil. Guo *et al.* (2008) reported that colonization and isolation rate of fungal endophytes in *Pinus tabulaeformis* (Pinaceae) in the Dongling Mountains, Beijing was influenced by season and tissues age but seasonal factors did not conspicuously influence the composition of endophytic assemblages. Colonization rate of fungal endophytes in leaves of *Theobromae cacao* was influenced by the canopy cover and leaf age (Arnold and Herre, 2003).

When compared to the result from other research, colonization rates for all three locations in Medan City were in the range between 55-96% which is wider than in oil palm plantations in Thailand (81-89%) (Pinruan *et al.*, 2010). Comparison with other palm species, the colonization rate showed varied results. Endophytic fungi in leaves of the *Trachycarpus fortunei* (Taylor *et al.*, 1999) plant and leaves of *Euterpe oleracea* (Rodrigues, 1994) have colonization rates only 23-57 and 21-30%, respectively. In Licuala plants (in Brunei and Australia), colonization rates of endophytic fungi were more higher, 81-87% (Frohlich *et al.*, 2000).

Endophytic fungi in the plant tissues generally come from the environment where these plants grow. They tend to enter the plant's natural opening passively or through water transport into tissues, colonize the epidermis and cortex tissues and proliferate in undergone aging plants (Promputtha *et al.*, 2007). In addition, fungal endophytes are rarely found in the meristematic area such as the root tips due to the presence of BAX inhibitor-1 gene (HvBI-1) which is responsible for inhibiting the growth of fungal hyphae (Deshmukh *et al.*, 2006). Gomez-Vidal *et al.* (2006) did an observation with Transmission Electron Microscopy (TEM) and found that fungal endophytes colonizing the intercellular spaces of the plant parenchymal tissues. The host plant sometimes induce a callus formation or a physical barrier and activate chemical defense like chitinase and peroxidase enzymes as a response mechanism of plants to fungal endophyte colonization (Yedidia *et al.*, 1999).

On the inhibition study, selected isolates were found to be dominated by *Trichoderma* because these fungi grew quickly and could compete with *G. boninense*. There have been several studies on the effectiveness of *Trichoderma* against *G. boninense*. Non-endophytic *Trichoderma* could reduce the incidence of stem rot diseases in oil palm crops with a disease incident rate of only 28.35% when compared with controls (Sundram *et al.*, 2008). As potential biocontrol agent, *Trichoderma* is also capable of forming endophytic symbiosis with plants. This fungus was reported to enter the host plant tissues from soil and colonized the tissues of *Theobroma cacao* (Bailey *et al.*, 2009). Thus, our study gives more evidence that *Trichoderma* is capable of forming endophytic symbiosis in plants as well as possessing a biocontrol agent quality like its non-endophytic relatives.

In antagonism phenomena, microscopic observation of selected fungal endophytes attached themselves to the the pathogen's hyphae and may produce metabolites or lytic enzymes that caused abnormalities hypae morphology such as hypae swelling, distortion, cytoplasm aggregation (Prapagdee *et al*., 2008) and early branch formation (Harris, 2008). Considering that most of the selected isolates were *Trichoderma*, this result is not surprising because mycoparasitism is one of the well-known pathogen control mechanism displayed by this genus; the other two being competitive for nutrients and antibiosis (Chet, 1987). Evidence of mycoparasitism phenomena against *G. boninense* was supported by the result of chitinolytic assessment. Coloidal Chitin Bromocresol Purple (CCBp) medium used coloidal chitin as carbon source. Coloidal chitin in the medium is not soluble and chitinase enzyme will degrade it into soluble products. Alteration of chitin solubility into their monomer (N-acetylglucosamine) will change pH of medium. Bromocresol purple (BCP) or 5',5"-dibromo-o-cresolsulfophthalein as pH indicator on CCBp medium that caused chitinolytic activity from fungal endophytes are more easily to be identified. This assessment is more simple and accurate compare than the observation of clear zones around the fungal colony (Agrawal and Kotasthane, 2012). In general, the result showed that all selected isolates caused the medium color to change as described above which signifies the production of chitinase. However, only *Trichoderma* isolates showed high chitinase activities indicating that they can be powerful antagonists against *G. boninense*.

CONCLUSION

As a conclusion, this study has succeeded in selecting a number of endophytic fungi as potential biocontrol agents against *G. boninense*. Our suggestion would be to focus on the *Trichoderma* isolates for field trials or further research. It is hoped that this research could open the way to solve the problem in oil palm plantations caused by *G. boninense*.

ACKNOWLEDGMENT

Acknowledgements are given to the Directorate General of Higher Education of the Republic of Indonesia, who provided a BPPS Doctoral Programs Scholarship and University of North Sumatera, Medan Indonesia for PhD dissertation financial support.

REFERENCES

Agrawal, T. and A.S. Kotasthane, 2012. Chitinolytic assay of indigenous *Trichoderma* isolates collected from different geographical locations of Chhattisgarh in Central India. SpringerPlus, Vol. 1. 10.1186/2193-1801-1-73

Arnold, A.E. and E.A. Herre, 2003. Canopy cover and leaf age affect colonization by tropical fungal endophytes: Ecological pattern and process in *Theobroma cacao* (Malvaceae). Mycologia, 95: 388-398.

Arnold, A.E., L.C. Mejia, D. Kyllo, E.I. Rojas, Z. Maynard, N. Robbins and E.A. Herre, 2003. Fungal endophytes limit pathogen damage in a tropical tree. Proc. Natl. Acad. Sci. USA., 100: 15649-15654.

Azevedo, J.L., W. Maccheroni Jr., J.O. Pereira and W.L. de Araujo, 2000. Endophytic microorganisms: A review on insect control and recent advances on tropical plants. Electron. J. Biotechnol., 3: 40-65.

Bailey, B.A., M.D. Strem and D. Wood, 2009. *Trichoderma* species form endophytic associations within *Theobroma cacao* trichomes. Mycol. Res., 113: 1365-1376.

Banerjee, D., 2011. Endophytic fungal diversity in tropical and subtropical plants. Res. J. Microbiol., 6: 54-62.

Bayman, P., L.L. Lebron, R.L. Tremblay and D. Lodge, 1997. Variation in endophytic fungi from roots and leaves of *Lepanthes* (Orchidaceae). New Phytol., 135: 143-149.

Bivi, M.R., M.S.N. Farhana, A. Khairulmazmi and A. Idris, 2010. Control of *Ganoderma boninense*: A causal agent of basal stem rot disease in oil palm with endophyte bacteria *in vitro*. Int. J. Agric. Biol., 12: 833-839.

Bonjar, G.H.S., P.R. Farrokhi, S. Aghighi, L.S. Bonjar and A. Aghelizadeh, 2005. Antifungal characterization of Actinomycetes isolated from Kerman, Iran and their future prospects in biological control strategies in greenhouse and field condition. Plant Pathol. J., 4: 78-84.

Cabral, D., L.J. Iannone, A. Stewart and M.V. Novas, 2009. The distribution and incidence of *Neotyphodium* endophytes in native grasses from Argentina and its association with environmental factors. New Zealand Grassland Association: Endophytes Symposium, pp: 79-82.

Carroll, G., 1988. Fungal endophytes in stems and leaves from latent pathogen to mutualistic symbiont. Ecology, 69: 2-9.

Chet, I., 1987. Trichoderma-application, Mode of Action and Potential as a Biocontrol Agent of Soilborne Plant Pathogenic Fungi. In: Innovative Approaches to Plant Disease Control, Chet, I. (Ed.). John Wiley and Sons, New York, pp: 137-160.

Deshmukh, S., R. Huckelhoven, P. Schafer, J. Imani and M. Sharma *et al*., 2006. The root endophytic fungus *Piriformospora indica* requires host cell death for proliferation during mutualistic symbiosis with barley. Proc. Natl. Acad. Sci. USA., 103: 18450-18457.

Frohlich, J., K.D. Hyde and O. Petrini, 2000. Endophytic fungi associated with palms. Mycol. Res., 104: 1202-1212.

Gao, F.K., C.C. Dai and X.Z. Liu, 2010. Mechanisms of fungal endophytes in plant protection against pathogens. Afr. J. Microbiol. Res., 4: 1346-1351.

Gazis, R. and P. Chaverri, 2010. Diversity of fungal endophytes in leaves and stems of wild rubber trees (*Hevea brasiliensis*) in Peru. Fungal Ecol., 3: 240-254.

Ghimire, S.R. and K.D. Hyde, 2004. Fungal Endophytes. In: Plant Surface Microbiolgy, Varma, A., L. Abbott, D. Werner and R. Hampp (Eds.). Springer-Verlag, Berlin, pp: 281-292.

Gomez-Vidal, S., L.V. Lopez-Llorca, H.B. Jansson and J. Salinas, 2006. Endophytic colonization of date palm (*Phoenix dactylifera* L.) leaves by entomopathogenic fungi. Micron, 37: 624-632.

Guo, L.D., G.R. Huang and Y. Wang, 2008. Seasonal and tissue age influences on endophytic fungi of *Pinus tabulaeformis* (Pinaceae) in the Dongling Mountains, Beijing. J. Integr. Plant Biol., 50: 997-1003.

Hanada, E.H., A.W.V. Pomella, H.S. Costa, J.L. Bezerra, L.L. Loguercio, J.O. Pereira, 2010. Endophytic fungal diversity in *Theobroma cacao* (cacao) and *T. grandiflorum* (cupuacu) trees and their potential for growth promotion and biocontrol of black-pod disease. Fungal Biol., 114: 901-910.

Harris, D., 2008. Branching of fungal hyphae: Regulation, mechanisms and comparison with other branching systems. Mycologia, 100: 823-832.

Hubbard, M., J. Germida and V. Vujanovic, 2012. Fungal endophytes improve wheat seed germination under heat and drought stress. Botany, 90: 137-149.

Khan, R., S. Shahzad, M.I. Choudhary, S.A. Khan and A. Ahmad, 2010. Communities of endophytic fungi in medicinal plant *Withania somnifera*. Pak. J. Bot., 42: 1281-1287.

Kumar, D.S.S. and K.D. Hyde, 2004. Biodiversity and tissue-recurrence of endophytic fungi in *Tripterygium wilfordii*. Fungal Divers, 17: 69-90.

Macia-Vicente, J.G., V. Ferraro, S. Burruano and L.V. Lopez-Llorca, 2012. Fungal assemblages associated with roots of halophytic and non-halophytic plant species vary differentially along a salinity gradient. Microb. Ecol., 64: 668-679.

Mejia, L.C., E.I. Rojas, Z. Maynard, S.V. Bael and A.E. Arnold *et al*., 2008. Endophytic fungi as biocontrol agents of *Theobroma cacao* pathogens. Biol. Control, 46: 4-14.

Pinruan, U., N. Rungjindamai, R. Choeyklin, S. Lumyong, K.D. Hyde and E.B.G. Jones, 2010. Occurrence and diversity of basidiomycetous endophytes from the oil palm, *Elaeis guineensis* in Thailand. Fungal Divers., 41: 71-88.

Prapagdee, B., C. Kuekulvong and S. Mongkolsuk, 2008. Antifungal potential of extracellular metabolites produced by *Streptomyces hygroscopicus* against phytopathogenic fungi. Int. J. Biol. Sci., 4: 330-337.

Promputtha, I., S. Lumyong, V. Dhanasekaran, E.H.C. McKenzie, K.D. Hyde and R. Jeewon, 2007. A phylogenetic evaluation of whether endophytes become saprotrophs at host senescence. Microb. Ecol., 53: 579-590.

Rodrigues, K.F., 1994. The foliar fungal endophytes of the Amazonian palm *Euterpe oleracea*. Mycologia, 86: 376-385.

Rodriguez, R.J., J.F. White Jr., A.E. Arnold and R.S. Redman, 2009. Fungal endophytes: Diversity and functional roles. New Phytol., 182: 314-330.

Shankar, N.B. and J. Shashikala, 2010. Diversity and structure of fungal endophytes in some climbers and grass species of Malnad Region, Western Ghats, Southern India. Mycosphere, 1: 265-274.

Skujins, J.J., H.J. Potgieter and M. Alexander, 1965. Dissolution of fungal cell walls by a streptomycete chitinase and â-(1 6 3) glucanase. Arch. Biochem. Biophy., 111: 358-364.

Stone, J.K., C.W. Bacon and J.F. White, 2000. An Overview of Endophytic Microbes Endophytism Defined. In: Microbial Endophytes, Bacon C.W. and J.F. White (Eds.). Marcel Dekker, New York, pp: 3-29.

Strobel, G. and B. Daisy, 2003. Bioprospecting for microbial endophytes and their natural products. Microbiol. Mol. Biol. Rev., 67: 491-502.

Sundram, S., F. Abdullah, Z.A.M. Ahmad and U.K. Yusuf, 2008. Efficacy of single and mixed treatments of *Trichoderma harzianum* as biocontrol agents of *Ganoderma* basal stem rot in oil palm. J. Oil Palm Res., 20: 470-483.

Suryanarayanan, T.S., G. Venkatesan and T.S. Murali, 2003. Endophytic fungal communities in leaves of tropical forest trees: Diversity and distribution patterns. Curr. Sci., 85: 489-492.

Taylor, J.E., K.D. Hyde and E.B.G. Jones, 1999. Endophytic fungi associated with the temperate palm, *Trachycarpus fortunei*, within and outside its natural geographic range. New Phytol., 142: 335-346.

Yedidia, I., N. Benhamou and I. Chet, 1999. Induction of defense responses in cucumber plants (*Cucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum*. Applied Environ. Microbiol., 65: 1061-1070.

Yurnaliza, R., H. Wibowo and K. Nurtjaja, 2008. Isolation and antagonistic activity of fungal endophyte from root of oil palm (*Elaeis Guineensis* Jacq.) against *Ganoderma Boninense* pat. Jurnal Biologi Sumatera, 3: 36-41.

Zaiton, S., M. Sariah and M.A. Zainal Abidin, 2006. Isolation and characterization of microbial endophytes from oil palm roots: Implication as biological control agents against *Ganoderma*. Planter, 82: 587-597.

Zaurov, D.E., S. Bonos, J.A. Murphy, M. Richardson and F.C. Belanger, 2001. Endophyte infection can contribute to aluminum tolerance in fine fescues. Crop Sci., 41: 1981-1984.

Zinniel, D.K., P. Lambrecht, N.B. Harris, Z. Feng and D. Kuczmarski *et al*., 2002. Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. Applied Environ. Microbiol., 68: 2198-2208.