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The present investigation was carried out with the objective of isolating fungal endophytes from Cherry tomato plant commonly cultivated in Nagaland, India. Forty fungal endophytes were isolated from 540 segments of different plant parts (leaf, stem and root) and at different growth stages (seedling, vegetative and maturity) and by using three different isolation media (Malt Extract Agar, Potato Dextrose Agar and Rose Bengal Agar). Out of 40 endophytes 13 could be identified at genus level. The most common endophytes isolated from different growth stages of seedling, vegetative and maturity were FE06 (*Fusarium* sp.), FE09 and FE29 with colonization frequency of 6.67%, 55.56% and 65.56%. Whereas the most common endophytes from different plant parts of root shoot and leaf were FE03, FE07 and FE29 with colonization frequency of 11.11%, 22.22% and 50.00%. MEA and PDA were found to be more promising as a culture medium for isolation.

Key words: Cherry tomato, colonization frequency, *Chaetomium*, diversity, fungal endophyte, *Fusarium, Pythium*

INTRODUCTION

It is an accepted characteristic of endophytes that they are not harmful to the host plant. Endophytes also include “bacteria to insects colonizing inside any organ of the plant with inconstant lifestyles” (Schulz and Boyle, 2005).Endophytic studies started with temperate plants but now it is common to find studies on tropical plants and plants from all the geographical regions of the world. (Suryanarayanan and Vijaykrishina, 2001; Azevedo and Araujo, 2007; Mishra *et al*. 2012).

Fungal endophytes have been reported from all parts including root, shoot and leaf of plant (Schulz and Boyle, 2005; Rodriguez *et al*. 2009).As a matter of fact, many endophytic microbes could be unculturable. They are found in all plant species known till date. It is believed that the endophytic fungi have been in close association with plant host for more than 400 million years (Krings *et al*.2007). Most of the endophytic fungi are members of Ascomycota, while some belong to true fungal groups Basidiomycota and Zygomycota and some in Oomycota (pseudofungi or protists).

Endophytic fungi are the storehouse of several secondary metabolites with immense beneficial role to plants where they exist (Li *et al*. 2005; Gunatilaka, 2006; Suryanarayanan *et al*.2009). The volatile organic compounds of some endophytic fungi exhibit killing effect on harmful pathogens like fungi and bacteria (Strobel, 2006; Mitchell *et al*. 2010). Endophytes are now known to promote plant growth, improve tolerance to biotic and abiotic stresses and improve overall fitness of plants in the ecosystem (Ting *et al*. 2008; Saikkonen *et al*.2010).

Presently endophytes are being explored for utilization as potential antagonists to manage plant pests including disease causing organisms (Backman and Sikora, 2008; Li *et al*.2014). Reports of endophytes from various crop plants from diverse geographical regions are forthcoming. In this respect, a need was felt to isolate and understand the diversity of fungal endophyte from cherry tomato (*Solanum lycopersicum* var. *cerasiforme*) that is quite commonly cultivated in Nagaland with the aim of discovering some potential fungi for various agricultural uses.

MATERIALS AND METHODS

The present study was carried out in the laboratory of Department of Plant Pathology, School of

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Agricultural Sciences and Rural Development, Nagaland University, Medziphema Campus situated at 25º45”43” N latitudes and 93º53”04” E longitudes at an elevation of 305 m above mean sea level.

Culture medium

Endophytic fungi of Cherry tomato plant were isolated using three different laboratory media which are described below.

Potato Dextrose Agar (PDA)

Potato Dextrose Agar (PDA) (peeled potato-20 g, dextrose-20 g, agar-agar – 20 g and distilled water- 1000ml) was prepared by peeling the potatoes following the method of Jahan *et al*. (2013). An amount of 200 g of peeled potatoes was weighed and the peeled potatoes were sliced into small pieces and boiled it in 500 ml of distilled water until it became soft. The potato extract was filtered through a muslin cloth in a beaker and 20 g of dextrose was added to it. In another 500 ml of distilled water 20 g of agar-agar was taken and allowed to boil till it dissolved. The two solutions were then mixed properly and made up to 1000 ml and were transferred to 250 ml of conical flask @150 ml/flask. The flasks were plugged with non-absorbent cotton and autoclaved at 15 psi pressure, 121o C temperature for 20 mins.

Malt Extract Agar (MEA)

Malt extract agar (MEA) (malt extract – 20 g, agar-agar – 20 g and distilled water- 1000 ml) was prepared as suggested by Nagamani *et al*. (2006) by weighing 20 g of malt extract and boiling it in 500 ml distilled water and in another 500 ml distilled 20 g of agar-agar was taken and allowed to boil till it dissolved. The two solutions were mixed properly and made up to 1000 ml and were transferred to 250 ml conical flask @150 ml/flask. The flasks were plugged with non-absorbent cotton and autoclaved at 15 psi pressure, 121^{0}C temperature for 20 mins.

Rose Bengal Agar medium

All the ingredients as suggested by Tsao (1964) – ie., dextrose- 10 g, yeast extract – 0.50 g, KH_2PO_4 - 0.50 g, K_2HPO_4 - 0.50 g, $MgSO_4.7H_20$ - 0.50 g, Peptone -0.50 g, Rose Bengal - 0.05 g, Agar-agar - 17.00 g and Streptomycin - 0.03 g were dissolved in distilled water and mixed along with the 17g agar and transferred into 250 ml conical flask @150 ml/ flask and autoclaved at 15psi pressure, 121^0 C temperature for 20 mins.

Isolation of fungal endophytes

Sample collection. The tomato plant samples for isolation and identification of endophytes were collected from the shade net house located at the department of plant pathology and brought to the laboratory. The isolation procedures were carried out inside the laminar air flow chamber in an aseptic condition.

Surface sterilization. The collected samples were washed in running water and surface sterilized by dipping in 70% ethanol for 1 min followed by dipping in 5% sodium hypochlorite solution for 5 min. The surface-sterilized plant samples were washed three times using sterile water to remove traces of sodium hypochlorite (Wiyakrutta *et al*. 2004).

Isolation procedure. After drying the samples, segments of approximately 1cm squares were cut and placed on Petri plates containing potato dextrose agar medium (PDA), Malt Extract Agar (MEA), Rose Bengal Agar, supplemented with streptomycin (100 mg/L) to suppress bacterial growth and incubated at 27°C for 4 days. The fungal colonies thus obtained were purified and maintained in PDA slants.

Data recording

The Petri plates were observed after 3-4 days of incubation. The parameter under which the fungal endophytic growths were observed is: colony colour, colony structure and colony number. The colonization frequency (CF) of a single endophyte species was calculated according to the formula given below (Petrini *et al*. 1982).

$$\text{Colonization frequency} = \frac{\text{Total no. of segments yielding } \geq 1 \text{ isolates}}{\text{Total no.of segments incubated}} \times 100$$

Characterization and identification

The pure cultures of isolates were observed under compound microscope for studying the morphological characters of the fungal hyphae and their breadth. Preliminary identification was made based on the cultural and morphological characteristics. Photo micrographs of each fungal endophyte de-

picting the characteristic features were taken and measurements of the conidia were taken at micrometer scale using the inbuilt camera software under 40X objective lens.

RESULTS AND DISCUSSION

Isolation of fungal endophytes at different growth stages and parts of tomato plant

In the present investigation fungal endophytes were isolated from different growth stages viz., seedling, vegetative, maturity and different parts (leaf, shoot and root) of tomato plant. A total of 40 fungal endophytes (FE01 to FE40) were isolated from 540 segments in the laboratory from different growth stages and different parts of tomato plant (Table1). Among the different growth stages, 24 fungal endophytes were isolated from seedling stage followed by 14 fungal endophytes in vegetative stages and 13 in maturity stage. Among the plant parts, highest number of fungal endophytes were isolated from shoot (27) followed by 15 in leaf and 13 in root. From different stages, isolated fungal endophytes like FE01, FE04, FE12, FE13, FE15, FE20, FE21, FE22 (*Fusarium* sp.), FE32 (*Aspergillus* sp.), FE33 (*Curvularia* sp.), FE37 (*Cladosporium* sp.) and FE38 were found only in seedling stages whereas, FE07, FE08, FE11 (*Fusarium* sp.), FE16, FE18 and FE19 were isolated only from vegetative stage. Fungal endophytes FE25, FE26, FE27, FE29, FE30, FE39 (*Fusarium* sp.) and FE40 were isolated from maturity stage. Among plant parts, fungal endophytes FE05, FE13, FE23, FE25, FE30, FE31 (*Verticillium* sp.) and FE35 (*Aspergillus* sp.) were found only in leaf whereas, FE01, FE04, FE18, FE20, FE21, FE24, FE26, FE27, FE29, FE30, FE32 (*Aspergillus* sp.), FE33 (*Curvularia*sp.), FE37 and FE38 were isolated only from shoot of tomato plant. Fungal endophytes FE08 (*Chaetomium* sp.), FE16, FE19, FE22 (*Fusarium* sp.), FE28 (*Fusarium* sp.), FE35 *(Aspergillus* sp.), FE39 (*Fusarium* sp.) and FE40 were isolated from root only.

From different growth stages of tomato plant, species richness of fungal endophytes was recorded highest from seedling stage (24). Previous report revealed the same result with highest species diversity of fugal endophytes in seedling stage (19) as compared to flowering (16) and fruiting stage (15) in chilli plant (Paul *et al*. 2012). From different plant parts of tomato plant highest species diversity of fungal endophytes was found in shoot (25). Similar results were also found by Zheng *et al*. (2013) and Li *et al*. (2014). They also reported highest species diversity of fungal endophytes from stem part of the plants *Camellia oleifera* and cotton.

While recording the occurrence of endophytes from different segments, their colonization frequencies (Tables 2 and 3) from different stages and plant parts were also calculated. Out of the 40 fungal endophytes 13 could be identified at genus level and 27 fungal endophytes were denoted by numbers as they were not found to be sporulating. The most common endophytes isolated from seedling stage were from FE11 (*Fusarium* sp.) and FE13 with colonizing frequency of 6.67%, and that from vegetative stage was FE09 with colonizing frequency of 55.56% followed by maturity stage (FE29 with colonizing frequency of 65.56%). Whereas the most common endophyte isolated from root was from FE03 with colonizing frequency of 11.11%, that from shoot was FE07 with colonizing frequency of 22.22% and from leaf was FE29 with colonizing frequency of 50.00%. Among all the isolated fungal endophytes FE29 was found to be predominant with higher colonizing frequency of 65.56% in different stages of tomato plant and 50.00% colonizing frequency in different parts of tomato plant. Fusarium sp. was the most dominant genus found in all the three different growth stages and plant parts of tomato plant. It is reported by Rodriguez *et al*. (2008) that *Fusarium culmurom* colonize almost all the non-embryonic tissue of coastal dunegrass (*Leymus mollis*).

It was also observed (Table2) that more numbers of endophytes were found in seedling stage (24) with less colonizing frequency but as the stages progress, in maturity stage (13), a smaller number of endophytes was found with higher colonizing frequency as compared with the seedling stage. Result of the present investigation showed that as the tissue aged, more colonizing frequency of the fungal endophytes was observed. This finding is in accordance with previous report on the endophyte colonizing frequency of *Enterpe oleracea* where it was extremely influenced by the age of the foliage. In the present study leaf was recorded with highest colonizing frequency of endophytes (Table 3) as compared to shoot and root. The highly possible reasons are that leaf has larger surface area, more exposed to outer environment and through which

Table 1: Fungal endophytes isolated at different growth stages and different parts of tomatoplant

Growth stages	Seedling	Vegetative	Maturity	Total
Plant parts Leaf	FE05, FE12, FE13, FE15, FE17, FE35 (*Aspergillus* sp.), FE23, FE31 (*Verticillium* sp.)	FE07, FE06 (*Fusarium* sp.), FE09, FE10 (*Fusarium* sp.)	FE14, FE23, FE25, FE30, FE31 (*Verticillium* sp.)	15
Shoot	FE01, FE04, FE09, FE12, FE14, FE15, FE03, FE20, FE24, FE32 (*Aspergillus* sp.), FE33 (*Curvularia* sp.), FE37 (*Cladosporium* sp.), FE36, FE10 (*Fusarium* sp.), FE21, MS38	FE07, FE09, FE11 (*Fusarium* sp.), FE17, FE18, FE24, FE10 (*Fusarium* sp.)	FE02 (*Pythium* sp.), FE24, FE26, FE27, FE29, FE30	25
Root	FE06 (*Fusarium* sp.), FE22 (*Fusarium* sp.)	FE02 (*Pythium* sp.), FE08 (*Chaetomium* sp.), FE11 (*Fusarium* sp.), FE16, FE19, FE35 (*Aspergillus* sp.), FE36	FE03, FE28 (*Fusarium* sp.), FE39 (*Fusarium* sp.), FE40	13
Total	24	14	13	

the airborne and water-dispersed spores can easily enter through the stomata (Gond *et al*. 2010).

Endophytes are ubiquitous and may grow within roots, stems and/or leaves (Stone *et al*., 2004). The result obtained from the present study differ from those findings by James and Mathew (2015) who isolated 42 fungi isolated from root and 26 from the shoot of tomato plant from Kerala. However, since external factors plays an important role in endophytic population, variations in endophytic population occurs due to samples collected from different agro-climatic zones. Fungal endophytes species communities are also vividly affected by the different types of plant tissues (Guo *et al*.2008) and also it may be due to competition between the same species; antagonism among the endophytes also leads to replacement of one species by another (Yan *et al*. 2015).

Isolation of fungal endophytes on different media

Three different media PDA (Potato dextrose agar), MEA (Malt extract agar) and (Rose Bengal agar) were used for the isolation of fungal endophytes from tomato plant and out of the three media highest number of colony (26) was recorded on MEA followed by PDA (25) and RBA (8) (Table 4). It was observed that use of MEA media produced 14 different unique colony followed by 11 in PDA, whereas RBA did not produce any uncommon colony. Stone *et al.* (2004) reported that malt extract was most commonly used for endophytes isolation and use of selective media leads to discovery and enumeration of different endophytic fungi.

Identification of fungal endophytes

The fungal endophytes isolated were characterized and an attempt was made to identify them based on their colony characters, hyphal septation, spore size and breadth of the hypha. Most of the endophytes isolated in the experiment did not produce spores but they showed distinct morphological or mycelia characteristics. Therefore, the endophytic fungi were named as FE01 (fungal endophyte) FE02, FE03 and so on. Out of 40 isolated fungal endophytes 13 were identified at genus level which are FE02 (*Pythium* sp.), FE06 (*Fusarium* sp.), FE08 (*Chaetomium* sp.), FE10

Table 2: Colonization frequency of fungal endophytes at different growth stages of tomato plant

Fungal Endophytes		Colonization frequency (%)		
		Seedling	Vegetative	Maturity
FE 01		2.22	0.00	0.00
FE 02	*(Pythium* sp.)	0.00	1.11	3.33
FE 03		0.00	0.00	11.11
FE 04		3.33	0.00	0.00
FE 05		2.22	0.00	0.00
FE 06	(*Fusarium* sp.)	4.44	15.56	0.00
FE 07		0.00	26.67	0.00
FE 08	*(Chaetomium* sp.)	0.00	7.78	0.00
FE 09		2.22	55.56	0.00
FE 10	(*Fusarium* sp.)	1.11	2.22	0.00
FE 11	(*Fusarium* sp.)	6.67	4.44	0.00
FE 12		2.22	0.00	0.00
FE 13		6.67	0.00	0.00
FE 14		3.33	0.00	3.33
FE 15		2.22	0.00	0.00
FE 16		0.00	1.11	0.00
FE 17		3.33	2.22	0.00
FE 18		0.00	2.22	0.00
FE 19		0.00	0.00	2.22
FE 20		1.11	0.00	0.00
FE 21		1.11	0.00	0.00
FE 22	*(Fusarium* sp.)	1.11	0.00	0.00
FE 23		2.22	0.00	0.00
FE 24		3.33	2.22	1.11
FE 25		0.00	0.00	3.33
FE 26		0.00	0.00	7.78
FE 27		0.00	0.00	1.11
FE 28	*(Fusarium* sp.)	0.00	0.00	7.78
FE 29		0.00	0.00	65.56
FE 30		0.0 0	0.00	32.22
FE 31	*(Verticillium* sp.)	1.11	0.00	1.11
FE 32	*(Aspergillus* sp.)	1.11	0.00	0.00
FE 33	*(Curvularia* sp.)	1.11	0.00	0.00
FE 34		1.11	0.00	0.00
FE 35	*(Aspergillus* sp.)	0.00	1.11	0.00
FE 36		0.00	1.11	0.00
FE 37	*(Cladosporium* sp.)	1.11	0.00	0.00
FE 38		1.11	0.00	0.00
FE 39	*(Fusarium* sp.)	0.00	0.00	1.11
FE 40		0.00	0.00	1.11

Table 3: Colonization frequency of fungal endophytes at different parts of tomato plant

	Fungal Endophytes	Colonization frequency (%)		
		Root	Shoot	Leaf
FE 01		0.00	2.22	0.00
FE 02	*(Pythium sp.)*	1.11	3.33	0.00
FE 03		11.11	3.33	0.00
FE 04		0.00	3.33	0.00
FE 05		0.00	0.00	2.22
FE 06	(*Fusarium Sp.*)	4.44	0.00	15.56
FE 07		0.00	22.22	4.44
FE 08	(*Chaetomium sp.*)	7.78	0.00	0.00
FE 09		0.00	13.33	44.44
FE 10	*(Fusarium sp.)*	0.00	2.22	1.11
FE 11	*(Fusarium sp.*	3.33	1.11	0.00
FE 12		0.00	1.11	1.11
FE 13		0.00	2.22	4.44
FE 14		0.00	3.33	3.33
FE 15		0.00	1.11	1.11
FE 16		1.11	0.00	0.00
FE 17		0.00	2.22	3.33
FE 18		0.00	1.11	0.00
FE 19		2.22	0.00	0.00
FE 20		0.00	1.11	0.00
FE 21		0.00	1.11	0.00
FE 22	*(Fusarium sp.)*	1 .11	0.00	0.00
FE 23		0.00	0.00	3.33
FE 24		0.00	7.78	0.00
FE 25		0.00	0.00	3.33
FE 26		0.00	7.78	0.00
FE 27		0.00	1.11	0.00
FE 28	*(Fusarium sp.)*	7.78	0.00	0.00
FE 29		0.00	15.56	50.00
FE 30		0.00	10.00	22.22
FE 31	*(Verticillium sp.)*	0.00	0.00	2.22
FE 32	*(Aspergillus sp.)*	0.00	1.11	0.00
FE 33	*(Curvularia sp.)*	0.00	1.11	0.00
FE 34		0.00	0.00	1.11
FE 35	*(Aspergillus sp.)*	1.11	0.00	0.00
FE 36		1.11	0.00	0.00
FE 37	*(Cladosporium sp.)*	1.11	1.11	0.00
FE 38		0.00	1.11	0.00
FE 39	*(Fusarium sp.)*	1.11	0.00	0.00
FE 40		1.11	0.00	0.00

Table 4: Fungal endophytes of tomato plant using different media

Media	Endophytes diversity on different media	No. of endophytes recorded
Potato dextrose agar (PDA)	FE01, FE03, FE04, FE05, FE06 (*Fusarium* sp.), FE07, FE08 (*Chaetomium* sp.), FE09, FE10 (*Fusarium*sp.)*, FE11 (*Fusarium* sp.), FE13, FE14, FE15*, FE21*, FE22 (*Fusarium* sp.), FE23*, FE27*, FE28 (*Fusarium* sp.)*, FE29, FE30, FE32 (*Aspergillus* sp.)*, FE36*, FE37 (*Cladosporium* sp.)*, FE39 (*Fusarium* sp.)*, FE40*	25
Malt extract agar (MEA)	FE02 (*Pythium*sp.)*, FE03, FE04, FE06 (*Fusarium* sp.), FE08 (*Chaetomium* sp.), FE09, FE11 (*Fusarium* sp.), FE12 *, FE13, FE14, FE16*,FE17*, FE18*,FE19*,FE20*, FE24,FE25*, FE26*, FE29, FE30, FE31 (*Verticillium* sp.)*, FE33 (*Curvularia* sp.)*, FE34*,FE35 (*Aspergillus* sp.)*, FE38*	26
Rose Bengal agar (RBA)	FE05, FE06 (*Fusarium* sp.), FE07, FE08 (*Chaetomium* sp.), FE09, FE13, FE22 (*Fusarium* sp.), FE24.	8

* indicate the unique colony obtained from each of the media (PDA and ME)

(*Fusarium* sp.), FE11 (*Fusarium* sp.), FE22 (*Fusarium* sp.), FE28 (*Fusarium* sp.), FE31 (*Verticillium* sp.), FE32 (*Aspergillus* sp.), FE33 (*Curvularia*sp.), FE35 (*Aspergillus* sp.), FE36 (*Cladosporuim* sp.) and FE39 (*Fusarium* sp.).Endophytes mostly belong to Ascomycota (Schulz and Boyle, 2005; Rodriguez *et al*., 2009). Some endophytic fungi have also been reported from Basidiomycota, Zygomycota and Oomycota.

Different types of *Fusarium* sp. were isolated from the roots of tomato plants in different crop areas in Columbia by Andrade-Linares (2011). Fungal endophytes like *Fusarium culmorum, Colletotrichum* spp. and *Curvularia protuberata* were isolated from roots, rhizomes, stems and leaves. Dark sepate endophytes like DSE48, DSE49 and *Leptodontidiumorchidicola* are also reported to be isolated from tomato plant (Rodriguez *et al*. 2008). Kim *et al*. (2007) also reported the isolation and identification of two *Chaetomium* sp. from tomato plant.

Several other endophytic fungi viz., *Nigrospora*sp., *Fusarium oxysporum, F. chlamydosporum, Chrysosporium* sp., *Trichoderma hamatum* and *T. pseudokoningii*(Obura,2010), *Metarrhiziu manisopliae* (Elena *et al*. 2011), *Colletotrichum gloeosporioides, Alternaria alternata, Cladosporium* sp. and *Penicillium* sp. (Larran *et al*. 2001) etc. were isolated from tomato plants.

However, fungal endophytes like *Trichoderma, Penicillium, Alternaria* and *Colletotrichum* reported by others were not isolated in the present investigation as endophyte composition of plant depends on host factors like plant type, plant age, plant tissue type and climatic and soil factors (Torres *et al*. 2011).

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REFERENCES

Andrade-Linares D. R. 2011. *Characterization of tomato root endophytic fungi and analysis of their effect on plant development, on fruit yield and quality on interaction with the pathogen Verticillium dahliae*. Ph.D Thesis. Institutional Repository of the University of Potsdam. http://nbn-resolving.org/urn:nbn:de.kobv:517-opus-51475. Accessed on 4/05/19.

Azevedo, J. L. and Araujo, W. L. 2007. Diversity and applications of endophytic fungi isolated from tropical plants. In*Fungi: multifaceted microbes*. (B.N.Ganguli,S.K.Deshmukh, Eds) pp. 189–207.

Backman, P. A. Sikora, R. A. 2008. Endophytes: an emerging tool for biological control. *Biol. Control.* **46**: 1-3

Elena, G. J., Beatriz, P. J., Alejandro, P., Lecuona, R. E. 2011. *Metarhiziumanisopliae* (Metschnikoff) Sorokin Promotes Growth and has Endophytic Activity in Tomato Plants. *Adv. Biol. Res*. **5**:22–27.

Gond, S. K., Verma, V. C., Mishra, A., Kumar, A., Kharwar, R. N.

2010. Role of fungal endophytes in plant protection. *Mycoscience* **53**: 113-121.

Gunatilaka, A. A. L. 2006. Natural products from plant-associated microorganisms: Distribution, structural diversity, bioactivity and implications of their occurrence. *J. Nat. Prod.* **69**: 509-526.

Guo, L., Chi, D., Ping, W. 2008. Diversity and ecological distribution of endophytic fungi associated with medicinal plants. *Life Sci.* **51**: 751-759.

Jahan, N., Sultana, S., Adhikary, S. K., Rahman, S., Yasmin. 2013. Evaluation of the growth performance of *Trichoderma harzianum* (Rafai.) on different culture media. *J. Agri. Vet.Sci.* **3**: 44-50.

James, D., Mathew, S. K. 2015. Diversity and distribution of endophytic microorganisms in tomato plants in Kerala. *Inter. J. Adv. Biol. Res*. **5**: 358-362.

Kim, H. Y., Choi, G. J., Lee, H. B., Lee, S. W., Lim, H. K., Jang, K. S., Son, S. W., Lee, S. O., Cho, K. Y., Sung, N. D., Kim, J. C. 2007. Some fungal endophytes from vegetable crops and their anti-oomycete activities against tomato late blight. *Lett. Appl. Microbiol*. **44**: 332-337.

Krings, M., Taylor, T.N., Hass, H., Kerp, H., Dotzler, N., Hermsen, E.J. 2007. Fungal endophytes in a 400-million-yr-old land plant: infection pathways, spatial distribution, and host responses. *New Phytol*. **174**: 648–657.

Larran, S., Monaco, C., Alippi, H. E. 2001. Endophytic fungi in leaves of *Lycopersicon esculentum* Mill. *World J. Microbiol.Biotechnol.* **17**:181–184.

Li, H. Y., Qing, C., Zhang, Y. L., Zhao, Z. W. 2005. Screening for endophytic fungi with antitumour and antifungal activities from Chinese medicinal plants. *World J. Microbiol. Biotechnol.* **21**: 1515-1519.

Li, Z.F., Wang, L.F., Feng, Z.L., Zhao, L.H., Shi, Y.Q., Zhu, H.Q. 2014. Diversity of Endophytic Fungi from Different Verticillium-Wilt-Resistant *Gossypium hirsutum* and Evaluation of Antifungal Activity Against *Verticillium dahliae In Vitro*. *J. Microbiol.Biotechnol*. **24**: 1149–1161.

Mishra, A., Gond, S. K., Kumar, A., Sharma, V. K., Verma, S. K., Kharwar, R. N.,Sieber, T. N. 2012. Season and tissue type affect fungal endophyte communities of the Indian medicinal plant *Tinospora cordifolia* more strongly than geographic location and their antimicrobial potential. *Microb. Ecol.* **64**:388–398.

Mitchell, A. M., Strobel, G. A., Moore, E., Robison, R., Sears, J. 2010. Volatile antimicrobials from *Muscodorcrispans*, a novel endophytic fungus. *Microbiology* **156**:270-277.

Nagamani, A., Manoharachary, C. and Kunwar, I. K. 2006. *Handbook of soil fungi*. I. K. International. Pvt. Ltd. New Delhi. pp. 34.

Obura. 2010. Root endophytic fungi of tomato and their role as biocontrol agents of root-knot nematodes *Meloidogyne incognita* (Kofoid and white) Chitwood and growth promotion in tomato plants (*Lycopersicon esculentum* Mill) Bruce Ochieng.Ph.D thesis, IPB Publications, Bogor Agricultural University. http://repository.ipb.ac.id/handle/123456789/43430

Paul, N. C., Deng, J. X., Sang, H. K., Choi, Y. P., Yu, S. H. 2012. Distribution and Antifungal Activity of Endophytic Fungi in Different Growth Stages of Chilli Pepper (*Capsicum annuum* L.) in Korea. *Plant Pathol. J.* **28**: 10-19.

Petrini, O., Stone, J., Carroll, F. 1982. Endophytic fungi in evergreen shrubs in western Oregon: a preliminary study. *Can. J. Bot*. **60**: 789-796.

Rodriguez, R. J., Henson, J., Van Volkenburgh, E., Hoy, M., Wright, L., Beckwith, F., Kim, Y. O., Redman, R. S. 2008. Stress tolerance in plants via habitat-adapted symbiosis. *Isme J*. **2**: 404-416.

Rodriguez, R. J., White, J., Arnold, A., Redman, R. 2009. Fungal endophytes: diversity and functional roles. *New Phytol.* **182**: 314-330.

Saikkonen, K., Saari, S., Helander, M. 2010. Defensive mutualism between plants and endophytic fungi? *Fungal Divers.* **41**: 101–113.

Schulz, B., Boyle, C. 2005. The endophytic continuum. *Mycol. Res*. **109**: 661-686.

Stone, J. K., Polishook, J. D., White Jr, J. F. 2004. Endophytic fungi. In*Biodiversity of Fungi: Inventory and Monitoring Methods*. (G.M. Mueller, G.F. Bills, M.S. Foster, Eds.),Elsevier Academic Press, New York, USA, pp. 241-270.

Strobel, G. 2006. *Muscodor albus* and its biological promise. Journal of Industrial *Microbiol.Biotechnol*. **33**: 514–522.

Suryanarayanan, T. S., Thirunavukkarasu, N., Govindarajulu, M. B., Sasse, F., Jansen, R. Murali, T. S. 2009. Fungal endophytes and bioprospecting. *Fungal Biol. Rev*. **23**: 9-19.

Suryanarayanan, T. S., Vijaykrishina, D. 2001. Fungal endophytes of aerial roots of *Ficus benghalensis*. *Fungal Divers.* **8**:155–161.

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

Torres, M. S., Tadych, M., James, F. White Jr., Bills, G. F. 2011. Isolation and identification of fungal endophytes. Department of Plant Biology and Pathology, Rutgers University, 59 Dudley Road, New Brunswick, NJ08901. USA.

Tsao, P. H. 1964. Effect of certain fungal isolation agar media on *Thielaviopsis basicola* and its recovery in soil dilution plates. *Phytopathology* **54**: 548-555.

Wiyakrutta, S., Sriubolmas, N., Panphut, W., Thongon, N., Danwisetkanjana, K., Ruangrungsi, N., Meevootisom, V. 2004. Endophytic fungi with anti-microbial, anti-cancer and anti-malarial activities isolated from Thai medicinal plants. *World J. Microbiol. Biotechnol*. **20**: 265–272.

Yan, J. F., Broughton, S. J., Yan, S. L., Garge, A. C. 2015. Do endophytic fungi grow through their host systematically? *Fungal Ecol*.**13**:53-59.

Zheng, Y., Qiao, X., Miao, C. 2013. Diversity, Distribution and biotechnological potential of endophytic fungi. *Annals of Microbiol*. **66**:529-542.