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THESIS

BIODIVERSITY OF HALOPHILIC FUNGI AND ANTAGONISTIC ACTIVITIES AGAINST SOME PLANT PATHOGENIC FUNGI

SIANGJEAW PIRIYAPRIN

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
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Sixty one soil samples, thirteen *Acacia* leaves samples and nine samples of organic residues were collected from 2 Provinces in Thailand on November 2008 to December 2010. For isolation of the fungus, soil samples were isolated using the soil plate and soil dilution plate methods on Gochenaur's glucose ammonium nitrate agar and potato dextrose agar supplemented with 10% sodium chloride and incubated at 28°C for 5-7 days. Fungal identifications were based on morphological characters under light and scanning electron microscopes and 18 S rDNA sequences. Four species of halophilic, endophytic fungi including *Fusarium equiseti*, *Fusarium* sp., *Lasiodiplodia pseudotheobromae* and *Nectria rigidiuscula*, were isolated from leaves of *Acacia ampliceps* Maslin (Family Fabaceae) obtained from the areas of highly saline soil (pH 9.2) at Amphoe Kham Thale Sor, Nakhon Ratchasima Province, Thailand. The fungi were characterized for their *in vitro* antagonistic activity and for enzyme production. *F. equiseti* and *N. rigidiuscula* were tested against seven species of plant pathogenic fungi in dual cultures on PDA. These two species inhibited 100% mycelium growth of *Phytophthora palmivora* and 80-92% mycelium growth of *Pythium aphanidermatum, Curvularia oryzae, Colletotrichum capsici* and *Rhizoctonia solani* but showed only moderate activities against *Helminthosporium oryzae* and *Alternaria brassicicola*. Regarding enzyme production, *F. equiseti* strongly digested lipid, whereas *N.rigidiuscula* strongly degraded phosphate and lipid and slightly degraded protein.

In another investigation, four species of alkaliphilic fungi including *Fomitopsis ostreiformis*, *Scytalidium hyalinum*, *Termitomyces cartilaginous* and *Trichoderma virens* were found from organic residues of *Acacia ampliceps* obtained from the moderately and highly saline soil (pH 8.7-9.6) at Amphoe Kham Thale Sor, Nakhon Ratchasima Province and Amphoe Ban Pai, Khonkean Province. In dual cultures test, these four species inhibited 67-100% mycelium growth of *Alternaria brassicicola*, *Helminthosporium oryzae*, *Colletotrichum capsici*, *Pythium aphanidermatum* and *Rhizoctonia solani*. In addition, *Trichoderma virens* inhibited 100% mycelium growth *of Phytophthora palmivora*. These organisms also possessed cellulase, protease, phosphatase and lipase activities. *T. virens* rapidly produced cellulase and showed strongly activities in degrading cellulose, phosphate and lipid. *In vitro* antagonistic teast revealed that *T. virens* suppressed *Phytophthora* foot rot and *Rhizoctonia* leaf blight of durian at the Orchard of Development Royal Project Center, Amphoe Thamai, Chantaburi Province. After foliar spray of *Trichoderma* inoculums (10^6/ml) 10 litre per durian tree every 15 days for 6 months, the rotten bark tissue of durian recovered dried and producing the healthy bark as were as javum leaves young. Furthermore, the *Trichoderma* hypha and conidia alive in the plant cells of cork and cortex inner tissue of the host plant layers of durian fibrous root.

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ACKNOWLEDGEMENTS

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Siangjeaw Piriyaprin

August 2014

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BIODIVERSITY OF HALOPHILIC FUNGI AND ANTAGONISTIC ACTIVITIES AGAINST SOME PLANT PATHOGENIC FUNGI

INTRODUCTION

Saline soil is a problem soil which have effect limiting or toxic for most living cells of plants and soil microorgamisms. Sodium is a very abundant cation in the nature of saline soil. In the high salt amounts condition which cause high osmotic pressure, cells living in natural saline systems must maintain lower water potential than their surroundings in order to survive and proliferate and at the same time adjust to increased concentrations of sodium ions in the cells. Halophilic microorganisnus have developed different strategies for counterbalancing osmotic pressure (Kogej *et al*., 2005). The mechanisms of salt tolerance in some halotolerant fungi show that the maintenance of positive turgor pressure at high salinity is mainly due to an increased production and accumulation of glycerol, trehalose and of other organic compatible solutes. These halotolerant fungi have been studied mostly in *Aspergillus nidulans*, *Rhodotorula mucilaginosa* and *Pichia guilliermondii* (Almagro *et al*., 2000; Gunde – Cimerman *et al*., 2005; Lahav *et al.*, 2002).

There are lots of soil beneficial fungi which are prominent role to decompose organic residues in soil for giving nutrient sources (Gilman, 1957; Lodge, 1993). A wide range of species of soil fungi in Thailand which isolated from various soil samples as forest soil, organic debris, animal dung and mangrove soil are considered important role as a potential source of new compounds (Manoch, 2004). The most common genera are *Aspergillus, Pencillium, Fusarium, Paecilomyces, Trichoderma, Curvularia, Sordaria, Gelasinospora, Chaetomium, Neosartorya, Emericella, Eupenicillium, Talaromyces, Thielavia, Achyla, Allomyces, Dictyuchus, Phytophthora* and *Pythium etc*. In the present, there are many reports which research concerning drng discovery from natural products extracted bioactive secondary metabolites from soil fungi for the pharmaceutical industry, have been regarded as “particularly creative fungi” (Dreyfuss and Chapela, 1994). In Thailand, soil fungi have been

explored for a variety of end-products: biocontrol, source of enzymes which studied on research and development of microbial products for bio-agriculture and noted success in using bioactive compounds from crude extracts of *Chaetomium* and *Trichoderma* species (Soytong, 2004; Chamswarng *et al*., 2001).

Abdel - Harfez (2004) isolated halophilic fungi from desert saline Saudi Arabian soil, the most frequent genera were *Aspergillus*, *Penicillium*, *Ulocladium*, *Cladosporium*, *Myrothecium*, *Scopulariopsis* and *Trichoderma*. Nagai *et al*. (1995) studied alkalophilic fungi or alkali-tolerants that can grow at pH 10 such as *Acremonium alternatum*, *Gliocladium cibotii*, *Phialophora geniculata*, *Scytalidium bicolor*, and *Stilbella annulata*. Rajankar *et al*. (2007) isolated soil beneficial fungi from saline soil in the area Amravati district, India. They found *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp., showing the ability to solubilize the inorganic insoluble phosphate. In Thailand, Kladwang *et al.* (2003) studied alkaline-tolerant fungi from various natural habitats at different locations to screen for the fungal species capable of producing alkaline-tolerant enzyme very useful in modern detergent industry. Frisvad (2005) stated that new lead compounds from fungi are often expected to be found in unusual habitate and there are less data on the possibility to find a new electrolyte producer in extreme environment. Thus it is very interesting to study halophilic, fungi in highly saline environment in the northeast of Thailand.

OBJECTIVES

1. To isolate beneficial halophilic fungi from saline soil.

2. To classified halophilic fungi by morphological characteristic and molecular analysis.

3. To study antagonistic test of halophilic fungi against two plant pathogenic fungi, *Alternaria brassicicola, Colletotrichum capsici, Curvularia oryzae, Helminthosporium oryzae, Phytophthora palmivora, Pythium aphanidermatum*, *Rhizoctonia oryzae, Rhizoctonia solani* and *Sclerotium rolfsii*

4. To study the production of enzymes as cellulase, protease, phosphatase and lipase from these halophilic fungi.

5. To study the application of halophilic fungus for biocontrol Phytophthora foot rot and Rhizoctonia leaf blight of durian Monthong variety *in vivo*.

LITERATURE REVIEWS

Wattle (*Acacia ampliceps*) is considered one of the most important economic leguminous, fast-growing tree in Australia which has been used as food, medicines, clubs, boomerangs and shields (Schöll *et al*., 2004). In the northeastern part of Thailand, *Acacia* plantation is useful in the salinity agricultural areas especially in the paddy field where agroforestry systems were employed to increase soil fertility by providing organic matter from falling senescence leaves. Tran *et al*. (2010) reported endophytic fungi colonised in the tissue of the phyllodues of *Acacia* spp. in Australia. There were identified as *Aureobasidium, Chaetomium* and Sordariomycetes through genetic analysis of ribosomal RNA genes. These fungi was examined in bioactivity and could exhibited antibacterial also produced amylase activity and were thus able to hydrolyse starch. Dong-Rui *et al.* (2012) reported *Fusarium solani* as an halophilic endophytic fungus from *Salicornia europaea* (Chenopodiaceae), a perennial dicot grows in various zones of intertidal salt marshes using molecular biology and morphology. In addition, they study the optimization of its antioxidative fermentation condition. Pérez *et al*. (2012) presented the first report in Cuba of cacao cushion gall disease caused by *Albonetria rigidiuscula* (*Fusarium decemcellulare* or *F. rigidiuscula* anamorph state) which had been previously reported as endophyte in forest trees in Cuba which induced vegetative and flower super production of primordia developing.

Salt wattle (*Acacia ampliceps* Maslin) grow on sandy or loamy alluvial soils with an alkaline reaction and is highly tolerant to salinity which distribute widespread in northwest Australia (Chapman and Maslin, 1992). *Acacia* plantation has potential for use in reclamation of salt-affected areas with a raised or high water table and dune stabilization. The abundant volume of fallen leaves from *Acacia* plantation, the soil is enhanced with the addition of organic fertilizer, which can remain on the ground tofunction as much and stimulating the activities of soil microorganisms which improve the soil structure and increase pH level value in soil for helping nutrients solubilization in the organic matter available to the plants (Schöll *et al*., 2004).

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According to Rungjindamai *et al*. (2008) reported *Fomitopsis* spp. isolation from residue of oil palm leaves. Many of these morphotypes were shown to be basidiomycetes as clamp connections were present and some produced basidia and basidiospores in culture. The Largest fungal assemblage was within the Fomitopsidaceae, four endophytic isolates clusteredwith *Fomitopsis* species (*F. ostreiformis, F. palustris*), two and three isolates grouped with *F. pinicola* and *F. meliae*, respectively.

Cholyklin (2009) had studied the diversity of endophytic basidiomycetes from the oil palm and found that *Fomitopsis ostreiformis* (Berk.) T. Hatt. was isolated from the oil palm leaves and petioles. *F. ostreiformis* had potential use for biological control organisms against the oil palm pathogen, *Ganoderma boninense* by producing bioactive secondary metabolite.

Oh *et al*. (2011) reported that in metal tolerance test, among the four metals tested, the fungus, *Fomitopsis palustris* showed the best resistant ability to copper representing 99.9% followed by lead, cadmium and arsenic representing 94.4%, 83.7% and 78.4% respectively. As a result of biodegradation test, the fungus effectively decomposed wood samples treated with copper based wood preservatives, causing mass loss up to 29.2%.

Steinkraus (1996) reported that *Termitomyces i*s a genus of the order *Agaricales* distinguished form other genera by its distinctive pseudorhiza and its obligate association with termite nests. Interest in these mushrooms strems not only from their well-known association with underground termite nest but also because of their edibility. Especially in Africa, were the mushrooms are gathred in bushels, most species of termitomyceo are highly walued and are considered superior to all other mushrooms. Quimio (1977) recorded rice bran, need press and corn meal can be recommended as good substrate for spawn production since the mycelium of Termitomyces cartilaginous grows readily and densely.

Helan Soundra Rani and Kalaiselvam (2013) reported the *in vitro* antibacterial activity of halophilic fungi extracts against human pathogens. Extracts of Alternaria alternata were highly active towards the pathogens *Escherichia coli*, *Salmonella typhi*, *Vibrio parahaemolyticus*, *Klebsiella oxytoca*. Crude extracts from *Cladosporium* sp showed activity against *Salmonella typhi*, *Klebsiella pneumoniae* and *Hortaea werneckii* fungus also showed inhibition against two pathogens *Escherichia coli* and *Vibrio parahaemolyticus*.

It was concluded that halophilic fungi from solar saltern are diverse, many produce compounds with antimicrobial activity and could be suitable sources of new antimicrobial natural products.

Gunde-Cimerman *et al.* (2009) reported extreme environments have for long been considered to be populated almost exclusively by prokaryotic organisms and therefore monopolized by bacteriologists. Solar salterns are natural hypersaline

environments characterized by extreme concentrations of NaCl, often high concentrations of other ions, high uv irradiation and in some cases extremes in pH. In 2000 fungi were first reported to be active inhabitants of solar salterns. Since then many new species and species previously known only as food contaminants have been discovered in hypersaline environments around the globe. The eukaryotic microorganism most studied for its salt tolerance is *Saccharomyces cerevisiae*. However, *S. cerevisiae* is rather salt sensitive and not able to adapt to hypersaline conditions. In contrast, some species like *Debaryomyces hansenii*, *Hortaea werneckii*, and *Wallemia ichthyophaga* have been isolated globally from natural hypersaline environments.

Frisvad (2005) stated that new lead compounds from fungi are often expected to be found in unusual habitats and there is little information on the possibility of discovering new electrolyte producers in extreme environments. An important first step in such efforts would be to identify and characterize halophilic, endophytic fungi from highly saline environments such as those in the northeast of Thailand.

Soil salinity have high levels of sodium and soil pH is greater than 8.4 which restrict water holding capacity, limits water infiltration, prevents drainage and degradation of vegetation (Franzen, 2013). There were some natural plant tolerance in highly saline soil which were ecological characterization such as *Buchannania siamensis* Miq, *Maytenus marcanii* Ding Hou, *Pluchea indica* (L.) Less. and *Acacia ampliceps* Maslin (Leksungnoen, 2006 ; Chapman and Maslin, 1992). Particularly, salt wattle (*Acacia ampliceps* Maslin) grow on sandy or loamy alluvial soils with an alkali reaction and is highly tolerant to salinity which distribute widespread in northwest Australia (Chapman and Maslin, 1992). The abundant volume of fallen leaves from salt tolerance natural vegetation, the soil is enhanced with the addition of organic fertilizer, which can remain on the ground to function as much and stimulating the activities of soil microorganisms which improve the soil structure and increase pH level value in soil for helping nutrients solubilization in the organic matter available to the plants (Schöll *et al*., 2004).

Gonsalves *et al*. (2012) reported halophilic fungi as *Aspergillus, Penicillium* and *Cladosporium* species were dominated in polyhaline Mandivi estuary which showed maximum growth at 10% salt. Helan Soundra Rani and Kalaiselvam (2013) reported the in vitro antibacterial activity of halophilic fungi extracts against human pathogens. Extracts of *Alternaria alternata* were highly active towards the pathogens *Escherichia coli, Salmonella typhi, Vibrio parahaemolyticus, Klebsiella oxytoca*. Crude extracts from *Cladosporium sp* showed activity against *Salmonella typhi*, *Klebsiella pneumoniae* and *Hortaea werneckii* fungus also showed inhibition against two pathogens *Escherichia coli* and *Vibrio parahaemolyticus*. There halophilic fungi had produced some compounds with antimicrobial activity and could be suitable sources of new antimicrobial natural products.

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Meyer *et al*. (1990) studied antagonistic bioassays of *Scytalidium fulvum* for controlling nematodes of soybean. The results reveal that *S. fulvum* had efficient mature yellow females nematode, *Heterodera glycines* Ichinohe and eggs from brown cysts 88.7 and 93.2% respectively.

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The soil fungus, *Penicillium citrinum* Thom. was isolated from rhizosphere of sugarcane in saline soil (Yadav *et al*., 2011). This fungal strain has ability to solubilize fixed form of phosphorns and induced systematic resistance in plants (Rodriguez *et al*., 1996; Whitelaw, 1999). Further more, the growth and phosphate solubiliztion of *P. citrinum* Thom. are very well effected by high concentration of salt and pH of the soil. The salinity effect on solubilization of tricalcium phosphate by *P. citrinum* Thom. was showed maximum significant in 1% $CaCl_2$ at pH 8.0 which solubilized tricalcium phosphate 455 micro mL^{-1} (Yadav *et al*., 2001). This fungus can provide great benefit in the available phosphate maintenance for crops in saline and alkaline soil which a large fraction of land arid and semiarid regions is affected by salinity in India as about 7.5 million of hectares.

Some strains of plant growth promoting fungi (PGPF) in saline soil are important to contribute plant growth by means of mechanism of nutrition solubilization and their acquition to plants production of hormone substances including preventing the attack of plant pathogen. Majority of the inorganic phosphorus applied to problem soil as a chemical fertilizer is rapidly fixed as insoluble forms (phosphates of iron, aluminum and calcium) and thus become unavailable to plants (Mittal *et al*., 2008). In addition, soils are often high in insoluble mineral and organic phosphates but deficient in available orthophosphate.

In a greenhouse study, mycorrhizal fungi had affected to increase salt tolerance of leguminous species *Strophostyles helvola* in estuarine areas. Some strains of mycarrhizal fungi can tolerate high salt concentration (Tsang and Maun, 1999). In the condition of non-containing miycorrhizal plants of the leguminous *S. helvola*, the dry weight of roots and shoots and leaf chlorophyll content of *S.helvola* plants decreased with an increase in the concentrations of salt solutions. Whereas in

the containing mycorrhizal plants of *S.helvola*, the results showed evidence of significantly higher chlorophyll content, shoot dry weight and number of root nodules than non – mycorrhizal plants. Tsang and Maun (1999) showed that high salt concentrations had a negative effect on the growth of *S. helvola* plants but the negative effects were partially mitigated by the presence of mycorrhizal fungi.

Some species of black yeasts have been isolated from hypersaline waters of solar salterns which were the group of halophilic yeasts as *Hortaea werneckii, Phaeotheca triangularis, Trimmatostroma salinum*, and *halotolerant* as *Aureobasidium pullulans* (Gunde – Cimerman *et al*., 2000). The salinity range of a brand optimum growth for *H.werneckii* in vitro was from 6 to 10 % sodium chloride (NaCl) and could tolerate up to 32 % NaCl (Butionar *et al*., 2005). For the *A.pullulans* as – halotolerant black yeast had grown very well without NaCl but could tolerate up to 17% NaCl in the growth medium (Zalar *et al*., 1999). Both *H.werneckii* and *A.pullelans* accumulate glycerol when grown in saline environment (Hernandez – Saavedra *et al.*, 1995 ; Petrovic *et al*. , 2002). Kogej *et al*., 2005 concluded that in saturation 5 % NaCl showing salt – adapted cells of *H.werneckii* and *A.pullulans* kept very low amount of internal Na^+ even when grown at high NaCl concentration and can be thus considered Na^+ excluders, suggesting the existence of efficient mechanisms for the regulation of ion fluxes and these fungi did not use K^+ and Na^+ for osmoregulation.

Soil fungi can be responsible for the resistance of soil aggregates to breakdown upon wetting. They produce large quantities of non water – soluble extracellular materials composed of polysaccharides, glycolipids or glycoproteins that bind soil particles into soil aggregate and stabilization. (Caesar and Cochran, 2001). Organic matter from no till management with plant residues returned as carbon source which promoted fungal activity and the production of more soil – binding agents; thus contributing increased soil aggregction and water infiltration, reduced run – off and soil erosion (Aspiras *et al*., 1971).

Nutrient recycling by microbial communities especially bacteria and fungi be used as a tool to enhance reforestation with mangrove seedling (Bashan and Holguin, 2002). A close microbe – nutrient plant relationship that functions as a major mechanism for recycling and conserving essential nutrients in the mangrove systems which transforms dead vegetation or organic residues into sources of nitrogen, phosphorus and other nutrients for the plant growth whereas plant – root exudates serve as a food source for the microorganisms living in the ecosystem. Mangrove ecosystems are rich in organic matter; however, in general, they are nutrient – deficient ecosystems, especially of nitrogen and phosphorus (Alongi *et al*., 1993). In tropical Australian – mangroves , bacteria and fungi constitute 91% at the total microbial biomass , whereas algae and protozoa represent only 7 and 2% respectively (Alongi, 1988).

Mallea (1992) reported that few microorganisms were able to grow in the Faraman pan which was a highly saline Mediterranean coastal pool, where the total dissolved salt content average 300 % with sodiumchloride as one of the main components. Fungi were collected from driftwood, and several Deuteromycetes and two Ascomycetes were found growing on the vegetation around the edges of the pan. These terrestrial fungi were able to develop than those of sea-water. Culture media as corn meal agar (CMA) was made up with 8 dilutions of pan water to give a salinity range of 5, 10, 35, 80, 100, 120, 150 and 300 % and one of distilled eater (CMA/DW). These media were used to isolate fungi which were *Acremonium strictwn, Alternaria alternata Arthrinium phaeospermum, Aspergillus* gr. *qlaucus, Aureobacterium pullulans, Camarosporium roumeguerii, Cladosporium* sp., *Fusarium* sp., *Gonatobotrys* sp., *Penicillium expansion* and *Penicillium olsonii*. Efficient mycelia production and sporulation under salinity condition occurred with the following four deuteromycetes. *Cladosporium* sp. grew readily in the presence of sea water *Pencillium olsonii* was found on the terminal leaves and stems of halophytes which developed rapidly on all the culture media and sporulated abundantly (from CMA/120). *Acremonium strictum* (syn. *Cephalosporium acremonium*) showed a broad tolerance to various salinities and spore production was always high which spore germination occurs at salinity levels of 27.5 %.

Deshmukh (2004) reported isolating keratinophilic fungi from the salt pans and their vicinity around Mumbai and Thane. Ten Species classified in six genera were recovered using horse hair as bait. The isolated species were reported in the following order of dominance : *Chrysosporium indicum* (12.0%), *Microsporum gypseum* complex (7.2 %), *C.tropicum* (5.6 %) *C.state* of *Ctenomyces serratus* (4. %), *Trichophyton terrestre* (3.2 %), *Malbranchea aurantiaca* (2.4 %), *C.fluviale* (1.6 %), *Uncinocarpus reesii* (1.6 %), *Malbranchea* sp. (0.8 %), and *T. mentagrophytes* (0.8 %).

Rajankar *et al*., (2007) isolated soil beneficial fungi from saline soil in the area Amravati district, India. In the present study 107 isolates were collected from saline affected area, among these samples, 33 (30.80 %) samples showed the ability to solubilize the inorganic insoluble phosphate. From the study it was observed that the fungi viz; *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp. have the more solubilizing ability of inorganic insoluble phosphate than bacteria, viz; *B.subtilis*, and *B.megatherium*. Hence the application of biofertilizer prepared by above mentioned fungi should be helpful to reduce the salinity of soil by neutralization phenomenon, because these microorganisms release the acid in very minute quantity in phosphate Solubilization.

Nagai *et al*., (1995) studied alkalophilic soil fungi. Most of the fungi isolated on ACMA, especially from the alkaline soils, were alkaline soils, were alkalophiles or alkali-tolerants that can grow at pH 10. *Acremonium alternatum*, *A. furcatum. Acremonium* sp. 6, *Gilocaldium cibotii* (YBLF 575), *Phialophora geniculata, Stachylidium bicolor* and *Stilbella annulata* were alkalophilic, of which *Acremonium* sp. 6 was the most pronounced alkalophile. Ability to grow under alkaline conditions, as well as under acidic condition, was common in many *Acremonium* species. The use of alkaline medium facilitates the isolation of alkalophilic soil fungi

Abdel-Harfez (2004) isolated halophilic fungi from desert saline soil. Twenty-five genera and sixty-eight species, in addition to one variety of each of *A. Chevalieri, A. Flavus* and *A. nidulans* were isolated from 40 soil sample collected from desert in

Saudi Arabia on 5% sodium chloride-Czapek' agar. The most frequent genera were *Aspergillus* (20 species + 3 varieties) , *Penicilium* (14 species), *Derchsiera* (2 species) and *Ulocladium* (3 species), followed by s*temphylium* (1 species), *Scopulariopsis* (2 species), *Trichoderma* (1 species) *Botryotrichum* (2 species), *Cladosporium* (3 species), *Myrothecium* (1 species) and *Alternaria* (1 species) From these genra *A. amstelodami, A.chevalieri, A. ruger, A ochraceus, P. brevi-compactum. P. cyano-fulvum, P. notatim, D. spicifera, U. consortiale, S. botryosum, S. brevicaulis, T. viride, B. piluliferum, C. herbarum, M. verrucaria* and *A. alternata* were the most common. The results obtained in this investigation reveal that the fungus flora of Saudi Arabian soils is of halophilic nature.

El-Meleigy *et al*. (2010) studied. Two halophilic fungal isolates named *Trichoderma piluliferum* fs. *Halophila* sp. and *Aspergillus restrictus* were isolated from Sharm El-Sheikh andfrom Raas Sader regions at Sinai-Egypt. They tolerates till 30 and 25% (W/V) NaCl (respectively). The former required at least 5% (W/V) NaCl, while the second grew poorly at salt free medium. Morphogenesis and ultrastructure of the two fungal isolates revealed that, 10-20 % NaCl concentration was the best for normal growth and cell ultrastructure and. It is worth to mention that this is the first record for true obligate halophilic fungal isolate (*Trichoderma piluliferum* fs. *Halophila* sp). The two fungal isolates could be used in many biotechnological applications.

Niture and Pant (2011) studied the efficient cell wall-degrading enzymes production of estuarine fungal isolate *Fusarium moniliforme*. The results revealed that. A pH tolerant strain of *Fusarium moniliforme* NCIM1276 with a saprophytic mode of nutrition was isolated from a coastal estuarine environment. Under laboratory condition, the fungus produced significant biomass between pH 3 and 9, and produced cell wall degrading enzymes such as pectinases (polygalacturonase and pectate lyase), carboxymethylcellulase, xylanase and amylase. The production of these enzymes by the isolate in liquid medium, semi-solid medium and in infected tomato and cauliflower plants tissue was investigated. In liquid medium, the production of cell wall-degrading enzymes was induced by appropriate substrates,

whereas the organism secreted all enzymes constitutively on wheat bran. The production of polygalacturonase, pectate lyase, carboxymethycellulase, xylanase and amylase was increased by 3, 2, 11 10 and 4-fold respectively on semi – solid medium containing wheat bran and orange pulp. Moreover when the fungus was allowed to infect tomato and cauliflower plants, the fungus was localized in the cortical tissues of the plants and sereted pectinases, carboxymethylcellulase and xylanase enzymes in the infected host tissue.

Manoch *et al.* (2009) found marine-derived fungi from marine sponges namely *Emericella variecolor, Eurotium cristatum, Curvularia lunata*, *Cladosporium varium* and *Acremonium* sp. They evaluate the biological activity metabolites, the ethyl acetate crude extracts of these fungi to tested against plant pathogenic fungi *In Vitro*. The result show that 10,000 ppm concentration of the crude extract of *Curvularia lunata* effectively inhibited mycelium growth of *Phytophthora palmivora*, *Pythium aphanidermatum*, *Colletotrichum capcisi* and *Sclerotium orlfs:i*. However the crude wxtracts of *Emericella variecolor* and *Acremonium* sp. were found to inhibit the mycelium growth of *Lasiodiplodia theobromae* and *Fusarium oxysporum* respectively. The crude extract of *Cladosporium varium* failed to inhibit all the tested fungi

The soil plate method (Warcup, 1950) yield common fungal species, whereas alcohol and heat treatments enables the recovery of ascomycetes and some rare hyphomycetes (Warcup and Baker, 1963; Nelson *et al*., 1964). A baiting technique (Manoch, 1998; Sudpro; Sudpro, 1999; Busarakum, 2002) is recommended for *Rhizoctonia, Pythium* and *Phytophthora* species. Soil and root washings yield a different mycota, ofter of slower growing fungi and those that do not sporulate readily on agar media. Gochenaur s glucose ammonium nitrate agar, with rose Bengal and streptomycin or 3 % malt extract agar, have been used to isolate soil fungi and heat resistant fungi (Gochenaur, 1964; Luangsa – ard *et al*., 2004; Manoch, 2004), whereas yeast glucose and yeast starch agars are recommended for thermophile and thermotolerant fungi (Manoch, 1993; Cruesrisawath, 1985; Kanjanamaneesathian, 1988).

There are three functional groups of soil fungi as decomposers, mutualists and mycorrhizal fungi (Jenkins, 2005). Decomposing fungi convert dead organic matter in immobilizing and ertain nutrients in the soil. Soil fungi develop mutually benificial relationships with plants by helping the plant to obtain nutrients such as phosphorus from the soil. Furthermore, the important roles of sol fungi are mycorestoration (Stanets, 2005). There fungi secrete enzymes and organic acids that degrade large molecules of dead plants into simpler molecules, which the fungi can reassemble into building blocks, such as polysaccharides, for cell walk. Moreover, fungi recycle carbon, hydrogen, nitrogen, phosphorus and minerals from dead plants into available nutrients for living plants. Mycorestoration is the we of soil fungi to repair or restore the weakened immune systems of environments. The practices of mycorestoration play in determining the balance of biological populations because of involving using soil fungi to filter water as mycofitration, to enact ecoforestry policy as mycoforestry or co-cultivation with food crops (mycogardening), to denature toxic wastes as mycoremediation and to control insect pests including plant pathogens as mycobiopesticide.

Rasul *et al*. (2009) studied immobilization and minealization of nitrogen during microbial use of sugarcane filter cake amended with glucose in saline and alkaline soil. Addition of glucose and ammonium adjust C/N ratio of 12.5 on filter cake decomposition and on the release of inorganic N from microbial residues formed initially Glucose and filter cake amendment increased microbial biomase C and N within 6 the and such an increase persisted. The fungal cell-menbrane component ergosterol initially showed a disproportionate increase in relation to microbial biomass C. Filter cake addition addition in saline and alkaline soil led to an immediate 5-fold increase in cellulase activity. At high salt concentrations hed effectual retardation or complete inhibition of nitrification. The addition of sugarcane filter cake and glucose in combination with NH_4, some filter cake – colonizing fungi in saline soil recovered immediately by rehydration during rewitting.

Organic matter are considered important sources of carbon and energy in soil and water ecosystems (Alef and Nanripieri, 1995). There are lots of soil benificial fungi which are prominent role to decompose organic residues in soil for giving nutrient sources (Pointing and Hyde, 2001). These fungi have closely – relationship to rhizosphere of various kinds of plant in cultivation areas (Sunantapongsak *et al*., 2002.) Furthermore, the fungi are capable of producing enzymes such as lignocellulase, protease, phosphatase and lipase for degradating organic residues in soil including releasing organic acids for solubilization of inorganic compounds as phosphate and potassium which transform to available plant nutrients (Whitelaw *et al*., 1999). Many soil fungi are native inhabitants of estuarine and alkaline soils (Rajankar *et al*., 2007; Karamchand *et al*., 2009). They play an important role in supplementing available nutrients to the plants. Fungi have able greater ability to solubilize or transform insoluble nutrients than the other soil microorganisms.

According to Yunianto *et al*. (2012) endophytic fungi, *Nectria rigidiuscula* was fermented in liquid media for three weeks, extracted using ethyl acetate, partitioned and evaporated to obtain ethyl acetate extract. The result indicated that cytotoxicity assay on MCF-7 breast cancer cells, *N. rigidiuscula* is potential as source of anti-breast compounds by the level 100 ppm of ethyl acetate extract could inhibit the viability of breast cancer cells 91.84% by fermentation. *N. rigidiuscula* an endophytic fungi Srikaya plants (*Annona squamosa*) was expected to have similar extracted metabolites as annonaceous acetogenin which has cytotoxic activity against cancer cells and lower toxicity compared to other cancer drugs.

Many higher plants may contain several endophytic microbes capable of producing secondary metabolites as bioactive natural compounds have promising potential applicability in medicine, agriculture and industry (Joseph and Priya, 2011).

Strobel and Daisy (2003) confirmed that endophytic fungi highly dependent on environmental conditions where the host lives as the different distribution patterns of fungal isolates into different orders between Brazil and Indonesia on one side and China on the other hand.

Hyakumachi (1994) reported that the saprophyte plant growth promoting fungus (PGPF) *Fusarium equiseti*, obtained from turfgrass rhizospheres, could enhance plant growth significantly and suppress several soil-borne disease caused by *Pythium* spp., *Rhizoctonia solani, Sclerotium rolfsii*, *Gaeumannomyces graminis* var. *tritici* and *Cochliobolus sativus*.

Maciá-Vicente *et al*. (2008) and Nitao *et al.* (2001) indicated that *F. equiseti* has been characterized as a natural root endophyte able to colonize plant roots and endowed with properties that could make it a promising candidate for the biological control and root pathogens and nematodes.

Horinouchi *et al*. (2007) reported (PGPF) plant growth promoting fungus; *Fusarium eqiuseti* (Corda) Sacc. was tested in hydroponic rock wool systems as potential biocontrol agent of Fusarium crown and root rot (FCRR) of tomato caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL). PGPF *Fusarium equiseti* proved the most effective organism in controlling FCRR, The numbers of colony-forming units of FORL per gram fresh weight of stems were significantly reduced (P=0.05) in plants treated with *F. equiseti*. Stem extracts from *F. equiseti*-treated and pathogen-challenged plants significantly inhibited the germination and germ tube length of FORL microconidia. Moreover, stem extracts from *F. equiseti*-treated plants (not treated with pathogen) significantly inhibited the germination of FORL microconidia and production of FORL budding-cells

Horinouchi *et al.* (2010) reported that the plant growth promoting fungus (PGPF), *Fusarium equiseti* effectively controlled *Fusarium* wilt of spinach caused by *F. oxysporum* f. sp. *spinaciae* by reducing in disease severity ranzed from 43.5 to 91.8%. *F. equiseti* not only suppressed the disease but also reduced pathogen population in the spinach roots because this fungus might induce the physiological changes in the composition of plant extracts and enhance defence mechanisms that are further triggered when the plant senses a potential pathogen in a phenomenon known as potentiation or conditioning.

Pinruan *et al.* (2010) reported the diversity of basidiomycetous endophytes from the leaves, petioles and rachides of oil palm, *Elaeis guineensis* in Trang Province. There were *Fomitopsis* cf. *meliae, Fomitopsis cf. ostriformis.* cf. *ostriformis, Fomitopsis cf. ostriformis* cf. *pimicola*, *Perenniporia* sp., *Pycnoporus sanguineus, Trametes lactenia* and *Schizophyllum commune*. These fungi produced poroid basidiomes in potato dextrose agar culture, while the remainder had clamp-connections,

Choeyklin (2009) studied the diversity of saprotrophic basidiomycetes from the the oil palm, *Schizophyllum commune* and *Fomitopsis ostreiformis* found as endophytic fungi. Cholyklin (2009) had studied the diversity of endophytic basidiomycetes from the oil palm and found that *Fomitopsis ostreiformis* (Berk.) T. Hatt. was isolated from the oil palm leaves and petioles. *F. astreiformis* had potential use for biological control organisms against the oil palm pathogen, *Ganoderma boninense* by producing bioactive secondary metabolite.

Su *et al.* (2012) reported the freeze-dried powder of liquid culture of *Pseudallescheria boydii* was extractd with ethanol and fractionated on silica gel column. The most active fraction was further purified by silica gel chromatography as 6-6'-bis (2HH-pyran-3-carbalde-hyde) ether which is a new natural product showing strong inhibitory activity against *Alternaria brassicicola*, black leaf spot of cabbage. The new antibiotic, a yellow oil with a molecular weight of 234, was named pseudallin which which had effect competitive saprophytic colonization of plant debris by *P. boydii* in soil. At 1000 ppm of extract antibiotic fractions inhibited germination of *A. brassicicola* conidia completely.

The new antibiotic pseudallin is a 6-6'-bis (2*H-pyran*-3-carbaldehyde) ether which is extracted from liquid culture of *Pseudallescheria boydii* and very effective in controlling black leaf spot of cabbage caused by *A. brassicicola* in the greenhouse, its potential of being developed into a commercial product and safely as a plant disease control agent (Ko *et al.*, 2010).

Michael *et al*. (2011) stated that there were limited researches dealing with the endophytic fungi in *Acacia* spp. Most of these have been on native Southern African *Acacia* species (Van der Linde *et al*., 2010). However, various die-back and stress-related diseases are caused by fungi that are knowna be latent pathogens that live as asymptomatic endophytes in healthy tissue. The fact that large numbers in healthy tissue. The fact that large numbers of fungi live within the tissues of healthy, asymptomatic trees was recognized only relatively recently. Some fungi produce compounds toxic to defoliating insect pests as has for instance been shown for some grassed. Some of the most-impartant stress related pathogens are members of the Botryosphaeraiceae, a well-known group of plant endophytes and Botryosphaeria canker and die-back can result in very severe diselase on Austratian *Acacia* species in plantations.

Basidiomycete endophytic fungi are asymptomal fungal infletions within tissues of healthy plants and can be dormant saprotrophs, latent pathogens as well as mutualistic symbionts. (Stone *et al*., 2000) The endophytic fungus as *Schizophyllum commune* from oil palm is a common wood inhabiting basidiomycete with a world wide distribution and grown commercially as an edible mushroom (Vikinesway *et al*,. 2007). *S. commune* was also isolated as a saprophyte and endophyte from a mangrove tree. More endophytic fungi were isolated from leaves than petioles and rachides, and this may be due to nutritional requirements, or the ability of the fungi to utilize different substrates. Most basidiomycete endophyte may become saprophytes when host plants senescence. *Fomitopsis spp.* as *F. melial, F. ostreiformis, F palustris* and *F. pinicola* were not found as saprobes of palms (Wilson, 2000).

Tran *et al.* (2010) reported endophytic fungi exist within the tissues of living plants which had closely the relationship between the plants and its endosphytes were symbiotic whereby the fungi colonized the internal tissues of the *Acacia* trees without any adverse effects in endophytes and their origins, their biodiversity, endophyte-host interactions, their role secondary metabolites. The fungal endophytes were readily isolated from the phyllodes of *Acacia* species as Austration native plants and fungi exhibit promising bioactive properties which may be on useful source of novel

bioactive compound. Endophytic fungi colonised in the tissue of the phyllodues of *Acacia baileyana, A. podalysiifolia and A. floribunda.* There were classified as *Aureobasidium, Chaetomium* and Sordariomycetes through genetic analysis of ribosomal RNA genes. These fungi was examined in bioactivity and could exhibited antibacterial also produced amylase activity and were thus able to hydrolyse starch.

The genus *Acacia* comprises over 1300 species of which nearly 1000 are found in Australia. The majority of Australian *Acacia* produces "leaves" or phyllodes of great variety. These phyllodes are not really leaves but are flattened leaf stalks, allowing the tree to survive stressful environments. The common name of genus *Acacia* is Wattle which is Family Fabaceae, Subfamily Mimosoideae. For the distribution and accurrence of *Acacia* species are widespread and abundant especially in arid and semi-arid areas. Most species are used widely as food (e.g. seeds are ground into flour and the gum is edible) and the wood has been traditionally made into clubs, spears, boomerangs and shields. Various species are used as medicines for narcotics and painkillers, to treat headaches, could and fevers, as antiseptics and bactericides and treat skin disorders by the indigenous people of Australia. In semi-arid areas of West Africa, *Acacia* is a large tree which is commonly used. The use of *Acacia* in the field and pasture lands for agroforestry systems in dry areas. Leaves of *Acacia* provide shade for cattle in the dry season, and fall at the beginning of the rainy season. This pattern prevents competition with the main crop for light water and nutrients.

Acacia plantation in agroforestry systems increases soil fertility by providing organic matter from falling senescence leaves, nitrogen fixation, a nutrient pump in its extensive root system and giving shade for cattle in the dry season. Furthermore, *Acacia* also provides feed for cattle in the form of fruits, leaves and young shoots. *Acacia* is leguminous plant and fast-growing tree which can be used as living fences and there is no problem in competition for water between the main crop and the shrubs (Schöll *et al*., 2004)

The abundant volume of fallen leaves from *Acacia* plantation, the soil is enhanced with the addition of organic fertilizer, which can remain on the ground to function as mulch and nitrogen-fixers also provide an extra amount of nitrogen. Organic matter from *Acacia* fallen leaves has a great capacity to retain nutrients and thus increases the cation exchange capacity in the soil, absorbing a lot of water in dry periods more water is available for the plants for a longer time and contributes soil aggregate formation which improve the soil structure especially for both sandy and clay soils becomes of having poor structure. Organic matter can bind hydrogen ion and thus prevent soils from becoming acidic to increase pH level value in soil and finally the growth and activities of soil microorganisms are stimulated which helps make the nutrients in the organic matter available to the plants (Schöll *et al*., 2004)

According to Rungjindamai *et al*. (2008) fungal endophytes were isolated from healthy leaves, rachis and petioles of the oil palm *Elaeis guineensis* in a Thai plantation. Many of these morphotypes were shown to be basidiomycetes as clamp connections were present and some produced basidia and basidiospores in culture. Based on ITS sequence analysis the two Agaricales strains grouped with *Schizophyllum* species and showed a close relationship with *S. commune*. The Largest fungal assemblage was within the Fomitopsidaceae, four endophytic isolates clustered with *Fomitopsis* species (*F. ostreiformis*, *F. palustris*), two and three isolates grouped with *F. pinicola* and *F. meliae*, respectively.

Currie and Hiratsuka (1996); Moltzan and Blenis (1999) reported biological control of western gall rust (WGR) on Ponderosa pine by using mycoparasite *Scytalidium uredinicola*. *S. uredinicola* is a destructive hyperparasite of WGR, which reduces the inoculums potential. This mycoparasite breaks down spore and the active rust sori of WGR, making infected area appear clumpy and stringy. Infected sori are a yellowish-green to whitish-gray in appearance, depending on the stage of infection. Hyphae of the mycoparasite are able to penetrate the wood tissue of the galls and destroy rust hyphae ot a depth of 300 μm below the sori.

Meyer *et al*. (1990) studied antagonistic bioassays were conducted on eggs from nematodes of soybean. The results reveal that *Phoma chrysantremicola* and *Verticillium ohlamydosporium* caused a decrease in the number of viable eggs, although no hyphae were observed colonizing live eggs. Heterodera glycines Ichinohe, the soybean – producing countries.

Fowler *et al.* (1999) repored utilization of endophytic fungi as *Epicoccum*, *Scytalidium* and *Ulocladium* isolates for antagonistic effecting to *Botrytis cinerea*, *B. cinerea* Pers, the fungal pathogen causing *Botrytis* root, frequenthy causes bunch rot significant losses in grape crops. The results showed that all fungal isolate tested reduced significanthy the development of *B. cinerea* conidiophores on inoculated grape rachii. These fungi had effected suppressing this plant pathogen by producing of antifiumgal compounds. In order for potential antagonists to reduce the level of overwintering *B. cinerea* inoculums on rachii, it is essential that they have the ability to survive and/or grown as well as or better than the pathogen in the winter temperature and moisture conditions. For the temperatures 10-20°C, conidisphore production of *B. cinerea* were greatest suppressed after treated rachii with three fungi.

According to John *et al*. (2010) *Trichoderma viride* was highly effective biocontrol agent against two fungal pathogens, *Fusarium oxysporum* f. sp. *adzuki* and *Pythium arrhenomanes*, infecting soybean. These pathogens heavly infect soybean and thus, influence growth from germination to all stages of plant development which are a main obstacle to obtain a high yield of soya during commercial cultivation. Application of chemical fungicide has been replaced by biocontrol agents because of the emergence of fungicide - resistance strains and public concerns regarding the health and environmental impacts of these chemicals. The results indicated that microscopic observation of dual cultured plates showed different interactions to inhibit pathogens growth such as coiling of *Trichoderma* around the pathogens. In mycoparasitic interactions, a diffusible factor released from the host before physical contact was responsible for induction of hydrolytic enzymes. And lectins in the host is cell wall caninduce *Trichoderma* coiling for producing appressorium – like structures. After penetrating with appressarium – like structures, *Trichoderma* hyphae grew

within the pathogen and the pathogen nuycelia was gdgraded. After 10 days of sowing soybean seeds, *Trichoderma* applied soil achieved the highest percentage of seed germination between 90-100 % whereas *Fusarium* applied higly aftected seed germination as 35% of seeds were germinated. After 6 weeks, control plants and *Trichoderma* treated plants were having 18 root nodules whereas the pathogen – infected plants were having fewer root nodules and smaller in size including the poor density of the root system. In conditions of control plants and *Trichoderma* applied plants had many big and pink colored nodules. Furthermore, the secondary metabolites such as auxin like compounds or auxin inducing substances by *Trichoderma* had affected the improved plant growth. The total chlorophyll pigent was lowest in pathogens infected plants. In generally, *Trichoderma* spp. Colonize plant roots and invade only in the surface layers of the root, funther penetration con be controlled by the plant defence reactions (Harman *et al*., 2004)

Alam *et al*. (2010) found that a novel eucalyptus root colonizing fungus, *Penicillium* sp. EU 0013 was applied to potting mix resulted in 78 and 74 % reduction of the diseases caused by *Fusarium exysporiu* in tomato and cabbage, respectively. Diseases severity and percent disease reduction rariec significantly ($P<0.05$) with the concentration of EU 0013. *Penicillum* sp. recovery from roots of tomato and cabbage varied 39 to 81% and 36 to 79% respectively.

Henrigues *et al.* (2009) reported a lot of genera fungi as *Acremonium, Aspergillus, Beauveria, Fusarium, Gliocladium, Scytalidium* and *Trichoderma* which have the symbiosis – cork oak interaction. These fungi contribute beetle population establishment and tree weakness. *Scytalidium* might act in the fungi colonies momagement within the galleries as following *P. cylindrus* host colonization process, symbiotic fungi may start to act as wood degrading, thus facilitating galleries excavation.

Morsy *et al.* (2009) reported that *Trichoderma viride* and *Bacillus subtilis* were most feasible biocontrol microorganisms suppress several pathogens like *Fusarium* In the field experiment, these antagonistic treated plant favoured greater

proliferation of rhizosphere microflora and higher dehydrogenase activity in the rhizosphere. Moreeover, the dual inoculation gave the highest records of growth patameters, fruit yields and plant nutrent content than individual one. Both of *T. viride* and *B. subtilis* strains reduced groth percentage of *F. solani* by 57.8 and 34.4 % respectively, comparing with the control. These entagonistic secrete hydrolytic enzymes or antifungal metabolites. *T. viride* secreted chitinase and ß 1, 3 glucanase in supernatants and *B. subtilis* secreted several antifungal metabolites such as subtilin, bacitracin, bacilli and bacillomycin which had on and inhibitory effect on fungal pathogens (Montealegre *et al*., 2005; Alippi and Monaco, 1994) Inoculaion with *T. viride* and *B. subtilis* significanthy increased survival rate compared with the *F. solani* infested soil from 35 to ranging between 73-70% respectively. Application of combination of these antagonistic had significanthy decreased disease severity in comparison with the dividual ones. The mechanism of *Trichoderma* and *Bacillus* action on pathogens attacked and binded the pathogenic organisms by sugar linkage and beginning to secrete extracellular enzyme as protease and lipase (Cal *et al.*, 2004). *Moreeaver*, these antagonistic produced phytohormones, vitamins and solubilizing mineral besides which promoted the tomato growth and fruit gield (Morsy, 2005).

In Thailand, there are two important diseases of durian; durian root and stem rot caused by *Phytophthora palmivora* Butler (Drenth and Guest, 2004) and durian leaf blight caused by *Rhizoctonia solani* Kuhn (Cuambot and Tangonan, 2001).

The scientific name of durian is *Durio zibethinus* L. and as a member of the plant order Malvales, family Malvaceae and tribe Durioneae (Salakpetch, 2009). Many people in Southeast Asia regarded durian to “The King of Fruits”. There are hundreds of durian cultivars. Most consumers express preferences for specific cultivars, which fetch higher prices in the market. The popular cultivars of durian that are favorite among the growers and consumers alike are four types : Monthong, Kanyao, Chanee and Kradumthong.

In 2013 of June, The Orchard Development Center of Royal Project, Amphor Thamai, Chanthaburi Province had the seriously devastation of durian diseases

especially *Phytophthora* root and stem rot as well as *Rhizoctonia* leaf blight. There were 72 durian plants which were attacked by the two fungal pathogens. The most important environmental factor influencing *Phytophthora*-related root and stem disease of durian and also *Rhizoctonia* leaf blight of was probably due to the water saturation in soil with low fertility (Duniway, 1979).

There were noxious weeds in durian plantation areas which cauld be the source of pathogens host ranges of both of durian foot rot and leaf blight pathogens. Soil samples were collected from under durian canopy which closed to the rotten foot stem of durian. The samples of rotten bark tissues from the tree and leaf blight infected leaves were also collected. *Phytopthora palmivora* and *Rhizoctonia solani* were isolated from these samples on potato dextrose agar (PDA).

1). *Phytophthora palmivora* Butler (Root, stem and fruit rot disease of durian)

This fungal pathogen is in Order Peronosporales and Family Pythiaceae. *Phytophthora* is soil-borne pathogen (Vawdrey, 2001) which can survive, either as a saprophyte or as dormant spores. The water saturated soil condition is known to favour the rapid formation of sporangia and infectious zoospores (Duniway, 1979). Generally, mycelium and zoospores of this fungus survive for only a few weeks, while chlamydospores may survive for 6 years, and oospores for 13 years (Erwin and Ribeiro, 1996).

The root and stem rot symptoms are major durian disease. The disease attacks the root system and rotting durian roots. Finally, the rotten roots become brown in color and if rot is serious, the durian leaves which are at the tip of branches become yellow and stop their development and fall down. The durian leaves at the base of the branch fall down later than the leaves at the end of the branch. Furthermore, a clear symptom is the development of a juicy spot at the bark tissue with liquid substance or water run off at the foot stem of the durian tree. When using a knife to cut this wound, liquid substance will gush out of it. The bark tissue of durian including the core of the trunk change to be a dark brown color. If trunk change to be

a dark brown color. If this symptom spreads around the entire foot of the tree, all leaves will fall down and eventually the tree will die. If the disease infects the durian leaves, there is brown spot appearance and the disease will spread into the frecit. Brown spots are usually visible on the rotting fruit and this brown spot will expand and eventually the rotted fruit will fall down. This symptom is often found at about a month before harvesting. The mature fruit having brown spot will split and other fungi can infect and destroy the fruit.

The role of environmental factors such as humidity, rain and drainage affect disease infection to durian tree. The soil characteristics with poor drainage are favorable for *Phytophthora* to produce sporangia which rapidly liberate infectious zoospores that can colonize durian roots (Duniway, 1979). Moreover, temperatures between 15 and 30^{o}C, relative humidities of 80 to 100% and high rainfall provide conditions conductive for disease development (Ahmad kanmil *et at*. 2004).

2). *Rhizoctonia salani* Kuhn (Leaf blight disease of durian)

This soil-borne fungal pathogen is in Order Cartharellales and Family Ceratobasidiaceae (Ogoshi, 1987). The fungus produces asexually and exists primarily as vegetative mycelium and then develops the typical brown sclerotia (1-2 mm) within 3 weeks in the culture (Cuambot and Tangonan, 2001). The characteristic right-angled mycelia measure 38.83<8.23 μm. The hyphae often branch at a 90^{o} angles. *R. solani* can survive for many years by producing sclerotia in soil and on plant tissue.

The leaf blight symptom of durian is characterized by the browning of the leaf along the tip and margin and progressing until the whole leaf become blighted. During the rainy season, leaf blight disease of durian often occurs on 3 to 5 year-old durian (Thuan *et al*. 2008). This fungus causes severe leaf web blight of durian from seedlings to mature plants because of heavy rainy southern part of Thailand (Preecha, 2011). The symptoms of durian leaf appear as large, pale brown, blighted lesions with

an irregular border. In highly humid condition, *Rhizotonia* yellowish white hypha appear on the lesions and affected durian leaves turn dark brown, wilt and fall down.

Phytophthora and Rhizoctonia are serious pathogens of durian that have the ability to attack the plant at various stages of its life cycle such as roots, stems, fruits and leaves of seedlings, young trees and mature trees (Guest *et al*. 2004; Prasun and Kenerley, 2010). The principles of *Phytophthora* and *Rhizoctonia* diseases of durian management practices available under the following; cultural practices, resistance breeding, biological control, fungicides and phosphorate (Drenth and Guest, 2004; Preecha, 2001).

For the biological control management there are several microbial biocontrol agents for suppressing two fungal pathogens such as *Gliocladium virens*, *Trichoderma virens*, *Trichoderma harzianum, Pseudomonas putida, Burkholderia glidioli* and *Bacillus polymyxa* (Shari, 1999; Aryantha and Guest, 2006; Mpika *et al*. 2009). *T. virens* used as biological control agents which rapidly grows and increasing biomass multiplication. *T. virens* produces two types of spore as conidia and chlamydospore. This fungus grows very well, especially producing conidia and vegetative mycelium. There is conidiophore which origin produces phialide for proliferating mass or cluster of conidia.

Application of *Trichoderma* bioagents (*T. harzianum* and *T. viride*) in vermicompost carrier (farmyard manure-coffee husk) under field condition was most effective in reducing rhizome rot and azhukal diseases of cardamom up to 52 to 69 percent (Vijayan *et al.* 2008). Solarization of FYM-Coffee husk based system was employed for biomass multiplication and sporulation of *Trichoderma* which produced maximum number of colony forming units 10^8/g.

For biomass multiplication of *Trichoderma viride* through solid state fermentation is an appropriate technology for high efficient quantity of spore biomass. Substrates of sugarcane bagasse were cheaper carrier and containing much more amount nutrition for growth of fungal agent *T.viride* under siolid state fermentation

(Chaudhari *et al*. 2001). The maximum spore production in sugarcane bagasse substrate fermentation was 10^9 CFU/g of substrate.

Application of 15 g/m^2 *Trichoderma harzianum* in lettuce seedling was significanthy increased compared to the control and effective in eliciting increased growth response only in one of the seedling characteristics in grasshouse condition (Bal and Altintas, 2008). The growth promotion effect in the presence of *T. harzianum* in the rhizosphere zone was due to increased root surface area allowing the roots to explore larger volumes of soil which had shown that micronutrients and insoluble phosphates became soluble and available for the plant growth.

The cell-free culture filtrates of bio-control agents, *Trichoderma virens* and *Trichoderma viride* inhibited completely infection in green house grown potato plants by *Phytophthaora infestans* causing late blight of potato. *T. viride* showed 45% of the plant infection (Chanderkala *et al*. 2012). The antagonist, *T. virens* had the potential of preventing or inhibiting the germination of *Phytophthora infestans* sporangia and also preventing the infection from *P. infestans.*

Several species of *Trichoderma* promoted growth and development of seedlings of vegetable and non-vegetable crops (Bal and Altintas, 2008) and induced defense responses and systemic resistance in addition to control of plant pathogens (Alfano *et al.*, 2007). *Trichoderma harzianum* is used as a successful biological control agent to control different soil-borne plant pathogen induding *Pythium* spp., *Rhizoctonia solani*, *Fusarium* spp., *Sclerotium rolfsii*, *Phytophthora* spp. etc (Harman *et al*., 2004).

Haque *et al*. (2012) studied the effect of *Trichoderma harzianum* supplemention with chemical fertilizer as biofertilizer for the growth and yield of mustard and tomato. The results revealed that three *Trichoderma* - enriched biofertitizers when supplementedwith N fertilizer significantly boosted up the growth and yield of mustard and tomato. Application rate of 50% N fertilizer along with 50% *Trichoderma* - enriched biofertilizers augmented 108 and 203% in mustard and

tomato, respectively. Utilization of *Trichoderma*-enriched biofertilizer could save at least 50% N fertilizer uses for mustard and tomato. Application of *Trichoderma* increased root dry weight of tomato and mustard which could increase the chance for acquisition of nutrients by exploitation of more volume of soils.

Hernández *et al*. (2014) studied the efficacy of biological control agents including *Trichoderma virens*, *T. longibrachiatum*, *T. asperellum* and *T. gamsii* against *Phytophthora parasitica* in roselle plants under field and greenhouse conditions. In the field condition, *T. longibrachiatum* treatment had 15% less incident of black shank and effected highest calyx dry weight. *T. virens* and *T. gamsii* increased height, fresh and dry weight of roselle plants infected with *F. oxysporum*. Temperature and relative humidity are factors for *Trichoderma* development. Chlamydospore germination of *Trichoderma* was appeared very well under optimal conditions of humidity more than 75% and temperature between 28-30ºC. Furthermore, nutrient source from organic matter is essential for the growth of filamentous *Trichoderma*. *Trichoderma* could adapt and suruive in extreme conditions of temperature, pH and salinity. Roselle plants treated with *T. virens*

Saran-Sunder *et al*. (2013) indicated that *Trichoderma* antagonistic spectrum is broad and con affect the growth of several plant pathogens, such as *Fusarium solani, Macrophomina phaseolina, Pythium aphanidermatum* and *Alternaria solani*.

Mukherjee *et al*. (2013) mentioned that *Trichoderma* antagonists had their different control mechomisms which consisted of mycoparasitism, antibiosis, competition for space and nutrients and induction of resistance.

Okoth *et al*. (2011) reported that *Trichoderma harzianum* combined with triple superphosphate and calcium ammonium nitrate increased seed germination of beans and corn including also increasing stem diameter, roots and shoots growth. Maize seeds coated with *Trichoderma* inoculums and planted on soils without fertilizer addition recorded the highest germination rate of 82.7% followed by seeds coated with the inoculum and planted in soils treated with manure (82.2%).

Azarmi *et al*. (2011) reported tomato seedling height, crown diameter, shoot fresh and dry weight, and root fresh and dry weight, as well as leaf number and total area of leaves were increased signific antly by applying *T. harzianum*. The concentration of Ca^{+2}, Mg^{+2}, P, Na^{+} and K^{+} increased in the shoot and root of tomato following the application of *T.harzianum* in the soil. Chlorophyll content in tomato seedling grown in *Trichoderma* sp. amended soil as well as in *Trichoderma* sp. and *T. harzianum* coated seed and increased leaf number and leaf area. *Trichoderma* soil amended treatment when compared to the control significantly reduced the Na^{+} concentration.

Ageeb and Mohamed (2012) revealed that the application of *Trichoderma harzianum* effected increasing in all measured parameters including growth parameters, chlorophyll content, starch content, nucleic acid content, total protein content and phytohormones content of maize plantation. *T.harzianum* secrete higher amounts of cellulases which is the most prominant emzyme system for the completely hydrolysis of cellulosic substrates and transforming monomeric glucose. *Trichoderma* application had important economical implications such as shortening the plant growth period and time including induced lignifications reduction for benificial efficient in enhancing fresh state of maize stalks.

Badar and Qureshi (2012) concluded that application of *Trichoderma hamatum* alone and in combination with rhizobial isolates found effective not only increasing the growth of sunflower plant by improving its root-shoot length and biomass but also increasing the organic and inorganic content of same plant. *T. hamatum* had affected to increase or improve mineral uptake by releasing available nutrients from soil and organic matter. This fungus increased chlorophyll content, carbohydrate and crude protein contents of experimental sunflower at 30^{th}-60^{th} day as compare to control. Moreover, statistically significant increase in nitrogen and phosphorus percentage content in plant was obtained after 30^{th} day of sunflower plantation.

MATERIALS AND METHODS

Materials

1. Isolation and identification of halophilic fungi

1.1 Materials for collected soil samples

1.1.1 shovel
1.1.2 permanent marker
1.1.3 plastic bags
1.1.4 rubber hand
1.1.5 camera notebook

1.2 Laboratory materials

1.2.1 permanent marker
1.2.1 forceps
1.2.3 fine needles
1.2.4 petri-dishes
1.2.5 test tubes
1.2.6 beakers
1.2.7 electric scale
1.2.8 hot air oven
1.2.9 autoclave
1.2.10 alcohol lamp
1.2.11 70% and 95% ethyl alcohol
1.2.12 slides and cover slips
1.2.13 distilled water
1.2.14 lactophenol
1.2.15 stereo microscope (SZ-PT Olympus)
1.2.16 light microscope (Carl Zeiss Scope A.J, BH-2 Olympus)

1.2.17 camera lucida

1.2.18 Scanning Electron Microscope (JEOL JSM 6400)

1.2.19 thermometer

1.2.20 water bath

1.2.21 oil emersion

1.2.22 cylinder

1.2.23 spatula

2. Preservation of halophilic fungi

2.1 sterilized soil

2.2 sterilized filter paper Whatman No.1

2.3 liquid paraffin

2.4 aluminium foil

2.5 paper bags

2.6 plastic bags

2.7 vial, size 1 dram.

2.8 petri-dishes

2.9 forceps

2.10 dessicater, electric dry cabined (WEIFO)

3 Antagonistic and enzyme activities test of halophilic fungi

3.1 pure culture of halophilic fungi and plant pathogenic fungi

3.2 petri-dishes

3.3 cock borer

3.4 fine needles

3.5 permanent marker

3.6 forceps

3.7 test tubes

3.8 beakers

3.9 erlenmeyer flask 50, 100, and 250 ml.

3.10 paper filtrate whatman No. 1

3.11 rotary evaporator

3.12 aluminum foil

3.13 tank chamber

3.14 agar media

3.15 electric scale

3.16 hot air oven

3.17 autoclave

3.18 alcohol lamp

3.19 ruler

3.20 cotton

3.21 distilled water

3.22 15% and 95% ethyl alcohol

3.23 incubator shaker

3.24 centrifuge

3.25 hot water bath

3.26 pipettes 1 ml and pipette tips

4. Media (Appendix)

4.1 Glucose Ammonium Nitrate Agar (GAN)

4.2 Potato Dextrose Agar (PDA)

4.3 Carboxymethyl Cellulose Agar (CA)

4.4 Skim Milk Agar (SMA)

4.5 Calcium Phosphate Agar (CPA)

4.6 Tributyrin Agar (TA)

4.7 Cellulose Liquid Media (CLM)

4.8 Skim milk Liquid Media (SMLM)

4.9 Calcium Phosphate Liquid Media (CPLM)

4.10 Tributyrin Liquid Media (TLM)

Methods

1. Fungal Isolation

Alkaliphilic fungi were isolated from moderately and highly saline soil at Amphoe Kham Thale Sor, Nakhonratchasima province and Amphoe Ban Pai, Khon Kaen province. Soil fungi were isolated using the soil dilution plate method on Gochenaur ' glucose ammonium nitrate agar, potato dextrose agar (PDA), carboxymethyl cellulose agar (CMA), skim milk agar (SMA) calciumphosphate agar (CPA) and tributyrin agar (TA). Concentration of 10% sodiumchloride was added in each culture media and incubated at room temperature (30 ^{0}C) for 7 days and then subculture onto fresh culture media for pure culture and identification.

The leaves or phyllodes of *Acacia appliceps* (Fabaceae) were collected from the areas of highly saline soil, Amphoe Kham Thale Sor, Nakhon Ratchasima province, Thailand, GPS 47 P 0815023 1664647 (Figure 1). Plant samples were placed in plastic bags, kept in ice box and brought to the laboratory. The surface sterilize method was employed to isolate endophytic fungi (Strobel, 2006 ; Li *et al*., 2005; Radu and Chen, 2002). A random sample from each plant consisting of an asymptomatic leaf was taken. Leaf portions were thoroughtly washed in sterile distilled tap water after which they were surface sterilized by submerging in 70% ethanol for 2 min. After drying, each leaf was divided into four segments and placed on 10% NaCl potato dextrose agar (PDA) supplemented with 50 mg L^{-1} streptomycin to suppress bacterial growth. All the plates were incubated at room temperature for 3-4 weeks/ Emerging fungi were transferred to fresh potato dextrose agar (PDA) plates, incubated for week, and periodically checked for purity. After isolation, the samples were kept in the herbarium at Kasetsart University.

The saline soil samples including organic residues of *Acacia appliceps*, *Fimbristylis schoenoides* and *Pluchea indica* (Figures 2) were collected from the areas of highly saline soil (pH 8.7-9.6), Amphoe Kham Thale Sor, Nakhon Ratchasima Province, Thailand, GPS 47 P 0814784 1664672 and 47P0813822

Figure 1 Wattle (*Acacia ampliceps*) or *Acacia* plantation are useful in the salinity areas especially in the paddy field ; (A) *Acacia* tree, (B) *Acacia* leaves and flower and (C) *Acacia* leaves and pods (arrow mark a and b).

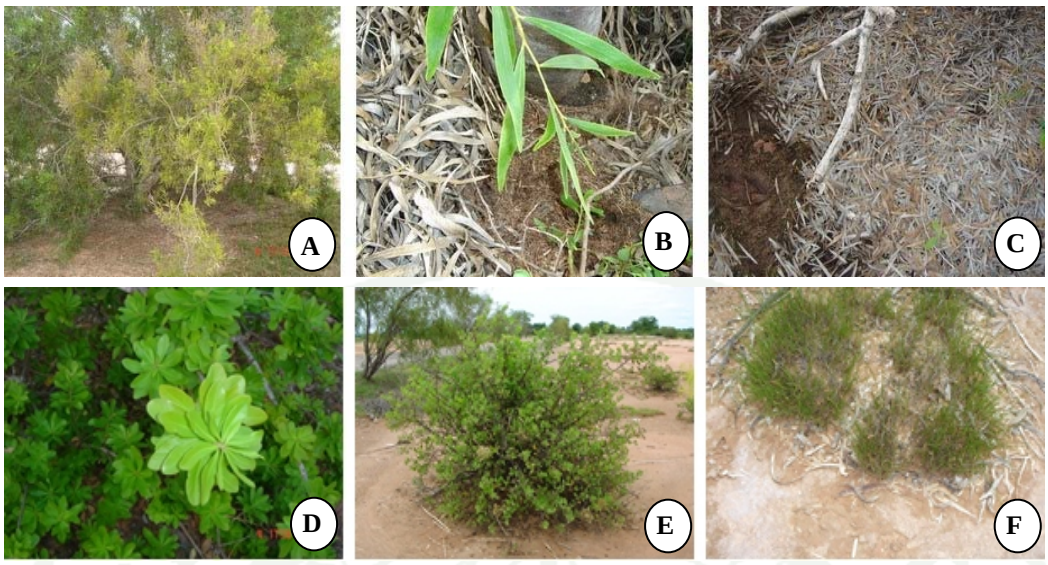


Figure 2 *Acacia* plantation contribute to increasing soil fertility (A-C) by providing lots of organic matter from falling senescence *Acacia* leaves. *Buchanania* (D), *Pluchea* (E) and *Fimbristylis* (F) trees have ability grow on highly saline soil.

1663637. The alkaliphilic fungus was isolated using the soil dilution plate method on 10% sodiumchloride, Gochenaur' glucose ammonium nitrate (GAN), potato dextrose agar (PDA), carboxymethyl cellulose agar (CMA), calciumphosphate agar (CPA) and yeast tributyrin agar (TA) supplemented with 50 mg L-1 streptomycin to suppress bacterial growth (Manoch, 2004 and Gochenaur, 1964). Concentration of 10% sodiumchloride was added in each culture media and incubate at room temperature (28°C) for 7 days and then subculture onto fresh culture media for pure culture and identification. Emerging fungi were transferred to fresh potato dextrose agar (PDA) plates, incubated for a week, and periodically checked for purity. After isolation, the samples were kept in the herbarium at Kasetsart University.

2. Identification of Halophilic Fungi

2.1 Macroscopic examination

Morphological characteristic of colonies were determined such as growth pattern, color, texture on different media, including CZA, CYA and MEA for 7 to 14 days, at 28˚C. Diameters of colonies were measured in millimeters, most effectively by transmitted light and from the reverse side.

Colony characteristics were examined under a stereo microscope and naked eyes. The microscope was used for assessing texture of colonies and the appearance of anamorph and conidial heads.

2.2 Microscopic examination

Microscopic characteristics were examined on a slide preparation using sterile distilled water and lactophenol as mounting media and examined under a light microscope (Carl Zeiss Scope.A.1). Camera lucida drawings were made. Photomicrographs of fungal structure were taken under stereo, light and scanning electron microscopes.

Study on ornamentation of ascospore was conducted using Scanning Electron Microscopy. Matured ascomata and ascospores of *Neosartorya* from dry culture agar media were transferred with a fine needle and placed onto double-stick transparent adhesive tape on aluminium stubs. The specimens were coated with gold for 5-7 min. and examined in a JEOL JSM 6400 scanning electron microscope (Manoch, 2004)

Identification was based on examination of the morphological characteristics under stereo, light and scanning electron microscopes. *Neosartorya* were identified following the research done in previous reports (Raper and Fennell,

1965; Hong *et al*., 2006, 2008; Horie *et al*., 2003; Peterson, 1992; Samson *et al*., 2007; Yaguchi *et al*., 2010).

The measuring and drawing of the macroscopic and microscopic characters were made under the light microscope using a drawing tube (Olympus CH) from 4x to 100x and stereomicroscope (Olympus SZ) equipped with eyepiece of 4x to 100x. Photographs of the halophilic fungi under the stereomicroscope were taken by using Nikon, P6000. Detail characters of reproductive structure under the light microscope (Olympus CH) were recorded by using Nikon (P6000) at the Department of Plant Pathology Kasetsart University.

3. Antagonistic Tests *in vitro*

The halophilic fungi were tested for antagonistic activity against 8 species of plant pathogenic fungi including *Alternaria brassicicola, Curvularia lunata, Colletotrichum capsici, Helminthosporium oryzae, Phytophthora palmivora, Pythium aphanidermatum, Rhizoctonia solani* and *Sclerotium rolfsii* (Figure 1). Young mycelium from the colony margin of the test fungi and the plant pathogens were cultivated at 28 ◦C, excised with a sterile cork borer (0.8 cm diam.), and placed on PDA 6 cm apart. All Petri-dishes were incubated at room temperature (28◦C) for 14 days. The inhibition levels were calculated by using the formula: G_1-G_2 / G1 x 100 where G_1 = colony radius of plant pathogenic fungus in the control and G_2 = colony radius of plant pathogenic fungus in the dual culture test (Intana *et at*., 2003). Each treatment was performed with three replicates.

Table 1 Species of plant pathogenic fungi from various disease fruits and vegetables used for antagonistic test

Plant pathogenic fungi	Host plant	Diseases
Alternaria brassicicola	*Brassica albograbra* (Chinese kale)	Leaf spot
Colletotrichum capsici	*Capsicum frutescens* (Chilli)	Anthracnose
Curvularia oryzae	*Oryza sativa* (Rice)	Leaf spot
Helminthosporium	*Oryza sativa* (Rice)	Leaf blight
Phytophthora palmivora	*Durio zibethinus* (Durian)	Foot and Root rot
Pythium aphanidermatum	*Brassica albograbra* (Chinese kale)	Damping-off
Rhizoctonia solani	*Durio zibethinus* (Durian)	Leaf blight
Sclerotium rolfsii	*Solanum tuberosum* (Potato)	Southern blight

4. Protein, Lipid, and Phosphate Transformation on Selective Solid Media

For the mineral transformation test, young mycelium of each the halophilic fungus was cut from the colony margin with sterile pasture pipette and placed in the center of a Petri-dish with either skim milk agar (SMA), calcium phosphate agar (CPA) or tributyrin agar (TA). The clear zone appearance around each halophilic fungus on each selective medium was recorded after cultivation for 14 days at 28°C.

5. Enzyme Actitity Test

The halophilic fungi were assessed for their enzymatic ability to degrade cellulase protease, phosphatase and lipase by growing on the substrates of filter paper, tyrosine, calcium phosphate and paranitrophenol, respectively (Alef and Nanripieri, 1995)

5.1 Cellulase assay:

At the beginning and every 3 days of incubation, the enzyme was extracted from the sample. For preparation of enzyme solution, 1 ml of dlude extract is suspended in 10 ml of distilled water and stood at room temperature for 30 minutes. Then solution samples were centrifuged at 15,000 rpm for 20 minutes and taking the supernatant was used as an enzyme solution. Cellulase activity was determined by the method described by Mandels and Sternberg (1976).

5.5.1 Filter paper degrading activity was measured by mixing 50 mg strip (1 x 6 cm) of whatman filter paper (No.1) with 0.5 ml of enzyme and 0.5 ml of 0.02 M sodium phosphate buffer (pH 6.0) incubated at 50 $^{\circ}$C for 1 hr. and reducing sugar was determined by reaction with 3 ml of dinitrosalicylic acid. The reducing sugar reduced the nitro of 3, 5 dinitrosalicylic acid to amino, thereby generating a reddish brown color for amino compounds which detected the absorbance by measuring with the spectrophotometer at wavelength 500 nm (nanometer). The enzyme activity was represented at the amount of reducing sugar released per minute pre g dry weight of material for filter paper.

5.5.2 Calibration for standard curve of filter paper activity : - Taking 0, 200, 400, 800 and 1,000 mg of glucose, then 3 ml of dinitrosalicylic acid was added in each concentration and boiling in the water bath at 10 minutes. After that, they were diluted with 20 ml of distillated water and measured at the wavelength 550 nm by spectrophotometer.

5.5.3 Preparation of dinitrosalicylic acid reagent :- Solution A as taking 7 g of phenol was dissolved in 15 ml of 10% NaOH solution and then addition of 70 ml distilled water. Preparing solution B by mixing 6.9 g sodium bicarbonate, 8.8 g dinitrosalicylic acid, 255 g sodium potassium tartate and 13.5 g NaOH were dissolved in 1180 ml distilled water. After that solution A and B were mixed and keeping prevention light condition.

5.2 Protease assay :

Enzymatic assay of protease activity was determined one unit hydrolysed casein to produce color equivarent to 1.0 μ mole of tyrosine per minute at pH 7.5 and 30 ºC. Generating color from enzymatic hydrolysis of casein with Folin &Ciocalteau's reagent was detected the absorbance by the spectrophotometer at the wavelength 600 nm.

5.3 Phosphatase assay:

For phosphatase activity analysis was determined by using para-nitrophenyl phosphate. The reaction yields para-nitrophenol at pH 7.2 and 37ºC which became an intense yellow soluble color and was measured at the wavelength 405 nm on a spectrophotometer.

5.3.1 Calibration for standard curve of phosphatase :- Taking 0, 0.2, 0.4, 0.6, 0.8 and 1.00 ml, of p-nitrophenol solution were added with 3.0, 2.8 2.6, 2.4, 2.2 and 2.0 ml of distilled water respectively. Then 8 ml of ethanol was added in each tube and mixed them. Addition 2 ml of 2 M tris solution mixed them for 15 sec after that measureed at the wavelength 400 nm by spectrophotometer.

5.3.2 Reagents preparation :-p-Nitrophenyl phosphate disodium salt was prepared by scale PNP 371.2 mg to 50 ml erlenmayer flask and added 20 ml distilled water. Tris maleate buffer 0.5 M (pH 6.5) was prepared by

(A) 2 M solution of Tris acid malate 24.2 g of Tris (hydroxymethyl) aminomethane + 23.2 g of maleic acid in 100 ml distilled water

(B) 2 M NaOH solution

(C) 25 ml + (B) 20 ml and adjust to 100 ml with distilled water

Tris (hydroxymethyl) aminomethane 2 M was prepared by 24.2 g. Tris (hydroxymethyl) aminomethane and dissolveed with 100 ml distilled water.

p-Nitrophenol 1 mM was prepared by scale 139.1 mg p-Nitrophenol and dissolveed with 1,000 distilled water.

5.4 Lipase assay :

For the lipase hydrolysis activity was determined by using para-nitrophenyl esters. The reaction mixture (para-mitrophenyl) solubilized in heptane at 30ºC for 2 h under magnetic stirring at 500 rpm. The absorbance of reaction mixture as para-nitrophenol was measured by colorimetric method at the wavelength 421 nm.

6. Antagonistic Test *In Vivo*

6.1 Biomass multiplication of *Thichoderma* inoculum

The fungal inoculums of *T. virens* were cultured on the substrate of agricultural grains and rice husk with the proportion of sorghum seeds: rice husk as 4:1 or substrate prepared from (20: 5 g of one set). The substrates of sorghum seeds-rice husk mixture (4:1 by weight) was placed in plastic bag (12x18 in inches size) and filled with 25 ml of distilled water (<40% moisture) and covered the opening with cotton before sterilization by autoclaving at the pressure 15 lb/inch^2 for 1 hr. After that, the pure culture of *T. virens* was inoculated in the substrate in plastic bag and incubated at 28^{o}C for 5 days (Figure 7). The sporulation of *T. virens* was recorded and assessment of spore counts was made by dilution plate technique (Pramer and Schmidt, 1956).

6.2 Appication of Trichoderma inoculum against durian foot rot (Figure 3 and 4) and leaf blight diseases (Figure 5 and 6) were foliar spray every 15 days for 6 months. Spraying of *Trichoderma* methods were applied durian true (Figure 8).

Figure 3 Comparison of foot rot symptom on durian (a) and healthy durian tree (b)

Figure 4 The rotten root of durian after the root system was attacked by *Phytophthora palmivora*

Figure 5 *Rhizoctonia* leaf blight of durian tree.

Figure 6 *Rhizoctonia* attacked the grass host and infected at the foot of the stem of durian tree.

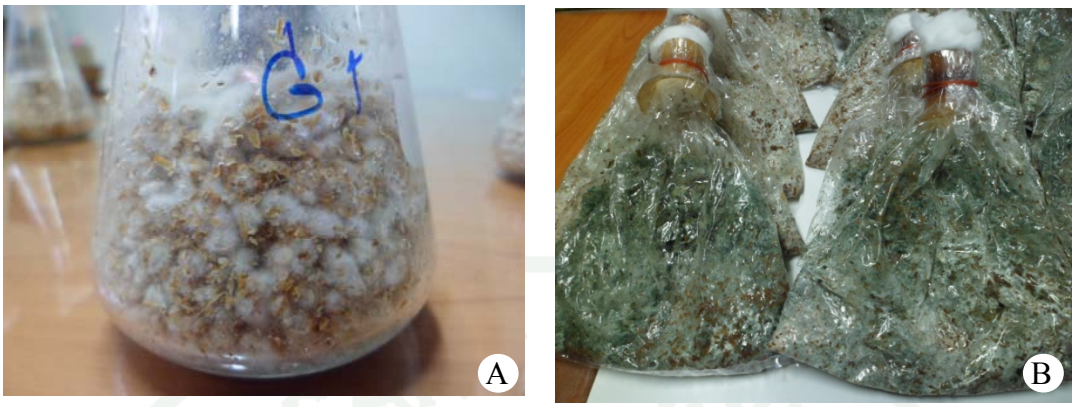


Figure 7 Biomass production of *Trichoderma virens*; (A) Starter of biomass cultivation of *T. virens* in sterilized sorghum with rice hust at 3 days after incubation, (B) *T. virens* biomass production of sorghum with rice husk at 5 days after incubation.

Figure 8 Spraying of *Trichoderma virens* using conidial suspension into the soil (A), on the leaves and stem of durian tree (B).

RESULTS AND DISCUSSION

1. Resource of halophilic fungi

Halophilic fungi were found from soil samples, rhizosphere of plant and healthy plants in alkali forest soil which collected from two provinces in Thailand. There were sixty one soil samples, falling *Acacia* leaves and healthy *Acacia* leaves.

1.1 Location of sample agricultural areas

In the northeastern of Thailand, Acacia plantation (*Acacia ampliceps*) are useful in the agricultural areas especially in the paddy field for agroforestry systems which promote to increasing soil fertility by providing organic matter from falling senescence leaves, nitrogen fixtion, a nutrient adsorption in its extensive root system. Morever, *Acacia* trees support giving shade for cattle in the dry season and also provides feed for cattle in the form of pods, leaves and young shoots.

There were various kinds of toleance salinity plants which grew around saline soils. *Acacia* tree had dominant in these areas. The salted value level of saline soils at Amphoe Kham Thale Sor, Nakhon Ratchasima Province and Amphoe Banphai, Khonkean Province in average of electrical conductivity as 6.19 dS/m and pH value as 9.2. (Table 2) Saline soils had low soil fertility because of comprising mostly of sand. The level of organic matter were very low as 0.47%. Salty soils have no structure and a lot of salt. White spots of salt appear on the surface where salt has accumulated. These groundsalter there the ground water is not very deep. There are problem for agriculture use. There salinity areas must have integrating promotion of soil fertility, having good irrigation and drainage system.

The low soil fertility in salinity areas had effect low soil nutrients. The plant nutrients in saline soil were not absortbed from the soil solution in the form of ions beause of no structure of salty soil. Therefore, The anount of macronutrients including micro nutrients were very low. In macronutrients, there werelow

phosphorus and potassium contents as 4.75 and 9.87 mg/kg. The micronutrients of calcium and magenium contents were 0.88 and 0.05 mmol/l where as the amount of sodium content was high as 65.19 m mol/l (Table 2).

However, *Acacia* plantation had benefit for promoting organic matter accunulation. Because of leguminous *Acacia* trees, they coutribute nitrogen fixation, increasing microbial activities and recycling of falling seneescence *Acacia* leaves in the beneath of Aacia trees. Wattles (*Acacia apmliceps*) were considered one of the most important economic leguminous fast growing tree in the Northeastern of Thailand which had tolerance salnity agricultural area.

Table 2 Chemical property of alkali soil analysis in low land areas at Amphoe Kham Thale Sor, Nakhon Ratchasima Province and Amphoe Banphai Khonkaen Province.

Soil samples	pH	ECe dS/m	Organic mater (%)	Extractable (mg/kg)		Soluble Cations (m mol/l)		
				P	K	Ca	Mg	Na
Alkali 1	9.0	6.19	0.70	4	11	0.88	0.05	64.21
A1kali 2	8.7	5.83	0.47	3	8	0.53	0.03	61.05
Alkali 3	9.6	6.50	0.28	6	7	1.05	0.07	67.66
Alkali 4	9.1	6.28	0.64	6	13	0.96	0.07	68.04
Alkali 5	8.9	5.94	0.52	4	8	0.52	0.05	60.38
Alkali 6	9.4	6.36	0.26	6	11	1.02	0.08	65.72
Alkali 7	9.6	6.20	0.39	3	13	1.02	0.03	67.09
Alkali 8	9.3	6.29	0.51	6	8	1.04	0.06	67.42
Average	9.2	6.19	0.47	4.75	9.87	0.88	0.05	65.19

Alkali 1-6 = Nakhon Ratchasima Province

Alkaili 7-8 Khonkean Province

Table 3 Isolates of Alkali fungi from highly saline soil at Amphoe Kham Thale Sor, Nakhon Ratchasima Province and Amphoe Banphai, Khonkean Province.

Fungal codes	Fungal species	Collection sources
KUFCM 1	*Aspergillus* sp.	Saline soil
KUFCM 2	*Aspergillus* sp.	Saline soil
KUFCM 3	*Curvularia* sp.	Saline soil
KUFCM 4	*Fomitopsis ostreiformis*	Residue of *Acacia* leaves
KUFCM 5	*Fusarium equiseti*	*Acacia* leaves
KUFCM 6	*Fusarium* sp.	*Acacia* leaves
KUFCM 7	*Lasiodiplodia pseudotheobromae*	*Fimbristylisn* leaves
KUFCM 8	*Lasiodiplodia pseudotheobromae*	*Buchanania* leaves
KUFCM 9	*Nectria rigidiuscula*	*Acacia* leaves
KUFCM 10	*Scytalidium hyalinum*	Residue of *Acacia* leaves
KUFCM 11	*Termitomyces* sp.	Residue of *Pluchea* leaves
KUFCM 12	*Termitomyces cartilagineus*	Residue of *Acacia* leaves
KUFCM 13	*Trichoderma virens*	Residue of *Acacia* leaves

KUFCM = Kasetsart University Fungal Collection of Alkali fungus

1.2 Occurrence of isolates of halophilic fungi

The alkali fungi were found from alkali or saline soil, residue of *Acacia* leaves and healthy *Acacia* leaves (Figure 9). There were 13 isolates of alkali fungi as the dominant species (Table 3). Two isolates of *Aspergillus* sp. (KUFCM 1 and KUFCM 2) and one isolate of *Curvularia* sp. (KUFCM 3) was collected from saline soils. The isolate of *Fomitopsis ostreiformis* (KUFCM 4) was collected from residue of falling senescence *Acacia* leaves and 2 isolates of *Fusarium equiseti* (KUFCMS) *Fusarium* sp. (KUFCM 6) as endophytic fungi were obtaned from healthy *Acacia* leaves. *Lasiodiplodia pseudotheobromae* (KUFCM 7-8) were isolated from *Fimbristylis* and *Buchanania* leaves. *Nectria rigidiuscula* (KUFCM 9) was also collected from healthy *Acacia* leaves. The alkali fungus, *Scytalidium hyalinum*

(KUFCM 10) was isolated from residue of falling senescence *Pluchea* and *Fimbristylis* leaves. The isolates of *Termitomyces* sp. (KUFCM 11) and *Termitomyces cartilagineus* (KUFCM 12) were collected from residue of falling senescence *Pluchea* and *Acacia* leaves respectively. The isolate of *Trichoderma virens* was collected from *Acacia* residue.

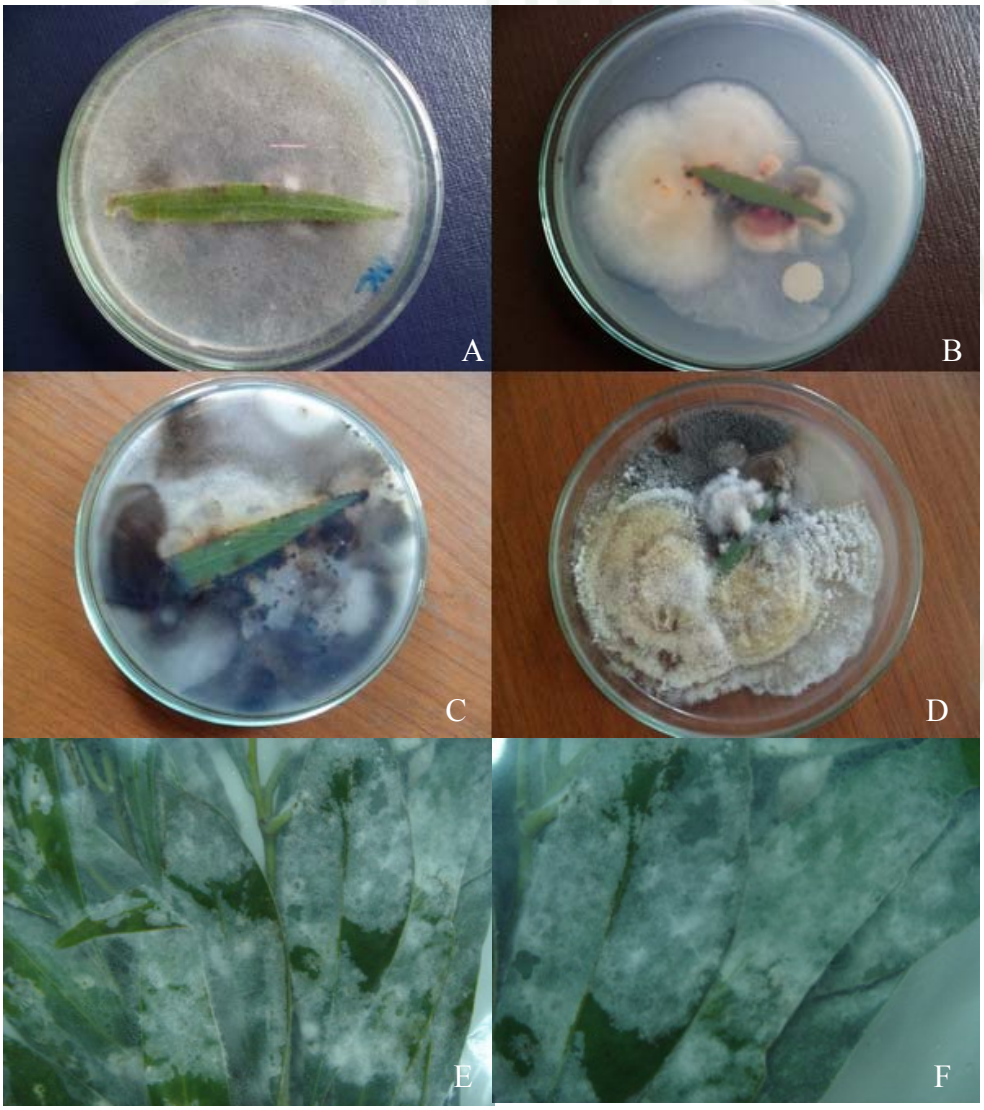


Figure 9 Halophilic endophytic fungi (alkaliphilic fungi) from healthy *Acacia* leaves on PDA supplemented with10% NaCl (A-D) and the growth of alkali fungi from the healthy *Acacia* leaves (E, F).

2. Fungal Isolate

2.1 Halophilic endophytic fungi

Five isolates comprising four species of halophilic endophytic fungi were found including one isolate each of *Fusarium equiseti*, *Fasarium* sp., *Nectria rigidiuscula*, and two isolates of *Lasiodiplodia pseudotheobromae* from healthy *Acacia* leaves (Figure 10). Genetic analysis of 18 S rDNA sequences was conducted to confirm the identity of the fungal species.

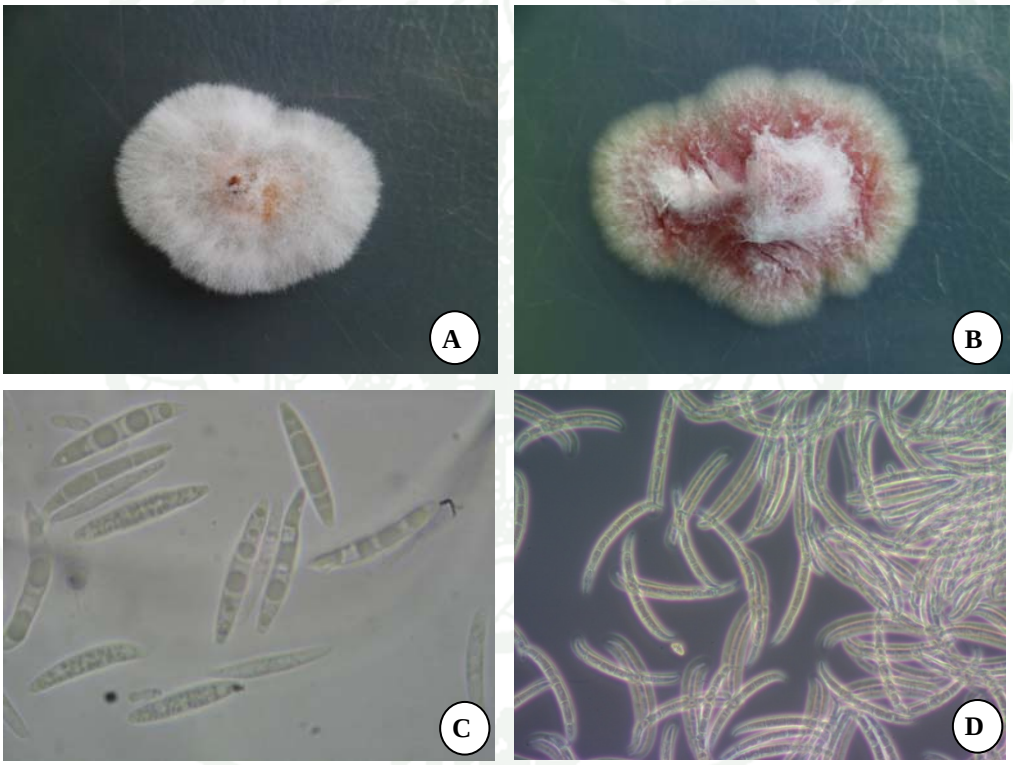


Figure 10 Colonies of *Fusarium equiseti* (A) and *Nectria rigidiuscula* (*Fusarium decemcellulare* anamorph) (B) on PDA for 5 days after incubation; macroconidia of *F. equiseti* and *N. rigidiuscula* (C, D).

In the present study, we found *Fusarium equiseti* and *Nectria rigidiuscula* (Ascomycota, Hypoceales, Nectriaceae) (Figure 5), which is similar to the report by Dong-Rui *et al.* (2012) who found *Fusarium solani* as an halophilic

endophytic fungus from *Salicornia europaea* (Chenopodiaceae), a perennial dicot grows in various zones of intertidal salt marshes. In contrast, Tran *et al*. (2010) found the Ascomycota endophytic fungi *Aureobasidium, Chaetomium* and Sordariomycetes from *Acacia* spp. in Australia.

Yunianto *et al*. (2012) recorded *N. rigidiuscula* an endophytic fungi of Srikaya plants (*Annona squamosa*) was expected to have similar extracted metabolites as annonaceous acetogenin which has cytotoxic activity against cancer cells and lower toxicity compared to other cancer drugs. In addition, Mandeel (2006) reported that *F. equiseti* produced thick-walled chlamydospores as the salinity increased between 5-20% NaCl. *F.equiseti* was also obtained from seeds of cowpea, *Vigna unduiculata* (L.) Walp. by remaining endophytic in the seeds as dormant mycelium or chlamydospores without causing disease (Rodrigues and Menezes, 2006).

2.1 Alkaliphilic fungi

Seven isolates comprising seven species of alkaliphilic fungi were found including one isolate each of *Fomitopsis ostreiformis*, *Scytalidium hyalinum*, *Termitomyces cartilaginous*, *Termitomyces* sp., and *Trichoderma virens*, *Curvularia* sp. and two isolates of *Aspergillus* sp. Genetic analysis of 28 SrDNA sequences was conducted to confirm the identity of the fungal species (Figure 11).

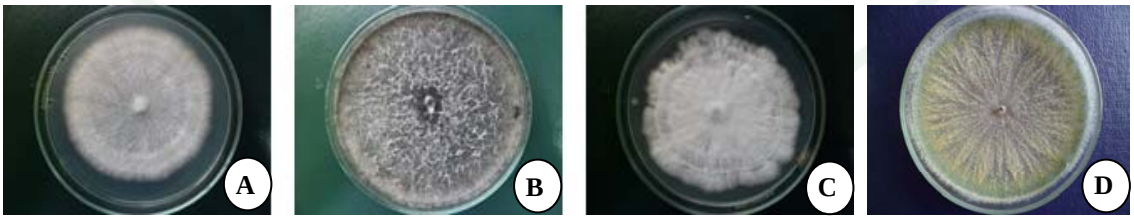


Figure 11 Colonies of *Fomitopsis ostreiformis* (A), *Scytalidium hyalinum* (B), *Termitomyces cartilagineus* (C) and *Trichoderma virens* (D) on PDA at 5 days after incubation at 28°C

According to Rungjindamai *et al*. (2008) reported *Fomitopsis* spp. isolation from residue of oil palm leaves. Many of these morphotypes were shown to be basidiomycetes as clamp connections were present and some produced basidia and basidiospores in culture. The largest fungal assemblage was within the Fomitopsidaceae, four endophytic isolates clustered with *Fomitopsis* species (*F. ostreiformis, F. palustris*), two and three isolates grouped with *F. pinicola* and *F. meliae*, respectively.

Tibuhwa (2012) reported *Termitomyce*s R. Heim, a basidiomycete fungus lives in a mutualistic symbiosis with termites of the subfamily Macrotermitinae. This study explored the cultural properties and macro-micromorphological characters including scanning electron microscopic studies of ten *Termitomyces* species collected from different parts of Tanzania. Pure cultures were isolated from the asexual fruit bodies growing on or near the termite mound by tissue culture techniques in three different media Ghosh, Hagem Modess, and Modified Malt Extract Agar. The results showed that *Termitomyces aurantiacus* (Heim) Heim and *Termitomyces* striatus Heim have unsmooth basidiospore, the character noted for the first time in this genus, and the two species might be conspecific. In cultures, while micromorphological characters remain undistinguishable between taxa, Macromorphological characters distinguished them in colour of the mat, growth rate, mycelia elevation and advancing zones as well as the mat texture. *T. saggitiformis* (Kalchbr. & Cooke) D.A. Reid, *T. titanicus* Pegler & Piearce species are reported for the first time in the country record and key to the studied species is supplied. This study suggests the redefining of the genus by omitting the smooth or adding unsmooth basidiospore character in the genus circumscription

3. *In Vitro* Antagonistic Effects of Halophilic Endophytic Fungi

Fusarium equiseti showed high efficacy in controlling mycelial growth of *P. aphanidermatum* (Figure 12A) and *P. palmivora* (Figure 12B),causing more than 90% inhibition (Table 4), but showed somewhat less efficacy in controlling mycelium growth of *Alternaria brassicicola* and *Curvularia oryzae* (Figure 12C-12D).

Nectria rigidiuscula Berk. & Broome effectively inhibited and overgrew more than 80% of the mycelial growth of *Pythium aphanidermatum, Phytophthora palmivora, Colletotrichum capsici* and *Helminthosporium oryzae* (Figures 12E-12H and Table 4).

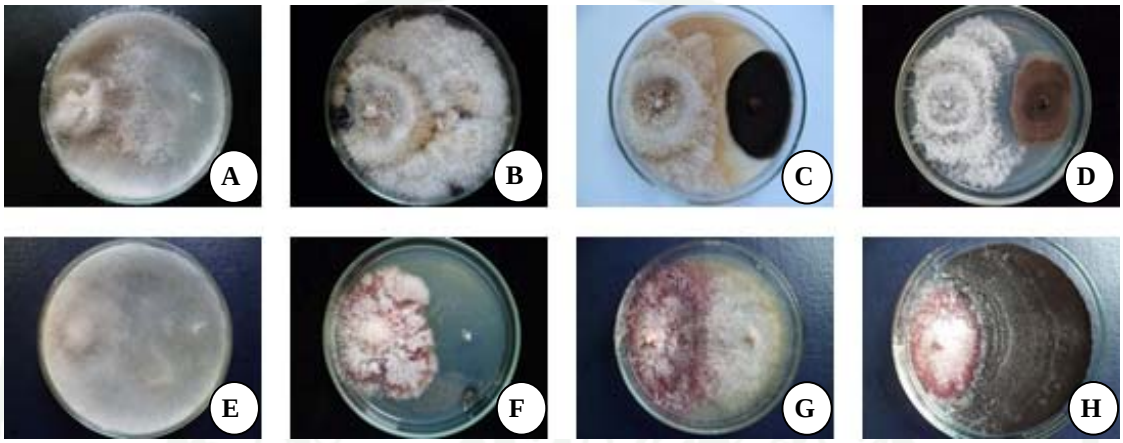


Figure 12 Dual culture test for antagonism between halophilic fungi (left) and plant pathogenic fungi (right) on PDA incubated for 7 and 14 days at 30°C: After incubation 7 days of the culture between *Fusarium equiseti* vs *Pythium aphanidermatum* (A), *Phytophthora palmivora* (B), *Alternaria brassicicola* (C) and *Curvularia oryzae* (D); After incubation 14 days of the culture between *Nectria rigidiuscula* vs *Pythium aphanidermatum* (E), *Phytophthora palmivora* (F), *Colletotrichum capsici* (G) and *Helminthosporium oryzae* (H).

SEM photomicrographs (Figures 13A-13D) showed that halophilic endophytic fungus, *Fusarium equiseti* suppresses the growth of *Rhizoctonia oryzae* by overgrowing, destroying encroachment, commission straps and absorption within the host mycelium. *F. equiseti* produced appressorium-like structures capable to destroy the plant pathogenic fungus, *Rhizoctonia oryzae*. Mode of action of *F.equiseti* against plant pathogen involved the mechanisms of competition as strong ability to complete with pathogen for nutrients, mycoparasitism, antibiosis or inhibitory, and lysis. *F. equiseti* produced some antibiotic substances and hydrolytic enzymes as cellulose,

protease, and lipase to inhibit the pathogen growth which degraded the mycelial cell wall of pathogen by growing along and penetrating into the host mycelial pathogen.

Horinouchi *et al.* (2010) reported that the plant growth promoting fungus (PGPF), *F. equiseti* effectively controlled Fusarium wilt of spinach caused by *F. oxysporum* f. sp. *spinaciae* by reducing in disease severity from 43.5 to 91.8%. *F. equiseti* not only suppressed the disease but also reduced the pathogen population in the spinach roots. This fungus might induce physiological changes in the composition of plant extracts and enhance defense mechanisms that are further triggered when the plant senses a potential pathogen in a phenomenon known as potentiation or conditioning. Macia-Vicente *et al*. (2009) recorded that *F. equiseti* reduced the mean root lesion length caused by the pathogen by producing toxins antagonistic to fungal pathogens and plant parasitic nematodes. Horinouchi *et al*. (2007) reported that *Fusarium equiseti* (Corda) Sacc., when tested in a hydroponic rock wool system, was a potential biocontrol agent of *Fusarium* crown and root rot of tomato, caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici.* Punja *et al.* (2008) found that *Fusarium equiseti* was the most effective organism in controlling *Fusarium* crown and root rot. In ginseng soil In British Columbia, *F. equiseti* was found prevalent in ginseng soil, straw mulch, and in ginseng root tissues and the fungus grew well at pH 7.2-7.8 suggesting a preference for alkaline conditions. According to Yunianto *et al*. (2012), *N. rigidiuscula* has potential as source of anti-breast cancer compounds since 100 ppm of ethyl acetate extract inhibited the viability of breast cancer cells 91.84% by liquid fermentation.

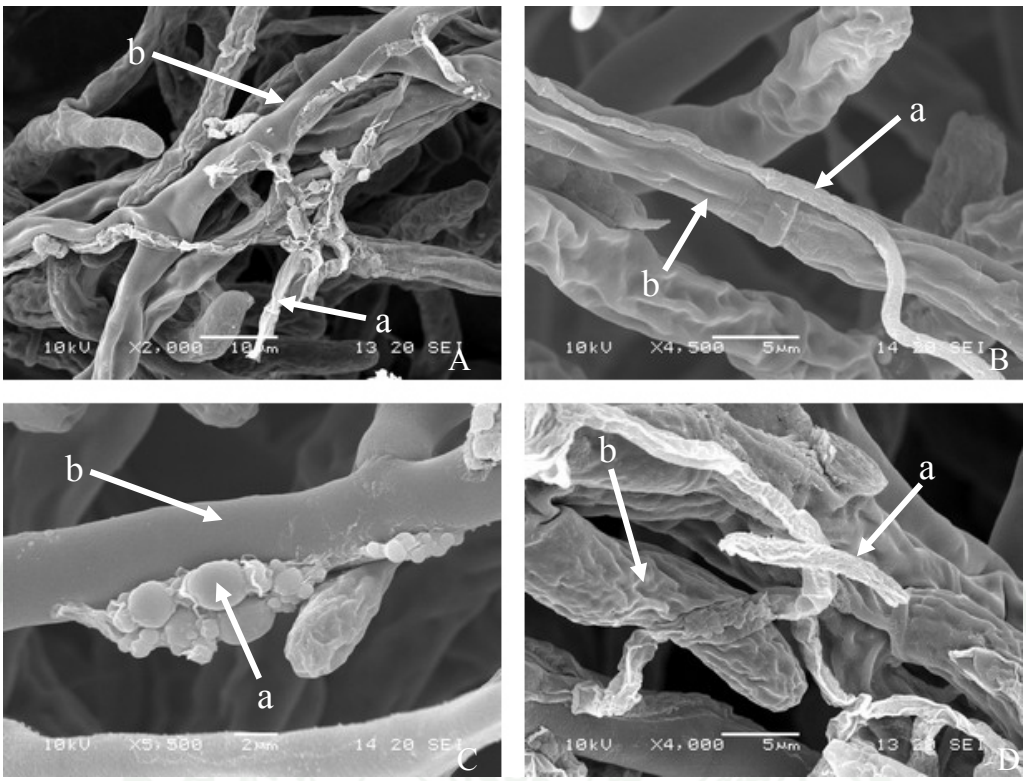


Figure 13 SEM photomicrograph showing antagonistic activity of the halophilic endophytic fungus, *Fusarium equiseti* (a) suppressing the mycelial growth of plant pathogenic fungus *Rhizoctonia oryzae* (b) by overgrowing or covering on the mycelial plant pathogen (A), parallel encroachment for absorping organic substance and degrading the cell wall of the mycelia pathogen (B), commission straps and absorption of organic substance within the host mycelium (C), and penetration of mycelia antagonistic fungus into the host mycelium (D).

4. *In Vitro* Antagonistic Effects of Alkaliphilic Fungi

Fomitopsis ostreiformis and *Scytalidium hyalinum* were the most effective species, inhibiting 100% of the pathogen's growth of *Alternaria brassicicola, Curvularia oryzae, Helminthosporium oryzae, Colletotrichum capsici, Pythium aphanidermatum* and *Rhizoctonia oryzae* (Figure 14; Table 5 and 6).

Termitomyces cartilaginous effectively inhibited 100% of the mycelial growth of *Alternaria brassicicola*, *Helminthosporium oryzae*, *Colletotrichum capsici*, *Pythium aphanidermatum* and *Rhizoctonia oryzae* but showed moderate efficacy in controlling 60% of the mycelial growth of *Curvularia oryzae* (Figure 14; Table 5 and 6).

SEM photomicrographs (Figures 17A-17D and 18A-18D) showed parasitism mechanism that alkaliphilic fungi, *Fomitopsis ostreiformis* and *Scytalidium hyalinum* suppressed the growth of *Rhizoctonia oryzae*. Finally, the mycelia structure of *R. oryzae* was damaged and collapsed. *Fomitopsis ostreiformis* suppressed the mycelia growth of *Rhizoctonia oryzae* by overgrowing including coiling around plant pathogen hyphae, destroying encroachment, commission straps and absorption within the host mycelium. (Figures 17A-17B). *F. ostreiformis* produced haustoria formation and penetrated the host hyphae of *R.oryzae* including subsequent dissolution of the host cytoplasm (Figure 17C-17D). This alkaliphilic fungus produced some enzymes as cellulase, protease and lipase to digest the plant pathogenic hyphae. *Scytalidium hyalinum* also inhibited the mycelial growth of *R. oryzae* by overgrowing and destroying encroachment and absorption within the host mycelium. (Figure 18A-18B). The rectangular conidia of this alkaliphilic fungus encroached the host hyphae of plant pathogen and absorped the cytoplasm of this pathogen. Furthermore, *S. hyalinum* alsoproduced cellulase, protease and lipase which had effected dissoloving the cell wall of plant pathogenic hypthae by breaking down the polysaccharide that was responsible for the rigidity of fungal pathogen cell wall (Figure 18C and 18D).

Cholyklin (2009) had studied the diversity of endophytic basidiomycetes from the oil palm and found that *Fomitopsis ostreiformis* (Berk.) T. Hatt. was isolated from

the oil palm leaves and petioles. *F. ostreiformis* had potential use for biological control organisms against the oil palm pathogen, *Ganoderma boninense* by producing bioactive secondary metabolite. And also involved the mechanisms of competition, mycoparasitism including antibiotic or toxin production.

Mukerji and Chamola (1999) reported Ectomycorrhizae are known to prevent growth of pathogenic fungi on root surface of host plants, Thereby protecting plants from the harmful effect of pathogens. Mycorhizal roots show increased respiration. Mycorrhization strengthens the cell wall by increasing lignification and production of other polysaccharide. The growth of pathogen is restricted or inhibited because of these. A stronger of vascular system will increase the flow of nutrient, impart greater mechanical strength and diminish derogatory effects from vascular pathogens.

Currie and Hiratsuka (1996); Moltzan and Blenis (1999) reported biological control of western gall rust (WGR) on Ponderosa pine by using mycoparasite *Scytalidium uredinicola*. *S. uredinicola* is a destructive hyperparasite of WGR, which reduces the inoculums potential. This mycoparasite breaks down spore and the active rust sori of WGR, making infected area appear clumpy and stringy. Infected sori are a yellowish-green to whitish-gray in appearance, depending on the stage of infection. Hyphae of the mycoparasite are able to penetrate the wood tissue of the galls and destroy rust hyphae ot a depth of 300 μm below the sori. Infections begin early in the season and are found in the developing sorus under the peridium, thus providing control before spores are released. For the dissemination of the mycoparasite *S. uredinicola*, it was found that *Epuraea obliques* (Coleoptera : Nitidulidae) was the most abundant invertebrate species on gall rust, with much of its like cycle occurring on the gall during sporulation. Since *S. uredinicola* is also present on the sporulating tissue during the same period, it has a close temporal association with the beetle.

Fowler *et al.* (1999) reported utilization of endophytic fungus as *Scytalidium* isolates for antagonistic effecting to *Botrytis cinerea*, *B. cinerea* Pers, the fungal pathogen causing Botrytis root, frequenthy causes bunch rot significant losses in grape crops. The results showed that all fungal isolate tested reduced significanthy the

development of *B. cinerea* conidiophores on inoculated grape rachii. This fungus had effected suppressing this plant pathogen by producing of antifugal compounds. In order for potential antagonist to reduce the level of overwintering *B. cinerea* inoculums on rachii, it is essential that *Scytalidium* sp. the ability to survive and/or grown as well as or better than the pathogen in the winter temperature and moisture conditions. For the temperatures 10-20°C, conidisphore production of *B. cinerea* were greatest suppressed after treated rachii with three fungi.

Stamets (2005) reported some mushroom is an antagonistic fungi as the clustered woodlover, *Hypholoma capnoides* (Fires) Quelet is an aggressive conifer stump decomposer. This mushroom grows on dead wood, primarily conifers and is an aggressive saprophyte competing well against several parasitic fungi. For mycorestoration potential, *H. capnoides* could be used for protecting against blights, for recycling stumps, and for enzyme production. Mycoforesters should carefully consider the judicious use of this fungus. Mycelium of *H. capnoides* over running and inhibit the growth of a culture of *Armillaria mellea*, a blight fungus that devastates thousands of acres of forests.

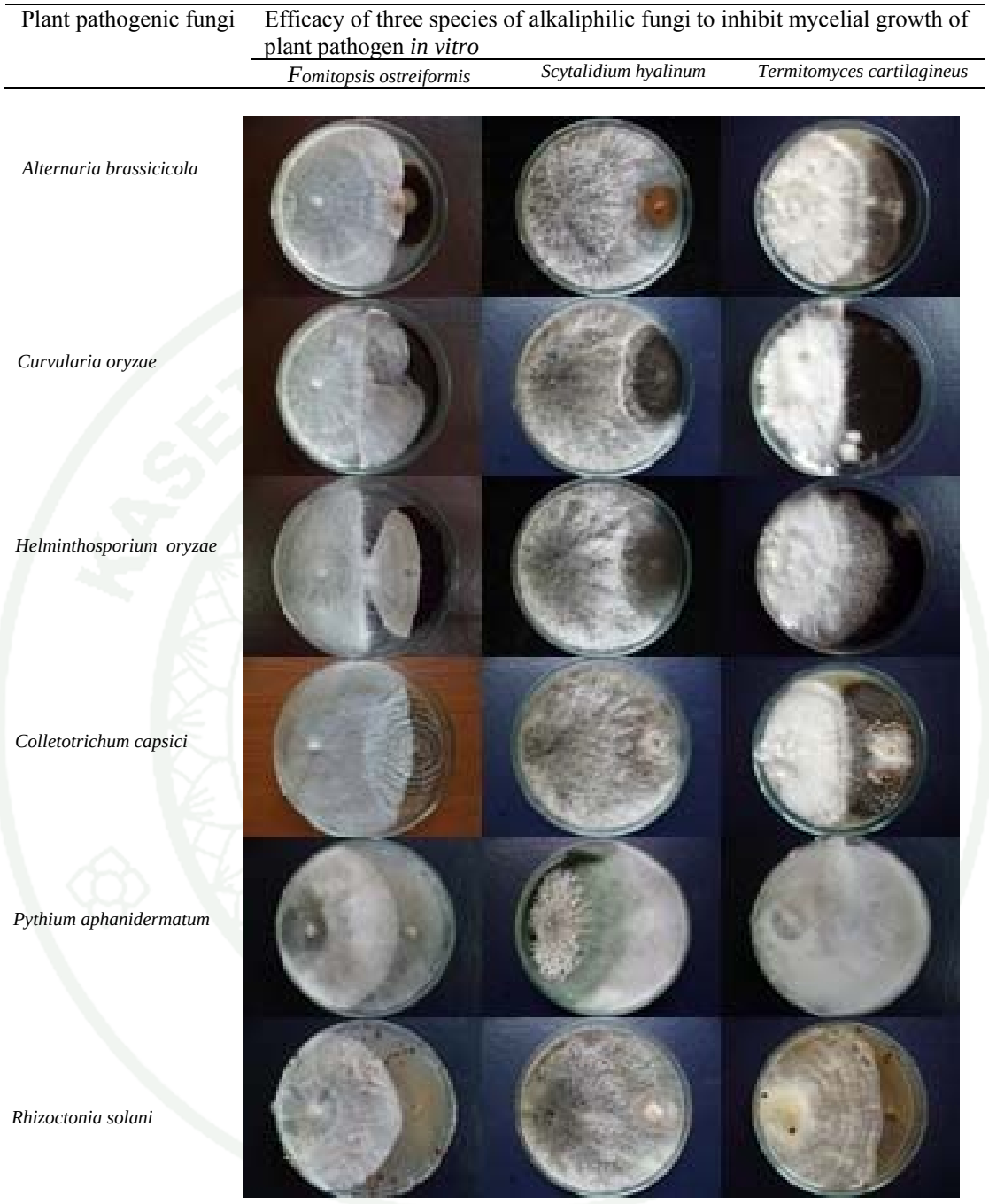


Figure 14 Antagonistic activity test of three species of alkaliphilic fungi (left) against six species of plant pathogenic fungi (right), incubated as dual culture on PDA at 28°C for 14 days

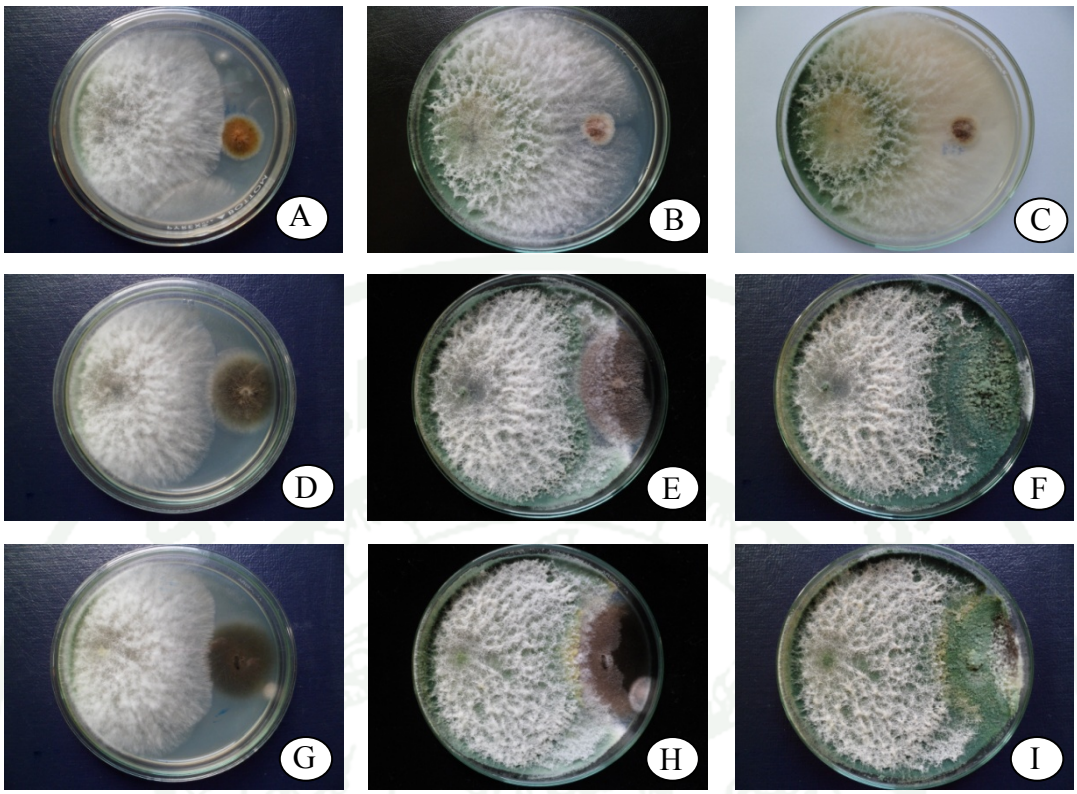


Figure 15 Antagonistic test between alkaliophilic fungi, *Trichoderma virens* (left) and plant pathogenic fungi (right) on PDA incubated for 7 days at 28ºC;
A-C. *Trichoderma virens* vs *Alternaria brassicola*
D-C. *Trichoderma virens* vs *Curvularia oryzae*
G-I. *Trichoderma virens* vs *Helminthosporium oryzae*

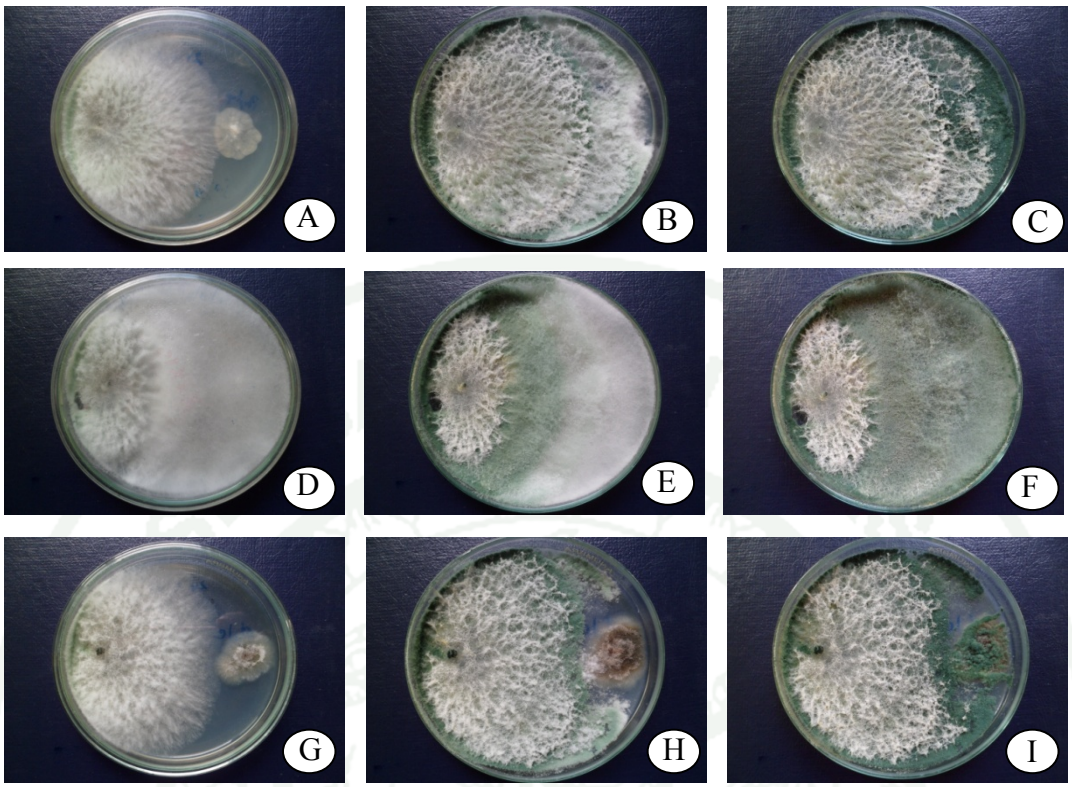


Figure 16 Antagonistic test between alkaliphilic fungus, *Trichoderma virens* (left) and plant pathogenic fungi (right) on PDA incubated for 7 days at 28ºC;
A-C. *Trichoderma virens* vs *Alternaria brassicola*
D-F. *Trichoderma virens* vs *Curvularia oryzae*
G-I. *Trichoderma virens* vs *Helminthosporium oryzae*

Table 4 Effect of halophilic endophytic fungi on mycelial growth of plant pathogenic fungi cultivated on PDA in dual culture at 28°C for 14 days.

Halophilic fungi	Mycelium growth inhibition (%)	
	Fusarium equiseti	*Nectria rigidiuscula*
1. *Alternaria brassicicola*	83.73c	60.03e
2. *Curvularia oryzae*	85.10c	83.43d
3. *Helminthosprium oryzae*	60.06d	86.76c
4. *Colletotrichum capsici*	82.16c	80.90d
5. *Phytopthrora palmivora*	100.00a	100.00a
6. *Pythium aphanidermatum*	92.63b	89.66b
7. *Rhizoctonia solani*	83.06c	82.00c

Values in the same column followed by the same latters are significantly different (Duncan's New Multiple Range Test, $P < 0.05$).

Table 5 Effect of alkali fungi on mycelial growth of plant pathogenic fungi cultivated on PDA as dual culture at 28°C for 14 days.

Alkali fungi	Mycelium growth inhibition (%)			
	Alternaria brassicicola	*Curvularia oryzae*	*Helminthosporium oryzae*	*Colletotrichum capsici*
1. *Fomitopsis ostreiformis*	100.0a	100.0a	100.0a	100.0a
2. *Scytalidium hyalinum*	100.0a	100.0a	100.0a	100.0a
3. *Termitomyces cartilagineus*	100.0a	60.7c	100.0a	100.0a
4. *Trichoderma virens*	100.0a	100.0a	100.0a	100.0a

Values in the same column followed by the same latters are significantly different (Duncan's New Multiple Range Test, $P < 0.05$).

Table 6 Effect of alkali fungi on mycelial growth of plant pathogenic fungi cultivated on PDA as dual culture at 28°C for 14 days.

Alkali fungi	Mycelium growth inhibition (%)		
	Phytophthora palmivora	*Pythium aphanidermatum*	*Rhizoctonia solani*
1. *Fomitopsis ostreiformis*	100.0a	100.0a	100.0a
2. *Scytalidium hyalinum*	100.0a	90.8a	98.6a
3. *Termitomyces cartilagineus*	100.0a	100.0a	100.0a
4. *Trichoderma virens*	100.0a	100.0a	100.0a

Values in the same column followed by the same latters are significantly different (Duncan's New Multiple Range Test, $P < 0.05$).

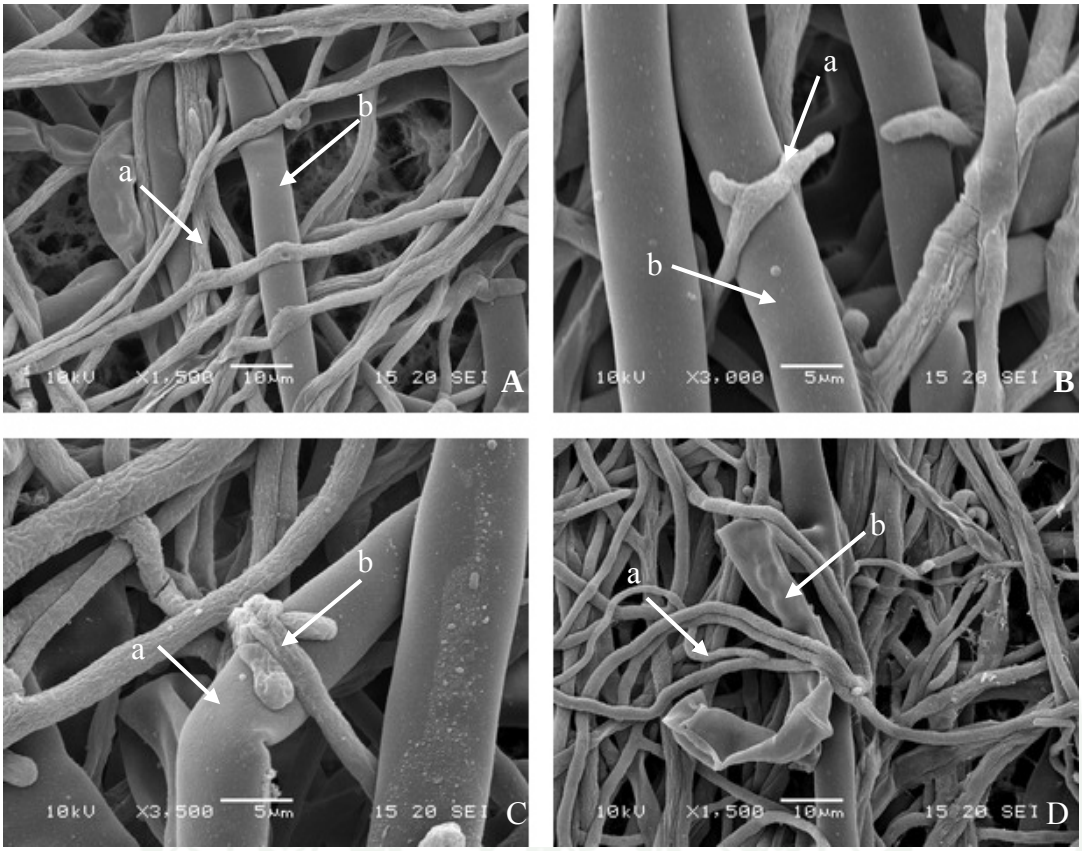


Figure 17 SEM photomicrographs showing antagonistic activity of alkaliphilic fungus, *Fumitopsis ostreiformis* (a) suppressing plant pathogenic fungus *Rhizoctonia solani* (b); by overgrowing or covering on the mycelial plant pathogen (A), mycelial growth of *F.ostreiformis* encroachment and commission straps including absorption of organic substances within the host mycelium (B and C), and mycelial plant pathogen was destroyed which had shown wilting mycelium (D).

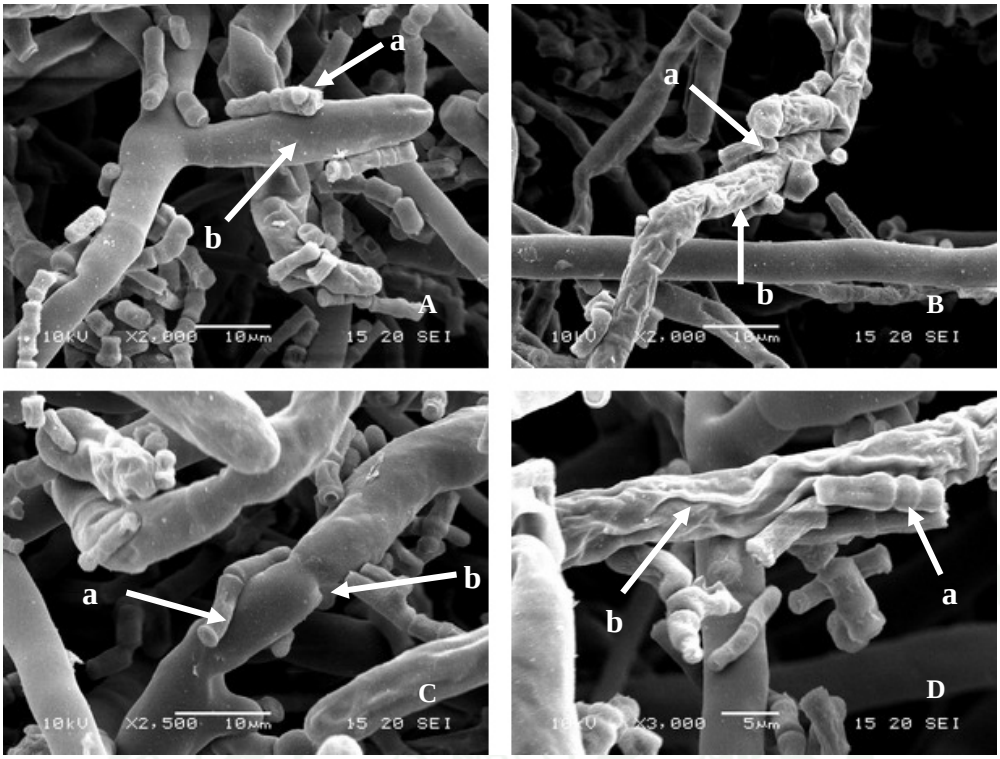


Figure 18 SEM photomicrographs showing antagonistic activity of alkaliphilic fungus, *Scytalidium hyalinum* (a) suppressing plant pathogenic fungus, *Rhizoctonia solani* (b) under scanning electron microscope; conidia of *S. hyalinum* commission straps encroaching contact the cell wall of the mycelia plant pathogen (A and C), conidia of alkaliphilic fungus strapped mycelia of plant pathogen and absorped organic substances within the host mycelium which had shown wilting mycelium of plant pathogen (B and D).

has been established that viable spores of this mycoparasite can be culture from the beetle's grass. WGR is most destructive in tree farms, plantations and nurseries. This disease can directly and more rapidly infect because it has no alternate host. The aeciospores clearly can reinfect pine. There are no telia or basidia spores. WGR is widespread throughout the Pacific Northwest affecting susceptible trees throughout their range, it is found on lodgepole, ponderosa and jack pine in natural forest.

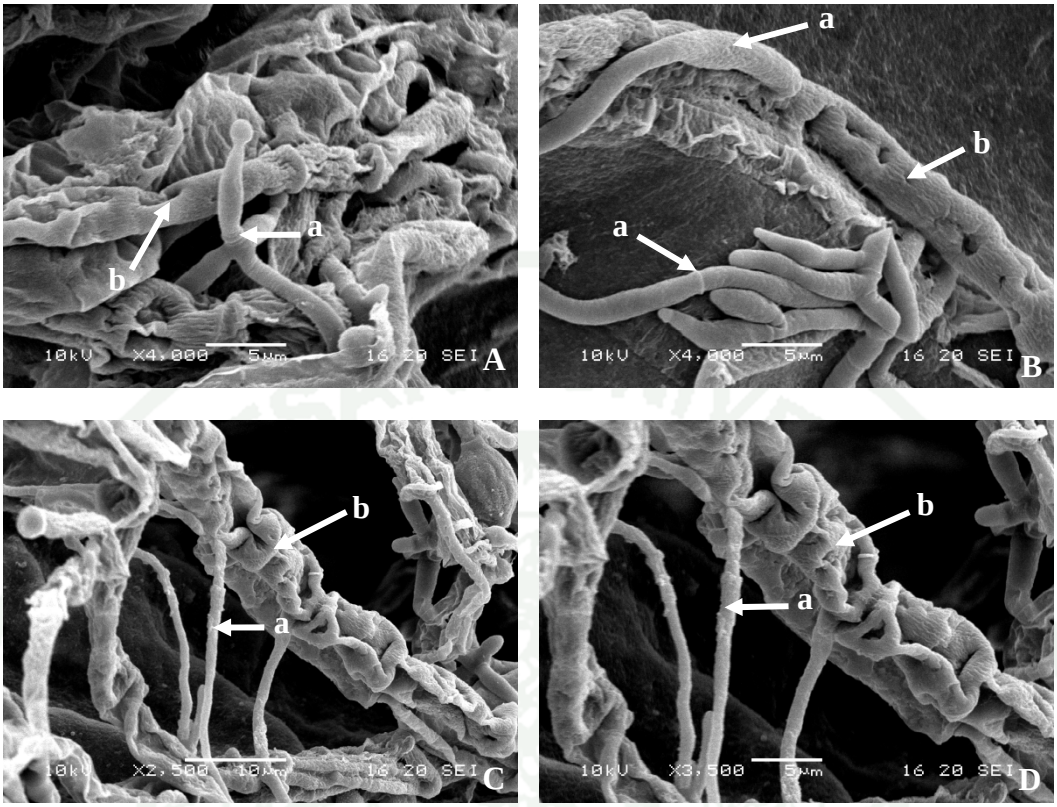


Figure 19 SEM photomicrograph showing antagonistic activity of the alkaliphilic fungus *Trichoderma virens* (a) destroying the mycelial growth of plant pathogenic fungus *Rhizoctonia solani* (b) by parallel encroachment and degrading the cell wall of the mycelial pathogen (A) and (B), *Trichoderma virens* (a) suppressing the mycelia growth of plant pathogenic fungus *Sclerotium rolfsii* (b) by penetration of mycelial antagonistic fungus into the host mycelium and absorption of organic substance within the host mycelium (C) and (D).

Mukerji and Chamola (1999) reported the ectomycorrhizae are known to prevent growth of pathogenic fungi on root surface of host plants. Moreover, the mycelium of ectomycorrhizal fungi had strengthen the liginification and polysaccharides which had affected to encroached and strapped the mycelium of plant pathogenic fungi.

The fungi, *Scytalidium hyalinum* and *Trichoderma virens* had highly efficient antagonists of plant pathogenic fungi (Table 5 and 6). *S. hyalinum* produced a compact mass of mycelium and overgrew on 100% the mycelium growth of *A. brassicicola, C. oryzae, H. oryzae, C. capsici* and *P. palmivora*. (Figure 2A-D) This fungus also inbibited more than 90% of the mycelium growth of *P. aphanidermatum, R. oryzae* and *S. rolfsii*. The fungus as *Trichoderma virens* was the most effective species, which succeeded in controlling 100% of the mycelium growth of all plant pathogenic fungi (Figure 15A-15I).

SEM photomicrographs (Figure 18A-18B) the alkali funfus *Scytalidium hyalinum* showed the actions of mycoparasitism, production of cell wall-degrading enzymes and nutrients completion. The mycelial *Scytalidium* had attacked and parasited to the hyphal of *Rhizoctonia oryzae*. Afterthat, the mycelia structure of *R.oryzae* was destroyed and the wilting of hyphae because of compression straps and nutrients absorbtion within the mycelia of plant pathogen by *S. hyalinum* (Figure 18C-18D). Fowler *et al.* (1999) reported utilization of endophytic fungus as *Scytalidium* isolates for antagonistic effecting to *Botrytis cinerea*, Pers, the fungal pathogen causing *Botrytis* root, frequenthy causes bunch rot significant losses in grape crops. reduced significanthy the development of *B. cinerea* conidiophores on inoculated grape rachii. This fungus had effected suppressing this plant pathogen by producing of antifugal compounds.

For the mechanism of *Trichoderma virens* action on *Sclerotium rolfsii* severity showed attaching and growing around the plant pathogen (Figure 19A-19D). The mycoparasite hyphae directly contacted and coiled around the cell wall of fungal pathogen and then penetrated in the host's cell wall (Chamswarng *et al*., 2001). Enzyme action from *T. virens* has destroyed the hyphal *S. rolfsii* to causing cell degradation (Figure 19A-19B). *Trichoderma* penetrating had affected the appearance of expose hole around the mycelia of *S.rolfsii* (Figure 19C-19D).

5. Degradation of Protein, Phosphate and Lipid by Halophilic Endophytic Fungi

For mineral transformation in selective solid media, *Fusarium equiseti* degraded lipid very well in 10% NaCl skim milk and tributyrin agar by showing a clear zone around the fungal colony (Figures 20A-20B). *Nectria rigidiuscula* degraded phosphate and lipid very well in 10% NaCl of calcium phosphate and tributyrin agar, but only slightly digested protein in skim milk agar, (Figures 20C-20E).

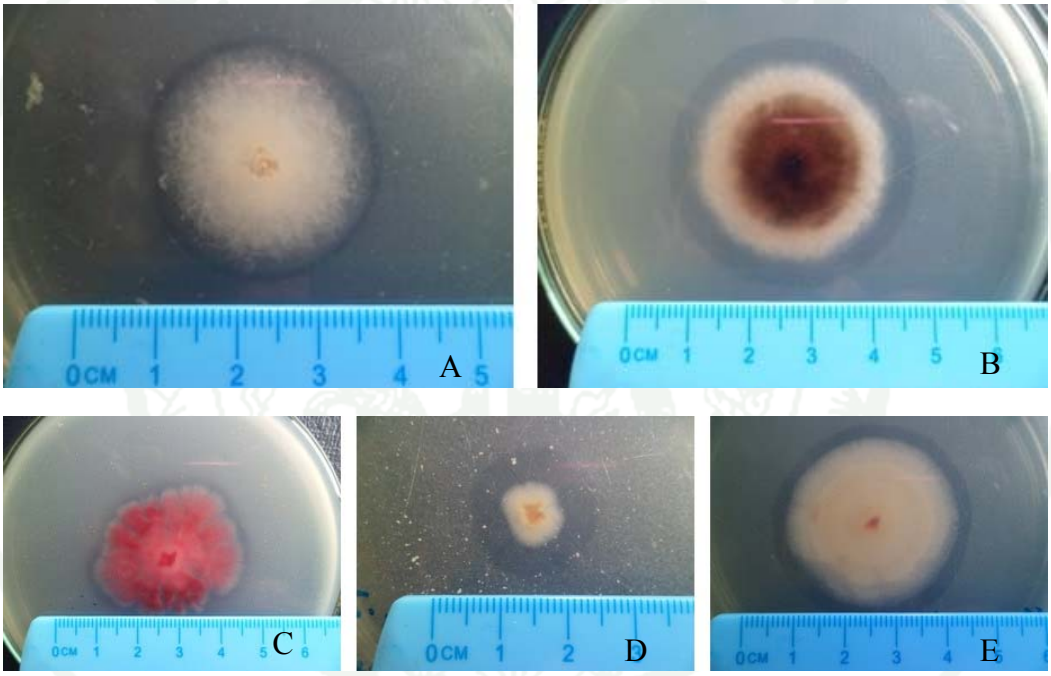
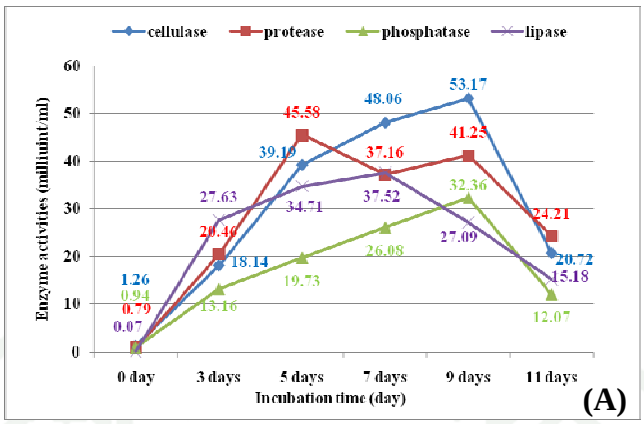
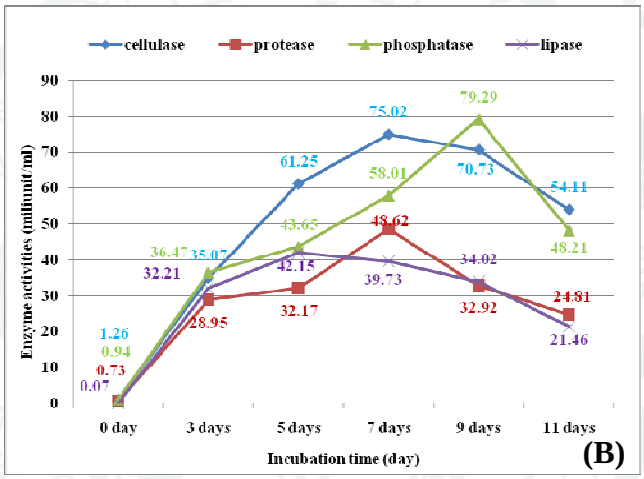


Figure 20 Colonies halophilic endophytic fungi and clear zone appearance in each selective media incubated for 7 days at 30°C; A, B) *Fusarium equiseti* on 10% NaCl skim milk agar and 10% NaCl tributyrin agar, respectively. C, D, E) *Nectria rigidiuscula* on skim milk agar, 10% NaCl phosphate agar and tributyrin agar, respectively.



Fusarium equiseti



Nectria rigidiuscula

Figure 21 Activities of cellulase, protease, phosphatase, and lipase produced by *Fusarium equiseti* (A) and *Nectria rigidiuscula* (B) during 11 days of incubation.

For enzyme activities of halophilic fungi, *F. equiseti* and *N. rigidiuscula* highly secreted lipase with 48.06 and 42.15 milliunit/ml (Figures 21A, 21B). The results showed some correlation as directly change between the width of clear zone appearance in the solid media tests and enzyme production during 11 days of incubation. Kladwang *et al*. (2003) reported that *Fusarium* isolates showed positive alkaline tolerant fungi isolation for alkaline enzyme production by using azurine dyed and cross-linked substrates which appeared a blue diffusion zone around the well indicates a strong enzyme activity.

6. Degradation of Protein, Phosphate and Lipid by Alkaliphilic Fungi

For mineral transformation in selective solid media, *Fomitopsis ostreiformis* could degraded phosphate and lipid very well in calcium phosphate and 10% NaCl tributyrin agar showing clear zone around the fungal colony (Figures 21A, 21B). *Scytalidium hyalinum* showed a lipid clear zone which indicated that significant degradation of lipid in 10% NaCl tributylin agar. *Termitomyces cartilagineus* showed a lipid clear zone which degraded lipid very well in 10% NaCl tributylin agar and moderately degraded calcium phosphate (Figures 22E-22F).

F. ostreiformis, *S. hyalinum* and *T. cartilagineus* secreted phosphatase at 67.97, 61.53 and 53.70 milliunit/ml respectively whereas secreted lipase at 28.92, 29.05 and 29.47 milliunit/ml, respectively (Figures 22A, 24B and 25A). There were some correlation between clear zone appearance and enzyme production during 11 days of incubation. Kang *et al*. (2002) recorded phosphate solubilizing ability of *Fomitopsis* sp. was capable of phosphate solubilization in saline conditions which was enhanced in the presence of 1% NaCl. The strain *Fomitopsis* sp. could thus be of great benefit in maintaining the available phosphate levels for crops in saline alkaline soils. Yoon and Kim (2005) reported the utilization of the brown-rot basidiomycete *Fomitopsis palustris* for degrading agriculture wastes and forest materials containing high levels of lignocelluloses by producing exocellulase as the major extracellular enzyme.

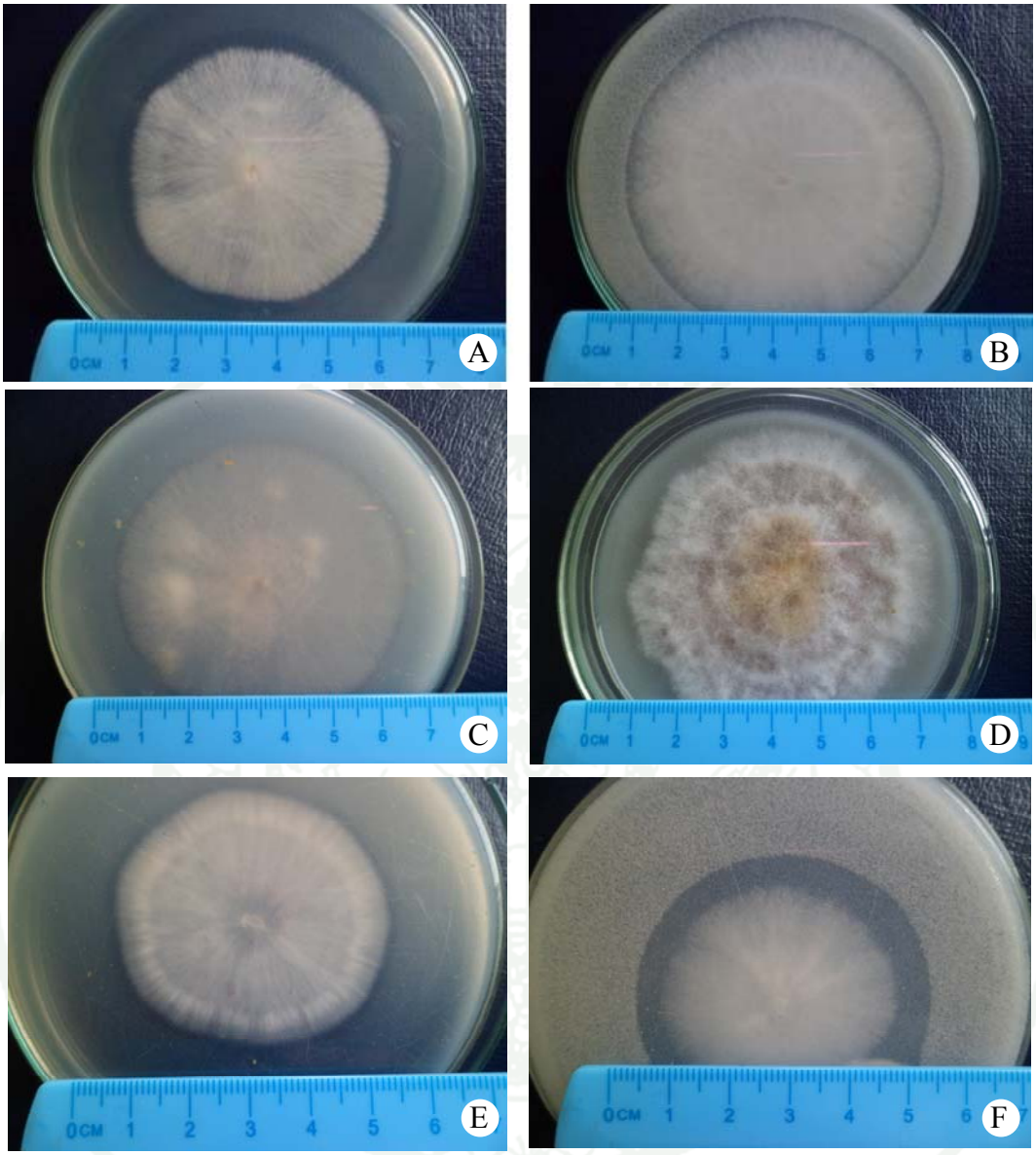


Figure 22 Colonies of alkaliphilic fungi and clear zone appearance in each selective media incubated for 7 days at 28ºC ; A, B) *Fomitopsis ostreiformis* on phosphate agar and 10% NaCl tributyrin agar, respectively. C, D) *Scytalidium hyalinum* on phosphate agar and 10% NaCl tributyrin agar, respectively. E, F) *Termitomyces cartilagineus* on phosphate agar and 10% NaCl tributyrin agar, respectively.

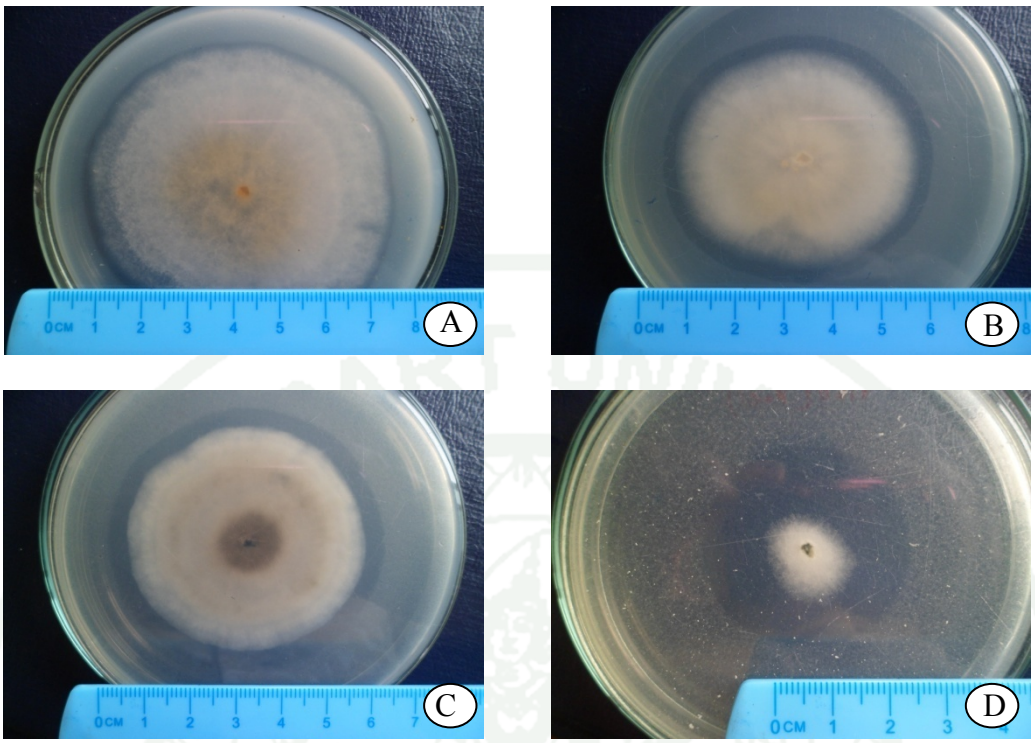
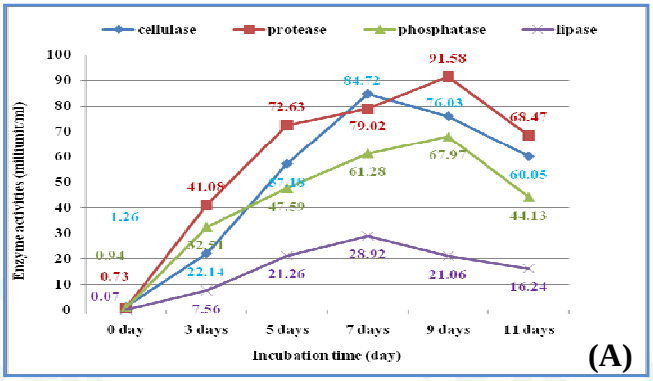


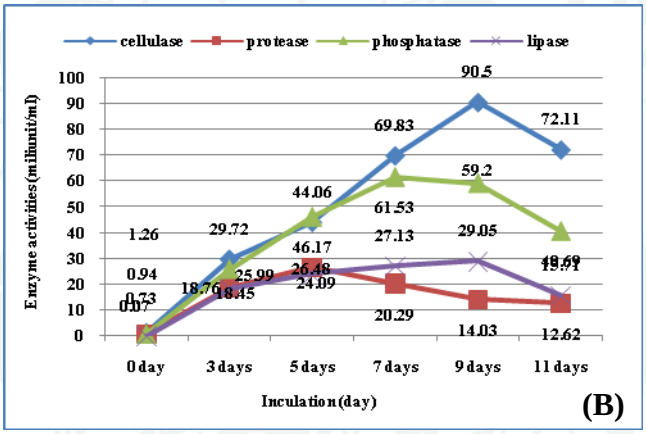
Figure 23 Colony alkaliphilic fungus, *Trichoderma virens* and clear zone appearance in each selective media incubated for 7 days at 28ºC, (A,B,C,D) *Trichoderma virens* on 10% NaCl phosphate agar, tributyrin agar and 10% NaCl tributyrin agar, respectively

Zeleke *et al*., (2013) reported Termites of the subfamily *Macrotermitinae* are known to live in an obligate symbiosis with *Termitomyces* mushrooms, although the exact benefit of the association is still debating. *Termitomyces* are believed to degrade lignocelluloses into smaller units which then can be used by the fungus-growing termites. In this study, extracts of the termite comb showed strong xylanase activity (8.27 ± 0.14 unit per g of dried comb) with no cellulase activity. Termite comb and wheat bran supported the growth of *Termitomyces* culture in solid state fermentations, in which culture-extracts showed strong xylanase activities (52.25 ± 1.98 and 37.38 ± 1.09 units per g of dried culture, respectively), yet no cellulase activities were detected. Furthermore, we observed that *Termitomyces* cultures were unable to grow on pure cellulose (Avicell). Hence, the isolated *Termitomyces* may be incapable of using cellulose in the studied termite nest. As the absence of cellulase activities in the extracts (both comb and culture) and the inability to grow on pure cellulose (Avicell) are unpredicted properties of the fungus, results of this study may add some important data on the ongoing debate for the association between *Macrotermitinae* termites and *Termitomyces* mushrooms.

Trichoderma virens had efficiency degraded calciumphosphate and lipid in 70% sea water phosphate agar and tributyrin agar (Figure 6 G-H). The another one as *T.virens* had also the high efficacy cellulase and protease secretion as 87.61 and 62.39 milliunit/ml and moderately in phosphatase and lipase activities as 24.07 and 19.20 milliunit/ml (Figure 25B). Production of various enzymes by estuarine fungi had effected degraded and destroyed cell wall of mycelia plant pathogenic fungi. The mechanism of *Trichoderma* action on pathogens attacked and binded the pathogenic organisms by sugar linkage and beginning to secrete extracellular enzyme as protease and lipase (Cal *et al*., 2004). Moreover, these antagonistic produced phytohormones, vitamins and solubilizing mineral besides which promoted the tomato growth and fruit gield (Bouziane *et al*., 2011; Srivastava *et al*., 2010).

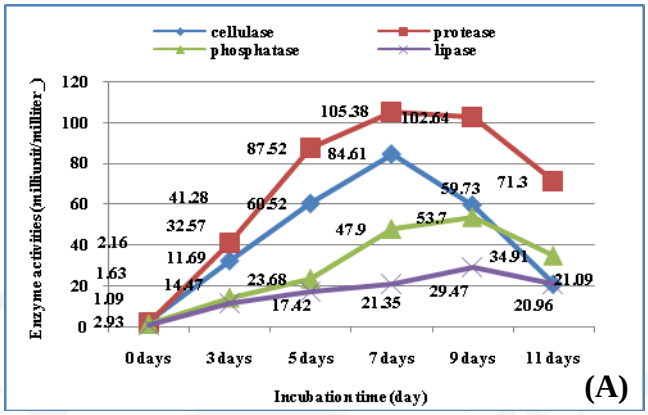


Fomitopsis ostreiformis

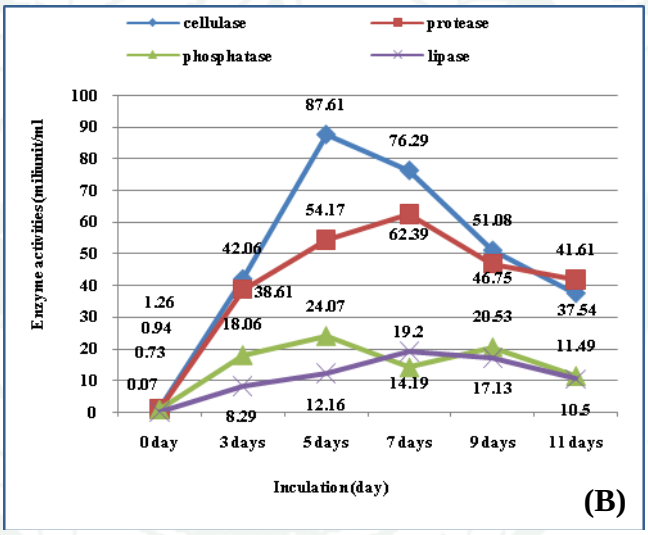


Scytalidium hyalinum

Figure 24 Activities of cellulase, protease, phosphatase and lipase produced by *Fomitopsis ostreiformis* (A) and *Scytalidium hyalinum* (B)during 11 days of incubation.



Termitomyces cartilagineus



Trichoderma virens

Figure 25 Activities of cellulase, protease, phosphatase and lipase produced by *Termitomyces cartilagineus* (A) and *Trichoderma virens* (B) during 11 days of incubation.

7. Biological control of Phythophthora foot rot and Rhizoctinia leaf bright of durian *in vivo*

T. virens showed high efficacy in suppressing mycelial growth of *Phytophthora palmivora* (Figure 27) and *Rhizoctonia solani* (Figure 28), causing 100% of inhibition on PDA, incubated for 3, 5 and 7 days at 28^{O}C. For the incubation time 7 days, *Trichoderma* mycelia overgrew the colonies of two plant pathogenic fungi which indicated interactions of nutrient competition and parasitism against plant pathogens. Highly efficient potential of *T. virens* has able to interaction as colonization of the rhizosphere in soil and protecting soil-borne plant pathogens including producing various enzymes such as cellulase proteinase phosphatase and lipase. These enzymes could degrade cell wall of plant pathogens and penetrate hyphal bioagents amd [emetrate hyphal bioagents for absorping organic substance within the host mycelium. Furthermore, *T. virens* promotes the plant growth and induces the defensive mechanisms for increasing plant resistance. For the experiment, *T. virens* was applied as biological agent for suppression of *Phytophthora* foot rot and *Rhizoctonia* leaf blight of durian, Monthong cultivar.

1. Evaluation of carrier media for biomass multiplication of *Trichoderma*

After inoculated with 7 mm. mycelia mat and incubated 28ºC for 5 days, the number of colony forming with was recorded. *T. virens* grew very well on the solid substrate media and produced sporulation at 5.7 x 10^{9} CFU/g dryweight of substrate (Figure 26).

This fungus grew very well in sorghum rice husk substrate and produced maximum number of colony forming units as 9.76 log no./g of media for 5-7 days of incubation (Table 7). The fifth day of cultivation was the appropriate incubation time for biomass multiplication of *T. virens* (Figure 29).

Table 7 Comparative sporulation of Trichoderma virens in cultivated sorghum with rice husk media during 9 days of incubation at 28ºC.

Incubation time (days)	Trichoderma virens sporulation (CFU/g of media)
0	2.4×10^2
1	4.8×10^4
2	2.9×10^6
3	3.8×10^7
4	9.2×10^8
5	5.7×10^9
6	5.1×10^9
7	6.0×10^9
8	5.4×10^9
9	4.2×10^9

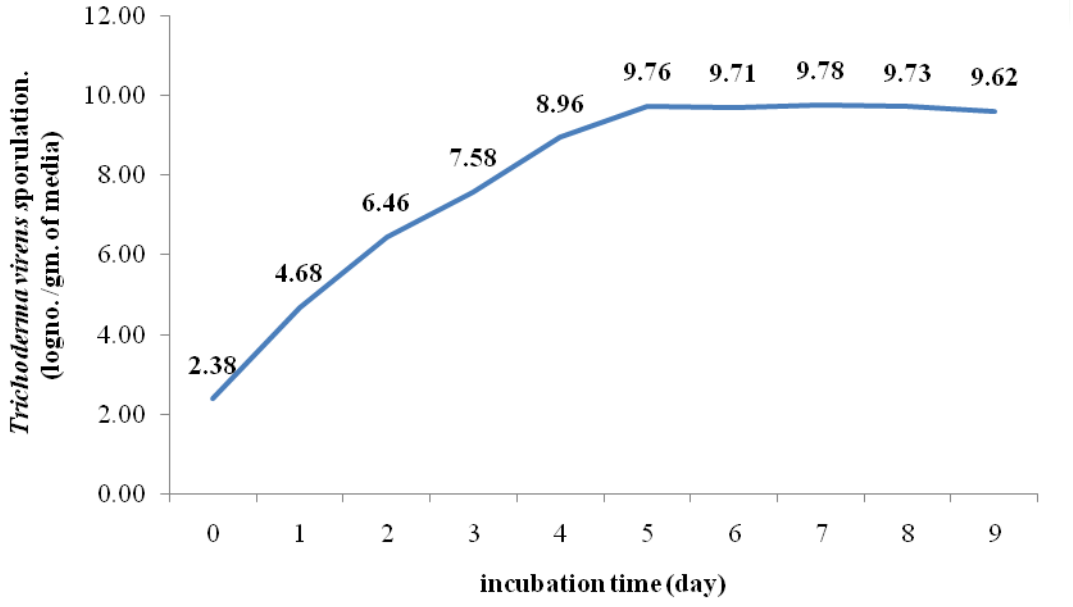


Figure 26 Comparative sporulation Trichoderma virens cultivated in sorghum with rice husk media during of incubation at 28ºC.

2. Evaluation of carrier media for producing bioagent of *Trichoderma*

The biomass of *T. virens* was carried out by incorporating sterilized compost carrier in the mixture using the proportion ratio of Trichoderma biomass to compost as 100 gm *Trichoderma* inoculums (by fresh weight) : 5 kgs sterilized compost. The mixture of *Trichoderma* compost were taken in airdried condition for 3-5 days. *Trichoderma* sporulation in compost carrier were evaluated as 10^8 CFU/gm dry weight, which readily applied in the field condition compost is the organic substance as a rich source of nutrients and energy or carbon source for the active population of beneficial soil microorganisms especially *Trichoderma*-enriched antagonist and biofertilizer (Guest *et al*. 2004).

3. Evaluation of *Trichoderma* biomass multiplication under liquid condition for the field experiment

Foliar spray method of *Trichoderma* bioagent was applied were prepared by water solubilization which siddolved 1 kg of *Trichoderma* compost in 200 litres water for 20 durian trees. Water vacuum machine was weed for the foliar spray preparation. The *Trichoderma* solution dosage was 10 litres per durian tree. Application usage of foliar spray in durian plantation areas were practiced by spraying in the soil beneath durian tree, spraying at or around the foot stem of durien tree and spraying at the whole leaves and branches including the stem of durian tree. Foliar spray timing of *Trichoderma* solution were spraying every 15 days in the durian plantation areas. The colonial number of *Trichoderma virens* in foliar spray solution were 10^7 CFU/ml. The results indicated that the symptom of foot or stem durian disease and leaf blight durian disease became developing healthy bark tissues and leaves formation of durian tree after *Trichoderma* foliar spray every 15 days for 4-6 months.

4. Effect of *Trichoderma* bioagent for controlling *Phytophthora* foot rot and Rhizoctonia leaf blight of durian in Vitro

The results revealed that *Trichoderma virens* had been shown to be potential biomass control agent to suppress soil-borne plant pathogen as *Phytophthora palmivora*, foot rot diseases of durian (Figure 30A-30B) and *Rhizoctonia solani*, leaf blight disease of durian (Figure 30C-30D).

SEM photomicrographs (Figure 31A-31B) showed that the alkaliphilic fungus, *T. virens* grew very well and transform multiplication of microbial and conidial biomass in dual culture test against *Phytophthora palmivora* on PDA for 7 days. This fungus rapidly increase sporulation by overgrowing, dertroying encrochment, commission straps and absorption within the host mycelium the hyphal *Trichoderma* penetrated into the *Phytophthora* hyphae and biomass multiplication of *T. virens* in conidial and mycelia forms. Finally, the hyphal *Phytophthora* was lyzed (Figure 31C-31D).

For the dual culture test of antagonistic interaction between *T. virens* and *Rhizoctonia solani*, SEM photomicrographs indicated that the bioagents showed against plant pathogens by involving the mechanisms of competition ability to fungal pathogen for nutrients. *T. virens* produced biomass multiplication of conidia whereas cailing hyphal *Rhizoctonia* and absorping liquid substance or protoplasm within the host mycelium (Figure 32A-32B). Hyphal lysis formation of R. solani was degraded which involved enzymatic production from *T. virens*. Enzyme activities of *T. virens* had an important role for lyzing cell wall of hyphal pathogens (Figure 32C-32D).

Biological activating and organic matter in soil is the prominent role the contribution for suppression of soil-born *Phytophthora* diseases (Halsalt and forrest, 1977). Mode of interaction for the inhibit growth of *Phytophthora* involed hyperparastism, antibiotic production, competition, enzyme production and induced resistance (Konam and Guest, 2002). The resulting biological control affected on *Phytophthora* had reduced differentiation and release of zoospores, decreasing

movement of zoospore from sporangium, reduced inoculums potential of pathogen, and increased lysis of hyphal cell walls pathogen (Figure 33A-33B).

The alkaliphilic fungus, *Trichoderma virens* produced highly concentration of enzyme production in liquid and solid state fermentation. The enzyme activation highest of cellulase, protease, phosphatase and lipase produced by *T. virens* were 87.61., 62.39,24.07 and 19.2 milliunit/ml during 5 to 7 days of incubation. Microbial cellulose enzyme activitive played a significant role in suppression of *Phytophthora* (Richter, 2009). Cellulase production from T. virens effected reducing and degrading vegetation hyphae of *Phytophthora* sporangia and chlamydospore although vegetative hyphae have a lower cellulose content than two propagules. Cellulase directly impacted *Phytophthora* cell wall which degraded the cellulosic component of this pathogen.

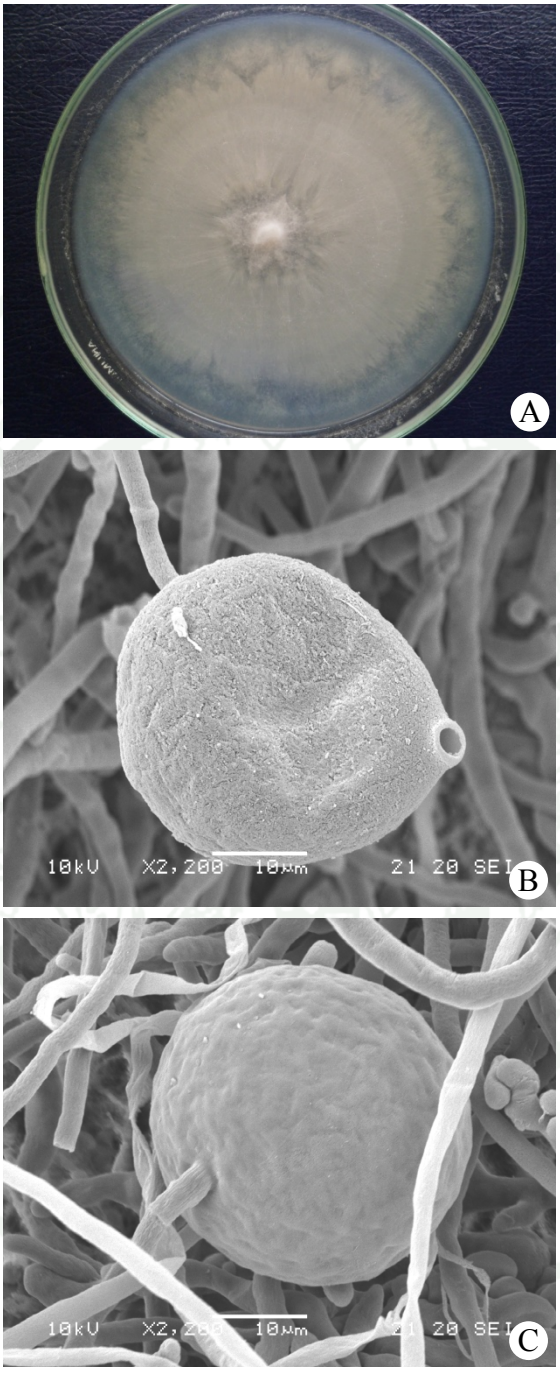
SEM photomicrograph showing the diseased bark tissure of durian, there were mycoparasitic interactions of Tricoderma virens against *Phytophora palmivora* by penetration of antagonist to the sporangia of pathogen. Furthermore, SEM photomicrograph showing potential habitat of *T. virens* alive in the diseased bark in spore formation tissues of durian in spore formation mycelia of *T. virens* colonized the bark tissues for protecting and destroging the host mycelium of *P. palmivora* (Figure 33A-33B and Figure 34). In addition to SEM photomicrograph showing mycotic potential habitat of spore formal *Trichoderma virens* alive in the root tissues of durian. The spore formation of *T. virens* could alive in the cell of cortex layer (Figure 35A-35D). After foliar spraing *Trichoderma* inoculum to durian tree every 15 days for 6 months, the bark tissues of durian tree became dried and production healthy bark tissue and leaves of durian formation (Figure 36B and 37B). The wound of bark brown color and falling leaf blight before spraying *Trichoderma* suspension (Figure 36A and 37A).

In *vitro* research of cellulase impaction on cell walls of *Phytophthora cinnamomi*, the results showed that addition of 10-25 unit/ml cellulase to *Phytophthora cultures* in soil extract impaired zoospores and chlamydospores

development (Department *et al*. 2001). Cellulase concentrations greater than 25 unit/ml had effected disruption of Phytophthora mycelium.

Phytophthora cell walls are consisted of primarily (80-90%, dry weight) of β-linked glucose polymers, proteins, lipids and other polysaccharides comprising the remainder of the wall mass (Broadbent and Baker, 1974). The β-glucan component is divided into cellulosic (primarily β-1, 4-linked) and non-cellulosic (primarily β-1, 3- and β 1, 6-linked) glucans. Cellulose content is higher in sporangial and cyst walls (chlamydospore walls) than in hyphal walls.Numerous antagonistic fungi against *Phytophthora*, in cluding *Gliocladium virens*, *Trichoderma viride* and *Chaetomium globosum*, are also known cellulase producers which has been shown to reduce production of sporangia, zoospores and chlamydospores by *P. cinnamomi* at concentrations of 10 units/ml or greater in soil extract. The organic fertilizers-amended mulch had effected inhabiting antagonist stimulation for reducing *Phytophthora* disease by the host mycelium lysis.

In addition, calcium content and soil pH level are factors the suppression of soil-borne *Phytophthora* diseased. The concentrations above 0.71 mM of calcium amendment in soil inhibited sporangia production in *Phythopthora* species whereas low calcium concentrations sporangial contributed numbers (Halsall and Forrester, 1977). Calcium concentration in soil has involved soil pH levels. The soil pH range between 5.0 and 6.0 has effected the favorable *Phytophthora* reproduction.



Figrue 27 SEM photomicrograph showing ; colony of *Phytophthora palmivora* on PDA incubated for 10 days at 28◦C. (A), sporangium of *Phytophthora palmivora*.(B), spherical chlamydospore of *Phytophthora palmivora* (C).

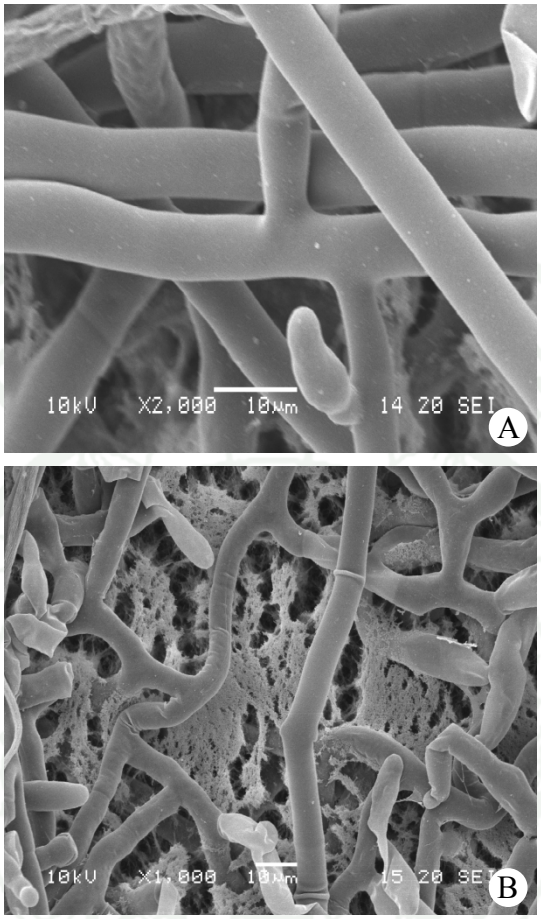


Figure 28 SEM photomicrograph of *Rhizoctonia solani* producing vegetative mycelium and the hyphae often branch at **right** 90º angles (A) and hyphal anatomosis (B).

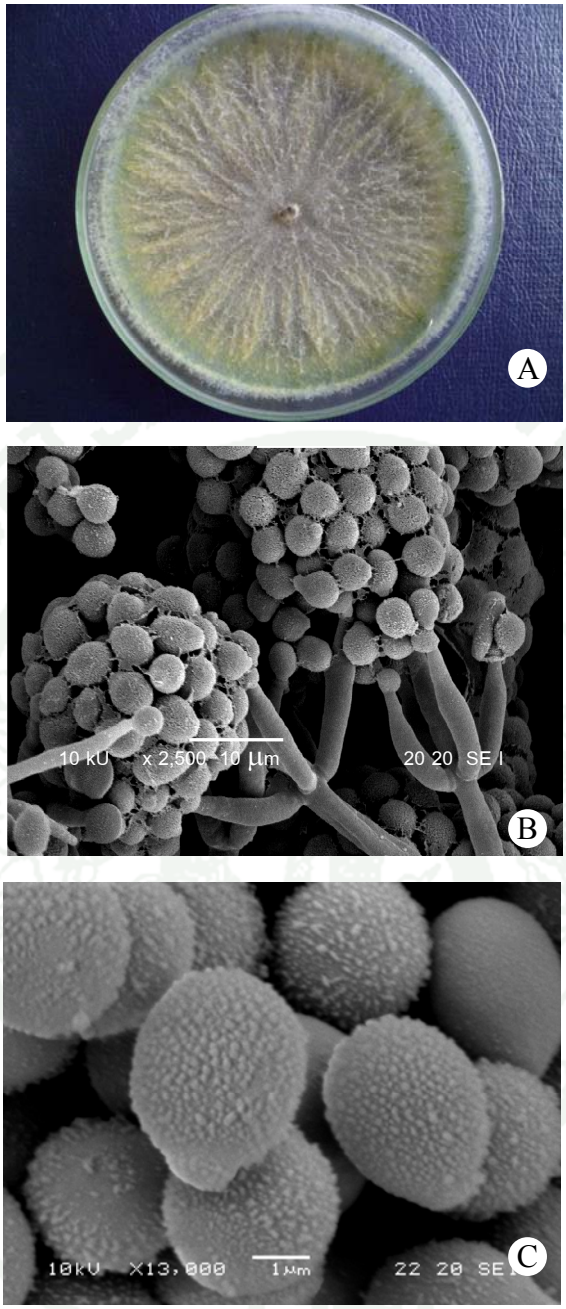


Figure 29 Colony of *Trichoderma virens* on PDA incubated for 5 days at 28°C (A), SEM photomicropraph showing; conidiophores, phialide s**,** conidia (B), roughy wall conidia of *Trichoderma virens* and high magnification of conidia (C).

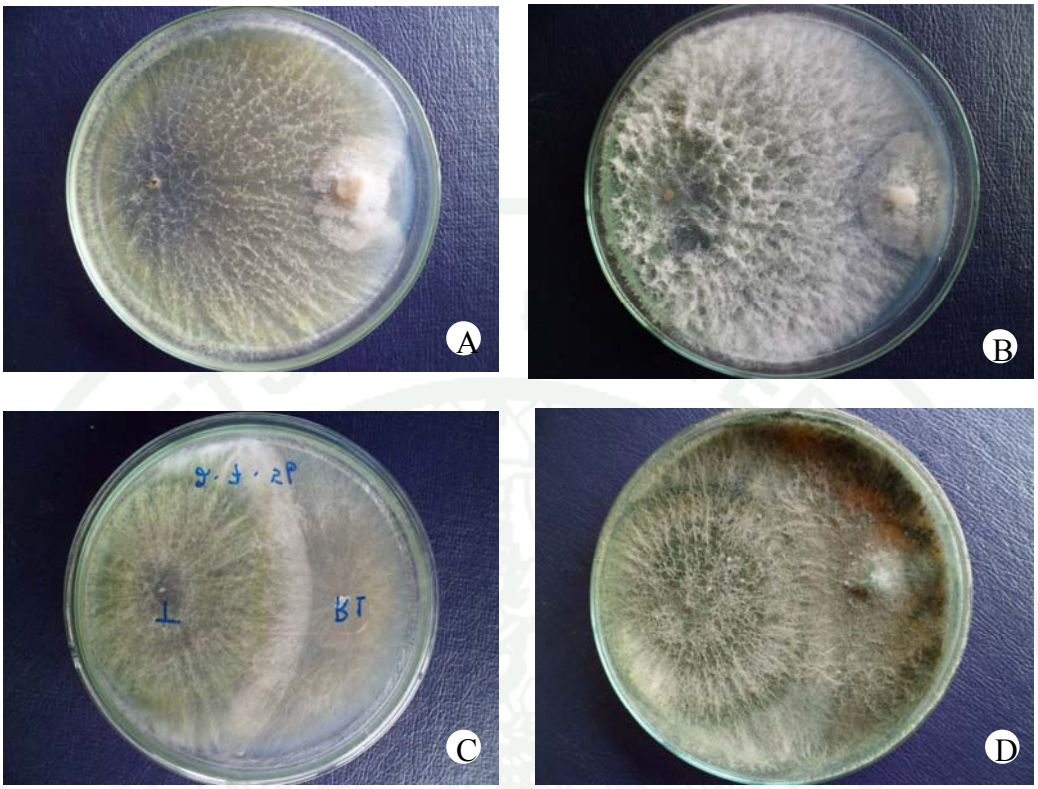


Figure 30 Alkaliphilic fungus, *Trichoderma virens* (left) aganinst *Phytophthora palmivora* (A) and *Rhizoctonia solani* (B) and plant pathogenic fungi (right) in dual culture on PDA incubated for 7 day at 28°C *Trichoderma virens* vs *Phytophthora palmivora* (A), (B) and *Rhizoctonia solani* (C).

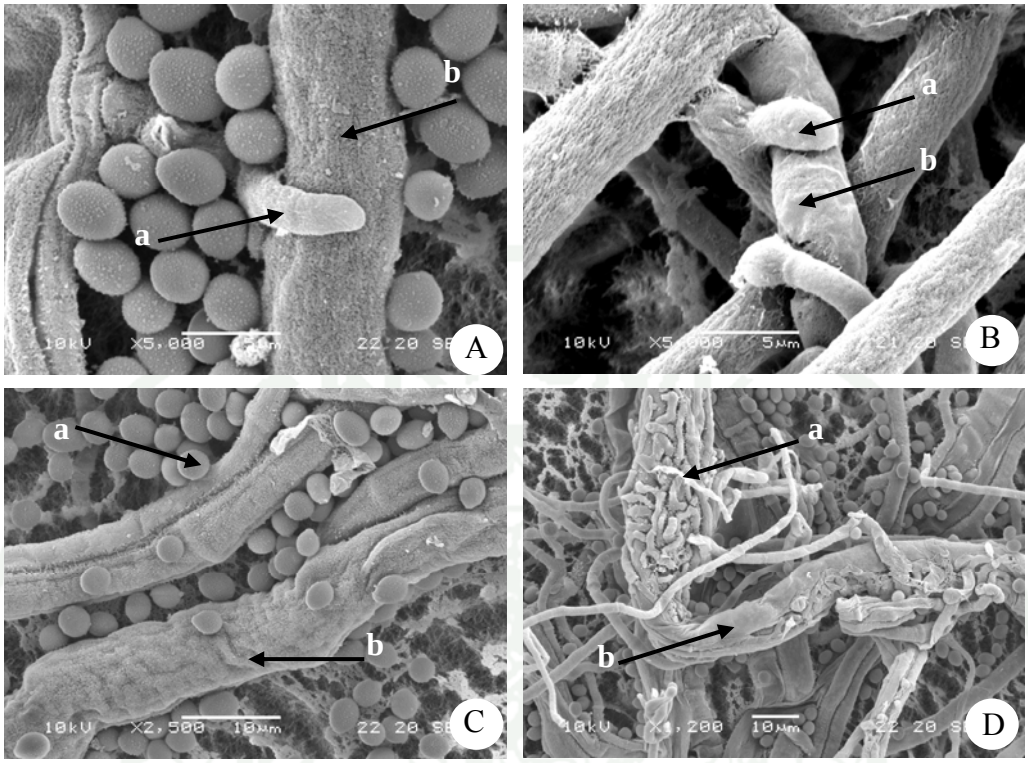


Figure 31 SEM photomicrograph showing antagonistic activity of the alkaliphilic fungus, *Trichoderma virens* (a) against *Phytophthora palmivora* (b); commission straps the host mycelium (A), (B), *Trichoderma* biomass of mycelium and conidia within the host mycelium (C), (D).

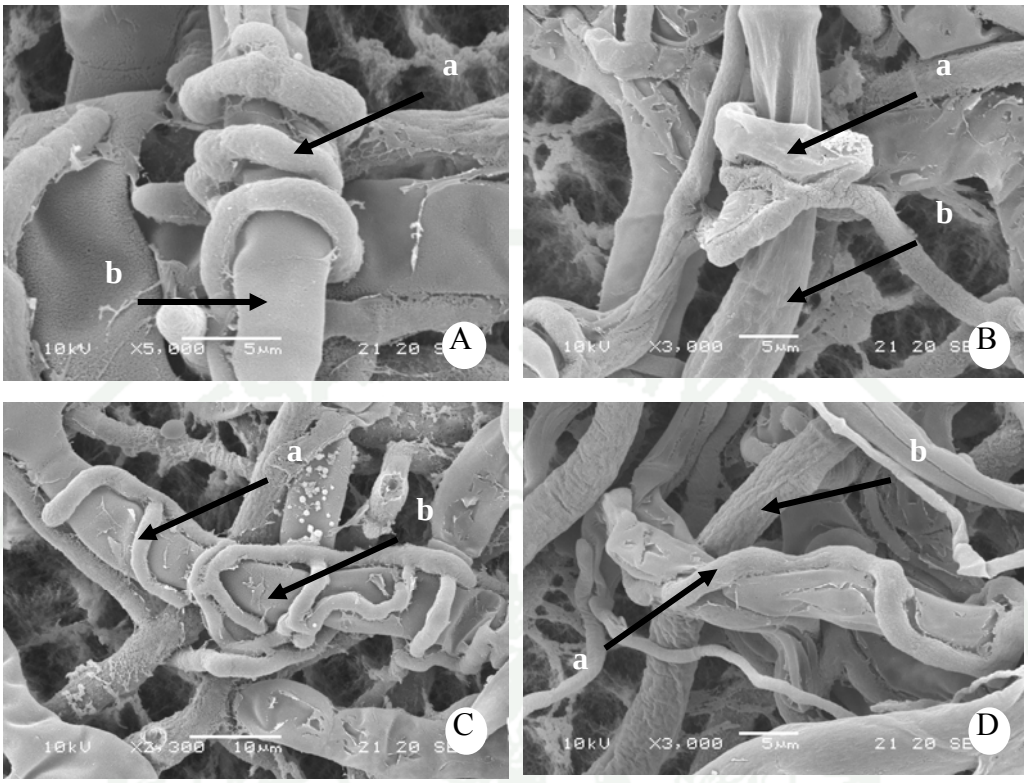


Figure 32 SEM photomicrograph showing antagonistic activity of alkaliphilic fungus, *Trichoderma virens* (a) against *Rhizoctonia solani* (b) by coiling around the pathogen hypha (A,B); and closely attaching on the hypha (C,D).

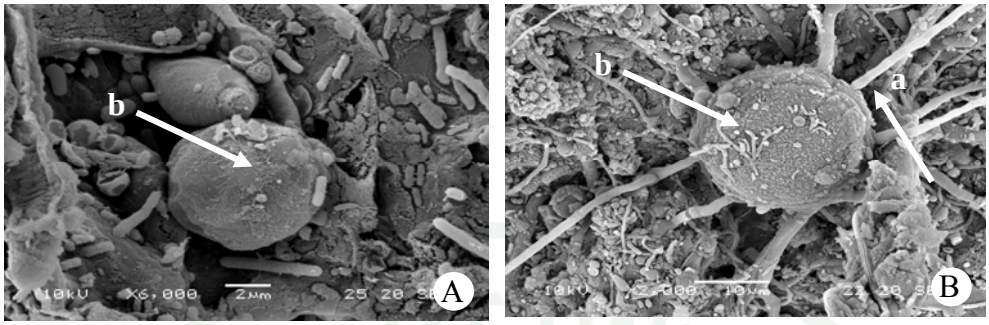


Figure 33 SEM photomicrograph showing mycoparasitic interaction hyphae of *Trichoderma virens* (a) against sporangium of *Phytophthora palmivora* (b) in the wound of durian; Antagonistic fungus parasitized the host sporangium (A), (B).

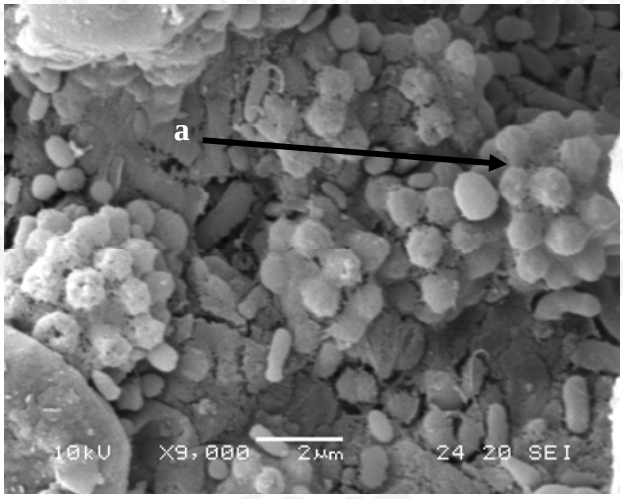


Figure 34 SEM photomicrograph showing potential habitat of *Trichoderma virens* alive in the diseased bark tissues of durian; *T. virens* produced spores in the bark tissues (a).

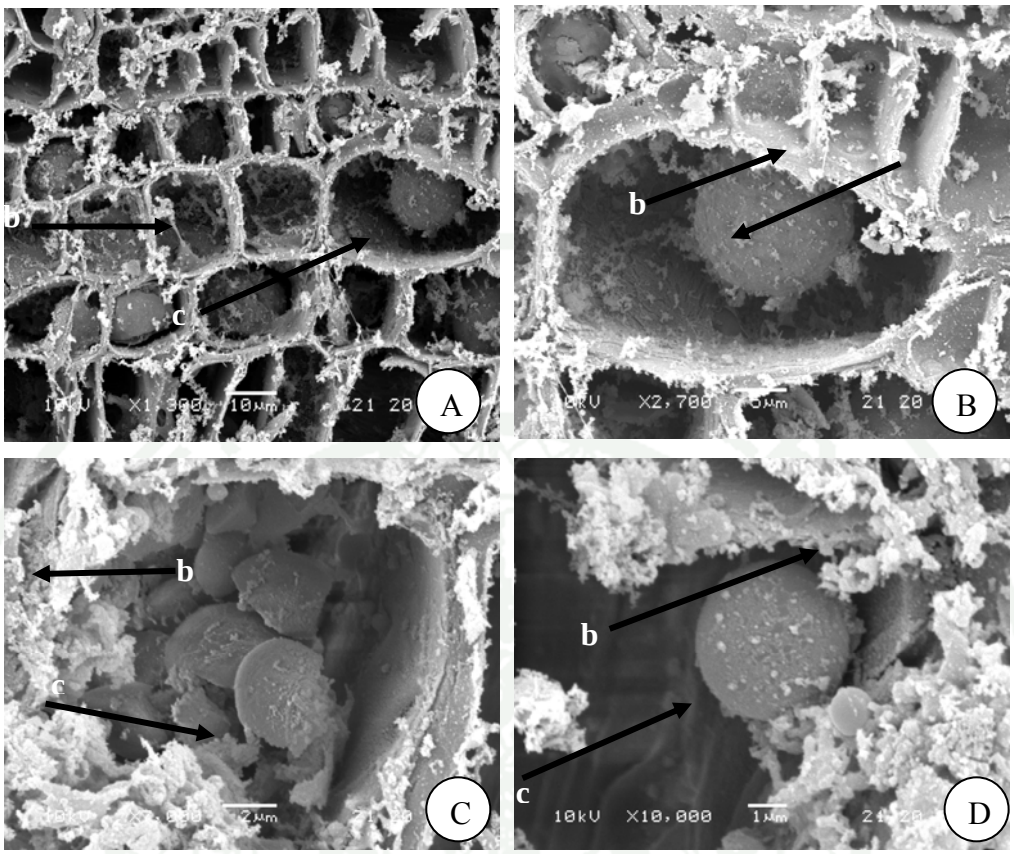


Figure 35 SEM photomicrograph showing mycoparasitic potential habitat of spore formation *Trichoderma virens* within the root cortex of durian: conidia (a) and cortex layers (b) spore of *Trichoderma virens* alive within the cell of cortex layer (A-D).

Figure 36 Comparison on foot rot symptom of durian before and after foliar spray with *Trichoderma* spore suspension at 10^6 CFU /ml every 15 days for 6 months ; the wound of bark tissue of durian was dark brown color before spraying *Trichoderma* (A) and the bark surface became dried and producing healthy bark after foliar spray application(B).

Figure 37 Comparison on *Rhizoctonia* leaf blight symptom of durian before and after foliar spray with *Trichoderma* spore suspension at 10^6 CFU/ml every 15 days for 4 months leaf; blight symptom of durian before spraying (A), durian leaves became developing healthy leaves after foliar spray application (B).

CONCLUSION

Two species of halophilic endophytic fungi namely, *Fusarium equiseti* and *Nectria rigidiuscula* were isolated from healthy leaves of *Acacia ampliceps* (Family Fabaceae) obtained from the areas of highly saline soil (pH 9.2) at Amphoe Kham Thale Sor, Nakhon Ratchasima Province. Identifications were based on morphological characters under light microscope and 28 S rDNA sequence. *In vitro* antagonistic activity test showed that *F. equiseti* and *N. rigidiuscula* suppressed more than 80% mycelial growth of *Alternaria brassicicola, Curvularia oryzae, Phytophthora palmivora* and *Pythium aphanidermatum.* In regard with enzyme production, *F. equiseti* and *N. rigidiuscula* strongly digested lipid.

Four species of alkaliphilic fungi including *Fomitopsis ostreiformis*, *Scytalidium hyalinum*, *Termitomyces cartilaginous* and *Trichoderma virens* were found from organic residues of salt tolerance natural vegetation of *Acacia ampliceps* Maslin, *Fimbristylis schoenoides* (Retz.) Vahl and *Pluchea indica* (L.) Less. obtained from the areas of medium and highly saline soil (pH 8.7-9.6) at Amphoe Kham Thale Sor, Nakhon Ratchasima Province and Amphoe Bampai, Khonkean Province.

In vitro antagonistic test showed that *Fomitopsis ostreiformis, Scytalidium hyalinum* and *Termitomyces virens* inhibited 100% mycelial growth of *Alternaria brassicicola, Curvularia oryzae, Helminthosporium oryzae, Colletotrichum capsici, Pythium aphanidermatum* and *Rhizoctonia solani,* whereas *Termitomyces cartilagineus* inhibited 100% mycelial growth of *A.brassicicola, H. oryzae, C.capsici, P. aphanidermatum* and *R. solani*, but only inhibited 60% mycelial growth of *Curvularia oryzae*. These four species of alkaliphilic fungi strongly digested Calcium phosphate and lipid under alkali condition but slightly degraded Calcium phosphate and lipid in normal condition.

In vitro and *in vivo* antagonistic activity test of *Trichoderma viride* against *Phytophthora* root rot and *Rhizoctonia* sheath blight of durian were conducted. The results revealed that after foliar spray with 10 litres of 10^6/ml spore suspension of *T.*

virens to a durian tree every 15 days for 6 months, the root and stem tissue were recovering and producing new and healthy tissues. SEM photomicrographs showed the hyphae and conidia of *T. viride* in the inner root cortex of durian.

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APPENDIX

1. Gochenaur's Glucose Ammonium Nitrate Agar (GAN)

NH_4NO_3	1.0	g
K_2HPO_4	1.0	g
$MgSO_4.7H_2O$	0.5	g
Rose Bengal	0.03	g
Yeast extract	1.0	g
Glucose	5.0	g
Agar	15.0	g
Streptomycin solution	30.0	ppm
Distilled water	1,000	ml

Addition of 30 ppm streptomycin sulfate 1.0 ml after finishing autoclaved culture media 175 ml

2. Cellulose Agar (Selective media for cellulolytic fungi)

	gram/1,000 ml	
Cellulose (CMC)	6.0	g
$(NH_4)_2SO_4$	2.0	g
KH_2PO_4	0.6	g
K_2HPO_4	0.4	g
$MgSO_4.7H_2O$	0.5	g
Yeast Extract	1.0	g
Agar	18	g
Trace element MC	1	ml
H_2O	1,000	ml

Addition of 30 ppm streptomycin sulfate 1.0 ml after finishing autoclaved culture media 175 ml

3. Calcium Phosphate Agar (Selective media for phosphate solubilizing fungi)

	gram/1,000 ml	
Glucose	10.0	g
$Ca_3(PO_4)_2$	5.0	g
$(NH_4)_2SO_4$	0.5	g
NaCl	0.2	g
$MgSO_4.7H_2O$	0.1	g
KCI	0.2	g
Yeast Extract	0.5	g
$MnSO_4.7H_2O$	a little	g
$FeSO_4.7H_2O$	a little	g
Agar	18	g
H_2O	1,000	ml

Addition of 30 ppm streptomycin sulfate 1.0 ml after finishing autoclaved culture media 175 ml

4. Skim Milk Agar (Selective media for proteolytic fungi)

	gram/1,000 ml	
Glucose	1	g
Skim Milk	5	g
K_2HPO_4	0.2	g
$MgSO_4.7H_2O$	0.2	g
$FeSO_4.7H_2O$	a little	
Agar	18	g
H_2O	1,000	ml

Addition of 30 ppm streptomycin sulfate 1.0 ml after finishing autoclaved culture media 175 ml

5. Selective media for lipolytic fungi

	gram/1,000 ml	
Yeast Extract	2	g
Peptone	3	g
T10 Tributyrin	1	g
Agar	18	g

Addition of 30 ppm streptomycin sulfate 1.0 ml after finishing autoclaved culture media

6. Potato Dextrose Agar

	gram/1,000 ml	
Potato	200	g
Deatrose	20	g
Distilled water	1,000	ml

Addition of 30 ppm streptomycin sulfate 1.0 ml after finishing autoclaved culture media 175 ml

7. Cellulose Liquid Media (Selective media for cellulolytic fungi)

	gram/1,000 ml	
Filter paper Whatman No1	6.0	g
$(NH_4)_2SO_4$	2.0	g
KH_2PO_4	0.6	g
K_2HPO_4	0.4	g
$MgSO_4.7H_2O$	0.5	g
Yeast Extract	1.0	g
Trace element MC	1	ml
H_2O	1,000	ml

Addition of 30 ppm streptomycin sulfate 1.0 ml after finishing autoclaved culture media 175 ml

8. Calcium Phosphate Liquid Media (Selective media for phosphate solubilizing fungi)

	gram/1,000 ml	
Glucose	10.0	g
$Ca_3(PO_4)_2$	5.0	g
$(NH_4)_2SO_4$	0.5	g
NaCl	0.2	g
$MgSO_4.7H_2O$	0.1	g
KCI	0.2	g
Yeast Extract	0.5	g
$MnSO_4.7H_2O$	a little	g
$FeSO_4.7H_2O$	a little	g
H_2O	1,000	ml

Addition of 30 ppm streptomycin sulfate 1.0 ml after finishing autoclaved culture media 175 ml

9. Skim Milk Liquid Media (Selective media for proteolytic fungi)

	gram/1,000 ml	
Glucose	1	g
Skim Milk	5	g
K_2HPO_4	0.2	g
$MgSO_4.7H_2O$	0.2	g
$FeSO_4.7H_2O$	a little	
H_2O	1,000	ml

Addition of 30 ppm streptomycin sulfate 1.0 ml after finishing autoclaved culture media 175 ml

10. Tributyrin Liquid Media (Selective media for lipolytic fungi)

	gram/1,000 ml	
Yeast Extract	2	g
Peptone	3	g
T10 Tributyrin	1%	

Addition of 30 ppm streptomycin sulfate 1.0 ml after finishing autoclaved culture media 175 ml

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