Chapter 1

Introduction

Plants are usually associated with diverse microorganisms. Endophyte microorganisms are those that colonize the healthy plant internal tissue (Stone J.K. *et al*., 2004). The meaning of term "endophyte" is as broad as its literal definition and spectrum of potential hosts and inhabitants. Endophytes are used for both bacteria and fungi (Schulz, B. *et al*., 2006). Modern usage of the term endophytic fungi in mycology refers to those fungi which live within leaves stems roots and other part of apparently healthy host plants and can not be seen in visually signs of infection (Stone J.K. *et al*., 2004). Dreyfuss and Chapela (1994) estimated that there may be at least one million species of endophytic fungi alone. It means that almost all plant species are usually infected with endophytic fungi.

Recently endophytic fungi have attracted the attention from many scientists in the world as estimated that such species may useful as sources of anticancer, antidiabetic, insecticidal and immunosuppressive compounds (Strobel, G. and Daisy, B., 2003).Endophytic fungi from medicinal plants can be used for the development of drugs. For example *Fusarium oxysporum* isolated from *Catharanthus roseus* plant can produce Vinblastine and Vincristine, excellent anti-cancer drugs (Kumar A. *et al*, 2013). Some species of endophytic fungi from Chinese medicinal plant *Actinidia macrosperma* can inhibit the tumour cells (Yin Lu *et al*, 2011). The differences in diversity and types of endophytic fungi depend on their host plants and changes in environment (Gange *et al*., 2007). For the beneficial role of endophyte in plants can be divided into two categories based on types of activity: growth promotion and disease control (Anu Rajan, S., 2012). It can directly enhance plant growth, increase the host plants' adaptability to such diverse conditions as dry, cold, and high-salt environments, and help to resist pathogen damage (Waller F., 2005) as well as being able to alter the community structure (Purahong W, Hyde KD., 2011) and antioxidant activity of the host plants (Hamilton CE., 2012). For example fungal endophyte, *Scolecobasidium humicola* isolated from the roots of tomato can increase the tomato biomass with the organic nitrogen sources (Rola S. Mahmoud and Kazuhiko Narisawa, 2013). They also can produce gibberellin and indoleacetic acid and promote host plant during stress condition (Waqas M. *et al*, 2012).

Orchids are monocotyledonous plants and taxonomically belong to Order Orchidales and Family Orchidaceae. There are 15000 to 25000 of orchid species distributed all around the world, mostly can found in tropical environment. They all can divide in 2 classifications as epiphytic orchids and terrestrial orchids. In Thailand, there are about 500 genus and 800 orchid species. The special hereditary characteristic of orchid species is having many tiny seed in the pod and there are not growing and no endosperm in embryo. So to be geminated, the orchids have to depend on some fungi species to be the energy and food sources such as many nutrients that important for growth (Thamasiri K., 2005).

Orchids are among the plant groups that have aroused most widespread interest among scientists and horticulturists, both for their study and use. They have been subject to particularly high commercial demand over the past 40 years for the beauty of their structure and their vividly colored flowers, steeped in symbolism and mystery. Some countries have declared orchid species as their national flowers (Clemente M., 2009). In Thailand orchids are the most valuable product for export and make money for country. Last years (2013) the export of orchids value 563,134,221 Bart, and the country that most import Thai orchids is China, Japan, U.S.A, India and Vietnam, respectively (Source: Office of Agriculture Economics).

Fungal endophytes have now been investigated in a large number of orchid species from around the world. The traditional approach to identify the fungal endophytes of orchids has been to isolate pelotons from orchid tissues and to maintain fungal colonies in pure culture. The fungi were then identified on the basis of anatomy and morphology including such features as nucleus number, hyphal cross wall structure and spore dimensions (Perkins *et al.* 1995). Worldwide, the fungi involved with orchids are almost all members of the phylum Basidiomycota group, however many do not produce sexual spores, and are consequently assigned to the form genus *Rhizoctonia* (Rasmussen 2002).Although results may vary with the species of orchid examined, it appears that under natural conditions a particular orchid species may associate with only a few or a single fungal species. For example the *Xylaria ssp.* and *Rhizotonia-like fungi* were isolate from root and leaves of genus *Lepanthes* (Bayman, P., *et al*., 1997). Bougoure J.J. and J.D.W. Dearnaley isolated the endophytic fungi form *Dipodium Variegatum* (Orchidaceae) found the *Russula* sp. and non-mycorrhizal Deuteromycetes.

This study aims to investigate the diversity of endophytic fungi by isolation from the roots and leave of 9 orchid varieties in Rajamangala University of Technology Tawan-ok Chantaburi campus.

State of Problem

At present the most farmers always use chemical matter such ass chemical fertilizers insecticide, cause effect to farmer’s health and many toxic matter contaminate to the agriculture products. To investigate for bioactive from nature is the main project of all scientists, to replace of chemical matter. This research project is to isolate endophytic fungi from various orchid varieties, that this is the first step to find the beneficial fungi for further analysis for bio-control in orchid production.

Objectives

1. To isolate the endophytic fungi form various orchid varieties;
2. To identify the species of endophytic fungi from various orchid varieties.

Location of Research

Biocontrol research Laboratory and Mycology Section, Faculty of Agricultural Technology, King Mongkut’s Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand.

Strong crops inter. Co. Ltd. Samut Prakarn province, Thailand.

During of the Research

The research will be conducted from October 2014 to September 2015.

Chapter 2

Review of Literature

The living together of unlike organisms is known as symbiosis. . The term symbiosis (from the Greek: *syn* "with" and *biosis* "living") can play a roll in different kind of associations. In natural ecosystems plants are potential hosts for a broad spectrum of microbes (usually bacteria and fungi), the microbes that live on surface of plants call as epiphytes and microbes that live inside tissue of plant call as endophytes. They can be broadly categorized into mutualism, commensalism and parasitism according to benefits for both partners (mutualistic symbiosis), microbial benefit not affecting the host plant (commensalistic symbiosis) or negative impact on host fitness (pathogenic or parasitic interaction) (Paszkowski, 2006).Endophytes are organisms that live inside the plant without apparent any symptom of infection (Gimenez, *et al*. 2007).

2.1 Endophytic fungi

2.1.1 Definition and classification of endophytic fungi

“Endophyte” was named by Debary (1866) to describe fungi ranging from virulent foliar pathogens to mycorrhizal root symbionts.The term endophyte comes from the Greek (*endo* “within” and *phyton* “plant”) that include a broad spectrum of plant endosymbionts from bacteria to insects colonizing inside any organ of the plant with variable life styles (Schulz and Boyle, 2005).Endophytes include an assemblage of microorganisms with different life style: some endophyte perform as saprophyte on dead or senescing tissue, mutualisms that can protect the host plant from pathogen or can promote growth the host plant, but also can be the latent pathogens and virulent pathogens at early stages of infection. These parasitic interactions may vary from mutualistic to commensalistic to latently pathogenic and exploitive. Phenotypes of the interactions are often plastic, depending on the genetic dispositions of the two partners, their developmental stage and nutritional status, but also on environmental factors (Schulz and Boyle, 2006).

Fungal endophytes are fungi that grow inside living plant tissues for at least part of their life cycle without causing disease symptoms(Stone *et al*., 2004). There is some debate over where the boundaries of this definition should exist (Schulz and Boyle, 2005). Generally, an endophytic relationship refers to a mutualistic relationship with a positive impact on the fitness of both organisms; however, antagonisms in species-specific interactions have been demonstrated (Saikkonen *et al*., 2004, 2006).

Based on phylogeny and life history traits, endophytic fungi were originally organized into two broad groups – clavicipitaceous endophytes (CEs) colonize shoots and rhizomes of a narrow host range of cool- and warm-season grasses (*Poaceae*) and non-clavicipitaceous endophyts (NCEs) have been isolated from shoots and/or roots of almost all sampled plants and are plentifully diverse. More recently, these groups have been divided into four distinct functional classes based on a number of different criterion related to life history. The classification into one of the four classes based on: host range (single species to highly ubiquitous), tissue colonization (localized to general), extent in plantcolonization (limited to extensive) and biodiversity (low to high), mode of transmission (vertical to horizontal) and host, most of them belonging to Ascomycota.(Rodriguez *et al*., 2009).

The CEs (class 1 endophytes) belong to the fungal teleomorphic genera *Epichloë* and *Balansia* correspond to the anamorphs *Neotyphodium* and *Ephelis*, respectively. They form systemic intercellular infections with a hyphal gradient along the plant axis and primarily transmitted vertically by seeds (Schulz and Boyle, 2005; Kuldau and Bacon, 2008).

The NCEs have been divided into three functional groups, class 2 class 3 and class 4. Class 2 endophytes broadly colonize both above and below ground tissues, but with limited biodiversity in individual plant hosts. They are both transmitted vertically and horizontally, sometime colonize non-habitat or habitat adapted benefits to the plant host. Endophytic fungi in this class belong to a few members of the *Agaricomycotina*, *Pucciniomycotina Basidiomycota* and also to *Pezizomycotina* (Ascomycota) such as *Phoma* sp., *Arthrobotrys* sp.,*Fusarium culmorum*, *Colletrichum sp.* and *Curvularia protuberata* that can also found in roots, rhizomes, stems and leaves (Rodriguez *et al*., 2009, Llorca *et al*., 2006).

NCEs class 3 the colonization has restricted to shoots, but with high biodiversity in individual tissues. They have been isolated broadly from tropical forest to boreal and Arctic plant populations. They are horizontally transmitted by wind, rain and insects (Higgins *et al*., 2007; Arnold, 2008; Feldman *et al*., 2008). The Ascomycota that isolated from leaves the most belonging to the *Sordariomycetes*, *Dothidiomycetes*, *Pezizomycetes*, *Leotiomycetes* and *Eurotiomycetes*, while *basidiomycetous* isolates belonging to *Agaricomycotina, Pucciniomycotina.* Their benefits to the plant seem to be more complex and non-habitat-adapted (Arnold *et al*., 2007; Higgins *et al*., 2007; Rodriguez *et al*., 2009.,Vega *et al*., 2010).

Class 4 endophytic fungi colonize only roots. They also known as Dark Septate Endophytes (DSEs), are easily distinguished by their highly melanized septate hyphae. These fungi are limited to the roots of their host, coexisting with mycorrhizal fungi but not growing outward into the rhizosphere. They have a broad host range and belong to different phylogenetic groups among Ascomycota and among non-mycorrhizal members of the order *Sebacinales* (Basidiomycota) (Weiss *et al*., 2004; Addy *et al*., 2005; Selosse *et al*., 2009; Schäfer and Kogel, 2009).

2.1.2 Ecology of fungal endophyte

Fungal endophyte species are found across diverse habitats in the majority of plant species (Vega, 2008).They can reside in root, stem, leaf or multiple tissues (Stone et al.. 2006; Yuan et al., 2009). As refer to many study of their diversity in many plants as follow.

Dandu Anitha *et al* (2013) isolated endophytic fungi from 5 medicinal plants viz *Boswellia ovalifoliolata, Pterocarpus santalinus, Shorea thumbuggaia, Syzygium alternifolium*. 14 fungal species, viz., *Fusarium oxysporum, Colletotrichum falcatum, Pestalotiopsis sp.* , *Aspergillus fumigatus, Aspergillus flavipes, Sterile mycelia, Penicillium senticosum, Gliocladium roseum,Phomopsis jacquiniana, Nigrospora sphaerica, Leptosphaeria sp., Phomopsis archeri* , *Alternaria alternata, Aspergillus niger* were isolated. They found that fungi were most diverse in leaves and root while in stems have low diverse of endophytic fungi. In opposite of this research and these plants Sun Y. et el. (2011) isolated endophytic fungi from *Suaeda* species, annual halophytes growing in soils with high salinity and high concentration of irons where non-halophytes are unable to thrive, found *Alternaria sp.*, Ascomycete sp., *Bipolaris* sp., *Camarosporium* sp., *Cladosporium* sp., *Fusarium* sp., *Penicillium* sp. and *Phoma sp.*the isolation frequency in stems was higher than in leaves.

Glenn A. and Bodri MS. (2012) isolated endophytic fungi from 4 species of the carnivorous pitcher plant genus Sarracenia: S. minor, S. oreophila, S.purpurea, and S. psittacina. Twelve taxa of fungi, 8 within the Ascomycota and 4 within the Basidiomycota, were identified. They speculate that endophyte-infected Sarracenia may benefit from their fungal associates by their influence on nutrient availability from within pitchers and, possibly, by directly influencing the biota within pitchers.

Sun Y. *et al.* (2011) in China isolated endophytic fungi from the stems and leaves of Suaeda sp. found 2 species i.e. *Alternaria* sp. and *Phoma* sp.

Saithong P. *et al.* (2010) from Thailand isolated the endophytic fungi from the bark of *Cephalotaxus mannii* (plum-yew) found 13 genera, viz. *Cladosporium* sp., *Acremonium* sp., *Trichoderma* sp., *Monilia* sp., *Fusarium* sp., *Spicaria* sp., *Humicola* sp., *Rhizoctonia* sp., *Cephalosporium* sp., *Botrytis* sp., *Penicillium* sp., *Chalaropsis* sp. and *Geotrichum* sp.

Kumar, D.S.S. and Hyde, K.D. (2004) reported that 60 taxa including 30 morphotypes were isolated from the different parts of the Chinese medicinal plant, *Tripterygium wilfordii*.The endophytic assemblages of *T. wilfordii* comprised a number of cosmopolitan species such as *Colletotrichum gloeosporioides, Guignardia* sp., *Glomerella cingulata, Pestalotiopsis* sp., *Phomopsis* sp. and *Phyllosticta* sp.The overall fungal community of *T. wilfordii* was moderately diverse. The fungal community from the twig xylem parts was most diverse, followed by leaves, twig bark, root xylem and flowers.

As follow study we can indicate that endophytic fungi ecology are very diverse, but there is a question that how can endophytic fungi colonize in plant tissue from one generation to one generation. About this question Yuan *et al.* (2009) conclude that endophytic fungi transfer from each plants by 2 ways. The first one is vertical transfer that direct transfer by seeds. The second is horizontal transfer that can transfer by air, rain and insect in environment who have high taxonomic and host diversity, and can broadly colonize all plants in an eco-system.

2.1.3 Secondary metabolites produced by edophytic fungi

Endophytes may produce new bioactive substances because of a continual metabolic interaction with their hosts and the environment and this metabolic interaction is important for the symbiosis of both partners (Schulz and Boyle, 2005; Suryanarayanan *et al*., 2009). Many researchers try to indicate for antimicrobial from many plants. Below there are many research about isolate antimicrobial substance from endophytic fungi.

From China Su Z-Z *et al*. (2013) investigated the dark septate endophyte (DSE) *Harpophora oryzae* in rice roots and its biocontrol potential in rice blast disease caused by *Magnaporthe oryzae*. The result showed that the colonization pattern of H. *oryzae* was consistent with the typical characteristics of DSEs. H. *oryzae* enhanced local resistance by reactive oxygen species (ROS) and high antioxidative level and induced OsWRKY45-dependent SA-mediated systemic resistance against rice blast. Yin Lu *et al*. (2012) isolated Endophytic fungi from *actinidia macrosporma* investigated for their bioactivity indicated that fungi from this plant exhibited toxicity against brine shrimp and antitumour. Liang H. *et al.* (2012) indicated that endophytic fungi from ornamental plant like *Ophiopogon japonicus* (Liliaceae) reported that this plants also produce metabolite inhibited *Staphylococcus aureus* and *Cryptococcus neoformans*. And in previous year Gao Y. *et al*. (2011) reported that 4 strains of endophytic fungi from roots of Pigeon Pea (*Cajanus cajan* (L.) Millsp.) were producing Cajaninstilbene acid (CSA, 3-hydroxy-4-prenyl-5-methoxystilbene-2-carboxylic acid), CSA is responsible for the prominent pharmacological activities in pigeon pea. The five endophytic isolates were characterized by analyzing the internal transcribed spacer (ITS) rRNA and b-tubulin genes. The 4 strains isolated from pigeon pea roots were found to be closely related to the species *Fusarium oxysporum* and another one was identified as *Neonectria macrodidym*.

While in Philippine Jeremy Martin O. Torres and Thomas Edison E. dela Cruz (n.d.) indicate that crude isolated endophytic fungi from *Canarium ovatum*show that *Colletotrichum* sp. PFE31 crude extract exhibited broad-spectrum antibacterial activity against representative Gram-positive, Gram-negative, and spore-forming bacilli. Melfei E. Bungihan*et al*. (2013) isolated Endophytic fungi from *Pandanus amaryllifolius.* The genera *Colletotrichum*, *Chaetomium, Diaporthe,Glomerella*, *Guignardia*, *Lasiodiplodia*, *Lulworthia*, *Phoma*, *Phyllosticta*, *Trichoderma,* and *Truncatella*. were identified by morphology. The selected species was use for antimicrobial, show that can against *Mycobacterium tuberculosis, Staphylococcus aureus, Eschericia coli,* and *Gordonia terrae*.

Oil-Seed Crop *Jatropha curcas* was selected for endophytic fungi in India by Susheel Kumar and Nutan Kaushik (2013). *Colletotrichum truncatum*, *Nigrospora oryzae*, *Fusarium proliferatum, Guignardia cammillae*, *Alternaria destruens*, and *Chaetomium sp.* were selected from leaf of

Jatropha curcas. Dual plate culture bioassays and bioactivity assays of solvent extracts of fungal mycelia showed that isolates of *Colletotrichum truncatum* were effective against plant pathogenic fungi *Fusarium oxysporum* and *Sclerotinia sclerotiorum*. Susheel Kumar, Nutan Kaushik and Peter Proksch (2013) isolated *Chaetomium globosum* from *Withania somnifera*. They used Ethyl acetate, hexane and methanol extracts method to show the control of *Chaetomium globosum* against *Sclerotinia sclerotiorum*. The result showed that Ethyl acetate and methanol extracts were more effective than hexane extract. Qadri M. *et al*. (2013) reported that endophytic fungi of selected plants from the Western Himalayas, India. Seventy two strains of endophytic fungi were isolated. Several strains of these endophytic fungi were showed that against E. coli and S. aureus. 24 endophytes inhibited three or more phytopathogens 17 fungi possessed immuno-modulatory activities with five of them showing significant immune suppression as demonstrated by the in vitro lymphocyte proliferation assay. Kumar A. *et al* (2013) isolated endophytic fungi from *Catharanthus roseus* plant and found a fungus that can produce vinblastine and vincristine, anti-cancer drugs, in appreciable amounts. That endophytic fungi have identified as *Fusarium oxysporum.*

In Thailand Tiamphet M. and Nuangmek W. (2013) isolated endophytic fungi from Eucalyptus and another 29 species of herbs. Two hundred and seventy endophytic fungi were isolated. Unknown, *Phomopsis* sp. and *Xylaria* sp. were the most common species. Dual culture method was used to screening for evaluation on the potential effectiveness of candidate biological control agents against rot disease of eucalyptus (K7) seedling caused by *Rhizoctonia solani*. Revealed that *Nodulisporium* sp. and *Muscodor* sp. were strong anti-fungal activity against *R. solani* and also have good result in green house. In the same year in southern Thailand Supaphon P et al. (2013) reported that seven isolates endophytic fungi from three seagrasses species, *Cymodocea serrulata*, *Halophilaovalis* and *Thalassia hemprichii*including *Hypocreales* sp. PSU-ES26 from *C. serrulata*, *Trichoderma* sp. PSU-ES8 and PSU-ES38 from *H. ovalis*, and *Penicillium* sp. PSU-ES43, *Fusarium* sp. PSU-ES73, *Stephanonectria* sp. PSU-ES172 and an unidentified endophyte PSU-ES190 from *T. hemprichii* exhibited strong antimicrobial activity against human pathogens with minimum inhibitory concentrations (MIC) of less than 10 μg/ml. The inhibitory extracts at concentrations of 4 times their MIC destroyed the targeted cells as observed by scanning electron microscopy. These results showed the antimicrobial potential of extracts from endophytic fungi from seagrasses.

In Taiwan Min Y.H. *et al*. (2012) isolated endophytic fungi from medical herbs of *Lauraceae* and *Rutaceae*. 156 isolates of endophytic fungi collected from twigs of medicinal plants of Lauraceae (67 isolates) and Rutaceae (89 isolates). The most common endophytes were in the taxa of *Colletotrichum*, *Guignardia*, *Hypoxyl*on, *Nigrospora*, *Phomopsis* and *Xylaria*, and the most common hosts were *Citrus* and *Zanthoxylum* of Rutaceae and *Cinnamomum* of Lauraceae. Endophytic fungi of *Lasmenia* sp. Isolate significantly reduced severity of anthracnose of Chinese cabbage caused by *Colletotrichum higginsianum* under greenhouse conditions.

In Korea Paul N. C. *et al*. (2011) reported that a total of 481 isolates were recoveredfrom the surface sterilized tissues of leaves, stems and roots of chili pepper (*Capsicum annuum* L.) plants in seedling, flowering and fruiting stages. Twenty one fungal genera were characterized, belonging to 16 Ascomycota and 5 Basidiomycota. *Penicillium* in seedling stage, *Fusarium* in flowering stage, *Colletotrichum* followed by *Fusarium, Alternaria* and *Xylaria* in fruiting stage was predominant and *Alternaria, Cladosporium* and *Fusarium* were common in all growth stages.Ninety phenotypes were evaluated for the antimicrobial activity against three major pathogens (*Phytophthora capsici*, *Colletotrichum acutatum* and *Fusarium oxysporum*) of chili pepper. Among them 16 isolates inhibited the growth of at least one test microorganisms. Three strains showed a broad spectrum antifungal activity and displayed strong inhibition against chili pepper pathogenic fungi.

In Brazil Hanada R.E. *et al*. (2010) isolated endophytic fungi from stems and branches of *Theobroma cacao* (cacao) and *Theobroma grandiflorum* trees growing in the Amazon region of Brazil. One hundred and three isolates that could be classified at least up to genus level were tested against P. *palmivora* in pods attached to cacao trees in the field. Results indicated that 70 % of isolates showed biocontrol effects to a certain extent, suggesting that culturable endophytic fungal biodiversity in this system is of a mostly mutualistic type of interaction with the host.

In Panama of USA Herre A.D. *et al*. (2007) isolated endophytic fungi from *Theobroma cacao* and showed that the endophytic fungi against pathogen (*Phytophthora palmivora*).

Endophytic fungi also can promote plant growth to growing up better. They are able to produce phytohormones such as Gibberelin (GAs) and indoleacetic acid (IAA) and mitigate abiotic stresses like salinity and drought. Some research of plant growth promotion of endophytic fung as follow.

Rola S. Mahmoud, Kazuhiko Narisawa (2013) Isolated endophytic fungi from roots of tomatos and cabbages. Two hundred and five isolates have showed that the most fungi dominat by *Fusarium sp.*and a new fungal endophyte, *Scolecobasidium humicola*, was identified as a common dark septate endophytic fungal (DSE) species under both natural and agricultural conditions. This fungus was found to grow endophylically in the roots of tomato seedlings.Two isolates of *S. humicola* have shown the ability to increase plant biomass with an organic nitrogen source. This finding is the first report of *S. humicola* as an endophyte and could help to improve plant growth with organic nitrogen sources.

Waqas M.*et al*. (2012) isolated endophytic fungi from the root of cucumber. 2 strains of fungal endophyte, *Phoma glomerata* LWL2 and *Penicillium* sp. LWL3, were selected and further to examine for their production of gibberlelins (GAs) and indoleacetic acid (IAA). The result showed that both fungi strain can promoted shoot length of two rice varieties, *Waito-C* and Dongjin-beyo rice, The cultures of *P. glomerata* and *Penicillium* sp. also contained IAA. The culture application and endophytic-association with host-cucumber plants increased the plant biomass and related growth parameters under sodium chloride and polyethylene glycol induced salinity and drought stress as compared to control plants.The endophytic symbiosis resulted in higher assimilation of essential nutrients like potassium, calcium and magnesium as compared to control plants during salinity stress. Endophytic-association reduced the sodium toxicity and promoted the host-benefit ratio incucumber plants as compared to non-inoculated control plants. The symbiotic-association mitigated stress by compromising the activities of reduced glutathione, catalase, peroxidase and polyphenol oxidase. Under stress conditions, the endophyte-infection significantly modulated stress through down-regulated abscisic acid, altered jasmonic acid, and elevated salicylic acid contents as compared to control. In conclusion, the two endophytes significantly reprogrammed the growth of host plants during stress conditions.

Khan S.A. *et al*. (2012) investigated plant growth promoting activity of roots inhabiting endophytic fungi in order to evaluate their role in the survival of host plants under extreme sand dune environment of coastal regions. One hundred and twenty two fungal isolates were collected from the roots of 9 sand dune plants and were screened for growth promoting secondary metabolites. The results showed that 101 fungal isolates (82.7%) promoted plant height and shoot length of *Waito-C* rice, while 21 fungal isolates (17.2%) inhibited growth attributes. The fungal isolate *Gibberella fujikuroi* along with distilled water and Czapek broth medium were used as control during the experiment. It was concluded that a major proportion of endophytic fungi inhabiting sand dune plants produce metabolites, which are helpful in plant growth and development.

Hamayun M. *et al*. (2010) isolated endophytic fungi from cucumber (Cucumis sativus L.) found 19 strains of fungi. Pure cultures of 19 endophytic fungi were tested for shoot length promotion of Waito-C rice to identify the GA production capacity of these fungal isolates. One of these isolate showed that increased shoot length of Waito-C, in comparison to control treatments.

2.1.4 Host benefit of infection endophytic fungi

Fungal endophytes, and their effects on plant metabolism, play a large role in phenotypic plasticity and environmental adaptability and likely played a unique role in selection and speciation in many host species (Rudgers *et al*., 2009).Plant can supply nutrients to endophyte, and endophyte will release active metabolites through metabolic activities. Those active metabolites can enhance the roots development and assist solubilizing phosphorus, with which would increase the ability of plants to absorb nutrients from the soil (Li, J.H., *et al*, 2008). Because of those activities, endophyte can promote host growth and supply resistance mechanism of host. As the study of Dai C.C. *et al*. (2008) who inoculated the endophytic fungi *fusarium sp.* strain E4 and E5 that isolated from medicinal plant, *Euphorbia pekinensis*, to the host plant in pot culture. The result exhibited significant increase in growth and all tested growth parameters, as compared to the control.

There is a wide spread phenomenon about the symbiotic relationships between endophytes and plants. But this relationships can switch between mutualistic and parasitic (Kogel, K.-H*et al*. 2006). While a single gene changes can alter the mechanism of endophyte. For example, E. *festucae* which is a wild-type grows systemically in intercellular spaces, can promote the grown and enhance the resistance of plant, but while a single-copy plasmid of a NADPH oxidase gene, noxA, was inserted in the coding region, the plant would lose its apical dominance, become severely stunted, show precocious senescence, and eventually die (Tanaka, A., *et al*, 2006). The same endophyte colonized in different host can react in the different lifestyles. Colletotrichum magna can cause anthracnose in cucurbit plants, but can grow asymptomatically when colonized in various non-cucurbit species(Kogel, K.-H*et al*. 2006).

2.2 Orchids

Orchids are the monocotyledon taxonomy in Order Orchidales, Family Orchidaceae. There are 15000 to 25000 of orchid species distributed all around the world, mostly can found in tropical environment. They all can divide in 2 classifications as epiphytic orchids and terrestrial orchids. In Thailand found 500 genus and 800 orchid species. The special hereditary characteristic of orchid species is having many tiny seed in the pod and there are not growing and no endosperm in embryo. So to be geminated the orchids have to depend on some fungi species to be the energy and food sources such as many nutrients that important for growth (Thamasiri K., 2005).

Orchids are among the plant groups that have aroused most widespread interest among scientists and horticulturists, both for their study and use. They have been subject to particularly high commercial demand over the past 40 years for the beauty of their structure and their vividly colored flowers, steeped in symbolism and mystery. Some countries have declared orchid species as their national flowers (Clemente M., 2009). In Thailand orchids are the most valuable product for export and make money for country. Last years (2013) the export of orchids value 563,134,221 Bart, and the country that most import Thai orchids is China, Japan, U.S.A, India and Vietnam, respectively (Source: Office of Agriculture Economics). It takes many interested in planting orchid for commercial from businessman and horticulturists. So when there are plentiful culture of orchids that's mean it's following by the distributed of disease in 2012 . There are many study that compiled the pathogen fungus on orchids include *Alternaria*sp., *Botrytis cineria*, *Cercospora*sp., *Cladosporium*sp., *Colletotrichum gloeosporioides*, *Curvularia eragrostidis*, *Diplodia*sp., *Drechslera*sp., *Fusarium*sp., *Nectria*sp., *Phyllosticta pyriformis*, *Pseudocercospora dendrobii*, *Phytophthora nicotianea* var. *parasitica*, *Phytophthora palmivora*, *Rhizotonia*sp., *Schlerotium rolfsii* and *Volutela* sp.(Thamasiri K., 2005; Sutthiwari P., 2006).

2.3 Previous study about endophytic fungi in orchids

Juan Chen *et al*. (2013) isolated endophytic fungi from 7 *dendrobium*sp. (*Orchidaceae*) to search for *Xylariaceous* fungi. The result showed that A total of 961 culturable endophytic fungi were isolated from seven species of *Dendrobium*.In the original 961 cultures, the xylariaceous taxa had the highest isolation frequency, 22.58% (217/961), followed by *Fusarium* at 10.71% (103/961), *Colletotrichum* at 6.55% (63/961), and *Phomopsis* at 4.37% (42/961).

Sawmya K. *et al*. (2013) compared the leaf and root endophyte assemblages of two orchids (*Bulbophyllum neilgherrense* and *Pholidota pallida*) from natural forests and greenhouse conditions. The result found that *Xylariaceae* sp. were consistently associated with leaf and root tissues, while *Guignardia* and *Pestalotiopsis* were found predominantly in the leaf tissues of both orchids. The endophyte assemblages showed that the endophytes exhibited distinct organ but little host specificity. Endophytic fungi have more divers in green house condition more than in forest condition. And the roots showed more diverse than in leaves.

Mangunwardoyo W, Suciatmih and Gandjar I. (2012) were isolated endophytic fungi from *Dendrobium crumenatum(*Pigeon orchid*)*and investigated antimicrobial activity against 5 pathogens namely *Candida albicans* ATCC 2091, *Candida tropicalis* LIPIMC 203, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 25923. The result showed that twelve species of endophytic fungi were identified from 60 samples obtained from *D. crumenatum*. namely *Cladosporium cladosporioides*, *C.sphaerospermum*, *Colletotrichum gloeosporioides*, *Colletotrichum* sp., *Curvularia brachyspora*, *Fusarium nivale*, *F.solani*, *Guignardia endophyllicola* (anamorf: *Phyllosticta capitalensis*), *Pestalotiopsis* sp., *Scolecobasidium* sp., *Westerdikella* sp., and *Xylohypha* sp. The dominant endophytic fungi was *G. endophyllicola*. For investigated the antimicrobial showed that *Fusarium nivale* was able to inhibit the growth of *C. albicans* and *C. tropicalis*. However, others specimens did not inhibit the tested bacterial, which are Gram-negative *E. coli* and Gram-positive *B. subtilis* and *S. aureus*.

Kasmir J. *et al*. (2011) isolated endophytic fungi from 4 species epithytic orchids namely *Bulbophyllum kaitiense* Reichebt. *Gastrochilus acaulis* (Lindley) *KuntzeDendrobium nanum* Hook.f and *Geodorum densiflorum* (Lam). Schltr. The two important fungi belonging to tricomaceae members *Aspergillus terreus* and *Penicillium aculeatum.*

Tempesta S. *et al*. (2011) isolated endophytic fungi to screening for *Pestalotiopsis* endophyte from leaves of two orchid species: Among the fungi isolates from orchids 29 strains of *Pestalotiopsis*sp. was discovered such as P. *sydowiana*, P. *photiniae*, P. *leucothoes*, P. *clavispora*, P. *crassiuscula*, P. *rhododendri*, P. *versicolor*, P. *photiniae,* P. *gracilis*, P. *aquatic*, P. *palmarum*, P. *theae*, P. *maculans*, P. *funerea*, P. *neglecta*, P. *disseminata*, P. *adusta*, P. *microspore*, P. *bacilia* and P. *vismiae.*

Valdes M. *et al.* (2011) investigated fungi from root of terrestrial orchid *Cypripedium irapeanum* found 10 isolates 8 strains of fungi : 3 identified as endophytic fungi, *Fusarium*sp. and *Cylindrocarpon*sp., 3 as micorhiza, *Sistotrema*sp., *Rhizoctonia solani* and *Epulorhiza*sp., 3 as dark septate endophytes, MUT 885 and *Phomopsis*sp., 1 as biocontrol fungus, *Gliocladium catenulatum.*

Juntawong J. *et al.* (2010) investigated edophytic fungi from terrestrial orchids *Geodurum* roots in three different seasons in deciduous forest in northern Thailand. The result showed that 198 isolates comprising to 13 genera. The major genera were *Tricoderma*sp. followed by *Fusarium*sp., the least were *Aspergillus*sp., *Colletotrichum*sp., *Eupenicillium*sp. and *Helicoma*sp. In rainy season showed high diversity of fungi more than dry and cold season. This study indicated that changes of physical factors in different seasons had effect on endophytic fungi diversity.

Gezgin Y. and R. Eltem (2009) were investigated in orchids from the Aegean and Mediterranean regions. Four species of orchids as follow *Anacamptis pyramidalis* (L.) L.C.M.Richard, *Orchis sancta* L., *Ophrys fusca* Link., and *Serapias vomeracea* subsp. *orientalis*

Greuter were isolated for endophytic fungi. The result showed that a total of 47 isolates, having genus characterisations as 44 (94%) isolates belonging to the genus *Fusarium*, 2 (4%) isolates belonging to the *Rhizoctonia* DC. ex Fr.-like fungi, and 1 (2%) isolate belonging to the genus *Papulaspora* Preuss, were found from the orchid root and tubers.

Tao G. *et al.* (2008) investigated endophytic fungi from terrestrial orchid *Bletilla ochracea*. There were many species of fungi have revealed in this study. Fourteen species were isolated from the leaves, *Alternaria* sp., *Ascomycete* sp., *Chaetothyriales* sp., *Cladosporium* sp., *Colletotrichum* sp., *Cryptococcus* sp., *Dioszegia* sp., *Epulorhiza* sp., *Gibberella* sp., *Herpotrichiellaceae* sp., *Leptosphaerulina* sp., *Mycosphaerella* sp., *Pestalotiopsis* sp. and *Phaeosphaeria* sp. Nine species were isolated from roots, *Cercospora* sp., *Cylindrocarpon* sp., *Exophiala* sp., *Nectria* sp., *Fusarium* sp., *Gibberella* sp., *Herpotrichiellaceae* sp., *Neonectria* sp. and *Sebacina* sp. As the result of this study micorrhiza fungi were isolated from leaves. The diversity within leaves was higher than that within roots. Fungal communities within leaf and root tissues were significantly different.

Ovando I. *et al.* (2005) screened endophytic fungi in roots of mature plants of epiphytic orchid Cattleya skinneri to test their potential to improve seed germination in C. skinneri and develop in acclimatizing plantlets of C. aurantiaca and Brassavola nodosa. One hudred and eight isolates belonging to 11 genera consist of *Tricoderma*sp., *Verticillium*sp., *Aspergillus*sp., *Epulorhiza*sp., *Fusarium* sp., *Monilliopsis*sp., *Pestalotiopsis* sp., *Botrytis*sp., *Penicillium*sp., *Penicillifer*sp. and *Tetracladium*sp. Eight strains in this result used for promote seed germination of C. *skinneri* and 3 strains used for promote plant growth of B. *nodosa*. None of these strains can promote both of experiments.

Bougoure J.J. and J.D.W. Dearnaly (2005) isolated endophytic fungi from roots of *Dipodium variegatum* found *Russula occidentalis*, R. *salaris*, R. *lepida*, *Verticillium*sp., *Tricoderma hamatum, Halocyphina villosa* and *Merismodes fasciculata.*

Chapter 3

Materials and Methods

The simple will be collected in greenhouse condition from Rajamangala University Technology Tawan-ok Chantaburi Campus, Chantaburi province, Thailand. Samples of Leaves and roots from 9 varieties of selected orchids will be packed in sterile polythene bags to keep moisture and bring to laboratory over 12 hours after collecting. The name of nine orchid varieties will be collected as follows:

1. *Grammatophyllum scriptum*
2. *Cymbidium dayanum* Rchb.F
3. *Dendrobium hercoglossum* Rchb.f.
4. *Dendrobium palpebrae* Lindl.
5. *Torenia fournieri* Lindl. ex Fourn.
6. *Doritis pulcherrima* var. buyssoniana
7. Dendrobium crumenatum Sw.
8. *Dendrobium friedericksianum* Rchb.f.
9. *Grammatophyllum specinocum* BL.

2. Isolation of endophytic fungi

The surface sterilization will be done following the procedure used by Helander *et al*. (2007) and Blumenstein (2010) with some modifications

• Cut approximately 0.5 cm^2long piece from each leaf and root of orchids; will try to remove epidermal tissues gently by razor blade from leaves and remove outer cuticle from root sections too.
• Thoroughly washed in sterile water to remove dirt;
• Dip the samples into 75% ethanol for 3 minutes;
• Dip the samples in 0.5% Na-hypochlorite solution for 2 minutes;
• Wash the samples in sterile water for 3 times;
• Dry the samples in sterile tissue paper until dehydrate.

The procedure will be carried out under a laminar hood in aseptic condition.After removing the excess water, the segments were placed on Petri plates (9 cm diam.) containing with WA (water agar). The plates were incubated at room temperature and fungal colonies will be regularly observed.The Petri dishes will be then periodically checked every day for fungal growth. Each time a seen new colony from inner leaf tissue and root segments, it will be sub-cultured on PDA (Potato Dextrose Agar) plates to make pure cultures.

3. Identification of endophytic fungi by morphological and physiological characterization

For characterization of the morphology of fungal isolates, slides will be prepared from cultures and will be stained with bromothymol blue reagent in lacto phenol and will be examined with a binocular compoundmicroscope. Identification will be based on morphological characteristics such as growth pattern, colony and hyphae, colour of colony and medium, surface texture, margin character, aerial mycelium, mechanism of spore production and characteristics and size of the spore (H. L. Barnett and B. B. Hunter, 1956).

The morphological characteristics will also be followed Crous *et al*. (2009) that used as criterion to the group all fungi. Each fungus will be observed and examined to the characteristics and will be assigned to a specific morphological group. To be part of the same group, a fungus will be grouped.

- Colour. The colony colour will be observed to discriminate the fungi. If a fungal colony will be seen more than one distinct colour, the description will done from the middle towards the outer part.
- Agar colour**.** Some isolates may possible be released metabolites or substances to change the colour of the agar where the colony is growing.
- Liquid drops. The presence and the colour of the liquid drops that some fungi produced on their surface will be recorded.
- Colony shapes. Each colony will be observed to grow in a certain way forming different shapes. The colony shapes will be distinct: crenate, entire edge, erose or dentate, fimbriate, lobate, radially striate with lobate edge, undulate, with rhizoids.
- Colony texture will be distinct as follow:**-** woolly, velvety, hilly, spongy, dry, furry and creamy.
- Spore characteristics will be noted. If the fungi would be developed a clear accumulation of spores on their surfaces and the colour of the spores will be observed.
- The growth rate will be observed as an additional characteristic in order to increase the common characteristic within a group.

Chapter 4

Results and Discussions

Many isolates can't identify the species because it doesn't produce spores. Twelve species belonging to Ascomycota were isolated from 9 orchid varieties. They are as follow:

1. Chaetomiumcochliodes

Habitat: *Grammatophyllum scriptum*, *Cymbidium dayanum*, *Dendrobium hercoglossumand Toreniafournieri*.

The colonies are quit slowly growing, for cultured on PDA medium in room temperature for 7 days have 7 cm in diameter, cottony and white in color initially and then become soft-green.C. *cochliodes*has distinctive small brown 'lemon' or 'football'shapedascospores.(Arx, J.A. von *et al*. 1986).

This species also isolated as endophytic fungi from *Cirsiumarvense*, (Gange AC. Et al., 2012). Li Gy et al. 2006 indicated thatepipolythiodio-xopiperazines and chaetocochinwere isolated from the ethyl acetate extract of the solid-state fermented rice culture of the fungus *Chaetomiumcochliodes*. Their structures were elucidated on the basis of spectroscopic analysis. Compounds 1, 3, and 4 exhibited significant cytotoxicity in vitro against cancer cell lines Bre-04, Lu-04, and N-04,

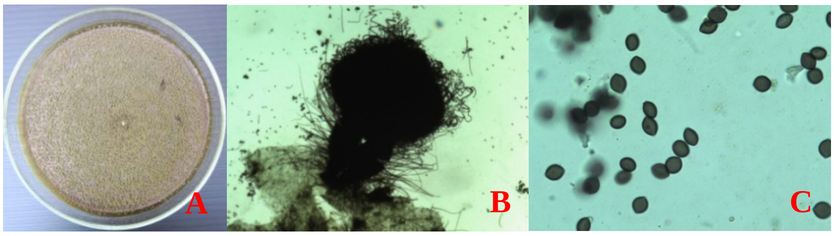

Fig. 1:*Chaetomium cochliodes***A**: The colony that culture on PDA 30 days, **B**The spores under microscops 10X**C**The ascospores under microscops 40X

2. Chaetomium cupreum

Habitat:*Grammatophyllum scriptum.*

The growth was quit slow, cultured on PDA medium for 7 days the growth have 4.4 cm in diameter. The hyphea are red and it produces the red color around mycelia.

Septate hyphae, perithecia, asci and ascospores are visualized. Perithecia are large, red color, fragile, globose to flask shaped and have filamentous, hair-like, brown to black appendages (setae) on their surface. Perithecia have ostioles (small rounded openings) and contain asci and ascospores inside (De Hoog *et al*., 2000).

This species was isolated as endophytic fungus from *Macleayacordata* and have ability to antifungal activity against *Rhizoctoniasolani*, *Botrytis cinerea*, *Pytiumultimum*, and antitumor activities against HL-60 and A549. It is toxic to *Artemiasalina* (Bi Z.M. *et al*, 2010).

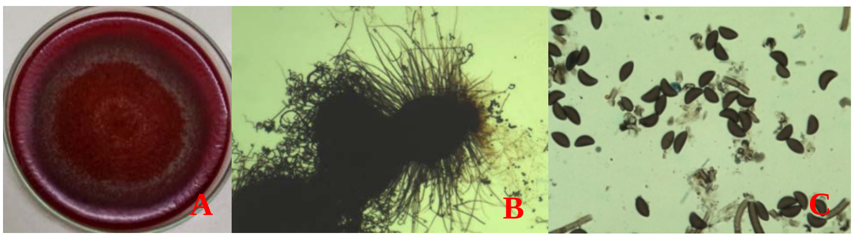

Fig. 2:*Chaetomium cupreum* **A**: The colony cultured on PDA 30 days**B**:The spores under

microscops 10X and **C:**Theascospores under microscops 40X

3. Xylaria sp.

Habitate: *Cymbidium dayanum, Dendrobiumhercoglossum, Doritispulcherrima, Dendrobiumcrumenatum, Dendrobiumfriedericksianum*and*Grammatophyllumspecinocum*.

The fungi have white mycelia;the growth was quit slowl, for cultured on PDA in room temperature for 7 days have 5.8 cm in diameter. They produce the stroma on 20th day but didn't produce ascus and ascospore.

Xylaria sp. was the common species that can isolated from other orchid varieties. Previous research also found that this species are most colonized in 7 *Dendrombium sp*. in China (Chen J. *et al*. 2013). Sawmya K. *et al*. (2013) also isolated *Xylariasp*. from *Bulbophyllumneilgherrense*and *Pholidotapallida*.

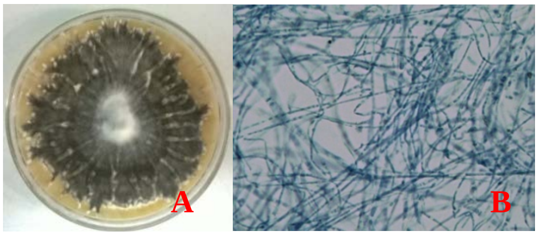

Fig. 3:*Xylaria* sp.**A**: Colony culture on PDA 30 days **B**: Mycelia of xylaria sp. under microscops 40X

*4. Colletotrichum*sp.

Habitats: *Cymbidium dayanum*, *Dendrobiumhercoglossum*, *Toreniafournieri*, *Dendrobium crumenatum* and *Grammatophyllumspecinocum*.

The growth on PDA medium 7 days have 8.5-9 cm, the mycelia initially white-grey and then become black-brown. The conidia have no color with rods shape(Busarakham K.,2001).

Previous study from India showed that the endophytic fungus, *Colletotrichum gloeosporiodes*was isolated from *Justiciagendarussa*, a medicinal plant have ability to produce Taxol that is a potent anticancer drug used widely in the treatment of a variety of cancers (GangadeviV. andMuthumary J., 2008).

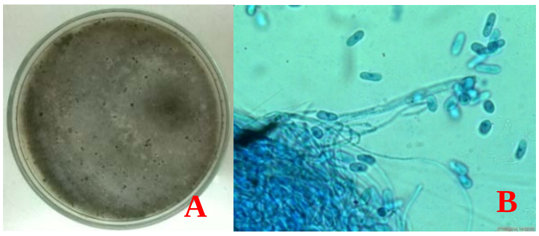

Fig. 4:*Colletotrichum* sp.**A**: The colony culture on PDA 30 days and **B:** The spores under microscope 40X

*5. Nigrospora*sp.

Habitat: *Dendrobiumhercoglossum*

Nigrospora grows quit fast and produces woolly colonies on potato dextrose agar (PDA) at room temperature, on 7thdays has 9 cm in diameter. Colorof the colony is white initially and then becomes gray with black areas and turns to black eventually from both front and reverse. Spores (conidia) are produced singly on swollen urn-shaped conidiophores and are egg-shaped to flattened-spherical, black, and often have an equatorial colourless line or germ slit.

This species also can isolated from deep sea invironment at Arabian Sea and The secondary metabolites produced from this organism showed potent antimicrobial and anticancer activities with immediate application to cosmetics and pharmaceutical industries. (Arumugam GK, *et al*, 2015)

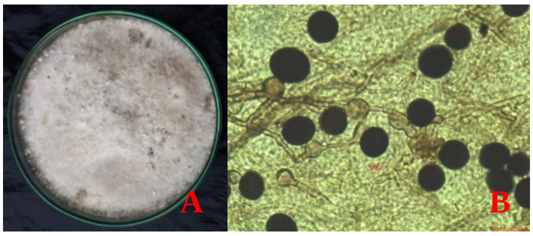

Fig. 5:*Nigrospora* sp. **A**: The colony culture on PDA 30 days**B:** The hyphea and spores under microscope 40X

6. Phoma sp.

Habitats:*Cymbidium dayanum*, *Doritispulcherrima* and *Grammatophyllumspecinocum*.

The hyphae were growing rapidly, for 2 days that cultured on PDA medium have 6.5 cm in diameter. The hyphae first growth have whitecolor and than become black-brown.Spores are colorless and unicellular. The pycnidia are black and depressed in the tissues of the host.

Phoma sp. are endophytic fungi that can isolated from many plants. Wang LW *et al*. (2012) isolated*phoma* sp. from chinese medicinal plant *Arisaemaerubescens* and showed that the bioactive metabolites from this species can inhibited the growth of four plant pathogenic fungi (*Fusariumoxysporium*,*Rhizoctoniasolani*, *Colletotrichumgloeosporioides*, and *Magnaportheoryzae*). This species also isolated from roots of cucumber in Korea and showed that have ability to produce Gibberellins and Indoleacetic Acidand Promotes Host-Plant Growth during Stress (Waqas M. *et al*., 2012).

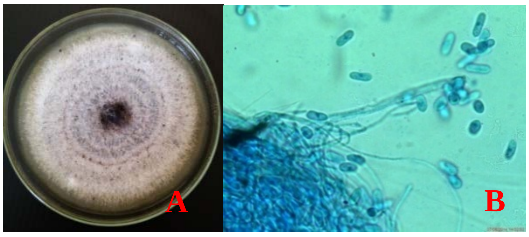

Fig. 6:*Phoma* sp1. **A**: The colony culture on PDA 30 days and **B:** The spores and mycelia under microscope 40X

7. Curvularia sp.

Habitats: *Grammatophyllum scriptum* and *Doritispulcherrima*.

The growth was quit fast for cultured on PDA medium for 2 days have 5.2cm in diameter. From the front, the color of the colony is white to pinkish grayinitially and turns to black as the colony matures. From the reverse, it is dark brown to black. Conidia are pale brown, with three or more transverse septa (phragmoconidia) and are formed apically through a pore (poroconidia) in a sympodially elongating geniculate conidiophore similar to *Drechslera*. Conidia are cylindrical or slightly curved, with one of the central cells being larger and darker. Germination is bipolar and some species may have a prominent hilum(McGinnis, M.R. 1980).

Zakaria L. *et al*. (2010) isolated this species from *Oryzasativa.* Aharwal R.P. *et al*. (2014) also isolated this fungus from *Calotropisprocera* (Linn.) R.Br. a widely used medicinal plant in India.

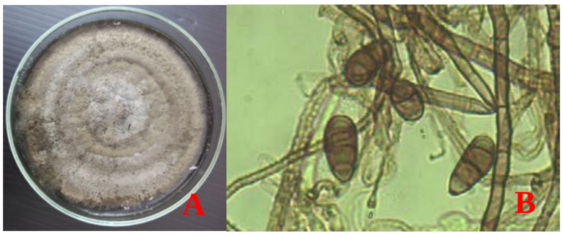

Fig. 7:*Curvularia* sp. **A**: The colony culture on PDA 30 days and
B: The spores under microscope 40X

9. Pestalotiopsis sp.

Habitat:*Dendrobiumfriedericksianum ,Cymbidium dayanumand Grammatophyllum scriptum.*

The mycelia are white in color. This species have quit slow of growing. For growing on PDA in room temperature for 7 days have 5.5cm in diameter. Conidiophores (annellides) produced within compact fruiting structures (aecervuli or pycnidia). Spores (conidia) 4 to 5 celled, with the two or three central cells dark brown, and with two or more apical appendages or hairs.

Pestalotiopsis sp. is the common endophytic fungi that can isolate from orchids and other plants. They also isolated from other orchid varieties from other country such as Tempesta S. *et al*. (2011) isolated endophytic fungi from leaves of *C. mrcranthum* and *O. adendrobium* also found 29 strains of *Pestalotiopsis*sp. Wei J.G*et al*. (2007) isolated this species that associated with plants of Podocarpaceae, Theaceae and Taxaceae in southern China also obtained 24 species.Kumaran RS*et al*. (2010) isolated *Pestalotiopsisver-sicolor* and *Pestaloti-opsisneglecta* as endophytic fungi from Japanese Yew tree, *Taxuscuspidata* and indicated that they can produce taxolconcentration induces increased cell death in cancer.

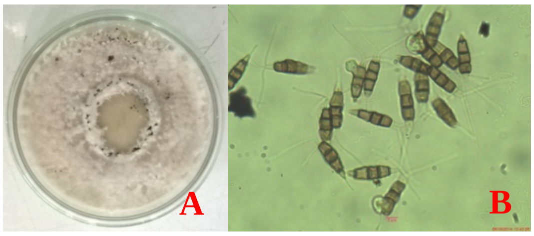

Fig. 9:*Pestalotiopsis* sp. **A**: The colony culture on PDA 30 days **B**:The conidia under microscops 40X

10. Corynascus sp.

Habitat: *Cymbidium dayanum*

The fungus have quit fast in growth. Only 3 days the mycelia have 9.0 cm in diameter. It has black-white mycelia, produce lots of black spores and inside spores there are lots of little ascospores.

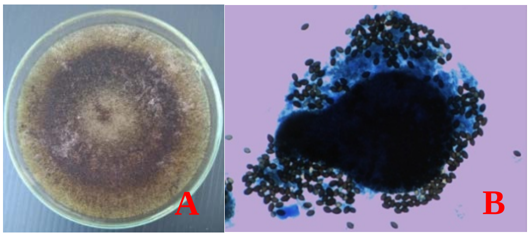

Fig. 10:*Corynascus* sp. **A**: The colony culture on PDA 30 days and
B: The spores and acospores under microscops40X

11. Cladosporium-like fungi

Habitat:*Grammatophyllum scriptum*, *Cymbidium dayanum*, *Dendrobiumpalpebrae*, *Doritispulcherrima*and *Dendrobiumcrumenatum*

The initial growth the mycelia have white color and then become black. The growth was quit fast. On 6th day of culture on PDA have 9 cm in diameter. The mycelia have branches and the lots of small spore.

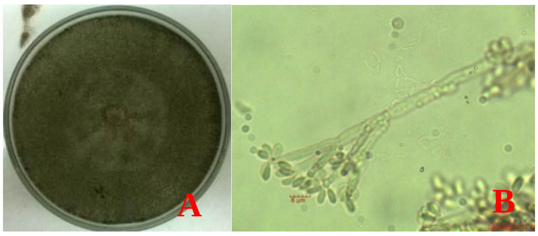

Fig. 11:*Cladosporium*-like fungi**A**: The colony culture on PDA 30 days and **B:** Thespores and mycelia under microscops 40X

12. Achaetomium sp.

Habitat:*Grammatophyllum scriptum*

The mycelia growth look like the yellow sheets and this fungus growth quit slow, cultured on PDA medium in room temperature for 7 days have 5.5 cm in diameter.The spores are large with yellow in color. Inside the spores have many ascospores.

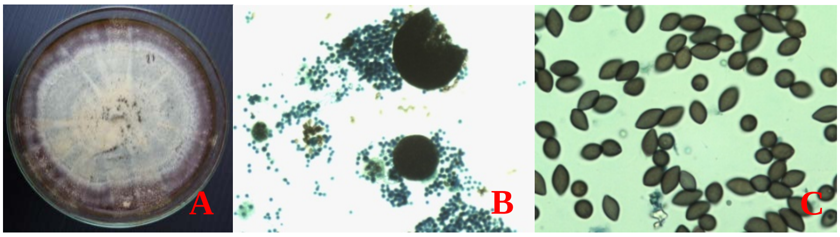

Fig. 12:*Achaetomium* sp.**A**: The colony culture on PDA 30 days **B:** The spores under microscops 10X and **C**: The ascospores under microscops 40X

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APPENDIX

Sour V., Phonpho S and Soytong K. (2015) Isolation of endophytic fungi from some orchid varieties. Journal of Agricultural Technology 11(5):1243-1254