CHAPTER 6

OPTIMIZATION OF ORCHID MYCORRHIZAL FUNGI ON MEDIA

6.1 Introduction

Orchid mycorrhizal fungi are extremely important for the establishment and growth of orchid in nature. In order to cultivate orchids for conservation and horticulture, it is desirable that effective fungal symbionts can be grown under controlled conditions to produce inoculum. It is well known that different ecological and physiological factors affect the growth of mycorrhizal fungi and also mycorrhiza synthesis and function in nature (Dasa *et al.* 2006). Temperature and pH are major factors that influence the occurrence and the growth of mycorrhizal fungi in their natural habitat (Marx *et al.* 1970; Dasa *et al.* 2006; Mei-ling *et al.* 2008). Effects of various temperature and pH values on the growth of mycelium of *Phebopus portentous* (Boletales), ectomycorrhizal fungi were evaluated and reported the optimal growth temperature and pH was 30°C and pH 4 respectively (Kumla *et al.* 2011). Moreover, the effects of temperature and pH on the growth of *Amanita caesarea* were studied and found that the largest radial growth was obtained at pH 6 - 7 and optimal temperature growth was 24 - 28°C depending on the isolate (Dasa *et al.* 2006). To produce an inocula of mycorrhizal fungi for cultivation of orchids, it is also necessary to determine the suitable media for the multiplication of vegetative hyphae and, sometimes, suitable carries for delivering the inoculum

to the end user. There is extensive knowledge on substrates for inoculum production of many ectomycorhizal fungi (Hung and Trappe, 1987; Douds *et al.* 2007; Lee *et al.* 2008) but this is much less information for orchid symbionts.

This study aimed to determine the optimal temperature and pH for growth of orchid mycorrhizal fungi and suitable media of selected mycorrhizal fungi for orchid cultivation, *ex vitro*.

6.2 Materials and methods

6.2.1 Effects of temperature on fungal growth on PDA

The orchid mycorrhizal fungi (Table 6.1) were cultured on PDA. A cock (diameter, 0.5 cm) of each fungal isolate was inoculated on to sterile cellophane over PDA in a Petri dish. There were 3 replicate Petri dishes of each fungal isolate for each temperature. The plates were incubated at 5 temperatures (20, 25, 30, 35, and 40°C) for 10 days. Then, the colony diameter and dry weight of each fungal colony were measured. The fungal dry weight was measured after being dried at 60°C for 48 hours.

6.2.2 Effects of pH on fungal growth on PDA

The same setup was used as in 6.2.1. Eight pH treatments (3, 4, 5, 6, 7, 8, 9, and 10) were established. The pH of PDA was adjusted using 6N NaOH and 6N HCl before autoclaved. The inoculated plates were incubated at 25°C for 10 days. Then, the diameter and dry weight of each fungal colony were measured. The fungal dry weight was measured after being dried at 60°C for 48 hours.

Table 6.1 Orchid mycorrhizal fungi isolated from roots of six terrestrial orchids (*Doritis pulcherrima, Eulophia spectabilis*, *Paphiopedilum bellatullum*, *Pecteilis susannae*, *Phaius tankervilleae*, and *Spathoglottis affinis*) and used in optimization of fungal growth temperature and pH

Orchids	Fungal isolates	Fungal taxon
Doritis pulcherrima	CMU-DP 506	*Epulorhiza* sp.
	CMU-DP 514	*Tulasnella* sp.
Eulophia spectabilis	CMU-STE 004	*Epulorhiza* sp.
	CMU-STE 003	*Gloeotulasnella* sp.
	CMU-STE 011	*Tulasnella* sp.
	CMU-STE 014	*Epulorhiza* sp.
Paphiopedilum bellatulum	CMU-SLP 007	*Epulorhiza* sp.
	CMU-SLP 008	*Epulorhiza* sp.
Pecteilis susannae	CMU-AUG 002	*Epulorhiza* sp.
	CMU-AUG 007	*Epulorhiza* sp.
	CMU-AUG 013	*Epulorhiza* sp.
	CMU-AUG 025	*Epulorhiza* sp.
	CMU-AUG 028	*Epulorhiza* sp.
	CMU-AUG 031	*Epulorhiza* sp.
	CMU-AUG 040	*Epulorhiza* sp.
Phaius tankervilleae	CMU-NUT 012	*Epulorhiza* sp.
	CMU-NUT 013	*Epulorhiza* sp.
Spathoglottis affinis	CMU-AU 211	*Epulorhiza* sp.
	CMU-AU 212	*Epulorhiza* sp.

6.2.3 Evaluation of suitable grain and potting media for fungal inoculum production

Three effective mycorrhizal fungi from chapter 4 (CMU- AUG 007, CMU-AUG 028 and CMU-STE 014) were selected for this experiment. The three effective mycorrhizal fungi were inoculated on the top of each grain and potting media (Table 6.2) contained in a test tube (16 × 150 mm). There were 3 replicate tubes of each fungal isolate per each medium. The inoculated test tubes were incubated at 30°C for 15 days. The growth of fungal mycelium was observed by measuring the length of fungal mycelium that grew into media from the inoculated point every 3 days.

Table 6.2 Grain and potting media used for evaluation of suitable material for fungal inoculum production

Grain media	Potting media
corn (*Zea mays*)	coconut coir
black bean (*Phaseolus mungo*)	coconut husk
kidney bean (*Phaseolus vulgaris*)	coconut coir with PDB
sorghum (*Sorghum bicolor*)	coconut husk with PDB

Coconut coir and husk with PDB was prepared by soaked the coconut coir and husk with PDB for 12 hours. For grain and potting media preparation, see Appendix C

6.3 Results

6.3.1 Effects of temperature on fungal growth on PDA

All fungal isolates had the highest fungal dry weight and the largest colony diameter after culturing on PDA at 30°C for 10 days (Figures 6.1 - 6.9).

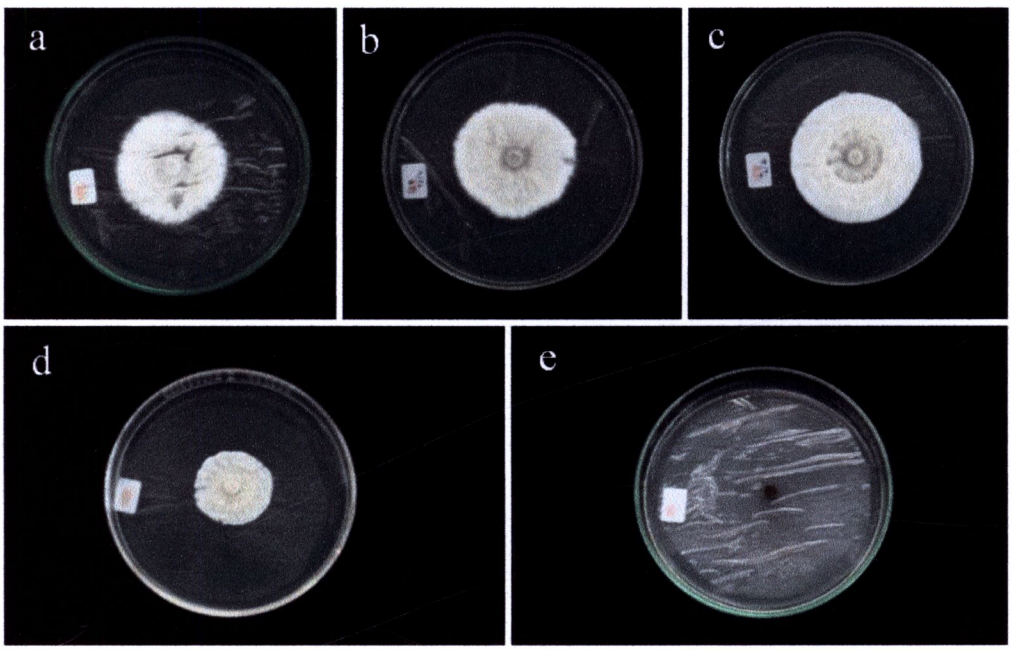

Figure 6.1 Effects of temperature on growth of fungal isolate CMU-AUG 028, *Epulorhiza* sp., after culturing on PDA and incubation at 20°C (a), 25°C (b), 30°C (c), 35°C (d) and 40°C (e) for 10 days.

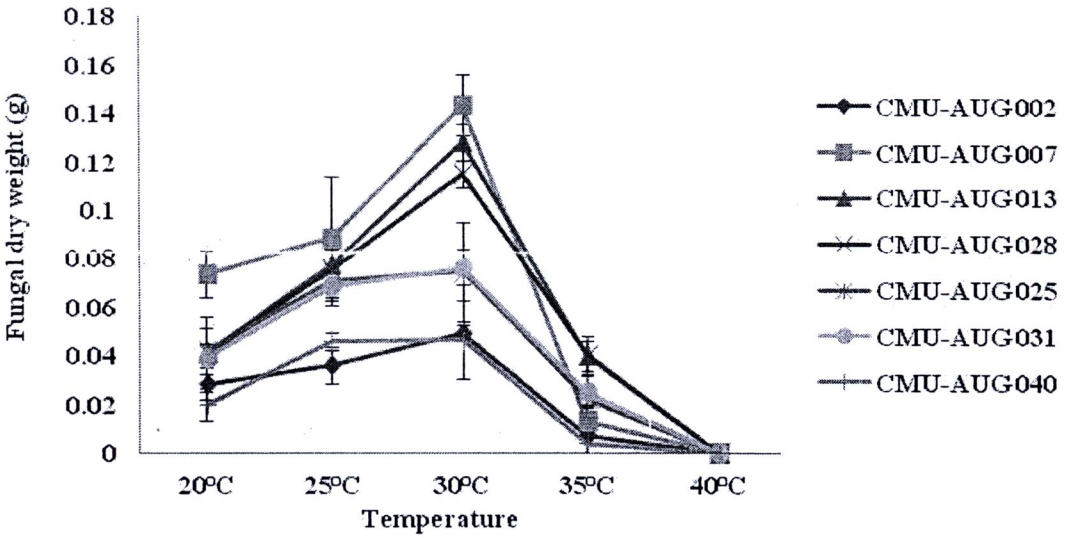

Figure 6.2 Effects of temperature on growth (dry weight) of orchid mycorrhizal fungi isolated from roots of *Pecteilis susannae* after cultured on PDA for 10 days. The results are means (n = 3) ± SE

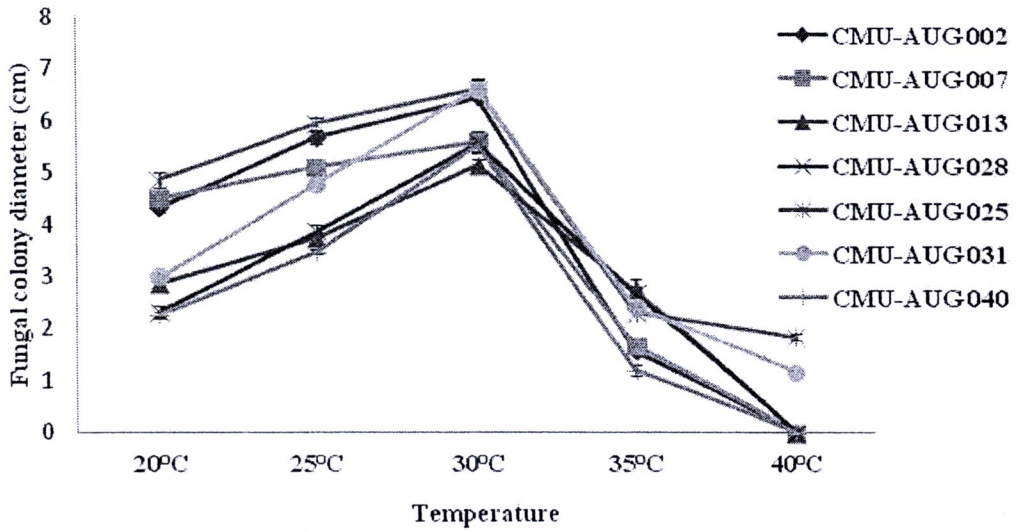

Figure 6.3 Effects of temperature on growth (colony diameter) of orchid mycorrhizal fungi isolated from roots of *Pecteilis susannae* after cultured on PDA for 10 days. The results are means (n = 3) ± SE

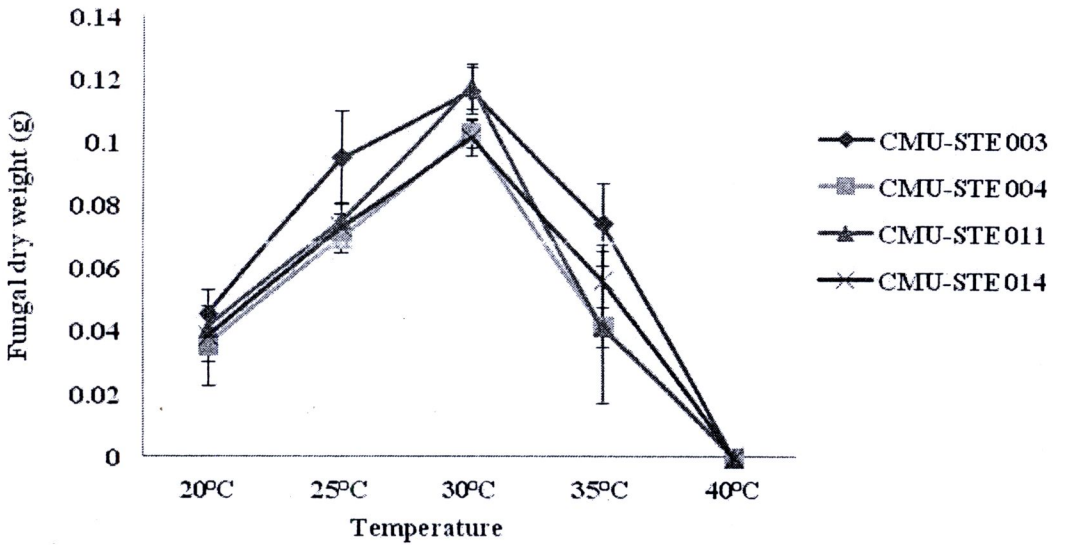

Figure 6.4 Effects of temperature on growth (dry weight) of orchid mycorrhizal fungi isolated from roots of *Eulophia spectabilis* after cultured on PDA for 10 days. The results are means (n = 3) ± SE

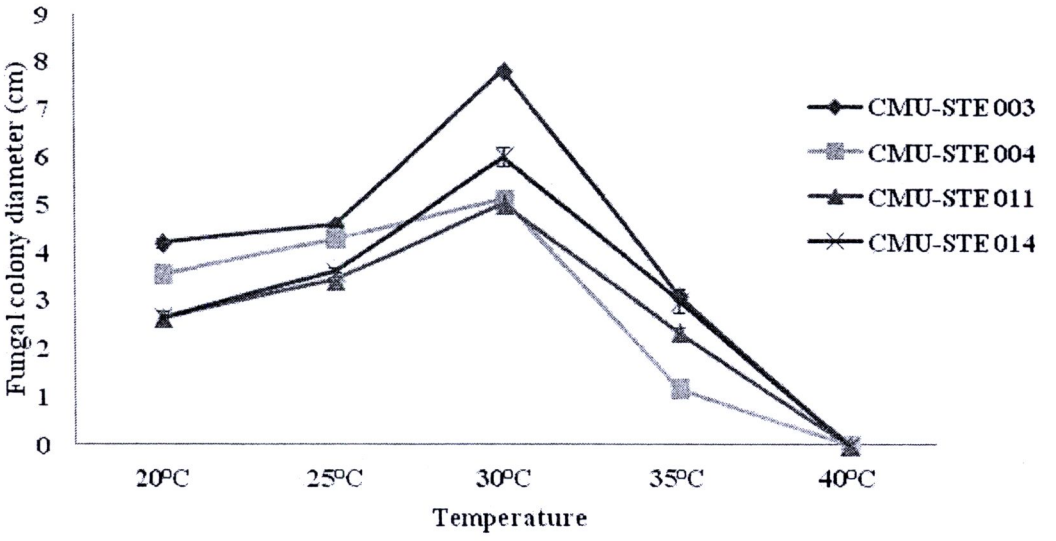

Figure 6.5 Effects of temperature on growth (colony diameter) of orchid mycorrhizal fungi isolated from roots of *Eulophia spectabilis* after cultured on PDA for 10 days. The results are means (n = 3) ± SE

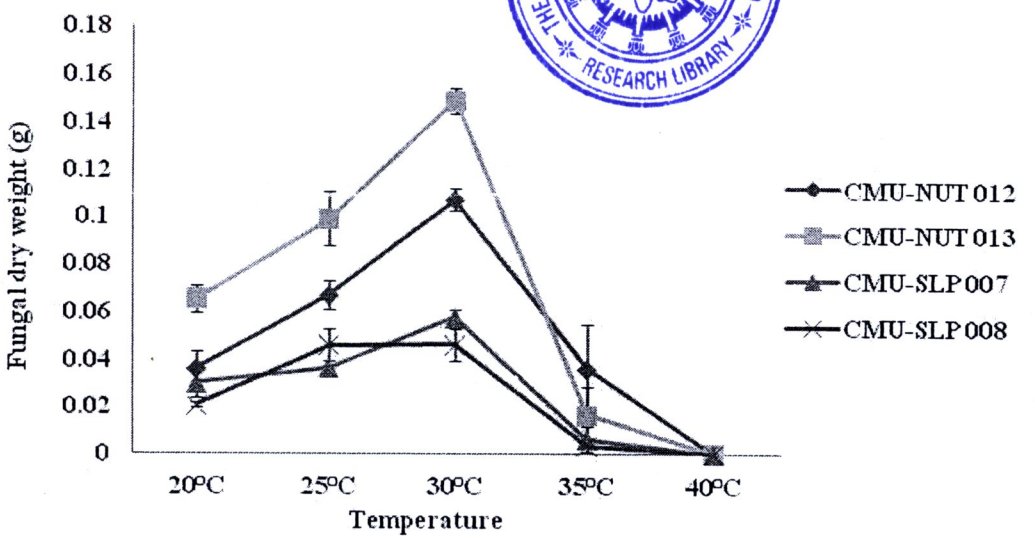

Figure 6.6 Effects of temperature on growth (dry weight) of orchid mycorrhizal fungi isolated from roots of *Paphiopedilum bellatullum* and *Phaius tankervilleae* after cultured on PDA for 10 days. The results are means (n = 3) ± SE

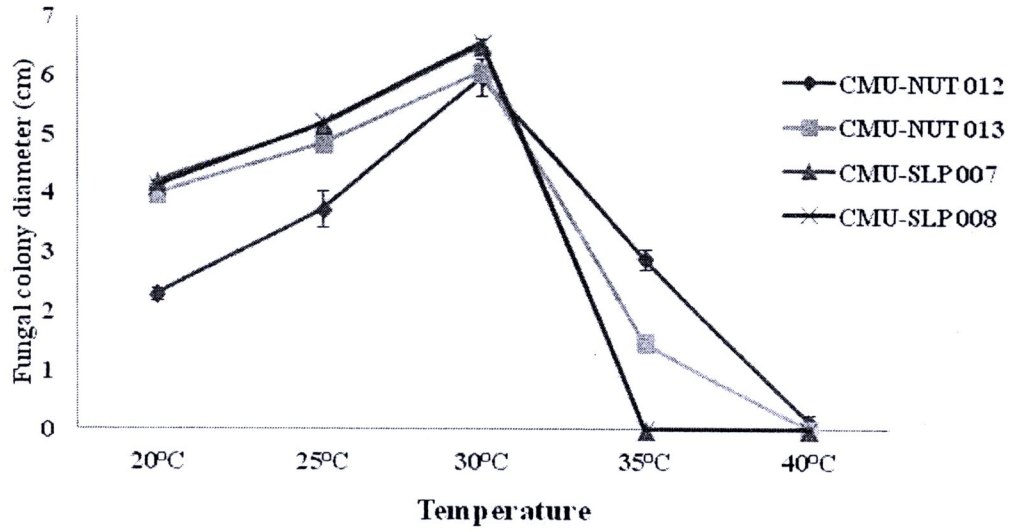

Figure 6.7 Effects of temperature on growth (colony diameter) of orchid mycorrhizal fungi isolated from roots of *Paphiopedilum bellatullum* and *Phaius tankervilleae* after cultured on PDA for 10 days. The results are means (n = 3) ± SE

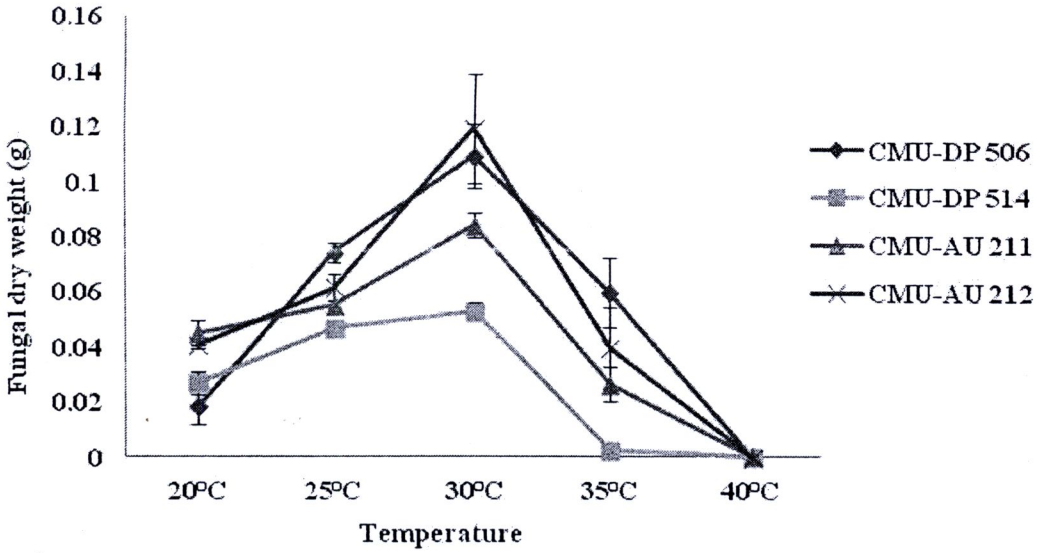

Figure 6.8 Effects of temperature on growth (dry weight) of orchid mycorrhizal fungi isolated from roots of *Doritis pulcherrima* and *Spathoglottis affinis* after cultured on PDA for 10 days. The results are means (n = 3) ± SE

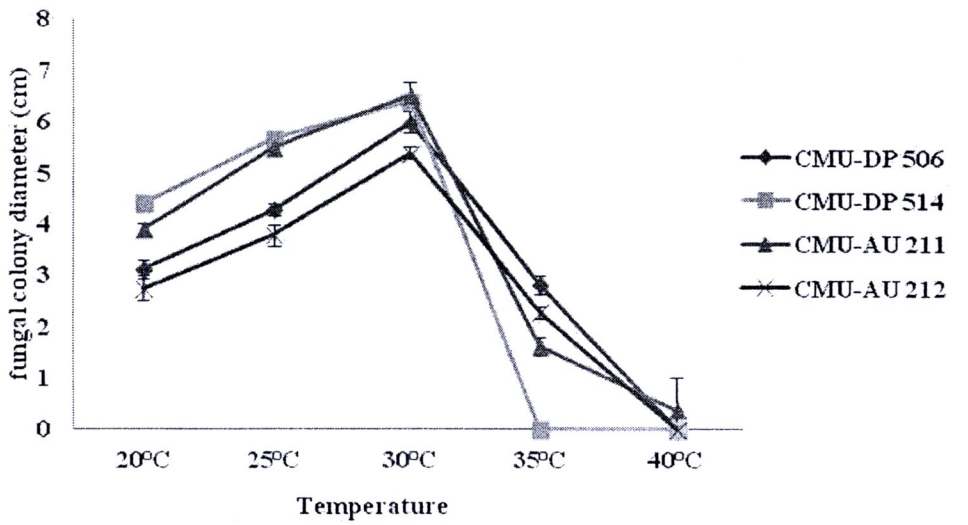

Figure 6.9 Effects of temperature on growth (colony diameter) of orchid mycorrhizal fungi isolated from roots of *Doritis pulcherrima* and *Spathoglottis affinis* after cultured on PDA for 10 days. The results are means (n = 3) ± SE

6.3.2 Effects of pH on fungal growth on PDA

All fungal isolates had the highest dry weight and the largest colony diameter after culturing on PDA adjusted pH to 6 – 8 and incubated at 25°C for 10 days (Table 6.3, Figures 6.10, 6.11 and 6.12).

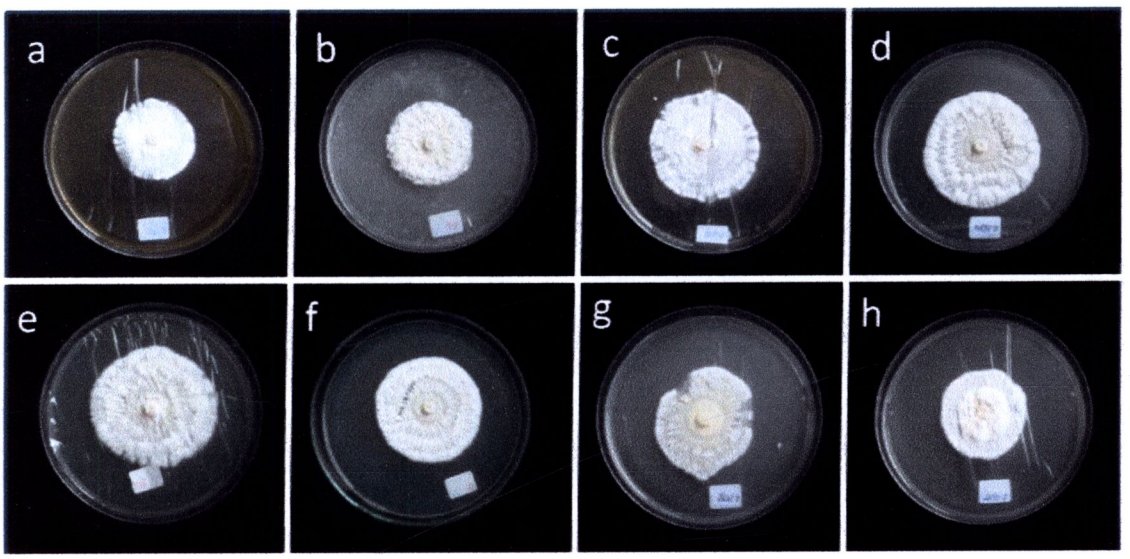

Figure 6.10 Effects of pH on growth of fungal isolate CMU-AUG 028, *Epulorhiza* sp., after cultured on PDA adjusted pH 3 (a), 4 (b), 5 (c), 6 (d), 7 (e), 8 (f), 9 (g) and 10 (h) and incubated at 25 °C for 10 days.

Table 6.3 The optimal growth pH evaluated from fungal dry weight and fungal colony diameter of orchid mycorrhizal fungi isolated from roots of six terrestrial orchids after cultured on PDA for 10 days.

Orchid	Fungal isolate	Fungal taxon	Optimal growth pH	
			Dry weight	Colony diameter
Doritis pulcherrima	CMU-DP 506	*Epulorhiza* sp.	7	7
	CMU-DP 514	*Tulasnella* sp.	6	6
Eulophia spectabilis	CMU-STE 003	*Gloeotulasnella* sp.	7	8
	CMU-STE 004	*Epulorhiza* sp.	8	8
	CMU-STE 011	*Tulasnella* sp.	8	8
	CMU-STE 014	*Epulorhiza* sp.	7	7
Paphiopedilum bellatulum	CMU-SLP 007	*Epulorhiza* sp.	8	8
	CMU-SLP 008	*Epulorhiza* sp.	8	8
Pecteilis susannae	CMU-AUG 002	*Epulorhiza* sp.	7	8
	CMU-AUG 007	*Epulorhiza* sp.	6	6
	CMU-AUG 013	*Epulorhiza* sp.	7	7
	CMU-AUG 025	*Epulorhiza* sp.	8	8
	CMU-AUG 028	*Epulorhiza* sp.	7	7
	CMU-AUG 031	*Epulorhiza* sp.	7	8
	CMU-AUG 040	*Epulorhiza* sp.	8	8
Phaius tankervilleae	CMU-NUT 012	*Epulorhiza* sp.	7	7
	CMU-NUT 013	*Epulorhiza* sp.	7	7
Spathoglottis affinis	CMU-AU 211	*Epulorhiza* sp.	7	7
	CMU-AU 212	*Epulorhiza* sp.	7	8

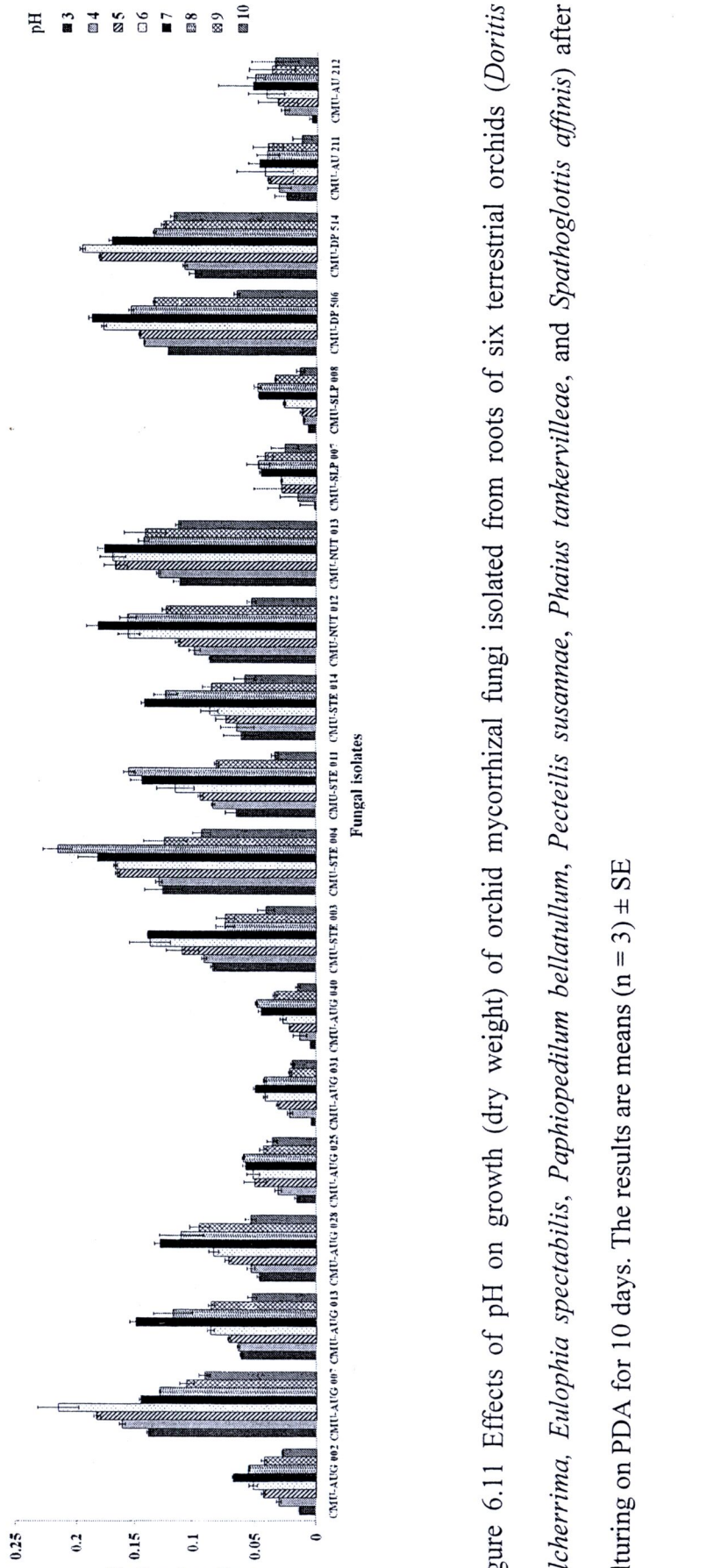

Figure 6.11 Effects of pH on growth (dry weight) of orchid mycorrhizal fungi isolated from roots of six terrestrial orchids (*Doritis pulcherrima, Eulophia spectabilis, Paphiopedilum bellatullum, Pecteilis susannae, Phaius tankervilleae*, and *Spathoglottis affinis*) after culturing on PDA for 10 days. The results are means (n = 3) ± SE

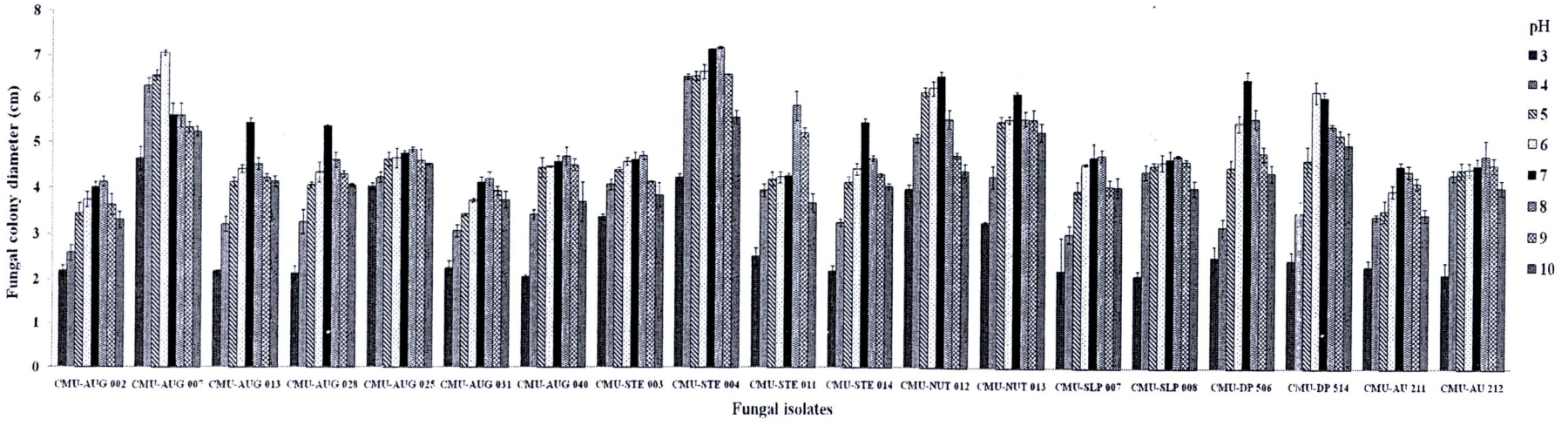

Figure 6.12 Effects of pH on growth (colony diameter) of orchid mycorrhizal fungi isolated from roots of six terrestrial orchids (*Doritis pulcherrima, Eulophia spectabilis*, *Paphiopedilum bellatullum*, *Pecteilis susannae*, *Phaius tankervilleae*, and *Spathoglottis affinis*) after culturing on PDA for 10 days. The results are means (n = 3) ± SE

6.3.3 Evaluation of suitable grain and potting media for fungal inoculum production

The coconut husk with PDB showed the best growth of all fungal mycelium (Figures 6.13 - 6.16).

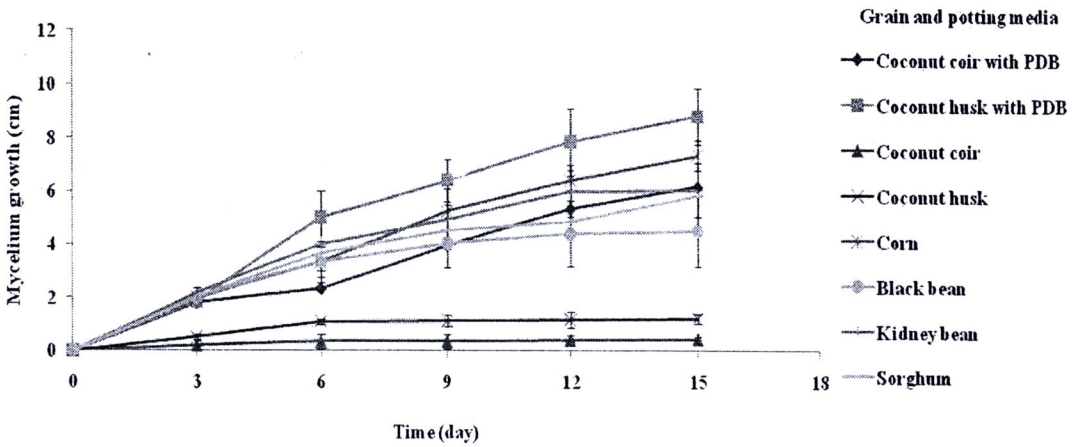

Figure 6.13 Mycelium growth of orchid mycorhizal fungi, *Epulorhiza* sp. (CMU-AUG 007), isolated from roots of *Pecteilis susanne* in grain and potting media after incubation at 30°C for 15 days. The results are means (n = 3) ± SE

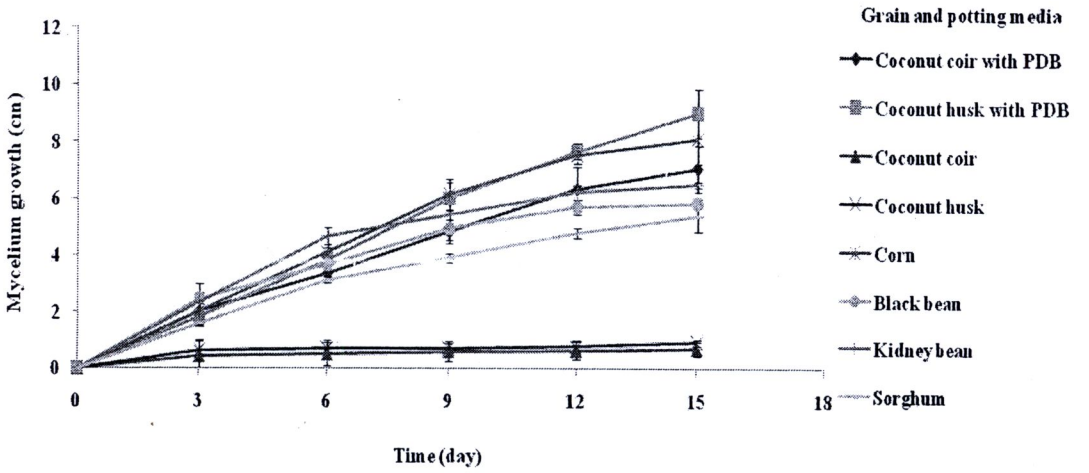

Figure 6.14 Mycelium growth of orchid mycorhizal fungi, *Epulorhiza* sp. (CMU-AUG 028), isolated from roots of *Pectelis susannae* in grain and potting media after incubation at 30°C for 15 days. The results are means (n = 3) ± SE

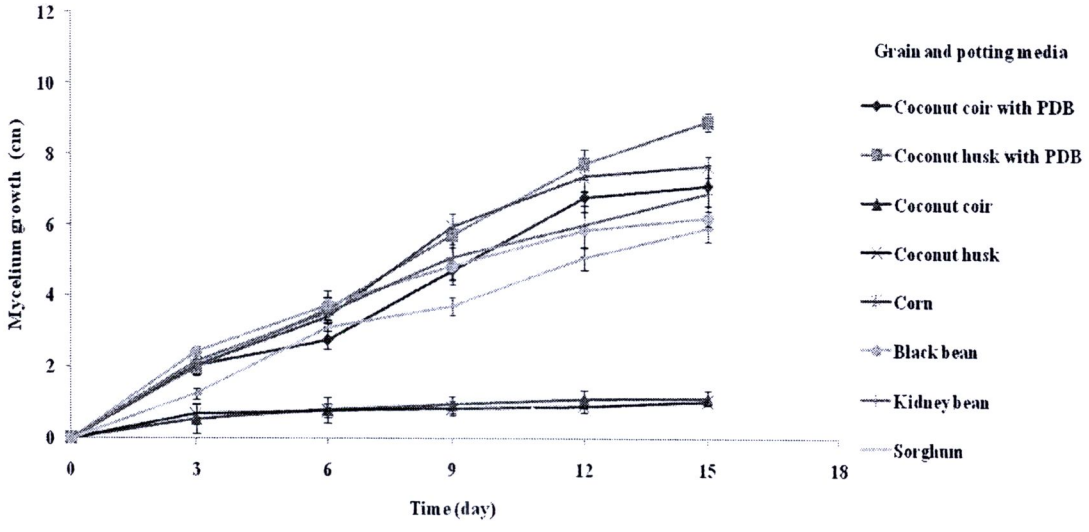

Figure 6.15 Mycelium growth of orchid mycorhizal fungi, *Epulorhiza* sp. (CMU-AUG 014), isolated from roots of *Eulophia spectabilis* in grain and potting media after incubation at 30°C for 15 days. The results are means (n = 3) ± SE

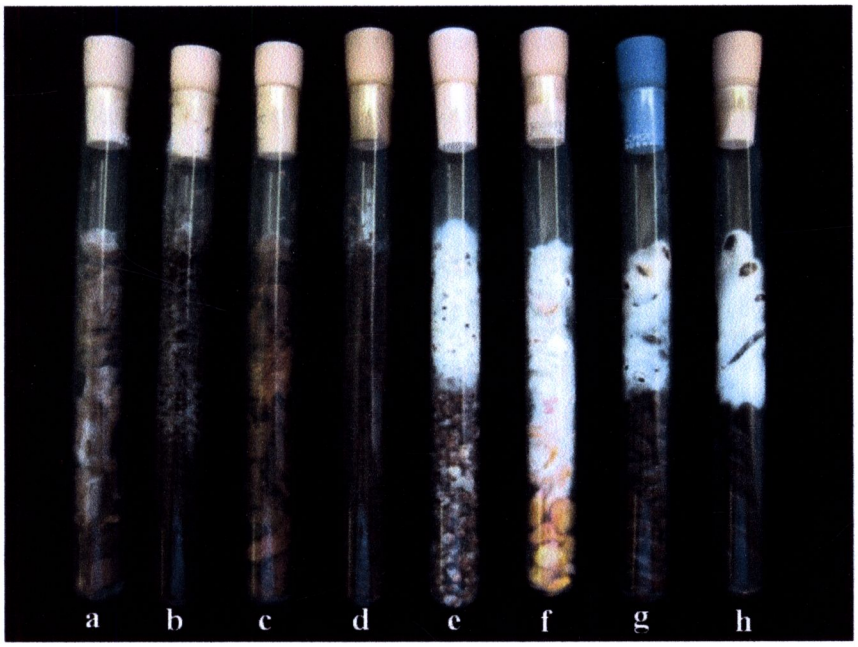

Figure 6.16 Growth of mycelium of orchid mycorrhizal fungi, *Epulorhiza* sp. (CMU-AUG 028), isolated from roots of *Pecteilis susannae*, in grain and potting media (a; coconut husk with PDB, b; coconut coir with PDB, c; coconut husk, d; coconut coir, e; sorghum, f; corn, g; black bean, and h; kidney bean) after incubation at 30°C for 15 days.

6.4 Discussion

This study described the effects of temperature and pH on the growth of orchid mycorrhizal fungi in pure culture and explored suitable grain and potting media for fungal inoculum to cultivate orchids in the green house or in the field. The results clearly showed that the growth of mycelium in pure culture was greatly influenced by temperature and pH, as observed in other studies (Sánchez *et al.* 2001; Yamanaka, 2003; Dasa *et al.* 2006; Kumla *et al.* 2011). Fungi generally grow well in acidic conditions (Yadav and Tripathi, 1991; Kim *et al.*

2005; Sanmee *et al.* 2010), but some species favor neutral to slightly alkaline conditions. Fries, (1956) reported that *Croprinus radiates*, *C. micaceus* and *C. ephemerus* collected from habitats with a high pH grew well above pH 8. In addition, El-Abyad and Webster, (1968) reported some carbonicolous species, that found fruit bodies on alkaline to neutral soil after fire, grew well at pH 6.2 – 8.2. This study showed that the optimal pH of the isolated mycorrhizal fungi were in the range 6 – 8, same as the pH of soils from the field sites. Biomass production and colony diameter of some mycorrhizal fungi showed different responses pH, no doubt because the colony diameter reflected the capability of planar growth, but biomass reflected three-dimensional growth (Jonbloed and Borst-Pauwels, 1990; Mei-ling *et al.* 2008).

For the effects of temperature on the growth of isolated mycorrhizal fungi, the optimal temperature (30°C) of all fungal isolates were similar to the optimal temperature of some tropical ectomycorrhizal fungi, for example, *Phlebopus portentus*, recovered from the northern part of Thailand (Sanmee *et al.* 2010; Kumla *et al.* 2011). In addition, this optimal temperature is quite similar to the average temperature (about 25 - 30°C) in the rainy season in northern Thailand. Therefore, our results indicated that the growth of mycorrhizal fungi was directly limited and affected from natural habitat and natural condition.

Generally, many fungi are able to grow in grain media and some potting media. This study showed that, of the media tested, coconut husk with PDB medium was most suitable. These results are quite similar to the using of potting media containing vermiculites and peat moss supplemented with nutrient medium, used for some ectomycorrhizal fungi (Trappe, 1977; Douds *et al.* 2007; Lee *et al.*

2008). Moreover, coconut husk and coir are widely used in orchid potting media (Brundrett *et al.* 2001). Thus, it seems possible to use coconut husk with PDB medium to produce fungal inoculum and then to use this as a potting media for orchid cultivation in a green house or field site. This will be investigated further in chapter 7.