CHAPTER 4

RESULTS

4.1 Characteristic and surface appearance of the longan fruit

Longan fruit consist of a tough pericarp, a thick edible translucent aril and single centrally located seed.

In the early stages of ontogeny; immature fruit the pericarp composes of abundant setae and exist in a deeply invaginated form (Figure 4.1a), with the pericarp limited to the exposed portions of each fold (Figure 4.1b). With seed development, subsequent aril development, pericarp invaginations were progressively lost with continuous pericarp structure formed 120 days after anthesis and cuticle micro-cracking first observed.(Figure 4.1c) and stomata were observed (Figure 4.1d). The pericarp is further expanded, total pericarp thickness decreases. Once mature the pericarp tightly encases both the seed and aril. Progressive thinning of the pericarp cuticle was observed during development, with thickness reduced from 1.65 mm at 74 days after anthesis to 0.42 mm in the mature tissue. Abundance of cuticle cracking of the pericarp surface was observed (Figure 4.1e, f).

The study of surface appearance of the harvested longan fruit, under stereo microscope, has shown that longan fruit skin was rough and uneven (Figure 4.2a). Under a SEM, it appeared that the surface of longan fruit consisted of trichomes. Abundance of cracking in the pericarp surface was also observed (Figure 4.2b). The filamentous fungi and fungal spore were observed from fruit surface. (Figure 4.3)

Mature longan fruit pericarp consists of three distinctive layers (Figure 4.4, 4.5). Epicarp, the outermost layer, consists of a discontinuous cuticle and trichomes, while

most part of the pericarp was the mesocarp, the inner layer. Most of mesocarp layer consisted parenchymatous cells, large intercellular spaces, groups of stone cells, a few idioblasts and distinct vascular elements. The inner most layer called endocarp is also referred to as the abaxial epidermal zone of the pericarp. This layer made up of small, thin-walled unsuberized epidermal cells and detached from the aril fruit when mature.

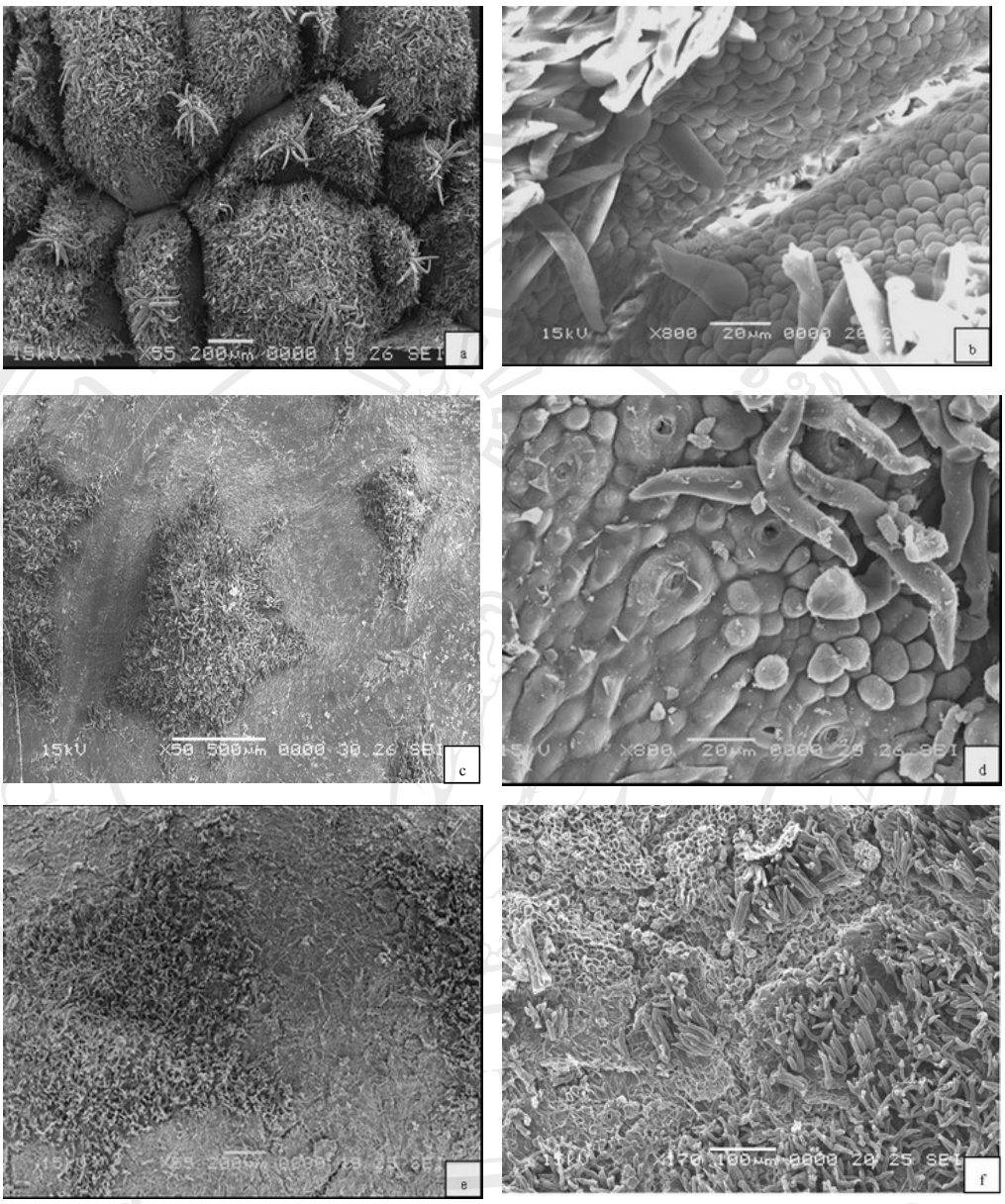

Figure 4.1 Scanning electron micrographs of longan pericarp surface structure at selected intervals during fruit development.

(a) 60 days after anthesis; the pericarp is deeply folded showing setae. Bar = 100µm

(b) 90 days after anthesis; pericarp micro-cracking first observed. Bar = 100µm

(c) 120 days after anthesis; cuticle micro- cracking first observed. Bar = 100µm

(d) 120 days after anthesis; stomata observed. Bar = 100µm

(e) 180 days after anthesis; fruit maturity show cuticle cracking observed. Bar= 100µm

(f) 194 days after anthesis; fruit maturity with abundance cuticle cracking is bserved. Bar = 100µm

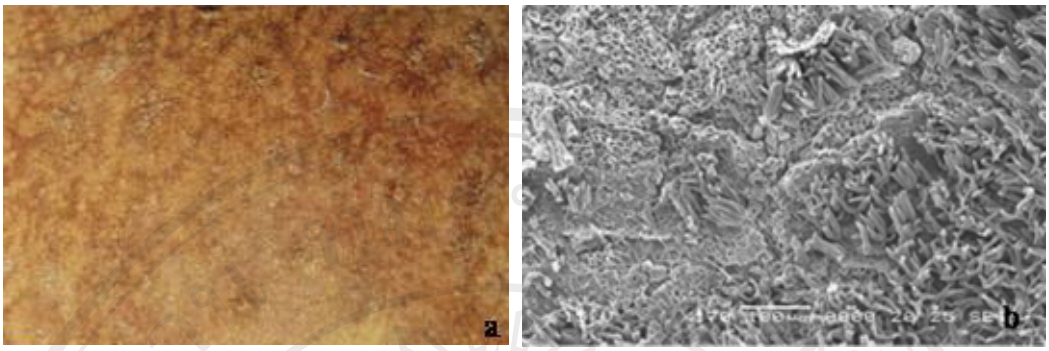

Figure 4.2 Longan fruit surface

(a) Stereo micrograph showed longan fruit skin is rough and uneven.

(b) Scanning electron micrographs; fruit maturity (194 days after anthesis) with abundance cuticle cracking is observed. Bar = 100μm

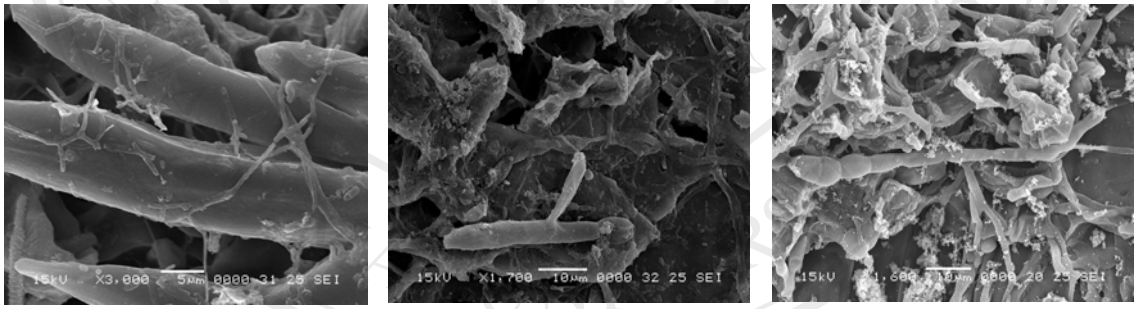

Figure 4.3 Scanning electron micrographs of longan fruit surface

(a) Filamentous fungi were found on trichomes. Bar = 5 μm

(b) Spore germinate was found. Bar = 10 μm

(c) Filamentous fungi were found on fragments cuticle. Bar = 10 μm

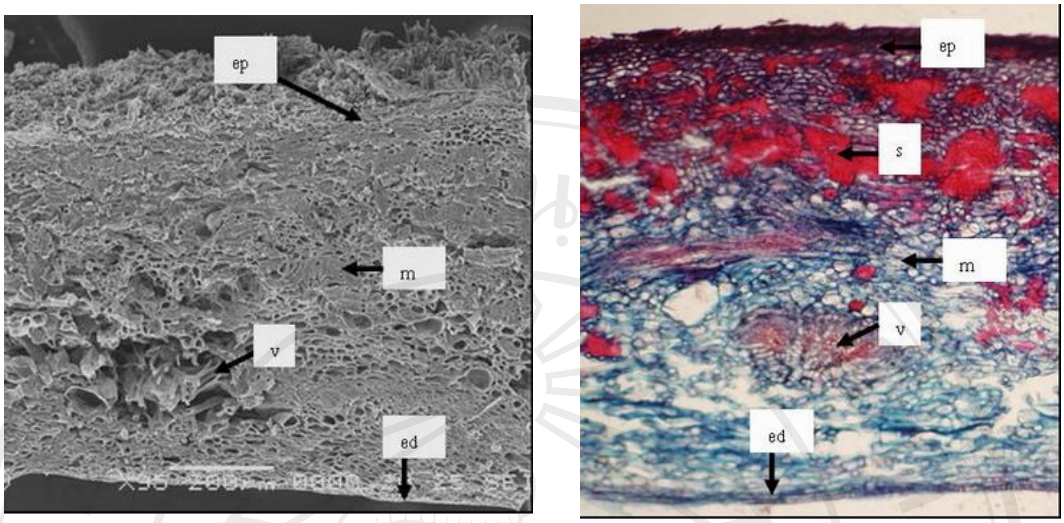

Figure 4.4 Cross-section of mature longan fruit pericarp 194 days after anthesis, illustrating the epicarp (ep); the middle mesocarp (m); the endocarp (ed) vascular tissue (v) and stone cell (s)

Left: Scanning electron micrograph. Bar = 200μm

Right: Light micrograph.

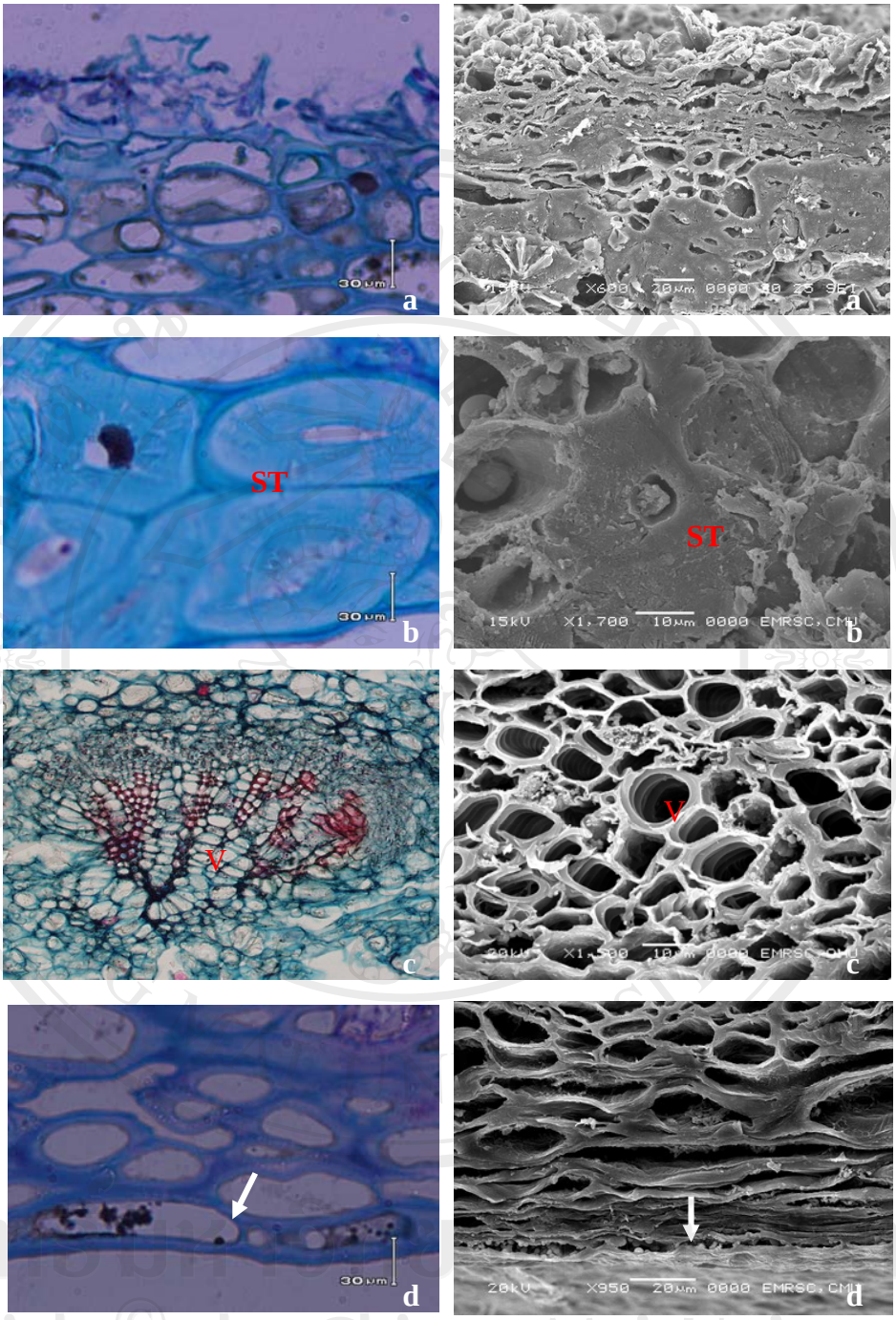

Figure 4.5 Cross-section of mature longan fruit pericarp 194 days after anthesis; (a) epicarp (b) stone cell were found abundance in mesocarp (c) vascular tissue in mesocarp (d) a single layer endocarp (white arrow)

Left: Light micrograph

Right: Scanning electron micrograph.

(ST = stone cell, V = vascular tissue)

4.2. Isolation and Identification of longan pathogenic fungi

Two hundred filamentous fungi specimens were isolated from the pericarp and stem-end of longan fruits. They were identified to 12 genera while 7 isolates were unidentified (Table 4.1). Followings are information of fungi isolates.

1. *Lasiodiplodia* sp. (Figure 4.6)

The colonies of *Lasiodiplodia* sp. on PDA usually filled the whole petri-dish (9 cm in diameter) with loose to moderately dense weft of light to dark grey hyphae and adhered to the petri-dish lid within 3 days. Microscopically, this fungi species was distinguished by large ellipsoidal conidia which were developed in black pycnidia. The immature conidia were hyaline and aseptate, while the mature one was a two-celled conidium, dark brown in color and septate.

2. *Fusarium* sp. (Figure 4.7)

On PDA, the colonies of *Fusarium* sp. were moderately deep, floccose, with white to pink mycelium in concentric rings often alternated with rings of cream or bluish grey sporodochia. Macroconidia were abundant for this fungi species, with 3-4 septa, typically canoe-shaped where several-celled slightly curved at the pointed ends. Microconidia were with 1-2 celled which are ovoid to oblong in shape.

3. *Penicillium* sp. (Figure 4.8)

Colonies of *Penicillium* sp. on PDA were very dense and radially sulcate with white velutinous mycelium. They were usually visible only at the margins. The formation of conidia was light to heavy and more commonly dark green in color.

Table 4.1 Genera of fungi isolated from the pericarp and stem-end of longan fruit (*Dimocarpus longan* Lour. cv. Daw.) using tissue transplanting method incubate for 2-3 days.

Taxa	pericarp	Stem-end
Aspergillus sp. LK1	+	+
Aspergillus sp. LP2	+	-
Cladosporium sp. CM	+	-
Colletotrichum sp.LP1	+	-
Fusarium sp.LP3	-	+
Lasiodiplodia sp. LP20	+	+
Mucor sp.CM	+	-
Penicillium sp. LK5	+	-
Pestalotiopsis sp. LYLP	+	+
Pestalotiopsis sp. LK4S	-	+
Pestalotiopsis sp. HCM20S1	-	+
Pestalotiopsis sp. HCM23P1	+	-
Pestalotiopsis sp. MLP	+	-
Phomopsis sp. LK8	+	+
Rhizopus sp. CM	+	-
Trichoderma sp. LK9	-	+
Verticillium sp. LK11	+	-
Unidentified LK1	+	-
Unidentified LK2	+	-
Unidentified LK10	+	-
Unidentified LK13	+	-
Unidentified LK15	+	-
Unidentified LK18	+	-

4. *Aspergillus* spp.

a. *Aspergillus* sp. LK1. (Figure 4.9)

The colonies of *Aspergillus* sp. LK1 on PDA were pale yellow in color, with white inconspicuous mycelium. The conidial heads of this fungal species were usually developed uniformly over the whole colony. The conidiophores, bearing phialides at the apex or radiating from the entire surface, were upright, simple and terminal in globose swelling. The 1-celled conidia were globose with hyaline color.

b. *Aspergillus* sp. LP2 (Figure 4. 10)

The colonies of *Aspergillus* sp. LP2 on PDA were plane and velutinous with white mycelium and black conidial heads. The conidiophores were developed from surface hypha with vesicles spherical shape and phialides over the whole surface. The 1-celled conidia were brown color with the wall conspicuously roughened. Conidial head developed in large and radiate.

5. *Cladosporium* sp. (Figure 4.11)

On PDA, the colonies of *Cladosporium* sp. were dense and velvety with olive color. The 1-2 celled conidiophores were dark, variable in shape and size which could be ovoid to cylindrical and irregular shapes.

6. *Colletotrichum* sp. (Figure 4.12)

On Malt Yeast Extract Agar (MYA), the colonies of *Colletotrichum* sp. were sparse basal layer of hyphae. The acervuli were readily seen on the agar surface. The conidia of this fungal species were single celled, cylindrical in shape and hyaline or brightly in color. However, the colonies of this fungus on PDA were white in color.

7. *Pestalotiopsis* sp. (Figure 4.13)

Colonies of *Pestalotiopsis* sp. on PDA were plane and floccose with white mycelium. The conidia were produced in flat, black acervuli, developed just beneath the agar surface. These conidia would open irregularly at maturity, filled with dense layers. The 5-celled conidia were fusiform where the central 3 cells were brown in color and the apical and basal cells were hyaline. The basal one was with a single usually unbranched spike-like appendage and the apical one was with two or more simple or branched spiky appendages.

8. *Phomopsis* sp. (Figure 4.14)

The colonies of *Phomopsis* sp. were white on PDA. Mycelium immersed, branched, septate, black pycnidium and conidia hyaline, fusiform.

9. *Trichoderma* sp. (Figure 4.15)

The colonies of *Trichoderma* sp. on PDA were flat and spread rapidly. The conidia were green in color. Small single celled conidia were produced from phialides which were arranged in irregular verticals, with the subterminal phialides developed more or less at right angles to the stipe.

10. *Verticillium* sp. (Figure 4.16)

The colonies of *Verticillium* sp. were white on PDA and often alternated with rings of green conidia. The conidiophores were slender shape and branched, or at least some of the branches were verticillate. The 1-celled conidia were hyaline and ovoid to ellipsoid in shape.

11. *Mucor* sp. (Figure 4.17a)

The colonies of *Mucor* sp. grew rapidly at 25-30°C and quickly cover the surface of the agar. Its fluffy appearance with a height of several centimeters

resembles cotton candy. From the front, the color was white initially and becomes grayish brown in time. Non-septate were with broad hyphae, sporangiophores, sporangia, while the spores were visualized. Sporangia were round, gray to black in color, and were filled with sporangiospores which were round or slightly elongated.

12. *Rhizopus* sp. (Figure 4.17b)

On PDA, the colonies of *Rhizopus* sp. covered or filled the whole Petri-dish with pale grey mycelium. This mycelium becames dark grey as the sporangia reach maturity.

13. Unidentified fungi isolates (species)

Among 7 unidentified fungi specimens obtained from rotten longan fruits in this experiment, 6 isolates were with septate mycelium and the conidia formation was not found.

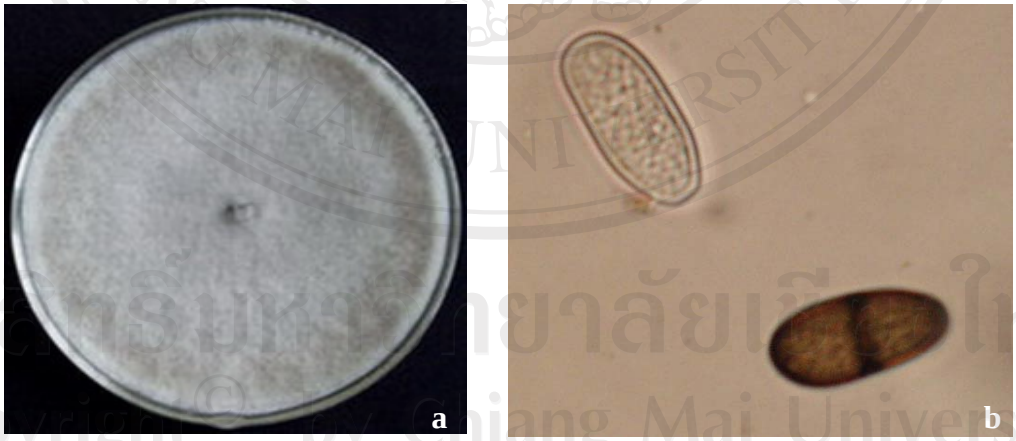

Figure 4.6 *Lasiodiplodia* sp. (a) colony on PDA, 3 days, 25 ^{0}C; (b) conidia

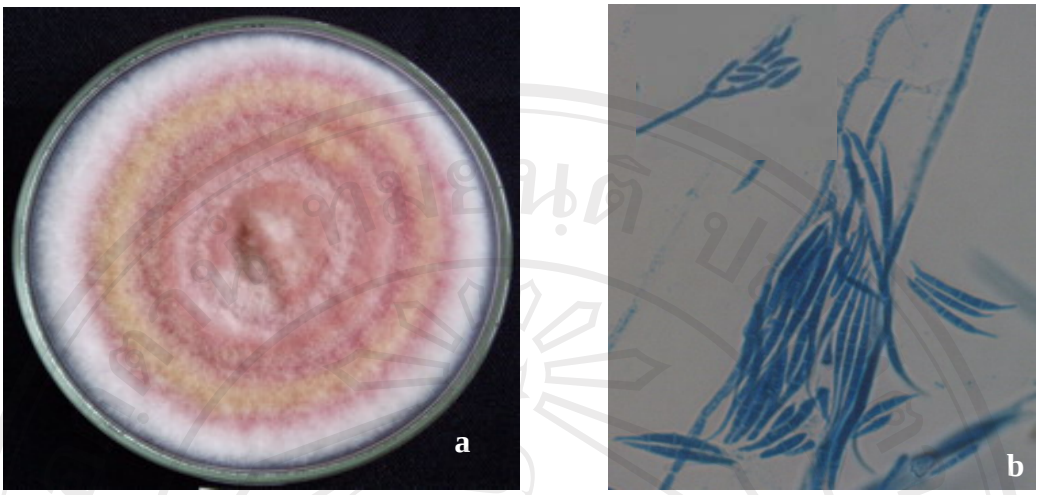

Figure 4.7 *Fusarium* sp. (a) colony on PDA, 7 days, 25 ^{0}C; (b) macroconidia and microconidia

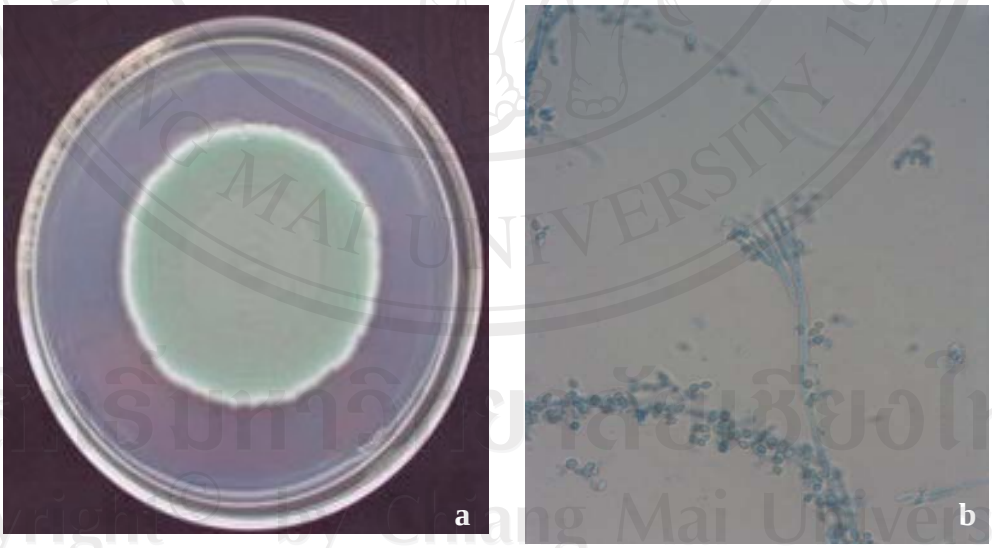

Figure 4.8 *Penicillium* sp (a) colony on PDA, 7 days, 25 ^{0}C; (b) a penicillus and conidia

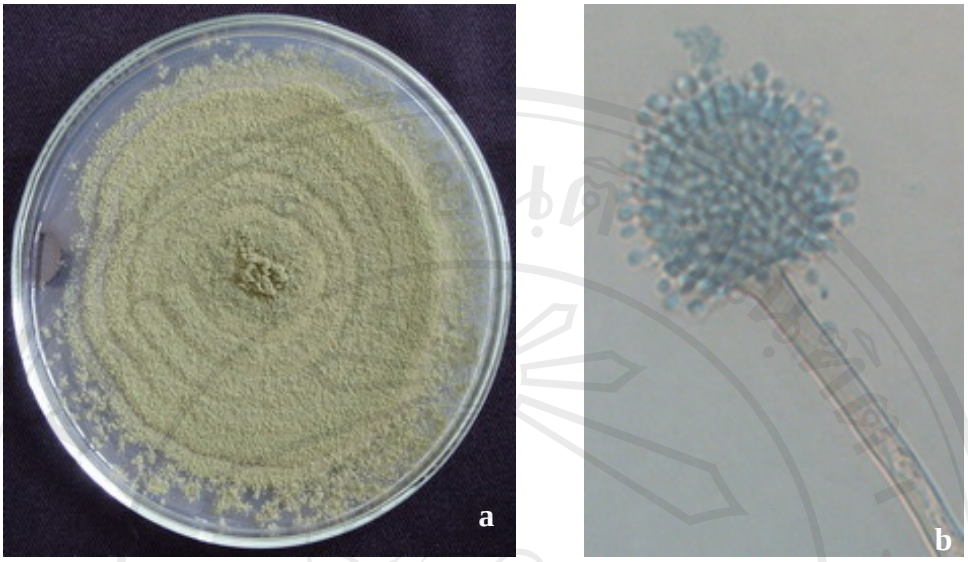

Figure 4.9 *Aspergillus* sp. LK1 (a) colony on PDA, 7 days, 25 ^{0}C; (b) a conidial head

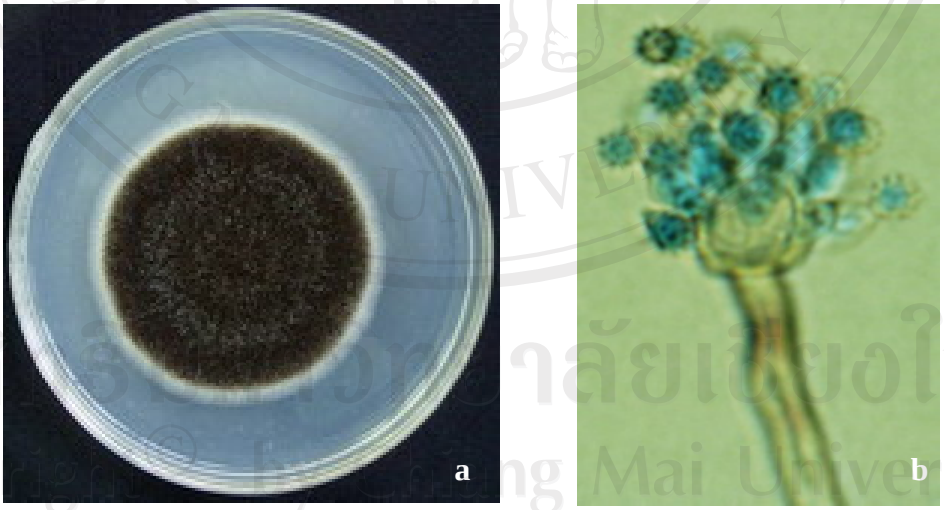

Figure 4.10 *Aspergillus* sp. LP2 (a) colony on PDA, 7 days, 25 ^{0}C; (b) a conidial head

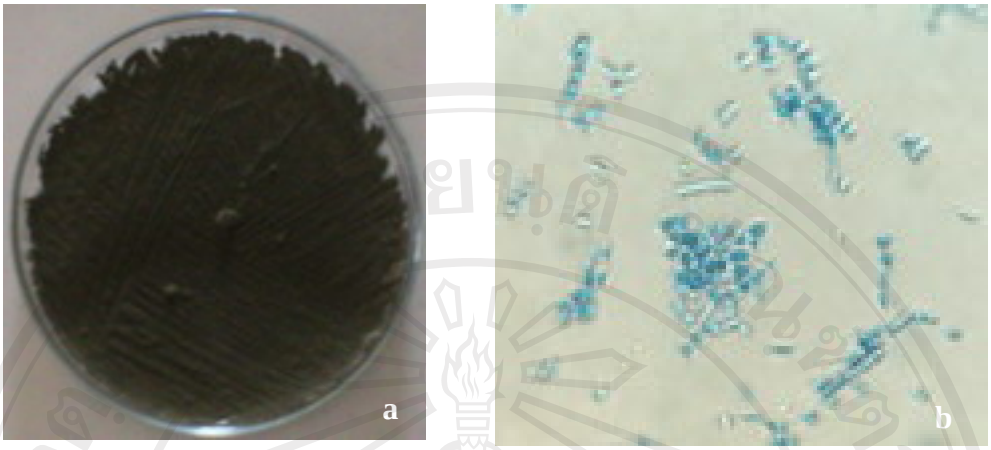

Figure 4.11 *Cladosporium* sp. (a) colony on PDA, 7 days, 25 ^{0}C; (b) conidia

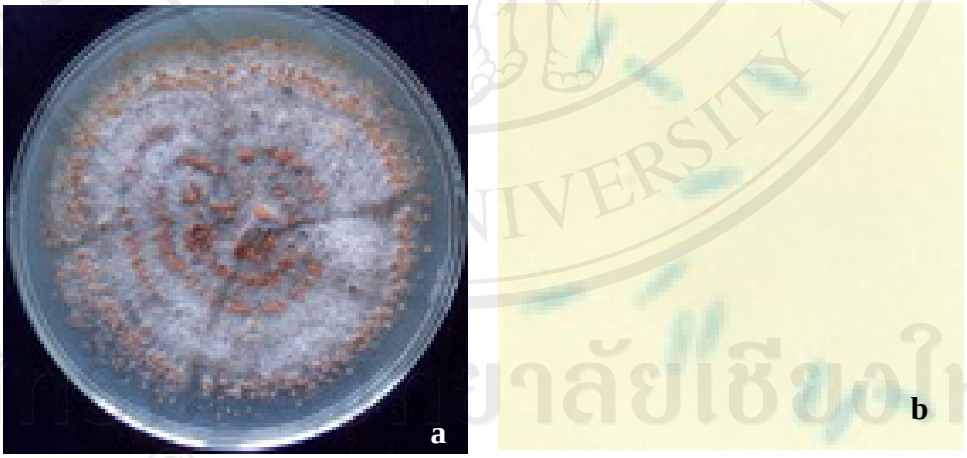

Figure 4.12 *Colletotrichum* sp. (a) colony on MYA, 7 DAYS, 25^{0}C; (b) conidia

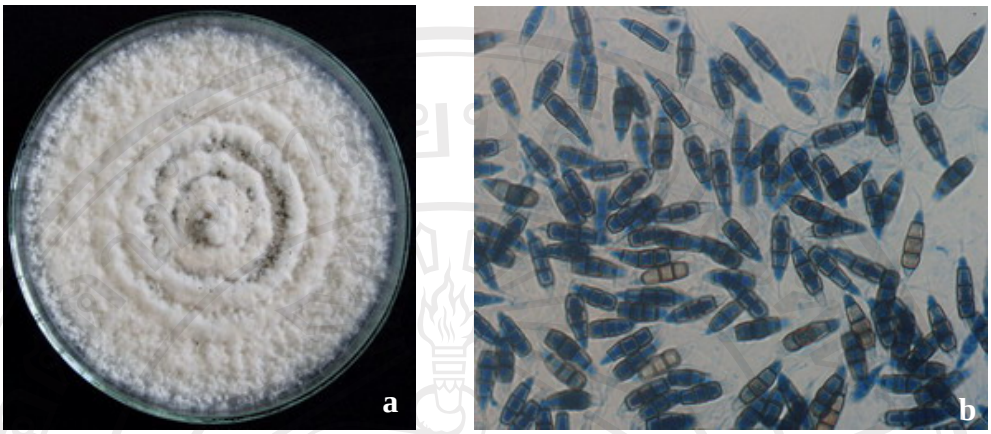

Figure 4.13 *Pestalotiopsis* sp. (a) colony on PDA, 7 days, 25 ^{0}C; (b) conidia

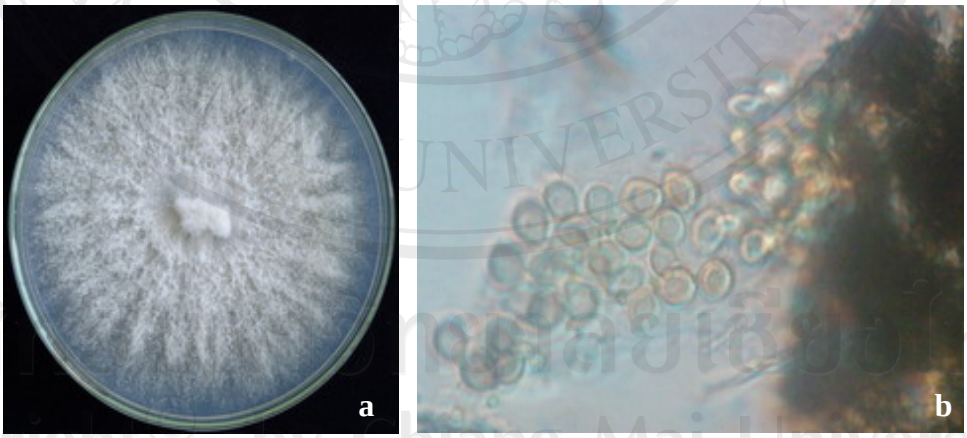

Figure 4.14 *Phomopsis* sp. (a) colony on PDA, 7 days, 25 ^{0}C; (b) conidia

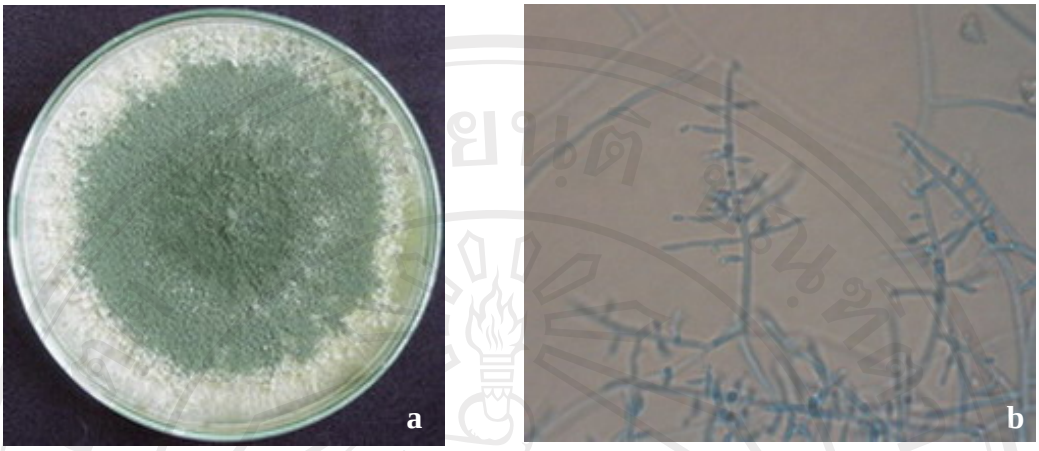

Figure 4.15 *Trichoderma* sp. (a) colony on PDA, 7 days, 25 ^{0}C; (b) conidia

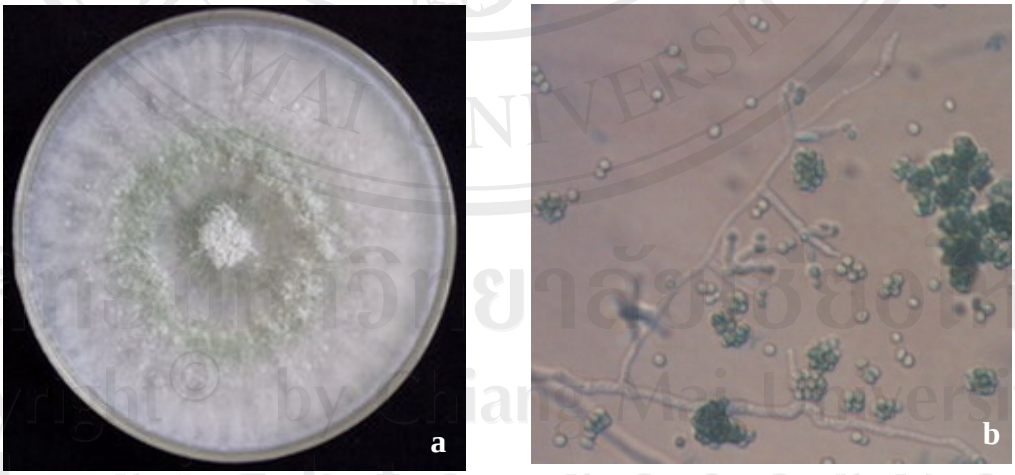

Figure 4.16 *Verticillium* sp. (a) colony on PDA, 7 days, 25 ^{0}C; (b) conidia

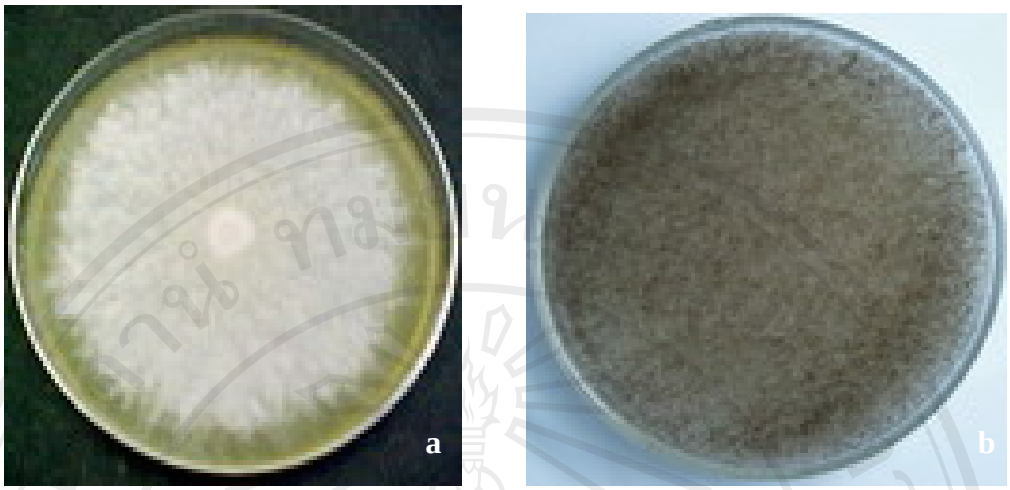

Figure 4.17 *Mucor* sp. (a) and *Rhizopus* sp.(b) colonies on PDA, 7 days, 25 ^{0}C

4.3 Pathogenicity of the isolated fungi and disease development

Various molds/fungi generally grew well on longan fruit. However, only a few of them were able to cause necrotic lesion and rot the fruit (Table 4.2). Among these, *Lasiodiplodia* sp. LP20, which was commonly isolated from the fruit peel and stem-end, was also indicated to be one of the most virulent pathogen of postharvest longan fruit.

After inoculated with *Lasiodiplodia* sp. LP20, the inoculated point (the fruit surface) was darkend as the result of tissue necrosis in 6 hours after inoculation. At 12 hours both sides of the pericarp were dark brown in color. Whole of the fruit was thoroughly decayed eventually in 48 hours (Figure 4.18). The fruit which was inoculated near the stem-end rotted faster than inoculated at the outer part of fruit peel. The rotting areas spreaded rapidly from the stem-end down to the other part of fruit peel and near by aril. The aril became yellowish in color. Softening of the tissue was detected in 24 hours (Figure. 4.19)

The inoculation of *Pestalotiopsis* sp. MLP isolate on longan fruit caused fruit rot disease where brown areas on the peels were found (Figure 4.21a, b). The whole fruits were covered with cottony mycelia within 5 days and abundant black conidial masses were found on day 5 (Figure 4.20c).

Figure 4.18 Disease symptoms on detached fruit 48 hours after being inoculated with *L. theobromae* LP20 on the fruit surface. Top: Un-inoculated (control) longan fruits, Bottom: Inoculated longan fruits.

Figure 4.19 Disease symptoms on detached fruit 48 hours after being inoculated with *L. theobromae* LP20 on the stem-end. Top: Un-inoculated (control) longan fruits, Bottom: Inoculated longan fruits.

Table 4.2 The pathogenicity of fungi isolated from longan fruits (*Dimocarpus longan* Lour. cv. Daw)

Genera	Inoculum	Disease Rating*	Incidence (%)
Aspergillus sp.LK1	Conidia	0	0
Aspergillus sp.LP2	Conidia	2	16.66
Cladosporium sp.CM	Conidia	2	20
Collettotrichum sp.LP1	Conidia	3	46.66
Fusarium sp. LP3	Mycelia	2	23.33
Lasiodiplodia sp. LP20	Mycelia	5	100
Mucor sp. CM	Conidia	0	0
Penicillium sp. LK5	Conidia	0	0
Pestalotiopsis sp. LYLP	Conidia	4	80
Pestalotiopsis sp. LK4S	Conidia	4	66.66
Pestalotiopsis sp. HCM20S1	Conidia	3	60
Pestalotiopsis sp. HCM23P1	Conidia	4	60
Pestalotiopsis sp. MLP	Conidia	4	90
Phomopsis sp. LK8	Mycelia	2	20
Rhizopus sp. CM	Conidia	0	0
Trichoderma sp. LK9	Conidia	2	16.66
Verticillium sp. LK11	Conidia	0	0
Unidentified LK1	Mycelia	0	0
Unidentified LK2	Mycelia	2	10
Unidentified LK10	Mycelia	0	0
Unidentified LK13	Mycelia	2	10
Unidentified LK15	Mycelia	0	0
Unidentified LK18	Mycelia	0	0
Unidentified LK19	Mycelia	0	0

Disease rating is the degree of severity, ranging from 0 to 5, which were observed on the fruit surface 48 hours after the fungus inoculation; 0 = no necrotic lesion, 5 = brown necrotic lesion of more than 2 cm in diameter. Incidence= number of disease longan fruits/number of normal longan fruit

Figure 4.20 Disease symptoms developed on detached longan fruits after being inoculated with *Pestalotiopsis* sp. MLP; (a, b) 48 hours after the inoculation; (c) abundant black conidial masses were found on day 5 after the inoculation.

4.4 Identification of a rot causing fungal isolates

From 4.3, *Lasiodiplodia* sp. LP20 was clearly the most virulent fungus isolate which rapidly cause necrotic lesion and rot the longan fruits. This fungus grew well on PDA plate with the rate of diameter increasing of about 2-3 centimeters per day; however, the fungus rarely produced spores on this medium. To induce sporulation, small pieces of sterilized grass leaf were spreaded on the surface of the full-grown colonies. On the other hand, this fungus produced its spores well on potato dextrose broth (Figure. 4.21). Their one-celled young spores were hyaline, while the two-celled mature spores were dark brown in color with thick walled (Figure. 4.22). The inoculated longan fruit peel observed under a SEM showed numerous hypha of this fungus (Figure. 4.23). The identification of this fungus was then made following von Arx (1981) and Sutton (1980). Based on morphology, DNA sequencing and blast search, *Lasiodiplodia* sp. LP20 was identified as *Lasiodiplodia theobromae* (Pat) Griff. & Maubl.

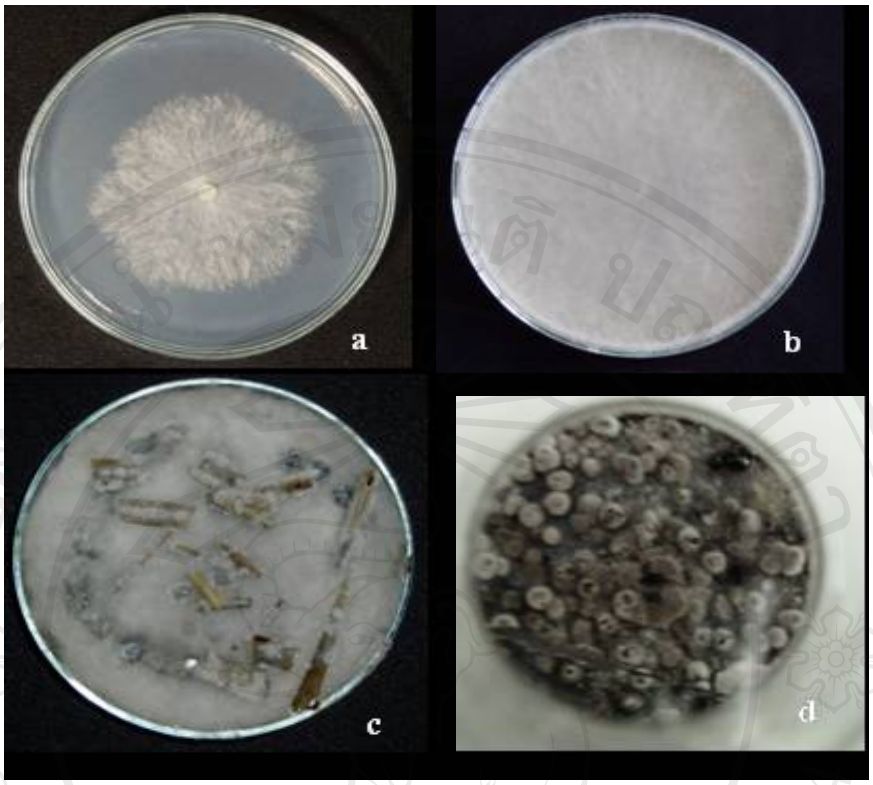

Figure 4.21 Colonial growth of *L. theobromae* LP20. (a) 1 day on potato dextrose agar (PDA), (b) 2 days on PDA, (c) 2 days after placing pieces of sterilized grass leaf on the PDA culture of (b), (d) 4 days on potato dextrose broth

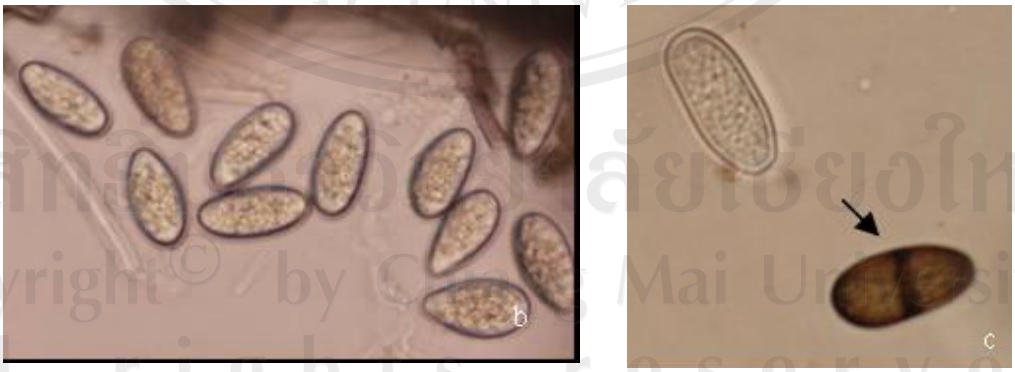

Figure 4.22 Light micrographs of *L. theobromae* LP20. (a) Immature, hyaline, aseptate conidia . (b) A mature, dark brown, septate conidium (arrow).

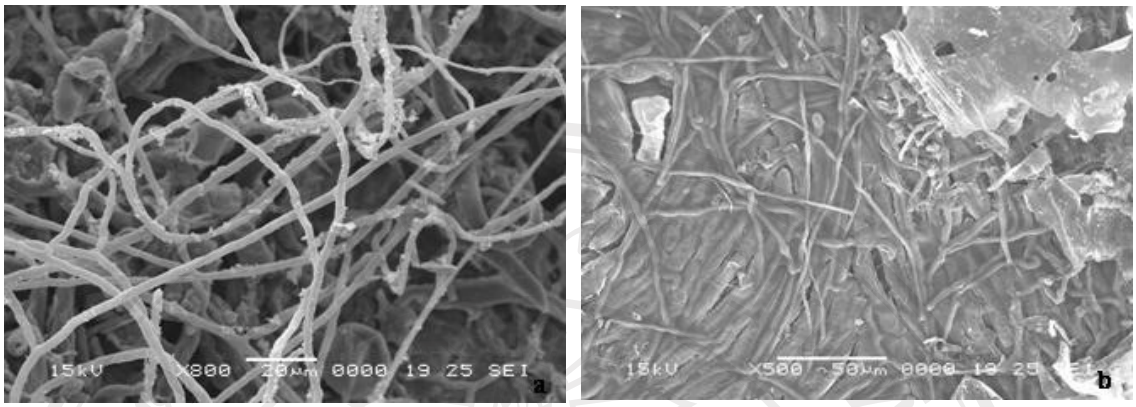

Figure 4.23 Scanning electron micrographs of *L. theobromae* LP20. (a) hypha on the exocarp of longan fruit observed 24 hours after the inoculation of *L. theobromae* LP20, indicated scale bar = 20µm, (b) hypha in the endocarp of longan fruit observed 24 hours after the inoculation, indicated scale bar = 50 µm

4.5 Infection process of *Pestalotiopsis* sp. MLP on the pericarp and stem-end of harvested longan fruits

From the examination of *Pestalotiopsis* sp. MLP spore germination on Millipore filtering-paper under a light microscope, it was found that the spores produced germ tube which had the length of more than haft of the spore width at hour 3 after the incubation of the experimental units. Long and branched hyphal were found at hour 24 (Figure.4.24).

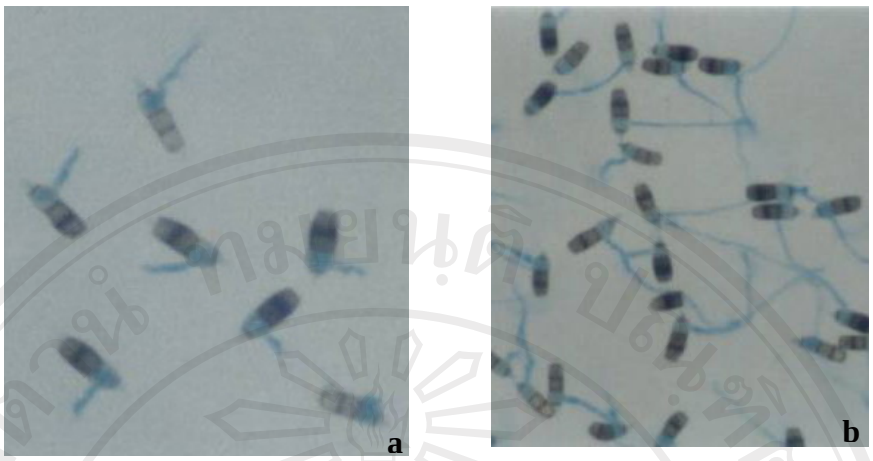

Figure 4.24 Light micrographs of germinated spores of *Pestalotiopsis* MLP. (a) spore germination was found at hour 3 after being incubated, (b) long and branching hyphae were found at 24 hours after the incubation.

Detection of the infection process

a. Examination by stereo microscopy

Pestalotiopsis sp. MLP mycelia appeared only two to three days after being inoculated and incubated in moist chamber. Infected longan fruits were rotten and the whole surfaces of all fruits were covered with white cottony mycelia and abundance of black conidial masses were found on day 5 (Figure 4.25).

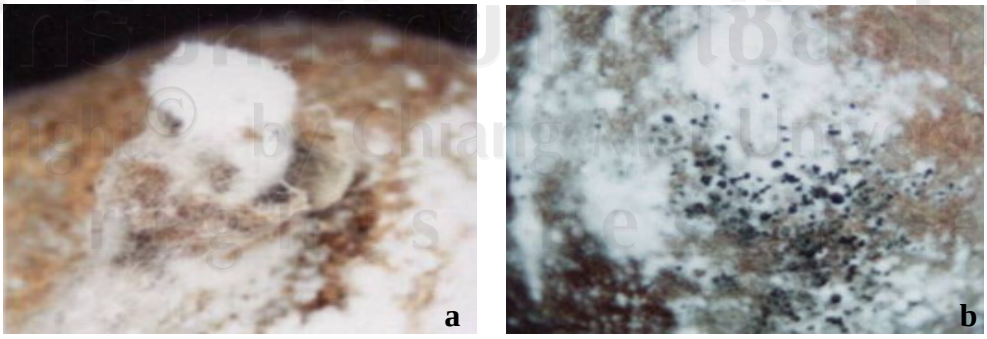

Figure 4.25 Stereo micrographs of longan fruit peel surfaces infected with *Pestalotiopsis* sp. MLP. (a) White cottony mycelia was clearly seen on stem-end, (b) abundant black conidial masses covered fruit peel surface on day 5

b. Examination by light compound microscopy

The germination of *Pestalotiopsis* sp.MLP spores was observed at hour 5 after the inoculation (Figure.4.26). The acervuli were found at 96 after the inoculation, respectively (Figure. 4.27).

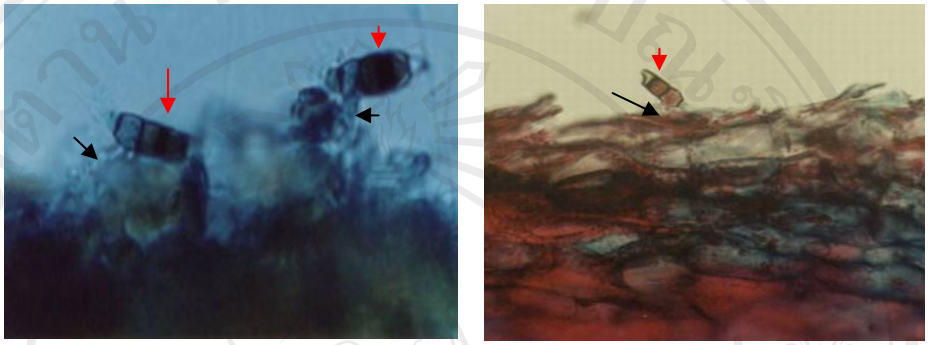

Figure 4.26 Spores germination of *Pestalotiopsis* sp. MLP was found germ tubes at 5 hours after the inoculation Left: Clearing method, Right: Paraffin section method (spores: red arrow, germ tube : black arrow)

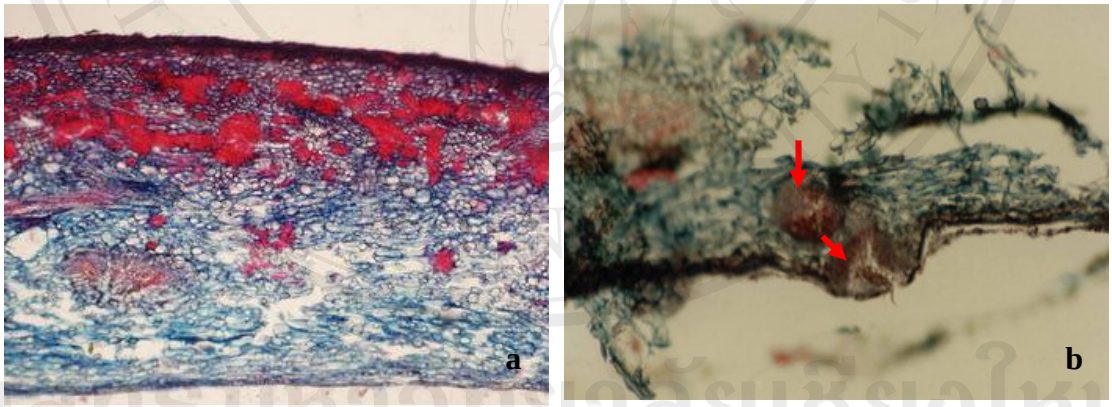

Figure 4.27 Cross-section view of the inoculated longan fruit peel with conidia of *Pestalotiopsis* sp. MLP, (a) 96 hours after the inoculation; the pericarp of the uninocutated fruit after dipping in sterilized water (control), (b) 96 hours after the inoculation; the pericarp of the inoculated fruit after dipping in the conidia suspension of *Pestalotiopsis* sp. MLP, acervuli (red arrows) were found in the endocarp of the inoculated longan fruit

c. Examination by scanning electron microscopy

Spores of *Pestalotiopsis* sp. MLP germinated after being inoculated on the surface of mature longan fruit for 3 hours (Figure 4.28). The conidia were observed on fruit surface among the cuticular fraction and the groups of trichomes (Figure 4.29). One conidium could produce one or two germ tubes. The appressorium formation was visible at hour 12 (Figure 4.30). After 24 hours, the first mycelium was found in inner endocarp of longan fruit (Figure 4.31). At 48 hours, some area on the fruit surface exposed the acervulus with conidia in black mucilaginous matrix (Figure 4.32).The whole fruit surfaces were covered with abundant mycelia on hour 48 (Figure 4.33). Then pericarp tissues invaded by mycelia were found in 96 hours (Figure 4.34).

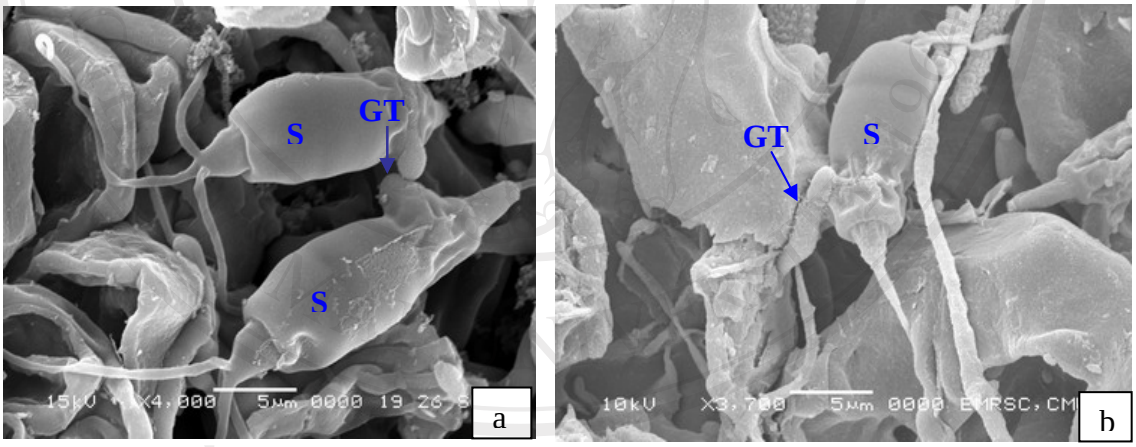

Figure 4.28 Scanning micrographs of germinated spore of *Pestalotiopsis* sp. MLP on the surface of longan fruit sampled, (a) spore germination and germ tube; 6 hours after inoculation, (b) spore germination and germ tube; 6 hours after inoculation (S=spore, GT= germ tube)

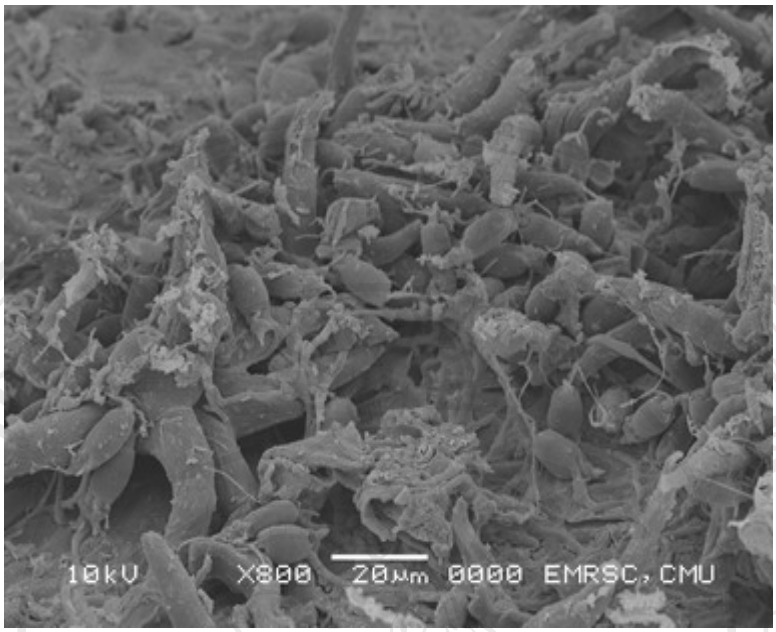

Figure 4.29 The conidia of *Pestalotiopsis* sp. MLP were observed on the longan fruit surfaces

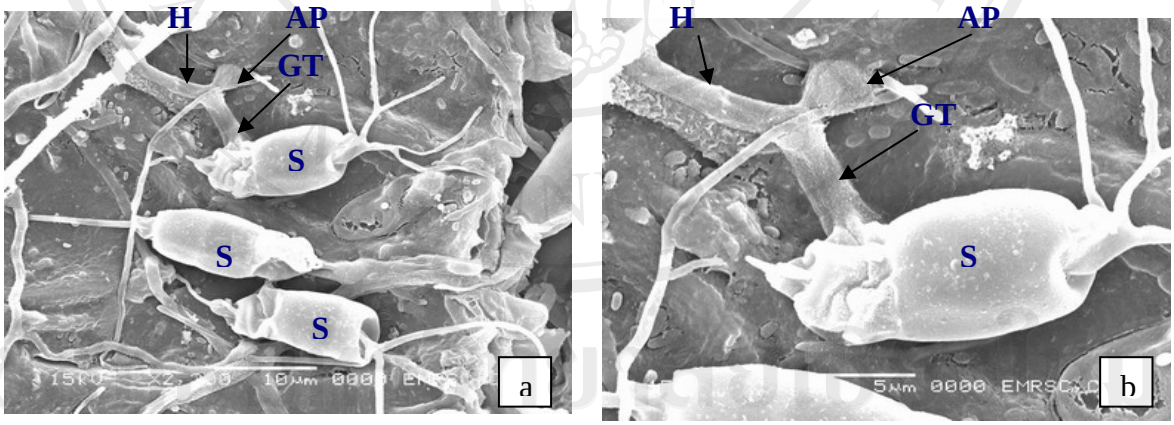

Figure 4.30 Scanning micrographs shows early infection stages of the *Pestalotiopsis* sp. MLP, (a) appressorium were found at hour 12 after the inoculation, (b) appressorium were found at hour 12 after the inoculation (GT=germ tube, S=spore, H=hypha, AP: appressorium)

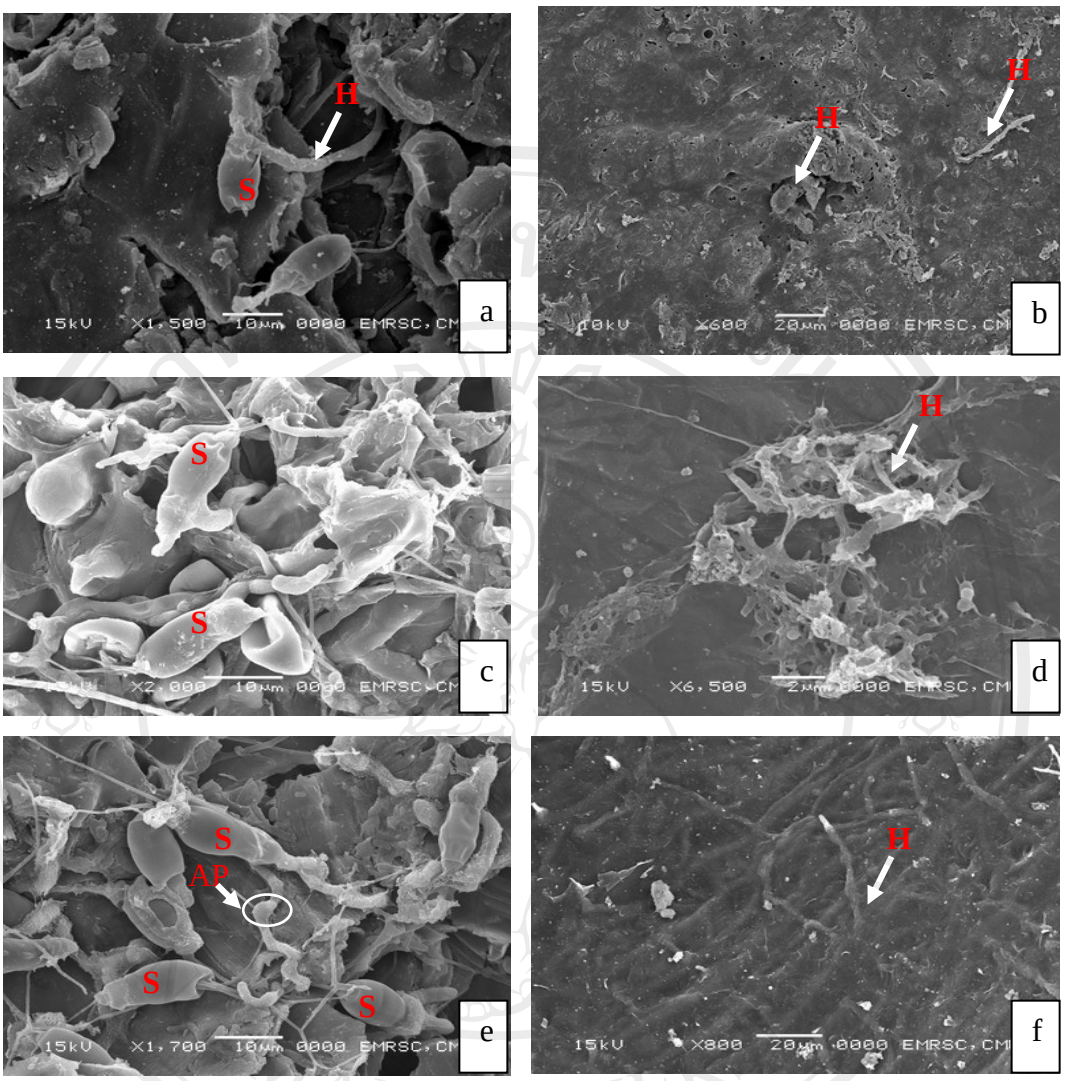

Figure 4.31 Scanning micrographs showed infection stages of the *Pestalotiopsis* sp. MLP, (a) Spores germinated through crack areas of the skin surface of longan fruit at 24 hours after the inoculation, (b) hyphae were found on the endocarp surface at 24 hours after the inoculation, (c) hyphae covered the whole area of the skin surface of longan fruit at 48 hours after the inoculation), (d) mycelia were found on the endocarp surface at 48 hours after the inoculation, (e) spores germinated through crack areas of the skin surface at 72 hours after the inoculation, (f) hyphae covered the whole areas of the endocarp surface of longan fruit at 72 hours after the inoculation (S = spore, H = hypha, GT= germ tube, = appressorium)

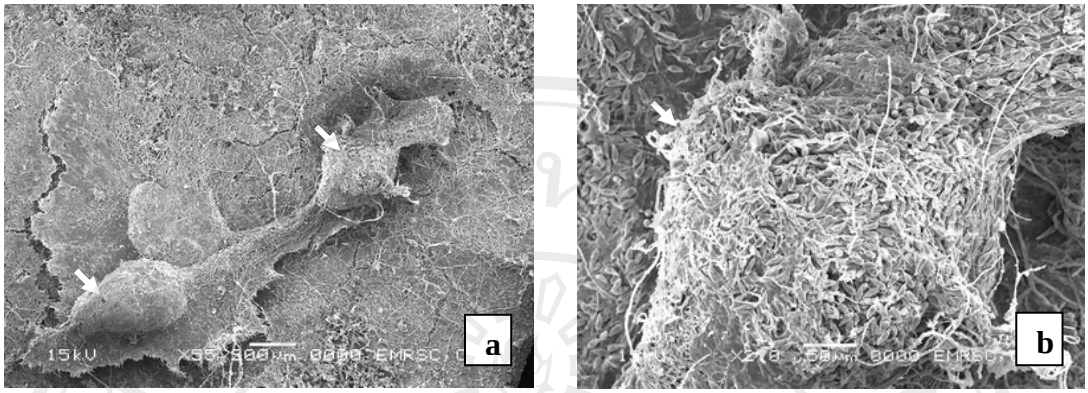

Figure 4.32 Scanning micrograph showed (a, b) droplet-like spore masses of *Pestalotiopsis* sp. MLP on the skin surface of infected longan fruit, 48 hours after the inoculation. (White arrow)

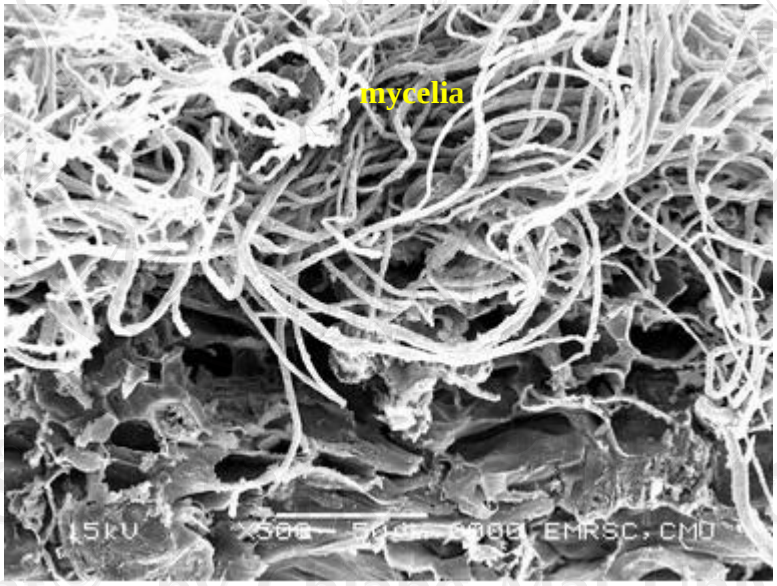

Figure 4.33 Scanning micrograph showed the skin surface of longan fruit well-covered with abundant mycelia of *Pestalotiopsis* sp. MLP at 48 hours after the inoculation.

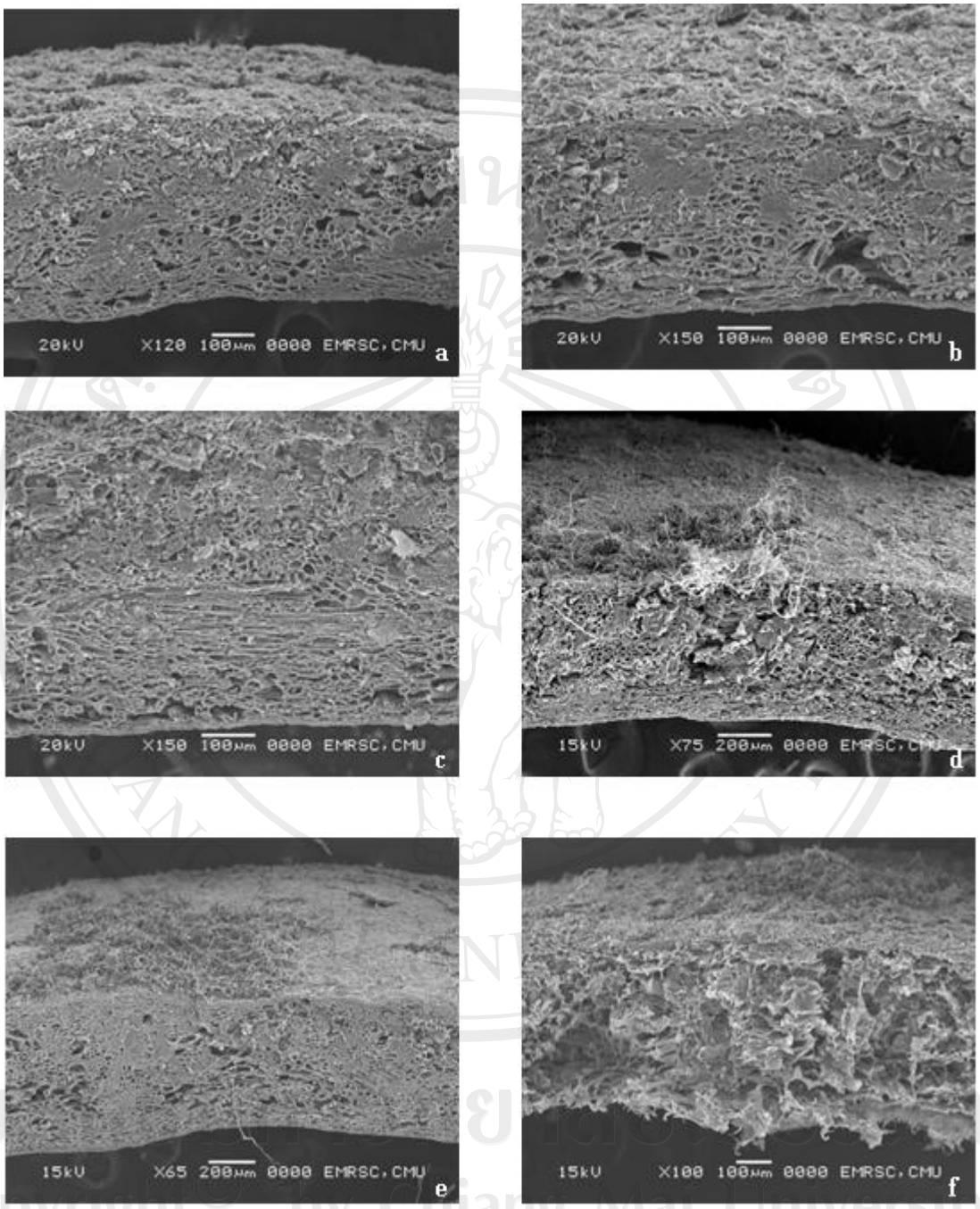

Figure 4.34 Scanning micrographs showed the processes of internal breakdown of fruit tissue after inoculated with *Pestalotiopsis* sp. MLP, (a) 6 hours, (b) 12 hours, (c) 24 hours, (d) 48 hours, (e) 72 hours and (f) 96 hours after the inoculation.

d. Transmission electron microscopy (TEM)

By transmission electron microscopy (TEM) study of the infection progress of *Pestalotiopsis* MLP, the appressorium and infection peg, signs of positive infection, were not found in the experimented longan fruit.

4.6 Sulfur dioxide fumigation effect on disease development of harvested longan fruit

4.6.1 Surface appearance changes of longan fruit fumigated with SO_2

The surface appearance examination of the harvested longan fruits fumigated with SO_2 found that the effect was presented in the skin surface of the fruits. The filamentous hypha of the epiphytic fungus on the outer skin of the fruits peels were blistered or swelled up compared to the epiphytic fungus hypha found on outer skin of non-fumigated fruits (Figure 4.35).

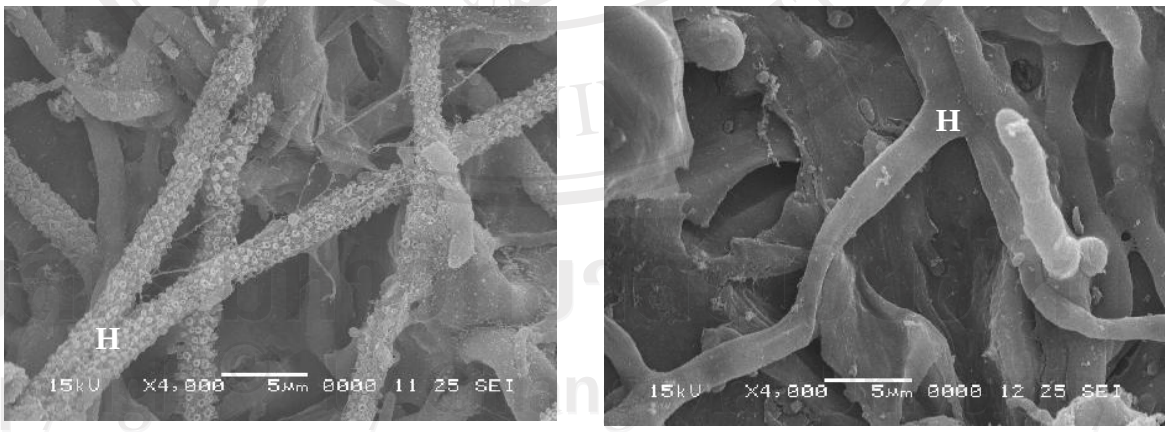

Figure 4.35 Scanning micrographs of the epiphytic fungal hypha on the outer skin texture of the peel of the SO_2 – fumigated longan fruit (left) compared to the ones of non-fumigated fruits (right). With the same magnification under SEM, the fungus hypha presented on the fumigated fruits were clearly blistered. (H = hypha)

4.6.2 Effect of SO_2 on disease development on the inoculated longan fruits

The examination carried out to study the effect of SO_2 on disease development on the inoculated longan fruits found that the SO_2-fumigation treatment had affected on the fungus spores and germ tubes where they became blistered (Figure 3.36). The SO_2-fumigation clearly had affected the growth of fungus on the outer skin of the infected longan fruits results the delayed of the fruit rotting, while the fruits with fungus inoculated and non–fumigation rotted within 2 days after the inoculation.

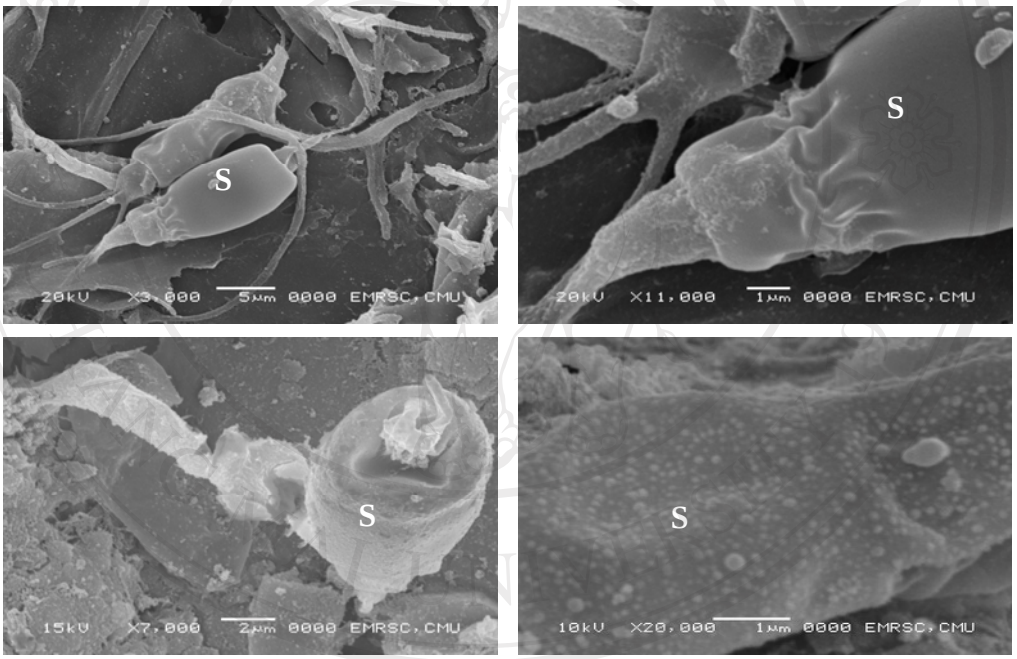

Figure 4.36 Scanning micrographs shows the blistered spores of *Pestalotiopsis* sp. MLP presented on the inoculated fruits with SO_2-fumigation. (S = spore)

Top: Spores of *Pestalotiopsis* sp. MLP presented on the inoculated fruits without SO_2-fumigation.(control)

Bottom: The blistered spores of *Pestalotiopsis* sp. MLP presented on the inoculated fruits with SO_2-fumigation.

4.6.3 Effect of SO_2 on fungal growth *in vitro*

The study of the fungal filamentous hypha (*Lasiodiplodia theobromae* LP20 and *Pestalotiopsis* sp. MLP)from the inoculated and SO_2-fumigated longan fruits under SEM-microscope, it was found that the hypha became deflated and, with EDX-analysis, crystallized materials with sulfur-component was also found (Figure 4.37). Furthermore, culture of these filamentous hypha of *Pestalotiopsis* sp. MLP obtained from the inoculated and SO_2-fumigated longan fruits was negative. On the other hand, the same application for *Lasiodiplodia theobromae* LP20 was positive where its filamentous hypha were re-cultured at 48 hours after being cultured onto PDA.

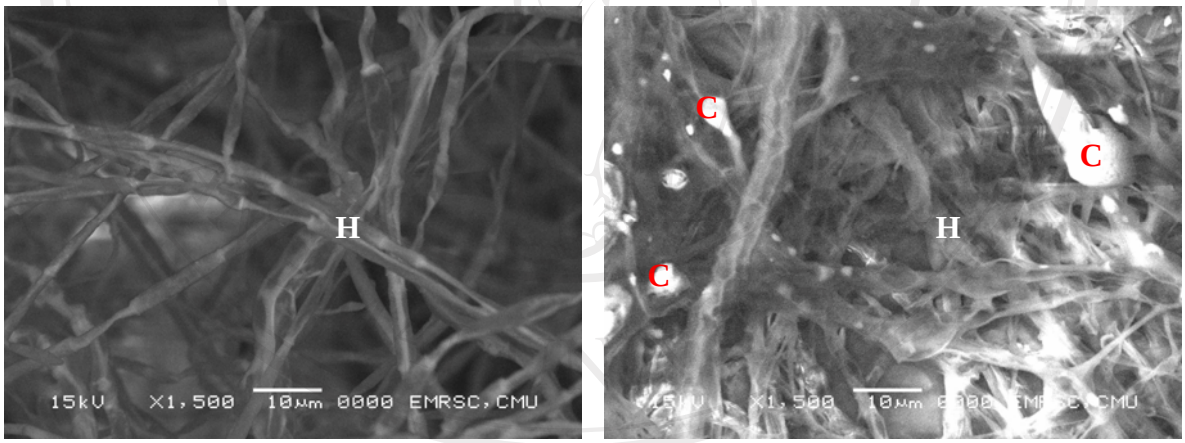

Figure 4.37 Scanning micrographs showed the filamentous hypha of *Pestalotiopsis* sp. MLP on cultured PDA (H= hypha, C = sulfur crystal on SO_2-fumigated hyphae)

Left: The inoculated fruits with non- SO_2-fumigation

Right: The inoculated fruits with SO_2-fumigation