CHAPTER 5

DISCUSSION

Obervation on the pericarp of the harvested longan fruit of Daw cultivar (*Dimocarpus longan* Lour. cv. Daw) revealed that outer skin surface was composed of 3 parts as mentioned in former study by Supawattanabodee (1997) and the longan in China (Qu *et al*.,2001). The outer skin surface of longan fruit was rough and uneven covered with scales and trichomes. Discontinuous cuticles were found and they were broken ones. Both filamentous hyphae of mold or fungus, yeast and bacteria were found on the outer skin surface of the fruit. These microorganisms, especially filamentous fungi (molds), might indicate the fast rotting of the fruits after being harvested. Fungi have been reported as the main cause of post-harvested longan fruit rot (Chaiwangsri, 1992; Nachaiwiang, 1994). Mahattanapak (1999) reported the reduction of a fungus-resisting substance; resocinal on the outer surface of the post-harvested longan fruit, while the same substance was found at very high content in the pre harvested fruits on the trees. Additionally, the component structure of the fruit skin could be also weaker after being harvested. The fruit skin of longan would generally get thinner as the fruit grows larger while the cuticles are reduced and the cuticle-thickness was also reduced and broken (Suwanakood, 2005). These could

allow the fruit to become more susceptible to rotting pathogens, where they can infect the fruit by penetrating through, and even hiding in, the broken parts of the cuticles. This obtained information can be beneficial for the development of post-harvested disease prevention of longan fruit.

Deterioration of longan fruit peel after harvest might be a contributing factor in changes that occur in the peel texture. These are the causes that stimulate the infection. For further application of this information will be used to find a way to protect longan fruit (after harvest) from microbial penetration and infection. This information will help us to make decisions regarding spraying with chemical agents. An experiment showed that there are a few stomata on the peel of mature longan fruit. Inducing the stomata to close by dipping the fruit in some safe food preservatives or in cold water before fumigation with SO_2 could possibly reduce the sulfur residues in its aril.

A number of filamentous fungi were isolated from the pericarp and stem-end of longan fruits using tissue transplanting method. After that, these isolated fungi were testing for their pathogenicity in longan fruit. However, only *Lasiodiplodia* and *Pestalotiopsis* have shown high pathogenicicty, and are able to penetrate the unwounded fruit directly. According to former studies, these two genera have been reported to be among common pathogens in longan fruits (Chaiwangsri, 1992; Nachaiwiang, 1994) as well as in other fruits (Pandey and Dwivedi 1987, Jonnson *et al*., 1994, Sangchote and Saoha 1998), both in pre-harvested and post-harvested stages. They could induce the fruit to rot quickly. Sardsud *et al*. (1998) had also reported that *Lasiodiplodia theobromae* and *Pestalotiopsis* were endophytic fungi in

longan tree, where another report by Suwanakood *et al* (2004) found the presence of *Pestalotiopsis* in both healthy and rotting longan fruits.

The present study had found that *Lasiodiplodia theobromae* LP20 was the most aggressive isolate in causing fruit rot of longan. Mycelia of *Lasiodiplodia theobromae* LP20 spread rapidly, over the peel tissue of both intercellular and intracellular. According to Nagaraja *et al*.(1971); Wang and Pinckard(1971); Adisa and Fajala (1982) this mold had high capability to produce large amount of wall lytic enzymes i.e. pectinase, cellulase and protease (Nagaraja *et al*., 1971; Wang and Pinckard, 1971; Adisa and Fajala, 1982). Cracking of the cuticle in cuticular layer provided spaces for molds to go into the peel. The molds penetrated through three layers of the peel quite easily within only 12 hours and rotted the whole fruit in one day (within 24 hours after inoculation). Hidden mold in the stem-end could grow through vascular duct (Srivastava and Urgapal, 1964). On inoculated fruit, the rot at the stem-end expressed very fast. The molds grew through hilum area to the peel around the stem-end and also the inner part of the fruit, softening the fruit aril in 24 hours. No different in disease development on stem-end and peel surface after inoculated with *Lasiodiplodia theobromae* LP20. This finding agreed with the report by Tongdee (1977) that most common fungi in rotten longan fruits were *Lasiodiplodia (Botryodiplodia)*, as well as, the report by Chaiwangsri (1992). *Lasiodiplodia* is the fungus with fast growing capacity. Their filamentous hyphae grow rapidly without producing spores unless stimulated. Fungus spore characteristics and properties were mainly used for the identification. Some earlier studies by Choomsan (1987) and Nantiya (1988) could not identify this fungus due to the lack of spore production. In the present study, in

order to induce spore production of this fungus, sterilized grass pieces were added onto the culture of *Lasiodiplodia* in PDA which helped to stimulate the spore production. Then, also by DNA sequencing it was confirmed that this fungus was *Lasiodiplodia theobromae* (Pat) Griff. & Maubl.

For *Pestalotiopsis* sp. MLP, it was also found that this fungus could induce fruit rot quickly. By the examination under a light transmitted microscope (LM), its spores germinated at 3 hours after suspending in sterilized distill water and placed on moist filtered paper. While, being on the longan fruit skin, the spores germinated at 5 hours after the inoculation. In addition, the examination under a scanning electron microscope (SEM) revealed that at 5-6 hours after inoculation the spores developed germ tubes up to half of the spore length. This finding indicated that spores of *Pestalotiopsis* MLP on the outer skin of longan fruit would develop germ tubes and also produce appressorium, the structure to stick to the fruit skin, at 5 hours after the inoculation. At 96 hours after the inoculation, most of acervuli, with many spores inside were found. However an infection peg produced from the appressorium in order to penetrate through the outer skin into the mesocarp was not found under a transmission electron microscope (TEM).

The present study has finally found that the virulent pathogenic fungi for longan fruit rot are *Lasiodiplodia theobromae* and *Pestalotiopsis* sp. MLP, respectively. Furthermore, the obtained information about the component structure of the longan fruit can eventually be beneficial for the development of the post-harvest disease prevention and problem solving.

Nowadays SO_2 is commonly applied to control saprophytic fungi on fruit surface and prevents browning on fruit peel (Underhill *et al*., 1992; *Han et al*., 1999;

Tongdee, 1994; Pan *et al.*, 1999). An examination on the mature fruit after being fumigated with SO_2 found abnormality of surface molds. Numerous bubbles were found on the surface of *in vitro* fumigated mycelia of *Lasiodiplodia theobromae* LP20 and *Pestalotiopsis* sp. MLP. Infected fruit artificially inoculated with *Lasiodiplodia theobromae* LP20 and *Pestalotiopsis* sp. MLP and fumigated with SO_2 rot slower than the control group (non SO_2 fumigation). In vitro fumigated mycelia of *Lasiodiplodia theobromae* LP20 was able to regrow in 48 hours after fumigation while *Pestalotiopsis* sp. MLP could not. *Lasiodiplodia theobromae* LP20 showed more tolerant to SO_2 than *Pestalotiopsis* sp. MLP. Magan (1994) reported that there were some yeasts and molds found in food and beverages appear to be very tolerant to $S0_2$.