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Ultrastructure studies of fungus *Colletotrichum gloeosporioides* cause of Anthracnose disease of mango by microscope technique.

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ABSTRAC

This research aimed to study the formation of *C. gloeosporioides* in mango cv. Nam Dok Mai Sri Thong with light microscope and transmission electron microscope. Due to the microscopy and transmission of electron microscopes, the microstructure of *C. gloeosporioides* causes of Anthracnose of Mango. The structure of the fungus found in the skin cells and the host tissue cells that have been destroyed and infection in the epidermis and tissue cells In mango fruit thickness found that conidial germination in parenchyma cells and epidermis cells.

INTRODUCTION

Microscope technique prepared for light and transmission electron microscopy can be divided into eight major steps: primary fixation, washing, secondary fixation, dehydration, infiltration with transitional solvents, infiltration with transitional resin, embedding and cutting [1,2,5]. The aim of the present investigation was to study ultrastructure of fungus *C. gloeosporioides* caused by anthracnose disease of mango.

C. gloeosporioides fungus is the cause of anthracnose disease occurring in fruit trees such as mangoes grown in hot and high humid conditions. Anthracnose fungus destroys plants at all stages of growth of the mango throughout the growing season. The fungus began to develop as new, destroying the mangoes, causing rotten fruit, with black brown spots on the mango fruit (Figure 1 and 2). Especially mangoes that are commonly eaten, usually with thin crusts that are easily infected. In the post-harvest period of mangoes such as Nam Dok Mai species the fungal infection results in loss of quality is a major obstacle to the delivery of both domestic and international markets. The early stages of fungal development during the infection process are essential for all *Colletotrichum* species and can be separated into stages including deposition of conidia on plant surfaces, attachment of conidia to those surfaces, germination of conidia, production of appressoria, penetration of the plant epidermis, growth and colonization of plant tissues, and production of acervuli and sporulation [3,6]. High temperature and high humidity are suitable for the growth of the pathogen, and the germination of the spores can be relatively high.

METHODOLOGY

Plant material

Mango is in the plant family Anacardiaceae. The mature mango fruits were collected from commercial orchards in Nakhon Ratchasima Province, the north - east Thailand during

In August 2018. The fungus began to develop as new, destroying the mangoes, causing rotten fruit, with black-brown spots on the Mango Nam Dok Mai Sri Thong fruit (Figure 1). The mature mango fruits were studied using a microscope technique.

Microscopy studies

Morphology of fungus *C. gloeosporioides* under optical microscopy.

Figure 1. Symptoms on ripe mango fruit.

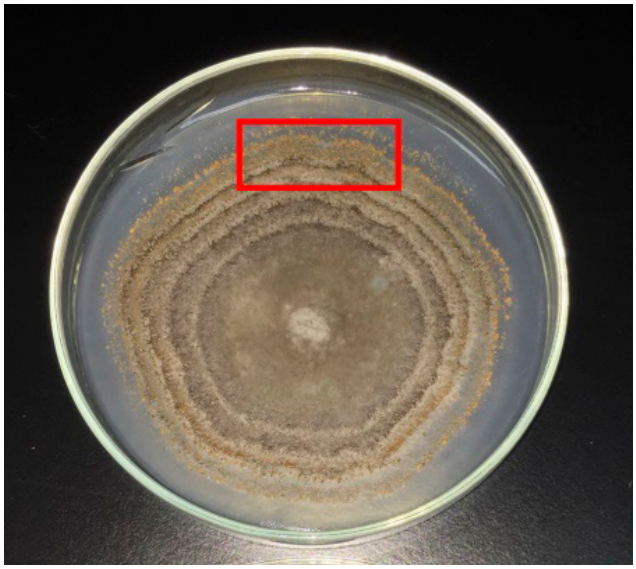

Figure 2. *C. gloeosporioides* grown showed on PDA.

Stereo microscopy observation

Mango Nam Dok Mai Sri Thong rotten fruit showed symptoms with black-brown spots when examined under a stereo microscope (Carl Zeiss; Stemi 2000-C) which was equipped with a photographic camera under normal bright-field imaging.

Light microscopy observation

The samples were placed on a glass slide with a drop of distilled water, then covered with a cover glass, and then examined under a light microscope (Carl Zeiss; Axiostar Plus), which was equipped with a photographic camera under normal bright-field imaging. The length and width of the conidia were measured from 100 positions and ranges, and the average is reported. The number of conidia was counted in 5 areas.

Ultrastructure under light microscopy

Specimens were cut into small pieces (1x 2 mm) and then were fixed in primary fixative containing 4% glutaraldehyde in 0.1 M sodium phosphate buffer pH 7.2 for 12 hours at 4 ˚C. After that, the specimens were washed 3 times (10 min per time) in the same buffer. After washing, the specimens were post-fixed in 2 % osmium tetroxide in distilled water for 2 hours and then washed with distilled water 3 times (10 min per time). The specimens were dehydrated in a graded series of acetone 20 - 100 % (10 min per series) [5]. The specimens were then embedded in Spurr's low viscosity epoxy resin. After polymerization at 70 ˚C for 8 hours using a formulation of Spurr's resin (1969) [2], the specimens were cut into pieces 1 µm thick using an ultramicrotome EM UC7 (Leica, Austria) with a glass knife, and mounted onto a glass slide. The specimens were stained in Toluidine blue 1% in borax at 85˚C for 1 minute and stained in Basic Fuchsin 1% in distilled water at room temperature for 2 minutes, then dried at 85 ˚C for 20 minutes, and closed with a cover slide. Thirty areas per specimen were examined under light microscope (Carl Zeiss; Axiostar Plus), which was equipped with a photographic camera under normal bright-field imaging.

Ultrastructure under Transmission Electron Microscopy

For transmission electron microscopy, the analysis procedure was the same as that of light microscopy. Ultrathin section (100 nm) specimens were prepared using a Leica ultramicrotome EM UC7 (Leica; Austria), and the specimens were mounted on copper grids. The specimens were stained with 5% aqueous uranyl acetate for 15 minutes and Reynold's lead citrate for 15 minutes [4] and examined under an HT7700 transmission electron microscope (Hitachi; Japan).

RESULTS AND DISCUSSION

Morphology of fungus *C. gloeosporioides* under optical microscopy

Stereo microscopy observation

Stereo microscopic observation on ripe mango fruit revealed that the juicy spots are small, slightly dimpled. The edge of the wound is not clear. A small and solid black blister was observed and arranged around the band. If there is moisture, high humidity, there will be a group of Conidia that resembles a thick orange liquid occurs (Figure 3).

Light microscopy observation

Morphology conidia of *C. gloeosporioides*

The conidia resemble fusiform to cylindrical with both ends acute, conidia size of *C. gloeosporioides* were examined under light microscope (Zeiss; Axiostar plus), which was equipped with a photographic camera under normal bright-field imaging (Figure 4A and 4B). Conidia-single celled 4.45 - 15.68 µm in length and 3.50 - 8.19 µm in width. The measurements are also listed in Table 1.

Ultrastructure of *C. gloeosporioides* in Mango Nam Dok Mai Sri Thong fruit.

Ultrastructure under light microscopy

Examination using light microscopy revealed infection vesicles in epidermal cells. The same fungal structures were found in the epidermal and parenchymal cells of the host [1]. In mango fruit thickness found that conidial germination is located in parenchyma cells and epidermis cells (Figure 5A and 5B).

Ultrastructure under Transmission Electron Microscopy

Examination using transmission electron microscopy revealed infection vesicles in epidermal cells. The same fungal structures were found in the epidermal and parenchymal cells of the host. revealed hyphae infection in parenchymal cells and the middle lamella is destroyed (Figure 6A and 6B). Transmission electron microscopy revealed show

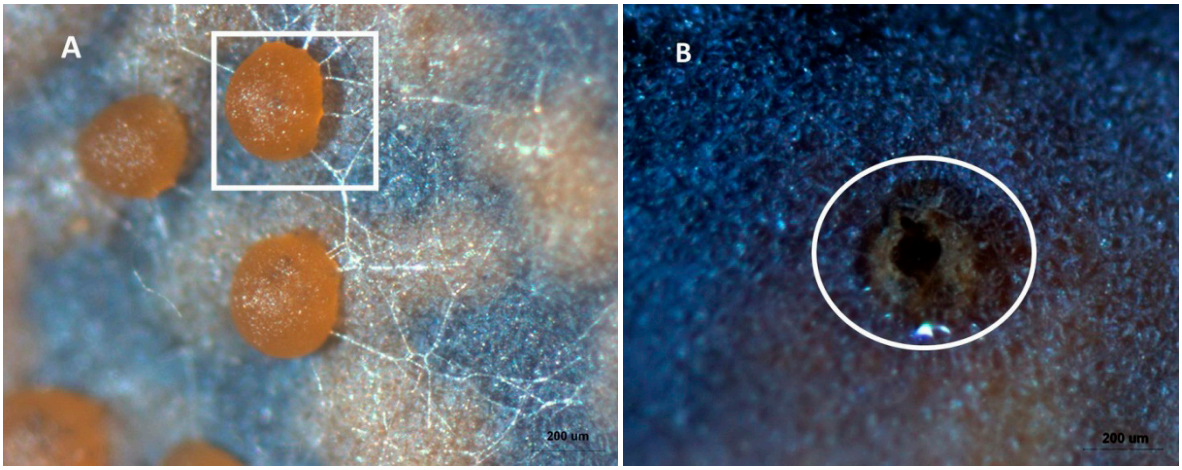

Figure 3. Microscopic photographs of infection on ripe mango fruit. The spots are small and a group of Conidia that resembles a thick orange liquid can be observed (square and circle).

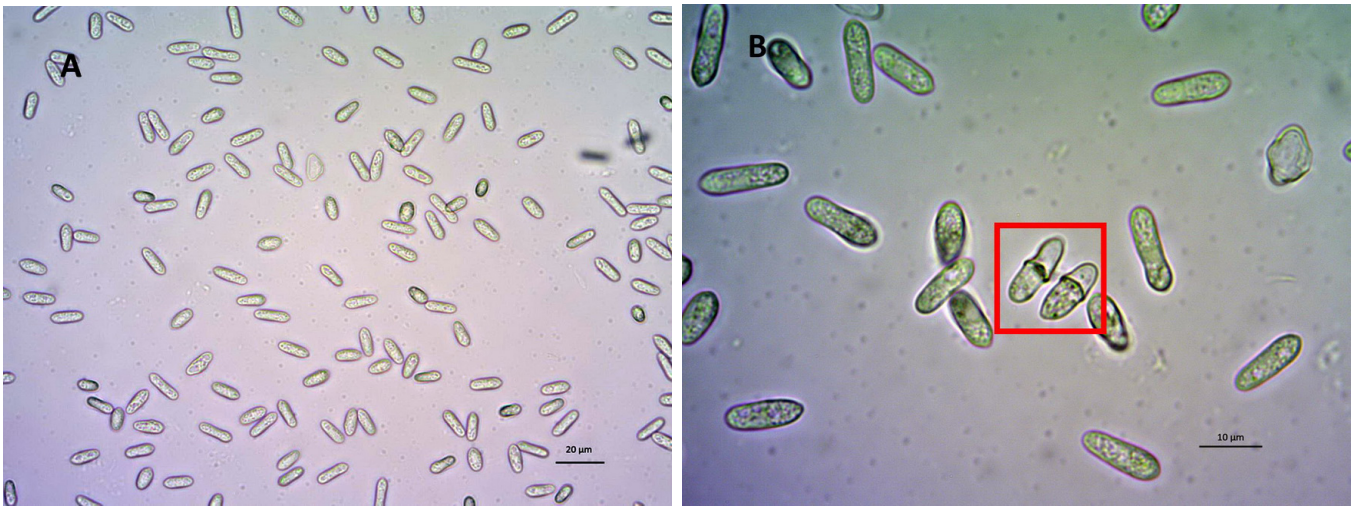

Figure 4. Light micrograph of *C. gloeosporioides* conidia A: Showing characteristics of conidial (40x). B: Showing conidial germination (square) (100x).

Table 1. Size of *C. gloeosporioides* conidia.

Sample size of C.gloeosporioides conidia	Range	Average
Length of conidia (µm)	4.45-15.68	12.50±1.83
Width of conidia (µm)	3.50-8.19	5.10±0.8

the colonization of *C. gloeosporioides* in mango fruits after infection hyphae inside the parenchymal cells. (Figure 7A, 7B and 8). In addition, light and transmission electron microscopy are proper techniques capable to the study of detailed morphologies and microstructure of the pathogen infection in plants.

CONCLUSION

The present study highlights that the infection process and the colonization are independent and have specific mechanisms, which vary according to the host-pathogen interaction, corroborating the observations of Emmett and Parbery [3]. In addition, light and transmission electron microscopy are proper techniques capable to the study of detailed morphologies and microstructure of the pathogen infection in plants.

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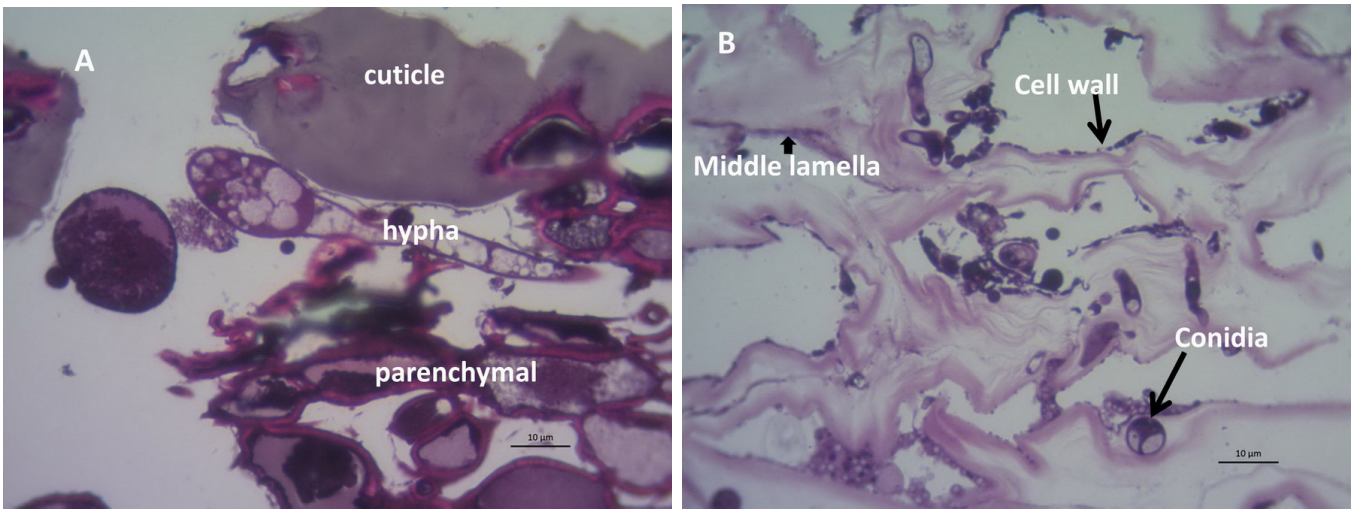

Figure 5. Light micrograph cross-section of mango fruit. A: Showing penetration of mango on epidermis cells by conidial germination of *C. gloeosporioides* (100x). B: Showing infection was found in parenchyma cells of the host. (100x).

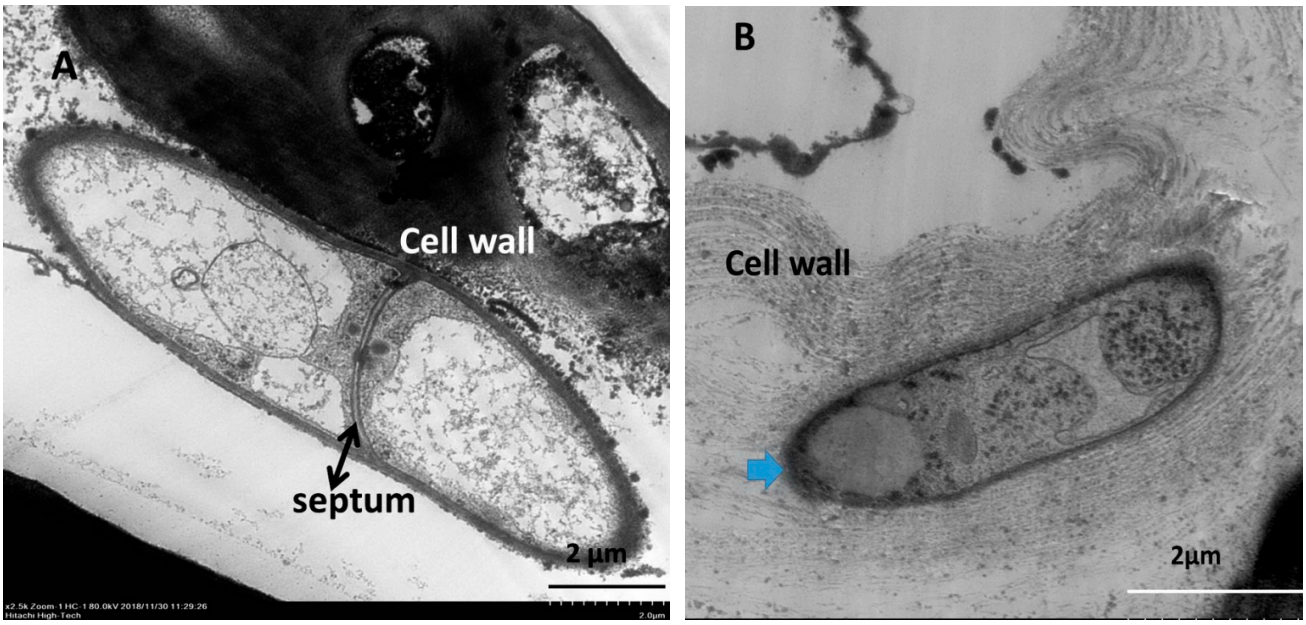

Figure 6. Transmission electron microscopy images showing the colonization of *C. gloeosporioides* in mango fruits after infection hyphae inside the parenchymal cells. A: Showing hypha infection in parenchymal cells. (magnification 2,500 x). B: Showing the infection of Hypha (arrow) in the cells, tissues and the middle lamella is destroyed. (magnification 4,000 x).

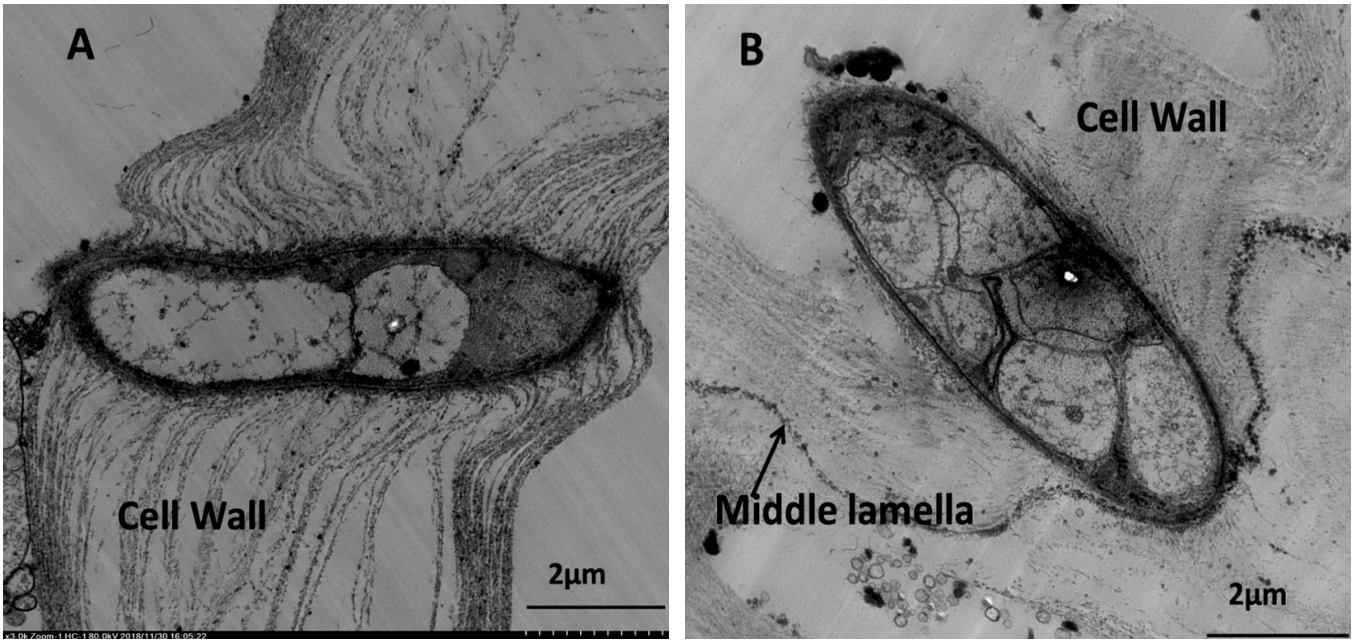

Figure 7. Transmission electron microscopy images showing the colonization of *C. gloeosporioides* in mango fruits after infection hyphae inside the parenchymal cells. A: Showing hypha infection in parenchymal cells. (magnification 3,000 x). B: Showing the infection of Hypha in the cells, tissues and the middle lamella is destroyed. (magnification 3,000 x).

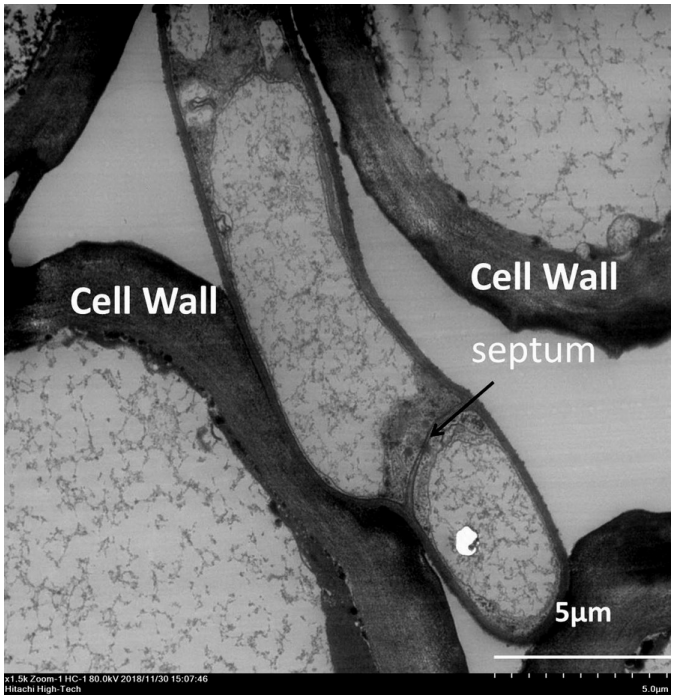

Figure 8. Transmission electron microscopy images showing the colonization of *C. gloeosporioides* in mango fruits after infection hyphae inside the parenchymal cells. (magnification 1,500 x).

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