Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2019 16(SI): 105-114. **OPEN ACCESS**

Plant-pathogen interaction between *Corynespora cassiicola* and *Carica papaya*

Mohammad Hailmi Sajili.[1], Kadir Jugah[2], Kamaruzzaman Sijam[2], Mahmud Tengku Muda[2], Noor Afiza Badaluddin[1], Mohammad Moneruzzaman Khandaker and Salmah Mohamed.

[1]School of Agriculture Science and Biotechnology, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin (UniSZA), Besut Campus, 22200 Besut, Terengganu, **Malaysia.**
[2]Plant Protection Department, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, **Malaysia.**

***Correspondence: mhailmi@unisza.edu.my** **Revised: 01 Oct. 2019, Accepted: 08 Oct. 2019 e-Published: 11 Nov. 2019**
Reviewed by: Dr. Nornasuha Yusoff, Dr. Norhayati Ngah

Prior research has indicated that *Corynespora cassiicola* was pathogenic to *C papaya* under optimum greenhouse conditions. The aims of this study were to examine the physical aspects of infection by *C. cassiicola* on the leaf surfaces of the resistant *C. papaya. Corynespora cassiicola* was isolated from infected *Carica papaya*, and maintained in the at 30 ^{o}C on V8 (half-strength) agar. Conidia were collected from 14 day old V8 (half-strength) agar plates by washing the surface with sterile distilled water and using a rubber spatula to rub for further experiment. At the end of the experiment, suggest ways to manage the infection. Observation of the infection was done by light microscopy which involved cross-section and leaf clearing method and also by scanning electron microscopy (SEM). Formation of many appressorium and 59.5% direct infection through direct infection (cuticle) instead of trough stomata were proved the *C. cassiicola* is great potential pathogen. The character of *C. cassiicola's* conidia germination was identified from both end and randomly from any septate. In Cross-section on both light and Scanning Electron Microscope shows the present of *C. cassiicola* part in the plant cell as proved that the infection was happened. The symptom of leaf spot disease was closed to leaf vein also proven by the compact of *C. cassiicola* mycelium view under SEM. Hence, as the pathogen seem to be major for the *C. papaya*, early detection was crucial for these diseases in order to control the dissemination.

Keywords: *Corynespora cassiicola*, Plant Disease, Disease, Leaf Spot Disease, Plant Pathology, Papaya

INTRODUCTION

The infection strategies of plant pathogenic fungi involves several stages such as attachment and germination of propagules, penetration of the host cell, differentiation of the germ tubes into specialized pre-penetration structures, and development of infection hyphae and colonization of plant tissue (Hailmi et al., 2011; Passos et al., 2010). At any of these stages, slight inherent or induced differences in morphology, biochemistry or physiology between plants can have a major effect on the establishment of a compatible interaction with a pathogen and hence expression of disease symptoms (Passos et al., 2010). Forming of appressorium was the main indication of penetration but penetration could happen with or without appressorium (Muzahid-E-Rahman et al., 2010).

Resistant reaction mechanisms restricting colonization of the resistant host may develop at

any stage of the infection process, and act simultaneously or sequentially depending on the morphology and the biochemistry of the plants as well as the environmental conditions Hailmi et al., (2011).

Infection process by *C. cassiicola* on papaya leaf was studied by light microscope, and Sccanning Electron Microscope (SEM) to provide information on the penetration of infections of *C. cassiicola* on the papaya leaf. Physical aspects of infection by *C. cassiicola* on the leaf surface of papaya were investigated to examine and to suggest way of control of the pathogen in the future.

MATERIALS AND METHODS

Plant pathogen interaction.

Fungal Culture

Corynespora cassiicola was isolated from diseased *Carica papaya*, and maintained in the dark under NUV light at 30 ^{o}C on V8 (half-strength) agar. Conidia were collected from 14 day old V8 (half-strength) agar plates by washing the surface with sterile distilled water and using a rubber spatula to rub and scrape the surface of the agar to dislodge the conidia. The conidia were counted with a haemocytometer and diluted to the required concentrations by adding sterile water.

Light Microscopy Study

Sample Preparation

Detached leaves of *C. cassiicola* (as susceptible host), rice (as resistant plant) and water agar (as control) were inoculated with 10 µl of conidial suspension at a concentration of < 10^4 and placed in petri plates containing sterile moistened filter paper to maintain the humidity. The inoculated leaves were incubated at 30 ^{o}C for 24 h in approximately 12 h darkness and 12 h natural light. After incubation, leaf sections were fixed on filter paper saturated with a formalin/alcohol/acetic acid (1:18:1 v/v/v) solution in plastic petri dishes sealed with Parafilm (American National Can, Greenwich, CT) for 2 h. The leaf sections were soaked for 42 to 48 h in a solution of chloral hydrate (200 g), distilled water (80 mL) and ethanol (250 mL) to decolorize the leaf sections. They were then preserved in scintillation vials containing 4 mL of lactophenol solution (20 g phenol, 20 mL lactic acid, 40 g glycerine, and 20 mL water). The sections were stained using cotton blue stain in lactophenol solution (100 mL lactophenol, 1 mL 1% aqueous cotton blue and 20 mL glacial acetic acid), and then mounted in glycerol.

The percentage of germination and number of appressoria formed were determined by counting the germinated conidia and appressoria in five ocular views per leaf section through a light microscope. A conidium was considered to have germinated if the length of its germ tube was half the length of the conidium itself and the blue-staining germ tube was visible.

Histology Study / Cross Section

Young detached leaves of the two hosts were inoculated with 10 uL conidial suspension with a concentration of 10^4/mL^3 conidia. The inoculated leaves were incubated for 12 h on moist filter paper. Leaf sections were fixed by soaking overnight at 4 °C in 3% glutaraldehyde in 0.05 M sodium phosphate, pH 6.8. After fixation, the sections containing conidial suspension were infiltrated with Xylene:Paraplast (75:25; 50:50, 25:75, 0:100 v/v) overnight in an oven at 75 ºC. The sections were then embedded in 100% wax and kept between 3 to 5 days in a freezer at 5 °C. The samples were sectioned with microtome and stained with 1% methylene blue.

Scanning Electron Microscopy study

Sample preparation

Leaves were inoculated with 10 µL conidial suspension with a concentration of 10^4/mL^3 conidia and incubated at 30 ºC for 12 h. Sections of the leaves were harvested for electron microscopic examination using standard techniques (Lee, 1993). The leaf sections were fixed with 2.5% gluteraldehyde in 0.05 M sodium phosphate, pH 6.8, for 4-6 h at 4 ºC, washed with sodium cacodylate buffer three times (10 min each wash). They were finally fixed in 1% osmium tetroxide for 1 h and washed with 0.1 M sodium cacodylate buffer as before.

The samples were dehydrated through a series of graded ethanol (10%, 20%, 30%, 40%, 50%, 70%, 80%, 90%, then 100%, 100% and 100% acetone). The samples were soaked for 10 min at each concentration except for 100% ethanol in which they were soaked for 15 min twice. Finally, they were soaked for 15 min in 100% acetone. The drying was completed by placing the samples in a flow of CO_2 in a Samdri-780-A critical point dryer (Tousimic Research Corp., Rockville, MD). The samples were mounted on aluminium stubs and coated with

Au/Pd using Hummer V sputter coater (Techinc, Alexandria, VA), and viewed and photographed under a scanning electron microscope (Philips XL30).

RESULTS

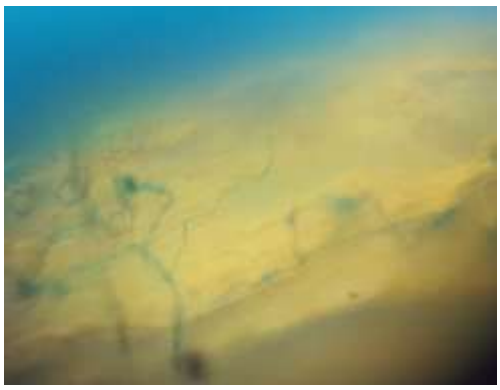
Microscopy Study

Light microscopy study was done to observe the plant-pathogen interaction during infection of the *C. cassiicola* on *C. papaya* using light microscope and scaning electron microscope (SEM).

Light Microscope

Leaf Clearing Methods

On leaf clearing methods of study (Figure 1), it was cearly shows the penetration was mainly on the adaxial midrib of the papaya leaf by forming of appressorium. Early colonization widely spread on the surface with all of the infection was closed to the veins of papaya leaf. Majority of the penetration was thought direct penetration instead of through stomata. But, most of the penetration was closed to subsidiary cell and guard cell of stomata.



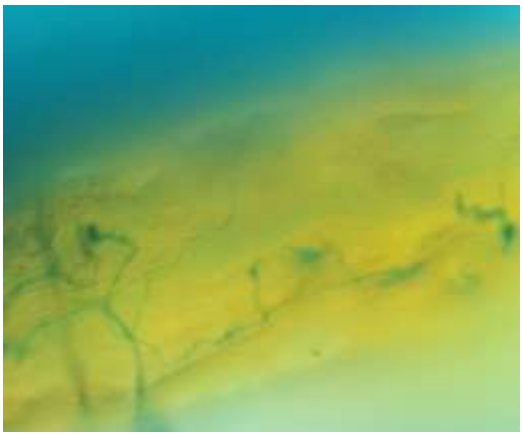
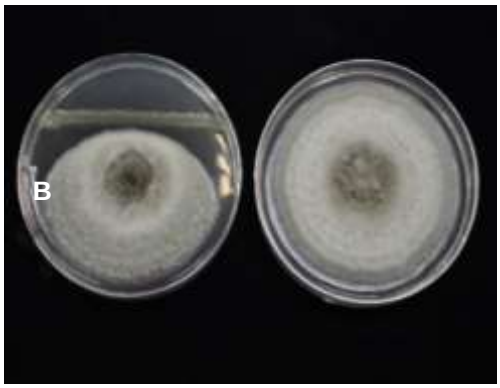


Figure 1: Conidia (A) of *C. cassiicola* on the leave (B & C) of *C. papaya* viewed under microscope and the mycelium of *C. cassiicola* within the leaf center veins tissue with 40x magnification.

Effect of Bacteria on the Mycelium and Conidia Germination

Effective bacteria isolates inhibited >80% the growth of *C.cassiicola* in antagonistic test, thus the mycelial disc of *C. cassiicola* was used and processed for light microscopy. Light microscopic investigation showed that hyphal morphology growth, hyphal tips of the fungus of *C. cassiicola* became swollen and malformed. Hphae were thickened with vacuolation compared to the control ones (Figure 2). Many swellings occurred in the hyphae, whereas, in the control, the hyphal walls were with no swellings or vacuolation (Figure 2).



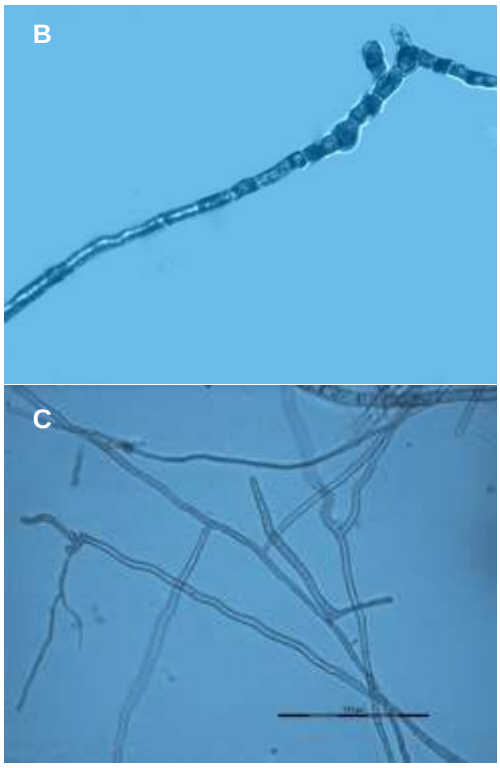


Figure 2: Character of fungi hypha of *C. cassiicola* treated with bacteria 8 days old on PDA.

Germination of Conidia of C. casscola

For every two hours, the length of hypha germinated from conidia of *C. cassiicola* was measured. The conidia started to germinate after two hours of incubation on WA. Application of the LCB stopped the germination of conidia onto WA for every 2 hours.

Average length of hypha reached to the half-length of conidia after 10 hours (Table 1). Mean of germination from 2 to 8 hour was ranged at 5.09 to 5.28 µm for every hour. The mean of germination increased at 10 hours which were the average increase to 6.50 µm an hour and 7.64µm an hour after 12 hour (Table 1, Figure 3).

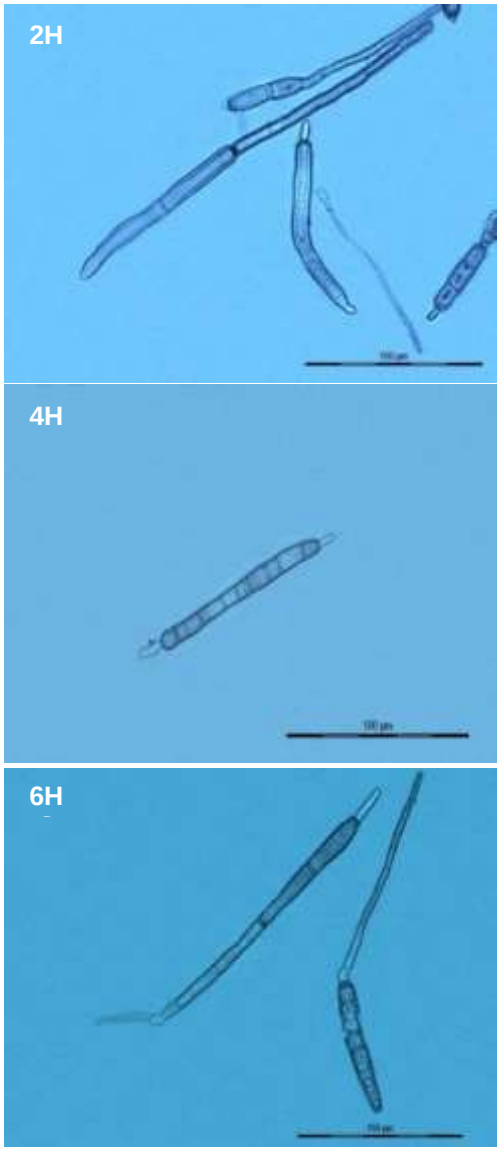
Germination of conidia was observed from both end of conidia septate and also from side part which was at the intermidate septate. From this study, it also proved that germinated from side of conidia only accured among short cinidia with 4 to 7 septates. For the long conidia (more than 7 septate) all of them were germinated form both end septate and there was no conidia germinated from intermidate septate.

Table 1: Germination of *C. cassiicola* for every two hours on WA view under microscope with 40x magnification.

Time (hour)	Mycelium lenght (Mean) µm	Germination / Hour (Mean)
2	10.52	5.26
4	20.68	5.17
6	31.65	5.28
8	40.69	5.09
10	65.01	6.50
12	91.69	7.64

*The conidia will considered germinated only if the length of hypha germinate half to the conidia length.

Conidia Germination Morphology



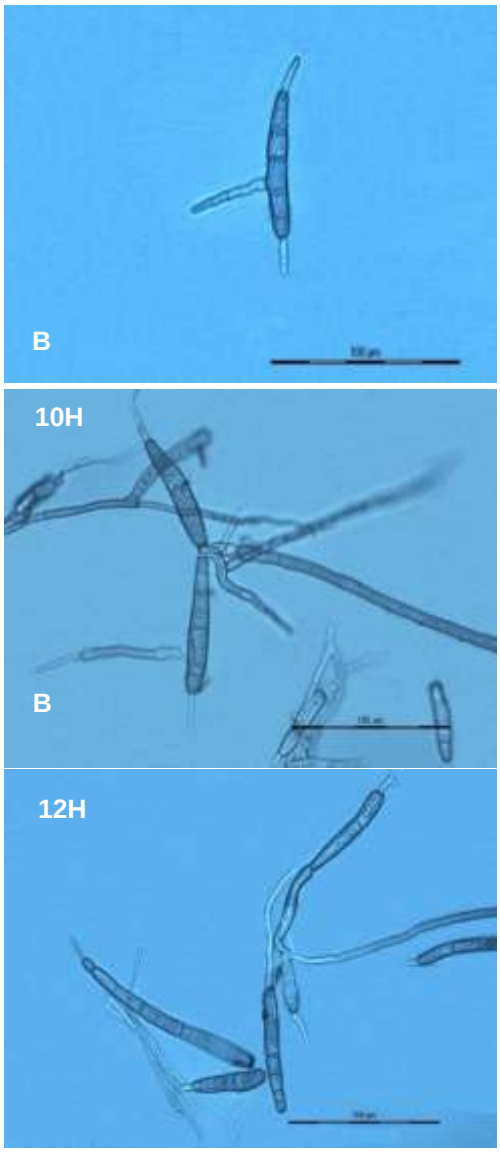


Figure 3: Germination of *C. cassiicola* every 2 hours for 12 hours in sterile distile water view under light microscope with 40x magnification.

Cross Sectioning

Cross section study was done to closely look into damage of plant cell on the *C. papaya* as a host plant of a pathogen (*C. cassiicola*). Figure 4A shows the damage of plant cell caused by an infection of *C. cassiicoa*. It was at the veins area which is the main area for *C. cassiicola* infections started. The cell were severely damaged compare to healty plant cell (Figure 4B) as a control.

Corynesporra cassiicola was also cause the damage to the others part of papaya leaf (Figure 5) when the disease spreaded on the leave of papaya. Damage of plant tissue especially on collenchyma, floem and xylem could be seen in clearly by cross sectioning study (Figure 5A, B, C) as compared to the healthy plant tissue (Figure 5C). Some of pathogen (*C. cassiicola*) part could be seen at damaged or infected tissue as shows by an arrow (Figure 5A, B, C). The damaged of upper epidermis anda lower epidermis will disturb the photosentisis process of the crops (papaya).

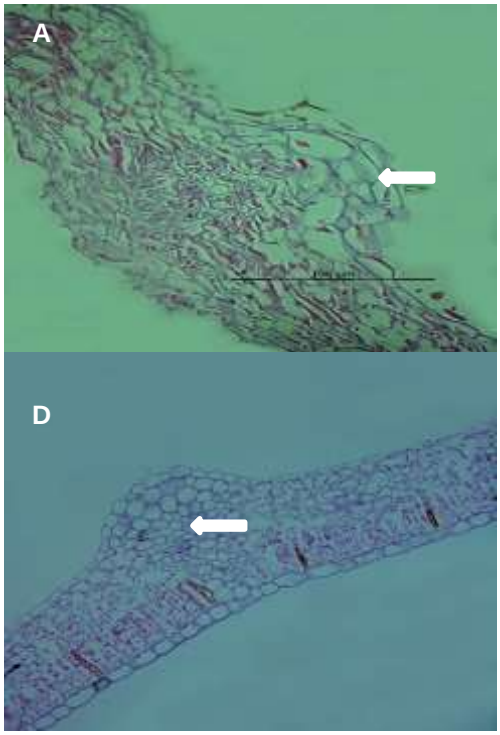
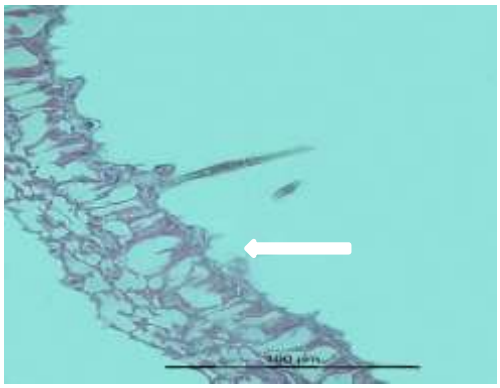


Figure 4: Cross section of infeccted and uninfected *C. papaya* leave view under light microscope with 10 X magnification.



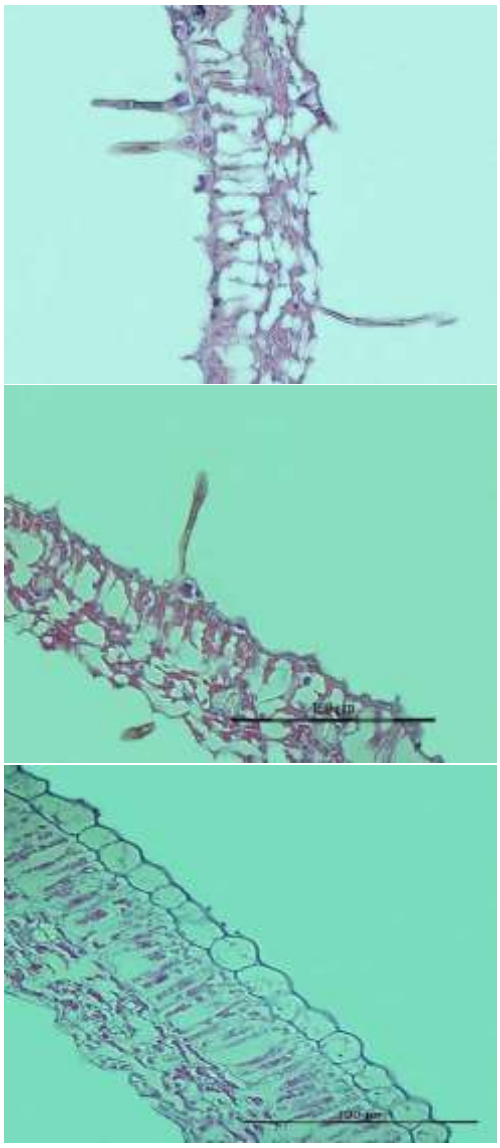


Figure 5: Cross section of treated and untreated *C. papaya* leaf view under light microscope with 10x magnification.

Scanning Electron Microscopy study

Observation of *C. cassiicola* under scanning electron microscope was done on conidia shape, colonization of *C. cassiicola* on papaya leave, cross section of infected leave, and mycelium collected from antagonism study.

The shape of *C. cassiicola* samples taken from infectious leave and agar media (PDA) was views under scanning electron microscope (SEM) and it were no difference on shape of *C. cassiicola* conidia's shape (Figure 6A,B). The colonization of the leave surface could be seen on inoculated leave (Figure 6C) of papaya compared to healthy papaya leave (Figure 6D).

Infection of C. cassiicola

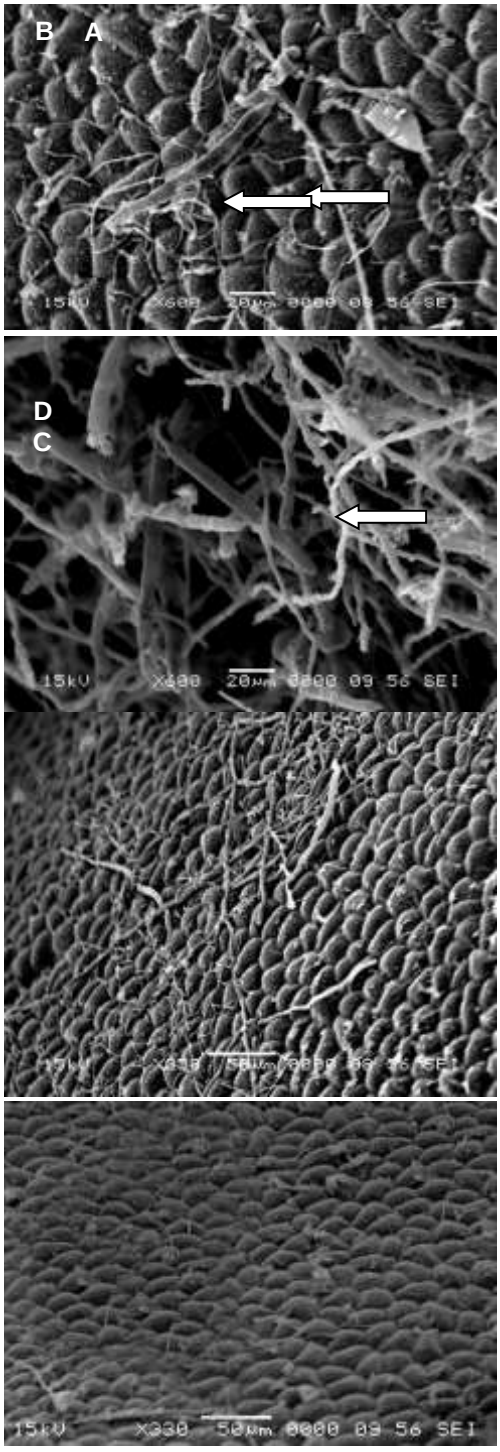


Figure 6: Monograph Conidia of *C. cassiicola* on the surface of papaya leaf (A) and on PDA half Strength (B). Monograph of *C. papaya* leaf surface uneffected (C), and infected (D) by *C.*

cassiicola **view under Scanning electron microscope with 330x and 350x time magnification respectively.**

Infection of C. cassiicola

Formation of appressorium (Figure 7A) is the sign of infection prosess into host plant (papaya). As the result, forming of appressorium was accepted as a direct penetration and it shows a high ability of the fungi to infecting plant. Meanwhile the penetration also occurred trought natural opening like stomata, hydatode or injury. Only penetration trought stomata was counted as natural opening in this study (Figure 7B).

Total 200 hypha end spot were observed. Result show that the fungal directly infection was higher then penetration trough natural opening. Out of 200 hypha end infection spot, 119 infection were through direct penetration and 81 were trought stomata and it was ststistically significant different (Figure 8).

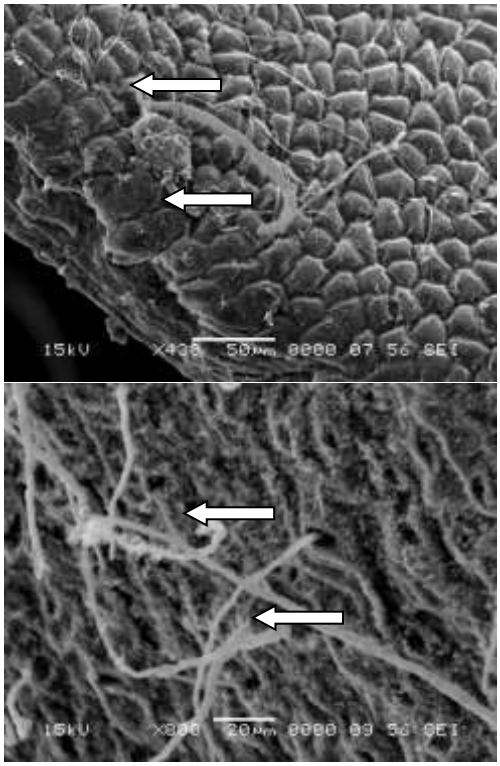


Figure 7: Monograph of Approsserioum formation during infection of *Corynespora cassiicola* on papaya leaf views under Scanning Electron microscope with x430 (A) time magnification. B = Monograph of Infection of *C. cassiicola* on the leaf of *C. papaya* trough natural opening (sstomata) after 10 days of inoculation view under Scanning Electron microscope with x800 time magnification.

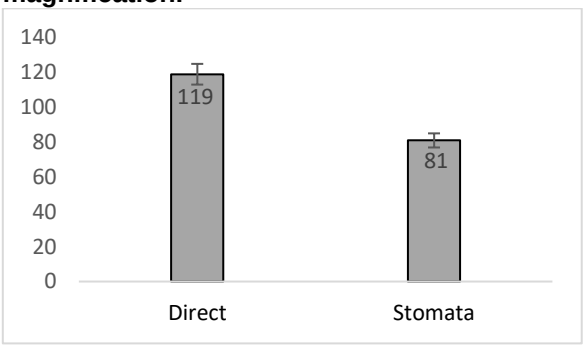


Figure 8: Methods of infection by *C. casssiicola* on the leaf of *C. papaya*.

Cross Sectioning

Cross sectioning was done in this study to prove and support the light microscope study on *C. cassiicola* infection into the plant cell. Damage of the plant tissue clearly seen (Figure 9A). The hypha of *C. cassiicola* could existed from the plant infected tissue (Figure 9A) compared to the uninfected or healthy plant tissue (Figure 9B). It was proved that an infection was not only on the surface of the papaya leave.

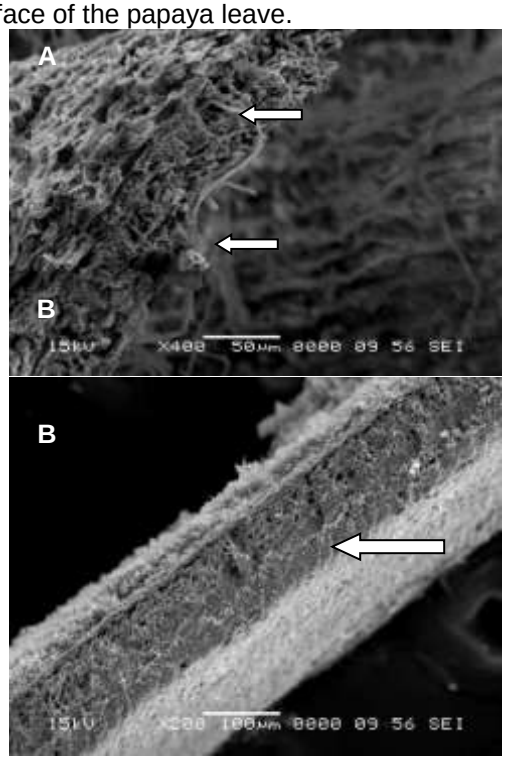


Figure 9: Infection of *C. cassiicola* ;(A) Normal or untreated leaf of papaya, (B) shows the mycelium of *C. cassiicola* as a treated leaf. Viewed under scanning electron microscope (JOEL-JSM5610LV)

Area of Infection (On papaya leaf)

Leaves of *Carica papaya* have been inoculated with *c. cassiicola* in the laboratory with 20 replicates. Even though the *C. cassiicola* inoculum was evenly spreaded on the leaf of papaya using drawing brush. All of replicates shows that the symptoms of infection by *C. cassiicola* were started at the veins of the papaya leaf (Figure 10). The symptoms were appears days after innoculation 3th with small yellow spot. The sign (mycelium) of *C. cassiicola* could be seen by naked eye after 6th days after inoculation.

The mycelium was higly compact along the veins (Figure 10A) and it was observed become lower as the distant far from the veins (Figure 10B). It was similar to the symptoms shows in the field where most of the spots was detected closed to the vein especially centre veins.

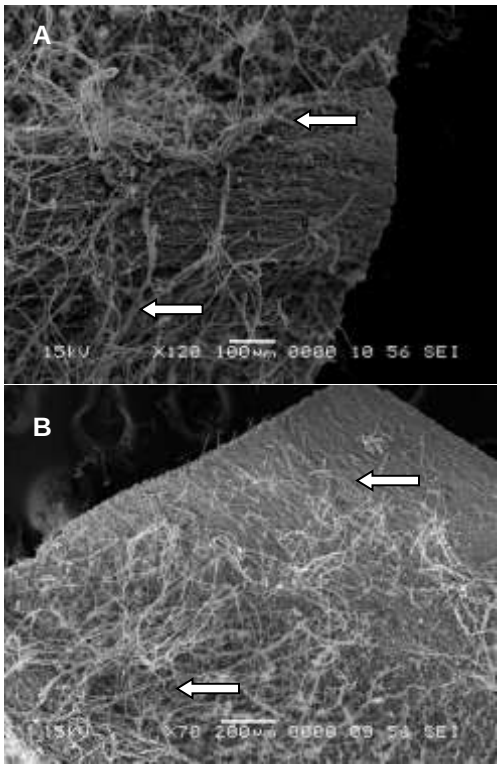


Figure 10: Area of infection of *C. cassiicola* on the leaf surface of C. papaya after 120h of inoculation in room temperature. Viewed under Philiph JOEL electron microscope.

Effect of C. cassiicola on Bacteria Treatment

Study also done on the character of the hypha (mycelium) at the antagonist zone (Figure 31A) of fungi and effective bacteria. First mycelium's character that could be seen was the compactness of the mycelium at the area of fungi-bacteria antagonist zone (Figure 31C, D). The mycelium was growth abnormally with character of stuntend or retarded compared to mycelium on untreated (Figure 11B).

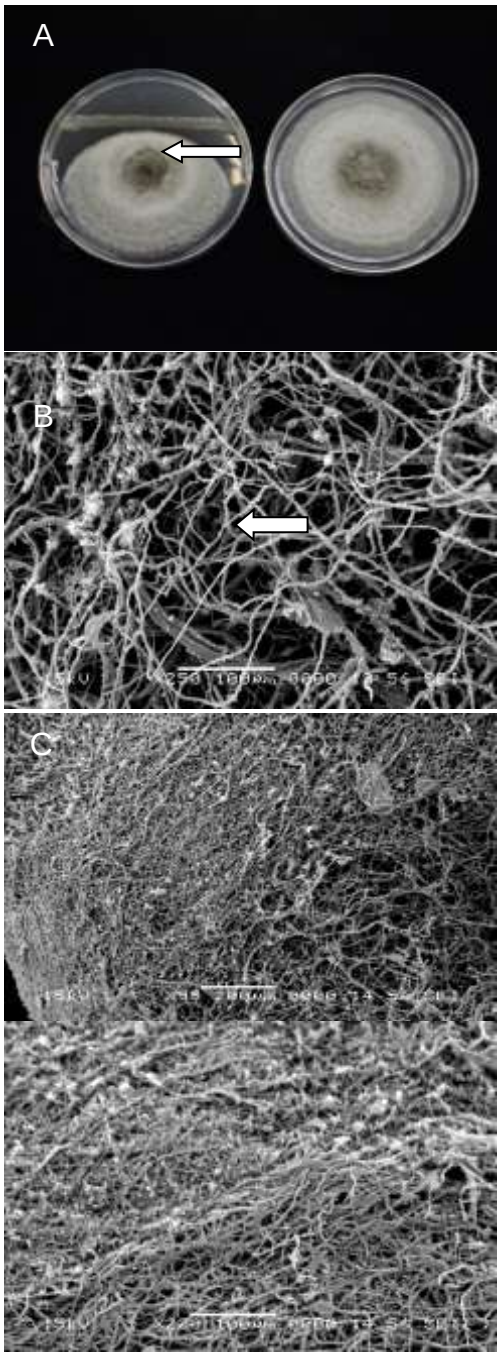


Figure 11: Mycelium of *C. cassiicola* taken from the colony on PDA without treatment (B) and mycelium of *C. cassiicola* taken from the treatment area (C & D); (A) shows the area that the mycelium taken from on PDA.

DISCUSSION

Light microscope study was prove that the infection was occurred on tha adaxial midrib instead of abaxial as reported by (Passos et al., 2010) Furthermore**,** preference for colonization could be typical for particular assiciation of host-pathogen Passos et al., (2010).

Histological observation involving fungus-plant (host) interaction were made on *C. papaya* leave inoculated with *C. cassiicola*, a pathogen of leaf spot disease of papaya. Ruined of collenchyma, floem and xylem tissue will derived to the failure of nutrient transportation which was showed by yellowing of the papaya leaf and fallen.

On light microscope study, effect of bacteria treatment on the growth of mycelium was observed. The study demonstrate a mycelium collected from the antagonistic area was retarded and growth in compact mycelium compared to the untreated *C. cassiicola* in both light and SEM study. Microscopic observation disclosed that the hypha of *C. cassiicola* become thickened, malformed as describe by (Begum et al., 2008) on *Colletrotrichum truncatum* and by (Rahman et al., 2007) on *Colletrotrichum gleosorioides*. Most of the hypha swellings up whereas not occurred in normal hypha **(**Begum et al., 2008; Rahman et al., 2007**)**. Meanwhile the ungerminated conidia was shringking and looks dried compared to normal and germinated conidia of *C. cassiicola*.

On SEM study, conidia of *C. cassiicola* shape was consistent on both artificial media and papaya leaf. Microscopic examination have demonstrated that between 24 to 48 h after inoculation, conidial infection and tissue penetration had already occured. It was different compared to *E. monoceras* which was at 8-12 hour (Hailmi et al., 2011), and *Exserohilum longirostratun* was infected plant at less than 24 h (Ng et al., 2012b), but the result was consistent to the result by (Passos et al., 2010) that reported *C. cassiicola* infection of *Lantana camara* after 24-48 h.

This study also proved that, infection of *C. cassiicola* on papaya leaf also consistent to the area of the sympthom (spot) emerged that was closed along the veins. The compactness of the mycelium was seen very close to the veins proved that the area was suitable for *E. cassiicola* penetration. Penetration was majority through the cuticle instead of stomata and this was consistent to the study by Ng et al., (2012b) on E. monoseras and also by Hailmi et al., (2011) on *Exserohilum monoseras* penetration on the *Echinocloa crusgalli* leaf. Instead, study by Clearly et al., (2013) mention that the penetration and colonization of the pathogenic fungi was mostly through stomata.

Other than that, formation of the appressorium proved that the penetration process occurred (Ng et al., 2012; Hailmi et al., 2011). Lack of sheath around the appressorium means the papaya leaf was susseptible to *E. cassiicola*. Resistant plant should produce more sheath for penetration than a susseptible plant (Hailmi et al., 2011)

CONCLUSION

Both Light microscope and SEM study as done on cross section of the infected and uninfected papaya leaf. The result was proved that the infection of the bacterial in the papaya leave by presents of the hypha in plant tissue. The hypha colonized the plant tissue by most fungal pathogens requires differentiation of specialized types of structure (Clearly at al., 2013). The present of hypha could be seen clearly in both light microscope and SEM study. Ruined plant tissue also clearly seen for treated papaya plant tissue compared to normal. Even some part of the *C. cassiicola* (hypha, conidia) were present on this cross section observation.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

The authors wish to express their gratitude to laboratory staff at Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin Besut Campus for their invaluable assistance and hospitability throughout the study.

AUTHOR CONTRIBUTIONS

MHS design and performed the experiment. Others author (KJ, KS, MTM, NAB, MMK and SM) read and approved the final version.

REFERENCES

Begum MM, Sariah M, Abidin MAZ, Puteh AB, & Rahman MA, 2008. Antagonistic potential of selected fungal and bacterial biocontrol agents against colletotrichum truncatum of soybean seeds. *Pertanika Journal of Tropical Agricultural Science*, *31*(1), 45–53.

Hailmi MS, Kadir J, Juraimi AS, & Norhayati MZ, 2011. Plant-Pathogen Interaction between Exserohilum monoceras with Oryza sativa and Echinochloa crus-galli *Hailmi. *Journal Of Agrobiotechnology*, *2*(0), 73–82.

Muzahid-E-Rahman M, Ali R, Kumar DT, & Delowar HM, 2010. Scanning Electron Microscopy Of Invasion Process. *Journal of Plant Protection Research*, *50*(3).

Ng SC, Kadir J, & Hailmi MS, 2012. Histological Study of the Interaction between Exserohilum Longirostratum, Barnyard Grass, and Rice var. MR219. *Pertanika J. Trop. Agric. Sci. 35*, *35*, 57–70.

Passos JL, Barbosa LCA, Demuner AJ, Barreto R W, King-Diaz B, & Lotina-Hennsen B, 2010a. Effects of Corynespora cassiicola on Lantana camara. *Planta Daninha*, *28*(2), 229–237.

Passos JL, Barbosa LCA, Demuner AJ, Barreto R W, King-Diaz B, & Lotina-Hennsen B, 2010b. Effects of Corynespora cassiicola on Lantana camara . *Planta Daninha*.

Rahman MA, Kadir J, Mahmud TMM, Abdul Rahman R, & Begum MM, 2007. Screening of antagonistic bacteria for biocontrol activities on Colletotrichum gloeosporioides in papaya. *Asian Journal of Plant Sciences*, *6*(1), 12–20. https://doi.org/10.3923/ajps.2007.12.20