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Influence of Culture Media, Temperature and pH on *Colletrotrichum gleosporioides*, Isolated from *Carica papaya* in Besut, Terengganu, Malaysia

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ABSTRACT

Colletotrichum gleosporioides is known as the causal agent of anthracnose disease. Economic losses are reported during postharvest where the disease incidence and severity was recorded as 90-98 % and 25-38 % respectively. *Colletotrichum* spp. were isolated from lesions of infected *Carica papaya* L. that showing the typical anthracnose disease symptoms in Besut, Terengganu, Malaysia. Three types of fungal cultures were obtained that are identified as *Colletotrichum* sp., *Fusarium* sp. and *Rhizoctonia* sp. One of the fungal obtained was confirmed as *Colletotrichum gleosporioides* based on conidial morphology and growth characteristics on PDA media. The pathogen under study varied in its ability to grow under different environmental conditions. However, isolate preferred temperature range of 20°C to 30°C for the growth on PDA media. *C. gloeosporioides* isolates grew well at pH values within range of pH6 to pH 7.

Keywords: *Colletotrichum gleosporioides, Fusarium* sp., *Rhizoctonia* sp., *Carica papaya,* Plant Disease, Fungi

ABSTRAK

Colletotrichum gleosporioides adalah agen penyebab penyakit yang sinonim dengan penyakit antraknos. Kerugian telah dilaporkan pada peringkat lepas tuai yang mana kejadian penyakit dan keterukkan penyakit direkodkan pada 90-98% dan 25-38% setiap satunya. *Colletotrichum* sp. telah dipencilkan dari *Carica papaya* L. yang menunjukkan tipikal simptom penyakit antraknos di Besut, Terengganu, Malaysia. Tiga jenis kulat telah berjaya dipencilkan dan dikenali sebagai *Colletotrichum* sp., *Fusarium* sp. and *Rhizoctonia* sp. Salah satu darinya telah dibuat pengenalpastian lanjut dan telah dikenalpasti sebagai *Colletotrichum gleosporioides* berdasarkan kepada kriteria morpologi spora dan pertumbuhan di atas media PDA. Patogen yang dikaji menunjukkan kepelbagaian tahap kemampuan di bawah perbezaan sekitaran. Walau bagaimana pun, suhu yang sesuai adalah diantara 20°C ke 30°C bagi pertumbuhan di atas media PDA. *C. gloeosporioides* juga dilihat tumbuh dengan baik pada pH antara pH 6 ke pH 7.

Kata Kunci: *Colletotrichum gleosporioides, Fusarium* sp., *Rhizoctonia* sp., *Carica papaya,* Penyakit tanaman, Kulat

INTRODUCTION

Colletotrichum sp. was renowned as a causal agent for anthracnose disease of *Carica papaya*. It was the main problem of papaya fruit in the field or post-harvest. The disease will downgrade the quality and value of the fruits makes farmers lost their profit. *Colletotrichum gleosporioides* is the causal agent for this disease. There are also cases where anthracnose disease in a crop is caused by several *Colletotrichum* species such as *C. acutatum*, *C. fragariae*, *C. gleosporioides* which was the causal agent for strawberry anthracnose (Smith and Black, 1990). Association of several *Colletotrichum* spp. may also cause different diseases other than anthracnose. For example, ripe rot of grape was caused by association of *C. acutatum* and *C. gleosporioides*. *Colletotrichum gloeosporium* is well-known as a causal agent for anthracnose disease in most of fruits in the tropics such as papaya, mango, guava and capsicum while banana is caused by *C. musae* (Smith and Black, 1990).

In papaya, anthracnose appears primarily as water-soaked spots that become sunken, turn brown to black, and enlarge to 5 centimeters or more in diameter (Freeman *et al.,* 1998). Pinkish-orange areas are formed by the conidial masses that cover the lesion center and these lesions are referred to as "chocolate spots" (Freeman *et al.,* 1998). As the fruit ripens, these spots rapidly enlarge up to 20 mm in diameter, to form the characteristic circular sunken lesions. Infected fruit is of much reduced quality and much of it becomes worthless (Rahman *et. al.*, 2007; Kadir *et al.*, 2008). Most significant economic losses are reported occur during post-harvest (Freeman *et al.,* 1998). Geographically, the climate of Malaysia is highly conducive to maintain and cause outbreaks of anthracnose all year round, thus, the development of management recommendations will be inevitable for anthracnose control (Mahmodi *et al.,* 2013). The objectives of the study were to identify the best media and optimum level of pH and temperature for fungi growth.

MATERIAL AND METHODS

Sampling of Anthracnose Fungi

Causal agent of anthracnose disease, *Colletotrichum* sp. was isolated from lesions of infected *Carica papaya* L. from a supermarket in Jerteh, Besut, Terengganu, Malaysia. The infected fruits were taken to the laboratory and isolated using the protocol as outlined by Cai *et al.*, (2009).

Pathogen Isolation

Infected part of fruit was cut into small pieces of 1cm^2 along with some healthy tissue. Then soaked into 10% sodium hypochlorite for 30 sec, 70% ethanol for 30 – 60s and washed with distilled water for 60s. Dried with sterile filter paper and immediately placed on PDA (Ng *et al.*, 2011; Hailmi *et al.*, 2011). Mix colonies of fungi isolates were then re-isolated to obtain pure culture for each plate. Plates were incubated at 30°C in incubator (Memmert-Germany). The observation on colony morphology was done by naked eyes and mycelium and conidia was viewed under light microscope.

Effect of Environmental Factors on Fungal Growth

Test of media on fungi

Difco's Potato Dextrose Agar (PDA), Difco's Nutrient Agar (NA), Difco's Corn Meal Agar (CMA), and HiMedia's Rose Bengal Chloramphenicol Agar (RBC) were prepared according to standard provided by supplier. Then 0.5-cm fungal disc was taken from 7 days old culture and transferred to the center of all media. The cultured media was incubated for 7 days at 30°C. There are four replicates for each treatment. Radial growth of each fungi was measured daily for 7 days or until the fungi growth at full plate in millimeter by using ruler.

Effect of Temperature on Growth of Fungi

Approximately 0.5 cm plug was taken from the margin of an actively growing culture of 7-day-old cultured and inoculated on PDA. The plug was placed at the center of petri dish and sealed with parafilm. Then, the fungi was incubated at four different temperatures of 20°C, 25°C, 30°C and 35°C in incubator with four replication. Radial growth of each fungi was measured daily for seven days or until the fungi growth at full plate. The measurement was taken in millimeter by using a ruler.

Effect of pH on Growth of Fungi

Difco's Potato Dextrose Agar (PDA) was used to prepare for 100 mL for each pH and the pH was adjusted accordingly by using HCL and NaOH. Then the solution was autoclaved and poured into Petri dishes, a 0.5cm fungal disc that taken from the periphery of 7-day-culture was transferred to the center of agar. pH 4, pH 5, pH 6, pH 7 and pH 8 were tested to determine the optimum pH condition for fungal growth. Then the petri dishes were incubated at 30°C with four replicates for each temperature. Radial growth was measured daily using ruler for 7 days.

RESULTS AND DISCUSSION

Effect of Different Media on Fungal Growth

Colony grows and appears differently on different type of media (Figure 1). It shows that *C. gloeosporioides* exhibit greater colony growth on PDA (6.54 ±1.30 cm) (Table 1). Both PDA and NA showed typical morphology of *C. gloeosporioides* with no significant different. PDA was mentioned to be the best media for mycelial growth (Maheshwari *et al.*, 1999; Salha *et al.*, 2008) and result of this study was consistent to the finding. *C. gloeosporioides* showed lowest growth on RBC (4.12±0.13 mm). Colony on CMA appeared to be denser with less visible mycelia. Meanwhile in RBC, no orange color was spotted on the media. Fungi are recognized and identified basically based on their phenotypes (Zain *et al.*, 2009). Findings of this study revealed that different culture media influenced the growth and colony character of test fungi (Figure 2). Out of four media tested in this study, PDA reproduced most visible colony morphology and selected to be used throughout this study.

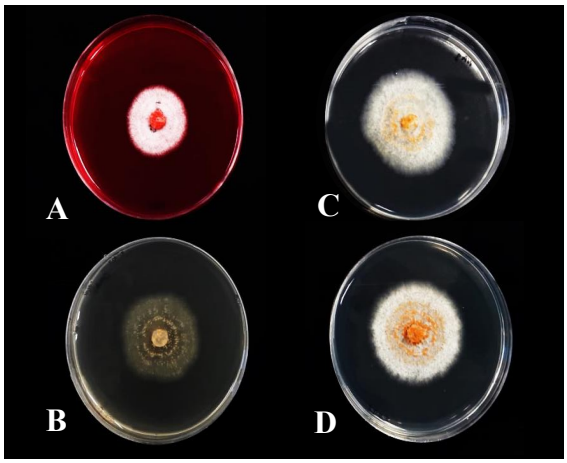

Figure 1 Colony of *C. gloeosporioides* in different types of media on 4th days, (A) RBC; (B) CMA; (C) NA; (D) PDA

Table 1 Means for different media in homogeneous subsets

Type of media	Means of Radial Growth (cm)
PDA	6.54[a]
NA	6.28[a]
CMA	4.46[b]
RBC	4.12[b]

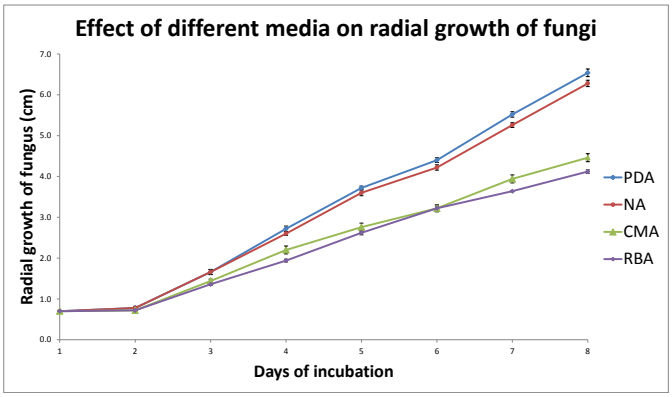

Figure 2 Effect of different media on radial growth of fungi

Effect of Different Temperature on Fungal Growth

Temperature has been proved has an influenced on the rate of fungal growth. Based on the result, fungi showed excellent growth rate in all temperature tested except 35 °C (Figure 2). *C. gloeosporioides* showed the best reading of radial growth in temperature of 25 °C with means 6.72 ± 0.148 cm. Fungi grown at the slowest rate in 35 °C (1.10 ± 0.173 cm) and after 4 days, the growth of fungi was completely inhibited (Figure 3). Overall, *C. gloeosporioides* could tolerate and grow in temperature range from 20 °C to 30 °C but the optimum temperature is 25 °C.

In an *in-vitro* study on growth of *C. gloeosporioides* by Hegde *et al.* (1990), maximum growth obtained with optimum temperature within the range of 25 – 35 °C. Pandey *et al.* (2012) also suggested that the most suitable temperature was within 15-35 °C range. But the result of this study showed that temperature of 35 °C inhibited

completely the growth of fungi (Table 2 and Figure 3). This was aligned to study by Slade *et al.* (1987), temperatures above 30 °C may have an inhibitory effect.

A study by Sangeetha (2003) showed that isolates of *C. gloeosporioides* grow best at temperature within 25-30 °C in the mango but sporulation was at an optimum range of 25-28 °C. *Colletrotrichum gloeosporioides* was reported to produce maximum radial mycelial growth at 25 °C after 6 day (Prabakar *et al.*, 2003), but in study by Pandey *et al.* (2012) maximum growth was achieved at 28 °C on 7th day after inoculation. As conclusion, the isolates of *Colletotrichum* sp. showed different results when exposed to different temperature conditions.

Table 2 Means for different temperature in homogeneous subsets

Temperature	Means of Radial Growth (cm)
20 °C	6.72[a]
25 °C	6.10[b]
30 °C	5.38[c]
35 °C	1.10[d]

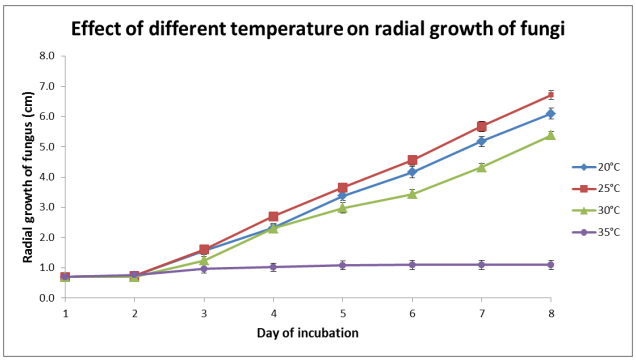

Figure 3 Effect of different temperature on radial growth of fungi

Effect of Different pH on Fungal Growth

Radial growth of fungi responded differently towards different pH levels tested. Fungal radial growth within 8 days on PDA and each point represent average of four replicates (Table 3). Growth of fungi was observed significantly higher at pH 7 with means 6.975 ± 0.0957 cm followed by pH 6 and pH 5. The most unfavorable pH condition for fungal growth was pH 8 with the lowest radial growth means (5.375 ± 0.168 cm) (Figure 4). Fungi growth at pH4 and pH8 were significantly less than the other pH levels tested. pH 6 and pH 7 does not show significantly different but have significantly higher with the rest of pH values tested (Table 3). pH4 does not significantly compared to both pH5 and pH8, but both pH5 and pH8.

Lilly and Barnett (1951) reported that pH of the medium determines the rate and amount of growth including many other life processes of fungi. A medium with a specific pH also said to favor the growth but be unfavorable for sporulation or other processes. Kumara *et al.* (2008) stated that a medium that was suitable for sporulation in most fungi was said to be medium having pH values within range of pH 5 to pH 6. In present study, *C. gloeosporioides* said to be better growth in pH values range within pH 6 to pH 7. They had also stated that fungi generally tolerate more acid than alkali and it was consistent to the result that shows the means of fungal radial growth in pH 4 (5.725 ±0.556 cm) was slightly higher than means in pH 8 (5.375 ± 0.486 cm). Similar observations were also reported with different species of *Colletotrichum* (Naik *et al.*, 1988). In another study by Pandey et al. (2012), optimal growth pH of 6 was reported for this species. Kumara and Rawal (2008) also reported that *C. gloeosporioides* isolates from papaya fruit crop grew well in a medium of pH 5.

Table 3: Means for different pH in homogeneous subsets

Values of pH	Means of Radial Growth (cm)
pH 4	5.725[bc]
pH 5	6.150[b]
pH 6	6.800[a]
pH 7	6.975[a]
pH 8	5.375[c]

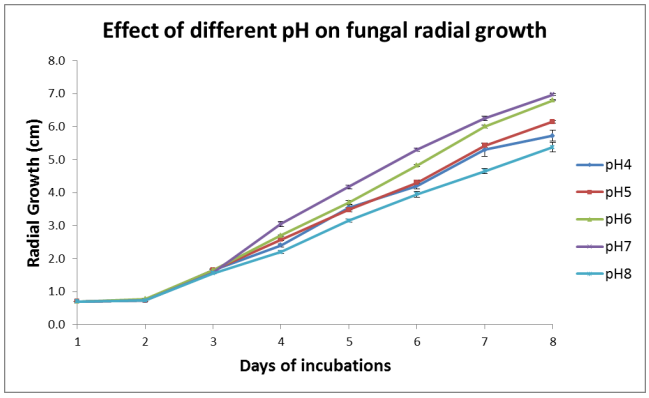

Figure 4: Effect of different pH on radial growth of fungi

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