



Faculty of Resource Science and Technology

**MORPHOLOGICAL AND PHYSIOLOGICAL
CHARACTERISTICS OF DIFFERENT ISOLATES OF
Pestalotiopsis spp.**

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This project is submitted in partial fulfilment of the requirement for the degree of Bachelor of
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Morphological and Physiological Characteristics of Different Isolates of *Pestalotiopsis* spp.

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ABSTRACT

Morphological and physiological characteristics of *Pestalotiopsis* spp. were studied. Six isolates of *Pestalotiopsis* isolated from *Shorea macrophylla*, ornamental and Rhododendron leaves and also from guava fruit. The identification of this fungus was done based on the morphological and physiological characteristics. The growth of *Pestalotiopsis* was tested on potato dextrose agar (PDA), potato carrot agar (PCA) and malt extract agar (MEA). The growth of this fungus at different temperature, pH, light conditions and macro elements were also examined. There was significant difference at $P < 0.05$ of effect of media on the growth of fungi. The rapid growth was recorded on PDA for isolates 1077 and 1091. The media showed no significant effect on colony growth of isolates 1214, 1213 and 1080. Isolate 1202 grew significantly faster on PDA and PCA compared on MEA. There was significant difference at $P < 0.05$ of effect of temperature on the growth of fungi. The optimum temperature of growth for isolates 1214, 1202 and 1213 was at 25°C. However, the optimum temperature for growth of isolate 1077, isolate 1080 and isolate 1091 was in a range of 15°C to 20°C, 20°C to 30°C and 15°C to 30°C respectively. Different isolate of *Pestalotiopsis* able to grow at the different pH, however, the optimum pH for growth varied depending on the isolate. Macro element significantly affected the growth of *Pestalotiopsis* isolates. *Pestalotiopsis* grew faster in dark condition compared to under light condition.

Key words: *Pestalotiopsis*, morphology, physiology, growth test and colony growth

ABSTRAK

Ciri-ciri morfologi dan fisiologi *Pestalotiopsis* spp. telah dikaji. Sebanyak enam pencilan telah dipencilkan daripada lokasi yang berbeza. Pengenalpastian kulat ini telah dijalankan berdasarkan ciri-ciri morfologi and fisiologi. Pertumbuhan *Pestalotiopsis* telah diuji di atas media agar dextrose kentang (PDA), agar kentang dan lobak (PCA) dan agar ekstrak malt (MEA). Pertumbuhan kulat ini pada suhu, pH, keadaan pencahayaan and elemen-elemen makro yang berbeza juga telah diuji. Terdapat perbezaan yang ketara pada $P < 0.05$ untuk kesan media ke atas pertumbuhan kulat ini. Pertumbuhan yang cepat telah dicatatkan di atas media PDA untuk pencilan 1077 dan 1091. Media menunjukkan tiada perbezaan ketara terhadap pertumbuhan koloni untuk pencilan 1214, 1213 dan 1080. Pencilan 1202 tumbuh dengan cepat di atas media PDA dan PCA jika dibandingkan dengan MEA. Terdapat perbezaan ketara pada $P < 0.05$ terhadap kesan suhu kepada pertumbuhan kulat. Suhu optima pertumbuhan untuk pencilan 1214, 1202 dan 1213 adalah pada 25°C. Walau bagaimanapun, suhu optima pertumbuhan untuk pencilan 1077, 1080 dan 1091 adalah pada 15°C ke 20°C, 20°C ke 30°C dan 15°C ke 30°C. Pencilan *Pestalotiopsis* yang berbeza berkeupayaan untuk tumbuh pada pH yang berlainan. Walau bagaimanapun, pH optima pertumbuhan adalah berbeza bergantung dengan pencilan. Elemen-elemen makro mempengaruhi pertumbuhan pencilan-pencilan *Pestalotiopsis*. *Pestalotiopsis* tumbuh dengan cepat dalam keadaan gelap berbanding dengan dalam keadaan bercahaya.

Kata kunci: *Pestalotiopsis*, morfologi, fisiologi, ujian pertumbuhan dan pertumbuhan koloni

CHAPTER 1

INTRODUCTION

Fungi are organisms that exist as a single cell or form a multicellular body called a mycelium. Most fungal cells are multinucleate and have cell walls composed of chitin. The principal criteria used in classification are the nature of the spores produced and the presence or absence of cross walls within the hyphae (Isaacs *et al.*, 1999).

There are various species of fungi found in Malaysia. They can be isolated from plant and soil from the forest, plant materials and cultivated crop. According to Kuthubutheen and Sepiah (1985), the common species of fungi isolated from the healthy leaves of fruit trees were *Colletotrichum*, *Fusarium*, *Botryopodia* and *Pestalotiopsis*.

Pestalotiopsis spp. is among the common fungi present in tropical region. It is a weak parasites and saprophytes that can be found in the tropics (Smith, 1988). Mordue (1980) stated that this species have been found in Malaysia, India, Nigeria, Australia, Brunei, Bangladesh and Hong Kong. For example, *P. funerea* have been found in plant family, Cupressaceae in France and *P. guepinii* were isolated from the Ericaceae especially in *Rhododendron* species (Smith, 1988).

According to Sepiah *et al.*, (2003) *Pestalotiopsis guipini* can be isolated from the Carambola (B17) in Malaysia that caused Pestalotiopsis rot. Besides that, *P. funerea* can be isolated from mangrove soil in Victoria and *P. triseti* were found in the glumes of *Brachypodium sylvaticum* from the Switzerland (Griffiths and Swart, 1974). From

the previous study, it shows that *Pestalotiopsis* spp. can be found in various countries, plant species and different types of plant materials.

The objective of the present study is to identify the morphological and physiological characteristics of different isolates of *Pestalotiopsis* spp. This study is very important due to the lack of published information on the morphological and physiological characteristics of this species in Sarawak.

CHAPTER 2

LITERATURE REVIEW

Pestalotiopsis spp. are anamorph, belongs to the members of Ascomycetes and the family of Amphisphaeriaceae. These fungi are parasitic or endophytic that lives on and in plant parts such as healthy leaves, dead plant matter and can be found in the soil (Raj, 1993).

There are different species of *Pestalotiopsis* spp. have been reported in the previous study. According to Sutton (1980) species of *Pestalotiopsis* spp. that have been studied includes *P. guepinii*, *P. gallica* and *P. natrassii*, *P. carssiusscula*, *P. dictaeta*, *P. disseminate*, *P. funereal*, *P. glandicola*, *P. karstenii*, *P. oxyyanthi* and *P. steyaertii*.

2.1 Morphological characteristics

The morphological characteristics of *Pestalotiopsis* spp. are different among this genus. They can be distinguished by their shape and colour of conidia, conidiophores, hyphae, mycelia, appendages and acervuli. Raj (1993) reported that, the conidia contains 4 to 5 celled, with the two or three dark brown central cells, and with two or more apical appendages or hairs. The conidiophores are produced within the compact fruiting structures.

According to Sutton (1980), the shape of conidia is fusiform, straight or slightly curved and has four euseptate. While, the conidiophores are hyaline, branched, cylindrical and septate at the base and above. The mycelia are branched, septate and pale brown. This genus has acervular, epidermal to subepidermal conidiamata. The

conidiogenous cells are holoblastic, annellidic, indeterminate, integrated, cylindrical and smooth.

Trapero *et al.* (2003) found that *P. maculans* isolated from nursery plant of *Arbutus unedo* have smooth and five celled of conidia, apical and basal cells were hyaline, three median cells were brown where the upper two cells were darker than the lower cells. The conidia were 22 to 30 μm and 5 to 9 μm . It consists of three apical appendages averaging 17 μm long and basal appendages averaging 6 μm .

Mordue (1980) stated that, *P. mangifera* conidia are fusiform, straight or slightly curved and slightly constricted at the septa. It consists of thin wall and hyaline apical and basal cells. The conidiophores formed from the upper cells of pseudoparenchyma, hyaline, cylindrical to lageniform, septate and branched at the base or above.

P. funerea have three, thick wall median cells capped by hyaline end cells. The hyaline basal cells bear an unbranched appendage a little longer than the basal cell. It has appendages arise from the hyaline apical cell and consists of four to six appendages (Griffiths and Swart, 1974).

According to Ploetz (2003), *P. versicolor* produced thick cottony, white to yellow mycelium and acervuli that are 0.5 mm in diameter and exude conidia in glistening, greenish black drops. It has fusiform conidia and three thick wall, dark median cells and thin wall, colourless apical and basal cells. It also consists of three bristles radiate from the apex of the spore, while a single bristle like pedicel protrudes from the basal cell giving the spore an insect like appearance.

2.2 Physiological characteristics

The physiological characteristics of *Pestalotiopsis* spp. are also important to be studied. They can be distinguished by the different growth requirement in different media, temperature and pH.

Deacon (1998) stated that PDA (Potato dextrose agar) and MEA (malt extract agar) are the suitable and standard media with pH 5 to 6 that contains high ratio of carbohydrates and nitrogen components that is used to support the fungi growth on solid media. According to Smith and Onions (1994), most fungi can grow well at temperature range from 10°C to 35°C and with the optimum temperature between 15°C to 30°C. While, the optimum pH are from the pH 3 to 7 but some can grow well at pH 2 or below. In addition, the growth media will affect the colony morphology and colour of the fungi.

Hopkins and McQuilken (2000) found that *P. sydowiana* can grow well on potato dextrose agar (PDA), dextrose agar (SDA), V8 juice agar (V8), malt extract agar (MEA) and Czapek Dox agar (CDA). Moreover, the suitable growth temperature was from 20°C to 25°C on PDA. However there were no growths or little growth occurring below 5°C or above 30°C. The hyphal extension occurred between pH 2.6-8.6 and the optimum values occurring at pH 5.5.

Mordue (1980) found that *P. mangiferae* can grow on various media that consists of carbon, nitrogen, sulphur and phosphorus. It requires growth temperature at 20°C to 30°C and the optimum of pH range from 5.5 to 6.

Mordue and Holliday (1971) found that *P. palmarum* that caused grey leaf blight on coconut produced white and fluffy colonies on the PDA and it can be identified by the hyaline, cylindrical to obovoid shape of conidiophores. According to Harrison and Jones (2003), the conidia are fusiform, straight and contain four septate with slight constrictions at septa.

Fungi require macro elements such as nitrogen, phosphorus and phosphate for internal and external physiology activities. According to Deacon (1980), nitrogen and phosphorus are the important macro elements for fungal activities and fungal interaction in nature.

Other macro elements for instances, magnesium and potassium are also required for fungal growth. According to Jennings and Lysek (1996) both are required as an activator of a number of key enzymes, and potassium is required to contribute to the appropriate ionic environment for enzyme functioning. In addition, they stated that, potassium can make a significant contribution to the internal osmotic potential.

In natural environment, fungi grow in light and dark condition. According to Deacon (1998), light influences fungi to produce circular zonation in some fungi and branching of the mycelia.

2.3 Diseases caused by *Pestalotiopsis* spp.

Pestalotiopsis have been reported as pathogen that causes various types of diseases in plant. The diseases include leaf spot, leaf blight, stem and fruit canker, fruit rot, postharvest fruit disease and stem end rot.

According to Sepiah *et al.* (2003), *Pestalotiopsis* rot is caused by the *P. guipini* on 'B17' of carambola. These diseases occur during the storage of carambola fruit. *P. guipini* is a weak pathogen and requires wound on carambola fruit to infect the fruit. The symptom appears after infection are light brown, circular lesions enlarging up to 2 cm in diameter and the fruit become soften. The white mycelium will cover the lesions and this produces dark masses of conidia (Sepiah *et al.*, 2003).

P. psidii is a weak pathogen that occurs in the woody tissues of twigs as an endophyte (Lim and Khoo, 1990). In Malaysia, this pathogen caused the *Pestalotiopsis* fruit canker on guava (Mordue, 1969). It has conidia with three apical appendages are produced in acervuli on leaves and fruit (Lim and Manicom, 2003).

CHAPTER 3

MATERIALS AND METHODS

3.1 Isolations of Fungi

The isolates of *Pestalotiopsis* spp. were obtained from the culture collection of University Malaysia Sarawak new collections of plant materials. The new isolations were done by using guava fruit bought from Satok, Kuching, *Shorea macropyhlla* and ornamental leaves which taken from Sabal Forest Reserve and Taman Sukma respectively.

Leaves were cut into 1x 1.5cm², and then soaked in 10 % Clorox for 5 to 10 minutes in the Petri dishes before washed with steriled distilled water thrice. The samples were dried on the filter paper and were transferred on the Water Agar or potato dextrose agar (PDA). The plated media were incubated at 25°C. The fungal colonies that were grown on the media were observed. Fungal isolations from guava fruit was done by inoculating a disease infected tissue onto PDA. *Pestalotiopsis* sp. obtained from the leaves and culture collection were recultured on the PDA for further study.

3.2 Morphological studies

The fungal isolates were cultured on MEA. The colour and shape of the colony on MEA were observed and recorded. Microscopic slides were prepared to examine the characteristics of hypha and conidia. A small portion of pycnidia was lifted using a needle and was transferred on the slide to observe the morphological characteristics through the microscope. A drop of lactophenol blue was used as stain. The cover slip

was placed gently on the specimen to avoid the present of air bubbles. The morphological characteristics of conidia such as length, number of cells, apical and basal appendages were observed and recorded.

3.3 Physiological studies

3.3.1 Growth tests on different media

The colonies of the *Pestalotiopsis* spp. from the pure culture (PDA) were cut in a small blocks size 3 mm in diameter. A block of agar containing the fungal mycelia was inoculated on PDA (potato dextrose agar), MEA (malt extract agar) and PCA (potato carrot agar) in Petri dishes. Three replicates were prepared for each of the media. The inoculated fungus was incubated at 25°C for a week. The diameter of the colony was observed and recorded everyday. The average diameter of colony was determined by measuring two diameters which were perpendicular to each other. Then, the average growth rates were determined.

$$\text{Average colony diameter, (D)} = \frac{d1 + d2}{2}$$

Average growth rate =

$$\frac{(D2 - D1) + (D3 - D2) + (D4 - D3) + (D5 - D4) + (D6 - D5) + (D7 - D6)}{N-1}$$

D = Average diameter for everyday

d = diameter of colony

N = Number of days

3.3.2 Growth tests at different temperature

Block of agar containing mycelia of the *Pestalotiopsis* sp. was inoculated onto PDA. Three replicates were prepared for each isolate and for each temperature. The isolate were incubated at 15°C, 20°C, 25°C, 30°C and 35°C. The average diameter for the colony were measured and recorded for seven days. The average diameter of the colony was calculated as above.

3.3.3 Growth tests at different pH

30 mL of potato dextrose broth (PDB) was placed in 150 mL conical flask and the pH was adjusted by adding HCl or NaOH. The tested pH values were 3, 4, 5, 6, 7, 8, and 9. The media were autoclaved at 15 psi and 120°C for 15 minutes. One block of agar containing mycelium of the tested *Pestalotiopsis* sp. was inoculated into the media. The inoculated media were incubated at 25°C for seven days. The mycelium of *Pestalotiopsis* spp. was filtered by using known weight of dried filter paper. The mycelium was dried for two days at 60°C. Then, dried weight of the mycelia was determined.

Dried weight of mycelium = $x - y$

x = dried weight mycelia + filter paper

y = dried weight of filter paper

3.3.4 Growth tests under different light conditions

Six isolates were cultured on PDA and three replicate were prepared for each isolates. The culture were wrapped in aluminium foil to exclude light and the others were placed under the fluorescence light (697.4 lux, 230 V). The diameter of colony was recorded for seven days and the average growth rate was measured. Average growth rates were calculated as above.

3.3.5 Effects of macro elements on the fungal growth

The effects of macro elements on the growth were determined by culturing the isolates in complete media as a control and on media without potassium, without nitrogen, without phosphate and without magnesium. The media were prepared as follows:

Complete medium

Agar powder (20g/L), Glucose (40g/L), Magnesium sulphate, $MgSO_4$ (1.25g/L), Potassium dihydrogen Orthophosphate, KH_2PO_4 (2.5g/L) and Potassium nitrate, KNO_3 (5g/L).

Without Potassium

Agar powder (20g/L), Glucose (40g/L), Magnesium sulphate, $MgSO_4$ (1.25g/L), Sodium dihydrogen Orthophosphate, Na_2HPO_4 (5g/L), Sodium nitrate $NaNO_3$ (5g/L)

Without Nitrogen

Agar powder (20g/L), Glucose (40g/L), Magnesium sulphate, MgSO_4 (1.25g/L), Potassium chloride, KCl (5g/L), Potassium dihydrogen Orthophosphate, KH_2PO_4 (2.5g/L).

Without Phosphate

Agar powder (20g/L), Glucose (40g/L), Magnesium sulphate, MgSO_4 (1.25g/L), Potassium chloride, KCl (5g/L), Potassium dihydrogen Orthophosphate, KH_2PO_4 (2.5g/L).

Without Magnesium

Agar powder (20g/L), Glucose (40g/L), Potassium nitrate, KNO_3 (5g/L), Potassium dihydrogen Orthophosphate, KH_2PO_4 (2.5g/L) and Potassium Sulphate, K_2SO_4 (2.5g/L).

Agar block of *Pestalotiopsis* was inoculated onto the media. Three replicate were prepared for each isolate. All the inoculated *Pestalotiopsis* were incubated at 25°C for seven days. Colony diameter of this fungus was calculated as above.

CHAPTER 4

DATA ANALYSIS

All data were analyzed by using SPSS windows version 11.0. One-way and two-way ANOVA and Tukey's test were used.

CHAPTER 5

RESULTS

5.1 *Pestalotiopsis* isolates

A total of six isolates were used in this study. Three isolates were from UNIMAS stock culture, 1077, 1080 and 1091 and the other three were isolated from *Shorea macrophylla* leaf (1214), guava fruit (1202) and ornamental leaf (1213). The isolates were listed in Table 1.

Table 1: *Pestalotiopsis* isolates from different plant origin and location

Isolates	Plant origin	Location
1214	<i>Shorea macrophylla</i> leaves	Sabal Forest Reserve
1202	Guava fruit	Satok
1213	Ornamental leaves	Taman Sukma
1077	Rhododendron	Gunung Murud
1080	Rhododendron	Gunung Murud
1091	Rhododendron	Gunung Murud

5.2 Morphological characteristics

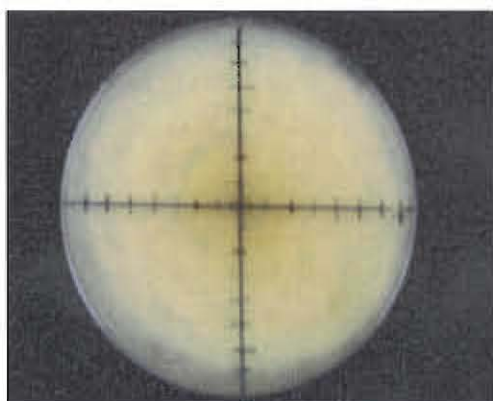
The characteristics of *Pestalotiopsis* showed some difference depending on the isolates and origins. The descriptions of the six isolates are as following:

Isolate 1214

On MEA, colony of upper surface was white and at reverse plate the colour was cream and slightly brown at the non-growing zone. Conidia were 20 μm in length and consist of 3 brown cells. Comprises of 2 or 3 apical appendages, each were 20 μm and consists of one basal appendage and was hyaline. The shape of colony was circular.



(a) Colony on MEA



(b) Reverses plate of MEA



(c) Conidia (100x)



(d) Smooth margin

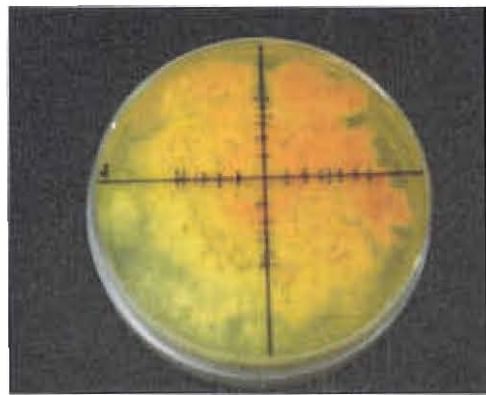
Figure 1: *Pestalotiopsis* isolate 1214 from *Shorea macrophylla*

Isolate 1202

Colony was white on MEA and and at reverse plate, the colour was yellow. Produced conidia on MEA after one to three weeks at room temperature (20-25°C). Conidia were 20 μm in length and consist of 4 cells. Comprises of 2 apical appendages, one basal appendage and both were hyaline in colour. The colony shape was irregular.



(a) Colony on MEA



(b) Reverse plate



(c) Conidia (100x)



(d) Black pycnidia

Figure 2: *Pestalotiopsis* isolate 1202 from guava

Isolate 1213

Colony on MEA was white both top and reverse surface of plate on MEA. Produced conidia on MEA after four weeks at 20°C 25°C. Consist of 2 apical appendages and one basal appendage, both was hyaline. The colony shape was circular.



(a) Colony on MEA



(b) Reverse plate



(c) Black pycnidia

Figure 3: *Pestalotiopsis* isolate 1213 from ornamental plant leaf

Isolate 1077

Initially, the colony on MEA was white and in two to three weeks produced yellowish pigmented on the colony. At reverse plate the colony was cream and produced brown circle around the margin. Conidia was 20 μm in length, cells were brown and consist of 3 cells. The conidia consist of 2 or 3 apical appendages and 1 basal appendage (4 μm). Require 6 weeks to produce conidia on MEA at 20°C. The colony shape was wavy.



(a) Colony on MEA



(b) Reverse plate of MEA



(c) Conidia (100x)



(d) Yellowish pigmented

Figure 4: *Pestalotiopsis* isolate 1077 from Rhododendron

Isolate 1080

Colony on MEA was white both site of the Petri dishes. Shape of colony was irregular and consists of rare mycelia. No conidia were formed during the presents study.



(a) Colony on MEA



(b) Reverse plate

Figure 5: *Pestalotiopsis* isolate 1080 from Rhododendron