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Full Length Article

Morphological and Molecular Characterization of *Phytophthora capsici*, the Causal Agent of Foot Rot Disease of Black Pepper in Sarawak, Malaysia

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Abstract

Sarawak is one of the largest exporters of black pepper (*Piper nigrum* L.) but the production of this crop is in the decline, because of the foot rot disease. The objective of this study was to determine the morphological and molecular characters of the *Phytophthora capsici* the causal agent of foot rot disease of black pepper in Sarawak. Thirteen major pepper growing areas were surveyed and confirmed for the incidence of foot rot disease. Ulu Sarikei (Sarikei) had the highest disease incidence (75%) followed by Pasai Siong (Sibu) (70%) and the lowest incidence at Tatau (Bintulu) (5%). The highest disease severity was at Ulu Sarikei (70%) followed by Pasai Siong (62%) and the lowest at Tatau (4%). Based on morphological characterization, the foot rot pathogen exhibited globose oogonia with paragynous antheridia, chlamydospore, torulose hyphae and lemon shaped sporangia with long pedicel. Molecular identification by using nested-PCR showed unique DNA fragment of *c*. 560 bp further confirmed that the causal agent of foot rot disease of black pepper in Sarawak was *P. capsici* Leonian.

Keywords: Foot rot disease; Morphological characterization; Disease incidence; Nested-PCR; *Phytophthora capsici*

Introduction

Black pepper (*Piper nigrum* L.), the "king of spices" is a traditional and historic spice crop, which has been used as spice since 4th century B.C. It was first brought into Malacca in 1583 by the Portuguese (Rahman, 1993). Black pepper is a wood climber and the cultivation of black pepper is mainly confined to India, Brazil, Indonesia, Malaysia, Thailand, Sri Lanka and Vietnam. Pepper crop cultivation gained popularity in Johor and Singapore during the early 19th century and it was widely planted in Sarawak since the mid-19th century. Currently, 99% (14 622 hectares) of the crop is grown in Sarawak and only 0.95% is grown in Peninsular Malaysia and 0.05% in Sabah (IPC, 2010). Sarawak black pepper has gained popularity in the world pepper market. However, disease infestation in orchards is still the biggest problem in black pepper cultivation.

Foot rot is one of destructive disease of black pepper in Sarawak and affects the total production of black pepper in Malaysia. Foot rot is a debilitating disease that effected plants to survive for several years and death of plants occur gradually over a period of 4 years (Ravindran, 2000). This disease is more severe in the orchards if nematodes such as *Radopholus similis* and *Meloidogyne incognita* are present together with the causal fungus (Anandaraj *et al*., 1996a, b). Nematodes has also been reported as a major constraint to the black pepper production. A survey conducted by Eng (2001) in 43 black pepper farms revealed that root-knot nematodes (RKN) were present in all farms, suggesting that black pepper is one of the important hosts of RKN. According to Eng (2001), no resistant cultivars to RKN is available in Sarawak.

At beginning, foot rot disease is also referred as 'slow wilt' (Nambiar and Sarma, 1982) as the slow decline in Malaysia (Kueh and Sim, 1992; Varughese and Anuar 1992) and 'Yellow disease' complex (De Waard, 1979; Zaragoza *et al*., 1991) or 'Yellows disease' in Indonesia (Sitepu and Kassim, 1991). Based on the etiological studies, slow decline or foot rot disease caused by *Phytophthora capsici* Leonian on black pepper in Malaysia were reported in Sarawak by Bong and Saad (1985) and Johor by Varughese and Anuar (1992).

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According to literature reviews, now this disease is known as *Phytophthora* foot rot disease or 'quick wilt disease' of black pepper. This disease has been reported throughout the world where the black pepper is grown (Anandaraj, 2000). In Java and Sumatera, the disease was first called foot rot by Muller (1937), who determined its aetiology and gave a full account of the symptoms and epidemiology. However, the disease is often colloquially referred to as 'quick wilt' or 'quick death' by farmers in many countries such as Vietnam (Truong *et al*., 2008). Miller (1953) has reported the severe outbreak of the disease in Sarawak. The foot rot disease shows the symptoms of collar rot, root rot and leaf disease as described and illustrated by Sarma *et al.* (1991).

Phytophthora foot rot is a highly destructive disease and invariably leads to low productivity of black pepper production. The disease incidence was first reported in Indonesia in 1885 (Erwin and Ribeiro, 1996). Since this report, the disease has become one of the major limiting factors of black pepper production wherever the crop is grown (Anandaraj, 2000). In Malaysia, there are no conclusive reports available on the effect of the disease in terms of economic loss or yield loss. However, Manohara *et al*. (2004) reported that the loss of vines due to the disease is generally from 5-20% but it is significantly higher in India (Shamarao and Siddaramaiah, 2002) and Indonesia (Sitepu and Mustika, 2000). According to Anandaraj *et al.* (1989), the disease has been reported to affect up to 95% of the vines in individual farms in certain countries.

Accurate and rapid identification of a pathogen is necessary for the appropriate management of the disease (Singh *et al*., 2006). Morphological characteristics are key for the identification and taxonomy of many fungal species such as *Fusarium* and *Phytophthora* (Mchau and Coffey, 1995; Sahar *et al*., 2012). The *Phytophthora* taxonomic system has been based on morphological characteristics of the globose oogonia with paragynous antheridia, chlamydospore, torulose hyphae and lemon shape of sporangia (Mchau and Coffey, 1995). Introduction of molecular methods, which are faster and sensitive than morphological identification, are also employed to identify phytopathogens until species level (De Biazio *et al*., 2008). The use of PCR, nested PCR and DNA sequence analysis of internal transcribed spacer (ITS) regions has become routine for the detection, identification, classification and phylogenetic analysis of many fungi at the species level (Taylor *et al*., 2000; Bowers *et al*., 2007). ITS regions sequences are highly variable in *Phytophthora*, and taxon-selective ITS amplification has been used to detect many fungal pathogens, such as *Fusarium* and *Phytophthora* (Bowers *et al*., 2007; Sahar *et al*., 2012).

Therefore, a study on the disease incidence, morphology and development of sensitive detection method of foot rot disease in Sarawak would be beneficial to avail new information to be used in the planning of disease management practices, for policy makers to formulate strategic plans, as a guide to researchers in prioritization of research programs and to promote awareness among growers due to inadequate information pertaining this disease in Malaysia.

Materials and Methods

Study Sites

Thirteen orchards in four divisions (Sarikei, Sibu, Kapit and Bintulu) of the Sarawak state were surveyed for foot rot disease incidence and severity between November 2010 and January 2011 in Sarawak (Table 1). Black pepper of Kuching variety had been planted in most of the commercial plantations, small-scale farms and backyard orchards were evaluated.

In Sibu, the survey was conducted at Pasai Siong, the orchards are located far away from settlement area. In Sarikei, studies were conducted in Repok and Ulu Sarikei areas, in Kapit and Bintulu, study sites were in Belaga and Tatau, respectively. Crop age ranged from 4 to 10 years. Thirteen points were assessed and at each location, one out of three sites was selected and the mean disease severity for the location was calculated.

Sampling and Data Collection

The methods of detection and delimiting surveys were used to determine the presence of foot rot disease in commercial and backyard orchards in Sarawak. The status of foot rot disease present was assessed randomly, at least 10% of the black pepper plants at a site in the field by means of disease intensity, which was measured through observation of disease incidence and severity of overall field symptoms of foot rot disease. Disease severity was determined using descriptive type assessment key with a 0-4 score scale represents; 0=0%, 1=1-30%, 2=31-50%, 3=51-75%, 4=76-100% (Kim and Hwang, 1992).

The incidence and severity of foot rot disease is also measured based on leaf defoliation symptom on black pepper plant. Through this method, 10 plants in every surveyed location were examined by random sampling. Disease severity was determined using descriptive type assessment key with a 0-4 score scale represented by 0=0%, 1=1-25%, 2=26-50%, 3 =51-75%, 4=76-100% (Abraham *et al*., 1996). The score scales were then converted to disease severity index for non-parametric measurements (Kim *et al*., 2000) and expressed as percentages:

$$DSI = \frac{\sum(ab)}{N \times K} \times 100$$

DSI = Disease Severity Index.

$\sum$ ab = Sum of the product of assessed plants with their corresponding score scale.

N = Total number of assessed plants.

K = Highest score scale.

Data on temperature and rainfall in the surveyed areas were obtained from the Malaysia Department of Meteorology (2011). Plant age data were obtained from the black pepper farmers.

Isolation and Morphological Characterization of *P. capsici*

P. capsici was isolated from infected black pepper roots collected from black pepper orchards in Sibu, Sarikei, Kapit and Bintulu. The root samples were placed in a beaker and washed gently using running tap water. Subsequently, the roots were cut into small sections of advancing root lesions (0.5-1.0 cm), followed by surface sterilization using 10% sodium hypochlorite for 30-60 sec. The roots were then rinsed in sterile distilled water in a beaker for 1 min. The roots were blotted to dry using sterile filter paper. The roots were then transferred to rose bengal agar (RBA) plates (Scharlau Chemie, Spain). The RBA plates were incubated in an incubator at 28±2°C for 72 h of continuous darkness. *P. capsici* colonies appeared on the plates were isolated and sub-cultured in potato dextrose agar (PDA; Merck KGaA, Germany) plates. Morphological characteristics observation such as mycelia structure and types of conidia produced were performed using compound microscope (Leica DM 2500, USA). Images were captured using camera microscope (Jenoptik Optical Systems GmbH, Germany).

Molecular Identification of *P. capsici*

DNA Extraction

Fungal mycelia were grown in potato dextrose broth (PDB; Fluka Analytical, Switzerland) and incubated at 25°C for 4-6 days until the mycelia covered the surface of PDB medium. The mycelia were then harvested in microcentrifuge tube. DNA was extracted according to Wong and Wong (2009). Genomic DNA was visualized by agarose gel electrophoresis for estimation of amount and quality. The DNA was stored at -20°C until use.

PCR Amplification

Specific primers for *P. capsici* (PC-1, 5'-GTCTTGTACCCTATCATGGCG-3′ and PC-2, 5′-CGCCACAGCAGGAAAAGCATT-3′) were used to amplify PCR products of 560 bp. Nested-PCR method was used as decribed by Zhang *et al.* (2006). PCR reaction volume of 25 µL contained 1 µL genomic DNA, 0.5 µm primers, 50 µm of each dNTP (Invitrogen, USA), 2.5 µL of 10X PCR buffer (Invitrogen, USA), 2 mM Mg^{2+} (Invitrogen, USA), and 1.25 U *Taq* DNA polymerase (Invitrogen, USA). Amplification was performed using thermal cycler programmed for one cycle at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 70°C for 30 s and 72°C for 30 s. A 7 min extension at 72°C completed the programme. Nested PCR included two rounds of amplification, initially using the universal primers ITS1 and ITS4 for the first round followed by the *P. capsici* specific primers PC-1 and PC-2 for the second round. In this study negative controls without template DNA were used in each experiment to test for contaminants.

Data Analysis

Disease incidence, severity data on foot rot and leaf defoliation data was randomly recorded in field. The recorded data was tested using analysis of variance (ANOVA) were arcsine transformed prior to analysis by descriptive statistics using SAS V9.2. Means were compared using Duncan's New Multiple Range Test (Steel and Torrie, 1980).

Results

Incidence and Severity of Foot Rot Disease of Black Pepper in Sarawak

Foot rot disease symptoms were observed mostly at the Kuching variety in all of the 13 orchards surveyed. Overall, disease incidence of foot rot disease occurred in the Sibu, Sarikei, Kapit and Bintulu Divisions. The mean on disease incidence was 46.31% and severity was 40.91%. Mean incidence on defoliation was 68% and severity was 30% (Table 2 and 3). Leaves yellowing symptom was observed on both the upper and lower leaf canopies at all sites surveyed. Subsequently, high disease incidence on foot rot symptom was recorded in orchard A, Ulu Sarikei (75%) followed by orchard C at Pasai Siong (70%), orchard A at Tatau (65%) and Repok (65%) on orchard A while the least incidence was recorded on orchard B at Repok (5%) and Tatau (5%). The characteristic of foot rot disease during survey was indicating the symptoms of foliar yellowing, defoliation and collar rot (. 1a, b and c).

Orchard A, the disease severity on foot rot symptoms at Ulu Sarikei was 70%, moderate severity was recorded at Pasai Siong (62%) i.e., orchard C and Tatau (62%) i.e., orchard A, while the lowest disease severity was recorded at Repok (4%) and Tatau (4%) i.e., orchard B. In terms of leaf defoliation, Kuching variety showed the highest disease incidence at Pasai Siong (90%) followed by Ulu Sarikei (80%), Repok (60%) and Tatau (60%). Belaga (50%) had the lowest incidence compared to other locations and similar trend was observed in disease severity at most of the locations surveyed (Table 4).

Number of trees with disease severity in terms of defoliation is shown in Table 3. At Pasai Siong none, slight, moderate and severe infections were observed while at Belaga and Tatau regions, none, slight and moderate infections on Kuching variety were observed. On the other hand, there was no severe infection in all locations (Sibu, Sarikei, Ulu Sarikei and Bintulu) and it shows that the disease incidence was not very much serious to induce any death of the black pepper plant.

Table 1: Locations covered during *Phytophthora* foot rot disease surveys in Sarawak, Malaysia

Location	Number of orchard
Sibu	4
Sarikei	5
Kapit	2
Bintulu	2
Total	13

Table 2: Distribution and severity of *Phytophthora* foot rot disease on black pepper in Sarawak, Malaysia (2011)

Division/Location	Orchard/ Variety	Plant Age (year)	Incidence (%) Mean*	Severity (%) Mean*
Sibu (Pasai Siong)	A/Kuching	10	55^{c}	48^{c}
	B/Kuching	10	62^{bc}	54^{bc}
	C/Kuching	10	70^{ab}	62^{ab}
	D/Kuching	6	35^{d}	26^{d}
Sarikei (Ulu Sarikei)	A/Kuching	6	75^{a}	70^{a}
	B/Kuching	10	35^{d}	28^{d}
	C/Kuching	6	55^{c}	50^{c}
Sarikei (Repok)	A/Kuching	10	65^{abc}	58^{bc}
	B/Kuching	4	5^{e}	4^{e}
Kapit (Belaga)	A/Kuching	10	55^{c}	48^{c}
	B/Kuching	8	20^{d}	18^{d}
Bintulu (Tatau)	A/Kuching	15	65^{bc}	62^{ab}
	B/Kuching	5	5^{e}	4^{e}
Mean			46.31	40.92
CV			30.57	31.98

Means within a column followed by the same letters are not significantly different at $p \leq 0.05$

*Data was arcsine transformation

Table 3: Number of black pepper plant with disease severity score of defoliation symptom in Sarawak, Malaysia (2011)

Location	Disease severity based on score scale (0-4)*				
	None (0)	Slight (1)	Moderate (2)	Severe (3)	Very severe (4)
Sibu (Pasai Siong)	1	4	3	2	0
Sarikei (Ulu Sarukei)	2	3	2	3	0
Sarikei (Repok)	4	3	2	1	0
Kapit (Belaga)	5	4	1	0	0
Bintulu (Tatau)	4	3	3	0	0

*None = 0%, Slight = 1-25%, Moderate = 26-50%, Severe = 51-75% and Very severe = 76 – 100% (Abraham *et al*., 1996)

Regardless of location monthly temperature was approximately similar, where Pasai Siong, Ulu Sarikei and Repok (26.5^{o}C) were on the same reading, while Belaga and Tatau was recorded to be 26.7^{o}C. The highest foot rot disease severity was observed at Ulu Sarikei (70%) followed by Pasai Siong (62%), Tatau (62%), Repok (58%) and Belaga (48%). The mean of annual rainfall during the survey year (2010), for Pasai Siong, Ulu Sarikei and Repok, was experiencing the same annual rainfall 3779.9 mm, while it was 4227.1 mm at Belaga and Tatau (Table 5).

Table 4: Disease incidence and severity of defoliation symptoms on black pepper plant in Sarawak, Malaysia (2011)

Location	Variety	Average plant age (Year)	Disease incidence (%)	Disease severity (%) (range)*
Sibu (Pasai Siong)	Kuching	9	90^{a}	40^{a} (0-3)
Sarikei (Ulu Sarukei)	Kuching	7	80^{b}	40^{a} (0-3)
Sarikei (Repok)	Kuching	7	60^{c}	25^{ab} (0-3)
Kapit (Belaga)	Kuching	9	50^{d}	15^{b} (0-2)
Bintulu (Tatau)	Kuching	10	60^{c}	30^{ab} (0-2)
Mean			68%	30%

Means within a column followed by the same letters are not significantly different at $p \leq 0.05$. *Disease Severity: 0-4 scale, where 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75% and 4 = 76-100% (Abraham *et al*., 1996)

Table 5: Comparison of foot rot disease severity, mean monthly temperature, mean annual rainfall and plant age at different locations in Sarawak, Malaysia (2011)

Location	Foot rot disease severity (%)*	Temperature (^{o}C)*	Rainfall (mm)*	Plant age (years)
Sibu (Pasai Siong)	[1]62^{ab}	26.5^{a}	3779.9^{a}	10
Sarikei (Ulu Sarikei)	70^{a}	26.5^{a}	3779.9^{a}	6
Sarikei (Repok)	58^{b}	26.5^{a}	3779.9^{a}	10
Kapit (Belaga)	48^{c}	26.7^{a}	4227.1^{a}	10
Bintulu (Tatau)	62^{ab}	26.7^{a}	4227.1^{a}	15

*Means within a column followed by the same letters are not significant different at $p \leq 0.05$

[1]The highest disease severity score at each location

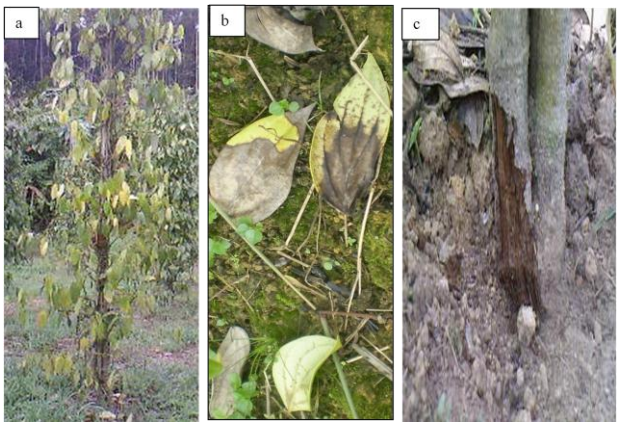

Fig. 1: Field symptoms of *Phytophthora* foot rot disease observed on infected black pepper in Sarawak; leaves yellowing (a), leaves defoliation (b) and collar rot symptoms (c)

Morphological Identification of *P. capsici*

Identification of *P. capsici* is mainly based on the morphology of sporangia. In the present study, *P. capsici* was successfully isolated and identified based on morphological characteristics. Fig. 2a, b, c and d shows the morphological characteristics of a pure fungal isolate.

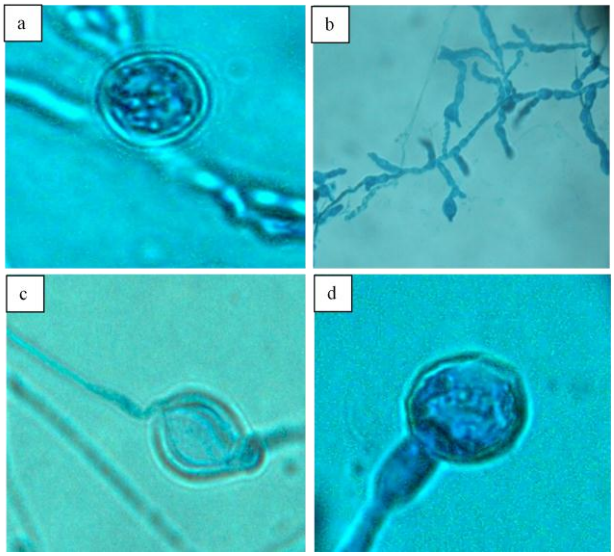

Fig. 2: Typical morphological characteristics of *Phytophthora capsici* isolated from infected black pepper root: Chlamysdospore (a); torulose hyphae (b); lemon shape sporangium with long pedicel (c) and globose oogonia with paragynous antheridia (d)

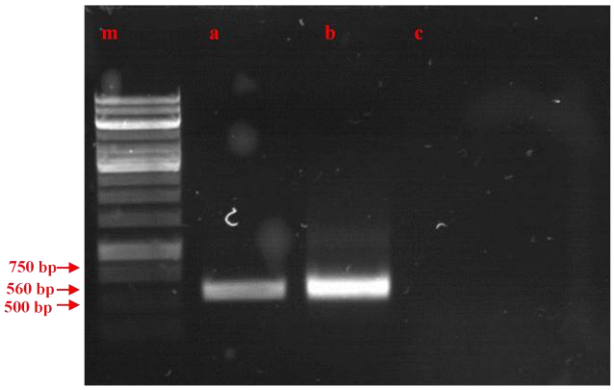

Fig. 3: Agarose gel electrophoresis of Nested PCR-amplified products of *Phytophthora capsici* isolates. Clear band at 560 bp was obtained after first round amplification using ITS1 and ITS4 primers (a). Specific band at 560 bp was obtained using specific primers PC-1 and PC-2 after second round amplification (b)., DNA marker using Generuler 1kb DNA Ladder (m) and negative control (c)

The characteristics of this isolate was typical of *P. capsici* such as globose oogonia with paragynous antheridia, chlamydospore, torulose hyphae and lemon shape of sporangia with long pedicels as described by Mchau and Coffey (1995).

Molecular Identification of *P. capsici*

Selected fungal isolates showing typical morphological characteristics of *P. capcisi* were further verified using nested-PCR. The ITS amplification product ranging from 500-750 bp were obtained for *Phytophthora* species by using primer ITS1 and ITS4. After second rounds of amplification by using primer PC-1 and PC-2, the specific primer was able to identify *P. capsici* by amplifying unique DNA fragment of *c.* 560 bp from selected isolates tested (Fig. 3).

Discussion

Phytophthora capsici is a diverse fungal species causing disease on a broad range of both temperate and tropical crops. In tropical countries as well as Malaysia, this fungal pathogen has been reported to cause destructive disease on a variety of hosts, such as cacao, rubber, sweet orange, chili, papaya, black pepper, and others. In this study, we conducted field surveys in Sarawak to determine the disease incidence and severity of foot rot disease in black pepper. The foot rot disease was detected in four major growing areas of black pepper in Sarawak i.e., Sibu, Sarikei, Kapit and Bintulu. It is difficult to confirm that the black pepper arising areas will be free from the disease. Efficient application of disease management program is urgently required together with precise information concerning the quantitative measurement of the disease (Nutter, 2001).

Based on the present findings, the highest percentage of disease incidence was recorded in Ulu Sarikei (75%) followed by Pasai Siong (70%). These major growing areas of black pepper also had high percentage of disease severity 62-70%. High percentage of disease incidence and severity in the surveyed areas could possibly due to co-infection by other pathogens such as nematodes and *Fusarium solani*. Based on the previous report, showed that the foot rot disease has been speculated to be a nematode-fungus complex involving either *R. similis* or *Meloidogyne* spp. and *Fusarium* spp. and *P. capsici* (Koshy, 1986).

The correlation between disease severity and surveyed location suggests the possibility of plantations being infested with pathogenic soil borne fungus long before they are replanted. Favorable microclimate conditions in the soil could promote the establishment of soil pathogens. This can be related to the significant correlation between temperature and rainfall in the surveyed areas. Relatively high rainfall during the monsoon with high soil moisture (>25%) and conducive temperature (22°C to 29°C) and suitable relative humidity (80%) are favourable for rapid multiplication of the fungus (Anandaraj *et al*., 1996b). Bong and Saad (1985) reported that black pepper *Phytophthora* is moisture loving and it will establish fast and reproduce rapidly in places where the water is plentiful. Moreover, occurrence of parasite nematode such as *R. similis*, which can complete their cycle within 25-30 days at temperature 21°C to 23°C (Koshy, 1986; Geetha, 1991) and *Meloidogyne* spp. which is widely distributed in tropical, subtropical and warm temperate regions. This could explain why foot rot disease of black pepper occurs more rapidly in this region based on the rainfall and temperature records.

In Pasai Siong, Ulu Sarikei and Repok, the mean of annual rainfall was 3,800 mm and annual temperature ranged between 25°C to 27°C. In Belaga and Tatau, annual rainfall recorded 4,300 mm and annual temperature was the ranged between 25°C to 27°C. This could explain why the highest percentage for both disease incidence and disease severity was recorded in Ulu Sarikei and Pasai Siong. Bong and Saad (1985) reported that the climatic conditions in Sarawak are favourable for *Phytophthora* disease development and the causal fungus also has a high vegetative production rate.

In Malaysia, foot rot disease of black pepper is caused by *P. capsici* was also shown the symptoms of decline on the black pepper plant. Based on field inspection, the collar rot infection occurs either at the collar or just above or below the soil level. Collar and root infection go unnoticed until the foliar yellowing symptom is recognized. The infection at initial stage starts as water soaked, which is the same as leaf infection. The lesions later turn to brown to dark brown in color within two to three days and later it appear as slimy dark patches. Young leaves become flaccid followed by yellowing and defoliation. The affected portion is wet discolored slimy emitting foul smell. Vascular discolorations observed in many cases but not consistently (Nambiar and Sarma, 1977). During the advanced stage of infection, the cortex gets disintegrated and peeled off. The infection of the collar gradually progresses downwards and spreads to the root system. This results in rotting of the root (Holliday and Mowat, 1963).

Root infections of the vines go unnoticed without any visible at aerial symptoms. The root infection starts at finer feeder roots (Holliday and Mowat, 1963), later it spreads to the main roots and the collar. The black pepper vines remain healthy until large portions of the roots are damaged. In the advanced stages of the root rot, foliar yellowing of the vine, and shedding of leaves, spikes and lateral branches are noticed. The amount of defoliation due to root rot infection is equal to root damage. The root loss to regeneration determines the spread of the decline and death of the vine. During the post monsoon season with depletion of soil moisture, the remaining root system is unable to support the vine, so the entire vine collapses with wilting and drying of leaves. Foliar yellowing, flaccidity, defoliation, breaking of the stems at nodal regions and spike shedding are the characteristic aerial symptoms of root rot and collar rot infections (Muller, 1936; Holliday and Mowat, 1963). The detail symptomatology of the disease has been described by Sastry (1982), Anandaraj *et al.* (1988) and Sarma *et al.* (1988).

In Malaysia, due to insufficient information on the morphology and molecular characteristics of *P. capsici* resulted in confusing identification of this pathogen and it was difficult to control in the orchards. According to previous study, all isolates of *Phytophthora* from cacao were classified as *P. palmivora* (Butl.) Butler (Brasier and Griffin, 1979). They were placed into four morphological groups i.e., MF1, MF2, MF3, and MF4. Detail study on the MF4 isolates lead to a new classification of the group. This group was then reconsidered to be *P. capsici* Leonian (Erwin and Ribeiro, 1996). The isolates, which were isolated from cacao, black pepper, and other tropical crops, some of which formed chlamydospores, were characterized as *P. capsici* in an amended description of the species (Tsao and Alizadeh, 1988). In the present study, pathogenic fungal isolated from infected black pepper exhibited classic morphological characteristic of *P. capsici* by producing globose oogonia with paragynous antheridia, chlamydospore, torulose hyphae and lemon shape of sporangia with long pedicels.

Accurate, sensitive and cost-effective detection and identification method is very crucial in managing this disease effectively. In the present study, specificity of the detection method was improved by using nested-PCR. The primer set PC-1 and PC-2 was able to amplify a unique DNA fragment of *c*. 560 bp. The nested-PCR method in this study provided consistent and reproducible results. Based on the previous study, showed that by using nested PCR, the sensitivity of detection will increased by factors of 10–1000 (Li and Hartman, 2003). Nested PCR assay therefore has great potential as a diagnostic tool for detecting and surveying phytopathogens in diseased plants, water and soil. Moreover, this method is very easy to use and requires minimal training.

Strengthening the future study on the management program of foot rot disease is truly recommended to find the best way on how to overcome the disease in the orchards as this major disease would cause major loss on black pepper production in Malaysia. Some recommendation from other research was avoiding planting black pepper in heavily or poorly drained areas and mulching black pepper plants which help in providing extra nutrition and at the same time increasing soil aeration. Practice sanitation in nurseries and fields also one of the best ways to control disease by removing severely infected leaves and clean up fallen plant debris. Meanwhile, planting leguminous ground cover in black pepper farms could provide nitrogen and prevent splashing of pathogen-infested soil onto foliage. Application of chemical pesticides to be kept at minimal if possible while green manures and organic matter should be employed to control soil-borne pathogens.

In conclusion, 13 major growing areas of black pepper in Sarawak, Malaysia were surveyed and confirmed of incidence of foot rot disease. The highest disease incidence was Sarikei and the lowest incidence was Bintulu. Based on morphological characterization, the foot rot pathogen exhibited of globose oogonia with paragynous antheridia, chlamydospore, torulose hyphae and lemon shape of sporangia with long pedicel. Moreover, molecular identification by using Nested-PCR showed unique DNA fragment of *c*. 560 bp, which was further confirmed that the causal agent of foot rot disease of black pepper in Sarawak was *P. capsici* Leonian.

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