BIODIVERSITAS
Volume 25, Number 11, November 2024
Pages: 4095-4104

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d251109

Inoculum prevalence, virulence, and morphometric variation of *Phytophthora palmivora* recovered from soil across cocoa plantations in East Luwu, Indonesia

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Manuscript received: 28 July 2024. Revision accepted: 9 November 2024.

Abstract. *Brugman E, Hardina N, Kuswinanti T. 2024. Inoculum prevalence, virulence, and morphometric variation of* Phytophthora palmivora *recovered from soil across cocoa plantations in East Luwu, Indonesia. Biodiversitas 25: 4095-4104.* In most cocoa plantations across Indonesia, the soilborne pathogen *Phytophthora palmivora* is a major concern for causing pod rot disease. This study aims to evaluate the inoculum prevalence, virulence, and morphological variation of *P. palmivora* in cocoa plantations across East Luwu. The pathogen was recovered from soil samples using the apple soil baiting method, and pure cultures were obtained on modified V8 juice agar media. Morphological observations included colony patterns and asexual features. Principal component analysis (PCA) was conducted to identify patterns in 12 measured morphometric variables. The virulence level was determined based on disease severity (%), latent period (days), and infection rate (day-unit^{-1}). Isolation via soil baiting confirmed the prevalence of *P. palmivora* in all soil samples (100%), confirming soil as a consistent source of inoculum in the cocoa plantation. Fifteen isolates exhibited varying sporangial shapes (ovoid, pyriform, and globose) and types (papillate, semi-papillate, and non-papillate), as well as globose intercalary and terminal chlamydospores with irregular collaroid, torulose, and loop hyphal swellings. The PCA plot grouped isolates into three clusters based on 12 morphometric variables, where all isolates from Mangkutana were included in the same cluster. The virulence test revealed that one isolate (KLN2) was avirulent, while the remaining isolates were pathogenic with low to medium virulence.

Keywords: *Phytophthora palmivora*, pod rot disease, soil inoculum, *Theobromae cacao*, V8 juice agar media

INTRODUCTION

Soilborne diseases caused by various pathogens, including fungi, oomycetes, bacteria, and nematodes, pose a significant threat to global crop production. These pathogens have a considerable impact on agriculture by directly reducing crop productivity and indirectly increasing cultivation costs (Oliveira and Bell 2022). Soilborne pathogens live and function in the soil for at least part of their life cycle. Depending on environmental conditions, available substrates, and competitive abilities, a soilborne pathogen can be dormant, parasitic, or saprophytic (Allen and Nehl 1997). Many persist in the soil through resilient resting structures such as cysts, chlamydospores, oospores, and sclerotia, which enable them to survive unfavorable conditions and periods without a host. When embedded in crop residues, these structures can last even longer (Mihajlović et al. 2017).

Among the most destructive soilborne pathogens are species from the genus *Phytophthora*. Most *Phytophthora* species are primary plant pathogens with hemibiotrophic or necrotrophic lifestyles, although the genus also includes saprophytic and opportunistic necrotrophic species (Perrine-Walker 2020; Matsiakh and Menkis 2023). *Phytophthora* is classified as an oomycete (a fungal-like microorganism). Oomycetes differ from fungi in several biological characteristics, for instance, their cell wall contains cellulose (1,3-β-glucans, some 1,6-β-glucans, and 1,4-βglucans) instead of chitin, as in true fungi. Fungal hyphae are septate, whereas oomycete hyphae are nonseptate (Latijnhouwers et al. 2003). *Phytophthora* has more than 200 well-described species and an estimated 200 to 400 other species that have not yet been discovered in natural ecosystems (Brasier et al. 2022; Chen et al. 2022). *P. palmivora* is a notable soilborne pathogen that has been reported to have a wide host range, capable of infecting more than 200 plant species.

P. palmivora, the primary causal agent of pod rot disease, has remained a major constraint on national cocoa production in recent years (Komalasari et al. 2018; Edy et al. 2019; Masanto et al. 2019). *P. palmivora* is also responsible for causing significant diseases in other plantation crops across the pantropical region, including oil palm, coconut, rubber, durian, and jackfruit (Lim and Chan 1986; Borines et al. 2014; Torres et al. 2016; Krishnan et al. 2019; Perrine-Walker 2020; Avinash et al. 2022). The severity and incidence of pod rot disease across cocoa-growing areas can vary depending on several factors, including humidity, rainfall, temperature, and inoculum density. Abiotic and biotic elements of the soil, along with soil management practices such as agrochemical applications and field sanitation, also might impact the prevalence of soilborne pathogens. In South Sulawesi, the second-largest cocoa-producing province in Indonesia, the severity of pod rot disease can reach up to 60% (Rosmana et al. 2010).

Phytophthora palmivora is a highly aggressive species. They are capable of reproducing asexually through motile zoospores and sexually through the production of oospores (Perrine-Walker 2020). *P. palmivora* is classified as heterothallic (self-sterile), possessing A1 and A2 mating types. A growth regulator produced by one thallus stimulates the other to produce gametangia (Tomura et al. 2017). Both A1 and A2 mating types have been identified in several *P. palmivora* populations in Indonesia, which contributed to their genetic diversity (Masanto et al. 2019; Brugman et al. 2022).

The asexual spores (sporangia, zoospores, chlamydospores) and sexual spores (oospores) of *P. palmivora* can infect all parts of the cacao plant at various growth stages, causing pod rot, stem canker, leaf blight, and cherelle wilt, with the most significant losses occurring when the pods are infected. Chlamydospores and oospores can survive outside the host plant, acting as the main inoculum source in the field. Oospores have been identified as a key, stable inoculum source in cocoa plantations in West Java, Indonesia (Purwantara 2008). As a soilborne pathogen that infects the above-ground parts of cocoa trees, several dispersal mechanisms for soil inoculum to the canopy or between canopies have been documented, including rain and insects. *P. palmivora* can be transmitted vertically from soil to plant surfaces via ants (*Iridomyrmex cordatus* Smith 1859) and termites (Rosmana et al. 2010). Once in the canopy, reservoirs of inoculum are established in cankers, infected flower cushions, and mummified pods (Guest 2007).

Due to the nature of soilborne pathogens, soilborne diseases are particularly difficult to predict, detect, diagnose, and successfully control. Information on soil inoculum availability, viability, virulence, and variation of *P. palmivora* in cocoa plantations is crucial to support strategies for mitigating soilborne diseases. This study focuses on East Luwu District, South Sulawesi, Indonesia a significant cocoa-producing region in South Sulawesi, where we aim to assess the availability and virulence of *P. palmivora* in soil inoculum and analyze its morphological variation across cocoa plantations.

MATERIALS AND METHODS

Sample collection

This research was conducted from September 2022 to February 2023. Exploration and sample collection occurred in three cocoa plantations in East Luwu District, South Sulawesi Province, Indonesia, specifically in the Kalaena, Mangkutana, and Angkona sub-districts. Laboratory experiments were conducted at the Plant Diseases Laboratory of the Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Hasanuddin, Makassar, Indonesia. Sample collection followed a random sampling method, with five sampling units identified in each plantation. In each sampling unit, soil samples were collected from three points around the tree and combined into a 250 g composite sample. Subsamples from this mixture were then subjected to baiting.

Isolation and morphology characterization

Isolation from soil samples was performed using the apple-baiting method, following Campbell's technique (Drenth and Sendall 2001). This method involved the insertion of soil into a hole made in the apple flesh (Granny Smith). The apple was then incubated in a plastic bag at 25°C for five days. The edge of the rotted area around the apple hole was aseptically cultured in a laminar airflow on modified Vegetable Juice 8 media (Hardina et al. 2020). The growing mycelium tips were observed and subcultured onto fresh V8 plates to obtain pure cultures. These pure isolates were cultured on V8 slant media and stored at 28±2°C for further use. Morphological characterization was conducted using a Nikon Eclipsed E100 optical microscope with an Optilab Advanced Plus digital camera. The morphological characteristics assessed included macroscopic structures (colony pattern and color), asexual structures (sporangia, chlamydospores, and mycelia), and sexual structures (oogonia, antheridia, and oospores). Each microscopic structure's size and measurement were determined using Image Raster 3.0.

Virulence test

The virulence test was conducted by inoculating *P. palmivora* isolates onto healthy, detached cocoa pods. Each isolate was tested on 3-5 cocoa pods. The cocoa pods used for the test were immature and between 5-7 months old. The inoculum consisted of a 7-day-old *P. palmivora* culture. Before inoculation, the cocoa pods' surfaces were cleaned of dirt and organic residue. The isolates were then extracted using a 0.5 mm diameter corkborer. Inoculation involved placing pieces of the inoculum on the pod surfaces, covering them with clear adhesive tape, and incubating them in the incubator (Memmert INE-40 Richmond Scientific) at 28°C for up to 10 days without light exposure. The progression of symptoms (lesion area) was observed daily. Parameters assessed in the virulence test included the latent period (days), lesion area (cm²), disease severity, and infection rate. The lesion area on the pod surface was calculated using modified transparent centimeter block. The scoring value of each category is based on the lesion area on cocoa pods surface (Score 0: No spots; Score 1: 0<X20; Score 2: 20<X<40; Score 3: 40<X<60; Score 4: 60<X<80; and Score 5: X>80). The disease intensity was calculated using the formula as follows:

$$\text{Disease severity} = \sum \frac{(\text{ni x vi})}{\text{N x V}} \times 100\%$$

Where: n: number of infected plants having the same score, v: severity score, Z: maximum rating scale number, N: total number of plants observed

The determination of the infection rate was calculated based on disease progression for polycyclic disease using the following formula:

$$r = \frac{2{,}3}{t}\left(Log\frac{X1}{1-X1} - Log\frac{X0}{1-X0}\right) Unit.Time^{-1}$$

Where: t: observation time interval, XO: pod rot disease severity value at the first observation, X1: pod rot disease severity value

The virulence level of *P. palmivora* isolates was determined using data on latency period, disease severity, and infection rate as shown in Table 1.

Principal Component Analysis (PCA) analysis

Principal Component Analysis (PCA) was conducted to reduce data complexity and highlight key variations to identify patterns and relationships between isolates. This analysis also helps visualize the main differences within the dataset, offering insights into the phenotypic variation among *P. palmivora* isolates. The variables analyzed included papilla length, pedicle length, sporangia length (L) (max; min; mean) and breadth (B) (max; min; mean), the L/B ratio, and chlamydospore diameter (max; min; mean), the L/B ratio, and chlamydospore diameter (maximum, minimum, mean).

Before the analysis, the data were standardized using the scale function in RStudio, which scales each variable to have a mean of 0 and a standard deviation of 1 to ensure that all variables are on the same scale and comparable. The PCA was conducted using RStudio version 4.0.5 (RStudio Team 2024), employing several R packages, including ggplot2 for visualization, stats for core PCA functions, proxy for calculating distance matrices, and cluster for clustering the PCA results. The principal components were extracted to summarize the variation in the dataset, and their contributions were examined.

RESULTS AND DISCUSSION

The general condition of the cocoa plantations

In this study, we examined the general condition at the sampling locations to understand the ecological state of the cocoa plantations. General information about the plantations is presented in Figure 1 and Table 2.

Field observations revealed that all the cocoa plantations are intercropped with various plants, including coconut, oil palm, banana, papaya, gamal (*G. sepium)*, and lamtoro (*L. leucocephala).* Each plantation uses coconut as a shade crop, and the Mangkutana cocoa plantation is adjacent to an oil palm plantation. Field sanitation is minimal or nonexistent, with plant residue and pod debris present in and around the plantation. According to interviews with local growers, no disease control measures using agrochemicals are implemented in any of the plantations.

Morphological variation

The number of sampling points in this study is 15, and we successfully recovered 15 *P. palmivora* isolates from all collected samples. The morphological characteristics observed included macroscopic features (colony pattern) and microscopic structures (sporangia, hyphal swelling, chlamydospores, and gametangia). The colony patterns of all collected isolates and the forms of the microscopic structures are shown in Figures 2 and 3, respectively.

Table 1. Criteria for *Phytophthora palmivora* virulence on cocoa pods

Score	a Latent period (day)	b Disease severity (cm^2)	c Infection rate (unit/time)	Virulence level
0	X>7	0	r= 0	Avirulent
1	5<X≤7	0<X≤40	0<r≤0,2	Low virulence
2	3<X≤5	40<X≤ 80	0,2<r≤0,4	Medium virulence
3	1<X≤3	>80	r>0,4	High virulence

Notes: Virulence score = a+b+c/3

Table 2. The general condition of the cocoa plantations in East Luwu, South Sulawesi, Indonesia

Observed parameter	Sampling location		
	Mangkutana	Kalaena	Angkona
Isolates	MKTN1, MKTN2, MKTN3, MKTN4, MKTN5	KLN1, KLN2, KLN3, KLN4, KLN5	AKN1, AKN2, AKN3, AKN4, AKN5
Location coordinate	Kasintuwu (-2.432, 120.807)	Kalaena Kiri (-2.461137,120.865421)	Taripa (-2.432462, 120.888845)
Altitude	115.6 m asl	72.8 m asl	101.6 m asl
Cocoa cultivar	Clone 45	Clone 45	Clone 45
Intercropping plants	Coconut, palm oil, papaya	Coconut, banana, *gamal* (*Gliricidia sepium* (Jacq.) Kunth)	Coconut, *lamtoro* (*Leucaena leucocephala* (Lam.) de Wit)
Sanitation	Occasional pruning and no field sanitation	Occasional pruning, field sanitation	Occasional pruning, no field sanitation, abundant plant residue
Fungicide application	None	None	None

Figure 1. Cocoa plantation in East Luwu. A. Mangkutana; B. Kalaena; C. Angkona

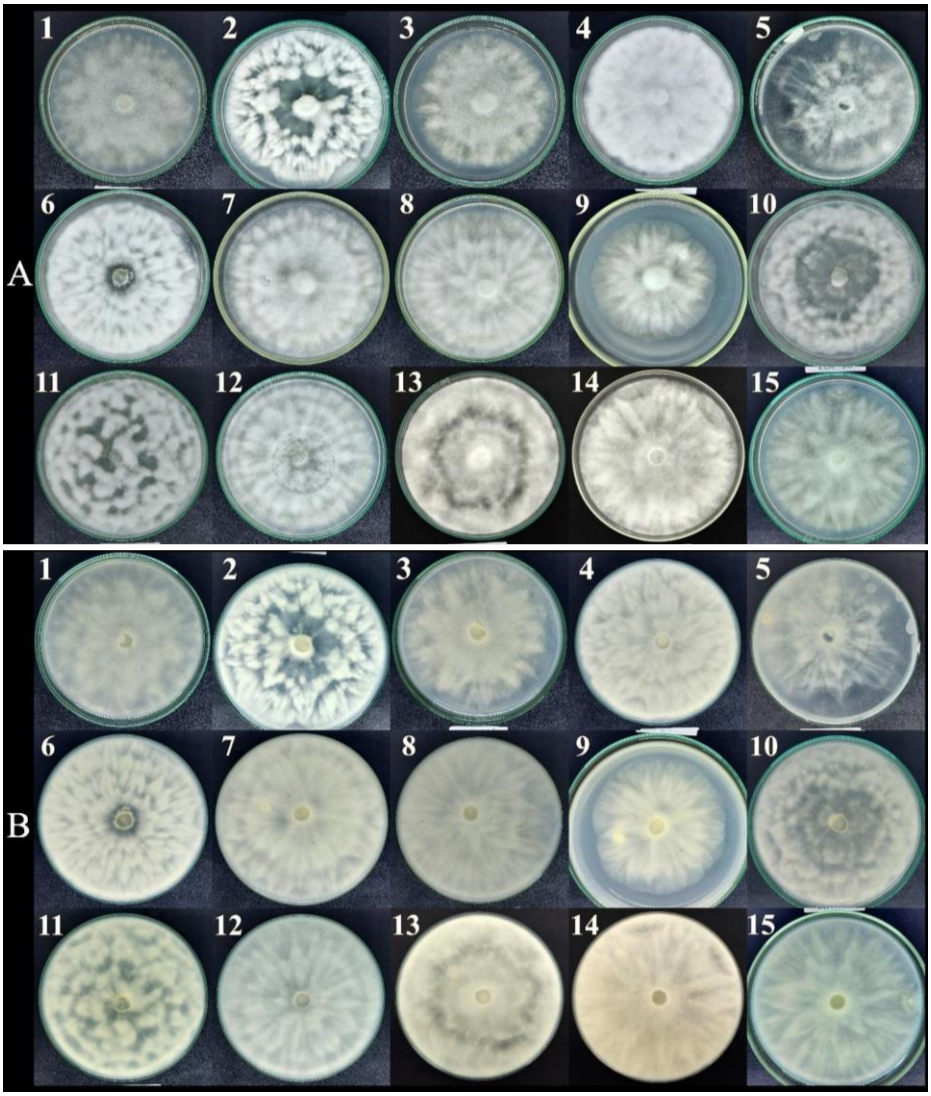

Figure 2. The colony patterns of *Phytophthora palmivora* isolates cultured from soil sample. A. Aerial view; B. reverse view; 1. MKTN1; 2. MKTN2; 3. MKTN3; 4. MKTN4, 5. MKTN5; 6. KLN1; 7. KLN2; 8. KLN3; 9. KLN4; 10. KLN5; 11. AKN1; 12. AKN2; 13. AKN3; 14. AKN4; 15. AKN5

The collection of *P. palmivora* isolates exhibited various colony patterns, including stellate, petaloid, rosette, stellate-striated, and no pattern, with aerial or cottony mycelia on V8 media. The mycelia are aseptate and hyaline. The observed asexual structures included chlamydospores and sporangia (papillate, semi-papillate, and non-papillate). All chlamydospores had a globose shape, while sporangia varied from ovoid to pyriform to globose. The chlamydospores were predominantly intercalary or terminal, located at the tips of mycelia. Hyphal swellings were also present in all isolates, mostly in irregular collaroid or torulose forms, with looped hyphal swelling observed in only one isolate (KLN 4). Interestingly, no gametangia were found in any of the isolates. The characteristics of the macroscopic and microscopic morphology of all collected isolates are presented in Table 3.

Table 3. Morphological characteristic of 15 *Phytophthora palmivora* isolates from East Luwu on V8 agar media at 14 days post-isolation

Isolates	Colony	Hyphal swelling	Sporangia		Chlamydospore		Gametangia
			Form	Type	Position	Abundance	
MKTN1	Petalloid	Torulose	Pyriform, ovoid	NP	I	Few	None
MKTN2	Cottony, petaloid	Torulose	Ovoid	P	I, T	Few	None
MKTN3	Stellate-striated	Irregular collaroid	Ovoid, pyriform	P	I	Abundant	None
MKTN4	Cottony, stellate	Irregular collaroid	Ovoid, pyriform	P	I	Abundant	None
MKTN5	Stellate	Irregular collaroid	Ovoid, globose	NP	I, T	Abundant	None
KLN1	Cottony, stellate	Torulose	Ovoid, globose	P	I, T	Abundant	None
KLN2	Cottony, stellate-striated	Irregular collaroid	Ovoid	P	I, T	Abundant	None
KLN3	Stellate	Irregular collaroid	Ovoid	P	I, T	Abundant	None
KLN4	Stellate	Loops	Ovoid, pyriform	P	I	Abundant	None
KLN5	Rossete	Irregular collaroid	Ovoid, pyriform	SP	I	Abundant	None
AKN1	Rossete	Irregular collaroid	Ovoid, globose	P	I	Abundant	None
AKN2	Stellate	Irregular collaroid	Pyriform	NP	I	Few	None
AKN3	No pattern	Irregular collaroid	Ovoid, globose, pyriform	P	I, T	Abundant	None
AKN4	Stellate	Torulose	Ovoid	P	I, T	Few	None
AKN5	Stellate	Torulose	Ovoid	P	I, T	Abundant	None

Notes: P: Papillate; SP: Semipapillate; NP: Nonpapillate; I: Intercalar; T: Terminal

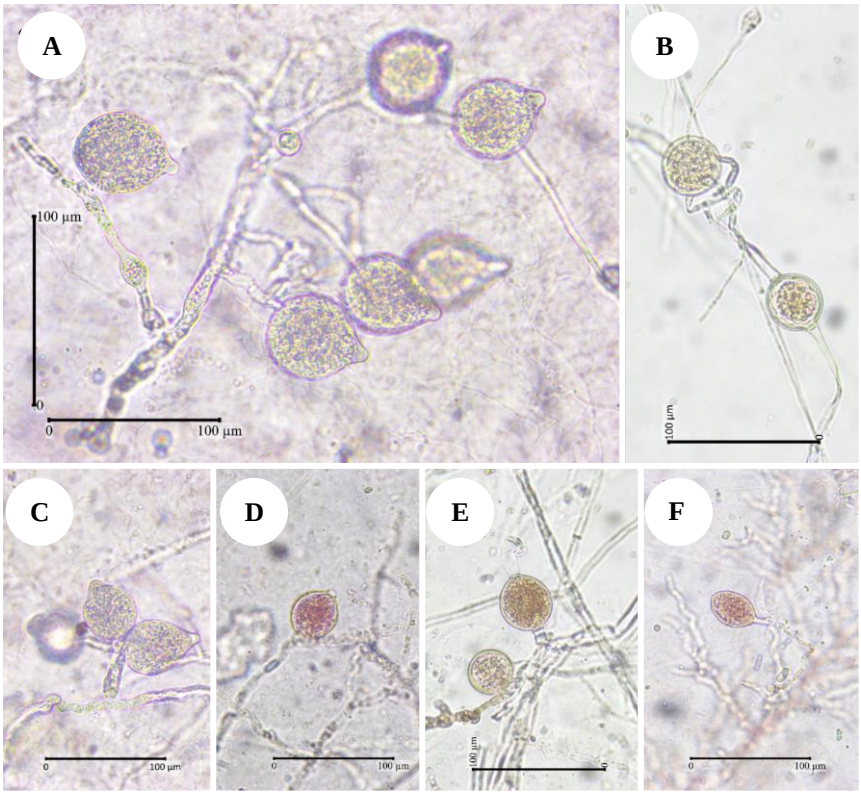

Figure 3. A. Sympodia and sporangia cluster; B. Intercalary chlamydospore; C. Pyriform papillate sporangia; D. Ovoid semipapillate sporangia; E-F. Nonpapillate sporangia. Scale bar: 100 µm

In addition to morphological observations, we also documented quantitative morphometric data, including the average papilla length, average pedicle length, sporangia length (maximum, minimum, and mean), sporangia breadth (maximum, minimum, and mean), L/B ratio, and chlamydospore diameter (Table 4). The range for papilla length is 3.65-8.31 µm, and for pedicle length, it is 3.76-13.07 µm. The mean sporangia length ranges from 41.55-60.90 µm, while the breadth ranges from 31.97-44.42 µm. The L/B ratio varies from 1.17 to 1.37, and the mean chlamydospore diameter ranges from 38.66 to 50.52 µm. The measurements are presented in Table 3. The morphometric data were then analyzed using principal component analysis, with the results visualized in a PCA scatter plot (Figure 4).

The PCA analysis of the morphometric data, illustrated in Figure 4, revealed three clusters. Interestingly, the isolates from Mangkutana were grouped into the same cluster (Cluster 3). Meanwhile, the isolates from Kalaena and Angkona were distributed across Clusters 1 and 2. The first principal component (PC1) accounts for 44.8% of the variation, while the second (PC2) explains 20.4%, resulting in a combined total of 65.2% of the variation depicted in the graph.

The virulence level of the isolates recovered from soil samples was evaluated based on disease severity, latent period, and infection rate. Disease severity ranged from 0-80%, the average latent period ranged from 2.7-12 days, and the infection rate ranged from 0.00-0.69 units per day. The virulence assessment showed that one isolate was categorized as avirulent, seven had low virulence, and seven had medium virulence (Table 5).

Table 4. Morphometric variation of 15 *Phytophthora palmivora* isolates from East Luwu, South Sulawesi, Indonesia

Isolates	Papilla length (µm)	Pedicle length (µm)	Sporangia						LB ratio	Chlamydospore diameter (µm)		
			Length/L (µm)			Breadth/B (µm)						
			Max	Min	Mean	Max	Min	Mean		Max	Min	Mean
MKTN1	NP	5.06	48.10	38.85	42.98	37.11	33.85	35.66	1.21	51.71	39.21	43.84
MKTN2	6.38	7.13	45.32	38.98	41.55	35.29	27.55	31.97	1.30	51.59	36.82	44.49
MKTN3	5.96	11.11	52.43	40.15	44.44	37.99	31.30	33.76	1.32	48.39	38.40	42.50
MKTN4	6.02	8.46	53.34	40.94	45.91	42.66	32.94	37.81	1.21	42.28	36.39	40.43
MKTN5	NP	4.57	49.24	43.58	46.32	42.34	33.02	38.92	1.19	44.85	36.70	39.71
KLN1	5.87	7.25	57.59	53.60	55.71	47.04	35.49	41.00	1.36	40.80	36.26	38.66
KLN2	7.78	8.83	54.08	49.33	51.89	44.50	40.84	42.52	1.22	45.75	35.88	42.17
KLN3	7.93	7.21	57.98	53.81	55.22	47.33	35.26	43.10	1.28	42.66	35.51	38.86
KLN4	7.25	3.86	57.57	40.37	49.80	42.76	34.99	40.18	1.24	51.10	42.12	47.64
KLN5	3.65	7.98	50.53	42.98	45.95	46.23	33.73	39.32	1.17	52.30	45.05	49.69
AKN1	7.34	3.76	53.54	37.13	46.22	46.97	32.32	38.87	1.19	53.85	43.21	50.52
AKN2	NP	6.22	75.04	50.01	60.90	63.33	36.28	44.41	1.37	45.86	36.99	41.88
AKN3	8.31	13.07	55.11	50.34	51.85	44.07	41.25	42.09	1.23	49.77	42.13	45.20
AKN4	6.46	8.12	54.87	50.91	53.23	46.56	33.53	40.83	1.30	48.76	39.46	42.97
AKN5	6.28	6.05	57.98	45.14	50.96	41.43	35.29	38.16	1.34	48.86	39.64	43.84

Notes: NP: Non papillate

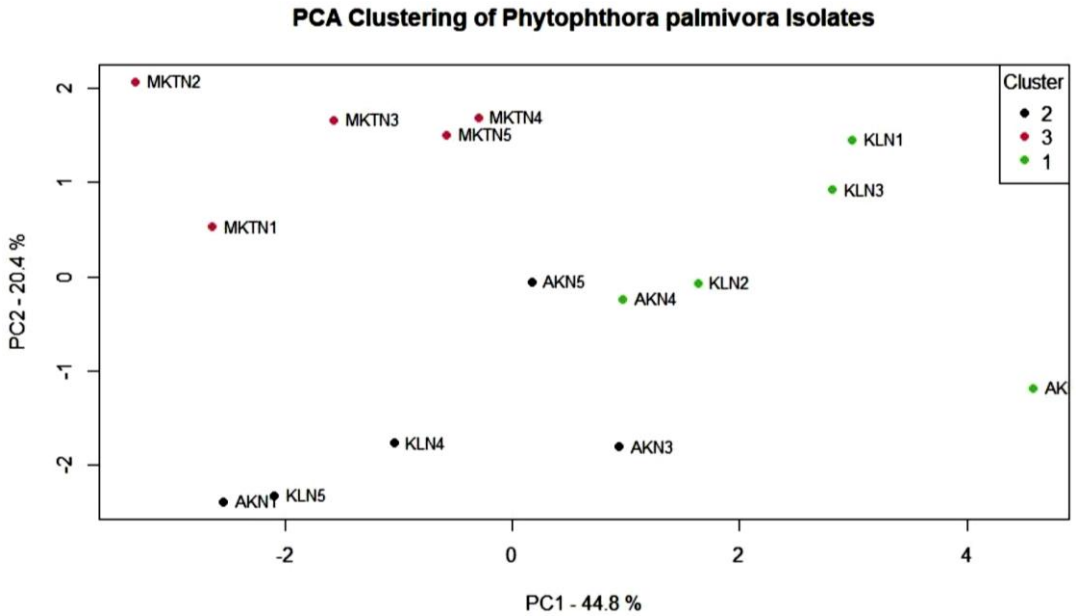

Figure 4. Principal component analysis on 12 morphometric variable of *Phytophthora palmivora* isolates from East Luwu

Table 5. Disease severity, latent period, infection rate, and virulence level of 15 *Phytophthora palmivora* isolates inoculated on detached cocoa pod

Isolate code	Disease severity (%)	Latent period (day)	Infection rate (UnitDay-1)	Virulence category
MKTN1	53	3.7	0.38	Medium virulence
MKTN2	80	3.7	0.69	Medium virulence
MKTN3	47	6.0	0.43	Medium virulence
MKTN4	27	4.5	0.41	Medium virulence
MKTN5	10	3.5	0.11	Low virulence
KLN1	20	3.5	0.12	Low virulence
KLN2	0	12.0	0.00	Avirulent
KLN3	20	4.0	0.12	Low virulence
KLN4	40	2.7	0.37	Medium virulence
KLN5	27	6.0	0.21	Low virulence
AKN1	7	7.0	0.49	Medium virulence
AKN2	47	3.0	0.22	Low virulence
AKN3	40	4.0	0.37	Low virulence
AKN4	27	3.7	0.09	Low virulence
AKN5	27	5.0	0.41	Medium virulence

Discussion

The soilborne nature of *P. palmivora* allows it to persist in soil for extended periods, serving as a source of inoculum that leads to a continuous cycle of infection. In this study, we evaluated soil samples from three cocoa plantations in East Luwu to understand the prevalence, virulence, and morphometric variation of *P. palmivora* recovered from the soil. We used the apple baiting method (Drenth and Sendall 2001) to detect the presence of *P. palmivora* and successfully isolated it from all soil samples, confirming its 100% prevalence. This finding aligns with Purwantara (2008) study, which identified soil as a significant and consistent source of *P. palmivora* inoculum in cocoa plantations throughout the year. Another study by Salamat et al. (2021) used flower baiting to identify the potential sources of *P. palmivora* from jackfruit seedlings nurseries; the result shows that *P. palmivora* was detected in soil from germination bed, potting mix, irrigation water, and roots of the seedlings. Our results also demonstrate that soil baiting is highly effective for detecting and isolating soilborne pathogens. The baiting conditions favor parasitic species that sporulate quickly, infect various bait species, achieve infection with a small number of zoospores, and grow rapidly on selective agar (Sarker et al. 2023).

A total of 15 representative isolates from each sampling site were further examined for morphological variation (Tables 3 and 4). The isolates exhibited a range of colony patterns, including stellate, stellate-striated, petaloid, rosette, and no distinc. The colony pattern of *Phytophthora* species typically varies significantly depending on the culture media and the specific isolates (Jadesha et al. 2022). Asexual structures observed included papillate, semi-papillate, and non-papillate sporangia, which displayed various shapes such as ovoid, globose, and pyriform. In most *Phytophthora* species, an apical papilla forms as the sporangia mature. During sporulation, the dissolution of this papilla provides an escape route (operculum) for the wall-less zoospores, which are expelled from the sporangium by turgor pressure. This process requires the sporangia to be immersed in liquid (Judelson and Blanco 2005; Perrine-Walker 2022). Therefore, Phytophthora species with papillate sporangia, like *P. palmivora*, are typically adapted to humid environments and tend to infect plants through rain splash or water transmission, meanwhile, non-papillate sporangia release zoospores in a more passive manner, possibly through the breakdown of the sporangium wall.

In this study, we also observed intercalary and terminal globose chlamydospores, as well as hyphal swelling, which was either a torulose or irregular collaroid in shape. Chlamydospores and hyphal swelling are produced by some *Phytophthora* species and can be useful structures for species identification (Abad et al. 2023a). Chlamydospores are formed from existing hyphal cell or conidia that develops a thickened wall and cytoplasm packed with lipid reserves. These thickened cell walls may be pigmented or hyaline and chlamydospores can appear as terminal, intercalary, globose, sub-globose, lobate, radiated, irregular, catenulated, or clustered. Hyphal swelling is a thin-walled structure showing various shapes and delimited by hyphae (Abad et al. 2023b). Detailed recognition of asexual and sexual structures is very important for correct understanding of the whole infectious process and disease development. Chlamydospores are resistant structures produced in nature under water or soil conditions that allow *Phytophthora* species to enter a reversible state of low metabolic activity under unfavorable environmental conditions. This structure promotes long-term persistence within the soil and assists long-distance dispersal facilitated by abiotic factors (Custer et al. 2022).

Overall, the colony patterns and asexual features found in this study align with previous *P. palmivora* research (Santoso et al 2015; Masanto et al. 2019; Kuswinanti et al. 2023). Isolates from Java, observed by Masanto et al. (2019), showed similar characteristics, producing papillate sporangia with varied shapes, including distorted, ellipsoid, globose, obpyriform, and ovoid, as well as terminal and

intercalary spherical chlamydospores. In another study, Motulo et al. (2007) identified *P. palmivora* colonies with rosaceous, stellate, and cottony patterns in 22 isolates across Indonesia. Their sporangia displayed a range of forms, including ovoid, limoniform, obturbinate, and obpyriform. A recent study by Kuswinanti et al. (2023) also reported that *P. palmivora* from South Sulawesi exhibited collaroid, torulose, loops, and irregular hyphal swelling with simple sympodia. These characteristics are shared by many other *Phytophthora* species, as *Phytophthora* is considered a biologically and structurally cohesive genus (Brasier et al. 2022). Regarding sexual structures, no gametangia were observed in any of the collected isolates in vitro. A similar observation was observed in *P. palmivora* isolates from three locations in South Sulawesi (Luwu, Gowa, and Pinrang) (Kuswinanti et al. 2023). This suggests that *P. palmivora* in South Sulawesi is heterothallic (self-sterile), with a predominant mating type in the population. Moreover, the mating type test conducted by Masanto et al. (2019) confirmed that all *P. palmivora* samples from Sulawesi were of the A2 mating type. Further research involving large-scale mating tests is needed to verify this result.

The quantitative morphometric data collected in this study, including papilla length (3.65-8.31 µm), pedicle length (3.76-13.07 µm), sporangia length (41.55-60.90 µm) and breadth (31.97-44.42 µm), L/B ratio (1.17-1.37), and chlamydospore diameter (38.66-50.52 µm), align with previous studies (Appiah et al. 2003; Hung et al. 2015; Masanto et al. 2019; Barboza et al. 2020; Rodríguez-Polanco et al. 2020; Wartono and Taufiq 2021). Principal component analysis (PCA) was conducted on 12 morphometric variables to identify grouping patterns among the isolates. The PCA yielded 12 components corresponding to the 12 morphometric variables. Projecting the data onto the first two principal axes, which accounted for 65.2% of the total variance (PC1 = 44.8%, PC2 = 20.4%), revealed three clusters. Isolates from Mangkutana are grouped exclusively into Cluster 3, while isolates from Kalaena and Angkona are scattered across Clusters 1 and 2. The distinct separation of *P. palmivora* isolates into three clusters suggests significant variation among the isolates, likely due to genetic diversity. The clustering of isolates from Mangkutana into a single group suggests that these isolates share specific characteristics, likely due to common environmental factors, agricultural practices, or common genetic origin potentially introduced throught a shared source of inoculum. The cocoa plantation in Mangkutana is intercropped with coconut and is adjacent to a palm oil plantation (Figure 1). Both coconut and oil palm are known to be susceptible hosts for *P. palmivora*, which causes significant diseases such as bud rot in these crops (Torres et al. 2016; Azni et al. 2019; Maizatul-Suriza et al. 2019; Palliyath et al. 2021). This proximity may influence the pathogen population dynamics, potentially leading to a more homogeneous population of *P. palmivora* in this area. These clustering results highlight the need for further research to understand the genetic or environmental factors that contribute to these observations.

In this study, the virulence of *P. palmivora* was assessed based on disease severity on detached cocoa pods, latent period, and infection rate. Among the isolates tested, one was avirulent, while the remaining isolates exhibited virulence (Table 5). The virulent isolates produced necrotic lesions on cocoa pods (Clone 45), with the initial symptoms appearing within 2 to 5 days post-inoculation. The first symptom of pod rot disease was the development of brownish spots on the pod surface, which rapidly expanded to cover the entire pod. As the disease progressed, the pathogen infected deeper pod tissues, eventually reaching the beans and causing them to rot (Guest 2007; Vanegtern et al. 2015). The average latent period of the isolates ranges from 2 to 12 days. Isolates from Mangkutana show the fastest average latent period, and most of them have medium virulence. The hemibiotrophic lifestyle of *P. palmivora* makes it particularly aggressive towards cocoa. To complete its lifecycle, the pathogen switches from a biotrophic to a necrotrophic phase. This shift is marked by the presence of necrotic plant tissues, extensive hyphal growth, and prolific formation of sporangia and chlamydospores within infected tissues (Carella et al. 2018; Perrine-Walker 2020).

The results of this study highlight that soil serves as a consistent source of inoculum in cocoa plantations. Therefore, disease management practices should focus on reducing soil-borne inoculum. In Indonesia, approximately 99.63% of cocoa plantations are managed by smallholder growers, while the remainder are managed by government and private estates (Statistic Indonesia 2023). Disease control mostly depends on basic practices like pruning and minimal sanitation. Our field observations in three cocoa plantations in East Luwu District, South Sulawesi, Indonesia showed minimal or no active disease management. While local growers occasionally prune trees, field sanitation is often neglected. Crop residues, including fallen leaves and empty pod cases, are frequently left piled around trees. These residues can harbor *P. palmivora* inoculum, facilitating its persistence in the soil and enhancing its transmission. Improving field sanitation is essential to reduce the pathogen's inoculum. Regularly collecting crop residues and composting them can accelerate residue breakdown and prevent the pathogen from persisting in the soil (Mihajlović et al. 2017; Panth et al. 2019). Regular pruning is also necessary to thin the canopy and increase light penetration, which can reduce favorable conditions for the pathogen. In addition, the application of biocontrol agents to the soil is an alternative to suppress soilborne pathogens through parasitism, antibiosis, competition for the host and nutrients, and induction of resistance. Several microbes that can be employed as biocontrol agents include *Trichoderma* spp., *Chaetomium* spp., *Bacillus* sp., and many more (Hung et al. 2015; Sriwati et al 2019; Geroche et al. 2024).

The findings of this study confirm that *P. palmivora* is prevalent in all soil samples, emphasizing that soil acts as the main and sustainable source of inoculum. The lack of adequate disease management practices, particularly poor field sanitation, further supports the persistence of inoculum in these cocoa plantations. Implementing proper sanitation practices is essential to reduce inoculum sources and should be a key component of integrated disease management strategies. The presence of resilient resting

structures, likely chlamydospores, highlights the pathogen's ability to survive in the soil over extended periods. Virulence testing revealed that 93.3% of isolates recovered from soil inoculum were pathogenic, with varying levels of virulence based on latent period, infection rate, and disease severity. Furthermore, Principal Component Analysis (PCA) of morphometric data indicated genetic diversity among the isolates and suggested geographical associations, particularly within the Mangkutana population. These findings underscore the importance of understanding pathogen diversity and its ecological and geographical distribution to develop effective management strategies for controlling pod rot disease in cocoa plantations.

ACKNOWLEDGEMENTS

The authors would like to thank the Faculty of Agriculture Universitas Hasanuddin, Makasar, Indonesia, for facilitating the financial support through the Junior Lecturer Research Grant (*Hibah Penelitian Dosen Muda Internal*) in 2022 (No: 7785/UN4.10/PT.01.02/2022). The authors would like to thank Sriwan for helping with sampling collection and field observation.

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