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MORPHOLOGY OF Phytophthora SP. CAUSING STEM CANKER IN NUTMEG AND THE ROLE OF ACTIVATED CHARCOAL AS A BIOLOGICAL CONTROL AGENT

MORFOLOGI *Phytophthora* sp. PENYEBAB KANKER BATANG TANAMAN PALA DAN PERAN ARANG AKTIF SEBAGAI AGENS PENGENDALI HAYATI

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ABSTRACT

Nutmeg (Myristica fragrans Houtt.) is a key Indonesian spice with high export value. However, in recent years, its productivity has decreased significantly due to the outbreak of stem cancer. This study aims to characterize the morphology of Phytophthora sp., the cause of stem cancer in nutmeg plants, and evaluate the effectiveness of activated charcoal from three types of raw materials: coconut shell, pine wood, and walnut shells in suppressing the development of the disease. The research was carried out through field surveys in three nutmeg centre villages on Ambon Island, namely Hatu, Seith, and Toisapu, laboratory observations at the BBP2TP Ambon Mycology Laboratory, and the Clinical and Biotechnology Laboratory of the Faculty of Agriculture UGM. Effectiveness testing was carried out in situ and in vitro. Morphological identification includes observation of sporangium, chlamydospora, and sporangiophore branching using a microscope. The results of the study show that coconut shell charcoal is more effective than pine and walnut charcoal. Phytophthora sp. found in nutmeg displays varying morphological characteristics and differs from previous species: Sporangia average size 17.9 × 11.42 µm, caducous, with simple simpodial branched sporangiophores, so it is suspected to be a new subspecies. Among the three activated charcoals tested, coconut shell proved most effective in reducing stem canker damage. The results may support the development of sustainable nutmeg stem canker control methods.

KATA KUNCI:
Arang aktif, ramah lingkungan, tanaman pala

ABSTRAK

Tanaman pala (*Myristica fragrans* Houtt.) merupakan salah satu komoditas rempah unggulan Indonesia yang memiliki nilai ekonomi dan ekspor yang sangat tinggi. Namun, dalam beberapa tahun terakhir, produktivitasnya mengalami penurunan signifikan akibat merebaknya penyakit kanker batang. Penelitian ini bertujuan untuk mengkarakterisasi morfologi Phytophthora sp. penyebab kanker batang pada tanaman pala dan mengevaluasi efektivitas arang aktif dari tiga jenis bahan baku tempurung kelapa, kayu pinus, dan tempurung kenari dalam menekan perkembangan penyakit tersebut. Penelitian dilaksanakan melalui survei lapangan di tiga desa sentra pala di Pulau Ambon, yaitu Hatu, Seith, dan Toisapu. Pengamatan laboratorium di Laboratorium Mikologi BBP2TP Ambon serta Laboratorium Klinik dan Bioteknologi Fakultas Pertanian UGM. Pengujian efektivitas dilakukan secara in situ dan in vitro. Identifikasi morfologi mencakup pengamatan sporangium, klamidospora, dan percabangan sporangiofor menggunakan mikroskop. Hasil penelitian menunjukkan bahwa arang tempurung kelapa lebih efektif jika dibandingkan dengan arang kayu pinus dan kenari. Hasil penelitian menunjukkan bahwa Phytophthora sp. yang ditemukan pada tanaman pala memiliki karakteristik morfologi yang berbeda dari spesies sporangia yang telah dikenal sebelumnya, berukuran rata-rata 17,9 × 11,42 µm, bersifat caducous, dengan sporangiofor bercabang simple simpodial sehingga diduga merupakan subspesies baru. Dari ketiga jenis arang aktif yang diuji, arang tempurung kelapa terbukti paling efektif dalam menekan intensitas kerusakan akibat kanker batang. Diharapkan temuan ini memberikan kontribusi nyata bagi pengembangan strategi pengendalian penyakit kanker batang pala yang ramah lingkungan.

1. INTRODUCTION

Nutmeg (*Myristica fragrans* Houtt./M. fragrans) is a strategic Indonesian spice commodity that significantly contributes to domestic trade and global exports, with Maluku recognized as a major production center. (Dumatubun *et al*., 2020;Woriwun *et al*., 2021). However, in the last decade, nutmeg productivity has declined sharply due to the increasing prevalence of stem canker, especially on the island of Ambon (Pesireron *et al*., 2019). The disease manifests as exudate discharge, necrotic tissue, and fatal attacks in severe cases (Sumbula *et al*., 2016; Febbiyanti *et al*., 2019).

Stem canker in nutmeg is linked to soil-borne *Fusarium* and *Phytophthora* infections. (Halimathussadiah *et al*., 2021). Pathogens commonly responsible for fruit rot (Defitri, 2017) can also infect stems and branches, inducing canker symptoms. (Mariadi *et al*., 2018). Conversely, canker-infected stems may act as inoculum sources for fruit rot (Fawke *et al*., 2015). A similar phenomenon has been widely reported in cocoa, where *Phytophthora palmivora* infestation causes the stems and branches to darken in colour, secrete reddish exudate, and leave a rust-like crust after drying (Motulo *et al*., 2020). Early symptoms are difficult to detect because they are covered by outer tissue or moss, but peeling the bark of the stem reveals reddish-brown inner tissue. These infections progress rapidly and severely damage productive stem tissue (Anandaraj *et al*., 2020;de Oliveira *et al*., 2023). Beyond nutmeg, stem canker impacts forestry, horticultural, and plantation crops (Hansen, 2015). The symptoms include discolouration of the bark of the stem, sap discharge, drying of tissues down to the xylem, and impaired flower and fruit production, which are generally caused by *Phytophthora* spp (Sumbula, Mathew, & Mini, 2016;(Cruz *et al*., 2024). Different *Phytophthora* species have distinct host ranges; for instance, *P. cinnamomi* infects *Pieris japonica*, whereas *P. infestans* is the primary pathogen of potatoes and tomatoes**.** In Indonesia, *P. capsici* is a serious threat to pepper cultivation (*Piper nigrum* L) (Rohmah *et al*., 2019;Chen *et al*., 2023). Meanwhile, *P. cactorum* is recognized as a major pathogen causing bleeding cankers, fruit rot, root rot, and dieback in diverse agricultural, horticultural, and forestry crops. First reported on maple trees in New Jersey in 1940, *P. cactorum* (Brown *et al*., 2021).

Unlike true fungi with chitin cell walls*, Phytophthora spp.* have *β-glucan*-rich walls and are major plant pathogens. The pathogen can survive for a long time through sexual oospores and vegetative chlamydospores, and it spreads through soil; zoospores infecting the roots and base of the stem in wet conditions; and air through wind- or rain-carried sporangia (McCarren *et al*., 2005). Disease development is enhanced by humid, poorly drained soils, with *P. citrophthora* implicated in orange gummosis (Rodrigues da Silva *et al*., 2021;Van Tran *et al*., 2023)

Beyond biological control (Singh & Faull, 2020), integrated management (El Khoury & Makkouk, 2010), solarisation (Hamdani & Susanto, 2020), and plant extracts (Panth *et al*., 2020) provide eco-friendly strategies against plant diseases. Despite various efforts, control of nutmeg stem canker by *Phytophthora* sp. remains inadequate and ecologically unfriendly (Febbiyanti *et al*., 2019). Activated charcoal from coconut shells, walnuts, and pine wood enhances soil properties, detoxifies compounds (Ardiwinata, 2020), and reduces soil-borne pathogens (Arshad *et al*., 2023). Although raw material properties affect charcoal performance, research on activated charcoal for nutmeg disease control in Ambon Island is still minimal. This study is crucial for developing an environmentally friendly control strategy for nutmeg stem canker applicable to farmers. It aims to characterize the morphology of *Phytophthora* sp. from nutmeg and to evaluate the effectiveness of activated charcoal derived from coconut, cypress, and walnut shells in inhibiting disease development.

2. MATERIALS AND METHODS

2.1 Field Survey and Sampling of Disease Plants

This research was carried out in three locations of smallholder nutmeg plantations on Ambon Island (Hatu, Seith, and Toisapu Villages) (Figure 1), and laboratory analysis was carried out at the BBP2TP Ambon Mycology Laboratory and the Clinical and Biotechnology Laboratory of the Faculty of Agriculture UGM.

2.2 Materials and Tools

The materials and tools used in the study consisted of three parts, namely: (a) Field survey: small shovels/hoes, knives/machetes, dissecting sets, petri dishes, water agar medium, parafilm, collection bottles, silica gel, sterile water, alcohol, portable cooling boxes, transparent plastic bags (ziplock), label paper, thermohygrometer, GPS, tissue paper, spray paint, cameras, binoculars, pruning scissors, and disinfectant wet wipes. (b) Laboratory observations: test tubes, Erlenmeyer, shakers, Petri dish, cutters, tissue paper, spertus, oose needles, sterile water, laminar flow, incubators, PDA, V8 Juice agar, apples, 70% alcohol, NaCl, shears mounting, methylene blue, immersion oil, and sick plant samples. (c) Activated charcoal effectiveness testing: charcoal from (coconut shells, firs, and walnut shells), aquaades, small baking sheets, cloth meters, iron brushes, sandpaper, scales, mortars, buckets, baking sheets, dissecting sets, hoes, machetes, grass forks, wire brushes, drums, shovels, wooden lace, water, gloves, masks, filters/1 mm mesh, microscopes, object glass, and cover glass.

2.3 Research Methods

2.3.1 Sampling of Disease Plant

Infected tissues were randomly sampled according to stem canker severity, representing 5% of the total nutmeg population. All equipment is sterilized using 70% ethanol before and after pickup. Samples are taken at the boundary between healthy and diseased tissue, then prepared in pairs, with one spare sample as a reference. Next, the sample is placed in a tightly sealed plastic bag and given a dry tissue or drying paper to absorb excess moisture. Samples were taken from plants with stem canker damage of differing severity, from mild to severe, in the field.

Figure 1. Map of the research location (✹) Hatu village (3°43'30.93"S 128°03'01.22"E), Seith (3°36'00.90"S 128°01'26.08"E), dan Toisapu (3°38'55.66"S 128°16'38.69"E).

2.3.2 Pathogen Isolation

Samples of infected stems from the site were cut into 5–10 mm, sterilized with alcohol, rinsed with water, dried, and planted in prepared Potato Dextrose Agar (PDA) and V8 Juice agar media. Soil-borne pathogens were isolated using a sequential dilution method up to 10^{-5}. From the final dilution, 1 ml of suspension was plated onto Potato Dextrose Agar (PDA) and V8 Juice agar, then incubated at 25–27 °C for 5–7 days. The baiting method was applied by injecting 10^{-1} and 10^{-2} dilution suspensions into sterile apples or by placing sterile apple pieces on nutmeg root soil in Petri dishes, followed by incubation for 5–7 days. Fungi growing on symptomatic apple tissue were transferred to PDA medium, cultured, and purified until a pure isolate was obtained (Dhingra & Sinclair, 1995). Sporangia formation was induced by incubating the cultures under alternating 12-hour light and dark cycles at 25–30 °C.

2.3.3 Sporangium/Spore Production

Single-spore isolation of *Phytophthora* sp. was performed by inducing sporangium formation on V8 juice agar, transferring sporangia to sterile water or saline to release zoospores, and suspending them in sterile water. The suspension was serially diluted to a low concentration, enabling spores to be separated individually**.** Single-spore isolation of *Phytophthora* sp. was conducted using two approaches: (1) streak plate, by spreading diluted spore suspensions on PDA or selective media with sterile needles until individual spores were separated; and (2) spread/pour plate, by distributing diluted suspensions evenly over the medium surface with an L-glass. Colonies derived from single spores were then transferred to slant agar for purification. Post-incubation, single-point colonies from individual spores appeared isolated from others.

2.3.4 Morphology Identification

Identification was based on colony type, hyphal swelling, chlamydospore features, sporangiophore morphology, caducity, and pedicel/papilla length. Microscopic observations employed an Olympus CX21 with ocular and objective micrometers, supported by a Canon EOS 600D camera.

2.4 Observation of Damage Intensity and Effectiveness of Activated Charcoal on Stem Canker Disease

Charcoal from coconut shells, pine wood, and walnuts was finely ground into powder, sieved with a 1-mesh filter, and weighed at 200 g each. Before treatment, the initial level of canker damage in nutmeg plants was assessed. Treatments with coconut shell, walnut, and pine wood charcoal were repeated three times at six-week intervals. Charcoal powder was mixed with sufficient water to form a paste, which was then applied to the stems of nutmeg plants affected by canker. Post-treatment, damage intensity reduction was re-evaluated at three sites. The severity of stem canker was calculated using the formula described by (Natawigena, 1989):

$$DS = \frac{\sum(n.v)}{N.Z} \times 100\% \quad (1)$$

Notes: DS = Disease Severity (%), n = Equal number of scores, v = score, N = number of samples observed, Z = highest score.

2.5 Data Analysis

The observed parameters included: (a) macroscopic pathogen identification colony surface colour, colony base colour, and colony shape, observed 6–10 days after inoculation; (b) microscopic pathogen identification sporangia shape and size, sporangiophore shape and length, sporangia and mycelium colour, chlamydospore production and diameter, sporangiophore branching, caducity, pedicel length, and papilla formation. Microscopic traits were documented immediately and

compared with the literature (Drenth & Guest, 2004); and (c) scoring calculations of stem canker (*Phytophthora* sp.) damage intensity, compiled based on field symptoms (Table 1).

3. RESULTS AND DISCUSSION

3.1 Observation of Disease Symptoms

Diseases cause changes in plant morphology, including form, size, colour, and texture. A single disease may produce multiple symptoms, such as discolouration, concave or protruding lesions, and cracks in branches, stems, or twigs, depending on the host, pathogen, environment, and disease stage. Pathogens are typically present in infected tissues or on tissue surfaces, appearing as fruiting bodies, sclerotia, or other specialized structures. Stem canker in nutmeg manifests as elongated black lesions, mucus discharge, and cracks with brown discolouration spreading downward. Initial nutmeg stem canker signs include dark bark lesions with surrounding discolouration and progressive leaf yellowing and drop. Wounds typically begin as small patches exuding foul-smelling mucus, then expand to penetrate the wood tissue, producing brown discolouration that contrasts with healthy tissue (Figure 2).

Table 1. Nutmeg Stem Canker Scoring (Purnomo, 2006)

Score	Descriptions
0	No. symptoms
1	Mild symptoms, lesions on the stem in the form of small dots, no change in leaf density
2	Moderate symptoms included trunk lesions with fluid exudation
3	Severe symptoms were marked by expanding trunk lesions with foul fluid exudation, tissue deformation, and 10–25% leaf density loss.
4	At very severe stages, the stem became hollow and fragile, with 25–35% leaf density reduction above the wound.
5	Dead Plants

Figure 2. Symptoms of nutmeg stem canker in the villages of Seith (a), Hatu (b), and Toisapu (c) (white arrows).

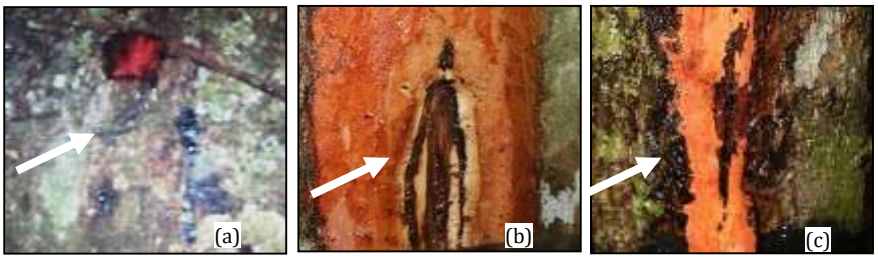


Figure 3. Brown wood tissue indicates stem canker symptoms in nutmeg trunks from Hatu (a), Seith (b), and Toisapu (c), highlighted with white arrows.

Under heavy infection, stems turn porous and break easily, while mould density escalates sharply. This occurs because damaged tissue provides space and nutrients for massive fungal reproduction before the host plant dies completely (Figure 3). Based on field observations, mild symptoms of nutmeg stem canker are characterized by small stem lesions, with leaf density around the infection site remaining unchanged. Moderate symptoms appear as elongated stem wounds with red fluid exudation. Severe symptoms involve wounds that lengthen and widen, releasing foul-smelling fluid when opened; underlying tissues change in shape and colour, and adjacent leaves turn yellow, dry, and fall, with a 10–25% reduction in leaf density. Very severe symptoms result in hollow, porous, and fragile stems, accompanied by a 25–35% decrease in leaf density, visible as reduced leaf volume compared to healthy stems.

At the research site, no nutmeg plants died from stem canker throughout the study. Plant mortality was more often caused by stem borer and termite attacks. This suggests that while stem canker significantly weakens plants, it does not directly cause death but instead predisposes them to secondary pest infestations.

3.2 Pathogen identification

Through repeated purification of field samples, six isolates were obtained from nutmeg stems and root-zone soil (Table 3).

3.2.1 Macroscopic Observations

The macroscopic characteristics of *Phytophthora* sp. observed in the three sample villages are summarized in Table 4, while its growth patterns on culture media are illustrated in Figure 4**.** Observed *Phytophthora* sp. colonies displayed diverse morphologies, from smooth surfaces without patterns to dense, flower-shaped growth. Colony formation of *Phytophthora* sp. isolates varied even within repeated observations of the same isolate number. No isolate exhibited a stable colony pattern; instead, multiple colony forms were often observed within a single isolate**.** Some nutmeg-origin *Phytophthora* colonies exhibited uneven edges, rose-like surfaces, and cloud- or cotton-like textures. Consistent with (Erwin & Ribeiro, 1996), *Phytophthora* sp. colonies generally display uneven white margins and rosaceous, stellate, or cottony types. Abundant mycelium and reproductive structures (sporangia/spores) formed on bark tissue surfaces, enabling pathogenic hyphae to penetrate vascular tissues (phloem and cambium). This process directly correlates with lesion expansion in the wood and increasing disease severity.

Besides *Phytophthora* sp., pathogens such as *Fusarium*, *Colletotrichum*, *Botrytis*, *Monilinia*, *Curvularia*, and *Acremonium* were detected, often in soil beneath infected nutmeg trees and within tissues under moist conditions, aided by mucus exudation.

Table 3. *Phytophthora* sp Isolation Results at All Sample Locations

Isolation Code[1)]	Origin of Isolate (villages)	Materials
SH	Hatu	Soil
TRH	Hatu	Bark trunk
SS	Seith	Soil
TRS	Seith	Bark trunk
Sto	Toisapu	Soil
TRto	Toisapu	Bark trunk

Note: SH (Soil_Hatu); TRH (Tree_Hatu); SS (Soil_Seith); TRS (Tree_Seith); STo (Soil_Toisapu); TRto (Tree_Toisapu).

Table 4. Macroscopic observations of *Phytophthora* of nutmeg origin

Origin of Isolate (Villages)	Isolate Code[a)]	Colony surface color	Base color of the colony	Colony form
Hatu	SH	white	white	Colony edges were jagged and thick, with rough, flower-like surfaces. Other colonies displayed smooth, ray-like patterns.
Hatu	TRH	white	White	Colony edges appeared jagged and thick, with rough, flower-like surfaces, while other colonies exhibited smooth, ray-like patterns.
Seith	SS	white	White	Colony edges were serrated and thick, with rough, flower-like surfaces, while other colonies exhibited smooth, ray-like patterns.
Seith	TRS	white	White	Colony edges were serrated and thick, with rough, flower-like surfaces, while other colonies exhibited smooth, ray-like patterns.
Toisapu	STo	white	White	Colony edges were serrated and thick, with rough, flower-like surfaces, while other colonies exhibited smooth, ray-like patterns.
Toisapu	TRTo	white	White	Colony edges were serrated and thick, with rough, flower-like surfaces, while other colonies exhibited smooth, ray-like patterns

Note: S=Isolate from the soil; TR = nutmeg tree roots.

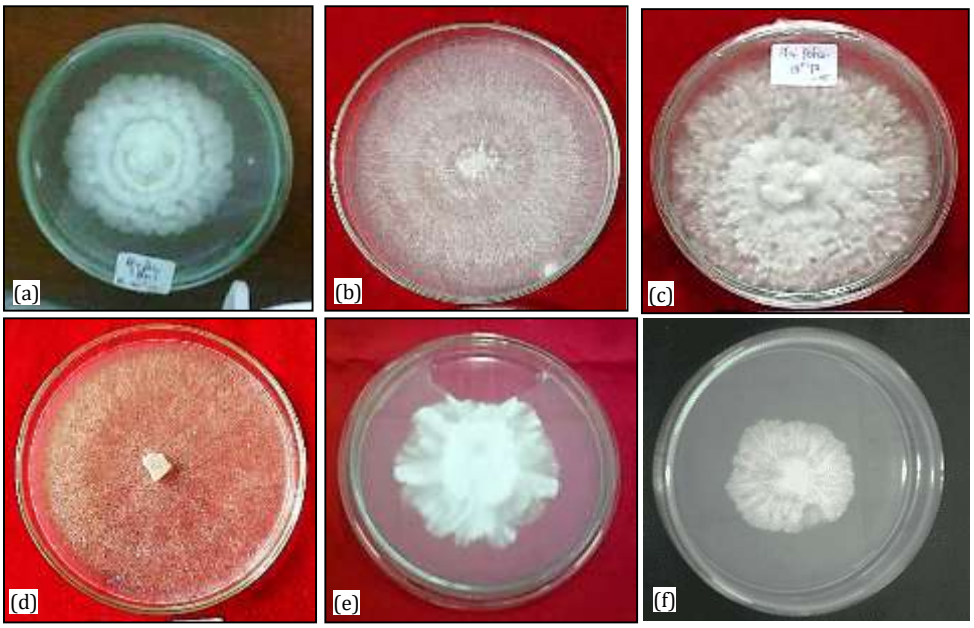


Figure 4. Growth of the original *Phytophthora* isolates: a=SH, b=TRH, c=SS, d=TRS, and e=STo, f=TRTo. Key: S=isolates from the soil; TR = tree root nutmeg.

3.2.2 Microscopic Observation

Microscopic examination revealed that pathogenic fungi grown at 25–27 °C developed hyaline, aseptate hyphae, with septa forming in older structures. These hyphae were multinucleated (coenocytic) (Figure 6). These hyphae are capable of producing sporangia asexually, as well as oospores through sexual reproduction. The formation of an antheridium, which produces male gametes, and an oogonium, which produces female gametes, characterizes the sexual cycle of this

organism. If both gametangia appear on the same thallus, the organism is homothallic; if they originate from different hyphae, it is heterothallic, as in *P. palmivora* (Scanu *et al*., 2021). Gametes are formed through meiosis, after which the antheridium penetrates the oogonium, allowing the fusion of their protoplasm in plasmogamy. Subsequently, the gamete nuclei unite in karyogamy to form a zygote, and the oogonium containing this zygote develops into an oospore (Carella *et al*., 2018). Microscopic observations of fungi cultured on PDA at 25–27 °C revealed hyaline, coenocytic hyphae without partitions; septa appeared only in older hyphae. These hyphae produced sporangia asexually and oospores sexually (Figure 6). Microscopic observations revealed that the sporangiophore branching pattern in *Phytophthora* is either simple sympodial or simple type (Figure 7).

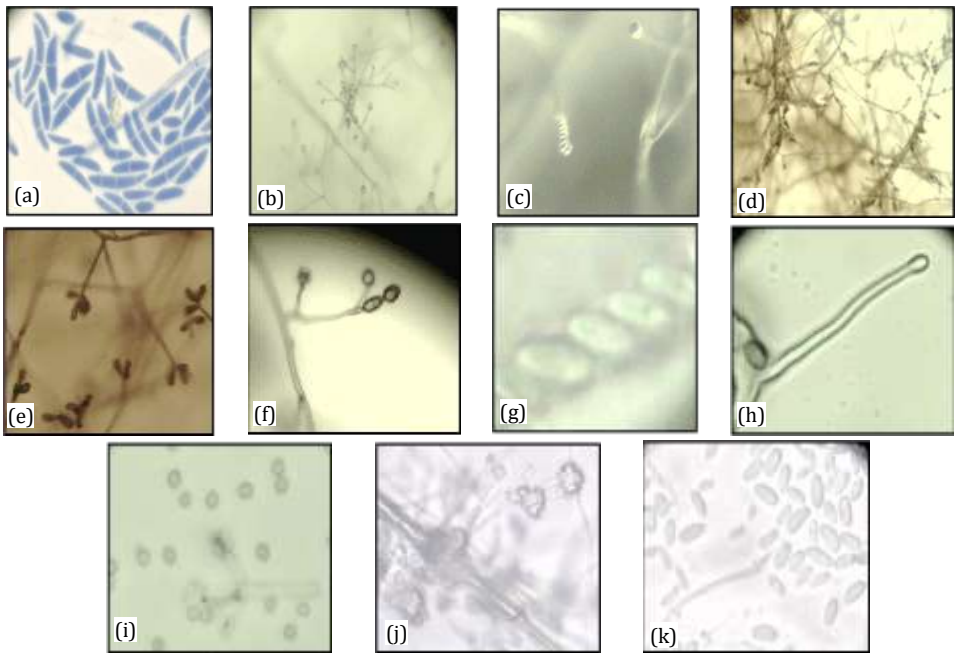


Figure 5. (a-d) *Fusarium* sp., (e-f) Curvularia sp., (g) *Monilinia* sp., (h) *Mucor* sp., (i) *Botrytis* sp., (j) *Acremonium* sp., (k) *Colletotrichum* sp., isolated from soil and nutmeg plant tissues. Description: (a),(g),(h),(i), and (k) 400x magnification; (b) - (f), (j) 100x magnification.

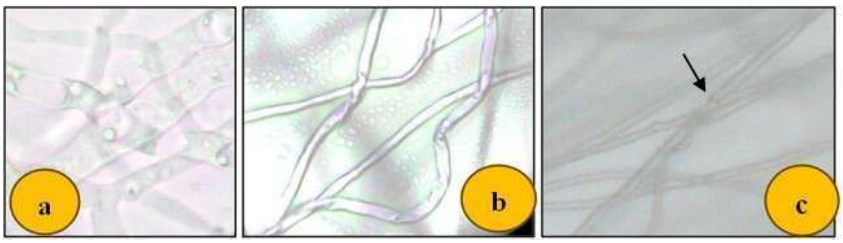


Figure 6. (a) Hyphae are not clear (hyalin), multi-nucleated (*coenocytic*), unblocked, and (c) form a barrier in the old hyphae (arrows). Description : (a) 400x magnification, (b)(c) 100x magnification.

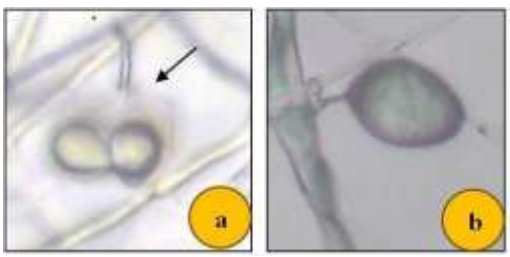


Figure 7. (a) and (b)Types of *branching in Phytophthora: simple simpodial.*

Hyphal swelling occurs at 28–30 °C, accompanied by abundant chlamydospore formation. Chlamydospores, measuring 7.5–18 µm in diameter, develop at terminal positions (Figures 8a–c). This feature distinguishes the isolate from *P. palmivora*, which rarely produces chlamydospores. In *P. lateralis*, hyphae do not swell, but thin-walled chlamydospores measuring 20–77 µm are readily formed. In contrast, sporangia of *Phytophthora austrocedrae* develop on unbranched sporangiophores, often accompanied by hyphal swelling. These sporangia are ovoid to obpyriform, conspicuously large, and caducous with pedicels 2–7 µm in length**.** In nutmeg plants, sporangia measured 9–25 × 5–17.5 µm (average 17.9 × 11.42 µm), contrasting with *P. palmivora*, which produces spherical chlamydospores 30–60 µm in diameter (Table 5).

The morphology of *Phytophthora* in nutmeg differs from that in forest and horticultural trees. Sporangia are papillate, ovoid to obpyriform (17.0–38.9 × 14.6–29.2 µm), and caducous (Table 6), unlike *P. katsurae*, which is non-caducous. Chlamydospores measure 7–18 µm, smaller than those of *P. katsurae*, while oogonia and antheridia were not observed throughout the study. This variation highlights species differences and parallels sporangial diversity reported in chilli peppers (Granke *et al*., 2011).

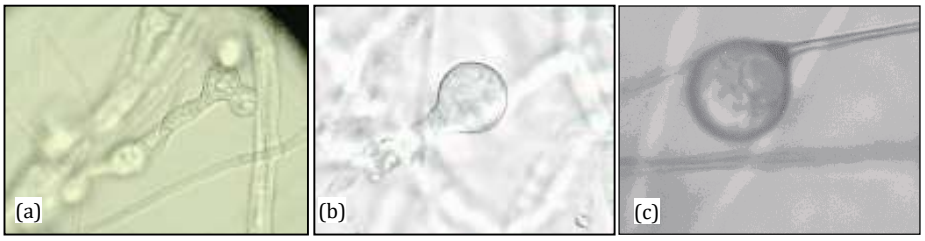


Figure 8. Swelling of hyphae in *Phytophthora* (a, b) and terminal chlamydospores (c).

Table 5. Results of observations of the characteristics of *Phytophthora* sp*.*

Origin of Isolate	Isolate[a]	Swelling of hyphae[b]	Sporangiosphore				
			Papilla [c]	Bentuk	Sporangiosphore branch	Caducity[d]	Pedicel length (µm)
Hatu	SH	+	+	Spherical, Ovoid, obpyriform	simple simpodial	+	5 – 7
Hatu	TRH	+	+	Spherical, Ovoid, obpyriform	simple simpodial	+	3 – 5
Seith	SS	+	+	Spherical, Ovoid, obpyriform	simple simpodial	+	2 – 3
Seith	TRS	+	+	Spherical Ovoid, obpyriform	simple simpodial	+	3 – 5
Toisapu	Sto	+	+	Spherical Ovoid, obpyriform	simple simpodial	+	3,5 -7
Toisapu	TRTo	+	+	Spherical Ovoid, obpyriform	simple simpodial	+	3,5 -7

Notes: [a] Isolation code (Table 2); b) + hyphae are swollen; c) + have papillae; d) + sporangium caducous.

Table 6. Results of observation of sporangia characteristics in *the Phytophthora* sp isolate

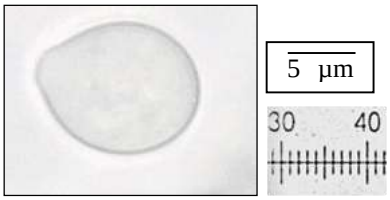
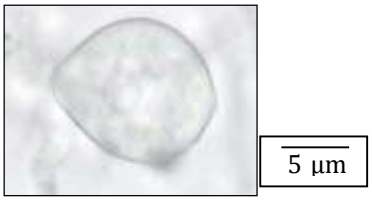
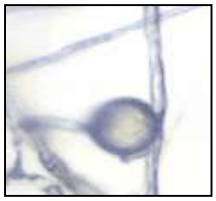
Origin of Isolate	Isolate Code[a]	Sporangium			
		Panjang (µm)	Average (µm)	Width (µm)	Average (µm)
1	2	3	4	5	6
Hatu	SH	15,0 – 22,5	18,75	5,00–17,5	11,25
Hatu	TRH	12,5 – 25,0	18,75	5,00–17,5	11,25
Seith	SS	20,0 – 22,5	21,25	5,00–17,5	11,25
Seith	TRS	9,00 – 25,0	17,00	7,00 17,5	12,25
Toisapu	STo	10,0 – 22,5	16,25	5,00–17,5	11,25
Toisapu	TRTo	9,00 – 22,5	15,75	5,00–17,5	11,25
Average	-	-	17,96	-	11,42

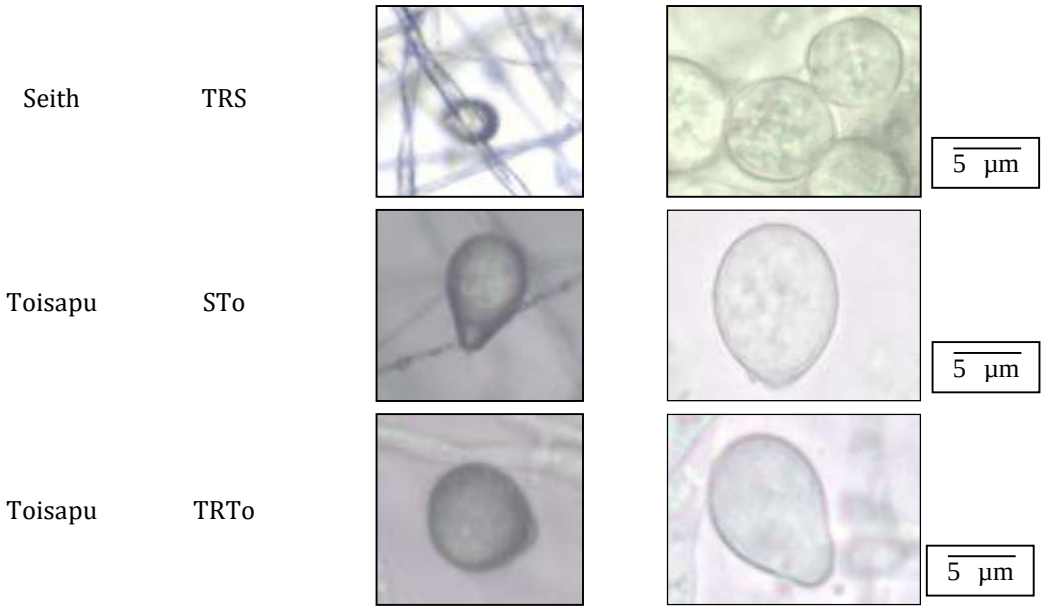
Notes: Isolation Code: SH and TRH (Hatu); SS and TRS (Seith); STo and TRTo (Toisapu); S=soil, TR=nutmeg tree root).

Sporangia of *P. nicotianae* are oblong to rounded, pear-shaped with distinct papillae, measuring 11–60 × 20–45 µm (mean 40.18 × 28.53 µm), borne on irregularly branched or sympodial sporangiophores. Chlamydospores are rounded, thick-walled, and formed intercalary or terminally. In *P. citrophthora*, sporangia are sponge-like, resembling orange fruit, with irregularly branched sporangiophores; colonies form petalate patterns on PDA and stellate or lanose on cornmeal agar. *P. palmivora* produces oblong sporangia and oospores 22–29 µm, sometimes lacking papillae, oval to pear-shaped, measuring 25–35 × 40–60 µm. Sporangia germinate directly via germ tubes or indirectly by releasing motile zoospores. Hyphae are aseptate when young, but septa appear in older hyphae as reproductive organs develop. Colonies are typically white with uneven margins, exhibiting rosaceous, stellate, and cottony forms (Motulo *et al*., 2020). According to the Forest *Phytophthora* of the World key (López-García, 2024) and EPPO Q-bank Phytophthora, no morphological traits of the nutmeg isolate correspond to previously reported species. Thus, *Phytophthora* sp. from nutmeg is suspected to represent a new subspecies, requiring molecular characterization to confirm its taxonomic status and pathogenic identity. Sporangium: *Phytophthora* sp*.* isolated from the soil around the roots of the nutmeg tree and those isolated from diseased tissues (Figure 9).

Fruit bodies of the pathogenic fungus emerge on the bark surface. Their increasing abundance indicates that infection has penetrated trunk tissues, damaging the inner bark where nutrient absorption occurs. Extensive external fruiting reflects internal tissue decay. As fruit bodies multiply, spore production intensifies, spreading through bark cracks. Consequently, small canker wounds expand rapidly, causing bark peeling, watery exudation, and ultimately tree death due to the disruption of food transport channels. Nutmeg trees affected by stem canker develop rotting, moist bark that attracts secondary fungi. Severely damaged trees thus host multiple fungal species, as the decayed tissues provide a favorable environment for diverse pathogens.

Table 7. Sporangium: *Phytophthora* sp. is *spherical, ovoid*, and *obpyriform*, and has prominent papillae.

Origin of Isolate	Isolates[a)]	Sporangium	
		40x magnification	400x magnification
Hatu	SH		
Hatu	TRH		
Seith	SS		



Notes: Sporangium shape with enlargement of 40 times (a) and 400 times (b), SH and TRH (Hatu); SS and TRS (Seith); STo and TRTo (Toisapu); S=soil, TR=nutmeg tree roots).

Table 8. Intensity of Stem Canker Disease Damage in Nutmeg Plants before and after activated charcoal treatment

Villages	Disease Intensity (%)		
	Before the treatment of activated charcoal	After activated charcoal treatment	Average
Hatu	16,67	6,67	12,22
Seith	13,33	6,67	10,00
Toisapu	16,67	10,00	13,33

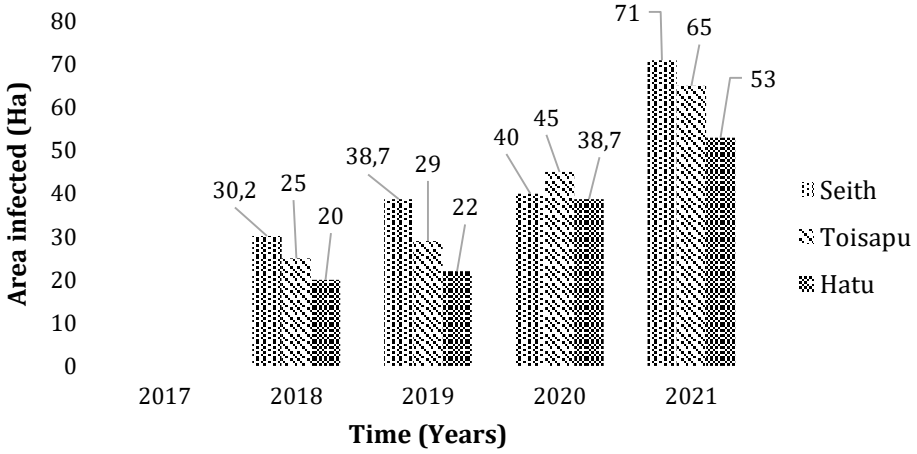


Figure 9. The extent of stem canker attacks in the 2018-2021 period in the villages of Seith, Hatu, and Toisapu. (Source: Plantation Crop Protection Development Unit, BB2TP, Ambon 2022).

3.4 The Effectiveness of Shell Charcoal to Suppress the Development of Nutmeg Stem Cancer

Average data from the Plantation Crop Protection Development Unit in Ambon (2017–2021) show a sharp increase in nutmeg stem canker incidence. The affected area expanded from 30.2 ha in 2018 to 38.7 ha in 2019, and surged to 71 ha in 2020 (Figure 9). Observations of damage intensity (IK) before and after charcoal treatment showed a clear reduction in disease severity (Table 8).

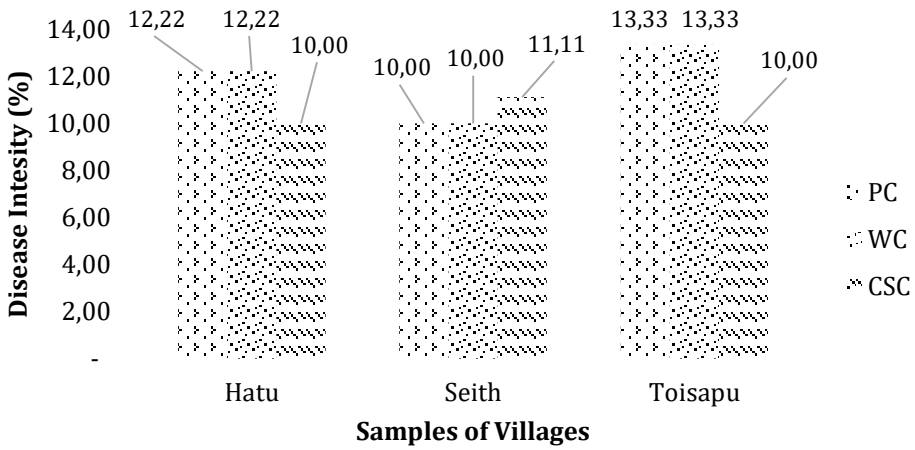


Figure 10. The intensity of damage after treating the three types of charcoal. Descriptions PC=pine charcoal, WC=walnut charcoal, and CSC = coconut shell charcoal).

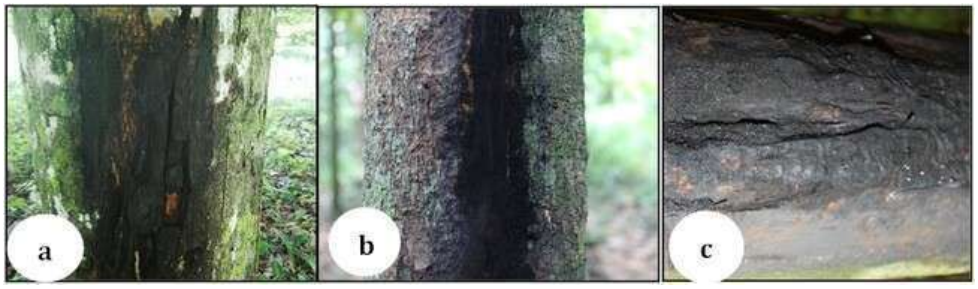


Figure 11. The results of the treatment of the three types of charcoal were (a) walnut shell charcoal (WC), (b) pine wood charcoal (PC), and (c) coconut shell charcoal (CSC) in the sample plants.

Monitoring of stem canker intensity (IK) in nutmeg plants at Hatu, Seith, and Toisapu villages showed significant improvement in plant health after activated charcoal treatment. At the start of observation, Hatu and Toisapu recorded the highest damage levels, reaching 16.67%. In Seith Village, the initial damage rate was relatively lower at 13.33%. Following activated charcoal treatment, damage intensity decreased across all sites, with Hatu Village showing a sharp decline to 6.67%. In Seith Village, the damage rate decreased from 13.33% to 6.67%, while in Hatu Village it dropped sharply from 16.67% to 6.67%. In Toisapu Village, the rate also declined from 16.67% to 10.00%, which remained the highest post-treatment level among the three sites. Overall, all villages showed reduced stem canker intensity after activated charcoal treatment, even though differences between locations were not statistically significant.

Figure 12. Charcoal paste application (a), after treatment (b), the rod wound begins to heal as bleeding from the wound stops (c).

Coconut shell charcoal adheres better than walnut shell and pine wood charcoal, which are more easily washed away. This advantage stems from its smoother, more stable pore structure that enhances adsorption capacity. Activated charcoal generally has a surface area of 300–3,500 m^2/g, with coconut shell charcoal reaching 350.5 m^2/g (BBP2TP, 2011). It's fine pores expand the contact area and accelerate adsorption through film diffusion, pore diffusion, and molecular attachment to the charcoal surface.

Control using coconut shell, pine wood, and walnut shell charcoal reduced liquid availability, thereby lowering the infection rate. With less fluid, zoospore production and release were suppressed, mycelial growth was inhibited, and chlamydospore formation was promoted. This restricted zoospore spread, slowed hyphal development, and diminished β-glucan reserves, ultimately hindering pathogen progression. The cell wall of *Phytophthora* is composed primarily of β-1,3 and β-1,6 glucans (80–90%), with cellulose as a minor component, along with sugars such as mannose and glucosamine (Fabre *et al*., 1984). The wall structure of *Phytophthora* varies by life phase, from thin walls in cysts to complex walls in sporangia and oospores (Erwin, 1995). Nutmeg stem canker control using three types of activated charcoal absorbs fluid from infected stems, reducing water availability. This limits the metabolism of *Phytophthora sp.*, since sporangia require water to produce and release zoospores (Pangestu *et al*., 2015). The study results showed that applying charcoal paste from all three types had a positive effect, as treated nutmeg plants exhibited recovery (Figure 12).

Activated charcoal paste plays an important role in environmentally friendly plant pathogen control. Unlike chemical fungicides or bactericides that directly kill pathogens, charcoal functions as an adsorbent, capturing toxic compounds, pathogenic enzymes, and exudates that support microbial growth. Its millions of fine pores act like sponges, absorbing excess moisture from stem wounds and stabilizing pH, thereby creating conditions less favorable for pathogen development. Applied to nutmeg stem canker, charcoal paste works in two main ways, namely as a physical protector of the wound surface and as a regulator of the microenvironment. Since stem-canker fungi thrive in wet, humid conditions, reducing moisture through adsorption effectively suppresses their growth (Zwart & Kim, 2012). Applying charcoal paste, especially from activated charcoal, provides an environmentally friendly method of controlling plant pathogens. Acting as an adsorbent, it captures toxic compounds, pathogenic enzymes, and exudates that promote fungal growth. Unlike chemical fungicides that directly kill pathogens, charcoal modifies the wound environment: its alkaline properties stabilize pH, while its porous structure absorbs excess moisture. This makes the wound area drier and less favorable for stem-canker fungi, which thrive in wet, acidic conditions (Lehmann & Joseph, 2024). By reducing water and toxins, charcoal limits vascular damage, enhances wound tissue recovery, and stimulates beneficial microbial activity. Thus, charcoal paste functions both as a physical protector and as a promoter of overall plant health.

The use of charcoal paste is not just a technical treatment, but a holistic approach that supports plant recovery through two mechanisms: (a) *Adsorption and Neutralization of Toxins*, Activated charcoal works through its extensive micropore structure to absorb toxin compounds from pathogens as well as harmful chemical residues such as pesticides and herbicides. By purifying the environment around the roots and stems, the concentration of toxic substances can be significantly suppressed. This not only frees the plant from environmental stress but also strengthens its natural immunity in the face of disease attacks; (b) *Optimization of Physical and Chemical Conditions of the Planting Medium*: Integrating charcoal powder (biochar) into the planting medium can transform the soil structure into a more crumbly and porous soil. Improved aeration and drainage are crucial to prevent root rot, which is often triggered by water-saturated soil conditions. In addition, biochar acts as a pH balancer, creating a stable and ideal growing space for plants to develop optimally; (c) *Beneficial Microbial Stimulation*: Charcoal pores provide a safe dwelling for beneficial

microorganisms, such as non-pathogenic bacteria and fungi. The presence of this microbiota helps the organic nutrient cycle become a form that plants can easily absorb. At the same time, they act as "natural protectors" that suppress pathogen populations through space and nutrient competition; (d) *Physical Protection on Plant Wounds*: In practical applications, charcoal paste serves as a "biological bandage" on injured stems or trimming marks. This layer becomes the primary physical barrier that prevents the entry of pathogens that cause stem canker. By keeping the wound area dry, charcoal paste minimizes excess moisture, which is usually the main trigger for parasitic fungal growth.

4. CONCLUSION

This study found that the main cause of stem canker in nutmeg plants on Ambon Island is the pathogenic fungus *Phytophthora* sp. The isolates found have distinctive morphological characteristics, such as sporangia measuring 17.96 × 11.42 µm, papillae, and the formation of chlamydospores. These characteristics do not fully match those of other widely known *Phytophthora* species, so it is suspected that this pathogen is a new subspecies of the Moluccas. The use of simple local ingredients such as activated charcoal from coconut shells, walnuts, and pine wood is an effective solution to control stem canker in nutmeg plants. This finding provides new hope for nutmeg farmers as an environmentally friendly and sustainable strategy for protecting plants.

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