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Endophytic fungi and their potential in controlling white root disease of cashew

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Abstract: White root disease is a significant disease of cashew caused by *Rigidoporus* sp. Five endophytic fungal isolates, namely AR31D (*Fusarium proliferatum*), AR42D (*Penicillium citrinum*), BR32C (*Trichoderma asperellum*), VNTB1 (*Chaetomium* sp.), and EAGS14 (*Curvularia lunata*), were assessed as the biocontrol agents against *Rigidoporus* sp. *in vitro* and *in planta*. The research objective was to obtain endophytic fungi that effectively control *Rigidoporus* sp. and their mechanisms. The *in vitro* test results showed that all isolates could inhibit *Rigidoporus* sp. and promote plant growth by producing volatile organic compounds, chitinase enzymes, and indole acetic acid. Meanwhile, only four isolates could solubilize phosphate with low-medium solubilization efficiency. The isolates successfully colonized the root of cashew saplings in 10–65%. The effectiveness of endophytic fungal isolates in controlling white root disease was determined by the mechanisms involved, such as resistance induction (increased activity of defense enzymes like polyphenol oxidase), chitinase enzyme production, indole acetic acid production, phosphate solubilization, and suppression of plant stress which observed from decreased malondialdehyde concentrations in saplings' roots. *Trichoderma asperellum* and *Chaetomium* sp. were the best isolates with the highest control effectiveness and stimulating plant growth.

Keywords: biological control; controlling mechanism; plant defense; *Rigidoporus* sp.

Cashew (*Anacardium occidentale* L.) has been grown on a plantation scale and has become a significant plantation commodity in some regions of Indonesia, such as Bali, Nusa Tenggara, and Sulawesi. This plant produces "true" fruit known as cashew nuts. Recently, market demand for cashew nuts has increased in domestic and international markets, especially Europe and America. In 2019/20, cashew nuts were the third most popular tree nut (after almonds and walnuts) and contributed 17% of global tree nut production (International Nut and Dried Fruit Council Foundation 2020). As one of the cashews nut-producing countries, Indonesia ranks 7th as the largest producer globally (Ministry of Trade of Indonesia 2016). Furthermore, the data released by the Directorate General of Plantations of the Ministry of Agriculture (2019) showed that the total area of the national cashew nut was 494 268 ha until 2018, which was dominated by smallholder plantations. Therefore, 99.8% of Indonesia's cashew nut production came from smallholder plantations.

Poor field management and sanitation are a weakness of smallholder plantations, so cultiva-

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tion disturbances such as pests and diseases spread quickly. As a result, productivity was not optimal. The problem often found in smallholder plantations of cashew in Indonesia is white root disease caused by *Rigidoporus* sp. In recent years, this disease has emerged in several central areas of cashew plantations in Indonesia, such as Bali, West Nusa Tenggara, and Southeast Sulawesi. The disease was found at all stages of plant growth, incredibly productive cashew trees under ten years (Taufik et al. 2021). However, the reduced yield caused by the disease has yet to be reported.

The control of white root disease, which infected several plants, has been carried out by farmers with various methods. Farmers in Bali still applied the eradication methods to control the disease in the cashew field. Meanwhile, the control of this fungus on rubber plants was generally carried out by applying some synthetic fungicides (sprinkled on the soil around the root neck) (Yulia et al. 2017). In Indonesia, fungicides with some active ingredients, such as triadimefon, triadimenol, and hexaconazole were commonly used in controlling the disease (Directorate General of Plantations 2015). Applying synthetic fungicides on a wide scale certainly requires a considerable cost and negatively impacts the ecosystem. Biological control is one of the methods of controlling fungal pathogens currently being researched, both in terms of effectiveness and long-term impact. Control can be done by utilising fungi outside the host or endophytic. So far, no studies have reported the effectiveness of endophytic fungi as biological control agents of cashew white root disease. According to Berg (2009), endophytic fungi usually live in the same plant tissue as bacteria or fungal pathogens, which are very suitable for biological control agents. Endophytic fungi play a role in plant growth both directly and indirectly and induce resistance to phytopathogens by producing biologically active metabolites (Anisha & Radhakrishnan 2017; Ye et al. 2020), thus having a beneficial effect on the host plant.

Latz et al. (2018) reported several mechanisms for endophytic fungi to act as biological control agents for plant pathogens. These mechanisms include inducing plant resistance, mycoparasitism, antibiosis, and competition. Therefore, endophytic fungi can be used as a new source of helpful biological control agents, especially in integrated disease control strategies (Rojas et al. 2020). This research aimed to obtain endophytic fungi which effectively control white root disease on cashew and examine their mechanism of action.

MATERIAL AND METHODS

The study was conducted at IPB University, Dramaga Campus, Bogor, Indonesia, from August 2020 to June 2021. *In vitro* tests between pathogens and endophytes were carried out at the Plant Mycology Laboratory, Department of Plant Protection. The effectiveness testing of endophytic fungi controlling *Rigidoporus* sp. was carried out in the greenhouse at Cikabayan Experimental Station.

Isolation and morphological identification of pathogenic fungus

Pathogenic fungus causing white root disease was taken from infected cashew plants from smallholder plantations in Kubu Subdistrict, Karangasem Regency, Bali Province, Indonesia (8°14'56.3"S 115°31'42.9"E). The pathogen was isolated from the plant roots showing signs and symptoms of being infected by this fungus.

Isolation of pathogenic fungi was carried out following Farhana et al. (2017) with modifications. Infected cashew roots were cleaned with running water to remove soil particles and dirt. The part showing typical symptoms was then cut. The root surface was sterilised by immersing it in a 1% natrium hypochlorite solution for 30 s and rinsing it with sterile distilled water three times. Five pieces of samples were dried and placed on potato dextrose agar (PDA) medium in a Petri dish, then incubated at 27 °C for 72 h to allow the mycelium to grow.

The morphological characters appearing on the surface of the medium, including macroscopic (colony shape and colour) and microscopic (hypha shape and colour, and other structures formed), were observed to identify the fungus. Identification was referred to the previous studies by Cui et al. (2009) and Sarmiento-S et al. (2016). Isolates showing the characteristics of *Rigidoporus* sp. were selected and transferred to a new PDA medium with pH 7. After obtaining pure cultures, isolate was recultured on PDA medium as stock for the tests.

Endophytic fungal cultures

The endophytic fungal isolates tested in this study were five collection isolates from the Laboratory of Plant Mycology, Department of Plant Protection,

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IPB University. All of the isolates were isolated from plant roots. Three isolates were isolated from wild peat vegetation of South Sumatra, namely AR31D (*Fusarium proliferatum*) from *Imperata silindrica*, AR42D (*Penicillium citrinum*) from *Scirpus* sp. and BR32C (*Trichoderma asperellum*) from *Carex sylvatica*. The fourth and fifth isolates were VNTB1 (*Chaetomium* sp.) isolated from *Vachellia nilotica* in Baluran National Park, East Java, and EAGS14 (*Curvularia lunata*) isolated from *Elaeis guineensis* Jacq. in the peatlands of South Sumatra. All five isolates used in this study have been deposited in the IPB Culture Collection, Department of Biology, Faculty of Mathematics and Natural Science, IPB University, Bogor, Indonesia.

Molecular identification of pathogenic and endophytic fungi

For molecular identification, DNA extraction was carried out by following the method of Abd-Elsalam et al. (2003). We used universal of ITS1 (forward) and ITS4 (reverse) primer pair (White et al. 1990) for DNA amplification. PCR was performed in a total volume of 30 µL containing 1.2 µL of each primer, 15 µL of Dream TaqTM Green PCR Master Mix (Thermo Scientific, USA), 11.1 µL of nuclease-free water, and 1.5 µL of the DNA template. The PCR amplified product was sent to First Base Asia (http://www.base-asia.com/) for DNA sequencing. The DNA sequences were compared with all entries in the GenBank using the Basic Local Alignment Search Tool (BLAST) program (https://blast.ncbi.nlm.nih.gov/).

Screening for antibiosis activity and fungal interaction during dual culture

The antibiosis test was carried out using the dual culture method (Li et al. 2007) with some modification in incubation time. A 4 mm diameter mycelial plug of endophytic fungus was taken from the edges of seven days-old fungal colonies and transferred to the centre of a 90 mm diameter Petri dish of potato dextrose agar (PDA). At the same time, two plugs of the fungal pathogen were transferred onto the dish on the left and right side of the endophytic plug at the same distance. The cultures were grown at 22 °C for seven days in the dark. The incubation time was changed from 1–3 weeks to seven days because the isolates tested had relatively faster growth. The antibiosis mechanism was characterized by a clear zone between the colony of endophytic fungi and *Rigidoporus* sp. This test was carried out with four repetitions on each isolate.

The interactions between *Rigidoporus* sp. and endophytic fungal isolates were classified using a categorization system developed by Wheeler and Hocking (1993): "(1) type A: mutual intermingling growth, the fungi grew into each other without any signs of interaction; (2) type B: mutual inhibition on contact or space between colonies small < 2 mm; (3) type C: inhibition of a fungus on contact, the inhibited fungus grew at a significantly reduced rate, while the inhibitor fungus grew at a reduced rate or unchanged; (4) type D: mutual inhibition at a distance > 2 mm; (5) type E: inhibition of a fungus on contact, the inhibitor species continuing to grow at a reduced rate through the inhibited fungus; (6) type F: inhibition of one fungus on contact or at a distance, the inhibitor fungus then continuing to grow at an unchanged rate through or over the inhibited colony."

Screening for volatile organic compounds production

Five endophytic fungal isolates were tested for their ability in producing volatile organic compounds (VOCs). The test was carried out with four repetitions on each isolate. Potato dextrose broth (PDB) (HiMedia Laboratories Pvt. Ltd, India) with addition of agar powder (HiMedia Laboratories Pvt. Ltd, India) was used as the culture medium of the fungi (24 g PDB and 15 g pure agar in 1 000 mL distilled water). In addition, we also modified the composition of culture medium by reducing the PDB concentration. Therefore, there were five variants of PDB (with the addition of 15 g pure agar in 1 000 mL distilled water for each level), namely 24 g, 19.2 g, 14.4 g, 9.6 g, and 4.8 g, respectively. The VOCs production test was carried out using two Petri dishes of the same diameter by cupping a Petri dish containing *Rigidoporus* sp. colonies onto a petri dish containing colonies of endophytic fungi (Raza et al. 2015; Maknunah & Sinaga 2018). After seven days of incubation at 22 °C in the dark, the VOCs production of endophytic fungi was carried out by comparing the diameter of *Rigidoporus* sp. in treatment with control. The inhibition (% *I*) was calculated using the following formula:

$$I = [(DC - DT)/DC] \times 100\% \quad (1)$$

where: *I* – inhibition rate; *DC* – diameter of control; *DT* – diameter of pathogen in treatment (Yuan et al. 2012).

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Screening for the chitinolytic activity of endophytic fungal isolates

The chitinolytic activity test was carried out following the method by Yadav et al. (2015) with four repetitions. The medium used was chitin agar (1.5 g yeast extract, 2 g colloidal chitin, and 20 g agar in 1 L of water). Endophytic fungal isolates were cultivated on chitin agar medium and then incubated at 22 °C for up to seven days. Each isolate in a Petri dish was stained with 0.1% congo red at 48 h after incubation to visualise chitinolytic activity. A clear zone around the isolate indicated the chitinase enzyme activity produced by endophyte. The chitinolytic activity was measured by the chitinolytic index, with the formula: diameter of the clear zone divided by the colony's diameter (Hartati et al. 2015).

Screening for indole acetic acid production of endophytic fungal isolates

The test of indole acetic acid (IAA) production by endophytic fungal isolates followed the method by Siri-Udom et al. (2019) with modifications to the incubation period after adding reagents. The test was carried out using Salkowski's reagent (1 mL of 0.5 M $FeCl_3$; 50 mL of 35% $HClO_4$) with four repetitions. The isolates were cultured in 5 mL of PDB liquid medium with L-tryptophan (2 mg/mL) with 150 rpm shaking in a shaker for five days. The suspension of the isolates was then filtered with two layers of sterile gauze to separate it from the mycelia. The filtrates were centrifuged at 10 000 rpm for 5 minutes. Then 1 mL of the supernatant was taken and added to 1 mL of Salkowski's reagent, then homogenised using a vortex. The suspensions were then stored for 30 min at room temperature (22 °C) in the dark. The pink appearance of the culture filtrate suggested that the endophytic fungal isolates produced IAA. The absorbance value of each sample was measured using a spectrophotometer at a wavelength of 530 nm. Then, IAA concentration was calculated using the linear regression equation obtained from the standard curve (average of four repetitions at 22 °C).

Screening for phosphate solubilization of endophytic fungal isolates

The ability of endophytic fungal isolates to solubilize phosphate was tested *in vitro* following the method by Adhikari and Pandey (2019) with four repetitions. Seven-day-old endophytic fungal isolates were cultured on Pikovskaya's agar (HiMedia Laboratories Pvt. Ltd, India) medium and incubated at room temperature (22 °C) for seven days. The clear zone appearing around the colony indicated that the isolates were able to solubilize phosphate. The phosphate solubilization index was determined by dividing the diameter of the clear zone with the diameter of the colony (Fankem et al. 2006). The phosphate solubilization index value obtained was then used to classify the solubilization efficiency (Hara & Oliveira 2005).

Root colonization test of endophytic fungal isolates

Root colonization test was carried out by following Muvea et al. (2014) with modifications. We used 30 seeds for each endophyte treatment. The surface of cashew seeds was sterilized with 1% NaOCl solution for 3 min and rinsed with sterile distilled water three times. The seeds were then immersed in conidia suspension (10^5/mL) of each isolate for 24 hours. For control, seeds were immersed in sterile water. The inoculated seeds were then dried on sterile tissue for 20 min, then sown in seedling trays with sterile black sand media and maintained at room temperature (25 °C). Endophytic fungal isolates were inoculated again by sprinkling the endophytic fungi suspension around the roots three weeks after sowing. Seeds were watered once a day in the morning until they reached the age of eight weeks.

Colonization of endophytic fungal isolates was confirmed by re-isolation on the roots of seedlings that thrived on obtaining the same endophytic fungi as previously inoculated. Isolation was done by randomly selecting five pieces of lateral roots and root hairs. The surfaces of the root pieces were sterilised with 1% NaOCl solution for 2 min and rinsed with sterile distilled water three times. The root pieces were air-dried and placed on the PDA medium in Petri dishes. Observation of the endophytic fungal colonies was confirmed by macroscopic and microscopic morphology.

***In planta* test of endophytic fungi in the greenhouse**

Plant material, endophytic fungal isolates, and pathogen inoculum. The cashew saplings used for testing in the greenhouse were healthy saplings of B 02 variety from the Cikampek Experi-

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mental Garden, Indonesian Spice and Medicinal Crops Research Institute, The Indonesian Agency for Agricultural Research and Development. Five-month-old saplings were taken to the greenhouse to be transplanted into larger polybags and cared for until the age of nine months before endophytic application. Endophytic fungal isolates were propagated by growing each isolate on a PDA medium at room temperature. Ten-day-old cultures were washed with sterile water to harvest the conidia. Meanwhile, the propagation of pathogenic inoculum *Rigidoporus* sp. was prepared by growing it on PDA medium in glass bottles together with 10 cm length wood sticks of cashew. The wood sticks were previously sterilised with 1% NaOCl solution for 3 min, rinsed three times with sterile water, and continued autoclaved for 15 min at 121 °C. A total of five pieces of sterile wood sticks were put into each glass bottle containing PDA medium, then sterilised again by autoclave. Three pieces of inoculum culture were cultured in the glass bottle and incubated until the bottles were filled with mycelia *Rigidoporus* sp. for three weeks.

Experimental design. The greenhouse experiment consisted of two trials with a completely randomized design. Each experiment consisted of six treatments with four replications. Each treatment in each replication consisted of three plants, so that the total number of cashew saplings tested in one experiment was 72. In the second experiment, six experimental groups of saplings were tested: (1) control. Saplings with no endophytic isolates treatment, challenged with *Rigidoporus* sp. (2) Saplings treated with *F. proliferatum*, challenged with *Rigidoporus* sp. (3) Saplings treated with *P. citrinum*, challenged with *Rigidoporus* sp. (4) Saplings treated with *T. asperellum*, challenged with *Rigidoporus* sp. (5) Saplings treated with *Chaetomium* sp., challenged with *Rigidoporus* sp. (6) Saplings treated with *C. lunata*, challenged with *Rigidoporus* sp.

While for the second experiment, the number of treatments was as same as the first experiment. The cashew saplings were only treated by endophytic fungal isolates without being challenged with *Rigidoporus* sp. Six experimental groups of saplings were tested: (1) control – saplings with no endophytic isolates; (2) saplings treated with *F. proliferatum*; (3) saplings treated with *P. citrinum*; (4) saplings treated with *T. asperellum*; (5) saplings treated with *Chaetomium* sp.; (6) saplings treated with *C. lunata*.

Endophytic application and inoculation of Rigidoporus sp. on saplings. In the second experiment, endophytic isolates were applied twice: one month before pathogen inoculation and one week after pathogen inoculation. The application of endophytic isolates before inoculation of pathogens was carried out as a preventive control. Endophytic isolates were expected to adapt first and successfully colonize plant root tissue for one month. In addition, the presence of these endophytic isolates earlier was expected to induce plant resistance to biotic stress. Furthermore, the application of endophytic isolates after pathogen inoculation is carried out as a curative control. Endophytic fungi were inoculated by sprinkling the conidial suspension near the sapling roots with a density of 10^5 conidia/mL. Inoculation of pathogenic *Rigidoporus* sp. was carried out one month after the first application of endophytic fungi. Three wood sticks of pathogen inoculum filled with mycelia of *Rigidoporus* sp. immersed as deep as ± 10 cm in the neck of the plant roots in a position parallel to the taproot. The daily water requirement of saplings was reduced in intensity to create a microclimate suitable for field conditions. The application of endophytic isolates in the first experiment was also carried out twice at an interval of one month.

Assessment of disease incidence and severity and effectivesness of disease control. Observation of disease incidence and severity in the second experiment was carried out at three months post inoculation (MPI) by observing the roots of cashew saplings. Scoring for white root disease severity was based on signs and symptoms: score 0 = root is free from *Rigidoporus* sp. infection, healthy plant; score 1 = root is covered with *Rigidoporus* sp. mycelium limited around the point of inoculation, healthy plant; score 2 = *Rigidoporus* sp. mycelium has spread widely on the root surface, healthy plant; score 3 = *Rigidoporus* sp. mycelium infects roots and basal stem, plant is symptomatic with wilting, pale, yellowing, or leaf defoliation; and score 4 = plant dies. Disease incidence (*DI*) and severity (*DS*) and control effectivesness (*CE*) were calculated following the formula below (Cooke 2006):

$$DI = n/N \times 100\% \quad (2)$$

where: n – number of saplings showing infected roots; N – total number of observed saplings.

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$$DS = [\Sigma(n_i \times v_i)/N \times Z] \times 100\% \quad (3)$$

where: n_i – number of infected cashew sapling in the certain v score; v_i – rating score; Z – maximum rating score.

$$CE = [(Sc - Se)/Sc] \times 100\% \quad (4)$$

where: Sc = disease severity of control; Se = disease severity of endophytic fungal treatment.

Observation of plant growth. Plant height and wet and dry root weight were measured as part of the plant growth observations. Plant height was measured from 0–4 months after the application of endophytic fungal isolates. The wet weight of the root was weighed when dismantling the plant. The roots were then dried at 60 °C for 48 h in an oven and weighed again.

Analysis of polyphenol oxidase activity of roots. Analysis of polyphenol oxidase (PPO) activity was carried out on plant roots in the first experiment (plants treated with endophytic isolates and inoculated with *Rigidoporus* sp.). The analysis was begun with protein extraction from cashew root, following Sukma et al. (2012). We evaluated three saplings for each treatment in each replication. The roots taken compositely were primary, secondary, and fibrous roots. In each treatment, a total of 0.5 grams of root tissue was added with cold phosphate buffer solution (50 mM pH 7) with a ratio of 1/4 (w/v). The scouring of root tissue was then centrifuged at a speed of 5 000 rpm and a temperature of 4 °C for 10 minutes. The supernatant was taken, and the total dissolved protein was determined by Lowry et al. (1951). PPO enzyme activity in each treatment was carried out following Mayer et al. (1966). The reaction mixture consisted of 1.5 mL of 0.1 M phosphate buffer, pH 6.5, 0.5 mL of the enzyme preparation extract, and 0.5 mL of 0.01 N catechol. The absorbance was measured by spectrophotometer at a wavelength of 495 nm every 30 s for 0–180 seconds. PPO activity was calculated as units of activity (UA) in which one unit of PPO was defined as the increase in absorbance value per unit time per protein weight.

Analysis of malondialdehyde concentration in roots. Measurement of malondialdehyde (MDA) levels followed the method of Wang et al. (2013) with some modifications. We evaluated three saplings for each treatment in each replication. The measurement stages are as follows. A total of 0.5 g of root sample was ground with the addition of 5 mL trichloroacetic acid (TCA) 5% (w/v). Then, the homogenate was centrifuged at 12 000 rpm for 5 min at 4 °C. 2 mL of supernatant from the extract formed was taken, mixed with 3 mL of 0.5% (w/v) thiobarbituric acid (TBA) in 5% (w/v) TCA. The mixture was incubated in a water bath at 80 °C for 30 min, then immersed in cold water to cool it. After that, it was centrifuged again at 12 000 rpm for 5 min at 4 °C. The last step was to measure the absorbance using a spectrophotometer with 450, 532, and 600 nm wavelengths. The MDA concentration was calculated using the following formula:

$$\text{MDA (µmol/g)} = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450} \quad (5)$$

Data analyses

The data obtained in each experiment were tabulated using Microsoft Office Excel 2019. All tests were carried out using a completely randomised design with four replications. The data obtained were analysed with the SAS program (version 9.1) through analysis of variance (ANOVA) and Tukey test (α 0.05).

RESULTS

***Rigidoporus* sp. from the infected root of cashew**

We successfully isolated the pathogenic fungus causing white root disease from the infected root tissue. A pure isolate identical to *Rigidoporus* sp. was obtained (Figure 1). Colonies growing on PDA medium were concentric and bright white with a fibrous surface. Colony diameter growth on the PDA medium was 11.4 mm per day at room temperature (22 °C). The microscopic observations showed that the hypha looked hyaline and septate. The fusoid cystidioles structure (like a pole) was present between hyphae. Fungi of the genus *Rigidoporus* are characterised by monomythic and septate hypha systems and the presence of cystidioles (Cui et al. 2009; Sarmiento-S et al. 2016).

Molecular identification of pathogenic fungus and endophytic fungal isolates

Based on molecular identification, the pathogenic isolate had 92.09% homology with *Rigidoporus* sp. E7081 from Java and *Rigidoporus* sp. E7089

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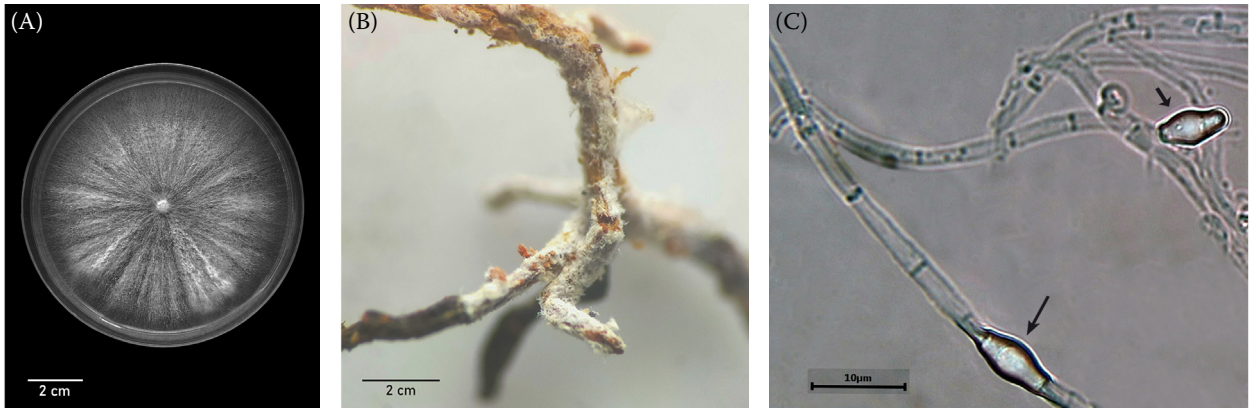

Figure 1. *Rigidoporus* sp. infecting root of cashew
(A) Colony on potato dextrose agar after six days, (B) rhizomorph infecting secondary root and (C) septate hyphae and cystidioles with 100 × 10 optical zoom

from Sumatra (Table 1). While, for endophytic fungal isolates, DNA sequencing confirmed that the isolates were *Fusarium proliferatum*, *Penicillium citrinum*, *Trichoderma asperellum*, *Chaetomium* sp., and *Curvularia lunata* (Table 2).

Screening for antibiosis activity and fungal interaction during dual culture

Five endophytic fungi isolates tested using the dual culture method showed no a clear inhibition zone (Figure 2). We observed three different interactions between the pathogen *Rigidoporus* sp. and five endophytic fungal isolates from the dual culture plates. In *F. proliferatum*/*Rigidoporus* sp. interaction, the mycelium of endophytic fungal breached into the colony of the pathogen. There was a weak yellowish pigmentation observed at the reverse of the dish on the fifth day post inoculation (Figure 2B). In *P. citrinum*/*Rigidoporus* sp. interaction, a yellowish pigmentation was also observed at the border of the pathogen (Figure 2C). These two interactions represented type E interaction–inhibition of a fungus on contact, the inhibitor species continuing to grow at a reduced rate. For *T. asperellum* and *C. lunata* , type F interaction was shown when co-cultivated with *Rigidoporus* sp. *T. asperellum* inhibited the pathogen growth by covering the pathogen colony (Figure 2D), while *C. lunata* overgrown the pathogen colony (Figure 2F). The interaction of *Chaetomium* sp./*Rigidoporus* sp. showed type C (Figure 2E), in which the pathogenic fungus grew at a significantly reduced rate, and the inhibitor fungus grew at a reduced rate.

Screening for volatile organic compounds production

Volatile organic compounds production test of endophytic fungal isolates on different composition of medium showed an inhibition for *Rigidopo-*

Table 1. Nucleotide homology of *Rigidoporus* sp. isolated from infected cashew tree with isolates in GenBank

Identity of GenBank isolates	Accession code	Query cover (%)	Homology (%)	Plant host/source
Rigidoporus sp. E7081	AJ537500.1	98	92.09	*Acacia mangium*/Java (INA)
Rigidoporus sp. E7089	AJ537410.1	98	92.09	*Acacia mangium*/Sumatra (INA)
Rigidoporus sp. DK4	JF701601.1	98	91.37	*Eucalyptus* spp./Malkapur (IND)

Table 2. Nucleotide homology of five endophytic fungal isolates compared to other fungi in GenBank

Isolate codes	Identity of GenBank isolates	Accession code	Query cover (%)	Homology (%)
AR31D	*Fusarium proliferatum* FMB-FR	MN368194.1	98	99.81
AR42D	*Penicillium citrinum* 32	MW014916.1	100	99.81
BR32C	*Trichoderma asperellum* ST8	MZ831506.1	99	100.00
VNTB1	*Chaetomium* sp. BAB-3301	KU504294.1	100	99.12
EAGS14	*Curvularia lunata*	LC317566.1	100	99.63

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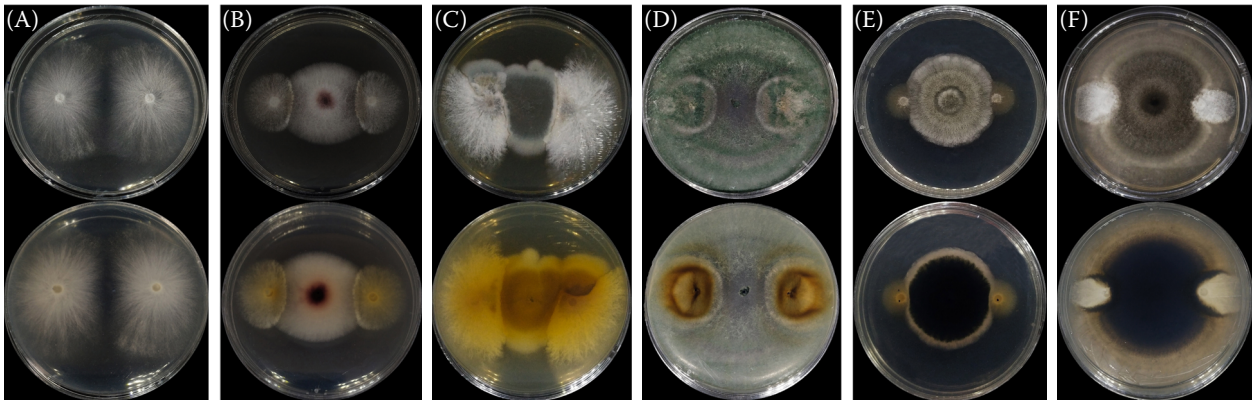

Figure 2. Antibiotic assay using dual culture method between endophytic fungal isolates against *Rigidoporus* sp. (A) Control, (B) *Fusarium proliferatum*, (C) *Penicillium citrinum*, (D) *Trichoderma asperellum*, (E) *Chaetomium* sp., and (F) *Curvularia lunata* in seven days after incubation

rus sp. Each endophytic fungus has a different level of inhibition at each level of the culture medium. The highest percentage of inhibition on *F. proliferatum* was produced on medium D (9.6 g PDB, 15 g agar powder), which was 60.70% and significantly different from medium E (4.8 g PDB, 15 agar powder) (Table 3). Like *F. proliferatum*, *Chaetomium* sp. produced the highest inhibition at medium D, which was 57.38% and significantly different from medium A (24.0 g PDB, 15 g agar powder) and medium B (19.2 g PDB, 15 g agar powder). Meanwhile, *P. citrinum* and EAGS14 produced the highest inhibition at medium A, i.e., 59.32% and 64.46%, respectively, but not significantly different from other culture media.

In *P. citrinum, T. asperellum*, and *C. lunata*, the higher concentration of PDB, the higher inhibition produced. It means that the concentration of the growth medium influenced the production of VOCs from the three endophytic fungal isolates. Medium with high nutrient concentrations caused the three isolates to grow optimally, thus producing higher amounts of VOCs. Meanwhile, in *F. proliferatum* and *Chaetomium* sp., the production of VOCs seemed to be optimal if the two isolates grew in nutrient-poor media conditions.

Screening for the chitinolytic activity of endophytic fungi

Five endophytic fungal isolates tested showed chitinolytic activity, which was indicated by the appearance of a clear zone around the culture (Figure 3). The formation of a clear zone in each isolate was observed from day 5 to day 7, where *F. proliferatum* had the clearest clear zone compared to other isolates. The chitinolytic index of each endophytic

Table 3. The effect of volatile organic compounds from endophytic fungal isolates grown on agar media to the growth of *Rigidoporus* sp.

Composition of culture medium*	Inhibition rate (%) of endophytic fungal isolates to *Rigidoporus* sp.				
	Fusarium proliferatum	*Penicillium citrinum*	*Trichoderma asperellum*	*Chaetomium* sp.	*Curvularia lunata*
24.0 g PDB, 15 g agar	53.68 ± 2.61^{ab}	59.32 ± 11.52^{a}	77.67 ± 3.09^{a}	42.20 ± 3.68^{c}	64.46 ± 4.51^{a}
19.2 g PDB, 15 g agar	66.22 ± 2.86^{a}	48.29 ± 13.30^{a}	58.23 ± 4.26^{b}	48.84 ± 2.31^{bc}	54.21 ± 12.21^{a}
14.4 g PDB, 15 g agar	53.36 ± 6.88^{ab}	54.15 ± 7.50^{a}	54.90 ± 6.99^{bc}	52.02 ± 2.62^{ab}	51.43 ± 15.00^{a}
9.6 g PDB, 15 g agar	60.70 ± 0.88^{a}	51.40 ± 6.01^{a}	48.34 ± 3.10^{c}	57.38 ± 6.02^{a}	56.55 ± 1.31^{a}
4.8 g PDB, 15 g agar	43.83 ± 11.39^{b}	53.43 ± 2.56^{a}	53.26 ± 2.35^{bc}	52.59 ± 2.00^{ab}	53.81 ± 2.60^{a}

PDB – potato dextrose broth

*Composition of culture medium in 1 000 mL distilled water

$^{a–c}$Data followed by the same lowercase letters in the same column show no-significant difference (Tukey test 5%)

Data are given as mean ± SD

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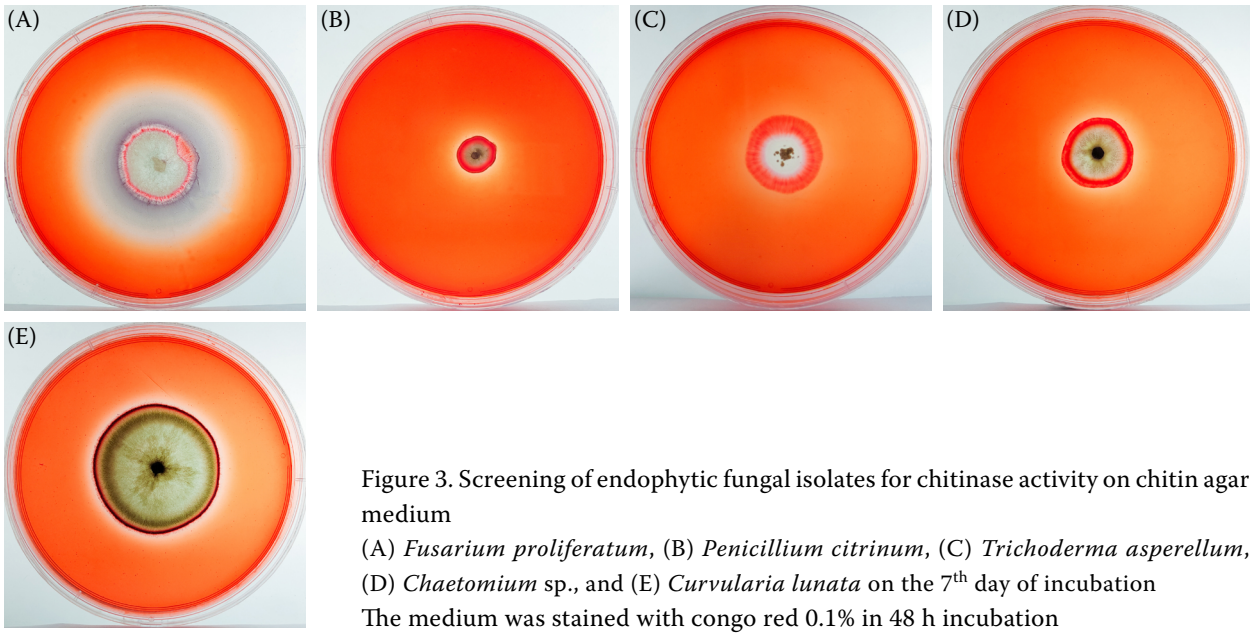

Figure 3. Screening of endophytic fungal isolates for chitinase activity on chitin agar medium
(A) *Fusarium proliferatum*, (B) *Penicillium citrinum*, (C) *Trichoderma asperellum*, (D) *Chaetomium* sp., and (E) *Curvularia lunata* on the 7th day of incubation
The medium was stained with congo red 0.1% in 48 h incubation

fungal isolate from day 5 to day 7 is presented in Table 4. Overall, the chitinolytic index in five endophytic fungal isolates did not significantly increase from day 5 to day 7. Based on statistical analysis, *F. proliferatum* was the isolate that had the highest chitinolytic index, followed by *Chaetomium* sp. Meanwhile, *P. citrinum*, *T. asperellum*, and *C. lunata* had chitinolytic indexes that were not significantly different.

Endophytic fungi were known to have chitinolytic activity by producing metabolites such as chitinase enzymes. Malto et al. (2019) reported that endophytic fungi could degrade chitin within 3–5 days after incubation and were potential sources of chitinase. Several groups of endophytic fungi have been shown *in vitro* to produce high chitinase activity, such as *Trichoderma* spp. (Dolatabad et al. 2017; Prasetyawan et al. 2017), *Chaetomium globosum* (Dolatabad et al. 2017). Rusmarini et al. (2017) proved that enzymes degrading cell walls such as chitinase play a significant role in inhibiting the growth of pathogenic fungi.

Screening for IAA production

Endophytic fungal isolates grown for seven days on PDB medium with the addition of L-tryptophan produced IAA at different concentrations. The filtrate of each endophytic fungus culture reacted with Salkowsky's reagent produced a red or pink color, which indicated the presence of IAA compounds. The highest average IAA production was found in *P. citrinum*, 63.10 ppm, and was statistically significantly different from the other four isolates (Figure 4). The lowest IAA production was by *Chaetomium* sp., 4.08 ppm, but not significantly different from *F. proliferatum*, *T. asperellum*, and *C. lunata*. According to Numponsak et al. (2018), L-tryptophan concentration, room temper-

Table 4. Chitinolytic index of endophytic fungal isolates

Endophytic fungal isolates	Chitinolytic index on days of incubation		
	5th	6th	7th
Fusarium proliferatum	1.46 ± 0.37^{a}	1.49 ± 0.39^{a}	1.54 ± 0.38^{a}
Penicillium citrinum	1.12 ± 0.02^{a}	1.13 ± 0.02^{a}	1.13 ± 0.03^{b}
Trichoderma asperellum	1.11 ± 0.04^{a}	1.12 ± 0.04^{a}	1.13 ± 0.04^{b}
Chaetomium sp.	1.18 ± 0.02^{a}	1.19 ± 0.02^{a}	1.20 ± 0.02^{ab}
Curvularia lunata	1.11 ± 0.03^{a}	1.11 ± 0.02^{a}	1.12 ± 0.02^{b}

^{a,b}Data followed by the same lowercase letters in the same column show no-significant difference (Tukey test 5%)
Data are given as mean ± SD

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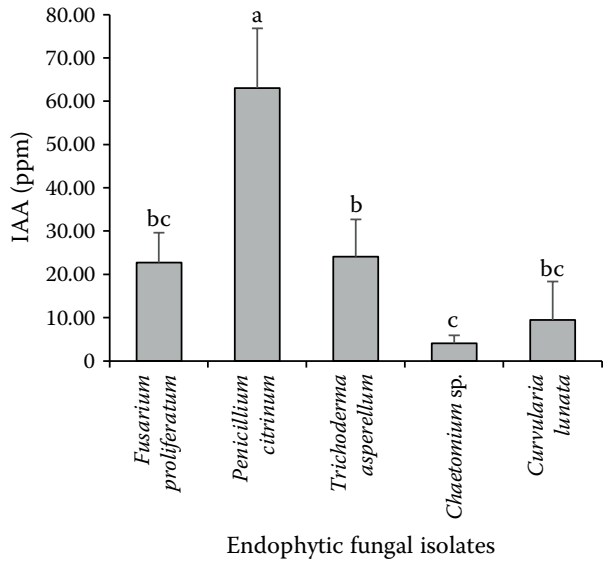

Figure 4. IAA production of endophytic fungal isolates
Data are mean of four replications with SD bar (mean ± SD)
[a–c]Bars followed with different letters are significantly different according to Tukey test 5%

ature, and incubation period of endophytic fungi affected IAA production.

Screening for phosphate solubilization of endophytic fungal isolates

The test results showed four isolates of endophytic fungi that could solubilize phosphate on Phikovskaya's agar medium, indicated by the appearance of a clear zone around the colony culture (Figure 5). One isolate, *T. asperellum*, did not have phosphate solubilization ability. The phosphate solubilization index and solubilization efficiency measured from days 5–7 are presented in Table 5. The solubilization efficiency of *F. proliferatum* was low from day 5 to day 7. Meanwhile, the solubilization efficiency of *P. citrinum* was medium on the 5–7th day. *Chaetomium* sp. had a low category of solubilization efficiency on the fifth and sixth days, then turned moderate on the seventh day. The highest phosphate solubilization index was 2.37 from *P. citrinum*, measured on the sixth day, and 2.12 from *C. lunata* on the seventh day, with a medium category of solubilization efficiency.

Root colonization by endophytic fungal isolates

The percentage of endophytic fungi colonization on roots and their effect on root length are presented in Table 6. Overall, the percentage of endophytic fungi colonization on root hairs was higher than in lateral roots. Statistical analysis showed that the highest percentage of root colonization in both root hairs and lateral roots was found in *T. asperelellum* treatment, 65% and 55%, respectively. In *C. lunata* treatment, the percentage of root hair colonization was 50% and 20% in lateral roots. While in *Chaetomium* sp. treatment, the percentage of root hair colonization was 45% and 25% on lateral roots. The high percentage of root colonization seems to be in line with root length. The average root length of seedlings in the treatment of *T. asperellum* was also the highest and significantly different from other isolates, 28.23 cm.

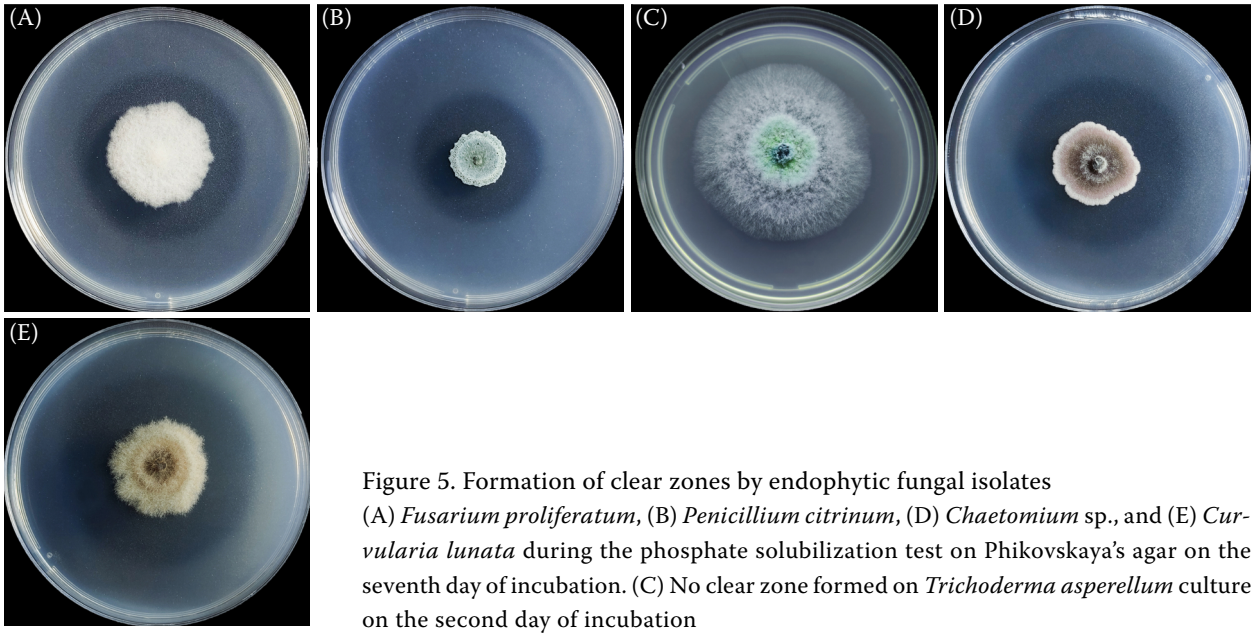

Figure 5. Formation of clear zones by endophytic fungal isolates
(A) *Fusarium proliferatum*, (B) *Penicillium citrinum*, (D) *Chaetomium* sp., and (E) *Curvularia lunata* during the phosphate solubilization test on Phikovskaya's agar on the seventh day of incubation. (C) No clear zone formed on *Trichoderma asperellum* culture on the second day of incubation

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Table 5. Phosphate solubilization index and efficiency of endophytic fungal isolates

Endophytic fungal isolates	Solubilization index and efficiency in days of incubation					
	5th		6th		7th	
	solubilization index	efficiency	solubilization index	efficiency	solubilization index	efficiency
Fusarium proliferatum	1.63 ± 0.10[b]	low	1.71 ± 0.09[b]	low	1.72 ± 0.11[c]	low
Penicillium citrinum	2.35 ± 0.25[a]	medium	2.37 ± 0.25[a]	medium	2.36 ± 0.13[a]	medium
Trichoderma asperellum	0.00 ± 0.00[c]	–	0.00 ± 0.00[c]	–	0.00 ± 0.00[d]	–
Chaetomium sp.	1.45 ± 0.09[b]	low	1.63 ± 0.17[b]	low	2.03 ± 0.07[b]	medium
Curvularia lunata	2.05 ± 0.11[a]	medium	2.10 ± 0.12[a]	medium	2.12 ± 0.15[b]	medium

[a–c]Data followed by the same lowercase letters in the same column show no-significant difference (Tukey test 5%)
Solubilization efficiency: low (IS < 2), medium (2 ≤ IS < 4) and high (IS > 4)
Data are given as mean ± SD

Table 6. Root length and percentage colonization of roots at two months post inoculation

Endophytic isolates treatment	Root length (cm)	Root colonization (%)	
		hairy root	lateral root
Fusarium proliferatum	23.63 ± 1.14[bc]	35.00[b]	15.00[ab]
Penicillium citrinum	24.70 ± 0.68[bc]	30.00[b]	10.00[b]
Trichoderma asperellum	26.43 ± 0.92[a]	65.00[a]	55.00[a]
Chaetomium sp.	25.28 ± 0.36[b]	45.00[ab]	25.00[ab]
Curvularia lunata	23.05 ± 0.52[c]	50.00[ab]	20.00[ab]
Control	15.65 ± 0.72[d]	0[c]	0[b]

[a–d]Data followed by the same lowercase letters in the same column show no-significant difference (Tukey test 5%)
Data are given as mean ± SD

Roots of seedlings inoculated with endophytic fungal isolates were longer than control (Figure 6). According to Pal et al. (2020), the successful colonization of root-origin endophytic fungi was often observed in the intercellular portions of the epidermis and the outer cortical regions in cross-sections. In some parts of the root, endophytes appear attached to the host cell wall or root epidermis.

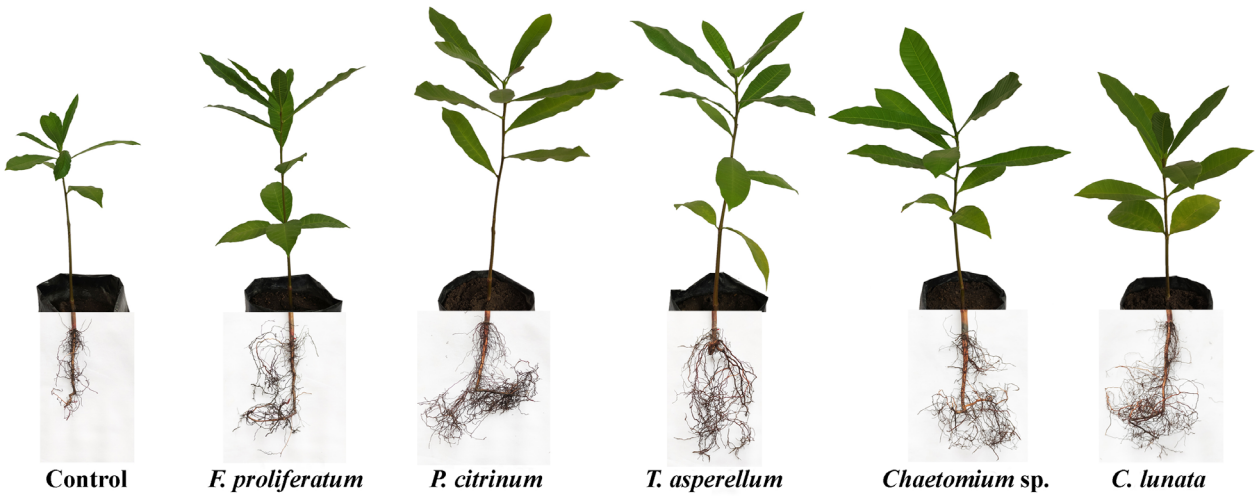

Figure 6. Effect of endophytic fungal isolates on cashew seedlings at two months post inoculation of colonization root test
Each plant represents four replications of six treatments

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In planta test of endophytic fungi in the greenhouse

Disease incidence and severity and effectiveness of disease control. The application of endophytic fungal isolates on cashew saplings was proven to suppress the incidence and severity of white root disease, although the average percentage of disease incidence was still above 50%. The average of disease incidence in *F. proliferatum*, *P. citrinum*, and *C. lunata* treatments were 91.67%, 66.67%, and 75.00%, respectively. The highest suppression of disease incidence occurred in saplings treated with *T. asperellum* and *Chaetomium* sp. with the same incidence percentage, 58.33%, and significantly different from the control (Table 7). Based on statistical analysis, the average percentage of disease severity in all treatments of endophytic fungal isolates was significantly different from the control. The lowest average percentage of disease severity was in *T. asperellum* treatment, 15.62%, followed by *Chaetomium* sp., *P. citrinum*, *C. lunata*, and *F. proliferatum* treatments with 18.75%, 29.16%, 31.24%, and 41.66%, respectively. Thus, the best control effectiveness was also shown by *Trichoderma asperellum* and *Chaetomium* sp., at 83.52% and 80.30%, respectively.

Measurement of PPO activity. The analysis results showed that the increase of PPO activity in plant roots inoculated with endophytic fungal isolates was higher than the control (inoculated with *Rigidoporus* sp. without endophytic inoculation) (Table 8). The highest PPO activity was found in *T. asperellum* treatment, reaching 0.096 UA/g, followed by *Chaetomium* sp. (0.050 UA/g), *F. proliferatum* (0.047 UA/g), *C. lunata* (0.037 UA/g), and *P. citrinum* (0.035 UA/g). The lowest PPO activity was control (0.014 UA/g), and it indicates that the treatment of endophytic fungal isolates increased PPO activity which functioned to inhibit the pathogen infection process.

Table 8. Polyphenol oxidase (PPO) activity of plant root inoculated with endophytic fungal isolates and *Rigidoporus* sp.

Treatment	Protein (mg/mL)	PPO (UA/g)
Fusarium proliferatum + *Rigidoporus* sp.	1.762 0	0.047
Penicillium citrinum + *Rigidoporus* sp.	4.818 0	0.035
Trichoderma asperellum + *Rigidoporus* sp.	1.732 1	0.096
Chaetomium sp. + *Rigidoporus* sp.	0.663 7	0.050
Curvularia lunata + *Rigidoporus* sp.	2.269 3	0.037
Control*	4.841 8	0.014

*Plants were inoculated with *Rigidoporus* sp. without endophytic fungal isolates

Measurement of malondialdehyde (MDA) concentration. The application of endophytic fungal isolates could suppress lipid peroxidation in plant roots, indicated by lower MDA concentrations in all plants treated with endophytic fungal isolates compared to control (Table 9). The highest MDA concentration was in control (endophytic isolate non-treated), while the lowest MDA concentration was found in *C. lunata* and *T. asperellum* treatments, which were 0.011 8 mol/g and 0.014 3 mol/g, respectively. The concentration of MDA in *P. citrinum*, *F. proliferatum*, and *Chaetomium* sp. were 0.021 4 mol/g, 0.042 7 mol/g, and 0.046 3 mol/g, respectively. According to Davey et al. (2005), MDA concentrations varied in response to biotic and abiotic stress in plants.

Table 7. Disease incidence and severity

Endophytic isolates treatment	Disease incidence (%)	Disease severity (%)	Control effectiveness (%)
Fusarium proliferatum	91.67 ± 6.66^{ab}	41.66 ± 16.67^{b}	56.82 ± 15.73^{b}
Penicillium citrinum	66.67 ± 27.22^{ab}	29.16 ± 17.35^{bc}	69.13 ± 19.04^{ab}
Trichoderma asperellum	58.33 ± 16.67^{b}	15.62 ± 4.96^{c}	83.52 ± 5.72^{a}
Chaetomium sp.	58.33 ± 16.67^{b}	18.75 ± 7.98^{bc}	80.30 ± 8.50^{ab}
Curvularia lunata	75.00 ± 16.67^{ab}	31.24 ± 4.17^{bc}	67.24 ± 5.37^{ab}
Control	100.00 ± 0^{a}	95.83 ± 4.81^{a}	0.00 ± 0.00^{c}

$^{a–c}$Data followed by the same lowercase letters in the same column show no-significant difference (Tukey test 5%)
Data are given as mean ± SD

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Table 9. Malondialdehyde (MDA) concentration of plant root inoculated with endophytic fungal isolates

Endophytic isolates treatment	MDA (µmol/g)
Fusarium proliferatum	0.042 7
Penicillium citrinum	0.021 4
Trichoderma asperellum	0.014 3
Chaetomium sp.	0.046 3
Curvularia lunata	0.011 8
Control*	0.079 7

*Without inoculation of endophytic fungal isolates

Plant growth. The results of the measurement of the sapling's height in each treatment are presented in Table 10. Height measurements were carried out from one to four MPI. In the second experiment (endophytic application and pathogen inoculation), statistical analysis showed that the mean of plant height of all endophytic fungi treatments at 1 MPI was not significantly different from control, as well as at 2 MPI. The sapling height of *T. asperellum* treatment was significantly different from control at 3 and 4 MPI but not significantly different from the other four endophytic fungi treatments. In the first experiment (the only endophytic application without pathogen inoculation), statistical analysis showed that two treatments were significantly different from control at 1 and 2 MPI, namely *F. proliferatum* and *Chaetomium* sp. Meanwhile, at 3 and 4 MPI, *Chaetomium* sp. treatment is the only treatment that is significantly different from control. The treatments of other endophytic isolates were not significantly different from each other.

The effect of the treatment of endophytic fungal isolates on the wet and dry weight of cashew root is presented in Table 11. The wet and dry weights of roots inoculated with endophytic fungi were higher than the control. In the first experiment, the highest wet and dry weight of roots was found in *Chaetomium* sp. treatment, 72.18 g, and 39.28 g, respectively. The second experiment had the highest wet and dry weights in *T. asperellum* treatment, 68.01 g, and 37.73 g, respectively.

DISCUSSION

We identified the causal agent of the disease, namely the fungus *Rigidoporus* sp., which is also the cause of white root disease in several other tropical plantation crops. This fungus infects plant roots with its mycelium threads (rhizomorphs). Rhizomorphs attach and spread along the root surface. According to Mohammed et al. (2014), this fungal rhizomorph

Table 10. Plant height after endophytic fungal isolates and *Rigidoporus* sp. inoculation in the greenhouse

Endophytic isolates treatment	Plant height (cm) at months post-inoculation (MPI)				
	0 MPI	1 MPI	2 MPI	3 MPI	4 MPI
Experiment 1 (without inoculation of *Rigidoporus* sp.)					
Fusarium proliferatum	62.67 ± 6.40^{a}	99.17 ± 17.18^{a}	108.50 ± 18.6^{a}	115.25 ± 21.30^{ab}	131.25 ± 26.48^{ab}
Penicillium citrinum	62.17 ± 12.43^{a}	90.09 ± 23.34^{ab}	101.42 ± 29.34^{ab}	115.17 ± 37.25^{ab}	135.50 ± 36.47^{ab}
Trichoderma asperellum	58.92 ± 8.77^{a}	85.50 ± 18.38^{ab}	94.58 ± 19.31^{ab}	103.00 ± 12.26^{ab}	115.67 ± 15.76^{ab}
Chaetomium sp.	63.58 ± 5.92^{a}	98.92 ± 12.05^{a}	109.17 ± 13.86^{a}	127.08 ± 23.46^{a}	151.17 ± 24.63^{a}
Curvularia lunata	54.58 ± 9.93^{a}	57.17 ± 13.14^{b}	62.92 ± 16.43^{b}	71.25 ± 18.66^{b}	93.50 ± 25.43^{b}
Control	54.25 ± 5.60^{a}	56.75 ± 6.38^{b}	59.75 ± 8.31^{b}	72.58 ± 9.67^{b}	88.42 ± 16.61^{b}
Experiment 2 (with inoculation of *Rigidoporus* sp.)					
Fusarium proliferatum	72.50 ± 13.91^{a}	103.08 ± 28.83^{a}	118.09 ± 28.00^{a}	130.67 ± 20.08^{ab}	144.92 ± 28.40^{ab}
Penicillium citrinum	68.58 ± 18.80^{a}	76.25 ± 23.65^{a}	87.17 ± 30.91^{a}	99.08 ± 31.75^{b}	111.67 ± 34.77^{ab}
Trichoderma asperellum	74.25 ± 6.14^{a}	110.00 ± 21.30^{a}	124.83 ± 18.56^{a}	149.92 ± 28.79^{a}	159.08 ± 26.75^{a}
Chaetomium sp.	74.67 ± 3.02^{a}	108.92 ± 11.42^{a}	122.17 ± 14.51^{a}	132.08 ± 19.70^{ab}	141.67 ± 20.16^{ab}
Curvularia lunata	68.42 ± 11.36^{a}	86.67 ± 13.93^{a}	98.17 ± 17.95^{a}	104.42 ± 17.88^{ab}	108.67 ± 19.76^{ab}
Control	68.75 ± 1.89^{a}	73.33 ± 3.16^{a}	79.84 ± 3.18^{a}	84.25 ± 3.18^{b}	95.17 ± 4.15^{b}

^{a,b}Data followed by the same lowercase letters in the same column show no-significant difference (Tukey test 5%)
Data are given as mean ± SD

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Table 11. Root weight after inoculation with endophytic fungal isolates and *Rigidoporus* sp.

Endophytic fungal isolates treatment	Root weight (g)	
	fresh weight	dry weight
Experiment 1 (without inoculation of *Rigidoporus* sp.)		
Fusarium proliferatum	44.36 ± 13.99^{ab}	20.47 ± 4.95^{ab}
Penicillium citrinum	46.46 ± 21.03^{ab}	22.02 ± 9.88^{ab}
Trichoderma asperellum	47.67 ± 9.65^{ab}	25.07 ± 7.92^{ab}
Chaetomium sp.	72.18 ± 43.36^{a}	39.28 ± 26.96^{a}
Curvularia lunata	32.38 ± 5.03^{ab}	15.67 ± 5.46^{ab}
Control	21.95 ± 3.51^{b}	10.15 ± 1.62^{b}
Experiment 2 (with inoculation of *Rigidoporus* sp.)		
Fusarium proliferatum	44.27 ± 9.21^{ab}	22.43 ± 6.65^{ab}
Penicillium citrinum	29.77 ± 17.82^{ab}	13.32 ± 8.50^{b}
Trichoderma asperellum	68.01 ± 35.57^{a}	37.73 ± 17.99^{a}
Chaetomium sp.	50.34 ± 30.46^{ab}	24.23 ± 15.12^{ab}
Curvularia lunata	25.82 ± 2.86^{ab}	11.67 ± 2.49^{b}
Control	19.13 ± 3.53^{b}	8.26 ± 1.33^{b}

^{a,b}Data followed by the same lowercase letters in the same column show no-significant difference (Tukey test 5%)
Data are given as mean ± SD

requires nutrients generally sourced from tree stumps, other dead wood, or live tree roots that have been successfully infected. According to Wu et al. (2017), fungi from the *Rigidoporus* group and other morphologically identical genera (such as *Physisporinus* and *Oxyporus*) have been widely reported as pathogens of plantation crops. The fungi in this group are wood decay fungi that have essential ecological functions and economic ones. Nonetheless, there has been no report of white root disease on cashew plants outside of Indonesia so far.

As a significant disease of cashew, the disease has long been in the spotlight of researchers. Various control methods have been conducted, but they have not been effective in controlling this disease. The study's findings revealed that five endophytic fungal isolates investigated in this study have characteristics that could be used as biological control agents. These five isolates have antagonistic roles against pathogenic *Rigidoporus* sp., according to the results of *in vitro* tests. Endophytic fungal isolates are potential biological agents, indicated by chitinolytic activity and the ability to produce VOCs. The isolates also can produce IAA and solubilizing phosphate, which are very important for plant growth.

There was not a clear inhibition zone on each isolate when co-cultured with *Rigidoporus* sp., indicating that the antagonism between endophytic fungal isolates and *Rigidoporus* sp. was most probably a result of direct contact and competition for nutrient uptake and space. Five endophytic fungal isolates had three types of interaction when co-cultivated with *Rigidoporus* sp. *T. asperellum* and *C. lunata* showed a more aggressive antagonistic mechanism than the other three isolates. The colony growth of the two isolates dominated the PDA medium and could even grow on the surface of the pathogenic colony so that the growth of *Rigidoporus* sp. be significantly hindered. This finding is attributed to the ability of the two isolates to produce lytic enzymes that can degrade the pathogen's cell wall and enable the isolates to colonize the pathogen's colony. It is similar to *Xylaria* sp. against *Fusarium solani*, reported by Hamzah et al. (2018). *F. proliferatum*, *P. citrinum*, and *T. asperellum* also produced yellow pigmentation when their mycelia contacted with *Rigidoporus* sp. in the dual culture plates. Some endophytic fungi produced natural pigments such as melanins by *Spissiomyces endophytica* (Suwannarach et al. 2019), red pigments by *Aspergillus versicolor* (Sibero et al. 2017) and *Talaromyces assiutensis* (Mishra et al. 2021). These pigments are linked to important functions such as antimicrobial activity.

Furthermore, endophytic fungi can produce a combination of VOC molecules that act as antifungals and pathogen defenses (Hung et al. 2015; Kaddes et al. 2019). Five isolates of endophytic fungi tested in this study produced VOCs with inhibitory abilities ranging from 42–77%. The isolate with the highest level of inhibition was *T. asperellum*. Several endophytic fungi have been reported to produce VOCs, including the strain *Trichoderma asperellum* T1 (Wonglom et al. 2020), which has been proven to suppress pathogen development, induce resistance and stimulate plant growth. Plaszkó et al. (2020) succeeded in identifying the composition of VOCs from the endophytic fungi *Fusarium* sp. and *Curvularia* sp., which is an antimicrobial compound and a carbon source. Previously, Zhang et al. (2014) found that the isolate *Fusarium oxysporum* CanR-46 was detected to produce VOCs that strongly inhibited *S. sclerotiorum* and *B. cinerea*. The endophytic fungus *Penicillium* sp. was also shown to produce VOCs with 59.72% inhibition against the pathogen *F. oxysporum* (Cosoveanu et al. 2016).

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The results of this study showed that all endophytic fungal isolates produced chitinase. The ability of the isolates to degrade chitin on chitin agar media depends on the type of endophytic fungus and the amount of chitinase enzyme it produces. Chitin is found in many groups of fungi, including Basidiomycetes, and is one of the components that make up mycelia and spores' membrane structure and cell walls (Elsoud & El Kady 2019). Fungal cell walls are an essential fungi part of the interaction of fungi with biotic and abiotic environments (Ehren et al. 2020), especially in fungal pathogens, so that the degradation of one of its constituent compounds becomes a controlling strategy in the field. When contact occurs, endophytic fungi will degrade the pathogen's cell wall by involving the hydrolytic enzymes it produces, such as chitinase. According to Kumar et al. (2018), besides improving the plant defense system because it degrades chitin as the main component of the cell wall of fungal pathogens, chitinase also increases plant growth and yield without any negative impact on plants.

Penicillium citrinum produced the highest levels of IAA, which was 63.10 ppm. Some endophytic fungi from the genus *Penicillium* were also reported as IAA producing fungi, such as *Penicillium roqueforti* (Ikram et al. 2018). *P. ruqueforti* induced resistance in wheat plants and limited heavy metal transfer from soil to plants by secreting IAA. Besides, Chand et al. (2020) reported that several endophytic fungi from the genus *Fusarium*, namely *F. tricinctum*, *F. solani*, and *F. oxysporum*, could synthesise IAA *in vitro* either with L-tryptophan at different concentrations or without L-tryptophan at all as a precursor.

In addition to producing IAA, the *in vitro* test showed that four isolates of endophytic fungi could solubilize phosphate in Phikovskaya's agar medium. Microorganisms (such as endophytic fungi) depended on their ability to produce organic acids to solubilize insoluble phosphate in soil (Adhikari & Pandey 2019). These organic acids decreased soil pH and converted the insoluble metal phosphate component to potassium or sodium, resulting in soluble phosphate salts. According to Varga et al. (2020), phosphorus (P) compounds were essential nutrients for plant growth, but they were relatively inaccessible to plants due to their chemical characteristics. Most of the P in the soil was available in the form of phosphate (usually a complex metal) so the compound was bound to organic and mineral matter. Therefore, the solubilizing of phosphate by endophytic fungi associated with plants is a necessary process, significantly increasing plant growth. Several previous studies had also proven that endophytic fungi could solubilize phosphate. Hakim et al. (2012) reported that the endophytic fungus *Trichoderma spirale* isolated from roots could solubilize phosphate *in vitro*. Priyadharsini and Muthukumar (2017) also reported that the endophytic fungus *Curvularia geniculate* from the roots of *Parthenium hysterophorus* could increase plant growth through phosphate solubilization.

The success of endophytic fungi colonization in root tissue in this study proved that endophytic fungi from roots were stable and had an incredible impact on the plants, even though they were introduced into root tissues that were not their original hosts. Geisen et al. (2017) reported that most endophytic fungi from root tissue were unlikely pathogenic to plants, whereas most endophytic fungi isolated from seeds were potentially pathogenic. The five endophytic fungal isolates used in this study were class-4 endophytic fungi based on Rodriguez et al. (2009) classification. Class-4 endophytic fungi come primarily from the ascomycetes group, and their colonization is limited to plant root tissue. Endophytes of this group also have a broad host range, high colonization frequency, and horizontal transmission. The ability of endophytic fungal isolates to colonize cashew roots appeared to be positively correlated with the suppression of white root disease. The percentage of root colonization in plants treated with *T. asperellum* was above 50% in both hair roots and lateral roots, in line with the lowest disease severity compared to other isolates. It is essential to conduct *in planta* experiments in a greenhouse to determine the effectiveness of endophytic fungal isolates in suppressing the pathogen *Rigidoporus* sp. on cashew saplings. This study proved that applying endophytic fungal isolates twice with a concentration of 10^5 conidia/mL was quite effective in suppressing the pathogen *Rigidoporus* sp. These results align with *in vitro* tests, which show that various control mechanisms can occur between endophytic fungal isolates and pathogens. The findings of this study also support the role of endophytic fungi in inducing enzymes involved in plant defense mechanisms, such as PPO, against invading pathogens. The increase in PPO activity in plant roots treated with endophytic fungal isolates was in line with the results of previous

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studies on plants infected with the pathogen. Yuan et al. (2017) reported that treatment of endophytic isolates CEF-818 (*Penicillium simplicissimum*) and CET-714 (*Leptosphaeria* sp.) increased PPO activity, resulting in improved resistance of cotton plants to Verticilium wilt disease. Bilal et al. (2017) reported that inoculation of endophytic isolate *Paecilomyces formosus* LHL10 in soybean plants significantly induced antioxidant activity such as PPO. Shobha and Mahadeva (2018) also found an increase in PPO activity in pepper plants inoculated with the endophytic fungal isolates *Trichoderma harzianum* Th16 and Th5. According to Li et al. (2017), the activity of enzymes related to plant resistance, such as PPO, has a positive relationship with the level of expression of defense genes.

Furthermore, endophytic fungal isolates have great potential to reduce the production of MDA compounds on cashew in the greenhouse. Plants under various stress conditions such as drought will induce oxidative stress caused by reactive oxygen species (ROS). ROS at high concentrations is free radicals that are very harmful to the organism. Increasing ROS cause oxidative damage to lipids, proteins, and DNA (Sharma et al. 2012). Lipids are one of the main cellular components susceptible to ROS, so lipid peroxidation measurements can be used to see the formation of these ROS. El-Beltagi and Mohamed (2013) stated that lipid peroxidation was a precise mechanism of cell damage so that it can be used as an indicator of oxidative stress in cells and tissues of plants. MDA is a secondary metabolite of lipid peroxidation that can be used as a marker of oxidative stress in plants (Ladhari et al. 2020). Lipid peroxidation can be measured by calculating the content of malondialdehyde (MDA), both from plant tissues such as roots (Savicka & Škute 2010), leaves (Morales & Munné-Bosch 2019), and all plant parts (Ladhari et al. 2020).

The application of endophytic fungal isolates on cashew saplings affected plant growth, such as plant height and root weight, although not all isolates had a significant effect. In general, the plant height and root weight of saplings inoculated by endophytic fungal isolates were better than controls. It is undoubtedly related to the production and induction of growth hormones such as IAA and endophytic fungal isolates' ability to solubilize phosphate. The results of this study indicate that the application of endophytic fungal isolates to perennial tropical plants such as cashew has a positive effect on increasing root biomass so that it can help plants absorb water and nutrients more optimally and increase plant fitness. According to an analysis conducted by Dastogeer (2018), the presence of endophytic fungi increased root biomass in dicotyledonous plants, and the effect was significantly higher than in monocotyledonous plants under non-stressed conditions. Furthermore, root biomass was significantly increased in perennial plants under non-stressed conditions.

In short, five endophytic fungal isolates tested have inhibition activity against *Rigidoporus* sp. *in vitro* and *in planta*, produce VOCs, have chitinolytic activity, produce IAA, and solubilize phosphate. The isolates that could suppress disease incidence and severity with the highest disease control effectiveness (above 80%) and stimulate plant growth are *T. asperellum* and *Chaetomium* sp. The effectiveness of endophytic fungal isolates is influenced by the increased activity of PPO as a defense enzyme, thereby inducing systemic resistance to white root disease and promoting plant growth. The endophytic fungal isolates also reduce the concentration of MDA, which is harmful to plants.

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