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Metabolites Profiling of *Penicillium citrinum* Recovered from Endophytic of Ramie (*Boehmeria nivea*) as a Potential Biocontrol Against Pathogenic Fungi

Kartika Sari[1], Vira Kusuma Dewi[2], Asri Peni Wulandari[1,3*], Nia Rossiana[1], Tati Herlina[4], Fitri Widiantini[2]

[1]*Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Sumedang, Indonesia*
[2]*Department of Plant Pests and Diseases, Faculty of Agriculture, Padjadjaran University, Sumedang, Indonesia*
[3]*Center for Bioprospection of Natural Fibers and Biological Resources, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Sumedang, Indonesia*
[4]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Sumedang, Indonesia*

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ABSTRACT

Endophytes are a potent source of bioactive compounds that mimic plant-based metabolites. Fungi *Fusarium* spp. and *Clonostachys rosea* have been identified as pathogenic microorganisms in ramie (*Boehmeria nivea*). The antifungal test was carried out using the diffusion method and the MIC50 and Minimum Fungicidal Concentration (MFC) values using the tested microbial pathogens were *Fusarium solani* isolate 3248941, *F. solani* isolate Colpat-359, *F. oxysporum* isolate N-61-2, dan *Clonostachys rosea* strain B3042. Identification of secondary metabolites of the extract was carried out using GC-MS. The chromatogram of GC-MS analysis of this ethyl acetate extract (EA) showed seven dominant chemical compounds with various biological activities. Ethyl asetate extract of *P. citrinum* showed inhibition zone ranged from 19.10–22.07 mm with strong-very strong category at 200 mg ml^{-1} concentration against the tested microbial pathogens. Results revealed that the significant MIC values were observed against *F. solani* isolate 3248941 and *Clonostachys rosea* strain B3042 by less than 6.3 mg ml^{-1} and against *F. solani* isolate Colpat-359 and *F. oxysporum* isolate N-61-2 with 12.5 mg ml^{-1} and 25 mg ml^{-1} respectively. Minimum Fungicidal concentrations (MFC) for EA of *P. citrinum* were also reported against *F. solani* isolate 3248941 and *C. rosea* strain B3042, by 12.5 mg ml^{-1}, whilst *Fusarium solani* isolate Colpat-359 by 25 mg ml^{-1} and *Fusarium oxysporum* isolate N-61-2 ,by 50 mg ml^{-1} respectively. Results of this study showed that *P. citrinum*, endophytic fungi of ramie, could be a promising source of compounds for antifungal agents.

1. Introduction

Ramie (*Boehmeria nivea*) is one of the natural fibers used as raw material in the textile industry. The potential for ramie development is quite attractive because of the high fiber requirement. The utilization of ramie fiber is now increasingly widespread for various industrial applications, especially for textiles and their derivative products (TPT). Hanifah and Kartiasih (2018) reported that the Indonesian textile industry uses cotton fiber as the primary raw material. However, during 2000–2014 domestic production could only reach 0.23% of the total domestic cotton fiber demand; 99.77% of the cotton was imported to require the total demand (Hanifah and Kartiasih 2018). Meanwhile, increasing cotton production will be difficult because cotton cultivation requires high production costs, high agronomy risk, a lack of quality seed varieties, and vulnerability to planthopper pests (Novarini and Sukardan 2015). Therefore, there is a need for alternative fibers other than cotton to meet the textile industry's fiber needs. Ramie can be an alternative substitute for cotton as a natural fiber. Ramie also has the main composition of cellulose and better characteristics than cotton, such as fibre length, fiber cell breadth, and tenacity (Banerjee *et al.* 2015). Data from Grand View Research (2021) shows that natural fibers lead the market for textile needs and account for more than 44% share of global

* Corresponding Author
E-mail Address: asri.peni@unpad.ac.id

revenue in 2020. It is estimated that the demand for natural fibers from plants to replace glass fiber for the composite product market in the world can reach 120,000 MT or $214.15 (Novarini and Sukardan 2015). Based on The Discover Natural Fiber, world natural fiber production in 2022 is estimated at 33.7 million tonnes. However, ramie fiber production is estimated at 60,000 tonnes. The need for ramie fiber supply requires readiness in the ramie cultivation system in the field to produce ramie with good fiber quality.

Ramie cultivation is experiencing problems with plant pathogenic microorganisms, that can reduce the quality and quantity of fiber yields. In China, the anthracnose by *Colletotrichum gloeosporioides* was found in approximately 10,000 ha of ramie plantation and caused yield losses in the range of 20 to 55% (Wang *et al.* 2011). Yu *et al.* (2016) reported that the brown root rot by *Pytium vexans* caused yield loss of more than 40% on ramie plantation fields in Hunan Province, China. Previous studies have also found several pathogenic microorganisms in ramie plants, such as *Colletotrichum gloeosporioides* and *Collectotrichum higginsianum*, which causes anthracnose (Wang *et al.* 2010; Wang *et al.* 2011), *Curvularia eragrostidis* leaf blight, *Cercospora boehmerae* causes leaf spot disease, and *Sclerotium* sp. ramie stem disease (Gawande *et al.* 2016).

Various studies have revealed that endophytic fungi have benefit for plant health (Triastuti 2020). *Penicillium citrinum* is an endophytic fungus isolated from plant tissue. Since 2015, experiments have been conducted to determine the ability of *P. citrinum* to increase plant growth and increase the absorption of plant nutrients (Surya Hakim and Yuwati 2020), protect plants against biotic stress (Waqas *et al.* 2015), and protect the host from pathogen attack through the production of antagonist compounds (Toghueo and Boyom 2020). *P. citrinum* endophytes have developed to produce antibiotic compounds and various biological activities have been reported in agriculture, pharmaceutical, and biotechnology applications (Toghueo and Boyom 2020). Previous studies have reported that *P. citrinum* has been isolated and produces metabolites for antibacterial, antifungal, and cytotoxic activities (Khamthong *et al.* 2012), anticancer, antiviral, antioxidant, anti-inflammatory, antidiabetic, anti-obesity, antibiotic, neuroprotective effects, insecticidal, and biocontrol activities (Toghueo and Boyom 2020).

Previous research has been carried out by Triani (2021), which showed the presence of endophytic microbes in various organs of the ramie plant, including roots, stems, leaves, and flowers. The antagonist test showed that all endophytic isolates had the potential to inhibit the growth of ramie pathogenic fungi. The selection of superior isolates with a percentage of 92.07% with the highest inhibition showed that the fungus *Penicillium citrinum* isolate MEBP0017 had the potential to be developed as an antifungal candidate for diseases in ramie plants. In this study, the antifungal test was conducted on the ethyl acetate extract of *Penicillium citrinum* strain MEBP0017 against the ramie pathogenic fungi, including *Fusarium* spp. and *Clonostachys rosea* strain B304. The crude extract was also analyzed for its compounds to determine the active components that have the potential as antifungals against ramie fungi pathogens.

2. Materials and Methods

2.1. Materials

Pure isolates of *Penicillium citrinum* strain MEBP0017 and isolates of pathogens *Fusarium* spp. and *Clonostachys rosea* were obtained from the collection of the Microbiology Laboratory, Department of Biology, FMIPA, Padjadjaran University. The pathogenic fungi of ramie that used in this research were *Fusarium solani* isolate 3248941, *Fusarium solani* isolate Colpat-359, *Fusarium oxysporum* isolate N-61-2, and *Clonostachys rosea* strain B3042. The medium growth that used were Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), and Sabouraud Dextrose Agar (SDA) [Oxoid], Media Roswell Park Memorial Institute (RPMI) 1640 GlutaMAX™ [Gibco, Life Technologies, New York, NY, USA], glucose RPMI-2%, 3-(Nmorpholino) propanesulfonic acid (MOPS), 0.9% NaCl, and Dhitane.

2.2. Extraction of Antimicrobial Metabolites of *Penicillium citrinum*

The extraction method adapted of Li *et al.* (2017) with modification. *Penicillium citrinum*, which has been isolated on a PDA medium for seven days at 28°C, is taken six petri dish measuring 5 × 5 mm using an ose and inserted into 100 ml Czapek's broth medium (30 g L^{-1} $NaNO_3$, 1.0 g L^{-1} KH_2PO_4, 0.5 g L^{-1} KCl, 0.5 g L^{-1} $MgSO_4$ 7.H_2O, 0.01 g L^{-1} $FeSO_4$ 7.H_2O). The samples were stirred using a rotary shaker at 200 rpm for seven days at 28°C (Li *et al.* 2017). 1,000 ml of 96% ethyl acetate (EtOAc) was added. The liquid extract was evaporated using a rotary evaporator to obtain a concentrated

extract of ethyl acetate *Penicillium citrinum* isolate MEBP0017. The extract was then stored at 4°C until used for analysis purposes (Parsaee *et al.* 2013).

2.3. Analysis of Gas Chromatography Mass Spectrometry (GC-MS)

Analysis of GC-MS based on Phuong *et al.* (2018) with modification. Analysis of the compound content in the extract was carried out using Gas Chromatography (Shimadzu Thermo with TG-SQC column; 15.0 m × 0.25 mm × 0.25 µm) and mass spectrophotometer. The initial column conditions were at 30°C and heated to 300°C at 10°C/5 min. High purity helium gas was used as the carrier gas which was conditioned to flow at a rate of 1.0 ml/min in split mode. The mass spectra were recorded in the range 35-400 with an electron impact ionization energy of 70 eV. Identification of compounds was carried out based on the retention time and the area of each peak that appeared and carried out using the Wiley/NIST Library software.

2.4. Antifungal Activity Test

Antifungal activity test was carried out based on Yanti *et al.* (2016). Antifungal activity was tested using the well diffusion method with a hole diameter of 6 mm (Balouiri *et al.* 2016). All pathogenic ramie fungi *Fusarium solani* isolate 3248941, *Fusarium solani* isolate Colpat-359, *Fusarium oxysporum* isolate N-61-2, and *Clonostachys rosea* strain B3042 were subcultured in 4% Sabouraud Dextrose Agar (SDA, Oxoid) for five days at 35°C. Inoculum suspended in a test tube containing 5 ml of sterile 0.9% NaCl solution and vortexed for 15 seconds at 2,500 rpm. The cell density was adjusted to the standard Mcfarland 0.5. After measuring the turbidity according to McFarland standards, pathogenic fungi were inoculated in SDA media by dipping a sterile cotton swab into the inoculum. SDA media inoculated with the test pathogenic fungi suspension is left for 5-15 minutes. Next, holes with a diameter of 6 mm were made in the SDA media using a sterilized cork borer. 50 µl of ethyl acetate extract *P. citrinum* with 100%, 80%, 60%, 40%, and 20% were dripped into each hole. Dithane 1% was used as a positive control and 0.9% NaCl as a negative control. Then the test samples were incubated at 37°C for 3 × 24 hours. The diameter of the clear zone formed around the well was measured with a caliper. The morphological changes of pathogenic fungi also observed with optical microscope (Olympus® model CH30). The category for the inhibition zone is based on Clinical and Laboratory Standart Institute: very strong for diameter (d) >20 mm; medium when (d) 15–19 mm; and weak when (d) <14 mm (Cockerill *et al.* 2012).

2.5. Determination of Minimum Inhibitory Concentration (MIC50%) and Minimum Fungicidal Concentration (MFC)

Determination of the MIC50 value was carried out using the microdilution method based on the method used by Berkow *et al.* (2020). The test was carried out qualitatively on a 96-well microplate with turbidity observations. Antifungal susceptibility testing using RPMI 1640 media (RPMI 2% Glucose Medium). The extract concentrations used to determine the MIC50% were 200–200,000 µg ml^{-1}. The extract inserted into a microtube with 3 ml of RPMI 1640 media and were vortexed until homogeneous. Then, 10 µl inoculum suspension of pathogenic fungi was added to each 96-well plate and were incubated at 28°C for 3 × 24 hours. After incubation, the presence (or absence) of growth was observed visually. The lowest concentration that no visible growth of pathogenic fungi was observed and defined as MIC.

To determine the MFC, the two lowest concentrations of inhibiting fungal pathogens and controls were used in the test. 20 L of the resulting extract with the concentration of MIC determination was sub cultured on Sabouraud dextrose agar (SDA) agar plates. After 72 h of incubation at 28°C, the MCF was determined based on growth control. The minimum fungicide concentration was considered the lowest extract concentration that inhibited the growth of the visible subculture (not producing fungal growth on the solid media used).

3. Results

3.1. GC-MS Analysis of *Penicillium citrinum* Isolate MEBP001 Ethyl Acetate Extract Metabolites

GC-MS chromatogram results of ethyl acetate extract indicated that were seven dominant compounds (Table 1). Seven dominant compounds ware obtained in retention time of 4.183, 18.910, 38.545, 39.084, 6.410, 38.614, 49.225 minutes. The dominant compounds in *Penicillium citrinum* isolate MEBP001 ethyl acetate extract were benzeneacetic acid (17.56%), 1-Hexacosanol (CAS)

Table 1. Compound content from ethyl acetate extract of *Penicillium citrinum*

Retention time	Compound name	Molecular weight (g mol^{-1})	Area (%)	Activity
18.910	Benzeneacetic acid	136	17.56	antioxidant (Abd El-Hady *et al.* 2014), antimicrobial (Kim *et al.* 2004), antitumor (Forte *et al.* 2013)
49.926	1-Hexacosanol (CAS) Hexacosanol-1	382	12.65	antifungal (Brito *et al.* 2022), antioxidant dan antitumor (Fouad *et al.* 2018)
39.084	1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0] nonane	210	9.92	antibacterial (Azman *et al.* 2017), antioxidant, anticancer (Ser *et al.* 2015), anti-biofilm (Nadar Rajivgandhi *et al.* 2020)
59.895	Ergost-25-ene-3,5,6,12-tetrol, (3.beta.,5. alpha.,6. beta.,12.beta.)-(CAS)	449	7.43	antibacterial (Akbar *et al.* 2020; Fajrih *et al.* 2022), antioxidant (Chernukha *et al.* 2021)
38.545	Hexadecanoic acid, methyl ester (CAS) Methyl palmitate	270	7.42	antifungal, antioxidant (Pinto *et al.* 2017), anti-inflammatory (Saeed *et al.* 2012)
4.183	Acetic acid, butyl ester (CAS) *n*-Butyl acetate	116	7.07	antimicrobial (Lens *et al.* 2016)
6.410	Ethanol, 2-butoxy-	118	6.80	antimicrobial (Abu-gharbia *et al.* 2020)

Hexacosanol-1 (12.65%), 1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonane (9.92%), Ergost-25-ene-3,5,6,12-tetrol, (3.beta.,5.alpha.,6.beta.,12.beta.)-(CAS) (7.43%), Hexadecanoic acid, methyl ester (CAS) Methyl palmitate (7.42%), Acetic acid, butyl ester (CAS) *n*-Butyl acetate (7.07%), and Ethanol, 2-butoxy-(6.80%).

3.2. Antifungal Activity of *Penicillium citrinum* Ethyl Acetate Extract

The antifungal activity of the ethyl acetate extract of *P. citrinum* against *Fusarium* spp. and *Clonostachys rosea* showed inhibitory activity against the growth of these pathogenic fungi (Table 2).

EA samples showed very strong inhibition activity (diameters >20 mm) for *F. solani* isolate 3248941, *F. oxysporum* isolate N-61-2, and *Clonostachys rosea* strain B3042 at concentration of 20%. The higher extract concentration can increase the inhibitory activity until it reaches the average clear zone in the diameter range of 29.05-31.46 mm.

3.3. MIC50 of Ethyl Acetate Extract Against Pathogenic Fungi of Ramie Plant

The results of the dilution analysis to show the MIC can be shown in Table 3. The MIC50 for *F. solani* isolate 3248941, *F. solani* isolate Colpat-359, *F. oxysporum* isolate N-61-2, and *Clonostachys rosea* strain B3042 were obtained respectively of 6.3, 12.5, 25, and 6.3 mg ml^{-1}.

3.4. Minimum Fungicidal Concentration (MFC)

The results of the MFC test are shown in Figure 1 by observing the growth of pathogenic fungi for 48 hours based on the control and treatment of the ethyl acetate extract of the *P. citrinum* metabolite at a concentration without colony growth. The MFC values of *Fusarium solani* isolate 3248941 and *Clonostachys rosea* strain B3042 could be determined at a concentration of 1.25% (12.5 mg L^{-1}), while *Fusarium solani* isolate Colpat-359 at a concentration of 2.5% (25 mg L^{-1}), and *Fusarium oxysporum* isolate N- 61-2 at a concentration of 5% (50 mg ml^{-1}).

Table 2. Inhibition zone of ethyl acetate extract *Penicillium citrinum* against pathogenic fungi of ramie plant

Samples	Concentration (%)	Inhibition zone diameter (mm)			
		Fusarium solani isolate 3248941	*Fusarium solani* isolate colpat-359	*Fusarium oxysporum* isolate N-61-2	*Clonostachys rosea* strain B3042
Ethyl acetate extract	100	30.57±2.13	29.05±1.61	29.80±0.34	31.46±0.57
	80	29.23±1.56	26.94±1.30	27.77±0.48	30.07±0.84
	60	25.43±2.29	24.70±1.47	25.19±0.50	27.97±1.55
	40	23.66±3.25	22.93±2.42	22.20±0.83	25.92±0.62
	20	21.31±0.56	19.10±1.10	20.80±0.74	22.07±0.04
Dithane (+)		31.46±0.61	33.97±1.04	33.81±1.32	35.04±1.04
NaCl (-)		-	-	-	-

Table 3. The results of MIC50 determination ethyl acetate extract of *Penicillium citrinum* against pathogenic fungi of ramie plant

Concentrations (mg/ml)	Pathogenic fungi of ramie plant			
	Fusarium solani isolate 3248941	*Fusarium solani* isolate colpat-359	*Fusarium oxysporum* isolate N-61-2	*Clonostachys rosea* strain B3042
0.2	+	+	+	+
0.4	+	+	+	+
0.8	+	+	+	+
1.6	+	+	+	+
3.1	+	+	+	+
6.3	-	+	+	-
12.5	-	-	+	-
25.0	-	-	-	-
50.0	-	-	-	-
100.0	-	-	-	-
200.0	-	-	-	-
Control (+)	+	+	+	+
Control (-)	-	-	-	-

Symbol (+): the liquid looks turbid in 96-well, which means the fungi is still growing. Symbol (-): the liquid in the 96-well begins to decrease in turbidity, which means the growth of pathogenic fungi is inhibited. Control (+): positive control containing fungi media and suspension. Control (-): negative control contained the extract according to the concentration

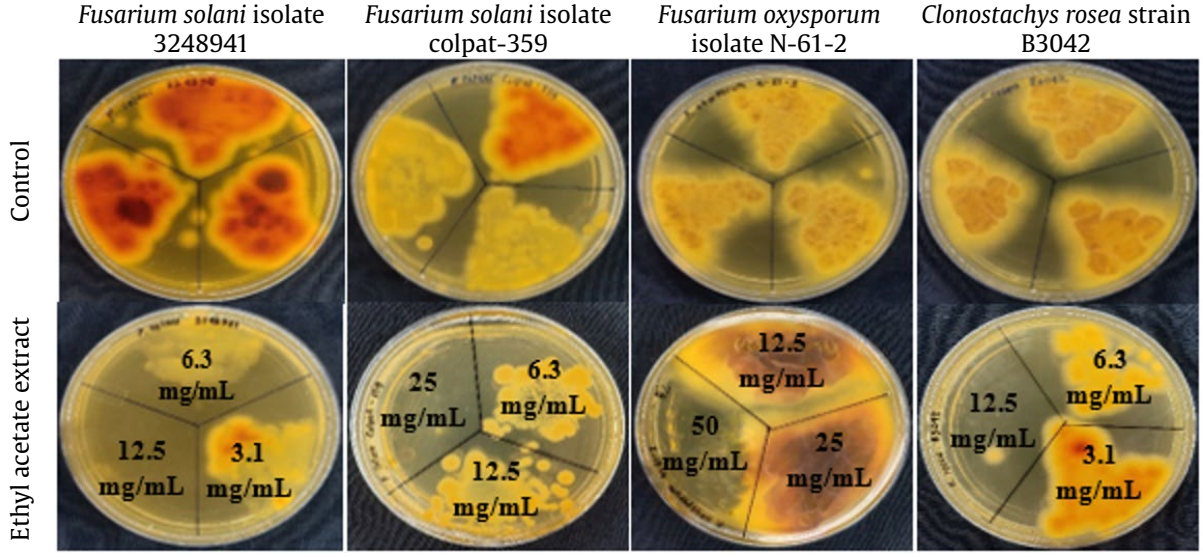

Figure 1. The results of MFC determination ethyl acetate extract of *Penicillium citrinum* against pathogenic fungi of ramie plant

3.5. Morphological Changes of Pathogenic Fungi of Ramie After Treatment of Extract

Confirmation of the observation of ramie pathogenic fungi by microscopic can be seen in Figure 2. Microscopy analysis was seen from the *in vitro* effect. Control treatments that were not given *P. citrinum* ethyl acetate extract against microscopic fungal pathogens under a microscope had long and clear healthy and clear hyphae and septate hyphae and abundant round to oval microconidia. The effect of treatment of *P. citrinum* ethyl acetate extract also affected the hyphae morphology of pathogenic ramie fungi based on microscopic changes (Figure 2). These observations showed morphological changes in which the hyphae looked dwarf and few, and the number of microconidia was less than before treatment with the extract.

4. Discussion

Research on the antifungal test of extract of endophytic fungi of ramie against ramie pathogenic fungi has not been widely reported. Previous research conducted by Sun *et al.* (2021) isolated and characterized ramie endophytic bacteria in Hunan, China, but not ramie endophytic fungi. Ramie endophytic bacteria exhibited antagonistic activity against fungal pathogens, including *Phytophthora capsici* (*Linum usitatissimum*), *Rhizoctonia solani* (*Solanum tuberosum*), *R. solani* (*Oryza sativa*), *Colletotrichum linicolum, Fusarium oxysporum f.* sp. line, *F. oxysporum f.* sp. cucumerinum owen and *Sclerotinia sclerotiorum* (Sun *et al.* 2021). Meanwhile,

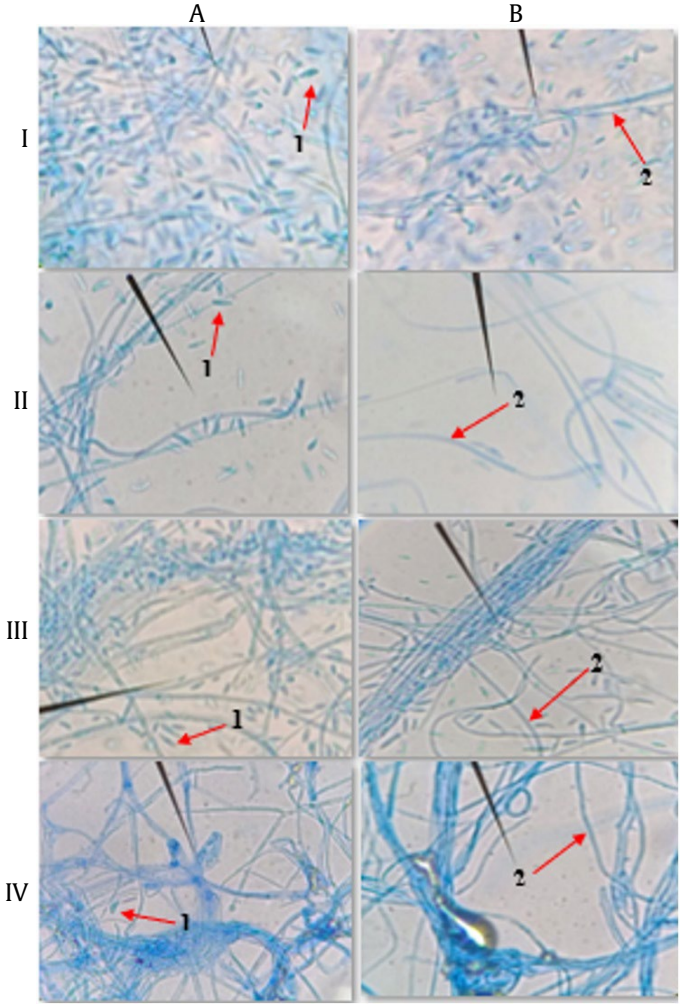

Figure 2. Microscopic observations after treatment of *P. citrinum* ethyl acetate extract to morphology changes in pathogenic fungi: (I) F*usarium solani* isolate 3248941, (II) F*usarium solani* isolate Colpat-359, (III) *Fusarium oxysporum* isolate N-61-2, and (IV) *Clonostachys rosea* strain B3042: (A) control growth, (1) microconidia; (B) ethyl acetate extract of *P. citrinum* after 72 hours, (2) hyphae

Triani (2021) has isolated and tested antifungal fungi from ramie endophytic fungi without an extraction process against ramie pathogenic fungi. The results showed that *Penicillium citrinum* MEBP0017 showed the most effective antifungal activity against ramie pathogenic fungi. However, in that study, the identification of secondary metabolites produced by ramie endophytic fungi has not been identified. Furthermore, in this study, *Penicillium citrinum* MEBP0017 extracted with ethyl acetate was used to increase the effectiveness of antifungals against ramie pathogenic fungi, including *Fusarium solani* isolate 3248941, *Fusarium solani* isolate Colpat-359, *Fusarium oxysporum* isolate N-61-2, and *Clonostachys rosea* strain B3042. In addition, the analysis of secondary metabolites produced from the ethyl acetate extract of *Penicillium citrinum* MEBP0017 was carried out.

The ethyl acetate extract of *P. citrinum* showed inhibition against *Fusarium* ssp. and *Clonostachys* sp. Based on the antifungal test results using the well diffusion method, the ethyl acetate extract showed very strong inhibition (inhibition zone diameter >20 mm) starting from the extract concentration of 20% (200 mg ml^{-1}) in the diameter range of 19.10±1.10 to 22.07±0.04 mm against ramie pathogens *Fusarium solani* isolate 3248941, F*usarium solani* isolate Colpat-359, *Fusarium oxysporum* isolate N-61-2, and *Clonostachys rosea* strain B3042. At higher concentrations, the inhibitory ability of the ethyl acetate extract was getting stronger until it reached an average inhibition zone of 29.05±1.61 to 31.46±0.57 mm. In a previous study conducted (Putri *et al.* 2019), bitter gourd (*Momordica charantia* L.) extract showed inhibition of the *Fusarium oxysporum*, with an average inhibition zone diameter of 4.25±0.95 mm (Putri *et al.* 2019). Another study also conducted by Febriantini *et al.* (2022) used a plant extract of *Psidium cattleianum* to inhibit the growth of *Fusarium solani*. The inhibition zone was 8.0–9.2 mm (Febriantini *et al.* 2022). Meanwhile, in this study, the ethyl acetate extract of *P. citrinum* produced a higher average clear zone value of 20.80±0.74 mm against the ramie pathogenic fungus *Fusarium oxysporum*, which means that the ethyl acetate extract has very high potential as an antifungal for *Fusarium oxysporum*.

The antifungal activity correlated with the ability of *P. citrinum* ethyl acetate extract from ramie endophytic fungi to inhibit the growth of pathogenic fungi. It is possible because of the influence of secondary metabolites in the ethyl acetate extract of *Penicillium citrinum* MEBP0017. The results of the GC-MS analysis show that there are various group of dominant compounds contained in the ethyl acetate extract of *P. citrinum*, namely esters, carboxylic acids, fatty alcohols, fatty acids, and alcohols which have various biological activities, such as antifungal (Brito *et al.* 2022; Pinto *et al.* 2017), antimicrobial (Abu-gharbia *et al.* 2020; Kim *et al.* 2004; Lens *et al.* 2016), antibacterial (Azman *et al.* 2017; Akbar *et al.* 2020; Fajrih *et al.* 2022), antitumor (Forte *et al.* 2013; Fouad *et al.* 2018), anti-inflammatory (Saeed *et al.* 2012), antioxidant (Abd El-Hady *et al.* 2014; Chernukha *et al.* 2021; Fouad *et al.* 2018; Pinto *et al.* 2017; Ser *et al.* 2015), anticancer (Ser *et al.* 2015), and anti-biofilm (Nadar Rajivgandhi *et al.* 2020). Among these compounds, several compounds have been reported to have antifungal activity. In a previous study, ethyl acetate extract of *Fuschia lycioides* containing 1-hexacosanol, a fatty alcohol group reported by Brito *et al.* (2022), can inhibit the growth of the fungus *Candida albicans*. In addition, Pinto *et al.* (2017) reported that vegetable oil containing methyl palmitate, a fatty acid methyl ester, can inhibit the growth of *Paracoccidioides* spp. and *Candida* ssp.

Based on the GC-MS analysis, one of the compounds reported to have antifungal properties is Hexadecaic acid methyl ester (Methyl palmitate) (Pinto *et al.* 2017). Hexadecaic acid methyl ester is a fatty acid that has antimicrobial properties with the mechanism of damaging the cell wall structure synergistically with various active compounds so that they can increase the effect of antimicrobial activity and can inhibit microbial growth (Asghar *et al.* 2011; Karunia *et al.* 2017; Noviyanti 2010; Padmini *et al.* 2010). In addition, 1-Hexacosanol (CAS) HEXACOSANOL-1 has been reported as an antifungal. These compounds are derivatives of alcohol that affect the mitochondrial membrane permeabilization of external and internal eukaryotic cells, causing cell death by apoptosis and necrosis (Brito *et al.* 2022). Moreover, it also has pro-oxidative action, leading to an inhibited performance in apoptosis and necrosis (Islam *et al.* 2018). Butyl acetate is a compound that reported antimicrobial properties (Lens *et al.* 2016). Butyl acetate is an organic solvent commonly used in cosmetics. Cosmetic products containing more than 5% butyl acetate can inhibit the growth of microorganisms physically and chemically. Butyl acetate also showed the highest antimicrobial and bactericidal activity against *Bacillus cereus* and *Serratia marcescens* (Abu-gharbia *et al.* 2020).

Further tests were carried out to determine the MIC50 value by the microdilution method. MIC50 was defined as the lowest concentration that no

visible growth of pathogenic fungi was observed. In this study, ethyl acetate extract of *P. citrinum* was used and the MIC50 value obtained was 6.3 mg ml^{-1} against *F. solani* isolate 3248941 and *Clonostachys rosea* strain B3042. The previous studies reported that *Penicillium* sp. extract had antifungal activity against various fungi. Khokhar *et al.* (2011) reported that *Penicillium* extracts inhibited the growth of *Fusarium oxysporum, Fusarium solani, Macrophomina phaseolina, Aspergillus japonicus var aculeatus*, and *Cladosporium cladosporioides* in the range of 25–68%. Research conducted by Ma *et al.* (2017) used extracts of *Penicillium* sp. fractionated R22 showed antifungal activity, especially fraction 1 of a 3-O-methylviridicatin compound against *Alternaria brassicae, Alternaria alternata,* and *Valsa mali* with a MIC value of 0.031 mg ml^{-1} (Ma *et al.* 2017). *In vitro* antifungal test carried out by Luo *et al.* (2019) showed that the endophytic *Penicillium citrinum* DBR-9 fraction suspected emodin inhibited plant pathogenic fungi with a MIC50 value of 0.141 mg ml^{-1} (Luo *et al.* 2019). The findings in this study indicated that the ethyl acetate extract of *P. citrinum* showed the effect of antifungal activity and was potentially used as biocontrol agent for pathogenic fungi of ramie plants.

In conclusion, the ethyl acetate extract of *Penicillium citrinum* isolate MEBP0017 metabolite had antifungal activity against pathogenic fungi on ramie plants, showing an inhibition zone from 19.10 to 22.07 mm, which showed a strong–very strong category of antifungal activity at a concentration of 200 mg ml^{-1}. The MIC50 value was obtained against the pathogenic fungi *Fusarium solani* 3248941 and *Clonostachys rosea* strain B3042 at 6.3 mg ml^{-1} and against *Fusarium solani* isolate Colpat-359 and *Fusarium oxysporum* isolate N-61-2 with 12.5 mg ml^{-1} and 25 mg ml^{-1} respectively. The antifungal effects are correlated with the secondary metabolites contained in the extract. The secondary metabolite analysis of ethyl acetate extracts showed that several active compounds have various biological activities, including antifungals. Hexadecanoic acid, 1-Hexacosanol, and butyl acetate are the active compounds that possibly inhibit the growth of ramie pathogenic fungi. Further studies can be conducted to determine the potential of fungi extracts containing these secondary metabolites against other pathogenic microbes.

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