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Phylloplane Yeasts on Cocoa and Their Abilities to Inhibit *Phytophthora palmivora* In Vitro

Khamir Filoplan pada Kakao dan Kemampuannya Menghambat *Phytophthora palmivora In Vitro*

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ABSTRACT

Cocoa is an economically valuable plantation commodity, but its cultivation often faces a significant challenge, which is caused by the *Phytophthora palmivora*. Phylloplane yeast has an important role in protecting plant surfaces from pathogen infection. The aim of the study was to assess the diversity of phylloplane yeasts found on cocoa fruits, and determine them as biological control agents for *P. palmivora.* The metode include isolating yeasts from young, old and rotten cocoa pods and testing them as antagonists against *P. palmivora*. The findings of this study yielded eight yeast isolates that were significant in inhibiting the growth of *P. palmivora*. These isolates spanned across six genera, including *Debaryomyces* sp., *Metschnikowia* sp., *Zygosaccharomyces* sp., *Candida* sp., *Wickerhamomyces* sp., and *Cryptococcus* sp. *Candida* sp.2 and *Wickerhamomyces* sp. as particularly promising species species that had a consistently resistant level of inhibitory effect, achieved percentage inhibition of 36.26% (10.8 mm) and 34.44% (9.6 mm), respectively.

Key words: antimicrobial activity, diversity, inhibitory effects, in vitro assay

ABSTRAK

Kakao merupakan komoditas perkebunan yang bernilai ekonomis, namun dalam pembudidayaannya sering kali menghadapi tantangan yang cukup besar yaitu penyakit busuk buah yang disebabkan oleh *Phytophthora palmivora*. Khamir filoplan memiliki peran yang penting dalam melindungi permukaan tanaman dari infeksi patogen. Tujuan penelitian ini ialah mengisolasi khamir filoplan pada buah kakao dan menentukan sebagai agens pengendali hayati untuk mengendalikan *P. palmivora*. Kegiatan meliputi isolasi khamir dari buah kakao muda, tua, dan busuk dan mengujinya sebagai antagonis *P. palmivora*. Temuan penelitian ini menghasilkan delapan isolat khamir yang signifikan dalam menghambat pertumbuhan *P. palmivora*, yaitu *Debaryomyces* sp, *Metschnikowia* sp, *Zygosaccharomyces* sp, *Candida* sp, *Wickerhamomyces* sp, dan *Cryptococcus* sp. Khamir *Candida* sp.2 dan *Wickerhamomyces* sp. sebagai spesies yang memiliki tingkat efek penghambatan yang resisten secara konsisten, mencapai persentase penghambatan masing-masing 36.26% (10.8 mm) dan 34.44% (9.6 mm).

Kata kunci: antimikroba, daya hambat, keanekaragaman, uji *in vitro*

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INTRODUCTION

Cocoa is an important pillar of Indonesia's agricultural sector, a high-value commodity with significant economic importance and a significant contribution to the national economy. Its role is crucial not only as a source of employment and income, but also as a generator of foreign exchange. Even though over 90% of cocoa production comes from smallholder plantations, Indonesia has secured its position as the world's sixth largest cocoa exporter, after Ivory Coast, Ghana, Ecuador, Cameroon and Nigeria (Marlina *et al*. 2023). Cocoa cultivation is disrupted by cocoa pod rot, a disease caused by *Phytophthora palmivora* (Merga 2022). This pathogen has caused severe economic losses, with reports of cocoa production losses of up to 90% on the island of Java (Sukorini *et al*. 2021). *Phytophthora palmivora* is a parasitic oomycete with a broad host range, capable of infecting over 200 different plant species (Perrine-Walker 2020). Its impact has been particularly severe in cocoa cultivation in Indonesia (Wang *et al*. 2020).

One of the critical challenges faced in the cultivation of cocoa is the lack of adequate sanitation practices for cocoa pods that show symptoms of *P. palmivora* infection. Failure to adequately address this issue can exacerbate the problem by serving as a reservoir for inoculum, allowing the pathogen to persist and spread within cocoa cultivation areas (Adeniyi and Asogwa 2023). A promising alternative approach to controlling *P. palmivora* is the use of biological agents isolated from healthy cocoa pods. Recent studies have yielded positive results, demonstrating the ability of microorganisms such as *Pseudomonas aeruginosa* and *Bacillus subtilis*, isolated from disease-free cocoa pods, to suppress cocoa pod rot (Khaeruni *et al*. 2023). In addition, saprophytic fungi isolated from cocoa pod shells have potential as biological control agents against *P. palmivora* (Delgado-Ospina *et al*. 2021). This offers hope for a more sustainable and environmentally friendly approach to cocoa pod rot management.

In addition, yeast strains isolated from natural sources have shown the ability to inhibit plant diseases both before and after harvest (Satianpakiranakorn *et al*. 2020). In the context of cocoa farming, exploring the yeast community living on the phylloplane—the aerial parts of cocoa plants—is a promising research opportunity. Given the urgent need to address the serious threat posed by *P. palmivora* to the cocoa industry in Indonesia, it is evident that research focusing on the role of phylloplane yeast strains isolated from cocoa pods in controlling this pathogen is an urgent and critical effort. Such research has the potential not only to reduce the losses faced by cocoa farmers, but also to introduce more sustainable and environmentally friendly practices in the control of cocoa pod rot.

MATERIALS AND METHODS

Phytophthora palmivora, Cultures Assessment through Pathogenicity Test.

Phytophthora palmivora isolates were obtained from the collection of the Department of Plant Pest and Disease, University of Brawijaya. To confirm that the isolates retained their pathogenicity and virulence, Koch's postulates were applied using V8 juice media adapted from Sukorini *et al*. (2021). In this process, healthy cacao fruits were used to test the ability of the P. *palmivora* samples to infect and induce symptoms.

Yeast Isolation

Samples of healthy cacao fruits were collected from the cacao plantation of PT. Perkebunan Nusantara XII, Bantaran Plantation, Penataran, Blitar, East Java. A 100 mL aliquot of distilled water was prepared in Erlenmeyer flask. Cocoa fruit samples were classified into young, mature, and rotten categories. For the young fruit category, samples were selected based on a length of 8 to 10 cm, ensuring that the fruits were at an early stage of development. In contrast, the mature fruit category included fruit that were 17 to 23 cm in length, indicating a fully developed stage suitable for standard cocoa production analysis. In addition, the rotten fruit category was distinguished not only by the same size criteria as the mature fruit,

i.e. a length of 17 to 23 cm, but also by the presence of decay symptoms, thus allowing the study of factors influencing fruit rot in cocoa production.

Before immersion, the cacao fruits were carefully processed; the outer skin of six healthy fruits was carefully cut into slices (2 cm × 3 cm). These sliced pieces of fruit skin were then placed in the Erlenmeyer flask containing the distilled water and shaken at 120 rpm for 24 hours. Dilutions were performed on the resulting soaking or shaking water at concentrations of 10^{-3}, 10^{-4}, and 10^{-5}. A 50 µL volume was used for growth in yeast malt agar (YMA) medium. Yeast strains were purified using streaking techniques on YMA media and then identified based on morphological characteristics such as color, shape, colony texture, colony edges, bud type, number of nuclei (Sukmawati *et al*. 2024).

Antagonism Assay

Each yeast isolate was plated on potato dextrose agar (PDA) medium in the center of a Petri dish, with a single inoculation replicate per dish (Khunnamwong *et al*. 2020). The control included the use of PDA medium without yeast inoculation, which served as a baseline to assess growth conditions and to identify any potential contamination or inherent growth on the medium itself. Colonies of *P. palmivora* were then placed approximately 3 cm from both the right and left sides for each yeast isolate on the PDA medium. The percent inhibition (PH) was calculated using the following formula:

$$PH = \frac{rp - rk}{rp} \times 100\%, \text{ with}$$

PH, inhibition percentage of yeast against *P. palmivora*; rp, colony radius of pathogen without yeast treatment (r1 + r2); rk, colony radius of pathogen with yeast treatment (r1 + r2); and r1 + r2, inhibition zone width caused by yeast against pathogens.

Antibiosis Assay

The antibiosis test was carried out using yeast malt broth medium (YMB: yeast extract (3 g), malt extract (3 g), peptone (5 g), glucose (10 g), and distilled water (1000 mL)) according to the method of Arini *et al*. (2021). Two different methods were used to evaluate the production of antibacterial compounds: YMB medium without *P. palmivora* (non-induced conditions) and YMB medium supplemented with *P. palmivora*. (induced conditions). This approach aimed to determine whether the antibiosis compounds produced by the yeast were induced or unaffected by the presence of *P. palmivora*.

Phytophthora palmivora was cultured in 100 mL of yeast malt broth (YMB) media and subjected to rotary shaking for 4 days. The culture was then inoculated with 5 mL yeast suspension (10^6) and incubated for seven days under rotary shaking conditions. Subsequently, filtration was conducted to separate yeast from the YMB medium extract. The initial filtration process utilized Whatman filter paper, with three repetitions performed. Sterilized bottles were used to collect the filtrate. Further filtration was carried out using a microfilter (0.22 µm). The obtained YMB medium extract was prepared for utilization in the paper-disc assay.

Screening and testing of antibiosis compounds were conducted using the paper-disc diffusion technique, involving the immersion of paper discs into the supernatant obtained from microfilter filtration. Next, the paper discs were placed onto media inoculated with *P. palmivora* and incubated at room temperature. Every two days, 300 µL of the medium extract was added. The clear zones surrounding the paper discs indicated the activity of antibiosis compounds capable of inhibiting the growth of *P. palmivora*. The percentage of colony inhibition was calculated using the following formula.:

$$P = \frac{r1 - r2}{r2} \times 100\%, \text{ with}$$

P, percentage of relative inhibition of pathogen growth; r1, radius of pathogen colony approaching to the disk paper; and r2, radius of pathogen colonies that stay away from the disk paper.

Experimental Design and Data Analysis

A completely randomized design (CRD) was used in the in vitro antagonist test, screening, and testing of antibiosis compounds. Nine treatments were included, with one control and eight yeast treatments. The yeast treatments comprised the following isolates: The following yeast isolates were used: *Debaryomyces* sp. (BM1), *Metschnikowia* sp. (BM3), *Zygosaccharomyces* sp. (BT1), *Candida* sp.1 (BT2), *Wickerhamomyces anomalus* (BT3), *Candida* sp.2 (BT4), *Candida orthopsilosis* (BB1), and *Cryptococcus* sp. (BB2). Each treatment was replicated three times to ensure the reliability of the results.

The variance analysis, or F-test at a 5% significance level, was used to determine whether there were statistically significant differences in the data obtained from the antagonist test and the paper-disc test. If the F-test results indicated significant differences, a post hoc analysis was conducted using the Duncan Multiple Range Test (DMRT) at the 5% significance level. This subsequent testing provided a more precise understanding of the differences between treatments.

RESULT

Pathogenecity Test

The pathogenecity test experiment definitively showed characteristic symptoms of black, well-defined lesions focused at the point of inoculation on the 4th day post-inoculation (Figure 1). *P. palmivora* hyphae are non-separated and hyaline. The sporangium has papillae at the tip and is shaped like a pear, while the chlamydospores are round. The sporangium of *P. palmivora* measures 44.60-50.89 µm long and 25.78-30.68 µm wide. The diameter of chlamydospores ranges from 24.56 to 34.33 µm. Inside the chlamydospores and sporangium, there are many black spots that resemble diplod nuclei. These black spots are zoospores produced asexually by *P. palmivora*. Zoospores are spores that have two flagella which function as a means of movement when conditions are damp and can later infect the fruit of healthy plants. Microscopic examination of these mycelia confirmed them as colonies of the *P. palmivora* pathogen. The temperature during the pathogenicity test process is 27–29 °C with humidity at 86–90%.

Characterization of Yeast

The antagonism test revealed eight yeast isolates that exhibited antagonistic behavior by producing antibiotic compounds. All isolates demonstrated the ability to create clear zones between *P. palmivora* colonies and yeast colonies. Eight distinct yeast isolates were identified through macroscopic and microscopic examination. Despite differences observed in the identification results, the general characteristics of the isolates, namely cream, white, and yellowish white colonies at 12 days after inoculation, were similar (Table 1).

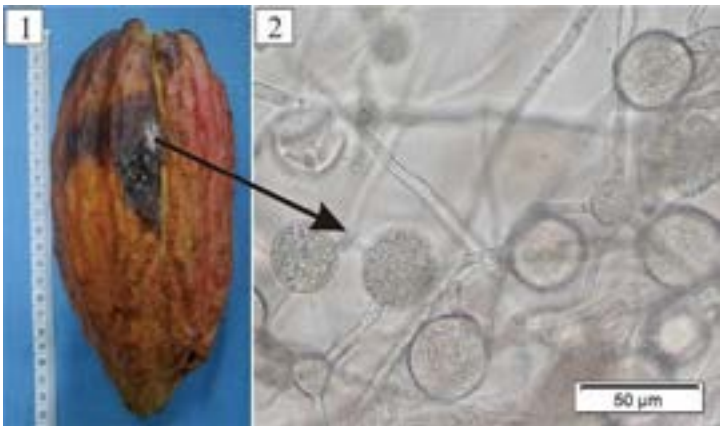

Figure 1 Pathogenicity test of *Phytophthora palmivora* on cocoa fruit. 1, Symptoms of cacao fruit rot; and 2, *Phythophtora palmivora* chlamydospores.

Table 1 Characteristics of yeast isolates from healthy cocoa pods

Cocoa's fruit sample	Name of isolate	Color of colony	Yeast cell (µm)	Shape	Number of nuclei	Pseudomycelia	Single cell and pair	Growth time* (day)
Young	*Debaryomyces* sp.	whitish to yellowish color	1.55–2.77	semi-oval to oval	single	formed	formed	3
	Metschnikowia sp.	cream color	2.73–2.81	round to oval	single	formed	formed	2
	Zygosaccharomyces sp.	whitish to yellowish	1.03–2.78	semi-oval to oval	single	formed	formed	4
Mature	*Candida* sp.1	white (top) brown to whitish-brown (back)	1.10–1.57	oval to elongated oval	single	formed	formed	3
	Wickerhamomyces sp.	cream color	2.00–2.49	elongated-oval	single	formed	formed	3
	Candida sp.2	whitish to yellowish	0.55–0.83	semi-oval to oval	single	true mycelia	formed	4
Fruit rot	*Candida* sp.3	cream color	2.00–7.00	round to oval	single	formed	formed	4
	Cryptococcus sp.	opaque white	0.52–0.97	semi-oval to oval	single	formed	formed	4

*The time required by yeast to form a solid line on YMA media

Debaryomyces sp., *Metschnikowia* sp., *Zygosaccharomyce*s sp., *Candida* spp., *Wickerhamomyces* sp., and *Cryptococcus* sp. exhibited a wide range of macroscopic and microscopic characteristics on YMA medium. Macroscopically, these yeasts displayed colors ranging from whitish to yellowish, with textures from dense to solid and colony shapes featuring irregular, grain-like structures to filamentous edges. It is notable that the formation of a solid line post-inoculation varied among species. *Metschnikowia* sp. formed a solid line as quickly as two days after inoculation, while *Cryptococcus* sp. took four days. Microscopically, cell sizes varied broadly, with shapes from oval to round and semi-oval. Budding patterns were primarily multipolar. It is notable that pseudomycelia were observed in some species, contributing to the filamentous appearance of colonies. Conversely, true mycelia were identified in *Candida* sp.2, showcasing the diverse reproductive and structural adaptations among these yeasts.

Antagonism Assay

The clear zones observed in the yeast treatments among the eight yeast isolates. Based on the F-test performed, the inhibition of yeasts on the growth of the pathogen *P. palmivora* at 4 days post-inoculation (dpi) showed significant differences. The results of the analysis showed a significant difference in the percentage of inhibition between the control treatment and all the yeast treatments. Among the yeast treatments, *Candida* sp.2 showed the highest inhibition percentage against *P. palmivora* at 36.26%, while *Zygosaccharomyces* sp. showed the lowest inhibition percentage at 22.51% (Table 2).

Antibiosis without *P. palmivora* Induction *in Vitro*

The study revealed the extent of antibiosis inhibition exhibited by phylloplane yeast species against *Phytophthora palmivora* under non-induced conditions, assessed five days post-inoculation using the paper-disk method. *Debaryomyces* sp. (BM1) and *Wickerhamomyces* sp. (BT3) demonstrated notable inhibitory effects, registering inhibition percentages of 4.94% and 8.08% respectively. Conversely, *Metschnikowia* sp. (BM3), *Candida* sp.1 (BT2), *Candida* sp.2 (BT4), *Candida* sp.3 (BB1), and *Cryptococcus* sp. (BB2) exhibited minimal to no inhibition against *P. palmivora*, with inhibition ranging from 0.00% to 3.95% (Table 3).

Antibiosis with *P. palmivora* Induction in vitro

The data from the study investigating the antibiosis inhibition of phylloplane yeast under induced conditions against *P. palmivora*, assessed five days post-inoculation using the paper-disk method, revealed distinct inhibitory patterns among the different yeast treatments. The control samples exhibited no inhibition (0.00%), while the treatments with *Debaryomyces* sp. (BM1), *Metschnikowia* sp. (BM3), *Zygosaccharomyces* sp. (BT1), *Candida* sp.1 (BT2), *Candida* sp.2 (BT4), *Candida* sp.3 (BB1), and *Cryptococcus* sp. (BB2) displayed varying degrees of inhibition, ranging from 10.51% to 19.47%. *Wickerhamomyces* sp. (BT3) demonstrated the highest inhibition percentage at 34.58%, clearly indicating its pronounced efficacy in inhibiting *P. palmivora* growth under induced conditions (Table 3).

Table 2 Inhibition of phylloplane yeast from cocoa against *Phytophthora palmivora* in the antagonism test after 4 days of inoculation

Treatment	Inhibition (%)
Control	0.00 a
Debaryomyces sp. (BM1)	33.40 b
Metschnikowia sp. (BM3)	27.62 b
Zygosaccharomyces sp. (BT1)	22.51 b
Candida sp.1 (BT2)	32.38 b
Wickerhamomyces sp. (BT4)	34.44 b
Candida sp.2 (BT4)	36.26 b
Candida sp.3 (BB1)	35.80 b
Cryptococcus sp. (BB2)	28.85 b

Note: Values in the same column followed by the same letter are significantly different based on Duncan' s multiple range test at α 5%.

Table 3 Antibiosis inhibition of phylloplane yeast against *Phytophthora palmivora* using the paper-disk method on induced and non-induced conditions

Treatment	Inhibition (%)	
	Non-induced conditions[a]	Induced conditions[b]
Control	0.00 a	0.00 a
Debaryomyces sp. (BM1)	4.94 a	10.51 ab
Metschnikowia sp. (BM3)	0.00 a	12.16 ab
Zygosaccharomyces sp. (BT1)	3.70 a	14.97 ab
Candida sp.1 (BT2)	1.28 a	16.20 ab
Wickerhamomyces sp. (BT3)	8.08 a	34.58 c
Candida sp.2 (BT4)	3.85 a	13.64 ab
Candida sp.3 (BB1)	3.95 a	19.47 b
Cryptococcus sp. (BB2)	0.00 a	13.64 ab

Note: [a]without *Phytophthora palmivora* induction; [b]with *Phytophthora palmivora* induction; Values in the same column followed by the same letter are significantly different based on Duncan' s multiple range test at α 5%.

DISCUSSION

The initial pathogenic testing in this study, following Azni *et al*. (2019) methodology, revealed infection symptoms on healthy fruits typically appearing after 3 days post-inoculation (dpi). Infections persist for 6 days, marked by the development of necrotic lesions characterized by a color change to brown, covered by whitish mycelia and sporangia on the fruit's surface. Eight distinct yeast isolates were identified, belonging to six genera: Debaryomyces sp., *Metschnikowia* sp., *Zygosaccharomyces* sp., *Candida* sp., *Cryptococcus* sp., *Wickerhamomyces* sp. These findings align with those presented by Tovar *et al.* (2021) also found the same yeast genera in cacao fruits in Maceo (Antioquia), San Vicente de Chucurí (Santander), and Rivera and Algeciras (Huila), Colombia.

Debaryomyces sp. colonies exhibit a yellowish-white color with a dense, slightly grainy texture and uneven edges. They have smooth and shiny surfaces, appearing whitish to yellowish with grain-like structures and a convex elevation when grown at 25 °C. *Debaryomyces* sp. cells are round to slightly elongated, sometimes appearing in pairs, with sizes ranging from 1.55-2.77 µm. They reproduce by producing multipolar buds and are commonly found in various natural substrates such as soil, fruits, and plants (Jacques *et al.* 2015).

Wickerhamomyces sp. is a cream-colored fungus with a granular texture and stringy edges on both the top and bottom of the petri dish. Thin fibers resembling true meselia are observed at the edge, which are actually false meselia under microscopic examination. The cells are elongated and oval-shaped, ranging from 2-2.49 µm, with a hyaline appearance and multipolar shoots of semi-round oval shape. On artificial media, *Wickerhamomyces* sp. appears cream-colored with a grainy and smooth texture, featuring round, ellipsoidal, or elongated cells ranging from 1.9-6.1 µm. It reproduces asexually through multiple bud formation from each cell and through the production of ascospores (Salazar *et al*. 2022).

Candida sp.1 is part of the *Candida* genus. When grown on solid media, it will show macroscopic characteristics of whitish to yellowish color, rough and smooth texture, and surface appearance. The yeast cells of *Candida* sp.1 were oval-elongated, single, hyaline, with a size ranging from 1-5 µm. They exhibited a multipolar budding pattern, capable of producing 1-3 buds on each cell. These microscopic characteristics are consistent with those of *Candida* spp. (Delgado-Ospina *et al*. 2020).

Metschnikowia sp. colonies are cream-colored with uneven edges, a dense texture, and few granules. The lower colony exhibits a cream color at the edges and brown in the center, with a flat height and smooth surface. Pseudomycelia are observable under microscopic observation, and colony solidification takes two days. *Metschnikowia* sp. cells are round to ovoid, colorless, with a size ranging from 2.73-2.81 µm and containing a single nucleus. They exhibit multilateral budding, forming semi-round to oval-shaped shoots. Colonies of *Metschnikowia* sp. grown at 25 ºC are shiny with a whitish to creamy color, grain-like texture, and raised elevation. The yeast cells are rounded to oval, occurring singly or in small groups, with sizes ranging from 2-8 µm and undergoing multilateral budding to produce 1-3 buds (Oztekin and Karbancioglu-Guler 2021).

Cryptococcus sp. colonies on YMA media have uneven edges and appear cloudy white with a smooth and flat surface. The growth of yeast is slow, taking four days to become solid lines. Cryptococcus sp. can produce basidiospores in its sexual cycle, but no form of basidiospores was observed. The cells of *Cryptococcus* sp. are semi-round to oval, ranging from 0.52 to 0.97 µm. They belong to the phylum Basidiomycota and are described as round to slightly elongated under microscopic observation, with sizes ranging from 0.5 to 7 µm (Neto *et al*. 2021).

Zygosaccharomyces sp. colonies on YMA media are yellowish white with a slightly rough and dense texture. The colony edge appears stringy, with smooth surfaces and flat elevations. Pseudomycelia are visible as clear filaments. Cell size ranges from 1.03-2.78 µm, with a semi-round to oval, and each cell can produce 1-3 shoots forming a semicircle. Each cell contains one nucleus and is colorless or hyaline. When grown at 25 °C, *Zygosaccharomyces* sp. exhibits a smooth texture, with a whitish to yellowish color and a grain-like appearance. The yeast cells are round to oval-elongated, with sizes ranging from 1 to 13 µm. Furthermore, *Zygosaccharomyces* sp. can form ascospores (Brysch-Herzberg *et al.* 2022).

Among the eight yeast isolates identified in the initial selection, the yeast genus most commonly found from plant surfaces and potentially serving as a bio-agent is *Candida* sp. (Fourie and Pohl 2019). The presence of clear zones separating the two colonies observed in yeast treatments, including *Debaryomyces* sp., *Metschnikowia* sp., *Zygosaccharomyces* sp., *Candida* sp.1, *Wickerhamomyces* sp., *Candida* sp.3, and *Cryptococcus* sp., is unmistakable evidence of the antibiosis antagonistic mechanism, which indicates the existence of antibiosis compounds (Sukmawati *et al*. 2020). Yeast exhibits reduced secretory ability and usually produces limited amounts of secondary metabolites compared with filamentous fungi (Zhang *et al.* 2020). However, there are several examples of metabolites, including volatile organic compounds (VOCs) and cytotoxic killing toxins, that have been observed to exhibit antifungal efficacy (Zhao *et al.* 2022).

The findings of this study are consistent with Gouka *et al.* (2022), indicating that phylloplane yeasts typically possess higher antagonistic capabilities due to their adaptability and faster growth. This advantage allows phylloplane yeasts to outcompete other microorganisms, including pathogens, for resources and living space, both *in vitro* and *in vivo*. According to Oztekin *et al.* (2023), an antagonist agent is considered effective if it can compete with other microorganisms or pathogens to acquire space, nutrients, and resources effectively, both *in vitro* and *in vivo*.

The yeast culture media extract in the non-induction treatment did not provide inhibition against *P. palmivora*. The percentage of inhibition value was the same as the control treatment (0%). It is clear that yeast metabolite compounds will not come out if pressure is not applied.

The yeast culture media extract (induction) treatment that exhibited the highest inhibition percentage against *P. palmivora* was *Wickerhamomyces* sp., with an inhibition rate of 32.22%. This indicates that the yeast

culture media extract from *Wickerhamomyces* sp. contains potent antibiosis compounds that can control or inhibit the development of *P. palmivora*, the causal agent of cocoa fruit rot (Kowalska *et al*. 2022). The ability of *Wickerhamomyces* sp. to produce killer toxins and other species in the genera *Pichia* and *Williopsis* has received significant attention due to their potential application in the field of plant protection. (Giovati *et al*. 2021). Killer toxins, also known as KTs or mycocins, are secreted proteins or glycoproteins that eliminate susceptible microorganisms. These toxins employ various mechanisms of action, typically involving interactions with specific surface receptors. Killer yeasts demonstrate immunity to the cytotoxic effects of their own killer toxins.

Candida sp. contains antibiosis compounds that clearly inhibit the growth of pathogenic colonies of *P. palmivora*. *Candida* UICC Y-533 has been proven to inhibit the growth of hyphae and sporulation of *Aspergillus niger* by up to 100% and reduce hyphae size by 3-85% (Dhiani 2012). Furthermore, *Candida* spp. can secrete β-1,3-glucanase, chitinase, and protease enzymes. These three enzymes work in concert to degrade the cell walls of pathogens by breaking the complex polysaccharide polymer bonds in the hyphae or spores of the pathogen into smaller subunits that can be used as a carbon source by yeast cells (Bar-Shimon *et al*. 2004).

The results of this study prove that phylloplane yeasts are an effective biological agent for controlling *P. palmivora*. The use of biological agents such as *Candida* sp.2 and *Wickerhamomyces* sp. for controlling *P. palmivora* is a sustainable and environmentally friendly alternative to chemical pesticides. Furthermore, the yeast's ability to produce effective antibiosis compounds that inhibit pathogen growth is a crucial aspect in the development of more natural control strategies. This study definitively supports efforts to maintain the sustainability of the cocoa industry and reduce the use of chemical pesticides with adverse environmental impacts.

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