Exploration and Selection of Potential Epiphytic Yeasts to Control *Fusarium Fujikuroi* cause of Bakanae Disease in Rice Plants

Rosiana Fadilah Hrp, [1)] Darnetty*, [1)]Eri Sulyanti, and [2)]Refilda

1) Department of Plant Protection, Andalas University, Padang, Indonesia.
2)Department of Chemistry, Andalas University, Padang, Indonesia.
*Corresponding author: darnetty_06@yahoo.com

ABSTRACT

Bakanae disease on rice is caused by *Fusarium fujikuroi* which can cause yield losses ranging from 3.0-95.4%. Epiphytic yeasts have been known as biological control agents for plant pathogens. The purpose of this study was to determine the potential of epiphytic yeast derived from rice leaves to control bakanae disease. The study consisted of several stages, namely: 1). Exploration of epiphytic yeasts in 3 districts in West Sumatra, namely Pesisir Selatan, Solok and Tanah Datar 2). Selection epiphytic yeasts based on morphological characteristics and pathogenicity 3). Testing the ability of epiphytic yeasts to control *F. fujikuroi* using the dual culture and Sealed Plate Methods 4). Testing epiphytic yeasts to control *F. fujikuroi* from infected rice seed and 5). Molecular identification of potential epiphytic yeast isolates for controlling *F. fujikuroi*. Forty-two yeast isolates were successfully explored, isolated and characterised. Then they were tested for their ability to suppress the growth of *F. fujikuroi* by using the dual culture method and 14 isolates were obtained which had an inhibitory power above 50%. Then, these 14 isolates were tested for their ability to produce volatile compounds and control *F. fujikuroi in vivo*. The percentage of inhibition of epiphytic yeast against *F. fujikuroi* using dual culture and Sealed Plate Methods were range from 55.12 – 86.00% and 32.35- 73.03% respectively. The results of *in vivo* study showed that treatment of rice seeds with epiphytic yeast isolates reduced ungerminated seeds, infected seedlings except (KS3, KTD8, KS6) and disease severity with the percentage of inhibition of (25.84-100%), (13.26-21.42%) and (31.77- 44.18%) respectively. There were 4 epiphytic yeast isolates that had high potential in suppressing the growth of *F. fujikuroi in vitro* and *in vivo* studies, namely KS2, KTD1, KTD6, and KS6, molecularly identified as *Candida parapsilosis, Moesziomyces antarcticus, Pseudozyma churashimaensis* and *Moesziomyces antarcticus* respectively.

Keywords: Biological control; Exploration; *Fusarium fujikuroi*; Screening.

INTRODUCTION

Bakanae disease is a rice disease that has been known for over a century; however, in recent decades its incidence and severity have increased in several rice-producing countries, including Indonesia. This disease needs serious attention because it is a threat to sustainable rice production in all rice-producing areas including Indonesia. The disease can cause crop losses ranging from 3% to 95.4%. The incidence and severity of this disease vary depending on the region and cultivar. Bakanae disease has had a significant impact in Asian countries and now this impact has spread to Europe (Gupta *et al*, 2015; Karthik and Qingyao, 2023). In Indonesia, this disease has begun to develop and needs to be watched out for. Darnetty and Sulyanti (2014) have reported that bakanae disease was found in rice plantations in West Sumatra, especially in the lowlands (Padang City) with an attack rate in some areas relatively high or reaching 20%. Infected seeds will grow into seedlings that experience abnormal elongation, yellowing leaves and root rot. In addition, seedlings can also experience stunting which can cause death. Seedlings that can survive until in the field will elongate and form grains earlier but are empty (Naeem *et al.,* 2016; Darnetty and Sulyanti, 2017)

Various control efforts have been made to reduce the level of attack, one of which is by using healthy or certified seeds. Soaking seeds with chemical compounds is also the most commonly adopted strategy to control pathogens, although it can support the occurrence of fungicide-resistant strains and is harmful to other organisms (Thobunluepop, 2009). Therefore, it is necessary to have other alternative controls that are more environmentally friendly such as the use of biological agents such as yeast (Irtwange, 2006). According to Kowalska *at al*, (2022), yeasts can be a good biocontrol agent because they quickly colonize plant surfaces, tolerate to wide temperature spectrum, use nutrients from various sources, produce no dangerous secondary metabolites and cause no adverse effects on the final food products.

Biocontrol of plant pathogens using yeasts have several mechanisms such as the production of volatile organic compounds (VOC), production of toxins, competition for space and nutrient compounds, production of lytic enzymes, induction of plant immunity and mycoparasitism (Kowalska *et al*, 2022; Hartati, *et al.* 2023). In most yeast species, a few mechanisms occur simultaneously, which enhance the antagonistic effect against phytopathogens. The results of the study by Contarino *et al* (2019) showed that alcohol (ethyl alcohol, 3-methyl-1-butanol and phenylethyl alcohol) and esters (ethyl acetate and isoamyl acetate) were found to be the main VOCs emitted by yeast strains, which had different production levels over a period of 16 days. Epiphytic yeasts isolated from phylloplane of various plant species have also been reported to increase plant growth by producing secondary metabolites of the hormone Indole Acetic Acid (IAA) (Limtong and Koowadjanakul, 2012), biofilm, siderophore, phosphate and zinc oxide solubilization (Nutaratat *et al*. 2014)

The success of epiphytic yeast in controlling plant pathogens has been widely reported. Rice seeds treated with *P. guilliermondii* R9, *M. pulcherrima* R23 and R26 isolated from rice seeds significantly reduced the infection rate of *F. fujikuroi,* compared to some commercial biofungicides. The four selected yeasts reduced the bakanae disease severity in rice plants grown in greenhouse trials (Matić *at al*, 2014). Thirteen epiphytic yeast strains derived from rice leaves showed antagonistic activity against the rice seed-borne pathogenic fungi *Culvularia lunata* and *Helminthosporium oryzae*. The epiphytic yeasts *Torulaspora indica* and *Wickerhamomyces anomalus* were able to control rice seedling rot caused by these two pathogenic fungi (Limtong *et al.* 2020). Result of study by Hermaleni*, at al.* (2022) showed that epiphytic yeast originating from chili leaves inhibited the growth of *Colletotrichum capsici,* cause of anthracnose disease of chili.

Five yeast species (*Candida oleophila, Aureobasidium pullulans, Metschnikowia fructicola, Cryptococcus albidus, Saccharomyces cerevisiae*) have been registered for the application as biocontrol products (Freimoser *at al*. (2019).

MATERIALS AND METHODS

Research Design

The selection of potential epiphyte yeasts to control *F. fujikuroi* was conducted using a Completely Randomized Design (CRD) consisting of 43 treatments and 2 replications. The treatments consisted of 42 epiphyte yeast isolates (results of exploration) and 1 control (without yeast). The testing was carried out using the dual culture method. The design used for the volatile compound formation test was also a Completely Randomized Design (CRD) with 15 treatments and 3 replications using the sealed plate method. The treatment consisted of 14 epiphytic yeasts, 1). KTD1, 2). KPS12, 3). KTD4, 4). KS2, 5). KPS8, 6). KTD12, 7). KS6, 8). KTD8, 9). KTD11, 10). KTD6, 11). KPS16, 13). KS3, 14). KTD9, 15). Control 1 (without yeast), with screening results showing an inhibition percentage of >50%. *In vivo* testing also uses the same design and treatment as the volatile compound test

Exploration and Isolation of Epiphytic Yeasts

Exploration of epiphytic yeasts was carried out in 3 districts in West Sumatra, namely Solok, Tanah Datar and Pesisir Selatan. The isolation method refers to Assis and Mariano (1999). Each healthy leaf was weighed as much as 10 grams and put into 100 ml of sterile aquadest, then shaken using a rotary shaker for 15 minutes at a speed of 120 rpm. The suspension obtained was made into a serial dilution up to 10^{-2}, then grown in Yeast Glucose Chloramphenicol Agar (YGCA) medium in a petri dish and incubated at room temperature for 3 days. Yeast purification was carried out using the Single spore method**.**

Morphological Characterization and Pathogenicity Test of Epiphytic Yeasts Isolates

Morphological characterization of yeast was carried out by observing yeast colonies in YGCA medium including (colony colour, colony edge, colony surface) and yeast cells under a light microscope including (cell shape, cell size, whether they form hyphae or pseudohyphae).

To confirm the non-pathogenic nature of the epiphytic yeast isolates, a pathogenicity assay was performed. A 20 µL suspension of yeast cells (10^7 cells mL^{-1}) was sprayed onto healthy 21-day-old rice plants, which had been cultivated without pesticide treatment (Hartati et al., 2014). The epiphytic yeast used is not a pathogen, as evidenced by the absence of symptoms such as necrosis, chlorosis and rot on leaves inoculated with epiphytic yeast.

Isolation and Identification of *F. fujikuroi*

Fusarium fujikuroi was isolated from the roots of rice plants exhibiting bakanae symptoms. The roots were cut into 1 × 1 cm pieces and surface-sterilized sequentially with 70% ethanol and 1% sodium hypochlorite for 1 minute each, followed by three rinses with sterile distilled water. The sterilized root segments were then air-dried on sterile filter paper and cultured on Potato Dextrose Agar (PDA) in Petri dishes. The cultures were incubated at room temperature for 7 days (Kazempour and Anvary. 2009). Emerging fungal colonies were transferred to fresh PDA plates and purified using the single-spore isolation method. Pure fungal isolates were morphologically characterized and identified according to the identification key described by Leslie and Brett (2006).

Propagation and Pathogenicity Test of *F. fujikuroi*

F. fujikuroi was cultured in Potato Dextrose Broth (PDB). A 5 mm-diameter fungal mycelial plug of *F. fujikuroi* was introduced into 100 ml of PDB in an Erlenmeyer flask and agitated using a rotary shaker at 150 rpm for 7 days. The spore concentration was determined using a Neubauer haemocytometer with serial dilution, reaching a density of 10^7 spores/ml.

A pathogenicity assay was performed by immersing 14-day-old rice seedlings after sowing in a liquid culture of *Fusarium fujikuroi* (10 ml) within test tubes, and the results were compared to seedlings treated with sterile distilled water. The earliest signs of bakanae disease in this

assay included excessive seedling elongation and leaves turning pale green to yellow, which appeared 4 days post-inoculation (Hrp *et al*., 2014).

***In Vitro* Evaluation of Antagonistic Activity of Epiphytic Yeast Isolates Against *Fusarium fujikuroi* Using Dual Culture and Sealed Plate methods.**

***a.* Dual Culture Method**

F. fujikuroi, with an initial culture diameter of 4 mm, and an epiphytic yeast isolate with a cell density of 10^7 cells/mL were co-cultured on the same PDA medium in a 9 cm Petri dish. *F. fujikuroi* was placed 3 cm from the edge of the Petri dish, while the epiphytic yeast isolate was inoculated 3 cm away from *F. fujikuroi* by streaking up with the length of 3 cm (Figure 1). A control treatment, without the epiphytic yeast, was incubated for 15 days until the Petri dish was fully colonized by *F. fujikuroi.* The colony area of *F. fujikuroi* was then measured, and the percentage of inhibition by the epiphytic yeast against *F. fujikuroi* was calculated using Formula 1.

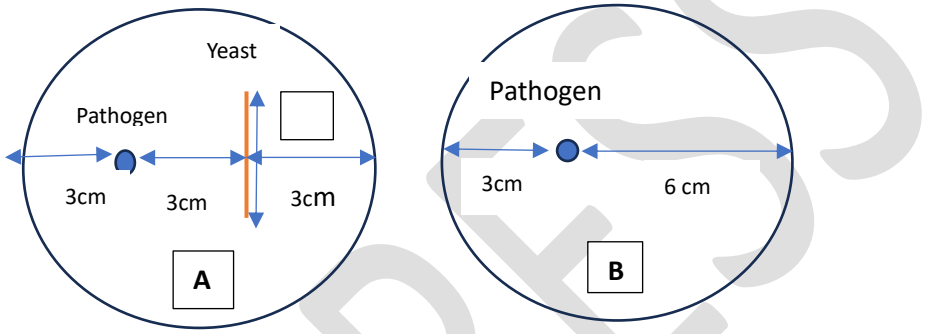

Figure 1. Sketch of the antagonistic interaction test using a dual culture method (A) Epiphytic yeast treatment (B) control.

Percentage of inhibition = $\frac{colony\ area\ of\ control - colony\ area\ of\ treatment}{colony\ area\ of\ control} x\ 100\%$ (Formula 1)

b. Sealed Plate Method.

The assessment of volatile compound formation was conducted using the Sealed Plate Method adapted from Huang *et al*. (2011) with slight modifications. This test was carried out by growing 5-day-old epiphytic yeast isolates by scratching 1 loop of the isolate right in the middle of a petri dish containing PDA medium and growing a piece of *F. fujikuroi* (4 mm diameter) from a 10-day-old pure culture right in the middle of another petri dish also containing PDA medium. Furthermore, the two petri dishes without lids, each containing epiphytic yeast isolates and *F. fujikuroi,* are cupped with the position of the petri dish containing *F. fujikuroi* below and the petri dish containing epiphytic yeast isolate on top, then covered using wrapping. The petri dish containing *F. fujikuroi* was cupped with a petri dish containing only PDA medium without epiphytic yeast used as a control (Figure 2). Observation of colony diameter of *F. fujikuroi* was carried out when the control petri dish was full. The percentage of inhibition was calculated using the Formula 2.

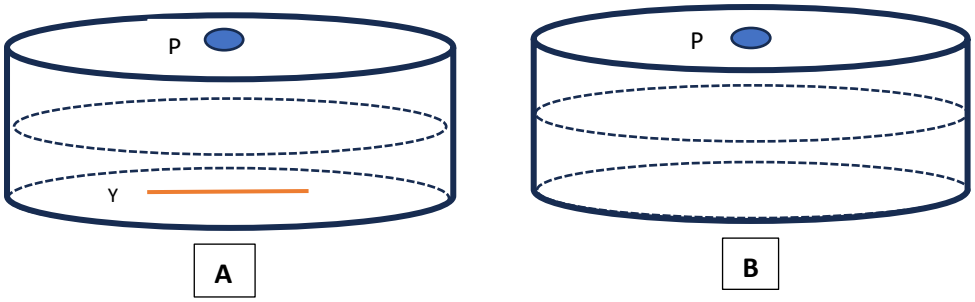

Figure 2. Sketch of antagonist test using Sealed Plate Method(A) Epiphytic yeast treatment (B) Control. P = Pathogen (*F. fujikuroi*), Y = Yeast

Percentage of inhibition = $\frac{Diameter\ colony\ of\ control - diameter\ colony\ of\ treatment}{Diameter\ colony\ of\ control} x\ 100\%$ (Formula 2.

Evaluation of Hyperparasitic Activity of Epiphytic Yeast Isolates Against *Fusarium fujikuroi*

This experiment was conducted to elucidate the hyperparasitic mechanism of epiphytic yeast in suppressing the growth of *Fusarium fujikuroi*. The interaction between the epiphytic yeast and *F. fujikuroi* was examined using the slide culture method. A 1 × 1 cm block of water agar (WA) medium was placed on a glass slide. A mycelial plug of *F. fujikuroi* was positioned on one side of the WA block, while the epiphytic yeast culture was streaked on the opposite side. The preparation was covered with a cover slip and placed in a sterile Petri dish. Microscopic observations of the interaction between the epiphytic yeast and *F. fujikuroi* were carried out using a light microscope at 4–6 days after incubation, following the method described by Hartati *et al*. (2015).

In Vivo* Evaluation of Antagonistic Activity of Epiphytic Yeast Isolates Against *Fusarium fujikuroi

Prior to treatment with epiphytic yeast isolates, rice seeds were artificially inoculated with *Fusarium fujikuroi* by immersing the seeds in a conidial suspension (10^7 conidia mL^{-1}) for 30 min. The inoculated seeds were subsequently soaked in a suspension of epiphytic yeast isolates (10^7 cells mL^{-1}) for 60 min, air-dried, and then planted. The planting medium consisted of a latosol soil–cow dung mixture (2:1, v/v) with a muddy texture, which had been sterilized using the Tyndallization method. For each treatment, 100 seeds were evenly arranged in a germination tray containing planting medium. Observations were conducted 21 days after planting to assess (1) the percentage of non-germinated seeds, (2) the percentage of infected seedlings, and (3) disease severity.

Molecular Identification of Potential Epiphytic Yeast Isolates as A Biocontrol Agent for *F. fujikuroi*

Molecular identification was performed on four promising epiphytic yeast isolates (KTD1, KS2, KS6, and KTD6) that had been previously screened through in vitro and in vivo assays, using the ITS1 and ITS4 primers.

RESULTS

The exploration and isolation of epiphytic yeasts from the leaf surfaces of rice plants collected from three regencies in West Sumatra resulted in a total of 42 isolates. These comprised 18 isolates from Pesisir Selatan Regency (KPS), 12 isolates from Tanah Datar Regency (KTD), and 11 isolates from Solok Regency (KS), as presented in Table 1. The isolates exhibited considerable morphological diversity, as indicated by variations in colony colour, colony shape, colony margin, elevation, and yeast cell morphology.

The antagonistic activity of epiphytic yeast isolates against *Fusarium fujikuroi* was evaluated *in vitro* using the dual culture method. The results showed that almost all epiphytic yeast isolates were able to inhibit the colony area of *F. fujikuroi* at varying levels, with the exception of isolates KS10, KS9, KPS17, and KS4. Fourteen epiphytic yeast isolates exhibited inhibition rates greater than 50%, namely KTD1, KPS12, KTD4, KS8, KS2, KPS8, KS3, KTD12, KTD9, KS6, KTD8, KPS16, KTD11, and KTD6. Among these, isolates KTD1 and KPS12 demonstrated the strongest antagonistic effects, resulting in the smallest fungal colony areas and inhibition percentages of 86.00% and 72.44%, respectively (Table 1). Representative images illustrating the colony area of *F. fujikuroi* in the presence of epiphytic yeast isolates are presented in Figure 3. The fourteen isolates that exhibited inhibition percentages greater than 50% were further evaluated for volatile compound production using the sealed plate method.

This study investigated the antagonistic mechanisms of epiphytic yeasts, particularly hyperparasitism. Microscopic examination demonstrated multiple hyperparasitic interactions with *F. fujikuroi*, including hyphal attachment and penetration, as well as morphological alterations such as hyphal swelling and constriction (Figure 4).

Table 1. The effect of epiphytic yeast isolates on the colony area of *F. fujikuroi* using the dual culture method at 15 days after inoculation (dai)

No.	Epiphytic yeast isolates	Colony area of *F. fujikuroi* (cm^2) ± SD	Percentage of inhibition (%) ± SD
1.	Control	63,58 ± 0,00 a	0,00 ± 0,00
2.	KS10	61,06 ± 3,88 ab	3,96 ± 2,07
3.	KS9	60,39 ± 3,93 abc	4,16 ± 1,51
4.	KPS17	55,90 ± 4,80 abcd	12,08 ± 2,27
5.	KS4	55,16 ± 2,20 abcd	13,24 ± 4,68
6.	KPS4	53,45 ± 0,35 bcde	15,93 ± 0,45
7.	KTD10	51,45 ± 3,11 cdef	19,07 ± 1,45
8.	KPS1	47,70 ± 12,16 defg	24,97 ± 4,41
9.	KTD13	47,61 ± 0,90 defg	25,11 ± 3,74
10.	KS5	45,15 ± 11,95 efgh	28,98 ± 3,81
11.	KPS9	45,03 ± 1,02 efgh	29,17 ± 6,51
12.	KPS13	44,64 ± 2,27 efghi	29,78 ± 4,90
13.	KTD7	43,20 ± 3,02 fghij	32,05 ± 2,53
14.	KPS7	42,14 ± 2,65 ghijk	33,72 ± 2,19
15.	KPS3	41,63 ± 1,79 ghijk	34,52 ± 6,34
16.	KPS15	41,17 ± 5,01 ghijk	35,25 ± 1,25
17.	KTD2	39,45 ± 0,31 ghijkl	37,95 ± 5,17
18.	KPS5	39,21 ± 1,38 ghijkl	38,32 ± 1,64
19.	KPS2	38,75 ± 11,38 ghijkl	39,05 ± 0,07
20.	KTD3	37,84 ± 0,74 hijklm	40,48 ± 3,91
21.	KPS18	37,46 ± 0,92 hijklm	41,08 ± 2,58
22.	KPS11	35,72 ± 3,19 ijklmn	43,81 ± 3,19
23.	KPS14	35,45 ± 2,75 jklmn	44,24 ± 9,58
24.	KPS6	35,22 ± 2,72 jklmno	44,60 ± 9,92
25.	KS11	34,93 ± 6,15 jklmno	45,06 ± 8,75
26.	KS1	33,35 ± 0,21 klmnop	47,54 ± 2,36
27.	KS7	32,07 ± 2,09 lmnop	49,55 ± 0,80
28.	KPS10	32,05 ± 8,65 lmnop	49,59 ± 0,05
29.	KTD5	31,95 ± 7,88 lmnop	49,74 ± 2,10
30.	KTD6	31,71 ± 2,40 lmnopq	50,12 ±13,01
31.	KTD11	31,60 ± 0,45 lmnopq	50,29 ± 8,65
32.	KPS16	29,52 ± 7,33 mnopqr	53,57 ± 1,71
33.	KTD8	29,25 ± 1,41 mnopqr	53,99 ± 0,02
34.	KS6	28,07 ± 3,02 nopqr	55,85 ± 2,33
35.	KTD9	27,61 ± 3,15 nopqr	56,57 ± 3,26
36.	KTD12	26,35 ± 0,77 opqrs	58,55 ± 3,09
37.	KS3	25,15 ± 1,27 pqrs	60,44 ± 1,69
38.	KPS8	24,56 ± 1,62 pqrs	61,37 ± 2,70

39.	KS2	22,83 ± 3,49	qrs	64,09 ± 1,58
40.	KS8	21,95 ± 3,81	rs	65,47 ± 1,00
41.	KTD4	21,50 ± 1,21	rs	66,18 ± 0,28
42.	KPS12	17,52 ± 2,44	st	72,44 ± 2,80
43.	KTD1	8,90 ± 0,32	t	86,00 ± 8,68

Numbers followed by the same lowercase letter in the same column are not significantly different according to the LSD test at the 5% level.

Figure 3. Colony area of *F. fujikuroi* treated with epiphytic yeasts isolates (KPS8, KS3, KTD12, KTD9, KTD11) using dual culture method at 15 days after inoculation (dai)

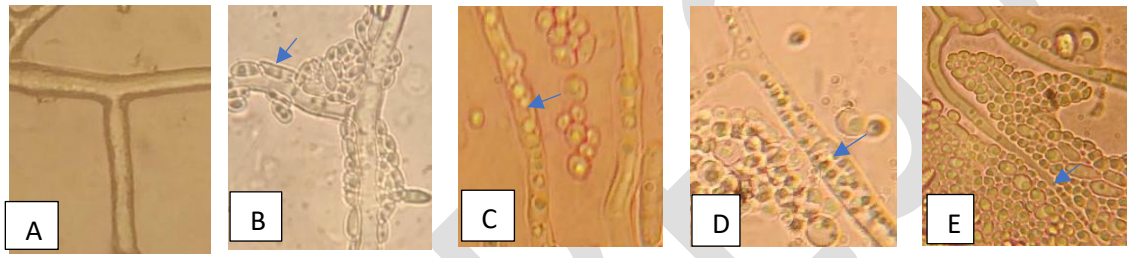

Figure 4. Hyperparasite mechanism of epiphytic yeast against *F. fujikuroi* A. *F. fujikuroi hyphae* (Control), B. Attachment of yeast cells to *F. fujikuroi* hyphae (KTD1,) C. Penetration of yeast cells to *F. fujikuroi* hyphae (KPS12), D. Penetration of yeast cells to *F. fujikuroi* hyphae and enlargement of *F. fujikuroi* hyphae (KTD4), E. Narrowing of *F. fujikuroi* hyphae (KS2)

All fourteen epiphytic yeast isolates tested produced volatile compounds, as indicated by their ability to inhibit the colony diameter of *Fusarium fujikuroi*, with varying levels of effectiveness. The inhibition percentages ranged from 32.35% to 80.00%. Nine isolates (KTD12, KTD1, KPS12, KS8, KTD4, KTD6, KS2, KTD1, and KS6) exhibited inhibition percentages ≥ 50%. Among these, isolate KS6 showed the highest inhibitory activity, with an inhibition rate of 80% (Table 2).

Table 2. The effect of epiphytic yeast isolates on the colony diameter of *F. oxysporum* using the sealed plate method at 15 days after inoculation

No.	Epiphytic yeast isolates	Colony diameter of. *F. fujikuroi* (cm) ± SD		Percentage of Inhibition (%)
1.	Control	9.00 ± 0.00	a	0.00
2.	KPS16	6.80 ± 0.55	b	32.35
3.	KPS8	6.50 ± 0.70	bc	38.46
4.	KTD8	6.50 ± 0.52	bc	38.46
5.	KS3	6.40 ± 0.72	bcd	40.62
6.	KTD9	6.30 ± 0.21	bcde	42.85
7.	KTD12	6.00 ± 0.63	bcde	50.00
8.	KTD1	5.70 ± 0.76	bcde	57.89

9.	KPS12	5.50 ± 0.05	bcde	63.63
10.	KS8	5.50 ± 0.70	bcde	63.63
11.	KTD4	5.50 ± 0.55	bcde	63.63
12.	KTD6	5.40 ± 0.42	cde	66.60
13.	KS2	5.20 ± 0.31	cde	73.03
14.	KTD11	5.10 ± 0.73	de	76.47
15.	KS6	5.00 ± 1.41	e	80.00

Numbers followed by the same lowercase letter in the same column are not significantly different according to the LSD test at the 5% level.

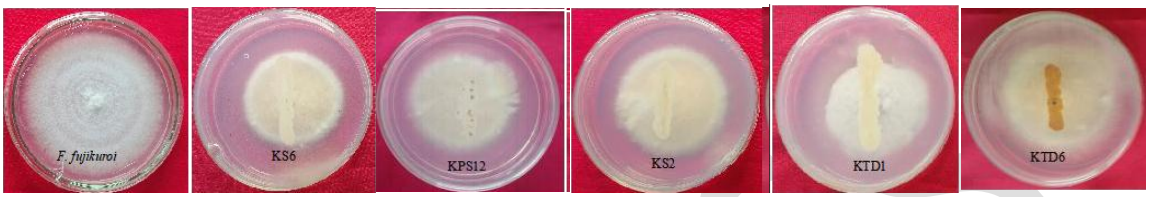

Figure 5. Colony area of *F. fujikuroi* colony treated with volatile compound of epiphytic yeasts isolates using sealed plate method at 15 dai

All rice seed treatments with epiphytic yeast isolates reduced the percentage of ungerminated seeds compared to the control, which showed 89% ungerminated seeds, except for isolates KPS12 and KTD4. The highest inhibition of ungerminated seeds was observed in treatments with isolates KS2 (100%) and KTD6 (71.91%) (Table 3). Seed treatment with epiphytic yeasts did not show a significant effect on the percentage of infected rice seedlings compared to the control, with infection levels ranging from 77 to 100% (Table 4). However, disease severity suppression in rice seedlings was observed in treatments with isolates KTD1, KTD12, KPS16, KTD6, and KS2, with inhibition percentages ranging from 34.00 to 44.18% (Table 5).

Table 3. The effect of epiphytic yeast isolates on ungerminated seeds by F. *fujikuroi*

No	Epiphytic yeast isolates	Ungerminated seeds (%) ± SD	Percentage of Inhibition (%)
1.	Control	89,00 ± 1,41 a	0.00
2.	KPS4	86,00 ± 2,82 a	3.37
3.	KPS12	80,00 ± 1,41 a	10.11
4.	KS8	66,00 ± 1,4 b	25.84
5.	KTD9	62,00 ± 8,48 bc	30.88
6.	KTD8	59,00 ± 5,65 bcd	33.70
7.	KPS8	54,00 ± 2,82 cde	39.92
8.	KTD11	50,00 ± 7,07 de	43.82
9.	KTD1	47,00 ± 4,24 ef	47.19
10.	KPS16	40,00 ± 5,65 fg	55.05
11.	KS6	40,00 ± 1,41 fg	55.05
12.	KTD12	36,00 ± 4,24 g	59.55
13.	KS3	34,00 ± 4,24 g	61.79
14.	KTD6	15,00 ± 5,65 h	71.91
15.	KS2	0,00 ± 0,00 i	100.00

Numbers followed by the same lowercase letter in the same column are not significantly different according to the LSD test at the 5% level.

Table 4. The effect of epiphytic yeast isolates on disease incidence in rice seedlings caused by *F. fujikuroi*

No.	Epiphytic yeast isolates	Infected seedling (%) ± SD		Percentage of inhibition (%)
1	KS3	100.00 ± 4.24	a	-2.04
2.	KTD 8	100.00 ± 8.48	a	-2.04
3.	KS6	100.00 ± 2.82	a	-2.04
4.	Control	98.00 ± 1.41	ab	0.00
5.	KTD4	95.00 ± 1.41	ab	3.06
6.	KS2	92.00 ± 4.24	abc	6.12
7.	KS8	91.00 ± 1.41	abcd	7.14
8	KPS12	90.00 ± 1.41	abcd	8.16
9.	KPS8	90.00 ± 2.82	abcd	8.16
10.	KTD6	90.00 ± 11.31	abcd	8.16
11.	KTD11	90.00 ± 12.72	abcd	8.16
12.	KTD9	89.00 ± 8.48	abcd	9.18
13.	KTD12	85.00 ± 11.31	bcd	13.26
14.	KPS16	79.00 ± 1.41	cd	19.38
15.	KTD1	77.00 ± 12.72	d	21.42

Numbers followed by the same lowercase letter in the same column are not significantly different according to the LSD test at the 5% level.

Table 5. The effect of epiphytic yeast isolates on disease severity of seedlings by *F. Fujikuroi*

No.	Epiphytic yeast isolates	Disease severity (%) ± SD		Percentage of inhibition (%)
1.	Control	93,62 ± 3,71	a	0.00
2.	KTD4	89,12 ± 2,29	ab	5.04
3.	KTD8	84,37 ± 6,79	abc	9.88
4.	KPS12	83,25 ± 2,47	abc	11.07
5.	KS6	75,50 ± 16,61	abcd	19.35
6.	KS8	74,62 ± 6,89	abcd	20.29
7.	KTD11	74,25 ± 16,61	abcd	20.69
8.	KTD9	72,00 ± 6,01	abcd	23.09
9.	KPS8	69,25 ± 10,60	abcd	26.03
10.	KS3	68,12 ± 23,51	abcd	27.23
11.	KTD1	63,87 ± 10,07	bcd	31.77
12.	KTD12	62,12 ± 11,49	bcd	36.00
13.	KPS16	61,87 ± 11,49	bcd	34.00
14.	KTD6	56,12 ± 24,92	cd	40.05
15.	KS2	52,25 ± 21,56	d	44.18

Numbers followed by the same lowercase letter in the same column are not significantly different according to the LSD test at the 5% level.

Four epiphytic yeast isolates (KS2, KS6, KTD1, and KTD6) exhibiting relatively high inhibitory activity against *Fusarium oxysporum* in both *in vitro* and *in vivo* assays were molecularly identified. The amplified nucleotide sequences ranged from 650 to 1,013 bp in length. Isolate KS2 showed 100% sequence similarity to *Candida parapsilosis* strain AMC_CP_002 (accession number KU961982.1) originating from India. Isolates KS6 and KTD1 shared 99.87% sequence similarity with *Moesziomyces antarcticus* voucher HMAS

248025 (accession number MK027038.1), originally isolated from *Echinochloa* and *Leersia* grasses (Poaceae) in China. Isolate KTD6 exhibited 99.85% sequence similarity to *Pseudozyma churashimaensis* strain OK39 (accession number AB704895.1), which was isolated from sugarcane in Japan (Table 6).

Table 6. BLAST analysis results of DNA sequences from epiphytic yeast isolates

Isolate Code	Length of nucleotide sequence (bp)	Similarity (%)	No.GenBank accession	Species
KS2	650	100.00	KU961982.1	*Candida parapsilosis* strain AMC_CP_002
KS6	1013	99.87	MK027038.1	*Moesziomyces antarcticus* voucher HMAS 248025
KTD1	900	99.87	MK027038.1	*Moesziomyces antarcticus* voucher HMAS 248025
KTD6	800	99.85	AB704895.1	*Pseudozyma churashimaensis* strain OK39

DISCUSSION

A total of 42 epiphytic yeast isolates were obtained from rice leaf exploration, consisting of 18 isolates from Pesisir Selatan Regency (KPS), 13 isolates from Tanah Datar Regency (KTD), and 11 isolates from Solok Regency (KS). Differences in isolate abundance among the regencies are likely influenced by environmental conditions, especially altitude, with Pesisir Selatan categorized as lowland, Tanah Datar as medium-altitude, and Solok as highland areas (Table 1). This finding is consistent with Limtong *et al*. (2012), who reported that epiphytic yeasts grow more rapidly at 30°C than at 23°C. A total of 42 epiphytic yeast isolates characterized as potential biological control agents were subsequently evaluated *in vitro* using the dual culture method. All tested epiphytic yeast isolates were capable of suppressing *F. fujikuroi* growth, with 14 isolates showing inhibition rates greater than 50% (Table 1 and Figure 3). The ability of epiphytic yeast isolates to suppress *F. fujikuroi* growth is related to their antagonistic potential as biocontrol agents. In this study, hyperparasitism was observed, as indicated by attachment to the hyphae, penetration, and morphological changes including narrowing and malformation of *F. fujikuroi* hyphae (Figure 4). This is in accordance with the research results of Hartati (2023) which stated that epiphytic yeast isolated from *Pseudozyma hubeiensis* leaves induced hyphal malformation in *Colletotrichum acutatum*, while yeast from *P. shanxiensis* caused hyphal entrapment of the same pathogen.

The ability of all tested epiphytic yeast isolates to produce volatile compounds was confirmed through their inhibitory effects on *F. fujikuroi* colony growth using the sealed plate method, with different inhibition levels observed (Table 2), indicating the antifungal nature of these compounds. This effect is attributed to the antifungal activity of volatile organic compounds (VOCs). Contarino *et al.* (2019) reported that yeasts are capable of producing various antifungal VOCs, including alcohols (ethanol, 2-methylpropanol, 3-methylbutanol, and 2-methylbutanol), organic acids (3-methylbutanoic acid), esters (2-methylpropyl hexanoate, 3-methylbutyl hexanoate, and 3-methylbutyl pentanoate), benzene derivatives (2-phenylethanol and 2-phenylethyl acetate), aldehydes (trans-cinnamaldehyde), and hydrazines (1,1-dimethylhydrazine). The variation in the inhibitory effect on *F. fujikuroi* growth may be attributed to differences in the types or concentrations of volatile compounds produced.

In vivo studies demonstrated that epiphytic yeast isolates significantly reduced the percentage of ungerminated seeds, disease incidence, and disease severity caused by *Fusarium fujikoroi* (Tables 3, 4, and 5). This suppression is likely attributable to multiple antagonistic mechanisms

exhibited by epiphytic yeasts, including competition for nutrients and space, hyperparasitism, antibiosis, and the production of antifungal volatile organic compounds (VOCs), as evidenced by the *in vitro* assay results. This is in accordance with statements by Punja and Utkhede (2003), Nutaratat *et al*. (2012), and Limtong *et al*. (2020) that epiphytic yeasts exhibit various antagonistic mechanisms, including competition for nutrients and space, secretion of lytic enzymes such as glucanase and chitinase, toxin production, emission of volatile organic compounds (VOCs), mycoparasitism, and induction of host resistance.

Based on *in vitro* and *in vivo* assays, four epiphytic yeast isolates with high biocontrol potential (KS2, KTD6, KS6, and KTD1) were selected. Molecular identification revealed that these isolates corresponded to *Candida parapsilosis* (KS2), *Moesziomyces antarcticus* (KS6 and KTD1), and *Pseudozyma churashimaensis* (KTD6). Despite the clear differences in antagonistic activity against *F. fujikuroi* colonies, molecular analysis confirmed that KTD1 and KS2 belong to the same species. Therefore, the variation in inhibitory performance is most likely due to strain-level (intraspecific) differences rather than species level (interspecific) Among these isolates, the epiphytic yeast isolate KS2 (*Candida parapsilosis*) showed the highest average effectiveness in suppressing the growth of *Fusarium fujikuroi* both *in vitro* and *in vivo*. This is partly due to the influence of the volatile compounds it produces, which can suppress the growth of *F. fujikuroi* by 73.03% (Table. 2). Shane *et al.*(2022) stated that the volatile compounds produced by *C. parapsilosis* were primarily characterized by significant abundances of ethanol; 1-butanol, 3-methyl-; 1-butanol, 3-methyl-, acetate; and phenylethyl alcohol. Other highly abundant compounds included the following fatty acid esters: butanoic acid, ethyl ester; propanoic acid, ethyl ester; and propanoic acid, 2-methyl, ethyl ester. Furthermore, Branco *et al*. (2023) reported that *Candida parapsilosis* exhibits high biocontrol potential, supported by its strong adhesion ability, efficient surface colonization, and biofilm formation, which are recognized as key virulence factors. According to Fallah *et al* (2016) the mycelial growth and fumonisin production of Fusarium isolates significantly decreased in the presence of *C. parapsilosis* in comparison with the control cultures. The percentage of mycelial growth inhibition ranged from 56.36% to 74.54%. The minimum and maximum decline in total fumonisin production was 12% and 78%, respectively

Isolate KTD6 (*Pseudozyma churashimaensis*) exhibited the highest average effectiveness, after KS2, in suppressing the growth of *Fusarium fujikuroi*. *P. churashimaensis* produces glycolipid biosurfactants, a mixture of mannosylerythritol lipids (MELs), including a novel tri-acetylated derivative (MEL-A2), from glucose (Morita *et al.,* 2011) and can stimulate plant resistance (Lee and Lee, 2017). Lee and Lee (2017) also reported that foliar application of a suspension of *P. churashimaensis* strain RGJ1 at a concentration of 10^8 cfu mL^{-1} significantly reduced disease severity caused by *Xanthomonas axonopodis* in cucumber Interestingly, the treatment also provided protection against several plant viruses, including cucumber mosaic virus, pepper spot virus, pepper mild mottle virus, and broad bean wilt virus under field conditions. Besides *Pseudozyma churashimaensis*, the production of mannosylerythritol lipids (MELs) has also been demonstrated in *Moesziomyces antarcticus* (Saika *et al*, 2019).

Although yield loss was not directly quantified in the present study, the observed effects on seed germination, seedling infection, and disease severity provide important insights into the potential impact of the disease at later growth stages. Reduced seed germination directly translates into lower plant population density, which is a primary determinant of yield potential in the field. Furthermore, early seedling infection and increased disease severity can impair plant vigor, delay development, and increase susceptibility to secondary infections, ultimately contributing to yield reduction. Previous studies have demonstrated that early-stage infections are strongly associated with substantial yield losses, particularly when disease pressure persists throughout the growing season. Therefore, the seedling-level responses observed in this study

can be considered early indicators of the potential field-level yield losses reported in the literature, supporting the relevance of our findings to phytopathological yield loss assessments.

CONCLUSION

A total of 42 epiphytic yeast isolates were obtained through exploration and characterization, comprising 18 isolates from Pesisir Selatan Regency (KPS), 13 from Tanah Datar Regency (KTD), and 11 from Solok Regency (KS). Of these, 14 isolates were selected as potential biocontrol agents against *Fusarium fujikuroi* based on *in vitro* dual culture and sealed plate assays, exhibiting inhibition rates of 50.12–86.00% and 32.35–80.00%, respectively. *In vivo* evaluation demonstrated that the selected epiphytic yeasts effectively reduced the proportion of ungerminated seeds and disease severity, with inhibition levels ranging from 3.37% to 100% and 5.40% to 44.18%. Based on combined *in vitro* and *in vivo* assessments, four isolates—KS2, KTD6, KS6, and KTD1—showed the highest potential to suppress bakanae disease. Molecular identification revealed that these isolates were *Candida parapsilosis*, *Pseudozyma churashimaensis*, *Moesziomyces antarcticus*, and *Moesziomyces antarcticus*, respectively.

ACKNOWLEDGMENT

The authors gratefully acknowledge the financial support provided by Andalas University through the 2024 UNAND Research Grant under the Master's Thesis Research Scheme (PTM), Contract No. 286/UN16.19/PT.01.03/PTM/2024.

REFERENCES

Assis, S.M.P. and R.L.R Mariano.1999. Antagonism of yeast to *Xanthomonas campestris* pv. *campestris* on cabbage phylloplane in field. Rev. Microbiol, 30: 191-195.

Branco, J., I.M. Miranda and G.R. Rodrigues. 2023. Mechanisms: A comprehensive review of key determinants. J. Fungi, 9 (80): 2-15.

Contarino. R., S. Brighina, B. Fallico, G. Cirvilleri, L. Parafati and C. Restuccia. 2019. Volatile organic compounds (VOCs) produced by biocontrol yeasts. Food Microbiology, 82:70–74.

Darnetty dan E. Sulyanti. 2014. Distribusi dan mating populasi (MPs) Fusarium yang berasosiasi dengan penyakit bakanae pada tanaman padi di Sumatera Barat. Prosiding Seminar Nasional dan Rapat Tahunan Dekan Bidang Ilmu Pertanian BKS-PTN Wilayah Barat. Hal 768-733.

Darnetty and E. Sulyanti. 2017. Respon beberapa varietas padi terhadap serangan *Fusarium fujikuroi* penyebab penyakit bakanae. Jurnal Proteksi Tanaman, 1(1): 18 -23.

Fallah, B. , F. Zaini, R. Daei Ghazvini. R. Kachuei, P. Kordbacheh, M. Safara and S. , Mahmoudi. 2016. The antagonistic effects of *Candida parapsilosis* on the growth of Fusarium species and fumonisin production. Curr Med Mycol, 2(1): 01-06

Freimoser, M.S., M.P. Rueda-Mejia, B. Tilocca and Q. Migheli. 2019. Biocontrol yeasts: mechanisms and applications. World Journal of Microbiology and Biotechnology, 35:154

Gupta,A.K., I.S. Solanki, B.M. Bashyal, Y., Singh and K. Srivastava. 2015. Bakanae of rice-an emerging disease in Asia. JAPS: Journal of Animal & Plant Sciences, 25(6): 1499-1514

Hartati, S., S. Wiyono, S. H. Hidayat and M. S. Sinaga. 2015. Mode of action of yeast-like fungus *Aureobasidium pullulans* in controlling anthracnose of postharvest chili. International Journal of Sciences: Basic and Applied Research (IJSBAR) 20 (2), 253 -263

Hartati, S., S. Wiyono, S. H. Hidayat, and M.S. Sinaga. 2023. Potency and mechanism of yeast-like fungus Pseudozyma in controlling Anthracnose on Chili. Agrosainstek, 7 (1): 8-16

Hermaleni, U., Darnetty, dan Yunisman. 2022. Potency of indigenous epiphytic yeast to control *Colletotrichum capsici* the cause of Anthracnose disease in red chili . Journal of Plant Protection, 6(2):55-65

Hrp, R.F., Darnetty and E. Sulyanti. 2024. Testing the ability of epiphytic yeasts from rice leaves to control Bakanae disease in rice plants. Agrotekma, 9(1): 11-18

Huang, R., G.Q. Li, J. Zhang, L.Yang, H.J. Che, D.H. Jiang and H.C. Huang. 2011. Control of postharvest Botrytis fruit rot of strawberry by volatile organic compounds of *Candida intermedia*. Phytopathology, 101(7), 859–869

Irtwange, S. V. 2006. Application of biological control agents in Pre- and Postharvest operations. Biological Control, 8 (3): 1–12

Karthik, C., and S.H.U. Qingyao. 2023. Current insights on rice (*Oryza sativa* L.) bakanae disease and exploration of its management strategies. Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology). 24(9):755-778

Kazempour .M.N and A. Anvary. 2009. Isolation of *Fusarium fujikuroi* antagonistic bacteria and cloning of its phenazine carboxylic acid genes. African Journal of Biotechnology 8 (23), 6506-6510

Kowalska, J., J. Krzymińska, and J. Tyburski. 2022. Yeasts as a potential biological agent in plant disease protection and yield improvement—a short review. Agriculture, 12(9):1404–1419

Lee, G., S.H. Lee, I. Kyung, and C. M. Ryu. 2017. Foliar application of the leaf-colonizing yeast *Pseudozyma churashimaensis* elicits systemic defense of pepper against bacterial and viral pathogens. Scientific Reports. **7**: 3943

Leslie, J. F., and B. A. Summerell. 2006. *The Fusarium laboratory manual*. Blackwell Publishing.

Limtong, S. and N. Koowadjanakul, 2012. Yeasts from phylloplane and their capability to produce indole-3-acetic acid. World J Microbiol Biotechnol, 28:3323–3335.

Limtong, S., P. Into, and P. Attarat, 2020. Biocontrol of rice seedling rot disease caused by *Curvularia lunata* and *Helminthosporium oryzae* by epiphytic yeasts from plant leaves. Microorganisms, 8(5):647-665

Matić, S., D, Spadaro, A, Garibaldi, and M.L. Gullino. 2014. Antagonistic yeasts and thermotherapy as seed treatments to control *Fusarium fujikuroi* on rice. Biological Control. 73, 59–67.

Naeem, M., M. Iqbal, N. Parveen, Q. Abbas, A. Rehman, and M. Sad. 2016. An over view of bakanae disease of rice. Environ. Sci, 16(2):270–277.

Nutaratat, P., N. Srisuk, P. Arunrattiyakorn, and S. Limtong. 2014. Plant growth- promoting traits of epiphytic and endophytic yeasts isolated from rice and sugar cane leaves in Thailand. Fungal Biol, 118: 683–694.

Punja, Z.K., and R.S. Utkhede. 2003. Using fungi and yeasts to manage vegetable crop diseases. TRENDS in Biotechnology, 21(9): 400–407

Saika, A., H. Koike, T. Yarimizu, T. Watanabe, H. Kitamoto and T. Morita. 2019. Deficiency of biodegradable plastic-degrading enzyme production in a gene-deletion mutant of phyllosphere yeast, Pseudozyma antarctica defective in mannosylerythritol lipid biosynthesis. Extremophiles 9(100)

Thobunluepop, P. 2009. The inhibitory effect of the various seed coating substances against rice seed borne fungi and their shelf-life during storage. Pakistan Journal of Biological Sciences. 12(16): 1102-1110.