BIODIVERSITAS
Volume 23, Number 7, July 2022
Pages: 3820-3826

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d230759

Screening of yeast isolates for the biocontrol of *Sclerotium rolfsii*

NURHALIMAH♥, ANTON MUHIBUDDIN, MUHAMMAD AKHID SYIB'LI
Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia.
Tel.: +62-821-11320039, ♥email: halimahnur514@gmail.com

Manuscript received: 29 May 2022. Revision accepted: 29 June 2022.

Abstract. *Nurhalimah, Muhibuddin A, Syib'li MA. 2022. Screening of yeast isolates for the biocontrol of* Sclerotium rolfsii. *Biodiversitas 23: 3820-3826. Sclerotium rolfsii* is a fungal pathogen that can reduce peanut production. Biological control with yeast antagonist offers an environment friendly control for this pathogen. The aim of this study was to isolate and identify yeast antagonists, and to study their antagonistic mechanism and potential to control the peanut disease caused by *S. rolfsii.* The study was conducted from October 2020 to May 2021. The study consisted of four stages: isolation and identification of yeasts, yeasts antagonist testing against *S. rolfsii,* molecular identification of yeast NH_4, and scanning electron microscope observation of NH_4 against *S. rolfsii.* Based on the research, four different yeast isolates were isolated, identified, and found able to be developed as a biological control agent to inhibit the growth of *S. rolfsii.* Identification showed that NH_1 was *Saccharomyces* sp. with inhibitory percentage of 9,26%, NH_2 was *Candida* sp. with 22,21% inhibition of, NH_3 *Pichia* sp. with 11,11% inhibition of, and NH_4 was *Wickerhamomyces* sp. with an inhibitory percentage of 32,96%. The results of the yeast antagonism mechanism showed that NH_1 (*Saccharomyces* sp.) and NH_3 (*Pichia* sp) had not showed any mechanism while, NH_2 (*Candida* sp.) had a competition and NH_4 (*Wickerhamomyces* sp.) had an antibiosis mechanism. NH_4 can suppress the growth of *S. rolfsii* by more than 30%, which has been identified as species of *Wickerhamomyces* sp. (NH_4) with 99.84% homology with *Wickerhamomyces anomalus* CBS 5759 with accession number MH545921.1.

Keywords: Biological control, peanut, *Sclerotium rolfsii*, yeast

INTRODUCTION

Peanut is an important economic, oil and feed crop in the world (Guclu et al. 2020), especially in Indonesia. This plant has a high economic value due to its high nutritional contents, especially in protein (Chairudin 2018). According to the USDA National Nutrient Database for Standard Reference, 100 g of raw peanuts has approximately 567 calories, 25.8% proteins (USDA 2015). About 85% of peanut available in Indonesia is used for food with a consumption level of 0.27 Kg/capita/year (Respati et al. 2014). Because of this, they often encounter several obstacles in their cultivation; one of which is the presence of plant diseases caused by different phytopathogens such as *Sclerotium rolfsii* Sacc. *S. rolfsii* can infect all parts of the plant and, resulting in a yield reduction of up to 30-80% (Sukamto and Wahyuno 2013). Stem rot is a soil borne fungal disease that destroys most peanut produce of the countries such as India, China and the United States (Jin et al. 2011).

The control of peanut plant diseases including caused by *S. rolfsii*, so far has been controlled by using synthetic fungicides (Khan and Javaid 2015). At present, available methods to control peanut southern stem rot include agricultural measures (such as deep ploughing, crop rotation and soil disinfection), treating soil, seeds and plants with fungicides, as well us using biological microorganisms (Lu et al. 2016). *S. rolfsii* is a devastating soil borne fungal pathogen that causes diseases in more than 500 plant species including chickpea, chili, bell pepper, etc. (Javaid et al. 2020; Khan et al. 2020; Jabeen et al. 2021). The continuous use of synthetic fungicides can cause various problems to plant animals and soils because these fungicides are difficult to degrade naturally (Dharmaputra et al. 2016). Problems appeared due to the use of synthetic fungicides that will rise the pathogen's resistance and have negative effects on ecological environment organisms (Yao et al. 2020). One approach that can be taken to control fungal pathogens in plants is the use biological control agents (Ali et al. 2020; Sharf et al. 2021), is one of the suitable safest way to avoid negative of using synthetic fungicides.

Biological control is an environmentally friendly strategy to control plant pathogens by using antagonistic microorganisms including bacteria and fungi (Khan and Javaid 2021, 2022; Javaid et al. 2021; Sharf et al. 2021). One of the antagonist microorganisms that can be used as biological control agents for plant diseases are yeasts, such as antagonist yeasts. The use of antagonistic yeasts as biological agents has given many advantages because it has anti-microbial properties and has a better capability to resist environmental stress (Widiastutik and Alami 2013). Several types of yeasts that can act as biological control agents are the yeasts *Pichia guilliermondii*, *Candida musae*, *Candida quercitrusa*, and *Issatchenkia orientalis* which are included in the antagonist yeast against the pathogenic fungus *Colletotrichum capsici* which causes anthracnose in chili peppers (Dharmaputra et al. 2016).

The biological control mechanism for plant diseases can be understand through the interaction between antagonistic microorganisms and plant pathogens. Each biological control agent shows an inhibitory mechanism that can not the same as the others (Loekas 2013). Several biological control mechanisms were used by antagonistic yeasts, including parasitism, antibiosis and competition.

Based on the mechanism, the use of antagonistic yeast isolated from peanuts has the potential as an alternative for controlling *S. rolfsii*. The biological control techniques by utilizing antagonist agents have the potential to reduce the negative impact of using fungicides (Nurlela et al. 2016). Therefore, this study aimed to isolate and identify yeasts that have the potential to be antagonistic yeasts and to determine the mechanism of the antagonists produced and their potential to control disease-causing pathogens in peanut plants caused by *S. rolfsii*.

MATERIALS AND METHODS

Isolation, identification, and purification of *Sclerotium rolfsii*

Sclerotium rolfsii was obtained from the collection Department of Pests and Plant Diseases, Universitas Brawijaya. *S. rolfsii* was cultured on Potato Dextrose Agar (PDA) media at room temperature (± 28ºC) and inoculated under dark conditions for 5-7 days and then used in antagonistic in vitro assay. Based on the research (Shofiana et al. 2015), symptomatic plant parts were cut ± 1 cm long, sterilized by washing with 1% sodium hypochlorite (NaOCl) solution for 1 minute and then soaking in 70% alcohol for 1 minute and repeated 2 times. After that, the sample pieces were rinsed with distilled water for 1 minute and repeated 2 times, then dried on sterile tissue paper. After drying, the sample pieces were planted on PDA media in Petri dishes and incubated for 5-7 days at room temperature or until the fungus grew to fill the Petri dish.

The fungus was identified to the genus level with the aid of observing characteristics of hyphae (color, shape and size) and clamp connections. The isolated fungus was transferred into a new PDA media using an ose needle to obtain a pre-culture. If the fungus that grew was still mixed with other fungi, it was purified again (Shofiana et al. 2015).

Exploration and morphological identification of yeast

Sampling for the isolation yeast was carried out in Wagir, Malang, Indonesia, with a diagonal sampling method. The age of peanut plants was 30-50 days after planting. The yeasts were isolated from the soil of peanut plants. About 10g of the soil sample was weighed, put into Erlenmeyer 250 mL containing 90 mL of sterile water, and homogenized on a rotary shaker at room temperature for 24 hours. Serial dilution was carried out as put 1 mL of suspension into test tube containing 9 mL of sterile water, and diluted with series 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} of dilutions. Each series of dilutions of 0.1 mL suspension were taken and subsequently, samples were plated on YMA (Yeast Malt Agar) media with the method of spread (spread plate) used stick L (Widiastutik and Alami 2014). Then incubated at room temperature for approximately 3 days and observed for macroscopic colony growth and transferred to new media until uniform pure isolates were obtained.

Yeast isolates were further purified and identified, such as cultural characteristics; colony color, shape and texture, asexual structures; shape and size of cells (Bhargava 2019). Purification to obtain pure isolates was carried out to selected colonies that growth dominant and had different colonies morphological characteristics from the others.

Antagonistic test of yeast against *Sclerotium rolfsii*

Antagonistic test of yeast isolates with *S. rolfsii* used a completely randomized design (CRD) with 4 treatments, namely the total isolated yeast isolates. Control treatment without yeast inoculation was also prepared as a comparison. Each treatment was repeated 3 times. Antagonistic test of yeast against *S. rolfsii* was carried out by the method of Shofiana et al. (2015). Yeast and *S. rolfsii* were grown on PDA media. Yeast is etched on PDA media in the middle of a Petri dish. Pure cultures of *S. rolfsii* were scratched on the right and left sides of the yeast with a distance of ± 3 cm and then incubated at room temperature and observed for 7 days by measuring the width of the yeast inhibition zone against *S. rolfsii* every day. The level of antagonist inhibition was calculated using the formula (Begum et al. 2008).

$$\text{PIRG} = \frac{R1-R2}{R1} \text{ x } 100\%$$

Where:

PIRG: The percentage of inhibition level on pathogen growth (%)

R1: The number of radii (R1 + R2) of pathogenic colonies without yeast treatment (control)

R2: The number of radii (R1 + R2) of pathogenic colonies treated with yeast

Molecular identification of yeast NH$_4$

Identification of antagonistic yeast based on molecular character was carried out at the genetics laboratory of PT. Genetica Science Indonesia (Tangerang, Indonesia). The identification steps started with DNA extraction, PCR amplification, purification of PCR products, and sequencing. DNA extraction was carried out using the CTAB method (according to the protocol issued by the company). Quantity test of extracted DNA was carried out using a NanoDrop ND-1000 Biocompare spectrophotometer. Open the software, then select Nucleid acid. Calibration was carried out before measurement using a blank in the form of 3 L sterile aquadest for 3 minutes. Enter 1µL of ddH2O then click blank. A 1µL DNA sample is inserted and closed then click Measure. The concentration of DNA is seen on a computer device.

Identification of the antagonist yeast was carried out by PCR using universal primers, namely NL1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and NL2 (5'-GGTCCGTGTTTCAAGACGG-3'). Polymerase Chain Reaction (PCR) was carried out in three stages, the initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, then annealing at

54°C for 30 seconds, and extension at 72°C for 1 minutes, followed by a final extension at 72°C for 10 minutes and a storage temperature of 4°C in the final stage.

Visualization of PCR results was carried out by electrophoresis method using 1% agarose gel. 5µL of PCR product was mixed with 1µL of safety loading dye and put into each well (Electroporator Bio-red Powerpac 300). 1Kb marker is used as a reference for the band size. Electrophoresis was carried out in 0.5% TBE buffer solution at 100 volts for 45 minutes. The results of PCR products were visualized using gel documentation under UV light. The PCR products were sequenced to determine the base sequence. The sequences obtained were analyzed by means of alignment with BLAST (Basic Local Alignment Search Tool) to determine the percentage of base pair similarity with the references found in Gen bank. The phylogenetic tree was constructed using MEGA X software using the neighbor-joining method and bootstrap analysis (1000 replicates) using the Kimura-2 method.

Scanning electron microscope (SEM) observation of NH_4 vs *Sclerotium rolfsii*

Scanning Electron Microscope (SEM) observations were carried out the sample of the antagonist test of NH_4 against isolates of the fungal pathogen *S. rolfsii*. Observations were made at the Bioscience Laboratory, Universitas Brawijaya, East Java, Indonesia. The sample that was taken depended on the results of the antagonist test that showed the highest inhibition of pathogens. Each antagonist mycelium was planted at one end of the agar plug and the pathogen at a distance of 3 cm. small pieces of agar were taken from the dual cultures at the interaction zone, while yeast and fungi had been at their early range of interaction. The pieces were coated with platinum and observed in a Hitachi TM3000 SEM. Scanning electron microscope (SEM) provided useful information on the shape, size and SEM can be used for basic as well as diagnostic purposes and produces images of biofilm samples by scanning the surface with a focused beam of electrons (Nazir et al. 2019).

Statistical analysis

The yeast antagonist test against the pathogen *S. rolfsii* was carried out using a Completely Randomized Design (CRD). All data were analyzed by ANOVA with a level of 5%. If it showed a significant effect, then proceed with the DMRT (Duncan Multiple Range Test) further test. All data obtained were processed using Microsoft Excel 2019 software, then presented in the form of graphs and tables. Statistical analysis used R-statistics software.

RESULTS AND DISCUSSION

Isolation and identification of yeast

The results of exploration, isolation and identification of yeasts obtained 4 different yeast isolates, consisting of *Saccharomyces* sp., *Candida* sp., *Pichia* sp., and *Wickerhamomyces* sp. The colonies of *Saccharomyces* sp. on PDA media were white, with a granular and dense texture, convex elevation, shiny surface and flat edges. Oval-shaped single cells with multilateral budding, 2.54-5.18 × 3.62-8.58 µm in size. Similarly, the *Candida* sp. colonies on PDA media were white, with a dense and granular texture, convex elevation, shiny surface and tassel edges. Round-shaped cells and cylindrical, single cell and group with multilateral budding, single cell sized between 2.85-4.47 × 4.39-9.91 µm. While, *Pichia* sp. colonies on PDA media were white, with a granular and dense texture, convex elevation, not shiny surface and flat edges; egg-shaped cell or cylindrical with multilateral budding, single cell size between 3.14-4.52 × 5.27-7.10 µm, the colonies of *Wickerhamomyces* sp. on PDA media were white, with a dense and granular texture, convex elevation, shiny surface and tassel edges. Oval or egg-shaped cells with multilateral budding, single cell size between 3.1-7.2 × 2.2-5.2 µm.

Antagonistic test of yeast against *Sclerotium rolfsii*

Based on the results of tests (Table 1), 4 yeast isolates were able to inhibit the growth of *S. rolfsii*, with varying degrees of inhibition. Yeasts having an average inhibition of below 30% in 7 days' incubation. The percentage inhibition of individual yeast isolates was observed as: *Saccharomyces* sp. NH_1 (9.62%), *Candida* sp. NH_2 (22.21%), *Pichia* sp. NH_3 (11.11%) and *Wickerhamomyces* sp. NH_4 (32.96%). The treatment of isolate NH_1 showed the lowest percentage of inhibition, while the highest percentage of inhibition during 7 days of incubation as shown in the treatment of isolate NH_4. The lowest percentage of yeast resistance to *S. rolfsii* was on day 8 of DAI.

In the antagonist test the treatment (control) without giving yeast isolate, the pathogen *S. rolfsii* was grown in a petri dish to the right and left sides, so that the growth became equal and spread (Figure 1.A). In the treatment of isolate NH_1 (*Saccharomyces* sp.) with the fungal pathogen *S. roflsii* (Figure 1.B) none type of the mechanism was observed between both pathogen and biocontrol agent. In the treatment of isolate NH_2 (*Candida* sp.), the growth of yeast and the pathogen *S. rolfsii* showed the competition in a petri dish (Figure 1.C). The treatment of isolate NH_3 (*Pichia* sp.) against *S. rolfsii* showed the antibiosis mechanism, it did produce an inhibitory mechanism against *S. rolfsii*. (Figure 1.D). In the treatment isolate NH_4 showed the results of the antibiosis mechanism (Figure 1.E), where the pathogen *S. rolfsii* could grow closer to the yeast but the hyphae that were closer to the yeast looked more transparent. In the treatment of isolate NH_4 (*Wickerhamomyces* sp) it was also seen that the yeast secreted a liquid or clear zone which was thought to be used to inhibit the growth of pathogens.

Molecular identification of yeast NH_4

Molecular identification of yeast NH_4 that act as antagonistic agents was carried out by using the Polymerase Chain Reaction (PCR) technique. Based on the results of macroscopic and microscopic identification, NH_4 was of the genus *Wickerhamomyces* sp. The results of DNA extraction showed that the quantitative value of DNA concentration in the NH_4 sample was 10.8 ng/µl with a purity level of 1.92. At the DNA amplification stage, it is a process of doubling DNA strands using PCR with repeated cycles. Visualization of the amplified DNA band was carried out using the TBE *agarose* electrophoresis method with a concentration of 1%. The results of the visualization are shown in (Figure 2). At the PCR amplification stage using universal primers NL1 and NL4, obtained a visualization of the PCR results gel (Figure 2) which shows a product band of 700 bp.

The Neighbor-joining method is the most frequently used in reconstructing phylogenetic trees (Figure 3). Boostrap analysis can be done by re-sampling from existing data to test the validity of the phylogenetic tree construction. The results of sequence analysis using BLAST showed that NH_4 had a similar base arrangement to the isolate *Wickerhamomyces anomalus* CBS 5759 with accession number MH545921.1 with a similarity level of 99.84%. A phylogenetic tree was created to analyze the relationship between isolates. The results of the phylogenetic tree showed that NH_4 was related to the isolate *Wickerhamomyces anomalus* CBS 5759.

Scanning electron microscope (SEM) observation of antagonist yeast NH_4 to *Sclerotium rolfsii*

The results of SEM observations (Figure 4), showed that the hyphae of *S. rolfsii* were malformed in certain parts (Figure 4.A). The state of malformed hyphae was a hyphal structure that damaged or a hyphae condition that has decreased hyphal size so that the hyphae appear to be thin and indented. Subsequently, the hyphae also showed the formation of protrusions and swell of the hyphae (Figure 4.B). In antagonism of *W. anomalus* to *S. rolfsii*. there was a degradation event of the pathogenic hyphae wall of *S. rolfsii*. resulted in flattened hyphae (Figure 4.C). Each mechanism has a different effect depending on the plant pathogen and the yeast. The effect of antagonistic yeasts on pathogenic fungi showed that hyphae were more resistant to attack by antagonists, because they were not completely lysed.

Discussion

In the present time, the use of synthetic that were not environmentally friendly in controlling plant disease is going limited. The continuous use of these materials can cause environmental damage and pathogen resistance. The control of plant diseases by the use of biological control using antagonistic microorganisms, one of them is yeast is a best alternate to overcome the problems caused by the use of synthetic fungicides. According to Herliyana et al. (2013) biological control using antagonist agents once can suppress the growth and development of pathogens for a relatively long period without causing environmental pollution.

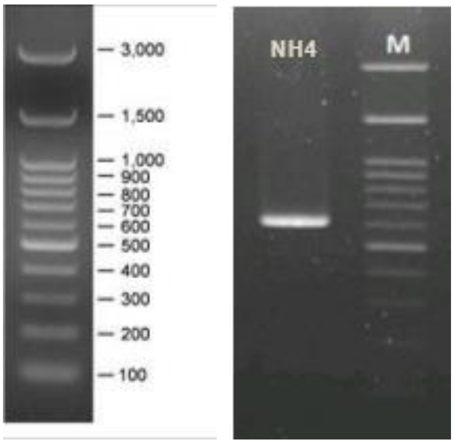


Figure 2. Visualization of PCR results with NL1 and NL4 primers

Table 1. The average percentage of yeast inhibition against *Sclerotium rolfsii* pathogen for 8 DAI (days after inoculation)

Treatments	Average percentage of inhibition *Sclerotium rolfsii* (%) days to-						
	2 DAI	3 DAI	4 DAI	5 DAI	6 DAI	7 DAI	8 DAI
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
NH_1	40.07±23.81	49.61±25.75	42.41±24.00	32.22±25.11	27.03±25.68	16.29±24.37	9.62±16.67
NH_2	60.9±5.84	62.98±5.19	60.47±3.55	45.18±6.31	37.40±4.20	31.10±2.94	22.21±4.84
NH_3	59.52±8.24	68.81±11.97	61.91±11.84	47.40±11.56	38.88±8.68	27.03±6.11	11.11±1.11
NH_4	56.34±9.09	65.39±14.55	63.15±19.08	54.81±17.58	47.03±18.67	41.47±20.19	32.96±23.33

A **B** **C** **D** **E**

Figure 1. Antagonism test between the antagonistic yeast against *Sclerotium rolfsii*. Control (A), NH_1 (B), NH_2 (C), NH_3 (D) and NH_4 (E)

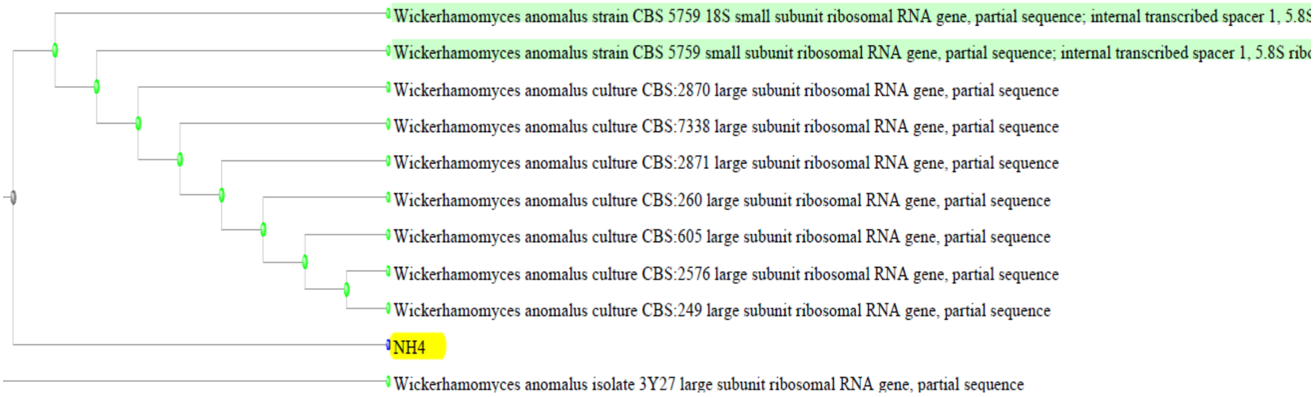
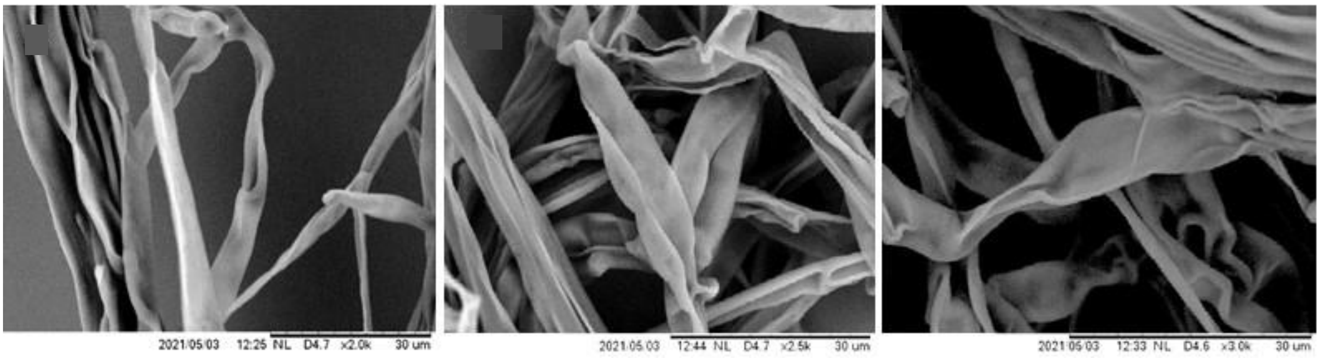


Figure 3. Phylogenetic construction of yeast NH_4 using the neighbor-joining method and bootstrap analysis (1000 replications) using the Kimura-2 method using Mega software



A **B** **C**

Figure 4. The SEM results of the yeast antagonist test with the pathogen *Sclerotium rolfsii* in the treatment of NH_4 with a magnification of 200, 250 and 300 times. hyphal malformation (A), swollen hyphae (B) and flat hyphae (C)

The results showed that *S. rolfsii* in peanuts can be controlled through biological control using antagonist yeast. Yeast obtained from the isolation have various types of yeast, including *Saccharomyces* sp., *Candida* sp., *Pichia* sp., and *Wickerhamomyces* sp. Several studies have shown that a large number of plant-beneficial microorganisms (e.g., yeasts, yeast-like fungi) protect plants (De Curtis et al. 2010), one of them is the use of yeast *Streptomyces* sp. in managing peanuts stem rot disease caused by *S. rolfsii* under the greenhouse conditions (Simi et al. 2016). Then the genus *Candida* is one of the yeasts grouped to the phylum *Ascomycota* where the yeast is grouped as a phylum that has the ability to antagonism (El-Tarabily et al. 2006). In addition, strains of *Wickerhamomyces anomalus* were also found to inhibit the growth of phytopathogenic fungi and showed antagonistic activity (Khunnamwong et al. 2019).

The results showed that the inhibition occurred between the yeast and the pathogenic fungus *S. rolfsii* had different results. The ability of antagonistic yeasts as biological agents can be shown by seeing the inhibition percentage from the antagonist mechanism produced. *Candida subhashii* was identified as a competitive and antagonistic soil yeast with potential as a novel biocontrol agent against plant pathogenic fungi (Hilber-Bodmer et al. 2017). However, in the end the efficiency of antagonism is influenced by several factors, for example; environmental conditions and each mechanism action depended on certain interactions between the pathogen and the antagonistic organism, but also between one of them and its host (Syed Ab Rahman et al. 2018).

Wickerhamomyces sp., had the highest inhibitory yield among other yeast isolates. From the results of molecular identification, NH_4 was identified as *Wickerhamomyces anomalus* CBS 5759 with a product band of 700 bp. According to Fatchiyah et al. (2011) the size of the amplification target is usually less than 1,000 base pairs (bp) or 1 kbp because the amplification results were more than 1 kbp have an inefficient process, so the long products susceptible to inhibitors that effected polymerase DNA enzyme work and longer time will be needed.

The antagonistic mechanism that occurs can be supported by the Scanning Electron Microscope (SEM) observations. It can be seen in this study that the hyphae were damaged at any existing magnification size. According to Shalehah (2017) that fungal hyphae colonized by yeast can experience changes in shape such as shrinking the size of the hyphae so that the hyphae look thin and curvy, hyphae swelling and the formation of protrusions on the hyphae. This is because the nutrients in the hyphae exposed to the outside, which resulted in inhibited metabolism in *S. rolfsii* (Hardianto 2018). Taley et al. (2013) reported that hyphae damage, mycoparasitism and eventually lysis of *Aspergillus flavus* hyphae were caused by *W. anomalus*. Each mechanism has a different effect depending on the plant pathogen and the yeast. The effect of antagonistic yeasts on pathogenic fungi showed that hyphae were more resistant to attack by antagonists, mainly because they were not completely lysed.

In this study, yeast isolates were able to control the pathogen *S. rolfsii*, especially the NH_4 yeast *Wickerhamomyces anomalus*. The use of yeast as an antagonist microorganism in this study was due to the ability of yeast to inhibit the growth of pathogenic fungi, which have different potentials for success. The use of pathogenic fungi *S. rolfsii* which is a soil borne pathogen can be suppressed by the use of antagonist yeasts. According to (Rachmawati 2017) based on the results of his research stated that the yeast *K. marxianus* was able to suppress the growth of *S. rolfsii* by 5.97% while the yeast *M. guilliermondii* was able to suppress the growth of the fungus *S. rolfsii* by 7.76% *in vitro*. In this study the yeast *Wickerhamomyces anomalus* was able to inhibit the growth of the fungus *S. rolfsii in vitro*. According to Campos-Martínez et al. (2016) *W. anomalus* isolated from avocado leaves was shown *in vitro* to have antagonistic activity against *C. gloeosporioides* caused by anthracnose disease in avocados.

In conclusion, the yeast isolated from the soil, namely the yeast *Wickerhamomyces anomalus* CBS 5759, had the highest inhibition and produced an antagonistic mechanism in the form of antibiosis, so it has the potential to be used as a biological control for soil borne diseases in peanuts.

ACKNOWLEDGEMENTS

The author would like to thank Yoga Lorensa Putra Yusa for his help during the survey and the collection of soil samples. The laboratory assistance provided by Fitria Sulistiya and Putri Dewi Sartika was greatly appreciated.

REFERENCES

Ali A, Javaid A, Shoaib A, Khan IH. 2020. Effect of soil amendment with *Chenopodium album* dry biomass and two *Trichoderma* species on growth of chickpea var. Noor 2009 in *Sclerotium rolfsii* contaminated soil. Egypt J Biol Pest Control 30: 102. DOI: 10.1186/s41938-020-00305-1.

Begum MM, Sariah M, Abidin MAZ, Puteh AB, Rahman MA. 2008. Antagonistic potential of selected fungal and bacterial biocontrol agents against *Colletotrichum truncatum* of soybean seeds. Pertanika J Trop Agric Sci 31 (1): 45-53.

Bhargava. 2019. Antimicrobial Resistance Surveillance and Research Network 2nd Edition. Indian Council of Medical Research. India.

Campos-Martínez A, Velázquez-del Valle MG, Flores-Moctezuma HE, Suárez-Rodríguez R, Ramírez-Trujillo JA, Hernández-Lauzardo AN. 2016. Antagonistic yeasts with potential to control *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. and *Colletotrichum acutatum* JH Simmonds on avocado fruits. Crop Prot 89: 101-104. DOI: 10.1016/j.cropro.2016.07.001.

Chairudin. 2018. Pengaruh varietas kacang tanah (*Arachis hypogeae* L.) dan dosis pengapuran terhadap penyakit busuk batang *Sclerotium rolfsii* Sacc. pada lahan gambut. Jurnal Agrotek Lestari 5: 1. DOI: 10.35308/jal.v4i1.636. [Indonesian]

De Curtis F, Lima G, Vitullo D, De Cicco V. 2010. Biocontrol of *Rhizoctonia solani* and *Sclerotium rolfsii* on tomato by delivering antagonistic bacteria through a drip irrigation system. J Crop Prot 29: 663-670. DOI: 10.1016/j.cropro.2010.01.012.

Dharmaputra OS, Sudirman LI, Misnawati MM. 2016. Potensi khamir sebagai agens pengendali hayati *Colletotrichum capsici*, cendawan penyebab antraknosa pada buah cabai. Jurnal Hortikultura Indonesia 7 (2): 91-101. DOI: 10.29244/jhi.7.2.91-101. [Indonesian]

El-Tarabily KA, Sivasithamparam K. 2006. Potential of yeasts as biocontrol agents of soil borne fungal plant pathogen and as plant growth promoters. Mycoscience 47: 25-35. DOI: 10.1007/S10267-005-0268-2.

Fatchiyah E, Arumingtyas L, Widyarti S, Rahayu S. 2011. Biologi Molekuler Prinsip Dasar Analisis. Erlangga, Jakarta. [Indonesian]

Guclu V, Aydogdu M, Basak M, Kizil S, Uzun B, Yol E. 2020. Characterization of a groundnut collection to stem rot disease caused by *Sclerotium rolfsii*. Aust Plant Pathol 49: 691-700 DOI: 1007/s13313-020-00748-y.

Hardianto. 2018. Optimalisasi fosfat untuk pertumbuhan dan kemampuan antagonis *Saccharomyces cerevisiae* terhadap *Fusarium* sp. [Skripsi]. Universitas Brawijaya, Malang. [Indonesian]

Herliyana E, Jamilah R, Taniwiryono D, Firmansyah M, 2013. Uji In-vitro pengendalian hayati oleh *Trichoderma* spp. terhadap *Ganoderma* yang menyerang sengon. Jurnal Silvikultur Tropika 4 (3): 190-195. [Indonesian]

Hilber-Bodmer M, Schmid M, Ahrens CH, Freimoser FM. 2017. Competition assays and physiological experiments of soil and phyllosphere yeasts identify *Candida subhashii* as a novel antagonist of filamentous fungi. J BMC Microbiol 17: 4. DOI: 10.1186/s12866-016-0908-z.

Jabeen N, Javaid A, Shoaib A, Khan IH. 2021. Management of southern blight of bell pepper by soil amendment with dry biomass of *Datura metel*. Plant Pathol J 103 (3): 901-913. DOI: 10.1007/s42161-021-00874-6.

Javaid A, Afzal R, Shoaib A. 2020. Biological management of southern blight of chili by *Penicillium oxalicum* and leaves of *Eucalyptus citriodora*. Intl J Agric Biol 23 (1): 93-102.

Javaid A, Ali A, Shoaib A, Khan IH. 2021. Alleviating stress of *Sclertium rolfsii* on growth of chickpea var. Bhakkar-2011 by *Trichoderma harzianum* and *T. viride*. J Anim Plant Sci 31 (6): 1755-1761. DOI: 10.36899/JAPS.2021.6.0378.

Jin P, Gao XY. 2011. Research and overview of southern blight. J Agric Catastrophol 1 (1): 14-22.

Khan IH, Javaid A. 2015. Chemical control of collar rot disease of chickpea. Pak J Phytopathol 27 (1): 61-68.

Khan IH, Javaid A. 2021. *In vitro* screening of *Aspergillus* spp. for their biocontrol potential against *Macrophomina phaseolina*. J Plant Pathol 103 (4): 1195-1205. DOI: 10.1007/s42161-021-00865-7.

Khan IH, Javaid A. 2022. DNA cleavage of the fungal pathogen and production of antifungal compounds are the possible mechanisms of action of biocontrol agent *Penicillium italicum* against *Macrophomina phaseolina*. Mycologia 114 (1): 24-34. DOI: 10.1080/00275514.2021.1990627.

Khan IH, Javaid A, Al-Taie AH, Ahmed D. 2020. Use of neem leaves as soil amendment for the control of collar rot disease of chickpea. Egypt J Biol Pest Control 30: 98. DOI: 10.1186/s41938-020-00299-w.

Khunnamwong P, Lertwattanasakul N, Jindamorakot S. 2019. Evaluation of antagonistic activity and mechanisms of endophytic yeasts against pathogenic fungi causing economic crop diseases. Folia Microbiol 65: 573-590. DOI: 10.1007/s12223-019-00764-6.

Loekas S. 2013. Pengantar Pengendalian Hayati Penyakit Tanaman. PT. Rajagrafindo Persada, Depok. [Indonesian]

Lu Y. 2016. Biological Control of *Bacillus amyloliquefaciens* 41B-1 on Peanut Stem Rot. Nanjing Agricultural University, Nanjing, China.

Nazir R, Zaffar MR, Amin I. 2019. Bacterial biofilms: the remarkable heterogeneous biological communities and nitrogen fixing microorganisms in lakes. In: Bandh SA, Shafi S (eds). Nowsheen Shameem Freshwater Microbiology. Srinagar, India. DOI: 10.1016/B978-0-12-817495-1.00008-6.

Nurlela, Hakim L, Ulim MA. 2016. Efektivitas beberapa agen antagonis dan cara aplikasinya untuk menekan pertumbuhan *Sclerotium rolfsii* pada tanaman kedelai *Glycine max* L. Merril. Jurnal Ilmiah

Mahasiswa Unsyiah 1 (1): 155-167. DOI: 10.17969/jimfp.v1i1.1012. [Indonesian]

Rachmawati S. 2017. Identifikasi molekuler dan karakterisasi fisiologi khamir serta uji potensi antagonismenya terhadap jabuh rebah semai (*Sclerotium rolfsii*). [Skripsi]. Universitas Brawijaya, Malang. [Indonesian]

Respati E, Hasanah L, Wahyuningsih S, Sehusman, Manurung M. 2014. III. Kacang Tanah (in Indonesia). In: Sabarella (eds). Buletin Konsumsi Pangan. Pusat Data dan Sistem Informasi Pertanian. [Indonesian]

Shalehah NA. 2017. Eksplorasi Khamir dan Bakteri sebagai Kandidat Agens Antagonis Penyebab Penyakit Busuk Pangkal Batang (*Botryodiploidi theobromae* Pat.). [Skripsi]. Institut Pertanian Bogor, Bogor. [Indonesian]

Sharf W, Javaid A, Shoaib A, Khan IH. 2021. Induction of resistance in chili against *Sclerotium rolfsii* by plant growth promoting rhizobacteria and Anagallis arvensis. Egypt J Biol Pest Control 31: 16. DOI: 10.1186/s41938-021-00364-y.

Shofiana RH. 2015. Eksplorasi jamur endofit dan khamir pada tanaman cengkeh (*Syzygium aromaticum*) serta uji potensi antagonismenya terhadap jamur akar putih (*Rigidopus micoporus*). [Skripsi]. Universitas Brawijaya, Malang. [Indonesian]

Simi J, Sajjalaguddam RR, Kumar KVK, Varshney R, Sudini HK. 2016. Assessing the prospects of Streptomyces sp. RP1A-12 in managing groundnut stem rot disease caused by *Sclerotium rolfsii* sacc. J Gen Plant Pathol 82: 96-104. DOI: 10.1007/s10327-016-0644-0.

Sukamto, Wahyono D. 2013. Identifikasi dan Kkarakterisasi *Sclerotium rolfsii* Sacc. penyebab penyakit busuk batang nilam (*Pogostemon cablin* Benth). Balai Penelitian Tanaman Rempah dan Obat 24 (1): 35-41. [Indonesian]

Syed Ab Rahman SF, Singh E, Pieterse CMJ, Schenk PM. 2018. Emerging microbial biocontrol strategies for plant pathogens. Plant Sci 267: 102-111. DOI: 10.1016/j.plantsci.2017.11.012.

Taley AA, El-Tras WF, Moussa SH, El-Agamy MA. 2013. Antifungal action of *Pichia anomala* against aflatoxigenic *Aspergillus flavus* and its application as a feed supplement. J Sci Food Agric 93 (13): 3259-3263. DOI: 10.1002/jsfa.6169.

USDA. 2015. National nutrient database for standard reference. United States Department of Agriculture. Agricultural Research Service, Release 27.

Widiastutik N, Alami NH. 2013. Isolasi dan identifikasi yeast dari rhizosfer *Rhizospora mucronata* Wonorejo. J Sains dan Seni Pomits 2 (1): 1-5. [Indonesian]

Widiastutik N, Alami NH. 2014. Isolasi dan identifikasi yeast dari rhizosfer *Rhizophora mucronata* Wonorejo. J Sains dan Seni Pomits 3: 1. [Indonesian]

Yao X, Zhang F, Qiao Z, Yu H, Sun S, Li X, Zhang J, Jiang X. 2020. Toxicity of thifluzamide in earthworm (*Eisenia fetida*). Ecotoxicol Environ Saf 188: 109880. DOI: 10.1016/j.ecoenv.2019.109880.