Potential of *Saccharomyces cerevisiae* to Control Stem Cancer Disease in Dragon Fruit Caused by *Neoscytalidium dimiatum*

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ABSTRACT

Neoscytalidium dimidiatum fungus is the cause of stem cancer disease on dragon fruit plant. This disease is a major disease of dragon fruit plants that is economically detrimental. The use of *Saccharomyces cerevisiae* is an environmentally friendly control alternative. This study aimed to determine the potential of *S. cerevisiae* in suppressing the growth of *N. dimidiatum* which causes dragon fruit vine cancer. The antagonistic tests of *S. cerevisiae* against *N. dimidiatum* were carried out *in vitro* and *in vivo*. The experimental design used in this research was a Completely Randomized Design (CRD) with 4 treatments and 6 replications. The treatments consisted of several different inoculation time of *S. cerevisiae* namely A (*S. cerevisiae* was inoculated 2 days after *N. dimidiatum* inoculation), B (*S. cerevisiae* was inoculated simultaneously with *N. dimidiatum* inoculation), C (*S. cerevisiae* was inoculated 2 days before *N. dimidiatum* inoculation) and D (Control, without *S. cerevisiae*). The results of the research showed that treatment B (application of *S. cerevisiae* simultaneously with inoculation of *N. dimidiatum*) and C (application of *S. cerevisiae* 2 days before inoculation of *N. dimidiatum* inoculation) inhibited the growth of *N. dimidiatum* in vitro and in vivo. The best treatment was C (the inoculation of *S. cerevisiae* 2 days before inoculation of *N. dimidiatum* with the persentage of inhibition by 57.8% and 87.88%. respectively.

Keywords: dragon fruit, hyperparasitism ,*Neoscytalidium dimidiatum,*

INTRODUCTION

The dragon fruit plant (*Hylocereus polyrhizus*) is native to the Americas. It belongs to the Cactaceae family. In Indonesia, dragon fruit began to be cultivated around 2000 in Jember, Jombang, Mojokerto, and Pasuruan. Currently, dragon fruit has been cultivated in almost all regions in Indonesia, with development centers in Riau Province, Riau Islands, Central Java, East Java, West Kalimantan, Central Kalimantan and West Sumatra (Jumjunidang *et al*., 2019). Dragon fruit productivity in Indonesia has declined over the past three years. In 2021, it was 73.85 tons/ha; in 2022, it was 58.11 tons/ha; and in 2023, it was 41.83 tons/ha (Ministry of Agriculture, 2024). One of the factors causing low dragon fruit productivity is the presence of disease. Some diseases frequently found in dragon fruit include soft rot caused by the fungus *Fusarium* sp. (Masyahit *et al*., 2009), black rot caused by *Fusarium* sp. and *Xanthomonas* sp. (Ezra *et al*., 2013), anthracnose caused by *Colletotrichum spp.* (Takahashi et al., 2008), and vine canker caused by *Neoscytalidium dimidiatum* (Sanahuja *et al*., 2016).

Vine canker is considered a major disease in dragon fruit plants. According to Jumjunidang *et al*. (2019), symptoms of this disease include the appearance of sunken white spots on the vines. The centre of the spots contains small, puncture-like holes. In advanced cases, the spots cover the entire surface of the vine and black fungal pycnidia are present. Under these conditions, the vines rot and dry out. This disease affects not only the vines but also the fruit. According to Dewi & Soekarno (2017), the incidence of dragon fruit vine canker disease can reach 98.3% - 100%, with a disease severity of 25.3% - 45.7% in cultivated areas.

To control the dragon fruit vine canker disease, farmers typically use synthetic fungicides or even no control at all. Continuous spraying of synthetic fungicides pollutes the environment, leaves harmful residues, and makes

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the pathogen resistant to them Therefore, environmentally friendly control methods are needed, one of which is the use of the antagonistic microorganism *Saccharomyces cerevisiae. S. cerevisiae* has been known as a biocontrol agent that suppresses the growth of pathogens by various mechanisms.

The antagonistic mechanisms of *S. cerevisiae* suppress the growth of pathogenic fungi through competition for space and nutrients, antibiosis by producing metabolic compounds, and parasitism (Nunes, 2012). S. cerevisiae isolates produced antifungal compounds, competed for nutrients, inhibited pathogen germination, and produced killer activity and hydrolytic enzymes when in contact with the fungus wall (Lopes *et al.,* 2015). The results of research by Liu *et al*., (2017) *showed* that three *S. cerevisiae* isolates produced antifungal compounds, inhibited *C. gloeosporioides* conidia germination and produced β-1,3-glucanase and chitinase.

S. cerevisiae was reported to be able to control several pathogens such as *Phytium aphanidermatum* which causes damping-off disease by means of a mycoparasitism mechanism (Benyagoub *et al*., 1996), *Aspergillus flavus* and *Aspergillus parasiticus* (Personsa *at al*., 2013). The ability of *S. cerevisiae* to suppress the growth of *N. dimidiatum* has never been reported. This study aimed to determine the potential of *S. cerevisiae* in suppressing the growth of *N. dimidiatum*, the cause of dragon fruit vine cancer.

MATERIALS AND METHODS

The experimental design used in this research was the Completely Randomized Design (CRD) consisting of 4 treatments and 6 replications. The treatments used were different inoculation times for *S. cerevisiae*, as described by Shofiana et al. (2015): 1). *S. cerevisiae* was inoculated 2 days after inoculation with *N. dimidiatum*, 2). *S. cerevisiae* was inoculated simultaneously with *N. dimidiatum*, C. *S. cerevisiae* was inoculated 2 days before inoculation with *N. dimidiatum*, and D. Control (without *S. cerevisiae*).

Isolation of *N. dimidiatum*

N. dimidiatum was isolated from dragon fruit vine showing symptoms of canker disease. Samples were collected in Tikalak Village, X Koto Singkarak District, Solok Regency. First, the symptomatic dragon fruit vines were cut approximately 1 cm in length, with half the diseased portion and half the healthy portion. The cut vines were surface sterilized by rinsing with sterile distilled water, soaking in a 5% NaOCl solution for 1 minute, then rinsing with sterile distilled water, and air-drying. The dried sterile vine cuttings were planted on PDA media and incubated at room temperature for 2 days. Then, the growing fungi were transferred to a new PDA medium and incubated at room temperature for 4 days and a pure culture was obtained.

Characterization and Identification of *N. dimidiatum*

Characterization was conducted to determine the macroscopic and microscopic characteristics of the fungus *N. dimidiatum*. Macroscopic characteristics include colony growth, colony colour, colony texture, and colony thickness. Microscopic characteristics include hyphae, conidia shape, and conidia colour. Then, it was identified based on macroscopic and microscopic characteristics referring to to Dy et al. (2022).

Pathogenicity Test for *N. dimidiatum*

Pathogenicity test is performed to fulfill Koh's postulates. Healthy dragon fruit shoots were cut into 20 cm lengths and then surface by spraying with sterile distilled water, 5% NaOCl solution, and then with sterile distilled water. The sterile dragon fruit vines were wounded using a sterile needle and then inoculated with *N. dimidiatum* fungus that was cut with a cork borer (5 mm). The sterile dragon fruit vines were placed in plastic bags, tightly sealed, and incubated until the symptom appeared

Preparation of Conidial Suspension

The *N. dimidiatum* suspension was prepared by pouring 10 ml of sterile distilled water into a Petri dish containing a 7-day-old *N. dimidiatum* isolate. The conidia were then removed using a sterile, soft brush, and the suspension was transferred to a test tube and homogenized using a vortex. The conidial density of *N. dimidiatum* was calculated using a Neubauer-improved haemocytometer. The conidial density used was 10^7 per ml/ suspension

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Antagonism test of *S. cerevisiae* against *N. dimidiatum in vitro*

Antagonism tests were conducted to determine the ability of *S. cerevisiae* to inhibit the growth of *N. dimidiatum* on PDA media. This test method, as described by Sa'adah (2018), involved growing *S. cerevisiae* and *N. dimidiatum* in the same medium (dual culture). *S. cerevisiae* was streaked in the centre of the petri dish for 3 cm, and *N. dimidiatum* was planted 3 cm from the edge of the petri dish (9 cm). The schematic of the in vitro antagonism test of *S. cerevisiae* against *N. dimidiatum* is shown in Figure 1.

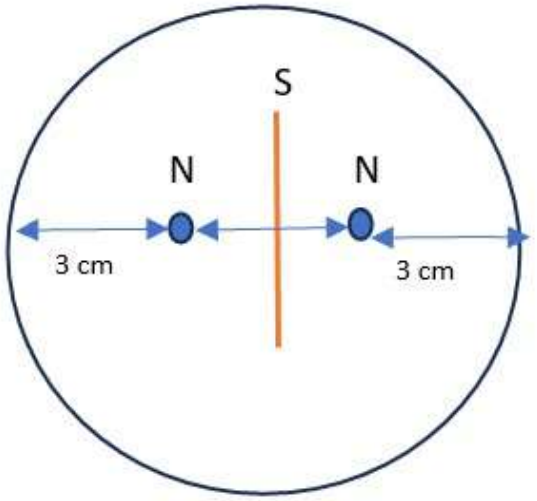

Figure 1. Diagram of the antagonism test using the dual culture method in a petri dish (Sa'adah, 2018): A) *N. dimidiatum*; B) *S. cerevisiae*.

RESULTS

1. Antagonism test of *S. cerevisiae* against *N. dimidiatum in Vitro*

The results of the antagonistic test of *S. cerevisiae* against *N. dimidiatum in vitro* show that *S. cerevisiae* can suppress the growth of colony area of *N. dimidiatum.* the area of N. dimidiatum colonies on treatment B (*S. cerevisiae* was inoculated simultaneously with *N. dimidiatum*) and C (*S. cerevisiae* was inoculated 2 days before *N. dimidiatum*) were not significantly different but were significantly from A (*S. cerevisiae* was inoculated 2 days after *N. dimidiatum*) and the control (without *N. dimidiatum*). The lowest colony area was found in treatment C (25.25 cm2) with the inhibition percentage of 57.80% (Table 1and Figure 2).

Table 1. Colony area of *N. dimidiatum* and the percentage of inhibition in various treatments with *S. cerevisiae* on PDA media 4 days after inoculation (dai)

Treatments	Colony area of *N.dimidiatum* (cm ^{2})	Percentage of Inhibition (%)
A *S. cerevisiae* was inoculated 2 days after *N. dimidiatum* inoculation	54.83 a	6.00
B *S. cerevisiae* was inoculated *S. cerevisiae* was inoculated simultaneously with *N. dimidiatum*	43.83 b	27.00
C *S. cerevisiae* was inoculated 2 days before *N. dimidiatum inoculation*	25.25 c	57.80
D. Control (without *N. dimidiatum)*	60.00 a	0,00

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

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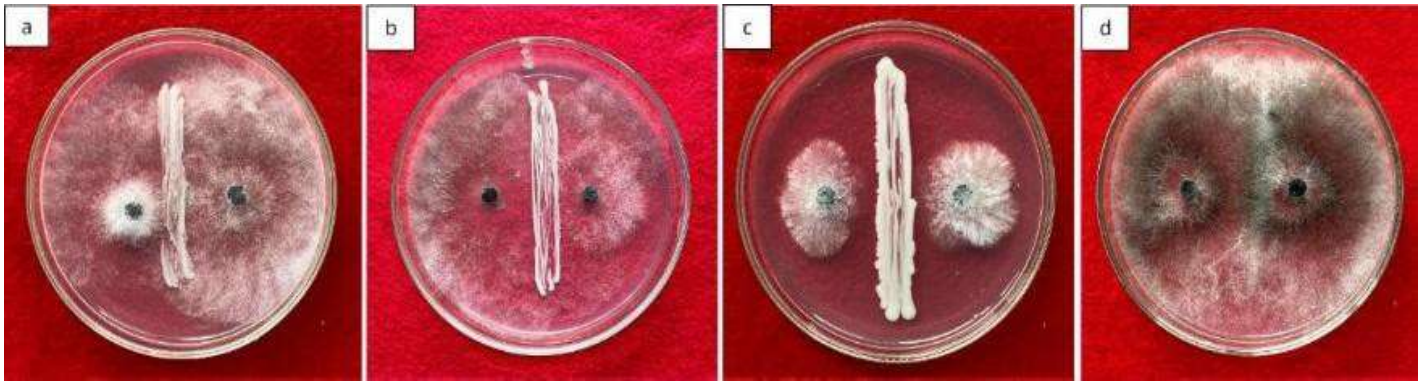

Figure 2. Colony area of *N. dimidiatum* on different treatments of *S. cerevisiae at* 4 days after inoculation. A (*S. cerevisiae* was inoculated 2 days after *N. dimidiatum* inoculation), B. *S. cerevisiae* was inoculated simultaneously with *N. dimidiatum*), C. (*S. cerevisiae* was inoculated 2 days before *N. dimidiatum* inoculation) and D. (Control, without *N. dimidiatum*)

The *N. dimidiatum* colonies treated with *S. cerevisiae* are white or grayish white while the control colonies were blackish. Besides that, the part of the *N. dimidiatum* colonies that are close to *S. cerevisiae* are thinner compared to the part of the colonies that are far from *S. cerevisiae* (Figure 2).

The antagonistic mechanisms of *S. cerevisiae* in suppressing the growth of *N. dimidiatum* include nutrient competition and hyperparasitism. Nutrient competition is characterized by inhibited fungal colony growth compared to the control (Figure 2). Meanwhile, the hyperparasitism mechanism was identified based on microscopic observations. *S. cerevisiae* cells adhere to the hyphae and conidia of *N. dimidiatum*, followed by penetration. Furthermore, *S. cerevisiae* also causes changes in the shape (malformation) of the hyphae. *S. cerevisiae* cells colonize the space, causing narrowing of the pathogenic hyphae. The hyperparasitism mechanism can be seen in Figure 3

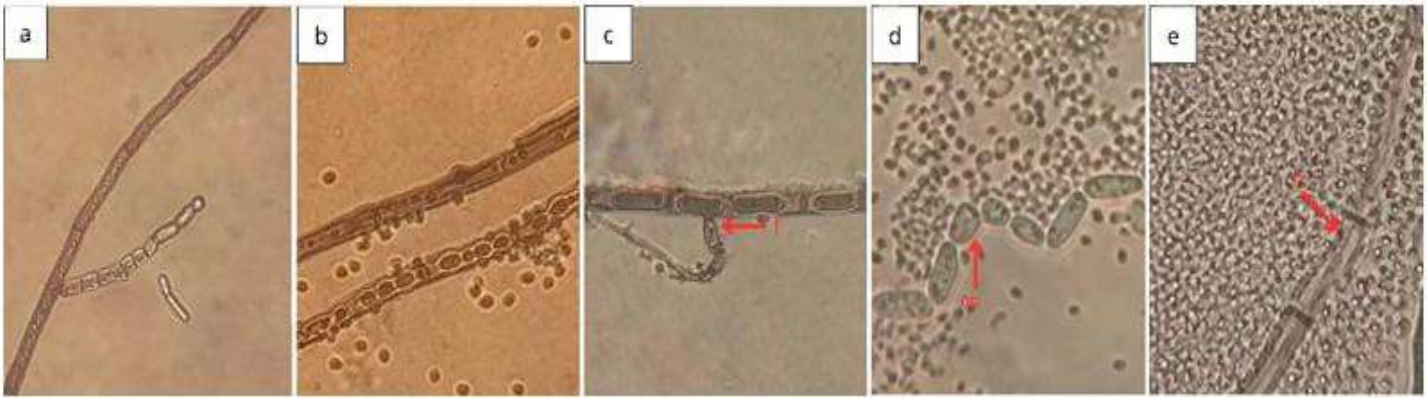

Figure 3. Mechanism of *S.* cerevisiae hyperparasitism against *N. dimidiatum*. a) Normal hyphae; b) Attachment and penetration; c) Hyphal malformation; d) Condia attachment and penetration; e) Hyphal constriction

2. Antagonist test of *S. cerevisiae* against *N. dimidiatum in vivo*

All of inoculated dragon fruit plants with *N.dimidiatum* showed symptoms on the second day after inoculation, but the area of symptoms is differed among treatments. Treatments B (*S. cerevisiae* was inoculated simultaneously with *N.dimidiatum*.and C (*S. cerevisiae* was inoculated 2 days before *N. dimidiatum* inoculation) are not significantly different from each other, but were significantly different from the control and A. The smallest symptom area was found in treatment C, namely 7.30 mm2 with an inhibition percentage of 87.88 % and followed by treatment B with the area of symptom and inhibition percentage of 12.79 mm2 and 78.78 % respectively (Table 2). The symptom of dragon fruit cancer disease at different treatments showed in Figure 4

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Table 2. The incubation period, incidence and area of cancer disease symptom of dragon fruit in various treatments with S. cerevisiae

Treatments	Incubation Time (day)	Disease Incedence (%)	Area of Symptom (mm^2)	Percentase of inhibition (%)
A. *S. cerevisiae* was inoculated 2 days after *N. dimidiatum* inoculation.	2	100	34.01 ab	43.57
B. *S. cerevisiae* was inoculated simultaneously with *N. dimidiatum*.	2	100	12.79 bc	78.78
C. S. cerevisiae was inoculated 2 days before *N. dimidiatum* inoculation.	2	100	7.30 c	87.88
D. Control (without *N. dimidiatum)*	2	100	60.28 a	0.00

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

Table 2, show that all inoculated dragon fruit plants showed symptoms on the second day after inoculation, but the area of symptoms are differed among treatments. Treatments B and C are not significantly different from each other, but were significantly different from the control and A. The smallest spot area was found in treatment C, namely 7.30 mm2 with an inhibition percentage of 87.88 % and followed by treatment B with the area of symptom and inhibition percentage of 12.79 mm2 and 78.78 % respectively. The symptom of dragon fruit cancer disease at different treatments showed in Figure 4

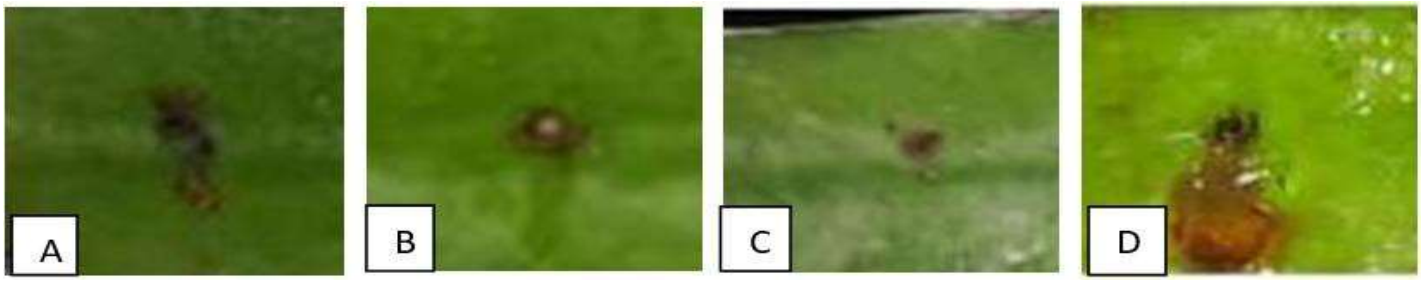

Figure 4. Symptom of dragon fruit cancer disease in various treatments with *S. cerevisiae.* A (*S. cerevisiae* was inoculated 2 days after *N. dimidiatum* inoculation), B. *S. cerevisiae* was inoculated simultaneously with *N. dimidiatum*), C. (*S. cerevisiae* was inoculated 2 days before *N. dimidiatum* inoculation) and D. (Control, without *N. dimidiatum*)

DISCUSSION

The results of the in vitro antagonist test showed that the application of *S. cerevisiae* at different times was able to inhibit the growth of *N. dimidiatum* with different levels of ability. Treatment C, inoculating *S. cerevisiae* 2 days before *N. dimidiatum* inoculation, showed the lowest fungal colony growth, at 25.25 cm2, with a relative inhibition rate of 57.8% (Table 1 and Figure 1). This inhibition occurred due to competition for nutrients between the *S. cerevisiae* and *N. dimidiatum*. In addition to suppressing fungal growth, *S. cerevisiae* also causes colonies to turn white, whereas the fungal mycelium is normally black. This occurs due to the volatile compounds produced by *S. cerevisiae*, namely isoamyl alcohol, ethyl acetate, and 2-phenylethanol, which have antifungal properties (Lopes *et al*., 2015). The *N. dimidiatum* colony close to the yeast cell was thin. Carstens *et al*. (2003) reported that S. cerevisiae produces the enzyme chitinase, which can degrade the cell walls of *N. dimidiatum*. This cell wall degradation activity causes the growth of fungal colonies to thin towards yeast cells (Figure 2b). The ability of *S. cerevisiae* to suppress the growth of *N. dimidiatum* is also due to its hyperparasitism mechanism. *S. cerevisiae* cells can attach to *N. dimidiatum* hyphae and conidia, followed by penetration (Figure 3). This attachment causes the hyphae to deform (malformation). Penetration occurs because *S. cerevisiae* can break down the pathogen's cell wall by producing the enzymes chitinase and β-gluconase (Peja *et al*., 2025)

S. cerevisiae can suppress the development of vine canker in vivo. *S. cerevisiae*-inoculated vines showed symptoms ranging from 7.30% to 34.01%, with an effectiveness rate of 87%. Treatments B and C suppressed the extent of vine canker symptoms by more than 50%. Data on the extent of disease symptoms can be seen in Table 5. This suppression is thought to be due to *S. cerevisiae* colonized rapidly of plant tissue (wounding points). This statement is supported by Fu *et al*. (2015), who stated that *S. cerevisiae* produces IAA, which functions as a signal in filamentation activity for invasion or colonization of plant tissue. In some yeasts, IAA also plays a role in the elongation of pseudohyphae to expand the nutrient absorption area. In addition to producing IAA, *S. cerevisiae* also produces extracellular polysaccharides that facilitate attachment to plant tissue and prevent pathogen colonization (Liu *et al*., 2022).

The best treatment in the in vivo antagonist test was C (*S. cerevisiae* was inoculated 2 days before inoculation of *N. dimidiatum)* with area of disease symptoms of 7.30 mm2 and the percentage of inhibition of 87.88%. This treatment can be used as a preventive measure for the occurrence of dragon fruit vine cancer. *S. cerevisiae* that is inoculated first can colonize the tissue at the point of injury so that the pathogen were difficult to penetrate the plant tissue.

CONCLUSION

Based on the research results, it can be concluded that treatment B (inoculation of *S. cerevisiae* simultaneously with inoculation of *N. dimidiatum*) and C (application of *S. cerevisiae* 2 days before inoculation of *N. dimidiatum* inoculation) inhibited the growth of *N. dimidiatum* in vitro and in vivo. The best treatment was C (the inoculation of *S. cerevisiae* 2 days before inoculation of *N. dimidiatum* with the persentage of inhibition by 57.8% and 87.88%. respectively.

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