

## Antifungal Activities of Yeast against *Fusarium sp.*

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**Abstract:** The antagonistic activities of yeast strains were studied against plant pathogenic fungi, *Fusarium sp.* The maximum production of antifungal activity of three yeast strain coded as B (isolated from banana), P (isolated from pine apple) and W (isolated from water melon) were detected and only 120hrs incubation period was chosen for more investigation as antifungal agent and examine the antifungal activities. The significant zones of inhibition were given by W on PDA plate culture for pathogenic fungi.

**Keywords:** antifungal activities, yeast, *Fusarium sp.*, well diffusion method

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### I. Introduction

Fungal plant diseases are one of the major concerns to agricultural food production. Soil borne pathogenic fungi such as *Pythium*, *Fusarium*, *Rhizotonia* and *Phytophthora* attack most of the economically important crops plants (either through seed root before germination or seedling after germination) resulting in loss of billion of dollars worldwide [1]. It has been estimated that total losses as a consequence of plant diseases reach 25% of the yield in western countries and almost 50% in developing countries. Of this, one third is due to fungal infection. Besides, the use of fungicides has been efficient in decreasing losses due to deterioration of food, but also generates health and environmental concerns mainly due to the carcinogenic and/or teratogenic properties of the compounds, and by their cumulative toxic effects. Besides, growth of moulds in food and animal feed leads to reduced nutritional values and production of allergenic spores and hazardous mycotoxins. Traditionally, fungicides have been used to deal with these problems, but factors such as consumer health and environmental concerns, resistance problems and a more strict legislation have made alternatives necessary. During recent decades, biological control of moulds has evolved as a possibility. Many yeasts and other microorganisms inhibiting plant pathogens have been reported, especially within the fruit and vegetable-producing sector, and several new products have reached the commercial market [2]. Biological control using microbial antagonists has emerged as one of the most promising alternatives, either alone or as part of an integrated control strategy to reduce synthetic fungicide inputs [99Ben]. Among the microorganisms that can have shown potential as biocontrol agents, yeasts show great potential due to their high colonization rate and ability to survive on the fruit surface for prolonged periods under different environmental conditions [3]. More than ten genera of yeasts have been used to control postharvest diseases, especially of fruits. Suppression of classes of fungal pathogens of fruits and foliage that are similar to those associated with soil borne fungal root pathogens, strongly suggests that yeasts also have potential for the biological control of diseases caused by soil-borne fungal plant pathogens, as is evident in reports of certain yeasts in suppressing some soil-borne fungal plant pathogens [4]. The advantage of using yeasts in biocontrol of foliar and postharvest diseases is caused by the fact that they are the major component of the epiphytic microbial community on the surface of leaves, fruits and vegetables. They are effective as biocontrol agents because they are phenotypically adapted to these niches and are able to effectively colonize and compete for nutrients and space on fruit and leaf surfaces [5]. The aim of this work is to screen antifungal activities of isolated yeast against *Fusarium sp.*

### II. Materials And Method

#### A. Materials

The chemicals and medias used in this research work were obtained from the Department of Biotechnology at Technological University (kyaukse) under Ministry of Education, Myanmar.

#### B. Methods

##### Cultivation of Target Plant Pathogens

The plant pathogenic fungi *Fusarium sp.* was provided from the Department of Biotechnology at the Mandalay Technological University. It was subcultured on potato dextrose (PDA) agar every month and stored at 4°C.

**Preparation of the Fungal Inoculums**

For examination of the antifungal activities of the yeast, the pathogen was first prepared from approximately 7 days old cultures grown on PDA media. The volumes of 200 ml potato dextrose broth (PDB) medium was used as the pathogen culture broth placing in conical flask and incubated on shaker at  $35 \pm 2^\circ\text{C}$  for one week.

**Preparation of the Yeast Inoculums**

The yeast strains coded as B (isolated from banana), P (isolated from pine apple) and W (isolated from water melon) were kindly provided also from the Department of Biotechnology at Technological University (kyaukse) under Ministry of Education, Myanmar. The yeast were first cultured for about 48 hours on PYG media. Each 48 hours old culture of yeast isolates were incubated on PYG broth at pH -7 and  $35 \pm 2^\circ\text{C}$  for 7 days on shaker to get the uniformity of colonization and to allow the production of metabolites into the media. Then 1ml of each yeast incubated broth are taken out from each culture starting from 72 hrs, 96 hrs, 120 hrs, 144 hrs and 168 hrs respectively. After that, the yeast broths were centrifuged at 8500 rpm for 15 minutes.

**In Vitro Detection of Antifungal Activities of the Yeast Strains**

The antagonistic activities of 3 yeast strains against the *Fusarium* sp. were examined by agar well diffusion method. To initiate the assay, the plates were prepared on PDA media and each broth cultures of the tested pathogens were swabbed on each plate and then made 8 mm wells. Then, 100  $\mu\text{l}$  of each resulting yeast supernatant were added into these wells for investigation of the antifungal activities. Then detected the inhibition zone diameters and examined daily for antifungal activities. These procedures were carried out immediately on each respective day after making the yeast supernatant to get the data more exactly. Inhibition was indicated when the inhibition zone appeared around the well that was added the yeast supernatant.

**Statistical Analysis**

Analysis of variance (ANOVA) and Tukey's test (SAS Institute, Cary, NC) were used to compare means of the inhibition zone diameters of the yeast strains.

**III. Results and Discussion****A. Inhibitory Effect by Using the Broth Culture**

The well diffusion method was used for the inhibition of the yeast strains against *Fusarium* sp. and the results of the zone diameters by Using 72 hrs, 96 hrs, 120 hrs and 144 hrs Incubate Yeast Broths were described in Table 1. In this work, taking into account concerning the antagonism based on antibiosis and competition for space and nutrients, the extracellular metabolites producing activity was tested by conducting the assays with the broths and supernatants of yeast cultures on PDA against the target fungi. All three strains showed the inhibitory effects on the tested fungi based mainly on mechanisms of antibiosis and competition for space and nutrients. The assays were carried out by varying the incubation time range from 72 hrs to 144 hrs of the isolated yeast cultures in PYG broth. According to the results described in tables, it was observed that the The assay on PDA solid medium (at  $35 \pm 2^\circ\text{C}$ ) using 120hrs incubated supernatant gave the highest inhibited activity against the target fungi. So, we should focus on the 120hrs incubated supernatants of all antagonistic yeasts.

Table 1. Results of the Inhibition Zone Diameters (mm) by 72 hrs, 96 hrs, 120 hrs and 144 hrs Incubate Yeast Broths

Yeast Strain	Inhibition Zone Diameter in mm (Mean $\pm$ SD) Supernatant Vs <i>Fusarium</i> sp.			
	72 hrs	96 hrs	120 hrs	144 hrs
B	14.33 $\pm$ 3.88	24.50 $\pm$ 6.08	27.33 $\pm$ 4.82	18.00 $\pm$ 6.14
P	17.00 $\pm$ 3.75	18.58 $\pm$ 7.48	18.58 $\pm$ 6.09	17.08 $\pm$ 5.35
W	16.75 $\pm$ 3.78	25.50 $\pm$ 5.85	28.25 $\pm$ 2.61	26.17 $\pm$ 2.24

**B. Inhibition Zone Diameters (mm) on *Fusarium* sp. for 120hrs Incubated Supernatants of the Yeast Strains**

The results of the inhibition zone diameters by using the supernatants of the effective yeast strains on 120hrs incubation time range against *F. oxysporum* were described in Tabe 2. Among these yeast strains having the effective biocontrol activities, the strain W isolated from watermelon showed the best inhibitory effect not

only on fungal pathogen longer days than other strains as well as the zone diameter. So, W should also be chosen as the biocontrol agent for target pathogenic fungi, *Fusarium* sp.

**Table 2.** Results of the Inhibition Zone Diameters (mm) Using 120 hrs Culture Yeast Broths

Yeast Strain	Inhibition Zone Diameter in mm (Mean±SD) Supernatant Vs <i>F. oxysporum</i>
B	23.41 ± 6.49 bc
P	24.33 ± 7.79 ab
W	25.29 ± 8.06 a

### Conclusion

According to this study, it could be suggested that the inhibition of fungal development was a promising alternative for biological control of plant diseases caused by fungal pathogen *Fusarium* sp. The yeast strains having more than one antagonistic event such as competition for nutrients and antibiosis were suitable not only to control the plant diseases but also to solve the environmental pollution problems due to the uses of chemical fungicides. Even though further studies are necessary to establish the most efficient means for the application of yeasts in commercial facilities, such antagonistic yeasts especially W are recommended to use as a promising strategy for plant diseases control purposes.

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