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## Application of *Saccharomyces cerevisiae* as a biocontrol agent against *Fusarium* infection of sugar beet plants

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**ABSTRACT** Applicability of *Saccharomyces cerevisiae* as a biocontrol agent of *Fusarium oxysporum* and as plant growth promoter was investigated. At 5 g L<sup>-1</sup> concentration, germination rate of the soaked seeds reached 85.83% in comparison with 54.00% for the untreated ones. Plant growth parameters, chlorophyll contents, TSS and sucrose percentages were also tested. Application of 5 g L<sup>-1</sup> of yeast resulted in a reduction of the pre- and post-emergence damping-off 6.67 and 11.67%, respectively. Survival of treated plants increased to 83.33% in comparison with 30.00% for the pots inoculated with the pathogen containing untreated seeds. Linear growth of *F. oxysporum* was inhibited with 39.52% and 50% by using 5 g L<sup>-1</sup> and 6.35 g L<sup>-1</sup> of the yeast, respectively.

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**KEY WORDS**

sugar beet  
*Saccharomyces cerevisiae*  
growth promotion  
damping-off  
biological control

Nowadays, a great attention has been focused on the possibility of using natural and safe agents for promoting growth of sugar beet and for inducing its resistance against different diseases. *Saccharomyces cerevisiae* is considered a new promising plant growth promoting yeast for different crops. It became in the last few decade a positive alternative to chemical fertilizers safely used for human, animal and environment (Omran 2000). Due to its cytokinin content, yeast treatments were suggested to play a beneficial role in cell division and cell enlargement (Natio et al. 1981). Yeast as a natural stimulator is also characterized by its richness in protein 47%, carbohydrates 33%, nucleic acid 8%, lipids 4%, and different minerals 8% such as Na, Fe, Mg, K, P, S, Zn, Mn, Cu, Si, Cr, Ni, Va and Li in addition to thiamin, riboflavin, pyridoxine, hormones and other growth regulating substances, biotin, B12 and folic acid (Nagodawithana 1991). Earlier reports explained the effect of yeast application on vegetative and fruit growth due to its richness in tryptophan which consider precursor of IAA (indole acetic acid) and on flower ignition due to its effect on carbohydrate accumulation (Warring and Philips 1973).

Biological control of different plant diseases was focused primarily using bacteria or filamentous fungi (Whipps 2001). So, application of yeasts as biocontrol agents acts as a new trend against different pathogens. Potential use of yeasts as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters were recent investigated by El-Tarabily and Sivasithamparam (2006). El-Tarabily (2004) reported that the fungal activities of *Rhizoctonia solani* dis-

eased sugar beet plants were well suppressed by using different yeasts. Wide variety of yeasts have been used extensively for the biological control of post-harvest diseases of fruits and vegetables (Punja 1997 and Zheng et al. 2003)), against moulds of stored grains (Pettersson et al. 1999) and to control powdery mildews (Urquhart and Punja 1997).

The aim of this study was to suppress the soil-borne pathogenic fungus *Fusarium oxysporum* by using *S. cerevisiae* as biocontrol agent and as a plant growth promoter of sugar beet plants.

### Materials and Methods

This investigation was carried out in experimental pots for sowing sugar beet (*Beta vulgaris* L.) during two successive seasons of 2006/2007 and 2007/2008 at Kafrelsheikh University, Egypt. The bio-compound used in this study is active dry yeast of *S. cerevisiae*. Yeast application was conducted as seed soaking, foliar spraying and as soil inoculation using three concentrations of 1, 2 and 5 g L<sup>-1</sup>.

### Test for plant growth promotion

Dry yeasts were well dissolved firstly in slight sugar solutions and cultivated for 12 h. Before application, the solutions were diluted to the required concentrations using sterile distilled water.

Seeds were soaked in defined yeast concentrations at overnight before sowing. Soaked seeds in pure water were acted as control. For foliar spraying or soil inoculation treatments, seeds were sown without soaking. Each treatment was represented by five replicates. Thinning was done after complete germination, leaving one plant per pot. After

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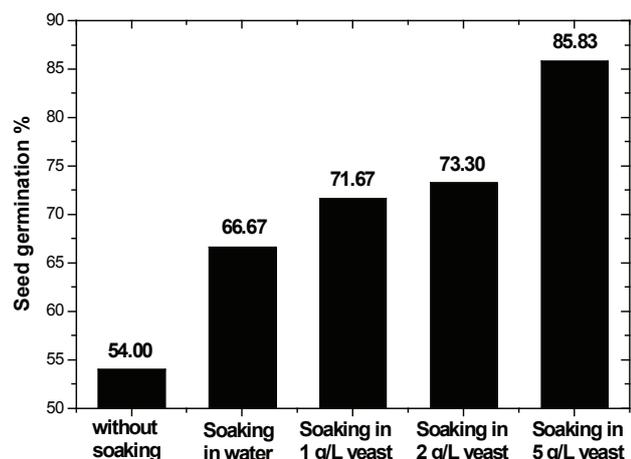


Figure 1. Effect of yeast application on germination rate of sugar beet seeds.

30 days from planting, shoots were sprayed and soils were dressed with yeast solutions for both application methods. This was regularly repeated about four week's intervals during the season.

Germination rate of the seeds was calculated at seedling stage. After 90 days from planting, photosynthetic pigments (chlorophyll a, b and total) were determined according to Moran (1982). Vegetative growth parameters like leaf area, root length, root diameter, fresh and dry weigh of roots and shoots were estimated at the season end. Using root juices, total soluble solids (TSS %) were measured via a hand refractometer. For sucrose content, 26 g of root samples were treated with 176 ml of basic lead acetate and filtered through filter papers according to the procedures of Supernova et al. (1979). Sucrose content was measured in the filtrate using a saccharometer.

### Biocontrol tests

#### Isolation of a *Fusarium* pathogen strain

Damping-off root samples were collected from different locations of Kafr El-sheikh governorate. After removing adhering soil particles with water, samples were cut into small pieces, surface sterilized (0.25% sodium hypochlorite, 4 min), washed several times with sterile distilled water, blotted between two sterilized filter papers and finally placed onto potato dextrose agar plates (PDA). Inoculated plates were incubated at 28°C for 3-7 days. Fungal isolates were microscopically examined and purified using the hyphal tip technique. Purified isolates were identified as *Fusarium oxysporum*. Isolates (identified as *Fusarium oxysporum*) were maintained on PDA slants at 4°C. Cultural, morphological, microscopic and pathological properties were considered to identify the fungal isolates ac-

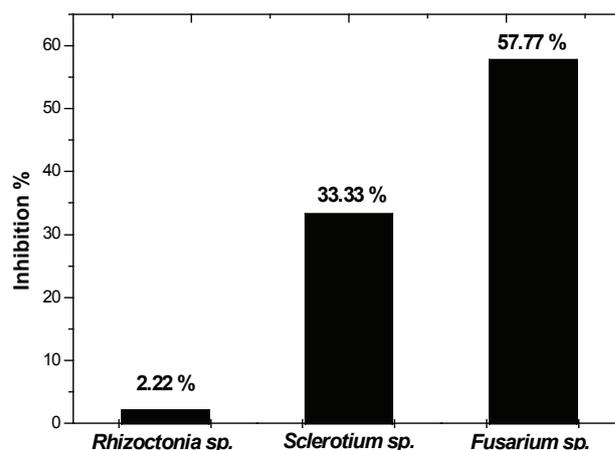


Figure 2. Antifungal activity of *S. cerevisiae* against *R. solani*, *S. rolfsii* and *F. oxysporum* F4.

ording to Ellis (1976) and Booth (1977). Among them F4 was the most virulent isolate.

#### Antagonism

Growth of *F.oxysporum* F4 (FOF4) was estimated in presence of 0, 1, 2 and 5 g L<sup>-1</sup> yeast on PDA medium. Plates were inoculated centrally with mycelial disks (0.5 cm in diameter) taken from 7 days old cultures. Diameters of the colonies (incubated at 28°C) were measured in every 24 hours. When growth of the fungal isolate had just covered the untreated plates, percentages of inhibition (I %) were calculated according to the formula of Topps and Wain (1957):

$$I \% = [(A - B)/A] \times 100$$

where, I % = percentage of inhibition, A = mean diameter growth in the control, B = mean diameter growth in a given treatment. Growth inhibition data were linear fitted and IC<sub>50</sub> value was calculated.

Efficiency of yeast application against damping-off disease in sugar beet seedlings was also evaluated. Pots of 30 cm diameter were filled with autoclaved clay soil mixed with the FOF4 inoculum one week before planting. FOF4 inoculum was prepared by growing on corn meal medium at 28°C for 15 days. Inoculum was added to the plotted soil at rate of 5% w/w (Hussein 1973). Infected soil was mixed thoroughly and moistens every day. Yeast concentrations of 1, 2 and 5 g L<sup>-1</sup>, adjusted at 0.70, 1.23 and 2.78 x 10<sup>8</sup> cell/ml, respectively, were applied at the time of planting as seed treatment. Each treatment was represented by 3 replicates. Pots were kept in greenhouse and watered when needed. Percentages of pre- and post-emergence damping off as well as survival plants were calculated up to 45 days from planting as follows:

$$\% \text{ of pre-emergence damping-off} = (\text{No. of non emerged seeds} / \text{No. of sown seeds}) \times 100$$

**Table 1.** Effect of yeast on vegetative growth of sugar beet plants during two successive seasons.

Treatments	Con g/L	Leaf area Cm <sup>2</sup>	Root parameters <sup>1,2</sup> Cm & <sup>3</sup> Kg			Dry weight %	
			<sup>1</sup> Length	<sup>2</sup> Diameter	<sup>3</sup> Fresh w.	Shoot	Root
2006/2007							
Control	0	103.0 a	17.33 ab	6.00 cde	0.23 bc	20.75 a	25.54 ab
	1	139.52 ab	17.33 ab	6.08 cde	0.23 bc	21.02 a	26.51 b
Seed soaking	2	145.16 b	18.33 ab	6.42 de	0.27 cd	28.55 c	26.74 b
	5	210.05 d	19.00 b	6.75 e	0.32 cd	28.79 c	28.41 c
Foliar spraying	1	137.32 ab	15.33 ab	5.83 cd	0.23 bc	28.57 c	24.65 a
	2	146.54 b	18.67 ab	5.50 bc	0.24 c	35.79 d	24.64 a
Soil inoculation	5	162.56 bc	19.00 b	5.83 cd	0.26 c	29.43 c	33.05 d
	1	192.99 cd	14.33 a	4.58 a	0.15 a	21.47 a	25.65 ab
LSD at 5%	2	208.87 d	15.33 ab	5.00 ab	0.17 ab	22.75 ab	26.92 bc
	5	250.28 e	16.67 ab	5.92 cd	0.22 bc	26.57 bc	36.98 e
2007/2008							
Control	0	139.42 ab	24.00 a	12.50 a	0.68abc	30.59 a	32.48 a
	1	117.74 a	23.00 a	12.00 a	0.41 a	44.34 d	34.77 a-d
Seed soaking	2	126.11 a	23.00 a	12.25 a	0.61 ab	43.15 d	33.48 ab
	5	212.17 cd	24.50 ab	20.00 c	1.21 b-e	51.17 e	36.57 cd
Foliar spraying	1	181.16bcd	25.17 ab	12.50 a	0.68 abc	37.91 c	34.30 abc
	2	184.90bcd	26.00 ab	16.50 bc	1.33 de	36.51 c	35.74 bcd
Soil inoculation	5	208.52 cd	28.00 b	19.00 c	1.30 cde	45.54 d	43.13 e
	1	163.47abc	25.50 ab	14.50 ab	0.82 a-d	31.10 ab	32.60 a
LSD at 5%	2	227.14 d	28.00 b	18.00 bc	1.58 e	34.88 bc	37.02 d
	5	294.32 e	34.00 c	19.00 c	1.67 e	38.61 c	46.88 f
LSD at 5%		49.00	3.19	3.71	0.58	3.80	2.34

% of post-emergence damping-off = (No. of killed seedlings/total No. of emerged seedlings) x 100

% of survival plants = (No. of un-infected plants/total No. of plants) x 100.

**Statistical analysis**

Data were statistically tested for one-way analysis of variance (ANOVA) using SPSS computer software program and Duncan's multiple range tests were applied for comparing means (Duncan 1955).

**Results and Discussion**

**Effect of yeast application on seed germination**

The effect of *S. cerevisiae* application can be seen in Figure 1. The most effective was the highest concentration (5 g L<sup>-1</sup>), where the seed germination reached to 85.83% after nine days from planting. This was in agreement with the data obtained by El-Emery (2004), who reported that germination rate of different seeds of barley, maize, pea and bean was great enhanced by using different yeast dilutions and

**Table 2.** Effect of yeast application on chlorophyll content of sugar beet leaves during two successive seasons.

Treatments	Con g/L	2006/2007			2007/2008		
		Chl a	Chl b	Chl a + b	Chl a	Chl b	Chl a + b
Control	0	16.37 a	5.40 a	21.77 a	12.72 a	3.28 a	16.00 a
	1	21.14 c	7.06 bc	28.20 c	12.60 a	3.12 a	15.72 a
Seed soaking	2	22.24 cd	7.68 bc	29.92 cde	13.40 a	3.39 a	16.79 a
	5	24.80 e	7.49 bc	32.29 f	14.11 a	4.31 a	18.42 a
Foliar spraying	1	19.40 b	6.55 abc	25.95 b	12.35 a	3.63 a	15.98 a
	2	22.71 cd	8.19 c	30.90 def	14.45 a	3.60 a	18.05 a
Soil inoculation	5	23.67 de	8.16 c	31.83 ef	16.69 b	4.70 a	21.39 b
	1	16.70 a	6.40 ab	23.10 a	13.95 a	3.38 a	17.34 a
LSD at 5%	2	17.32 a	6.14 ab	23.46 a	17.81bc	4.57 a	22.38 bc
	5	22.07 cd	7.42 bc	29.49 cd	19.49 c	4.60 a	24.09 c
LSD at 5%		1.50	1.49	1.86	2.20	1.71	2.48

**Table 3.** Effect of yeast on yield quality of sugar beet plants.

Treatments	Conc. g/L	% of yield quality	
		TSS	Sucrose
Control	0	22.20 d	20.50 bc
	1	18.73 b	22.15 cd
Seed soaking	2	21.87 d	22.63 d
	5	22.63 d	25.10 e
Foliar spraying	1	18.83 bc	19.45 ab
	2	24.47 e	20.54 bc
Soil inoculation	5	26.47 f	23.24 d
	1	15.13 a	17.78 a
LSD at 5%	2	19.80 c	20.16 b
	5	22.80 d	25.54 e
		0.99	1.70

growth of plumules, rootlets and cotyledon enlargement was also stimulated.

### Effect of yeast on the vegetative growth

Table 1 shows the effect of yeast on the morphological characteristics investigated: changes were more pronounced with increasing yeast concentrations.

Due to the use of 5 g L<sup>-1</sup> yeast in the soil, leaf area was more than twofold increased in both seasons, indicating enhanced cell division rate and cell enlargement. Regarding root parameters, their length, diameter and fresh weight were significantly enhanced during the second season compared to the first one, indicating a quite establishment with the experimental conditions. The highest percentage of shoot dry weight of 51.17% was achieved in the second season due to seed treatment with 5 g L<sup>-1</sup> of yeast solution. Results indicate that soil application was the most suitable technique enhancing leaf area, root length, root diameter and root fresh and dry weight clearly, while the best dry weight of shoots were achieved via seed soaking in the used biofertilizer before planting. These are in agreement with data obtained by Muller and Leopold (1966), who demonstrated that enhancing effect of yeast application might be due to yeast cytokinins enhancing the accumulation of soluble metabolites. The enhancing effect of yeast on germination rate and on the vegetative growth parameters was strongly supported by Entian and Fröhlich (1984). They stated increased enzyme activity regulating catabolic productions in eukaryotic cells treated with *S. cerevisiae*.

### Effect of yeast application on chlorophyll content

Table 2 shows that yeast application resulted in higher leaf pigment content in compared to the control. Irrespective the application technique, increasing in pigments formation of Chlorophyll a, b and their total was obtained via increasing yeast concentration during the tested seasons. Such increase

**Table 4.** Effect of different concentrations of *S. cerevisiae* against *F. oxysporum* causing damping-off in sugar beet seedlings.

Treatments	% Disease expressions		
	pre emer- gence damping-off	post emergence damping-off	plants survival
Un-infected (control)	5.00 a	6.08 a	92.50 d
Infected	40.00 c	50.00 b	30.00 a
Infected + 1 g L <sup>-1</sup>	20.00 b	12.50 a	70.00 bc
Infected + 2 g L <sup>-1</sup>	30.00 bc	13.10 a	60.00 b
Infected + 5 g L <sup>-1</sup>	6.67 a	11.67 a	83.33 cd
L. S. D. at 5 %	12.51	22.31	20.59

in photosynthetic pigments formation could be attributed to the role of yeast cytokinins delaying the aging of leaves by reducing the degradation of chlorophyll and enhancing the protein and RNA synthesis (Castelfranco and Beale 1983).

### Effect of yeast application on the yield quality

Effect of yeast treatment on the total soluble solids (TSS) and sucrose content are presented in Table 3. The highest TSS values were achieved via foliar spraying using 5 and 2 g L<sup>-1</sup> of yeast concentrations. The greatest sucrose values were 25.54% and 25.10% obtained via soil inoculation and seed treatment.

### Evaluation of *S. cerevisiae* as biocontrol agents

As a biological control agent, *S. cerevisiae* was tested *in vitro* against various soil-borne fungi of *Rhizoctonia solani*, *Sclerotium rolfsii* and *F. oxysporum* F4, causing severe symptoms in sugar beet seedlings. Data presented in Figure 2 show various inhibitory effect against these fungi. *F. oxysporum* F4 proved to be the most sensitive (57.77% growth inhibition), in comparison with *Rhizoctonia solani* and *Sclerotium rolfsii*, growth inhibition of 33.33% and 2.22%, respectively. These results are similar as of El-Tarabily (2004) and Madi et al. (1997): they reported that *R. solani* and *S. rolfsii* were effectively suppressed by some plant growth-promoting yeasts, respectively.

So, sensitivity of *F. oxysporum* F4 was also tested against 0, 1, 2, and 5 g L<sup>-1</sup> of yeast concentrations. Linear growth of *F. oxysporum* F4 was inhibited with 39.52% by using 5 g L<sup>-1</sup> of *S. cerevisiae*. To achieve 50% inhibition (IC<sub>50</sub>), data were fitted (R<sup>2</sup> = 0.999) and 6.35 g L<sup>-1</sup> of yeast are required. These results were supported by Attyia and Youssry (2001). They stated suppressed radial growth of *Macrophomina phaseolina* and *Fusarium solani* by using the local isolate of *S. cerevisiae*.

Based on these results, the potential of *S. cerevisiae* as biocontrol agent against *Fusarium oxysporum* F4 causing sever damping-off symptoms of sugar beet was evaluated in Table 4.

The lowest percentages of pre- and post-emergence damping-off were recorded by 5 g L<sup>-1</sup> yeast concentration with the higher survival rate of plants of 83.33%. It indicates that *F. oxysporum* F4 was suppressed strongly by 5 g L<sup>-1</sup> of *S. cerevisiae*. A similar behavior was observed by Hassan and Abd El-Rehim (2002) for controlling onion neck rot disease. They observed that, increasing yeast concentration (0.05 to 0.1%) leads to increasing reduction of the disease incidence. *Fusarium spp* was also inhibited and seed germination of watermelon was induced by using several taxa included yeast genera as plant growth promoters and as biocontrol agents (Lokesh et al. 2007). Antagonistic activity of the ascomycetous yeast strain *Pichia anomala* against *Fusarium spp* contaminated barley grains was also reported by Laitila et al. (2007) under industry conditions. So, this study is considered one of the first successful attempts using *S. cerevisiae* as promising biocontrol agents.

The results of our study indicate that *S. cerevisiae* have strong potential as plant growth promoters and as biocontrol agents of the soil-borne fungal plant pathogen *F. oxysporum* causing damping-off symptoms in sugar beet seedlings.

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