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Application of Yeast and Its Metabolities in Biological Control

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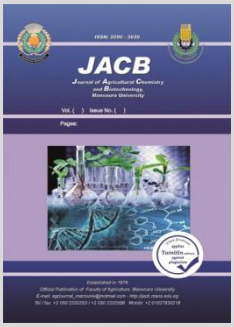

ABSTRACT

This study suggest the possibility of using *Saccharomyces cerevisiae* as an alternative treatment in the food industries to inhibit phytopathogenic fungi. Four phytopathogenic fungi isolated from fungal soils as: *Macrophomina phaseolina*, *Sclerotium rolfsii*, *Rhizoctonia solani* and *Fusarium solani*. In the study a commercial dry yeast was tested to suppress the growth of phytopathogens. Also, yeast was tested by standerd characterizations as *S. cerevisiae*. *In vitro*, by antagonism *S. cerevisiae* reported the significantly best maximum growth inhibition zone with *R. solani* and *F. solani* were obtained 8.85 cm and 7.35 cm, respectively. In this work, we highlight the principal mechanisms (e.g., production of volatile organic compounds and lytic enzymes) utilized by yeast as biocontrol agents (BCAs) *S. cerevisiae* against the common pathogenic found as soil borne fungi caused damping-off disease. The tested yeast is potential to produce ß-1,3- glucanase, exochitinase, HCN and IAA as mode of action for its metabolites as antifungal activity, but it failed to produce cellulase. *In vivo*, application of 6g L^{-1} concentration from yeast was the best and significantly treatment in decreased damping-off disease as well as significantly increased survival plants with all tested fungal pathogens. However, further investigations of yeast with large scale trials are needed to lead to a possible formulation and commercial use in biological control.

Keywords: antagonism, lytic enzymes , *S. cerevisiae*, volatile organic compounds

INTRODUCTION

Yeast as a natural stimulator is 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). There are several fungal pathogens in soil named soil-borne fungi that cause different plant diseases. The most important these pathogens are belong to the genera of Alternaria, Aspergillus, Botrytis, Fusarium, Mucor, Penicillium and Rhizopus. From many pioneering works, other researchers are using microbial strains (bacteria or fungi) for biological control towerds plant pathogens. (Barkai-Golan, 2001), while others antagonistic microorganisms used are usually yeasts. A new act may be used against different phytopathogens is yeasts as considerd biocontrol agents. Yeasts as plant growth stimulator are most potential use for control soil-borne fungal such as *Fusarium solani* and *Rhizoctonia solani* causing plant diseases were found by El-Tarabily and Sivasithamparam (2006). For a long time on simple nutrients and on dry surfaces yeasts are able grow and colonize roots (Chanchaichaovivat *et al.* 2007). Shalaby and El-Nady (2008) reported that applicability of dry yeast of *Saccharomyces cerevisiae* as a biocontrol agent and as plant growth stimulator. Yeasts are the highly antagonistic activity against fungal pathogens when observed reduction of moulds (Olstrope and Passooth 2011). In addition, yeast as *S. cerevisiae* has been used as a biocontrol agent because it is cheap, easy, safe for environmental or no toxicological for human or plant and cultivated for large-scale (Mari *et al.* 2016). The main biocontrol microbes are used against filamentous fungal pathogens: yeasts, bacteria and fungi (Zhao *et al.* 2022). As a result, biological control methods, which are based on living microorganisms to reduce the population or to inhibit the growth of pathogens, have arisen as a safe alternative (Giseli *et al.* 2024).

Grzegorczyk *et al.* (2017) observed that the mechanism used by the antagonistic yeast was the production of VOCs to inhibit the growth of the fungal pathogens. Afify and Ashour (2018) reported that important groups of microorganisms were development of plant growth through its role in biological control of some phytopathogenic fungi by producing various antifungal substances. Recantly, *S. cerevisiae* may be the key antifungal substance, because produced volatile organic compound (2-phenylethanol 2-PE) (Xixi *et al.* 2024).

Under field conditions, several pathogenic fungi attack growing sugar beet plants causing serious diseases (El-Kholi 2000). El-Tarabily (2004) found that the yeasts was suppressed some phytopathogens, especially when Shalaby and El-Nady (2008) showed yeast application was conducted as sugar beet seeds soaking. Understanding BCAs importance and mode of action is a necessary step in order to reduce the determental effect of harmful fungi in the agriculture and food industry, by achieving a more sustainable and safer control of them (Giseli *et al.* 2024).

Therefore, this work was conducted to estimate the suppression of growth phytopathogenic fungi by *S. cerevisiae* as well as determination of yeast metabolities. In laboratory study was applied to explain the relationship between antagonism mechanisms of yeast and their biocontrol potential. And in glasshouse experiment was to evaluate potential antagonists yeast against four fungal pathogens caused sugar beet damping-off.

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MATERIALS AND METHODS

Source of yeast

A commercial dry yeast was used as biocontrol agent. YDP medium was used (yeast extract 1%, peptone 2%, dextrose 2% and agar 1.8%) for yeast growth.

Preparetion of yeast

Fresh preparations from dry commercial formula of yeast (100 g) was dissolved in sterile distilled water as suspension was diluted to 10 1iter before *in vitro* application. Yeast application was prepared as seed soaking using three concentrations of 1 , 3 and 6 gL^{-1}.

Curltural, morphological and biochemical characterizations of yeast

The yeast preparation growth was checked for cultural, morphological, biochemical and physiological characters. For cultural, like color, surface and margin etc. For morphological such as cell shape, gram reaction, endospore formation and Motility test. Also, biochemical characterization catalase, coagulase etc…, IMViC (Indole, Methyl Red, Vougas-Proskaur, Citrate) tests and sugar fermentation tests were carried out. Finally, yeast was inoculated in YDP broth medium for determination its growth and stability at different temperature (20, 25, 30, 37 and 45ºC) for 24 h..

Phytopathogenic fungal isolates

Four soil borne phytopathogenic fungi (*Macrophomina phaseolina* (Tassi), *Sclerotium rolfsii* (Sacc.), *Rhizoctonia solani* and *Fusarium solani*) were obtained from Plant Pathology Research Institute, Agric. Res. Center (ARC), Giza, Egypt.

Antagonism

Growth of each fungal pathogen was estimated in presence of fresh preparations from (three concentrations of 1 , 3 and 6 gL^{-1}) dry commercial formula yeast on PDA medium. In the control treatment, a disc of the pathogen only was placed in dish. There were three plates (replicates) for each treatment. The control plates when growth of the each pathogen was covered, the antagonistic effects of tested yeast concentrations were determined by measuring the free inhibition zone (cm) after about 5-8 days (Topps and Wain 1957).

Yeast metabolities produced

Medium as yeast malt broth (YMB broth) with glucose as the sole carbon source *S. cerevisiae* was cultured. Culture media (100ml) was incubated at 28ºC for 3 days on shaker, filtrate culture as supernatant was used for metabolic assays.

1.Hydrolytic enzymes production

The ability of *S. cerevisiae* to degrade ß-1,3- glucan it is indicator of ß-1,3- glucanase activity. A reaction mixture was added 1gm of ß-1,3- glucan (Sigma) + 2 gm of agar in 100ml distilled water and melting by shaken, then poured in petri dishes and left to solidify. After that, were made pores on agar and inoculated by stable amount of filtrate on each pore and left the plates for 20-30 min. After that it was added with congo-red (1%), (which stain the polysaccharides with red color and does not react with monosacchaaride) left for 30 sec if enzyme produced color of stain developing to clear zone. For the chitinase detected the same method was used. But only replace glucan by chitin (chitin is soluble in boiling water).

For the cellulase assay also the same method was used, but added 0.5% Na-carboxymethyl cellulose (CMC) as substrate for cellulase assay. Congo-red was used as the indicator when added to the plates. Cellulase production when clear zone is appeared (Lingappa and Lockwood 1962).

2.Hydrogen Cyanide (HCN) production

According to Bakker and Schippers (1987) the method was used for production of hydrocyanic acid (HCN).

Indole Acetic Acid (IAA) production

The production of IAA was detected according to Ehmann (1977). Yeast was grown using (YMD broth) medium. After yeast were grown broth was centrifuged. Salkowski reagent were added when a pink color observed this indicate the presence of indole acetic acid.

Glasshouse trail

Sterilized pots (35 cm. in diameter) were used for experiment in glasshouse for sowing sugar beet (*Beta vulgaris* L.) at ARC, Egypt. Pots were filled sterilized clay soil by autoclave and mixed with the fungal pathogen one week before planting individually. Each pathogen inoculum was grown on sorghum medium to the plotted soil at rate of 5% w/w and soil was moistens every day (Hussein 1973). *S. cerevisiae* application was used as seed soaking using different concentrations of 1, 3 and 6 gL^{-1} (10^8 cell/ml) respectively. Seeds was soaked in water as a control. Each treatment was repeated by four replicates. The following parameters were calculated during planting:

1.% of pre-emergence damping-off= (No. of non emerged seeds/ No. of sown seeds) x 100
2.% of post-emergence damping-off= (No. of killrd seedlings/ total No. of emerged seedlings) x 100
3.% of survival plants = (No. of un-infected plants/ total No. of plants) x 100

Statistical analysis

ANOVA were used to analyse the obtained data and to evaluate significant differences between the treatments (P=0.05).

RESULTS AND DISCUSSION

Characterizations of biocontrol agent

Curltural, morphological and biochemical characterizations of yeast are shown in Table (1&2). The colonies of yeast on YM agar plates were large sized, cream colored and smooth surface. Under light microscope the slide culture showed a typical oval shape cells and the gram positive nonspore-forming (Table 1). In addition, in Table (2) is shown to biochemical examinations, indicated that the commercial yeast was grown at different temperature after 24 h. up to 30ºC and showed no viability at 37ºC and 45ºC. This commercial dry yeast belonging as *S. cerevisiae* was studied to use as biocontrol agent. The characters of the yeast as morphological, physiological and biochemical were applied in yeast taxonomy by the standerd methods (Yarrow 1998). Regarding the growth on the opithelial cells, the morphology was unicellular in all cases and no hyphae were observed. Also, in biochemical tests *S. cerevisiae* should be the only one capable of using pectin, cellobiose, trehalose and raffinose in aerobic conditions (Pilar *et al.* 2021 & Prem *et al.* 2023).

Table 1. Colonial and morphological characteristics of commercial yeast

Character	Commercial yeast
Colony shape and color	Smooth cream colonies
Colony size	large
Cell shape	Oval shape
Gram-reaction	+
Endospore formation	-
Motility test	-

(+): positive test ; (-): negative test

Table 2. Biochemical characterization of commercial yeast

Test	Commercial yeast
Catalase	+
Coagulase	-
Methyl Red	+
Vougas-Proskaur	-
Indole	-
Citrate	-
Urease	-
Oxidase	-
Hydrogen sulphide	+
Starch hydrolysis	-
Arabinose	Acid + Gas
D-mannitol	Acid + Gas
Glucose	Acid + Gas
Galactose	Acid + Gas
Fructose	Acid + Gas
Lactose	Acid + Gas
Sucrose	Acid +Gas
Maltose	Acid + Gas
growth at different temperature after 24 h.	
20ºC	+
25ºC	+
30ºC	+
37ºC	-
45ºC	-

(+): positive reaction ; (-): negative reaction

Antagonism between *S. cerevisiae* and fungal pathogens

The test of antagonism between the bioagent as *S. cerevisiae* and four fungal pathoges (*M. phaseolina*, *S. rolfsii*, *R. solani* and *F. solani*), on Petri dishs containing PDA, revealed that all concentrations of *S. cerevisiae* used could able to suppress the fungal growth as proved by the production inhibation zone surrounding yeast growth compared with the plates of control. The best significantly maximum growth of *S. cerevisiae* 6 gL^{-1} for inhibition zone with *R. solani* and *F. solani* were obtained 8.85 cm and 7.35 cm, respectively (Table 3). Antagonistic microorganisms have been used by many workers for controlling soil-borne plant pathogens (Afify and Ashour 1995 & Afify and Ashour 2025). Bioagents were screened for its antagonistic activity against pathogens by reduction of moulds (Olstrope and Passooth 2011). Key substance of antifungal mechanism by *S. cerevisiae* it produce 2-phenylethanol (2-PE) (Xixi *et al*. 2024).

Table 3. Antagonistic activity different concentrations of *S. cerevisiae* against pathogenic fungi

Treatments	Growth of pathogens(cm)[a]			
	M. phaseolina	*S. rolfsii*	*R. solani*	*F. solani*
S. cerevisiae 1 gL^{-1}	0.89	1.13	3.80	3.33
S. cerevisiae 3 gL^{-1}	1.45	1.90	5.35	5.50
S. cerevisiae 6 gL^{-1}	1.98	2.16	8.85	7.35
C[(b)](only pathogen)	9.00	9.00	9.00	9.00
LSD($P< 0.05$)	0.86	1.31	1.09	1.34

[(a)] Mean of three replicates

[(b)] Control *S. cerevisiae* was absent

Yeast metabolities detection

Results showed that hydrolytic enzymes were produced with tested *S. cerevisiae* . ß-1,3- glucanase chitinase and enzymes were positive when the clear zone was observed in medium . *S. cerevisiae* were able to produce ß-1,3-glucanase and exochitinase when clear zones contained (Table 4). It was reported that the extensive production of extracellular lytic enzymes by the antagonistic yeast, especially ß-1,3-glucanase and chitinase may provide an important mechanism for its antifungal potentiality, either by enhancing nutrient competition with other degradation (Scherm *et al.* 2003 & Pilar *et al.* 2021). While, cellulase enzyme was not produced by the *S. cerevisiae*. Similar results were reported by Allpress *et al.* (2002). Pilar *et al.* (2021) reported that *S. cerevisiae* should be cpable of using cellobiose. In the same Table (4) yeast as *S. cerevisiae* showed positive results with HCN and IAA. These products protect plants from phytophytopathogenic fungi by mounting up the antifungal substance- like HCN (Kremer and Souissi 2001). In addition, growth regulators such as auxin-like substance IAA improved plant growth (Hameeda *et al*. 2008 & Afify and Ashour 2018). Such these metabolities (enzymes) produced by safety microoganisms can be very safely and easily used in plant protection for the inhibition of fungal pathogens (Afify and Ashour 2025).

Table 4. Detection of yeast activity by *S. cerevisiae* metabolities

Metabolities	*S. cerevisiae*
ß-1,3- glucanase	+
Chitinase	+
Cellulase	-
HCN	+
IAA	+

Effect of different concentrations from *S. cerevisiae* on damping-off disease caused with fungal pathogens

These results based that the high concentration of *S. cerevisiae* is the best potential as biocontrol agent towards four fungal pathogens causing damping-off disease of sugar beet. This is report considered as one of the first successful attempts using *S. cerevisiae* for biological control these agreement with Shalaby and El-Nady (2008).

1.Effect of different concentrations from *S. cerevisiae* on damping-off disease caused with *M. phaseolina*

Data in Table (5) means important treatments with *S. cerevisiae* as 6 g L^{-1}, because reduced damping-off and increased survival plants (78%). Campo *et al.* (1994) reported that several microorganisms when applied as seed treatments inhibited *M. phaseolina* growth and increased plant emergence from 12 to 100%.

Table 5. Evaluation of the efficacy of three concentrations from *S. cerevisiae* against *M. phaseolina*

Treatments	Damping-off %		Survival plants %
	pre-emergence (15 days)	post-emergence (45 days)	
M.phaseolina + *S. cerevisiae* 1 gL^{-1}	15.0	38.0	62.0
M.phaseolina + *S. cerevisiae* 3 gL^{-1}	11.0	26.5	73.5
M.phaseolina + *S. cerevisiae* 6 gL^{-1}	10.0	22.0	78.0
Untreated (control)	3.0	10.0	90.0
M.phaseolina only	35.0	60.0	40.0
LSD ($P< 0.05$)	12.51	20. 31	18.68

2.Effect of different concentrations from *S. cerevisiae* on damping-off disease caused with *S. rolfsii*

Data in Table (6) are evaluated in pot experiments that damping-off increased with post emergence (40%) with low concentration from yeast (1 g L^{-1}), and increased survival plants (76%) with high concentration from yeast (6 g L^{-1}). These results means that concentration of yeaste are very

important to inhibit fungal pathogens. **Ashour and Afify (1999a)** showed consistent *in vitro* antagonism against *F. oxysprum, R. solani* and *S. rolfsii*. *In vivo* experiments applied several strains as bioagents used showed different levels of efficiency in increasing the surviving seedlings in a greenhouse tests and yield in field conditions.

Table 6. Evaluation of the efficacy of three concentrations from *S. cerevisiae* against *S. rolfsii*

Treatments	Damping-off %		Survival plants %
	pre-emergence (15 days)	post-emergence (45 days)	
S. rolfsii + *S. cerevisiae* 1 gL^{-1}	19.0	40.0	60.0
S. rolfsii + *S. cerevisiae* 3 gL^{-1}	12.0	25.5	74.5
S. rolfsii + *S. cerevisiae* 6 gL^{-1}	10.0	24.0	76.0
Untreated (control)	3.0	10.0	90.0
S. rolfsii only	35.0	55.0	45.0
LSD (P< 0.05)	11.30	21.10	17.60

3.Effect of different concentrations from *S. cerevisiae* on damping-off disease caused with *R. solani*

In Table (7) the data showed that the effect of release concentration of yeast at the rate of 6 gL^{-1} can be released sutvival plants (80%), at the same time decreased damping-off disease during 15 and 45 days (8.0 and 20.0%) respectively. In a greenhouse test in soil infested with *R.solani* and *M. phaseolina* isolates all the bioagents strains were effective in increasing the percentage of surviving seedlings (Ashour and Afify 1999b).

Table 7. Evaluation of the efficacy of three concentrations from *S. cerevisiae* against *R. solani*

Treatments	Damping-off %		Survival plants %
	pre-emergence (15 days)	post-emergence (45 days)	
R. solani + *S. cerevisiae* 1 gL^{-1}	12.0	35.0	65.0
R. solani + *S. cerevisiae* 3 gL^{-1}	10.0	22.5	77.5
R. solani + *S. cerevisiae* 6 gL^{-1}	8.0	20.0	80.0
Untreated (control)	3.0	10.0	90.0
R. solani only	30.0	60.0	40.0
LSD (P< 0.05)	11.15	21.13	19 86

4.Effect of different concentrations from *S. cerevisiae* on damping-off disease caused with *F. solani*

Results in Table (8) indicate that all treatments reduced damping-off and increased healthy plants and with soil infected by pathogen. *S. cerevisiae* with 6 g L^{-1} (90%) was the most increase survival plants%, followed by *S. cerevisiae* 3 g L^{-1} (82.5%). It is indicated that yeasts (*S. cerevisiae* and *P. albicans*) applied were significantly reduced disease on sugar beet plants compared with control ons (El-Sayed and Farrag 2011).

Table 8. Evaluation of the efficacy of three concentrations from *S. cerevisiae* against *F. solani*

Treatments	Damping-off %		Survival plants %
	pre-emergence (15 days)	post-emergence (45 days)	
F. solani + *S. cerevisiae* 1 gL^{-1}	11.0	30.0	70.0
F. solani + *S. cerevisiae* 3 gL^{-1}	8.0	17.5	82.5
F. solani + *S. cerevisiae* 6 gL^{-1}	6.0	10.0	90.0
Untreated (control)	3.0	10.0	90.0
F. solani only	40.0	65.0	35.0
LSD (P< 0.05)	21.27	22.30	20.17

CONCLUSION

This work provides important for further application by a commercial dry yeast of *S. cerevisiae* to safely and most potential for phytopathogenic fungi. The results indicated that *S. cerevisiae* must be further studied to control fungal pathogens caused damping-off disease. Therefore, these results very important as basis for use *S. cerevisiae* as a cheap potential option for reducing fungal pathogens causing plant diseases. However, further works needed a possible formulation and commercial use of yeast with large scale in biological control.

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تطبيق منتجات الخميرة فى المقاومة الحيوية

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الملخص

تقدم هذه الدراسة إقتراح بإمكانية إستخدام خميرة الخباز التجارية والمستخدمة فى إعداد الغذاء كعامل مقاوم لكثير من فطريات التربة الممرضة للنبات والمسببه لمرض موت بادرات النباتات. وقد تأكدنا من خصائص نمو المستعمرات وبالفحص الميكروسكوبى والإختبارات البيوكيمياتية والفسيولوجية أن هذه الخميرة التجارية هى السكاروميسس سيرفيسيا ومعمليا أمكن بإختبار التضاد أنها تستطيع تثبيط نمو الفطريات المسببة لموت البادرات والمعزولة من نباتات بنجر السكر المصابة. سجلت نتائج تضاد الخميرة لأربعة من الفطريات الممرضة وهى ماكروفومينا فاصولينا , إسكروشيم رولفسياي وريزوكتونيا سولانى وفيوزاريوم سولانى وكانت أعلى مناطق خالية من النمو الفطرى معنوية فى التثبيط مع فطرى ريزوكتونيا سولانى وفيوزاريوم سولانى (٨,٨٥ و ٧,٣٥سم) على الترتيب. كما تم تقدير مواد التضاد التى يمكن أن تنتجها هذه الخميرة وسجلت النتائج أن الخميرة تنتج بعض الإنزيمات المحللة لجدر خلايا الفطريات الممرضة مثل إنزيم ١و٣ بيتا جلوكونيز وإنزيم الكيتينيز ولكنها فشلت فى إنتاج إنزيم السليليز كما وجد أن هذه الخميرة تنتج سيانيد الهيدروجين الذى يوقف نمو كثير من الفطريات الممرضة للنبات بالإضافة إلى إنتاجها لإندول حمض الخليك وهو من المواد المشجعة لنمو النباتات وبالتالى تساعد النبات على مقاومة الفطريات الممرضة. وفى تجربة تطبيقية بزراعة نبات بنجر السكر فى أصص بالبيوت الزجاجية وبمعاملة بذور البنجر بتركيزات مختلفة من محلول الخميرة (١و٣و٦ جرام خميرة / لتر من الماء المقطر والمعقم) وعدوى التربة فى الأصص بالفطريات الممرضة الأربعة منفردة كلا مع تركيزات الخميرة الثلاثة المختلفة مع وجود معاملة الكنترول بدون أى إضافة ومعاملة أخرى بالفطر الممرض بمفرده مع الأخذ فى الإعتبار أربعة مكررات لكل معاملة . أظهرت النتائج فى التجربة أن أفضل القيم معنوية كان التركيز العالى من الخميرة (٦ جرام/ لتر) وذلك فى زيادة النسبة المئوية للبادرات الباقية على قيد للحياة بالإضافة إلى إنخفاض النسبة المئوية لموت البادرات ,هذا فى كل حالات العدوى بالفطريات الأربعة المختلفة. لذا توصى الدراسة بأن لابد من التطبيق بشكل كبير وإستخدام وتجهيز الخميرة كمصدر رخيص وآمن فى المقاومة الحيوية.