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Biocontrol potential of *Saccharomyces cerevisiae* Meyen ex E.C. Hansen against *Fusarium culmorum* (Wm. G. Sm.) Sacc induced crown rot in wheat

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Biocontrol potential of *Saccharomyces cerevisiae* Meyen ex E.C. Hansen against *Fusarium culmorum* (Wm. G. Sm.) Sacc induced crown rot in wheat

Abstract: *Fusarium* crown rot (FCR), caused by *Fusarium culmorum*, (Wm.G.Sm.) Sacc is a destructive wheat disease that severely affects root and crown tissues, leading to plant weakening, reduced grain yield, and significant economic losses. This study investigates the biocontrol potential of *Saccharomyces cerevisiae* Meyen ex E.C. Hansen strains (Sc1, Sc2, Sc3) against *F. culmorum* (F19A isolate) through multiple antagonistic mechanisms. Direct confrontation assays revealed significant fungal growth inhibition, with Sc2 exhibiting the highest effect (57.53 %). Volatile compounds produced by *S. cerevisiae* further suppressed fungal expansion, with Sc1 demonstrating the strongest impact (49.6 %). Additionally, yeast treatments reduced conidial germination, with Sc3 achieving the highest inhibition rate (26.48 %). Beyond antifungal activity, *S. cerevisiae* strains enhanced wheat growth under pathogen stress by improving stem elongation, and root development. Enzymatic and biochemical analyses confirmed their ability to degrade fungal cell walls through chitinase and β-1,3-glucanase activity. Additionally, they exhibited plant growth-promoting traits such as phosphate solubilization, siderophore production, and indole-3-acetic acid (IAA) synthesis. The combined effects of antifungal action and plant growth enhancement highlight *S. cerevisiae* as a promising and sustainable strategy for controlling *Fusarium* crown rot in wheat. This eco-friendly approach could serve as an alternative to chemical fungicides, reducing environmental risks while improving crop resilience and productivity.

Key words: biocontrol, *Fusarium culmorum, Fusarium* crown rot, *Saccharomyces cerevisiae*, wheat.

Potencial bionadzora s kvasovko *Saccharomyces cerevisiae* Meyen ex E.C. Hansena proti fuzarijski gnilobi pšenice, ki jo povzroča gliva *Fusarium culmorum* (Wm.G.Sm.) Sacc

Izvleček: Fuzarijska gniloba (FCR), ki jo povzroča gliva *Fusarium culmorum* (Wm.G.Sm.) Sacc., je uničujoča bolezen pšenice, ki močno prizadene tkiva korenin in nadzemnih delov, kar povzroči oslabitev rastlin, zmanjša pridelek zrnja in znatne gospodarske izgube. Ta raziskava preučuje biokontrolni potencial sevov kvasovke *Saccharomyces cerevisiae* Meyen ex E.C. Hansen (Sc1, Sc2, Sc3) proti glivi *F. culmorum* (izolat F19A) prek več antagonističnih mehanizmov. Testi neposredne konfrontacije so pokazali znatno zaviranje rasti gliv, pri čemer je sev Sc2 pokazal največji učinek (57,53 %). Hlapne spojine, ki jih proizvaja kvasovka so dodatno zavirale širjenje gliv, pri čemer je sev Sc1 pokazal najmočnejši učinek (49,6 %). Poleg tega so tretiranja s kvasovkami zmanjšala kalitev konidijev, pri čemer je sev Sc3 dosegel največjo inhibicijo (26,48 %). Poleg protiglivnega delovanja so sevi S. cerevisiae okrepili rast pšenice pod stresom patogenov z izboljšanjem dolžinske rasti stebla in razvoja korenin. Encimske in biokemične analize so potrdile njihovo sposobnost razgradnje celičnih sten glive z aktivnostjo hitinaze in β-1,3-glukanaze. Poleg tega so pokazali lastnosti, ki spodbujajo rast rastlin, kot so solubilizacija fosfata, proizvodnja siderofor in sinteza indol-3-ocetne kisline (IAA). Kombinirani učinki protiglivičnega delovanja in pospeševanja rasti rastlin poudarjajo uporabo kvasovke *S. cerevisiae* kot obetavno in trajnostno strategijo za nadzor fuzarijske gnilobe pšenice. Ta okolju prijazen pristop bi lahko služil kot alternativa kemičnim fungicidom, saj bi zmanjšal okoljska tveganja in hkrati izboljšal odpornost pridelka in produktivnost.

Ključne besede: bionadzor, *Fusarium culmorum*, fuzarijska gniloba, *Saccharomyces cerevisiae*, pšenica

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1 INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important grain crops cultivated in various regions worldwide, serving as a primary food source for humans and animals (Singh et al., 2016). However, its cultivation faces numerous challenges, among which fungal diseases significantly affect yield and quality. *Fusarium* species are among the most often encountered pathogens from major agricultural crops, including wheat, causing substantial agricultural and economic losses (Kuzdralinski et al., 2014). *Fusarium* crown rot (FCR) is particularly destructive in arid and semi-arid regions, significantly impacting plant health and productivity (Kazan and Gardiner, 2018). It is characterized by necrosis and dry rot in the crown and basal stem tissues, root decay, and fungal colonization of vascular tissues, leading to plant weakening, tiller abortion, and the formation of white heads with shriveled kernels. Early infection appears on seedlings as brown lesions, that later extend to the crown, leaf sheaths, and stems, restricting water and nutrient transport within the plant. The primary causal agents of FCR: *Fusarium culmorum*, *F. pseudograminearum* O'Donnell & T. Aoki and *F. graminearum* Schwabe are of great economic significance in wheat production globally (Chakraborty et al., 2006; Scherm et al., 2012). Beyond its impact on plant growth, several *Fusarium* species infecting cereals produce toxic compounds such as trichothecenes, including deoxynivalenol (DON) and nivalenol (NIV), that accumulate in plant tissues and pose serious health risks to humans and animals when contaminated products are consumed (Özdemir, 2022). Although chemical control remains one of the most direct and effective methods for managing plant diseases, excessive pesticide use threatens environmental and human safety due to residue accumulation in soil and crops. These risks highlight the need for alternative, sustainable strategies for disease management. Biological control has emerged as a promising approach for suppressing *Fusarium* infections while promoting plant growth. Beneficial microorganisms, including *Trichoderma* spp, *Bacillus* spp, and various yeast species, have demonstrated significant efficacy in controlling fungal pathogens (Feng et al., 2023). Among these, *Saccharomyces cerevisiae*, stands out as a potential biocontrol agent due to its ability to inhibit *Fusarium* growth and mitigate its pathogenic effects. This yeast is readily available, cost-effective, and widely used not only in food industries but also for improving soil fertility and promoting plant growth (Lonhienne et al., 2014). Studies have indicated the biocontrol potential of various yeast species, with certain isolates effectively inhibiting soil-borne fungal pathogens (Abd Elkader et al., 2012). *Saccharomyces cerevisiae* has also been successfully employed as a bioagent against root rot pathogens under greenhouse conditions, reinforcing its role in biological control. Moreover, this yeast exhibits antifungal activity against *Fusarium* through multiple mechanisms, including competition for nutrients, secretion of bioactive compounds, and induction of plant defense responses (Muccilli & Restuccia, 2015). Notably, its ability to produce enzymes and volatile organic compounds, positions it as a promising candidate for reducing fungal infections in wheat (El-Tarabily & Sivasithamparam, 2006). Based on these findings, this study aims to evaluate the biocontrol potential of *S. cerevisiae* strains against *F. culmorum* through *in vitro* and growth chamber experiments. It also investigates principal antifungal mechanisms, including chitinase and β-glucanase activities, as well as hydrocyanic acid HCN, and siderophore production, phosphate solubilization, and indole-3-acetic acid (IAA) synthesis, all contributing to fungal inhibition and plant growth promotion.

2 MATERIALS AND METHODS

2.1 BIOLOGICAL MATERIALS

The fungal pathogen *Fusarium culmorum* F19A isolate used in this study was isolated from an infected wheat crown, which showed symptoms of *Fusarium* crown rot. The isolate was purified, and morphologically identified at the Applied Microbiology Laboratory, Ferhat Abbas Setif 1 University, Algeria. The internal transcribed spacers of ribosomal DNA (ITS) were amplified us-ing two primers ITS1/ITS4 (CTTGGTCATTTAGAG-GAAGTAA/ TCCTCCGCTTATTGATATGC) (White et al., 1990). Sequencing confirmed its identity; the generated sequence was deposited in the National Center for Biotechnology Information (NCBI) under the accession number PV123206, and its pathogenicity on durum wheat was confirmed in the study of Segmane et al. (2025).

Three strains of *Saccharomyces cerevisiae* (Sc1, Sc2, Sc3), were used for the biocontrol agents. These strains were obtained from commercially available bread yeast and selected for their potential antagonistic activity against fungal pathogens. The yeast strains were cultured on yeast extract-peptone-dextrose agar (YPD) at 30 °C for 48 hours to ensure optimal growth. After incubation, yeast cells were harvested, and stock suspensions were prepared in YPD broth, then stored at 4 °C. Before experimental use, the yeast strains were reactivated on YPD agar under the same conditions to ensure metabolic activity and viability.

2.2 EFFECT OF *SACCHAROMYCES CEREVISIAE* ON MYCELIAL GROWTH OF *FUSARIUM CULMORUM*

The antagonistic potential of the three *S. cerevisiae* strains (Sc1, Sc2, Sc3) against the *F. culmorum* F19A isolate was asessed using the dual culture technique as described by Gupta et al. (2001) with some modifications. A 5 mm mycelial plug from the *F. culmorum* colony, grown for 7 days, was placed at the center of Petri dishes containing PDA medium. The yeast strains were streaked as two parallel lines, each 2.5 cm away from the fungal colony, using 48-hour-old yeast cultures.

For the control plates, only the *F. culmorum* culture plug was placed in the center without yeast inoculation. All plates were incubated at 28 °C for 5 days. Each test was performed in triplicate. The fungal growth inhibition rates were determined by comparing the treated plates with the controls using Vincent›s formula (1947):

$$I\,(\%) = \frac{(C-T)}{C} \times 100$$

Where:

I = Mycelial growth inhibition percentage (%).

C = Radial growth of the fungal mycelium in the control plates (without yeast).

T = Radial growth of the fungal mycelium in the presence of *S. cerevisiae* strains.

2.3 INHIBITION OF *FUSARIUM CULMORUM* GROWTH BY VOLATILE COMPOUNDS PRODUCED BY *SACCHAROMYCES CEREVISIAE*

The effect of volatile compounds produced by *S. cerevisiae* strains on the growth of *F. culmorum* isolate was evaluated using the indirect confrontation method of Daami-Remadi and El Mahjoub (2001). A 5 mm diameter discs of the pathogen were placed at the center of Petri dishes containing PDA, while the *S. cerevisiae* strains were cultured on YPD agar medium in separate plates. The two Petri dishes were stacked, with the dish containing *S. cerevisiae* at the bottom and the one containing *F. culmorum* on top, then sealed tightly with parafilm to prevent the dispersion of volatile compounds. The plates were incubated in darkness at 28 °C for 5 days. The control was prepared by stacking a dish containing an *F. culmorum* disc with a lower dish containing only YPD agar medium without yeast. Each test was performed in triplicate. After incubation, the radial growth of the pathogen was measured, and the percentage of inhibition was calculated using the same formula applied in the Dual Culture Assay.

2.4 EFFECT OF *SACCHAROMYCES CEREVISIAE* ON CONIDIAL GERMINATION OF *FUSARIUM CULMORUM*

An *in vitro* study was conducted using micro-well plates to evaluate the inhibitory effect of the three *S. cerevisiae* strains on the germination of macroconidia and microconidia of *F. culmorum* isolate, following a modified protocol by Li et al. (2022).

Preparation of *S. cerevisiae* and *F. culmorum* suspensions: *F. culmorum* F19A isolate was cultured on synthetic nutrient-poor agar (SNA) for 10 days at 25 °C to induce sporulation. Conidia were harvested by scrubbing the colony surface with 10 ml of sterile distilled water containing 0.1 % Tween 20 to enhance conidia separation. The suspension was then filtered through two layers of sterile muslin to remove hyphal fragments. The conidial concentration was determined using a Malassez counting chamber and adjusted to 10^5 conidia ml^{-1} using sterile distilled water (Su et al., 2012). The three yeast strains (Sc1, Sc2, and Sc3) were cultivated in YPD medium and incubated at 28 °C for 48 hours under constant shaking at 150 rpm. The yeast cells were then harvested by centrifugation at 10,000 rpm for 10 minutes, washed with sterile physiological saline water (0.85 NaCl), and resuspended to a final concentration of 10^8 cells ml^{-1}. The concentration was confirmed by measuring OD_{600} using a spectrophotometer, with necessary dilutions made to standardize all suspensions.

Conidial germination assay: A 75 µl aliquot of each yeast suspension (10^8 cells ml^{-1}) was added to randomly selected wells in a micro-well plate. Immediately, 75 µl of the *F. culmorum* conidial suspension (10^5 conidia ml^{-1}) was introduced into the same wells.

For the control treatments, 75 µl of sterile physiological saline water was mixed with 75 µl of the conidial suspension. Each treatment, including controls, was performed in triplicate.

The plates were incubated at 28 °C for 18 hours. After incubation, samples from each well were observed microscopically, and germinated conidia were counted among 100 observed conidia per replicate. Conidia were considered germinated when the germ tube length was equal to or exceeding the spore diameter (Klosowski et al., 2018).

Calculation of conidial germination inhibition (CGI, %):

$$CGI\,(\%) = \frac{(N_t - Ng)}{N_t} \times 100$$

where:

N_t represents the total number of conidia examined.

N_g represents the total number of germinated conidia.

2.5 EFFECT OF *SACCHAROMYCES CEREVISIAE* ON *FUSARIUM CULMORUM* IN THE GROWTH CHAMBER

The experiment was conducted in a growth chamber following the protocol of Ren et al. (2015) with major modifications. Sterilized soil was used, autoclaved twice at 121 °C for 20 minutes to ensure complete sterilization. Soil inoculation was performed by adding five PDA discs (5 mm diameter) containing the *F. culmorum* isolate per experimental unit (seedling tray with alveolates cells). A negative control (without *F. culmorum*) and a positive control (with *F. culmorum* only) were included.

After surface sterilization in 2 % sodium hypochlorite (NaOCl) for 5 minutes, followed by rinsing three times with sterile distilled water, durum wheat (*Triticum turgidum* subsp. *durum*, 'Vitron') seeds were pre-germinated for two days at 22 °C, then two seeds were planted per experimental unit in the infected soil. The three strains of *S. cerevisiae* were introduced as a biocontrol treatment, applied by watering 10 ml of yeast suspension (10^8 cells ml^{-1}) around the roots. The control treatments were watered with the same volume of sterile water to maintain uniform moisture conditions. The experimental units were randomly distributed, with three replicates per unit.

Disease assessment and plant growth measurements: Disease severity was evaluated 20 days after inoculation using a 0–4 scale as follows:

0 = No symptoms, healthy stem base.

1 = Lesions affecting less than 25 % of the stem base circumference.

2 = Lesions covering 26–75 % of the stem base circumference.

3 = Lesions covering more than 75 % of the stem base circumference.

4 = Complete plant rot and seedling death.

Additionally, plant growth parameters were measured, including stem length and the longest root length.

2.6 ENZYMATIC ACTIVITIES OF *SACCHAROMYCES CEREVISIAE* STRAINS

Production of chitinase and β-1,3-glucanase: The enzymatic activity of chitin degradation was assessed following the modified method of Renwick et al. (1991). Three plates containing chitin medium (contained (g l^{-1}): chitin 4, KH_2PO_4 0.3, $KHPO_4$ 0.7, NaCl 0.3, $MgSO_4 \cdot H_2O$ 0.5, yeast extract 0.2, $MnCl_2$ 0.001, $FeSO_4$ 0.01, $ZnSO_4 \cdot 7H_2O$ 0.07, and agar 20), were inoculated using the stab inoculation method with freshly grown colonies (48 hours) of three *S. cerevisiae* strains. To evaluate β-1,3-glucanase production, an additional medium was prepared under the same conditions (contained (g l^{-1}): peptone 5, yeast extract 5, barley flour 10, and agar 20) (Teather and Wood, 1982). All plates were incubated at 28 °C for five days. After incubation, the chitin medium plates were stained with lugol solution (Djellel and Larous, 2018), while enzymatic activity in the β-1,3-glucanase detection medium was revealed by staining with 0.1 % Congo Red. The formation of clear halos around the colonies in both media indicated positive enzymatic activity.

2.7 BIOCHEMICAL ACTIVITIES OF *SACCHAROMYCES CEREVISIAE* STRAINS

Production of hydrocyanic acid (HCN): The ability of *S. cerevisiae* strains to produce HCN was assessed following the method described by Trivedi et al. (2008). Each strain was individually inoculated onto Petri dishes containing Trypticase Soy medium supplemented with 4.4 g l^{-1} of glycine, using a platinum loop. A sterile filter paper was placed inside the lid of the Petri dish in an inverted position and impregnated with a yellow solution composed of picric acid and sodium carbonate. The Petri dish was then sealed with parafilm and incubated upside down at 28 °C for five days. HCN production was determined by observing a color change in the filter paper, transitioning from yellow to brown or reddish-brown.

Solubilization of phosphate: Phosphate solubilization was assessed using a modified method of Zaidi et al. (2006). The three *S. cerevisiae* strains were inoculated onto Pikovskaya's agar using stab inoculation. The inoculated plates were incubated at 28 °C for 5 days. Phosphate solubilization was evaluated based on the formation of halo zones around the colonies.

Production of siderophores: *Saccharomyces cerevisiae* strains' ability to produce siderophores was evaluated using the Chrome Azurol S (CAS agar) assay, a qualitative universal method described by Schwyn and Neilands (1987). The CAS blue medium was inoculated by stab inoculation using active colonies of the three yeast strains, and the plates were then incubated at 28 °C for 7 days in the dark. A color change in the medium from blue to yellow around the colony indicated positive siderophore production, according to Khunnamwong et al. (2020).

Production of indole-3-acetic acid (IAA): The ability of three strains of *S. cerevisiae* to produce indole-3-acetic acid (IAA) was assessed by culturing 100 µl of each isolate in tubes containing 10 ml of YPD medium supplemented with 5 g l^{-1} of tryptophan, with three replicates per isolate.

After 72 hours of incubation at 28 °C, the cultures were centrifuged at 5000 rpm for 20 minutes. Then, 1 ml of the supernatant was mixed with 2 ml of Salkowski's reagent, which consists of 50 ml of 35 % perchloric acid and 1 ml of 0.5 M $FeCl_3$ solution. The IAA concentration was determined using the colorimetric method described by Loper and Schroth (1968), by measuring absorbance at 530 nm after 30 minutes of incubation at room temperature in the dark, using a standard calibration curve for IAA solution (Bric et al., 1991).

2.8 STATISTICAL ANALYSIS

The data were analyzed using SPSS version 26. One-way ANOVA was performed to test the differences between treatments, followed by Tukey's HSD test to determine significant differences between groups at a 5 % significance level with a 95 % confidence interval.

3 RESULTS AND DISCUSSION

3.1 EFFECT OF *SACCHAROMYCES CEREVISIAE* ON MYCELIAL GROWTH OF *FUSARIUM CULMORUM*

After 5 days of incubation at 28 °C, the *S. cerevisiae* strains exhibited inhibitory effects on the radial growth of *F. culmorum* F19A isolate compared to the control (Fig.1). The mean colony diameter of *F. culmorum* in the control plates was 8.4 cm, while it was reduced to 3.6 cm in the presence of Sc1, 3.56 cm with Sc2, and 3.9 cm with Sc3. Consequently, the inhibition rates were 57.14 %, 57.53 %, and 53.57 %, respectively, indicating varying degrees of antifungal activity among the tested strains.

Statistical analysis revealed significant differences among the treatments ($F = 656.968$, $p < 0.001$), confirming that the yeast strains significantly affected fungal colony growth. Tukey's HSD test identified two statistically distinct groups: the control formed a separate group with the highest colony diameter, whereas the yeast-treated groups (Sc1, Sc2, Sc3) clustered together, exhibiting significantly lower colony diameters. This indicates a strong antifungal effect of *S. cerevisiae* strains against *F. culmorum* isolate (Tab.1).

3.2 INHIBITION OF *FUSARIUM CULMORUM* MYCELIAL GROWTH BY VOLATILE COMPOUNDS PRODUCED BY *SACCHAROMYCES CEREVISIAE*

The effect of volatile compounds produced by three *S. cerevisiae* strains on the growth of *Fusarium culmorum* isolate, was evaluated using the indirect confrontation method. The results showed a significant reduction in the average colony diameter compared to the control, which had a mean diameter of 8.4 cm. In contrast, the colony diameters for the yeast-treated groups were 4.23 cm (Sc1), 5.26 cm (Sc2), and 5.93 cm (Sc3). This reduction corresponded to inhibition percentages of 49.6 % (Sc1), 37.29 % (Sc2), and 29.36 % (Sc3). One-way ANOVA revealed significant differences between treatments ($F = 7.161$, $p = 0.012$), indicating that the yeast strains had varying effects on *F. culmorum* growth. Post hoc Tukey's test confirmed that the inhibitory effects of Sc1 ($p = 0.009$) and Sc2 ($p = 0.041$) were statistically significant compared to the control, while Sc3 did not show a significant difference ($p = 0.111$). The homogeneous subsets analysis further indicated that Sc1 and Sc2 formed a distinct group, separate from Sc3 and the control, suggesting that these two strains had the strongest inhibitory effects. These results indicate that *S. cerevisiae* strains can produce volatile compounds that inhibit *F. culmorum* growth, though the degree of inhibition varies depending on the yeast strain (Tab.1).

3.3 EFFECT OF *SACCHAROMYCES CEREVISIAE* ON CONIDIAL GERMINATION OF *FUSARIUM CULMORUM*

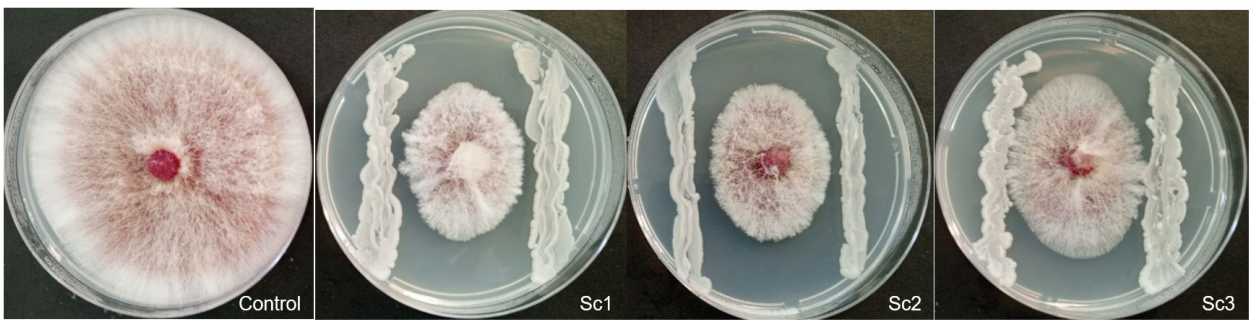

Figure 1: Inhibition of *F. culmorum* mycelial growth by *S. cerevisiae* strains (Sc1, Sc2, Sc3) after 5 days of incubation at 28 °C.

Table 1: Effect of *S.s cerevisiae* strains (Sc1, Sc2, Sc3) on the growth of *F. culmorum* (F19A) in direct and indirect confrontation, and on conidia germination

Treatment	Colony diameters (cm) Direct confrontation-	Colony diameters (cm) Indirect confrontation-	Conidia germination %
Control F19A	8.4 ± 0.1[b]	8.4 ± 0.1[b]	70.33 ± 8.75[a]
F19A + Sc1	3.6 ± 0.26[a]	4.23 ± 1.59[a]	57.21 ± 4.76[a]
F19A + Sc2	3.56 ± 0.11[a]	5.26 ± 0.45[a]	65.08 ± 4.75[a]
F19A + Sc3	3.9 ± 0.1[a]	5.93 ± 1.58[ab]	51.69 ± 11.41[a]

Means values + standard deviations followed by different letters within the same column are significantly different according to Tukey's HSD test ($p < 0.05$)

The results showed a variation in the effect of *S. cerevisiae* strains on the conidial germination of *F. culmorum* F19A isolate after 18 hours of treatment. The highest germination rate was observed in the control treatment (70.33 %), followed by treatment with strain Sc2 (65.08 %), then with strain Sc1 (57.21 %), and the lowest germination rate was observed with strain Sc3 (51.69 %). Strain Sc3 recorded the highest inhibition rate of 26.48 %, while the inhibition rates for Sc1 and Sc2 were 18.61 % and 7.45 %, respectively. Some deformities in conidia were also observed, suggesting an impact of the yeast strains on conidial growth. The results of ANOVA showed an *F-value* of 3.245 and a *p-value* of 0.081, indicating no significant difference between the treatments at a 5 % significance level. However, potential differences were observed at the 10 % significance level. Multiple comparisons (Tukey HSD) showed no significant differences between the treatments ($p > 0.05$), showing that the effect of the treatments was not large enough to cause a significant difference in conidial germination (Tab.1).

3.4 EFFECT OF *SACCHAROMYCES CEREVISIAE* ON *FUSARIUM CULMORUM* IN THE GROWTH CHAMBER

The results showed significant differences between the different treatments in plant growth parameters and disease severity (Fig.2). Wheat inoculated with *F. culmorum* isolate was negatively affected, while some *S. cerevisiae* strains improved growth and reduced the harmful effect of the pathogen. Regarding plant growth parameters, the negative control exhibited the highest stem length (5.60 cm), indicating normal growth in the absence of *F. culmorum* isolate impact. Wheat inoculated with *F. culmorum* and *S. cerevisiae* strain Sc2 ranked second (4.48 cm), showing improved growth compared to wheat inoculated with *F. culmorum* only, which had the shortest stems (2.75 cm). For root length, wheat in the negative control had the longest roots (16.03 cm), followed by wheat inoculated with *F. culmorum* and *S. cerevisiae* strain Sc1 (12.68 cm), while wheat inoculated with *F. culmorum* only had the shortest (6.65 cm), reflecting the negative impact of *F. culmorum* F19A on root development. Severity measurements showed that the negative control exhibited no infection (0), while wheat inoculated only with *F. culmorum* F19A showed the highest severity (4). Combined inoculation with *S. cerevisiae* strains reduced severity to varying degrees: Sc1 (2.5), Sc2 (2), and Sc3 (3), indicating a partial protective effect of the yeasts against the pathogen.

The results of ANOVA showed a significant effect of treatments on stem length ($F = 10.857$, $p = 0.001$), indicating notable differences between groups. No significant effect on root length ($F = 3.022$, $p = 0.071$). The most substantial effect was on disease severity ($F = 26.4$, $p < 0.001$), confirming a strong influence of treatments. Tukey's multiple comparison test identified specific differences between treatments. In stem length, positive control significantly differed from the negative control ($p = 0.01$) and wheat inoculated with F19A isolate and Sc3 strain ($p < 0.05$). For the root length, no significant differences were detected between treatments. Therefore, all treatments except the positive control exhibited significant increases in disease severity ($p < 0.01$). Tukey's test showed homogeneous groups that reflect the effect of each treatment on plant growth (Tab.2).

3.5 ENZYMATIC ACTIVITIES OF *SACCHAROMYCES CEREVISIAE* STRAINS

All *S. cerevisiae* strains exhibited enzymatic activity for chitinase and β-1,3-glucanase, with β-1,3-glucanase production being significantly higher in all strains. For chitinase, strain Sc2 showed the highest activity with a halo diameter of 1.5 mm, while Sc1 and Sc3 exhibited weak activity with values of 0.8 mm and 0.83 mm, respectively. In contrast, β-1,3-glucanase activity was considerably higher, with Sc1 displaying the largest halo (3.8 mm), followed by Sc2 (3.6 mm) and Sc3 (3.1 mm). These results indicate that the strains have a greater capacity for

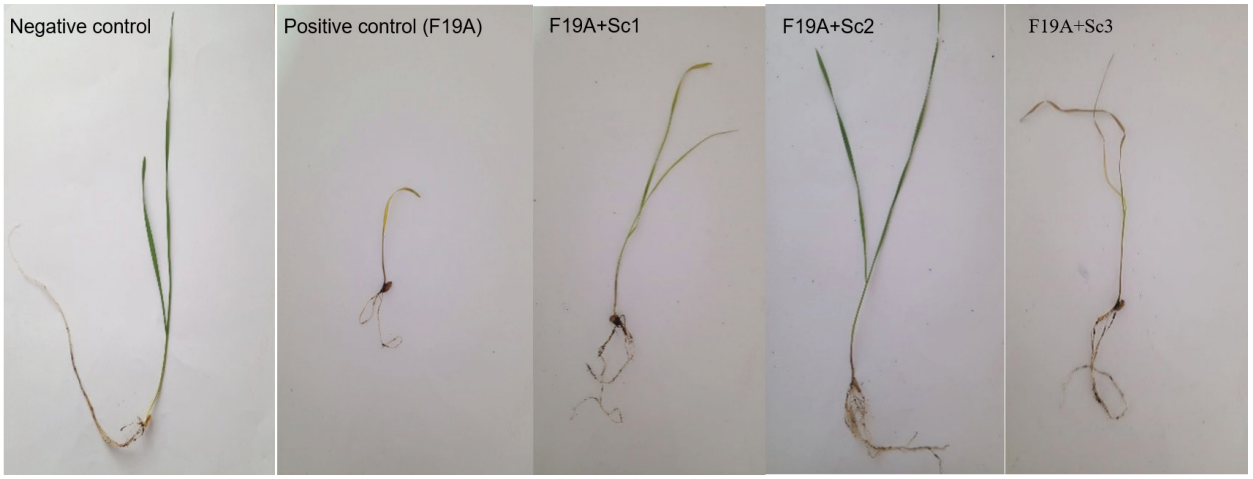

Figure 2: Effect of *F. culmorum* F19A isolate on wheat plants: Comparison between negative control, positive control (F19A), and treatment with *S.s cerevisiae* strains (Sc1, Sc2, and Sc3).

β-1,3-glucanase production compared to chitinase production.

3.6 BIOCHEMICAL ACTIVITIES OF *SACCHAROMYCES CEREVISIAE* STRAINS

None of the tested *S.s cerevisiae* strains exhibited hydrocyanic acid (HCN) production, as no color change was observed on the filter paper, and the results were similar to the uninoculated control. Regarding phosphate solubilization, strain Sc1 demonstrated the highest solubilization ability, with a halo diameter of 3.6 cm, followed by strain Sc2 with 3.1 cm, while strain Sc3 showed the lowest activity with 2.5 cm. For siderophore production, strain Sc2 exhibited the highest production with a halo diameter of 1.7 cm, while both Sc1 and Sc3 showed the same diameter of 1.6 cm. Concerning indole-3-acetic acid (IAA) production, strain Sc3 exhibited the highest concentration at 0.9 μg ml^{-1}, followed by strain Sc2 at 0.72 μg ml^{-1}, whereas strain Sc1 showed the lowest production at 0.47 μg ml^{-1}. Based on these results, strain Sc1 demonstrated the highest ability for phosphate solubilization, while Sc2 was the most efficient in siderophore production. Meanwhile, Sc3 exhibited the highest production of indole-3-acetic acid (IAA). Overall, Sc2 can be considered the most promising strain due to its strong performance in siderophore production and phosphate solubilization, along with its moderate IAA production.

3.7 DISCUSSION

Fungal infections caused by *Fusarium* and crop contamination with mycotoxins represent major challenges in agricultural production, as prevention remains difficult even with the adoption of optimal agricultural practices. Given the need for effective pathogen control strategies, increasing interest has been directed toward using microorganisms as biological control agents, driving research efforts to enhance their efficiency and expand their global application (Podgórska-Kryszczuk et

Table 2: Mean Values and Tukey's Significant Difference Test for Growth Parameters and Disease Severity in Wheat Seedlings Inoculated with *F. culmorum* F19A isolate and Treated with *S. cerevisiae* strains (Sc1, Sc2, and Sc3).

Treatment	Stem length (cm)	Root length (cm)	Severity
Control	5.6 ± 0.8^{c}	16.03 ± 0.95^{b}	0 ± 0^{a}
Control + F19A	2.75 ± 0.05^{a}	6.65 ± 1.05^{a}	4 ± 0^{c}
Sc1 + F19A	3.8 ± 0.61^{ab}	12.68 ± 3.61^{ab}	2.5 ± 0^{b}
Sc2 + F19A	4.48 ± 0.4^{bc}	11.21 ± 1.87^{ab}	2 ± 0.5^{b}
Sc3 + F19A	3.13 ± 0.76^{ab}	11.8 ± 6.15^{ab}	3 ± 1^{bc}

Means values + standard deviations followed by different letters within the same column are significantly different according to Tukey's HSD test ($p < 0.05$).

al., 2022). In this context, the present study aimed to evaluate the sensitivity of *F. culmorum*, the causal agent of *Fusarium* crown rot in wheat, to yeast strains *S. cerevisiae*, through experiments conducted *in vitro* and in the growth chamber. Applying yeasts as biological control agents is a promising approach to combating various pathogens. Previous studies, such as those by El-Tarabily and Sivasithamparam (2006), have demonstrated the role of yeasts in controlling soil-borne fungal pathogens and promoting plant growth (Shalaby and El-Nady, 2008), supporting the broader implementation of yeasts in biological control strategies. Several studies have demonstrated the efficacy of *S. cerevisiae* in inhibiting mycelial growth and reproductive structures of different *Fusarium* species. Some yeast strains exhibited strong effects on fungal growth and spore production, while others had a limited impact. Our study aligns with these studies, as *S. cerevisiae* strains showed significant inhibitory effects on *F. culmorum* in direct and indirect confrontation assays. In direct confrontation, strain Sc2 recorded the highest inhibition rate of *F. culmorum* mycelial growth among the three tested strains, reaching 57.53 %. This result is consistent with the findings of Kamel et al. (2016), where *S. cerevisiae* inhibited *F. oxysporum* Schlecht. emend. Snyder & Hansen mycelial growth by 60 %. Similarly, Shalaby & El-Nady (2008) reported that *S. cerevisiae* suppressed *F. oxysporum* mycelial growth by 57.77 %, indicating that yeast can be effective against various *Fusarium* species, albeit with varying degrees of inhibition. Regarding indirect confrontation through volatile compounds, strain Sc1 in our study exhibited the highest inhibition rate at 49.6 %. This highlights the role of yeast-emitted volatile compounds in reducing fungal growth, aligning with the results of Podgórska-Kryszczuk et al. (2022), who reported that *S. cerevisiae* strongly inhibited *F. culmorum* mycelial growth, whereas its effect on *F. graminearum* was weaker. Additionally, strain Sc3 proved to be the most effective in inhibiting spore germination, with an inhibition rate of 26.48 %. This is consistent with the study by Zhao et al. (2019), that showed that *S. cerevisiae* Y-912 reduced *F. graminearum* spore germination to 1.93 % at a concentration of 1×10^9 cells ml^{-1}. These results indicate that some yeast strains can directly interfere with the fungal life cycle by preventing reproduction. Furthermore, Ather et al. (2018) demonstrated the strong efficacy of *S. cerevisiae* in inhibiting *F. semitectum* Berk. & Ravenel, mycelial growth by 93 %, indicating the yeast's high potential for fungal biocontrol. In contrast, Schisler et al. (1995) found that *S. cerevisiae* did not affect the conidial germination of *F. sambucinum* Fuckel, showing that fungal response varies depending on the yeast strain used. Regarding the impact of *S. cerevisiae* on fungal growth and toxin production, Armando et al. (2013) found that *S. cerevisiae* strain RC016 completely inhibited *F. graminearum* mycelial growth under all tested conditions, whereas strain RC008 exhibited variable effects depending on pH and temperature. These results support our study, where some yeast strains were more effective than others in inhibiting *F. culmorum*, emphasizing that efficacy varies according to environmental conditions and yeast strain specificity. Additionally, our study results align with Kamel et al. (2016) and Podgórska-Kryszczuk et al. (2022) results, as all studies confirmed that *S. cerevisiae* possesses plant growth-promoting traits through auxin production, phosphate solubilization, and siderophore secretion, in addition to its enzymatic activity in degrading the cell wall of pathogenic fungi. *Saccharomyces cerevisiae* strains exhibited varying levels of auxin and siderophore production, with some strains excelling in these traits, which is consistent with the findings of Kamel et al. (2016), who observed differences in these abilities among isolates. Furthermore, our study confirmed that the tested isolates were incapable of producing HCN, aligning with previous findings that indicated this mechanism is not utilized by *S. cerevisiae* in fungal inhibition (Ahmad et al., 2008). Regarding enzymatic activity, our results confirmed that all tested strains exhibited activity for both chitinase and β-1,3-glucanase, with β-1,3-glucanase production being higher in all strains. These results agree with Podgórska-Kryszczuk et al. (2022), who found that yeasts with antifungal activity against *Fusarium* exhibited greater β-1,3-glucanase activity compared to chitinase, confirming that this mechanism may play a key role in inhibiting *F. culmorum* and other pathogenic fungi. The present study confirmed the positive effect of certain *S. cerevisiae* strains on the growth of wheat infected with *F. culmorum*, as some isolates contributed to improving stem and root length, and reducing disease severity. However, none of the strains were able to fully restore growth to normal levels, indicating that their effect was more preventive or mitigating rather than curative. Variations in strain effectiveness were observed, with some strains, such as Sc2, being more effective in promoting growth. This could be attributed to their ability to produce cell wall-degrading enzymes or stimulate growth through phytohormone production. These results are also consistent with Podgórska-Kryszczuk et al. (2022), who reported differences among yeast isolates in their ability to inhibit *Fusarium* growth on wheat grains. Furthermore, our results also showed that strain Sc1 had the highest phosphate solubilization ability, Sc2 exhibited strong siderophore production, and Sc3 was the most efficient in IAA production. These results align with previous studies (Kamel et al., 2016), which reported variability among *S. cerevisiae* and other yeast strains in their ability to solubilize phosphate and produce siderophores.

Finally, these findings indicate that certain *S. cerevisiae* strains could be a promising approach for mitigating the negative effects of *F. culmorum* on wheat. However, their effectiveness depends on the specific strain used, necessitating further research to determine optimal application conditions, such as yeast concentration and method of application in agriculture.

4 CONCLUSIONS

This study demonstrates the potential of *Saccharomyces cerevisiae* as a biocontrol agent against *Fusarium culmorum*, highlighting its capacity to inhibit fungal growth through direct and indirect mechanisms. The findings indicate variability in the effectiveness of different yeast strains, with some exhibiting significant inhibition of mycelial growth, spore germination, and disease severity. Additionally, *S. cerevisiae* displayed plant growth-promoting traits, including auxin production, phosphate solubilization, and enzymatic degradation of fungal cell walls, reinforcing its applicability in sustainable agriculture. However, the efficacy of yeast-based biocontrol is influenced by strain-specific characteristics and environmental conditions. Further research is required to optimize application parameters, assess long-term effects, and conduct field trials to validate laboratory results. Integrating *S. cerevisiae* into agricultural management strategies presents a viable and environmentally sustainable alternative for controlling *Fusarium* infections in wheat, potentially reducing reliance on chemical fungicides while enhancing crop health and productivity.

5 DATA AVAILABILITY STATEMENT

This research received no external or public funding. The data generated during this study were obtained using the author's own resources. The datasets are available from the corresponding author upon reasonable request.

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