

Biocontrol potential of yeast isolates compared with chemical fungicide against *Fusarium* wilt of lentil

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Wilt of lentil caused by *Fusarium* sp is one of the most destructive biotic factors limiting lentil (*Lens culinaris* Medik.) production in South Asia and is capable of causing total crop failure under favourable conditions. Sustainable management of this pathogen necessitates the integration of biological and chemical control methods. In the present study, three yeast isolates- YDP27 (*Meyerozyma caribbica*), YDP41 (*Candida oleophila*), and YZ7 (*Saccharomyces cerevisiae* YJM 451) and their consortium, along with the fungicide Nativo (tebuconazole 50% + trifloxystrobin 25% w/w) were evaluated against the *Fusarium* wilt pathogen under both *in vitro* and *in vivo* conditions. Under *In vitro* condition, the fungicide achieved 100% inhibition of mycelial growth at 50 and 100 ppm concentration and 68.60% inhibition at 5 ppm concentration. Among the yeast isolates, the consortium was the most effective with 34.97% inhibition of mycelial growth followed by YZ7 (22.42%), YDP27 (19.28%) and YDP41 (13.67%). Under *in vivo* conditions, the fungicide provided the highest disease inhibition of 62.75% over the control whereas the yeast consortium, YZ7, YDP27 and YDP41 achieved 18.26%, 11.58%, 7.57% and 5.08% disease control respectively. The compatibility test revealed that YDP27 exhibited higher tolerance to tebuconazole 50% + trifloxystrobin 25% w/w at 1, 5, and 10 ppm concentrations, as concentrations increased (20 and 30 ppm), the growth of the yeast isolates were completely inhibited. The finding indicated that the consortium has strong efficacy under *in vitro* but very less effective under *in vivo* condition. Therefore, another new efficient strain must be investigated to combat the disease.

Keywords : Biological control, Fungicide, *Fusarium* wilt, *Lens Culinaris*, Yeast isolates.

INTRODUCTION

Lentil (*Lens culinaris*) is a valuable source of protein, as well as an important component of local and international trade. It ranks as the world's fourth most significant pulse crop after beans, peas and chickpeas. Lentil is believed to have been domesticated in the fertile crescent region, with secondary centres of diversity in South Asia (India, Pakistan) and Central Asia.

The profitable cultivation of lentil is hindered by numerous fungal diseases, which cause significant crop losses at all growth stages, from planting to harvest and even during storage.

The most important diseases that cause an economic loss of lentil are Collar rot (*Sclerotium rolfsii*), Anthracnose (*Colletotrichum truncatum*), *Fusarium* wilt (*Fusarium oxysporum* f. sp. *lentis*), Ascochyta blight (*Ascochyta lentis*), Stemphylium blight (*Stemphylium botryosum*), Root rot (*Rhizoctonia solani*), Rust (*Uromyces viciae-fabae*), White mold (*Sclerotinia sclerotiorum*) (Kumar et al. 2013). The pathogens associated to wilting of the lentil plants are *Fusarium*, *Pythium*, *Phytophthora*, *Rhizoctonia*, *Sclerotium* and *Verticillium* sp. Infected plants typically exhibit stunting, wilting, chlorosis, necrosis and defoliation, often leading to plant death. The initial symptoms of *Fusarium* wilt often include the yellowing of a single leaflet or twig and slight wilting of lower leaves on a single stem (Madhavi

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et al. 2006). Among the wilt-causing pathogens, *Fusarium* sp. plays a crucial role in wilting in lentil crops. At each stage of growth, including seed, seedling, blooming and crop maturity in stem and root, *Fusarium* sp. affects lentil and causes seed rot, stem rots, damping off, wilt and root rot. Yield losses may vary depending on growth stage. Complete loss of the crop (100%) is possible if the pathogen strikes during the seedling and pre-pod stages. In contrast, losses around 60-70% can be seen when infections happen during flowering and pod formation stages of the plant (Garkotiet *al.* 2014). This disease was first reported in Hungary (Fleischmann, 1937) and has since been documented in numerous countries including the United States (Wilson and Brandsberg, 1965), the Soviet Union (Kotava *et al.* 1965), Syria (Bayya *et al.* 1986) and Turkey (Bayya *et al.* 1998) and in most of the countries in the world except in Australia (Tosi and Cappelli, 2001).

Heavy crop losses from *Fusarium* wilt have led to the use of chemical fungicides which pose environmental risks and can harm mammals. Additionally, fungicides may lead to fungal resistance, reducing their effectiveness. According to Shalaby and El-Nady (2008), yeast isolate *Saccharomyces cerevisiae* inhibited *F. oxysporum* growth by 39.52% with a dose of 5 g/L. The study aims to evaluate the yeast isolates as bio-control agents as sustainable alternatives to fungicides for managing lentil wilt disease, with a focus on identifying effective options for crop protection.

MATERIALS AND METHODS

Experimental site

The field experiments were conducted over two consecutive seasons at the experimental site of the All India Coordinated Research Project (AICRP) on Fruits, Mondouri, Nadia, West Bengal. Pooled data from both seasons are presented.

Isolation and identification of the wilt pathogen

Lentil plants showing characteristic wilting symptoms were collected from the infected fields.

The root portions were excised, cut into small pieces sized 5 mm each and surface-sterilised by rinsing in 0.1% (w/v) mercuric chloride solution for 30 s, followed by three times washing with sterile distilled water. Each surface sterilized pieces of roots were subsequently plated onto Potato Dextrose Agar (PDA) and incubated at $28 \pm 1^\circ\text{C}$ for seven days. Emerging colonies exhibiting characteristic white-to-pale mycelium were purified by single hyphal tip transfer technique and identified as *Fusarium* sp. on the basis of morphological and cultural characteristics. Pure cultures were maintained on PDA slants at 4°C for further use.

Details of the treatments

Three biocontrol yeast isolates- YDP27 (*Meyerozyma caribbica*, GenBank accession no. MN873568), YDP41 (*Candida oleophila*, GenBank accession no. MT341913) and YZ7 (*Saccharomyces cerevisiae* YJM451, GenBank accession no. KT459474) were collected from the Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal. All of the aforementioned yeast isolates were mixed to prepare a yeast consortium. The evaluated chemical fungicide was Nativo (tebuconazole 50% + trifloxystrobin 25% w/w; Bayer CropScience). For *in vitro* assays, the fungicide was tested at three concentrations (5, 50, and 100 ppm) using the poisoned food technique (Ramaiah and Garampalli, 2015).

In vitro antagonistic assay

To study the antagonism of yeast isolates against wilt pathogen (*Fusarium* sp.) in the dual culture technique (Badalyan *et al.* 2002), 5 mm mycelial discs of *Fusarium* sp. were inoculated at one side of Petri dish containing PDA and on the other side different yeast isolates were streaked. Then the Petri dishes were incubated for 7 days in BOD incubator at a temperature of $28 \pm 1^\circ\text{C}$. The first observation on the pathogen mycelial growth was recorded after 72 hours of incubation, with subsequent observations being taken at 48 hours interval up to 9 days after inoculation (DAI). The percentage inhibition of mycelial growth compared to the untreated control was calculated using the formula used by Lotfi *et al.* (2021):

Percent inhibition (%) = $[(C - T) / C] \times 100$

Where, C= radial growth in the control (cm); T= radial growth in the treated plate (cm).

In vivo disease management

Yeast formulations and the fungicide were applied to the collar region of lentil plants at the first visible appearance of the disease symptoms. The fungicide was dissolved in sterile distilled water at the recommended dose of 250–300 g/ha and applied in the field (Saha *et al.* 2018). For the yeast preparation, each isolate was mass-cultured in the urea-molasses liquid medium (Tamizharasiet *al.* 2005). Yeast cell suspensions were prepared where cell count was adjusted to 6×10^7 cells/ml using a haemocytometer and sprayed twice at seven days apart. The untreated control plots were sprayed with only sterile distilled water. The Percent Disease Index was scored on a 1-9 scale given by Chandra *et al.*, 2020 (Table 1) on different days.

Table 1: Disease rating scale for *Fusarium* wilt

Score	Mortality Percentage	Disease Reaction
1	No symptoms on any plant	Resistant
3	10% or less mortality	Moderately Resistant
5	11-20% mortality	Tolerant
7	20-50% mortality	Moderately susceptible
9	51% or more mortality	Susceptible

Percent Disease Index (PDI) were calculated using the formula given by Juber *et al.* (2014) and percentage disease control over the control was computed as:

Percent disease control (%) = $[(C - T) / C] \times 100$

Where, C= PDI in control plots, T = PDI in treated plots.

Compatibility assay

The compatibility of each yeast isolate was evaluated with the fungicide (tebuconazole 50% + trifloxystrobin 25% w/w) under *in vitro* conditions. Yeast Extract Potato Dextrose Agar (YEPDA) was amended with the fungicide at five concentrations (1, 5, 10, 20 and 30 ppm). Each yeast isolate was streaked onto the amended YEPDA medium and colony growth was recorded at 24h intervals up to 96 h.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) using standard statistical procedures. Percent Disease Index (PDI) data were subjected to angular (arcsine) transformation prior to analysis to stabilise variance both original and transformed values.

RESULTS AND DISCUSSION

In vitro efficacy testing of yeast isolates and chemical fungicide against *Fusarium* sp.

The efficacy of three yeast isolates, their consortium and the chemical fungicide against *Fusarium* sp under *in vitro* conditions is presented in (Table 2 and Fig. 1.) The fungicide (tebuconazole 50% + trifloxystrobin 25% w/w) was the most effective treatment achieving complete inhibition (100%) of mycelial growth of the pathogen at 50 ppm and 100 ppm concentration while significant inhibition of 68.60% achieved at 5 ppm concentration. Among the yeast treatments, the yeast consortium showed the highest percent inhibition of 34.97% followed by YZ7 (22.42%), YDP27 (19.28%) and YDP41 (13.67%) compared to control treatment.

In vivo management of *Fusarium* wilt using yeast isolates and chemical fungicide

Among the treatments evaluated under *in vivo* condition, highest efficacy was found in tebuconazole 50% + trifloxystrobin 25% w/w providing 62.75% disease control over the control (Table 3). Among the yeast isolates, yeast consortium showed 18.26% disease control followed by YZ7 (11.58%), YDP27 (7.57%) and YDP41 was the least effective treatment with 5.08% disease control over the control (Table 3).

Compatibility test of different yeast isolates with chemical fungicide (tebuconazole 50% + trifloxystrobin 25% w/w)

The compatibility test of the three yeast isolates on YEPDA medium amended with increasing concentrations of the fungicide (tebuconazole 50% + trifloxystrobin 25% w/w) are presented in (Table 4 and Fig. 2). Isolate YDP27 showed the

Table 2: Efficacy of yeast isolates and chemical fungicide against *Fusarium* sp under *in vitro* conditions:

Treatments	Radial growth at different days of interval (cm)				Percent inhibition over control (%)
	3 DAI	5 DAI	7 DAI	9 DAI	
YDP27 (<i>M. caribbica</i>)	1.30 ^{cd}	1.56 ^c	2.33 ^d	3.60 ^d	19.28
YDP41 (<i>C. oleophila</i>)	1.46 ^d	2.16 ^d	3.53 ^e	3.85 ^e	13.67
YZ7 (<i>S. cerevisiae</i>)	1.33 ^{cd}	1.49 ^c	2.20 ^d	3.46 ^d	22.42
Yeast Consortium	1.21 ^c	1.41 ^c	1.55 ^c	2.90 ^c	34.97
Tebuconazole 50% + trifloxystrobin 25%w/w at 5 ppm	0.40 ^b	0.86 ^b	1.10 ^b	1.40 ^b	68.60
Tebuconazole 50% + trifloxystrobin 25%w/w at 50 ppm	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	100.00
Tebuconazole 50% + trifloxystrobin 25%w/w at 100 ppm	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	100.00
Control	1.76 ^e	3.20 ^e	4.20 ^f	4.46 ^f	0
C.D. at 5%	0.16	0.154	0.16	0.183	
SE(m) (±)	0.053	0.051	0.053	0.061	

*Mean of three replications. Means with different letters differ significantly (DMRT, p d" 0.05).

Table 3. Management of *Fusarium* wilt of lentil using biocontrol yeast and chemical fungicide under *in vivo* conditions

Treatments	Percent Disease Index (%)			Percent Disease Control (%) (C-T) x100/C
	Before 1 st spray	7 days after 1 st spray	7 days after 2 nd spray	
YDP27 (<i>M. caribbica</i>)	22.16 (28.08) ^b	41.25 (39.96) ^d	66.83 (54.83) ^d	7.57
YDP41 (<i>C. oleophila</i>)	19.98 (26.55) ^a	44.60 (41.90) ^e	68.63 (55.94) ^e	5.08
YZ7 (<i>S. cerevisiae</i>)	21.90 (27.90) ^b	38.23 (38.19) ^b	63.93 (53.09) ^c	11.58
Yeast Consortium	26.98 (31.29) ^e	39.53 (38.96) ^c	59.10 (50.24) ^b	18.26
Tebuconazole 50% + trifloxystrobin 25%w/w	24.10 (29.40) ^d	26.08 (30.71) ^a	26.93 (31.26) ^a	62.75
Control	23.19 (28.79) ^c	48.89 (44.36) ^f	72.31 (58.25) ^f	0
C.D. at 5%	2.771705	2.42091	2.535532	
SE(m) (±)	0.899523	0.785676	0.822876	

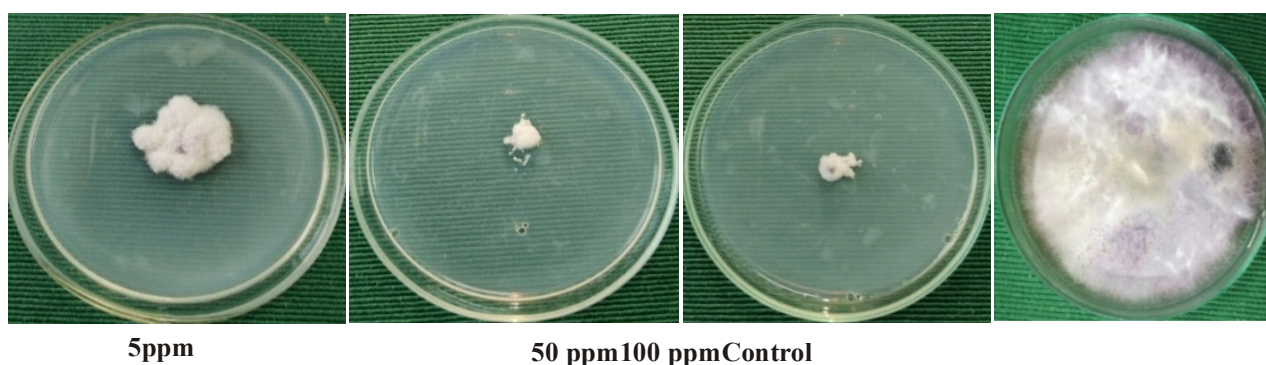
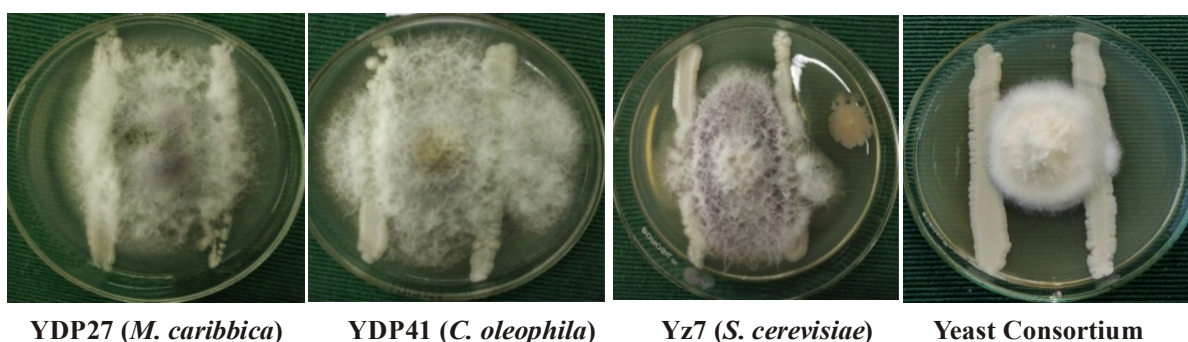
*Mean of three replications. Angular transformed values in parentheses. Means with different letters differ significantly (DMRT, p d" 0.05).

highest tolerance to the fungicide showing moderate growth at 1 ppm, fair growth at 5 ppm, poor growth at 10 ppm and complete inhibition at 20 and 30 ppm concentration of the fungicide. YDP41 showed sufficient growth at 1 ppm but was inhibited at 10 ppm and above concentrations. YZ7 was the most sensitive among the isolates with no growth at 5 ppm and above concentrations.

Lentil is one of the most important pulse crops suffering heavily from *Fusarium* wilt every year. Enhancing lentil production while suppressing the disease following suitable control measures is the most critical task. Yeast, a unicellular eukaryotic microorganism is a well-known member of the phyla Ascomycota and Basidiomycota, exhibiting distinctive biocontrol capabilities against the plant

Table 4 : Growth of yeast isolates at increasing concentrations of the fungicide (tebuconazole 50% + trifloxystrobin 25% w/w) under *in vitro* conditions

Yeast Isolates	Growth of yeasts at different concentrations of the fungicide					Control
	1 ppm	5 ppm	10 ppm	20 ppm	30 ppm	
YDP27 (<i>M. caribbica</i>)	Moderate Growth	Fair Growth	Poor Growth	No Growth	No Growth	Profuse Growth
YDP41 (<i>C. oleophila</i>)	Sufficient Growth	Moderate Growth	No Growth	No Growth	No Growth	Profuse Growth
YZ7 (<i>S. cerevisiae</i>)	Fair Growth	No Growth	No Growth	No Growth	No Growth	Profuse Growth



Tebuconazole 50% +Trifloxystrobin 25%w/w

Fig 1: Antagonistic test of the yeast isolates and poisoned food technique of the fungicide against *Fusarium* sp.

diseases (Tahir *et al.* 2022). In our experiment (in *vitro* as well as *in vivo*) different yeast isolates (YZ7, YDP27, YDP41) and their consortium exhibited antagonistic potentiality against *Fusarium* wilt. In both conditions, yeast consortium showed highest efficacy compared to individual isolates (YZ7, YDP27, YDP41). But it was noted that their efficacy was reduced under *in vivo* condition compared to *in vitro* condition. Probably the reason behind this was the impact of different environment factors on their antagonistic activities. Similarly, the strain

Pichia anomala 0732-1 sprayed on Hami melon leaves, reduced the disease incidence of bacterial fruit blotch by 82.3 % (Wang *et al.* 2009); the *Rhodotorula* sp. LD-19 reduced the severity of tomato soft rot by 21.2 % (Gomes *et al.* 2005) and three strains of *Cryptococcus* suppressed the population of *Erwinia amylovora* (Burrill) Winslow by 65 % in the detached stigmas of apple flowers (Pusey *et al.* 2009). But the yeast isolates are not as effective as chemical fungicide. Therefore, there is a need of further investigation on efficient strain of yeasts for better result

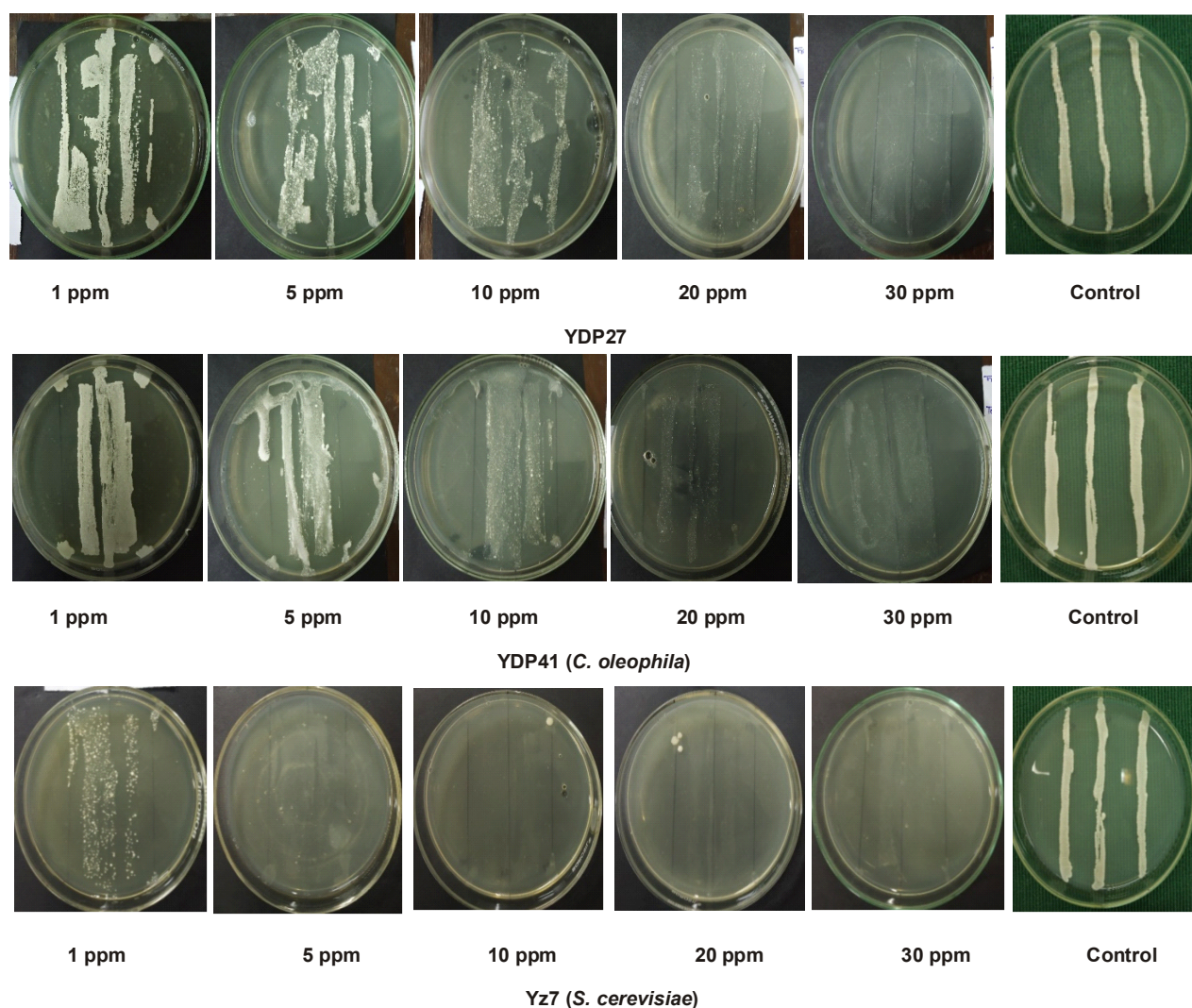


Fig. 2 : Compatibility test between different yeast isolates and chemical fungicide (tebuconazole 50% + trifloxystrobin 25% w/w) under *in vitro* condition as evidenced by growth responses of the different fungi

against the pathogen. Generally, biocontrol yeast competes against pathogenic fungi for nutrients and limited space on the surface of the fruit and plant roots. This phenomenon was observed during the antagonistic reaction among the yeast isolates. Similar result was reported by Shudeet *et al.* (2022) where they isolated 344 yeast epiphytes from cereal and weed plants and evaluated against *Fusarium graminearum*. Among them, twelve showed promising antagonistic activity. In contrast, the fungicide was the most effective treatment in both *in vitro* and *in vivo* conditions having mode of action of inhibition of mitochondrial respiration by blocking transfer of electron (complex III) by trifloxystrobin whereas tebuconazole disrupts ergosterol biosynthesis by inhibiting the cytochrome P450 enzyme (14 α -

demethylase) (Bag, 2009). The complete inhibition of mycelial growth at 50 ppm under *in vitro* and 62.75% disease control under *in vivo* conditions confirms the suitability of this fungicide against *Fusarium* wilt in lentil. Obviously, fungicides are better than bio control agents. But they have limitation in their use because their indiscriminate application leads to development of resistance in the pathogen population. In compatibility test, it was found that all the yeast isolates were unable to grow at higher concentrations of this fungicide. So, the yeasts or other beneficial microbes cannot be applied just after spraying of this fungicide to control the disease. The findings were similar to Uribe-Gutiérrez *et al.* (2021) where they found that the yeast isolate *Rhodotorula mucilaginosa*

Lv316 when combined with commonly used fungicides at lower concentrations provided valuable insights for its integration into disease management practices.

CONCLUSION

This study is about biological control activities of yeast against *Fusarium* sp. Here the yeast isolates and their consortium played a beneficial role against *Fusarium* wilt of lentil both in laboratory and field condition like other beneficial microorganisms (*Trichoderma* sp. and *Bacillus* sp.). However, their disease control efficacy remains inferior to chemical fungicides. In contrast, the fungicide (tebuconazole 50% + trifloxystrobin 25% w/w) remains the most reliable treatment for managing *Fusarium* wilt and is recommended for use against the disease where it poses an economic threat to lentil production. The yeast isolates are also showing negative compatibility with increasing concentrations of the fungicide.

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DECLARATION

Conflict of Interest. Authors declare no conflict of interest.

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