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# Characterization of endophytic yeast and its suppressive effect on root rotting fungi of tomato under neem cake soil amendment

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## Abstract

**Background:** The exposure of crops to a variety of fungal and bacterial pathogens leads to huge economic losses. Different strategies are being adapted to control these diseases among which the application of chemicals fungicide is common. However, these chemicals are posing a serious threat to the environment. For biological management of root rot disease of tomato and better fruit quality, studies were conducted on the possible use of endophytic yeast as a biocontrol agent.

**Results:** Endophytic yeasts were isolated from healthy plants and identified. Identification of selected isolates was confirmed on the basis of 18S rDNA gene sequencing. They were evaluated for suppressive effect on root rotting fungi in vitro and also in vivo on tomato plants, used alone or under neem cake soil amendment. Seventy-six isolates of yeasts were evaluated against root rotting fungi *Fusarium oxysporum*, *F. solani*, *Rhizoctonia solani* and *Macrophomina phaseolina* using dual culture plate assay. Seventy-five isolates were found to suppress radial growth of *F. oxysporum*, *F. solani* and *M. phaseolina* by producing zones of inhibition or lysing the fungal hyphae. However, none of the isolates was found to inhibit *R. solani* in vitro. Most of the isolates also caused nematicidal activity at varying degree against *Meloidogyne javanica*. All test isolates produced indole acetic acid in vitro and solubilized phosphorus. In pots and field plot experiments, test isolates of yeasts were able to suppress root rotting fungi on tomato in natural soil and soil amended with neem cake with enhancement of growth of tomato plants. Yeasts were also found to ameliorate the plant resistance through enhancing polyphenolic contents, salicylic acid and antioxidant activity.

**Conclusions:** Endophytic yeasts were found effective against root rot disease of tomato and could be used as a potential biocontrol agent for the management of soil-borne diseases of tomatoes.

**Keywords:** Endophytic yeast, Neem cake, Biological control, Root rot, Tomato plant

## Background

Antagonistic microbes have emerged as a practical substitute to the chemical fungicides. They are used to suppress the survival or activity of a pathogen resulting in reduction in the incidence of diseases. Yeasts have been used in industries for thousands of years. Its several

strains have now been recognized as potential biocontrol agents against plant diseases (Fareed et al. 2019). *Pichia membranifaciens* is reported to inhibit *Rhizopus stolonifer* on nectarine (Fan and Tian 2000), while *Cryptococcus albidus* suppressed *Penicillium expansum* in apples and pears (Tian et al. 2002). Another research indicated that plant growth enhancing and biocontrol ability of *S. cerevisiae* make it a strong competitor of *Fusarium oxysporum* which causes damping-off symptoms in sugar beet seedlings (Shalaby and El-Nady 2008). Species of *Fusarium* are necrotrophic pathogens of winter

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wheat that contaminate crops through their mycotoxins (Baliukoniene et al. 2011). Yeasts of genera *Cryptococcus*, *Rhodotorula* and *Saccharomyces* are pronounced to have deleterious effects on *Fusarium sporotrichioides* to some satisfactory degrees (Wachowska et al. 2013). Similarly, epiphytic yeasts have been reported to reduce post-harvest *Penicillium* rot of citrus (Habiba et al. 2019).

Tomato (*Lycopersicon esculentum* Mill.), widely consumed vegetable crop, is known for their nutritional values. The production of good quality tomatoes is highly favored by the diversified climatic conditions in Pakistan throughout the year, but natural obstacles are always there. Tomato plants are always subjected to many bacterial, fungal, viral and nematocidal attacks (Agrios 2005) but major yield reduction has resulted from fungal pathogens (Stone et al. 2000). This crop has a short life and is highly prone to mycotic diseases (Chohan et al. 2017). Greater losses were observed, when tomato crops were affected by both fungi and root knot nematodes (Parveen et al. 2020). The use of synthetic fungicide seems to be the best-known technique for the management of soil-borne diseases. Application of pesticides, no doubt, caused effective suppression of diseases resulting in the improvement of crop yield and quality (Sultana et al. 2011). However, the regular use of these chemicals may produce collateral problems. One of the major threats is the contamination of the environment with toxic elements which directly or indirectly affects human and animal health. Continuous application of fungicides to the crops also increases the pathogen resistance to such chemicals in particular soil environments (Tupe et al. 2014). These treatments are mostly non-specific, i.e., not only affect target pathogens but also other beneficial microorganisms (Ranganathswamy et al. 2013).

Aim of the application of any biocontrol agent is to not only suppress the disease but also to get high crop yield with good quality. A good and effective biocontrol agent has both the inhibitory effect on the pathogen as well as plant growth promoting ability. Many of the bioagents including yeast have been reported earlier as effective antagonists with positive impacts on plant growth (Moin et al. 2020). In a previous report, the biocontrol potential of endophytic yeasts against root rotting fungi of sunflower was reported (Fareed et al. 2019). The present research describes the efforts for further isolating the indigenous endophytic yeasts from healthy plants and determining their biocontrol potential against root rotting fungal pathogens of tomato in soil under neem cake amendment and their plant growth promoting ability. The report also describes the role of endophytic yeasts in enhancing the nutrient uptake and increasing antioxidant activity; salicylic acid and phenolic content status in plants.

## Methods

### Isolation and identification of endophytic yeast

Healthy plants (5 from each location) were collected from Karachi University and Malir, Karachi, Pakistan, and isolation of endophytic yeasts was made within 24 h in the laboratory. Stem, roots and leaves were separately cut into 2–3 cm long sections. The adhered debris and epiphytic microorganisms were removed by washing the cuttings with sterile water. They were then subjected to consecutive 1 min washes with 1% sodium hypochlorite, 70% ethanol and sterile distilled water. Surface-disinfected tissue was aseptically deliquesced with homogenizers. Serial dilution was made up to  $10^{-6}$  and 0.5 mL from the last two dilutions was spread on YM medium plates, and then incubated for 5–7 days at  $25 \pm 1$  °C. The yeast isolates grown were initially examined for their morphological characteristics based on colony color, texture and some physiological and biochemical assays (Kurtzman et al. 2011).

### Molecular identification of potential yeasts

The DNA of freshly cultured yeast was extracted by using Biobasic Kit, Canada, as described by the manufacturer. ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (3'-TCC TCC GCT TAT TGA TAT GC-5') primers were used for the amplification of rDNA on a thermal cycler (BioRad, USA). The amplified products were submitted to BGI Genomic Services (Shenzhen, Guangdong, China) for sequencing. For edition and alignment of sequences BioEdit software (Version 7.2.6) was used. Yeast isolates were identified by using BLAST and finally sequences were submitted to NCBI Genbank.

### In vitro antifungal activity of endophytic yeasts

Newly isolated yeasts along with previously tested isolates (Fareed et al. 2019) were tested for antifungal activity in vitro against four root rotting fungi of tomato using dual culture plate assay. The Petri plates were poured with sterilized potato dextrose agar (PDA) and after solidification, the medium was streaked with yeast isolates on one side. A disk (5 mm) of test fungi viz., *Fusarium oxysporum*, *F. solani*, *M. phaseolina* and *R. solani* was positioned on the side of plates opposite to each yeast streak. The plates were kept at 28 °C for 3–7 days. The inhibitory effect of yeasts was assessed by measuring zones of inhibition produced by yeasts against test fungi. The experiment was conducted twice with three replicates.

### Cell free culture filtrates of yeast and their nematocidal activity in vitro

The test yeast isolates were grown in YM broth in 250-ml flasks and incubated for 5 days. The broth was filtered over Whatman filter paper. The culture filtrate was

then exposed to chloroform vapors to kill yeast cells, if any. Aqueous suspension (1 ml) of freshly hatched second juvenile (20 juveniles) was transferred in glass cavity blocks with 1 ml of a yeast culture filtrate. Each test was kept with 3 replicates at  $26 \pm 5$  °C for 48 h and the nematode's mortality was recorded (Noreen et al. 2015). The experiment was repeated once.

#### Indole acetic acid (IAA) production by potential isolates of endophytic yeasts

The yeast isolates were grown in YM broth containing 0.1% (w/v) L-tryptophan and then incubated in the dark for 5 days. Broth was centrifuged at 4000 rpm for 15 min and one ml supernatant was mixed with 2 ml of Salkowski's Reagent. O.D. was recorded at 530 nm on a spectrophotometer (Shimadzu, Japan, UV 1800) after 30 min, when color was developed (Gordon and Weber 1951). IAA production was calculated against the calibration curve using IAA. The experiment was conducted twice with 3 replicates.

#### Phosphate solubilization activity of potential yeast isolates

The ability of yeast to solubilize inorganic phosphorus was determined in vitro by using Pikovskaya's agar medium (Pikovskaya 1948) in triplicate and repeated once. The fresh cultures of yeast isolates were inoculated on plates containing solid medium and left to incubate for 5 days at 25 °C. The solubilization of phosphorus was specified by the production of distinct halos around yeast colonies.

#### Clay pots experiment

Sandy loam soil was amended with neem cake [Sigma Energy (pvt) Ltd, Karachi] at 1% w/w and transferred to 12 cm clay pots at 1 kg per pot. A natural infestation of *Fusarium* spp. (3000 cfu g<sup>-1</sup> of soil), 3–13% colonization of *R. solani*, and 3–9 sclerotia g<sup>-1</sup> soil of *M. phaseolina* were found by using techniques described by Nash and Snyder (1962), Wilhelm (1955) and Sheikh and Ghaffar (1975). Pots were watered daily for 2 weeks. After decomposition of organic matter, 25 ml aqueous suspension (10<sup>8</sup> cells/ml) of endophytic yeasts viz., *Debaryomyces hansenii* (Y-17), *D. hansenii* (Y-34), *Rhodotorula mucilaginosa* (Y-54) and *Meyerozyma guilliermondii* (Y-62), grown in YM broth for 1 week, was drenched into each pot. Four tomato (*Lycopersicon esculentum* Mill) seedlings were transplanted into each pot and each treatment was replicated 4 times. In another set, yeast suspensions were inoculated in un-amended soil. Carbendazim (25 mL of 200 ppm) served as positive control, while untreated plants served as control. The pots were kept with daily watering for 45 days. Observation on the effect of yeast isolates on the incidence of

fungal infection and plant growth was examined after 45 days. Plant growth parameters (shoot length and shoot weight; root length and root weight) were recorded. To determine the infestation of root rot fungi, tap roots after washing were cut into pieces (1 cm), sterilize with 1% bleach and transferred onto PDA plates (amended with streptomycin (0.2 g ml<sup>-1</sup>) and Penicillin (100,000 units ml<sup>-1</sup>). Root rot fungi emerged from each piece after 5 days (at 25 °C) were identified and infection (%) for each fungus was calculated as described by Noreen et al. (2015). The experiment was repeated once, next year in similar conditions.

#### Field experiment

The antagonistic ability of the endophytic yeasts was also investigated under field conditions, where (2 × 2 m) plots of the experimental field of Crop Diseases Research Institute (CDRI) (PARC, Karachi University Campus, Karachi) was amended by neem cake. The neem cake was mixed in the soil at 70 g per 2 m row and allowed to decompose by watering on alternate days. Twelve healthy and equal sized seedlings of tomato were planted in each row. Each row was inoculated by 250 ml of yeast suspensions viz; *Debaryomyces hansenii* (Y-17), *D. hansenii* (Y-34) and *Meyerozyma guilliermondii* (Y-62). All the treatments were replicated 4 times. Plots without any treatment served as control, while carbendazim (100 ml of 200 ppm per meter row was drenched) served as positive control. Data were recorded after 45 and 90 days by uprooting four tomato plants from each treatment.

#### Estimation of biochemical parameters of leaf

##### Determination of phosphorus from leaf samples

Leaf phosphorus was measured by the following method used by Noreen et al. (2019). One g oven-dried leaf sample (dried at 120 °C for 24 h) was crushed and homogenized in 2N-HCl. The extract was digested for 60 min and then filtered (Whatman No.1 filter paper). Digested filtrate (10 ml) and freshly prepared Barton reagent (10 ml) were mixed in a 100-ml flask. Final volume was made 100 ml with distilled water. Absorbance of phosphorus vanado-molybdate complex was recorded against blank at 420 nm after 30 min and concentration of phosphorus was calculated in mg/g (Crous et al. 2019).

##### Estimation of polyphenols

The extraction of oven-dried leaves was done with ethanol (96% v/v). After centrifuging the extracts at 3000 rpm for 20 min, supernatants were used for analyzing polyphenols and antioxidants.

The Folin–Ciocalteu assay described by Rahman et al. (2017) was applied for the quantification of total phenols

from plant leaves. Sodium bicarbonate ( $\text{Na}_2\text{CO}_3$ ) 2% of 2 ml was added to 100 mL of extract (10 mg/ml) and left untouched for 2 min at room temperature. After adding 100  $\mu\text{L}$  Folin–Ciocalteu reagents (50%), the mixture was incubated in a dark place for 30 min. The test samples were subjected to a spectrophotometer for determining their absorbance at 720 nm. Standard curve obtained from Gallic acid was used for the estimation of total phenol contents in the samples.

#### Estimation of salicylic acid (SA)

Salicylic acid in leaf samples was determined by using the method of Warriar et al. (2013), where 0.1 ml of chilled ethanolic leaf extracts was mixed with 3 mL of 0.1% ferric chloride. The absorbance was noted at 540 nm on the spectrophotometer. The quantity of SA ( $\mu\text{g ml}^{-1}$ ) was calculated as  $\text{mg g}^{-1}$  dried samples, where 100 mg of SA in 100 mL of ethanol was used for the standard curve.

#### Estimation of antioxidant activity

An aliquot of leaf sample (200  $\mu\text{l}$ ) was mixed with 800  $\mu\text{l}$  of 10 Mm Tris HCl buffer (pH 7.4), then 30  $\mu\text{M}$  DPPH (dissolved in DMSO) was added to the mixture. After mixing, the mixture was kept in the dark at room temperature. DPPH in ethanol served as control. The absorbance was taken at 517 nm against blank after 1 min and then at 30 min after keeping in dark. Free radical scavenging ability was calculated as described by Rahman et al. (2016).

#### Data analysis

Statistical software, CoStat (CoHort Software, USA) was used to determine the analysis of variance (ANOVA) and the significant level at ( $p < 0.05$ ) was calculated using least significant difference (LSD).

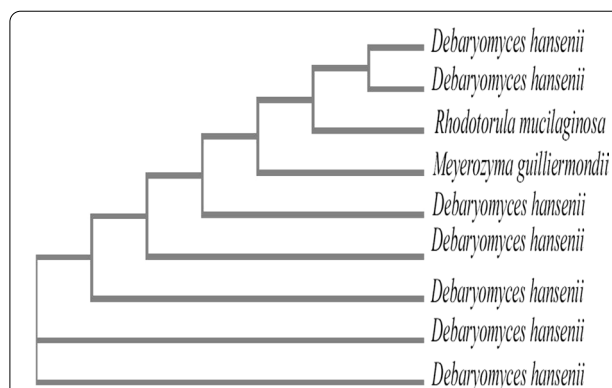
## Results

#### Isolation and identification of endophytic yeasts

A total of 76 isolates of endophytic yeasts were isolated and attentively identified (Additional file 1: Table S1).

#### Molecular identification of promising isolates

The potential isolates of endophytic yeasts were identified as *Rhodotorula mucilaginosa* (MT949216), *Debaryomyces hansenii* (MT949217), *Debaryomyces hansenii* (MT949218), *Debaryomyces hansenii* (MT949219), *Debaryomyces hansenii* (MT949220), *Meyerozyma guilliermondii* (MT949221), *Debaryomyces hansenii* (MT949222), *Meyerozyma guilliermondii* (MT949223) and *Debaryomyces hansenii* (MT949224) by molecular biology technique (Fig. 1).



**Fig. 1** Endophytic yeasts identified on the basis of 18S rDNA sequencing showing their ancestral relationship. Phylogenetic tree was generated using parsimony, neighbor-joining and maximum likelihood analysis of aligned sequences with different sets of cluster

#### In vitro antifungal activity of endophytic yeasts against root rotting fungi

The yeast isolates tested for their antifungal activity, showed varying degrees of inhibition against test fungi. Out of 76 isolates, 75 inhibited the mycelial growth of 3 tested fungi viz; *F. solani*, *F. oxysporum* and *M. phaseolina*, while none of the isolates inhibited *R. solani*. Among these isolates, some produced significant zones of inhibition around fungal growth. Lysis of fungal hyphae was also caused by some yeast isolates like: Y-37, Y-39, Y-41, Y-42, Y-43, Y-44, Y-45, Y-46, Y-47, Y-48, Y-49, Y-51, Y-53, Y-64 and Y-69 (Additional file 1: Table S1). Maximum inhibition of *F. solani* growth was observed by the yeast culture Y-34, Y-62 and Y-66. Yeast isolates Y-9, Y-25, Y-34, Y-38 and Y-62 produced maximum growth inhibition of *F. oxysporum*, whereas proliferation of *M. phaseolina* was greatly affected by isolates Y-5, Y-9, Y-34, Y-38 and Y-62 (Fig. 2; Additional file 1: Table S1).

#### In vitro nematicidal activity of cell free culture filtrates of endophytic yeasts

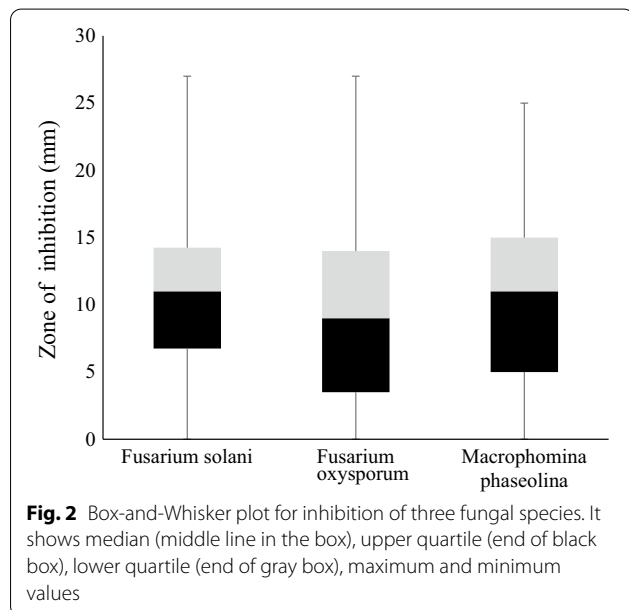
Culture filtrates of different yeast isolates showed a nematicidal activity against root knot nematodes, juveniles at varying degrees. Complete killing of juvenile's (100%) was exhibited by yeast isolates viz; Y- 5, Y-9, Y-29, Y-30, Y-34 and Y-66 within 24 h, of exposure, while yeast isolates Y-10, Y-21, Y-31, Y-65 and

**Table 1** Production of indole acetic acid (IAA) and phosphate solubilization by some potential endophytic yeast isolates in vitro

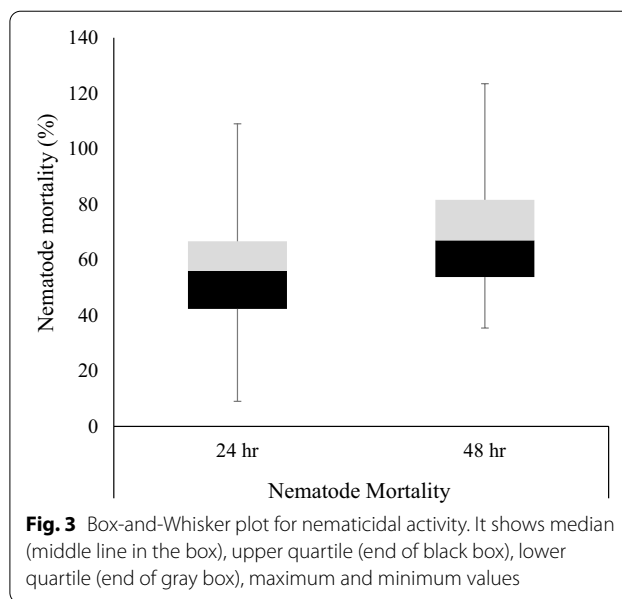
Yeast isolates	Concentration of IAA ( $\mu\text{g/ml}$ )	Phosphate solubilization
Y-1	7.36	–
Y-5	7.2	+
Y-9	6.5	+
Y-10	6.9	+
Y-17	9.21	+
Y-23	3.73	+
Y-31	1.29	–
Y-34	8.58	+
Y-38	2.66	+
Y-54	7.6	+
Y-58	2.25	+
Y-62	9.2	+
Y-66	8.7	+
Y-67	8.2	+
Y-72	4.3	+
LSD <sub>0.05</sub>	0.39 <sup>a</sup>	

+, clear zone formation; –, no zone formation

<sup>a</sup> Mean values for IAA in columns showing differences greater than LSD values are significantly different at  $p < 0.05$

**Fig. 2** Box-and-Whisker plot for inhibition of three fungal species. It shows median (middle line in the box), upper quartile (end of black box), lower quartile (end of gray box), maximum and minimum values

Y-67 killed the juveniles completely (100%) after 48 h, whereas several yeast isolates were able to kill more than 50 nematodes within 48 h (Fig. 3; Additional file 1: Table S1).

**Fig. 3** Box-and-Whisker plot for nematocidal activity. It shows median (middle line in the box), upper quartile (end of black box), lower quartile (end of gray box), maximum and minimum values

#### In vitro indole acetic acid (IAA) production by potential isolates of endophytic yeasts

The concentration of IAA produced by the yeast isolates was within the range from 1.29  $\mu\text{g/ml}$  to 9.21  $\mu\text{g/ml}$ . Maximum IAA (9.2  $\mu\text{g/ml}$ ) was found to be produced by Y-17 and Y-62 as compared to other yeasts. Y-31 produced relatively the lowest IAA concentration (Table 1).

#### Phosphate solubilization activity by potential yeast isolates

Out of 15 yeast isolates tested for phosphate solubilizing ability, 12 were found to be efficient in this activity by producing clear halos around yeast colonies (Table 1).

#### Clay pots experiment

Plant growth was significantly enhanced by the application of endophytic yeasts either alone or along with neem cake. Significant increase at  $p < 0.05$  in shoot length, shoot weight, root length and root weight were acquired by the plants treated with *M. guilliermondii* along with neem as compared to the non-treated control plants and plants having individual treatments of carbendazim and neem (Table 2). Treatments of plants with yeasts and neem cake significantly reduced the incidence of fungal pathogens in the root system. Complete suppression of *F. solani* was observed by the treatment of *M. guilliermondii* alone, followed by the minimum infection in *D. hansenii* (Y-17) treatment alone and in *D. hansenii* (Y-34) combination with neem cake. *M. phaseolina* and *F. oxysporum* were completely suppressed by *M. guilliermondii* and combined treatment of carbendazim and

**Table 2** Effect of endophytic yeast isolates on vegetative growth of tomato plants in natural soil and soil amended with neem cake in pots experiment—season I and II

Treatments	Growth parameters							
	Shoot length (cm)		Shoot weight (g)		Root length (cm)		Root weight (g)	
	S-I	S-II	S-I	S-II	S-I	S-II	S-I	S-II
Control	22.93	19.35	4.05	3.88	11.49	14.63	0.95	0.19
Carbendazim (Cd)	23.56	18.9	5.27	3.6	16.68	17.24	1.88	1.74
Neem cake (NC) @ 1%	26.31	22.7	5.87	4.85	21.06	13.35	2.79	2.04
Cd + NC	26.68	23.75	5.47	3.49	15.81	15.5	3.07	2.03
<i>Debaryomyces hansenii</i> (Y-17)	37.07	25.5	7.35	5.47	23.8	15.6	3.72	1.04
Y-17 + NC	33.5	25.4	7.08	5.1	24.37	19.75	3.55	1.53
<i>D. hansenii</i> (Y-34)	37.73	28.64	7.99	6.24	23.75	19.98	4.0	1.92
Y-34 + NC	38.92	27.02	7.33	5.93	21.06	16.23	3.41	1.21
<i>Rhodotorula mucilaginosa</i> (Y-54)	31.4	27.16	7.58	5.78	26	19.35	3.66	2.76
Y-54 + NC	34.25	23.5	6.94	4.54	20.25	16.53	2.97	1.59
<i>Meyerozyma guilliermondii</i> (Y-62)	34.37	30.29	6.9	5.1	20.5	20.83	3.37	2.15
Y-62 + NC	40.5	29.38	8.05	5.13	25	21.63	3.86	1.57
LSD <sub>0.05</sub>	6.4 <sup>a</sup>	7.78 <sup>a</sup>	0.99 <sup>a</sup>	2.45 <sup>a</sup>	3.82 <sup>a</sup>	6.41 <sup>a</sup>	0.91 <sup>a</sup>	1.28 <sup>a</sup>

S-I, season I; S-II, season II

<sup>a</sup> Mean values in column showing differences greater than LSD values are significantly different at  $p < 0.05$ **Table 3** Effect of endophytic yeast isolates on *Fusarium solani*, *F. oxysporum*, *Macrophomina phaseolina* and *Rhizoctonia solani* on tomato plants in natural soil and soil amended with neem cake in pots experiment—season I

Treatments	Infection %			
	<i>F. solani</i>	<i>F. oxysporum</i>	<i>M. phaseolina</i>	<i>R. solani</i>
Control	100	68.7	93.7	87.5
Carbendazim (Cd)	31.2	50	18.7	37.5
Neem cake (NC) @ 1%	18.7	37.5	62.5	18.7
Cd + NC	50	0	50	25
<i>Debaryomyces hansenii</i> (Y-17)	6.2	18.7	12.5	62.5
Y-17 + NC	25	6.2	18.7	37.5
<i>Debaryomyces hansenii</i> (Y-34)	18.7	12.5	43.7	31.2
Y-34 + NC	18.7	0	12.5	43.7
<i>Rhodotorula mucilaginosa</i> (Y-54)	62.5	31.2	43.7	56.2
Y-54 + NC	68.7	43.7	25	68.7
<i>Meyerozyma guilliermondii</i> (Y-62)	0	6.2	0	25
Y-62 + NC	68.7	50	6.2	56.2
LSD <sub>0.05</sub>	Treatments = 16.5 <sup>a</sup>		Pathogens = 9.5 <sup>b</sup>	

<sup>a</sup> Mean values in column showing differences greater than LSD values are significantly different at  $p < 0.05$ <sup>b</sup> Mean values in rows showing differences greater than LSD values are significantly different at  $p < 0.05$ 

neem cake, respectively. Minimum infection by *R. solani* was produced by the individual treatment of neem cake followed by *M. guilliermondii* (Table 3).

In season II (repeated experiment), *M. guilliermondii* combined with neem cake and alone produced significantly highest shoot length, shoot weight, root length and root weight (Table 2). Treatments of plants with yeasts in

neem cake amended soil significantly reduced the incidence of fungal pathogens in the root system. Significant suppression of *F. solani* was observed by the treatment of *M. guilliermondii* with neem cake. *D. hansenii* (Y-17) with neem cake showed significant reduction in infection of *F. oxysporum*. *R. mucilaginosa* (Y-54) with neem

**Table 4** Effect of endophytic yeast isolates on *Fusarium solani*, *F. oxysporum*, *Macrophomina phaseolina* and *Rhizoctonia solani* on tomato plants in natural soil and soil amended with neem cake in pots experiment—season II

Treatments	Infection %			
	<i>F. solani</i>	<i>F. oxysporum</i>	<i>M. phaseolina</i>	<i>R. solani</i>
Control	93.7	75	81.2	75
Carbendazim (Cd)	75	68.7	18.7	62.5
Neem cake (NC) @ 1%	43.7	18.7	56.2	75
Cd + NC	75	62.5	50	0
<i>Debaryomyces hansenii</i> (Y-17)	6.2	43.7	75	68.7
Y-17 + NC	18.7	6.2	18.7	50
<i>Debaryomyces hansenii</i> (Y-34)	12.5	12.5	50	31.2
Y-34 + NC	43.7	56.2	25	18.7
<i>Rhodotorula mucilaginosa</i> (Y-54)	25	31.2	43.7	62.5
Y-54 + NC	31.2	50	6.2	50
<i>Meyerozyma guilliermondii</i> (Y-62)	0	37.5	12.5	18.7
Y-62 + NC	6.2	43.7	12.5	31.2
LSD <sub>0.05</sub>	Treatments = 16.4 <sup>a</sup>		Pathogens = 9.4 <sup>b</sup>	

<sup>a</sup> Mean values in column showing differences greater than LSD values are significantly different at  $p < 0.05$

<sup>b</sup> Mean values in rows showing differences greater than LSD values are significantly different at  $p < 0.05$

cake showed significant suppression of *M. phaseolina* (Table 4).

#### Field plot experiment

Tomato plants treated with endophytic yeasts either alone or along with neem cake showed better plant growth than control plants. Combined treatment of *M. guilliermondii* and neem resulted in maximum positive effect

on plant growth. These plants also produced the greatest number of fruits as compared to untreated plants after 90 days but it was statistically non-significant. Maximum weight was gained by the fruits of plants treated with *D. hansenii* (Y-17) alone (Table 5). Infection by root rotting fungi was also significantly reduced by the application of endophytic yeasts. Within 45 days, minimum incidence of *F. solani* was detected in the roots of plants treated

**Table 5** Effect of endophytic yeast isolates on vegetative growth of tomato plants in natural soil and soil amended with neem cake in field plot experiment

Treatments	Growth parameters											
	Shoot length (cm)		Shoot weight (g)		Root length (cm)		Root weight (g)		No. of fruits/plant		Weight of fruits/plant (g)	
	Time (days)											
	45d	90d	45d	90d	45d	90d	45d	90d	45d	90d	45d	90d
Control	18.5	32.56	15	54.12	9.5	17.31	1.62	3.38	–	6.25	–	14.28
Carbendazim (Cd)	21.63	34.31	18.13	30.23	11.56	18.38	0.99	2.04	–	0.5	–	4.21
Neem cake (NC) @ 1%	23.63	39.56	22.06	51.22	16.25	22.38	1.71	3.76	–	2.25	–	5.96
Cd + NC	22.19	40.9	48.56	64.42	15.75	23.44	1.92	3.94	–	1.63	–	6.19
<i>Debaryomyces hansenii</i> (Y-17)	27.5	34.93	21.38	35.39	11.39	21.44	1.63	2.52	–	2.25	–	16.93
Y-17 + NC	30.06	46	56.63	78.84	16.13	27.19	1.97	4.56	–	5.33	–	15
<i>Meyerozyma guilliermondii</i> (Y-62)	28.5	34.06	14.75	28.67	9.38	17.13	0.76	1.88	–	0.58	–	2.53
Y-62 + NC	38	57.25	94.56	141.89	20.19	31.31	3.38	7.22	–	8.3	–	4.24
<i>Debaryomyces hansenii</i> (Y-34)	31.31	44	34.4	51.32	15.75	23.94	1.88	3.64	–	4.5	–	5.64
Y-34 + NC	35.75	49.37	63.68	84.57	14.5	27.69	4.99	5.38	–	4.4	–	10.52
LSD <sub>0.05</sub>	1.77 <sup>a</sup>	14.4 <sup>a</sup>	5.13 <sup>a</sup>	61.03 <sup>a</sup>	3.88 <sup>a</sup>	7.9 <sup>a</sup>	NS	NS	–	NS	–	NS

<sup>a</sup> Mean values in column showing differences greater than LSD values are significantly different at  $p < 0.05$

with *D. hansenii* (Y-17) alone followed by the individual treatment of *M. guilliermondii* but after 90 days combined treatment of *M. guilliermondii* and neem produced least *F. solani* infection. No infection by *F. oxysporum* was found in the treatments of *D. hansenii* (Y-17 and Y-34) and *M. guilliermondii* alone in 45 days but after 90 days it was little increased but still the least infection as compared to control plants. *D. hansenii* (Y-17) and combined treatment of *M. guilliermondii* and neem completely suppressed *M. phaseolina* within 45 days but after 90 days its least incidence was observed by the treatment of *M. guilliermondii* and *D. hansenii* (Y-34) with neem. *Rhizoctonia solani* was greatly suppressed by carbendazim alone and with neem in 45 days but after 90 days least infection was observed in the treatment of *M. guilliermondii* alone followed by the combined treatment of *D. hansenii* (Y-17) and neem (Table 6).

#### Estimation of biochemical parameters of leaf

##### Phosphorus content

Phosphorus content was significantly increased in the plants treated with yeast alone or along with neem cake. After 45 days, maximum phosphorus absorption was detected in the plants that were treated with *M. guilliermondii* along with neem cake, followed by the combined treatment of *D. hansenii* (Y-17) and neem. After 90 days, *D. hansenii* (Y-17) plus neem showed the highest uptake of phosphorus in contrast to control plants and other treatments (Table 7).

**Table 7** Effect of endophytic yeast isolates on salicylic acid and phosphorus uptake by tomato plants in natural soil and soil amended with neem cake in field plot experiment

Treatments	Salicylic acid ( $\mu\text{g g}^{-1}$ )		Phosphorus (mg/g)	
	45 days	90 days	45 days	90 days
Control	2.6	3.75	0.62	0.99
Carbendazim (Cd)	3.29	6.45	0.76	0.49
Neem cake (NC) @ 1%	3.38	6.54	0.94	0.83
Cd + NC	3.56	7.41	0.72	0.65
<i>Debaryomyces hansenii</i> (Y-17)	2.6	6.76	1.21	0.38
Y-17 + NC	2.76	7.44	1.68	2.22
<i>Meyerozyma guilliermondii</i> (Y-62)	5.6	9.35	1.25	0.38
Y-62 + Neem	4.4	7.1	1.97	2.14
<i>Debaryomyces hansenii</i> (Y-34)	3.58	5.94	1.09	0.91
Y-34 + Neem	4.77	6.65	0.92	0.86
LSD <sub>0.05</sub>	0.46 <sup>a</sup>	0.53 <sup>a</sup>	0.015 <sup>a</sup>	0.76 <sup>a</sup>

<sup>a</sup> Mean values in column showing differences greater than LSD values are significantly different at  $p < 0.05$

##### Salicylic acid

After 45 days, the level of salicylic acid (SA) was much increased in the plants treated with *M. guilliermondii* alone, followed by the combined treatment of *D. hansenii* (Y-34) and neem. Highest level of SA was produced by the treatment of *M. guilliermondii* alone after 90 days,

**Table 6** Effect of endophytic yeast isolates on *Fusarium solani*, *F. oxysporum*, *Macrophomina phaseolina* and *Rhizoctonia solani* on tomato plants in natural soil and soil amended with neem cake in field plot experiment

Treatments	Infection %								
	<i>F. solani</i>		<i>F. oxysporum</i>		<i>M. phaseolina</i>		<i>R. solani</i>		
	Time (days)								
	45d	90d	45d	90d	45d	90d	45d	90d	
Control	100	93.7	56.2	62.5	81.2	75	93.7	87.5	
Carbendazim (Cd)	56.2	81.2	12.5	25	62.5	31.2	18.7	56.2	
Neem cake (NC) @ 1%	43.7	62.5	6.2	12.5	12.5	37.5	31.2	56.2	
Cd + NC	87.5	62.5	18.7	12.5	6.25	37.5	6.2	56.2	
<i>Debaryomyces hansenii</i> (Y-17)	18.7	37.5	0	18.7	0	25	43.7	37.5	
Y-17 + NC	75	43.7	25	6.2	31.2	25	56.2	31.2	
<i>Meyerozyma guilliermondii</i> (Y-62)	37.5	50	0	6.2	12.5	25	50	25	
Y-62 + NC	56.2	25	12.5	6.2	0	6.2	25	37.5	
<i>Debaryomyces hansenii</i> (Y-34)	43.7	37.5	0	6.2	37.5	18.7	31.2	37.5	
Y-34 + NC	50	62.5	18.7	6.2	6.2	0	43.7	56.2	
LSD <sub>0.05</sub>	Treatment = 11.9 <sup>a</sup>				Pathogens = 7.5 <sup>b</sup>				Time = 5.3 <sup>c</sup>

<sup>a</sup> Mean values for treatment in column showing differences greater than LSD values are significantly different at  $p < 0.05$

<sup>b</sup> Mean values for pathogen in rows showing differences greater than LSD values are significantly different at  $p < 0.05$

<sup>c</sup> Mean values for time in rows showing differences greater than LSD values are significantly different at  $p < 0.05$



**Table 8** Effect of endophytic yeast isolates on the polyphenols and antioxidant activity of tomato plants in natural soil and soil amended with neem cake in field plot experiment

Treatments	Polyphenols (mg ml <sup>-1</sup> GAE)		Antioxidant (%)			
	45 days	90 days	45 days		90 days	
			1 min	30 min	1 min	30 min
Control	4.43	5.45	12	19.6	18	35.9
Carbendazim (Cd)	6.53	9.53	23.1	40.6	29	42
Neem cake (N) @ 1%	9.48	13.8	31.8	46.8	39.8	52.9
Cd + NC	7.44	9.69	23.6	28.7	24.3	37.2
<i>Debaryomyces hansenii</i> (Y-17)	8.34	12.1	35.2	39.1	41.2	54.6
Y-17 + NC	7.41	11.3	28	41.7	32	38.6
<i>Meyerozyma guilliermondii</i> (Y-62)	10.17	15.6	36.3	58.1	58.1	74.8
Y-62 + NC	7.21	10.31	32.6	49.5	52.2	60.8
<i>Debaryomyces hansenii</i> (Y-34)	6.63	9.56	19.5	28.5	35.2	49.3
Y-34 + NC	7.35	10.19	16.7	32.5	31.1	38
LSD <sub>0.05</sub>	0.59 <sup>a</sup>	0.53 <sup>a</sup>	3.9 <sup>a</sup>	1.9 <sup>a</sup>	1.7 <sup>a</sup>	1.7 <sup>a</sup>

<sup>a</sup> Mean values in column showing differences greater than LSD values are significantly different at  $p < 0.05$

followed by the combined treatment of *D. hansenii* (Y-17) and neem cake (Table 7).

#### Total phenolic contents

*Meyerozyma guilliermondii* alone treatment produced the highest phenolic contents within the 45 and 90 days interval, followed by the treatment of neem cake alone (Table 8).

#### Antioxidant activity

At day 45, antioxidant activity was much enhanced in the plants treated with *M. guilliermondii* alone, followed by the combined treatment of *M. guilliermondii* and neem cake. The same treatments exhibited the highest antioxidant activity in the plants after 90 days (Table 8).

#### Discussion

Among the major threats to food security are soil-borne pathogens including fungal phytopathogens and parasitic nematodes (Parveen et al. 2020). Emergence of endophytes as potential biocontrol agents for the management of plant diseases has opened a new window for research. In the present study, endophytic yeasts isolated from different healthy plants showed suppressive effect against root rotting fungi including *F. solani*, *F. oxysporum*, *M. phaseolina*, *R. solani*, and *M. javanica*, a root knot nematode in vitro. There are reports that yeast strains produce certain glycoproteins with antagonistic and fungicidal properties (Suzzi et al. 1995). They produced toxins, inhibitory to other microorganisms also known as “Killer Phenomenon.” This killer property was previously associated with only *Saccharomyces cerevisiae* but now many

other yeast genera have been reported to produce killer toxins, such as *Cryptococcus*, *Candida*, *Hanseniaspora*, *Debaryomyces*, *Hansenula*, *Pichia*, *Kluyveromyces*, *Tilletiopsis*, *Sporidiobolus* and *Zygosaccharomyces* (Santos et al. 2000). The ability of several killer yeasts to antagonize filamentous pathogenic fungi is also well recorded (Li et al. 2016). The well-known killer strain of *Saccharomyces cerevisiae* has been used as a positive biocontrol agent against *F. oxysporum* (Shalaby and Nady 2008), *F. solani* and *M. phaseolina* (Attyia and Youssry 2001). Liu et al. (2018) reported the successful antagonistic activity of *S. cerevisiae* against *Colletotrichum gloeosporioides*, a pre-harvest anthracnose agent of grapes. The killer strains may possibly produce certain metabolites like volatile compounds and hydrolytic enzymes which contribute to their antagonistic activity (Li et al. 2016). Antagonistic metabolites produced by endophytic yeasts need further investigation. In this study, several isolates on endophytic yeasts besides producing zones of inhibition also caused lysis of fungal hyphae. Yeasts produce different levels of hydrolytic enzymes such as chitinases and glucanases (Saligkarias et al. 2002), which have antagonistic effects on phytopathogenic fungi (Langner and Göhre 2016). Similarly, *Candida* and *Kluyveromyces* species have been reported to produce alkaline proteases (Agrawal and Kotasthane 2012). Alkaline proteases inhibitor is known to possess activity against phytopathogenic fungi (Vernekar et al. 1999).

In the present study, the endophytic yeasts applied as soil-drench individually or in soil amended with neem cake suppressed root rot pathogens of tomato. Akhtyamova and Sattarova (2013) reported antagonistic activity

of yeasts against different pathogens. Abd El-Kader et al. (2012) reported *S. cerevisiae* as an effective bio-agent against root rot pathogens under green-house conditions. Similarly, Fareed et al. (2019) reported suppression of root rot of sunflower by the endophytic yeasts. The antagonistic properties of yeasts have opened the door for their use as natural biocontrol agents both in pre- and post-harvest crops diseases (Habiba et al. 2019).

Phosphorus, a major micronutrient, is required by both plants and microorganisms for their growth and productivity. Unfortunately, this phosphorus is not found in free form in soil (Yang et al. 2010). Microorganisms play their important role in solubilizing inorganic phosphate to soluble form and making it available for plant uptake. Use of phosphate solubilizing microagents provides an eco-friendly and sustainable method for dissolving both fertilizer phosphorus and bond phosphorus (Khan et al. 2007). In the present study, all the tested endophytic yeast isolates showed their phosphate solubilizing ability. Many yeasts belonging to different genera have previously been reported as positive phosphate solubilizers like: *Saccharomyces*, *Rhodotorula*, *Hansenula*, *Kloeckera* and *Debaryomyces* species (Varsha et al. 2010).

Utilization of organic amendments along with biological agents has become a promising practical approach. These organic amendments not only enhance crop productivity but also exhibit antagonistic behavior to plant pathogens. Several crop management programs have successfully improved the crop yields by the application of mustard cake, cotton cake, neem cake, castor cake and sesame cake as organic amendments (Shafique et al. 2016). In the present study, the neem cake amendment to the soil, either alone or in combination with endophytic yeast significantly enhanced vegetative growth and reduced fungal infections. Neem cake is used for its fungicidal and nematicidal effects as it has been reported (Shafique et al. 2016).

In this research, the antioxidant activity in the crop plants after treatment with yeasts was examined. The plants grown in soil amended with neem cake and yeast strains showed high levels of phenolic compounds and antioxidant activity than in the untreated plants. According to reports, total phenolic compounds increased in tomatoes by organic fertilizers (Toor et al. 2006) and there is a direct relationship of phenolic content with the antioxidant activity of plants (Kumar et al. 2008). The improved status of these compounds by the activity of antagonistic yeasts resulted in amelioration of plant defense mechanisms against the root rotting fungi and resulted in the improved growth of tomato plants. SA is a phenolic compound which behaves like a plant hormone by stimulating many physiological, biochemical and molecular processes and protecting it under stress

conditions (Khan et al. 2013). Salicylic acid is a multifaceted hormone to combat diseases (Vlot et al. 2009). Application of *P. churashimaensis* as foliar spray was proved to be effective in disease suppression by stimulating plant defense mechanisms in pepper (Lee et al. 2017). Most probably, high SA activity in infected plants may increase H<sub>2</sub>O<sub>2</sub> levels which results in the activation of systemic acquired resistance (SAR) against pathogens (Tian et al. 2007).

## Conclusions

Many mycelial fungi and numerous bacteria have been reported as best biological antagonists against various plant pathogens. It is concluded from the study that endophytic yeasts can be utilized as potential biological antagonists against many root rotting fungi. The yeasts not only showed significant suppressive effects against these plant pathogens but also promoted plant growth. The improved status of antioxidant activity, phosphorus uptake, phenolic compounds and salicylic acid in treated plants ensured the potentiality of yeasts to combat successfully with the pathogens by inducing systemic acquired resistance in the host plants.

## Abbreviations

rDNA: Ribosomal deoxyribonucleic acid; IAA: Indole acetic acid; FAO: Food and Agriculture Organization; YM: Yeast malt; DNA: Deoxyribonucleic acid; BGI: Beijing Genomics Institute; BLAST: Basic Local Alignment Search Tool; NCBI: National Center for Biotechnology Information; PDA: Potato dextrose agar; O.D: Optical density; Cfu: Colony-forming unit; CDR: Crop Diseases Research Institute; PARC: Pakistan Agricultural Research Council; Min: Minutes; ml: Milliliter; v/v: Volume/volume; rpm: Revolutions per minute;  $\mu$ l: Microliter; nm: Nanometer; SA: Salicylic acid; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; LSD: Least significant difference.

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s41938-021-00493-4>.

**Additional file 1: Table S1.** Growth inhibition of root rotting fungi *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani* and *F. oxysporum* in dual culture plate assay and mortality of root knot nematode *Meloidogyne javanica* by the endophytic yeast isolates

## Authors' contributions

AJ, FU and AR isolated endophytic yeasts and performed experiments on tomatoes. HF and MI confirmed the identification of yeast using molecular biology techniques. AJ and HF analyzed and interpreted the results and wrote the manuscript. SE conceived and designed experiments, supervised research work and improved the manuscript. All authors read and approved the final manuscript.

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## Availability of data and materials

Data will be provided on request.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

No conflict of interest declared.

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