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RESEARCH ARTICLE

Biocontrol activity of yeast and AM fungi against *Fusarium oxysporum f. sp. Lycopersici*

Gomathy Muthukrishnan[1*], Sabarinathan KG[2*], Ananthi K[3], Rajakumar D[4], Pranab Dutta[5], Monika Soni[6], Maitrayee Dutta Swargiary[6], Lipa Deb[7], Madhusmita M[7], Ejilane J[8] & Ajkar A[1]

[1]Department of Soil Science & Agricultural Biochemistry, V. O. Chidambaranar Agricultural College & Research Institute, Killikulam, Thoothukudi 628 252, Tamil Nadu, India

[2]Department of Agricultural Microbiology, Agricultural College & Research Institute, Madurai 625 104, Tamil Nadu, India

[3]Department of Crop Physiology, Agricultural College & Research Institute, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

[4]Department of Agronomy, V. O. Chidambaranar Agricultural College & Research Institute, Killikulam, Thoothukudi 628 252, Tamil Nadu, India

[5]School of Crop Protection, Central Agricultural University, Umiam 793 103, Meghalaya, India

[6]Department of Bio-Sciences, Assam Don Bosco University, Tapesia Gardens, Sonapur 782 402, Assam, India

[7]Department of Plant Pathology, Central Agricultural University, Umiam 793 103, Meghalaya, India

[8]Department of Agricultural Microbiology, ICAR-Krishi Vigyan Kendra, Tamil Nadu Agricultural University, Vellore 632 104, Tamil Nadu, India

*Correspondence email - gomathy.m@tnau.ac.in, sabarinathan@tnau.ac.in

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Abstract

Fusarium oxysporum f. sp. lycopersici, the causal agent of *Fusarium* wilt in tomato, posed a significant threat to tomato cultivation. This study investigated the biocontrol potential of yeast isolates and Arbuscular Mycorrhizal Fungi (AMF) against this pathogen. Soil and phyllosphere samples were collected from tomato fields in Tamil Nadu, India, leading to the isolation of 120 yeast strains using serial dilution and leaf imprinting techniques. Morphological characterization grouped the isolates with 35 unique colonies were selected for further analysis. Molecular characterization identified three key yeast isolates: *Rhodosporidium toruloides* (Y2), *Moesziomyces antarcticus* (Y14) and *Pichia kudriavzevii* (Y16). Under pot culture conditions, the combined application of AMF and yeast isolates significantly reduced *Fusarium* wilt incidence. Treatment T5 (AMF liquid-based inoculum + soil yeast Y14) exhibited the highest root colonization (86.66 %), maximum spore load (10397 spores) and minimal disease incidence (11 %), with an 89 % disease reduction compared to the control. This treatment also enhanced tomato plant growth metrics, including height (121.00 cm), root length (61.10 cm) and antioxidant enzyme activity (peroxidase: 0.392 min/g, polyphenol oxidase: 0.791 min/g). Yield parameters were also improved, with maximum fruit weight (19.45 g) and fruit count (6.24) observed in T5-treated plants.

Keywords: AM fungi; *Fusarium oxysporum* f. sp. *Lycopersici*; *Moesziomyces antarcticus; Rhizophagus irregularis;* yeast

Introduction

Tomato (*Solanum lycopersicum* L.) was one of the most widely cultivated and economically important vegetable crops globally. However, its productivity was significantly constrained by soil borne pathogens, notably *Fusarium oxysporum f. sp. lycopersici,* which caused *Fusarium* wilt and led to substantial yield losses. The pathogen invades the plant's vascular system, causing wilting, yellowing and often plant death (1). Traditional control strategies involving chemical fungicides posed ecological risks and could lead to the development of resistant pathogen strains. Hence, there was growing interest in sustainable, eco-friendly alternatives such as microbial inoculants.

Arbuscular mycorrhizal fungi (AMF) played a pivotal role in enhancing plant health by improving nutrient uptake, modifying root architecture and inducing systemic resistance. *Rhizophagus irregularis*, a well-studied AMF species, was shown to modulate defense gene expression and activate immune signaling pathways in solanaceous crops. AMF colonization primes the plant immune system, a phenomenon referred to as mycorrhiza-induced resistance (MIR), which strengthens plant defense against both biotic and abiotic stresses.

On the other hand, plant growth-promoting yeasts (PGPYs) such as *Moesziomyces bullatus* have recently gained attention for their ability to suppress phytopathogens via competitive exclusion, siderophore production and secretion of antifungal enzymes (2). Beyond growth promotion, these yeasts stimulated the plant's innate immune responses, a phenomenon termed immune priming, similar to ISR (Induced Systemic Resistance) (3).

Meanwhile, AM fungi formed symbiosis with plant roots that improved the uptake of all nutrients and conferred resistance against pathogens (4). Their combined application showed promise in suppressing *Fusarium* wilt, offering an integrated approach to sustainable agriculture.

Despite growing evidence on the individual benefits of AMF and yeasts, their combined effects remained underexplored, particularly in vegetable cropping systems challenged by soil pathogens. Most studies to date examined either AMF or PGPYs. Furthermore, the mechanistic synergy between AMF mediated nutrient acquisition and yeast-mediated pathogen suppression was not adequately documented, especially in the context of integrated disease management.

There was a paucity of data on the integrated use of AMF and PGPYs, such as *Rhizophagus* and *Moesziomyces*, in managing soil-borne diseases in tomato under greenhouse conditions. No prior studies thoroughly investigated the dynamic interactions between these bioinoculants across phenological stages of the crop.

Materials and Methods

Isolation and purification of yeast

Yeast isolates were isolated using Yeast Extract Peptone Dextrose (YEPD) agar (5). Phyllosphere leaves were washed and used for isolation of yeast. Both leaf and soil samples were plated on YEPD media by following leaf imprinting and serial dilution technique respectively. Petri plates were incubated for 48 hr at room temperature. Based on the morphological characters, yeast colonies were selected and purified using YEPD medium.

Morphological characterization

The morphological characters such as colony and cell morphology, gram staining were studied as described (6).

Colony and cell morphology

The morphological characteristics of yeast colonies, such as size, color, surface, form, margin and elevation were observed by culturing the isolates on YEPD agar plates. After Gram staining, morphological characteristics such as cell shape, cell arrangement and budding pattern were identified under a microscope.

Gram staining

Gram's staining was performed on all yeast isolates collected from rhizosphere and phyllosphere of tomato (7). Yeast isolates were thinly smeared on a glass slide, air dried and then heat fixed. After heat fixing, primary strain crystal violet was applied and allowed to dry for 1 min before being rinsed with tap water. The mordant Lugol's iodine was added for 30 sec before being washed off with tap water. Following the application of the mordant, the decolorizer (alcohol) was added and washed off with tap water for 30 sec. The counter stain safranin was the applied for 30 sec and rinsed using tap water. After air drying, the slides were examined under a microscope.

ITS sequencing of yeast isolates

Polymerase Chain Reaction (PCR) was carried out with total volume of 50 µL using Emerald Amp® GT PCR master mix by using genomic fungal DNA as the template. The intermediate 5.8S ribosomal gene, along with ITS 1 and ITS 4 regions, was amplified using ITS 1 and ITS 4 primers. The PCR conditions, included an initial denaturation at 94 °C for 5 min, followed by 30 cycles denaturation process at 94 °C for 30 sec, annealing at 59 °C for 30 sec and extension at 70 °C for 2 min. A final extension performed at 72 °C for 7 min. The reaction took place in an Eppendorf tube and was carried out using Mastercycle Gradient PCR machine. The PCR products were resolved by electrophoresis in 1 % agarose gel. The PCR products were purified by using FavorPrep GEL/PCR purification kit and sequencing was done.

The primers used for amplification of ITS region were:

ITS1-5'-CTTGGTCATTTAGAGGAAGTAA-3' (forward primer)

ITS4- 5'-TCCTCCGCTTATTGATATGC -3' (reverse primer)

Phylogenetic tree construction

The isolates were identified through BLAST analysis and sequencing using GenBank. A phylogenetic tree was constructed using MEGA 6 software.

Evaluation of bio control potential of AM fungi and yeast against *Fusarium oxysporum f. sp. lycopersici* through pot experiment

The experiment was conducted under pot culture in green house conditions by adapting Completely Randomized Block Design (CRBD) at VOC Agricultural College and Research Institute, Killikulam. The sterilized potting soil was prepared with combination of red soil: sand: FYM at 1:1:1 ratio. The pure culture of pathogen was mass cultured in sand-maize medium and the inoculum was incorporated at a rate of 5 g per pot. Five tomato seeds (PKM 1) were sown in the pots (30 cm in diameter) with 5 replications and both inoculated and uninoculated control plant were maintained. The yeast obtained from the rhizosphere soil of tomato (soil yeast Y14) *M. antarcticus* Y14) (39 x 10^{10}) was mass cultured and used for seed treatment (Table 1). AMF inoculum was available in the Microbiology Unit at VOC AC & RI, Killikulam. *R. irregularis* (AMF) vermiculite based inoculum was applied at the rate of 16 g per plant at the time of sowing (containing 1420 spores) and *R. irregularis* liquid inoculum was applied at the rate of 8000 spores/mL at the time of sowing. During vegetative, flowering and fruit setting stages of tomato Percent Disease Incidence (PDI) was calculated using the formula

$$PDI = \frac{\text{Number of wilted plants}}{\text{Total Number of plants observed}} \times 100$$

Table 1. Treatment details

T. No	Treatments
T1	*F. oxysporum* + AMF vermiculite based inoculum (*R. irregularis*)
T2	*F. oxysporum* + AMF liquid based inoculum (*R. irregularis*)
T3	*F. oxysporum* + soil yeast Y14 (*M. antarcticus)*
T4	*F. oxysporum* + AMF vermiculite based inoculum + soil yeast Y14
T5	*F. oxysporum* + AMF liquid based inoculum + soil yeast Y14
T6	Chemical treatment with Carbendazim 50 % WP @ 2 g kg^{-1}
T7	Pathogen inoculated control
T8	Uninoculated control

Estimation of AM fungal (*R. irregularis)* colonization in roots (8)

Tomato plants (both inoculated and uninoculated) were uprooted and washed thoroughly with water. The roots were cut into 1 - 2 cm segments and treated with FAA solution. Bleaching of roots was carried out in 10 % KOH, which was kept in autoclave for 10 min, followed by repeated washing with water. The root bits were then immersed in 30 % hydrogen peroxide solution for 15 min. Excess alkali was removed by washing and

the roots were acidified with 3 % HCl for 5 min and then decanted. Staining was done with 0.05 % Tryphan Blue stain for 10 min. Ten root segments were arranged on a slide and observed through stereo zoom microscope for the presence of vesicles, arbuscules and external mycelium.

$$\text{AMF colonization percentage} = \frac{\text{Total Number of root bits with AM infection}}{\text{Total Number of root bits examined}} \times 100$$

Enumeration of AM fungal (*R. irregularis)* spores in soil

Wet sieving and decanting technique

This was one of the most popular techniques for isolation of AM Fungal spores from soil compared to other methods. This wet sieving and decanting technique was used for sieving the different coarse particles of soil and retaining the different AM fungal spores (250 µm to 38 µm) (9). One hundred gram of soil were mixed with 100 mL of distilled water in the 500 mL beaker. The soil mixture was thoroughly agitated to free the AM fungal spores from soil particles. After getting clear suspension supernatant was decanted through different standard sieves (280 µm - 80 µm). By using a stereo zoom microscope the number of spores was observed.

Biometric observations of tomato plant

The biometric observation were recorded during vegetative, flowering and fruit setting stages of tomato

Shoot length

Shoot length was measured in each plant and the recorded mean value was expressed in cm (10).

Root length

Root length was measured in each plant root (10). The recorded mean value was expressed in cm.

Analysis of defense related enzyme activity

Enzyme extraction

Root sample of tomato (1 g) were treated with 2 mL of 0.1 M sodium phosphate buffer with the pH of 7.0 at 4 °C and centrifuged at 15000 rpm for 20 min. Enzyme were extracted using the same buffer used for Peroxidase (PO) and Poly Phenol Oxidase (PPO) analysis.

Peroxidase (PO)

PO enzyme analyzed was done through the protocol as described in earlier report (11). Tomato roots (1 g) were grounded with 0.1 M phosphate buffer (pH 7.0) and centrifuged at 15000 rpm for 15 min. A 0.1 mL aliquot of enzyme extract was added to 1.5 mL of pyrogallol and 0.5 mL of 1 % hydrogen peroxide. Absorbance was recorded at 420 nm at 30 sec intervals for 3 min at room temperature (min^{-1} mg^{-1} of fresh tissue).

Poly Phenol Oxidase (PPO)

PPO estimation was estimated using the method as described in an earlier study (12). One gram of tomato root sample was macerated by adding 2 mL of buffer (Sodium phosphate - pH 6.5) at 4 °C. The homogenate was centrifugated at 12000 rpm for 15 min at 4 °C. The resulting supernatant was used as the enzyme source for PPO activity analysis. The reaction mixture was prepared by adding 200 µL of plant extract and 1.5 mL of 0.1 M sodium phosphate buffer at pH 6.5. To this 0.01 M catechol was added and the enzyme activity was measured calorimetrically. Absorbance changes were recorded at 470 nm and expressed as min^{-1} mg^{-1} of fresh tissue.

Total phenols

Root samples of tomato (1 g) were grounded with 80 % methanol (10 mL) and placed on shaker at 10000 rpm for 15 min. The methanol extract (1 ml) was mixed with 5 mL distilled water and 250 µL of 1N of Folin-Ciocalteu (FC) reagent, then incubated at 25 °C. The enzyme mixture was retained in a water bath 25 °C for 1 hr. Absorption was measured at 725 nm using a spectrophotometer. Total phenols were calculated based on standard curve obtained from FC reagent and expressed as min^{-1} mg^{-1} of fresh tissue (13).

Yield characters of tomato

Number of branches

All the branches originating from the main stem on each tomato plant were counted. The branch count was recorded for each plant and expressed as the mean (14).

Number of fruits

Each tomato plant was inspected and the total number of mature fruits was counted. The fruit count was recorded for each plant and expressed as the mean (14).

Weight of tomato

Each tomato plant was observed and the mature fruit was harvested. The fruit weight was recorded and expressed as the mean in grams (14).

Statistical analysis

The data were subjected to analysis of variance (ANOVA) as per the methods described in AGRES.

Results

Collection of soil and phyllosphere samples and isolation of yeast from tomato

Soil and leaf samples were collected from AC & RI, Killikulam, Thoothukudi district and Agricultural College and Research Institute, Madurai. A total of 120 yeast isolates were obtained from both rhizosphere soil and phyllosphere of tomato through serial dilution and leaf imprinting technique in various media *viz.,* Yeast Extract Peptone Dextrose agar (YEPD).

Morphological characterization of yeast isolates

Yeast colonies were examined for the morphological characteristic. The colonies exhibited dull white to pink coloration. Yeast colonies were mostly circular in shape, flat and raised at the centre. Most of the yeast isolates produced smooth colonies. Cell shape, cell arrangement, budding were also studied. The observation revealed that the yeast cells were spherical in shape, predominantly single and budding was observed under microscope. Based on the morphological characteristic, similar colonies were grouped and colonies showing distinct morphological featured were selected separately. Thus, a total of 35 yeast isolates were selected for further study. All the isolated yeast colonies were tested for Gram's reaction.

Molecular characterization of yeast isolates

Based on the morphological characters, 3 yeast isolates *viz.,* Y2, Y14, Y16 were selected for molecular characterization and also for further studies.

The NCBI database was used to compare the sequences and BLAST was performed to identify closely related species. The results of molecular sequences revealed that Y2 yeast isolate was identified as *Rhodosporidium toruloides,* Y14 as Moesziomyces *antarcticus* and Y16 as *Pichia kudriavzevii.* (Plate 1, 2). All 3 yeast isolates were obtained from rhizosphere soil sample of tomato.

Evaluation of bio control potential of AM fungi (AMF) and plant growth promoting yeast against *F. oxysporum f. sp. lycopersici* under pot culture experiment

Soil properties

The greenhouse grown tomato crop was cultivated in red soil (sandy loam, pH 6.4), with temperatures ranging from 25 - 32 °C during the day with the relative humidity at 70 - 80 %. These conditions are known to support optimal AMF colonization, which prefer slightly acidic, well aerated soils with moderate moisture content.

Estimation of root colonization percentage and spore load (per 100 g of wet soil) of AM Fungi

The root colonization percentage of AMF was examined at vegetative, flowering and fruit setting stages of tomato. Among the various treatments, the treatment T5 (*F. oxysporum* + AMF liquid-based inoculum + Soil yeast Y14) recorded the highest root colonization percentage of 86.66 % with the spore load of 10397 spores/ 100 g of soil. T4 (*F. oxysporum* + AMF vermiculite-based inoculum + soil yeast Y14) showed the percentage of 80.00 % with the spore load of 8446 nos which was followed by treatment T2 (*F. oxysporum* + AMF liquid-based inoculum (*R. irregularis*) (76.66 %) (8433 nos) as shown in Table 2 and Fig 1.

Biocontrol potential

All the treatments significantly reduced the disease incidence except pathogen inoculated and uninoculated control. Among the 8 treatments, in the fruit setting stage, treatment T5 (*Fusarium oxysporum* + AMF liquid-based inoculum + Soil yeast Y14) recorded minimum disease incidence of 11 % and percent disease over control of 89 %. It was followed by Treatment T4 (*Fusarium oxysporum* + AMF vermiculite-based inoculum + soil yeast Y14) which showed 33 % and a percent disease reduction over control of 67 %. Treatment T3 (*Fusarium oxysporum* + AMF liquid-based inoculated with *Rhizophagus irregularis*) recorded a disease incidence 88.66 % and percent disease reduction over control of 11.34 %, which was statistically on par with the treatment *F. oxysporum* + AMF vermiculite-based inoculated with *R. irregularis*). A 100 % disease incidence was observed in pathogen inoculated control (Fig. 2).

Tomato plant biometrics

The results of tomato plant biometrics revealed that the Treatment T5 (*F. oxysporum* + AMF liquid-based inoculum + Soil yeast Y14) at the fruit- setting showed significant increase in plant height (121.00 cm) and root growth (61.10 cm) followed by Treatment T4 (*F. oxysporum* + AMF vermiculite-based inoculum + soil yeast Y14) (Table 3; Fig 3, 4).

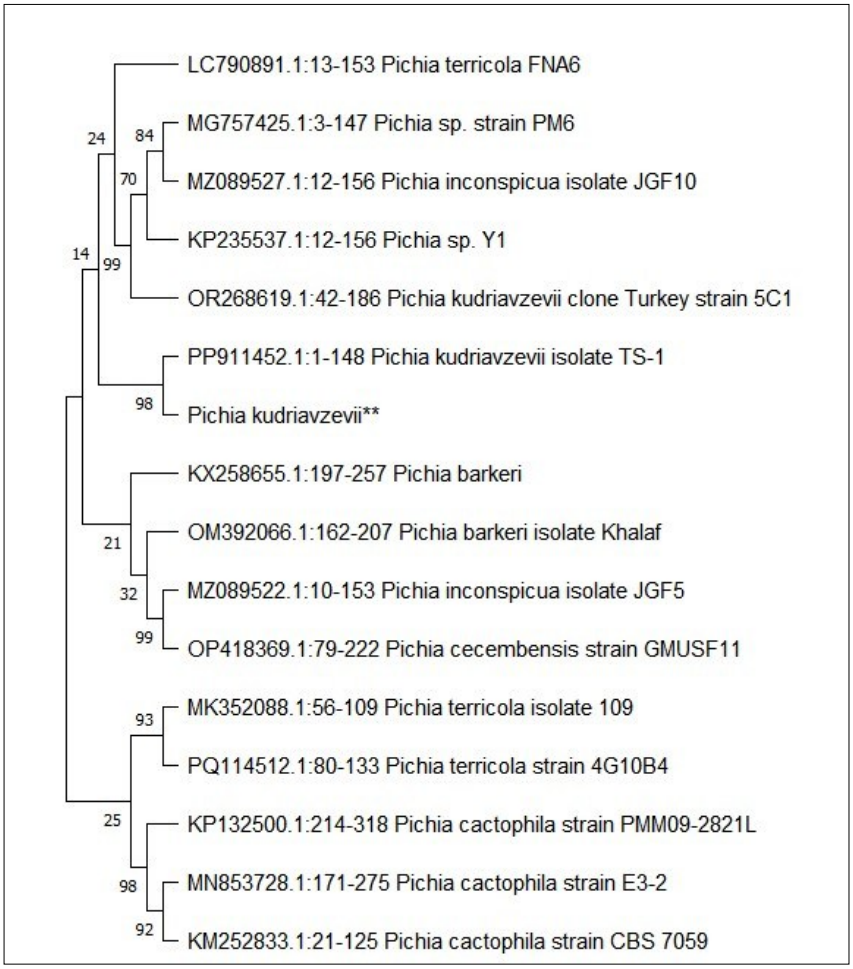

Plate 1. ITS based phylogenetic tree of the yeast isolate Y16 *Pichia kudriavzevii* from tomato.

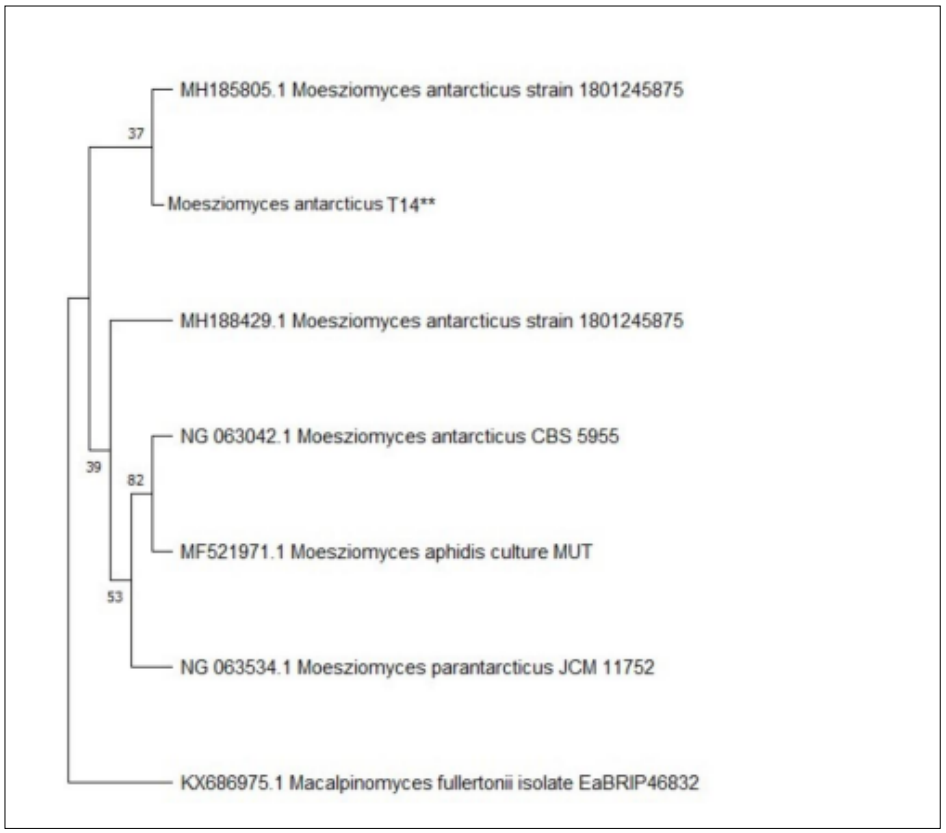

Plate 2. ITS based phylogenetic tree of the yeast isolate *Moesziomyces antarticus* from tomato.

Table 2. Assessment of root colonization percentage and enumeration of AM fungal spores in soil under challenge inoculation with *F. oxysporum f. sp. Lycopersici*

Treatments	AM fungal root colonization (%)			AM fungal spore load in 100 g of wet soil (nos)		
	Vegetative stage	Flowering stage	Fruit setting stage	Vegetative stage	Flowering stage	Fruit setting stage
T1	36.66^{c}	53.33^{ab}	70.66^{c}	1223^{c}	3323^{bc}	6426^{b}
T2	43.33^{bc}	60.00^{a}	76.66^{bc}	3290^{a}	7355^{ab}	8433^{a}
T3	10.33^{d}	23.33^{b}	23.33^{d}	700^{d}	780^{cd}	822^{c}
T4	46.66^{b}	60.00^{a}	80.00^{ab}	2313^{b}	6324^{ab}	8446^{a}
T5	53.33^{a}	66.66^{a}	86.66^{a}	3587^{a}	8428^{a}	10397^{a}
T6	10.00^{d}	10.00^{b}	23.33^{d}	30^{e}	58^{d}	75^{d}
T7	10.00^{d}	10.00^{b}	10.00^{e}	10^{e}	21^{d}	28^{d}
T8	10.00^{d}	10.00^{b}	10.00^{e}	100^{c}	155^{d}	310^{d}
SEd	1.20	35.37	2.11	106.66	2.80	60.10
CD (0.05)	2.40	77.08	4.60	212.67	5.60	120.20

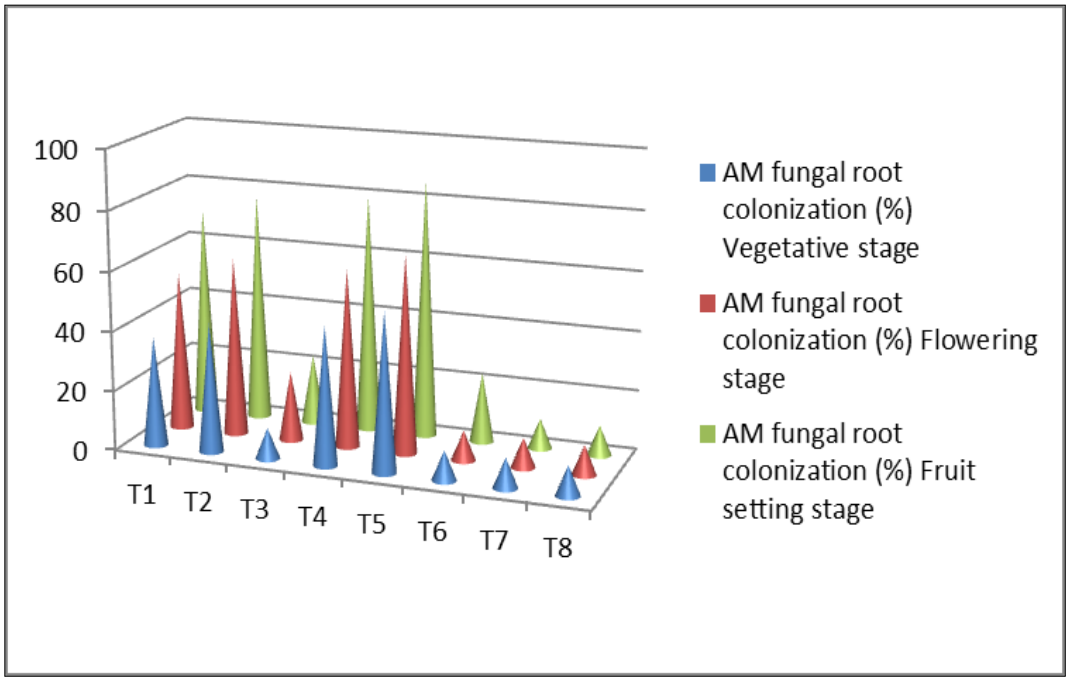

Fig. 1. Assessment of root colonization percentage under challenge inoculation with *Fusarium oxysporum f. sp. Lycopersici.*

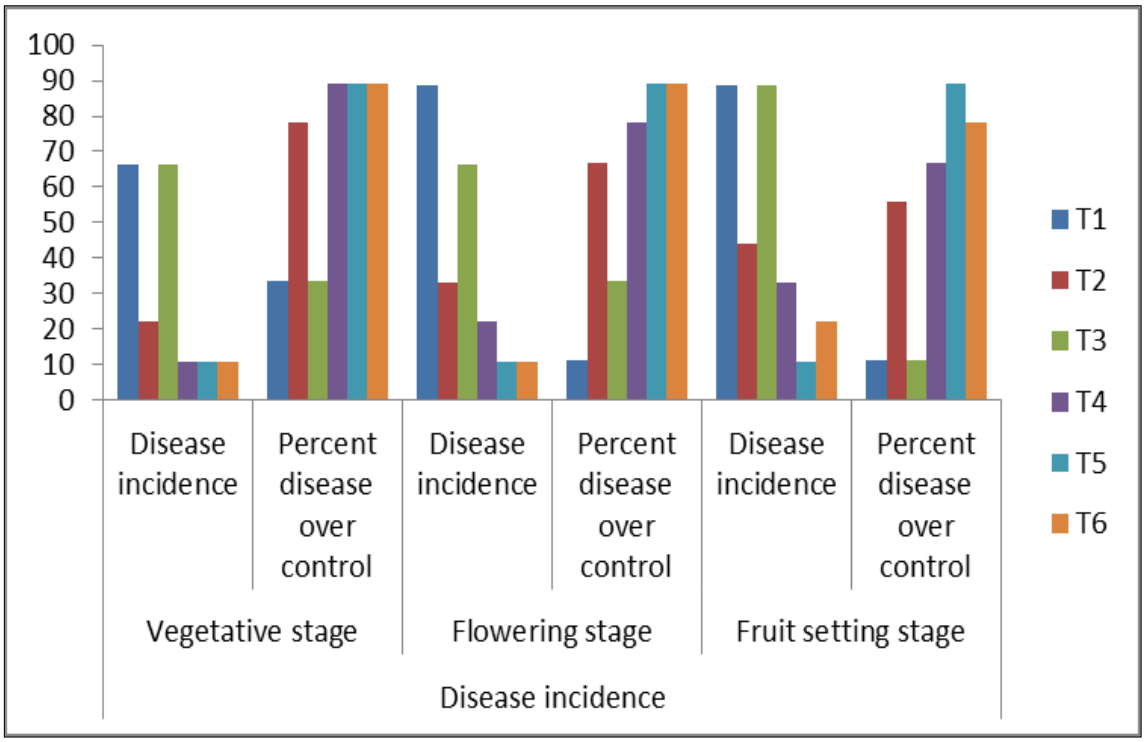

Fig. 2. Effect of AM fungi and plant growth promoting yeast on the disease incidence of tomato under challenge inoculation with *F. oxysporum f. sp. Lycopersici.*

Table 3. Effect of AM fungi and yeast on biometric observations of tomato under challenge inoculation with *F. oxysporum f. sp. lycopersici*

Treatments	Vegetative stage (cm)		Flowering stage (cm)		Fruit setting stage (cm)	
	Shoot length	Root length	Shoot length	Root length	Shoot length	Root length
T1	30.94^{bc}	20.90^{bc}	54.19^{bc}	27.60^{bc}	109.73^{bc}	49.80^{bc}
T2	31.76^{b}	21.19^{b}	55.41^{b}	28.72^{b}	115.62^{b}	50.01^{bc}
T3	29.55^{c}	19.89^{c}	53.25^{c}	27.54^{bc}	113.28^{b}	48.62^{c}
T4	31.58^{b}	21.40^{b}	59.94^{a}	28.00^{b}	116.76^{b}	52.80^{b}
T5	33.61^{a}	24.54^{a}	59.00^{a}	31.21^{a}	121.00^{a}	61.10^{a}
T6	29.60^{c}	21.30^{b}	55.88^{b}	27.38^{bc}	105.62^{c}	47.30^{c}
T7	15.28^{e}	9.22^{e}	32.11^{e}	13.52^{e}	75.20^{e}	28.25^{e}
T8	25.74^{d}	12.48^{d}	45.91^{d}	21.68^{d}	97.52^{d}	40.88^{d}
SEd	0.50	0.45	1.20	0.47	2.80	0.90
CD (0.05)	1.00	0.90	2.40	1.44	5.60	1.80

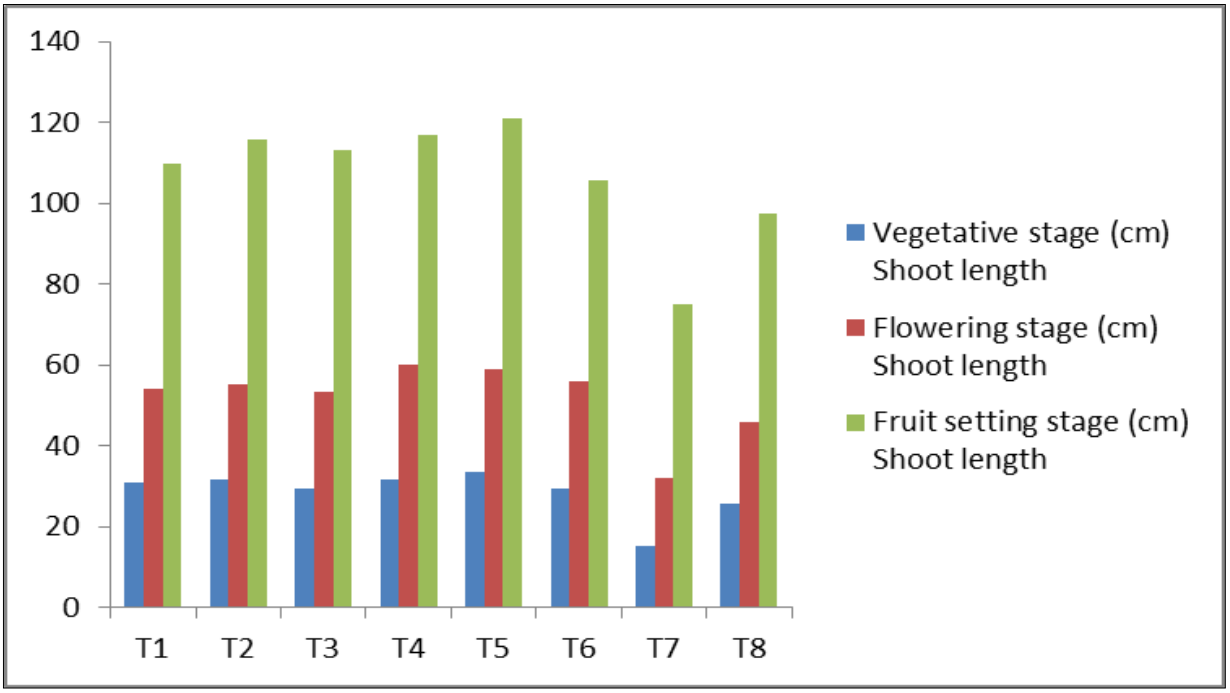

Fig. 3. Effect of AM fungi and yeast on shoot length (cm) of tomato under challenge inoculation with *F. oxysporum f. sp. lycopersici.*

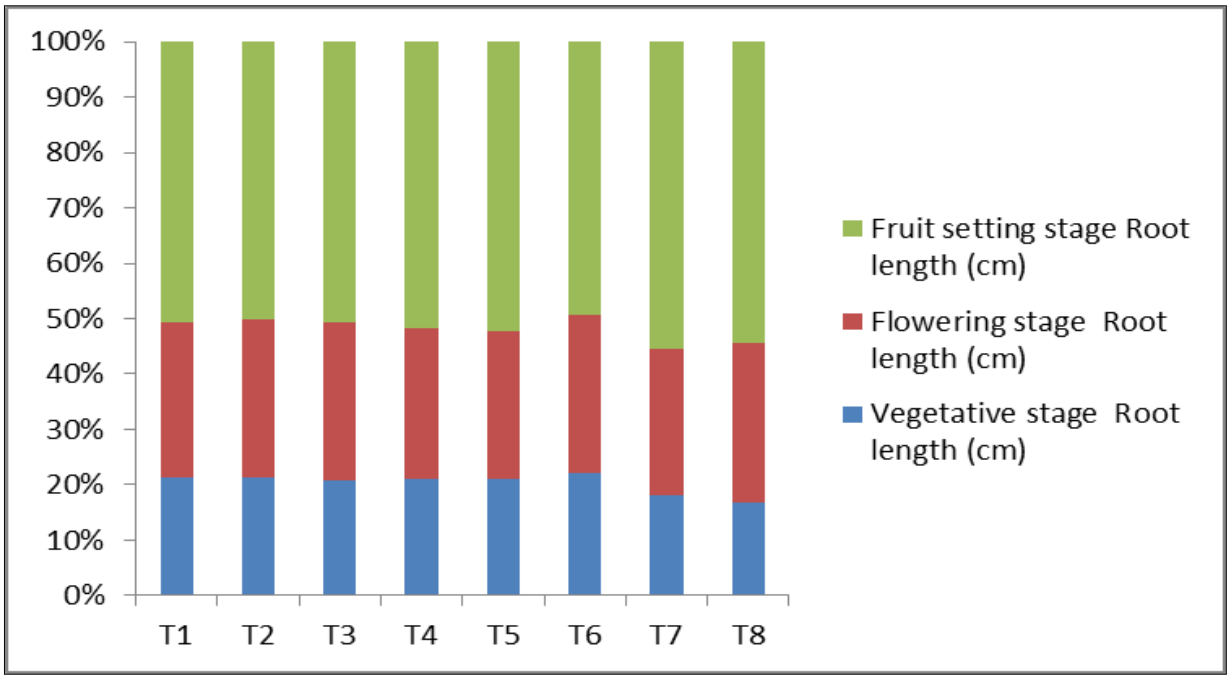

Fig. 4. Effect of AM fungi and yeast on root length (cm) of tomato under challenge inoculation with *F. oxysporum f. sp. Lycopersici.*

Activity of antioxidant enzymes and Total Phenol

The experimental results revealed that Peroxidase, Polyphenol Oxidase and Total Phenol accumulation showed an increasing trend upto fruit setting stage. The activity of antioxidant enzymes and Total Phenol were significantly different between the treatments. Among the treatments, at the time of fruit -setting, Treatment T5 recorded highest induction: Peroxidase (0.392 µmol product/ min/ g fresh weight), Polyphenol Oxidase (0.791 µmol product/ min/ g fresh weight) and Total Phenols (0.381 µmol product/ min/ g fresh weight). This was followed by the Treatment T4 which recorded Peroxidase activity of 0.358 µmol product/ min/ g fresh weight, Polyphenol Oxidase activity of 0.624 µmol product/ min/ g fresh weight and Total Phenols of 0.353 µmol product/ min/ g fresh weight (Table 4).

Yield parameters

Number of branches

Maximum number of branches was recorded in T5, followed by T6 in the fruit setting stage of tomato 10.28 and 10.15 respectively and minimum branches were recorded in control 7.23.

Number of fruits

Treatment T5 (6.24 fruits) recorded maximum number of fruits followed by T6 (6.11 fruits) and were found to be on par with each other. The minimum number of fruits was observed in control (2.34 fruits).

Weight of fruits

Maximum fruit weight was recorded in T5 that received *F. oxysporum* + AMF liquid-based inoculum + Soil yeast Y2 (19.45 g) followed by T6 inoculated with *F. oxysporum* + AMF vermiculite-based inoculum + soil yeast Y14 (19.02 g). The minimum fruit weight was observed in control (10.23 g).

Discussion

Isolation of yeast

In this research work, a total of hundred and twenty yeast colonies were isolated from soil and phyllosphere of tomato crops. Yeast colonies were obtained from field soil and phyllosphere of sugarcane in earlier reports provided foundational insight into soil yeast diversity whereas newer studies highlight functional strains

Table 4. Antioxidants enzyme and total phenol activity of tomato as induced by AM fungi and plant growth promoting yeast under challenge inoculation with *F. oxysporum f. sp. lycopersici*

Treatments	Peroxidase activity (µmol product/min/g fresh weight)			Polyphenol Oxidase activity (µmol product/min/g fresh weight)			Total Phenol (µmol product/min/g fresh weight)		
	Vegetative stage	Flowering stage	Fruit setting stage	Vegetative stage	Flowering stage	Fruit setting stage	Vegetative stage	Flowering stage	Fruit setting stage
T1	0.211^{c}	0.222^{c}	0.322^{c}	0.430^{c}	0.453^{c}	0.510^{c}	0.220^{c}	0.262^{b}	0.280^{b}
T2	0.288^{b}	0.344^{b}	0.328^{c}	0.483^{b}	0.497^{c}	0.620^{b}	0.220^{c}	0.262^{b}	0.290^{b}
T3	0.170^{d}	0.132^{d}	0.102^{e}	0.152^{e}	0.130^{e}	0.100^{e}	0.160^{d}	0.142^{d}	0.126^{d}
T4	0.280^{b}	0.311^{b}	0.358^{b}	0.463^{b}	0.535^{b}	0.624^{b}	0.261^{a}	0.311^{a}	0.353^{a}
T5	0.315^{a}	0.374^{a}	0.392^{a}	0.578^{a}	0.710^{a}	0.791^{a}	0.262^{a}	0.258^{b}	0.381^{a}
T6	0.099^{e}	0.085^{e}	0.061^{f}	0.085^{f}	0.074^{f}	0.061^{f}	0.079^{e}	0.052^{e}	0.057^{e}
T7	0.033^{f}	0.032^{f}	0.046^{f}	0.056^{f}	0.052^{f}	0.036^{f}	0.120^{d}	0.088^{d}	0.062^{e}
T8	0.083^{e}	0.061^{e}	0.050^{f}	0.072^{f}	0.063^{f}	0.052^{f}	0.070^{e}	0.060^{e}	0.043^{e}
SEd	0.004	0.005	0.004	0.007	0.006	0.009	0.001	0.004	0.006
CD (0.05)	0.009	0.010	0.009	0.015	0.014	0.019	0.003	0.009	0.014

such as *M. bullatus*, which exhibited strong antagonism against phytophathogens and support plant health under abiotic stress (15–17). Different kinds of yeast isolates were obtained from masau fruits *viz*., *Saccharomyces cerevisiae, Pichiapastoris* and *Aureobasidium pullulans* (18).

Morphological characterization of yeast isolates

Upon morphological identification, the isolated yeast colonies exhibited variations in culture morphology. Colonies were white in colour or pink due to pigmentation and most of the yeast cells were circular, flat and raised at centre in shape. The surface area of the yeast isolates exhibited rough and smooth characters. The margin of the yeast isolates was sharply defined. Uder microscopic observation yeast cells appear spherical, elongated in shape and budding of was also observed. Similar types of colonies were reported in earlier studies, where the isolates were spherical and moist in appearance (19). The isolated yeast colonies were oval to round in shape, dull white to white in colour and some exhibited pigmented (20). Yeast colonies obtained from the phyllosphere of rice were found to produce pink pigments and round in shape (21).

The source of isolation of yeast contributed to variation in cultural characteristics. Out of the 7 yeast isolates, 5 were milky white in color and slimy in consistency, while 2 were watery appearance. Some isolates had round or oval shape with blue colored colonies (22). The occurrence of different forms of colonies exhibits dimorphic growth, development of hyphae and other taxonomic traits were documented (23). Some genera of yeasts, such as *Phaffia, Rhodosporidium* and *Sporidiobolus* produced pigments like red, orange and yellow (24). Phenotypic and molecular characterization of the yeast isolates were conducted and the phenotypic traits were identified in *Candida krusei* (25).

Based on similar morphological characters, colonies with similar were grouped together and those with distinct morphology were selected separately. Thus, a total of 35 colonies were chosen for further study. Yeast colonies isolated form rhizosphere soil of tomato were designed as Y1 to Y20, while isolates from phyllosphere of tomato were designed as Y21 to Y35.

Molecular identification of yeast isolates

Based on the above characters such as morphological characterization, 3 yeast isolates were selected for molecular characterisation to determine their genus and species. Molecular characterization results revealed that Y2, Y14 and Y16 yeast isolates as *Rhodosporidium toruloides, Moesziomyces antarcticus, Pichia kudriavzevii* respectively and were chosen for further studies .

Pot culture evaluation of bio control potential of AM Fungi and plant growth promoting yeast against *F. oxysporum f. sp. lycopersici*

Assessment of root colonization percentage and enumeration of AM fungal spores in soil under challenge inoculation with *F. oxysporum f. sp. lycopersici*

The maximum root colonization percentage (86.66 %) and spore load (10397 nos) was observed in T5, followed by T4 and T2. Similar results were reported, where the combined application of G*lomus mosseae* + *Acaulospora laevis* + *Trichoderma viride* showed a higher colonization percentage (87 yeast %) and a greater number of spores in soil (92 spores/100 g soil) (26). The combined inoculation of *Pseudomonas fluorescens* + *Trichoderma viride* + mycorrhizae recorded higher root colonization percentage and showed the maximum numbers of spores in soil (27).

In this present study, the combined application of AMF liquid-based inoculum and soil yeast resulted in a higher root colonization percentage and spore load that might have been due to the synergistic effect of the AMF inoculum and soil yeast. Higher mycorrhizal root dependency in host plant stimulated various defense related enzymes such as peroxidase and polyphenol oxidase against pathogen attack (28).

Biometrics of tomato under challenge inoculation with *F. oxysporum f. sp. lycopersici*

In the present study, maximum plant growth parameters *viz.,* shoot and root length were observed in the treatment T5, which received *F. oxysporum* + AMF liquid-based inoculum + Soil yeast Y14. Similarly, the combined application of AM fungi + *T. viride* + *P. fluorescens* significantly improved the growth parameters and phosphorus content (29).

Disease incidence

In the study, among the 8 different treatments, treatment T_5 (*Fusarium oxysporum* + AMF liquid-based inoculum + Soil yeast Y14) recorded the minimum percent disease incidence (11 %) at the time of fruit setting stage. Several reports have shown that root colonization of AM fungi conferred resistance to several root pathogens (30).

Similar studies reported that co-inoculation of mycorrhizal fungi and *T. harzianum* against pigeon pea wilt disease resulted in a lower disease severity percentage compared to control plant (29). The reduced stem rot incidence might have been due to *T. viride* and *P. fluorescens,* which played a role in mycoparasitism against pathogen attack (31).

Antioxidants enzyme and total phenol activity of tomato as induced by AM fungi and plant growth promoting yeast under challenge inoculation with *F. oxysporum f. sp. lycopersici*

Peroxidase activity in plants has been correlated with many disease resistance mechanisms in host pathogen interaction and the Polyphenol Oxidase enzyme catalyzes the phenols to quinones (32). It also played a major role in disease resistance in host plant (33). The results of the present study indicated that there was increased activity of different antioxidant enzymes (PO, PPO) and Total Phenol in the treatment applied with AMF liquid-based inoculum and soil yeast Y14 along with the pathogens (T5).

AM fungal symbiosis constantly renders the plant with increased resistance to pathogen infection. This might have been the reason for the increased production of antioxidant enzymes in the mycorrhizal inoculated plants, which helped overcome the stress created by the *F. oxysporum* in tomato plant.

Inoculation of *G. fasciculatum* against *S. rolfsii* under pot culture condition resulted in higher mycorrhizal root dependency, which induced the different antioxidant enzymes such as Peroxidase, Polyphenol Oxidase and Total Phenol activity. The greater accumulation of these compounds in host plant led to enhanced resistance against *S. rolfsii* in groundnut (34).

These results were consistent with earlier investigations

in which the single and combined inoculation of *G. fasciculatum* + *Macrophomina phaseolina* causing charcoal root rot in groundnut under greenhouse condition showed that reduction in disease severity was due to more root colonization of AMF in groundnut and enhanced biochemical activities such as peroxidase, polyphenol oxidase and total phenols (35). Onion plants treated with *T. viride* + *P. fluorescens* + AMF also exhibited higher level antioxidant enzymes and total phenol activity.

Effect of AM fungi and plant growth promoting yeast on yield parameters of tomato under challenge inoculation with *F. oxysporum f. sp. lycopersici*

In the present study, maximum yield parameters were obtained in treatment T5 which received *F. oxysporum* + AMF liquid-based inoculum + Soil yeast Y14, followed by T4 *F. oxysporum* + AMF vermiculite-based inoculum + Soil yeast Y14. The combined inoculation of AM fungi and soil yeast performed better than individual inoculations. The outcome demonstrated a synergistic effect of between 2 organisms, which established successfully in the rhizosphere, where they effectively controlled *F. oxysporum* and aided the plant in achieving maximum yield. Additionally, the combined treatment stimulated higher production of antioxidant enzymes that helped the plant to cope with the environmental stress, particularly the biotic stress rendered by *F. oxysporum*.

Moreover, it was noted that *R. irregularis*, a well-characterized AMF, has been increasingly associated with enhanced nutrient uptake, improved root morphology and strong disease resistance in solanaceous crops like tomato and chilli. These interactions have been found to be not only species-specific but also influenced by plant phenology and soil microbiota composition, as shown in more recent integrated omics-based assessments (36).

Thus, incorporating bioinoculants such as yeast and AM fungi offers a promising strategy for sustainable vegetable cultivation, punder pathogen pressure from *Fusarium oxysporum f. sp. lycopersici*.

Conclusion

The study demonstrated that the combined application of AM fungi and plant growth-promoting yeast isolates significantly enhanced tomato plant growth, suppressed *F. oxysporum* f. sp. *lycopersici* and improve overall crop productivity. Among all treatments, the combined application of AMF liquid-based inoculum and yeast isolate Y14 (*M. antarcticus*) was the most effective. This treatment resulted in the highest root colonization percentage (86.66 %) and spore load (10397 spores per 100 g soil), while also achieving the lowest disease incidence (11 %) representing an 89 % reduction compared to the control. It also produced improved plant biometrics including the highest plant height (121.00 cm), root growth (61.10 cm) and enhanced yield parameters, such as the number of branches, fruits and total fruit weight. Furthermore, the treatment induced significantly higher activity of antioxidant enzymes - Peroxidase (0.392 µmol product/ min/ g fresh weight), Polyphenol Oxidase (0.791 µmol product/ min/ g fresh weight) and increased Total Phenol content (0.381 µmol product/ min/ g fresh weight) in this treatment suggest a robust induced defense mechanism against *F. oxysporum*.

These findings highlighted the potential of AMF and beneficial yeast isolates as sustainable biocontrol agents and plant growth promoters in tomato cultivation. Their use can reduce the reliance on chemical fungicides and fertilizers, paving the way for eco-friendly and cost-effective agricultural practices.

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Authors' contributions

GM contributed to experimental design, performed experiments and drafted the initial manuscript. SKG contributed to experimental design and performed experiments. SK and RD performed experiments. PD carried out data analysis and molecular studies. MS, MDS and LD proofread the initial version and made necessary corrections. MM and EJ contributed to data analysis and molecular studies. AA contributed to experimental design, performed experiments and co-wrote the first draft of the manuscript. All authors contributed to the article and approved the final submitted version.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflicts of interest.

Ethical issues: None

References

1. Chitwood-Brown J, Vallad GE, Lee TG, Hutton SF. Breeding for resistance to *Fusarium* wilt of tomato: A review. Genes. 2021;12 (11):1673. https://doi.org/10.3390/genes12111673
2. Kumar A, Verma S, Sharma S. *Rhizophagus irregularis* enhances growth and defense responses in tomato against *Fusarium* wilt under greenhouse conditions. Mycorrhiza. 2022;32(2):183–93. https://doi.org/10.1007/s00572-021-01058-5
3. Freimoser FM, Rueda-Mejia MP, Tilocca B, Migheli Q. Biocontrol yeasts: mechanisms and applications. World J Microbiol Biotechnol. 2019;35(10):154. https://doi.org/10.1007/s11274-019-2729-1
4. Weng W, Yan J, Zhou M, Yao X, Gao A, Ma C, et al. Roles of arbuscular mycorrhizal fungi as a biocontrol agent in the control of plant diseases. Microorganisms. 2022;10(7):1266. https://doi.org/10.3390/microorganisms10071266
5. Nassar AH, El-Tarabily KA, Sivasithamparam K. Promotion of plant growth by an auxin-producing isolate of the yeast *Williopsis saturnus* endophytic in maize (*Zea mays* L.) roots. Biol Fertil Soils. 2005;42:97–108. https://doi.org/10.1007/s00374-005-0008-5
6. Kurtzman CP, Fell JW. Yeast systematics and phylogeny-implications of molecular identification methods for studies in ecology. In: Biodiversity and ecophysiology of yeasts. Springer; 2006. p. 11–30. https://doi.org/10.1007/3-540-30985-3_2
7. Gerhardt P. Manual of methods for general bacteriology. Am Soc Microbiol. 1981.
8. Phillips JM, Hayman DS. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc. 1970;55 (1):158–IN118. https://doi.org/10.1016/S0007-1536(70)80110-3
9. Gerdemann JW, Nicolson TH. Spores of mycorrhizal *Endogone*

species extracted from soil by wet sieving & decanting. 1963.

10. Sairam RK, Rao KV, Srivastava GC. Differential response of wheat genotypes to long-term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Sci. 2002;163 (5):1037–46. https://doi.org/10.1016/S0168-9452(02)00278-9

11. Hammerschmidt R, Nuckles EM, Kuc J. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiol Plant Pathol. 1982;20(1):73–82.

12. Mayer AM. Catechol oxidase: enzymic liberation from sugar beet chloroplasts. Phytochem. 1966;5(6):1297–301.

13. Zieslin N, Ben-Zaken RJ. Peroxidase activity and presence of phenolic substances in peduncles of rose flowers. Plant Physiol Biochem. 1993;31(3):333–39.

14. Saha J, Brauer EK, Sengupta A, Popescu SC, Gupta K, Gupta B. Polyamines as redox homeostasis regulators during salt stress in plants. Front Environ Sci. 2015;3:21. https://doi.org/10.3389/fenvs.2015.00021

15. Slavikova E, Vadkertiova R. The diversity of yeasts in the agricultural soil. J Basic Microbiol. 2003;43(5):430–36. https://doi.org/10.1002/jobm.200390061

16. Stolz I, Herrfurth C, Wensing A. *Moesziomyces bullatus*: A yeast-like biocontrol agent inducing systemic resistance and inhibiting fungal pathogens. Front Microbiol. 2021;12:665503. https://doi.org/10.3389/fmicb.2021.665503

17. Nasanit R, Tantirungkij M, Limtong S. The assessment of epiphytic yeast diversity in sugarcane phyllosphere in Thailand by culture-independent method. Fungal Biol. 2015;119(12):1145–57. https://doi.org/10.1016/j.funbio.2015.08.011

18. Nyanga LK, Nout MJ, Gadaga TH, Theelen B, Boekhout T, Zwietering MH. Yeasts and lactic acid bacteria microbiota from masau (*Ziziphus mauritiana*) fruits and their fermented fruit pulp in Zimbabwe. Int J Food Microbiol. 2007;120(1-2):159–66. https://doi.org/10.1016/j.ijfoodmicro.2007.06.021

19. Okerentugba PO, Ataikiru TL, Ichor T. Isolation and characterization of hydrocarbon utilizing yeast (HUY) isolates from palm wine. Am J Mol Biol. 2016;6(2):63–70. https://doi.org/10.4236/ajmb.2016.62007

20. Jeyashri M, Gomathy M, Sabarinathan KG, Subhashini R, Suresh S. Screening of phyllosphere yeast of rice for the production of enzymes and solubilisation of minerals. Int J Curr Microbiol App Sci. 2019;8(8):465–72. https://doi.org/10.20546/ijcmas.2019.808.056

21. Muthukrishanan G, Munisamy J, Gopalasubramaniam SK, Kizaharael SS, Dharmaraj R, Nath DJ, et al. Impact of foliar application of phyllosphere yeast strains combined with soil fertilizer application on rice growth and yield. Envl Microb. 2024;19:102.

22. Terryana RT, Ilmiyah N, Setyawati I, Haryati T, Mulya K, Riyanti EI, et al. Morphological, physiological and molecular identification and characterization of yeast isolated from Indonesian fruits and woods. AIP Conf Proc. 2021;2331:040007.

23. Kurtzman C, Fell JW, Boekhout T. The yeasts: A taxonomic study. Elsevier; 2011.

24. Ebabhi AM, Adekunle AA, Okunowo WO, Osuntoki AA. Isolation and characterization of yeast strains from local food crops. J Yeast Fungal Res. 2013;4(4):38–43.

25. Yadav A, Suri VK, Kumar A, Choudhary AK. Effect of AM fungi and phosphorus fertilization on P-use efficiency, nutrient acquisition and root morphology in pea (*Pisum sativum* L.) in an acid Alfisol. J Plant Nutr. 2018;41(6):689–701. https://doi.org/10.1080/01904167.2017.1416115

26. Al-Askar A, Rashad Y. Arbuscular mycorrhizal fungi: A biocontrol agent against common. Plant Pathol J. 2010;9(1):31–38.

27. Tanwar A, Aggarwal A. Impact of arbuscular mycorrhizal fungi and other bioinoculants on growth promotion in *Linum usitatissimum* L. J Indian Bot Soc. 2011;90(3–4):216–23.

28. Spagnoletti FN, Cornero M, Chiocchio V, Lavado RS, Roberts IN. Arbuscular mycorrhiza protects soybean plants against *Macrophomina phaseolina* even under nitrogen fertilization. Eur J Plant Pathol. 2020;156(3):839–49. https://doi.org/10.1007/s10658-020-01905-w

29. Dehariya K, Shukla A, Sheikh IA, Vyas D. *Trichoderma* and arbuscular mycorrhizal fungi-based biocontrol of *Fusarium udum* Butler and their growth promotion effects on pigeon pea. J Agric Sci Technol. 2015;17(2):505–17.

30. Druzhinina IS, Seidl-Seiboth V, Herrera-Estrella A, Horwitz BA, Kenerley CM, Monte E, et al. *Trichoderma*: The genomics of opportunistic success. Nat Rev Microbiol. 2011;9(10):749–59. https://doi.org/10.1038/nrmicro2637

31. Hammerschmidt R, Nuckles EM, Kuc J. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiol Plant Pathol. 1982;20(1):73–82. https://doi.org/10.1016/0048-4059(82)90025-X

32. Deborah SD, Palaniswami A, Vidhyasekaran P, Velazhahan R. Time-course study of the induction of defense enzymes, phenolics and lignin in rice in response to infection by pathogen and non-pathogen. J Plant Dis Prot. 2001;4(2):204–16.

33. Doley K, Dudhane M, Borde M. Effects of arbuscular mycorrhizal fungi against *Sclerotium rolfsii* in groundnut (JL-24). Indian J Fundam Appl Life Sci. 2015;5(1):293–302.

34. Doley K, Jite PK. Disease management and biochemical changes in groundnut inoculated with *Glomus fasciculatum* and pathogenic *Macrophomina phaseolina* (Tassi) Goid. Plant Sci Feed. 2013;3(2):21–26.

35. Yuvarani R, Mohan KR, Balabaskar P, Kumar KS. Induction of defense-related enzyme in onion by using combined application of fungal and bacterial biocontrol agents with AM fungi against *Fusarium oxysporum* f. sp. *cepae*. Plant Arch. 2020;20(1):21–24.

36. Chen H, Lin W, Liu L. Deciphering AMF-plant-microbiome interactions in vegetable systems through integrated omics approaches. Sci Total Environ. 2023;857:159654. https://doi.org/10.1016/j.scitotenv.2022.159654

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