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Impact of bioinoculants on growth enhancement, physicochemical characteristics, biochemical profiles, and enzymatic defense mechanisms in tomato (*Lycopersicum esculentum*)

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The excessive use of fungicides in agriculture causes challenges like pathogen resistance, soil and water contamination, and potential health risks. Sustainable options like *Pseudomonas* spp. and yeast are being explored as bioinoculants to promote plant growth and inhibit fungal proliferation. 87 isolates, comprising 36 fluorescent *Pseudomonas* spp. and 51 yeast isolates were obtained from healthy fruits and vegetables. Yeast (YFSL) and *Pseudomonas* (PFSL) isolates significantly ($p < 0.05$) inhibited the in-vitro growth of *Fusarium solani* and *Drechslera sp.* Experiments in a screen house for 90 days used a randomized block design to study the effects of bioinoculants on plant and fruit health. Moreover, plants and fruits treated with these bioinoculants showed increased levels of salicylic acid (66.14%), total phenolic content (59.67%), chlorophyll (24.31%), carbohydrates (40.38%), phosphorus (0.24%), and antioxidant activity (90%). The treatments displayed higher levels of plant defensive enzymes, chitinase (0.09 mg/h/protein) and β-1-3-glucanase (0.093 mg/h/protein). The increased concentrations of antioxidant enzymes like SOD (0.07 U/L), POD (0.23 U/L), and APX (0.24 U/L) were also observed in the fruits of bio-inoculated plants. However., the difference in results was non-significant ($P \leq 0.05$). This study demonstrates the Efficacy of bioinoculants in improving plant growth, compositional characteristics, and antioxidant activities while reducing losses in tomato plants and fruits.

Keywords Antioxidant enzymes, Fluorescent *Pseudomonas* Spp., Defensive enzymes, Bioinoculants, Salicylic acid, Tomato

Abbreviations

YFSL	Yeast isolate name
PFSL	Pseudomonas isolate name
PGPR	Plant growth-promoting bacteria
HPLC	High-performance liquid chromatography
NYDA	Nutrient yeast dextrose agar
UV	Ultraviolet
KB medium	King's B medium
PDA medium	Potato dextrose agar medium
P	Phosphorous
SA	Salicylic acid
TPC	Total phenolic content

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DPPH	2-2-diphenyl-1-pirchlhydrazyl
APX	Ascorbate peroxidase
POD	Peroxidase
SOD	Superoxide dismutase
CHI	Chitinase
TSS	Total soluble solids
pH	Negative logarithm of hydrogen ion concentration
TA	Titratable acidity
ANOVA	Analysis of variance
LSD	Least significant difference

The tomato plant's yield and quality are affected deleteriously due to the soil-borne fungal pathogens inhabiting the field. Various fungal diseases of tomato plants have been reported, including *Alternaria* stem canker (*Alternaria alternata*), anthracnose (*Colletotrichum coccodes*), early blight (*Alternaria solani*), charcoal rot (*Macrophomina phaseolina*), *Fusarium* root rot and crown rot (*Fusarium oxysporum* f. sp. *radices-lycopersici*), *Fusarium* wilt (*Fusarium oxysporum* f. sp. *Lycopersici*), and *Rhizoctonia* diseases (*Rhizoctonia solani*)[1,2]. The tomato production has considerably decreased during the past five years in Pakistan is not only the result of the biotic factors, but various socio-economic factors like, fertilizers application, farmers' education, farmers' income, and seed rate has strongly impacted agriculture productivity[3]. Fungal infections in the field along with other biotic factors, abiotic factors, and post-harvest infections cause the loss of 30–35% of the worldwide tomato losses[4].

To mitigate the proliferation of fungi, farmers use synthetic fungicides as a primary tool to manage field disease, but their overuse and dependence lead to the emergence of pathogens resistant to fungicides, and their long-lasting effects on the environment and public health have raised concerns[5]. Hence, to mitigate the detrimental effects of chemical fungicides, the utilization of bioinoculants or biocontrol agents presents a sustainable alternative approach. Past studies on bacteria[6,7], yeast[8] and Ref.[9], and the combined application of yeast and bacteria[10,11] proved them as a potential substitute for chemical fungicides. The mixtures of bioinoculants together are reported to upgrade the potential forces against many plant pathogens[12]. It is highly possible that in most cases where biocontrol occurs in nature is a consequence of multiple antagonists, rather than a single antagonist with a high population[13]. However, it has also been reported in some cases that mixtures of biocontrol agents are ineffective in controlling the disease compared with separate antagonists[14].

Biocontrol yeasts have their capability as antagonists with low cultivation requirements, and limited risks of biosafety[15]. The action mechanisms of yeast involve nutrient competition[16], enzyme secretion[17], toxin production[18], volatile organic compound production[19], mycoparasitism[20], and induction of resistance[21]. Among plant growth-promoting rhizobacteria (PGPR), fluorescent *Pseudomonas* constitutes a major part of native microflora inhabiting the surface of fresh vegetables and they take over the role of keeping the quality and safety of fresh fruits and vegetables[22]. The common mechanisms associated with fluorescent *Pseudomonas* against plant pathogens, besides the production of siderophore, are the production of antifungal metabolites and induction of systemic resistance in plants[23,24].

Biocontrol agents present effective alternatives to synthetic plant nutrition and protection agents by enhancing soil fertility, mitigating pest pressures, and alleviating microclimate changes[25] These plant-beneficial microorganisms, certain strains of arbuscular mycorrhizal fungi and rhizobacteria have demonstrated significant beneficial effects on tomato plant growth parameters, including plant height, biomass, and fruit yield[26,27]. Furthermore, the biochemical responses of plants to microbial inoculation can enhance photosynthetic efficiency, increase chlorophyll content, and stimulate the production of secondary metabolites essential for stress adaptation[28].

Moreover, microbial inoculants can activate the defensive enzymatic activities of plants, thereby enhancing their resistance to biotic and abiotic stress. Plant defenses often involve the upregulation of key enzymes, such as peroxidases, polyphenol oxidases, and phenylalanine ammonia-lyase, which play crucial roles in the synthesis of defensive compounds and the detoxification of reactive oxygen species[29]. By enhancing these enzymatic activities, bioinoculants can boost the plant's innate defense mechanisms against pathogens and environmental stressors[30].

This study aims to systematically investigate the biocontrol potential of yeast and fluorescent *Pseudomonas* spp., both individually and in combination on plant health (growth parameters, infection percentage), fruit quality including compositional parameters (% weight loss, diameter, firmness, total soluble solids, titratable acidity, pH), biochemical parameters (salicylic acid content, total polyphenol content, DPPH, chlorophyll content, phosphorus, carbohydrates), defense-related enzymes (Chitinase activity, glucanase activity), and antioxidant enzymatic activity (APX, POD, SOD). By elucidating the multifaceted roles of bioinoculants, we aim to provide insights into sustainable agricultural practices that enhance resilience and productivity in tomato cultivation.

Materials and methods
Chemicals and reagents
All chemicals i.e. Magnesium Sulfate heptahydrate (> 99.0% Sigma Aldrich), Iron(III) chloride (> 97.0% Sigma Aldrich), L-Methionine (> 98.0%, HPLC grade, Sigma Aldrich), Nitro tetrazolium blue chloride (> 90.0%, HPLC grade, Sigma Aldrich), Calcium phosphate dibasic (> 98.0-105.0% Sigma Aldrich), Manganese(II) sulfate monohydrate (> 98.0% Sigma Aldrich), Sodium phosphate dibasic dihydrate (> 98.5–101.0% Sigma Aldrich), Urea (> 99.0-100.5% Sigma Aldrich), Ammonium sulphate (> 99.0% Sigma Aldrich), Calcium phosphate (> 98.0-105.0% Sigma Aldrich), Ammonium vanadate (Sigma Aldrich), Ammonium molybdate tetrahydrate (> 81.0–83.0% Sigma Aldrich), L-Tryptophan (> 98.0%, HPLC grade, Sigma Aldrich), Phenol red (Sigma

Aldrich), Hexadecyltrimethylammonium bromide (Sigma Aldrich), Potassium phosphate dibasic (> 98.0% Sigma Aldrich), Iron(II) sulfate heptahydrate (> 99.0% Sigma Aldrich), Congo red (> 35.0% Sigma Aldrich), Agar Oxoid (Tech no.2), Ethanol (99.0% Sigma Aldrich), Methanol (> 99.0% Sigma Aldrich).

Sample collection for fungal pathogen isolation
For the isolation of post-harvest fungal pathogens, tomato fruits with visible disease symptoms of fungi such as white cottony growth for *Fusarium* spp. and brown to blackish brown growth for *Drechslera* spp. were collected from the local vegetable markets of Karachi.

Isolation and identification of pathogenic fungi
The direct plating technique[31] was used for the isolation of pathogenic postharvest fungi. Tiny pieces from the margin of diseased areas were cut and placed on the plates containing Potato dextrose agar (PDA) medium. Fungal pathogens were identified based on their morphological characteristics including colony color, conidia size, shape, and septation of mycelium and conidia using literature including Dematiaceous hyphomycetes[32] and Handbook of Tropical Soil Biology[33].

Isolation of biocontrol agents
Sample collection for biocontrol isolation
For the biocontrol isolation healthy, disease-free fruits and vegetables (Banana, Guava, Pomegranate, Sapodilla, Orange, Lemon, Chili, Tomato, and Brinjal) were collected from the supermarket in Karachi.

Isolation of epiphytic yeast
Epiphytic yeast was isolated from fresh fruits and vegetables within 24 h of sample collection. A 2 g sample from the surface of the fruit was homogenized using 20 mL of phosphate buffer (0.05 M, pH 6.5) with a pestle and mortar. The sample mixture (300μL) was transferred onto petri plates containing nutrient yeast dextrose agar (NYDA) medium. The plates were incubated for 2 d at 24 °C[34].

Identification of epiphytic yeast
Epiphytic yeast was identified by studying the morphological characteristics suggested by Kurtzman et al.[35]. Different biochemical tests including urea hydrolysis[36], pectinase[37], β-1,3-glucanase[38], and phosphate solubilization[39] were performed on all yeast isolates to confirm their biocontrol potency.

Isolation of epiphytic fluorescent Pseudomonas spp
The surfaces of the fruit samples were sterilized, and 2 g of the samples were homogenized in 20 mL of phosphate buffer (0.05 M, pH 6.5) with the help of thistle mortar. 100 μL from each fruit sample was transferred to a Petri dish having Gould's S1 medium[40] and incubated. King's B (KB) agar medium was used to culture and store the bacterial colonies that fluoresced when exposed to UV light[41].

Identification and biochemical tests for epiphytic fluorescent Pseudomonas spp
The identification tests for the fluorescent *Pseudomonas* spp. were carried out based on their growth behavior at different temperatures viz., growth at 41℃ and 4℃[42], arginine metabolism test[43], levan formation test[44], and gelatin liquefaction test[45].

In-vitro antifungal studies
The inhibitory effect of yeast and fluorescent *Pseudomonas* spp. isolates against pathogenic fungi were assessed by placing mycelial discs (5-mm circular plugs) of test fungi on one end of the plate. In contrast, each yeast and fluorescent *Pseudomonas* spp., isolate was streaked on the other end of the plate[46]. Control plates were inoculated only with a disc of the respective fungus. Plates with dual cultures were incubated at room temperature for 4 to 7 days.

In-vivo study
Effective isolates of yeast and fluorescent *Pseudomonas* spp., which inhibited the growth of *Drechslera* sp. and *F. solani in vitro*, were selected for In-vivo studies.

Seed sample collection and purchasing
Tomato seeds (Variety; Taj seeds) were collected and bought from the local fruit market at Superhighway, Karachi, Pakistan.

In-vivo screen house experimentation
Clay pots (diameter 17 cm top, 12 cm base) were used for the screen house experiment in 2 sets. Each set comprised ten treatments each treatment replicated three times. The soil was sterilized in an autoclave (121℃, 15 Pi, for 30 min) before use, and aqueous suspensions (25 mL) of different yeast (10^6cfu/mL) and fluorescent *Pseudomonas* spp. (10^8cfu/mL) isolates were poured into the treatment pot separately and in combinations. Ten seeds of tomato (*Lycopersicon esculentum Mill*) were sown into the pots, after germination five seedlings showing proper growth were kept and the rest were uprooted. During the experiment, in the positive control, 25 mL of fungicide Topsin-M (200 ppm) was used, and all plants were watered regularly with regular water. The ambient temperature varied between 30 and 40 °C, and the plants were exposed to three to six hours of sunlight while being partially shaded. The plants of Set 1were harvested at day 45 to screen the biocontrol efficacy on inhibition of fungal infection, plant growth parameters, biochemical, antioxidant, and activation of defense mechanism of

the plant. Plants of Set 2 were kept for 90 days and allowed to reach the fruiting stage to screen the biocontrol efficacy on the nutritional composition, including the fruit's physicochemical, biochemical, antioxidant, and defensive enzymes.

Screening of biocontrol efficacy on plant growth
The growth parameters of tomato plants (weight and length of roots and shoots) were noted. To assess root infection caused by fungi, root plating was performed as the plant roots were cut into 1 cm pieces, washed and sterilized with 1% sodium hypochlorite solution (2–3 min), and placed onto PDA plates supplemented with antibiotics, penicillin (100,000 unit/L) and streptomycin (0.2 g/L). Fungi (white cottony colony for *Fusarium* spp. and brown to blackish brown colonies for *Drechslera* spp.) emerging from infected roots were noted and infection (%) was calculated[47].

Screening of biocontrol efficacy on biochemical parameters of tomato leaf
The following biochemical parameters were estimated from the leaves of plants.

Estimation of chlorophyll (CHL) content
Fresh leaves (1 g) were homogenized in 10 mL acetone (80%) and centrifuged at 5000–10,000 rpm for 5 min. The supernatant was collected, and the absorbance of the supernatant was taken at 645 nm and 663 nm while acetone served as blank. Total chlorophyll content was calculated[48].

Total Chl content (mg/ (g.fr wt) = ((20.2×A645) + (8.02×A663))/(1000×W) ×V.

Whereas A = Sample absorbance (nm), V = Extract volume in milliliters, W = Weight of plant sample (g).

Estimation of carbohydrate content
The phenol sulphuric acid method was used to quantify the carbohydrates present in the leaf sample[49]. Oven-dried leaves (1 g) were crushed in distilled water (10 mL) and centrifuged at 4000 rpm for 10 min. 1 mL of supernatant and 3 mL of freshly prepared anthrone reagent were mixed. Test tubes were covered and placed in a water bath and cooled down by using an ice bath. Finally, the optical density was recorded at 680 nm via a UV-spectrophotometer.

Quantification of phosphorus (P)
The leaf phosphorus content was estimated by following protocol[50]. Oven-dried sample of leaves (0.5 g) was digested in 10 mL HCl (2 N) for 1 h. The solution was filtered, and 1 mL filtrate was mixed with 1 mL of ammonium vanado-molybdate reagent (freshly prepared). After incubation of 30 min at room temperature absorbance was noted at 410 nm. Potassium dihydrogen phosphate was used as a standard calibration curve in ppm.

Total Phosphorus (ppm) = P×A/W×50/V.

Whereas P = Phosphorus value using standard curve (ppm), A = Volume of digested sample (mL), V = Digested sample volume used for estimation (mL), W = Sample weight (g).

Quantification of salicylic acid (SA)
Ferric Chloride (0.1%) was used for the quantification of salicylic acid[51]. Samples of leaf were crushed in ethanol followed by centrifugation (10,000 rpm) for 10 min. Aliquots (0.1 mL) were mixed with 3 mL of ferric chloride (0.1%). The absorbance was recorded at 540 nm by spectrophotometer.

Estimation of total phenolic content (TPC)
Folin–Ciocalteu method was utilized for the quantification of total phenolic content based on the following procedure[52], where gallic acid served as a standard phenolic compound. Briefly, 100µL of the filtered leaf extracts (in 96% ethanol) were mixed with 500 µL of Folin-Ciocalteu (0.2 N) and incubated for 5 min, later 400 µL of sodium carbonate (7.5%) was added and further incubated for 90 min. The absorbance was noted at 765 nm.

Antioxidant activity (%) by DPPH method
To measure free radical scavenging activity, we used the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method[53]. We prepared a 0.1mM solution of DPPH using methanol. To test the antioxidant activity, we mixed 2 mL of ethanolic extract of the plant/fruit with 2 mL of the DPPH solution. We then measured the absorbance immediately at 1 min and then again after 30 min (while incubated in the dark at 25 °C) at 517 nm. For the control, we used water or ethanol instead of the tested sample, and methanol instead of DPPH was used as the blank.

DPPH% = [1-((Asample-Asampleblank)/Acontrol)] × 100.

Whereas $A_{control}$ = Abs of the control (DPPH solution only), A_{sample} = Abs of the test sample (Solution mixture of sample and DPPH solution), $A_{sample\ blank}$ = Abs of plant sample only.

Screening of antioxidant enzyme levels in tomato leaf
The antioxidant enzymes including Ascorbate peroxidase (APX), Peroxidase (POD), and Superoxide dismutase (SOD) were estimated from tomato leaf obtained from the screen-house experiment as below,

Ascorbate peroxidase (APX)
The assay of ascorbate peroxidase was carried out by mixing phosphate buffer (700 µL, pH 5) with L-ascorbic acid (100 µL, 0.5 mM), and H_2O_2 (100 µL, 0.15 mM). Finally, 100 µL of enzyme extract was added to the mixture and the absorbance was noted repeatedly after 30 s till 90 s at 290 nm. Mean values were used for the calculation of APX activity[54].

Peroxidase (POD)
For the peroxidase (POD) activity, enzyme extract (100 μL) was incubated with phosphate buffer (0.1 M), pyrogallol (0.1mM), and H_2O_2 (5mM) for 5 min at 25 °C. 1 mL of H_2SO_4 (2.5 N) was added to the mixture to stop the reaction. The absorbance was noted at 420 nm against blank where water was substituted in place of enzyme extract[55].

Superoxide dismutase (SOD)
SOD activity was figured out by using nitro blue tetrazolium (NBT). Enzyme extract (100 μl) was incubated with phosphate buffer (1 mL, pH 5), 300 μL of methionine (22μM), distilled water (1 mL), and 100 μL of NBT (20μM) under ultraviolet light for 15 min. Finally, 0.6μM riboflavin (100μL) was added to the mixture which served as a substrate, and the absorbance was recorded at 560 nm. Mean values were used for the calculation of SOD activity[56].

Screening of biocontrol efficacy on defense-related enzymes of tomato leaf
The discussion below covers the details of the defensive enzymes found in the leaves of the tomato plant.

Chitinase (CHI) activity
For chitinase (CHI) activity, as suggested by Lucas-Bautista et al.[57], the extraction of enzymes was done accordingly. For sample preparation, 0.3 g polyvinyl polypyrrolidone (PVPP) and tissue samples (10 g) were ground together in 30 mL buffer of sodium acetate (50 mM, pH 5.0) at 4℃ and centrifuged for 30 min (17,000 g). The supernatant was collected and stored for enzyme assay. Chitinase activity was assayed by incubating enzyme extract (1 mL) with 2 mL of dye-labeled carboxymethyl chitin (2%, prepared in sodium acetate buffer) for 1 h (37℃). 1 M HCl (1 mL) was utilized to stop the reaction. The reaction mixture was centrifuged. The supernatant was collected and utilized for absorbance noted at 550 nm. The specific enzyme activity was expressed as l mol product h^{-1} mg^{-1} protein[58].

β-1, 3 glucanase activity
β-1, 3-Glucanase activity was assayed by the dinitro salicylate method with some modification to the method suggested by Wang et al.[59]. Enzyme extract (250μL) was incubated with 250μL of laminarin (0.5%) 1 h at 37℃. Sterilized distilled water (200 μL) was added to 50 μL of the reaction mixture. The blank was a mixture of the crude enzyme extract with laminarin without incubation. 250 μL of 3, 5-dinitro salicylate was added to the mixture to stop the reaction and placed in a hot water bath for 5 min. The final volume of the solution was made up to 4 mL and the amount of reducing sugars was observed at 500 nm. The specific activity of β-1, 3-glucanase was expressed as the formation of 1 μm glucose equivalents h^{-1} mg^{-1} protein.

Screening of biocontrol efficacy on physicochemical quality of fruit
The physicochemical properties studied on tomato fruit are described below:

Weight
The weight of tomato fruits was measured on the day of harvest with weight balance and expressed in grams (g).

Diameter
Vernier caliper was used to measure the diameter of tomato fruits on the day of harvest and expressed in centimeters (cm).

Firmness
The firmness of fruits was measured after harvest using a penetrometer GY-3 and expressed in Newton (N).

Total soluble solids (TSS)
A hand refractometer (Atago Co., Tokyo, Japan) was used to measure the total soluble solids content of tomato fruits in percent (%)[60].

pH
The pH Meter (JENCO 6173 pH) was used to find the pH of tomato fruit after harvest.

Titratable acidity (TA)
Tomato juice (5 mL) was titrated against NaOH (0.1 N), while phenolphthalein was used as an indicator. According to a standard method, data was represented in % citric acid[61].

$$\% \text{ Citric Acid} = (V \times N \times W_{meq} \times 100)/Y,$$

Whereas V = NaOH solution utilized for titration, N = Normality of NaOH solution (0.1 N), W_{meq} = Milliequivalent of citric acid (0.064), Y = Weight of the Sample (g/mL).

Statistical analysis
The software, IBM SPSS Statistics (version 26.0) was employed for the analysis of data obtained during experimentation. Representation of data was done as Mean ± Standard deviation. To determine the differences between treatments, a Least Significant Difference (LSD) test was performed at a significance level of $P \le 0.05$.

Results

Isolation and identification of pathogenic fungi

From the diseased tomato fruit, the postharvest fungal pathogens including *Drechslera* sp. (colony color; brown to blackish brown, conidia size and shape; solitary, cylindrical rounded at the ends, septation; pseudo septation) and *Fusarium solani* (colony color; white to loam-yellow; conidia size and shape; large, curved conidia, both ends rounded to tenpin like base, septation; Septate) were obtained (Supplementary Fig. S1A and S1B).

Isolation of bioinoculants

51 epiphytic yeast isolates (YFSL-1, YFSL-2,....YFSL-51) and 36 isolates of *Pseudomonas (PFSL-1*, *PFSL-2*, *PFSL-3*,........., PFSL-35, PFSL-36) were isolated from the disease-free fruits and vegetables including, tomato, lemon, papaya, mango, orange, green chili, grapefruit, banana, melon, grapefruit, orange, sapodilla, apple, and pomegranate) which were gathered from fields and supermarkets in Karachi, Pakistan (Supplementary Table S1, S2 & Supplementary Fig. S2A-D).

Morphological identification and biochemical characterization of yeast

Morphological studies were performed on the 56 isolates of epiphytic yeast (Table 1). The yeast isolates were analyzed based on the texture, color, and surface of the colonies they produced on the NYDA medium. The colonies showed diverse textures: 2 isolates had brittle colonies, 32 had butyrous colonies, and 17 had viscid colonies. The colors of the colonies varied as well: YFSL-1 had a creme-colored colony, YFSL-22 had a pinkish-white colony, YFSL-28 had a brownish-white colony, and YFSL-41 had an orange colony. Furthermore, 17 isolates had yellow-brown colonies, and 30 had pale white colonies. In terms of surface morphology, 34 isolates had dull colonies, while 17 had glistening colonies. (Table 2). For the confirmation of the biocontrol potency of yeast isolates in-vitro biochemical tests were performed (Supplementary Table S3). 5 yeast isolates (YFSL-21, YFSL-32, YFSL-3, YFSL-34, and YFSL-38) showed hydrolysis of urea by the change in color of urea medium from yellow to pink within half an hour of incubation period whereas, rest of the yeast isolates exhibited positive results after 24 h of incubation at 37℃ (Supplementary Fig. S3). Pectinase activity was shown positive by 8 yeast isolates namely YFSL-2, YFSL-9, YFSL-13, YFSL-16, YFSL-19, YFSL 30, YFSL-32 and YFSL-39 (Supplementary Fig. S4), The positive β-1,3-glucanase activity was shown by 47 yeast isolates (Supplementary Fig. S5). Five yeast isolates, YFSL-3, YFSL-10, YFSL-23, YFSL-32, and YFSL-45, showed phosphate solubilization activity (Supplementary Fig. S6).

Differentiation among different species of fluorescent *Pseudomonas*

Various tests were conducted to differentiate between the parasitic and saprophytic fluorescent *Pseudomonas* species. These tests included the arginine metabolism test, growth at 4 °C and 41 °C, leaven formation, and gelatin liquefaction. The arginine metabolism test showed positive results for all *Pseudomonas* species. The isolates displayed saprophytic characteristics by turning Thornley's medium pink. In the temperature tests, isolates cultured at 41 °C showed positive results, while isolates cultured at 4 °C showed no growth, indicating negative results. The gelatin liquefaction test was performed to detect the presence of gelatinase enzymes, a characteristic of *Pseudomonas aeruginosa* and *P. putida*. Out of 20 isolates, 20 showed positive results, while 16 isolates did not liquefy. All isolates tested negative for fructose polymer formation (Table 2; Supplementary Fig. S7A-B).

Key

+ Positive activity. – No.

Arginine metabolism	+	Saprophytic
Growth at 41 ℃	+/–	*P.aeruginosa/P. putida/P.fluoresence*
Growth at 4 ℃	–/+	*P.aeruginosa/P. putida/P.fluoresence*
Levan formation	–/+	*P. aeruginosa/P. putida/P. fluoresence*
Gelatin liquefaction	+/–	*P.aeruginosa/P. putida/P.fluoresence*

Biochemical tests including urease, protease, β-1,3-glucanase, and phosphate solubilization activities were performed to confirm the biocontrol potency of the f. *Pseudomonas* isolates (Supplementary Table S4). The results of 5 fluorescent *Pseudomonas* isolates namely PFSL-7, PFSL-9, PFSL-17, PFSL- 21, and PFSL-36 showed positive urease activity (Supplementary Fig. S8). However, none of the *Pseudomonas* showed positive pectinase activity. 24 fluorescent *Pseudomonas* isolates showed positive β-1,3-glucanase activity (Supplementary Fig. S9), and seven isolates of fluorescent *Pseudomonas* PFSL-9, PFSL-18, PFSL-20, PFSL-21, PFSL-23, PFSL-30, and PFSL-32, showed positive phosphate solubilization activities (Supplementary Fig. S10).

In-vitro antifungal studies

In-vitro antifungal activities of all yeast and fluorescent *Pseudomonas* isolates were studied using the dual culture method against *Drechslera* sp. and *Fusarium solani* isolated from diseased tomato fruit.

Isolates	Texture	Color	Surface
YFSL-1	Butyrous	Creme	Dull
YFSL-2	Viscid	Yellow-brown	Glistening
YFSL-3	Viscid	Yellow-brown	Glistening
YFSL-4	Butyrous	Pale White	Dull
YFSL-5	Butyrous	Pale White	Dull
YFSL-6	Butyrous	Pale White	Dull
YFSL-7	Butyrous	Pale White	Dull
YFSL-8	Butyrous	Pale White	Dull
YFSL-9	Viscid	Yellow-brown	Glistening
YFSL-10	Butyrous	Pale White	Dull
YFSL-11	Viscid	Yellow brown	Glistening
YFSL-12	Butyrous	Pale White	Dull
YFSL-13	Viscid	Yellow brown	Glistening
YFSL-14	Butyrous	Pale White	Dull
YFSL-15	Butyrous	Pale White	Dull
YFSL-16	Viscid	Yellow brown	Glistening
YFSL-17	Viscid	Yellow brown	Glistening
YFSL-18	Butyrous	Pale White	Dull
YFSL-19	Viscid	Yellow brown	Glistening
YFSL-20	Viscid	Yellow brown	Glistening
YFSL-21	Viscid	Yellow brown	Glistening
YFSL-22	Brittle	Pinkish white	Dull
YFSL-23	Butyrous	Pale White	Dull
YFSL-24	Butyrous	Pale White	Dull
YFSL-25	Butyrous	Pale White	Dull
YFSL-26	Butyrous	Pale White	Dull
YFSL-27	Butyrous	Pale White	Dull
YFSL-28	Brittle	Brownish white	Dull
YFSL-29	Viscid	Yellow brown	Glistening
YFSL-30	Viscid	Yellow brown	Glistening
YFSL-31	Butyrous	Pale White	Dull
YFSL-32	Viscid	Yellow brown	Glistening
YFSL-33	Viscid	Yellow brown	Glistening
YFSL-34	Butyrous	Pale White	Dull
YFSL-35	Butyrous	Pale White	Dull
YFSL-36	Butyrous	Pale White	Dull
YFSL-37	Viscid	Yellow brown	Glistening
YFSL-38	Butyrous	Pale White	Dull
YFSL-39	Viscid	Yellow brown	Glistening
YFSL-40	Viscid	Yellow brown	Glistening
YFSL-41	Butyrous	Orange	Dull
YFSL-42	Butyrous	Pale White	Dull
YFSL-43	Butyrous	Pale White	Dull
YFSL-44	Butyrous	Pale White	Dull
YFSL-45	Butyrous	Pale White	Dull
YFSL-46	Butyrous	Pale White	Dull
YFSL-47	Butyrous	Pale White	Dull
YFSL-48	Butyrous	Pale White	Dull
YFSL-49	Butyrous	Pale White	Dull
YFSL-50	Butyrous	Pale White	Dull
YFSL-51	Butyrous	Pale White	Dull

Table 1. Colonial characteristics of yeast isolates.

Isolates	Arginine metabolism	Growth at 41 ℃	Growth at 4 ℃	Levan formation	Gelatin liquefaction
PFSL-1	+	+	–	–	–
PFSL-2	+	+	–	–	–
PFSL-3	+	+	–	–	+
PFSL-4	+	+	–	–	–
PFSL-5	+	+	–	–	+
PFSL-6	+	+	–	–	–
PFSL-7	+	+	–	–	–
PFSL-8	+	+	–	–	+
PFSL-9	+	+	–	–	–
PFSL-10	+	+	–	–	+
PFSL-11	+	+	–	–	+
PFSL-12	+	+	–	–	–
PFSL-13	+	+	–	–	–
PFSL-14	+	+	–	–	–
PFSL-15	+	+	–	–	+
PFSL-16	+	+	–	–	–
PFSL-17	+	+	–	–	+
PFSL-18	+	+	–	–	+
PFSL-19	+	+	–	–	+
PFSL-20	+	+	–	–	–
PFSL-21	+	+	–	–	–
PFSL-22	+	+	–	–	+
PFSL-23	+	+	–	–	+
PFSL-24	+	+	–	–	+
PFSL-25	+	+	–	–	+
PFSL-26	+	+	–	–	+
PFSL-27	+	+	–	–	+
PFSL-28	+	+	–	–	+
PFSL-29	+	+	–	–	+
PFSL-30	+	+	–	–	+
PFSL-31	+	+	–	–	–
PFSL-32	+	+	–	–	+
PFSL-33	+	+	–	–	+
PFSL-34	+	+	–	–	–
PFSL-35	+	+	–	–	–
PFSL-36	+	+	–	–	–

Table 2. The identification tests including arginine metabolism, growth at 41 ℃, growth at 4 ℃, levan formation, and gelatin liquefaction on epiphytic f. *Pseudomonas* Spp.

In-vitro antifungal studies of yeast
The yeast isolates evaluated for their antifungal activity showed variable degrees of inhibition against *Drechslera* and *Fusarium solani*. Most of them were able to inhibit the growth of pathogenic fungi. YFSL-3 (25.9 ± 2.3 mm) showed maximum zone of inhibition against *Drechslera* sp., followed by YFSL-13 (25.4 ± 2.3 mm), YFSL-35 (24.6 ± 1.1 mm), YFSL-43 (23.6 ± 1.5 mm), YFSL-42 (23 ± 2.6 mm), YFSL-41 (22.3 ± 3.2 mm), YFSL-40 (22 ± 1.7 mm), YFSL-10 (21.8 ± 2.1 mm), YFSL-34 (21.6 ± 0.5 mm), YFSL-5 (20.9 ± 0.1 mm), YFSL-2 (20.8 ± 3 mm), YFSL-39 (20.6 ± 0.5 mm), and YFSL-44 (20.6 ± 3.7 mm). The most effective isolate against *F. solani* was YFSL-45 (45.3 ± 2 mm) followed by YFSL-19 (42 ± 1 mm), YFSL-42 (39 ± 1 mm), YFSL-36 (38 ± 1 mm), YFSL-37 (37.3 ± 1.5 mm), YFSL-44 (37.3 ± 2 mm) and lastly YFSL-31 (33.3 ± 2.5 mm) (Table 3; Supplementary Figs. S11 & S12).

In-vitro antifungal activities of fluorescent Pseudomonas spp
Table 4 and Supplementary Fig. S13- S14 shows the in-vitro growth inhibition of *Fusarium solani* and *Dreschlera* sp. The fluorescent *Pseudomonas* spp. isolates namely PFSL-9 (24.77 ± 2.49 mm) have shown maximum inhibition against *Drechslera* sp. followed by PFSL-32 (24.44 ± 2.95), PFSL-34 (23.44 ± 1.67 mm), PFSL-23 (23.77 ± 3.56 mm), PFSL-33 (23.33 ± 1.66), and PFSL-22 (23.22 ± 2.82 mm). Maximum inhibition of *F. solani* was shown by fluorescent *Pseudomonas* sp., isolate namely PFSL-9 (30.11 ± 2.71 mm) followed by PFSL-5 (29.22 ± 1.07 mm), PFSL-33 (27.33 ± 2.45 mm), PFSL-22 (27.22 ± 3.27 mm), PFSL-26 (25.88 ± 3.89 mm), PFSL-

| Isolates | Zone of inhibition (mm) | |
	Drechslera spp.	*Fusarium solani*
YFSL-1	15 ± 1.3	17.6 ± 0.3
YFSL-2	20.8 ± 0.3	32 ± 0.7
YFSL-3	25.9 ± 2.3	41.3 ± 1.5
YFSL-4	15.2 ± 1.5	23.3 ± 2.8
YFSL-5	20.9 ± 0.1	24.3 ± 0.5
YFSL-6	13.7 ± 0.1	25.6 ± 2.5
YFSL-7	9.5 ± 1.8	25 ± 1
YFSL-8	10.8 ± 0.6	27.6 ± 1.5
YFSL-9	8.4 ± 0.5	30 ± 1
YFSL-10	21.8 ± 2.1	24.6 ± 1.5
YFSL-11	18.7 ± 0.5	35.3 ± 3.5
YFSL-12	11.7 ± 0.9	25.3 ± 3.2
YFSL-13	25.4 ± 2.3	23.3 ± 3.7
YFSL-14	14 ± 2.1	18 ± 1
YFSL-15	17.9 ± 0.1	23.6 ± 3
YFSL-16	14.2 ± 0.5	28.6 ± 1.5
YFSL-17	16.4 ± 2.2	25.6 ± 1.1
YFSL-18	17.6 ± 0.9	25 ± 1.1
YFSL-19	9.6 ± 0.6	42 ± 1
YFSL-20	5.3 ± 0.5	29.6 ± 1.5
YFSL-21	15.9 ± 1.8	26 ± 3
YFSL-22	17.4 ± 1.8	23 ± 2
YFSL-23	17.6 ± 1.5	23 ± 2.6
YFSL-24	20 ± 1	31.3 ± 3.2
YFSL-25	18.6 ± 2.5	23.6 ± 2
YFSL-26	16 ± 0.5	21.6 ± 1.5
YFSL-27	18 ± 2.6	25.6 ± 2
YFSL-28	15.3 ± 0.5	23 ± 2.6
YFSL-29	10 ± 1.7	21.8 ± 4.1
YFSL-30	18.6 ± 2	30 ± 2
YFSL-31	19 ± 1.5	33.3 ± 2.5
YFSL-32	18.3 ± 0.5	32.6 ± 1.5
YFSL-33	19.6 ± 1.5	31.3 ± 2
YFSL-34	21.6 ± 0.5	18.3 ± 3
YFSL-35	24.6 ± 1.1	26.6 ± 2.5
YFSL-36	18.6 ± 3.5	38 ± 1
YFSL-37	18 ± 2	37.3 ± 1.5
YFSL-38	19.6 ± 2.3	35.3 ± 1.5
YFSL-39	20.6 ± 0.5	32.6 ± 2
YFSL-40	22 ± 1.7	30.3 ± 2.3
YFSL-41	22.3 ± 3.2	30.6 ± 2
YFSL-42	23 ± 2.6	39 ± 1
YFSL-43	23.6 ± 1.5	30.6 ± 3.2
YFSL-44	20.6 ± 3.7	37.3 ± 2
YFSL-45	17.6 ± 0.9	45.3 ± 2
YFSL-46	17.4 ± 1.8	36.6 ± 2
YFSL-47	15.3 ± 0.5	29.6 ± 2.5
YFSL-48	19 ± 1.5	28.6 ± 1.5
YFSL-49	18 ± 2	26 ± 2.6
YFSL-50	18.7 ± 0.5	24.6 ± 2
YFSL-51	11.7 ± 0.9	30.3 ± 2
LSD$_{0.05}$	0.000[1]	0.000[1]

Table 3. In-vitro antifungal activity of yeast isolates against pathogenic fungi (mm). Each value is mean ± S. D (Standard deviation) of 3 replicates. *YFSL* represents yeast spp. isolates. [1]Mean values in the column showing differences greater than LSD values are significantly different at $p < 0.05$.

Isolates	*Drechslera* sp.	*F. solani*
PFSL-1	20 ± 0.10	23.55 ± 3.40
PFSL-2	20.77 ± 2.84	20.44 ± 3.71
PFSL-3	17.44 ± 2.07	22.22 ± 2.22
PFSL-4	18.55 ± 3.40	21.11 ± 2.03
PFSL-5	20.22 ± 2.06	23.66 ± 2.06
PFSL-6	18.44 ± 1.20	19.44 ± 4.50
PFSL-7	19.77 ± 3.93	21.44 ± 3.91
PFSL-8	17 ± 2.45	19.66 ± 2.00
PFSL-9	24.77 ± 2.49	30.11 ± 2.71
PFSL-10	21.66 ± 3.57	25.44 ± 2.07
PFSL-11	20.88 ± 3.58	20.11 ± 3.44
PFSL-12	18.77 ± 2.63	25.55 ± 3.50
PFSL-13	23.22 ± 1.12	17.22 ± 2.28
PFSL-14	18.55 ± 1.25	20.55 ± 0.06
PFSL-15	18.11 ± 3.52	19.77 ± 3.03
PFSL-16	19.55 ± 2.25	12.11 ± 0.44
PFSL-17	17.11 ± 3.82	19.77 ± 1.09
PFSL-18	19.44 ± 2.03	22.22 ± 2.28
PFSL-19	15.55 ± 3.50	14.66 ± 2.39
PFSL-20	16.11 ± 1.61	21.55 ± 1.24
PFSL-21	16.77 ± 2.24	25.77 ± 2.11
PFSL-22	23.22 ± 2.82	27.22 ± 3.27
PFSL-23	23.77 ± 3.56	17.88 ± 1.96
PFSL-24	14.88 ± 3.48	16.11 ± 2.67
PFSL-25	20.88 ± 3.26	21.22 ± 2.41
PFSL-26	21.33 ± 3.08	25.88 ± 3.89
PFSL-27	22.22 ± 1.39	21.66 ± 3.08
PFSL-28	22.22 ± 3.38	20 ± 3.57
PFSL-29	22.55 ± 2.07	25 ± 3.24
PFSL-30	19.77 ± 1.72	19.44 ± 0.92
PFSL-31	23.22 ± 3.42	19.55 ± 2.79
PFSL-32	24.44 ± 2.95	29.22 ± 1.07
PFSL-33	23.33 ± 1.66	27.33 ± 2.45
PFSL-34	23.44 ± 1.67	24.11 ± 2.26
PFSL-35	21.44 ± 3.17	24.88 ± 2.47
PFSL-36	22.55 ± 2.35	23 ± 1.50
LSD$_{0.05}$	0.000[1]	0.000[1]

Table 4. *In-vitro* antifungal activity of fluorescent *Pseudomonas* spp. isolates against pathogenic fungi zone of inhibition (mm). Each value is mean ± S. D (Standard deviation) of 3 replicates. *PFSL* represents fluorescent *Pseudomonas* spp. isolates. [1]Mean values in the column showing differences greater than LSD values are significantly different at $p < 0.05$.

21 (25.77 ± 2.11 mm), PFSL-12 (25.55 ± 3.50 mm) and (25.44 ± 2.07 mm) by PFSL-10 (Table 4; Supplementary Fig. S13 & S14).

In-vivo screen house experimentation

Using yeast and fluorescent *Pseudomonas* spp. individually and in combinations (YFSL-3, YFSL-45, PFSL-9, PFSL-32, YFSL-3 + PFSL-32, YFSL-45 + PFSL-9) as bioinoculants in soil drenching improved plant growth and biochemical activity in tomato plants.

One set of pots was harvested after 45 days for the analysis of physical and biochemical plant parameters and the second set was harvested after 90 days for the analysis of physical and biochemical fruit characteristics. Details are mentioned below,

Assessing the efficacy of bioinoculants on the growth parameters of tomato plants grown under screen house conditions

At 45 d harvest, the physical growth parameters of tomato plants showed significant results ($p < 0.05$) in the individual and combined treatments of biocontrol (yeast and fluorescent *Pseudomonas* spp.). The treatments YFSL-3 + PFSL-32 and YFSL-45 + PFSL-9 (53.83 ± 6.05 cm and 50.22 ± 7.55 cm) showed maximum shoot length in comparison with positive control (35.77 ± 6.22 cm). The highest shoot weight (16.37 ± 9.16 g) was shown in treatment YFSL-3 + PFSL-32 in comparison with positive control (5.79 ± 1.80 g), followed by the treatments YFSL-45 + PFSL-9 and YFSL-45 + PFSL-32 (10.73 ± 3.43 g and 10.06 ± 4.58 g). The root length was maximum in treatment PFSL-9 (15.94 ± 4.51 cm) compared to the control and positive control (11.94 ± 2.35 cm and 12.61 ± 1.83 cm), followed by PFSL-32, YFSL-3 + PFSL-9 and YFSL-3 + PFSL-32 (15.77 ± 3.03 cm, 15.50 ± 3.14 cm and 15.50 ± 3.14 cm). However, in comparison to the control and positive control (0.43 ± 0.26 g and 5.43 ± 0.15 g), the treatments YFSL-3 + PFSL-32 (1.59 ± 0.15 g) and YFSL-45 + PFSL-9 (0.96 ± 0.33 g) showed the maximum root weight of plants (Table 5; Supplementary Fig. S15).

Efficacy of bioinoculants on the root infection % in tomato plants under screen house conditions

The biocontrol treatments suppressed the growth of *Fusarium solani* effectively. However, in comparison to the control (25 ± 2.04%), treatments YFSL-45 (5.56 ± 1.35%), PFSL-32 (8.33 ± 2.50%), YFSL-45 + PFSL-9 (8.33 ± 3.17%), YFSL-3 + PFSL-32 (16.66 ± 2.67%), and YFSL-45 + PFSL-32 (16.66 ± 4%) showed the highest suppression (Fig. 1).

Efficacy of bioinoculants on the biochemical properties in tomato plants under screen house conditions

Based on the observations made during the experiment, it was found that the application of yeast and fluorescent *Pseudomonas* spp. (used both individually and in mixtures) on tomato plants had a positive effect on the physiochemical properties of the tomato leaves. The treatment YFSL-3 showed the highest chlorophyll content (31.902 ± 3.41 mg/g. fr. wt), followed by treatments PFSL-9 (31.397 ± 7.66 mg/g. fr. wt) and YFSL-45 + PFSL-9 (29.473 ± 5.18 mg/g. fr. wt). These treatments showed a significant increase in chlorophyll content as compared to the control and positive control (25.66 ± 0.85 mg/g. fr. wt and 27.895 ± 0.85 mg/g. fr. wt). Similarly, treatments YFSL-3 + PFSL-32 showed the highest amount of carbohydrates (2.92 ± 0.064 µg/mL), followed by treatments YFSL-45 + PFSL-9 (2.91 ± 0.101 µg/mL) and YFSL-3 + PFSL-9 (2.74 ± 0.052 µg/mL). These treatments showed a significant increase in carbohydrate content as compared to the control (2.08 ± 0.05 µg/mL) and positive control (2 ± 0.01 µg/mL). The treatments PFSL-32, YFSL-3 + PFSL-9, and YFSL-45 showed the highest phosphorus content (2.415 ± 0.252 mg/g, 2.1 ± 0.424 mg/g, and 2.088 ± 0.179 mg/g, respectively), as compared to the control

Growth parameter (45 days)				
Treatments	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)
Control	35.22^d ± 3.51	4.64^d ± 2.03	11.94^b ± 2.35	0.43^c ± 0.16
Positive control	35.77^d ± 6.22	5.79^{cd} ± 1.80	12.61^b ± 1.83	0.53^c ± 0.15
YFSL-3	40.72^{cd} ± 6.70	7.39^{bcd} ± 2.66	12.50^b ± 2.06	0.77^{bc} ± 0.25
YFSL-45	43.11^{bcd} ± 3.49	6.55^{bcd} ± 3.12	10.11^b ± 2.52	0.39^c ± 0.16
PFSL-9	43.12^{bcd} ± 4.26	7.40^{bcd} ± 3.67	15.94^a ± 3.51	0.46^{bc} ± 0.15
PFSL-32	43.38^{bcd} ± 5.99	7.97^{bcd} ± 3.31	15.77^a ± 3.03	0.72^{bc} ± 0.21
YFSL-3 + PFSL-9	44.27^{bc} ± 5.71	6.42^{bcd} ± 2.07	15.50^a ± 3.14	0.59^{bc} ± 0.13
YFSL-3 + PFSL-32	53.83^a ± 4.05	16.37^a ± 4.16	15.50^a ± 3.14	1.59^a ± 0.15
YFSL-45 + PFSL-9	50.22^{ab} ± 5.55	10.73^b ± 3.43	12.61^b ± 1.61	0.96^b ± 0.33
YFSL-45 + PFSL-32	47.16^{abc} ± 4.23	10.06^{bc} ± 3.58	11.72^b ± 2.33	0.81^{bc} ± 0.21
LSD$_{0.05}$	1.931^1	1.096^1	0.687^1	1.192^1

Table 5. Biocontrol efficacy on the vegetative growth of tomato plants under screen house condition. Data are reported as mean ± standard deviation, derived from the analysis of three replicates per sample. Letters highlight statistically significant differences among analyzed samples, based on a one-way ANOVA test ($p < 0.05$). Different letters within columns indicate significant differences according to Duncan's Multiple Range Test ($P \leq 0.05$) Whereas, *Control* sterilized distilled water, *Positive control* Topspin M@ 200ppm, *YFSL*-Yeast, *PFSL*- fluorescent *Pseudomonas* spp., *YFSL + PFSL*-combinations of yeast and fluorescent *Pseudomonas*.
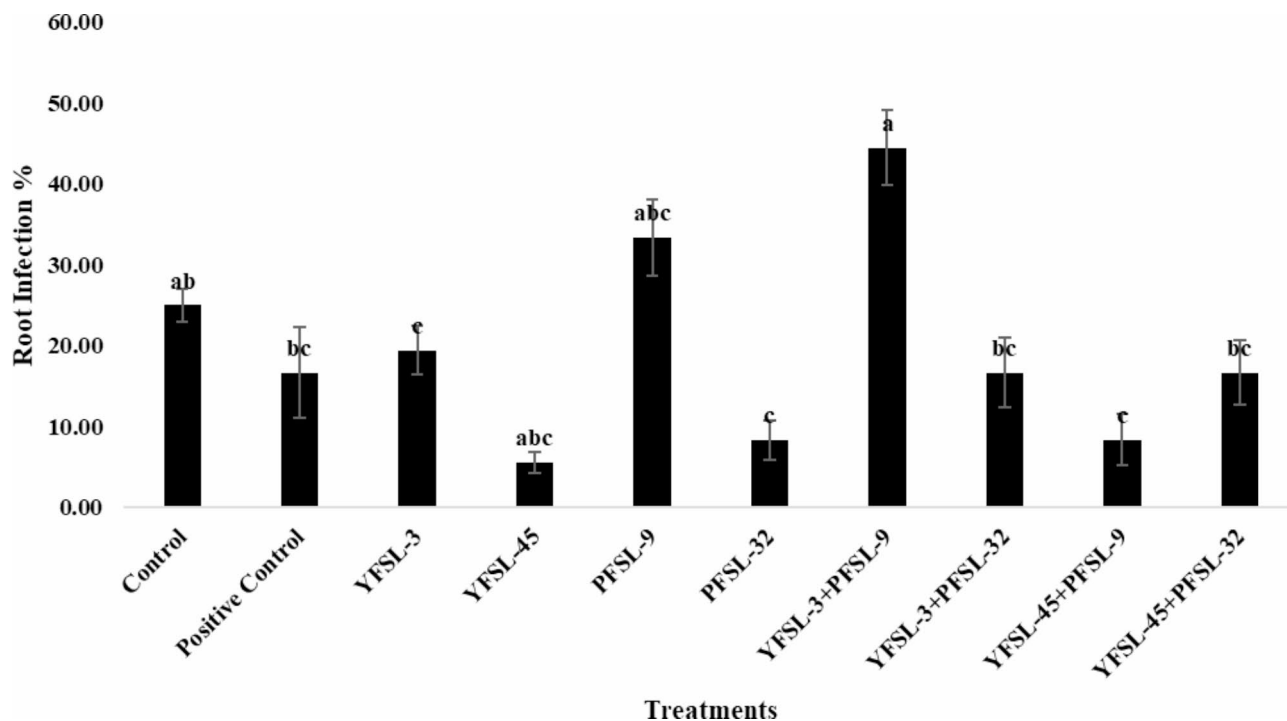
Fig. 1. Graphical representation of root infection % (LSD$_{0.05}$: *F. solani*[1] = 0.014[1]) inhibited in tomato plants grown under screen house inoculated with bioinoculants separately and in combinations. Data are reported as mean ± standard deviation, derived from the analysis of three replicates per sample. Letters highlight statistically significant differences among analyzed samples, based on a one-way ANOVA test ($p < 0.05$). Different letters within columns indicate differences according to Duncan's Multiple Range Test ($P \leq 0.05$). Whereas *Control* sterilized distilled water, *Positive control* Topsin M@ 200ppm, *YFSL*-Yeast, *PFSL*- fluorescent *Pseudomonas*, *YFSL + PFSL*-combinations of yeast and fluorescent *Pseudomonas* spp.

(1.855 ± 0.092 mg/g) and positive control (2.041 ± 0.165 mg/g). The treatments YFSL-45, YFSL-3 + PFSL-32 and YFSL-3 + PFSL-9 (13.66 ± 0.57 µg/g, 11.73 ± 0.20 µg/g and 11.3 ± 0.50 µg/g) showed the highest salicylic acid (SA) content, as compared to the control (7.06 ± 0.15 µg/g) and positive control (8.93 ± 0.11 µg/g). The maximum total phenol content was noted in treatment YFSL-45 + PFSL-32 (0.99 ± 0.00 µg/g), followed by YFSL-3, PFSL-32, and YFSL-3 + PFSL-32 (0.87 ± 0.03 µg/g and 0.84 ± 0.01 µg/g). These treatments showed a significant increase in TPC as compared to the control and positive control (0.62 ± 0.01 µg/g and 0.81 ± 0.00 µg/g). The maximum antioxidant activity was shown in treatment PFSL-9 and YFSL-45 (89.71 ± 1.07% and 89.43 ± 0.59%), followed by treatment YFSL-45 + PFSL-32 (88.86 ± 0.86%). These treatments showed a significant increase in antioxidant activity in comparison to the control (67.99 ± 0.12%) as shown in Table 6.

Efficacy of bioinoculants on the antioxidant enzymatic activities in tomato plants under screen house conditions
Tomato plants treated with biocontrol showed higher concentrations of antioxidant enzymes in their leaves compared to the control and positive control. The maximum activity of superoxide dismutase (SOD) was found in treatment PFSL-9 (0.082 ± 0.003U/L), followed by treatments YFSL-3 + PFSL-32 (0.077 ± 0.004 U/L), YFSL-45 + PFSL-9, YFSL-45 + PFSL-32 and YFSL-45 (0.074 ± 0.003 U/L). In contrast, the control had an activity of 0.068 ± 0.005 U/L. The treatment PFSL-9 (0.037 ± 0.003U/L) showed maximum activity of peroxidase (POD) in comparison to control (0.016 ± 0.004 U/L), followed by treatments YFSL-3 + PFSL-9 and YFSL-45 + PFSL-9 (0.032 ± 0.001 U/L and 0.031 ± 0.00 U/L). Similarly, treatment YFSL-3 + PFSL-32 (0.28 ± 0.07 U/L) exhibited highest activity of ascorbate peroxidase (APX), followed by treatments YFSL-3 + PFSL-9 and YFSL-45 + PFSL-9 (0.27 ± 0.02 U/L and 0.26 ± 0.08 U/L), when compared to the control (0.119 ± 0.03U/L) (Fig. 2).

Efficacy of bioinoculants on the defensive enzymatic activity in tomato plants under screen house conditions
The levels of defensive enzymes were tested, and the maximum activity of Chitinase was observed in treatment YFSL-45 (0.093 ± 0.002 mg/h/protein) as compared to the control (0.063 ± 0.001 mg/h/protein). Following that, the treatments YFSL-3 + PFSL-32, YFSL-3, PFSL-32, and YFSL-3 + PFSL-9 (0.084 ± 0.001 mg/h/protein, 0.077 ± 0.001 mg/h/protein, 0.074 ± 0.000 mg/h/protein and 0.072 ± 0.001 mg/h/protein) were observed. On the other hand, when compared to the control (0.072 ± 0.003 mg/h/protein), the concentration of β-1,3-Glucanase was observed to be the highest in the following treatments YFSL-45 + PFSL-32, YFSL-45 + PFSL-9 and YFSL-3 + PFSL-32 (0.093 ± 0.001 mg/h/protein, 0.084 ± 0.003 mg/h/protein and 0.075 ± 0.004 mg/h/protein) (Fig. 3).

| Treatments	Chlorophyll (mg/g.fr.wt)	Carbohydrates (µg/mL)	Phosphorus (%)	Salicylic acid (µg/g)	TPC (µg/g)	Antioxidant activity (%)
Control	25.66[c]±0.85	2.08[e]±0.05	0.18[cd]±0.00	7.06[g]±0.15	0.62[g]±0.01	78.6[g]±10.58
Positive control	27.89[bc]±0.85	2.00[e]±0.01	0.20[bc]±0.01	8.93[f]±0.11	0.81[b]±0.00	52.34[cd]±2.70
YFSL-3	31.90[a]±3.41	2.31[c]±0.19	0.19[bcd]±0.00	10.33[de]±0.11	0.87[c]±0.03	82.64[f]±2.17
YFSL-45	21.87[c]±0.24	2.11[de]±0.11	0.20[b]±0.01	13.66[a]±0.57	0.77[e]±0.00	59.17[ab]±0.64
PFSL-9	31.39[a]±7.66	2.20[cd]±0.15	0.20[bc]±0.02	10.3[de]±0.36	0.83[d]±0.00	59.88[a]±1.42
PFSL-32	26.01[c]±3.05	2.23[cd]±0.13	0.24[a]±0.02	10.06[e]±0.05	0.84[d]±0.01	93.69[d]±1.53
YFSL-3 + PFSL-9	27.36[bc]±3.32	2.74[b]±0.05	0.21[b]±0.04	11.3[bc]±0.50	0.74[f]±0.02	85.32[c]±7.27
YFSL-3 + PFSL-32	27.19[bc]±1.23	2.92[a]±0.06	0.18[d]±0.02	11.73[b]±0.20	0.84[d]±0.01	46.04[d]±0.44
YFSL-45 + PFSL-9	29.47[ab]±5.18	2.91[a]±0.10	0.19[bcd]±0.01	10.86[cd]±0.05	0.83[d]±0.00	31.42[e]±0.44
YFSL-45 + PFSL-32	25.65[c]±4.30	2.69[b]±0.09	0.19[bcd]±0.01	8.96[f]±1.05	0.99[a]±0.00	59.88[b]±0.355
LSD$_{0.05}$	0.822[1]	0759[1]	0.189[1]	0.383[1]	0.198[1]	1.285

Table 6. Effect of bioinoculants on the biochemical properties of tomato plants under screen house condition. Data are reported as mean ± standard deviation, derived from the analysis of three replicates per sample. Letters highlight statistically significant differences among analyzed samples, based on a one-way ANOVA test ($p < 0.05$). Different letters within columns indicate significant differences according to Duncan's Multiple Range Test ($P \leq 0.05$) Whereas, Control- sterilized distilled water, Positive control- Topsin M@ 200ppm, *YFSL*-Yeast, *PFSL*- fluorescent *Pseudomonas* spp., *YFSL + PFSL*-combinations of yeast and fluorescent *Pseudomonas*.
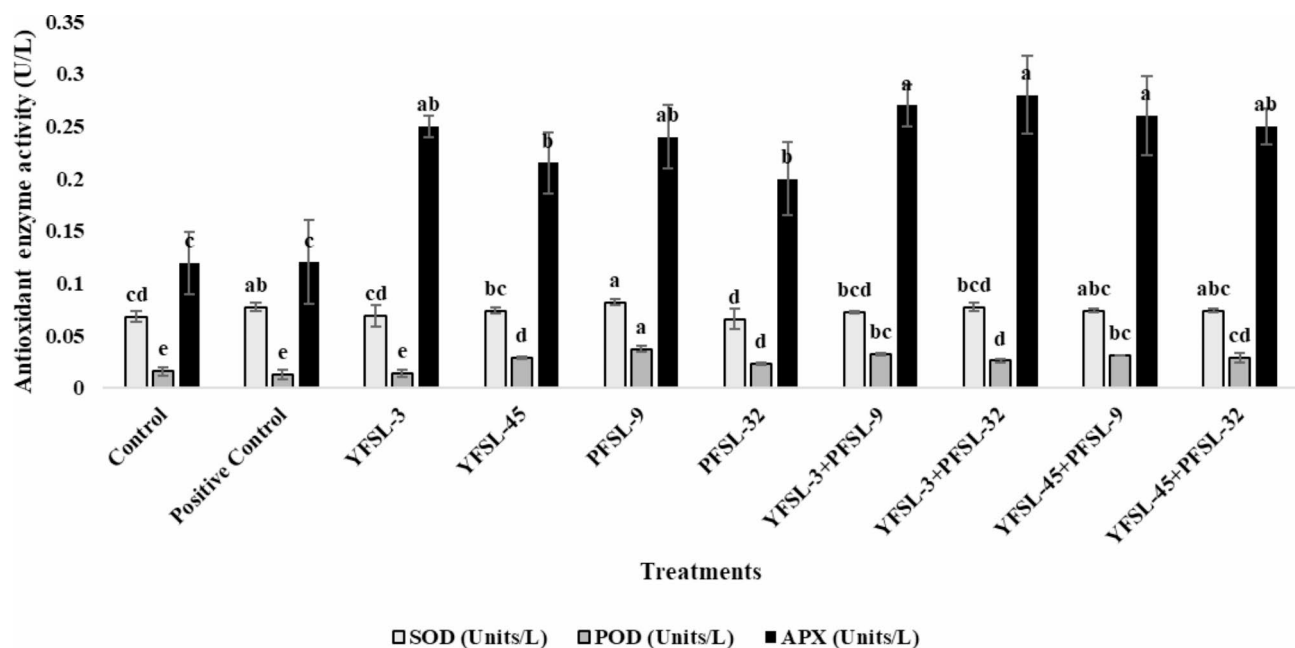
Fig. 2. The graphical representation of the antioxidant (SOD, POD, APX) enzymatic activity of tomato plants grown under screen house inoculated with bioinoculants separately and in combinations. Data are reported as mean ± standard deviation, derived from the analysis of three replicates per sample. Letters highlight statistically significant differences among analyzed samples (LSD$_{0.05}$ SOD = 0.0021, POD = 0.0031, APX = 0.0221), based on a one-way ANOVA test ($p < 0.05$). Different letters within columns indicate differences according to Duncan's Multiple Range Test ($P \leq 0.05$). Whereas *Control*- sterilized distilled water, *Positive control* Topsin M@ 200ppm, *YFSL*-Yeast, *PFSL* fluorescent *Pseudomonas*, *YFSL + PFSL* combinations of yeast and fluorescent *Pseudomonas* spp.

Efficacy of bioinoculants on the physiochemical properties of fruit under screen house conditions
Bioinoculants of yeast and fluorescent *Pseudomonas* spp. showed improved fruit quality and enhanced biochemical and enzymatic activity in tomato fruit obtained under screen house conditions. The efficacy of bioinoculants on the tomato fruit was seen in the treatments. Greater weight was seen in treatment YFSL-3 and YFSL-45 (26.65 ± 10.33 g and 26.60 ± 4.87 g) as compared to control (25.86 ± 6.69 g) and positive control (22.05 ± 4.19 g). The highest diameter was observed in YFSL-3 (13.94 ± 3.35 cm) followed by control (13.63 ± 2.75 cm). The firmness of fruit was found highest in treatments YFSL-3 + PFSL-32, YFSL-45 + PFSL-9, and YFSL-45 + PFSL-32, with values of 2.66 ± 0.15 N, 2.53 ± 0.05 N and 2.36 ± 0.45 N, respectively. These
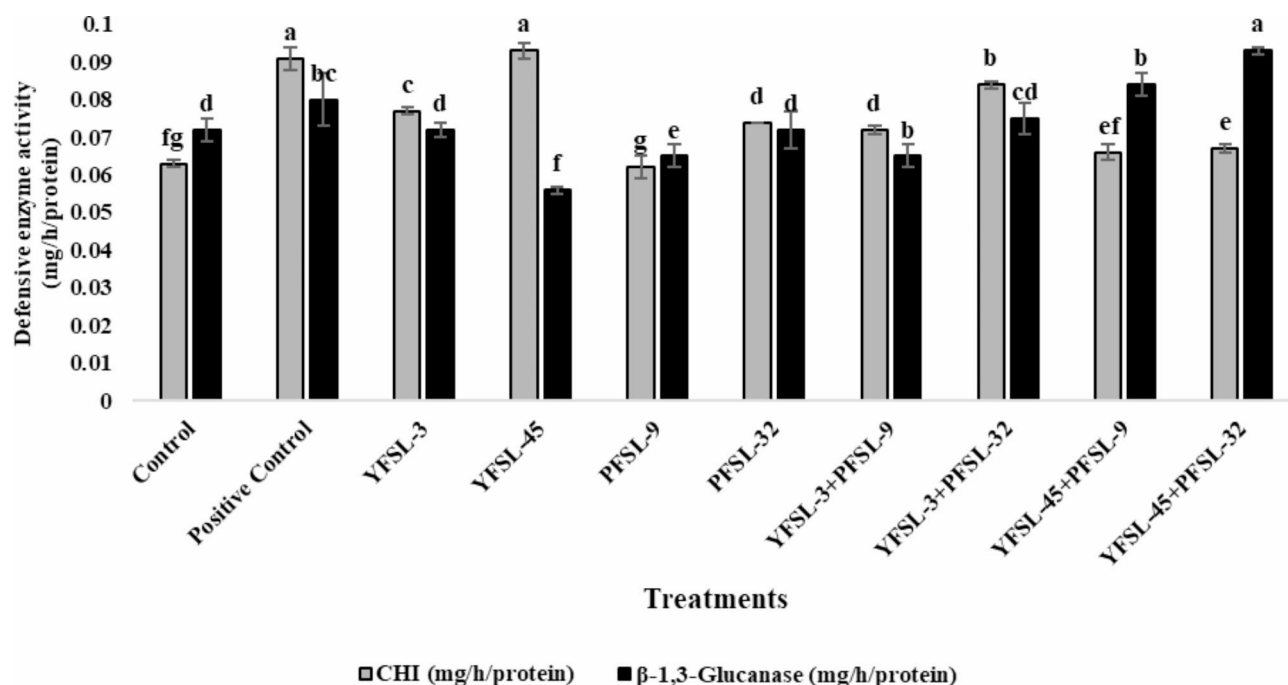
Fig. 3. The graphical representation of the antioxidant (β-1-3-Glucanase, Chitinase) enzymatic activity of tomato plants grown under screen house inoculated with bioinoculants separately and in combinations. Data are reported as mean ± standard deviation, derived from the analysis of three replicates per sample. Letters highlight statistically significant differences among analyzed samples (LSD$_{0.05}$ CHI = 0.003, β-1-3-Glucanase = 0.003), based on a one-way ANOVA test ($p < 0.05$). Different letters within columns indicate differences according to Duncan's Multiple Range Test ($P \leq 0.05$). Whereas *Control* sterilized distilled water, *Positive control* Topsin M@ 200ppm, *YFSL*-Yeast, *PFSL* fluorescent *Pseudomonas*, *YFSL + PFSL* combinations of yeast and fluorescent *Pseudomonas* spp.

values were compared to control (2.2 ± 0.2 N) and positive control (2.5 ± 0.2 N). Biocontrol treatment resulted in a lower pH compared to untreated fruits. The lowest pH value was shown in treatment YFSL-3 + PFSL-32 (3.90 ± 0.01), followed by treatments YFSL-3 + PFSL-9 and PFSL-32 (3.94 ± 0.005 and 3.96 ± 0.005), whereas the control had a pH of 4.09 ± 0.01. In terms of TSS, treatment YFSL-3 + PFSL-9 showed the least amount of TSS at 5.86 ± 0.41%, compared to control (6.93 ± 0.64%) and positive control (7.06 ± 0.11%). The treatments, YFSL-45 + PFSL-32 and YFSL-45 + PFSL-9 (6 ± 1% and 6.13 ± 0.57%), also showed lower TSS values. TA % in treatments YFSL-45 + PFSL-9 (0.25 ± 0.00%CA) followed by YFSL-45 + PFSL-32 (0.14 ± 0.02%CA), YFSL-3 + PFSL-32 (0.27 ± 0.04%CA) were observed least as compared to control (0.37 ± 0.005%CA) at same maturity stage (Table 7).

Efficacy of bioinoculants on the biochemical parameters of tomato fruit under screen house conditions
Fruits obtained from the plants treated with yeast and fluorescent *Pseudomonas* spp. showed improved biochemical parameters as compared to control and positive control. The maximum amount of salicylic acid (SA) was found in treatment YFSL-45, (3.69 ± 0.69 μg/g), as compared to the control and positive control (0.76 ± 0.10 μg/g and 1.06 ± 0.16 μg/g,) respectively. The following treatments also had high levels of SA: PFSL-32, PFSL-9, YFSL-45 + PFSL-9, and YFSL-3 + PFSL-32 (1.44 ± 0.10 μg/g, 1.40 ± 0.12 μg/g, 1.36 ± 0.06 μg/g and 1.36 ± 0.08 μg/g). In comparison to the control (0.09 ± 0.06 μg/g), the maximum content of total phenols was seen in treatments YFSL-45 + PFSL-9 and PFSL-9 (0.16 ± 0.00 μg/g and 0.12 ± 0.00 μg/g). The highest activity of antioxidants was found in treatments YFSL-3 + PFSL-9 (62.31 ± 5.87%), compared to the control and positive control (25.37 ± 6.06% and 35.47 ± 6.31%). The following treatments also showed high activity of antioxidants: YFSL-3 + PFSL-32, YFSL-45 + PFSL-32, and YFSL-45 + PFSL-9 (60.65 ± 1.73%, 60.49 ± 0.81%, and 58.96 ± 7.22%) (Table 8).

Efficacy of bioinoculants on antioxidant enzymatic activities in tomato fruit under screen house conditions
The bio-inoculated plants yield fruits with improved levels of antioxidant enzymes as compared to control. Treatment YFSL-45 (0.0091 ± 0.001 U/L) showed maximum activity of peroxidase (POD) as compared to control (0.0072 ± 0.0004 U/L) and other treatments Maximum activity of superoxide dismutase (SOD) was shown by treatment PFSL-9 (0.072 ± 0.003 U/L) followed by YFSL-45 (0.064 ± 0.003U/L) and YFSL-3 (0.059 ± 0.01 U/L) in comparison with control (0.058 ± 0.005 U/L) whereas treatment YFSL-3 (0.25 ± 0.01 U/L) showed a maximum concentration of ascorbate peroxidase (APX) when compared to the control (0.19 ± 0.03 U/L), followed by treatments PFSL-32 (0.20 ± 0.05 U/L) and YFSL-45 + PFSL-32 (0.20 ± 0.17 U/L) while Treatment YFSL-45

Physiochemical properties of tomato fruit under screenhouse						
Treatments	Weight (g)	Diameter (cm)	Firmness (N)	pH	TSS (Brix %)	Titratable acidity (%CA)
Control	25.86^a ± 6.69	13.63^{ab} ± 2.75	2.20^{abc} ± 0.20	4.09^b ± 0.01	6.93^{ab} ± 0.64	0.37^c ± 0.00
Positive control	22.05^{ab} ± 4.19	12.79^{abc} ± 1.42	2.50^b ± 0.20	3.90^f ± 0.01	7.06^{ab} ± 0.11	0.67^b ± 0.02
YFSL-3	26.65^a ± 3.33	13.94^a ± 3.35	1.90^c ± 0.26	4.06^b ± 0.00	7.53^a ± 0.80	0.38^c ± 0.01
YFSL-45	26.60^a ± 4.87	13.32^{ab} ± 0.64	2.26^{abc} ± 0.66	3.98^c ± 0.00	6.80^{ab} ± 0.72	0.39^c ± 0.00
PFSL-9	18.12^{ab} ± 5.67	11.98^{abc} ± 0.92	2.03^{bc} ± 0.30	4.12^a ± 0.00	6.53^{ab} ± 1.60	0.74^a ± 0.02
PFSL-32	15.79ab ± 4.37	9.53^{cd} ± 1.44	2.33^{abc} ± 0.30	3.96^d ± 0.00	5.53^b ± 0.70	0.32^d ± 0.03
YFSL-3 + PFSL-9	15.02^{ab} ± 2.67	9.67^{cd} ± 0.92	1.96^{bc} ± 0.05	3.94^e ± 0.00	5.86^b ± 0.41	0.37^c ± 0.02
YFSL-3 + PFSL-32	19.72^{ab} ± 2.05	11.32^{abc} ± 0.57	2.66^a ± 0.15	3.90^f ± 0.01	6.26^{ab} ± 0.46	0.27^e ± 0.04
YFSL-45 + PFSL-9	15.01^{ab} ± 3.40	10.1^{bcd} ± 2.9	2.53^b ± 0.05	3.99^c ± 0.01	6.13^{ab} ± 0.57	0.25^e ± 0.00
YFSL-45 + PFSL-32	11.22^b ± 3.33	7.49^d ± 1.04	2.36^{abc} ± 0.45	3.98^c ± 0.00	6^{ab} ± 1	0.14^f ± 0.02
LSD$_{0.05}$	0.091^1	0.006^1	0.108^1	0.000^1	0.133^1	0.000^1

Table 7. Biocontrol efficacy on the physiochemical properties of tomato fruit under screen house condition. Data are reported as mean ± standard deviation, derived from the analysis of three replicates per sample. Letters highlight statistically significant differences among analyzed samples, based on a one-way ANOVA test ($p < 0.05$). Different letters within columns indicate differences according to Duncan's Multiple Range Test ($P ≤ 0.05$). Whereas *Control-* sterilized distilled water, *Positive control-* Topsin M@ 200ppm, *YFSL-*Yeast, *PFSL-* fluorescent *Pseudomonas*, *YFSL + PFSL-*combinations of yeast and fluorescent *Pseudomonas* spp.

Treatments	SA (µg/g)	TPC (µg/g)	Antioxidant activity (%)
Control	0.76^d ± 0.10	0.09^{bc} ± 0.06	25.37^e ± 6.06
Positive control	1.06^c ± 0.16	0.081^c ± 0.0	35.47^{de} ± 6.31
YFSL-3	1.34^{ab} ± 0.06	0.084^c ± 0.0	43.99^{cd} ± 0.91
YFSL-45	3.69^c ± 0.39	0.082^c ± 0.0	49.27^{abc} ± 0.60
PFSL-9	1.40^{ab} ± 0.12	0.12^b ± 0.0	35.01^{de} ± 3.80
PFSL-32	1.44^a ± 0.10	0.084^c ± 0.0	47.32^{bcd} ± 8.60
YFSL-3 + PFSL-9	1.28^{ab} ± 0.04	0.086^c ± 0.0	62.31^a ± 5.87
YFSL-3 + PFSL-32	1.36^{ab} ± 0.08	0.089^c ± 0.0	60.65^{ab} ± 1.73
YFSL-45 + PFSL-9	1.36^{ab} ± 0.06	0.16^a ± 0.00	58.96^{ab} ± 7.22
YFSL-45 + PFSL-32	1.2^{bc} ± 0.17	0.088^c ± 0.0	60.49^{ab} ± 0.81
LSD0.05	0.571^1	0.001^1	5.153^1

Table 8. Efficacy of bioinoculants on biochemical parameters of tomato fruit under screen house conditions. Data are reported as mean ± standard deviation, derived from the analysis of three replicates per sample. Letters highlight statistically significant differences among analyzed samples, based on a one-way ANOVA test ($p < 0.05$). Different letters within columns indicate differences according to Duncan's Multiple Range Test ($P ≤ 0.05$). Whereas *Control* sterilized distilled water, *Positive control* Topsin M@ 200ppm, *YFSL* Yeast, *PFSL* fluorescent *Pseudomonas*, *YFSL + PFSL* combinations of yeast and fluorescent *Pseudomonas* spp.

(0.0091 ± 0.001 U/L) showed maximum activity of peroxidase (POD) as compared to control (0.0072 ± 0.0004 U/L) and other treatments as shown in Fig. 4.

Efficacy of bioinoculants on defensive enzymatic activity in tomato fruit under screen house conditions
The chitinase activity was significantly higher in the YFSL-45 treatment (0.083 ± 0.002 mg/h/protein) compared to the control (0.053 ± 0.001 mg/h/protein). Following YFSL-45, treatments YFSL-3 + PFSL-32, YFSL-3, PFSL-32, and YFSL-3 + PFSL-9 exhibited chitinase activities of 0.074 ± 0.001 mg/h/protein, 0.067 ± 0.001 mg/h/protein, 0.064 ± 0.000 mg/h/protein, and 0.062 ± 0.001 mg/h/protein, respectively The highest concentration of the enzyme β-1-3-Glucanase was observed in treatment YFSL-45 + PFSL-32 (0.073 ± 0.001 mg/h/protein), surpassing the control (0.052 ± 0.003 mg/h/protein). This was followed by treatments YFSL-45 + PFSL-9 and YFSL-3 + PFSL-32, which exhibited concentrations of 0.064 ± 0.003 mg/h/protein and 0.055 ± 0.004 mg/h/protein, respectively, as depicted in Fig. 5.

Discussion
Bioinoculants or biocontrol agents are administered to plants to improve their growth, fruit yield, resistance to biotic and abiotic stress, and quality attributes of fruits and vegetables. In the present study, the *in-vitro* studies, epiphytic yeasts, and fluorescent *Pseudomonas* spp., isolated from different healthy vegetables and fruits showed suppressive effect against *Drechslera* sp. and *Fusarium solani* isolated from diseased tomatoes. These antagonistic
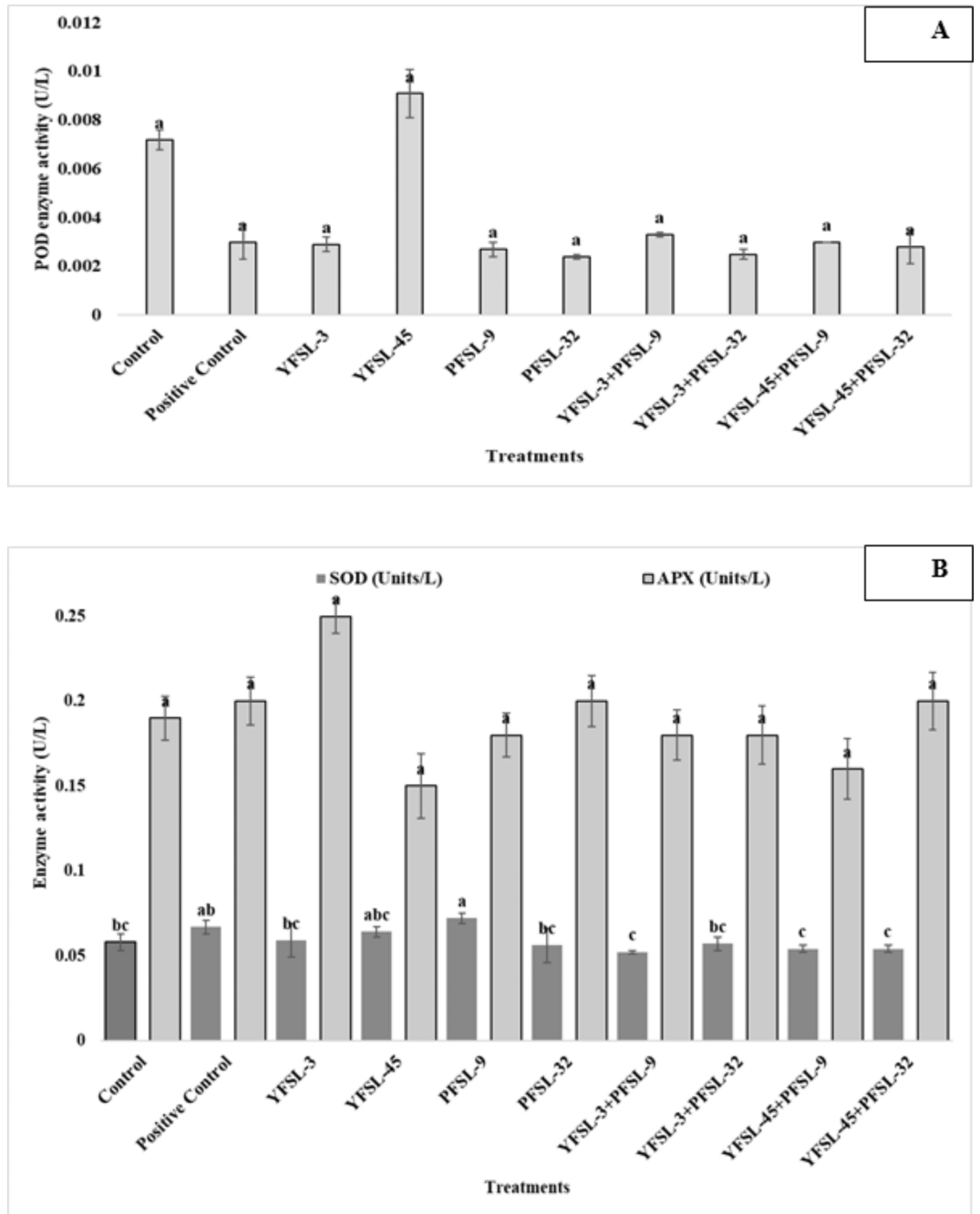
Fig. 4. The graphical representation of the enzymatic activity of tomato fruit grown under screen house inoculated with biocontrol agents separately and in combinations. Figure (**A**) Biocontrol efficacy of different isolates of yeast (YFSL) and fluorescent *Pseudomonas* spp. (PFSL) on the antioxidant enzyme (POD LSD$_{0.05}$=0.001) activity of tomato fruit grown under screen house. Figure (**B**) Biocontrol efficacy of different isolates of yeast (YFSL) and fluorescent *Pseudomonas* spp. (PFSL) on the antioxidant (SOD LSD$_{0.05}$= 0.003 and APX LSD$_{0.05}$=0.024) activity of tomato fruit grown in a screen house. Data are reported as mean $\pm$ standard deviation, derived from the analysis of three replicates per sample. Letters highlight statistically significant differences among analyzed samples, based on a one-way ANOVA test ($p < 0.05$). Different letters within columns indicate differences according to Duncan's Multiple Range Test ($P \leq 0.05$). Whereas *Control* sterilized distilled water, *Positive control* Topsin M@ 200ppm, *YFSL* Yeast, *PFSL* fluorescent *Pseudomonas*, *YFSL + PFSL* combinations of yeast and fluorescent *Pseudomonas* spp.
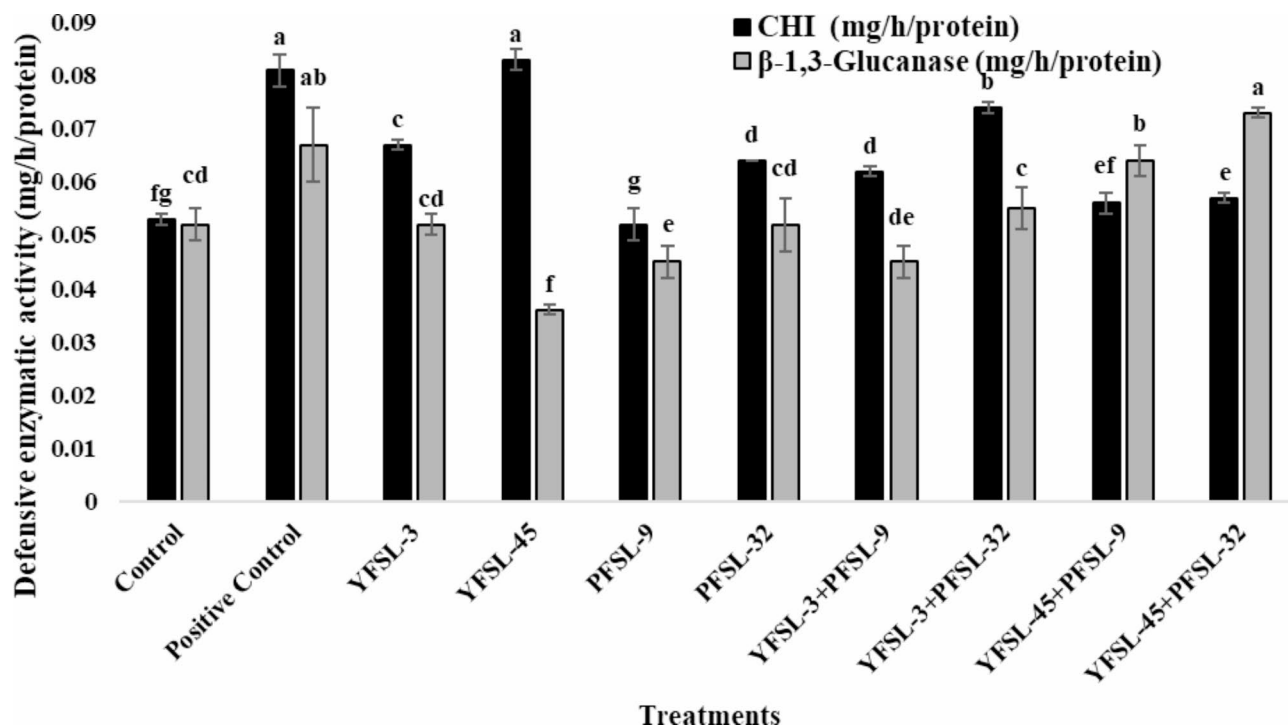
Fig. 5. The graphical representation of the defensive enzymatic activity (β-1-3-Glucanase & Chitinase) of tomato fruit grown under screen house inoculated with bio-inoculants individually and in combinations (LSD$_{0.05}$β-1-3-Glucanase = 0.004, CHI = 0.0.003). Data are reported as mean ± standard deviation, derived from the analysis of three replicates per sample. Letters highlight statistically significant differences among analyzed samples, based on a one-way ANOVA test ($p < 0.05$). Different letters within columns indicate differences according to Duncan's Multiple Range Test ($P ≤ 0.05$). Whereas *Control* sterilized distilled water, *Positive control* Topsin M@ 200ppm, *YFSL* Yeast, *PFSL* fluorescent *Pseudomonas*, *YFSL + PFSL* combinations of yeast and fluorescent *Pseudomonas* spp.

yeast isolates secrete diffusible antifungal substances which may retard the growth of fungal mycelium and form a zone of inhibition in dual cultures[62]. Whereas the attribute of producing secondary metabolites by fluorescent *Pseudomonas* is known for their growth retarding potential against phytopathogens[63] and the production of 2, 4-diacetyl phloroglucinol, a well-known secondary metabolite and antibiotic as well as phenazine derivative which is active against soil-inhabiting fungal pathogens including *Fusarium* and *Verticillium dahlia*[64,65].

In the *in-vivo* studies, the effective isolates of epiphytic yeast and fluorescent *Pseudomonas* spp. were used as soil drench separately as well as in combinations; it enhanced the plant's height and weight along with the root length and weight, the improved shoot and root growth is possibly attributed to the ability of fluorescent *Pseudomonas* to produce plant growth regulators[66]. Prior findings also reported vegetative shoot growth improvement in yeast-treated sugar beet plants[67]. Reduction in the root infection percentage by *F. solani* was observed in biocontrol-treated plants as compared to untreated plants, however, plants that were treated with yeast isolates showed the highest suppression against root-infecting fungi, *F. solani*. The ability of antagonist yeast to reproduce rapidly at the site of infection as well as attachment to, the tissues of the host plants and spores and hyphae of the pathogens might have retarded the growth of pathogenic fungi[68]. The antagonistic yeast also changes their gene expression and metabolism which often leads to the formation of the biofilm[69]. Microorganisms could change associations with pests and pathogens, and they can function as effective bioinoculants and act as plant growth promoters which enhances their value[70]. The combination of yeast and fluorescent *Pseudomonas* spp. was more operative than using each biocontrol agent individually which is like previous findings[71].

In this study, the effectiveness of yeast and fluorescent *Pseudomonas* spp. on physical and biochemical properties including antioxidant enzymes and defense-related enzymes of tomato plant and fruit under screen house conditions were studied. The biocontrol-treated plants showed elevated chlorophyll content which is supported by earlier reports[72–74]. The elevation in the content of chlorophyll in leaves might be due to cytokinins and some nutrients fixed by the BCAs in the soil. The treated tomato plants showed elevated levels of Phosphorus which may suggest the ability of the microorganism to solubilize phosphorus to provide plants as phosphorus is not readily available for plants in the absorbable form[75]. The utilization of microorganisms capable of solubilization of Phosphorus paves a new gateway for dissolving both fertilizer phosphorus and bond phosphorus.

In the present study, plants treated with epiphytic yeast and fluorescent *Pseudomonas* spp. showed a higher content of salicylic acid (SA), indicating their efficacy in mediating systemic resistance under stress conditions. Furthermore, tomato fruits exhibited an increase in SA content when grown in screen house conditions. Salicylic

acid is a phenolic complex that behaves like a plant hormone and promotes various physiological, biochemical, and molecular processes while protecting plant cells[76].

The present study portrays that biocontrol-treated plants and fruits showed higher antioxidant activities and total phenolic content under screen-house conditions. However, the combined treatment of yeast and fluorescent *Pseudomonas* spp. showed elevated levels of antioxidant activities in tomato fruits as compared to the individual bioinoculants. Phenols are an eminent class of secondary metabolites, and the total phenolic content of plants and their antioxidant activity is correlated, enabling them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers because of their redox properties. It is believed that the content of 1–3, 1-6-β-D-glucan, and protein fractions found in the cell wall mainly constitutes the antioxidant activity of yeast[77]. Moreover, it is reported[78] that the anti-oxidative activity of the *Saccharomyces cerevisiae* yeast cell wall proteins greatly exceeds that of β-glucan.

Tomatoes fruits from plants treated with fluorescent *Pseudomonas* spp. showed better fruit firmness compared to control and positive control by slowing down the process of ripening in order to enhance the shelf life. Firmness of fruit and its degree of maturity are interlinked, usually, as the fruit reaches maturity, a decrease in its firmness is observed. During ripening, the cell wall structure loosens and breaks down due to the solubilization and depolymerization of pectin[79].

In this study, titratable acidity and pH were seen less in tomato fruit obtained from individual and combined biocontrol-treated plants as compared to the fruits obtained from control (only water) and positive control fungicide-treated plants. The two most important quality attributes of the fruit are its pH and titratable acidity which are interlinked with each other. The indication of the pH in fruits is determined by their acid content, it has been reported that the pH of the fruit increases with the increase in the fruit's ripening stage. It is commonly believed that the decrease in TA during fruit maturation and over-ripening is due to a reduction in citric acid levels[80]. The acceptability and quality of fruit are determined by its titratable acidity[81].

All treatments resulted in increased total soluble solids (TSS) content. However, the combination of yeast and fluorescent *Pseudomonas* spp. resulted in the least increase in TSS as compared to untreated plants. These increases in TSS content may also be due to the improvement in chlorophyll content and other physiochemical activities[82]. In our study, we observed that the TSS content was lower in plants treated with biocontrol in comparison to untreated plants, along with lower pH and titratable acidity which are like the previous findings[83].

Several enzymatic and non-enzymatic antioxidants protect plants from the scavenging of free radicals[84]. In the present study, the treatment showed increased concentration of both antioxidant enzymes, (SOD, POX, and APX) as well as enzymes related to the defense system of plants (β-1-3-glucanases and Chitinases) in plants as well as fruit under screen house. The increased concentrations of SOD, POX, and APX in plants treated with bioinoculants might indicate the activation of the defense system against pathogens as compared to control (sterilized water) as it is also addressed in the previous findings that the stressed plants show their mechanism of tolerance by several physicochemical approaches, which includes many enzymatic complexes, such as superoxide dismutase (SOD), peroxidases (POX), ascorbate peroxidase (APX)[85]. During plant pathogen association, the generation of active oxygen species or the scavenging of active oxygen species is suppressed by the development of an antioxidant system of defense in plants which protects them against the damages caused by oxidative stress[86]. These enzymes take part in detoxifying active oxygen species rapidly (superoxide anion (O_2^-); hydrogen peroxide (H_2O_2) singlet oxygen (O^-) into water. This rapid conversion inhibits the toxic effect of active oxygen species (AOS) on the host plant. Plants induce resistance with defense enzymes, such as Chitinase and β-1,3-Glucanase[87]. Chitinase and β-1,3-glucanase manage the disintegration of cell wall components in sequence such as chitin and β-1,3-glucans[88]. The utilization of the antagonistic yeast and fluorescent *Pseudomonas* spp. as bioinoculants against pre-harvest infections in tomatoes proved them as an effective candidate, and alternative to chemical fungicides with no environmental hazards.

Conclusions

This study examined the bio-regulatory effects of epiphytic yeast and fluorescent *Pseudomonas* on fungal infections and losses in tomato plants. The findings indicated that the application of these agents, both individually and in combination, enhances the physical and chemical parameters of tomato plants. This includes increased total chlorophyll, carbohydrates, phosphorus, salicylic acid, total phenolic content, and antioxidant and defense-related enzymes. The use of these bioinoculants not only improves plant quality but also presents an opportunity for sustainable tomato production. Overall, the results suggest that these bioinoculants can significantly enhance both the quality and quantity of tomatoes, contributing positively to global food security.

Data availability

Data is provided within the manuscript or supplementary information files.

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Author contributions

A.A and S.S: Experimentation, original draft writingH: Research supervisor/ principal investigator, Conceptualization, and final revisions A.R: facilitation and guidance for in-vitro studies, reviewer S.N : co-investigator, provide a revision to the written draftK.S: co- provides resources and a revision to the written draft.

Declarations

Competing interests

The authors declare no competing interests.

Experimental research on plants including the collection of plant material

The authors declare that the cultivation of plants and the study conducted in the screen house located at the Biological Research Center (BRC), University of Karachi (UoK), complies with all relevant institutional, national, and international guidelines and treaties.

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The authors declare that the seed specimens used in this study are publicly accessible seed materials.

Additional information

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