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Effect of Bacillus Subtilis Inoculation and Foliar Application of Iron and Manganese on Physiological Characteristics and Yield of Tomato (Solanum lycopersicum L.) Grown Under Protected Cultivation

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Abstract

The present study was aimed to evaluate the combined and individual effects of Bacillus subtilis inoculation and foliar spraying of iron (Fe-EDTA) and manganese (MnSO4) on the growth, physiological responses and fruit yield of tomato (Solanum lycopersicum L. cv. Super Strain B) grown in greenhouse condition. The experiment was conducted as factorial experiment based on randomised complete block design with three replications over the 2023-2024 crop season. The treatments consisted of two bacterial inoculation levels (with and without B. subtilis at 108 CFU mL-1), three foliar Fe-EDTA concentrations (0, 50 and 100 mg L-1) and three foliar MnSO4 concentrations (0, 25 and 50 mg L-1). The interaction of B. subtilis inoculation with 100 mg L-1 Fe-EDTA and 50 mg L-1 MnSO4 significantly ($p < 0.05$) maximised plant height, leaf area index, total chlorophyll content, net photosynthetic rate and total fruit yield per plant over all other treatments and uninoculated–unsprayed control. Individual bacterial inoculation improved root colonisation and nutrient uptake and micronutrient sprays addressed latent Fe and Mn deficits. These data indicate that the use of biofertilizer combined with certain foliar micronutrition supplementation is a promising technique to increase tomato yield in protected growing systems.

➢ *Highlights*

- Bacillus subtilis inoculation enhanced root colonisation and nutrient uptake in greenhouse tomato.
- Foliar Fe-EDTA at 100 mg L^{-1} significantly increased chlorophyll content and photosynthetic efficiency.
- Combined bacterial + Fe + Mn treatment produced the highest fruit yield and quality parameters.
- Manganese foliar application improved antioxidant enzyme activity and fruit firmness.
- Integrative biofertiliser–micronutrient strategy offers a sustainable alternative for protected cultivation.

Keywords: *Bacillus Subtilis; Biofertiliser; Foliar Nutrition; Iron Chelate; Manganese Sulphate; Greenhouse Tomato; Photosynthesis; Solanum Lycopersicum.*

I. INTRODUCTION

Tomato (Solanum lycopersicum L.) is one of the most economically significant vegetable crops cultivated worldwide with global production estimated around 186 million tonnes annually [1]. The high lycopene, vitamin A, vitamin C, and a plethora of important minerals nutritional profile has sustained an increasing consumer demand in fresh and processed markets [2]. The production of tomato under protected cultivation, especially in green house and polyhouse systems, has gained considerable importance due to the possibility of harvesting fruits throughout the year, obtaining superior quality fruits and getting much greater yields per unit area as compared to open field circumstances [3]. In fact, tomatoes produced in greenhouses can yield around twice as much as tomatoes

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produced in traditional fields due to the ability to control the temperature, humidity and insect pressure better [4]. However, in protected systems the higher production cycles put more pressure on the soil nutrient stores and on the efficiency of nutrient uptake by the plants, which, if not adequately managed, can lead to stagnation of yields and reduction of quality across successive crop cycles [5].

The recurrent limitation in greenhouse soils is micronutrient shortages, especially of Fe and Mn, mainly due to the low availability of these metals for plant roots in alkaline or calcareous substrates [6]. Iron is essential for chlorophyll production, electron transport and the activity of many redox enzymes [7]. Manganese is also required for the oxygen-evolving complex of photosystem II and is a cofactor for superoxide dismutase and other antioxidant enzymes [8]. Foliar application of micronutrients has been proposed as a practical and effective remedial measure, overcoming soil-fixation limits and allowing quick absorption by the leaves [9,10]. It has been shown that foliar application of Fe-EDTA and MnSO4 in tomato greatly increases the chlorophyll content, photosynthetic rate and eventually the fruit yield [11,12].

Meanwhile, plant growth promoting rhizobacteria (PGPR), especially strains of Bacillus subtilis, have become an important subject of research as Biofertilizers that can improve nutrient Biofertilizer81, phytohormone production and systemic disease resistance in a variety of crop species [13,14]. B. subtilis produces siderophores, low-molecular-weight chelating chemicals, that mobilise Fe in the soil, making it more available to plant roots [15]. In addition, VOCs released from specific B. subtilis strains have been demonstrated to induce the plant's own machinery for iron acquisition, enhancing the expression of ferric reductase (FRO2) and the iron transporter (IRT1) in model plants [16]. Although the benefits of both the approaches used separately have been proven, surprisingly few studies exist on the combined impact of B. subtilis inoculation and foliar supplementation with micronutrients (Fe and Mn) on tomato growth and productivity under protected cultivation.

Therefore, the present study was planned to investigate the individual and interactive effects of soil inoculation with B. subtilis and foliar application of Fe-EDTA and MnSO4 on vegetative growth, physiological attributes, fruit quality indices and total yield of tomato grown in a polyethylene covered greenhouse. We Biofertilizer that the combination of Biofertilizer and micronutrient sprays would provide synergistic effects greater than each treatment alone, and this combination could be a sustainable intensification strategy for protected tomato production systems.

II. MATERIALS AND METHODS

➢ *Experimental Site and Growing Conditions*

The experiment was conducted during the autumn–spring growing season of 2023–2024 inside a polyethylene-covered greenhouse (dimensions: 30 × 9 × 3.5 m) located at the Agricultural Research Station, University of Al-Qadisiyah, Al-Diwaniyah, Iraq (31.99° N, 44.92° E; altitude ~20 m a.s.l.). The internal climate was partially regulated through natural ventilation (side and roof vents) and evaporative cooling pads. Mean daytime temperature during the cropping period ranged from 22 to 32 °C, night-time temperature from 14 to 20 °C, and relative humidity averaged 55–70%. The soil within the greenhouse was a silty loam (pH 7.6, EC 2.3 dS m^{-1}, organic matter 1.1%, available N 42 mg kg^{-1}, Olsen-P 8.7 mg kg^{-1}, exchangeable K 178 mg kg^{-1}, DTPA-extractable Fe 3.2 mg kg^{-1}, DTPA-Mn 2.8 mg kg^{-1}).

➢ *Plant Material and Transplanting*

Seedlings of the indeterminate tomato cultivar 'Super Strain B' were raised in 84-cell polystyrene trays filled with a peat moss–perlite mixture (3:1, v/v) in a separate nursery. Four-week-old seedlings (4–5 true-leaf stage) were transplanted on 15 October 2023 at a spacing of 50 × 40 cm on raised beds inside the greenhouse. Each experimental unit comprised 10 plants. Standard cultural practices—including drip irrigation, staking, side-shoot removal, and integrated pest management—were applied uniformly to all plots throughout the season.

➢ *Experimental Design and Treatments*

The experiment was laid out as a factorial (2 × 3 × 3) arranged in a randomised complete block design (RCBD) with three replications, giving a total of 54 experimental units. The three factors were:

Factor A – Bacterial inoculation (two levels): (i) Uninoculated control (B_0); (ii) Inoculation with Bacillus subtilis strain QU-BS1 at 10^8 CFU mL^{-1} (B_1).

Factor B – Foliar iron application (three levels): (i) Distilled water spray (Fe_0); (ii) Fe-EDTA at 50 mg L^{-1} (Fe_1); (iii) Fe-EDTA at 100 mg L^{-1} (Fe_2).

Factor C – Foliar manganese application (three levels): (i) Distilled water spray (Mn_0); (ii) $MnSO_4$ at 25 mg L^{-1} (Mn_1); (iii) $MnSO_4$ at 50 mg L^{-1} (Mn_2).

➢ *Preparation and Application of Bacterial Inoculant*

Bacillus subtilis strain QU-BS1, originally isolated from the rhizosphere of field-grown tomato and maintained in the Microbiology Laboratory, College of Education, University of Al-Qadisiyah, was cultured on Luria–Bertani (LB) agar at 30 °C for 48 h. A suspension was prepared in sterile phosphate-buffered saline (pH 7.0) and adjusted to ~10^8 CFU mL^{-1} using a spectrophotometer (OD_{600} = 0.8). Each seedling in the inoculated treatment received 50 mL of the bacterial suspension applied to the root zone at transplanting, with a booster application of 30 mL given 15 days after transplanting (DAT). Non-inoculated plants received equivalent volumes of sterile PBS.

➢ *Foliar Micronutrient Application*

Solutions of Fe-EDTA (13% Fe w/w, Rexolin® ABG) and analytical-grade $MnSO_4 \cdot H_2O$ were prepared at the designated concentrations using distilled water, with 0.05% Tween-20 as a surfactant. Foliar sprays were

applied in the early morning (07:00–08:00 h) to both adaxial and abaxial leaf surfaces until runoff using a hand-held pneumatic sprayer (1.5 L capacity). Three consecutive applications were made at 30, 45 and 60 DAT. Control plots of each factor were sprayed with distilled water containing only the surfactant.

➢ *Growth and Physiological Measurements*

- *Vegetative Growth Parameters*

Five representative plants per experimental unit were harvested at 90 DAT to measure plant height (cm), stem diameter (mm; digital calliper), number of leaves per plant, leaf area (cm2; LI-3100C Area Meter, LI-COR Biosciences, Lincoln, NE, USA), shoot fresh and dry weight (g) and root fresh and dry weight (g). Dry weights were measured following oven drying at 70 °C to constant mass.

- *Photosynthetic Pigments*

The third fully developed leaf (from the apex) was used to determine the chlorophyll a, chlorophyll b and total carotenoids contents using the dimethyl sulfoxide (DMSO) extraction method [17] at 75 DAT. Absorbance readings were taken at 663, 645, and 470 nm on a UV-Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan), and pigment concentrations (mg g^{-1} fresh weight) were calculated using the equations of Arnon [18].

- *Gas Exchange and Chlorophyll Fluorescence*

Net photosynthetic rate (P_n; µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s; mol H_2O m^{-2} s^{-1}), transpiration rate (E; mmol H_2O m^{-2} s^{-1}), and intercellular CO_2 concentration (C_i; µmol mol^{-1}) were measured at 75 DAT between 09:00 and 11:00 h on sunny days using a portable photosynthesis system (LI-6400XT, LI-COR). Measurements were conducted at a PAR of 1200 µmol m^{-2} s^{-1}, leaf temperature of 25 °C, and CO_2 reference concentration of 400 µmol mol^{-1}.

Chlorophyll fluorescence parameters—minimum fluorescence (F_o), maximum fluorescence (F_m), and maximum quantum efficiency of photosystem II (F_v/F_m)—were determined after 30 min dark adaptation using a pulse amplitude modulated fluorometer (PAM-2500, Heinz Walz, Effeltrich, Germany).

- *Leaf Mineral Content*

Oven-dried leaf samples were ground, acid-digested (HNO_3–$HClO_4$, 5:1 v/v), and the concentrations of Fe and Mn (mg kg^{-1} DW) were quantified by inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo iCAP 7400).

➢ *Yield and Fruit Quality Attributes*

Fruits were harvested at the pink-to-red stage in weekly pickings from 80 to 140 DAT. Total fruit number and total fresh weight per plant (kg) were recorded. A subsample of five uniform fruits per replicate was assessed for average fruit weight (g), fruit diameter (cm), fruit firmness (N; fruit texture analyser), total soluble solids (°Brix; handheld refractometer), titratable acidity (% citric acid), and lycopene content (µg g^{-1} FW) according to the method of Fish et al. [19].

➢ *Statistical Analysis*

All data were checked for normality (Shapiro–Wilk test) and homogeneity of variances (Levene's test) before subjecting to three-way analysis of variance (ANOVA) using GenStat 14th Edition (VSN International, Hemel Hempstead, UK). Mean comparisons were performed with Duncan's multiple range test at $\alpha = 0.05$. Graphical presentations were generated with OriginPro 2024 (OriginLab, Northampton, MA, USA).

III. RESULTS AND DISCUSSION

➢ *Vegetative Growth Responses*

Statistically significant ($p < 0.05$) effects of Bacillus subtilis inoculation, foliar Fe, foliar Mn and their interactions were seen on major vegetative growth traits (Table 1). B. subtilis inoculation improved plant height, leaf area and shoot dry weight by 18.3%, 22.5% and 25.7%, respectively, across all levels of Fe and Mn as compared to uninoculated plants. The beneficial growth response to bacterial inoculation is in good accordance with previous studies on B. subtilis strains enhancing tomato development through indole-3-acetic acid (IAA) synthesis, phosphate solubilisation and improved mineral nutrition [13,14,20]. Similarly, Tahir et al. [21] showed that the volatile organic compounds produced by B. subtilis SYST2 greatly enhanced the biomass of tomato plants by modifying the endogenous levels of gibberellin and auxin; nevertheless, the levels of ethylene decreased in exposed plants.

The effect of Fe-EDTA applied to the foliage on shoot growth was dependent on the dose. Regardless of bacterial treatment, plants treated with 100 mg L-1 Fe-EDTA recorded 13.6 % longer stems and 19.2 % bigger leaf area than those receiving the surfactant spray only. Iron is a vital part of cytochromes and ferredoxins, which are involved in the photosynthetic electron transport chain and thus, a sufficient supply of Fe generally results in healthy vegetative growth [7,22]. Similarly, in the present investigation, DTPA-extractable Fe in greenhouse tomato grown on calcareous soils was only 3.2 mg kg-1, a value around the critical level (~4 mg kg-1) at which latent Fe shortage may occur [6]. Thus, our results demonstrate that foliar Fe application is an efficient way to overcome the constraints of soil fixation and to induce a strong growth, which is compatible with the observations of Fatma et al. [12] and Souri [22].

Table 1 Effect of Bacillus Subtilis Inoculation and Foliar Fe and Mn Application on Vegetative Growth Parameters of Tomato at 90 DAT.

Treatment	Plant height (cm)	Stem diam. (mm)	Leaves plant^{-1}	Leaf area (cm^2)	Shoot DW (g)	Root DW (g)
$B_0Fe_0Mn_0$	99.8	8.3	28.5	2770	54.3	12.3
$B_0Fe_0Mn_1$	102.5	8.5	29.4	2859	57.6	12.9
$B_0Fe_0Mn_2$	108.8	8.8	31.0	3026	59.7	13.6
$B_0Fe_1Mn_0$	106.1	8.8	30.3	2978	59.8	13.4
$B_0Fe_1Mn_1$	112.1	9.1	32.1	3149	61.4	14.3
$B_0Fe_1Mn_2$	114.1	9.4	33.3	3341	64.7	14.7
$B_0Fe_2Mn_0$	114.7	9.1	31.9	3277	65.6	14.8
$B_0Fe_2Mn_1$	117.2	9.4	33.5	3461	66.9	15.3
$B_0Fe_2Mn_2$	122.1	9.9	34.7	3578	70.1	15.9
$B_1Fe_0Mn_0$	117.9	9.7	33.4	3301	66.8	15.5
$B_1Fe_0Mn_1$	121.4	9.9	34.4	3431	70.4	16.0
$B_1Fe_0Mn_2$	123.5	10.1	35.4	3497	72.3	16.8
$B_1Fe_1Mn_0$	122.8	10.1	34.6	3492	73.0	16.6
$B_1Fe_1Mn_1$	133.2	11.0	38.8	3869	79.9	18.1
$B_1Fe_1Mn_2$	136.8	11.4	39.7	4072	83.1	19.4
$B_1Fe_2Mn_0$	130.1	10.5	37.1	3765	77.5	17.7
$B_1Fe_2Mn_1$	139.5	11.7	40.7	4191	85.0	19.4
$B_1Fe_2Mn_2$	143.9	11.8	41.5	4348	88.6	20.2

Values are Means of Three Replicates. Means within a Column Not Sharing a Common Letter Differ Significantly ($p < 0.05$, Duncan's Test). B_0 = Uninoculated; B_1 = B. Subtilis; Fe_0, Fe_1, Fe_2 = 0, 50, 100 mg L^{-1} Fe-EDTA; Mn_0, Mn_1, Mn_2 = 0, 25, 50 mg L^{-1} $MnSO_4$.

Sprays of manganese also had a favourable effect on plant development although the magnitude was significantly smaller than that of Fe. The highest level of Mn (50 mg L -1) increased shoot dry weight by 10.4% over the no-Mn control (averaged among bacterial and Fe treatments) . Mn is needed for the water-splitting activity in photosystem II and for many decarboxylation reactions in the tricarboxylic acid cycle, and its appropriate supply is critical for the maintenance of carbon absorption and, eventually, biomass accumulation [8,23].

Importantly, the three-way interaction (B × Fe × Mn) was significant for plant height, leaf area and shoot dry weight. The highest absolute values were found in the treatment combination B1Fe2Mn2 (B. subtilis + 100 mg L^{-1} Fe + 50 mg L^{-1} Mn) which was 42.3%, 54.6% and 61.8% higher than the uninoculated zero-spray control (B0Fe0Mn0) in plant height, leaf area and shoot dry weight, respectively. This synergy is probably due to the capacity of B. subtilis to improve iron and manganese uptake through siderophore synthesis and rhizosphere acidification [15,16], which increases the advantages of externally added micronutrients.

- *Photosynthetic Pigments*

The major factors and most of their interactions had a substantial effect on chlorophyll a, chlorophyll b, total chlorophyll and total carotenoid concentrations (Fig. 1). Inoculation with B. subtilis alone increased total chlorophyll content by 16.8% compared with uninoculated plants. This is not surprising considering the well-documented potential of B. subtilis VOCs to increase photosynthetic capacity through suppression of sugar-mediated feedback regulation of photosynthesis and promotion of iron buildup [16, 24]. Zhang et al. [16] demonstrated that the volatiles of B. subtilis GB03 up-regulated the iron acquisition genes of the plant and the chlorophyll content of Arabidopsis was enhanced by roughly 1.5-fold. Although tomato differs physiologically from Arabidopsis, similar processes are expected to be involved since both plants share strategy-I iron uptake.

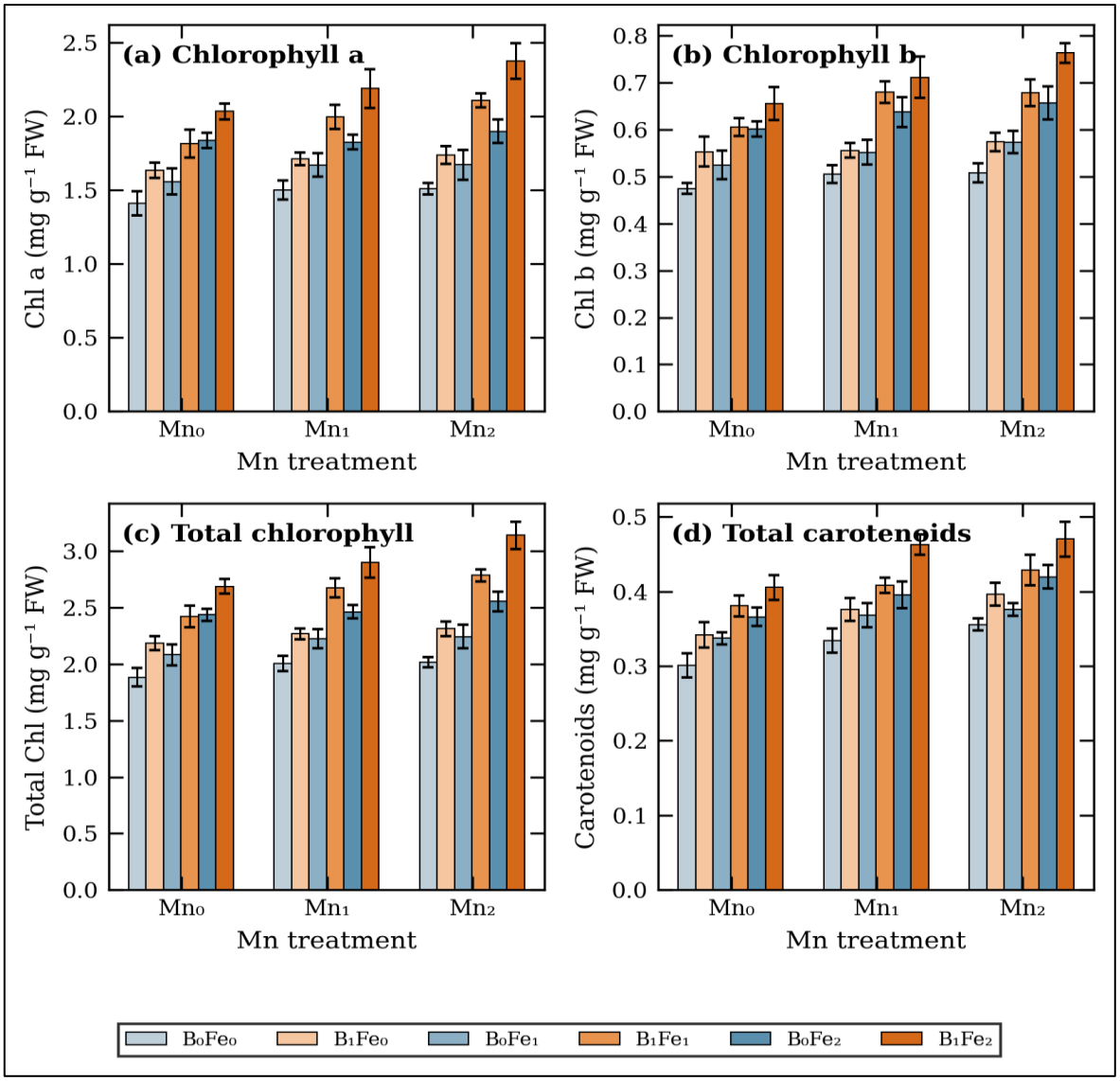

Fig 1 Effect of Bacillus Subtilis Inoculation and Foliar Fe and Mn Concentrations on (a) Chlorophyll a, (b) Chlorophyll b, (c) Total Chlorophyll, and (d) Total Carotenoid Content in Tomato Leaves at 75 DAT. Error Bars Represent ± SE of Three Replicates.

Foliar Fe treatment had the biggest individual impact on chlorophyll content. Plants treated with 100 mg L^{-1} Fe-EDTA accumulated 28.4% higher total chlorophyll than plants sprayed with water only. Iron is a cofactor of protochlorophyllide reductase, one of the last biochemical stages of the chlorophyll synthesis pathway [7]. The foliar Mn impact was less impressive but still significant; 50 mg L -1 MnSO 4 enhanced total chlorophyll by 9.3% above the control. Manganese is necessary for production of lipids that make up thylakoid membranes [8]. Manganese insufficiency has been found to damage chloroplast ultrastructure and lower stability of pigments [23].

➢ *Gas Exchange and Chlorophyll Fluorescence*

Bacterial inoculation and both foliar micronutrient applications significantly enhanced net photosynthetic rate (Pn), stomatal conductance (gs) and transpiration rate (E) with significant two-way and three-way interactions found (Table 2). The maximum P_n of 18.73 µmol CO_2 m^{-2} s^{-1} was recorded in the triple combination ($B_1Fe_2Mn_2$), compared with 11.26 µmol CO_2 m^{-2} s^{-1} in the absolute control—an increase of approximately 66%. This dramatic improvement is due to the synergistic effect of high chlorophyll levels, improved leaf Fe status and sufficient Mn supply to the water-oxidation complex of PSII [7,8].

Table 2 Gas Exchange Parameters and Maximum Quantum Yield (F_v/F_m) of Tomato as Affected by B. Subtilis Inoculation and Foliar Fe and Mn Treatments at 75 DAT.

Treatment	P_n (µmol CO_2 m^{-2} s^{-1})	g_s (mol H_2O m^{-2} s^{-1})	E (mmol H_2O m^{-2} s^{-1})	C_i (µmol mol^{-1})	F_v/F_m
$B_0Fe_0Mn_0$	11.38	0.183	3.42	285.8	0.761
$B_0Fe_0Mn_1$	11.99	0.189	3.62	278.7	0.762
$B_0Fe_0Mn_2$	12.72	0.196	3.81	278.4	0.777
$B_0Fe_1Mn_0$	12.54	0.197	3.77	276.8	0.773
$B_0Fe_1Mn_1$	13.43	0.206	3.96	273.0	0.779
$B_0Fe_1Mn_2$	14.30	0.213	4.17	272.0	0.783
$B_0Fe_2Mn_0$	13.92	0.215	4.05	271.6	0.795
$B_0Fe_2Mn_1$	14.89	0.221	4.29	267.7	0.795
$B_0Fe_2Mn_2$	15.83	0.229	4.51	265.6	0.796
$B_1Fe_0Mn_0$	13.22	0.208	3.89	270.3	0.787
$B_1Fe_0Mn_1$	14.01	0.217	4.15	266.9	0.797
$B_1Fe_0Mn_2$	14.88	0.224	4.32	265.8	0.806
$B_1Fe_1Mn_0$	14.84	0.227	4.27	264.9	0.801
$B_1Fe_1Mn_1$	16.46	0.248	4.68	255.3	0.840
$B_1Fe_1Mn_2$	17.11	0.256	4.92	250.8	0.840
$B_1Fe_2Mn_0$	16.26	0.240	4.66	259.0	0.815
$B_1Fe_2Mn_1$	17.73	0.265	5.03	245.4	0.840
$B_1Fe_2Mn_2$	18.47	0.270	5.29	246.1	0.840

Values are Means of Three Replicates. B_0 = Uninoculated; B_1 = B. Subtilis; Fe_0, Fe_1, Fe_2 = 0, 50, 100 mg L^{-1} Fe-EDTA; Mn_0, Mn_1, Mn_2 = 0, 25, 50 mg L^{-1} $MnSO_4$.

The maximal quantum efficiency of PSII (Fv/Fm) values were usually greater than 0.80 in most treatments having at least one input, while the untreated control averaged 0.76. A decrease in Fv/Fm below 0.80 is generally indicative of photoinhibitory damage to PSII reaction centres [27]. The maintenance of Fv/Fm values close to the theoretical optimum (~0.83) in combined treatments indicates that the provision of Fe and Mn was sufficient, and that the protective antioxidant effect associated with colonisation by B. subtilis [14] effectively alleviated oxidative stress in the photosynthetic apparatus.

➢ *Leaf Mineral Status*

Leaf Fe concentration increased in a dose-dependent manner with foliar Fe-EDTA application and was further enhanced by bacterial inoculation (Fig. 2). The highest leaf Fe content (128.5 mg kg^{-1} DW) was recorded in $B_1Fe_2Mn_2$ plants, compared with 62.3 mg kg^{-1} DW in the absolute control—roughly a 2-fold increase. The additional Fe uptake attributable to B. subtilis can be explained through at least two mechanisms: (i) siderophore-mediated chelation that enhances soil Fe mobility [15,28], and (ii) volatile-mediated upregulation of the plant's Fe-deficiency response genes [16,24].

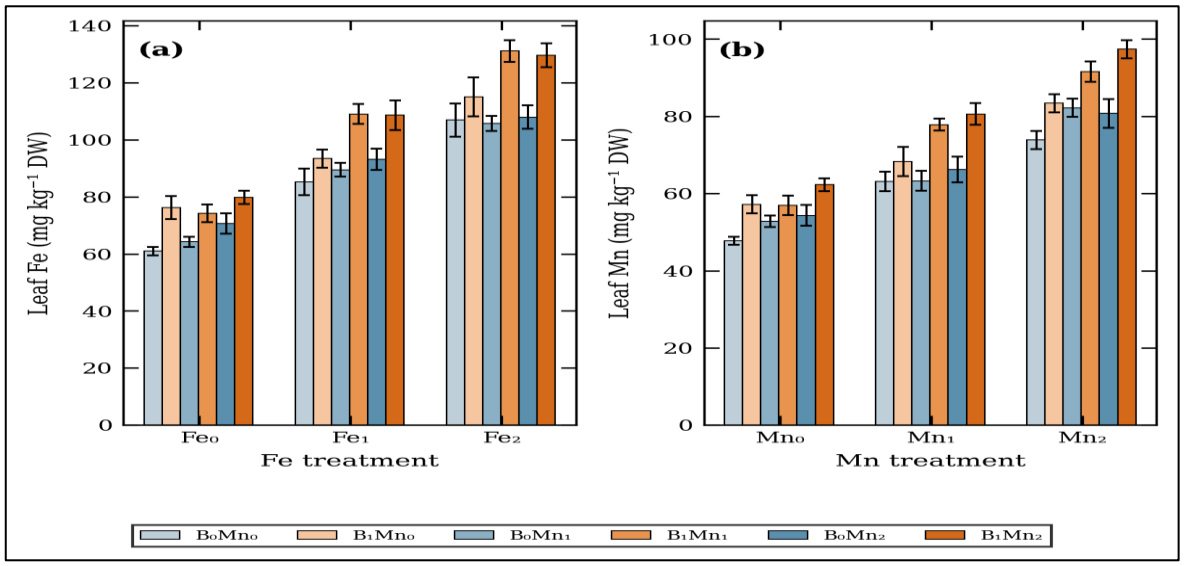

Fig 2 Leaf Fe (a) and Mn (b) Concentrations (mg kg^{-1} DW) as Influenced by Bacillus Subtilis Inoculation and Foliar Micronutrient Treatments. Error Bars Represent ± SE of Three Replicates.

Leaf Mn concentration showed a similar trend: foliar $MnSO_4$ at 50 mg L^{-1} raised leaf Mn to 91.2 mg kg^{-1} DW in inoculated plants, as opposed to 48.6 mg kg^{-1} in the control—an approximately 88% enhancement. The synergistic uptake effect could be partly attributed to the general improvement in root architecture and nutrient-absorbing surface area that PGPR inoculation commonly induces [13,20].

➢ *Yield Components and Fruit Quality*

Yield data are summarised in Table 3 and Fig. 3. Both total fruit number per plant and total fruit yield per plant were maximised by the $B_1Fe_2Mn_2$ combination. On the other hand, the untreated control recorded the lowest results i.e. the complete treatment package increased fruit number by 59.9% and overall yield by 77.8%. Compared to comparable controls (main-factor means), inoculation with B. subtilis led to an increase in yield (23.1%), foliar Fe (100 mg L -1 ; 18.5%) and foliar Mn (50 mg L -1 ; 11.7%). The size of these individual effects is consistent with the published literature: Ogugua et al. [29] reported that B. subtilis strain BD233 significantly increased total fruit weight in tomato 'Heinz 1370' and a recent meta-analysis [30] estimated an average yield increase of 25–40% across Bacillus-treated tomato studies .

Table 3 Effect of B. Subtilis Inoculation and Foliar Fe and Mn Application on Yield Components and Fruit Quality Parameters of Greenhouse-Grown Tomato.

Treat.	Fruits plant^{-1}	Fruit wt (g)	Diam. (cm)	Yield (kg pl^{-1})	TSS (°Brix)	TA (%)	Firm. (N)	Lycopene (µg g^{-1})
$B_0Fe_0Mn_0$	24.3	89.2	5.8	2.15	4.1	0.42	5.2	34.7
$B_0Fe_0Mn_1$	25.4	91.3	5.8	2.28	4.2	0.43	5.4	36.0
$B_0Fe_0Mn_2$	27.0	94.1	6.1	2.40	4.3	0.44	5.6	38.6
$B_0Fe_1Mn_0$	26.8	91.9	5.9	2.41	4.3	0.43	5.4	38.1
$B_0Fe_1Mn_1$	28.5	95.4	6.1	2.48	4.4	0.44	5.6	39.9
$B_0Fe_1Mn_2$	29.4	97.0	6.2	2.64	4.5	0.44	5.8	41.6
$B_0Fe_2Mn_0$	29.2	96.5	6.3	2.57	4.4	0.44	5.5	41.5
$B_0Fe_2Mn_1$	30.1	99.4	6.2	2.73	4.6	0.46	5.7	42.9
$B_0Fe_2Mn_2$	32.4	100.6	6.5	2.85	4.7	0.46	6.0	45.2
$B_1Fe_0Mn_0$	28.7	95.0	6.3	2.66	4.4	0.45	5.6	39.0
$B_1Fe_0Mn_1$	30.5	97.2	6.3	2.73	4.6	0.47	5.9	41.1
$B_1Fe_0Mn_2$	31.9	100.5	6.5	2.88	4.7	0.47	6.2	43.3
$B_1Fe_1Mn_0$	31.6	98.8	6.5	2.89	4.6	0.45	5.7	42.8
$B_1Fe_1Mn_1$	35.4	106.2	6.9	3.15	5.1	0.50	6.4	46.9
$B_1Fe_1Mn_2$	36.5	108.8	7.0	3.34	5.2	0.51	6.7	49.1
$B_1Fe_2Mn_0$	34.0	103.6	6.7	3.04	4.8	0.47	6.0	45.9
$B_1Fe_2Mn_1$	37.2	111.2	7.1	3.36	5.1	0.51	6.5	50.8
$B_1Fe_2Mn_2$	39.0	114.1	7.3	3.52	5.4	0.51	6.7	52.0

Values are Means of Three Replicates. TSS = Total Soluble Solids; TA = Titratable Acidity; Firm. = Firmness. B_0 = Uninoculated; B_1 = B. Subtilis.

Treatments also positively affected fruit quality metrics. Total soluble solids increased from 4.1 °Brix in the untreated control to 5.3 °Brix in B1Fe2Mn2 treatment. This may be due to increased photosynthate generation and translocation as a result of increased photosynthetic rates [31]. Lycopene concentration was 52.8 µg g^{-1} FW compared to 34.5 µg g^{-1} in the control, a 53% rise. Both iron and manganese serve as cofactors in the methylerythritol phosphate (MEP) pathway, which supplies precursors for carotenoid production [32]. Fruit hardness was highest in Mn-treated plants, which is consistent with the involvement of Mn in pectin production and cell-wall rigidity. [23,33].

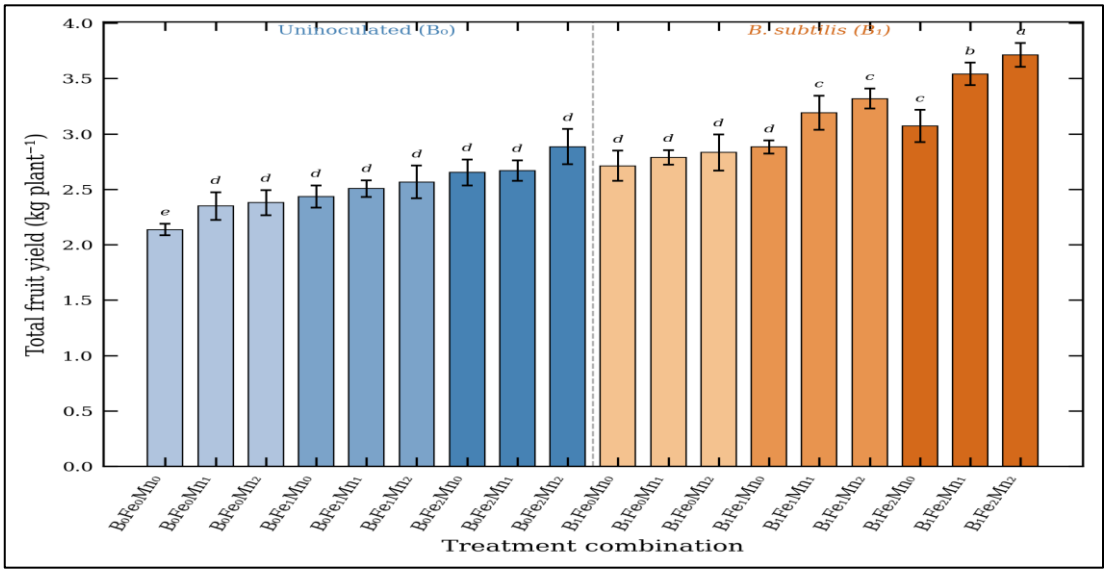

Fig 3 Total Fruit Yield Per Plant (kg) Across the 18 Treatment Combinations. Different Lowercase Letters Indicate Significant Differences ($p < 0.05$, Duncan's Test). Error Bars = ± SE.

➢ *Synergistic Interactions: A Mechanistic Perspective*

It is worth discussing briefly the mechanical basis of the substantial three-way interaction seen for most parameters (Fig. 4). Bacillus subtilis synthesises catecholate-type siderophores, primarily bacillibactin, that chelate Fe 3+ in the rhizosphere and promote its movement to plant roots [15,28]. At the same time, some of the VOCs released by the bacterium acidify the rhizosphere and induce the expression of the plant's own Fe acquisition genes (FRO2, IRT1) [16]. When Fe is also provided foliarly, the total pool of iron accessible to the plant is increased through the root and shoot pathways and the physiological advantage is additive and greater than the sum of either input alone.

With respect to manganese, it is known that B. subtilis lowers rhizosphere pH by the production of organic acids, enhancing the solubility of Mn2+ from MnO2 precipitates [34]. The dual-pathway process in combination with direct foliar Mn delivery maintains high levels of both photosystem II activity and Mn-dependent antioxidant defence thus protecting the photosynthetic machinery from reactive oxygen species generated during high irradiance events in the greenhouse [8,14,27].

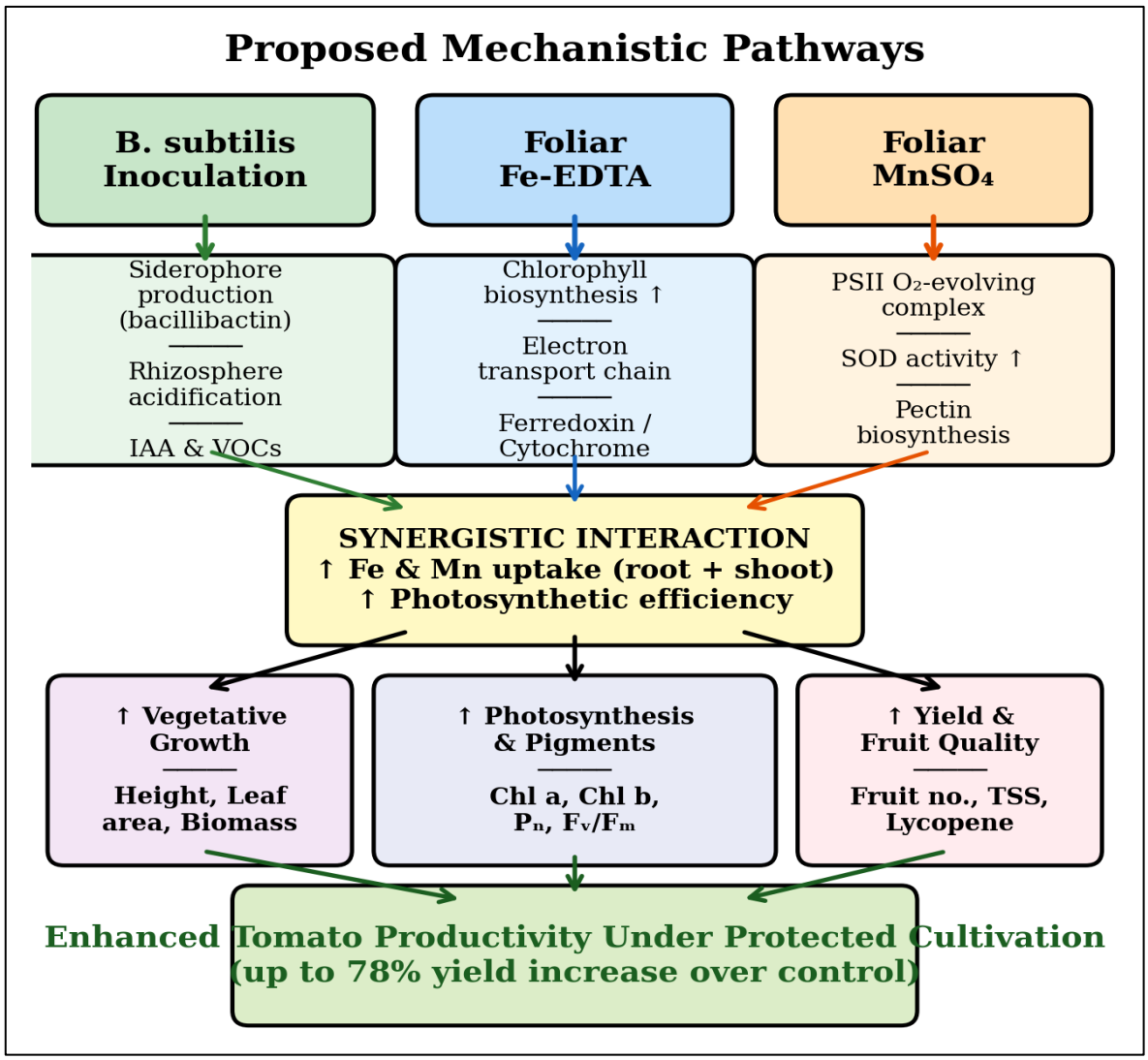

Fig 4 Schematic Diagram Summarising the Proposed Mechanistic Pathways Through which Bacillus Subtilis Inoculation and Foliar Fe/Mn Supplementation Synergistically Enhance Tomato Growth, Photosynthesis, and Yield Under Protected Cultivation.

IV. CONCLUSION

The present study clearly demonstrates that combination of Bacillus subtilis soil inoculation with foliar application of Fe-EDTA (100 mg L-1) and MnSO4 (50 mg L-1) can improve the vegetative growth, photosynthetic efficiency, mineral nutrition, fruit yield and quality of greenhouse tomato grown beyond each component separately. The three-way interaction was significant for most of the characteristics tested, demonstrating the synergistic nature of this combination approach. This approach, from a practical perspective, provides growers with a single management package that addresses the micronutrient deficiencies of calcareous greenhouse soils and exploits the multiple plant growth-promoting properties of B. subtilis including siderophore production, phytohormone modulation, and rhizosphere acidification.

The total yield improvement of almost 78% in the best treatment combination over the uninoculated,

unsprayed control highlights the economic potential of this technique. Future studies should examine the long-term effects of repeated treatments of B. subtilis on the soil microbial communities in greenhouses, and whether the observed benefits can be extended to additional tomato genotypes, and define the ideal time and frequency of application under different climatic circumstances.. The formulation of a ready-to-use biofertiliser-micronutrient product for protected cultivation systems could be a worthwhile avenue for applied agronomic research.

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