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Comparative effects of nano-iron, salicylic acid, and Rizolex fungicide on the growth and biochemical traits of tomato plants (*Solanum lycopersicum* L.)

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Abstract

This study aimed to evaluate the effects of nano-iron and salicylic acid on the physiological and biochemical traits of tomato (*Solanum lycopersicum* L.) in both laboratory and pot experiments. The results showed that the nano-iron significantly enhanced plant growth parameters, increased chlorophyll and carbohydrate contents, and reduced free proline and hydrogen peroxide accumulations, indicating improved photosynthetic efficiency and reduced oxidative stress. Additionally, the laboratory experiments demonstrated that the nano-iron improved nutrient uptake and stimulated metabolic activities, positively affecting cell integrity and antioxidant enzyme activity.

Salicylic acid increased fresh and dry weights of shoots and root systems, elevated chlorophyll and carbohydrate contents, and reduced proline and hydrogen peroxide, reflecting enhanced metabolic processes and physiological performance.

In contrast, the chemical fungicide Rizolex had limited impact on physiological traits, showing only slight increases in fresh and dry weight and chlorophyll content, with minimal effect on carbohydrates or oxidative stress indicators.

Overall, nano and natural treatments were more effective than chemical fungicides in promoting tomato growth and physiological efficiency, and combining nano-iron with salicylic acid could provide synergistic effects to enhance productivity and plant performance.

Keywords: Biochemical responses, nano-iron, physiological performance, salicylic acid, tomato (*Solanum lycopersicum* L.)

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops worldwide and in Iraq. It belongs to the family Solanaceae and represents a fundamental pillar of Iraqi agricultural production, as it is widely cultivated and significantly contributes to supporting the local economy and achieving food security. Data from the Food and Agriculture Organization (FAOSTAT, 2025) ^[21] indicate that tomato production in Iraq faces increasing challenges due to adverse environmental conditions and the widespread incidence of pests and plant diseases.

With the growing environmental awareness of the harmful effects of chemical pesticides on human health and soil quality, it has become necessary to move toward sustainable and eco-friendly alternatives. Among these alternatives, salicylic acid is considered an effective compound that stimulates systemic acquired resistance mechanisms in plants, thereby enhancing their ability to withstand pathogenic stresses (Abass, 2017) ^[1]. In addition, nanotechnology applications, such as nano-iron, have demonstrated significant potential in enhancing plant immunity and improving resistance to both biotic and abiotic stresses (Abd Oun, 2023) ^[4].

Treating tomato plants with silver nanoparticles under field conditions resulted in a positive effect, represented by an increase in the internal content of chlorophyll and carotenoid pigments, as well as an enhancement in the activity of the enzyme polyphenol oxidase, indicating their role in improving the physiological efficiency of the plant (Mahawar *et al.*, 2020) ^[35].

An experiment was conducted to study the effect of foliar spraying with salicylic acid on some physiological traits and the productivity of cherry tomato plants (cv. Batil) using four levels of salicylic acid. The results showed that the high concentration of salicylic acid (150) achieved significant increases in all studied traits, including NPK content, leaf area, and yield (AL-Mafargy *et al.*, 2020) [19]. This study aims to evaluate the efficiency of both nano-iron and salicylic acid in improving tomato seed germination parameters, as well as to assess the physiological responses and potential phytotoxic effects of these treatments on tomato plant growth.

Materials and Methods

Effect of Nano-Iron Oxide, Salicylic Acid, and Rizolex on Tomato Seed Germination under Laboratory Conditions.

Salicylic acid and nano-iron particles (30-50 nm) were obtained from Skyspring Nanomaterials Inc. (USA). The fungicide was obtained from Greenriver Industry Co., Ltd., a Chinese company specialized in the manufacture of agricultural pesticides. Tomato seeds (cv. Asma F1) were obtained from Green Gold Company.

The experiment was conducted in the laboratories of the Department of Plant Protection, College of Agriculture, University of Misan. Tomato (*Solanum lycopersicum* L.) seeds of the cultivar Asma F1 were used. Seeds were surface-sterilized with 10% sodium hypochlorite solution for 5 min, thoroughly rinsed with distilled water, and air-dried. Ten seeds were placed in each Petri dish lined with sterile filter paper and evenly distributed. Five milliliters of treatment solutions were added as follows: nano-iron oxide at concentrations of 100, 200, and 300 ppm; salicylic acid at concentrations of 0.5, 1.0, 1.5, 2.0, and 2.5 ppm; and the fungicide Rizolex at concentrations of 5, 10, and 20 ppm, in addition to a control treatment (distilled water). The Petri dishes were incubated at 25 ± 2 °C.

After 10 days of incubation, the following germination parameters were recorded:

1. **Peak value of germination (PV):** Calculated according to Czabator (1962) [17].
2. **Mean germination time (MGT):** Calculated following Ellis and Roberts (1981) [20].
3. **Mean daily germination (MDG):** Calculated using the equation of Czabator (1962) [17].
4. **Germination percentage (GP):** Calculated as:

$$GP (\%) = (\text{Number of germinated seeds} / \text{Total number of seeds}) \times 100.$$
5. **Germination rate (GR):** Calculated according to Czabator (1962) [17].
6. **Germination value (GV):** Calculated as:

$$GV = PV \times MDG \text{ (Czabator, 1962) [17].}$$
7. **Plumule and radicle length (cm):** Five normal seedlings were randomly selected from each treatment, and the lengths of plumule and radicle were measured and averaged (AOSA, 1983).
8. **Tolerance index (TI):** Calculated according to Iqbal and Rahmati (1992) [28].
9. **Seedling vigor index (SVI):** Calculated using the equation of Abdul-Baki and Anderson (1973) [3].

Effect of Nano-Iron Oxide, Salicylic Acid, and Rizolex on Tomato Plant Growth under Pot Conditions.

The pot experiment was conducted using plastic pots with a capacity of 3 kg, filled with sterilized soil. Tomato seedlings

(cv. Asma F1) were transplanted at a rate of three seedlings per pot, with three replicates per treatment.

Plants were treated with nano-iron oxide at concentrations of 100, 200, and 300 ppm, followed by salicylic acid at concentrations of 1, 1.5, and 2 ppm, and the fungicide Rizolex at concentrations of 5, 10, and 20 ppm, in addition to an untreated control. Treatments were applied as foliar sprays until runoff. Plants were maintained under greenhouse conditions and irrigated regularly.

At the end of the experimental period, the following growth, physiological, and biochemical parameters were evaluated:

Fresh and Dry Weight of Shoot and Root Systems (g)

Fresh weight was measured by harvesting three plants per treatment, cutting them at the soil surface, and immediately weighing them using a sensitive balance. For dry weight determination, samples were oven-dried at 60 °C for 72 h until constant weight and then weighed.

Chlorophyll pigments

Chlorophyll a, chlorophyll b, and total chlorophyll contents were determined according to Arnon (1949). Briefly, 200 mg of fresh leaf tissue was homogenized in 8 mL of 80% acetone, centrifuged at 3000 rpm for 10 min, and absorbance was recorded at 645 and 665 nm using a spectrophotometer. Pigment concentrations were calculated using the equations of Ranganna (1977).

Free proline content

Proline content was determined following Bates *et al.* (1973) [14]. 0.5 g of fresh leaf tissue was homogenized in 10 mL of 3% sulfosalicylic acid, centrifuged at 6000 rpm for 10 min, and reacted with acid ninhydrin reagent. After incubation at 100 °C for 1 h, absorbance was measured at 520 nm, and proline concentration was expressed as $\mu\text{mol g}^{-1}$ fresh weight.

Hydrogen peroxide (H₂O₂) content

Hydrogen peroxide content was determined according to Sergiev *et al.* (1997) [46]. Leaf tissue (0.5 g) was extracted in 0.1% trichloroacetic acid, centrifuged at 13,000 rpm for 15 min, and the supernatant was reacted with potassium phosphate buffer and potassium iodide. Absorbance was recorded at 390 nm, and H₂O₂ concentration was calculated using a standard curve.

Total carbohydrate content

Total carbohydrate content was determined according to Dubois *et al.* (1956) [18]. Dried leaf tissue (0.1 g) was extracted with 80% ethanol, reacted with phenol and concentrated sulfuric acid, and absorbance was measured at 490 nm using a spectrophotometer.

Experimental design and statistical analysis

Data were subjected to analysis of variance (ANOVA) under a completely randomized design (CRD) with three replicates. Means were compared using the Least Significant Difference (LSD) test at $p \leq 0.01$ for the laboratory experiment and $p \leq 0.05$ for the pot experiment.

Results and Discussion

Effect of nano-iron oxide on tomato seed germination parameters in petri dishes

The results presented in Table 1 revealed a significant effect of nano-iron oxide concentrations on all germination

parameters compared with the control treatment. The concentration of 300 ppm recorded the highest peak value of germination (0.96 seeds day⁻¹) compared with the control (0.60 seeds day⁻¹), indicating stimulation of early germination. In addition, the mean germination time (MGT) decreased significantly to 22.33 days at this concentration, compared with 27.33 days in the control, reflecting acceleration of the germination process.

The mean daily germination (MDG) reached its maximum value (1 seed day⁻¹) at 300 ppm, accompanied by the highest germination percentage (100%), whereas the control recorded 90%. Similarly, germination rate and germination value increased significantly, reaching 4.46 and 0.93, respectively, at 300 ppm, indicating enhanced germination efficiency and uniformity.

Regarding early seedling growth, the plumule and radicle lengths were significantly higher at 300 ppm (27.38 and 14.11 cm, respectively) compared with the control (7.62 and 4.43 cm, respectively), demonstrating a pronounced stimulatory effect on seedling development. Furthermore, the tolerance index (TI) and seedling vigor index (SVI)

increased markedly to 318.3 and 4149, respectively, at 300 ppm, compared with 100 and 1084.8 in the control. All differences among treatments were statistically significant at $p \leq 0.01$.

These results can be attributed to the unique physicochemical properties of nano-iron particles, including large surface area, high reactivity, and enhanced solubility, which facilitate their interaction with cellular membranes and internal cellular components, thereby improving physiological processes related to seed germination and early growth (Feng *et al.*, 2022)^[22]. Moreover, seed priming with metallic nanoparticles stimulates enzymatic activities such as amylase and protease, promoting the mobilization of stored reserves required for embryo development and germination (Abid *et al.*, 2022)^[5].

These findings are consistent with those reported by Madusanka *et al.* (2024), who demonstrated a biphasic effect of nano-iron oxide on seed vigor index, with significant enhancement observed at 100 ppm, suggesting improved germination and metabolic activity through multiple regulatory mechanisms.

Table 1: Effect of nano-iron oxide on tomato seed germination parameters in petri dishes

Treatments (ppm)	Peak value of germination	Mean germination time (days)	Mean daily germination (seeds day ⁻¹)	Germination percentage (%)	Germination rate	Germination value	Seedling length (cm)	Mean plumule length (cm)	Plumule length (cm)	Mean radicle length (cm)	Radicle length (cm)	Tolerance index	Seedling vigor index
Control	0.60	27.33	0.66	90.00	3.63	0.56	7.62	0.06	0.667	0.733	4.433	100.0	1084.8
100	0.66	26.66	0.96	90.00	3.73	0.63	8.61	0.047	0.600	0.827	5.013	113.1	1226.1
200	0.73	26.33	0.96	90.66	3.82	0.76	13.76	0.056	0.527	0.827	7.093	160.0	1889.8
300	0.96	22.33	1.00	100.0	4.46	0.93	27.38	0.067	0.650	1.387	14.11	318.3	4149
LSD (0.01)	0.27	3.91	0.094	7.69	0.69	0.17	6.74	0.016	0.117	0.808	6.77	15.65	14.604

Effect of salicylic acid on tomato seed germination parameters in petri dishes

The results showed that salicylic acid concentrations positively affected all germination and early growth parameters of tomato seeds. Peak germination reached its highest value at 2 ppm (1.93 seeds/day) compared to the control (1.633 seeds/day), indicating stimulation of early germination. Mean germination time (MGT) decreased from 31.667 seeds/day in the control to 25 seeds/day at the highest concentration, while mean daily germination and germination percentage increased, reaching 96.667% at 2 ppm (Table 2).

Germination rate and value also improved at the highest concentration, reflecting activation of physiological functions and enhanced germination efficiency. Seedling

and radicle lengths increased from 3.82 and 3.127 cm in the control to 6.447 and 4.267 cm, respectively, indicating stimulation of overall seedling and radicle growth. Quality indicators showed that the tolerance index increased from 100 to 136.5 and the vigor index from 602 to 1036 at 2 ppm, with no significant differences between 2 and 2.5 ppm.

Salicylic acid regulates plant growth, flowering, and nutrient uptake, affects ethylene production and stomatal movement, enhances photosynthetic efficiency, and modulates the activity of several enzymes (Yusuf *et al.*, 2013). Another study on the Rio Grande cultivar showed that seed soaking in salicylic acid did not negatively affect germination, but promoted the formation of secondary roots at 1 and 0.01 μ M concentrations (Dzib-Ek *et al.*, 2021)^[19].

Table 2: Effect of salicylic acid on germination parameters of tomato seeds in petri dishes

Treatment (ppm)	Peak Germination Value	Mean Germination Time (seeds/day)	Mean Daily Germination (seeds/day)	Germination (%)	Germination Rate (days)	Germination Value	Seedling Length (cm)	Mean Plumule Length (cm)	Plumule Length (cm)	Mean Radicle Length (cm)	Radicle Length (cm)	Tolerance Index	Seedling Vigor Index
Control	1.633	31.667	20	86.667	1.967	30.133	3.82	0.7	1.247	1.5	3.127	100	602
0.1	1.733	29.333	18	86.667	2.3	30.667	4.46	0.8	1.44	1.7	3.313	105.9	673
0.5	1.733	29.333	17.333	90.0	2.667	32.667	5.467	0.9	1.707	1.7	3.773	120.7	831.6
1	1.8	28.33	18	90.0	2.7	32.667	6.04	0.96	1.887	1.933	3.853	123.2	890.4
1.5	1.8	25	19.333	90.0	2.883	34.933	6.433	1.02	2.02	2.037	4.0	127.9	939
2	1.93	25	17	96.667	2.983	35.2	6.447	1.9	2.62	2.207	4.267	136.5	1036
2.5	1.90	26	17.333	94.0	2.98	34.991	6.44	1.8	2.61	2.2	4.26	135.1	1030
LSD (0.01)	0.32	5.93	3.243	4.21	1.319	2.73	0.385	0.1	0.154	0.244	0.668	7.708	30.159

Effect of Rizolex on tomato seed germination in petri dishes

The results indicated that Rizolex concentrations progressively improved all germination parameters of

tomato seeds. Peak germination increased from 0.7 in the control to 0.8, 0.9, and 1.3 at 5, 10, and 20 ppm, respectively, while mean germination time (MGT) decreased from 10 seeds/day in the control to 8.67 seeds/day

at the highest concentration. Daily germination rate, germination percentage (from 90% to 100%), germination speed, and germination value also increased as shown in Table 3.

Early seedling growth improved, with increases in seedling, shoot, and radicle lengths as the concentration increased. The highest tolerance and vigor indices were recorded at 20 ppm, confirming the effectiveness of Rizolex in enhancing

early germination, seedling growth, and seed vigor.

These improvements are attributed to the role of Rizolex in enhancing initial germination conditions, accelerating seedling emergence, reducing embryonic stress, and promoting seedling health and vegetative growth (Selim, 2019)^[38]. The active ingredient, Tolclofos-methyl, acts as a protective fungicide, preventing early infections and producing healthier, stronger seedlings.

Table 3: Effect of Rizolex pesticide on germination parameters of tomato seeds in petri dishes

Treatment (ppm)	Peak Germination Value	Mean Germination Time (seeds/day)	Mean Daily Germination (seeds/day)	Germination (%)	Germination Rate (days)	Germination Value	Seedling Length (cm)	Mean Plumule Length (cm)	Plumule Length (cm)	Mean Radicle Length (cm)	Radicle Length (cm)	Tolerance Index	Seedling Vigor Index
Control	1.633	31.667	20	86.667	1.967	30.133	3.82	0.7	1.247	1.5	3.127	100	602
0.1	1.733	29.333	18	86.667	2.3	30.667	4.46	0.8	1.44	1.7	3.313	105.9	673
0.5	1.733	29.333	17.333	90.0	2.667	32.667	5.467	0.9	1.707	1.7	3.773	120.7	831.6
1	1.8	28.33	18	90.0	2.7	32.667	6.04	0.96	1.887	1.933	3.853	123.2	890.4
1.5	1.8	25	19.333	90.0	2.883	34.933	6.433	1.02	2.02	2.037	4.0	127.9	939
2	1.93	25	17	96.667	2.983	35.2	6.447	1.9	2.62	2.207	4.267	136.5	1036
2.5	1.90	26	17.333	94.0	2.98	34.991	6.44	1.8	2.61	2.2	4.26	135.1	1030
LSD (0.01)	0.32	5.93	3.243	4.21	1.319	2.73	0.385	0.1	0.154	0.244	0.668	7.708	30.159

Effect of nano-iron oxide on fresh and dry weight of tomato plants in pots

The results (Table 4) indicated that nano-iron oxide concentrations (100, 200, 300 ppm) significantly influenced the fresh and dry weights of both shoot and root systems in tomato plants. The highest concentration (300 ppm) recorded the greatest fresh weight (290 mg for shoots and 142.2 mg for roots) and dry weight (19 mg for shoots and 3 mg for roots), with statistically significant differences compared to the control.

Nano-iron oxide is considered a promising alternative to conventional iron supplements in agriculture, as it enhances plant growth, increases fresh and dry weight, and stimulates secondary metabolite production by improving iron uptake and activating physiological processes within the plant (Irum *et al.*, 2020). Another study on soybean showed that 0.75 ppm of nano-iron oxide significantly increased fresh and dry weights of leaves and pods (Sheykhabglou *et al.*, 2010)^[39].

Table 4: Effect of nano-iron oxide on fresh and dry weight of shoot and root systems of tomato plants

Treatment (ppm)	Fresh Weight of Shoot (mg)	Fresh Weight of Root (mg)	Dry Weight of Shoot (mg)	Dry Weight of Root (mg)
Control	282	33	13	2.33
100	130	37	9	1.33
200	190	77	12.7	2.33
300	290	142.2	19	3
LSD 0.05	60	83.7	3.6	0.9

Effect of nano-iron oxide on biochemical components of tomato leaves

Chlorophyll content

Treatment with nano-iron oxide at concentrations of 100, 200, and 300 ppm significantly increased chlorophyll a, chlorophyll b, and total chlorophyll. The highest values were recorded at 300 ppm, with chlorophyll a, chlorophyll b, and total chlorophyll measuring 19.989, 6.663, and 26.652 mg g⁻¹, respectively, compared to 16.530, 5.510, and 22.040 mg g⁻¹ in the control. This indicates enhanced photosynthetic efficiency and improved leaf pigment composition (Broadley *et al.*, 2012; Briat *et al.*, 2015; Mai & Bauer, 2016; Singh *et al.*, 2024)^[16, 15, 32, 40].

Free proline content

Nano-iron oxide treatment resulted in a significant reduction in leaf free proline content. The lowest value was observed at 300 ppm (0.570 μmol g⁻¹) compared to 0.607 μmol g⁻¹ in the control, indicating reduced oxidative stress and better physiological status of the plants. This reflects the activation

of osmotic adjustment and stress adaptation mechanisms (Alhaithloul, 2023; Singh *et al.*, 2024)^[8, 40].

Hydrogen peroxide (H₂O₂) content

H₂O₂ levels decreased significantly with nano-iron treatment, reaching 0.850 mmol g⁻¹ at 300 ppm, compared to 1.295 mmol g⁻¹ in the control. This reduction is associated with enhanced activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), contributing to decreased oxidative damage in plant tissues (Manzoor *et al.*, 2023)^[34].

Carbohydrate content

The highest carbohydrate content was observed at 300 ppm (16.32 mg g⁻¹), significantly higher than the control (11.05 mg g⁻¹). This increase is likely due to enhanced photosynthetic performance, higher chlorophyll content, and improved iron uptake, which together stimulate sugar accumulation in plant tissues (Yoon *et al.*, 2019; Ghaseminezhad *et al.*, 2022)^[44, 24]. Table (5).

Table 5: Effect of nano-iron oxide on chlorophyll, proline, hydrogen peroxide, and carbohydrate content of tomato seedlings

Treatment (ppm)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)	Proline ($\mu\text{mol/g}$)	Hydrogen Peroxide (mM/g)	Carbohydrates (mg/g)
Control	16.530	5.510	22.040	0.607	1.295	11.05
100	16.071	5.357	21.428	0.627	1.215	10.91
200	18.423	6.141	24.564	0.577	1.092	14.37
300	19.989	6.663	26.652	0.570	0.850	16.32
LSD (0.05)	0.18	0.18	0.32	0.04	0.06	0.85

Effect of salicylic acid on fresh and dry weight of tomato plants

Results showed that salicylic acid at concentrations of 1, 1.5, and 2 ppm significantly affected the fresh and dry weight of both shoot and root systems. The highest increase was observed at 2 ppm, with fresh weight reaching 259m g for shoots and 148.3m g for roots, and dry weight reaching 52.7 mg for shoots and 3.3m g for roots, compared to the control values of 107 and 31.7m g (fresh weight) and 14 and 2m g (dry weight) for shoots and roots, respectively (Table 6).

Salicylic acid enhances fresh and dry weight by stimulating essential metabolic processes, including improved photosynthetic efficiency and protein synthesis, which directly contribute to biomass accumulation in both shoots and roots (Van *et al.*, 2022)^[2].

Table 6: Effect of salicylic acid on fresh and dry weight of shoot and root systems of tomato plants

Treatment (ppm)	Fresh Weight (mg)		Dry Weight (mg)	
	Shoot	Root	Shoot	Root
Control	107	31.7	14	2
1	124	12	7	1.3
1.5	139	99	12	1.7
2	259	146.3	25.7	3.3
LSD (0.05)	57	26.6	4.2	1

Effect of salicylic acid on biochemical traits of tomato plants

Chlorophyll content

Salicylic acid at concentrations of 1, 1.5, and 2 ppm significantly increased chlorophyll a, chlorophyll b, and total chlorophyll in tomato leaves. The highest values were observed at 2 ppm, with chlorophyll a, b, and total chlorophyll measuring 28.161, 9.387, and 37.548 mg g⁻¹, respectively, compared to the control values of 16.527,

5.509, and 22.0368 mg g⁻¹. This enhancement is attributed to improved CO₂ assimilation, photosynthetic rate, and mineral uptake, which collectively improve plant growth and photosynthetic efficiency (Khan, 2003; Szepesi, 2005; Anwar *et al.*, 2025)^[29, 41, 11].

Free proline content

Treatment with salicylic acid reduced leaf free proline levels. The lowest content was recorded at 2 ppm (0.527 $\mu\text{mol g}^{-1}$) compared to 0.607 $\mu\text{mol g}^{-1}$ in the control. This decrease results from the regulation of proline metabolism, where biosynthetic enzymes (γ -glutamyl kinase and pyrroline-5-carboxylate reductase) are downregulated, and proline oxidase activity is increased, enhancing proline degradation. This reflects improved stress adaptation and metabolic balance (Misra & Saxena, 2009)^[36].

Hydrogen peroxide (H₂O₂) content

Salicylic acid at 2 ppm significantly reduced H₂O₂ levels to 0.817 mmol g⁻¹ compared to 1.295 mmol g⁻¹ in the control. This reduction indicates a mitigation of oxidative stress, consistent with previous studies showing SA decreases H₂O₂ accumulation and MDA levels, acting as a signaling molecule in stress responses (Husen *et al.*, 2018; Hao *et al.*, 2014)^[27, 26].

Carbohydrate content

Salicylic acid at 2 ppm increased carbohydrate content to 16.95 mg g⁻¹ compared to 11.05 mg g⁻¹ in the control. This is due to enhanced enzymatic activities and metabolite transport, as well as improved photosynthesis. Similar effects were observed in other plants, where SA application significantly increased carbohydrates in leaves, stems, and roots, supporting growth and energy supply (Amin *et al.*, 2007; Hashish *et al.*, 2015)^[10, 25]. (Table 7).

Table 7: Effect of salicylic acid on chlorophyll, proline, hydrogen peroxide, and carbohydrate content of tomato seedlings

Treatment (ppm)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)	Proline ($\mu\text{mol/g}$)	Hydrogen Peroxide (mM/g)	Carbohydrates (mg/g)
Control	16.527	5.509	22.036	0.607	1.295	11.05
1	19.479	6.493	25.972	0.650	1.201	11.23
1.5	23.961	7.987	31.948	0.563	1.085	13.16
2	28.161	9.387	37.548	0.527	0.817	16.95
LSD (0.05)	0.63	0.24	0.87	0.05	0.07	0.92

Effect of Rizolex fungicide on growth traits of tomato plants

Fresh and dry weight of shoot and root

Statistical analysis (Table 8) showed that Rizolex at concentrations of 5, 10, and 20 ppm significantly affected the fresh and dry weight of both shoot and root. The highest concentration, 20 ppm, resulted in a significant increase in fresh weight, recording 213.3 g and 44.3 g for the shoot and

root, respectively, and dry weight of 19.0 g and 2.00 g for the shoot and root, respectively.

The positive effect of Rizolex on both fresh and dry weight is attributed to its role in promoting early growth of roots and stems (Vuyyuru *et al.*, 2018)^[43]. Other studies also reported that Rizolex treatment enhanced plant growth traits compared to the control. For dry weight, treated plants reached 7 g per plant, while fresh weight showed a balanced

increase compared to other treatments (Korayem *et al.*, 2020) [30].

Table 8: Effect of Rizolex on fresh and dry weight of shoot and root systems of tomato plants

Treatment (ppm)	Fresh Weight (mg)		Dry Weight (mg)	
	Shoot	Root	Shoot	Root
Control	288.0	38.3	14.3	23.3
5	104.0	28.0	9.0	1.33
10	145.0	24.0	12.7	1.67
20	213.3	44.3	19.0	2.00
LSD (0.05)	298.1	11.8	2.7	1.3

Effect of Rizolex fungicide on biochemical traits of tomato plants grown in pots

Chlorophyll content

Statistical analysis (Table 9) showed significant differences among Rizolex concentrations (5, 10, and 20 ppm) in their effect on chlorophyll a, chlorophyll b, and total chlorophyll content. The concentration of 20 ppm recorded the highest chlorophyll a, b and total chlorophyll (17.616, 5.872 and 23.488 mg g⁻¹ respectively) compared with the control (16.530, 5.510 and 22.040 mg g⁻¹ respectively). These improvements are attributed to the indirect role of Rizolex in enhancing water and nutrient uptake, protecting chloroplasts, and reducing physiological stress, thereby promoting photosynthesis. Similar findings were reported by Lakshmanan *et al.* (2007) [31], who observed a marked increase in chlorophyll pigments at 25 ppm.

Free proline content

Rizolex treatments significantly affected free proline levels, with the lowest value recorded at 20 ppm (0.563 µmol g⁻¹),

compared with 0.607 µmol g⁻¹ in the control. This reduction indicates decreased stress intensity and improved physiological balance. Rizolex may transiently increase abscisic acid (ABA), influencing amino acid metabolism and proline turnover, thereby enhancing stress tolerance (Manivannan *et al.*, 2007) [33]. Previous studies also reported a 30-75% reduction in proline content in tomato plants treated with recommended doses of Rizolex (Selim & Selim, 2019) [38].

Hydrogen peroxide (H₂O₂) content

Statistical results (Figure 8) indicated that Rizolex significantly reduced leaf H₂O₂ levels, with the lowest concentration recorded at 20 ppm (1.084 mmol g⁻¹) compared with 1.295 mmol g⁻¹ in the control. This decrease reflects enhanced antioxidant defense mechanisms and reduced oxidative damage. Rizolex has been shown to limit ROS accumulation and protect membrane integrity, thereby improving plant physiological status (Velikova *et al.*, 2000; Selim & Selim, 2019) [42, 38].

Carbohydrate content

Rizolex treatments significantly influenced carbohydrate content; however, no significant difference was observed between the 20 ppm treatment (10.27 mg g⁻¹) and the control (11.05 mg g⁻¹). This response may be related to reduced sink strength and limited translocation of sugars from leaves to growing tissues, resulting in carbohydrate retention in leaves (Zabalza *et al.*, 2004) [45]. In contrast, Mystkowska (2023) [37] reported that Rizolex increased total and reducing sugars in potato tubers, indicating enhanced carbohydrate accumulation under certain growth conditions. (Table 9).

Table 9: Effect of Rizolex on chlorophyll, proline, hydrogen peroxide, and carbohydrate content of tomato seedlings

Treatment (ppm)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)	Proline (µmol/g)	Hydrogen Peroxide (mM/g)	Carbohydrates (mg/g)
Control	16.530	5.510	22.040	0.607	1.295	11.05
5	15.576	5.192	20.768	0.643	1.269	8.49
10	16.413	5.471	21.884	0.603	1.122	9.25
20	17.616	5.872	23.488	0.563	1.084	10.27
LSD	0.66	0.21	0.87	0.05	0.05	0.78

Conclusion

The study revealed that nano-iron and salicylic acid significantly enhanced tomato growth, increased chlorophyll and carbohydrate contents, and reduced proline and hydrogen peroxide accumulations, indicating improved physiological performance and reduced oxidative stress. In contrast, the Rizolex fungicide showed limited effects on physiological traits, slightly increasing some parameters such as fresh and dry weight and chlorophyll content, while its impact on carbohydrates, proline, and hydrogen peroxide was minimal.

It is recommended to use nano-iron or salicylic acid to enhance growth and physiological efficiency, limit the use of Rizolex to disease control only, and consider combining nano-iron with salicylic acid for potential synergistic effects to improve growth and productivity.

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