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Morpho-physiological adaptations in grafted tomato plant under concomitant infestation of *Fusarium oxysporum* and *Meloidogyne incognita*

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ABSTRACT

This study evaluated the impact of rootstocks, scions, and their interactions on tomato growth, physiology, yield, fruit quality, and disease resistance under Fusarium-nematode infested conditions. At early stages, non-grafted scions (R0) showed higher plant height (up to 28.56 cm at 60 DAT), but at final harvest, grafted plants with RB5 rootstock reached the greatest height (146.25 cm) compared to RB3 (144.79 cm) and R0 (142.42 cm). RB5 also significantly increased leaf area (33.70 sq. cm with polyhouse LC scion) and leaf relative water content (71.25%), outperforming RB3 and non-grafted plants. Carotenoid content peaked at 1.35 mg/g in RB5 × Pusa Ruby, and chlorophyll content reached 2.95 mg/g in RB5 × Polyhouse LC. Yield attributes were highest with RB5: 6.76 trusses per plant, 45.73 fruits per plant, average fruit weight of 50.67 g, and total fruit yield of 257.66 q/ha, surpassing RB3 and R0. Fruit quality improved with RB5, showing highest total soluble solids (5.80 °Brix) and ascorbic acid (20.25 mg/100g), while acidity remained stable (~0.31%). Disease parameters revealed RB5 strong resistance, with the lowest root galls (12.50), egg masses (7.17), soil nematode population (41.85/200 cc), and fusarium wilt incidence (27.50%), compared to high susceptibility in R0 (galls 124.42, egg masses 104.25, nematodes 284.55, wilt 91.67%). Scion effects were less marked and interactions mostly non-significant, indicating rootstock RB5 as the key factor for enhanced growth, yield, quality, and disease tolerance in nematode-infested conditions.

Key words: Scion, rootstock, trusses, grafting, nematode, fruit quality.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.), a vital crop in the Solanaceae family, originated in Peru, Ecuador, and the Galapagos Islands, with domestication rooted in Mexico. In India, tomatoes are cultivated across 849,000 hectares, producing 20.4 million tonnes at a productivity of 24.07 t/ha (Indiastat, 10). Haryana contributes with 22,870 hectares and 400,599 tonnes (Indiastat, 10). Tomato adapts well to varied climates and is grown in open fields and greenhouses, the latter enabling off-season and large-scale production of indeterminate varieties. Tomatoes vary in colour, size, and shape, and are rich in water, vitamins A and C, minerals, and antioxidants like lycopene and beta-carotene compounds linked to reduced risks of cancer and heart disease. Lycopene acts as a strong antioxidant, while tomatine, a fungicidal alkaloid present in immature fruits, holds breeding significance (Friedman, 6). However, tomato production is threatened by biotic stresses, notably root-knot nematodes (*Meloidogyne incognita*) and Fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici*). These pathogens often act synergistically, intensifying wilt symptoms and causing yield losses up to 50% or more (Ghazalibiglar, 7). Excessive reliance on chemicals poses environmental and health hazards, prompting the need for sustainable alternatives. Grafting, the union of a scion and a rootstock, is an effective eco-friendly method for enhancing resistance to soil-borne pathogens and environmental stresses. Resistant rootstocks improve root development, water and nutrient uptake, and physiological efficiency, boosting photosynthesis and plant vigour (Yetışır *et al*., 19). Grafted plants exhibit enhanced height, leaf area, relative water content, and often yield better fruit quality compared to non-grafted plants. They also display improved hormonal and nutrient balance, contributing to overall resilience. Despite several reports on grafting as a strategy against soil-borne diseases, limited studies have addressed the simultaneous challenge of *Fusarium oxysporum* and *Meloidogyne incognita* in tomato. Furthermore, physiological and biochemical responses of grafted plants under this dual stress remain underexplored. The present study addresses this gap by evaluating resistant rootstocks under concomitant infestation, aiming to develop a sustainable and cost-effective approach to disease management.

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MATERIALS AND METHODS

The experiment was conducted at the screen-house of Department of Nematology, CCS Haryana Agricultural University (CCSHAU), Hisar, (29º 10'N latitude and 75 46'E longitude) from November 2022 to May 2023. Two resistant genotypes, RB3 and RB5 (*Solanum torvum*), were selected as rootstocks for grafting. The scion varieties used were Pusa Ruby (S1), Punjab Gaurav (S2), and Polyhouse Local Cultivar (S3). These were grafted onto the selected rootstocks (RB3 and RB5), resulting in six graft combinations: RB3 × S1, RB3 × S2, RB3 × S3, RB5 × S1, RB5 × S2, and RB5 × S3. Plants were grown in soil pre-infested with *Fusarium oxysporum f. sp. lycopersici* and *Meloidogyne incognita,* (Saharan *et al*., 17). A conidial suspension of *Fusarium oxysporum f. sp. lycopersici* (1 × 10^6 conidia/ml) was prepared and applied by soil drenching, while freshly hatched second-stage juveniles (J2) of *M. incognita* obtained from surface-sterilized egg masses using the Modified Baermann Funnel Technique, were inoculated at a rate of 1000 J2 per kg of soil. The experiment was laid out in Completely Randomized Design (CRD) with four replications for each combination. Five random tomato plants per replication were tagged for data collection. Growth and physiological parameters such as plant height (at 30, 60, 90 DAT and at harvest), and leaf area, relative water content, chlorophyll, and carotenoid content at first harvest were measured using standard tools (measuring scale, leaf area meter, spectrophotometer). Yield attributes including number of trusses, fruits, fruit size (length and width), fruit weight, total and marketable yield were recorded. Quality parameters such as total soluble solids, ascorbic acid, and titratable acidity were analyzed following standard protocols. TSS was estimated using a digital ATAGO refractometer (0–50 °Brix) from juice of red-ripe fruits. Ascorbic acid content was determined by the 2,6-dichlorophenol indophenol titration method (AOAC, 1) and expressed as mg/100 g fresh weight. Titratable acidity was measured according to AOAC (2) by titrating juice samples with 0.1 N NaOH using phenolphthalein as indicator. Nematode infection was assessed by counting root galls and egg masses per root system, and nematode population using stereoscopic binocular microscope with the help of counting dish and finally the nematode population/ 200cc soil was calculated. Fusarium wilt incidence was calculated as percentage of affected plants. The original data was analyzed using two-factor analysis with the help of OPSTAT software.

RESULTS AND DISCUSSION

The study evaluated plant height under Fusarium-nematode stress with different rootstocks and scions (Table 1). At 30 DAT, non-grafted scions (R0) showed the highest plant height (13.79 cm), followed by grafted scions with rootstock RB3 (12.04 cm) and RB5 (10.67 cm), with no significant interaction. At 60 DAT, non-grafted scions (R0) again outperformed others (28.56 cm), while RB3 (25.94 cm) slightly outperformed RB5 (25.22 cm), with a significant interaction observed. By 90 DAT, non-grafted scions continued to grow the tallest (88.17 cm), while RB3 reached 80.67 cm and RB5 77.71 cm, with no significant interaction. At the final harvest, non-grafted scions (R0) reached 142.42 cm, while grafted scions with RB5 (146.25 cm) outgrew those with RB3 (144.79 cm). A significant difference in plant height between rootstocks was found (Factor B = 4.23), but no significant interaction was observed. Rootstock selection influenced plant height primarily at later growth stages. Reduced height at early growth stages may be due to grafting shock, but compatible grafts later enhance growth and development. The study also found that rootstock RB5 significantly increased leaf area and leaf relative water content (LRWC) compared to RB3 and non-grafted plants. RB5 combined with Polyhouse LC (S3) showed the largest leaf area (33.70 sq. cm) and highest LRWC (71.25%), while non-grafted scions had the smallest leaf areas and lowest water content (Fig. 1a and 1b). Treatment effects were significant for both traits, but rootstock–scion interactions were not, indicating rootstock primarily drives these improvements. For carotenoid content, RB5 rootstock combinations exhibited the highest levels, especially RB5 × Pusa Ruby (1.35 mg/g), while RB3 and non-grafted plants showing lower values. Both treatment and rootstock had significant effects, but their interaction did not. Similarly, chlorophyll content was highest with RB5, particularly RB5 × Polyhouse LC (2.95 mg/g), followed by RB3 and lowest in non-grafted scions (Fig. 2a and 2b). Treatment effects were significant, while rootstock and interaction effects were not, highlighting the dominant role of treatment in chlorophyll accumulation.

The evaluation of different rootstocks and scions under Fusarium-nematode infested conditions revealed that rootstock RB5 consistently outperformed RB3 and non-grafted plants (R0) across multiple important growth and yield parameters (Table 3). Specifically, RB5 resulted in the highest number of trusses per plant, indicating better vegetative development, with a mean of 6.76 compared to 6.39 for RB3 and 6.10 for R0. Although the number of fruits per truss did not significantly differ among treatments, RB5 showed a trend towards higher fruit counts. Fruit size parameters were notably improved by RB5, which produced the longest fruits (up to 4.66 cm with Punjab Gaurav scion) and the widest fruits

Table 1: Effect of rootstocks, scions and their interactions on plant height (cm) at 30, 60, 90 DAT and at harvest under fusarium-nematode infested conditions.

Rootstock (A)/Scion (B)	Pusa Ruby (S1)	Punjab Gaurav (S2)	Polyhouse LC (S3)	Mean A
30 DAT				
RB5	10.88	10.63	10.50	10.67
RB3	11.75	11.25	13.13	12.04
R0 (Non-grafted)	12.63	13.25	15.50	13.79
Mean B	11.75	11.71	13.04	
CD at 5% Level of Significance	Factor A (treatment)= 1.40	Factor B (rootstock)= NS	Factor A×B= NS	
60 DAT				
RB5	25.67	25.17	24.83	25.22
RB3	24.50	27.33	26.00	25.94
R0 (Non-grafted)	25.33	28.00	32.33	28.56
Mean B	25.17	26.83	27.72	
CD at 5% Level of Significance	Factor A (treatment)= 1.31	Factor B (rootstock)= 1.31	Factor A×B= 2.27	
90 DAT				
RB5	79.50	77.63	76.00	77.71
RB3	82.25	80.75	79.00	80.67
R0 (Non-grafted)	92.00	88.00	84.50	88.17
Mean B	84.58	82.13	79.83	
CD at 5% Level of Significance	Factor A (treatment)= 1.95	Factor B (rootstock)= 1.95	Factor A×B= NS	
At harvest				
RB5	150.25	146.50	142.00	
RB3	149.00	144.88	140.50	
R0 (Non-grafted)	144.75	143.25	139.25	
Mean B	148.00	144.88	140.58	
CD at 5% Level of Significance	Factor A (treatment)= NS	Factor B (rootstock)= 4.23	Factor A×B= NS	

(average 4.32 cm), surpassing both RB3 and R0. These increases in fruit dimensions reflect enhanced fruit development associated with this rootstock. Yield components also favoured RB5, which supported the greatest number of fruits per plant (45.73) and the heaviest total fruit weight per plant (2.32 kg). Correspondingly, average fruit weight was highest in RB5 plants at 50.67 g, indicating not only quantity but also quality improvements. Most importantly, total fruit yield and marketable yield were significantly higher with RB5, reaching 257.66 q/ha and 241.96 q/ha respectively, compared to lower yields in RB3 and R0 plants. These differences were statistically significant, as indicated by the critical difference values calculated for the treatments. While scion choice showed some influence on growth and yield traits, the interaction effects between scions and rootstocks were generally not significant, suggesting that the rootstock's role was the primary driver of improved plant performance under Fusarium-nematode stress. The study examined the effects of rootstocks and scions on fruit quality under Fusarium-nematode stress. The highest TSS was recorded with RB5 × Polyhouse LC (5.80 °Brix), followed by RB5 × Punjab Gaurav (5.73 °Brix) and RB3 × Polyhouse LC (5.65 °Brix). Non-grafted scions showed lower TSS (Table 2). Statistical analysis showed significant effects of rootstock and scion, but no interaction. RB5 × Polyhouse LC had the highest ascorbic acid (20.25 mg/100g), with non-grafted scions showing the lowest. The highest acidity was observed in RB5 × Pusa Ruby (0.311%), and the lowest in non-grafted Pusa Ruby (0.279%) (Table 2). No significant effects were observed from rootstock, scion, or interaction for acidity. Grafting plays a critical role in enhancing tomato growth and yield by utilizing vigorous rootstocks that improve nutrient and water uptake, disease tolerance, and overall plant resilience

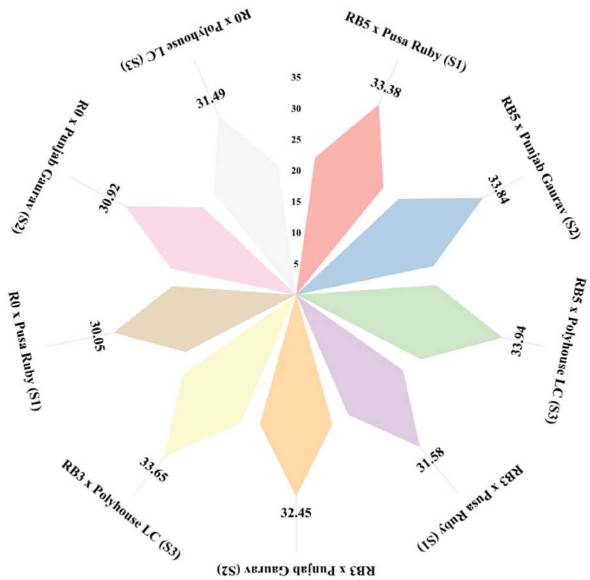

Fig. 1a. Leaf area (cm^2) across different rootstock and scion combinations under fusarium-nematode infested conditions. CD at 5% level of significance; Factor A (treatment)= 0.75; Factor B (rootstock)= 0.75; Factor A×B= NS.

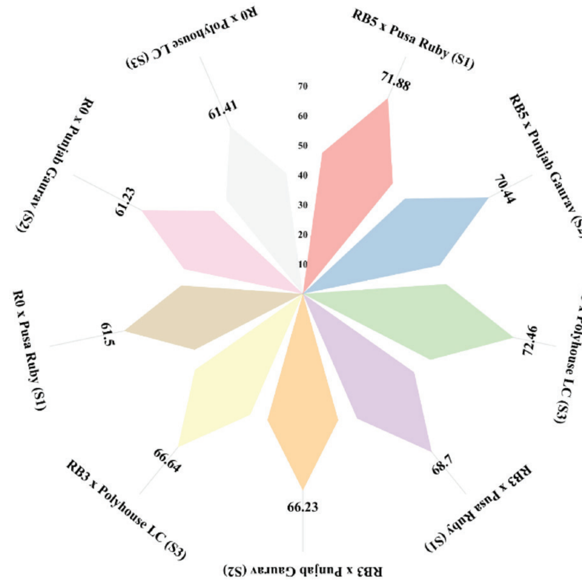

Fig. 1b. Leaf relative water content (%) across different rootstock and scion combinations under fusarium-nematode infested conditions. CD at 5% level of significance; Factor A (treatment)= 4.93; Factor B (rootstock)= NS; Factor A×B= NS.

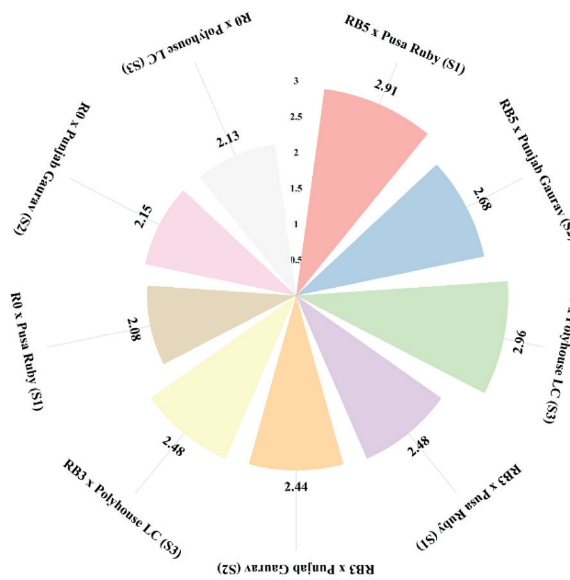

Fig. 2a. Chlorophyll content in leaves (mg/g) across different rootstock and scion combinations under fusarium-nematode infested conditions. CD at 5% level of significance: Factor A (treatment): 0.17; Factor B (rootstock): NS (No significant difference); Factor A×B (Interaction): NS (No significant interaction).

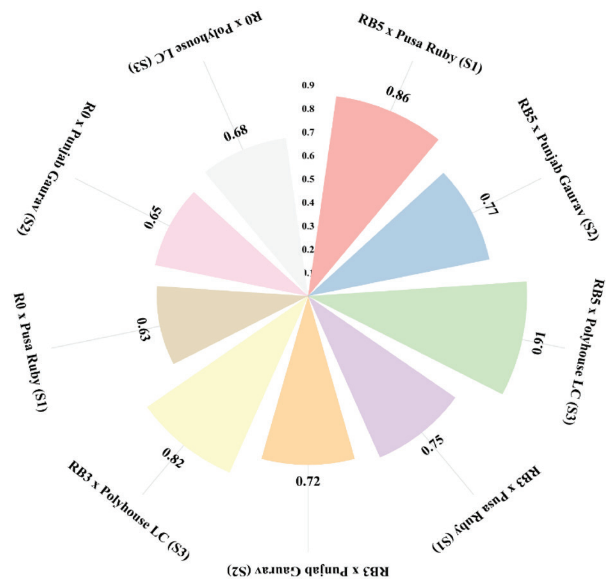

Fig. 2b. Carotenoid content in leaves (mg/g) across different rootstock and scion combinations under fusarium-nematode infested conditions. CD at 5% level of significance: Factor A (treatment): 0.05; Factor B (rootstock): 0.05 and Factor A×B (Interaction): NS (No significant interaction).

Table 2: Effect of rootstocks, scions, and their interactions on TSS content (°Brix), ascorbic acid content (mg/100g), and acidity (%) of fruits under fusarium-nematode infested conditions.

Rootstock (A)	Scion (B)	TSS (°Brix)	Ascorbic acid (mg/100g)	Acidity (%)
RB5	Pusa Ruby (S1)	5.43	18.95	0.311
	Punjab Gaurav (S2)	5.73	18.20	0.295
	Polyhouse LC (S3)	5.80	20.25	0.301
	Mean (A)	5.65	19.13	0.302
RB3	Pusa Ruby (S1)	5.23	17.65	0.292
	Punjab Gaurav (S2)	5.60	17.20	0.282
	Polyhouse LC (S3)	5.65	19.95	0.288
	Mean (A)	5.49	18.27	0.287
R0 *(Non-grafted)*	Pusa Ruby (S1)	4.83	15.43	0.279
	Punjab Gaurav (S2)	5.28	15.25	0.285
	Polyhouse LC (S3)	5.38	17.10	0.282
	Mean (A)	5.16	15.93	0.282
Mean (B)	Pusa Ruby (S1)	5.16	17.34	0.294
	Punjab Gaurav (S2)	5.53	16.88	0.287
	Polyhouse LC (S3)	5.61	19.10	0.290
CD at 5%	Factor A	0.23	1.37	NS
	Factor B	0.23	1.37	NS
	Interaction (A×B)	NS	NS	NS

Table 3: Effect of rootstocks, scions, and their interactions on various yield attributing traits of tomato under fusarium-nematode infested conditions.

Parameter	RB5 (S1)	RB5 (S2)	RB5 (S3)	RB3 (S1)	RB3 (S2)	RB3 (S3)	R0 (S1)	R0 (S2)	R0 (S3)	CD Factor A (treatment)	CD Factor B (rootstock)	CD Factor A×B (Interaction)
Number of trusses per plant	6.83	6.80	6.65	6.54	6.35	6.28	6.29	6.08	5.94	0.40	NS	NS
Number of fruits per truss	6.75	6.56	7.00	6.43	6.38	6.69	6.35	6.30	6.45	NS	NS	NS
Fruit length (cm)	3.86	4.66	3.94	3.35	4.15	3.83	3.24	3.95	3.51	0.25	0.25	NS
Fruit width (cm)	4.37	4.29	4.31	4.30	3.97	4.24	4.10	3.76	3.95	0.31	NS	NS
Number of fruits per plant	46.10	44.54	46.55	42.10	40.38	41.76	39.90	38.27	38.22	0.31	NS	NS
Weight of fruits per plant (kg)	2.31	2.27	2.39	2.07	1.99	2.18	1.88	1.85	1.93	0.16	NS	NS
Average fruit weight (g)	50.00	50.75	51.25	48.88	49.38	52.25	47.00	48.50	50.50	1.39	1.39	NS
Total fruit yield (q/ha)	256.11	251.67	265.22	229.34	221.17	242.30	208.82	206.00	214.22	17.48	NS	NS
Marketable fruit yield (q/ha)	241.11	234.06	250.72	213.88	203.29	226.14	186.75	180.33	194.30	11.34	11.34	NS

(Marsic and Osvald, 13). Although grafted plants initially exhibit slower growth and delayed flowering due to stress from the graft union, they eventually surpass non-grafted plants in height, biomass, and yield at later stages (Hossain *et al*., 8; Caradonia *et al*., 5). The higher biomass observed in grafted plants compared to non-grafted ones can be attributed to several well-documented mechanisms. Vigorous rootstocks develop more extensive and deeper root systems, which enhance water and nutrient uptake and ensure efficient translocation to the scion. In addition, grafting improves photosynthetic efficiency by increasing chlorophyll content and leaf area, thereby providing greater assimilate availability for growth. Rootstocks also influence hormonal regulation by supplying growth-promoting hormones (e.g., auxins, cytokinins, gibberellins) that stimulate cell division and shoot development. Moreover, grafted plants generally exhibit better tolerance to biotic and abiotic stresses, which reduces resource loss to defense and allows greater allocation toward biomass accumulation. The rootstock RB5 showed superior performance by significantly increasing plant height, number of trusses, fruit length and width, fruit number per plant, and total fruit yield by 10 to 22% under fusarium-nematode infested conditions. This improvement is attributed to enhanced root vigor, better nutrient absorption, and hormonal regulation from the rootstock (Sharma *et al*., 18). Fruit quality parameters such as total soluble solids (TSS), ascorbic acid content, and titratable acidity were significantly higher in grafted plants, improving both nutritional value and marketability (Pogonyi *et al*., 14; Ibrahim *et al*., 9; Caradonia *et al*., 5). Physiological traits such as greater leaf area, higher chlorophyll concentration, elevated relative leaf water content, and increased carotenoid accumulation in grafted tomato plants collectively point toward improved photosynthetic efficiency and superior stress adaptation. An expanded leaf area and enhanced chlorophyll content contribute to greater light interception and assimilation capacity, while elevated relative water content reflects better root-mediated water uptake and retention, ensuring sustained turgor under stress. Similarly, higher carotenoid levels not only aid in light harvesting but also function as antioxidants that protect photosynthetic machinery from oxidative damage. Together, these physiological modifications enhance carbon assimilation, energy balance, and stress resilience, ultimately translating into stronger growth and improved yield performance in grafted tomatoes (Rahmatian *et al*., 15; Bikdeloo *et al*., 4).

This study evaluated the influence of different rootstocks and scions on nematode infestation and fusarium wilt incidence in tomatoes grown under fusarium-nematode infested conditions. Among the rootstocks tested, RB5 consistently demonstrated superior resistance, showing the lowest number of root galls (12.50), egg masses (7.17), soil nematode population (41.85 per 200 cc), and fusarium wilt incidence (27.50%). In contrast, non-grafted plants exhibited the highest susceptibility with gall counts averaging 124.42, egg masses 104.25, nematode populations 284.55, and wilt incidence reaching 91.67%. RB3 showed intermediate levels for all parameters. Statistical analysis confirmed that rootstock choice significantly affected all measured parameters, while scion type only significantly influenced egg mass counts. No significant interactions between rootstock and scion were detected (Table 4), indicating their effects operate independently. These findings underscore the pivotal role of resistant rootstocks such as RB5 in effectively mitigating the dual threats of root-knot nematodes and *Fusarium oxysporum* wilt, thereby safeguarding tomato health and productivity regardless of the scion employed. The pronounced suppression of root galling and egg mass formation by up to 90%, along with a substantial reduction in soil nematode populations, indicates not only direct protection of the host plant but also a broader contribution to limiting inoculum build-up and subsequent pathogen dissemination in the soil (Baidya *et al*., 3). Likewise, the reduction of Fusarium wilt incidence by nearly 70% in grafted plants highlights the ability of RB5 rootstock to function as both a physical and physiological barrier to vascular infection, while simultaneously improving the efficiency of water and nutrient uptake (King *et al*., 12; Jabnoun-Khiareddine *et al*., 11). Beyond disease suppression, grafting also conferred significant agronomic advantages, including an extension of crop duration by approximately 12%, which facilitated prolonged harvesting and translated into higher cumulative yields (Rashid *et al*., 16; Baidya *et al*., 3). Collectively, these results reinforce the potential of resistant rootstocks as a sustainable and integrated strategy for managing soilborne pathogen complexes in tomato production.

In summary, grafting tomato plants onto resistant rootstocks such as RB5 provides a sustainable approach to managing the *Fusarium–Meloidogyne* complex, while improving growth, yield, fruit quality, and physiological resilience. The enhanced performance of RB5 is linked to its vigorous root system that optimizes nutrient uptake, water relations, and hormonal balance, thereby reducing dependence on chemical controls and strengthening crop tolerance under biotic stress.

Table 4: Effect of rootstocks, scions, and their interactions on nematode infestation and fusarium wilt incidence under fusarium-nematode infested conditions.

Rootstock (A)	Scion (B)	Number of galls per root system	Number of egg masses per root system	Final nematode population (per 200 cc soil)	Fusarium wilt incidence (%)
RB5	Pusa Ruby (S1)	14.50	9.50	45.01	30.00
	Punjab Gaurav (S2)	9.75	4.25	37.24	25.00
	Polyhouse LC (S3)	13.25	7.75	43.31	27.50
	Mean (A)	12.50	7.17	41.85	27.50
RB3	Pusa Ruby (S1)	42.50	34.25	52.01	62.50
	Punjab Gaurav (S2)	39.00	30.25	48.89	57.50
	Polyhouse LC (S3)	41.00	28.25	50.54	60.00
	Mean (A)	40.83	30.92	50.48	60.00
R0 *(Non-grafted)*	Pusa Ruby (S1)	133.75	114.25	291.00	95.00
	Punjab Gaurav (S2)	111.25	90.50	277.80	87.50
	Polyhouse LC (S3)	128.25	108.00	284.85	92.50
	Mean (A)	124.42	104.25	284.55	91.67
Mean (B)	Pusa Ruby (S1)	63.58	52.67	129.34	62.50
	Punjab Gaurav (S2)	53.33	41.67	121.31	56.67
	Polyhouse LC (S3)	60.83	48.00	126.23	60.00
CD at 5%	Factor A (Rootstock)	9.57	8.45	13.90	8.99
	Factor B (Scion)	NS	8.45	NS	NS
	Factor B (Scion)	NS	NS	NS	NS

AUTHORS' CONTRIBUTION

Conceptualization of research (IA and KY); Designing of the experiments (IA and KY); Contribution of experimental materials (IA and KY); Execution of field/lab experiments and data collection (KY and AK); Analysis of data and interpretation (SS, KY, K and PP); Preparation of the manuscript (KY, SKD, AS, DS and SS)

DECLARATION

The authors declare that there is no conflict of interest.

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