



Effect of *Trichoderma*, Organic Manure and Inorganic Fertilizer on Growth, Flowering and Post-harvest Parameters in Rose cv. Top Secret

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: <https://doi.org/10.9734/ijpss/2025/v37i65497>

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://pr.sdiarticle5.com/review-history/137324>

Original Research Article

Received: 28/03/2025
Accepted: 30/05/2025
Published: 31/05/2025

ABSTRACT

The present investigation was conducted from 2023 to 2025 at the Horticulture Research Farm and Post-harvest Laboratory, Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, featuring nine nutrient treatments that included various combinations of farmyard manure (FYM), vermicompost and recommended fertilizer doses (RDF). These were divided into two groups, viz., *Trichoderma* inoculated plants and *Trichoderma* uninoculated plants, to assess their effects on growth, flowering and postharvest quality of rose cv. Top Secret, under open ventilated polyhouse conditions. The experiment was conducted using a Completely

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Cite as: Singh, Mandeep, Anil K. Singh, Anjana Sisodia, Anurag Srivastav, and Arun Kumar Maurya. 2025. "Effect of *Trichoderma*, Organic Manure and Inorganic Fertilizer on Growth, Flowering and Post-Harvest Parameters in Rose Cv. Top Secret". *International Journal of Plant & Soil Science* 37 (6):167-77. <https://doi.org/10.9734/ijpss/2025/v37i65497>.

Randomized Design (CRD) with nine different treatments, each replicated five times. The treatments included: T₁ (100% RDF), T₂ (100% FYM), T₃ (100% Vermicompost), T₄ (75% FYM + 25% Vermicompost), T₅ (50% FYM + 50% Vermicompost), T₆ (25% FYM + 75% Vermicompost), T₇ (50% FYM + 50% RDF), T₈ (50% Vermicompost + 50% RDF) and T₉ (Control, no fertilizer), each examine with and without *Trichoderma* inoculation. Significant differences were recorded across all measured parameters. The maximum plant height (107.08 cm) and the highest number of leaves (189.76) were found in T₇ with *Trichoderma* inoculated and flower production (27.69 flowers/plant) was also in the same. Early bud initiation (26.14 days), the longest vase life (16.12 days) occurred in T₇ with *Trichoderma* inoculation and the highest anthocyanin content (620.66 mg/100g) occurred in T₃ with *Trichoderma* inoculation. These findings suggest that integrated nutrient management using a combination of organic and inorganic fertilizers, along with *Trichoderma*, can significantly enhance the growth, flowering and postharvest quality of rose cv. Top Secret under protected cultivation.

Keywords: *Trichoderma*; vermicompost; organic fertilizers; protected cultivation; anthocyanin; inoculation; inorganic fertilizers.

1. INTRODUCTION

Roses are widely recognized as symbols of love and peace, serving as a natural means of communication between humans and nature. Their appealing shape, size, fragrance and vibrant colours, along with their gradual blooming process and long-lasting freshness, make them highly valued for commercial cultivation to meet both domestic and international demand (Pandey, 2024). Rose (*Rosa hybrida* L.), roses belong to the Rosaceae family and have a basic chromosome number of $n = 7$. Many species within the *Rosa* genus, particularly those of Asian origin, are diploid with $2n = 14$ chromosomes. However, modern cultivated roses are typically tetraploid with $2n = 28$ chromosomes (Singh and Sisodia, 2017). Roses are native to various regions, including the Himalayas, West Asia, China, Japan, Europe and North America. The *Rosa* genus comprises about 200 species, but only a select few have significantly contributed to the development of modern roses. These key species include *Rosa gallica*, *R. damascena*, *R. chinensis*, *R. foetida*, *R. gigantea*, *R. moschata*, *R. multiflora* and *R. wichuriana*. Modern roses are categorized into different groups such as Hybrid Teas (HT), floribundas, climbers, miniature roses and shrub roses (Kumar et al., 2023). Roses have been cultivated since ancient times and continue to hold their title as the "King of Flowers" among cut flowers. Within the Rosaceae family, classification is based on fruit type, dividing it into four subfamilies: *Spiraeoideae*, *Amygdaloideae*, *Maloideae* and *Rosoideae*. The *Rosa* genus belongs to the *Rosoideae* subfamily (Longhi et al., 2014). In horticulture, the natural fertility of the soil has historically determined its production. A rich

source of nutrients, farmyard manure (FYM), vermicompost and poultry manure are examples of organic inputs that improve the physical and chemical characteristics of soil, promoting long-term soil health and sustainable crop production. This study was conducted to assess the ability of organic manures and vermicompost as substitute nutrient sources due to the growing expense of chemical fertilizers, which are frequently utilized to satisfy the nutritional requirements of horticultural crops (Sendhilnathan et al., 2019). *Trichoderma* fungi are recognized as effective bio stimulants with a global presence, thriving in diverse environments such as soil, decaying wood and especially the rhizosphere. They produce a range of bioactive compounds that facilitate interactions with plants and microbes. Through mechanisms like hyper parasitism and antibiosis, *Trichoderma* spp. suppresses pathogens including bacteria, viruses and *Fusarium* fungi (Wojtkowiak et al., 2006). Additionally, studies (Benitez, 2004; Swierczynska, 2011; Poveda, 2021) suggest they can boost plant defences against insect pests. These fungi secrete enzymes (e.g., cellulases, proteases), antibiotics, volatiles and plant growth regulators, enhancing both plant health and soil biology (Kosicka, 2014, Bohra & Kumar, 2014).

2. MATERIALS AND METHODS

The present experiment was conducted in a naturally ventilated polyhouse at the Horticulture Research Farm and Post-harvest Laboratory, Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, during the years 2023-2025. The experimental site is situated at 25° 15'

North latitude and 82° 59' East longitude, with an altitude of 103 meters above mean sea level, near the banks of the river Ganges. The region experiences a humid subtropical climate with significant seasonal temperature variations. The maximum temperature was recorded as 47.2 °C to the minimum. The soil had a pH of 7.2 and was rich in organic carbon and nitrogen. The experiment was conducted on the rose cultivar Top Secret, an attractive red rose variety chosen for its high market demand and suitability for polyhouse conditions. Uniformly healthy plants with consistent height and bud development (2 years old) were selected for research work. The experiment was laid out in a Complete Randomized Design (CRD) involving 9 different treatments (including various combinations of organic and inorganic fertilizers) on rose plants, where each treatment was divided into 2 groups: half the plants were inoculated with *Trichoderma* @25ml/plant, and the other half were left uninoculated. This design allowed for the comparison of the *Trichoderma* inoculation effect across various treatment conditions on rose plants. The nine treatments were T₁ (100% RDF), T₂ (100% FYM), T₃ (100% Vermicompost), T₄ (75% FYM + 25% Vermicompost), T₅ (50% FYM + 50% Vermicompost), T₆ (25% FYM + 75% Vermicompost), T₇ (50% FYM + 50% RDF), T₈ (50% Vermicompost + 50% RDF) and T₉ (Control, without fertilizer application). Plants were spaced at 30 cm × 30 cm using a single row system on raised beds. Fertilizers were applied in different proportions according to the treatments. The required quantities for FYM were 100% (2.08 kg/m²), 75% (1.56 kg/m²), 50% (1.04 kg/m²) and 25% (0.52 kg/m²), while for vermicompost, they were the same. Urea was applied at 100% (2 g/plant) and 50% (1 g/plant), whereas Single Super Phosphate (SSP) was given at 100% (1 g/plant) and 50% (0.5 g/plant). Observations were recorded on various growth, flowering and postharvest parameters. Results thus obtained were subjected to statistical analysis as suggested by Panse and Sukhatme (1978).

3. RESULTS AND DISCUSSION

Growth parameters: Plant height and number of leaves in rose were significantly influenced by various nutrient treatments (Tables 1 and 2). *Trichoderma* alone showed inconsistent effects, but when combined with organic and inorganic nutrients, it boosted early growth. The highest plant height was recorded in T₇ (50% FYM + 50% RDF with *Trichoderma* inoculation) at

107.08 cm, followed by T₃ (100% Vermicompost with *Trichoderma* inoculation) at 103.90 cm and T₇ (50% FYM + 50% RDF without *Trichoderma* inoculation) at 99.26 cm. *Trichoderma* has the ability to trigger systemic defence responses in plants, enhancing their resistance to pests and pathogens (Poveda et al., 2020 and Abdel et al., 2024). Similar trends were observed by Singh and Jauhari (2005), Singh (2006) using *Azotobacter* and others (Patel et al., 2017; Kumar et al., 2022), attributing growth improvement to better nutrient uptake, enhanced soil conditions and microbial activity.

The number of leaves per plant is a vital marker of rose growth and yield. The study showed significant variation across treatments, with the highest leaf number recorded during the first flush T₇ (50% Vermicompost + 50% RDF with *Trichoderma* inoculation) showed the maximum at 189.76, followed by T₈ (50% Vermicompost + 50% RDF with *Trichoderma* inoculation) at 182.92 and T₃ (100% Vermicompost with *Trichoderma* inoculation) at 178.5. It could also be attributed due to fact that after proper decomposition and mineralization, the farmyard manure supplied available nutrients directly to the plant and also had solubilizing effect on fixed form of nutrients in soil (Sinha et al., 1981). The beneficial effect of farmyard manure on growth and flowering in rose might be due to additional supply of plant nutrients as well as improvement in physical and biological properties of the soil (Majumdar et al., 2002 and Qasim et al., 2023). Similar trends were observed by Singh and Jauhari (2005), Singh and Singh (2010), Boshra et al. (2012) and Bhat and Shepherd (2006), who highlighted the role of manure types in influencing leaf in plant.

Flowering parameters: Early flowering is crucial for the commercial floriculture industry. Bud initiation in roses varied significantly with different nutrient treatments shown in Table 2, and *Trichoderma* inoculation, with the earliest recorded in the third flush. The fastest bud initiation was observed in T₇ (50% FYM + 50% RDF with *Trichoderma* inoculation) at 26.14 days, followed by T₃ (100% Vermicompost with *Trichoderma* inoculation) at 27.14 days and T₈ (50% Vermicompost + 50% RDF with *Trichoderma* inoculation) at 27.83 days. *Trichoderma* significantly influences bud initiation in rose flowers. Organic manures like farmyard manure and vermicompost improve soil structure, microbial activity and nutrient availability, leading to healthier plants and earlier

bud initiation. The supporting findings by Preethi *et al.* (1999) and Singh (2005), who also noted the positive role of microbial inoculants in plant development.

Bud diameter, a key indicator of rose growth and yield, varied significantly with nutrient treatments and *Trichoderma* inoculation. The largest diameter was recorded in the first flush under T₇ (50% FYM + 50% RDF with *Trichoderma* inoculation) at 26.76 mm, followed by T₈ (50% Vermicompost + 50% RDF with *Trichoderma* inoculation) at (25.27 mm). Using organic manures makes the soil healthier and provides more nutrients, helping buds grow steadily. Inorganic fertilizers offer quick nutrients for fast bud growth, especially potassium. *Trichoderma* increases nutrient uptake and boosts plant defences (Khuong *et al.*, 2024), which together enhance the size of rose buds and the quality of the flowers. The similarly result found by Preethi *et al.* (1999) and Singh (2005), who emphasized the role of nutrient and microbial interactions in plant development.

The number of flowers per plant is a key yield factor in roses. This study showed significant variation across treatments, with the first flush producing the most flowers. The highest number was in T₇ (50% FYM + 50% RDF with *Trichoderma* inoculation) at 27.69, followed by T₈ (50% Vermicompost + 50% RDF with *Trichoderma* inoculation) at 26.37 and T₃ (100% Vermicompost with *Trichoderma* inoculation) at 26.29. All flowering and yield characteristics were enhanced by the application of organic manure. This improvement can be attributed to both chemical and biological factors. Chemically, as organic manures decompose, they release organic compounds into the soil, enriching its nutrient profile. Biologically, they serve as a food source for beneficial soil microorganisms, thereby enhancing microbial activity and increasing nutrient availability to plants. These findings are in line with the results reported by Kolambe (2008), Rathva (2011) and Naik *et al.* (2008). Furthermore, Lambat and Pal (2012) observed that the highest number of flowers per plant was recorded in treatments involving neem cake, phosphate-solubilizing bacteria (PSB) and *Azotobacter*.

Flowering duration, an important trait for rose yield, varied significantly with nutrient treatments shown in Table 3 (Fig. 2) and with *Trichoderma* application. The longest duration was observed in the third flush under T₇ (50% FYM + 50 %

RDF with *Trichoderma* inoculation) at 7.31 days, followed by T₃ 100% Vermicompost with *Trichoderma* inoculation (7.31 days) and T₈ 50% Vermicompost+ 50% RDF with *Trichoderma* inoculated (7.04 days) indicating the positive impact of organic inputs and *Trichoderma* on prolonged blooming. Organic inputs strengthen petals and retain moisture. Inorganic nutrients like potassium boost petal durability and *Trichoderma* induces systemic resistance, delays senescence. The results are consistent with previous studies, including that of Bhalla *et al.* (2006a or b), who reported similar findings in *Gladiolus* cv. Red Beauty and standard carnation. Likewise, Vishen (2005) observed an extended flowering duration in tuberose with the combined application of *Azotobacter*, FYM and vermicompost.

Postharvest parameters: Vase life, a key indicator of postharvest quality in roses, varied significantly with nutrient treatments and *Trichoderma* inoculation shown in Table 4 and Fig. 1. The longest vase life was recorded in T₇ (50% FYM + 50 % RDF with *Trichoderma* inoculation) at 16.12 days, followed by 15.86 days in the second flush of the same treatment, and 15.84 days in T₃ (100% Vermicompost with *Trichoderma* inoculation). *Trichoderma* promotes systemic resistance and enhances root health, increasing nutrient uptake and stress resilience, which slows down aging and prolongs vase life (Sisodia *et al.*, 2024). Notably, potassium and calcium strengthen cell walls and lessen petal aging, aiding in the preservation of flower freshness after harvest. Similar findings were reported by Bhor (2010) and Trivedi *et al.* (2016). Anzu-Man-Ara *et al.* (2022) observed that the use of a combination of soil, coco dust, vermicompost and leaf compost increases the vase life of flower.

Anthocyanins protect rose petals from UV damage and increase under stress, contributing to both stress tolerance and postharvest quality. This study found significant variation in anthocyanin content across treatments shown in Table 4, with the highest in the first flush under T₃ (100% Vermicompost with *Trichoderma* inoculation) at 620.66 mg/100g, followed by T₃ without inoculation (598.30 mg/100g) and the second flush of T₃ 100% Vermicompost with inoculation (558.30 mg/100g) and the second flush of T₃ 100% Vermicompost with inoculation (558.30 mg/100g). Organic manures such as farmyard manure and vermicompost help make the soil healthier (Paul and Mandi, 2024), and

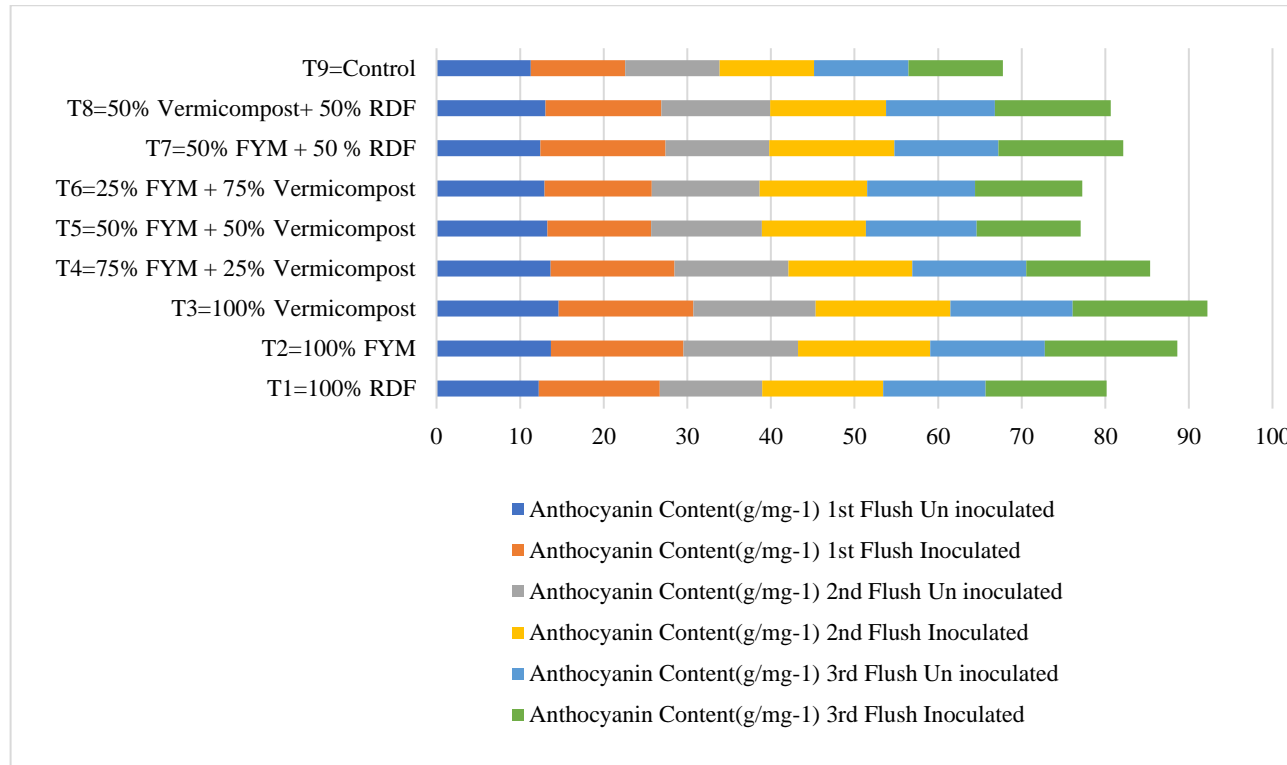


Fig. 1. Effect of *Trichoderma*, organic manure and inorganic fertilizer on anthocyanin content (g/mg-1) in rose petals

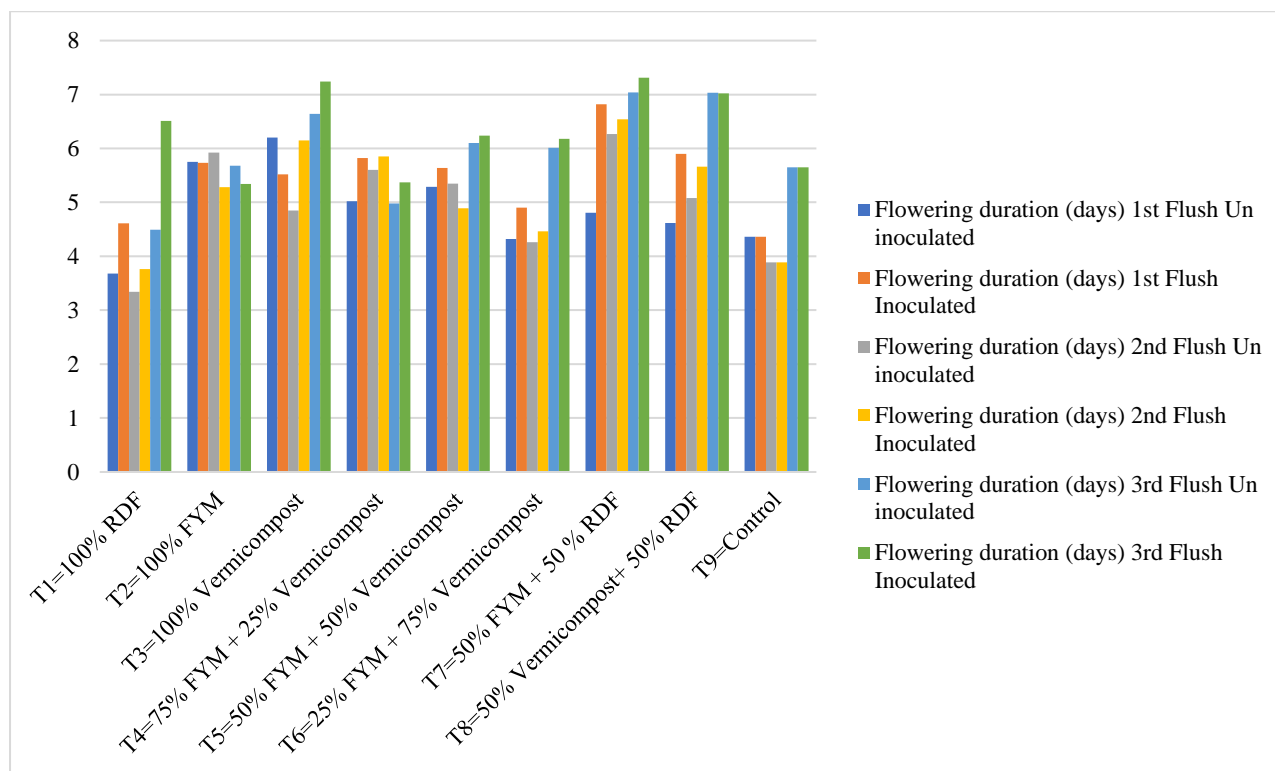


Fig. 2. Effect of Trichoderma, organic manure and inorganic fertilizer on flowering duration in rose plant

Table 1. Effect of *Trichoderma*, organic manure and inorganic fertilizer on growth parameters in rose

Treatment <i>Trichoderma</i> Nutrients →	Plant height (cm)						Number of leaves per plant					
	1 st Flush		2 nd Flush		3 rd Flush		1 st Flush		2 nd Flush		3 rd Flush	
	Un inoculated	Inoculated	Un inoculated	Inoculated	Un inoculated	Inoculated	Un inoculated	Inoculated	Un inoculated	Inoculated	Un inoculated	Inoculated
T ₁ =100% RDF	62.08	76.72	45.48	48.12	42.92	43.21	126.41	126.08	74.86	77.46	75.23	79.60
T ₂ =100% FYM	79.36	89.36	48.30	51.96	48.57	55.60	154.60	137.41	74.80	76.58	81.63	79.84
T ₃ =100% Vermicompost	88.46	103.90	53.36	56.36	55.78	51.07	167.98	169.53	83.20	81.77	77.91	80.71
T ₄ =75% FYM + 25% Vermicompost	90.70	96.94	55.39	57.01	52.57	47.07	138.97	131.95	72.26	74.40	79.69	75.29
T ₅ =50% FYM + 50% Vermicompost	88.58	68.02	53.06	52.30	50.46	52.06	143.23	150.96	73.60	84.60	82.77	77.02
T ₆ =25% FYM + 75% Vermicompost	95.22	89.56	47.38	51.73	54.04	56.61	145.65	154.32	74.06	78.20	77.85	74.71
T ₇ =50% FYM + 50 % RDF	99.26	107.08	46.29	58.44	47.70	58.83	173.24	189.76	73.34	86.84	76.80	88.40
T ₈ =50% Vermicompost+ 50% RDF	97.72	93.78	48.96	56.58	45.90	46.96	178.56	182.92	71.95	83.87	74.26	84.52
T ₉ =Control	59.30	59.30	40.38	40.38	37.66	37.66	102.51	102.51	61.69	61.69	69.07	69.07
Mean	84.52	87.18	48.73	52.54	48.40	49.90	147.91	149.16	73.30	77.71	77.24	78.91
C.D. at 5%												
T	NS		NS		NS		NS		0.38		0.23	
N	9.77		8.55		9.24		6.13		2.68		2.36	

Table 2. Effect of *Trichoderma*, organic manure and inorganic fertilizer on flowering parameters in rose

Treatment <i>Trichoderma</i> Nutrients →	Day to bud initiation						Bud diameter (mm)					
	1 st Flush		2 nd Flush		3 rd Flush		1 st Flush		2 nd Flush		3 rd Flush	
	Un inoculated	Inoculated	Un inoculated	Inoculated	Un inoculated	Inoculated	Un inoculated	Inoculated	Un inoculated	Inoculated	Un inoculated	Inoculated
T ₁ =100% RDF	47.64	41.97	39.44	35.86	28.94	29.69	21.40	22.88	19.68	21.40	15.78	17.86
T ₂ =100% FYM	39.82	39.25	34.12	34.86	32.22	29.22	22.60	24.11	21.80	20.69	20.24	17.04
T ₃ =100% Vermicompost	45.06	42.60	36.30	35.02	32.94	26.14	22.44	24.95	20.31	22.88	15.81	20.52
T ₄ =75% FYM + 25% Vermicompost	42.47	43.20	37.45	32.40	33.33	32.39	22.60	23.19	21.64	20.92	17.22	18.72
T ₅ =50% FYM + 50% Vermicompost	45.32	44.50	42.20	38.00	29.52	28.56	21.35	24.49	20.68	21.38	16.06	19.67
T ₆ =25% FYM + 75% Vermicompost	42.56	42.79	34.80	35.60	31.23	29.23	21.35	23.75	18.28	21.34	16.46	16.46
T ₇ =50% FYM + 50 % RDF	42.32	37.11	32.86	31.40	34.60	27.60	21.83	25.76	20.50	25.17	16.54	19.70
T ₈ =50% Vermicompost+ 50% RDF	43.46	37.25	36.42	34.41	29.05	27.83	23.57	26.27	19.48	23.14	16.19	18.12
T ₉ =Control	48.07	48.07	39.29	39.29	36.08	36.08	20.20	20.20	22.15	22.15	12.26	12.26
Mean	44.08	41.93	36.99	35.20	31.99	29.64	21.92	23.96	20.50	22.34	16.29	17.82
C.D. at 5%												
T	1.98		1.64		1.75		0.33		0.92		1.38	
N	4.21		3.49		3.73		1.36		1.95		2.94	

Table 3. Effect of *Trichoderma*, organic manure and inorganic fertilizer on flowering parameters in rose

Treatment <i>Trichoderma</i> Nutrients →	Flowering duration (days)						Number of flowers per plant					
	1 st Flush		2 nd Flush		3 rd Flush		1 st Flush		2 nd Flush		3 rd Flush	
	Un inoculated	Inoculated	Un inoculated	Inoculated	Un inoculated	Inoculated	Un inoculated	Inoculated	Un inoculated	Inoculated	Un inoculated	Inoculated
T ₁ =100% RDF	3.68	4.61	3.34	3.76	4.49	6.51	22.38	22.51	12.70	13.30	15.64	18.69
T ₂ =100% FYM	5.75	5.73	5.92	5.28	5.68	5.34	23.94	25.69	13.75	13.77	19.58	18.28
T ₃ =100% Vermicompost	6.20	5.52	4.85	6.15	6.64	7.24	24.33	26.37	13.89	14.70	20.03	20.05
T ₄ =75% FYM + 25% Vermicompost	5.02	5.82	5.60	5.85	4.98	5.37	22.59	23.93	13.71	14.08	16.77	18.49
T ₅ =50% FYM + 50% Vermicompost	5.29	5.64	5.35	4.89	6.10	6.24	22.14	25.74	14.10	14.03	16.51	17.51
T ₆ =25% FYM + 75% Vermicompost	4.32	4.90	4.26	4.46	6.01	6.18	23.24	25.09	15.45	16.05	14.18	17.43
T ₇ =50% FYM + 50 % RDF	4.81	6.82	6.27	6.54	7.04	7.31	24.26	26.69	15.71	17.02	18.63	22.59
T ₈ =50% Vermicompost+ 50% RDF	4.62	5.90	5.08	5.66	7.03	7.02	23.11	27.29	13.98	14.38	17.12	21.49
T ₉ =Control	4.36	4.36	3.89	3.89	5.65	5.65	21.61	21.61	11.35	11.35	15.82	15.82
Mean	4.89	5.37	4.95	5.16	5.96	6.33	23.06	25.02	13.82	14.30	17.14	18.93
C.D. at 5%												
T	0.37		0.15		0.24		0.61		0.42		1.08	
N	0.78		0.71		0.87		1.30		0.89		2.29	

Table 4. Effect of *Trichoderma*, organic manure and inorganic fertilizer on postharvest parameters in rose

Treatment <i>Trichoderma</i> Nutrients →	Vase life (days)						Anthocyanin Content(g/mg ⁻¹)					
	1 st Flush		2 nd Flush		3 rd Flush		1 st Flush		2 nd Flush		3 rd Flush	
	Un inoculated	Inoculated	Un inoculated	Inoculated	Un inoculated	Inoculated	Un inoculated	Inoculated	Un inoculated	Inoculated	Un inoculated	Inoculated
T ₁ =100% RDF	12.25	14.47	11.80	12.60	12.93	14.26	395.57	403.89	353.57	349.89	373.67	390.16
T ₂ =100% FYM	13.70	13.83	13.42	14.40	15.11	15.24	435.65	547.54	391.65	473.54	382.27	433.72
T ₃ =100% Vermicompost	12.62	15.12	14.32	14.14	15.45	15.84	598.30	620.66	558.30	528.66	530.27	518.28
T ₄ =75% FYM + 25% Vermicompost	13.64	14.82	13.22	13.92	14.41	14.84	353.01	402.50	348.81	356.50	386.10	411.12
T ₅ =50% FYM + 50% Vermicompost	13.26	12.42	13.20	11.87	13.55	13.35	506.65	463.12	486.65	423.12	422.34	404.94
T ₆ =25% FYM + 75% Vermicompost	12.92	12.83	12.80	12.64	13.13	14.19	466.82	431.38	466.82	407.33	468.05	494.22
T ₇ =50% FYM + 50 % RDF	14.62	16.12	14.60	15.20	14.32	15.54	527.58	517.24	397.58	346.68	465.91	494.01
T ₈ =50% Vermicompost+ 50% RDF	13.05	14.84	12.40	14.14	13.50	14.58	489.59	464.78	461.59	451.18	416.95	446.29
T ₉ =Control	11.29	11.29	10.61	10.61	12.23	12.23	307.67	307.67	283.67	283.67	329.29	329.29
Mean	13.04	14.08	12.93	13.26	13.85	14.45	453.43	462.08	416.51	402.29	419.43	435.78
C.D. at 5%												
T	0.53		0.31		0.47		NS		NS		NS	
N	1.13		1.38		1.00		79.01		57.94		58.93	

add important micronutrients like magnesium and iron, which are necessary for making chlorophyll and anthocyanin. Potassium also helps in developing pigments by boosting the metabolic processes that are linked to flavonoid production (Lallawmzuali, 2023). Similar observations were reported by Ahmed *et al.* (2011). Furthermore, Latif and Mustafa (2019) examined the effect of biofertilizers and carbolizers on the growth of *Gerbera jamesonii*, reporting an anthocyanin concentration of 30.11 mg/100 g in flower petals.

4. CONCLUSION

The current study assessed the impact of different nutrient combinations and *Trichoderma* inoculation on the growth, flowering and postharvest characteristics of rose cv. Top Secret in polyhouse conditions. The findings indicate that using either (T₇) 50% FYM + 50% RDF in inoculation or (T₈) 50% vermicompost + 50% RDF in inoculation with *Trichoderma* found the best results. These nutrient combinations led to notable enhancements in plant height, leaf count, bud initiation, flower yield, vase life and anthocyanin content. The combined effects of organic, inorganic nutrients and *Trichoderma* significantly contributed to improved overall plant health, flower quality and postharvest durability, surpassing the benefits of individual treatments and promoting sustainable cultivation under diverse agro-climatic conditions.

ACKNOWLEDGEMENT

The authors sincerely appreciate the support and resources provided by the Institute of Agricultural Sciences, BHU, Varanasi and acknowledge the valuable assistance from the Department of Horticulture (Floriculture and Landscaping), Institute of Agricultural Sciences, BHU, Varanasi.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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