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Efficacy of bioagents on seed germination, vigour, and seedling development in muskmelon (*Cucumis melo*) hybrid

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ABSTRACT

Improving seed germination and seedling vigour in muskmelon (*Cucumis melo* L.) remains a significant challenge, especially under variable field conditions that hinder early crop establishment. The use of selected bioagents offers a potential strategy to enhance germination efficiency, seedling growth, and vigour. The experiment was conducted during October 2024 at the Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajinagar, Maharashtra to assess the effects of 36 different individual and combined treatments of bioagents, including *Trichoderma* spp., *Pseudomonas fluorescens*, *Bacillus* spp., and *Rhizobium japonicum*. The bioagents and their combinations significantly improved seed germination and vigour index, although some treatments showed inhibitory effects. Among the 36 treatments, the combined treatment $T_1 + T_3$ (*Trichoderma viride* and *Trichoderma harzianum*) recorded the highest seed germination (99.67%), followed by T_4 (*Trichoderma harzianum*, 99%). The combined treatment $T_4 + T_6$ (*Trichoderma harzianum* and *Pseudomonas fluorescens*) achieved the highest seedling length (20.12 cm) and vigour index (1745.10), closely followed by T_4 (17.22 cm; 1705) and $T_2 + T_4$ (*Trichoderma viride* and *Trichoderma harzianum*, 17.53 cm; 1680.60), indicating a synergistic effect on early seedling growth. The lowest germination was observed in the control (T_0), with inhibitory effects noted in $T_7 + T_8$ (*Pseudomonas fluorescens* and *Bacillus subtilis*), $T_7 + T_{11}$ (*Pseudomonas fluorescens* + *Rhizobium japonicum*), T_{10} (microbial consortium), and T_8 (*Bacillus subtilis*). These findings suggest that specific combinations of compatible bioagents can significantly enhance muskmelon seedling vigour under laboratory conditions.

Keywords: Bioagents, Muskmelon, *Pseudomonas fluorescens*, Seed germination, *Trichoderma,* Vigour index

Muskmelon (*Cucumis melo* L., 2n = 2x = 24) is an important fruit vegetable cultivated widely across tropical, subtropical, and temperate regions. Major centres of diversity include Iran, Uzbekistan, Afghanistan, China, and India (Ojo 2021, Kaur *et al.* 2025). India is one of the leading producers, with extensive cultivation in Uttar Pradesh, Madhya Pradesh, Punjab, Rajasthan, and Haryana (Pansare and Dhakne 2024). Despite its economic importance, muskmelon cultivation faces several challenges, including poor seed germination, weak seedling growth, and low vigour, particularly during off-season conditions. The optimum temperature for germination (25–35°C) often drops during January–February, resulting in patchy germination and poor seedling establishment. In addition, soil-borne pathogens such as *Fusarium oxysporum* f. spp. *melonis*, *Erwinia tracheiphila*, *Pythium* spp., *Rhizoctonia* spp., and *Monosporascus cannonballus* further constrain crop performance (Ojo 2021, Schuh and Grabowski 2022, Liu *et al.* 2024). Seed treatment has been shown to improve germination and seedling vigour under stress. Among various methods, bio-priming with beneficial microorganisms has gained attention as an eco-friendly and sustainable approach. *Trichoderma* species are well recognised as biocontrol agents due to their antagonistic activity against pathogens and growth-promoting potential (Yedidia *et al.* 1999, Harman 2011, Patil *et al.* 2023). Similarly, rhizobacteria such as *Pseudomonas fluorescens*, *Bacillus* spp., and *Rhizobium japonicum* enhance plant growth through phytohormone production and nutrient mobilisation (Vinale *et al.* 2008, Saleemi *et al.* 2017). Considering these advantages, the present study was carried out to evaluate the effects of individual and combined fungal and bacterial bioagents on muskmelon seed germination, seedling growth, and vigour index. The primary objective was to identify effective, synergistic microbial combinations that enhance early seedling performance and support sustainable seed enhancement practices under laboratory conditions.

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MATERIALS AND METHODS

The experiment was conducted during October

2024 at the Seed Pathology and Fungal Biotechnology Laboratory, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajinagar (19°54'10.71" N, 75°18'26.17" E; at an elevation of 575 m amsl), Maharashtra. To improve seed germination and vigour in muskmelon, seeds underwent 36 different treatments, both individually and in combination, involving bioagents, viz. *Trichoderma* spp., *Pseudomonas fluorescens*, *Bacillus* spp., and *Rhizobium japonicum* (Table 1). Both treated and untreated (control) seeds were tested for germination percentage, seedling length, and vigour index under laboratory conditions. Observations were recorded using standard procedures, and the data were statistically analysed to determine the significance of the treatment effects.

Sources of bio-control agents: *Trichoderma* spp., *Pseudomonas fluorescens*, *Bacillus subtilis*, *Rhizobium japonicum*, microbial consortium, and other bio-products used in this study were from various research institutes and commercial suppliers. A muskmelon hybrid, Ellora Sindhu-596, was used for seed treatment against bioagents (individual and combinations) with different Colony Forming Units (CFUs).

Selection of bioagents and their preparation: The experiment comprised 36 treatments, including 8 individual bioagents and 28 combinations of bioagents. The muskmelon seeds used in the study were six months old and had been stored at 5–10°C. Seeds without cracks or visible deformities were selected for the experiment. The seeds were surface-sterilised by immersing them in a 1% sodium hypochlorite solution for 15 min, followed by three washes with sterile distilled water, and subsequently dried under aseptic conditions. Germination papers were placed in large petri dishes and moistened with sterile distilled water. In each replicate, 100 surface-sterilised seeds were randomly selected for the treatment.

Sources of bioagents: T_1 and T_{11}, Maharashtra State Seeds Corporation Ltd., Akola, Maharashtra; T_2 and T_7, IPL biologicals Ltd. Gurugram, Haryana; T_3 and T_6, Katyayani Krishi Direct, Bhopal, Madhya Pradesh; T_4, Titan Agritech Ltd. Bhiwadi, Rajasthan; T_5, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra; T_8, Seed Pathology and Fungal Biotechnology Laboratory, Dept. of Botany, Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajinagar, Maharashtra; T_9, Indian Farmers Fertiliser Cooperative Ltd.; T_{10}, ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru, Karnataka; and T_{12}, Vardesian Life Sciences, Cary, North Carolina, USA.

Seed treatment: Seed coating was performed using a spore suspension formulated with 2% (w/v) starch, which acted as an adhesive to ensure even adherence of propagules to the seed surface. A batch of 300 dry muskmelon seeds (10 g) was treated with 1 g of bioagent formulation for 1–2 min, covering a total of 36 treatments: 8 individual bioagents, 24 binary combinations, 4 additional treatments, and one untreated control. The control seeds were immersed in sterilised distilled water, following the procedure described by Ben-Jabeur *et al.* (2019). After treatment, all seeds were air-dried under laminar airflow to promote complete coating and prevent microbial contamination. For the *in vitro* germination assay, treated seeds were placed aseptically in sterile petri dishes lined with germination paper pre-moistened with sterilised distilled water. The dishes were then incubated at 25°C under controlled laboratory conditions. For combination treatments, spore suspensions were prepared by mixing the respective bioagents at half of their individual concentrations while maintaining the recommended total CFU level for the treatment. This approach ensured balanced microbial loading and avoided excessive inoculum that could interfere with early seed physiological processes.

Table 1 Details of the bioagents used for the muskmelon seed treatment

Treatments	Bioagents	CFU value	Strain no.	Accession no.	Trade name	Formulation type
T_1	*Trichoderma viride* *	2×10^6	IIHR TV-5	ITCC No.-688	Mahabeej	Powder
T_2	*Trichoderma viride* *	2 v 10^9	IPL/VT/101	NA	Sanjeevani	Powder
T_3	*Trichoderma harzianum* *	5×10^8	NA	NA	Katyayani	Powder
T_4	*Trichoderma harzianum* *	2×10^6	IIHR-TH-2	ITCC NO.-6888	Trich-Peph	Powder
T_5	Mix *Trichoderma* *	NA	NA	NA	Biomix	Powder
T_6	*Pseudomonas fluorescens* *	5×10^8	NA	NA	Katyayani	Powder
T_7	*Pseudomonas fluorescens* *	1×10^8	IPL/PS-01	MTCC 5727	Sanjeevani	Powder
T_8	*Bacillus subtilis*	2×10^6	NA	NA	NA	Liquid
T_9	NPK Consortia biofertilizer *	5×10^7	NA	NA	Liquid Consortia	Liquid
T_{10}	Microbial Consortium *	10^8	NA	NA	Arka Microbial Consortium	Liquid
T_{11}	*Rhizobium japonicum* *	1×10^8	NA	NA	Mahabeej	Liquid
T_{12}	Nutritional supplement with PGPR*	NA	NA	NA	SDM	Powder

*, Commercial product; NA, Not Available; CFU, Colony forming unit. Commercial formulations were used for some bioagents, some manufacturers did not provide strain and accession numbers.

Seed germination assay: The experiment was arranged in a completely randomised design (CRD) with 37 treatments (36 treatments + 1 control), each replicated three times. Each replication consisted of five petri dishes, with 20 seeds sown/dish, totaling 100 seeds/replication. Experimental plates were kept in a growth chamber maintained at 25 ± 1°C with alternating 12 h light and dark cycles, and observations of seed germination and seedling development were recorded at 3, 5, and 7 days after coating (Ben-Jabeur *et al.* 2019). Observations included days to plumule initiation, days to radicle initiation, mean shoot and root length (seedling length), and vigour index. Germination percentage was determined by applying the standard formula:

$$\text{Germinational (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds used}} \times 100$$

Seedling parameters: As described by Febri Doni *et al.* (2014), shoot length was measured from the base of the primary leaf to the base of the hypocotyl, while root length extended from the primary root tip to the hypocotyl base. Measurements were recorded in centimetres (cm). A total of 15 healthy seedlings were randomly selected on the 7th day to assess shoot and root lengths using a standard scale.

Vigour index: The value was derived by using the formula below (Abdul Baki and Anderson 1973):

$$\text{Vigour Index (VI - I)} = \text{Germination (\%)} \times \text{Seedling length (mean shoot length + mean root length)}$$

Statistical analysis: The collected data were analysed using one-way analysis of variance (ANOVA), and the least significant difference (LSD) test was employed to identify significant differences ($p \leq 0.05$) among the treatments. Statistical analyses were performed using the Agricolae package in R Studio (Mendiburu 2020). Correlation analysis was conducted using the Metan package in R Studio software (Olivoto and Lucio 2020).

RESULTS AND DISCUSSION

The impact of various bioagent treatments on seedling growth parameters of muskmelon including shoot length, root length, total seedling length, germination percentage, and seedling vigour index (VI-I), was found to be significant at $p \leq 0.05$ (Table 2). Significant differences among treatments were observed for all parameters, indicating that inoculating different bioagents either alone or in combination had varying effects on seedling performance. Most of the bioagent-treated seeds showed significant improvement over the control (T_0). The results revealed substantial variation in seedling growth, germination percentage, and vigour index among treatments, both individually and in combinations. Germination percentage showed wide variation among treatments, ranging from 72.67% (T_0)–99.67% ($T_1 + T_3$). The highest germination was recorded in the combined treatment $T_1 + T_3$ (*Trichoderma viride* + *T. harzianum*), followed closely by T_4 (*T. harzianum*; 99.00%), $T_1 + T_6$ (*T. viride* + *Pseudomonas fluorescens*; 98.33%), and $T_2 + T_4$ (*T. viride* + *T. harzianum*; 96.33%). These treatments exhibited significantly higher germination than the untreated control (T_0), which recorded only 72.67%. Sharma *et al.* (2023) reported that *Trichoderma harzianum* significantly improved seed germination and vigour in cucumber. Similar growth-promoting effects of *Trichoderma* spp., *Pseudomonas fluorescens* and other bioagents were reported in squash landraces by Neji *et al.* (2024), confirming that bioagent application is essential for improving seed metabolic activation and early seedling establishment. Shoot and root lengths also varied significantly among treatments. The maximum shoot length (9.58 cm) was recorded in $T_4 + T_6$ (*T. harzianum* + *P. fluorescens*), followed by T_4 (8.40 cm) and T_1 (7.63 cm). Root length was highest in $T_6 + T_{11}$ (*P. fluorescens* + *Rhizobium japonicum*; 10.55 cm), followed closely by $T_4 + T_6$ (10.54 cm). These results demonstrated the strong growth-promoting ability of *Pseudomonas fluorescens*, particularly when combined with either *T. harzianum* or *R. japonicum*. These treatments exhibited a positive interaction between the bioagents, resulting in a more robust root-shoot development (Fig. 1, 2 and 3). The highest seedling length (20.12 cm) was observed in $T_4 + T_6$, followed by $T_2 + T_4$ (17.53 cm) and T_4 (17.22 cm) (Supplementary Fig. 1). In contrast, T_8 (*Bacillus subtilis*) and T_{10} (Microbial consortium) exhibited the shortest shoot (2.39 cm, 1.86 cm, respectively) and root length (3.41 cm, 3.01 cm, respectively) producing total seedling lengths of only 5.80 cm and 4.88 cm, respectively. It indicates a suppressive influence of the *Bacillus subtilis* and microbial consortium treatment on seedling length. Poveda and Eugui (2022) reported that co-inoculation of *Trichoderma viride* and *T. harzianum* with plant growth-promoting bacteria such as *Pseudomonas fluorescens*, *Rhizobium* spp., *Bacillus* spp., and *Azotobacter* spp. significantly enhanced

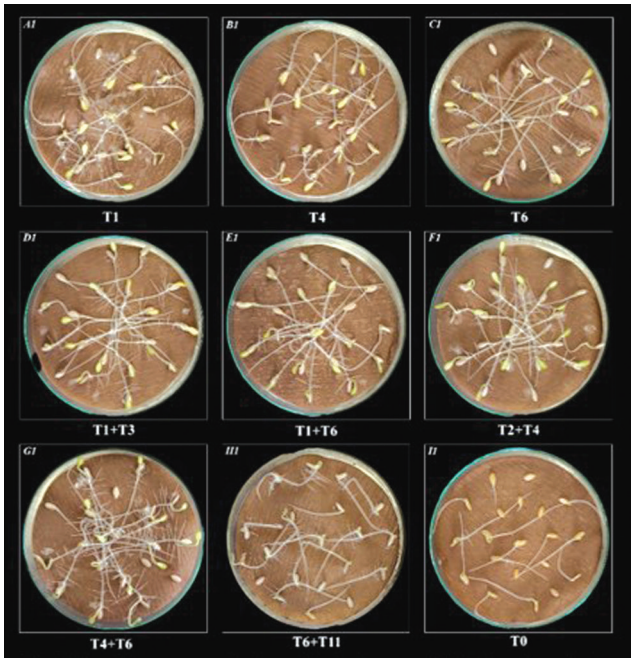

Fig. 1 Effect of bioagents on muskmelon seedling development at three days after sowing.

Table 2 Effect of bioagents and their combinations on seed germination, seedling growth, and seedling vigour in muskmelon

Sr. No.	Treatments	Mean length (cm)			Mean germination (%)	Vigour index (VI-I)
		Shoot	Root	Seedling		
1.	T_1	7.63^{cd}	8.29^{g}	15.91^{d}	95.33^{cd}	1518.97^{e}
2.	T_2	3.31^{qr}	5.22^{m}	8.53^{n}	85.33^{lm}	734.67^{stu}
3.	T_3	4.40^{lmno}	5.01^{mn}	9.41^{m}	94.6^{7d}	891.17^{q}
4.	T_4	8.40^{b}	8.82^{ef}	17.22^{bc}	99.00^{ab}	1705.00^{ab}
5.	T_5	5.25^{i}	7.33^{ij}	12.57^{h}	90.67^{fgh}	1137.23^{l}
6.	T_6	7.41^{d}	9.65^{bc}	17.07^{bc}	82.67^{n}	1409.23^{fg}
7.	T_7	6.44^{efg}	8.41^{fg}	14.85^{f}	90.33^{gbi}	1340.27^{hij}
8.	T_8	2.39^{t}	3.41^{p}	5.80^{p}	89.67^{hi}	521.50^{v}
9.	T_9	5.09^{ij}	7.62^{hij}	12.71^{h}	95.33^{cd}	1209.87^{k}
10.	T_{10}	1.86^{u}	3.01^{p}	4.88^{q}	84.67^{lm}	416.90^{w}
11.	T_{11}	4.86^{ijkl}	7.55^{ij}	12.41^{hi}	85.67^{kl}	1062.33^{mn}
12.	T_{12}	3.93^{op}	4.95^{mno}	8.89^{mn}	85.33^{lm}	760.20^{rst}
13.	$T_1 + T_3$	6.76^{e}	9.92^{b}	16.68^{c}	99.67^{a}	1662.03^{bc}
14.	$T_1 + T_4$	2.57^{st}	3.07^{p}	5.65^{p}	89.33^{ij}	492.93^{v}
15.	$T_1 + T_6$	6.65^{ef}	9.03^{de}	15.67^{de}	98.33^{b}	1540.43^{e}
16.	$T_1 + T_7$	4.31^{mno}	6.33^{kl}	10.64^{l}	90.00^{hi}	959.30^{op}
17.	$T_1 + T_8$	2.85^{rst}	5.93^{l}	8.78^{mn}	89.33^{ij}	784.10^{rs}
18.	$T_1 + T_{11}$	3.15^{qr}	4.61^{no}	7.76^{o}	88.33^{j}	684.37^{u}
19.	$T_2 + T_3$	3.98^{op}	4.55^{no}	8.53^{n}	90.67^{fgh}	770.27^{rst}
20.	$T_2 + T_4$	8.07^{bc}	9.47^{bcd}	17.53^{b}	96.33^{c}	1685.60^{ab}
21.	$T_2 + T_6$	6.27^{fgh}	7.73^{hi}	14.00^{g}	91.67^{ef}	1280.53^{j}
22.	$T_2 + T_7$	6.79^{e}	8.12^{gh}	14.91^{f}	92.33^{e}	1370.40^{ghi}
23.	$T_2 + T_8$	2.93^{rs}	6.10^{k}	9.03^{mn}	90.33^{ghi}	817.17^{r}
24.	$T_2 + T_{11}$	7.75^{cd}	9.25^{cde}	17.00^{bc}	95.33^{cd}	1616.80^{cd}
25.	$T_3 + T_6$	4.61^{jklm}	7.17^{j}	11.78ij	90.67^{fgh}	1066.30^{m}
26.	$T_3 + T_7$	6.72^{ef}	9.07^{de}	15.79^{de}	91.33^{efg}	1441.10^{f}
27.	$T_3 + T_8$	5.23^{i}	9.95^{b}	15.17^{ef}	86.67^{k}	1315.70^{ij}
28.	$T_3 + T_{11}$	4.12^{no}	6.59^{k}	10.71^{kl}	84.33^{m}	906.63^{pq}
29.	$T_4 + T_6$	9.58^{a}	10.54^{a}	20.12^{a}	86.67^{k}	1745.10^{a}
30.	$T_4 + T_7$	5.23^{i}	6.16^{k}	11.39^{jk}	95.33^{cd}	1085.67^{lm}
31.	$T_4 + T_8$	5.97^{gh}	9.12^{de}	15.09^{ef}	91.67^{ef}	1386.33^{fgh}
32.	$T_4 + T_{11}$	3.10^{qr}	4.49^{o}	7.59^{o}	94.33^{d}	717.10^{tu}
33.	$T_6 + T_8$	5.79^{h}	9.50^{bcd}	15.29^{def}	95.33^{cd}	1448.27^{f}
34.	$T_6 + T_{11}$	6.58^{ef}	10.55^{a}	17.13^{bc}	91.67^{ef}	1575.63^{de}
35.	$T_7 + T_8$	3.55^{pq}	9.20^{cde}	12.75^{h}	81.33^{o}	1041.90^{mn}
36.	$T_7 + T_{11}$	4.94^{ijk}	7.48^{ij}	12.42^{hi}	80.67^{o}	999.50^{no}
37.	T_0	4.52^{klm}	6.59^{k}	11.11^{jk}	72.67^{p}	802.10^{r}
Mean		5.2159	7.2914	12.5076	90.0811	1132.5025
SEM		0.210	0.221	0.321	0.51	27.73
CV (%)		5.68	4.29	3.63	0.81	3.46

Lowercase letters indicate significant differences at $p \leq 0.05$ (LSD). T_1, *Trichoderma viride*; T_2, *Trichoderma viride*; T_3, *Trichoderma harzianum*; T_4, *Trichoderma harzianum*; T_5, Mix *Trichoderma*; T_6, *Pseudomonas fluorescens*; T_7, *Pseudomonas fluorescens*; T_8, *Bacillus subtilis*; T_9, NPK Consortia biofertiliser; T_{10}, Microbial Consortium; T_{11}, *Rhizobium japonicum*; T_{12}, Nutritional supplement including PGPR; T_0, Control.

plant growth responses. Their review highlighted that synergistic interactions between fungi and bacteria improve nutrient uptake, stimulate phytohormone production, and strengthen plant developmental processes. An increase in seed germination percentage and seedling length enhances seedling vigour, implying that uniform emergence ultimately improves physiological and metabolic activity during early growth stages. The maximum vigour index (1745.10) was obtained in combined treatment $T_4 + T_6$, followed by T_4 (1705.00) and $T_1 + T_3$ (1662.03), while the minimum was observed in T_{10} (416.90) and T_8 (521.50). Microbial consortium (T_{10}) and *Bacillus subtilis* (T_8) exhibited comparatively lower effectiveness in enhancing seedling vigour in muskmelon. The enhanced vigour index (VI-I) in these treatments was precisely associated with increased seedling length and germination percentage, reflecting the potential of these bioagents or their combinations to boost seedling performance (Fig. 2 and 3). The observed superiority of the combined treatment $T_4 + T_6$ confirmed the established synergistic relationship between these two different bioagents. The independent study of Vij *et al.* (2022) and Kumari *et al.* (2025), demonstrated that the co-inoculation of *T. viride* or *T. harzianum* with *P. fluorescens* provided the maximum increment in seedling vigour index and growth parameters in cabbage and chickpea, respectively, surpassing the performance of either microbe applied alone. Furthermore, this synergy is often linked to the potentiation of plant-beneficial responses, where the bacterial component plant growth promoting bacteria (PGPBs) upregulates the effector functions of the fungal antagonist (Guzman-Guzman *et al.* 2024). This suggests that the combined application is not simply additive but triggers muskmelon seedling development. During the analysis, it was observed that not all microbial combinations were synergistic. Certain combinations such as $T_1 + T_4$, $T_1 + T_{11}$, $T_4 + T_{11}$, and $T_3 + T_{11}$ displayed reduced effectiveness compared to the performance of these microbes individually. These outcomes likely reflect functional incompatibilities, competition for root colonisation sites, or production of inhibitory metabolites. In contrast, combinations of *Trichoderma viride*, *T. harzianum*, and *Pseudomonas fluorescens* especially $T_4 + T_6$, $T_1 + T_3$, and $T_2 + T_4$ consistently produced superior growth responses, highlighting strong compatibility and complementary mechanisms. Earlier studies also supported these findings. Jaiman *et al.* (2020), Pawar *et al.* (2023), and Sharma *et. al.* (2023) reported that seed treatment with *Trichoderma harzianum*, *T. viride*, and *Pseudomonas fluorescens* significantly enhanced germination, seedling length, and overall vigour index in tomato, brinjal, onion, chilli, cabbage and Cucumber. Their results corroborated the present findings, highlighting the broad-spectrum efficacy of these bioagents across different crops.

The emergence of radicle, root hairs, and plumule in muskmelon seeds was strongly influenced by the application of individual and combined bioagents (Table 3). The treatments displayed substantial variation in the timing and progression of emergence events, reflecting the differing

Table 3 Effect of bioagents on radicle, root hair, and plumule emergence in muskmelon

Sr. no.	Treatments	Days to radicle emergence	Days to root hair emergence	Days to plumule emergence
1	T_1	2.00	3.00	3.26
2	T_2	2.50	-	8.33
3	T_3	2.50	-	5.63
4	T_4	2.00	3.00	3.58
5	T_5	2.50	5.00	4.78
6	T_6	2.00	3.00	4.25
7	T_7	2.50	4.44	4.50
8	T_8	4.66	-	8.50
9	T_9	2.50	4.33	5.38
10	T_{10}	2.50	4.43	5.31
11	T_{11}	2.50	5.75	5.17
12	T_{12}	3.00	6.00	8.20
13	$T_1 + T_3$	2.00	3.32	3.85
14	$T_1 + T_4$	4.00	5.00	8.67
15	$T_1 + T_6$	2.00	3.10	4.05
16	$T_1 + T_7$	2.50	-	7.00
17	$T_1 + T_8$	2.50	-	8.33
18	$T_1 + T_{11}$	3.00	5.00	7.75
19	$T_2 + T_3$	2.50	-	7.13
20	$T_2 + T_4$	1.50	4.58	3.58
21	$T_2 + T_6$	2.00	4.50	4.94
22	$T_2 + T_7$	2.50	4.33	4.53
23	$T_2 + T_8$	3.00	-	7.13
24	$T_2 + T_{11}$	2.00	3.56	4.05
25	$T_3 + T_6$	3.00	5.00	5.55
26	$T_3 + T_7$	2.00	3.44	4.21
27	$T_3 + T_8$	2.50	3.47	5.18
28	$T_3 + T_{11}$	3.00	-	7.07
29	$T_4 + T_6$	2.00	3.00	3.41
30	$T_4 + T_7$	2.00	4.89	5.11
31	$T_4 + T_8$	3.00	7.00	6.69
32	$T_4 + T_{11}$	2.50	4.50	8.60
33	$T_6 + T_8$	2.50	4.78	6.47
34	$T_6 + T_{11}$	2.50	3.67	7.78
35	$T_7 + T_8$	3.00	6.33	8.06
36	$T_7 + T_{11}$	2.50	4.87	6.69
37	T_0	2.50	4.20	6.42

Sign "-" indicates No emergence.

T_1, *Trichoderma viride*; T_2, *Trichoderma viride*; T_3, *Trichoderma harzianum*; T_4, *Trichoderma harzianum*; T_5, Mix *Trichoderma*; T_6, *Pseudomonas fluorescens*; T_7, *Pseudomonas fluorescens*; T_8, *Bacillus subtilis*; T_9, NPK consortia biofertiliser; T_{10}, Microbial consortium; T_{11}, *Rhizobium japonicum*; T_{12}, Nutritional supplement including PGPR; T_0, Control.

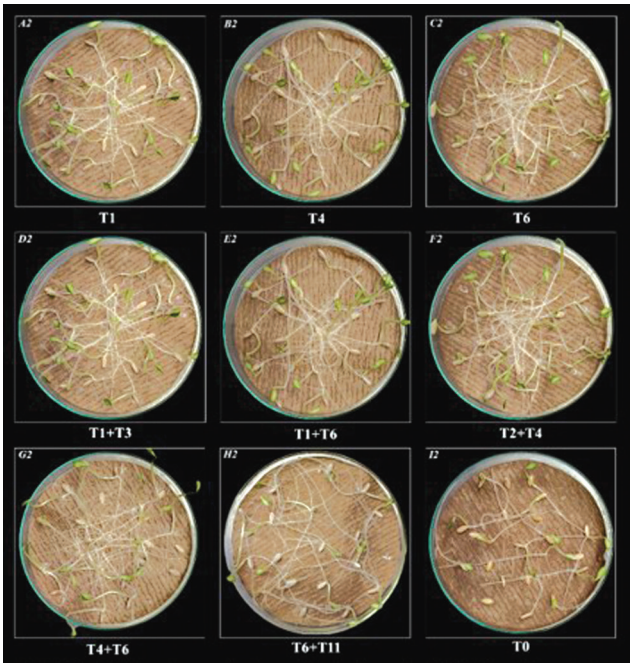

Fig. 2 Effect of bioagents on muskmelon seedling development at five days after sowing.

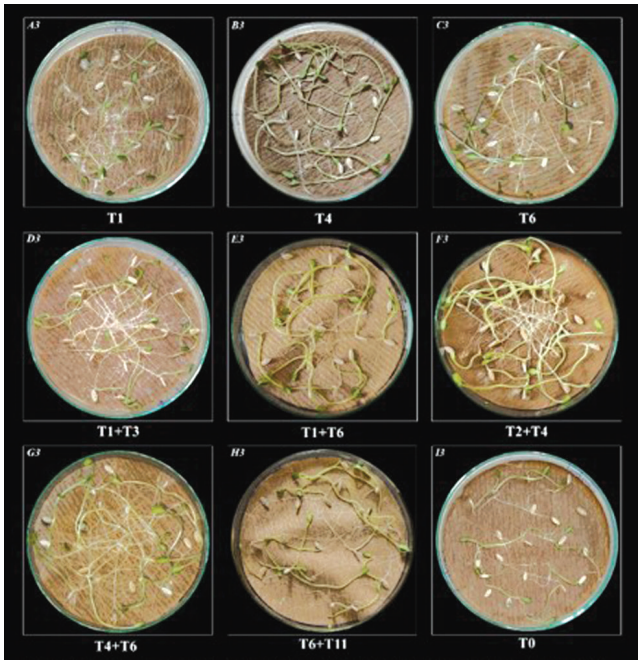

Fig. 3 Effect of bioagents on muskmelon seedling development at seven days after sowing.

physiological responses triggered by microbial compatibility, antagonism, and inoculum composition. The earliest radicle emergence was recorded in the $T_2 + T_4$ treatment (1.50 days), indicating a rapid activation of early germination processes and highlighting the synergistic interaction between *Trichoderma viride* and *Trichoderma harzianum*. Other treatments, viz. T_1, T_4, T_6, $T_1 + T_3$, $T_1 + T_6$, $T_2 + T_6$, $T_3 + T_7$, and $T_4 + T_6$ also promoted early radicle emergence (2.00 days), further emphasising the beneficial role of *Trichoderma* spp. and *Pseudomonas fluorescens* in stimulating radicle protrusion. These bioagents are known to produce growth-promoting metabolites, induce cell wall loosening, and improve water uptake, which may explain their consistent performance. In contrast, delayed radicle emergence was observed in treatments T_8 (4.66 days) and $T_1 + T_4$ (4.00 days), suggesting potential microbial incompatibility, nutrient competition, or stress-inducing biochemical interactions that hindered early seed physiological activity. The control (T_0) exhibited moderate radicle emergence at 2.50 days, serving as a baseline for comparison. Root hair initiation showed a similarly diverse pattern across treatments. Rapid root hair formation (≤3 days) was observed in T_1, T_4, T_6, and $T_4 + T_6$, signifying efficient stimulation of early root architecture. Enhanced root hair density contributes to improved nutrient absorption and root-soil contact, which in turn supports vigorous seedling development. These effects were clearly reflected in the seven-day root development pattern (Fig. 3). In contrast, delayed root hair emergence was evident in treatments such as $T_4 + T_8$ (7.00 days) and T_{12} (6.00 days), indicating suboptimal microbial compatibility or disruptive interactions that affected early root differentiation. Notably, several treatments completely suppressed root hair formation, including T_2, T_3, T_8, $T_1 + T_7$, $T_1 + T_8$, $T_2 + T_3$, $T_2 + T_8$, and $T_3 + T_{11}$. The absence of root hair initiation suggests strong antagonistic interactions, possibly due to inhibitory metabolites, competition for root exudates, or imbalanced colonisation patterns on the seed surface. Plumule emergence showed further differentiation among treatments. The earliest plumule emergence was recorded in $T_4 + T_6$ (3.41 days), followed closely by T_1 (3.26 days), T_4 (3.58 days), and $T_2 + T_4$ (3.58 days). These treatments demonstrated significant enhancement of shoot emergence, reflecting efficient coordination of microbial signals that promote shoot apex activation and cell division. Conversely, the slowest plumule emergence occurred in treatments $T_1 + T_4$ (8.67 days), T_8 (8.50 days), T_2 (8.33 days), and $T_1 + T_8$ (8.33 days), highlighting the detrimental effects of incompatible microbial combinations. The untreated control (T_0) showed plumule emergence at 6.42 days, marking an intermediate response. Microbial inoculum levels also played a critical role in determining treatment efficiency. Bioagents such as T_1 (*Trichoderma viride*, 2×10^6 CFU/g), T_4 (*Trichoderma harzianum*, 2×10^6 CFU/g), and T_6 (*Pseudomonas fluorescens*, 5×10^8 CFU/g), whether applied singly or in combinations such as $T_4 + T_6$ or $T_2 + T_4$, consistently improved radicle emergence, root hair formation, and plumule initiation. In contrast, treatments involving T_8 (*Bacillus subtilis*, 2×10^6 CFU/g), T_{10} (Microbial consortium, 1×10^8 CFU/mL), T_{11} (*Rhizobium japonicum*, 1×10^8 CFU/mL), and several incompatible combinations demonstrated inconsistent or suppressed emergence patterns, revealing the importance of optimising CFU and microbial compatibility for successful seed treatment. These findings confirmed that early seedling emergence in muskmelon depends heavily on microbial compatibility and optimised inoculum levels (CFU). Among

all treatments, bioagents involving *Trichoderma* spp. and *Pseudomonas fluorescens* showed the highest and most consistent enhancement of radicle, root hair, and plumule emergence. This demonstrates their potential as effective bioagents for seed treatment, improving early seedling establishment and crop vigour in muskmelon.

Although different CFU levels of bioagents were applied, the treatments produced diverse outcomes because each microbial strain exhibited unique biochemical activity, colonisation ability, and compatibility behaviour. Treatments such as T_4 (*Trichoderma harzianum*, 2×10^6 CFU/g), T_1 (*T. viride*, 2×10^6 CFU/g), and T_6 (*Pseudomonas fluorescens*, 5×10^8 CFU/g) consistently enhanced germination, seedling growth, and vigour, both individually and in combination, indicating strong compatibility and complementary plant growth-promoting activities in muskmelon. *T. viride* at 2×10^6 CFU/g (T_1) was highly compatible with *T. harzianum* at 5×10^8 CFU/g (T_3). In contrast, the higher inoculum of *T. viride* (T_2, 2×10^9 CFU/g) performed best with the lower CFU of *T. harzianum* (T_4, 2×10^6 CFU/g), demonstrating the importance of balanced microbial ratios. Synergistic interactions were evident in combinations such as $T_4 + T_6$, $T_2 + T_4$, $T_1 + T_3$, and $T_1 + T_6$. Commercially, these optimised microbial treatments offer an effective seed bio-priming strategy that improves seed germination, strengthens early establishment, and enhances seedling vigour in muskmelon.

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