

Response of Indian Mustard to Azotobacter Priming and Vermicompost: A Comparative Analysis

A study investigating the responses of Indian mustard (*Brassica juncea*) to biopriming with Azotobacter, incorporation of vermicompost and their comparative analysis was performed in 2024 Rabi season in new alluvial zone. The research involved ten genotypes of Indian mustard and four treatments in completely randomized design. Treatments were designed as seeds sown in field soil, Azotobacter-primed seeds in field soil, seeds sown in vermicompost mixed with field soil, and Azotobacter-primed seeds in vermicompost with field soil. Eight key parameters were considered i.e. germination rate, seedling fresh and dry weight, seedling length, vigour index I and II, proline, and chlorophyll content. Results established improvements in germination and other growth parameters, particularly with the treatment combining Azotobacter priming and vermicompost, which presented the highest values across most genotypes. The performance of TM 306 - 1 and TBM 143 genotypes had produced the best results. The results emphasize the potential for utilizing biofertilizers and organic amendments in sustainable mustard cultivation, providing an effective substitute for chemical fertilizers.

ABSTRACT

Keywords: Azotobacter, Vermicompost, Seed Priming, Indian Mustard

1. INTRODUCTION

Among the oilseeds, Indian mustard, from the Brassicaceae family, is a significant oilseed crop as it occupies a very vital place in the Indian agriculture scenario. It ranks second to groundnut in both area and production and is responsible for about 80% of the total rapeseed-mustard production. The mustard seeds are rich in nutrients, having an oil content that ranges from 38 to 50% and comprising erucic acid, linoleic acid, and oleic acid (Bater Dabi *et al.*, 2001; Gantait *et al.*, 2024; Janaki *et al.*, 2022; Kaushik *et al.*, 2024). The adverse effects of chemical fertilizers on Indian agriculture, both on soil and human health are manifold. The long-term effects of synthetic fertilizer usage have resulted in soil degradation, including reduced fertility and increased soil pH, so that at one point in time, it might turn unproductive land (Bhokare P. R. & Wankhade R. R., 2024; Dube *et al.*, 2024). Excessive use of chemical fertilizers also leads to the contamination of the soil with the metals, like cadmium and lead, which impose extensive environmental and health hazards (Dash *et al.*, 2022). Farmers are complaining that synthetic fertilizers not only detract nutritional quality from the crops but also taste bad, in addition to causing health problems such as hemoglobin disorders and chronic health issues because of high nitrate levels (Nichols, 2023). To solve the problem, biopriming with bio-fertilizers like Azotobacter and some organic amendments like vermicompost are good areas to explore as these provide sustainable and eco-friendly alternatives. Biopriming, a sort of seed treatment, refers to soaking seeds in a solution containing a beneficial microorganism. Microorganisms like bacteria or fungi colonizes and, in some cases, penetrate the seed coat (Gantait *et al.*, 2024; Govind *et al.*, 2024). Free living nitrogen fixing bacteria i.e. Azotobacter can convert atmospheric nitrogen into an available form from which plants can derive. Seed germination

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and seedling vigor are enhanced by growth-promoting chemical compounds produced by *Azotobacter* (Bater Dabi *et al.*, 2001; Janaki *et al.*, 2022; Kaushik *et al.*, 2024). Vermicompost refers to organic fertilizer formed from the digestion of organic waste materials by the earthworms. It is nutrient-rich semi-bulky organic fertilizer containing high concentrations of macro and micronutrients. Vermicompost is a good additive to soil because it improves the soil quality, soil fertility and microbial activity (M. Kumar *et al.*, 2023; Singh *et al.*, 2018). The present experiment has been conducted to compare the germination, seedling vigor, chlorophyll and proline content of different varieties when primed and exposed to vermicompost.

2. MATERIALS AND METHODS

The research investigated the impacts of ten genotypes (G) and four treatments (T) on several seedling growth and physiological metrics. The experiment was arranged in a completely randomized design with three replicates. Parameters including germination, seedling fresh and dry weight, seedling length, vigor index I and II, proline content, and chlorophyll content were evaluated to determine the growth potential and resilience of the crop under various treatments. The genotypes were BRM4(G₁), BRM13(G₂), BRM 14(G₃), Varuna(G₄), JD 6(G₅), PM 25(G₆), PM 29(G₇), TM 306-1(G₈), TBM 204(G₉) and TBM 143(G₁₀). The four treatments are as following. Seeds sown in field soil(T₁), Azotobacter primed seeds sown in field soil(T₂), Seeds sown in vermicompost + field soil(T₃), Azotobacter primed seeds sown in vermicompost + field soil(T₄). 50 seeds were placed in each sterilized plastic container left in open condition. In case of vermicompost treatment 50% of vermicompost and 50% of field soil was used. For Azotobacter seed priming at 5:1 ratio of Azotobacter to seed was maintained and seeds were soaked for one hour then dried. During the time of experiment maximum and minimum temperatures were 31.8°C and 12.4°C respectively, maximum, and minimum relative humidity were 78% and 54% respectively with 8.1 hours of average bright sunshine hours. After seventh day seedlings from the container were counted and germination(%) was calculated by dividing the number of seeds germinated by the total seeds planted, then multiplied by 100. Ten seedlings were picked gently from container after 7th day and seedling length was measured using a centimeter scale. Average data was presented in centimeter(cm). For seedling fresh weight five random seedlings were taken out from each container and their weight was measured in a weighing balance and average was calculated. To obtain seedling dry weight they were put in hot air oven till constant temperature was achieved. After that weights of five dry seedlings were observed using a weighing balance and average was calculated. The vigor index I was assessed to evaluate the overall vigor and health of the seedlings under controlled conditions. The vigor index I was calculated using the formula given by Abdul-Baki and Anderson(1973)[Vigor Index I=Germination Percentage x Average seedling length(cm)]. The vigor index II is a critical parameter for evaluating seedling vigor, providing insights into the overall health and growth potential of plants. The vigor index II was calculated using the formula given by Abdul-Baki and Anderson(1973)[Vigor Index II=Germination Percentage x Mean dry weight of seedlings(mg)]. Proline content was determined spectrophotometrically by adopting the ninhydrin method of Bates *et al.* (1973). Total chlorophyll was estimated following Arnon's method (Arnon, 1949). Statistical analysis was done using OPSTAT (Sheoran *et al.*, 1998).

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Germination(%)

Among the genotypes highest mean germination was shown by G₃ (99.43%), G₆ (98.87%) followed by G₅ (98.68%), while the lowest mean germination was recorded for G₈ (94.31%). The treatment with the highest germination was T₄ (99.02%) followed by T₃ (97.07%), and the lowest was T₁ (95.00%). The interaction of genotypes and treatments showed the three highest germination rates for G₃

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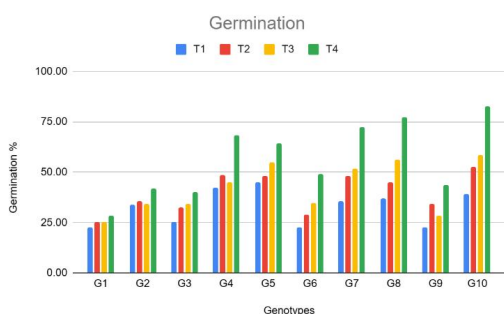
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$\times T_4$ (100.00%), $G_6 \times T_4$ (100.00%), and $G_5 \times T_4$ (100.00%). The difference between T_4 and T_3 was not statistically significant.

Fig 1 Bar graph showing percentage of germination against different treatments

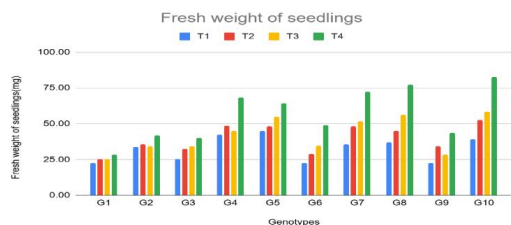


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3.1.2 Fresh weight of seedlings(mg)

Fig 2 Bar graph showing Fresh weight of seedlings against different genotypes



Genotypes G_{10} (0.861mg), G_8 (0.833 mg), and G_7 (0.607 mg) had the highest seedling fresh weight while G_4 (0.453 mg) had the lowest. Among treatments T_4 (0.801 mg) and T_3 (0.632 mg) had the highest fresh weight of seedling with T_1 (0.520 mg) being the lowest. Among interaction effect $G_{10} \times T_4$ (1.147 mg), $G_8 \times T_4$ (1.015 mg), and $G_8 \times T_3$ (1.192 mg) had the most seedling fresh weight. G_{10} and G_8 were significantly different from G_7 , but there was no significant difference between G_{10} and G_8 . The difference between T_4 and T_3 was significant, suggesting that T_4 provides a notable improvement in fresh weight.

Table 1 :Fresh weight of seedlings(mg) and germination

Table 1	Germination(%)					Fresh weight of seedlings(mg)				
	T ₁	T ₂	T ₃	T ₄	Mean G	T ₁	T ₂	T ₃	T ₄	Mean G
G ₁	96.75	97.25	97.25	99.00	97.56	0.646	0.555	0.592	0.760	0.638
G ₂	95.00	95.75	96.00	98.70	96.36	0.594	0.470	0.657	0.751	0.618
G ₃	99.00	99.50	99.25	100.00	99.43	0.440	0.465	0.595	0.770	0.568
G ₄	93.25	95.75	96.00	98.75	95.93	0.335	0.478	0.421	0.579	0.453
G ₅	98.00	98.00	98.75	100.00	98.68	0.414	0.574	0.525	0.752	0.566

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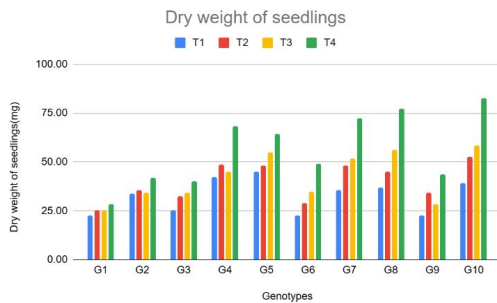
G₆	98.25	98.25	99.00	100.00	98.87	0.478	0.514	0.548	0.884	0.606
G₇	93.25	95.25	97.50	99.25	96.31	0.534	0.631	0.604	0.660	0.607
G₈	92.50	93.50	94.00	97.25	94.31	0.551	0.574	1.192	1.015	0.833
G₉	95.25	97.25	97.25	98.00	96.93	0.513	0.722	0.386	0.689	0.578
G₁₀	88.75	92.50	95.75	99.25	94.06	0.692	0.802	0.803	1.147	0.861
Mean T	95.00	96.30	97.07	99.02		0.520	0.579	0.632	0.801	
	Factor G	Factor T	Factor G X T			Factor G	Factor T	Factor G X T		
C.D(5%)	NS	NS	NS			0.040	0.025	0.079		
SEm(±)	2.112	1.336	4.224			0.014	0.009	0.028		
G- Genotypes, T- Treatments, CD- Critical Difference, SEm(±)- Standard Error of Mean, NS- Non significant										

3.1.3 Dry weight of seedlings(mg)

In case of seedling dry weight best genotypes were G₈ (0.086 mg), G₁₀ (0.085 mg), and G₆ (0.076 mg), while the worst one was G₃ (0.051 mg). Among treatments, T₄ (0.093 mg) and T₃ (0.064 mg) had the highest, with T₁ (0.054 mg) had the lowest value. The best three interactions were G₈ × T₄ (0.125 mg), G₆ × T₄ (0.115 mg), and G₁₀ × T₄ (0.110 mg). both genotypes and treatments showed significant difference but G₈, G₁₀, and G₆ exhibited non-significant difference.

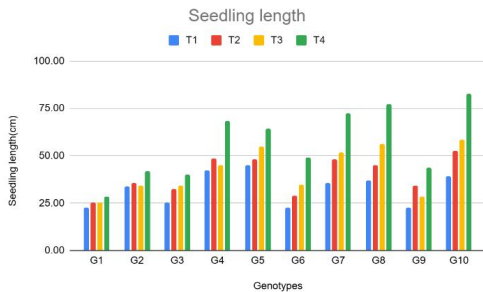
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Fig 3 Bar graph showing Dry weight of seedlings against different genotypes



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Fig 4 Bar graph showing Seedling length against different genotypes



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3.1.4 Seedling length(cm)

G₈ (18.408 cm), G₁₀ (16.804 cm), and G₅ (14.892 cm) recorded maximum seedling length but G₃ (12.233 cm) was the lowest. In case of treatments T₄ (16.023 cm) and T₂ (14.900 cm) had the highest, with T₁ (13.270 cm) had the lowest seedling length after 7 days. Both genotypes and treatments were significant and G₈ was significantly different from G₁₀ and G₅, while the latter two did not differ significantly from each other. The difference between T₄ and T₂ is significant, highlighting that T₄ strongly enhances seedling length.

Table 2 : Dry weight of seedlings(mg) and Seedling length(cm)

	Dry weight of seedlings(mg)					Seedling length(cm)				
	T ₁	T ₂	T ₃	T ₄	Mean G	T ₁	T ₂	T ₃	T ₄	Mean G
G ₁	0.057	0.060	0.060	0.064	0.060	13.267	14.400	14.067	15.100	14.208
G ₂	0.047	0.050	0.052	0.084	0.058	11.733	13.067	11.767	16.367	13.233
G ₃	0.040	0.044	0.044	0.077	0.051	11.600	12.467	11.733	13.133	12.233
G ₄	0.049	0.053	0.055	0.075	0.058	11.700	13.667	12.533	14.000	12.975
G ₅	0.054	0.059	0.061	0.086	0.065	12.967	15.800	14.900	15.900	14.892
G ₆	0.057	0.060	0.073	0.115	0.076	13.633	14.033	13.967	16.067	14.425
G ₇	0.055	0.079	0.082	0.083	0.075	10.967	15.233	11.133	15.367	13.175
G ₈	0.062	0.069	0.090	0.125	0.086	17.067	17.233	19.433	19.900	18.408
G ₉	0.053	0.077	0.057	0.113	0.075	13.167	15.900	14.333	17.067	15.117
G ₁₀	0.071	0.090	0.070	0.110	0.085	16.600	17.200	16.083	17.333	16.804
Mean T	0.054	0.064	0.064	0.093		13.270	14.900	13.995	16.023	
	Factor G	Factor T	Factor G X T			Factor G	Factor T	Factor G X T		
C.D(5%)	0.018	0.011	NS			2.058	1.302	NS		
SEm(±)	0.006	0.004	0.012			0.730	0.461	1.459		

G- Genotypes, T- Treatments, CD- Critical Difference, SEm(±)- Standard Error of Mean, NS- Non significant

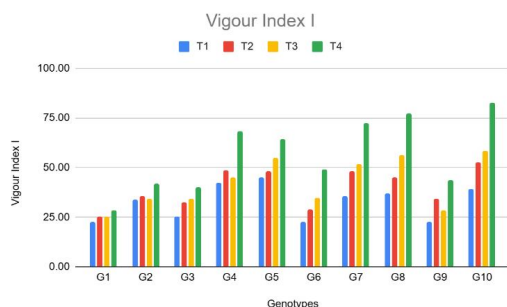
Comment [SS16]: When measuring the length of a seedling in centimeters, **standard measurements:** Report up to **one decimal place** (e.g., 5.2 cm). **Higher precision:** Use up to **two decimal places** if the instrument and experimental requirements demand it (e.g., 5.23 cm). How to measure the length in cm with 3 decimal value?

3.1.5 Vigour Index I

The best vigour index I was presented by T₄ (1,585) and T₂ (1,429), and T₁ (1,255) was the lowest. Best genotypes for high vigour index I was G₈ (1,736), G₁₀ (1,580), and G₅ (1,471), while the worst was G₇ (1,270). The highest interactions were G₈ × T₄ (1,927), G₈ × T₃ (1,819), and G₁₀ × T₄ (1,724). both treatments and genotypes were significant and G₈, G₁₀, and G₅ also showed significant difference among each other. The difference between T₄ and T₂ is highly significant, indicating a substantial effect of T₄ on vigour index I.

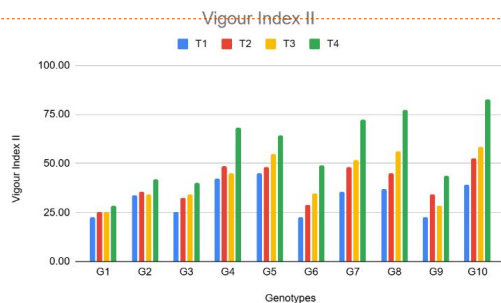
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Fig 5 Bar graph showing Vigour Index I



3.1.6 Vigour Index II

Fig 6 Bar graph showing Vigour Index II



Comment [SS18]: If you conduct experiments in triplicate, you must include error bars in the plotted graph.

The genotypes for vigour index II were G₈ (8.247), G₁₀ (8.053), and G₆ (7.514), with G₃ (5.146) being the lowest. For treatments T₄ (9.198) and T₃ (6.257) were the highest, and T₁ (5.156) was the lowest. The three highest interactions were G₈ × T₄ (12.152), G₆ × T₄ (11.457), and G₁₀ × T₄ (10.896). Both genotypes and treatments were significant, with G₈ being significantly different from G₁₀ and G₆, while G₁₀ and G₆ did not differ significantly. The difference between T₄ and T₃ was significant, reinforcing T₄'s superior performance in vigor improvement.

Table 3 :Comparison between Vigour Index I and Vigour Index II

Table 3	Vigour Index I					Vigour Index II				
	T ₁	T ₂	T ₃	T ₄	Mean G	T ₁	T ₂	T ₃	T ₄	Mean G
G ₁	1,253	1,386	1,362	1,496	1,374	5.577	5.865	5.784	6.344	5.892
G ₂	1,114	1,252.0	1,129	1,618	1,278	4.423	4.797	5.017	8.258	5.624
G ₃	1,146	1,242	1,157	1,319	1,216	4.041	4.392	4.416	7.733	5.146
G ₄	1,083	1,300	1,209	1,381	1,243	4.571	4.714	5.283	7.335	5.476
G ₅	1,282	1,546	1,469	1,588	1,471	5.293	5.785	5.966	8.412	6.364
G ₆	1,333	1,371	1,384	1,601	1,423	5.528	5.872	7.199	11.457	7.514
G ₇	1,024	1,455	1,081	1,522	1,270	5.121	7.498	8.003	8.281	7.226
G ₈	1,583	1,614	1,819	1,927	1,736	5.734	6.429	8.672	12.152	8.247
G ₉	1,257	1,531	1,399	1,669	1,464	4.980	7.443	5.530	11.109	7.266

G₁₀	1,472	1,588	1,538	1,724	1,580	6.295	8.318	6.702	10.896	8.053
Mean T	1,255	1,429	1,355	1,585		5.156	6.111	6.257	9.198	
	Factor G	Factor T	Factor G X T			Factor G	Factor T	Factor G X T		
C.D(5%)	206.239	130.437	NS			1.747	1.105	NS		
SEm(±)	73.110	46.239	146.220			0.619	0.392	1.239		
G- Genotypes, T- Treatments, CD- Critical Difference, SEm(±)- Standard Error of Mean, NS- Non significant										

3.1.7 Proline content(μmol/ g FW)

In case of proline content of seedlings highest values were observed in G₇ (56.311 μmol/ g FW), G₈ (46.200 μmol/ g FW), and G₄ (44.600 μmol/ g FW), with G₅ (28.567 μmol/ g FW) being the lowest. Also, in treatments T₄ (40.801 μmol/ g FW) and T₃ (40.071 μmol/ g FW) were the highest, and T₂ (38.511 μmol/ g FW) was the lowest. The three highest interactions were G₇ × T₄ (77.656 μmol/ g FW), G₆ × T₄ (45.744 μmol/ g FW), and G₈ × T₄ (34.878 μmol/ g FW). The results showed significant differences between G₇ and the other groups (G₈ and G₄), while G₈ and G₄ were not significantly different. But treatments were not significant.

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Fig 7 Bar graph showing total chlorophyll content

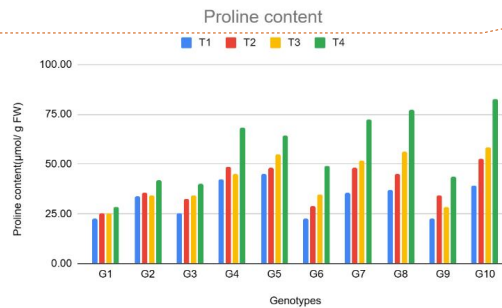
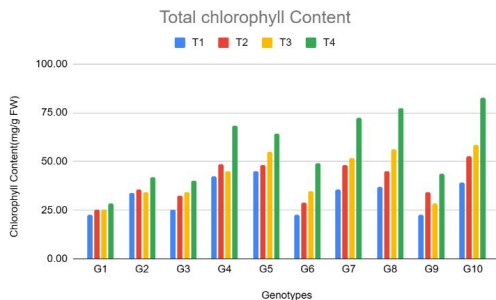


fig8 Bar graph showing total proline content

Comment [SS20]: If you conduct experiments in triplicate, you must include error bars in the plotted graph.

3.8 3.1.8 Total chlorophyll Content(mg/g FW)

T₄ (56.840 mg/g FW) and T₃ (42.290 mg/g FW) showed the highest chlorophyll content with T₁ (32.640 mg/g FW) being the lowest. For genotypes G₁₀ (58.300 mg/g FW), G₈ (53.900 mg/g FW), and G₇ (52.000 mg/g FW) had the highest total chlorophyll content values, while G₁ (25.375 mg/g FW) was the lowest. The three highest interactions were G₁₀ × T₄ (82.800 mg/g FW), G₈ × T₄ (77.400 mg/g FW), and G₇ × T₄ (72.400 mg/g FW). Factors Genotype, treatment and their interaction were significant. Significant differences were observed among G₁₀, G₈, and G₇. The difference between T₄ and T₃ was significant, indicating T₄ greatly enhances chlorophyll content.

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Table 4 Total chlorophyll and Proline Content

Table 4	Proline content(μmol/ g FW)					Total chlorophyll Content(mg/g FW)				
	T ₁	T ₂	T ₃	T ₄	Mean G	T ₁	T ₂	T ₃	T ₄	Mean G
G ₁	36.20	31.66	30.53	27.28	31.42	22.70	25.20	25.10	28.50	25.37

G ₂	22.36	36.92	41.11	32.62	33.25	33.80	35.40	34.20	41.80	36.30
G ₃	38.33	30.11	36.97	58.35	40.94	25.40	32.40	34.10	40.20	33.02
G ₄	52.80	44.00	41.97	39.62	44.60	42.50	48.60	45.10	68.40	51.15
G ₅	33.06	30.15	28.06	22.97	28.56	45.10	48.20	54.80	64.20	53.07
G ₆	23.43	44.54	51.16	45.74	41.22	22.80	28.90	34.70	48.90	33.82
G ₇	48.66	44.82	54.10	77.65	56.31	35.70	48.20	51.70	72.40	52.00
G ₈	54.63	49.57	45.71	34.87	46.20	36.80	45.20	56.20	77.40	53.90
G ₉	45.63	42.01	44.07	43.30	43.75	22.50	34.20	28.40	43.800	32.22
G ₁₀	37.30	31.30	26.98	25.56	30.28	39.10	52.70	58.60	82.80	58.30
Mean T	39.24	38.51	40.07	40.80		32.64	39.90	42.29	56.84	
	Factor G	Factor T	Factor G X T			Factor G	Factor T	Factor G X T		
C.D(5%)	5.773	NS	11.545			2.627	1.661	5.253		
SEm(±)	2.046	1.294	4.093			0.931	0.589	1.862		
G- Genotypes, T- Treatments, CD- Critical Difference, SEm(±)- Standard Error of Mean, NS- Non significant										

3.2 Discussion

Germination study indicated higher mean values for treatments G₃, G₆, and G₅, especially under T₄, which consistently demonstrated greater germination rates. But the interactions between genotype and treatment was non-significant which means the treatments did have an effect but their influence was largely consistent among genotypes. Many studies reported the positive effect of vermicompost on the germination and growth of mustard seedlings and plants (Merta, 2023; Haque & Ali, 2020; Reza, 2023; Reza *et al.*, 2022). The improved germination upon vermicompost application could be attributed to several factors. Vermicompost has many available mineral nutrients, humic substances, and plant growth-promoting agents such as auxins, which are known to improve seed germination and seedling growth (Bhattacharya *et al.*, 2019; Pathma & Sakthivel, 2012). Vermicompost helps in increasing the porosity, aeration, and water retention capabilities which enhance the germination and growth of mustard plants (Sarma & Gogoi, 2015; Merta, 2023). Azotobacter further increases seed germination in crops by ensuring plant health through nitrogen fixation, phosphate solubilization, and growth hormone production. Together, these factors lead to optimum growth, increased vigor, and effective germination (Abbas *et al.*, 2024). Azotobacter has been found effective in promoting seed germination in several crops of paddy (Chennappa *et al.*, 2017a), wheat (Siliniet *et al.*, 2012), buckwheat, winter wheat (Roi *et al.*, 2022), and beetroot (Kurdish *et al.*, 2008).

The fresh and dry weight of the seedlings indicated that genotypes G₁₀, G₈, and G₇ performed to the best under T₄ conditions. The genotypes that showed a significant improvement in growth, based on fresh weight measurements under T₄ condition were G₁₀ and G₈. In addition, the treatments together with the genotypes significantly affected seedling length; under T₄ conditions, G₈ was the best combination. Vermicompost significantly increases the fresh weight and dry weight of seedlings, especially for tomatoes and pepper plants (Brace, 2017). Riwandiet *al.* (2023) showed that vermicomposting gave a great influence on both fresh and dry weights in maize seedlings. Shoot fresh weights were increased over 23% for wheat inoculated with Azotobacter strain Azo-8, and increases over 23% in shoot dry weights along with marked improvements in root biomass have also been reported (Singh *et al.*, 2013). In addition to the above, *Vigna radiata* seedlings have shown a 20.07% increase in fresh weight and little over 62% increase in dry weight through Azotobacter inoculation (Munnaza *et al.*, 2012). Scientists show that the addition of vermicompost to growth medium can bring a change in seedling height to cucumbers from 1.9% to about 18.6%, related to leaf area and fresh weight increase (Jankauskienė and others, 2022).

The vigour indices (I and II) reconfirmed the superiority of G₈, G₁₀, and G₆. Most importantly, G₈ under T₄ recorded the maximum values. By enhancing seed germination, promoting disease resistance, and enhancing the overall health of the plants, vermicompost improves vigour index of crops (Mohite *et al.*, 2024). Research conducted by Bajaj (2023) disclosed that the tomato plants' vigour index has increased with altered levels of vermicompost, indicating a positive effect of vermicompost on the crop's growth. The culture filtrate of *Azotobacter salinestris*(GVT-1) has improved the vigour index of paddy seeds, thus enhancing growth and seedling germination rates in crops (Chennappa *et al.*, 2017b).

Comment [SS22]: Full organism name must be in italic.

The proline content was variable from one genotype to another, with a maximum content of G₇ and G₈ met under T₄ treatment. This indicates an increased possibility for genotypes to develop physiological resistance toward such stresses. In high correlation, some genotypes recorded very high chlorophyll contents which are very vital for photosynthesis, that is, G₁₀, G₈, G₇ under T₄ conditions. These results confirmed those genotypes as most suitable for maximizing treatment benefits towards better physiological output. Mixed inoculation with different *Azotobacter* strains on wheat seedlings has been studied, which increased proline level and growth parameters under osmotic stress, shown to be significantly related to drought resistance (Liu *et al.*, 2013). Various *Azotobacter* strains have reported improving the physiological attributes such as proline synthesis in maize grown on saline soils and hence its usefulness toward osmotic adjustment and alleviation of stress in plants (Abdel Latef *et al.*, 2020). Research suggests that addition of vermicompost resulted in increase in proline concentration, which is the major osmotic regulator that helps plant in overcoming abiotic problems like drought and salinity (Bokobana *et al.*, 2020; Hosseinzadeh *et al.*, 2017). During water stress conditions, 30% vermicompost induced a 39% increase in proline content of chickpea seedlings (Hosseinzadeh *et al.*, 2017). In fact, when tomato seedlings are subjected to vermicompost-leachate especially during heat and moisture stresses, there are increased proline levels (Chinsamy *et al.*, 2014). Researches have well put vermicompost as an important source of macro- and micronutrients which henceforth augments plant nutrition and at the same time improves chlorophyll concentration, as commonly exhibited by *Capsicum annum* and other vegetable crop seedlings (Kamalkant Yadav *et al.*, 2014; Theunissen, 2010). Kumar *et al.* (2016) observed improvement in the photosynthetic pigments of *Jatropha* by *Azotobacter* and arbuscular mycorrhizal fungus. A treatment of *Azotobacter* in wheat plants indicated a very high increase in the total chlorophyll content (mg g⁻¹) (El-zawawy *et al.*, 2023). The chlorophyll content has been shown to be increased with the inoculation of *Azotobacter*, either alone or with *Rhizobium*, in black gram (*Vigna mungo*) as compared to the control (Tiwari *et al.*, 2017).

Comment [SS23]: Full name of plant scientific name must be in italic.

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The combination, vermicompost with *Azotobacter*, produced positive improvements in crop growth, yield, and nutrient uptake in many crops. This is a blend of the benefits of vermicompost from organic matter and nitrogen-fixing *Azotobacter*, which enhances plant growth parameters in crops such as chili, strawberry, and maize, more than that by either one of the components, or chemical fertilizers alone (Kalpana, 2019; Shirkhani&Nasrolahzadeh, 2016; Tripathi *et al.*, 2015). It also helps in increasing the availability and uptake of essential nutrients such as nitrogen, phosphorus, and potassium in plants and a much better nutrient content in crops such as rice and wheat (Ghadimi *et al.*, 2021; V. Kumar & Singh, 2001; Rather & Sharma, 2009) reducing the use of chemical fertilizer, being among the sustainable agricultural practices towards environmental sustainability (Rather & Sharma, 2009; Shirkhani&Nasrolahzadeh, 2016). The presence of *Azotobacter* in vermicompost, therefore, helps improve the microbial activity of the soil, which translates to a better soil structure and health, thus benefiting long-term crop productivity (Ghadimi *et al.*, 2021; Mal *et al.*, 2021).

4. LIMITATIONS

Although the study establishes the benefits of *Azotobacter* seed priming and vermicompost integration in Indian mustard cultivation, but it is limited because it focused only on seedling stages in a controlled setup. Exactly similar results may not be replicated in a field trial also without exploring long term effects of treatments on seed yield and plant health. So further field trials and cost-benefit analysis is needed.

5. CONCLUSION

The study highlights the importance of combining genotype selection with advanced treatment techniques for improving mustard cultivation. Genotypes TM 306-1(G₈) and TBM 143(G₁₀) are the top performers, especially when paired with treatment Azotobacter primed seeds sown in vermicompost + field soil(T₄), which provides favorable conditions for nutrient uptake, growth, and stress resilience. Farmers can improve germination rates, seedling vigor, and stress tolerance by selecting these genotypes and applying T₄. These genotypes are adaptable to varying conditions, making them ideal for cultivation in diverse agro-climatic regions.

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