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Comparative Analysis of Growth Responses in Indian Mustard to Azotobacter Priming and Vermicompost for Sustainable Cultivation

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Authors' contributions

This work was carried out in collaboration among all authors. Author AM designed the study, performed the statistical analysis, wrote the protocol, and first draft of the manuscript. Authors Ananya Baidya and Achuyta Basak managed the analyses of the study. Author MSAM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

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 at Bidhan Chandra Krishi Viswavidyalaya, Nadia district, West Bengal. 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.

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 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 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 earthworms. It is a nutrient-rich semi-bulky organic fertilizer containing high concentrations of macro and micronutrients. Vermicompost is a good additive to soil because it improves 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 in new alluvial zone at Bidhan Chandra Krishi Viswavidyalaya, Nadia district, West Bengal. 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 BRM 4(G_1), BRM13(G_2), BRM 14(G_3), Varuna(G_4),JD 6(G_5), PM 25(G_6), PM 29(G_7), TM 306-1(G_8), TBM 204(G_9) and TBM 143(G_{10}). The four treatments are as follows- Seeds sown in field soil(T_1), Azotobacter primed seeds sown in field soil(T_2), Seeds sown in vermicompost + field soil(T_3), Azotobacter primed seeds sown in vermicompost + field soil(T_4). 50 seeds were placed in each sterilized plastic container and left in open condition. In case of vermicompost treatment 50% of vermicompost and 50% of field soil was used. For Azotobacter seed priming a 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^{0}C and 12.4^{0}C respectively, maximum, and minimum relative humidity were 78% and 54% respectively with 8.1 hours of average bright sunshine hours. After the 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 the 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× 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 × 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 G_3 (99.43%), G_6 (98.87%) followed by G_5 (98.68%), while the lowest mean germination was recorded for G_8 (94.31%). The treatment with the highest germination was T_4 (99.02%) followed by T_3 (97.07%), and the lowest was T_1 (95.00%). The interaction of genotypes and treatments showed the three highest germination rates for $G_3 \times T_4$ (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.

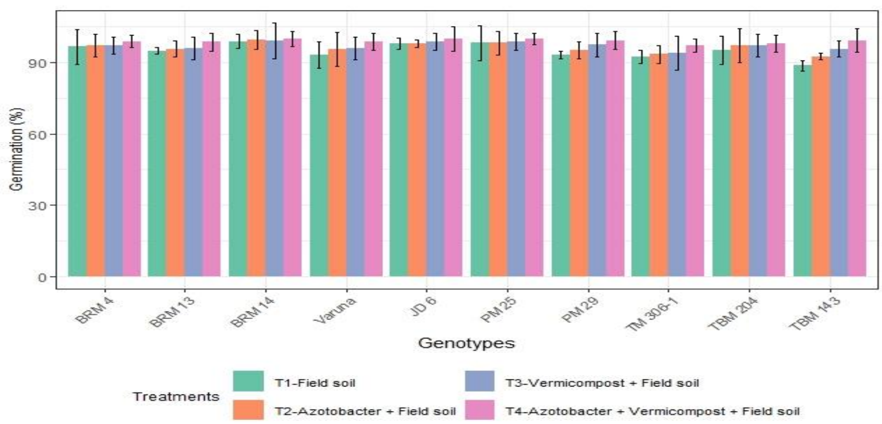

Fig. 1. Comparative analysis of germination (%) across different Indian mustard genotypes (G1-G10) under four treatments (T1-T4). Error bars represent Standard Error of Means [SEm(±)]. Values are means of three replicates

3.1.2 Fresh weight of seedlings (mg)

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 seedlings with T_1 (0.520 mg) being the lowest. Among interaction effects $G_{10} \times T_4$ (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.

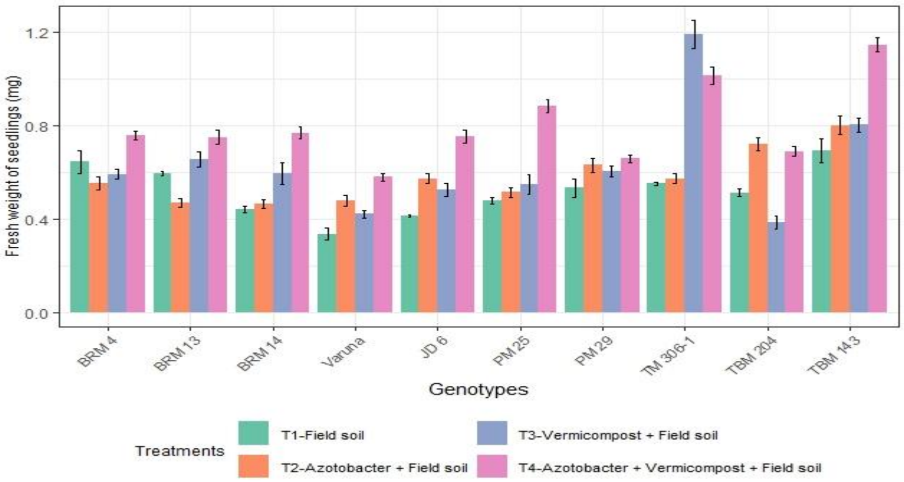

Fig. 2. Comparative analysis of fresh weight of seedlings (mg) across different Indian mustard genotypes (G1-G10) under four treatments (T1-T4). Error bars represent Standard Error of Means [SEm(±)]. Values are means of three replicates.

Table 1. Effect of Azotobacter priming and vermicompost treatments on germination (%) and fresh weight of seedlings (mg) in different Indian mustard genotypes

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
G_6	98.25	98.25	99.00	100.00	98.87	0.478	0.514	0.548	0.884	0.606
G_7	93.25	95.25	97.50	99.25	96.31	0.534	0.631	0.604	0.660	0.607
G_8	92.50	93.50	94.00	97.25	94.31	0.551	0.574	1.192	1.015	0.833
G_9	95.25	97.25	97.25	98.00	96.93	0.513	0.722	0.386	0.689	0.578
G_{10}	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		

[a] *G_1: BRM 4; G_2: BRM13; G_3: BRM 14; G_4: Varuna; G_5: JD 6; G_6: PM 25; G_7: PM 29; G_8: TM 306-1; G_9: TBM 204; G_{10}: TBM 143*

[b] *T_1: Seeds sown in field soil; T_2: Azotobacter primed seeds in field soil; T_3: Seeds in vermicompost + field soil; T_4: Azotobacter primed seeds in vermicompost + field soil*

[c] *CD: Critical Difference*

[d] *SEm(±): Standard Error of Mean e NS: Non-significant*

3.1.3 Dry weight of seedlings (mg)

In the case of seedling dry weight best genotypes were G_8 (0.086 mg), G_{10} (0.085 mg), and G_6 (0.076 mg), while the worst one was G_3 (0.051 mg). Among treatments, T_4 (0.093 mg) and T_3 (0.064 mg) had the highest, with T_1 (0.054 mg) having the lowest value. The best three interactions were $G_8 \times T_4$ (0.125 mg), $G_6 \times T_4$ (0.115 mg), and $G_{10} \times T_4$ (0.110 mg). Both genotypes and treatments showed significant difference but G_8, G_{10}, and G_6 exhibited non-significant difference.

3.1.4 Seedling length (cm)

G_8 (18.408 cm), G_{10} (16.804 cm), and G_5 (14.892 cm) recorded maximum seedling length but G_3 (12.233 cm) was the lowest. In the case of treatments T_4 (16.023 cm) and T_2 (14.900 cm) had the highest, with T_1 (13.270 cm) having the lowest seedling length after 7 days. Both genotypes and treatments were significant and G_8 was significantly different from G_{10} and G_5, while the latter two did not differ significantly from each other. The difference between T_4 and T_2 is significant, highlighting that T_4 strongly enhances seedling length.

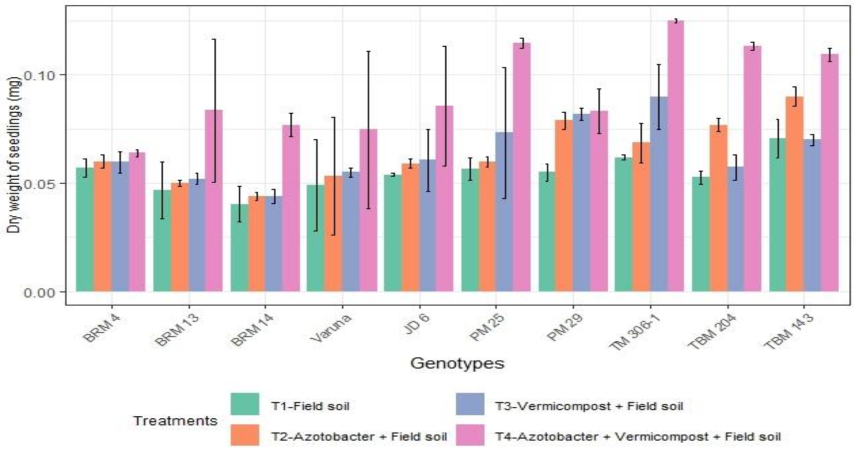

Fig. 3. Comparative analysis of dry weight of seedlings (mg) across different Indian mustard genotypes (G1-G10) under four treatments (T1-T4). Error bars represent Standard Error of Means [SEm(±)]. Values are means of three replicates.

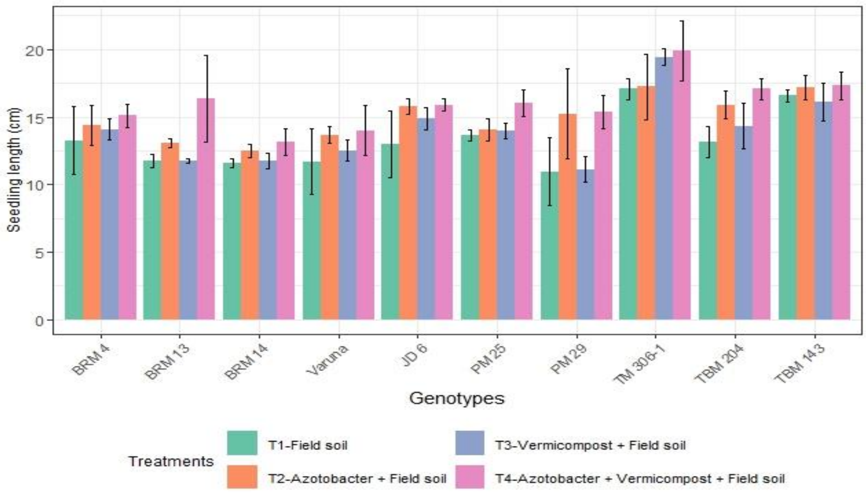

Fig. 4. Comparative analysis of seedling length (cm) across different Indian mustard genotypes (G1-G10) under four treatments (T1-T4). Error bars represent Standard Error of Means [SEm(±)]. Values are means of three replicates

Table 2. Effect of Azotobacter priming and vermicompost treatments on dry weight of seedlings (mg) and seedling length (cm) in different Indian mustard genotypes

Table 2	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	13.26	14.40	14.06	15.10	14.20
G_2	0.047	0.050	0.052	0.084	0.058	11.73	13.06	11.76	16.36	13.23
G_3	0.040	0.044	0.044	0.077	0.051	11.60	12.46	11.73	13.13	12.23
G_4	0.049	0.053	0.055	0.075	0.058	11.70	13.66	12.53	14.00	12.97
G_5	0.054	0.059	0.061	0.086	0.065	12.96	15.80	14.90	15.90	14.89
G_6	0.057	0.060	0.073	0.115	0.076	13.63	14.03	13.96	16.06	14.42
G_7	0.055	0.079	0.082	0.083	0.075	10.96	15.23	11.13	15.36	13.17
G_8	0.062	0.069	0.090	0.125	0.086	17.06	17.23	19.43	19.90	18.40
G_9	0.053	0.077	0.057	0.113	0.075	13.16	15.90	14.33	17.06	15.11
G_{10}	0.071	0.090	0.070	0.110	0.085	16.60	17.20	16.08	17.33	16.80
Mean T	0.054	0.064	0.064	0.093		13.27	14.90	13.99	16.02	
	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		

[a] G_1: BRM 4; G_2: BRM13; G_3: BRM 14; G_4: Varuna; G_5: JD 6; G_6: PM 25; G_7: PM 29; G_8: TM 306-1; G_9: TBM 204; G_{10}: TBM 143
[b] T_1: Seeds sown in field soil; T_2: Azotobacter primed seeds in field soil; T_3: Seeds in vermicompost + field soil; T_4: Azotobacter primed seeds in vermicompost + field soil
[c] CD: Critical Difference
[d] SEm(±): Standard Error of Mean e NS: Non-significant

3.1.5 Vigour index I

The best vigour index I was presented by T_4 (1,585) and T_2 (1,429), and T_1 (1,255) was the lowest. The best genotypes for high vigour index I was G_8 (1,736), G_{10} (1,580), and G_5 (1,471), while the worst was G_7 (1,270). The highest interactions were $G_8 \times T_4$ (1,927), $G_8 \times T_3$ (1,819), and $G_{10} \times T_4$ (1,724). both treatments and genotypes were significant and G_8, G_{10}, and G_5 also showed significant differences among each other. The difference between T_4 and T_2 is highly significant, indicating a substantial effect of T_4 on vigour index I.

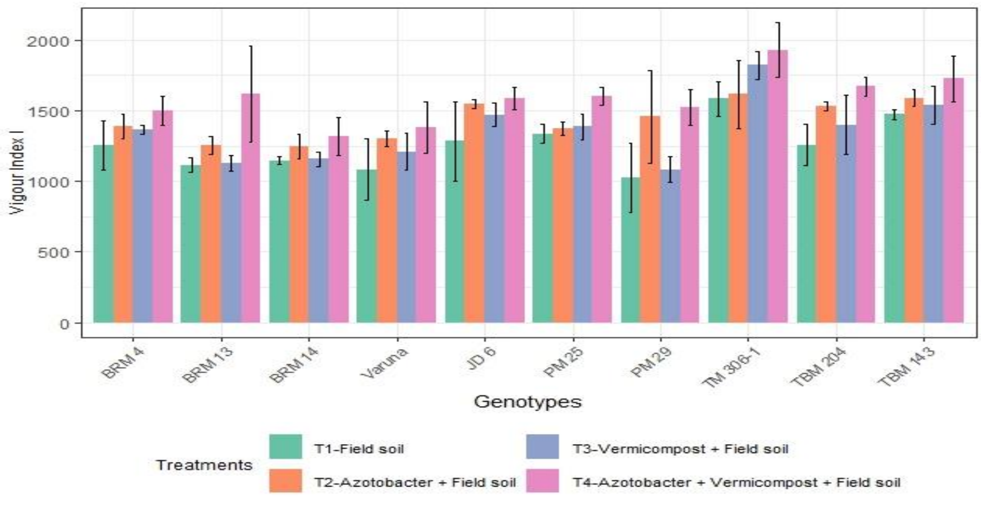

Fig. 5. Comparative analysis of vigour index I across different Indian mustard genotypes (G1-G10) under four treatments (T1-T4). Error bars represent Standard Error of Means [SEm(±)]. Values are means of three replicates

3.1.6 Vigour Index II

The genotypes for vigour index II were G_8 (8.247), G_{10} (8.053), and G_6 (7.514), with G_3 (5.146) being the lowest. For treatments T_4 (9.198) and T_3 (6.257) were the highest, and T_1 (5.156) was the lowest. The three highest interactions were $G_8 \times T_4$ (12.152), $G_6 \times T_4$ (11.457), and $G_{10} \times T_4$ (10.896). Both genotypes and treatments were significant, with G_8 being significantly different from G_{10} and G_6, while G_{10} and G_6 did not differ significantly. The difference between T_4 and T_3 was significant, reinforcing T_4's superior performance in vigor improvement.

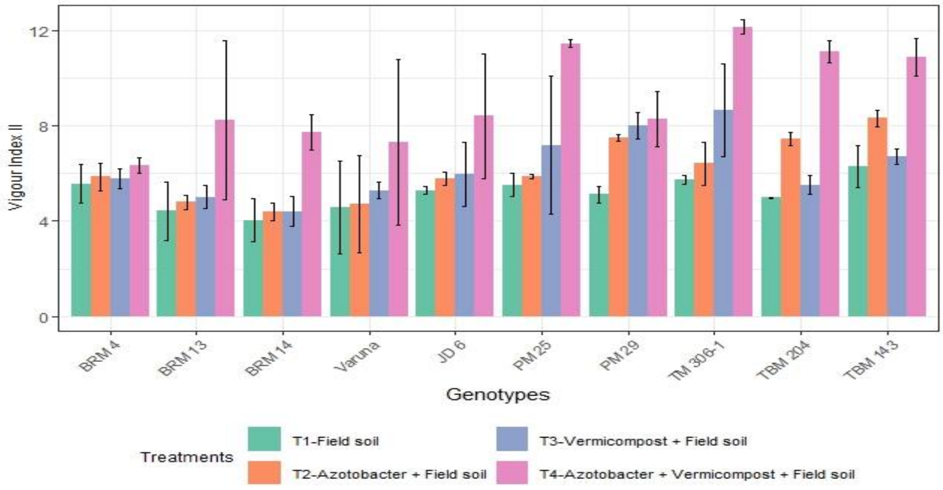

Fig. 6. Comparative analysis of vigour index II across different Indian mustard genotypes (G1-G10) under four treatments (T1-T4). Error bars represent Standard Error of Means [SEm(±)]. Values are means of three replicates

Table 3. Effect of Azotobacter priming and vermicompost treatments on Vigour Index I and Vigour Index II in different Indian mustard genotypes

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
G_{10}	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		

[a] *G_1: BRM 4; G_2: BRM13; G_3: BRM 14; G_4: Varuna; G_5: JD 6; G_6: PM 25; G_7: PM 29; G_8: TM 306-1; G_9: TBM 204; G_{10}: TBM 143*
[b] *T_1: Seeds sown in field soil; T_2: Azotobacter primed seeds in field soil; T_3: Seeds in vermicompost + field soil; T_4: Azotobacter primed seeds in vermicompost + field soil*
[c] *CD: Critical Difference*
[d] *SEm(±): Standard Error of Mean e NS: Non-significant*

3.1.7 Proline content (µmol/ g FW)

In the case of proline content of seedlings, highest values were observed in G_7 (56.311 µmol/ g FW), G_8 (46.200 µmol/ g FW), and G_4 (44.600 µmol/ g FW), with G_5 (28.567 µmol/ g FW) being the lowest. Also, in treatments, T_4 (40.801 µmol/ g FW) and T_3 (40.071 µmol/ g FW) were the highest, and T_2 (38.511 µmol/ g FW) was the lowest. The three highest interactions were $G_7 \times T_4$ (77.656 µmol/ g FW), $G_6 \times T_4$ (45.744 µmol/ g FW), and $G_8 \times T_4$ (34.878 µmol/ g FW). The results showed significant differences between G_7 and the other groups (G_8 and G_4), while G_8 and G_4 were not significantly different. However, treatments were not significant.

3.1.8 Total chlorophyll Content (mg/g FW)

T_4 (56.840 mg/g FW) and T_3 (42.290 mg/g FW) showed the highest chlorophyll content with T_1 (32.640 mg/g FW) being the lowest. For genotypes, G_{10} (58.300 mg/g FW), G_8 (53.900 mg/g FW), and G_7 (52.000 mg/g FW) had the highest total chlorophyll content values, while G_1 (25.375 mg/g FW) was the lowest. The three highest interactions were $G_{10} \times T_4$ (82.800 mg/g FW), $G_8 \times T_4$ (77.400 mg/g FW), and $G_7 \times T_4$ (72.400 mg/g FW). Factors Genotype, treatment, and their interaction were significant. Significant differences were observed among G_{10}, G_8, and G_7. The difference between T_4 and T_3 was significant, indicating that T_4 greatly enhances chlorophyll content.

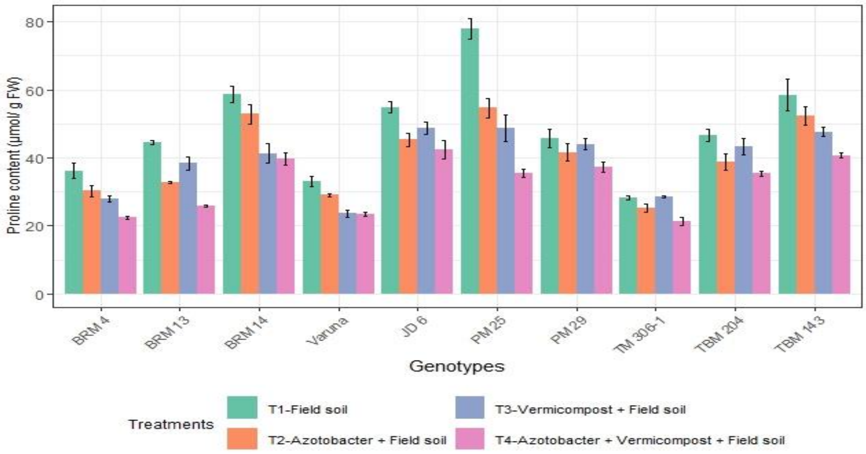

Fig. 7. Comparative analysis of proline content (µmol/ g FW) across different Indian mustard genotypes (G1-G10) under four treatments (T1-T4). Error bars represent Standard Error of Means [SEm(±)]. Values are means of three replicates

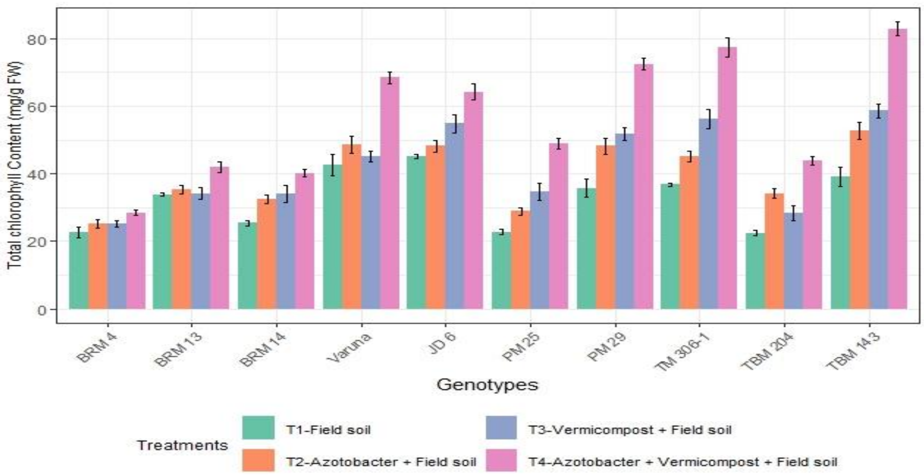

Fig. 8. Comparative analysis of total chlorophyll Content (mg/g FW)across different Indian mustard genotypes (G1-G10) under four treatments (T1-T4). Error bars represent Standard Error of Means [SEm(±)]. Values are means of three replicates

Table 4. Effect of Azotobacter priming and vermicompost treatments on proline content (µmol/ g FW) and total chlorophyll Content (mg/g FW) in different Indian mustard genotypes

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		

[a] G_1: BRM 4; G_2: BRM13; G_3: BRM 14; G_4: Varuna; G_5: JD 6; G_6: PM 25; G_7: PM 29; G_8: TM 306-1; G_9: TBM 204; G_{10}: TBM 143
[b] T_1: Seeds sown in field soil; T_2: Azotobacter primed seeds in field soil; T_3: Seeds in vermicompost + field soil; T_4: Azotobacter primed seeds in vermicompost + field soil
[c] CD: Critical Difference
[d] SEm(±): Standard Error of Mean e NS: Non-significant

3.2 Discussion

The germination study indicated higher mean values for treatments G_3, G_6, and G_5, especially under T_4, which consistently demonstrated greater germination rates. However, the interactions between genotype and treatment were 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 (Silini 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 G_{10},G_8, and G_7 performed to the best under T_4 conditions. The genotypes that showed a significant improvement in growth, based on fresh weight measurements under T_4 conditions were G_{10} and G_8. In addition, the treatments together with the genotypes significantly affected seedling length; under T_4 conditions, G_8 was the best combination. Vermicompost significantly increases the fresh weight and dry weight of seedlings, especially for tomatoes and pepper plants (Brace, 2017). Riwandi et al. (2023) showed that vermicomposting had a great influence on both fresh and dry weights in maize seedlings. Shoot fresh weights were increased by over 23% for wheat inoculated with Azotobacter strain Azo-8, and increases by 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 a 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 G_8, G_{10}, and G_6. Most importantly, G_8 under T_4 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).

The proline content was variable from one genotype to another, with a maximum content of G_7 and G_8 met under T_4 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 vital for photosynthesis, that is, G_{10}, G_8, G_7 under T_4 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 been reported to improve 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 the addition of vermicompost resulted in an increase in proline concentration, which is the major osmotic regulator that helps plants in overcoming abiotic problems like drought and salinity (Bokobana et al., 2020; Hosseinzadeh et al. al, 2017). During water stress conditions, 30% vermicompost induced a 39% increase in the 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). Researchers 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 is 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).

The combination of 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). In Amaranthus, this combination of vermicompost and Azotobacter was conducive to early emergence and higher germination percentages, which led to the development of more vigorous seedlings (Yadav et al., 2024). Furthermore, the combined application of Azotobacter and plant-based composts, such as Moringa, has been demonstrated to enhance growth parameters and nutrient levels in various crops, further supporting the synergistic benefits of Azotobacter when utilized in conjunction with organic soil amendments(Albureikan, 2024). It also helps in increasing the availability and uptake of essential nutrients such as nitrogen, phosphorus, and potassium in plants and 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 benefitting long-term crop productivity (Ghadimi et al., 2021; Mal et al., 2021).

4. CONCLUSION

The study highlights the importance of combining genotype selection with advanced treatment techniques for improving mustard cultivation. Genotypes TM 306-1(G_8) and TBM 143(G_{10}) are the top performers, especially when paired with treatment Azotobacter primed seeds sown in vermicompost + field soil(T_4), 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 T_4. These genotypes are adaptable to varying conditions, making them ideal for cultivation in diverse agro-climatic regions.

5. LIMITATIONS

Although the study establishes the benefits of Azotobacter seed priming and vermicompost integration in Indian mustard cultivation, 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 are needed.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors 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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