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Effect of bio fertilizers and organic manure on growth and yield of Indian spinach

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Abstract
During the summer of 2021-2022, a study at the Organic Farming Research and Training Centre examined the effects of organic manure and biofertilizers on the growth and yield of Indian spinach. The experiment revealed significant effects using a Factorial Randomized Block Design with three bio-fertilizers (*Azotobacter*, PSB, *Azospirillum*) and two organic manure FYM levels. *Azotobacter* had the shortest germination period (5.26 days), while *Azospirillum* had the best growth characteristics. The 100% RDN via FYM treatment outperformed in all growth and yield parameters. *Azospirillum* had higher yield characteristics such as fresh weight of leaves (21.91 g) and total yield per hectare (20.38 t). *Azospirillum* had the highest available nitrogen according to soil analysis. Economic analysis revealed that *Azospirillum* and the 100% RDN through FYM treatment resulted in higher returns and benefit-cost ratios. In conclusion, *Azospirillum* bio-fertilizer and 100% RDN through organic manure FYM exhibited superiority in the growth and yield of Indian spinach compared to other treatments.

Keywords: Yield, bio fertilizers, FYM, organic manure, *Azospirillum*, *Azotobacter*, PSB

Introduction
In the context of yield and yield attributing parameters, it was observed that different parameters were significantly influenced by both bio-fertilizers and organic manure. *Azospirillum* treatment resulted in the highest yield and yield attributes, including fresh weight of leaves per plant (33.03 g), yield per plot (13.90 kg), and yield per hectare (20.35 t), compared to *Azotobacter* and PSB treatments. Among different levels of organic manure (FYM), the treatment with 100% Recommended Daily Nutrient (RDN) through FYM outperformed the 75% RDN through FYM and control treatments in all yield and yield attributes, including fresh weight of leaves per plant (22.05 g), yield per plot (12.58 kg), and yield per hectare (20.74 t). The interaction effect of bio-fertilizer and organic manure on quality parameters of spinach was found to be non-significant.

Various quality parameters were also significantly influenced by both bio-fertilizers and organic manure. *Azospirillum* treatment recorded higher values for quality parameters, including chlorophyll content (37.39 SPAD value), ascorbic acid value (61.72 mg 100-1 g), and iron content (21.95 mg 100-1), compared to *Azotobacter* and PSB treatments. Among different levels of organic manure (FYM), the treatment with 100% RDN through FYM outperformed the 75% RDN through FYM and control treatments in all quality parameters, including chlorophyll content (39.16 SPAD value), ascorbic acid value (61.09 mg 100-1 g), and iron content (22.02 mg 100-1 g). The interaction effect of bio-fertilizer and organic manure on growth attributes of spinach was found to be non-significant.

The vegetation indices (NDVI) value was highest in the *Azospirillum* treatment (0.81) compared to *Azotobacter* (0.77) and PSB treatments (0.77). Among different levels of organic manure (FYM), the highest vegetation indices (NDVI) value was found in the treatment with 100% RDN through FYM (0.87). The cost of cultivation for the *Azospirillum* treatment was Rs. 1,03,666.67/ha, recording maximum values for gross monetary returns (Rs. 2,57,715.34/ha), net monetary returns (Rs. 1,54,048.68/ha), and B: C ratio (2.48). Among different levels of organic manure (FYM), the treatment with 100% RDN through FYM had the highest cost of cultivation (Rs. 1,10,833.33/ha) but also recorded maximum gross monetary returns (Rs. 2,35,246.10/ha), net monetary returns (Rs. 1,24,412.77/ha), and B: C ratio (2.36).

Materials and Methods

The study examining the impact of bio-fertilizers and organic manure on the growth and yield of Indian spinach was conducted at the Organic Farming Research and Training Centre, Vasantrao Naik Marathwada Krishi Vidhyapeeth, Parbhani, Maharashtra, during the summer season of 2021-2022. The experimental design employed was a Factorial Randomized Block Design (FRBD), comprising twelve treatment combinations with three replications.

Factor-A Bio fertilizer (B)

The crop is tried with three bio fertilizers and one control condition as

B_1	:	*Azotobacter*
B_2	:	PSB
B_3	:	*Azospirillum*
B_4	:	Control

Factor-B Organic manure level (L)

Two levels of nitrogen through organic manure (FYM) along with the control is tried in the experiment,

L_1	:	Control
L_2	:	75% RDN through FYM
L_3	:	100 % RDN through FYM

Treatment combination was mentioned in table below

Sr. No	Treatment name	Treatment combination	Interaction details
1.	T_1	B_1L_1	*Azotobacter* + Control
2.	T_2	B_1L_2	*Azotobacter* + 75% RDN through FYM
3.	T_3	B_1L_3	*Azotobacter* + 100 % RDN through FYM
4.	T_4	B_2L_1	PSB + Control
5.	T_5	B_2L_2	PSB + 75% RDN through FYM
6.	T_6	B_2L_3	PSB + 100 % RDN through FYM
7.	T_7	B_3L_1	*Azospirillum* + Control
8.	T_8	B_3L_2	*Azospirillum* + 75% RDN through FYM
9.	T_9	B_3L_3	*Azospirillum* + 100 % RDN through FYM
10.	T10	B_4L_1	Control + Control
11.	T11	B_4L_2	Control + 75% RDN through FYM
12.	T12	B_4L_3	Control + 100% RDN through FYM

The recommended nutrient dosage for spinach cultivation is 80:40:40 NPK (Nitrogen, Phosphorus, Potassium) in kilograms per hectare. When applying nutrients through Farmyard Manure (FYM), only the nitrogen component was considered, and FYM was added to the specific plot in the required proportion. FYM is known to contain 0.52% nitrogen, 0.2% phosphorus (P2O5), and 0.5% potassium (K2O).

For the experiment, bio-fertilizers such as *Azotobacter*, PSB (Phosphate Solubilizing Bacteria), and *Azospirillum* were obtained from the Department of Agricultural Chemistry and Soil Science, Vasantrao Naik Marathwada Krishi Vidhyapeeth, Parbhani.

The bio fertilizers were applied in pattern as showed below:

Time of application-	Dose
1. Bio-priming: For 12 hour prior to sowing	1 ml/10 ml of water
2. Drenching: At 15 days interval	20 ml/1 litre of water
3. Spraying: At 15 days interval	10 ml/1 litre of water

The seeds of spinach (*Beta vulgaris* L.) variety All Green were obtained from market.

The plant growth observations like plant height, number of leaves, and weight of leaves per plant, Yield Per Plot recorded from the selected observational plants.

Result and Discussion

The current investigation aimed to assess the impact of bio-fertilizers and organic manure on the growth and yield of Indian spinach during the summer season of 2022. The results for each aspect are presented with statistical inferences, and efforts have been made to establish causal relationships among various parameters significantly influenced by different treatments. Relevant findings from previous research are also cited to support the results of the present study.

Table 1 presents data on the days required for potential germination of spinach seeds. Significant differences were observed due to the use of bio-fertilizers and organic manure. Bio-fertilizers significantly influenced the days to potential germination, with *Azotobacter*-treated seeds requiring the minimum time (5.26 days), followed by *Azospirillum* (5.43) and PSB (5.50), while the control group took the maximum time (5.93 days) for potential germination. *Azotobacter*, known for excreting phytohormones like auxin and gibberellin, demonstrated the shortest germination time, supporting similar findings reported by Devi *et al*., (2022).

Days to potential germination were also significantly influenced by organic manure FYM levels (RDN kg ha^{-1}). The minimum days to potential germination (5.39) were observed when 100% RDN was applied through FYM, comparable to 75% RDN through FYM (5.55), while the control treatment took 5.74 days. Increasing levels of organic manure correlated with a decrease in the days to potential germination, likely due to enhanced microorganism activity and the release of plant hormones and phytochemicals.

The interaction effect of bio-fertilizer and organic manure on days to potential germination was found to be non-significant.

The germination percentage of spinach seeds treated with different bio-fertilizers and sown under various levels of organic manures was significantly influenced. The highest germination percentage was observed in seeds treated with *Azotobacter* (90.18), followed by *Azospirillum* (88.39), while untreated seeds showed the lowest germination percentage (84.85). PSB recorded a germination percentage of 86.76. The

superior germination percentage with *Azotobacter* treatment may be attributed to its ability to fix extra nitrogen from the atmosphere and produce growth-promoting materials, resulting in enhanced metabolic activities and higher germination, consistent with similar observations in tomatoes reported by Reddy *et al*., (2018) [13].

The germination percentage of spinach seeds was significantly influenced by organic manure FYM levels (RDN kg ha^{-1}). The highest seed germination (89.84%) was observed when 100% RDN was given through FYM, followed by (87.51%) when 75% RDN was given through FYM. The increase in germination percentage correlated with the increased percentage of organic manure levels FYM (RDN kg ha^{-1}). This improvement may be attributed to the enhanced physical and chemical properties of the soil, providing better aeration, increased metabolic activities, and the release of growth-promoting and dormancy-breaking hormones. These results align with Sharma and Agarwal (2014) [14] in spinach.

The number of leaves per plant of spinach was significantly influenced by different bio-fertilizers. The significantly maximum mean number of leaves per plant was recorded with *Azospirillum* (12.74) treatment, which was significantly superior over *Azotobacter* (11.47) and PSB (11.01) treatments. The number of leaves per plant was highest in the *Azospirillum* treatment, potentially due to increased nutrient uptake, nitrogen fixation, and involvement in protein synthesis, leading to improved growth. Similar findings were reported in tomatoes by Reddy *et al*. (2018) [13] and in spinach by Krishna *et al*. (2022) [10].

Among organic manure FYM levels (RDN kg ha^{-1}), the number of leaves per plant was significantly influenced. The significantly maximum number of leaves per plant was recorded with the application of 100% RDN through FYM (12.34), significantly superior over 75% RDN through FYM (11.33) and the control (10.11). This increase in leaf number could be attributed to the availability of nutrients in higher levels along with growth substances throughout the crop growth period, consistent with findings in palak by Jha and Jana (2009) [9].

The mean plant height of spinach was significantly influenced by different bio-fertilizers. The significantly maximum plant height was recorded with *Azospirillum* (29.17) treatment, significantly superior over *Azotobacter* (24.95) and PSB (23.46) treatments. The height of the plant was significantly maximum in the *Azospirillum* treatment, likely due to increased nutrient uptake, nitrogen fixation, and involvement in protein synthesis. Similar results were reported in tomatoes by Reddy *et al*. (2018) [13] and in spinach by Krishna *et al*. (2022) [10].

Among organic manure FYM levels (RDN kg ha^{-1}), the mean height of the plant was significantly influenced. The significantly maximum mean height of the plant was recorded with the application of 100% RDN through FYM (27.24), significantly superior over 75% RDN through FYM (25.05) and the control (22.62). The increased application of nitrogen to spinach favored vegetative growth, increasing plant vigor and shoot growth rate, resulting in improved growth attributes. These results align with Thapa *et al*. (2021) [16] in spinach.

The interaction effect of bio-fertilizer and organic manure on germination percentage, number of leaves per plant, and plant height was found to be non-significant. Data on mean leaf area recorded at first, second and third cutting of the crop are presented in Table 4. Mean leaf area recorded at 1st, 2nd, and 3rd cutting was 30.83, 51.76 and 79.29 respectively. The rate of increase in mean leaf area was observed with each successive cutting.

The mean leaf area of spinach was significantly influenced by different bio-fertilizers. The significantly maximum leaf area was recorded with *Azospirillum* (61.26 cm2) treatment, significantly superior over *Azotobacter* (55.89 cm2), PSB (53.51 cm2), and control (45.19 cm2) treatments. *Azospirillum* treatment, known for increased nutrient uptake, nitrogen fixation, and involvement in protein synthesis, resulted in the highest leaf area. Similar findings were reported in tomatoes by Reddy *et al*. (2018) [13] and in spinach by Krishna *et al*. (2022) [10].

Mean leaf area was also significantly influenced by organic manure FYM levels (RDN kg ha^{-1}). The significantly maximum mean leaf area was recorded with 100% RDN (60.32) given through FYM, significantly superior over 75% RDN (54.51) given through FYM and the control (47.05). The increase in leaf area may be attributed to the adequate nitrogen and other nutrient supply through organic manure, leading to higher metabolic activity, synthesis of carbohydrates, and phytohormones. Similar results were observed in palak by Sharma and Agarwal (2014) [14].

The days required for horticultural maturity were significantly influenced by different bio-fertilizers. The significantly minimum days for horticultural maturity were observed under PSB (32.55 days) treatment, significantly superior over *Azospirillum* (33.77 days) and *Azotobacter* (34.28 days), while control required significantly maximum days (35.18). PSB treatment might have increased phosphorous availability, which plays a crucial role in energy storage and transfer, contributing to a decrease in the duration for horticultural maturity. Similar results were reported in fenugreek by Govind *et al*. (2016).

Days required for horticultural maturity were also significantly influenced by organic manure FYM levels (RDN kg ha^{-1}). Significantly maximum days required for horticultural maturity were recorded for the control treatment (35.54), while significantly minimum days (32.15) were observed when 100% RDN was given through FYM, with 75% RDN through FYM requiring (34.15). Increased levels of FYM (RDN kg ha^{-1}) provided easy release of different major and micro nutrients, resulting in early maturity and more cuttings. Similar results were reported in amaranth by Chaudhary *et al*. (2018) [19].

The mean length of the shoot did not show a significant interaction effect between bio-fertilizer and organic manure.

The average yield per plot was significantly influenced by different treatments. The total yield per plot recorded at the 1st, 2nd, and 3rd cutting were (2.25), (3.85), and (6.14) kg plot-1, respectively. The yield per plot was significantly maximum with *Azospirillum* (13.87 kg) treatment, significantly superior over *Azotobacter* (13.04 kg), PSB (11.94 kg), and control (10.21 kg). The highest yield in the *Azospirillum* treatment may be attributed to increased nutrient uptake, nitrogen fixation, and involvement in protein synthesis. Similar results were reported in spinach by Krishna *et al*. (2022) [10].

The yield per plot was also significantly influenced by different levels of organic FYM (RDN kg ha^{-1}). Significantly maximum total yield per plot (14.11 kg) was recorded when

100% RDN was given through FYM, significantly superior over 75% RDN (12.38 kg) through FYM and control (10.27 kg). Organic manure acts as a chelating agent and regulates the availability of micronutrients, increasing growth and yield. Similar results were observed in spinach by Jabeen *et al*. (2018) [8]. The interaction effect of bio-fertilizer and organic manure on average yield per plot was found to be non-significant.

Table 1: Effect of bio fertilizers and organic manure on days to potential germination of spinach

Treatments			Days to potential germination	Germination percent (%)
Factor A: Bio fertilizer(B):				
B_1	:	*Azotobacter*	5.26	90.18
B_2	:	PSB	5.5	86.76
B_3	:	*Azospirillum*	5.43	88.39
B_4	:	Control	5.93	84.85
S.Em.±			0.099	1.56
C.D. @ 5%			0.29	NS
Factor-B: Organic manure level (L):				
L_1	:	Control	5.74	85.29
L_2	:	75% RDN through FYM	5.55	87.51
L_3	:	100 % RDN through FYM	5.39	89.84
S.Em.±			0.086	1.35
C.D. @ 5%			0.25	NS
Interaction				
S.Em.±			0.172	2.7
C.D. @ 5%			NS	NS

Table 2: Effect of bio fertilizers and organic manure on number of leaves plant^{-1} of spinach

Treatments			Number leaves plant^{-1}			
			1st cutting	2nd cutting	3rd cutting	Mean
Factor A: Bio fertilizer(B)						
B_1	:	*Azotobacter*	7.81	12.29	14.33	11.47
B_2	:	PSB	7.69	11.60	13.76	11.01
B_3	:	*Azospirillum*	8.11	14.61	15.50	12.74
B_4	:	Control	7.21	10.24	12.04	9.82
S.Em.±			0.13	0.23	0.37	
C.D. @ 5%			0.38	0.67	1.09	
Factor-B: Organic manure level (L)						
L_1	:	Control	7.41	10.99	11.95	10.11
L_2	:	75% RDN through FYM	7.70	12.34	13.96	11.33
L_3	:	100 % RDN through FYM	8.01	13.23	15.81	12.34
S.Em.±			0.11	0.19	0.32	
C.D. @ 5%			0.33	0.58	0.95	
Interaction						
S.Em.±			0.23	0.39	0.64	
C.D. @ 5%			NS	NS	NS	
Mean			7.70	12.18	13.90	

Table 3: Effect of bio fertilizers and organic manure on height of plant (cm) of spinach

Treatments			Height of plant (cm)			
			1st cutting	2nd cutting	3rd cutting	Mean
Factor A: Bio fertilizer(B)						
B_1	:	*Azotobacter*	24.01	25.24	25.63	24.95
B_2	:	PSB	22.14	24.01	24.25	23.46
B_3	:	*Azospirillum*	28.30	29.38	29.85	29.17
B_4	:	Control	21.70	22.52	22.66	22.29
S.Em.±			0.86	0.76	0.76	
C.D. @ 5%			2.52	2.23	2.23	
Factor-B: Organic manure level (L)						
L_1	:	Control	21.74	22.92	23.22	22.62
L_2	:	75% RDN through FYM	24.14	25.36	25.67	25.05
L_3	:	100 % RDN through FYM	26.23	27.59	27.90	27.24
S.Em.±			0.74	0.66	0.65	
C.D. @ 5%			2.18	1.94	1.93	
Interaction						
S.Em.±			1.488	1.317	1.41	
C.D. @ 5%			NS	NS	NS	
Mean			24.03	25.28	25.59	

Table 4: Effect of bio fertilizers and organic manure on leaf area (cm^2) of spinach

Treatments			Leaf area (cm^2)			
			1st cutting	2nd cutting	3rd cutting	Mean
Factor A: Bio fertilizer (B)						
B_1	:	*Azotobacter*	31.61	52.56	83.52	55.91
B_2	:	PSB	32.59	53.48	74.49	53.56
B_3	:	*Azospirillum*	35.03	61.71	87.06	61.27
B_4	:	Control	24.12	39.34	72.11	45.19
S.Em.±			1.01	1.50	1.61	
C.D. @ 5%			2.96	4.41	4.73	
Factor-B: Organic manure level (L)						
L_1	:	Control	28.49	45.02	67.67	47.06
L_2	:	75% RDN through FYM	31.01	52.09	80.45	54.52
L_3	:	100 % RDN through FYM	33.02	58.20	89.77	60.33
S.Em.±			0.875	1.301	1.397	
C.D. @ 5%			2.57	3.82	4.10	
Interaction						
S.Em.±			1.75	2.60	2.79	
C.D. @ 5%			NS	NS	NS	
Mean			30.84	51.77	79.29	

Table 5: Effect of bio fertilizers and organic manure on days required for horticultural maturity of spinach

Treatments			Days required for horticultural maturity
Factor A: Bio fertilizer(B):			
B_1	:	*Azotobacter*	34.28
B_2	:	PSB	32.55
B_3	:	*Azospirillum*	33.77
B_4	:	Control	35.18
S.Em.±			0.586
C.D. @ 5%			1.72
Factor-B: Organic manure level (L):			
L_1	:	Control	35.54
L_2	:	75% RDN through FYM	34.15
L_3	:	100 % RDN through FYM	32.15
S.Em.±			0.51
C.D. @ 5%			1.49
Interaction			
S.Em.±			1.02
C.D. @ 5%			NS

Table 6: Effect of bio fertilizers and organic manure on yield $plot^{-1}$ (kg) of spinach

Treatments			Yield $plot^{-1}$ (kg)			
			1st cutting	2nd cutting	3rd cutting	Total yield
Factor A: Bio fertilizer(B)						
B_1	:	*Azotobacter*	2.44	4.08	6.52	13.04
B_2	:	PSB	2.2	3.62	6.12	11.94
B_3	:	*Azospirillum*	2.58	4.32	6.97	13.87
B_4	:	Control	1.8	3.38	5.03	10.21
S.Em.±			0.061	0.065	0.14	
C.D. @ 5%			0.18	0.19	0.45	
Factor-B: Organic manure level (L)						
L_1	:	Control	1.76	3.21	5.3	10.27
L_2	:	75% RDN through FYM	2.26	3.88	6.24	12.38
L_3	:	100 % RDN through FYM	2.76	4.5	6.85	14.11
S.Em.±			0.05	0.06	0.09	
C.D. @ 5%			0.15	0.17	0.26	
Interaction						
S.Em.±			0.10	0.11	0.18	
C.D. @ 5%			NS	NS	NS	
Average yield per cutting			2.25	3.85	6.14	

Conclusion

In summary, the comprehensive analysis of growth attributes in spinach cultivation revealed significant impacts of bio-fertilizers and organic manure. *Azotobacter* treatment demonstrated the shortest days to potential germination (5.26) and the highest germination percentage (90.18%), underscoring its efficacy in promoting early and robust plant establishment. *Azospirillum* treatment exhibited superior

growth attributes, including increased number of leaves per plant, greater plant height, larger leaf area, and longer shoot length compared to *Azotobacter* and PSB treatments. Conversely, PSB treatment resulted in the minimum days required for horticultural maturity (32.55 days), emphasizing its potential for accelerating the spinach crop's development. Moreover, the application of 100% recommended dose of nitrogen through farmyard manure (FYM) consistently outperformed the 75% RDN through FYM and control treatments across all growth attributes. Moving on to yield parameters, *Azospirillum* treatment emerged as the optimal bio-fertilizer, recording higher values for fresh weight of leaves per plant, yield per plot, and yield per hectare in comparison to *Azotobacter* and PSB treatments. Similarly, the 100% RDN through FYM exhibited superior performance in yield attributes compared to the 75% RDN through FYM and control treatments. The interaction effect of bio-fertilizer and organic manure on quality parameters was found to be non-significant, highlighting the individual impact of each factor on spinach cultivation. Overall, the findings underscore the potential of *Azospirillum* and 100% RDN through FYM in optimizing both growth attributes and yield parameters, contributing to the overall success of spinach cultivation.

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