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Influence of bio-fertilizers and organic manure on the quality parameters, yield and yield attributes of Indian spinach

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Abstract

The study conducted at the Organic Farming Research and Training Centre, Vasantao Naik Marathwada Krishi Vidhyapeeth, focused on the impact of bio-fertilizers and organic manure on Indian spinach during the summer season of 2021-2022. Employing a Factorial Randomized Block Design, three bio-fertilizers (*Azotobacter*, PSB, *Azospirillum*) and two organic manure FYM levels (75% and 100% RDN through FYM) were evaluated, totaling twelve treatments. Observations on growth, yield, quality, soil analysis, and economic factors were recorded and statistically analyzed. The results highlighted significant effects of bio-fertilizers and organic manure on growth, yield, and quality parameters. *Azotobacter* demonstrated the shortest germination period (5.26 days) and highest germination percentage (90.18%). *Azospirillum* exhibited optimal growth attributes, including number of leaves (12.74), plant height (29.17 cm), leaf area (61.27 cm²), and shoot length (27.80 cm). PSB led to the minimum days to horticulture maturity (32.55). The treatment with 100% RDN through FYM showed favorable results in various growth parameters and days to maturity. *Azospirillum* resulted in superior yield attributes, including fresh weight of leaves (21.91 g), total plot yield (13.87 kg), and total yield per hectare (20.38 t). Similarly, the 100% RDN through FYM displayed higher values for yield parameters (fresh weight, total plot yield, total yield per hectare). Quality parameters, such as chlorophyll content, ascorbic acid value, and iron content, were notably higher with *Azospirillum* and the 100% RDN through FYM treatment. Soil analysis revealed maximum available nitrogen with *Azospirillum*, phosphorus with PSB, and potassium with PSB treatment. The highest monetary returns and benefit-cost ratio were achieved with *Azospirillum* and the 100% RDN through FYM treatment. In summary, *Azospirillum* bio-fertilizer and 100% RDN through organic manure FYM exhibited superior effects on the growth, yield, and quality of Indian spinach compared to other bio-fertilizers and organic manure levels.

Keywords: Bio fertilizers, FYM, organic manure, *Azospirillum*, *Azotobacter*, PSB, quality parameters

Introduction

Beta vulgaris var. *bengalensis* Hort., commonly referred to as palak, is a member of the Chenopodiaceae family, possessing a chromosome count of 2n=18 (Purohit, 1970) [13]. Primarily cultivated in tropical and subtropical regions (Veeraragavathatham, 1998) [18], this leafy vegetable is renowned for its nutritional attributes. Rich in folic acid, vitamins, polyphenols, flavonoids, and natural antioxidants, spinach beet leaves contain substantial amounts of vitamin A (9770 I.U.), vitamin C (70 mg/100 g), and various minerals including calcium, phosphorus, and iron. The nutritional profile further includes 268.60 mg of nitrogen, 49.68 mg of phosphorus, 141.68 mg of potash, 368.00 mg of calcium, 42.32 mg of iron, 50.24 mg of ascorbic acid, and 52.00 g of carotene per 100 g of the edible portion. In trace amounts, oxalic acid, cholesterol, and calories are present, earning it the epithet "Mines of Minerals".

The utilization of organic manures enhances the quality of harvested produce by providing essential nutrients to plants and improving the physical, chemical, and biological properties of the soil. To achieve sustainable production, the application of plant nutrients through organic manures, such as farmyard manure, vermicompost, sheep manure, and bio-fertilizers, presents an alternative method (Wu and Ma, 2015) [19]. Farmyard manure, in particular, contributes to soil structure improvement and increased soil biomass (Dauda *et al.*, 2008) [5]. These organic manures serve as significant sources of micro and macro nutrients, fostering a robust organic foundation that enhances soil structural properties like bulk density, porosity, aeration, and water holding capacity. They also regulate soil pH, maintain soil health, promote biological diversity and soil micro-flora, reduce nutrient leaching, and increase nutrient availability and uptake, thereby facilitating sustainable vegetable production devoid of harmful residues

(Acharya and Mandal, 2002) [1].

Bio-fertilizers, consisting of microorganisms capable of converting nutrients from non-usable to usable forms through biological processes, offer an additional avenue for nutrient supplementation. These microorganisms, including nitrogen-fixing species like *Azotobacter* and *Azospirillum*, as well as phosphate-solubilizing bacteria, contribute to improved plant nutrition (Gajbhiye *et al.*, 2003) [6]. *Azospirillum* spp. and *Azotobacter*, specifically, exhibit the ability to associate with the root systems of various crop plants, fixing nitrogen and resulting in yield increases. *Azospirillum* inoculation promotes better vegetative growth and reduces the need for nitrogenous fertilizers by 10-20% (Sukhada, 1999) [16], even under stressful conditions such as drought. Depending on the crop, phosphorus fertilizer application can be reduced by 25-50%, with significant nutrient content increases in leaves and substantial savings in phosphorus without compromising plant yield (Sukhada, 1999) [16].

Given the aforementioned considerations, coupled with the dearth of spinach-specific information, the importance of spinach in human nutrition necessitated comprehensive research on the application of bio-fertilizers and organic manures, detailing their forms and application methods for spinach crop enhancement. In anticipation of future advancements in agriculture, spectral response analysis of plant phenotypes through spectroradiometers, particularly the calculation of the normalized difference vegetation index (NDVI), emerges as an effective and widely utilized approach for high-throughput phenotyping. Spectroradiometers, capable of acquiring data across broad ranges of electromagnetic radiation (350–2500 nm), play a pivotal role in monitoring drought, forecasting agricultural production, and aiding in the prediction of fire zones and desert offensive maps.

Considering the aforementioned factors and the significance of spinach for human health, this study was initiated to investigate the impact of bio-fertilizers and organic manure on the yield and quality of Indian spinach.

Material and Method

The current study, focusing on 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, Vasant Rao 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, each with three replications.

Factor-A Bio fertilizer (B)

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

B ₁	:	<i>Azotobacter</i>
B ₂	:	PSB
B ₃	:	<i>Azospirillum</i>
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 ₁ L ₁	<i>Azotobacter</i> + Control
2.	T ₂	B ₁ L ₂	<i>Azotobacter</i> + 75% RDN through FYM
3.	T ₃	B ₁ L ₃	<i>Azotobacter</i> + 100% RDN through FYM
4.	T ₄	B ₂ L ₁	PSB + Control
5.	T ₅	B ₂ L ₂	PSB + 75% RDN through FYM
6.	T ₆	B ₂ L ₃	PSB + 100% RDN through FYM
7.	T ₇	B ₃ L ₁	<i>Azospirillum</i> + Control
8.	T ₈	B ₃ L ₂	<i>Azospirillum</i> + 75% RDN through FYM
9.	T ₉	B ₃ L ₃	<i>Azospirillum</i> + 100% RDN through FYM
10.	T ₁₀	B ₄ L ₁	Control + Control
11.	T ₁₁	B ₄ L ₂	Control + 75% RDN through FYM
12.	T ₁₂	B ₄ L ₃	Control + 100% RDN through FYM

The recommended nutrient dosage for spinach cultivation is 80:40:40 (NPK) kilograms per hectare. When applying nutrients through Farmyard Manure (FYM), only the nitrogen component was considered. The required amount of FYM was added to the designated plot, accounting for the fact that FYM contains 0.52% nitrogen, 0.2% P₂O₅, and 0.5% K₂O.

Bio-fertilizers, specifically *Azotobacter*, Phosphate Solubilizing Bacteria (PSB), and *Azospirillum*, necessary for the experiment, were sourced from the Department of Agricultural Chemistry and Soil Science at Vasant Rao 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

Seeds of the All Green variety of spinach (*Beta vulgaris* L.) were procured from the market. The total chlorophyll content was assessed using a "Chlorophyll Spade Meter" (Minolta SPAD 502, Konica Inc. Tokyo, Japan) on fully expanded leaves from five selected observational plants, and the average mean was determined. Ascorbic acid levels were determined following the standard procedure outlined by Rangana (1995) [14] using the formula:

Ascorbic acid = (Titre value x Dye factor x Volume made up x 100) / (Volume of filtrate taken x Wt. of sample taken)

The iron content of the leaves was quantified in the diacid digest of plant tissues using Atomic Absorption spectrophotometry, following the method described by Bhuvaneswari *et al.* (2015) [2].

Normalized Difference Vegetation Index (NDVI) was calculated using the formula:

NDVI = (NIR-RED / NIR + RED) where NIR represents reflection in the near-infrared spectrum and RED represents reflection in the red range of the spectrum. This index ranges from -1.0 to 1.0, with negative values primarily associated with clouds, water, and snow, and values close to zero predominantly linked to rocks and bare soil.

Post-harvest, individual plot sampling was conducted to estimate available nutrient content. Expenditure on various inputs and labor costs were documented based on recent market rates for the inputs required for each treatment. The data was subjected to analysis of variance.

The cost of cultivation (Rs./ha) for each treatment was calculated, taking into account the current prices of inputs, cultivation charges, labor, land, and other expenses associated with that specific treatment. The total crop value was determined in rupees based on prevailing market rates during the harvest period. Gross returns (Rs./ha) were calculated by multiplying the total yield from a hectare with the prevailing market price of the produce. Net returns per hectare were determined by subtracting the cost of cultivation from gross returns per hectare. The benefit-cost ratio was computed using the formula:

Benefit: cost ratio = Gross monetary return (Rs) / Cost of production (Rs).

Result and Discussion

The data presented in Table 1 indicates a significant influence of different treatments on the yield per hectare. The average yield per hectare during the 1st, 2nd, and 3rd cuttings were recorded as 3.31 t, 5.66 t, and 9.03 t ha⁻¹, respectively. The calculation of yield per hectare was based on the average yield per plot and the total number of plots per hectare for each treatment. A noticeable increase in average yield per hectare was observed with each successive cutting. The influence of various bio-fertilizers on the average yield per hectare was also significant. *Azospirillum* treatment exhibited the highest total yield per hectare (20.38 t), followed by *Azotobacter* (19.15 t), PSB (17.54 t), and the control (14.99 t). The superiority of *Azospirillum* in total yield might be attributed to its capacity to fix atmospheric nitrogen, stimulate protein synthesis, enhance gibberellic acid and indole acetic acid activity, and increase dehydrogenase activity, ultimately improving growth characteristics. These findings align with the results reported by Krishna *et al.* (2022) [11] in spinach.

Regarding organic manure, significantly higher total yield per hectare (20.72 t) was recorded when 100% Recommended

Daily Nutrient (RDN) was provided through Farmyard Manure (FYM). This was superior to the application of 75% RDN through FYM (18.19 t) and the control (15.08 t). The positive impact of organic manure on yield is attributed to its role as a chelating agent, regulating micronutrient availability and promoting nutrient uptake, thus enhancing growth and yield. These results are consistent with the findings of Jabeen *et al.* (2018) [9] in spinach. The interaction effect of bio-fertilizer and organic manure on average yield per hectare was found to be non-significant.

Table 2 presents data on the quality characteristics of spinach influenced by bio-fertilizer and organic manure. The mean chlorophyll content of the crop (33.36 SPAD units) was significantly influenced by different treatments. Among the bio-fertilizers, *Azospirillum* treatment demonstrated the highest mean chlorophyll content (37.39), significantly superior to *Azotobacter* (35.90) and PSB (34.46) treatments. The mean chlorophyll content in PSB treatment was 34.45, while the control exhibited the lowest mean chlorophyll content at 33.08. The higher chlorophyll content in the *Azospirillum* treatment can be attributed to its ability to fix atmospheric nitrogen, stimulate protein synthesis, and enhance various growth-promoting activities, as reported by Krishna *et al.* (2022) [11] in spinach.

Among different levels of organic manure (FYM), significantly maximum mean chlorophyll content was recorded at 100% Recommended Daily Nutrient (RDN) through FYM (39.16), which was significantly superior to 75% RDN through FYM (35.14) and the control (31.31). The interaction effect of bio-fertilizer and organic manure on chlorophyll content was found to be non-significant. Singh *et al.* (2014) [15] observed similar improvements in chlorophyll content in chili with the addition of organic manures.

The mean ascorbic acid value of the crop (53.50 mg 100-1 g) was significantly influenced by different treatments. Among the bio-fertilizers, *Azospirillum* treatment exhibited the highest ascorbic acid value (61.72 mg 100-1 g), significantly superior to *Azotobacter* (55.21 mg 100-1 g) and PSB (52.45 mg 100-1 g) treatments. The highest ascorbic acid value in the *Azospirillum* treatment can be attributed to increased nutrient uptake, nitrogen fixation, and various growth-promoting activities. These results are consistent with the findings of Krishna *et al.* (2022) [11] in spinach. Among the organic manure levels (FYM), significantly highest ascorbic acid value (61.1 mg 100-1 g) was recorded at 100% RDN through FYM, which was significantly superior to 75% RDN through FYM (54.22 mg 100-1 g) and the control (45.18 mg 100-1 g). Increased levels of organic manures were associated with higher levels of ascorbic acid, in line with the findings of Jabeen *et al.* (2018) [9] in spinach. The interaction effect of bio-fertilizer and organic manure on ascorbic acid value was found to be non-significant.

The mean iron content of the crop (18.78 mg 100-1 g) was significantly influenced by different treatments. Among the bio-fertilizers, *Azospirillum* treatment recorded the highest iron content (21.94 mg 100-1 g), significantly superior to *Azotobacter* (18.53 mg 100-1 g) and PSB (18.57 mg 100-1 g) treatments. The highest iron content in the *Azospirillum* treatment may be attributed to increased mineralization of nutrients due to the application of *Azospirillum*. These results align with the findings of Thampi *et al.* (2019) [17] in spinach.

Among different levels of organic manure (FYM), the highest iron content was recorded (22.02 mg 100-1 g) when 100%

Recommended Daily Nutrient (RDN) was provided through FYM. This was significantly superior to 75% RDN through FYM (18.80 mg 100⁻¹ g) and the control (15.52 mg 100⁻¹ g). The increase in iron content with increased application of organic manure is attributed to the presence of organic carbon in FYM, acting as a source of energy for soil microorganisms. This, upon mineralization, releases organic acids that decrease soil pH, improving iron availability. These findings align with the results reported by Thampi *et al.* (2019) [17] in spinach. The interaction effect of bio-fertilizer and organic manure on iron content was found to be non-significant.

In terms of vegetation indices (NDVI), the mean value was 0.74 and significantly influenced by different treatments. The highest mean vegetation indices were recorded with *Azospirillum* (0.81), which was on par with *Azotobacter* (0.77) and PSB treatments (0.77), while the control exhibited the lowest mean vegetation indices (0.61). Among organic manure levels (FYM), the highest mean vegetation indices (0.87) were recorded when 100% RDN was provided through FYM. This was significantly superior to 75% RDN through FYM (0.78) and the control (0.57). The interaction effect of bio-fertilizer and organic manure on vegetation indices was found to be non-significant.

The economic analysis in Table 4 indicates that the cost of cultivation did not vary significantly among different bio-fertilizer treatments. The minimum cost of cultivation (Rs. 1,00,444/ha) was observed under control conditions, and it was slightly higher in all three bio-fertilizer treatments (Rs. 1,03,666.67/ha). Among different levels of organic manure (FYM), the maximum cost of cultivation (Rs. 1,18,712.33/ha) was recorded when 100% RDN was provided through FYM, exceeding the cost of cultivation for 75% RDN through FYM (Rs. 1,11,440.6/ha) and control (Rs. 99,833.33/ha). The higher cost with increased organic manure levels is due to the additional cost of FYM, which is comparatively more expensive than bio-fertilizers.

Gross monetary returns were significantly influenced by bio-

fertilizer treatments. The maximum gross monetary returns (Rs. 2,57,715.34/ha) were observed under *Azospirillum* treatment, while the minimum (Rs. 1,77,545.49/ha) was in the control treatment. For *Azotobacter* and PSB treatments, gross monetary returns were (Rs. 2,13,533.55/ha) and (Rs. 2,08,596.97/ha), respectively. Gross monetary returns were highest in the *Azospirillum* treatment, attributed to the higher yield of spinach per hectare with the use of *Azospirillum*. Similar trends were reported by Ganeshe *et al.* (2000) [7] in okra. Among different levels of organic manure (FYM), the maximum gross monetary returns (Rs. 2,54,100/ha) were recorded when 100% RDN was provided through FYM, exceeding the returns for 75% RDN through FYM (Rs. 2,23,373.44/ha) and control (Rs. 1,84,423.97/ha).

Net monetary returns followed a similar trend, with the maximum (Rs. 1,54,048.68/ha) under *Azospirillum* treatment and the minimum (Rs. 77,101.04/ha) in the control treatment. Net monetary returns for *Azotobacter* and PSB treatments were (Rs. 1,09,866.88) and (Rs. 1,04,930.31), respectively. Among different levels of organic manure (FYM), the maximum net monetary returns (Rs. 1,35,388.3/ha) were recorded when 100% RDN was provided through FYM, exceeding the returns for 75% RDN through FYM (Rs. 1,11,930.8/ha) and control (Rs. 84,850.6/ha).

The Benefit-Cost (B: C) ratio was significantly influenced by bio-fertilizer treatments. The maximum B: C ratio (2.48) was observed under *Azospirillum* treatment, while the minimum (1.76) was in the control treatment. B: C ratios for *Azotobacter* and PSB treatments were (2.05) and (2.01), respectively. Among different levels of organic manure (FYM), the maximum B: C ratio (2.14) was recorded when 100% RDN was provided through FYM, exceeding the ratio for 75% RDN through FYM (2.00) and control (1.84). In summary, the use of *Azospirillum* as a bio-fertilizer and providing 100% RDN through FYM resulted in higher yields, improved quality parameters, and better economic returns in spinach cultivation.

Table 1: Effect of bio fertilizers and organic manure on yield ha⁻¹ (t) of spinach

Treatments		Yield ha ⁻¹ (t)				
		1 st cutting	2 nd cutting	3 rd cutting	Total yield	
Factor A: Bio fertilizer(B)						
B ₁	:	<i>Azotobacter</i>	3.58	5.99	9.58	19.15
B ₂	:	PSB	3.23	5.32	8.99	17.54
B ₃	:	<i>Azospirillum</i>	3.79	6.35	10.24	20.38
B ₄	:	Control	2.64	4.96	7.39	14.99
		S.Em.±	0.09	0.1	0.22	
		C.D. @ 5%	0.26	0.28	0.67	
Factor-B: Organic manure level (L)						
L ₁	:	Control	2.58	4.71	7.79	15.08
L ₂	:	75% RDN through FYM	3.32	5.7	9.17	18.19
L ₃	:	100% RDN through FYM	4.05	6.61	10.06	20.72
		S.Em.±	0.08	0.08	0.13	
		C.D. @ 5%	0.23	0.24	0.39	
Interaction						
		S.Em.±	0.15	0.16	0.26	
		C.D. @ 5%	NS	NS	NS	
		Average yield per cutting	3.31	5.66	9.03	

Table 2: Effect of bio fertilizers and organic manure quality parameters of spinach crop

Treatments		Quality parameters			
		Chlorophyll content (SPAD value)	Ascorbic acid value (mg 100 ⁻¹ g)	Iron content (mg 100 ⁻¹ g)	
Factor A: Bio fertilizer(B)					
B ₁	:	Azotobacter	35.90	55.22	18.54
B ₂	:	PSB	34.46	52.46	18.58
B ₃	:	<i>Azospirillum</i>	37.39	61.72	21.95
B ₄	:	Control	33.08	44.61	16.07
		S.Em.±	0.61	1.3	0.80
		C.D. @ 5%	1.78	3.81	2.35
Factor-B: Organic manure level (L)					
L ₁	:	Control	31.32	45.19	15.52
L ₂	:	75% RDN through FYM	35.14	54.22	18.80
L ₃	:	100% RDN through FYM	39.16	61.09	22.02
		S.Em.±	0.52	1.12	0.69
		C.D. @ 5%	1.54	3.30	2.04
Interaction					
		S.Em.±	1.05	2.25	1.39
		C.D. @ 5%	NS	NS	NS
		Mean	33.36	53.50	18.78

Table 3: Effect of bio fertilizers and organic manure on effect of bio fertilizers and organic manure on vegetation indices (NDVI)

Treatments		Vegetation indices (NDVI)	
Factor A: Bio fertilizer(B):			
B ₁	:	<i>Azotobacter</i>	0.77
B ₂	:	PSB	0.77
B ₃	:	<i>Azospirillum</i>	0.81
B ₄	:	Control	0.61
		S.Em.±	0.02
		C.D. @ 5%	0.07
Factor-B: Organic manure level (L):			
L ₁	:	Control	0.57
L ₂	:	75% RDN through FYM	0.78
L ₃	:	100% RDN through FYM	0.87
		S.Em.±	0.02
		C.D. @ 5%	0.06
Interaction			
		S.Em.±	0.04
		C.D. @ 5%	NS
		Mean	0.74

Table 4: Effect of bio fertilizers and organic manure on economics of spinach production

Treatments		Cost of cultivation (Rs./ha)	Gross monetary returns (Rs./ha)	Net monetary returns (Rs./ha)	Benefit: Cost Ratio	
Factor A: Bio fertilizer(B)						
B ₁	:	Azotobacter	1,03,666.6	2,13,533.55	1,09,866.88	2.05
B ₂	:	PSB	1,03,666.6	2,08,596.97	1,04,930.38	2.01
B ₃	:	<i>Azospirillum</i>	1,03,666.6	2,57,715.34	1,54,048.68	2.48
B ₄	:	Control	1,00,444.4	1,77,545.49	77,101.04	1.76
Factor-B: Organic manure level (L)						
L ₁	:	Control	99,833.33	1,84,423.97	84,850.6	1.84
L ₂	:	75% RDN through FYM	1,11,440.6	2,23,373.44	1,11,930.8	2.00
L ₃	:	100% RDN through FYM	1,18,712.3	2,54,100.6	1,35,388.3	2.14

Conclusion

In conclusion, the study revealed significant impacts of bio-fertilizers and organic manure on the yield, quality parameters, growth attributes, and economic aspects of spinach cultivation. *Azospirillum* treatment emerged as the most effective bio-fertilizer, displaying superior performance in yield parameters such as fresh weight of leaves plant-1, yield per plot, and yield per hectare, as well as quality parameters like chlorophyll content, ascorbic acid value, and iron content. Similarly, the application of 100%

recommended dose of nitrogen through farmyard manure (FYM) demonstrated the highest values across various yield and quality attributes compared to 75% RDN through FYM and control. Additionally, *Azospirillum* treatment exhibited the highest Normalized Difference Vegetation Index (NDVI), indicating enhanced vegetative growth. Economically, *Azospirillum* treatment and 100% RDN through FYM resulted in the highest gross monetary returns, net monetary returns, and benefit-cost ratio, highlighting their potential for maximizing economic returns in spinach cultivation. The

findings underscore the importance of bio-fertilizers and organic manure in sustainable agriculture, promoting both crop productivity and economic viability.

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