



Microbial Enriched Biochar Levels and Fertilizer Doses on Soil Chemical Properties under Spinach (*Spinacia oleracea*) Production

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Intensive cultivation of vegetables and imbalanced fertilization depleted the soil nutrients and caused low yield with poor quality crops. A novel microbial enriched biochar and fertilizer combination could alleviate the soil and nutrient stress. Therefore, a field experiment was conducted with 14 treatments [4 levels of biochar (5, 7.5, 10, 15 t ha⁻¹), 3 doses of fertilizers (50, 75, 100% of recommended) along with without biochar and absolute control] in spinach production. This study was conducted with randomized block designed and replicated three times in trial plot at BAIF, Central Research Station, Pune during 2020-2021. Maximum growth parameters (plant height, leaf length, leaf width and petiole length) were recorded in (T4) with Biochar @ 10 tha⁻¹ + 75% of RDF followed by in (T7) Biochar @ 7.5 tha⁻¹ + 50% of RDF. Whereas, the highest Spinach yield (18.58 tha⁻¹) was recorded under (T4) Biochar @ 10 tha⁻¹ + 75 % of RDF followed by (T3) Biochar @ 7.5 tha⁻¹ + 75 % of RDF recorded (17.93 tha⁻¹). The treatment (T1) @ 100 % RDF has registered as superior from an economic point of view. Furthermore, the minimum values of growth and yield were recorded under absolute control. Based on the above results it is concluded that the combined application of microbial enriched biochar (10t ha⁻¹) and fertilizers (75% of recommended dose) enhanced the nutrient availability to spinach for maximum growth and yield.

Keywords: Spinach; yield; biochar; growth and nutrient.

1. INTRODUCTION

Spinach has a long history of use as both medicinal and an edible plant [1]. It contains phyto-nutrients, with large amount of vitamins (A, C, B-complex such as folate), mineral (Potassium, manganese, calcium, magnesium, and copper) and anti-oxidants which protects the body and fights carcinogenic cells and it is low in calories and fats [2].

Inorganic fertilizers have played a significant role in increasing crop production since the “green revolution” [3] however, they are not a sustainable solution for maintenance of crop yields [4]. Long-term overuse of mineral fertilizers may accelerate soil acidification, affecting both the soil biota and biogeochemical processes, thus posing an environmental risk and decreasing crop production [5]. Organic amendments, such as biochar and compost could therefore be useful tools to sustainably maintain or increase soil organic matter, preserving and improving soil fertility and crop yield.

Biochar is a carbon-rich material obtained from thermochemical conversion (slow, intermediate, and fast pyrolysis or gasification) of biomass in an oxygen-limited environment. It can be produced from a range of feedstock, including fire wood of forest trees and agriculture residues, such as straw, nut shells, rice hulls, cotton stalk pellets, tree bark, and switch grass [6]. Biochar has been described as a possible tool for soil fertility improvement, potential toxic element adsorption, and climate change mitigation [7].

Indeed, several studies have shown that Biochar application to soil can improve soil physical quality in terms of bulk density (BD), and soil moisture holding capacity and in chemical properties it improves soil pH and Soil Organic Carbon (SOC) [8], enhance plant nutrient availability and correlated growth and yield [9] and reduce greenhouse gas emissions through C sequestration [10]. Therefore, experiment was conducted on microbial enriched biochar levels and fertilizer doses on soil chemical properties under Spinach (*Spinacia oleracea*) production. This could help to overcome the constraints related to nutrient uptake, moisture retention, soil organic matter and microbial population under spinach production.

2. MATERIALS AND METHODS

The study was conducted at BAIF, Central Research Station, Urulikanchan, Ta Haveli Dist. Pune Maharashtra, India. The land having clay type of soil with properly managed cultivable land was selected for conducting the field trial. The analysis of soil carried out to understand the chemical properties of soil before conducting the trial and after harvesting of crop (Table-4). Initially, the land was deep ploughed and laid out the plot adapting the RBD design The plot size of 4 x 3 m² were prepared in which farmyard manure @ 10 tons per ha was spread equally. The var. Allgreen (Spinach) was sown in the row to row distance of 15 cm on 08th April 2021. The recommended dose of fertilizer (RDF) 40:40:40 kg/ha⁻¹ considered and accordingly the dose of fertilizers were reduced as mentioned in treatment details (Table-1). Considering the climatic condition, a five time irrigation was given during the entire growth period. The Spinach was harvested after 45 days of sowing (23rd May 2021).

Table 1. Treatment details

Sr #	Treatments Details
T 1	100 % Recommended dose of fertilizer (RDF)
T 2	Biochar @ 5 t ha ⁻¹ + 75 % RDF
T 3	Biochar @ 7.5 t ha ⁻¹ + 75 % RDF
T 4	Biochar @ 10 t ha ⁻¹ + 75 % RDF
T 5	Biochar @ 15 t ha ⁻¹ + 75 % RDF
T 6	Biochar @ 5 t ha ⁻¹ + 50 % RDF
T 7	Biochar @ 7.5 t ha ⁻¹ + 50 % RDF
T 8	Biochar @ 10 t ha ⁻¹ + 50 % RDF
T 9	Biochar @ 15 t ha ⁻¹ + 50 % RDF
T 10	Biochar @ 5 t ha ⁻¹ + 100 % RDF
T 11	Biochar @ 7.5 t ha ⁻¹ + 100 % RDF
T 12	Biochar @ 10 t ha ⁻¹ + 100 % RDF
T 13	Biochar @ 15 t ha ⁻¹ + 100 % RDF
T 14	Control (No fertilization)

The Subabul (*Leucaena leucocephala*) is used as a fodder for goat and cattle feeding by local community in the villages. Besides its fodder value many a time the remained wood biomass is used as fire wood. The alternate option to make use of subabul wood may be the preparation of biochar which will be intern used as soil amendment for enriching the soil organic carbon etc. By adapting proper combustion method using kiln biochar can be produced up to 50-55 % with the production cost of Rs.15 per kg.

The extended microbial culture of *Rhizobium*, *Phosphate Solubilizing Bacteria* (PSB) and

Table 2. Effects of different microbial enrich biochar levels in combination with NPK on growth of Spinach

Tr #	Treatment Details	Plant Height (cm)	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Leaf: petiole (Length basis)	Average no. of leaves/ plant
1	100 % Recommended dose of fertilizer (RDF)	32.7	13.4	10.2	18.5	0.72	11
2	Biochar @ 5 t ha ⁻¹ + 75 % RDF	36.4	14.7	10.4	21.6	0.68	11
3	Biochar @ 7.5 t ha ⁻¹ + 75 % RDF	30.0	13.2	9.3	17.3	0.77	11
4	Biochar @ 10 t ha ⁻¹ + 75 % RDF	34.6	13.7	9.3	20.0	0.69	11
5	Biochar @ 15 t ha ⁻¹ + 75 % RDF	30.1	13.4	8.6	17.6	0.76	11
6	Biochar @ 5 t ha ⁻¹ + 50 % RDF	35.7	14.4	9.7	21.1	0.69	10
7	Biochar @ 7.5 t ha ⁻¹ + 50 % RDF	37.9	15.0	9.7	21.6	0.70	11
8	Biochar @ 10 t ha ⁻¹ + 50 % RDF	34.2	13.6	8.9	20.3	0.67	12
9	Biochar @ 15 t ha ⁻¹ + 50 % RDF	38.4	16.3	11.8	24.0	0.68	12
10	Biochar @ 5 t ha ⁻¹ + 100 % RDF	34.2	14.2	9.3	22.4	0.63	10
11	Biochar @ 7.5 t ha ⁻¹ + 100 % RDF	33.9	14.0	9.5	20.4	0.69	11
12	Biochar @ 10 t ha ⁻¹ + 100 % RDF	31.5	12.5	8.8	22.9	0.55	10
13	Biochar @ 15 t ha ⁻¹ + 100 % RDF	30.1	13.6	9.4	19.6	0.69	10
14	Control (No fertilization)	22.8	8.8	7.7	12.7	0.69	7
	SE(m)±	3.099	1.037	0.721	0.865	0.048	0.661
	CD at 5 %	N.S.	N.S.	N.S.	2.528	N.S.	N.S.

Table 3. Effects of different microbial enrich biochar levels in combination with NPK Fertilization on yield and economics of Spinach

Tr #	Treatment Details	Yield (tha ⁻¹)	Gross Monetary Returns (Rsha ⁻¹)	Cost of cultivation (Rsha ⁻¹)	Net Monetary Returns (Rsha ⁻¹)	Benefit : cost ratio
1	100 % Recommended dose of fertilizer (RDF)	16.81	252083	67274	184809	3.75
2	Biochar @ 5 t ha ⁻¹ + 75 % RDF	17.22	258333	120411	137923	2.15
3	Biochar @ 7.5 t ha ⁻¹ + 75 % RDF	17.93	268958	150973	117985	1.78
4	Biochar @ 10 t ha ⁻¹ + 75 % RDF	18.58	278625	181536	97089	1.53
5	Biochar @ 15 t ha ⁻¹ + 75 % RDF	16.03	240500	242661	-2161	0.99
6	Biochar @ 5 t ha ⁻¹ + 50 % RDF	15.53	232917	112418	120499	2.07
7	Biochar @ 7.5 t ha ⁻¹ + 50 % RDF	16.25	243792	142981	100811	1.71
8	Biochar @ 10 t ha ⁻¹ + 50 % RDF	17.89	268292	173543	94749	1.55
9	Biochar @ 15 t ha ⁻¹ + 50 % RDF	16.74	251167	234668	16499	1.07
10	Biochar @ 5 t ha ⁻¹ + 100 % RDF	15.20	228042	127491	100551	1.79
11	Biochar @ 7.5 t ha ⁻¹ + 100 % RDF	15.46	231833	158954	72880	1.46
12	Biochar @ 10 t ha ⁻¹ + 100 % RDF	16.10	241458	189516	51942	1.27
13	Biochar @ 15 t ha ⁻¹ + 100 % RDF	15.27	229083	250641	-21558	0.91
14	Control (No fertilization)	3.50	52500	35320	17180	1.49
	SE(m)±	0.75	11,379.49		12,383.66	0.09
	CD at 5 %	2.21	33,262.61		36,197.84	0.263

Table 4. Soil chemical analysis

Tr #	Treatment Details	pH	EC	OC (%)	N (kg/ha)	P (kg/ha)	K (kg/ha)
	Initial soil status	7.34	0.48	0.61	134	30	215
After harvesting							
T 1	100 % Recommended dose of fertilizer (RDF)	7.52	0.42	0.58	139	36	231
T 2	Biochar @ 5 t ha ⁻¹ + 75 % RDF	7.26	0.44	0.66	125	31	230
T 3	Biochar @ 7.5 t ha ⁻¹ + 75 % RDF	7.49	0.39	0.64	130	32	225
T 4	Biochar @ 10 t ha ⁻¹ + 75 % RDF	7.37	0.48	0.68	125	34	223
T 5	Biochar @ 15 t ha ⁻¹ + 75 % RDF	7.48	0.47	0.65	138	38	238
T 6	Biochar @ 5 t ha ⁻¹ + 50 % RDF	7.3	0.47	0.52	140	28	225
T 7	Biochar @ 7.5 t ha ⁻¹ + 50 % RDF	7.48	0.44	0.57	126	25	215
T 8	Biochar @ 10 t ha ⁻¹ + 50 % RDF	7.45	0.4	0.76	128	29	235
T 9	Biochar @ 15 t ha ⁻¹ + 50 % RDF	7.41	0.48	0.6	127	34	230
T 10	Biochar @ 5 t ha ⁻¹ + 100 % RDF	7.36	0.39	0.52	130	43	240
T 11	Biochar @ 7.5 t ha ⁻¹ + 100 % RDF	7.48	0.47	0.58	132	31	231
T 12	Biochar @ 10 t ha ⁻¹ + 100 % RDF	7.42	0.42	0.69	134	33	237
T 13	Biochar @ 15 t ha ⁻¹ + 100 % RDF	7.44	0.47	0.6	135	29	230
T 14	Control (No fertilization)	7.57	0.35	0.5	136	27	230

Tricoderma was prepared and again 20 liter of extended microbial solution was mixed in 80 liter of water for treating 100kg of biochar. This will helps to reduce the cost of bio fertilizers for treating the biochar. The treated biochar was dried under shade for a day and applied to the field as per the doses 5, 7.5, 10 and 15 tha^{-1} separately. At the same time chemical fertilizers applied before sowing of the crop as per the dose mentioned in the Table-1. The growth observations like Plant height, leaf length, width and petiole length were recorded before 2 days of harvesting of crop from each treated plots.

The change in the soil chemical properties before and after harvest of the crop was analyzed in the laboratory from the collected soil samples. The data was statistically analyzed using MS-Excel (2010) and OPSTAT software [11].

3. RESULTS AND DISCUSSION

3.1 Influence of Biochar on Growth Parameters of Spinach

The data presented in Table 2. Clearly indicates that, except petiole length, there is a non-significant variation in other growth parameters due to application of different doses of Biochar and fertilizers. However, the study shows that, a higher plant growth was recorded in treatment T_9 (Biochar @ 15 t ha^{-1} + 50 % RDF) in plant height (38.4 cm), Leaf length (16.3 cm), Leaf width (11.8 cm), was recorded over RDF. The observation made on leaf: petiole ratio shows that there was non-significant effect due to different level of treatments which might be attributed to slow release of nutrients through Biochar, leading to better growth of spinach. The growth parameters recorded are near to the results of Chat et al., [12] and Roy et al., [13] in spinach.

3.2 Effects of Different Levels of Biochar on Spinach Yield

The data pertaining to leaf yield and economics of experiment are presented in Table 3. The effect of microbial enriched biochar levels on Spinach yield is indicated positively due to combined application of different levels of microbial enriched biochar and chemical fertilizers. However, the application of microbial enriched biochar @ 10 tha^{-1} + 75 % of RDF (T_4) has given significantly higher yield (18.58 tha^{-1}) over the RDF. But in control plot growth and yield parameters were recorded minimum values

over all the treatments. However, study needs to be continued at least for three seasons to come out with confined conclusion. The above findings are closer to the observation made by Ansari [14], Canali et al., [15] and Patel et al., [16].

3.3 Benefit Cost Ratio of the Study

The data pertaining to economics of each treatment viz., cost of cultivation, gross income, net income and benefit: cost ratio has shown in Table 3. The maximum yield 18.58 tha^{-1} was recorded in T_4 and also the highest gross monetary return of Rs. 278625/- ha^{-1} was calculated. But cost of cultivation of Rs. 250641/- was higher in treatment T_{13} (Biochar @ 15 tha^{-1} + 100 % RDF) followed by T_5 (Biochar @ 15 tha^{-1} + 75 % RDF) Rs. 240500/- ha^{-1} , in T_9 (Biochar @ 15 tha^{-1} + 50 % RDF) Rs. 234668/- ha^{-1} . It is mainly because of use of higher levels of microbial enrich biochar. Whereas, in treatment T_{14} lowest gross income Rs. 52500/- ha^{-1} was recorded as the levels of microbial enrich biochar is not used. By considering all inputs, the benefit: cost ratio was calculated and shows higher in T_1 (100 % RDF) i.e. 3.75, due to no cost of microbial enrich biochar.

3.4 Effects of Different Levels of Biochar Application on Soil Properties

It has been reported that the application of biochar to soil improves nutrient availability, when the effects vary with biochar types/doses and soil types [17]. The effects of biochar on soil nutrients can have high adsorption capacity and can reduce nutrient loss and increase soil fertility [18]. The study shows that Nitrogen was increased from 134 to 140 kg ha^{-1} in T_6 (Biochar @ 5 t ha^{-1} + 50 % RDF) and Phosphorus, Potassium was increased from 30 to 43 kg ha^{-1} , 215 to 240 kg ha^{-1} in T_{10} (Biochar @ 5 t ha^{-1} + 100 % RDF) respectively. Similarly the soil organic carbon (SOC) percent was increased from 0.61 to 0.76 in treatment T_8 (Biochar @ 10 tha^{-1} + 50% of RDF). The increased SOC is near to the observation made in the study by [19] and [20] using Biochar at the rate of (5 tha^{-1}). The reason for increase in SOC may be due to presence of stable carbon in the Biochar which led to increase in soil carbon [21].

4. CONCLUSION

It can be concluded that spinach is highly responsive to combined application of different

levels of Biochar and inorganic source of nutrition. Application of microbial enriched biochar @ 10 tha⁻¹ with 75% of RDF (T4) given maximum yield (18.58 tha⁻¹).

Considering the soil health, the consistent use of chemical fertilizer alone will not be recommended. Using chemical fertilizer along with the Microbial enriched biochar will be the more benefit in terms of moisture holding, increased soil organic carbon. However, the study need to be continued for at least for the three seasons to draw a precise conclusion related to yield and soil properties.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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