

**Effect of mulberry stalk biochar on soil nutrients status and productivity of mulberry
(*Morus alba L.*)**

Abstract

Biochar as an organic amendment improves soil attributes, with a potentially significant effect on soil fertility. The main objective of this study was to quantify the effect of biochar addition on nutrients status in mulberry cultivated soil. A field experiment was conducted in the mulberry crop to know the effect of soil application of mulberry stalk biochar on nutrients build up in soil at farmer's field at Sidlaghatta (TQ), Chikkaballapura District during 2020-2021 (rabi2020, summer and kharif-2021). A randomized block design was employed with eight treatments replicated thrice T₁: Control (NPK375:140:140 kg ha⁻¹ alone) T₂: POP (FYM (25 t ha⁻¹) + NPK 375:140:140 kg ha⁻¹) T₃: Soil application of biochar @ 5 t ha⁻¹ T₄: Soil application of biochar @ 7.5 t ha⁻¹ T₅: Soil application of biochar @ 10 t ha⁻¹ T₆: Soil application of biochar @ 5 t ha⁻¹ + FYM @ 10 t ha⁻¹ T₇: Soil application of biochar @ 7.5 t ha⁻¹ + FYM @ 10 t ha⁻¹ T₈: Soil application of biochar @ 10 t ha⁻¹ + FYM @ 10 t ha⁻¹ NPK is common for all the treatments. Combined soil application of biochar @ 10 t ha⁻¹ and FYM @ 10 t ha⁻¹ (T₈) increased the leaf yield (13.07 t ha⁻¹) by 21 % over control (10.45 t ha⁻¹). Significantly higher soil primary, secondary and micronutrients were recorded in T₈ (soil application of biochar @ 10 t ha⁻¹ + FYM @ 10 t ha⁻¹) followed by treatment T₇ which received soil application of biochar @ 7.5 t ha⁻¹ + FYM @ 10 t ha⁻¹. The lower soil primary, secondary and micronutrients were recorded in T₁ which is devoid of biochar. The findings revealed that utilization of mulberry stalk as a biochar has positive effect on the improvement of nutrient status and it could partly replace chemical fertilizers and promote organic farming in a circular economy concept.

Key words: Biochar, leaf yield, primary, secondary nutrients

Introduction

“Mulberry is deep rooted foliage yielding and fast growing perennial crop grown for its leaf and is the sole food for silkworm (*Bombyx mori* L.) rearing. It is grown under varied climatic conditions ranging from temperate to tropics. In India, most states have taken up sericulture as an important agro-industry. Though mulberry cultivation is practiced in various climates, the major area is in tropical zone covering Karnataka, Andhra Pradesh and Tamil Nadu states, with about 90%. In the sub-tropical zone, West Bengal, Himachal Pradesh and North-Eastern states have major areas under mulberry cultivation”. (Mahesh *et al.*, 2020). “The sustainable production of mulberry leaf is entirely dependent on the maintenance of the soil fertility of mulberry garden through the periodical application of organic sources and inorganic fertilizers in required quantities. Leaf quality and quantity not just impact the silkworm growth and development but also impact the cocoon production and quality of raw silk”. (Rohela *et al.*, 2020)

“Soil organic matter (SOM) is the heart of soil cannot be overlooked as it is a vital source of energy and nutrients for the soil macro- and micro- organisms and the plants as well. SOM is important to soil fertility because it contains at least 95% of the total nitrogen and sulphur along with 20 to 75% of phosphorus in the soil surface” (Steiner *et al.*, 2007). “Soil organisms, including microorganisms, use soil organic matter as food. As they break down the organic matter, any excess nutrients (N, P and S) are released into the soil in the forms that plants and organisms can use”. (Johnston *et al.*, 2009)

According to Lehmann and Joseph (2009), “biochar is becoming a popular alternative to organic amendments that is being applied to soils to increase and sustain soil productivity”. The use and functions of biochar in soils have been recently reviewed by Sohi *et al.*, (2010) and potential mechanisms of achieving agricultural benefits by biochar – soil application by Atkinson *et al.*, (2010). “Application of biochar carbon allows cycling of nutrients back into the agricultural soils and sequestering carbon in a recalcitrant form” (Steiner, 2010).

“Biochar alters soil structure, increases among others, water retention capacity, CEC, sorption capacity, base saturation percentage, surface area, microbial activity, pH of acid soils *etc.* That is why biochar is gaining huge acclamation of the environmentalists, scientists and researchers nowadays to mitigate climate change and to enhance nutrient management”. (Steiner, 2010)

“Biochar is a source of organic amendment/manure that is receiving attention by researchers all over the world” (Lehmann *et al.*, 2003). “The process of biochar production under controlled oxygen is known as pyrolysis and it results in a very stable carbon (C)-rich material not only capable of improving physical and chemical soil properties but also increasing soil carbon storage on a large scale. Among soil organic amendments, biochar is considered as a more stable nutrient source than others” (Chan *et al.*, 2007). “Organic C content in biochar has been reported up to 90 percent depending upon its feedstock, which enhances C sequestration in soil” (Lehmann and Joseph, 2009).

Biochar is a carbon rich material, in association with porous characteristics and high surface area which are favourable to accumulating soil moisture, increase the porosity, reduces the bulk density and to promote the formation of soil aggregation. All the above soil physical improvement can provide a good environment for the growth of plants. Furthermore, biochar is an ideal acidic soil amendment which can improve the pH of acidic soil. It contains nutrient element which can be directly released into soil, and its surface charge and functional groups are conducive to soil nutrient retention, such as the reduced leaching of NH_4^+ and NO_3^- , PO_4^{3-} , therefore improve the efficiency of nutrient elements.

“Moreover, due to the ability of biochar to persist in the soil over a long period of time as it is recalcitrant to decomposition, it can provide desirable benefits to crops over several seasons” (Steiner *et al.*, 2007; Major *et al.*, 2010). The sustainable production of mulberry leaf and cocoon crop is entirely dependent on the maintenance of the soil fertility of mulberry garden through the periodical application of organic sources and inorganic fertilizers in required quantities. Though lot of mulberry stalk has been generated throughout the year and for its management aspects, the present study is proposed against this back drop with the broad aim to produce biochar from the mulberry stalks and this could have a dramatic impact on our society and on agriculture worldwide. Thus keeping in view the above facts, the current study was undertaken to assess the impact of biochar on mulberry leaf yield and nutrients build up in soil.

Material and methods

The experiment was carried out during 2020-2021 (rabi2020, summer and kharif-2021 2020-2021) in farmer's field at Sidlaghatta (TQ), Chikkabalapura District, Karnataka, India, which falls under Eastern Dry Zone of Karnataka (Agro climatic Zone No. 5) and is situated at 13o 36' North latitude 77o 43.49'East longitude and at an altitude of 915 meters above the mean sea level. Victory 1 (V1) variety planted at a spacing of 90 x 60 cm. The experiment was laid out in randomized complete block design and replicated thrice with 8 treatments and the test crop was mulberry. The treatment details are given below

T₁: Control (NPK375:140:140 kg ha⁻¹ alone)

T₂: POP (FYM (25 t ha⁻¹) + NPK 375:140:140 kg ha⁻¹)

T₃: Soil application of biochar @ 5 t ha⁻¹

T₄: Soil application of biochar @ 7.5 t ha⁻¹

T₅: Soil application of biochar @ 10 t ha⁻¹

T₆: Soil application of biochar @ 5 t ha⁻¹ + FYM @ 10 t ha⁻¹

T₇: Soil application of biochar @7.5 t ha⁻¹ + FYM @ 10 t ha⁻¹

T₈: Soil application of biochar @ 10 t ha⁻¹ + FYM @ 10 t ha⁻¹

NPK is common for all the treatments

The physical and chemical characteristics of the top 0-15cm depth of the soil of the experimental site are summarized in Table 1.

Table 1: Initial physico-chemical properties of the experimental site

Particulars	Content
Texture	Sandy loam
Bulk density (Mg m ⁻³)	1.34
Aggregate stability (%)	52.53
MWHC (%)	32.60
Soil pH (1:2.5)	6.64
EC (dS m ⁻¹) (1:2.5)	0.21
Organic carbon (g kg ⁻¹)	0.40

Available nitrogen (kg ha ⁻¹)	261.37
Available phosphorus (P ₂ O ₅ kg ha ⁻¹)	35.84
Available potassium (K ₂ O kg ha ⁻¹)	210.26
Available sulphur (ppm)	15.82
Exchangeable calcium [cmol(p ⁺) kg ⁻¹]	4.52
Exchangeable magnesium [cmol(p ⁺) kg ⁻¹]	1.85
DTPA extractable iron (mg kg ⁻¹)	12.66
DTPA extractable copper (mg kg ⁻¹)	1.56
DTPA extractable manganese (mg kg ⁻¹)	4.91
DTPA extractable zinc (mg kg ⁻¹)	0.83
Hot water-soluble boron (mg kg ⁻¹)	0.33

Biochar used for the study

“Biochar is the C-rich solid product resulting from the heating of biomass in an oxygen-limited environment. Due to its highly aromatic structure, biochar is chemically and biologically more stable compared with the organic matter from which it is made. Generally, the properties of biochar vary widely, depending on the source of biomass used and the conditions of production of biochar” (Lehman and Joseph, 2009). The mulberry stalk generated as waste residue after leaf harvest in the farmer’s field is converted to biochar through pyrolysis by local method of farmers practice. The mulberry stalk biochar was ground to fine powder and made to pass through a sieve of 2 mm and used in the present investigation as a soil conditioner. “The biochar was characterised by various standardized analytical procedures for its specific physico-chemical properties such as bulk density, water holding capacity, pH, EC and total elementary composition” [43] (Table 2).

Table 2: Methods employed for the analysis of biochar

Sl. No	Parameter	Method	Reference
1.	pH	Potentiometry	Jackson (1973)
2.	EC (dS m ⁻¹)	Conductometry	Jackson (1973)

3.	MWHC (%)	Keen's cup method	Piper (2002)
4.	Bulk density (g/cc)	Keen's cup method	Piper (2002)
5.	Total carbon (%)	Dry combustion method (CHNS, LECO)	Page <i>et al.</i> 1982
6.	Nitrogen (%)	Kjeldahl digestion and distillation method	Jackson (1973)
7.	Phosphorus (%)	Diacid digestion and vanadomolybdate method	Jackson (1973)
8.	Potassium (%)	Diacid digestion and flame photometer method	Jackson (1973)
9.	Calcium (%)	Complexometric titration method	Jackson (1973)
10.	Magnesium (%)	Complexometric titration method	Jackson (1973)
11.	Sulphur (ppm)	0.15 % CaCl ₂ extraction and Turbidity	Black (1965)
12.	Iron (ppm)	Diacid digestion and Atomic Absorption Spectrophotometry	Lindsay and Norwell (1978)
13.	Copper (ppm)		
14.	Manganese (ppm)		
15.	Zinc (ppm)		
16.	Boron (ppm)	Diacid digestion and Azomethine-H method	Gupta (1967)

The physico-chemical characteristics of the mulberry stalk biochar used in this study are presented in the Table 3.

Table 3: Physico-chemical characteristics of mulberry stalk biochar

Parameters	Value
Bulk density (Mg m ⁻³)	0.32
WHC (%)	93.14
pH (1: 2.5)	8.53
EC (dS m ⁻¹) (1: 2.5)	0.39

Total carbon (%)	69.37
Nitrogen (%)	0.89
Phosphorus (%)	0.22
Potassium (%)	0.65
Calcium (%)	0.96
Magnesium (%)	0.48
Sulphur (%)	0.18
Iron (ppm)	493
Manganese (ppm)	94.1
Zinc (ppm)	34.59
Copper (ppm)	20.55
Boron (ppm)	33.5

Results and discussion

Leaf yield of mulberry

Application of FYM and different levels of biochar significantly influenced the leaf yield and the values ranged from 568.45 to 691.37 g plant⁻¹ and 10.45 to 13.07 t ha⁻¹ in pooled data (Table 4).

Combined application of biochar and FYM had profound influence on leaf yield of mulberry in all the three crops cuttings. The pooled mean data showed marked significant differences with respect to leaf yield and the highest leaf yield was being recorded in T₈ (13.07 t ha⁻¹) and the next best treatment was T₇ (12.61 t ha⁻¹) and increased by 21 and 20 % respectively, over the control. Treatments which received biochar @ 10, 7.5 and 5 t ha⁻¹ recorded significantly higher leaf yield of 12.13 (T₅) (12 and 16%, respectively), 11.83 (T₄) (8.9 and 13%, respectively) and 11.62 (T₃) (6.9 and 11%, respectively) t ha⁻¹ over treatments T₂ (10.86 t ha⁻¹) which received POP (FYM @ 25 t ha⁻¹ + NP₂O₅ K₂O 350:140:140 kg ha⁻¹) and T₁

(10.45 t ha⁻¹) which received NPK alone. Among different treatments, with increased level of biochar application increased the leaf yield.

“The treatment which received biochar @ 10 t ha⁻¹ + FYM @ 10 t ha⁻¹ recorded higher number of leaves and thereby higher leaf yield. Increased rate of biochar application increased leaf yield due to increased availability of nutrients” (Ahmed *et al.*, 2017 and Lamani *et al.*, 2024). This might be due to increase in rate of biochar which increases the moisture content and nutrient supply in soil. Increase in leaf yield with application of biochar can be attributed to increased CEC of soil, pH and base saturation, available P, nutrient retention and increased plant-available water and also due to better partitioning and migration of the total available photosynthates to economic yield. Such responses with application rates were reported by Major *et al.* (2010), Fasiha and Devakumar (2022) and Zwieten *et al.* (2010). “Addition of more nutrients through combination of biochar, FYM and inorganic fertilizers resulted in higher grain and stover yield. Many research workers have reported that biochar-induced yield increases in the sugarcane crop, rice and maize production” [Chen *et al.*, (2010), Ogawa and Okimori (2010)].

Table 4: Effect of mulberry stalk biochar on leaf yield of mulberry at different crop cutting seasons

Treatments	First crop cutting		Second crop cutting		Third crop cutting		Pooled mean	
	Leaf yield (g plant ⁻¹)	Leaf yield (t ha ⁻¹)	Leaf yield (g plant ⁻¹)	Leaf yield (t ha ⁻¹)	Leaf yield (g plant ⁻¹)	Leaf yield (t ha ⁻¹)	Leaf yield (g plant ⁻¹)	Leaf yield (t ha ⁻¹)
T ₁	367.73	6.99	612.18	11.34	725.43	13.01	568.45	10.45
T ₂	375.57	7.14	641.55	11.90	736.55	13.55	584.56	10.86
T ₃	394.59	7.31	699.08	12.95	789.41	14.62	627.69	11.62
T ₄	403.84	7.48	711.03	13.17	801.36	14.84	638.74	11.83
T ₅	416.79	7.72	728.72	13.49	819.05	15.17	654.85	12.13
T ₆	425.45	7.87	743.76	13.77	834.09	15.45	667.77	12.36
T ₇	433.21	8.09	757.85	14.03	848.18	15.71	679.75	12.61
T ₈	442.94	8.20	770.39	14.64	860.76	16.37	691.37	13.07
S.Em±	5.73	0.04	7.76	0.29	8.48	0.29	6.54	0.20
CD @ (5 %)	17.38	0.13	23.54	0.88	25.73	0.90	19.84	0.61

T ₁ : Control (NPK alone)	T ₅ : Soil application of biochar @ 10 t ha ⁻¹
T ₂ : POP (FYM @ 25 t ha ⁻¹ + NP ₂ O ₅ K ₂ O 350:140:140 kg ha ⁻¹)	T ₆ : Soil application of biochar @ 5 t ha ⁻¹ + FYM @ 10 t ha ⁻¹
T ₃ : Soil application of biochar @ 5 t ha ⁻¹	T ₇ : Soil application of biochar @ 7.5 t ha ⁻¹ + FYM @ 10 t ha ⁻¹
T ₄ : Soil application of biochar @ 7.5 t ha ⁻¹	T ₈ : Soil application of biochar @ 10 t ha ⁻¹ + FYM @ 10 t ha ⁻¹

Soil nutrient status after the harvest of mulberry crop

Primary nutrient

The nutrient status *i.e.*, available N, P₂O₅ and K₂O, of the soil after the harvest of mulberry was analysed and results are presented in Table 5.

Combined application of biochar and FYM had profound influence on primary nutrients status after harvest of mulberry in all the three crops cuttings (Table 5). In case of pooled data, significantly higher available nitrogen (294.78 kg ha⁻¹), phosphorus (47.22 kg ha⁻¹) and potassium (235.06 kg ha⁻¹) content was recorded in T₈ (soil application of biochar @ 10 t ha⁻¹ + FYM @ 10 t ha⁻¹) and on par with T₇ (N:291.59 kg ha⁻¹, P₂O₅:45.47 kg ha⁻¹ K₂O: 230.80 kg ha⁻¹) received soil application of biochar @ 7.5 t ha⁻¹ + FYM @ 10 t ha⁻¹. The lower available nutrient contents were recorded in T₁ (N: 261.80 kg ha⁻¹, P₂O₅: 35.24 kg ha⁻¹ K₂O: 209.46 kg ha⁻¹) which is devoid of biochar.

The higher status of soil available nitrogen under biochar applied with different doses of fertilizers were ascribed due to more release and additive effect of mineralization and their uptake, there by the higher yields under these treatments. Nitrogen retention could be improved as biochar provides micro habitat. Biochar improved the soil physical properties which might have altered the inorganic nitrogen pool. Biochar might also reduce the leaching losses due to its higher surface area.

Biochar application can reduce nutrient leaching from soil with resulting increase in fertilizer use efficiency. Various other studies indicated that addition of biochar recorded higher available nitrogen, phosphorus and potassium of soil by Widowati *et al.* (2012), Khan *et al.*, (2014) and Widowati & Asnah (2014). According to Venkatesh *et al.* (2018) observed application of pigeon pea biochar increased in soil available nitrogen 26 percent over the initial soil available nitrogen (109.4 kg ha⁻¹). Lehmann (2007) reported that biochar alters the N dynamics in soil. With biochar application to soil increased the availability and rate of mineralization of organic N which indicates the ability of biochar as a slow release N fertilizer by Chan and Xu (2013), Singh *et al.*, (2018) and Steiner *et al.* (2008).

The increase in soil phosphorus content as compared to initial was ascribed due to P content of biochar and also due to its effect on biotic activity. The favourable effect of biochar on phosphorus availability was also reported by Venkatesh *et al.* (2018), who reported that application of pigeon pea biochar increased 14 % availability of phosphorus over the initial soil (11.4 kg ha⁻¹). Abewa *et al.* (2014) reported that biochar application improves available phosphorus due to synergetic effect of biochar and fertilizer. Possible explanation for increased phosphorus content on biochar application includes some capacity

of the biochar to retain anions and also presence of soluble and exchangeable phosphate in biochar, it is a modifier of soil pH and ameliorator of P complexing metals (Al^{3+} , Fe^{3+}) and promoter of microbial activity and hastening P mineralization. Such increase in available P_2O_5 content with biochar addition was also reported by Laird *et al.*, (2010); Novak *et al.*, (2009); Parvage *et al.*, (2013) and Hass *et al.*, (2012). Chan *et al.* (2007) and Chan *et al.* (2008) also reported the increase in available phosphorus in soil after the application of biochar.

With increase in levels of biochar increased the potassium content of soil at harvest in post harvested soil which may be due to the high concentration of K found in the biochar (Chan *et al.*, 2007). The immediate beneficial effects of biochar additions on nutrient availability are largely due to higher potassium (Lehmann *et al.*, 2003). The biochar contains high ash and itself has more amount of potassium content compared to other major nutrients, so by the application of ash rich biochar to soils increased the potassium content significantly.

Table 5: Effect of mulberry stalk biochar on major nutrients of soil at different crop cutting seasons

Treatments	First crop			Second crop			Third crop			Pooled mean		
	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O
	(Kg ha ⁻¹)											
T ₁	266.95	36.49	212.13	261.84	35.04	208.93	256.62	34.20	206.32	261.80	35.24	209.46
T ₂	270.37	37.39	213.26	265.26	36.60	210.06	260.37	35.19	208.45	265.33	36.39	210.59
T ₃	279.31	40.31	221.26	274.40	39.38	218.06	268.97	38.01	216.12	274.23	39.23	218.48
T ₄	283.39	42.22	224.17	278.27	41.75	220.90	273.06	39.92	218.96	278.24	41.30	221.34
T ₅	287.57	43.20	226.41	282.49	42.98	223.14	277.24	40.84	221.20	282.43	42.34	223.58
T ₆	291.42	46.17	230.26	286.09	44.24	226.99	280.75	42.18	225.05	286.09	44.20	227.44
T ₇	296.77	47.56	233.63	291.58	45.19	230.36	286.43	43.66	228.42	291.59	45.47	230.80
T ₈	299.92	49.13	237.98	294.83	46.76	234.58	289.59	44.88	232.64	294.78	47.22	235.06
S.Em±	2.74	0.94	2.37	2.86	0.80	2.37	2.80	0.87	2.42	2.80	0.78	2.32
CD@ (5 %)	8.33	2.86	7.20	8.68	2.45	7.19	8.50	2.64	7.43	8.49	2.36	7.04

T ₁ : Control (NPK alone)	T ₅ : Soil application of biochar @ 10 t ha ⁻¹
T ₂ : POP (FYM @ 25 t ha ⁻¹ + NP ₂ O ₅ K ₂ O 350:140:140 kg ha ⁻¹)	T ₆ : Soil application of biochar @ 5 t ha ⁻¹ + FYM @ 10 t ha ⁻¹
T ₃ : Soil application of biochar @ 5 t ha ⁻¹	T ₇ : Soil application of biochar @ 7.5 t ha ⁻¹ + FYM @ 10 t ha ⁻¹
T ₄ : Soil application of biochar @ 7.5 t ha ⁻¹	T ₈ : Soil application of biochar @ 10 t ha ⁻¹ + FYM @ 10 t ha ⁻¹

Secondary nutrients

Application of biochar at different quantities significantly influenced the exchangeable Ca status of soil (Table 6). In case of pooled data, highest exchangeable calcium status in the soil [5.85 cmol (p+) kg⁻¹] was recorded in T₈ treatment with soil application of biochar @ 10 t ha⁻¹ + FYM @ 10 t ha⁻¹ followed by T₇ treatment which received biochar @ 7.5 t ha⁻¹ + FYM @ 10 t ha⁻¹ [5.68 cmol (p+) kg⁻¹]. Lowest exchangeable calcium status of soil [4.71 cmol (p+) kg⁻¹] was recorded in control (T₁).

Significant differences were found between treatments with respect to exchangeable magnesium status. Significantly higher [2.77 cmol (p+) kg⁻¹] exchangeable magnesium was recorded in soil application of biochar @ 10 t ha⁻¹ + FYM @ 10 t ha⁻¹ (T₈) treatment, whereas significantly lower magnesium value of 1.87 cmol (p+) kg⁻¹ was found in T₁ treatment (control).

Application of biochar at different quantities significantly influenced the available sulphur status of soil (Table 5). The available sulphur status in soil showed significant difference among the treatments. However, the highest available sulphur status (21.97 mg kg⁻¹) was found with application of biochar @ 10 t ha⁻¹ + FYM @ 10 t ha⁻¹ (T₈). The lower sulphur status (17.35 mg kg⁻¹) was found in control (T₁). Combined application of biochar and FYM had profound influence on secondary nutrients status after harvest of mulberry in all the three crops cuttings.

Significant effect of application of different levels of biochar on secondary nutrients in soil was observed. Exchangeable bases such as Ca and Mg status in soil varied significantly with application of varied levels of biochar combined with FYM due its high cation exchange capacity. Increase in exchangeable bases in soil can be attributed to release of basic cations from biochar. Most of the Ca, Mg, K, P and plant micronutrients in feedstock are partitioned into the biochar ash fraction during pyrolysis. Ash in biochar rapidly releases free bases such as Ca, Mg and K to the soil solution thereby not only increases soil pH but also exchangeable bases. Such observations were also noticed by Lehmann *et al.* (2003) and Chan *et al.* (2008).

Sulphur status in soil varied significantly with application of different levels of biochar with FYM. This may be due the contribution of available sulphur to soil after the mineralization of organic sulphur in biochar and also due to application of FYM. The results suggest that biochar also improves the bioavailability of sulphur; which mainly depends on mineralization of organic forms of sulphur in soil (De Luca *et al.*, 2009, Ranjita bai *et al.*, 2023 and Faruque *et al.*, 2017).

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Table 6: Effect of mulberry stalk biochar on secondary nutrients of soil at different crop cutting seasons

Treatments	First crop			Second crop			Third crop			Pooled mean		
	Ca	Mg	S	Ca	Mg	S	Ca	Mg	S	Ca	Mg	S
	[cmol (p ⁺) kg ⁻¹]		(mg kg ⁻¹)	[cmol (p ⁺) kg ⁻¹]		(mg kg ⁻¹)	[cmol (p ⁺) kg ⁻¹]		(mg kg ⁻¹)	[cmol(p ⁺) kg ⁻¹]		(mg kg ⁻¹)
T ₁	4.88	1.93	17.70	4.70	1.87	17.32	4.53	1.83	17.03	4.71	1.87	17.35
T ₂	4.92	2.10	18.29	4.74	1.98	17.91	4.62	1.92	17.62	4.76	2.00	17.94
T ₃	5.28	2.35	19.68	5.09	2.22	19.30	4.96	2.12	19.01	5.11	2.23	19.33
T ₄	5.37	2.46	20.17	5.14	2.37	19.78	5.06	2.25	19.50	5.19	2.36	19.82
T ₅	5.49	2.54	20.68	5.31	2.45	20.29	5.16	2.35	20.01	5.32	2.45	20.33
T ₆	5.63	2.65	21.17	5.51	2.53	20.78	5.42	2.43	20.50	5.52	2.54	20.82
T ₇	5.80	2.74	21.47	5.68	2.61	21.08	5.56	2.54	20.80	5.68	2.63	21.12
T ₈	5.96	2.88	22.25	5.83	2.77	21.97	5.76	2.65	21.69	5.85	2.77	21.97
S.Em±	0.10	0.07	0.34	0.09	0.07	0.36	0.11	0.06	0.35	0.10	0.07	0.35
CD@ (5 %)	0.32	0.22	1.04	0.30	0.21	1.11	0.33	0.19	1.07	0.31	0.21	1.07

T ₁ : Control (NPK alone)	T ₅ : Soil application of biochar @ 10 t ha ⁻¹
T ₂ : POP (FYM @ 25 t ha ⁻¹ + NP ₂ O ₅ K ₂ O 350:140:140 kg ha ⁻¹)	T ₆ : Soil application of biochar @ 5 t ha ⁻¹ + FYM @ 10 t ha ⁻¹
T ₃ : Soil application of biochar @ 5 t ha ⁻¹	T ₇ : Soil application of biochar @ 7.5 t ha ⁻¹ + FYM @ 10 t ha ⁻¹
T ₄ : Soil application of biochar @ 7.5 t ha ⁻¹	T ₈ : Soil application of biochar @ 10 t ha ⁻¹ + FYM @ 10 t ha ⁻¹

Micronutrients

A significant difference was found with respect to micronutrients like Fe, Zn, Mn, Cu and B in soil after harvest of mulberry crop due to imposition of different treatments (Table 7 and 8).

Combined application of biochar and FYM had profound influence on micronutrient status after harvest of mulberry in all the three crops cuttings. In case of pooled data, significantly higher Fe (16.34 mg kg⁻¹), Zn (1.00 mg kg⁻¹), Mn (6.13 mg kg⁻¹), Cu (1.71 mg kg⁻¹) and B (0.44 mg kg⁻¹) content was recorded in T₈ (soil application of biochar @ 10 t ha⁻¹ + FYM @ 10 t ha⁻¹) and on par with T₇ (Fe: 15.99 mg kg⁻¹, Zn: 0.98 mg kg⁻¹, Mn: 6.00 mg kg⁻¹, Cu: 1.69 mg kg⁻¹ and B: 0.42 mg kg⁻¹) received soil application of biochar @ 7.5 t ha⁻¹ + FYM @ 10 t ha⁻¹. The lower micronutrient contents were recorded in T₁ (Fe: 12.88 mg kg⁻¹, Zn: 0.82 mg kg⁻¹, Mn: 5.04 mg kg⁻¹, Cu: 1.59 mg kg⁻¹ and B: 0.31 mg kg⁻¹) which is devoid of biochar. It was noticed that micronutrient status of soil increased with increased levels of biochar and FYM. Increase in available micronutrient status in soil treated with biochar and FYM might also be due to enhanced solubilisation of native minerals. As pH of soil and micronutrients availability was negatively correlated, immobilization of micronutrient occurs in soil. But the micronutrient status in soil increased with the application of biochar in combination with FYM. This may be due to the mineralization of micronutrients from organic matter and the release of micronutrients during decomposition of organic manures. Increase in the status of micronutrients at harvest of crop might be due to higher availability of the plant nutrients from the soil nutrient reservoir and additional quantity of nutrients supplied through farm yard manure (Sharma and Dixit, 1987).

The higher content of available Fe was found in the treatment which received biochar @ 7.5 t ha⁻¹ this might be due good retention power of biochar which retained large amount of Fe in soil (Amonette and Joseph, 2009) and (Shankar *et al.*, 2012).

Prasanna (2012) found that significantly highest zinc content in soil was recorded with the application of biochar @ 2 t ha⁻¹ + RDF + FYM @ 10 t ha⁻¹, followed by the treatment receiving biochar @ 1 t ha⁻¹ + RDF + FYM @ 10 t ha⁻¹. The increased available zinc status in soil under different doses of biochar in combination with FYM application might be due to the addition of organics. The organic materials form chelates and increase the availability of zinc.

The available manganese status might be increased because of enhanced solubilisation of manganese due to reduction potential of manganese and non-complexation by organic

ligands. Lentz and Ippolito, 2011 supported the significant increment in available manganese on biochar addition as biochar acts as a source of manganese.

Slight increase in copper status with the addition of biochar and FYM might be due to high extractability of micronutrients and Cu is strongly chelated by organic carbon and is less subjected to adsorption process. Similar observations were made by Bandara *et al.* (2017). There was an increase in Cu status with biochar application. Application of biochar might have increased the soluble organic carbon; thereby resulting in the mobilization of Cu. Beesley and Marmioli (2011) also reported dependence of Cu content on soluble carbon and pH.

Boron content in the biochar amended plots was higher than in the control treatment which was probably due to the lower absorption of boron by the plants under the conditions of increasing soil pH. A similar relationship was found by Hu and Brown (1997), according to whom the availability of boron to plants decreases with increasing soil pH and therefore its soil content increases.

Table 7: Effect of mulberry stalk biochar on DTPA extractable iron, zinc and manganese of soil at different crop cutting seasons

Treatments	First crop			Second crop			Third crop			Pooled mean		
	Fe	Zn	Mn	Fe	Zn	Mn	Fe	Zn	Mn	Fe	Zn	Mn
	mg kg ⁻¹											
T ₁	13.02	0.87	5.12	12.87	0.81	5.03	12.74	0.79	4.97	12.88	0.82	5.04
T ₂	15.25	0.98	5.71	15.10	0.93	5.68	14.97	0.91	5.51	15.11	0.94	5.63
T ₃	15.15	0.96	5.65	15.00	0.90	5.52	14.87	0.88	5.44	15.01	0.91	5.54
T ₄	14.82	0.94	5.42	14.67	0.88	5.35	14.54	0.86	5.23	14.68	0.89	5.33
T ₅	14.26	0.93	5.39	14.11	0.85	5.27	13.98	0.83	5.11	14.12	0.87	5.26
T ₆	15.92	1.00	5.95	15.77	0.95	5.81	15.52	0.93	5.73	15.74	0.96	5.83
T ₇	16.13	1.02	6.14	15.98	0.97	5.99	15.85	0.95	5.86	15.99	0.98	6.00
T ₈	16.48	1.05	6.25	16.33	0.99	6.11	16.20	0.97	6.04	16.34	1.00	6.13
S.Em±	0.40	0.01	0.08	0.37	0.01	0.07	0.35	0.01	0.09	0.36	0.01	0.08
CD @ (5 %)	1.21	0.04	0.26	1.13	0.03	0.23	1.07	0.03	0.30	1.10	0.03	0.26

T ₁ : Control (NPK alone)	T ₅ : Soil application of biochar @ 10 t ha ⁻¹
T ₂ : POP (FYM @ 25 t ha ⁻¹ + NP ₂ O ₅ K ₂ O 350:140:140 kg ha ⁻¹)	T ₆ : Soil application of biochar @ 5 t ha ⁻¹ + FYM @ 10 t ha ⁻¹
T ₃ : Soil application of biochar @ 5 t ha ⁻¹	T ₇ : Soil application of biochar @ 7.5 t ha ⁻¹ + FYM @ 10 t ha ⁻¹
T ₄ : Soil application of biochar @ 7.5 t ha ⁻¹	T ₈ : Soil application of biochar @ 10 t ha ⁻¹ + FYM @ 10 t ha ⁻¹

Table 8: Effect of mulberry stalk biochar on copper and boron content of soil at different crop cutting seasons

Treatments	First crop		Second crop		Third crop		Pooled mean	
	Cu	B	Cu	B	Cu	B	Cu	B
	mg kg ⁻¹							
T ₁	1.60	0.32	1.59	0.31	1.57	0.30	1.59	0.31
T ₂	1.70	0.40	1.66	0.38	1.62	0.37	1.66	0.38
T ₃	1.68	0.39	1.67	0.39	1.61	0.35	1.65	0.37
T ₄	1.65	0.37	1.63	0.34	1.60	0.32	1.63	0.34
T ₅	1.63	0.35	1.60	0.32	1.59	0.31	1.61	0.33
T ₆	1.72	0.44	1.67	0.39	1.63	0.38	1.67	0.40
T ₇	1.74	0.46	1.69	0.41	1.65	0.40	1.69	0.42
T ₈	1.76	0.48	1.71	0.43	1.67	0.42	1.71	0.44
S.Em±	0.009	0.01	0.01	0.009	0.01	0.01	0.008	0.009
CD@ (5 %)	0.02	0.03	0.03	0.02	0.02	0.03	0.02	0.02

T ₁ : Control (NPK alone)	T ₅ : Soil application of biochar @ 10 t ha ⁻¹
T ₂ : POP (FYM @ 25 t ha ⁻¹ + NP ₂ O ₅ K ₂ O 350:140:140 kg ha ⁻¹)	T ₆ : Soil application of biochar @ 5 t ha ⁻¹ + FYM @ 10 t ha ⁻¹
T ₃ : Soil application of biochar @ 5 t ha ⁻¹	T ₇ : Soil application of biochar @ 7.5 t ha ⁻¹ + FYM @ 10 t ha ⁻¹
T ₄ : Soil application of biochar @ 7.5 t ha ⁻¹	T ₈ : Soil application of biochar @ 10 t ha ⁻¹ + FYM @ 10 t ha ⁻¹

Conclusion

Biochar, a high carbon solid made from mulberry stalks, can be suited to the crop, soil type and management system to maximise the benefit. Soil application of biochar and FYM have more stimulating effect on the soil nutrient status. Application of FYM, and different levels of biochar significantly influenced the leaf yield and the values ranged from 10.45 to 13.07 t ha⁻¹ in pooled data. Among all the treatments imposed, soil application of biochar @ 10 t ha⁻¹ + FYM @ 10 t ha⁻¹ recorded higher soil nutrient status compared to control. From the present investigation, it is observed that combined application of biochar and FYM has significantly increased the crop productivity and improved the nutrients status in the soil.

References

1. ABEWA, A., YITAFERU, B., SELASSIE, Y. G. AND AMARE, T., 2014, The Role of Biochar on Acid Soil Reclamation and Yield of Teff (*Eragrostis tef*) in Northwestern Ethiopia. *J. Agri. Sci.*, **6**(1): 1-12.
2. AHMED, F., ISLAM, M. S. AND IQBAL, M. T., 2017, Biochar amendment improves soil fertility and productivity of mulberry plant. *Eurasian J. Soil Sci.*, **6**(3): 226-237.
3. AMONETTE, J. E. AND JOSEPH, S., 2009, Characteristics of biochar: micro chemical properties. Chapter 3: In: Lehmann J, Joseph S (Eds.) Biochar for environmental management science and technology. *Earth scan, London*, pp: 33-52.
4. ATKINSON, C. J., FITZGERALD, J. D. AND HIPPS, N. A., 2010, Potential mechanism for achieving agricultural benefits from biochar application to temperate soils: A review. *Plant and soil*. **337**: 1-18.
5. BANDARA, T., INDIKA, H., PRASANNA, K., MIHIRI, S., GAMINI, S., NISHANTA, R., VITHANAGE, M. AND YONG, S., 2017, Role of woody biochar and fungal- bacterial co-inoculation on enzyme activity and metal immobilization in serpentine soil. *J. Soils Sediments*, **17**(3): 665-673.
6. BEESLEY, L. AND MARMIROLI, M., 2011, The immobilization and retention of soluble arsenic, copper, cadmium and zinc by biochar. *Environ. Pollut.*, **159**: 474-480.
7. CHAN, K. Y. AND XU, Z., 2013, Biochar: nutrient properties and their enhancement. *Biochar environ. Manag. Sci. Technol.*, pp: 67-84.
8. CHAN, K.Y., VAN ZWIETEN, L., MESZAROS, I., DOWINE, A. AND JOSEPH, S., 2007, Agronomic values of green waste biochar as a soil amendment. *Australian J. Soil Res.*, **45**(8): 629–634.

9. CHAN, K.Y., VAN ZWITETEN, L., MESZAROS, I., DOWNIE, A. AND JOSEPH, S., 2008, Using poultry litter biochars as soil amendments. *Aust. J. Soil Res.* **46**:437–444.
10. CHEN, Y., SHINOGI, Y. AND TAIRA, M., 2010, Influence of biochar use on sugarcane growth, soil parameters and ground quality. *Aust. J. Soil Res.* **48**: 526-530.
11. DE LUCA, T. H., MACKENZIE, M. D. AND GUNDALE M. J., 2009, Biochar effects on soil nutrient transformations. Biochar for environmental management science and technology. *Earthscan, London*, pp:72-87.
12. FASIHA AND DEVAKUMAR, A. S., 2022, Characterization of corncob biochar produced through the gasification process for application as soil amendment. *Mysore J. Agric. Sci.*, **56**(1): 100-107.
13. FARUQUE, A., ISLAM, M. S. AND IQBAL, M. T., 2017, Biochar amendment improves soil fertility and productivity of mulberry plant. *Eurasian Journal of Soil Science*, **6**(3), 226-237.
14. HASS, A., JAVIER, M. G., ISABEL M. L., HARRY, W. G., JONATHAN J. H. AND DOUGLAS G. B., 2012, Chicken manure biochar as liming and nutrient source for acid appalachian soil. *J. Environ. Qual.*, **41**(4): 1096-1106.
15. HU, H. N. AND BROWN, P. H., 1997, Absorption of boron by plant roots. *Plant and Soil*, **193**: 49–58.
16. JOHNSTON, A. E., POULTON, P. R. AND COLEMAN, K., 2009, Soil organic matter: its importance in sustainable agriculture and carbon dioxide fluxes. *Advances in agronomy*, **101**: 1-57
17. KHAN, K. T., CHOWDHURY, M. T. A AND HUQ, S. I., 2014, Application of biochar and fate of soil nutrients. *Bangladesh J. Sci. Res.*, **27**(1): 11-25.
18. LAIRD, D. A., FLEMING, P., DAVIS, D. D., ROBERT, H., WANG, B. AND KARLEN, D. L., 2010, Impact of biochar amendments on the quality of a typical midwestern agricultural soil. *Geoderma*, **158**: 443-449.
19. LAMANI, H. D., PRAKASHA, H. C, NAVEEN, D. V., KALYANA MURTHY, K. N. AND RINKU VERMA, 2024, Effect of Mulberry Stalk Biochar, FYM and Humic Acid on Growth, Yield and Quality of Mulberry (*Morus alba* L.). *Int J. Plant and Soil Sci.*, **36**(4):174-183.
20. LEHMANN, J. AND JOSEPH. S., 2009, Biochar for Environmental Management. *Biochar Environ. Manag.Sci. Technol.*, 907p.

21. LEHMANN, J., 2007, A handfull of carbon. *Nature*, **447**: 143-144.
22. LEHMANN, J., KERN, D. C., GERMAN, L. A., MCCAN, J., MARTINS, G. C., MOREIRA, A., 2003. Soil fertility and production potential. *The Netherlands*. pp: 105–124
23. LENTZ, R. D. AND IPPOLITO, J. A., 2011, Biochar and manure affect calcareous soil and corn silage nutrient concentrations and uptake. *J. Environ. Qual.*, **41**: 1033 – 1043.
24. MAHESH, D. S., MUTHURAJU, R., VIDYASHREE, D. N., NARAYANASWAMY, T. K. AND SUBBARAYAPPA, C. T., 2020, Effect of Silkworm Pupal Residue Protein (SPRP) and Silkworm Pupal Residue Extract (SPRE) Spray on Major and Micronutrients Status of Mulberry. *Ind. J. Pure App. Biosci*, **8**(1): 110-114.
25. MAJOR, J., RONDON, M., MOLINA, D., RIHA, S. J. AND LEHMANN, J., 2010, Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. *Plant Soil*, **333**:117–128.
26. NOVAK, J. M., BUSSCHER, W. J., LAIRD, D. L., AHMEDNA, M., WATTS, D. W. AND NIANDOU, M. A. S., 2009, Impact of biochar amendment on fertility of a southeastern coastal plain soil. *Soil Sci.*, **174**: 105-112.
27. OGAWA, M. AND OKIMORI, Y., 2010, Pioneering works in biochar research, Japan, *Aust. J. Soil Res.*,**48**: 489–500.
28. PARVAGE, M. M., BARBRO, U., ERIKSSON, J., JEFFERY, S. AND HOLGER, K., 2013, Phosphorus availability in soils amended with wheat residue char. *Biol. Fert. Soils*, **49**(2): 245-250.
29. PRASANNA, K. S., 2012, Studies on the impact of biochar on nutrient dynamics in soil, growth and yield of maize. *M.Sc. (Agri.) Thesis*, Univ. Agric. Sci., Bengaluru.
30. SHANKAR, K., SREEDEVI, S., SUMATHI, M., SHANKAR AND REDDY, N. N., 2012, Comparison of nutritional quality of organically versus conventionally grown tomato. *Indian J. of Hort.*, **69** (1): 86-90.
31. SHARMA, R.A. AND DIXIT, B. K., 1987, Effect of nutrient application on rainfed soybean. *J. Indian Soc. Soil Sci.*, **35**: 452-455.
32. SINGH, C., TIWARI, S., GUPTA, V. K AND SINGH, J. S., 2018, The effect of rice husk biochar on soil nutrient status, microbial biomass and paddy productivity of nutrient poor agriculture soils. *Catena*, **171**: 485-493.

33. SOHI, S., LOEZ-CAPEL, E., KRULL, E. AND BOL, R., 2010, Biochar's roles in soil and climate change. A review of research needs. CSIRO and Water Science, Report 5/9: 64.
34. STEINER, C. GLASER, B. TEIXEIRA, W. G., LEHMANN, J. AND BLUM, W. Z. W., 2008, Nitrogen retention and plant uptake on a highly weathered central Amazonian Ferralsol amended with compost and charcoal. *J. Plant Nutr. Soil Sci.*, **171**(6):893–899.
35. STEINER, C., 2010. Biochar in Agricultural and Forestry Application. In: Biochar from agricultural and forestry residues – A complimentary use of “Waste” Biomass. U. S. – Focused Biochar Report: Assessment of Biochar's Benefit for the United States of America, Colorado, USA, pp. 7.
36. STEINER, C., TEIXEIRA, W. G., LEHMANN, J., NEHLS, T., MACEDO, J. L. V. AND BLUM, W. E. H., 2007, Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. *Plant and Soil*, **291**:275–90.
37. RANJITHA BAI, H., GOWDA, R., GOWDA, M., NAIKA, R., KADALLI, G. G. and ARUNKUMAR., B. R., 2023, Effect of levels of mulberry shoots biochar, Farm yard manure and NPK fertilizer on soil properties under tree mulberry garden.
38. ROHELA, G. K., SHUKLA, P., KUMAR, R. AND CHOWDHURY, S. R., 2020, Mulberry (*Morus* spp.): An ideal plant for sustainable development. *Trees, Forests and People*, **2**, 100011.
39. VENKATESH, G. SAMMI REDDY, K., GOPINATH, K. A., VISHA KUMARI, V. AND CHARY, R. G., 2018, Biochar for enhancing the soil, water and crop productivity in rainfed areas. *Rainwater Management for Climate Resilient Agriculture in Drylands*: 496-523.
40. WIDOWATI., UTOMO, W. H., GURITNO, B. AND SOEHONO, L. A., 2012, The effect of biochar on the growth and N fertilizer requirement of Maize (*Zea mays* L.) in green house experiment. *J. Agric. Sci.*, **4**(5): 255-262.
41. WIDOWATIL, W. AND ASNAH, A., 2014, Biochar can enhance potassium fertilization efficiency and economic feasibility of maize cultivation. *J. Agric. Sci.* **6**(2): 24-36.
42. ZWIETEN, V. L., KIMBER, S., DOWNIE, A., MORRI, S., PETTY, S., RUST, J. AND CHAN, K.Y., 2010, A glass house study on the interaction of low mineral ash biochar with nitrogen in a sandy soil. *Aust. J. Soil Res.*, **48**:569-576.

43. Nandini R, Prakasha HC, Subbarayappa CT, Kadalli GG, Anand MR, Umashankar N. Impact of recycling of mulberry stalk as Biochar for improving soil condition of mulberry cultivated soil. The Pharma Innovation Journal. 2022:501-7.

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