

Recycling of mulberry stalk as biochar and its effect on uptake of nutrients by mulberry

Abstract

A field experiment was conducted in the mulberry crop to know the effect of soil application of mulberry stalk biochar on nutrients uptake by mulberry at farmer's field at Sidlaghatta (Tq), Chikkabalapura District. A randomized block design was employed with eight treatments and three replications. A randomized block design was employed with eight treatments replicated thrice. Results revealed that, Combined soil application of biochar @ 10 t ha⁻¹ and FYM @ 10 t ha⁻¹ (T₈) recorded higher leaf yield per hectare (13.07 t ha⁻¹) and it was on par with T₇ (12.61 t ha⁻¹) which received soil application of biochar @ 7.5 t ha⁻¹ and FYM @ 10 t ha⁻¹. The lowest leaf yield of 10.45 t ha⁻¹ was recorded in control which was devoid of biochar. Among different treatments, significantly higher uptake of primary nutrients (Nitrogen, phosphorus and potassium of 90.68, 9.07 and 47.10 kg ha⁻¹, respectively) secondary nutrients (calcium, magnesium and sulphur of 44.48, 17.26 and 9.75 kg ha⁻¹, respectively) and micronutrients (iron, manganese, zinc, copper and boron of 748.20, 153.22, 139.34, 60.92 and 54.25 g ha⁻¹, respectively) by mulberry were recorded in the treatment (T₈) which received soil application of biochar @ 10 t ha⁻¹ + FYM @ 10 t ha⁻¹ and followed by T₇ which received biochar @ 7.5 t ha⁻¹ + FYM @ 10 t ha⁻¹ and found superior over other treatments. The control with no biochar application recorded lower nutrients uptake by mulberry crop. The findings revealed that utilization of mulberry stalk as a biochar has positive effect on nutrient uptake by mulberry crop and it could partly replace chemical fertilizers and promote organic farming in a circular economy concept.

Key words: Mulberry, leaf yield, biochar, nutrient uptake

Introduction

Mulberry, the sole nourishment plant for silkworm, *Bombyx mori* L. plays a vital role in the growth and development of silkworm and in turn the silk production. Leaf quality and quantity not just impact the silkworm growth and development but also impact the cocoon production and quality of raw silk. It is grown under varied climatic conditions ranging from temperate to tropics. 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.

Imprudently disposed and burning of a huge amount of agricultural waste including crop residues and animal manure is devastating our environment by emitting gases like carbon dioxide (CO₂) and methane (CH₄). Recycling and value-added utilization of agricultural residues through combining technologies such as anaerobic digestion and pyrolysis could double the recoverable energy, close the nutrient recycle loop and ensure cleaner agricultural production. The conversion of waste biomass into biochar could help to mitigate CO₂ emissions, reduce the generation of CH₄ emissions, and increase the carbon sequestration in the soil for sustainable climate-smart agriculture.

“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. 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 has a high surface area and a porous structure, which increases its CEC. CEC refers to the soil's ability to retain and exchange positively charged ions (cations) such as calcium (Ca²⁺), magnesium (Mg²⁺), potassium (K⁺), and ammonium (NH₄⁺), which are essential nutrients for plant growth. By increasing CEC, biochar can retain nutrients in the soil, preventing leaching, and making them more available to plants” (Lehmann & Joseph, 2009). The porous structure of biochar allows it to hold water like a sponge, which can improve soil water retention. This can be particularly beneficial in sandy soils or in regions with limited

water availability. By holding onto moisture, biochar creates a more favorable environment for root growth and nutrient uptake (Jeffery *et al.*, 2011).

Biochar can serve as a habitat for beneficial soil microbes. These microbes play crucial roles in nutrient cycling and can help break down organic matter into forms that are more readily available to plants. By enhancing microbial activity, biochar indirectly promotes nutrient availability and uptake by plants (Winsley, 2007). Biochar can help reduce nutrient losses through leaching and volatilization by adsorbing nutrients and holding them in the root zone. This prevents them from being washed away by rainfall or lost to the atmosphere, thereby increasing their availability for plant uptake (Glaser *et al.*, 2002). In view of this, the current study was undertaken to assess the impact of biochar on productivity and nutrient uptake by mulberry crop.

Material and methods

The experiment was carried on 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 13° 36' North latitude 77° 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 test crop was mulberry. Total 3 crop cutting were taken. The treatment details are given below

T₁: Control (NPK 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 ($\text{P}_2\text{O}_5 \text{ kg ha}^{-1}$)	35.84
Available potassium ($\text{K}_2\text{O kg ha}^{-1}$)	210.26
Available sulphur (ppm)	15.82
Exchangeable calcium [$\text{cmol}(\text{p}^+) \text{ kg}^{-1}$]	4.52
Exchangeable magnesium [$\text{cmol}(\text{p}^+) \text{ kg}^{-1}$]	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 biochar used for the study was obtained from mulberry stalk and after the production, the biochar was ground and

sieved through a 2mm sieve. The physico-chemical characteristics of the mulberry stalk biochar used in this study are presented in the Table 2.

Table 2: 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

Table 3: Methods employed for the analysis of plant samples

1	Total nitrogen	Micro Kjeldahl method	Tandon (1998)
2	Total phosphorus	Vandomolybdo phosphoric yellow colour method	
3	Total potassium	Flame photometer method	
4	Total calcium and magnesium	Complexometric titration	Piper (2002)
5	Total sulphur	Turbidimetric method	Tandon (1998)
6	Micronutrients (Fe, Mn, Cu and Zn)	Atomic absorption spectrophotometer method	Page <i>et al.</i> (1982)
7	Boron	Azomethine- H method	Jones and Case (1990)

Studies on nutrient uptake by mulberry

Nutrient contents in leaf were analysed as per the standard procedures [Black (1965), Jackson (1973), Lindsay and Norwell, (1978)] and ultimately the nutrient uptake was calculated by using the formula,

$$\text{Nutrient uptake (kg ha}^{-1}\text{)} = \frac{\text{Nutrient concentration (\%)} \times \text{biomass (kg ha}^{-1}\text{)}}{100}$$

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 and Fig. 1).

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) while the lower values was recorded in control (10.45 t ha⁻¹). Treatments which received biochar @ 10, 7.5 and 5 t ha⁻¹ recorded significantly higher leaf yield of 12.13 (T₅), 11.83 (T₄) and 11.62 (T₃) t ha⁻¹ compared to 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. 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), Zwieten *et al.* (2010) and Fasiha and Devakumar (2022) and 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

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

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

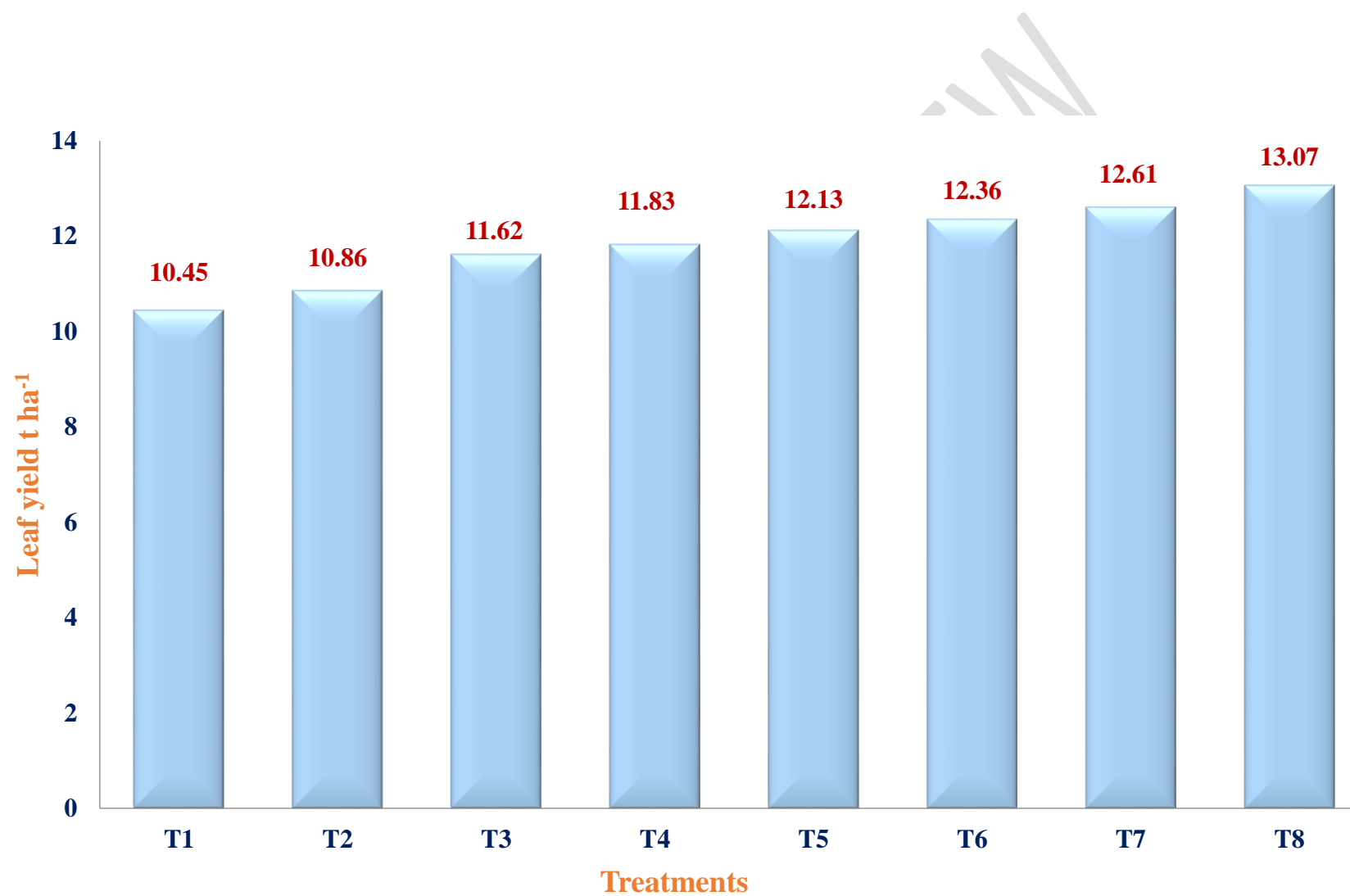


Fig. 1: Effect of mulberry stalk biochar on leaf yield of mulberry (pooled mean)

Uptake of nutrients

Primary nutrients uptake (kg ha⁻¹)

Combined application of biochar and FYM had profound influence on uptake of nitrogen, phosphorus and potassium by mulberry in all the three crops cuttings (Table 5 and Fig. 2).

The pooled mean data showed marked significant differences with respect to uptake of nitrogen, phosphorus and potassium and the highest uptake of nitrogen, phosphorus and potassium being recorded in T₈ (90.68, 9.07 and 47.10 kg ha⁻¹) and the next best treatment was T₇ (87.40, 8.78 and 45.45 kg ha⁻¹, respectively) while the lower values were recorded in control (66.17, 5.66 and 31.99 kg ha⁻¹, respectively).

The increase in N uptake due to application of biochar and FYM was due to increase in leaf yield, effective root system and increased concentration of nutrients in soil solution as well as better soil physical environment coupled with sufficiency of water and nutrients helped in better uptake of water and nutrients. This clearly indicated that biochar application helped to absorb nitrogen efficiently high. Major *et al.* (2010) also supported that total nutrient uptake by the maize crop increased with the application of biochar. Similarly, Nigussie *et al.* (2012) found that uptake of N by plants was increased with biochar application.

The changes in uptake were similar to the changes in the leaf yield. Inclusion of organic materials such as biochar found to bring about changes in nutrient availability and its uptake. Biochar being a component of soil organic carbon with high CEC and organic carbon content provides information on the nutrient fixation and release through ion exchange reaction besides acting as a nutrient reservoir and soil conditioner. This is in line with the findings of Novak *et al.* (2009) and Hossain *et al.* (2010) who found that biochar application had resulted in increased nutrient availability in soils and increased nutrient uptake in plants and also Uzoma *et al.* (2011) reported that nutrient uptake by maize grain was significantly increased with higher biochar applications.

Addition of biochar to soil has shown definite increases in the availability of major cations and phosphorus as well as in total nitrogen concentrations. Glaser *et al.* (2002), Lehmann *et al.* (2003) and Van Zwieten *et al.* (2010) reported “similar effect of biochar on N uptake in which it was observed that application of biochar significantly increased uptake of plant nitrogen”.

Biochar with different doses and in combination with FYM showed highest value as compared to respective RDF. Thus, the data revealed that, the application of biochar with FYM was better than the fertilizer application alone and increased the uptake of phosphorus from soil. The improvement in soil physical condition caused due to addition of organics is beneficial

for enhanced uptake. The organics also help in enhancing nutrients available in soil by reducing fixation of phosphorus, which improves the efficient use of added phosphorus.

In the present study biochar application increased nutrient uptake in mulberry over control, which could be due to the fact that biochar can capture high amounts of exchange cations (Lehmann *et al.*, 2003) because of its high porosity and surface/volume ratio and can improve plant nutrients and uptake. Biochar addition had positive effects on plant phosphorus nutrition. Atkinson *et al.* (2010) reviewed several mechanisms which can enhance availability and plant uptake of P after biochar addition to soil. It acts as source of soluble phosphorus salts and exchangeable phosphorus forms, avoids phosphorus precipitation by modifying soil pH (bonding or sorbing elements which precipitate phosphorus) or enhance microbial activity leading to changes in phosphorus availability. Similarly, Nigussie *et al.* (2012) found that uptake of phosphorus by plants were increased with biochar application. Xu *et al.* (2014) also reported that application of rice straw biochar observed a cumulative uptake of nitrogen by 10.8 to 15.4 per cent, phosphorus by 23.4 to 38.6 per cent, K by 32 to 33.2 per cent in successive four cropping season of rice-wheat system due to improvement in physical and chemical properties of soil.

Higher nutrient concentration in mulberry in biochar treatments might be due to favourable soil physical and chemical conditions that might have increased the availability of nutrients with application of biochar which has high organic load. The increase in nutrient concentration may also be due to higher nutrient content coupled with better vegetative growth in these treatments. The increase in microbial activity due to application of biochar could also be the other reason for the highest nutrient uptake in biochar treated soils. The ash content of biochar helps for the immediate release of the occluded mineral nutrients like Ca, K and N for crop use.

An increase in N, P and K content with FYM and biochar application may be due to the fact that added FYM and biochar served as store house of several macro and micronutrients which are released during the process of mineralization. In addition to release of plant nutrients from the organic matter, the organic acids formed in the decomposition process also release the native nutrients in soil and increases the availability to plants. Lehmann *et al.* (2003) also observed an increase in P concentration in plants with increasing biochar application. The increase in K uptake in biochar amended soils might also be attributed to the presence of K rich ash in the biochar.

Major *et al.* (2010) reported that total nutrient uptake by the maize crop increased with the application of biochar. Similarly, Nigussie *et al.* (2012) found that uptake of N, P and K by plants were increased with biochar application.

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

Treatments	First crop cutting			Second crop cutting			Third crop cutting			Pooled mean		
	N	P	K	N	P	K	N	P	K	N	P	K
	Kg ha ⁻¹											
T ₁	42.99	3.68	21.41	72.41	6.18	34.71	83.10	7.10	39.84	66.17	5.66	31.99
T ₂	44.23	3.95	22.10	76.97	6.52	37.34	87.81	7.47	42.60	69.67	5.98	34.01
T ₃	48.45	4.30	24.78	85.04	7.62	43.94	95.03	8.61	48.95	76.17	6.84	39.23
T ₄	50.10	4.45	25.13	87.06	7.84	43.09	98.12	8.83	49.56	78.43	7.04	39.26
T ₅	52.06	4.89	26.03	91.05	8.55	45.53	101.92	9.61	51.17	81.68	7.68	40.91
T ₆	53.98	5.14	27.53	94.47	8.99	48.19	105.28	10.08	54.04	84.58	8.07	43.25
T ₇	55.70	5.59	28.96	97.51	9.79	50.68	108.97	10.95	56.72	87.40	8.78	45.45
T ₈	57.88	5.82	30.49	101.99	10.12	52.40	112.17	11.25	58.42	90.68	9.07	47.10
S.Em±	1.24	0.09	0.78	1.67	0.35	1.35	1.98	0.36	1.38	1.34	0.24	1.00
CD @ (5 %)	3.77	0.29	2.39	5.08	1.06	4.11	6.01	1.09	4.20	4.08	0.75	3.04

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

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

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

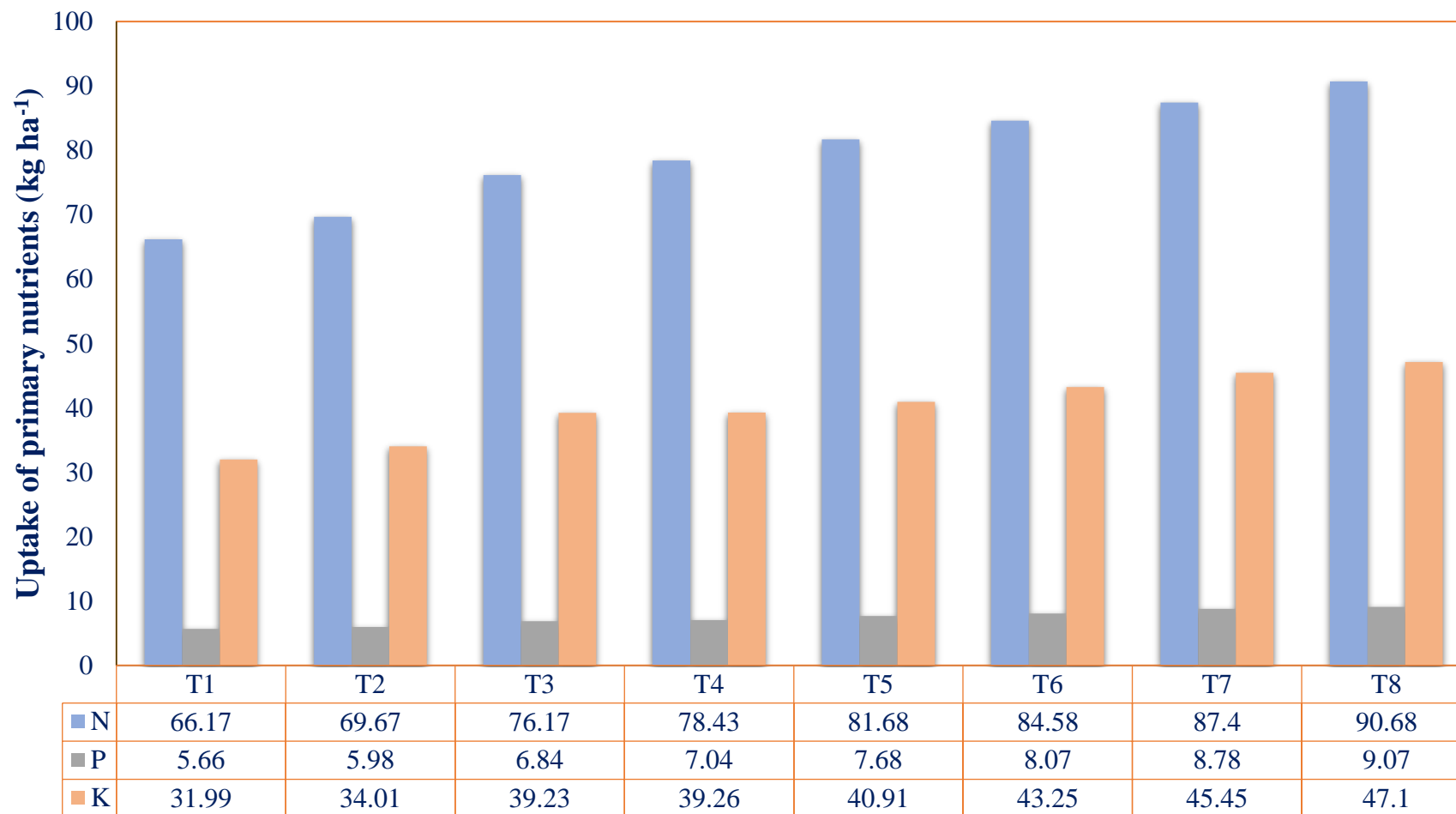


Fig. 2: Effect of mulberry stalk biochar on uptake of primary nutrients by mulberry (pooled mean)

Secondary nutrients uptake (kg ha⁻¹)

The results pertaining to the effect of different levels of biochar along with FYM on Ca, Mg and S concentration and uptake of nutrients are presented in Table 6. The concentration and uptake in leaves differed significantly due to imposition of different treatments.

The results of pooled mean data indicated that significantly higher uptake of calcium (44.48 kg ha⁻¹), magnesium (17.26 kg ha⁻¹) and sulphur (9.75 kg ha⁻¹) was recorded in T₈ and lowest uptake of calcium, magnesium and sulphur was recorded in T₁ (31.28, 12.43 and 5.42 kg ha⁻¹) treatment. Among the different treatments, the treatment with application of biochar @ 10 t ha⁻¹ (T₅) recorded higher uptake of calcium (39.42 kg ha⁻¹), magnesium (15.33 kg ha⁻¹) and sulphur (8.15 kg ha⁻¹) and it was on par with T₄ (38.45, 14.78 and 6.96 kg ha⁻¹) and superior over T₃ (36.81, 14.27 and 6.69 kg ha⁻¹), T₂ (33.49, 13.10 and 6.04 kg ha⁻¹) and T₁ (31.28, 12.43 and 5.42 kg ha⁻¹) treatments.

Increase in calcium, magnesium and sulphur uptake was observed with increasing the level of biochar in combination with FYM and significantly higher Ca, Mg and S uptake was recorded with the application of biochar @ 10 t ha⁻¹ + FYM @ 10 t ha⁻¹ (T₈). Gaskin *et al.* (2008) found that application of pine chip biochar along with fertilizer significantly increased Ca content of maize plant compared to alone application of biochar. Similar type of result also found by Chan *et al.* (2007) and reported that application of poultry litter biochar increased the calcium (0.138 g pot⁻¹) and magnesium uptake (0.033 g pot⁻¹) of radish plants and significant increases were found only at higher application of poultry litter biochar @ 50 t ha⁻¹. Positive effect of biochar application on crop growth, yield and uptake (radish and common bean) has also been reported by several workers [Lehmann *et al.*, (2003), Chan *et al.*, (2007) and Asai *et al.*, (2009)]. Rondon *et al.* (2007) stated that application of biochar @ 60 g kg⁻¹ recorded highest sulphur uptake of 9.5 mg pot⁻¹ in common bean and application of biochar increased soil available nutrients and plant productivity (Sohi *et al.*, (2009).

Mg uptake in mulberry was fairly increased with increased application of biochar along with FYM. Similar types of results were obtained by Lehmann *et al.* (2003) and they observed that application of biochar along with fertilizer significantly recorded higher Mg content (15.3 mmolc kg⁻¹) in rice plant compared to alone application of fertilizer. Further, similar results found by Uzoma *et al.* (2011) in sandy soil reported that application of cow manure biochar @ 10, 15 and 20 t ha⁻¹ along with fertilizer significantly increased the uptake of Ca and Mg in maize and the highest Ca uptake of 0.91 kg ha⁻¹ and Mg uptake of 4.99 kg ha⁻¹ was recorded with application of cow manure biochar @ 20 t ha⁻¹. Similarly, Chan *et al.* (2008) reported that application of poultry litter biochar at rates of 10, 25, 50 t ha⁻¹ along with N fertilizer significantly increased

the sulphur content *i.e.*, 0.78 per cent, 0.83 per cent and 0.79 per cent, respectively in radish plant compared to control.

Increase in uptake of secondary nutrients might be due to higher biomass production. The higher content of calcium in biochar enhanced the cation exchange capacity of soils. This phenomenon in soil increased the uptake of calcium and magnesium by maize and these results are in accordance with the results of Nigussie *et al.* (2012). Further, Fox *et al.* (2012) described that in biochar amended soil, the higher number of bacterial colonies, improve S and P uptake through enhanced nutrient mobilization.

Application of biochar and FYM which contains high amount of Ca, Mg and S helped in increasing the secondary nutrients uptake. Nutrient uptake is a function of nutrient content and biomass production. Increased rate of application of biochar increased biomass production which obviously increased the nutrient uptake. These results are in accordance with findings of Xu *et al.* (2014) and Vecstaudza *et al.* (2018).

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

Treatments	First crop cutting			Second crop cutting			Third crop cutting			Pooled mean		
	Ca	Mg	S	Ca	Mg	S	Ca	Mg	S	Ca	Mg	S
	Kg ha ⁻¹											
T ₁	20.94	7.10	3.19	33.95	13.81	5.88	38.96	16.39	6.74	31.28	12.43	5.42
T ₂	22.03	7.68	3.63	36.64	14.58	6.61	41.80	17.04	7.55	33.49	13.10	6.04
T ₃	23.31	8.69	4.34	40.92	15.89	7.45	46.21	18.22	8.42	36.81	14.27	6.69
T ₄	24.31	9.07	4.97	42.80	16.31	7.75	48.24	18.95	8.73	38.45	14.78	6.96
T ₅	25.09	9.70	5.36	43.87	17.08	9.07	49.30	19.22	10.19	39.42	15.33	8.15
T ₆	26.14	10.23	6.15	45.74	17.79	10.05	51.29	20.49	11.27	41.06	16.17	9.02
T ₇	27.09	10.92	6.57	47.35	18.23	10.51	53.07	21.07	11.76	42.50	16.74	9.42
T ₈	28.13	11.17	7.09	49.52	18.94	10.86	55.80	21.68	12.14	44.48	17.26	9.75
S.Em±	0.41	0.30	0.23	1.22	0.37	0.21	1.34	0.35	0.24	1.08	0.34	0.18
CD @ (5 %)	1.25	0.91	0.70	3.70	1.13	0.64	4.08	1.06	0.72	3.29	1.03	0.56

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
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Micronutrients uptake (g ha⁻¹)

Effects of mulberry stalk biochar on micronutrients uptake by mulberry are presented in Table 7 and 8. From the results, it is observed that the uptake of micronutrients was significantly affected by the treatments in all the three crop cuttings. The increasing levels of biochar significantly increased the uptake of Fe, Zn, Mn, Cu and B in mulberry crop.

The pooled mean data showed marked significant differences with respect to uptake of micronutrients and the highest uptake of iron, manganese, zinc, copper and boron being recorded in T₈ (748.20, 153.22, 139.34, 60.92 and 54.25 g ha⁻¹, respectively) and the next best treatment was T₇ (732.23, 145.59, 135.08, 59.22 and 52.72 g ha⁻¹, respectively) while the lower values was recorded in control (615.66, 111.94, 81.99, 45.51 and 35.12 g ha⁻¹, respectively).

The application of biochar differed micronutrient content and uptake by mulberry crop significantly in different treatments. In general, application of biochar improved the content and uptake of nutrients by mulberry. Application of biochar at higher rates, in comparison to control significantly increased the content and uptake of micronutrients. Higher uptake of these micronutrients might be due to higher biomass production which was recorded due to increased level of biochar along with FYM. Application of biochar is accompanied by increase in soil pH and reduced mobility of micronutrients. But in presence of plant, which actively releases organic compounds in rhizosphere may mobilize the micronutrients.

Lehmann *et al.* (2003) also noticed “higher uptake of P, K, Ca, Zn, and Cu by the plants with increased level of biochar additions due to reduced leaching losses and increased fertilizer use efficiency”. “Increase in iron uptake by mulberry might be due to addition of biochar and FYM, chelated the iron and prohibit the oxidation of iron and keeping it to available form. At maturity, N, P, K, B, and S uptake was enhanced significantly in plants” (Qaswar *et al.*, 2020). “This uptake was higher with the co-application of biochar and synthetic fertilizers. The reason is that the combined doses of biochar and inorganic fertilizers release more nutrients for plants uptake than synthetic fertilizers alone” (Iqbal *et al.*, 2019). “Biochar application along with B and S increases the accumulation of N, P, K, B, and S in the soil, thereby improving soil fertility and thus increasing plant nutrient absorption” (Yadav *et al.*, 2019).

Similar findings were also reported by Antonio *et al.* (2013), Willis *et al.* (2016) and Jatav *et al.*, (2018) who have reported that the uptake of iron, copper, zinc, manganese and boron (micronutrients) increased significantly with graded dose of biochar application.

Conclusion

Soil application of biochar and FYM have more stimulating effect on nutrients uptake by mulberry. Among all the treatments imposed, soil application of biochar @ 10 t ha⁻¹ + FYM @ 10 t ha⁻¹ recorded higher uptake values compared to control. Treatments T₄, T₅ and T₆ which received biochar @ 5, 7.5 and 10 kg ha⁻¹, respectively increased the uptake of nutrients over control. From the present investigation, it is observed that combined application of biochar and FYM has significantly increased the nutrients uptake by mulberry.

Table 7: Effect of mulberry stalk biochar on uptake of iron, zinc and manganese by mulberry at different crop cutting seasons

Treatments	First crop cutting			Second crop cutting			Third crop cutting			Pooled mean		
	Fe	Zn	Mn	Fe	Zn	Mn	Fe	Zn	Mn	Fe	Zn	Mn
	g ha ⁻¹											
T ₁	384.76	54.63	70.63	682.41	88.55	120.79	779.80	102.78	144.42	615.66	81.99	111.94
T ₂	392.33	58.18	72.80	696.34	96.77	125.85	793.59	112.65	147.64	627.42	89.20	115.43
T ₃	444.10	75.27	85.95	776.35	131.66	147.23	875.59	149.05	170.59	697.68	118.66	134.59
T ₄	428.16	70.85	82.80	753.75	124.77	141.15	849.50	143.16	165.48	677.14	112.92	129.81
T ₅	414.75	64.87	78.57	734.40	113.21	135.20	829.30	130.17	159.09	659.48	102.75	124.29
T ₆	456.94	81.17	89.27	799.29	141.46	154.60	896.38	161.00	176.10	717.54	127.88	139.99
T ₇	466.43	85.41	92.72	816.55	149.41	162.35	913.72	170.41	181.70	732.23	135.08	145.59
T ₈	479.38	88.50	97.99	833.57	154.53	171.27	931.67	174.99	190.42	748.20	139.34	153.22
S.Em±	6.72	2.10	1.77	10.34	3.99	4.00	10.62	3.69	3.63	8.97	2.49	2.34
CD @ (5 %)	20.40	6.39	5.39	31.38	12.12	12.13	32.21	11.21	11.02	27.21	7.56	7.11

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

Treatments	First crop cutting		Second crop cutting		Third crop cutting		Pooled mean	
	Cu	B	Cu	B	Cu	B	Cu	B
	g ha ⁻¹							
T ₁	30.61	22.10	49.63	38.55	56.28	43.71	45.51	35.12
T ₂	31.69	24.67	52.73	42.19	59.00	48.12	47.80	38.33
T ₃	35.20	31.09	61.56	54.39	68.57	61.13	55.11	48.87
T ₄	34.27	29.17	60.34	51.37	66.74	57.90	53.79	46.15
T ₅	33.03	27.33	58.52	48.44	64.84	54.70	52.13	43.49
T ₆	36.80	32.21	64.39	56.41	70.89	63.26	57.36	50.63
T ₇	38.18	33.59	66.44	58.78	73.03	65.79	59.22	52.72
T ₈	39.06	34.69	68.84	60.23	75.88	67.83	60.92	54.25
S.Em±	0.35	0.74	1.42	1.17	1.58	1.46	1.16	1.10
CD @ (5 %)	1.07	2.26	4.32	3.55	4.81	4.44	3.54	3.36

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
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References

- Antonio J A, Pablo S, Vidal B, Jose T, Maria-Del C C, Antonio G Rafael V. Enhanced wheat yield by biochar addition under different mineral fertilization levels. *Agron. Sustainable Devleop.* 2013; 33: 475–484.
- Asai H, Samson B K, Stephan H M, Songyikhangsuthor K, Homma K, Kiyono Y, Inoue Y, Shiraiwa T, Horie T. Biochar amendment techniques for upland production in Northern Iaso on soil physical properties, leaf SPAD and grain yield. *Field Crop Res.* 2009; 111: 81-84.
- Atkinson C J, Fitzgerald J D, Hipps N A. Potential mechanism for achieving agricultural benefits from biochar application to temperate soils: A review. *Plant and soil.* 2010; 337: 1-18.
- Black C A. Methods of soil analysis part-1 physical and mineralogical properties. *Agronomy Monograph No.9.* American society of agronomy, Madison, Wisconsin, USA. 1965; 7: 18-25.
- Chen Y, Shinogi Y, Taira M. Influence of biochar use on sugarcane growth, soil parameters and ground quality. *Aust. J. Soil Res.* 2010; 48: 526-530.
- Fasiha, Devakumar A S. Characterization of corncob biochar produced through the gasification process for application as soil amendment. *Mysore J. Agric. Sci.* 2022; 56(1): 100-107.
- Fox A, Cullen R, Kwapinski W, Schmalenberger A. Bacterial mobilization of sulfur and phosphorous in biochar amended soils. *Commun. soil sci. plant anal.* 2012; 5 :49–50.
- Gaskin J W, Steiner C, Harris K, Das K C, Bibens B. Effect of low temperature pyrolysis conditions on biochars for agricultural use. *American Soc. Agric. Biol. Eng.*, 2008; 51 (6): 2061-2069.
- Glaser B, Lehmann J, Zech W. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal—a review. *Biol. Fertil. Soils.* 2002; 35:219–230.
- Hossain M K V, Strezov K, Chan Y, Nelson P F. Agronomic properties of wastewater sludge biochar and bioavailability of metals in production of cherry tomato (*Lycopersicon esculentum*). *Chemosphere.* 2010; 78:1167-1171.
- Iqbal A, He L, Khan A, Wei S, Akhtar K, Ali I, Jiang L, Munsif F, Zhao Q, Jiang L. Organic manure coupled with inorganic fertilizer: An approach for the sustainable production

- of rice by improving soil properties and nitrogen use efficiency. *Agron.* 2019; 9 (10):651.
- Jackson M L. 1973, Soil chemical analysis. Prentice Hall of India, New Delhi. 1973; 1: 485-486.
- Jatav H S, Singh S K, Singh Y, Kumar O. Biochar and sewage sludge application increases Yield and micronutrients uptake in rice. *Commun. Soil Sci. Plant Anal.* 2018; 10(3): 1532-2416.
- Jeffery S, Verheijen F G A, Van Der Velde M, Bastos A C A. quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. *Agriculture, Ecosystems & Environment.* 2011; 144(1): 175-187.
- Jones J B, Case V W. Sampling, handling an analysing plant tissue sample. In: R.L. Westerman (ed), soil sestring an plant analysis. 3r Ed. Soil Sci. Soc. Am., Inc. Madison, WI. 1990; pp: 389-447.
- Lehmann J, Joseph S. Biochar for Environmental Management. *Biochar Environ. Manag. Sci. Technol.* 2009; 907p.
- Lehmann J, Pereira Da Silva J R J, Steiner C, Nehls T, Zech W, Glaser B. Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. *Plant and Soil.* 2003; 249: 343-357.
- Lindsay W L, Norwell W A. Development of DTPA soil test for zinc, iron, manganese and copper. *Soil Sci. Soc. Amer. J.* 1978; 42: 421-428.
- Major J, Rondon M, Molina D, Riha S J, Lehmann J. Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. *Plant Soil.* 2010; 333:117–128.
- Nigussie A, Kissi E, Misganaw M, Ambaw G. Effect of biochar application on soil properties and nutrient uptake of lettuces (*Lactuca sativa*) grown in chromium polluted Soils. *American Eurasian J. Agric. Environ. Sci.* 2012; 12: 369-376.
- Novak J M, Busscher W J, Laird D L, Ahmedna M, Watts D W, Niandou M A S. Impact of biochar amendment on fertility of a south eastern coastal plain soil. *Soil Sci.* 2009; 174: 105-112.
- Ogawa M, Okimori Y. Pioneering works in biochar research, Japan, Aust. *J. Soil Res.* 2010; 48: 489–500.

Page A L, Miller R H, Kenay D R. Methods of Soil Analysis, part–II, Soil Science Society of America, Inc, Publishers, Madison, Wisconsin, USA. 1982.

Piper C S. Soil and Plant Analysis, Hans Publishers, Bombay, India. 2002; Pp. 85-87.

Qaswar M, Jing H, Ahmed W, Dongchu L, Huimin Z, Huimin Z, Cai A, Lisheng L, Yongmei X, Jusheng G. Yield sustainability, soil organic carbon sequestration and nutrients balance under long-term combined application of manure and inorganic fertilizers in acidic paddy soil. *Soil and Tillage Res.* 2020; 198:104569.

Rondon M, Lehmann J, Ramirez J, Hurtado M. Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with bio-char additions. *Biol. Fert. Soils.* 2007; 43: 699-708.

Sohi S, Loez-Capel E, Krull E, Bol R. Biochar's roles in soil and climate change. A review of research needs. CSIRO and Water Science. 2009; Report 5/9: 64.

Tandon H L S. Methods of analysis of soils, plants, water and fertilizers. Fertilizer development and consultation organization, new delhi. 1998; pp:42-144.

Uzoma K C, Inoue M, Andry H, Fujimaki H, Zahoor A, Nihihara E. Effect of cow manure biochar on maize productivity under sandy soil condition. *Soil Use Manag.* 2011; 27: 205–212.

Van Zwieten, L, Kimber S, Morris S, Chan K Y, Downie A, Rust J, Joseph S, Cowie A. Effects of biochar from slow pyrolysis of paper mill waste on agronomic performance and soil fertility. *Plant and Soil.* 2010; 327: 235–246.

Vecstaudza D, Lelde G L, Galina M, Raimonds K, Turs S, Vilhelmine S, Solvita S, Christoph S, Olga M. The impact of wood-derived biochar on the survival of *Trichoderma* spp. And growth of *Secale 27 ulber* L. in sandy soil. *Biocontrol Sci. Tech.*, 2018; 28(4): 341–358.

Willis G, Moreblessing M, Farai M, Tonny P. Comparative short-term effects of sewage sludge and its biochar on soil properties, maize growth and uptake of nutrients on a tropical clay soil in Zimbabwe. *J. Integrative Agric.* 2016; 15(6): 1395–1406.

Winsley P. Biochar and bioenergy production for climate change mitigation. *New Zealand Sci. Rev.* 2007; 64: 5-10.

- Xu G, Wei L L, Sun J N, Shao H B, Chang S X. What is more important for enhancing nutrient bioavailability with biochar application in to a sandy soil. *Ecol. Eng.* 2014; 52: 119-124.
- Yadav S K, Benbi D K, Prasad R. Effect of continuous application of organic and inorganic sources of nutrients on chemical properties of soil. *Int. J. Current Microbio. App. Sci.* 2019; 8 (4):2455–63.
- Zwieten V L, Kimber S, Downie A, Morri S, Petty S, Rust J, Chan K Y. A glass house study on the interaction of low mineral ash biochar with nitrogen in a sandy soil. *Aust. J. Soil Res.* 2010; 48:569-575.

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