

## Biochar amendment improves soil fertility and productivity of mulberry plant

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### Abstract

Biochar has the potential to improve soil fertility and crop productivity. A field experiment was carried out at the experimental field of Bangladesh Sericulture Research and Training Institute (BSRTI), Rajshahi, Bangladesh. The objective of this study was to examine the effect of biochar on soil properties, growth, yield and foliar disease incidence of mulberry plant. The study consisted of 6 treatments: control, basal dose of NPK, rice husk biochar, mineral enriched biochar, basal dose + rice husk biochar and basal dose + mineral enriched biochar. Growth parameters such as node/meter, total branch number/plant, total leaf yield/hectare/year were significantly increased in basal dose + mineral enriched biochar treated plot in second year compared with the other fertilizer treatments. In second year, the total leaf yield/hectare/year were also 142.1% and 115.9% higher in combined application of basal dose + mineral enriched biochar and basal dose + rice husk biochar, respectively, than the control treatment. The soil properties such as organic matter, phosphorus, sulphur and zinc percentage were significantly increased with both the (mineral enriched and rice husk) biochar treated soil applied with or without recommended basal dose of NPK than the control and only the recommended basal dose of NPK, respectively. Further, the lowest incidences of tukra (6.4%), powdery mildew (10.4%) and leaf spot (7.6%) disease were observed in second year under mineral enriched biochar treated plot than the others. The findings revealed that utilization of biochar has positive effect on the improvement of soil fertility and productivity as well as disease suppression of mulberry plant.

**Keywords:** Soil fertility, systemic resistance, pyrolysis, decomposition, pruning.

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### Introduction

Biochar is a source of organic fertilizer that is receiving attention by researchers all over the world (Lehmann et al., 2003). The process of biochar production 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 C storage on a large scale (Sohi et al., 2010; Kookana et al., 2011). 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% depending upon its feedstock, which enhances C sequestration in soil (Lehmann and Joseph, 2009).

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Different types of biochar can be used for improving soil properties and increasing agricultural production. This includes rice-husk and mineral enriched biochar. Application of biochar to soil help improve yield and its components of different crops. A study conducted by [Agboola and Moses \(2015\)](#) showed that the growth and yield of soybean [*Glycine max* (L.) Merr.] was increased due to addition of rice husk biochar. Likewise, soil properties such as soil pH, organic C, soil nitrogen (N), calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) changes due to the application of rice husk biochar in soil. Similarly, [Gebremedhin et al. \(2015\)](#) reported that biochar significantly increased grain and straw yields of wheat (*Triticum aestivum* L.) by 15.7 and 16.5%, respectively, over the NPK application.

Biochar amendment may not only improve soil properties but also suppress disease infestation on various crops. [Mercado-Blanco and Bakker \(2007\)](#) found that biochar addition to the potting medium of strawberry (*Fragaria* sp) plants suppressed foliar diseases caused by fungi having *Pseudomonas* in several crops. Several studies observed that biochar amended soil help proliferate plant growth promoting rhizobacteria/fungi (PGPR/F) which are known to promote plant health in addition to plant growth either by directly controlling plant pathogens or through the potentiating of plant systemic resistance responses against diseases. [Elad et al. \(2011\)](#) demonstrated that addition of biochar to soil increased beneficial microorganism populations that promote plant growth and improve resistance against biotic stresses. However, the impact of biochar on mulberry (*Morus alba*) plant production with common disease incidence and changes in soil physical and chemical properties is not known yet in Bangladesh. Therefore, the focus of this investigation was to quantify the response of rice husk and mineral enriched biochar on changes in soil properties, yield components and diseases incidence of mulberry plant. We hypothesize that mulberry yield will be the highest and incidence of common mulberry disease will be the lowest in mineral-enriched biochar treatment compared with other treatments. Likewise, soil physical and chemical properties will be changed due to long-term application of biochar in soil.

## Material and Methods

### Experimental location

The experiment was conducted at the experimental field of Bangladesh Sericulture Research and Training Institute (BSRTI), Rajshahi, Bangladesh (24° 22' 29" North and 88° 37' 3.84" East). On the basis of Agro-Ecological Zone (AEZ), BSRTI, Rajshahi falls under the Active Ganges Floodplain-10 and High Ganges River Floodplain-11.

### Soil condition

The soil of the experimental plot was mainly loamy in nature, having alkaline characteristics with pH ranging from 8.2 to 8.6 in water. As a consequence of this alkalinity, the soil was poor in K and available P. Both C and N levels were low in uncultivated as well as in the cultivated plots. N level was not in balance with C. This was more prominent in the farm area where mulberry was cultivated for years. Toxic metals were present in traces, but they were well below the harmful levels. The average two years pre-treated basic physicochemical properties of the experimental soil are presented in Table 1.

Table 1. Average two years data of the experimental soil before applied the treatments

Soil pH in H <sub>2</sub> O	Nitrogen (%)	Phosphorus (mgkg <sup>-1</sup> )	Potassium (cmol kg <sup>-1</sup> )	Sulphur (mgkg <sup>-1</sup> )	Zinc (mgkg <sup>-1</sup> )	Organic matter (%)
8.4	0.1	13.9	0.1	12.6	0.9	1.1

### Sample plant material

Mulberry plant was used as a sample planting material for this experiment and it is perennial, deep rooted and hardy in nature. Due to these characteristics, mulberry is grown in a wide range of soil and agro-climatic conditions. The mulberry variety BM-9 and square high bush (plant to plant and row to row distance is 3ft and plant height is 1.5 ft) plantation system was used for this study.

### Taxonomy of mulberry plant

Mulberry belongs to the genus *Morus*. Mulberry is highly heterozygous and out breed in nature [Vijayan et al. \(2012\)](#). Different workers from different corners of the world classified the genus *Morus* L. in different ways. [Linnaeus \(1753\)](#) recognized seven species under the genus *Morus* L. presently of which only five, namely *Morus alba*, *Morus nigra*, *Morus rubra*, *Morus. india* and *Morus tartarica* fit in to the circumscription of the

genus. Based on female inflorescence and fruit character, French Botanist Bureau (1873) recognized five species, 19 varieties and 11 sub-varieties of the genus *Morus*.

### Experimental condition

In this experiment, three-years old and high-bush plantation system mulberry garden was used. In Bangladesh, generally four commercial silkworm rearing seasons is followed in a year. On the basis of silkworm rearing seasons mulberry garden was pruned four times in a year each after three months interval. In this experiment, the pruning was done for two years from January 2014 to December 2015.

### Experimental design

The experiment was arranged in a randomized complete block design with three replications, where the plot size was 8 m long × 3 m wide. Each plot had 20 plants and fertilizer management was done for each replicated plot. The same experiment was repeated for two years.

### Treatments

There were six treatments for this experiments which are as follows:

(i) Control

No fertilizer was applied.

(ii) Recommended basal dose

The recommended basal dose of BSRTI is 305 kg N, 105 kg K and 66 kg P/hectare/year with four split doses each after three months interval.

(iii) Biochar (Rice husk)

Only the rice husk biochar was applied at a time @ 1 t/hectare/year. In this case, the 2nd dose was applied after one year interval.

(iv) Biochar (Mineral enriched)

Only the mineral enriched biochar was applied at a time @ 1 ton/hectare/year. In this case, the 2nd dose was applied after one year interval.

(v) Recommended basal dose + Biochar (rice husk)

BSRTI recommended basal dose at four split doses each after three months interval in a year and 1 t rice husk biochar/hectare/year at a time was applied for mulberry cultivation.

(vi) Recommended basal dose + Biochar (mineral enriched)

BSRTI recommended basal dose at four split doses each after three months interval in a year and 1 t mineral enriched biochar/hectare/year at a time was applied for mulberry cultivation.

### Chemical properties of biochar

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). In our experiment, two types of biochar such as rice husk and mineral enriched biochar were used, which were collected from China. The chemical properties of the applied biochar are provided in Table 2.

Table 2. Properties of rice husk and mineral enriched biochar used in the experiment

Elements	Rice husk biochar	Mineral enriched biochar
pH	8.0	8.9
Total N (%)	1.7	2.1
Total P (%)	0.2	0.5
Total K (%)	0.2	1.1
OC (g/kg)	54.0	67.0
Total Na (%)	0.2	0.6
S (mg/kg)	0.2	0.4
Fe (mg/L)	7.8	8.7
Ca (mg/L)	213.0	273.0
Cu (mg/L)	0.1	0.1
Al (mg/L)	0.9	1.0
Mn (mg/L)	4.4	5.1
Ash (%)	50.3	39.7

## Experimental procedure

The treatments were randomly assigned in the experimental plot for each replication. In each year and every case according to the treatment, all the fertilizers such as basal, rice husk biochar, mineral enriched biochar, basal + rice husk biochar and basal + mineral enriched biochar were applied 15 days after pruning (DAP) on the mulberry plant when sprouting was started. Both the biochar (rice husk and mineral enriched) were applied through surface application method in the root zone area of plant and was then incorporated into soil. In flooding system three times irrigation per crop season @ 1.5 to 2.0 acre inch water per irrigation at an interval of 15-30 days was done and other intercultural practices such as digging cum weeding and insect-pest control were done as per requirement for each cropping season.

## Data collection

According to the treatments data were collected for the growth parameters, percentage of disease incidence and soil properties for each two years.

## Growth parameters

For determining the growth impact data were collected for the following parameters:

### (i) Node/meter/plant

The number of node/meter was determined by counting the number of node/meter manually for each of the plant each after 90 days of pruning.

### (ii) Total branch number/plant

The number of branch/plant was determined by counting the number of branch manually for each of the plant each after 90 days of pruning.

### (iii) Total branch height/plant (cm)

All the branch height for a plant was measured by the measuring tape and adding each after 90 days of pruning

### (iv) Total leaf weight/plant (g)

All the green leaves of a plant except shoot were weighted using a weighting balance each after 90 days of pruning.

### (v) Total leaf yield (t/hectare /year)

At maturity of leaf (after 80 days of pruning) the total green leaf yield/ha/year was determined by the following formula:

$$\text{Leaf yield (t / hectare / year)} = \frac{\text{Leaf weight (g) of m}^2 \text{ plant} \times \text{Number of crop season / year} \times 10000 \text{ m}^2}{1000 \times 1000}$$

## Disease incidence

The occurrence of disease incidence/plant was recorded for three consecutive years after 60–65 days of pruning by randomly selecting 10 plants in each replication. Foliar diseases such as powdery mildew (*Phyllactinia corylea*) and leaf spot (*Pseudocercospora mori*) and tukra (*Meconellicoccus hirsutus*) were recorded during this period. Studies on percent disease incidence (PDI) with respect to powdery mildew, leaf spot and tukra diseases were recorded and the data during both the years were pooled and analyzed. The percent disease incidence (PDI) was calculated following the formula suggested by [Rai and Mamatha \(2005\)](#).

$$\text{Percent Disease Incidence (PDI)} = \frac{\text{Number of diseased leaves on each plant}}{\text{Number of total leaves on each plant}} \times 100$$

## Soil analysis

Soil was collected from approximately the top 55 cm depth of the experimental plots. According to the treatments for each cropping year, the collected soil sample was homogenized, air-dried for 72 hours, ground and sieved to pass a 2 mm sieve. The initial soil pH was determined before incubation experiment in deionised water using a soil-to-solution ratio of 1:2.5. Soil organic matter content was determined by ([Walkley et al., 1934](#)) multiplying the percent value of organic C with the conventional Van-Bemmelen factor of 1.724 ([Piper, 1950](#)). The N content of the soil sample was determined by distilling soil with alkaline  $\text{KMnO}_4$  solution ([Subhaiah and Asija, 1956](#)) and the distillate was collected in 20 ml of 2% boric acid solution with methylred and bromocresol green indicator and titrated with 0.02 N  $\text{H}_2\text{SO}_4$  ([Podder et al., 2012](#)). Soil available S (mg  $\text{kg}^{-1}$ ) was determined by calcium phosphate extraction method with a spectrophotometer at

535 nm (Petersen, 1996). The soil available K was extracted with 1N NH<sub>4</sub>OAC and determined by an atomic absorption spectrometer (Biswas et al., 2012). The available P of the soil was determined by spectrophotometer at a wavelength of 890 nm. The soil sample was extracted by Olsen method with 0.5 M NaHCO<sub>3</sub> as outlined by Huq and Alam (2005) and the Zn in the soil sample was measured by an atomic absorption spectrophotometer after extracting with DTPA (Soltanpour and Workman, 1979).

### Statistical analysis

Statistical analysis was conducted using Genstat 12.1 for Windows (Lawes Agricultural Trust, UK) and Statistix 10 software. Treatment means were separated by the least significant difference (LSD) at the 0.05% level of significance (Steel and Torrie, 1984). Mulberry plant growth and composition data were analyzed by a one-way analysis of variance (ANOVA) for the main effect of mulberry plant growth and Sigma Plot 12.5 version was used for representing the growth results in a figure form. Disease parameters were statistically analyzed and mean values were evaluated by DMRT test.

## Results

### Effect of fertilizer application on bulk soil properties

The effect of treatment application on the soil properties after harvesting of mulberry leaf are presented in Table 4. The addition of different fertilizer as a soil amendment significantly ( $P < 0.001$ ) increased organic matter, P, S and Zn contents in soil. In contrast, only organic matter ( $P < 0.001$ ) and S ( $P < 0.05$ ) contents were significantly increased for the cropping season and the effect of season  $\times$  treatment was also significant for S ( $P < 0.01$ ) and Zn ( $P < 0.001$ ) contents in soil (Table 3). The highest level of organic matter (2.0%) was found in the second year due to the application of mineral enriched biochar @ 1 t/hectare/year, the highest level of P (30.7%) was obtained in the second year for rice husk biochar @ 1 t/hectare/year, the highest S content 9.3% was found in second year for mineral enriched biochar @ 1 t/ hectare//year + recommended basal fertilizer dose and the highest Zn content was recorded 1.8% in the second year for rice husk biochar @ 1 t/hectare/year (Table 4). The soil properties such as pH, N and K level were not influenced by the cropping season, treatment and season  $\times$  treatment effect (Table 3).

Table 3. Level of significance for the main and interaction effect on season and treatment for soil properties

Sources of variation	Soil pH	Organic matter (%)	N (%)	P (%)	K (%)	S (%)	Zn (%)
Season	ns	***	ns	ns	ns	*	ns
Treatment	ns	***	ns	***	ns	***	***
Season $\times$ treatment	ns	ns	ns	ns	ns	**	***

Table 4. Effect of different fertilizers on soil properties by year after leaf harvest in mulberry plant

Treatments	pH		Organic matter (%)		N (%)		P (%)		K (%)		S (%)		Zn (%)	
	1 <sup>st</sup> year	2 <sup>nd</sup> year	1 <sup>st</sup> year	2 <sup>nd</sup> year	1 <sup>st</sup> year	2 <sup>nd</sup> year	1 <sup>st</sup> year	2 <sup>nd</sup> year	1 <sup>st</sup> year	2 <sup>nd</sup> year	1 <sup>st</sup> year	2 <sup>nd</sup> year	1 <sup>st</sup> year	2 <sup>nd</sup> year
Control	8.2	8.3	1.1	1.1	0.1	0.1	18	17.7	0.2	0.2	8.9	8.4	1.1	1.0
Basal fertilizer	8.2	8.1	1.2	1.2	0.1	0.1	28.5	28.2	0.2	0.2	9.0	9.2	1.4	1.4
Rice husk biochar	8.3	8.3	1.1	1.1	0.1	0.1	30.5	30.7	0.2	0.2	8.6	8.9	1.8	1.8
Mineral enriched biochar	8.2	8.4	1.9	2.0	0.03	0.05	27.2	27.3	0.2	0.2	7.3	7.5	0.8	0.8
Basal fertilizer + rice husk biochar	8.2	8.4	0.7	0.8	0.03	0.04	21.3	21.6	0.2	0.2	7.9	8.1	0.7	0.7
Basal fertilizer + mineral enriched biochar	8.3	8.4	0.8	0.8	0.03	0.05	23.2	23.4	0.2	0.2	9.1	9.3	0.5	0.5

### Effect of fertilizer application on growth and yield

#### Node/meter

The two-way interaction between season and fertilizer treatment was significant ( $P < 0.05$ ) for node/meter of mulberry plant (Table 5). Among the two cropping seasons, the highest number of node/meter (24.5) was found in the second year for the fertilizer treatment of mineral enriched biochar @ 1 t/hectare/year with the recommended basal dose of BSRTI (Table 6). In contrast, the control had the lowest number of node/meter (18.5) in the second year compared with other treatments (Figure 1).

Table 5. Significance levels from for the main and interactive effect on season and treatments for growth and yield contributing characteristics of mulberry plant

Source of variation	Node Per meter	Total branch number per plant	Total branch height per plant (cm)	Total leaf weight per plant (gm)	Total leaf yield/ ha/yr (mt.)
Season	***	***	***	***	***
Treatments	***	***	***	***	***
Season × Treatments	*	n.s.	***	***	***

Table 6. Average two years values of growth and yield contributing characteristics for fertilizer treatments

Treatments	Node per meter		Total branch number per plant		Total branch height per plant (cm)		Total leaf weight per plant (gm)		Total leaf yield/ ha/yr (mt)	
	1 <sup>st</sup> year	2 <sup>nd</sup> year	1 <sup>st</sup> year	2 <sup>nd</sup> year	1 <sup>st</sup> year	2 <sup>nd</sup> year	1 <sup>st</sup> year	2 <sup>nd</sup> year	1 <sup>st</sup> year	2 <sup>nd</sup> year
Control	20.0	18.5	8.7	8.3	686.4	697.9	336.6	320.5	21.5	20.5
Basal	20.3	22.5	10.3	10.7	788.0	785.0	411.9	422.5	26.4	27.0
Biochar (Rice husk)	20.2	22.7	11.3	12.7	898.0	916.3	433.6	550.6	27.7	35.0
Biochar (Mineral enriched)	21.0	23.2	12.3	14.0	935.7	1229.0	446.3	648.6	28.6	41.5
Basal + Biochar (Rice husk)	21.2	23.5	13.0	16.3	1006.3	1230.0	447.2	692.1	28.6	44.3
Basal + Biochar (Mineral enriched)	23.5	24.5	15.8	17.2	1121.3	1361.3	486.2	775.8	31.1	49.6

### Total branch number per plant

Our results showed that the effect of cropping year and fertilizer treatments were significantly ( $P < 0.001$ ) increased the total branch number per plant (Table 5). The average highest total branch number per plant (17.2) was found in 2<sup>nd</sup> year for the biochar (Mineral enriched) @ 1 t/hectare/year with recommended basal dose of BSRTI treated plot as compared to others fertilizer treatments. (Table 5, Table 6, Figure 2).

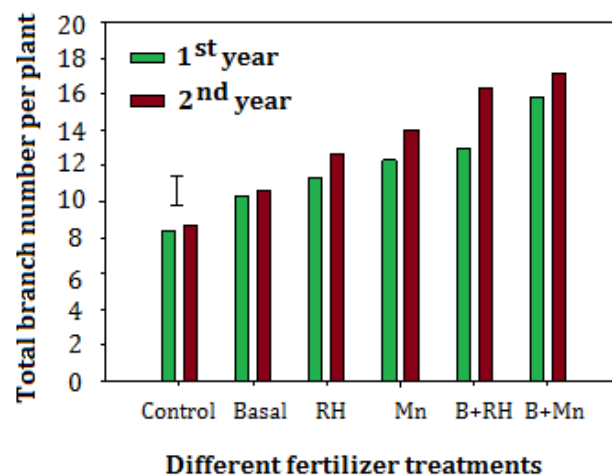
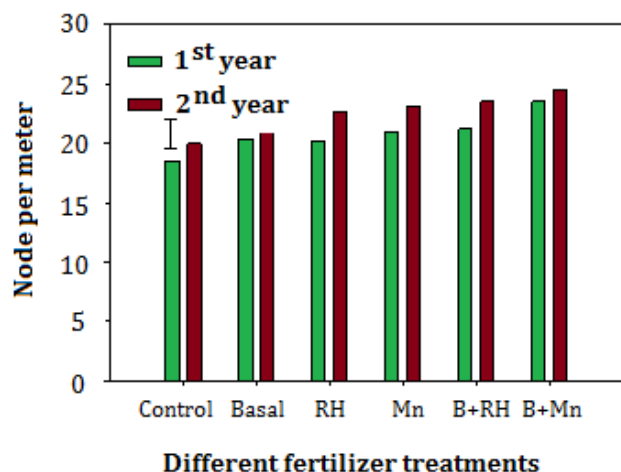


Figure 1. Effect of various fertilizer treatments on node per meter of mulberry plant. Vertical bar represent LSD ( $P \geq 0.05$ ) for season and treatment interaction. RH indicates rice husk biochar, Mn indicates mineral enriched biochar and B means BSRTI recommended basal fertilizer

Figure 2. Effect of various fertilizer treatments on total branch number per plant. Vertical bar represent LSD ( $P \geq 0.05$ ) for season and treatment interaction. RH indicates rice husk biochar, Mn indicates mineral enriched biochar and B means BSRTI recommended basal fertilizer

### Total branch height per plant (cm)

Interestingly, the effect of cropping season, treatments application and interactive effect of different fertilizer treatments and cropping seasons on the total branch height per plant were significantly ( $P < 0.001$ ) changed with various amendments (Table 5). However, the highest average total branch height per plant was obtained 1361.3 cm in 2<sup>nd</sup> year due to application of biochar (Mineral enriched) @ 1 t/hectare/year with recommended basal dose of BSRTI followed by the rest of the treatments (Table 5, Table 6, Figure 3).

### Total leaf weight per plant (gm)

Interestingly, the total leaf weight per plant was significantly ( $P < 0.001$ ) increased by the seasons, treatments and season  $\times$  treatments (Table 5). The maximum average total leaf weight per plant was recorded 775.8 gm in 2<sup>nd</sup> year for the treatment of biochar (Mineral enriched) @ 1 t/ hectare/year with recommended basal dose (@ 660 kg urea, 330 kg TSP and 210 kg MP per /hectare/year) of BSRTI as compared to other treatments. (Table 5, Table 6, Figure 4).

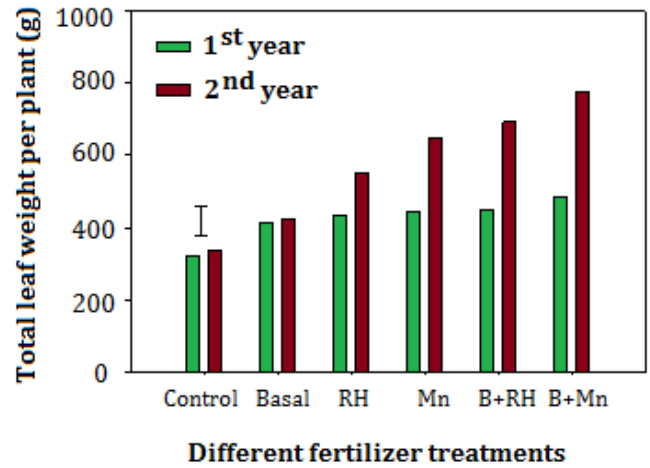
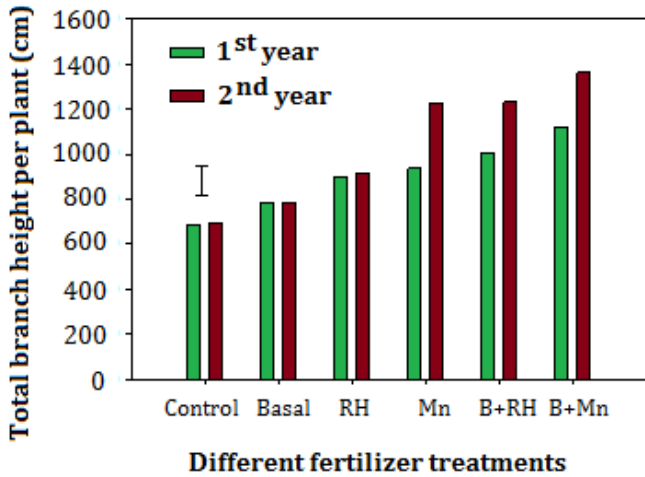


Figure 3. Effect of various fertilizer treatments on total branch height per plant. Vertical bar represent LSD ( $P \geq 0.05$ ) for season and treatment interaction. RH indicates rice husk biochar, Mn indicates mineral enriched biochar and B means BSRTI recommended basal fertilizer

Figure 4. Effect of various fertilizer treatments on total leaf weight per plant. Vertical bar represent LSD ( $P \geq 0.05$ ) for season and treatment interaction. RH indicates rice husk biochar, Mn indicates mineral enriched biochar and B means BSRTI recommended basal fertilizer

### Total leaf yield/ha/yr (mt.)

According to the results of the present study among the two cropping years the total leaf yield per hectare per year was significantly ( $P < 0.001$ ) influenced by the cropping seasons, treatments and season  $\times$  treatments (Table 3). However, application of biochar (Mineral enriched) @ 1 t/hectare//yr with recommended basal dose (@ 660 kg urea, 330 kg TSP and 210 kg MP per hectare/year) of BSRTI gave the maximum average total leaf yield per hectare per year 49.6 mt. in 2<sup>nd</sup> year followed by rest of the treatments. On the contrary, the lowest average total leaf yield per hectare per year was 20.5 mt. in 2<sup>nd</sup> year for control treatment (Table 5, Table 6, Figure 5).

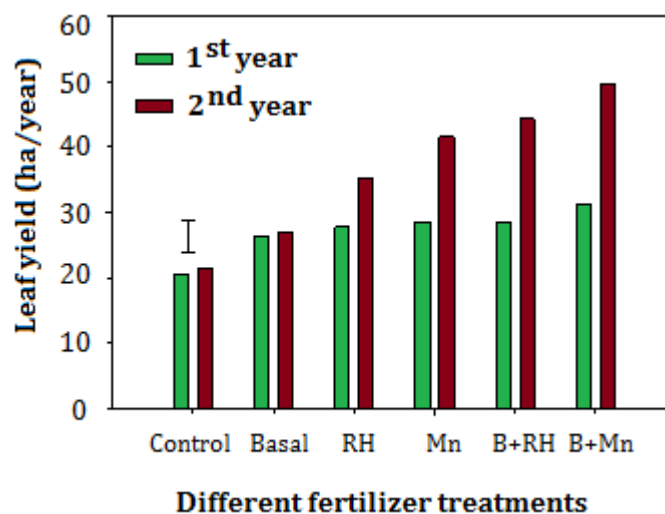


Figure 5. Effect of various fertilizer treatments on leaf yield of mulberry plant. Vertical bar represent LSD ( $P \geq 0.05$ ) for season and treatment interaction. RH indicates rice husk biochar, Mn indicates mineral enriched biochar and B means BSRTI recommended basal fertilizer

## Incidence of common mulberry diseases under organic fertilizers management

Common mulberry diseases regulated by organic fertilizer management. In general, mineral rich biochar has fewer incidences of common diseases as compared to other fertilizer management practices. The incidence percentage of Tukra disease was highest in control treatment for the first year. However, lowest incidence was found both in mineral enriched biochar as well as combination of recommended basal dose plus mineral enriched biochar. Moreover, incidence of Tukra disease in first year did not differ significantly ( $P > 0.05$ ) by the basal, rice husk biochar and recommended basal dose plus rice husk enriched biochar fertilizer treatments (Table 7). Similarly, in second year the highest occurrence of Tukra disease (17.1%) also found for the control treatment and lowest incidence was observed for the mineral enriched biochar as well as combination of recommended basal dose plus mineral enriched biochar. However, in second year the Tukra disease incidence was not also differ significantly ( $P > 0.05$ ) by the basal, rice husk biochar and basal dose plus rice husk biochar fertilizer treatments (Table 7).

Table 7. Average two years incidence percentage of common diseases under different fertilizer treatments

Treatments	Mean diseases incidence percentage					
	Tukra		Powdery mildew		Leaf spot	
	1 <sup>st</sup> year	2 <sup>nd</sup> year	1 <sup>st</sup> year	2 <sup>nd</sup> year	1 <sup>st</sup> year	2 <sup>nd</sup> year
Control	12.1 <sup>a</sup>	17.1 <sup>a</sup>	17.1 <sup>a</sup>	20.1 <sup>a</sup>	13.4 <sup>a</sup>	15.6 <sup>a</sup>
Basal dose	9.8 <sup>b</sup>	10.2 <sup>b</sup>	13.8 <sup>b</sup>	11.0 <sup>b</sup>	11.5 <sup>bc</sup>	12.5 <sup>b</sup>
Biochar (Rice Husk)	9.3 <sup>b</sup>	9.4 <sup>b</sup>	12.5 <sup>bc</sup>	10.2 <sup>b</sup>	11.8 <sup>ab</sup>	10.8 <sup>c</sup>
Biochar (Mineral enriched)	6.4 <sup>c</sup>	7.1 <sup>c</sup>	10.4 <sup>c</sup>	7.9 <sup>c</sup>	7.6 <sup>d</sup>	7.9 <sup>d</sup>
Basal + biochar (Rice Husk)	9.5 <sup>b</sup>	10.5 <sup>b</sup>	11.3 <sup>c</sup>	10.0 <sup>b</sup>	10.0 <sup>c</sup>	10.2 <sup>c</sup>
Basal + biochar (Mineral enriched)	6.1 <sup>c</sup>	7.2 <sup>c</sup>	10.8 <sup>c</sup>	8.3 <sup>c</sup>	10.3 <sup>bc</sup>	9.1 <sup>c</sup>

In case of Powdery mildew disease in first year the maximum incidence percentage was found (17.1%) in control treatment. In contrast, the minimum occurrence of powdery mildew disease was recorded both for the mineral enriched biochar as well as combination of recommended basal dose plus mineral enriched biochar treatments. Similarly, in first year the basal, rice husk biochar and recommended basal dose plus rice husk biochar treatments also did not significantly ( $P > 0.05$ ) differ (Table 7). Likewise, in second year the highest occurrence of powdery mildew disease was also observed for the control treatment but the lowest incidence was recorded for mineral enriched biochar as well as combination of recommended basal dose plus mineral enriched biochar. The rest of the treatments like basal, rice husk biochar and basal dose plus rice husk biochar were not significantly ( $P > 0.05$ ) differ for the powdery mildew occurrence percentage (Table 7).

The highest incidence of leaf spot disease was found in control as well as rice husk biochar in first year. In contrast, the lowest occurrence of leaf spot disease was observed only for the mineral enriched biochar but the other treatments like basal, basal plus rice husk biochar and basal plus mineral rice biochar were not significantly ( $P > 0.05$ ) differ among them (Table 7). In second year regarding the incidence percentage the maximum occurrence of leaf spot disease was also recorded for control treatment and minimum occurrence was found for the mineral enriched biochar treatment. However, the treatments basal, basal plus rice husk enriched biochar and basal plus mineral enriched biochar were not significantly ( $P > 0.05$ ) differ for the incidence percentage of leaf spot disease (Table 7).

## Discussion

### Changes of soil nutrient concentration response to different fertilizers

Soil nutrient availability increased due to biochar amendment within soil. Biochar application improved soil nutrient availability by rising of soil pH, organic matter, phosphorus, sulphur and zinc contents percentage in within soil (Table 4). Though the soil pH was not significantly increased but the average soil pH was increased due to the soil treated with only the biochar (mineral enriched and rice husk). Our results indicate that most of soil chemical properties increased due to biochar amendment within soil except nitrogen and potassium. Similarly, a study conducted by [Oguntunde et al. \(2004\)](#) found that the soil treated with biochar significantly increased soil pH, electrical conductivity (EC), exchangeable Ca, Mg, K, Na and P in compared to the control soil. They also found that the relative K change observed up to 329% while organic C and total N decreased by 9.8% and 12.8%, respectively. They recommended that organic C and total N were highly correlated ( $P < 0.01$ ) and both the parameters significantly ( $P < 0.05$ ) depended on clay contents in soils. In



addition due to the dominating effect of biochar residues while its reduction in soil organic C and total N contents depend on the effects at the severe fire during pyrolysis. The soil pH, available P, electrical conductivity, base saturation, exchangeable K, Ca, Mg and Na were significantly higher in biochar treated soil than the control soil. Likewise, [Dume et al. \(2016\)](#) found that biochar increased the soil pH, electric conductivity (EC), cation exchange capacity (CEC), organic carbon (OC), organic matter (OM), total nitrogen (TN), exchangeable cations and available phosphorus of the soil. They speculated that in biochar had a displacement of exchangeable acidity and high buffering capacity consequentially soil pH increased, high organic matter contents and enhanced decomposition rate when added biochar in soil in terms enriched the soil organic matter and also biochar attributed the improvement of soil pH which ultimately reduce the activity of Fe and Al resulting the phosphorus availability increased in soil due to the Biochar treated soil.

Our speculation is that biochar directly contains high amount of soluble nutrients (macro and micro), ash containing organic matter and some exchangeable elements. Besides, biochar itself has a cation exchange and high buffering capacity with high decomposition rate of organic matter. As a result the soil treated with the biochar both as a single dose or combined application with recommended basal dose of BSRTI absorbed maximum nutrients as per requirements, organic matter decomposition rate increased resulting improved the soil status and significantly increased the soil properties than the other treatments.

### **Biochar amendments enhances mulberry plant growth and yield**

Biochar amendments along with BSRTI recommended basal dose improved the growth and yield of mulberry plant. Our findings showed that the total leaf yield per hectare per year were respectively increased 142.1 % and 115.9 % in addition of 1 ton mineral enriched biochar and 1 ton rice husk biochar in soil respectively with BSRTI recommended basal dose of NPK than the control treatment. However, there was no study available in literature about combined application of biochar and inorganic fertilizer for growth and yield of mulberry plant. Some studies available in literature for combined application of inorganic fertilizer and biochar. [Igarashi \(2002\)](#) reported that rice husk biochar application increased the yield of maize, soybean and peanut in Indonesia. Likewise, [Lehmann et al. \(2006\)](#) found that addition of high rates of biochar in the tropical environment have been associated with increase plant uptake of P, K, Ca, Zn and Cu. This could be due to the fact that increased nutrient uptake from combined biochar and inorganic fertilizer can result mulberry plant growth and yield. Likewise, biochar (mineral enriched and rice husk) with combination of recommended basal dose of BSRTI may more capable to improve the growth and development of mulberry plant. It could be due to the reason that biochar (mineral enriched and rice husk) contains most of the plant nutrients (macro and micro). Regardless of that our biochar amendment increased some of the soil properties like organic matter, sulphur, zinc and phosphorus content in soil which may improve the soil texture, water holding capacity, soil aggregation, soil moisture retention. This causes improved nutrient uptake status by mulberry plants. As a result, mulberry plant growth and yield increased due to biochar amendment.

### **Effect of time on biochar decomposition**

Biochar addition along with BSRTI recommended basal dose increased the mulberry leaf yield in the second year comparatively to the first year. Our findings showed that biochar treated plot along with BSRTI recommended basal dose produced highest leaf yield 49.6 metric ton/hectare/year in 2<sup>nd</sup> year which was comparatively high than the 1<sup>st</sup> year leaf yield 31.1 metric ton/hectare/year. Likewise, [Major et al. \(2010\)](#) found that a single application of 20 t ha<sup>-1</sup> biochar to a Colombian savanna soil resulted in an increase in maize yield by 28 to 140% as compared with the unamended control in the 2<sup>nd</sup> to 4<sup>th</sup> years after application. They stated that decomposition rate of biochar can increase in 4<sup>th</sup> year as compared to 2<sup>nd</sup> year that results increase in maize production. However, our speculation is that combined application of biochar with BSRTI, recommended basal dose provides a suitable habitat for a large and diverse group of soil microorganisms in respect of point in time. This could be due to the reason that microbial decomposition of biochar and nutrients availability comparatively high in second year than the first year. As a result the growth and development of mulberry plant was comparatively advanced in second year than the first year in biochar treated plot with BSRTI recommended basal dose of NPK fertilizers than the others.

### **Application of biochar in soil reduces common diseases in mulberry plant**

Biochar application to soil diminishes the incidence of common diseases in mulberry plant. Our findings showed that the use of mineral enriched biochar within soil reduced about 50% the occurrence of common mulberry diseases (Table 7). This reduction of diseases happens could be due to the availability of all

essential macro and micro nutrients within mineral rich biochar. Our speculation can be verified by [Huber and Graham \(1999\)](#). They stated that essential nutrients are vital for plant growth, development, soil microbial activities and most important factors for diseases control. They also mentioned that all the essential nutrients can affect disease severity of plant.

Further, common diseases like Tukra, Powdery mildew and Leaf spot were occurred simultaneously both two years in our mulberry plant. Tukra, powdery mildew and leaf spot occurrence was significantly high in control where no inputs were applied. In first year significantly the lowest average Tukra, Powdery mildew and Leaf spot disease incidence percentage were 6.1, 10.4 and 7.6 respectively under mineral enriched biochar and in second year the lowest average incidence percentage were 7.1, 7.9 and 7.9 respectively. Mineral enriched biochar may be due to increased levels of soil microbial activity leading to increased competition and antagonism in the rhizosphere may be contributing factors for reduction of Tukra, Powdery mildew and Leaf spot disease incidence.

Very few studies have addressed for the potential of Biochar soil amendment to impact plant resistance to disease pathogen. However, similarly [Elad et al. \(2010\)](#) found that the severity of diseases caused by necrotrophic (*Botrytis cinerea*) and biotrophic (*Oidiopsis sicula* (originally referred to according to its teleomorph name: *Leveillula taurica*)) foliar pathogens in pepper and tomato was significantly reduced in biochar-amended treatments. Likewise, [Harel et al. \(2012a; 2012b\)](#) found that Biochar addition to the potting medium of strawberry plants suppressed foliar diseases caused by fungi having very different infection strategies: necrotrophic (grey mould, *Botrytis cinerea*), semi-biotrophic (anthracnose, *Colletotrichum acutatum*), and biotrophic (powdery mildew, *Podosphaera aphanis*). Our speculation, Biochar influence microbial populations which increase in beneficial microorganisms that directly protect against pathogens by producing antibiotics, by out-competing the pathogens, or by grazing on the pathogens. In addition, chemical compounds in the residual tars that are added to the soil with the biochar may have direct toxic effects on soil pathogens which is more or less closely related with the findings of [Graber et al. \(2010\)](#). They identified a number of biochar compounds that are known to adversely affect microbial growth and survival. These include ethylene glycol and propylene glycol, hydroxy-propionic and butyric acids, benzoic acid and o-cresol, quinones (recorsinol and hydroquinone), and 2-phenoxyethanol. Low levels of these toxic compounds could suppress sensitive components of the soil microbiota thereby resulting in proliferation of resistant microbial communities. [Graber et al. \(2010\)](#) also isolated a number of bacteria with high 16S rRNA gene sequence identity to known biocontrol agents, induced resistance agents and growth promoters (15 out of 20 total isolates) from the root zone of biochar-amended pepper plants where promotion of plant growth and induction of systemic resistance against fungal foliar diseases occurred simultaneously ([Elad et al., 2010; Graber et al., 2010](#)). Besides, [Bernardet and Bowman \(2006\)](#) also reported that biochar strongly induced the bacteroidetes-affiliated genus flavobacterium, which is widely distributed in nature; commonly possess an arsenal of extracellular enzymes such as proteinases and chitinases which enable them to degrade bacteria, fungi, insects, and nematode constituents. Our another rumor that the applied biochar improved soil nutrient and produces direct antibiotic against micro organisms, which reduced the common diseases incidence. Similarly, [Graber et al., \(2010\)](#) reported biochar improved plant nutrition and Microorganisms which excel at degrading toxic organic contaminants generally are more resistant to a variety of toxic organic compounds. Also, antibiotic and volatile organic compound producers are often resistant to a multitude of antibiotics ([Nodwell, 2007; Laskaris et al., 2010](#)). Antibiotic producers (*Pseudomonas mendocina* and *P. aeruginosa* strains) were identified in biochar-amended soil ([Graber et al., 2010](#)).

## Conclusion

This study demonstrated that the application of biochar in soil either with or without BSRTI recommended basal dose of N P K fertilizers can improve soil fertility and productivity, decrease the disease incidence and increase the leaf productivity in mulberry plant. The mulberry leaf production, soil properties and disease incidence rate was significantly differed from first year to second year due to addition of Biochar in soil either with or without BSRTI recommended basal dose. However, the highest leaf yield was found in combined application of Biochar (mineral enriched) with BSRTI recommended basal dose. Comparatively the lowest incidence of powdery mildew, tukra and leaf spot disease were found in second year than first year. Further study will be conducted for wheat plant to quantify the effect of biochar for soil fertility and crop productivity.

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