International Journal of Plant & Soil Science

Volume 36, Issue 4, Page 174-183, 2024; Article no.IJPSS.113818
ISSN: 2320-7035

Effect of Mulberry Stalk Biochar, FYM and Humic Acid on Growth, Yield and Quality of Mulberry (*Morus alba* L.)

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Authors' contributions

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

Article Information

DOI: 10.9734/IJPSS/2024/v36i44466

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/113818

Original Research Article

Received: 24/12/2023
Accepted: 28/02/2024
Published: 01/03/2024

ABSTRACT

A field experiment was conducted in a mulberry crop to investigate the impact of soil application of mulberry stalk biochar with FYM and foliar spray of humic acid on the growth, yield and quality of mulberry at a farmer's field in Mylandlahalli Village, Chintamani Taluk, Chikkaballapura District. The experiment was set up in a randomized complete block design with eight treatments, each replicated thrice. Result of the experiment revealed that among the various treatments applied, the combined application of biochar with FYM and humic acid (T_8: Soil application of biochar @ 10 t ha^{-1} + FYM @10 t ha^{-1} + 2% Humic acid foliar spray) had significant effect on plant height (196.10 cm), number of shoots per plant (15.06), number of leaves per shoot (31.78), leaf yield (26.22 t ha^{-1}),

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crude protein (20.28%) and lower crude fibre (1.56%). The next best treatments were T_7 (Soil application of biochar @ 7.5 t ha^{-1} + FYM @10 t ha^{-1} + 1% foliar spray of humic acid) and T_6 (Soil application of biochar @ 5 t ha^{-1} + FYM @10 t ha^{-1} + 0.5% Humic acid foliar spray) and these treatments were statistically comparable but significantly superior to the package of practice (POP).

Keywords: Mulberry; biochar; humic acid; growth; yield; quality; combined application; cultivation; agricultural residues; mulberry plants.

1. INTRODUCTION

The cultivation of mulberry (*Morus alba* L.) plays a pivotal role in the sericulture industry, serving as the primary feed for silkworms (*Bombyx mori*). The success of sericulture is intricately linked to the health and productivity of mulberry plants, making it imperative to explore sustainable agricultural practices that enhance growth, yield and quality. In this context, the integration of biochar and humic acid into mulberry cultivation emerges as a promising avenue, as these amendments have shown considerable potential in improving soil health and plant performance in various cropping systems (Srinivasarao et al., 2013).

Biochar, a carbon-rich material produced through the pyrolysis of organic matter, has gained attention for its ability to enhance soil structure, water retention and nutrient availability. Mulberry stalk biochar, derived from the agricultural residues of mulberry plants, not only serves as a valuable waste management strategy but also presents an opportunity to harness the beneficial properties of biochar for mulberry cultivation. Ali et al. (2018) observed that the application of biochar showed that the addition of mulberry stalk biochar to the soil has the potential to influence plant growth, yield and the overall quality of mulberry leaves, thereby impacting the sericulture industry positively.

Humic acid, a complex mixture of organic molecules derived from the decomposition of plant and animal residues, is another amendment known for its positive effects on soil fertility and plant development. The unique chemical composition of humic acid contributes to improved nutrient uptake, root development and stress tolerance in plants. Integrating humic acid into mulberry cultivation holds promise for enhancing the physiological processes of mulberry plants, potentially leading to increased productivity and improved leaf quality.

The synergy between mulberry stalk biochar and humic acid in agricultural systems has been a subject of growing interest, driven by the need for sustainable and environmentally friendly practices. These amendments, when applied together, have the potential to create a conducive soil environment that promotes optimal root development, nutrient assimilation and overall plant growth. Additionally, the combination of biochar and humic acid may contribute to enhanced water-use efficiency, an essential factor in regions where water resources are limited.

Despite the potential benefits, there is a paucity of comprehensive studies focusing on the joint influence of mulberry stalk biochar and humic acid on mulberry cultivation. This research aims to address this gap by systematically investigating the impact of different levels of mulberry stalk biochar and humic acid on the growth parameters, yield and quality of mulberry plants. Through a randomized complete block design with carefully selected treatments and replications, we aim to provide valuable insights into the synergistic effects of these amendments on mulberry cultivation.

2. MATERIALS AND METHODS

The experiment was conducted on a farmer's field located in Mylandlhalli Village Chintamani Taluk, Chikkaballapur District, Karnataka, India. This region is part of the Eastern Dry Zone of Karnataka, which is designated as Agroclimatic Zone No. 5. The field is situated at 13^o 36' North latitude and 77^o 43.49' East longitude, with an altitude of 915 meters above mean sea level. The experiment was designed using a randomized complete block design, replicated three times and consisted of eight treatments. Mulberry (Victory V1 variety) was chosen as the test crop for the study. The treatment details are provided below.

"A surface soil sample (0-15 cm depth) was collected before the experiment began. The initial soil status of the experimental site of the experimental plot was sandy loam with a pH of 6.64 and an electrical conductivity of 0.21 dS m^{-1}" [1].

List 1. The treatment details are provided below

Treatments	Details
T_1	Control
T_2	POP (FYM (25 t ha^{-1}) + N :P_2O_5 :K_2O 350:140:140 kg ha^{-1})
T_3	Soil application of biochar @ 5 t ha^{-1} + 0.5% Humic acid foliar spray
T_4	Soil application of biochar @ 7.5 t ha^{-1} + 1% Humic acid foliar spray
T_5	Soil application of biochar @ 10 t ha^{-1} + 2% Humic acid foliar spray
T_6	Soil application of biochar @ 5 t ha^{-1} + FYM @ 10 t ha^{-1} + 0.5% Humic acid foliar spray
T_7	Soil application of biochar @7.5 t ha^{-1} + FYM @ 10 t ha^{-1} + 1% Humic acid foliar spray
T_8	Soil application of biochar @ 10 t ha^{-1} + FYM @ 10 t ha^{-1} + 2% Humic acid foliar spray

** NPK 350:140:140 kg ha^{-1} is common for all the treatments*

2.1 Production of Biochar

The mulberry stalk generated as waste residue after leaf harvest in the farmer's field was collected and air dried. Producing biochar from mulberry stalks via pyrolysis is an eco-friendly process. Mulberry stalks, a readily available agricultural byproduct, are first prepared by cutting them into uniform pieces. These prepared stalks are loaded into a pyrolysis unit designed for oxygen-free conditions. The pyrolysis process heats the stalks, breaking them down into biochar (a stable carbon-rich material), bio-oil (a liquid byproduct) and syngas (a gas mixture). After carbonization, the biochar was collected and ground to a fine powder and used for the field experiment.

2.2 Characterization of Biochar

"The biochar was characterised by various standardized analytical procedures for its specific physicochemical properties such as bulk density, water holding capacity, pH, EC and total elements composition" [1]. The powdered mulberry stalk biochar was tested for various chemical parameters and findings are shown in Table 1.

The data shown in Table 1 revealed that the mulberry stalk biochar has recorded a bulk density of 0.34 Mg m^{-3} and a water holding capacity of 95.05 per cent. "The chemical composition of biochar was found to be alkaline in nature with a pH of 8.53 and an electrical

Table 1. Characterization of physicochemical properties of mulberry stalk biochar

Physical properties	
Parameters	**Value**
Bulk density (Mg m^{-3})	0.34
WHC (%)	95.05
Chemical properties	
pH (1:10)	8.53
EC (1:10) dS m^{-1}	0.39
C (%)	72.18
N (%)	0.83
P (%)	0.35
K (%)	0.98
Ca (%)	0.68
Mg (%)	0.43
S (%)	0.15
Fe (mg kg^{-1})	493
Mn (mg kg^{-1})	98.02
Zn (mg kg^{-1})	38.68
Cu (mg kg^{-1})	29.09
B (mg kg^{-1})	33.5

conductivity of 0.39 dS m^{-1}. The total carbon content of 72.18 per cent was recorded and nitrogen, phosphorus and potassium were recorded at 0.83, 0.35 and 0.98 per cent, respectively. It also recorded a good amount of calcium, magnesium and sulphur with the tune of 0.68, 0.43 and 0.15 per cent, respectively. It also recorded appreciable quantities of iron, zinc, manganese, copper and boron to an extent of 493, 38.68, 98.02, 29.09 and 33.5 mg kg^{-1}, indicating sustainability for improving physic-chemical properties in the soil" [1]

3. RESULTS AND DISCUSSION

3.1 Growth Parameters

Plant height**:** Plant height in mulberry crop was significantly influenced by soil application of biochar in combination with FYM and humic acid, values ranged (Table 2) from 145.63 to 196.10 cm at harvest.

A perusal of the data revealed that the plant height increased progressively from 30 DAP up to the harvest stage. Among the different treatments, the highest plant height at 30 DAP (102.24 cm), 45 DAP (130.38 cm) and 60 DAP (161.24) was recorded in the treatment T_8 with combined soil application of biochar @ 10 t ha^{-1} + FYM @ 10 t ha^{-1} + 2% foliar spray of humic acid which was significantly superior to all other treatments except T_7 (soil application of biochar @ 7.5 t ha^{-1} + FYM @ 10 t ha^{-1} + 1% foliar spray of humic acid) and T_6 (soil application of biochar @ 5 t ha^{-1} + FYM @ 10 t ha^{-1} + 0.5% foliar spray of humic acid) compared to package of practise (POP). The lowest plant height at 30 DAP (71.67 cm), 60 DAP (103.78 cm), 90 DAP (128.73 cm) and at harvest (145.63 cm) was recorded in control (NPK alone). Application of biochar in combination with FYM and humic acid increased the plant height with an increase in crop period. The soil application of biochar in combination with FYM and humic acid showed an increase in plant height at all growth stages. Application of biochar and humic acid to the soil promotes vegetative growth by improving photosynthetic pigment production, rapid synthesis of protoplasm and increase in cell size which ultimately influence the vegetative growth and which is expressed morphologically through an increase in plant height. The beneficial effects of biochar application were attributed to a shift in the microbial community to plant growth promoting rhizobacteria and fungi agrees with Dong et al. [2] and Graber et al. [3]. Kalyani et al. [4] also reported that the combination of organic manure with biochar increased the plant height and number of leaves of beans, fenugreek and mint compared to compost alone application.

Number of shoots per plant: Application of biochar with FYM and humic acid significantly influenced the number of shoots per plant at different growth stages of mulberry and data is presented in Table 3.

Table 2. Effect of mulberry stalk biochar on plant height (cm) of mulberry at different growth stages

Treatments	30 DAP	45 DAP	60 DAP	Harvest
Control ($N:P_2O_5:K_2O$ 350:140:140 kg ha^{-1})	71.67	103.78	128.73	145.63
POP (FYM (25 t ha^{-1}) + N :P_2O_5 :K_2O 350:140:140 kg ha^{-1}))	96.61	113.10	146.32	163.95
Soil application of biochar @ 5 t ha^{-1} + 0.5% Humic acid foliar spray	94.95	108.23	141.65	160.33
Soil application of biochar @ 7.5 t ha^{-1} + 1% Humic acid foliar spray	96.70	113.35	146.78	165.44
Soil application of biochar @ 10 t ha^{-1} + 2% Humic acid foliar spray	96.88	116.08	150.97	168.51
Soil application of biochar @ 5 t ha^{-1} + FYM @ 10 t ha^{-1} + 0.5% Humic acid foliar spray	98.66	121.15	156.09	182.79
Soil application of biochar @7.5 t ha^{-1} + FYM @ 10 t ha^{-1} + 1% Humic acid foliar spray	100.90	125.24	159.07	189.90
Soil application of biochar @ 10 t ha^{-1} + FYM @ 10 t ha^{-1} + 2% Humic acid foliar spray	102.24	130.38	161.24	196.10
S.Em±	2.47	3.07	3.92	4.51
CD @ (5%)	7.49	9.32	11.90	13.67

** NPK 350:140:140 kg ha^{-1} is common for all the treatments DAP- Days After Pruning*

Table 3. Effect of mulberry stalk biochar on the number of shoots per plant at different growth stages

Treatments	30 DAP	45 DAP	60 DAP	Harvest
Control (N:P_2O_5:K_2O 350:140:140 kg ha^{-1})	9.63	9.90	10.71	8.31
POP (FYM (25 t ha^{-1}) + N :P_2O_5 :K_2O 350:140:140 kg ha^{-1})	10.02	10.87	11.48	8.51
Soil application of biochar @ 5 t ha^{-1} + 0.5% Humic acid foliar spray	9.23	9.63	10.51	9.49
Soil application of biochar @ 7.5 t ha^{-1} + 1% Humic acid foliar spray	9.67	10.97	11.60	9.84
Soil application of biochar @ 10 t ha^{-1} + 2% Humic acid foliar spray	9.87	11.39	11.83	10.29
Soil application of biochar @ 5 t ha^{-1} + FYM @ 10 t ha^{-1} + 0.5% Humic acid foliar spray	10.69	11.82	12.07	10.76
Soil application of biochar @7.5 t ha^{-1} + FYM @ 10 t ha^{-1} + 1% Humic acid foliar spray	11.08	12.10	13.62	11.33
Soil application of biochar @ 10 t ha^{-1} + FYM @ 10 t ha^{-1} + 2% Humic acid foliar spray	11.49	13.50	14.56	11.93
S.Em±	0.27	0.29	0.32	0.34
CD @ (5%)	0.83	0.87	0.96	1.04

** NPK 350:140:140 kg ha^{-1} is common for all the treatments DAP- Days After Pruning*

The number of shoots per plant at 30 DAP ranged from 9.63 to 11.49. The highest number of shoots per plant (11.49) was recorded in the treatment T_8 with the application of biochar @ 10 t ha^{-1} + FYM @ 10 t ha^{-1} + 2% foliar spray of humic acid which was on par with the treatment T_7(11.08) and T_6 (10.69). The lowest number of shoots per plant was recorded in control (9.63).

Different treatments significantly influenced the number of shoots per plant in mulberry at 60, 90 DAP and at harvest. The treatment T_8 which received soil application of biochar @ 10 t ha^{-1} + FYM @ 10 t ha^{-1} + 2% foliar spray of humic acid at 30 DAP (11.49), 45 DAP (13.50), 60 DAP (14.56) and at harvest (15.06) significantly recorded the highest number of shoots per plant and it was at par with T_7 and T_6 but significantly superior to other treatments. The significantly lower number of shoots at 30 DAP (9.63), 45 DAP (9.90), 60 DAP (10.71) and at harvest (11.10) was recorded in control (NPK alone). The highest numbers of shoots were recorded in treatment (T_8) biochar @ 10 t ha^{-1} + FYM @ 10 t ha^{-1} + 2% foliar spray of humic acid. This might be attributed to responses to improved physical character of the soil in a way that benefits root growth and or nutrient and water retention and acquisition provided by the biochar with FYM and humic acid application. This confirmed the findings of Qassem et al. [5] in cucumber.

Number of leaves per shoot: The number of leaves per shoot in mulberry crop differed significantly at 30, 45, 60 DAP and at harvest due to soil application of biochar at different levels (Table 4).

The number of leaves per shoot increased gradually with the advancement of crop growth stages and the significantly highest number of leaves per shoot (15.18, 19.60, 25.68 and 31.78, respectively) at 30, 45, 60 DAP and at harvest were recorded in the treatment T_8 followed by T_7 and T_6. The lowest number of leaves at 30 DAS (10.45), 45 DAS (14.58), 60 DAS (20.77) and at harvest (26.64) was recorded in control. “The increase in the number of leaves might be due to the increased uptake of nutrients in the plants leading to enhanced chlorophyll content and carbohydrate synthesis that led to the increase in cell division and enlargement of the cell size which helped in increased plant height, stem girth and number of leaves. This significant increase in vegetative growth” Ameta et al. [6]. Foliar spray of humic acid improves plant metabolic activity, physiological and structural function *viz* increased photosynthesis and respiration rate, oxidative phosphorylation, protein synthesis and enzymatic reactions Al-madhagi [7] thereby increasing plant vigour and production of metabolites responsible for cell division, cell elongation and cell differentiation. In addition, applied humic acid might have provided some essential nutrient elements which were absorbed through leaves.

Table 4. Effect of mulberry stalk biochar on the number of leaves per shoot at different growth stages

Treatments	30 DAP	45 DAP	60 DAP	Harvest
Control ($N:P_2O_5:K_2O$ 350:140:140 kg ha^{-1})	10.45	14.58	20.77	26.64
POP (FYM (25 t ha^{-1}) + N :P_2O_5 :K_2O 350:140:140 kg ha^{-1}))	13.00	17.24	22.69	28.99
Soil application of biochar @ 5 t ha^{-1} + 0.5% Humic acid foliar spray	12.13	16.09	22.15	28.37
Soil application of biochar @ 7.5 t ha^{-1} + 1% Humic acid foliar spray	13.04	17.43	23.55	29.16
Soil application of biochar @ 10 t ha^{-1} + 2% Humic acid foliar spray	13.45	17.64	23.83	29.91
Soil application of biochar @ 5 t ha^{-1} + FYM @ 10 t ha^{-1} + 0.5% Humic acid foliar spray	14.37	18.46	24.55	28.21
Soil application of biochar @7.5 t ha^{-1} + FYM @ 10 t ha^{-1} + 1% Humic acid foliar spray	14.65	18.65	24.77	30.85
Soil application of biochar @ 10 t ha^{-1} + FYM @ 10 t ha^{-1} + 2% Humic acid foliar spray	15.18	19.60	25.68	31.78
S.Em±	0.35	0.46	0.62	0.75
CD @ (5%)	1.05	1.39	1.88	2.28

** NPK 350:140:140 kg ha^{-1} is common for all the treatments   DAP- Days After Pruning*

3.2 Yield of Mulberry

Application of different levels of biochar in combination with FYM and humic acid significantly influenced the leaf yield and the values ranged from 21.14 to 26.22 t ha^{-1} and data is presented in Fig. 1.

Combined soil application of biochar @ 10 t ha^{-1} and FYM @ 10 t ha^{-1} (T_8) recorded higher leaf yield per hectare (26.22 t ha^{-1}) and it was on par with T_7 (25.31 t ha^{-1}) and T_6 (24.42). The lowest leaf yield of 21.14 t ha^{-1} was recorded in control which was devoid of biochar. Among different treatments, with increased level of biochar in combination with FYM and humic acid application increased the leaf yield. This might be due to an increase in the rate of biochar which increases the moisture content and nutrient supply in soil. An increase in leaf yield with the 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 Fasiha and Devakumar [8].

3.3 Quality of Mulberry Leaf

Varied amounts of crude protein and crude fibre contents were noticed in mulberry crop among different treatments (Fig. 2). However, the crude protein increased and the crude fibre content decreased with increased levels of biochar application in combination with FYM and humic acid. Maximum crude protein (20.28%) and minimum crude fibre (1.56%) were recorded in T_8 (Soil application of biochar @ 10 t ha^{-1} + FYM @ 10 t ha^{-1} + 2% foliar spray of humic acid). The next best treatment was T_7 which showed 19.87% of crude protein and 1.74 % of crude fibre followed by T_6 which recorded 19.42 per cent of crude protein and 1.94 of crude fibre percentage. The improvement in nutritive value (more protein; less fibre) as a result of soil amendment with biochar is especially promising. A long term field demonstration in Canada, in which biochar was applied at 3.9 t $ha^{-1}$to mixed grass-clover forage plots Husk and Major [9] Ty et al. [10] demonstrated "increased yields of forage (4.1%) in the third year and associated increases in nutritive value (Crude protein increased by 10%, crude fibre decreased by 5.9%). These changes in botanical composition would explain part of the changes in nutritive value. Plant fibre refers to the cell-wall constituents of hemicelluloses, cellulose and lignin. The Neutral Detergent Fibre (NDF) values represent the total fibre fraction that makes up cell walls. For forage quality, the lower the NDF value, the better (-5.9% with biochar in this case). Fat (+5.3%) and starch (+2.9) content are both higher in plants from the biochar-amended plot, contributing to the higher overall plant nutrient energy value. The higher starch content is most likely associated with the lower fibre content".

Fru et al. [11] and Rohitha et al. [12] reported that "the increase in protein content in mulberry might be due to the increased availability of N and its uptake and storage in the leaf and also due to the recalcitrant nature of the biochar which was influenced by the high pyrolysis temperatures during the production process".

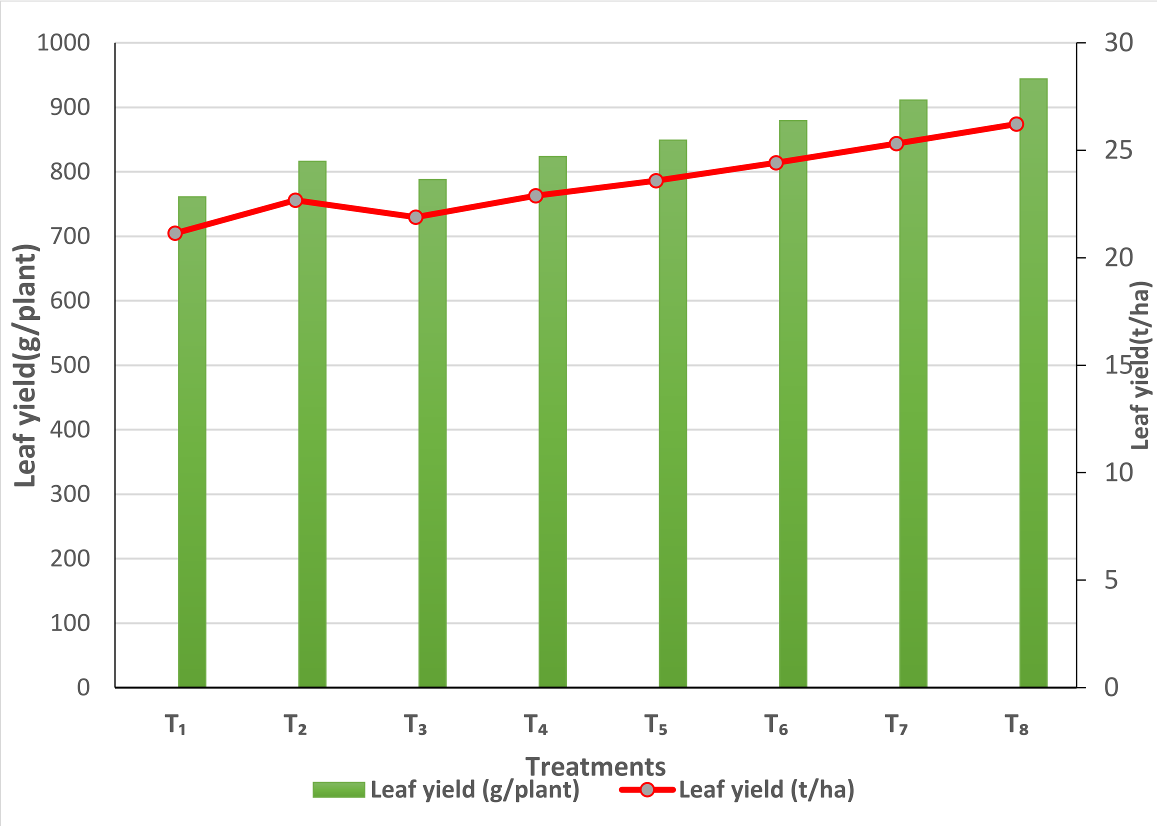

Fig. 1. Effect of soil application of mulberry stalk biochar on leaf yield of mulberry

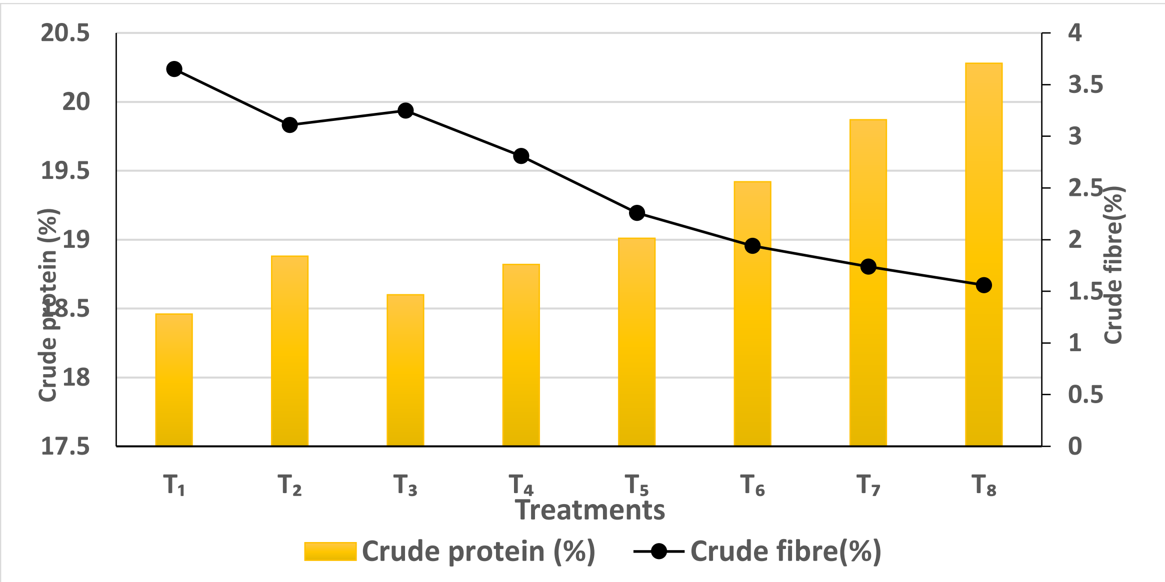

Fig. 2. Effect of soil application of mulberry stalk biochar on crude protein and crude fibre of mulberry leaf

3.4 Chlorophyll Content (mg g^{-1})

Data on the chlorophyll content of mulberry at different growth stages is presented in Table 4 and Fig 3 indicates that the application of biochar in combination with FYM and humic acid recorded significantly higher chlorophyll meter readings than POP treatments.

Significantly higher chlorophyll content (3.21, 4.96, 5.72 and 6.99 mg g^{-1}) was recorded in the treatment (T_8) which received soil application of biochar @ 10 t ha^{-1} and FYM @ 10 t ha^{-1} + 2% Humic acid foliar spray in the first, second and third crop cuttings compared to POP. Treatment T_8 was on par with treatment T_7 (3.14, 4.69, 5.58 and 6.83) and T_6 (3.04, 4.38, 5.41 and 6.75) The lower chlorophyll content was recorded in the first crop in control (NPK alone) (2.22, 3.42, 4.75 and 6.20). The chlorophyll content of leaves is a practical indicator of both potential photosynthetic productivity and general plant vigour, which is related to the N concentration in green plants. Biochar and humic acid application positively affects chlorophyll content and related parameters such as increased activity of PS II and facilitates electron transport, which boosts the total photosynthetic performance index. The increases in chlorophyll content are most likely linked to better availability of macro (K) and micronutrients (Fe, Mn, Zn) in the biochar and humic acid that play a fundamental role in the biosynthesis of chlorophyll and other pigments involved in the photosynthetic activity Netto et al., [13] and also application of biochar to soil might have mineralized most of its N and made plant available. "Nitrogen is a limiting nutrient in crop production and an increase in availability of N will increase plant height, number of shoots, number of leaves and chlorophyll content" [14].

Significantly higher SPAD reading upon application of humic acid might be attributed to increased chlorophyll content due to efficient absorption and uptake of N and Mg from soil which are essential components of chlorophyll. The increase in chlorophyll content increases the photosynthetic rate and efficiency of mulberry ultimately leading to better growth and yield of the crop. A similar increase in chlorophyll content upon application of humic acid was documented Avinash [15] in capsicum; Tejada et al. [16] Chaitra [17] in groundnut; Kiran [18] in maize.

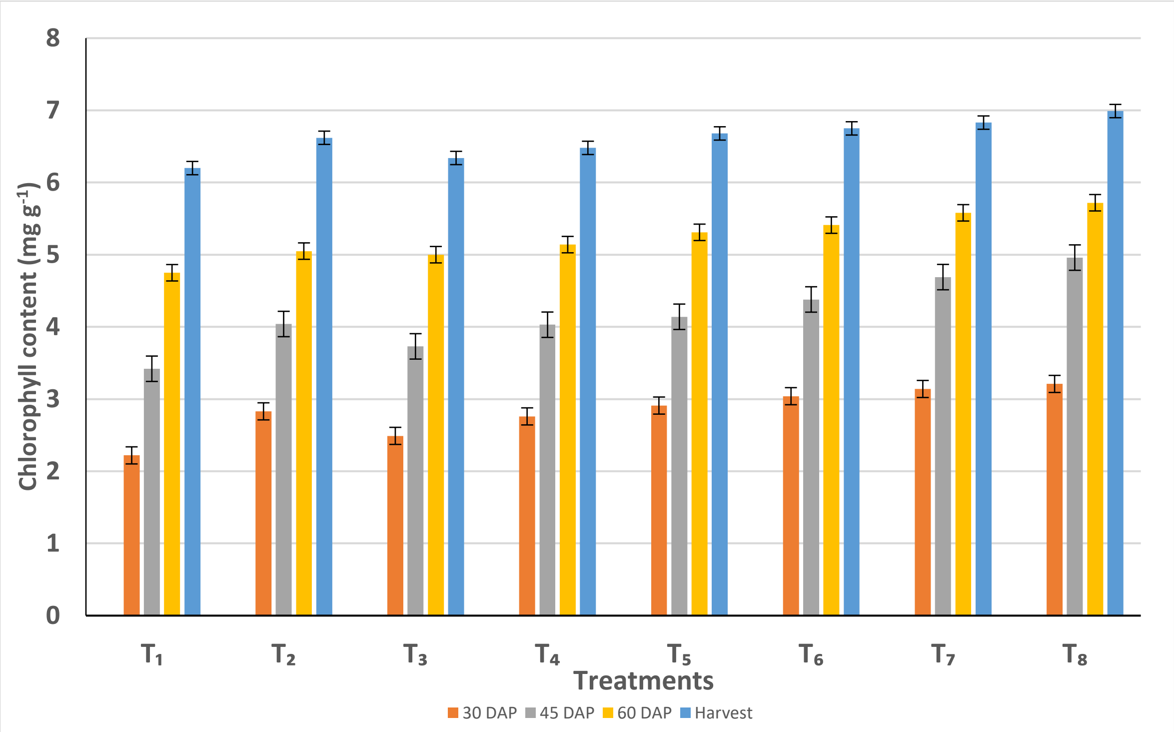

Fig. 3. Effect of mulberry stalk biochar on chlorophyll content (mg g^{-1}) of mulberry at different growth stages

4. CONCLUSION

Among the various treatments evaluated, the combination of biochar applied at a rate of 10 t ha^{-1}, FYM at 10 t ha^{-1} and 2% foliar spray of humic acid resulted in the most significant improvements in growth, yield and quality compared to the package of practise (POP). The findings underscore the effectiveness of integrating biochar and FYM applications in enhancing mulberry production. Specifically, the synergistic effects of biochar and FYM contributed to substantial improvements in growth and yield attributes. Furthermore, humic acid foliar spray accentuated these benefits, leading to superior quality outcomes. Overall, the combined approach of biochar and FYM application, complemented by humic acid supplementation, emerged as a promising strategy for optimizing mulberry cultivation, offering significant potential for enhancing agricultural productivity and sustainability.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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