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Research article

ENHANCING GROWTH OF SPINACIA OLERACEA BY *TYPHA ANGUSTATA* BIOCHAR

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ABSTRACT: Biochar as a kind of organic matter used as soil amendment to improve soil structures and fertility qualities. *Typha angustata* is an aquatic weed causing serious problems in drainage channels and conversion of it in to biochar is one of the ways to make it useful. In this study, biochar was prepared using batch reactor and the effects on soil and plant growth parameters were analyzed. Biochar was added to soil at 0, 50, 100 and 150g/kg and transpiration rate (E), stomatal conductance (gs), net photosynthesis (Pn) was measured in field using infra-red gas analyzer (IRGA). After 45 days biochar significantly increased the studied parameters. Results further showed that microbial biomass nitrogen was increased 41.67 % in treatment as compared to control. Total nitrogen, total organic carbon and organic matter were increased in soil amended with biochar, whereas pH and EC of soil were decreased due to the application of biochar. The results of the study were enormous helpful in identifying the potential of biochar of T*ypha angustata* to increase yield of *Spinacea oleracea.*
Key Words: Biochar, Spinach, *Typha angustata.*

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INTRODUCTION

Vegetables are significantly important in the human diet (Anjana *et al*., 2007). Leafy vegetable like Spinach (*Spinacea oleracea* L.) is an important source of minerals, vitamin A, vitamin C and minerals especially iron and it is low calorie food as well (Toledo *et al*., 2003). Spinach *(Spinacia oleracea L.)* is greatly responsive to nitrogen fertilization (Cantliffe, 1992; and it is one of the highest NO3 accumulator plants, due to a very efficient uptake system and inefficient reductive systems (Maynard et al., 1976) but clearly documented as high oxalate containing plant (Honow and Hesse, 2002). Moreover, it represents a good source of vitamins as well as minerals such as calcium and magnesium (NRC, 1984).

Application of chemical fertilizers alone to sustain high yield had not been successful because crop response to applied fertilizer depend on soil organic matter (Agboola and Omueti, 1982). Biochar in combination with fertilizer or alone could be an effective treatment to increase the plant growth and soil health (Chan *et al*., 2008). Plant dry matter has been well reported to increase by the application of biochar derived from wood chips (Glaser *et al.*, 2007). Biochar can act as a soil conditioner enhancing plant growth by supplying and, more importantly, retaining nutrients and by providing other services such as improving soil physical and biological properties (Glaser *et al*., 2002; Lehmann *et al.*, 2003). Other nutrient contents of biochar and Carbon depending on the type of supply of organic used (Gaskin *et al*., 2008). Crop yields increase when biochar apply to soil, reduces the leaching of nutrient sand stimulate soil microbial activity (Kolb *et al*., 2009; Jeffery *et al*., 2010; Singh *et al.*, 2010). Studies reported the influence of biochar on early stages of plant growth such as on seed germination and seedling growth.

Biochar application to agricultural soils has the potential for long term C sequestration, due to the stability of biochar in soil environments. Biochar is composed of a range of different forms of C, from recalcitrant aromatic ring structures, which can persist in soil for millennia, to more easily degradable aliphatic and oxidized C structures, which mineralize to CO_2 more rapidly through degradation by biotic and abiotic oxidation (Schmidt and Noack, 2000; Cheng *et al.*, 2006; Liang *et al.*, 2008). Increased CO_2 emissions, following biochar addition to soil, have been attributed to increased mineralization rates in the biochar-amended soil due to mineralization of applied biochar C (Major *et al*., 2010a; Smith *et al*., 2010) or enhanced soil organic C mineralization. Bokashi, Mokusakueki and Biochar have been found having positive effects on soil fertility and crop production.

Carbon sequestration is mainly done by production of biochar and this sequestered carbon is advantageous for soil amendment and high soil fertility (Day *et al.,* 2004). Soil amendment of biochar can also enhance crop yield resulting motivation of soil activities. This modification in the soil microbial community can consequently manipulate alterations in crop development in soil amend with biochar (Graber *et al*., 2010; Kolton *et al*., 2011). However, there has been limited attention paid to the influence of biochar on the important process of biological N_2 fixation in leguminous plants.

Biological carbon is a sensitive component of soil organic matter, which can serve as a vulnerable indicator of changes in soil environment (Goberna *et al*., 2006). MBC changes more quickly as compared to total organic matter due to change in land management practices (Yan *et al*., 2003) uses as environmental measures. It also acts as soil available nutrient and assimilates in microbial body with vegetation restoration from degraded soil which losses due to physical and chemical changes in soil ecosystem (Yuan *et al.*, 2011).

Typha angustata is a developing wild plant and causes severe harms in the water carriage ways of Pakistan. It is usually dispersed in water logged areas and along drainage/irrigation channels threatening to impede the flow of water and enhance evaporation.

Researchers documented this plant as beneficial in term of food, bedding, roofing and parts have been used to make baskets, rope, paper and shoes. Typha ointment has been used for treatment of measles, snakebite and many other disorders (Anon, 1981). Of all natural plants, Typha have been called the most helpful emergency foodstuff. They are also a source of pulp, paper and fiber (Morton, 1975). As a wide variety of plant biomass could be utilized for production of biochar so converting Typha angustata in to biochar could be an option to make it useful. Keeping in view the importance of this fertilizer in plants the current assessment was designed to assess the impact of *Typha angustata* biochar on the growth of *Spinacea oleracea* L and relationship of biochar application with soil organic carbon and soil properties, consequently the study would lead to solve the environmental issue through this problematic plant.

MATERIALS AND METHODS

Biochar Preparation

Biomass of *Typha angustata* was used for biochar production. For this purpose, *typha* plants were grown in the field of PMAS Arid Agriculture University Rawalpindi Pakistan. After collection, the selected were air dried for 9-10 days before pyrolysis. Biochar was prepared by pyrolysis in Batch Reactor System (Baggio *et al*., 2009). The 5 kg biomass of *typha* leaves was used to produce 2 kg biochar. Wood required for biochar preparation was 80 kg. Biochar was placed overnight for cooling and was removed next day. Then the biochar was crushed and passed through 2 mm sieve for final application.

Pot Experiment

A pot experiment was carried out in Research Area of Department of Environmental Sciences during winter, 2013. Plastic pots were taken and filled with 3kg of field soil. Seeds of *Spinacia oleracea* were sown in these pots. These seeds were grown for 2 months. After sowing soil analysis was done with the interval of 15 days.

Biochar Application

Biochar was applied in soil to observe the effect on plant growth. Randomized complete design was used as experimental design with three replications (CRD). The treatments were:

$T_{1=}$Control (0 g/kg soil) $T_{2=}$Biochar (50 g/kg soil)
$T_{3=}$Biochar (100 g/kg soil) $T_{4=}$Biochar (150 g/kg soil

Soil Analysis

pH

pH was measured by using multimeter (Model: $MM40^+$).

Electrical Conductivity (EC)

EC was measured by using multimeter (Model: $MM40^+$).

Organic matter

One gram air dried soil sample was taken. 20 ml concentrated sulphuric acid and 10 ml 1 N potassium dichromate was added. Suspension was mixed and left for 30 minutes. After that 10 ml concentrated orthophosphoric acid and 200 ml distilled water was added. Allowed the mixture to cool. Diphenylamine indicator was added in the form of drops. Soil samples were titrated against ferrous ammonium sulphate solution, until color changed from violet-blue to green. Two blanks were also prepared containing all reagents but no soil (Walkley, 1947). It was measured by formula

% organic matter = 1.724 × Total Organic Carbon %

Total Organic Carbon

Total organic carbon was analyzed by extraction and titration methods (Nelson *et al.,* 1982).

Soil sample was extracted with K_2S0_4 at 25 rpm for 60 min using horizontal shaker. After shaking, soil suspension was centrifuged at 2000 rpm for 10 min followed by filtration with filter paper to obtain soil free filtrate. Four ml soil extract was transferred in 100 ml conical flasks for titration. One ml of 0.066 M $K_2Cr_2O_7$ and 5 ml concentrated H_2SO_4 was added with continuous shaking. After cooling and adding 0.3 ml indicator, soil extract was titrated with acidified ferrous ammonium sulphate solution. The color of solution changed from green /violet to red. Blank titrant was also prepared as sample except adding soil extract.

TOC (%) calculated as:

$$TOC\ (\%)=\{(A*M*0.003/g*(E/S)*100$$

Where A is Blank sample, M is Molarity of Ferrous ammonium sulphate, g is soil used for extraction, E is Extraction volume, S is Sample volume.

Microbial Biomass Nitrogen (MBN)

Microbial biomass nitrogen was measured by modified method of rapid microwave (MW) irradiation and extraction method (Islam and Weil 1988) and colorimetric determination (Brookes *et al*., 1995).

Soil sample was divided into two sets of 10 gm of air dried soil. One set was exposed to 800 J g^{-1} energy Micro Wave oven (MW). After Micro Wave (MW) and cooling both Micro Waved (MW) and Un-Micro Waved (UMW) soil was extracted with 0.5 M K_2SO_4 (pH 7.O) using a horizontal shaking at 250 rpm for 60 min. After shaking , soil suspensions centrifuged at 2000 rpm for 10 min followed by filtration with (Whatman No.42 nitrate-free filter paper) to obtain free solution.

Soil extract of 2 ml of each from MW and UMW sample was taken and mixed with 1ml of 5% salicylic acid by continuous mixing. After half an hour 10 ml solution of sodium hydroxide was added in test tube mixed and kept for 60 minutes for the development of color. Spectrophotometer was used to measure the absorbance of sample and standard at 410 nm. An absorbance graph was plotted against standard concentration to calculate concentration of unknown samples. Using formula nitrate nitrogen of both microwave and un microwave was calculated.

The MBN was calculated as:

$$MBN = (MW\ N-UMW\ N)*1.46$$

Where MWN is nitrate nitrogen measured in microwave soil samples and UMWN is nitrate nitrogen measured in un-microwaved soil sample.

Total Nitrogen

Analysis of the total nitrogen was performed by method of Anderson and Ingram (1993). Reagents of specific amount i.e. 14 g Lithium to 350ml hydrogen peroxide and 0.42 g selenium powders to prepare digestion mixture. H_2SO_4 (Conc.) 420 ml was added while cooling on ice bath. In digestion tube soil was taken. 4.4 ml of digestion mixture was added and digested for 2 hours at 360 ºC temperature until it change to colorless. 50 ml water was added after cooling no sediment will be dissolved at last and increased up to 100 ml volumetrically with distilled water. A clear solution was taken for analysis.

Reagent N_1, reagent N_2 and NH_4^+-N Standard were prepared. For preparation of reagent N_1 in 750 ml of water, Sodium Citrate 25 g, Sodium Salicylate 34 g and 25 g Sodium Nitrate was added together. Reagent N_2 was prepared by dissolving 30g NaOH in 750 ml water. Preparation of NH_4^+-N standard was done by dissolving dry Ammonium Sulphate of 4.714 g in water and made final volume to 1000 ml. The other standards with concentration of 1, 1.5, 2, 2.5, 3, ppm were prepared from stock solution of NH_4^+-N.

Sample was colorimetrically determined by taking standards and 0.1 ml of digested sample into test tube. 5 ml Reagent N_1 5 ml was added into it placed for 15 minutes, in the same way 5 ml of the reagent N_2 was added into test tube and let it stand for change in color.

Determination was done by recording absorbance of sample and standard spectrophotometrically at 655 nm. Concentration of sample was calculated by plotting a graph between standard concentrations and standard absorbance. Following relation was used for measurement of corrected concentration:
C= Corrected value of concentration, W= weight of sample and V = Extract volume,

$$\text{Nitrogen \%} = \frac{C \times V}{W} \times 0.0001$$

Physiological Parameters

Transpiration Rate (E)

Rate of transpiration was measured weekly between 09:00 am and 12:00 pm with Infra Red Gas Analyzer IRGA (CIRAS 2). Rate of transpiration was measured to find out how much transpires.

Stomatal Conductance (gs)

Stomatal conductance (gs) was measured weekly between 09:00 am and 12:00 pm with Infra Red Gas Analyzer. The upper most fully expended leaf of each plant in all the treatment was used for conductance measurement. The leaf was held in the curvette of the gas analyzer and readings were noted.

Net Photosynthesis (Pn)

As net Photosynthesis of *Spinacia oleracea* for each treatment was measured weekly by using infrared gas analyzer (CIRAS-2). Net photosynthesis is a difference between total amount of photosynthesis and transpiration.

Plant Height

Height was measured from base to apex of the plant by using measuring tape. Plant height was also measured after fifteen days.

No. of Leaves

Number of leaves per plant were counted within different interval of days

Statistical Analysis

Through MS Excel 2007 one way analysis of variance (ANOVA) was used to analyze the data statistically at 5% level of significance.

RESULTS AND DISCUSSION

Soil Analysis

A study was conducted to determine the growth of *Spinacia oleracea* by applying biochar of *Typha angustata*. Soil was analyzed with the duration of 15, 30, and 45 days for the following parameters (e.g. pH, Electrical Conductivity, Microbial Biomass Nitrogen, Organic Matter and Total Organic Carbon).

pH

pH variation of soils in *Spinacia oleracea* with different biochar treatments compared to control is presented in Fig 1. pH variation between control and biochar treatments was differed significantly ($P<0.05$) and demonstrated a remarkable deccrease in pH under biochar treatments. pH increased in T_3 (10% biochar) after 30 days. The pH of soil was 6.9 initially in T3 and reached to 6.83 at the end of 45 days.

In T_1 (0 g/kg soil), maximum increase was observed after 30 day with 7.5 pH and maximum decrease was observed on 45 day with 7.1 pH. In T_2 (50 g/kg soil), maximum increase was observed on 15 day with 7.3 pH and decrease was observed on 45 day 6.9 pH. As above results showed decrease in pH under 15% biochar concentration but this decreased comparatively more than control, which showed a greater value after 30 days. Many studies show that biochar can change soil internal properties, include pH and bulk density (Tyron, 1948; Glaser *et al*., 2002; Lehmann *et al*., 2003; Gundale and DeLuca, 2006; DeLuca *et al*., 2006). Biochar amendment can develop soil productiveness, (by rising accessibility soil nutrients hence rising plant yield) (Lehmann *et al*., 2003; Blackwell *et al*., 2009).

Electrical Conductivity (EC)

The figure 2 demonstrated the variation in electrical conductivity of soil under different biochar treatments. Non significance difference was observed among the treatments ($p > 0.05$). Statistical analysis showed non significant variations with the interval of days (p=1.33). As observed by Nigussie *et al*. (2012) in their study biochar increases electrical conductivity of soil. Biochar contain high surface area for water retention and also increase the soil electrical conductivity.

All treatments T_2, T_3 and T_4 showed increasing trend with greater biochar concentrations. In T_2 (50 g/kg soil), maximum increase was observed on 45 day with 456 $\mu Scm^{-1.}$ In T_3 (100 g/kg soil), maximum increase was observed after 30 day with 600 $\mu Scm^{-1.}$ In T_4 (150 g/kg soil), maximum increase was observed on 45 day with 735 μScm^{-1}. Treatment T_1 (control), showed decline in EC with increasing continuous biochar concentrations. Tryon, (1948); Mikan and Abrams, (1995); Topoliantz *et al.* (2002) also reported the increase in EC in their studies with increased time duration.

Microbial Biomass Nitrogen (MBN)

The figure 3 demonstrated the variation in MBN of soil under different biochar treatments. Significance difference was observed among the treatments ($p > 0.05$). During the experiment reading were taken after fifteen days interval. Graphical representation depicts that biochar amended treatment shows slightly increase in the MBN of the soil over the control treatment and it shows that MBN of the soil increase with the time intervals. Herai *et al.* (2006) found that MBN in the soil is statistically significant with the biochar amendment.

All treatments T_2, T_3 and T_4 showed increasing trend with greater biochar concentrations. In T_2 (50 g/kg soil), maximum increase was observed on 45 days with 36.95 % MBN. In T_3 (100 g/kg soil), maximum increase was observed on 30 days with 43.78 % MBN. In T_4 (150 g/kg soil), maximum increase was observed on 30 day with 41.67 % MBN. Biochar concentration showed high MBN after 30 day as compared to 45 day. Treatment T_1 (control), showed decline in MBN with increasing continuous biochar concentrations. Variations in nitrogen and microbial biomass nitrogen also related to the substrate (Nguyen, 2000) as substrate provide restricted sites for herbicide adherence (Johnson *et al*., 1995). An increase in nitrogen content is the result of microbial need for survival (Nadi *et al*., 2012).

As above results showed increase in MBN under 10 % biochar concentration but this increased comparatively less than control, which showed a greater value at 30 days this value was comparatively less than T_2, T_3 and T_4.

Total Nitrogen

The results showed that total nitrogen were statistically significant among the biochar treatments (p>0.05). Figure 4 showed that total nitrogen uptake was increased with greater biochar concentrations with 15% biochar. Treatment T_4 (150 g/kg) plant uptake maximum N 2.4% whereas control (without biochar) plant uptake 1%. This showed that biochar enhance the plant nutrients uptake and promote growth. Pietikainen *et al*. (2000) found in their studies that biochar enhance In control (0%) biochar, plants uptake less nitrogen as compared to T4 (15%) biochar showed in Figure 4. In T_2 (50 g/kg soil), increase was observed on 30 day with 1.29 % N. In T_3 (100 g/kg soil), increase was observed on 45 day with 2.1 % N. In T_4 (150 g/kg soil), maximum increase was observed on 45 day with plant nitrogen uptake to promote growth and increase productivity. as compared to T_1 (control), showed increased in N with increasing continuous biochar concentrations. These results showed that biochar increased the N uptake of plants significantly with higher biochar concentration. Similar results were observed by Nigussie *et al*. (2012) on lettuces and Chan *et al*. (2010) on raddish grown in biochar amended soil. Van Zwieten *et al*. (2010) also reported that uptake biochar amount in soil increasing plant N significantly.

Organic Matter

Change in organic matter content during the experiment as biochar degradation progressed in control (0% biochar) showed in figure 5. Organic matter differed significantly ($P < 0.05$) in all treatments. Significant variations (p=0.0003) in organic matter was observed among the treatments 45 days. Control (without biochar) showed no increased in organic matter. In T_2 (5%), T_3 (10%), and T_4 (15%) increased in organic matter was 0.8%, 0.9%, and 1.1% respectively. T_4 with more biochar concentration showed a greater increase in organic matter at T_4 treatment as compared to other treatments.

The greater concentration of organic matter develops pore space distribution and the soil aggregation and also improved the conductivity and soil water retained capacity (Saxton and Rawals, 2006). Biochar has high porous structure that increased the potential to absorb organic molecules and also improves soil structure and soil stability. The figure 5 showed the increased in organic matter content in biochar amended soil during 30 days under 10% biochar. The organic matter content was 0.9% at the start of experiment, which further increased upto 1.4%, 1.6% and 1.6% respectively in treatments T_2, T_3 and T_4. Results showed that the soil with different biochar applications contained more organic matter in biochar treatments as compared soil without biochar. Chan *et al*. (2007) found similar result in their experiment. Kimetu *et al.* (2008) reported that increased organic matter also increased the crop productivity and improve the soil structure.

Total Organic Carbon

Figure 6 shows the affect of different concentrations of biochar on the total organic carbon (TOC) of soil. Statistical analysis showed non-significant relationship between the concentration of biochar and total organic carbon (p= 0.66). Total organic carbon of the control treatment showed no significant difference with increase in number of days. Biochar application can improve plant development and civilizing, chemical and physical characteristics of soil.

Chemical characterictics include (i.e., total organic carbon, nutrient accessibility), physical characteristics incude (i.e., permeability, water holding capacity), and soil properties, all characteristics are contributing to enhance production of crops (Glaser *et al*., 2002; Lehmann and Rondon, 2006; Yamato *et al*., 2006).

After 15 days the TOC was 2.4 % which was increased slightly to 2.6 % after 30 days and 3.36 % after 45 days. With the increase in total organic carbon microbial biomass also increases (Gosai *et al*., 2010; Gu *et al*., 2009). In T_4 the value of TOC was increased more than $T_{3,}$ T_2 and control. Total organic carbon in T_4 was 2.5 % and increased to 3.5 % from 15 days to 30 days. It was increased to 3.45 % after 45 days (50 % as compared to control) in T_4.

Plant Analysis

Transpiration Rate

Transpiration rate in *Spinacia oleracea* at different biochar treatments compared to control is graphically presented in figure 7. Transpiration rate differed non significantly (P > 0.05) in all treatments. At the 15 days transpiration rate in *Spinacia oleracea* was 1.3 mmol. $m^{-2}s^{-1}$ in control, at 5 % biochar rate of transpiration was 1.5 mmol. $m^{-2}s^{-1}$ at 10% biochar transpiration rate was 2.3 mmol. $m^{-2}s^{-1}$ sudden improved, at 15 % biochar rate of transpiration was 1.7 mmol. $m^{-2}s^{-1}$ gradual decreased, at 15 days transpiration rate was increased in 10 % biochar treatment.

Transpiration rate was higher in treatment T_4 (15 % biochar) then other treatments as increased the growth of plants and also increased the transpiration and photosynthesis rate. There was highly significant difference occurred in rate of transpiration in 15 % biochar treatment. Biochar increased transpiration rate of plants. The effect of continuous biochar treatment increased transpiration rate of plants. The effect of continuous biochar on the rate of transpiration on *Spinacia oleracea* is graphically represented.

At 15 days, rate of transpiration of T_4 was 1.7 mmol. m^{-2} s^{-1} which increased to 2.7 mmol. m^{-2} s^{-1} at 45 days. During 15 days, the plant exibit the positive response and transpiration rate was increased 2.3 mmol m^{-2} s^{-1} in T_3 (10% biochar) decreased 1.7 mmol. m^{-2} s^{-1} in T_4 (15% biochar). But at 30 and 45 days, transpiration rate slowly increased in T_4 (15% biochar) where it shows rapid growth in plants, it is observed that increased in the transpiration rate as the plant growth increased. Transpiration metabolism adaptivity was improved in rice under different treatments of biochar (Weimieng and Wenfu, 2011).

Stomatal Conductance

Stomatal conductance (gs) in *Spinacia oleracea* with different biochar treatments compared to control is graphically presented in figure 8. Stomatal conductance between control and biochar treatments was differ significantly (P<0.05) which demonstrated a remarkable increase in stomatal conductance under biochar treatments. Stomatal conductance increased in T4 (15 % biochar) after 45 days. The stomatal conductance of plant was 65 mmol m^{-2} s^{-1} initially in T4 and reached to 89 mmol m^{-2} s^{-1} at the end. Plant with greater biochar concentrations showed better stomatal conductance as reported by Gindaba *et al.* (2004). The stomatal conductance of control was 65 mmol m^{-2} s^{-1} initially after 15 days and T4 (15 % biochar) showed 89 mmol m^{-2} s^{-1} at the ending of the experiment when plant growth was increased. A plant species which can endure higher levels of biochar concentration shows better stomatal conductance as reported by (Gindaba *et al*. 2004).

That increased in stomatal conductance also causes increased in net photosynthesis and transpiration. It indicated that stomatal conductance shows relation the rate of transpiration. Jones (1997) observed that stomata exist in middle position and provide a way for the exchange of carbon dioxide and also loss of water from plants. Stomata provide main short way to control both transpiration and photosynthesis. *Spinacia oleracea* howed increased in stomatal conductance with greater concentration of biochar treatments.

Net Photosynthesis

Net photosynthesis in *Spinacia oleracea* with different biochar treatments compared to control is graphically presented in Figure 9. Statistical analysis showed the significant difference (P < 0.05) for net photosynthsis between control and biochar treatments. There was a significant different occurred (*p*=0.00) within application of biochar.

Initially, the net photosynthesis rate (pn) was o.5 mmol m^{-2} s^{-1} increased to 1.6 mmol m^{-2} s^{-1}. Comparison with the control (0 % biochar), 15 % biochar plants showed highly rate in net photosynthesis. *Spinacia oleracea* is water consuming plant so it shows high rate of net photpsynthesis.

Wang *et al.* (2007) determine that net photpsynthesis rate of R. *peseudoaccacia* decreased as soil water potential reduced. In the start of the experiment plants shows even photosynthesis rate at 0% biochar. But in 15% biochar plants as the biochar leval increases net photosynthesis increased because stomata increased the amount of water vapor and exchange of carbon dioxide, so as water level rises net photosynthesis also increased. Under well watered condition, genotypes transpired more water, maintained more opened stomata and high water use before flowering (Aniya and Herzog, 2003).

Plant Height

Changes in plant height of plant under control (0 % biochar) with different biochar application in soil were showed in figure 10. Statistical analysis showed that plant with different biochar application differs significantly (*P*<0.05). At the start, the palnt height of plant under 10 % biochar was 12 cm^2 and 14 cm^2 at the end showed rapid increase.

All trearments T_1 (control), T_2 (5%), T_3 (10%) and T_4 (15%) showed slightly increase in height during 4 weeks and then showed a major shifts in last 2 weeks under mild water stress. Treatment T_2 showed minute increased in height.

Statistical analysis showed that plant subjected to mild continuous water stress with different biochar 10 % biochar was 12 cm^2 and 14 cm^2 at the end showed rapid increase. Widowati *et al*. (2012) found that plant height and diameter increased by applying biochar in soil. Hoshi (2001) also demonstrated that biochar application increase 15 % in volume and 35% increase in height of tea trees. Maximum height (18 cm^2) was attainted in T_4. These results are similar to Chartzoulakis *et al.* (2002). Shao *et al.* (2008) reported increased in plant height as biochar concentration increases.

Number of Leaves

Changes in plant leaves of plant under control (0 % biochar) with different biochar application in soil were showed in figure 11. Statistical analysis showed that plant leaves with different biochar application differs significantly ($P<0.05$). Analysis showed significant increased in leaves of plant under 15 % biochar treatment ($p=0.024$).

At the start, the leaves of plant under 10 % biochar were 20 and 35 at the end showed rapid increase. All treatments T_1 (control), T_2 (5%), T_3 (10%) and T_4 (15%) showed increasing trend with greater biochar concentrations. T_1 (control), T_3 (10%) and T_4 (15%) showed increase in leaves after 15 days. Treatment T_2 showed increased in leaves at the interval of 15 days. In T_2, maximum increase was observed on 45 day with 35 leaves and decrease was observed on 15 day with 20 leaves. In T_3, maximum increase was observed on 45 day with 40 leaves. In T_4, maximum increase was observed on 45 day with 52 leaves and decrease was observed on 15 day with 28 leaves.

In addition, biochar is long term benefit for soil fertility and production of biomass and thus guarantees a highly recalcitrant to microbial decomposition (Steiner et al., 2007). Similarly number of the leaves produced was significantly high for the biochar treatment than for the earthworm treatment because leaf turnover was higher in presence of biochar than in presence of earthworms (Noguera et al., 2012). The figure 11 represented the effect of 15% biochar on leaves of plant. The plants of 15% biochar treatments showed rapid increased at the start 28 leaves showed and at the end 52 leaves were observed. Maximum leaves 52 were shown in T_4 after 45 days.

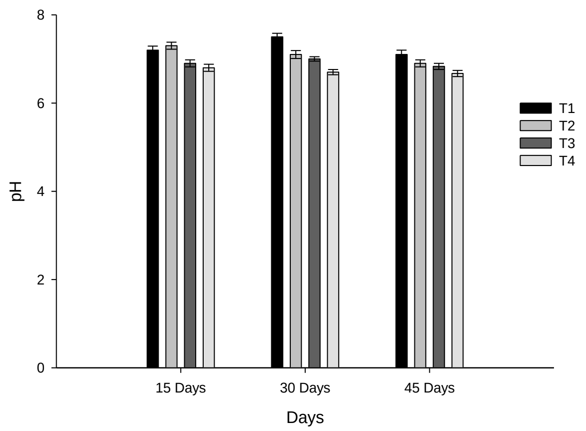

Fig 1: Variation of pH in soil amended with biochar at different application rate

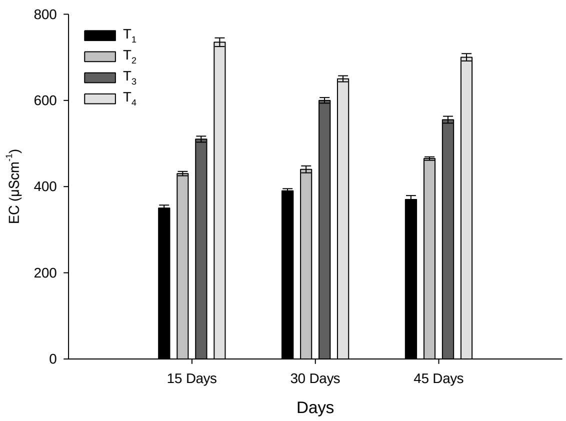

Fig 2: Variation of EC (µScm-^{1}) in soil amended with biochar at different applications rate

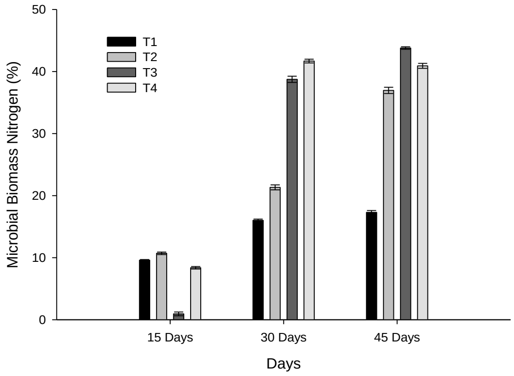

Fig 3: Variation of microbial biomass nitrogen (%) in soil amended with biochar at different biochar applications rate

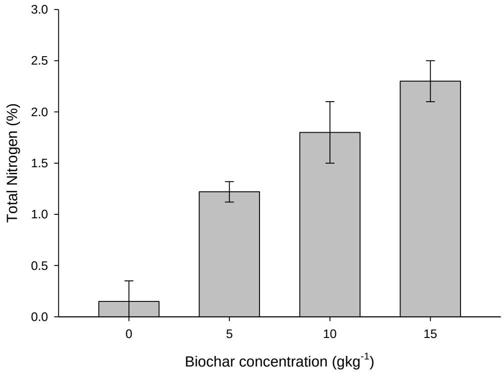

Fig 4: Variation of Total nitrogen (%) in soil amended with biochar at different applications rate

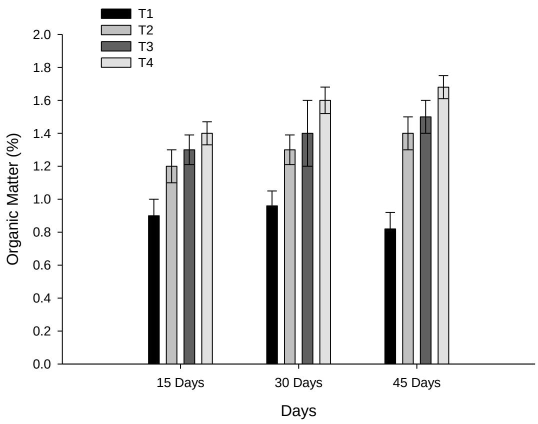

Fig 5: Variation of Organic Mattar (%) in soil amended with biochar at different application rate

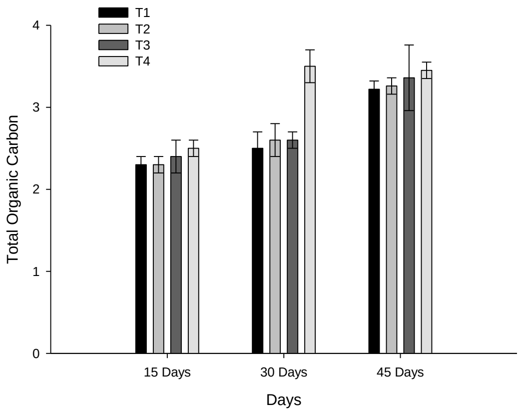

Fig 6: Variation of Total Organic carbon (%) in soil amended with biochar at different applications rate

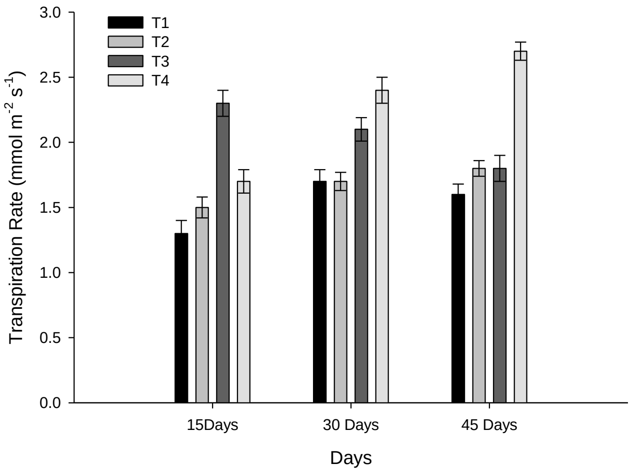

Fig 7: Variation of Transpiration rate (mmolm^{-2} s^{-1}) in *Spinacia oleracea* with different biochar applications in soil after 15 days interval

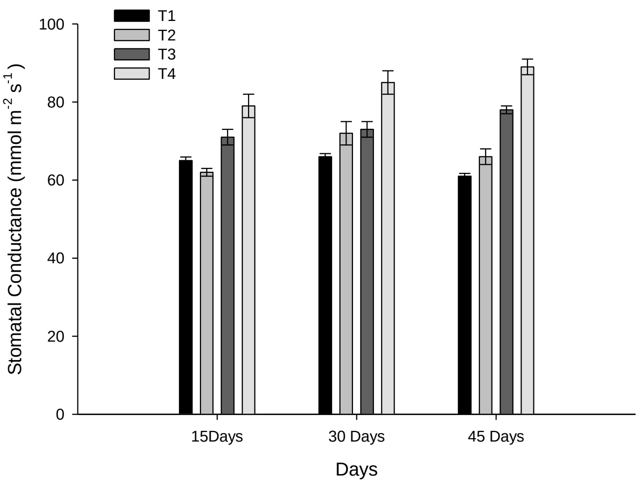

Fig 8: Variation of stomatal conductance (mmol m^{-2} s^{-1}) in *Spinacia oleracea* amended with different biochar applications in soil after 15 days interval

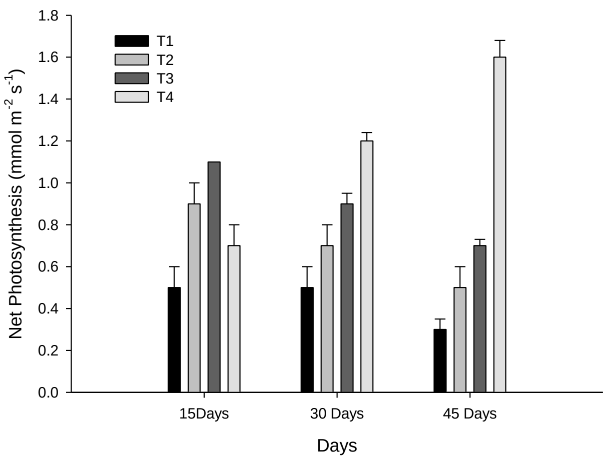

Fig 9: Variation of Net photosynthesis (mmol m^{-2} s^{-1}) *in Spinacia oleracea* with different applications in soil at intervals of 15 days

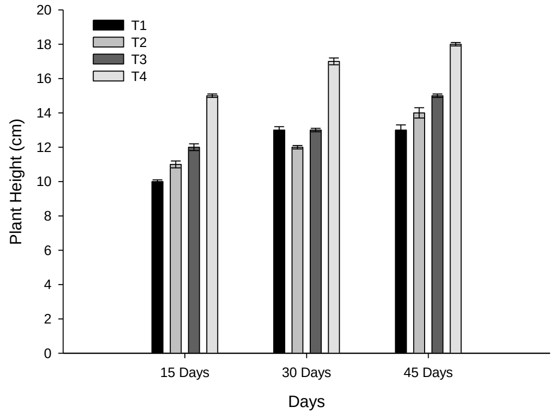

Fig 10: Variation of height in *Spinacia oleracea* biochar amended with biochar at different applications rate

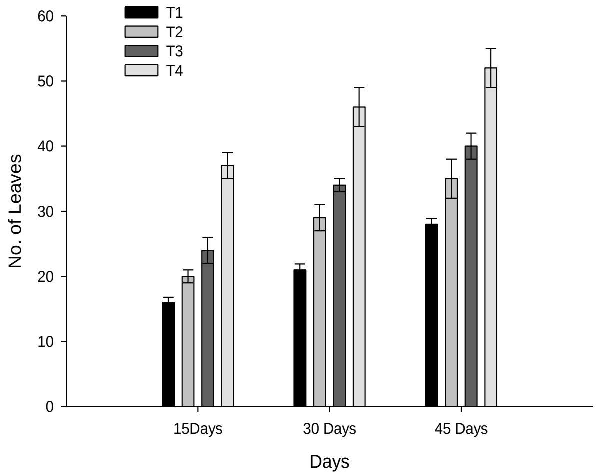

Fig 11: Variation of Number of leaves in *Spinacia oleracea* amended with biochar at different applications rate

Plate 1: Application of biochar before seed sowing

Plate 2: Measurement of physiological parameters of *Spinacia oleracea* by IRGA

Plate 3: Application of biochar applied to the *Spinacia oleracea* plants

Plate 4: Effect of biochar application on *Spinacia oleracea* leaves

SUMMARY

Biochar is a type of charcoal produced from biomass by pyrolysis. Soil modification with biochar is evaluated globally as a means to enhance productiveness of soil and to evaluate climate change. The present study was conducted to evaluate the consequences of biochar on soil physiology and also on plant growth. Pot experiment was carried out using *Spinacia oleracea* by applying biochar treatments and soil was amended with 3 biochar ratios in field area of Department Environmental Sciences, PMAS-AAUR. Changes in soil physiology as well as effect on plant growth were examined. Soil parameters, Microbial biomass nitrogen (MBN) were increased in biochar plants as compared to control plants. A continuous decreasing trend was observed in pH. There was no significant difference recorded in EC of soil in all the treatments after 15 days up to 45 days. Biochar positively affected the soil by increasing total organic carbon, soil organic matter, and total nitrogen were also increased in biochar plants as compared to control plants. Transpiration rate, Stomatal conductance, and Net photosynthesis were increased in 15 % biochar treatment with time. These parameters were also decreased in control plants (0 % biochar) with time but increased in biochar plants. *Spinacia oleracea* height and number of leaves were increased due to application of biochar.

The increase in plant growth parameters showed that biochar application have significant effect on plants growth. The addition of biochar in soil improved the cation exchange capacity that resulted in more efficient nutrient availability to plant. Hence plant growth was increased due to increased soil fertility.

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