

## EFFECT OF DAIRY MANURE DERIVED BIOCHAR ON MICROBIAL BIOMASS CARBON, SOIL CARBON AND *VITIS VINIFERA* UNDER WATER STRESS CONDITIONS

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### Abstract

Water is a key factor for plant growth and development and plants require an adequate amount of water for their optimum growth. But, in some cases plants grow better under a period of moderate water stress. *Vitis vinifera* is often subjected to severe water stress during hot, dry summers. Soil amendment is one of the methods to overcome drought stress. Biochar has been popularized due to its potential role in many fields like better crop yield, C sequestration, increasing microbial biomass carbon and soil carbon. In the present study, two rates of biochar (2g and 5g kg<sup>-1</sup> soil) were investigated with two levels of water stress (35% and 70%). A considerable increase was observed in leaf area, plant height, net photosynthesis, transpiration rate and stomatal conductance when 35% stress was maintained along with the addition of large amount of bio char respectively. Moreover, highly significant results were found in plants treated with biochar as compared to control among all treatments. *Vitis vinifera* showed resistance having 5gkg<sup>-1</sup> biochar with 35% water stress for all parameters. Soil carbon was 4.9% in 70 % stress and 5gkg<sup>-1</sup> biochar except microbial biomass carbon which was 0.11% having moderate water stress (35%) and high biochar rate (5gkg<sup>-1</sup>). Results indicate that dairy manure derived biochar can be used to promote plant growth specifically of *Vitis vinifera* and increase microbial biomass carbon and soil carbon in soil. In future dairy manure derived biochar can be used for enhancing plant growth and improving soil properties under water stress conditions.

**Key words:** Biochar, Microbial biomass carbon, Photosynthesis, Soil carbon, Stomatal conductance, *Vitis vinifera*, Water stress.

### Introduction

Waste management, environmental degradation, productivity of biomass carbon and soil carbon under water stress are the growing concerns of the present age. Anthropogenic activities like emissions of greenhouse gasses (GHGs) can lead to climate change and consequently affect growth, reproduction, establishment, survival and distribution of plant species on the globe. These activities have quite damaging effects on various essential biochemical processes of plants. Multi dimensional applications of biochar make it suitable for uses in sustainable agriculture and waste management practices (Zwieten *et al.*, 2010).

Different environmental conditions affect plant growth and productivity. For instance different kinds of stresses such as salinity and water stress have negative impact on plants. Whenever the transpiration rate becomes too high and the roots of plants receive limited amount of water, hence plants face water stress (Anjum *et al.*, 2011). Water potential reduces water stress in the root zone and water deficiency created for plants (Bohnert and Sheveleva, 1998). Plants have a potential impact on microbial activity as they produce organic carbon (Antisari *et al.*, 2010). Microorganisms play a vital role in biogeochemical cycling but alteration in chemical properties of soil affects the microbial community (Beck *et al.*, 1984). Soil microbial biomass carbon has a much faster turnover rate comparing with total organic matter and it is the total amount of microbiological components of soil (Jekinson *et al.*, 2004).

*Vitis vinifera*, a species of *Vitis*, with wide spread production is used throughout the world for utilization

and manufacturing processes. It can tolerate abiotic stresses including drought. Torres *et al.*, (2002) reported that it is considered as both fruit and wine and its key by-product, the grape pomace is extensively studied. Industrial processing of its grapes leads to products or by-products as *Vitis vinifera* leaves. The relation between plant and soil is intimate. They influence the soil by active processes of their living parts and by passive effects of their litter (Gobat *et al.*, 2004).

In the current study, dairy manure was used in making biochar. Dairy manure derived biochar has great importance in remediation as well as in agronomic purposes. Manure and biosolids are significant because they release nutrients which also contribute in emissions of greenhouse gases (Smith *et al.*, 2008). Dairy manure can be recycled by composting process which ensures the biochar stabilization and the sanitization (Bernal *et al.*, 1998). Biochar is a type of charcoal which is formed in limited supply of oxygen by thermal decomposition of organic matter and is used in the improvement of soil (Lehmann and Joseph, 2009). Addition of biochar increases the soil quality, sequestering carbon dioxide (CO<sub>2</sub>) and remediating the polluted soils. It actively removes CO<sub>2</sub> and the possibility of mitigation of climate change has been rising due to the biochar application to agricultural soil (Major *et al.*, 2010). Furthermore, biochar surfaces have capacity to sorb dissolved organic carbon, hydrocarbons, pesticides and heavy metals from soil.

Biochar affects a number of microbe mediated processes such as nutrient cycling, GHGs emissions and organic matter decomposition by improving the growth of microorganisms. It affects microbial community because

of high porosity, sorption and cation exchange capacity (Lehmann *et al.*, 2011). To improve soil water carrying capacity, water penetration and infiltration, soil's water content, hydraulic conductivity, soil aeration and nutrient holding property, biochar is a fine way (Baronti *et al.*, 2014). In this paper the effect of two rates of biochar i.e.  $2\text{gkg}^{-1}$  and  $8\text{gkg}^{-1}$  on microbial biomass carbon and soil carbon and changes on *Vitis vinifera* plant due to two levels of water stress i.e. 35% and 70% were evaluated.

## Materials and Methods

**Biochar preparation:** Biomass of dairy manure was used for biochar production. Firstly, biomass was air dried for one week before subjection to pyrolysis. Biochar was

prepared by pyrolysis at  $400^{\circ}\text{C}$  according to the procedure described by Baggio *et al.*, (2009).

## Biochar Analyses

**pH and electrical conductivity:** Biochar was desiccated, ground and converted into fine powder form. An aqueous extract was prepared by using biochar-water ratio of 1:10 respectively for the determination of electrical conductivity and pH of biochar (Ellen *et al.*, 2010).

**Ash and moisture content:** Initially, biochar heated for 4 hours at  $800^{\circ}\text{C}$ . Loss in weight (ash content of biochar) was observed after the heating process (Ellen *et al.*, 2010). Furthermore, moisture content of biochar was estimated (ASTM 1762-84, 2007) using the following relation:

$$\% \text{ Moisture} = \frac{\text{Weight of air-dried biochar (g)} - \text{Weight of oven-dried biochar (g)}}{\text{Weight of oven-dried biochar}} \times 100$$

**Pot experiment and water treatment:** A pot experiment was carried out using plastic pots filled with 4kg of field soil. Cuttings of *Vitis vinifera* were transplanted having a height of 24cm in these pots. These cuttings were grown for 1 month. Water treatment was given after transplantation of cuttings of *Vitis vinifera* and pots were maintained at 35% and 70% stresses (Sankar *et al.*, 2008). Polythene bags were used to cover plant pots in order to prevent the water loss due to root respiration and moisture loss through soil evaporation as well. Portable weighing balance was used to weigh plants on daily basis, while moisture was maintained daily. After three weeks, the pots were subjected to stress and continuously 35% and 70% of daily loss of water from all the plants under stress was returned till the end of experiment. The experiment was continued up to five weeks after stress to get the results of plants having nearly zero stomatal conductance (Table 1).

was selected and marked as first day. Then same leaves used for physiological parameters till the end of the experiment. Leaf was desiccated, dust was removed and it was kept in infrared gas analyzer (IRGA) for further measurements (Fang *et al.*, 2012).

## Soil analyses

**Electrical conductivity and pH of soil:** Soil (used for pots) was analyzed for electrical conductivity (EC) and pH by glass electrode method respectively (Page, 1982). Sample (50 gram of soil) was air dried and suspension was prepared by adding water in it based on 1:10 soil to water ratio. Furthermore, a saturation paste was prepared and extracted using a vacuum pump. A multimeter (Model: MM 40+) was employed for the estimation of pH and EC of soil extract.

**Soil carbon:** Determination of total organic carbon (TOC) in soil was performed using FAS titration method (Walkley, 1947). Organic matter analysis was done using dichromate digestion method that was further processed using the following equation for TOC calculation.

$$\text{TOC in soil} = 1.334 \times \text{Oxidizable organic carbon}$$

**Microbial biomass carbon:** A heating and extraction technique known as rapid microwave irradiation and extraction method was used for the calculation of microbial biomass carbon (Islam and Weil, 1998). Soil sample of 10g was arranged in two sets and dried using oven equivalent of field moist soil, were taken in two 50mL tightly screwed polypropylene tubes. One set was kept in 50mL tubes with a small hole in screw-top (homogenize vapour pressure) and second set was primarily irradiated to  $400\text{Jg}^{-1}$  and then agitated for an even and uniform mixing to be exposed for next irradiation at  $400\text{Jg}^{-1}$  so as to reduce heat intensification within the moist soil. Moreover, soil samples were placed in a desiccator to lower the temperature for 30 min. Both sets of soils were extracted with 25mL of 0.5M Potassium Sulfate ( $\text{K}_2\text{SO}_4$ ) (pH 7.0) by using an orbital shaker for 60 min at 250 rpm. Suspensions of soil were subjected to centrifugation at 2000 rpm for 10 minutes after shaking

**Table 1. Treatments with stress and non-stress conditions.**

Treatments	Water level (%)	Biochar (g/kg)
T <sub>1</sub> (control)	0	0
T <sub>2</sub>	35	0
T <sub>3</sub>	70	0
T <sub>4</sub>	0	2
T <sub>5</sub>	35	2
T <sub>6</sub>	70	2
T <sub>7</sub>	0	5
T <sub>8</sub>	35	5
T <sub>9</sub>	70	5

## Plant growth parameters

**Plant height and leaf area:** Height of all plants was recorded from base to top of stem via measuring tape. Height of the plants was measured after every two weeks (Fang *et al.*, 2012). Leaf area was measured by leaf area measuring meter after every week.

**Physiological parameters of plant:** Stomatal conductance, net photosynthesis and transpiration were recorded every week (8:00 am to 10:00 am) using "CIRAS-2 Portable Photosynthetic System". For this, fully expanded upper leaf

and then filtered to obtain soil free filtrates. Consequently, Microbial biomass carbon in the extract examined by rapid Microwave (MW) oxidation and titration method. 4mL of extract mixed with 1mL of 0.0667 M Potassium Dichromate ( $K_2Cr_2O_7$ ) and 5mL conc. Sulfuric acid ( $H_2SO_4$ ). Extracts were titrated with acidified ferrous ammonium sulfate solution till end point.

$$MBC \text{ (mg C/ kg of soil)} = (MWC_{\text{ext}} - C_{\text{ext}}) * 2.46$$

$MWC_{\text{ext}}$  = Extractable carbon in post microwaved soil,

$C_{\text{ext}}$  = Extractable carbon in field-moist soil

### Statistical analysis

Different statistical tools were applied with Two Way Analysis of Variance (ANOVA) keeping level of significance at 5% in MS Excel 2007. ANOVA was carried out using SPSS14.0 statistical software.

### Results

**Soil analysis:** Physico chemical parameters of soil were studied. Total organic carbon (TOC) of soil was determined prior to start of the experiment and after stress final reading of control and stressed plants with different amounts of biochar was taken at the 5<sup>th</sup> week. The effect of dairy manure derived biochar on the TOC is graphically presented in Fig. 1. Graphical representation shows that biochar treated plants (T8, T9) significantly enhanced the TOC of soil upto 3.9% and 4.9 % respectively even under drought conditions over the control treatment. The results for TOC in soil in plants which were treated with biochar as compared to control were found to be highly significant ( $p \leq 0.01$ ).

Microbial Biomass Carbon (MBC) was determined before and after the experiment and initial and final readings were also taken. The effect of biochar on MBC is graphically represented in Fig. 2. Results show that biochar treatments significantly increased MBC of the soil as compared to control. MBC of the biochar treated soil with no stress reached up to 0.19% while the MBC of control soil was 0.05%. Likewise, MBC of stressed plants reached upto 0.11% which was higher than the control soil.

**Biochar Analysis:** Biochar pH was noted to be 9.3 (alkaline), ash content was 10.6% and moisture content 23.8% respectively. Additionally, the electrical conductivity was  $67 \mu\text{Scm}^{-1}$  and bulk density of biochar was 0.1.

**Plant height and leaf area:** Plant height was determined weekly after applying stress. Effect of biochar treatment on plant height compared with control is presented in Fig. 3. Results for plant height were highly significant ( $p \leq 0.01$ ) in plants subjected to biochar treatment than control. Plant height reached maximum of 51.56cm in plants with no water stress and 5g/kg biochar as compared to the plants with 35% and 70% water stress. With 35% stress, plant height was recorded to be 47cm with higher amounts of biochar while with 70% stress plant height was higher (40cm) with similar higher amounts of biochar (as the treatment of Biochar was given at the rate of none, 2 g/Kg, and highest of 5g/Kg weight of soil). Height of non stressed plants was relatively better than plants under stress. Hence, biochar application rate of 5g/kg was more

found to be effective as compared to other application rates. Similar observations were recorded in stressed plants with 35% water stress + 5g/kg biochar was found extra useful soil amendment than other rates.

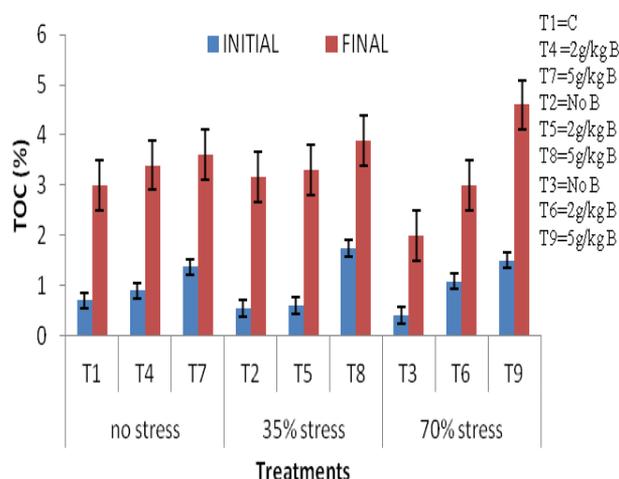


Fig. 1. Effect of biochar on total organic contents of the soil ( $p \leq 0.01$ ). C: Control, B: Biochar

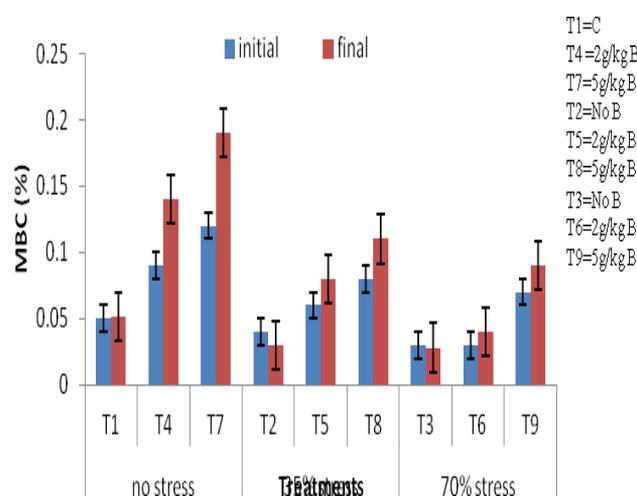


Fig. 2. Effect of biochar on microbial biomass carbon (%) of the soil ( $p \leq 0.01$ ). C: Control, B: Biochar

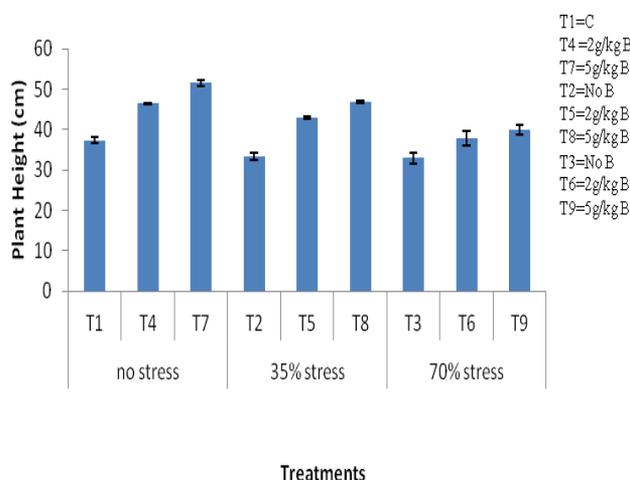


Fig. 3. Effect of biochar on the plant height (cm) of plants ( $p \leq 0.01$ ). C: Control, B: Biochar

Leaf area recorded at seventh day after stress treatment (DAS) and compared between all biochar treated plants and control is shown in Fig. 4. Significant results ( $p \leq 0.01$ ) were observed in applied treatments with increasing trends. Whereas, highest leaf area ( $87.2\text{cm}^2$ ) was recorded in T7 as compared to T1 and T4 having leaf area ( $64.4\text{cm}^2$  and  $70\text{cm}^2$ ), respectively. In the same manner, similar trend was observed in stressed treatments and leaf area was found to be high in T8 as well at 35% stress ( $74\text{cm}^2$ ).

**Physiological Parameters of Plant:** A comparative analysis between stomatal conductance of biochar treatments and control was done (Fig. 5). Highly significant ( $p \leq 0.05$ ) results were obtained in applied treatments. The stomatal conductance was found to be extremely high ( $405.33\text{mmolH}_2\text{O m}^{-2}\text{s}^{-1}$ ) in  $5\text{g/kg}$  biochar as compared to plants having  $2\text{g/kg}$  biochar. On the contrary, in stressed plants up to three weeks, it

reduced very slowly. After third week an unexpected decline was noticed in stomatal conductance with increasing stress. Biochar exhibited constancy in rigorous stress and hence stomatal conductance considerably reduced, while in treatment without biochar it reached value closer to zero at sixth week of the experiment.

Figure 6 is depicting highly significant results ( $p \leq 0.05$ ) for the transpiration rate of plants treated with biochar under stress compared with control. In stressed plants the transpiration rate was higher ( $3.8\mu\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$ ) in moderate water stress and high biochar rate ( $5\text{gkg}^{-1}$ ). It was reduced slowly but when plants were given with 35% and 70% water stress after three weeks, a prompt decline was noticed in the transpiration rate at this stage. At the end of the experiment, the plants with no biochar application showed trend (close to zero) for transpiration rate similar to stomatal conductance.

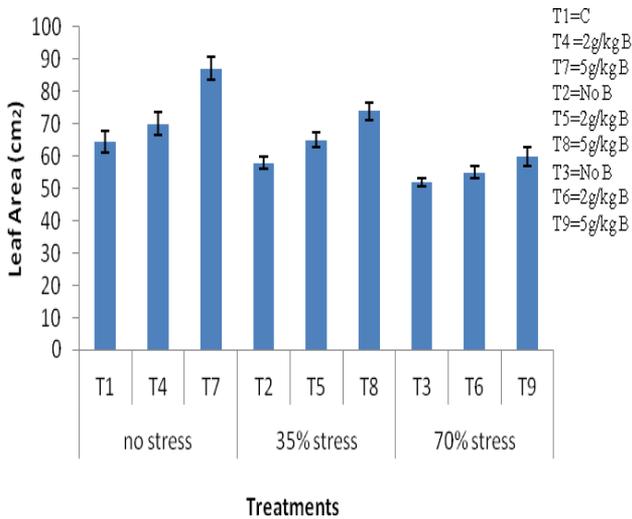


Fig. 4. Effect of biochar on the leaf area ( $\text{cm}^2$ ) of plants ( $p \leq 0.01$ ). C: Control, B: Biochar

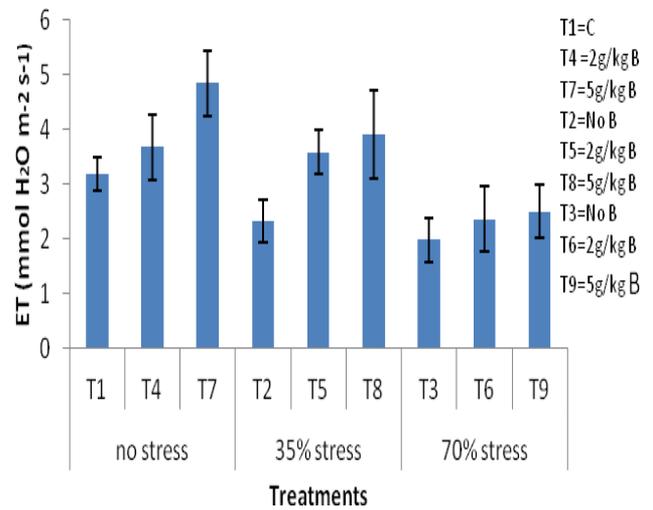


Fig. 6. Effect of biochar on the transpiration ( $\text{mmolH}_2\text{O m}^{-2}\text{s}^{-1}$ ) of plants ( $p \leq 0.05$ ). C: Control, B: Biochar

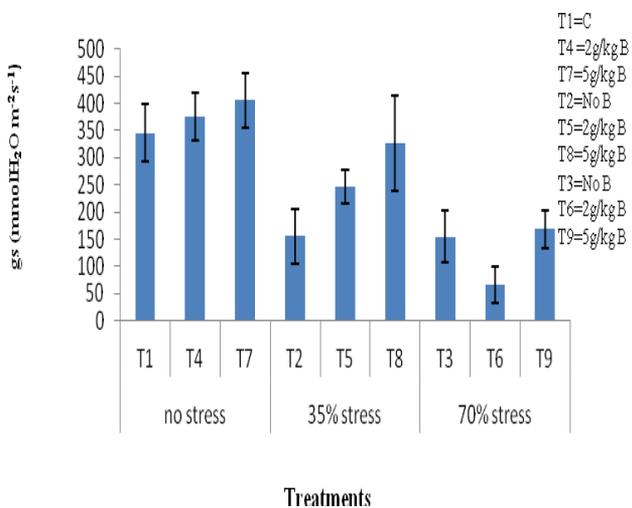


Fig. 5. Effect of biochar on the stomatal conductance ( $\text{mmolH}_2\text{O m}^{-2}\text{s}^{-1}$ ) of plants ( $p \leq 0.01$ ). C: Control, B: Biochar

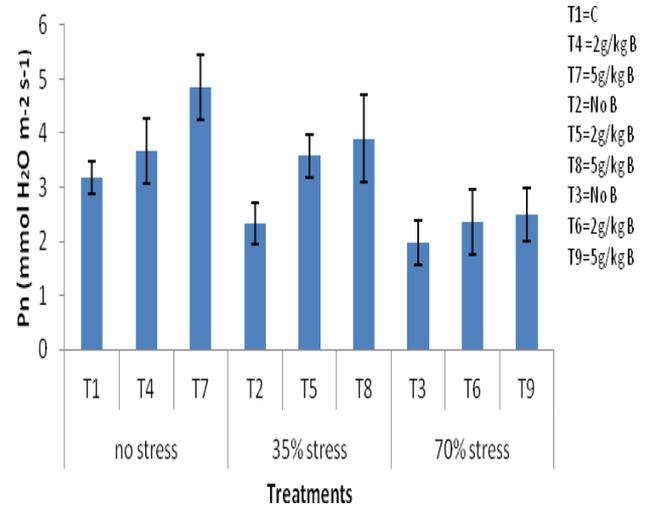


Fig. 7. Effect of biochar on the photosynthesis ( $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ ) of plants ( $p \leq 0.01$ ). C: Control, B: Biochar

All plants were also observed for photosynthesis rate during the whole experiment and results were highly significant ( $p \leq 0.01$ ) for all application rates. Photosynthetic rate of control and biochar treated plants were compared and is shown in Figure 7. Photosynthesis rate was higher in 5gkg<sup>-1</sup> biochar and 35% stress (8.05µmol CO<sub>2</sub> m<sup>-2</sup>s<sup>-1</sup>) as compared to that of control (3.808µmolCO<sub>2</sub>m<sup>-2</sup>s<sup>-1</sup>) respectively.

## Discussion

Water significantly affect the rate of plant development specifically growth parameters and 50% reduction was witnessed when plants were subjected to very little quantity of water (McGiffen *et al.*, 1992). Biochar can be efficiently used to keep water in the soil, so water supply may elevated under water stress situations (Blackwell *et al.*, 2009). García *et al.*, (2008) demonstrated that organic matter amendment considerably increase the TOC of soil and also decreases with the passage of time. It shows that Total Organic Carbon increase with increasing amount of biochar at a specific level under drought conditions. Pascal *et al.*, (1999) reported that organic wastes work as carbon, nutrient and energy source, keeping the Microbial Biomass Carbon standardized in comparison with control soil. Microbial Biomass Carbon was highly significant ( $p \leq 0.01$ ) in biochar treated soil as compared to control soil. Jenkinson *et al.*, (2004) report that addition of organic matter increases the TOC and MBC of soil as well. A considerable decrease in their values is evident with the passage of time but still remain higher as compared to control soil.

In the present study, when field capacity was maintained; leaf area showed an increasing trend continuously up to three weeks. Afterwards, there was less increase in all stressed plants with 8g biochar as compared to the plants with high amount of biochar (5g/kg). Similarly, Masinde *et al.*, (2006) has been reported that in 35% and 70% water stressed conditions; 5g/kg biochar is found to be having sufficient moisture to increase both leaf area and transpiration rate.

It has been reported that stomatal conductance was reduced under water stress in different plant species (Batool *et al.*, 2015; Fathi *et al.*, 2018; Gindaba *et al.*, 2005). There is an increase in stomatal conductance with biochar treatment in comparison to control (Solaiman *et al.*, 2010). In contrast, Kusvuran (2012) observed constant decrease in stomatal conductance of melon plant (*Cucumis melo*) without any alteration in soil. Reduced leaf area of stressed plants resulted in decline in the rate of transpiration (Borrell *et al.*, 2000). In contrast, Liu and Stutzel (2002) reported reduction in stomatal conductance as another cause for the decline in transpiration rate instead of decrease in leaf area. Transpiration rate of plants grown in soil with no amendment was zero rather than a bit higher in biochar treated plants even under drought at the end of experiment. It indicated that biochar has provided this tolerance to sustain under water stressed environment. Similar observations were recorded in a study in which plants were kept at 35% and 70% for non-stress conditions (Solaiman *et al.*, 2010). On the other hand, photosynthesis rate also

reduced in *R. pseudoaccacia* species (Wang *et al.*, 2007). Moreover, Solaiman *et al.*, (2010) reported photosynthetic rate with increasing trends in clover plants with biochar treatment under water deficient conditions. Ippolito *et al.*, (2012) revealed that 3-7% of moisture content is increased due to 2% application of biochar that further improved photosynthesis rate as well.

## Conclusion

A substantial increase was observed in all the plant physiological constraints (Leaf area, plant height, stomatal conductance, transpiration rate and net photosynthesis) when 35% stress was maintained along with large amount of biochar and highly significant results were found specifically in plants with biochar treatment as compared to control among all treatments. *Vitis vinifera* showed resistance having 5gkg<sup>-1</sup> biochar with 35% water stress for all the parameters. Soil carbon was found to increase 4.9% in 70 % stress and 5gkg<sup>-1</sup> biochar except microbial biomass carbon which was 0.11% having moderate water stress (35%) and high biochar rate (5gkg<sup>-1</sup>). The study results clearly indicate that dairy manure derived biochar can be used to promote plant growth specifically of *Vitis vinifera* and increase microbial biomass carbon and soil carbon in the soil. In future dairy manure derived biochar can be used to improve soil properties and for enhancing growth in plants exposed to water stress conditions.

## “Compliance with Ethical Standards”

This article does not contain any studies with human participants or animals performed by any of the authors.

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