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EVALUATING THE EFFECTS OF BIOCHAR AMENDMENTS ON DROUGHT TOLERANCE OF SOYBEAN (*GLYCINE MAX* L.) USING RELATIVE GROWTH INDICATORS

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Abstract

Drought is the extremely critical environmental limitation that restricts crucially the leaf growth, stem elongation and overall plant growth rate. In the agricultural lands biochar has its remarkable role for sustainable soil management strategies and potential tool for mitigating drought. In the present research work, the impact of acacia wood shaving (AWB), sugarcane bagasse (SBB), wheat (WSB) and rice straw (RSB) biochar at two amendment levels (i.e., 10 and 20 t/ ha) under three different moisture conditions i.e., optimum moisture (OM) at 75± 2% of FC, deficit moisture (DM) at 50 ± 2% of FC and severe moisture deficit (SMD) at 40 ± 2% of FC on growth parameters of soybean in poor sandy loam (SL) soil and clay loam (CL) soil were investigated. We focused on individual growth components along with specific growth measurements such as NAR (net assimilation rate) and RGR (relative growth rate) in different growth stages. Moisture deficit conditions reduced all shoot and leaf parameters during first harvest interval of vegetative growth and effects were more negative at reproductive growth during third harvest interval. Biochar in CL soil mostly caused higher increase in shoot and leaf growth parameters on relative basis than control. While, under water deficit conditions, most influential amendments were acacia (sp. *nilotica*) wood shavings biochar (10 t/ ha) and sugarcane bagasse associated with higher growth in shoot and increases shoot length, shoot fresh as well as dry biomass, number of leaves, leaf fresh and leaf dry biomass, leaf area, net assimilation rates and relative growth rates of soybean. Consequently, sugarcane bagasse and wood biochar can be valued as the most promising tools for recovery of growth under moisture deficit conditions.

Key words: Moisture regimes; Organic amendments; Harvest interval; Relative growth; Net assimilation.

Introduction

Water deficiency becomes the major problem of arid regions throughout the globe (Forouzani & Karami, 2011). World-wide drought scenarios has been intensified from the last few decades which induces huge agricultural corners for its drastic effects on plant developmental processes (Sivakumar *et al*., 2011; Alvi *et al*., 2022; Arain *et al*., 2022). Moreover, extensive agricultural practices in arid regions deteriorated the soil structure with inadequate water holding capacity which further intensify the consequences of drought (Rawls *et al*., 2003). Hence, for agricultural system to be really sustainable, maintenance of agricultural soil is of prime importance and this task can be achieved by using organic amendments as compost, manures, straws, sawdust, sewage sludge and biochar (Scotti *et al*., 2015). However, in comparison to other organic amendments, addition of biochar in the soil is a very promising, advanced and safest practice with vast environmental and agricultural interests with waste biomass reduction (without discharge of toxic gases as carbon monoxide) including by-product in the form of energy production, carbon restructuring in to a more stable form, and refurbishes soil physical properties with increased water holding capacity for longer durations (Lehmann & Joseph, 2009; Yu *et al*., 2013). Biochar is prepared from waste organic material by heating it at a very high temperature (usually above 250°C) under anaerobic conditions in order to convert it into aromatic carbon structures with spongy nature (Pandey, 2009). However, properties, interactions of biochar material with soil and ultimate effects on plants usually depend upon the principal material used for its production (Mukherjee *et al*., 2011). Most of the recent work usually focused on the biochar influence on soil properties and require further investigation with respect to the influence of different feedstock based biochar on plant growth and development under optimum and water stressed conditions in diverse soils media.

Soybean (*Glycine max*) considered amongst the potential agronomic crops for the yield of high quality oil and protein on commercial basis (Singh & Shivakumar, 2010). Cultivation of soybean is practiced across the globe, including Asia, many parts of North America and South America (Kumudini, 2010). Variations in photoperiod, temperature and moisture provisions affects the growth and yield of soybean (Mundstock & Thomas, 2005). However, insufficient moisture supply crucially affects soybean growth by disrupting vital physiological processes (Hamayun *et al*., 2010; Souza *et al*., 2013). Water requirement of soybean varies from 450–800 mm based up on crop management pactices, soil media and climatic conditions. Soybean water demands usually increases with plant development and peaking during reproductive growth (Farias *et al*., 2007). But under arid conditions due to insufficient and random rainfall events, soybean crop usually suffers from moisture deficit conditions (as low as 50-40% moisture content of FC) during its critical growth phases which resulted in overall growth and yield reduction (GHosH *et al*., 2000). Relative growth estimation is the most crucial approach for determining plant size, its ultimate survival and reproduction. Hence, plant growth analysis served as a most promising tool for determining plant responses to various fertilizers and environmental conditions (Khatoon *et al*., 2000). So, the current research work was designed to improve our understanding about the influence of biochar amendments (derived from different feed stocks) under drought on soybean's relative growth attributes and deals with shoot length, root growth, leaf area, their fresh weights and dry biomass production, relative growth rates and net assimilation rates on relative basis per unit time.

Material and Method

Four types of biochar were produced from *acacia* (Sp. *nilotica*) wood shavings, sugarcane bagasse, rice and wheat straw. After drying at 80°C the raw biomass were crushed and filled in the small containers (stainless steel) then filled containers were placed inside the larger containers and fitted within the bench top fixed bed (temperature controlled) biochar production unit. Biochar were produced at 450°C for three hours using slow pyrolysis method and N_2 was used as a carrier gas. Physicochemical characteristics of acacia (Sp. *nilotica*) wood shaving (AWB), sugarcane bagasse (SBB), wheat straw (WSB) and rice straw (RSB) biochar respectively were as follows: pH was 7.7, 7.4, 8.3 and 8.7, electrical conductivity (mSm^{-1}) was 4.4. 5.2, 5.9 and 6.8, water holding capacity ($g.g^{-1}$) was 1.7, 1.1, 1 and 0.62, fixed carbon (%) was 53.3, 60, 7.69 and 9.31, volatile matter (%) was 33.7, 43.2, 62.7 and 71.7, ash content (%) was 3.5, 6.4, 20 and 28% (Jahan *et al*., 2019).

Two levels (i.e., 10 and 20 t/ ha) of AWB, SBB, RSB and WSB biochar were supplemented within the SL and CL soils in the pots (12 inch). Characteristics of CL and SL soils are presented in Table 1.Biochar in the pots were incubated for six months in order to improve its association with soil and moisture retention ability. Soybean Var. NARC II (acquired from NARC, Islamabad) was used in the study. Three moisture regimes i.e., optimum moisture (OM) at 75± 2% of FC, deficit moisture (DM) at 50 ± 2% of FC and severe moisture deficit (SMD) at 40 ± 2% of FC were imposed at vegetative (V4) growth and at reproductive (R3) development.

Plant sampling had been carried out in all treatments from the day of first water deficit condition imposition at vegetative stage (V4) with an interval of 15 days. A total of 4 harvests had been taken which contributes to three harvest interval i.e. first harvest interval (2nd – 1st harvest includes deficit irrigation at vegetative stage), second harvest interval (3rd – 2nd harvest includes normal irrigation vegetative to early reproductive stage) and third harvest interval (4th – 3rd harvest includes deficit irrigation at Reproductive stage to maturity). Sampling was done by randomly selecting three plants from all treatments and the roots were washed with gentle steam of tap water. Afterwards, plant samples were partitioned into individual parts i.e., root, stem and leaves for growth studies. Shoot and root length (in centimeters) measurements were made using meter rod and the relative growth values of soybean from all treatments were calculated by the equation given by Radford (1967).

$$Relative\ increase\ in\ shoot\ or\ root\ length\ \left(\frac{cm}{day}\right) = \frac{Log_e L_2 - Log_e L_1}{T_2 - T_1}$$

Fresh weight data was noted by weighing the root and shoot fresh biomass after harvesting and afterwards heated in the oven (70°C) for three days till their weights becomes constant. Root and shoot fresh and dry weights on relative basis was determined by the following formulas:

$$\text{Relative increase in shoot or root fresh weight}\ \left(\frac{\text{g}}{\text{day}}\right) = \frac{\text{Log}_\text{e}\text{Fw}_2 - \text{Log}_\text{e}\text{Fw}_1}{\text{T}_2 - \text{T}_1}$$

$$\text{Relative increase in shoot or root Dry weight}\ \left(\frac{\text{g}}{\text{day}}\right) = \frac{\text{Log}_\text{e}\text{Dw}_2 - \text{Log}_\text{e}\text{Dw}_1}{\text{T}_2 - \text{T}_1}$$

Growth of No. of leaf per plant per day, relative leaf area in addition to leaf fresh and dry biomass was determined by the method of Radford (1967).

$$\text{Relative increase in No. of leaves} = \frac{\text{Log}_\text{e}\text{NL}_2 - \text{Log}_\text{e}\text{NL}_1}{\text{T}_2 - \text{T}_1}$$

Measurements regarding leaf area was taken with the help of ImageJ v.1.51 64-bit (NIH, Bethesda, MD, USA) software by setting scanning scale as a fraction of dpi/ (px/cm) and 1dpi = 0.393701 px/ cm. Leaf area measurements were taken by using a polygon selection tool and relative increase in leaf area was determined by working on the formula as mentioned below:

$$R\text{elative increase in leaf area} = \frac{\text{Log}_\text{e}\text{L}_2 - \text{Log}_\text{e}\text{L}_1}{\text{T}_2 - \text{T}_1}$$

Leaf fresh weight data was recorded and relative increase in leaf fresh biomass was determined:

$$\text{Relative increase in leaf fresh weight}\ \left(\frac{\text{g}}{\text{day}}\right) = \frac{\text{Log}_\text{e}\text{Fw}_2 - \text{Log}_\text{e}\text{Fw}_1}{\text{T}_2 - \text{T}_1}$$

Fresh leaves from all the treatments were heated in the oven (at 70°C) for 72 hours. After taking dry weights from all treatments, increase in dry biomass per day was determined as follows:

$$Relative\ increase\ in\ leaf\ dry\ weight\ \left(\frac{g}{day}\right) = \frac{Log_e Dw_2 - Log_e Dw_1}{T_2 - T_1}$$

RGR was evaluated by the formula of Radford (1967) as mentioned below:

$$RGR\ \left(\frac{g}{day}\right) = \frac{Log_e W_2 - Log_e W_1}{T_2 - T_1}$$

Table 1. Characteristics of sandy loam (SL) and clay loam (CL) soil.

	SL Soil	CL Soil
EC $mScm^{-1}$	0.8	1.5
pH	8.1	8.2
Organic Matter (%)	0.77	0.77
Available Nitrogen (%)	0.039	0.039
Available Phosphorus (mg Kg^{-1})	3.3	2.7
Available Potassium (mg Kg^{-1})	79	98

NAR (g $cm^{-2}day^{-1}$) was determined by the method of Gregory (1917).

$$NAR\ or\ E\ \left(\frac{mg}{cm^2}\right) = \frac{1}{Log_e L_2 - Log_e L_1} \times \frac{W_2 - W_1}{T_2 - T_1}$$

For statistical significance data was analysed on IBM SPSS (Ver. 21) by using GLM (General Linear Model) for univariate analysis. Statistical significance was determined for soil and biochar treatments by using ANOVA at 95% confidence interval. Percentage difference between treatments was estimated by Post hoc Duncan's test as a measure of significance between treatments. Standard deviation of means was marked as errors bars in the figures. Column represents the mean values of the treatments and different alphabets on the column in the figures represents significant variations from control under deficit irrigation i.e., DM and SMD.

Results

Relative shoot growth of soybean in different treatments is presented in Fig. 1a and 1b for first harvest interval (HI). In both CL and SL soil, all biochar treatments enhanced relative shoot length in contrast to the plants facing deficit moisture (DM) and severe moisture deficit (SMD) conditions without biochar and maximum shoot length i.e., 79% larger in 10 t/ ha AWB under DM irrigation regime and 1.7 times more in 20 t/ ha AWB under SMD regime within CL soil. while in coarse textured SL soil appreciably improved shoot length per day was recorded in 10 t/ ha AWB i.e., 84-113% enhanced under DM and SMD regimes. During second HI of only OM (optimum moisture) regime maximum improvement of shoot length was observed with biochar in CL soil in contrast to SL soil. During third HI under DM conditions highest gain in shoot lengths were assessed within AWB (under both concentrations) in CL soil (Fig. 1c), while in SL soil (Fig. 1d) highest relative increase was found in RSB (10 t/ ha) and WSB (20 t/ ha) in contrast to those plants which received deficit irrigation without biochar.

During first HI upon receiving DM irrigation soybean plants attained 56-106% improved shoot fresh weight in 10 t/ ha AWB and 44-100% improved in 10 t/ ha SBB than plants under DM regime without biochar in CL and SL soil (Fig. 2a and 2b) respectively. During second HI of only OM regime (i.e., 75% in all treatments), maximum improvement of soybean shoot fresh weights on relative basis was recorded in 10 and 20 t/ ha RSB (23-31% respectively) in CL soil than control. During third HI in SL soil highest relative gain in fresh weights of soybean shoots was traced in both concentrations of AWB under DM in contrast to alone DM regime. While SBB (10 and 20 tones ha^{-1}) and AWB (10 t/ ha) amendments caused highest relative gain in fresh weights of soybean shoots under all moisture regimes in CL and SL soils (Fig. 2c and 2d).

In CL soil in contrast to alone DM and SMD maximum gain in dry weights of shoots were 53-65% under DM regime and 52-89% under SMD regime in SBB (20 t/ ha) and AWB (10 t/ ha) respectively (Fig. 3a). While in SL soil (Fig. 3b) under OM regime 32% and 19% enhanced shoot dry weights per day was recorded in 10 t/ ha and 20 AWB respectively than respective controls. During second HI, 120-175% higher gain in dry biomass of soybean was traced in RSB (20 t/ ha) and AWB (20 t/ ha) which received SMD followed by OM than other amendments in CL soil. During third HI mostly all biochar treatments (Fig. 3c and 3d) had relatively high shoot dry weights increments under SMD and DM irrigation than without biochar with SMD and DM irrigation.

During first HI 78-83% highest relative increase in root length was recorded in10 t/ ha SBB and 20 t/ ha RSB under DM irrigation than control (without biochar under DM regime) in SL soil (Fig. 4b) while in CL soil (Fig. 4a) 21-36% highest relative enhancement in root length was noticed within both concentrations of SBB under SMD irrigation in contrast to control (under SMD regime). Relative increase in root length for second HI of OM irrigation regime was non-significant between treatments in both types of soils. During third HI (Fig.4c and 4d) deficit irrigation regimes of DM and SMD have no considerable variations in soybean root length among different amendments in both types of soil.

Relative gain in fresh weights of soybean roots in SL soil (Fig. 5b) during first HI was found to be appreciably higher in AWB (10 t/ ha), SBB (10 t/ ha) and WSB (10 t/ ha) under DM irrigation i.e., 95%, 105% and 140% respectively than control under DM conditions, while in CL soil (Fig. 5a) considerably higher gain in fresh weights of roots were traced in the plants of AWB (10 t/ ha) amendments i.e., 96-138% under DM and SMD regimes than under alone DM regime. During third HI maximum relative gain in fresh weights of soybean roots were noticed in SBB (10 t/ ha) under DM regime i.e., 89% high than alone DM in SL soil (Fig. 5d) while AWB (10 t/ ha) under DM regime had 100% higher gain in fresh weights of roots on relative basis than alone DM in CL soil (Fig. 5c).

During first HI in CL soil (Fig. 6a) maximum relative (48-74%) gain in dry weights of soybean roots were recorded with both concentrations of AWB under DM and SMD regimes than controls, while in SL soil (Fig. 6b) SBB (10 t/ ha) under DM regime had 89% higher relative gain in dry weights of roots than alone DM regime. During second HI 60-65% higher relative gain in dry weights of soybean roots were recorded in SBB (20 t/ ha) and RSB (10 t/ ha) which were exposed with SMD irrigation previously and received OM regime during second HI in CL soil while in SL soil 35-39% high relative gain in dry weights of roots were noticed in WSB (10 and 20 t/ ha) amended soil which received DM regime during first harvest interval and OM regime during second HI. During third HI (Fig. 6c and 6d) highest relative gain in dry weights of roots were found in AWB (10 t/ ha) under DM and SMD regimes.

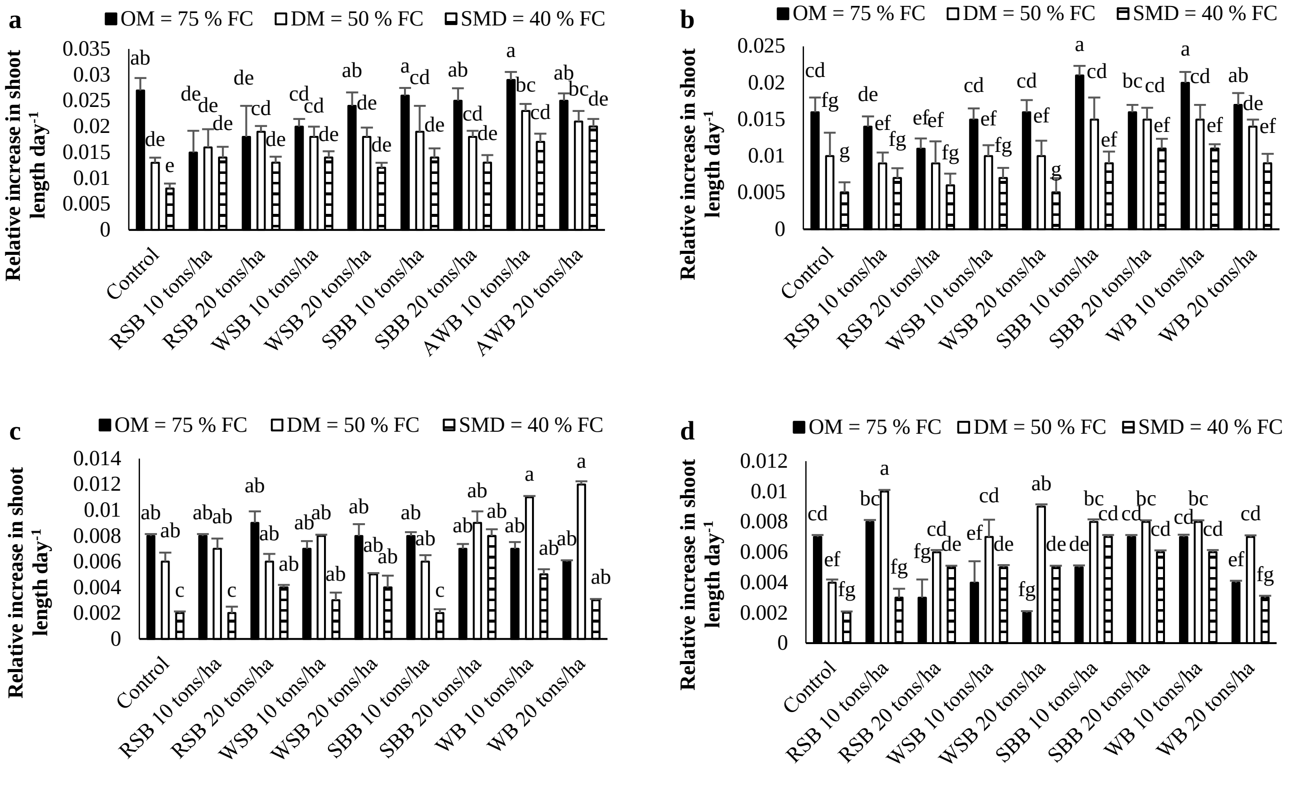

Fig. 1. Relative Increase in Shoot Length Day^{-1} during **a)** 2nd – 1st harvest interval in CL **b)** 2nd – 1st harvest Interval in SL **c)** 4th – 3rd harvest Interval in CL **d)** 4th – 3rd harvest interval in SL

RSB = Rice Straw Biochar WSB = Wheat Straw Biochar SBB = Sugarcane Bagasse Biochar
AWB= Wood Shaving Biochar CL = Clay Loam Soil SL =Sandy Loam Soil

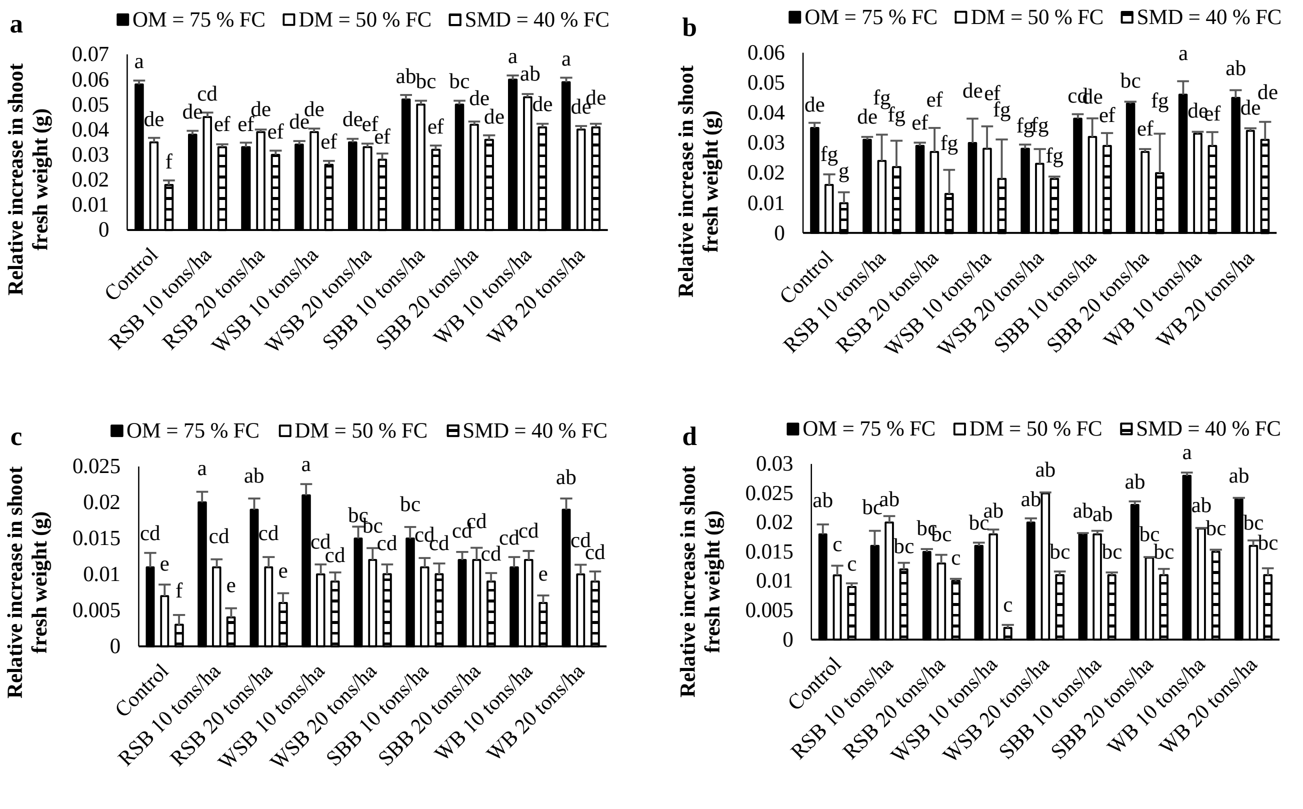

Fig. 2. Relative Increase in Shoot Fresh weight day^{-1} during **a)** 2nd – 1st harvest interval in CL **b)** 2nd – 1st harvest Interval in SL **c)** 4th – 3rd harvest Interval in CL **d)** 4th – 3rd harvest interval in SL

RSB = Rice Straw Biochar WSB = Wheat Straw Biochar SBB = Sugarcane Bagasse Biochar
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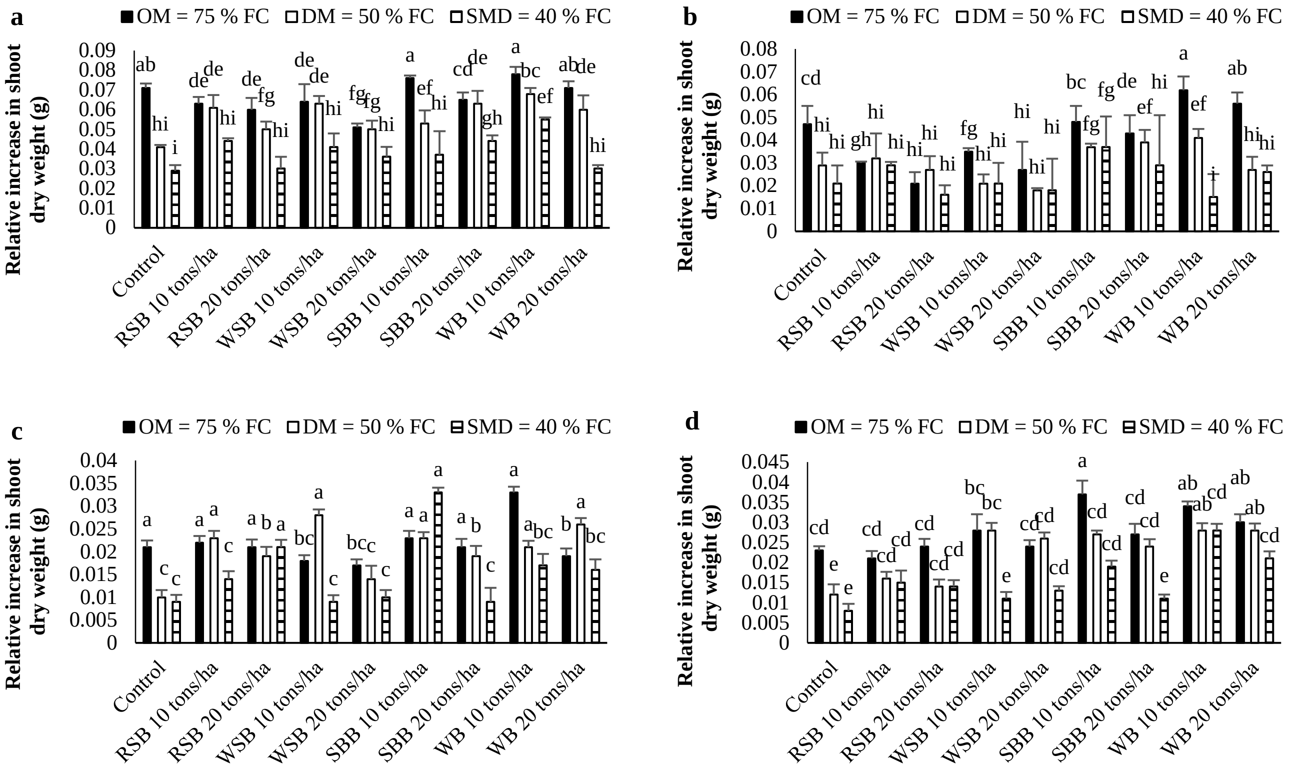

Fig. 3. Relative Increase in Shoot Dry weight day^{-1} during **a)** 2nd – 1st harvest interval in CL **b)** 2nd – 1st harvest Interval in SL **c)** 4th – 3rd harvest Interval in CL **d)** 4th – 3rd harvest interval in SL
RSB = Rice Straw Biochar WSB = Wheat Straw Biochar SBB = Sugarcane Bagasse Biochar
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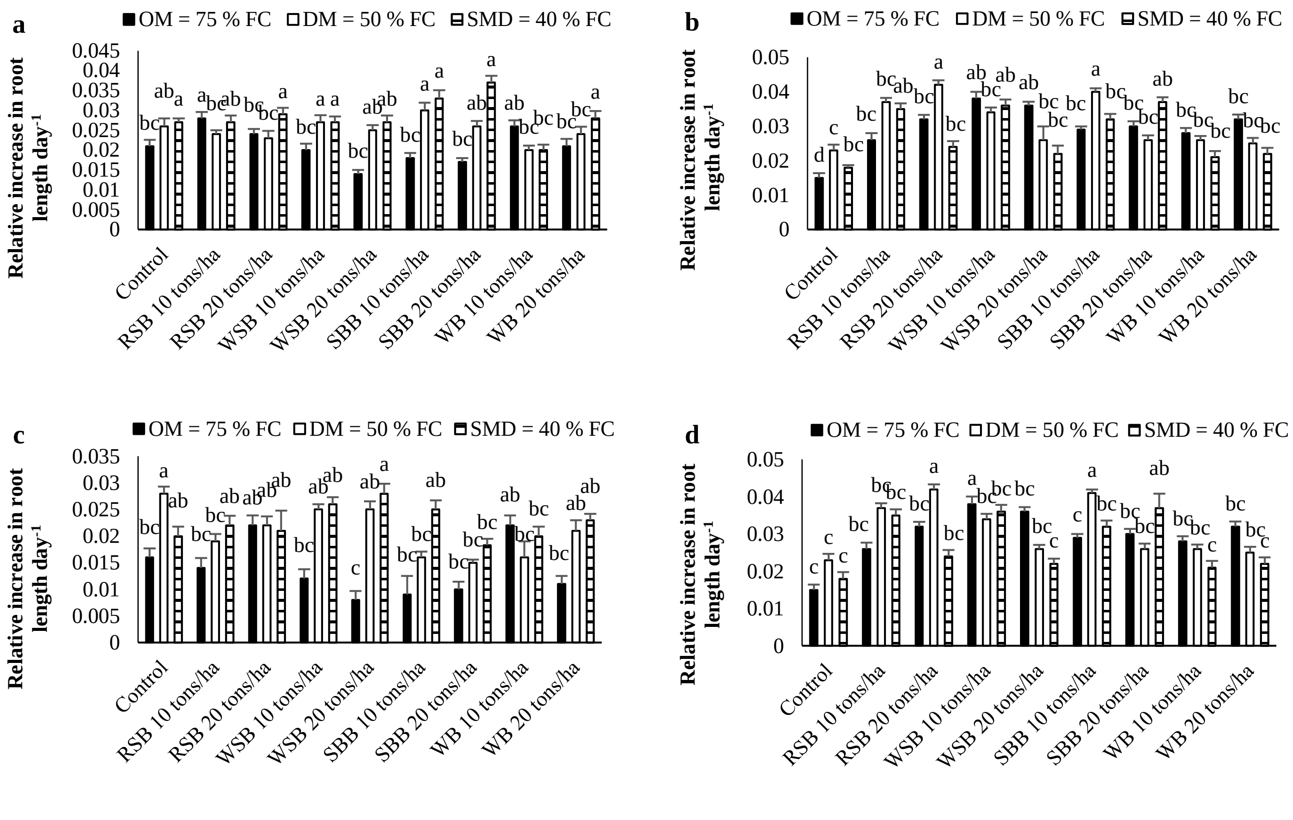

Fig. 4. Relative Increase in Root Length Day^{-1} during **a)** 2nd – 1st harvest interval in CL **b)** 2nd – 1st harvest Interval in SL **c)** 4th – 3rd harvest Interval in CL **d)** 4th – 3rd harvest interval in SL
RSB = Rice Straw Biochar WSB = Wheat Straw Biochar SBB = Sugarcane Bagasse Biochar
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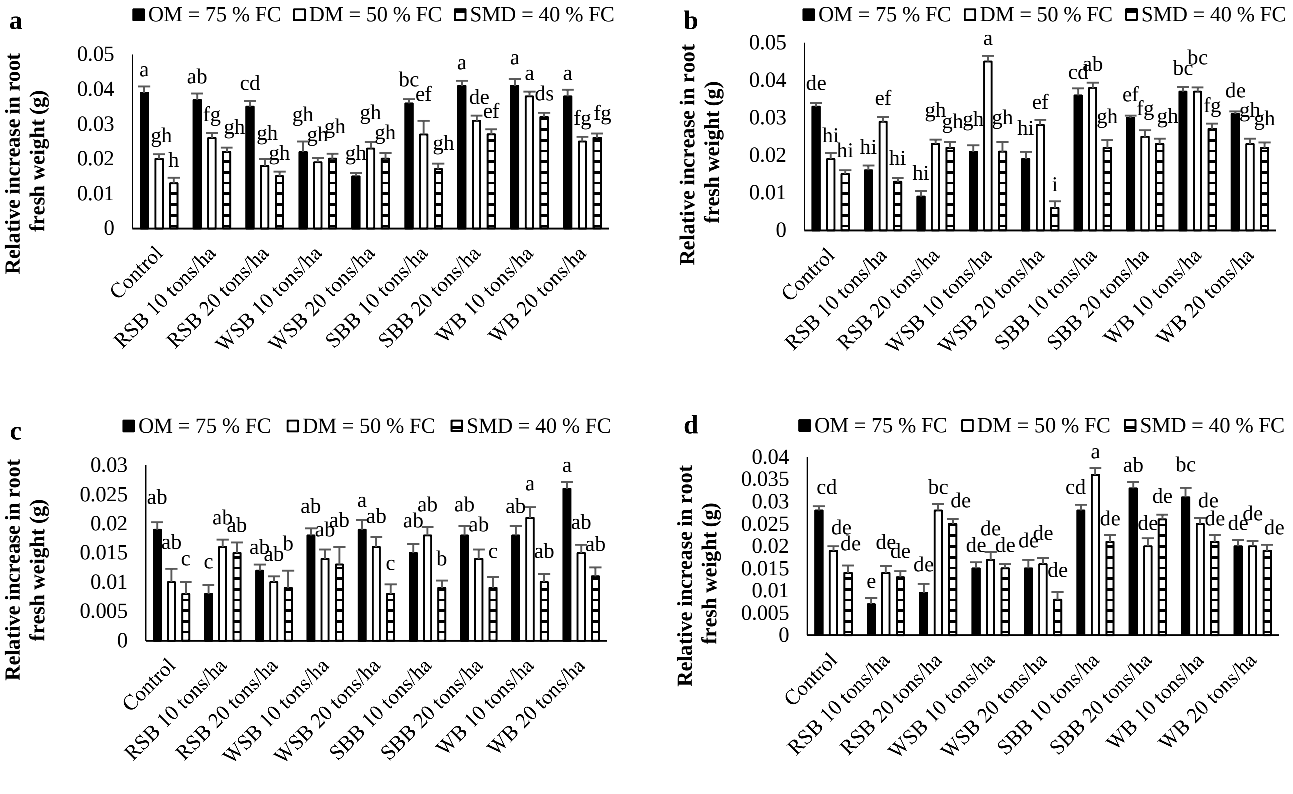

Fig. 5. Relative Increase in Root Fresh Weight Day^{-1} during **a)** 2nd – 1st harvest interval in CL **b)** 2nd – 1st harvest Interval in SL **c)** 4th – 3rd harvest Interval in CL **d)** 4th – 3rd harvest interval in SL

RSB = Rice Straw Biochar WSB = Wheat Straw Biochar SBB = Sugarcane Bagasse Biochar
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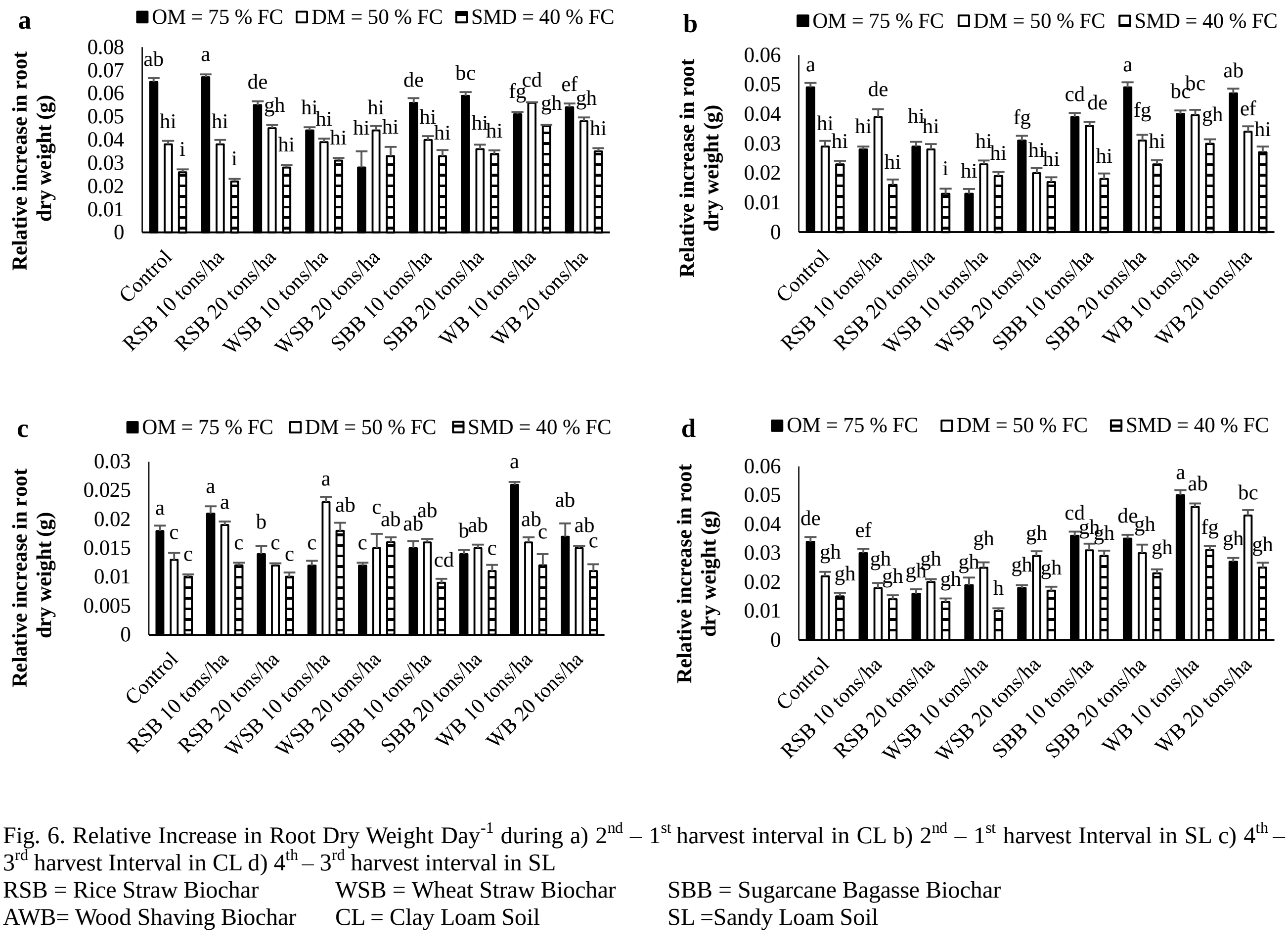

Fig. 6. Relative Increase in Root Dry Weight Day^{-1} during a) 2nd – 1st harvest interval in CL b) 2nd – 1st harvest Interval in SL c) 4th – 3rd harvest Interval in CL d) 4th – 3rd harvest interval in SL

RSB = Rice Straw Biochar WSB = Wheat Straw Biochar SBB = Sugarcane Bagasse Biochar
AWB= Wood Shaving Biochar CL = Clay Loam Soil SL =Sandy Loam Soil

During first HI most biochar treatments (except RSB with both application rates under moisture deficit regimes) in CL soil (Fig. 7a) had considerably higher relative increase in soybean No. of leaf than those without biochar under SMD and DM regimes. Both levels of AWB and SBB under OM and DM regimes had considerably improved No. of leaves than respective control. During third HI high relative increase in No. of leaves per day (Fig. 7c) was recorded in most of the biochar treatments in CL soil while in SL soil (Fig. 7d) only 10 t/ ha AWB had considerably improved No. of leaves under SMD irrigation in contrast to control.

Maximum relative gain in fresh weights of leaves during first HI were noted in CL soil as shown in Fig. 8a and 8b. Considerably higher relative achievement in fresh weights of leaves were recorded in soybean within AWB (20 t/ ha) i.e., 24 % elevated from control (under OM regime) in CL soil while under DM regime highest relative increase of 39-41% was noted in AWB (10 t/ ha) in SL and CL soil. During second HI of OM irrigation regime, plants of CL and SL soil (with biochar amendments) which received water stress previously gained highest relative increase in leaf fresh weights. During third HI (Fig. 8c) relative gain in fresh weights of leaves were 49%, 58% and 82% in 10 t/ ha AWB under DM regime, 10 t/ ha WSB under DM regime and RSB 10 t/ ha under OM regime in CL was noticed.

Figure 9a and 9b represents relative gain in dry weights of leaves in first HI. Under DM irrigation, 10 t/ ha AWB had highest relative gain in dry weights of leaves in comparison to all other biochar treatments in both types of soils. During second HI of only OM irrigation regime most treatments which were exposed previously to deficit irrigation had high relative gain in fresh weights of leaves particularly in 10 t/ ha AWB and 10 t/ ha SBB in both CL and SL soils. During third HI (Fig. 9c and 9d) relative gain in dry weights of leaves were higher in most biochar amendments in CL soil than those without biochar under DM and SMD irrigations.

In CL soil (Fig. 10a) during first HI under DM irrigation considerably higher relative gains of leaf area were evaluated in soybean growing in 10 t/ ha AWB amendment under OM regime i.e., 12% improved from control plants while in SL soil (Fig. 10b) 10 t/ ha SBB had 75% higher relative gains of leaf area under DM regime than its respective control. Relative gains of leaf area during 2nd HI of only one irrigation regime (OM regime) differ non-significantly between different treatments in CL soil while in SL soil most biochar treatments had high relative increase than those without biochar. During third HI in CL soil (Fig. 10c) in contrast to SL soil (Fig. 10d) under SMD irrigation more drastic reduction in relative growth of leaf area was found in both concentrations of WSB, 20 t/ ha RSB and alone SMD irrigation.

Net assimilation rate (NAR) during first HI was recorded to be considerably higher in the plants of AWB (20 t/ ha) amended CL soil (Fig. 11a) under OM conditions i.e., 25% improved than respective control. However, under DM irrigation AWB (10 t/ ha) had 77% higher NAR than DM regime while in SL soil (Fig. 11b) maximum NAR was found in WSB (10 t/ ha) under OM irrigation i.e., 2.4 times higher from control plants. While SBB (both concentrations) had 1.2-2 times higher NAR under SMD and DM irrigation respectively. NAR during second HI differ non-significantly with OM irrigation conditions in both types of soils. During third harvest interval under DM irrigation 2-2.3% high NAR was noticed in treatment 20 t/ ha AWB and 20 t/ ha SBB than DM irrigation alone in SL soil (Fig. 11d). In CL soil (Fig. 11c) under DM irrigation 3.2 times higher NAR than DM regime was found in 10 t/ ha WSB and almost all biochar treatments had high NAR than control under SMD irrigation.

Relative growth rate (RGR) during first HI was found to be appreciably higher in the plants of AWB (10 t/ ha) amendment in CL soil under OM, DM and SMD irrigation regimes i.e., 0.11, 1.11 and 1.26 times of their respective control. Similarly in SL soil (Fig. 12b) 10 t/ ha AWB had maximum RGR i.e., 18.11, 32 and 68% high than control SMD, OM and DM respectively. RGR during second HI of only OM regime of FC in all treatments was significantly higher. Figure 12c and 12d depicts RGR during third HI. Noticeably higher RGR was found under OM, DM and SMD irrigation regimes in SBB (10 and 20 t/ ha) and AWB (10 and 20 t/ ha) amended SL soil (Fig. 12d). While in CL soil (Fig. 12c) plants of 10 t/ ha AWB amendment under OM, DM and SMD irrigation conditions had considerably higher RGR than their respective controls.

Discussion

Water is equally essential quantitatively as per its qualitative requirements; it mostly constitutes the protoplasmic structures, and extreme water deficiency could even cause changes in the cellular framework and decreased physiological activity which causes reduction in growth and tissue water content (Paul, 1983). However, biochar in the soil improve its moisture holding potential and serve as a valuable approach to improve moisture availability to plants under drought (Akhtar *et al*., 2014). Both moisture deficit regimes DM and SMD regimes caused decline in the shoot growth parameters i.e., shoot lengths, shoot fresh and dry weights. Since shoot biomass is the most crucial indicator of growth after facing stress (Hamlyn *et al*., 1989) and it inhibited as soon as the plant roots receives the signal of water deficit conditions prior to decrease of water potential in the above ground parts of plants (Gowing *et al*., 1990). Growth regulating signals from roots during stress conditions could be involved in reduced shoot lengths and abscisic acid (ABA) could be one of them which is sourced by roots and limit shoot growth (Sharp & LeNoble, 2002). Reduction in relative gains of dry weights of shoot under water limmited conditions could be due to decreased growth and productivity which usually accompanied by reduced cell expansion and division by the loss of turgidity (Kiani *et al*., 2007; Hussain *et al*., 2009). It was also reported earlier that water deficit conditions decreased shoot biomass (fresh and dry weights) of *Tagetes erecta* L. (Asrar & Elhindi, 2011), and in *Salvia miltiorrhiza* L. (Liu *et al*., 2011). Synergistic impacts of *Acacia* (Sp. *nilotica*) wood shaving biochar (AWB) particularly 10 t/ ha under DM regime on relative increase in shoot length, shoot fresh and dry weights of soybean could be due to greater potential of wood nature biochar to improve soil physical structure, soil nutrition which consequently enhance crop growth (Lehmann & Joseph, 2009; Zimmerman, 2010).

On relative basis high increase in shoot length, shoot fresh and dry weight under water deficit regimes could be attributed to biochar's high moisture holding ability (Asai *et al*., 2009; Major *et al*., 2010). Erlier, it was reported that biochar (derived from *Lantana camara plant biomass at 450°C)* application in *Abelmoschus esculentus* increased plant height under moisture stress (Batool *et al*., 2015), similarly soybean growth was enhanced by pelleted broiler litter (PBL) biochar (Sanvong & Nathewet, 2014), shoot fresh weight enhanced by Lantana biochar (6 t/ ha) amendment (Berihun *et al*., 2017) and vigorous growth triggered an enhanced shoot dry biomass by Lantana biochar (18 t/ ha) was also reported earlier (Shamshuddin *et al*., 2004; Berihun *et al*., 2017). However not all biochar have beneficial influence in the soil some biochar contain toxic substances that affect plant growth negatively (Jones & Stewart, 1997). So, the impact of biochar on plant growth and development mainly influenced by the type of biochar, its rate of application, and most importantly on soil physical as well as chemical properties (Alburquerque *et al*., 2014).

During first harvest interval moisture deficit regimes triggered enhancement in root lengths in contrast to control plants growing in both types of soils but at reproductive stage during third harvest interval there was found a substantial reduction in relative increase in root length under moisture deficit conditions. Moisture limited conditions had less injurious impact on roots of soybean in contrast to shoots this response might be due to root plasticity towards moisture stressed environment during early stages of growth, hence more carbon allocation occurs towards the roots which causes increased root ratio in contrast to aerial part which ultimately enhanced root parameters as root length, weight, volume and root hairs which are associated with water stress tolerance (Liu *et al*., 2005). Though, moisture scarcity causes substantial reduction in dry biomass of *Salvia miltiorrhiza* roots (Liu *et al*., 2011). Previously, Salim (2016) also reported that with the amendment of plant origin biochar (at a rate of 2% and 5%) enhanced root length and root fresh biomass of *Triticum aestivum*. Increased root and plant growth of soybean under moisture stressed environment in biochar amended soil could be due to its potential to enhance water holding capacity, nutrient availability and better ion exchange capacity of soil. As biochar soil applications facilitate availability of vital growth nutrients as K, P, N, Ca and Mg (Nigussie *et al*., 2012; Walter & Rao, 2015), which are obligatory associated with plant development (Changxun *et al*., 2016), as K^+ associated with osmotic adjustment, stomatal functioning and biochemical regulation under drought (Wyn Jones *et al*., 1979).

Moisture deficit conditions affect all growth parameters of leaf during first harvest interval of vegetative growth and affects were more negative at reproductive growth during third harvest interval. Previous literature also clarify that moisture stress associated with reduced leaf area due to turgor losses, by reduction in cell enlargement which caused reduced growth rates of leaves, decreased No. of leaves and hence reduced vegetative as well as reproductive growth by decreased photo-assimilation (Hsiao, 1973; Farooq *et al*., 2010), early senescence could also be involved (Nooden, 1988). During second harvest Interval when water deficit plants were irrigated to OM, the relative increase in all leaf parameters were high in contrast to previous harvest interval. It was also reported earlier that rapid growth occurs following a moisture deficit period because metabolites accumulated under water stress by inhibition of cell enlargement are used in rapid cell growth and new cell wall synthesis after turgor maintenance (Paul, 1983). Most biochar treatments in CL soil had high relative growth of leaves at OM regime and under water deficit conditions mostly sugarcane bagasse and wood biochar (10 t/ ha) had high relative gains in No. of leaves, fresh and dry weights of leaves and leaf photosynthetic area. Hence, increase in leaf growth parameters under water stress could be due to beneficial modification in soil surface area and pore space density by biochar which ultimately influence positively soil water holding capacity and plant available water content (Verheijen *et al*., 2010; Case *et al*., 2012). So, biochar application under moisture deficit conditions could be a valuable approach for improved water use efficiency for plants (Akhtar *et al*., 2014). It was also reported earlier that leaf morphological adjustments might be associated with moisture stress tolerance and hence regulation of leaf area may be greatly advantageous to provide survival benefits to soybean under water deficit conditions (Manavalan *et al*., 2009). One of such adjustments in plants is to increase the No. of epidermal cells which resulted in increased leaf area under mild water stress but under extreme water deficit conditions the number of epidermal cells also increases but due to arrest in cell enlargement leaf area couldn't increase (Bunce, 1977). Relatively high increase in leaf parameters by biochar may be due to increased leaf area which promotes plant photosynthetic capacity and higher carbon gains (Carter *et al*., 2013).

Plant's circadian photosynthetic activity is reflected in terms of net assimilation rate which causes the accumulation of carbon content in the plants and ultimately leads to constructive physiological changes (Shipley, 2002). As vegetative stage of plant is characterized by intensive growth under appropriate moisture conditions, which consequently raised plant biomass due to increased net assimilation rate, similarly proper irrigation during early reproductive stage is associated with greater yields of plants (Cakir, 2011). Plants with fast growth rate usually have high net assimilation rates (Li *et al*., 2016). NAR is also strongly associated with relative growth rate (RGR) across woody as well as herbaceous plants (Shipley, 2006; Poorter & Nagel, 2000).

Relative growth rate of soybean under different irrigation regimes during three harvest interval revealed that during first and third harvest interval (Fig. 12a and 12d) moisture deficit condition (DM & SMD regimes) sufficiently reduce relative growth rate than those under OM irrigation. However, most biochar treatments resulted in high relative growth rate in CL soil than SL soil. Second harvest interval with OM irrigation regime in all treatments marked with high relative growth rate in most treatments. Soybean respond to moisture deficit regimes by increased root biomass and reduced RGR and leaf area in contrast to those under well-watered regimes (OM irrigation) this could be due to proportionally more allocation of photo assimilates to roots than to shoots and leaves (Fernández *et al*., 2002).

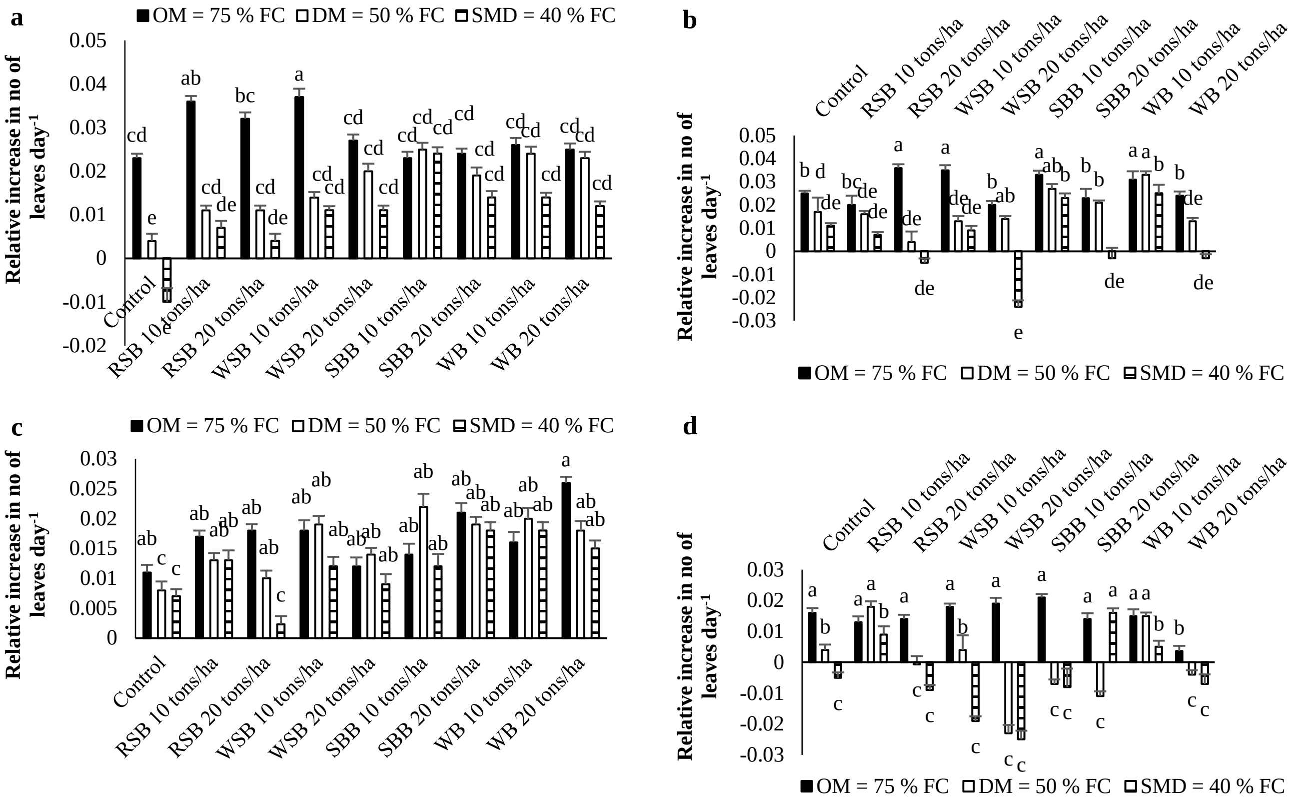

Fig. 7. Relative Increase in No. of Leaves Day^{-1} during a) 2nd – 1st harvest interval in CL b) 2nd – 1st harvest Interval in SL c) 4th – 3rd harvest Interval in CL d) 4th – 3rd harvest interval in SL

RSB = Rice Straw Biochar WSB = Wheat Straw Biochar SBB = Sugarcane Bagasse Biochar
AWB= Wood Shaving Biochar CL = Clay Loam Soil SL =Sandy Loam Soil

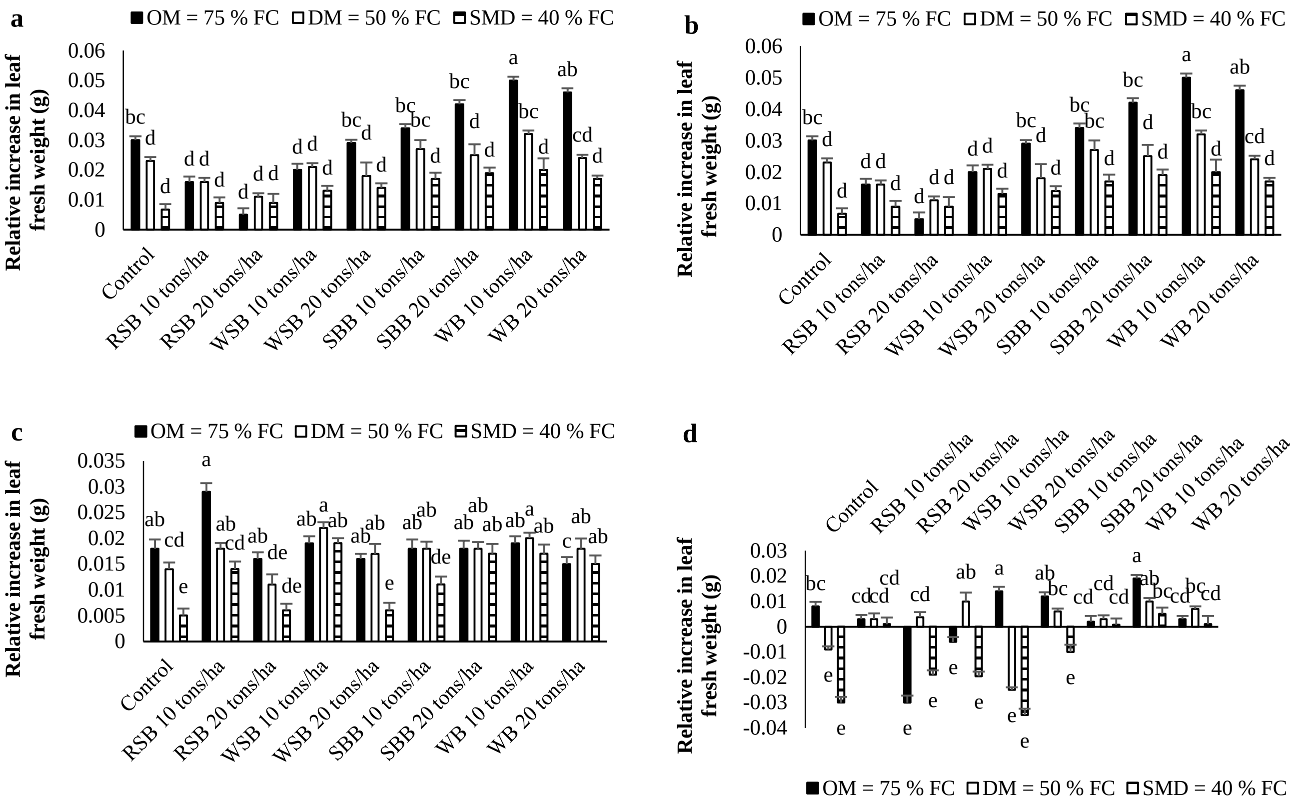

Fig. 8. Relative Increase Leaf Fresh Weight Day^{-1} during a) 2nd – 1st harvest interval in CL b) 2nd – 1st harvest Interval in SL c) 4th – 3rd harvest Interval in CL d) 4th – 3rd harvest interval in SL

RSB = Rice Straw Biochar WSB = Wheat Straw Biochar SBB = Sugarcane Bagasse Biochar
AWB= Wood Shaving Biochar CL = Clay Loam Soil SL =Sandy Loam Soil

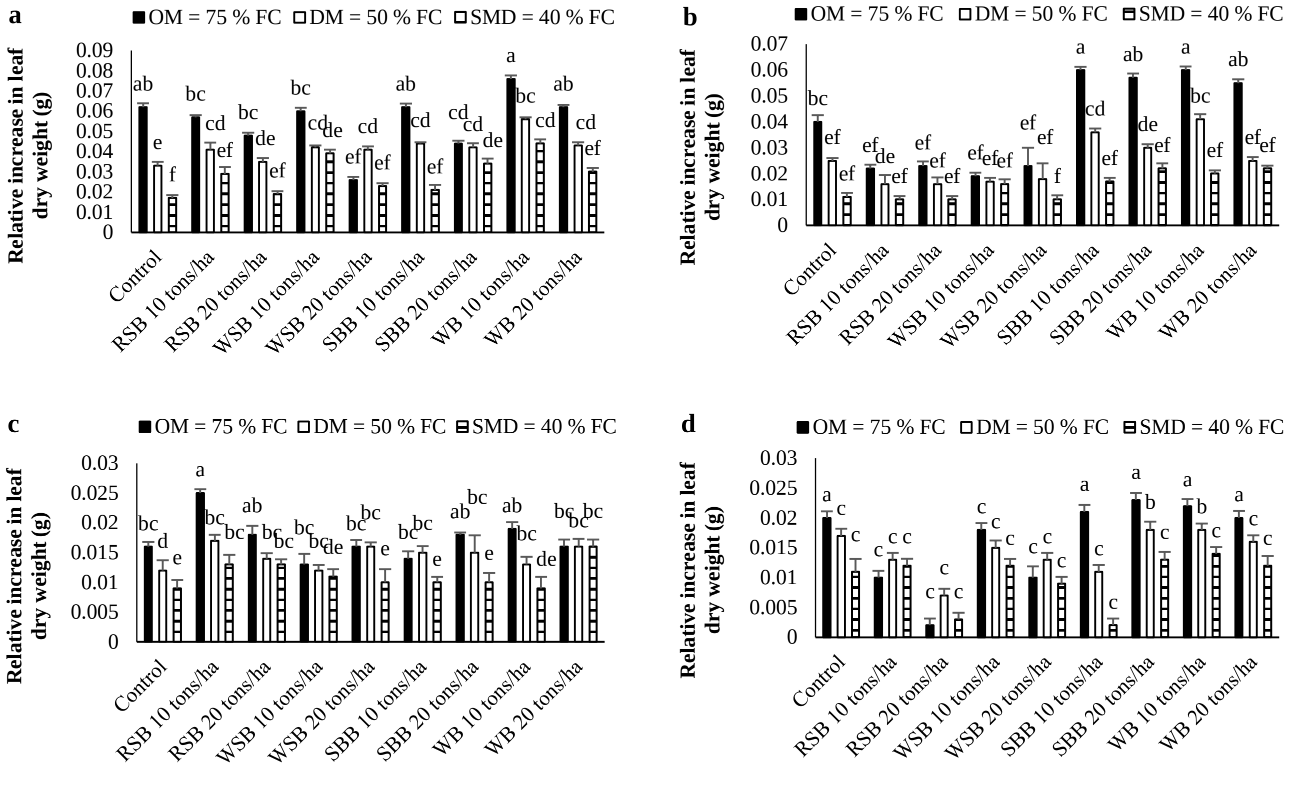

Fig. 9. Relative Increase in Leaf Dry Weight Day^{-1} during a) 2nd – 1st harvest interval in CL b) 2nd – 1st harvest Interval in SL c) 4th – 3rd harvest Interval in CL d) 4th – 3rd harvest interval in SL

RSB = Rice Straw Biochar WSB = Wheat Straw Biochar SBB = Sugarcane Bagasse Biochar
AWB= Wood Shaving Biochar CL = Clay Loam Soil SL =Sandy Loam Soil

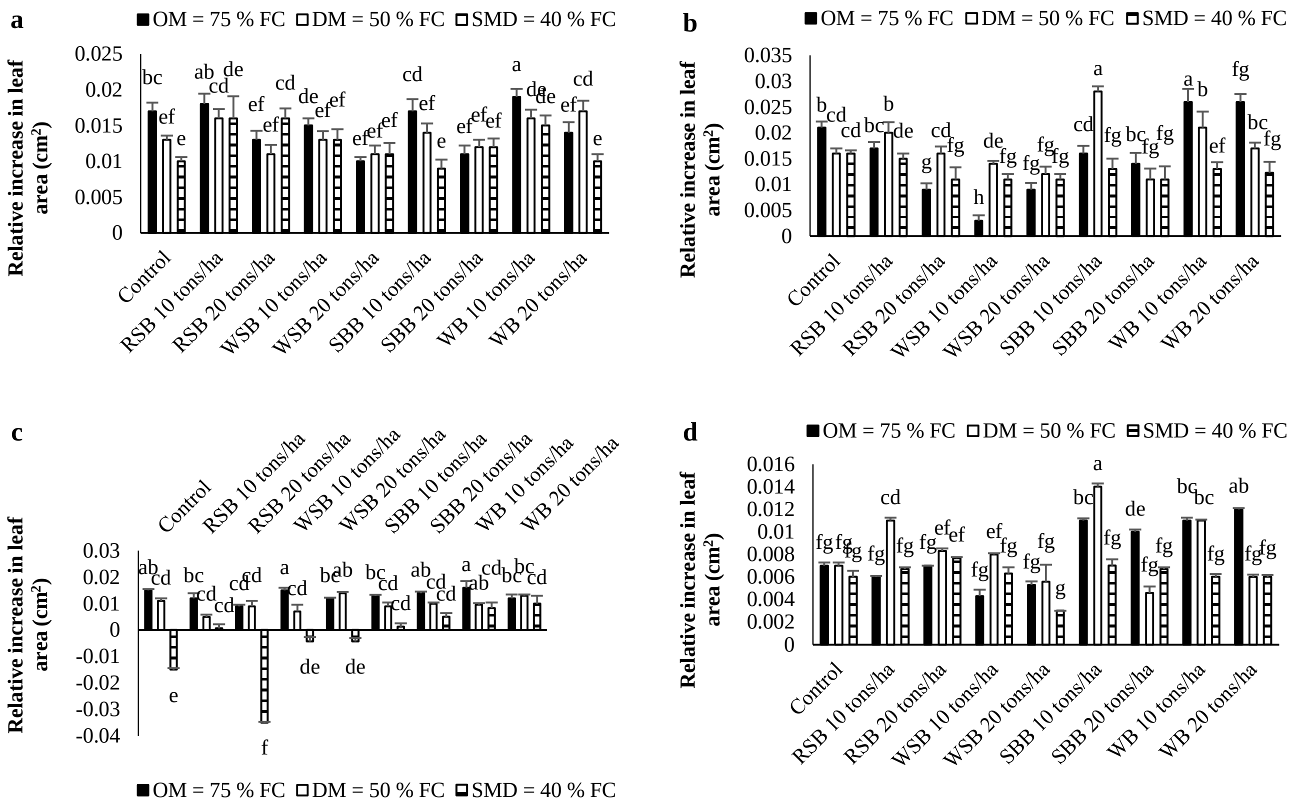

Fig. 10. Relative Increase in Leaf Area Day^{-1} during a) 2nd – 1st harvest interval in CL b) 2nd – 1st harvest Interval in SL c) 4th – 3rd harvest Interval in CL d) 4th – 3rd harvest interval in SL

RSB = Rice Straw Biochar WSB = Wheat Straw Biochar SBB = Sugarcane Bagasse Biochar
AWB= Wood Shaving Biochar CL = Clay Loam Soil SL =Sandy Loam Soil

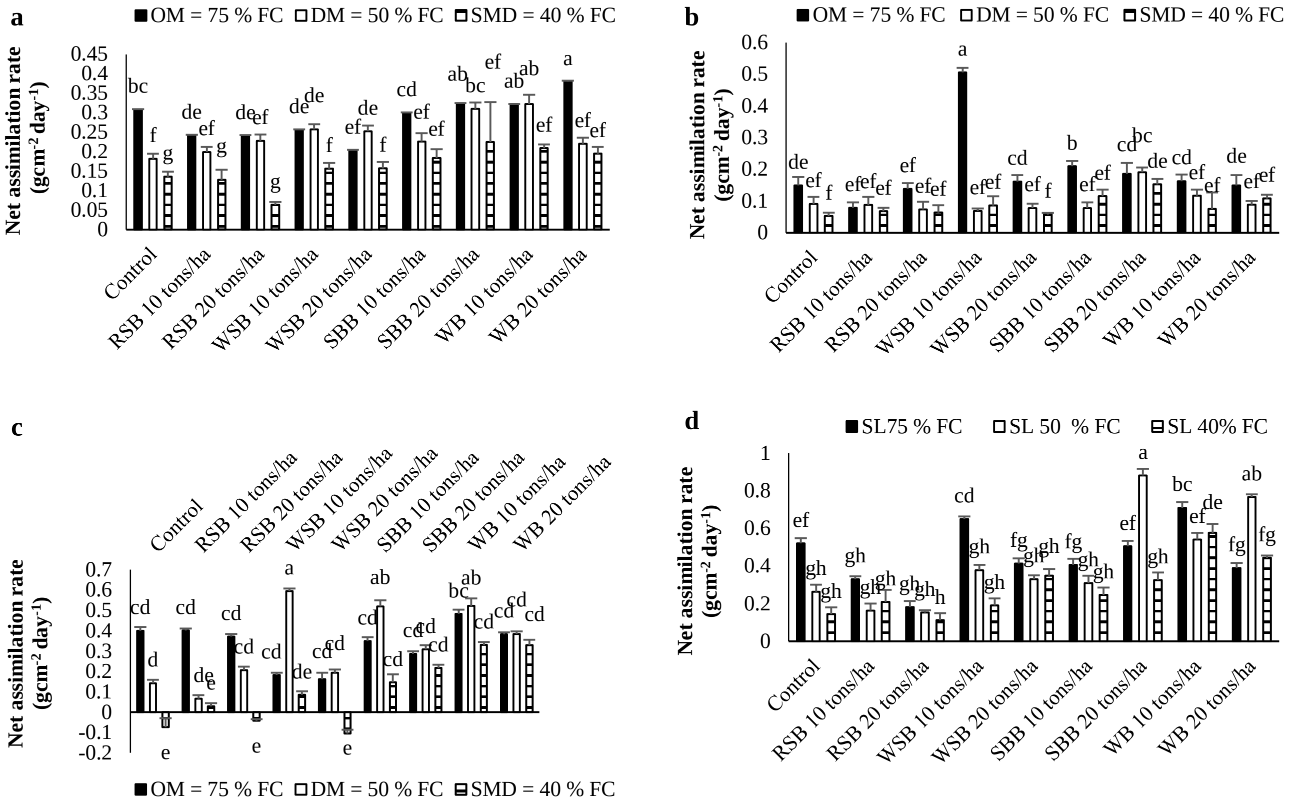

Fig. 11. Net Assimilation Rate (NAR) during first during **a)** 2nd – 1st harvest interval in CL **b)** 2nd – 1st harvest Interval in SL **c)** 4th – 3rd harvest Interval in CL **d)** 4th – 3rd harvest interval in SL

RSB = Rice Straw Biochar WSB = Wheat Straw Biochar SBB = Sugarcane Bagasse Biochar
AWB= Wood Shaving Biochar CL = Clay Loam Soil SL =Sandy Loam Soil

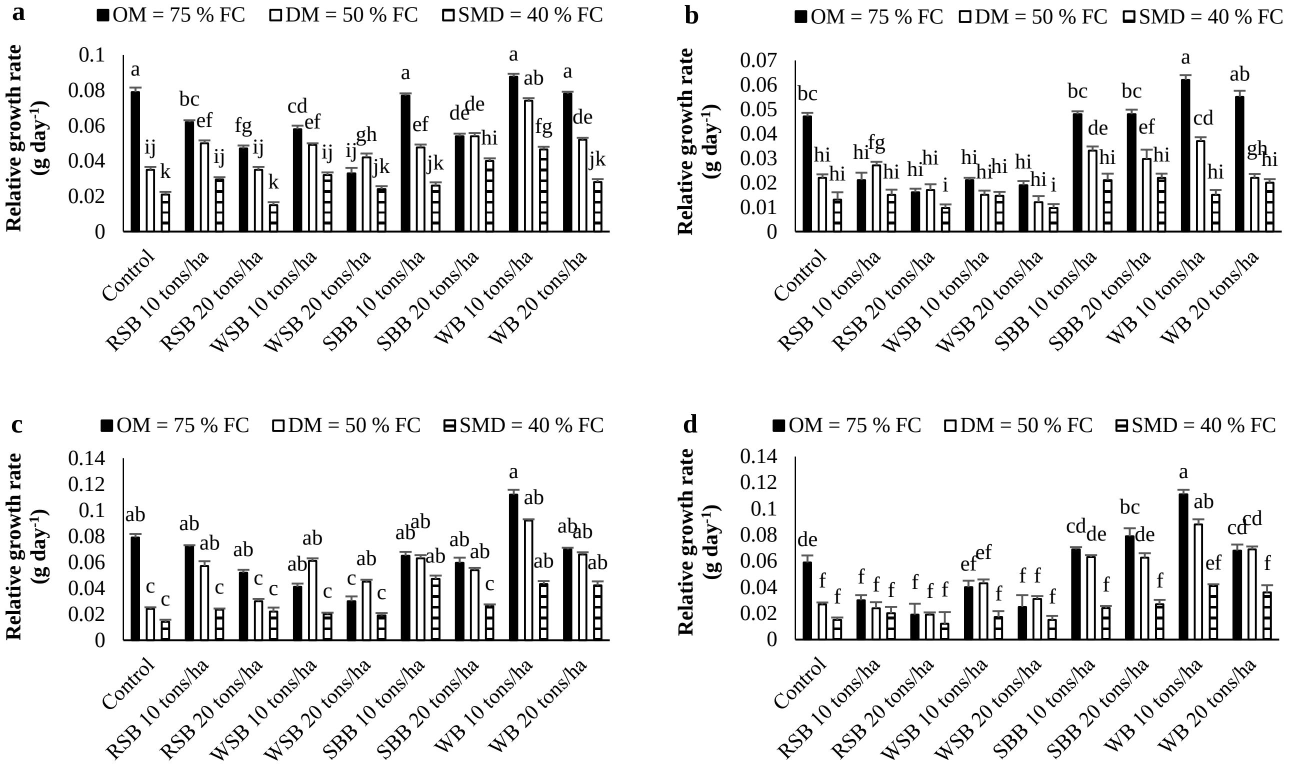

Fig. 12. Relative Growth Rate (RGR) during a) 2nd – 1st harvest interval in CL b) 2nd – 1st harvest Interval in SL c) 4th – 3rd harvest Interval in CL d) 4th – 3rd harvest interval in SL

RSB = Rice Straw Biochar WSB = Wheat Straw Biochar SBB = Sugarcane Bagasse Biochar
AWB= Wood Shaving Biochar CL = Clay Loam Soil SL =Sandy Loam Soil

Conclusion

Moisture deficit condition declined soybean growth attributes as relative gain in shoot length, fresh and dry weights of shoots, leaf area, No. of leaves, fresh and dry weights of leaves, net assimilation rate and hence relative growth rate. Most biochar treatments had high relative growth under control soil under both normal and deficit irrigation regimes. However, acacia (sp. *nilotica*) wood biochar amendment particularly 10 t/ ha under moisture deficit regimes caused relatively high growth in soybean. Positive influence of biochar suggests its potential role for persistent beneficial soil modification which favors its practical implementation in commercial agriculture. Thus, the present work needs to be repeated under wider range of field conditions for further recommendations.

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