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Research Article

Amending Soil with Rhizobium carrying Biochar Ameliorates Drought Stress on *Phaseolus vulgaris*

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Abstract: As a consequence of climate change/global warming earth's agriculture output is under rigorous stress. There is a growing need to develop strategies to cope with these abiotic stresses. Biochar exhibiting many beneficial qualities appeared to alleviate these problems by improving soil fertility by adding carbon and preventing nutrient losses etc. Biochar can also enhance BNF and could be used as a carrier for rhizobium by providing a suitable microenvironment. The current study is aimed to find the ameliorative potential of different biochar types to be used as rhizobium carriers for *Phaseolus vulgaris* L. exposed to drought stress. Both types of biochar were analyzed for physico-chemical and morphological parameters. Presence of Silicon content remains the key finding for rice husk biochar which was absent in *Lantana* biochar. Increased C, K, and Ca weight percentages were found in *Lantana* biochar as compared to their proportions for rice husk biochar. On the contrary, the oxygen content was higher in rice husk biochar as compared to that in *Lantana. Phaseolus* seeds were used for the pot experiment where stress treatment was applied by FTSW (Fractionable Transpirable Soil Water) technique. One isolated strain along with two types of biochar carrier was applied to the plants in combination with water stress treatment. Plants were analyzed for growth and physiological parameters including plant height, leaf area, biomass, photosynthesis, transpiration rate, stomatal conductance, and water use efficiency, where rice husk biochar responded better than the one obtained from *Lantana.* Plants responded positively for all the growth as well as physiological parameters when treated in combination with the inoculum for both stress levels i.e., 100 % and 60 % field capacity F.C. The present study advocates rice husk biochar for its ability to enhance tolerance in *Phaseolus* against drought stress through its role as an inoculum carrier contributing suitable habitat for the microorganism.

Keywords: Biochar, *Phaseolus vulgaris,* Rhizobium carrier, drought, arid climate

1. INTRODUCTION

Sustainability of global agriculture is inherently linked with climatic transformations. Shift in weather patterns, rainfall intensity, and frequency has diverse effects on ecological systems especially soil health [1]. These worldwide environmental changes are a matter of great concern due to their potential effects on our future ecosystems [2] and demand thoughtful learning along with the quantification of the anthropogenic processes involved [3]. Fahad *et al.* [4] have emphasized on employing various mitigation strategies to combat climate change. The prolonged drought can pose a wide range of abiotic stresses for plants [5–6]. Therefore, plants exhibit a vast range of morphological and physiological variations in response to such water-deficit situations [7]. The plant regulates its diverse mechanisms ranging from leaf area reduction to root/shoot ratio increase, stomatal conductance, or osmotic adjustment [8–10] to cope with the water stress condition.

Many rhizobial strains show tolerance towards certain stress effects thus forming effective (N_2-fixing) symbiotic association with their host legumes under heat, salt, and acid stress environments. However, the stress tolerance

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levels vary with strains depending on their natural habitats. It becomes difficult for rhizobium to infect root hairs at temperatures greater than 40 °C, while nitrogenase activity also decreases even at 35 °C [11]. Similarly, nitrogen assimilation was also found to be limited by low temperatures [12]. To sustain the rhizobia in the soil different carrier materials have been used for the inoculation of legumes with reasonable beneficial results in protecting the bacteria and enhancing the survival of inoculants for longer durations. The use of organic carriers offers additional benefits over chemicals of physical carriers of nutrient provision to the soil.

Biochar could be used as an imperative intervention for increasing soil organic pool for the reason of organic matter deficiency being considered as a major factor limiting plant growth. In addition to this, biochars from different feedstocks exhibit divergent effects on soil fauna diversity, activity, and transport owing mainly to the indirect changes in the chemical properties of soil. Studies have documented positive influences of biochar addition on soil productivity enhancing nutrient uptake, water retention capacity and soil structure, etc. [4,13]. Biochar could also be employed in the reduction of soil acidity by exploiting its alkaline nature [14–15] and could also benefit alkaline soils by utilizing some modification techniques [16]. Biochar augments nutrient availability thus being effectively engaged in getting nutrient uptake benefits increasing the soil pH, P, K, and Ca whereas subsiding free Al [17–18]. Owing to its high porosity and surface-to-volume ratio it easily captures exchangeable cations.

Biochar has been found to be used for the improvement of soil fertility and simultaneously it can also create an opportunity to carry plant-growth-promoting rhizobacteria (PGPR). Its unique characteristics are responsible for making it conducive for being used as an inoculum carrier. Rondon *et al.* [19] have obtained increased BNF results by common beans with increasing rates of biochar additions.

The bean crop is generally grown in rain-fed conditions therefore drought stress could be a common problem. The problem is further exacerbated if the soil is shallow and low in essential nutrients and organic matter [20–21]. Nitrogen fixation by common beans has been found to be limited by environmental stresses like drought and adverse soil conditions. It becomes difficult for rhizobium to infect root hairs at temperatures greater than 40 °C, while nitrogenase activity also decreases even at 35 °C [22]. Moisture stress has been observed to have a greater impact on nodulation and nodule activity of legumes with varying adaptabilities at rhizobial strains level. In this context the different carrier materials have been used for the inoculation of legumes with reasonable beneficial results.

Rhizobium-legume compatibility is very much significant for getting successful inoculation benefits. If any legume crop is grown for the first time in certain area, there is a possibility that rhizobium strains which are compatible with that specific legume may not inhabit that soil. Sometimes many rhizobia already exist in soils, but often these rhizobia are fewer in number or not effective enough for attaining desired nitrogen fixation and increased yield. Such problems can be alleviated by inoculating with compatible strains of rhizobium and spectacular expected yield increase benefits can be achieved.

Keeping in view the multiple benefits of biochar we want to manipulate and optimize biochar properties as an inoculum carrier for certain rhizobial strains which have not been fully acknowledged in the past. The current research aims to evaluate the suitability of biochar as rhizobial carrier material. The present study has been planned to apply biochar (obtained from two different feedstocks) as a rhizobium carrier for *Phaseolus vulgaris* L. to achieve the following objectives. 1) Comparative assessment of different types of biochar (prepared from rice husk and *Lantana*) as an improved type of inoculum carrier specifically for *P. vulgaris* L. and 2) Comparison of the growth and physiological responses of *P. vulgaris* L. grown in drought stress under different biochar treatments.

2. MATERIALS AND METHODS

Biochar from two different feedstocks (i.e., Rice Husk and *Lantana camara* L.) was used as inoculum carrier for *P. vulgaris* L. to cope against drought stress.

2.1. Isolation of Rhizobial Strains

Few healthy plants of *P. vulgaris* L. were brought from Molen Gol valley near Chitral city, KP province, Pakistan to the laboratory for isolation of rhizobium strains. The collected specimens were compared with the herbarium collection at Herbarium of the Quaid-i-Azam University Islamabad (ISL) and the Herbarium of Pakistan Museum of Natural History Islamabad. A voucher specimen was also deposited in the Herbarium (ISL) against the Herbarium collection Number: ISL-A573 (This is available in the Angiosperms section of the Herbarium at the Department of plant sciences. Nodules of freshly uprooted *P. vulgaris* L. plant were used for the isolation. Healthy pink nodules were selected for the isolation of nodule-associated bacteria (NAB). Individual nodules were dissected from the roots. Subsequently, these were surface disinfected. These surface-disinfected nodules were then crushed. The obtained bacterial suspensions were streaked on YEM-agar medium and incubated at 28 °C. Each colony was then purified by streaking repetitively on YEM agar plates. All obtained pure isolates were sustained on YEM media and well-preserved at 4 °C [23].

2.2. Morphological Characterization and Gram Staining

After 2, 4 and 6 days of incubation at 28 °C, different colonies were analyzed for morphological (form, size, margins, elevation, texture and opacity) and biochemical characterization. Gram staining was performed for all the obtained isolates and finally observed under microscope (magnification: 100X) with immersion oil.

2.3. Biochemical Characterization (Confirmatory Tests for Rhizobium)

The isolated colonies were also characterized for their biochemical characteristics [24] mentioned below and specifically their growth on YEM (Yeast Extract Mannitol) Agar medium incorporated with Congo red dye [25]. Isolates that developed white colored colonies (with very low absorption of dye were considered as rhizobium. The isolated colonies were also grown on YEM medium containing Bromothymol Blue (0.00125 mg/kg) for the detection of acid growth reaction and growth responses [26].

For further confirmation of being gram-negative and lactose fermenters, all the isolates were streaked on MacConkey agar medium as well as Eosine Methylene Blue (EMB) agar medium. Development of pink colonies (due to acid production) on MacConkey agar medium was taken as positive result indication for lactose fermenters while on EMB dark purple colored colonies indicated the positive results.

For further confirmation for nitrogen utilization the isolates were grown on minimal salt agar medium (MSA) containing ammonium sulphate. Triple Sugar Iron (TSI) media was used to perform TSI test in order to check production of H_2S gas and carbohydrate fermentation by the isolates. Color alterations in slants (red/yellow) and the butts were observed and used as indication of specific carbohydrate fermentation, acid and gas production.

All isolated strains were screened for oxidase test using Kovac's reagent prepared according to [24]. For the detection of enzyme catalase, catalase test (using 1-2 drops of 3 % H_2O_2) was performed with isolated colonies (24-48 hrs. incubation). For the differentiation of isolates on the basis of urease activity, urea agar base was utilized. After incubation the inoculated slants were observed within 2-6 hrs. for valid rapid urease-positive results. While for delayed urease reaction observations were taken after 24-48 hrs. In case of positive urease reaction exhibited by the isolates intense pink color development was observed.

All the isolated strains were tested for their citrate utilization ability (as carbon source) using slants of simmon citrate agar medium. Fluid thioglycolate medium designated to check the aero-tolerance of the isolates was used. All the test isolates were stab-inoculated on motility indole urea medium in order to check for motility. Diffused growth/turbidity was considered as indicator for motile organisms while growth along stab line was taken for non-motile ones.

2.4. Preparation of Biochar

Biochar of rice (*Oryza sativa*) husk was prepared by the pyrolysis in the muffle furnace at 300 °C with the rise in temperature of 17 °C per minute. After removing the sample from furnace, it was (kept in a desiccator) weighed and stored in air tight plastic containers. Small pieces (5-8 cm, dried at 105 °C for 1 hour) of *Lantana* (stem and branches) were pyrolyzed at 450 °C for 20 minutes. Finally, the prepared biochar was crumpled into small particles before use [27–28].

2.5. Biochar Analyses

Biochar was analyzed after preparation for following physico-chemical parameters and morphological analyses for surface morphology, phase and structural properties (Table 1).

2.6. Analyses of Soil used for Pot Experiment

Soil (used for the pot experiment) was characterized for its electrical conductivity, moisture content [30], pH, texture [35] and organic matter [36]. All the measurements were taken both before and after the pot experiment.

2.7. Screening of Rhizobial Strains

The screening for the efficient rhizobial strains was carried out in two parts. Initially the nodule forming strains were screened which were then used for their stability on biochar.

2.8. Screening of Nodule Forming Strains

Pure cultures were tested for nodule formation in *P. vulgaris* L. displaying a potential to enhance nodulation, growth and yield of legume plants when co-inoculated with Rhizobium. This study genetically characterizes bacteria isolated from bean root nodules in Cuba and investigates the effect of Rhizobiume *Pseudomonas* co-inoculation on common bean (*P. vulgaris* L.) [23]. Seeds of *P. vulgaris* L. were treated with 10 % ethanol solution and rinsed with sterile distilled water. The sterile seeds were placed on sterile paper towel for 4-5 days. After germination five seedlings were planted in pots filled with sterile sand for jar experiment. At time of sowing each seedling was dipped in broth solution containing isolated strains separately (three replications). The duration of jar experiment was six weeks. After six weeks plants were uprooted and checked for nodule formation. Strains which showed development of healthy nodules in bean plants were used for further experimentation.

2.9. Inoculum Stability on Biochar

Inoculum was prepared by inoculating strain (performing good in screening test) in 10 ml YEM broth [37] and incubated in shaking incubator (150 rpm) overnight at 28 °C [38]. Sterile biochar (Rice husk & *L. camara*) was added in this broth culture in 1:10 ratio (1 g biochar in 10 ml culture) and placed on an orbital shaker (150 rpm) at 28 °C [39]. Optical density of these combinations (inoculum + carriers) at 600 nm was observed at regular intervals to get substantial results. All the procedures were conducted under strict aseptic conditions.

2.10. Effect of Stabilized Inoculant on Seed Germination

The biochar inoculum combinations were tested for their efficacy in germination of *Phaseolus* seeds. Seeds were surface sterilized with mercuric chloride (0.1 %) for 2 min., then thoroughly rinsed and soaked in distilled water for six hours. Soaked

Table 1. Details of methods used for analytical parameters

Parameters	Methods
Moisture content (%)	Gravimetric method [29]
pH	pH meter [30]
Electrical Conductivity (µS/cm)	EC meter [30]
Ash content (g)	Weight loss after heating method [31]
Bulk density (g/cm^3)	ICARDA manual [32]
Nutrients (N, P, K, Ca, Mg)	ICP-OES [31] Nutrients in weight and atomic percentage were also determined by EDS
Scanning electron microscopy	MIRA3 TESCON scanning microscope
Energy dispersive x-ray spectroscopy surface functional groups	IRTracer-100 Fourier Transform Infrared Spectrophotometer (Shimadzu) [33]

seeds were then mixed with the combination of biochar and inoculum. After mixing, ten seeds from each combination were placed on sterile filter paper and incubated at 25 °C for 1-2 weeks. The detail of the treatments applied is given as follows:

C: Control (without Biochar and strain)
CS: (Control with strain)
B_1 S: (Biochar of rice husk + strain)
B_2 S: (Biochar of *L. camara* L. + strain)

All the treatments were applied in three replications.

2.11. Pot Experiment

Pot experiment was conducted to check the efficacy of the formulated biochar inoculums combination to increase the water use efficiency in *Phaseolus*. The experiment was conducted with the germinated seedlings [27].

2.11.1. Experimental Setup

For each pot (~5 kg capacity & dimension: 18 cm high × 22 cm diameter) one percent combination (biochar + inoculums) was mixed to the soil (4 kg air dried, ground & sieved) on weight basis and germinated seeds (Control) were planted in pots. After six weeks of plantation (after nodule formation) water stress was applied to the plants by maintaining their field capacities (F.C.) at 100 % and 60 % [40]. The same treatments were used in well watered condition by returning 100 % transpired water to the plants.

2.11.2. Application of drought stress

An evening before the start, stressed plants were soaked with water up to their saturation levels and were then left to drain out. The next day the plant pots were weighed. But before weighing, all plants were concealed within polythene bags which were tied to the bottom of the shoot in order to avoid the evaporative loss. Afterwards, each plant was weighed daily and required percentage of the daily weight loss was returned to it by adding distilled water in order to maintain its field capacity. This practice was continued till their stomatal conductance reached zero.

Following method of Masinde *et al*. [41], the initial pot weight referred to the weight of a pot at 100 percent water holding capacity (WHC) while final pot weight referred to the weight of that pot when the transpiration of the stressed plants was almost zero in comparison with the well-watered plants. Daily values of FTSW (Fractionable Transpirable Soil Water) for each pot in the water deficit treatment on each day for each pot was calculated using the following formula, Where

$$\text{Daily FTSW} = \frac{\text{daily pot weight} - \text{final pot weight}}{\text{initial pot weight} - \text{final pot weight}} \quad \text{(eq. 1)}$$

The stress was imposed to evaluate the resistance of *P. vulgaris* and biochar was applied (along with rhizobial strain). Detail of stress treatments is given in Table 2.

2.12. Measurement of Morphological and Physiological Parameters

The plants under pot trial were subjected to various morphological (plant height, plant biomass, leaf area) after 21st and 42nd day after stress (DAS) treatment [27, 41, 42].

All the plants of control and stress treatments were measured for the changes in their physiological parameters i.e., net photosynthesis (Pn), stomatal conductance (gs), transpiration rate (Tr) in morning

Table 2. Application of drought stress treatments

Treatments	**Water Levels (%)**	**Biochar**	**Inoculation**
T_1	100	0	0
T_2	60	0	0
T_3	100	Rice Husk	0
T_4	60	Rice Husk	0
T_5	100	*Lantana camara*	0
T_6	60	*Lantana camara*	0
T_7	100	Rice Husk	Inoculated
T_8	60	Rice Husk	Inoculated
T_9	100	*Lantana camara*	Inoculated
T_{10}	60	*Lantana camara*	Inoculated
T_{11}	100	0	Inoculated
T_{12}	60	0	Inoculated

with (IRGA) Infra-Red Gas Analyzer (CIRAS-2 Portable Photosynthesis System, software version 1.70). For measurement of these physiological parameters the uppermost fresh and fully expanded leaves were placed in the cuvettes of IRGA to obtain the values [43]. Water use efficiency (WUE) was calculated by dividing net photosynthesis by transpiration rate as done by Jianlin *et al*. [44], where

$$WUE = Pn/Tr \quad \text{(eq. 2)}$$

2.13. Statistical Analysis

All data were statistically analyzed by analysis of variance (ANOVA) in SPSS version 16.0 and graphed using OriginPro software. All the treatments were compared using least significant differences at the significance level of $P < 0.05$ to determine the effects on plant growth and physiological properties. While factorial experiments was used to establish the significance of the results, and individual means were tested by LSD.

3. RESULTS

The study was aimed to explore the ameliorative potential of biochar (derived from two different feedstocks) acting as rhizobium carrier to be used for *P. vulgaris* L. exposed to drought stress.

3.1. Physicochemical Analyses of Biochar

The results of physic-chemical characterization of biochar prepared from two different feedstocks are presented in Table 3.

3.2. Morphological Characterization of Biochar

3.2.1. Scanning Electron Microscopy (SEM)

The SEM images of rice husk at magnifications: 200 x, 500 x, 1.00 kx, 5.00 kx is shown in (Figure 1).

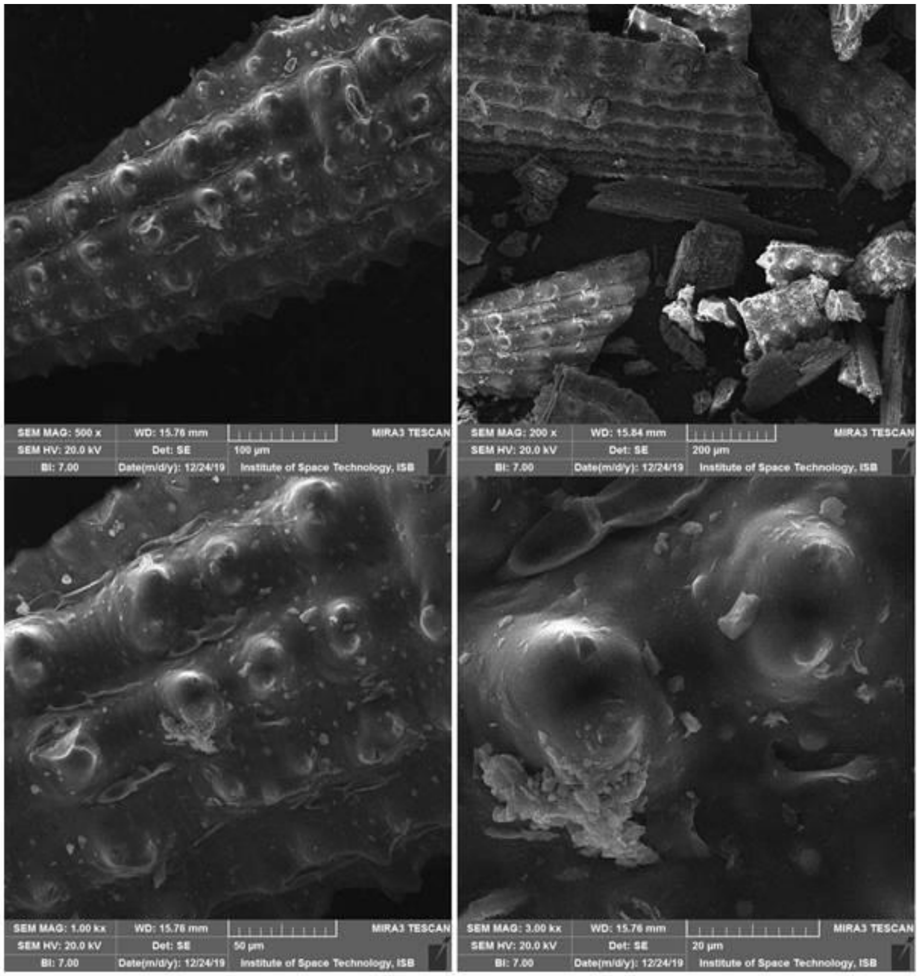


Fig. 1. Scanning electron microscope (SEM) images of rice husk biochar

Table 3. Physicochemical analyses of biochar prepared from two feedstocks

Parameters	**Biochar (Rice husk)**	**Biochar (*Lantana camara*)**
Moisture content %	24.08±0.01	0.88±0.01
pH	6.05±0.01	6.64±0.01
Electrical Conductivity (µS/cm)	898±0.01	1894±0.01
Ash content (g)	0.91±0.01	0.52±0.01
Bulk density (g/cm^3)	0.24±0.02	1.44±0.02
Nitrogen (%)	2.651±0.01	0.724±0.01
Phosphorus (%)	0.029±0.01	0.317±0.01
Potassium (%)	0.197±0.02	0.621±0.02
Calcium (%)	0.047±0.01	0.181±0.01
Magnesium (%)	0.018±0.01	0.986±0.01

While the SEM images of *Lantana* biochar at magnifications: 200 x, 500 x, 1.00 kx, 5.00 kx is shown as under (Figure 2).

3.2.2. Energy-Dispersive X-Ray Spectroscopy (EDS)

In addition to SEM, information regarding the

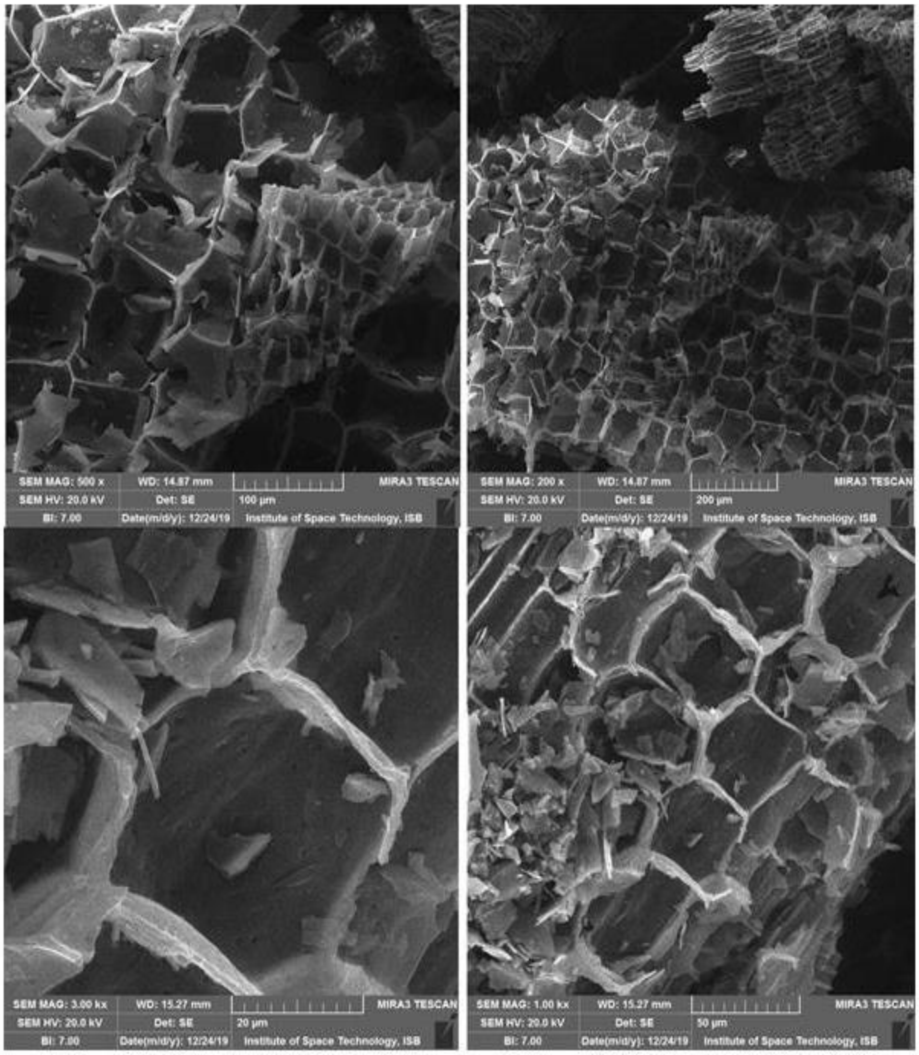


Fig. 2. Scanning electron microscope (SEM) images of *Lantana* biochar

spectral and chemical characteristics of the two biochars was also obtained through energy disruptive X-ray spectroscopy analysis. The noticeable result for rice husk is the presence of Si content with 7.45 % which was absent in *Lantana* biochar. *Lantana* biochar was richer in carbon, potassium and calcium with weight percentages of 77.8 %, 2.15 % and 0.67 % respectively as compared to the proportions of these contents) in rice husk biochar. Oxygen content (33.40 %) was found to be higher in rice husk biochar as compared to 19.20 % in *Lantana* (as shown in Figure 3a and 3b).

3.3. Surface Functionality by Fourier Transform Infrared Spectroscopy (FTIR)

All the FTIR measurements of the biochar samples were taken over the wavenumber range of 4500-400 cm^{-1} and 16 co-added scans, with spectra in transmittance units (%). The detailed description of chemical bonding, peak ranges/band ranges for both the biochars are presented in Table 4 showing the following surface functional groups: aromatic C=N bond, hydroxyl, carboxyl, N–H bond etc.

3.4. Soil Analyses

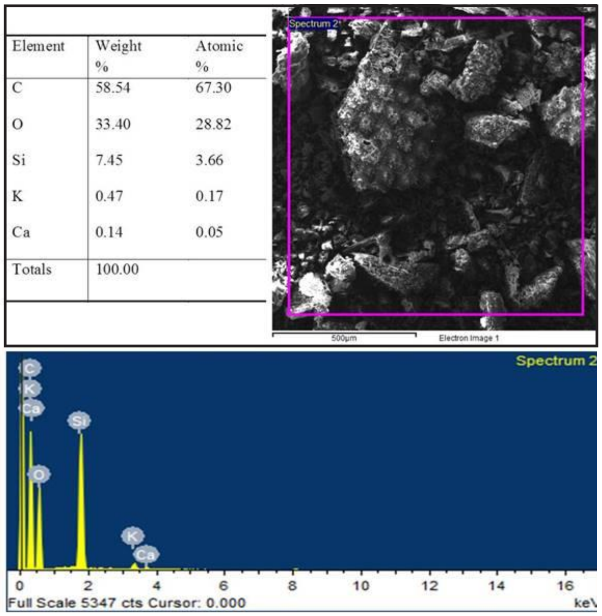
The pH of control soil remained unchanged while pH of soils of the treatments (i.e. rice husk biochar, *Lantana* biochar, rice husk biochar + inoculum, *Lantana* biochar + inoculum and inoculum) was changed (i., 8.9, 8.5, 7.8, 7.7 and 6.9 respectively). Noticeable changes in the electrical conductivity were also observed i.e., from 3.0 µS/cm to 13.0, 10.0, 6.8, 5.6 and 8.5µS/cm respectively in soils with treatments mentioned above. Moisture content and organic matter of pot soil also showed increasing percentages (Table 5).

3.5. Results of Pot Experiment

Results of morphological and physiological parameters are described as under:

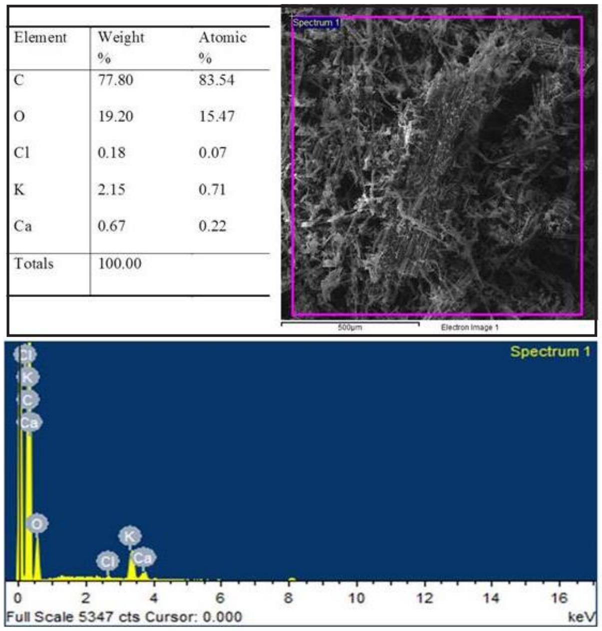
3.5.1. Plant Height

The plant height was measured at twenty one and forty two days after stress and the contrast between the treatments under stressed (60 % F.C.) and non-stressed (100 % F.C.) conditions and the controls is depicted in Figure (4a). Highly significant (P ≤ 0.01) results for these height measurements were recorded in plants treated with biochar as compared to the control. Length of plants was better in the non-stressed (100 % F.C.) plants as compared to the plants at 60 % F.C. The rice husk biochar treated plants (35 cm) were taller plants treated with *Lantana* biochar (23 cm) and the controls (29 cm). Similar was the case of 60 % F.C. more plant height



Element	Weight %	Atomic %
C	58.54	67.30
O	33.40	28.82
Si	7.45	3.66
K	0.47	0.17
Ca	0.14	0.05
Totals	100.00	

Fig. 3a. EDS Spectra of rice husk biochar showing elemental weight percentages.



Element	Weight %	Atomic %
C	77.80	83.54
O	19.20	15.47
Cl	0.18	0.07
K	2.15	0.71
Ca	0.67	0.22
Totals	100.00	

Fig. 3b. EDS Spectra of *Lantana* biochar showing elemental weight percentages.

Table 4. The functional groups existing in two biochar samples (i.e. rice husk and Lantana) determined by FTIR spectrogram.

Wave number (cm^{-1})	**Rice husk biochar**	***Lantana* biochar**	**Vibration characteristics**
3902-3703	–	+	
3614-3570	–	+	
3643-3599	+	–	
3599-3565	+	–	
3376-3342	+	–	O-H stretching
3036-2830	–	+	C-H stretching
2929-2818	+	–	C-H stretching
2384-2335	–	+	
2383-2305	+	–	
2260-2238	+	–	C≡C stretching
2210-2151	–	+	C≡C stretching
1881-1859	+	–	
1847-1836	+	–	
1680-1635	–	+	N-H bond C=O stretching
1580-1435	+	–	Methyl C-H asymmetric Carboxylic acid
1554-1499	–	+	Carboxylic acid
1488-1444		+	Methyl C-H asymmetric
1435-1357	+	–	Nitro compound stretching
1260-1204	–	+	Aryl-O stretching
1212-1178	+	–	C-N stretch of tertiary amine
1133-1066	+	–	Primary amine of C-N stretch Secondary amine of C-N stretch
1104-1038	–	+	Primary amine of C-N stretch Secondary amine of C-N stretch
1000-883	–	+	Substituent of aromatic rings
877-844		+	Substituent of aromatic rings
732-654	+	–	Substituent of aromatic rings
622-588	–	+	Substituent of aromatic rings
542-520	+	–	
498-486	+	–	

Table 5. Results of soil analyses after pot experiment.

Treatments	**Moisture (%)**	**pH**	**EC (µS/cm)**	**Soil Texture**	**Organic matter (%)**
Control	1.2±0.1	7.1±0.01	4.0±0.01	Sandy Loam	0.5±0.03
Rice husk biochar	15±0.02	8.9±0.01	14.0±0.01	Sandy Loam	1.9±0.07
Lantana biochar	16±0.01	8.5±0.01	9.0±0.01	Sandy Loam	2.0±0.01
Rice husk biochar + inoculum	26±0.03	7.8±0.005	7.0±0.01	Sandy Loam	3.5±0.05
Lantana biochar + inoculum	30±0.07	7.7±0.01	5.0±0.01	Sandy Loam	3.1±0.01
Inoculum	15±0.06	6.8±0.01	8.5±0.01	Sandy Loam	4.0±0.01

was observed with rice husk biochar as compared to control and with the addition of *Lantana* biochar. Therefore, rice husk biochar treatment was seen to show nominal growth in plant height than others.

Biochar along with inoculum of isolated strain has been found to have a significant effect on the height. This comparison between control and combinations of biochar and inoculum treatments is presented in the Figure (4b). Elevated measurements in the height were observed with biochar and inoculum added plants as compared to the plants with no soil amendment and inoculum were smeared showing highly significant (at P ≤ 0.0) effects for all the treatments.

While comparing treatments: 100 % F.C + without biochar + without inoculum, 100 % F.C. + rice husk biochar + inoculum and 100 % F.C + *Lantana* biochar + inoculum the plant showed considerable increase throughout the experiment. Nevertheless, the observed increase was higher in plants with BR + I (33 cm). In case of more stressed plants i.e. with 60 % F.C, BR + I treatment proved to be more beneficial for height increase in a similar fashion. Inoculation also has substantial influence on the height of the bean plants. The comparison between control plants and plants with inoculum (isolated strain) addition for the parameter of plant height is presented in Figure (4c).

Inoculum treatment has also enhanced plant height with high significance as compared to the plants with no added inoculum. With all these treatments i.e., 100 % F.C. + no added inoculum, 60 % F.C. + no added inoculum, 100 % F.C. + inoculum and 60 % F.C. + inoculum, considerable height escalation was observed in inoculum added plants (32.6 cm) as compared to the control (28.9 cm) plants.

3.5.2. Plant Biomass

Biomass measurements of the control and biochar treatments in terms of dry matter is presented in (Figure 5a) showing higher values of all non-stressed plants which were sustained with 100 % field capacity. While among these treatments (i.e., BR and BL) dry matter was found to be higher (3 g) with BR treatment as compared to the stressed (60 % F.C.) and non-stressed ones (100 % F.C).

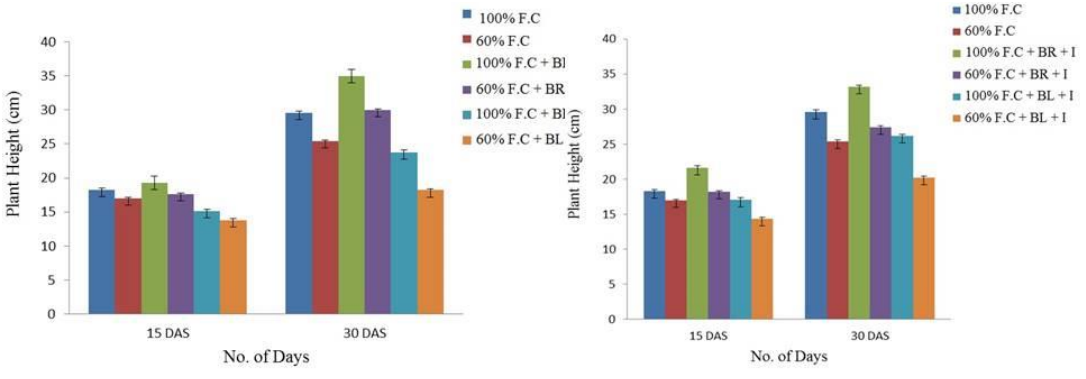


Figure 4a: Comparison of control and biochar treatments

Figure 4b: Comparison of control and biochar with inoculum treatments

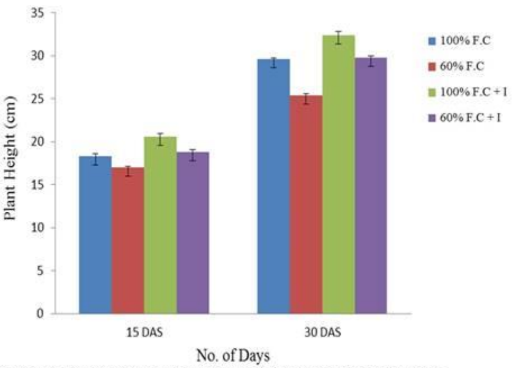


Figure 4c: Comparison of control and inoculum treated plants

Fig. 4 (a, b, c). Changes in plant height of control and treatments under stressed (60 % F.C) and non-stressed (100 % F.C) conditions

Comparison of control and the combinations of biochar and inoculum addition treatments is shown in (Figure 5b) where maximum value of dry matter (i.e., 2 g) was obtained at 100 % F.C. with BR + I. While in case of stressed plants with 60 % F.C. less value was observed. Figure (5c) is presenting the comparison of control and inoculum treatments. It is obvious from the figure that biomass increased in non-stressed conditions and comparatively higher values of dry matter (0.5 g) than control plants.

3.5.3. Leaf Area

Measurements for leaf area are presented in (Figure 6a) depicting highly significant ($P \leq 0.01$) results. As seen in figure all the treatments (BR and BL), which were retained at 100 % field capacity, showed increasing trends but with BR at 100 % F.C, 120 cm^2 leaf area was obtained which was the maximum value. Similar is the case of stressed treatments (i.e., 60 % F.C., 60 % F.C. + BR, 60 % F.C. + BL) where higher measurements were acquired with the addition of rice husk biochar.

It is noteworthy that greater values of leaf area were observed in all the plants kept at 100 % F.C. as compared to the stressed ones (60 % F.C.). Outcomes of combination of two types of biochar and inoculum were established while comparing all the treated plants with the controls (Figure 6b). When we look at the leaf area measurements of all the plants maintained at 100 % field capacity along with the treatments of biochar (BR and BL) and inoculum addition, higher values were obtained with BR + I. Therefore, at 100 % F.C rice husk biochar + inoculum was found to be more effective in increasing this growth parameter as compared to others. Furthermore, the same rice husk biochar at 60 % F.C. displayed similar increasing trend. Apart from biochar, inoculum addition alone was found to be effective for treated plants as depicted in Figure (6c).

3.5.4. Stomatal Conductance (gs)

Measurements for stomatal conductance (gs)

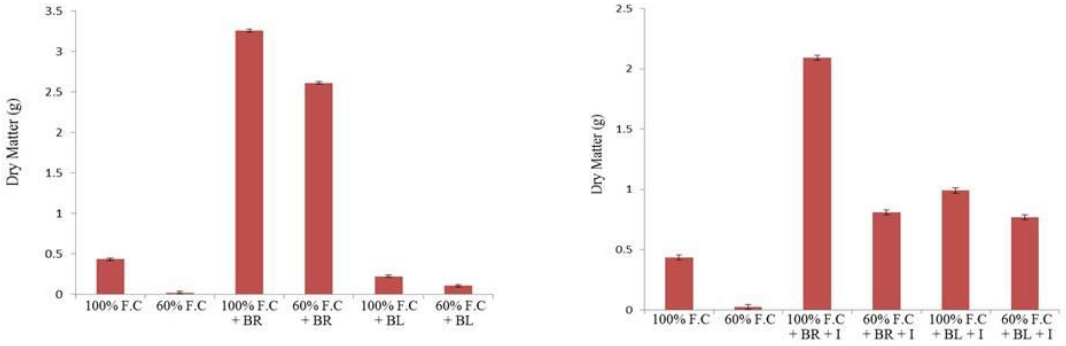


Figure 5a: Comparison of control and biochar treatments

Figure 5b: Comparison of control and biochar with inoculum treatments

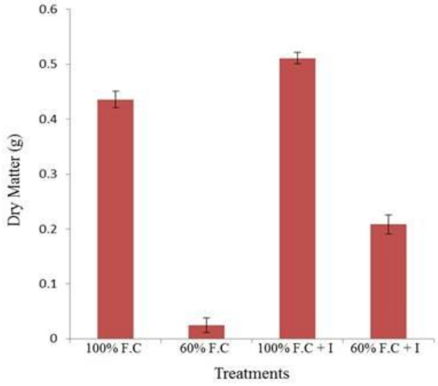


Figure 5c: Comparison of control and inoculum treated plants

Fig. 5 (a, b, c). Changes in dry matter of control and treatments under stressed (65 % F.C) and non-stressed (100 % F.C) conditions.

in all treated and control plants are shown in (Figure 7a). Highly significant ($P \leq 0.01$) differences were observed while comparing all the treatments. In case of non-stressed plants (i.e., 100 % F.C. + BR, 100 % F.C. + BL), stomatal conductance measurements persisted at constant value till week 6 due to daily maintenance of field capacity (100 %). It's noteworthy that the stomatal conductance was higher (365 mmol H_2O $m^{-2}s^{-1}$) with the addition of rice husk biochar in contrast to BL where it was 310 mmol H_2O $m^{-2}s^{-1}$. While, in case of stressed plants (i.e., with 60 % F.C, 60 % F.C. + BR, 60 % F.C. + BL) the values for stomatal conductance decreased very slowly in initial three weeks. After that when the stress was increased, a swift drop was observed in the values of stomatal conductance. Hence, biochar provided stability at such extreme stress level and thus stomatal conductance decreased upto 70 and 61 mmol H_2O $m^{-2}s^{-1}$ in BR and BL treatments, respectively contrasting to the treatments with no biochar additions where it touched closer to zero value till the sixth week.

Besides the treatments with biochar of two different feedstocks, the amalgamation of biochar with inoculum of the isolated strain also exhibited significant results ($P \leq 0.01$) for the responses in the stomatal conductance (Figure 7b). Just like biochar responses, similar effects were found when inoculum was added along with the two biochars. At 100 % filed capacity the stomatal conductance maintained almost at same readings throughout the experimental time period for all the treatments (0 % B + 0 % I, BR + I, BL + I). On the contrary, in case of stressed treatments (60 % F.C) for all the treatments (0 % B + 0 % I, BR + I, BL + I) the value of stomatal conductance declined till zero for 0 % B+ 0 % I as compared to the others where it didn't touch the figure of zero till the end of experiment. As a result, biochar in combination with inoculum contributed in increasing plants' resistance to drought stressed conditions.

Inoculum addition alone also had affirmative effects in improving the stomatal conductance (Figure 7c) with highly significant differences at

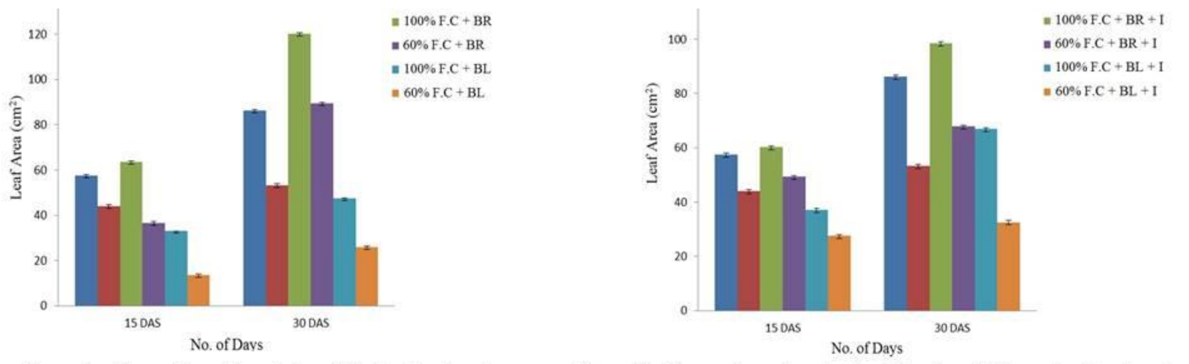


Figure 6a: Comparison of control and biochar treatments

Figure 6b: Comparison of control and biochar with inoculum treatments

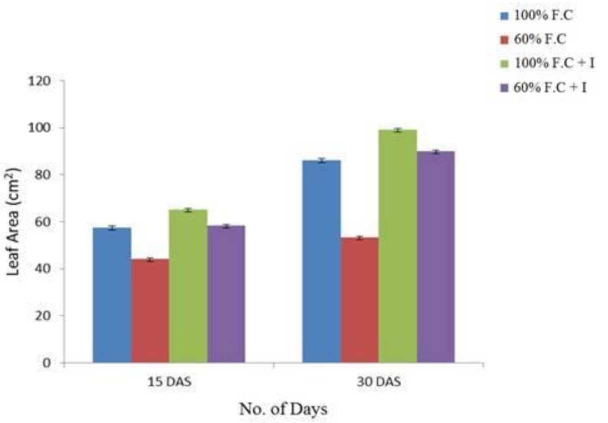


Figure 6c: Comparison of control and inoculum treated plants

Fig. 6 (a, b, c). Changes in leaf area measurements of control and treatments under stressed (65 % F.C) and non-stressed (100 % F.C) conditions.

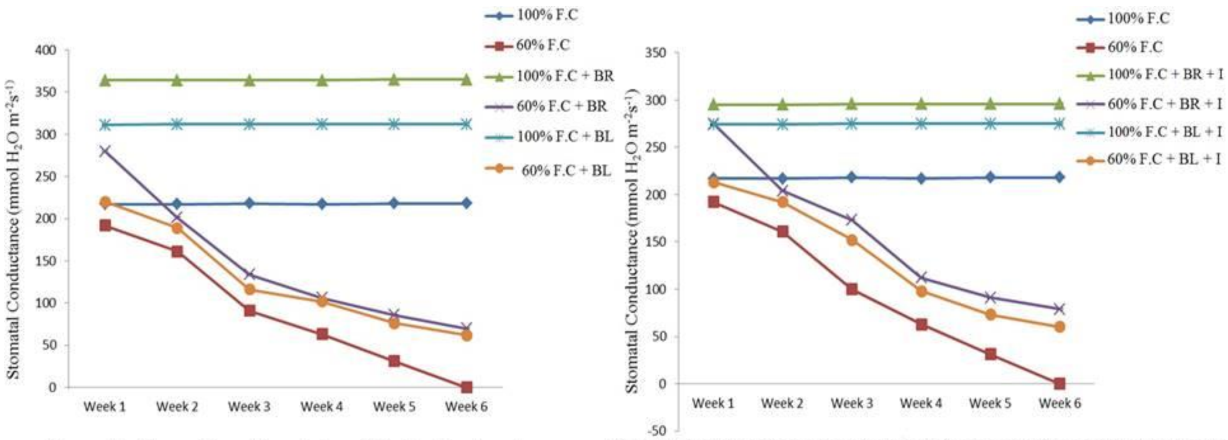


Figure 7a: Comparison of control and biochar treatments

Figure 7b: Comparison of control and biochar with inoculum treatments

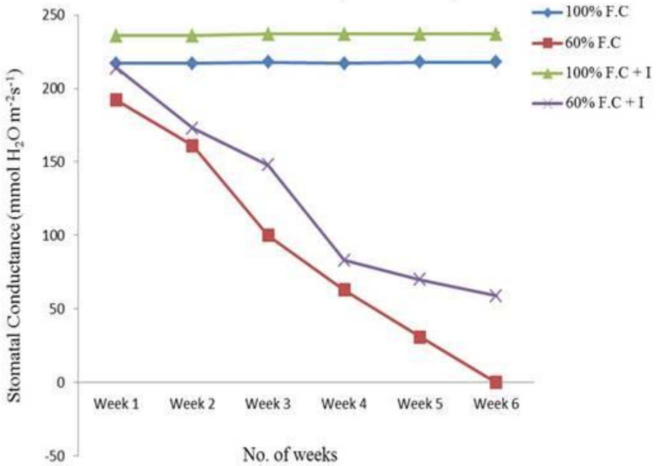


Figure 7c: Comparison of control and inoculum treated plants

Fig. 7 (a, b, c). Changes in stomatal conductance of control and treatments under stressed (65 % F.C) and non-stressed (100 % F.C) conditions.

$P \leq 0.01$ among all the treatments. No noticeable changes in stomatal conductance were observed in plants maintained at 100 % field capacity and inoculum treatment. Additionally, higher value of stomatal conductance was obtained for inoculum added plants (235 mmolH_2O $m^{-2}s^{-1}$) than the control plants (215 mmol H_2O $m^{-2}s^{-1}$). In contrast to this, a gradual reduction in the stomatal conductance value of stressed plants (60 % F.C. + I & 60 % F.C) till the third week. After that a rapid drop up to zero was seen in plants at 60 % F.C. with no inoculum addition in contrast to the inoculum treated plants where the value was 60 mmol H_2O $m^{-2}s^{-1}$.

3.6. Net Photosynthesis

Responses in the form of net photosynthesis of control and biochar treated plants have also shown highly significant differences. At 100 % F.C. for the treatments T1, T3 & T5, no significant change was witnessed in the photosynthesis rates. Comparatively higher rates were witnessed in T3 (5 µmol CO_2 $m^{-2}s^{-1}$) than 2.5 µmol CO_2 $m^{-2}s^{-1}$ for T1 and 4.5 µmol CO_2 $m^{-2}s^{-1}$ for T5. On the other hand at 60 % field capacity an increasing trend was noticed with two types of biochar as compared to control (without biochar addition) where the rate decreased at week six. Photosynthesis rate was higher with rice husk biochar and further increased from 4.5 to 7 µmol CO_2 $m^{-2}s^{-1}$ from start till the end (Figure 8a). It is obvious from the results that rice husk biochar has significantly influenced the photosynthetic rate under stress conditions as an effective treatment.

Photosynthetic rates were also compared for the plants with biochar treatments along with inoculum additions (Figure 8b) and significant ($P \leq 0.01$) results were observed for all the comparisons. Higher photosynthetic rates were witnessed in plants maintained at 100 % field capacity with the treatment combinations of biochar and inoculum as compared to the controls. In case of plants with 100 % F.C., rice husk biochar addition plus inoculum was found to be more effective than the others. In contrast to this, a gradual rise in the photosynthesis rate was witnessed in plants with 60 % F.C. with biochar inoculum treatments (T_8 & T_{10}) than control (T_2). Similarly, rice husk biochar along with inoculum has also shown better effects than *Lantana* biochar.

Photosynthesis rates of control plants and inoculated plants were also compared with significant results ($P \leq 0.01$) shown in (Figure 8c). Very little alteration was detected in the photosynthesis rates of plants which were kept at 100 % field capacity (T_1 and T_{11}) throughout the pot experiment. But inoculated plants showed higher rates of photosynthesis (4 μmol CO$_2$ m^{-2}s^{-1}) than the control plants (2.6 μmol CO$_2$m^{-2}s^{-1}). While for the plants at 60 % F.C. (T_2 and T_{12}) the photosynthesis rates were higher. In plants with inoculum treatment, it increased from 2.6 to 5 μmol CO$_2$ m^{-2}s^{-1} and in control plants the value decreased from 2.5 to 0.7 μmol CO$_2$ m^{-2}s^{1}.

3.7. Transpiration Rate (Tr)

Plants of the pot experiment were compared for their transpiration rates of control and biochar treatments (Figure 9a) with highly significant ($P \leq 0.05$) results. In case of all non-stressed plants (i.e. T_1, T_3 & T_5) there appeared no significant change in transpiration rate throughout the experimental period, as the 100 % field capacity was sustained daily. On the contrary, in stressed plants (i.e. T_2, T_4 & T_6) a slow reduction was seen till three weeks. After that a rapid decline was observed. The values changed from 3.7, 5.1, 4.0 and 2.9 to 4, 3.6 and 3.2 in plants of T_2, T_4 & T_6, respectively. Finally, the value nearly touched zero value at the termination of experiment in plants without biochar addition.

Significant impact was witnessed on the rate of transpiration of plants which were treated

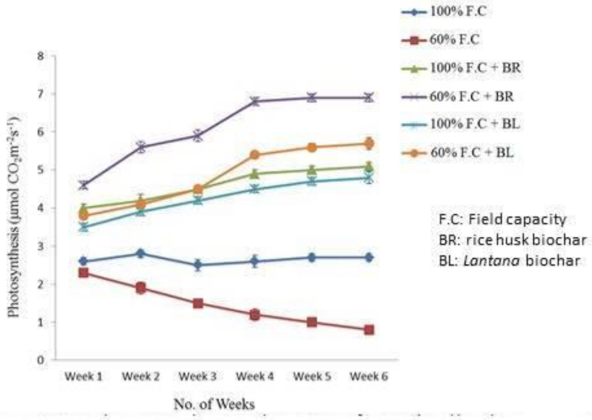


Figure 8a: Comparison of control and biochar treatments

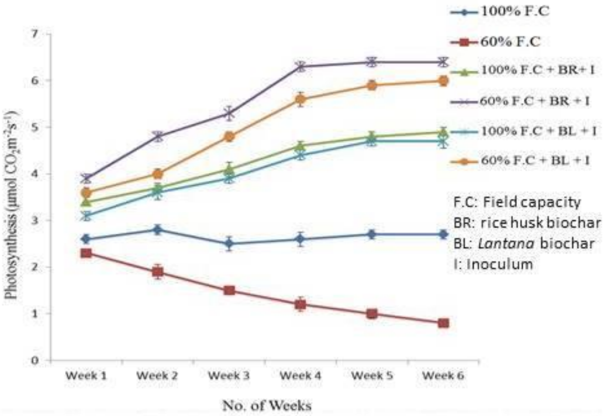


Figure 8b: Comparison of control and biochar with inoculum treatments

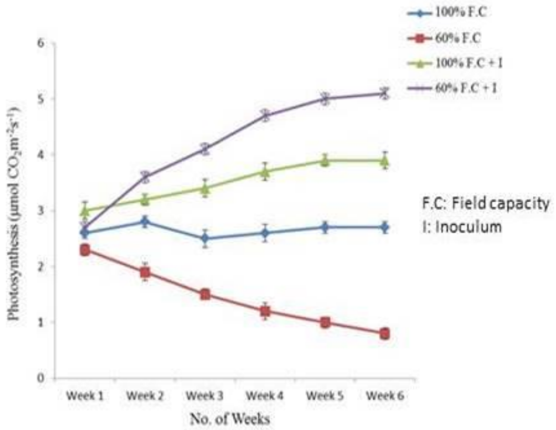


Figure 8c: Comparison of control and inoculum treated plants

Fig. 8 (a, b, c). Changes in photosynthetic rates of control and treatments under stressed (65 % F.C) and non-stressed (100 % F.C) conditions with combination of biochar and inoculum. The comparison between control and this combination treatment is depicted in (Figure 9b) with obvious significant results ($P \leq 0.01$). In case of treatments at 100 % field capacity (T_1, T_7 & T_9) no substantial changes were observed during the course of experiment. Whereas in case of T_2, T_8 & T_{10} kept with 60 % F.C. the transpiration rate gradually declined till the third week with sudden decrease afterwards with proximate zero values at the end.

The inoculum-biochar combination treatments also showed significant results ($P \leq 0.01$) on the transpiration rates of the plants when compared with the controls (Figure 9c). Similar trends were experienced by the plants maintained at 100 % F.C (T_1 & T_{11}) with almost constant transpiration rates. However, stressed plants at 60 % F.C (T2 & T_{12}) showed a gradual decreasing transpiration rates and the values further reached proximate zero values at week 6.

3.8. Water Use Efficiency (WUE)

Water use efficiency was also calculated simply by dividing net photosynthesis (Pn) by transpiration rate (Tr) and the obtained values for the control plants and biochar treated plants were compared (Figure 10a) at P value of ≤ 0.01. As depicted in previous comparisons rice husk biochar has shown improved results for the water use efficiency of Bean plants as compared to the controls. In case of treatments T_1, T_3 & T_5, which were kept with 100 % field capacity minor changes, were observed in the obtained water use efficiency values weekly till the end of the experiment. But it improved in plants with biochar treatments as compared to less value for the controls. For 60 % F.C different trends were observed for the treatments T_2, T_4 & T_6 as increased values were calculated. Plants with rice husk biochar treatment have shown the maximum value for WUE i.e. 4.7 mmol mol^{-1} as compared to

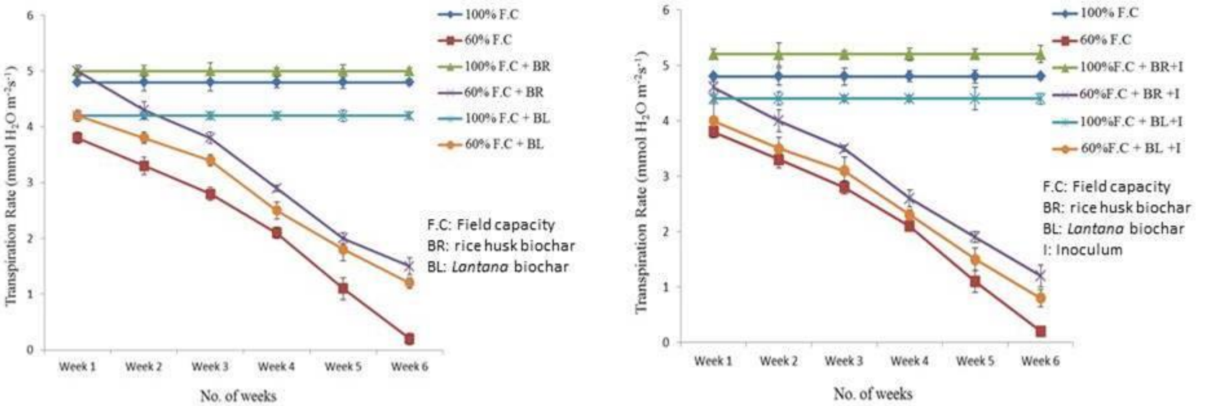


Figure 9a: Comparison of control and biochar treatments

Figure 9b: Comparison of control and biochar with inoculum treatments

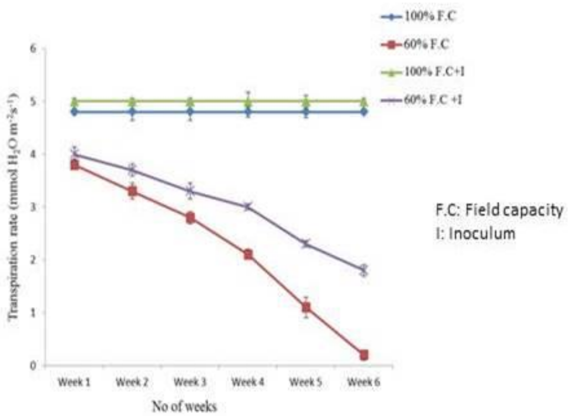


Figure 9c: Comparison of control and inoculum treated plants

Fig. 9 (a, b, c). Changes in transpiration rates of control and treatments under stressed (65 % F.C) and non-stressed (100 % F.C) conditions control and BL (1.5 and 3.7 mmol mol^{-1}).

Figure 10b is presented for the comparison of WUE between control and the combined treatment of biochar and inoculum additions and it is showing highly significant results ($P \leq 0.01$) for all the treatments. Plants which were returned with 100 % transpired water i.e. T_1, T_7 & T_9 similar trends were observed with maximum calculated value of WUE for T_7. In contrast to this, the other group of plants which were returned with 60 % transpired water (i.e. T_2, T_8 & T_{10}), water use efficiency did increase during the course of experiment with similar WUE improvements in plants with rice husk biochar along with inoculum additions.

The third comparison between control and inoculum applied treatment for the parameter of water use efficiency (WUE) is shown in (Figure 10c) with highly significant results ($P \leq 0.01$). Water use efficiency varied slightly in plants maintained at 100 % field capacity for both controls and inoculum treatments. But in case of plants with 60 % F.C significant rise in WUE values were observed for T_2 & T_{12} throughout all the weeks of experiment. However comparatively higher efficiency was seen in inoculated plants rather than control plants.

4. DISCUSSION

The present study was designed with basic aim of evaluating the ameliorative potential of biochar as a rhizobial carrier for the bean plant with specific focus on increasing tolerance against drought stress by improving its water use efficiency. Another aspect of the study was to compare two types of biochars which were obtained from rice husk and a wild invasive shrub *L. camara.* The study also tried to assess the potential of this carrier material for the delivery of isolated strain and assisting the plant in achieving tolerance against water stress.

The addition of biochar provides habitat fulfilling one of the basic microbial requirements.

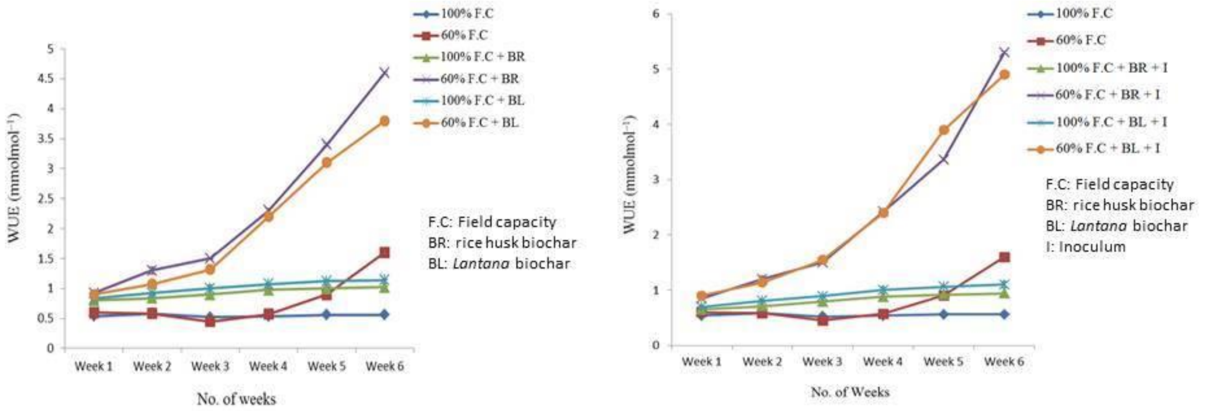


Figure 10a: Comparison of control and biochar treatments

Figure 10b: Comparison of control and biochar with inoculum treatments

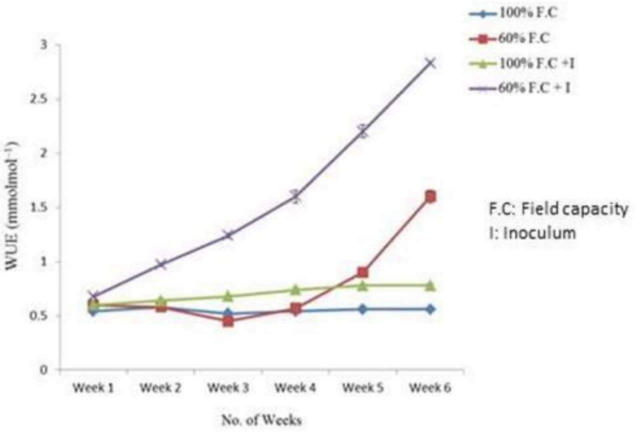


Figure 10c: Comparison of control and inoculum treated plants

Figure 10 (a, b, c). Changes in water use efficiency (WUE) of control and treatments under stressed (65 % F.C) and non-stressed (100 % F.C) conditions.

Jaafar *et al*. [45] confirms our hypothesized potential habitat contribution of different types of biochar, for soil microorganisms, owing to their high porosity and surface area as indicated by scanning electron microscopy micrographs. The high porosity of biochar allows it to maintain water, nutrients and also microbes for a considerable long duration [46].

Scanning electron microscopy along with EDS provides valuable information related to the spectral as well as chemical characteristics of biochar [47]. The analysis showed highly heterogeneous microstructure of biochar upto the magnifications of 5.00 kx. Presence of silicon (Si=7.45 %) mineral agglomerates in rice husk biochar presents typical structure having biomass origin. Rice husk biochar exhibited high degree of macro-porosity with contents of carbon (58 %), oxygen (33 %), potassium (0.47 %) and calcium (0.14 %). On the other hand, the results of SEM-EDS analysis for *Lantana* biochar indicated that these particles consisted of comparatively higher percentage of carbon content (77 %), potassium (2015 %), calcium (0.67 %), chlorine (0.18 %) and lesser oxygen (19 %).

Silicon is one the non-essential elements, that is considered to boost plant growth by improving certain physiological processes in them [48]. Datnoff *et al.* [49] documented inefficient tolerance of both biotic and abiotic stresses when plants were grown in Si-deficient environment. However, if provided with Si, plants showed high tolerance by retaining leaf water potential, stomatal conductance and photosynthetic activity. So it comes in the category of functional nutrients [50] and is regarded as essential (agronomically) for achieving sustainable crop productivity. Silicon plays a similar role for rice and other crops of grass family. Large amount of silicon (in the form of silica gel ($SiO_2.nH_2O$) is accumulated by *Oryza sativa.* This accumulated Si performs the functions of cell wall support, reduction water losses through evapotranspiration, imparting resistance against pathogens and heavy metal and drought tolerance etc. Rice crop specifically takes up greater quantities of Si (even more than essential elements). Rice husk is rich in lignin in combination with opaline silica (apporx. 20 %) and its confirmed by our EDS analysis results for rice husk biochar. Varela Milla *et al.* [33] applied rice husk biochar achieving improved nutrient conditions in Ultisol and increased plant biomass production as compared to wood biochar.

Mostly FTIR transmission analyses provide information about the organic functional groups representing the organic components present in bichar. The peak in the band range 3376-3342 cm^{-1} is expected due to hydrogen bonded hydroxyl groups stretching, designating phenols and alcohols. It may be due to any retained water in the BR sample which is found absent in BL. Another vibration i.e. of C–H stretch between 3036-2830 cm^{-1} indicated presence of alkene groups in BL. A peak observed at 2929-2818 cm^{-1} demonstrates the presence of the aliphatic C–H stretching vibrations.

Stretching band of 2260-2238 cm^{-1} represents C≡C group in BR and 2210-2151 cm^{-1} indicate the same group in BL. The N–H bond with peak range of 1680-1635 cm^{-1} was present in the case of BL. The band at 1580-1435 cm^{-1} is attributed to carboxylate functional group and C–H asymmetric band in BR. Presence of such oxygenated groups have been found to be efficient nutrient adsorbers for the plants capable of releasing these nutrients controlled by various pH driven mechanisms. Presence of variable surface groups enables biochar particles to harbor both positive and negatively charged agri-inputs [51]. The biological effectiveness could further be upgraded through conversion into nan-forms.

The FTIR spectrum of BL was also characterized with the presence of inorganic functional groups as evident by the peaks around 1680-1635 cm^{-1} which could be due to the presence of C=O stretching of aromatic rings. Purakayastha *et al.* [52] suggested that highly stable biochar might have great potential for long-standing carbon sequestration in the soil specifically attributed to the stronger surface groups of aromatic C–C stretching.

Rhizobium strains are reported to have great potential for plant growth promotion and nutrient procurement. Nevertheless, the persistence and proliferation is greatly affected by drought. Protection against this environmental stress could be achieved by different carrier materials such as biochar and their shelf life could further be prolonged. A similar study by Ardakani *et al.* [53]

compared different materials as potential carrier for the survival and viability of bacterial inoculants over an experimental period of six weeks. They proposed wood biochar as efficient inoculum carrier. Saranya *et al*. [54] also compared biochar from two different feedstock and declared coconut shell biochar as better microbial inoculant carrier in sustaining highest number of viable cells.

Besides known qualities of biochar for assisting plants in increasing tolerance against water stress conditions, it is expected to act as efficient carrier for certain rhizobial strains. The present study is based on this hypothesis of ameliorative potential of biochar (obtained from two different feedstocks) as rhizobial carrier besides its role in granting drought tolerance to a leguminous plant (*P. vulgaris*). During the last part of the study, biochar from two different feedstocks were compared in attaining the assigned objectives for all the morphological and physiological parameters. Sandy loam textured pot soil was collected from dry sub-tropical conditions characterizing distinctive water scares and stressed environment for the experimental plants. It was low in organic matter content with neutral pH having electrical conductivity of 4.0 µS/cm. Rice husk biochar alone as well as in combination with the inoculant gave encouraging responses in improving all the studied parameters of the plant.

Our findings regarding plant height evaluation, rice husk biochar was found to be influencing the plant height positively more than *Lantana* biochar. Studies by Dong *et al*. [55] and Win *et al*. [56] have also found significant increases in shoot length as a result of biochar amendment in soil. The results of another study by Pratiwi and Shinogi [57] revealed enhanced shoot height by the application of commercial rice husk biochar that greatly support our results. These findings have further elucidated that such progressive outcomes are partially accredited to the nutrients contribution of the biochar with high electrical conductivity values that represent the quantity of water soluble nutrients. The changes in our EC values are comparable to these studies. Even at 60 % field capacity plant height was more with the addition of BR as compared to control and that of with the addition of *Lantana* Biochar coinciding with the findings of Shashi *et al*. [58].

The rate of increase in leaf area of plants usually decrease due to reduction in the quantity of water provided as reported by McGiffen *et al*. [59] who observed 50 % drop in the leaf area increase as they decreased the amount of water. In the present study a significant rise in the leaf area was seen till the three weeks and afterwards the leaf expansion of all the droughty plants (at 60 % field capacity) began to decline in comparison to the expansion of all the control i.e. well watered plants. In a similar study conducted by Głodowska *et al.* [60] biochar-coated seeds showed significant higher fresh weight, leaf area and plant height compared to the other treatments.

A greater increase in biomass production was observed with the application of fertilizer and rice husk biochar [33]. A similar output was achieved in plant biomass with the application of biochar along with fertilizer [61]. Głodowska *et al*. [60] also found significant higher fresh and dry weight in maize crop when seeds were coated with biochar along with inoculum treatment. Bio-char additions by Rondon *et al*. [19] were found to significantly increase (39 %) total biomass production with the biochar addition to the N-fixing bean. This achievement of getting better biomass production by N-fixing beans is mainly due to greater leaf biomass as a result of biochar and inoculum treatments [19]. A comparable finding of improvements in dry weight with the inoculum addition treatment for the controls as well as stressed plants, was done by Jones *et al*. [62] in which he concluded that dry matter reduction in the stressed plants was observed due to stumpy transpiration and lesser leaf area.

Win *et al*. [56] have supported the impression that biochar do promote the survival of inoculated bacteria performing the role of a stable shelter for them. Furthermore, if crops are provided with inoculum in combination with the biochar, significant yield enhancements could be achieved utilizing its property of acting as a carrier. A review by Sashidhar *et al*. [51] suggested varying inoculum responses of different types of biochar prepared from different feedstocks with varying process settings. Biochar has a supplementary advantage that enables to be utilized as inoculum carrier ensuring survival of the microbes for maximum duration [63].

Purakayastha *et al*. [52] have advocated rice biochar for its ability to restore bio-fertility of soils by enhancing their microbial activities. According to them the rice biochar has being found to be fairly labile in the soil contributing to the microbial biomass proliferation supporting our better results with rice husk biochar. Being a major rice grower continent, Asia gets copious amount of rice residues with the estimates of about 560 million tons of straw and 112 million tons in the form of husk. These huge quantities of residues could be efficiently utilized or in other words recycled in the form of biochar and in return be benefitted by its enormous qualities [33] with additional benefit of getting rid of winter smog problem caused by rice residue burning. This could be the reason that makes husk biochar the most cost effectively considered biochar for a long time.

Limited studies have been conducted about the synergistic utilization of biochar and rhizobacteria. Hafez *et al*. [64] conducted a field study on alleviating water deficit in rice crop using biochar and rhizoacteria in combination with soil treatments i.e. control, biochar, PGPR as well as combination of PGPR with biochar especially in the field. According to their results this integrative use augmented the physicochemical properties of the soil. Significant increase in the stomatal conductance occurred with decreasing proline content in *Oryza sativa* being treated simultaneously with PGPR and biochar confirming the efficacy of our study approach for the bean plant. Studies have shown that the photosynthetic machinery is impaired under drought stress and application of PGPR have been found to overcome this impairment along with the enhancement in the chlorophyll content and shoot length [65].

Supporting our findings with reduced conductance in water stress treatment, Galmés *et al.* [66] compared different plant species for reduction in stomatal conductance under water deficient environment. On the contrary, a plant showing higher rates of stomatal conductance even under drought conditions depicts its greater tolerance level [67]. Studies have linked such closure response of stomata to the moisture content of the soil than the leaf water status.

According to Jones *et al*. [62], there is a linear relation between the decline in transpiration rate and dry matter reduction. Signals are transmitted by the roots towards the shoots with a measure of reduced water consumption through transpiration reduction enabling plants to grow in drying soil. This transpiration decline can be achieved either by reduction in leaf expansion or by leaf shedding in case of severe drought stress [68]. Liu and Stuzel [69] attributed such transpiration declines as the major cause of reduction in stomatal conductance. Transpiration rates of control plants reached zero at the end as compared to a bit higher with biochar and inoculum treatments. This shows that biochar and inoculum has enabled plants to respond better in water deficient conditions.

Our findings of improvements in photosynthetic rates of bean plant in response to the biochar application under drought stress conditions are confirmed by the studies of Solaiman *et al*. [70]. In accordance with Akhtar *et al*. [71], biochar amendment specifically prepared from rice husk had positive influence on the photosynthesis, transpiration rate, stomatal conductance and water use efficiency in relation to respective *Lantana* biochar and non-biochar control treatments. Hattori *et al*. [72] found by bluegrass under drought stress with the application of Si and concluded that it happened due to improved water uptake, root growth and leaf erectness contributed by Silicone.

Similar to our results for getting enhancement in water use efficiency with biochar applications, Romdhane *et al*. [73] concluded about getting considerable reduction in drought sensitivity and improved water use efficiency in maize plant. Water use efficiency (WUE) depicts the performance of a crop facing any environmental constraint. Several studies have demonstrated similar results of contribution of declined transpiration rate (Tr) and increased photosynthetic rate (Pn) towards water use efficiency [74–75]. In our experiment, we achieved WUE enhancement through reduction in transpiration rate and escalation in photosynthesis rate.

5. CONCLUSION

Water, in certain quantity, is the basic requirement of all the life forms existing on planet earth for optimal growth and survival. Water use efficiency (WUE) acts as the most crucial factor that can limit plant growth. Current scenarios of rising temperatures (contributed mostly due to anthropogenic reasons) with the consequences of climate change are contributing severe weather patterns with flash floods, droughts and threatened watersheds are going towards extreme water scarcity. Soil amendments like biochar have proven their vital role as one of the paramount methods in overcoming stress due to drought conditions. Along with its contributions as soil amendment, biochar was evaluated for its ameliorative potential as microbial carrier for rhizobial strain in *Phaseolus* plants exposed to water stressed condition. The present study advocates rice biochar for its ability to enhance tolerance in *Phaseolus* against drought stress through its role as inoculum carrier contributing suitable habitat for the microorganism. The huge quantities of rice residues produced in Pakistan could be utilized efficiently in the form of biochar and could solve the problem of biomass burning and the resultant smog. In the arid regions of Pakistan, where drought is prevalent, application of rice-husk biochar can significantly enhance the plants' tolerance against this climate stress by improving their water use efficiencies. In conclusion the synergistic use of rice husk biochar and rhizobacteria could be a promising strategy in improving growth and productivity of *P. vulgaris* L. under drought stress as compared to the biochar prepared from *Lantana camara*.

The beneficial role of biochar prepared from crop residues of the same plant (i.e., *P. vulgaris*) invites further research to explore its true potential for growth as well as grain yield enhancements of this plant using isolated strain inoculum in water deficit areas focusing on plant's physiological responses. Comparative evaluation of biochar over other known carrier materials like peat and lignite for the isolated strains could be done. The inoculation effects on Nitrogen retention and BNF by *P. vulgaris* with different application rates of biochar as well as for long term applications in the field could further be explored. Study could be focused on micronutrient uptake of the bean plant as affected by biochar inoculum combination.

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7. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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