

Biochar effect to enhance nodulation and suppress root pathogenic fungi in chickpea

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Abstract

The aim of the current research was to explore the impact of biochar and *Mesorhizobium ciceri* to enhance nodulation and antagonistic expression against soil-borne pathogens namely *Phytophthora medicaginis*, *Fusarium oxysporum* and *F. solani* of chickpea (*Cicer arietinum* L.). Chickpea plants grown in vermiculate medium were treated with 5% green waste biochar, nitrate, *M. ciceri* and one was synergetic treatment (biochar + *M. ciceri*) which showed significant difference from other treatments and control plants producing an average 60 nodule in each plant with 57.90 mg average weight as compare to *M. ciceri*, green waste biochar and nitrate treated plant producing 31, 39 and 7 nodules per plant with a weight of 39.5, 46.5 and 35.6 mg nodule⁻¹, respectively after 60 days of growth. Control plants produced no nodules in first and 2nd harvest but in 3rd harvest, just 2 nodules per plant were observed with 30.5 mg weight. Synergistic treatment also showed a significant difference in shoot length, fresh weight and dry weight but primary root length was small with a cluster of feeding roots. Combination of both the treatments completely inhibited the colony development of chickpea pathogen after three days. Colony diameter of *F. solani*, *F. oxysporum* and *P. medicaginis* was calculated just 0.86, 0.99 and 0.64 cm as compared to control treatment having colony diameter 3.98, 4.45 and 2.80 cm, respectively, after a week.

Keywords: Antagonistic, biochar, *Fusarium*, nodulation, pathogen, rhizobacteria.

Introduction

Chickpea is the world's second most widely grown legume. India is leading to Australia and Pakistan, which are producing 8,832,500, 813,300 and 751,000 metric tonnes chickpea annually, respectively (FAO, 2014), paving against malnutrition, food security and global livelihood generation in rainfed areas of the developing countries. Excessive use of inorganic fertilizers and poor nitrogen area based have no more balance nutrition availability (Hakoomat *et al.*, 2004) Intensive use pesticides and herbicides to control insect pest and weeds are leading to deterioration in soil health as well as unvalued production which also causing endocrine loss of immunity and behavioral changes in human being (Shahjahan *et al.*, 2015). It's necessary practicing to increase the organic matter content to balanced stream of plants nutrients (Gupta *et al.*, 2014).

Despite its high production potential in arid areas of Punjab, fungal root diseases are the major bottlenecks in chickpea productivity. *Fusarium oxysporum* f. sp. *ciceri* causing wilt, *Macrophomina phaseolina* (dry root rot), *Rhizoctonia solani* (wet root rot), *F. solani* (black root rot), *Phytophthora* root rot and damping off cause major losses and thwart farmers from realizing the potential yield of chickpea crops. Root infecting pathogenic fungi involves mainly *F. oxysporum* f. sp. *ciceris*, *F. solani*, and *Phytophthora* spp. The pathogens are both seed and soil borne and can survive in soil, even

in the absence of its host, for six years (Haware *et al.*, 1996; Ayyub *et al.*, 2003). Due to the prolonged nature of survival of the pathogens, cultural control such as crop-rotation is not feasible and chemical control is not only costly, but it also imposes serious implications on the environment.

Biochar having sequential carbon have the ability to enhance the fertility of soil (Graber *et al.*, 2010; Asai *et al.*, 2009). Rhizobia are recognized to capture atmospheric nitrogen by symbiotic association with legumes crops (Wielbo *et al.*, 2010; Margaret *et al.*, 2011) as *Mesorhizobium ciceri* LMS-1 strain was tested for more nodulation and enhancing the yield in chickpea (Nascimento *et al.*, 2012). Several types of experiments have shown the efficacy of biochar against pathogens and increasing the productivity of soil to increase the yield of different crops pepper, tomato, maize, wheat and rice (Meller Harel *et al.*, 2012; Joseph *et al.*, 2013). Biochar can protect soil from root pathogenic fungi as bean crops were tested to control root pathogenic fungi (Jaiswal *et al.*, 2015). So, in this study, we are first time reporting the effect of biochar to increase the nodulation in chickpea. Furthermore, we are also reporting the synergistic effect of biochar with *M. ciceri* to mycelial growth in-vitro conditions.

Materials and Methods

Biochar and plant growth medium

Biochar prepared from green waste at a high treatment temperature of 450 °C in the pyrolysis system, was used throughout the research. GW Biochar was grounded into a powder of <0.5 mm particles and stored in sealed containers. The physical and chemical characteristics of the biochar were reported in a previous study (Graber *et al.*, 2013). Unless otherwise stated, autoclaved pots and grade 2 vermiculite were used when sterile growth conditions were necessary, 5% GW Biochar was mix with vermiculite for biochar treatment experiment.

Plant growth conditions

In all experiments conducted, WT chickpea was used. Plants were grown in controlled glasshouse conditions (28 and 24 °C, day and night, respectively, with a 16 h day length). Seeds were surface-sterilized using 70% (v/v) ethanol for 10 s followed by rinsing five times with sterile water, sown in sterile vermiculite in 4 L pots. Plants were watered daily and supplemented with a B & D nutrient solution (Broughton and Dilworth, 1971) twice a week.

Rhizobium and chickpea fungal pathogens growth conditions

M. ciceri (isolated from the chickpea growing area at the Department of Primary industries, NSW, Australia) was grown for 48 h at 28 °C in Yeast Mannitol Broth (YMB; Somerville and Kahn, 1983). The isolated strain was confirmed by 16s rDNA sequence. Cultures were diluted with water to a final concentration of OD600 = 0.01 prior to inoculating plants. Approximately 150 mL of this final concentration was applied per pot if necessary. Chickpea pathogen *P. medicaginis*, *F. oxysporum* and *F. solani* (isolated from NSW and Queensland chickpea growing area) were cultured on PDA liquid medium, 25 °C with 150 rpm.

Effect of biochar treatment on chickpea growth

Four different treatments (*M. ciceri*, Biochar, *M. ciceri* + Biochar and nitrate) were used in this

experiment, water as a control. All these seedlings were watered with a without nitrate B & D nutrient solution. Two mM potassium nitrate was additionally supplied for nitrate treatment twice a week. *M. ciceri* was inoculated on the third day after germination. In each pot, four plants were grown, and each treatment has three duplicates.

Plants were harvested after 20 days, fresh weight, dry weight, root length (primary root), shoot length, number of nodules and weight of nodule were measured for each plant. Data were analyzed using an analysis of variance (one-way ANOVA) procedure for independent samples to test for statistically significant differences using SPSS (SPSS Inc., Chicago, IL, USA). Standard errors (SEs) of the means were calculated and make a diagram in Excel.

Pathogen inoculation, and disease evaluation

Chickpea pathogen *P. medicaginis*, *F. oxysporum* and *F. solani* were inoculated with sterilized vermiculite, non-inoculated as a control were used for Biochar, *Mesorhizobium ciceri*, and the combination of *Mesorhizobium ciceri* + Biochar treatment, which were tested as antagonistic effect against root pathogenic fungi of chickpea. Disease severity was observed after 15 days of germination.

Efficacy of the experiment was determined through poison food technique method. When PDA media was poured into Petri plates, were mixed with different treatments (Biochar, *M. ciceri* and *M. ciceri* + Biochar). Each treatment was replicated four-time and control Petri plates were not mixed with any kind of treatment to compare the colony growth of pathogen with poisoned and unpoisoned. More than 10 days old fungal inoculum was inoculated in each labelled Petri plate and were incubated at room temperature. Data was taken after 5 days and the mycelial growth diameter was calculated from the centre and whole data was statically analyzed to check the significant relationship between the different treatment and control.

Table 1: Colony diameter of fungal pathogens on different media.

Treatment	Colony diameter (cm)		
	<i>F. oxysporum</i>	<i>F. solani</i>	<i>Phytophthora sp.</i>
Biochar	1.43 b	1.20 b	1.13 b
<i>Mesorhizobium</i>	1.21 c	1.18 b	1.14 b
Biochar + <i>Mesorhizobium</i>	0.99 d	0.86 c	0.64 c
Control	4.45 a	3.98 a	2.80 a

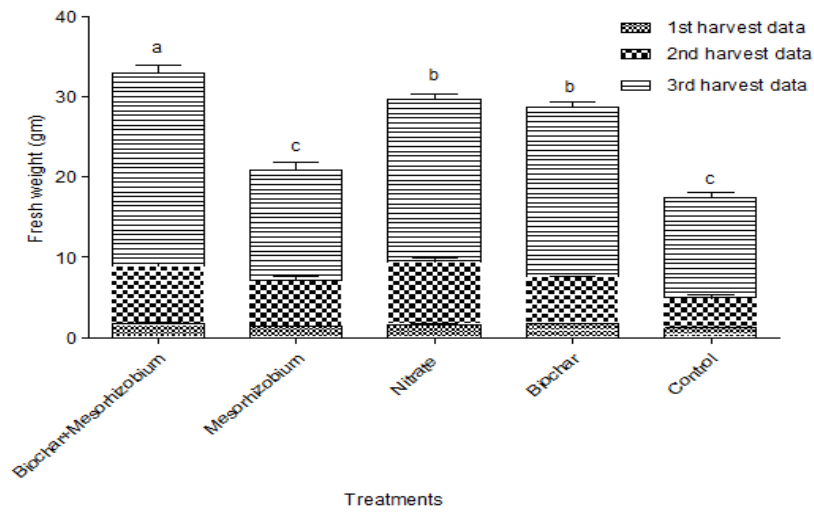


Fig. 1. Fresh weight of chickpea plants with different treatments in different harvest after 20 days interval .

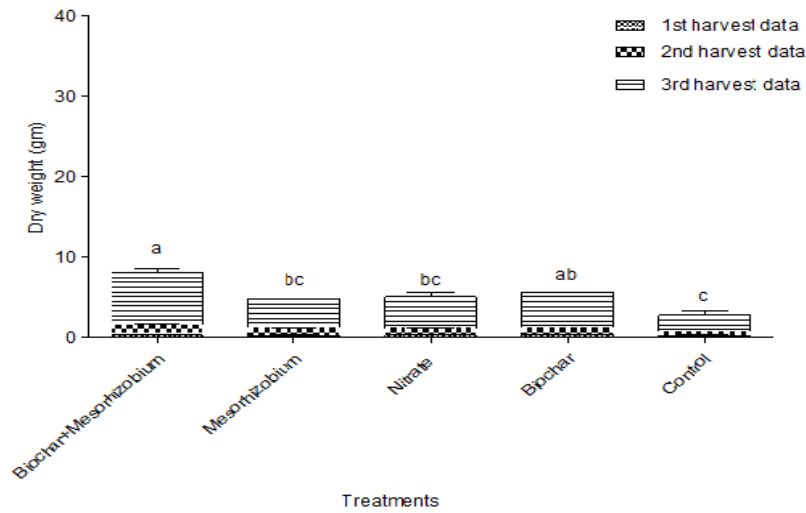


Fig. 2. Dry weight of chickpea plants with different treatments in different harvest after 20 days interval.

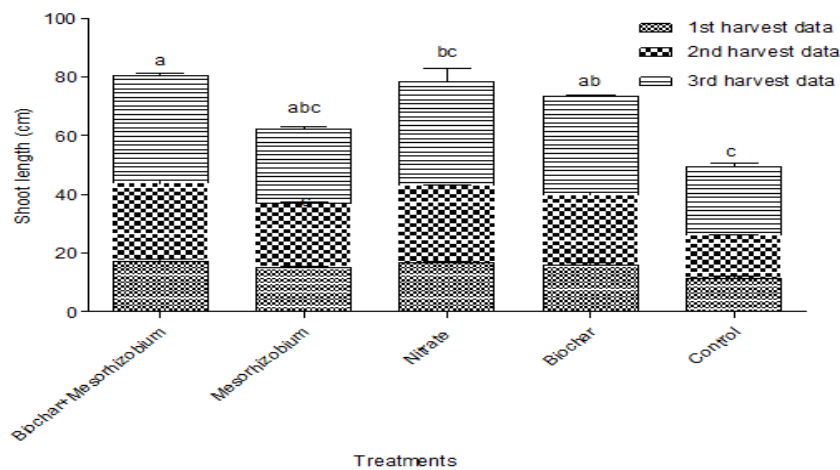


Fig. 3. Shoot length of chickpea plants with different treatments in different harvest after 20 days interval.

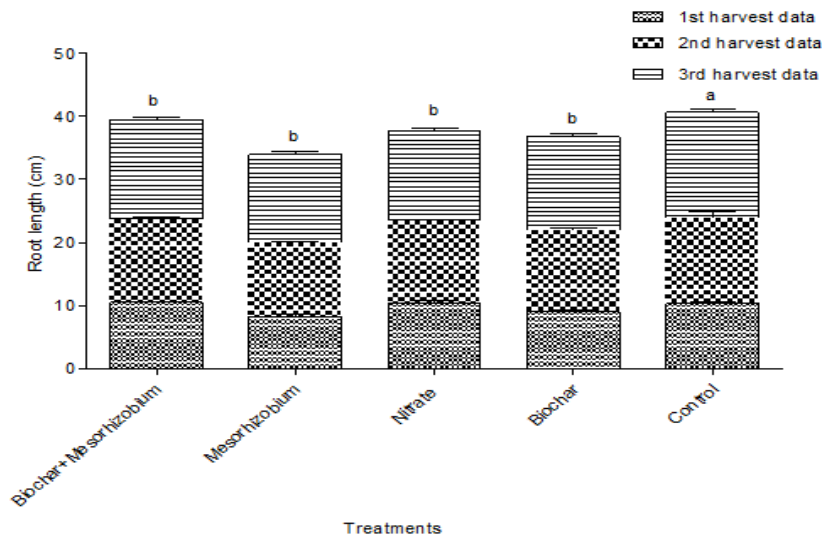


Fig. 4. Root length of chickpea plants with different treatments in different harvest after 20 days interval.

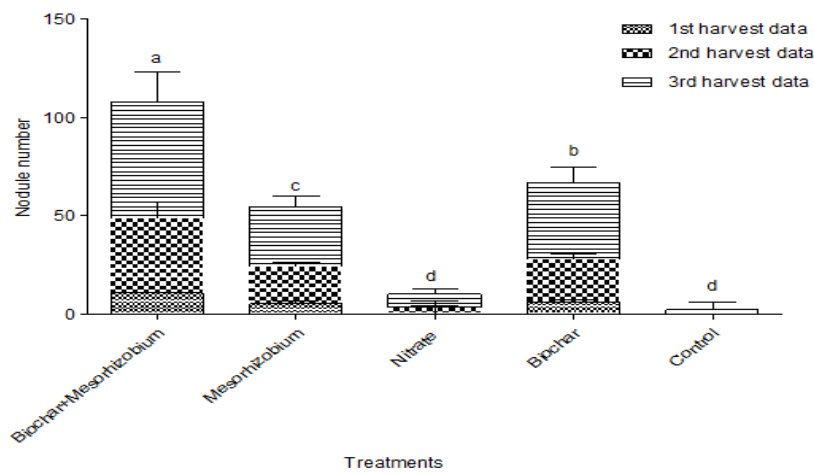


Fig. 5. Number of nodule development in chickpea plants with different treatments in different harvest after 20 days interval.

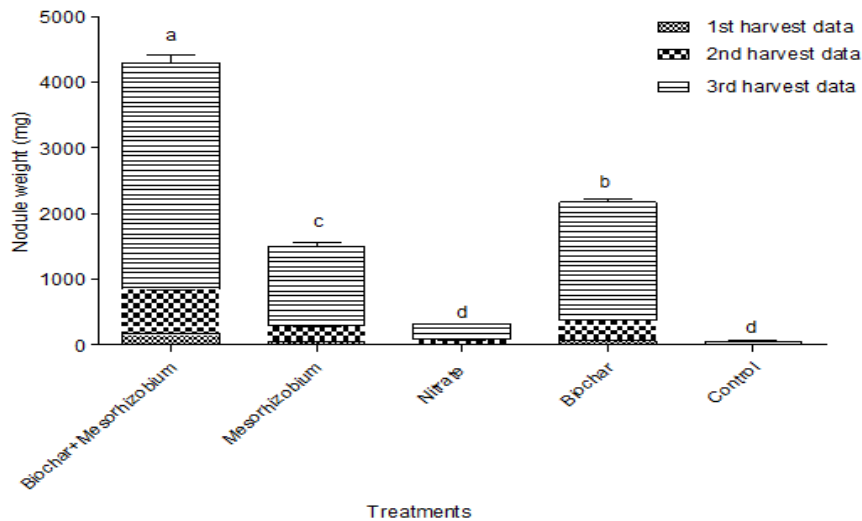


Fig . 6. Nodule weight of chickpea plants with different treatments in different harvest after 20 days interval.

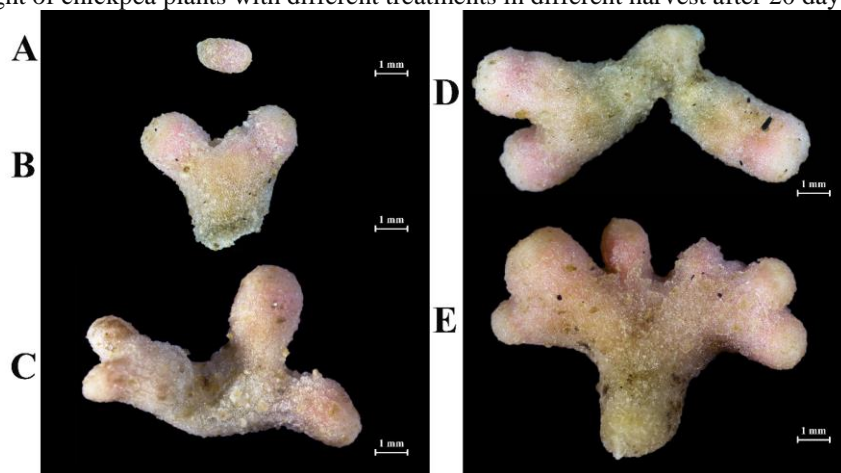


Fig. 7. Size of nodule, developed in chickpea plants with different treatments as in A)- Nodule of control (Water) treated chickpea plants B)- Nitrate treated nodule C)- *Mesorhizobium cicer* treated nodule D)- biochar treated nodule and in E)- Symbiotic (biochar + *M. ciceri*) treated nodule after 60 days.



Fig. 8. Chickpea plants after 20 and 40 days.

Results and Discussion

We have found significant differences among

bio-fertilizers (Biochar & *M. ciceri*), inorganic treatment (Nitrate) and control in biomass

production and nodule development as Fig. 1 described the effect of treatments on vegetative growth development, statistical data analysis after the first harvest (20 days) for fresh weight raised three groups among the five treatments but means of shoot length are not significantly different from another same after 40 days data Nitrate and Mesorhizobium + biochar shows no noteworthy difference but significant difference from other treatments, Biochar and rhizobium treated plants were approximately same means but all treatments were substantial weight difference from the nitrogen-free plants act as control plants. But after 60 days significant difference between the different treatments. Control treatment and the combination of biochar with *M. ciceri* means have a noteworthy difference in fresh weight which was 24.27 g and 12.71 g respectively but biochar and nitrate treatments have no significant difference with 19.18 g and 20.47 g. In the treatment of *M. ciceri* fresh weight is not augmented significantly with control plants. Dry biomass was calculated after 72 hours oven dried after each harvest, first harvest statistical data was categorized in “a” and “b” in which the means are not significantly different from each other as with proceeding of an experiment in 2nd harvest means of treatment were categorized in a, b and c.

Symbiotic treatment of biochar + *M. ciceri* yield maximum as compare to *M. ciceri*, biochar and nitrated treated plants which all are categorized in the same category, but control plants just treated were as least category. After 60 days all treatments means were significantly different among each other with 0.05 value of alpha and 0.5846 critical value of comparison. *M. ciceri* + biochar yields maximum dry weight 6.48 g and *M. ciceri*, nitrate and biochar produced 3.59 g, 3.92 g and 4.49 g, respectively which is significantly different from the 2.10 g dry weight of control plants (Fig. 2).

Data of shoot length shown in Fig. 3 and root length shown in fig 4 correlative results, in case of shoot length biochar + *M. ciceri* treated plants showed maximum but minimum in root length. The maximum length of shoot is in biochar + *M. ciceri* which is followed by nitrate, biochar, *M. ciceri* and control plant but in case of root maximum length is observed in control plants followed by biochar + *M. ciceri*, biochar, nitrate and *M. ciceri* treated plants.

The prime objective of the research was an evaluation of different treatments to excel the nodulation in chickpea for this as same data was taken from different treatments and replication in three harvests after 20 days interval. In first harvest few nodules were observed in symbiotic treatments (biochar + *M. ciceri*), biochar and rhizobium treated plants but very small in size and less in weight, nitrate and control plants were unable to augmented nodule in first 20 days with the extension of experiments 20 to 40 days symbiotic treatment show excel in nodule development and after 60 days a

massive number of heavy nodule was observed in biochar + *M. ciceri* plants and nodule number were lagging biochar, *M. ciceri*, and nitrate treated plants. In 3rd harvest control plants also produce few tiny nodules. Mean value of nodule number and nodule weight in each treatment is described by fig 5 and fig 6 respectively. All vegetative data was calculated after 20 days interval in three harvest as in figure 7 showed the development of nodules and other vegetative characters. In last harvest nodule developed with different treatments were examined under compound microscope and nodule developed in response to biochar and rhizobia were significantly more size as compare to control and other treatments as shown in Fig. 8.

In the second part of the experiment antagonistic effect of biochar, *M. ciceri*, and symbiotic effect of both treatments was confirmed against *F. oxysporum* pv. *ciceris* (BRIP 61614 and 61615), *F. solani* pv. and *ciceris* (BRIP 61615 b) and *P. medicaginis*, root pathogen of chickpea. Biochar + *M. ciceri* were shown best potential antifungal effect. Data was taken after 2 to 7 days. Control Petri plates having no poison were completely chockfull with pathogen within a week but pathogen in treated plates was slowly grown up to for three days and then colony growth was restricted for further mycelial development. Colony growth data was statically analyzed. The experiment is repeated three times and each time four replication of each treatment was applied, in the following table mean value from all experiments and replication was analyzed.

It is a need of time to increasing demand for convincing information on the impact of sustainable agricultural development. Here were reported an ultimate solution to curtail chemical with organic material which strengthens the holistic approaches that have been historically tested, traditionally practised, culturally integral, economically viable, socially responsible, environmentally sustainable and agreeable as a policy. Study of Nelson *et al.* (2010) emphasise the role of fertilizer to get more yield as per increasing demand for agriculture produce from last four decades. So, biochar has been already reported as a noteworthy substitute which has a significant impact on symbiotic microbial community structure in soil and the rhizosphere (Lehmann *et al.*, 2011). Biochar was also reported to prompt an increase in the relative abundances of bacterial phyla and genera with antagonistic activity use as biocontrol (Kolton *et al.*, 2011)

Biochar is successfully tested as a bio-fertilizer in low rain area of Western Australia in grain crops which crease grain yield through abundance of microbial biomass (Zakaria *et al.*, 2010) in our research biochar is used in two treatments, in first treatment 5% green waste biochar was used and in other treatment biochar was

applied with *M. ciceri*. In individual treatment of biochar a greater number of nodule is chickpea plants were observed and nodule were weighty as compare to nitrate and *M. ciceri* treated plants as Singh *et al.* (2012) reported that biochar as nitrogen source which significantly increased the yield of legumes crops through heat tolerance by increasing the water holding capacity of soil and increasing antagonistic microbial colony. So current research was purposed in the vermiculate medium due to more aeration, water holding capacity and nutrients free medium as described by the previous study conducted by Indrasumunar and Gresshoff (2013) which rustled that vermiculate is suitable medium to study the effect of different treatment on nodulation development. Nascimento *et al.* (2012) also describeth the use of vermiculate medium where chickpea plants (CHK3226) were inoculated with the same species microbes known as *M. ciceri* and demonstrate the 127% increase in the nodule number and also increase the biomass up to 125% which indicate that deaminase production by *Mesorhizobium* which may provide cognate legume with lot of benefits which also helpful to crop for the suppression of *Pythium* pathogen which causes damping off in chickpea.

M. ciceri resulted as antagonistic effect in current study against *F. oxysporum*, *F. solani* and *P. medicaginis* as different plant promoting rhizobacteria have already success stories to control different pathogen in different crops like *Pythium* in cucumber was successfully managed with the use of PGPR and potato crop was protected against soft rot disease caused by *Erwinia* spp. as Hao *et al.* (2007) and Toklikishvili *et al.* (2010) described that ACC producing bacteria can inhibit the development of crown gall causing pathogen in tomato and castor crops. Targore *et al.* (2013) examined the effect of rhizobacteria in field experiment which increases the nodule number and weight of nodule, application of rhizobacteria also showed its positive effect in enhancing all the yield attribute parameters, grain and straw yields. Biochar was also evaluated by Meller Harel *et al.* (2012) against the same type of air and soil borne pathogen of pepper, strawberry and tomato crops. Biochar also reduced the disease curve in m maple and oak plants which induced resistance against *B. cinerea* (Zwart and Kim, 2011, 2012). Study of El Oirdi *et al.* (2011) reviled the production of ethylene in response to biochar and application which induce resistance to grey mould being ET- dependent.

Literature and our research emphasis the use of symbiotic treatments, biochar with *M. ciceri* act as carrier and trigger the effect of microbes and yield double as compare to other treatments and spokes *et al.* (2011) describes that biochar efficiently use as standard carries as compare to peat moss because it commonly contains a large number of adsorbed volatile organic compounds that directly

effect on the colonization of rhizospheric microbes through habituating them in nano microspores. biochar addition had could increase the density of microbes as inoculum which was determined by the experiment of (Downie *et al.*, 2009; Hardie *et al.*, 2014) by quantification of total 16S rRNA genes, viable microbial association with biochar increase their abundance through bio-stimulation process (Chen *et al.*, 2013) and recently interfering of biochar with microbes was accessed as different biochar have different degree of quorum sensing compounds (Masiello *et al.*, 2013).

In conclusion, it is shown here for the first time in chickpea excellent use of green water biochar with *M. ciceri* to excel the nodulation development which also enhances the vegetative growth of plants and showed broad-spectrum antagonistic response against the disease-causing agent in an in-vitro study. In further in-vivo experiment results will give an ultimate solution to curtail chemical fertilizer & fungicides with organic and microbe's base product.

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