Madras Agric. J., 98 (4-6): 158-164, June 2011
https://doi.org/10.29321/MAJ.10.100266

Influence of Biochar and *Azospirillum* Application on the Growth of Maize

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A pot culture experiment was conducted to assess the influence of different levels of acacia wood based Biochar with *Azospirillum* strain AZ 204 on the growth of maize variety PEHM5 in the black cotton soil. The results revealed that the addition of biochar to soil with *Azospirillum* inoculation significantly increased the plant-microbial responses and the nutrient status of both plant and amended soil. *Azospirillum* and total diazotrophs population in the rhizosphere significantly increased with Biochar application at all the stages of crop growth. Furthermore, a significant increase of the native mycorrhizal response to Biochar with *Azospirillum* application was also observed in terms of root colonization of maize.

Keywords: *Azospirillum*, Biochar, Maize, *Mycorrhiza.*

Biochar is a newly emerging carbon rich by-product obtained by the pyrolysis of biomass (from agricultural and forestry sources) especially wood during electrical power generation in power plants (Day *et al.*, 2005). Quality of biochar differs based on the sources of wood used for power generation. Biochar is widely recognized nowadays in conjunction with soil management and C sequestration issues (Steiner *et al.*, 2004). Recent studies show that biochar amendments at rates varying from 0.5 to 135 t ha^{-1} are indeed capable of improving nutrient availability and microbial activity in soil, resulting in a significant increase (up to 34%) in yield when applied to crops like rice, sorghum, corn, various beans (soybean, common bean, cow pea), banana and vegetables such as carrots (Lehmann *et al.*, 2003). Moreover, with increasing rates, plant responses was positive until 30 t ha^{-1} of biochar, wherein a maximum was reached, above which growth response was negative for beans with application of 0 to 93 t ha^{-1} (Pietikainen *et al*., 2000). Apparently biochar provides a suitable habitat for a large and diverse group of soil microorganisms (Topoliantz *et al.,* 2005). Root infection by arbuscular mycorrhiza significantly increased by adding 1 kg m^{-2} of biochar in alfalfa in a volcanic ash soil that related very well with the growth of alfalfa being 40-80% greater after the application (Ishii and Kadoya, 1994).

Although, there has been some research on the effect of wood based biochar addition on the soil microbiological community (Steiner *et al*., 2004), there is relatively no information on the effects of biochar application with bioinoculants like *Azospirillum* that aid in increasing the soil fertility by acting as a best plant growth promoter by N_2 fixation in soil. Since, biochar addition not only affect the microbial populations and activity in soil, but also plant-microbe interactions through their effects on nutrient availability and modification of habitat. With appropriate application rates of biochar and supplementary nutrient additions by using available bioinoculants like *Azospirillum*, nutrient input to agricultural systems can be increased without decreasing plant productivity. Such a soil management system may be in the context of mixed legume-cereal intercropping or of agroforestry with woody legumes. Soil nitrogen stocks and eventually nitrogen availability can be increased and be made available to the non-legumes in a rotational system. Moreover, majority of the south Indian soils are low in organic carbon and as there is a current need to increase the carbon content of soil for sustainable agriculture and replenish the soil health, which can be achieved innovatively using the co-generated biochar from electrical power generation plant using wood as raw material. The present investigation was undertaken to find out the influence of biochar and *Azospirillum* application on the growth of maize as well as beneficial soil microbes like total diazotrophs and native arbuscular mycorrhizal fungal population.

Materials and Methods

A pot culture experiment was performed in completely randomized block design (CRD) with three replications for each treatment at Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore. Acacia wood based Biochar was tested at different levels (0, 5, 10, 15, 20 and 25 t ha^{-1}) with and without *Azospirillum* inoculation. The

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physio-chemical properties of biochar used in present study were presented in Table 1. Each earthen pot (1' x 1') was filled with 8 kg of field soil collected from Eastern Block of Tamil Nadu Agricultural University farm located at Coimbatore. The soil used was saline in pH (8.52), low in organic carbon (0.56 %), low in available N (91 mg kg^{-1}soil), medium in available P (6.05 mg kg^{-1}soil) and high in available K (229 mg kg^{-1}soil). According to the

Table 1. Physio-chemical properties of *Acacia* wood based Biochar used in this study

Property	Values
Water holding capacity (%)	200
Moisture (%)	20-30
Bulk density (g cm^{-1} cube)	1.62
Porosity (%)	73.33
pH	7.00
Total Carbon (%)	84
Total Hydrogen (%)	2.30
Total Oxygen (%)	10.70
Total Nitrogen (%)	0.01
Total Ash (%)	3.24
Oxides of Al, Fe, Ca, Si, Hg, Ar, Se	Nil

treatments, biochar was applied on the surface soil. *Azospirillum* (AZ 204) (containing 72 x 10^9 cfu ml^{-1} of broth) was seed inoculated with maize just before sowing. The recommended doses of fertilizers (135:62.5:50 kg of N, P_2O_5 and K_2O ha^{-1}) were applied as urea, single super phosphate (SSP) and murate of potash (MOP). The entire P and half of N were applied as basal. Remaining N and K was applied in two splits at 30 and 45 days after sowing. Soil and plant samples were collected during 30, 60 and 90 days after sowing.

Observations on growth parameters like shoot length (cm $plant^{-1}$) and root length (cm $plant^{-1}$) were recorded. From the collected rhizosphere soil samples, *Azospirillum* population was enumerated by MPN technique using Dobereiner N-free malic acid semisolid medium (Baldani and Dobereiner, 1980). Total diazotroph population was estimated by serial dilution and plating method using Watanabe and Barraquio medium (1979) and the results were expressed as cfu g^{-1} dry weight of the soil. Additionally, arbuscular mycorrhizal (AM) root colonization by the method described by Philip and Hayman (1970) was carried out and the results were expressed as per cent root colonization. Also, nutrient status of soil samples for organic carbon (Chromic acid wet digestion method, Walkley and Black, 1934); available nitrogen (Micro-Kjeldhal digestion, Piper, 1966); available phosphorous (HCl extract volumetric method, Jackson, 1973) and available potassium (Flame photometry, Standford and English, 1949) were estimated at 120 days after crop growth. Nitrogen (Semi automatic kjeldahl distillation, Humphries, 1956); phosphorous (Vanadomolybdate colorimetric method, Jackson, 1973) and potassium (Flame photometer, Jackson, 1973) contents of plant samples were also determined. The data were subjected to statistical scrutiny as per the methods detailed by Panse and Sukhatme (1985).

Results and Discussion

Crop growth response

A critical examination of the data presented in Table 2 indicated the significant influence of biochar on shoot length of maize at all the stages of crop

Table 2. Influence of Biochar and *Azospirillum* on shoot length of maize (cultivar PEHM5)

Biochar levels	Shoot length (cm $plant^{-1}$)					
	30 DAS		60 DAS		90 DAS	
	- AZ 204	+AZ 204	- AZ 204	+AZ 204	- AZ 204	+AZ 204
Uninoculated control	62	64	106	110	114	118
Biochar (5 t ha^{-1})	73 (17.7)	74 (19.3)	113 (6.6)	118 (11.3)	125 (9.6)	126 (10.5)
Biochar (10 t ha^{-1})	75 (20.9)	75 (20.9)	127 (19.8)	130 (22.6)	135 (18.4)	139 (21.9)
Biochar (15 t ha^{-1})	75 (20.9)	76 (22.5)	129 (21.6)	131 (23.5)	136 (19.2)	144 (26.3)
Biochar (20 t ha^{-1})	77 (24.1)	78 (25.8)	132 (24.5)	134 (26.4)	146 (28.0)	151 (32.4)
Biochar (25 t ha^{-1})	78 (25.8)	79 (27.4)	140 (32.0)	147 (38.6)	165 (44.7)	170 (49.1)
CD (P < 0.05)						
Biochar	0.48**		8.01**		7.57**	
Azospirillum	0.28**		NS		NS	
Biochar x *Azospirillum*	NS		NS		NS	

Values represent mean of three replications; Data in parantheses represent per cent increase over control; '- AZ204' - without *Azospirillium* inoculation, '+AZ204' - with *Azospirillum* inoculation; **- significant at p<0.01, NS - not significant.

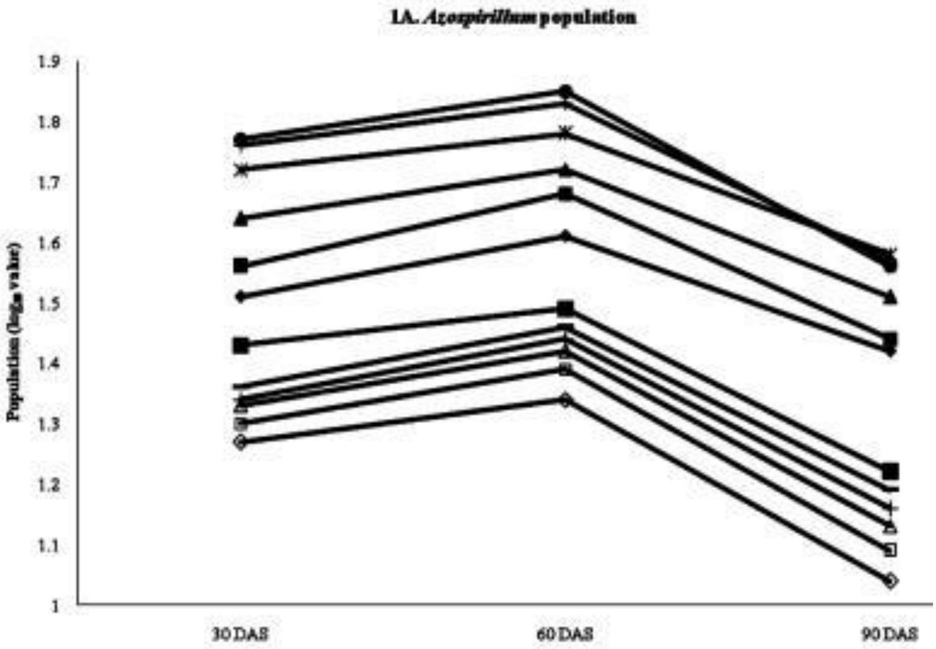
growth. Application of biochar recorded 17.7 to 25.8 % increase in shoot length with the maximum of 78 cm plant^{-1} at 30 days after sowing. When *Azospirillum* was applied along with biochar the shoot length was still enhanced to the level of 19.3 to 27.4 % over uninoculated control. The interaction effect was not observed significantly. Influence of biochar at varying levels was observed significant on shoot length at all the three stages of plant growth. At 90 days, the response of biochar both with and without *Azospirillum* was to the tune of 44.7 and 49.1 % respectively at higher levels of biochar (25 t ha^{-1}) application. Root length was significantly influenced with the application of biochar either alone or with *Azospirillum* inoculation (Table 3). At each level of biochar application, the increase in

Table 3. Influence of Biochar and *Azospirillum* on root length of maize (cultivar PEHM5)

Biochar levels	Root length (cm plant^{-1})					
	30 DAS		60 DAS		90 DAS	
	- AZ 204	+AZ 204	- AZ 204	+AZ 204	- AZ 204	+AZ 204
Uninoculated control	21	23	23	25	24	25
Biochar (5 t ha^{-1})	23 (9.52)	26 (23.8)	25 (8.6)	27 (17.3)	27 (12.5)	29 (20.8)
Biochar (10 t ha^{-1})	24 (14.2)	27 (28.5)	29 (26.0)	31 (34.7)	32 (33.3)	33 (37.5)
Biochar (15 t ha^{-1})	26 (23.8)	28 (33.3)	31 (34.7)	32 (39.0)	34 (41.6)	35 (45.8)
Biochar (20 t ha^{-1})	31 (47.6)	33 (57.1)	34 (47.8)	36 (44.0)	41 (70.8)	42 (75.0)
Biochar (25 t ha^{-1})	35 (66.6)	38 (80.9)	45 (95.6)	49 (113.0)	49 (104.1)	50 (108.3)
CD (P < 0.05)						
Biochar	0.56**		4.00**		4.18**	
Azospirillum	0.32**		NS		NS	
Biochar x *Azospirillum*	NS		NS		NS	

Values represent mean of three replications; Data in parantheses represent per cent increase over control; '- AZ204' - without *Azospirillium* inoculation, '+AZ204' - with *Azospirillum* inoculation; ** - significant at p<0.01, NS - not significant.

root length was observed and it ranges from 23 to 35 cm plant^{-1} without *Azospirillum* and 26 to 38 cm plant^{-1} with the inoculation of *Azospirillum*, recording 9.5 to 66.6 % and 23.8 to 80.9 % increase over control respectively at 30 days of plant growth. The similar trend was maintained upto 90 days. Maximum root length was recorded at 90 days with the inoculation of biochar and *Azospirillum* (50 cm plant^{-1}). The results were supported by Lehmann et al. (2002) who had found that, plant growth response was



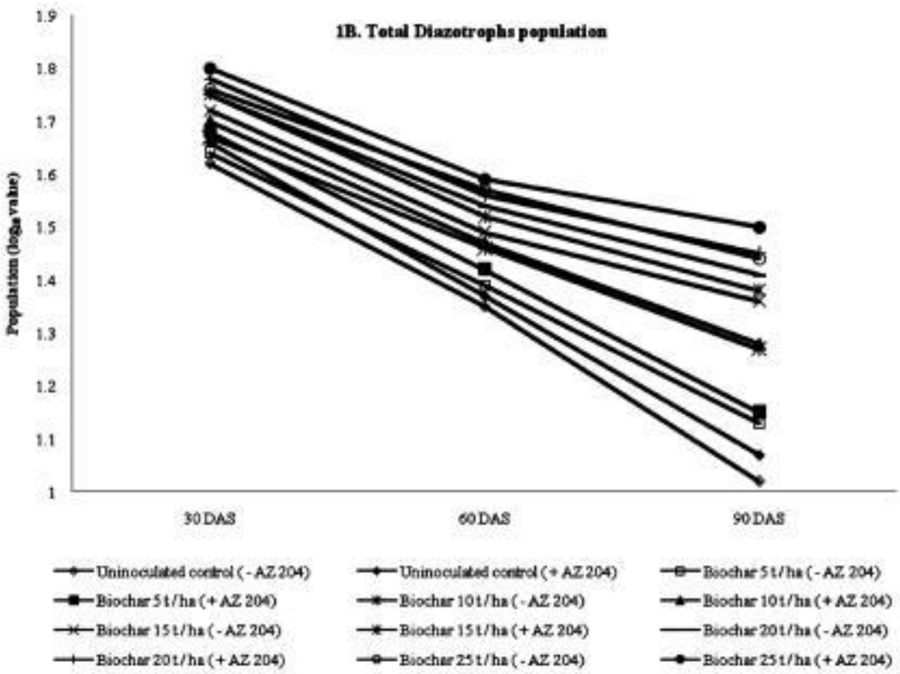


Fig. 1. Influence of Biochar on the *Azospirillum* and total diazotrophs populations in the rhizosphere of maize

positive for rice, sorghum, beans (soyabean, common bean, cowpea, moongbean), banana and vegetables with increasing rates of biochar application upto 61.4 t ha^{-1}.

Rhizosphere microbial population

Pertaining to the results presented in Fig. 1A, it was evident that application of biochar either alone or with *Azospirillum* inoculation significantly influenced the rhizosphere population of *Azospirillum*. At each level of biochar application, especially at higher levels of 25 t ha^{-1}, the increase in native population of Azospirillum was observed and it ranges from 1.27 $\log_{10}$ cfu g^{-1} of soil to 1.43 $\log_{10}$ cfu g^{-1}of soil without *Azospirillum* and 1.5 $\log_{10}$ cfu g^{-1} of soil to 1.77 $\log_{10}$ cfu g^{-1} of soil with the inoculation of *Azospirillum* at 30 days of plant growth. Thereafter an increase was noticed upto 60 days followed by a decline during 90 days of sowing. Maximum population of *Azospirillum* was recorded at 60 days with the inoculation of biochar (at 25 t ha^{-1}) alone which was 1.49 $\log_{10}$ cfu g^{-1} of soil and it was further increased to 1.85 $\log_{10}$ cfu g^{-1} of soil when it was combined with the application of *Azospirillum.* Biochar application stimulated the native population of *Azospirillum* to the tune of 11.2 to 17.3 % over control throughout the period of study. Application of biochar registered increase in total diazotrophs with the maximum of 1.76 $\log_{10}$ cfu g^{-1} of soil at 30 days after sowing (Fig 1B). When *Azospirillum* was amended along with biochar, the rhizosphere diazotrophs population was further enhanced to 1.8 $\log_{10}$ cfu g^{-1} of soil. Thereafter a decline was registered both at 60 and 90 days after planting. Though the population of diazotrophs was declined

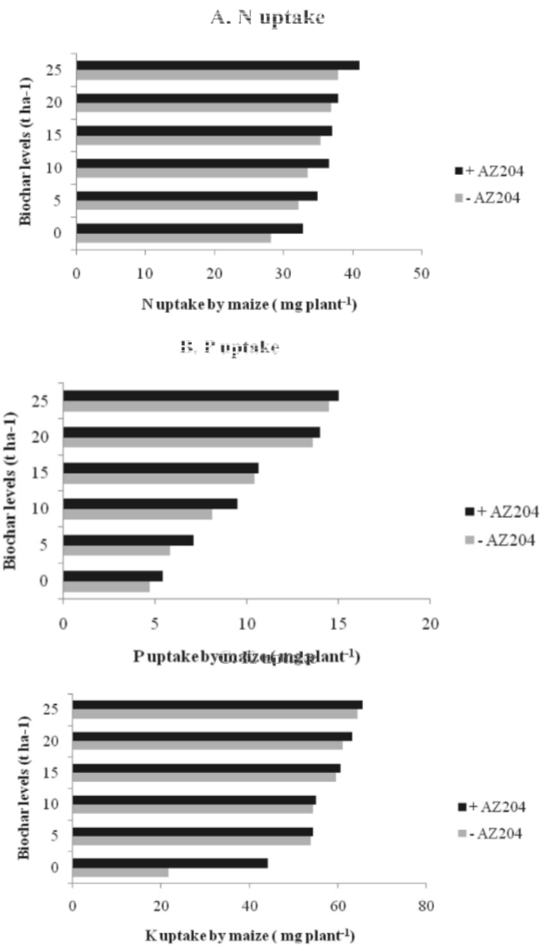


Fig 2. Influence of Biochar and *Azospirillum* application on nutrient uptake in maize (cultivar PEHM5)

after 30 days, the magnitude of response of biochar towards diazotrophic population was enhanced. At 30 days, enhancement in population was 8 % over control and it was enhanced to 38.6 % over control at 90 days. Applicaton of biochar along with *Azospirillum* recorded the population increase of 12.3 to 42.6 % over control from 30 to 90 days of plant growth. This shows the significant influence of biochar on total diazotrophs population at all the three stages of plant growth. There were no significant differences registered with respect to the interaction effect of biochar and *Azospirillum* at all stages of the crop growth.

These results are in accordance with findings of Zackrisson *et al.* (1996) and Steiner et al. (2004). Zackrisson *et al.* (1996) found that biochar provided a suitable habitat for a large and diverse group of soil microorganisms due to a high surface area in forest soils. Moreover Steiner *et al*. (2004) confirmed that increased rates of biochar especially 62 and 93 t ha^{-1} to a highly weathered soil not only enhanced microbial populations and activity in soil up to 45 %, but also favoured the plant-microbe interactions through their effects on nutrient availability and modification of habitat. Increased rates of biochar application to soil were able to increase the net microbial population and nutrient retention up to 50-72 % in the soil, thereby resulting in increased crop yield. Enhanced microbial population size, as well as population composition noticed in this experiment by higher levels of biochar application of 25 t ha^{-1} with *Azospirillum* amendments might be due to the reason that, biochar itself serves as a source of reduced carbon compounds, nutrients and as a refugee for any biochar colonizing soil bacteria, including total diazotrophs and *Azospirillum*. Some indications exist from soils that are rich in biochar that microbial community composition, species richness and diversity change with greater biochar concentration in soil as supported by Lehmann *et al*. (2003), whose finding depicts that even small amount (7.9 t ha^{-1}) of biochar in Anthrosol of the Amazon basin significantly enhanced microbial growth rates and biomass when nutrients were supplied by fertilizer.

Mycorrhizal responses to biochar in soil

The results presented in Table 4 tend to indicate a sort of positive influence of biochar on native AM root colonization of maize at all the stages of crop growth. Application of biochar at varying levels had a significant influence on root colonization at all three stages of plant growth. Biochar application recorded root colonization of 21 to 35 % at 30 days after planting. When *Azospirillum* was applied along with biochar the root colonization of maize by native AM fungi was still enhanced upto 37 %. The similar trend was maintained upto 90 days. The interaction effect was not observed significant. At 90 days, the response of biochar both with and without *Azospirillum* reached its peak of 59 and 60 % respectively at higher levels of biochar (25 t ha^{-1}) application. The response of biochar on the native AM fungi is in conformation with the latest report of Harvey *et al.* (1976) and Ishii and Kadoya (1994). It was found that AM fungi responded more positively to biochar additions than to additions of other types of organic material. Xie *et al.* (1995) and Cohn et al. (1998) reported that biochar increased the population size of Rhizobium due to the production of certain metabolites that induce flavanoid production in nearby plants (legumes) that ultimately increased root colonization of plant roots by AM fungi. In addition to chemical signals, biochar may also adsorb compounds toxic to microbial growth (Wallstedt *et al.,* 2002) especially for mycorrhizal fungi, which might had aided in elevated mycorrhizal responses to biochar at the level of 25 t ha^{-1} in soil with *Azospirillum* inoculation.

Table 4. Influence of Biochar and *Azospirillum* on root colonization of maize (cultivar PEHM5) by native AM fungi

Biochar levels	AM Root Colonization (%)					
	30 DAS		60 DAS		90 DAS	
	- AZ 204	+AZ 204	- AZ 204	+AZ 204	- AZ 204	+AZ 204
Uninoculated control	18	20	32	35	39	41
Biochar (5 t ha^{-1})	21	25	37	41	43	45
Biochar (10 t ha^{-1})	23	29	41	44	47	48
Biochar (15 t ha^{-1})	24	32	43	48	49	50
Biochar (20 t ha^{-1})	28	34	46	50	52	54
Biochar (25 t ha^{-1})	35	37	48	50	59	60
CD (P < 0.05)						
Biochar	5.31**		3.46**		3.97**	
Azospirillum	3.07*		NS		NS	
Biochar x *Azospirillum*	NS		NS		NS	

Values represent mean of three replications; '- AZ204' - without *Azospirillium* inoculation, '+AZ204' - with *Azospirillum* inoculation; ** - significant at p<0.01, * - significant at p<0.05, NS - not significant.

Nutrient uptake

With reference to the uptake of nutrients, biochar application had exhibited significant influence on uptake of macronutrients, and it was further improved by the inoculation of *Azospirillum* (Fig 2). Combination of 25 t ha^{-1} of biochar and *Azospirillum* registered 1to 2 fold increase in N uptake (Fig 2A), 2 to 4 fold increase in P uptake (Fig 2B) and 3 fold increase in K uptake of maize over a period of 120 days after planting (Fig. 2C). It is witnessed that addition of biochar (25 t ha^{-1}) and *Azospirillum* increased the crop yield due to enhanced uptake of nutrients through higher availability of macro nutrients, improved soil conditions and microbial activity. The results are in agreement with the findings of Steiner *et al.* (2007) and Steiner *et al.* (2007) reported a cumulative yield increase of rice and sorghum on a Brazilian Oxisol of approximately 75% after three repeated biochar applications of 7 t ha^{-1} over two years. They also reported a progressive increase in beneficial effects of biochar over time like increased NPK availability in soil. Chan *et al.* (2007) found that

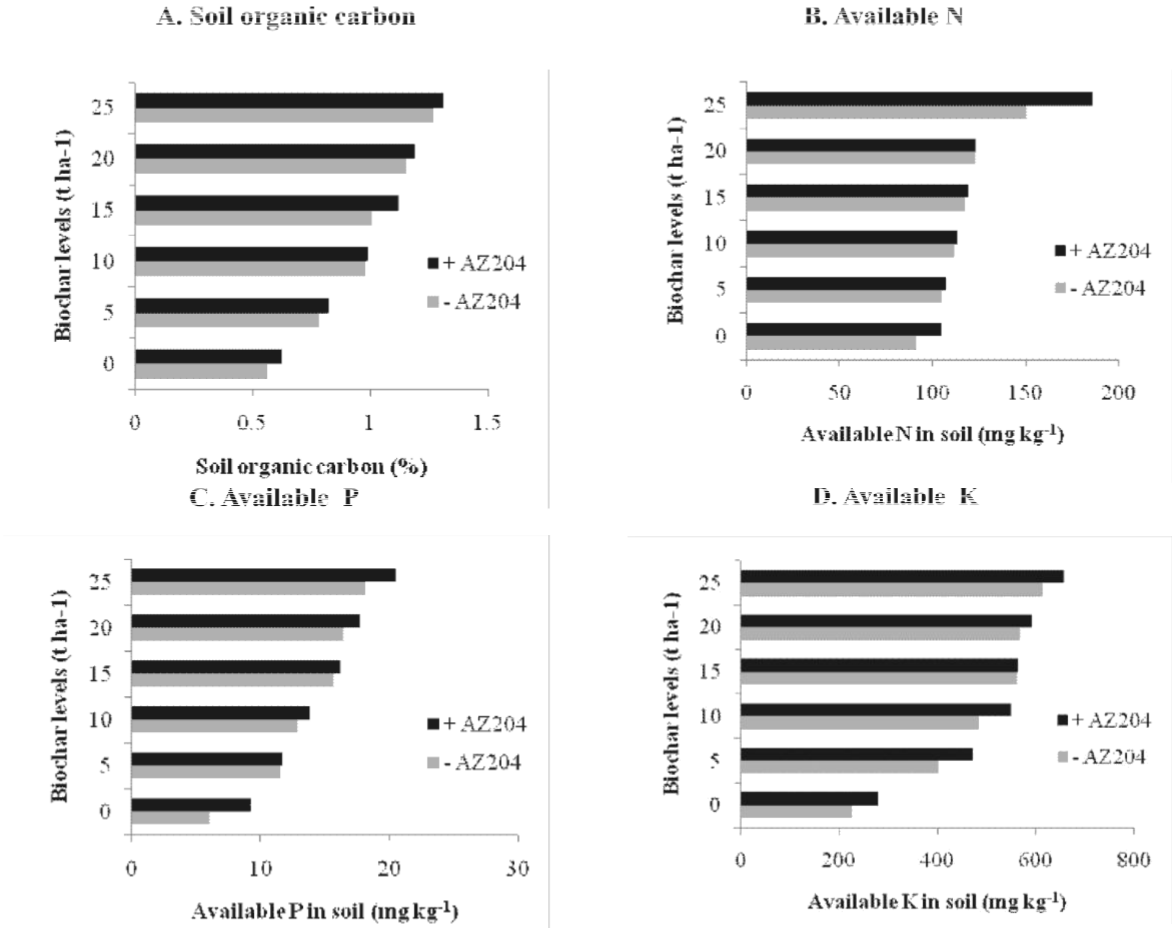


Fig 3. Influence of Biochar and *Azospirillum* application on soil nutrient status

100 t ha^{-1} of biochar application increased the yield of radish to 266% over control in Alfisol. Also, biochar rate of 20 t ha^{-1} recorded an increase in maize yield of about 28.1, 30.2 and 140 % during 1^{st}, 2^{nd} and 4^{th} year respectively, when applied consecutively with inorganic fertilizers (Chan *et al.*, 2007).

Soil nutrient status

Biochar application resulted in significantly greater available nutrients in the soil (Fig. 3A-D). Biochar application recorded soil organic carbon (SOC) content of 0.56 to 1.27 %. When *Azospirillum* was applied along with biochar, the SOC in soil reached a pinnacle of 1.31 %, i.e. nearly 133.92 % increase over control. Maximum available NPK in soil recorded at 120 days after crop growth with the addition of biochar alone was 151, 18.1 and 614 mg kg^{-1} soil respectively and it was further increased to 186, 20.5 and 658.5 mg kg^{-1} soil respectively with the application of *Azospirillum*. Increase in available N, P, K in soil was 65.9, 199.1 and 168.1% over control. Application of biochar along with *Azospirillum* recorded the available nutrient increase of 104.3, 238.8 and 187.5 % N, P, K over control at 120 days of plant growth. The results are in conformity with the findings of Lehmann et al. (2002) who reported that biochar addition resulted in higher C, P, K and Zn availability, and to a lesser extent Ca and Cu along with greater stabilization of organic matter, concurrent slower nutrient release from added organic matter and better retention of cations due to greater cation exchange capacity which results in immediate beneficial effects of biochar additions for soil nutrient availability resulting in plant nutrient uptake, increased crop growth and yield attributes. These results suggests two scenarios pertaining to the enhanced crop growth response with the application of biochar at 25 t ha^{-1} either alone or with *Azospirillum* inoculation; one is enriched availability of nutrients especially carbon and potassium in the soil by higher level of biochar application. The other is enhanced nitrogen in the soil by *Azospirillum* inoculation which would have resulted in higher absorption of nutrients with developing healthy root system and better production of photosynthates which consequently resulted in vigorous vegetative growth leading to higher crop yield.

Conclusion

The present experimental results point to an exciting fact that biochar act as a soil conditioner and habitat for soil microorganisms. Moreover, biochar application with *Azospirillum* inoculation can enhance the soil microbial activity and fertility that ultimately favours the maximized crop growth response.

Acknowledgement

Authors are grateful to Incrops Enterprise Hub, University of East Anglia, UK for their financial aid and Dr. P. Venkatachalam, Professor and Head, Department of Bioenergy, TNAU, Coimbatore for providing biochar.

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Received: April 4, 2011; Accepted: June 10, 2011