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Effect of fertilizers, biochar and humic acid on soil enzymes at different stages of maize growth

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Abstract
A field study was carried out at college farm, College of Agriculture, Rajendranagar, Hyderabad, Andhra Pradesh during *Kharif* 2013 to find out the influence of humic acid and biochar on soil enzymes of maize rhizosphere at different stages. The experiment was laid out in a Randomized Block Design and replicated thrice with three factors comprised of factor-I (fertilizers- 100 % RDF and 75 % RDF), Factor-II (biochar levels- 0, 5 and 7.5 t ha^{-1}) and Factor-III (humic acid levels of 0 and 30 kg ha^{-1}). Dehydrogenase activity at 30 DAS was significantly higher the activity being 16.19 µg TPF g^{-1} soil day^{-1} with 75% NPK in combination with humic acid. While, at 60 DAS and harvest, significantly higher activity was noticed with biochar @ 5 t ha^{-1} along with 100 % NPK and 75% NPK respectively with the corresponding activities of 20.72 and 5.594 µg TPF g^{-1} soil day^{-1}. Combined application of 75% NPK, 7.5 t ha^{-1} of biochar and 30 kg ha^{-1} of humic acid could result in a significantly higher acid phosphatase which was on par with recommended NPK along with biochar @ 7.5 t ha^{-1} at all the stages of assay. Alkaline phosphatase activity highest at 30 and 60 DAS (48.93 and 158.6 µg *p*-nitro phenol g^{-1} soil h^{-1}) with 100 % NPK along with biochar @ 7.5 t ha^{-1}, while at harvest stage higher activity (37.31 µg *p*-nitro phenol g^{-1} soil h^{-1}) was shown by 75% NPK alone.

Keywords: biochar, humic acid, enzymes, maize, rhizosphere

Introduction
Maize (*Zea mays* L.) is an important food and feed crop which ranks third after wheat and rice in the world. It is a multipurpose crop that provides food for humans, feed for animals (especially poultry and livestock) and raw material for the industries. India is the fifth largest producer of maize in the world contributing 3 per cent of the global production (Arif *et al*., 2012) [2]. This crop has much higher grain protein content than our staple food rice. Current concerns about global food security combined with the need to develop more sustainable agricultural systems and reduced greenhouse gas emissions necessitate many changes in agricultural management. Central to this tenet is the need for replenished soil organic matter reserves to sustain nutrient cycling; and improved WUE that help to mitigate climate change. Since excessive application of chemical fertilizers may affect soil health and sustainable productivity, it's imperative to search for possible alternate organic source that can sustain soil health and crop production (Jones *et al*., 2012) [5].

The application of biochar to agricultural land is receiving increasing attention as an intervention strategy for the sequestration of carbon and as a means of improving soil quality and nutrient cycling thereby aiming at reduced fertilizer use (Richard *et al*., 2012) [7].

Climate change and global warming have worldwide adverse consequences. Biochar production and its use in agriculture can play a key role in climate change mitigation and help improve the quality and management of waste materials coming from agriculture and forestry. Biochar is a carbonaceous material obtained from thermal decomposition of residual biomass at relatively varying temperatures and under oxygen limited conditions (pyrolysis). Biochar is currently a subject of active research worldwide because it can constitute a viable option for sustainable agriculture due to its potential as a long term sink for carbon in soil and benefits for crops (Alburquerque *et al*., 2013) [1]. Studies suggest that biochar sequesters approximately 50% of the carbon available within the biomass feedstock being pyrolyzed (Kelsi Bracmort, 2010) [6]. Humic substances are major components of organic matter, have both direct and indirect effects on plant growth (Sangeetha *et al.*, 2006) [8].

Humic acid (HA) improves the physical chemical and biological properties of the soil and influences plant growth. Because of its molecular structure, it provides numerous benefits to crop production. This present investigation is planned to integrate biochar with humic acid to evaluate its efficacy as a fertility amendment at varied fertiliser levels to check the enzyme activity.

Materials and Methods

This experiment was conducted during *kharif*, 2013 at the College Farm, Acharya N.G Ranga Agricultural University, Rajendranagar, Hyderabad situated at 17^{o}19$'$ N latitude, 78^{o}23$'$ E longitude and at an altitude of 542.6 m above mean sea level falls under the Southern Telangana agro-climatic zone of Andhra Pradesh. Dehydrogenase activity in the soil was determined by the procedure given by Cassida *et al.* (1964) [3]. The method involves spectrophotometric determination of the Tri phenyl formazon (TPF) produced when 1 g of soil was incubated with 50 mg calcium carbonate, 2.5 ml of distilled water and 1 ml of 3% 2, 3, 5- tri phenyl tetrazolium chloride (TTC) for 24 hours at room temperature. Acid and alkaline phosphatase activities in the soil were determined by the procedures as described by Tabatabai and Bremner (1969) [10] and Eivazi and Tabatabai (1977) [4]. The method involves spectrophotometric determination of the *p*-nitro phenol released when 1 g of soil incubated for 1 hr at 30^o C with 0.2 mL toluene, 4 mL of universal buffer (of pH 6.5 for assay of acid phosphatase activity and pH 11.0 for assay of alkaline phosphatase activity) and 1 mL of 0.25 M *p*-nitro phenyl phosphate prepared in the respective buffers.

Results and Discussion

Dehydrogenase activity

Fertiliser and humic acid levels interacted positively and significantly at 30 DAS. Dehydrogenase activity was significantly higher the mean activity being 16.19 µg TPF g^{-1} soil day^{-1} with 75% NPK in combination with humic acid. The reduced level of 75 percent NPK at any level of biochar and humic acid resulted in a significantly higher dehydrogenase activity as compared to recommended NPK. At this level of fertilisers, the integration with humic acid did not show any significant effect at 0 and 5 t ha^{-1} of biochar levels, while at 7.5 t ha^{-1} of biochar, there was a significant difference the values being 8.57 and 14.55 µg TPF g^{-1} soil day^{-1}. While at recommended level of NPK, significant influence of humic acid was derived at lower levels of biochar application (Table 1).

While, at 60 DAS significantly higher activity was noticed with biochar @ 5 t ha^{-1} along with 100 % NPK of 20.72 µg TPF g^{-1} soil day^{-1} against to recommended NPK alone (8.69 µg TPF g^{-1} soil day^{-1}). The interaction of fertilisers with biochar and humic acid resulted in significant increase in dehydrogenase activity. At both the levels of NPK, significantly higher dehydrogenase activity was shown by the treatment receiving biochar at 5 t ha^{-1} the corresponding activities being 20.72 and 19.06 µg TPF g^{-1} soil day^{-1} (Table 2). At harvest, significantly higher activity was noticed with biochar @ 5 t ha^{-1} along with 75% NPK with the activity of 5.594 µg TPF g^{-1} soil day^{-1} as against treatment receiving recommended NPK along with biochar @ 5 t ha^{-1} and humic acid@ 30 kg ha^{-1}. Similar results were obtained by Sellamuthu *et al.* (2003) [9]. The interaction of fertilisers with biochar and humic acid resulted in significant differences in dehydrogenase activity. Integration with humic acid resulted in a decreased enzyme activity at all combinations of fertilisers and biochar. Biochar at 5 t ha^{-1} was significantly superior without humic acid with the activities being 5.42 and 5.59 µg TPF g^{-1} soil day^{-1} respectively at 100 and 75% NPK levels (Table 3).

Table 1: Dehydrogenase activity (µg TPF g^{-1} soil day^{-1}) in the soil at 30 DAS of maize as influenced by fertiliser, biochar and humic acid levels and their interaction

Treatments	BC @ 0 t ha^{-1}			BC @ 5. 0 t ha^{-1}			BC @ 7.5 t ha^{-1}			Fertiliser Mean
Fertiliser levels	HA_1	HA_2	Mean	HA_1	HA_2	Mean	HA_1	HA_2	Mean	
100% NPK	10.62	13.57	12.10	11.21	15.42	13.32	11.66	12.18	11.92	12.44
75% NPK	16.02	16.36	16.19	11.87	13.17	12.52	8.57	14.55	11.56	13.42
Mean	13.32	14.96	14.15	11.54	14.29	12.92	10.11	13.36	11.74	12.93
CV (%)	11.93									
CD at 5% level	Fert. = NS Biochar = NS Humic acid = NS				Fert. x biochar = NS Fert. x humic acid = 1.41 Biochar x humic acid = 1.71 Fert. x biochar x humic x acid = 2.44					

Table 2: Dehydrogenase activity (µg TPF g^{-1} soil day^{-1}) in the soil at 60 DAS of maize as influenced by fertiliser, biochar and humic acid levels and their interaction

Treatments	BC @ 0 t ha^{-1}			BC @ 5. 0 t ha^{-1}			BC @ 7.5 t ha^{-1}			Fertiliser Mean
Fertiliser levels	HA_1	HA_2	Mean	HA_1	HA_2	Mean	HA_1	HA_2	Mean	
100% NPK	8.69	10.08	9.39	20.72	10.77	15.75	15.80	9.38	12.59	12.58
75% NPK	16.75	12.54	14.65	19.06	10.14	14.60	12.78	11.8	12.29	13.85
Mean	12.72	11.31	12.02	19.89	10.455	15.17	14.29	10.59	12.44	13.21
CV (%)	7.26									
CD at 5% level	Fert. = 0.66 Biochar = 0.81 Humic acid = 0.66					Fert. x biochar = 1.14 Fert. x humic acid = N.S Biochar x humic acid = 1.14 Fert. x biochar x humic x acid = 1.62				

Table 3: Dehydrogenase activity (µg TPF g^{-1} soil day^{-1}) in the soil at harvest of maize as influenced by fertiliser, biochar and humic acid levels and their interaction

Treatments	BC @ 0 t ha^{-1}			BC @ 5. 0 t ha^{-1}			BC @ 7.5 t ha^{-1}			Fertiliser Mean
Fertiliser levels	HA_1	HA_2	Mean	HA_1	HA_2	Mean	HA_1	HA_2	Mean	
100% NPK	4.495	3.75	4.12	5.42	3.50	4.46	4.62	3.72	4.17	4.25
75% NPK	5.22	3.71	4.47	5.59	3.65	4.63	4.53	3.75	4.15	4.41

Mean	4.85	3.73	4.29	5.50	3.58	4.54	4.58	3.73	4.16	4.33
CV (%)	3.28									
CD at 5% level	Fert. = 0.09 Biochar = 0.12 Humic acid = 0.09				Fert. x biochar = 0.17 Fert. x humic acid = 0.13 Biochar x humic acid = 0.17 Fert. x biochar x humic x acid = 0.24					

Acid phosphatase activity

The interaction of fertilisers with biochar and humic acid was found significant. Recommended NPK, when applied along with biochar @ 5 t ha^{-1} significantly higher acid phosphatase activity of 53.42 µg *p*-nitro phenol g^{-1} soil h^{-1}. However, the influence of the interaction was ambiguous and not specific at 30 DAS (Table 4). At 60 DAS the interaction of fertilisers with biochar and humic acid was found significant. Reduced NPK, when applied along with biochar @ 5 t ha^{-1} significantly gave lower acid phosphatase activity of 83.1 µg *p*-nitro phenol g^{-1} soil h^{-1} (Table 5). Combined application of 75% NPK, 7.5 t ha^{-1} of biochar and 30 kg ha^{-1} of humic acid could result in a significantly higher acid phosphatase assay of 148.5 µg *p*-nitro phenol g^{-1} soil h^{-1} which was on par with recommended NPK along with biochar @ 7.5 t ha^{-1}. The interaction between fertilisers, biochar and humic acid was found significant at harvest. Significantly lower acid phosphatase activity of 25.6 µg *p*-nitro phenol g^{-1} soil $h^{-1}$was obtained with recommended NPK alone. When biochar was not supplemented, irrespective of humic acid addition the enzyme activity in both fertiliser levels was at a par. At 7.5 t ha^{-1} level of biochar, the enzyme activity was significantly higher without humic acid for 100% NPK and with humic acid for 75% NPK the activities being 35.9 and 35.1 µg *p*-nitro phenol g^{-1} soil h^{-1} respectively (Table 6).

Table 4: Acid phosphatase activity (µg p-nitro phenol g^{-1} soil h^{-1}) in the soil at 30 DAS of maize as influenced by fertiliser, biochar and humic acid levels and their interaction

Treatments	**BC @ 0 t ha^{-1}**			**BC @ 5. 0 t ha^{-1}**			**BC @ 7.5 t ha^{-1}**			**Fertiliser Mean**
Fertiliser levels	HA_1	HA_2	Mean	HA_1	HA_2	Mean	HA_1	HA_2	Mean	
100% NPK	44.39	49.64	47.02	53.42	43.32	48.37	50.40	48.49	49.45	48.28
75% NPK	51.50	51.03	51.27	47.31	51.01	49.16	46.15	50.87	48.51	49.65
Mean	47.94	50.33	49.14	50.36	47.16	48.77	48.27	49.68	48.98	48.96
CV (%)	1.73									
CD at 5% level	Fert. = 0.58 Biochar = N.S. Humic acid = 0.58				Fert. x biochar = N.S Fert. x humic acid = 0.83 Biochar x humic acid = 1.020 Fert. x biochar x humic x acid = 1.44					

Table 5: Acid phosphatase activity (µg p-nitro phenol g^{-1} soil h^{-1}) in the soil at 60 DAS of maize as influenced by fertiliser, biochar and humic acid levels and their interaction

Treatments	**BC @ 0 t ha^{-1}**			**BC @ 5. 0 t ha^{-1}**			**BC @ 7.5 t ha^{-1}**			**Fertiliser Mean**
Fertiliser levels	HA_1	HA_2	Mean	HA_1	HA_2	Mean	HA_1	HA_2	Mean	
100% NPK	94.1	110.2	102.1	130.7	107.3	119.0	140.0	125.9	132.9	118.0
75% NPK	115.2	127.0	121.1	83.1	130.0	106.5	99.3	148.5	123.9	117.1
Mean	104.6	118.6	111.6	106.9	118.6	112.7	119.6	137.2	128.4	117.6
CV (%)	7.68									
CD at 5% level	Fert. = N.S Biochar =7.65 Humic acid = 6.25				Fert. x biochar = 10.82 Fert. x humic acid = 8.84 Biochar x humic acid = N.S Fert. x biochar x humic x acid =15.31					

Table 6: Acid phosphatase activity (µg p-nitro phenol g^{-1} soil h^{-1}) in the soil at harvest of maize as influenced by fertiliser, biochar and humic acid levels and their interaction

Treatments	**BC @ 0 t ha^{-1}**			**BC @ 5. 0 t ha^{-1}**			**BC @ 7.5 t ha^{-1}**			**Fertiliser Mean**
Fertiliser levels	HA_1	HA_2	Mean	HA_1	HA_2	Mean	HA_1	HA_2	Mean	
100% NPK	25.6	29.4	27.5	29.1	30.3	29.7	35.9	30.1	33.0	30.1
75% NPK	26.5	32.9	29.7	27.4	33.2	30.3	31.6	35.1	33.4	31.1
Mean	26.1	31.1	28.6	28.2	31.8	30.0	33.8	32.6	33.2	30.6
CV (%)	4.04									
CD at 5% level	Fert. = 0.85 Biochar =1.04 Humic acid = N.S			Fert. x biochar =1.48 Fert. x humic acid =1.21 Biochar x humic acid =1.48 Fert. x biochar x humic x acid =2.09						

Alkaline phosphatase assay

The interaction of fertilisers with biochar and humic acid was found significant. Significantly higher alkaline phosphatase activity was facilitated with recommended NPK along with biochar @ 7.5 t ha^{-1}, while lower value with 75 percent NPK along with biochar @ 5 t ha^{-1} the corresponding values being 48.93 and 41.47 µg *p*-nitro phenol g^{-1} soil h^{-1}. The interaction of fertilisers with biochar and humic acid was found significant (Table 7). Recommended NPK along with humic acid @ 30 kg ha^{-1} significantly gave higher alkaline phosphatase activity of 160.9 µg *p*-nitro phenol g^{-1} soil h^{-1}. These results were on par with treatments receiving recommended NPK along with biochar @ 7.5 t ha^{-1}. Significantly lower alkaline phosphatase assay of 144.0 µg *p*-

nitro phenol g^{-1} soil h^{-1}was observed in treatments receiving 75 percent NPK along with humic acid @ 30 kg ha^{-1}. The interaction of fertilisers with biochar and humic acid was found significant (Table 8). 75 percent NPK alone significantly gave higher alkaline phosphatase assay of 37.31 µg *p*-nitro phenol g^{-1} soil h^{-1}. These results were on par with treatments receiving 75 percent NPK along with biochar @ 7.5 t ha^{-1}. Significantly lower alkaline phosphatase assay of 25.21 µg *p*-nitro phenol g^{-1} soil h^{-1} was observed in treatments receiving recommended NPK alone (Table 9).

Table 7: Alkaline phosphatase activity (µg p-nitro phenol g^{-1} soil h^{-1}) in the soil at 30 DAS of maize as influenced by fertiliser, biochar and humic acid levels and their interaction

Treatments	**BC @ 0 t ha^{-1}**			**BC @ 5. 0 t ha^{-1}**			**BC @ 7.5 t ha^{-1}**			**Fertiliser Mean**
Fertiliser levels	HA_1	HA_2	Mean	HA_1	HA_2	Mean	HA_1	HA_2	Mean	
100% NPK	45.02	42.06	43.54	46.59	44.38	45.49	48.93	45.33	47.13	45.39
75% NPK	46.30	42.08	44.19	41.47	41.80	41.64	42.30	43.82	43.06	42.96
Mean	45.66	42.07	43.87	44.03	43.09	43.56	45.61	44.57	45.09	44.17
CV (%)	2.02									
CD at 5% level	Fert. = 0.62 Biochar = 0.75 Humic acid = 0.62				Fert. x biochar = 1.07 Fert. x humic acid = 0.87 Biochar x humic acid = 1.07 Fert. x biochar x humic x acid = 1.518					

Table 8: Alkaline phosphatase activity (µg p-nitro phenol g^{-1} soil h^{-1}) in the soil at 60 DAS of maize as influenced by fertiliser, biochar and humic acid levels and their interaction

Treatments	**BC @ 0 t ha^{-1}**			**BC @ 5. 0 t ha^{-1}**			**BC @ 7.5 t ha^{-1}**			**Fertiliser Mean**
Fertiliser levels	HA_1	HA_2	Mean	HA_1	HA_2	Mean	HA_1	HA_2	Mean	
100% NPK	154.5	160.9	157.7	156.5	144.2	150.3	158.6	150.9	154.7	154.2
75% NPK	158.1	144.9	151.5	156.9	146.5	151.7	157.3	144.0	150.6	151.2
Mean	156.3	152.9	154.6	156.7	145.3	151.0	157.9	147.4	152.7	152.7
CV (%)	1.10									
CD at 5% level	Fert. =1.17 Biochar =1.43 Humic acid =1.17				Fert. x biochar = 2.02 Fert. x humic acid =1.65 Biochar x humic acid = 2.02 Fert. x biochar x humic x acid = 2.86					

Table 9: Alkaline phosphatase activity (µg p-nitro phenol g^{-1} soil h^{-1}) in the soil at harvest of maize as influenced by fertiliser, biochar and humic acid levels and their interaction

Treatments	**BC @ 0 t ha^{-1}**			**BC @ 5. 0 t ha^{-1}**			**BC @ 7.5 t ha^{-1}**			**Fertiliser Mean**
Fertiliser levels	HA_1	HA_2	Mean	HA_1	HA_2	Mean	HA_1	HA_2	Mean	
100% NPK	25.21	26.60	25.91	27.84	32.52	30.19	27.79	36.52	32.16	29.42
75% NPK	37.31	28.97	33.14	36.37	33.64	35.01	36.53	31.06	33.80	33.99
Mean	31.26	27.78	29.53	32.11	33.08	32.60	32.16	33.79	32.98	31.70
CV (%)	3.82									
CD at 5% level	Fert. = 0.83 Biochar =1.02 Humic acid = 0.83				Fert. x biochar = N.S Fert. x humic acid = 1.18 Biochar x humic acid = 1.45 Fert. x biochar x humic x acid = 2.05					

Conclusion

Biochar application at either 5 or 7.5 t ha^{-1} encouraged the dehydrogenase activity; the effect was more pronounced at 75% NPK level. Combined application of 75% NPK, 7.5 t ha^{-1} of biochar and 30 kg ha^{-1} of humic acid could result in a significantly higher phosphatase which was on par with recommended NPK along with biochar @ 7.5 t ha^{-1}at all the stages of assay.

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