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Effect of chemical fertilizers and microbial inoculations on soil properties in cassava (*Manihot esculenta*) growing Vertisols of Tamil Nadu

A C HRIDYA[1] and G BYJU[2]

Central Tuber Crops Research Institute, Sreekariyam, PO Box 695 017, Thiruvananthapuram, Kerala

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ABSTRACT

Cassava (*Manihot esculenta* Crantz) is an important subsidiary food and industrial raw material in the tropics. Considering the importance of the crop, an on farm experiment was conducted to study the effect of NPK fertilizer rate and biocontrol agents (*Trichoderma* and *Pseudomonas fluorescens*) and biofertilizers (*Azospirillum*, AM fungi and phosphorus solubilising bacteria) on soil chemical, biochemical and microbial biomass carbon in cassava growing Vertisols of Tamil Nadu during 2008 and 2009. The study was conducted in split plot design with two levels of NPK fertilizer as main plot treatments and eight microbial inoculations as subplot treatments. *Azospirillum* with *Trichoderma* (170.58 kg/ha), AM fungi with *Trichoderma* (57.85 kg/ha) and *Trichoderma* alone (473.70 kg/ha) significantly increased available nitrogen, phosphorus and exchangeable potassium by reducing the amount of NPK. *Pseudomonas fluorescens* with *Trichoderma* at 50 per cent recommended NPK rate increases the available iron in soil. The AM fungi with *Trichoderma* significantly increased available manganese and zinc compared to other inoculations at 50 per cent recommended NPK rate. *Azospirillum* with *Trichoderma* at the recommended NPK rate increased the urease enzyme activity (835.21 mg urea hydrolysed/g soil/h) compared to other treatments. The soil application of all cultures at 50 per cent recommended NPK rate increased soil dehydrogenase and β glucosidase enzyme activities. Interaction effect showed significantly higher microbial biomass carbon in AM fungi with *Trichoderma* at 50 per cent recommended NPK rate (3792.45 μg/g soil) and was on par with soil application of all cultures at 100 per cent and 50 per cent recommended rate. In general microbial inoculations at 50 per cent recommended rate gave on par or significantly higher results compared to uninoculated control at recommended NPK rate.

Key words: Available nutrients, Biocontrol agents, Biofertilizers, Microbial biomass carbon, Soil enzymes

In India, cassava (*Manihot esculenta* Crantz) is mainly grown in the southern part of Tamil Nadu, where it is grown mostly in irrigated Vertisols. The productivity of cassava in Tamil Nadu is 40 tonnes/ha on an average, which is the highest among the world averages. The application of mineral fertilizers is the most advantageous and the fastest way to increase crop yields. The reduced use of synthetic agrochemicals, to maintain soil fertility by alternative means is the subject of investigation. Microbial studies indicated that combination of microorganisms interact with each other synergistically, providing nutrients, removing inhibitory products and stimulating each other through physical or biochemical activities that may enhance some beneficial aspects of their physiology (Bashan 1998). The challenge is to continue sustainable agricultural crop production through minimization of harmful effects of fertilization (Duarah *et al.* 2011). The effect of NPK rate and biocontrol agents and biofertilizers on yield and nutrient uptake of cassava was reported (Hridya *et al.* 2012). The purpose of the experiment was to study the influence of NPK fertilizer rates, biocontrol agents and biofertilizers on soil properties such as chemical, biochemical and microbial biomass carbon in Vertisols under cassava.

[1]Research Scholar, [2]Principal Scientist (e mail: byju_g@yahoo.com), Division of Crop Production

MATERIALS AND METHODS

In order to study the effect of NPK rates and microbial inoculations on soil properties, on farm experiments were conducted at Panamarathupatty, Salem district, Tamil Nadu, India (11.39° N latitude; 78.12° E longitude; 180 m above mean sea level) during 2008 and 2009. The soil of the experimental location is classified as clayey, smectitic, hyperthermic, udic, haplusterts. The soil is alkaline with pH 8.31. The organic carbon is 3.7 g/kg. The available nitrogen, phosphorus and exchangeable potassium were 97.18, 12.88 and 337.79 kg/ha respectively. The cultivar H-226 released from Central Tuber Crops Research Institute was used for planting. The experimental field has been under continuous cassava cultivation for the past 10 years.

The experiment was conducted in split plot with three replications Main plots were two rates of NPK application, namely recommended (100: 50: 100 kg/ha N: P_2O_5: K_2O) and 50 per cent of the recommended rate (50: 25: 50 kg/ha N: P_2O_5: K_2O) (Nair *et al.* 2004). Fertilizers were applied as basal dressing within one week after planting and as top dressing at 45-60 days after planting. Fertilizers were thoroughly mixed and spread equally on the mounds. Sub plots were eight treatments of different combinations of biocontrol agents (*Trichoderma* and *Pseudomonas fluorescens*) and/or biofertilizers (*Azospirillum*, AM fungi and P solubilising bacteria) applied at the rate of 5 kg/ha as given in Table 1.

Soil samples were collected at the active growth stage of cassava which is at 3 months after planting. At the active growth stage cassava is most responsive to the added supplements. The soil samples were air dried and sieved through 2 mm sieve to analyse soil chemical properties such as pH (Byju 2001), organic carbon (Walkley and Black 1934), available nitrogen (Page *et al*. 1982), available phosphorus (Olsen *et al*. 1954), exchangeable potassium (Knudsen *et al.* 1982), calcium and magnesium and available micronutrients such as Fe, Mn, Zn, Cu and B (Byju 2001). Analysis of soil enzymes such as urease, β glucosidase and dehydrogenase were conducted in fresh soil samples. A modified assay for urease activity based on Broadbent *et al*. (1958) was used. Dehydrogenase activity (2,3,5-triphenyl tetrazolium chloride reductase activity) was determined according to Casida *et al*. (1964). β Glucosidase activity was determined according to the method suggested by Eivazi and Tabatabai (1988). Soil microbial biomass carbon (MBC) was estimated by the chloroform fumigation extraction method described by Vance *et al*. (1987).

The results of analysis were statistically analysed using SAS (2002). The LSD was used at the 0.05 level of probability to test difference between treatment means. Analysis of variance was performed on the different properties studied to determine the effects of NPK rates, biocontrol agents and/or biofertilizers and their interaction.

Table 1 Details of the sub plot treatments used in the on farm experiment

Treatment No.	Treatment details
1	Uninoculated control
2	*Trichoderma* soil application
3	*Pseudomonas fluorescens* with *Trichoderma* soil application
4	*Azospirillum* with *Trichoderma* soil application
5	AM fungi with *Trichoderma* soil application
6	P solubilising bacteria with *Trichoderma* soil application
7	Soil application of *Pseudomonas fluorescens*, *Azospirillum*, AM fungi and P solubilising bacteria and *Trichoderma*
8	Sett treatment *of Pseudomonas fluorescens*, *Azospirillum,* AM fungi, and P solubilising bacteria and soil application of *Trichoderma*

RESULTS AND DISCUSSION

Major and secondary nutrients

The mean effects of NPK rates and biocontrol agents and biofertilizers on major and secondary nutrients at 3 months after planting cassava are given in Table 2. The mean effects of NPK rates and biocontrol agents and biofertilizers were not significant on soil pH and organic carbon. The available nitrogen was significantly increased at the recommended NPK rate whereas available phosphorus and exchangeable potassium showed on par results at the two NPK rates. *Azospirillum* with *Trichoderma* increased the available nitrogen (170.58 kg/ha) in soil by 56.76 per cent over uninoculated (108.81 kg/ha) control. The AM

Table 2 Effects of NPK rates and biocontrol agents and biofertilizers on soil chemical characteristics at 3 months after planting (Mean of 2 years)

Treatment	pH	Organic carbon (%)	Available N	Available P	Exchangeable K	Available Ca	Available Mg
			kg/ha			mg 100/g	
NPK rate							
Recommended rate	8.47	0.42	149.71	47.60	438.45	261.03	255.95
50% recommended rate	8.45	0.39	137.23	44.27	415.16	247.58	240.35
LSD (P=0.05)*	NS	NS	11.81	4.10	35.41	NS	7.81
Biocontrol agents and biofertilizers							
Control	8.45	0.40	108.81	33.84	384.17	224.32	234.25
Trichoderma	8.57	0.41	136.34	40.53	473.70	256.88	256.08
Pseudomonas	8.50	0.37	140.74	52.53	411.23	251.34	257.56
Azospirillum	8.55	0.38	170.58	46.03	437.63	252.68	248.56
AM Fungi	8.41	0.39	150.04	57.85	428.11	281.54	251.33
PSB*	8.42	0.40	147.56	53.12	415.92	256.35	247.91
All - soil	8.37	0.43	152.59	43.82	448.61	258.46	247.49
All–sett treatment	8.50	0.42	141.94	41.10	414.37	255.48	242.01
LSD (P=0.05)*	NS	NS	12.27	0.80	13.41	13.22	10.69

*PSB – Phosphorus solubilising bacteria; *LSD (0.05) - Least significant difference at 0.05 probability level fungi with *Trichoderma* inoculation increased the available phosphorus (57.85 kg/ha) by 70.95 per cent over uninoculated control (33.84 kg/ha). *Trichoderma* inoculation increased exchangeable potassium (473.70 kg/ha) by 23.3 per cent over the uninoculated control (384.17 kg/ha). The AM fungi with *Trichoderma* significantly increased the available calcium (281.54 mg 100/g) than other treatments. The treatment was 25.50 per cent higher over uninoculated control (224.32 mg 100/g). The mean effects showed that biocontrol agents and biofertilizers significantly increased available magnesium over uninoculated control (234.25 mg 100/g). Available magnesium was highest in *Pseudomonas* with *Trichoderma* and was on par with other inoculations except sett application of all cultures.

The interaction effects of NPK rates and biocontrol agents and biofertilizers on major and secondary nutrients at 3 months after planting cassava are given in Fig 1. The interaction effects showed that microbial inoculations such as *Azospirillum* with *Trichoderma,* AM fungi with *Trichoderma* and *Trichoderma alone* significantly increased major nutrients such as available nitrogen, available phosphorus and exchangeable potassium respectively at 100% (recommended dose of fertilizer) RDF and was on par with same at the 50% RDF. The major nutrients in the microbial treated plots at 50 per cent recommended rate were significantly increased or were on par with uninoculated control at the recommended rate.

The interaction effects corroborated that AM fungi with *Trichoderma* significantly increased available calcium than other treatments at two NPK rates. Microbial inoculations at two NPK rates showed on par results on available calcium. Microbial inoculations at 50 per cent recommended NPK rate was on par with uninoculated control at recommended NPK rate whereas AM fungi with *Trichoderma* at the 50 per cent recommended rate gave significantly higher results compared to uninoculated control at the recommended NPK rate.

The interaction effects corroborated that, at the recommended NPK rate, highest available magnesium was observed in *Trichoderma* alone (268.27 mg 100/g) and was on par with *Pseudomonas fluorescens* with *Trichoderma*, AM fungi with *Trichoderma*, PSB with *Trichoderma* and soil application of all cultures (Fig 2). Least available magnesium was observed in uninoculated control at 50 per cent recommended NPK rate. Microbial inoculations except *Trichoderma* alone resulted in on par results at the two NPK rates. Microbial inoculations at 50 per cent recommended NPK rate was on par with uninoculated control at the recommended NPK rate and was significantly higher compared to uninoculated control at 50 per cent recommended NPK rate.

Azospirillum with *Trichoderma* enhanced the available nitrogen at 50 per cent recommended NPK rate. The stimulatory effect exerted by *Azospirillum* has been attributed to several mechanisms including biological nitrogen fixation (Okon and Itzigsohn 1995). The AM fungi with *Trichoderma* reduced the use of inorganic fertilizers by increasing the available phosphorus at 50 per cent

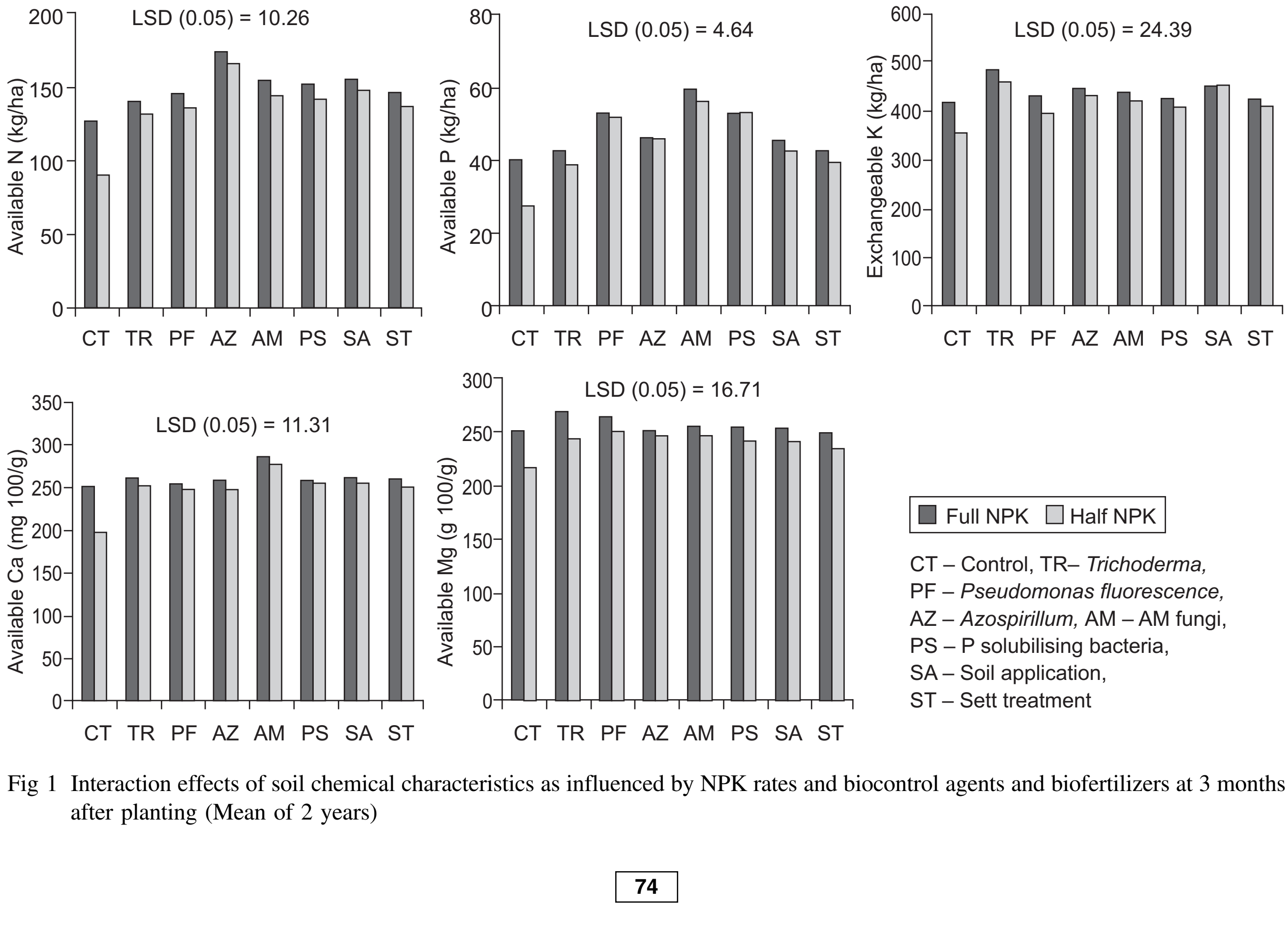

Fig 1 Interaction effects of soil chemical characteristics as influenced by NPK rates and biocontrol agents and biofertilizers at 3 months after planting (Mean of 2 years)

recommended NPK rate. Field inoculation with effective AM isolates may increase yields and decrease the fertilizer phosphorus requirements (Howeler and Sieverding 1983). *Trichoderma* alone at 50 per cent recommended NPK rate significantly increased exchangeable potassium because *Trichoderma* has the strong capacity to mobilize the soil nutrients including potassium (Benitez *et al*. 1998). The results showed that AM fungi with *Trichoderma* increased available calcium in soil at recommended NPK rate. The AM fungi effectively released cations like calcium that would otherwise bind to form complex with phosphates (Bradgett 2005).

Microbial inoculations at 50 per cent recommended rate is sufficient to increase major and secondary nutrients when compared to that at recommended NPK rate. The microbial inoculations proved to be beneficial by reducing the chemical fertilizer rate and increasing soil nutrients. Sundara *et al*. (2002) reported that PSB reduced the required phosphorus dosage by 25 per cent and Young *et al*. (2003) observed reduction in 50 per cent chemical by exploring the microbial potential to augment soil fertility.

Available micronutrients

The mean effects of NPK rates and biocontrol agents and biofertilizers on soil available micronutrients at 3 months after planting are given in Table 3. The statistical scrutiny of the data indicated that the mean effect of two NPK rates on available iron, manganese and boron was not significant. The mean effect of biocontrol agents and biofertilizers showed that *Pseudomonas fluorescens* with *Trichoderma* significantly increased the available iron (22.86 µg/g) by 38.04 per cent over uninoculated control (16.56 µg/g). The AM fungi with *Trichoderma* significantly increased available manganese and zinc compared to other inoculations. The treatment was 29.47 per cent higher over uninoculated control (10.11 µg/g). The treatment was 69.69 per cent higher over uninoculated control (1.03 µg/g). The soil application of all cultures (3.19 µg/g) significantly increased available copper and was 26.56 per cent higher over uninoculated (2.56 µg/g) and was on par with sett application of all cultures (3.14 µg/g). The *Trichoderma* alone (1.47 µg/g) significantly increased the available boron and was on par with *Pseudomonas* with *Trichoderma* (1.34 µg/g). Inoculated treatments significantly increased the available micronutrients in soil at 3 months after planting cassava over uninoculated control.

Table 3 Effects of NPK rates and biocontrol agents and biofertilizers on soil available micronutrients at 3 months after planting (Mean of 2 years)

Treatment	Available Fe	Available Mn	Available Zn	Available Cu	Available B
			µg/g		
NPK rate					
Recommended rate	21.59	12.84	1.67	2.96	1.05
50% recommended rate	19.31	11.19	1.33	2.81	0.93
LSD (P=0.05)*	NS	NS	0.11	0.18	NS
Biocontrol agents and biofertilizers					
Control	16.56	10.11	1.03	2.56	0.41
Trichoderma	21.61	11.39	1.54	3.02	1.47
Pseudomonas	22.86	11.58	1.45	2.95	1.34
Azospirillum	21.56	12.10	1.42	2.94	0.87
AM fungi	20.72	13.09	1.75	2.81	0.82
PSB*	20.07	12.12	1.48	2.89	0.79
All - soil	20.01	12.17	1.59	3.19	1.19
All–sett treatment	20.20	12.13	1.75	3.14	0.99
LSD (P=0.05)*	1.13	0.86	0.15	0.28	0.21

*PSB - Phosphorus solubilising bacteria

The interaction effects of NPK rates and biocontrol agents and biofertilizers showed that available iron in soil was significantly higher in the treatment *Pseudomonas* with *Trichoderma* and was on par with *Trichoderma* alone, *Azospirillum* with *Trichoderma* and AM fungi with *Trichoderma* at recommended NPK rate and *Pseudomonas* with *Trichoderma* at 50 per cent recommended NPK rate (Fig 2). The interaction effects showed that AM fungi with *Trichoderma* significantly increased the available manganese and zinc in soil. Available manganese and zinc at the two NPK rates was on par in the AM fungi with *Trichoderma* inoculated plots. The soil application of all cultures at the 50 per cent recommended NPK rate increased the available copper and was on par with that at the recommended NPK rate and *Trichoderma* alone at recommended NPK rate. *Trichoderma* alone significantly increased available boron at two NPK rates and was on par with *Pseudomonas fluorescens* with *Trichoderma* and soil application of all cultures at the recommended NPK rate. All microbial inoculations at the recommended NPK rate showed on par results with that at 50 per cent recommended NPK rate on available boron. All microbial inoculations at 50 per cent recommended NPK rate was significantly higher compared to uninoculated control at recommended NPK rate on available boron.

Pseudomonas fluorescens with *Trichoderma* effectively increased available iron in soil by reducing the amount of NPK fertilizer rate. The siderophores produced by *Pseudomonas fluorescens* helped plant growth by supplying iron through an iron-chelating function (Crowley *et al*. 1991). The AM fungi with *Trichoderma* increased available manganese and zinc in soil compared to other treatments. Similar results were observed by Nogueira *et al*. (2007) and Manjunath and Habte (1988). Soil application of all cultures significantly increased available copper, but *Trichoderma* alone showed similar results at recommended NPK rate. Reports showed that *Trichoderma* possess a range of different mechanisms to solubilise copper. It produces diffusible metabolites that solubilise copper (Altomare *et al*. 1999).

Results showed that microbial inoculations increased

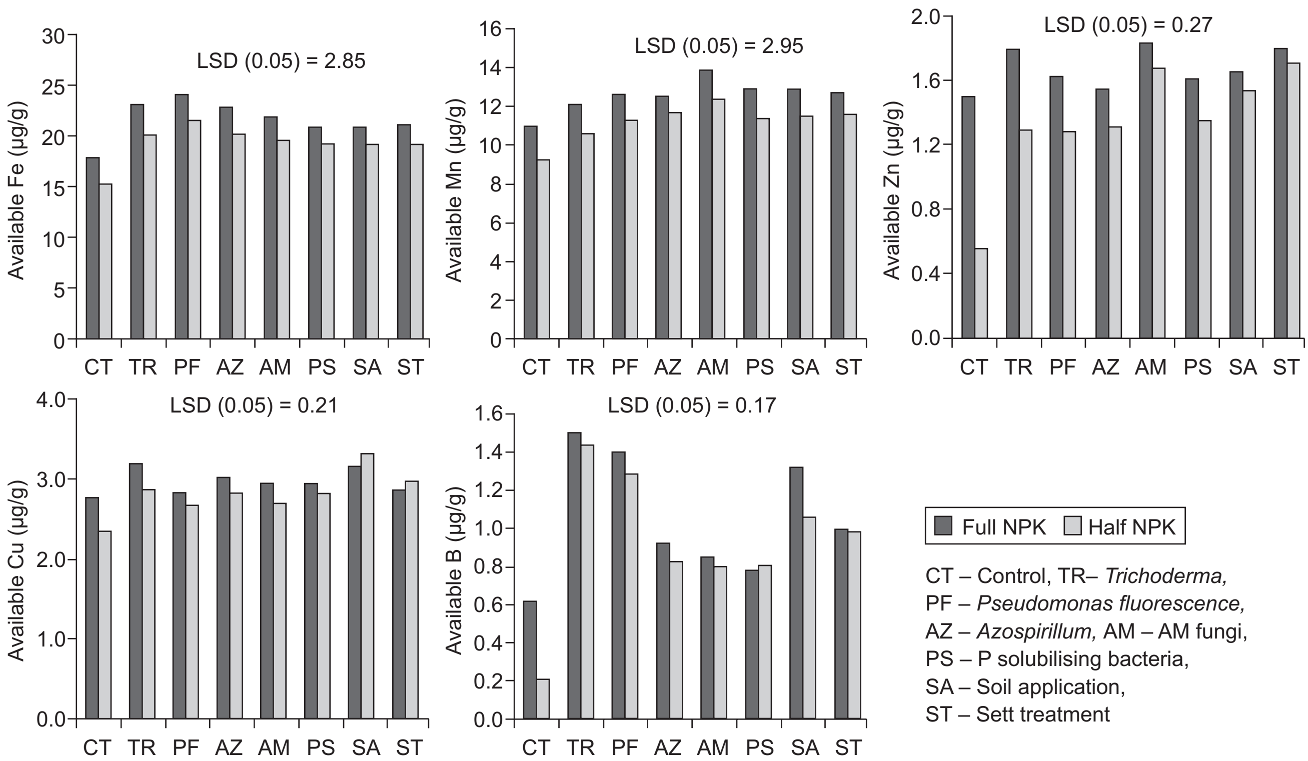

Fig 2 Interaction effects of soil available micronutrients as influenced by NPK rates and biocontrol agents and biofertilizers at 3 months after planting (Mean of 2 years)

available micronutrients at the 50 per cent recommended NPK rate over uninoculated control at the recommended rate. Report by Nogueira *et al.* (2007) showed that AM fungi at the lower phosphorus level significantly increased available iron and manganese compared to uninoculated control at the lower and higher phosphorus levels.

Soil enzyme activities and microbial biomass carbon

The mean effect of NPK rates and biocontrol agents and biofertilizers on soil enzyme activities and microbial biomass carbon at 3 months after planting are given in Table 4. The mean effect of NPK rates depicted any significant difference on the soil urease and dehydrogenase enzyme activity and microbial biomass carbon. The mean effect of biocontrol agents and biofertilizers showed that soil urease enzyme activity was significantly higher in the treatment *Azospirillum* with *Trichoderma* (823.85 mg urea hydrolysed/g soil/h). The treatment was 89.51 per cent higher over the uninoculated control (434.72 mg urea hydrolysed/g soil/h). Soil dehydrogenase enzyme activity was significantly increased by soil application of all cultures (91.45 mg TPF/g soil). The treatment was 116.04 per cent higher over the uninoculated control (42.33 mg TPF/g soil). Soil application of all cultures significantly increased the β glucosidase enzyme activity (55.20 mg PNP/g soil) by 73.80 per cent over uninoculated control (31.76 mg PNP/g soil). The soil application of all cultures (3716.00 mg/g soil) significantly increased microbial biomass carbon and was on par with AM fungi with *Trichoderma* (3665.70 mg/g soil). Microbial inoculations increased the soil enzyme activity and microbial biomass carbon over uninoculated control.

The interaction effects of NPK rates and biocontrol agents and biofertilizers showed that *Azospirillum* with *Trichoderma* at the recommended NPK rate increased the urease enzyme activity compared to other treatments. The dehydrogenase enzyme activity was significantly increased by soil application of all cultures at 50 per cent recommended NPK rate and was on par with soil application of all cultures at recommended NPK rate and AM fungi with *Trichoderma* at the 50 per cent recommended NPK rate. Soil application of all cultures increased the β glucosidase enzyme activity than other treatments at 50 per cent recommended NPK rate. At lower rates of NPK *Trichoderma* alone, *Pseudomonas fluorescens*, PSB with *Trichoderma* and soil and sett application of all cultures significantly increased the enzyme activity compared to higher NPK rates. Soil enzyme activities at the 50 per cent recommended NPK rate was significantly increased over uninoculated control at the recommended rate, reducing the amount of fertilizer to get better enzyme activities.

Interaction effect depicts significantly higher microbial biomass carbon was observed in AM fungi with *Trichoderma* at 50 per cent recommended NPK rate (3792.45 mg/g soil) and was on par with soil application of all cultures at the recommended rate and 50 per cent recommended rate. Microbial inoculations such as AM fungi with *Trichoderma* and PSB with *Trichoderma* resulted in significantly lower microbial biomass carbon at the recommended rate compared to 50 per recommended NPK rate, whereas

Table 4 Effects of NPK rates and biocontrol agents and biofertilizers on soil enzyme activities and microbial biomass carbon at 3 months after planting (Mean of 2 years)

Treatment	Urease (mg urea hydrolysed/ g soil/h)	Dehydro-genase (µg TPF*/ g soil)	β Glucosidase treatment (µg PNP*/ g soil)	MBC* (mg/g soil)
NPK rate				
Recommended rate	701.37	71.68	40.81	3106.02
50% recommended rate	692.76	73.86	45.4	3085.30
LSD (P=0.05)*	NS	NS	1.33	NS
Biocontrol agents and biofetilizers				
Control	434.72	42.33	31.76	2511.10
Trichoderma	713.94	72.49	33.79	2932.60
Pseudomonas	748.36	75.69	38.50	3122.80
Azospirillum	823.85	69.91	43.81	3065.80
AM Fungi	651.47	88.17	51.73	3665.70
PSB*	629.28	65.44	52.63	3136.00
All - soil	790.44	91.45	55.20	3716.00
All–sett treatment	784.85	76.68	49.48	2615.3
LSD (P=0.05)*	22.57	3.71	1.97	79.45

*PSB - Phosphorus solubilising bacteria; *MBC - microbial biomass carbon; TPF - Triphenyl formazan; PNG - p-nitrophenyl β-D glycopyranoside

Trichoderma alone gave significantly lower results at 50 per cent recommended NPK rate. All the inoculations at 50 per cent recommended NPK rate was significantly increased microbial biomass carbon compared to uninoculated control at the recommended NPK rate.

Azospirillum with *Trichoderma* increased urease enzyme activity in soil. Perotti and Pedello (1999) observed that *Azospirillum* increased urease enzyme activity in soil. The AM fungi with *Trichoderma* increased soil dehydrogenase enzyme activity and microbial biomass carbon at 50 per cent recommended NPK rate. Vazquez *et al.* (2000) reported that inoculation of mycorrhizal fungi increased the microbial population and enzyme activities in the plant rhizosphere. The AM fungi and *Trichoderma* are compatible and are potentially used in agricultural systems and enhanced microbial biomass carbon (Kim *et al.* 1998).

At 50% RDF soil application of all cultures increased enzyme activities such as dehydrogenase and β glucosidase and microbial biomass carbon. Earlier research showed that NPK application decreased the enzyme activity in soil and because NPK amended to soil contributed to cause toxic effect on soil microbes (Tilak *et al*. 2005, Duarah *et al*. 2011).

On the basis of the results from the field experiment conducted in Tamil Nadu, it is observed that available nutrients soil enzyme activities and microbial biomass carbon are improved if microbial inoculations are applied (5 kg/ha) along with NPK fertilizer at half the recommended rate (50: 25: 50 kg/ha N: P_2O_5: K_2O).

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