The Andhra Agric. J 70 (1): 126-134, 2023
10.61657/aaj.2023.20

Changes in Soil Enzyme Activity and Microbial Population with Incorporation of Crop Residue along with Microbial Consortium in Black Cotton Soils

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ABSTRACT

An incubation experiment was conducted to study the decomposition and its influence on soil enzyme activity and microbial populations with residue incorporation along with microbial consortium for 90 days in poly house. The soil enzyme activity has increased in residue treated pots either alone or in combination with microbial consortia along with starter dose of N and P fertilizers as decomposition accelerators than in treatment that received RDF. The soil enzyme activity and microbial populations assayed at different intervals were significantly increased by the application of crop residue along with microbial consortia. Among the treatments, the highest microbial populations were recorded with the treatment $T_{7,}$ which received crop residue @1.5 t ha^{-1} + Microbial consortium@2 kg t^{-1}+ urea 3 kg t^{-1} + SSP 15 kg t^{-1} of residue incorporated was at par with T_6 and T_3 during the experimentation.

Key words: *Crop residue, Microbial consortium, Soil enzyme activity and Soil micro flora*

Returning crop residues to soil is an effective method for sustaining soil organic matter concentration, enhancing biological activities and increasing nutrient availabilities (Smith *et al.,* 1992). The recycling of crop residues has the advantage for converting the surplus residues into useful product for meeting the nutrient requirement for soil microorganism as well as succeeding crops crop residues when incorporated directly into the soil accelerate the biochemical and biological components of the carbon cycle in soil (Maithani *et al*., 1998). As residues decompose, they also serve as a source of metabolic energy and nutrients for microorganisms and soil arthropods, contributing to recycle nutrients in the soil, and thus help to increase biodiversity and the activity of beneficial organisms in the soil (Tian and Brussaard 1993).

Crop residue incorporation treatments may also lead to a large input of cellulose and up to 15 per cent of the organic C in crop residues can be cellulose and cellulose derivatives (Stevenson, 1982). Unlike fresh herbaceous material, straw has a low content of easily absorbable sugars and proteins, but straw is rich in cellulose and hemicelluloses (Henriksen and Breland, 1999). Hence crop residues decomposition depends on the adequate colonization and growth of microorganisms producing extracellular cellulases and hemicellulases. The role of fungi is particularly significant in this process. The objective of this study was to monitor the changes in soil enzyme activities and microbial population when korra crop residue with wider C:N ratio was added along with microbial consortium to black cotton soil.

MATERIAL AND METHODS

Study location

An incubation study was conducted at green house of Agricultural Research Station, Amaravathi of Acharya N.G.Ranga Agricultural University of Andhra Pradesh during 2017-18. The bulk surface soil collected from field number 4 was used for incubation experiment. Black cotton soil from field was processed and filled in 20 kg capacity cement pots. The korra crop residue was used for incorporation in soil @1.5 t ha^{-1} after chopping in to 3-4 cm size the except in control (T_1) and RDF (T_8). Total eight treatments including control were employed and each treatment was replicated thrice

by following completely randomized block design. Turnings at weekly intervals were given and the residue was allowed for aerobic decomposition for 90 days and maintained at 60 per cent water filled pore space throughout incubation. Microbial consortium consists of decompo.A (fungal consortium of *Pleurotous ostreatous*, *Phanerochaete chrysosporium*, yeast and *Trichoderma*), decompo.B(bacterial consortium of *Bacillus sp*, *Lactobacillus* sp and *Pseudomonas sp*) developed at Agricultural Research Station, Amaravathi.The details of the treatment are as follows.

TREATMENTS:

T1 Absolute control
T2 Crop residue@1.5 t ha-1
T3 Crop residue@1.5 t ha-1+ 3.0 kg Microbial consortia
T4 Crop residue@1.5 t ha-1 +1.5 kg urea + 7.5 kg SSP
T5 Crop residue@1.5 t ha-1 +3.0 kg urea + 15 kg SSP
T6 Crop residue@1.5 t ha-1+3.0 kg Microbial consortia + 1.5kg urea+ 7.5 kg SSP
T7 Crop residue@1.5 t ha-1+ 3.0 kg Microbial consortia + 3.0 kg urea + 15 kg SSP
T8 RDF (20-50-0-40) of N,P_2O_5 and S ha^{-1}

Parameter assessments

Soil samples were collected at 15 days interval after incubation were analysed for assessing enzyme activities and microbial population in each treatment during the decomposition of korra crop residue. Dehydrogenase activity in the soil sample was determined using 2, 3, 5 triphenyltetrazolium chloride incubated at 28 ± 0.5^{0}C for 24 hours. TPF formed was extrapolated from the standard curve drawn in the range of 10 ìg to 90 ìg TPF mL^{-1} and expressed as ìg TPF g^{-1} soil h^{-1}. Alkaline phosphatase activity was estimated by modified universal buffer (pH 11) incubated at 37^0 C and expressed in ìg p-nitrophenol g^{-1} soil h^{-1}. Cellulase activity was estimated by incubation with substrate carboxy methy cellulose (CMC) (0.7%) in an acetate buffer (pH 5.5) at 50^{0}C for 24 hours. Urease activity was estimated by quantifying the rate of release of NH_4^+ from the hydrolysis of urea. After incubation for 12 hours at 37^0 C the NH_4^+- N released was determined by distilling suspension with sodium hydroxide (Na OH) for five minutes. Urease activity was expressed in terms of NH_4^+ - N released g^{-1} soil h^{-1}.Population dynamics of the microbes also was estimated by taking into account the microbes population at different stages.

RESULTS AND DISCUSSION

Enzyme activity

Urease: The urease activity estimated at 15 days intervals during incubation was significantly influenced by the treatments. The data presented in table 1 revealed that the urease activity was increased in all the treatments except T_1 and T_8 up to 45 days after incubation and then steadily decreased. Among the treatments, the treatment T_8 (RDF) recorded significantly the highest urease activity at 15 DAI whereas, the lowest was recorded in absolute control (T_1). The treatment T_7 which received crop residue along with microbial consortia and starter dose of N and P fertilizer recorded the highest activity from 30 to 90 DAI. The per cent increase in urease activity in T_7 over control was 92.0 at 45 DAI .The treatment T_3 which received microbial consortium and crop residue recorded higher urease activity than the treatment which received only crop residue (T_2) at all stages of incubation. The similar increase in urease activity with residue application was reported by Behera *et al.* (2021).Among the integrated treatments, the treatments which received higher starter dose of inorganic nitrogen (T_5 and T_7) recorded higher activity when compared to the treatments which received lower dose (T_4 and T_6). Added organic matter in the presence of inorganic nitrogen promoted biological and microbial activities and accelerated the breakdown of organic substances. The highest organic matter levels in the crop residue treatments might have provided a more favourable environment for the accumulation of enzymes in soil matrix, since soil organic constituents were important in forming stable complexes with free enzymes. Low enzymatic activity in absolute control might be due to less availability of substrate. Results are in tune with findings of Kumari *et al.* (2016).

Dehydrogenase: The results presented in table 2 showed an increase in dehydrogenase activity up to 45 days of incubation and then decreased. The

significantly highest dehydrogenase activity was recorded in T_7 treatment (54.0 µg TPF g^{-1} soil d^{-1}) which received crop residue @1.5 t ha^{-1} along with 3.0 kg of microbial consortium and urea 3.0 kg +15 kg SSP at 45 days after incubation and this was on par with the treatments T_6 and T_3. The highest dehydrogenase activity in T_7 was attributed that application of crop residue along with microbial consortium might have created conducive environment for decomposition of organic matter, which stimulated the activity of soil microorganisms resulting in an increase in dehydrogenase enzyme activity of the soil . The results are in corroboration with the findings of Bajpai *et al.* (2006) and Prasad *et al.* (2010). Similarly the highest dehydrogenase activity was also recorded by Simon and Czako (2014) with application of straw along with cattle slurry and NPK fertilizer. The lowest values were recorded in treatment T_1 (absolute control).Incorporation of crop residue along with microbial consortium in T_7 at 45 days after incorporation had improved the dehydrogenase activity by 34.32 per cent over control, which gradually declined with further period of incubation.

It is evident that soil enzymatic activity is strongly connected with soil organic matter content. The higher organic matter level had provided enough substrate to support higher microbial biomass, hence higher enzyme production. Several authors reported positive correlation between organic matter and dehydrogenase activity content (Moeskops *et al.*, 2010 and Li *et al.*, 2019). Chandra (2011) reported that the crop residue incorporation resulted in significantly higher dehydrogenase activity of 65 µg of TPF g^{-1} soil 24 h^{-1} in silty clay loam soil over 100per cent NPK in wheat (60 µg of TPF g^{-1} soil 24 h^{-1}).

Table 1. Effect of incorporation of korra crop residue on urease activity of soil during incubation

Treatment details	Urease activity (µg NH_4^+ g^{-1} soil h^{-1})					
	15 DAI	**30 DAI**	**45 DAI**	**60DAI**	**75DAI**	**90 DAI**
T_1: Absolute control	12.33	13	12.5	12.67	11.67	11
T2: Crop residue @ 1.5 t ha-1	14.67	15	15.57	14.2	13.67	13
T_3: T_2 + 3.0 kg Microbial consortium	16	18.33	20.77	17.9	17.67	17
T_4: T_2 + 1.5 kg Urea + 7.5 kg SSP	17	17.17	17.5	16.33	14.67	13.47
T_5: T_2 + 3.0 kg Urea + 15 kg SSP	18	17.57	17.93	16.73	15	14
T_6: T_3 + 1.5 kg Urea + 7.5 kg SSP	17.67	19.33	22.67	19.67	18.67	17.53
T_7: T_3 + 3.0 kg Urea +15 kg SSP	19.33	23.63	24	20.67	19.33	18.8
T_8: RDF (20-50-40) of N,P_2O_5 & S kg ha^{-1}	25	20.33	20	19.33	16.67	15
SE(m)±	0.56	0.56	0.58	0.61	0.47	0.46
CD(0.05)	1.69	1.69	1.73	1.84	1.41	1.37
CV(%)	5.33	5.4	5.52	5.99	5.14	5.29

*DAI-Days after incubation

Alkaline Phosphatase: The results of alkaline phophatase activity assayed at 15 days interval during incubation are presented in table 3 and the data indicated that the phosphatase activity was significantly influenced by the treatments throughout the incubation period. In all the treatments the activity was increased progressively up to 60 days after incubation and then the activity was decreased. Among the treatments the treatment T_6 which received integration of crop residue, microbial consortia and starter dose of N and P fertilizers recorded highest activity at all intervals of incubation and it was significantly superior over T_1,T_2, T_5 and T_8 and was at par with T_3 and T_7. These results suggested that incorporation of crop residue is important in transformation of phosphorus and vital for maintaining the plant available pool of soil phosphorus. The treatment T_6 had about 26.74 per cent higher activity compared with absolute control (T_1) at 60 days of incubation. The improved soil organic matter status with crop residue addition might have led to an increased alkaline phosphatase activity. Mineralization is mediated by the enzyme

phosphatases. The phosphatase enzyme cleaves phosphorus from organic substrates. *Bacillus, Pseudomonas, Aspergillus and Penicillium* can synthesize this enzyme. Similarly Zhang *et al.* (2016) reported a significant increase in mean phosphatase activity after four years of incorporation of maize straw in soils with a pH of 8.5 in semi arid region of China. The results were in corroboration with the findings of Singh *et al.* (2018)

The treatment $T_{8,}$ which received recommended dose of fertilization recorded the lowest activity. At the end of the incubation time, higher phosphatase values than those of the control soil were detected in all the treatments amended with crop residue.

In contrast Akmal *et al.* (2012) reported that activity was more in fertilized treatments than in unfertilized control. Alkaline phosphatase activity was very low in treatments not receiving microbial consortium when compared to treatments receiving microbial consortium. Low levels of activity represent a lag phase and increased activity is associated with microbial growth (Louise and Teresa**,** 2005). This was evidenced by a strong positive correlation between alkaline phosphatase activity and microbial population (r= 0.854**) at 60 DAI.

Table 2. Effect of incorporation of korra crop residue on dehydrogenase activity of soil during incubation

Treatment details	Dehydrogenase activity (µg TPF g^{-1} soil d^{-1})					
	15 DAI	**30 DAI**	**45 DAI**	**60DAI**	**75DAI**	**90 DAI**
T_1: Absolute control	40.2	41	41.5	40	39	38.67
T2: Crop residue @ 1.5 t ha-1	44	45.07	46.47	41	40	40.33
T_3: T_2 + 3.0 kg Microbial consortium	50	51	51.07	43.67	43	41.67
T_4: T_2 + 1.5 kg Urea + 7.5 kg SSP	47	48	47	41.67	41	39
T_5: T_2 + 3.0 kg Urea + 15 kg SSP	48.6	49.33	48	42	41.33	40.33
T_6: T_3 + 1.5 kg Urea + 7.5 kg SSP	51.33	52	53.13	44	43.33	42.33
T_7: T_3 + 3.0 kg Urea +15 kg SSP	52	53	54	47	45.33	43.33
T_8: RDF (20-50-40) of N,P_2O_5 & S kg ha^{-1}	43.67	44.67	45.33	40	39	38.67
SE (m)±	1.55	1.58	1.61	1.35	1.22	1.21
CD (0.05)	4.64	4.75	4.84	4.11	3.67	3.2
CV (%)	5.67	5.72	5.82	5.61	5.07	4.55

*DAI-Days after incubation

Table 3. Effect of incorporation of korra crop residue on alkaline phosphatase activity of soil during incubation

Treatment details	Alkaline phosphatase activity (µg p-nitrophenol g^{-1} soil h^{-1})					
	15DAI	**30 DAI**	**45 DAI**	**60DAI**	**75DAI**	**90 DAI**
T_1: Absolute control	80.33	81	84	86	78	70
T2: Crop residue @ 1.5 t ha-1	93.33	94.67	96	97	81.33	77
T_3: T_2 + 3.0 kg Microbial consortium	95	97	98	99	84	82
T_4: T_2 + 1.5 kg Urea + 7.5 kg SSP	86.33	87	89	92	80	78.33
T_5: T_2 + 3.0 kg Urea + 15 kg SSP	83	86	87	88	74	71.67
T_6: T_3 + 1.5 kg Urea + 7.5 kg SSP	98	101	105.33	109	90	82.67
T_7: T_3 + 3.0 kg Urea +15 kg SSP	96	100	104	108	90	83.33
T_8: RDF (20-50-40) of N,P_2O_5 & S kg ha^{-1}	77	78	80	82	71	68
SE (m)±	2.58	2.89	2.7	3.34	2.7	2.13
CD (0.05)	7.74	8.66	8.08	10	8.08	6.39
CV (%)	5.05	5.52	5.02	6.07	5.76	4.81

*DAI-Days after incubation

Table 4. Effect of incorporation of korra crop residue on cellulase activity of soil during incubation

Treatment details	Cellulase activity (µg glucose g^{-1}soil d^{-1})					
	15 DAI	**30 DAI**	**45 DAI**	**60 DAI**	**75 DAI**	**90 DAI**
T_1: Absolute control	29	29.17	30.28	30.53	30.67	30.33
T2: Crop residue @ 1.5 t ha-1	30.33	30.67	31.67	32	33.67	32
T_3: T_2 + 3.0 kg Microbial consortium	32	34.67	35	36	38.9	36
T_4: T_2 + 1.5 kg Urea + 7.5 kg SSP	30.67	31.67	32	33	34	32.67
T_5: T_2 + 3.0 kg Urea + 15 kg SSP	31.67	32.33	33.33	34	35	34.33
T_6: T_3 + 1.5 kg Urea + 7.5 kg SSP	33.33	34.47	35.67	36.33	39	37.67
T_7: T_3 + 3.0 kg Urea +15 kg SSP	34.67	35	36	37	41	39
T_8:RDF (20-50-40) of N,P_2O_5 & S kg ha^{-1}	29.33	29.67	29	29.17	29.67	29
SE (m)±	1	0.72	0.77	0.73	0.73	1.11
CD (0.05)	3	2.15	2.31	2.18	2.2	3.31
CV (%)	6.38	6.03	6.34	5.75	5.64	5.82

*DAI-Days after incubation

Cellulase activity: The data presented in table 4 showed a significant influence of korra crop residue incorporation on cellulase activity. The activity was improved when compared to the initial in all the treatments except T_1 and T_8. The activity was increased with days of incubation up to 75 days in all residue applied treatments. The highest cellulose activity was recorded in treatment T_7, which received crop residue @ 1.5 t ha^{-1} along with 3.0 kg microbial consortia and 3.0 kg urea + 15 kg SSP and this was on par with the treatments T_6 which received crop residue @ 1.5 t ha^{-1} along with 3.0 kg microbial consortia and 1.5 kg urea + 7.5 kg SSP and T_3 which received crop residue @ 1.5 t ha^{-1} + MC@ 2 kg t^{-1} of residue. The lowest was recorded in absolute control followed by treatment which received only inorganic fertilizer.

The highest cellulase activity in korra residue treatments was mainly due to the cellulose content (31.8%) of the crop residue used in the present study. Thus incorporation of plant residues into soils leads to a large input of cellulose to soils. The degradation of cellulose releases glucose and soluble cellodextrins, which are readily available C source for microbial growth. The decrease in cellulose activity after 75 DAI might be due to reduction in cellulose content in soil. The treatment T_7 is significantly superior in breaking down cellulose and at par with other two treatments that received microbial consortium.

Similarly, Sinsabaugh and Linkins (1993) reported that the concentration of cellulase in soil was highly correlated with the cellulose content. Kanazawa and Miyashita (1987) established a correlation between cellulase activity and mass loss from decomposing leaf litter in forest soils of Japan.

The higher activity in T_3, T_6 and T_7 might be due to application of microbial consortium which stimulates enzyme activities in the soil. This was linked to the increased soil organic carbon and microbial biomass after fertilization. The results are in agreement with those of Zhu *et al.* (2019). In residues that are rich in cellulose, N addition stimulates decomposition rate (Hobbie, 2000) and cellulose degrading enzyme activity (Keeler *et al*., 2009).

Microbial Population

The influence of incorporation of korra crop residue along with chemical fertilizers on microbial population *viz*., bacteria, fungi and actinobacteria estimated at 15 days interval during incubation is presented in table 5. The perusal of the data revealed that there was an improvement in soil microbial population *viz.*, total bacteria, fungi and actinomycetes over the initial population after incubation except in T_1. Significantly the highest number of soil microbial

population was found in treatment T_7 which received integration of crop residue, microbial consortium and higher doses of starter dose of N and P fertilizer at all intervals. This was followed by T_6 and T_3. The improvement in soil microbial population might be due to the addition of crop residue which might have added a fair quantity of organic matter to the soil, which in turn acted as a substrate for the multiplication and development of microbes either over the initial and absolute control. Similarly, increased trend in soil microbes due to incorporation of residues was also reported by Nagar *et al*. (2016).

At 60 DAI, the maximum bacterial population was recorded in treatment T_7 which was at par with T_6 and T_3 and then decreased with duration of incubation. The activity and growth of microorganisms in earlier and later stages of incubation were limited by the availability of N due to application of korra crop residue with low N content. The higher bacterial population in T_7, T_6 and T_3 was apparently due to external addition of microbial consortium culture dominated by bacterial isolates. Residue retention promotes the microbial abundance compared to residue removal Govaerts *et al*. (2006) and Noya *et al*. (2013). At 75 DAI fungal population was highest in treatment T_7 and then decreased with duration of incubation. This might be due to the action of fungi on decomposed products of bacteria. The fungal population in the soil was relatively less efficient metabolically than the bacteria and actinobacteria in all the treatments throughout the incubation. This also implies that compared with the fungal population, the bacterial population is probably more active metabolically under the semi-arid conditions. Similar increase in population of bacteria was also reported earlier by Singh *et al.*, 2019 in residue applied treatments.

The actinobacterial population has increased with duration of incubation up to 45 DAI and decreased later with the days of incubation. The highest population of actinobacteria was recorded in treatment T_7 and it was at par with treatments T_6 and T_3. The increase in actinobacterial population might be due to availability of substrate as food simple sugars, which decreased later due to competition from bacterial groups. Chandra (2011) reported that crop residue incorporation increased the population of total bacteria (8.2 log10 CFU g^{-1}), fungi (4.88 log10 CFU g^{-1}) and actinobacteria (3.69 log10 CFU g^{-1}) in soil compared to 100 per cent NPK (8.03, 4.76 and 3.69 log10 CFU g^{-1}, respectively).

The microbial populations and enzyme activities were significantly influenced by the application of crop residue along with microbial consortia. The population of bacteria, fungi and actinobacteria were significantly increased in treatment T_7 which received integration of crop residue, microbial consortium and starter doses of inorganic N and P fertilizer.The highest urease, dehydrogenase, alkaline phosphatase and cellulase activities were also recorded in T_7 treatment.

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Table 5. Effect of incorporation of korra crop residue on microbial population of soil during incubation

Treatment details	Bacteria (CFUx$10^6 g^{-1}$ soil)						Fungi (CFUx10^4 g^{-1} soil)						Actinobacteria (CFUx$10^5 g^{-1}$ soil)					
	15 DAI	30 DAI	45 DAI	60 DAI	75 DAI	90 DAI	15 DAI	30 DAI	45 DAI	60 DAI	75 DAI	90 DAI	15 DAI	30 DAI	45 DAI	60 DAI	75 DAI	90 DAI
T_1: Absolute control	10	12	13	15	10	8	8	8	8	8	7	7	18	17	17	17	17	17
T2: Crop residue @ 1.5 t ha-1	12	18	20	22	12	11	11	12	15	16	18	9	19	24	23	22	21	20
T_3: T_2 + 3.0 kg Microbial consortium	23	32	34	37	32	15	16	17	24	25	26	12	23	26	25	24	23	22
T_4: T_2 + 1.5 kg Urea + 7.5 kg SSP	21	23	24	26	18	12	12	14	17	18	20	11	21	25	23	22	21	20
T_5: T_2 + 3.0 kg Urea + 15 kg SSP	22	25	26	28	20	13	13	15	20	21	22	12	22	24	24	23	22	21
T_6: T_3 + 1.5 kg Urea + 7.5 kg SSP	26	33	35	38	33	16	17	19	25	26	27	14	24	27	26	25	24	23
T_7: T_3 + 3.0 kg Urea +15 kg SSP	29	34	36	39	34	17	18	20	27	28	29	15	25	28	27	26	25	24
T_8: RDF (20-50-40) of N,P_2O_5 & S kg ha^{-1}	14	16	18	19	12	10	10	10	10	10	10	9	23	21	20	20	19	18
SE (m)±	0.68	0.76	0.66	0.91	0.76	0.55	0.54	0.58	0.65	0.87	0.65	0.54	0.75	0.74	0.82	0.89	0.74	0.79
CD (0.05)	2.03	2.29	1.97	2.74	2.29	1.66	1.62	1.73	1.94	2.6	1.94	1.62	2.26	2.21	2.45	2.67	2.21	2.37
CV (%)	6.05	5.51	4.24	5.6	6.66	5.27	7.13	6.96	6.13	7.89	5.63	8.41	6.09	5.31	6.12	6.89	5.83	6.33

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Received on 04.09.2022 and Accepted on 10.02.2023