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Enzyme activities and biological fertility index of the soils of coconut based cropping systems in mid laterite soils of Kerala (AEU 9)

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Abstract
Enzymes in the soil are closely related to the physical, chemical and biological characteristics of the soil and regulate the formation of soil fertility, organic matter, nutrient mineralization and cycling in nature. As soil contains several enzymes the objective of the conducted experiment was to estimate dehydrogenase, acid phosphatase, glucosidase, protease, catalase and amylase in five coconut based cropping systems *viz.*, Coconut+Fodder, Coconut+Banana, Coconut+Pepper, Coconut+Tuber and Coconut+Vegetable under organic and conventional farming systems and find out the biologically fertile cropping system using enzyme activity number (EAN) and enzyme kinetics for good cropping system in mid-laterite soils of Kollam district of Kerala AEU 9. The dehydrogenase, acid phosphatase and catalase activity were found to be higher in Coconut + Banana under organic farming system, glucosidase and amylase activity was more in Coconut + Pepper under organic farming system and protease activity was found to be higher in Coconut + Tuber cropping system under organic management system. Coconut + Banana under organic farming situation was found to be best cropping system compare to other cropping systems.

Keywords: coconut based cropping system, dehydrogenase, acid phosphatase, glucosidase, protease, catalase, amylase, enzyme activity number

1. Introduction
Enzymes in soils mainly come from plants, soil, animals and microorganisms which are connected covalently, cross linked, copolymerized, adsorbed and included in the microcapsules of soil particles (Girish and Ajit, 2011) [12]. Soil enzymes have been reported as useful soil quality indicators due to their relationship to soil biology, being operationally practical, sensitive, integrative, ease to measure and described as "biological fingerprints" of past soil management, and relate to soil tillage and structure (Utobo and Tewari, 2014) [22]. Soil enzymes play an important role in formation, converting and decomposition of organic matter to the plant digestible forms, decomposition of xenobiotics, involved in the nitrogen and other elements cycle and life cycling of soil microorganisms (Dick and Tabatabai, 1992) [9]. Soil enzyme activities have been used as indicators in evaluation of soil quality, climate changes, destruction and toxification in ecosystems. Soil enzyme activities vary seasonally and have been related to soil physico-chemical characters, microbial community structure, vegetation, disturbance and succession (Caldwell, 2005) [5].

Soil dehydrogenase enzymes are one of the main components of soil enzymatic activities participating in and assuring the correct sequence of all the biochemical routes in soil biogeochemical cycles. Among all enzymes in the soil environment, dehydrogenases are one of the most important, and are used as an indicator of overall soil microbial activity, because they occur intracellular in all living microbial cells (Salazar *et al.*, 2011) [17]. Dehydrogenase is an enzyme that oxidises soil organic matter by transferring protons and electrons from substrate to acceptors. This enzyme is considered to exist as an integral part of intact cells but do not accumulate extracellularly in the soil (Das and Varma, 2011) [7].

Acid phosphatases catalyze non-specific hydrolysis of inorganic phosphate from phosphate monoesters in pH ranges from 4 to 6 and plays a major role in the supply and metabolism of phosphate in plants (Tabaldi *et al.*, 2007) [19]. Organic phosphorous is abundant in soil and can contribute to the P nutrition of plants and microbes following hydrolysis and release of free phosphate. This process is catalysed by phosphatase enzyme, which are secreted into soil (Miller *et al.*, 2001) [16]. P compounds in soil are generally mineralized by enzymes, collectively called “phosphatases” that catalyzes the hydrolysis of esters and anhydrides of phosphoric acid.

Jn B-glucosidase enzyme plays an important role in soils because it is involved in catalyzing the hydrolysis and biodegradation of various β-glucosidase present in plant debris decomposing in the ecosystem (Martinez and Tabatabai 1997) [15]. Enzyme involved in cellulose degradation, plays an important role in the soil organic carbon cycle (Esen, 1993) [11].

Protease activity is an indicator of the biological capacity of soil for the enzymatic conversion of the substrate, which is independent of the extent of microbial activity and also have an important role in the ecology of microorganisms in the ecosystem

(Burns, 1982) [4]. Protease in soil plays a significant role in nitrogen mineralization, more active in soils with a high water and humus content forest soils and landfills.

Catalase decomposes peroxide and its activity depends from organic oxygen concentration, microbial biomass, changes in CO_2, and depends from dehydrogenase, amidase, glucosidase and esterase activity in soils (Burns, 1982)[4]. Soil catalase activity higher under well-aerated condition (Brzezinska *et al*., 2005) [3].

The α-amylases are synthesized by plants, animals, and microorganisms, whereas, β-amylase is synthesized mainly by plants (Thoma *et al*., 1971) [21]. Amylase widely distributed in plants and soils and it plays a significant role in the breakdown of starch, which converts starch like substrates to glucose and oligosaccharides, which converts starch to maltose (Thoma *et al*., 1971) [21].

2. Materials and Methods

Soil samples were collected from five different coconut based cropping systems under two farming systems *viz.*, organic and conventional farming systems in mid-laterite soils of Kollam district of Kerala (Agro Ecological Unit) AEU 9. In all soil samples activities of dehydrogenase, acid phosphatase, glucosidase, protease, catalase and amylase were estimated.

The dehydrogenase activity was measured by the method described by Casida *et al.*, 1964 [6]. The dehydrogenase activity was estimated using 3 % 2, 3, 5 - triphenyl tetrazolium chloride (TTC). The concentration of dehydrogenase in the sample was obtained by plotting standard graph drawn by using tri phenyl formazon (TPF) as standard. The enzyme activity was expressed in µg of TPF released g^{-1} soil 24 h^{-1}.

The acid phosphatase activity of soils was measured by the method outlined by Tabatabai and Bremner (1969) [20]. The acid phosphatase activity was estimated using 0.05 M p- nitrophenyl phosphate. The enzyme activity was expressed in µg of p-nitrophenol released g^{-1} soil h^{-1} on dry weight basis at 37 ^{0} C at pH 6.5.

Eivazi and Tabatabai (1988) [10] method was used for estimating activity of glucosidase in soil samples by using 0.5 M p-nitrophenyl β– glucopyranoside. The glucosidase activity was expressed in µg pnp D- glucosidase g^{-1} soil h^{-1} x 10^{-4}.

The activity of protease of soils was estimated by Kunitz M. (1947) [14] method and is expressed in µM of amino nitrogen hydrolysedg^{-1} soil h^{-1}.

Soil catalase activity by Bach A.N. and Zubkova S.M. Method and activity was expressed in % of H_2O_2 hydrolysed g^{-1} soil h^{-1}.

Bernfeld (1955) [2] outlined procedure for estimating activity of amylase in soil samples.

Biological Fertility Index, for the different combinations of treatments were computed based on the activity of five different enzymes *viz.*, dehydrogenase, catalase, acid phospha0tase, protease and amylase proposed by Beck (1984) through enzyme activity Number. The Enzyme Activity Number for the different treatments was computed using the formula.

EAN = 0.2{TPF+ catalase (%)/10 + phenol (µg)/40 + amino-N (µg)/40 + amylase (%)/20}.

Statistical Analysis

The data generated from the experiments were subjected to the Analysis of variance as per the design, Factorial CRD and their significance was tested using F test (Snedecor and Cochran, 1975) [18]. Critical difference (CD) was calculated at 0.05% probability levels using statistical tools.

3. Results and Discussion

From the study activity of dehydrogenase was highest in coconut+Banana (462.52 µg of TPF released g^{-1} of soil 24 h^{-1}) cropping system under organic farming system (Table. 1). Organic system of cultivation might have contributed to the spurt of microbial population in the soil especially bacteria thus resulting in higher dehydrogenase activity. Similar increase in dehydrogenase activity with the addition of FYM /vermicompost was reported by Wells *et al.* (2000) [24] and the lowest dehydrogenase activity was observed in conventional farming system of Coconut + Tuber (101.25 µg of TPF released g^{-1} of soil 24 h^{-1}) (Fig. 1).

The highest acid phosphatase activity was noticed in organic farming system of Coconut + Banana (80.35 µg of p-nitrophenol released g^{-1} of soil 24 h^{-1}) (Table. 1 & Fig. 2). The highest activity of acid phosphatase noticed in these treatments might be attributed to the low available P status. Versaw and Harrison (2002) [23] reported that whenever there is a signal indicating P deficiency in the soil, acid phosphatase secreation from plant roots is increased to enhance the solubilisation and remobilization of PO_4 thus influencing the ability to cope with P stressed condition.

The highest β- glucosidase activity was noticed in Coconut+Pepper (4.1 µg pnp D- glucosidase g^{-1}soil h^{-1}X 10^{-4}) and Coconut + Tuber (Table. 1). In general the organic system of cultivation was found to be favourable for the activity of β-glucosidase. This is also supported by the results showing that microbial growth favouring the release of β- glucosidase in this kind of environment (Dick, 1994) [8] and the lowest β-glucosidase activity was observed in conventional farming system of Coconut+Vegetable (1.53 µg pnp D- glucosidase g^{-1} soil h^{-1}X 10 $^{-4}$) (Fig. 3).

The highest activity of protease in Coconut+Tuber (188.84 µM of amino nitrogen hydrolyzed g^{-1} of soil h^{-1}) cropping system recorded the highest value for protease activity (Table. 1 & Fig. 4). This may be attributed to highest status of available K, mineralizable nitrogen, organic carbon and water holding capacity.

Catalase activity in soils is considered an indicator of aerobic microbial activity and has been related to both the number of aerobic microorganisms and soil fertility. The highest catalase activity was observed in Coconut+Banana (2.85 % of H_2O_2 hydrolyzed g^{-1} of soil h^{-1}) cropping system (Table. 1), which was on par with Coconut + Pepper (2.64 % of H_2O_2 hydrolyzed g^{-1} of soil h^{-1}) while, the lowest activity of catalase was noticed in Coconut + Vegetable (Fig. 6).

From the investigation carried it was observed that the highest activity of amylase 13.82 µM of maltose g^{-1} of soil was reported in Coconut+Pepper (Table. 1 & Fig. 5) under organic system of management. Griffiths *et al*. (1999) [13] observed similar trends when studying the effect of varying rates of C substrates on amylase activity.

With regard to the EAN as proposed by Beck (1984) [1], from Table. 2, it is observed that the highest EAN was noticed in Coconut + Banana main effects, Coconut+Banana (94.01) under organic system of cultivation. It is obvious from the study, the highest values for dehydrogense (462.52 µg of TPF released g^{-1}

of soil 24 h^{-1}), acid phosphatase (80.35 µg of p- nitrophenol released g^{-1} of soil 24 h^{-1}) and catalase (3.9% of H_2O_2 hydrolysed g^{-1} of soil hr^{-1}) noticed in Coconut+Banana under organic system might have contributed to highest enzyme activity.

Table 1: Enzyme activities of the soils of coconut based cropping systems AEU 9

Treatments	Dehydrogenase (µg of TPF released g^{-1} of soil 24 h^{-1})	Acid phosphatase (µg of p nitro phenol released g^{-1} of soil 24 h^{-1})	Glucosidase (µg pnp D-glucosidase g^{-1} soil h^{-1} x 10^{-4})	Protease (µM of amino nitrogen hydrolysed g^{-1} of soil h^{-1})	Catalase (% of H_2O_2 hydrolysed g^{-1} of soil h^{-1})	Amylase (µM of maltose g^{-1} of soil)
Coconut + Fodder (C_1)	221.46	52.44	3.06	84.94	2.02	8.98
Coconut + Banana (C_2)	326.92	56.23	2.51	165.84	2.85	9.44
Coconut + Pepper (C_3)	276.42	42.19	3.64	149.7	2.64	9.7
Coconut + Tuber (C_4)	212.87	55.95	3.27	174.98	2.32	9.16
Coconut + Vegetable (C_5)	184.3	45.76	2.12	172.17	1.96	9.01
Organic Farming (F_1)	344.49	59.89	3.37	163.22	3.05	12.31
Conventional Farming (F_2)	144.29	41.14	2.47	135.84	1.67	6.21
C_1F_1	311.83	56	3.51	99.34	2.54	9.8
C_2F_1	462.52	80.35	2.8	186.37	3.9	12.52
C_3F_1	368.38	47.26	4.1	161.46	3.54	13.82
C_4F_1	321.49	58.68	3.75	188.84	2.74	12.7
C_5F_1	258.22	57.13	2.72	180.07	2.52	12.7
C_1F_2	131.08	48.87	2.61	70.54	1.5	8.16
C_2F_2	191.32	32.11	2.23	145.32	1.8	6.36
C_3F_2	184.45	37.11	3.18	137.95	1.74	5.58
C_4F_2	101.25	53.2	2.79	161.11	1.9	5.62
C_5F_2	110.37	34.37	1.53	164.27	1.4	5.32
CD- C (0.05)	21.66	6.29	0.14	19.05	0.23	0.25
CD- F (0.05)	13.69	3.98	0.06	12.05	0.14	0.16
CD- C F(0.05)	30.63	8.91	0.14	NS	0.32	0.35

Table 2: Enzyme Activity Number of the soils of coconut based cropping systems AEU 9

Treatments	EAN
Coconut + Fodder (C_1)	45.13
Coconut + Banana (C_2)	66.59
Coconut + Pepper (C_3)	56.39
Coconut + Tuber (C_4)	43.85
Coconut + Vegetable (C_5)	38.1
Organic Farming (F_1)	70.19
Conventional Farming (F_2)	29.83
C_1F_1	63.31
C_2F_1	94.01
C_3F_1	74.92
C_4F_1	65.69
C_5F_1	53.03
C_1F_2	26.95
C_2F_2	39.18
C_3F_2	37.87
C_4F_2	22
C_5F_2	23.17
CD- C (0.05)	4.33
CD- F (0.05)	2.74
CD- C F(0.05)	6.13

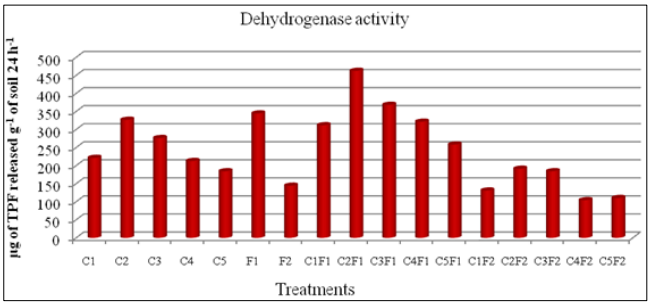

Fig 1: Dehydrogenase activity of the soils under coconut based cropping system AEU 9

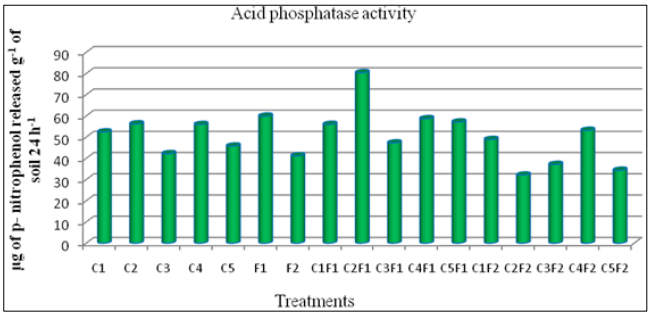

Fig 2: Acid phosphatase activity of the soils under coconut based cropping system AEU 9

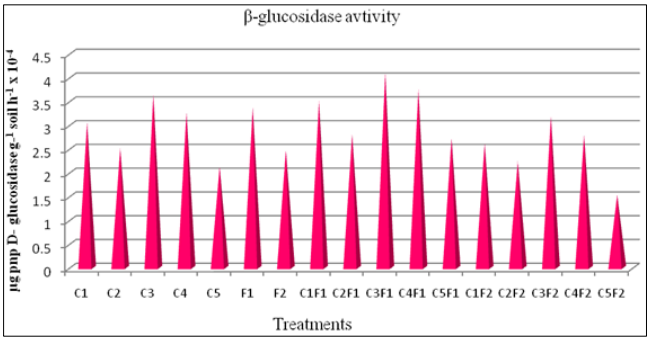

Fig 3: β-glucosidase activity of the soils under coconut based cropping system AEU 9

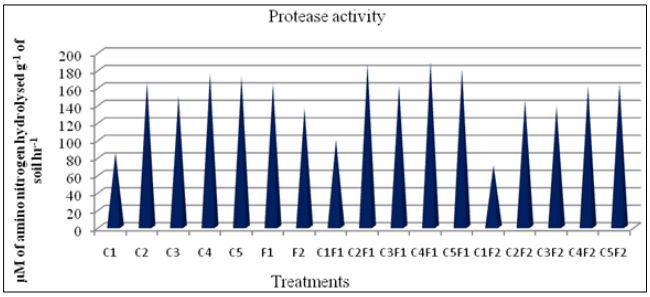

Fig 4: Protease activity of the soils under coconut based cropping system AEU 9

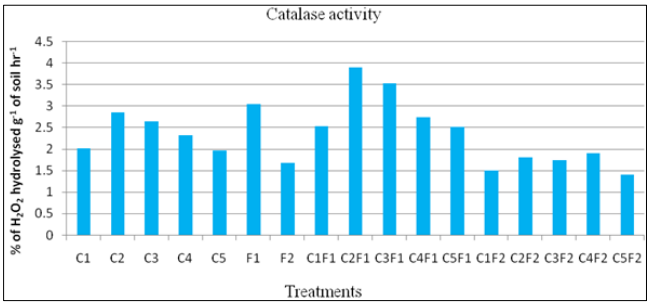

Fig 5: Catalase activity of the soils under coconut based cropping system AEU 9

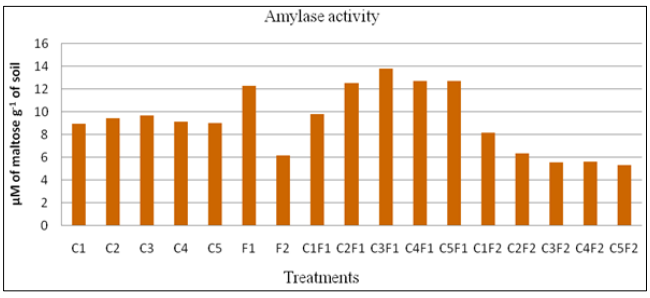

Fig 6: Amylase activity of the soils under coconut based cropping system AEU 9

4. Conclusion

From the study, it is concluded that, under the various cropping systems and farming systems Coconut + Banana under organic system recorded highest enzyme activity of dehydrogenase, acid phosphatase and catalase and also enzyme activity number (EAN) higher for Coconut + Banana under organic system of management, thus making it biologically fertile and sustainable.

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