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Comparative Effect of Compost Manure and Inorganic Fertilizer on Selected Enzymatic Activities of Cocoa Seedling Rhizosphere

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ABSTRACT

Soil enzymes activities have been proposed to evaluate the sustainability and economic effects of agricultural practices, and even to diagnose the soil categories. In this study, the effect of soil amendment on the activities of urease and dehyrogenase in the rhizospheres of cocoa seedlings were analyzed. Cocoa seedlings were self-grown in nursery located at the Orchard of the Faculty of Agriculture, University of Benin and Amelonado variety Tc1-Tc8 pods were used. The seeds were prepared, pre-germinated and planted in bags containing 5 kg top soil. Organic fertilizers (compost poultry manure and cow dung) and inorganic fertilizer was applied to the soil surrounding the seedlings at one month after planting. The sowing soil and the rhizosphere of the cocoa seedlings at one month to four month were collected and analyzed. The physical and chemical analysis of the sowing soil and the organic contents of the organic fertilizers were investigated and the activities of the enzymes: dehydrogenase and urease were assayed using standard procedures. It was observed that the Nitrogen: Phosphorus:Potassium (NPK) content of the poultry manure (4.11:33.43:6.00) was higher than that of the cow dung (1.75:0.84:5.52). The enzymatic assay showed soil amended with poultry manure having the highest dehydrogenase (11.58 – 12.34) and urease (15.80 -16.90) activities; while that of NPK (10.46 – 10.53) and (14.32 -14.52) recorded the least enzymatic activity respectively.

1. Introduction

Theobroma cacao L. (Cocoa) is a preferentially alogamous tropical woody species in the Malvaceae family [1]. Cocoa is a commodity produced in the developing countries of the tropics and when fermented and processed, the beans produce one of the most desired flavours in the world - chocolate. The geographical origin of cocoa is South America [2]; however cocoa is now grown in some 50 tropical countries. Cocoa was first grown in the western region of Nigeria in 1890 and gained prominence rapidly such that by 1965, Nigeria became the second largest producer in the world [3]. The production of cocoa in Nigeria has witnessed a downward trend since the early 1970s due to numerous factors such as ageing trees, ageing farmers, wrong application of recommended agronomic techniques, effects of pests and diseases and deficiencies in macro and micro nutrients in the soils [4]. Soil quality has been known to have a strong effect on cocoa tree growth [5, 6, 7, 8]. To achieve high productivity, cocoa requires a soil abundant in

nutrients [9]. Soil organic matter can play a crucial role in maintaining soil fertility [10]. Soils are the habitat and resource for a large part of global biodiversity: over one- fourth of all living species on earth are strict soil or litter dwellers [11] and microorganisms are responsible for most biological transformations that result to the development of nutrients in the soil [12]. According to a general view, the rhizosphere includes plant roots and the surrounding soil. This is a definition coined more than hundred years ago by Hiltner [13]. The rhizosphere inhabiting microorganisms compete for water, nutrients and space and sometimes improve their competitiveness by developing an intimate association with plant [14].

1.1 Enzyme activity in the rhizosphere

The overall enzyme activity of the rhizosphere as well as bulk soil can depend on enzymes localized in root cells, root remains, microbial cells, microbial cell debris, microfaunal cells and the related cell debri; free extracellular enzymes or enzymes adsorbed onto or occluded into the soil colloids [15]. Enzyme activity in the rhizosphere can be of intracellular origin, released after microbial cell disuption or root cell sloughing [16]. Root exudates represent a carbon-rich substrate for the rhizosphere microorganisms. Roots also release inorganic compound such as CO2, inorganic ions, protons and anions as a consequence of the root metabolic activity.

1.2 Urease

Urease enzyme is responsible for the hydrolysis of urea fertilizers applied to the soil. The ability to produce urease is widespread among microbial populations; its use in agricultural soils has also been severally reported especially in nitrogen volatilization [17, 18, 19, 20].

1.3 Dehydrogenase

Dehydrogenase occurs in all viable microbial cells and is considered as an indicator of oxidative metabolism and total microbial activity in soils [21, 22]. This enzyme functions as a measurement of the metabolic state of soil microorganisms [23]. Dehydrogenase activity (DHA) is one of the most adequate, important and one of the most sensitive bioindicators, relating to soil fertility [24].

2. Methodology

2.1 Nursery and seedling preparation

Amelonado variety Tc1-Tc8 pods purchased from Cocoa Research Institute of Nigeria (CRIN) was used. Pods were opened longitudinally with a knife within 3 days of purchase and good beans were selected from the middle only of the pods, the surrounding pulp was removed using saw dust, the beans were washed afterwards. Each bean were singly placed on a moisted tray and covered under humid condition and sprouting was noticed within 24 hr. Then the emerging part of the germinating beans were inserted in the centre of the soil in a pre-filled polythene bag and adequate watering and weeding followed for the 4 month period of cultivation. Seedlings were generated with methods described by Adeyemi *et al.* [25].

2.2 Collection of fertilizers

Poultry droppings were collected from the Farm House, University of Benin, while fresh cow dung was collected from the Cattle Market in Aduwawa, Benin City. The inorganic fertilizer N.P.K 14-14-14 manufactured by Olam Industries was used.

2.3 Manure composting

The compost pile of poultry droppings and cow dung self-heated to temperatures > 55°C in the central core of the pile on a slab for 4 weeks; at 9 weeks the pile was turned for even distribution of heat and sparely watered. The pile reheated to > 50 - 55°C for one week, and then gradually cooled to ambient temperature by 13 weeks. The pile was allowed to cure for an additional 3 weeks before the compost was air-dried and stored in covered containers. Composite samples were obtained according to standard methods [26].

2.4 Application of fertilizer

The fertilizer application rate for cocoa seedling of 10 kg/ha for inorganic fertilizer and 2.5 t/ha for organic fertilizer [27, 28] was applied around the seedling at 1 month after planting (MAP) as described by Ooi and Chew [29].

2.5 Soil sample collection

A 50 g of the sowing soil was collected and the Root Adhering Soil (RAS) of seedlings were collected every month through 4 months after planting (MAP) [30].

2.6 Soil analysis

2.6.1 Physical properties

Particle size distribution was determined by hydrometer method using sodium hexametaphosphate (calgon) as a dispersant [31].

2.6.2 Chemical properties

i. Soil pH: Soil pH was determined electronically using a glass electrode pH meter in water as modified by Jones, 2001 [32].

ii. Total Nitrogen: Total nitrogen in the soil was determined using the macro Kjeidahl method [33].

iii. Organic Carbon: Organic carbon was determined using dichromate wet oxidation method as modified by Eno *et al*. [34].

iv. Available Phosphorus: Available phosphorous was determined by Bray P.1 method [35]. The P concentration in the extract was determine calorimetrically by the vandadomolybdate method and read by spectrophometer meter at wavelength of 400mm.

vi. Exchangeable Cations: Exchangeable Ca, Mg, K and Na were determined by method of Chapman, 1965 [36].

vii. Exchangeable Acidity: Exchangeable acidity was determined by leaching the soil with potassium chloride (KCL solution) and the extract titrated with standard solution hydroxide solution [34].

viii. Effective Cation Exchange Capacity (ECEC): ECEC was determined by summation of exchangeable cations and exchangeable acidity [37].

ix. Base Saturation: This was determined by summation of exchangeable cation divided by effective cation exchange capacity multiply by 100

2.7 Dehydrogenase activity

Dehydrogenase activity (DHA) was determined using the classical TTC method by Pepper and Gerba, 2004 [38]. To 6 g of sieved soil, 30 mg glucose, 1 ml of 3% TTC (2,3,5-triphenyltetrazoliumchloride) solution and 2.5 ml pure water were added. The samples were incubated for One (1) week at 27 ^{0}C in the dark. The formation of TPF (1, 3, 5 triphenylformazan) was determined spectrophotometrically and results were expressed as µg TPF g-1 dry soil.

2.8 Urease activity

The method of Kandeler and Gerber, 1988 [39] was followed to analyze soil urease activity. A 5g soil was taken into 250ml conical flask and 10ml of urea solution was added along with 20ml buffer solution (citric acid, KOH, NaOH) having pH 6.7. The solution was filtered after incubating at 37°C for 24 hours and then 3ml of filtrate was taken into 50 ml flask. Contents were mixed in the flask after adding 20 ml of water and 4 ml of mixed reagent (Phenol + NaOH) in it. Then 4ml of sodium hypochlorite solution was added, mixed and volume was made up to 50ml with distilled water. The absorbance of blue color was checked at 578 nm through spectrophotometer and results were expressed as µg g (soil)$^{-1}$ h^{-1}.

2.9 Statistical analysis

The data collected were analyzed using analysis of variance (ANOVA) and means were separated using Genstat statistical package 10th edition (Turkey test) LSD at the 5% level of significance.

3. Results and Discussion

Table 1: Chemical and physical analysis of sowing soil

Properties	Values
pH (water)	4.75
Organic Carbon (g/kg)	13.45
Total Nitrogen (g/kg)	0.43
Available Phosphorus (mg/kg)	4.52
Potassium (cmol/kg)	0.41
Cacium (cmol/kg)	1.49
Magnesium (cmol/kg)	0.22
Sodium (cmol/kg)	0.18
Exchangeable Acidity (cmol/kg)	1.77
Effective Cation Exchange Capacity (ECEC)	4.07
Base saturation (%)	56.51
Sand (g/kg)	885
Silt (g/kg)	60
Clay (g/kg)	55
Textural class	Sandy loam

Table 2: Chemical analysis of organic fertilizers

Properties	Poultry manure	Cow dung
pH (water)	6.73	6.40
Organic Carbon (g/kg)	14.40	38.20
Total Nitrogen (g/kg)	4.11	1.75
Available Phosphorus (mg/kg)	33.43	0.84
Potassium (cmol/kg)	6.00	5.52
Cacium (cmol/kg)	13.71	2.60
Magnesium (cmol/kg)	18.33	1.93
Sodium (cmol/kg)	0.35	0.20

Table 3: Enzymatic activity of soil samples

S/No.	**Treatment**	**Time**	**Dehydrogenase** (µg TPF g-1 dry soil)	**Urease** (µg g $(soil)^{-1}$ h^{-1})
1	Control	C	10.22	14.22
2		c1	10.43	14.37
3		c2	10.53	14.85
4		c3	10.55	14.52
5	Cow dung	cd1	11.42	15.31
6		cd2	11.18	15.4
7		cd3	10.85	15.31
8	NPK	npk1	10.46	14.32
9		npk2	10.53	14.46
10		npk3	10.48	14.57
11	Poultry manure	pm1	12.34	16.9
12		pm2	11.65	16.7
13		pm3	11.58	15.8

Key:
cd = cow dung manure cd 1,cd 2 and cd 3 = rhizosphere of soil amended with cow dung after 1 months, 2 months and 3 months respectively. Control = sowing soil c1, c 2, c 3 = un-amended soil after 1month , 2months and 3months respectively. Npk 1, 2 and 3 = rhizosphere of soil amended with NPK after 1 month, 2 and 3 months respectively. Pm = compost poultry manure pm1, 2 and 3 = rhizosphere of soil amended with poultry manure after 1 month , 2 months and 3 months respectively.

Table 4: means for dehydrogenase and urease activities

Treatment	**Dehydrogenase**	**Urease**
Cow dung	10.56a	14.53a
Control	10.86a	15.13a
Poultry manure	10.95a	15.23a
NPK	11.11a	15.19a

Means in same column followed by same letter(s) are not significantly different $P \leq 0.05$ using Turkey Test.

Nitrogen contents of the sowing soil was 0.43g/kg which is adequate for cocoa since the values were higher than the critical level (0.09%) of nitrogen for cocoa cultivation according to Egbe *et al*., 1989 [40]. While potassium value of 0.4 cmol/kg was above the critical level of 0.03 cmol/kg [41]; implying that large amounts of K required for good cocoa cultivation are available in these soils. The value obtained for exchangeable Ca (1.49 cmol/kg) were inadequate for cocoa production as it was below the critical value of 5 cmol/kg [42].

Comparing poultry manure to cow dung, the former had higher nutrient (N, P, K, Ca, Mg and Na) values in all but organic matter. The reason for cow dung having a higher value of organic matter than poultry manure is probably because of the dietary content of the cow dung and its digestion in the four – chambered digestive system of the cow. While the compositions of poultry manure (grains, saw dust or wood shavings) is probably the reason for its richness.

Due to the sensitivity to the environmental changes, soil enzymes activities have been proposed to evaluate the sustainability and economic effects of agricultural practices, and even to diagnose the soil categories [43, 44]. The result of the enzymatic assay in Table 3 show the soils amended with organic fertilizers having higher values for urease (15.40 – 16.90) and dehydrogenase (10.85 – 12.34) compare to the NPK amended and the control soil (14.22 – 14.83) and (10.43 – 10.55) respectively. Organic management increases overall enzyme activity in soil [45, 46]. Soils amended with poultry manure had the highest enzymatic activities, followed by cow dung. However, the control and the soil amended with inorganic fertilizer had about the same level of enzymatic activities; probably because of the pre-existing enzymatic activities in the sowing soil. In their study Moeskops *et al*., 2010 [46] comparing the effect of organic and conventional farming practice on soil microbial dynamics: found a strong negative impact of intensive chemical fertilizer and pesticide use on soil enzymes activities.

Enzymes in soil especially dehydrogenase are highly associated with the microbial biomass, which in turn affects the decomposition of organic matter. Zagal et al. [47] reported that dehydrogenase activity is strongly influenced by soil management. Similarly, incorporation of organic materials into soil promotes microbial activity and also soil urease activity [48, 49, 50, 51, 52]. Poultry manure (PM) is an excellent organic fertilizer as it contains high N, P, K and other essential nutrients [53]. It has been reported to supply P more readily to plants than other organic sources [54]. This is evident in the results of the chemical analysis of the organic fertilizers, where poultry manure had not only higher N-P-K values than cow dung but of other nutrients (Ca, Mg and Na). However, the effects of soil amendment on the enzymes were not statistically significant.

4. Conclusion

There are obvious benefits of application of organic manure to agricultural soil over inorganic fertilizers; as it profits enzymatic activities in the rhizosphere which further influences plant health and soil fertility.

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