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Effect of Inorganic and Organic Amendments on Yield of Cocoyam (*Colocasia esculenta*), and on Soil Properties

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Abstract The response of cocoyam *(Colocasia esuclenta* (L.) Schott) to various treatments(Twenty combinations of Treatments) of inorganic and organic amendments were studied under field conditions in 2011 and 2012 growing seasons at farm of the Regional Centre of Central Tuber Crops Research Institute, (CTCRI), Bhubaneswar. Odisha, India. Various enzymatic activities were studied for optimization cornel production. The physio chemical properties of soil quality with effect of integrated use of lime, biological, inorganic and organics on soil micro-flora were statistically analyzed for cocoyam production in lowland of Eastern India.

Keywords: *Colocasia esculenta, cormels, soil fertility, dehydrogenase activity, alkaline phosphatase, fluorescein diacetate hydrolysis assay*

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1. Introduction

Cocoyam (*Colocasia esculenta* (L.) Schott.) a member of the *Araceae* family is a subsistence and emergency food source in many parts of the world Taro or cocoyam (*Colocasia esculenta*) is a tropical food crop with high potential because of the high yield of the roots (or corms) and foliage (IFA 1992). Soils condition and its health status are vital for the agricultural production. This needed for long-term studies at fixed sites for monitoring changes in fertility status of soil. Long-term experiments in India suggested that under continuous cropping, changes in soil fertility due to imbalanced fertilization may be recognized as one of the important factor that limits crop yields. Application of synthetic fertilizers towards an increase in agricultural production of farming system is well known. But their injudicious use exhibits a detrimental effect on soil health (Kanwar and Katyal 1997). Bush fallow which had been an efficient, balanced and sustainable system for soil productivity and fertility restoration in the past is presently unsustainable due to high population pressure and other human activities which have resulted in reduced fallow period (Steiner K.G. 1991). Continuous cropping and long-term fertilization are liable to change soil properties and crop production, depending upon the type of management practices. Long-term fertilizer experiments provide best possible means to study changes in soil properties, dynamics of nutrient processes and future strategies for maintaining soil health. Therefore, the present study was undertaken.

2. Materials and Methods

2.1. Experimental Site

A long term field experiment has been laid out in the farm at Regional Centre of Central Tuber Crops Research Institute (CTCRI), Bhubaneswar during 2006 to study the effect of soil amendments, biological, inorganic and organic sources on soil fertility, yield and quality of *Colocasia esculent* in an acid alfisol and the soils have been used for the present investigation after harvest of 1st cropping cycle of *Colocasia esculenta*.

2.2. Field Experiment

A field experiment was conducted in an acidic alfisol at farm of the Regional Centre of Central Tuber Crops Research Institute,Bhubaneswar, Govt of India, during 2011-2012 to study the effect of integrated use of lime, biological, inorganic and organic sources on *Colocasia esculenta.*

2.3. Treatment Details

Control; 100% N; 100% P; 100% K; 100% NP; 100% NK; 50% NPK; 100% NPK; 150% NPK; FYM +100% NPK (based on soil test i.e. 85:15:57 kg NPK ha^{-1}); FYM + lime + ½ NPK; Green manure + Lime + ½ NPK; FYM+ ½ NPK + $ZnSO_4$; FYM + lime + ½ NPK + $ZnSO_4$; FYM + ½ NPK + B; FYM + lime + ½ NPK + B; FYM + ½ NPK + $MgSO_4$;FYM + lime + ½ NPK + $MgSO_4$; FYM + ½ NPK + VAM; FYM + lime + ½ NPK + VAM.

The experimental soil is sandy loam, acidic (pH 5.51), non saline (0.58 dS m^{-1}), and having 0.33% organic carbon, 0.075% total N, 141.75, 12.32 and 217.84 kg ha^{-1} of available nitrogen (N), phosphorus (P) and potassium (K), respectively. Lime and Farmyard manure were applied well in advance of planting. Cowpea was raised up to 45 days, chopped, and incorporated the respective plots. Vesicular Arbuscular Mycorrhiza (VAM) culture along with FYM and sand was broad casted on a raised bed and planted the cormels separately, grown up to 45 days, uprooted and transplanted in the respective treatments along with other treatments. A fertilizer dose of 80-60-80 kg N, P_2O_5 and K_2O ha^{-1} in the form of urea, single super phosphate and muriate of potash were applied in respect of 100% NPK. One-third of N, entire P_2O_5 and ½ K_2O at before planting, ⅓ N and ½ K_2O at 45 days after planting (DAP), and the balance ⅓ N at 75 DAP were applied as per the treatments. Colocasia (*cv* Muktakeshi) cormels were raised in a nursery and the seedlings were planted at a spacing of 50 x 30 cm. All the inter cultural practices were followed as per the schedule and the crop was harvested at maturity i.e. 165 days after planting of the seedlings. Yield parameters like number of cormels/plant, average cormel weight, cormel yield per plot were recorded at harvest. Tuber samples (cormels) were collected at harvest, washed thoroughly, and used for estimation of bio-chemical constituents like starch, total sugars, and dry matter. Per cent yield response was computed as

$$\text{Yield response }(\%) = \frac{(\text{Treatment yield} - \text{Control yield})}{\text{Control yield}} \times 100$$

2.4. Collection and Processing of Soil Samples

Soil samples were collected from individual treatments of the experiment after harvest of the crop at a depth of 0.30 m. Soil samples were collected by digging a V - shaped hole in the soil, after clearing the surface litter and placed in polythene bags, and labeled. Less than a teaspoonful of soil is actually used for the laboratory analysis. That small amount must represent the entire area for which the recommendation is to be made. Collected soil samples were shade dried, pounded, sieved with 2.0 mm sieve and used for estimation of physicochemical properties by using standard procedures as outlined by (Jackson 1973). Fresh soil samples from individual plots in all the three replications were collected pooled together, removed the gravel, stubbles, roots and other waste materials, sieved with 0.5 mm sieve, properly labeled, preserved in refrigerator at 4°C and the compound samples used for enumeration of microbial counts and estimation of enzyme activities.

2.5. Collection and Processing of Plant Samples for Proximate Composition

The cormel samples were collected at harvest, washed thoroughly with distilled water, oven dried at 60°C, and dry weights were recorded. Total sugars were estimated in the alcohol filtrate and starch was determined in the residue as per the procedure outlined by (Moorthy and Padmaja 2002).

2.6. Isolation and Identification of Bacteria

The soil samples were passed through a sieve (1.7 mm mesh) to remove large pieces of debris and vegetation. The bacteria were originally isolated by plating dilutions of soils in saline solution (0.9% NaCl) on nutrient agar and incubated at 37°C for 48 h. The developed colonies were counted in plates and the average number of colonies per three plates was determined. The number of total bacteria (CFU) per gram dry weight soil was determined. Individual colonies of bacteria which varied in shape and color were picked up and purified by streaking on nutrient agar. The bacterial isolates were kept on nutrient agar at 4°C and re-cultured in every 4 weeks. The bacterial isolates were identified on the basis of classification schemes published in Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984).

3. Determination of Enzyme Activities

3.1. Dehydrogenase Enzymatic Activity (DHA)

Soil DHA determination in soils was first initiated by (Lenhard, G., 1956). Since then it has been widely used because of its simplicity as compared to other quantitative methods. The method was later on modified (Casida, L.E., Jr., D.A. Klein and T. Santoro, 1964). (Von Mersi, W. and F. Schinner, 1991). 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 biogeochemical cycles (Ladd, J.N., 1985). It has also found that measurement of changes in soil enzyme activities may provide a useful index of changes in soil quality (Visser, S. and D. Parkinson, 1992).

3.2. Different Method Used for Measurement of Dehydrogenase Activity

Use of Tetrazolium Salts: Dehydrogenase assays based on the reduction of 2, 3, 5- triphenyltetrazolium chloride (TTC) to the creaming red-colored formazan (TPF), have been used to determine microbial activity in soil Water-soluble tetrazolium salts are the preferred oxidants because they form water-insoluble colored formazans which can be measured spectrophotometrically. Soil was Prepared with $CaCO_3$ and dispensed in three tubes as 6 g each. The total volume of fluids added to the soil was 3.5 ml; this included any fluids added during pre incubation of the soil. Most substrate solution concentrations were 1% dextrose solutions. All of these were sterilized by autoclaving and 1 ml was added to the soil at the time of TTC addition. However, the substrates extracellular in the soil. Measurement of dehydrogenase were added as 0.5 ml of a double-strength solution if there was danger of exceeding the 3.5-ml fluid volume limit.TTC (Calbiochem, San Diego, Calif.) was prepared as a 3% aqueous solution and was sterilized by passage through 0.30-, um membrane filter (Millipore Corp., Bedford, Mass.). Each tube received 1 ml of TTC, except for the soil blank, which received water instead. After addition, the water, substrate and TTC were simultaneously mixed through the soil with a sterile glass rod; then rubber stoppers were inserted and the tubes were incubated at 37°C at different incubation period of 6 hrs, 12 hrs, & 24 hrs. Upon completion of incubation, the tubes could be extracted immediately. Each soil sample was transferred with methanol to a funnel containing Whatman no. 5 filter paper (W. and R. Balston, Ltd.) placed on a 100-ml graduated cylinder. Additional portions of methanol were passed through the soil until 50 ml of methanol, containing the formazan, had been collected in the graduated cylinder. If the filtrate passing from the funnel still had a red color, additional methanol was passed through the soil until all formazan had been extracted and corrections in calculations were made for the additional methanol. The red methanolic solutions of the formazan were read at 485 nm against the extract from the non-TTC soil blank by using a Spectronic 20 colorimeter (Bausch and Lomb, Rochester, N.Y.). The values obtained were compared against a formazan (Calbiochem) standard curve prepared with methanol and they are reported as milligrams of formazan per gram of soil.

3.3. Assay of Acid and Alkaline Phosphatase

Due to relative importance of phosphomonoesterases in soil organic P mineralization and plant nutrition, their assay in soil assumes more importance. The enzymes are classified as acid and alkaline phosphomonoesterases, because they show optimum activities in their respective pH ranges. Alkaline phosphomonoesterases is contributed both by plant roots and soil-inhabiting microbes. Of the various methods available for assay of phosphomonoesterases activity in soils, the method developed by Tabatabai and Bremner (1969) is the most rapid, accurate and precise. It involves colorimetric estimation of the p-nitrophenol related by phosphomonoesterases activity, when the soil is incubated with buffer (at pH 6.5 and 11 for acid and alkaline phosphomonoesterases activities, respectively) sodium p-nitrophenyl phosphate solution and toluene. Alkaline phenol has a yellow colour, allowing it to be estimated colorimetrically. The $CaCl_2$-NaOH treatment described for extraction of p-nitrophenol after incubation serves to stop the phosphomonoesterases activity, to develop yellow color and to provide quantitative recovery of p-nitrophenol from soils.

3.4. Fluorescein Diacetate Hydrolysis Assay

The fluorescein diacetate (FDA) hydrolysis assay measures the enzyme activity of microbial populations and can provide an estimate of overall microbial activity in an environmental sample. The assay is considered non-specific because it is sensitive to the activity of several enzyme classes including lipases, esterases, and proteases. Activity of these enzymes results in the hydrolytic cleavage of FDA (colorless) into fluorescein (fluorescent yellow-green). In the FDA assay, the environmental sample is mixed with FDA and buffer and incubated with shaking for 1 – 2 hours. The intensity of the resulting yellow-green color is indicative of the amount of enzymatic cleavage of the FDA molecule and the overall enzymatic activity in the sample. Quantification of enzyme activity is performed by assessing the intensity of color formation using spectrophotometry. Five standard solution of fluorescein diacetate were prepared by the following procedure (Green *et al*., 2006).

4. Result and Discussion:

4.1. Effect of Inorganic, Biological and Organic Sources on Soil Fertility, Yield and Proximate Composition of *Colocasia Esculenta*

4.1.1. Effect of single and balanced fertilization

The results in Table 1 revealed that application of recommended doses of N has recorded highest cormel yield (8.49 t ha^{-1}) followed by K (7.97 t ha^{-1}) and P (7.90 t ha^{-1}). Among the three major nutrients, single application of recommended doses of N, P and K showed a yield response of 28, 19, and 20 per cent, respectively over that of control, while the combined application of NK showed higher yield response (59.8%) rather than NP (57.8%) over control. (Table 1)

Table 1. Effect of lime, inorganic, biological and organic sources on yield of Colocasia esculenta

Treatments	Cormels/ plant	Av. cormel weight (g)	Cormel yield (kg ha^{-1})	Yield response (%)
Control	6.47	21.95	6637.7±155.15	-
100%N	7.80	24.78	8491.7±82.41	27.9
100%P	7.33	25.14	7898.3±132.77	19.0
100%K	7.67	23.89	7971.7±38.69	20.1
100%NP	9.83	26.40	10476.7±77.69	57.8
100%NK	9.60	25.04	10608.3±106.29	59.8
50%NPK	9.40	22.28	9113.3±159.08	37.3
100%NPK	10.53	30.63	13138.3±406.20	97.9
150%NPK	11.17	32.47	14297.7±959.38	115.4
FYM+100%NPK[a]	11.07	32.25	14163.3±200.48	113.4
L+ FYM +½NPK	10.70	27.45	11053.3±260.34	66.5
L+GM+½NPK	10.80	29.16	12365.0±183.76	86.3
FYM+½NPK+$ZnSO_4$ [b]	10.07	31.86	14263.3±152.28	114.9
L+FYM +½NPK+$ZnSO_4$[b]	11.40	32.07	14691.7±140.04	121.3
FYM+½NPK+B[c]	9.53	29.84	13593.3±138.42	104.8
L+FYM +½NPK+B[c]	9.70	30.69	13933.3±261.48	109.9
FYM+½NPK+$MgSO_4$[c]	10.40	30.59	14108.3±52.551	112.5
L+ FYM+½NPK+$MgSO_4$[c]	10.77	32.75	14273.3±168.56	115.0
FYM +½NPK+VAM	10.33	29.45	13968.3±234.78	110.4
L+FYM+½NPK+VAM	10.90	30.38	14041.7±120.67	111.5

a=Based on soil test; L=Lime; b=10kg ha^{-1}; GM=Green manure; c=2.5kg ha^{-1}.Result of one-way ANOVA was not significant; *Result of ANOVA was significant at 0.05 probability level.

Balanced application of recommended doses of NPK showed a yield response of 98% over control. All other yield attributes like number of cormels/plant, average weight of cormel and cormel yield were significantly increased due to application of balanced doses of NPK rather single or combined application of N and K.

4.1.2. Effect of Graded Doses of NPK

Application of graded doses of NPK showed an increasing trend of cormel yield of Colocasia. The yields were increased significantly due to sub optimal and optimal doses of NPK and it was not significantly increased due to super optimal doses of NPK. It was observed that the increase in cormel yield was pronounced to be 37, 98 and 115% with respect to 50, 100 and 150% of the recommended doses of NPK over control. The tuber (cormel) yields of Colocasia were increased considerably due to application of super optimal doses of NPK fertilizers in low and marginal soils, where it is being cultivated extensively. Similar findings were reported by (Halavatau *et al*. 1998).

4.1.3. Effect of Organic Manures

In-situ incorporation of green manure along with half of the recommended doses of NPK and lime has recorded an increase of 86% of cormel yield over control followed by lime + FYM + ½ NPK (67%). However, green manuring combined with lime and ½ NPK showed an increase of 36% of cormel yield over that of 50% NPK. Incorporation of FYM + lime + ½ NPK showed an increase of 21% cormel yield in comparison to 50% NPK, indicating that green manuring had positive response on yield parameters of Colocasia, which might be due to higher retention and availability of all the essential nutrients as well as improvement in soil physical and biological properties (Kamara and Lahai 1997). The varying yield response to the organic sources might be attributed to the nature and amount of nutrients present in the manures, their decomposition and nutrient release pattern in the soils (Mubarik Ali 1999). Application of lime in combination with inorganic and organic sources had favorable effect on total biomass and the effect of organic sources is more pronounced when they applied in combination with lime rather than their sole application. The results emphasized that lime had profound influence when it applied in combination with organic sources in comparison to inorganic fertilizers might be ascribed to enhanced nutrient transformations and improvement in soil physical properties that contributed in augmenting the crop yields (Ossom and Rhykerd 2008).

4.1.4. Effect of Zn, B and Mg

Significantly highest cormel yield (14.69 t ha^{-1}) was recorded due to integrated application of lime + FYM + ½ NPK + $ZnSO_4$ with a yield response of 121, 61 and 12 per cent over that of control, 50% NPK and 100% NPK, respectively. However, combined application of lime + FYM + ½ NPK + $MgSO_4$ recorded an increase of 115, 57 and 9 per cent cormel yield over that of control, half and full doses of NPK, respectively. It was observed that conjunctive use of lime, FYM, ½ NPK and $ZnSO_4$ showed relatively higher yield response (2.8%) over that of super optimal doses of NPK. The per cent yield response was found highest with respect to Zn (33) followed by Mg (29) and B (26) over that of lime + FYM + ½ NPK. (Figure 1).

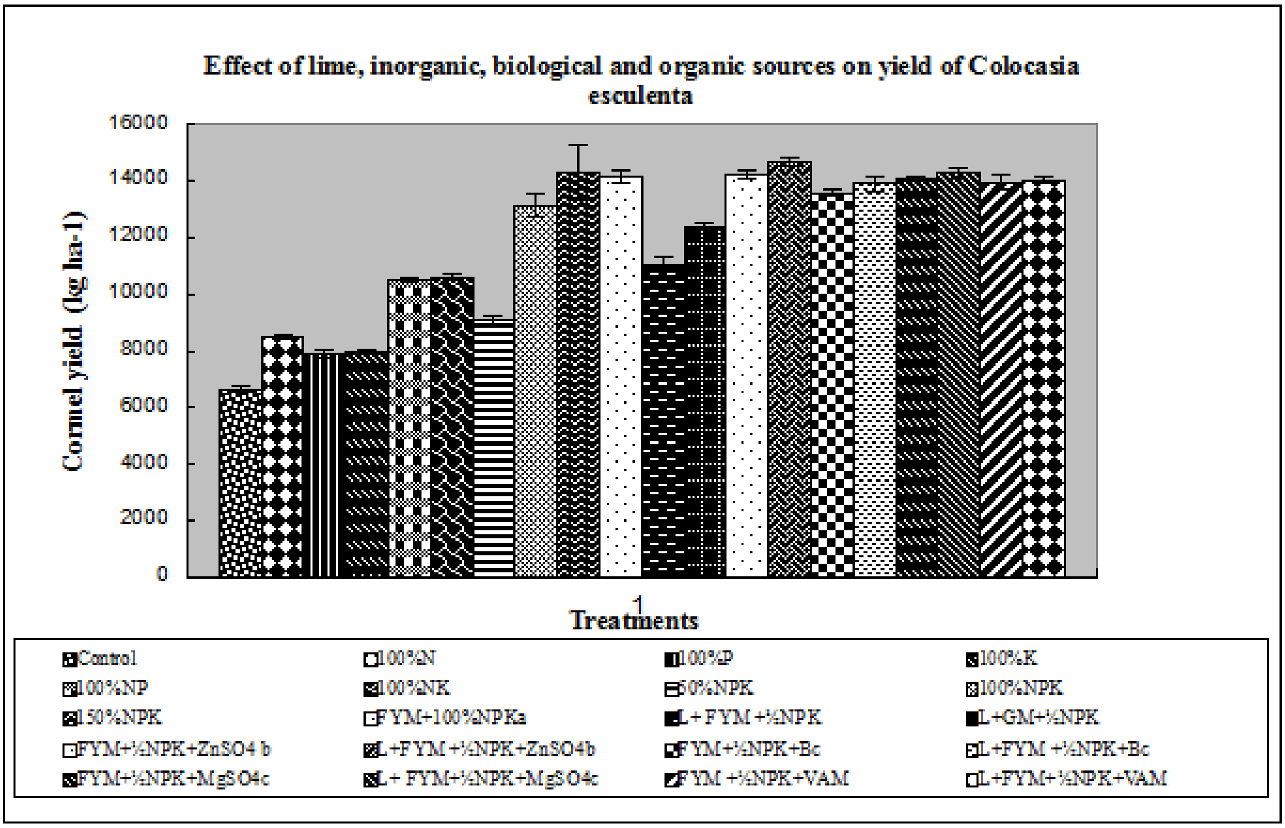

Figure 1. Effect of lime, inorganic, biological and organic sources on yield of *Colocasia esculenta*

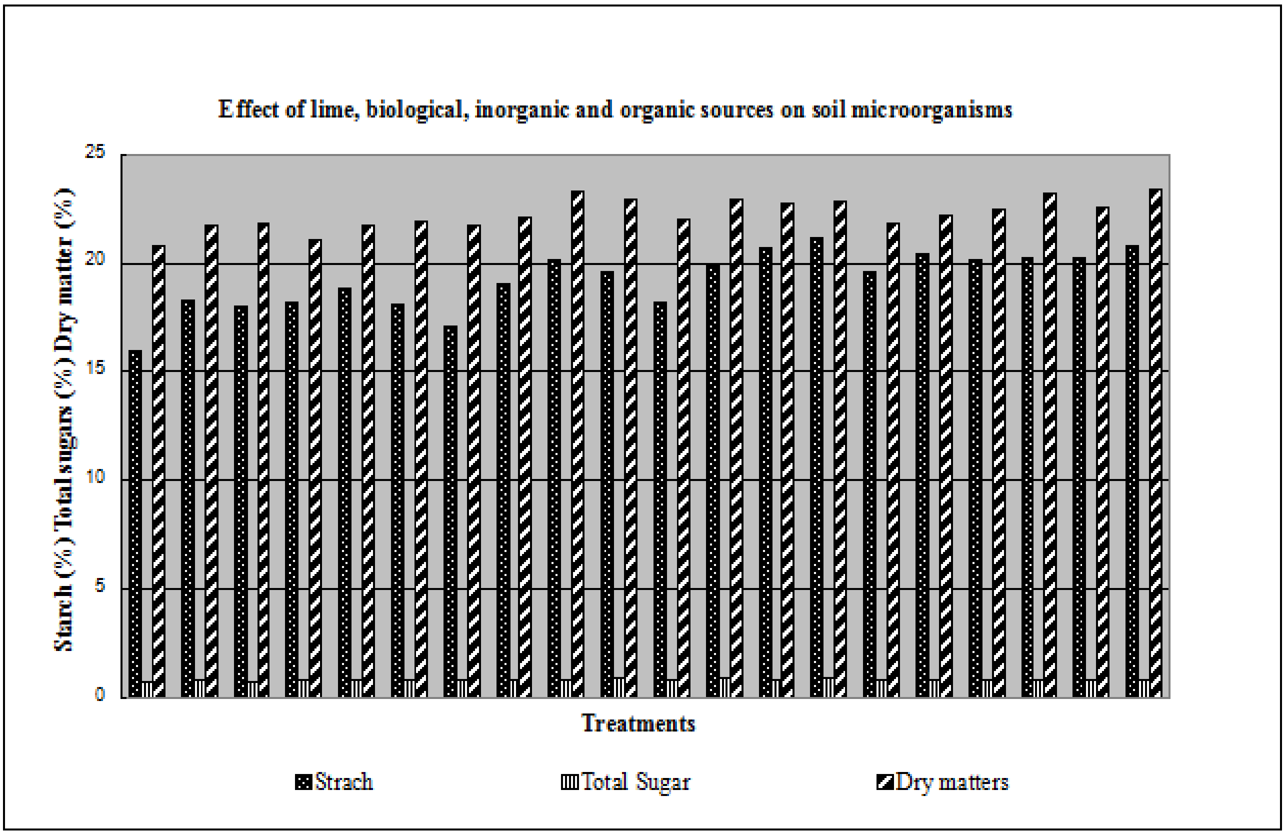

Figure 2. Effect of lime, biological, inorganic and organic sources on soil microorganisms

4.1.5. Effect of VAM

Inoculation of VAM fungi combined with half of the recommended doses of NPK and FYM recorded a cormel yield of 13.97 t ha^{-1} with an increase of 110, 53, and 6 per cent over that of control, 50% NPK and 100% NPK, respectively. However, incorporation of VAM in combination, when applied along with organic manures. (Figure 3)

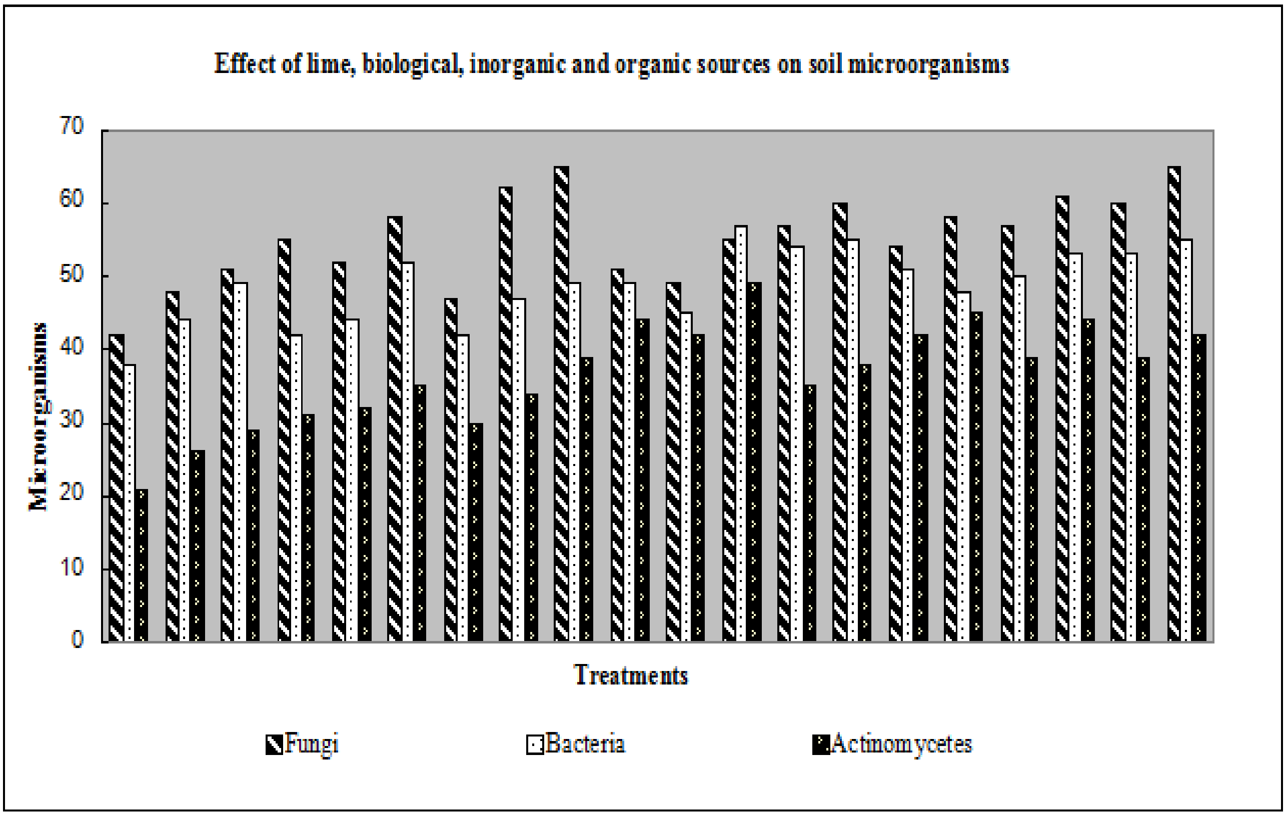

Figure 3. Effect of lime, biological, inorganic and organic sources on soil microorganisms

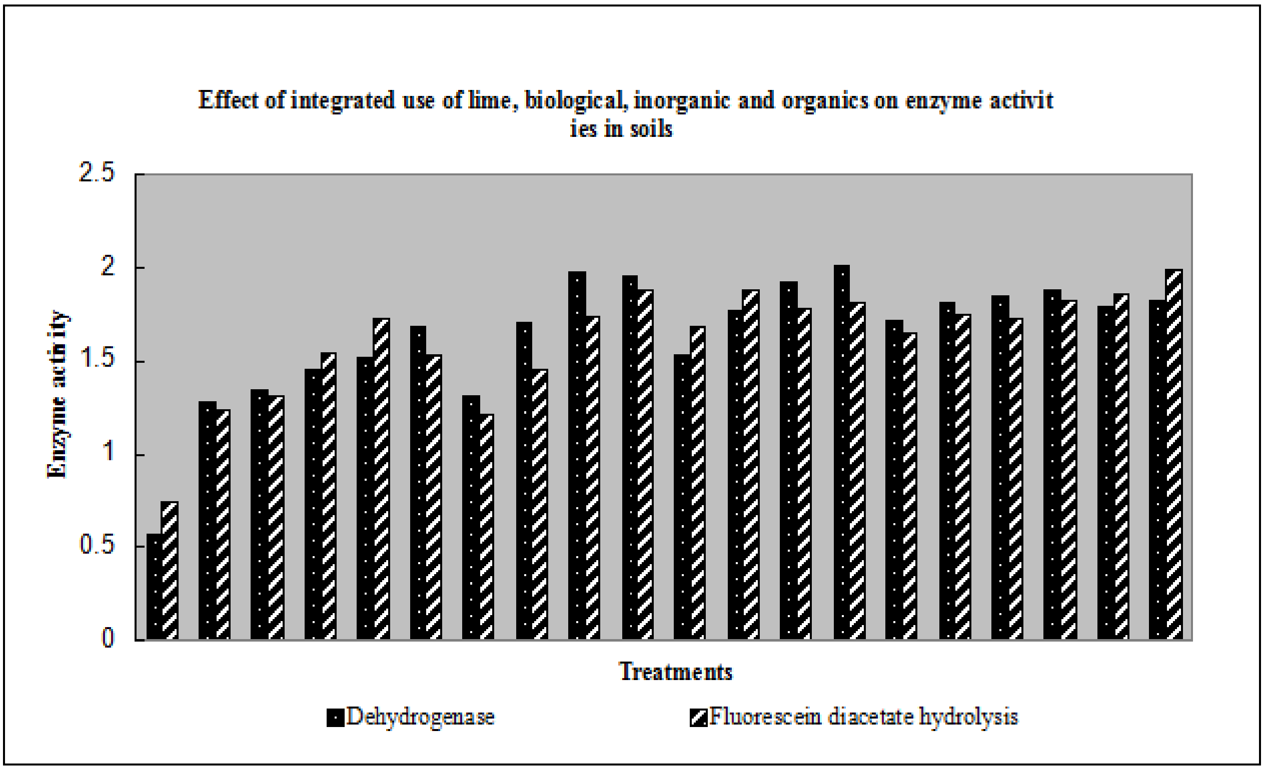

Figure 4. Effect of integrated use of lime, biological, inorganic and organics on Enzyme activities in soils

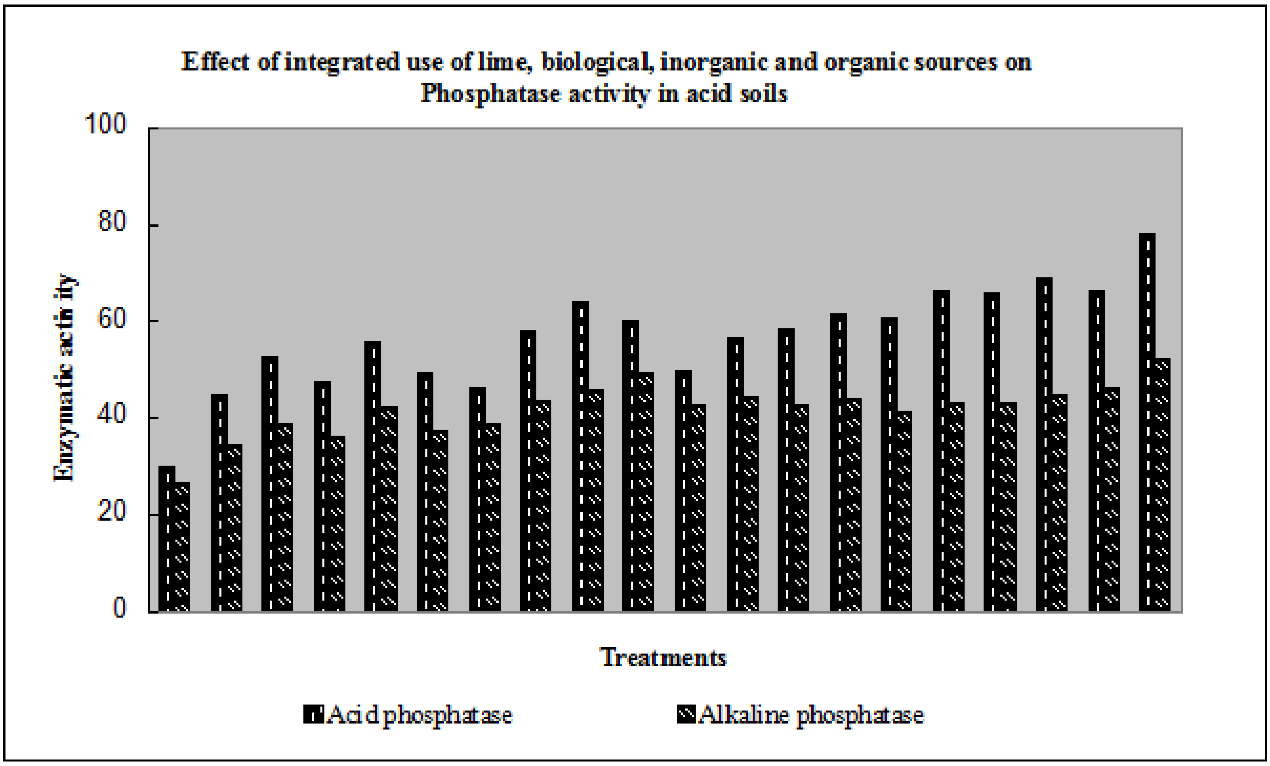

Figure 5. Effect of integrated use of lime, biological, inorganic and organic sources on phosphatase activity in acid soils

4.2. Effect of Inorganic, Biological and Organic Sources on Proximate Composition.

Significantly highest starch (21.18%, fresh wt. basis) content was recorded due to integrated application of lime + FYM + ½ NPK + $ZnSO_4$ (Table 2) with an increase of 32% over control followed by lime + FYM + ½ NPK + VAM (20.80%). Balanced application of NP, NK, and NPK showed higher starch content over that of single application of recommended doses of N, P, and K. Addition of graded doses of NPK showed an increase of 7, 19 and 26% starch over control. Among the organics, *in-situ* incorporation green manure in combination with lime and ½ NPK showed higher starch (19.87%) followed by lime + FYM + ½ NPK (18.21%). Fungal inoculation of the seedlings with VAM showed an increase of 14% starch over that of lime + FYM + ½ NPK.

Total sugars varied from 0.75 – 0.89% irrespective of treatments with highest sugars being due to incorporation of green manure in combination with lime and ½ NPK. Addition of micronutrients in combination with lime, NPK and FYM showed relatively higher sugars than the inorganic and organic manures. Significantly highest dry matter (23.40%) was recorded due to integrated application of lime + FYM + ½ NPK + VAM at par with 150% NPK (23.28%) and lime + FYM + ½ NPK + $MgSO_4$ (23.18%). No significant variation of dry matter was observed due to sole application of N, P and K as well as combined application of NP, NK and NPK. (Table 2)

Table 2. Effect of lime, biological, inorganic and organic sources on soil microorganisms

Treatments	Starch (%)	Total sugars (%)	Dry matter (%)
Control	16.01	0.75	20.84
100% N	18.31	0.79	21.75
100% P	18.00	0.77	21.86
100% K	18.20	0.84	21.08
100% NP	18.87	0.83	21.76
100% NK	18.12	0.85	21.95
50% NPK	17.06	0.79	21.78
100% NPK	19.06	0.85	22.11
150% NPK	20.14	0.85	23.28
FYM + 100% NPK	19.56	0.88	22.91
L + FYM + ½ NPK	18.21	0.86	22.01
L + GM+ ½ NPK	19.87	0.89	22.93
FYM + ½ NPK + $ZnSO_4$	20.69	0.87	22.79
L + FYM +½ NPK + $ZnSO_4$	21.18	0.88	22.83
FYM + ½ NPK + B	19.60	0.81	21.79
L + FYM + ½ NPK + B	20.44	0.82	22.16
FYM + ½ NPK + $MgSO_4$	20.17	0.83	22.51
L + FYM + ½ NPK + $MgSO_4$	20.26	0.85	23.18
FYM + ½ NPK + VAM	20.24	0.81	22.58
FYM + L + ½ NPK + VAM	20.80	0.83	23.40

L=Lime; GM=Green manure

4.3. Effect of Inorganic, Biological and Organic Sources on Soil Quality

4.3.1. Physico-Chemical Properties

The soil pH after harvest of colocasia during 2011-12 ranged from 4.97 to 6.10 (Table 3). Application of NPK alone showed decreasing trend of pH, whereas integrated use of inorganics and organic sources considerably improved the soil pH. Application of lime and higher amount of organic sources, which can buffer pH and thus, countering the soil acidity. *In-situ* incorporation of green manure along with lime and 50% NPK showed an increase of 0.69 units of pH over control. Relatively higher status of soil pH was observed under 100% NPK when applied in combination with organic manures as compared to application of NPK alone since organic matter has high cation exchange capacity and it facilitated retention of exchangeable bases (Svotwa *et al.*, 2007); (Ossom and Rhykerd, 2008).

Table 3. Effect of lime, inorganic, biological and organic sources on residual soil fertility

Treatments	pH (1:2)	EC (dS m^{-1})	Org. C (%)	Total N (%)	Available nutrient (kg ha^{-1})		
					N	P	K
Control	5.18	0.124	0.290	0.0762	143.0	82.88	145.0
100% N	4.97	0.206	0.422	0.0852	175.6	89.60	150.9
100% P	5.21	0.186	0.343	0.0834	154.3	100.80	179.3
100% K	5.37	0.236	0.356	0.0849	161.8	94.08	219.2
100% NP	5.18	0.305	0.462	0.0886	178.1	107.52	193.2
100% NK	5.24	0.309	0.409	0.0913	185.7	96.32	228.4
50% NPK	5.46	0.169	0.394	0.0872	158.1	92.14	160.4
100% NPK	5.39	0.327	0.515	0.0914	178.4	114.24	260.3
150% NPK	5.32	0.477	0.541	0.0926	184.4	123.20	272.3
FYM + 100% NPK	5.58	0.216	0.528	0.0908	188.2	90.32	274.3
L + FYM + ½ NPK	5.75	0.197	0.541	0.0912	163.1	95.84	202.0
L + GM + ½ NPK	5.97	0.198	0.634	0.0937	164.3	105.28	236.1
FYM + ½ NPK + $ZnSO_4$	5.46	0.158	0.462	0.0871	195.7	93.26	210.8
L + FYM + ½ NPK + $ZnSO_4$	5.63	0.189	0.607	0.0885	217.0	98.33	239.3
FYM + ½ NPK + B	5.47	0.185	0.568	0.0869	161.8	103.04	193.2
L + FYM + ½ NPK + B	5.59	0.244	0.603	0.0877	179.4	109.25	213.2
FYM + ½ NPK + $MgSO_4$	5.67	0.266	0.555	0.0892	190.7	114.24	236.5
L + FYM + ½ NPK + $MgSO_4$	6.10	0.307	0.687	0.0910	233.3	118.07	158.6
FYM + ½ NPK + VAM	5.52	0.163	0.564	0.0883	191.9	110.76	249.7
FYM + L + ½ NPK + VAM	5.83	0.169	0.594	0.0890	227.0	128.12	265.1

L=Lime; GM=Green manure

The soils are non saline (EC ranged from 0.12 - 0.48 dS m^{-1}). Organic carbon content varied from 0.29 to 0.69% (mean of 0.50%). Continuous cropping without fertilization or manuring of the soil led to reduction in organic C content from 0.36 to 0.29% (in control). Significantly highest organic carbon (0.69%) was recorded due to integrated application of lime + FYM + ½ NPK followed by lime + green manure + ½ NPK (0.63%) and lime + FYM + ½ NPK + $ZnSO_4$ (0.61%). Graded doses of NPK significantly improved the organic carbon content of the soils. Incorporation of green manure along with lime and ½ NPK showed higher org. C (0.63%) rather than lime + FYM + ½ NPK (0.54%). Apart from yield gains, green manuring adds organic matter, improves soil physical properties and neutralizes soil pH (Hartemink and O'Sullivan, 2001).

Total N in the soils varied from 0.0762 to 0.0937% with a mean value of 0.0882%. Significantly highest total N (0.0937%) was observed due to application of super optimal doses of NPK. Addition of organics along with inorganics showed significant improvement in total N content of the soils. This could be attributed to N mineralization pattern of these organics and indirect influence on physico-chemical characteristics of the soil (Singh *et al*., 2002). Long term application of organic manure combined with NPK fertilizers increased the contents of soil organic matter, N, P, and K, and improved the soil physico-chemical properties as well as enzyme activities.

Addition of nitrogenous fertilizers tends to increase the available N status of the soil by 11, 25 and 29% in respect of 50, 100 and 150% NPK over control. Significantly highest available N (233 kg ha^{-1}) was recorded due to integrated application of lime + FYM + ½ NPK + $MgSO_4$ at par with lime + FYM + NPK + VAM (227 kg ha^{-1}). Incorporation of organics along with 50% NPK and lime has moderately aggravates the available N status by 3.2 and 4.0 per cent due to FYM and green manure, respectively over that of 50% NPK. The humus produced from the organic manures on their decomposition, can supply almost all the essential nutrients slowly but steadily.

To the growing crops besides direct supply from the inorganic fertilizers contributed in improvement of available nutrient status of the soil. The magnitude of increase in available P under 50, 100 and 150% NPK was 11, 38 and 49 per cent over control. Significantly highest available P was observed due to integrated application of lime + FYM + ½ NPK + VAM (128.1 kg ha^{-1}) followed by 150% NPK (123.2 kg ha^{-1}). Incorporation of organics in combination with lime and half of the recommended doses of NPK showed relatively higher available P over that of 50% NPK. Increase in available P content of the soil attributed by decomposition of organic manures which could have enhanced the labile P in the soil by complexing Ca, Mg & Al (Subramanian and Kumaraswamy, 1989) and solubilization of phosphate rich organic compounds through release of organic acids upon decomposition of organic matter and chelation of organic anions with Fe & Al resulting into effective solubilization of inorganic phosphates into soil (Subba Rao, 1999). Significantly highest available K (274.3 kg ha^{-1}) content in the soil was observed due to combined application of FYM and recommended doses of NPK based on soil test followed by 150% NPK (272.3 kg ha^{-1}) and lime + FYM + ½ NPK + VAM (265.1 kg ha^{-1}). It seems that the crop requirements were partly met from the released K and both the applied K and released K brought out available K build up in the soil. Addition of lime in combination with limited doses of NPK and organic manures showed a marginal increase in available K over that of inorganic and organic sources. The differential release pattern of non-exchangeable K from the soil reserve besides variation in K uptake by the crop will be held responsible for such differences in the available K status of the soil (Svotwa *et al*. 2007). Among the organics an increase of 47 and 26 per cent of available K was observed due to incorporation of green manure along with lime + ½ NPK and lime + FYM + ½ NPK, respectively over that of 50% NPK (Table 3).

4.4. Effect of Integrated Use of lime, Biological, Inorganic and Organics on Soil Micro-Flora

Incorporation of organic sources had profound influence on microbial populations. Organic matter acts as store house for various essential nutrients as well as it provides congenial environment for growth and multiplication of various microbial communities in the soil. The organic matter in soil being the chief source of energy and food for most of the soil organisms, it has great influence on the microbial population. Organic matter influence directly or indirectly on the population and activity of soil microorganisms. It influences the structure and texture of soil and thereby activity of the microorganisms. Fungal (VAM) inoculation of Colocasia in combination with optimum doses of NPK and organic manure improved various microbial colonies in the soil; however, FYM application in combination with NPK and $MgSO_4$ further enhanced the microbial counts in the soil (Table 4).

Table 4. Effect of lime, biological, inorganic and organic sources on soil microorganisms

Treatments	Fungi (1 x 10^4 cells g^{-1} soil)	Bacteria (1 x 10^5 cells g^{-1} soil)	Actinomycetes (1 x 10^4 cells g^{-1} soil)
Control	42	38	21
100% N	48	44	26
100% P	51	49	29
100% K	55	42	31
100% NP	52	44	32
100% NK	58	52	35
50% NPK	47	42	30
100% NPK	62	47	34
150% NPK	65	49	39
FYM + 100% NPK	51	49	44
L + FYM + ½ NPK	49	45	42
L + GM + ½ NPK	55	57	49
FYM + ½NPK + $ZnSO_4$	57	54	35
L + FYM + ½ NPK + $ZnSO_4$	60	55	38
FYM + ½NPK + B	54	51	42
L + FYM + ½ NPK + B	58	48	45
FYM + ½ NPK + $MgSO_4$	57	50	39
L + FYM + ½ NPK + $MgSO_4$	61	53	44
FYM + ½ NPK + VAM	60	53	39
FYM + lime + ½ NPK + VAM	65	55	42

L=Lime; GM=Green manure

Application of lime showed positive influence on growth and multiplication of soil microbes in the soil. Incorporation of lime enhances the soil fertility by providing better atmosphere for microbial growth. It has marginal effect on soil microflora. Application of lime in combination with inorganic and organic sources had favorable effect on total biomass and the effect of organic sources is more pronounced when they applied in combination with lime rather than their sole application. Incorporation of lime along with organic manures showed greater effect on microbial counts in the soil as pH had a definite influence/effect on quantitative and qualitative composition of soil microbes. Most of the soil bacteria, blue-green algae, diatoms and protozoa prefer a neutral or slightly alkaline reaction between pH 4.5 and 8.0 and fungi grow in acidic reaction between pH 4.5 and 6.5 while actinomycetes prefer slightly alkaline soil reactions. Soil reactions also influence the type of the bacteria present in soil. Of all the treatments, application of super optimal doses of NPK showed higher counts of fungi (65 x 10^4 cells g^{-1} soil), whereas *in-situ* incorporation of green manure combined with lime + ½ NPK has recorded higher population of total bacteria (57 x 10^5 cells g^{-1} soil) and total actinomycetes (49 x 10^4 cells g^{-1} soil). Among the organic sources, *in-situ* incorporation of green manure had significant influence on various soil microbes as compared to FYM, which may be ascribed to greater build up of organic matter with the green manuring. Application of micro and secondary nutrients in combination with soil amendments, organic manure and optimum doses of NPK enhanced the population of total fungi, bacteria and actinomycetes rather than sole application of inorganic chemical fertilizers.

4.4.1. Effect of Integrated Use of Lime, Inorganic, Biological and Organic Sources on Dehydrogenase Activity

Perusal of the data in (Table 5) revealed the significant improvement in enzyme activities over control. Application of optimum doses of K has shown higher biological activity rather than P and K. Increased doses of NPK enhance the biological activity of soil.

Table 5. Effect of integrated use of lime, biological, inorganic and organics on Enzyme activities in soils

Treatments	Dehydrogenase (µg TPF hr^{-1} g^{-1} soil)	Fluorescein diacetate hydrolysis assay (µg g^{-1} hr^{-1})
Control	0.574	0.749
100% N	1.276	1.241
100% P	1.349	1.315
100% K	1.461	1.548
100% NP	1.517	1.725
100% NK	1.682	1.537
50% NPK	1.319	1.218
100% NPK	1.702	1.453
150% NPK	1.981	1.741
FYM + 100% NPK	1.961	1.887
L + FYM + ½ NPK	1.534	1.684
L + GM + ½ NPK	1.769	1.882
FYM + ½ NPK + $ZnSO_4$	1.927	1.780
L + FYM + ½ NPK + $ZnSO_4$	2.012	1.819
FYM + ½ NPK + B	1.714	1.652
L + FYM + ½ NPK + B	1.816	1.755
FYM + ½ NPK + $MgSO_4$	1.853	1.730
Lime + FYM + ½ NPK + $MgSO_4$	1.884	1.832
FYM + ½ NPK + VAM	1.798	1.864
FYM + lime + ½ NPK + VAM	1.829	1.986

L=Lime; GM=Green manure

Among the organic sources, incorporation of green manure along with lime and 50% NPK showed higher dehydrogenase activity (1.1.769 µg TPF hr^{-1} g^{-1} soil) in comparison to FYM (1.534 µg TPF hr^{-1} g^{-1} soil). Of all the treatments, integrated application of lime + FYM + ½ NPK + $ZnSO_4$ has recorded highest dehydrogenase activity (2.012 µg TPF hr^{-1} g^{-1} soil) followed by 150% NPK (1.981 µg TPF hr^{-1} g^{-1} soil) and FYM + 100% NPK based on soil test (1.961 µg TPF hr^{-1} g^{-1} soil). Fungal inoculation with VAM showed relatively higher response in terms of soil micro-flora and dehydrogenase activity over that of lime + FYM + ½ NPK and lime + green manure + ½ NPK. Addition of lime and organic matter enhanced the dehydrogenase activity rather than application of inorganic fertilizers, which may be due to increased microbial population and enhanced biological activity. These results are in agreement with the findings of (Casida *et al*. 1964); (Lal *et al*. 1996).

4.4.2. Effect of Integrated Use of Lime, Inorganic, Biological and Organic Sources on Fluorescein Diacetate Hydrolysis Assay

Of all the treatment combinations, fungal inoculation with VAM in combination with lime + FYM + 50% NPK has recorded highest Fluorescein Diacetate activity (1.986 µg g^{-1} hr^{-1}) followed by FYM + 100% NPK (based on soil test) (1.887 µg g^{-1} hr^{-1}) and lime + green manure + ½ NPK (1.882 µg g^{-1} hr^{-1}). Increased doses of NPK application showed an increasing trend of FDA. Integrated application of lime + FYM + ½ NPK + $MgSO_4$ has shown highest FDA activity (1.832 µg g^{-1} hr^{-1}) in comparison to lime + FYM + ½ NPK + $ZnSO_4$ (1.819 µg g^{-1} hr^{-1}). Increased doses of inorganics nutrients showed an increasing trend of dehydrogenase and FDA in the soils. However, long-term application of inorganic fertilizers (NPK) alone decreased the soil enzyme activities and continuous cropping without addition of any source of nutrients (control) further deteriorates the soil quality in terms of microorganisms and enzymes activities. Liming and organics incorporation stimulate the bioactivity and resulted higher values of FDA activity rather than non-limed plots. These results are in accordance to the findings of (Zelles *et al*. 1987). FDA hydrolysis is often used as an indicator of microbial activity and is correlated with microbial respiration (Schnürer and Rosswall, 1982). As such it is a simple, non-specific, but sensitive technique that can be used to estimate relative levels of microbial activity in soils, and has been recommended as a useful parameter to assess soil quality (Dick, 1997). The increased microbial activity (dehydrogenase, Fluorescein diacetate, and phosphatase) due to addition of organic amendments in combination with inorganic chemical fertilizers and micronutrients, which may be ascribed to greater availability of substrates that support such activities as well as the cofactors of several enzymes were highly influenced by supplementing of micronutrients (Zablotowicz *et al*., 1998; (Kremer and Jianmei Li, 2003).

4.4.3. Effect of Integrated Use of Lime, Inorganic, Biological and Organic Sources On phosphatase Activity

The results in (Table 6) revealed that application of graded doses of NPK showed an increasing trend of both acid phosphatase and alkaline phosphatase over the control. Application of optimum doses of P and NP has shown highest phosphatase activities in comparison to single application of N & K and combined application of NK. Integrated use of recommended doses of NPK based on soil test values and FYM has recorded highest acid phosphatase and alkaline phosphatase (60.32 and 49.45 µg PNP g^{-1} soil h^{-1}, respectively) over that of 100% NPK (49.24 and 37.52 µg PNP g^{-1} soil h^{-1}, respectively. *In-situ* incorporation of green manure along with ½ NPK and liming showed higher acid and alkaline phosphatase activities (56.68 and 44.67 µg PNP g^{-1} soil h^{-1}) in comparison to lime + FYM + ½ NPK (49.75 and 42.61 µg PNP g^{-1} soil h^{-1}, respectively), indicating that the lime had greater influence on phosphatase activity, similar to the findings of (Srinivas and Saroja Raman 2000).

Table 6. Effect of integrated use of lime, biological, inorganic and organic sources on phosphatase activity in soil

Treatment	Acid phosphatase (µg p-nitrophenol g^{-1} soil h^{-1})	Alkaline phosphatase (µg p-nitrophenol g^{-1} soil h^{-1})
Control	30.16	26.71
100%N	44.86	34.46
100%P	52.61	38.92
100%K	47.63	36.14
100%NP	55.67	42.13
100%NK	49.24	37.52
50%NPK	46.29	38.74
100%NPK	58.21	43.84
150%NPK	64.12	45.69
FYM+100%NPK	60.32	49.45
L+FYM+½NPK	49.75	42.61
L+GM+½NPK	56.68	44.67
FYM+½ NPK+$ZnSO_4$	58.52	42.90
L+FYM+½NPK+$ZnSO_4$	61.39	44.19
FYM + ½ NPK+B	60.52	41.31
L+FYM+½NPK+B	66.08	43.34
FYM+½NPK+$MgSO_4$	65.79	43.22
L+FYM+½NPK+$MgSO_4$	68.97	45.13
FYM +½NPK+VAM	66.28	46.28
FYM +L+½ PK+VAM	78.19	52.16

L=Lime; GM=Green manure

Among all the treatment combinations, application of half of the recommended doses of NPK in combination with lime + FYM + VAM recorded highest acid phosphatase activity (78.19 µg PNP g^{-1} soil h^{-1}) and alkaline phosphatase activity (52.16 µg PNP g^{-1} soil h^{-1}) and application of $ZnSO_4$ decreases the phosphatase activities. Combined application of lime + FYM + ½ NPK + $MgSO_4$ has shown relatively higher acid phosphatase (68.97 µg PNP g^{-1} soil h^{-1}) and alkaline phosphatase activity (45.13 µg PNP g^{-1} soil h^{-1}) in the soils of the present investigation. Application of lime had positive effect on both acid phosphatase and alkaline phosphatase activities as it improves the soil pH, which can limit the enzyme-mediated reaction rates by affecting the maximum activities of enzymes, and the solubility of substrates and cofactors (Dick *et al*., 1988). The activities of both alkaline and acid phosphatase are closely related to soil pH, with acid phosphatase dominating in acid soils, and alkaline phosphatase in alkaline soils, and the results of the present study are in concurrent with the findings of (Eivazi and Tabatabai 1977).

A long term field experiment was laid out at Regional Centre of Central Tuber Crops Research Institute, Bhubaneswar, Orissa for five years (2006-11) during kharif (Rainy season) in an acidic alfisol to study the effect of integrated application of soil amendments (lime), inorganic chemical fertilizers (N, P, K, Mg, Zn, B), biological (VAM), and organic sources (FYM, green manuring) on soil quality, yield, quality of Sweet potato (*Ipomoea batatas* L) and in the same plots Colocasia (*Colocasia esculanta* L) was grown during kharif season, 2011-12. The yield data for 2011-12 indicated that single application of recommended doses of N, P and K showed a yield response of 28, 19, and 20 per cent, respectively over that of control, while the combined application of NK showed higher yield response (59.8%) rather than NP (57.8%) over control. The increase in cormel yield was pronounced to be 37, 98 and 115 per cent with respect to 50, 100 and 150% of the recommended doses of NPK over control. Among the organic sources, in-situ incorporation of green manure along with half of the recommended doses of NPK and lime has recorded an increase of 86% of cormel yield over control followed by lime + FYM + ½ NPK (67%). However, green manuring combined with lime and ½ NPK showed an increase of 36% of cormel yield over that of 50% NPK. Of all the treatment combinations, integrated application of lime + FYM + ½ NPK + $ZnSO_4$ recorded significantly highest cormel yield (14.69 t ha^{-1}) with a yield response of 121, 61 and 12 per cent over that of control, 50% NPK and 100% NPK, respectively The per cent yield response was found highest with respect to Zn (33) followed by Mg (29) and B

(26) over that of lime + FYM + ½ NPK. Fungal inoculation with VAM in combination with half of the recommended doses of NPK and FYM recorded a cormel yield of 13.97 t ha^{-1} with an increase of 53 and 6 per cent over that of 50% NPK and 100% NPK, respectively. Significantly highest starch (21.18%, fresh wt. basis) content was observed due to integrated application of lime + FYM + ½ NPK + $ZnSO_4$ followed by lime + FYM + ½ NPK + VAM (20.80%). Total sugars varied from 0.75 – 0.89% irrespective of treatments with highest sugars being due to incorporation of green manure in combination with lime and ½ NPK. Significantly highest dry matter (23.40%) was found due to integrated use f lime + FYM + ½ NPK + VAM at par with 150% NPK (23.28%). Incorporation of organic sources along with chemical fertilizers showed higher population of fungi, bacteria, actinomycetes and dehydrogenase activity; however, lime addition has further improved the microbial counts in these acidic alfisols. Addition of micronutrient fertilizers ($ZnSO_4$ & Borax) and secondary nutrients ($MgSO_4$) along with half of the recommended doses of NPK, lime and organic manure had significant effect on soil microbes. Incorporation of lime enhances the soil physical environment and provide better atmosphere for microbial growth. Graded doses of NPK enhance the biological activity (DHA) of the soil. Integrated application of lime + FYM + ½ NPK + $ZnSO_4$ has recorded highest dehydrogenase activity (2.012 µg TPF hr^{-1} g^{-1} soil) followed by 150% NPK (1.981 µg TPF hr^{-1} g^{-1} soil). Inoculation with VAM fungi in combination with lime + FYM + half of the recommended doses of NPK has recorded highest Fluorescein Diacetate activity (1.986 µg g^{-1} hr^{-1}) followed by FYM + 100% NPK (1.887 µg g^{-1} hr^{-1}). Integrated application of lime + FYM + ½ NPK + $MgSO_4$ has shown relatively higher FDA activity (1.832 µg g^{-1} hr^{-1}) rather than lime + FYM + ½ NPK + $ZnSO_4$ (1.819 µg g^{-1} hr^{-1}). Among the treatment combinations, application of half of the recommended doses of NPK in combination with lime + FYM + VAM recorded highest acid phosphatase activity (78.19 µg PNP g^{-1} soil h^{-1}) and alkaline phosphatase activity (52.16 µg PNP g^{-1} soil h^{-1}) Integrated use of lime with optimum doses of NPK has recorded highest acid phosphatase activity (57.76 µg p-nitrophenol g^{-1} soil h^{-1}) and alkaline phosphatase (42.08 µg p- nitrophenol g^{-1} soil h^{-1}) over that of 100% NPK (53.68 and 39.20 µg p- nitrophenol g^{-1} soil h^{-1}, respectively). Integrated use of soil test based NPK doses and FYM has recorded highest acid phosphatase and alkaline phosphatase (60.32 and 49.45 µg PNP g^{-1} soil h^{-1}, respectively) over that of 100% NPK (49.24 and 37.52 µg PNP g^{-1} soil h^{-1}, respectively). Green manuring combined with ½ NPK and liming showed higher acid and alkaline phosphatase activities (56.68 and 44.67 µg PNP g^{-1} soil h^{-1}) over that of lime + FYM + ½ NPK. Fluorescein Diacetate activity and alkaline phosphatase activities had significantly higher relationship with total actinomycetes, whereas dehydrogenase and acid phosphatase had highly significant relationship with total fungi, Decrease of soil acidity had higher significant relationship with alkaline phosphatase activity ($r = 0.632^{**}$) rather than acid phosphatase activity ($r = 0.603^{**}$). Available P in the soil showed significant relationship with acid phosphates activity ($r = 0.828^{**}$) followed by alkaline phosphatase activity ($r = 0.683^{**}$). Organic carbon showed highly significant relationship with cormel yield ($r = 0.76^{**}$), starch ($r = 0.71^{**}$) and dry matter ($r = 0.65^{**}$) as the application of organic amendments improved the organic matter status of the soil. Dehydrogenase activity showed highly significant relationship with yield and bio-chemical constituents of Colocasia. Incorporation of organic residues along with lime showed greater FDA hydrolytic, dehydrogenase, and acid phosphatase activities than the soils received in organics alone or no source of nutrients. Soil pH showed positive and significant relationship with cormel yield, starch and dry matter, emphasizing that the increased soil pH due to addition of lime has contributed in enhanced crop yields as well as quality of Colocasia. Conjunctive use of soil amendments, balanced chemical fertilizers and organic manures improves the soil physio-chemical and biological properties and thus produces sustainable crop yields.

5. Conclusion

The Study revealed that the response of cocoyam (Colocasia esuclenta (L.) Schott) to various treatments(Twenty combinations of Treatments) of inorganic and organic amendments promotes yield. Various enzymatic activities were studied for optimization cornel production. The physio chemical properties of soil quality with effect of integrated use of lime, biological, inorganic and organics on soil micro-flora were optimized for cocoyam production in lowland of Eastern India.

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