

THE INFLUENCE OF COW DUNG AND INORGANIC FERTILIZER ON POPULATIONS OF ACTINOMYCETES IN ALFISOL OF SUDAN SAVANNA

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ABSTRACT

The experiment for the determination of the Influence of Cow Dung and Inorganic Fertilizer on Alfisol was laid down at Samaru for more than 60 years to determine whether the yield potential of tested crops could be maintained under continuous cultivation and the best combination of organic and inorganic fertilizers needed to achieve this. The experiment consisted of 4 treatments (Cow Dung, Nitrogen, Phosphorus, and Potassium) each at 3 levels (0, 1, and 2) applied annually in all possible combination. Each combination had one replicates only and there were 81 plots arranged in 9 blocks. The laboratory experiment involves an analytical study of the number of actinomycetes in the soil as influence by organic and inorganic fertilizers. Generally, soil amendments have been found to affect actinomycetes numbers to about 60% increased. Where phosphorus has been continuously applied at 2 and 1 levels, the actinomycetal counts were observed to be higher than where no amendments were made. The fertilization with N.P.K, particularly nitrogen in form of $(NH_4^+)_2 SO_4$ resulted in acidification of the soil and strong development of actinomycetes. Actinomycetes were found to help in the decomposition of organic matter in to humus and release of nitrogen. However, there is still much to be done in inorganic fertilizer on actinomycetal counts.

KEYWORDS: *Dung, Actinomycetal counts and Inorganic fertilizer.*

INTRODUCTION

The soils forms a complex mosaic of micro-habitat within which it is difficult to determine the individual factors which control the population density of the micro-organisms. The quantities of micro-organisms in the soil are so numerous that their population cannot be determined with precision in any given soil samples. As such estimation, not measurement is accomplished (Burgess and Raw, 1967). A total number of individual species of micro-organisms constitutes the majority of living organisms and by weight, makes up a significant of the earth biomass. The agricultural importance lies in their capacity to break down and transform the organic and inorganic materials of the soil making nutrients available to crops (Skinner, 1975).

Soil contains five major groups of micro-organisms: the bacteria, actinomycetes, fungi, algae, and protozoa. However, most important of these are actinomycetes, their number increase in the presence of decomposing organic matter. As a rule there are more tolerant to acidity and their numbers decline at pH 5.0. The most conductive range of pH is between 6.5 and 8.0 Actinomycetes, group of micro-organism combining the straits of bacteria and fungi (Sivakumar, 2008). The actinomycetes are on the whole a fairly well-defined group and are usually regarded as intermediate between the bacteria and fungi. However, they are regarded to be more closely related to bacteria than to fungi. In the sizes of their cells and spores, and in the absence of aerial mycelia they correspond to bacteria, they possess nucleoids like bacteria

(Petras, 1959). Chitin or cellulose compounds, characteristics of the cell walls of true fungi, do not occur in actinomycetes (Avery and Blank, 1954) their cell walls are polymers of sugars, amino sugars and a few amino acids. The sensitivity of the actinomycetes to phages and antibiotics also places them with the bacteria. They are likely to occur on tough of organic material and compost, its pleasant, earthy smell, they are especially important in the formation of humus. They liberate carbon (C), Nitrate nitrogen (NO_3) and ammonia nitrate (NH_4), making nutrients available to plants (Katherine, 2009). Application, of phosphorus resulted in the increase in actinomycetal count than application of nitrogen and potassium (Rangaswami and Venkatesan, 1966).

Actinomycetes are characterized by filamentous or rod and coccus structure and the presence of lateral protuberances. They are all gramm-positive actinomycetes are widely found in soil, in the silt of bodies of water, in the air, and plant remains. Among the actinomycetes there are also pathogenic forms which cause actinomycosis, tuberculosis and diphtheria (McGraw-Hill, 2004). A majority of actinomycetes feed on protein or non protein organic matter. Among the actinomycetes there are autographs, as well as forms for waxes, resins, paraffin and petroleum can be use as the source of carbon. For them nitrates ammonium salts, urea, amino acids and other substances can be use as the source of nitrogen. Actinomycetes live under the most diverse conditions, aerobic and anaerobic situation.

Actinomycetes participate in diverse soil processes (ammonium fixation, decomposition of cellular tissue and the synthesis and decomposition of humus) (Sylvia *et al*, 2005). There are particularly well adapted to survived in harsh environments, water logging of soil is unfavorable for the growth of actinomycetes, whereas deserted soils of arid and semi-arid zones sustain sizeable population, probably due to the resistance of spores to desiccations. (Thomas, 2008). The aims and objectives of this study is to determined whether the influence of cow dung and inorganic fertilizer on population of actinomycetes in Alfisol of Sudan Savanna and to maintain yield potential of tested crops under continuous cultivation.

MATERIALS AND METHODS

The experiment was solely a laboratory research. It involved microbiological studies of soils and routine soils analysis. The work was conducted in the soil microbiology laboratory of Department of Soil Science, Faculty of Agriculture, Ahmadu Bello University, Zaria (Dilution plate method is used in the study).

Experimental Site

Site description. The trial was conducted at the research farm of the Institute of Agricultural Research (IAR Samaru, Zaria). Samaru is located at latitude 11° 11'N and longitude 7°38'E in the Northern Guinea Savanna of Nigeria at an altitude of 686 meter above sea level. The soils are developed from drift sand overlaying basement complex (Klinkenberg and Higgins, 1968) and classified as Alfisol (Awujoola, 1979, IAR, 1987). Long term average annual rainfall is 1060mm confined to between May and October. Global radiation is evenly distributed throughout the year, ranging from 440 cal cm⁻² day⁻¹ in August to 550 cal cm⁻² day⁻¹ in April and May (Kowal, 1972).

Methodology

In this experiment four treatments (Cow Dung, Nitrogen, Phosphorus, and Potassium) each at three levels (0, 1, 2) were applied annually in all possible combinations, each combination has one replicate only and there are 3 x 3 x 3 x 3 or 81 plots arranged in 9 blocks (a 3⁴ factorial design with one replicate). Each plot is about 0.25ha. The experimental field has been cropped since 1970 with a rotational cropping system involving cereals, fibres and legumes.

The rate fertilizers and manures treatment during the experimental period (1970 - 2005) are presented in Table 1, in all cases level rates exactly half of level 2, while level 0 is nil, Until 1989, the source of nitrogen fertilizer was ammonium nitrate (CAN) due to increased in the acidity of the soil. Before 1991, fertilizer rates were lower but rates of dung have remained unchanged. As from 1997 the phosphate fertilizer was half that of 1991 to correspond to the officials AERLS ext, Bull. 11, 1993. Individual's plots have received the same treatment combinations annually from 1970 to the present. All crops residues and weeds etc are removed from the field each year after the harvest of experiment and burnt in nearby trenches.

Also the spraying of the trace elements Zn, Mo, B, and Cu was carried out in 1987 and in the same cropping season, lime (CaCO₃) was applied.

In 2001 and 2002 four sub-treatments were super-imposed, these are:

- (1) Sub-soiling to depth of 40cm + liming 2.5tons/ha.
- (2) Sub-soiling only to a depth of 40cm.
- (3) Liming only at the same rate as (1)
- (4) No sub-soiling and liming

Cotton was planted in 2005, cropping season before soil sampling was carried out.

Table 1: Rate of Fertilizer/Manuring Treatments used During the Experimental Period 1970 - 2005 (kg/ha)

Fertilizer Element and Manure	Treatment Level			Year(s)
Dung (FYM)	0	2500	5000	1970 - 2005
Nitrogen (N)	0	24	48	1970 - 2005
	0	67.5	135	1992 - 2005
	0	33.5	67	2002
	0	9	18	1970 - 2005
Phosphors (p)	0	27	54	1992 - 1998
	0	13.5	27	1999 - 2005
Potassium (k)	0	14.5	29	1970 - 2001
	0	29	58	2002 - 2005

Soil Sample

Top soils (0-15cm) were sampled from nine different plots under study having Cow Dung and NPK application ratio as shown in table below:-

Table 2: Cow Dung and NPK Application Ratio

Plot	Cow Dung	Nitrogen	Phosphorus	Potassium
1	0	2	0	0
2	0	0	0	0
3	0	1	0	0
4	0	0	2	0
5	1	0	0	0
6	0	2	0	1
7	2	0	0	0
8	0	0	0	2
9	0	0	1	0

During sampling, large pieces of plant materials and other impurities were handpicked. The collected soil samples were then put in polyethene bags and stored for subsequent analysis.

Sterilization Of Bottles, Water, Petri-Dishes, And Pipettes

This step was carried out in order to eliminate all micro-organisms that might cause contamination.

The water sterilization was done by putting the water in a large glass bottle and then taken into an autoclave and the pressure of the autoclave

adjusted to 15 pounds per square inches for minutes to allow for pressure development. The sterilization of the Petri-dishes, bottles, and pipettes was done by enclosing the Petri-dishes in a sterilized can taken to an oven together with the bottles. The temperature of the oven was adjusted to 160°C and left for one and half hours.

Preparation of Media

Dextrose-nitrate agar was used as a media for actinomycetal counts. This was prepared by mixing appropriately the following materials.

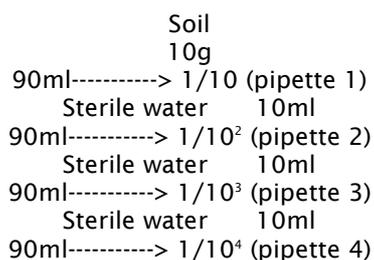
Materials	Amount
Dextrose (glucose)	1.0g
Di-potassium hydrogen phosphate	0.1g
Sodium nitrate	0.1g
Potassium chlorite	0.1g
Magnesium sulphate	0.1g
Agar powder	15.0g

One litre of water was added, but about 500ml of the water was added first then the flask together with the media was placed on a hot plate until all the bubbles started to appear after which the remaining 500ml of water was added to make up to volume (one litre). The whole flask was then returned to the hot plate. The suspension was constantly stirred to ensure thorough mixing of the materials and the mixture was left on the hot plate to boil. After the mixture has started boiling it was removed from the hot plate, allowed to cool a little and then put into the autoclave for sterilization at 15 pounds per square inches for 15 minutes.

Procedure for Dilution and Total Microbial Counts

Dilution Procedure

10g of each of the soil sample was weight and suspended in 90ml. of sterile water to obtain a dilution of 1:10. This suspension was shaken vigorously for about five minutes. The coarse particles were allowed to settle and 10ml was withdrawn aseptically with a sterile pipette and then discharged in to the second dilution bottle containing 90ml of sterile water. This second suspension comprised dilution 10². The dilution and plate inoculation steps were completed according to the following diagram.



Sterile water 10ml
 90ml-----> 1/10⁵ (pipette 5)
 Sterile water 10ml
 90ml-----> 1/10⁶ (pipette 6)
 Sterile water 10ml
 90ml-----> 1/10⁷ (pipette 7)
 Sterile water 10ml
 90ml-----> 1/10⁸ (pipette 8)
 Sterile water
 1ml
 Delivered to each plate

Actinomycetal Counts Procedure

For each soil sample, eight different dilutions were set as above i.e. 10⁻¹ - 10⁻⁸. Each dilution was assigned to five Petri-dishes. One milliliter of each soil dilution was delivered by a separate sterile pipette into five Petri-dishes and 15ml of the prepared media was added to each Petri-dishes.

A set of control was provided in this procedure, 1ml of sterile water and 15ml of the media were poured into five Petri-dishes. This is

to enable us check the sterility of the media. After all the plate inoculation steps had been accomplished, the plates were incubated in the inverted position for 10 days before colony counting. This was to allow the colonies to become more differentiated. The temperature used was 28°C. After incubation, plates with well soil were distributed colonies were selected and counted and the number of actinomycetes per gram of calculated using the expression below:-

$$\text{Number of actinomycetes/of soil} = \frac{\text{Average Count} \times \text{dilution factor}}{\text{Dry weight of a gram of soil}}$$

For actinomycetal count the dilution factor used was 10⁸.

RESULTS

Table 4: Multiple Range Tests for Actinomycetal Counts in Soil

Soil	Counts	Means
9	4	1.40 x 10 ⁸ a
8	4	1.03 x 10 ⁸ b
3	4	9.93 x 10 ⁷ bc
4	4	9.00 x 10 ⁷ bc
5	4	7.65 x 10 ⁷ bcd
1	4	6.95 x 10 ⁷ cd
6	4	6.90 x 10 ⁷ cd
7	4	4.75 x 10 ⁷ de
2	4	3.55 x 10 ⁷ e

- (1) Methods: 95 percent LSD intervals
- (2) Soil means followed by similar letters are not significantly different at 5 percent probability level by Duncan's multiple range test (D.M.R.T).

RESULTS AND DISCUSSIONS

Effect of Dung N.P.K on the Actinomycetal Counts in Soil

The mean actinomycetal counts recorded are presented in table 4, the results indicated that there was significant difference amongst the treatments. The highest number of actinomycetes observed for soil 9 which was significantly different from numbers of actinomycetes on other soils. Soil 2 which had not been receiving any amendments since 1949 had the lowest number of actinomycetes. However, it was not significantly different from the numbers in soil 7 which has been receiving 5 tons of Dung all through the period of the experiment.

Differences amongst soils, 8, 3, 4 were not significant and so were those amongst 3, 4,

5, 1, and 6. Similar numbers of actinomycetes in soils 5, 1, 6, and 7 were statistically at par. Actinomycetes are affected directly by the presence of available carbon and their numbers are especially great in soils rich in organic matter. In general, sites high in carbonaceous material and humus have larger numbers than habitat poor in organic matter. Amendments of soil with organic nutrients such as crop residues and animal manures increase the abundance of actinomycetes. The population might reach even up to 10⁸ per gram of soil with crop residues turnover (Alexander, 1977). However, the results presented in table 6 contradict these findings. Soil 7 which received high organic matter (Table 2) recorded very low actinomycetal counts (Table 6). Also treatment 5, recorded low number of actinomycetes.

Rangaswami and Venkatesan (1966) in their study of the effect of manuring on the microbial population of paddy soil observed an enormous increase in population of actinomycetes as a result of application of FYM. However, there was a gradual decrease in population afterwards. This might explain the low actinomycetal counts recorded in soils 7 and 5 which have been receiving dung since 1949. The high number of actinomycetes observed for soil 4 and 9 agrees with findings of Rangaswami and Venkatesan, (1966) who reported that application of phosphorus resulted in increase growth of actinomycetes than application of nitrogen and potassium.

The soils were generally acidic (Table 3) which has depressing effect on the growth of microbes especially bacterial (Myskow and Zieba, 2001). They showed that that fertilization with N.P.K particularly N in the forms of $(\text{NH}_4^+)_2\text{SO}_4$, resulted in the acidification of the soil, strong development of bacteria and actinomycetes.

SUMMARY AND CONCLUSION

Generally, in this study manures and fertilizers affects actinomycetes numbers to certain extent. Application of phosphorus proved more important than application of nitrogen and potassium. On the other hand, the application of

phosphorus relatively increased the numbers of actinomycetes in the soil. The numbers of fungi in the soil upon fertilization gave no definite trend. Soil actinomycetes are seen as producers of soil fertility through the decomposition of organic matter, producing humus, nitrates, phosphorus and compound of Ca, K and other elements of great importance. However, the application of fertilizers and manures to the soil strongly affects microbial decomposition. Some conclusion can be drawn from the study.

- Although certain formulations may adversely affect a specific group of micro-organism, manures and fertilizers generally increase numbers and species of actinomycetes.
- Continuous application of Dung to the soil is advocated since it produced marked effect on the actinomycetal count in the soil.
- There is usually a variation in the number of actinomycetes in appearance and dominance following manuring and fertilization.
- They help in the decomposition of organic matter into humus and release of nitrogen.

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