Improving soil fertility and performance of tomato plant using the anaerobic digestate of Tithonia diversifolia as Bio-fertilizer

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ABSTRACT

Prolonged application of agrochemicals has caused soil dehydration and destruction of plant tissues as well as spillage of excess fertilizers into water bodies. This study was designed to investigate improvement in soil fertility and performance of tomato using bio-fertilizer obtained from the anaerobic digestate of Tithonia diversifolia (Mexican sunflower) shoot. It also compared bio-fertilized, chemical-fertilized and non-fertilized plants. On the bio-fertilizer and soil, microbial analysis was carried out to determine the subsisting microbial diversities. Tomato plants were raised in twenty four pots, each containing containing 10 kg soil. Bio-fertilizer was applied at varying rates of 1000, 800, 600, 400 and 200 ml in three pots each; chemical fertilizer was also applied into three pots as positive control, while three pots served as negative control. A comparison was made across the three treatments with respect to productivity which was measured in plant height, leaf length and number of branches. Potted plants treated with 1000 ml of bio-fertilizer had the highest plant growth rate which was followed by those treated with 800 ml of bio-fertilizer. The plants to which chemical fertilizer was applied showed same increase in plant height and leaf length as the potted plant amended with 400 ml of bio-fertilizer. There was no distinct increase in height of the negative control and yellowing of leaves was observed two weeks after transplanting. The results of this study shows that bio-fertilizer is very effective in enhancing plant growth as it facilitates activities of beneficial microorganism.

Keywords: Bio-Fertilizers; Chemical Fertilizer; Microorganisms; Tomato Plant.

1. INTRODUCTION

Soil fertility and exchange of nutrient is an important factor to improving the produce of plants. Prolonged use of chemical fertilizer (inorganic fertilizer) has readily increased the adverse effect of soil intoxication and also water pollution (Owamah et al., 2014). Bio-fertilizer contains active and beneficial microorganism which helps to secrete plant growth hormones, improve soil fertility, breakdown complex nutrients making it readily available in simpler form for utilization and also inhibiting further colonization of pathogenic organism to plants (Johansen et al., 2013).

Bio-fertilizer are substances carrying living microbes which inhabit the rhizosphere of a plant as well as promoting its growth by increasing the major food requirements, breaks down complex molecule for easy utilization and also produce growth stimuli. Bio-fertilizers can be
produced from plant extracts, decomposed urban waste, and various microbial mixtures with unidentified constituents (Hari et al., 2010). These fertilizers are of great economic importance because of its reduced expenses and also an inexhaustible source of plant nutrients thus supplements use of artificial fertilizers on farmlands. They also enhance plants productivity and soil as a result of the various microbial activities on the plants roots. Rhizobium provides plants with nitrogen, promotes soil sustainability and also minimizes environmental pollution. There are different types of microbial bio-fertilizers which serves various purpose for plant growth, this includes:

- Nitrogen fixing bio-fertilizers (Rhizobium, Bradyrhizbium, Azobacter, Azospirillum, klebsiella).
- Phosphorus solubilizing bio-fertilizers (PBS) (Bacillus, Pseudomonas and Aspergillus)
- Phosphate mobilizing bio-fertilizers (mycorrhiza)
- Plant growth promoting bio-fertilizers (Pseudomonas spp.) (Hari et al., 2010).

Bio-fertilizer are mostly dominated by nitrogen fixers, potassium and phosphorus solubilizers they may also possess the presence of some mold or fungi. The interaction of micro-organism to plant varies independently, bacteria (example Rhizobium) for instance interacts with the plants roots of leguminous plant (symbiosis) whereas, Rhizobacteria inhabits the root surface or the rhizosphere of some specific plants.

Bacteria responsible for the utilization of phosphorus for plants growth are capable of producing organic acid which helps to break down insoluble phosphorus to soluble form, this makes nutrient (phosphorus) easily accessible which in turn promotes rapid growth (Gupta, 2014). Fungi associated with plants are responsible for the accumulation of nutrients and also influences growth by preventing diseases caused by pathogen, this feature is mostly associated with the vesicular arbuscular mycorrhiza fungi VAM (Gupta, 2004).

Bio-fertilizer has shown great economic importance in supplying nutrients, micro elements, organic materials and growth hormones at a cheaper rate necessary for the survival of plants (Mohammed et al., 2014).

Beneficial microorganisms contribute to the soil enrichment. This is because they absorb phosphorus for their own nutritional requirement, making available the soluble form of phosphorus for easy absorption by plants roots. In the solubilization of phosphorus for plants nutrient, Pseudomonas, Micrococcus, Bacillus, Flavobacterium, Sclerotium, Aspergillus, Penicillium and Fusarium have been identified as an active biological agent to promoting the availability of soluble phosphorus in the soil (Pindi et al., 2012). Micrococcus spp. phosphate solubilizing strain has multivalent characteristics in making nutrients available, this includes the phosphate solubilizing capacity and siderophore formation (Dastager et al., 2010). Also, Aspergillus fumigatus and Aspergillus niger two fungi spp. were isolated from a decayed cassava peel and was found to convert decayed cassava to phosphate bio-fertilizers (Ogbo, 2010). Gluconic and 2-ketogluconic acids produced by Burkholderia vietnamiensis (stress tolerant bacteria), also promote phosphate solubilization (Park et al., 2010). In the isolation of Enterobacter and Burkholderia from the rhizosphere of sunflower, they produced siderophores and indolic compounds (ICs) which has the ability to solubilize phosphate (Ambrosini et al., 2012). Potassium solubilizing microorganisms also known as (KSM) are responsible for making available potassium in the soil, these organisms include Aspergillus, Bacillus and Clostridium (Mohammadi et al., 2012). The symbiotic association of plants roots
with fungi helps to satisfy nutrients demands (Kogel et al., 2011) thus promoting growth and prevents invasion of pathogenic organism and environmental stress (Lamabam et al., 2011). This symbiotic relationship results in phosphate uptake by the hyphae from the ambience into internal cortical mycelia. Phosphate is ultimately transferred to the cortical root cells of the plant (Smith et al., 2011). Finally, Besides the impact of nitrogen, growth-promoting substances and vitamins produced by algae Cylindrospermum musicola are also involved in promoting root growth and yield of rice plants (Venkataraman et al., 1967).

Food is essential for survival, over population has led to shortage of food supply. As a result of this, agriculturists sorted for a faster medium to provide food for the overcrowding population and so chemical fertilizer were introduced. In the long run, continuous use of chemical fertilizers has led to so many catastrophes, it has being observed that (chemical fertilizer kills helpful soil microorganisms responsible for plant productivity thus making the soil chemical dependent for nutrients). Also, the consistent use of chemical fertilizer has helped to build up harmful residues and caused environmental pollution due to run-off from irrigation or rain it has also caused soil to dry up resulting in caking or hardening, thereby reducing the arability of the soil. This has resulted into land infertility and crop destruction (Greenback, 2011).

Bio-fertilizer having the activities of having micro-organism when administered on plant helps to promote the growth and productivity (Youssef et al., 2014). The breakdown of the substrate that forms the bio-fertilizer is achieved by the presence of organism in the cow rumen. During digestion, the substrates is broken down into methane, carbon dioxide and bio-fertilizer (Oloyede, 2015). The bio-fertilizer derived from anatomically digested organic substrates increases crop yield making available necessary nutrients (This is done by converting large polymers of plants nutrients into simpler forms), production of plant growth hormones, reduced cost and expenses (khoroso et al., 2012), thus provides a better preference of organic fertilizers to the use of artificial fertilizers which are more expensive and causes environmental hazards to water bodies and toxic effects on the human populace (khoroso et al., 2012).

2. MATERIALS AND METHODS

2.1 Materials

Soil used was obtained from Landmark University Teaching and Research Farms, Omu-Aran, Kwara State. The tomato seeds was purchased in the Teaching and Research Farms as well. Bio-fertilizer used was the digestate of Tithonia diversifolia. Also, NPK 15:15:15 inorganic fertilizer was obtained Teaching and Research Farm, plants pot, watering cans.

Reagents

Regents includes crystal violet, Grams iodine, acetone, safranin, distilled water, ethanol, blood plasma, Mannitol, Glucose, lactose, fructose, phenol red and blood.

Media

The media used includes: Czapedox agar CDA, nutrient agar, and Blood agar, sugars, Cysteine Lactose Electrolyte Deficient Agar CLED
Equipment and Apparatus

The apparatus used were: 500 ml measuring cylinder, 500 ml conical flasks, test tubes, glass slides, cover slips, staining rack, filter paper, flat bottom universal bottles, sterile Petri dishes, needles and syringes, pipette, cotton wool, inoculating loop, markers, aluminum foil, spatula, labelling tape, laminar air flow cabinet, laboratory incubator 37 oC (DNP-9052), compound microscope, autoclave (Yx-280A), Ohaus electrical weighing balance (PA512), centrifuge, refrigerator, Durham tubes, photometer.

2.2 Methods

Soil intended for planting, tomato seeds, bio-fertilizer were collected and analysed in two different ways:

a. Microbial analyses and characterization (soil and bio-fertilizer)

b. Physiochemical analysis (Soils nutrient analysis).

A portion of land at the Landmark University Teaching and Research was selected. A nursery bed was prepared; water was applied on the soil to soften the ground and to allow proper germination. The tomato seeds were planted and watered twice daily (morning and evening).

Two weeks before transplanting, 10kg of soil was transferred into 24 experimental pots each. The soil in 18 pots were individually mixed with the anaerobic digestate of *Tithonia diversifolia* at different ratios ranging from 1000, 800, 600, 400 and 200 (ml) in 3 pots each. This pot were separated and aligned differently from the other pots having the positive (applied with NPK two weeks after planting) and negative control (with no application of fertilizer). 30 days after nursery, the growing seedlings were transplanted into the pot (including pots containing the bio-fertilizer) and were watered twice daily. The data on leaf length, plants height and number of branches were recorded every three days of measurement. The application of fertilizer was done once in twelve days (Mona, 2011).

3. RESULTS

The following were the results obtained from the physicochemical analysis of the soil. In the table listed below, the analysis shows the quantity of nitrogen, phosphorus, potassium and other elements in the soil sample.
### Table 1: Physicochemical analysis of soil before planting

| Parameter          | Soil |
|--------------------|------|
| Nitrogen (N) mg/L  | 16   |
| Phosphorus (P) mg/L| 2.4  |
| Potassium (K) mg/L | 3.6  |
| Calcium (Ca) mg/L  | 60   |
| Magnesium (Mg) mg/L| 36   |
| Copper (Cu) mg/L   | 1.95 |
| Zinc (Zn) mg/L     | 15   |
| Iron (Fe) mg/L     | 3.4  |
| Aluminium (Al) mg/L| 0.33 |
| Nitrate (NO₃) mg/L | 1.5  |
| Ammonium (NH₄) mg/L| 0.35 |
| Phosphate (PO₄) mg/L| 74.4 |
| Manganese (Mn) mg/L| 0.012|
| Sulphate (SO₄) mg/L| 61   |

3.1 Microbial analysis on the bio-fertilizer

### Table 2: Analyses of the morphological features of micro-organisms isolated from Bio-fertilizer using different agar for the identification of bacterial growth.

| Agar used               | Morphology                                      | Organism selected |
|-------------------------|-------------------------------------------------|-------------------|
| Nutrient agar (general  | a) Mucoid and round growth with a slightly       | Klebsiella        |
| purpose agar)           | raised elevation.                              |                   |
|                         | b) Circular growth having a smooth texture.     | Escherichia coli  |
| CLED                    | Sub culture of (a) Slimy growth with a smooth   | Klebsiella        |
|                         | and opaque appearance.                         |                   |
| MacConkey               | Sub culture of (a) Large Round and pink mucoid  | Klebsiella        |
|                         | growth which are slightly raise and translucent having a complete margin |       |
|                         | Sub culture of (b). Pink tiny colonies, circular in shape having a shiny appearance with a slightly raised margin | Escherichia coli |
3.2 Data on plants growth

Table below shows the various data collated on plant growth on the first day and the last day of bio-fertilizer application on each pots (positive control are pots applied with chemical or inorganic fertilizer while negative control are pots applied with no fertilizer at all).

Table 3: Data on plants growth (first bio-fertilizer application)

| Application of bio-fertilizer in (ml) | Plants height(cm) | Leaf length(cm) | Number of branches |
|---------------------------------------|-------------------|----------------|-------------------|
| 1000                                  | 12± 0.01          | 2.0±0.02       | 6±0.03            |
| 800                                   | 11± 0.01          | 1.5±0.02       | 5±0.02            |
| 600                                   | 10± 0.03          | 1.3±0.01       | 5±0.02            |
| 400                                   | 9.1± 0.01         | 1.2±0.02       | 4±0.01            |
| 200                                   | 6.0± 0.02         | 1.2±0.01       | 3±0.01            |
| Negative control                      | 7.1± 0.02         | 1±0.01         | 2±0.01            |
| Positive control                      | 9.0± 0.01         | 1.2±0.02       | 6±0.02            |

Table 4: Data on plant growth (six days after bio-fertilizer application)

| Application of bio-fertilizer in (ml) | Plants height (cm) | Leaf length(cm) | Number of branches |
|---------------------------------------|--------------------|----------------|-------------------|
| 1000                                  | 14.5±0.01          | 2.2±0.02       | 13±0.01           |
| 800                                   | 13.0±0.02          | 1.9±0.03       | 9±0.01            |
| 600                                   | 11.9±0.01          | 1.6±0.01       | 8±0.02            |
| 400                                   | 10.2±0.02          | 1.4±0.03       | 7±0.03            |
| 200                                   | 7.4±0.02           | 1.3±0.01       | 6±0.03            |
| Negative control                      | 7.0±0.03           | 1.2±0.01       | 4±0.01            |
| Positive control                      | 9.6±0.01           | 1.4±0.03       | 7±0.02            |

Table 4 illustrates the progressive changes that occurred after six days of bio-fertilizer application. It was observed that the pot applied with 1000 ml still maintained the highest value of plants heights, leaf length and number of branches. Pot applied with 800 ml also had an improvement as well. It was observed that the negative control (pot without any fertilizer) had the lowest plant height while plant applied with 200 ml of bio fertilizer had a drastic increase in height from 6.2 to 7.4 cm, leaf length from 1.1 to 1.4 cm and number of branches from 3 to 4.

Table 5: Data on plant growth (three days after the reapplication of bio-fertilizer)

| Application of bio-fertilizer in (ml) | Plants height | Leaf length | Number of branches |
|---------------------------------------|---------------|-------------|--------------------|
| 1000                                  | 21.6±0.03     | 3.3±0.02    | 22±0.02            |
| 800                                   | 17.2±0.01     | 2.8±0.02    | 10±0.02            |
| 600                                   | 17±0.01       | 2.6±0.02    | 9±0.01             |
| 400                                   | 15.2±0.03     | 2.2±0.01    | 10±0.02            |
| 200                                   | 10±0.02       | 2.1±0.03    | 9±0.01             |
| Negative control                      | 8.3±0.02      | 1.8±0.03    | 10±0.03            |
| Positive control                      | 16.3±0.01     | 2.1±0.02    | 11±0.01            |

Table 5 shows the progressive increase three days after the reapplication of bio-fertilizer on each pots. Height of 21.6 cm was recorded with pot containing 1000 ml, there was also an increase on leaf length from 2.9 to 3.3 cm. It was also observed that the number of branches
increased respectively (from 20 to 22). Pots having 800 and 600 ml had the same value for plant’s height with a difference of 0.2 cm in pot containing 800 ml of bio fertilizer. The negative control showed the lowest value in plant’s height and leaf length still.

Table 6: Data on plant growth (fourteen days after the reapplication of bio-fertilizer)

| Application of bio-fertilizer in (ml) | Plants height | Leaf length | Number of branches |
|---------------------------------------|--------------|------------|--------------------|
| 1000                                  | 29.5±0.01    | 4.5±0.02   | 30±0.02            |
| 800                                   | 22.1±0.01    | 4.2±0.02   | 17±0.01            |
| 600                                   | 20±0.02      | 4±0.01     | 14±0.02            |
| 400                                   | 19.8±0.03    | 4±0.02     | 14±0.02            |
| 200                                   | 18.5±0.02    | 3±0.01     | 13±0.03            |
| Negative control                      | 9.2±0.01     | 2.4±0.01   | 10.1±0.01          |
| Positive control                      | 19.5±0.02    | 3.7±0.01   | 15±0.01            |

After fourteen days of reapplication of bio-fertilizer, it was revealed by table 6.0 that the negative control had a very little increase in plants height of 9.1 to 9.2 cm with no increase in the number branches and leaf length. In contrast to this, pot having 1000 ml showed a drastic increase in height from 26.5 to 29.5 cm. 800 and 400 ml followed suit as well as positive control.

![Figure 1](image.png)

**Fig 1:** Comparison between the bio-fertilized plants in different ranges to the positive and negative control

The figure above explains the growth Pattern of each plant applied with different quantities of bio-fertilizer, it also makes comparison with the positive control and the bio-fertilizer plants. Bio-fertilized plant with 1000 ml showed a distinction in plant height, increased number of branches and leaf length respectively. Second to the highest value of leaf length, branch number and plants heights was the pot applied with 800 ml of bio-fertilizer, this Trend was followed by pot having the 400 ml as well. It was observed that the positive control had the
same value of plant negative control showed no progression in its growth as it remained stagnant for 2 days.

4. DISCUSSION

The application bio-fertilizer improved the soil fertility as well as growth of the tomato plant. The physicochemical analysis made on the soil revealed the available nutrients in the present in the soil before planting. Also, the microbial analysis from the bio-fertilizer helped to identify active organisms present, posing as an active decomposer and also serving as inoculum for the bio-fertilizer. This organism actively provided nutrients necessary for plants growth. In the soil, the presence of *Bacillus* spp helped phosphorus solubilisation. The pot to which 1000 ml of bio-fertilizer was applied had an improved growth pattern with the highest plant’s heights, broad leaves and increased number of branches. This trend was followed by the pot applied with 800 ml of bio-fertilizer with a significant variation in plant height and number of leaves in contrast with other pots applied with 600, 400 and 200 ml of bio-fertilizer (Ramakrishna *et al.*, 2012). The plants applied with chemical fertilizer showed an increase in plant height but leaves showed a little increase in its length. The negative control (pot applied with no fertilizer) indicated no progressive increase in height, yellowing of leaves was observed two weeks after transplanting.

Also, the phosphorus solubilization activities of both *Bacillus* and *Aspergillus* essential for early plants productivity contributed to the performance of tomato. Hence, phosphate solubilizing bacteria plays a vital role in the utilization of unavailable phosphate resulting in soil enrichment by producing nutrients and organic acids (Napoleon *et al.*, 2012).

The physicochemical analysis of the soil and the plant was not carried out due to time constrain.

5. CONCLUSION AND RECOMMENDATION

In conclusion, this study has shown that bio-fertilizer are very effective for plant growth as they contain the activities of beneficial microorganism which helps to break down complex organic nutrients to simpler forms thus promoting the growth plant and ensuring continuous enrichment of the soil.

It is therefore recommended that bio-fertilizer should be effectively utilized to enhance environmental friendly sustainable farming practices by reducing excessive amount of chemical fertilizer application.

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