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Comparing the effect of biological amendments and chemical fertilizer on bacterial and fungal population

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ABSTRACT

Amelioration of degraded soils has triggered interest regarding alternatives to supply nutrient and improve microbial diversity through the integrated use of organic manure and biofertilizers. In this light, a screenhouse experiment set in a completely randomized design (CRD) with seven (7) treatments; *Bacillus thuringiensis* (5 ml); digestate (22.4 t ha^{-1}); 50% of digestate + NPK 15:15:15 (11.2 & 3.1 t ha^{-1}); Digestate + *B. thuringiensis* (22.4 t ha^{-1} & 5 ml); Digestate + *B. thuringiensis* + *Glomus mosseae* 22.4 t ha^{-1}, 5 ml & 500 g); positive control (NPK 15:15:15) and negative control with no treatment, replicated three (3) times was done. This research was carried out in the screen house of the department of soil science and land management, Faculty of Agriculture, University of Benin, with the aim of evaluating the agronomic value of anaerobic digestate (derived from cattle rumen content waste), inoculants of *B. thuringiensis* and *G. mosseae* in comparison with chemical fertilizer (NPK 15:15:15), while determining its' effect on soil microbial properties, growth and yield of tomato. The results showed that application of NPK 15:15:15 at a rate of 6.1 t ha^{-1} caused a significant decrease in number of leaves of the tomato plant with a total of 50 and 56 leaves/plant in pots treated with NPK 15:15:15, and a combination of digestate and NPK respectively at 8 WAT. At 8 WAT, total organic carbon of the soil was best improved in soil with a treatment of digestate, bacteria and mycorrhiza inoculum (61%), and was also significantly improved in the positive control (63%), with the pots with no treatments having the least amount of organic carbon (1.8%). Total nitrogen was highest in treatments of digestate and both inoculums at 5.7% and least in soils with a combination of digestate and NPK 15:15:15 (0.3%). Soil microbial population of bacteria and fungi increased by 20% and 43% respectively. These findings indicate that a combination of anaerobic digestate, *B. thuringiensis* and *G. mosseae* applied at 22.4 t ha $^{-1}$, 5 ml and 500 g respectively is best suited for tomato cultivation and is recommended for optimum growth and yield of tomato in the study area

1.0 Introduction

Soil fertility has been a major problem limiting crop production especially vegetables in dry land areas. In the past years, inorganic fertilizer was advocated for crop production to ameliorate low inherent fertility of soils. Although these chemical fertilizers serve as an important contributor to the increase in world agricultural productivity over the past decades, the negative effects of chemical fertilizer on the soil and environment limit its usage in sustainable agricultural system. In this regard, recent efforts have been channeled more towards the production of 'nutrient rich high-quality food' in sustainable comportment to ensure biosafety.

One of such possibilities is the use of organic manure and microbial-based biofertilizers. The need to use renewable forms of biological amendments for soil improvement, crop production and reduced fertilizer cost has triggered the search for new opportunities to improve nutritional content of the soil as that is the key agricultural component of crop productivity and sustainable agro-ecosystems (Singh *et al.,* 2016).

Bio-fertilizers are organic substances which make use of microorganisms to increase the fertility of soil. They are environment friendly, low cost and non-bulky agricultural inputs which play a significant role in plant nutrition as a supplementary and complementary factor to mineral nutrition and resistance to diseases. *Bacillus thuringiensis* and the mycorrhizal fungi, *Glomus mosseae* as biofertilizers contain active microbes which supply or make different nutrients available to plants (Pathak and Kumar, 2016).

B. thuringiensis can directly promote the growth of plants by producing phytohormones and can also indirectly induce growth by suppressing plant diseases through the production of siderophores which binds iron. *G. mosseae* maintains phosphorus and nitrogen uptake ultimately helping in plant development at higher and lower phosphorus levels under different irrigation regimes (Liu *et al*., 2014). Anaerobic digestate derived from cattle rumen content, provides crops with readily available nitrogen, and improves the sustainability of farming by reducing emissions of greenhouse gases associated with fertilizer manufacture, and by reconnecting nutrient cycles. Other benefits of digestate includes; increased soil nutrient availability due to increased soil microbial activity, soil structure improvements and root development and increased soil water availability. In light of these, this research was designed to determine the effect of different levels of digestate (CRC) combined with *B. thuringiensis*, *G. mosseae* and NPK 15:15:15, on soil chemical and microbial properties, growth, and yield of tomato.

2.0 Materials and Methods

2.1 Study Area

The experiment was carried out in the screen house of the Department of Soil Science and Land Management with 06°24'0.10"N latitude and 05°37'28.2"E longitude at the Faculty of Agriculture Research and Experimental Farm, Faculty of Agriculture, University of Benin, Edo state.

2.2 Experimental Design

Pots were filled with 18 kg soil and set in a completely randomized design with seven (7) treatments replicated three times. Treatments included; *Bacillus thuringiensis* (5 ml); Digestate (22.4 t ha^{-1}); Digestate (11.2 t ha^{-1}) + NPK 15:15:15 (3.1 t ha^{-1}); Digestate (22.4 t ha^{-1})+ *B. thuringiensis* (5 ml); Digestate (22.4 t ha^{-1}) + *B. thuringiensis* (5 ml)+ *Glomus mosseae* (500 g); positive control (NPK 15:15:15) and negative control with no fertilizer application.

2.3 Sources of Material Used

Tomato seeds (F1 Cobra 26) were obtained from a seed company (Technisem). *G. mosseae* used in the study was obtained from the soil laboratory of Rubber Research Institute of Nigeria (RRIN), Iyanomo, Edo state. Cattle rumen content derived anaerobic digestate (D) was obtained from the National Centre for Energy and Environment, University of Benin. *B. thuringiensis* was obtained from the soil laboratory, Department of Soil Science and Land Management, University of Benin.

2.4 Sample Analysis

Composite soil samples were collected from each pot at 8 weeks after transplanting and analyzed for routine chemical content, microbial and genotypic properties. Microbial counts and identification were based on standard techniques as described by Cowan and Steel (1970).

CRC was air dried, pulverized and subjected to laboratory analysis to determine its heavy-metal content, nutrient content, microbial load, and its identification was also carried out. *G. mosseae* used in the study was obtained from soil laboratory of Rubber Research Institute of Nigeria (RRIN), Iyanomo, Edo state, Nigeria and the mycorrhizal spore count was determined using wet-sieving and decanting technique (Gerdemann and Nicolson, 1963).

2.5 Data Collection and Analysis

Tomato seedlings were raised in a nursery and transplanted after twenty-one (21) days using the ball of earth method of transplanting. CRC (22.4 t ha^{-1}) was applied into the selected pots, mixed with the soil, and allowed to homogenize for three (3) weeks prior to transplanting. Selected pots were also inoculated with 5 ml of *B. thuringiensis* and 500 g of *G. mosseae* at the time of transplanting.

Plant growth data was collected within 2-8 weeks after transplanting. Three (3) plants were randomly selected per pot for determination of growth and yield parameters which includes plant height (cm), number of branches, number of leaves, yield, and soil temperature. Plant height was determined with a meter rule at the distance from soil level to the terminal bud; number of leaves, branches and fruits were determined by visual counting, soil temperature was measured by inserting a thermometer probe into the soil.

Analysis of Variance (ANOVA) procedure was carried out to determine the difference in parameters using GENSTAT software 12th edition. Mean values were compared using the Least Significant Difference (LSD) at 5% level of probability.

3.0 Result

Table 1 shows the result of the nutrient analysis done on both the anaerobic digestate while Table 2 shows the routine analysis of the composite soil before planting

was a no significant increase (P<0.05) in pH across all

Table 1. Nutrient content of CRC

Sample	Nutrient					
	(mg/kg)			(g/100g)		
	Nitrogen	Phosphorus	Potassium	Organic matter	Crude protein	Crude fibre
CRC	315	679	807	785	1.05	29.46

Chemical Properties of Soil at 8 Weeks After Transplanting (WAT)

Table 2. Chemical analysis of soil before planting

Treatment	pH	TOC	TN	PO_4	Na	Ca	Mg	K	CEC	EC	Sand	Silt	Clay
		%		mg/kg		cmol/kg						g/kg	
Sample	4.17	1.95	0.21	18.10	0.09	0.96	0.49	0.08	4.12	121	800.2	40.3	150.7

treatments. As pH was slightly raised in soil with *B. thuringiensis* (B), DB and DBG with a pH of 4.6, 4.6 and 4.8, respectively and highest in DN and D (5.3 and 6.7). With the ideal pH range for optimum growth of tomato being 5.6 – 6.8, the treatments balanced the pH level. Phosphorus availability was not significantly different ($P<0.05$) across all treatments and highest in soils with *B. thuringiensis* treatment (B) (107.1 mg/kg) followed by DN and D (57.7 and 50.7 mg/kg respectively) and least in DB (37.3 mg/kg).

There was a significant increase ($P<0.05$) in TOC across the pots, but highest in soils with NPK 15:15:15 treatment (+Control) and pots with a combination of digestate, *B. thuringiensis* and *G. mosseae* with a significant value of 63.1% and 61.1% respectively, and lowest in DN (2.35%), D (2.1%) and -Control (1.84%).

Basic cations (Ca, K, Mg and Na) were not significantly different ($P<0.05$) across all the treatments. Calcium increased incredibly by almost 90% when compared with the initial amount before planting, with D having the highest amongst the treatments (8.46 cmol/kg). The concentration of exchangeable K and Mg were adequate for tomato production with a concentration of Mg and K highest in DBG (3.2 and 0.20 cmol/kg respectively).

Table 3. Chemical Properties of Soil at 8 WAT

§WAT: weeks after transplanting; B: *B. thuringiensis;* DB: *Cattle rumen derived anaerobic digestate + B. thuringiensis;* DBG: *cattle rumen derived anaerobic digestate+ B. thuringiensis + G. mosseae;* DN: *cattle rumen derived anaerobic digestate+ NPK 15:15:15* D: *cattle rumen derived anaerobic digestate;* +Control: *NPK 15:15:15;* -Control: *No fertilizer application*

Number of Branches

Treatment	pH	TOC	TN	PO_4	Na	Ca	Mg	K	CEC	EC	Sand	Silt	Clay
		%		mg/kg		cmol/kg						g/kg	
+ Control	5.5a	63.1b	5.33b	44.0	0.80a	3.54a	2.7c	0.13	6.96b	376b	918.7	18.2a	17.1ab
- Control	b	1.84a	0.32a	a	0.51b	9.2b	1.1ab	a	10.8c	126a	b	63.3b	98.6c
	7.1			45.7				0.13			853.3		
	3a			a				a			b		
B	4.6a	5.03a	0.47a	107.	0.15a	1.46a	0.5a	0.13	2.81a	192a	895.3	57.2b	110.5ab
DB	4.6a	3.23a	0.33a	1c	0.42a	2.9a	2.4bc	a	5.91b	533b	903.7	35ab	85.7c
DBG	4.8a	61.1b	5.68b	37.3	0.34b	3.52a	3.2c	0.13	7.67b	c	b	33.1a	126.a
DN	5.3a	2.35a	0.27a	a	0.12a	2.53a	0.7c	a	3.51a	558c	906.1	b	12.2a
D	b	2.10a	0.36a	49.1	0.17a	8.46b	0.7a	0.20	9.94c	535b	b	40.3a	113.7ab
	6.7			a				a		c	888.5	b	
	bc			57.7				0.23		181a	b	103c	
				a				a			783.3		
				50.7				0.17			a		
				a				a					
LSD(5%)	0.8	2.91	0.55	13.6	0.065	2.19	0.97	0.02	1.29		54.10	23.35	26.9
	9			4						112.6			

Soil Microbial Properties at 8 WAT

Table 4 shows the microbial population of bacteria and fungi colonies isolated from soil the soil at 8 WAT. Microbial analysis revealed a total of 374 and 276 colonies of bacteria and fungi organisms respectively were present in the soil. **B** and **DN** was observed to have the highest colonies of bacterial organisms (90 and 90 cfu/g) while the least number of colonies were observed in **+Control** (34 cfu/g) and **-Control** (10 cfu/g). As shown in Table 5, bacteria isolates identified biochemically and morphologically included *Bacillus* sp, *Micrococcus* sp, and *Enterobacter sp*. Fungal organisms identified culturally included *Aspergillus flavus, Mucor sp* and *Fusarium sp.* with the highest number of colonies observed in **-Control** (56 Cfu/g), **DBG** (48 Cfu/g), and **D** (44 Cfu/g) number of colonies were observed in soils with only NPK 15:15:15 treatment (**+Control**).

Table 4. Microbial population of the soil (Cfu/g)

	Bacteria	Fungi
Treatment	10^5 (Cfu/g)	10^3 (Cfu/g)
B	90	31
DB	44	32
DBG	69	48
DN	90	46
D	47	44
+Control	34	17
-Control	10	56

Table 5. Biochemical Characteristics of Isolated Bacterial Organisms

ISOLATE	GRAM R x N	CAT	OXI	COA	URE	IND	CIT	POSSIBLE ORGANISMS
A	-	+	-	-	-	-	+	*Enterobacter Sp*
B	+	+	-	-	+	-	+	*Micrococcus sp.*
C	+	+	-	-	+	-	+	*Bacillus sp.*
F	-	-	-	-	-	-	+	*Bacillus subtilis*
G	+	+	-	-	+	-	-	*Micrococcus sp.*

§**CAT:** Catalase, **OXI:** Oxidase, **COA:** Coagulase, **URE:** Urease, **IND:** Indole, **CIT:** Citrate

Effect of Treatments on Growth Parameters from 2 – 8 Weeks After Transplanting (WAT)

At 8 WAT, D recorded the highest number of branches with 15 branches/ pot. This was followed by DBG (14 branches/ pot). There was a significant (P<0.05%) decrease in the number of branches of B, DN and +Control between 6-8 WAT.

Figure 2: Effect of Treatments on Number of Leaves from 2 – 8 WAT

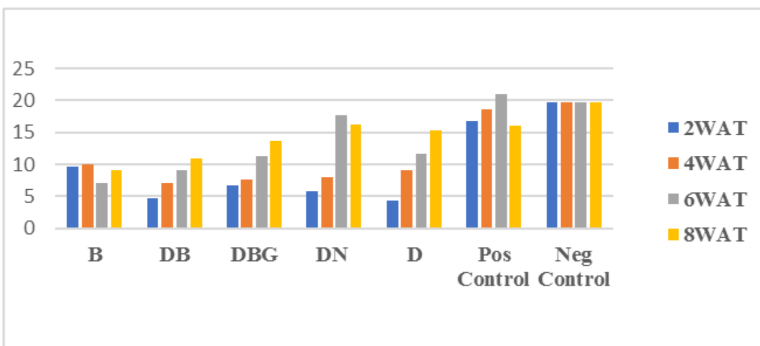
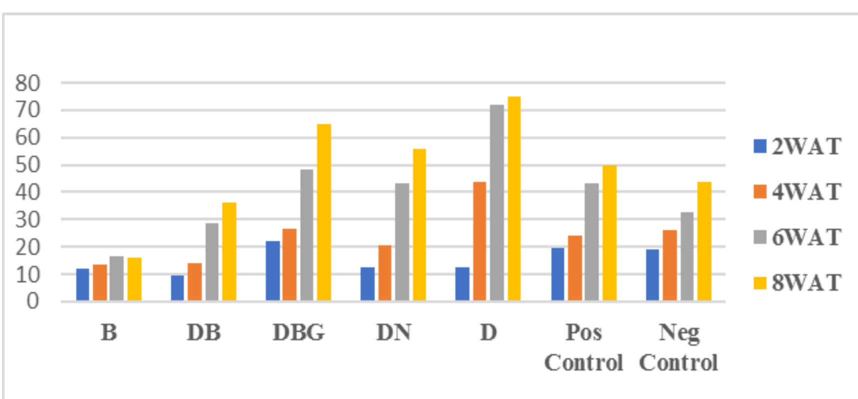


Figure 1: Effect of Treatments on Number of Branches from 2 – 8 WAT

Number of Leaves

There was a significant (P<0.05%) increase in the number of leaves in all treatment except B which had a decrease at 8 WAT from 17 to 14 leaves/ pot as a result of wilting of some leaves. DBG and D were recorded to have the highest number of leaves with a value of 65 and 75 leaves/ pot, respectively. Meanwhile, plants with NPK 15:15:15 treatment (+Control) showed a significant difference as +Control had a total of 45 leaves/ pot at 8 WAT.



Plant Height

There was a proportional decrease in height of the tomato plant across all the treatments within 2 – 8 WAT, with DBG having the tallest plant (103 cm), followed by the negative control (90 cm), and +control (59 cm). Plant height in DBG increased significantly (P<0.05%) within the duration of the experiment (47 cm to 103 cm). The significant response of the plant to a combination of digestate, *B. thuringiensis* and *G. mosseae* (DBG) could be due to high organic matter content and N, P and K content of the experimental treatments

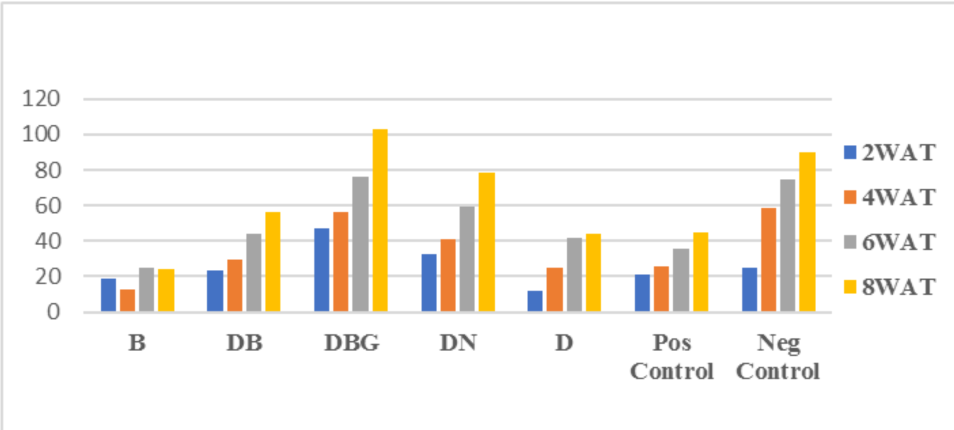


Figure 3: Effect of Treatments on Plant Height from 2 – 8 WAT

Soil Temperature

Soil temperature varied relatively across the treatments. Temperature ranged from 23^{o}C to 28^{o}C. DB observed the highest temperature at 2 WAT (26.1^{o}C) which was however lowered over the weeks (25^{o}C) and the lowest temperature (23^{o}C) was observed in plants with digestate (D).

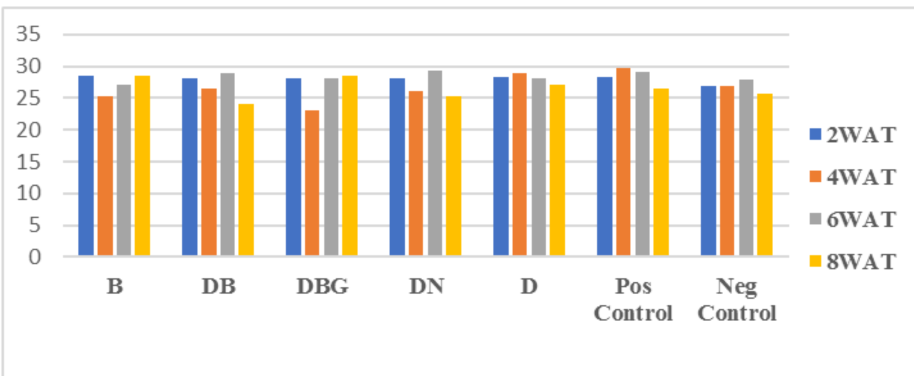


Figure 4: Effect of Treatments on Soil Temperature from 2 – 8 WAT

4.0 Discussion

Tomato plant inoculated with *Bacillus thuringiensis* alone observed a slow growth rate in height, branches and leaves throughout the experiment. It was also observed in one of the replicates that the tomato plant showed wilting symptoms and eventually died within four (4) weeks after planting. This agrees with the findings of Cristian *et* al., (2013) about *B.thuringiensis* having limited beneficial features as biofertilizer. Tomato plants with anaerobic digestate and *B. thuringiensis* treatment showed an initial slow growth rate within the first two (2) weeks after application of treatment in the height, branches and leaves of the plants. However, the growth rate increased from the third week. Pots with tomato plants inoculated with *B.thuringiensis*, Mycorrhiza and anaerobic digestate observed a rapid growth rate with significant increase in the height and number of leaves. This treatment was observed to have given the best result compared to the other treatments used in the experiment. This could be due to the strong synergistic relationship between mycorrhiza (*G. mosseae)* and *B. thuringiensis*. This agrees with the findings of Vafadar *et al.,* (2014) who reported that the effect of mycorrhizal and PGPR increased all growth parameters.

Pots with tomato plants anaerobic digestate and NPK15:15:15 (50% respectively) treatment showed an increased growth rate in the height, number of branches and leaves. It was observed in this experiment that NPK 15:15:15 (50%) in combination with anaerobic digestate (50%) had a better increase in height and leaves than plants with 100% of the same treatments. This shows that combination of organic and inorganic fertilizer produce better result than when used alone. This agrees with the findings of Paul and Mannan (2009). Pots with tomato plant with anaerobic digestate (100%) treatment showed an increase in the height, number of branches and leaves of the plant. It was observed that this treatment produced a better result in the number of leaves compared to when used in combination (50%) used. This is due to its excellent fertilizer potential which has been shown from several studies (Nkoa, 2014).

From the result of physical and chemical analysis carried out on the soil before planting, it was observed that the initial soil sample had a pH of 4.17 which was acidic for tomato growth. However, at the end of the experiment, there was an increase in the pH to a slightly acidic soil across the various treatments. Hence, the pH became favorable for the growth of tomato. **B** was observed to have the highest amount of available phosphorus (107.1 mg/kg). This implies that *Bacillus thuringiensis* improved the amount of phosphorus in the soil.

5.0 Conclusion

The result obtained from the research shows that all treatments applied, both individually and combined increased microbial population (bacteria and fungi) and growth parameters (Height, branches, and leaves) of the tomato plant. However, it was observed that the treatments with biofertilizers gave better results when compared to the treatment with chemical fertilizer. There was also a general improvement in the physical and chemical properties of the soil with a noticeable change in pH from acidic to slightly acidic which is required for tomato growth. The combination of these biofertilizers (anaerobic digestate, *B. thuringiensis* and *G. mosseae*) applied at the rate of 22.4 t ha^{-1}, 5 ml and 500 g respectively, can be recommended for environmentally friendly and optimum cultivation of tomatoes in the study area.

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