

## Responses of Okra and Soil Microbial Population Changes to the Application of Tithonia Manure

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**Abstract:** Yield reduction in okra production is mostly associated with cultivation of marginal lands or continuous land cultivation. Hence, the need for sustainable approach to resuscitating soil fertility through *Tithonia diversifolia* manure application may alter microbial population. Two soil depths [Topsoil (0-15 cm) and Subsoil (15-30 cm)], three levels of *Tithonia* manure applications (control, half recommended and recommended at 120 kg N/ha) and three okras varieties (Clemson spineless, LD88 and NHAe 47-4) were evaluated in completely randomized design replicated thrice. Soil bacterial and fungal colonies were higher in the topsoil compared to the subsoil and increased with increase in level of tithonia manure application under topsoil, while the inverse was observed under subsoil condition for bacteria colony-forming units and mycorrhizal spore count. Under topsoil, LD88 variety treated with recommended rate of tithonia manure had the highest biomass dry matter (79.4 g). Significantly higher leaf area (395.92 mm<sup>2</sup>) and biomass dry matter (66.89 g) were produced by NHAe 47-4 and Clemson spineless treated with recommended rate of tithonia manure compared to their respective controls (181.37 mm<sup>2</sup> and 29.63 g) under subsoil. Varieties LD88 and Clemson spineless combined with recommended rates of tithonia manure were suggested for topsoil and subsoil conditions, respectively.

### Introduction

Okra [*Abelmoschus esculentus* L. (Moench)] is a vegetable cultivated for its fibrous fruits or pods in the tropical and warm climates of the world [1]. The immature fruits when harvested, are eaten or cooked in a variety of ways. Similarly, the leaves and older immature pods that are yet to become fibrous are also cut into slices, sun-dried and ground and used in soups during the dry season when fresh fruits are scarce [2]. According to Adeboye and Oputa [3], the fruit provides significant quantities of and vitamin C, proteins sugars as well as essential and non-essential amino acids similar to soybean. These constituents are often considered lacking in the diet of developing countries. Its medicinal value has also been reported by Siesmonsma and Hamon [4]. The most important production regions in Africa are Ghana, Burkina Faso and Nigeria [5]. Jamala *et al.* [6] reported that the production and economic value of okra as a vegetable in Nigeria have improved significantly over the years, with various varieties being used by farmers to satisfy market demand for okra. According to Osabohien and Ogunbiyi [7], the increase in production was linked to an increase in harvested land area from 200,000 ha in 1980 to 1,859,900 ha in 2015 with an average yield of 2.1 t/ha to 1.11 t/ha, respectively. The reduction in yield per unit land area is mainly associated to the cultivation of marginal land or depletion in soil fertility status due to continuous land cultivation, thereby reducing soil nutrient status and affecting soil microbial diversity and population. Hence, the need for a sustainable approach to resuscitating soil fertility.

Soil improvement through the application of inorganic fertiliser and organic manure have been well documented. Most researchers have reported significant okra fruit yield increase from fertiliser application. Akande *et al.* [8] reported 2.5 t/ha of organic and 60 kg N NPK fertiliser as optimum for okra yield. Sanni *et al.* [9] reported that 25 t/ha poultry manure significantly enhanced okra growth

and yield. Jama *et al.* [10] also reported soil fertility improvement when *Tithonia* was used as a green manure for crop yield increase, while Santos *et al.* [11] reported the effects of mineral and organic fertilisers on the production and quality of okra. The utilization of *Tithonia diversifolia* (an invasive weed) as green manure or compost to improve soil fertility thereby enhancing crop productivity has been reported [12,13]. However, how they influence the microbial population thus increasing crop productivity has not received adequate research attention.

Soil is a large habitat for huge diversity of microorganisms. Besides many animals (from invertebrates such as worms and insects) to mammals (like rabbits, rodents and badgers), Bacteria, Actinomycetes and Fungal population are found to exist in the soil [14,15]. These biological lives in the soil has been suggested to have ideal ratios in productive soils [14]. Many factors are involved in controlling the soil microbial density, among which plant species/varieties, soil types and level of fertility are important [16,17,18]. The soil microbial population encourages soil ecological processes which helps to determine soil fertility status [19]. Therefore, this study evaluates the response of different varieties of okra and microbes to *Tithonia* manure under two soil conditions.

## Materials and Methods

The experiment was conducted in 2019 at the screen house of the Department of Agronomy, University of Ibadan, Ibadan, Nigeria, with Longitude 7° 27' N and Latitude 3° 81' E. It was a 2×3×3 factorial arranged in a completely randomised design with three replicates. The factors were: two soil depths (Topsoil and Subsoil at 0-15 and 15-30 cm), three levels of manure (*Tithonia diversifolia*) applications (control, 50% recommended and recommended rate of 120 kg N/ha [20] and three okra varieties (Clemson spineless, LD88 and NHAe 47-4). The evaluation was repeated twice.

1	2	3	4	5	6	7	8	9	Rep 1
T <sub>1</sub> N <sub>1</sub> V <sub>3</sub>	T <sub>1</sub> N <sub>0</sub> V <sub>3</sub>	T <sub>2</sub> N <sub>1</sub> V <sub>1</sub>	T <sub>1</sub> N <sub>1</sub> V <sub>2</sub>	T <sub>2</sub> N <sub>0</sub> V <sub>2</sub>	T <sub>1</sub> N <sub>½</sub> V <sub>2</sub>	T <sub>1</sub> N <sub>½</sub> V <sub>1</sub>	T <sub>1</sub> N <sub>½</sub> V <sub>3</sub>	T <sub>2</sub> N <sub>1</sub> V <sub>2</sub>	
18	17	16	15	14	13	12	11	10	Rep 2
T <sub>1</sub> N <sub>0</sub> V <sub>1</sub>	T <sub>2</sub> N <sub>1</sub> V <sub>3</sub>	T <sub>1</sub> N <sub>1</sub> V <sub>1</sub>	T <sub>2</sub> N <sub>½</sub> V <sub>1</sub>	T <sub>2</sub> N <sub>0</sub> V <sub>3</sub>	T <sub>1</sub> N <sub>0</sub> V <sub>2</sub>	T <sub>2</sub> N <sub>½</sub> V <sub>3</sub>	T <sub>2</sub> N <sub>0</sub> V	T <sub>2</sub> N <sub>½</sub> V <sub>2</sub>	
19	20	21	22	23	24	25	26	27	Rep 3
T <sub>2</sub> N <sub>½</sub> V <sub>2</sub>	T <sub>1</sub> N <sub>1</sub> V <sub>1</sub>	T <sub>1</sub> N <sub>0</sub> V <sub>1</sub>	T <sub>1</sub> N <sub>0</sub> V <sub>3</sub>	T <sub>1</sub> N <sub>½</sub> V <sub>2</sub>	T <sub>2</sub> N <sub>1</sub> V <sub>1</sub>	T <sub>2</sub> N <sub>½</sub> V <sub>3</sub>	T <sub>1</sub> N <sub>1</sub> V <sub>3</sub>	T <sub>2</sub> N <sub>1</sub> V <sub>3</sub>	
36	35	34	33	32	31	30	29	28	Rep 3
T <sub>1</sub> N <sub>½</sub> V <sub>1</sub>	T <sub>2</sub> N <sub>0</sub> V <sub>2</sub>	T <sub>2</sub> N <sub>0</sub> V <sub>3</sub>	T <sub>1</sub> N <sub>½</sub> V <sub>3</sub>	T <sub>1</sub> N <sub>0</sub> V <sub>2</sub>	T <sub>2</sub> N <sub>0</sub> V <sub>1</sub>	T <sub>2</sub> N <sub>1</sub> V <sub>2</sub>	T <sub>2</sub> N <sub>½</sub> V <sub>1</sub>	T <sub>1</sub> N <sub>1</sub> V <sub>2</sub>	
37	38	39	40	41	42	43	44	45	Rep 3
T <sub>1</sub> N <sub>0</sub> V <sub>3</sub>	T <sub>1</sub> N <sub>1</sub> V <sub>1</sub>	T <sub>1</sub> N <sub>½</sub> V <sub>1</sub>	T <sub>2</sub> N <sub>1</sub> V <sub>1</sub>	T <sub>1</sub> N <sub>0</sub> V <sub>2</sub>	T <sub>1</sub> N <sub>0</sub> V <sub>1</sub>	T <sub>2</sub> N <sub>½</sub> V <sub>2</sub>	T <sub>1</sub> N <sub>1</sub> V <sub>3</sub>	T <sub>2</sub> N <sub>1</sub> V <sub>3</sub>	
54	53	52	51	50	49	48	47	46	Rep 3
T <sub>2</sub> N <sub>1</sub> V <sub>2</sub>	T <sub>1</sub> N <sub>½</sub> V <sub>3</sub>	T <sub>1</sub> N <sub>½</sub> V <sub>2</sub>	T <sub>2</sub> N <sub>0</sub> V <sub>3</sub>	T <sub>2</sub> N <sub>½</sub> V <sub>3</sub>	T <sub>2</sub> N <sub>½</sub> V <sub>1</sub>	T <sub>2</sub> N <sub>0</sub> V <sub>2</sub>	T <sub>2</sub> N <sub>0</sub> V <sub>1</sub>	T <sub>1</sub> N <sub>1</sub> V <sub>2</sub>	

**Figure 1:** The experimental layout of the study

Soil samples (Topsoil and Subsoil) were collected from the Department of Agronomy farm along Parry Road, University of Ibadan, Ibadan. The soils were taken from the depth of 0-15 cm (Topsoil) and 15-30 cm (Subsoil) to represent marginal and depleted soils, respectively. The samples were collected and bulked separately. The bulked samples were air-dried, sieved through a 2 mm mesh to remove gravel and debris before weighing 5 kg soil into each of the black polythene bags. Representative samples were taken for chemical analyses (pH, available phosphorus, organic carbon, exchangeable bases and total nitrogen in order to determine the nutrient status of the soil) and particle size analysis properties as shown in Table 1 using standard procedures [21], while the nutrient compositions of the tithonia manure are shown in Table 2. The evaluation was repeated using part of the soils earlier collected for the study.

**Table 1:** Pre-cropping physical and chemical properties of the soils (Topsoil and Subsoil) used for the Experiment

Soil properties	Soil depths	
	Topsoil (0-15 cm)	Subsoil (15-30 cm)
pH	7.1	6.8
Total Nitrogen (g/kg)	1.46	1.32
Organic Carbon (g/kg)	13.98	10.72
C/N ratio	9.99	7.82
Available P (mg/kg)	7	5
Exchangeable bases (cmol/kg)		
Ca	10.8	6.54
Mg	9.42	0.38
K	0.63	0.34
Na	0.43	0.33
Basic micro-elements (mg/kg)		
Fe	70	82
Mn	170	125
Cu	0.86	0.59
Zn	1.29	1.1
Particle size distribution (g/kg)		
Sand	896	872
Silt	40	60
Clay	64	68

**Table 2:** Major nutrient elements in Tithonia manure

Nutrient elements	Percentage (%)
Org. M	24.04
Nitrogen (N)	1.74
Phosphorus (P)	0.82
Potassium (K)	3.92
Ca	3.07
Mg	0.005
C	14.00
C:N	8:1

(Source: Olabode *et al.*, 2007)

**Microbial population determinations:** The soil microbial population determinations were determined by counting the colony forming units before and after the study. The technique used was serial dilution method and plated on Nutrient agar (NA-Oxoid) for bacterial, and Potato Dextrose Agar (PDA-Oxoid) for fungal isolation [22]. After incubation, the colony forming units were counted, calculated and expressed in CFU/g of the soil. The analysis was carried out at the Soil Microbiology laboratory of the Department of agronomy, University of Ibadan, Ibadan, Nigeria.

### Sources of materials and management

Three varieties of okra were used in this study (Clemson spineless, LD88 and NHAe 47-4) were obtained from the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria. *Tithonia diversifolia* leaves used as organic manure was collected from the research plot of the Department of Agronomy. The tithonia leaves were air-dried for 2 weeks and ground into a semi-powdery form,

before incorporating it according to the treatments on the 5 kg polythene bag of soil. The pots were arranged conforming to the design. Three seeds were sown, and 1 plant maintained per bag at 2 weeks after sowing. The pots were watered every othered day throughout the period of the experiment.

**Data Collection:** The following growth parameters were taken fortnightly for a period of 10 weeks; Plant height was measured from the soil level to the shoot apex of the plant with the aid of meter rule; Number of leaves (by counting the number of leaves on the plant); Leaf area (using a graph method with the correction factor of 0.62 as described by Musa and Usman [23]; and dry matter of okra biomass was determined by drying the sample to constant weight in an oven at 70°C.

**Data Analysis:** All data collected were analysed using Analysis of Variance (ANOVA) with the use of GenStat Release 10.3DE version. Significant means were separated using Least Significant Difference (LSD) at 5% probability level.

## Results

The values of chemical soil properties in the topsoil were higher than those observed in the subsoil except for Fe with no significant difference observed among the parameters considered (Table 1). However, the total nitrogen in the topsoil was 10.6% higher compared to that of subsoil. Topsoil and Subsoil were in the neutral soil pH range, but differed slightly with no significance observed. Available P was 40.0% higher in topsoil compared to the subsoil. Fe was 17.1% higher in subsoil compared to the topsoil. Organic carbon in the topsoil was 30.4% higher compared to that of the subsoil. Magnesium was extremely higher (95.9%) in topsoil compared to the subsoil. Manganese was 36.0% higher compared to the subsoil. Calcium in Topsoil was 65.1% higher compared to the subsoil. Sodium was 30.0% higher in the topsoil than in subsoil while, Potassium was 46.0% higher in topsoil compared to the subsoil.

The compositions of sand, silt and clay in the topsoil (0-15 cm depth) and the subsoil (15-30 cm depth) indicated that they belong to the textural classifications of loamy sand and sandy soil, respectively. The physical properties in both soils did not differ significantly, even though they belong to different soil textural classification.

## Colonies of bacteria before and after planting on topsoil and subsoil

The bacteria colony-forming units found before the planting of okra was lower in the topsoil compared to the subsoil (Table 3). The bacteria colony-forming units were higher before the planting of okra than after the introduction of okra in the topsoil and subsoil conditions. With respect to bacteria colony-forming units after tithonia manure application, the higher the rate of application, the more the bacteria colony forming units in the topsoil, with significantly higher value observed in the recommended rate of tithonia manure application. However, the reverse was observed in the subsoil, with higher bacteria colony forming units observed in the treatment without tithonia manure application and the lowest observed in the recommended rate of application. In the topsoil, there was a reduction in the values of bacteria colony forming units before planting compared to bacteria colony forming units after okra planting in the treatment without tithonia application. However, the values were similar in the subsoil. The application of the recommended rate of tithonia manure had the highest bacteria colony-forming units in the topsoil.

**Table 3:** Microbial population density as influenced by soil types, manure application and period of sampling

Soil types	Manure Application	Microbial sampling period	Bacteria colony forming units ( $\times 10^5$ )/g Soil	Mycorrhizal Spore Count/50g Soil	Fungi Colony forming units ( $\times 10^3$ )/g soil
Topsoil	-	BP	3.30c	48.00e	8.00a
	N <sub>0</sub>	AP	2.20e	39.00f	4.00d
	N <sub>1/2</sub>	AP	3.80b	43.00g	7.00ab
	N <sub>1</sub>	AP	4.50a	55.00d	6.00bc
Subsoil	-	BP	3.60bc	66.00a	5.00cd
	N <sub>0</sub>	AP	3.60bc	60.00b	4.00d
	N <sub>1/2</sub>	AP	2.80d	57.00c	6.00bc
	N <sub>1</sub>	AP	2.00e	44.00f	7.00ab
SE			0.12	0.59	0.41

BP = Before planting; AP = After planting; N<sub>0</sub>= Control; N<sub>1/2</sub>= half recommended; N<sub>1</sub>= Recommended; In a column, figures with same letter(s) do not differ significantly, while figures with dissimilar letter differ significantly according to DMRT at 5% level of probability

### **Mycorrhizal spore count before and after planting on topsoil and subsoil**

The total mycorrhizal spore count was higher in the subsoil compared to the observed value in the topsoil (Table 3). With respect to the topsoil, the amount of mycorrhizal spore before planting was higher compared to the no tithonia manure application. The values of mycorrhizal spore count after the planting of okra increased with an increase in the application of tithonia manure with the highest value observed at the recommended rate. In the subsoil, however, mycorrhizal spore count was higher before planting compared to the values observed after planting. The values observed after okra planting reduced with the increase in the application rate of tithonia manure in the subsoil. Comparatively, the mycorrhizal spore count observed was higher in the treatments under subsoil compared to those under the topsoil.

### **Fungi colonies before and after planting on topsoil and subsoil**

Topsoil had 5.5% higher numbers of fungal colonies compared to the subsoil (Table 3). The initial number of fungi colonies in Topsoil was higher compared to the number of colonies observed after the applications of *Tithonia diversifolia* manure. However, fungi colonies observed after planting was higher in half the recommended rate compared to the recommended rate, while the lowest fungi colonies were observed in the treatment with no manure application. Under the subsoil conditions, fungi colonies before planting of okra were only higher compared to the value observed in the treatment without tithonia manure application. However, the fungi colonies increased with increase in tithonia manure applications.

### **Responses of Okra varieties to *Tithonia diversifolia* manure under topsoil and subsoil conditions**

Growth parameters of okra under the two soil (Topsoil and Subsoil) conditions were shown in Table 4. Across the rate of tithonia manure applications, the plant height of okra was higher under the topsoil conditions compared to the subsoil conditions. Similarly, LD88 okra variety had a higher plant height across the tithonia manure applications under topsoil with no significant difference observed among treatments. However, the highest plant height was observed under LD88 okra variety and the application of the recommended rate of tithonia manure. With respect to subsoil conditions, Clemson spineless variety of okra improved plant height compared to the other varieties across the tithonia manure rates of applications. Significantly higher difference ( $p \leq 0.05$ ) in plant height were observed at half the recommended and recommended rates of *Tithonia diversifolia* manure compared to no application treatments under subsoil conditions.

**Table 4:** Growth parameters of okra at 8 weeks after planting and biomass dry matter under two soils (Topsoil and Subsoil) conditions

Treatments	Plant height (cm)		Number of leaves		Leaf area (cm <sup>2</sup> )		Biomass dry matter (g)	
	Topsoil	Subsoil	Topsoil	Subsoil	Topsoil	Subsoil	Topsoil	Subsoil
N <sub>0</sub> V <sub>1</sub>	62.87a-c	43.80d	4.67b	5.00a	293.70	181.37d	59.50a-c	29.63d
N <sub>0</sub> V <sub>2</sub>	59.00b-d	39.03e	5.00ab	4.00bc	325.68	237.85cd	52.52bc	16.88e
N <sub>0</sub> V <sub>3</sub>	49.10d	34.30f	5.00ab	4.00bc	320.07	241.10cd	49.85c	27.29d
N <sub>½</sub> V <sub>1</sub>	66.57ab	64.10ab	5.33ab	4.67ab	299.81	300.85bc	67.93a-c	50.45b
N <sub>½</sub> V <sub>2</sub>	68.77ab	64.63ab	5.67a	4.00bc	369.75	322.50a-c	70.20a-c	40.80c
N <sub>½</sub> V <sub>3</sub>	64.03ab	47.40d	5.00ab	3.33c	315.25	313.89a-c	68.76a-c	40.95c
N <sub>1</sub> V <sub>1</sub>	69.23ab	67.87a	5.00ab	3.33c	297.77	382.06ab	78.74a	66.89a
N <sub>1</sub> V <sub>2</sub>	70.33a	63.10b	5.00ab	4.33ab	317.08	314.19a-c	79.40a	54.24b
N <sub>1</sub> V <sub>3</sub>	52.73cd	56.87c	5.33ab	3.33c	296.75	395.92a	71.65ab	50.79b
SE	3.48	1.34	0.28	0.23	30.46	30.12	6.88	3.12

N<sub>0</sub> = No organic manure addition; N<sub>½</sub>= Addition of Tithonia manure at half recommended rate; N<sub>1</sub>= Addition of Tithonia manure at recommended rate; V<sub>1</sub>= Clemson spineless; V<sub>2</sub>= LD88; V<sub>3</sub>= NHAe 47-4; In a column, figures with same letter(s) or without letters do not differ significantly, while figures with dissimilar letter differ significantly according to DMRT at 5% level of probability

There was no significant difference observed in the leaf area with respect to okra varieties and *T. diversifolia* manure application under Topsoil. However, the highest leaf area was observed in LD88 treated with half the recommended rate of manure application while the lowest was observed in NHAe 47-4 variety treated with the recommended tithonia manure rate. With respect to Subsoil, Clemson spineless variety of okra plants treated with the recommended rate of manure had significantly ( $p \leq 0.05$ ) higher leaf area in compared to the controls.

Clemson spineless and LD88 varieties of okra treated with the recommended rates of tithonia manure did not differ significantly in the biomass dry matter produced, but varied significantly from the treatments without manure applications under topsoil condition. Under subsoil condition, Clemson spineless treated with the recommended rate of tithonia manure produced significantly biomass dry matter compared to the other treatments under subsoil condition, with the lowest value observed in LD88 without manure application.

## Discussion

The classification of the Topsoil and Subsoil as loamy sand and sandy soil, respectively indicated that the soils have low water holding capacity. According to Akpan-Idok [24], loamy sand lacks adsorption of capacity for basic plant nutrients and water. The pH is within the range considered favourable for crop production especially vegetables [25]. The relatively higher Fe content in subsoil and small increase in organic carbon and basic cations in the topsoil may account for the slight difference in soil pH. This is similar to the observations of Mielki *et al.* [26] that low soil pH tends to have more available Fe content than higher pH soils for plant growth. Furthermore, the soil organic carbon contents were also below the critical values ( $< 30$  g/kg) [27]. Also, the N contents were also below the critical value ( $< 1.5$  g/kg). The low level of organic carbon and nitrogen for lower soil depth (15-30 cm) compared with 0-15 cm depth could be attributed to the availability of organic matter in the topsoil in the form of litters which tends to improve the organic carbon and nitrogen after decomposition [28]. Similarly, higher concentrations of exchangeable bases in the topsoil compared to the subsoil was also reported by Day and Ludeke [29]. The differences were associated to the fact that the soil at lower depth may be the bank of these nutrients, but are at the nonexchangeable form. The topsoil must have experienced more intense weathering which helped to realise the nutrients into exchangeable for enhanced crop performance. Also, the roots of trees could act as suction pump by absorbing nutrients from the sub soil and realise them as litter to the soil surface which latter realise nutrients lock after decomposition. This may explain the higher organic carbon in the topsoil than the

subsoil. This was in support of Carbon and N concentrations decreased with soil depth [30,31]. Although, both soils were not significantly different with respect to physical properties, but the lower concentrations in clay and silt in the topsoil compared to subsoil indicated the movement of the particles to the lower horizon. This was confirmed by Adugna and Abegaz [32] and Sanaullah et al. [33] that many topmost *soils* contain a relatively low amount of silt and *clay* in the surface layer resulting from the downward movement of the particles with drainage/percolating waters.

It was expected that the fertility status of the soils could be improved and enhanced through the application of *Tithonia diversifolia* manure. This plant is a weed that grows quickly and has become an option as an affordable substitute to expensive inorganic fertilisers [12,13]. The biomass from *T. diversifolia* breaks down easily and releases nutrients quickly which improves soil fertility status by activating soil microbes and increasing their population. This was supported by the C:N ratio of the manure used in the study.

The reduction in the bacterial population under the subsoil condition may be attributed to the lower organic C which is an index for the fertility status of the soil. Reports have shown that soil fertility status down the soil horizon have effects on the bacterial population [16]. The horizon with higher organic matter tended to have more bacterial population than the lower horizons with minimum organic matter. Similarly, the differences in the soil textural classification of the soils (topsoil and subsoil) might have influenced the variations in the bacterial population count. The higher bacterial count in the topsoil compared to the subsoil was in line with Naveed *et al.* [17] reports. According to their studies, soil texture affects the water content and soil nutrient status, thus affecting the bacteria population and their metabolic activity. Soil organic C content is the predominant factor that influences the microbial functional diversity [18]. The higher the soil organic C content the more the microbial diversity. According Mohammad [34], the correlation coefficient between soil microbial population and organic C was 0.79. Improvement in the bacterial community was ascribed to the fact that soil organic C provides energy to microbes, thus soil with higher SOC content has higher microbial biomass and functional diversity. The finding confirmed that the bacterial population was significantly affected by organic C, yielding a higher bacterial population in topsoil with finer soil texture than the subsoil.

Ranjard and Richaume [35] reported 40-70% of the bacteria were located in the 2-20 cm depth. Consequently, the assessment of microbial characteristics under diverse soil conditions can improve our perception of the influence of soil properties on microbes. Soil microbial population varied with the different soil conditions. The increase in microbial populations counts in the topsoil compared to the subsoil is supported by Bhattarai *et al.* [16] that microbial population decreases with respect to different soil conditions, depth or horizon.

The diversity and abundance of mycorrhizal spore count may be strongly influenced by cultivation, temperature, soil pH, soil moisture, soil mineralogy and soil organic matter. In this study, the mycorrhizal spore count was higher in the topsoil compared to the subsoil. A similar result was reported by Mafaziya and Madawala [36], that mycorrhizal spore richness was higher in fertile soil than in degraded soil. Furthermore, there was a reduction in mycorrhizal spore count after the planting of okra as well as with the increase in tithonia manure application under subsoil conditions. Reports have shown that tithonia manure is relatively higher in P content compared to poultry manure [12], which may mask the effect of the mycorrhizal fungi effectiveness in its major role of improving P nutrient uptake from the phosphorus pools for crop development. This is similar to Prasad *et al.* [37] report that high P source may be detrimental to mycorrhizal colonization and limit the sporulation of the fungi. Also, plants allocate more photosynthate to the shoots and leaves and less to the roots and mycorrhizal fungi under improved nutrient status resulting in reduced mycorrhizal sporulation.

The alteration in the soil microbial population resulting from impacts fertiliser or manure application may result in the modification of the soil environment thereby promoting or decreasing plants growth and yield. The application of tithonia manure improved the fungi colony-forming units in both the topsoil and subsoil conditions. Several studies confirmed that organic matter had a great influence on fungal abundance [34,38]. Similarly, increase in the quantity of nitrogen has been reported to affect the fungal number in a positive manner [18]. The application of the tithonia manure

must have improved the soil N content, thus increasing the fungal colony-forming units in the topsoil and subsoil. Higher total nitrogen availability increased sporulation and hyphal growth rate [18]. Numerous studies have revealed the influence of soil pH on the distribution of fungal populations [18,39]. Fungi are able to grow in a wide range of soil pH, although they prefer a slightly acidic pH condition [18].

Among other factors that affect the increase or decrease in microbial population are types of crops and the types of microorganisms. Similarly, the microbial decomposition plays a significant role in mineralization and immobilization processes of applied organic waste materials [39]. A crop plant required a good and favourable soil conditions and environmental situation to give full potential yield. However, the improper nutrient availability due to soil biological properties may results in lower crop production even under good management practices [39]. *Tithonia diversifolia* manure has been shown to increase plant yields and the soil nutrients of N, P, and K [10]. The results have also shown that the application of *T. diversifolia* manure increased microbial population, thus improving plant height, number of leaves, leaf area and biomass dry matter in all the three varieties. The improvement of okra performance through fertiliser application have been reported. Akande *et al.* [8] and Santos *et al.* [11] have reported the response of okra to organic and inorganic fertilisers. Furthermore, Jama *et al.* [10] reported the importance of tithonia in crop improvement, while Jeptoo *et al.* [40] reported the positive effect of tithonia manure on the carrot. Olabode *et al.* [12] attributed the yield improvement ability of tithonia manure to the high nutrient content with respect to P and K, which are essential for enhancing okra yield.

The ability of tithonia manure in crop yield enhancement was associated to the improvement in soil physical and chemical qualities, thus increasing yield [10,12,13]. The LD88 variety of okra performed better than Clemson spineless and NHAe 47-4 in all the observed parameters under topsoil condition. Muhammad *et al.* [20] reported a similar observation that LD88 variety of okra had better growth performance than NHAe 47-4. Under subsoil condition, Clemson spineless and NHAe 47-4 performed better with respect to plant height and leaf area respectively. These differences in growth performance may be associated with their genetic attributes. According to Ayoub *et al.* [41], differential growth of crops under similar environmental conditions is normally due to differences in the genetic make-up of these crops. However, studies have shown that crop varieties vary in their response to treatments [12,20]. Furthermore, the interaction and complimentary role of fertiliser application and variety in influencing the ability of okra cultivars to express its potentials in terms of growth, development and yield has been reported by Jamala *et al.* [10] and Muhammad *et al.* [20]. The study indicated the interdependence of okra varieties with tithonia manure under the different soil conditions. These may account for the better response observed in the LD88 treated with the recommended rate of tithonia manure than the other varieties under topsoil conditions. Similarly, the increase in Clemson spineless treated with the recommended rate of tithonia manure under subsoil may indicate that the variety has the ability to respond better to manure application under poorer soil conditions.

## Conclusions

This study enunciated the role of tithonia manure on microbial population density and different okra varieties under two soil conditions. It was revealed that the bacterial forming units, mycorrhizal spore count and fungal forming units under topsoil and subsoil were lower after the planting of okra compared to the mycorrhizal spore count before the introduction of okra. The application of tithonia manure improved all the microbial populations with the increase in application levels under the topsoil and subsoil conditions, except for mycorrhizal spore count under the subsoil condition. The LD88 and Clemson spineless varieties of okra treated with the recommended rate of tithonia manure performed better with respect to the observed growth parameters under topsoil and subsoil conditions. It is concluded that the applications of recommended rates of tithonia manure were better for LD88 and Clemson spineless varieties of okra under topsoil and subsoil, respectively.



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