Microbial Diversity of Water Hyacinth and Cow Dung Bio-compost used for the Growth of Tomato Plant (*Solanum lycopersicum* L.)

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Abstract: Water hyacinth is an aquatic weed that is difficult to manage due to its rapid and extensive growth rate. Composting is a promising technique widely used for the management of organic wastes. This study evaluated the application of water hyacinth and cow dung composts as bio-fertiliser on tomato plant. Water hyacinth and cow dung were composted for 20 days in five different proportions of cow dung, water hyacinth, cow dung and water hyacinth (CW) at ratio (1:1, 1:2, 2:1) respectively. Physicochemical properties and microbial load of the composts were determined. Bacterial and fungi isolates were isolated and identified using standard methods. Composts were applied to sterile soil after which tomatoes were transplanted. Agronomic parameters such as plant height, number of leaves, stem girth and leaf area were determined after eight weeks of transplanting. Results showed that cow dung only had the highest total bacterial count of 27.2 x 10^6 cfu/g while water hyacinth only had the least bacterial count of 5.0 x 10^6 cfu/g. Cowdung and water hyacinth (1:1) and cowdung only recorded had the highest and lowest total fungal count of 12.8 x 10^6 cfu/g and 2.0 x 10^6, respectively. pH ranged from 5.8 – 7.8 while temperature ranged from 25.67-40.5°C. The isolated and identified bacteria were *Bacillus subtilis, Campylobacter jejuni, Citrobacter koseri, Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli, Klebsiella oxytoca, Pseudomonas aeruginosa* and *Staphylococcus saprophyticus.* The identified fungal isolates include: *Aspergillus flavus, A. fumigatus, A. niger, Candida albicans, Penicillium* sp. and *Saccharomyces* sp.Cowdung: water hyacinth at ratio 1 to 2and water hyacinth only showed the highest and least support for tomato plants, respectively. All the agronomic parameters analysed were significantly higher($P\leq 0.05$)in all the treatments than the control. This study revealed that compost of cow dung and water hyacinth could be used to improve the growth of tomato.

Keywords: Compost, Cow dung, Tomato, Water hyacinth, Microbial load

INTRODUCTION

Water hyacinth (*Eichhornia crassipes*) is a free floating aquatic plant which is rooted in the mud (Rai, 2009). The plant causes several problems including obstruction of waterways, destruction of wildlife resources and oxygen depletion (Montoya *et al.* 2013).Water hyacinth has been recognized as the most harmful aquatic weed in the world due to its negative effects on people's livelihoods and waterways (Wilson *et al.,* 2005).Cow dung manure on the other hand, is an indigestible plant material released from the intestine of a cow (Dijkstra *et al.,* 2011).It is a renewable resource, rich in nutrients, minerals, used as fertilizer, often supplement organic matters and improve soil conditions (Umsaku *et al*., 2010; Ukpabi *et al.,* 2017).

Composting is a process that involves microbial decomposition of organic matter from the biodegradable wastes to form a non-degradable humus-like substance. The result of composting is a biologically stable and humic end product that contains stabilized carbon, nitrogen and other nutrients in the organic fraction (Chang and Chen, 2010). During composting, microorganisms aerobically metabolize organic substrates and release carbon dioxide, water vapour and a large amount of heat (Prasad *et al*., 2013).

The control of water hyacinths is very difficult due to regeneration from fragments of stems and seed that can remain viable for more than six years (Gunnarsson and Petersen, 2007). Composting had been a promising technique for waste management. The organic substrates of water hyacinth can be biodegraded and stabilized by composting and the final compost products could be utilized as bio-fertilizer or soil conditioner.

Incorporation of compost into soil also provide biological control of diseases caused by soil-borne plant pathogens and reduce the severity of disease caused by foliar plant pathogens (Coventry *et al.*, 2005; Popoola *et al.,* 2014).

Water hyacinth and cow-dung are environmental wastes. Water hyacinth competes with other aquatic plants for nutrients and also hinders fishing and transportation on water. It is therefore imperative to convert these wastes to wealth. Hence, the objective of this study was to explore the ability of water hyacinth and cowdung compost to improve tomato plant.

MATERIALS AND METHODS

Water hyacinth was collected from *Majidun* River at Ikorodu, Lagos State, Nigeria. It was identified taxonomically and authenticated by Dr. Oyelakin of the Department of Pure and Applied Botany, Federal University of Agriculture, Abeokuta, Nigeria. A sample of each identified plant was deposited for future reference and was given voucher number FHA-0091.Cow dung was collected from the COLANIM Farm, Federal University of Agriculture, Abeokuta and tomato (Beske cultivar) seeds were obtained from Institute of Agricultural Research and Training (IART), Ibadan, Nigeria.

Preparation of Compost

This was carried out as described by Muoma (2016) with little modification. The water hyacinth was shredded and mixed with cow dung in five different proportions with triplicates in each proportion composition. The prepared proportions include: Water hyacinth only (250g), cow dung only (250g), water hyacinth and cow dung at a ratio of 1:1 (250g of water hyacinth and 250g of cow dung), water hyacinth and cow dung at a ratio of 1:2 (250g of water hyacinth and 500g of cow dung), water hyacinth and cow dung at a ratio of 2:1 (500g of water hyacinth and 250g of cow dung). Fifty millilitres of distilled water was added to each proportion and mixed thoroughly. Each mixture was kept for twenty days with the addition of water and mixing on daily basis. Samples were taken at 5 days interval for physicochemical and microbiological analyses.

Determination of Physical and chemical Properties of the Compost

The temperature was determined using mercury in glass thermometer; pH, electrical conductivity and organic matter were determined using the method ofKalamdhad and Kazmi (2009); total nitrogen was determined using the Kjeldahl method (Prasad *et al*., 2013); available phosphorus was determined using APHA (2005); nutrient elements (Na, Mg, Ca, K) were determined using flame photometer(Baeta *et al.,*2006).

Isolation and Identification of Bacterial and Fungi Isolates from the Compost

Samples of the composts were 5-fold serially-diluted using sterile distilled water. Using pour plate method, 1 mL inoculum was inoculated on Plate Count Agar (PCA), Nutrient Agar (NA) and Potato Dextrose Agar (PDA). The PCA and NA plates were incubated at 37 °C for 24 hr while PDA plates were incubated at 28 °C for 72 hr. Distinct colonies were counted and sub-cultured to obtain pure cultures. Bacterial isolates were identified based on their morphological and biochemical characteristics with reference to Bergey’s Manual (Akintokun and Taiwo, 2016) while the fungal isolates were identified based on their cultural and morphological characteristics with reference to Oloyede *et al.* (2016).

Green house Experiments

Determination of Biofertilizer activity of the compost

Topsoil was collected (0-10 cm) from DURFARMS, Federal University of Agriculture, Abeokuta, Nigeria and then autoclaved at 160 °C for 1 hr. Nurseries of the tomato seeds were raised for 3 weeks using the sterile soil. Then 100g of each compost was mixed with 3 kg sterile soil in plastic planting pots. Tomato seedlings were transplanted into each pot and the pots were watered twice daily with distilled water. The experiment was laid out in a completely randomized design with triplicates treatments. Agronomic parameters such as the plant height was measured with a measuring tape, number of leaves were recorded by physical counting of leaves, leaf area was determined by measuring the length and breadth of the leaf and was calculated using the formular L×B×0.851 (Fascella *et al.,* 2009) and stem girth was measured using a vernier calliper, at two weeks interval after transplanting for eight weeks(Mashavira *et al.,* 2015).

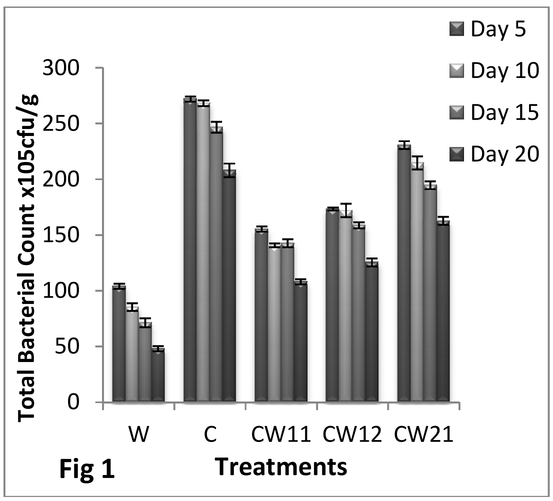
Data Analysis

Data were analysed using statistical package for social sciences (SPSS)version 16.0.

RESULTS AND DISCUSSION

Bacterial and Fungal Loads

In all the treatments, cow dung had the highest bacterial count of 27.2 x 10^6 ± 0.24 cfu/g at Day 5 while water hyacinth had the least bacterial count of 5.0 x 10^6 ± 0.3 cfu/g (Figure 1). The highest fungal count of 12.8 x 10^6 ± 1.03 cfu/g was recorded in experimental set up with water hyacinth and cow dung at (1:1) in Day 5 while cow dung also had the least fungal count of 2.0 x 10^6 ± 0.83 cfu/g after 20 days of composting (Figure 2).



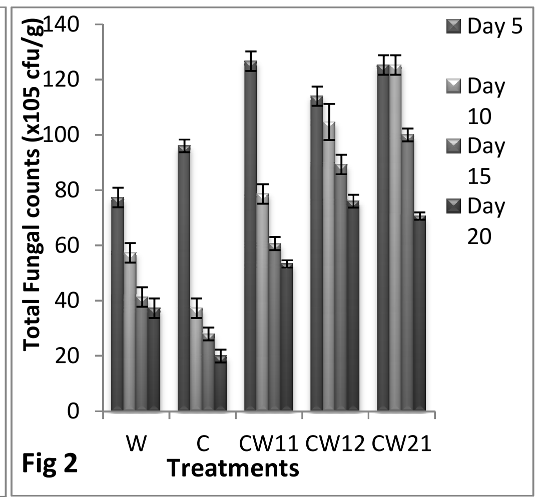


Fig. 1: Bacterial counts of composts **Fig. 2: Fungal counts of composts**

Key: W- Water Hyacinth Only; C- Cow dung Only; CW11- Cow dung + Water Hyacinth (1:1), CW12- Cow dung + Water Hyacinth (1:2); CW21- Cow dung + Water Hyacinth (2:1)

Physical and chemical properties of the composts

The pH of the prepared composts ranged from 5.8 to 7.8 withCW12 (cow dung and water hyacinth at 1:2) having the highest pH value of 7.8 while water hyacinth had the least value of 5.8. The highest pH values in all the treatments were obtained at Day 15 with the exception of CW21(cow dung and water hyacinth at 2:1) which recorded the highest pH value at Day 20 (Figure 3a). The highest temperature of 40.5 °C was also recorded by CW12 while water hyacinth only had the least temperature (25.67 °C). The highest temperature values in all the treatments were obtained at Day 20 of composting (Figure 3b).Table 1 showed the results of conductivity, organic carbon, phosphorus, nitrogen, sodium, calcium, potassium and magnesium as analysed from the prepared composts. Cow dung had the highest conductivity value of 8.37 µS/cm, organic carbon (1.96 %) and phosphorus value of 10.6 mg/kg. The CW11 compost recorded the highest nitrogen value of 0.19 %.

The nutrients involving: sodium, calcium, potassium and magnesium were found to contain higher values in cow dung (0.39 cmol/kg), CW21 (3.86 cmol/kg), CW12 (0.71 cmol/kg) and CW21 (1.84 cmol/kg), respectively.

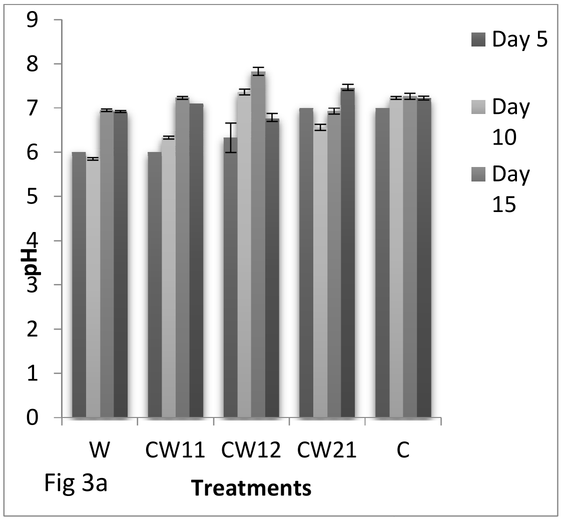


Fig. 3a: Analysis of pH in water hyacinth and cow dung composts

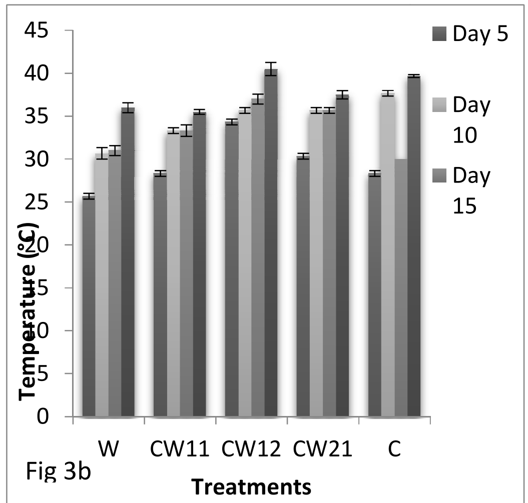


Fig. 3b: Analysis of temperature in water hyacinth and cow dung composts

Key: W- water hyacinth only; C- cow dung only; CW11- cow dung + water hyacinth (1:1) CW12- cow dung + water hyacinth (1:2); CW21- cow dung + water hyacinth (2:1)

Table 1: Analysis of the physical and chemical properties of water hyacinth and cow dung composts

Properties	Cow dung	Water hyacinth	CW11	CW21	CW12
Electrical conductivity (µS/cm)	8.37	3.60	3.75	6.67	4.75
Organic Carbon (%)	1.96	1.50	2.54	1.90	1.22
Nitrogen (%)	0.18	0.14	0.19	0.18	0.14
Phosphorus (mg/kg)	10.6	5.47	4.01	8.08	8.08
Sodium	0.39	0.36	0.24	0.35	0.30
Calcium	3.66	2.19	2.21	3.86	2.87
Potassium	0.36	0.27	0.39	0.51	0.71
Magnesium	1.80	0.64	0.79	1.84	0.73

Identification of microorganisms from the prepared composts of water hyacinth and cow dung

The identified bacteria from all the composts include: *Bacillus subtilis, Campylobacter jejuni, Proteus mirabilis, Citrobacter koseri, Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli, Klebsiella oxytoca, Pseudomonas aeruginosa* and *Staphylococcus saprophyticus*. Among these isolates, only *Bacillus subtilis, Enterobacter aerogenes* and *Pseudomonas aeruginosa* survived after the 20 days composts (Table 2). Regarding the identified fungal isolates, *Aspergillus flavus, A. fumigatus, A. niger, Candida albicans, Penicillium* sp. and *Saccharomyces* sp were obtained. Among these fungi, only *A. flavus, A. niger, C albicans* and *Penicillium* sp. were able to survived after the 20th day of composting (Table 3).

Table 2: Bacterial isolates succession in composts treatments over time

Treatments	Days of Composting			
	5 days	**10 days**	**15 days**	**20 days**
Water hyacinth	*Bacillus subtilis, Campylobacter jejuni, Citrobacter koseri, Enterobacter aerogenes, Klebsiella oxytoca, Pseudomonas aeruginosa*	*B. subtilis, C. jejuni, Enterobacter aerogenes, K. oxytoca, P. aeruginosa*	*B. subtilis, C. jejuni, Enterobacter aerogenes, P. aeruginosa*	*B. subtilis,Enterobacter aerogenes.*
Cow dung	*Bacillus subtilis, Proteus mirabilis, Campylobacter jejuni, Enterobacter aerogenes Enterococcus faecalis, Escherichia coli, Klebsiella oxytoca, Pseudomonas aeruginosa, Staphylococcus saprophyticus*	*B. subtilis, C. jejuni, Enterobacter aerogenes Enterococcus faecalis, E. coli, K. oxytoca, P. aeruginosa, Staph. saprophyticus*	*B. subtilis, Enterobacter aerogenes, Enterococcus faecalis, E. coli, P. aeruginosa, Staph. saprophyticus*	*B. subtilis, Enterobacter aerogenes, P. aeruginosa*
Cow dung and Water hyacinth (1:1)	*Bacillus subtilis, Citrobacter koseri, Enterobacter aerogenes, Enterococcus faecalis, Klebsiella oxytoca, Pseudomonas aeruginosa, Staphylococcus saprophyticus*	*B. subtilis, Enterobacter aerogenes, Enterococcus faecalis, K. oxytoca, P. aeruginosa, Staph. Saprophyticus*	*B. subtilis, Enterobacter aerogenes, Enterococcus faecalis, P. aeruginosa, Staph. Saprophyticus*	*B. subtilis, Enterobacter aerogenes,*
Cow dung and Water hyacinth(1:2)	*Bacillus subtilis, Campylobacter jejuni, Citrobacter koseri, Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli, Klebsiella oxytoca, Pseudomonas aeruginosa, Staphylococcus saprophyticus*	*B. subtilis, C. jejuni, Enterobacter aerogenes, Enterococcus faecalis, E. coli, K. oxytoca, P. aeruginosa, Staph. Saprophyticus*	*B. subtilis, Enterobacter aerogenes, P. aeruginosa, Staph. Saprophyticus*	*B. subtilis, Enterobacter aerogenes, P. aeruginosa*
Cow dung and Water hyacinth (2:1)	*Bacillus subtilis, Campylobacter jejuni, Citrobacter koseri, Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli, Klebsiella oxytoca, Pseudomonas aeruginosa, Staphylococcus saprophyticus*	*B. subtilis, C. jejuni, Citrobacter koseri, Enterobacter aerogenes, Enterococcus faecalis, E. coli, K. oxytoca, P. aeruginosa, Staph. saprophyticus*	*B. subtilis, Enterobacter aerogenes, P. aeruginosa, Staph. Saprophyticus*	*B. subtilis, Enterobacter aerogenes, P. aeruginosa*

Table 3:Fungal isolates succession in composts treatments over time

Treatments	Days of compost			
	5 days	**10 days**	**15 days**	**20 days**
Water hyacinth	*Aspergillus flavus, A. niger, Candida albicans, Penicillium* sp., *Saccharomyces* sp.	A. flavus, *A. niger,* C. *albicans, Penicillium* sp., *Saccharomyces* sp.	. *A. flavus, A. niger, Penicillium* spp., *Saccharomyces* spp.	*A. flavus, A. niger, Penicillium* sp,
Cow dung	*Aspergillus fumigatus, A. niger, Candida albicans, Penicillium* sp.	*A. fumigatus, A. niger, Penicillium* sp	*A. fumigatus, A. niger, Penicillium* sp.	*A. niger, Penicillium* sp.
Cow dung and Water hyacinth (1:1)	*Aspergillus flavus, A. fumigatus, A. niger, Candida albicans, Penicillium* sp., *Saccharomyces* sp.	*A. flavus, A. fumigatus, A. niger, Penicillium* sp., *Saccharomyces* sp.	*A. flavus, A. fumigatus, A. niger, Penicillium* sp., *Saccharomyces* sp.	*A. flavus, A. niger, Penicillium* sp.,
Cow dung and Water hyacinth (1:2)	*Aspergillus flavus, A. niger, Candida albicans, Penicillium* sp., *Saccharomyces* sp.	*A. flavus, A. niger, C. albicans, Penicillium* sp., *Saccharomyces* sp.	*A. flavus, A. niger, C albicans, Penicillium* sp., *Saccharomyces* sp.	*A. flavus, A. niger, C albicans, Penicillium* sp
Cow dung and Water hyacinth (2:1)	*Aspergillus flavus, A. niger, Candida albicans, Penicillium* sp., *Saccharomyces* sp.	*A. flavus, A. niger, Penicillium* sp., *Saccharomyces* sp	*A. flavus, A. fumigatus, A. niger, Penicillium* sp., *Saccharomyces* sp.	*A. flavus, A. niger, Penicillium* sp.

Bio-fertilizer effect of the compost on tomato plant

The recorded tomato plant height, stem girth and the leaf area were fully developed in all the treatments after 8 weeks of plantation. The CW 12 performed better in all the experiments followed by CW11.The least activity from the different compost ratios or treatments was observed in (CW 21) at 2 to 6 weeks of planting and at 8 weeks, least activity was observed in cow dung compost. CW12 entered the flowering stage at 4 weeks and at 6 weeks, soil amended with cow dung and water hyacinth composts entered the fruiting stage except for the control (without compost), although, compost from cowdung only had delayed flowering up to the 8th week(Fig 4a to 4d).

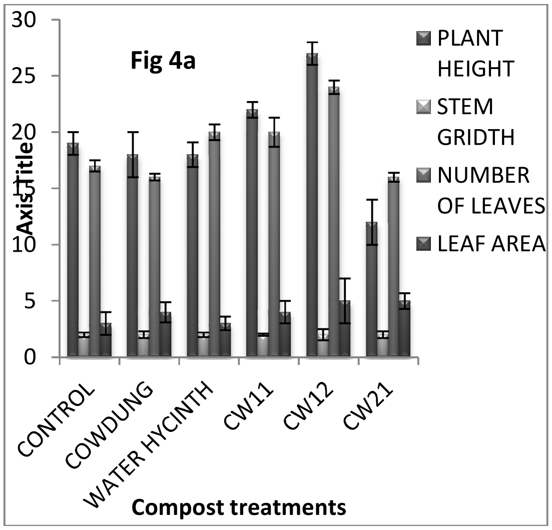


Fig. 4a: Biofertilizer effect of the composts on tomato plant after two weeks

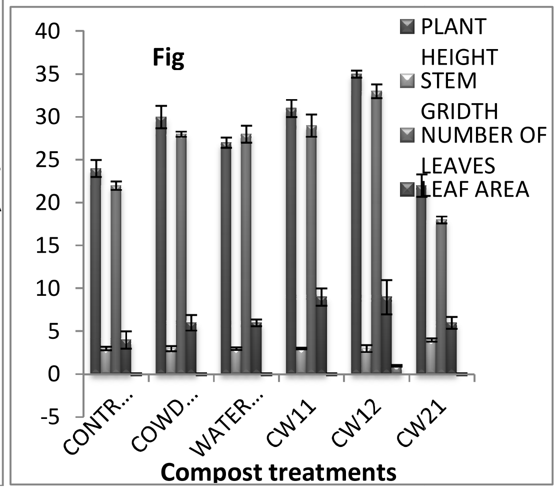


Fig. 4b: Biofertilizer effect of the composts on tomato plant after four weeks

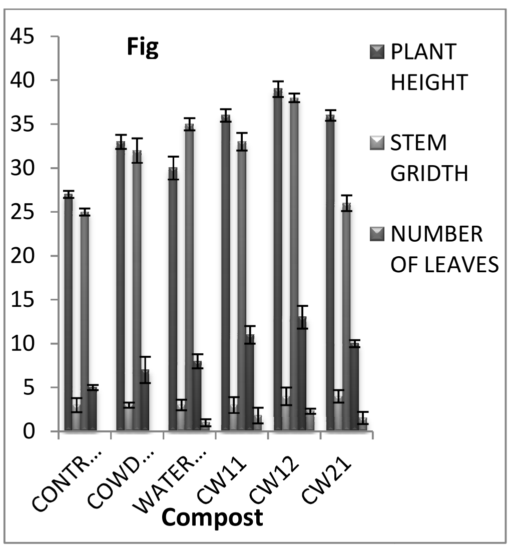


Fig. 4c: Biofertilizer effect of the composts on tomato plant after six weeks

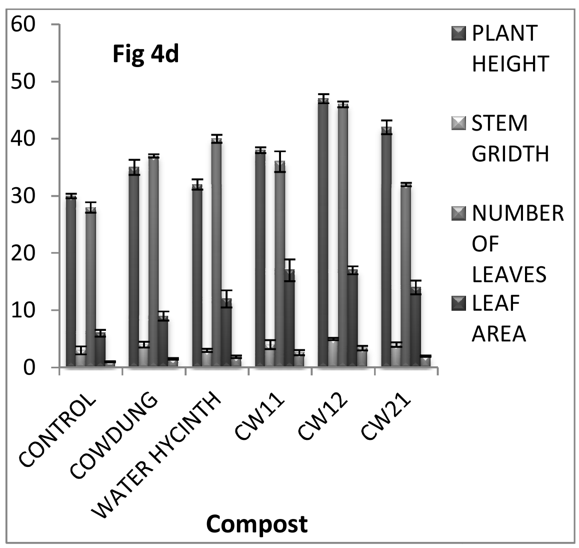


Fig. 4d: Biofertilizer effect of the composts on tomato plant after eight weeks

DISCUSSION

Low crop productivity and decrease in soil fertility could arise due to continuous cultivation on a particular farmland (Belay, 2015). Nevertheless, an important source of macro and micronutrients that enhance plant growth, increases the activity of useful microorganisms, increase uptake of N, P and K by plant roots resulting to increase yield of crops could be achieved through the application of organic manure as compost (Salama, 2002; Siam, 2008;Ganesh *et al*., 2012).

In the present study, bacterial and fungal counts were higher in all the compost treatments. In a similar study, Vishan *et al*. (2013) reported a higher total bacterial count of 1.71 x 10^{12} cfu/g and total fungal count of 8.90 x 10^{7} cfu/g from water hyacinth composted for 21 days.

Similarly, highest bacterial and fungal countsin cow dung and CW11 composts was in relation to the work done by Patidar *et al.* (2013). Dhal *et al.* (2012) reported pH range of 6.2 to 8.2, during the composting of water hyacinth using cattle manure and saw dust as bulking agent. Ko *et al.*(2008), however suggested that as composting proceeds, the organic acids become neutralized and compost material tends toward a neutral pH. The increase in pH in the compost from Day 5 to Day 20 could possibly be linked to the microbial decomposition of organic compounds in the compost coupled with the liberation of ammonium (NH^{4+}).

The reduction in the pH by some compost ratio could be attributed to the production of organic acids into the compost leading to further reduction in the pH. This however was also reported by Wichuk and McCartney(2010).The high temperature observed during composting was attributed to higher content of easily biodegradable organic matter (Kalamdhad and Kazmi, 2009). The highest temperature obtained in this study was lower compared to 56.7 °C reported by Dhal *et al.* (2012) which could be due to the variation of the used substrates. In addition, Prasad *et al.* (2013) reported that the increase in temperature with time in the compost was due to the metabolism of the organic substrates and release of carbon dioxide, water vapour and a large amount of heat.

The electrical conductivity obtained in this study is similar to 2.2 - 7.3 µS/cm reported by Dhal *et al.* (2012) while the nutrient elements were lower than those (Ca: 4.7-25.4 cmol/kg; Mg: 3.7-11.2 cmol/kg; K: 9.7-84.1 cmol/kg) reported by Islam and Toyota (2004). Higher electrical conductivity usually slow down rooting process and reduce the transportation of water and nutrients into the plants (Singh and Kalamdhad, 2013).

The available phosphorus and total nitrogen obtained in this study were lower than those of Prasad *et al.* (2013) which reported 22.5-53.7 % and 50.0- 66.7 % available phosphorus and total nitrogen respectively. It was also observed that as the compost days increases, the number of micro-organisms decreases and also some of the microorganisms identified at day5 disappeared after 20 days of the compost. This could be due to their metabolic activities and nutrient depletion as the number of days increases. Vishan *et al.* (2013) reported the isolation of spore-forming bacteria, mesophilic bacteria and fungi from compost of water hyacinth, cow dung and saw dust. Also, *Bacillus subtilis* and *Pseudomonas aeruginosa* isolated from the compost had been proven to be an effective biocontrol agents in the previous work of Akintokun and Taiwo (2016). Belay (2015) also documented that compost used on farmland have beneficial effects on the soil and crop yield.

The tomato treated with compost commenced flowering at 4^{th} week while the soil without compost was still at the vegetative stage. This however, corroborates with the reports of Mashavira *et al.* (2015) and Taguiling, (2013) that documented that compost amendment reduces the maturity period of plants.

The ability of the compost made from water hyacinth and cowdung to improve the tomato plant was in line with the report given by Popoola*et al.* (2014) who stated that organic substrates of water hyacinth can be biodegraded and stabilized by composting and the final compost products could be utilized as bio-fertilizer or soil conditioner.

CONCLUSION

This study revealed that compost of water hyacinth and cow dung at the different combinations or ratios could be used to improve the growth of tomato plant. Hence, incorporation of water hyacinth and cow dung compost into soil for amendment helps in converting some of the environmental nuisance or waste like the cowdung and water hyacinth into wealth due to the microbial action on this waste and their ability to aid the tomato plant growth.

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