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ARTICLE

Postharvest Quality and Safety of Potted Greenhouse Tomato Grown on Forest Soil-Biosolids substrate, Blended with NPK Fertilizer

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ABSTRACT

Studies on the effects of biosolids (BS) amended substrate on food quality and safety in tomato production have not been adequately addressed. The objective of this study was to investigate the influence of composted BS and NPK fertilizer on post-harvest quality and safety of potted greenhouse tomato *Solanum lycopersicum* L. Potted tomatoes "Maxim F1" were grown in a randomized complete block design with four replications. Inorganic fertilizer NPK (17:17:17 fertilizer was applied at 0, 100 kg ha^{-1} (5g per pot) and 200 kg ha^{-1}; (10g per pot), BS was applied at 0%, 10%, 20%, 30%, and 40% v/v, in all possible combinations. Tomato were harvested and analyzed for ascorbic acid, chlorophylls, carotenoids and total phenolic compounds; weight loss, fruit firmness, titratable acidity and total soluble solids, as well as heavy metals and microbial contaminants. Results revealed that tomato fruit at 10% BS in combination with NPK fertilizer at 100 kg ha^{-1} had the highest β-carotene (6.1 mg 100 g^{-1}), lycopene (26.1 mg 100 g^{-1}), ascorbic acids (128.0 mg 100 g^{-1}), total phenolic acids (13.2 mg 100 g^{-1}), total soluble solids (17%). However, the same rates produced tomato fruit with lower titratable acidity (2.2%) and had heavy metal residues within the permissible level, according to International EPA standards on biosolids utilization for food crops production. Similarly, no trace of pathogenic bacteria; *Salmonella, Escherichia coli, Staphylococcus* was observed on the harvested tomato. This study reveals at BS 10% with NPK fertilizer at 100 kg ha^{-1} substrate as a better option of plant nutrient source for quality and safe greenhouse tomato production.

1. Introduction

Biosolids are organic materials that result from the treatment of domestic sewage in a wastewater treatment [28]. World production of biosolids is ever increasing due to global population rise and social progress, particularly in developing countries [31]. Although technologies to manage, transform, or reuse biosolids are continuously being developed, their safe agricultural use is also considered a sustainable option. They are, not only rich in nutrients but also contain significant level of contaminants such as pathogens and pollutants [10]. However, for use in crop production, they are stabilized to reduce or eliminate pathogens and manage volatile organic solid. Thus, application of biosolids as a fertilizer in crop cultivation is a common practice in many countries [28]. When applied to land, biosolids can improve crop

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yields through fertilization, increase soil water storage, improve soil quality, avert greenhouse gas emissions, and accelerate carbon sequestration by improving the capacity of the soil to store carbon [8].

The use of biosolids has been reported to enhance secondary metabolites in tomatoes *Solanum lycopersicum* L especially carotenoids, of which lycopene is the most abundant in the ripe fruit, accounting for approximately 80-90% of the total pigments [22]. Lycopene is an antioxidant which protects cells from oxidative damage and helps to decrease the risk of chronic diseases such as coronary heart diseases and cancer. Environmental conditions and growing medium fertility can affect fruit lycopene content [19]. Similarly, α-, β-, γ-, δ-carotene, zeaxanthin, lutein, neurosporene, phytoene, and phytofluene, which has clinically been proven as natural anti-carcinogenic and health related compounds in tomatoes, is directly dependent on nutritional application on tomato[9]. Previous studies have shown that tomato plants accumulate phenolic compounds as defense mechanism under certain stress conditions, such as low N availability. Ribas-Agustí *et al.*[41] also suggested that fruit quality in terms of weight, diameter, brix and phenolic content can be achieved by partially replacing mineral N fertilizer with composted biosolids. An increase in tomato phenolic content was observed after organic amendment, biosolids, was added to the substrate[7]. In a different study, Tzortzakis *et al.*[48], working on greenhouse pepper *Capsicum annum* production reported that use of biosolids substrate, increased total phenols and fruit lightness. However, it reduced fruit acidity but did not affect fruit dry matter content, firmness, green colour, total soluble sugars and EC of peppers. In another study by Perez-Espinosa *et al.*[40], titrable acidity, and soluble solids in fresh fruit seemed to increase with sewage sludge application. Mostly these organic fertilizers have been known to supply both macro and micronutrients. Among the micronutrients, boron and zinc play an important role in improving the yield and quality of tomato in terms of secondary metabolites and chlorophyll content [40].

In another hand, product safety in the use of biosolids is of great concern and especially on production of fresh produce. The rates of biosolids application in agriculture have been guided by the amount of trace element in the samples used [35]. Trace elements are of particular concern in regard to their effects on human and animal health. United States EPA (2002) has analyzed the risks of heavy metals and trace elements to humans, animals, plants, and soil organisms from exposure to pollutants in biosolids via different pathways for applied biosolids. The application of biosolids ceases, if it is estimated that the cumulative loading limit is exceeded [13].

Microbial contamination in biosolids is mainly of faecal origin [35]. However, as most of the contaminating micro-organisms are heat-sensitive, they are eliminated during composting, leading to a faecal pathogen-free end product. For persistent microbes, D'Addabbo *et al.* [11] proposed sanitization, through employing solarization of the media. Strauch [42] quantified the pathogens of concern and their evolution during composting. Pathogens found in biosolids can be viruses, bacteria, protozoa or helminths. These micro-organisms are potential contaminants because of their pathogenicity and are indicators of faecal contamination. Decrease in faecal contamination indicators and elimination of faecal pathogens was reported by De´portes *et al.* [12], when biosolid were left before use over period of one year and all were eliminated. The objective of this work was to determine the effect of biosolids blended with forest soil and NPK fertilizer on post-harvest quality and safety of greenhouse tomato.

2. Materials and Methods

2.1 Experimental Site

This study was conducted for two growing seasons (season 1: January to May 2018 and season 2: June to November 2018) at the Horticulture Research and Teaching Field, Egerton University, Njoro, Kenya. The site is located on latitude 0 23' S and longitude 35 35' E in the lower highland III (LH3) agro ecological zone at an altitude of 2238 m above sea level [25]. A tunnel-shaped greenhouse measuring 8 m by 60 m by with a height of 3 m and covered with UV stabilized polyethlene sheet gauge 150 µm from Amiran Kenya Ltd Nairobi, Kenya. The air temperatures inside the greenhouse during the experiment were 24.5 ± 0.9 °C and 13.3 ± 4 °C during the day and night, respectively. The average day and night relative humidity inside the greenhouse were 55.6 ± 9.6% and 80.8 ± 3.6%, respectively.

2.2 Biosolids and Forest Soil Sample Collection, Substrate Preparation and Analysis

Biosolids (BS) were collected from a lagoon pond at Egerton University Wastewater Treatment Plant and forest soil (typically tropical forest soil), within the same locality. Before blending with NPK (17:17:17) rates, the substrates for tomato production were prepared by mixing the biosolids and forest soil (BS: FS at rates of 0, 10, 20, 30, and 40% (v/v). Samples from each rate, were comprehensively analysed in Laboratory at Kenya Plant Health Inspectorate Services (KEPHIS), Kitale, Kenya. This was to determine the physico-chemical characteristics and nu-

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trient levels of the substrates (Table 1).

Table 1. Physico-chemical characteristics of the substrate used for tomato transplant production

Characterization/ substrates	FS	BS 10%	BS 20%	BS 30%	BS 40%
Bulk density (g cm^{-3})	1.7	1.6	1.5	1.3	1.3
Porosity (%)	35.9	39.6	43.4	50.9	50.9
Moisture content (%)	25.8	34	40.8	42.8	44.5
EC (mS m^{-1})	2.6	3.2	3.6	4.4	5.1
pH	7.4	6.2	6.6	6.5	6.4
Organic matter (g kg^{-1})	157.7	197.8	196.7	210	209.8
C:N	21.3	19.7	15.4	9.6	12.7
Total Carbon (mg g^{-1})	91.7	115.0	114.4	122.1	122.0
Total N (g kg^{-1})	4.3	5.9	7.4	12.9	9.6
Total P (mg k g^{-1})	69.1	83	90.3	101	95.9
K (mg kg^{-1})	132.5	412.3	419.9	427.8	422.4
Ca (mg kg^{-1})	21.9	24	22.8	29.5	27
Mg (mg kg^{-1})	131.1	126.1	117.7	119.1	113.8
Na (mg kg^{-1})	62.9	254.8	342.1	252.8	348.3
Mn (mg kg^{-1})	69.6	530.4	524.8	539.4	553.9
Fe (mg kg^{-1})	27	2490	2473.9	2479.1	2471.5
Zn (mg kg^{-1})	4.7	47.4	44	44	45.9
Cu (mg kg^{-1})	4.4	12.2	12.7	10.3	12.7
Cd (mg kg^{-1})	0.0023	0.0128	0.0115	0.0117	0.0122
Pb (mg kg^{-1})	109.6	2.8	2.1	5.1	3.1

Key: FS (Forest soil), BS (Boisolids).

2.3 Experimental Design and Treatments

The experiment was as arranged in a split-plot in a randomized complete block design, replicated four times. Inorganic fertilizer NPK (17:17:17) at three levels: 0, 100 kg ha^{-1} (5g per pot) and 200 kg ha^{-1}; (10g per pot) was the main plot factor while Biosolids mixing rates in forest soil (FS) at five levels: 0, 10%, 20%, 30% and 40% (v/v) constituted the subplot factor. Four blocks each with 15 treatment combinations were separated by a 0.7 m path. Each experimental unit had 10 potted tomato plants in two rows. Using khaki paper bags, tomato samples for post-harvest analysis were harvested at breaker stage from the six pots in the middle of each plot. They were arranged in the laboratory according to the design in the field.

2.4 Postharvest Analysis

After harvesting tomato from the greenhouse, thirty fruits were randomly selected from each treatment, for further postharvest analysis. Fruits were placed on the shelf for ten days to ripen uniformly. Within the period of those ten days, data for various postharvest data were collected at interval of 0, 5 and 10 days after harvest. The average temperatures inside the laboratories shelves at Egerton were 22 ± 4 °C and 21 ± 0.9 °C for day and night respectively. The average relative humidity inside the laboratory was 55 ± 2.3% and 60 ± 3.6%, for day and night, respectively.

Tomato secondary metabolites and other quality aspects were done in the Molecular Biotechnology laboratory at Egerton University, Kenya. For extraction, 4 tomatoes were randomly picked from each substrate treatments, cut into small pieces, blended into a paste using pestle and mortar and duplicated 3 times.

2.4.1 Ascorbic Acid Content

Ascorbic acid was determined by titration with 2, 6-dichlorophenolindophenol dye. Ten grams of fresh tomato fruit sample were extracted in 30 mL of 5% oxalic acid using a pestle and mortar, and then filtered (Whatman No.1 filter paper). Standard indophenol solution was prepared by dissolving 0.05 g of 2, 6-dichlorophenol-indophenol in distilled water then diluted to 100 mL and filtered. Ascorbic acid standard solution was prepared by dissolving 0.05 g of pure ascorbic acid in a small volume of 5% oxalic acid solution and then diluted to 250 mL with the same oxalic acid solution. Ten milliliter of the ascorbic acid standard solution was then titrated with the indophenol solution to a slight pink end point. Ten milliliters of oxalic acid were titrated as a blank. The amount of ascorbic acid corresponding to one milliliter of indophenol solution was then calculated. Ten milliliters of the filtered sample extract were pippeted into a 50 mL flask and made to the mark with the 5% oxalic acid solution. The standard indophenol solution was used for titrating 10 mL of the filtrate. The ascorbic acid content was expressed in mg per 100 g sample. Using the formula;

Ascorbic acid = C×V× (DF/WT)

Where C = ascorbic acid (mg); V= Volume of dye used for titration of diluted samples (mL)

DF = dilution factor, WT= sample weight (g).

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2.4.2 Total Phenolic

The total phenolic content (TPC) was measured according to Genovese *et al*. (2008)[18]. The samples were extracted in proportions of 1:20 (m/v) with methanol, using a homogenizer for 1 minute. The sample was re-extracted in the same ratios. The supernatants were filtered using filter paper No. 1 and the volume made up to 50 mL. An aliquot (1 mL) of supernatant was oxidized with 1 mL of Folin-Ciocalteu's reagent and neutralized by 1 mL of 20% sodium carbonate. The reaction mixture was incubated for 30 minutes at ambient temperature and absorbance was measured at 745 nm using a UV/Vis Spectrophotometer Model: U-T6, (Shanzhai) Co. Ltd, and China. Total phenolic content was obtained using a calibration curve of garlic acid (1 mg/mL) as standard. Total phenolics were expressed in mg equivalents of garlic acid per 100 grams of the sample (mg GAE/100 g).

2.4.3 Determination of Lycopene, β-carotene and Chlorophylls

Lycopene and β-carotene and chlorophyll were extracted as described by Fish *et al*. [15] using acetone/hexane (4:5). The samples were analysed by UV-Vis spectrophotometer, for β-carotene (453 nm), lycopene (505 nm), and chlorophyll a (663 nm) and b (645 nm). Carotenoids and chlorophylls were calculated and expressed as µg/g DM.

For calculating β-Carotene and Lycopene, the formula for their content was = *(Ex x V)/FW,*

Where Ex; absorbance depending on the carotenoids, V; volume of the solution (25 ml) and FW; the fresh weight of the sample.

Total carotenoids = (450 x V x 4)/FW

Where E450 is the absorbance at 450nm, Vis the volume of the solution (25ml), 4 is a constant and FW is the fresh weight of the sample.

Chlorophyll a = {(10.1 x E663) - (10.1 x E645) x V}/FW

Chlorophyll b = {(16.4 x E645 - 1.01 x E663) x V}/FW

Where E663 and E645; the absorbance of chlorophyll a and b respectively, V; the volume of the solution and FW; the fresh weight of the sample done according to Gogo et al.[20].

2.4.4 Weight Loss

Weight loss was determined from harvested tomato fruits on the individual biosolids treatments. A random sample of five fruits was drawn from each treatment lot and weighed from harvest. The same fruits were kept open at room temperature and re-weighed after every two days until the tenth day and results presented as percent weight loss based on the initial weight.

2.4.5 Fruit Firmness

The determination of tomato fruit firmness was done from harvested tomato fruits. Using a random sampling, ten fruits drawn from each treatment lot and data collected on a period of 0, 5 and 10 days. Fruit firmness (kgF cm^{-2}) was taken from each fruit using a hand-held penetrometer (FT327; Shangai Precision and Scientific Instrument Co., China), with bore size no. 12 and the means were computed for each treatment lot according to Otieno *et al.* [39].

2.4.6 Total Soluble Solids

Fruits total soluble solids (TSS) was determined by extracting juice by squeezing and then centrifuged to obtain a homogenized sample. TSS was determined using a hand held refractometer (RHB; Shangai Precision and Scientific Instrument Co., China) as per the procedure described by Tigchelaar [47]. Results from the treatments were reported as % TSS.

2.4.7 Titratable Acidity

Five milliliters of tomato juice was diluted with 50 mL of distilled water and titrated against 0.1 N NaOH solution using phenolphthalene indicator The volumes of NaOH titre required to change the indicator from colourless to pink were recorded and multiplied by a factor of 0.064, the acid factor for the predominant acid in tomato (citric acid), to estimate the TA levels according to the formula of Tigchelaar [47]:

$$Acid\ (\%) = Titer \times acid\ factor \times 100\ 10\ mL\ of\ tomato\ juice$$

2.4.8 Heavy Metal Analysis

Determination of heavy metal contaminants on tomato fruits was done in the soil and tissue analysis Laboratory at Kenya Plant Health Inspectorate Services (KEPHIS), Kitale, Kenya. This was to assess the presence of Pb Cd, Cu, Zn and Ni. Ripe and ready to eat tomato were blended and wet digestion method was employed. Five ml of blended tomato juice sample was taken and transferred to 100mL conical flask. An aliquot of 4 mL HNO_3, and 0.5 mL H_2O_2 was added and special containers packed and placed into a microwave for digestion. The resultant solution was transferred to 50 mL volumetric flask diluted by Internal Standard Solution (ISTD) Ge, Rh, T1 at 50 ppb for nitric acid digestion. The Inductively Coupled Plasma Mass Spectrometry (ICP-MS) conditions were adjusted, calibrated by blank solution. Then for better operating conditions, the ICP-MS was adjusted to nebulizer gas flow

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0.91 L/min, radio frequency (RF) 1200 W, lens voltage 1.6V, cool gas 13.0L/min, and auxiliary gas 0.70 L/min and finally metals contents of the fruits were analysed by ICP-MS, method adopted by Musa and Lal [36].

2.4.9 Microbial Load

Microbial contamination study was done in the Food Science Microbiology laboratory at, Egerton University, Kenya. This study was based on safety aspect of the tomato fruits ready for consumption, targeting the existence of *Salmonella* sp, *Escherichia coli, Staphylococcus* spp as contaminants. This was done to determine specific microbes mentioned, in the fruit when biosolids is used as a plant nutrient. Ripe tomatoes from different rates of BS were used for microbial load analysis. Using random selection, four tomatoes samples were picked from each plot, washed thoroughly in running water, and then rinsed in double distilled water. The tomatoes fruits were cut in to small pieces and 25 g of the pieces were blended using a juice blender into a tomato paste. Samples were tested for the presence/absence of biological contaminants such as *Salmonella* spp, *Escherichia coli, Staphylococcus* sp. Using manufacturers procedure, three types of selective media were prepared for the inoculation of the specific bacteria of concern: *Salmonella spp in* Salmonella Shigella Agar, *Escherichia coli* in Eosin Methylene Blue Aga and *Staphylococcus* in Baivd Parker Agar. For the counting of bacteria, 1 mL of homogenate was aseptically transferred onto plate count nutrient agar (Oxoid, England) in triplicates. The plates were incubated at 37 °C for 24 hours under aerobic atmosphere. After incubation and isolation, the number of colonies were determined by a colony counter and recorded as colony-forming unit (CFU) g^{-1} and total viable count (TVC) g^{-1} of the growing medium. The evaluation of microbial cellular content in samples were determined by counting of plates. The existence of pathogenic microbial organisms like *Salmonella sp*., *Escherichia coli* and *Staphylococcus sp.* was determined in each sample in triplicates, method used by Otieno *et al*. [38].

2.5 Data Analysis

Data analysis was carried out using SAS statistical package version 9.1 (SAS Institute, Cary Inc., 2001). Numerical data were subjected to analysis of variance (ANOVA) at $p \leq 0.05$. Means for significantly different treatments were separated using Tukey's test at $p \leq 0.05$. Data are presented in table and graphs as means ± standard deviations.

3. Results

3.1 Ascorbic Acid

The ascorbic acid content of tomato fruits was significantly influenced by substrates used for production. Generally, ascorbic acid content started to reduce from the time of harvest. The interactive effect of BS at the rates of BS 10% in combination with 5g per plant of NPK showed a consistency in high ascorbic acid content throughout the 10 days storage period (Figure 1A). However, beyond BS 20%, combining the substrate with NPK fertilizer did not improve ascorbic acid content in the tomato fruit.

3.2 Total Phenolic Acids

Total phenolic acids of the fruits generally decreased during the storage period. Regarding responses to the substrate rates, phenolic acids was significantly higher in NPK 5g per plant with BS 10% compared to the control and other substrates observed before 5 Days after harvest (DAH). At 5 DAH, similar trend was observed with NPK 100 kg ha^{-1} and BS 10% registering higher phenolic acids compared to the control and the higher rates both BS and NPK combined. This was consistent throughout 10-day period (Figure 1B).

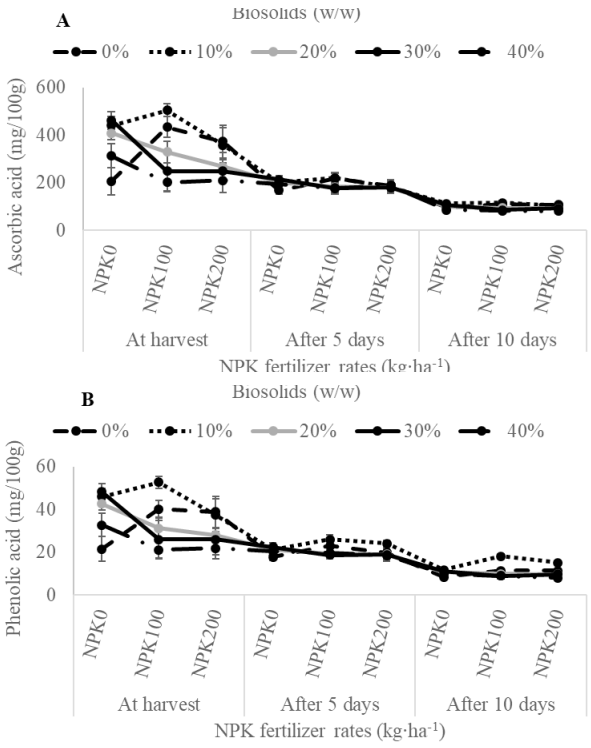

Figure 1. Effect of NPK and biosolids rates on tomato ascorbic acid and phenolic acid contents during room temperature storage

Note:
**Values represent means ± standard deviations.*

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3.3 Beta β-carotene Content

The substrate significantly influenced the fruit tomato β-carotene. Generally, tomato β-carotene content increase with days in the shelf. The results showed the substrate significantly influenced the fruit tomato β-carotene from 5 DAH. At both 5 DAH and 10 DAH, interactive effect of BS 10% and NPK 5g per plant registered higher fruit β-carotene content than control and other higher rates but not significantly different from BS 30% in for forest soil (Figure 2A).

3.4 Tomato Lycopene Content

Tomato lycopene content generally increased during the 10-days storage period. Fruit lycopene content generally increased with shelf-life. For the fruits produced in BS without NPK fertilizer, 10%, 20% and 30% were significantly higher in lycopene content than control and BS at 40%. However, the interactive effect between BS 10% with fertilizer 5g per plant, registered higher lycopene content consistently throughout the 10 DAH. Any rate of BS above 10% did not increase lycopene content of the fruits in combination with fertilizer (Figure 2B).

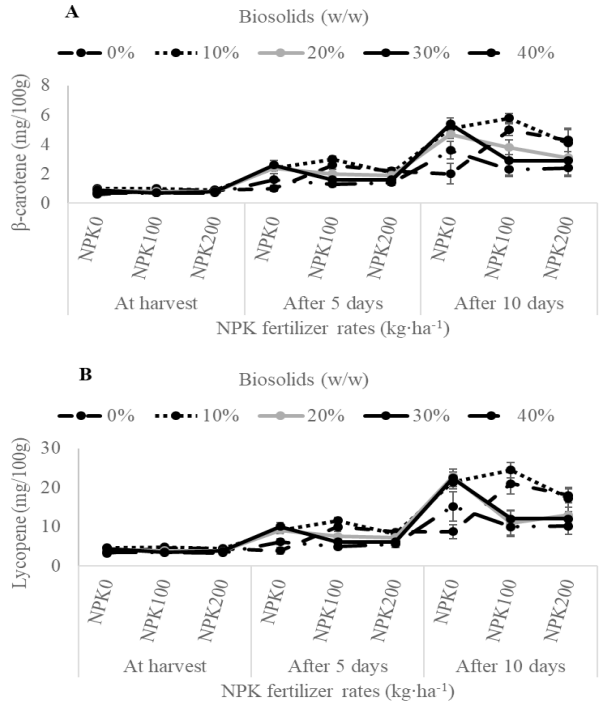

Figure 2. Effect of NPK and biosolids rates on tomato β-carotene and lycopene contents during room temperature storage

Notes:
**Values represent means ± standard deviations.*

3.5 Tomato Chlorophyll a and b

Generally, the total chlorophyll of tomato decreased with storage days as were significantly influenced by BS rates and when combined with NPK fertilizer. From BS at 10% to 30%, chlorophyll a content of the fruits were higher than control and BS at 40%. While on substrate combined with 5g per plant of NPK, only BS at 10% showed significantly higher chlorophyll a (Figure 3A). A similar response was observed in chlorophyll b content of the tomato fruits during the ten days period (Figure 3B). Similarly, any rate of BS above 10% with NPK fertilizer did not show a significant increase in both chlorophyll a and b.

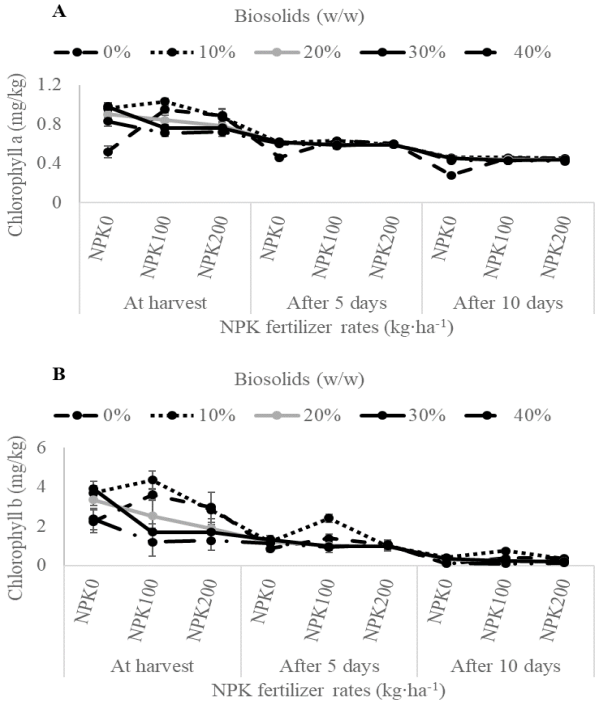

Figure 3. Effect of NPK and biosolids rates on tomato chlorophyll a and b contents during room temperature storage

Note:
**Values represent means ± standard deviations.*

3.6 Fruit Weight Loss

Tomato weight loss was significantly influenced by various rates of biosolids and fertilizer. Forest soil (control) showed significantly ($p<0.05$) higher percentage in rate of weight loss within the 10 days of storage. The same trend was observed in BS at 20% and 30% with higher percentage in weight loss compared to the rest of the substrates (Figure 4A). Interactive effect of the BS and NPK fertilizer was evident as from day after harvest where lowest rate of weight loss was observed on BS 10% combined with 5g per plant.

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3.7 Fruit Firmness

Generally, fruit firmness decreases with increase in days after harvest. The interactive effect of BS at 10% and NPK at 5g per plant was observed as the best rate of the substrate on tomato fruit firmness and this was consistent within the 10-days period in the shelf (Figure 4B).

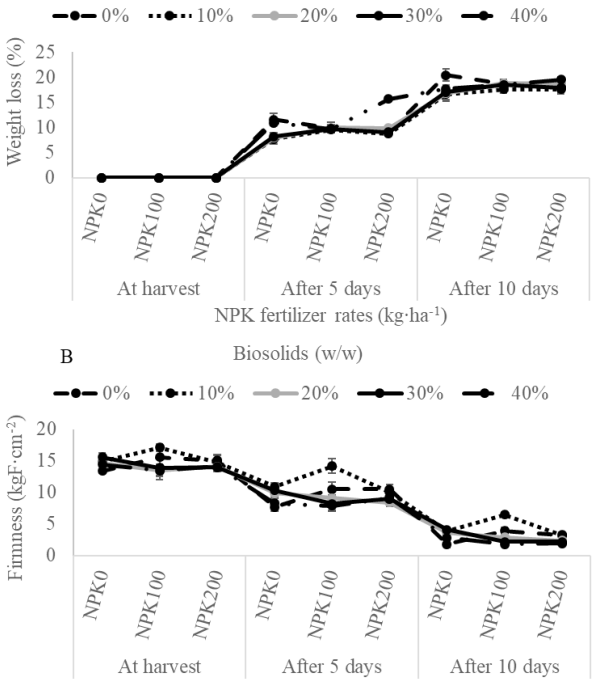

Figure 4. Effect of NPK and biosolids rates on tomato weight loss and fruit firmness during storage at room temperature

Note:
**Values represent means ± standard deviations.*

3.8 Total Soluble Solids

The TSS generally increased within the 10-days in the shelf (Figure 5A). The combination of BS at 10% and NPK at 5g per plant was significantly higher than the control. While BS rate alone were best at 30%, although not significantly different from the combination of BS and fertilizes aforementioned above.

3.9 Titratable Acidity

The results showed a general decrease in fruit Titratable acidity (TA) from different substrate rates. Titratable acidity was significantly higher on the fruits produced from substrate biosolids 20 to 40% and those blended with NPK 10g per pot compared to the rest of the substrates (Figure 5B). This trend was consistent throughout the 10-days of tomato postharvest.

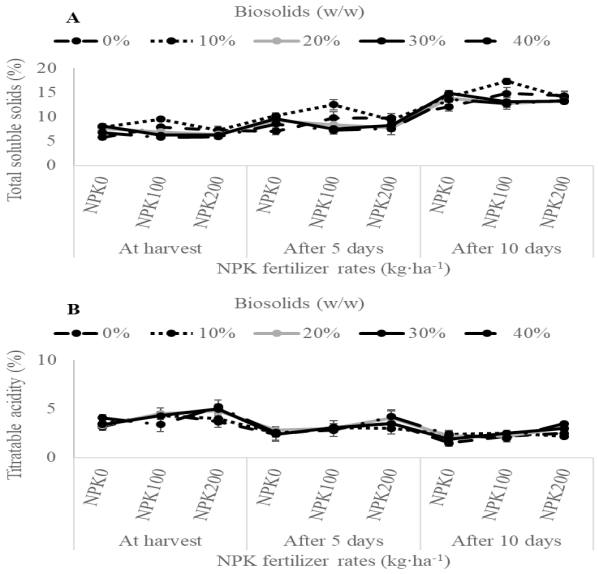

Figure 5. Effect of NPK and biosolids rates on tomato total soluble solids and titratable acidity during room temperature storage

Note:
**Values represent means ± standard deviations.*

3.10 Heavy Metal

The rates of biosolids substrate differently influenced the presence of heavy metals in the fruits. Rates of BS at 20% to 40% showed higher significance Zn, Ni, Cu, Cd, and Pb compared to the control, and BS at 10%. Nevertheless, all were still below the standard stipulated by the EPA (Table 2). The interactive effect of BS and NPK fertilizer reduce the amount of BS required for most of the post-harvest quality to boisolids rate of 10% and NPK 5g per pot.

3.11 Microbial Load on the Tomato Fruits

In the tomato fruit extracts the targeted bacteria; were *Salmonella, Escherichia coli, Staphylococcus* as food poisoning main contaminants associated with wastewater. As was observed in the biosolids substrates rates, the absence of the aforementioned bacteria, similar observation was made on the fruits grown on the biosolids substrates used in the greenhouse production; there was absence of those specified bacterial contaminants in the tomato (Table 3).

4. Discussion

Consumers have become increasingly concerned about the safety and quality of food products they are consume. The present study evaluated the effect of blended biosolids (BS) on postharvest quality of tomato. Various pre-harvest factors including plant nutrition are known to influence the phytochemical properties, which may improve post-harvest quality of horticultural crops including tomato.

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Table 2. Effect of NPK and biosolids rates on tomato heavy metal content during production

		µg·kg^{-1}				
NPK	**Biosolid**	**Zn**	**Ni**	**Cu**	**Cd**	**Pb**
NPK_0	0%	3.5±0.3^{c}*	3.1±0.1^{c}	3.5±0.3^{b}	2.3±0.1^{b}	5.0±0.3^{b}
	10%	3.9±0.2^{bc}	3.4±0.1^{bc}	3.7±0.4^{b}	2.4±0.3^{b}	5.2±0.4^{b}
	20%	4.7±0.2^{ab}	4.3±0.1^{ab}	4.8±0.5^{a}	3.2±0.3^{a}	6.0±0.3^{a}
	30%	4.8±0.2^{ab}	4.5±0.2^{ab}	5.1±0.3a	3.4±0.2^{a}	6.4±0.3^{a}
	40%	5.1±0.2^{a}	4.9±0.2^{a}	5.3±0.5^{a}	3.5±0.4^{a}	6.6±0.2^{a}
NPK_5	0%	3.5±0.2^{c}	3.4±0.1^{bc}	3.8±0.7^{ab}	2.4±0.3^{b}	5.3±0.5^{b}
	10%	3.7±0.4^{bc}	3.6±0.4^{bc}	3.8±0.5^{ab}	2.5±0.3^{b}	5.3±0.6^{b}
	20%	4.1±0.3^{b}	3.6±0.4^{bc}	4.3±0.1^{ab}	2.5±0.4^{b}	5.6±0.1^{ab}
	30%	4.2±0.2^{b}	4.0±0.2^{ab}	4.2±0.2^{ab}	2.8±0.1^{ab}	5.4±0.3^{ab}
	40%	4.7±0.2^{ab}	4.5±0.3^{ab}	4.9±0.6^{a}	3.3±0.4^{a}	6.5±0.3^{a}
NPK_{10}	0%	3.5±0.3^{c}	3.5±0.2^{bc}	3.6±0.4^{b}	2.3±0.2^{b}	5.6±0.1^{ab}
	10%	3.7±0.3^{bc}	3.6±0.4^{bc}	4.2±0.3^{ab}	2.8±0.2^{ab}	5.7±0.2^{ab}
	20%	3.8±0.5^{bc}	3.9±0.4^{ab}	3.9±0.4^{ab}	2.6±0.2^{ab}	5.6±0.2^{ab}
	30%	4.3±0.4^{b}	4.1±0.1^{ab}	4.7±0.4^{a}	3.1±0.2^{a}	5.8±0.3^{ab}
	40%	4.4±0.4^{b}	4.2±0.1^{ab}	4.9±0.4^{a}	3.3±0.3^{a}	6.0±0.1^{a}
	EPA^{y}	200.0	60.0	100.0	1.0	150.0
	WHO/FAO^{Z}	100.0	67.0	73.0	0.2	0.3

Notes:
**Means ± standard deviation followed by the same letter within a column are not significantly different according to Tukey's test at $p \leq 0.05$. ^{y}Maximum ceiling values of heavy metals(mg kg^{-1}) for agricultural land application according to New South Wales EPA [13]. ^{Z}Source = Codex Alimentarius Commission FAO/WHO (mg kg^{-1}) WHO [51].*

Table 3. Effect of NPK and biosolids rates on microbial contaminant after harvest

NPK	**Biosolid**	**TVC (PCA)**	**CFU (MAC)**	***E. coli* (EMB)**	***Salmonella* (BPA)**	***Staphylococcus* (BPA)**
NPK_0	0%	TNTC	4.5	-ve	-ve	-ve
	10%	121x 10^{-6}	TNTC	-ve	-ve	-ve
	20%	NG	NG	-ve	-ve	-ve
	30%	TNTC	TNTC	-ve	-ve	-ve
	40%	77x10^{-6}	107.5	-ve	-ve	-ve
NPK_5	0%	67x10^{-6}	20	-ve	-ve	-ve
	10%	TNTC	48.5	-ve	-ve	-ve
	20%	6.5x 10^{-6}	6.5	-ve	-ve	-ve
	30%	5x10-6	5	-ve	-ve	-ve
	40%	16x10^{-6}	113	-ve	-ve	-ve
NPK_{10}	0%	TNTC	TNTC	-ve	-ve	-ve
	10%	TNTC	51	-ve	-ve	-ve
	20%	TNTC	28.5	-ve	-ve	-ve
	30%	88x10^{-6}	44.5	-ve	-ve	-ve
	40%	TNTC	6	-ve	-ve	-ve

Key:
TVC- Total Viable Count, CFU- Colony Forming Units, EMB- Eosin Methylene Blue (E. coli), Mac- MaCconkey Agar, BPA- Bairvd Parker Agar (Staphylococcus sp), BPA- Bairvd Parker Agar (Salmonella sp). TNTC- Too Numerous to Count, No Growth, -ve = Absent.

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Ilupeju *et al.* [23] noted that pre-harvest factors including soil fertility influenced ascorbic acid content in tomato. Results from the present study have demonstrated that application of BS 10% combined with NPK 5g per pot produced fruits with higher ascorbic acid content. This was a contribution of the available nutrient and organic matter in both BS independently and also when NPK added to make a blended BS substrate. Similar to this study, Taghavi *et al.* [43] reported in their findings the impact of fertilization and composts, on water and nutrient supply to the plant and its influence in the nutritional composition, higher ascorbic acid content on tomato fruit. Among the elements found in the substrates as mentioned by Otieno *et al.,* [38], boron and zinc were significantly higher in the FS and BS in combination as a substrate at different rates, which subsequently enhanced quality to tomato fruits. Boron is among the very essential trace elements that plays a big role in the synthesis of one of the bases for RNA formation. Boron has been shown to influence ascorbic acid content of tomato fruit [44].

The current study demonstrates that the use of BS 10% and NPK 5g per pot demonstrated higher ascorbic acid content in tomato fruits. However, at BS 10% when the rate of NPK were doubled in the substrate, ascorbic acid content reduced. This could have been due to the antagonistic effect of these elements in excess in the BS substrate. A different study has shown that K, Mg and Ca can antagonize in nutrient solution when in excess [6]. The antagonistic effect of increased Mg levels on the K uptake was reported to be due to differences in their ionic mobility [26]. Similarly, high K concentrations in the nutrient solution may result in Mg deficiencies in the plant tissue and vice versa. In the current results, the reduction in ascorbic acid with increased rate to up to10g of NPK per plant may have resulted from the above-mentioned antagonistic effects of those elements at higher concentration. In a similar argument, whenever Na dominates the media, K is normally over taken by the latter element and therefore may not be available for the growing crops. Reduction of ascorbic acid in the present work especially at higher rate of BS and NPK could also be related to higher N in the substrate, which is associated with more vegetative growth, which led to shading effect of tomato fruits. Development of ascorbic acid in tomato fruit also depends on exposure to light for its accumulation in fruits. In the current study this phenomenon was probably the reason for low content of ascorbic acid registered on fruits in higher rates of BS and NPK observed.

The content of fruit phenolic acid reflected nutritional characteristics of the different substrates tested. As common with many plant secondary metabolites fruit phenolic acids are affected by different growth environments including soil properties such mineral nutrients, salinity and drought [2]. The result of the current study demonstrates a wide range phenolic acid content in tomato within the 10 days of storage period. The significant presence of phenolic acids in fruits raised in BS rates from 10 to 30% without NPK and BS at 10% with NPK 5g per plant, was not only due to availability of mineral nutrients in the substrates but also favored by a suitable pH (6.2 to 6.5 and EC range (3.2 to 4.4 dS m^{-1}), as earlier reported by Otieno et al. [38].

The present study reported higher content of β-carotene in BS at 10% with combined NPK at 5g per pot. Carotenoids have been reported to possess provitamin A activity Tang [45], a precursor of vitamin A essential for promotion of general growth, maintenance of visual function [19], and N being one of the essential elements for their production. In regards to β-carotene, Bojović and Stojanovic [5] demonstrated that carotenoid content depended on the presence and ratio of macronutrients especially N as one of the most essential elements and its deficiency decreases its accumulation. Whereas in the present study, β-carotene content reduced with further increasing rates of BS, and when rates of NPK was doubled. Salt stress in the substrate has been indicated as one of the factors leading to N deficiency [6]. Similarly, lower content of β carotene in fruits from BS at 10% to 40% in without NPK was due to lower N, P and K in different rates of substrate as was reported by Otieno *et al.*[38]. This result is in conformity with those reported Khavari-Nejad *et al.* [29] on the reduction of β carotene in tomato due to deficiency of N and P deficiency. However, López-Ráez and Bouwmeester [32] reported another scenario that P starvation can induce changes in gene expression of some carotenoids including β carotenes and compounds derived from them in tomato roots.

In the current study, tomato fruit lycopene content generally increased in all the substrates with days on the shelf, indicating that the fruit accumulated more of this important secondary metabolite, with shelf-life. Lycopene represents 60-74% of the tomato fruit carotenoids and of other tomato products and content is affected by many pre-harvest factors such as plant nutrition[39]. Biosolids consist of humic acids after degradation, and is able to release plant nutrients by decomposition. Lycopene content plays a key role in tomato appearance and attractiveness to consumer, a part from its immense health benefits[30]. Color is a key quality indicator of tomato fruit. Lycopene in tomato is responsible for the redness, and β-carotene for orange coloration. Deeper red color in tomato is an indication of predominant existence of lycopene which an important antioxidative compound. Nutrition as a pre-

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harvest factor has been known for carotenoid development leading to high lycopene content in tomatoes. In the current study, BS at 10% to 30%, and interactive effect of BS at 10% and 5g of NPK per plant resulted in increase of lycopene content, which consistently increased within 10 days of tomato post-harvest period. Higher nutrient content in the tested biosolids probably enhanced soil moisture status and nutrient availability such as N, P, K, Mg, B Zn, Cu and Mo[38], leading to biosynthesis of carotenoids which are responsible for tomato fruit color especially lycopene.

Chlorophyll content in a tomato fruit is normally affected by pre-harvest factors, of which the main one is nutrition. At post-harvest the chloroplast content may not increase but degrade into chromoplast which, eventually turn into carotenoids [21]. Thus, in the present study, generally, the total chlorophyll of tomato decreased with storage days as were significantly influenced by biosolids rate as well as NPK fertilizer. The present results demonstrated that at harvest, there was higher chlorophyll in fruits produced at BS rates of at 20% to 40%, than FS. This implied there was higher N in organic in the substrate rates as reported by Otieno *et al*. [38]. While on substrate with 5g of NPK, only BS at 10% showed significantly higher chlorophyll a and b. It was demonstrated that combination of forest soil and biosolids as substrate may not only contribute to organic matter but also Mg, Fe, Cu and Zn in the nutritional pool. Magnesium and iron are essential mineral elements for plant growth and also the development of cell structural component. Iron in particular ranges at the upper limit of the micronutrient category with approximately 2 μmol/g plant dry weight and plays an important role as an activator of many biochemical and enzymatic processes [21]. In regards to the current study, Cu and Zn were found to be in sufficient quantity in BS at 10% combined with NPK at 100 kg ha^{-1}. This probably resulted to the higher plant chlorophyll content as observed in the study. Our results are in agreement with Alves *et al.* [1] who demonstrated that concentration of organic fertilizer in the soil and their association with doses of biofertilizer influenced the gas exchange and SPAD chlorophyll content in tomato plants. However, the higher dose of biofertilizer reduced chlorophyll content of tomato plants, which led to the reduction of gas exchange hence reduced photosynthesis.

Weight loss and fruit firmness were influenced by use of BS and NPK fertilizer. Calcium plays a crucial role in cell division and the maintenance of cell permeability and cell wall integrity, all of which directly influence factors such as firmness and shelf-life [16]. Calcium has also been shown to improve cell membrane integrity [37], thus lead to reduction in fruit water loss and consequently weight loss and quality. The present study has demonstrated the benefits of using BS at 10% and with 100 kg ha^{-1} of NPK fertilizer in improving the post-harvest quality of tomato fruits in terms of weight loss reduction as influenced by the use of BS. Fruit firmness may be considered side by side with fruit weight loss since they are affected by plant cell wall integrity. This may have been due to the presence of Ca as indicated to be within the range of pH 6.2 to 7 [44]. Calcium plays a crucial role in cell division and the maintenance of cell permeability and cell wall integrity, all of which directly influence factors such as firmness and shelf-life [16], also known to improve cell membrane integrity [37]. The results of the current study are in conformity with study by Otieno *et al*. [39], who reported that higher rates soil *Lippia kituensis* Vatke and *Ocimum gratissimum* L. used as substrate organic amendment produced tomato with firm fruits compared to the lower rates of organic amendments used. Firmness of fruits goes together with water loss and nutrients. In the present study, weight loss was reduced by use of biosolids at higher rates.

Biosolids are rich in N and organic matter which holds the mineralized plant nutrients together in a substrate. Addition of NPK availed N in the current study which played a significant role in photosynthesis, hence production of carbohydrates and subsequently sugar formation during ripening. Chlorophyll and carotenoid content formation at the vegetative stage of the crop may contribute to the quality of tomato at post-harvest. This is from the carbohydrates production [4], leading to higher TSS in fruits. This concurs with the present study regarding the inverse relationship between soil nitrogen concentration and fruit TSS [4]. Nitrogen seems to increase not only vegetative growth but also yield in expense of fruit quality. Wang *et al.* [50], confirms decrease of TSS with increasing N concentration in the substrate due to salt stress situation and antagonism among the major plant element especially in the higher BS rates blended with double NPK fertilizer.

Application of BS at 20% to 40% blended with 100 kg ha^{-1} of NPK resulted in increased TA, and this was indicative of salt stress with elevated EC of the substrate. Higher EC was observed in the substrate with biosolids at 20% and higher, blended with NPK at 200 kg ha^{-1}. Mardi et al. [34] made similar observations. Thus, higher TA observed in fruits produced from BS at higher rates of BS blended with NPK is attributed to this scenario. Contrary to the findings of the present study, Tzortzakis and Economakis [49] observed a reduction of TA in tomato production with use of perlite, pumice and their mixtures compared with pure soil treatments. This scenario may be alluded to the fact that soilless substrate normally has narrow range of buffering capacity as opposed to soil-based substrate, and this may lead to essential and trace

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element deficiency for crop quality. In a different study, Fathy *et al.* [15] reported that when humic acid was applied to an apricot crop, which led to an increase in the content of TSS and decreasing fruit acidity, a positive constituent of biosolids. Under high available soil moisture, the root may absorb more water, resulting in a reduction in the TSS by water dilution as observed in BS at 40%.

The heavy metal concentration in fresh commodities has been a subject of great concern to consumers. Several factors such as metal concentrations in soils, soil pH, cation exchange capacity, organic matter content, types and varieties of crops have been reported to affect the uptake of heavy metals [33]. It is generally accepted that the metal concentration in soil is the dominant factor as far as residues are concerned [27]. The concentration of heavy metals in plants is often positively correlated with the abundance of these elements in soils or substrate. In the present study, it was observed that at BS at 10% with 100 kg ha^{-1} of NPK is a suitable rate of production, since it registered heavy metals concentration below the maximum allowable limit, according to standards of New South Wales, EPA[13]. These were Zn (3.7 µg kg^{-1}), Ni (3.6 µg kg^{-1}), Cu (3.8 µg kg^{-1}), Cd (2.5 µg kg^{-1}) and Pb (5.3 µg kg^{-1}). In this regard, other authors have also reported the presence of these micro-nutrient concentration in biosolids[24]. Our results are similar to those of Bagdatlioglu, et al.[3], who demonstrated that the concentrations of Cu, Zn, Fe, Pb, and Cd, which were within safety baseline levels for human consumption (in reference to permissible limits of heavy metals as per FAO/WHO [51], These were as follows: Cd (0.1 mg g^{-1}), Pb (0.2 mg g^{-1}), As (0.1 mg g^{-1}), Hg (0.03 mgg^{-1}), Cu (40 mg kg^{-1}), Zn (0.60 mg kg^{-1}), Fe (5.0 mg kg^{-1}.Biosolids therefore are among the most important organic fertilizer rich in micronutrients (trace elements) such as, Zn, Mn, Cu, Fe, B, Cl, Mo and Ni, which are essential to crop growth, yield and quality. As various studies have shown, not all heavy metals pose risks in crop production systems. Some heavy metals are useful in plant physiological processes especially Zn, Cu and Ni [3]. Many enzymes in plant need Zn for their activity and it may also be required for chlorophyll biosynthesis in other plants [44]. Copper, like Fe is also associated with enzymes involved in redox reactions such as plastocyanin, which is involved in electron transfer during the light reaction of photosynthesis [46]. Nickel is another heavy metal that plays a key role in the production of secondary plant metabolites that influence resistance to diseases [17]. In another study, it has been shown that tomato respond well with Ni at 30 mg kg^{-1} and this level may increase quality of fruit; auxin and gibberellin content [17]. Others are non-essential metals; arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg), not required for normal biological function [44].

The results on microbial contamination of the tomato fruits produced in this study indicated the absence of the targeted microbes. This was not surprising because previous analyses had shown that targeted microbes *Salmonella sp., Escherichia coli* and *Staphylococcus* were absent in all the dry BS substrates [38]. The absence of microbial contaminants can be explained from the fact that microbe community in biosolids are faecal in nature, most being parasite on animals, therefore survival in the plant system was not possible except by external contamination. Additionally, they are heat-sensitive, and their survival may have been reduced during decomposition, solarization and storage of the substrate for six months before planting crops. Strauch [42] also reported reduction of *Salmonella, Shigella, Streptococci* and *Escherichia coli*) in sludge during decomposition. Concerning decrease in faecal contamination, De´portes *et al.* [12] reported disappearance of faecal pathogens, when biosolids were left before use over period of one year. This probably rendered the BS substrates used in this study free from targeted pathogen in the tomato fruits produced by the use of substrate.

5. Conclusion

Based on data from the present study, use of biosolids blended in forest soil and 5g per plant of NPK, is a viable means of improving greenhouse tomato postharvest quality through improving substrate status; a practice, which may also be used in integrated nutrient management strategies. This work has revealed that for safe production of quality tomato fruit, forest soil blended with BS 10% and 5g per plant of NPK resulted in fruits with higher postharvest quality. While the study lays a good foundation on improving greenhouse tomato performance, further study using other organic sources as amendments and various greenhouse tomato varieties could be studied to ascertain the outcome of our results on product quality and safety.

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