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Investigating the antioxidant Content of Sweet Pepper in Response to Fertilisers

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ABSTRACT

A pot experiment was conducted to compare the vegetative performance, yield and antioxidant content of *Capsicum Annuum L.* (Sweet pepper) in response to three sources of fertilizers i.e. organic (cow manure compost; 5% N: 3% P_2O_5:1% K_2O), inorganic (NPK; 12% N, 24%P, 12%K) and a source of bio-organic fertilizer containing two strains of bacteria, *Lactobacillus acidophilus and Saccharomyces cerevisiae.* The treatments were set up in a completely randomized design, with four (4) treatments each having three (3) replicates. The green mature sweet peppers were harvested from the respective treatments and were tested for the different secondary metabolites. The Inorganic fertilizer treatment gave better results for all growth and reproductive performances compared to the other treatments. Total carotenoid content (TCC), total flavonoid content (TFC) and total phenolic content (TPC) were higher as a result of the organic fertilizer treatment. The results indicated that there is an excellent potential for enhancing antioxidant compounds of sweet pepper for economic production using organic fertilization, for these two treatments that resulted in increased antioxidant constituents.

Keywords: Phenolic, Flavonoids, Carotenoids, Biofertilizer, Antioxidant, Metabolites

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INTRODUCTION

Sweet pepper (*Capsicum annuum L.*) is one of the most popular and favorite vegetable crops cultivated worldwide for local market and consumption (Young et.al., 2002). Sweet pepper (solanaceous family) can be grown throughout the year (Kabura et.al., 2008). They differ from common hot peppers in size and shape of the fruits, capsaicin content and usage. Due to its high yielding nature and nutritional values, this crop has been ranked as the third most important vegetable crop in world agriculture, after tomato and onion (Islam et. al., 2011). Over 25 percent of the world's population consumes peppers every day (Namiki et. al., 1990). Sweet peppers are an excellent source of bioactive compounds such as vitamins, provitamins and antioxidants compounds such as phenols, carotenoids, flavonoids etc (Hervert-Hernandez et.al., 2010). Capsanthin, lutein and violaxanthin are abundant carotenoids in sweet peppers. Carotenoids may have potential benefits to protect against cancer (Howard et. al., 2000). Adequate intake may also improve eye health (Flores et. al., 2009). Quercetin and luteolin are two beneficial for the prevention of chronic diseases, such as heart disease and cancers. Sweet peppers also have anticancer effects (Martinez et. al., 2007; Sun et. al, 2007; Matsufuji et.al., 2007; Marin et. al., 2004). Peppers contain high levels of Vitamin C, compared to other fruits such as citrus and also vegetables (Deepa et.al., 2007). Antioxidant compounds in pepper offer protection to cells against oxidative damage and thus prevent the development of common degenerative diseases such as cancer, cardiovascular disease, cataract, diabetes, Alzheimer's and Parkinson's (Matsufuji et.al., 2007; Kaur et.al., 2001; Gorinstein et.al., 2009; Goni et.al., 2010). The international market for pepper is continuously growing. It grew from 17,289,616 metric tons in 1997 to 26,056,900 metric tons in 2007. These compounds are produced as a chemical defense mechanism for plants to protect themselves from pests and diseases and from the ultraviolet light via the formation of free radicals (Matkowski et.al., 2006; Sarikurkcu et.al., 2009).

common polyphenols synthesized by sweet peppers and

Table 1: Nutritional Value of Sweet peppers (Green, Raw).

Principle	Mean Nutrient Value per 100g fresh weight
Vitamin C, Total ascorbic acid	80.4 mg
Lutein+Zeaxanthin	341 µg
Carotene, beta	208 µg
Carotene, alpha	21 µg
Quercetin	2.2 mg
Luteolin	4.7 mg
Vitamin A, IU	370 IU

Source: https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/food-surveys-research-group/docs/foodlink/ (Accessed 27 January 2020).

Free radicals, produced as a result of oxidation, lead to chain reactions that may damage cells. This process has been implicated in the etiology of several ailments caused in humans, such as diabetes, cancer and other cardiovascular diseases (Trombino et. al., 2004). Sweet peppers rich in antioxidants will prevent these diseases. Table 1 shows the components of sweet peppers and their nutritional values.

The antioxidant content in Sweet pepper can be affected by external factors such as temperature (Seeja et.al., 2014), light, compost, fertilizers, etc.

Conventional crop production utilizes chemical fertilizers. These fertilizers are manufactured from minerals and synthetic chemicals. These sources of fertilizer have successfully resulted in increased plant growth, development and yields. The source of fertilizer and the level of the application directly influence the quantities of nutrients available in plant's tissues and indirectly influence their physiology and the biosynthesis of secondary compounds (Heaton et.al., 2001). Fertilizers are said to reduce the level of secondary compounds and also the excess application of these synthetic fertilizers may increase the accumulation of heavy metals in plant tissues and compromises fruit nutrition value and edible quality (Shimbo et. al., 2001). The use of organic and bio-organic fertilizers in crop production has claimed to increase the production of secondary metabolites, inclusive of antioxidants. This may result in organically grown foods being more health-promoting than conventionally grown foods. Literature review reveals that there is minimum information on the influence of different sources of fertilizers on the potential activity of carotenoids, phenolics and flavonoids as natural antioxidants from sweet pepper.

The value and type of fertilizers have direct influences on the level of available nutrients and indirect influences on the plant's physiology and also the biosynthesis of secondary compounds in plant tissues. Studies have shown that chemical fertilizers cause plants to detract from producing defensive compounds, which includes antioxidants (Halweil et. al., 2007). Inorganic fertilizers reduce the protein content of crops, and carbohydrate quality of such crops get degraded. Over-application of macronutrient such potassium has been reported to significantly decrease Vitamin C, Carotene content and antioxidant compounds in vegetables (Toor et. al., 2006; Aminifard et. al., 2013).

In order to determine the effect of compost on antioxidant compounds and fruit quality of sweet pepper (*Capsicum annum L.),* an experiment was conducted in an open field. Compost treatments positively affected fruit antioxidant compounds of pepper (antioxidant activity, total phenolic and carbohydrate content). But, no significant difference was found in total flavonoid content between compost and control treatments. Thus, these results showed that compost has a strong impact on fruit quality and antioxidant compounds of pepper plants under field conditions (Aminifard et. al., 2013; Cloete et. al., 2009).

The effects of maturation and type of agricultural practice (organic or conventional) on the ascorbic acid, total carotenoid and total phenolic contents and colour parameters of sweet peppers (Capsicum annuum cv. *Almuden*) grown in a controlled greenhouse were determined (López et.al, 2007). Levels of vitamin C, phenolic compounds and carotenoids increased during ripening, with red sweet peppers having higher contents of these bioactive compounds. Moreover, peppers grown under organic culture had higher vitamin C, phenolic and carotenoid levels than those grown under conventional culture. With respect to colour parameters, organic red peppers had higher values of lycopene, giving them a higher intensity of red colour (Lopez et. al., 2007). Antioxidant properties of sweet peppers have been noted (Sun et.al., 2007). The effect of fertilizers on the antioxidant activity of other plants has also been reported (Abdou et. al., 2010; Naguib et. al., 2012; Kapoor et. al., 2007).

The aim of this study was to investigate the effects of inorganic, organic and bio-organic fertilizers on the yield and antioxidant content of sweet peppers. This study will help farmers to maximize profits and improve their role in sustainable agriculture since in recent times, most consumers are demanding higher quality and safer food and are highly interested in organic products. This study will also edify crop producers on the role fertilization plays on the potential health benefits of these products. The general public, which includes consumers will also benefit because the healthy regime of crop produce will be available for consumption, which may play a vital role in common disease prevention. Institutions such as the National Agricultural Research and Extension Institute (NAREI), Health organizations, and the University of Guyana will benefit because this research may be the baseline for finding future research of this nature.

METHODOLOGY

Sample site

This research was conducted at Section C, Turkeyen, Georgetown, The IAST building Food and Drug Laboratory, University of Guyana, Turkeyen, East Coast Demerara in the months of April-July 2018.

Experimental Design

A pot experiment was conducted using a Completely Randomized Design (CRD) for statistical comparison. The data collected were analyzed by Analysis of Variance (ANOVA), using the Statistix10 Software. There were four treatments, three replicates, resulting in twelve (12) experimental units. Each experimental unit was comprised of three (3) plants. The total numbers of plants used in this experiment were thirty-six (36).

Treatment Design

The four experimental treatments that were used are: Treatment 1 (T1): Control, Treatment 2 (T2): Inorganic, Treatment 3 (T3): Organic and Treatment 4 (T4): Biofertilizer

Experimental Management

Materials and Equipment

Plant Material

The Bull Horn sweet pepper variety was used for this experiment, six (6) weeks old seedlings were obtained from a local supplier.

Agricultural Conditions

Ithaca sandy loam soil was sourced from Mahaicony. A sample was extracted for a soil chemical analysis. The soil was treated with an herbal fungicide (Fungidote), which contains neem and garlic extracts. Seven (7) kg of soil was placed in each pot used for the experiment.

Fertilizers Treatments

Control

The soil in this treatment was left untreated.

Mineral NPK fertilizer (12:24:12)

Inorganic fertilizers were applied by the basal application method at weeks two (2), four (4) and six (6) after transplanting. This was done at equivalent rates of 221 kg/ha.

Organic fertilizer

Cow manure compost was sourced and a chemical analysis was conducted at the Guysuco Laboratory, fertilizer was mixed thoroughly incorporated two (2) weeks before transplanting. This was done at the rate of 0.28 kg per each replicate.

Bio-organic fertilizer (Inno-care, 50% *Lactobacillus acidophilus* and 50% *Saccharomyces cerevisiae*)

Inno-care biofertilizer contained (50% *Lactobacillus acidophilus* and 50% *Saccharomyces cerevisiae*). 15mL of biofertilizer was added to 5 gals of water, along with 1lb of sucrose. The solution was stirred vigorously, sealed and stored out of direct sunlight for one week. The solution was applied to the roots of seedlings, using a root dipped method, at transplanting. The solution was then injected near the roots of each plant, two (2), five (5) and eight (8) weeks, after transplanting at the level (10ml /plant). The bio-fertilizer was manufactured by Health 2000 (H2K), and was made available by a local distributor, Green to life farm Enterprise. It contained a mixture of generally recognized as Safe Microorganisms-Bacillus Coagulants and *Saccharomyces cerevisiae*, and beneficial botanical extracts and biochemical.

Transplanting

Transplanting was done on the same day for each treatment.

Care

Plants were watered as required and weeds were removed manually. While pest was controlled manually and also with the use of a mixture of neem extract, soap-water and oil.

Parameters of Interest

These include Plant height at maturity, Days to 50% flowering, Total yield per treatment, Soil chemical characteristics before and after cropping, Total Flavonoid Content, Total Phenolic content, Total Carotenoids content.

Plant Height at Maturity

A meter rule was used to measure plant height at maturity,

Table 2. Soil analysis before and after cropping.

Sample Description	pH	N(mg/kg)	P(mg/kg)	K(mg/kg)
Before Planting	**6.2**	**1475**	**11.6**	**1260**
T_1	**6.0**	**1170**	**9.2**	**957.22**
T_2	**5.7**	**6950**	**26.9**	**3872**
T_3	**7.3**	**3322**	**13.53**	**1422**
T_4	**7.1**	**2450**	**16.01**	**753**

the rule was placed at ground level and the height from base to the highest point of the plant was recorded.

Days to 50% Flowering

This was determined by recording the number of days following transplanting until 50% of plants for the treatments had at least one (1) flower open.

Total Yield per Treatment

The total number and total weight of fruits for each treatment were determined to establish the total yield per treatment.

Preparation of plant samples for chemical analysis

Green mature sweet peppers were harvested from the respective treatments and were placed and sealed in zip lock bags. Peppers were transported immediately to the Food and Drug Laboratory, washed, sliced and removed peduncles and seeds. Only the Pericarp was used for the different analysis.

Preparation of fresh plant acetone extract

10g of frozen sweet pepper tissues were grounded in 10ml of 80% acetone using a mortar and Pestle. The extract was filtered using Whatman No.1 filter paper. The sample was then placed in centrifuge tubes before placing it in a centrifuge for ten (10) minutes at 3000rpm. The pellet was discarded and the supernatant was taken to conduct the different analysis.

Determination of total carotenoids content (TCC)

The supernatant was made up to a known volume of 10ml using petroleum ether extract. The absorbance values were read at 480nm in UV-Spectrophotometer. Samples were analyzed in triplicates.

Determination of total flavonoid content (TFC)

The total flavonoid content (TFC) of fresh sweet pepper extracts was spectrophotometrically determined by the aluminum chloride method using quercetin as standard to prepare a calibration curve (Quettier et al., 2000). The sample contained 1ml of the acetone extract solution and 2% 〖AlCl〗_3 dissolved in methanol. Incubated for 1 hr. at room temperature, samples were measured at 415 nm and expressed as mg quercetin equivalents (QE)/g fresh weight. Samples were analyzed in triplicates.

Determination of total phenolic content (TPC)

The total phenolic content (TPC) of fresh sweet pepper extracts was spectrophotometrically determined by Folin Ciocalteau reagent assay using Gallic acid as a standard compound to prepare a calibration curve (20-200mg/l) according to (Spanos,1990). The total phenolic content of samples was measured at 765 nm and expressed as mg Gallic acid equivalents (GAE)/g fresh weight. All samples were analyzed in triplicates.

Data Analysis

Data were analyzed using Statistix10 Software. ANOVA, with a 95% confidence interval, was used to analyze all data recorded. Tables and graphs were generated using Microsoft Excel (Skoog et. al., 1998; 2014; Daniel et. al., 2003; Lane et. al., 2015).

RESULTS AND DISCUSSION

The chemical properties of the soil used in the experiment are presented in Table 2. The data indicated that the soil was moderately acidic at the initial stage with no fertilizer application. However, after the source of the respective fertilizer were applied as different treatments, it was observed that the soil sample extracted from the inorganically fertilized soil, had decreased pH level as opposed to the soil samples extracted from the treatments that consisted of organic matter and biofertilizer solution. The latter two had elevated the pH. The NPK fertilizer treatment significantly decreased the soil pH, whereas the organic manure and biofertilizer treatments significantly increased the soil pH (Table 2). Soil nitrogen content increased after the NPK fertilizer was added to the soil, the application of the organic manure and biofertilizer solution treatment also resulted in an increase in nitrogen content but was lower than the NPK fertilizer treatment. The available phosphorus in the soil increased by more than

Table 3: LSD All-Pairwise Comparison Testing for Days to 50% Flowering**.**

TREATMENTS	MEAN	HOMOGENOUS GROUP
Control (T_1)	**69.778±1.48**	**A**
Inorganic (T_2)	**29.444±2.5**	**D**
Organic (T_3)	**36.333±4.9**	**C**
Bio fertilizer (T_4)	**47.333±7.2**	**B**

All values are means of three replicates and are significantly different at p≤0.05±standard deviation.

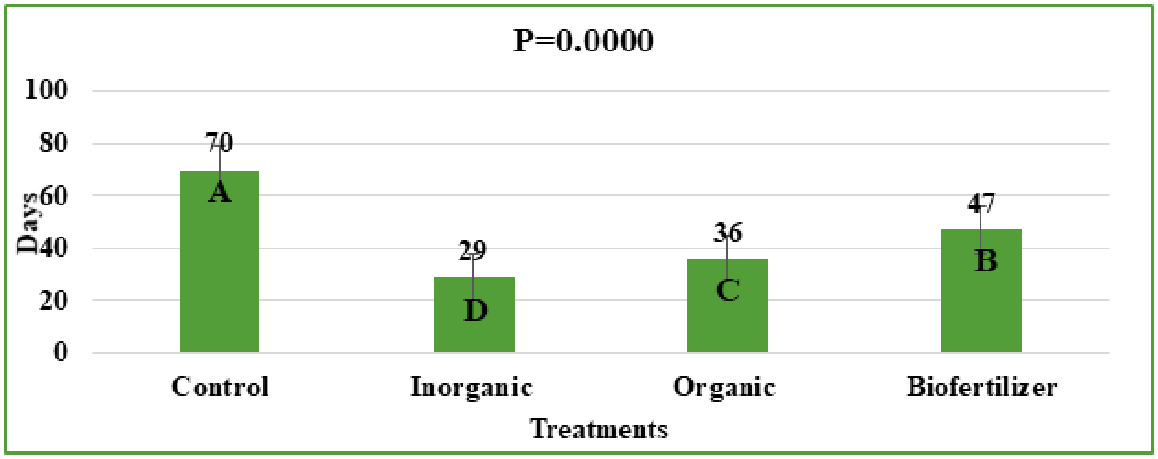

Figure 1: Days to 50% Flowering.

Table 4: LSD All-Pairwise Comparison Testing for Plant Height at Maturity.

TREATMENT	MEAN	HOMOGENOUS GROUPS
Control (T_1)	**29.000±6.95**	**C**
Inorganic (T_2)	**51.444±7.31**	**A**
Organic (T_3)	**36.889±5.01**	**B**
Bio fertilizer (T_4)	**42.111±6.99**	**B**

All values are means of three replicates and are significantly different at p ≤ 0.05±standard deviation. All values with the same letters are not significantly different.

50% for inorganic and bio-fertilizer treatments. Similarly, exchangeable potassium concentrations were significantly increased for inorganic and organic treatments but decrease for bio-fertilizer treatment.

Table 3 and Figure 1 show the effect of the different sources of fertilizers on the number of days to 50% flowering of Sweet pepper is shown in. Analysis of variance, ANOVA, showed significant differences existing amongst the treatments studied, since P < 0.05. The application of the inorganic fertilizer treatment triggered flowering at the earliest date. This may be as a result of the soil having a higher level of available macronutrients including phosphorus, which is essential for stimulating and enhancing bud set and development, seed formation and blooming, hence the ability of plants in this treatment to attain 50% flowering at an earlier date as opposed to the other treatments. It has been observed that organic and bio-fertilizers have the ability to decrease days to flowering in sweet peppers, after transplanting (Aliyu, 2002). Thus, the order of testing for days to 50% flowering, based on treatments: inorganic > organic > bio-fertilisers > control.

There were significant differences among treatment means with respect to plant height at maturity (Table 4 and Figure 2) as P value < 0.05. The highest plant mean height of (51.4 ± 7.31 cm) was induced by the inorganic fertilizer treatment. The application of organic and bio-organic fertilizer did show a fair increase in mean height when compared to the control treatment. Significantly, maximum plant height using the inorganic fertilizer may be due to an increase in fertilization of macronutrients in sufficient amount to facilitate the increase in biomass and development of sweet pepper plants. Also, this source of

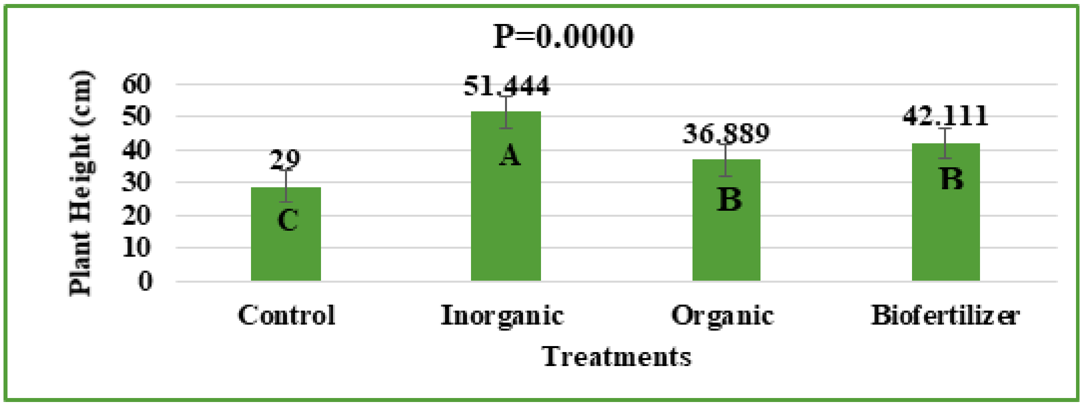

Figure 2. Plant Height at Maturity.

Table 5: LSD All-Pairwise Comparison Testing for Total Yield per Treatment.

TREATMENTS	MEAN	HOMOGENOUS GROUPS
Control (T_1)	**19.044±15.352**	**B**
Inorganic (T_2)	**52.244±37.971**	**A**
Organic (T_3)	**42.502±34.688**	**AB**
Bio fertilizer (T_4)	**51.000±32.652**	**A**

All values are means of three replicates and are significantly different at p≤0.05±standard deviation. All values with the same letters are not significantly different.

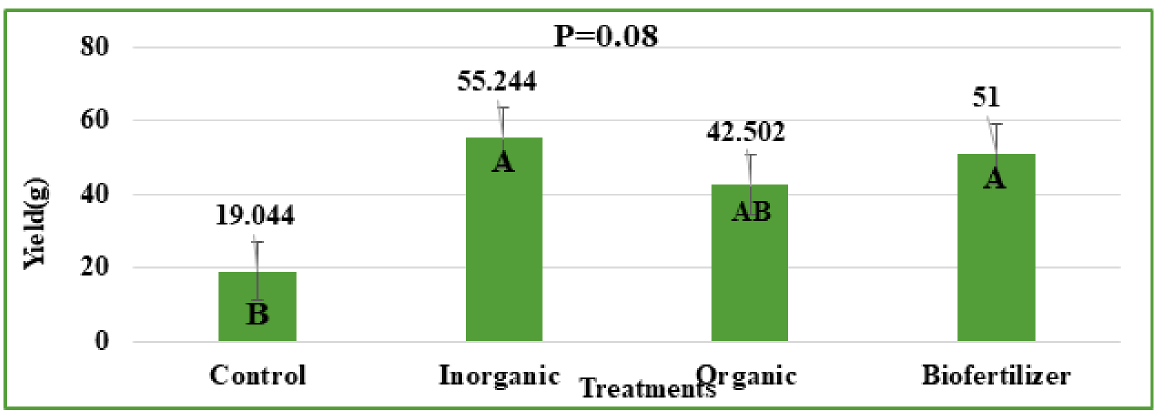

Figure 3: Total Yield per Treatment.

fertilizer is water-soluble and readily available for uptake by plant roots. Higher concentration of potassium ion has an important influence on the increase in plant height, potassium catalyzes the synthesis and translocation of carbohydrate. The biofertilizer showed a fair amount of increase in plant growth. Inoculation of sweet pepper with *Saccharomyces cerevisiae* as a bio-fertilizer led to the enhancement of plant growth (Botha et. al., 2009). Thus, the order of decreasing plant height at maturity, based on the various treatments, follow the sequence: inorganic > bio-fertilizer> organic > control.

The mean separation of the different treatments (Table 5 and Figure 3) shows that there were no significant differences existing between the treatments studied, as it relates to the total yield per treatment, P-value greater than 0.05. Despite relatively low yields in the control treatment, which is possibly due to wide variability in crop yields. The application of inorganic, organic and biofertilizer treatments significantly increased crop yield. The increase in yield in the biofertilizer treatment may be explained by the processes carried out the strains of bacteria introduced to plants, such as N fixation, P solubilizing and the production of plant growth regulators such as GAA and IAA (Tomar et. al., 1998; Naguib et. al., 2012).

As indicated in Table 6 and Figure 4 the concentration of total carotenoids in the green sweet peppers was relatively

Table 6. Effect of Inorganic, Organic and Bio fertilizers on TCC of green Sweet pepper.

TREATMENTS	MEAN	HOMOGENOUS GROUP
Control (T_1)	0.008±0.225	BC
Inorganic (T_2)	0.00345±0.052	C
Organic (T_3)	0.0423±0.033	A
Bio fertilizer (T_4)	0.0135±0.0154	B

All values are means of three replicates and are significantly different at p≤0.05±standard deviation. All values with the same letters are not significantly different.

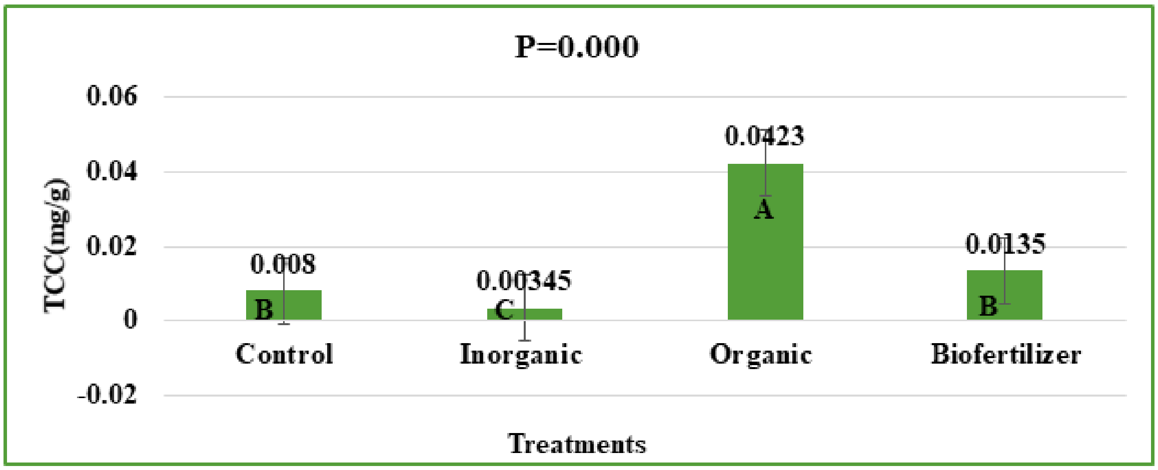

Figure 4: Total Carotenoids Content (TCC).

Table 7: Effect of Inorganic, Organic and Bio fertilizers on TFC fresh, green Sweet pepper.

TREATMENTS	MEAN	HOMOGENOUS
Control (T_1)	6.087±1.148	D
Inorganic (T_2)	10.727±2.125	C
Organic (T_3)	29.029±0.745	A
Biofertilizer (T_4)	18.713±3.179	B

All values are means of three replicates and are significantly different at p≤0.05±standard deviation. All values with the same letters are not significantly different.

low. However, with reference to the mean values amongst the treatments studied, a significant difference existed, P-value < 0.05. The application of organic and biofertilizer to the soil had an extremely positive effect on the synthesis of carotenoids in sweet pepper. Alternatively, the addition of the inorganic fertilizer appears to hinder or slow down the process of carotenoids synthesis. The TCC was promoted significantly, with the use of organic fertilizer. Thus, TCC in decreasing order, based on the individual treatments, follows the sequence: organic > bio-fertilizer> control > inorganic

Table 7 and Figure 5 indicates that, with respect to TFC, there were significant differences existing among the treatments studied, p-value < 0.05. The application of organic and bio-organic fertilizers significantly increased the TFC of green sweet peppers in comparison to the control treatment. This increase reaches more than 50%. The production of flavonoids in fresh sweet pepper tissues appears to not be promoted by the inorganic treatment. Higher nitrogen application decreases the level of flavonoids in solanaceous crops (Stewart et.al., 2005). This phenomenon may be explained by the PCM (Protein Competition Model), where the contingent predicted that when biomass increases in response to elevated nitrogen nutrition, the production of compounds such as flavonoids will decline because partitioning to flavonoids and other secondary metabolites synthesis. Thus, with respect to the treatments, TFC follow the sequence: organic > biofertiliser > inorganic > control.

The total phenolic content increased significantly (p < 0.05)

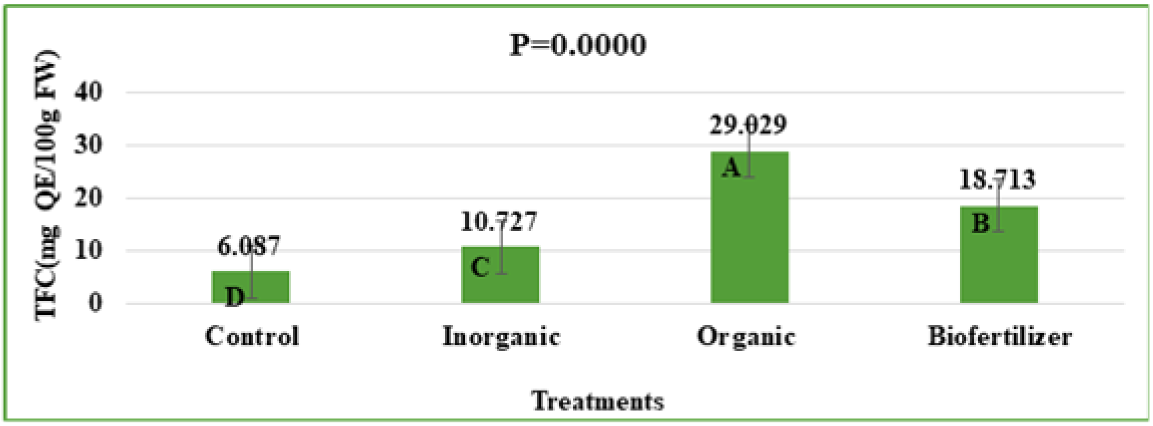

Figure 5: Total Flavonoid Content (TFC).

Table 8: Effect of Inorganic, Organic and Bio fertilizers on TPC fresh, green Sweet pepper.

TREATMENTS	MEAN	HOMOGENOUS GROUPS
Control (T_1)	**14.067±0.061**	**D**
Inorganic (T_2)	**20.397±1.665**	**C**
Organic (T_3)	**60.49±6.559**	**A**
Bio fertilizer (T_4)	**44.063±8.866**	**B**

All values are means of three replicates and are significantly different at p≤0.05±standard deviation.All values with the same letters are not significantly different.

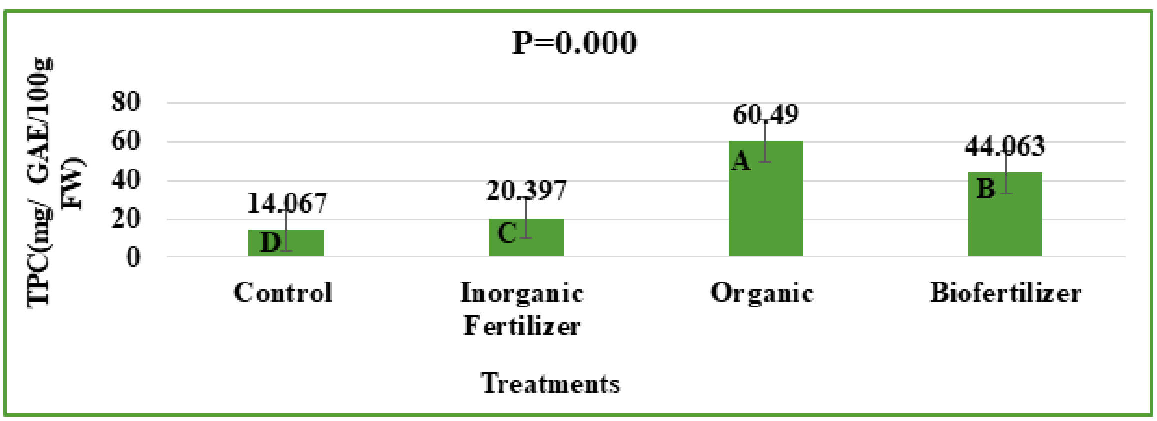

Figure 6. Total Phenolic Content (TPC).

(Table 8 and Figure 6) in response to the application of the organic and bio-organic fertilizers as opposed to the other treatments. Results indicate that these fertilizers have a stimulatory effect on the accumulation of phenolics in sweet pepper. The higher concentrations of total phenolics can be explained by the role of organic fertilizers in the biosynthesis which catalyzes the acetate shikimate pathway, resulting in higher phenolics (Zheng et. al., 2001). Thus, the Total Phenolic Content, TPC, with respect to the treatments, follow the sequence: organic > bio-fertilizer> inorganic > control.

Plate 1(a) shows the flowering stage of sweet pepper and Plate1 (b) shows the green mature stage of sweet pepper.

CONCLUSION

It can be concluded that the inorganic treatment was superior amongst all treatments studied as it relates to all measured reproductive and vegetative growth parameters. However, sweet pepper antioxidant content was favoured by the use of organic and bio-organic fertilizer treatments.

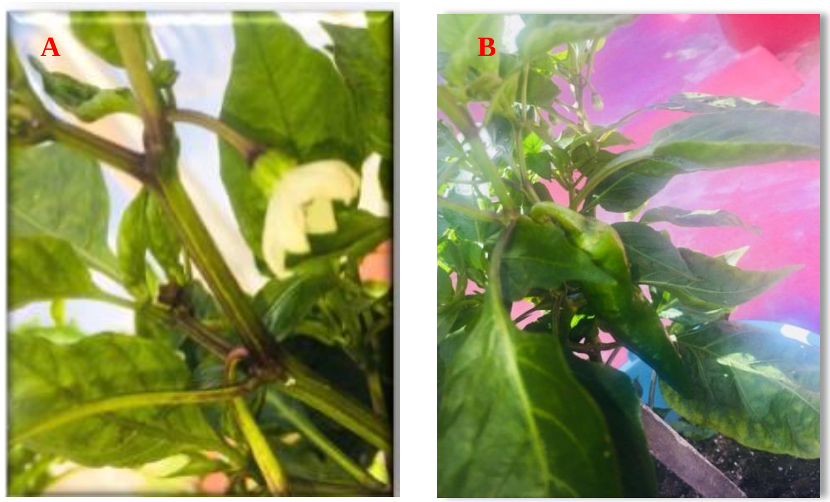

Plate 1(a) Flowering Stage of Sweet pepper (b) Green Mature Stage of Sweet pepper

Therefore, the organic and bio-organic fertilizer may be recommended to farmers and kitchen garden cultivators, since they will result in enhanced total carotenoids, flavonoids and phenolics which all possess antioxidant properties and are beneficial to human health.

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