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EFFECTS OF ORGANIC AND CHEMICAL FERTILIZERS ON THE CONTENT OF MAJOR PHENOLIC COMPOUNDS OF CARROT

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ABSTRACT

Carrot is a popular winter vegetable rich in health-promoting phenolic compounds. In this study, different chemical and organic fertilizers were used during cultivation to observe the effect on major phenolic compounds (total phenols, total flavonoids, and proanthocyanidins) of carrot roots. Total five treatments which are F1=100% organic fertilizer, F2= 100% chemical fertilizer, F3= 75% chemical + 25% organic fertilizer, F4= 50% chemical + 50% organic fertilizer (of recommended dose) and F5= local control with three replications were applied in the experiment designed in Randomized Completed Block Design (RCBD). The contents of total phenols varied significantly in response to the chemical and organic fertilizer treatment. The highest amount of total phenols (10.549 mg kg^{-1}) was found in F2 treatments where 100% chemical fertilizer of the recommended dose was applied, and the lowest amount (7.126 mg kg^{-1}) was found in F5 treatment where no fertilizer was applied. The content of total flavonoids and proanthocyanidins did not vary significantly in response to the fertilizer treatments. Based on the current study, it is not proven that organically fertilized carrot contains more amount of health-promoting phenolic compounds than chemically fertilized carrot.

KEY WORDS : Phenolic compounds, Chemical fertilizer, Organic fertilizer, Carrot

INTRODUCTION

Carrot (*Daucus carrota* L.) is a biennial plant of Apiaceous family. It is a root vegetable and winter crop, commonly orange in color, though purple, black, red, white and yellow colored cultivars are also found. It is cultivated throughout the temperate, tropical and subtropical regions of the world (Bore and Som 1990) while extensively cultivated in Europe, Asia, North Africa, North America and South America (Thompson and Kelly, 1957). In Bangladesh, carrot is grown during winter season, specially from mid-November to early December when temperature ranges from 11.7° to 28.9°C (Alin, 1974; Rashid, 1993).

Carrot has excellent nutritional properties (Yawalkar, 1992) and important medicinal values (Sadhu, 1993). Major bioactive compounds found in carrot consist of phenolic, phytosterol, triterpene and polyacetylen (Singh *et al.,* 2012). It has been reported that carrots are rich in phenolic acids, such as p-hydroxybenzoic, caeic, and chlorogenic, as well as in anthocyanins, a class of avonoids (Goncalves *et al.,* 2010). Carrot has been used as anti-diarrhea, anti-infection, anti-high blood cholesterol, anti-inflammation, anti-seizure, anti-fungal anti-bacteria and anti-cancer (Chatatikun and Chiabchalard, 2013).

It contains carotene (10 mg/100 g), thiamin (0.04 mg/100 g), riboflavin (0.05 mg/100 g) and also serves as a source of carbohydrate, protein, fat, minerals, vitamin-C and calories (Yawalkar, 1992).

Blindness in children due to vitamin-A deficiency is a problem in some countries, especially in the rice dependent countries of Asia (de Carvalho, *et al.*, 2014). Carrot which is rich in vitamin-A may contribute to overcome this problem in Bangladesh

Phenolics prevent oxidative degradation of lipids and thereby enhance the excellence and nutritional value of food. Phenolics can perform a wide spectrum of biochemical activities such as antioxidant, anti-mutagenic, anti-carcinogenic as well as ability of modifying gene expression (Chandra *et al.*, 2014). Flavonoids are the most common and widely distributed group of plant phenolic compounds, occurring virtually in all plant parts, particularly in those plant cells which are associated with photosynthesis (Saied *et al.*, 2015).

Although, it is well agreed that depleted soil fertility is a major constraint for higher crop production in Bangladesh and declining of the yield of several crops, however, indiscriminate use of chemical fertilizer could changes physical, chemical and biological properties of soil which might affect the fertility in some soils are observed (Jahiruddin and Satter, 2010). On the other hand, organic matter content of the soil in Bangladesh is below 1% in about 60% of cultivable lands compared to an ideal minimum value of 3%[14]. Organic matters play a vital role in maintaining soil fertility and crop production and maintenance of soil fertility is prerequisite for long term sustainable agriculture. Chemical hazards in different vegetables, specially fresh consumable crops like carrot, due to the use of synthetic fertilizer has been increasing day by day (Islam, 2005; Fernandes *et al.*, 2012). Thus, the use of organic fertilizers over chemical one, is preferable, and the demand of organic products is growing steadily. Moreover, improved animal welfare, environmental protection, human health, and taste and freshness are the most important reasons for the increasing demand of organic food products (Wier *et al.*, 2008).

However, the concentration of phenolic compounds available in carrot roots can be affected by multiple factors including the cultivar, storage conditions and temperature, fertilizer application, nutrients availability, processing procedures, and various biotic and abiotic stress factors (Manach *et al.*, 2014; Alasalvar 2005).

Considering the fact that use of organic fertilizer in crop production is more acceptable than use of chemical fertilizer due to environment and health concern, the study is aimed to observe the influence of chemical fertilizers and organic fertilizers on the total phenolic compounds and flavonoids content (total phenols, flavonoids and proanthocyanidins) of carrot roots from nutritional point of view. This research was also aimed to find out the suitable fertilization practice for carrot production in terms of total phenols, flavonoids and proanthocyanidins content in Bangladesh.

MATERIALS AND METHODS

Plant materials, treatments and experimental design

The experiment was carried out at the Farm of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh (23°75' N, 90°34' E) during November 2018 to March 2019. Information regarding average monthly maximum and minimum temperature, rainfall, relative humidity and soil temperature as recorded by the Bangladesh Meteorological Department (climate division) Agargaon, Dhaka, during the period of study have been presented in Table 1.

The soil of the experimental area was medium high land type and belongs to the Modhupur Tract and AEZ No. 28 (FAO, 1988; UNDP, 1988). The carrot seeds of TAKII and CO., LTD. Kyoto, Japan were collected from Alamgir Seed House, Siddique Bazar, Dhaka. The experiment was a one factorial designed to study the effects of different fertilizer

Table 1. Monthly average record of air temperature, rainfall, relative humidity and sunshine of the experimental site during the period from November 2018 to March 2019

Month	Air temperature (ºC)		Relative humidity (%)	Total rainfall (mm)	Sunshine (hr.)
	Maximum	Minimum			
November, 2018	29.6	19.2	77	34.4	6.2
December, 2018	26.4	14.1	69	15.8	5.5
January, 2019	25.1	12.5	68	9.9	5.1
February, 2019	26.8	15.7	78	30.2	5.9
March, 2019	27.9	17.1	79	32.5	6.3

doses (recommended by Chowdhury and Hassan, 2003) while it consists of five different treatments as following: F1 (100% organic fertilizer), F2 (100% chemical fertilizer), F3 (75% chemical fertilizer + 25% organic fertilizer), F4 (50% chemical fertilizer + 50% organic fertilizer) and F5 (local control). The total area of the experimental plot was 108m^2 (12m x 9m), which was divided into three equal blocks and each block was divided into 5 unit plots. The size of each plot was 1.5m^2 (1.5m x 1m). Good agricultural practices were maintained from planting to harvesting of carrots. After harvesting, fresh and cleaned roots were selected randomly from each plot followed by washing, air drying and storage at 40 °C.

Extracts preparation

40 g weighed cut pieces with water (1:1 ratio) was blended first, followed by mixing of blended mixture and methanol (1:1 ratio), and 2-3 minutes vortexing. Then the sample was kept in rest for 5-6 minutes which caused a layer of supernatant up on the 50 ml tube. Then the supernatant was taken into a 20 ml tube and centrifuged at 2000 rpm for 5 minutes. Then the clean extract was taken into a tube for chemical analysis.

Chemical analysis

The chemical analysis of the extracted samples was done at the food processing and preservation laboratory, Faculty of Engineering, Hajee Mohammad Danesh Science & Technology University, Bangladesh.

Determination of total phenols content (TPC)

Determination of the total phenol contents (TPC) of each sample was done according to the method of Saikia[23]. Briefly, 0.5 ml of extracted sample was taken in a falcon tube and mixed with 0.5 ml Folin-Ciocalteu's reagent and mixed thoroughly. The mixture was allowed to react with 1 ml of 7.5% Na_2CO_3 to the test tube for neutralization. Then 8 ml of distilled water was added and vortexed for 20 seconds. After the mixture was allowed to stand for 35 min in a dark place at room temperature followed by centrifugation for 10 min at 4000 rpm. Then, absorbance of the supernatant was taken at 725 nm using spectrophotometer (UV/VIS, UV-1800). The concentration of the total phenolics was calculated as µg/g of gallic acid equivalent by using an equation obtained from gallic acid calibration curve.

Determination of total flavonoids content (TFC)

Total flavonoids content was measured by colorimetric method following (Kim *et al,* 2003) with little modification. Briefly, extracted sample (1 ml) was diluted with 4 ml distilled water and 0.3 ml of 5% $NaNO_2$ was added to it. After 5 min the samples were allowed to react with 0.3 ml of 10% $AlCl_3$ and incubate for 1 min in a room temperature. Then 2 ml of 1 M NaOH and 2.4 ml of distilled water were added and vortexed for 20 seconds. The mixture was centrifuged at 4000 rpm for 5 min and kept in the dark for 15 min at room temperature. Finally, absorbance of the supernatant was taken at 510 nm using spectrophotometer (UV/VIS, UV-1800). Catechin was used to make the calibration curve and the total flavonoid content expressed as µg of catechin per gram of sample.

Determination of Proanthocyanidins

The assay was performed as according to the published method of (Price *et al,* 1978) with slight modifications. In a test tube containing about 1 ml of extracted sample was added to 5 ml of 0.5% vanillin-HCl reagent (0.5%, w/v vanillin in 4% concentrated HCl in methanol). Then the mixture was incubated for 20 min at room temperature. Then absorbance was read at 500 nm using spectrophotometer (UV/VIS, UV-1800) and a standard curve using catechin and expressed as µg of catechin equivalent to per gram of sample.

Statistical analysis

The data obtained for total phenols, total flavonoids and proanthocayanidins were statistically analyzed by using "Statistics 10" computer package program to find out the significance of difference for fertilizer treatments on contents of total phenols, total flavonoids and proanthocyanidins in carrot. Pairwise comparisons among the values were done by LSD test at 5% level of probability.

RESULTS AND DISCUSSION

Effects of organic and chemical fertilizer on total phenols content of carrot

The amounts of total phenol found from carrot extraction under different fertilizer treatment have been presented in Table 2.

Total phenol contents in carrot root varied significantly due to the application of different doses of fertilizer (cowdung and chemical). The amounts

Table 2. Content of total phenols (mg kg^{-1}) in carrot roots under different fertilizer treatment

Fertilizer treatment	Total phenols (mg kg^{-1})
F1	7.126 ± 0.353 c
F2	10.549 ± 1.969 a
F3	10.127 ± 0.644 ab
F4	9.377 ± 2.329 abc
F5	7.876 ± 1.116 bc
$LSD_{(0.05)}$	2.2926

Values are given as mean ± SD. Different letters in column indicates statistically significant difference and same letters in column indicates no significant difference by LSD at 5% level of probability

of total phenols in carrot root extraction ranged from 7.126 mg kg-1 to 10.549 mg kg-1. The highest amount of total phenols (10.549 mg kg-1) was found in F2 fertilizer treatment where 100% chemical fertilizer of recommended dose was applied, and the lowest amount of total phenols (7.126 mg kg-1) was found in F1 fertilizer treatment where 100% cowdung of recommended was applied as organic fertilizer. The amounts of total phenols found in F3 which is combination of 75% chemical fertilizer + 25% cowdung of recommended dose, and F4 which is combination of 50% chemical fertilizer + 50% cowdung of recommended dose are statistically identical with F2 treatment. The amount of total phenols found in F5 treatment where no fertilizer was applied is 7.876 mg kg-1, which is statistically similar to those of F1, F3 and F4 treatments. Whereas the highest amount of yield of carrot (4.03 kg) was found in F3 treatment, and the lowest amount of yield (3.06 kg) was found in F5 treatment. The contents of total phenols in carrot did not influenced by the yield of carrot. It was reported that, carrot yield has no significant influence on its total phenol contents (Zakir, *et al*, 2012). The application chemical fertilizer influenced positively to the contents of total phenols of carrot root, though the combination of chemical and organic fertilizer treatments shown statistically similar results to that of sole chemical fertilizer treatment. Carrot cultivated in organic methods contained significantly lower amount of vitamin-C and â-carotene than those of cultivated in conventional method (Bendev *et al*, 2009). It was reported that there is no significant difference in phenol contents between organically and chemically cultivated carrots (Sink, *et al*, 2017), but the present study shows that application of chemical fertilizers significantly increased the amount of total phenol contents in carrot.

Table 3. Contents of total flavonoids in carrot root under different fertilizer treatment

Fertilizer treatment	Total flavonoids (mg kg^{-1})
F1	6.1928 ± 1.6814 a
F2	5.1158 ± 0.9327 a
F3	5.3850 ± 1.6814 a
F4	4.3080 ± 2.0328 a
F5	5.3850 ± 0.9327 a
$LSD_{(0.05)}$	2.8655

Values are given as mean ±SD. There is no significant difference among the values under each treatment by LSD at 5% level of probability

Effects of organic and chemical fertilizers on total flavonoids content of carrot

The amount of total flavonoids found from carrot extraction under different fertilizer treatment have been presented in Table 3.

Contents of total flavonoids in carrot extraction not varied significantly under different fertilizer treatments (chemical and organic). The amount of total flavonoids ranged from 4.3080 mg kg^{-1} to 6.1928 mg kg^{-1}. The highest amount of total flavonoids (6.1928 mg kg^{-1}) was found in F1 treatment where 100% cowdung of recommended dose was applied as organic fertilizer treatment, and the lowest amount of total flavonoids was 4.3080 mg kg^{-1} found in F4 treatment where combination 50% cowdung + 50% chemical fertilizers was applied. The contents of total flavonoids under all treatment were statistically similar to each other by LSD at 5% level of significance. This result indicates that, fertilizer treatments have no comparative significant influence on total flavonoids content of carrot roots. Fertilizer treatments whether chemical or organic had no comparative significant influence on total flavonoids content in onions (Sink, *et al*, 2017; Soltoft, *et al*, 2010).

Effects of organic and chemical fertilizer on proanthocyanidins content of carrot

Content of proanthocyanidins found in carrot extraction under different fertilizer treatments have been presented in Table 4.

Contents of proanthocyanidins under different fertilizer treatments did not vary significantly by LSD at 5% level of probability. The amount of proanthocyanidins found in root extraction ranged from 4.835 mg kg^{-1} to 11.343 mg kg^{-1}. The highest amount of proanthocyanidins (11.343 mg kg^{-1}) was found in F5 where no fertilizer was applied, and the lowest amount of proanthocyanidins (4.835 mg kg^{-1})

Table 4. Contents of proanthocyanidins in carrot root under different fertilizer treatment.

Fertilizer treatment	Proanthocyanidins (mg kg^{-1})
F1	4.991 ± 6.641 a
F2	4.835 ± 3.627 a
F3	7.383 ± 1.344 a
F4	6.759 ± 6.251 a
F5	11.343 ± 4.445 a
$LSD_{(0.05)}$	7.7168

Values are given as mean ±SD. There is no significant difference among the values under each treatment by LSD at 5% level of probability

was found in F2 treatment where 100% chemical fertilizer of recommended dose was applied. However, all the values found under different fertilizer treatment was statistically similar to each other. Which means either chemical fertilizer or organic fertilizer, had no influential impacts over one-another on proanthocyanidins contents of carrot root.

CONCLUSION

The findings of the current study indicate that organic fertilizer (cowdung) has no significant influence in increasing the contents of total phenols, flavonoids, and proanthocyanidins in carrot roots. Rather, in F1 treatment where 100% organic fertilizer were applied, the amount of total phenols was significantly low. It was observed that application of chemical fertilizers significantly increase the contents of total phenols in carrot roots. The highest amount of total phenols was found in F2 where 100% chemical fertilizer was applied. Though in mix fertilizers (chemical + organic) treatments, amounts of total phenols were statistically non-significant to that of F2 treatment. Thus, the use of organic fertilizer with chemical fertilizer will reduce the intensity of use of chemical fertilizers in one hand, and on the other hand it is cost-effective for farmers. However, considering the growing food demands it is difficult to replace chemical fertilizers with organic fertilizers.

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