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Full Length Article

Compost and Synthetic Fertilizer Affect Vegetative Growth and Antioxidants Activities of *Moringa oleifera*

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Abstract

Moringa oleifera Lam, a highly valuable food commodity that has wonderful range of medicinal uses and possessing high nutritional value. A pot study was conducted to evaluate the combined effects of compost and N:P:K (21:17:17) on growth and antioxidative activities of moringa under greenhouse conditions. Compost and N:P:K fertilizers were applied separately and in combination including control (no fertilizer), 100 g Compost, 2 g NPK, 2 g+100 g (NPK+ Compost), 4 g +200 g (NPK+ Compost) and 6 g+300 g (NPK+ Compost). It was found that when moringa plants were fertilized with 2 g of NPK, it produced maximum plant height (44.79 cm), stem girth (6.33 mm), leaf score (342.40), number of branches (16.07) and crude protein (9.76 mg/g dry weight) contents. Carbohydrate, phenolics, flavonoids (291.25, 16.61, 2.019 mg/g dry weight) respectively were recorded in highest quantity in 100 g compost treated plant. The compost (100 g) treated plants also displayed promising antioxidant activities in terms of the IC_{50} and $IC_{o\cdot5}$ values. Therefore, it is concluded that the compost and NPK treatments can effectively improve the vegetative growth, biochemical, phytochemical and antioxidant activities of moringa plant. © 2017 Friends Science Publishers

Keywords: *Moringa oleifera*; Compost; Vegetative growth; Biochemical; Phytochemical analysis; Antioxidant activities

Introduction

The awareness of *Moringa oleifera* for the South Asian countries is very important because of its nutritive and medicinal importance. *M. oleifera* is capturing attention of plant, livestock and nutritional scientists to cultivate under various climatic conditions due to its higher biomass production and nutritive profile (Nouman *et al*., 2016). Moreover, it has been previously recorded that intake of moringa as fodder does not have toxic impact on animal health (Makkar and Becker, 1996; Furo and Ambali, 2011), which indicates its effective utilization (Asaolu *et al*., 2012; Adegun and Aye, 2013). Moringa leaves are very nutritious which are vital for animal health and growth improving milk and meat production (Alonso-Diaz *et al*., 2010; Dela Cruz, 2012). Moringa is believed to be a rich source of proteins, phytochemicals, and antioxidants (Nouman *et al*., 2016). The presence of phenolic compounds improves its antioxidant potential which provides health benefits to its consumers either livestock or human beings inducing resistance to many diseases (Anwar *et al*., 2007). For these benefits, moringa is being considered as live-stock fodder in many parts of the world (Nouman *et al*., 2014). Moringa crop yields high dry matter (DM) production between 4.2–8.3 tons ha^{-1}, depending on the landraces, seasons, and ecological zones when harvested between 40–75 days cutting intervals (Sánchez *et al*., 2006). It has been well consented that moringa crop can be harvested at each 30 days obtaining maximum biomass at 30 cm cutting height (Basra *et al*., 2015). Moreover, due to its fast growth and deep rooting system, it can grow under diverse climatic conditions like subtropical and dry tropical with low water availability and moderate saline conditions while varying soil conditions also support moringa growth with the exception of water logged conditions with a slight change in its nutritional quality and antioxidant activities.

Antioxidants are those substances that defer or obstruct oxidative damage even present in very little amounts compared to an oxidizable substrate (Halliwell and Gutteridge, 1989). These compounds influence the mechanism of lipid peroxidation due to variation in their form of action. Hence, disease prevention in live-stock can be done with the help of antioxidants effectively by neutralizing free radicals or by inhibiting their produced damages (Argolo *et al*., 2004). These activities have been recorded previously in moringa under different climatic, geo

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graphical and soil conditions (Nouman *et al*., 2014). Most of the antioxidant activities in the plants are attributed due to the existence of highly potential chemical compounds like phenolic acids (Aqil *et al*., 2006). Moreover, the plants produce many bioactive compounds with good antioxidant potential like ascorbic acid, benzoic acid, carotenoids, etc. Among these, β-carotene and ascorbic acid are being excessively used in pharmaceutical and food manufacturing industries (Mccall and Frei, 1996).

There is no doubt that moringa is a fast growing species but covering the increasing demands of live-stock, its productivity should be increased, which can be brought up with the application of fertilizers (Animashaun *et al*., 2013). As described earlier, different moringa cultivars provide varying protein content, phytochemicals and antioxidant activities depending upon soil and ecological conditions. PKM-1 is the only moringa variety which was developed for its leaf biomass and fresh pods. A very few studies are available so far on its biomass production and phytochemicals or antioxidant activities. With the reference of a pre-experiment observation, it was noted that PKM-1 moringa exhibits less number of leaves as compared to its wild landraces (Sauveur, 2001). The recent study was designed with the hypothesis that PKM-1 moringa biomass and quality traits can be improved with the application of synthetic fertilizers and compost.

Materials and Methods

Experimental Details

The seed material hybrid PKM-1 of *M. oleifera* used in this study was purchased from Javeed Ibrahim Fatta KMC, enterprises, Mumbai, India during 2016 and stored in a dry and air tight container at (room temperature) till further use.

The experiment was conducted in the greenhouse of the Department of Biological and Environmental Sciences, Dongguk University, Ilsan campus, Goyang, South Korea during April–July 2016 to investigate the individual and combined effect of different doses of N:P:K (21:17:17) and compost on growth and quality of PKM-1 *M. oleifera*. Soil used for experiment was loamy in texture with electrical conductivity (ECe) 1.10 dS m^{-1}, pH 5.0, organic matter contents 11.0 g kg^{-1} soli, available P 365 mg kg^{-1} soil, and available Ca, Mg, K were 2.10, 1.0, and 1.32 $cmol^{+}$ kg^{-1}soil, respectively.

Moringa plants were fertilized with six fertilizer treatments in Completely Randomized Design (CRD) replicated three times. The six treatments were control (without compost and NPK fertilizer), 100 g Compost, 2 g NPK, 2 g+100 g (NPK+ Compost), 4 g +200 g (NPK+ Compost) and 6 g+300 g (NPK+ Compost). Each pot was filled with 6 kg of soil from field and pot size was 20 × 24 cm. The constituents of compost used in this study were organic matter more than 30%, poultry manure 50%, cattle manure 5%, pork manure 10% and sawdust 35%.

Cultural Practices

The seeds of hybrid PKM-1 *M. oleifera* were sown in the second week of April in green house in germination trays. Germination was completed in two weeks and equal sizes of seedlings were later transplanted into pots at the rate of one plant per pot in first week of May. There were 30 plants per block (i.e., 5 plants per treatment level in a block). There were 90 plants in three replications.

Application of Treatments

Fully prepared and mature compost was applied one week before transplanting the seedlings into the pots. Compost 100, 200 and 300 g with combination of N:P:K (21:17:17) 2, 4 and 6 g per pot according to the treatments were applied except control. After application of compost two weeks old emerged seedlings were transplanted into the pots. Weeds were controlled by pulling them with hands and spider mites attacked on the plants which were controlled by spraying with water. Crops were maintained up to ten weeks.

Vegetative Growth Parameters

The vegetative growth of moringa seedlings was estimated by measuring plant height (cm), stem girth (mm), number of leaves and number of branches per plant. The data of growth parameters were taken for five weeks starting from second week after transplanting. The plant height was determined using meter rule, stem girth by Vernier caliper while the number of leaves and number of branches per plant were determined by counting.

Biochemical and Phytochemical Analysis

M. oleifera leaves were harvested for biochemical (protein and carbohydrates), and phytochemical (total phenolics and total flavonoid) assays. Moreover, antioxidant activities (DPPH radical scavenging, ABTS radical scavenging, reducing power and nitric oxide scavenging) were also investigated in the treated plants. The fresh biomass was first shade dried followed by oven drying at 45°C till constant weight was achieved. Dried leaves were taken in a clean mortar and pestle and liquid nitrogen was added into it and further ground into a fine powder properly. The formed powder was preserved into the test tube at -4°C in a refrigerator for further use. Fine powder of 0.5 g moringa leaves was taken in a clean mortar and pestle, about 3 mL of distilled water was added, mixed it properly. Then centrifuged it at 1000 rpm for 5 min, collected the supernatant, made up the volume to 5 mL. The extract was frozen at -20°C for further use.

The protein contents of the extracts were determined using the Folin Ciocalteu phenol reagent following the standard method described by Lowry with slight modifications (Lowry *et al*., 1951). A Multiskan™ GO

Microplate Spectrophotometer (A product of Thermo Fishier Scientific Company, USA) was used to make sample and standard readings at 660 nm against the reagent blank using bovine serum albumin (BSA) as reference. The protein content was calculated as BSAmg^{-1} g^{-1} of dry leaves.

The phenol-sulphuric acid method was used to determine the carbohydrate contents (Dubios *et al*., 1956). The absorbance was observed at 490 nm using a Multiskan^{TM} GO Microplate Spectrophotometer (A product of Thermo Fishier Scientific Company, USA) against the reagent blank. The carbohydrate contents were calculated as standard glucose equivalents SG mg^{-1} $g^{-1}$of dry plant leaves.

The method described by Singleton and Rossii (1965) was adopted to determine total phenolic contents with slight modifications using microplate spectrophotometer (Multiskan^{TM} GO, Thermo Fishier Scientific Company, USA). The phenolic content was calculated as gallic acid equivalents GAE g^{-1} of dry plant matter.

Flavonoid contents of the extracts were calculated by using the protocol described by Chang *et al*. (2002) with sight modifications. The absorbance of the reaction mixtures was observed against blank at 420 nm using a Multiskan^{TM} GO Microplate Spectrophotometer (A product of Thermo Fishier Scientific Company, USA).

Preparation of Extract for Antioxidant Assays

About 0.5 g fine powder of moringa dry leaves of each sample was mixed with 30 mL of methanol and kept for 48 h with continuous shaker at room temperature which was later filtered through Whatman's filter paper No. 2. The filtrate was then transferred to petri dish for oven dry at 45^0C. The dry powder was collected and stored in clean dry glass bottles at 4°C till further use. The yield percentage was calculated by the following equation:

$$\text{Yield (\%age)} = \frac{\text{Final weight of sample}}{\text{Initial weight of sample}} \times 100$$

The yield of dry samples in methanol solvent were 16.0, 13.0, 15.5, 17.0, 16.0, and 18.0% for treatments control, 100 g Compost, 2 g NPK, 2 g+100 g (NPK+ Compost), 4 g +200 g (NPK+ Compost) and 6 g+300 g (NPK+ Compost), respectively.

Antioxidant Assays

The 2, 2-diphenyl-2-picrylhydrazylhydrate (DPPH) free radical scavenging activity of moringa dry leaves was evaluated according to the standard procedure as described by Patra *et al*. (2017) with slight modification using a Multiskan^{TM} GO Microplate Spectrophotometer (A product of Thermo Fishier Scientific Company, USA) and the absorbance was noted at 517 nm.

The 2, 2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radical scavenging potential of moringa dry leaves was evaluated by a standard procedure described by Patra *et al*. (2017) reading the absorbance at 734 nm using a Multiskan^{TM} GO Microplate Spectrophotometer (A product of Thermo Fishier Scientific Company, USA).

The nitric oxide scavenging activity of moringa dry leaves was calculated by the procedure described by Patra *et al*. (2015) and the absorbance was read at 540 nm using a Multiskan^{TM} GO Microplate Spectrophotometer (A product of Thermo Fishier Scientific Company, USA).

The results of DPPH, ABTS and nitric oxide radical scavenging activities were presented as IC_{50} values.

The ferric reducing antioxidant potential (FRAP) is used to assess the total antioxidant power of bioactive compounds (Benzie and Strain, 1996).The reducing power of moringa dry leaves was determined using the standard method described by Sun *et al*. (2011) and the absorbance was noted at 700 nm and data were presented in terms of absorbance value with respect to concentration of extracts. The results were expressed in terms of $IC_{0.5}$ values (concentration of moringa extract required to get 0.5 O.D. value) measured by regression analysis.

Statistical Analysis

Data were analyzed statistically through one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Tests using SPSS version 2 (IBM Corp., USA). Statistical significance was accepted at < 0.05. Collected data were expressed as mean ± standard error of mean (SEM).

Results

Growth Parameters of Moringa

Application of compost and NPK increased the height of *M. oleifera* over the weeks (Table 1). Although the plant height was increased in all the treatments during all weeks but tallest plant of 44.79 cm after ten weeks was produced with the application of 2 g NPK, while the shortest plant of 5.32 cm after two weeks was observed in control treatment. Other treatments 2 g+100 g (NPK+Compost), 4 g +200 g (NPK+ Compost), and 6 g+300 g (NPK+ Compost) also increased plant height during 10^{th} week as 44.78, 41.13 and 38.30 cm, respectively. The differences in height yield between NPK, compost and control plant were statistically significant ($p < 0.05$).

The stem girth has the tendency to rise as growth progressed regardless of fertilizer application (Table 2). All treatments improved stem girth statistically at all weeks except at 4^{th} week. The highest stem girth (6.33 mm) after ten weeks was observed in plant that received 2 g of NPK while the lowest stem girth (1.99 mm) was observed after two weeks in control plant. There is no significant differences ($p<0.05$) statistically between the stem girth values observed in compost and all other treatments except control at 2, 8 and 10 weeks which is significantly ($p<0.05$) different.

Table 1: Responses of height (cm) of moringa to compost and N:P:K fertilizer applications

Treatments	Weeks after transplanting				
	2	4	6	8	10
T_1= control	5.32±0.34b	5.97±0.47d	6.77±0.26d	9.38±0.45c	18.57±1.38c
T_2= 100 g compost	7.03±0.45a	8.42±0.51c	11.79±0.46c	22.20±2.38b	31.97±1.09b
T_3= 2g NPK	8.00±0.65a	10.60±0.28a	19.42±1.19a	30.42±1.24a	44.79±1.11a
T_4= 2 g+100 g (NPK + Compost)	7.70±0.78a	9.69±0.36ab	16.90±1.42b	29.73±2.66a	44.78±5.60a
T_5= 4g +200 g (NPK + Compost)	7.32±0.70a	9.56±0.53ab	16.31±0.99b	29.45±4.58a	41.13±2.90a
T_6= 6g+300 g (NPK + Compost)	7.18±0.31a	9.27±0.25bc	15.95±0.43b	28.79±2.74a	38.30±2.77ab

Values followed by the same letter were not significantly different at the 5% level of significance

Table 2: Responses of stem girth (mm) of moringa to compost and N:P:K fertilizer applications

Treatments	Weeks after transplanting				
	2	4	6	8	10
T_1= control	1.99±0.03b	2.43±0.09a	2.54±0.17c	2.86±0.30b	3.34±0.40b
T_2= 100 g compost	2.69±0.08a	2.83±0.41a	2.94±0.21bc	4.40±0.62a	6.03±0.65a
T_3= 2g NPK	2.70±0.07a	2.90±0.08a	3.72±0.28a	4.71±0.32a	6.33±0.55a
T_4= 2 g+100 g (NPK+ Compost)	2.70±0.06a	2.85±0.19a	3.54±0.55ab	4.64±1.12a	6.29±1.58a
T_5= 4g +200 g (NPK+ Compost)	2.69±0.22a	2.84±0.52a	3.04±0.38abc	4.60±0.84a	6.31±0.60a
T_6= 6g+300 g (NPK+ Compost)	2.70±0.13a	2.84±0.11a	3.24±0.14abc	4.46±0.14a	6.29±0.23a

Values followed by the same letter were not significantly different at the 5% level of significance

Table 3: Responses of number of leaves of moringa to compost and N:P:K fertilizer applications

Treatments	Weeks after transplanting				
	2	4	6	8	10
T_1= control	19.20±0.66d	37.47±1.84c	65.06±2.62c	100.93±4.82c	175.93±4.16b
T_2= 100 g compost	23.53±1.64c	52.60±3.82b	90.00±6.15b	124.40±10.37b	214.73±26.30a
T_3= 2g NPK	28.87±0.74a	63.27±4.26a	118.06±2.07a	169.20±9.27a	342.40±18.78a
T_4= 2 g+100 g (NPK+ Compost)	27.00±0.33ab	57.33±3.44ab	112.47±5.08a	167.47±5.94a	340.87±17.44a
T_5= 4g +200 g (NPK+ Compost)	26.20±1.40a	55.53±2.69ab	114.00±6.10a	165.93±13.28a	336.00±21.17a
T_6= 6g+300 g (NPK+ Compost)	26.47±1.22ab	57.20±4.33ab	107.67±3.17a	163.07±8.78a	336.47±7.54a

Values followed by the same letter were not significantly different at the 5% level of significance

Table 4: Responses of *Moringa oleifera* number of branches to compost and N:P:K fertilizer applications

Treatments	Weeks after transplanting				
	2	4	6	8	10
T_1= control	4.27±0.25c	5.93±0.19c	7.93±0.34b	9.73±0.62b	11.67±0.38c
T_2= 100 g compost	4.67±0.09ab	6.87±0.68b	9.73±0.90a	11.80±0.99a	13.73±0.90b
T_3= 2g NPK	6.00±0.28a	7.93±0.25a	10.87±0.62a	13.07±0.62a	16.07±1.05a
T_4= 2 g+100 g (NPK+ Compost)	5.27±0.38b	7.87±0.41a	10.40±0.85a	13.00±0.57a	15.40±0.57a
T_5= 4g +200 g (NPK+ Compost)	5.20±0.49b	7.67±0.50ab	10.27±0.19a	12.93±0.47a	15.47±0.68a
T_6= 6g+300 g (NPK+ Compost)	5.13±0.17a	7.60±0.19ab	10.40±0.16a	12.87±0.08a	15.53±0.14a

Values followed by the same letter were not significantly different at the 5% level of significance

Average leaf score per plant has the tendency to increase in all treatments over the weeks irrespective of the fertilizer used (Table 3). NPK 2 g produced the highest number of leaves (342.40) at week 10 while the lowest leaves of 19.20 were obtained from control plant at week 2. This attribute also improved by all the treatments statistically at10th week.

Application of compost and NPK increased the number of branches per plant across all treatments over the weeks (Table 4). Average highest number of branches (16.07) at week 10 was produced by 2 g NPK which was statically similar to other treatments except sole application of compost, while the average lowest number of branches per plant (4.27) at week 2 was observed in control plant.

Biochemical and Phytochemical Analysis

Biochemical and phytochemical composition of *M. oleifera* leaves were significantly (P<0.05) affected by fertilizer and compost application. NPK significantly increased the protein content of moringa leaves. Carbohydrate, phenolic and flavonoid contents in moringa leaves were significantly higher with the application of compost compared to other treatments. The highest protein content (9.76 mg/g dry weight) was observed in plants sown in pot having 2 g NPK fertilizer. While highest carbohydrate, phenolic and flavonoid contents (291.25, 16.61, 2.019 mg/g dry weight, respectively) were recorded in plants raised from soil having 100 g compost (Table 5).

Table 5: Effects of compost and N:P:K fertilizer on biochemical and phytochemical contents of Hybrid PKM1 (*M. oleifera)* under greenhouse condition

Treatments	(mg/g dry weight)			
	Proteins	Carbohydrates	Phenolics	Flavonoids
T_1= control	6.87±0.06d	76.21±2.99e	11.90±0.21d	0.041±0.01e
T_2= 100g compost	7.64±0.09c	291.25±4.33a	16.61±0.20a	2.019±0.13a
T_3= 2g NPK	9.76±0.22a	82.10±1.96e	13.64±0.30c	1.183±0.00b
T_4= 2 g+100 g (NPK+Compost)	9.02±0.16b	151.67±2.51b	16.28±0.24a	0.132±0.03e
T_5= 4g +200g (NPK+ Compost)	7.95±0.36c	121.25±3.25c	15.14±0.04b	0.820±0.02d
T_6= 6g+300g (NPK+ Compost)	8.56±0.39b	125.03±4.83c	15.26±0.29b	1.054±0.03c

Values followed by the same letter were not significantly different at the 5% level of significance

Table 6: Free radical scavenging and reducing power of *M. oleifera* dry leaves (µg/mL)

Treatments	DPPH Assay (IC_{50}) *	ABTS Assay (IC_{50}) *	NOX Assay (IC_{50}) *	Reducing Power ($IC_{0.5}$) **
T_1= control	50.40	26.01	322.94	831.67
T_2= 100g compost	46.51	24.16	557.42	986.00
T_3= 2g NPK	51.10	29.16	482.02	1240.75
T_4= 2 g+100 g (NPK+Compost)	46.80	25.27	430.23	1240.00
T_5= 4g +200g (NPK+ Compost)	48.57	42.77	441.08	1653.33
T_6= 6g+300g (NPK+ Compost)	48.21	41.58	595.87	826.67
BHT	32.50	35.73	248.70	258.95

***** IC_{50}: Concentration of extract (µg/mL) showing 50% scavenging potential; ****** $IC_{0.5}$: Concentration of extract (µg/mL) showing 0.5 O.D. values at 700 nm

Antioxidant Activities

Moringa dry leaves exhibited 70.20, 72.57, 67.10, 72.44, 70.21 and 72.30% DPPH free radical scavenging potential at 100 µg/mL in control, 100 g Compost, 2 g NPK, 2 g+100 g (NPK+ Compost), 4 g +200 g (NPK+ Compost) and 6 g+300 g (NPK+ Compost) treatments, respectively and the reference compound BHT exhibited 90.97% scavenging at 100 µg/mL (Fig. 1A). The IC_{50} values of treatments control, 100 g Compost, 2 g NPK, 2 g+100 g (NPK+ Compost), 4 g +200 g (NPK+ Compost) and 6 g+300 g (NPK+ Compost) were 50.40, 46.51, 51.10, 46.80, 48.57 and 48.21 µg/mL, respectively, whereas IC_{50} value of BHT was 32.50 µg/mL (Table 6).

ABTS free radical scavenging potentials of moringa dry leaves were 88.21, 87.19, 81.77, 85.70, 83.20, and 83.13% in control, 100 g Compost, 2 g NPK, 2 g+100 g (NPK+ Compost), 4 g +200 g (NPK+ Compost) and 6 g+300 g (NPK+ Compost) treatments at 100 µg/mL, respectively (Fig. 1B). The IC_{50} values of 26.01, 24.16, 29.16, 25.27, 42.77, and 41.58 µg/mL were observed in treatments control, 100 g Compost, 2 g NPK, 2 g+100 g (NPK+ Compost), 4 g +200 g (NPK+ Compost) and 6 g+300 g (NPK+ Compost), respectively and BHT, taken as the reference compound exhibited 35.73 µg/mL IC_{50} value (Table 6).

The results exhibited that the treatments control, 100 g Compost, 2 g NPK, 2 g+100 g (NPK+ Compost), 4 g +200 g (NPK+ Compost) and 6 g+300 g (NPK+ Compost) showed nitric oxide activity of 15.62, 9.49, 10.81, 11.92, 10.91, and 8.39% at 100 µg/mL, respectively and the reference compound BHT exhibited 20.51% inhibition at 100 µg/mL (Fig. 1C). The IC_{50} values of NOX activity observed in treatments control, 100 g Compost, 2 g NPK, 2 g+100 g (NPK+ Compost), 4 g +200 g (NPK+ Compost) and 6 g+300 g (NPK+ Compost) were 322.94, 557.42, 482.02, 430.23, 441.08 and 595.87 µg/mL, respectively whereas in case of BHT it was 248.70 µg/mL (Table 6).

M. oleifera dry leaves exhibited a high reducing power activity of 0.059, 0.051, 0.037, 0.04, 0.03 and 0.06% in treatments control, 100 g Compost, 2 g NPK, 2 g+100 g (NPK+ Compost), 4 g +200 g (NPK+ Compost) and 6 g+300 g (NPK+ Compost) at 100 µg/mL, respectively and the reference compound BHT had value of 0.19 µg/mL at 100 µg/mL (Fig. 1D). The $IC_{0.5}$ values of treatments control, 100 g Compost, 2 g NPK, 2 g+100 g (NPK+ Compost), 4 g +200 g (NPK+ Compost) and 6 g+300 g (NPK+ Compost) were 831.67, 986.00, 1240.75, 1240.00, 1653.33 and 826.67 µg/mL, respectively whereas $IC_{0.5}$ value of BHT was 258.95 µg/mL (Table 6).

Discussion

Both organic and inorganic fertilizers supply nutrients to the plants which are essential for their growth and development. But, each contains different ingredients with composition and plants utilize these nutrients in different ways. In present study, the vegetative growth of moringa was best supported by the application of NPK (2 g) as indicated by higher height, stem girth and maximum number of leaves and branches among the treated plants (Table 1-4). According to Anamayi *et al.* (2016), NPK proved more superior to cow dung manure because it produced better attributes such as leaves score, stem girth and plant height in *M. oleifera* in comparison with other applications. The supply of nitrogen and phosphorus to moringa trees would be beneficial for root development and leaf canopy growth as described previously (Fugile, 1999). According to

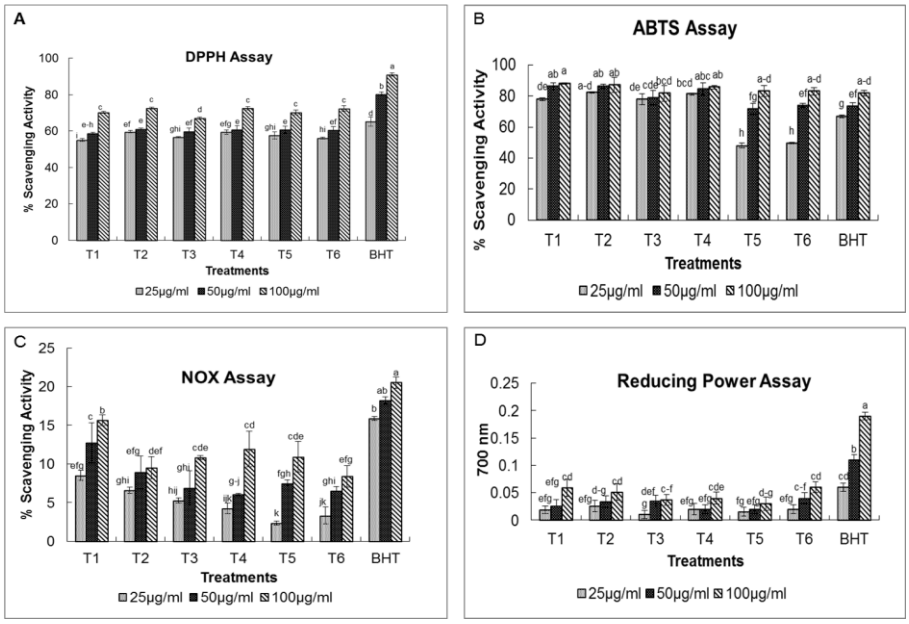

Fig. 1: Antioxidant potential of methanol extract of dry leaves of moringa and standard reference compound BHT. A: DPPH radical scavenging assay; B: ABTS radical scavenging assay; C: Nitric oxide scavenging assay; D: Reducing power assay. Values followed by the same letter were not significantly different at the 5% level of significance

Fagbenro (2001), *Ceiba pentandra*, *Parkia biglobosa* and *Gmelina arborea* plants showed positive response to NPK 15:15:15 fertilizer. The plant height, stem girth, leaves count and number of branches of plants from pot supplied with 2 g NPK were significantly higher than other treatments across all the weeks. The plant of highest height (44.79 cm), highest stem girth (6.33 mm), maximum leaves count (342.40) and maximum number of branches (16.07) at week 10 in present study is higher than that obtained from control (18.57 cm, 3.34 mm, 175.93, 11.67, respectively) as reported by Makinde (2013).

Ainika and Amans (2011) reported that vegetable amaranth has been given positive response with respect to its vegetative growth and vegetative yield parameters to the application of NPK fertilizer. The fertilizer NPK gave much better results on vegetative growth of hybrid *M. oleifera* (PKM-1) as compared to compost because it produced better attributes like plant height, stem girth, leaves count and number of branches than its counter parts produced. Probably the NPK fertilizer provided the quick supply of macronutrients (N, P, K) to the plants as compared to compost from which the slow release of nutrients took place. Due to that reason the vegetative growth was much better than compost. Other treatment combinations (NPK+compost) also improved these attributes in addition to NPK alone but most of NPK in these combinations may be lost due to leaching and sufficient increase in these attributes would not be observed as compared to NPK alone.

Protein contents increased at all treatments compared to control. Plants produced maximum protein contents (9.76 mg/g/dry weight) that were treated with NPK (2 g pot^{-1}) compared to control and all other treatments as supported by Stephen *et al.* (2014) who concluded that *Amaranthus* species grown with NPK significantly had higher protein contents. The higher protein in moringa plant is consistent with the result of Makinde (2013). The increased nitrogen to the soil by N:P:K (15:15:15) fertilizer increased the uptake by the moringa resulting in increased protein. On the other hand, compost as an organic fertilizer can be a good source of microorganism, while others serve as a source of food for microorganism, can help to stimulate microbial communities (Raviv *et al*., 2005). The highest carbohydrate contents (291.25 mg/g dry weight) were produced in plant obtained from pot having 100 g compost. These results are supported by the C/N balance hypothesis. When N is quickly available, as a result plants are trending primarily to make high N-content containing compounds (e.g., proteins for growth).When N availability is reduced, plants would make more carbon-containing compounds such as starch, cellulose, and non-N-containing secondary metabolites such as phenolics and terpenoids (Haukioja *et al*., 1998). As different fertilizers would be the source of different nutrients so it could produce different C/N ratios in plants and this in turn could lead to different secondary metabolites production (Montagu and Goh, 1990).

Different fertilizers and their rates affected phenolic and flavonoid contents. The application of compost increased the production of phenolics and flavonoids contents in moringa (Table 5). The availability of nitrogen should influence the amounts of phenolics more strongly than terpenoids because phenolics are produced in the same shikimic acid pathway as aromatic amino acids as suggested

previously (Muzika and Pregitzer, 1992). Therefore, it can be suggested that the phenolics concentration in plants can get decreased at high nitrogen availability and vice versa (Haukioja *et al*., 1998). Recently, Hakkinen and Torronen (2000) noticed maximum phenolic compounds in organically grown strawberry in comparison with other strawberry cultivars cultivated inorganically. The production of carbohydrate, total phenolics and total flavonoids was positively correlated indicated by the previous studies done on *Labisia pumila* (Ibrahim *et al*., 2013; Jaafar *et al*., 2012).

On the basis of IC_{50} values the highest antioxidant activities of moringa were observed in plants treated with 100 g compost. The effects on DPPH are ascribed to compost and NPK fertilizer (Fig. 1A). The highest DPPH value was found under compost (100 g) application (72.57 µg/mL) at 100 µg/mL and lowest under NPK (2 g) fertilization (67.10 µg/mL). The results revealed that the application of organic manure can increase the radical scavenging activity in *M. oleifera* where as it could be reduced significantly by the application of inorganic fertilizer. BHT, which was taken as a reference standard compound (positive control), it showed concentration dependent activity (Fig. 1A). Another significant finding of present study is that organic manure (100 g compost) was advantageous for the increase of the antioxidant potential of moringa. A synergic effect on DPPH radical scavenging activity produced by the combination of phenolics and ascorbic acid would have been observed as supported by Murakami *et al.* (2003).

The high ABTS radical scavenging activity exhibited in moringa might be due to the presence of a number of functional groups or the stereo-selectivity of the radicals presented in moringa dry leaves (Fig. 1B) (Adedapo *et al*., 2008). Nitric oxide has been reported as an unstable radical that produces different molecules of high reactivity such as NO_2, N_2O_4 and N_3O_4 when reacted with oxygen molecules, producing different physiological disorders such as fragmentation of DNA, lipid peroxidation and cell damage in the body (Santiso *et al*., 2012).

The reducing power of *M. oleifera* was affected by different fertilizers and their rates. The highest reducing potential was observed under organic fertilization. Antioxidant activity of moringa was evaluated by the capability to reduce 2, 4 and 6-tripyridyl-s-triazine (TPTZ)-Fe (III) complex to TPTZ-Fe (II). The antioxidant component of dietary polyphenols could be evaluated by the FRAP assay (Luximon-Ramma *et al*., 2005). The reducing power of moringa (Fig. 1D) could be ascribed having different kinds of potential antioxidant rich compounds (Srivastava *et al*., 2006). The importance of phenolic compounds is clear that these compounds act as electron donors in free radical reactions due to their scavenging ability (Heo *et al*., 2006). The findings of the present study was in accordance with Wang *et al.* (2003) who reported that elevated CO_2 concentration increased the free radical scavenging power of strawberry. The highest production of carbohydrate, phenolics, flavonoids and increased antioxidant activities were observed in compost (100 g/pot) treated moringa seedlings as supported by the previous literature (Ibrahim and Jaafar, 2012).

Conclusion

The NPK fertilizer and compost are valuable sources for vegetative growth, biochemical, phytochemical and antioxidant activities of hybrid *M. oleifera* (PKM 1) because they have greatly improved performance of treated plants over the control. However, treatment (2 g NPK) with respect to vegetative growth proved more superior to compost and all other treatments. Furthermore, the compost may be an effective source of organic fertilizer to increase the expression of primary and secondary metabolites as well as antioxidant activities than NPK fertilizer alone. Moringa seedlings treated with 100 g compost showed higher phenol, flavonoids, carbohydrate contents and antioxidant activities.

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