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Effect of compost and NPK fertilizer on improving biochemical and antioxidant properties of *Moringa oleifera*

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ABSTRACT

Organic and inorganic fertilizers play a significant role in improving the nutritional quality of Moringa plant (*Moringa oleifera*). A field experiment of six treatments (control; 5 t/ha Compost; 120 kg/ha NPK; 50 + 50%/ha (NPK + Compost); 10 + 50%/ha (NPK + Compost); and 50 + 10%/ha (NPK + Compost) with three replications was conducted in a randomized complete block design in order to evaluate the effect of the application of compost and NPK fertilizer in various combinations on the biochemical and antioxidant potential of the Moringa plant. The results showed a significant influence of the compost and NPK fertilizer in various combination on the biochemical and antioxidant properties of the plant. Higher amount of carbohydrate, phenolic and flavonoid contents (406.24, 45.23 and 1.69 mg g^{-1} dry wt., respectively) were observed in the plant treated with 50 + 50%/ha (NPK + Compost). Protein content (27.91 mg g^{-1} dry wt.) was improved by the application of 120 kg/ha NPK fertilizer. The antioxidant properties such as, DPPH, ABTS and nitric oxide were analyzed as percentage scavenging and half maximal effective concentration (EC_{50}) whereas ferric-reducing antioxidant potential (FRAP) was determined in terms of absorbance at 700 nm and $EC_{0.50}$. The half maximal effective concentrations in combined application of NPK and compost (50 + 50%/ha (NPK + Compost)) were the lowest among the fertilizer treatments, resulting in EC_{50} value of 27.61, 17.18, 104.24 μg mL^{-1} for DPPH, ABTS and nitric oxide assays, respectively whereas in case of the FRAP assay the $EC_{0.50}$ value as found out to be 292.35 μg mL^{-1}.

1. Introduction

Moringa oleifera is a medium-sized tree, growing to about 10 m in height, commonly known as drumstick tree, Horseradish tree or Ben tree and belongs to the family Moringaceae (Adediran et al., 2003). All parts of the tree possess a wonderful range of medicinal and nutritional properties (Fahey, 2005; Mishra et al., 2011). Its leaves are nutritious and rich in minerals and vitamins especially vitamin C, vitamin B_6 and provitamin A as beta carotene, calcium and magnesium (Bharali et al., 2003). The plant grows in natural environment, however, organic and inorganic fertilizers are necessary for its optimum growth. Both fertilizers supply nutrients that are essential for its optimum performance.

Inorganic or synthetic fertilizer has significantly supported the ever increasing population of the world and it has been estimated that, half of the population depends on the artificial nitrogen fertilizer used for their food requirements (Erisman et al., 2008). These inorganic fertilizers play a vital role in the commercial and subsistence farming for the growth of various crops (Masarirambi et al., 2010), because their application in the field is easier and they absorbs quickly by the crops. The synthetic fertilizers are used in a number of conventional agriculture system contains various macronutrients which dissolve quickly in the soil and supply the nutrients to the plants in large amounts (Masarirambi et al., 2010).

It is obvious from the epidemiological studies that food rich in natural antioxidants are helpful in protecting the body from various degenerative diseases such as cancer, coronary heart diseases and Alzheimer's disease (Pezzuto and Park, 2002). Hence, the intake of antioxidants in the body can be increased by the use of dietary sources and food those are rich in natural oxidants (Soong and Barlow, 2004). In old times, synthetic antioxidants like BHA and BHT prevailed as common food additives, but presently their use has be limited due to the safety concerns (Sun and Chi-Tang, 2005) and the use of natural antioxidants have become popular and encouraged (Wilson, 1999). Plants are considered as the best sources for natural antioxidants such as tocopherols, flavonoids, vitamin C and different phenolic compounds (Laandrault et al., 2001). Many species including the legumes and vegetables have been discovered as the rich source of natural antioxidants (Ismail et al., 2004). Moringa is also an important source of natural antioxidants along with proteins and numerous phytochemicals (Anwar et al.,

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2007; Nouman et al., 2016; Olson et al., 2016). In this context, the present study was conducted with the hypothesis that biochemical and antioxidant potential of Moringa might be improved by the use of compost and NPK (21:17:17) fertilizer under agro-climatic conditions of South Korea. Hence, the optimum combination of the compost and NPK in the soil was investigated followed by the estimation of the antioxidant potential of the Moringa leaves.

2. Materials and methods

2.1. Study area and experiment design

The study was conducted during the month of April–October 2016 in the experimental fields of Dongguk University, Ilsan Campus, Goyang, Republic of Korea which geographically lies between 37° 39′ 23″ N and 126° 50′ 05″ E. The average temperature, humidity and rainfall at that time were 28 °C, 78.2%, 1312 mm, respectively. The soil texture was loamy with an electrical conductivity (ECe) of 1.10 ds m^{-1}, pH 5.0, organic matter contents 11.0 g kg^{-1} soil, available phosphorus (P) 365 mg kg^{-1} soil, and available calcium (Ca), magnesium (Mg), potassium (K) were 2.10, 1.0, and 1.32 $cmol^{+}$ kg^{-1} soil, respectively. The fully decomposed and prepared compost, commercially available in the market was used in the current investigation and it contained, poultry manure 50%, pork manure 10%, cattle manure 5%, sawdust 35% and organic matter more than 30% (as mentioned by the manufacturer).

The Randomized Complete Block Design (RCBD) with six treatments and three replications was fitted into the experiment. The recommended rates of NPK used for Moringa is 120:100:100 kg/ha, and recommended rate of compost used for Moringa is 5 t /ha. For NPK fertilizer, the rate was 10 and 50% of 120,100 and 100 kg/ha and for compost it was 10 and 50% of 5 t/ ha. The treatments were as follows – T1 [control (i.e., no fertilizer)], T2 [5 t ha^{-1} Compost], T3 [120 kg ha^{-1} NPK], T4 [50 + 50% ha^{-1} (NPK + Compost)], T5 [10 + 50% ha^{-1} (NPK + Compost)], and T6 [50 + 10% ha^{-1} (NPK + Compost)]. Moringa hybrid PKM-1 seeds were sown in the first week of April 2016 in germination trays and one month, old seedlings of equal size were obtained. After germination, 75 seedlings per plot were later transplanted into the field.

The compost and NPK fertilizers were applied to the plots before transplanting the seedlings. Each plot was completely covered with black plastic mulching paper to save the seedlings from strong winds. Equal size of seedlings was transplanted into the plots by making holes into the plastic paper. The plot size for vegetative growth of the plant was 3.3 × 2.1 m^2 (6.93 m^2), with plant to plant distance of 22 cm and row to row distance of 42 cm, respectively. The irrigation was applied to the crops as required. The cultural practices were also maintained and the crop was retained from May to October. The Moringa plants were harvested in the month of October and after that various analysis were performed. At that stage the maximum plant height was 65.02 cm.

2.2. Biochemical analysis

The fresh leaves were harvested and shade dried followed by oven drying at 45 °C till constant weight was achieved. A clean mortar and pestle containing liquid nitrogen was used to grind the dry plant material which was stored at −20 °C in the refrigerator. For biochemical analysis, 3 ml of distilled water was added into the fine powder of 0.5 g Moringa leaves in a clean mortar and pestle and mixed properly. Then it was centrifuged at 1000 rpm for 5 min. After centrifugation, the supernatant was collected, tand distilled water added to it to make up the volume to 5 ml. It was then kept in a freezer at −20 °C for further analysis.

2.2.1. Protein measurement

The protein content of the extracts were determined using the standard procedure of Folin–Ciocalteu phenol reagent assay (Lowry et al., 1951). A UV–VIS spectrophotometer (Multiskan GO, Thermo Scientific, Waltham, MA, USA) was used to take the absorbance of both the sample and standard at 660 nm against the reagent blank using bovine serum albumin (BSA) as reference. The protein content was calculated as BSA mg g^{-1} of dry leaves.

2.2.2. Carbohydrate measurement

The carbohydrate contents were determined by the phenol-sulfuric acid method (Dubios et al., 1956). The absorbance was observed at 490 nm using a UV–VIS spectrophotometer against the reagent blank. The carbohydrate contents were calculated as standard glucose equivalents SG mg g^{-1} of dry plant leaves.

2.2.3. Phenolic content measurement

The method described by Singleton and Rossi (1965) was adapted to measure the total phenolic content using UV–VIS spectrophotometer. The absorbance of the reaction mixtures was observed against blank at 765 nm. The phenolic contents were calculated as gallic acid equivalents GAE mg g^{-1} of dry plant matter.

2.2.4. Flavonoid measurement

Flavonoid contents of the extracts were determined using the protocol described by Chang et al. (2002). The absorbance of the reaction mixtures was observed against blank at 420 nm using a UV–VIS spectrophotometer.

2.2.5. Antioxidant measurement

Moringa dry leaves fine powder of about 0.5 g of each sample was mixed with 30 mL of methanol and kept for 48 h in an electrical shaker with continuous stirring at room temperature which was later filtered through Whatman No. 2 filter paper. The filtrate was then transferred to petri dish for oven dry at 45 °C. The collected dry powder was stored in clean dry glass bottles at 4 °C.

The yield percentage was calculated by the following equation:

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

The yield of dry samples in methanol solvent were 19.0, 18.50, 15.50, 15.0, 18.0, and 17.0% for treatments [T1 — control; T2 — 5 t ha^{-1} Compost; T3 — 120 kg ha^{-1} NPK; T4 — 50 + 50% ha^{-1} (NPK + Compost); T5 — 10 + 50% ha^{-1} (NPK + Compost); and T6 — 50 + 10% ha^{-1} (NPK + Compost), respectively.

2.2.5.1. DPPH scavenging activity measurement. The DPPH (2, 2-diphenyl-1-picryl-hydrazyl) free radical scavenging activity of Moringa dry leaves were evaluated according to the standard procedure as described by Patra et al. (2017) with slight modification using a UV–VIS spectrophotometer. The absorbance of the reaction mixture was noted at 517 nm. The DPPH scavenging activity was measured by the following equation:

$$\text{Scavenging percentage}(\%) = \frac{Abs_{control} - Abs_{treatment}}{Abs_{control}} \times 100. \quad (1)$$

where, $Abs_{(control)}$ or $Abs_{(treatment)}$ is the absorbance of the control and the treatment, respectively.

The half maximal effective concentrations (EC_{50}) values were also calculated from the resluts.

2.2.5.2. ABTS scavenging activity measurement. The ABTS (2, 2-azino-bis (3-ethylbenzthiazoline 6-sulphonic acid) radical scavenging potential of Moringa dry leaves was calculated by a standard procedure described by Patra et al. (2017) reading the absorbance at 734 nm using a UV–VIS

spectrophotometer. The scavenging activity was calculated using Eq. (1). The half maximal effective concentrations (EC_{50}) values were also calculated from the results.

2.2.5.3. Nitric oxide scavenging activity measurement. The nitric oxide scavenging activity of Moringa dry leaves was determined by the procedure described by Patra et al. (2015) and the absorbance was read at 540 nm using a UV–VIS spectrophotometer. The scavenging activity was calculated using Eq. (1). The half maximal effective concentrations (EC_{50}) values were also calculated from the results.

2.2.5.4. Ferric reducing antioxidant activity measurement. The ferric 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 using UV–VIS spectrophotometer. The data were presented in terms of absorbance value with respect to concentration of the extract. The half maximal effective concentrations ($EC_{0.5}$) values were also calculated from the results.

2.3. Data collection and statistical analysis

All collected data were analyzed subjected to one-way analysis of variance (ANOVA) using SPSS version 2 (IBM Corp USA). Duncan's Multiple Range Tests was used to compare the means. The statistical significance was accepted at $P < .05$.

3. Results

A significantly higher amount of total protein (27.91 mg g^{-1} dry wt) was recorded in the leaves harvested from treatment T3 supplemented with NPK at the rate of 120 kg ha^{-1} (Table 1; $P < .05$). While treatment T4 receiving NPK and compost at the rate of 50 + 50% showed significantly higher values of biochemical contents compared to control and other treatments. The highest carbohydrate, phenolic and flavonoid contents (406.24, 45.23 and 1.69 mg g^{-1} dry wt, respectively) were recorded in treatment T4 (Table 1; $P < .05$).

Moringa dry leaves DPPH scavenging activity and standard reference BHT (positive compound) ware presented in Fig. 1. Moringa dry leaves exhibited 93.56, 95.25, 94.77, 95.57, 94.45 and 94.36% DPPH free radical scavenging potential in control (T1), 5 t ha^{-1} compost (T2), 120 kg ha^{-1} NPK (T3), 50 + 50% ha^{-1} (NPK + Compost) (T4), 10 + 50% ha^{-1} (NPK + Compost) (T5), and 50 + 10% ha^{-1} (NPK + Compost) (T6) treatments, respectively at 100 μg/mL and 90.70% for the reference compound BHT (Fig. 1). Higher value of DPPH free radical scavenging potential was recorded in the extract of Moringa dry leaves harvested from treatment T4 (Fig. 1). The DPPH free radical scavenging potential was exhibited in terms of EC_{50} values and the treatment T4 showed promising DPPH scavenging potential EC_{50} value of 27.61 μg mL^{-1} as compared to control and all other treatments and the reference standard, BHT (Table 2).

Table 1
Effects of NPK and compost on biochemical contents of Moringa.

Treatments	Proteins	(mg g^{-1} dry weight)		
		Carbohydrates	Phenolics	Flavonoids
T_1	24.26 ± 0.54d	225.97 ± 7.80f	39.46 ± 0.43c	1.33 ± 0.007d
T_2	25.56 ± 0.80c	260.51 ± 6.94e	44.22 ± 1.68ab	1.58 ± 0.06b
T_3	27.91 ± 0.38a	359.60 ± 8.57c	41.96 ± 0.27bc	1.38 ± 0.02cd
T_4	27.48 ± 0.60ab	406.24 ± 4.21a	45.23 ± 1.09a	1.69 ± 0.04a
T_5	26.28 ± 0.22bc	323.53 ± 9.58d	43.87 ± 1.58ab	1.49 ± 0.04c
T_6	27.38 ± 0.45ab	380.42 ± 8.63b	44.02 ± 1.35ab	1.59 ± 0.03b

$T_{1=}$ Control, $T_{2=}$ 5 t ha^{-1} Compost, $T_{3=}$ 120 kg ha^{-1} NPK, $T_{4=}$ 50 + 50% ha^{-1} (NPK + Compost), $T_{5=}$ 10 + 50% ha^{-1} (NPK + Compost), $T_{6=}$ 50 + 10% ha^{-1} (NPK + Compost). Values followed by the same letter were not significantly different at the 5% level of significance.

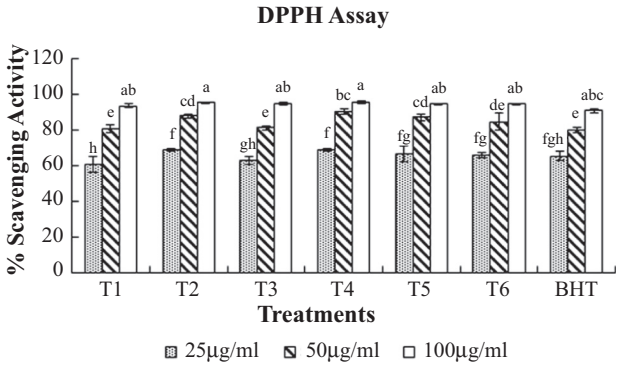

Fig. 1. Antioxidant potential of methanol extract of dry leaves of Moringa and standard reference compound BHT. DPPH radical scavenging assay. Values followed by the same letter were not significantly different at the 5% level of significance. T1 = Control, T2 = 5 t ha^{-1} Compost, T3 = 120 kg ha^{-1} NPK, T4 = 50 + 50% ha^{-1} (NPK + Compost), T5 = 10 + 50% ha^{-1} (NPK + Compost), T6 = 50 + 10% ha^{-1} (NPK + Compost).

Moringa dry leaves ABTS free radical scavenging activity and the BHT taken as the positive control is shown in Fig. 2. The highest ABTS free radical scavenging activity in terms of EC_{50} values was exhibited by the treatment T4 with EC_{50} 17.18 μg mL^{-1} as compared to all other treatments (Table 2). The nitric oxide scavenging activity of Moringa dry leaves and BHT as reference compound is represented in Fig. 3. The results of nitric oxide antioxidant potential in terms of EC_{50} values was displayed by the treatment T4 with 104.24 μg mL^{-1} EC_{50} as compared to all other treatments (Table 2). Moringa dry leaves reducing power and the standard reference compound BHT as the positive control, is represented in Fig. 4. The ferric reducing antioxidant potential in terms of $EC_{0.50}$ value was depicted by the treatment T4 (292.35 μg mL^{-1}) as compared to all other treatments (Table 2).

4. Discussion

Moringa oleifera plant is a valuable source of nutraceutical food with miraculous properties in humans and animals (Ganguly et al., 2010). So, information about biochemical contents of leaves can be helpful in making strategies for proper use. In South Korea it is not possible to get the seeds of Moringa due to temperate climate. The Moringa plants were harvested in the month of October 2016, and after that various analysis were performed on the leaves which is generally eaten as raw or in dried form as tea. At that stage the maximum plant height was 65.02 cm.

Maximum protein contents in plants that were treated with 120 kg ha^{-1} N:P:K 21:17:17 (T3) was obtained compared to the control and all other treatments (Table 1). Amaranthus species grown with NPK fertilizer significantly had higher protein contents as reported by Stephen et al. (2014). Similarly, significant differences ($P < .05$) in

Table 2
Free radical scavenging and reducing power of Moringa dry leaves (μg mL^{-1}).

Treatments	DPPH assay (EC_{50})[a]	ABTS assay (EC_{50})[a]	NOX assay (EC_{50})[a]	Ferric reducing power ($EC_{0.5}$)[b]
T_1	33.45	20.30	161.27	383.08
T_2	28.37	18.32	116.48	327.80
T_3	32.38	19.84	130.55	383.23
T_4	27.61	17.18	104.24	292.35
T_5	29.50	19.38	127.00	412.58
T_6	30.41	22.15	145.78	356.14
BHT	32.51	35.72	247.98	259.68

$T_{1=}$ Control, $T_{2=}$ 5 t ha^{-1} Compost, $T_{3=}$ 120 kg ha^{-1} NPK, $T_{4=}$ 50 + 50% ha^{-1} (NPK + Compost), $T_{5=}$ 10 + 50% ha^{-1} (NPK + Compost), $T_{6=}$ 50 + 10% ha^{-1} (NPK + Compost). Values followed by the same letter were not significantly different at the 5% level of significance.

[a] EC_{50}: Concentration of extract (μg/mL) showing 50% scavenging potential.

[b] $EC_{0.5}$: Concentration of extract (μg mL^{-1}) showing 0.5 O.D. values at 700 nm.

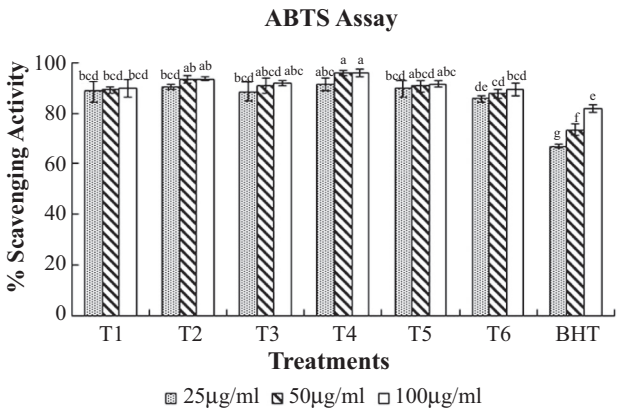

Fig. 2. Antioxidant potential of methanol extract of dry leaves of Moringa and standard reference compound BHT. ABTS radical scavenging assay. Values followed by the same letter were not significantly different at the 5% level of significance. T1 = Control, T2 = 5 t ha^{-1} Compost, T3 = 120 kg ha^{-1} NPK, T4 = 50 + 50% ha^{-1} (NPK+ Compost), T5 = 10 + 50% ha^{-1} (NPK + Compost), T6 = 50 + 10% ha^{-1} (NPK + Compost).

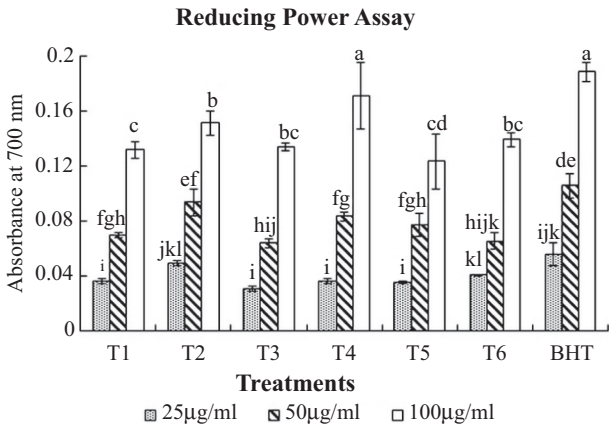

Fig. 4. Antioxidant potential of methanol extract of dry leaves of Moringa and standard reference compound BHT. Reducing power assay. Values followed by the same letter were not significantly different at the 5% level of significance. T1 = Control, T2 = 5 t ha^{-1} Compost, T3 = 120 kg ha^{-1} NPK, T4 = 50 + 50% ha^{-1} (NPK + Compost), T5 = 10 + 50% ha^{-1} (NPK + Compost), T6 = 50 + 10% ha^{-1} (NPK + Compost).

carbohydrate content were observed as function of fertilizer applications. Combined application of NPK and compost (50 + 50%) (T4), displayed a significant increase in the carbohydrate content and the results are supported by Haukioja et al. (1998) who reported that according to C/N balance hypothesis, when N is readily available, plants would primarily make compounds with high N content (e.g., proteins for growth). When N availability is limited, metabolism changes more towards carbon-containing compounds such as starch, cellulose, and non-N-containing secondary metabolites such as phenolics and terpenoids. These results are also supported by Marzeh et al. (2012) who reported that application of compost in tomato and cucumber showed an increment in results over the plants treated without compost. EI-Sherbeny et al. (2005) found that the application of compost in Montana plants showed an increase in plant pigments and total carbohydrate over plants treated with NPK fertilizer. Significant differences in the phenolic and flavonoid contents of the plants were recorded by the combine application of NPK and compost in the soil. Plants treated with NPK and compost (50 + 50%) (T4) in combination, depicted higher phenolic and flavonoid content as compared to the control and all other treatments (Table 1). These findings are in accordance with Ahmed et al. (2013) who cited that the application of bio-fertilizer alone or with combination of nitrogenous fertilizer increased carbohydrate and flavonoid contents in the plant species. These findings were also in accordance with Ibrahim and Jaafar (2011) who reported that *L. pumila* which displayed the lowest N and high C/N was found to accumulate higher total phenolics and flavonoids.

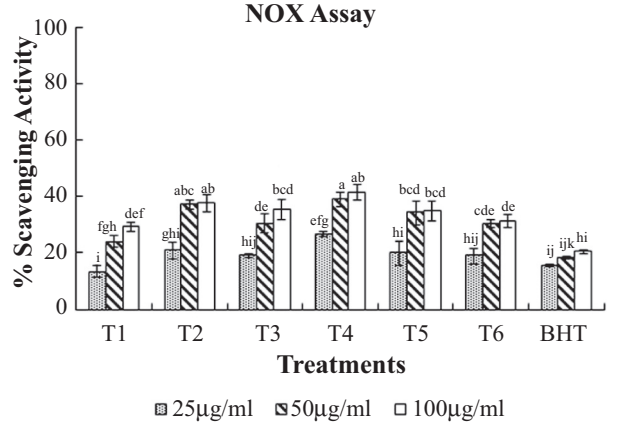

Fig. 3. Antioxidant potential of methanol extract of dry leaves of Moringa and standard reference compound BHT. Nitric oxide scavenging assay. Values followed by the same letter were not significantly different at the 5% level of significance. T1 = Control, T2 = 5 t ha^{-1} Compost, T3 = 120 kg ha^{-1} NPK, T4 = 50 + 50% ha^{-1} (NPK + Compost), T5 = 10 + 50% ha^{-1} (NPK + Compost), T6 = 50 + 10% ha^{-1} (NPK + Compost).

On the basis of half maximal effective concentration (EC_{50}) values, the highest DPPH potential of Moringa were observed in plants treated with 50 + 50% (NPK + compost) (T4) with EC_{50} value of 27.61 µg mL^{-1} (Table 2). A synergic effect on DPPH radical scavenging activity produced by the combination of phenolic and ascorbic acid would have been observed as supported by Murakami et al. (2003). The food industry and the agricultural scientists mostly use ABTS assay to measure the antioxidant potential of foods. This assay is used to estimate the relative ability of antioxidant to scavenge the ABTS compared with various reference standard (Fitriana et al., 2016). In the present study, the high ABTS radical scavenging activity (EC_{50} = 17.18 µg mL^{-1}) exhibited by the Moringa plant might be due to the presence of a number of functional groups or the stereo-selectivity of the radicals presented in the plant (Table 2; Fig. 2) (Adedapo et al., 2008). The findings of this study are the scientific proof to use *M. oleifera* leaves in daily diet to prevent diseases and depicted that *M. oleifera* leaves are good source of natural antioxidant (Fitriana et al., 2016). Nitric oxide activity (NOX) 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 (Cheng et al., 2011; Santiso et al., 2012). The positive NOX potential of the Moringa plant further proves its importance as an effective antioxidant (Table 2; Fig. 3). Previous literature have described that the reducing power of bioactive compounds is directly related to assess antioxidant activity (Siddhuraju et al., 2002). The lowest $EC_{0.50}$ values that reflects its high reducing power was observed in the extract of Moringa dry leaves treated with combined application of compost and NPK (50 + 50%) fertilizer (T4). Significant variation in the reducing power was observed for all fertilizer treatments analyzed (Fig. 4). These results are comparable to those of the double reducing power in Hsian tsao leaf gum (Lai et al., 2001), which has been reported as an important source of natural antioxidants. The differences in the accumulation of biochemical and antioxidant activities might be due to variability in N and C/N ratio.

5. Conclusion

The present study has revealed that application of compost and N:P:K 21:17:17 (50 + 50%) in combination (T4), had significant effects on biochemical and antioxidant potential of *Moringa oleifera* compared to sole application of compost and NPK except protein which was highest in

120 kg ha^{-1} NPK (T3) treated plants. The production of carbohydrate, phenolic, flavonoid and antioxidant properties was highest under combine application of compost and NPK (50 + 50%) treatment. Generally, it is recommended that in agro climatic conditions of South Korea, Moringa leaves should be harvested during August–October to get maximum levels of biochemical and antioxidant potential and related health benefits.

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