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ALTERATION IN ANTIOXIDANT POTENTIAL OF *Spinacia oleracea* IN RESPONSE TO SELECTED PLANT GROWTH REGULATORS

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The spinach (*Spinacia oleracea*) plants treated with certain seed priming (bio-fertilizer and Humic acid) and foliar treatments (Humic acid, *Moringa* leaf extract, 6-Benzyl amino purine etc.) were tested for total phenolic content and the antioxidant activity. Methanolic extracts of all spinach samples were assessed performing three different protocols including Folin-Ciocalteu, reducing power and DPPH radical scavenging assays. TPC value ranged 4.678-13.236 mg GAE/g of dry matter. Reducing power assay showed values (absorbance at λ_{max}=700nm) in the range of 0.351-1.874 at 10 mg/mL extract concentration. The range of IC_{50} values in DPPH radical scavenging assay was 0.499-1.063 μg/mL extract concentration. The one way ANOVA under CRD showed significant differences among treatments. Among various plant growth regulators, fresh *Moringa* leaf extract proved as the potent enhancer of antioxidant activity of spinach leaves.
Keywords: Spinach, growth regulators, radical scavenging potential, total phenolic contents, reducing power

INTRODUCTION

Provision of quality food is the top notch requisite in current arena of malnutrition and under nourishment (Odhav *et al*., 2007). Vegetables are nature blessed rich source of minerals, fibers and vitamins (Shyamala *et al.,* 2005; Mukhtar, 2008). In addition, they provide strength to immune system of human body due to their antioxidant potential. Leafy vegetables have unique appraisal in this regard as in most of the cases leaves serve as rich source of phenolic compounds, vitamins, iron and other minerals (Murcia *et al.,* 2009).

Organic farming reflects the quality and efficacy of food but it sometimes leads to conventional unhealthy agricultural practices (Mahdi *et al.,* 2010). Recently, trend to increase crop productivity by using different growth regulating substances like nitrogenous or phosphatic fertilizers, hormones and other organic/ inorganic compounds as seed treatment, foliar or soil application are getting popularity day by day (Ibrahim *et al.,* 2007). Plant growth regulating substances promote growth and development of plant at low doses but showing contrary effects at elevated doses. They play their part in the regulation of plant responses performing either antagonistic or synergistic activity by working in abiotic and biotic stress (Lobna *et al.,* 2011).

Leafy vegetables like spinach, coriander, mint, broccoli, cabbage etc. are part of daily diet and reportedly help in reducing a number of deficiencies like inflammation, cardiovascular, neurodegenerative and various sorts of cancers (Wong *et al.,* 2006). Moreover, availability of fibre content and bound water help human body against diabetic, histaminic, carcinogenic, hypolipidemic (lipid-lowering drugs) (LLD) and bacterial disorders (Souzan and Abd *et al.*, 2007). Antioxidant property of such vegetables is also an important contributing factor to overcome such deficiencies and disease tolerance (Hait-Darshan *et al.,* 2009).

Spinach is a commonly cultivated leafy green vegetable around the globe serving as an important raw material for food industry. This has been reported to possess significant antioxidant activity (Hunter and Fletcher, 2002) including many water soluble polyphenolic compunds (Edenharder *et al.,* 2001), flavonoids, ascorbic acid and tocopherols (El-Qudah, 2008). Antioxidant potential of leafy vegetables is found enhanced in response to a variety of plant growth regulators (PGRs) (Cecilia *et al*., 2011). Attempts to get bulk of leaf yield from these vegetables attract attention to find out impact of PGRs on leaf quality. However, sound reporting for nutritive status of vegetables in response to such treatments is felt lacking.

The determination of antioxidant activity of spinach is not a new practice. A lot of work has been done in this regard. However, the data regarding implementation of PGR in growth of spinach is yet insufficient. Therefore, the conducted research was based on assessment of antioxidants present in leaves of spinach (*Spinacia oleracea*) in response to various organic as well as inorganic PGRs.

MATERIALS AND METHODS

***Sample*:** The seeds of the vegetable, *Spinacia oleracea* were collected from Ayub Agricultural Research Institute (AARI),

Faisalabad. The vegetable was grown in the pots (Thermopore plastic, 4.5 x 7.5 inches) placed at the vegetable section of Institute of Horticultural Sciences, University of Agriculture, Faisalabad. The plants growth regulators (PGRs) selected were Bio-fertilizer (*BF*), Humic acid (*HA*), *Moringa* leaf extract (*MLE*) and 6-Benzyl amino purine (*6-BAP*). Triplicate samples were grown in order to collect sufficient data for the fulfillment of the statistical work. PGRs were applied according to the protocol mentioned in Table 1.

***Chemicals and reagents*:** The purchase of Catechin, Folin-Ciocalteu reagent, Gallic acid, Linoleic acid, 6-Benzyl amino purine was made from Sigma Chemical Company (St. Louis, MO, USA). The remaining reagents and chemicals were procured from E. Merck (Darmstadt, Germany).

***Extraction*:** The extraction process was accomplished following the procedure as proposed by Sultana *et al*. (2007). The leaves samples were collected through destructive sampling, washed with running tap water followed by distilled water, sun-dried and crushed in a grinding mill (Tector–Cemotec 1090 sample mill, Hognas, Sweden). Ground leaf samples (20g) were extracted with 200mL of aqueous methanol (80% v/v) in an orbital shaker for about 8-10h at ambient temperature (25°C). The filtration was accomplished to separate the extract from the insoluble portion using Whattman No. 1 filter paper. Additionally one to two solvent runs was performed in order to get complete separation of extracts. The filtrate was then concentrated under conditions of reduced pressure in a rotary evaporator at 45°C and preserved in a refrigerator for further group of analyses**.**

Determination of total phenolic content (TPC): The concentration of total phenolics was examined spectrophotometrically employing the Folin–Ciocalteu reagent protocol as reported by Chaovanalikit and Wrolstad (2004). In this assay, dry mass of leaves extract (50 mg) was mingled first with 0.5 mL of Folin-Ciocalteu reagent and then with 7.5 mL of deionized water. The whole mixture was placed at room temperature for about 10 min. After this, 1.5 mL of 20% sodium carbonate was also added. The mixture was then heated in a water bath at 40°C for 20 min and then it was cooled in an ice bath. At the end, the absorbance was read at 755 nm with a spectrophotometer (Hitachi U-2001, model 121-0032). Amount of TP was calculated by means of designing a calibration curve of gallic acid (10- 100 ppm) (R^2= 0.9986). The TPC results were shown with reference to gallic acid equivalents (GAE) per dry matter. Three replicates were assessed to get data in average.

***Reducing power assay*:** The reducing power assay for extracts was performed according to the method as demonstrated by Yen *et al.* (2000), with slight modification. In this procedure, equal volume of all leaf extracts (2.5-10.0 mg/mL) was brought separately into sodium phosphate buffer (5.0 mL, 0.2 M, pH 6.6) along with potassium ferricyanide (5.0 mL, 1.0 %). After this, the incubation of whole mixture was done at 50°C for about 20 min. After addition of trichloroacetic acid (5.0 mL, 10 %), the mixture was centrifuged at 980×*g*at 5°C in a refrigerated centrifuge (CHM-17; Kokusan Denki, Tokyo, Japan) for at least 20 min. The upper layer (5.0 mL) of the solution was then diluted with distilled water (5.0 mL) along with ferric chloride (1.0 mL, 0.1%). Finally absorbance was measured at 700 nm (Hitachi U-2001, model 121-0032). The experiment was performed thrice and results then averaged.

***DPPH Assay*:** The antioxidant potential of the methanolic extract of spinach leaves were assessed following the procedure as reported by Bozin *et al*. (2006) with slight modification, exploring their scavenging dynamics using 2,2-diphenyl-1- picrylhydrazyl stable radical. The samples of varying concentrations (0.01-1000 mg/mL) were brought in contact to equal volume of 90 μM DPPH solutions and

Table 1. Plant growth regulators and their mode of application

Treatment PGR	Mode of application	Concentration applied
T_0 = Control	———	———
T_1 = Humic Acid	Foliar application	10%, 25%, 30%
T_2 = *Moringa*Leaf Extract (*MLE*)	Foliar application (1:30 times diluted with water)	Fresh, 1 Month Old, 2 Month Old 25 ppm, 50 ppm, 75 ppm
T_3 = 6-Benzyl amino purine (*6- BAP*)	Foliar application	
T_4 = Biofertilizer	Seed priming	Soaking in slurry for ½ h and drying
T_5 = Humic Acid	Seed priming	6h, 9h and 12h, dipping with seed and solution ratio (1:5), respectively

- Two set of treatment runs were used *i.e.* the absence and presence of soil applied urea
- Fertilizers were mixed in soil/ applied before sowing
- Foliar application was applied after 15 days starting 25-30 DAE @ 160 L/hectare

the final volume was made 4 mL with methyl alcohol (95%). The sample without extract served the blank. The absorbance of the blank and all the extracts solutions were read at 515 nm at room temperature which was followed by decrease in absorbance value using a spectrophotometer (Hitachi U-2001, model 121-0032)**.**

The percent radical scavenging activity was measured by the following formula

Percent Inhibition =100- (A_{Blank}– A_{Sample}/A_{Blank})

Where A_{Blank} is the absorbance of the control not including the test compounds, and A_{Sample} denotes the absorbance of the extracts to be tested.IC_{50}, the concentration of extracts required to neutralize 50% solution of DPPH radicals, were calculated by plotting the percent inhibition versus extract concentration.

***Statistical analysis*:** The samples of all the crude methanolic extracts were statistically analyzed. All samples were run in triplicate and data was reported as mean ± SD. Data was analyzed using one way ANOVA at 5% significance level.

RESULTS AND DISCUSSION

***Yield of extract*:** Extraction strategy is used to recover a list of desired bio-actives from plants. It is employed in varying conditions of experimental parameters *i.e.* chemistry of material to be extracted, pH, temperature and choice of solventetc (Pinelo *et al.*, 2005). However, present study has denoted the role varying PGRs in bringing differences in extract yield. The percent yield of different methanolic extracts of spinach leaves was observed in the range of 16.75-36.00 g/100g of dry matter as shown in Table 2. The greater extract yield was shown by fresh *MLE* treated spinach sample and minimum by control, both in the absence and presence of urea. Maximum magnitude of extract yield from samples exposed to foliar treatment in the absence of urea was observed by *MLE* followed by *6-BAP* 75 ppm and *HA* 10% treated samples and least by *HA* 30%. The similar trend was also observed in soil conditioned with urea.

Whereas, maximum extraction yield from samples subjected to seed priming without urea was detected by the sample treated with *HA* 6h (P) followed by *HA* 9h (P) and *HA* 12h (P). The bio-fertilizer showed low yield. Similar results were also observed in urea presence in soil.

Table 2. Extract yield and Total phenolic contents (TPC) of spinach influenced by different plant growth regulators

Treatments	Percent Yield (g/100g DM)		Total Phenolic Content (mg GAE/gDM)	
	A_X	B_Y	A_X	B_Y
Control	18.81 ± 0.24^{e}	16.75 ± 0.28^{d}	5.312 ± 0.091^{bc}	4.678 ± 0.720^{d}
HA 10%	$30.25\pm0.19_{A}^{ab}$	$21.67\pm0.24_{A}^{bc}$	$9.969\pm0.054_{A}^{b}$	$8.709\pm0.121_{A}^{ab}$
HA 25%	$22.90\pm0.34_{B}^{d}$	$20.41\pm0.29_{B}^{c}$	$7.371\pm0.089_{B}^{b}$	$8.055\pm0.131_{B}^{b}$
HA 30%	$18.91\pm0.27_{C}^{e}$	$17.81\pm0.32_{C}^{d}$	$6.849\pm0.104_{C}^{bc}$	$7.653\pm0.196_{C}^{b}$
MLE fresh	$36.00\pm0.53_{A}^{a}$	$26.78\pm0.48_{A}^{a}$	$13.236\pm0.215_{A}^{a}$	$10.295\pm0.152_{A}^{a}$
MLE 1MO	$29.80\pm0.45_{B}^{b}$	$19.22\pm0.46_{B}^{c}$	$8.816\pm0.131_{B}^{b}$	$6.171\pm0.153_{B}^{c}$
MLE 2MO	$22.29\pm0.33_{C}^{d}$	$17.02\pm0.35_{C}^{d}$	$6.046\pm0.081_{C}^{bc}$	$4.686\pm0.064_{C}^{d}$
6-BAP 25ppm	$28.24\pm0.43_{C}^{b}$	$16.94\pm0.46_{C}^{d}$	$5.465\pm0.072_{C}^{c}$	$4.336\pm0.082_{C}^{d}$
6-BAP 50ppm	$31.20\pm0.46_{B}^{ab}$	$19.37\pm0.44_{B}^{c}$	$7.124\pm0.086_{B}^{bc}$	$7.287\pm0.114_{B}^{bc}$
6-BAP 75ppm	$34.40\pm0.51_{A}^{a}$	$26.25\pm0.49_{A}^{a}$	$10.393\pm0.123_{A}^{b}$	$8.820\pm0.091_{A}^{b}$
BF (P)	18.80 ± 0.28^{e}	19.41 ± 0.27^{c}	6.831 ± 0.112^{bc}	5.849 ± 0.052^{c}
HA (P) 6h	$25.52\pm0.38_{A}^{c}$	$23.67\pm0.35_{A}^{b}$	$9.302\pm 0.144_{A}^{b}$	$6.392\pm0.123_{A}^{bc}$
HA (P) 9h	$24.65\pm0.36_{B}^{cd}$	$22.38\pm0.29_{B}^{b}$	$8.142\pm 0.618_{B}^{b}$	$6.214\pm0.079_{B}^{bc}$
HA (P) 12h	$22.62\pm0.34_{C}^{d}$	$20.70\pm0.23_{C}^{c}$	$5.308\pm 0.112_{C}^{b}$	$5.493\pm0.171_{C}^{c}$

Values are means ± SD, samples of each plant material analyzed individually in triplicate (P <0.05)

HA=Humic acid; *MLE*=*Moringa*leaf extract*; 6-BAP* =6-Benzyl amino purine; *BF*=Bio-fertilizer; P=Seed Priming; A = Presence of urea; B = Absence of urea, while X and Y letters in the subscripts within the row showed their significant effects.

Small alphabets in superscripts within the column showed significant difference among treatments while capital alphabet in subscript within the column showed significant differences among different concentrations of individual PGR.

In overall comparison, samples treated with foliar supplements and grown in soil without urea demonstrated more extraction yield comparatively. The difference in the extract yield among samples might be due to the nature of PGRs to which the samples were subjected. Comparatively better results with *MLE* might be due to the reason that it is affluent source of zeatin, humic acid, pro-vitamin A, vitamins B, C and E, minerals (particularly iron) and some S-containing amino acids (methionine and cysteine) than rest of treatments (Foidle *et al.,* 2001; Nagar *et al.,* 2006) and hence served as good plant growth regulator (Nouman *et al.,* 2012).

Cytokinins and their derivatives (*e.g.6-BAP*) are being extensively employed exogenously as active PGRs. In their presence, endogenous level of cytokinins in plants is enhanced. They also effectively encourage many multi-developmental mechanisms which occur in day light (Merillon *et al.,* 1991). Humic substances (HS) have important organic constituents which assure maximum benefits to growing plant. Their indirect influences include improvement of various chemical, physical and biological constituents. The diluted humic fractions have been proved to show maximum activity (Nardi *et al*., 2007). The other benefits associated with HS includes increased microbial nutrition (Clapp *et al*., 2001), enhanced rate of respiration and enzyme activities and stimulation of hormonal actions along with energy associations (Nardi *et al*., 2007; Zancani *et al*., 2009).

Increased extract yield received from *BF* against control in seed priming treatments present the beneficial association of microorganism with soil particles resulting better supply of nitrogen and phosphorus from soil to the growing plant. The gained momentum hence cut down the toxicity caused by environmental pollutants. Therefore, stability of plant is highly boosted in view of yield and nutritional status.

Present study has neglected the use of urea fertilizer for getting good percentage yield. The reason might be associated with relevant soil toxicology because of some factors (physical/chemical) which result in disagreeable effects on composition of soil *i.e.* degradation, acidity and nutrient leaching etc. (Agbede, 2010).

***Total phenolic content (TPC)*:** Plant phenolic compounds are highly appreciated in daily human intakefor possession of antioxidant ability. Agronomic conditions,nature of test crop and medium of solvent extraction influence amount of phenolic compounds in varying plant parts (Naseri and Sharafzadeh, 2013). Total phenolic contents of all the crude methanolic extracts of spinach were evaluated as mg GAE/g of dry matter. The samples subjected to different PGRs showed significant differences in their total phenolic content as mentioned in Table 2. The overall observed TPC ranged 4.678-13.236 mg GAE /g of dry matter. Among foliar treatments in the absence of urea, the sample treated with fresh *MLE* exhibited the maximum value and *6-BAP* 25ppm the least. The similar trend was seen in the presence of soil applied urea.

While studying seed priming effect in the presence as well as absence of urea, the sample primed with *HA* 6h (P) possessed the maximum TPC and *HA* 12h (P) haunted the bottom value. The drift perceived is described below:

HA 6h (P) >*HA* 9h (P) > bio-fertilizer >*HA* 12h (P)

Results showed that in the seed priming mode of PGR application, *HA* increased TPC in the presence and absence of urea. Humic substances are reported to help in nitrate uptake from soil and facilitate water use efficiency. In addition, they serve to enhance various microbial and enzymatic processes (Ulukan, 2008).

Overall comparison is that the samples with foliar conducts possessed more TPC as compared to those of seed priming. The absence of urea in soil exhibited higher TPC comparatively. The logic might be deduced that the rate of photosynthesis remained continuous and enhanced due to appropriate and timely supply of nutrients via application of plant growth regulators (Nardi *et al.,* 2002). The higher value of TPC shown by *MLE* treated sample might be attributed to the presence of multi-nutritional components along with auxins, cytokinins etc. in the *Moringa* leaf extract. The phytochemicals present in *MLE* are a great precursor of bioactive components, (leutin, zeatin, humic acid, micronutrients etc.) loaded in antibiotic, anticancer and antioxidant potential (Bellostas *et al.,* 2010).

The higher TPC drawn from cytokinins derivatives against control is linked with their signalling actions through which they control cytokinesis along-with growth and other developmental mechanisms within plant cells. Moreover, resultant cell differentiation at increased rate facilitates the provision of more sites for polyphenols production. The higher TPC observed in samples treated with *HA* versus control is attributed to direct effect that accelerates nutrient uptake along with relevance penetration into cell membranes. Present study has also evaluated that effective concentrations of PGRs provide stimulatory effects for producing plants secondary metabolites including polyphenols and these findings are similar to those reported by Clapp *et al*. (2001).

The observed difference in TPC received from *BF* treatment against control is associated with certain induced mechanisms which result in producing various secondary metabolites including antibiotics and hormone inducing ability. Besides this, antagonistic activity like uptake of some pathogens is also prevented (Boraste *et al.,* 2009).

***Reducing power assay*:** Reducing potential is frequently linked with the ability of a substance retaining antioxidant potential to act as good electron donor (Yildirim *et al.,* 2000; Dorman *et al.,* 2003). There are various available reports about the existence of correlation between reducing ability and antioxidant potency of botanical extracts. During evaluation of reducing potential, all the methanolic extracts

showed increasing reducing trend with respect to increase in concentration. The reducing values were perceived in the range of 0.351-1.874 at 10.0 mg/mL extract concentration as shown in Table 3. The maximum value was exhibited by the sample treated with bio-fertilizer in the urea fitted soil. All the experimental extracts showed significant differences in reducing abilities affected by different treatments. Among foliar application of PGRs in the soil deprived of urea, the higher reducing ability was demonstrated by *MLE* fresh and the least by *6-BAP* 25ppm treated sample.

While assessing the reducing potential of samples subjected to seed priming, bio-fertilizer yielded the maximum reduction potential with and without soil urea supplementation. Overall, samples subjected to seed priming dominated in the reducing ability in comparison to those of foliar application of PGRs. The reason might be the difference in rate of fertilizer use efficiency and in turn uptake efficacy of essential soil nutrients in presence of variety of PGRs (Hirel *et al.,* 2011). Bio-fertilizers are reported as eco-friendly and contain beneficial composition of microorganisms that help to detoxify the pest borne diseases, have the ability to fix atmospheric nitrogen, decompose organic wastes and make utilization of essential plant nutrients and important enzymes with specificity to boost up plant growth via production of plant growth regulating substances (Singh *et al.,* 2011). They facilitate the renewal of soil fertility through plentiful supply of beneficial microorganisms and play a key role in the enhancement of antioxidant potential of plants. Hanan *et al*. (2011) reported the efficacy of bio-fertilizer applied on soybean plants alone and in combination with inorganic media and found that the maximum antioxidant value was exhibited by bio-fertilizer + inorganic media.

***DPPH Radical Scavenging Assay*:** DPPH assay stands as one of the reliable methods in order to screen the plant extracts for their antioxidant potential (Sanjukta and Ghosh, 2012). The DPPH free radical was used to evaluate the free radical scavenging activity of bioactive compounds inmethanolic extracts of spinach. The ability of plant extract to scavenge the free radical depends on the dose utilized. The percentage inhibition increases with increasing concentration of extract which is depicted by decrease in absorbance value. The antioxidants present in the spinach leaf extract capture the free radical via donation of hydrogen which causes the change in color of solution from purple to yellow (Derwich *et al.,* 2011). The structure of the

Table 3. Reducing power and IC_{50} value of spinach influenced by different plant growth regulators

Treatment	Reducing Power (10mg/mL)		IC_{50} Value (μg/mL)	
	A_X	B_Y	A_X	B_Y
Control	0.351 ± 0.006^{e}	0.481 ± 0.01^{e}	1.063 ± 0.016^{e}	0.946 ± 0.014^{d}
HA 10%	$1.011\pm0.013_{A}^{bc}$	$1.348\pm0.032_{A}^{b}$	$0.625\pm0.011_{A}^{b}$	$0.672\pm0.013_{A}^{ab}$
HA 25%	$0.927\pm0.011_{B}^{bc}$	$1.118\pm0.021_{B}^{bc}$	$0.847\pm0.012_{B}^{cd}$	$0.806\pm0.015_{B}^{bc}$
HA 30%	$0.795\pm0.015_{C}^{bc}$	$1.097\pm0.030_{C}^{bc}$	$0.909\pm0.013_{C}^{d}$	$0.926\pm0.017_{C}^{d}$
MLE fresh	$1.257\pm0.017_{A}^{ab}$	$1.547\pm0.025_{A}^{ab}$	$0.499\pm0.008_{A}^{a}$	$0.619\pm0.011_{A}^{a}$
MLE 1MO	$0.751\pm0.016_{B}^{cd}$	$0.721\pm0.034_{B}^{d}$	$0.658\pm0.012_{B}^{b}$	$0.733\pm0.012_{B}^{ab}$
MLE 2MO	$0.691\pm0.022_{C}^{cd}$	$0.567\pm0.043_{C}^{de}$	$0.694\pm0.010_{C}^{b}$	$0.849\pm0.012_{C}^{bc}$
6-BAP 25ppm	$0.564\pm0.008_{C}^{d}$	$0.814\pm0.015_{C}^{cd}$	$0.684\pm0.011_{C}^{b}$	$0.699\pm0.013_{C}^{ab}$
6-BAP 50ppm	$0.884\pm0.015_{B}^{bc}$	$1.237\pm0.029_{B}^{b}$	$0.667\pm0.009_{B}^{b}$	$0.685\pm0.007_{B}^{ab}$
6-BAP 75ppm	$1.162\pm0.018_{A}^{a}$	$1.421\pm0.034_{A}^{ab}$	$0.523\pm0.010_{A}^{a}$	$0.627\pm0.017_{A}^{a}$
BF (P)	1.445 ± 0.025^{a}	1.874 ± 0.038^{a}	0.767 ± 0.010^{b}	$0.792\pm0.013_{A}^{bc}$
HA (P) 6h	$0.794\pm0.014_{C}^{bc}$	$0.859\pm0.026_{C}^{cd}$	$0.632\pm0.009_{A}^{b}$	$0.676\pm0.007_{A}^{ab}$
HA (P) 9h	$0.890\pm0.010_{B}^{bc}$	$1.063\pm0.039_{B}^{bc}$	$0.806\pm0.011_{B}^{bc}$	$0.886\pm0.017_{B}^{cd}$
HA (P) 12h	$1.175\pm0.021_{A}^{a}$	$1.149\pm0.019_{A}^{bc}$	$0.819\pm0.012_{C}^{bc}$	$0.919\pm0.015_{C}^{cd}$

Values are means ± SD, samples of each plant material analyzed individually in triplicate (P <0.05)

HA=Humic acid; *MLE=Moringa*leaf extract*; 6-BAP* =6-Benzyl amino purine; *BF*=Bio-fertilizer; P=Seed Priming; IC=Inhibitory concentration; A = Presence of urea; B = Absence of urea, while X and Y letters in the subscripts within the row showed their significant effects.

Small alphabets in superscripts within the column showed significant difference among treatments while capital alphabet in subscript within the column showed significant differences among different concentrations of individual PGR.

antioxidant molecule in extract is also important for its ability to donate protons (Silva *et al.,* 2005). The extract concentration required to inhibit 50% of the amount of DPPH utilized denotes the IC_{50} (Atanassova *et al.,* 2011). The observed range of IC_{50} values was 0.499-1.063 μg/mL concentration with maximum value shown by control as denoted in Table 3. Amongst various foliar treatments, the sample influenced by fresh *MLE* and *HA* 30% showed the lowest and the highest values, respectively.

During assessment of seed priming results with and without addition of urea in soil, the lowest IC_{50} value was seen in case of *HA* 6h (P) followed by bio-fertilizer, *HA* 9h (P) and *HA* 12h (P).

Overall comparison elaborated that urea absence in addition to foliar application of PGRs showed good radical scavenging activity as reflected by lowering of the IC_{50} value. Olajire and Azeez (2011) reported percent radical inhibition value of methanolic leaves extract of spinach as 57.3±0.49 which falls in the range of current study. Exogenous application increment the endogenous nutrient profile (Li *et al.,* 2005), increase plant stress against the environmental stress (Kim *et al.,* 2009) and the yield per unit area by stimulating plant vegetative growth (Ibrahim *et al.,* 2007). The reason might be linked to the production of substantial amount of different compounds in fresh *MLE* exhibiting good antioxidant potential with confirmation of high nutrients, low oxalate and glucosinolate content presence (Perry *et al.,* 1999).

***Conclusions*:** The results obtained from spinach samples subjected to *in vitro* assays grown against organic and inorganic media proved as database for the exhibition of higher antioxidant activity in comparison to control. Amongst different PGRs, fresh *MLE* treated sample gave enhanced antioxidant action followed by *6-BAP* and *HA*. Overall, all PGRs exhibited positive response on the antioxidant potential of spinach making it more potent food and medicinal source for both developed and developing nations.

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