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Modification in Antioxidant Potential of *Coriandum sativum* Using Selected Plant Growth Regulators

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Plant growth regulators were employed on coriander (*Coriandum sativum*) to evaluate their possible effects on total phenolic content and antioxidant attributes. Applied plant growth regulators were humic acid, *Moringa* leaf extract, 6-benzyl amino purine in foliar and bio-fertilizer and humic acid in seed priming mode at varying dose rate. The treatments were applied in two soil environment, urea presence (experiment A) and urea absence (experiment B). The observed total phenolic content variations ranged as: 2.929-10.156 mg GAE/g of DM, reducing power estimated as: 0.613-1.952 (at 10.00 mg/mL extract concentration) while DPPH$^{\bullet}$ scavenging ability as: 0.354-0.979 µg/mL. *Moringa* leaf extract excelled in total phenolic content and DPPH$^{\bullet}$ assay, while, bio-fertilizer remained dominated in reducing power assay. Overall experiment A showed improved results than experiment B. Antioxidant activity was found to be enhanced significantly ($P < 0.05$) with exogenously applied treatments of plant growth regulators. However, *Moringa* leaf extract showed comparatively improved effects.

Keywords: Coriander leaf extracts, Humic acid, 6-Benzyl amino purine, *Moringa* leaf extract, Bio-fertilizer, Antioxidant activity.

INTRODUCTION

The role of organic farming cannot be neglected from view of quality crop production as the running unhealthy strategies commonly employed in agriculture are destabilizing farming systems[1]. Now-a-days, the goal to access valuable crop yield is accomplished by practicing non-destructive and economical agricultural practices which aim to ensure crop productivity. In recent years, it is a common practice to implement plant growth regulating compounds which might include growth hormones, inorganic fertilization and plants extracts *etc*. These growth enhancers are employed in various exogenous modes *i.e*. seed priming/foliar conducts, seed/soil application *etc*[2]. Such valuable substances benefit plants promoting their growth and proper differentiation/development while applied even at low concentration/dose. Their higher concentrations might lead to inhibit growth effects[3]. Therefore, it is preferred to avoid use of synthetic soil/foliar additives, preservatives, synthetic fertilizers and pesticides to avoid environmental contamination and maintaining appropriate farming strategies[4].

Plant growth regulators (PGRs) of natural/synthetic origin are gaining continued appraisal in agriculture farming systems since long. The practice of implementation of PGRs in agriculture is ensuring promising nutrient status besides improved yield of plants. The exogenously applied PGRs are also reported to show promising effects through appropriate modulation of concerned physiological, metabolic and other biochemical responses including cell division and differentiation, enlargement, germination, seed dormancy, leaf senescence and organogenesis *etc*[5]. Moreover, they also develop adaptations against the changing situations of climate during certain stresses and hence regulate the nutrient and water flow between source and its sink[6].

The health of human beings is reflected from not only the quality but also the quantity of the food taken. Vegetables express fresh and edible food portions in raw, partial and fully cooked manner. These treasured features are required to maintain biological, psychological and physiological machinery inside body. They also make body's immune system more viable and strong as decorated with combination of antioxidants, dietary fibre, minerals and vitamins. They also contain less fats and proteins[7].

Leafy green vegetables like coriander, spinach, mint, cabbage, broccoli *etc*. are routinely incorporated in daily diet. Moreover, they reportedly contribute to reduce varying health deficiencies and disorders (mainly cardiovascular, neurodegenerative, inflammation and breast cancers *etc*.) which are created as severe results of active oxygen species (AOS). Additionally, access of fiber along with bound water makes sure defending body against certain histaminic, diabetic, carcinogenic, microbial and hypolipidemic disorders. Antioxidants received from coriander and other leafy vegetables are considered as an important contributor to combat such deficiencies and increase resistance towards disease[8].

The present study was designed to appreciate the role of selected PGRs showed towards possible effects on antioxidant potential of coriander leaves. The outcomes of study might intend to reduce environmental destabilization and economically reducing gap between need and demand.

EXPERIMENTAL

The coriander, (*Coriandum sativum*) plant sample were grown-up in thermo-pore pots (dimensions: 4.5" × 7.5"), placed at vegetable section, Institute of Horticultural Sciences, University of Agriculture, Faisalabad. The selected PGRs were *Moringa* leaf extract (MLE), humic acid (HA), 6-benzyl amino purine (6-BAP) and bio-fertilizer (BF). Coriander leaf samples were subjected to two sprays in foliar mode, while seed priming agents were applied before sowing. The mature leaf samples were collected at peak maturity stage. The applications of PGRs according to developed procedure are designed in Table-1.

6-Benzyl amino purine, humic acid, gallic acid and Folin-Ciocalteu reagent were purchased from Sigma Chemicals (St. Louis, MO, USA). However, rest of chemicals and reagents were taken from Merck (Darmstadt, Germany).

Sun-dried leaves of all samples (10 g each) were chopped and subjected to extraction with aqueous methanol (80 % v/v) in the orbital shaker (Gallenkamp, UK) till 8-9 h at room temperature. Further, filtration of extracts was accomplished using filter paper (Whatman No. 1); after it they were concentrated in the rotary evaporator in reduced pressure at 40-45 °C. Concentrated viscous extracts were kept at 4 °C till analyzed[9].

A previously reported Chaovanalikit and Wrolstad procedure[10] was adopted to determine total phenolic content. Concisely, 0.5 mL of folin-ciocalteu reagent was added individually in 50 mg of each methanolic extract. After it, 7.5 mL of de-ionized water was added in each mixture. After a duration of 10 min, 1.5 mL of sodium carbonate (20 % w/v) was also added. All the extract mixtures were then heated at 40-45 °C on a water-bath for about 20 min followed by immediate ice cooling. Absorbance was taken at 755 nm with the help of spectrophotometer. Amount of total phenolic content were calculated using gallic acid calibration curve ranging from 10-100 ppm ($R^2 = 0.9986$) and results were expressed as mg gallic acid equivalents (GAE) mg/g of DM.

Antioxidant attributes of all extracts were evaluated *via* a list of antioxidant assays.

Reducing ability of all extracts was investigated using method implemented by Yen *et al.*[11]. All concentrated viscous extracts (2.5-10 mg/mL) were put to be analyzed in view of determination of their reducing power. Each extract was supplemented with sodium phosphate buffer (0.5 mL, 0.2 M, pH 6.6). After that, all extract mixtures were subjected to incubation at 50 °C for about 20 min after adding 0.5 mL of potassium ferricyanide (1.0 %) into each extract. Then 5 mL of trichloro acetic acid (10.0 %) was added in all mixtures and they were centrifuged for 8-10 min at 980 ×g. The upper phase was separated from the solution and diluted with distilled water (5 mL) and 1 mL of ferric chloride (0.1 %). Absorbance was read with the spectrophotometer at 700 nm.

Bozin *et al.*[12] method was employed in order to analyze DPPH free radical scavenging capability of the extracts. Entire extracts (0.01 to 10.00 mg/mL) were scrutinized to determine their free radical scavenging potential. To each extract, 1 mL of 90 µM DPPH solution was incorporated and the volume was made upto 4 mL by using 95 % methanol. All the extracts mixtures were placed for about 1h at room temperature. Absorbance was noted at 515 nm in spectrophotometer. Inhibition of DPPH free radical (%) by extracts was taken as follows.

$$\% \text{ Inhibition} = \frac{A(\text{Blank}) - A(\text{Sample})}{A(\text{Blank})} \times 100$$

Results were reported as mean ± S.D of threereplicates. Data was analyzed by one way analysis of variance (ANOVA) and Duncan's Multiple Range (DMR) test to observe difference of means using Minitab statistical software (version 13) at 95 % confidence level.

RESULTS AND DISCUSSION

The percent extract yield of coriander samples ranged 6.54-26.67 g/100 g DM (Table-2). Although all samples gave higher yield than control, but, the highest value was seen in sample treated with *Moringa* leaf extract-A in experiment A. Overall, higher values were received among foliar conducts in the following order: *Moringa* leaf extract > 6-benzyl amino purine > humic acid. Whereas, selected seed priming conducts (P) recorded the trend as: humic acid 6 h > bio-fertilizer > humic acid 9 h > humic acid 12 h in both experiment A and B.

The essence drawn from whole comparison showed the dominance of foliar conducts in both experiments (A and B). The greater magnitude received from *Moringa* leaf extract matters as blessed with appreciative bio-actives like vitamins (A, B, C, E); minerals (mainly Fe), amino acids (having sulphur groups), humic acid and cytokinins (specifically zeatin). These attributes authenticate it as a potent PGR[13]. There might exist the possibility of denaturing of components in *Moringa* leaf extract as it becomes old. Results showed that effect of humic

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 % (HA-1), 25 % (HA-2), 30 % (HA-3)
T_2 = Moringa leaf extract	Foliar application (1:30 times diluted with water)	Fresh (MLE-1), 1 Month Old (MLE-2), 2 Month Old (MLE-3)
T_3 = 6-Benzyl amino purine	Foliar application	25 ppm (6-BAP-1), 50 ppm (6-BAP-2), 75 ppm (6-BAP-3)
T_4 = Bio-fertilizer	Seed priming (P)	Soaking in slurry for ½ h and drying
T_5 = Humic acid	Seed priming (P)	HA 6 h, HA 9 h and HA 12 h, dipping with seed and solution ratio (1:5), respectively

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

TABLE-2
EXTRACT YIELD AND TOTAL PHENOLIC CONTENTS (TPC) OF CORIANDER LEAF EXTRACTS INFLUENCED BY DIFFERENT PLANT GROWTH REGULATORS

Treatments	Percent Yield (g/100g DM)		Total Phenolic Content (mg GAE/g of Extract)	
	A_X	B_Y	A_X	B_Y
Control	10.06 ± 0.24^{e}	6.54 ± 0.28^{d}	3.841 ± 0.091^{e}	2.929 ± 0.720^{d}
HA-1	$21.22 \pm 0.19_{A}^{b}$	$12.32 \pm 0.24_{A}^{ab}$	$6.850 \pm 0.054_{A}^{c}$	$6.678 \pm 0.121_{A}^{b}$
HA-2	$12.49 \pm 0.34_{B}^{d}$	$10.23 \pm 0.29_{B}^{bc}$	$4.592 \pm 0.089_{B}^{d}$	$3.765 \pm 0.131_{B}^{cd}$
HA-3	$12.21 \pm 0.27_{B}^{d}$	$8.28 \pm 0.32_{C}^{c}$	$4.051 \pm 0.104_{B}^{de}$	$3.684 \pm 0.196_{B}^{cd}$
MLE-1	$26.67 \pm 0.53_{A}^{a}$	$13.65 \pm 0.48_{A}^{a}$	$10.156 \pm 0.215_{A}^{a}$	$8.674 \pm 0.152_{A}^{a}$
MLE-2	$14.58 \pm 0.45_{B}^{cd}$	$10.78 \pm 0.46_{B}^{bc}$	$6.390 \pm 0.131_{B}^{c}$	$6.578 \pm 0.153_{B}^{b}$
MLE-3	$12.67 \pm 0.33_{B}^{d}$	$9.17 \pm 0.35_{BC}^{c}$	$6.198 \pm 0.081_{B}^{cd}$	$4.743 \pm 0.064_{C}^{c}$
6-BAP-1	$12.32 \pm 0.43_{B}^{d}$	$9.49 \pm 0.46_{B}^{c}$	$5.601 \pm 0.072_{C}^{d}$	$4.078 \pm 0.082_{BC}^{cd}$
6-BAP-2	$15.53 \pm 0.46_{B}^{cd}$	$10.98 \pm 0.44_{B}^{b}$	$6.793 \pm 0.086_{B}^{c}$	$6.180 \pm 0.114_{A}^{bc}$
6-BAP-3	$23.48 \pm 0.51_{A}^{b}$	$12.72 \pm 0.49_{A}^{ab}$	$7.737 \pm 0.123_{A}^{bc}$	$7.981 \pm 0.091_{A}^{ab}$
BF(P)	20.28 ± 0.28^{bc}	10.28 ± 0.27^{bc}	7.848 ± 0.112^{bc}	3.920 ± 0.052^{cd}
HA 6 h (P)	$21.61 \pm 0.38_{A}^{b}$	$11.01 \pm 0.35_{A}^{b}$	$8.503 \pm 0.144_{A}^{bc}$	$4.429 \pm 0.123_{A}^{c}$
HA 9 h (P)	$15.83 \pm 0.36_{B}^{c}$	$9.81 \pm 0.29_{B}^{c}$	$5.384 \pm 0.618_{B}^{d}$	$4.136 \pm 0.079_{A}^{cd}$
HA 12 h (P)	$11.93 \pm 0.34_{c}^{d}$	$7.80 \pm 0.23_{C}^{cd}$	$4.361 \pm 0.112_{B}^{de}$	$3.878 \pm 0.171_{B}^{cd}$

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; A = Presence of urea; B = Absence of urea, while X and Y letters in the subscripts within the row showed significant difference between experiments. 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

acid decreased with its increasing concentration. The diluted concentration is reported as active fraction. On its way to shoot, they feed microbes, enhance respiration and stimulate enzyme activities and corresponding hormonal actions[14]. The low yield received from experiment B might be associated with increased impact of eco-against factors (chemical/physical soil degradation, soil acidity and nutrient leaching *etc*.) while comparing with natural PGRs possessing eco-friendly nature[15].

Total phenolic content of coriander's leaf samples as undergone by different PGRs treatments are shown in Table-2. Significant differences ($P < 0.05$) were seen in all treated samples. total phenolic content varied 2.929-10.156 mg GAE/g of DM. In the experiment A, among all PGRs concentrations applied as foliar treatment, the higher value was estimated for *Moringa* leaf extract-1 treated sample and the lower by humic acid-3. The perceived tendency of total phenolic content regarding foliar effectiveness PGRs application was shown below:

Moringa leaf extract-1 > 6-benzyl amino purine-3 > humic acid-1 > 6-benzyl amino purine-2 > *Moringa* leaf extract-2 > *Moringa* leaf extract-3 > 6-benzyl amino purine-1 > humic acid-2 > humic acid-3 (experiment A)

The similar trend was also recorded for experiment B. For evaluating effectiveness of seed priming treatments, the maximum value of total phenolic content was shown by humic acid 6 h followed by bio-fertilizer treated samples. However, the lower limit was attained by humic acid 12 h.

Results showed that all PGRs produced promising results than control. The reason for higher total phenolic content from *Moringa* leaf extract treated sample might be influenced with higher nutritive concern of *Moringa* leaves. This, in addition, behaves as marvelous bio-pesticide along with valuable foliar supplement. Furthermore, it is highly emphasized with appropriate range of minerals including Na, Mn, Mg, K, P, Ca, S, Zn, Fe, Cu, *etc*.[16]; vitamins like A, B complex, C and E; and many growth regulating constituents like leutin, auxin, humic acid and zeatin *etc*. The *Moringa* leaf extract is also in-built with valuable flavonoids *i.e*. kaempherol and quercitin *etc*. These aspects therefore assist *Moringa* leaves to serve as blessed natural antioxidant and growth enhancer[17]. Valuable phenolics in 6-benzyl amino purine treatments could possibly be credited to its varying physiological attributes. They are also extensively reported exogenous PGRs and known to elevate the endogenous cytokinin level of plants. The enhanced endogenous level is seemed to be concerned with diverse actions. They ease cell division/cell differentiation, proceed apical dominance, mediate shoot development, preserve leaf senescence and contribute in apical meristem formation of shoot. They further activate floral development, break bud dormancy and accelerate seed germination. They excite plants secondary metabolites like indolic alkaloids and betacyanins. The greater total phenolic content in humic acid treatment against control might be due to higher enzymatic antioxidants, preparing plants against resistance to stress (chilling, drought *etc*.) and disease[18].

The existence of reductones in medicinal plant extracts reflect antioxidant potential by reducing ferric ions (Fe^{+3}) into ferrous ions (Fe^{+2}). Plant extracts show electron donation and reduce oxidized intermediates undergoing varying mechanisms in lipid peroxidation making themselves potent antioxidants. The reducing potential attained from selected leaf extracts of coriander was measured at selected extract concentrations (2.5-10.00 mg/mL). All extracts showed increased reducing power with greater concentration[19].

The reducing potential of coriander extracts as influenced by selected PGRs was estimated as 0.613-1.952 (absorbance at $\lambda_{max} = 700$ nm), 10.00 mg/mL extract concentration (Table-3). Although, all extracts showed higher reducing power than control but the maximum absorbance was seen by *Moringa* leaf extract-A treatment in experiment A. The obtained values were found to be correlated with Garg *et al*.[20]. They reported reduction potential of coriander leaf extract as 0.84 ± 0.15.

TABLE-3
REDUCING POWER AND IC_{50} VALUE OF CORIANDER LEAVES INFLUENCED BY DIFFERENT PLANT GROWTH REGULATORS

Treatments	Reducing Power (10 mg/mL)		IC_{50} Value (µg/mL)	
	A_X	B_Y	A_X	B_Y
Control	0.741 ± 0.006^{e}	0.613 ± 0.011^{de}	0.694 ± 0.016^{de}	0.979 ± 0.014^{d}
HA-1	$1.509 \pm 0.013_{A}^{b}$	$1.298 \pm 0.032_{A}^{b}$	$0.526 \pm 0.011_{A}^{c}$	$0.579 \pm 0.013_{A}^{a}$
HA-2	$1.118 \pm 0.011_{B}^{c}$	$0.921 \pm 0.021_{B}^{cd}$	$0.601 \pm 0.012_{B}^{cd}$	$0.682 \pm 0.015_{B}^{b}$
HA-3	$1.019 \pm 0.015_{B}^{cd}$	$0.765 \pm 0.030_{B}^{d}$	$0.655 \pm 0.013_{B}^{d}$	$0.726 \pm 0.017_{C}^{b}$
MLE-1	$1.792 \pm 0.017_{A}^{ab}$	$1.632 \pm 0.025_{A}^{a}$	$0.354 \pm 0.008_{A}^{a}$	$0.544 \pm 0.011_{A}^{a}$
MLE-2	$1.361 \pm 0.016_{B}^{bc}$	$0.838 \pm 0.034_{B}^{d}$	$0.560 \pm 0.012_{B}^{c}$	$0.652 \pm 0.012_{B}^{b}$
MLE-3	$0.774 \pm 0.022_{C}^{e}$	$0.671 \pm 0.043_{B}^{d}$	$0.674 \pm 0.010_{B}^{d}$	$0.704 \pm 0.012_{C}^{b}$
6-BAP-1	$1.269 \pm 0.008_{B}^{c}$	$1.053 \pm 0.015_{B}^{c}$	$0.645 \pm 0.011_{C}^{d}$	$0.678 \pm 0.013_{B}^{b}$
6-BAP-2	$1.318 \pm 0.015_{B}^{c}$	$1.173 \pm 0.029_{B}^{bc}$	$0.589 \pm 0.009_{B}^{cd}$	$0.631 \pm 0.007_{B}^{b}$
6-BAP-3	$1.569 \pm 0.018_{A}^{b}$	$1.409 \pm 0.034_{A}^{b}$	$0.416 \pm 0.010_{A}^{ab}$	$0.557 \pm 0.017_{A}^{a}$
BF(P)	1.952 ± 0.025^{a}	1.460 ± 0.038^{bc}	0.582 ± 0.010^{cd}	$0.609 \pm 0.013_{A}^{b}$
HA 6 h (P)	$1.378 \pm 0.014_{A}^{bc}$	$1.277 \pm 0.026_{A}^{bc}$	$0.441 \pm 0.009_{A}^{b}$	$0.556 \pm 0.007_{A}^{a}$
HA 9 h (P)	$1.192 \pm 0.010_{B}^{c}$	$1.258 \pm 0.039_{A}^{bc}$	$0.619 \pm 0.011_{B}^{d}$	$0.678 \pm 0.017_{B}^{b}$
HA 12 h (P	$1.174 \pm 0.021_{B}^{c}$	$1.073 \pm 0.019_{B}^{c}$	$0.666 \pm 0.012_{B}^{d}$	$0.691 \pm 0.015_{B}^{b}$

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 subs-cripts within the row showed significant difference between experiments. 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

The drift regarding reducing potential among the foliar growth regulators was: *Moringa* leaf extract > 6-benzyl amino purine > humic acid. In seed priming conducts, the greater reduction potential was depicted by the sample treated with bio-fertilizer, however, least value was gained through humic acid 12 h.

In overall evaluation, seed priming conducts showed pronounced effects on reduction ability while compared to foliar conducts of PGRs. Bio-fertilizers are valuable due to eco-friendly nature, possess beneficial microorganisms which show synergic action and detoxify many pest related disorders. Moreover, they utilize essential bio-actives, ease faster decay of organic wastes and enhance nitrogen fixation in atmosphere. They replenish fertility in soil by providing plentiful favorable micro-organisms and therefore prove as main enhancer of anti-oxidant potential. Hannan *et al.*[21] also reported higher anti-oxidant activity while treating soybean plants with bio-fertilizer (alone and with fertlizer).

$DPPH^{\bullet}$ assay deals with scavenging of free radicals and is a reliable way for assessment of antioxidant potency of plant extracts percent scavenging increases with higher extract dose. Extracts donate hydrogen to DPPH radical making its hydrazine derivative, converting purple shaded solution into yellow ones. The structure of molecule of plant also imparts its role in enhancing proton donation[22].

The observed IC_{50} values (0.354-0.979 µg/mL) for coriander extracts are demonstrated in Table-3. All PGRs treated samples showed lower value of IC_{50} against control. Amongst selected foliar applications in experiment A, the lowest value was revealed by *Moringa* leaf extract-1, followed by 6-benzyl amino purine-3 and humic acid-1 treated sample. Similarly, comparable drift was also reflected from experiment B.

While considering seed priming treatments, the observed trend in both experiments (A and B) estimated as: humic acid 6 h followed by bio-fertilizer, humic acid 9 h and humic acid 12 h.

Previously, $DPPH^{\bullet}$ scavenging action of measured from coriander seeds extracted with acetone was estimated as 19.01-44.42 %[23]. The varying results might be attributed in view of different solvent and different nature and concentrations of PGRs applied.

It can be concluded from the current study that $DPPH^{\bullet}$ scavenging potential enhanced by implementation of exogenous application of PGRs. Plants also showed improved behavior against certain environmental stress, leading to stimulation of vegetable growth as well as yield in unit area. The motive of the assay might also be linked to greater natural production of suitable amount of bio-active compounds in fresh *Moringa* leaf extract treated sample, relying it a good source of plant growth regulating substances[24].

Conclusion

The acquired results from coriander leaf extracts grown against certain natural and synthetic media, served as catalogue for the exhibition of overall higher antioxidant potential against control. Among varying applied PGRs, extracts treated with *Moringa* leaf extract displayed superior performance followed by 6-benzyl amino purine and humic acid. Conclusively, all PGRs positively responded towards antioxidant potential of coriander extracts, proving it a safer food source and valuable medicinal herb for individuals of developed/developing nations.

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