

Boosting the growth of rocket plants in response to the application of *Moringa oleifera* extracts as a biostimulant

Mona M. Abdalla

Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt.

messam_9156@hotmail.com

Abstract: *Moringa oleifera* plant is an outstanding and impressive source of nutrition and medication. It grows in a wide range of tropical and semi-arid climates, making it a viable solution for areas affected by food shortages and populations susceptible to malnutrition. Accordingly, rocket (*Eruca vesicaria subsp. sativa*) plants were foliar sprayed with the aqueous extracts of leaves and twigs of *Moringa oleifera* at rates of 1,2 and 3%. Among these concentrations, fertilization of rocket plants with 2% leaf and 3% twig extracts potentially increased all measured growth criteria (plant height, fresh and dry herb weight), photosynthetic rates, stomatal conductance, the amounts of each of chlorophyll a, b, carotenoids, total sugars, total protein, phenols, ascorbic acid, N, P, K, Ca, Mg, Fe as well as the contents of growth promoting hormones (auxins, gibberellins and cytokinins). Reversibly, bio-organic manuring with both *Moringa* extracts at all concentrations applied negatively reduced the levels of each of lipid peroxidation and abscisic acid as well as the activities of the antioxidant enzymes (catalase, peroxidase and superoxide dismutase). Thus, it is concluded that *Moringa oleifera* leaf and twig extracts can be recommended to be used effectively by farmers as a bio-organic fertilizer for various crops due to its high productivity, easy preparation, highly nutritive and antioxidant effect, low cost and environmentally friendly.

[Mona M. Abdalla. **Boosting the growth of rocket plants in response to the application of *Moringa oleifera* extracts as a biostimulant.** *Life Sci J* 2014;11(11):1113-1121]. (ISSN: 1097-8135). <http://www.lifesciencesite.com>. 189

Keywords: antioxidants, gas exchange, growth, metabolites, photosynthetic pigments, phytohormones.

1. Introduction:

Eruca vesicaria subsp. sativa (rocket or arugula) is an economically important leafy vegetable commonly found in the Mediterranean region, southern Europe and Central Asia (Pignone, 1997). Beside its culinary uses, rocket is also considered a medicinal plant, the extract of both plant and seed possesses diversified therapeutic properties including anti-hyperlipidemic, anti-hyperglycemic, anti-nephrolithiatic, antiulcer, anti-scorbutic, stimulant, stomachic and diuretic oil seed (Hungard et al, 1988; Pignone, 1997; Bukhsh et al, 2007; Alqasoumi et al, 2009). In addition, rocket has shown great potential as a green manure for controlling pathogenic fungi and parasitic nematodes as it contains chemicals with high biocidal activity that mimic synthetic fumigants (Ekaterini et al, 2006). The dependency on the use of inorganic fertilizers as a source of plant nutrients by farmers and their high cost is further associated with land and soil degradation and environmental pollution (Phiri, 2010). Thus, there is continuous need to search for alternative safe natural sources of plant nutrients. *Moringa oleifera* is one such alternative, being investigated to ascertain its effect on growth and yield of crops and thus can be promoted among farmers as a possible supplement or substitute to inorganic fertilizers (Phiri, 2010). Moreover, several researches have indicated that *Moringa oleifera* Lam (family: Moringaceae) is a highly valued plant with

multipurpose effects (Yang et al, 2006; Anwar et al, 2007; Adebayo et al, 2011; Moyo et al, 2011; Mishra et al, 2011). The tree ranges in height from 5 to 10m (Morton, 1991). It is found wild and cultivated in many countries of the tropics and subtropics (Morton, 1991). It is considered as one of the world's most useful trees, as almost every part of the tree has an impressive effect for food, medication and industrial purposes (Khalafalla et al, 2010; Adebayo et al, 2011; Moyo et al, 2011). Different parts of this plant contain a profile of important minerals, proteins, vitamins, β -carotene, amino acids and various phenols and provides a rich and rare combination of zeatin with several flavonoid pigments (Nagar et al, 1982; Siddhuraju and Becker, 2003; Anwar et al, 2007; Oluduro, 2012), so it is a good source of natural antioxidant compounds (Anwar et al, 2007; Jacob and Shenbagaraman, 2011; Dehshahri et al, 2012; Pakade et al, 2013). *Moringa* seeds can be eaten fresh or cooked, or it can be pressed into sweet, non-desiccating oil of high quality (30-40% of seed weight), commercially known as "Ben oil". Moreover, its unique property is the ability of the dry crushed seed and seed press cake which contain certain polypeptides to serve as a natural coagulants with antibacterial and antifungal activities, thus it has a compelling water purifying power (The Wealth of India, 1962; Ndabigengesere and Narasiah, 1998; Anwar et al, 2007). Concerning its medicinal value, it

acts as cardiac and circulatory stimulants, possesses antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, anti-diabetic, hepatoprotective, antibacterial and antifungal activities and are being employed for the treatment of different ailments in the indigenous system of medicine particularly in South Asia (**The Wealth of India, 1962; Morimitsu et al, 2000; Siddhuraju and Becker, 2003; Anwar et al, 2007; Moyo et al, 2011; Mishra et al, 2011; Jacob and Shenbagaraman, 2011**). Besides the medicinal values of this plant, there has been earlier reports by **Fuglie (2000)** that the leaf extract of *Moringa oleifera* accelerated growth of young plants, strengthened plants, improved resistance to pests and diseases, increased leaf duration, increased number of roots, produced more and larger fruits and generally increased yield by 20 and 35%. Several recent investigations were undertaken aiming to increase both the growth parameters measured as plant height, number of leaves, leaf area, fresh and dry weight, number of tillers, shoot vigor, root length, germination percentage and yield represented as fruit number and dry weight by foliar application of *Moringa* leaf extracts at different rates (**Prabhu et al, 2010; Balakumbahan and Rajamani, 2010; Phiri, 2010; Nouman et al, 2011; Yasmeen et al, 2013**).

Although various parts of *Moringa oleifera* plant extracts are known to possess diverse medicinal and biological activities on human and animals but little is known scientifically about its effect as a bio-organic fertilizer on the hormonal, metabolic and antioxidant potential on plants. Therefore, the present study was planned to explore the comparative and dose-dependent effect of the aqueous extracts of *M. oleifera* leaf and twigs in sustaining the growth, metabolic, hormonal and antioxidant activities of a leafy vegetable plant, rocket (*Eruca vesicaria subsp. sativa*).

2. Materials and Methods:

Plant material and treatments

The seeds of rocket (*Eruca vesicaria subsp. sativa* c.v. Balady) were obtained from the Agricultural Research Center, Giza, Egypt. They were then planted in plastic pots (20 cm in diameter), filled with equal amount of clay loamy soil under controlled greenhouse conditions (relative humidity 35-50% \pm 2, average day/night temperature 25/18 $^{\circ}$ C \pm 2) and irrigation was done following the common agricultural practice. The pots were arranged into 7 sets, 20 pots/set. The first set remained untreated to serve as control while the other 6 sets were fertilized with the biostimulant at different rates as follows: 0 (untreated control), 1L, 2L, 3L, 1T, 2T, 3T (*M. oleifera* leaf and twig extracts, each at 1, 2 and 3% respectively). After one week from planting and before foliar treatment,

thinning was done so as to leave 10 plants /pot. The aqueous extract of *M. oleifera* tender leaves and twigs were prepared at the rates of 1, 2 and 3% using distilled water. The chemical analysis of both extracts were investigated by **Fuglie (2000) and Moyo et al (2011)** and represented in table (1). Foliar spraying of both extracts (leaves and twigs) were done twice at 7 and 14 days after planting (DAP). The plants were harvested at 50 DAP. Ten plants were randomly selected for the measurement of growth criteria and gas exchange rates. Another samples were taken (5 replicates/ treatment) and either oven dried for determinations of carbohydrates, nitrogen, phenols, ascorbic acid, total proteins and minerals or rapidly frozen for the estimations of antioxidant enzyme activities, photosynthetic pigments and phytohormonal levels.

Measurement of gas exchange rates

Photosynthetic rate and stomatal conductance were measured using an open gas portable photosynthesis system (LI-6400, LICOR, BioSciences, USA). Measurements were performed on sunny days under light conditions and between 9.00 and 12.00h on the upper most fully expanded leaves of 10 plants randomly chosen per treatment and expressed on a leaf area basis (**Renault et al, 2001**).

Chemical analysis

Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined spectrophotometrically (**Metzner et al, 1965**). Carbohydrate fractions were extracted, clarified and determined as total sugars (TS) (**Dubois et al, 1965**). Total proteins were estimated by Lowry's method (**Lowry et al, 1951**). Ascorbic acid, a scavenger of oxyradicals was assayed by the method described by **Roe and Keuther (1953)** where ascorbate is converted to dehydro- ascorbate by the treatment with activated charcoal. Dehydro-ascorbic acid then reacts with 2,4-dinitrophenyl hydrazine to form osazones, which dissolves in sulphuric acid to give an orange colored solution, whose absorbance can be measured spectrophotometrically at 540nm. Total phenols were determined in the ethanolic extract following the method described by **Simons and Ross (1971)** using folin reagent. For the assay of antioxidant enzymes (catalase, CAT; peroxidase, POD; superoxide dismutase, SOD), fresh material were extracted following the method of **Guerrier and Strullu (1990)**. The activities of CAT and POD were determined according to **Chance and Maehly (1955)**. CAT activity were determined by measuring the decomposition of H₂O₂, by following the decline in its absorbance at 240nm for 3min. POD activity was assayed by measuring the oxidation of guaiacol and the increase in absorbance at 470nm was recorded in 3min. The activity was defined as OD/min/mg FW.

SOD activity was assayed by the nitrobluetetrazolium (NBT) modified method from that described by **Dhindsa et al (1980)**. One unit of SOD was defined as that being contained in the volume of extract that caused a 50% inhibition of the SOD-inhibitable fraction of the NBT reduction. The level of lipid peroxidation in tissues was measured by the determination of nmole of malonodialdehyde (MDA) formed using an extraction coefficient of $155\text{nmole L}^{-1}\text{cm}^{-1}$. MDA was determined using 20% trichloroacetic acid containing 0.5% thiobarbituric acid (TBA) reaction and the developed color was extracted with 2ml n-butanol and the absorbance was measured at 530nm. The value for the non-specific absorption at 600nm was subtracted (**Zhao et al, 1994**). Mineral elements were extracted from tissues similar to that of **Chapman and Pratt (1961)**. Phosphorus was determined following the method described by **Humphries (1956)**. Potassium was estimated photometrically according to **Williams and Twine (1960)**. Calcium, magnesium and iron were determined by atomic absorption spectrophotometer according to **A.O.A.C. (1984)**. Total-N was determined by micro-Kjeldahl, Tector model 1026 after digestion in sulphuric acid (**Horwitz, 2002**).

Hormonal analysis

Growth regulators were estimated by collecting fresh samples in cold redistilled 95% ethanol in glass stoppered brown jars and kept in a deep freeze ready for the further analysis process. The method of extraction was essentially adopted by **Wasfy et al (1974)**. The fraction of the ethanol extract was carried out according to the method described by **Shindy and Smith (1975)**. The acidic fraction contains the acidic hormones (IAA, GA₃ and ABA) while the aqueous fraction comprised the cytokinins. The growth promoters (auxins, gibberellins, cytokinins) and the growth inhibitors (abscisic acid) were quantified using high performance liquid chromatography (HPLC) according to the method adopted by **Muller and Hilgenberg (1986)**.

Statistical analysis

Morphologic and gas exchange values (photosynthetic rate and stomatal conductance) were means \pm Standard errors of 10 replicates while those of chemical and hormonal analysis were means \pm standard errors of 5 replicates. Significant differences were calculated using student's (t) test. SPSS version 15 was performed for multiple comparisons.

3. Results and Discussion:

Growth parameters

Results presented in table(2) showed that *Moringa oleifera* leaf and twig extracts at 2% and 3% respectively significantly enhanced the height of rocket plants as comparable to untreated and differently treated plants. Also the highest percentage

increase in fresh herb weight of (68.1) and (61.7) per plant was recorded from the treatments 2L (2% leaf extract) and 3T (3% twig extract) respectively while the lowest fresh herb weight was obtained in the untreated control plants. Similarly, the maximum percentage increase in dry herbage weight of (51.5) and (42.6) was recorded from the same treatments 2L and 3T and were significantly different from other treatments and control. It was thus apparent that these two treatments (2L and 3T) among the other five treatments caused maximization of yield which is the ultimate goal to be achieved in any crop management aspect. Consistent results were obtained by **Prabhu et al (2010)** and **Balakumbahan and Rajamani (2010)** by applying *Moringa* leaf extract at 2 and 4% on sacred basil and senna plants respectively. They found that foliar spraying of *Moringa* leaf extract at 2% was more effective than 4% and raised all measured growth parameters above control plants (plant height, number of leaves, leaf area, leaf area index, fresh and dry weight, number of pods, number of branches, dry leaf yield and dry pod yield). In another trial, **Culver et al (2012)** realized that *Moringa* leaf extract significantly increased growth and yield of tomato in both green house and field. It increased dry matter yield, root dry matter weight and plant height. The possible reason for this acceleration of growth might be due to the enriched content of *Moringa* leaf and twig extracts of crude proteins (43.5%) and growth promoting hormones i.e. auxins and cytokinins (**Makkar and Becker, 1996; Moyo et al, 2011**). Proteins are essential for the formation of the protoplasm while growth hormones favored rapid cell division, cell multiplication and enlargement.

Gas exchange measurements, photosynthetic pigments, carbohydrate and soluble protein contents

When rocket plants were treated with 2% *Moringa* leaf extract (2L) they manifested an optimum photosynthetic rate and stomatal conductance ($12.6\ \mu\text{mol CO}_2\ \text{m}^{-2}\ \text{s}^{-1}$) and ($0.263\ \text{mol H}_2\text{O}\ \text{m}^{-2}\ \text{s}^{-1}$) respectively followed by 3% *Moringa* twig extract (3T) while, on the contrary, untreated control plants showed the least effect among the other treatments ($8.94\ \mu\text{mol CO}_2\ \text{m}^{-2}\ \text{s}^{-1}$) and ($0.112\ \text{mol H}_2\text{O}\ \text{m}^{-2}\ \text{s}^{-1}$) successively (table 3). These results were compatible with those of **Amaya-Carpio et al (2009)** and **Karanatsidis and Berova (2009)** using different organic fertilizers who attributed these increases in the photosynthetic ability in plants in response to organic fertilization due to various factors namely the efficiency of PSII, the stability of chloroplast ultrastructure, the enhanced rate of CO₂ absorption by leaf cell and its fixation. It was prominent from table (3) that application of *Moringa* leaf extract at the medium dose (2L) exhibited the highest

concentrations of photosynthetic pigments, namely, chlorophyll a, chlorophyll b and carotenoids as compared to those of untreated control plants. In addition, spraying rocket plants with the twig extract at three rates 1, 2 and 3% elicited gradual increases in the amounts of these photosynthetic pigments but these increases were comparably less than those of *Moringa* leaf extract. Corroborative data were obtained using different types of organic fertilizers as compost, vermicompost, animal manure, seaweeds and *Moringa* leaf extract (Amujoyegbe *et al*, 2007; Noori *et al*, 2010; Abdalla and El-Khoshiban, 2012; Yasmeen *et al*, 2013). The present knowledge ascertained that *Moringa* (leaves, seeds, pods) contains appreciable amounts of specific plant pigments with demonstrated potent antioxidant properties such as the carotenoids (lutein, alpha-carotene, beta-carotene, xanthin) and chlorophyll (Owusu, 2008). Besides that, the leaves have high nutritional potentialities of several macro elements as Mg (Yameogo *et al*, 2011), a constituent of chlorophyll, both would account for the increase in the amounts of chlorophyll a and chlorophyll b in *Eruca* plants. Rocket plants treated with the four concentrations of *Moringa* leaf and twig extracts (1L, 2L, 2T and 3T) showed significant accumulations of total sugars and total proteins and the maximum increments of both were obtained when the plants were treated with 2% leaf extract (2L), while the other two concentrations (3L and 1T) manifested marginal increases in both total sugars and total proteins above those of untreated control plants (table 3).

Among the two *Moringa* extracts examined, the leaf extract was found superior than the twigs towards the values of both total sugars and total proteins in *Eruca* plants. Foidl *et al* (2001) reported that foliar spraying of some plant leaves with *Moringa* extract produced some notable effects as overall increase in plant yield between 20-35% and higher sugar and mineral levels. The enhanced accumulations of both total protein and total sugars in rocket plants in response to treatments with *Moringa* leaf and twig extracts were due to the high protein, sugar and starch content of the entire *Moringa oleifera* plant (table 1) and which make it of great scientific and agricultural interest (Foidl *et al*, 2001; Yameogo *et al*, 2011).

Antioxidant compounds and antioxidant enzyme activities

With increasing rates of *Moringa* leaf and twig extracts, either progressive declines in each of peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) activities and the levels of lipid peroxidation (measured as nmole of MDA formed) or gradual increases in phenol and ascorbic acid (AA) amounts were observed so as to reach minimum and maximum increments at 2L and 3T respectively as

comparable to those of untreated plants (table 4). Data in table (4) clearly manifested that *Eruca* plants sprayed with *Moringa* twig extract attained higher antioxidant enzyme activities (SOD,POD,CAT) and MDA level and lower antioxidant compound levels (phenols and AA) more than those treated with the leaf extract which reflects the booster effect of the leaf extract as an antioxidant. Concurrent with these results, it was found that foliar spraying of *Triticum aestivum* plants with *Moringa* leaf extract increased the levels of POD, CAT and AA (Yasmeen *et al*, 2013). Total phenols comprise the largest group of plants secondary metabolite. They can directly react with superoxide anions and lipid peroxyl radical and consequently inhibit or break the chain of lipid peroxidation (Rajanandh and Kavitha, 2010). Ascorbic acid (AA) is a familiar molecule because of its dietary significance, it is not only an important antioxidant, it also appears to link flowering time, developmental senescence, programmed cell death and responses to pathogens through a complex signal transduction network (Nicholas, 1978; Talreja, 2011). They induces better root and shoot growth, maintain higher leaf moisture content and lower disease incidence in both normal and stressful environments (Zhang and Schmidt, 1999). Under normal circumstances, the reactive oxygen molecules (ROS) such as O_2^- , H_2O_2 and OH are continuously generated and detoxified by the antioxidant compounds (phenols, flavonoids, carotene and AA) and antioxidant enzymes (SOD,POD,CAT) present in the tissues. Thus, there is an equilibrium between the ROS generated and the antioxidant present. However, owing to ROS overproduction and /or inadequate antioxidant defense, this equilibrium is hampered favoring the ROS upsurge that culminates in oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA (Sreelatha and Padma, 2009).

Moringa oleifera aqueous extract possessed an excellent source of a wide spectrum of dietary antioxidant as phenols, flavonoids, β -carotene, AA, α -tocopherol (vitamin E) and antioxidant enzymes (SOD, POD and CAT). Thus, the extract exhibited strong scavenging effect of 2, 2-diphenyl-2-picrylhydrazyl (DPPH) free radical, superoxide, nitric oxide radical and inhibition of lipid peroxidation as comparable to many reference antioxidant extracts of various leafy vegetables and fruits (Dasgupta and De, 2006; Sreelatha and Padma, 2009; Jacob and Shenbagaraman, 2011; Dehshahri *et al*, 2012; Pakade *et al*, 2013). Thus, this extract can prevent oxidative damage to major biomolecules and afford significant protection against oxidative damage through favoring the accumulation of higher levels of AA and phenols which, in turn, rendered the activities

of antioxidant enzymes and lipid peroxidation in rocket plants under investigation to minimum levels.

Inorganic nutrient contents

It is evident from table (5) that there were positive correlations between the increased rates of both extracts of *Moringa* applied up to 3% and the accumulation of both major (N, P, K, Ca and Mg) and minor elements (Fe) estimated, in the present study, in rocket plants. The uptake and accumulation of these nutrients in rocket plants were significantly higher due to fertilization with the leaf extract more than the twigs. Several comparable studies confirmed the current data. For instance, **Schuphan (2005)**, **Noori et al (2010)**, **Sivakumar and Ponnusami (2011)** and **Abdalla and El-Khoshiban (2012)** realized increased uptake and accumulations of some nutritive elements as N, K, Ca, Mg, P as well as Fe in roots and shoots of several plants under investigation as a consequence of organic fertilization from different sources (plant and animal source) including *Moringa* leaf extract. **Sivakumar and Ponnusami (2011)** indicated that organic manures are fairly good source of nutrients which boosted plants to uptake progressively beneficial elements, to increase the leaf nutrient status and eventually attain optimum growth and productivity. Bio-organic fertilization is supposed to accelerate the nutrient uptake through the tested rocket plant by increasing the permeability of root membranes for electrolytes, preventing their fixation in the soil and increasing their mobility. Different parts of *Moringa oleifera* plants have been reported to be a rich source of important minerals as Ca, Mg, K, Fe, Zn, P, S, Cu, Mn, Se and Na which can be valorized for a balanced nutrition of populations (**Yameogo et al, 2011; Moyo et al, 2011**).

Hormonal analysis

The changes in the phytohormonal levels of untreated and biofertilized rocket plants are presented in table (6). The contents of growth promoting hormones (auxins, gibberellins and cytokinins) increased progressively above those of the corresponding controls in a dose related manner so as to reach maximum levels either at 2L or 3T in response to fertilization with *Moringa* leaf and twig extracts respectively. Reversibly, supplementing rocket plants with both leaf and twig extracts negatively reduced the abscisic acid (ABA) values either markedly (1L, 2L, 2T, 3T) or marginal (3L, 1T) below those of untreated plants (fig.4). Noteworthy in the current work, the above results of different values of phytohormones accommodated well with those of growth criteria due to foliar spraying of rocket plants with *Moringa* leaf and twig extracts at three rates 1, 2 and 3%. Consistent results were obtained by **Makkar and Becker (1996)**, **Anwar et al (2007)** and **Yasmeen et al (2012)**. They isolated growth promoting hormones (zeatin and auxins) from *Moringa* leaf extract and used this extract as a foliar spray to accelerate growth of different plants. Results showed that foliar treatment with *Moringa* extract increased flowering, dry matter, fruit weight, produced larger flowers and fruits, increase number of grains / spike, the weight of thousand grains and consequently higher yield at harvest time, greater number of shoots per plant and higher percentage of sugars and minerals and eventually caused plants to be firmer and more resistant to pests and diseases (**Foidl et al, 2001; Yasmeen et al, 2012**).

Table 1: Chemical composition of *Moringa oleifera* leaf and twig aqueous extracts. Values listed are expressed as g/ 100g.d.wt.

Chemical components	leaves	twigs
Water	5.9	3.8
Protein	27.2	17.8
Lipids	17.1	8.9
Total sugars	38.6	21.3
Fiber	19.2	21.2
Calcium	2.00	6.9
Magnesium	0.37	1.7
Potassium	0.013	7.4
Sodium	non defined	non defined
Iron	0.028	0.016
Phosphorus	0.20	0.139
Vitamin A(β -carotene)	0.016	0.006
Vitamin B ₁ (thiamine)	0.0026	0.0020
Vitamin B ₂ (riboflavin)	0.021	0.019
Vitamin B ₃ (nicotinic acid)	0.008	0.006
Vitamin C(ascorbic acid)	0.017	0.017
Vitamin E(tochopherol acetate)	0.113	0.011

Table 2. The potential of *Moringa oleifera* leaf and twig aqueous extracts on growth attributes of rocket plants.

Treatment	Plant height(cm)	F.wt/plant(g)	D.wt/plant(g)
0	17.8±2.3	11.12±1.7	1.36±0.32
1L	19.6±2.6	12.34±2.2	1.41±0.48
2L	23.6±3.2	18.69±1.9	2.06±0.63
3L	18.4±3.0	14.34±1.8	1.56±0.36
1T	18.0±2.1	11.22±1.3	1.33±0.28
2T	18.8±1.8	12.89±1.5	1.55±0.30
3T	22.3±1.9	17.98±1.9	1.94±0.57

*The mean difference is significant at the 0.05 level

Table 3: The potential of *Moringa oleifera* leaf and twig aqueous extracts on the gas exchange, photosynthetic pigment, total sugars and total proteins of rocket plants.

Treatment	Photosynthesis $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$	Stomatal Conductance $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$	Chlorophyll a mg/g f wt	Chlorophyll b mg/g f wt	Carotenoids mg/g f wt	Total proteins mg/g d wt	Total sugars mg/g d wt
0	8.94±0.30	0.112±0.02	6.08±0.21	3.40±0.16	1.03±0.13	10.06±0.48	7.63±0.23
1L	10.6±0.41	0.176±0.03	6.98±0.23	3.86±0.28	1.38±0.24	12.67±0.56	9.29±0.37
2L	12.6±0.28	0.263±0.09	8.23±0.31	5.02±0.19	2.43±0.19	14.93±0.68	11.67±0.52
3L	11.0±0.34	0.187±0.06	7.63±0.41	4.09±0.32	1.91±0.23	11.28±0.71	8.23±0.61
1T	10.2±0.32	0.164±0.03	6.35±0.36	3.71±0.46	1.16±0.37	10.87±0.69	7.98±0.43
2T	10.9±0.26	0.180±0.01	7.12±0.29	4.18±0.28	1.89±0.35	12.96±0.49	8.74±0.32
3T	11.7±0.44	0.208±0.08	7.97±0.25	4.87±0.27	2.01±0.28	13.52±0.67	10.18±0.29

*The mean difference is significant at the 0.05 level

Table 4: The potential of *Moringa oleifera* leaf and twig aqueous extracts on the antioxidant compounds and antioxidant enzyme activities of rocket plants.

Treatment	Phenol (mg/g.d.wt)	Ascorbic acid (mg/g.d.wt)	Lipid peroxidation (MDA/g.f.wt)	CAT (mg/g.f.wt /hr)	POD (mg/g.f.wt /hr)	SOD (mg/g.f.wt/ hr)
0	8.70±0.24	0.393±0.08	2.39±0.16	398±3.6	685±6.3	4.82±0.31
1L	9.38±0.36	0.482±0.04	1.93±0.19	301±3.8	618±3.8	3.33±0.67
2L	11.84±0.49	0.698±0.07	1.06±0.21	196±4.2	428±2.9	2.77±0.39
3L	10.03±0.69	0.503±0.09	2.00±0.18	238±4.9	563±4.8	3.61±0.27
1T	8.93±0.36	0.401±0.03	2.17±0.20	384±6.3	596±5.1	4.39±0.48
2T	9.76±0.29	0.514±0.01	1.64±0.17	263±2.9	518±6.6	3.53±0.32
3T	11.36±0.67	0.602±0.07	1.19±0.16	206±3.1	472±4.6	2.96±0.17

*The mean difference is significant at the 0.05 level

Table 5: The potential of *Moringa oleifera* leaf and twig aqueous extracts on the inorganic nutrient contents of rocket plants. Values listed are expressed as mg/g.d.wt.

Treatment	N	P	Mg	K	Ca	Fe
0	24.0±3.2	4.8±0.3	3.67±0.2	9.73±0.31	14.5±0.32	0.66±0.001
1L	29.8±2.9	5.6±0.1	4.18±0.1	11.61±0.42	27.1±0.41	0.84±0.003
2L	41.3±1.8	7.9±0.4	5.76±0.3	16.90±0.23	48.3±0.25	1.31±0.004
3L	35.4±2.9	5.9±0.6	4.02±0.4	12.80±0.33	38.6±0.17	1.05±0.005
1T	27.3±3.9	4.9±0.2	3.99±0.3	10.36±0.23	21.9±0.14	0.79±0.004
2T	32.4±2.2	5.7±0.1	4.68±0.2	11.84±0.26	34.6±0.48	0.92±0.005
3T	38.9±2.1	6.4±0.3	5.01±0.4	14.68±0.19	43.6±0.51	1.18±0.006

*The mean difference is significant at the 0.05 level

Table 6: The potential of *Moringa oleifera* leaf and twig aqueous extracts on the phytohormonal contents of rocket plants. Values listed are expressed as mg/ kg.f.wt.

Treatment	Auxins	Gibberellins	Cytokinins	Abcisic acid
0	12.7±0.21	11.6±0.17	19.6±0.22	10.8±0.11
1L	13.6±0.15	12.1±0.16	22.4±0.18	8.70±0.13
2L	15.8±0.18	17.9±0.20	27.3±0.16	7.60±0.12
3L	12.9±0.13	14.0±0.13	24.1±0.23	9.90±0.16
1T	13.0±0.16	12.5±0.12	21.8±0.19	10.0±0.14
2T	13.5±0.17	13.8±0.16	23.6±0.16	9.20±0.15
3T	14.3±0.19	15.6±0.21	25.6±0.14	8.40±0.12

*The mean difference is significant at the 0.05 level

4. Conclusions

Overall, based on the current results, *Moringa* leaf and twig extracts can be used at rates 2% and 3% respectively to stimulate the biomass production of *Eruca* plants, enhanced photosynthetic pigments, total sugars, total proteins, growth promoting hormones (auxins, gibberellins, cytokinins) and various essential mineral elements (N, K, Ca, Mg, P, Fe). In addition, the present results support the potent antioxidant activity of *Moringa* extracts as it successfully increased the phenol and AA contents whereas decreased the activity of each of SOD, CAT, POD and lipid peroxidation levels in rocket plants, which adds one more positive attribute to its known pharmacological properties and hence its use in traditional system in medicine.

Furthermore, the presence of high levels of inorganic and organic matter in the extracts of this plant, its easy preparation and its ecofriendly nature suggest the greater practical importance of *Moringa* extracts. Thus, with the current thrust on sustainable agriculture and organic farming, the use of *Moringa oleifera* extract should grow in popularity and led to development of a large number of *Moringa* extract products that can be employed either as root tips, soil drenches or foliar sprays. Subsequently, it is concluded that this tree has the potential to improve nutrition, boost food security and foster rural development.

Acknowledgement

The author is thankful to the Biotechnology Department, National Research Center, Dokki, Giza, for performing mineral analysis by atomic absorption spectrophotometer and hormonal analysis by high performance liquid chromatography (HPLC). Sincere thanks and obligations are also extended to Dr. Raifa A. Hassanein, Professor of Plant Physiology, Faculty of Science, Ain Shams University, for her valuable advice and to my husband who encouraged me throughout this work.

References:

1. Abdalla M.M. and El-Khoshiban, N. 2012. The palliative effect of bio-organic fertilizer on lead pollution in *Lycopersicum esculentum* plants. *J. Basic App. Sci.* 8: 1-12.
2. Adebayo, A.G., Akintoye, H.A., Olufolaji, O.O., Aina, M.T., Olatunji, M.T. and Shokalu, A.O. Assessment of organic amendments on vegetative development and nutrient uptake of *Moringa oleifera* Lam in the nursery. *Asian J. Plant Sci.* 2011; 10 (1):74-79.
3. Alqasoumi, E., Al-Sohaibani, M., Al-Howiriny T., Al-Yahya M., Rafatullah S. Rocket (*Eruca sativa*): A salad herb with potential gastric anti-ulcer activity. *World J.* 2009; 15(6):1958- 65. [Gastroenter. 15. info@eruca-sativa.com](mailto:info@eruca-sativa.com).
4. Amaya-Carpio, L., Davies, J.F.I., Fox, T. and He, C. Arbuscular mycorrhizal fungi and organic fertilizer influence photosynthesis, root phosphatase activity, nutrition and growth of *Ipomea carnea* sp. *Fistulosa. Photosynth.* 2009; 47, 1:1-10.
5. Amujoyegbe, B.J., Opabode, J.T., Olayinka, A. Effect of organic and inorganic fertilizer on yield and chlorophyll content of maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.) Moench. *Afr. J. Biotechnol.* 2007; 6, 16: 1869 -1873.
6. Anwar, F., Latif, S., Ashraf, M. and Gilani, A.H. *Moringa oleifera*: A food plant with multiple medicinal uses. *Phototherapy Res.* 2007;21: 17-25.
7. A. O.A. C. Official Method of Analysis of the Association of Official Analytical Chemists, 14thed, Published by the Association of Official Analytical Chemists, PO Box, 540, Benjamin Franklin Station, Washington, dc. 20044. 1984.
8. Balakumbahan, R. and Rajamani, K. Effects of biostimulants on growth and yield of Senna (*Cassia angustifolia* var. KKM1). *J. Hort Sci. Orn. Plants.* 2010; 2(1): 16-18.
9. Bukhsh, E., Malik, S.A. and Ahmad, S.S. Estimation of nutritional value and trace element contents of *Carthamus oxyacantha*, *Eruca sativa* and *Plantago ovata*. *Pak. J. Bot.* 2007; 39(4): 1181-1187.
10. Chance, B. and Maehly, A.C. Assay of catalases and peroxidases. *Method Enzymol.* 1955; 2: 764-775.
11. Chapman, H.D. and Pratt, P.F. Methods of analysis for soils, plants and water. Univ. of California, Div. Agr. Sci. Agro. J., 1961; 66: 412- 421.
12. Culver, M., Fanuel, T. and Chiteka, A.Z. Effect of *Moringa* extract on growth and yield of tomato. *Green J. Agr. Sci.* 2012; 2(5): 207- 211.

13. Dasgupta, N. and De, B. Antioxidant activity of some leafy vegetables of India: A Comparative study. *J. Food Chem* 2006; 2: 1-3.
14. Dehshahri, S., Wink, M., Afsharypuor, S., Asghari S. and Mohagheghzadeh, A. Antioxidant activity of methanolic leaf extract of *Moringa peregrina* (Forssk.) Fiori. *Res. Pharma Sci* 2012; 7(2): 111- 118.
15. Dhindsa, R.S., Dhindsa, P.P. and Thorpe, T.A. Leaf senescence correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.* 1980; 33: 93-101.
16. Dubois, M., Cilles, K.A., Hamilton, J.K., Rober, P.A. and Smith F. Colorimetric method for determination of sugars related substances. *Anal. Chem.* 1965;28: 350-365.
17. Ekaterini, R. Performance of arugula (*Eruca sativa*) as a green manure and trap crop for fungal pathogens and parasitic nematode suppression in potato. *Amer. Phytopath. Soc. Abst.* 2006; 96, S97:1-2.
18. Foidl, N., Makkar, H.P.S. and Becker, K. The potential of *Moringa oleifera* for agricultural and industrial uses. In: Proceedings of the International Workshop "What development potential for *Moringa* products", Dar-es-Salaam, Tanzania. 2001; pp:47-67.
19. Fuglie, L. J. The Miracle Tree: *Moringa oleifera*: Natural Nutrition for the Tropics. The multiple Attributes of *Moringa*. 2000; pp: 172.
20. Guerrier, G. and Strullu, D.G. Development laxes embryonnaire de pois pourvus des reserves. *Cand. J. Bot.* 1990; 68: 742-746.
21. Horwitz, W. Official methods of AOAC. International Gaithersburg, Maryland, USA., 2002. pp:2077-2417.
22. Humphries, E.C.. Mineral component and ash analysis. In: (Peach, K., Tracey, M.V. Ed., Modern Methods of Plant Analysis: Springer-Verlag, Berlin. 2002) 1956; pp:1-148.
23. Hungard, B.L., Goldstein, D.B., Villegas, F. and Cooper, T. The ganglioside GM 1 reduces ethanol induced phospholipase activity in synaptosomal preparation from mice. *Neurochem. Int.*, 1988;25:321-325.
24. Jacob, S.J.P. and Shenbagaraman, S. Evaluation of antioxidant and antimicrobial activities of the selected green leafy vegetables. *Int. J. Pharm. Tech. Res.* 2011; 3(1): 148-152.
25. Karanatsidis, G. and Berova, M. Effect of organic-N fertilizer on growth and some physiological parameters in pepper plants (*Capsicum annum* L). *Biotechnol and Biotechnol E.Q.* 2009; 23: 254-257.
26. Khalafalla, M.M., Abdellatef, E., Dafalla, H.M., Nassrallah, A.A., Aboul-Enein, K.M., Lightfoot, D.A., El-Deeb, F.E. and El-Shemy, H.A. Active principle from *Moringa oleifera* Lam leaves effective against two leukemias and a hepatocarcinoma. *Afr. J. Biotechnol.* 2010; 9(49):8467-8471.
27. Lowry, O.H., Rosenbrough, N.J. and Farr, A.. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 1951; 193:265-275.
28. Makkar, H.P.S. and Becker, K.. Nutritional value and anti-nutritional components of whole and ethanol extracted of *Moringa oleifera* leaves. *Anim. Feed Sci. Technol.* 1996 1951; 63:211-228.
29. Metzner, H., Rau, H. and Senger, H. Untersuchungen zur synchronisierbarkeit ein zeiner pigment. Mangol Mutanten von *Chlorella*. *Planta.* 1965; 65: 186-191.
30. Mishra, G., Singh, P., Verma, R., Kumar, S., Srivastava, S., Jha, K.K. and Khosa, R. L. Traditional uses, phytochemistry and pharmacological properties of *Moringa oleifera* plant: An overview. *Der Pharmacia Lettre. Scholars Research Library* 2011; 3(2):141-164.
31. Morimitsu, Y., Hayashi, K., Nakagama, F., Horio, K.U. and Osawa, T. Antiplatelet and anticancer isothiocyanates In Japanese horseradish, wasabi. *Bio Factors*, 2000;13:271-276.
32. Morton, J.F. The horseradish tree, *Moringa pterigosperma* (Moringaceae). A boon to arid lands. *Econ. Bot.* 1991; 45: 318-333.
33. Moyo, B., Masika, P.J., Hugo, A. and Muchenje, V. Nutritional characterization of *Moringa (Moringa oleifera* Lam) leaves. *Afric. J. Biotechnol.* 2011;10(60), 12925-12933.
34. Muller, P. and Hilgenberg, W. Isomers of zeatin and zeatin riboside in club root tissue: evidence for trans-zeatin biosynthesis by plasma diophora brassicae. *Physiol. Plant.* 1986; 66:245-250.
35. Nagar, P.K., Leyer, R.I. and Sircar, P.K. Cytokinins in developing fruits of *Moringa pterigosperma* Gaertn. *Physiol. Plant.* 1982; 55:45-50.
36. Ndabigengesere, A. and Narasiah, K.S. Quality of water treated by coagulation using *Moringa oleifera* seeds. *Water Res.* 1998; 32:781-791.
37. Nicholas, L. The function and metabolism of ascorbic acid in plants. *Annals Bot.* 1978;1996: 661-669.
38. Noori, J.M., Ardalan, M.M., Nezami, M.T., Cherati, A. and Alizadeh, G. Effect of organic fertilizers (compost Amol, vermicompost, animal manure) and manganese on chlorophyll concentrations and some of nutrient concentrations of soybean (*Glycine max* L). OFIS conference, Islamic Azad University of Bojnord. 2010.
39. Nouman, W., Siddiqui, M.T. and Basra, S.M.A. *Moringa oleifera* leaf extract: An innovative priming tool for rangeland grass. *Turk. J. Agric. For.* TUBITAK, 2011; 35: 1-11.
40. Oluduro, A.O. Evaluation of antimicrobial properties and nutritional potentials of *Moringa oleifera* Lam. leaf in South Western Nigeria. *Mal. J. Microbiol.* 2012; 8(2): 59- 67.
41. Owusu, D. Phytochemical Composition of *Ipomea batatus* and *Moringa oleifera* leaves and crackers from Underutilized Flours. M. Sc. Thesis. Department of Biochemistry and Biotechnology, Faculty of Bio Science, College of Science, Kwame Nkrumah, University of Science and Technology. 2008.
42. Pakade V., Cukrowska, E. and Chimuka, L. Comparison of antioxidant activity of *Moringa oleifera* and selected vegetables in South Africa. *S. Afr. J. Sci.* 2013; 109(3/4):1-5.
43. Phiri, C. Influence of *Moringa oleifera* leaf extracts on germination and early seedling development of major

- cereals. *Agric. Biol. J. of N. Amer.* 2010; 1(5): 774-777.
44. Pignone, D. Present status of rocket genetic resources and conservation activities in Padulosi, S. and Pignone, D. (Eds). Rocket, a Mediterranean crop for the world. *Inter. Plant Genetic Res. Inst. Rome*, 1997; pp: 2-12.
 45. Prabhu, M., Kumar, A.R. and Rajamani, K. Influence of different organic substances on growth and herb yield of sacred basil (*Ocimum sanctum* L). *Ind. J. Agric. Res.* 2010; 44(1): 48-52.
 46. Rajanandh, M.G. and Kavitha, J. Quantitative estimation of β -sitosterol, total phenolic and flavonoid compounds in the leaves of *Moringa oleifera*. *Int. J. of Pharm. Tech. Res.* 2010; 2(2): 1409-1414.
 47. Renault, S., Croser, C., Franklin, J.A., Zwiazek, J.J., Mackinnon, M. Effect of consolidated tailings water on red-osier dogwood (*Conus stolonifera* michx) seedlings. *Environ Poll.* 2001; 113:27-33.
 48. Roe, J.H. and Keuther, C.A. The determination of ascorbic acid in whole blood urine through 2, 4-nitrophenyl hydrazine derivative of dehydro-ascorbic acid. *J. Biol. Chem.* 1953; 147:399-407.
 49. Schuphan, W. Nutritional value of crops as influenced by organic and inorganic fertilizer treatments. *Plant Foods Human Nut.* 2005; 23(4): 333-358.
 50. Siddhuraju, P. and Becker, K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agro-climatic origins of drumstick tree (*Moringa oleifera* Lam). *J. Agric. Food Chem.* 2003; 15:2144-2155.
 51. Shindy, W.W. and Smith, O. Identification of plant hormones from cotton ovules. *Plant Physiol.* 1975; 55:550-554.
 52. Simons, T.S. and Ross, A.F. Changes in phenol metabolism associated with system resistance to tobacco mosaic virus in Samsun NN tobacco. *Phytopath.* 1971; 61, 1261-1268.
 53. Sivakumar, V. and Ponnusami, V. Influence of spacing and organics on plant nutrient uptake of *Solanum nigrum*. *Plant Arch.* 2011; 11(1), 431-434.
 54. Sreelatha, S. and Padma, P.R. Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. *Plant Foods Hum. Nut.* 2009; 64: 303-311.
 55. Talreja, T. Biochemical estimation of three primary metabolites from medicinally important plant *Moringa oleifera*. *Int. J. Pharm. Sci. Rev. Res.* 2011; 7 (2), 186-188.
 56. The Wealth of India (A Dictionary of Indian Raw Materials and Industrial Products). Raw Materials, Vol.6, L-M; Council of Scientific and Indus. Res.: New Delhi, pp: 425-429. 1962.
 57. Wasfy, W., Shindy, L.R. and Orrin, E.S. Identification of plant hormones from cotton ovules. *Plant Physiol.* 1974; 55, 550-560.
 58. Williams, S. and Twine, M. Flame photometric method for sodium, potassium and calcium, in: Peach, K. and Tracey, M.V.: ed., *Modern Methods of Plant Analysis*, Springer-Verlag, Berlin. Vol.5, p. 3-5. 1960.
 59. Yameogo, C.W., Bengaly, M.D., Savadogo, A., Nikiema, P.A. and Traore, S.A. Determination of chemical composition and nutritional values of *Moringa oleifera* leaves. *Pak. J. of Nut.* 2011; 10 (3), 264-268.
 60. Yang, R.Y., Chang, L.C., Hsu, J.C., Weng, B.B.C., Palada, M.C., Chadha, M.L. and Levasseur, V. Nutritional and Functional Properties of *Moringa* leaves-from germplasm, to plant, to food, to health, in *Proceedings of the International Workshop "Moringa and other highly nutritious plant sources: strategies, standards and markets for a better impact on nutrition in Africa, Accra, Ghana, Nov.16-18, 1-9. 2006.*
 61. Yasmeen, A., Basra, S.M.A., Ahmad, R and Wahid, A. Performance of late sown wheat in response to foliar application of *Moringa oleifera* Lam. leaf extract. *Chilean J. Agric. Res.* 2012; 72(1): 32-41.
 62. Yasmeen, A., Shahzad, M.A.B., Wahid, A., Farooq, M. Nouman, W., Rehman, H. and Hussain, N. Improving drought resistance in wheat (*Triticum aestivum*) by exogenous application of growth enhancers. *Int.J. Agric. Biol.* 2013; 15(6): 1307- 1312.
 63. Zhao, S.J., Xu, C.C. and Zou, Q. Improvements of the method for measurement of malondialdehyde in plant tissue. *Plant Physiol.* 1994; 30: 207-210.
 64. Zhang, X., Schmidt, R. Biostimulating turfgrass. *Ground Maintenance.* [http:// license. icopyright. net](http://license.icopyright.net), 1-4. 1999.