

## MAIN SULPHUR CONTENT IN ESSENTIAL OIL OF *ERUCA SATIVA* AS AFFECTED BY NANO IRON AND NANO ZINC MIXED WITH ORGANIC MANURE

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Profitable prospective of rocket plant is progressively growing recently. Hence an experiment was conducted in open field to evaluate the effect of chicken manure, nano iron (Fe), nano zinc (Zn) and combination of them on morphological, fresh weight and seed yield, photosynthetic rate, transpiration rate, water use efficiency and chemicals constituents represented in macro and micro elements, plant pigments, total phenolics, total carbohydrate, alkaloids, tannins, flavonoids, ascorbic acid, crude protein, total fatty acids, indole acetic acid (IAA) and abscisic acid (ABA) hormones, oil seed yield and methylthiobutyl-isothiocyanate as main sulphur content in essential oil of *Eruca sativa* Mill. compared to chemical fertilisers (NPK) as control. Results revealed that, nano Fe and Zn treatments either alone or in combination with manure had the upper hand, where significantly increased almost all parameters under study in comparison with control. The outcomes of present research gave emphasis to global warning about pollution of chemical fertilisers and safety production.

Key words: chemicals constituents, nano Fe and Zn, oil yield, photosynthetic rate, rocket plant

Rocket salad (*Eruca sativa* Mill.) is a well known fresh vegetable that belonged to the Brassica family. The consumption of vegetables as fresh salad has been increased worldwide because of the health consciousness. In Egypt, about 5,000 fadden (one fadden equal 4,200 m<sup>2</sup>) are cultivated with rocket plants (FAO 2000). Leaves of rocket are mainly used as an astringent, antiphlogistic, diuretic, tonic, depurative, emollient, laxative, digestive, stimulant, and rubefacient (Yaniv *et al.* 1998). Besides its importance as green salad available all over the year, rocket seeds contain oil which is promising to be medical oil (Khoobchandani *et al.* 2010). Similar to other Brassica crops, rocket salad is known for various phytochemical metabolites such as polyphenols, vitamin C (Kim *et al.* 2006; Martinez-Ballestra *et al.* 2008). It is rich in iron, potassium and sulfur also

contains elevated levels of proteins and vitamins A (Porto *et al.* 2013).

Chemical fertilisation has met with success only in some situations, and negative effects of chemical fertilisers application such as a high probability of leaching, spray drift (wind), volatilization, and high energy consumption for their creation, the risk of toxic build up of chemicals such as arsenic, cadmium, and uranium in the soil. Long-term use of chemical fertiliser can change the soil pH, hurt helpful microbial ecosystems, enhance pests, stimulation of the vegetative growth and reduction of soil water storage on plant growth in semi-arid region have been reported (Rai *et al.* 2015).

Several reports state the possibility of improving nutritional quality, polyphenols, antioxidants, anthocyanins, vitamins, and minerals in vegetables

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by the organic fertilisers (Peck *et al.* 2006). Among agronomical practices, the advantages of the utilization of organic manure for improving the physico-chemical properties of soil have been documented (Rudrappa *et al.* 2006). Numerous researchers showed the importance of manure on increasing crop production. Manure is loaded in nutrients and can provide all macronutrients (N, P, K, Ca, Mg and S) essential for plant growth, as well as micronutrients, furthermore, it creates suitable environment for the activity of soil microorganisms (Fageria 2012).

Micronutrients are nutrients required by plants in small quantities throughout their life to organize a range of physiological functions. Although these elements are used in very small amounts, they are important to plant development and profitable crop production as the macronutrients. The soil in semi-arid regions is characterized by poor structure, low organic carbon content, high pH, low cation exchange capacity and salinity/alkalinity problems, and these conditions increase micronutrient deficiencies (Marschner & Rengel 2012). Among the micronutrients iron and zinc are essential for plant growth and food production. Fe is involved in the production of chlorophyll, photosynthesis, mitochondrial respiration, hormone biosynthesis (ethylene, gibberellic acid and jasmonic acid) production (Marschner & Rengel 2012). Besides, zinc is a necessary component of various enzyme systems for energy production, protein synthesis; it maintains the structural integrity of biomembranes and growth regulation (Alloway 2008; Marschner & Rengel 2012).

In recent years, some researchers tried to examine the potential of nanotechnology to improve efficiency of micronutrients uptake that result in the development of efficient new nano fertiliser delivery systems for field application (Naderi & Danesh-Shahraki 2013; Rameshaiah & Jpallavi 2015). Nano-formulated fertilisers can release nutrients more slowly in cooperation with other fertilisers which may lead to improvement of nutrient use efficiency. The application of zinc nano fertiliser on pearl millet improved some properties such as shoot length, root length, chlorophyll content, total soluble leaf proteins and plant dry biomass. The grain yield at crop maturity was improved by 37.7% due to application of zinc nano-fertiliser in pearl millet (Tarafdar *et al.*

2014). Faizan *et al.* (2017) concluded that presence of nano Zn enhanced the antioxidant systems and improved photosynthetic efficiency in tomato. The best fertiliser treatment for obtaining high seed yield was identified in sulfur plus nano zinc on chickpea (Sabaghnia & Janmohammadi 2017). In *Brassica napus* (Zakerin *et al.* 2014) showed that, the maximum grain yield and oil yield were obtained from nano Zn + Fe foliar application on barley.

The present research aims to investigate alternative source of chemical fertilisers using application of manure, and combination of manure fertiliser with foliar application of nano-iron and zinc on morphological, yield, chemical compositions represented in Methyl-thiobutyl-isothiocyanate (molecular weight: 161.3, C<sub>6</sub>H<sub>11</sub>N S<sub>2</sub>) as a main component of essential oil and has been used in variety of medical (particularly breast and testis cancer), industry and cosmetic fields, beside fixed oil constitutes from seed yield of rocket plants.

## MATERIAL AND METHODS

Present investigation was done at Kafr Tuhurmis, El-Giza Governorate, with the latitude of 30.021074 and the longitude is 31.173145. Category with the Gps of 30° 1' 15.8664" N and 31° 10' 23.3220" E. Elevation is a 24 meters height, Egypt. For four continues repeated seasons during 2015 and 2016, every two cuts represented as one season. Mechanical and chemical analyses of soil were performed at Soil & Water and Environment Research Institute, Agriculture Research Center (A.R.C) according to Richards (1954) and Jackson (1973) shown in Table1.

### *Plant material, transplant and harvest dates*

Seeds of (*Eruca sativa* Mill.) were obtained from experimental farm of Faculty of Pharmacy, Cairo University, and planted in an open area with a distance of 40 cm between rows, and 30 cm spacing between plants in plots with 3 × 5 m<sup>2</sup>. The seeds were planted on February 5<sup>th</sup> and the first cut was taken on March 14<sup>th</sup>, while the second cut was taken on April 15<sup>th</sup> 2015, both two cuts considered as the first season. The seeds were planted again on February 5<sup>th</sup>, the first cut was taken on March 15<sup>th</sup>, whereas the second one was taken on April 16<sup>th</sup> 2016; both two cuts considered as the second season.

T a b l e 1

Some physical and chemical characteristics of clay soil used for growing *Eruca sativa* plant

| Parameter                    | Value |
|------------------------------|-------|
| Physical characteristics     |       |
| Texture                      | Clay  |
| Clay [%]                     | 40.50 |
| Silt [%]                     | 35.10 |
| Fine sand [%]                | 21.00 |
| Coarse sand [%]              | 3.40  |
| pH                           | 7.88  |
| EC [dS/m]                    | 1.63  |
| Organic matter [%]           | 1.70  |
| Chemical characteristics     |       |
| Soluble cations [meq/l]      |       |
| Ca <sup>++</sup>             | 7.22  |
| Mg <sup>++</sup>             | 2.98  |
| K <sup>+</sup>               | 0.33  |
| Na <sup>+</sup>              | 6.22  |
| Soluble anions [meq/l]       |       |
| Cl <sup>-</sup>              | 3.50  |
| SO <sub>4</sub> <sup>-</sup> | 2.45  |
| Available N [ppm]            | 30.10 |
| Available P [ppm]            | 22.50 |

### Land Preparation

Before planting, the soil was first mechanically ploughed and planked twice till the soil surface has been settled, then plots established.

T a b l e 2

Some chemical analysis of used chicken manure used

| Parameter                  | Value |
|----------------------------|-------|
| Total macronutrients [%]   |       |
| N                          | 2.60  |
| P                          | 0.70  |
| K                          | 1.15  |
| Total micronutrients [ppm] |       |
| Fe                         | 26.5  |
| Zn                         | 34.3  |
| Mn                         | 16.0  |
| Cu                         | 28.5  |
| pH                         | 7.60  |
| C/N ratio                  | 19.7  |
| Organic matter [%]         | 62.4  |

### Chemical fertilisers added

Chemical fertilisers as recommended dose according to Ministry of Agriculture and Land Reclamation were added at the rate of 50 kg/fed. (fadden equal 4,200 m<sup>2</sup>) of calcium superphosphate (15.5%) after 10 days from planting, while 50 kg/fed. ammonium sulphate (20.5%) and 20 kg/fed. of potassium sulphate (40%) were added after every cut.

### Chicken manure added

As Ministry of Agriculture and Land Reclamation recommended 5 tons/fed. chicken manure was added to the soil one week before plating.

### Nano Fe and Zn preparation and addition

Nano iron was prepared with little modify from magnetite (Fe<sub>3</sub>O<sub>4</sub>) of Fe<sup>3+</sup> and Fe<sup>2+</sup> at a molar ratio of 3:2 by reduction–precipitation in aqueous ammonia (0.3 mol/L) and coated tetramethyl ammonium hydroxide under vigorous stirring for 2 h using magnetic stirring, precipitate was then separated by magnetic filtration using a permanent magnet, and washed with distilled water until a neutral pH was obtained according to Qu *et al.* (1999). While nano zinc was prepared from aqueous solution of zinc sulfate and sodium hydroxide solution was added slowly in a molar ratio of 1:2 under vigorous stirring for 8 h. The precipitate obtained was filtered and washed thoroughly with ionized water. The precipitate was dried in an oven at 100°C, then expose to 15 psi of pressure for 6 hours (Daneshvar *et al.* 2007).

TEM (transmission electronic microscope) Figure 1 (a & b) was done in TEM lab (FA-CURP) Faculty of Agriculture Cairo University Research Park to determine the nano size.

Both Fe and Zn nano particles at concentrations 20 and 40 ppm were applied to *Eruca sativa* plants as foliar at 10, 17 and 24 days after planting in both seasons.

### Treatments

- NPK fertilisers (recommended doses) as control,
- Chicken Manure,
- Nano Fe and Zn,
- Nano Fe and Zn + chicken manure.

### Data recorded

- A. Plant growth parameters upon cuts
- Plant height [cm],

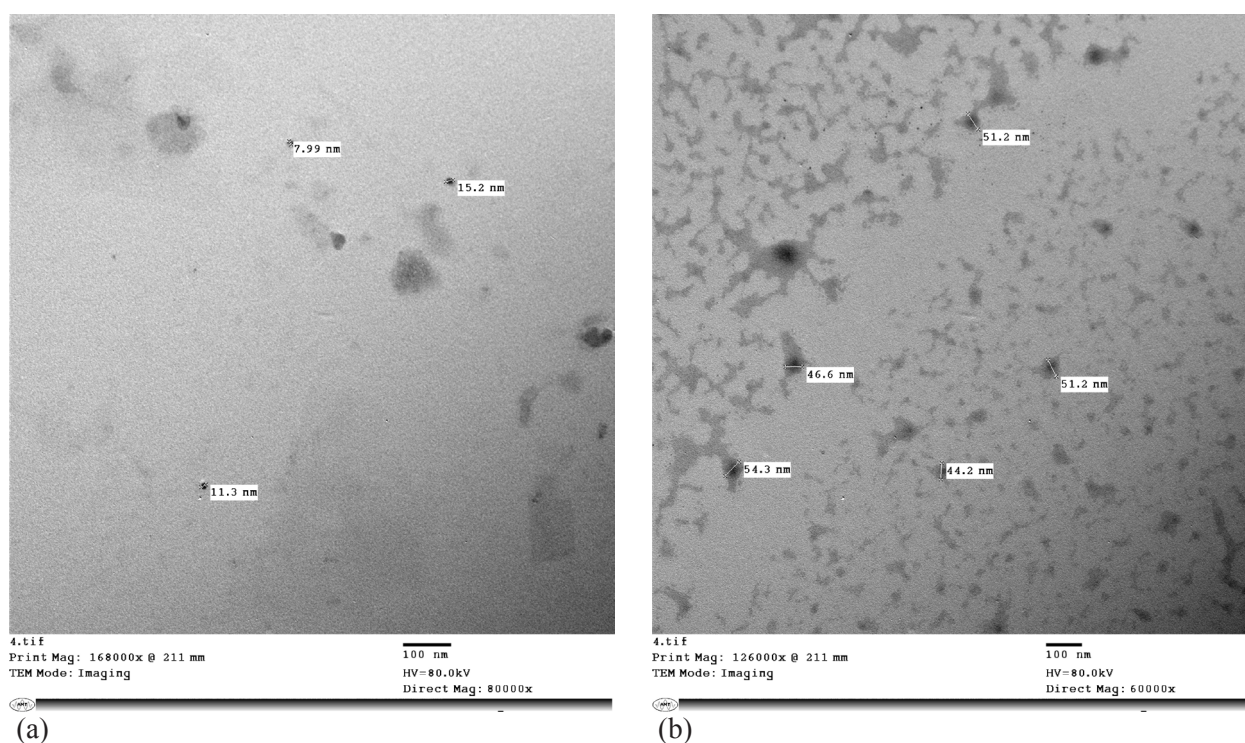


Figure 1. Zinc nano particles (a) – Iron nano particles (b)

- Leaf area [m<sup>2</sup>],
- Plant fresh weight [g],
- Plant dry weight [g],
- Yield fresh weight [t/fadden],
- Seed yield [kg].

*Ash percentage* was determined quantitatively by grinding 2 g of leaves sample and combusting the material in a muffle furnace at 550°C to constant weight. The total ash content was calculated using the following formula:

$$\% = \frac{A \times 100}{B}$$

where: A – weight of the ash in gram, B – weight of the anhydrous raw material in gram.

*Moisture content* was determined according to Tsand & Frutani (1989).

## B. Chemical analysis

*The total nitrogen* content of the dried leaves was determined by using the modified- micro-Kjeldahl

method as described by Helrich (1990). The nitrogen percentage was multiplied by 6.25 to estimate the crude protein percentages in leaves.

*Phosphorus* in leaves was determined calorimetrically by using the chloro-stannous molybdophosphoric blue color method in sulphuric acid according to Jackson (1973).

*Potassium* concentrations in leaves were determined by using the flame photometer apparatus (CORNING M 410, Germany).

Concentration of Ca, Mg, Fe, Mn, Cu, Ni, Pb and Zn were determined using Atomic Absorption Spectrophotometer with air-acetylene fuel (Pye Unicam, model SP-1900, US).

*Plant pigments – total chlorophylls and carotenoids* content were measured by spectrophotometer and calculated according to the equation described by Moran (1982).

*Total carbohydrates* in plant leaves were determined by phosphor molybdic acid method according to Helrich (1990).

*Total phenolic* contents of the extracts were determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method (Singleton & Rossi 1965).

*Vitamin – C* content as ascorbic acid [mg] was estimated in leaves fresh weight according to Helrich (1990) method using 2,6-dichloro phenolindophenol.

*The sugars* were separated on a chromatographic column filled with cation exchange resin (sulfonated polystyrene-divinylbenzene copolymer in the form of  $\text{Ca}^{2+}$ ). The mobile phase consisted of an aqueous solution of calcium disodium ethylene diamine tetraacetate. The eluted constituents were detected by a refractometric detector and determined by the external standard method.

*Total flavonoids* were determined using the method of Meda *et al.* (2005).

*Tannin contents* were determined using Folin-Ciocalteu reagent method as described by Chahardehi *et al.* (2009)

*Endogenous phytohormones* – the analysis was performed according to Fales and Jaouni (1973) for the determination of indole-acetic acid (IAA) and abscisic acid (ABA). The quantification of the endogenous phytohormones was carried out with Ati-Unicumgas–liquid chromatography, 610 Series, equipped with flame ionization detector according to the method described by Vogel (1975).

*Net photosynthesis, stomatal conductance and water use efficiency*

Measurements of net photosynthesis on an area basis [ $\mu\text{mol CO}_2 \text{ m}^2/\text{s}$ ], leaf stomatal conductance [ $\text{mol H}_2\text{O m}^2/\text{s}$ ], and water use efficiency of five different leaves per treatment was monitored using a LICOR 6400 (Lincoln, Nebraska, USA) infrared gas analyzer (IRGA). Light intensity (Photosynthetically active radiation, PAR) within the sampling chamber was set at  $1,500 \mu\text{mol m}^2/\text{s}$ , using a Li-6400-02B LED light source (LI-COR). The  $\text{CO}_2$  flow into the chamber was maintained at a concentration of  $400 \mu\text{mol/mol}$  using an LI-6400-01  $\text{CO}_2$  mixer (LI-COR).

*Essential oil in leaves*

The leaves from *E. sativa* were hydro-distilled for 3 hours with a Likens–Nickerson-type apparatus, using diethyl ether to get yellowish oil yield.

*Gas chromatography*

GC–MS was carried out with a Hewlett-Packard with a flame ionization detector (FID) on a capillary

column (TC-WAX FFS fused silica  $60 \text{ m} \times 0.25 \text{ mm}$  i.d.). The column temperature was programmed from  $60^\circ\text{C}$  to  $240^\circ\text{C}$  at a rate of  $3^\circ\text{C}/\text{min}$  and held at  $240^\circ\text{C}$ . The injector and detector temperatures were  $250^\circ\text{C}$  and  $280^\circ\text{C}$ , respectively.

*Fixed oil content [% of the seeds]*

Fixed oil was extracted from seeds by using a Soxhlet apparatus. The oil percentage was determined according to the methylation (change fixed oil into fatty acid) and GLC analysis was also recorded by GC according to Kinsella *et al.* (1977).

*GLC of fatty acid methyl esters*

Separation of fatty acid methyl esters was carried out using capillary column, which contained 15% diethyl glycol succinate (DEGS). The injector port and flame ionization detector were set at  $240^\circ\text{C}$ . The flow rate of carrier gas, nitrogen, was  $10 \text{ ml}/\text{min}$ . The gas chromatograph (Perkin-Elmer model 8310) had a temperature program from  $100$  to  $190^\circ\text{C}$  with interment rate of  $7^\circ\text{C}/\text{minute}$ . The initial and final time were identified according to their retention time compared to those of authentic samples.

*Statistical analysis*

The experimental design was randomized complete blocks design with ten replicates. The data were analysed using ANOVA at 5% significance level, the difference between treatments, then analysed using DMRT (Duncan Multiple Range Test) at level 5% (Duncan 1955).

## RESULTS AND DISCUSSION

*Growth parameters and yield*

Data related to growth parameters of the rocket plants using different types of fertilisers presented in Table 3 showed that, nano Fe and Zn combined with manure treatments had significantly stimulated growth characteristics with the highest values compared to all other treatments. The integrative nano Fe + Zn + manure application significantly increased plant height, leaf area, plant fresh and dry weight, moisture and ash percentage over either control (recommended dose of NPK fertilisers) or other treatments, and consequently the yield of rocket plants during both seasons.

According to Table 3, it is clear that mixed nano Fe and Zn with manure would not only result in high production of both fresh weight and seed yield but also improved the other desirable agronomic traits for market processes (plant height, leaf area, fresh weight and moisture). Moreover, the combination treatment significantly exceeded the growth parameter, and consequently the seed yield of rocket plants. This integrative addition exceeded seed yield by 26.6 and 14% as compared to NPK fertiliser in both seasons, respectively.

Results of the current study show that vegetative growth parameters were considerably improved by both manure and nano-micronutrient fertilisers. The association of manure and nano Fe + Zn fertilisers may complement the lack of some nutrients of manure fertiliser. Organic manures applied in integration with the inorganic fertilisers gave higher yield than sole chemical fertilizers (Badawi *et al.* 2005; Sarwar *et al.* 2008). The beneficial effects of

these treatments on rocket plant are due to nutrients availability to be absorbed by plant roots and improvement of soil's physical, chemical and biological properties as evident by higher water retention, decreased soil pH, and increased soil organic.

The findings of present research are consistent with those of El-Ghamry *et al.* (2009) who found that, farmyard manure plays an important role in supplying some essential plant nutrients. Bala *et al.* (2014) reported that the beneficial role of nano-fertilisers on growth of chickpea had significant effects compared to control without nano-fertilisers. Furthermore, Amirnia *et al.* (2014) have emphasized the positive effects of some micronutrient and macronutrients nano-fertilisers (iron, phosphorus and potassium) on saffron (*Crocus sativus* L.) production. Liu *et al.* (2006) reported that, nano-particles application was safe for wheat production and has some economic benefits and has also shown that nanoparticles get into plant cells through either sto-

T a b l e 3

Effect of NPK, manure and nano Fe and Zn on growth characters of rocket plants during two seasons

| Treatment             | Plant height [cm]       |                   | Leaf area [cm <sup>2</sup> ] |                    | Fresh weight /plant [g] |                    | Dry weight [g/plant] |                    |
|-----------------------|-------------------------|-------------------|------------------------------|--------------------|-------------------------|--------------------|----------------------|--------------------|
|                       | F1                      | F2                | F1                           | F2                 | F1                      | F2                 | F1                   | F2                 |
| NPK                   | 24.3 <sup>a</sup>       | 22.5 <sup>b</sup> | 67.16 <sup>b</sup>           | 69.43 <sup>b</sup> | 30.79 <sup>b</sup>      | 32.85 <sup>b</sup> | 7.32 <sup>b</sup>    | 8.41 <sup>b</sup>  |
| Manure                | 21.1 <sup>b</sup>       | 23.1 <sup>b</sup> | 66.85 <sup>b</sup>           | 70.20 <sup>b</sup> | 31.58 <sup>b</sup>      | 33.62 <sup>b</sup> | 8.61 <sup>b</sup>    | 8.55 <sup>b</sup>  |
| Nano Fe + Zn          | 20.7 <sup>b</sup>       | 22.2 <sup>b</sup> | 68.02 <sup>b</sup>           | 70.13 <sup>b</sup> | 32.45 <sup>b</sup>      | 33.92 <sup>b</sup> | 7.12 <sup>b</sup>    | 8.68 <sup>b</sup>  |
| Nano Fe + Zn + manure | 25.3 <sup>a</sup>       | 30.3 <sup>a</sup> | 71.11 <sup>a</sup>           | 75.42 <sup>a</sup> | 40.35 <sup>a</sup>      | 43.77 <sup>a</sup> | 10.36 <sup>a</sup>   | 12.36 <sup>a</sup> |
| Treatment             | Moisture [% dry matter] |                   | Ash [% dry matter]           |                    | Yield F. W. [t/fed.]    |                    | Seed yield [kg/fed.] |                    |
|                       | F1                      | F2                | F1                           | F2                 | F1                      | F2                 | F1                   | F2                 |
| NPK                   | 6.14 <sup>b</sup>       | 7.12 <sup>b</sup> | 16.22 <sup>b</sup>           | 17.31 <sup>b</sup> | 11.12 <sup>b</sup>      | 12.14 <sup>b</sup> | 310.2 <sup>c</sup>   | 364.5 <sup>c</sup> |
| Manure                | 7.24 <sup>b</sup>       | 7.15 <sup>b</sup> | 17.25 <sup>b</sup>           | 18.28 <sup>b</sup> | 11.51 <sup>b</sup>      | 12.73 <sup>b</sup> | 340.2 <sup>b</sup>   | 369.7 <sup>b</sup> |
| Nano Fe + Zn          | 6.18 <sup>b</sup>       | 7.14 <sup>b</sup> | 16.19 <sup>b</sup>           | 17.44 <sup>b</sup> | 11.14 <sup>b</sup>      | 12.79 <sup>b</sup> | 308.5 <sup>c</sup>   | 365.2 <sup>c</sup> |
| Nano Fe + Zn + manure | 8.15 <sup>a</sup>       | 9.23 <sup>a</sup> | 21.15 <sup>a</sup>           | 23.45 <sup>a</sup> | 14.29 <sup>a</sup>      | 15.30 <sup>a</sup> | 392.8 <sup>a</sup>   | 416.5 <sup>a</sup> |

Means with the same letter in a column are not significantly different by DMRT at level 5%

F1 – first season; F2 – second season

matal or vascular system. Our results also showed that the highest seed yield was related to nano Zn + Fe manure as compared to manure fertiliser alone. Nanoparticles with small size and large surface area are expected to be the ideal material for use as a fertiliser in plants. When materials are transformed to a nano-scale, their physical, chemical and biological characteristics as well as catalytic properties even more increased (Mazaherinia *et al.* 2010). In this subject Eichert *et al.* (2008) suggested that, stomatal pathway is highly capacitive because of its large size exclusion limit and its high transport velocity. These scientific reports support the present hypothesis of nanoparticle penetration plant cell through stomatal opening and natural nano-pore which may enhance plant cell metabolic activities hence, lead to higher plant production (Mosanna & Behrozyar 2015).

*Plant pigments, carbohydrates, phenols, alkaloids, tannins and flavonoids concentrations*

It is evident from Table 4, that manure fertiliser combined with nano Fe + Zn, lead to significant increases in the mean values of chlorophyll, carotenoids, carbohydrates, phenols, alkaloids, tannins and flavonoids concentrations compared with the NPK treatment (control). Total chlorophyll statistically increased by (29.5 and 25.4%) over control during both seasons, respectively, while the increase

was bigger than double in concentration of carotenoids. It reached (128 and 120%) during both seasons compared with control. For the carbohydrates and phenols percentage, the presented data revealed that the combined treatment of nano Fe + Zn + manure enhanced the accumulation by (30.3–27.8%) and (31.9–30.4%), respectively as compared to control plants during the two seasons. The same trend was found for alkaloids, tannins and flavonoids percentage.

Previous researches indicated that, fertilisation type had an influence on the phyto-nutritional quality of crops. Chemical fertilisers are believed to reduce the antioxidant levels while organic fertilisers were proven to enhance the antioxidant content in plants (Dumas *et al.* 2003). In tomato, the presence of zinc oxide nano-particles improved the antioxidant systems and photosynthetic efficiency as well (Faizan *et al.* 2017). The increase in total carbohydrates may be due to the increase of photosynthesis (Figure 2) as a result of increase in photosynthetic pigments content in leaves (Table 4). Manure appears to be a source of a number of essential elements that may play an important role in plant metabolism, notably the most significant function would appear to involve carbohydrate metabolism and photosynthesis (Tisdale & Nelson 1975). These results are in agreement with those obtained from Leclerc *et al.*

T a b l e 4

Effect of NPK, manure and nano Fe and Zn on chemical characters of rocket plants during two seasons

| Treatment             | Total chlorophyll<br>F.W. [mg/g] |                    | Total carotenoids<br>F.W. [mg/g] |                   | Total carbohydrates<br>[%] |                    | Total phenols<br>[%] |                    | Total alkaloids<br>[%] |                    | Tannins<br>[%]     |                    | Total flavonoids<br>[%] |                    |
|-----------------------|----------------------------------|--------------------|----------------------------------|-------------------|----------------------------|--------------------|----------------------|--------------------|------------------------|--------------------|--------------------|--------------------|-------------------------|--------------------|
|                       | F1                               | F2                 | F1                               | F2                | F1                         | F2                 | F1                   | F2                 | F1                     | F2                 | F1                 | F2                 | F1                      | F2                 |
| NPK                   | 28.42 <sup>c</sup>               | 30.51 <sup>c</sup> | 3.03 <sup>c</sup>                | 3.58 <sup>c</sup> | 17.43 <sup>b</sup>         | 19.42 <sup>c</sup> | 58.29 <sup>c</sup>   | 61.58 <sup>c</sup> | 10.43 <sup>c</sup>     | 12.46 <sup>c</sup> | 51.42 <sup>b</sup> | 48.11 <sup>c</sup> | 20.52 <sup>b</sup>      | 18.41 <sup>c</sup> |
| Manure                | 30.58 <sup>b</sup>               | 32.71 <sup>b</sup> | 4.88 <sup>b</sup>                | 5.61 <sup>b</sup> | 18.39 <sup>b</sup>         | 21.52 <sup>b</sup> | 63.29 <sup>b</sup>   | 66.41 <sup>b</sup> | 12.82 <sup>b</sup>     | 13.71 <sup>b</sup> | 47.33 <sup>c</sup> | 50.30 <sup>b</sup> | 20.39 <sup>b</sup>      | 22.58 <sup>b</sup> |
| Nano Fe + Zn          | 34.21 <sup>b</sup>               | 37.08 <sup>a</sup> | 4.57 <sup>b</sup>                | 5.49 <sup>b</sup> | 17.58 <sup>b</sup>         | 21.17 <sup>b</sup> | 60.72 <sup>c</sup>   | 62.35 <sup>c</sup> | 12.24 <sup>b</sup>     | 12.44 <sup>c</sup> | 45.97 <sup>c</sup> | 51.21 <sup>b</sup> | 22.68 <sup>a</sup>      | 22.79 <sup>b</sup> |
| Nano Fe + Zn + manure | 36.79 <sup>a</sup>               | 38.26 <sup>a</sup> | 6.92 <sup>a</sup>                | 7.89 <sup>a</sup> | 22.75 <sup>a</sup>         | 24.83 <sup>a</sup> | 76.91 <sup>a</sup>   | 80.31 <sup>a</sup> | 14.09 <sup>a</sup>     | 14.85 <sup>a</sup> | 52.67 <sup>a</sup> | 53.27 <sup>a</sup> | 23.95 <sup>a</sup>      | 25.78 <sup>a</sup> |

Means with the same letter in a column are not significantly different by DMRT at level 5%  
F1 – first season; F2 – second season

(1991) on carrot and Soliman & Mahmoud (2013) on *Adansonia digitata* L. Zn application in wheat could alleviate the oxidative stress of wheat through transcriptional regulation of multiple defense pathways, such as antioxidant enzymes and flavonoid secondary metabolism (Ma *et al.* 2017). Organic fertilisation has a stimulatory effect on accumulation of phenolics in fennel; it is well known that, the higher concentrations of phenolics can be explained by the role of organic fertilisers inducing the acetate shikimate pathway, resulting in higher production of flavonoids and phenolics (Sousa *et al.* 2008). Furthermore, the chlorophyll concentration noticeably responded to nutrients management and the highest value was obtained by combined application of manure and nano Fe plus Zn. There is a close relationship between the capacity of photosynthetic carbon dioxide fixation and the chlorophyll concentration. Therefore, the increase in the chlorophyll concen-

tration can be considered equivalent to the increased source strength represented in the export rate of a photo-assimilates from the source tissue (White *et al.* 2016).

#### Photosynthetic rate, transpiration rate and water use efficiency

Data of photosynthetic rate, transpiration rate and water use efficiency of the rocket plants fertilised with NPK, manure fertiliser or nano Fe + Zn individually and in combination with manure are presented in Figure 2. It was cleared that application of nano Fe + nano Zn fertilisers mixed with manure fertiliser significantly increased photosynthetic rate and water use efficiency (WUE) as compared to control in both seasons. Although, the application of NPK or manure treatments or nano Fe plus nano Zn individually were observed to show in significant differences for photosynthetic rate and water use efficiency. Application of nano Fe + nano Zn mixed with manure fertiliser significantly increased WUE which reached 5.26 and 5.32  $\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$  as compared with control (2.10 and 2.3  $\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$ ) in both seasons, respectively.

This might be due to the greater yield obtained where the capacity of the plant to store the moisture would also increase its water use and converting into the yield per unit of water applied (Behesh-ti & Fard 2010). The inorganic amendments were also found to have greater WUE over the control. Karasahin (2015) reported that, greater water use efficiency by the use of inorganic fertilisers than the organic manures. Nitrogen fertiliser increases the efficiency of water use by wheat (Deng *et al.* 2004). Positive effect on WUE was reported with Subhan *et al.* (2017) who found that, the inorganic fertilisers gave significant higher total dry matter, grain and straw yield and also due to the greater grain yield. The calculated WUE was greater in the NPK treatment as compared with organic manure; it's worth mentioning that, WUE is important parameters in the water scarce areas.

Enhancement values in photosynthetic rate 34.3 and 25.8% (Figure 2) with same previous mixed application may be due to increasing of leaf area (5.9 and 8.6%) (Table 3) and chlorophyll content (29.5 and 25.4%) over control (Table 4). Meanwhile, leaf numbers and leaf area are noticeably affected by

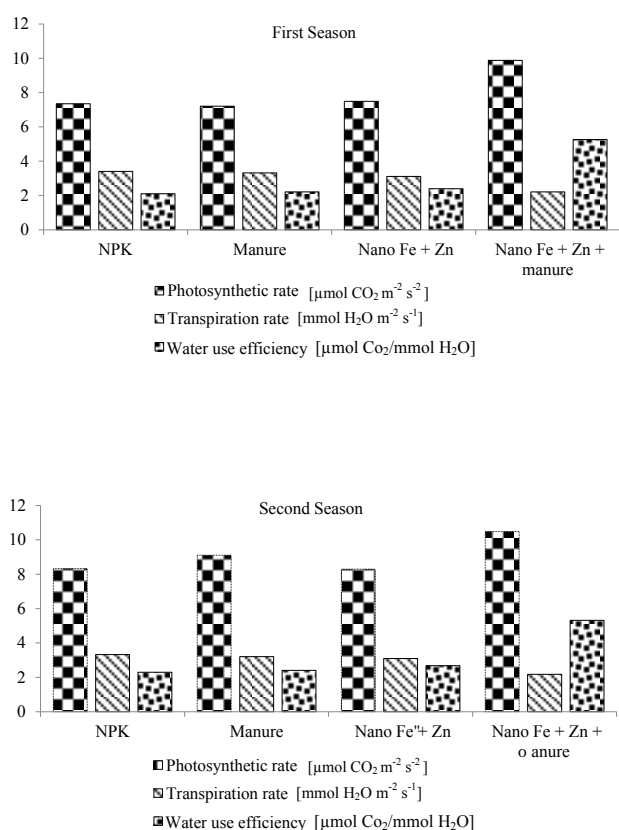


Figure 2. Effect of NPK, manure and nano Fe, nano Zn treatments on photosynthetic rate, transpiration rate and water use efficiency of *Eruca sativa* plant during two seasons

both organic and inorganic fertilisers. The leaves are the most important photosynthetic organ and assimilate supplying source that play critical roles in light interception, evapotranspiration and significant response to fertilisers (Pandey & Singh 2011). Increasing leaf area can directly affect the rate of photosynthetic (Evans & Sadler 2007). The chlorophyll contents were enhanced by (20.71%), and the net photosynthetic rate was promoted by (31.87%) as compared with the control in spinach plants (Gao *et al.* 2006).

#### Macro and micro nutrients concentrations

When investigated Tables 5 and 6, we could found that a significant increase in N, P, and K concentrations were recorded as a result of nano Fe + nano Zn + manure application over control (NPK). In both seasons, combination treatment showed an increase in N (61 and 35%), P (33 and 55%), K (55 and 49%), respectively compared to control treatments. Also the same treatment showed an increase concentrations of N (29.7 and 27.6%), P (71 and 65.5%), and K (113.6 and 106.8%), respectively compared to manure treatment alone. In addition to these results, with combination treatment Mg content was increased by 25 and 20% as compared to NPK treatment. Generally, addition of nano Fe + nano Zn alone led to reduce the accumulation of all macro-elements in comparison with other treatments. The results of combined treatment application as well as individual treatment of manure or NPK were increased Ca content significantly than the results of addition of nano Fe + nano Zn alone.

Meanwhile, Mn, Fe, Cu, Zn, Pb and Ni exhibited an increasing trend in response to manure application (Table 6).

Several recent articles have articulated the benefits of nano-scale micronutrients to crop yield enhancement as well as the accumulation of nutrients. For example, Nasri *et al.* (2010) stated that, the use of iron and zinc nano-nutrients caused 12% increment in amount of nitrogen in corn grain, indicating the role of these elements in increasing nitrogen percentage. Raliyaan & Tarafdar (2013) and Raliya *et al.* (2016) reported that increases in plant growth, yield, and nutrient content upon treatment with nano ZnO. Similarly, Subbaiah *et al.* (2016) demonstrated that, nano ZnO increased growth, yield, and Zn content of maize. Still, the agronomic effectiveness of nano practical compared to non-nano forms of micronutrients has yet to be fully resolved (Mahmoud *et al.* 2017).

On the contrary, according to Trinchera *et al.* (2008) chemical fertilisation is able to supply greater amounts of nitrogen to plants in a brief period to improve better metabolism without needing for additional activity of enzymes, however, in case of plants receiving the organic fertilisers, may face a condition of slowly releasing the available nitrogen. This slow release of N may be considered as adverse environmental conditions due to nutrient deficiency.

#### Main sulphur content, glucose, fructose, ascorbic and crude protein contents

Going with main sulphur content represented in

T a b l e 5

Effect of NPK, manure and nano Fe and Zn on macro-elements of rocket plants during two seasons

| Treatment             | N [%]             |                   | P [%]             |                   | K [%]             |                   | Mg [%]            |                   | Ca [%]            |                   |
|-----------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                       | F1                | F2                | F1                | F2                | F1                | F1                | F2                | F1                | F2                | F1                |
| NPK                   | 2.33 <sup>b</sup> | 2.97 <sup>c</sup> | 0.27 <sup>b</sup> | 0.31 <sup>b</sup> | 3.30 <sup>b</sup> | 3.61 <sup>b</sup> | 0.12 <sup>b</sup> | 0.15 <sup>b</sup> | 1.25 <sup>a</sup> | 1.36 <sup>a</sup> |
| Manure                | 2.89 <sup>b</sup> | 3.15 <sup>b</sup> | 0.21 <sup>c</sup> | 0.29 <sup>b</sup> | 2.19 <sup>c</sup> | 2.78 <sup>c</sup> | 0.15 <sup>a</sup> | 0.17 <sup>a</sup> | 1.26 <sup>a</sup> | 1.38 <sup>a</sup> |
| Nano Fe + Zn          | 1.94 <sup>c</sup> | 2.85 <sup>c</sup> | 0.19 <sup>c</sup> | 0.25 <sup>c</sup> | 2.17 <sup>c</sup> | 2.58 <sup>c</sup> | 0.10 <sup>b</sup> | 0.13 <sup>c</sup> | 1.24 <sup>b</sup> | 1.35 <sup>b</sup> |
| Nano Fe + Zn + manure | 3.75 <sup>a</sup> | 4.02 <sup>a</sup> | 0.36 <sup>a</sup> | 0.48 <sup>a</sup> | 4.68 <sup>a</sup> | 5.75 <sup>a</sup> | 0.15 <sup>a</sup> | 0.18 <sup>a</sup> | 1.34 <sup>a</sup> | 1.49 <sup>a</sup> |

Means with the same letter in a column are not significantly different by DMRT at level 5%  
F1– first season; F2 – second season

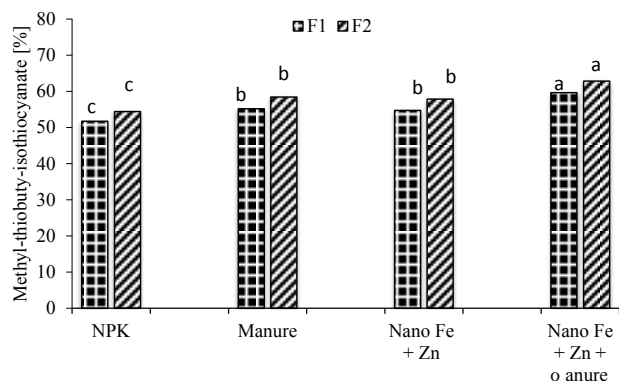


Figure 3. Effect of NPK, manure and nano Fe and Zn treatments on main sulphur content  
F1 – first season; F2 – second season

methyl-thiobutyl-isothiocyanate (Figure 3), glucose and fructose contents (Table 7), significantly increased due to application of either manure fertiliser alone or nano Fe + Zn or combination treatment over control. It was found that, combination treatment had a profound effect on methyl-thiobutyl-isothiocyanate (15.3 and 15.5%) during both seasons, respectively in comparison to control plants. Also the maximum improvements in ascorbic and crude protein were observed due to manure fertiliser and combination treatment applied of manure and nano Fe + Zn over both control and nano Fe + Zn individually. The integrative treatment increased crude protein, glucose and fructose contents (61 and 36%), (31 and 30%), (90 and 98.5%), respec-

T a b l e 6

Effect of NPK, manure and nano Fe and Zn on micro-elements of rocket plants during two seasons

| Treatment             | Mn [ppm]           |                    | Fe [ppm]           |                    | Cu [ppm]          |                   | Zn [ppm]          |                   | Pb [ppm]          |                   | Ni [ppm]          |                   |
|-----------------------|--------------------|--------------------|--------------------|--------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                       | F1                 | F2                 | F1                 | F2                 | F1                | F2                | F1                | F2                | F1                | F2                | F1                | F2                |
| NPK                   | 103.1 <sup>c</sup> | 107.3 <sup>c</sup> | 90.2 <sup>c</sup>  | 99.4 <sup>c</sup>  | 22.6 <sup>a</sup> | 19.3 <sup>a</sup> | 28.3 <sup>c</sup> | 30.5 <sup>c</sup> | 9.33 <sup>b</sup> | 7.21 <sup>b</sup> | 0.12 <sup>b</sup> | 0.11 <sup>b</sup> |
| Manure                | 110.8 <sup>b</sup> | 111.5 <sup>b</sup> | 112.3 <sup>b</sup> | 114.1 <sup>b</sup> | 20.8 <sup>b</sup> | 23.3 <sup>a</sup> | 31.7 <sup>b</sup> | 33.4 <sup>b</sup> | 11.7 <sup>a</sup> | 13.5 <sup>a</sup> | 0.21 <sup>a</sup> | 0.13 <sup>a</sup> |
| Nano Fe + Zn          | 104.4 <sup>c</sup> | 104.7 <sup>d</sup> | 110.2 <sup>b</sup> | 113.3 <sup>b</sup> | 22.1 <sup>a</sup> | 20.7 <sup>a</sup> | 34.5 <sup>a</sup> | 36.4 <sup>a</sup> | 7.12 <sup>c</sup> | 6.09 <sup>b</sup> | 0.10 <sup>c</sup> | 0.09 <sup>c</sup> |
| Nano Fe + Zn + manure | 112.4 <sup>a</sup> | 118.7 <sup>a</sup> | 114.8 <sup>a</sup> | 117.9 <sup>a</sup> | 24.9 <sup>a</sup> | 24.8 <sup>a</sup> | 35.4 <sup>a</sup> | 38.7 <sup>a</sup> | 12.5 <sup>a</sup> | 13.2 <sup>a</sup> | 0.19 <sup>a</sup> | 0.14 <sup>a</sup> |

Means with the same letter in a column are not significantly different by DMRT at level 5%

F1 – first season; F2 – second season

T a b l e 7

Effect of NPK, manure and nano Fe and Zn on chemical constituents of rocket plants during two seasons

| Treatment             | Ascorbic acid [mg/100 g] |                    | Crude protein [%]  |                    | Glucose [%] [g/100 g F.W.] |                   | Fructose [%] [g/100 g F.W.] |                   |
|-----------------------|--------------------------|--------------------|--------------------|--------------------|----------------------------|-------------------|-----------------------------|-------------------|
|                       | F1                       | F2                 | F1                 | F2                 | F1                         | F2                | F1                          | F2                |
| NPK                   | 48.55 <sup>c</sup>       | 51.82 <sup>c</sup> | 14.56 <sup>c</sup> | 18.50 <sup>c</sup> | 1.16 <sup>c</sup>          | 1.24 <sup>c</sup> | 1.26 <sup>c</sup>           | 1.4 <sup>c</sup>  |
| Manure                | 50.11 <sup>b</sup>       | 53.71 <sup>b</sup> | 18.06 <sup>b</sup> | 20.1 <sup>b</sup>  | 2.2 <sup>b</sup>           | 2.46 <sup>b</sup> | 1.96 <sup>b</sup>           | 2.24 <sup>b</sup> |
| Nano Fe + Zn          | 47.38 <sup>c</sup>       | 50.94 <sup>c</sup> | 12.12 <sup>d</sup> | 17.81 <sup>c</sup> | 4.4 <sup>a</sup>           | 3.68 <sup>b</sup> | 2.14 <sup>a</sup>           | 1.76 <sup>c</sup> |
| Nano Fe + Zn + manure | 54.92 <sup>a</sup>       | 56.38 <sup>a</sup> | 23.43 <sup>a</sup> | 25.12 <sup>a</sup> | 4.74 <sup>a</sup>          | 5.02 <sup>a</sup> | 2.56 <sup>a</sup>           | 2.78 <sup>a</sup> |

Means with the same letter in a column are not significantly different by DMRT at level 5%

F1 – first season; F2 – second season

tively compared with NPK for both seasons.

The increase in sulphur, ascorbic, crude protein, glucose and fructose contents may be due to advantageous effects of the manure and nano Fe + Zn resulting in extra release of nutrients in an accessible form for plant uptake, which resulted in higher efficiency of the photosynthesis process, increasing synthesis of afore mentioned chemical constituents.

Results in Figure 4 revealed that, manure fertilisation had a great impact on formation of free fatty acids content, meanwhile application of either manure only or nano Fe + Zn + manure resulted in a significant increase in free fatty acids content of rocket seed as compared to NPK treatment during the two seasons. The highest lauric, oleic and behenic acids contents were observed with both manure fertiliser alone and nano Fe + Zn + manure in 1<sup>st</sup> and 2<sup>nd</sup> seasons as compared with NPK. While, the highest accumulation of palmitic, eicosenoic and erucic acids were obtained with combination

treatment compared with other treatments. As for the individual treatment of nano Fe + nano Zn, produced the highest percentage of linoleic acid in the second season, whereas the combination treatment produced the best amount in the first season.

The increase in essential oil productivity with manure alone or combination treatment compared with control treatment could be explained on the basis of available elements, vitamins, hormone-like substances, amino acids and sugars, which have a significant effect on the physiological or biochemical processes within the plant and consequently boost essential oil yield. These results coincide with those obtained by Mahmoud (2012) on yarrow plant (*Achillea millefolium*) who stated that application of compost mixed with zeolite, humic acids and biofertilisers increased oil yield. In sunflowers under non-manure conditions, the application of the nanofertilisers could not affect the oil content, while the application of nano Zn and Fe under manure conditions significantly improved oil yield (Janmohammadi *et al.* 2016). Other researchers found that combination of the organic sources with inorganic nutrients gives higher oil content in rapeseed and Indian mustard (Tripathi *et al.* 2011). It has been revealed that nutrient management can affect the fatty acids biosynthesis pathway in oil crops and it seems that under nutritional imbalance a large proportion of photosynthates are diverted to protein formation. Deficiency of carbohydrates may stimulate the degradation fatty acids to acetyl co-enzyme A and it leads to decreased oil content (Marschner & Rengel 2012). Also, Mahmoud *et al.* (2017) concluded that application of organic fertilisers, natural soil amendments and chemical fertilisers (nano form) produced higher growth characteristics, chemical composition and oil yield in comparison with results derived from chemical fertilisers NPK on caraway plant.

#### Phytohormones concentrations

The results of hormonal analysis represented in abscisic acid (ABA) and indole acetic acid (IAA) in rocket plant as affected by different treatments are displayed in Figures 5 and 6. The increase of growth parameter was associated with high level of growth promoter (IAA) and low level of ABA. Hence it was found that nano Fe + nano Zn under manure fertiliser treatment gave significantly the highest content

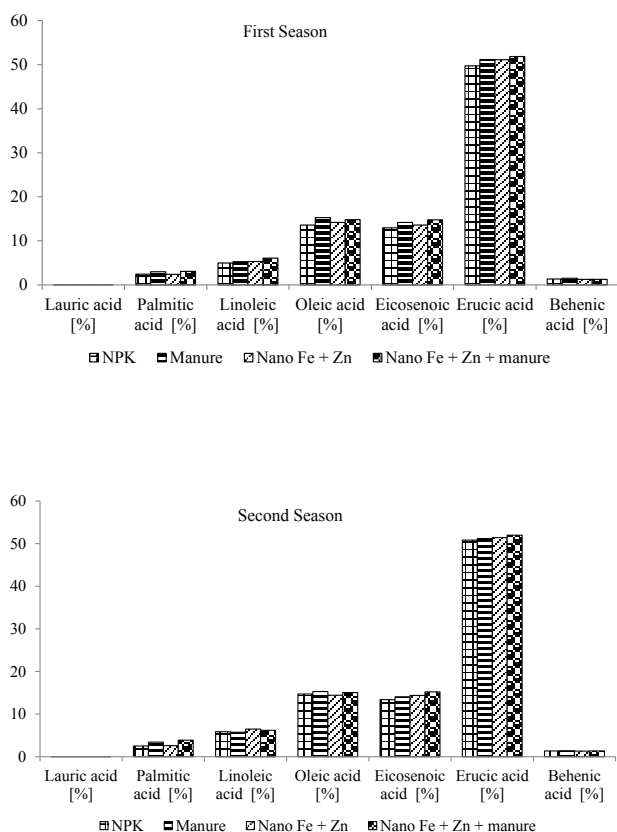


Figure 4. Effect of NPK, manure and treatments on percentage of some fatty acids content of seed oil of rocket plants during two seasons

of IAA over all other treatments including control (NPK) in both season. The increases were mainly (52 and 92.7%), respectively over control in the 1<sup>st</sup> and 2<sup>nd</sup> seasons.

It was established that, zinc was considered as an essential micronutrient for normal growth, development, and health of plants and human. Zinc enhances cation-exchange capacity of the roots, which in turn enhances absorption of essential nutrients, especially nitrogen which is responsible for higher protein content. Zinc plays a vital role in carbohydrate and proteins metabolism as well as it controls plant growth hormone IAA. Zn is also an essential component of dehydrogenase, proteinase, and peptides enzymes as well as promotes starch formation, seed maturation and production (Fageria *et al.* 2002). Besides, it controls the synthesis of indole acetic acid, a phytohormone which intensely regulates the plant growth, as well as necessary for chlorophyll synthe-

sis and carbohydrate formation (Vitosh *et al.* 1994). With reference to manure that may contains beneficial microorganisms which increase crop growth by secretion and synthesized phytohormones (Yamada & Xu 2001).

Concerning ABA content, in the first season, both nano Fe + Zn under manure fertiliser treatment and control treatment (NPK) significantly resulted in the lowest ABA content compared with manure fertiliser alone and nano Fe + Zn alone. In contrast, in the second season, it was clear that nano Fe + Zn mixed with manure significantly resulted in the lowest ABA in comparison with all other treatments. The above-mentioned results are in consonance with those obtained by Soliman and Mahmoud (2013) on *Adansonia digitata* L. The stimulated effects of mixed treatment may be attributed to the production of hormones like substances from manure fertiliser. As mentioned by Mehnaz *et al.* (2001), who reported that IAA produced by rhizobacteria as plant growth promoting is believed to increase root growth and root length, resulting in better nutrient uptake present in organic substances.

Marek and Skorupska (2001) provided evidence that *Bacillus* sp. produced hormones, which effectively reverse a chemical-induced inhibition of stem growth, working together in the presence of organic fertilisers.

## CONCLUSIONS

The achieved results from this investigation are sturdily verify that mixture of nano Zn and Fe with manure were boosted growth characters, yield whether seed oil or leaves fresh weight and chemical constituents with concern on main sulphur methyl-thiobutyl-isothiocyanate compound of rocket plant besides antioxidant compounds (ascorbic acid, total phenolics, total flavonoids and carotenoids) as compared to commercial fertilisers NPK. Particularly if we took into consideration that *Eruca sativa* plant enters variety of high economic pharmaceutical and cosmetics industries. Consequently, it could conclude that nano and organic fertilisers could replace chemical for improving the quality of the produced crops in addition to decreasing the production costs and environmental pollution.

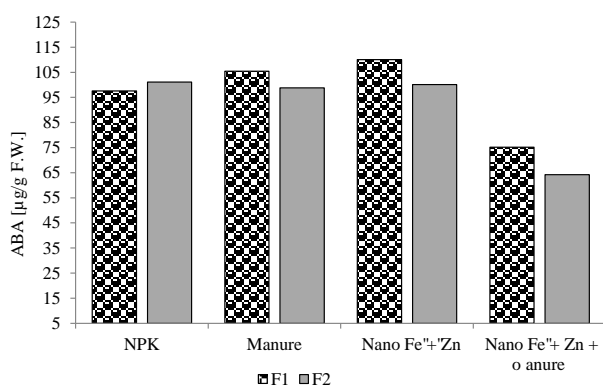


Figure 5. Effect of NPK, manure and nano Fe and Zn on ABA concentration of rocket plants during two seasons F1 – first season; F2 – second season

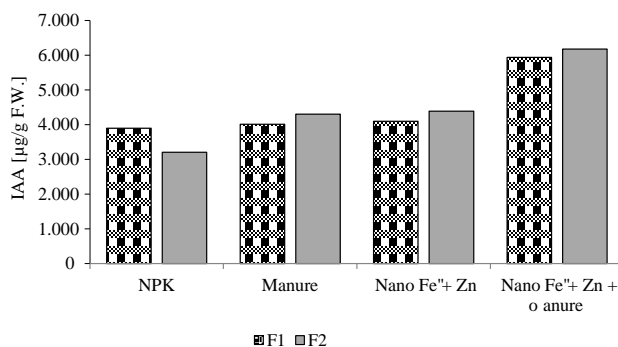


Figure 6. Effect of NPK, manure and nano Fe and Zn on IAA concentration of rocket plants during two seasons F1 – first season; F2 – second season

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