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# Anticancer Activity, Antioxidant Activity, Mineral Contents, Vegetative and Yield of *Eruca sativa* Using Foliar Application of Autoclaved Cellular Extract of *Spirulina platensis* Extract, Comparing to N-P-K Fertilizers.

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## ABSTRACT

Two field experiments of rocket (*Eruca sativa* Mill) were conducted during the two seasons of 2015 and 2016 at the Experimental Station Farm of the Faculty of Agriculture, Alexandria University, at Abeis, Alex. Governorate, A.R.E, to study the effect of autoclaved cellular content of *Spirulina platensis* as a foliar spray, comparing to N/P/K chemical fertilizer, on vegetative growth, yield, antioxidant, mineral contents and the cytotoxic effect of rocket cultured after harvested against the human cancer cells A549, and HepG2. Seeds of rocket were sown on 25th of March, in both seasons. Four treatments were investigated in this experiment; three treatments from extract of *S. platensis* (5%, 10%, and 15%) and the fourth one was NPK fertilization as a control treatment. The results indicated that the highest significant plant height was observed with the foliar spraying *of S. platensis* at the rate of 15 % (43.33 and 43.63 cm, respectively). However, *S. platensis* (5 %) exhibited the lowest significant plant height (35.00 and 36.66 cm, respectively). The highest significant number of leaves was recorded with *S. platensis* at the rate of 5 % (8.33 and 9.00, respectively). On the other hand, N-P-K control treatment showed the lowest significant number of leaves (6.67 and 7.00, respectively). Also, the results indicated that treatment of *S. platensis* 10 % was responsible for the highest significant total yield, in both seasons (2.32 and 2.29 kg/m$^2$, respectively), followed by *S. platensis* 5 % (2.03 and 2.08 kg/m$^2$, respectively).On the other side, the treatment of *S. platensis* 5% exhibited the highest positive significant antioxidant activities of all five assays, except for DPPH. In general, under the conditions of this study, it could be concluded that, the foliar spraying of 5% conc. *S. platensis* three times (10, 18 and 26 days after sowing), achieved the best significant quantitative (vegetative growth and yield) and qualitative (total antioxidant activity) characteristics of rocket plants. Interestingly, this concentration achieved anticancer activity (61.3 %) against hepatocellular carcinoma cell line (HepG2). In addition, this treatment is very safe for human, decreased the applied NPK-dose by 100 %. This in turn would reduce the costs, environmental pollution and improving the human health.

**Keywords:** *Eruca sativa, S. platensis,* vegetative growth, yield, mineral contents, Anticancer activity, antioxidant activity,

## INTRODUCTION

Rocket (*Eruca sativa* Mill*)* is an annual vegetable crop belonging to the mustard family (*Brassicaceae*). Common names include salad rocket, garden rocket, arugula (English); salatrauke (German); eruca (Spanish), roquette (French), rucola (Italian), and garger (Arabic). Rocket is traditionally grown in Italy, Portugal, Egypt, and Turkey (Dolezalova *et al.,* 2013). The sprouts and leaves of *E. sativa* are widely used in salads due to their hot pungent taste and can add as a savor to the salad. Interestingly, it contains high levels of antioxidants which has been shown to have powerful anti-cancer properties (O'Hare *et al.,* 2005). Moreover, rocket is the richest natural source of a compound called phenylethylisothiocyanate (PEITC), which gives the plant its unique peppery flavor. Rocket is used in many ways other than a food, like seed production and subsequent oil extraction. It contains glucosides, mineral salts and vitamin C. Therefore, it considered to be an excellent stimulant, diuretic, antiscorbutic, stomachic, cytoprotective, antiulcer and activities antisecretory (Dolezalova *et al.,* 2013). More recently, results of Alruwaih (2016) pointed out that *E. sativa* has a wide spread medicinal use as an astringent, diuretic, digestive, emollient, tonic, depurative, laxative, rubefacient, and stimulant purposes.

Cyanobacterium *Spirulina platensis*, blue green algae, are considered one of the most important sources of nitrogen fixation and have the ability to convert their nitrogen content into bioavailable forms of ammonium required for plant growth. These microorganisms play an important role in enhances the crop productivity, due to their unique composition and metabolic products (Grzesik and Romanowska-Duda, 2015). It is very well known that the using of chemical fertilizer is negatively affecting plant, soil, and environment. Therefore, in recent years, there is a global interest to increasing organic farming to reduce this adversely affects (Uysal *et al.,* 2015).In recent years, more and more worldwide concern has been obtained on the use of *Cyanobacteria* to intensify organic agriculture production. The use of *Cyanobacteria* as biofertilizers has many advantages, such as environmentally friendly, increases crop productivity, improve plant physiological activity, has useful potential in practice and has high economic value, although literature concerning this issue is insufficient (Sahu *et al.,*2012).

*Cyanobacterium*, a filamentous blue green algae, has wide range of potential applications, due to their metabolic products and their unique chemical and biochemical composition, such as food supplements, animal feeds, lipids Omega 3 production, enzymes, biomass, polymers, toxins, pigments, wastewater treatment, and green energy products (Wuang *et al.,* 2016). *Spirulina* is culture in alkaline environmental conditions and this priority prevents the external contamination by pathogenic bacteria and fungi, which make it suitability for environmental applications. *Spirulina* has also been suggested as a good alternative source to chemical fertilizers as well as a good protein supplement in livestock feeds. The world production of *Spirulina* is estimated to be about 3000 ton/year (Habib *et*

*al.,* 2008). Furthermore, A great number of substances and bioactive compounds extracted *S. platensis* can influence plant growth and development *S. platensis* have been reported to contain nutrients, sugar, and amino acids, and to benefit plants by producing growth-promoting bioregulators, vitamins, amino acids, and other secondary metabolites (Markou and Nerantzis, 2013). *Spirulina platensis*, a blue green microalga, has been used since ancient times as a source of food because of its high protein content (65%) and nutritional value. Lipids isolated from *S. platensis* have been shown to contain high levels of polyunsaturated fatty acids, including linolenic acid which is a precursor of arachidonic acid; this cyanobacteria contains, also, several kinds of sterols (Bensehaila *et al.,* 2015). Many different extraction processes of *S. platensis*, and even others microalgae, as a foliar spray applications were investigated by many authors; dried cells by 90% ethanol (Kumar *et. al.,* 2014), dry cells with 0.1% Tween solution (Abd El Baky *et al*., 2014), wet weight cells by physical extraction (freeze-thaw), or dry cells by hot water at 90°C/400 rpm for different extraction time (1h, 2h, 3h or 4h), and dry cells by autoclaving (121°C, 30min) for the extraction of *S. platensis* polysaccharides as biostimulant of plant growth, as recorded by Elarroussia *et al.* (2016).The autoclaving process, as a microalgae extraction method, is occurring damage in microalgal cell wall. As a result of this process, all the extra cellular contents of microoalgal cellsare releasing outside the cell wall and diffused in the liquidmedium contain the autoclaved cells. On the other hand, this extraction process, also, causes some damage of some phytochemical pigments of *S. platensis*.

In this study, we evaluate the effect of autoclaved cellular content of *Spirulina platensis* as a foliar spray, comparing to N/P/K chemical fertilizer, on vegetative growth, yield, antioxidant and mineral contents of *Eruca sativa*.

## MATERIALS AND METHODS

### Field Experiment of Eruca sativa

The field experiment of rocket *(Eruca sativa* Mill) was conducted during the two seasons of 2015and 2016 at the Experimental Station Farm of the Faculty of Agriculture, Alexandria University, at Abeis, Alex, governorate; A.R.E. Seeds of rocket were sown on 25$^{th}$ of March, in both seasons, to evaluate the effect of autoclaved cellular content of *Spirulina platensis* as a foliar spray, comparing to N/P/K chemical fertilizer, on vegetative growth, yield, antioxidant and mineral contents of *Eruca sativa*. Before executing the experiment, some physical and chemical properties of the two experimental soils (up to 30 cm depth) were analyzed according to Page (1982), as presented in Table (1). A commercial rocket seed of local Egyptian cv. Balady was used in this experiment.

### Experimental Design

Four treatments were investigated in this experiment; three treatments from crude extract of autoclaved cellular content of *S. platensis* (5%, 10%, and 15%) and the fourth one was NPK fertilization as a control treatment. The experimental layout was a Randomized Complete Blocks Design with three replications. The plots area was 12.5 m² (5 m² long and 2.5 m² width). Three doses of growth biostimulant were added 10, 18 and 26 days after sowing. N-P-K fertilization was carried out according to the recommendations for commercial production of rocket salad plant as outlined by Ministry of Agriculture and Land Reclamation-Arab Republic of Egypt. All experimental units received identical doses of nitrogen, phosphorus and potassium at the rate of 5.25, 3 and 1.25 kg/100 m2, orderly. Ammonium sulphate (20.5% N), calcium super phosphate (15.5 % P2O5) and potassium sulphate (48% K2O) were the respective forms of fertilizers. Nitrogen fertilizer was broadcasted at three applications; 7, 15 and 21 days after sowing. Phosphorus fertilizer was broadcasted during soil preparation. Potassium fertilizer was banded at one application at 15 days after sowing.

**Table 1. Soil's physical and chemical properties of the experimental sites in the two seasons of 2015 and 2016 before cultivation.**

| | Season 2015 | Season 2016 |
|---|---|---|
| Physical properties | | |
| Sand % | 31.5 | 30.9 |
| Silt % | 27. 4 | 26.3 |
| Clay % | 41. 1 | 42.8 |
| Soil texture | Clay loam | Clay loam |
| Chemical properties | | |
| pH | 7.85 | 7.95 |
| Soluble cations (meq/ l) | | |
| $Ca^{++}$ | 2.11 | 2.20 |
| $Mg^{++}$ | 1.90 | 1.97 |
| $Na^{+}$ | 2.73 | 2.65 |
| $K^{+}$ | 0.46 | 0.36 |
| Soluble anions (meq/ l) | | |
| $HCO_3^{-}$ | 1.67 | 1.78 |
| $Cl^{-}$ | 2.00 | 1.90 |
| $SO_4^{--}$ | 3.41 | 3.31 |
| Total N % | 0.16 | 0.15 |
| Available P (mg/l) | 0.32 | 0.27 |

### Methods of *Spirulina platensis*

### Spirulina source, growth conditions and culture characterizations

*Spirulina platensis* was obtained from National Institute of Oceanography and Fisheries (NIOF), Alexandria Branch and cultured at Microalgae Lab., Invertebrates Aquaculture Lab., Aquaculture Division, NIOF. *S. platensis* strain was maintained in 500 mL Erlenmeyer flasks containing 100 mL sterilized Zarrouk's medium (Zarrouk, 1966) at 28±2 ºC, pH 9.5, continuous illumination using cool white fluorescent tubes (3000 Lux /24 h.). The culture of *S. platensis* was carried out in carboys 20 L (three replicates) under controlled conditions of temperature (28±2 ºC), illumination (3000 Lux /24 h.), continuously aeration using Zarrouk medium. For *S. platensis* density determination, 2 mL of culture suspension was collected and the cell optical density was measured at 660 nm using a spectrophotometer using Cecil 2000. Samples for analysis and foliar spray preparation were taken atlate exponential phase.

### S. platensis Biochemical Composition Analysis

For *S. platensis* biochemical composition analysis, 10 ml of culture water samples of each

treatment medium were centrifuged (3000 rpm/20 min.) and preserved at frozen temperature (-20°C) until analysis. Total proteins were extracted according to (Rauch, 1981) and determined according to (Hartree, 1972). Total carbohydrates were extracted according to (Myklestad and Haug, 1972) and determined according to (Dubois *et al.,* 1956). Total lipid was calculated according to (Bligh and Dyer, 1959).

**S. platensis Foliar Spray Preparation**

3 L of *S. platensis* cultured in carboy (three replicates) were taken atlate exponential phase, centrifuged at 3000 rpm/20 min. and washed twice with distilled water and preserved frozen at -20 °C until foliar spray preparation. 1 g of frozen *S. platensis* pellets (three replicates) was resuspended in 1L distilled water, shaking vigorously and autoclaving for 20 min, cooling and preserved at -5 °C until using Autoclaved cellular content of *S. platensis* (crude extract) was diluted into three concentrations: 5%, 10% and 15% (50 ml, 100 ml, and 150 ml, respectively, of crude extract dissolved in l L distilled water and shake vigorously) to use as foliar spray as growth biostimulant, in comparison to NPK fertilization as a control.

**Growth and yield parameters of Eruca sativa**

Plants were harvested (cut) two times per both seasons; the first cut was done after 35 days from sowing, and the second cut was after 4 weeks after the first one. Total yield calculated as kg/ m². Plants were randomly chosen from the different treatments and their growth parameters were measured based on plant height (cm), number of leaves, dry weight of leaves, total yield.

**Nutrient Contents of S. platensis and Eruca sativa**

Total leaf's of rocket (Chlorophyll a, b and total carotene (µg/g) were determined according to Dere *et al.* (1998). Nutrient contents (N, P and K) of crude extract of autoclaved cellular content of *S. platensis* (as percentage of dry weight of cells), as well as for rocket *E. sativa* samples (as percentage of dry weight basis of the leaves) were performed. Total nitrogen and phosphorus contents were determined calorimetrically using spectrophotometer at 662 and 650 nm, according to Evenhuis (1976). Potassium was determined by atomic absorption spectrometry as described by Cottenie *et al.* (1982).

**Mineral content of S. platensis**

Sodium (Na), magnesium (Mg), lead (Pb) and cadmium (Cd) of crude extract of autoclaved cellular content of *S. platensis* were determined by atomic absorption spectrometry as described by Cottenie *et al.* (1982).

The sun dried samples of two seasons (2015 and 2016), for each treatment, were mixed, and ground well. One g dry powder sample of each treatment of *E. sativa* (three replicates for each treatment) was extracted with 10 ml methanol for 24 h at room temperature and filtrate using Whatman No. 1 filter paper and preserved at -5 °C until March 2017 for the determination of antioxidants and anticancer Activities. Free radical scavenging activity of methanolic extract against DPPH radicals was determined according to the method described by Kumar *et al.* (2014). The total antioxidant activity (TAC) was determined according to the method of Phosphomolybdate assay using ascorbic acid (µg/ml) as standard (Ahmed *et al.,* 2012). The total phenolic content (TPC) was determined by using the Folin–Ciocalteu method as modified by Kumar *et al.* (2014). Total flavonoid content (TVC) was determined according to the method of Chang *et al.* (2002) and expressed in mg/g of Quercetin as standard. Nutrient, mineral contents and biochemical com position of *S. platensis* (as % of dw) are shown in Table (2).

**Table 2. Nutrient, Mineral Contents and Biochemical Composition of *S. platensis*** (as % of dw).

| **Protein (%)** | **57.56±2.93** |
|---|---|
| Lipid (%) | 4.21±1.24 |
| Carbohydrate (%) | 9.19±3.85 |
| N (%) | 4.86 |
| P (%) | 1.64 |
| K (%) | 1.10 |
| Mg (ppm) | 15.43 |
| Zn (ppm) | 0.018 |
| Na (ppm) | 187.08 |
| Fe (ppm) | 2.50 |
| Cu (ppm) | 0.17 |

**Anticancer Activities of Eruca sativa**

**Cell culture:**

Culture was maintained in DMEM medium (in case of lung cancer cell line A549), RPMI medium (in case of hepatocellular carcinoma cell line HepG2). All media were supplemented with 10% fetal bovine serum and incubated at 37oC in 5 %$CO_2$ and 95% humidity. Cells were sub-cultured using trypsin versene 0.15 %. All cell lines were purchased from Vacsera (Giza, Egypt).

**Cell viability assay:**

After 24 h of seeding 10000 cells per well in case of A549 and HepG2 cell lines (in 96 well plates), the medium was changed to serum-free medium containing a final concentration of the extracts of 100 µg/ml in triplicates. The cells were treated for 48 h. 100 µg/ml. Doxorubicin was used as positive control and 0.5 % DMSO was used as negative control. Cell viability was determined using the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay as described by Mosmann (1983). The equation used for calculation of percentage cytotoxicity:

$$(1- (av(x) / (av(NC)))* 100$$

**Where** Av: average, X: absorbance of sample well measured at 595 nm with reference 690 nm, NC: absorbance of negative control measured at 595 nm with reference 690.

**Determination of $IC_{50}$ values**

In case of highly active extracts possessing high cytotoxicity on cancer cell lines, different concentrations were prepared for dose response studies.

**Statistical Analysis**

Statistical analysis was performed using analysis of variance (ANOVA). Differences among means were considered significant at $p<0.05$ multiple range of post hoc comparisons were performed using the least significant difference (LSD) to resolve the differences among the means of replication according to of Duncan using SPSS

program. The results of anticancer activities were used to calculate the IC50 values of each extract using probity analysis and utilizing the SPSS computer program (SPSS for windows, statistical analysis software package / version 9 / 1989 SPSS Inc., Chicago, USA).

## RESULTS AND DISCUSSION

**Vegetative growth and yield of *Eruca sativa***

Regarding to the data presented in Table (3), the foliar spraying with *S. platensis* at 5% had positively significant effects on plant height, numbers of leaves, chlorophyll *a* and *b*, comparing to N-P-K control treatment, in the two tested seasons. In this respect, the highest significant plant height, in two seasons of 2015 and 2016, was observed with the treatment of *S. platensis* at the rate of 15 % (43.33 and 43.63 cm, respectively). However, *S. platensis* (5 %) exhibited the lowest significant plant height (35.00 and 36.66 cm, respectively). Furthermore the highest significant number of leaves was recorded with *S. platensis* at the rate of 5 % (8.33 and 9.00, respectively), followed by *S. platensis* 15 %(8.33 and 8.00, respectively) and *S. platensis* 10 % (7.33 and 7.67, respectively). On the other hand, N-P-K control treatment showed the lowest significant number of leaves (6.67 and 7.00, respectively). On the other side, both *S. platensis* 5 % and 15 % recorded the highest mean values of Chlorophyll *a* and *b,* comparing to other experimented treatments in both seasons, as shown at Table (3). The promoting effects of *S. platensis* on vegetative growth rocket *(Eruca sativa)* plants could be related to that algae extract as a new bio-fertilizer containing macronutrients as well as micronutrients, growth regulator hormones, polyamines, proteins and vitamins applied to improve nutritional status and vegetative growth (Abd El-Migeed *et al.,* 2004; Abd El-moniem and Abd-Allah, 2008 and Spinelli *et al.,* 2009). The mechanisms effect of algae on cell metabolism are mainly through the physiological action of major and minor nutrients, amino acids, vitamins, and also growth regulators affect cellular metabolism in treated plants leading to enhanced growth (Abd El-Motty *et al*., 2010).

The data clarified the differences due to the studied factor on the total yield shown in the same table. The results indicated that treatment *S. platensis* 10 %was responsible for the highest significant total yield, in both seasons (2.32 and 2.29 kg/m$^2$, respectively), followed by *S. platensis* 5 % (2.03 and 2.08 kg/m$^2$, respectively). Interestingly, the lowest total yield was observed by N-P-K control treatment in both seasons (1.60 and 1.65 kg/m$^2$, respectively). The superiority in total yield resulted from *S. platensis* foliar application owes directly to the positive effects on vegetative growth and Chlorophyll *a* and *b*(Table 3and 4) uptake of N, P and K (Table 4) to go forward and accelerates the photosynthetic rate, consequently, increased total yield. Similar results were recorded by Abd El Baky *et al*. (2014), who investigated the effect of aqueous extracts of *Spirulina*, prepared from 20 g dry weight cells /L in 0.1% Tween solution, in order to increase salt tolerance of Wheat plants, and they concluded that the aqueous extracts dry cells of *Spirulina* from could be used as a promising plant growth enhancer for treating wheat plants irrigated with brackish water. Optimization of the nutrient fixing and nutrient toxicity, enabling every element to play its role a harmony with other nutrients, which leads, in turn, to the best dry matter accumulation. Also, Ali and Mostafa (2009) reached to a positive response as a result of the effect of foliar spray or soil application methods of potassium-humate and *Spirulina platensis* (individually or combined) as bio-organic fertilizer on sesame yield and its attributes.

**Nutrient contents of *Eruca sativa***

Results showed that the *S. platensis* 5 % and 15 % exhibited the highest mean values of Chlorophyll *a* and *b,* comparing to other treatments, in both seasons. The highest significant N content (%) was obtained with N-P-K control treatment (1.44 and 1.48 %, in the first and second season, respectively), followed by *S. platensis* 15% (1.41 and 1.39 %, respectively), and finally, *Spirulina* 5% (1.33 and 1.37 %, respectively). Meanwhile, the lowest significant N content was obtained with *Spirulina* 10 % (1.20 and 1.29 %, in both seasons, respectively).

The data in Table (4), clearly, indicated that foliar spraying of *Spirulina* at the rate of 10% and 15% reflected positive effect on leaf's phosphorus content as compared with other treatments, in both seasons. All *Spirulina* concentrations ( 5%, 15% and 10 % ) had higher leaf potassium content comparing to N-P-K control treatment, in both seasons. These results are in agreement with those reported by Mohsen *et al*. (2016), who stated that the foliar application of cyanobacterial extracts impacted significantly on growth parameters and mineral contentsof lettuce plants. Hegab *et al.* (2005) reported that algae extract have a positive effect on fruit setting, yield and fruit quality of Balady orange trees. Abd El-Moniem and Abd-Allah (2008) mentioned that spraying algae extract at 50% improved yield, fruit quality of Williama banana plants.

**Antioxidant activity of *Eruca sativa***

*E. sativa* is originally found in the Mediterranean and Middle Eastern countries. Flavonoids and Phenolic compounds are the major phytochemicals found in different parts of rocket *E. sativa*, which contribute to its antioxidant properties. Glucosinolates, sulfur-containing plant secondary metabolites, are responsible for the characteristic bitter taste of rocket *E. sativa* and were found to have anticarcinogenic,antibacterial, anticancer and antioxidant activities (Bell and Wagstaff, 2014 and Alruwaih, 2016). *E. sativa* is also a source of vitamins A, C, and K, minerals such as calcium and iron, and phytonutrients including carotenoids (Garg and Sharma, 2014).

In this study, antioxidant activities were investigated using five different assays; DPPH, total antioxidant activity using phosphomolybdate assay, total phenolic content, total flavonoid assay and total Carotene. Among all investigated five assays, treatment *S. platensis* 5% achieved the highest positive significant antioxidant activities of all five assays, except DPPH it was *S. platensis* 15% and the first season of N-P-K control treatment.

The highest significant activity of DPPH radical scavenging was observed at two seasons of S. platensis 15% (63.40 and 63.16 %, respectively), followed by N-P-K Control treatment (62.56 and 62.20 %, respectively), and followed by the first season of S. platensis 5% (55.40 %). The highest significant total antioxidant activity, using phosphomolybdate assay, was recorded by the two seasons of treatment S. platensis 5% (52.47 and 52.72 mg/g, respectively) and the lowest significant was achieved by the two seasons of N/P/K Control treatment (42.45 and 42.15 mg/g, respectively).The two seasons of treatment S. platensis 5%exhibited the highest significant total phenolic content (114.88 and 114.84 mg/g, respectively), followed by N-P-K Control treatment (106.51 and 106.19 mg/g, respectively) and S. platensis 10% (103.14 and 102.88 mg/g, respectively). Meanwhile, in the two seasons, the treatment of S. platensis 15%showed the lowest significant total phenolic content (83.33 and 83.33 mg/g, respectively), as shown in Table 5. Our results in agreement with those optioned by Michael *et al*. (2011), who cited that E. sativa has powerful bioactive components, like antioxidant components, that may be effective in increasing human health and preventing cancer.

According to Table (5), treatment of S. platensis 5%recorded the highest significant total flavonoid content (2975.4 and 2975.2 µg/g, respectively), followed by N-P-K Control treatment (2934.0 and 2934.8 µg/g, respectively in both seasons. However, foliar application of S. platensis at the rate of 15% and S. platensis 10% showed the lowest significant total flavonoid content. Michael *et al*. (2011) isolated and identifiednine natural flavonoid compounds from aqueous extract of Eruca sativa fresh leaves. In terms of antioxidant compounds, rocket (Eruca sativa) is a good source of carotenoids, which play a very important role among natural antioxidants (Martinez-Sanchez *et al*., 2008).

In the present study, in the two seasons, the treatment of *S. platensis* 5% recorded the highest significant total carotene (2.64 and 2.67 µg/g, respectively), as shown in Table 5.

**Table 3. Vegetative growth and yield of *Eruca sativa* treated with different foliar spray concentrations of S. platensis, comparing to N/P/K fertilizers**

| Treatments* | N/P/K (Control) | | 5% | | 10% | | 15% | |
|---|---|---|---|---|---|---|---|---|
| Seasons | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 |
| Plant Height(cm) | 39.66±1.55$^{b}$ | 39.00±1.00$^{b}$ | 35.00±0.45$^{c}$ | 36.66±1.45$^{c}$ | 40.66±0.40$^{b}$ | 42.66±0.77$^{a}$ | 43.33±1.45$^{a}$ | 43.63±1.09$^{a}$ |
| No. leaves | 6.67±0.58$^{b}$ | 7.00±1.00$^{b}$ | 8.33±0.58$^{a}$ | 9.00±1.00$^{a}$ | 7.33±1.15$^{ab}$ | 7.67±0.58$^{ab}$ | 8.33±0.58$^{a}$ | 8.00±1.00$^{ab}$ |
| Total yield (kg/m$^2$) | 1.60±0.20$^{c}$ | 1.65±0.12$^{c}$ | 2.03±0.02$^{a}$ | 2.08±0.14$^{a}$ | 2.32±0.12$^{a}$ | 2.29±0.16$^{a}$ | 1.78±0.20$^{bc}$ | 1.81±0.10$^{b}$ |

*** Values followed by the same alphabetical letter(s) in common, within a particular group of means in each character, no significantly differ, using Revised L.S.D test at probability 0.05**

**Table 4. Leaf chlorophyll and nutrient content (%) of *Eruca sativa* treated with different foliar spray concentrations of *S. platensis*, comparing to N/P/K fertilizers**

| Treatments* | N/P/K (Control) | | 5% | | 10% | | 15% | |
|---|---|---|---|---|---|---|---|---|
| Seasons | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 |
| Chlorophyll a (µg/g) | 23.79±0.04$^{a}$ | 23.82±0.03$^{a}$ | 23.87±0.08$^{a}$ | 23.89±0.12$^{a}$ | 23.86±0.11$^{a}$ | 23.89±0.13$^{a}$ | 23.61±0.09$^{b}$ | 23.64±0.05$^{b}$ |
| Chlorophyll b (µg/g) | 21.30±0.21$^{b}$ | 21.14±0.23$^{b}$ | 23.45±1.10$^{a}$ | 23.44±0.13$^{a}$ | 23.05±0.67$^{a}$ | 23.14±0.57$^{a}$ | 23.03±0.97$^{a}$ | 23.01±0.08$^{a}$ |
| N | 1.44±0.03$^{a}$ | 1.48±0.07$^{a}$ | 1.33±0.13$^{ab}$ | 1.37±0.02$^{ab}$ | 1.20±0.10$^{b}$ | 1.29±0.09$^{b}$ | 1.41±0.04$^{a}$ | 1.39±0.06$^{ab}$ |
| P | 0.741±0.01$^{b}$ | 0.753±0.01$^{b}$ | 0.633±0.01$^{c}$ | 0.641±0.02$^{c}$ | 0.762±0.02$^{a}$ | 0.771±0.01$^{a}$ | 0.764±0.01$^{a}$ | 0.767±0.02$^{a}$ |
| K | 0.172±0.02$^{c}$ | 0.179±0.02$^{c}$ | 0.243±0.01$^{a}$ | 0.245±0.01$^{a}$ | 0.213±0.02$^{b}$ | 0.217±0.04$^{b}$ | 0.243±0.03$^{a}$ | 0.238±0.02$^{b}$ |

*** Values followed by the same alphabetical letter(s) in common, within a particular group of means in each character, no significantly differ, using Revised L.S.D test at probability 0.05**

**Table 5. Antioxidant activity of *Eruca sativa* treated with different foliar spray concentrations of *S. platensis*, comparing to N/P/K fertilizers**

| Treatments* | N/P/K (Control) | | 5% | | 10% | | 15% | |
|---|---|---|---|---|---|---|---|---|
| Seasons | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 | 2015 | 2016 |
| DPPH(% inhibition) | 62.56±1.29$^{a}$ | 62.20±1.06$^{ab}$ | 55.40±1.75$^{ab}$ | 54.83±2.95$^{bc}$ | 48.28±6.30$^{b}$ | 47.36±4.53$^{c}$ | 63.40±5.60$^{a}$ | 63.16±5.83$^{a}$ |
| Total Antioxidant (mg/g) | 42.45±6.12$^{b}$ | 42.15±6.38$^{b}$ | 52.47±.46$^{a}$ | 52.72±1.75$^{a}$ | 41.92±3.46$^{b}$ | 41.80±3.60$^{b}$ | 45.38±2.46$^{b}$ | 45.41±2.14$^{ab}$ |
| Total Phenol (mg/g) | 106.51±7.51$^{a}$ | 106.19±8.19$^{a}$ | 114.88±3.60$^{a}$ | 114.84±3.16$^{a}$ | 103.14±8.72$^{a}$ | 102.88±9.88$^{a}$ | 83.33±2.02$^{b}$ | 83.33±1.67$^{b}$ |
| Total Flavonoid (µg/g) | 2934.0±66.1$^{a}$ | 2934.8±66.4$^{a}$ | 2975.4±7.5$^{a}$ | 2975.2±10.8$^{a}$ | 2614.0±138.9$^{b}$ | 2612.9±137.6$^{b}$ | 2424.4±249.4$^{b}$ | 2424.7±245.9$^{b}$ |
| Total Carotene(µg/g) | 2.39±0.15$^{ab}$ | 2.43±0.22$^{ab}$ | 2.64±0.30$^{a}$ | 2.67±0.35$^{a}$ | 2.27±0.05$^{ab}$ | 2.29±0.09$^{ab}$ | 2.11±0.19$^{b}$ | 2.12±0.19$^{b}$ |

*** Values followed by the same alphabetical letter(s) in common, within a particular group of means in each character, no significantly differ, using Revised L.S.D test at probability 0.05**

**Anticancer activity of *Eruca sativa***

The cytotoxic effect of rocket *(Eruca sativa)* cultured using three different foliar spray concentrations (5%, 10% and 15%) of *S. platensis*, comparing to N-P-K chemical fertilizers, using Doxorubicin as a positive control, against the human cancer cells A549 (lung cancer cell line) and HepG2 (hepatocellular carcinoma cell line) were shown in Table (6). Cultures of different cell lines were treated with extracts first at one concentration of 100 µg/ml and the results showed that, comparing to N-P-K chemical fertilizers, *S. platensis*5% is the only concentration possessed high cytotoxic effect (60.63 %) against Hepatocellular carcinoma (HepG2), with $IC_{50}$ value 86.5µg/ml, as shown in Table (7), in the same time, this treatment did not show positive effect against lung cancer cell line (A549). This results agreement with Mohd-Syahril *et al*. (2011), who reported that the crude extract of *S. platensis* has anticancer effect on liver cancer cell line (HepG2).Our results confirm that the use of *S. platensis* improves the ability of plants to treat cancer, compared to chemical fertilizers.

**Table 6. Cytotoxicity of tested samples (100 *µg/ml*) on tow human tumor cell lines: Lung carcinoma (A549) and hepatocellular carcinoma (HepG2).**

| Treatments | Cytotoxicity% | |
|---|---|---|
| | A549 | HepG2 |
| 5% | 6.43 ±0.12$^{c}$ | 60.63±0.72$^{b}$ |
| 10% | 11.90±0.10$^{b}$ | 27.33±0.75$^{c}$ |
| 15% | 3.30±0.35$^{d}$ | 13.60±0.40$^{e}$ |
| N-P-K | 0.33±0.58$^{e}$ | 25.30±0.72$^{d}$ |
| Doxorubicin | 87.33±0.61$^{a}$ | 88.85±0.91$^{a}$ |

**Results are represented by means of three replicates.**

**Table 7. In vitro cytotoxic activity ($IC_{50}$ µg/ml) of the more active sample against HepG2 cell lines after48 hours.**

| N0. | Sample code | $IC_{50}$µg/ml HepG2 |
|---|---|---|
| 1 | 5% Spirulina | 86.5±0.157 |

Results are represented by means of three replicates.

## CONCLUSION

Rocket (*Eruca sativa*) is traditionally grown in Egypt and is extensively used as food in most Egyptian traditional dietary. Until now, no available data about the quality and quantity of this important vegetable cultured in Egypt. Organic vegetable is a rapidly growing industry over the world. The current study investigate the quality and quantity of rocket (*Eruca sativa*) cultured using chemical fertilizers (N-P-K) in comparing to bioorganic fertilizer composed from three different foliar spray concentrations (5%, 10% and 15%) prepared from autoclaved cellular content, as an extraction method, of wet cells of *Spirulina platensis.* Bioorganic fertilizer is very safe for human, animal, environment get lower pollution; reduce soil salinity via decrease mineral usage fertilization, and saving fertilization cost. The results of the present study concluded that foliar spray concentration of 5% *S. platensis* resulted in the best significant quantity (vegetative growth and yield) and quality (total antioxidant activity) of *Eruca sativa*. Interestingly, this concentration achieved anticancer activity (61.3 %) against hepatocellular carcinoma cell line (HepG2).

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## المضادات السرطانية-مضادات الأكسدة -محتوى العناصر المعدنية النمو الخضرى والمحصول الكلى فى نبات الجرجير بإستخدام الرش الورقى بالتعقيم الخلوى لطحلب الأسبيرولينا مقارنة بالتسميد المعدنى

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أجريت التجربة الحقلية لنبات الجرجير خلال الموسمين 2015 و 2016بمزرعة الكلية في منطقة أبيس للبحوث الزراعية، جامعة الإسكندرية، لدراسة تأثير المحتوى الخلوى المعقم من طحلب *Spirulina platensis* كرش ورقي مقارنة بالأسمدة المعدنية وتأثيرذلك على كلا من النمو الخضرى والمحصول الكلى - مضادات الأكسدة - محتويات العناصر المعدنية - والتأثير السام على الخلايا المسرطنة للكبد والرئة A549و HepG2 فى نبات الجرجير بعد حصاده. وقد تم إستخدام ثلاث تركيزات من طحلب الأسبيرولينا (5-10-15%) وإستخدام التسميد المعدنى كمعاملة الكنترول .وقد أظهرت النتائج ان التركيز 15%قد أعطى أعلى تأثير معنوى بالنسبة لإرتفاع النبات فى كلا الموسمين (33,43-63,43على الترتيب) بيمنا أعطى تركيز 5% أقل تأثير منعنوى لإرتفاع النبات (66,36-35,36على الترتيب). كما أوضحت النتائج أن أعلى تأثير معنوى لعدد الأوراق للنبات كان لتركيز الطحلب 5% (33,8-9على الترتيب) فى كلا الموسمين بينما كان أقل تأثير معنوى لعدد أوراق النبات كان للتسميد المعدنى (67,6-7على الترتيب) فى كلا الموسمين . أوضحت النتائج أن التركيز 10%من طحلب الأسبيرولينا أعطى أعلى تأثير معنوى من المحصول الكلى (32,2 -29 ،2 كيلوجرام للمتر المربع) فى كلا الموسمين على الترتيب يليها معاملة 5% قد أعطت (03، 2 -08 ، 2كيلوجرام للمتر المربع) فى كلا الموسمين على الترتيب ،أيضا أوضحت النتائج أن معاملة 5%من الأسبيرولينا قد أعطت اعلى تأثير معنوى فى جميع البيانات الخاصة بمضادات الأكسدة فيما عداDPPH . وعموما يمكن التوصية بأن أفضل المعاملات تحت الظروف البيئية لهذه الدراسة المعاملة بتركيز 5% من طحلب الأسبيرولينا (رشا 3 مرات بعد 10 و 18 و 26 يوم من الزراعة)، قد أعطت أفضل النتائج لصفات النمو الخضرى والمحصول الكلى وايضا مقاومتها ضد الخلايا المسرطنة للكبد HepG2 فقد اعطت أعلى تأثير ضد الخلايا المسرطنة بنسبة 3، 61 %. بالإضافة الى ان تلك المعاملة (سالفة الذكر) قد وفرت 100 % من الجرعة السمادية وبالتالى قللت التكاليف وقللت فرصة تلوث البيئة وبالتالى تحسن فى الصحة العامة.