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IMPORTANCE OF BIOFERTILIZATION IN REDUCING THE REQUIRED OF MINERAL FERTILIZER AMOUNTS IN RELATION TO GROWTH, SOME PHYSIOLOGICAL PARAMETERS AND YIELD OF ROSELLE

Salwa AR. Hammad[1] and Taghrid A. Hegazy [2]

1-Agric. Botany Dept., Faculty of Agric., Minufiya Univ., Shibin El-Kom, Egypt.
2- Horticulture Dept., Faculty of Agric., Minufiya Univ., Shibin El-Kom, Egypt.

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ABSTRACT: ***Two field experiments were carried out at the Faculty of Agriculture, Minufiya University during 2008 and 2009 seasons to study the influence of some biofertilizers (phosphorein, nitrobein or effective microorganisms "EM") alone or in combination with five levels of recommended mineral fertilizer (0.0, 25, 50, 75 and 100% NPK) for improving growth, some physiological and chemical aspects, yield and its quality of roselle plants (Hibscus sabdariffa L. cv. Sabahia 17). The results indicated that most studied characters of growth, photosynthetic pigments, total soluble sugars, total carbohydrates, total phenols, minerals in leaves, yield and its quality showed significant increases by the application of biofertilizers singly or in combination with mineral fertilizers compared with control plants. As regard to the interaction, the best results were owing to addition of phosphorein followed by nitrobein compared to the treatment of 100% of recommended mineral fertilizer. The best results of N, P, K (% and uptake) of roselle leaves were recorded in plants treated with nitrobein, phosphorein and EM, respectively. Moreover, biofertilizers plus mineral fertilizers up to 75% of recommended dose gave the highest values of number and fresh weight of fruits / plant, fresh and dry weight of sepals / plant while its reduction of plants received 100%. The highest values of seed yield / plant was recorded in plants received 100% of mineral fertilizer with biofertilizers. Moreover, biofertilizers plus different rates of mineral fertilizer enhanced the concentration of anthocyanin, total phenols, total soluble solids and vitamin C in sepals. Phosphorein was superior in most traits than nitrobein or EM. From the previous results it can be recommended to applying 50 or 75% of recommended mineral fertilizer with phosphorein or nitrobein to obtain good yield with high active substances. Also, the use of biofertilizers with the recommended dose (100% NPK) to obtain the highest values of seed yield / plant.***

Key words***: Biofertilizers, roselle, growth, sugars, phenols, mineral, yield and its quality.***

INTRODUCTION

Medicinal plants occupied a prominent economic position because of the

continuous increasing demand for these medicinal is products from the local and foreign markets. Among these medicinal roselle plant (*Hibiscus sabdariffa* L.) is a member of family Malvacea and has a common name in Egypt as Karkadeh. Its purplish sepals (calyx and epicalyx) are the most economic parts of the plant which is considered a very popular beverage due to its effect on lowering and / or adjusting the blood pressure without producing any side effect (Faraji and Tarkhani, 1999). It is rich in ribioflavin, niacin, calcium and iron. The sepals and petals are potentially a good source of antioxidant agents as anthocyanins, ascorbic acid and total phenols (Raifa *et al.,* 2005 and Abdalla and Lotfy, 2008). Recently, the biological activities of anthecynin, such as antioxidant activity protection from atherosclerosis and anticarcinogenic activity have been investigated, and shown to have some beneficial effects in the treatment of diseases (Tasi *et al.,* 2002). The seeds are eaten in some parts of Africa, and also have been roasted as a substitute for coffee (Bengaly *et al.,* 2006).

Mineral fertilizer play an essential role in the growth of medicinal plants. Many problems of environmental pollution have resulted from excessive application of mineral fertilizers in the traditional farming system (Swiader, 1984). To confront this problem, it was necessary the use untraditional fertilizer. Biofertilization has become more and more important, it becomes a positive alternative to mineral fertilizer (Hussain *et al.,* 1999). Biological fertilizers are generally, based on altering the rhizospher flora by seed or soil inoculation with certain organisms, capable of inducing beneficial effects on a compatible host-bio-fertilizers mainly comprise nitrogen fixer, phosphate dissolvers or Vesicular Arbuscular Mycorrhizae and silicate bacteria. These organisms may affect their host plant by one or more mechanisms such as nitrogen fixation, production of growth promoting substance or organic acids, enhancing nutrient uptake or protection against pathogens (Bashan *et al.,* 1989).

Moreover, application of effective microorganisms is introduced to the natural farming system, where it contains lactic bacteria, Actinomyces and various other bacteria and fungi (Higa, 1994). EM application have been proved effective in many aspects and played important roles in promoting crop production and purifying the environment. Furthermore, EM is effective not only in fertilizing, but also in improving soil properties, stimulating of a crop and increasing tolerance (HO and Hwan, 2000).

Thus, this investigation was conducted to study the optimum dose of mineral fertilizer (NPK) applied substitute with biofertilizer to obtain the highest production of vegetative growth, yield and its components as well as the compositional quality as antioxidative activity of sepals of Egyptian roselle plants.

MATERIALS AND METHODS

Two filed experiments were carried out at the experimental farm of the Faculty of

Agriculture, Shibin El-Kom, Minoufiya University during the two successive summer seasons of 2008 and 2009. The study aimed to investigate the effects of some biofertilizers applied alone or in combination with different rates of recommended mineral fertilizer (0.0, 25, 50, 75 and 100% NPK) on reducing the required of mineral fertilizer amounts, the plant growth, physiological and chemical consistituents, yield and its quality of roselle.

Seeds of roselle (*Hibiscus sabdariffa* L. cv. Sabahia 17) were obtained from Horticultural Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt and sown on 15th February of both seasons. The soil was prepared and divided into plots (2 × 2 m), and each plot included two rows and 10 plants per hill with a distance of 50 cm apart from each row. Some physical and chemical properties of soil used were determined according to Jackson (1967), and are given in Table (1). After one month from the planting date, the plants were thinned to two plants per hill.

Table (1): Physical and chemical properties of the soil.

Property	Volume
a) Physical properties:	
Sand (%)	5.63
Silt (%)	43.60
Clay (%)	49.07
$CaCO_3$ (%)	1.70
Soil texture	Silty clay
b) Chemical properties:	
pH	7.58
E.C (mmohs / cm)	0.52
C.E.C (mg / 100 g)	30.20
Organic matter	1.56
Soluble ions	(mg/100 gm soil)
<u>Cations:</u>	
Ca^{++}	30.2
Mg^{++}	1.30
Na^{+}	1.01
K^{+}	1.21
<u>Anions:</u>	
CO_3^{-}	–
HCO_3^{-}	1.1
Cl^{-}	1.5

The used biofertilizers included nitrogen fixing bacteria (NFB) and phosphate dissolving bacteria (PDB). Strains of NFB (*Azospirillum* sp., *Azotobacter* sp. and *Klebsiella* sp.) under commercial name "Nitrobein", whereas PDB (*Bacillus megaterium*) under commercial name "phosphorein" were mixed with seeds (10 g / kg seeds) immediately before sowing. Effective microorganisms (EM) contain group of beneficial microorganisms containing about 80 species (Kato *et al.,* 1999). EM solution was added 2 liter / fed. (25 cm / liter / plot) with irrigation water three times. The first dose with sowing and other ones were added every 30 days. All biofertilizers were obtained

from Biofertilization Unit, Agriculture Research Center of Giza, Egypt.

Nitrogen fertilizer in the form of urea (46% N) at the rate of 200 kg/fed. (200 g / plot), calcium superphosphate (15.5% P_2O_5) and potassium sulphate (50% K_2O) were added at the rate of 100 kg / fed. (100 g / plot) as the recommended dose of NPK fertilizer. Mineral fertilizers were directly applied as soil application treatment in two equal portions at 45 and 60 days after sowing respectively. The several recommended agricultural practices were followed.

The experiment included 15 treatments which were all possible combination of three biofertilizers and five mineral fertilizers as the following 0.0, 25% NPK (50 : 25 : 25 g / plot), 50% NPK (100 : 50 : 50 g / plot), 75% NPK (150 : 75 : 75 g / plot) and 100% NPK (200 : 100 : 100 g / plot). The design of the experiment was split-plot. Mineral fertilizer was arranged randomly as the main plot (A), whereas biofertilizer was distributed randomly as a sub plots (B). All treatments were arranged with six replications.

After 90 days from sowing, six plants were randomly selected from each treatment and the following data were recorded:

1. **Vegetative growth parameters: plant height (cm), number of branches and leaves / plant, leaf area (cm^2) / plant using the dry weight method described by Aase (1978). Leaves, stems and roots were separated and dried in an electric oven at 70°C for 72 hrs and dry weights were measured in gms.**

2. Chemical constituents:

a. Photosynthetic pigments (Chl. a, b and carotenoids) were estimated in fresh leaves as described by Witham *et al.* (1971).

b. Total soluble sugars and total carbohydrates were estimated in dry leaves using the method described by Dubois *et al.* (1956).

c. Total phenols were determined in dried leaves following the method of Snell and Snell (1953).

d. Mineral concentration: N was measured in dry leaves, using micro-kjeldahl method according to Ling (1963). P was determined as mentioned in A.O.A.C. (1990). K was estimated using flamephotometer method described by Chapman and Pratt (1961), then both concentrations (%) and uptake (mg/plant) were calculated.

3. Yield and its components:

At harvest time October 2nd in two seasons, ten plants were randomly taken from each plot and the following data were recorded; number and fresh weight of fruits / plant, fresh and dry weight of sepals / plant and seed yield / plant.

4. Yield quality:

a. Total anthocyanin in fresh sepals was determined by using the method of Fuleki and Francis (1968) and developed by Du and Francis (1973).

b. **Total phenols** in dried sepals were estimated as above mentioned for dry leaves.

c. **Total soluble solids (T.S.S%),** The percentage of T.S.S in fresh sepals were estimated by a hand refractometer according to A.O.A.C (1990).

d. **Vitamin C concentration** (ascorbic acid), in fresh sepals was estimated by titration with 2, 6 dichlorophenol endophynol according to A.O.A.C (1990).

All obtained data were subjected to statistical analysis with the help of COSTAT-C Program, and the L.S.D. at 5% level was calculated according to Snedecor and Cochran (1972).

RESULTS AND DISCUSSION

1. Vegetative growth:

The results given in Table (2) clearly show a significant increase in plant height, number of branches and leaves / plant, leaf area as well as dry weights of leaves, stem and root / plant of roselle plants due to applying of biofertilizers (phosphorein, nitrobein, EM) during the growing seasons compared to control. The maximum values were obtained as a results of application of phosphorein followed by nitrobein. These results are agreement with those obtained by Noel *et al.* (1996) who indicated that non-symbiotic N_2-fixing bacteria, Azotobacter and Azospirillum strains produced adequate amounts of IAA and cytokinins, which increased the surface area per unit of root length and enhanced the uptake of nutrients from the soil. Moreover, the P-solubilizing bacteria has an important factor in raining mineral-P efficiency in the soil due to continuous solubility during plant growth period (El-Dahtory *et al.,* 1989). Desouky (2006) found that, the treatment of rose plant of Azotobacter or *Bacillus* sp. resulted in a considerable improvement of plant height, number of leaves / plant and dry weight. Similar results were obtained by Fathy *et al.* (2008) on roselle. In addition, the positive effect of the EM may be due to gibberellins which produced from *Aspergillus niger* fungi presented in EM (El-Bahrawy, 1983). In this respect Kato *et al.* (1999) reported that, the promotion of root development by EM application may be due to the effect of plant growth regulators (auxins, gibberellins and kinetin like substance) produced by inoculated microbes. Zaki and Salama (2006) on cucumber and Hammad (2010) on wheat came to the same conclusion.

The data in the same Table indicated that, plants showed a gradual increase in the above mentioned growth characters in response to increasing the doses of NPK compared to control in both seasons. These results are in accordance with those found by El-Beheidi *et al.* (2006) on tomato. Also Gardener *et al.* (1985) declared that P is an essential component of the energy

Table 2

transfer compounds, genetic information system, cell membranes and phosphoproteins. K is essential for cell division and the development of meristematic tissue (Mengel and Kirkby, 1978).

Regarding the interaction between biofertilizers and different rates of mineral fertilizer, the best growth parameters were noticed when phosphorein applied with the recommended dose of mineral fertilizer (pho. + 100% NPK) in both seasons. In this regard, it was observed that, there were no significant differences for number of branches / plant. Such results are in agreement with El-Beheidi *et al.* (2006) on tomato, Swaefy *et al.* (2007) on peppermint.

2. Chemical constituents:

a. Photosynthetic pigments:

Regarding the effect of fertilization treatments on photosynthetic pigments, the data recorded in Table (3) and illustrated in Fig. (1) showed that chl. a + b and carotenoids of roselle leaves were significantly increased as a result of fertilization treatments compared to control. Plants received 75% or full dose of chemical fertilizer (NPK) to each biofertilizer were recorded the highest increase in most cases, compared to that recorded with plants received each biofertilizer alone. Generally plants treated with EM only had the lowest values compared to those treated with phosphorein or nitrobein. This effect may be due to the certain microorganisms in EM culture such as photosynthetic and N-fixing bacteria, which enhanced the plant photosynthetic rates as reported by Xu *et al.* (2001). According the enhancing of nutrients uptake processes by microorganisms to augment the extent of the availability of nutrients in a form which can be easily assimilated and may be reflect on the biosynthesis of chlorophyll in tomato leaves (El-Beheidi *et al.,* 2006). The obtained results are in harmony with those obtained by Hammad (2009) on spinach and Hammad (2010) on wheat.

b. Total soluble sugars and total carbohydrates:

The presented data (Table 3) showed that, biofertilizers either alone or combined with mineral fertilizer were found to be a good agent in stimulating total soluble sugars (TSS) and total carbohdyates (TC) of roselle leaves in the two growing seasons compared to control. Moreover, there is a significant increase in TSS and TC due to increasing the doses of mineral fertilizer compared to untreated ones or treated with EM only. Moreover, phosphorein followed by nitrobein with the full recommended dose of NPK gave the best results. In case of combinations, EM + 25% NPK produced the lowest values in TSS and TC in both seasons. The superiority of the bioferitlizers of phosphorein and for nitrobein may be due to the release of the fixed phosphorus from the soil, and fixing nitrogen, hence increasing the concentration and availability of these elements in root zone. Phosphorus play a great role in enlargement cell division as well as the synthesis of

Table (3)

nucleic acids. Nitrogen also enhances protein synthesis, division and enlargement of cells as well as it is important for the photosynthetic processes (Abdalla *et al.* 2001). In this connection, Hammad (2009) found that, the heights values of TSS and TC in spinach leaves were obtained due to fertilizing the plants with mineral-N at 30 kg/fed. plus nitrobein. These results are in accordance with those found by Midan and Sorial (2006) on lettuce.

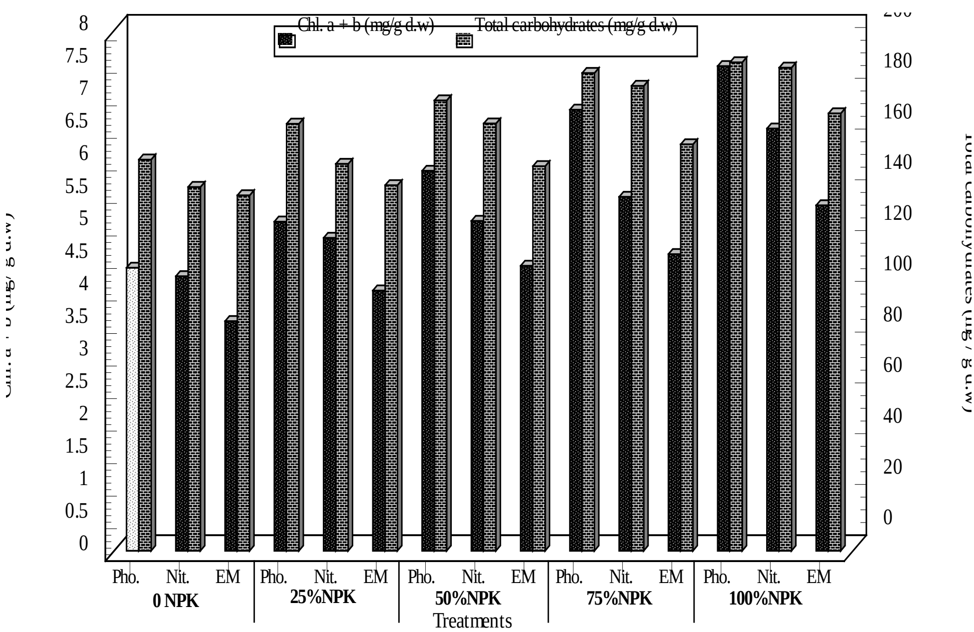

Fig. (1). Total chlorophyll and total carbohydrates in roselle leaves as affected by bio- and mineral fertilizers during 2008 season.

c. Total phenols:

Concerning the effect of chemical and biofertilization treatments on total phenols concentration, the presented data (Table 3) showed that the application of two and third doses of mineral fertilizer gave the highest values in both seasons compared to control. In most cases, using biofertilizers combined with 75% or 100% of recommended dose of NPK gave the best results compared to that recorded with plants received each biofertilizer only. In this respect phosphorein was superior over the other

ones. Moreover, the lowest values of total phenols were obtained from plants treated with EM combined with 25% or 50% of NPK compared with untreated ones. These results are in harmony with those obtained by Hammad and El-Gamal (2004) on pepper nad Hammad (2010) on wheat.

d. Mineral concentrations:

Data listed in Table (4) indicate that both NPK percentage and uptake in roselle leaves were significantly increased with adding biofertilizers or mineral fertilizer compared to control plants. Applying nitrobein with the full dose of mineral fertilizer resulted maximum values of N% and uptake. Moreover, using phosphorein with 100% of NPK gave the best results of P% and uptake of roselle leaves. In addition, the highest K concentration was obtained from applied EM + 100% NPK treatment followed by nitrobein + 100% NPK in both seasons. Biofertilizer enhanced N, P, K% and uptake owing to N_2-fixation by bacteria (Nour El-Dein *et al.,* 2005). In this regard Salem *et al.* (2007) found that applying biofertilizers alone or combined with mineral (NP) significantly affected N, P and K concentration of rosemary plants grown in sandy and calcareous soils. In this respect Sherif *et al.* (1997) pointed out that phosphate dissolving bacteria presses the ability to bring insoluble phosphate to be in soluble forms secreting organic acids such as formic, acetic and lactic acids. Such acids lower the pH and bring about the dissolution of bonds forms of phosphate and render them available for growing plants. Release of organic and inorganic acids and increasing O_2 evolution due to phosphate dissolving microorganisms and microbial types, reduce soil pH leading to more availability of P and other nutrients ready for plants. The release of plant growth hormones may increase root system development which improve absorbing of nutrient from soil (Singh and Kapoor, 1999).

3. Yield and its components:

In both seasons, results in Table (5) show a significant increase in number of fruits, fresh weight of fruits / plant, fresh and dry weights of sepals / plant as well as seed yield / plant due to mineral fertilizer levels compared to control. In this respect, the highest values of fresh weight of fruits and fresh and dry weight of sepals / plant were recorded at 75% of NPK treatment, meanwhile the highest values of No. of fruits and seed yield were recorded at 100% NPK. Moreover, application of biofertilizers gave a significant increase in yield and its components compared to uninoculated control. Significant differences in yield and its components were observed due to the interaction between bio-and chemical fertilizer. Generally, using 75% of NPK in addition to each biofertilizer gave the highest values of the pervious characters except seed yield / plant which recorded the highest values in plants received 100% of NPK with biofertilizers. In this connection phosphorein was superior

Table 4

Table 5

over nitrobein or EM. On the other hand, EM was more effective than nitrobein for the seed yield/plant at all levels of chemical fertilizers. The application of NPK particularly 50 and 75% ratio by biofertilizers produced significant increases in yield and its components than that obtained by applying full dose of NPK with or without biofertilizers. This means that inoculation with biofertilizers can reduce mineral fertilizers application without reducing productivity (Pol, 1998). Improving plant growth and yield productivity may be attributed to different mechanisms of action including: a) the production of secondary metabolites such as antibiotic, hydrogen cyanid and plant hormones like substances, b) the production of siderophores, c) antagonism to soil borne root pathogens, d) phosphate solubilization and e) dinitrogen fixation (Salem *et al.,* 2007). The application of EM promotes root growth and activity, and enhances photosynthetic efficiency and quality, which resulted in increasing grain yield of sweet corn (Xu-Huilian and Xu, 2000). This effect might be due to the release of nutrients from organic matter when EM was applied as (Yadav, 1999). Moreover, it could be concluded that the causal phenomenon of these results has been attributed also to the interaction between microbes and plants, which enhanced the plant productivity as reported by (Ahmed *et al.,* 2004) on faba bean. Similar results were obtained by Swaefy *et al.* (2007) on peppermint and Hammad (2010) on wheat.

4. Yield quality:

a) Total anthocyanin:

It is evident from Table (6) that significant increase was obtained in anthocyanine concentration of sepals as affected by applying bio-and mineral fertilizer as compared with control in both seasons. Phosphorein or nitrobein + full dose of NPK gave significantly higher values as compared to EM. The previous results agree with the findings of Fathy *et al.* (2008) who found that, inoculation of roselle seed before planting with VA-mycorrhizae + phosphorein increased anthocyanin concentration compared to control.

b) Total phenols in sepals:

Data presented in the same Table show that a gradual increase in total phenols concentration of sepals was observed with increasing NPK doses compared to control. Moreover, the interaction between bio-and mineral fertilizer recorded a significant increase in total phenols which was the highest with plants received full dose of NPK combined with phoephorein meanwhile, the lowest values were recorded with the application of EM compared with other biofertilizer. Also roselle plants treated with 50% NPK + phosphorein gave significantly higher values in total phenols concentration than those receiving 100% NPK. In this respect Abdalla and Lotfy (2008) reported that roselle sepals contain active compound and antioxidant agents

Table 6

as anthocyanin, total phenols and vitamin C. The stimulated effect of biofertilizer application on roselle phenol content may attributed to its role on the physiological and biochemical process in plants (Salem *et al.,* 2007).

c) Total soluble solids:

Data given in Table (6) demonstrated significant increases in total soluble solids in fresh sepals due to the use of bio-fertilizers alone or its combined with different rates of NPK fertilizer as compared with the control. Using phosphorein or nitorbein with 75% NPK gave the highest values in this respect, meanwhile applied EM was more effective when combined with 100% NPK. Similar results were obtained by Ali and Selim (1996) on tomato. The stimulatory affect of biofertilizer on total soluble solids may be due to the involvement in phytohormones production (Noel *et al.,* 1996).

d) Ascorbic acid (vitamin C):

In both seasons vitamin C concentration in sepals tended to increase by the application of biofertilizers compared to control. Such promoting effect gave the best values due to interaction between phosphorein and the full dose of NPK. These results are in agreement with those of Gabr *et al.* (2001) and Hammad and El-Gamal (2004) on pepper.

From this study, it can be concluded that the combination of biofertilizers (phosphorein, nitrobein or EM) with 50% or 75% of recommended mineral fertilizer gave the best results. Hence, it can be recommend to use such of these combinations in order to increase the crop productivity and to reduce the soil pollution resulted from excess of mineral fertilizer.

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أهمية التسميد الحيوى فى تقليل الكميات المطلوبة من التسميد المعدنى وعلاقته بالنمو وبعض الصفات الفسيولوجية والمحصول فى الكركديه

سلوى عبد الرحمن حماد[١] ، تغريد عاطف حجازى[٢]

(١) قسم النبات الزراعى – كلية الزراعة – جامعة المنوفية . شبين الكوم

(٢) قسم البساتين – كلية الزراعة . جامعة المنوفية . شبين الكوم

الملخص العربى :

أجريت تجربتان حقليتان بكلية الزراعة جامعة المنوفية خلال موسمى ٢٠٠٨ ، ٢٠٠٩ لدراسة أهمية بعض الأسمدة الحيوية (فوسفورين ، نيتروبين ، المخصب الحيوى "EM") منفردة أو بالإشتراك مع خمس معدلات من الأسمدة المعدنية الموصى بها (صفر، ٢٥ ، ٥٠ ، ٧٥ ، ١٠٠% ن فو بو) لتحسين النمو وبعض الصفات الفسيولوجية والكيماوية والمحصول وجودته فى نباتات الكركديه . وأشارت النتائج المتحصل عليها زيادة معنوية فى معظم الصفات المدروسة بالنمو وصبغات التمثيل الضوئى والسكريات الكلية الذائبة والكربوهيدرات الكلية والفينولات الكلية والمحتوى المعدنى فى الأوراق والمحصول وجودته باستخدام الأسمدة الحيوية منفردة أو مشتركة مع الأسمدة المعدنية بالمقارنة بنباتات الكونترول . أفضل النتائج فيما يتعلق بالتفاعل تحققت بإضافة الفوسفورين يليه النيتروبين عند المقارنة مع المعاملة ١٠٠% من الجرعة الموصى بها من التسميد المعدنى . أيضاً أفضل النتائج لـ ن ، فو ، بو (% ، الممتص) فى أوراق الكركدية قد سُجلت فى النباتات المعاملة بالنيتروبين ، الفوسفورين، EM على التوالى . علاوةً على ذلك أدى استخدام الأسمدة الحيوية مع معدلات الأسمدة المعدنية حتى ٧٥% من الجرعة الموصى بها إلى الحصول على أعلى القيم لعدد ووزن الثمار الغضة / نبات ، الوزن الغض والجاف للسبلات بينما نقصت عند المعدل ١٠٠% . وسُجلت أعلى القيم لوزن بذور النبات باستخدام الأسمدة الحيوية مع ١٠٠% من السماد المعدنى . علاوةً على ذلك أدى استخدام الأسمدة الحيوية مع المعدلات المختلفة من التسميد المعدنى إلى زيادة فى تركيز الأنتوسيانين والفينولات الكلية والمحتويات الصلبة الكلية وفيتامين ج فى السبلات . وقد تفوق الفوسفورين على النيتروبين ، EM فى معظم الصفات المدروسة . من النتائج السابقة يُمكن التوصية باستخدام ٥٠% أو ٧٥% من التسميد المعدنى مع الفوسفورين أو النيتروبين لإنتاج أعلى محصول مع زيادة فى المواد النشطة . أيضاً استخدام الأسمدة الحيوية مع الجرعة الموصى بها (١٠٠% ن فو بو) للحصول على أعلى قيم لمحصول البذور / نبات .

Importance of biofertilization in reducing the required of mineral……..

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Table (2): Vegetative growth parameters of roselle plants as affected by bio- and mineral fertilizers during 2008 and 2009 seasons.

Treatment \ Character		2008 season							2009 season						
		Plant height (cm)	Number of branches / plant	Number of leaves / plant	Leaf area (cm^2) / plant	Dry weight of leaves (g)/plant	Dry weight of stem (g)/plant	Dry weight of root (g)/plant	Plant height (cm)	Number of branches / plant	Number of leaves / plant	Leaf area (cm^2) / plant	Dry weight of leaves (g)/plant	Dry weight of stem (g)/plant	Dry weight of root (g)/plant
Pho.		50.13	5.33	52.60	266.8	5.97	3.88	0.94	51.53	4.80	51.93	284.70	6.00	4.38	1.02
Nit.		47.53	5.0	47.66	209.7	4.34	3.44	0.63	48.86	4.46	47.93	228.10	5.02	3.64	0.75
EM		39.53	2.2	33.46	172.2	3.08	2.44	0.53	42.27	2.73	39.53	189.18	3.64	2.86	0.64
0.0		37.88	3.44	36.22	157.62	3.29	2.05	0.43	41.33	3.22	38.22	179.73	3.81	2.51	0.51
25% NPK		43.00	3.77	39.44	182.1	4.43	2.65	0.56	43.44	3.55	41.66	208.03	4.39	3.03	0.69
50% NPK		45.44	4.22	43.66	216.8	4.32	3.21	0.70	47.11	3.88	45.89	237.73	5.04	3.51	0.78
75% NPK		48.44	4.55	48.33	239.3	4.70	3.75	0.82	50.22	4.44	50.22	263.50	5.45	4.02	0.91
100% NPK		53.88	4.88	55.22	285.5	5.58	4.59	0.97	55.66	4.89	56.33	281.10	5.73	5.05	1.13
0.0	Pho.	43.00	4.67	43.00	213.9	4.42	2.48	0.65	45.67	4.00	45.00	225.10	4.82	2.90	0.71
	Nit.	40.33	4.33	40.00	154.8	3.26	2.20	0.38	42.33	3.67	39.33	171.50	3.61	2.55	0.47
	EM	30.33	1.33	25.67	104.5	2.20	1.49	0.28	36.00	2.00	30.33	142.60	3.01	2.10	0.36
25%	Pho.	48.00	5.00	46.00	224.6	4.73	3.30	0.81	47.33	4.33	48.00	244.1	5.13	3.65	0.91
	Nit.	45.00	4.67	42.33	192.9	4.08	2.68	0.47	44.66	4.00	41.66	211.4	4.45	3.00	0.62
	EM	36.00	1.67	30.00	128.7	2.50	1.97	0.41	38.33	2.33	35.33	168.6	3.60	2.44	0.55
50%	Pho.	50.00	5.33	49.67	275.4	5.77	3.69	0.92	51.33	4.67	50.33	290.1	6.05	4.11	0.98
	Nit.	48.00	5.00	46.67	218.9	4.30	3.49	0.64	49.00	4.33	48.33	234.2	5.11	3.62	0.73
	EM	38.33	2.33	34.67	156.3	2.88	2.46	0.56	41.00	2.67	39.00	188.9	3.75	2.81	0.64
75%	Pho.	53.00	5.67	54.67	290.2	6.04	3.43	1.08	55.66	5.33	55.0	327.2	6.85	5.0	1.11
	Nit.	50.33	5.33	52.33	237.5	4.55	3.92	0.75	52.33	5.0	51.66	256.4	5.68	4.02	0.88
	EM	42.00	2.67	38.00	190.1	3.52	2.92	0.64	42.66	3.0	44.0	206.9	3.84	3.06	0.75
100 %	Pho.	56.67	6.0	69.67	330.0	6.93	5.50	1.23	58.00	5.67	61.33	337.20	7.15	6.23	1.46
	Nit.	54.00	5.67	57.00	244.5	5.52	4.91	0.94	56.00	5.33	58.66	267.30	6.04	5.02	1.08
	EM	51.00	3.0	39.00	281.9	4.29	3.37	0.75	53.33	3.67	49.00	238.90	4.00	3.91	0.91
LSD at 5%		A=0.82 B=0.83 AB=1.84	A=0.46 B=0.40 AB=N.S	A=2.99 B=1.76 AB=3.93	A=2.56 B=2.25 AB=5.03	A=0.48 B=0.34 AB=0.76	A=0.02 B=0.02 AB=0.04	A=0.008 B=0.006 AB=0.01	A=0.78 B=0.52 AB=1.15	A=0.47 B=0.49 AB=N.S	A=0.85 B=0.57 AB=1.27	A=4.40 B=1.80 AB=4.02	A=0.19 B=0.11 AB=0.08	A=0.14 B=0.11 AB=0.08	A=0.02 B=0.01 AB=0.02

Table (3): Physiological parameters in roselle plants as affected by bio- and mineral fertilizers during 2008 and 2009 seasons.

Character / Treatment		2008 season					2009 season				
		Chl. a + b (mg/g d.w)	Carotenoids (mg/g d.w)	Total soluble sugars (mg/g d.w)	Total carbohydrates (mg/g d.w)	Total phenols (mg caticol / 100 g d.w)	Chl. a + b (mg/g d.w)	Carotenoids (mg/g d.w)	Total soluble sugars (mg/g d.w)	Total carbohydrates (mg/g d.w)	Total phenols (mg caticol / 100 g d.w)
Pho.		6.32	2.87	17.53	176.00	16.93	5.46	3.09	17.68	181.1	17.54
Nit.		5.42	2.59	16.04	167.50	16.11	5.18	2.85	15.67	171.1	15.86
EM		4.52	1.79	13.74	153.91	14.18	4.45	2.37	13.32	163.2	14.24
0.0		4.74	1.95	13.81	145.80	14.44	3.98	2.27	13.04	153.9	14.14
25% NPK		4.97	2.19	14.86	154.50	14.86	4.29	2.50	14.88	158.1	15.12
50% NPK		5.53	2.46	15.54	166.60	15.35	5.11	2.87	15.80	170.1	15.59
75% NPK		5.70	2.58	16.78	177.10	16.45	5.26	2.95	16.51	185.3	16.59
100% NPK		6.16	2.89	17.86	185.00	17.59	6.52	3.27	17.55	191.7	17.95
0.0	Pho.	4.35	2.38	14.90	154.10	15.49	4.50	2.47	15.04	161.1	16.01
	Nit.	4.22	2.10	14.55	143.30	14.50	3.93	2.35	13.10	152.4	13.68
	EM	3.53	1.38	12.00	140.00	13.35	3.41	2.00	11.00	148.2	12.75
25%	Pho.	5.06	2.66	16.30	168.20	15.67	4.85	2.75	17.05	165.4	16.72
	Nit.	4.81	2.43	14.80	152.40	15.17	4.23	2.60	14.95	157.8	15.44
	EM	4.0	1.50	13.50	144.00	13.76	3.88	2.15	12.66	151.2	13.22
50%	Pho.	5.84	2.85	17.42	177.4	16.23	5.61	3.00	17.69	184.2	17.00
	Nit.	5.07	2.65	15.17	168.3	15.82	5.23	2.88	16.01	165.9	15.68
	EM	4.38	1.88	14.03	151.5	14.00	4.51	2.41	13.71	160.2	14.11
75%	Pho.	6.78	3.02	19.05	188.2	17.61	6.64	3.31	18.91	195.4	18.01
	Nit.	5.44	2.75	16.90	183.1	17.06	6.00	3.00	16.42	188.2	16.87
	EM	4.56	2.00	14.40	160.1	14.68	5.14	2.55	14.22	172.3	14.91
100%	Pho.	7.45	3.43	20.00	192.4	19.68	7.71	3.95	19.75	199.3	20.00
	Nit.	6.49	3.03	18.80	190.3	18.00	6.60	3.20	17.91	191.5	17.65
	EM	5.31	2.22	14.78	172.3	15.11	5.25	2.68	15.01	184.3	16.20
LSD at 5%		A=0.006 B=0.004 AB=0.009	A=0.03 B=0.02 AB=0.13	A=0.07 B=0.05 AB=0.08	A=1.48 B=1.50 AB=3.36	A=0.09 B=0.06 AB=0.14	A=0.23 B=0.11 AB=0.24	A=0.19 B=0.14 AB=0.29	A=0.11 B=0.12 AB=0.28	A=1.32 B=0.94 AB=2.11	A=0.37 B=0.28 AB=0.61

Table (4): Minerals concentration in roselle plants as affected by bio- and mineral fertilizers during 2008 and 2009 seasons.

Treatment \ Character		2008 season N (%)	2008 season N uptake (mg / plant)	2008 season P (%)	2008 season P uptake (mg / plant)	2008 season K (%)	2008 season K uptake (mg / palnt)	2009 season N (%)	2009 season N uptake (mg / plant)	2009 season P (%)	2009 season P uptake (mg / plant)	2009 season K (%)	2009 season K uptake (mg / palnt)
Pho.		2.41	13.57	0.49	2.80	2.74	15.47	2.64	16.06	0.48	2.93	2.79	16.97
Nit.		3.45	15.08	0.41	1.83	3.05	13.44	3.48	17.43	0.38	1.96	3.05	16.52
EM		2.89	9.32	0.32	1.04	3.78	12.11	2.90	10.37	0.34	1.26	3.68	13.54
0.0		2.51	7.98	0.33	1.17	2.68	8.65	2.65	10.02	0.32	1.28	2.61	11.43
25% NPK		2.67	9.98	0.37	1.45	2.94	10.76	2.80	11.97	0.35	1.59	2.98	12.90
50% NPK		2.87	12.09	0.40	1.83	3.09	13.02	2.99	14.86	0.38	1.96	3.15	15.38
75% NPK		3.12	14.53	0.45	2.16	3.45	15.58	3.16	16.92	0.44	2.46	3.40	18.08
100% NPK		3.41	18.70	0.50	2.84	3.78	20.32	3.43	19.31	0.51	2.96	3.71	20.58
0.0	Pho.	2.04	9.02	0.45	1.99	2.48	10.96	2.37	11.42	0.41	1.98	2.39	11.52
	Nit.	2.95	9.62	0.31	1.01	2.62	8.54	3.10	11.19	0.30	1.08	2.45	13.75
	EM	2.56	5.63	0.23	0.51	2.94	6.47	2.48	7.46	0.26	0.78	3.00	9.03
25%	Pho.	2.22	10.50	0.47	2.22	2.57	12.16	2.41	12.36	0.45	2.31	2.60	13.34
	Nit.	3.04	12.40	0.36	1.47	2.86	11.67	3.25	14.46	0.33	1.47	2.95	13.13
	EM	2.75	6.87	0.27	0.67	3.39	8.47	2.75	9.90	0.28	1.01	3.40	12.24
50%	Pho.	2.45	14.14	0.49	2.83	2.72	15.69	2.66	16.09	0.48	2.90	2.83	17.12
	Nit.	3.35	14.40	0.42	1.81	2.94	12.64	3.41	17.42	0.36	1.84	3.06	15.63
	EM	2.81	8.09	0.30	0.86	3.65	10.51	2.91	10.91	0.31	1.16	3.62	13.57
75%	Pho.	2.63	15.88	0.51	3.08	2.88	17.39	2.77	18.97	0.52	3.56	3.00	20.55
	Nit.	3.85	17.52	0.47	2.13	3.38	15.38	3.68	20.90	0.41	2.32	3.26	18.52
	EM	2.90	10.21	0.36	1.27	4.11	14.47	3.05	11.71	0.39	1.50	3.95	15.17
100%	Pho.	2.71	18.78	0.56	3.88	3.05	21.14	3.00	21.45	0.55	3.93	3.15	22.52
	Nit.	4.08	22.52	0.50	2.76	3.48	19.21	3.97	23.98	0.51	3.08	3.57	21.56
	EM	3.45	14.80	0.44	1.89	4.81	20.63	3.33	12.52	0.47	1.88	4.42	17.68
LSD at 5%	A	A=0.12	A=0.48	A=0.01	A=0.15	A=0.14	A=0.36	A=0.03	A=0.49	A=0.01	A=0.16	A=0.09	A=0.36
	B	B=0.06	B=0.46	B=0.02	B=0.07	B=0.04	B=0.27	B=0.02	B=0.59	B=0.007	B=0.16	B=0.08	B=0.35
	AB	AB=0.13	AB=1.03	AB=0.03	AB=0.17	AB=0.07	AB=0.60	AB=0.04	AB=1.10	AB=0.01	AB=0.36	AB=0.17	AB=0.79

Table (5): Yield and its components of roselle plants as affected by bio- and mineral fertilizers during 2008 and 2009 seasons.

Character / Treatment		2008 season					2009 season				
		Number of fruits / plant	Fresh weight of fruits (g/plant)	Fresh weight of sepals (g/plant)	Dry weight of sepals (g/plant)	Seed yield (g/plant)	Number of fruits / plant	Fresh weight of fruits (g/plant)	Fresh weight of sepals (g/plant)	Dry weight of sepals (g/plant)	Seed yield (g/plant)
Pho.		82.46	235.2	214.1	82.08	29.16	77.86	236.2	223.00	77.62	32.52
Nit.		74.26	180.2	199.1	73.70	21.59	68.06	182.6	221.90	66.3	24.51
EM		55.66	142.6	146.4	57.36	19.05	60.60	157.2	137.60	52.5	21.68
0.0		49.00	135.4	108.8	50.60	17.56	49.78	137.8	95.33	45.84	18.57
25% NPK		64.11	164.8	176.3	64.60	18.69	63.78	171.6	180.30	59.95	22.02
50% NPK		72.78	177.9	205.7	74.83	23.56	71.33	191.6	217.60	67.78	25.39
75% NPK		81.89	247.4	236.7	85.30	26.81	78.00	249.3	251.40	78.54	31.13
100% NPK		86.22	204.5	205.2	79.90	30.22	81.33	209.7	226.20	75.26	34.05
0.0	Pho.	62.67	171.7	124.0	59.73	20.81	58.67	177.6	110.70	52.80	21.11
	Nit.	50.33	127.3	110.1	47.03	16.67	49.33	133.5	98.60	45.10	18.55
	EM	34.00	107.9	92.5	45.10	15.22	41.33	102.3	76.70	39.63	17.05
25%	Pho.	74.33	205.2	211.9	71.16	21.72	70.67	217.7	229.9	68.65	25.10
	Nit.	69.67	166.5	195.2	69.96	16.67	65.33	150.5	218.5	63.75	18.83
	EM	48.33	122.7	121.9	52.81	17.70	55.33	146.6	92.6	47.46	22.15
50%	Pho.	85.33	217.7	253.3	89.90	28.31	81.67	247.5	258.3	79.23	31.43
	Nit.	75.33	169.6	233.1	77.11	19.22	71.33	166.4	245.9	72.31	21.11
	EM	57.67	134.1	130.8	57.53	21.64	61.00	160.9	148.6	51.82	23.65
75%	Pho.	100.33	335.6	271.8	100.50	35.56	93.67	305.3	309.5	99.20	40.10
	Nit.	93.33	247.5	256.8	93.83	20.81	79.33	257.4	277.7	79.23	25.23
	EM	65.00	159.2	181.5	61.60	24.06	71.00	185.3	166.8	57.20	28.07
100 %	Pho.	89.67	246.1	209.8	89.25	39.43	84.67	233.1	206.8	88.25	45.85
	Nit.	82.67	190.2	200.4	80.70	21.88	75.00	205.2	270.3	71.12	26.18
	EM	73.67	177.6	205.6	69.83	29.36	74.33	190.8	201.6	66.41	30.12
LSD at 5%		A=0.88 B=0.94 AB=2.10	A=6.31 B=4.21 AB=9.41	A=3.72 B=2.20 AB=4.92	A=1.58 B=1.37 AB=3.07	A=0.95 B=0.81 AB=1.81	A=0.64 B=0.52 AB=1.17	A=2.19 B=1.56 AB=3.50	A=1.67 B=1.09 AB=2.43	A=1.28 B=0.98 AB=2.20	A=1.58 B=0.59 AB=1.32

Table (6): Yield quality of roselle plants as affected by bio- and mineral fertilizers during 2008 and 2009 seasons.

Character / Treatment		2008 season				2009 season			
		Anthocyanin (mg/100 g f.wt)	Total phenols (mg caticol / 100 g d.wt)	Total soluble solids (%)	Vitamin C (mg/g f.wt)	Anthocyanin (mg/100 g f.wt)	Total phenols (mg caticol / 100 g d.wt)	Total soluble solids (%)	Vitamin C (mg/g f.wt)
Pho.		23.28	29.42	14.86	17.25	23.46	30.76	14.64	17.06
Nit.		20.83	25.81	12.68	16.34	20.58	26.78	12.56	15.49
EM		17.94	24.39	12.84	13.81	17.73	25.24	11.76	11.74
0.0		18.54	22.65	10.44	10.68	17.86	23.76	10.60	10.44
25% NPK		19.44	25.30	12.66	15.24	18.88	26.43	11.86	14.82
50% NPK		20.22	26.25	13.73	16.27	20.17	27.13	13.26	15.31
75% NPK		21.65	28.00	16.13	17.48	22.02	29.23	14.66	17.21
100% NPK		23.58	30.48	16.00	19.32	24.00	31.43	14.52	19.38
0.0	Pho.	21.04	25.14	11.60	11.70	20.09	26.61	12.20	11.52
	Nit.	18.57	22.08	10.80	11.52	18.20	23.22	10.40	10.62
	EM	16.01	20.75	8.80	8.82	15.30	21.45	9.20	9.18
25%	Pho.	21.41	27.45	13.40	17.48	21.15	28.71	12.80	16.20
	Nit.	20.01	24.66	12.40	15.30	19.40	25.90	11.60	15.66
	EM	16.91	23.81	12.20	12.96	16.11	24.68	11.20	12.60
50%	Pho.	22.87	28.31	14.40	18.36	23.05	29.31	14.20	18.54
	Nit.	20.75	25.55	12.80	17.10	20.00	26.33	13.20	13.95
	EM	17.05	24.91	14.00	13.36	17.48	25.76	12.40	13.50
75%	Pho.	24.67	30.72	17.60	18.90	25.11	32.72	17.20	19.08
	Nit.	21.55	27.85	16.40	18.36	21.20	28.75	14.00	18.00
	EM	18.75	25.44	14.40	15.20	19.75	23.00	12.80	14.58
100%	Pho.	26.45	35.48	17.20	19.80	27.90	36.48	16.80	19.98
	Nit.	23.30	28.91	16.00	19.44	24.11	29.70	13.60	19.26
	EM	21.00	27.07	14.80	18.72	20.06	28.11	13.20	18.90
LSD at 5%		A=0.09 B=0.09 AB=0.20	A=0.67 B=0.79 AB=0.53	A=0.28 B=0.22 AB=0.67	A=0.08 B=0.07 AB=0.16	A=0.66 B=0.39 AB=0.88	A=0.93 B=0.62 AB=1.38	A=0.14 B=0.21 AB=0.47	A=0.19 B=0.11 AB=0.25