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Response of spinach (*Spinacia olerasea* L.) to algae extract under different nitrogen rates

Doaa M.R. Abo- Basha, Rasha R.M. Afify and Hanan H. Abdel-Kader

Plant Nutrition Department, *National Research Centre, 33 El-Buhouth St., (former El- Tahrir St.,) Dokki, Giza, Egypt. Postal Code: 12622*

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ABSTRACT

Two pot experiments were carried out during the two successive seasons of 2016/2017 and 2017/2018, at the green house of National Research Centre, Dokki, Egypt, to select the suitable rates of nitrogen or application methods of algae extract to increase nitrogen use efficiency as well as maximizing yield and improving quality of Spinach plants grown in sandy loam soil. The experiment included three rates of mineral nitrogen fertilizer (60, 80 and 100% of recommended mineral N rate) and application of algae extract as a foliar spray or to soil application. The results showed that applications of algae extract to soil significantly increased the vegetative growth parameters and its components as well as contents of N, P, K, Fe, Mn, Zn, Cu and protein content, as well as leaf chlorophyll content compared to untreated plants. Also, Spinach plants which have been fertilized at the rate 80% or 100% of the nitrogen recommended rate (NRR) significantly enhanced plant growth, yield parameters and its components compared to fertilization with 60% of NRR. The recommended 80% NRR combined as a foliar application of algae extract had a favorable effect for improve vegetative growth and its quality. The application of microbial inoculants (bio-fertilizers or algae extract) is a promising technology to enhance the use of conventional inorganic fertilizers and for future sustainable farming systems in view of the need to more efficiently use available nitrogen (N). Algae extract save 20% from nitrogen requirement of Spinach plants.

***Keywords*:** Spinach production, Algae extract, nitrogen fertilizers

Introduction

Spinach (*Spinacia olerasea L.*,) vegetable is among the oldest vegetable crops in the world and success stories of its cultivation are attributed to the plant genetic physiological flexibility that allows for adaption to wide range of environments. The nutritional value of this vegetable is highly rated (Khandaker *et al.* 2008). Its high rate nutritional value coupled with easy environmental adaption make the vegetable crop suitable for improve in vegetable food security and reducing micro nutrients malnutrition globally (Soleymani *et al*. 2011a, b). Research has shown that vegetable cultivation can be improved under good soil management and ideal fertilization (Moradi *et al*. 2010 and Ogbaji *et al*. 2018).

Modern agricultural management is heavily dependent on the fertilizers for promotion of crop production, but the massive use of inorganic and chemical-based fertilizers currently available may be a serious threat to human health and environment.

Nitrogen has many functions in plant life being responsible for the biosynthesis of enzymes, nucleoproteins, amino acids, protein, sugars, polypeptides, chlorophylls and encourage cell division (Marschner 1995). In this respect, mineral nitrogen fertilizer enhanced plant growth (El-Desuki *et al.* 2010), leaf chlorophyll content and yield and its components (Danesh *et al.* 2012).

The application of microbial inoculants (Algae) is a promising for future sustainable farming systems in view of the need to more efficiently use available nitrogen (N).Algae are classified into three groups; namely green, brown and red based on their pigments. Different forms of algae have been reported to produce beneficial effects on some vegetable crops (Abdel-Mawgoud *et al*. 2010). They are a natural bio active materials rich in minerals, protein, lipids, carbohydrates, vitamins and microelements (Co, B, Mo, Zn, Cu). Algae extract as foliar application were recommended for increasing the growth parameters of potato (Awad *et al*. 2006), tomato (Nour *et al*. 2010), green

Corresponding Author: Doaa M.R. Abo- Basha, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt. E-mail: dr.doaa_nrc@yahoo.com

gram(Pramanick *et al*. 2013) and garlic plants (Shalaby and El-Ramady2014), Also, some researcher revealed that an improve in Photosynthetic pigments of bean, potato and snap bean (Latique *et al*. 2013) additionally, Mohsen 2012 reported that, an increment of number of pods per plant and total yield of green gram, cucumber and garlic plants reduction of chemical fertilizers became very important due to increasing their prices, also less ground water pollution as well as for human health.

The aim of this study is to examine the role of algae in minimize the amount nitrogen fertilizer as well as improving yield and quality of Spinach plant which grown under sandy loam soil conditions.

Materials and Methods

Soil analysis:-

Soil samples were taken from Research and Production Station, National Research Centre, Nobaria site, Beheara Governorate, Delta Egypt. Particle size distribution was determined as described by Blackmore *et al.* (1972). Soil pH, EC, cations and anions, organic matter, $CaCO_3$, total nitrogen as well as available phosphorus and Potassium were determined according to Black *et al.* (1982).

Some physical and chemical properties of the tested soil are shown in Table 1

Table 1: Some physical and chemical properties of used soil (Mean of two seasons).

Particle size distribution			**Texture class**	**pH (1:2.5)**	**EC (dSm^{-1}) (1:5)**		**$CaCO_3$ %**	**OM %**	
Sand %	**Silt %**	**Clay%**							
69.7	**12.2**	**17.8**	Sandy loam	7.65	2.24		2.2	0.28	
Cations and Anions (meq/L)								**Available Macronutrients (mg-100g soil)**	
Na^+	**K^+**	**Ca^{++}**	**Mg^{++}**	**CO_3^{--}**	**HCO_3^-**	**CL^-**	**SO_4^{--}**	**N**	**P**	**K**
11.53	1.41	5.60	3.82	------	4.11	9.33	8.71	6.22	0.85	11.32

Plant material and experimental work:-

Two pot experiments were conducted during two successive seasons, 2016/2017 and 2017/2018 at Wire house, National Research Centre, to evaluate the effect of algae on minimize the amount of nitrogen fertilizer as well as improving Spinach yield and quality. At 8th and 17th of December 2016/2017 and 2017/2018, seeds of Spinach (*Spinacia olerasea L*.,) were sown in plastic pots of eight Kg capacity. Pots were filled with sandy loam soil taken from Nobaria farm. All pots were received the recommended phosphorus and potassium fertilizers (1.5 g/pot supper phosphate 15 % P_2O_5 and 2.4 g/pot potassium sulphate 48% K_2O). Ten seeds of Spinach were sown and thinned to five plants per pot (after two weeks from sowing).

Nitrogen Fertilizer (Ammonium sulphat 20.5%) was used at 100%, 80% and 60% from recommended dose.

Preparation of Algae extract: - The used algae in this study *Spirulina platensis* as a photosynthetic and multicellular blue green micro alga which grows in wide range fresh, marine and brackish water (Marrez *et al*. 2014). The source of fresh algae was Algal Biotechnology Unit, NRC, Egypt. After 21 and 36 days from sowing were added to soil or as a foliar (at 2 mL/L) with all nitrogen treatments beside control plants (with tap water).

Measurement of vegetative growth and yield:-The growth parameters of Spinach such as plant height, leaves fresh and dry weights of Spinach were recorded according FAO (1980).

Measurement of nutritional status:- Macronutrients (N, P and K) and micronutrient (Fe, Mn, Zn and Cu) in Spinach were determined according to Black *et al* (1982).

Protein percentage: - was calculated by multiplying nitrogen content by 6.25.

Nitrate content:- in Spinach leaves was determined according to Faithfull (2002).

Measurement chlorophyll content:- Chlorophyll a, chlorophyll b and chlorophyll a+b in Spinach leaves were determined according to Lichtenthalr and Wellburn (1983).

Statically analysis:-

All data were statistically analyzed by using factorial completely randomized design. The means were compared using the least significant difference test (LSD at 5% level) according to Gomez and Gomez (1984**)**.

Results and Discussion

1. Growth and yield Characteristics:-

1.1. Effect of nitrogen rates:-

Data in Table (2) indicate that, all growth and yield parameters i.e., plant height (cm), leaf fresh yield (g/plant) and dry weight (g/plant) recorded the highest values with 100%NRR followed by 80%NRR followed by 60% NRR. These results are in harmony with those obtained by Marschner (1995) who stated that, the stimulative effect of nitrogen on different plant growth parameters may owe much to that nitrogen is an essential element for building up protoplasm, amino acids and protein which promote cell division. Also, nitrogen plays a vital contribution in several biochemical processes related to plant growth.

Erman *et al.* (2009) added that application of nitrogen to plants significantly enhanced vegetative growth characters.

1.2. Effect of algae extract:-

Present data in Table (2) show that, both foliar and soil application of algae extract significantly increased Spinach growth and yield characters compared with control (nitrogen levels alone). Algae extract soil application with 100% nitrogen recorded the greatest 31.77 cm, 30.13 g/plant and 5.11g/plant in plant height, leaf fresh yield and dry weight of Spinach respectively. Algae extract had a significant promotive effect of the studied parameters of Spinach under different rates of nitrogen compared with untreated plants. It is clear that Algae extract enhance the studied growth parameters especially with 100% or 80% NRR. Algae extract with 60% NRR gave the lowest values.

Table 2: Effect of Algae Extract and Nitrogen Fertilizer Rates on vegetative growth of Spinach plant (Mean of two seasons)

Treatments		**Plant height (cm)**	**Leaf fresh yield (g/ plant)**	**Leaf dry weight(g/plant)**
Algae	**NRR%**			
Control	**60**	19.98 f	19.89 f	3.35 f
	80	22.68 e	21.72 e	4.30 d
	100	25.82 d	20.88 ef	3.80 e
Mean		**22.83 C**	**20.83 B**	**3.82 B**
Soil application	**60**	26.86 cd	24.85 c	4.60 c
	80	30.31 ab	27.12 ab	4.71 ab
	100	31.77 a	30.13 a	5.11 a
Mean		**29.65 A**	**29.65 A**	**4.81 A**
Foliar application	**60**	27.58 cd	23.74 d	4.31 d
	80	28.66 bc	27.97 b	4.61bc
	100	28.51 bc	28.96 ab	4.74 ab
Mean		**28.25 B**	**26.89A**	**4.55 A**
Mean of NRR	**60**	**24.81C**	**22.82 C**	**4.09 B**
	80	**27.22 B**	**25.60 B**	**4.54 A**
	100	**28.70 A**	**26.66 A**	**4.57 A**

The values in the column having the same letter(s) are not significantly different at P= 0, 05 using LSD test.
*NRR: Nitrogen Recommended Rates

Algae extract save 20% from nitrogen requirement of Spinach plants.These results are agree with those obtained by Attememe (2009) who pointed that, The increscent effect of Algae extract on Spinach plant growth and yield may be attributed to the auxin content of algae extract. On the other hand, *Spirulina platensis* contains macronutrients (N, P and K) which are essential elements for plant growth and development. Confirm these results Gollan and Wright (2006) who reported that Algae extract increase plant height, shoot and root fresh and dry weights of Pea plant. Genaidy *et al*. (2015) added that Algae extract gave the great benefits on supplementing Spinach plants with their requirements from mineral nutrients.

2. Nutritional Status:-

2.1. Macronutrients:-

2.1.1. Effect of nitrogen rates:-

Data in Table (3) reveal that the addition of NRR 100% significantly increases the content of nitrogen, phosphorous, potassium andtotal protein followed by 80% NRR. While60 % NRR gave the lowest values.

These results are good agreement with those obtained by El-Desuki *et al*. (2010) who showed that, the content of N, P and K as well as total protein of Pea plants significantly increased as increasing nitrogen rates in plant media (100% NRR).

Table 3: Effect of Algae Extract and Nitrogen Fertilizer Rates on Macro nutrient content and Protein of Spinach plants (Mean of two seasons).

Treatments		Macronutrient content (%)			Protein	NO_3 (mg/Kg)
Algae	NRR%	N	P	K		
Control	60	2.25f	0.51e	2.64 f	14.06f	291 e
	80	2.68e	0.53 d	2.76 e	16.75 e	353 b
	100	2.93c	0.59bc	2.81 cd	18.31c	416 a
Mean		**2.62 B**	**0.54 B**	**2.74 C**	**16.37 B**	**353A**
Soil application	60	2.73d	0.48 c	2.75 e	17.06 d	273 g
	80	3.21 a	0.68 a	3.11 a	20.06 a	298 d
	100	3.26 a	0.73 ab	3.15 a	20.37 a	311 c
Mean		**3.03 A**	**0.63 A**	**3.03 A**	**19.16 A**	**294 B**
Foliar application	60	2.64 e	0.57c	2.71 e	16.50 e	258 h
	80	3.11 b	0.58 c	2.91 b	19.44 b	283 f
	100	3.16 b	0.62 b	3.09 b	19.75 b	291 e
Mean		**2.94 A**	**0.59 AB**	**2.90 B**	**18.36 A**	**277 C**
Mean of NRR	60	**2.54 B**	**0.55 B**	**2.75 B**	**15.89 B**	**339 A**
	80	**3.00 A**	**0.61 A**	**2.93 A**	**17.99 A**	**312 B**
	100	**3.05 A**	**0.63 A**	**2.95 A**	**19.11 A**	**274 C**

The values in the column having the same letter(s) are not significantly different at P= 0, 05 using LSD test.
*NRR: Nitrogen Recommended Rates

2.1.2. Effect of algae extract:-

Data in Table (3) show that, Algae extract addition as foliar or to soil had a significantly positive effect on Spinach N, P, K and total protein. These results are in harmony with those obtained by Abou El-Khair *et al*. (2010) who stated that the spraying of blue green algae extract to plants showed increases in N and P percentages. The effect of algae extract on garlic plants showed increases in N and P percentage. Confirm these results Genaidy *et al*. (2015). Latique *et al*. (2013) added that, Algae extract significantly improving biochemical characters of proteins content in *phaseolas vulgaris* plants. The addition of Algae extract to soil gave N, P, K and total proteins in Spinach plants higher than adding Algae extract as a foliar especially with 100% NRR followed by 80% NRR. Data also

reveal that 60% NRR gave the lowest values. It is clear that no significantly affected by 100% or 80% with Algae extract.

2.2. Micronutrients:-

2.2.1. Effect of nitrogen rates

Data in Table (4) show that the rate of nitrogen 100 % NRR gave the highest values of the studied micronutrients (Manganese, Zinc, Caber and Iron) in Spinach leaves followed by 80% N while the level of 60% N recorded the lowest ones in two seasons. These results are agree with those obtained by Marrez *et al*. (2014) who found that *Spirulina platensis* is a rich source of potassium and contains amounts of Ca, Cu, Fe, Mg, Mn, P and Zn which have a great role in cell division and enlargement and induce the Photosynthesis and in turn reflected a great shoot growth. Confirm these results Lopez *et al.* (2008).

2.2.2. Effect of algae extract:-

Data in Table (4) indicate that Spinach plants which treated with Algae extract as foliar resulted the highest content of Micronutrients Fe (511 mg/Kg), Mn (48.32 mg/Kg), Zn (69.32 mg/Kg) and Cu (8.22 mg/Kg) respectively, followed by Algae extract to soil compared with untreated plants. These results are in harmony with those obtained by Islam *et al*. (2011). Data in Table (4) also indicated that the two applications of Algae extract (to soil or foliar) significantly increased the content of micronutrients (Fe, Mn, Zn and Cu) in Spinach leaves especially with 100% NRR followed by 80% NRR followed by 60% NRR. These results are agree with those obtained by Vignesh *et al.* (2012).

Table 4: Effect of Algae Extract and Nitrogen Fertilizer Rates on Micronutrient content of Spinach plants (Mean of two seasons).

Treatments		Fe	Mn	Zn	Cu
Algae	**NRR%**	**(mg/Kg)**			
Control	**60**	485 f	39.23 h	53.15 i	7.82 f
	80	498 cd	39.67 g	62.54 e	8.15 cd
	100	504 bcd	41.35 e	68.32 b	8.27 a
Mean		**496 B**	**40.08 B**	**61.34 C**	**7.08 C**
Soil application	**60**	496 de	40.51 f	58.73 h	7.93 e
	80	508 ab	45.78 d	61.41 g	8.21 b
	100	511 a	48.32 a	69.32 a	8.22 b
Mean		**505 A**	**44.87 A**	**63.15B**	**8.12 B**
Foliar application	**60**	491 ef	41.35 e	62.31 f	8.10 d
	80	506 abc	46.12 c	67.15 d	8.19 c
	100	508 ab	47.21 b	68.23 c	8.21 b
Mean		**502A**	**44.89 A**	**65.90 A**	**8.17 A**
Mean of NRR	**60**	**490 B**	**40.38 C**	**58.07 C**	**7.95 C**
	80	**504 A**	**43.86 B**	**63.70 B**	**8.19 B**
	100	**508 A**	**45.63 A**	**68.63 A**	**8.24 A**

The values in the column having the same letter(s) are not significantly different at P= 0, 05 using LSD test.
*NRR: Nitrogen Recommended Rates

3. Nitrate Content:-

3.1. Effect of nitrogen rates:-

Data in Table (3) show that as nitrogen fertilizer in plant media increased, the nitrate in Spinach leaves content significantly increased. The addition of 100%NRR for Spinach plants had the highest NO_3 content followed by 80% from NRR while 60% from NRR gave the lowest one. These results are in harmony with those obtained by Marrez *et al.* (2014).

3.2. Effect of Algae extract:-

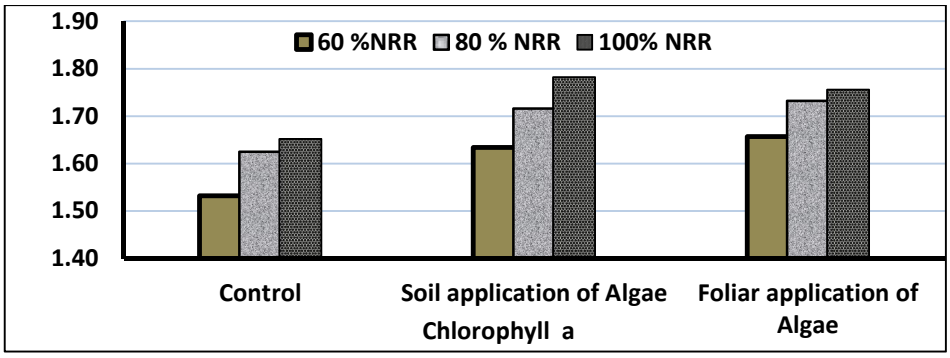
Data in Table (3) indicate that, nitrate content in Spinach leaves recorded the lowest values with the foliar application. While Spinach plants which treated with Algae extract to soil resulted nitrate content higher than foliar addition compared with untreated plants. These results are agree with those obtained by Hanafy *et al.* (2000) and Doaa (2009) found a significant decrease in nitrate accumulation of Spinach plant treated with biofertilizers.

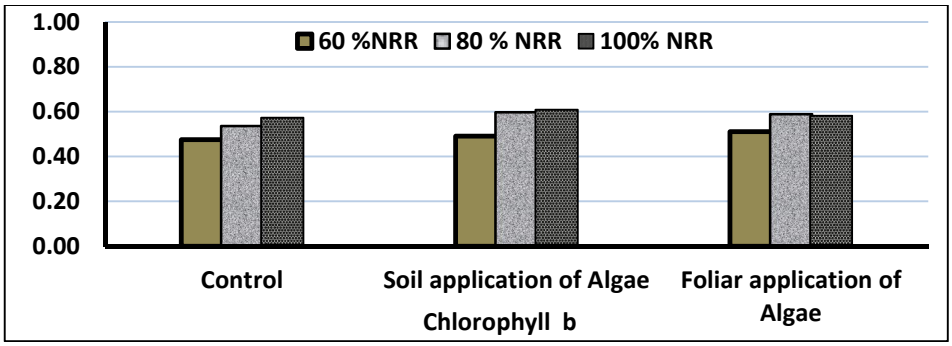
4. Chlorophyll Content:-

4.1. Effect of nitrogen rates:-

It is obvious from data in Fig. (1) that as increasing nitrogen level in plant media the chlorophyll content in Spinach leaves significantly increased.

Data clearly indicate that, both chlorophyll(a) and chlorophyll (b) as well as chlorophyll (a+b) contents in Spinach leaves recorded the greatest values with 100% NRR followed by 80% NRR while 60 % NRR gave the lowest ones. These results are in harmony with those obtained by Thirmaran *et al*. (2009).





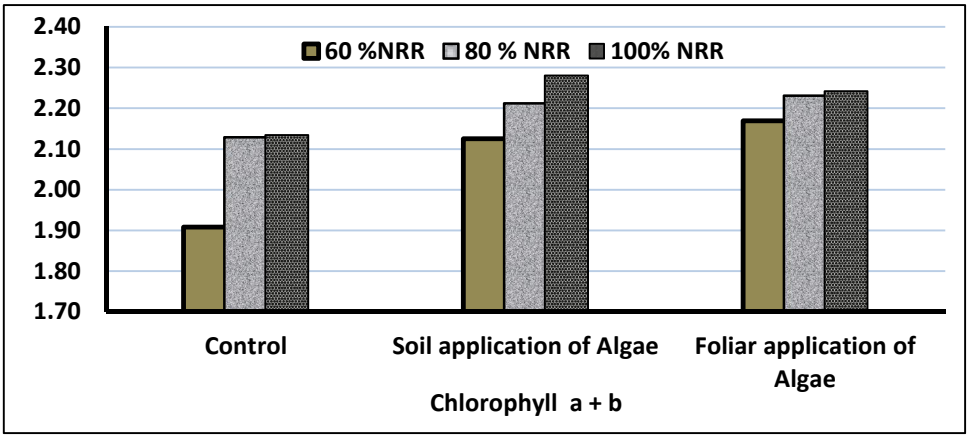


Fig.1: Effect of Algae Extract and Nitrogen Fertilizer Levels on Chlorophyll content of Spinach plants (Mean of two seasons).

4.2. Effect of Algae extract:-

Figure (1) clearly indicates that Algae extract significantly increased total chlorophyll content (chlorophyll a, chlorophyll b and chlorophyll a+b) in Spinach leaves tissue with plants which treated with Algae extract compared with untreated plants. Spinach plants which treated with Algae extract as soil application gave the highest values of total chlorophyll (a, b and a+b) of Spinach leaves especially with 100%NRR and 80% from NRR followed by plants which treated with Algae as a foliar. These results are in agreement with those obtained by Prasanna *et al.* (2008) who stated that using algae extract with chemical nitrogen fertilizers gave the highest values of chlorophyll in wheat leaves. Thomas (1996) reported that Algae extract contains cytokinens which induced the physiological activities and chlorophyll content in plant leaves. This will positively reflect in the photosynthesis rate and synthesized of chlorophyll.

Conclusion

Nitrogen fertilizer at recommended dose by Agriculture Ministry gave the highest growth, leaf yield and minerals content of Spinach compared with 80% and 60% from NRR. Algae extract application to soil gave the highest value followed by Algae extract application as a foliar. Nitrate content in Spinach leaves recorded the lowest figures with algae as a foliar followed by the application to soil. Algae extract save 20% from nitrogen requirement of Spinach.

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