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INTERACTION EFFECTS BETWEEN NITROGEN LEVELS AND BIOFERTILIZER INOCULATION METHODS ON OKRA GROWTH, YIELD AND PODS QUALITY.

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ABSTRACT

Two field trials were conducted at Sakha Horticultural Research Station Farm during the successive summer seasons of 2012 and 2013. The aim of the present study was to evaluate the effect of three nitrogen fertilization levels (60, 30 and 15 Kg N fed^{-1}) and two biofertilizer inoculation methods (liquid and tablets) on okra (Eskandrani var.) growth, yield and pods quality. 60 kg N fed^{-1} and liquid biofertilization method had the highest values of number of leaves / plant (36.19 and 37.52), stem length (96.84 and 110.14 cm), leaf area (409.93 and 425.8 cm^{2}), leaf dry weight (21.09 and 21.2 %), number of pods plant^{-1} (69.41 and 77.38), early yield (2.24 and 2.66 ton fed^{-1}) total yield (6.156 and 6.536 ton fed^{-1}) and nitrate concentration in okra dry pods (102.4 and 123.2 mg kg^{-1}). The interaction between application of 25% of the recommended nitrogen and tablets biofertilizer inoculation method had the lowest values of the previous parameters. Increasing nitrogen fertilization levels led to increase P% in okra leaves and pods either with or without biofertilization. Increasing nitrogen levels plus biofertilizer led to increase No_3 in leaves and pods.

INTRODUCTION

Okra is an annual herb and vegetable crop grown throughout the tropical and subtropical parts of the world. In Egypt, okra cultivated as a sole crop or intercropped with cotton and maize. Okra plays an important role in the human diet by supplying carbohydrates, proteins, fats, minerals and vitamins that are usually deficient in the table food. Okra requires heavy fertilization for its potential production (Avant and Manohar, 2001). Smil (2000) reported that use of mineral fertilizers can improve crop yield and soil pH, total nutrients content and nutrient availability, but its use is limited due to scarcity, high cost and nutrient imbalance. Indiscriminate use of inorganic fertilizers leads to nutrient imbalance in soil causing negative effect on soil properties and micro flora. Hence, there is need to reduce the use of chemical fertilizers. Significantly higher yield of okra was recorded at 150 kg ha^{-1} (Mubashir *et al*., 2010). Nitrate concentration increased up to 200% with increasing N. An excessive nitrate accumulation was recorded at 200 kg ha^{-1} Early season okra had significantly higher nitrate (10%) than late season (Mubashir *et al*., 2010). Vegetables are considered to be a high source of nitrate accumulation (Yimin *et al*., 1992). Vegetables are important dietary source of nitrate for human nutrition, increasing nitrate accumulation due to indiscriminate applications of N is becoming a public health concern. The toxic effects of nitrate are related to its endogenous conversion to nitrite which is related to methaemoglobinaemia, gastric cancer and other diseases (Santamaria, 2006). Excessive nitrate can also block iodide uptake of the sodium iodide symporter in a competitive manner (Tonacchera *et al*., 2005).

Mixing organic or bio and mineral fertilizers may be a sound soil fertility management strategy in many countries. A part from enhancing crop yields, the practices has a greater beneficial residual effect that can be derived from use of either organic or bio or mineral fertilizers applied alone. Akande *et al*., (2003) reported that combined use of ground rock phosphate applied together with poultry manure significantly improved growth and yield of okra compared to application of each material separately. Akande *et al*., (2010) found that complementary application of 2.5 tons organic based fertilizer and 60 Kg N as N P K 20:10:10 was most favored for okra growth and yield, which produced the highest values of plant height, stem circumference and leaf area, fruit yield and okra leaf N and P contents. Application of 6 ton ha^{-1} of plant residues increased the soil N, P, K, Ca, Mg, DM; pod N, P, K, Ca, Mg and ash, root length and pod yield of okra (Emmanuel, 2007).

Biofertilizers are the carrier- based preparations containing beneficial microorganisms in a viable state intended for seed or soil application. Biofertilizers are less expensive, eco-friendly viable and sustainable, and improves crop growth, yield and quality of produce. Biofertilizers fix appreciable amount of atmospheric nitrogen in soil, enhance plant growth by production of organic acids and growth substances and make available the complex phosphorus to the plant, which may cause an appreciable reduction in consumption of inorganic fertilizers [Avant and Manohar, (2001), Ghuge, *et al*., (2015) and Zaidi, *et al.,* (2015)].

The objective of the present study is to investigate the effect of nitrogen fertilizer levels and methods of biofertilizers application on okra growth, yield and nitrate accumulation.

MATERIALS AND METHODS

Two field experiments were conducted at Sakha Horticultural Research Station Farm 30^0 56N in latitude and 31^0 05 E in longitude during the successive summer seasons 2012 and 2013 to study the effect of nitrogen fertilizer levels and two methods of free nitrogen fixing biofertilizers application on yield and growth parameters of okra (*Abelmoschus esculentus* L.).

Three nitrogen fertilizer levels used were: 1) 100% of the recommended dose (60 kg N fed^{-1}) according to the recommendations of the Egyptian Ministry of Agriculture, 2) 50% of the nitrogen recommended dose (30 kg N fed^{-1}) and 3) 25% of the recommended dose (15 kg N fed^{-1}). The nitrogen was applied as ammonium sulphat fertilizer 20.5% N in three equal doses with the first, second and third irrigation. Effective strain of *Azospirillum sp*. as free nitrogen fixer and plant promoting hormone release was prepared as liquid (first method) and incorporated with Guar flour as tablets application (second method). Eskandrani var. was used, recommended seeds rate (8 kg fed^{-1}) was sown on 7th and 12th of April in the first and second seasons, respectively, on rows 60 cm apart and 30 cm between the hills. Three seeds were sown in the hill and plants were thinned to one plant after 21days from sowing.

The experimental plot was 3m in width (5 rows) and 4 m in length that equal to 12 m^2. Randomized complete blocks design was used with four replicates. All the treatments received 200 Kg P_2O_5 fed^{-1} as single superphosphate 15.5% P_2O_5 as well as 100 Kg K_2O fed^{-1} as potassium sulphate, 48% K_2O after soil preparation. The other agriculture practices i.e., irrigation, insect and disease control and weeding were done as recommended. Soil samples from the experimental farm were collected for some physical and chemical characteristics analyses according to Jackson (1967), some soil characteristics are presented in Table 1. Growth parameters i.e., plant height, number of leaves/plant, leaf dry weight (g), leaf area (cm^2) and number of branches/plant were estimated. Yield and yield components i.e., number of pods/plant, early yield (ton/fed.), total yield (ton/fed.) average weight of 100 fresh pods (g) and fruit dry weight percentage. Both P percentage and No_3 (mg/kg dry wt.) in the leaves and pods were also estimated. The samples of plant leaves and fruit were oven-dried at 65^{o}C for 48 h. phosphorus was determined colormeterically according to Snell and Snell, (1967). Nitrate in the dry plant samples was extracted by 2% acitic acid, determined according to Singh, (1988).

The obtained results were statistically analyzed according to Gomez and Gomez (1984).

Table 1: Some physical and chemical characteristics for the experimental soil.

Season	Particle size distributed (%)			Texture	pH	EC (dSm-1)	O. M (%)	Available nutrients (mg kg^{-1})		
	Sand	Silt	Clay					N	P	K
2012	53.0	25.4	21.6	Clayey	8.1	2.4	1.89	33	7.2	300
2013	49.0	26.0	25.0	clayey	8.3	2.6	1.75	30	6.8	285

pH in 1:2.5 soil: water suspension EC in soil paste extract N in 1M Kcl extract
P in 0.5 N $NaHco_3$ extracts K in 1 M ammonium acetate extract

RESULTS AND DISCUSSION

Data presented in Table 2 show highly significant increase in number of leaves/ plant due to the treatments. The highest values (36.19, 37.52) were obtained with 100% N+ liquid biofertilizer inoculation treatment in the first season and 100% N+ tablets biofertilizer inoculation in the second season. On the other hand, the lowest values (21.49, 22.83) were recorded with 25% N+ tab. biofertilizer inoculation in both seasons. No significant differences between 100% N+ liquid biofertilizer and 100% N+ tab. biofertilizer were detected.

Table 2: Effect of nitrogen fertilizer levels and biofertilizer inoculation methods on some vegetative growth parameters (no, of leaves/plant, stem length, leaf area and no. of branches) of okra plants.

Treatments	No. of leaves / plant		Stem length (cm)		Leaf area (cm^2)		No. of Branches/plant	
	2012	2013	2012	2013	2012	2013	2012	2013
1- 100%N mineral + Liquid-biofertilizer	36.2 a	37.5 a	96.8 a	110.1 a	409.9 a	425.8 a	6.0	5.6
2- 50%N mineral + Liquid-biofertilizer	34.1 a	34.8 b	92.8 abc	97.4 b	373.5 b	377.9 b	5.0	5.0
3- 25%N mineral + Liquid-biofertilizer	26.9 c	28.6 c	89.6 c	92.5 bc	327.3 c	335.7 c	5.0	5.0
4- 100%N mineral + Tablets-biofertilizer	35.2 a	37.3 a	96.6 a	109.9 a	379.6 b	402.0 ab	5.0	5.3
5- 50%N mineral + Tablets-biofertilizer	31.2 b	26.5 d	89.8 c	87.4 cd	317.5 c	312.4 cd	5.0	5.0
6- 25%N mineral + Tablets-biofertilizer	21.5 d	22.8 e	71.5 d	80.7 d	296.4 d	300.8 d	4.0	4.0
7- 100%N mineral only (control)	34.1 a	35. 5 b	94.0 ab	100.3 b	370.1 b	380.9 b	4.0	4.0
F. test	**	**	**	**	**	**	Ns	Ns
LSD. $_{0.05\%}$	3.156	1.667	5.442	7.821	17.211	28.86		

Stem length and leaf area parameters show the same trend of number of leaves/ plant , where the highest values of stem length (96.84 , 110.14, cm) and leaf area (409.93 , 425.8, cm^2) were obtained with 100% of the recommended nitrogen dose + liquid biofertilizer inoculation , and there is no significant difference between biofertilizer inoculation methods. The lowest values of stem length (71.5 and 80.71 cm) and leaf area (296.4 and 300.75 cm^2) were observed with 25% of nitrogen recommended dose + tablets biofertilizer inoculation. No significant difference was observed in number of branches / plant due to treatment used in both seasons. The previous data may be due to okra needed heavy nitrogen fertilization, any decrease in available nitrogen negatively affected okra growth, the second reason is the soil has more available nitrogen before sowing. The liquid biofertilizer was superior than tablet, one because the liquid distribution in the root zone was better than the tablets. These results are in agreement with those obtained by Akande *et al.*, (2010) and Mubashir *et al*. (2010).

Data presented in Table 3 indicate that nitrogen fertilization rate and biofertilizer inoculation methods significantly affected okra leaf dry weight, percentage in both seasons. The highest values (21.09 and 21.2 %) were obtained with 100% of the nitrogen recommended dose + liquid biofertilizer inoculation method in the first and second seasons, respectively. No significant difference between 100% N+ liquid biofertilizer inoculation treatment and 100% N+ tablet biofertilizer inoculation in the leaf dry values in both seasons. On the other hand, the lowest leaf dry values (18.89 and 18.93 %) were recorded with 25% of the recommended nitrogen fertilizer + tablets biofertilizer inoculation treatment.

Table 3: Effect of nitrogen fertilizer levels and biofertilizer inoculation methods on leaf dry weight, average weight of 100 fresh pods and fruit dry weight of okra plants.

Treatments	Leaf dry wt. (%)		Average wt. of 100 fresh pods (g)		Fruit dry wt.(%)	
	2012	2013	2012	2013	2012	2013
1- 100%N mineral + Liquid-biofertilizer	21.09 a	21.20 a	956.0 a	960.0 a	11.22	11.30
2- 50%N mineral + Liquid-biofertilizer	20.45 ab	19.73 b	946.0 a	950.0 a	11.18	11.15
3- 25%N mineral + Liquid-biofertilizer	19.28 bc	19.31 b	927.0 a	925.0 a	10.68	10.71
4- 100%N mineral + Tablets-biofertilizer	21.00 a	21.15 a	932.0 a	933.0 a	11.19	11.20
5- 50%N mineral + Tablets-biofertilizer	19.70 bc	19.62 b	878.0 b	880.0 b	10.89	10.60
6- 25%N mineral + Tablets-biofertilizer	18.89 c	18.93 b	870.0 b	871.0 b	10.55	10.53
7- 100%N mineral only (control)	19.88 abc	20.08 ab	940.0 a	945.0 a	11.06	11.20
F. test	*	**	**	**	NS	NS
LSD. $_{0.05\%}$	1.242	1.097	33.203	35.82		

Nitrogen levels and biofertilizer inoculation methods had highly significant effect on average weight of 100 fresh pods in both seasons. Therefore, the lowest records of that parameter were obtained from using either 50 or 25% of fertilizers + tablets biofertilizer in comparison with the remainder treatments that did not significantly differ in between.

No significant effects of nitrogen levels and biofertilizer inoculation methods were recorded on okra fruit dry weight % in both seasons. The previous data may be due to that available nitrogen was less in the soil and okra needed a heavy nitrogen fertilization, as well as liquid inoculation had good chance for distribution through the root zone comparing with the tablets inoculation. These results are in harmony with those obtained by Smil, (2000) and Akande *et al*., (2010).

Data presented in Table 4 and Figs.1 and 2 show that nitrogen fertilizer levels and biofertilizer inoculation method had significant effects on number of pods plant^{-1}, early yield (ton fed^{-1}) and total yield (ton field $^{-1}$) in both seasons. The highest number of pods plant^{-1} (69.41 and 77.38), early yield (2.24 and 2.66 ton field $^{-1}$) and total yield (6.16 and 6.54 ton fed^{-1}) were obtained with the treatment of 100% of the recommended nitrogen fertilizer plus liquid biofertilizer inoculation method in both seasons. On the other hand, the lowest number of pod plant^{-1} values (48.45 and 50.0), early yield (1.51 and 1.35 ton fed^{-1}) and total yield (3.95 and 4.56 ton fed^{-1}) were produced with the treatment of 25% of the recommended nitrogen fertilizer rate plus tablets biofertilizer inoculation method in both seasons. No significant differences were detected between 50% of the nitrogen fertilizer recommended dose plus liquid biofertilizer inoculation method treatment and the treatment of 100% of the recommended nitrogen fertilizer dose without biofertilizer inoculation were recorded on the most parameters studied in Table 4.

The previous results clearly show that biofertilizer inoculation enhanced growth parameters and okra yield, 50% N chemical fertilizer plus biofertilizer had similar yield as that of 100% chemical fertilizer, and liquid inoculation was superior to Tablets inoculation method. These results are in agreement with those obtained by Shaheen *et al*., (2007) who reported that fertilization of okra plant, by chemical fertilizer solely gained less plant growth values as well as yield of pods if compared with 50% of the recommended chemical nitrogen fertilizer plus the inoculation by biofertilizer.

Data presented in Table 5 show that nitrogen fertilizer levels clearly affected Phosphorus percent in okra leaves; the highest value (0.6%) was obtained with 100% of the nitrogen fertilizer dose plus biofertilizer inoculation with any method. This may be due to two reasons, the first is nitrogen chemical fertilizer affected soil pH at the micro zone of application, the second one is biofertilizer activation produced organic acids, gases and substrates that positively affects phosphorus availability, hence, phosphorus uptake increased. Nitrate in the leaves showed similar trend as that of phosphorus .These results are in agreement with those obtained by Akande *et al*.,(2010) and Mubashir *et al*., (2010).

Table 4: Effect of nitrogen fertilizer levels and biofertilizer inoculation methods on number of pods per plant, early yield and total yield of okra plants.

Treatments	No. of pods /plant		Early yield (ton/fed)		Total yield (ton/fed)	
	2012	2013	2012	2013	2012	2013
1- 100%N mineral + Liquid-biofertilizer	69.41 a	77.38 a	2.24 a	2.66 a	6.16 a	6.54 a
2- 50%N mineral + Liquid-biofertilizer	63.29 ab	70.90 bc	2.09 ab	2.20 b	5.89 ab	6.00 ab
3- 25%N mineral + Liquid-biofertilizer	57.50 bc	62.15 de	1.82 c	1.82 c	4.86 c	5.47 c
4- 100%N mineral + Tablets-biofertilizer	67.35 a	73.50 ab	1.99 b	2.24 b	5.85ab	6.23 a
5- 50%N mineral + Tablets-biofertilizer	54.79 cd	59.09 e	1.54 d	1.52 d	4.71 c	4.94 d
6- 25%N mineral + Tablets-biofertilizer	48.45 d	50.00 f	1.51 d	1.35 d	3.95 d	4.56 d
7- 100%N mineral only (control)	64.21 ab	67.50 cd	1.99 b	2.08 b	5.47 b	5.70 bc
F. test	**	**	**	**	**	**
LSD. $_{0.05\%}$	7.126	5.427	0.1716	0.2299	0.4240	0.4552

Fig 1: Effect of nitrogen fertilizer levels and biofertilizer inoculation methods on early yield of okra plants.

Fig 2: Effect of nitrogen fertilizer levels and biofertilizer inoculation methods on total yield of okra plants.

Table5: Effect of nitrogen fertilizer levels and biofertilizer inoculation methods on P% and No_3, mg kg^{-1} / dry matter, of okra leaves and pods in 2013 season.

Treatments	Okra leaves		Okra pods	
	P (%)	NO_3 (mg kg^{-1} dry matter)	P (%)	NO_3 (mg kg^{-1} dry matter)
1- 100%N mineral + Liquid-biofertilizer	0.60 a	208.0 a	0.68 a	102.4 b
2- 50%N mineral + Liquid-biofertilizer	0.36 c	128.8 b	0.60 b	80.0 c
3- 25%N mineral + Liquid-biofertilizer	0.33 c	116.8 c	0.24 c	80.0 c
4- 100%N mineral + Tablets-biofertilizer	0.60 a	207.83 a	0.68 a	65.6 d
5- 50%N mineral + Tablets-biofertilizer	0.40 c	128.5 b	0.26 c	46.4 e
6- 25%N mineral + Tablets-biofertilizer	0.35 c	110.4 d	0.19 d	46.4 e
7- 100%N mineral only (control)	0.48 b	208.0 a	0.68 a	123.2 a
F.test	**	**	**	**
$LSD._{0.05\%}$	0.054	2.187	0.037	2.667

Concerning P% and No_3 contents in pods, data presented also in Table, 5 clearly show that nitrogen fertilizer levels resulted in the highest values with the highest nitrogen fertilizer level with or without biofertilizer inoculation. No clear differences of P% between the treatments of 100% N+ liquid inoculation, 100% N+ tablets inoculation and 100% N without biofertilizer inoculation. This may be due to that the high nitrogen level increased available N in the soil, hence, increased N uptake and accumulation as well as microorganisms activation which led to increase of P uptake and accumulation.

Nitrate concentration in dry pods was increased with increasing nitrogen levels. The highest NO_3 values were recorded with 100% of recommended nitrogen fertilizer plus liquid biofertilizer inoculation followed by 100% N without biofertilizer inoculation (102.4 and 123.2 mg kg^{-1}, respectively). These values of nitrate are safe if no other sources of nitrate in the diet or drinking. These results are in agreement with those obtained by Knany and Abdalla (2006) on cucumber; Santamaria, (2006) and Mubashir et al. (2010)on carrot and okra.

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تـأثير التفاعل بـين مستويات النتـروجين وطرق اضـافة الاسمدة الحيويـة علـى نمـو ومحصول وجودة قرون الباميا.

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****معهد بحوث الاراضى و المياه و البيئه – مركز البحوث الزراعية – مصر .**

اقيمت تجربتـان حقليتـان بمزرعه محطة بحوث البساتين بسخا خلال الموسمين الصيفين٢٠١٢/٢٠١٣ وتهدف الدراسه الى تقييم تأثيرثلاثه مستويات للتسميد الازوتى (٦٠ و ٣٠ و ١٥ كجم ن/فدان) وطريقتين لاضافة السماد الحيوى (اضافه فى صوره سائله او فى صوره اقراص) على محصول ونمو وجودة قرون الباميا صنف اسكندرانى.

ويمكن تلخيص النتائج المتحصل عليها فى الآتى:-

- أعطى التفاعل بين اضافة ١٠٠% من الموصى به من التسميد الازوتى (٦٠ وحدة ن/ فدان) وإضافة الأسمدة الحيوية فى صورة سائلة أعلى قيم لعدد الأوراق للنبات (٣٦.١٩ و ٣٧.٥٢) وطول الساق (٩٦.٨٤ و ١١٠.١٤سم) وأكبر مساحة ورقيه (٤٠٩.٩٣ و ٤٢٥.٨سم^2) وأعلى نسبة وزن جاف للورقة (٢١.٠٩ و ٢١.٢%) وعدد القرون للنبات (٦٩.٤١ و ٧٧.٣٨) والمحصول المبكر (٢.٢٤ و٢.٦٦ طن/فدان) والمحصول الكلى (٦.١٥٦ و ٦.٥٣٦ طن/فدان) وتركيز النترات فى القرون الجافة للبامية (١٠٢.٤ و ١٢٣.٢ ملى جرام/كجم)
-أعطى التفاعل بين اضافة ٢٥% من المعدل الوصى به من التسميد الأزوتى و التلقيح بالسماد الحيوى بطريقة الأقراص اقل القيم فى كل القياسات السابقة.
- أدت زيادة التسميد الأزوتى إلى زيادة تركيز الفوسفور فى أوراق وقرون البامية فى وجود أوعدم وجود التسميد الحيوى.
- أدت زيادة التسميد الأزوتى مع التسميد الحيوى إلى زيادة تركيز النترات فى الأوراق والقرون.