Effect of Nitrogenous Fertilization, A- mycorrhizal Inoculation and Effective Microorganisms on Sunflower Productivity

Radwan, F. I., M. A. Gomaa, E. E. Kandil and M. K. A. Al- Akarmi
Plant Production Department, Faculty of Agriculture (Saba Basha), Alexandria University, Egypt.

ABSTRACT: Two filed experiments were carried out at the Experimental Farm of the Faculty of Agriculture (Saba Basha) at Abees region, Alexandria University, Alexandria, Egypt, during the two growing seasons 2014 and 2015 to study the effect of nitrogenous fertilization, A-mycorrhizal inoculation, and effective microorganism (EM) on sunflower cv. Sakha 53 productivity and its oil quality. Experimental design was split- plot with three replicates. The main results could be summarized as follows: 1) The application of nitrogen fertilizer at 96 kg N/ha., significantly increased stem diameter, head diameter, head weight, number of seeds/head, 100-seed weight, seed yield (ton/ha), straw yield (ton/ha.), biological yield (ton/ha.), Harvest index (%) and Oil yield (ton/ha.), 2) A-Mycorrhizal inoculation + foliar application of EM biofertilizer, significantly, increased yield and its components, as well as, oil yield (ton/ha.), chemical composition (N, P and K) and crude protein content in both seasons, 3) interaction between nitrogen fertilizer of 96 kg/ha and A-mycorrhizae + EM gave the highest values of all yield and its components compared and control treatment (without N fertilizer) with control (without biofertilizer) in both seasons. Also, oil yield (ton/ha.), chemical composition (N, P and K) and crude protein was, significantly, increased due to application 96 kg/ha. + A-mycorrhizae + EM. The present investigation suggest the need for more studies concerning the effect of mineral and biofertilizer, as well as, applying NPK on sunflower plants under different environmental conditions using different types of soils especially newly reclaimed soil, to reach the optimum combination of mineral and biofertilizer to achieve the highest yield and quality of seed oil content.
Key words: sunflower; nitrogen; A-mycorrhizae; Effective microorganisms; yield; oil.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important annual crops in the world grown for edible oil. It receives considerable attention in Egypt due to its short growing season and it can be grown well under the low fertility soils in the newly reclaimed areas. So, sunflower could be one of the main suggested oil crops to solve edible vegetable oil shortage in the country. Seeds contain 24-49 % oil and the cake contains 25-35% of protein (Henen, 2011).

Nitrogen plays an important role in plant growth and it is considered as the most important fertilizer element needed for maximum yield in most field crops, as well as, sunflower, and it should be applied at the optimum level to obtain the highest seed yield (Abou-Khadrah, *et al.,* 2002).

Mycorrhizae (VAM) fungi increases significant amount of nutrients to the plants such as copper, zinc, phosphorus and sulphur by producing widely extended hyphal network on the upper or lower side of the soil layer (Tilak and Singh, 1994).

In addition, biofertilization is one of the most important factors used to produce products free from mineral fertilizer that cause environmental pollution problems and high rates of it lead to decrease in the potential activity of micro flora and the mobility of organic matters. Hence, the attention has been focused on the researches of bio-fertilization as a safe attemative for the chemical fertilizers **(**Namvar *et al.,* 2012). Also, bio-fertilizers play vital role for increasing the number of microorganisms and accelerate certain microbial process in the rhizosphere of inoculated soil plants which can change the available form of nutrients into plants (Abou-Khadrah *et al*., 2002; Bassal, 2003 and Radwan *et al.,* 2015). Biofertilizers, significantly, increased yield attributes of sunflower, viz. thalamus diameter, weight of thalamus, filled seed/capitulum, and 100 seed weight (g), as well as seed biological yields and oil content. The combined inoculation of PSB + VAM + *Azotobacter* recorded higher values of these parameters, as compared to PSB + *Azotobacter* and VAM + inoculation (Patra *et al.,* 2013). Also, Pramanik and Bera (2013) concluded that inoculation of biofertilizers, significantly, increased test weight, weight of thalamus, number of filled seeds per capitulum as well as seed yield, biological yield and oil content of sunflower. The combined inoculation of PSB +VAM + Azotobacter recorded higher seed yield over Azotobacter, PSB + Azotobacter and VAM + Azotobacter inoculation.

Therefore, the objective of this study was to evaluate the effect of nitrogen levels and biofertilizers on yield and quality of sunflower (*Helianthus annuus* L.).

MATERIALS AND METHODS

Two field experiments were conducted at the Experimental Farm, the Faculty of Agriculture (Saba- Basha) at Abees region, Alexandria University, Egypt, during 2014 and 2015 seasons. The experiments were carried out to study the effect of nitrogenous fertilizer levels and biofertilizer on yield and quality of sunflower "*Helianthus annuus*, L." cv. Sakha 53.

The experimental design was split- plot with three replicates. Three nitrogen fertilizer levels (Control = without N applied, 48kg N/ha. and 96 kg N/ha) were allocated to main plots while four biofertilization treatments (without biofertilizer (control), Mycorrhizal inoculation, Effective microorganisms (EM) and "Mycorrhizal inoculation + EM") were allocated to sub- plots in both seasons.

Analysis of physical and chemical properties of the experimental soil site for the depth of (0 - 30 cm) as shown in Table (1) were carried out according to the methods reported by Page *et al*. (1982).

Table (1). Some physical and chemical properties of the experimental soil in 2014 and 2015 seasons.

Soil properties		
	Season	
	2014	2015
A) Mechanical analysis :		
Clay %	38	37
Sand %	32	33
Silt %	30	30
Soil texture	**Clay loam soil**	
B) Chemical properties		
pH (1 : 1)	8.20	8.31
E.C. (dS/m)	2.80	2.70
1)Soluble cations (1:2) (cmol/kg soil)		
K^+	1.52	1.54
Ca^{++}	9.4	8.7
Mg^{++}	18.3	18.5
Na^{++}	13.50	13.8
2)Soluble anions (1 : 2) (cmol/kg soil)		
$CO_3^{--} + HCO_3^-$	2.90	2.80
Cl^-	20.4	19.80
$SO_4^{—}$	12.50	12.60
Calcium carbonate (%)	6.50	7.00
Total nitrogen %	1.00	0.91
Available phosphate (mg/kg)	3.70	3.55
Organic matter (%)	1.41	1.40

Before planting sunflower seed were inoculated with A-mycorrhizal fungi (*Glomus mocrocarpum*) strain from Plant Production Department, Faculty of Agriculture (Saba Basha), Alexandria University, at a rate of 250 spores of infected roots and was mixed with seeds. The effective microorganisms (EM) sprayed as foliar application at two times after three weeks from planting date and the second spray was after two weeks from the first one at the rate of 4.8 litter/ha. The EM was produced by the General Organization for Agric. Equalization Foundation, Ministry of Agriculture, Egypt.

Nitrogen fertilizer treatments were applied in the form of ammonium nitrates (33.5 % N) at the rates of (control= no N fertilizer added, 48 and 96 kg N/ha) after thinning and before the first irrigation after planting, Phosphorus fertilizer was applied in the form of calcium super phosphate (15.5% P_2O_5) during soil preparation.

Each plot consisted of 5 ridges 3 m long and 60 cm apart with 30 cm space between plants. Two ridges were used to determine seed yield and its components. Sunflower “Sakha 53” seeds were sown in 28th and 26th June of the two growing seasons 2014 and 2015, respectively. In the first and second

season, Egyptian clover (*Trifolium alexandrinum* L.) and wheat (*Triticum aestivum*, L.) were the preceding crops in both seasons.

Hoeing was practiced before the first and second irrigation. The plants were thinned to secure one plant per hill after 10 days from planting other cultural practices for growing sunflower were carried out as recommended.

Recorded data:

A. Yield and yield components:

At harvest, two guarded plants were taken from the 2nd and 3th ridges in each plot to determine the following parameters

- Stem diameter (cm).
- Head diameter (cm).
- Head weight (g).
- Number of seeds/head.
- 100-seed weight (g).
- Seed yield (ton/ha).
- Straw yield (ton/ha.).
- Biological yield (ton/ha.).
- Harvest index (HI %).

B. Oil yield (ton/ha) and chemical compositions:

Oil percentage was determined using duplicated seed samples each of about two grams. Seed samples were oven dried at 65 to 70 ℃ for 24 hours. After weighting the seed samples seeds subjected to a constant pressure of 20000 pounds/square inch using a carve laboratory press which as described by A.O.A.C (1980).

Approximately 70% of the oil in the seed was extracted. The crushed seeds were then placed in oven with solvent petroleum ether stopper and allowed to stand a dry at 35℃. Two changes of solvent were applied at 24 hours intervals. Then oven dried for 24 hours at 65 to 70 ℃ and weighted. The loss in weight of seeds removed by pressing and solvent extraction were combined and oil % was then calculated as follows.

$$\text{Oil \%} = \frac{weight\ \ of\ \ oil}{weight\ of\ \ seed} \times 100$$

Oil yield (ton/ha) was determined by multiplying seed yield (ton/ha.) by seed oil percentage.

C. Chemical composition

Total nitrogen was determined in digested plant material colorimetrically by Nessler`s method **(**Chapman and Pratt, 1978). Protein content was calculated as N % x 6.25. Phosphorus was determined by the Vanadomolyate yellow method as given by Jackson (1973) and the intensity of colour developed was read in spectrophotometer at 405 nm. Potassium was determined

according to the method described by method Jackson (1973) using Beckman Flame photometer.

All the data collected were subjected to standard statistical analysis of variance ANOVA and LSD values to test the differences among the studied treatments means according to Gomez and Gomez (1984). The treatment mean was compared using the least significant differences (L.S.D.) test at 5% level of probability by using the split- model as obtained by CoStat 6.311, (2005) as statistical program.

RESULTS AND DISCUSSION

A) Yield and yield component

The obtained results given in Tables (2 and 3) showed, clearly, that nitrogen fertilizer levels exhibited significant effects on all estimated traits during the two cropping seasons of the study. Notably, increasing nitrogen fertilizer levels resulted in a significant increase in stem diameter, head diameter, head weight, number of seeds/head, 100- seed weight, seed, straw and biological yields (ton/ha), as well as, harvest index and oil yield (ton/ha).

These findings might be attributed to more adsorption of nutrition with reflect more growth substance more cell division and enlargement more of tissues and organs and plant elongation. Also, the nitrogen fertilizer may increase the synthesis of endogenous phytohormones which cause the formation of a big active root system which allow more nutrients uptake. The previous results agreed, more or less with the findings obtained by Abou Khadrah *et al.* (2002), Bassal (2003), El-Sadek (2005) and Radwan *et al.* (2015).

With regard to the effect of biofertilization on sunflower yield and its components. The results are shown in Tables (2 and 3). It could be concluded that inoculation of sunflower seeds with A-mycorrhizal + foliar application of EM encourages the increase of stem diameter, head diameter, head weight, number of seeds/head, 100- seed weight, seed, straw and biological yields (tons/ha), as well as, harvest index and oil yield (tons/ha) when compared with the control treatment in both seasons. This may be due to the effect of A-mycorrhizal inoculation + EM (foliar biofertilizer) which plays an important role in the increasing the nutrients that reflected on enhancing the growth of sunflower characteristic. Also, it could be attributed to the role of plant phytohormones such as IAA, GAs and CKs which promote plant growth, cell division, breaking the opical dominances, hence encouraging the phytosynthesis and assimilate accumulation **(**El-Khawas, 1990). These results are in harmony with the results obtained by Awad (2004), El-Temssah (2008), Henen (2011) and Patra *et al.* (2013).

The interaction between nitrogen fertilizer levels and bio-fertilization was significant for yield and its components in both seasons.

The highest mean values of seed yield (ton/ha.) character were recorded by application of 96 kg N/ha., with inoculation A- Mycorrhizae + Em in both seasons **(**Table 5).

B) Oil %, protein content (%) and chemical compositions

Increasing nitrogen fertilizer levels, significantly, brought an increase for oil %, protein content (%) and (N, P and K %) during both seasons.

The means of oil yield, protein % and N, P and K percentages for seed of sunflower plants increased due to increase nitrogen fertilizer levels up to 96 kg N/ha. Similar results were reported by Mohamed (2003), Ali (2004) and Nasim *et al.* (2012).

Data in Table (4) indicated that percentages of oil, nitrogen, phosphorus, potassium and crude protein, significantly, increased by inoculation of sunflower seed with A-mycorrhizal + EM (biofertilizer) when compared with the control of biofertilizer in both seasons. This may be due to the effect of mixture of A-mycorrhizal + EM (biofertilizer) of the ability of host plant to uptake insoluble nutrients. Particularly nitrogen, phosphorus and potassium and some microelements. These results are in agreement with those reported by Al-Karaki (2006) and Mai and Shamsuddin (2010).

The interaction between nitrogen fertilizer levels and biofertilizer was significant on protein content (%), oil content (%) and N, P and K% in both seasons.

Generally, the application of 96 kg/ha., with inoculation A- mycorrhizal and EM gave the best vegetative growth, yield and chemical composition as well as oil %, oil yield for sunflower variety in Alexandria conditions.

Table (2).Stem diameter (cm), head diameter (cm), head weight (g), number of seeds/head, 100-seed weight of sunflower cv. "Sakha 53" as affected by N-levels and bio-fertilization in 2014 and 2015 seasons.

Treatments	Stem diameter (mm)		Head diameter (cm)		Head weight (g)		Number of seeds/head		100- seed weight (g)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
A) Nitrogen fertilization levels (kg/ha.)										
0 (Control)	27.11	26.85	20.30	20.20	782.41	777.41	820.41	816.41	5.66	5.59
48	28.71	28.30	20.99	20.86	888.16	841.91	856.33	846.25	6.17	6.08
96	31.50	30.99	23.14	23.55	928.33	921.58	891.66	884.00	6.60	6.52
L.S.D. at 0.05	0.43	0.28	0.11	0.24	4.66	10.12	5.64	5.43	0.02	0.04
B)Bio-fertilization										
Without biofertilizer	24.65	24.37	19.06	18.91	640.77	631.77	788.88	784.55	5.37	5.30
Mycorrhizae	28.84	28.22	20.97	20.97	781.55	774.44	845.00	833.88	5.86	5.79
EM	30.56	30.25	22.67	22.43	867.33	865.66	878.88	871.77	6.47	6.37
Mycorrhizae + EM	32.38	32.01	24.00	23.83	1108.88	1116.00	911.77	905.33	6.88	6.79
L.S.D. at 0.05	0.28	0.23	0.30	0.34	4.56	11.83	3.12	5.41	0.03	0.03
Interaction										
Ax B	*	*	*	*	*	*	*	*	*	*

- * : Significant difference at 0.05 level of probability.

Table (3). Seed yield (ton/ha), straw yield (ton/ha), biological yield (ton/ha), harvest index (%) and oil yield (ton/ha) of sunflower cv. "Skaha 53" as affected by N-levels and bio-fertilization in 2014 and 2015 seasons.

Treatments	Seed yield (ton/ha)		Straw yield (ton/ha)		Biological yield (ton/ha)		Harvest index (%)		Oil yield (ton/ha)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
A) Nitrogen fertilization levels (kg/ha.)										
0 (Control)	3.59	3.50	6.48	6.53	10.07	10.10	35.55	35.23	1.44	1.44
48	3.88	3.87	6.84	6.92	10.75	10.80	36.06	35.78	1.97	1.73
96	4.17	4.23	7.17	7.18	11.34	11.42	36.72	36.98	1.98	1.98
L.S.D. at 0.05	0.01	0.01	0.02	0.03	0.05	0.03	0.10	0.13	0.01	0.04
B) Bio-fertilization										
Without biofertilizer	3.19	3.16	5.99	6.03	9.20	9.20	34.69	34.35	1.21	1.18
Mycorrhizae	3.69	3.69	6.88	6.41	10.07	10.11	36.57	36.48	1.60	1.58
EM	4.17	4.24	7.33	7.28	11.50	11.72	36.21	36.13	1.97	1.96
Mycorrhizae + EM	4.48	4.47	7.63	7.59	12.11	12.06	36.97	37.02	2.14	2.14
L.S.D. at 0.05	0.006	0.006	0.01	0.02	0.05	0.02	0.04	0.14	0.01	0.03
Interaction										
Ax B	*	*	*	*	*	*	*	*	*	*

- * : Significant difference at 0.05 level of probability.

Table (4).Protein content (%), oil content (%), macronutrients (N, P and K percentage) of sunflower cv. "Skaha 53" as affected by N-levels and bio-fertilization in 2014 and 2015 seasons.

Treatments	Protein (%)		Oil (%)		N (%)		P (%)		K (%)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
A) Nitrogen fertilization levels (kg/ha.)										
0 (Control)	19.04	18.23	40.66	39.88	3.04	2.99	0.615	0.607	2.61	2.61
48	20.26	19.84	44.50	44.60	3.24	3.17	0.639	0.633	2.81	2.79
96	20.98	20.72	47.31	46.49	3.36	3.31	0.713	0.705	3.12	3.08
L.S.D. at 0.05	0.22	0.23	2.68	1.58	0.05	0.03	0.002	0.006	0.01	0.04
B) Bio-fertilization										
Without biofertilizer	17.50	17.28	37.64	37.88	2.80	2.76	0.574	0.568	2.47	2.39
Mycorrhizae	19.51	19.03	43.01	42.78	3.12	3.04	0.648	0.640	2.70	2.68
EM	20.90	20.74	46.76	46.11	3.35	3.31	0.676	0.671	2.94	2.91
Mycorrhizae + EM	22.44	21.99	49.30	47.85	3.59	3.52	0.724	0.714	3.27	3.32
L.S.D. at 0.05	0.27	0.20	1.75	1.20	0.04	0.03	0.003	0.006	0.02	0.03
Interaction										
Ax B	*	*	*	*	*	*	*	*	*	*

- *: Significant difference at 0.05 level of probability.

Table (5). Interactions between nitrogen fertilizer levels and biofertilizer inoculation on seed yield/ha during 2013/2014 and 2014/2015 seasons.

Treatments		Seasons	
N- fertilizer levels (kg/ha.)	**Biofertilizer**	**2013/2014**	**2014/2015**
0	Uninoculated	2.99	2.94
	Mycorrhizae	3.45	3.44
	EM	3.82	3.80
	Myco + EM	4.09	4.07
48	Uninoculated	3.18	3.16
	Mycorrhizae	3.51	3.53
	EM	4.31	4.29
	Myco + EM	4.52	4.51
96	Uninoculated	3.39	3.37
	Mycorrhizae	4.10	4.09
	EM	4.39	4.63
	Myco + EM	4.82	4.82
LSD at 0.05		0.009	0.01

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الملخص العربي

تأثير التسميد النتروجينى والتلقيح بالميكوريزا والكائنات الدقيقة النافعة على إنتاجية عباد الشمس

فتحى ابراهيم رضوان – محمود عبد العزيز جمعة – عصام إسماعيل قنديل – محمد خليفة العكرمى

قسم الانتاج النباتي – كلية الزراعة (سابا باشا) – جامعة الاسكندرية

أجريت تجربتان حقليتان بالمزرعة البحثية لكلية الزراعة سابا باشا – جامعة الاسكندرية – مصر – خلال موسمى الزراعة ٢٠١٤ و ٢٠١٥ ، لدراسة تأثير التسميد النتروجينى والتلقيح بالميكوريزا والكائنات الحية المفيدة على إنتاجية وجودة محصول عباد الشمس "صنف سخا ٥٣" وصممت التجارب باستخدام تصميم القطع المنشقة مرة واحدة في ثلاثة مكررات وكان ملخص النتائج كما يلى:

١-أدت إضافة التسميد النتروجينى حتى ٩٦ كجم نتروجين/هكتار الى زيادة معنوية لقطر الساق ، قطر الرأس ، وزن الرأس ، عدد البذور/الرأس ، وزن ١٠٠ بذرة (الجم) ، محصول البذور (طن/هكتار) ، محصول القش (طن/هكتار) ، المحصول البيولوجى (طن/هكتار) ، دليل الحصاد (%) ن ومحصول الزيت (طن/هكتار) والمكونات الكيميائية لمحصول عباد الشمس (النسبة المئوية للبروتين ، الزيت ، النتروجين ، الفوسفور ، البوتاسيوم فى البذور) خلال موسمى الدراسة.

٢-حقق التليقح بالميكوريزا مع الرش بالكائنات الحية الدقيقة النافعة زيادة معنوية لجميع صفات المحصول ومكوناته وأيضاً محصول الزيت والمكونات الكيميائية خلال موسمى الزراعة.

٣-التداخل بين التسميد النتروجينى حتى (٩٦ كجم/هكتار) مع التلقيح بالميكوريزا والرش بالكائنات النافعة أعطى أعلى قيم لجميع صفات المحصول ومكوناته مقارنة مع المعاملة بالكنترول (بدون تسميد سواء النتروجينى او الحيوي) في كل من الموسمين. أيضاً النسبة المئوية للزيت ومحصول الزيت للزيت والمكونات الكيماوية.

التوصية:

يوصى البحث باستخدام التسميد النتروجينى بمعدل ٩٦ كجم ن/هكتار مع التسميد الحيوي للحصول على أعلى إنتاج لوحدة المساحة من محصول البذور لعباد الشمس صنف سخا ٥٣ وذلك تحت ظروف التجربة.