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Bio and mineral-N fertilization of Sunflower (*Helianthus annuus* L.) Grown on Sandy Soil Using ^{15}N Technique

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Abstract

Biofertilization using *Azotobacter chroococcum* (B_1), *Azospirillum brasilense* (B_2) and *Bacillus megaterium* (B_3), were assessed vs. Mineral-N fertilization using ammonium sulphate with 2% ^{15}N atom excess on sunflower grown on a virgin sand during 2014 summer season. Plants grew for 45 days. The highest plant height (64.87 cm) with an increase about 93.6% over the non-fertilized was given by the *Azospirillum* bacteria combined with the high N rate (N_3B_2). Total chlorophyll ranged from 17.8% by (N_2B_1) to 34.9% by (N_3B_0). The highest dry matter yield of 44.20 kg ha^{-1} was by N_3B_0. The highest N uptake was given by plants receiving high N without biofertilization (N_3B_0) with an increase 141.4%. The highest fertilizer N recovery of 18.45% was by high N without biofertilization (N_3B_0) with an increase 99.7%.

Keywords: N and Biofertilization, Fertilizer N recovery, ^{15}N-Isotope dilution, Sunflower.

Introduction

Sunflower (*Helianthus annuus L.*) is an important edible vegetable oil that ranks the fourth after soybean, palm oil and canola as a source of edible oil in the world **(USDA 2008)**. In Egypt, the cultivated area of sunflower is limited in Nile Valley and the delta due to the competition with other strategic summer crops. However, it could be cultivated on newly reclaimed soils in the desert area, which represents 96% of Egypt total area **(El-Sayed, 2012).**

Biofertilizers are organic products containing specific micro-organisms in concentrated forms, derived from the soil root zone (Rhizosphere**)** **(Mishra and Dadhich, 2010)**. They are considered as an important environment friendly sustainable agricultural practices, with low cost inputs; mainly including nitrogen fixing bacteria (*Azotobacter sp, Azospirillum sp etc.*) and phosphate solubilizing bacteria (*Bacillus megtherium*) **(Sharma and Namdeo, 1999).** The beneficial effect of biofertilizers inoculation on sunflower has been reported by several investigators. The obtained results by **Keshta and El-Kholy (1994)** indicated that the application of biofertilizers as a source of N_2 fixing bacteria on sunflower increased plant height, total chlorophyll, dry matter yield and N uptake.

Nitrogen is one of the most important nutrients to plant growth **(Li et al., 2015**). Plant need huge amount of nitrogen to form protein and nucleic acids. Usually N is consumed and supplied in the chemical form (**Mohamed, 2003).** The effect of mineral and organic fertilizers on sunflower was widely investigated **(Diacono et al., 2013 and Obour et al., 2017)**.

Osman and Awed (2010) in their study on sunflower showed that, increasing nitrogen level from 72 kg to 144 kg N ha^{-1} significantly increased all yield components.

This work aimed at tracing the contribution of bacterial inoculants and N fertilization rates on sunflower plants grown on poor fertile sand soil to recognize the best management combinations.

Materials and Methods

A field experiment to assess biofertilization and mineral fertilization on Sunflower (*Helianthus annuus* L.*) was* conducted at the Experimental Field of Soil and Water Research Department, Nuclear Research Center, Atomic Energy Authority, Abu-Zaable, Egypt during 2014 under drip irrigation system. The soil was sand. A randomized complete block design was used in the experiment with two factors. **Factor** N fertilization (N): with 4 treatments: unfertilized (N_0),105 kg N ha^{-1} (N_1), 140 kg N ha^{-1} (N_2) and 175 kg N ha^{-1} (N_3). **Factor** biofertilization (B): with 4 treatments: non-fertilized (B_0), fertilization with ***Azotobacter chroococcum*** (B_1), fertilization with ***Azospirillum brasilense*** (B_2) and fertilization with ***Bacillus megaterium*** (B_3), where B_1 and B_2 being free-living N_2-fixers and B_3 a P-dissolver ; each inoculum obtained from Agriculture Research Center, Giza, Egypt and was mounted on a peat moss carrier. A saccharide solution was used as a material for sticking the inoculants on seed surface three hours before seeding. Seeding was in rows (63 cm apart) with two seeds per hill 30 cm between hills. The plot area was 10 m^2 (1.25 m× 8.0 m). The crop exhibited no sign of insect or pest attack or disease incidence; therefore, no protection measures were applied. Compost provided by the Faculty of Agriculture, Moshtohor was used as a basal treatment (21 Mg ha^{-1}) for the experimental field before cultivation (45 days before cultivation). Compost properties are presented in Table 1. Compost analyses were done according to methods cited by **Carter and Gregorish (2008).**

Seeds were sown on April 15th, 2014 and plants were allowed 45 days growth (i.e. to May 29th, 2014).

Table 1. Main properties of organic compost used in the study.

Nutrients 'mgkg^{-1}', organic matter, ash, moisture (g kg^{-1}), pH, EC and C/N ratio of compost						
N	**P**	**K**	**Fe**	**Mn**	**Zn**	**Cu**
21.0	10.3	21.1	4.1	0.5	0.3	0.2
Ph (1:2.5)	**EC** (1:2.5) (dS m^{-1})	**Organic matter**	**Ash**	**Organic carbon**	**C/N ratio**	**Moisture** (g kg)
		g kg^{-1}				
7.4	5.2	643	332	207.0	9.9	22.6

A micro-plot was allocated where ^{15}N ammonium sulphate with 2% ^{15}N atom excess was used for ^{15}N isotope assessment. All plots received P, K and micronutrients as recommended by bulletin of the Ministry of Agriculture and Reclamation. N (as ammonium sulphate, 207 g N kg^{-1}) was applied in two equal splits (2 and 4 weeks after sowing).P was at 24 kg P ha^{-1} (as Ca-superphosphate, 68 g P kg^{-1}) during soil preparations, while K was at 25 kg K ha^{-1} (as K-sulphate, 415 g K kg^{-1}) 4 weeks after sowing. Soil properties (**Table 2)** were determined according to **Carter and Gregorich (2008)**. Fertilizer N recovery (FNR) was calculated as follows:

FNR= {kg N ha^{-1} derived from fertilizer ÷ kg N ha^{-1} fertilizer rate} x 100

Table 2. Main properties of soil of the experiment**.**

pH (1:2.5)	**EC*** (dS m^{-1})	**$CaCO_3$** (g kg^{-1})		**Organic matter** (g kg^{-1})	
7.12	0.27	0.0		0.3	
Available nutrients **(mg kg^{-1})			**Total nutrients** (g kg^{-1})		
K	**P**	**N**	**K**	**P**	**N**
1.0	0.1	0.3	0.2	2.0	5.0
Particle size distribution (%)					
Clay	**Silt**	**Sand**		**Texture**	
0.0	2.0	98.0		Sand	

* In paste extract. **Extracts of: KCl for N; $NaHCO_3$ for P; NH_4-acetate for K; soil texture according to the International Soil Texture Triangle (Moeys 2014)

Samples of plants were analyzed according to methods cited by **Estefan *et al.* (2013).** ^{15}N analysis was carried out using automated emission spectrometer (Fischer NOI-6 PC).The portion of nitrogen derived from fertilizer (%Ndff) present in the relevant plant was calculated in view of the ^{15}N atom excess (^{15}N a.e.) in materials **(IAEA, 2008)**. The equation is as follows:

%Ndff = (^{15}N a.e. in plant ÷ ^{15}N a.e. in fertilizer) x 100

% FNR = Ndff (kg ha^{-1}) / rate of applied N (kg ha^{-1}) x100

Results and Discussion

Plant height:

The lowest height of 33.5 cm was given by the untreated B_0N_0 plants while the highest of 64.9 cm was given by those of B_2N_3 which received *Azospirillum brasilense* bacteria and the high N rate, with a relative increase of 93.6%. The main effect of biofertilization shows averages of 3.40, 1.63 and 12.68% due to B_1, B_2 and B_3, respectively (Table 3). Biofertilization showed positive effect only in presence of mineral N fertilization. The main effect on mineral N fertilization shows a pattern of $N_2>N_3>N_1>N_0$ with highest plant height given by N_2 followed by N_3 then N_1 with increases averaging 39.8, 49.3 and 48.4 % due to N_1, N_2 and N_3 respectively. **Chantal et al. (2018)** found that the high N, P and K level gave the highest sunflower plant high with 142.6 cm. These results support those of **Mostafa and Abo-Baker (2010)**, who reported increased sunflower growth due to higher rate application of nitrogen. Similar experimental results were attained by **Shah and Khanday (2005)** as well as **Sarkar and Mallick (2009)**.

Table 3. Response to N inorganic fertilization and N or P biofertilization of sunflower: plant height after 45-day (cm)

Inorganic Fertilization (N)	Biofertilization (B)				mean
	B_0	**B_1**	**B_2**	**B_3**	
N_0	33.50	34.37	31.17	55.67	**38.68**
N_1	52.40	53.50	52.50	57.90	**54.08**
N_2	62.00	58.00	53.80	57.17	**57.74**
N_3	51.17	59.97	64.87	53.60	**57.40**
mean	**49.77**	**51.46**	**50.58**	**56.08**	

LSD: 0.05 = N: 0.61 ; B: 0.61 ; NB: 1.21

Notes: B_0: without biofertilization – B_1: Azotobacter; B_2: Azospirillum; B_3: *Bacillus megaterium* …… N_0, N_1, N_2 and N_3 = 0, 73.5, 98.0 and 122.5 kg N ha^{-1} (as ammonium sulphate) respectively.

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Total chlorophyll:

Application of N singly increased the total chlorophyll in plant leaves. Plants receiving N gave high total chlorophyll in plant leaves (Table 4). The increase in ranged from 17.8% by N_2B_1 to 34.9% by N_3B_0. Therefore, high N gave the highest total chlorophyll indicating a need for high N fertilization in order to obtain high chlorophyll in plant. In this case, soil should be reached to field capacity most of time to save the micro-organisms from these unsuitable conditions. The main effect on N fertilization shows a pattern of $N_3>N_2>N_1>N_0$ with highest plant height given by N_3 followed by N_2 then N_1. Increase averaged 30.55, 35.12 and 37.59 % due to N_1, N_2 and N_3, respectively. Biofertilization showed positive effect only within absence of N. the main effect of biofertilization shows a decrease averaged 1.36, 3.48 and 2.71% due to B_1, B_2 and B_3, respectively as shown in Table 2. The chlorophyll amounts were observed with in-creased nitrogen doses. While the value obtained from 30 g N $tree^{-1}$ and 60 g $tree^{-1}$ doses were higher than the control application, the highest chlorophyll dose was observed in 90 g N $tree^{-1}$ dose. While the lowest chlorophyll was found in control, it was followed by the 30 g N $tree^{-1}$ dose and the highest values were observed in the same groups of 60 g N $tree^{-1}$ and 90 g N $tree^{-1}$ (**Erdinç, 2018).** It concluded that total chlorophyll was increased by increasing the rate of nitrogen

Table 4. Response to N inorganic fertilization and N or P biofertilization of sunflower: total chlorophyll after 45-day

Inorganic Fertilization (N)	Biofertilization (B)				mean
	B_0	**B_1**	**B_2**	**B_3**	
N_0	29.20	27.97	26.00	27.20	**27.59**
N_1	35.07	37.47	36.37	35.17	**36.02**
N_2	37.87	34.40	37.17	36.70	**37.28**
N_3	39.40	36.77	37.07	38.60	**37.96**
mean	**35.38**	**34.90**	**34.15**	**34.42**	

LSD: 0.05 = N: 0.27 ; B: 0.27 ; NB: 0.54
See footnotes of Table 3

Dry matter yield:

The pattern of response (Table 5) was rather similar to that of total chlorophyll. Application of *Bacillus Megaterium* (singly) under no N application (N_0B_3) gave the lowest dry matter yield of 5.53 kg ha^{-1}, while the highest was 44.20 kg ha^{-1} (an increase of 699%), given by the high N non-biofertilized (N_3B_0). The main effect of N fertilization shows a pattern of $N_3>N_1>N_2>N_0$ with highest plant height given by N_3 followed by N_1 then N_2. Increases averaged 91.9, 36.9 and 143.7 % due to N_1, N_2 and N_3, respectively. Such a pattern of response was particularly evident under conditions of no biofertilization or under the P-dissolver *Bacillus Megaterium*. **Nasim *et al.* (2011)** observed that, with increasing N rate to sunflower, there was an increase in plant growth.Under biofertilization with the N-fixers there was little or no difference between the inorganic N-applied treatments. The main effect of biofertilization shows no positive response due to biofertilization. There were decreases averaging 35.0 31.8 and 31.5% due to B_1, B_2 and B_3, respectively. This shows that the biofertilizer organisms caused a depletion of soil available N leading to a decrease in plant growth and consequently N uptake. There should be enough available nutrients in the soil to make biofertilizer microorganisms increase plant growth.

Table 5. Response to N inorganic fertilization and N or P biofertilization of sunflower: Dry matter yield (kg ha^{-1}) in plants 45-day

Inorganic Fertilization (N)	Biofertilization (B)				mean
	B_0	B_1	B_2	B_3	
N_0	19.54	8.94	12.19	5.53	**11.55**
N_1	25.14	17.89	21.32	24.29	**22.16**
N_2	18.56	21.10	22.29	13.75	**18.93**
N_3	44.20	20.87	17.53	29.98	**28.15**
mean	**26.86**	**17.20**	**18.33**	**18.39**	

LSD: 0.05 = N: 3.93 ; B: 3.93 ; NB: 7.85
See footnotes of Table 3

N uptake

Response of N uptake resembled that of the yield (Table 6). The lowest N uptake of 9.72 kgha^{-1} was given by application of *Bacillus Megaterium* under no N fertilization (N_0B_3), while the highest of 65.36 kg ha^{-1} (which surpassed the lowest by 572%) was given by the non-biofertilized high N (N_3B_0). The main effect of N fertilization shows a pattern of N_3>N_1>N_2>N_0. Increases averaging 91.9, 36.9 and 143.7 % due to N_1, N_2 and N_3, respectively. Such a pattern of response was particularly true under conditions of no biofertilization or under the P-dissolver *Bacillus Megaterium*. Under biofertilization with the N-fixers little or no difference between the inorganic fertilizers was noted. The main effect of biofertilization shows no positive response with the three applied biofertilizers, with all being similar in effect. Dcreases caused by the biofertilizers averaged 35.0, 31.8 and 31.5% due to B_1, B_2 and B_3, respectively. This indicates that the biofertilizer organisms depleted available N in the soil (which is sand nearly devoid of available N). These results agree with those obtained by **Ahmed and El-Araby (2012)** and **Nadeem *et al.* (2014)**

Table 6: Response to N inorganic fertilization and N or P biofertilization of sunflower: N uptake (g ha^{-1}) in plants 45-day

Inorganic Fertilization (N)	Biofertilization (B)				mean
	B_0	B_1	B_2	B_3	
N_0	27.08	15.64	19.12	9.72	**17.89**
N_1	41.09	33.59	33.58	38.31	**36.64**
N_2	29.19	34.45	36.44	25.85	**31.48**
N_3	65.36	36.47	27.21	36.52	**41.39**
mean	**40.68**	**30.04**	**29.09**	**27.60**	

LSD: 0.05 = N: 2.62 ; B: 2.62 ; NB: 5.23
See footnotes of Table 3

Nitrogen derived from fertilizer (Ndff):

Ndff (Table 7) was lowest of 9.06 kgha^{-1}given by the medium-N *Bacillus Megaterium* biofertilized treatment (N_2B_3).The highest of 22.61 (surpassing the lowest by 149.6%) was given by the high-N non-biofertilized N_3B_0 treatment. Under condition of no biofertilization or *B. Megaterium*, the highest Ndff was given by the treatment of highest N dose. Under biofertilization with the N-fixers the medium N treatment showed highest Ndff. High Ndff indicates high efficient use of fertilizer N. A combination of biofertilizers such as N_2-fixers or P-dissolvers along with the soluble fertilizer N would enhance the positive effect of N fertilization **(Hekal, 2015)**.

Table 7. Response to N inorganic fertilization and N or P biofertilization of sunflower: Ndff (kg ha^{-1}) in plants 45-day

Inorganic Fertilization (N)	Biofertilization (B)				Mean
	B_0	B_1	B_2	B_3	
N_1	13.50	7.75	9.80	12.12	**10.79**
N_2	9.67	15.48	16.22	9.06	**12.61**
N_3	22.61	13.43	11.57	16.83	**16.11**
mean	**15.26**	**12.22**	**12.53**	**12.67**	

See footnotes of Table 3. Values are averages and no statistical analysis was done.

Fertilizer nitrogen recovery (FNR):

Fertilizer N recovery (FNR) represents the portion of N derived from fertilizer in relation to the rate of applied N. Table 8 shows that the lowest FNR of 9.24% was obtained in the plants given the medium N *B. Megaterium* (N_2B_3) and the second lowest of 9.87 % was obtained by the medium N non-biofertilized treatment (N_2B_0). The highest of 18.45% (surpassing the lowest by 99.7%) was given by the high-N non-biofertilized N_3B_0 treatment. Under condition of no biofertilization or *B. Megaterium*, the highest FNR was given by high or low dose of N. Under biofertilization with the N-fixers the medium N treatment showed highest FNR. The direct method on ^{15}N add is the most adequate to determine the recovery efficiency of N derived from fertilizer **(Araújo et al., 2018)**.

Table 8. Response to N inorganic fertilization and N or P biofertilization of sunflower: ^{15}N recovery (%) in plants 45-day

Inorganic Fertilization (N)	Biofertilization (B)				Mean
	B_0	**B_1**	**B_2**	**B_3**	
N_1	18.37	10.54	13.33	16.49	**14.68**
N_2	9.87	15.80	16.55	9.24	**12.86**
N_3	18.45	10.96	9.44	13.74	**13.15**
Mean	**15.56**	**12.43**	**13.11**	**13.16**	

See footnotes of Table 2. Values are averages and no statistical analysis was done.

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التسميد النيتروجينى المعدنى والحيوى لنبات دوار الشمس المنزرع فى أرض رملية بإستخدام تقنية ن[15]

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أجريت تجربة خلال الموسم الزراعى صيف 2014 بمزرعة قسم بحوث الأراضى والمياه – مركز البحوث النووية – هيئة الطاقة الذرية – أبوزعبل – مصر لمتابعة تأثير اللقاحات البكتيرية المختلفة ومعدلات التسميد المعدنى بالنيتروجين على نباتات دوار الشمس المنزرعة فى تربة رملية فقيرة (بكر). تم إنبات النباتات حتى عمر 45 يوم وتم قياس إستجابتها لعوامل التجربة التى آثرت على صفات النمو والكلوروفيل ومحصول المادة الجافة وامتصاص واستعاضة النيتروجين. كانت صورة السماد المعدنى النيتروجينى هى سلفات الأمونيوم والتى تحتوى على 2% وفرة من الذرات المرقمة بالنظير الثابت للنيتروجين. تفوقت المعاملة التى تلقت المعدل العالى من النيتروجين وبكتريا الازوسبيريللم (N_3B_2) بنسبة زيادة حوالى 93.6% فى طول النبات حيث أنها أعطت أعلى طول للنبات (64.87 سم). تراوحت الزيادة فى الكلوروفيل الكلى من 17.8% بواسطة N_2B_1 إلى 34.9% بواسطة N_3B_0. كان أعلى محصول مادة جافة هو 44.20 كجم هكتار−1 بواسطة N_3B_0 (إلى حد ما يمكن مقارنتها مع تلك الموجودة فى الكلوروفيل الكلى). أعلى إمتصاص نيتروجينى كان بواسطة النباتات التى تلقت المعدل العالى من النيتروجين ولم تتلقى تسميد حيوى (N_3B_0) بنسبة زيادة حوالى 141.4%. كان أعلى إسترجاع نيتروجينى هو 18.45% بواسطة N_3B_0 (المعدل للنيتروجين ولم تتلقى تسميد حيوى) بنسبة زيادة حوالى 99.68%.