Journal of
NUCLEAR
Technology in Applied Science
ISSN 2314-8209 e-ISSN 2314-8217

J. Nucl. Tech. Appl. Sci, Vol. 3, No. 3, PP. 157 : 169 (2015)

Response of Sunflower (*Helianthus annuus L.*) to N-application and Biofertilization with Assessment of Fertilizer N Recovery by ^{15}N Versus Subtraction Methods

Abdel-Salam[1], A. A.;Soliman[2], S. M.;Galal[2], Y. G. M.;Zahra[1], W. R.;Moursy[2], A. A. and Hekal[2], M. A.

Received: 01/07/2015
Accepted: 12/07/2015
Available on line: 30/07/2015

E.mail:solimanreh@yahoo.com
& Alyabsalam@yahoo.com

ABSTRACT

A factorial field experiment was conducted on sunflower (*Helianthus annuus* L.) grown on a sand soil (98% sand) supplied the different combinations of 4 N rates of 0, 105, 140 and 175 kg N ha^{-1} i.e. N_0, N_1, N_2, and N_3 respectively - as $(NH_4)_2SO_4$ - and 4 biofertilization inoculation (B) of none, ***Azotobacter chroococcum, Azospirillum brasilense*** and ***Bacillus megaterium***. i.e. B_0, B_1, B_2, and B_3 respectively. Labeled ammonium sulphate with 2% ^{15}N atom excess was used for ^{15}N assessment. All plots were supplied with 21 Mg compost+24 kg P+80 kg K ha^{-1}. Non-treated plants gave 0.534 Mg seeds ha^{-1} while the treated ones - especially those of N or N + biofertilizers - gave increases of up to 403% (N_2B_1). Main effect response patterns were: N: $N_3>N_2>N_1$, for B: $B_1\geq B_3\geq B_2$. Seed oil content in the N_0B_0 treatment was 222 gkg^{-1} increased reaching as high as 445 gkg^{-1} by N_2B_3; with N main effect of $N_2>N_3>N_1$ and B main effect of $B_2>B_3>B_1$. Seed oil yield was113 kg ha^{-1} by N_0B_0 increased to as high as 1105 kg ha^{-1} by N_2B_1 with main effects of $N_2>N_3>N_1$ and $B_3\geq B_2>B_1$. Uptake of N (in total plant parts of roots+stems+leaves+discs+seeds) increased by N application; averages for non-N were 18.1 kg ha^{-1} 18.5,14.7, 17.4 by N_0B_0, N_0B_1, N_0B_2, and N_0B_3 respectively; increased considerably by up to 667% (N_3B_3) upon N application. Plants recovered a portion of fertilizer N of 19.6 to 40.9% by N_1B_1 and N_2B_1 respectively as determined by ^{15}N technique, but 27.7 to 59.6% respectively as calculated by subtraction of non-N from N treatments. The subtraction estimation considerably exceeded the ^{15}N determined ones by + 39.5% to as high as + 194.6% indicating a non-real estimation of recovered fertilizer-N in crops. Thus, in studies using non-tracer techniques, estimation of uptake of fertilizer N could be erroneous. The reason in the current study could

KEYWORDS

Inoculation, Nitrogen rate, ^{15}N isotope, N recovery, Oil yield, Sunflower.

1. Soil and Water Department, Faculty of Agriculture, Moshtohor, Benha University.
2. Soil and Water Research Department, Nuclear Research Center, Atomic Energy Authority, Egypt.

most certainly be a greater volume of root system expansion of N-treated plants, causing more uptake of non-fertilizer-N than in the no-N-treated ones.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) of composite family *Asteraceae*, belongs to the genus *Helianthus*. It is an annual crop, with a large yellow inflorescence containing small flowers that give rise to achenes containing a kernel rich in oil **(Kamal and Bano, 2009).** It was first cultivated by natives of Central and North America, and was introduced into Europe in the 16th century; and its cultivation extended worldwide during the 19th and 20th centuries (becoming the fourth largest vegetable oil crop after palm, soybean and rapeseed) ,with main production countries of USA, Ukraine and Argentina **(Salas *et al.,* 2014)**. Its oil is edible, rich in oleic (omega-9), linoleic (omega-6) and linolenic (omega-3) acids (two of which - oleic and linoleic - are unsaturated fatty (FA) acids; also contains saturated palmitic and stearic FAs **(Simpson *et al.,* 1989),** all of which are useful to human **(Hu *et al.,* 2001).**

World production of sunflower oil is about 36 million Mg and represents about 8% of the total world oil production **(FAO, 2012).** Consumption of edible oil in Egypt is much higher than the production; a production of about 40 thousand Mg was far less than a consumption of about 380 thousand Mg **(FAO, 2006)**. Increasing the productivity as well as the cultivated area of this crop would help in narrowing such a gap in Egypt. Some high-oil cultivars containing as high as 470 to 530 g oil kg^{-1} in seeds are now grown worldwide replacing low-oil cultivars of 380 to 470 g kg^{-1} **(Izquierdo *et al.,* 2008).**

Although vegetable oils are used primarily in human diet, they hold considerable potential for a wide range of use Vegetable oils could substitute petroleum-derived materials as fuels, lubricants, and petro-chemicals **(Metzger and Bornsheuer, 2006).** Oil quality and yield depend on plant genotype as well as other factors **(Blum, 1997 and Reddy *et al.,* 2003)**. Growth, development, and spatial distribution of plants are restricted by various environmental stresses including water deficit **(Boyer, 1982)** and low fertility **(Bhattacharyya *et al.,* 2008).** Biofertilizers can increase soil fertility, and N-biofertilizers can increase the proportion of fatty acids and the ratio of unsaturated / saturated fatty acids **(Choudhury and Kennedy, 2004).** Soil microorganisms can increase availability of many nutrients through increasing their solubility **(Bentley and Chasteen, 2002).**

The direct ^{15}N labelling technique (DLT) is most suitable for following up the pathways of fate of applied fertilizer-N in the soil plant system **(Hood *et al.,* 2008)**. Under tropical field conditions, the use of this method with legume residues is scarce **(Vanlauwe *et al.,* 1998)**. Total soil N includes different pools that can deliver mineral N during the growing period of the crop. They include pools of available soluble mineral as well as organic N, microbial N and non-living labile organic N. Usually the part of N derived from the fertilizer is very low and is diluted within the pools. Crop N requirement may vary according to fertilization **(Makowski *et al.*, 1999)** and addition of nutrients other than N may affect fertilizer N use efficiency **(de Wit, 1992).** The objectives of the current study are to assess the response of sunflower to fertilizer-N application, either singly or in combination with biofertilizers, and the implications on seed and oil production. Fertilizer N recovery using the ^{15}N tracer technique versus the subtraction method will also be assessed.

MATERIALS AND METHODS

A field experiment was conducted on sunflower plants (cv. Sakha 53) at the experimental farm of the Nuclear Research Center (NRC), of the Atomic Energy Authority (AEA), Abou-Zaable, Egypt during the 2014 summer growing season under drip irrigation system on a sand soil (Table 1) using a randomized complete block design with two factors. **Factor N**: inorganic fertilization 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 B**:

biofertilization 4 treatments: un-biofertilized (B_0); biofertilized through seed inoculation with *Azotobacter chroococcum* (B_1); *Azospirillum brasilense* (B_2) and *Bacillus megaterium* (B_3), where $B_1$and B_2 being free-living N_2-fixers and B_2 a P-dissolver. In each plot (10m^2), a micro-plot was allocated where ^{15}N ammonium sulphate with 2% ^{15}N atom excess "a.e." was used for ^{15}N isotope studies. All plots received the recommended rates of 24 kg P (ordinary Ca-superphosphate) during soil preparations and 80 kg K ha^{-1} (K-sulphate) in two equal splits (4 and 7 weeks after sowing) as recommended by **MALR (2014)**. Also, a foliar spray was done at 1200 L ha^{-1} of 1300 mg L^{-1} for each of Fe, Mn, Zn and Cu as chelates (10% one week after sowing, 30% 3 weeks, 30% 5 weeks and 30% 6 weeks later). N content in the commercial ammonium sulphate fertilizer used in the experiment was 207 g N kg^{-1} while P content in the Ca-superphosphate fertilizer was 68 g P kg^{-1}, and K in the K-sulphate fertilizer was 400 g K kg^{-1}. Seeds were sown on April 15th, 2014 and growth lasted 95 days. Compost (Table 2) was applied during land preparation (45 days before seeding), as a basal treatment to the experiment field.

Table (1) *Physical and chemical properties of soil of the experiment field.*

pH	EC* (dS m^{-1})	$CaCO_3$ (g kg^{-1})	Organic matter (g kg^{-1})	Saturation % (SP) (w/w)
7.23	3.14	0.0	0.3	21.47
Soluble ions* (mmolc L^{-1})				
Na^+	6.8		CO_3^{2-}	0.0
K^+	3.6		HCO^{3-}	9.3
Ca^{2+}	14.6		Cl^-	8.5
Mg^{2+}	6.4		SO_4^{2-}	13.6

Available nutrients **(mg kg^{-1})						
N	P	K	Fe	Mn	Zn	Cu
5.0	2.0	0.2	25.8	0.5	1.4	1.4
Total nutrients (g kg^{-1})						
N	P	K	Fe	Mn	Zn	Cu
0.30	0.04	1.00	2.20	0.01	0.10	0.20

Particle size distribution (%)			
Sand	Silt	Clay	Texture
98.0	2.0	0.0	Sand

** In paste extract.*

***Extracts of: KCl for N; Na2HCO3 for P; NH4-acetate for K and DTPA for Fe, Mn, Zn and Cu. Soil pH in soil water suspension 1:2.5 (w/v).*

Table (2) *Chemical properties of the compost used in the experiment.*

Total nutrients (g kg^{-1}) ,pH , EC, C/N ratio and contents of organic matter, ash and moisture						
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	332.0	643.0	207.0	9.9	22.6

Gregorich (2008) and plants were analyzed according to methods cited by **Estefan *et al.* (2013).** Oil in seeds was analyzed using *n*-hexane as a solvent (**Akaranta and Anusiem, 1996).**

Analysis of $^{15}N/^{14}N$ ratio:

Plant samples were taken and subjected to ^{15}N analysis using emission spectrometer (Fischer NOI-6PC). Determination of the portion of nitrogen derived from fertilizer (%Ndff) which represents the N which is derived from the fertilizer relative to the total N (i.e. N-uptake) found in the relevant plant part(s) was carried out. Also determination was done to calculate fertilizer N recovery (%FNR) which represents the portion of N derived from the fertilizer, found in the relevant plant part(s), relative to the rate of fertilizer N applied to the soil. The equations for the two parameters are as follows:

%Ndff = (^{15}N% a.e. in sample of the relevant part ÷ ^{15}N% a.e. in fertilizer) x 100

% FNR = {Ndff (kgha^{-1}) in the relevant part ÷»rate «of added N (kgha^{-1})} x 100

RESULTS AND DISCUSSION

Sunflower seed yield:

Treatments receiving N (either singly or in combination with any of the three biofertilizers) resulted in high yields ranging from 1.168 (N_1B_1) to as high as 2.685 (N_2B_1) as compared with the 0.534 kgha^{-1} given by the non-treated, i.e. increases of 119 to 403 % respectively (Table 3). The treatments not given N gave very low yields. The low yields given by the un-biofertilized, non-N-fertilized or the biofertilized non-N-fertilized plants indicate that soluble N-fertilizer is needed for such poor fertile soil. **Soleimanzadeh *et al.*) 2010 (**obtained low sunflower seed yield by solely biofertilization with low-N application under moisture stress. The high yields obtained in the current experiment by treatments supplied with N, particularly in combination of biofertilization, is a manifestation of a positive effect of presence of enough soluble N combined in combination with biofertilization.

Fertilizer N increased yield with a pattern of N main effect as follows: $N_3>N_2>N_1>N_0$ with considerable increases averaging 221.7, 373.8 and 380.0% due to N_1, N_2 and N_3 respectively indicating a progressive increase with most positive effect being caused by the highest N rate followed by the medium rate then the lowest one. Application of N fertilizers to seed oil crops is vital for obtaining high seed yields **(El-Habbasha *et al.*, 2013).** The progressive increase obtained with increased N addition occurred, in particular, in presence of the N_2-fixer (B_2: the ***Azospirillum*** biofertilizer) as well as in presence of the P-dissolver (B_3: the ***B. megaterium*** bio-fertilizer). In absence of biofertilization, however, nearly all treatments given N were of comparable yields with no significant differences between them. This is a manifestation of an interaction caused by biofertilization affecting the response to N. The indication is as follows: "bio-fertilizers should be present in order for the response to N becomes progressive with increasing the rate of N-application.

Seed inoculation with the N_2-fixers or the P-dissolvers gave positive response but only in presence of the mineral fertilizer-N. The biofertilization main effect showed a pattern of $B_1 \geq B_3 \geq B_2 > B_0$ with increases averaging 23.1, 12.2 and 15.0% by B_1, B_2 and B_3 respectively indicating most positive effect being caused by the N_2-fixers of ***Azotobacter*** followed by the P-dissolvers of ***B. megaterium***, then by the N_2-fixers of ***Azospirillum***. The interaction caused by N status in soil affecting the response to biofertilization, reveals that the positive response to bio-fertilizers was particularly true only where there was fertilizer N in the soil. ***Azotobacter*** surpassed the others in presence of the medium N rate whereas ***Azospirillum*** surpassed the others in presence of the high N rate.

Table (3) *Response of sunflower to bio and inorganic fertilization: seed yield (Mg ha^{-1}).*

Inorganic N Fertilization (N)	Biofertilization (B)				mean
	B_0	B_1	B_2	B_3	
N_0	0.534	0.498	0.296	0.352	**0.420**
N_1	1.514	1.168	1.357	1.367	**1.351**
N_2	1.626	2.685	1.685	1.966	**1.990**
N_3	1.443	1.948	2.475	2.198	**2.016**
mean	**1.279**	**1.574**	**1.435**	**1.471**	
LSD: 0.05 = N = 0.161; B = 0.161; NB = 0.321					

Notes: B_0: without biofertilization – B_1: inoculation with *Azotobacter*; B_2: inoculation with *Azospirillum*; B_3: inoculation with *Bacillus megaterium* …… N_0, N_1, N_2 and N_3 = 0, 105, 140 and 175 kg N ha^{-1} (as ammonium sulphate) respectively.

Sunflower seed oil content:

All treatment combinations receiving nitrogen caused increases in oil content (Table 4). Contents of the N-treated plants gave seeds with oil contents ranging from 306.1 gkg^{-1} (N_1B_0) to as high as 445.4 gkg^{-1} (N_2B_3), as compared with 221.8 g kg^{-1} for the non-treated plants (N_0B_0). The lowest increases were given by the ***Azospirillum*** or ***B. megaterium*** given singly. The increases of treatment combinations receiving N ranged from 38.0% (N_1B_0) to as high as 100.1% (N_2B_3). Low oil content where N was not applied is an indication of the vital need for N addition to this poorly fertile sandy soil (Table 1). The greater oil content given by the N-treatments as a result of combining N with biofertilizers reflects the positive cumulative effect of N + biofertilizers.

The main effect of N fertilization shows a pattern of $N_2>N_3>N_1>N_0$. The increases averaged 72.2, 69.1 and 44.4% for N_2, N_3 and N_1 respectively indicating most positive effect being caused by the medium N rate followed by the highest rate then by the lowest one. It seems that the highest N rate favored vegetative growth at the expense of oil accumulation in seeds. Such pattern occurred in presence of B_0 or B_3 but not in presence of B_1 where the pattern was $N_3>N_2>N_1>N_0$ (i.e. progressive increase with progressive N rate); or in presence of B_2 where the pattern was $N_2>N_1=N_3>N_0$ (i.e. a near progressive increase with progressive N rate). Oil content in sunflower seeds in other studies carried out by **Akbari *et al.* (2011)** showed increases in response to application of mineral inorganic N. Therefore in the current study, the progressive increase obtained with increased N addition occurred particularly in presence of the ***Azotobacter*** N_2-fixerand to some extent in presence of the ***Azospirillum*** N_2-fixeras well. In presence of ***B. megaterium*** (the P- dissolver), however the highest response was by the medium N rate. Such was the nature of the interaction caused by biofertilization affecting the response to N application. It is necessary to bio-fertilize sunflower, particularly with ***Azotobacter,*** in order to benefit from increased N application.

The main effect of shows that biofertilizers increased oil content with a main pattern of $B_2>B_3>B_1>B_0$.The increases averaged 16.9, 21.2 and 18.8% by B_1, B_2 and B_3 respectively indicating most positive effect being caused by the ***Azospirillum*** followed by the ***B. megaterium*** P-dissolver, then by the ***Azotobacter.*** Such a pattern of effect of positive response to biofertilizers did not occur under all conditions of N indicating an interaction caused by N status affecting the response to biofertilizers. Only under conditions of the low N rate (N_1) that the pattern was in line with that of the main effect. Under no N application (N_0), the pattern was $B_1>B_2>B_3>B_0$ exhibiting superiority of the ***Azotobacter*** over ***Azospirillum*** with both being superior to ***B. megaterium.*** Under conditions of N_2 the pattern

was $B_3>B_2>B_1>B_0$ indicating superiority of ***B. megaterium*** over the two N_2 fixers. Under conditions of N_3 the pattern was $B_1\geq B_3>B_2>B_0$ indicating similar superiorities of ***Azotobacter*** and ***B. megaterium*** over ***Azospirillum***. The superiority of ***B. megaterium*** over the other two biofertilizers in presence of medium or high N indicates need for ample available N for the ***B. megaterium*** to cause high oil content in seeds.

In the current study, the high oil content obtained by N, particularly in combination of biofertilization, is a manifestation of the positive effect of readily soluble N sources combined with biofertilization. **Zheljazkov *et al*. (2008)** obtained low seed oil content, but high oil yield upon N application to sunflower.

Table (4) *Response of sunflower to bio and inorganic fertilization: seed oil content (g kg^{-1}).*

Inorganic N Fertilization (N)	Biofertilization (B)				mean
	B_0	B_1	B_2	B_3	
N_0	221.8	268.2	251.1	215.3	**239.1**
N_1	306.1	310.5	404.7	359.7	**345.2**
N_2	361.4	414.4	426.1	445.4	**411.8**
N_3	336.6	440.1	403.9	436.3	**404.2**
mean	**306.5**	**358.3**	**371.4**	**364.2**	
LSD: 0.05 = N = 5.7; B = 5.7; NB = 11.4					

Notes: B_0: without biofertilization – B_1: inoculation with *Azotobacter*; B_2: inoculation with *Azospirillum*; B_3: inoculation with *Bacillus megaterium* …… N_0, N_1, N_2 and N_3 = 0, 105, 140 and 175 kg N ha^{-1} (as ammonium sulphate) respectively.

Sunflower oil yield:

Of the different treatment combinations, only the ones receiving N with or without biofertilizers increased the yield of oil (Table.5). Increases ranged from 103% (N_1B_3) to 876% (N_2B_1). The non-treated plants showed a yield of 113.2 kg ha^{-1}. There were two treatment combinations which caused a decrease in oil yield. They were both non-N-fertilized but biofertilized either with ***Azospirillum*** (a decrease of 42.0%) or with ***B. megaterium*** (a decrease of 30.8%). The only treatment receiving biofertilizer non-combined with N which caused increased oil yield was that of the ***Azotobacter*** causing 23.9% increase, indicating higher efficiency over the other two when applied singly.

The main effect of N fertilization showed a pattern of $N_2>N_3>N_1>N_0$ with increases averaging 371.8, 752.9 and 717.0% due to N_1, N_2 and N_3 respectively indicating most positive effect being by the medium N rate followed by the highest rate then the lowest one. Only inabsence of biofertilization or in presence of B_1 the pattern was in line with the main effect. However, in presence of either B_2 or B_3 the pattern was $N_3>N_2>N_1>N_0$. This indicates a progressive oil yield increase caused by a progressive N application only in presence of either ***B. megaterium*** or ***Azospirillum*.** Such is the interaction caused by biofertilization affecting response to N. **Li *et al.* (2014)** stressed the importance of providing adequate N supply in the later growth stages of sunflower for obtaining high oil yield.

Biofertilization main effect showed a pattern of $B_3\geq B_2\geq B_1>B_0$ with increases averaging 38.9, 42.8 and 45.4% by B_1, B_2 and B_3 respectively indicating rather similar general effects of the three biofertilizers. However there was an interaction caused by N affecting the response to biofertilization. Under no N application there was an increase by the ***Azotobacter*** biofertilizer only, and decreases by the each of the other two, probably indicating heavy removal of available soil N caused by them thus competing with plant roots. Under N_1 conditions the only positive re-

sponse was that of ***Azospirillum*** whereas B***. megaterium*** caused no effect and ***B. megaterium*** caused a decrease. Under N_2 conditions the pattern was $B_1>B_3>B_2>B_0$, and under N_3 it was $B_3\geq B_2>B_1>B_0$. Such interaction means that all biofertilizers caused positive effect only where N was present. Besides, the ***Azotobacter*** biofertilizer was the most superior followed by ***B. megaterium*** in presence of N_2 whereas ***B. megaterium*** was the most superior followed by ***Azospirillum*** in presence of N_3.

Therefore it could conclude that low oil yield in absence of N reflects low fertility of sandy soils, and a combination of biofertilization with N application is most effective.

Table (5) *Response of sunflower to bio and inorganic fertilization: oil yield (kg ha^{-1}).*

Inorganic Fertilization (N)	Biofertilization (B)				mean
	B_0	B_1	B_2	B_3	
N_0	113.2	140.3	65.7	78.3	**99.4**
N_1	465.4	353.9	601.0	455.7	**469.0**
N_2	585.6	1104.7	777.0	923.8	**847.8**
N_3	526.6	750.4	970.3	1001.3	**812.1**
mean	**422.7**	**587.3**	**603.5**	**614.8**	
LSD: 0.05 = N = 28.3; B = 28.3; NB = 56.7					

Notes: B_0: without biofertilization – B_1: inoculation with *Azotobacter*; B_2: inoculation with *Azospirillum*; B_3: inoculation with *Bacillus megaterium* …… N_0, N_1, N_2 and N_3 = 0, 105, 140 and 175 kg N ha^{-1} (as ammonium sulphate) respectively.

N uptake by plant:

The N uptake in all plant parts where fertilizer N was not applied did not exceed an average of about 18 kg N ha^{-1} (Table 6). The uptake obtained upon N application was high and the increase by N-fertilization given an increase averaging about 57 kg N ha^{-1} (Table 7) indicating an average rise of about 316% upon N application. The higher N uptake of N by the N-treatments, was particularly marked where biofertilization was combined with N application indicating enhancement due to the biofertilization microorganisms **(Soleimanzadeh *et al.*, 2010, Choudhury and Kennedy, 2004 and Bently and Chasteen, 2002).**

Table (6) *Uptake of N (kgha^{-1}) in sunflower plant parts under no N fertilization.*

Plant part	Biofertilization				mean
	B_0	B_1	B_2	B_3	
Roots	1.56	2.22	2.74	2.74	**2.38**
Stems	2.42	2.96	1.52	2.78	**2.42**
Leaves	4.47	5.14	4.72	5.68	**5.00**
Discs	4.10	3.60	3.00	2.81	**3.38**
Seeds	5.52	4.59	2.63	3.42	**4.04**
Total	**18.07**	**18.51**	**14.67**	**17.43**	
LSD: 0.05 = N = 28.3; B = 28.3; NB = 56.7					

Notes: B_0: without biofertilization – B_1: inoculation with *Azotobacter*; B_2: inoculation with *Azospirillum*; B_3: inoculation with *Bacillus megaterium*

Fertilizer N recovery (FNR) as determined by subtraction calculation versus the ^{15}N tracer technique:

Fertilizer N recovery (FNR) is the recovery of fertilizer N obtained in the plant parts. It is "the N derived from fertilizer found in the fertilized plants in all plant parts" calculated as a portion of the amount of fertilizer-N applied to the soil expressed as a percentage of fertilizer N. It could be calculated where no ^{15}N tracers are used - by subtracting N uptake (kg ha^{-1}) in the non-fertilized plants from that in the fertilized ones (Tables 7 and 8). Where ^{15}N tracer is used, the recovery could be obtained directly by (Tables 9 and 10). The amount of fertilizer N recovered by the two methods varied considerably.

Table (7) *Response of sunflower to bio and inorganic N-fertilization: Plant uptake of N derived from fertilizer (kg ha-1) as calculated by the subtraction method.*

Inorganic N-Fertilization (N)	Biofertilization (B)									
	B_0	B_1	B_2	B_3	Mean	B_0	B_1	B_2	B_3	Mean
	Roots					Stems				
N_1	12.47	4.92	21.91	20.15	14.86	5.84	4.12	4.72	6.45	5.28
N_2	8.23	18.32	13.41	3.78	10.94	8.27	13.54	6.93	6.59	8.83
N_3	12.06	5.9	15.85	47.83	20.41	5.51	7.1	7.27	7.57	6.86
Mean	10.92	9.71	17.06	23.92	**15.40**	6.54	8.25	6.31	6.87	**6.99**
	Leaves					Discs				
N_1	6.02	7.33	13.74	14.45	10.39	3.71	5.99	5.63	5.62	5.24
N_2	23.07	15.51	16.25	10.60	16.36	6.5	14.16	9.02	8.91	9.65
N_3	6.27	18.58	10.4	20.68	13.98	5.09	8.01	9.12	8.75	7.74
Mean	11.79	13.81	13.46	15.24	**13.58**	5.10	9.39	7.92	7.76	**7.54**
	Seeds					Whole plant				
N_1	10.73	6.76	13.22	12.2	10.73	38.77	29.12	59.22	58.87	46.50
N_2	11.43	21.55	14.13	16.01	15.78	57.50	83.08	59.74	45.89	61.55
N_3	7.94	18.55	22.7	17.63	16.71	36.87	58.14	65.34	87.32	61.92
Mean	10.03	15.62	16.68	15.28	**14.40**	44.38	56.78	61.43	64.03	**56.66**

Notes: B_0: without biofertilization – B_1: *Azotobacter*; B_2: *Azospirillum*; B_3: *Bacillus megaterium* …… N_1, N_2 and N_3 = 105, 140 and 175 kg N ha^{-1} (as ammonium sulphate) respectively.(values are means of replicates with no statistical analysis).

Table (8) *Response of sunflower to bio and inorganic N-fertilization: % recovery of fertilizer N as calculated by the subtraction method.*

Inorganic N-Fertilization (N)	Biofertilization (B)									
	B_0	B_1	B_2	B_3	Mean	B_0	B_1	B_2	B_3	Mean
	Roots					Stems				
N_1	11.88	4.69	20.87	19.19	14.15	5.56	3.92	4.50	6.14	5.03
N_2	5.88	13.09	9.58	2.70	7.81	5.91	9.67	4.95	4.71	6.31
N_3	6.89	3.37	9.06	27.33	11.66	3.15	4.06	4.15	4.33	3.92
Mean	8.22	7.05	13.17	16.41	**11.21**	4.87	5.88	4.53	5.06	**5.09**
	Leaves					Discs				
N_1	5.73	6.98	13.09	13.76	9.89	3.53	5.70	5.36	5.35	4.99
N_2	16.48	11.08	11.61	7.57	11.68	4.64	10.11	6.44	6.36	6.89
N_3	3.58	10.62	5.94	11.82	7.99	2.91	4.58	5.21	5.00	4.42
Mean	8.60	9.56	10.21	11.05	**9.85**	3.69	6.80	5.67	5.57	**5.43**
	Seeds					Whole plant				
N_1	10.22	6.44	12.59	11.62	10.22	36.92	27.73	56.40	56.07	44.28
N_2	8.16	15.39	10.09	11.44	11.27	41.07	59.34	42.67	32.78	43.97
N_3	4.54	10.60	12.97	10.07	9.55	21.07	33.22	37.34	58.55	37.54
Mean	7.64	10.81	11.88	11.04	**10.34**	33.02	40.10	45.47	49.13	**41.93**

Notes: B_0: without biofertilization – B_1: *Azotobacter*; B_2: *Azospirillum*; B_3: *Bacillus megaterium* …… N_1, N_2 and N_3 = 105, 140 and 175 kg N ha^{-1} (as ammonium sulphate) respectively.(values are means of replicates with no statistical analysis)

Table (9) *Response of sunflower to bio and inorganic N-fertilization: Plant uptake of N derived from fertilizer (kg ha^{-1}) as determined by the ^{15}N tracer method).*

Inorganic N-Fertilization (N)	Biofertilization (B)									
	B_0	B_1	B_2	B_3	Mean	B_0	B_1	B_2	B_3	Mean
	Roots					Stems				
N_1	5.57	1.65	8.07	5.31	5.15	2.35	2.15	1.83	3.69	2.51
N_2	6.46	7.43	7.67	3.63	6.30	3.41	5.24	3.37	3.81	3.96
N_3	4.05	2.12	3.54	4.89	3.65	3.57	3.19	3.74	4.26	3.69
Mean	5.36	3.73	6.43	4.61	**5.03**	3.11	3.53	2.98	3.92	**3.38**
	Leaves					Discs				
N_1	7.03	7.23	11.49	11.55	9.33	2.60	2.16	2.93	2.66	2.59
N_2	8.69	7.57	8.06	10.53	8.71	4.27	7.23	4.30	3.19	4.75
N_3	11.22	12.38	16.61	15.81	14.01	4.28	4.45	4.66	4.56	4.49
Mean	8.98	9.06	12.05	12.63	**10.68**	3.72	4.61	3.96	3.47	**3.94**
	Seeds					Whole plant				
N_1	10.25	7.39	10.79	6.03	8.62	27.80	20.58	35.11	29.24	28.18
N_2	7.48	13.24	10.41	6.18	9.33	30.31	40.71	33.81	27.34	33.04
N_3	7.84	9.68	10.31	6.28	8.53	30.96	31.82	38.86	35.80	34.36
Mean	8.52	10.10	10.50	6.16	**8.82**	29.69	31.04	35.93	30.79	**31.86**

Notes: B_0: without biofertilization – B_1: *Azotobacter*; B_2: *Azospirillum*; B_3: *Bacillus megaterium* …… N_1, N_2 and N_3 = 105, 140 and 175 kg N ha^{-1} (as ammonium sulphate) respectively.(values are means of replicates with no statistical analysis)

Table (10) *Response of sunflower to bio and inorganic N-fertilization: % recovery of fertilizer N (by ^{15}N tracer method).*

Inorganic N-Fertilization (N)	Biofertilization (B)									
	B_0	B_1	B_2	B_3	Mean	B_0	B_1	B_2	B_3	Mean
	Roots					Stems				
N_1	5.30	1.57	7.68	5.06	4.90	2.24	2.04	1.74	3.52	2.39
N_2	8.61	9.90	10.23	4.84	8.40	2.43	3.74	2.41	2.72	2.83
N_3	6.75	3.54	5.90	8.15	6.09	2.04	1.82	2.14	2.43	2.11
Mean	6.89	5.00	7.94	6.02	**6.46**	2.24	2.53	2.10	2.89	**2.44**
	Leaves					Discs				
N_1	6.70	6.88	10.94	11.00	8.88	2.47	2.06	2.79	2.53	2.46
N_2	6.21	5.41	5.76	7.52	6.23	3.05	5.16	3.07	2.28	3.39
N_3	6.41	7.08	9.49	9.04	8.01	2.44	2.54	2.66	2.61	2.56
Mean	6.44	6.46	8.73	9.19	**7.70**	2.65	3.25	2.84	2.47	**2.81**
	Seeds					Whole plant				
N_1	9.76	7.04	10.28	5.74	8.21	26.47	19.59	33.43	27.85	26.84
N_2	9.98	17.65	13.88	8.24	12.44	30.28	40.86	35.35	25.60	33.02
N_3	13.07	16.13	17.18	10.47	14.21	30.71	31.11	37.37	32.70	32.97
Mean	10.94	13.61	13.78	8.15	**11.62**	29.15	30.52	35.38	28.72	**30.94**

Notes: B_0: without biofertilization – B_1: *Azotobacter*; B_2: *Azospirillum*; B_3: *Bacillus megaterium* …… N_1, N_2 and N_3 = 105, 140 and 175 kg N ha^{-1} (as ammonium sulphate) respectively.(values are means of replicates with no statistical analysis)

A comparison assessing the two determinations shows that the subtraction method values considerably exceeds those determined by the ^{15}N tracer technique. Such overestimation ranged from 39.5% to as high as 194.6% indicating a non-real estimation of recovered fertilizer-N when calculated by subtraction. The percent recovery of fertilizer N in plant ranged from about 20% (N_1B_1) to 41% (N_2B_1) with an overall average of 31% using the ^{15}N tracer, and from 21% (N_3B_0) to 59% (N_3B_3) with an overall average of 42% using the subtraction calculation. Such variation between the two methods of determination is most certainly due to the erroneous supposition behind the adoption of the subtraction method. This supposition presumes that the amount of non-fertilizer-N (the N originated from the soil) present in the in the plants grown in fertilized soil is equal to the N uptake in the plants grown on the non-fertilized soil. However, since plants grown on fertilized soil would most certainly be of greater growth (including greater root system), it follows that more volume of soil is explored by their roots. Therefore fertilized plants would have much greater amounts of non-fertilizer-N originating from soil compared with those of the non-fertilized. Hence erroneous overestimation of uptake of fertilizer Noccurs if calculated by subtraction. These results are similar to those reported by **Harmsen and Garabet (2003)** on California.

Nitrogen fertilizer should be applied at rates enough to allow marked increase in sunflower growth and seed yield as well as seed oil content and seed oil yield. 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. The percent recovery of fertilizer N in plant ranged from an overall average of about 31% using the ^{15}N tracer to 42% using the subtraction calculation.

REFERENCES

- **Akaranta, O. and Anusiem, A.C.I. (1996):** A bio re-

source solvent for extraction of castor oil. *Industrial Crops and Products*, 5: 273.

- **Akbari, P.; Ghalavand, A.; Modarres-Sanavy, A.M. and Agha-Alikhani, M. (2011):** The effect of biofertilizers, nitrogen fertilizer and farmyard manure on grain yield and seed quality of sunflower (*Helianthus annuus* L.). *J. Agric. Tech.*, 7: 173.
- **Bentley R., and Chasteen, T.G. (2002):** Microbial methylation of metalloids: Arsenic, antimony and bismuth. *Microbial. Mol. Biol. Rev.*, 66: 250.
- **Bhattacharyya, R.; Kundu, S.; Ved-Prakash, A. and Gupta, H.S. (2008):** Sustainability under combined application of mineral and organic fertilizers in a rainfed soybean–wheat system of the Indian Himalayas.*Euro. J. Agron.*, 28: 33.
- **Blum, A. (1997):** Crop responses to drought and the interpretation of adaptation. *In*: Belhassen, I. (Ed.), Drought tolerance in higher plants: Genetical, physiological, and molecular biological analysis. Kluwer Academic Publishers, Dordrecht, the Netherlands.
- **Boyer, J.S. (1982):** Plant productivity and environment. *Sci*., 218: 443.
- **Carter, M.R. and Gregorich, E.G. (2008):** Soil sampling and methods of analysis. Canada. *Soc. Soil Sci.,* 2nd Ed.
- **Choudhury, A. and Kennedy, I.R. (2004):** Prospects and potentials for systems of biological nitrogen fixation in sustainable rice production.*Bio. Fert. Soils.,* 39: 219.
- **de Wit, C.T. (1992):** Resource use efficiency in agriculture. *Agric. Syst*., 40: 125.
- **El-Habbasha, S.F.;Taha, M.H. and Jafar, N.A. (2013):** Effect of nitrogen fertilizer levels and zinc foliar application on yield, yield attributes and some chemical traits of groundnut. Res. *J. Agri. Bio. Sci.*, 9: 1.
- **Estefan, G.;Sommer, R. and Ryan, J. (2013):** Methods of soil, plant and water analysis: A manual for the West Asia and North Africa regions. International Center for Agricultural Research in the Dry Areas (ICARDA), 3rd Ed.
- **FAO (2006):** FAO Statistical year book, FAO, Rome, Italy.
- **FAO (2012):** Crop yield response to water. FAO Fert. Plant Nut. Bulletin No. 33.Food and Agriculture Organization (FAO) of the United Nations, Rome, Italy.
- **Harmsen, K. and Garabet, S. (2003):** A comparison of theisotope-dilution andthe difference method for estimatingfertilizer nitrogen recovery fractions incrops. III. Experimental. *Nuclear J. App. Sci.*, 51: 237.
- **Hood, R.; Van Kessel, C. and Vanlauwe, B. (2008):** Use of tracer technology for the management of organic sources.*In*IAEA (ed) Guidelines on nitrogen management inagricultural systems. *International Atomic Energy Agency-Training Course Series (IAEA-TCS) 9, Vienna, Austria.*
- **Hu, F.B.; Manson, J.E. and Willett, W.C. (2001):** Types of dietary fat and risk of coronary heart disease: a critical review. *J. Am. Coll. Nutr.*, 20: 5.
- **Izquierdo, N.G.; Dosio, G.A.A.; Cantarero, M.; Luján, J. and Aguirrezábal, L.A.N. (2008):** Weight per grain, oil concentration, and solar radiation intercepted during grain filling in black hull and striped hull sunflower hybrids. *Crop Sci.,* 48: 688.
- **Kamal, J. and Bano, A. (2009):** Efficiency of allelopathy of sunflower (*Helianthus annuus* L.) on physiology of wheat (*Triticumaestivum* L.). *Dep. Plant Sci., Faculty Bio. Sci. Quaid-I-Azam Uni. Islamabad, Pakistan.*
- **Li, S.; Tuo, D. and Duan, Y. (2014):** Nutrient imbalance following over- and under-application of some nutrients has restricted sunflower production in Northwest China. *International Plant Nutrition Institute (IPNI),* 98: 15.
- **Makowski, D.; Wallach, D. and Meynard, J.M. (1999):** Models of yield, grain protein, and residual mineral nitrogen responses to applied nitrogen for winter wheat. *Agron. J.*, 91: 377.
- **MALR (2014):** Cultivation and production of sunflower. Bulletin *Ministry of Agriculture and Land Reclamation (MALR)*, *Egypt*, 1315: 16.
- **Metzger, J.O. and Bornsheuer, U. (2006):** Lipids as renewable resources: Current state of chemical and biotechnological conversion and diversification. *Appl. Micro. Biotech*., 71: 13.
- **Reddy, B.V.S.; Sanjana-Reddy, P.; Bidinger, F. and**

Blümmel, M. (2003): Crop management factors influencing yield and quality of crop residues. *Field Crop Res.*, 48: 57.

- **Salas, J.J.; Martinez-Force, E.; Harwood, J.L.; Venegas-Caleron, M.; Aznar-Moreno, J.A.; Moreno-Perez, A.J.; Ruiz-Lopez, N.; Serrano-Vega, M.J.; Graham, I.A.; Mullen, R.T. and Garces, R. (2014):**mBiochemistry of high stearic sunflower:A new source of saturated fats. Prog. *Lipid Res*., 55: 30.
- **Simpson, B.W.; Mcleod, C.M. and George, D.L. (1989):** Selection for high linoleic acid content in sunflower (*Helianthus annuus* L.). *Aust. J. Exp. Agric.*, 29: 233.
- **Soleimanzadeh, H.; Habibi, D.; Ardakani, M. R.; Paknejad, F. and Rejali, F. (2010):** Response of sunflower (*Helianthus annuus* L.) to drought stress under different potassium levels. *World App. Sci. J.*, 8: 443.
- **Vanlauwe, B.; Sanginga, N. and Merckx, R. (1998):** Recovery of leucaena and dactyladenia residue nitrogen[15] in alley cropping systems. *Soil Sci. Soc. Am. J.*, 62: 454.
- **Zheljazkov, V.D.; Vick, B.A.; Ebelhar, M.W.; Buehring, N.; Baldwin, B.; Astatkie, T. and Miller, J.F. (2008):** Yield, oil content, and composition of sunflower (*Helianthus annuus* L.) grown at multiple locations in Mississippi. *Agron. J.*, 100: 635

مجلة
التقنيات النووية
فى العلوم التطبيقية
مجلد 3 ، عدد 3 ، ص 157 : 169 ، (2015)

إستجابة دوار الشمس للتسميد النيتروجيني والحيوي مع تقييم إسترجاع النيتروجين السمادي باستخدام النظير المستقر(N^{15}) ومقارنتها بطريقة الطرح

على أحمد عبدالسلام[1]، سليمان محمد سليمان[2]، يحيي جلال محمد جلال[2]، وسام رشاد زهرة[1]، أحمد عبدالمنعم مرسى[2]، محمد أشرف هيكل[2]

أجريت تجربة حقلية على محصول دوار الشمس المنزرع فى الأرض الرملية (٩٨٪ رمل) وتم معاملته بأربع مستويات من التسميد النيتروجينى وهى بدون 175 ،(N2) 140 ،(N1)105 ،(N0) (N3) كجم نيتروجين/هكتار فى صورة سلفات النشادر، وعوملت البذور بمعاملات القاح البكتيرى وهى بدون (B0)، الأزوتوباكتر Azotobacter chroococcum المثبتة للنتروجين (B1) ، الأزوسبيريللم Azospirillum brasilense المثبتة للنتروجين (B2) وبكتيريا الباسيلس مجاتيريم Bacillus megaterium المذيبة للفوسفات (B3). إستخدم سماد سلفات النشادر المرقم بالنظير الثابت ن^{15} (٢٪ وفرة من الذرات المرقمة). تلقت جميع القطع التجريبية ٢١ ميجا جرام (طن متري) كمبوست + ٢٤ كجم فوسفور P + ٨٠ كجم بوتاسيوم K للهكتار. أعطت النباتات الغير معاملة ٠,٥٣٤ ميجاجرام بذور/هكتار بينما أعطت النباتات المعاملة بالنيتروجين سواء فى وجود أو غياب التسميد الحيوى زيادة وصلت إلى ٤٠٣ ٪ (N2B1). الأثر العام لمستويات التسميد النيتروجينى هو «N3>N2>N1» أما بالنسبة للأثر العام للتسميد الحيوى فهو «B1≥B3≥B2». محتوى الزيت فى بذور المعاملة N0B0 كان ٢٢٢ جم/كجم وقد إزداد إلى ٤٤٥ جم/كجم بواسطة المعاملة N2B3 . الأثر العام لمستويات التسميد النيتروجينى هو«N2>N3>N1» أما بالنسبة للأثر العام للتسميد الحيوى فهو «B2>B3>B1» ومحصول الزيت كان ١١٣ كجم/هكتار بواسطة المعاملة N0B0 قد إرتفع إلى ١١٠٥ كجم/هكتار بواسطة المعاملة N2B1 حيث كان الأثر العام لمستويات التسميد النيتروجينى هو «N2>N3>N1» وللتسميد الحيوى كان «B3>B2>B1» و إمتصاص النيتروجين فى جميع أجزاء النبات من جذور وسيقان وأوراق وأقراص وبذور قد زاد بالأمداد النيتروجينى مقارنة بمعاملات غياب النيتروجين سواء فى وجود أو غياب التسميد الحيوى بمقدار ١٨,١، ١٨,٥، ١٤,٧، ١٧,٤٪ بواسطة N0B3، N0B2، N0B1، N0B0 على التوالى، وزاد الإمتصاص كثيراً إلى ٦٦٧٪ (N3B3) بواسطة الإمداد النيتروجينى. إمتصت النباتات نسبة تراوحت بين ١٩,٦٪ (في معاملة N١B١) إلى ٤٠,٩٪ (في معاملة N٢B١) من نيتروجين السماد في أجسامها طبقا للقياسات بإستخدام النظير المستقر ن^{15}، ولكن كانت النسب الموازية و المحسوبة بطريقة الطرح (طرح قيم الغير مسمد من قيم المسمد وهي المستخدمة في حالة عدم استخدام النظير المستقر) أعلى بكثير(٧,٧ ٪ و٩,٦ ٪ لنفس المعاملتين). ويعزي ذلك إلي إمتصاص النباتات المسمدة لكميات من نيتروجين التربة أكثر من مثيلاتها الغير مسمدة نظرا لتشعب النباتات المسمدة خلال حجم من التربة أكبر مما للنباتات الغير مسمدة مما يجعل طريقة الطرح غير معبرة تماما عن امتصاص الحقيقي لنتروجين السماد و تعطي قيما أعلي من الحقيقة.

1. قسم الأراضى، كلية الزراعة، مشتهر، جامعة بنها
2. قسم بحوث الأراضى والمياه، مركز البحوث النووية، هيئة الطاقة الذرية، أبوزعبل 13759، مصر