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1.1. EFFECT OF NITROGEN, BIOGAS SLUDGE MANURE AND BIO-FERTILIZER ON GRAIN NITROGEN UPTAKE AND YIELD OF WHEAT (*Triticum aestivum*,L.)

Nasser Kh. B. El-Gizawy

Department of Agronomy, Faculty of Agriculture, Benha University, Egypt. PO Box 13736, e-mail: Nasser.elgizawy@fagr.bu.edu.eg

ABSTRACT

A field study was carried out in the Agricultural Research and Experimental Center, Faculty of Agriculture at Moshtohor, Kalubia Governorate, Benha University, during 2006/07 and 2007/08 seasons. The aim was to assess influence ofnitrogen (N) fertilization, biogas sludge (BS) manure and bio-fertilizer (BF) on grain nitrogen uptake (Nup) and yield of wheat. Three N levels (0, 40 and 80 kg N/fed), two biogas sludge levels (0, 3 tons/fed) and two bio-fertilizer treatments (with and without) were used in split-spilt plot design with four replications. The results indicated that the highest N level (80 kg N/fed) was more effective for increasing all studied traits i.e. number of tillers and spikes/m^2, plant height, spike length, number of spikelets and grains/spike; 1000-grain weight, grain yield/fed., grain N content , grain protein content (GPC%) and grain N uptake.The application of 40 and 80 kg N /fed increased the grain yield over the control treatment by 45.4 and 65.0% in 2006/07 season, 40.0 and 66.5% in 2007/08 season, being 42.6 and 65.8% in the combined analysis. Application 3 tons biogas sludge manure/fed significantly increased grain yield, its components and grain N uptake. Grain yield increased due to adding 3 tons/fed over the check treatment by 10.2, 18.7 and 14.5 % in the 1st, 2nd seasons and combined analysis, respectively. Bio-fertilization significantly increased number of tillers and spikes/m^2, plant height, spike length, 1000- grain weight, grain yield/fed, N%, GPC% and grain N uptake compared without bio-fertilization in both seasons and combined analysis. Significant interaction effects (in combined analysis) were found between nitrogen levels and biogas sludge manure on spike length and number of tillers/m^2. Also, interaction was found between nitrogen levels and bio-fertilizer

on grain nitrogen uptake as well as between biogas sludge manure and bio-fertilizer on number of tillers /m^2.

INTRODUCTION

Wheat (*Triticum aestivum*, L.) is one of the most important cereal crops in terms of area and production. Also, wheat is considered as the first leading cereal crop in the world, due to its position as a staple food for the majority of the world population. In Egypt, wheat is the most important food crop and provides almost 35 % of the total food calories of the Egyptian people.

Egyptian soils are known to be poor in available nitrogen due to their low content of organic matter and the small amounts of organic manures added to them annually. Therefore, applying the optimum N level and suitable N carrier are most important means for raising the yield of most crops. The increase of N carriers prices, during last years in addition to the hazards of soil pollution resulting from excessive N application have increased the interest for improving plant efficiency in using nitrogen. Many researchers have found that nitrogen application is an important factor for wheat production.Salwau (1994) showed that soil application of nitrogen level up to 75 kg N/fed significantly increased plant height, number of spikes/m^2, spike characters, grain yield/fed and protein content in wheat grain. The soil application of 30, 45, 60 and 75 kg N/fed increased grain yield by 77.76 % , 106.22 %, 132.22 % and 122.78 %, respectively over the control in combined analysis. Sawires (2000) mentioned that application of 100 kg N/fed gave the tallest plants of wheat and highest values of number of tillers and spikes /plant ,number of grains/spike ,weight of grains/spike ,1000- grain weight, grain and straw yields/fed. The increase in grain and straw yields/fed amounted to 25.0 and 38.84 %, respectively by application of 100 kg N/fed than those received 40 kg N/fed.

Several investigators found that increasing grain protein and grain N uptake by applying higher fertilizer N rates is relatively not efficient, especially under dry soil conditions (Gauer *et al*. 1992). Sowers, *et al.* (1994) found that the application of high N rates may result in poor N uptake and low N use efficiency

(NUE) due to excessive N losses. The same trend was reported by Lopez-Bellido *et al.* (1998), Camara *et al*., (2003) and Staggenborg *et al.,(*2003). While, Ma *et al*.(2004) used 50, 100, 150 and 200 kg N/ha and showed that no significant differences in grain yield were detected among N fertilizer rates although higher N rates generally led to higher yield.

Biogas sludge manure as an organic fertilize, plays a very important role in plant nutrition as well as in soil conservation. It is not only a carrier and source of nutrients, but it also serves as an excellent soil conditioner. It improves the physical condition of the soil by improving texture, moisture-holding capacity, and aeration. It increases the buffering capacity of the soil. It combines with inorganic soil constituents to prevent their loss by leaching but releases them for the use of the plants. It stimulates the growth of the microorganisms. It retards the irreversible fixation of nutrients, prevents soil erosion and smoothes out temperature fluctuations. A more effective use of the nutrients in manure reduces the need for mineral fertilizers. This decreases costs for the farmer and also results in energy-savings since fossil raw materials and energy are needed to manufacture mineral fertilizers. Results of several studies have reported that using organic manure improved soil fertility as well as increasing wheat grain yield, number of tillers and spikes/m^2, number of grains/spike, GPC and nitrogen use efficiency (Mowafy, 2002 and Shafshak et al,2003)

Today, application of bio-fertilizer is considered as a promising alternative for mineral fertilizers and decreasing agricultural costs, maximizing crop yield due to providing them with an available nitrogen source and growth promoting substances. Free-living nitrogen-fixing bacteria *i.e. Azotobacter chroococcum* and *Azospirillum lipoferum,*were found tohave not only the ability to fix nitrogen but also the ability torelease phytohormones similar to gibberellic acid and indoleacetic acid, which could stimulate plant growth, absorptionof nutrients, and photosynthesis (Fayez *et al.,* 1985). Hussein and Radwan 2001 demonstrated that inoculated wheat seeds with bio-fertilizer significantly increased the grain yield/fed , harvest index, 1000-grain weight and spike grain weight by 6.5 , 3.4 , 2.1 and 8.8% , respectively , rather than non-biofertilized treatments. Mahmoud and Mohamed 2008 concluded that bio-fertilizers Cerealien®

stimulated wheat growth and yield. Badran, 2009 found that highly significantly increased by inoculation of wheat grains either by Nitrobin® or Microbin® ,compared with un-inoculated ones, in both seasons. El-Gizawy, 2009 demonstrated that inoculated wheat grains with bio-fertilizer significantly increasedgrain yield/fed, its component, GPC%, grain NPK uptake, compared with non-inoculated.

The objective of this study was to investigate the effect of nitrogen (N) fertilization, biogas sludge (BS) manure and bio-fertilizer (BF) on grain nitrogen uptake (Nup) and yield of wheat.

MATERIALS AND METHODS

Experimental site description and soil properties

Two field experiments were carried out at the Agricultural Research and Experimental Center, Faculty of Agriculture Benha Univ., during the two successive seasons 2006/07 and 2007/08 on wheat cv. Sakha 93 .Soil texture was clay. The preceding crop in both seasons was maize. Soil samples were taken from the surface 20 cm before treatment applications in both seasons (Table, 1).

Table1.Characteristics of the surface 20 cm of soil in experiment fields before treatment applications.

Soil properties	**1^{st} season**	**2^{nd} season**
Physical analysis		
Sand, %	24.5	24.4
Silt, %	19.5	20.3
Clay, %	56.0	55.3
Texture	Clay	Clay
Chemical analysis		
Organic matter %	1.87	1.88
pH $_{\text{water 1:2 ratio}}$	7.80	8.00
EC,ds /m	1.30	1.66
$CaCO_3$ %	2.55	2.78
Available nutrients (*m*g/kg)		
N (Nitrate-N) KCl-extractable	18.32	16.45
P($NaHCO_3$-extractable)	10.55	10.65

K(neutral extractable)	NH_4-acetate	243.00	255.00

Treatments and experimental design

Each experiment included 12 treatments which were the combinations of three N levels (0, 40 and 80 kg N/fed), two biogas sludge manure treatments (0 and 3 tons/fed) and two bio- fertilization (BF) (with and without). The treatments were arranged in a split-split plot design with four replicates. The main plots were occupied by N levels, the first sub plots were devoted for biogas sludge levels and bio-fertilizer were assigned to the second sub- plots. The sub-sub plot area was 10.5 m^2 (3m long and 3.5 m apart).

Crop management practices

The plots were sown on 25 and 15 of November in the first and second season, respectively. Wheat grains, just before sowing, were inoculated with a non-symbiotic N fixing *Azotobacter chroococcum* and *Azospirillum lipoferum,* under the commercial name Nitrobein® 500g/fed. Nitrobein inoculums which contains Azotobacter and Azospirillum microorganisms, at a concentration of 10^7 colony forming unit/g. Coating of wheat grains was conducted as recommended by the Egyptian Ministry of Agriculture, Giza, Egypt. Nitrobein® is produced by Bio-fertilizers Unit, General Organization of Agriculture Equalization Fund, Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. Super phosphate at the rate of 30 kg P_2O_5/fed was applied before seeding. Biogas sludge manure was supplied by biogas Training Center at Moshtohor village and added during land preparation. Chemical analysis of biogas manure is presented in Table (2).Nitrogen fertilizer in the forms of ammonium nitrate (33.5 % N) was applied in two equal rates before the first and second irrigation in both seasons. Weeds were controlled during the growing seasons by specific herbicide Brominal W 24 % (3,5-dibromo-4-hydroxybenzonitrile) at the rate 1.0 L/fed at the 2- to 4- leaf stage (after 21 days from sowing in both seasons). Weeds that germinated subsequently were removed by hand. The normal practices for growing wheat were followed as recommended for the region.

Table 2. Chemical properties of biogas manure.

Biogas manure parameters	2006/07	2007/08
Density (g/cm^3)	0.53	0.54
Moisture content **%**	26.5	28.3
Organic matter (%)	46.43	48.45
Electrical conductivity (ds/m)	2.51	2.63
pH(1:2.5 water suspension)	7.55	7.50
Total N (%)	1.43	1.38
Total P (%)	0.467	0.446
Total K (%)	0.672	0.760

Data collected

At harvest, (ripening stage Zadoks 92; Zadoks et al., 1974), plants in 0.5 m^2 were taken to determine the number of tillers and spikes/m^2.Then, ten fertile tillers from each sub-sub plot were randomly chosen to estimate plant height, spike length, number of spikelets/spike, number of grains/spike and 1000 – grain weight. Grain yield was recorded on the whole plot basis. Nitrogen (N) % in grain was determined by the microkjeldahl method according to A.A.C.C. 2000. Grain protein content (GPC %) was calculated as N% X 5.7 on dry weight basis. Nitrogen uptake in grain (kg/fed) was calculated according to Craswell and Godwin 1984 as follows: Nup = grain yield x N% in grain.

Statistical analysis

Data were statistically analyzed according to Steel and Torrie (1980) for split-split plot experiments for each year and then as a combined experiment. Before conducting a combined analysis over years, error variance was tested for homogeneity by Bartlett test using the MSTAT-C Statistical Software Package (Michigan State University, 1983). Where the F- test showed significant differences among means Duncn's multiple range test (1955) was performed at the 0.05 level of probability to compared means.

RESULTS AND DISCUSSION

Effect of growing seasons

Data in Table (3) showed that there were not significant seasonal effects *(P<0.05*) existed for all characters studied except number of grains/spike, 1000-grain weight, grain yield/fed and grain N uptake. Higher mean values for these traits were detected in the second season. Such superiority could be due to earlier planting date in the second season.

Table 3. Mean values of seasonal effect.

Traits	Growing seasons		F test
	2006/07	**2007/08**	
Number of tillers /m^2	408.8	413.0	N.S
Number of spikes /m^2	393.1	396.9	N.S
Plant height (cm)	96.6	95.8	N.S
Spike length (cm)	10.5	10.2	N.S
Number of spikelets/spike	18.3	18.8	N.S
Number of grains/spike	59.5	61.0	**
1000-grain weight (g)	37.4	37.7	**
Grain yield (kg/fed)	2352.0	2456.2	*
Grain N content (%)	1.55	1.56	N.S
Grain protein content (%)	8.85	8.93	N.S
Grain N uptake (kg/fed)	37.2	39.4	*

**, ** indicates significant at P < 0.05 and 0.01, respectively; N.S = non significant*

Effect of Nitrogen rates

Data presented in Table (4) show that all studied traits were significantly increased (*P<0.05*) with increasing N fertilization rates. The higher N level (80 kg N/fed) was more effective for increasing all studied traits i.e. number of tillers and spikes/m^2, plant height, spike length, number of spikelets and grains/spike; 1000-grain weight, grain yield/fed., N% , GPC and grain N uptake. Nitrogen at 80 kg N/fed produced the maximum grain yield and proved significantly superior to lower levels. The application of 40 and 80 kg N /fed increased the grain yield over the control treatment by 781.3 and 1118.8 kg/fed in the 1st

season and by 725.0 and 1206.2 kg/fed in the 2nd season. Being 753.1 and1162.5 kg/fed in the combined analysis. These increases correspond to 45.4 and 65.0% in 2006/07 season, 40.0 and 66.5% in 2007/08 season, being 42.6 and 65.8% in the combined analysis. The favorable effect of N fertilization on plant height and number of spikes/m^2 which are associated with number of tillers/plant may explain the role of nitrogen for stimulating cell division and elongation, consequently internodes

Table 4 . Yield, it components and grain N uptake as affected by Nitrogen fertilizer levels.*

Characters	Nitrogen fertilizer levels (kg/fed)								
	2006 / 07 season			2007 / 08 season			Combined analysis		
	Control	40	80	Control	40	80	Control	40	80
Number of tillers /m^2	388.6c	411.9b	426.0a	400.5c	413.3b	425.1a	394.5c	412.6b	425.5a
Number of spikes /m^2	372.4c	395.0b	412.1a	383.1c	396.1b	411.5a	377.7c	395.5b	411.8a
Plant height (cm)	88.7c	96.9b	104.2a	86.1c	95.4b	105.8a	87.4c	96.1b	105.0a
Spike length (cm)	9.2b	11.0a	11.2a	8.4c	10.4b	11.7a	8.8c	10.7b	11.5a
Number of	17.8a	18.3a	18.8a	17.4b	19.1a	20.0a	17.6c	18.7b	19.4a
spikelets/spike	53.5c	61.0b	64.0a	56.8c	61.1b	65.1a	55.2c	51.1b	64.5a
Number of grains/spike	34.0c	38.1b	40.1a	35.8c	39.9b	43.4a	34.9c	39.0b	41.7a
1000-grain weight (g)	1718.7c	2500.0b	2837.5a	1812.5c	2537.5b	3018.7a	1765.6c	2518.7b	2928.1a
Grain yield (kg/fed)	1.40c	1.58b	1.66a	1.40c	1.53b	1.75a	1.40c	1.55b	1.71a
Grain N content (%)	8.02c	9.02b	9.50a	8.02c	8.74b	10.02a	8.02c	8.88b	9.76a
Grain protein content (%)	24.3c	39.7b	47.5a	25.6c	39.1b	53.4a	25.0c	39.4b	50.5a
Grain N uptake (kg/fed)									

*,** *indicates significant at P < 0.05 and 0.01, respectively*

as shown in the chemical analysis of the manure (Table 2). Similar results were obtained by Mowafy, 2002 and Shafshak et al,2003 who found that using organic manure increasing wheat grain yield, number of tillers and spikes/m^2, number of grains/spike and GPC %.

Effect of bio-fertilization

Data presented in Table 6 demonstrated that inoculated wheat grains with bio-fertilizer significantly increased *(P<0.05*) numbers of tillers and spikes/m^2, plant height, spike length, spikelets/spike, grains/spike and 1000-grain weight, grain yield/fed, GPC%, grain N uptake compared with non-inoculated. Increases in grain yield/fed due to inoculating wheat grains with bio-fertilizer compared with control were 9.5, 11.6 and 10.5 % in the 1st, 2nd seasons and combined average, respectively. The relative positive effect of bio-fertilizer treatment on yield criteria may be attributed to their N_2-fixing activity and the production of plant growth promoting substances such as IAA, gibberellins and cytokinine-like substances (El-Demerdash et al., 1992) as well as mineralization of certain macro and micronutrients (El-Shanshoury, 1995). Similar findings were reported by Hussein and Radwan 2001 recorded that inoculated wheat grains with bio-fertilizer significantly increased the grain yield/fed , harvest index, 1000-grain weight and spike grain weight by 6.5 , 3.4 , 2.1 and 8.8%, respectively, rather than non-biofertilized treatments. Mahmoud and Mohamed 2008 and Badran, 2009 concluded that bio-fertilizers stimulated wheat growth and grain yield.

Interaction effects

Significant interaction effects *($P<0.05$*) were found among nitrogen levels and biogas sludge manure on spike length and number of tillers/m^2 in combined analysis (Table 7). Also, in combined analysis interaction were found among nitrogen levels and bio-fertilizer on grain nitrogen uptake (Table 8) as well as between biogas sludge manure and bio-fertilizer on number of tillers /m^2 (Table9).

Data in Table (7) indicated that the highest values of spike length (11.83 cm) and number of tillers/m^2 (433.2) were obtained from 80 kg N/fed when fertilized with biogas manure at 3 tons/fed.

Data illustrated in Table (8) recorded that maximum grain nitrogen uptake/fed (55.6 kg/fed) was obtained from 80 kg N /fed and inoculated with bio-fertilizer.

Data presented in Table (9) indicated that highest values of number of tillers/m^2 (421.5) was obtained from 3 tons biogas sludge manure and inoculated with bio-fertilizer. This increase represented 4.59 % as compared to the control (without Biogas manure and Non-inoculated).

Table 6.Yield, it components and grain N uptake as affected by bio-fertilizatio

Characters	Bio-fertilization								
	2006 / 07season			2007/08 season			Combined analysis		
	without	with	F test	without	with	F test	without	with	F test
Number of tillers /m^2	403.7	413.9	**	408.7	417.2	**	406.2	415.6	**
Number of spikes /m^2	386.4	399.8	**	391.0	402.9	**	388.7	401.4	**
Plant height (cm)	94.4	98.7	**	93.3	98.2	**	93.9	98.5	**
Spike length (cm)	10.1	10.8	*	9.7	10.6	**	9.9	10.7	**
Number of	17.6	19.0	**	18.1	19.6	**	17.8	19.3	**
spikelets/spike	55.7	63.3	**	58.5	63.6	**	57.1	63.4	**
Number of	35.6	39.1	**	38.4	41.0	**	37.0	40.1	**
grains/spike	2245.8	2458.3	**	2320.8	2591.6	**	2283.3	2525.0	**
1000-grain weight (g)	1.48	1.61	*	1.48	1.65	**	1.48	1.63	**
Grain yield (kg/fed)	8.48	9.21	*	8.45	9.40	**	8.47	9.31	**
Grain N content (%)	34.0	40.4	**	35.1	43.7	**	34.5	42.1	**
Grain protein content (%)									
Grain N uptake (kg/fed)									

*,** *indicates significant at* $P < 0.05$ *and 0.01, respectively*

Table7. Wheat criteria as affected by the interaction between nitrogen levels and biogas sludge manure (in combined analysis)*.

N fertilizer levels Kg/fed	Biogas sludge manure	
	Control	3 tons/fed.
Spike length (cm)		
control	8.04 d	9.70 c
40	10.50 b	10.95 b
80	11.16 ab	11.83 a
Number of tillers/m2		
control	391.0 c	398.1 c
40	410.1 b	415.0 b
80	417.9 b	433.2 a

* ***Note**: Means with different alphabets indicate significant difference between treatments by Duncan's multiple range test at* $p \leq 0.05$.

Table8. Grain N uptake kg/fed of wheat as affected by the interaction among N fertilizer levels and bio-fertilizer (in combined analysis)*.

N fertilizer levels Kg/fed	Bio-fertilization	
	without	with
control	22.3 e	27.6 d
40	35.9 c	42.9 b
80	45.3 b	55.6 a

***Note**: Means with different alphabets indicate significant difference between treatments by Duncan's multiple range test at p≤ 0.05.*

Table 9. Number of tillers /m^2 of wheat as affected by the interaction among biogas sludge manure and bio-fertilizer (in combined analysis)*.

Biogas sludge manure	Bio-fertilization	
	without	with
control	403.0 c	409.6 b
3 tons/fed	409.4 b	421.5 a

** Note: Means with different alphabets indicate significant difference between treatments by Duncan's multiple range test at p≤ 0.05.*

CONCLUSION

It could be concluded that under the conditions of the current experiment, the highest level of the three factors separately led to increased grain yield, its components and grain N uptake.

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