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ROLE OF NON-SYMBIOTIC N_2-FIXING BACTERIA IN OPTIMIZING THE MINERAL NITROGEN FERTILIZER USE OF WHEAT PRODUCTION

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ABSTRACT

Two field trails were carried out at Sakha Agricultural Research Station Farm through the two successive growing seasons of 2006/2007 and 2007/2008 to study the effects of fertilization with five nitrogen levels of 0, 25, 50, 75 and 100 kg N/fed. and applying *azospirillum* bacteria as effective biofertilizer on yield, yield components, nitrogen percentage and grain protein content of wheat .Split plot design was used, the main plots were assigned by N levels and the sub plots were assigned by biofertilization. The obtained results can be summarized as follows:

Inoculation with biofertilizer high significantly increased wheat grain yield from 1.32 to 2.13 ton fed.$^{-1}$ in the first season and from 1.31 to 2.2 ton fed^{-1} in the second season.

Increasing nitrogen fertilizer rates high significantly increased grain yield. The highest mean values of 2.26 and 2.6 ton fed^{-1} were obtained with 100 kg N fed^{-1} in the first and second season, respectively.

Obtained data clearly illustrated that the mean values of straw yield tons/fed. were increased by increasing nitrogen does up to 100 kg/fed. The highest mean values were recorded and they are 3.03 and 3.12 ton/fed. in the first and second growing seasons, respectively. On the contrary, the lowest mean values were recorded under the control treatment (without nitrogen) of 1.32 and 1.28 ton/fed. in the two growing seasons.

The mean values of nitrogen and protein percentages were increased by increasing both of mineral and biofertilizer applications.

The mean values for plant height were increased by increasing application of both mineral and biofertilizer. The mean values under biofertilizer application were 96.36 and 97.23 cm. But under non-application treatments the mean values were 62.12 and 61.04 cm in the first and second seasons, respectively. Also, application of mineral fertilizer up to 100 kg/fed. increased wheat plant height. The highest mean values were recorded with 100 kg N fed.$^{-1}$.

The mean values of 1000 grain weight, number of tillers and number of spikes were incased by increasing nitrogen dose up to 100 kg N/fed. Also, application of biofertilization led to increasing the studied parameters in comparison with non-application treatments.

Keywords: Fertilization-*Azospirillum*-protein content-Wheat (*Triticum aestivum* L.).

INTRODUCTION

Increasing of mineral fertilizers price, the attention by clean agriculture production and environment pollution led to great attention by biofertilizers. The problems of environmental nitrogen enrichment is most likely to solved by reducing the inputs of synthetic nitrogen fertilizers through the creation of cereal that like legumes, are able to fix nitrogen. In legumes rhizobia present in tracellularly in vesicles in the cytoplasm and irretum, the bacteria provide the plant with biologically fixed nitrogen. Recently, we have

demosntrated using novel inoculation condition with very low number of bacterial that cells at the root mersistems of maize while rice, wheat and other non-legume crop can be colonized intracellularly by the non-rizobial non nodulating nitrogen-fixing bacterium, gluconacetobacter, dizotrophicus, that accursnaturally in sugar cane *G. diazotrophcus*.

Many investigators have used biofertilizers from bacteria origin successfully to minimize the nitrogen fertilizers dose. Abd El-Aziz *et al.* (1989) showed that associative N_2 fixing bacteria were effective in reducing N fertilizer for wheat expressing nitrogen-fixing bacteria is present in membrane-bounded compartments in the cytoplasm of cells of the root meristems of the target cereal and non legume species, similar to the intracellular at the likelihood of adequate growth and yield of maize for example, with reduced inputs of synthetic nitrogen fertilizers we are determining the extent nitrogen fertilizer. Patra *et al.* (1989) used *Azotobacter* or *Azospirillum* inoculation.They concluded that using 40 kg N/ha + , Azotobacter were the most economic fertilizer application for obtaining the highest wheat grain yield. These results are in a great harmony with those obtained by Omar *et al.* (1991), Mitkees *et al.* (1996) who reported that biofertilization with mixture of N_2 fixing bacteria (*Azospirillum, Bacillus* and *Azotobacter*) added to the soil with different considerable parts of mineral nitrogen fertilizer by about 2/3 and 1/3 of the recommended nitrogen in old and new lands, respectively was the best treatment. El-Wakil *et al.* (2004) demonstrated increasing nitrogen fertilizer levels from 60 kg N/fed. up to 90 kg N/fed. significantly increased plant height, spike length, number of spikes/plant, number of grains/spike, number of spikes/m^2, 1000-grain weight and grain and straw yield/fed.

The aim of this work is to investigate applying biofertilizers on yield and its components of wheat in clay soils under surface irrigation system comparing with applying nitrogen fertilizer only without application of biofertilizers.

MATERIALS AND METHODS

Two field trails were carried out at Sakha Agricultural Research Station farm during the two successive growing seasons of 2006-2007 and 2007-2008 to study the effect of fertilization with different nitrogen levels of 0, 25, 50, 75 and 100 kg N/fed. and applying *Azospirillum* as biofertilizer on wheat (variety Sakha 94). Yield, components, nitrogen percentage and protein content in grains of wheat plants.

Surface soil samples were taken before conducting experiment to make some physical and chemical analysis:

Table (1): Chemical analysis of soil.

Season	Total N	EC dSm^{-1}	pH	O.M	Soluble anions meq/L				Soluble cation meq/L				Mechanical analysis			Stru-cture
					CO^{--}_3	HCO^{--}_3	Cl^-	SO^{--}_4	Ca^{++}	Mg^{++}	Na^+	K^+	Silt	Clay	Sandy	
1st	0.11%	2.1	7.5	1.3	0.0	2.6	5.6	11.00	.5	1.2	12.5	0.4	25.3	56.1	20.3	Clay
2nd	0.13%	3.2	7.8	1.8	0.0	3.1	5.0	26.3	6.4	4.3	24.3	0.5	26.6	53.7	21.3	Clay

Split plot design with three replicates was used in this current study. The main plots were randomly assigned by five nitrogen fertilization levels. The subplots were assigned by applying biofertilizers *Azospirillum* application with nitrogen values were randomly distributed as follows:

1.A. N-Fertilization treatments:

1. Without nitrogen fertilization (check).
2. 25 kg N/fed.
3. 50 kg N/fed.
4. 75 kg N/fed.
5. 100 kg N/fed.

2.B. Biofertilizer treatments:

1. Without biological fertilizer.
2. By using biological fertilizer.

The inoculation was performed by coating wheat grains with each of inoculum individually at the rate of 200 g biofertilizer was mixed with 75 kg grain, sticking substance (Arabic gum 5%) was used to sticking the inoculant materials with grains just before sowing.

Wheat (variety Sakha 94) was sown 7th, December 2006 and 2007 growing seasons. Plot area was (3.5 x 3 m), which contained 15 rows 3.5 m in length and 20 cm apart. Nitrogen fertilizers used as form of urea (46.5% N) was applied on two doses with the first and second irrigation. Phosphorus fertilizer was add for all treatment as recommended before sowing (farming treatments) as calcium super phosphate (15.5% P_2O_5). Also potassium was added as recommended which was added in two equal does, the first does was applied during preparing the soil and the second one was applied after one month from sowing. At harvest time, ten guarded plants were taken at random from the middle of each plot to estimate data of yield components. The grain yields and straw were calculated for each plot, the following data were recorded as plant height, number of tillers/plant, number of spike/plant, spike length, number of grains/spike, 1000-grain weight, grain yield (ton/fed.) and straw yield (ton/fed.). Wheat grain samples were taken, oven dried and fine ground and digested using sulphuric-perchloric acids method according to Jackson (1967). Nitrogen was determined by Kjldahel method according to Black *et al.* (1965). Protein was calculated by maltybling N% by 5.7. Data obtained statistically analysis using Snedecor and Cocchran (1989).

RESULTS AND DISCUSSION

Effect of fertilization with mineral and biofertilizers on:

1. Grain yield ton/fed.:

Presented data in Table (2a & b and 3) and Figs. (1, 2) illustrated that the mean values of wheat grain yield were highly significant affected by application of both mineral and biofertilizers. Application of biofertilizers had a great effect on wheat gain yield, where the highest mean values were recorded under biofertilization in comparison with non-application treatments. The mean values under application were 2.13 and 2.20 ton/fed. But under non-application treatments the mean values were less than above mentioned values and they were 1.32 and 1.31 ton/fed. in the first and second seasons, respectively.

Table (2a): Effect of inoculation with bio-fertilizer on grain yield in both seasons.

N level	Biofertilizer	Grain yield (ton/fed.)		Increased by inoculation (%)	
		1st	2nd	1st	2nd
Zero	Without	1.22	1.21	9.01	8.26
	With	1.33	1.31		
25	Without	1.37	1.43	8.75	6.99
	With	1.49	1.53		
50	Without	1.59	1.61	6.28	6.21
	With	1.69	1.71		
75	Without	1.84	1.85	5.90	5.9
	With	1.93	1.96		
100	Without	2.00	2.4	5.00	4.16
	With	2.1	2.50		

Table (2b): Effect of mineral and bio-fertilizers on grain yield and straw yield of wheat (ton fed^{-1}) in both seasons.

Treatments	Grain yield		Straw yield	
	1st	2nd	1st	2nd
A. N-levels kg fed^{-1}				
Control	1.22 d	1.21 d	1.32 e	1.28 e
25	1.51 c	1.51 c	1.78 d	1.81 d
50	1.67 b	1.70 b	2.20 c	2.06 c
75	1.91 b	1.95 b	2.52 b	2.66 b
100	2.26 a	2.6 a	3.03 a	3.12 a
F-test	**	**	**	**
L.S.D. 5%	0.045	0.039	0.022	0.042
1%	0.063	0.055	0.032	0.059
B. Biofertilizer				
Without	1.32	1.31	1.24	1.22
With	2.13	2.20	2.99	3.00
F-test	**	**	**	**

Table (3): Effect of mineral fertilizer on grain yield in both seasons.

N level	Biofertilizer	Grain yield (ton/fed.)		Increased by inoculation (%)	
		1st	2nd	1st	2nd
Zero	Without	1.32	1.31		
	With	1.22	1.21	8.19	8.26
25	Without	1.22	1.21	23.7	24.79
	With	1.51	1.51		
50	Without	1.22	1.21		
	With	1.67	1.70	36.9	40.16
75	Without	1.22	1.21		
	With	1.91	1.95	56.6	61.2
100	Without	1.22	1.21		
	With	2.26	2.6	85.2	114.8

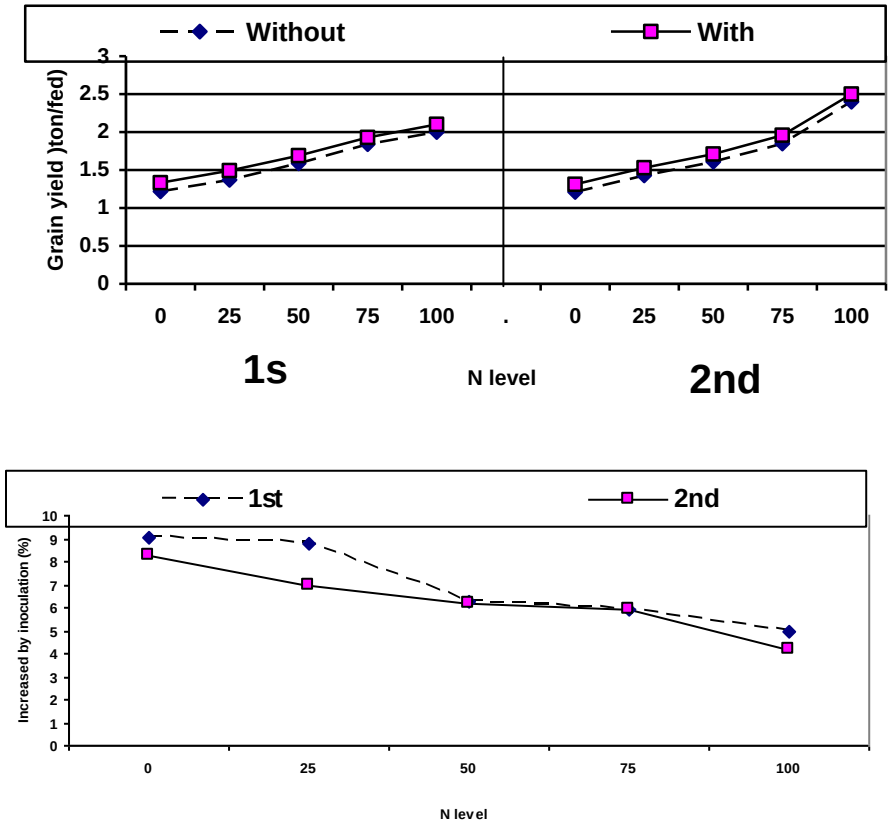

Fig. (1): Effect of inoculation with bio-fertilizer on grain yield in both seasons

Also, data in the same Table showed that by increasing application of nitrogen fertilization increased wheat grain yield in the two seasons, where the highest mean values were recorded under the highest fertilization dose of 100 kg N/fed. in comparison with the other doses and the mean values are 2.26 and 2.6 ton/fed. in the first and second seasons, respectively. These results are in a great harmony with those obtained by El-Aggory *et al.* (2001), Saleh (2003) and El-Wakil *et al.* (2004), who concluded that increasing nitrogen dose from 60 to 90 kg N/fed. increased wheat grain yield/fed.

2.Straw yield (ton/fed.):

Results in Table (4 and 5) and Figs. (1, 2) clearly illustrated that applying of I and biofertilizers and mineral fertilizers had a high significant effect on wheat straw yield in the two seasons. Under non-application treatments the mean values of straw yield were 1.24 and 1.22 ton/fed. but under application treatments the mean values were 2.99 and 3.00 ton/fed. in the first and second seasons, respectively **.**

Also, data in the same Table showed that by increasing nitrogen application increased the values of wheat straw yield were increased. The highest mean values were recorded under the highest nitrogen dose (100 kg N/fed.) in the two seasons but increasing rate under adding biofertilizers was more than applying nitrogen fertilizers only. The highest mean values under nitrogen application only are 3.03 and 3.12 ton/fed. in the first and second seasons, respectively. These results are in agreement with those obtained by El-Wakil *et al.* (2004).

Table (4): Effect of inoculation with bio-fertilizer on straw yield in both seasons.

N level	Biofertilizer	Straw yield (ton/fed.)		Increased by inoculation (%)	
		1st	2nd	1st	2nd
Zero	Without	1.23	1.23	7.3	5.6
	With	1.32	1.3		
25	Without	1.65	1.71	6.6	5.2
	With	1.76	1.8		
50	Without	1.99	1.99	5.51	5.51
	With	2.10	2.1		
75	Without	2.46	2.59	2.43	2.31
	With	2.52	2.65		
100	Without	3.00	3.07		
	With	3.03	3.12	1.00	1.6

Table (5): Effect of mineral fertilizer on straw yield in both seasons.

N level	Biofertilizer	Straw yield (ton/fed.)		Increased by inoculation (%)	
		1st	2nd	1st	2nd
Zero	Without	1.24	1.22		
	With	1.32	1.28	6.45	4.91
25	Without	1.24	1.28	34.8	41.40
	With	1.78	1.81		
50	Without	1.24	1.28	53.03	60.93
	With	2.02	2.06		
75	Without	1.24	1.28	90.90	107.8
	With	2.52	2.66		
100	Without	1.24	1.28	129.5	146.0
	With	3.03	3.12		

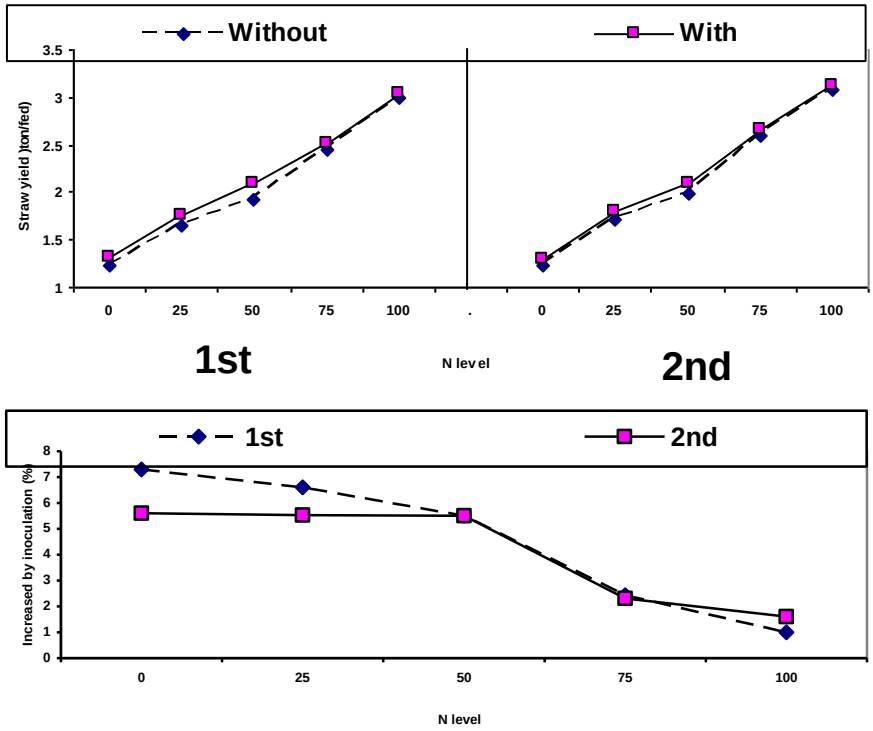

Fig. (2): Effect of mineral fertilizer on grain yield in both seasons.

Nitrogen and protein percentage:

Data presented in Table (6) showed that the mean values of N% was high significantly affected by applying biofertilizers and mineral fertilizers. Under application of biofertilizers the mean values were higher (1.88 and 1.99%) than those obtained under non-application (1.87 and 1.88%) treatments in the two seasons. Also, by increasing nitrogen rate the mean values of N% was increased up to 100 kg N/fed. application in both growing seasons i.e., 1.66, 1.76, 1.88, 1.9 and 2.0 in the first season and 1.64, 1.79, 1.85, 1.95 and 2.0 the second season due to 0, 25, 50, 75 and 100 kg N/fed., respectively. These results are in a great harmony with those obtained by El-Wakil *et al.* (2004)

1000 grain weight, number of tillers/plant and number of spikes/plant:

Data presented in Table (7)showed that the mean values of the three studied parameters were clearly affected by application of mineral and biofertilizer in both seasons. The highest mean values were recorded under application of biofertilizers comparing with non application ones. Also, by increasing nitrogen dose up to 100 kg N/fed. increased the mean values, they were 42.44 and 42.31 gram in the first and second seasons respectively in 1000 grain weight. No. of spikes/plants increased also the mean values were 3.24 and 3.3 in the first and second seasons.

Table (6): Effect of mineral and bio-fertilizers on nitrogen (%) and protein % of wheat in both seasons.

Treatments	N (%)		Protein (%)	
	1st	2nd	1st	2nd
A. N-levels				
Control	1.66 e	1.64 e	9.48 e	9.36 e
25	1.76 d	1.79 d	10.05 d	10.16 d
50	1.88 c	1.85 c	10.75 c	10.56 c
75	1.90 b	1.95 b	10.83 b	11.15 b
100	2.00 a	2.00 a	11.42 a	11.39 a
F-test	**	**	**	**
L.S.D. 5%	0.029	0.023	0.165	0.14
1%	0.040	0.032	0.229	0.19
B. Biofertilizer				
Without	1.87	1.88	10.66	10.74
With	1.88	1.99	10.73	11.34
F-test	**	**	**	**

Table (7): Effect of mineral and bio-fertilizers on 1000 grain weight (g) and No. of spikes /plant and No. of tillers/plants of wheat in both seasons.

Treatments	1000 grain weight		No. of spikes/ plant		No of tillers/plant	
	1st	2nd	1st	2nd	1st	2nd
A. N-levels						
Control	15.03 e	14.93 e	1.08 d	1.01 e	1.12 d	1.03 d
25	24.03 d	25.42 d	1.13 d	1.14 d	1.20 d	1.18 d
50	36.06 c	33.02 c	2.07 c	2.01 c	2.03 c	2.01 c
75	42.08 b	42.02 b	3.09 b	3.11 b	3.14 b	3.23 b
100	42.44 a	42.31 a	3.24 a	3.3 a	3.14 a	3.72 a
F-test	**	**	**	**	**	**
L.S.D. 5%	0.043	0.029	0.089	0.046	0.114	0.247
1%	0.059	0.040	0.123	0.064	0.158	0.343
B. Biofertilizer						
Without	19.07	18.91	2.07	1.98	2.03	2.02
With	35.01	35.91	2.21	2.31	2.43	2.53
F-test	**	**	**	**	**	**

Wheat plant height (cm):

In both seasons the mean values of wheat plant height were clearly affected by application of mineral and biofertilizers. The mean values under application of biofertilizers were 96.36 and 97.23 cm, but under non-application the mean values were less and they were 62.12 and 61.04 cm in the first and second seasons, respectively (Table 8). Data in the same Table clearly showed that by increasing nitrogen dose up to 100 kg N/fed. increased the mean values, they were 95.58 and 97.46 cm in the first and second growing seasons, respectively. Similar results were obtained by Hussein *et al.* (1993).

Table (8): Effect of mineral and bio-fertilization on plant height (cm) of wheat in both seasons.

Treatments	Plant height	
	1st	2nd
A. N-levels		
Control	63.82 b	54.7 d
25	55.2 b	56.4 d
50	59.62 b	60.36 c
75	84.40 a	88.51 b
100	95.58 a	97.0 a
F-test	**	**
L.S.D. 5%	11.47	8.8
1%	15.93	11.97
B. Biofertilizer		
Without	62.12	61.04
With	96.36	97.23
F-test	**	**

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دور البكتيريا المثبتة للازوت لاتكافليا فى ترشيد استخدام الاسمدة الازوتية المعدنية فى انتاج القمح

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نفذت تجربتان حقليتان فى مزرعة محطة البحوث الزراعية بسخا خلال موسمى النمو 2006-2007م ، 2007-2008م بمنطقة شمال ووسط الدلتا لدراسة تأثير التسميد النيتروجينى وإضافة الايزوسبريليم على المحصول ومكوناته والنسبة المئوية للنيتروجين والبروتين لمحصول القمح بمنطقة شمال وسط الدلتا.

حيث استخدمت خمسة مستويات من التسميد النتروجينى هى صفر ، 25 ، 50 ، 75 ، 100 كجم للفدان. واستخدمت معاملاتان للتسميد الحيوى هما (1) اضافة سلالة نشطة من البكتريا المثبة للازوت لاتكافليا (2) بدون اضافة.

واستخدم تصميم القطع المنشقة لتنفيذ التجربة حيث شغلت معاملات مستويات النتروجين القطع الرئيسية وشغلت معاملات التسميد الحيوى للقطع الشقية.

النتائج التى تم التوصل إليها يمكن تلخيصها كما يلى:

- تحت المعاملات التى لم يحدث بها إضافة الايزوسبريليم بزيادة التسميد النيتروجينى حتى 100كجم نيتروجين /فدان سجلت أعلى القيم لمحصول الحبوب وكانت القيم 2.26 ، 2.60 طن/للفدان فى الموسم الأول والثانى على الترتيب أوضحت النتائج أن أقل القيم سجلت تحت المعاملة بدون إضافة (كنترول) فى كلا موسمى الدراسة وكانت النتائج 1.22 ، 1.21 طن/فدان فى الموسم الأول والثانى على الترتيب.
- بإضافة الايزوسبريليم متوسط القيم بالنسبه لمحصول الحبوب زادت بالمقارنة بإضافة النيتروجين فقط حيث كانت القيم 2.20 ، 2.16 طن/فدان بالمقارنة بعدم الإضافة حيث كانت القيم 1.31 ، 1.32 فى الموسم الأول والثانى على الترتيب.
- أوضحت النتائج أن متوسط القيم بالنسبه لمحصول القش زادت بزيادة جرعة السماد النيتروجينى حتى 100 كيلو جرام نيتروجين/فدان وكانت القيم 3.03 ، 3.12 طن/فدان فى الموسم الأول والثانى على الترتيب على العكس من ذلك سجلت أقل القيم تحت المعاملات بدون إضافة وكانت القيم 1.32 ، 1.28 طن/فدان على الترتيب.
- أوضحت النتائج كذلك أن النسبة المئوية للبروتين والنيتروجين زادت بزيادة التسميد النيتروجينى والحيوى فى كلا موسمى الدراسة.
- متوسط القيم لطول نبات القمح زادت بزيادة التسميد الحيوى والنيتروجين حيث كانت القيم تحت إضافة التسميد الحيوى 96.36 ، 97.23سم وفى حالة بدون إضافة 62.12 ، 61.04سم فى الموسم الأول والثانى على الترتيب أيضا إضافة السماد النيتروجينى حتى 100 كيلو جرام نيتروجين/فدان أدى إلى زيادة طول النباتات حيث سجلت أعلى القيم.
- قيم الوزن للـ 1000 حبه ، عدد الفروع وكذلك السنابل زادت بزيادة التسميد النيتروجينى حتى 100كجم/فدان وكذلك إضافة السماد الحيوى عمل على زيادة الصفات المدروسة مقارنة بعدم الإضافة.