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RESPONSE OF WHEAT TO BIOFERTILIZER INOCULATION UNDER DIFFERENT LEVELS OF INORGANIC NITROGEN

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ABSTRACT

Two field experiments were conducted at El-Serw Agricultural Research Station, Damietta Governorate during the two seasons 2001/2002 and 2002/2003 to investigate the influence of mineral nitrogen fertilizer levels and biofertilizers, Serialin and/or Phosphorien, and accordingly their interactions on wheat growth, yield and its attributes as well as N and p uptake of wheat grain and straw and also grain protein content. A split plot design with four replicates was used. The obtained results could be summarized as follows:

- Raising meniral nitrogen fertilizer level from 45 to 60 and 75 kg N/fed. resulted in significant increases in plant height, number of tillers/plant, spike length, 1000-grain weight, grain and straw yields/fed. and protein content of grain. Also N and P uptake of grain and straw were significantly increased in both seasons.
- Inoculation of wheat grain with biofertilizers, Serialin and/or Phosphorein, resulted in marked increase in plant height, spike length, 1000 grain weight, grain and straw yields/fed., protein content of grain, N and p uptake of both grain and straw, compared with that uninoculated treatment in both seasons.
- Dual inoculation with Serialin and Phosphorein gave the highest values, while the lowest values were recorded with the uninoculated treatment.
- The interaction between mineral nitrogen levels and biofertilization had a significant effect on grain and straw yields/fed. In both seasons, N uptake of grain and straw in the second season. Also, P uptake of grain and straw in the second season.
- Although, the maximum wheat yield and its components were recorded with the dual inoculation with Serialin and Phosphorein at the highest N rate (75 kg N/fed.), rate of 60 kg N/fed. with the dual inoculation was most compatible, since it has the highest wheat yield over uninoculation control.

Finally, it could be concluded that inoculation wheat grains with dual inoculants Serialin and Phosphorein with using 80% of recommended dose (i.e. 60 kg N/fed.) is the recommended treatment for maximizing wheat yield and net return as well as reducing mineral fertilization and soil pollution.

INTRODUCTION

Wheat (*Triticum aestivum L*.) is one of the most important grain crops in Egypt. Any effort to increase wheat yield to face the increasing gab between wheat production and consumption, is highly appreciated. This could be achieved by applying recommended cultural practices such as using bio and chemical fertilizers.

Nitrogen is considered as one of the limiting factors to achieve the yield of wheat crop. Raising N fertilizer up to 75 kg N/fed. increased plant height, number of tillers, spike length, 1000 grains weight, grain and straw yields as

well as grain protein content (Morsy, 1993, Sultan et al., 1994 and Hamed, 1998).

Microbial inoculation of cereal crops by certain free-living-N_2-fixing bacteria and bacteria solubilizing phosphorus had a great important as a new technology, as it minimizes the amount of applied chemical fertilizer and reduce the costs of crop production as well as reducing soil pollution. Several free-living bacteria species can fix atmospheric nitrogen such as Azotobacter and Asospirillum which are prepared in commercial packets as biofertilization such as Serialin and Phosphorein which contain bacteria solubilizing soil phosphorus. Ahmed (1995) stated that Azotobacters enhanced wheat plant height, tillering, yield components, grain and straw yields. The same trend was observed by Sharief et al.,(1998), Rabie et al., (1995) and Kotb, (1998). Metwally (2000) reported that using biofertilizers Serealin and Phosphorein significantly increased grain and straw yields and NPK uptaked by wheat plants.

Thus, the aim of this study was to investigate the response of wheat crop to seed inoculation with Serialin and/or Phosphorein in presence of different levels of N fertilizer and accordingly their effects on wheat yield and its component as well as N and P uptake of wheat grains and straw, also, the protein content of grains.

MATERIAL AND METHODS

Two field experiments were performed at El-Serw Agricultural research station, Damietta Governorate during the two successive seasons 2001/2002 and 2002/2003 using wheat crop (*Triticum aestivum, L*.) variety Sakha 93.

The investigation was aimed to study:

1- The influence of microbial inoculation , using free-living N-fixing bacteria and/or bacteria solubilizing phosphorus, on growth characteristics, yield and yield components as well as nitrogen and phosphorus uptake of grain and straw.
2- The optimum level of mineral nitrogen fertilizer to obtain the maximum yield and the highest quality of wheat under the influence of microbial inoculation.

Soil samples were taken from the surface layer (0-30 cm depth) and analyzed for physical-chemical properties (Table1) as described by Page (1982). The experimental design was split plot with four replicates, the plot area was 10.5 m^2, where the preceding crop was cotton in the two seasons. The main plots were assigned to nitrogen fertilizer levels (45, 60 and 75 kg n/fed.). While, Biofertilizer treatments were randomly distributed in the sub plots as follows:

I_{0-} Control treatment (uninoculated)
I_{1-} Inoculation with biofertilizer Serialin (this product contains efficient strains of free living nitrogen fixing bacteria)

I_{2-} Inoculation with biofertilizer Phosphorein (this product contains efficient strains of bacteria solubilizing phosphorus)

I_{3-} Combined inoculation with both Serialin and Phosphorein.

Phosphorus fertilizer was applied to all experimental plots in one dose pre-planting wheat in the form of mono calcium superphosphate (15% P_2O_5) at the rate of 15 kg P_2O_5/fed.. Seed inoculation was carried out using Serialin and /or Phosphorein kindly obtained from General Organization Equalization fund, Ministry of Agriculture, Egypt. Inoculation was performed by coating the calculated weight of wheat seeds with the certain biofertilizer, then the coated seeds were air dried and sown immediately. Nitrogen fertilizer in the form of ammonium nitrate (33% N) was applied in two equal doses, one dose being applied before the first irrigation, while the remaining dose was applied before the second irrigation. The other agronomic practices of wheat cultivation were done as recommended. At harvest, plant height (cm), number of productive tillers/plant, spike length (cm), 1000-grains weight (g), grain and straw yields (tons/fed.), were recorded. Dry samples of grain and straw from each plot were ground and wet digested with H2SO4-HClO4 mixture as described by Jackson (1973). N% and P% were determined according to Black, Part- 2 (1982). Also, N and P uptake of grain and straw were calculated. Crude protein content of grain was estimated according to A.O.A.C. method (1980). The obtained data were statistically analyzed according to Snedecor and Cochran (1989). Finally the economic advantage of biofertilizers was done.

Table (1): Some physical and chemical properties of the experimental soil

Soil properties	1st season	2nd season
Soil texture	Clayey	Clayey
Soil PH (1:2.5 water susp.)	8.1	8.0
EC (soil paste at 25 c0), dS/m	4.3	3.8
O.M%	1.1	1.2
$CaCO_3$ %	1.6	1.3
Available-N ppm	29	33
Available-P ppm	10.2	12.1

RESULTS AND DISCUSSION

Wheat yield and its components:

A- effect of Nitrogen fertilizer levels:

Data presented in Tables (2 and 3) revealed significant increases in plant height, number of productive tillers per plant, spike length, 1000-grains weight, grain and straw yields with increasing nitrogen fertilizer rate up to 75 kg N/fed. in both seasons, when compared with addition of 45 or 60 kg N/fed. These increases mean that nitrogen is one of the most important components of cytoplasm nucleic acids and chlorophyll, so nitrogen has an important role in encouraging cell elongation, cell division and consequently increasing

vegetative growth and activation of photosynthesis process which enhance the amount of metabolites necessary for building plant organs which reflect increases in grain and straw yields. These results are in accordance with those obtained by Attallah and EL-Karamity, (1997); Ibrahim (1998); El-Moursy (1998); El-Naggar (1999) and Metwally (2000). The same data showed that protein content of wheat grains was increased significantly with increasing nitrogen fertilizer rate. This may be due to increasing N availability at critical stages of spike initiation and the development of plant metabolism which resulted in increasing synthesis of amino acids and finally increasing protein content of wheat grains. The same trend was reported by Darwiche (1994); Kotb (1998) and Metwally (2000).

B- Effect of microbial inoculation:

Data illustrated in Tables (2 and 3) clearly indicated that plant height, number of tillers, spike length, 1000 grain weight, grain and straw yields were significantly affected by biofertilization treatments in both seasons. Results showed that seed inoculation with serialin significantly increased all values of wheat yield and its attributes, The increase of grain yield was surpassed the control treatment (without inoculation) by 9.9 and 7.8% in the two seasons respectively, while the increase of straw yield was surpassed the control treatment by 5.8 and 5.5% in the two seasons, respectively.

With regard to the effect of inoculation with phosphorein, it was found that wheat yield and its attributes were also increased due to phosphorein biofertilizer application. The grain yield was higher than control by 7.6 and 3% in the two seasons, respectively, while the straw yield was increased than the control treatment by 5 and 2.7% in the two seasons, respectively.

Concerning the effect of combined inoculation with Serialin and Phosphorein, the same data revealed that the maximum grain and straw yields were obtained at combined inoculation with the two inoculants. The increment percentages of grain yield were 15 and 14.1% over control in the two seasons, respectively, while they were 9.5 and 9.4% over control with the respect of straw yield.

Also, data indicated that protein content of grains increased significantly with using Serialin and/or Phosphorein inoculants in both seasons. The highest values of protein content were obtained with using Serialin and Phosphorein in combination, while control treatment (uninoculation) gave the lowest values. These results could be attributed to: 1- The availability of more N fixation by free living bacteria which present in biofertilizer (serialin).2-The availability of more phosphorus due to bacteria solubilizing phosphorus which present in biofertilizer (Phosphorein). 3- The production of growth regulators substances such as indol acetic acid, gibberellins, pyrodoxine and others which stimulate plant growth and subsequently affect wheat yield and its attributes. The results are in agreement with those obtained by Ahmed (1995), Omar et al. (1996), Kotb (1998), Shareif et al. (1998) and Metwaly (2000).

Table (2): Some yield components as affected by Inoculation and nitrogen fertilizer levels in the two seasons

Treatments	Plant height, (cm)		No. of tillers Per plant		Spike length, (cm)		1000 grain weight, g	
A-N rate (kg N/fed.)	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
45	100.0	102.4	6.5	7.5	13.12	13.13	51.7	52.4
60	104.5	106.7	7.58	7.67	14.65	14.57	54.3	55.4
75	108.3	111.3	8.33	8.67	15.24	15.02	56.5	57.6
LSD at 5%	2.38	2.14	0.85	0.83	0.35	0.33	0.42	0.43
B-inoc.								
1-Control	100.7	103.5	6.78	7.33	13.83	13.77	52.9	53.6
2-Serialin	104.7	107.7	7.67	7.89	14.42	14.06	54.7	55.9
3-phosph.	103.0	105.1	7.11	7.44	14.23	14.30	53.7	54.4
4-2+3	108.7	110.1	8.33	8.44	14.86	14.82	55.7	56.6
LSD at 5%	2.06	2.96	0.94	--	0.31	0.28	0.38	0.29
A * B	--	--	--	--	--	--	--	--

Table (3): Grain and straw yields and protein content of grain as affected by inoculation and nitrogen fertilizer rates in the two seasons

Treatment	Grain yield, ardab/fed.		Straw yield, ton/fed.		Protein content of wheat grain%	
A-N rate (kg N/fed.)	1st season	2nd season	1st season	2nd season	1st season	2nd season
45	14.09	14.35	3.71	3.82	10.26	10.49
60	17.07	17.36	4.29	4.33	10.61	10.69
75	19.56	19.61	4.59	4.68	11.05	11.00
LSD at 5%	0.030	0.034	0.035	0.029	0.20	0.15
B-inoc.						
1-Control	15.62	16.07	3.99	4.03	9.92	10.16
2-Serialin	17.18	17.33	4.22	4.25	10.85	11.05
3-phosph.	16.81	16.54	4.19	4.14	10.37	10.39
4-2+3	18.01	18.48	4.37	4.41	11.42	11.34
LSD at 5%	0.024	0.033	0.021	0.020	0.08	0.16
A * B	* *	*	*	* *	--	--

*** significant at 5%, ** significant at 1%**

Nitrogen and Phosphorus uptake of wheat plant:

A-Nitrogen fertilizer level effect:

The results illustrated in Table (4) showed that increasing the rate of applied nitrogen fertilizer gradually increased the amount of nitrogen and phosphorus uptaked by both wheat grains and straw at maturity stage. The highest amounts of N and P uptaked were recorded by application of 75 kg N/fed. This might be attributed to the role of nitrogen nutrient in increasing the root surface per unit of soil volume and also the high capacity of the plant

supplied with N in building metabolites which increases the dry matter content and subsequently increases nutrients uptake by wheat plants.

B- Microbial inoculation effect:
Concerning the biofertilizer influence on nutrients uptaked by grains and straw, the same data clearly indicated that seed inoculation with Serialin and /or Phosphorin significantly increased all values of nitrogen and phosphorus uptaked by wheat grains and straw. Dual inoculation with Serialin and Phosphorein gave the highest values in the two seasons, while control treatment (uninoculated) gave the lowest values. This positive effect of inoculation upon nutrients uptake could be attributed to high efficiency of bacteria presence in the biofertilizer Serialin to fix atmospheric N and also to the more amount of soluble phosphorus released by bacteria present in Phosphorein, as well as to produce some biological active substances, which help in increasing the root biomass and indirectly help in increasing nutrients uptake by wheat plants. These results are in agreement with those obtained by Kotb (1998), Abd El-Salam and Sarhan (1999) and Metwally (2000).

The interaction effect:

Concerning the interactions among the inoculation treatments and nitrogen rates, data in Tables (5 and 6) demonstrated that adding 75 kg N/fed. under the treatment of inoculation with Serialin and Phosphorein, recorded the highest values of grain and straw yields in the two seasons, N uptake of grain and straw and P uptake of grain and straw in the second season. In addition, it could be noticed that, treated plants with nitrogen fertilizer at the rate of 60 kg N/fed. and dual inoculation with Serialin and Phosphorein gave results generally similar or more than those of the treatment of application of 75 kg N/fed without inoculation. Therefore, it seems from this treatment that saving 15 kg N/fed by using biofertilizer technique could be minimizes the production costs and pollution factors.

Table (4): Nitrogen and Phosphorus uptake of grain and straw as affected by inoculation and nitrogen fertilizer rates in the two seasons

Treatments	N uptake of grain, kg/fed.		N uptake of straw, kg/fed.		P uptake of grain, kg/fed.		P uptake of straw, kg/fed	
A-N rate (kg N/fed.)	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
45	35.95	37.22	9.62	9.91	6.69	7.00	9.83	9.75
60	45.02	46.26	12.24	12.35	8.64	8.75	12.17	12.29
75	53.68	53.59	14.59	14.78	10.25	10.19	13.55	13.68
LSD at 5%	0.94	0.71	0.56	0.25	0.23	0.13	0.21	0.40
B-inoc.								
1-Control	38.60	40.54	11.22	11.53	7.46	7.74	10.68	10.41
2-Serialin	46.36	47.62	12.16	12.30	8.58	8.42	11.80	11.50
3-phosph.	43.40	42.67	11.76	11.72	8.65	8.56	12.60	11.98
4-2+3	51.17	51.84	13.52	13.78	9.41	9.62	12.96	13.35
LSD at 5%	0.39	0.60	0.54	0.28	0.75	0.21	0.28	0.40
A * B	--	* *	--	*	--	*	--	*

*** significant at 5%, ** significant at 1%**

Table (5): grain and straw yields as affected by the interaction between inoculation and N fertilizer rates in the two seasons

Treatments	1st season			2nd season		
Rate of N fertilizer	**45**	**60**	**75**	**45**	**60**	**75**
Grain yield, ardab/fed						
Inoculation	12.93	15.74	18.19	13.42	16.16	18.64
1-Control (unin.)	14.15	17.42	19.97	14.35	17.82	19.81
2-Serialin	13.95	16.88	19.61	13.81	16.83	19.98
3-Phosphorein	15.34	18.63	19.45	15.81	19.61	20.40
4-2 + 3						
LSD at 5%	0.033			0.036		
Straw yield, ton/fed.						
1-control (unin.)	3.58	4.02	4.38	3.61	4.06	4.42
2-Serialin	3.76	4.28	4.61	3.75	4.33	4.65
3-Phosphorein	3.68	4.31	4.58	3.63	4.28	4.49
4-2 + 3	3.82	4.52	4.58	3.86	4.53	4.82
LSD at 5%	0.030			0.036		

Economic advantage of biofertilization use:

It is important to evaluate the economics of using biofertilization practices (Serealin and Phosphorein) on wheat crop and this is done in Table (7).

The cumulative income of the two inoculants are estimated according to the average of the results of the two seasons.

Generally, using both Serialin and Phosphorein individually resulted in net profit under using the three levels of nitrogen fertilkizers than that using nitrogen fertilizer alone. However, the highest net return (LE 638) was recorded by using the two inoculants together with 60 kg N/fed.

Therefore, the low cost of biofertilization practices will obtain increased income, conserve the environment and in the same time reducing soil pollution hazards.

Table (6): N and P uptake of grain and straw as affected by the interaction between inoculation and N fertilizer rates in the second season

Treatments	Wheat grain			Wheat straw		
Rate of N fertilizer	**45**	**60**	**75**	**45**	**60**	**75**
N uptake, kg N/fed.						
Inoculation	33.3	40.8	47.7	9.4	11.8	13.7
1-Control (unin.)	37.9	49.9	55.6	10.1	12.1	15.4
2-Serialin	35.1	43.3	49.7	9.5	11.9	13.9
3-Phosphorein	42.8	51.8	61.3	11.2	14.1	16.9
4-2 + 3						
LSD at 5%	**0.64**			**0.27**		
P uptake, kg P/fed.						
1- Control	6.5	7.8	9.3	8.7	10.6	11.9
2- Serialin	6.9	8.8	10.4	9.8	11.7	13.0

3- Phosphorein	6.6	8.6	10.0	9.8	12.4	13.5
4- 2 + 4	7.8	9.5	11.4	10.8	13.9	15.4
LSD at 5%	**0.20**			**0.25**		

Table (7): Economics of biofertilization use

Treatments		*Wheat grain yield (ardab/fed)	Yield increase (ardab/fed)	Value of grain yield increase (L E)	*Wheat straw yield (ton/fed)	Straw Yield increase (ton/fed)	Value of straw yield increase (L E)	Total value of Yield increase (L E)
Ino.	N fert.							
Control	45	13.18	----	----	3.60	---	---	---
	60	15.95	----	----	4.04	---	---	---
	75	18.42	----	----	4.40	---	---	---
Ser.	45	14.25	1.07	176.55	3.75	0.15	36.0	212.0
	60	17.62	1.67	275.55	4.30	0.26	62.4	337.0
	75	19.89	1.47	242.55	4.63	0.23	55.2	298.0
Phos.	45	13.88	0.70	115.5	3.65	0.05	12.0	128.0
	60	16.85	0.90	148.50	4.30	0.26	62.4	210.0
	75	19.65	1.23	202.95	4.54	0.14	33.6	237.0
Ser.	45	15.52	2.40	396.0	3.84	0.24	57.6	453.0
+phos	60	19.12	3.17	523.1	4.52	0.48	115.2	638.0
	75	19.93	1.51	249.2	4.60	0.20	48.0	297.0

***Each value in this table is an average of the results of the two seasons**
Prices: wheat grain= L.E165/ardab, wheat straw= L.E240/ton

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استجابة القمح للتسميد الحيوي تحت مستويات مختلفة من التسميد النتروجيني المعدني

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أقيمت تجربتان حقليتان في محطة بحوث السرو الزراعية – محافظة دمياط , خلال الموسمين المتتاليين ٢٠٠١/٢٠٠٢ ، ٢٠٠٢/٢٠٠٣ لدراسة تأثير مستويات التسميد النتروجيني وكذلك التسميد الحيوي بالسريالين والفوسفورين وأيضا التفاعل بينهما على نمو وانتاج محصول القمح ومكوناته وأيضا الكمية الممتصة من النتروجين والفوسفور بواسطة الحبوب والقش وكذلك محتوى الحبوب من البروتين. استخدم التصميم الاحصائي القطع المنشقة في أربع مكررات.
كانت النتائج المتحصل عليها كالأتي:

١- أدت زيادة التسميد الأزوتي من ٤٥ الى ٦٠ و ٧٥ كجم/ف الى زيادة معنوية في أطوال النباتات – عدد الأشطاء/النبات – طول السنبلة – وزن الالف حبة – محصول الحبوب والقش/ فدان – نسبة البروتين في الحبوب وكذلك الكمية الممتصة من
كل من النتروجين والفوسفور بواسطة الحبوب والقش في كلا الموسمين.
٢- ادى استخدام كلا من السريالين والفوسفورين الى زيادة معنوية في المحصول ومكوناتة مقارنة بمعاملة الكنترول (بدون تلقيح).
٣- أيضا أدى التلقيح بالسريالين والفوسفورين معا الى الحصول على اعلى القيم بينما أعطت معاملة الكنترول (بدون تلقيح) أقل القيم في كل الصفات المدروسة.
٤- كان التفاعل بين معدلات التسميد النتروجيني ومعاملات التسميد الحيوى معنويا على المحصول من الحبوب والقش في كلا الموسمين وكذلك على الكمية الممتصة من النتروجين والفوسفور في الموسم الثاني فقط.
٥- رغم أن استخدام التسميد النتروجيني بمعدل ٧٥ كجم نتروجين/فدان مع التسميد الحيوي أعطى أعلى القيم لمحصول الحبوب والقش نجد أن استخدام ٦٠ كجم نتروجين/فدان مع التلقيح الحيوي (سريالين + فوسفورين) أحرز أعلى عائد اقتصادى مما أدى الى توفير حوالي ١٥ كجم نتروجين/فدان, لذلك يمكن التوصية باستخدام ٦٠ كجم نتروجين/فدان مع التلقيح الحيوي من كل من السريالين والفوسفورين وذلك توفيرا لاستخدام السماد المعدني وتقليلا للتلوث في النظام البيئي الزراعي.