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RESPONSE OF SOYBEAN TO RHIZOBIUM INOCULATION AND SOME MICRONUTRIENTS ADDED BY SEED COATING

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ABSTRACT

Two field trials on sobyean (*Glycin max* L.) were conducted at Sakha Agricultural Research Station in 2000 and 2001 seasons. This research was designed to study the effect of Rhizobium inoculation and adding Zn, Mn, Fe, separately or as mixture by using seed coating method on sobyean.

The most important findings could be summarized as follows:

1. Inoculation with Rhizobia and/or fertilization with micronutrients significantly increased number and weight of nodules and dry matter of plants.
2. Nitrogen content of dry matter and seeds recorded significant increases.
3. The highest seed yield 1.7 and 1.6 ton/fed. for 2000 and 2001 seasons, respectively were obtained by adding the mixture of Zn, Mn, Fe using coating seed method and rhizobium inoculation.
4. Results confirm the importance of inoculation soybean seeds since it is cheaper and more effective for ensuring adequate N for soybean than application of combined nitrogen fertilizer.

INTRODUCTION

Sobyean (*Glycin max* L.) is one of the most important leguminous crops in Egypt. Soybean seeds have the higher protein among leguminous crops. It occupies an intermediate position in oil percentage between legumes and oil seeds. So, it serves as a major and excellent source of oil and protein for human and livestock consumption.

Because of sobyean atmospheric fixing capacity, it should play a large role in crop growing systems especially since it is excellent break crop. It is only necessary for its price to be sufficiently higher for it to be on a par with the cereals.

Several workers proved that inoculation of sobyean with *Bradyrhizobium japonicum* markedly increased nodulation e.g., El-Essawi *et al.,* 1990, Salama and Ghonema, 1990, Hegazy *et al.*, 1990, Hegazy *et al.,* 1993, Abadi *et al.,* 1995 and Ghobrial *et al.,* 1995. Also, special attention should be given to the status of micronutrients to cover the requirements of sobyean plants.

Alluvial soils of the Northern Delta region in Egypt thought to be deficient to micronutrients and subsequent low productivities of most crops cultivated there.

Application of such elements have historically been made to soil and foliage. additional method recently have been developed including seed coating (Osman *et al.,* 1991).

The current research was conducted to study the single application of Zn, Fe, Mn and mixture application of them using the methods of seed coating in presence of inoculation of rhizobia on sobyean yield in slightly alkaline soil.

MATERIALS AND METHODS

Two field trails on sobyean (*Glycin max* L.) Clarck variety were carried out during the two successive seasons of 2000 and 2001 at Sakha Agricultural Station. The soil was clayey in texture, with organic matter 1.63%, total nitrogen content of 0.097%. The pH of soil-water suspension (1: 2.5) forms of N, P, Fe, Mn and Zn nutrients were found as follows:

Available-N (Amm. sulfate extract. N)	65 ppm
Available-P (Olsen extractable-P)	11.51 ppm
Available-Fe (DTPA-Fe)	13.50 ppm
Available-Mn (DTPA-Mn)	11.61 ppm
Available -Zn (DTPA-Zn)	9.17 ppm

All the soil characteristics were determined according to the standard methods mentioned by Black (1965), Chapman and Pratt (1961) and Jackson (1958).

Leghaemoglobin content in nodules was measured as described by Tu *et al.* (1970).

A split-plot design with 4 replicates was used to study the two factors, rhizobium inoculation occupied the main plots and the treatments of micronutrients application with coating seeds were arranged to the sub-plots. The plot area was 12 m^2.

Inoculation with *Bradyrhizobium japonicum* was carried out by mixing three effective strains (1577, RJG and SB6) while kindly supplied by Department of Soil Microbiology, Sakha Agric. Res. Station, Unit of Biofertilizer. Inoculation with those strains for seeds that treated with micronutrients only, but the control treatment was uninoculated seeds of soybean (coated and non coated, inoculated and non inoculated) were sown in rows 60 cm apart and hills spaced 20 cm. The recommended N-and P rates of (20 kg N/fed. as urea 46% and 30 kg P_2O_5/fed. as single superphosphate 15% P_2O_5) were added at sowing.

Seed coating treatments were done at the rate of 2 g of the micronutrients compounds per one kg seeds. Triton B material was used as spreadler starker in all treatments.

Data of nodules number and weight, dry matter of plants at 50 and 90 days, from planting, nitrogen content in seeds as well as in shoots after the two stages (50 and 90 days) and seed yield were recorded. The statistical analysis (for all data) was carried out according to Snedecor and Cochran (1971). The mean values for factors studied and significant interaction were compared using L.S.D. (0.05) test.

RESULTS AND DISCUSSION

The data presented in Table 1 (A) show that the soil of the two sites in both seasons, of experimentation had not planted with soybean before. That is because the uninoculated plots did not form any nodule.

It is also clear from the data that micronutrients significantly affected the number of nodules. Zinc and manganese gave highest values as compared to the control. Ferrous achieved higher number of nodules than Zn and Mn. While the highest number of nodules was obtained by using Zn + Mn and Fe together. This result had the same trend in both season under this study. Similar findings were confirmed with Hegazy *et al.* (1990) and Hegazy *et al.* (1993).

Table 1 (B) show that dry weight of nodules was significantly increased due to the application of micronutrients. In general data of dry weight of nodules was significantly increased and took the same trend of the number of nodules. This was true in the two periods of sampling (50 and 90 days of planting). These results also were achieved in the two successive seasons of the experimentation.

At seed in Table 2 (A) inoculation of soybean seeds significantly increased the dry weight of shoots as compared to the uninoculated one. These results were obtained in the two ages of plants during the two seasons.

Also, it is clear from the data in Table 2(A) that application of micronutrients significantly increased the dry weight of shoots. Zinc significantly increased the dry weight of shoots over the control. Mn gave higher values over that obtained by Zn and control. Fe gave higher values over the control, Zn and Mn. While the highest values of dry weight of shoots were obtained by using the mixture of the three micronutrients (Zn + Fe + Mn).

Table 2(B) presents the nitrogen content (%) in sobyean shoots at the two ages of plant. It is clear from the data that results took the same trend of that obtained from the dry weight of shoots. Since the inoculated plants which received the mixture of (Zn + Mn + Fe) gave the highest values of N (%) in both seasons in both ages of plants.

Concerning the seed yield which is the most important character data in Table 3 revealed that seed yield was increased due to the application of Zn over the control treatment. Further increase was obtained by using Mn over the control and Zn. Fe application gave higher seed yield more than using Zn or Mn separately and over the control also. The highest seed yield was obtained when Zn + Mn + Fe were used as mixture of them.

The results in both seasons had the same trend (1.70 and 1.63 ton/fed.) in 2000 and 2001, respectively.

It should be mentioned in here, that a significant effect of interaction on seed yield between inoculation and micronutrients application. Since the above mentioned seed yield was achieved by the application of micronutrients (Zn + Mn + Fe) when plants were inoculated by Rhizobia.

Regarding nitrogen content is seed (%) data revealed significant increases due to the application of micronutrients either separately or in mixture.

The highest values were obtained when micronutrients (Zn + Mn + Fe) were used as mixture under the inoculation.

These results were true in both seasons and in harmony with those obtained by several workers i.e. (Hegazy *et al.*, 1990 and Abadi, Dawlat *et al.* (1995).

Table (1A): Effect of Rhizobium inoculation and seed coating with Zn, Fe, Mn on number of nodules at 50 and 90 days in 2000 and 2001 season.

	First stage (50 days) 2000			First stage (50 days) 20001			Second stage (90 days) 2000			Second stage (90 days) 2001		
	Uninoc.	Inoc.	Mean	Uninoc.	Inoc.	Mean	Uninoc.	Inoc.	Mean	Uninoc.	Inoc.	Mean
Control	0	35.67	17.84	0	29.33	14.67	0	57.67	28.84	0	51.33	25.67
Zn	0	36.00	18.00	0	31.00	15.50	0	52.67	26.34	0	51.0	25.50
Mn	0	34.67	17.34	0	31.67	15.84	0	55.67	27.84	0	47.00	23.50
Fe	0	38.67	19.34	0	37.00	18.50	0	59.33	29.67	0	56.33	28.17
Zn + Mn + Fe	0	45.00	22.50	0	42.00	21.00	0	69.67	34.84	0	66.33	33.18
L.S.D.			3.765			2.201			4.580			3.920

Table (1B): Effect of Rhizobium inoculation and seed coating with Zn, Fe, Mn on dry weight of nodules (g.) at 50 and 90 days in 2000 and 2001 season.

	First stage (50 days) 2000			First stage (50 days) 2001			Second stage (90 days) 2000			Second stage (90 days) 2001		
	Uninoc.	Inoc.	Mean	Uninoc.	Inoc.	Mean	Uninoc.	Inoc.	Mean	Uninoc.	Inoc.	Mean
(B)			þ									
Control	0	0.157	0.079	0	0.150	0.075	0	0.350	0.175	0	0.313	0.157
Zn	0	0.147	0.074	0	0.147	0.074	0	0.337	0.167	0	0.314	0.157
Mn	0	0.160	0.080	0	0.153	0.077	0	0.343	0.172	0	0.327	0.164
Fe	0	0.177	0.089	0	0.163	0.082	0	0.407	0.204	0	0.357	0.179
Zn + Mn + Fe	0	0.197	0.099	0	0.183	0.092	0	0.457	0.229	0	0.407	0.204
L.S.D.			0.042			0.023			0.016			0.023

Table (2A): Dry weight of sobyean shoots (g.) in the two periods (50 and 90 days) in seasons of 2000 and 2001.

	First stage (50 days) 2000			First stage (50 days) 2001			Second stage (90 days) 2000			Second stage (90 days) 2001		
	Uninoc.	Inoc.	Mean	Uninoc.	Inoc.	Mean	Uninoc.	Inoc.	Mean	Uninoc.	Inoc.	Mean
Control	12.32	15.68	14.00	11.81	14.63	13.22	16.80	27.40	22.10	16.26	26.30	21.28
Zn	12.47	15.67	14.07	11.93	14.19	13.06	17.30	27.57	22.44	16.73	26.73	21.73
Mn	13.23	15.90	14.57	12.42	14.60	13.51	17.50	27.87	22.69	16.87	27.43	22.15
Fe	13.73	16.27	15.00	12.73	15.17	13.95	17.83	28.57	23.20	17.30	28.17	22.74
Zn + Mn + Fe	13.88	16.82	15.35	13.20	16.70	14.95	18.60	30.33	24.47	17.83	29.87	23.85
L.S.D.	0.701	0.295	0.418	0.861	0.372	0.526	0.023	0.0612	0.016	0.023	0.09	0.013

Table (2B): Nitrogen percent in shoots of soybean in the two stages (50 and 90 days) in seasons of 2000 and 2001.

	First stage (50 days) 2000			First stage (50 days) 2001			Second stage (90 days) 2000			Second stage (90 days) 2001		
	Uninoc.	Inoc.	Mean	Uninoc.	Inoc.	Mean	Uninoc.	Inoc.	Mean	Uninoc.	Inoc.	Mean
Control	1.90	2.34	2.12	1.88	2.34	2.11	2.32	2.90	2.61	2.30	2.90	2.60
Zn	1.91	2.36	2.14	1.88	2.34	2.11	2.32	2.91	2.62	2.31	2.91	2.61
Mn	1.88	2.37	2.13	1.89	2.35	2.12	2.34	2.89	2.62	2.32	2.88	2.60
Fe	1.92	2.41	2.17	1.92	2.40	2.16	2.36	3.02	2.69	2.34	3.01	2.68
Zn + Mn + Fe	1.94	2.51	2.23	1.93	2.48	2.21	2.38	3.13	2.76	2.39	3.06	2.73
L.S.D.	0.302	0.436	0.616	0.343	0.164	0.231	0.014	0.009	0.04	0.091	0.012	0.017

Table (3): Response of seed yield of soybean to inoculation and micronutrients as well as nitrogen content % in seeds of soybean.

	Seed yield ton/fed. 2000			Seed yield ton/fed. 2001			N_2 in seeds 2000			N_2 % in seeds 2001		
	Uninoc.	Inoc.	**Mean**	Uninoc.	Inoc.	**Mean**	Uninoc.	Inoc.	**Mean**	Uninoc.	Inoc.	**Mean**
Control	1.080	2.109	1.598	1.068	2.020	1.544	4.69	6.08	5.39	4.67	6.07	5.37
Zn	1.096	2.115	1.603	1.080	2.030	1.549	4.70	6.09	5.40	4.68	6.09	5.37
Mn	1.099	2.122	1.611	1.090	2.098	1.594	4.70	6.12	5.40	4.68	6.09	5.39
Fe	1.123	2.188	1.656	1.114	2.108	1.611	4.73	9.17	5.45	4.70	6.14	5.42
Zn + Mn + Fe	1.167	2.237	1.702	1.133	2.125	1.629	4.77	6.27	5.52	4.77	6.21	5.49
L.S.D.	0.022	0.065	0.090	0.018	0.034	0.048	0.035	0.351	0.497	0.031	0.347	0.491

Data of leghaemoglubin content of nodules were determined and presented in Table 4. It is well known that the concentration of these component is correlated with N_2-fixing ability of nodules. (Bergersen and Turner, 1975). Also, nitrogen fixation consists of iron protein. Data in Table 4 show that leghaemoglubin content increased due to application of micronutrients. It is clear that Zn increased leghaemoglubin over the control. Also, it is obvious that application of the and Fe alone achieved significant increases over zinc alone. While the highest leghaemoglubin content was recorded by the application of the three micronutrients (Zn, Fe and Mn).

Table (4): **Legheamoglobin content of root nodules of soybean in 2000 and 2001 seasons.**

	After 50 days 2000			After 50 days 2001		
	Un.	In.	Mean	Un.	In.	Mean
Control	0	1.07	0.54	0	0.98	0.49
Zn	0	1.10	0.55	0	1.02	0.51
Mn	0	1.18	0.59	0	1.06	0.53
Fe	0	1.21	0.61	0	1.15	0.58
Zn + Mn + Fe	0	1.39	0.70	0	1.29	0.65
	0	1.19		0	1.10	

L.S.D. at 0.05 = 0.023

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استجابة فول الصويا للتلقيح بالريزوبيا والتسميد ببعض العناصر الصغرى المضافة بطريقة تغليف البذره

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أقيمت تجربتان حقليتان على محصول فول الصويا بمحطة البحوث الزراعية بسخا فى موسمى ٢٠٠٠ ، ٢٠٠١م لدراسة تأثير التلقيح البكتيرى بالريزوبيا وإضافة عناصر صغرى هى الزنك والحديد ، والمنجنيز منفردة أو فى مخلوط يجمع بين العناصر الثلاث ؛ باستخدام طريقة تغليف البذره ويمكن تلخيص أهم النتائج المتحصل عليا كما يلى:

١- التلقيح البكتيرى بالسلالات الفعالة لفول الصويا إيجابيا واقتصاديا لتحقيق احتياجات فول الصويا من عنصر النيتروجين وأفضل من إضافة الأسمدة النيتروجينية المعدنية.

٢- زادت جميع صفات محصول فول الصويا التى تمت دراستها نتيجة لإضافة عناصر الزنك أو الحديد أو المنجنيز منفردة أو إضافتها مجتمعة تحت ظروف التلقيح البكتيرى مقارنة بمعاملة المقارنة.

٣- كان أعلى محصول فول الصويا هو ١,٧ ، ١,٦ طن/فدان لموسمى ٢٠٠٠/٢٠٠١ على التوالى تم الحصول عليه بإضافة خليط من العناصر الصغرى الثلاث زنك + حديد + منجنيز بطريقة تغليف البذره تحت ظروف التلقيح البكتيرى.

٤- تؤكد النتائج أهمية التلقيح البكتيرى لمحصول فول الصويا للحصول على أعلى محصول وضرورة إرشاد المزارعين للقيام بهذه العملية الزراعية الناجحة ذات العائد الاقتصادى المفيد.