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RHIZOBIUM-SOYBEAN SYMBIOSIS AS AFFECTED BY SOURCE AND QUALITY OF WATER USED IN IRRIGATION

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ABSTRACT

An experiment in cement rough was conducted at Sakha Agric. Res. Station during two successive seasons (2000 and 2001) to explore symbiotic performance of two strains of *Bradyrhizobium japonicum* (1577 and 110) used to inoculate soybean cultivar crowford, irrigated with high quality of tap water and low quality of sewage and drainage water or their mixture. Results were recorded after 50 and 90 days from planting time. Effect of inoculation on nodules formation was very clear by soybean plants, irrigated with tap water and inoculated with any of two rhizobial strains. Strain 1577 achieved higher values of N_2-fixation parameters, as well as growth measurement of soybean plants than strain 110. On contrary soybean plants irrigated with sewage or drainage water didn't show any response to inoculation. While a little improvement was observed for soybean plants received a mixture of drainage and tap water, indicating that failure of nodule formation could be attributed to quality of water used in irrigation. Concerning the grain yield and their total N-content, irrigation with tap water gave the highest improvements being 105.57 for the seed yield and 156.92% for nitrogen content over control, while those received sewage and drainage water recorded a very less improvements. In the case of irrigation with sewage water, lower values of grain yield were obtained than the control (-1.71%).

For these reasons, the research was directed towards identifying some chemical and biological characteristic of wastewater used in irrigation, which it could be concluded that the failure of *Rhizobium*-soybean symbiosis is a resultant expression of three interacting factors: high concentration levels of heavy metals, high density of parasites, microbial pathogens and high amount of nitrogen especially in sewage water.

Key words: *Bradyrizobium japonicum* – drainage water- sewage water- soybean

INTRODUCTION

Since earliest time water has become a decisive factor for people's life. Water supply was and remains as a central challenge for humanity. The problem of sewage and drainage water has become acute since the Industrial Revolution and the resulting progress, which alongside its many impressive accomplishments, has discharged tremendous amount of waste and pollution. In Egypt, over many years domestic sewage and industrial wastes have been flow to the sea, polluting the country's beaches, surface water sources and ground water, contaminating wells and rendering them unusable.

Due to continuous gap between available volume of water and those needed in some countries, where the urge to continue on agricultural development, reuse of wastewater is becoming a basic component in their water policy. As a result of short supply of water in Northern Regions of Delta in Egypt, farmers are accustomed to use raw sewage and drainage water as the only choice that can provide an alternative means for water supplies for irrigation of various crops especially in summer seasons.

Unfortunately the quality of these water in addition to above mentioned effect has also two great drawbacks: (1) Release of heavy metals into the soil (plant environment) which are increased gradually due to continuous and frequencies of irrigation cycles. (2) Carrying high density of microbial load of human and animal pathogens.

Fixation of atmospheric nitrogen by microorganisms is a more metal-sensitive process. Thus N_2-fixation by free living bacteria and cyanobacteria decreases when soil metal concentration are blow EC limits, because of a decrease in the numbers of these microorganisms in soil (McGrath and Hirsch, 1989). *Symbiotic* N_2*-fixation by legumes with the rhizobia* is also inhibited, depending on the species from *Rhizobiaceae* derived. Most of the evidence for these is for the clover-*Rhizobium legumniosarum* bv. *Trifolii* association where decreased clover yields and N_2-fixation are associated with reduced survival of the bacteria in contaminated soils, (McGrath *et al.*, 1988 and Zahran, 1999).

In light of these information, our investigation was initiated to measure the symbiotic performance of *Bradyrhizobium japonicum* on soybean plant irrigated with raw sewage and drainage water, aimed for clarifying to what extend this quality of waste water can be affect biological N_2-fixation in comparison with high quality of tap water.

MATERIALS AND METHOD

Two experiments in cement roughs (lyzemeter 80 x 80 x 80 cm) were carried out at Sakha Agric. Res. Station, Kafr El-Sheikh Governorate, Egypt. The roughs were filled uniformly with clayey soil.

Soybean seeds (*Glycine max.* L.): Cultivar crowford was obtained from legume crop Section, Agric. Res. Center, Giza.

***Bradyrhizobium japonicum*:** Two strains, 1577 (Germany) and 110 (Nif-Tal USA) were obtained from the Stock Culture Collection of Dep. of Soil Microbiology, Sakha Agric. Res. Station.

Seeds inoculation: Cultures were maintained on yeast extract mannitol (YEM) agar slant (Vincent, 1970). Each rhizobial strain was grown in YEM broth on rotary shaker at 25 °C. Prior to inoculation, plate dilution count of viable cells were made (initial number of cells used for inoculation was about 3 X 10^9 cells/ml). Sex-days old culture from every strain was added to the fine nitragen peat, at the rate of 1:1 (v/w) before planting. Nitrogen fertilizer in the form of urea (46% N) was added at rate of 20 kg N/fed. to all inoculated treatments. Phosphorus in the form of ordinary superphosphate was applied at the rate of 30 kg P_2O_5/fed.

Source and quality of irrigation water: Three different sources of irrigation water (high quality of tap water, and low quality of sewage and drainage water), were used. Irrigation regime included weekly irrigation cycles during the sex week's period following sowing. Thereafter all treatment received biweekly irrigation.

Chemical analysis: Total nitrogen of shoots and seeds were determined by macro-Kjeldahl (Ward and Johnston, 1962). Level of heavy metals concentrations in experimental soils were determined according to the methods described by Chapman and Pratt (1961).

Microbiological characteristics of irrigation water: Coliform group bacteria: (Total and Fecal)as well as Salmonella and Shigella bacteria were counted on MacConkey's bile salt agar and S.S. agar media, respectively according to Difco Manual (1977). While Protozoa and other parasites were examined according to Monica-Cheesbrough(1991).

Data were statistically analyzed according to Snedecor and Cochran (1967), using LSD for comparison between means.

RESULTS AND DISCUSSION

The effect of irrigation with tap, sewage and drainage water on the symbiotic performance of *Bradyrhizobium japonicum* and the growth parameters of soybean plants could be discussed in the following points:

(1) Number and dry weight of nodules:

The effect of inoculation on nodules number as observed after 50 and 90 days from planting was cleared with all plants irrigated with tap water. In the non-inoculated treatment, the roots were free of nodules, so it is therefore the vital importance to continue inoculation of soybean with a mixture of effective, competitive and persistent strains of *Bradyrhizobium japonicum*, if good nodulation has to be achiveved. It is also noticed that soybean plants irrigated with sewage or drainage water didn't show any response to inoculation as shown in Table (1).

Regarding to tap water irrigation, analysis of the data presented in Table (1) revealed that no significant differences between the two rhizobial strains applied in inoculation treatment, however strain 1577 was more active to form nodules, where as, it achieved 33.0 nods./plant after 50 days of planting in comparison with strain 110 (28.0 nods./plant). A continuous increase in nodules number was found by aging of plants from 50 to 90 days. The relative improving percent was 71.0 and 80.46% in season 2000 and 2001, respectively. These differences in nodules number was reflected on the total dry weight of nodular tissue, as strain 1577 still recorded higher dry weight (175 mg/plant-average of two seasons) than strain 110 (135 mg/plant). The corresponding values were 380 and 335 mg/plant respectively after 90 days of planting time.

It is worthy to mention that the interactions between irrigation treatments and rhizobial strain showed a positive correlation in season 2000, while in season 2001, this interaction was insignificant for dry weight of nodular tissues.

It is also clear from the obtained data that irrigation with a mixture of drainage and tap water led to a very less improvement in nodules formation with average means 6 nods./plant. Such results indicated that determinant factors for nodule inhibition may be attributed to factors related with quality of irrigation water. This could be demonstrated in vitro using plate technique, (Fig. 1), where every rhizobial cells come into contact with sterilized sewage, drainage and tap water. A clear inhibition zones were detected around wells supplemented with sewage or drainage water only, not around tap water.

Table (1): Nodulation of soybean (No./plant) at 50 and 90 days after sowing as influenced by inoculation with *B.japonicum* strains, 1577(A) or 110 (B) and irrigation with tap, sewage and drainage water.

Treatments	Season, 2000				Season, 2001			
	50 days		90 days		50 days		90 days	
	Nods. No.	Nods. D.W. Mg	Nods. No.	Nods. D.W. mg	Nods. No.	Nods. D.W. mg	Nods. No.	Nods. D.W. mg
Tap water,(T)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T+ St.A	32.7	180.0	57.7	400.0	28.7	170.0	50.0	360.0
Sewage water,(S)+St.A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Drainage water,(D)+St.A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S+T(v:v)+St.A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
D+T(v:v)+St.A	8.0	10.0	9.0	20.0	4.7	10.0	3.3	20.0
T+St.B	28.3	140.0	46.7	350.0	23.0	130.0	43.3	320.0
S+St.B	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
D+St.B	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S+T(v:v)+St.B	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
D+T(v:v)+St.B	4.3	10.0	6.3	10.0	4.0	10.0	6.7	20.0
L.S.D.,0.05:								
Strains,(Sts)	N.S	N.S	1.97	N.S	0.894	N.S	N.S	N.S
Treatments,(Trs)	1.22	0.006	1.14	0.007	1.694	0.081	2.393	0.192
Sts × Trs	1.73	0.015	2.79	0.017	1.594	N.S	5.861	N.S

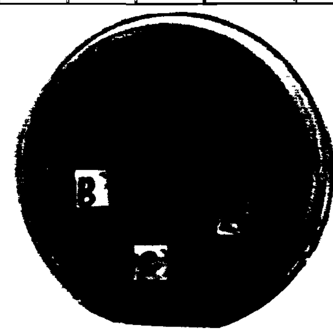

Fig. 1: Inhibitition zones of *Bradyrhizobium japonicum* strain 1577 by sterilized sewage (A), drainage (B) and tap water (C).

With these unfortunate results, research was directed towards identifying some chemical and biological characteristics of sewage and drainage water used in irrigation system from which the following could be concluded:

1- Data presented in Table-2 showed that the concentration levels of heavy metals estimated in the experimental soils after the first and second season were more or less than the inhibitory effect on soil microorganisms, but it seems propaply that rhizobial cells may exposed to higher concentration of heavy metals presented in sewage or drainage water once irrigation is carried out. It is well known that there are many factors operate in soils, including sorption and chelation by organic matter or precipitation reactions which led to complete different bioavailability, once these metals are added to the soil through irrigation treatments. For these reasons Sanders *et al.* 1987 mentioned that the measurements of the amount of metals in soil solution which bathes plant root and soil microorganisms may be a better indicator of exposure. It is well known that heavy metals can persist in the soil over long periods and have ecotoxicological effects on plants, (Skujins *et al.*1986), soil microorganisms, (Ernst, 1990) and on rhizobial populations, (Reddy *et al.* 1983). Results reported herein coincided with those obtained by Coppola *et al.* (1988); Giliar *et al.* (1989 and 1993); Baath (1989); Eivazi (1990); Chaudri *et al.* (1992) and Zahran (1999). It is also pointed from the obtained results (Table 3) that irrigation with sewage water that contains high amount of nitrogen in a form of nitrate (average 28.0ppm) and ammonia (43.8ppm) was enough to inhibit nodulation process, (Eskew *et al.* 1989)

2- The second quality criteria which may reduce the number of introduced rhizobial cells in plant rizosphere is the presence of high density of different pathogens in sewage and drainage water. Data presented in Table 3 showed that the initial counts of total and fecal coliform were on the average (sewage and drainage water) 2.9 $x10^4$ and 7.25 x 10^3 cfu/ml, respectively, beside abundance number of protozoa was found in the tested samples. It is worthy to note that the above mentioned pattern of the tested groups of microorganisms represent a part of microbial load present in sewage and drainage water, which expected to be attain highest values especially in the late stages of plant growth due to irrigation cycles. The antagonistic effect towards rhizobia by other microorganisms was reviewed by Danso *et al.* (1973) and Danso and Alexander (1975) who mentioned that each protozoa was able to consume thousands cells of *B.japonicum*. It is also noted that number of total and fecal coliform (Table 3) may resulted in actual risk to pubic health ($\geq$ 1000 cell/100 ml), according to the guidelines presented by WHO (1973) for the quality of wastewater use in agriculture. Therefore, precaution must be taken against the possible spread of some diseases in the environment.

Table (2): concentrations of heavy metals (ppm) in the experimental soils irrigated with tap, sewage or drainage water, after the two experimental seasons.

Heavy metals	Tap water			Sewage water			Drainage water		
	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)
Pb	2.50	2.51	2.50	5.11	9.02	7.07	3.60	5.10	4.35
Mn	7.40	7.38	7.39	16.12	21.05	18.59	13.02	16.78	14.90
Zn	1.35	1.33	1.34	5.22	8.02	6.62	3.12	6.11	4.62
Cd	0.08	0.08	0.08	0.56	0.99	0.78	0.15	0.32	0.24
Ni	0.55	0.55	0.55	1.01	3.62	2.31	0.78	2.51	1.65
Cu	0.08	0.08	0.08	1.97	2.82	2.39	1.18	2.35	1.77

(1) After the first season, (2) after the second season and (3) average.

Table (3): Some chemical and biological properties of sewage and drainage water used in the study.

Parameters	Sewage Water	Drainage water
NO_3 -N (ppm)	28.0	12.0
NH_4^+-N(ppm)	43.8	24.6
Parasities:		
Ascaris	++	ND
Protozoa	++++	++
Paramecium	++	ND
Bacteria:		
Total coliform*	$5.2x10^4$	$2.4x10^3$
Fecal coliform*	$8.3x10^3$	$6.2x10^3$
Salmonella & Shigella*	3.3	ND

ND= not detected *Colony forming units per ml.

2- Dry matter yield and nitrogen content:

Dry matter of soybean plants was strongly influenced by the quality of water used in irrigation and rhizobial strains (Table 4). Irrigation with tap water and inoculation by rhizobial strain 1577 or 110 increased shoot dry matter and total nitrogen content. Strain 1577 gave higher values of shoot dry matter and nitrogen content after 50 days of planting being 17.29 g/plant and 415.75 mg N/plant (average of two seasons). The correspond figures for strain 110 were 16.6 g for dry matter and 386.8 mg for nitrogen/plant. Such variation in the effectiveness between the tested rhizobial strains might be due to the genetic factors govering the relation between host plant and rhizobial cells (Alwi *et al.*, 1989 and Ghobrial *et al.*, 1995).

In respect to inoculated plants, irrigated with sewage or drainage water, shoots dry weight and total nitrogen content showed slight improvements after 50 days of planting in comparison with control. However, drastic effect on shoots dry weight and total nitrogen content was observed after 90 days of planting, (Table 4). Such effect could be attributed to phototoxic of heavy metals on plant growth (Sandman and Borger, 1983); decreasing of photosynthetic and transpiration rate (Tornabene *et al.* 1979; Chander and Brookes, 1991). Heavy metals have an inhibition effect on plant chlorophyll synthesis (Anna-Majbalsberg, 1989).

3-Seeds yield and N-content:

To evaluate the effect of water quality used in irrigation on grains yield and their nitrogen content, (Table 5) the percentage of changes were calculated, (Table 6). Data revealed that the inoculated treatments and irrigated with tap water gave the highest improvements in soybean productivity as well as their total nitrogen content compared with absolute control (uninoculated and irrigation with tap water). The average increase was about 105.57 for the seed yield and 156.92 % for nitrogen content. On contrary irrigation treatments including sewage and drainage water led to a very less improvements in seed yield and nitrogen content and beyond irrigation treatments with sewage water, lower values of grain yield were obtained than the control (-1.71%).

Table (4): Shoots dry weight(D.W) per plant and their nitrogen content as influenced by inoculation with *B.japonicum* strains, 1577(A) or 110 (B) and irrigation with tap, sewage and drainage water.

Treatments	Season, 2000						Season, 2001					
	50 days			90 days			50 days			90 days		
	D.W (g)	N content %	N content mg	D.W (g)	N content %	N content mg	D.W (g)	N content %	N content mg	D.W (g)	N content %	N content mg
Tap water,(T)	11.80	1.84	217.18	18.43	2.35	433.11	11.35	1.85	209.98	18.04	2.33	420.33
T+ St.A	17.57	2.42	425.2	27.66	3.10	857.5	17.00	2.39	406.3	25.13	3.07	771.5
Sewage water,(S)+St.A	14.67	1.93	283.1	16.43	2.27	373.0	13.17	1.95	256.8	15.72	2.24	352.2
Draing water,(D)+St.A	15.67	2.03	318.1	17.00	2.34	397.8	13.82	2.02	279.2	16.21	3.34	379.3
S+T(v:v)+St.A	15.20	2.16	328.3	18.03	2.42	436.3	13.95	2.08	290.2	17.23	2.41	415.2
D+T(v:v)+St.A	16.13	2.19	353.2	18.51	2.62	485.0	14.58	2.17	316.4	17.25	2.56	441.6
T+St.B	16.93	2.34	396.2	26.80	3.02	809.4	16.27	2.32	377.4	26.07	2.98	776.9
S+St.B	13.70	1.87	256.2	15.94	2.21	352.3	12.52	1.74	217.9	15.84	2.18	345.3
D+St.B	14.67	2.00	293.4	16.46	2.42	398.3	12.98	1.97	255.7	16.23	2.39	387.9
S+T(v:v)+St.B	14.93	2.10	313.5	16.72	2.39	399.6	13.76	2.07	284.8	16.55	2.35	388.9
D+T(v:v)+St.B	15.57	2.16	336.3	17.57	2.67	469.1	14.24	2.14	304.7	17.14	2.54	435.4
I.S.D.,0.05:												
Strains,(Sts)	N.S	N.S	--	N.S	N.S	--	N.S	0.048	--	N.S	0.041	--
Treatments,(Trs)	0.302	0.027	--	0.793	0.068	--	0.221	0.033	--	0.71	0.028	--
Sts × Trs	1.350	0.049	--	1.122	0.067	--	0.313	0.046	--	1.004	0.039	-

Table (5): Seeds yield (g/Lyzemeter) and their nitrogen content as influenced by inoculation with *B.Japonicum* strains, 1577(A) or 110 (B) and irrigation with tap, sewage and drainage water.

Treatments	Season, 2000			Season, 2001		
	Yield	N content		Yield	N content	
	(g)	%	(g)	(g)	%	(g)
Tap water,(T)	133.69	4.66	6.23	129.32	4.62	5.97
T+ st.A	270.76	5.84	15.81	173.80	5.82	15.94
Sewage water,(S)+St.A	133.66	4.86	6.50	130.80	4.81	6.29
Drainage water,(D)+St.A	136.73	4.93	6.74	133.30	4.90	6.53
S+T(v:v)+St.A	147.50	5.27	7.77	142.40	5.25	7.48
D+T(v:v)+St.A	150.80	5.43	8.19	145.43	5.35	7.78
T+St.B	266.80	5.77	15.39	270.03	5.76	15.55
S+St.B	129.43	4.80	6.21	123.13	4.77	5.87
D+St.B	131.03	4.90	6.42	127.90	4.87	6.23
S+T(v:v)+St.B	143.10	5.18	7.41	138.20	5.17	7.14
D+T(v:v)+St.B	146.17	5.41	7.91	141.37	5.36	7.58
L.S.D.,0.05:						
Strains,(Sts)	3.53	0.18	--	1.12	0.04	--
Treatments,(Trs)	0.95	0.02	--	1.17	0.14	--
Sts × Trs	1.35	0.05	--	1.56	0.20	--

Finally, it s hardly to determine which any factor profoundly influence the failure of nodulation and inhibition of plant growth. Is it related to the phytotoxicity exerted by heavy metals present in sewage and drainage water? And which any metal was more toxic? Or related to high density of microbial load of pathogens in wastewater that compete soil microbial population and rhizobial inoculants? Or related to high amount of total nitrogen that inhibit nodulation process? Although under this circumstances, the plants have a greater tendency to replace the fixation process but this was true at early stages of plant growth not at late stages as clarified from the obtained results (Table 6).

Table (6): Percentage of changes in nitrogen fixation parameters of shoots and seeds as compared with control treatment (uninoculated and irrigated with tap water)

Treatments	Shoots				Seeds	
	After 50 days		After 90 days		After 130 days	
	Dry weight	N-content	Dry weight	N-content	Dry weight	N-content
Tab water + Sts.	46.37	87.90	44.86	88.37	105.6	156.9
Sewage water + Sts.	16.76	18.71	-12.35	-16.65	-1.71	1.93
Drainage water +Sts.	23.41	34.20	-9.65	-8.41	0.56	6.23
Tap+sewage water+Sts.	24.92	42.45	-6.05	-3.91	8.59	22.13
Tap+drainage water+Sts.	30.71	53.43	-3.37	7.27	10.98	28.93

Each parameter was mean of the two strains during two seasons.

It seems that there are other factors present in wastewater that may decrease the absorbing capacity of the plant root system, consequently it did not help the plant to accumulate more nitrogen. Therefore, it is necessary to reappraise the limits for application of untreated sewage and drainage water in irrigation system especially in northern region of Delta, and measures needed to protect the soil microbes and their activity against phytotoxicity and adverse effects on plant growth as a result of continuous irrigation with this low quality of wastewater. Further studies are needed for the most appropriate techniques for further disinfection of the effluents obtained from the wastewater treatment, plants to be more safe from the hygienic stand point.

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العلاقة التكافلية بين الريزوبيا وفول الصويا ومدى تأثرها بنوعية مياه الري.

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اجريت تجربة في احواض اسمنتية بمحطة البحوث الزراعية بسخا– كفر الشيخ خلال موسمي ٢٠٠٠ ، ٢٠٠١ لدراسة تثبيت النيتروجين تكافليا بواسطة سلالتين من ريزوبيا فول الصويا (براديريزوبيم جابونيكم أرقام ١٥٧٧ ، ١١٠). وقد تم تلقيح نبات فول الصويا بكل سلالة على حدة واستخدمت مياه الحنفية ومياه المجاري ومياه الصرف الزراعي أو خليط منهما في الري. وقد بينت النتائج المتحصل عليها بعد ٥٠ ، ٩٠ يوم من الزراعة تأثير التلقيح بأي من السلالتين على تكوين العقد الجذرية في المعاملات المروية بماء الحنفية.
السلالة ١٥٧٧ أعطت قيم عالية لقياسات تثبيت النيتروجين وكذلك نمو نبات فول الصويا مقارنة بالسلالة ١١٠. وعلى النقيض من ذلك فان النباتات التي رويت بماء المجاري أو ماء الصرف لم تعطي أي استجابة للتلقيح بأي من السلالتين ولكن حدث تحسن طفيف لنباتات فول الصويا التي رويت بخليط من ماء الحنفية وماء الصرف وهذا دل على أن الفشل في تكوين العقد الجذرية قد يكون بسبب نوعية مياه الري. وفيما يتعلق بمحصول الحبوب ومحتواها من النيتروجين فان الري بماء الحنفية أعطى أعلى قيم (١٠٥,٥٧? بالنسبة لمحصول البذور و ١٥٦,٩٢? من محتواها من النيتروجين زيادة على معاملة الكنترول) في حين أن المعاملات التي رويت بماء المجاري أو ملء الصرف الزراعي سجلت تحسن قليل جدا والري بماء المجاري أعطى انتاج قليل من البذور مقارنة بالكنترول (– ١,٧١?).

ولهذه الاسباب فان البحث توجه الي معرفة بعض الصفات الكيمائية والبيولوجية للماء المستخدم في الري والتي امكن منها معرفه أن فشل ريزوبيا فول الصويا في التكافل يرجع الى ثلاثة عوامل هي: التركيز العالي من المعادن الثقيلة والكثافة العالية من الطفيليات والميكروبات المرضية والمحتوى العالي من النيتروجين خصوصا في ماء المجاري.