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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 9 (10): 1960-1964, 2006
ISSN 1028-8880

Effect of Treated Municipal Waste water and Rhizobia Strains on Growth and Nodulation of faba Bean (*Vicia faba* L. cv. Hassawi)

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Abstract: Scarcity of good quality irrigation water in the Kingdom of Saudi Arabia has resulted in a search for alternative sources for irrigation water. An experiment using municipal effluent in growing faba bean (*Vicia faba* L. cv. Hassawi) inoculated with four different strains of *Rhizobium* leguminosarum biovar. Viciae (Hass-1, Hass-2, 2500 and 3385), at various concentrations. The results indicated that the faba bean growth was good with neither signs of nutrient deficiency nor signs of toxicity as a result of the use of the different levels of municipal wastewater effluent. The results also indicated that *R. leguminosarum* strains formed effective symbiosis with faba bean under effluent and well water irrigation treatments. Effluent significantly increased shoot, root and nodule growth (fresh and dry weights), stem and root lengths and nodules/plant. N_2 fixation activity and nitrogen content of plant tissues were significantly affected by both effluent and rhizobia treatments. The results of the study revealed that the quality of the treated municipal wastewater effluent was suitable for irrigation of faba bean. The maximum improvement in faba bean growth was recorded with 100% effluent application. Thus, application of effluent was found comparatively more effective than well water in enhancing growth of faba bean. The results also revealed that inoculation of faba bean seedling with local strains (Hass-1 and Hass-2) was more effective in enhancing plant growth than introduced strains (2500 and 3385).

Key words: Faba bean, nodulation, rhizobia, waste water

INTRODUCTION

Saudi Arabia is predominantly an arid country and the shortage of irrigation water, is one of the limiting factors for maximizing cropping intensities and obtaining higher crop yield. The rainfall is highly variable and is not available for subsequent agricultural activities because of inadequate storage. The water supplies from the existing surface irrigation systems are not enough to meet crops water requirements. Increasing industrialization and urbanization in major cities in the Kingdom led to an increase in the availability and volume of effluent water (Al-Solaimani *et al*., 2002). The use of treated municipal wastewater effluent for irrigation of crop plants offers an opportunity to conserve water resources in arid areas such as Saudi Arabia (El-Nennah *et al*., 1982; El-Hassanin *et al*., 1993; El-Kadi and El-Quallaf, 1995). Effluent waste water treatments process contains organic matter and nutrients that can improve agricultural productivity of soils (Mbila *et al*., 2001; Wang *et al*., 2002; Chaudhary *et al*., 2004). Farmers in Al-Hassa area have expressed their concerns about the agricultural use of treated municipal wastewater effluent. This practice may affect the sustainability of agricultural land and may have environmental consequences as well.

Effluent water is now used for the restoration of degraded land and the growth of vegetation having commercial and environmental value (Dighton and Jones, 1991). The key for safe irrigation is the quality of water. Many standards have been set by different institutions to control the quality of water for irrigation. The concentration and composition of dissolved elements in water determines its quality for irrigation with wastewater treatment improve technology, most effluents produced by public treatment projects in the Kingdom of Saudi Arabia are of high quality.

Legumes have been suggested as appropriate crops for the enhancement of bioproductivity and the reclamation of marginal lands, because these plants not only yield good fodder, protein-rich seeds and fruits, but they also enrich soil nitrogen in symbiotic association with *Rhizobium* (Alexander, 1984; Ferreira and Castro, 1995; Sprent, 1999; Hungria and Vargas, 2000; El-Komy, 2005). Legume plants may utilize wastewater effluent and uptake heavy metals through extensive root system. Thus, these plants serve as effective biological sieves, inhibiting contamination of ground water sources (Karpiscak, 1996; Wang *et al*., 2002; Chaudhary *et al*., 2004; Junior *et al*., 2005). Nodulation is adversely affected by salinity, which can adversely affect legume growth or

reduce crop yield (Ferreira and Castro, 1995; Cordovilla *et al*., 1995; El-Komy, 2001; Katerji *et al*., 2003; Goormachtig *et al*., 2004). Nodulation and nitrogen fixation in legume-Rhizobium associations are adversely affected by water quality can preclude legume establishment and growth, or reduce crop yield (Mohammad *et al*., 1991; Ferreira and Castro, 1995; Goormachtig *et al*., 2004, Junior *et al*., 2005).

Unsuccessful symbiosis may be due to failure in the infection process because of the effect of water quality on the establishment of rhizobia (Singleton and Bohlool, 1984). Legumes and the process of nodule initiation are both more sensitive to osmotic stress than are rhizobia (Russell, 1976; Tu, 1981; Velagaleti *et al*., 1990; Katerji *et al*., 2003; El-Komy, 2001, 2005; Goormachtig *et al*., 2004).

Scarcity of good quality irrigation water in the Kingdom of Saudi Arabia has resulted in a search for alternative sources for irrigation water. Treated municipal wastewater is one of the main sources to be used for agricultural purposes, which could compensate partially the water needs of the Kingdom. Therefore, the aim of this study was to quantify the growth of faba bean seedlings nodulation and N_2 fixation in response to the effect of four different rhizobia strains under different levels of municipal effluent application.

MATERIALS AND METHODS

A pot experiment was carried out using plastic pots inside wire-proof green house at the Agricultural and Veterinary Training and Research Station of King Faisal University, Al-Hassa, Saudi Arabia during the period from March to September 2005, to assess the effects of irrigation with treated municipal wastewater effluent (Table 1) and inoculation with a number of salt tolerant *Rhizobium* strains (Table 2) on faba bean (*Vicia faba* L.) cv. Hassawi seedling growth. The station is located on the Delegia area about 15 km south-east of the University Campus.

The treatment combinations consisting of five irrigation treatments included: I_1: irrigation with treated municipal effluent (100%), I_2: irrigation with treated municipal effluent (75%)+well water (25%), I_3: irrigation with treated municipal effluent (50%)+well water (50%), I_4: irrigation with treated municipal effluent (25%)+well water (75%) and I_5: irrigation with well water (100%) as well as inoculation with four different strains of salt-tolerant *Rhizobiumleguminosarum* biovar viciae, namely Hass-1, Hass-2, 2500 and 3385.

The experiment was laid out as a complete randomized block design with five replications in 5×4 factorial arrangement. The experimental soil in each pot is loamy sand, slightly alkaline (pH 8.1), having low organic matter (0.45%), available nitrogen (234.32 kg ha^{-1}) and available phosphorus (13.20 kg ha^{-1}) but sufficient potash (254.76 kg ha^{-1}). The soil was previously heat sterilized in metal buckets at 125°C for an hour on each of 3 successive days (Abdel-Wahab *et al*., 2002). Pots were sterilized by washing thoroughly with 95% (v/v) ethanol, sterilization was necessary to eliminate possible contamination by bacteria other than the treatments.

Three grams of Rhizobium were mixed with 150 g of faba bean seeds for each strain. The seeds were first sprinkled with little water to moisture the seed surface and the inoculums to be mixed with the seeds. Seeds were sown just after inoculation to prevent killing of bacteria by sunlight. The pot size was 25 cm in diameter and 30 cm deep. The pots were filled with 6 kg of soil to which urea and super phosphate were added at the rate of 0.3 and 1.5 g per pot respectively.

Faba bean seeds were sown at the rate of 5 seeds/pot. The seeds were covered with 0.5 cm layer of sterilized soil. After two weeks seedlings were thinned to 3 per pot. The plants were irrigated as per treatment two times per weeks keeping the level of wetness in the soil to that at field capacity.

Data were collected when plants were 75 days old. The plants were removed by digging up the roots, the

Table 1: Chemical composition of treated municipal wastewater effluent and well water used for irrigation. Mean of three replicates+SE

Quality parameters	Effluent	Well water
pH	7.62±0.03	7.53±0.04
EC (dS m^{-1})	3.55±0.05	2.74±0.03
Total solids (g L^{-1})	3.81±0.02	0.13±0.002
Total dissolved solids (g L^{-1})	1.75±0.12	0.17±0.01
Total suspended solids (g L^{-1})	0.86±0.03	0.04±0.011
Biological oxygen demand (mg L^{-1})	68.7±0.87	3.0±0.71
Sodium (mg L^{-1})	27.3±0.21	13.0±0.40
Potassium (mg L^{-1})	17.3±0.11	4.26±0.23
Calcium (mg L^{-1})	12.9±0.02	0.04±0.002
Magnesium (m L^{-1})	3.4±0.01	6.8±0.002
NH_4-N (mg L^{-1})	32.50±0.61	2.13±0.001
NO_3-N (mg L^{-1})	26.5±1.12	6.38±0.001
PO_4-P (mg L^{-1})	12.96±0.72	0.57±0.02
Copper (mg L^{-1})	0.025±0.01	0.03±0.001
Iron (mg L^{-1})	0.026±0.02	0.02±0.007
Manganese (mg L^{-1})	0.026±0.01	0.020±0.007
Zinc (mg L^{-1})	0.038±0.02	0.030±0.002

Table 2: *Rhizobium leguminosraum* biovar viciae strains used in the experiment

Strain	Source
Hass-1	Strain isolated in 1995 in Al-Hassa, Saudi Arabia, from a saline soil grown previously with faba bean.
Hass-2	Strain isolated in 1995 in Al-Hassa, Saudi Arabia, from soil previously grown with faba bean and irrigated with saline drainage water.
2500	Strain received from USDA *Rhizobium*culture collection at Beltsville, MD and first tested for N_2 fixation ability in Al-Hassa.
3385	Strain received from Agricultural Research International Center, Ministry of Agriculture, Giza, Egypt.

roots were thoroughly rinsed with water, blotted dry on filter paper and nodules picked, counted and kept on ice. Observation included stem and root length, stem, root and nodules fresh and dry weights, nodules/plant, nitrogen content of plant tissues and nitrogenase activity. Dry weights were recorded after drying samples for 24 h at 70°C. The experiment was repeated twice under identical conditions and thus the value of each replicate is the mean of the data for two plants. Nitrogenase activity was determined by acetylene reduction on the entire root systems using a gas chromatograph equipped with a column of porpak as described by Cordovilla *et al.* (1995).

The obtained data were subjected to statistical analysis in accordance with the statistical analysis system (SAS Institute, 2003). The general linear model was used to perform analysis. Analysis of variance was conducted including the Least Significant Difference (LSD) test to compare means. Statistical tests were performed at a 0.05 level of significance.

RESULTS AND DISCUSSION

Application of sewage water to the agricultural lands serves dual purpose, firstly it is an economical way of disposal and secondly it provides plant nutrients and organic matter to the soil and increases its productivity (Mbila *et al.*, 2001; Wang *et al.*, 2002; Chaudhary *et al.*, 2004).

Table 3: Number, fresh and dry weights of nodules/plant N_2 (C_2H_2) fixation and nitrogen in plant tissues as influenced by treated municipal wastewater effluent and rhizobia strains. Mean of three replicates±SE

		Weight			
Treatment	Number	Fresh	Dry	Specific nodule activity (μmol C_2H_4 g h^{-1})	Total nitrogen accumulation in plant shoots (%)
Irrigation water (%)					
100 effluent	26.0	0.093	0.020	35.8	6.59
75 effluent	23.0	0.083	0.018	30.7	6.40
50 effluent	21.0	0.076	0.016	26.2	6.25
25 effluent	18.0	0.073	0.014	23.7	5.83
Well water	14.0	0.064	0.014	11.9	4.90
LSD. (0.05)	2.0	0.002	N.S.	2.4	0.13
Rhizobia strains				33.4	6.37
Hass-1	28.0	0.113	0.026	29.2	6.27
Hass-2	22.0	0.101	0.021	23.1	5.93
2500	17.0	0.074	0.018	16.9	5.44
3385	15.0	0.023	0.006	2.7	0.09
LSD (0.05)	1.7	0.009	0.002		

N.S. = No significant difference.

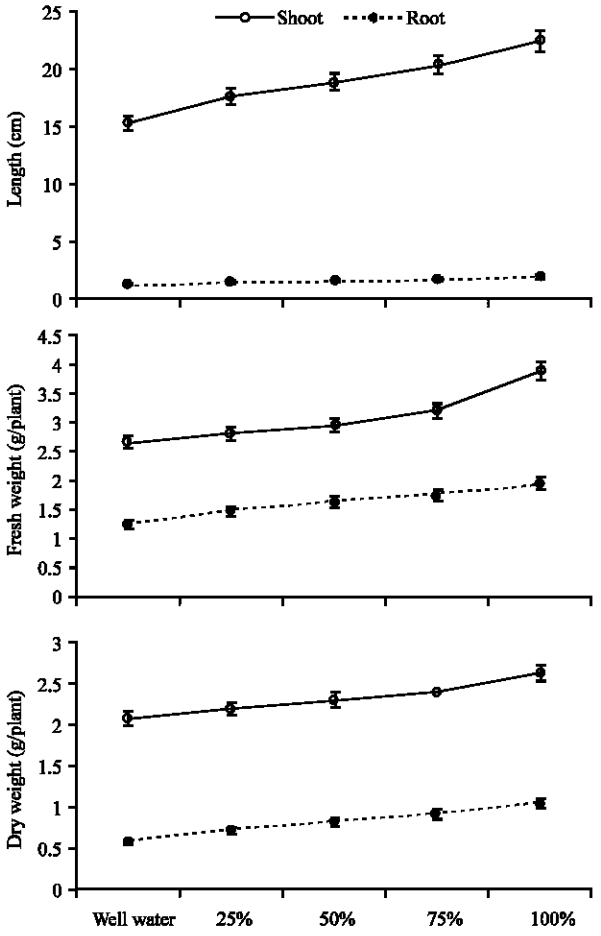

Fig. 1: Shoot and root lengths, fresh and dry weight of faba bean as influenced by different effluents. Mean of three replicates±SE

Growth attributes: Both effluent and *Rhizobium* treatments caused significant variation in all the measured parameters, irrespective of some minor fluctuations (Fig. 1,2 and Table 3). The value of all growth attributes were higher where faba bean was grown with increasing amount of effluent as well as when seeds were inoculated with local strains specially with Hass-1. During the study period, relative growth attributes were generally greatest for 100 and 75% effluent treatments, intermediate for the 50% effluent treatment and least for the two remaining treatments i.e. the 25%. Effluent and well water, although all differences between effluent treatments compared to well water were significant (Fig. 1,2 and Table 3).

Comparing across *Rhizobium*strains, strong patterns in growth attributes were apparent. Suggesting greatest effect of local strains (Hass-1 and Hass-2) on faba bean

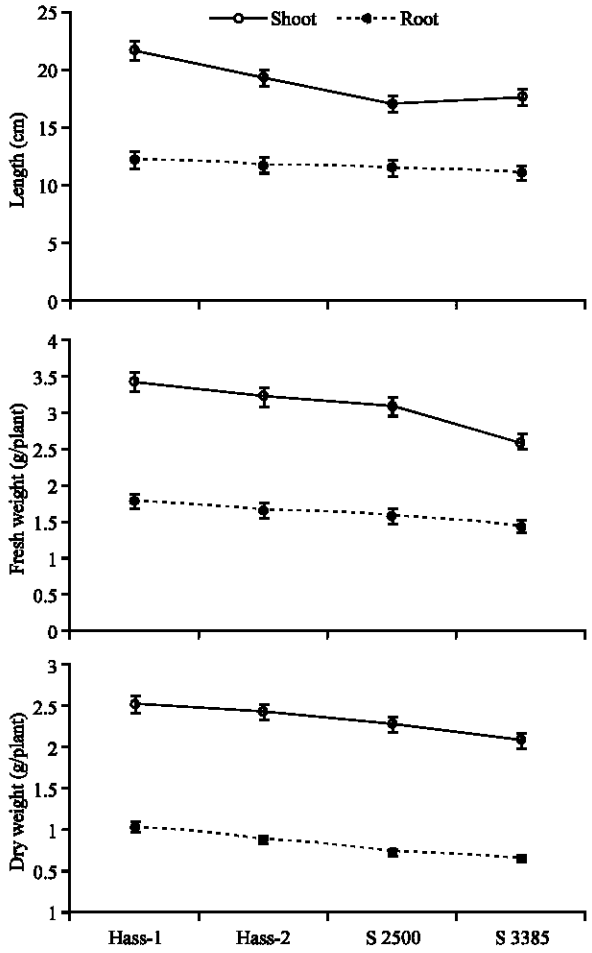

Fig. 2: Shoot and root lengths, fresh and dry weight of faba bean as influenced by different rhizobia strains. Mean of three replicates±SE

growth (Table 3). Maximum stem and root growth and nodulation were recorded for the local strains inoculated faba bean plants and least for faba bean plants inoculated with introduced strains (Fig. 1,2 and Table 3). Differences between strains were significant for all parameters studied. Seedling biomass was significant greater for seedling inoculated with Hass-1 compared to the other three rhizobia strains. Hass-1 appeared to exhibit high activity in nodulation and nitrogen fixation which may be due to its high adaptability to soil condition in Al-Hassa. These findings are in agreement with several previously published data (El-Nennah *et al*., 1982; El-Hassanin *et al*., 1993; El-Kadi and Quallaf, 1995).

Nitrogen fixation: Table 3 shows the effects of effluent and rhizobia strains on nitrogen fixation of faba bean seedling. The data shows that the application of effluent significantly increased the nitrogen fixation. These results suggest that more of the nitrogen in the effluent is used to establish seedlings in earlier stages independent to nitrogen fixation which is reflected in better seedling growth and higher number of nodules with larger sizes. At the sandy soils of Al-Hassa, the soil was very low in available nitrogen and fertilizer N appeared to be leached quickly by irrigation.

The four rhizobia strains exhibited quite different proportional responses in $N(C_2H_2)$ fixation. The best symbiotic performance in terms of nodule mass, $N_2(C_2H_2)$ fixation and nitrogen accumulation in shoots was achieved by inoculation with local strains (Table 3). The highest rate of N_2 fixation resulted from inoculation with strain Hass-1, which allowed an increase of 15.3% of nitrogen accumulated in faba bean shoots in relation to plants inoculated with the strain 3385. The two local strains used in this study showed higher nodulation activity than introduced strains (Table 3). Effluent irrigation increased N accumulation regardless of strain used for inoculation (Table 3). 100% treatment accumulated more N in the shoot of faba bean seedlings than all other treatments. The results in Table 3 further show that 100% effluent irrigated faba bean exhibited the largest increases in fixation and accumulation of nitrogen, when locally isolated rhizobia strains were used, probably because introduced strains had less $N_2(C_2H_2)$ fixation efficiency under Al-Hassa conditions(El-Nennah *et al*., 1982; El-Kadi and Quallaf, 1995; El-Hassanin *et al*., 1993; El-Komy, 2001, 2005).

Generally, the results of this experiment indicate that locally isolated strains of R. leguminosarum inoculated faba bean plants fixes much greater N_2 than when introduced strains are used. The results of this study also suggest that differences in seedling growth caused by effluent irrigation strongly influence nodulation, nitrogen fixation and nitrogen accumulation in faba plant tissues.

ACKNOWLEDGMENT

The author is grateful to the Deanship of Scientific Research, King Faisal University, Al-Hassa, Saudi Arabia for its financial support of this study. I extend a special thanks to my colleagues in the Biology Department, College of Science, King Faisal University for their extensive logistical and moral support during the course of this research project.

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