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Biofertilization Efficiency Affected by Quality of Irrigation Water and their Effect on Microbial Activity and Soybean Plant Growth

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AT GREEN house, Soil Science, Department, Faculty of Agriculture, Menoufia University , Shibin El-Kom, Menoufia Governorate, Egypt during growing summer season of 2022 this study was carried out as a pots experiment. This study was established to evaluate the efficiency of biofertilizers affected by quality of irrigation water and their effect on biological activity, N-fixation and soybean (Glycine max L.) plant growth. The used biofertilizers were RUK- 3407 *Bradyrhizobium japonicum* (R1) and ARC-SWERI-4 *Bradyrhizobium japonicum* (R2). Also, the used resources of irrigation water were well water (WW), treated agricultural drainage water (DW) and treated sewage water (SW) and two mixed water (1:1 and 2:1) of DW and SW, respectively. With the same biofertilizer and according to the found nodules number and its weights (mg/ plant), the used resources of irrigation water takes the order WW > DW > DW+SW (2:1) > DW+SW (1:1) > SW.As well as with all irrigation water resources, these measurements increased significantly with biofertilization inoculation, where found increases in the plants inoculated by R2 that were higher than those resulted from inoculation with R1. Also, similar effect was found with length roots and shoots (cm) as well as fresh and dry weights (g/ plant) of soybean plants value. Concentricity (%) and uptake (mg ⁄ plant) of N, P and K by shoots of soybean plants varied significantly within the studied treatments. The highest content of N, P and K was found in the plants irrigated by WW, while the lowest were found in the plants irrigated by SW. Also, the contents in the plants fertilized by R2 were higher than these inoculated by R1. Nitrogenase activity appeared to have a high response for irrigation water quality and biofertilization. With the same biofertilizers, the highest value of nitrogenase was found in the soil irrigated by WW, while the lowest one was found in the soil irrigated by SW. As well as, inoculation with R2 increased nitrogenase activity than that from inoculation with R1. This means that, biofertilizers inoculation is very important with irrigation using low quality resources of water irrigation.

Keywords: Soybean, Water quality, Biofertilization, Nodulation and Nitrogenase activity.

1. Introduction

Soybean (*Glycine max* L.) Merill a grain legume is regarded as a marvellous crop because of its geminate characteristics *viz*., high percentage of protein (40-43%) and oil (20%) (Anwar *et al.*, 2010 and Coskan and Dogan., 2011). Oad *et al*., (2002) indicated that *rhizobium japonicum* indicated a favorable change as to growth and seed yield of soybean. A lot of several microbial biofertilizers are plenty for agricultural usage and they increase plant growth and yield and improve soil properties, owing to the addition of useful microbial inoculants to soil as well as by stimulation of soil microorganisms (El-Yazeid *et al*., 2007).

The application of bio-fertilizers, namely *Bradyrhizobial* inoculant and PSB fertilizer, yielded notable increases in the upsurge of nitrogen by 13.64 to 30.00% and phosphorus by 17.17 to 41.12%, compared to traditional agricultural practices as disclosed by *Son et al*., (2007). Contrastingly, Dikand *et al*., (2012) revealed considerably lower results with regards to dry matter, N and grain yield, nodulation efficiency along with nitrogen fixation of soybean when employing solely N-fertilizers as compared to any *bradyrhizobium* inoculations.Moreover, it was discerned that a level of Nitrogen fertilization as high as 50 mg N kg^{-1} soil had detrimental impacts on soybean growth health whereas adopting a combined application strategy consisting only of merely 10 mg N kg^{-1} soil defined as an "N starter" coupled with inoculation via any strain of *bradyrhizobium* substantially improved key parameters such as soybean growth measured through plant height at harvest stage among other

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metrics like nodules per plant count including nodule magnitude per plant ratio besides general fresh weight statistics. Furthermore, integrating a dual-inoculation approach incorporating *Bradyrhizobium japonicum* (TAL-378) along with PSB significantly boosted parameters such pre-harvest shoot height during late flowering phase followed by early pod setting period apart from number seeds produced for each pod keeping pods per plant in check while delivering optimum seed yield ha^{-1} accompanied by efficient nutrient soaking in contrast against control environments according to insights published by Argaw (2012). Inferior quality of water can precipitate the breakdown of the infection process and is identified as a paramount factor leading to unsuccessful symbiosis. Legume trees have the capacity to intake wastewater effluent along with heavy metals through their root systems, thus playing a potent role as biological filters and constraining contamination from infiltrating groundwater resources. The legume-Rhizobium linkage particularly involving nodulation and nitrogen fixation is susceptible to water quality; hence substandard water quality could hinder legume development and deplete crop yield. Both legumes and the nodulation procedure exhibit heightened sensitivity towards osmotic stress in comparison to Rhizobium strains (Junior *et al*., 2005; Daudin and Sierra, 2008; Marie, 2019, Tantawy *et al*., 2019b). The treated wastewater contains organic matter and nutrients that can perfection soil and crop productivity. Presently, wastewater is being used for the return of degraded land and growth of commercial and environmental crops (Al-Fredan, 2006). After first treatment, the wastewater becomes safe for irrigation of non-food crops, such as tree plantations, and forestlands (Hassan *et al*., 2006; and Tabari and Salehi, 2009). Prior studies pointed that using sewage effluent for irrigation reform the soil properties and some growth parameters of forest trees, such as biomass potential, biomass allocations, specific gravity of the wood, fiber length, and volumetric shrinkage (Guang *et al*., 2010 and Ali *et al*., 2011).

Diazotrophic microorganisms are able to fix atmospheric N through biological nitrogen fixation (BNF) process (Raja *et al.,* 2006 and Mia and Shamsuddin, 2010). This process helps the agriculture sector especially for minimizing the cost of inorganic nitrogen fertilizer. It is due to the capability of microorganisms to transform atmospheric N into ammonia (NH_3) which is applicable by plants. Thus, most of the studies in this field of research were made to improve the N_2 fixation activities (Rebah *et al.,* 2007). The most significance factor for effective BNF process is appearance of nitrogenase enzyme that catalyse the fixation process (Sur *et al.,* 2010). The reaction involved is as follows (Giller, 2001):

$N_2 + 8H^+ + 8e^- + 16ATP$ Nitrogenase $2NH_3 + H_2 + 16ADP + 16Pi$

The nitrogenase enzyme catalyzes the reduction of Nitrogen (N_2) into Ammonia (NH_3), a process including both protons and electrons as well as yielding an evolution of Hydrogen (H_2). Accordingly, assessing N_2 fixation activities is conveniently achieved via H_2 production, which presents minimal disturbance to plant matter during analysis (Kumari and Kumar, 2018 and Kour *et al*., 2020).

The aims of this study may be listed in the following points.

1- Study the individual and combined effect of both irrigation water quality and type of biofertilizers on a: soybean plant growth and its content of nutrients and b: Microbial and enzyme activities.
2- Study the effect of irrigation water quality on the efficiency of biofertilization.

2. Materials and methods

Soil location and soil sampling

This study was executed on alluvial soil of the Experimental Farm, Faculty of Agriculture, Menoufia University, Shibin El-Kom, Menoufia Governorate, Egypt (Latitude 30° 33' 25.2659" N) (Longitude 31° 0' 49.3334" E (Altitude "MSL" 16m Horizontal Accuracy 49m, Vertical Accuracy 17m. It was established to evaluate the effect of irrigation water quality (treated waste water) and inoculation with *Bradyrhizobium japonicum* strains individually and in combination on microbial activity and the productivity of soybean plant (*Glycine max* L.) (Giza 111) **(Figure 1).**

Soil specimens (0-30 cm depth) were taken from the experimental soil, air-dried, ground, mixed, sieved through a 2 mm sieve and kept. A portion of this sample was taken and analyzed for some physical and chemical properties and its content of total and available N, P and K according to the methods obtained by Cottenie *et al*., (1982), Page *et al.*, (1982) and Klute (1986). Data are recorded in Table (1).

Soybean seeds

Seeds of soybean (Giza 111) were taken from Crops Institute Research, Agricultural Research Center (ARC), Egypt.

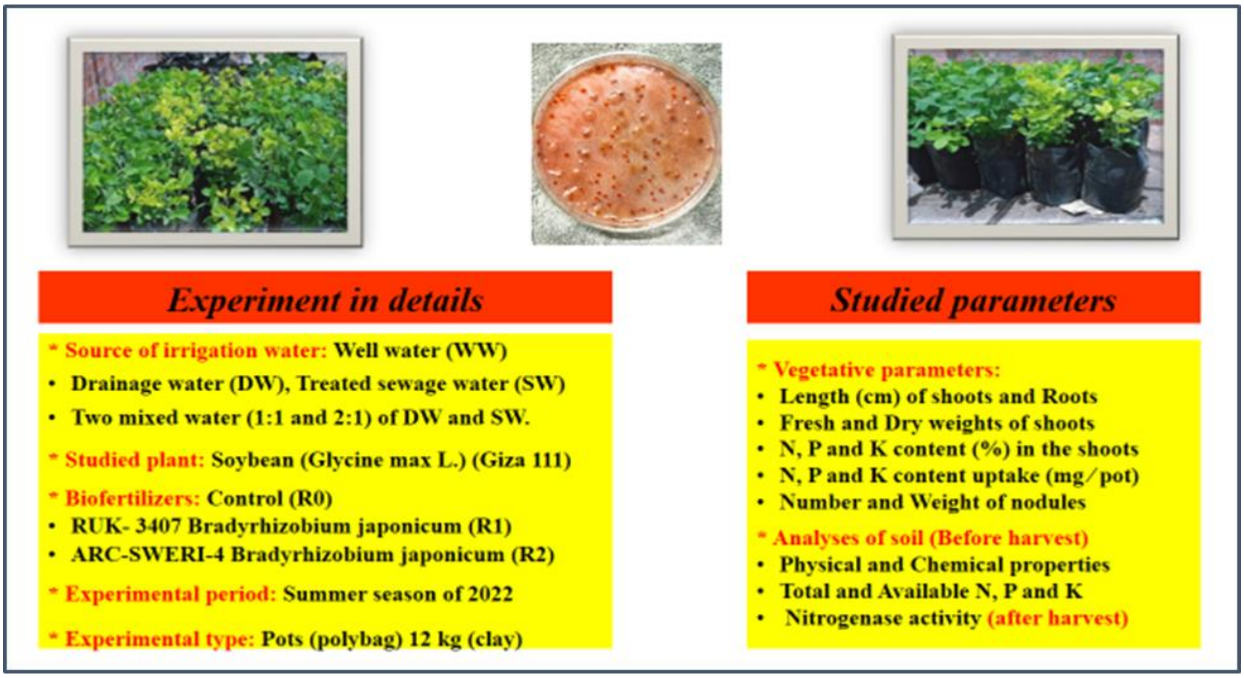


Fig. 1. Summary of the experimental performance.

Table 1. Some physical and chemical properties of the studied soil and its content of some nutrients.

a. Physical properties

Particles size distribution (%)				Field Capacity
Sand	Silt	Clay	Textural class	
33.7	23.6	42.7	Clay loam	26.59

b. Chemical properties

Organic matter (%)	$CaCO_3$ (%)	pH (1:2.5) soil:water suspension	EC, 1:5 (soil:water) extract dS m^{-1}	Soluble ions (cmolc kg^{-1} soil)							
				Cation				Anion			
				Ca^{2+}	Mg^{2+}	K^+	Na^+	CO_3^{2-}	HCO_3^-	Cl^-	SO_4^{2-}
1.9	2.8	7.3	0.6	0.8	0.6	0.2	1.3	0.0	0.5	1.7	0.7

c. Nutrient contents (mg kg^{-1})

N		P		K	
Total	Available (extracted with 1 N KCL)	Total	Available (extracted with 0.5 M $NaHCO_3$)	Total	Available (extracted with 1 N ammonium acetate)
160	59.13	100	9.66	610	271.03

Biofertilizers

The used biofertilization in this study was carried in two *Bradyrhizobium* strains of soybean plants which namely RUK- 3407 *Bradyrhizobium japonicum* (R1) and ARC-SWERI-4 *Bradyrhizobium japonicum* (R2) from Agricultural Microbiology Research Department, Soils, Water and Environment Institute Research, Agricultural Research Center (ARC), Egypt. Inoculum for these experiments was received by growing *Bradyrhizobium* strains in Yeast Mannitol Broth (YMB) medium (Vincet 1970). All strains were maintained in YMB and cultured routinely at 28 ^{0}c in YMB on a rotary shaker operating at 65 cycles min^{-1} overnight . The cells were washed once in sterile distilled water and the cell suspensions adjusted to 2.8×10^7 cell ml^{-1} based on OD readings (an OD595 of 1 is equivalent to 10^9 cells ml^{-1}). All bacterial strains were obtained by the following mixtures (Table, 2):

Table 2. *Bradyrhizobium japonicoum* used in the experiment.

Strain	Source
R1	(RUK- 3407 *Bradyrhizobium japonicum*) Rothamested Experimental Station, Herpenden, -U.k.
R2	(ARC-SWERI-4 *Bradyrhizobium japonicum*) Agricultural Research Center (ARC), Soils Water and Environment Research Institute, Unit of Biofertilizers Production Giza, Egypt. Location of Strain Isolate: Sudan – Damazen.

Irrigation water resources

In this study, three resources of irrigation water varied in their chemical composition were used. These resources were well water (WW) which collected from artizian well at Experimental Farm Faculty of Agricultural "Soil Science Department" Menoufia University, Shibin El-Kom, Egypt, treated agricultural drainage water (DW)and treated sewage water (SW) which taken from, Eltahreer region, Behaira Governorate, Egypt**.** Separately. Water sample were collected according to the standard methods of American Public Health Association (APHA,1985). The following steps were carried out during sampling: 1-Water samples were collected from the middle of selected region. 2- All samples were collected at morning time. 3- pH and electric conductivity were measured immediately in the water stream itself. 4- Water sample of each resource was analyzed for its chemical composition using the procedures explained by Cottenie *et al*., (1982) and Page *et al.,* (1982). Among these parameters are biological oxygen demand (BOD) is used as a measure of quantity of oxygen required for oxidation of biodegradable organic matter present in the sample by aerobic biochemical action (standard methods) (APHA,1985). The chemical oxygen demand (COD) test is used to measure the content of organic matter of a sample. The oxygen equivalent of organic matter that can be oxidized is measured by strong chemical oxidizing agent (potassium dichromate) in an acidic medium. Chemical oxygen demand (COD) was measured by potassium dichromate method according to ISO, (1990). Biological oxygen demand after five days (BOD) was determined by using dilution and seedling method according to ISO, (1990). Data are recorded in Table (3).

In addition, two mixed water of DW and SW at rate of 1:1 and 2:1, respectively were used in this study in irrigation.

Table 3. Chemical composition of the studied irrigation water resources.

Irrigation water resources	pH	EC dSm^{-1}	Soluble ions meq /l								(mg \l)	(mg\l)
			Cation				Anion					
			Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}	CO_3^{2-}	HCO_3^{-}	Cl^{-}	SO_4^{2-}	BOD	COD
WW	7.10	1.10	2.7	1.9	0.6	5.8	0.00	0.80	6.50	3.70	3.00	10.00
DW	7.54	1.99	1.08	0.88	0.83	17.2	0.00	1.17	13.18	5.64	30.33	200.00
SW	6.70	5.20	11.00	1.00	0.40	39.60	0.00	1.80	30.0	20.20	90.00	320.00
DW+SW (1:1)	6.90	3.30	5.24	0.90	0.50	26.32	0.00	1.14	20.00	11.55	55.54	270.00
DW+SW (2:1)	7.10	2.66	4.36	0.97	0.70	20.61	0.00	1.34	16.40	8.90	40.37	240.00

Greenhouse experiment

The study was undertaken in greenhouse of Soil Science Department, Faculty of Agriculture Menoufia University as pot experiments using plastic pots (54 pots) of 30 cm inter diameter and 60 cm depth. In the first, the pots were sterilized by washing well with 95% (v⁄v) ethanol, sterilization was requested to remove possible contamination by bacteria other than the treatments. Each pot was filled with 12 kg of the sieved soil. This experiment was carried out during the grown summer season of 2022.

The pots were divided into three main groups, (18 main group) representative inoculation treatments (without and with R1 and R2 as biofertilizers. The pots of each main group were divided into five subgroups (3 pot ⁄ sub groups) representing the treatments of irrigation water resources (WW, DW, SW, DW+SW"1:1" and DW+SW"2:1").

Three grams of Rhizobium of each strain were blended with 150 g of soybean seeds. The seeds were directly scattered 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, where each pot was planted by six uninoculated or inoculated seeds. All pots were irrigated (irrigation water treatments) at moisture content of soil field capacity

(26.59%). After completely seeds germination (15 days), the plants of each pot were thinned to four plants.

According to the recommendation of Egyptian Ministry of Agriculture for soy bean, all pots were fertilized before planting, with superphosphate (15.5 % P_2O_5) at a rate of 200 kg/fed (feddan = 4200 m^2) (2.40 g pot $^{-1}$). As well as all pots were supplied with potassium sulphate (48%K_2O) at a rate of 100 kg/fed (1.20 g pot $^{-1}$), and ammonium nitrate (33% N), at a rate of 25 kg / fed (0.30 g pot $^{-1}$). Irrigation process was carried out every three days.

After 60 days of sowing, whole plants of each replicate were taken separately. The harvested plants of each pot were divided into shoots and roots, washed well with tap water and distilled water to remove the soil particles from the roots, and weighed to record the fresh weight (g pot $^{-1}$). Then root nodules created on the roots were counted and recorded for each plant. Some vegetative growth parameters including shoots and root length (cm) as well as shoots, root and nodules fresh and dry weights were recorded. After that, the plant samples (roots and shoots) were oven – dried at 70 ^{0}C for 48 hrs. Weighted to found the dry matter yield.

Separately, 0.2 g of each oven – dried plant sample (roots and shoots) was digested in 5 ml mixture 3:1 of concentrated H_2SO_4 and $HClO_4$ on sandy plate at 250 ^{0}C up to obtain on clear digest (Chapman and Pratt,1961). The clear digest was diluted up to 50 ml using distilled water. The digest content of N was determined using semi Microcheldahl procedure as described by Cottenie *et al*., (1982). Also, the content of P was determined colourimetrically following the method of Cottenie *et al*., (1982). As well as the content of K was determined as described by Jackson (1973).

Acetylene Reduction by Detached Nodules

Acetylene Reduction by detached nodules. Nodules were elected from the root such that each set to be compared had nodules of equal size. Almost 50 mg freshly selected nodules were weighed, were sliced once with a clean razor blade, and were placed in in 50 ml vials each containing 30 ml of semi-solid YEM medium and the vials were then sealed with serum and left for 36 hours after the inoculation (Somasegaran and Hoben, 1994). The vials were stoppered with serum caps and filled with 10% (by volume) of high rarity acetylene (C_2H_2) gas by a hypodermic syringe; ethylene (C_2H_4) manufacture was determined after 1- and 24-hour incubations in the dark at 28°C. Vials without C_2H_2 were also included to determine the endogenous C_2H_4. At the latest of incubation time before analysis, the vials were thoroughly shaken in order to equilibrate the head space above the medium trapping ethylene. Accommodation of the samples, their incubation and analysis were performed as described by Rao *et al.*, (1983). For limitation of C_2H_4, 0.5 ml of the gas phase from each tube (three replications) were injected into a gas chromatograph (a Hewlett Packard 5890A gas chromatograph equipped with a-ionization detector and a packed column, 1.83 m long, 0.318 cm i.d., stainless steel, packed with HayeSep N; Supelco). Nitrogenase activity (means of three replicates) was expressed as µmoles of C_2H_4, formed ml^{-1} of isolates per 24h and was then calculated by the following formula:

$$\mu mole\ C_2H_4/ml\ culture = \frac{R\ X\ Container\ volume(vial)}{Time\ of\ incubation \times Inj.Vol. \times D \times 22.4} \times 100$$

Where:

Inj. Vol = injection volume

R= GC reading D=Volume of the medium (ml)

All values obvious on a nodule fresh weight basis can easily be converted to a nodule dry weight basis by using the ratio fresh weight: dry weight =5:1. The results were represented as µmole C_2H_4 ∕ gm dry nodule ∕ hr.

Statistical Analyses

The studied treatments (15 treatments) were distributed within the experimental units in spilt randomized block design (Two-way experiment with interaction, data analyzed using CoStat statistical analysis Program (version 6.4) with three replicates (45 units).

3. Results and Discussion

1. Nodulation activity

Data in Table (4) show both individual and combined effect of irrigation water quality and biofertilization on nodules number per plant formed on the roots of soybean plant at old of 70 days. These data clear that, nodules number greatly affected by the used resources of irrigation water, where according the found nodules number with the same biofertilizer, these water resources takes the order WW > DW > DW+SW (2:1) > DW+SW (1:1) > SW. This order show that, using DW and SW resulted in a significant decrease of formed root nodules, where with the same treatment of biofertilization the highest decrease was found with the plants irrigated by SW. For example in the plants fertilized by R1, the rate of these decreases in compared with the WW data were

- 45.14, - 70.02, - 61.78, - 51, 73 with water resources of DW, SW DW+SW (1:1) and DW+SW (2:1) respectively. These findings attributed mainly to the effected of irrigation water quality on both soil properties (especially soil pH, EC and the content of some toxic materials) and plant healthy ,where increase in soil pH and EC reduced the microbial activity in the root zone of growing plants (Junior *et al.*, 2005 and Daudin and Sierra, 2008) and AbdElAzeem and Hokam,2014).Also, Tantawy *et al.*,(2019b) found a significant decrease in the nodules number formed on the roots of common bean plants under calcareous soil condition with the increase in irrigation water salinity. Also, the high decrease in the nodules number with irrigation by SW because of the increases in soil content of toxic elements which associated by noxious effect on plant growth (Al-Fredan, 2006 and Ali *et al*., 2011).

Regarding to the effect of biofertilization on the nodules numeral made up of the roots of soybean plants, the data in Table (4) show that, with all irrigation water resources used in this study, inoculation using R1 and R2 as a biofertilizer resulted in a significant increase in the nodules number, where the found increases resulted from R2 inoculation were higher than these resulted from inoculation with R1. For example, with WW, the nodules number were 6.07, 46.60 and 54.15 ⁄ plant with the treatment of R0, R1 and R2, respectively. With the same resource of irrigation water, the found decrease rate in nodules number in compared with those found with WW was lower in the inoculated plants than these found in the non-inoculated plants. These findings mean that inoculation by biofertilizers reduced the harmful effect of low-quality irrigation water on microbial activity (Abd El Azeem and Hokam, 2014, Omara *et al* ., 2017, Marie, 2019 and Tantawy *et al*., 2019b).

Table 4. Individual and combined effect of irrigation water quality and biofertilizer on number of nodules and root nodules weight (mg ⁄ plant) of Soybean.

Irrigation water resources	Number of nodules ⁄ plant				Root nodules weight (mg ⁄ plant)			
	R 0	**R1**	**R2**	**Mean**	**R 0**	**R1**	**R2**	**Mean**
WW	6.07k	46.66b	54.35a	35.69a	3.25h	26.33bc	29.00a	19.53a
DW	3.33l	30.75e	42.29c	25.46b	2.86h	24.67c	27.00b	18.18b
SW	1.82m	8.54j	12.41i	7.59e	0.59i	7.67g	8.83g	5.70e
DW+SW(1:1)	2.32lm	16.12h	25.03f	14.49d	0.86i	14.00e	16.90d	10.59c
DW+SW(2:1)	2.93lm	22.11g	36.23d	20.42c	1.52hi	10.88f	13.36e	8.59d
Mean	3.29c	24.84b	34.06a	20.73	1.82c	16.71b	19.02d	12.52
ANOVA RESUITS								
A	***				***			
B	***				***			
A.B	***				***			

WW, DW, and SW = Well, drainage and sewage water, A: water resources, B: Biofertilizers, A.B: Interaction between A, B.

In addition, the data in Table (4) obtain a significant effect of both individual and combined treatments of water quality and inoculation with biofertilizers on nodules weight as mg ⁄ plant. With the same treatment of biofertilizers inoculation, the highest weight of nodules was found in the plants irrigated by WW followed by that result from irrigation by DW and the lowest one was found in the plants growing on the soil irrigated by SW. These results are in good relation with the effect of the used irrigation water resources on soil bio-chemical properties and plant growth as explained before that by Marie (2019) and Tantawy *et al*.,(2019b). The nodules weights ranged between 2.25, 26.33 and 29.00 mg ⁄ plant in the irrigated plants by WW to 0.50, 7.67 and 8.83 mg ⁄ plant in the plants irrigated by SW with inoculation by R0, R1 and R2, respectively. The decrease in nodulation activity in the root zone with the increase in salinity of irrigation water was pointed out before that by Marie, 2019 and Tantawy *et al*., (2019b).

As well as the data in Table (4) cleared the positive and increase effect of biofertilizers applications with the same resource of irrigation water, the highest nodules weight was found in the plants inoculated by R2, this explains the high efficiency of R2 in compared R1.

The differences in the efficiency of biofertilizers on nodulation activity under calcareous and alluvial soil planted by faba bean plants were pointed out before that by Raverkar and Tilak (2002) and El Noamany (2014). Therefore if low quality water used in irrigation application of biofertilizers is very important to decrease the harmful resulted from these water resources. Also, inoculation of soy beans with rhizobia across the world is evaluated to be in the range of 12-20 x 10^6 $year^{-1}ha^{-1}$ which results in the establishment of a large rhizobial population in the rhizosphere thereby improving nodulation and nitrogen fixation (Senevirante *et al*., 2000).

2- Shoots and roots length

Lengths of both shoots and roots of soybean plant takes as a vegetative growth parameter influenced by the studied treatments of water quality and biofertilization (El-Saied and Rashwan 2021). The found data in Table (5) obtain a greater effect of the study transactions on the length of soybean shoots and roots where their appeared wide range which ranged from 39.76 and 15.20 cm in the plants irrigated by SW without any biofertilization (R0) to 93.89 and 44.41 cm with the combined treatment of WW and R2, respectively. This means that, the lowest length of soybean shoots was found with the individual treatment of SW, while the highest value was found in the plants the combined treatment of WW and R2. With the same treatment of biofertilization and based on the found lengths of shoots and roots, the used irrigation water resources take the order: WW> DW > DW+SW (2:1) > DW+SW (1:1) > SW. This trend reflect the effect of the used water qualities on different soil properties and plant growth (Tantawy *et al*., 2019b). Daudin and Sierra (2008) and Marie (2019) mentioned that, low water efficiency can lead to deficiency of the infection process and deemed the original source for ineffective symbiosis.

Table 5. Individual and combined effect of irrigation water quality and biofertilizer on length (cm) of shoots and Roots of Soybean plant.

Irrigation water resources	Shoots length (cm / plants)				Root length (cm / plants)			
	R 0	**R1**	**R2**	**Mean**	**R 0**	**R1**	**R2**	**Mean**
WW	71.56e	88.06b	93.89a	84.50a	37.34c	42.07ab	44.40a	41.27a
DW	67.97f	76.13d	84.80c	76.30b	30.41e	36.45cd	40.41b	35.76b
SW	39.76k	50.98i	62.21gh	50.98e	15.20i	19.44h	20.45h	18.36e
DW+SW(1:1)	61.39h	71.38e	77.09d	69.95c	20.93h	24.82g	27.12fg	24.29d
DW+SW(2:1)	47.30j	63.50g	68.26f	59.69d	28.43ef	34.38d	37.28c	33.36c
Mean	57.60c	70.01b	77.25a	68.29	26.46c	31.43b	33.93a	30.61
ANOVA RESUITS								
A	***				***			
B	***				***			
A.B	***				ns			

WW, DW, and SW = Well, drainage and sewage water, A: water resources, B: Biofertilizers, A.B: Interaction between A, B

Also, data in Table (5) show the significant increase effect of biofertilizers (R1 and R2) inoculation on the length of soybean shoots and roots, where the found lengthes of soybean plants (shoots and roots) in the plants inoculated by R2 were higher than these found with treatment of R1. For example, with WW, the lengthes of shoots and roots were 88.06 and 42.07 cm with R1 inoculant and were 93.89 and 44.40 cm with R2 inoculant respectively. Before that, the difference effect of inoculation on vegetative growth parameters (faba bean and wheat plants) was obtained by El Noamany (2014). Singh (2005) and Son and Giang (2006) showed that inoculation of seeds with *Bradyrhizobium* strain gave significantly taller plants with more nodules, pods/plants, grains/pod and seed weight than uninoculation seeds. Yield of soybean increased by virtue of inoculation.

3. Fresh and dry matter yields

The data of fresh and dry matter yield (FY and DY) as g / pot of soybean shoots listed in Table (6) show a wide response to the quality of irrigation water resource and inoculation with biofertilizers individually and in combination, where this response were significant. With the same treatment of inoculation, the highest FY and DY were found in the plants irrigated by WW followed by these found in the plants irrigated by DW, while the lowest values were associated the irrigation by SW. For example, without inoculation of biofertilizers (R0), the obtained weights of FY and DY were 173.57 and 80.79, 132.53 and 60.37; and 115.58and 52.49 g / pot with irrigation water resources of WW, DW and SW, respectively. This mean that, increase salinity level of irrigation water resulted in a decrease of both FY and DY of soybean (shoots) plant. In this respect, Omara *et al.,* (2018), Marie (2019) and Tantawy *et al*., (2019b) obtained on similar results.

In addition, inoculation with R2 resulted in a more increase of FY and DY of soybean (shoots) plants (Table, 6), where this increase was significant. The increases in FY and DY as a result of biofertilizers inoculation were found with the tested resources of irrigation water. These findings means that, inoculation of biofertilizers improved the environment of root zone such as increase in the nutrients availability and microbial activity (Raverkar and Tilak., 2002 and Son *et al*., 2007).For example, FY was ranged between 173.57 and 115.58; 207.30 and 140.60; and 224.33 and 154.01 g ⁄ pot and DY was ranged between 80.29 and 52.49; 100.91 and 68.18; and 116.41 and 75.65 g ⁄ pot with irrigation water resource of WW and SW, respectively.

4. The content of macronutrients

Shoots of soybean plant concentration (%) and uptake (mg ⁄ plant) affected by irrigation water quality and biofertilizers inoculation were determined and the obtained data are listed in Tables (7 and 8). These data show a wide range in N, P and K contents (% and mg ⁄ pot) depending on water quality and of biofertilizer. With the same treatment of biofertilizers, the high percent (%) of N,P and K in the shoots were found in the plants irrigated by SW followed by those in the plants irrigated by DW+SW(2:1) and the lowest values were found with use WW in irrigation . For example, N,P and K percent in the plants non received biofertilizer were 0.98 , 1.50 and 2.14 % for N; 0.48 , 0.61 , 0.66 % for P and 0.70 , 1.18 and 1.24 % for K with WW,DW and SW, respectively. These findings are in harmony with the used water resources content of these nutrients. Before that, Basiouny (2016) and Rabie (2019) reported that, the plants irrigated by sewage water have a high content (%) of N, P and K compared by those found in the plants irrigated by agricultural drainage water or Nile River water. They added that, the plants (roots and shoots) irrigated by sewage water (un or treated) have a high content of N,P ,K, other nutrients and trace elements compared with those found in the same plants irrigated by agricultural drainage water and Nile water at Menoufia Governorate, Egypt.

In addition, data in Table (7) clear that, with the same resource of irrigation water there are a significant increase in soy bean plants (shoots) content (%) of N, P and k as a result of biofertilizers applications with wide variations within these increases which varied from biofertilizer to another.

Table 6. Individual and combined effect of irrigation water quality and biofertilizer on fresh and dry weights of soybean shoots (g ⁄ pot).

Irrigation water resources	Fresh weights (g ⁄ pot)				Dry weights (g ⁄ pot)			
	R 0	R1	R2	Mean	R 0	R1	R2	Mean
WW	173.57d	207.30b	224.33a	201.73a	80.29ef	100.91b	116.41a	99.20a
DW	132.35i	170.54de	184.48c	162.46b	60.37k	84.43d	93.18c	79.33b
SW	115.58k	140.60h	154.01g	136.73e	52.49m	68.19j	75.65h	65.44e
DW+SW(1:1)	121.59j	162.21f	170.23e	151.34c	57.84l	76.37gh	83.14de	72.45c
DW+SW(2:1)	110.76l	154.30g	162.10f	142.39d	54.75lm	72.11i	79.11fg	68.66d
Mean	130.77c	166.99b	179.03a	158.93	61.15c	80.40b	89.50a	77.02
ANOVA RESUITS								
A	***				***			
B	***				***			
AB	***				***			

WW, DW, and SW = Well, drainage and sewage water, A: water resources, B: Biofertilizers, A.B: Interaction between A, B

Table 7. Individual and combined effect of irrigation water resources and biofertilizer on N, P, K content (%) in the shoots of soybean plants.

Irrigation water resources	N (%)				P (%)				K (%)			
	R0	R1	R2	mean	R0	R1	R2	mean	R0	R1	R2	mean
WW	0.98i	1.63ef	2.17d	1.59e	0.48i	0.60gh	0.81cd	0.63c	0.70g	0.77g	0.90efg	0.79c
DW	1.50fg	2.50c	3.11b	2.37b	0.61gh	0.80cd	0.90ab	0.77a	1.18cde	1.37abc	1.48ab	1.34a
SW	2.14d	3.17b	3.50a	2.94a	0.66fg	0.84bc	0.96a	0.82a	1.24bcd	1.50ab	1.61a	1.45a
DW+SW(1:1)	1.35gh	2.19d	2.56c	2.03c	0.56hi	0.74def	0.76cde	0.69b	0.98cdef	1.18cde	1.26b	1.14b
DW+SW(2:1)	1.18hi	1.85e	2.27d	1.77d	0.51i	0.66fg	0.68efg	0.62c	0.82fg	0.98defg	1.10b	0.97b
mean	1.43c	2.27b	2.72a	2.14	0.56c	0.73b	0.82a	0.71	0.98b	1.16a	1.27a	1.14
ANOVA RESUITS												
A	***				***				***			
B	***				***				***			
AB	*				*				ns			

WW, DW, and SW = Well, drainage and sewage water, A: water resources, B: Biofertilizers, A.B: Interaction between A, B

As well as, with the same water resource, shoots content (%) of N, P and k in the plants inoculated by R2 were higher than those found with R1 inoculant. For example, the percent of in the plants irrigated by SW were 2.14, 0.66 and 1.24 % with R0, 3.17, 0.84 and 1.50 % with R1 and 3.50, 0.96 and 1.61 % with

R2, respectively. This example show the high importance of use biofertilizers when using low quality irrigation water in irrigation. These data are in similar before those obtained by Marie (2019).

On the other hand, data in Table (8) show reversible effect of the tested water resource on N, P and K uptake (mg ⁄ pot) by shoots of soybean plants with the three treatments of biofertilization (R0, R1 and R2). This mean that, the uppermost values of N, P and K uptake were found in the plants irrigated by WW and the minimum values were found in the plants irrigated by SW. These findings resulted from the high yields of shoots for the plants irrigated by WW than those in the plants irrigated by SW (Table, 6), or by other words these findings are attributed to the dilution effect of the yields on the elements content (Marschener, 2002). For example, in the shoots of the plants non resieved biofertilization N, P and K uptake were 786.84, 385.39 and 562.03 mg ⁄ pot in the plants irrigated by WW were 1123.29, 346.43 and 650.88 mg ⁄ pot with SW use in the irrigation . The effect of biofertilizers application on the uptake of N, P and K by soybean plants was similar with that recorded with its effect on the concentration (%) of these nutrients. This means that, biofertilization resulted in a significant increase of N, P and K with a superior increase effect of R2 compared with R1. For example, with SW treatments N, P and K uptake were 786.84, 385.39 and 562.03 mg ⁄ pot with R0 1644.83, 605.46 and 77.01 mg ⁄ pot with R1 and 2526.1, 942.92 and 1047.69mg ⁄ pot with R2 respectively. Nitrogen absorption has risen by 13.64 to 30.00 % and phosphorus absorption has risen by 17.17 to 41.12 % under the usage of bio-fertilizer (Bradyrhizobial inoculant and PSB fertilizer) in comparison with farmers' method (Son *et al*., 2007) and El-Ghamry *et al* ., (2018). Also, Dikand *et al.*, (2012) indicated that dry matter, N and germ harvest, nodulation and nitrogen fixation in N-fertilized soybean were the least in comparison with any *bradyrhizobium* strain. It was found that Nitrogen fertilization at 50 mg N kg^{-1} soil was damaging to soybean, but blending the application of 10 mg N kg^{-1} soil as N starter with any *bradyrhizobium* strain inoculation made significant improvement to soybean growth, N yields, nodulation, nitrogen fixation and grain yields.

Table 8. Individual and combined effect of irrigation water resources and biofertilizer on N, P and K content uptake (mg ⁄ pot) by shoots of soybean plants.

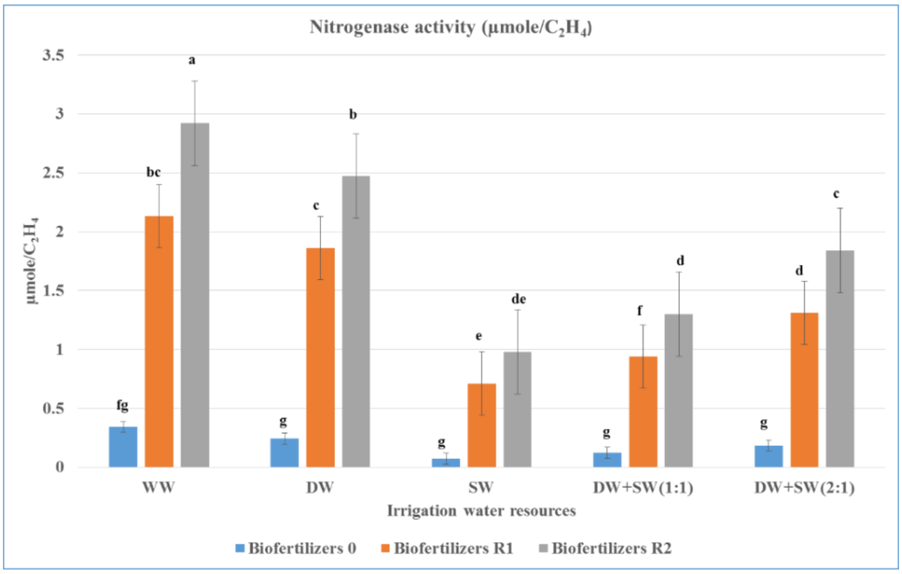
Irrigation water resources	N (%)				P (%)				K (%)			
	R0	R1	R2	mean	R0	R1	R2	mean	R0	R1	R2	mean
WW	789.3^{gh}	1649.0^{d}	2522.0^{b}	1653.4^{b}	385.3^{i}	605.3^{ef}	947.0^{a}	645.9^{a}	562.4^{hi}	776.9^{efgh}	1048.1^{bcd}	795.8^{b}
DW	883.1^{g}	2139.3^{c}	2899.6^{a}	1974.0^{a}	369.9^{ij}	674.8^{cd}	838.6^{b}	627.8^{a}	710.4^{fgh}	1156.6^{abc}	1378.8^{a}	1081.9^{a}
SW	1108.6^{f}	2155.0^{c}	2682.6^{b}	1982.1^{a}	340.6^{ij}	572.0^{efg}	725.5^{c}	546.1^{b}	642.5^{ghi}	1027.3^{bcd}	1221.3^{ab}	963.7^{a}
DW+SW(1:1)	773.5^{gh}	1677.0^{d}	2134.0^{c}	1528.1^{c}	322.2^{jk}	565.0^{fg}	629.3^{de}	505.5^{c}	558.6^{hi}	897.1^{def}	1019.8^{bcd}	825.2^{b}
DW+SW(2:1)	648.0^{h}	1333.0^{e}	1795.6^{d}	1258.9^{d}	279.2^{k}	457.2^{h}	540.7^{g}	425.7^{d}	448.9^{i}	949.3^{cde}	866.9^{defg}	755.0^{b}
mean	840.5^{c}	1790.6^{b}	2406.8^{a}	1679.3	339.4^{c}	574.9^{b}	736.2^{a}	548.4	584.5^{c}	961.4^{b}	1107.0^{a}	884.3
ANOVA RESUITS												
A		***				***				***		
B		***				***				***		
A.B		***				***				ns		

WW, DW, and SW = Well, drainage and sewage water, A: water resources, B: Biofertilizers, A.B: Interaction between A, B.

5- Nitrogenase activity

The data in the Fig (2) show nitrogenase activity rhizosphere of soybean plants at 70 old day under clay soil conditions affected by both irrigation water quality and types of biofertilization. These data clear that nitrogenase activity (µmole C_2H_4 ⁄ gm dry nodule ⁄ hr) appeared high response to the studied treatments. i.e. quality of irrigation water and type of biofertilizer, where its recorded values ranged between 0.07 µmole C_2H_4 ⁄ gm dry nodule ⁄ hr in the soil irrigated by SW without biofertilizers inoculation (R0) and 2.92 µmole C_2H_4 ⁄ gm dry nodule ⁄ hr in the soil irrigation by WW and inoculation by R2 . This range clear the greater effect of the studied two factors on biological activity in general and on nitrogenase activity in more specific (Bashan and de- Bashan 2005) and El-Gaafarey *et al*., (2020).

With the same type of biofertilizers data in Fig (2) show that, according to the found values of nitrogenase activity affected by the used irrigation water resources, these resources takes the order WW > DW > DW+SW(2:1) > DW+SW(1:1) > SW. This order effect the chemical properties of these resources on soil microorganisms (types and activity). Also, this order show decrease in nitrogenase activity with increase in water salinity. Before that, Marie (2019) and Tantawy *et al*., (2019b) found similar decrease effect of water salinity on biological activity.



WW, DW, and SW = Well , drainage and sewage water, A:water resources , B : Biofertilizers , A.B : Interaction between A ,B

Fig. 2. Individual and combined effect of irrigation water quality and biofertilizer on nitrogenase activity (µmole/C_2H_4) **of soybean plants.**

With the same resource of irrigation water inoculation of biofertilizers resulted in a significant increase of nitrogenase activity, where these increases with R2 were higher than those resulted from R1 inoculation. For example, in the soil irrigated by WW, nitrogenase activity recorded values of 0.34, 2.13 and 2.92 (µmole C_2H_4 ⁄ gm dry nodule ⁄ hr) with the treatments of R0, R1, and R2, respectively. These findings means that, the harmful effected of low-quality irrigation water may be decreased using biofertilization as mentioned before by (Bashan and de- Bashan 2005).

3. Conclusion

This study concluded that biofertilizers inoculation is very important with irrigation using low quality resources of water irrigation. As the best biofertilizer was R2. The contents of N, P and K in the plants fertilized by R2 were higher than these inoculated by R1, also the highest content of N, P and K was found with the plants irrigated by WW. Similar results were obtained with length of roots and shoots (cm), fresh and dry weights (g/plant) of soybean plants. Additionally, the highest value of nitrogenase was found in the soil irrigated by WW. As well as, inoculation with R2 increased nitrogenase activity than inoculation with R1. So, we recommend using WW (well water) with R2 ARC-SWERI-4 *Bradyrhizobium japonicum* in soybean plants.

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تأثير كفاءة التسميد الحيوى بنوعية مياه الرى وتأثيرهما على النشاط الحيوي ونمو نبات فول الصويا

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أجريت هذه الدراسة كتجربة أصص بصوبة قسم علوم الأراضي، كلية الزراعة، جامعة المنوفية، مصر، خلال موسم نمو صيف 2022، وذلك لدراسة تأثير نوعية مياه الرى على كفاءة التسميد الحيوى وكذلك تأثيرها على النشاط الحيوى وتثبيت النيتروجين ونمو نبات فول الصويا. واستخدم فى التجربة نوعان الأسمده الحيوية(اللقاحات الميكروبية) كمحفزات للنمو RUK- 3407 *Bradyrhizobium japonicum* (R1) and ARC-SWERI-4 *Bradyrhizobium japonicum* (R2) والمنتجان بقسم الميكروبيولوجيا الزراعية، معهد بحوث الأراضي والمياه والبيئة، مركز البحوث الزراعية، مصر. وكانت مصادر مياه الرى المستخدمة هى ماء بئر وماء صرف زراعى وصرف صحى معالج هذا بالإضافة الى خليط بين الماء العالج بنسبة خلط 1:1 و1:2 على الترتيب.

وأظهرت نتائج الدراسة التأثير المعنوى لنوعية مياه الرى والتسميد الحيوى على عدد العقد الجذرية المتكونة ووزنها (جم / نبات) وكذلك طول السوق والجذور (سم) والوزن الطازج والجاف لسوق نبات فول الصويا حيث تم ترتيب مصادر مياه الرى طبقا للقيم المتحصل عليها لهذه القياسات مع نفس السماد الحيوى على النحو التالى ماء بئر > ماء صرف زراعى > ماء صرف زراعي: ماء صرف صحى 2: 1 > ماء صرف زراعي: ماء صرف صحى 1: 1 > ماء الصرف الصحي. وكذلك فإنه مع نفس مصدر ماء الرى كانت هذه القيم أعلى فى النباتات الملقحة باللقاح الميكروبي R2 مقارنة بتلك الملقحة باللقاح الميكروبي R1.

كما أظهرت النتائج بأن كلا من التركيز % والممتص (ملليجرام / نبات) من النيتروجين والفوسفور والبوتاسيوم بسوق نباتات فول الصويا إختلافات معنوية مع معاملات الدراسة حيث أعلى قيم هذا المحتوى موجودا فى النباتات المروية بماء البئر بينما أقلها فى النباتات المروية بماء الصرف الصحى وكذلك أظهرت النباتات الملقحة باللقاح الميكروبى R2 محتوى أعلى من هذه العناصر مقارنه بتلك الموجودة فى سوق نباتات فول الصويا الملقحة باللقاح الميكروبى R1.

كما أظهر نشاط إنزيم النيتروجينيز إستجابة كبيرة لنوعية مياه الرى والتسميد الحيوى حيث سجل الرى بماء البئر أعلى قيمه لنشاط إنزيم النيتروجينيز بينما كانت أقل قيمه فى الأراضى التى تم ريها بماءالصرف الصحى . وكذلك فقد صاحب التلقيح باللقاح الميكروبى R2 تزايد نشاط انزيم النيتروجينيز مقارنة باللقاح الميكروبى R1. هذا يعنى أنه من الضرورى إضافة الأسمده الحيويه عند استعمال ماء رى منخفض النوعية.

الكلمات المفتاحية: كفاءة التسميد الحيوي، نوعية مياه الرى, نبات فول الصويا.