EFFECTS OF BIOGATION TECHNIQUE ON BIOFERTILIZERS PERFORMANCE AND TOMATO YIELD

ABSTRACT

Two field experiments were conducted for two successive seasons (2005-2006) at El-Bustan Experimental Farm belonging to Faculty of Agriculture, Ain Shams University to evaluate the effects of biogation as an alternative for the traditional fertigation technique. The two biofertilizers *Azotobacter chroococcum*, *Saccharomyces cerivesiae* or their mixture were used as substrates for experimentation. Densities of both organisms in the phyllosphere and rhizosphere along with the rhizospheric activities and yield of tomato grown in sandy soil under surface drip and solid-set sprinkler irrigation systems were taken as parameters for evaluation schedule.

Results revealed that dual inoculation enhanced the population density of each of the tested biofertilizers compared with single application under both irrigation systems. The population densities were also higher in rhizosphere than in phyllosphere compartment. These findings were positively correlated with CO_2 evolution rates and nitrogenase activities in the rhizosphere. Biogation via drip irrigation system gave better tomato yield than that obtained from solid-set sprinkler or un-inoculated treatment. Data speculated that 25% of the applied mineral fertilizers could be saved without any significant crop reduction of tomato with the application of biogation technique under sandy soil conditions.

Keywords: Biofertilizers, *Azotobacter chroococcum*, *Saccharomyces cerivesiae*, Sprinkler irrigation, Drip irrigation, Rhizosphere, Phyllosphere, N_2-fixation, Tomato yield.

INTRODUCTION

Tomato crop is one of the most key-commodity crops in Egypt at which about 464 491 feddan were cultivated in 2004 with a total yield of 7 640 818 ton/fed **(Ministry of Agriculture and Land Reclamation, Economic Affairs Sector, 2005)**. Chemigation is usually used to ensure stable productivity of tomato and other economical crops but it becomes undesirable due to its potential hazardous to environment and humans. Moreover, deficiency of pressurized irrigation systems due to distributors clogging and enhancing friction head losses due to chemical participation on pipe lines represent a serious problem for operation and maintenance of the system, as well as, its impacts on crop yields reduction and attributed quality parameters **(Replogle, 2000; Arafa *et al.*, 2004 and Hagag and Matter, 2005)**. Therefore, pressurized irrigation systems mangers normally inject acids into their systems to prevent chemical precipitation and distributor's damage and clogging.

Much research is addressed at improving understanding of the diversity, dynamics, and significance of rhizosphere microbial populations and their co-operative activities **(Barea *et al.*, 2005)**. Beneficial root-colonizing rhizosphere bacteria, the PGPR, are known to participate in many important biological activities, such as the biological control of plant pathogens, nutrient cycling, and/or plant growth

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enhancement (**Barea *et al.*, 2004; Zahir *et al.*, 2004**). Therefore, Biofertilization with selected strains of PGPR are being used as seed inoculates or foliar spray for a range of economical crops (**Sahin *et al.*, 2004; Zahir *et al.*, 2004**).

Applying biofertilizers via irrigation water, i.e. biogation is thought to be an alternative technique for chemigation with the consideration of the use of appropriate injector, properly designed and operated irrigation system and optimized microbial dose. The system could be particularly important under sandy soil condition where water economy and utilization of microbial activities are two main factors affecting crop performance.

No technically, economically and environmentally feasible studies focused on application possibility of the alternative technique; evaluation and performance consideration exists under field conditions. Therefore, the aims of this field study were to: 1) evaluate biogation system as an alternative for fertigation technique; 2) schedule phase-out of biogation for improving tomato yield as an example of important economical crop; and 3) compare the effect of irrigation systems manipulating two types of microbial inoculants (*Azotobacter chroococcum* and *Saccharomyces cerivesiae* or their mixture) along with biogation uniformity in relation to tomato productivity.

MATERIALS AND METHODS

Soil and irrigation water

Experiments were conducted in two successive growing seasons (2005 and 2006) at newly reclaimed sandy soil of the Experimental Farm of Fac. Agric., Ain Shams Univ., at El-Bustan belonging to Al-Bahira governorate. Physiochemical characteristics of the soils and quality of irrigation water analyzed in the Soil Dept. Lab., Fac. Agric., Ain Shams Univ., are given in **Tables (1 and 2).**

Table (1): Some physicochemical properties of the used soil

Soil depth (cm)	Particle Size Distribution %				F.C. %	W.P. %	B.D. g/cm^3	Texture Class
	C. sand	F. sand	Silt	Clay				
0 – 30	52.8	41.4	4.1	1.7	9.4	4.3	1.68	Sandy
30 – 60	50.0	43.5	5.0	1.5	8.5	4.4	1.57	Sandy
60 – 120	52.0	42.0	4.3	1.7	9.2	4.4	1.55	Sandy

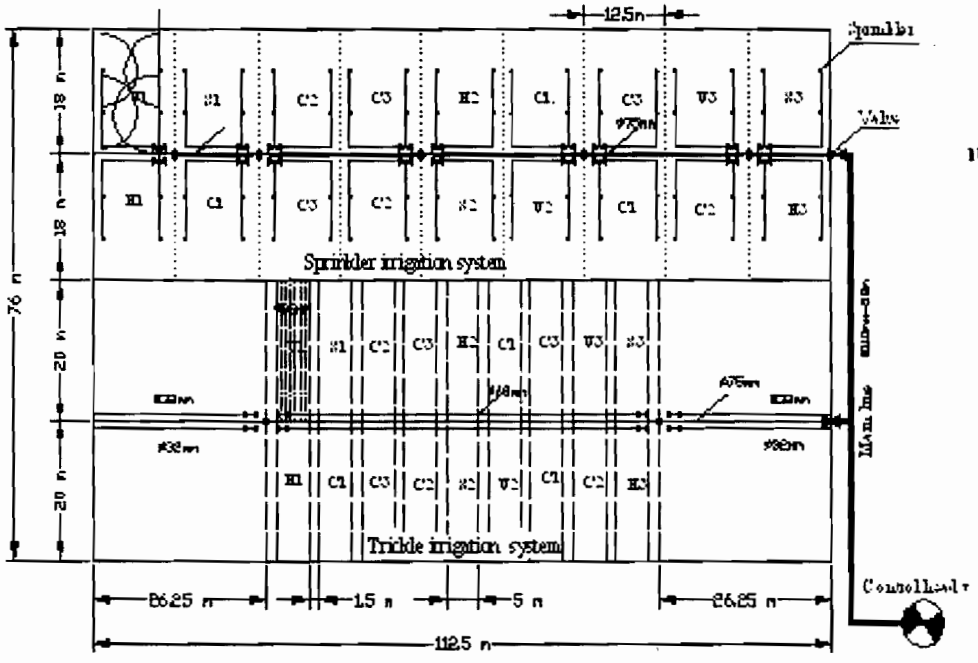
Soil depth (cm)	pH 1:2.5	EC dS/m	Organic carbon (%)	Total nitrogen (ppm)	Soluble cations, meq/l				Soluble anions, meq/l			
					Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^{-}	So_4^{--}	Cl^{-}
0 – 30	8.2	1.27	0.08	23	2.9	2.8	5.1	0.6	--	3.6	2.0	6.1
30 – 60	8.3	1.22	0.07	22	2.9	2.1	5.2	0.7	--	3.7	2.1	6.3
60 – 120	8.3	1.30	0.06	20	3.0	2.0	5.4	0.7	--	4.3	2.4	6.9

Table (2):Some chemical data on irrigation water used in El-Bustan experimental farm

pH	EC dS/m	Soluble cations, meq/l				Soluble Anions, meq/l			SAR
		Ca^{++}	Mg^{++}	Na^{++}	K^{+}	HCo_3^{-}	$So_4^{=}$	Cl^{-}	
7.74	0.55	1.03	0.74	8.01	0.42	1.95	4.52	3.73	8.51

Irrigation system

Two pressurized irrigation systems were used for experimental evaluation as shown in **Fig (1)**. The system included, 1) solid-set sprinkler irrigation system with sprinkler flow rate 1.0m^3/h at 2.5bar operating pressure. Sprinklers are fixed at 12x12 m spacing (4 sprinklers were placed for each experimental plot); 2) surface drip irrigation system with 16 mm nominal diameter PE laterals, 0.5 m emitter spacing and 20m lateral length. Emitters were GR built-in dripper types with 4 lph flow rate at 1.0 bar operating pressure.



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Tomato seedlings

Tomato seeds (*Lycopersicon esculentum,* cv. GS) were obtained from USA. Seedlings trays contained a mixture of peat moss and vermiculite (1:1) and the mixture was treated by fungicide (Benlate) as 1 gram per liter. The trays were hardened by subjecting them to open field for 10 days before transplanting. Tomato seedlings were transplanted to the main field in double rows (25 cm plant spacing) in the middle of February for the two successive growing seasons 2005 and 2006.

Biofertilizers

Two strains of *Azotobacter chroococcum* (AC) and *Saccharomyces cerevisiae* (UBFSC) were kindly provided from the Biofertilizers Unit, Fac. Agric., Ain Shams Univ., Cairo. Egypt.

EXPERIMENTAL TECHNIQUE

Preparation of microbial inocula

Heavy cell suspension of *Azo. chroococcum* strain (AC) was obtained by growing the active culture for 5 days on modified Ashby's N-deficient medium **(Abd-El-Malek and Ishac, 1968)** under stirring conditions (150 rpm) at 30±2C°. Active culture of *Sac. cerivesiae* was prepared by inoculating malt extract broth medium **(Wickerham, 1951)** with shaking on a rotary shaker for five days at 30±2C°. Obtained inocula were suspended in sterile distilled water to obtain a standard dense population of the organisms. Biofertilizers inocula were prepared for individual stain or in mixture (1:1 v/v). The suspension of individual inoculating microorganism was adjusted to contain ca. $9x10^{-9}$ cells ml^{-1} to be used as standard inocula and the mixture inoculant was prepared prior to application to give similar cells concentration. Microbial inocula were added in a 100 L tank and injected via irrigation water. Single or dual inocula were added in the drip or sprinkler irrigation system at the following growth stages of tomato i.e., one month after transplanting, at full bloom and at the beginning of fruit set stages.

Treatments and application

Developed seedlings were subjected seven biogation treatments were adopted as follows:

1. Surface drip irrigation with inoculation with *Azo. chroococcum*
2. Surface drip irrigation with inoculation with *Sac. cerevisiae*
3. Surface drip irrigation with *Azo. chroococcum* + *Sac. cerevisiae*
4. Solid-set sprinkler irrigation with inoculation with *Azo. chroococcum*
5. Solid-set sprinkler irrigation with inoculation with *Sac. cerevisiae*
6. Solid-set sprinkler irrigation with inoculation with *Azo. chroococcum* + *Sac. cerevisiae*
7. Surface drip and solid-set sprinkler irrigation with conventional fertigation technique (as control).

Microbiological criteria

Effect of biogation treatments on the densities of microbial inoculants

The effect of three biogation treatments tested on the densities of microbial inoculants was determined. Biogation treatments were compared with the traditional fertigation processes in the experimental region. Each of the biogation treatments was tested at 100%, 75% and 50% of the recommended requirement of the N nutrient (100 kg-N per feddan for fertigation and $9x10^{-9}$ cells ml^{-1} for biogation); traditional units of N was applied to compensate plant requirement. All fertilizers requirements had been scheduled based on available nutrients in the soil. Other agronomic and plant protection practices were considered according to the recommendation of the Horticulture Research Institute, ARC, Ministry of Agriculture and Land Reclamation. Three replicates were made for each treatment. Venturi injector was used for injecting biofertilizers and chemicals.

Rhizosphere soil as well as leaves samples were taken from fertigated and biogated treatments during the 3 tomato growth stages. For these purpose, seedlings were carefully uprooted, avoiding tearing of root hairs and packed in paper bags. Samples of whole plants were directly transferred to the laboratory for microbiological analysis. Ten grams of rhizosphere soil were suspended in 90 ml sterilized physiological saline solution in 250ml conical flask, thoroughly shacked for 10 min. and dilution series up to $6x10^{-9}ml^{-1}$ were prepared. The leaves samples representing various parts of the plant e.g. top, leaf and sheath were taken from the collected seedlings. Ten discs ($1cm^2$ in diameter) from the samples were shaken in a 100ml of sterilized physiological saline solution and shaken vigorously for 15 minutes then dilution series up to $6x10^{-9}ml^{-1}$ were made. The discs were picked out and their dry weights were determined at 80°C for 48h.

Samples were subjected for determination of densities of total microbial flora on soil extract agar medium **(Page *et al.*, 1982)** with decimal plate count technique, Azotobacters spp. on modified Ashby's liquid N-deficient medium **(Abd-El-Malek and Ishac, 1968)** using Most probable number (MPN) technique. Densities of Yeasts on Malt extract agar medium **(Wickerham, 1951)** with decimal plate count technique. Incubation was carried out at 30 ±2C° for one week and 4 days in respective order. The biological activity of rhizosphere soil as rates of CO_2 evolution was determined according to **Alef and Nannipieri (1995)**. N_2-ase activity in rhizosphere soil and on surfaces of detached leaves were estimated according to the method described by **Schollhorn and Burris (1967)** and **Murty (1984)** respectively using acetylene reduction assay (ARA).

Biofertilizers densities uniformity

To estimate the biofertilizer inoculants densities uniformity in either single or dual inoculation treatments in flowing water, 50 ml of irrigated water containing biofertilizers was collected at 3 different points (first, middle and end) of dripping hoses or in vessels under solid-set sprinkler irrigation systems. Azotobacters and yeasts populations were counted in collected samples as indicated above.

Engineering criteria

Irrigation water requirements of tomato crop under El-Bustan region conditions were calculated and scheduled based on CropWat 4.1 computer program

(Table 3); The reference of evapotranspiration (ET_o) was calculated based on climatic data of El-Bustan Weather station and the crop coefficient (Kc) values were used according to **FAO (1984).**

Table (3): Water consumptive use for tomato crop under El-Bustan site conditions

Month	Kc	ET_o mm/day	Water Consumptive Use, ET_c m^3 fed/ day
February	0.35	2.5	4.54
March	0.75	2.5	10.07
April	1.25	2.7	17.35
May	1.25	3.6	17.70
June	0.75	5.5	16.17

Irrigation requirement was dependent on water application efficiency of the irrigation systems.

The required measurements and calculations for evaluating the alternative technique (Biogation) compared with conventional one (fertigation) and its impacts on tomato yield had been conducted.

Statistical uniformity was calculated with the following equations for the tested pressurized irrigation systems **(ASAE, 2002)**:

i- for drip irrigation system:

$$U_s = 100(1 - \frac{s_q}{\bar{q}_i})$$

Where

U_s = statistical uniformity
S_q = standard deviation of emitter flow rate (lph)
q_i = average emitter flow rate of the i^{th} treatment

ii- for solid = set sprinkler irrigation:

$$U_S = 1 - \frac{\sum |x_i - \bar{x}|}{n\,\bar{x}}$$

Where

x_i= single observation of application rate as depth (mm)
x = average of the individual observation of the i^{th} to n^{th}

To reduce experimental error and protection against the subjective assignment of treatment, a complete randomization procedure was used. As consequence of individual trials and combination of orders between the treatments and experimental units and subunits were randomly chosen.

Bioengineering criteria

Bioengineering criteria i.e. irrigation systems performance analysis and biogation efficiency were estimated. At harvest time (with 4 picks harvested) the fruit yield of tomato was taken for determining some response criteria i.e., yield production (calculated during the harvesting period) (Mgram per feddan).

Table (4): Applied biofertilizer (*Azo. chroococcum* and *Sac. cerivesia*) densities and uniformity through the biogation technique as response to the application methods and irrigation systems' performance under sandy soils conditions.

Irrigation system	Sample zone	Biofertilizer application			
		Single		Dual	
		Azo. chroococcum	*Sac. cerivesia*	*Azo. chroococcum*	*Sac. cerivesia*
Surface drip	First	19.50	17.25	19.13	17.93
	Middle	18.53	16.81	18.01	17.18
	End	19.46	17.14	18.55	17.79
DU*		**95.00**	**92.37**	**94.60**	**93.15**
Solid-set sprinkler	First	11.57	10.48	11.82	12.62
	Middle	12.82	10.89	13.15	12.86
	End	12.61	11.99	13.20	12.22
DU		85.00	84.30	86.11	88.15
BDU **		**55.39**	**53.50**	**45.91**	**40.25**

* DU: Distribution uniformity of irrigation water

** BDU: Biological distribution uniformity enhancement as a ratio of average population densities of drip and solid-set sprinkler irrigation systems

Initial inoculation 10^9 cell ml^{-1}

MPN azotobacter in the rhizosphere: cells x 10^4 g^{-1} dry soil; in the phyllosphere: cells x 10^4 cm^2 leaf surface

Count of *Sac. cerivesia* in the rhizosphere: cells x10^4 cfu g^{-1} dry soil; in the phyllosphere cells x10^4 cm^2 leaf surface.

L.S.D. at 0.05

Surface drip irrigation systems'		
Biofertilizer inoculants (A):	1.555	0.601
Irrigation (B):	NS	1.210
Interaction AX B:	NS	NS
Solid-set sprinkler irrigation systems'		
Biofertilizer inoculants (A):	0.601	0.239
Irrigation (B):	NS	NS
Interaction AX B:	NS	0.232

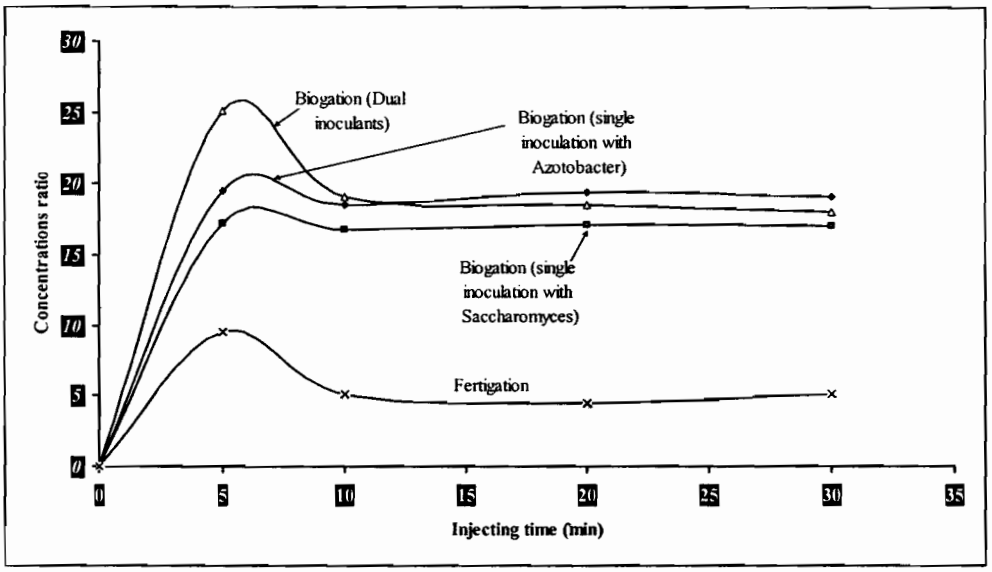

Fig. (2): Performance analysis of venturi device under different chemigation techniques and inoculant methods.

Statistical analyses

The least significant differences values (LSD) at 5% of probability were used to compare the differences between means of treatments. Data were statistically analyzed according to **Costat software (1985)**.

RESULTS AND DISCUSSION

Irrigation systems and biogation technique performance analysis

Irrigation systems' performance analysis had a significant impact on a crop yield production and costs. However, water is wasted when the irrigation system performance is low. Moreover, when fertilizers (minerals and/or biofertilizers) are being delivered in the irrigation water, their distribution uniformity and activity behavior are depending on the ability of the system to deliver water uniformly. Data in **Table (4)** revealed that there is significant effect of applying biofertilizers via different pressurized irrigation systems at single or dual application. The single inoculant was significantly effective under both irrigation systems. The dual inoculants, on the other hand, proved to be effective under solid-set sprinkler irrigation system. It is worth to mention that the interaction between solid-set sprinkler irrigation system and dual biofertilizer inoculants had a significant influence on Azotobacters and yeasts densities. On the other hand, results indicated that surface drip irrigation is more uniform and efficient than solid-set sprinkler irrigation system. However, it improved the microbial densities of the applied biofertilizers with about 55.39 and 45.91% for *Azo. chroococcum* and by about 53.5 and 40.25% for *Sac. cerivesia* when applied in a single and dual treatments respectively for tomato crop under sandy soil conditions.

With regard to the tested pressurized irrigation systems' performance analysis, results based on the field experimental data speculated that there is a significant effect of the applied biofertilizer application methods (single and dual) on the distribution efficiency under surface drip (92.37–95%) and solid-set sprinkler (84.3–88.15%). Regarding the interaction effect between the irrigation system and applied biofertilizer type, data revealed that there is significant effect on the microbial distribution uniformity.

With respect to the applicator performance analysis, data in **Fig. (2)** speculated that there is stability in applying biofertilizers (biogation) concentration ratio from the initial concentration in the storage tank more than that of the traditional mineral fertilizers (fertigation) within the application time. This may be due to the high accumulation of mineral fertilizers in the storage tank with time compared with biogation technique.

The above mentioned results are in agreement with those of **Abdel-Aziz (1998); Singh *et al.*, (2002) and Chieng and Ghaemi (2003).** Thus modifying the technique to apply microbial inoculants in a liquid form to the main water tank could give remarkable effects. The final concentration of the inoculants should be calculated on basis of the inoculants movement and distribution through the pressurized irrigation systems to keep the required concentration in the root zone.

Table (5): Effects of biofertilization with *Azo. chroococcum* and *Sac. cerivesia* and inoculation timing on total microbial densities, rates of CO_2 evolution, N_2-ase activity, Azotobacters and yeasts in rhizosphere of tomato grown plants under surface drip irrigation system.

Inoculation time	Applied techniques	Biofertilizer inoculants	Total microbial flora ($x10^5$ cfu cm^2 leaf surface)	Rate of CO_2 evaluation (μg^{-1} soil h^{-1})	N_2-ase activity (nmol C_2H_4 $plant^{-1}$ h^{-1})	Azotobacters ($X10^4$ cells g^{-1} dry soil)	Yeasts ($x10^5$ cfu g^{-1} dry soil)
One month after transplanting	Fertigation	Un-inoculated	70.65	12.59	13.13	2.11	4.32
	Biogation	*Azo. chroococcum*	190.26	16.57	68.70	11.55	4.80
		Sac. cerivesia	223.57	18.39	32.51	11.66	7.17
		Mixture	253.88	23.56	50.16	11.57	7.15
At full bloom stage	Fertigation	Un-inoculated	83.91	14.86	14.22	2.71	4.89
	Biogation	*Azo. chroococcum*	207.15	19.51	89.68	14.09	5.72
		Sac. cerivesia	317.38	25.17	45.10	11.91	7.55
		Mixture	366.88	33.47	72.97	11.91	7.73
At the beginning of fruit set	Fertigation	un-inoculated	95.36	15.21	15.08	2.78	5.10
	Biogation	*Azo. chroococcum*	350.34	22.23	128.14	16.43	5.75
		Sac. cerivesia	412.40	22.62	59.62	12.61	8.23
		Mixture	533.26	36.37	119.34	12.22	8.18
L.S.D. at 0.05							
Biofertilizer inoculants (A):			35.807	2.089	3.732	2.923	0.385
Time of inoculation (B)			36.870	3.766	4.307	2.532	0.596
Interaction: A X B			36.191	2.112	3.772	NS	NS

Table (6): Effect of the alternative biogation technique and inoculation time on total microbial densities, Azotobacters, Yeasts and N_2-ase activity in the phyllosphere of tomato plants under solid-set sprinkler irrigation system in sandy soil conditions.

Inoculation time	Applied techniques	Biofertilizer inoculants	Total microbial flora ($x10^5$ cfu cm^2 leaf surface)	N_2-ase activity (nmol C_2H_4 $plant^{-1}$ h^{-1})	Azotobacters ($X10^4$ cells g^{-1} dry soil)	Yeasts ($x10^5$ cfu g^{-1} dry soil)
One month after transplanting	Fertigation	Un-inoculated	19.12	1.31	0.13	0.88
	Biogation	*Azo. chroococcum*	90.67	14.88	6.82	7.00
		Sac. cerivesia	121.56	6.89	7.25	10.17
		Mixture	180.55	12.21	7.79	6.96
At full bloom stage	Fertigation	Un-inoculated	21.67	2.03	0.13	1.02
	Biogation	*Azo. chroococcum*	128.68	18.97	9.49	7.37
		Sac. cerivesia	221.29	9.46	10.83	10.53
		Mixture	244.41	17.17	7.83	10.12
At the beginning of fruit set	Fertigation	un-inoculated	25.89	2.43	0.13	1.03
	Biogation	*Azo. chroococcum*	204.81	25.53	8.20	7.97
		Sac. cerivesia	289.30	12.84	8.42	11.14
		Mixture	376.30	21.32	8.37	8.74
L.S.D. at 0.05						
Biofertilizer inoculants (A):			12.248	4.727	0.823	0.629
Time of inoculation (B)			12.054	4.855	0.763	0.607
Interaction: A X B:			12.381	NS	0.832	0.636

Effects of fertigation vs. biogation on microbial densities performance in the rhizosphere and phyllosphere of tomato grown under pressurized irrigation systems

Data presented in Tables (5 and 6) clearly show that microbial inoculation and application time with biofertilization significantly influencing microbial densities and biological activates under both irrigation systems. Interaction between microbial inoculation and its addition time significantly influenced densities of total microbial flora, CO_2 output and N_2-ase activity under both irrigation systems. Use of dual inoculant appeared to be significantly effective for total microbial flora, CO_2 output and densities of yeasts under the drip irrigation system (Table 4). But, using *Sac. cerivesia* inoculant alone or mixed with Azotobacters led to a significant increase of the yeasts densities. Using of *Azo. chroococcum* inoculant, on the other hand, led to a significant increase in the densities of yeasts and vise versa. Applied dual inoculants resulted in a significant increase of total microbial flora, N_2-ase activity and Azotobacters densities. The addition of the inoculant at the beginning of fruit set period resulted in a significant increase of total microbial flora, CO_2 output, N_2-ase activity and densities of Azotobacters and yeasts.

Microbial populations and their biological activities were gradually increased with plant age. The N_2-ase activity increased from 50.2 to 73 and then to 119.3 with dual inocula. Consequently, the N_2-ase activity might be attributed to the effect of exudation of carbon compounds that show a special importance for growth of N_2-fixing Azotobacters. Such results may support the concept that these bacteria produce growth regulating compounds which may improve plant productivity through hormonal stimulation besides N_2-fixation **(Hirsch *et al.*, 1997)**.

Under the sprinkler irrigation system (Table 6), the use of the dual inoculant appeared to be significantly effective for total microbial flora, N_2-ase activity and densities of Azotobacters. The use of *Sac. cerivesiae* inoculant alone resulted in a significant increase of the yeasts counts. The addition of the inoculant at the beginning of fruit set period resulted in a significant increase of total microbial flora and N_2-ase activity. The addition of the inoculant during full bloom stage led to a significant increase in the densities of Azotobacters and yeasts.

Soil analysis showed that the virgin sandy soil used in this study contained low organic carbon (0.08%) and nitrogen (23ppm) content. Therefore, this type of soil needs to be inoculated with the biofertilizers in order to increase the efficiency of biological activities. By spreading the applications over time, the bacteria are present in the soil environment for a longer period and therefore maintain biological activities for a longer period of time. Generally, microbial populations and their biological activities gradually increased with plant age.

Inoculants of mixed cultures of beneficial microorganisms have considerable potential for controlling the soil microbiological equilibrium and, thus, providing a more favorable conditions for plant growth and protection **(Vessey, 2003)**. In addition, the use of biofertilizers may have a range of benefits such as nitrogen fixation **(Kennedy *et al.*, 2004)**, mobilizing phosphate **(Girgis, 2006)** and micronutrients, through the production of organic acids, secreting growth promoting factors **(Guierrez-Manero *et al.*, 2001)**, increasing amino acids content **(Schank *et al.*, 1981)**, and increasing water and mineral uptake from the soil **(Sarig *et al.*, 1984)**. In the present work, biofertilizers may increased the concentration of simple organic molecules such as sugars, free amino acids and total soluble phenols which play a role in regulation of plant osmosis and consequently led to better plant growth and yield. The beneficial effect of inoculation with *Sac. cerivesiae* gave a significant increase in the various characteristics of inoculated plants.

Table (7): Effect of the alternative biogation technique regimes on the densities of *Azo. chroococcum* and *Sac. cerivesia* under different pressurized irrigation systems.

Applied technique	Applied amount percentage of N	Surface drip				Solid-set sprinkler			
		Biofertilizer inoculants							
		Single		Dual		Single		Dual	
		Azo. chroococcum	*Sac. cerivesia*	*Azo. chroococcum*	*Sac. cerivesia*	*Azo. chroococcum*	*Sac. cerivesia*	*Azo. hroococcum*	*Sac. cerivesia*
Fertigation	**Recommended dose**	2.57	5.41	4.60	3.95	2.10	10.43	2.25	11.89
Biogation	**50**	12.11	9.63	16.35	8.74	7.83	7.69	9.12	9.35
	75	21.46	15.87	24.98	13.79	12.76	12.38	18.21	14.73
	100	28.35	20.29	30.09	20.63	16.58	16.56	21.06	17.88

Initial inoculation 10^9 cell ml^{-1}
MPN azotobacter in the rhizosphere: cells x 10^4 g^{-1} dry soil; in the phyllosphere: cells x 10^4 cm^2 leaf surface
Count of *Sac. cerivesia* in the rhizosphere: cells x10^4 cfu g^{-1} dry soil; in the phyllosphere
cells x10^4 cm^2 leaf surface.

L.S.D. at 0.05

Biofertilizer inoculants (A):	0.681
Biogation (B):	3.537
Irrigation (C):	0.343
Interaction:	
A X B	7.391
A X C	13.302
B X C	NS

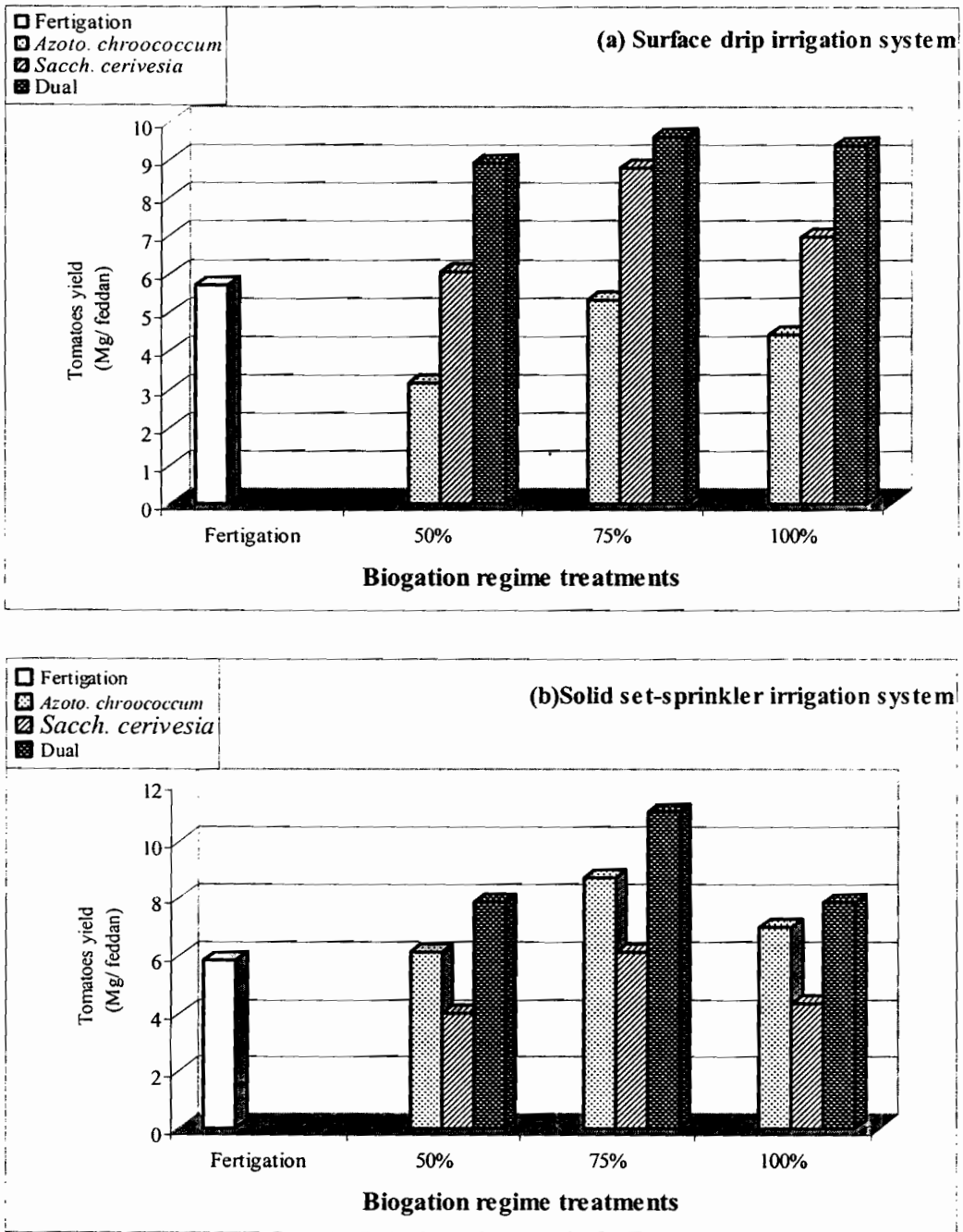

Fig (3): Tomato yield in response to the biofertilizers application under surface drip (a) and solid-set sprinkler (b) irrigation systems.

Biogation influence on Azotobacters and yeasts densities in tomato phyllosphere and rhizosphere under different pressurized irrigation systems

Table (7) shows that the type of irrigation system, the microbial inoculation as well as the biogation percentage significantly influenced the density of Azotobacters and yeasts. Dual inoculation with 100% biogation resulted in a significant increase in Azotobacters and yeasts densities. It is worth to mention that a gradual decrease was observed in Azotobacters and yeasts densities which was parallel to the decrease of the biogation percentage up to un-inoculated treatment (100% fertigation). Drip irrigation system significantly influenced Azotobacters and yeasts densities in biogation more than in sprinkler irrigation system. The influence of interaction between inoculum type and irrigation system, and between inoculum type and biogation significantly influenced the densities of Azotobacters and yeasts. Thus, it is obvious that the use of dual inoculant at 100% biogation under drip irrigation system gave the most significant densities of Azotobacters and yeasts.

Repeated use of biofertilizers as foliar application can replace the use of costly plant growth stimulants. Azotobacters cells grow and multiply by utilizing the carbon source in the leaf exudates and play a significant role as highly competitive colonizers in the phyllosphere due to its N_2-fixing ability and their ability to secrete plant growth promoting substances **(Tsavkelova *et al.*, 2006)**, B-group vitamin, ammonia and antifungal metabolites could benefit the plant in a multi-dimensional way including decreasing needs to chemical nitrogen fertilizers and/or increasing nitrogen use efficiency. In this concern, **Mohandas (1987)** found that inoculating tomato seedlings with *Azotobacter* resulted in high increase in leaf area, dry weight, nitrogen and phosphorus contents and yield.

Saccharomyces cerivesiae was also shown to produce growth promoting substances **(El-Kholy and Omar, 2000)** and synthesize antimicrobial and other useful substances such as cytokinins, hormones, B-vitamins and enzymes that promote active root cell division **(Abdul Khaliq, 2006)**. The effects of spraying dry yeast on growth and yield economical crops were investigated **(Ahmed, 2002 and Tartoura, 2002).** In treatments this study, the finding that densities of microorganisms were higher in the dual inoculation may reflect higher biological activity in the rhizosphere zone of plant grown under that treatment which could be reflected on yield over control as shown by other studies **(Hanafy *et al.*, 2000 and Omar and El-kattan, 2003)**.

Tomato yield in response to biogation technique under surface drip and solid-set sprinkler irrigation systems

Data presented in Fig. (3)

revealed that tomato yield responded to biogation technique and its management criteria. With respect to the capability of biogation technique for improving commodity crops as tomato, data revealed that yield has been enhanced by about 16.14 and 17.25% under drip and solid-set sprinkler irrigation system, respectively. This could be attributed to available nitrogen and growth promoting substances provided for growing plants by the tested biofertilizers. This finding is obviously reported with the dual inoculation treatment of *Azo. chroococcum* and *Sac. cerevisiae* where the highest records of tomato yield under both tested pressurized irrigation systems were recorded. Moreover, the treatment of 75% of the

biofertilization dose substituting the nutrient requirement mineral N was the best treatment. However, tomato yield ranged from 9.61% to 11.03 Mgram/feddan under pressurized irrigation systems. This may be due to the positive effects of biofertilizer inocula including N_2-fixation, increasing the availability of nutrients in the rhizosphere, enhancing root growth and morphology and promoting other beneficial microbes **(Vessey, 2003)**.

CONCLUSION AND FUTURE WORK

The development of biogation as an alternative technique for improving yield productivity under sandy soil conditions had been evaluated and resulted in a considerable increase in tomato yield over the traditional application (fertigation). However, the behavior of the applied biofertilizers with respect to the irrigation systems has been slightly differed from single and dual inoculation method. Biogation appeared to be an economically, technically and environmentally feasible alternative technique for enhancement of tomato yield productivity and reduce the amounts of added mineral fertilizers by about 25 percent of the crop nutrient requirements.

More studies are needed to determine the actual requirements and other related management criteria for biogation technique under different physical field resources, i.e. soil salinity, low water quality and stress conditions.

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تأثير تقنية الرى الحيوى على اداء الاسمدة الحيوية و محصول الطماطم

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تم اجراء تجربتين حقليتين فى ارض رملية حديثة الاستصلاح خلال الموسمين الزراعيين ٢٠٠٥ / ٢٠٠٦ فى مزرعة البستان التابعة لكلية الزراعة - جامعة عين شمس بمحافظة البحيرة بهدف تقييم امكانية استخدام تقنية الرى الحيوى مقارنة بالرى التسميدى بحقن بعض الميكروبات المستخدمة كسماد حيوى و المتمثلة فى الازوتوباكتر كروكوكم و السكارووميسس سرفيسيا (الخميرة) تحت نظامى الرى الضغطى التنقيطى السطحى و الرى بالرش لتقدير سلوك الاسمدة الحيوية مجال الدراسة من حيث كثافتها العددية و نشاطها الحيوي بمنطقة الجذور و انعكاس ذلك على محصول الطماطم.

وقد اظهرت النتائج ان نظام الرى الضغطى مع التلقيح المختلط ادى الى تحفيز تواجد وانتشار كلا من الاسمدة الحيوية المستخدمة مقارنة بالتلقيح المنفرد لهذه الاسمدة. كما لوحظ زيادة كثافة اعداد الميكروبات الكلية المتواجدة بمنطقة الجذور (الريزوسفير) و التى كانت مواكبة لمعدلات انطلاق ثانى اكسيد الكربون و نشاط انزيم النيتروجينيز فى ريزوسفير الطماطم. وبالنسبة للمردود البيئى فقد اشارت النتائج إمكانية تخفيض كميات الأسمدة المعدنية المستخدمة تحت ظروف الرى التسميدى التقليدى بمعدل ٢٥% من الأحتياجات السمادية لمحصول الطماطم عند استخدام تقنية الرى الحيوى دون حدوث اضرار معنوية فى إنتاجية الفدان.