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EFFECT OF BIO-FERTILIZERS AND SALINITY LEVELS OF IRRIGATION WATER ON THE GROWTH AND CHEMICAL CONTENT OF MANGROVE PLANT (*Avicennia marina*)

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ABSTRACT

Outdoor pot experiment was carried out to study the effect of inoculation with the active strains of *Azotobacter chrooccum, Bacillus megatheium var. phosphaticum* (PDB),VA mycorrhizal spores and their mixture in the presence of sea water i.e. A- tap water ,B- 1:2 sea-tap water (Ec = 15000ppm), C- 1:1 sea-tap water (Ec 22500ppm) and D- sea water (Ec 45000 ppm) on the total microbial counts, non symbiotic nitrogen fixing bacteria, phosphate dissolving bacteria (PDB) counts, count of mycorrhizal spores, growth parameters of mangrove plants at Desert Research Center (DRC). The results showed that, the highest significant increase recorded with irrigation water of 15000 ppm for nitrogen fixing bacteria and mycorrhizal spores, but for PDB the increases was at all different salinity levels. For nitrogen and phosphorus %, the highest significant increases were recorded with mixed inoculation and increased as salinity level increase for individuals or mixture inoculation. Growth parameters of mangrove plants expressed as plant height, root length, number of branches, number of leaves and stem diameters were higher with inoculated treatment as individual or as mixture than control especially when it irrigated with 1 sea water: 1 tap water (22500 ppm).

Keyword: Azotobacter, Azospirillum, Phosphate dissolving bacteria (P.D.B.), microhyzae, Mangrove plant.

INTRODUCTION

Mangroves is a vast and abundant hydro halophyte belonging to several plant families, inhabit along the east cost of the red sea. Mangrove forests are considered as open ecosystems connecting upland terrestrial and coastal ecosystems. Contributors to the geo. aquatic food chain, Mangrove forests are important for biomass production and coast line protection (Wekher *et al*., 2007; Fa-Yuanwang *et al*., 2004). In context our studies on microbiological aspects of ecosystem adaptation of mangroves to low salinity and fresh water. The objective of this study is to evaluate the effect of inoculation of Mangrove plants by azotobacter, phosphate dissolving bacteria, AM mycorrhiza and their combination and salinity levels of irrigation water on the growth parameters and nutrients content.

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MATERIALS AND METHODS

Outdoor pot experiment was conducted at Desert Research Center, Cairo Egypt to evaluate the effect of different bio-fertilizer treatments, salinity levels through irrigation water prepared by diluted seawater and their interaction on growth, yield and mineral contents in mangrove plants grown on soil collected from El-Asher of Ramadan El-Sharkia governorate. The soil is characterized as sandy in texture, mildly alkaline, non saline, non calcareous and low organic matter content (Table 1).

The experimental treatments were five biofertilizer treatments, i.e. a- non inoculation, b- inoculation with *Azotobacter chroococcum* c- inculcation with PDB strain d- inculcation with VA mycorrhizal, e-mixture of (b, c, d) .and four salinity levels prepared by using sea water as a source of salinity i.e. a- tap water ,b- 1:2 sea-tap

Table 1. Some chemical, physical and biological characteristics of the experimental soil

Variable	Value	Variable	Value
Ec ds/m, paste	1.65	Silt+ clay%	3.0
PH paste	7.4	Texture class	sand
OM %	0.05	Total microbial counts x 10^6	25 cfu/g soil
Total-N ppm	14	Azotobacter counts x 10^2	0.093 cfu/g soil
$CaCO_3$ %	2.62	Azospirilla counts x 10^2	0.45 cfu/g soil
Sand %	97	P- dissolving bacteria x 10^2	6 cfu/g soil
		VA mycrohizal count	0.0 / 100g soil

water (Ec = 15000ppm), c- 1:1 sea-tap water (Ec 22500ppm) and d- sea water (Ec 45000 ppm). Each treatment was replicated four times.

Metallic ten pots 35 cm in diameter and 50 cm in depth were used. Every pot contained 20 kg of air dried soil and planted with mangrove seedlings age two months and 30cm in height. Thirty rhizosphere soil samples from different site at red sea gulf cultivated with mangrove plants were used for microbial isolation. The high activity isolation of both azotobacter from Sharm El-madfua and phosphate dissolving bacteria (PDB) from Sharm El-Bahari were used in this experiment .The total nitrogen fixed by *Azotobacter chroococum* and PDB activity isolated from the rhizosphere of mangrove plants in different sites were determined and presented in Table 2.

Heavy cell suspension was prepared by growing them. Separately on Ashby's medium (Abdel-Malek and Ishac, 1968) and modified Bunt and Rovara medium (Abdel- Hafez 1966, Ravolanirina *et al.*, 1987) for seven day's at 28 $\pm$ 2°C. Suspension of cells of these strains containing $\simeq 10^3$ cells ml^{-1} was used as standard inocula. The third inoculated with VA mycorrhizal spores (100 spore/ plant) were isolated from the soil by the wet sieving and decanting method (Gerdemann and Nicolson 1963). Pot cultures were maintained on onion plants for six months in sterilized soils (Sharma *et al.*, 1996). The fourth inoculated with all the previous, while the fifth was the control

After 9 months from planting ,samples for growth parameters i.e. plant hight, number of branches and leaves, root length and frish weight and stem diameter were recorded. The aerial part of plants was subjected to chemical analysis to determine chlorophyll content using the appropriate method described by Chapman and Pratt (1961). Nitrogen content was determined by Kjeldahl method described by Jackson (1958) while P content was determined by ascorbic acid method as described by Watanab and Olsen (1965). Samples of rhizosphere soils of mangrove plants were collected and subjected microbiological analysis using Counting of azospirilla on semi-solid malate medium (Dobereiner, 1978), azotobacters on Ashb'y medium (Abdel- Malek and Ishac, 1968). Estimates of numbers of azotobacters and azospirilla by MPN technique were calculated using Cochrhan's Tables (Cochrhan, 1950) and PDB on modified Bunt and Rovara medium (Abedel-Hafez, 1966; Ravolanirina *et al.*, 1987).

RESULTS

Effect of Inoculation and Salinity Levels on Microbial Activity

The analysis of microbial counts i.e. total microbial counts, non symbiotic nitrogen fixing bacteria (azotobacter and azospirilla), and phosphate dissolving bacteria were detected after 9 months from seedling. The data are presented in Table 3.

Total microbial counts

Initial total microbial counts in soil were 25x 10^6 cfu/g dry soils (Table1). Generally speaking, just cultivation non inoculation treatment (control) enhanced the microbial growth to be doubled than that of uncultivated soil (initial statement soil).

Table 2. Total nitrogen fixed by *Azotobacter chroococum* and PDB activates isolated from the rhizosphere of mangrove plants collected from different sites at red sea gulf (each value average of five replicates)

Site	Total N. (ppm)	P-dissolving activity		
		Zone diameter/cm (z)	Colony diameter/cm (c)	C/Z cm
Marsa Shaeb	169	1.9	1.4	0.5
Sharm El-Bahari	144	3.1	2.0	1.1
Sharm El- madfua	190	2.0	1.1	0.9
South El- Houser	154	2.8	1.8	1.0

Table 3. Microbial density in the rhizosphere of mangrove plants as affected by inoculation with Azotobacter, PDB and VA mycorrhiza and salinity levels of irrigation water

Treatments	Salinity levels (ppm				Mean
	T.C	15000	22500	45000	
	A- Total microbial count counts x 10^6 cfu/g dry soils				
Control	65	127	71	68	83
Azotobacter	146	257	152	150	176
PDB	133	232	136	132	158
Mycorrhiza	112	210	120	120	141
Mixture	180	298	187	183	212
Mean	127	225	133	131	
LSD 5%	**Bio 26 – salinity 20 - interaction 47**				
	B- Densities of azotobactors counts x 10^4 cell/g soil				
Control	0.24	0.34	0.28	0.26	0.28
Azotobacter	0.70	0.95	0.72	0.70	0.77
PDB	0.45	0.56	0.47	0.47	0.49
Mycorrhiza	0.36	0.48	0.45	0.44	0.43
Mixture	1.20	1.80	1.30	1.20	1.37
Mean	0.59	0.82	0.64	0.61	
LSD 5%	**Bio 0.2 – salinity 0.05 - interaction 0.31**				
	C- Densities of azospirillia counts x 10^4 cell/g soil				
Control	0.20	0.33	0.22	0.22	0.24
Azotobacter	0.66	0.92	0.68	0.66	0.73
PDB	0.44	0.54	0.45	0.44	0.46
Mycorrhiza	0.32	0.40	0.42	0.40	0.39
Mixture	1.10	1.70	1.20	1.10	1.27
Mean	0.54	0.77	0.59	0.56	
LSD 5%	**Bio 0.22 – salinity 0.04- interaction 0.3**				
	D- Densities of PDb counts x 10^3 cfu/g soils				
Control	50	100	60	52	66
Azotobacter	150	240	164	153	177
PDB	122	205	139	126	148
Mycorrhiza	106	189	125	109b	132
Mixture	202	282	211	205a	225
Mean	126	203	140	129	
LSD 5%	**Bio 32 – salinity 15- interaction 46**				

Another increase in counts either was associated with the use of biofertilizer in the form of single strains or mixed culture. The highest count was associated with mixed culture to be 180 x 10^6 cfu/g dry soil followed in descending order by Az > PDB = VAMyc. With respect to salinity levels, the highest significant numbers were recorded in treatment irrigated by saline water contained 15000 ppm followed by 22500 ppm $\simeq$ 45000 ppm and tap water in descending order. The enhancement in microbial activity is a good parameter for many soil improvement indices. For example, Azotobacers produce growth promoting substances which enhance plant growth and proliferate lateral roots and root hairs which increase nutrient absorbing surface.

Also, inoculation with PDB resulting in affective solublization and utilization of phosphate, but several conditions could control this criterion, such as salinity, competition and symbiosis.

Non symbiotic nitrogen fixing bacteria

The initial densities for azotobacter and azospirilla were 0.093, 0.45 x 10^2 cell g^{-1} dry soil Table (1). The densities of azotobacter and azospirilla originated from the rhizosphere of mangrove plants ranged from 0.24 to 1.80 and 0.20 to 1.70 x 10^4 cell g^{-1} dry soil respectively.It seams that inoculation with *Azotobacter chroocumcum* is essential as energizing dose, while occurrence of (PDB) is cooperative agent especially in mixed inoculation.Also, azospirilla densities showed the same trend as azorobacters and the highest significant increase recorded with mixed inoculation at 15000 ppm salinity of irrigation water.

Phosphate dissolving bacteria (PDB)

The initial count of PDB was 6 x 10^2 cell soil, Table (1). However, their counts tended to increase in all treatments rather than the control by using different type of Biofertilizers. The highest counts were recorded in treatments inoculated with mixed inoculation at 15000 ppm as salinity level. The mixed inoculation recorded the highest significant increase at all different salinity levels.

VA mycorrhizal counts

The initial count of mycorrhizal spores was (zero spores 100 g^{-1} soils) Table 4 show that treatments inoculated with mixed inoculations (Azotobacter, PDB and mycorrhiza) had the highest significant number of mycrorrhizial spores in mangrove rhizosphere than all individual treatments at all salinity levels especially at the treatment irrigated by 15000 ppm as salinity level.From these data inoculation with *azotobacter choococum* or PDB enhanced the spore formation of mycorrhiza. In case of colonization rate, data are significant only at 15000 salinity level. The treatments received mixed inoculation, had the highest significances.

Effect of Inoculation and Salinity Levels on Growth Parameters

Growth parameters of mangrove plants (Table 5) expressed as plant height, root length, number of branches and leaves and stem diameters were higher with inoculated treatments by *Azotobacter,* PDB and VA mycorrhiza as individual or as mixture than non inoculated treatment (control), especially when it irrigated with irrigation water contained 22500 ppm salts.

Inoculated treatments had also significantly higher photosynthetic rate in terms of chlorophyll content,and fresh weights. Data also show that plant height decreased as salinity levels increased and the opposite direction was recorded with root length. Also biofertilization increased plant height and root length especially with mixed inoculation more than individuals. Number of branches and leaves increased significantly by using biofertilizers especially with mixed inoculation if compared with individuals.

Inoculation increased the resistance to salinity levels, whereas number of branches and leaves decreased with increasing salinity levels as represented in Table 5. Also, roots and shoots fresh weights, Table (5) were affected by salinity levels and biofertilization as single or as mixture. Weight of roots or shoots increased as salinity increased without biofertilizer 22500 ppm and inoculated treatments recorded the highest significant increase with mixed inoculation at 22500 ppm. Stem diameters increased as salinity level increased for inoculated one and the highest significant increase was recorded with mixed inoculation.

Table 4. Number of mycorrhizal spores in the rhizosphere mangrove plants and rate of colonization in mangrove roots as influenced by different salinity levels

Treatment	No. VA-M spors/ 100g				Colonization %			
	Salinity levels				Salinity levels			
	T.C	15000	22500	45000	21a	26b	35a	42a
Mycorrhiza	280b	318b	320b	260b	24a	38a	33a	45a
Mixture	320a	360a	340a	290a	23	32	34	44
Mean	300	339	330	275	21a	26b	35a	42a
LSD 5%	T= 7 S=. 13 T*S= 20				T= 1 S=. 4 T*S= 5			

Table 5. Growth parameters of mangrove plants as affected by Azotobacter, phosphate dissolving bacteria, VA mycorrhizae inoculation and irrigation water salinity

Treatments	Salinity levels			
	T.C	15000	22500	45000
		A- Plant height (cm)		
Control	65.3	46.3	58.0	47.3
Azotobacter	75.7	63.7	71.0	53.3
PDB	69.0	61.7	68.3	51.0
Mycorrhiza	74.7	57.0	73.0	53.7
Mixture	84.0	82.3	75.3	64.7
Mean	73.7	62.2	69.1	54.0
LSD 5%		Bio 1.5 – salinity 3.9 - interaction 7.5		
		B- Root length (cm)		
Control	33.3	38.3	42.7	35.0
Azotobacter	49.0	45.0	54.7	39.7
PDB	34.7	39.0	45.0	45.7
Mycorrhiza	36.0	50.0	51.7	42.7
Mixture	38.3	53.3	56.0	48.3
Mean	38.3	45.1	50	42.3
LSD 5%		Bio 1.4 – salinity 2.3 - interaction 4.5		
		C- Number of branches		
Control	2.3	0.0	1.7	0.0
Azotobacter	4.3	1.7	2.7	0.3
PDB	3.3	1.3	2.0	1.7
Mycorrhiza	3.0	4.3	3.0	2.3
Mixture	6.3	5.0	3.0	4.3
Mean	3.9	2.5	2.5	1.7
LSD 5%		Bio 0.5 – salinity 0.4 - interaction 13		
		D- Number of leaves		
Control	18.3	13.0	22.7	16.0
Azotobacter	20.3	24.0	28.7	16.3
PDB	28.3	27.0	24.0	18.7
Mycorrhiza	29.3	21.7	30.7	22.0
Mixture	32.7	30.7	32.0	24.7
Mean	25.8	233	27.6	19.5
LSD 5%		Bio 1.5 – salinity 1.4 - interaction 3.9		
		E- Root fresh weight(g /plant)		
Control	31.0	49.6	72.2	44.6
Azotobacter	37.6	42.3	119.5	77.5
PDB	43.1	55.7	83.9	55.0
Mycorrhiza	35.5	115.8	73.0	83.6
Mixture	63.9	149.2	138.9	95.3
Mean	423.2	92.5	97.5	71.2
LSD 5%		Bio 11 – salinity 11.1 - interaction 23.6		
		F- Shoot fresh weight(g/plant)		
Control	50.6	59.6	87.5	80.9
Azotobacter	83.0	79.0	114.0	91.5
PDB	66.0	63.4	98.0	88.6
Mycorrhiza	62.0	116.0	125.0	87.2
Mixture	90.4	184.6	145.0	102.0
Mean	70.4	100.5	113.9	90.0
LSD 5%		Bio 10.3– salinity 8.7 - interaction 26.8		
		G-Stem diameter (cm)		
Control	3.8	4.1	4.6	4.4
Azotobacter	3.9	5.7	4.7	4.8
PDB	4.3	4.1	4.9	4.5
Mycorrhiza	4.5	5.3	5.3	4.9
Mixture	4.6	5.9	5.4	5.0
Mean	4.2	5.2	5.0	4.7
LSD 5%		Bio 0.1 – salinity 0.2 - interaction 0.4		

Effect of Inoculation and Salinity Levels on Chemical Contents

Data presented in Table 6 show that, Chlorophyll content was decreased as salinity level increase than the control treatment. While for inoculated treatments it increased as salinity levels increase and the highest significant values was recorded with mixed inoculation. Nitrogen and phosphorus content increased significantly with increasing salinity levels and using biofertilizers as a single or as a mixture inoculation. The highest significant increase recorded with mixed inoculation and was increased as salinity level increased for individuals or mixture inoculation.

DISCUSSION

The previous results indicated that ,mangrove is a novel crop for salty soils which can contribute to arid- zone management, inoculation with N. fixer bacteria are an important source of available nitrogen in salt soil (Rueda-Puente *et al.*, 2003). Also Bacteria found on mangrove roots and promoting mangrove growth has high ability to fix nitrogen and solubilize phosphate also produce plant growth hormones (IAA) which raise the possibility of using bacterial species growth of mangrove in salty soils, while mangrove seedlings has the ability to absorb salts from soil which help in decreasing salinity and helping in soil reclamation. (Kandasamy Kathiresan and Masilamani Selvam, 2006 and Puente *et al.*, 2004).

There is an increased recognition today that mycorrhizas fungi associations may substantially contribute to the life not only individual plants but of whole communities, the nutrient balances of which, in all but fertile environments are dependent on and integrated by the mycorrhizal fungi (Reid *et al.* 1993). Mycorrhizas are also functionally implicated in plant tolerance to various types of physical and chemical stresses in soils (Marschner, 1995) including soils- water salinity (Ruiz-Lozano *et al.*, 1996). Whether AM the dominant form of mycorrhizas of the natural climax communities of nitrogen mineralizing, phosphorus deficient, low organic soils in tropical climates, plays a role in community development and stress tolerance of mangroves (Anjan Sengupta and Subhenda Chaudhuri, 2002).

The results of the Biofertilization study showed that inoculated mangrove seedlings with AM- mycorrhizae, phosphate dissolving bacteria and Azotobacter had significantly higher photosynthetic and growth rates in terms of plant height, roots length, number of branches, stem diameters, number of leaves, chlorophyll contents, root and shoot fresh weight (Martins *et al.*, 1997). Two main reasons have been offered to explain the increased photosynthetic rates, a nutritional effect due to increased N, P and K absorption (Reid *et al.,* 1993) and a non-nutritional effect, suggesting that photosynthetic rate are related to demand for carbohydrates and constitutes. The source- sink concept basis for carbon metabolism (Vodnik and Gogala 1996). Improved nutrient status of inoculated plants, especially P and N nutrition is a well known phenomenon (Finlay *et al.*, 1996 and Fa-Yuanwang *et al.*, 2004).

According to Wallander and Nylund (1992). P-deficiency increases the carbohydrate pool in plants which in non mycorrhizal plants should increase chloroplast starch and thus down regulate photosynthesis. Consequently, in mycorrhizae plants, where P increases and there is a drain of carbon compounds to the fungus photosynthesis should increase since there is no carbohydrate accumulation to inhibit photosynthesis.

It was therefore decided that foliar N and P may be better indicators of the average nutrient status of mangroves. The data available for the study provided an opportunity to test the relationships between N, P plant content and biofertilizations. It is clear that inoculation with azotobacter, phosphate dissolving bacteria and AM- mycorrhizae together were more effective in increasing plant N, P contents (Kevin and Wellington, 2003) and biological yield.

Table 6. Effect of inoculation of Mangrove plant with Azotobacter, phosphate dissolving bacteria and VA mycorrhizae on phosphorus, Nitrogen and Chlorophyll content

Treatments	Salinity			
	0	15000	22500	45000
	Chlorophyll content			
Control	51.8	49.1	49.1	47.9
Azotobacter	56.2	52.4	55.9	56.0
PDB	53.6	51.2	55.7	49.5
Mycorrhiza	56.7	55.7	56.2	56.3
Mixture	57.9	59.2	58.6	57.9
Mean	55.3	53.5	55.1	53.5
LSD 5%	**Bio 0.7 – salinity 0.3 - interaction 2.2**			
	Nitrogen content %			
Control	0.79	0.70	0.88	1.05
Azotobacter	1.23	1.23	1.58	1.58
PDB	1.05	1.14	0.88	1.40
Mycorrhiza	1.23	1.05	1.23	1.58
Mixture	1.75	1.93	1.58	1.58
Mean	1.21	1.21	1.23	1.44
LSD 5%	**Bio 0.08 – salinity 0.05 - interaction 0.25**			
	Phosphorus content %			
Control	0.228	0.301	0.289	0.266
Azotobacter	0.236	0.316	0.291	0.273
PDB	0.250	0.320	0.291	0.275
Mycorrhiza	0.240	0.324	0.292	0.296
Mixture	0.258	0.324	0.296	0.300
Mean	0.242	0.317	0.292	0.282
LSD 5%	**Bio 0.002– salinity 0.015 - interaction 0.019**			

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تأثير الأسمدة الحيوية ومستويات ملوحة ماء الرى على النمو والمحتوى الكيميائى لنبات المانجروف

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فى تجربة أصص تم دراسة تأثير التلقيح بأكفاء العزلات من الأزوتوباكتر كروكوكم، باسيلس ميجاتيرم وجراثيم الميكورهيز ١ على نمو نبات المانجروف الذى يروى بمياه مالحة بتركيزات مختلفة من الأملاح وعلى العدد الكلى للميكروبات وأعداد بكتريا الازوتوباكتر والبكتريا المذيبة للفوسفات وجراثيم الميكورهيز ١ فى بيئة النمو بعد الحصاد. وأظهرت نتائج الدراسة أن أفضل زيادة معنوية تم تسجيلها مع الرى عند ١٥٠٠٠ جزء فى المليون للأزوتو باكتر والميكورهيز ١بينما أعطت البكتريا المذيبة للفوسفات أفضل زيادة معنوية عند مستويات مختلفة من الملوحة. أما بخصوص النسبة المئوية للنيتروجين والفوسفور، تم تسجيل أعلى استجابة معنوية مع مخلوط من الميكروبات المستخدمة وزادت النسبة مع زيادة مستويات الملوحة للمعاملات الفردية والمخلوط. وقد أعطت قياسات النمو للمانجروف وهى عبارة عن طول النبات – طول الجذر – عدد الفروع والأوراق وقطر السيقان أعلى قيمة فى التلقيح الفردى والمخلوط أكثر من الكنترول خاصة عند الرى بمياه البحر مخلوط مع الماء العذب عند ٢٢٥٠٠ جزء فى المليون.