INFLUENCE OF MICROBIAL INOCULATION ON GROWTH OF SOME SOFTWOOD SEEDLINGS IRRIGATED BY BRACKISH WATER

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ABSTRACT

This study examined growth characteristics and wood properties of baldcypress (*Taxodium disticum* Rich.), calabrian pine (*Pinus brutia* Tenore) and cypress (*Cuprssus sempervirens* L.) as affected by both various microbial inoculations and brackish water treatment throughout the period from 10th April 2007 to 30th November 2008. The results of this study can be concluded that brackish water generally, decreased the activity of bacteria, fungus and actinomycetes in the planting soil of the three tree kinds through decreases the counts of these abovementioned microbes. Also, the microbial inoculations that used in this study decreased the soil bacteria, fungus and actinomycetes with different variations among them. Baldcypress was the most sensitive tree species to brackish water where all seedlings were died completely under the treatments of tested microbial inoculation. On the other hand, the recorded data indicated that the addition of *Azotobacter chrococcum* PGPR to the seedlings and soil gave the highest growth values for cypress seedlings. Also, inoculation the planting soil with *Bacillus polymixa* improved the growth parameters of calabrian.

Key words: Microbial inoculation, *Azotobacter, Bacillus*, PGPR, conifer, softwoods, brackish water.

INTRODUCTION

Water scarcity is a growing global problem challenging sustainable development and expansion of cultivated areas. Egypt is one of the countries facing this problem, due to its limited water resources represented mainly by its fixed share of the Nile water (55.5×10^9 m^3) and its aridity which is the general characteristics of the country. Conversely, brackish Water is available at shallow depths in the western and eastern Deserts and at the borders of the Nile valley. The average salinity of such water varies from 3000 to12000 ppm. Else more, according to National Health and Medical Research Council and Agricultural and Resource Management

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Council of Australia and New Zealand, (1996) the brackish water has an EC value ranged 1000- 5000 mg l^{-1}.

Softwoods or (conifers) actually refer to a group of different species of trees from which the wood is harvested like baldcypress, calabrian pine and cypress. They are fast-growing, can be easily cultivated, and produce relatively straight trunks, which makes harvesting and processing much less expensive. Conifers are also used in the manufacture of fiberboard and paper.

The term biofertilizers or which can be more appropriately called 'microbial inoculants' can be generally defined as a preparation containing live or latent cells of efficient strains of nitrogen fixing or phosphate solublization microorganisms used for application of seed, soil or composting areas with the objective of increasing the numbers of such microorganisms and accelerate certain microbial process to increase the extent of the availability of nutrients in a soil which can be easily assimilated by plant. Bio-fertilizers are natural fertilizers which are microbial inoculants of bacteria, algae, fungi alone or in combination and they augment the availability of nutrients to the plants. The use of bio-fertilizers, in preference to chemical fertilizers, offers economic and ecological benefits by way of soil health and fertility (NIIR Board, 1984). Free-living plant growth-promoting rhizobacteria (PGPR) can be used in different ways when plant growth improvement is necessary. The most intensively researched use of PGPR has been in agriculture and horticulture where these bacteria may be used to facilitate the growth of various plants, especially under stressful conditions (Wang *et al.* 2000 and Grichko and Glick 2001). Rajendran and Devaraj (2004) concluded that better nutrient uptake on *Casuarina equisetifolia* Forst was estimated in combined inoculation of Azospirillum + Phosphobacterium + AM + Frankia in respect of the nutrients such as N, P, K, Ca and Mg.

Recently developing areas of PGPR usage include forest regeneration and phytoremediation of contaminated soils. As the mechanisms of plant growth promotion by these bacteria are unraveled, the possibility of more efficient plant-bacteria pairings for novel and practical uses will follow (Lucy *et al.*, 2004). *Azotobacter* is an interesting plant growth promoting rhizobacterium (PGPR). Dobereiner and pedrosa, (1987) obtained large increases in plant growth for a variety of dicotyledonous and monocotyledonous plants grown in pots in natural soil inoculated with *Azotobacter paspali*. They concluded that indole acetic acid, gibberellins and cytokinens are the plant growth factors that promoted plant growth and plant growth promotion is derived mainly form a general effect on root growth and function.

Since most soils of Egypt has immobilize phosphate ions into unavailable forms. There are more microorganisms with a strong ability to decompose inositol phosphate in the soil which solubilize mineral bound phosphates by the excretion of chelating organic acids that lower the soil pH and bring about the dissolution of bound forms of phosphate.

The objective of this study was assessing the effect of various bacterial inoculants with brackish water on the growth and wood properties of baldcypress (*Taxodium disticum* Rich.), calabrian pine (*Pinus brutia* Tenore) and cypress (*Cuprssus sempervirens* L.) seedlings as well as, the microbial activity in their soils.

MATERIALS AND METHODS

Site and treatments

Experiments have been done during the period from 10th April 2007 to 30th November 2008 in open-field of private nursery in King- Mariut (30°58.18'N; 29°42.69'E), 50 Km south west of Alexandria. Two-year-old baldcypress (*Taxodium disticum* Rich.), calabrian pine (*Pinus brutia* Tenore) and cypress (*Cuprssus sempervirens* L.), as soft wood trees, were chosen for this study. The tree seedlings were transplanted on April 10, 2007 in large volume polyethylene bags had a diameter of 50cm and a depth of 60cm and filled with 30 Kg of sandy loam soil (58, 26 and 16% of sand, loam and clay respectively), and its chemical analysis is shown in (Table a). All the seedlings were watered with tap water for 30 days before starting the treatments afterward, seedlings were inoculated by drench 20 ml of microbial inoculation suspensions into the planted media.

The three microbial strains were supplied by microbiology Department, Soils, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt. Azotobacter was grown in Ashby's agar medium otherwise, both *Bacillus* varieties were grown in King's B. medium (Atlas, 1995).

The studied microbial inoculations were: (1) Control, (2) *Bacillus megatherium var. phosphaticum* as phosphobacteria, (3) *Bacillus polymixa* and (4) *Azotobacter chrococcum* PGPR, both as nitrogen fixing bacteria, that were chosen to test its growth-promoting ability when inoculated on the above mentioned softwood tree species under two types of irrigation water: (1) tap water and (2) brackish water (from a deep-well that is 25m far from the soil surface) where its chemical analysis is given in (Table b).

Measurements

At the end of study the microbial activity was measured through measuring the microbial count in the planting soil. Stem height and diameter (above ground level) of all seedlings were measured as well as, the number of first-order branch (number of main branches) of the seedlings were counted at the end of experiment. Furthermore, two random seedlings of each replicate were destructively harvested at the end of study period (30th November, 2008) then both fresh and dry biomass of leaves, stems and roots were determined.

Table (a): Chemical analysis of the planting soil

Soluble cations (mg l^{-1})				Soluble anions (mg l^{-1})				$CaCO_3$	pH	E.C.
K^+	Na^+	Mg^{2+}	Ca^{2+}	SO_4^{2-}	Cl^-	HCO_3^-	CO_3^{2-}	%		dS m^{-1}
0.305	2.04	0.24	1.1	0.935	1.3	1.45	-	32.5	8.0	0.35

Table (b): Average of chemical analysis of the irrigation brackish water throughout two successive seasons

Soluble cations (mg l^{-1})				Soluble anions (mg l^{-1})				E.C.
K^+	Na^+	Mg^{2+}	Ca^{2+}	SO_4^{2-}	Cl^-	HCO_3^-	CO_3^{2-}	dS m^{-1}
0.795	48.40	9.80	5.00	21.99	36.40	5.60	-	6.36

Afterward, these components were dried in oven for 72 hours at 50°C for leaves and 80 for stems and roots. Sample of 3-cm was cut from each stem at soil surface. The sample was divided into two further samples, one longed 2-cm was used to determine the wood density and the remained of 1-cm to determine the fiber length. Wood density was determined based on the fresh volume and oven- dry weight according to the American Society for Testing and Materials (1999). The samples were portioned into small segments, having radial and tangential dimension of 2 x 5 mm, respectively in order to determine the wood fiber length. These segments were macerated in a 1:1 (v:v) solution of glacial acetic acid and 30% hydrogen peroxide at 60°C for 48 hours, then rinsed several times with distilled water (Franklin, 1945). A drop of macerated tissue from each sample was placed on a microscope slide then, stained with Safrinin dye and covered. The slide was placed in a slide holder and projected onto a screen. Fifty whole fibers per sample were measured to the nearest 1mm.

Statistical analysis

Both the various microbial inoculations and the types of irrigation water combined and replicated three times in a factorial randomized complete blocks design. Each replicate was represented with three seedlings to reduce the error of the replicate mean. Since baldcypress seedlings were completely failed at brackish water treatment therefore, the data of this species under this treatment was terminated from the statistical analysis but the data of tap water treatment (control) was exhibited only. Data were analyzed by two ways ANOVA randomized complete blocks design for cypress and calabrian pine to examine the effect of both microbial inoculations and the types of irrigation water and as one way ANOVA randomized complete blocks design for baldcypress specie to examine the microbial inoculations only. Afterward, means were compared by Duncan's multiple range tests at 0.05 probability using CoStat software, (1990).

RESULTS AND DISCUSSION

1- Effects on planting soil

Generally, brackish water decreased the activity of bacteria, fungus and actinomycetes in the planting soil through decrease the counts of these microbes (Table 1).

1-a. Baldcypress

Data in Table (1) exposed that various microbial inoculation affected the different microbial counts in planting soil of baldcypress seedlings. *Bacillus polymixa* recorded the highest bacteria count (117.0 x 10^3 g^{-1} soil) conversely, both of *Azotobacter chrococcum* and *Bacillus megatherium var. phosphaticum* minimized bacteria counts, with the same significant level, in the planting soil of baldcypress seedlings comparing with non-inoculated soil (control).

Table (1): Different microbial counts (number x 10^3 g^{-1} soil) in the soil of three softwood seedlings treated with various microbial inoculations and brackish water after 20 months.

Treatments	Baldcypress			Cypress			Calabrian pine		
	Tap water	Brackish water		Tap water	Brackish water	Mean	Tap water	Brackish water	Mean
				Bacteria					
Control	113.60	66.50	90.05b	155.40	76.20	115.80a	36.00	8.50	22.25b
B. mega.	82.20	20.60	39.07c	124.00	60.20	92.10c	14.10	18.20	16.15c
B. polmx.	149.00	85.00	117.00a	180.00	31.50	105.75b	85.10	18.20	51.65a
A.chrococcum	64.20	18.30	41.25c	80.30	27.40	53.85d	25.00	10.00	17.50c
Mean	96.08a	47.60b		134.93a	48.83b		40.05a	13.73b	
				Fungus					
Control	8.70	5.70	7.20a	14.30	8.20	11.25a	12.20	2.00	7.10b
B. mega.	3.30	1.00	2.15d	5.50	3.50	5.30b	2.00	1.00	1.50d
B. polmx.	5.00	0.30	2.65c	5.00	3.50	4.25b	0.10	19.00	9.55a
A.chrococcum	7.10	2.30	4.70b	6.10	4.50	5.30b	7.10	0.10	3.60c
Mean	6.03a	2.33b		7.73a	4.93b		5.53a	5.53a	
				Actinomycetes					
Control	33.30	18.40	25.85a	14.30	10.50	12.40c	19.20	13.50	16.35a
B. mega.	7.00	3.50	5.25d	10.60	7.00	8.80d	6.00	4.60	5.30d
B. polmx.	16.70	10.40	13.55c	17.20	11.50	14.35b	16.10	8.60	12.35b
A. hrococcum	35.00	9.00	22.00b	18.50	17.20	17.85a	12.30	9.30	10.80c
Mean	23.00a	10.33b		15.15a	11.55b		13.40a	9.00b	

Means followed by a similar letter within a column (a, b, c) or row (x, y) are not significantly different at the probability level 0.05 using Duncan's Multiple Range Test.

On the other hand, fungus and actinomycetes counts of planting soil were decreased when inoculated by various microbial inoculations with similar trend. Table (1) showed that inoculated the soil of baldcypress seedlings with *B. megatherium var. phosphaticum* recorded the more little counts of bacteria and actinomycetes (2.15×10^3 g^{-1} soil and 5.25×10^3 g^{-1} soil, respectively) comparing with control. Subsequently, *Bacillus polymixa* inoculation decreased counts of both fungus and actinomycetes of the planting soil (2.65×10^3 g^{-1} soil and 13.55×10^3 g^{-1} soil, respectively). Then, *Azotobacter chrococcum* slightly decreased the counts of both fungus and actinomycetes of the planting soil (4.70×10^3 g^{-1} soil and 22.00×10^3 g^{-1} soil, respectively).

1-b. Cypress

Table (1) revealed that various microbial inoculations decreased bacteria counts in the planting soils of cypress seedlings. Therefore, the planting soil that inoculated with *Azotobacter chrococcum* recorded the more little count of bacteria followed by *B. megatherium var. phosphaticum* then *Bacillus polymixa* (53.85x 10^3 g^{-1} soil, 92.10x 10^3 g^{-1} soil and 105.75x 10^3 g^{-1} soil, respectively). Likewise, various microbial inoculations decreased fungus counts in the planting soils of cypress seedlings in comparing with control with the same significant level. On the contrary, actinomycetes counts were rise when soil of cypress seedlings was treated with *Azotobacter chrococcum* and *Bacillus polymixa* inoculations that recorded 17.85x 10^3 g^{-1} soil and 14.35x 10^3 g^{-1} soil, respectively whereas, *B. megatherium var. phosphaticum* decreased actinomycetes counts in planting soil of cypress seedlings (8.80x 10^3 g^{-1} soil) in comparison with control.

1-c. Calabrian pine

Inoculation the soil of calabrian pine with *Bacillus polymixa* only encouraged the growth of soil bacteria and fungus through increasing their count numbers (Table 1), whereas *Azotobacter chrococcum* and *Bacillus megatherium var. phosphaticum* were minimized the counts of soil bacteria and fungus, in comparing with control. Contrary, all used inoculations decreased the count of soil actinomycetes whereas addition of *Bacillus polymixa, Azotobacter chrococcum* and *Bacillus megatherium var. phosphaticum* recorded 12.35x 103 g-1 soil, 10.80x 103 g-1 soil and 5.30x 103 g-1 soil, respectively.

2- Effects on vegetative growth

Table (2) revealed that various microbial inoculations influenced the growth of the three softwoods, with significant differences among treatments for seedlings height, diameter at the base level, number of branches and maximum extend of roots. Otherwise, all baldcypress seedlings irrigated with brackish water were died.

2-a. Baldcypress

Azotobacter chrococcum and *Bacillus polymixa* had significant effects on height growth of baldcypress seedlings which irrigated with tap water therefore, the seedling heights were 60.74 and 54.60%, respectively more than control whereas, *B. megatherium var. phosphaticum* was not different significantly.

Table (2): Influence of various microbial inoculation and brackish water on growth characteristics of three softwood seedlings after after 20 months

Treatments	Baldcypress		Cypress			Calabrian pine		
	Tap water	Brackish water	Tap water	Brackish water	Mean	Tap water	Brackish water	Mean
Height (cm)								
Control	81.50b	-	119.50c	93.00d	106.25b	92.00a	82.50a	87.25a
B. mega.	89.00b	-	119.50c	102.00d	110.75b	90.50a	82.00a	86.25a
B. polmx.	126.00a	-	133.50b	126.00bc	129.75a	91.00a	84.00a	87.50a
A.chrococcum	131.00a	-	149.00a	121.50bc	135.25a	98.50a	83.50a	91.00a
Mean			130.38x	110.63y		93.00x	83.00y	
Diameter (cm)								
Control	1.43a	-	1.46a	0.93c	1.20b	1.27b	1.01c	1.14b
B. mega.	1.50a	-	1.45a	0.79c	1.12b	1.35ab	1.42a	1.39a
B. polmx.	1.76a	-	1.45a	1.25b	1.35a	1.30b	1.10c	1.20b
A.chrococcum	1.77a	-	1.47a	0.91c	1.19b	1.34ab	1.04c	1.19b
Mean			1.46x	0.97y		1.32x	1.14y	
Number of branches / plant								
Control	14.50a	-	100.00a	49.00c	74.50a	6.67c	5.00d	5.84c
B. mega.	16.00a	-	102.00a	62.00b	82.00a	6.67c	9.50b	8.09b
B. polmx.	15.00a	-	100.00a	57.50b	78.75a	13.67a	5.33d	9.50a
A.chrococcum	17.50a	-	103.00a	61.00b	82.00a	9.00b	6.67c	7.84b
Mean			101.25x	57.38y		9.00x	6.63y	
Max. extend of roots (cm)								
Control	67.50c	-	53.00d	50.00e	51.50c	62.50cd	50.50d	56.50b
B. mega.	85.50c	-	85.00b	52.00de	68.50b	77.00bc	59.50cd	68.25ab
B. polmx.	74.5b	-	91.00a	63.00c	77.00a	65.0cd	86.50ab	75.75ab
A.chrococcum	103.00a	-	54.00d	50.00e	52.00c	99.00a	74.00bc	86.50a
Mean			70.75x	53.75y		75.88x	67.63y	

Means followed by a similar letter within a column (a, b, c) or row (x, y) are not significantly different at the probability level 0.05 using Duncan's Multiple Range Test.

Conversely, all microbial inoculations had not significant effects comparing with control on diameter at base line and number of branches for the survival seedlings that irrigated by tap water. *Azotobacter chrococcum* was significantly effective in regard to maximum extend of seedling roots that extended for 52.60% more than non- inoculated seedlings.

2-b. Cypress

Inoculation with both *Azotobacter chrococcum* and *B. polymixa* had the same level of significant as well as, promoted the height of cypress seedling by 27.3 and 22.1%, respectively in comparing with non-inoculated seedlings. *B. megatherium* had the subsequent rank followed by *A. chrococcum* and *B. polymixa* that promoted the height of cypress seedlings by 4.2% than control. Also, *B. polymixa* recorded the significant wider diameter of the seedlings (12.5% more than control) whereas the other inoculations were not differing significantly while, Number of branches did not affected by using any microbial inoculations. Moreover, *B. polymixa* recorded the significant max. extend of roots of the seedlings (49.52% more than control).

Brackish water negatively affected the growth of cypress seedlings where height, diameter, number of branches and max. extend of roots were reduced by 15.15, 33.56, 43.33 and 24.03% lower than tap water, respectively.

Positive effect of using microbial inoculation on cypress seedlings under irrigation with brackish water was obvious with *B. polymixa* on height, diameter and max. extend of roots although effect of the three inoculations under irrigation by brackish water were significantly similar for number of branches.

2-c. Calabrian pine

Data in Table (2) revealed that height growth of calabrian pine seedlings was not affected significantly by the tested inoculations during the study period, probably since pine is a slow-growing species. On the other hand, *B. megatherium var. phosphaticum* improved the diameter growth by 21.93% more than control whereas, the other microbial inoculations were not differed significantly. Additionally, magnitude number of branches was emerged with *B. polymixa* inoculation (62.67% more than control) followed by both *B. megatherium* and *A. chrococcum* (38.53 and 34.25% more than control, respectively) with the same level of significance.

Height, diameter at base line and number of branches of calabrian pine seedlings grown under irrigation with brackish water were depressed compared with those irrigated by tap water. Therefore, brackish water decreased height, diameter at base line and number of branches by 10.75, 13.64 and 26.33% lower than tap water, respectively.

Whereas the three inoculations had similarly significant effect on seedling height under irrigation by brackish water therefore, *B. megatherium var. phosphaticum* had the superior effect on both diameter at base line and number of branches under irrigation by this quality of water.

These results are compatible with Woitke *et al.*, (2004) that *Bacillus subtilis* induces plant resistance to stress and produces plant hormones for growth improvement. Also, the promoting effect of *Azotobacter* *chrococcum* (PGPR) on cypress height growth was matched with Wang *et al.* (2000) and Grichko and Glick (2001) and this effect may be due to that azotobacter synthesizes various phytohormones, including auxins and cytokinins (Patten and Glick 1996). In this context, Glick (1995) and Glick *et al.* (1999) mentioned that PGPR can have an impact on plant growth and development in two different ways, indirectly or directly. The indirect promotion of plant growth occurs when bacteria decrease or prevent some of the deleterious effects of a phyto-pathogenic organism by one or more mechanisms. On the other hand, the direct promotion of plant growth by PGPR generally entails providing the plant with a compound that is synthesized by the bacterium or facilitating the uptake of nutrients from the environment. Enhancement effect of PGPR on cypress seedlings growth under brackish water possibly due to that number of PGPR contain the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, as cited by Glick *et al.* (1998) and Shah *et al.* (1998), and this enzyme can cleave the plant ethylene precursor ACC, and thereby lower the level of ethylene in a stressed plant. In addition, plants that are treated with ACC deaminase-containing PGPR are dramatically more resistant to the deleterious effects of stress ethylene that is synthesized as a consequence of stressful conditions such as high salt (Mayak *et al.*, 2004).

3- Effects on biomass

3-a. Baldcypress

Tables (3-1)) and (3-2) showed that *B. polymixa* had superior effect on fresh and dry weights of baldcypress followed by *A. chrococcum* whereas, *B. megatherium var. phosphaticum* had not significant differences comparing with non inoculated seedlings. Increasing in fresh and dry weights were 1.9 and 1.7-folds for leaves, 3.5 and 3.4-folds for stem and 1.2 and 1.5-folds for roots, more than control respectively.

3-b. Cypress

Data in Tables (3-1) and (3-2) revealed that various microbial inoculation were promising in promoting the biomasses yield of cypress seedlings. *A. chrococcum* recorded the magnitude significant fresh leaves, stem and roots biomass (153.10, 268.85 and 125.38 g seedling^{-1}, respectively) as well as, the highest dry biomass of the aforementioned parts (82.49, 83.40 and 96.91 g seedling^{-1}, respectively). Additionally, *B. megatherium var. phosphaticum* had the subsequent highest fresh weight of leaves as well as, highest dry weight of leaves and stem whereas, *B. polymixa* recorded the next magnitude significant fresh and dry weights of roots.

As a whole, irrigated cypress seedlings with brackish water significantly minimized fresh biomass of leaves, stem and roots as 105.46, 70.70 and

218.00 g seedling^{-1}, respectively whereas, dry biomass of the former parts were decreased to 52.62, 49.31 and 60.84 g seedling^{-1}, respectively.

Table (3-1): Influence of various microbial inoculation and brackish water on above and below-ground biomass of three softwood seedlings after 20 months

Treatments	Baldcypress		Cypress			Calabrian pine		
	Tap water	Brackish water	Tap water	Brackish water	Mean	Tap water	Brackish water	Mean
Leaves fresh weight (g seedling^{-1})								
Control	22.50c	-	108.50c	85.50d	129.52c	131.63ef	127.40f	129.52c
B. mega.	24.15c	-	134.90b	113.10c	177.45b	143.97e	210.93b	177.45b
B. polmx.	41.80a	-	105.30c	114.85c	212.18a	229.60a	194.75c	212.18a
A.chrococcum	28.10b	-	197.80a	108.40c	136.74c	171.57d	101.90g	136.74c
Mean			136.63x	105.46y		169.19x	158.75y	
Leaves dry weight (g seedling^{-1})								
Control	10.80b	-	64.02c	43.61d	53.82d	80.47f	73.03g	76.75c
B. mega.	12.10b	-	87.69b	55.25cd	71.47b	88.80e	124.13b	106.47b
B. polmx.	18.08a	-	60.02cd	57.43cd	58.73c	136.65a	118.60c	127.63a
A.chrococcum	13.48b	-	110.77a	54.20cd	82.49a	101.63d	61.20h	81.42c
Mean			80.63x	52.62y		101.89x	94.24y	
Stem fresh weight (g seedling^{-1})								
Control	27.90d	-	140.50bc	67.60e	104.05b	92.37c	91.83c	92.10c
B. mega.	30.95c	-	155.60b	56.40e	105.50b	98.33c	149.60a	123.97b
B. polmx.	96.45a	-	135.80c	92.55d	114.18b	160.55a	150.90a	155.73a
A.chrococcum	73.65b	-	183.50a	67.25e	125.38a	116.20b	89.45c	102.83c
Mean			153.85x	70.70y		116.86x	120.45x	
Stem dry weight (g seedling^{-1})								
Control	16.75d	-	84.30c	37.18f	60.74c	62.07bcd	57.47cd	59.77c
B. mega.	19.19c	-	105.81b	38.35ef	72.08b	67.27bc	91.13a	79.20b
B. plmx.	56.14a	-	84.20c	47.15e	65.68c	99.25a	93.85a	96.55a
A.chrococcum	45.66b	-	119.28a	74.55d	96.91a	75.75b	45.73d	62.24c
Mean			98.39x	49.31y		76.09x	72.80x	

Means followed by a similar letter within a column (a, b, c) or row (x, y) are not significantly different at the probability level 0.05 using Duncan's Multiple Range Test.

While, the three inoculations had positive effect on fresh and dry weights of cypress leaves when associated with irrigation by brackish water however, *B. polymixa* recorded the highest stem fresh weight and *A. chrococcum* increased dry weight of stem as well as fresh and dry weights of their roots when interacted with brackish water.

3-c. Calabrian pine

Results of biomass showed significant differences among the three inoculations (Tables 3-1 and 3-2). *B. polymixa* recorded the magnitude significant fresh biomass and dry biomass of leaves, stem and roots that were 63.82, 69.09 and 69.91% for fresh and 66.29, 61.54 and 82.70% for dry biomass more than control, respectively.

Table (3-2): Influence of various microbial inoculation and brackish water on above and below-ground biomass of three softwood seedlings after 20 months

Treatments	Baldcypress		Cypress			Calabrian pine		
	Tap water	Brackish water	Tap water	Brackish water	Mean	Tap water	Brackish water	Mean
			Root fresh weight (g seedling^{-1})					
Control	375.45d	-	193.70cd	230.90bc	212.30c	184.50e	256.50c	220.50d
B. mega.	388.60c	-	195.80de	228.90bcd	212.35c	315.30b	284.30bc	299.80b
B. polmx.	447.00a	-	230.10bc	264.50b	247.30b	190.70de	558.60a	374.65a
A.chrococcum	437.05b	-	390.0a	147.70e	268.85a	221.00d	270.50c	245.75c
Mean			252.40x	218.00y		342.48x	227.88y	
			Root dry weight (g seedling^{-1})					
Control	112.64d	-	54.24de	60.04d	57.14b	40.59c	48.74c	44.67d
B. mega.	124.35c	-	60.70cd	64.09cd	62.40b	78.83b	56.86c	67.84b
B. polmx.	166.08a	-	113.10a	39.88e	76.49a	51.49c	111.72a	81.61a
A.chrococcum	134.10b	-	87.44b	79.35bc	83.40a	50.83c	59.51bc	55.17c
Mean			78.87x	60.84y		69.21x	55.44y	

Means followed by a similar letter within a column (a, b, c) or row (x, y) are not significantly different at the probability level 0.05 using Duncan's Multiple Range Test.

Also, it was observed that, irrigated calabrian pine seedlings with brackish water significantly minimized fresh biomass of leaves and roots as 169.19 and 227.88, respectively whereas, dry biomass of the former parts were decreased to 94.24 and 55.44 g seedling^{-1} , respectively. On the other hand, both fresh and dry biomasses of calabrian pine were not affected significantly by brackish water.

Interaction of inoculation bacteria with irrigation by brackish water showed that the *B. megatherium var. phosphaticum* was the most effective one for the huge fresh and dry biomass.

The abovementioned results are compatible with Rajendran and Devaraj, (2004) that the maximum total biomass, after 24 months from planting, of *Casuarina equisetifolia* Forst inoculated with different biofertilizers were obtained in the combined application of *Azospirillum*, phosphobacterium, AM and *Frankia*.

4- Effects on total chlorophyll

4-a. Baldcypress

Table (4) showed that only the seedlings which irrigated by tap water were alive to the end of the study, on the other hand the seedlings that irrigated with brackish water died completely. Hence, *Bacillus polymixa* had the highest ability to increase the total chlorophyll content of baldcypress seedlings whereas, the seedlings that inoculated with *B. megatherium var. phosphaticum* had the poorest total chlorophyll content after the study period.

Table (4): Influence of various microbial inoculation and brackish water on the total chlorophyll content (mg $100g^{-1}$ F.W. of leaves) of three softwood seedlings after 20 months

	Baldcypress		Cypress			Pine		
	Tap water	Brackish water	Tap water	Brackish water	Mean	Tap water	Brackish water	Mean
Control	137.55b	-	100.76de	86.23f	93.50d	39.60c	34.93cd	37.27b
B. mega.	56.47d	-	127.41ab	118.06c	122.74b	65.14b	40.66c	52.90a
B. polmx.	147.71a	-	103.72d	95.92e	99.82c	83.84a	24.74d	54.29a
A.chrococcum	128.68c	-	133.52a	124.83bc	129.18a	64.62b	43.70c	54.16a
Mean		-	116.35x	106.26y		63.30x	36.01y	

Means followed by a similar letter within a column (a, b, c) or row (x, y) are not significantly different at the probability level 0.05 using Duncan's Multiple Range Test.

4-b. Cypress

Data in Table (4) revealed that the inoculated microbes not only enhance the growth and increase the yield of cypress seedlings, but also reflected on the total chlorophyll content. Therefore, *A. chrococcum* had the highest effect on the total chlorophyll content for cypress seedlings followed by *B. megatherium var. phosphaticum* then *B. polymixa* which were 38.16, 31.27 and 6.76%, respectively more than with control.

Also, the seedlings irrigated by brackish water had worst effect on the total chlorophyll content that decreased by 8.67% comparing with the seedlings irrigated by tap water.

Furthermore, the seedlings that inoculated with *A. chrococcum* and irrigated by tap water had the highest chlorophyll content 133.52 mg $100g^{-1}$ F.W.), however the seedlings which were not inoculated and irrigated by brackish water recorded the least total chlorophyll content (86.23 mg $100g^{-1}$ F.W.).

4-c. Calabrian pine

It can be seen from Table (4) that the used microbial inoculations had significant effect on enhancement the total chlorophyll content for calabrian pine seedlings comparing with control without significant differences among them. Moreover, the seedlings irrigated by brackish water decreased its content of total chlorophyll content by 43.11% in comparing with these irrigated by tap water.

According the interaction between microbes effect and irrigation quality, it seemed that the seedlings which inoculated with *B. polymixa* and irrigated by either tap water or brackish water, recorded the highest and the least total chlorophyll content (83.84 and 24.74 mg $100g^{-1}$ F.W., respectively)

5- Effect on wood properties

5-a. Baldcypress

Table (5) revealed that *B. megatherium var. phosphaticum* and *B. polymixa* inoculations significantly increased the wood density of baldcypress by 4.13 and 2.07%, respectively more than control. Whereas, *A. chrococcum* did not affect the wood density significantly.

Table (5): Influence of various microbial inoculation and brackish water on wood density and fiber length of three softwood seedlings after 20 months

Treatments	Baldcypress		Cypress			Calabrian pine		
	Tap water	Brackish water	Tap water	Brackish water	Mean	Tap water	Brackish water	Mean
	Density (g cm^{-3})							
Control	0.339c	-	0.602	0.511	0.557a	0.629	0.574	0.602a
B. mega.	0.353a	-	0.587	0.485	0.536b	0.606	0.585	0.596a
B. plmx.	0.346b	-	0.618	0.458	0.538b	0.618	0.598	0.610a
A.chrococcum	0.335c	-	0.590	0.489	0.540b	0.613	0.615	0.608a
Mean			0.600x	0.486y		0.606x	0.602x	
	Fiber length (mm)							
Control	1.40a	-	1.30	1.28	1.29a	1.52	1.25	1.39a
B. mega.	1.19b	-	1.32	1.28	1.30a	1.48	1.19	1.33a
B. plmx.	1.42a	-	1.15	1.16	1.16b	1.52	1.14	1.33a
A.chrococcum	1.19b	-	1.09	0.99	1.04c	1.21	1.33	1.27a
Mean			1.22x	1.18x		1.43x	1.23y	

Means followed by a similar letter within a column (a, b, c) or row (x, y) are not significantly different at the probability level 0.05 using Duncan's Multiple Range Test.

Conversely, both *B. megatherium var. phosphaticum and A. chrococcum inoculations* significantly, shortened the fiber length of baldcypress wood by 15.00% in comparing with non-inoculated seedlings. While inoculation the seedlings of baldcypress with *B. polymixa* significantly, not affected fiber length in comparison with control.

5-b. Cypresss

The results in Table (5) showed that all inoculations significantly reduced the density of cypress wood slightly by 3.77, 3.41 and 3.05% less than non- inoculated seedlings for *B. megatherium var. phosphaticum*, *B. polymixa* and *A. chrococcum*, respectively. As well as, *B. polymixa* and *A. chrococcum* shortened the fiber length of cypress wood by 10.08 and 19.38%, respectively less than non- inoculated seedlings.

Also, brackish water significantly lighten the density of cypress wood by 19.00% in regard with seedlings irrigated by tap water whereas, fiber length was not affected significantly by brackish water.

5-c. Calabrian pine

Table (5) demonstrated that there were no significant differences between the three inoculations and its relative control after the study period according their effect on wood density and fiber length.

Likewise, brackish water was not differed significantly with tap water according their effect on wood density only whereas, fiber length was shortened by 13.99% when seedlings irrigated by brackish water.

The above mentioned results are agree with the findings of Jesus and Brouard, (1988) that after seven years there were no differences in wood density of the eucalypts in *Eucalyptus-Leucaena* mixtures. On the other hand, Gentle *et al.*, (1968) found reduction in wood density of radiate pine with nitrogen fertilization. Also, in their partial summary of the effects of specific gravity on wood properties, covering 44 studies and 16 species of conifers, Zobel and Van Buijtenen (1989) assessed that the nitrogen fertilization cause a mild to severe decrease in specific gravity.

RECOMMENDATION

Sandy loam soils that irrigated by brackish water (up to 6.36 ds/m) could be inoculated with *Azotobacter chrococcum* PGPR to enhance the growth of cypress seedlings. Also, calabrian pine seedlings which irrigated by abovementioned water quality could be inoculated with *Bacillus polymixa* to improve their growth.

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الملخص العربى

تأثير اللقاحات الميكروبية على نمو أنواع مختلفة من شتلات الأخشاب الناعمة المروية بالماء المالح

فى هذه الدراسة تم تقييم النشاط الميكروبى بالتربة وصفات النمو وخصائص الخشب لشتلات أشجار كل من التاكسوديم والصنوبر والسرو والتى عوملت بأنواع متعددة من اللقاحات الميكروبية ورويت بمياه مالحة خلال الفترة من ١٠ أبريل ٢٠٠٧ وحتى ٣٠ نوفمبر ٢٠٠٨.

يمكن تلخيص نتائج هذه الدراسة فيما يلى:-

- مياه الرى المالحة بصفة عامة قللت من نشاط البكتريا والفطريات والأكتينوميسيتس الموجودة بتربة الأنواع الثلاث من الأشجار وذلك من خلال قلة اعداد هذه الميكروبات المذكورة آنفاً. كذلك فإن اللقاحات الميكروبية المستخدمة فى هذه الدراسة قللت من العد الميكروبى لكل من البكتريا والفطريات والأكتينوميسيتس الموجودة بالتربة وذلك بتأثيرات متفاوتة فيما بينها.
- التاكسوديم كان أكثر الأنواع المنزرعة حساسية تجاه نوعية مياه الرى حيث ماتت جميع الشتلات المعاملة بالمياه المالحة تحت الأنواع المتعددة من اللقاحات الميكروبية.
- من جهة أخرى أوضحت النتائج المتحصل عليها بأن تلقيح تربة شتلات السرو بلقاح PGPR *Azotobacter chrococcum* أعطى أعلى قيم للنمو.
- أيضاً إضافة لقاح *Bacillus polymixa* فى تربة النمو لشتلات الصنوبر أدى إلى تحسن صفات النمو.

لذلك نوصى بإمكانية إضافة لقاح *Azotobacter chrococcum* للتربة الجيرية الرملية والتى تروى بمياه مالحة حتى ٦,٣٦ ds/m وذلك لتحسين نمو شتلات السرو بها. كذلك فأن شتلات الصنوبر والتى تروى بنفس نوعية المياه المذكورة سابقاً يمكن تحسين نموها بإضافة لقاح *Bacillus polymixa* للتربة المنزرعة بها.