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Effect of Different Bio Fertilizers and Soil Media on Growth and Chemical Composition of *Eucalyptus camaldulensis* in North Africa

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Abstract: Two pot experiments were conducted at the nursery of the Ornamental Horticulture, Department, Horticulture Research Institute, A.R.C, Giza, Egypt. to study the effect of different Bio fertilizers (*Azotobacter chroococcum*, *Bacillus circulans* and Arbuscular mycorrhizal fungi AMF) and soil media (sand, sand:caly and clay) and their interactions on growth, chemical compositions, microbiological parameters and enzymatic activity in rhizosphere of the river red gum (*Eucalyptus camaldulensis* Dehnh) seedlings during two consecutive seasons of 2011 and 2012. The results in both seasons pointed out that the mixture of *Azotobacter chroococcum, Bacillus circulans* and Arbuscular mycorrhizal fungi (AMF) and/or clay and sand+clay significantly increased plant height, stem diameter, number of leaves, leaf area, fresh and dry weights of shoots and roots, chlorophyll a,and b, total carbohydrates, N, P, K% compared with the seedlings grown in sand medium and un-incoulated control. Also the results revealed that this treatment was recorded increase of total microbial count, *Azotobacter chroococcum* count and *Bacillus circulans* count. The highest significant increase in percentages of colonization of AM fungi (%), enzyme activity (dehydrogenase and nitrogenase), percentage of organic carbon and organic matter were recorded in the treatment inoculated with the mixed microbial treatment rather than that of individual and dual treatments in two seasons.

Key words:*Eucalyptus camaldulensis* · Bio fertilizers · Soil types · Vegetative growth · NPK · Microbiological parameters · Enzymatic activity

INTRODUCTION

Eucalyptus camaldulensis Dehnh. (The River Red Gum), Family Myrtaceae is an evergreen tree. It is fast growing and usually grows to 40 - 45 meters in height, depending on its location [1]. River red gum is often planted for shade, windbreak, ornamental, amenity purposes and as a source of nectar to produce high-quality honey [2]. Eucalyptus leaves are a traditional aboriginal herbal remedy. The essential oil found in the leaves is a powerful antiseptic, especially when it is old and is used all over the world for relieving coughs and colds, sore throats and other infections. The oil can be used internally in the treatment of diarrhea and bladder inflammation.

Growing media are considered major factors in controlling the physiological pattern as well as the morphological traits of many ornamental plants. In this regard, many authors agreed that sandy soil is usually considered the cheapest and most readily available material [3]. Soil fertility means the soil capacity to supply the plants with their requirements from nutrients, water and air along the growth season [4].

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Biofertilizers, include symbiotic nitrogen fixers *Rhizobium* sp., non-symbiotic, free living nitrogen fixers *Azotobacter, Azospirillum* etc., Mycorrhiza, cellulolytic microorganisms and organic fertilizers. Biofertilizers have important roles in agriculture. Application of biofertilizers results in increased mineral and water uptake, root development, vegetative growth and nitrogen fixation [5]. Phosphorus solubilizing biofertilizers / microorganisms (bacteria, fungi, mycorrhiza etc.) converts insoluble soil phosphate into soluble forms by secreting several organic acids and under optimum conditions. They liberate growth promoting substances and vitamins and help to maintain soil fertility, improve physical properties of soil, soil health in general and help in the bio-control of disease [6].

The objective of this work was to investigate the effect of different biofertilizers (*Azotobacter chroococum, Bacillus circulans* and Arbuscular Mycorrhizae fungi (AMF), individual or in combination) and soil media (sand, sand + clay (1:1) and clay) on growth and chemical composition of *Eucalyptus camaldulensis* plants in order to produce high quality seedlings, during a relatively short period.

MATERIALS AND METHODS

This study was carried out at the nursery of the Ornamental Horticulture, Department, Horticulture Research Institute, Agricultural Research Center, Giza, during the two successive seasons of 2011 and 2012.

Plant Material: The seedlings of *Eucalyptus camaldulensis* were obtained from the Horticulture Research Station at El-Qanater El-Khaireya, Kalyobia Governorate, Ministry of Agriculture, Egypt. The seedlings were 23 - 25 cm height with 24 - 26 leaves, 1.2 - 1.5 mm diameter.

The uniform seedlings were transplanted to plastic pots (30 cm diameter) filled with three types of soil (sand, sand + clay (1 : 1 v/v) and clay) one seedling/ pot on April 17th in the first and second seasons, respectively. All the control plants were fertilized with NPK at a rate of 1.6 g/pot urea 46% N, 4.0 g/pot calcium superphosphate 15.5% P_2O_5 and 1.0 g/pot potassium sulphate 48% K_2O. Just before transplanting, the seedlings were inoculated with mixed genera of Arbuscular Mycorrhizae fungi (AMF) (*Glomus*, *Gigaspora* and *Acaulospora* spp) once at the rate of 5 gm/pot (500 spore/g), in two seasons.

The plants inoculated with *Azotobacter chroococcum* (Az) were fertilized with NPK at a rate of 1.1 g/pot urea (as 2/3 dose), 4.0 g/pot calcium superphosphate and 1.0g/pot potassium sulphate. While the treatments of *Bacillus circulans* (Bc) were fertilized with1.6 g/pot urea, 4.0 g/pot calcium super phosphate and 0.65 g/pot potassium sulphate (2/3 dose), whereas the treatments inoculated with AM fungi fertilized at a rate of 1.6 g/pot urea, 1.33 g/pot calcium superphosphate (1/3 dose) and 1.0 g/pot potassium sulphate, respectively. The bacterial inocula biofertilizers were applied for two times in each season, at a rate of 5 ml / pot after transplanting and after 45 days, respectively as soil drench, which obtained by Soils Water and Environment Research Institute, Agriculture Research Center (ARC), Giza, Egypt.

Soil Types: The represented soil type samples (sand, sand+ clay 1:1 v/v and clay) were collected from different localities in Egypt. The collected soils were air dried, crushed and prepared to physical and chemical properties determinations. The main soil characteristics of the three types could be summarized as follows: total sand 86.5%, 16.8, silt 4.3 and clay 9.2 while the pH and EC values were 7.82 and 6.12dS/m^{-1}, respectively in the sandy soil type. The corresponding values of these parameters were 65.1% for the total sand, silt 18.7% and 16.2% for clay while for pH and EC were 7.95 and EC 7.42 dS/m^{-1} for the soil type 2 (1:1 sand: clay). On the other hand, the total sand was 31%, silt 35.6, clay 33.4, pH 7.3 and EC 3.4dS/m^{-1} in the clayey soil (type 3). Particle size distribution was determined according to the method described by Dewis and Freitas [7]. The electrical conductivity (EC) as well as soluble cations and anions were determined in saturated soil paste extract according to Jackson [8].

Treatments: Control (without fertilizers), Az, Bc, AMF, Az + Bc, Az + AMF, Bc + AMF and Mixture of (Az + Bc + AMF). These treatments were repeated in the three types of soil.

The Layout of the Experiment: This experiment was designed using a factorial in completely randomized blocks design. Factor (ST) was the soil types and Factor (B) was the biofertilizers treatments with 24 treatments with 3 replicates. (5 pots/ replicate), 8 bio-fertilizer treatments, including the control repeated in 3 soil types (sand, sand + clay and clay).

Data Recorded: After 180 days from planting the following data were recorded: plant height (cm), stem diameter (mm), number of leaves/ plant, leaf area (cm^2),fresh and dry weights of shoots and roots (g/ plant).

Chemical Composition: Photosynthetic pigments (in fresh leaves) after 180 days were determined qualitatively as described by Saric *et al.* [9], total carbohydrates (%) were determined according to Dubois *et al*. [10] and total N, P and K percentages (%) were determined in dry leaves of Eucalyptus plants at 180 days according to Pregl [11], King [12] and Isaac and Kerber [13], respectively.

Microbiological Parameters: Total microbial count, *Azotobacter spp.* and *B. circulans* were counted on Bunt and Rovira medium [14], Modified Ashby's medium [15] and Aleksandrov's medium [16], respectively. The percentage of AMF colonization was also estimated at 60 and 120days from planting according to the method described by Philips and Hayamn [17].

Enzymatic Activity: dehydrogenase activity (µg TPF/g dry soil/day) and nitrogenase activity (nmole C_2H_2 /g rhizosphere/hour values) in the rhizosphere were assayed at 60 and 120days from planting according to Thalmann [18] and Somasegaran [19], respectively. Organic carbon (%) and organic matter (%) were also determined according to the methods described by Page *et al*. [20] and Jackson [8].

Statistical Analysis of Data: The statistical analysis was carried out according to Snedecor and Cochran [21]. L.S.D. at 0.05% was used to compare the differences between treatments.

RESULTS AND DISCUSSION

Effect on Vegetative Growth: Data on vegetative growth (plant height, stem diameter, number of leaves/ plant, leaf area, fresh and dry weights of shoots and roots) of *Eucalyptus camaldulensis* plants as affected by biofertilizers and soil type are shown in Tables (1, 2). In both seasons, all the treatments of biofertilizers significantly increased the vegetative growth characters over the control plants. The mixture of Az, Bc and AMF was the best treatment in increasing the vegetative growth after 180 days from planting. These results agreed with those obtained by Raja and Kumari [22], Shinkafi and Aduradola [23]; El-Khateeb *et al*. [24].

Concerning the effect of the soil type, the data revealed that the best soil was clay, which significantly increased the vegetative growth characters after 180 days from planting in the first and second seasons. The shortest plants are found in the sand medium. Similarly, the best plant height have been reported by EL-Mahrouk *et al.* [25], Azza *et al.* [26] ; El-Assaly [27].

Regarding the effect of the interaction between the biofertilizers and the soil types, it is observed that all the biofertilizers treatments significantly increased the plant height in the different soils over the control plants in the two seasons. Applying the mixture of the three biofertilizers treatments to the plants grown in clay soil resulted in the tallest plants in the both seasons, respectively. The control plants which grow in the sand had the shortest plants after 180 days from planting without fertilization (control) in the both seasons, respectively.

Effect on Chemical Constituents: The data in Tables (3, 4) revealed that application of the mixture of the biofertilizers (Az + Bc + AMF) resulted in the highest content of chemical constituents (chlorophylls a, b, carotenoids content, total carbohydrates, N, P and K %) in both seasons, respectively. While the least contents were determined in the leaves of the plants received AMF only in both seasons, respectively. These results agreed with those found by Vijayakuinari and Janardhanan [28]; Kumudha and Gomathinayagam [29]; Meenakshisundaram *et al*. [30]. The increasing in phosphorus percentage due to the application of the biofertilizers may be as a result of the phosphorus solubilizing biofertilizers microorganisms (bacteria, fungi, mycorrhiza) which convert insoluble soil phosphate into soluble forms by secreting several organic acids and under optimum conditions [6]. Similar increases in N, P and K % of plants fertilized with biofertilizers were reported by other workers [30-32].

Regarding the effect of soil type on chemical constituents, the data pointed out that the plants grown in sand + clay medium had the greatest content of chemical constituents, in the two seasons. The plants grown in the sand contained the least content of chemical constituents in both seasons, respectively. Similar increase in the contents of chlorophyll in the leaves as a result of treatment has been reported by previous studies [26, 33].

Table 1: Effect of biofertilizers, soil types and their interactions on vegetative growth of *Eucalyptus camaldulensis* plants after 180 days from planting during the seasons of 2011 and 2012

	1st season Soil Types (ST)				2nd season Soil Types (ST)			
Biofertilizers (B)	Sand	Sand+Clay (1:1 v/v)	Clay	Mean	Sand	Sand+Clay (1:1 v/v)	Clay	Mean
				Plant height (cm)				
Control	101.33	106.73	112.54	106.87	104.40	107.63	114.20	108.74
Az	121.10	136.00	141.10	132.73	124.97	137.40	140.63	134.33
Bc	121.13	144.93	158.30	141.45	123.47	146.23	158.90	142.87
AMF	116.13	140.40	146.53	134.35	117.74	142.70	146.10	135.51
Az+Bc	135.83	149.83	156.43	147.36	138.63	150.27	158.17	149.02
Az+AMF	149.90	170.87	182.40	167.72	153.00	171.37	181.87	168.75
Bc+AMF	144.40	167.27	182.00	164.56	144.40	167.90	183.03	165.11
Mixture of all	159.77	177.80	191.63	176.40	163.83	177.23	192.10	177.72
Mean	131.20	149.23	158.87		133.81	150.09	159.38	
LSD (0.05) ST	0.85				1.05			
B	1.40				1.71			
STX B	2.42				2.97			
				Stem diameter (mm)				
Control	4.25	4.70	4.77	4.57	4.40	4.83	4.87	4.70
Az	5.06	5.28	5.68	5.34	5.16	5.38	5.68	5.41
Bc	4.76	5.22	5.63	5.20	4.93	5.42	5.70	5.35
AMF	4.91	5.32	5.75	5.33	5.21	5.55	5.75	5.50
Az+Bc	5.08	5.19	5.53	5.27	5.39	5.53	5.53	5.48
Az+AMF	5.08	5.48	5.88	5.48	5.32	5.62	5.88	5.61
Bc+AMF	5.22	5.58	5.96	5.59	5.32	5.88	5.96	5.72
Mixture of all	5.73	6.00	6.50	6.08	5.83	6.10	6.77	6.23
Mean	5.01	5.35	5.71		5.20	5.54	5.77	
LSD (0.05) ST	0.21				0.16			
B	0.35				0.26			
STX B	0.61				0.45			
				Number of leaves/ plant				
Control	86.33	90.00	90.00	88.78	86.00	90.00	90.30	88.77
Az	90.33	95.00	93.33	92.89	91.00	95.60	94.00	93.53
Bc	87.33	95.00	95.33	92.55	88.00	95.40	95.40	92.93
AMF	90.33	93.00	91.33	91.55	90.60	95.70	95.60	93.97
Az+Bc	93.33	100.00	97.33	96.89	95.50	100.00	97.30	97.60
Az+AMF	95.33	103.00	100.33	99.55	95.50	100.00	101.30	98.93
Bc+AMF	90.33	97.00	110.33	95.89	95.70	102.00	105.50	101.07
Mixture of all	100.33	115.00	110.33	108.55	100.30	108.00	115.00	107.77
Mean	91.71	98.50	97.29		92.83	98.34	99.30	
LSD (0.05) ST	0.17				0.42			
B	0.27				0.87			
STX B	0.47				1.38			
				Leaf area (cm^2)				
Control	12.18	12.79	12.10	12.36	11.50	12.00	12.03	11.84
Az	12.30	12.68	12.70	12.56	12.51	13.31	13.43	13.08
Bc	13.70	13.66	13.85	13.74	14.53	14.61	15.61	14.92
AMF	14.74	14.40	16.65	15.26	14.61	14.50	16.61	15.24
Az+Bc	16.68	16.84	16.74	16.75	15.71	16.00	16.31	16.01
Az+AMF	15.33	16.80	15.90	16.01	16.21	16.31	16.70	16.41
Bc+AMF	15.46	15.73	17.10	16.10	16.31	16.56	17.06	16.64
Mixture of all	16.76	17.56	17.90	17.41	17.80	17.81	18.00	17.87
Mean	14.64	15.06	15.37		14.90	15.14	15.72	
LSD (0.05) ST	0.74				0.28			
B	1.21				0.46			
STX B	2.09				0.79			

Az*: Azotobacter Chroococcum*, Bc:*Bacillus circulance*, AMF: Mycorrhizae, Mixture of all: Az + Bc+ AMF

Table 2: Effect of biofertilizers, soil types and their interactions on vegetative growth of *Eucalyptus camaldulensis* plants after 180 days from planting during the seasons of 2011 and 2012

	1st season Soil Types (ST)				2nd season Soil Types (ST)			
Biofertilizers (B)	Sand	Sand+Clay (1:1 v/v)	Clay	Mean	Sand	Sand+Clay (1:1 v/v)	Clay	Mean
				Shoot fresh weight (g)				
Control	60.15	66.75	75.81	67.57	70.04	71.14	69.58	70.26
Az	78.00	90.63	105.93	91.52	96.03	97.83	95.12	96.33
Bc	84.40	102.69	115.20	100.76	106.22	107.39	104.79	106.13
AMF	91.53	106.53	121.60	106.55	111.56	113.24	110.45	111.75
Az+Bc	105.66	120.78	136.00	120.81	125.86	127.56	124.75	126.06
Az+AMF	121.20	131.00	139.00	130.40	133.47	134.29	132.72	133.49
Bc+AMF	112.80	121.32	145.98	126.70	131.33	134.67	130.90	132.30
Mixture of all	130.20	143.00	153.00	142.07	146.02	147.03	145.04	146.03
Mean	97.99	110.34	124.07		115.07	116.64	114.17	
LSD (0.05) ST	0.56				0.46			
B	0.91				0.82			
STX B	1.58				1.37			
				Shoot dry weight (g)				
Control	20.05	22.25	25.25	22.52	23.34	23.70	23.19	23.41
Az	26.00	30.21	35.31	30.51	32.01	32.61	31.71	32.11
Bc	28.31	34.23	38.40	33.65	35.43	35.82	34.97	35.41
AMF	30.51	35.51	40.52	35.51	37.18	37.74	36.81	37.24
Az+Bc	35.22	40.26	45.31	40.26	41.94	42.51	41.57	42.01
Az+AMF	40.40	43.66	45.60	43.22	44.16	44.33	43.90	44.13
Bc+AMF	37.60	40.44	48.66	42.23	43.78	44.89	43.63	44.10
Mixture of all	43.40	47.60	50.00	47.00	48.20	48.40	47.87	48.16
Mean	32.69	36.77	41.13		38.25	38.75	37.96	
LSD (0.05) ST	0.44				0.43			
B	0.68				0.65			
STX B	1.61				1.33			
				Root fresh weight (g)				
Control	25.00	33.10	45.20	34.43	31.40	48.00	53.00	44.13
Az	31.00	44.00	53.70	42.90	41.00	59.40	65.40	55.27
Bc	27.00	43.60	56.00	42.20	46.80	66.00	74.00	62.27
AMF	32.00	50.00	62.00	48.00	53.00	71.60	75.20	66.60
Az+Bc	41.00	56.00	65.30	54.10	66.00	82.10	94.00	80.70
Az+AMF	45.00	60.50	71.00	58.83	52.50	80.00	87.60	73.37
Bc+AMF	43.20	68.10	73.70	61.67	54.60	83.70	88.50	75.60
Mixture of all	48.00	79.30	85.00	70.77	62.80	86.30	93.00	80.70
Mean	36.53	54.33	63.99		51.01	72.14	78.84	
LSD (0.05) ST	0.32				0.21			
B	0.66				0.34			
STX B	1.35				0.58			
				Root dry weight (g)				
Control	5.50	6.60	9.00	7.03	6.00	9.40	10.40	8.60
Az	6.00	8.30	10.60	8.30	8.00	11.60	12.80	10.80
Bc	6.40	8.80	11.20	8.80	9.10	13.00	14.60	12.23
AMF	6.80	10.00	12.40	11.20	10.40	14.00	15.00	13.13
Az+Bc	8.20	11.20	13.00	10.80	13.00	16.20	18.60	15.93
Az+AMF	9.00	12.00	14.20	11.73	10.20	15.50	17.20	14.30
Bc+AMF	8.60	13.60	14.60	12.27	10.60	16.40	17.40	14.80
Mixture of all	9.66	15.80	17.00	14.15	12.20	17.00	18.70	15.97
Mean	7.62	10.79	12.75		9.94	14.14	15.59	
LSD (0.05) ST	0.47				0.67			
B	0.72				0.87			
STX B	1.39				1.20			

Az*: Azotobacter Chroococcum*, Bc:*Bacillus circulance*, AMF: Mycorrhizae, Mixture of all: Az + Bc+ AMF

Table 3: Effect of biofertilizers, soil types and their interactions on chemical constituents in *Eucalyptus camaldulensis* plants during the seasons of 2011 and 2012

	1st season				2nd season			
	Soil Types (ST)				Soil Types (ST)			
Biofertilizers (B)	Sand	Sand+Clay(1:1 v/v)	Clay	Mean	Sand	Sand+Clay (1:1 v/v)	Clay	Mean
				Chlorophyll a(mg/g fresh leaves after 180 days)				
Control	0.82	1.06	1.43	1.10	0.78	1.08	1.40	1.09
Az	0.75	1.05	0.96	0.92	0.77	1.03	0.98	0.93
Bc	1.10	1.10	0.93	1.04	1.13	1.11	0.95	1.06
AMF	0.67	1.10	0.76	0.84	0.70	1.13	0.78	0.87
Az+Bc	1.11	1.06	1.00	1.06	1.13	1.05	1.02	1.07
Az+AMF	1.01	1.11	0.98	1.03	1.04	1.11	1.00	1.05
Bc+AMF	0.98	0.79	0.81	0.86	1.02	0.81	0.88	0.90
Mixture of all	1.14	1.02	1.17	1.02	1.17	1.04	1.19	1.13
Mean	0.95	1.04	1.01		0.97	1.04	1.03	
LSD (0.05) ST	0.03				0.03			
B	0.05				0.06			
STX B	0.09				0.09			
				Chlorophyll b(mg/g fresh leaves after 180 days)				
Control	0.26	0.40	0.31	0.32	0.29	0.42	0.33	0.35
Az	0.38	0.43	0.26	0.36	0.45	0.45	0.28	0.40
Bc	0.50	0.66	0.20	0.45	0.48	0.68	0.22	0.46
AMF	0.27	0.42	0.19	0.29	0.30	0.45	0.20	0.32
Az+Bc	0.46	0.40	0.46	0.44	0.46	0.42	0.48	0.45
Az+AMF	0.25	0.46	0.36	0.36	0.29	0.49	0.37	0.39
Bc+AMF	0.43	0.41	0.22	0.35	0.45	0.43	0.23	0.37
Mixture of all	0.86	0.60	0.48	0.65	0.82	0.68	0.53	0.67
Mean	0.43	0.47	0.31		0.44	0.50	0.33	
LSD (0.05) ST	0.02				0.02			
B	0.03				0.03			
STX B	0.06				0.05			
				Carotenoids(mg/g fresh leaves after 180 days)				
Control	0.48	0.49	0.48	0.48	0.48	0.49	0.47	0.48
Az	0.37	0.43	0.45	0.42	0.40	0.45	0.48	0.44
Bc	0.40	0.42	0.50	0.44	0.42	0.45	0.51	0.46
AMF	0.35	0.37	0.47	0.40	0.37	0.35	0.49	0.40
Az+Bc	0.40	0.42	0.44	0.42	0.42	0.45	0.46	0.44
Az+AMF	0.47	0.45	0.49	0.47	0.49	0.43	0.48	0.47
Bc+AMF	0.44	0.40	0.44	0.43	0.46	0.42	0.46	0.45
Mixture of all	0.22	0.33	0.42	0.32	0.24	0.30	0.47	0.34
Mean	0.39	0.41	0.46		0.41	0.42	0.48	
LSD (0.05) ST	0.06				0.05			
B	0.09				0.08			
STX B	0.16				0.15			

Az*: Azotobacter Chroococcum*, Bc:*Bacillus circulance*, AMF: Mycorrhizae, Mixture of all: Az + Bc+ AMF

Table 4: Effect of biofertilizers, soil types and their interactions on chemical constituents in *Eucalyptus camaldulensis* plants during the seasons of 2011 and 2012

	1st season Soil Types (ST)				2nd season Soil Types (ST)			
Biofertilizers (B)	Sand	Sand+Clay (1:1 v/v)	Clay	Mean	Sand	Sand+Clay (1:1 v/v)	Clay	Mean
Total carbohydrates content (g/100g dry leaves after 180 days)								
Control	21.00	29.40	30.30	26.90	21.90	29.00	31.50	27.47
Az	26.00	31.00	32.10	29.70	26.30	30.90	33.00	30.07
Bc	26.20	34.20	35.80	32.07	27.00	34.20	36.10	32.43
AMF	31.00	33.00	36.50	33.50	30.90	33.40	36.61	33.64
Az+Bc	35.20	34.70	39.00	36.30	35.80	35.10	39.70	36.87
Az+AMF	35.00	36.00	38.80	36.60	35.00	36.00	38.90	36.63
Bc+AMF	34.00	35.60	38.70	36.10	34.33	34.00	38.00	35.65
Mixture of all	36.60	39.30	44.00	39.97	38.10	40.00	43.90	40.67
Mean	30.63	34.15	36.90		31.17	34.15	37.21	
LSD (0.05) ST	1.40				1.28			
B	2.28				2.46			
STX B	3.96				3.79			
Nitrogen % after 180 days								
Control	0.912	0.904	1.020	0.945	1.004	0.906	1.147	1.019
Az	0.949	0.992	1.090	1.010	0.949	0.992	1.090	1.010
Bc	0.900	0.935	1.130	0.988	0.970	0.935	1.130	1.012
AMF	0.965	0.924	0.994	0.961	1.014	0.924	0.994	0.977
Az+Bc	1.140	1.210	1.180	1.177	1.230	1.210	1.180	1.207
Az+AMF	0.932	1.190	1.340	1.154	1.092	1.190	1.340	1.207
Bc+AMF	0.910	1.000	1.100	1.003	0.944	1.000	1.100	1.015
Mixture of all	1.440	1.570	1.520	1.510	1.456	1.657	1.550	1.554
Mean	1.019	1.091	1.172		1.082	1.102	1.191	
LSD (0.05) ST	0.09				0.06			
B	0.13				0.78			
STX B	0.16				0.17			
Phosphorus % after 180 days								
Control	0.190	0.198	0.197	0.195	0.182	0.200	0.197	0.193
Az	0.186	0.191	0.199	0.192	0.193	0.197	0.201	0.197
Bc	0.192	0.197	0.206	0.198	0.199	0.206	0.200	0.202
AMF	0.234	0.239	0.258	0.244	0.228	0.240	0.251	0.240
Az+Bc	0.177	0.188	0.192	0.186	0.186	0.195	0.190	0.190
Az+AMF	0.253	0.261	0.270	0.261	0.257	0.260	0.285	0.267
Bc+AMF	0.278	0.285	0.305	0.289	0.280	0.290	0.299	0.290
Mixture of all	0.290	0.297	0.321	0.303	0.295	0.300	0.327	0.307
Mean	0.225	0.232	0.244		0.228	0.236	0.244	
LSD (0.05) ST	0.002				0.002			
B	0.002				0.003			
STX B	0.004				0.004			
Potassium % after 180 days								
Control	1.820	2.640	3.110	2.523	1.880	2.700	3.170	2.583
Az	1.720	3.010	3.400	2.710	1.750	2.990	3.690	2.810
Bc	1.890	2.960	3.480	2.777	1.940	3.050	3.230	2.740
AMF	2.480	3.180	3.710	3.123	2.370	3.210	3.770	3.117
Az+Bc	2.730	3.830	3.550	3.370	2.790	3.790	3.790	3.457
Az+AMF	2.660	3.420	3.800	3.293	2.750	3.380	3.880	3.337
Bc+AMF	2.740	3.690	3.780	3.403	2.880	3.800	3.810	3.497
Mixture of all	2.860	3.780	3.880	3.507	2.960	3.880	3.910	3.583
Mean	2.363	3.314	3.589		2.415	3.350	3.656	
LSD (0.05) ST	0.85				0.42			
B	0.21				0.40			
STX B	0.61				0.46			

Az*: Azotobacter Chroococcum*, Bc:*Bacillus circulance*, AMF: Mycorrhizae, Mixture of all: Az + Bc+ AMF

Table 5: Effect of biofertilizers and soil types on microbial population in the soil of *Eucalyptus camaldulensis* plants during the seasons of 2011 and 2012

	1st season			2ed season		
	Soil Types (ST)			Soil Types (ST)		
Biofertilizers (B)	Clay	Sand+Clay(1:1 v/v)	Sand	Clay	Sand+Clay(1:1 v/v)	Sand
			Azotobacter chroococcum (CFU $\times 10^5$/g dry soil) after 60 day			
Cantrol	0.04	0.06	0.08	0.06	0.10	0.12
Az	0.16	0.22	0.30	0.20	0.30	0.41
Bc	0.05	0.10	0.10	0.08	0.10	0.16
AMF	0.06	0.09	0.08	0.08	0.11	0.10
Az+Bc	0.19	0.23	0.27	0.23	0.33	0.33
Az+AMF	0.23	0.35	0.40	0.27	0.40	0.35
Bc+AMF	0.06	0.15	0.22	0.09	0.20	0.34
Mixture of all	0.33	0.53	0.59	0.40	0.61	0.65
			Azotobacter chroococcum (CFU $\times 10^5$/g dry soil) after 120 day			
Cantrol	0.10	0.21	0.61	0.15	0.30	0.66
Az	0.57	0.61	0.91	0.61	0.65	0.97
Bc	0.13	0.23	0.73	0.23	0.30	0.79
AMF	0.31	0.43	0.83	0.36	0.47	0.91
Az+Bc	0.41	0.51	0.85	0.47	0.61	0.95
Az+AMF	0.65	0.69	1.00	0.70	0.72	1.31
Bc+AMF	0.44	0.51	0.90	0.61	0.71	1.77
Mixture of all	0.76	0.80	1.10	0.85	0.85	1.83
			Bacillus circulans (CFU $\times 10^5$/g dry soil) after 60 days			
Cantrol	41.8	53.1	34.4	43.2	54.2	40.4
Az	64.7	71.2	40.1	68.7	79.3	46.6
Bc	81.1	144.4	72.2	88.0	156.3	75.6
AMF	50.1	66.7	38.9	55.4	67.6	42.1
Az+Bc	88.7	95.4	73.3	91.4	100.0	88.4
Az+AMF	54.4	67.9	65.6	63.1	71.9	72.7
Bc+AMF	91.4	96.8	81.3	100.0	110.3	91.2
Mixture of all	99.3	100.0	93.7	110.3	131.0	93.0
			Bacillus circulans (CFU $\times 10^5$/g dry soil) after 120 days			
Cantrol	37.8	48.9	51.1	48.9	106.7	55.6
Az	45.6	54.4	68.9	57.8	134.4	166.6
Bc	54.4	76.7	87.8	61.1	91.1	167.8
AMF	33.3	52.2	74.4	38.9	104.4	187.8
Az+Bc	35.6	73.3	86.7	65.6	144.0	171.1
Az+AMF	42.2	57.8	78.9	43.3	97.8	162.2
Bc+AMF	41.1	53.3	58.9	52.2	118.9	174.0
Mixture of all	40.0	61.1	71.1	36.7	151.1	198.9

Az: *Azotobacter Chroococcum*, Bc: *Bacillus circulans*, AMF: Mycorrhizae, Mixture of all: Az + Bc+ AMF

As far as the effect of the interaction between the biofertilizers and the soil type, the data indicated that, in both seasons, the highest content of chemical constituents were found in the leaves of the control plants which grown in clay medium. Addition of AMF alone to the plants grown in the sand gave the least content of chemical constituents. Similar increases in the contents of chlorophyll in the leaves as result of treatment have been reported by other studies [25, 34].

Regarding the interaction between the soil type and the biofertilizers, the data revealed that, in the two seasons the highest content of chemical constituents was

Table 6: Effect of biofertilizers and soil types on mycorrhizal colonization (%) in the soil of *Eucalyptus camaldulensis* plants during the seasons of 2011 and 2012

	1st season Soil Types (ST)				2ed season Soil Types (ST)			
Biofertilizers (B)	Clay	Sand+Clay (1:1 v/v)	Sand	Mean	Clay	Sand+Clay (1:1 v/v)	Sand	Mean
Mycorrhizal colonization (%) after 60 days								
Cantrol	7.00	22.00	30.00	19.67	10.00	30.00	32.00	24.00
Az	11.00	25.00	35.00	23.67	15.00	35.00	40.00	30.00
Bc	17.00	30.00	37.00	28.00	27.00	40.00	43.00	36.67
AMF	40.00	50.00	55.00	48.33	45.00	55.00	60.00	53.33
Az+Bc	25.00	27.00	35.00	29.00	30.00	35.00	44.00	36.33
Az+AMF	45.00	65.00	70.00	60.00	50.00	70.00	75.00	65.00
Bc+AMF	45.00	65.00	75.00	61.67	45.00	72.00	75.00	64.00
Mixture of all	65.00	75.00	80.00	73.33	70.00	78.00	83.00	77.00
Mean	31.88	44.88	52.13		36.50	51.88	56.50	
LSD (0.05) ST	0.59				0.82			
B	0.97				0.73			
ST X B	1.68				1.08			
Mycorrhizal colonization (%) after 120 days								
Cantrol	30.00	33.00	40.00	34.33	30.00	34.00	42.00	35.33
Az	30.00	39.33	43.00	37.44	35.00	45.00	50.00	43.33
Bc	35.00	42.33	51.00	42.78	35.00	50.00	53.00	46.00
AMF	52.00	69.33	75.00	65.44	60.00	70.00	75.00	68.33
Az+Bc	41.00	42.33	53.00	45.44	51.00	53.00	60.00	54.67
Az+AMF	60.00	72.33	86.00	72.78	75.00	80.00	90.00	81.67
Bc+AMF	61.00	73.33	85.00	73.11	70.00	80.00	90.00	80.00
Mixture of all	70.00	84.33	90.00	81.44	75.00	100.00	100.00	91.67
Mean	47.38	57.04	65.38		53.88	64.00	70.00	
LSD (0.05) ST	1.24				1.43			
B	2.03				1.68			
ST X B	3.51				1.75			

Az: *Azotobacter Chroococcum*, Bc: *Bacillus circulans*, AMF: Mycorrhizae, Mixture of all: Az + Bc+ AMF

determined in the leaves of the plants after 180 days received the mixture of the three biofertilizers (Az + Bc + AMF) and grown in the sand. Growing the plants in the clay medium and fertilized with AMF alone resulted in the least content of chemical constituents. These results are in agreement with those found by some investigators [25, 27]. These results agree with other studies [26, 34].

Microbiological Parameters: Data presented in Tables (5, 6 and 7) indicated that sand soil exhibited the least most probable microbial population, mycorrhizal colonization (%) and enzymatic activities (dehydrogenase and nitrogenase) compared to other soil types whereas, the clay recorded the highest value.

After 60 days, the mixture treatment recorded higher on microbial population, mycorrhizal colonization (%) and enzymatic activities (dehydrogenase and nitrogenase) more than other treatments and the clay soil obtained the highest value with the same treatment, during two seasons, respectively. After 120 days of transplanting during the first and second seasons, the mixture of all treatment still recorded the highest microbial population, mycorrhizal colonization (%) and enzymatic activities comparing that to control and other treatments. Clay soil recorded the optimum value at first and second seasons, respectively.

Our findings matched with those obtained by prior studies [35, 36] who stated that free-living diazotrophic bacteria like *Azotobacter* play an important role in maintaining the soil health. Inoculation with mixture of microorganisms increases rhizospheric microbial community particularly bacteria and free living N2 fixing *Azotobacter* spp. as well as mycorrhizal fungi. Similar result was also reported [37]. The increase of *B. circulans* populations in clay loam soil refereed to the process of inoculation with mixture of microorganisms including (*B. circulans* + *Az. chroococcum* + AM fungi) where they have the ability solubilize weatherable minerals through excretion of organic acids such as α-keto glutaric acid.

Table 7: Effect of biofertilizers and soil types on Dehydrogenase and Nirtogenase activities, in the soil of *Eucalyptus camaldulensis* during the seasons of 2011 and 2012

	1st season Soil Types (ST)				1st season Soil Types (ST)			
Biofertilizers (B)	Sand	Sand+Clay (1:1 v/v)	Clay	Mean	Sand	Sand+Clay (1:1 v/v)	Clay	Mean
Dehydrogenase activity(µg TPF/g dry soil/day) after 60 day								
Cantrol	10.77	18.00	23.77	17.51	10.97	18.40	23.90	17.76
Az	17.13	22.47	37.77	25.79	17.27	22.60	37.93	25.93
Bc	14.57	29.80	42.27	28.88	14.83	30.20	42.30	29.11
AMF	15.80	25.30	39.40	26.83	15.97	25.87	39.53	27.12
Az+Bc	27.13	56.40	65.57	49.70	27.13	57.00	65.63	49.92
Az+AMF	35.20	67.50	69.77	57.49	35.33	68.10	70.03	57.82
Bc+AMF	37.07	71.50	84.03	64.20	37.20	72.03	84.30	64.51
Mixture of all	50.00	87.30	99.87	79.27	50.83	87.57	100.40	79.60
Mean	26.02	47.30	57.80		26.19	47.72	58.00	
LSD (0.05) ST	0.39				0.23			
B	0.63				0.38			
STX B	1.10				0.65			
Dehydrogenase activity(µg TPF/g dry soil/day) after 120day								
Cantrol	15.73	25.33	32.17	24.41	16.27	25.33	32.37	24.66
Az	21.93	33.47	42.67	32.69	21.87	33.47	42.67	32.67
Bc	18.00	40.60	55.93	38.18	18.40	40.60	56.07	38.36
AMF	22.83	31.17	50.10	34.70	23.27	31.17	50.57	35.00
Az+Bc	28.70	59.53	72.50	53.58	28.90	59.53	72.50	53.64
Az+AMF	33.73	51.43	85.60	56.92	34.20	51.43	85.60	57.08
Bc+AMF	36.50	77.20	93.27	68.99	38.33	83.50	93.53	71.79
Mixture of all	54.30	96.13	118.60	89.68	54.83	96.33	118.90	90.02
Mean	28.97	51.86	68.86		29.51	52.67	69.03	
LSD (0.05) ST	0.75				1.31			
B	1.23				2.13			
STX B	2.13				3.70			
Nirtogenase activity(nmol C_2H_4/g rhizosphere/hour) after 60 days								
Cantrol	2.11	13.51	22.31	12.64	3.00	17.34	35.30	18.55
Az	33.31	55.11	77.66	55.36	40.20	63.21	81.55	61.65
Bc	27.11	44.80	65.33	45.75	30.10	50.17	69.71	49.99
AMF	23.11	37.60	54.11	38.27	31.00	40.67	60.21	43.96
Az+Bc	47.18	67.30	81.55	65.34	51.22	73.66	87.77	70.88
Az+AMF	47.31	70.30	90.11	69.24	55.14	77.81	92.66	75.20
Bc+AMF	45.21	52.13	77.31	58.22	50.11	67.31	80.25	65.89
Mixture of all	100.35	213.00	270.00	194.45	110.30	255.00	299.40	221.57
Mean	40.71	69.22	92.30		46.38	80.65	100.86	
LSD (0.05) ST	0.15				0.18			
B	0.24				0.29			
STX B	0.42				0.51			
Nirtogenase activity(nmol C_2H_4/g rhizosphere/hour) after 120days								
Cantrol	6.25	24.12	73.81	34.73	7.77	44.77	76.18	42.91
Az	85.62	138.00	165.99	129.87	92.13	106.73	188.00	128.95
Bc	61.25	124.12	145.50	110.29	64.58	144.33	166.94	125.28
AMF	33.06	79.81	97.28	70.05	48.94	123.45	96.51	89.63
Az+Bc	116.23	370.14	406.41	297.59	124.26	411.20	448.58	328.01
Az+AMF	123.57	265.90	325.42	238.30	123.06	292.44	355.60	257.03
Bc+AMF	75.61	166.21	300.00	180.61	87.94	199.86	328.60	205.47
Mixture of all	213.84	435.21	550.61	399.89	242.05	551.50	566.30	453.28
Mean	89.43	200.44	258.13		98.84	234.29	278.34	
LSD (0.05) ST	0.08				0.09			
B	0.14				0.14			
STX B	0.24				0.25			

Az: *Azotobacter Chroococcum*, Bc: *Bacillus circulans*, AMF: Mycorrhizae, Mixture of all: Az + Bc+ AMF

This organic compound could exert a selective influence in soil microbial communities through a multiplication of α-keto glutarate catabolizing microorganisms [38]. Soil microorganisms use this organic acid to solubilize and release potassium that improved the plant growth and yield. In addition to that, microorganisms attached to mineral surface can also create microelements were concentrations of acidity and redox activity can be substialy elevated [39]. Also, these results also confirmed by Serra-Wittling *et al.* [40] who pointed out to the prevalence of a significant relationship between dehydrogenase activity, organic matter and soil microbial counts. They also added the activity of dehydrogenase enzyme was highly correlated with CO_2 release proteolytic activity and nitrification potentialities.

Obviously the response of the rhizosphere of *Eucalyptus* plants to inoculation with mixture of all in clay soil led to an increase of nitrogenase activity, this is because the highest values of nitrogenase activity recorded through the all periods was due to the favorable effects of the combination between microorganisms and organic matter represented in soil where mixed diazotrophes and AM mycorrhizae increased the production of some growth regulators, auxins and vital enzymes involving nitrogenase. Where the N_2 ase efficiency increases with increasing the efficiency of increase the nitrogen fixing bacteria [41].

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