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Effect of Biofertilizer, Cell Stabilizer and Irrigation Regime on Rosemary Herbage Oil Yield and Quality

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Abstract: A pot trial was accomplished at the greenhouse of the National Research Centre, Egypt, during the two successive seasons of 2004 and 2005, to study the influence of irrigation intervals on rosemary plant growth, yield and chemical composition. Also to estimate the effectiveness of using cell stabilizer, biofertilizer on rosemary plant production under different irrigation intervals grown in two soil types (sandy and clay) Recorded data assured that exposing rosemary plant to water stress led to a decrease in growth parameters at different cuts where plants were more affected in sandy soil than in clay. The percentage reduction in plant height, number of branches/plant, fresh and dry weights/plant reached 20.6, 52.3, 57.9 and 45.2% respectively for plants grown in clay soil, while it reached 43.5, 43.1, 68.8 and 47.0%, respectively for plant grown in sandy soil. Slight improvent in rosemary growth character was observed when using cell stabilizer (salicylic acid) and biofertilizer (*Azotobacter* vinelandii) treatments at different cuts. Also, prolongating water of irrigation to three weeks had reduced the oil content at the first cut under different treatments, while a neglicable reduction was observed at the second cut and the highest oil content was recorded by plants treated with biofertilizer at normal irrigation 0.75% followed by plants treated with cell stabilizer at irrigation extension of three weeks 0.67 %. The N,P,K contents in rosemary herb were affected by water stress, where P percentage was reduced, while the percentage increase in K ranged between 1.47 to 5.7% and N content showed an increase only under biofertilizer treatment. Also some of the essential 0.1 components were influenced by extension of irrigation interval as they recorded an acute increase such as alpha – pinene, β-pinene, Limonene, 1,8 – cineole, Linalool, campor, β-terpineol, borneol, terpinen 4-ol, carvone, thymol, carvacrol, linalylacetate, geranylacetate, β-caryophyllene, caryophyllene oxide, others increased when treated with cell stabilizer or biofertilizer and other components were not affected by any of above mentioned treatments.

Key words: Rosemary plant, water stress, nutrient composition, biofertilizer, cell stabilizer, growth, oil yield, essential oil.

INTRODUCTION

Rosemary (*Rosmarinus officinalis*) is a member of Lamiaceae family. Rosemary extract had been widely used as topical applications for wound-healing, antiaging and disease treatments. Such plants produce flavonoid compound with phenolic structures such phytochemicals are highly reactive with other compounds such as reactive oxygen species and biological macromolecules to neutralize free radicals or initiate biological effects. Hsu,[1]. Montero *et al.*[2] added that the antioxidant activity of rosemary extract was more effective at protecting lipid and protein oxidation was prevented.

Recently, attention has been paid to the possibility of cultivating medicinal and aromatic plants to diversify agricultural production by a new productive trend which would raise farm income and create new job opportunities, especially in virgin lands with low fertility and limited water supply.

Water scarcity was found to be one of the most detrimental effects on crop yield Delfine *et al.*[3]. So application of salicylic acid cell stabilizer is needed for its dual effect on plants grown under limited water supply, as it acts as an antitranspirant and antioxidant. Also, application of biofertilizers is needed to produce a clean product free of harmful agrochemicals for human safety Lampkin[4] and support plant growth under water stress condition.

The present work aimed to production of rosemary plant characterized by high quality devoid of achrochemicals through the application of a biofertilizer. Also to study the effect of soil types, water prolongation and salicylic acid cell stabilizer use on plant growth, yield and chemical composition.

MATERIALS AND METHODS

A pot trial was conducted during the two consecutive seasons of 2003 and 2004 at the Greenhouse of the

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National Research Centre, Dokki, Cairo, Egypt. Rosemary plant transplants were planted in plastic pots of 30cm diameter which were filled with two different types of soils. Some pots were filled with uncultivated low fertile sandy soil with the following characteristics: clay 8.19%; silt 7.04%, sand 84.58%, pH 7.41, EC 3.7 dSm^{-1} and the other pots were filled with clay soil with the following properties : clay 46.5%; silt, 33.11%; sand 18.7%; organic matter, 0.57% total nitrogen, 0.024%, calcium carbonate, 1.12%; pH, 7.66, EC 1.3 dSm^{-1}.

The pots were separated into three sets, the first and the second sets received recommended dose of ammonium nitrate, super-phosphate and potassium sulfate of (200, 150 and 50 kg/fed) respectively. The third set received biofertilizers using *Azotobacter vinelandii* liquid culture of 3.4 x 10^7 cell/ml at the rate of 5 ml/seedling which was applied to the soil during transplanting. The experiment was carried out in three replicates and the experimental design was laid out in a randomized complete block design.

Detail of experimental treatments
Irrigation interval + recommended dose of fertilizers:

- Normal irrigation (I_1)
- Irrigation every 7 days (I_2)
- Irrigation every 14 days (I_3)
- Irrigation every 21 days (I_4)

Salicylic acid (cell stabilizer) foliar application of 1 g/L used under irrigation intervals + recommended dose of fertilizers :

- Normal irrigation (I_1)
- Irrigation every 7 days (I_2)
- Irrigation every 14 days (I_3)
- Irrigation every 21 days (I_4)

Biofertilizer use (Azotobacter sp) was added to the soil once during transplanting under different irrigation intervals :

- Normal irrigation (I_1)
- Irrigation every 7 days (I_2)
- Irrigation every 14 days (I_3)
- Irrigation every 21 days (I_4)

At each cut, plants were sampled for determining and recording plant height/plant (cm) plant fresh and dry weight (g) number of branches/plant.

The percentage of nitrogen, phosphorus and potassium in plant leaves were determined according to the method described by A.O.A.C.[5]. For estimation of oil % and extraction of essential oil, samples of dry leaves for each replicate at each cut in both seasons were subjected to hydro-distillation. The extraction was carried out for three hours by water distillation using Clevenger apparatus according to Guenther[6].

The essential oil constituents were analyzed and determined in the samples. The dehydrated oil of each treatment was separately subjected to GLC analysis.
The relative peak area for each constituent as relative percent of the essential oil. The identification of these compounds was achieved by matching their retention times with those of authentic samples injected with the same conditions.

The combined data of the two seasons were statistically analyzed according to the procedure of Sendecor and Cochran[7] where the means of the studied treatments were compared using L.S.D. test at 0.05 significance level.

RESULTS AND DISCUSSIONS

Growth and yield: Results obtained in Table 1 indicated that prolongation of irrigation led to a significant decrease in plant height, number of branches, fresh and dry weights/plant at the first cut which was more affected in sandy soil than in clay, compared to that of normal irrigation.

The percentage decrease in plant height at the first cut reached 43.5% in sandy soil and 20.6% in clay soil when irrigation extended for three weeks. Also the number of branches decreased by 52.3% and 43.1% for plants grown in clay and sandy soil respectively. Moreover the fresh weight/plant (g) at the first cut decreased by 57.9% in clay soil and 68.8% in sandy soil at irrigation extension of three weeks. Similarly the dry weight/plant (g) was reduced by 45.2% and 47.0% for plants grown in clay soil and sandy soil respectively.

Application of cell stabilizer didn't show an improvement in plant growth parameters for plants grown in both types of soils. On the contrary biofertilizer application showed some improvement for plants grown in clayey soil rather than sandy soil. The interaction between soil types and different treatments was highly significant for plant height, number of branches/plant and fresh weight/plant (g).

At the second cut Table 2, the prolongation of irrigation to three weeks reduced each of number of branches/plant, fresh and dry weight/plant for plants grown in both types of soils, although plant height (cm) of plants grown in clay soil was not badly affected as that of plants grown in clay soil.

The use of cell stabilizer was more effective than that of the first cut, in stabilizing plant growth parameters as indicated in Table 2, which was more promising for plants grown in clayey soil than in sandy soil. On the other hand biofertilization treatment was not effective at different irrigation intervals except for plant height (cm) where it

Table 1: Effect of irrigation intervals, cell stabilizer and biofertilizer use on growth of rosemary plant grown in two soil types at the first cut (combined analysis of two years).

		Plant height (cm)		Number of branches/plant		Fresh weight/plant (g)		Dry weight/plant (g)	
Treatment	Irrigation interval	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
Control	I_1	29.00	26.00	42.67	21.67	28.70	19.83	14.85	08.58
	I_2	26.67	23.33	23.00	22.67	26.08	14.03	12.40	08.79
	I_3	26.33	20.00	23.00	17.67	23.26	08.86	11.52	05.44
	I_4	23.00	14.67	20.33	12.33	12.08	06.33	08.15	04.57
Cell stabilizer	I_1	29.33	28.33	43.33	21.33	38.57	17.83	22.08	10.42
	I_2	28.33	23.33	26.67	19.33	30.14	20.46	17.56	08.97
	I_3	27.00	21.33	24.33	18.67	22.80	16.00	13.42	06.63
	I_4	24.33	20.33	23.67	13.67	13.78	09.65	08.20	06.55
Biofertilization	I_1	38.00	30.00	46.67	27.00	40.65	22.95	23.10	12.70
	I_2	30.67	23.67	28.67	22.00	31.56	21.63	17.17	11.86
	I_3	28.00	23.00	24.33	18.67	20.37	14.74	14.32	07.61
	I_4	26.00	17.67	24.33	14.33	18.39	12.28	10.42	06.33
L.S.D. at 5%		A= 0.931 B= 2.281 AxB= 3.226		A= 0.916 B= 2.244 AxB= 3.173		A= 0.916 B= 2.245 AxB= 3.174		A= 0.755 B= 1.848 AxB= 2.615	

I_1: normal irrigation I_2: irrigation ever 1 week I_3: irrigation every 2 weeks I_4 = irrigation every 3 weeks
S_1 : clay soil S_2: sandy soil
A: soil types B: different treatments AxB = interaction between soil types and treatments

Table 2: Effect of irrigation intervals, cell stabilizer and biofertilizer use on growth of rosemary plant grown in two soil types at the second cut (combined analysis of two years).

		Plant height (cm)		Number of branches/plant		Fresh weight/plant (g)		Dry weight/plant (g)	
Treatment	Irrigation interval	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
Control	I_1	23.66	22	10	6.33	61.9	45.79	17.7	14.3
	I_2	27.67	18.33	9	5	51.2	39.87	13.02	10.8
	I_3	26	15.33	7.67	5	47.86	35.2	12.05	10.5
	I_4	23.67	10	4.67	4.33	47.9	25.8	10.72	8.08
Cell stabilizer	I_1	30	24	12	10.67	85.3	60.1	31.3	17.6
	I_2	25	20.33	11	7.67	71.3	57.9	28.5	14.5
	I_3	24.66	17	8.33	6.67	60.7	50.72	21.5	15.95
	I_4	28.67	16.67	7.67	6	51.12	36.5	19.65	10.3
Biofertilization	I_1	29	23	12.67	10.67	84	68.7	28.52	17.9
	I_2	28.67	19	8.33	7.33	60.2	49.8	21.56	13.5
	I_3	31.67	18.33	7	5.33	45.8	32.2	17.93	12.9
	I_4	18.67	16.67	7.67	5.33	42.2	31.43	13.43	9.7
L.S.D. at 5%		A= 0.951 B= 2.329 AxB= 3.294		A= 0.567 B= 1.431 AxB= 1.964		A= 1.648 B= 4.036 AxB = 5.708		A= 0.813 B= 1.991 AxB= 2.816	

I_1: normal irrigation I_2: irrigation ever 1 week I_3: irrigation every 2 weeks I_4 = irrigation every 3 weeks
S_1: clay soil S_2: sandy soil
A: soil types B: different treatments AxB = interaction between soil types and treatments

Table 3: Effect of irrigation intervals, cell stabilizer and biofertilizer use on growth of rosemary plant grown in two soil types at the third cut (combined analysis of two years).

		Plant height (cm)		Number of branches/plant		Fresh weight/plant (g)		Dry weight/plant (g)	
Treatment	Irrigation internal	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
Control	I_1	18	18.33	22	17	26.89	19.04	17.98	13.89
	I_2	20	14.67	18	9.33	23.4	15.45	15.8	11.5
	I_3	18	12.67	14.33	9.33	17.09	14.38	11.7	10.55
	I_4	13.33	9.33	8.33	6	14.6	10.76	9.15	7.95
Cell stabilizer	I_1	23.33	20.33	24	20	41.56	25.8	25.27	15.4
	I_2	21.33	18	20.67	14	30.32	18.6	19.9	13.9
	I_3	20.33	15.67	15.67	10	20.33	15.88	14.79	12.4
	I_4	18.33	15.67	9.33	8.67	17.9	12.9	10.82	9.14
Biofertilization	I_1	30	21.33	26.33	23.67	43.36	30.36	26.13	18.11
	I_2	24	18.67	19.33	17.33	33.4	19.96	21.32	15.75
	I_3	20.67	16.67	14.33	11	25.12	17.3	19.9	12.7
	I_4	19.33	17.33	10.33	8.33	14.1	13.6	11	10.58
L.S.D. at 5%		A= 0.604 B= 1.479 AxB= 2.093		A= 0.730 B= 1.789 AxB= 2.529		A= 1.083 B= 2.653 AxB= 3.752		A= 0.617 B= 1.512 AxB= 2.139	

I_1: normal irrigation I_2: irrigation ever 1 week I_3: irrigation every 2 weeks I_4 = irrigation every 3 weeks
S_1: clay soil S_2: sandy soil
A: soil types B: different treatments AxB = interaction between soil types and treatments

Table 4: Effect of irrigation intervals, cell stabilizer and biofertilizer use on essential oil percentage of rosemary plant grown in two soil types at the different cuts.

		First cut 25/3/2004		Second cut 10/9/2004		Third cut 10/2/2005	
Treatment	Irrigation internal	S_1	S_2	S_1	S_2	S_1	S_2
Control	I_1	0.64	0.41	0.68	0.58	0.59	0.32
	I_2	0.53	0.43	0.67	0.86	0.51	0.31
	I_3	0.75	0.54	1.18	0.67	0.65	0.47
	I_4	0.61	0.5	0.67	0.65	0.52	0.52
Cell stabilizer	I_1	0.931	0.4	0.95	0.66	0.6	0.39
	I_2	0.61	0.38	0.8	0.61	0.6	0.32
	I_3	0.69	0.39	0.88	0.4	0.61	0.41
	I_4	0.67	0.36	0.93	0.37	0.67	0.43
Biofertilization	I_1	0.84	0.47	1.5	0.61	0.75	0.41
	I_2	0.83	0.48	1.5	0.85	0.61	0.33
	I_3	0.64	0.55	1.37	0.83	0.63	0.42
	I_4	0.67	0.51	1.01	0.88	0.61	0.56

I_1: normal irrigation I_2: irrigation every week I_3: irrigation every 2 weeks
I_4: irrigation every 3 weeks
S_1: clay soil S_2: sandy soil

Table 5: Effect of irrigation intervals, cell stabilizer and biofertilizer use on N,P,K percentage of rosemary plant grown in different soil types.

Treatment	Irrigation internal	N % Clay	N % Sand	P % Clay	P % Sand	K % Clay	K % Sand
Control	I_1	11.07	11.59	0.16	0.24	1.56	1.75
	I_2	12.19	12.76	0.21	0.2	1.72	1.36
	I_3	12.76	11.07	0.28	0.68	1.59	1.08
	I_4	8.72	11.54	0.27	0.44	1.99	0.58
Cell stabilizer	I_1	10.31	12.19	0.35	0.22	2.94	1.43
	I_2	8.63	12.76	0.24	0.14	6.06	2.2
	I_3	10.22	9.85	0.44	0.15	3.78	0.9
	I^4	11.26	3.56	0.18	0.2	3.32	1.6
Biofertilization	I_1	10.22	8.35	0.19	0.24	3.26	1.16
	I_2	12.44	11.59	0.54	0.16	2.54	1.84
	I_3	6.75	12.76	0.21	0.18	3.78	1.1
	I_4	4.04	12.14	0.4	0.14	5.7	1.47

I_1: normal irrigation I_2: irrigation every week I_3: irrigation every 2 weeks I_4: irrigation every 3 weeks

Table 6: Relative percent of main oil constituents in rosemary plant as affected by cell stabilizer and biofertizer use at different irrigation intervals.

	Percentage						
	Normal irrigation (control)	Cell stabilizer			Biofertilization		
Compound	I_1	I_2	I_3	I_4	I_2	I_3	I_4
a-pinene	3.31	5.8	3.88	5.9	5.19	4.11	5.23
β-pinene	-	0.24	0.53	1.37	0.5	1.24	1.61
Limonene	20.49	2.95	8.92	10.06	9.32	9.72	10.67
1,8 cineole	18.59	29.52	20.92	16.62	19	18.44	17.85
Linalool	5.3	7.38	11.07	5.39	11.39	6.02	5.23
Camphor	3.74	5.13	8.39	3.36	3.07	3.37	2.59
β-terpineol	15.3	15.27	11.57	11.41	14.45	11.51	12.38
Borneol	3.53	9.7	1.12	1.11	4.93	1.88	2.63
Terpinen 4-ol	1.13	7.37	4.49	2.06	1.39	2.6	2.43
Carvone	2.54	2.01	2.84	2.87	2.94	2.37	1.04
Thymol	4.04	1.02	0.47	0.74	2.15	1.79	1.03
Carvacrol	1.17	0.44	0.9	1.29	1.48	3.15	2.55
Linalylacetate	8.35	1.12	0.84	1.49	1.54	3.28	1.33
Bromylacetate	3.11	1.22	4.62	1.11	4.63	1.05	0.89
Geranylacetate	1.42	0.74	4.63	5.68	1.53	5.64	3.83
β-caryophyllene	1.19	1.59	5.97	5.51	0.75	4.89	1.61
Caryophyllene oxide	1.95	-	0.45	2.07	1.15	1.98	2.12

I_1: normal irrigation I_2: irrigation every week
I_3: irrigation every 2 weeks I_4: irrigation every 3 weeks

showed little improvement. The interaction between soil types and treatments were highly significant for plant fresh weight (g), followed by plant height (cm), dry weights/plant (g), then the least was for the number of branches/plant.

At the third cut, plants grown in clay and sandy soils Table 3 showed a reduction in all growth parameters at irrigation interval every three weeks even under cell stabilizer and biofertilizer treatments. The most significant reduction was detected by fresh weight/plant (g) and the least by plant height (cm).

The influence of water prolongation, cell stabilizer and biofertilizer use on essential oil percentage of rosemary plant grown in sandy and clay soils at different cuts was shown in Table 4. It was clear that prolongating water of irrigation to three weeks had reduced the oil content for plants grown in clay soil at the first cut under different treatments, while at the second cut a neglicable reduction in oil content was observed under different treatments as compared to their controls. However at the third cut the highest oil content was observed by plants treated with biofertilizer at normal irrigation 0.75% followed by plants treated with cell stabilizers at irrigation extension of three weeks 0.67%. On the other hand plants grown in sandy soil showed an opposite trend at the first and second cut where the oil content increased when increasing water stress interval without treatments and also when using biofertilizers under water water stress. While at the third cut the soil content increased with increasing irrigation interval to three weeks the highest recorded was observed when using biofertilizers where it reached 0.56%.

Chemical composition: Rosemary nutrient contents N,P,K percentages were affected by soil type, irrigation regime, biofertilizer and cell stabilizer use. Data presented in Table 5 showed that the irrigation intervals every two weeks attained the maximum content of P for plants grown in different soil types and without any treatments, while that of K was attained at irrigation extension of three weeks, while N content reached maximum level at irrigation interval every two weeks 12.76% for plants grown in clay soil and at irrigation interval of one week for plants grown in sandy soil.

Cell stabilizer use Table 5, under water stress condition led to an increase use in N content for plants grown in clay soil only and also increased K content although P was reduced for plants grown in both types of soils. However, biofertilization treatment did not show a remarkable effect on rosemary nutrient content, where both N and P contents were reduced at irrigation extension of three weeks while that of K increased reaching 5.7 for plants grown in clay soil and 1.47 in sandy soil.

Analysis of rosemary essential oil Table 6 indicated that the most abundant components were (alpha – pinene, β-pinene, Limonene, 1,8-cineole, Linalool, campor, β-terpineol, borneol, terpinen 4-ol, carvone, thymol, carvacrol, linalylactate, Geranyl acetate, β-caryophyllene, caryophyllene oxide).

Different components were influenced by prolongating irrigation water to three weeks, also cell stabilizers and biofertilizer use but were not affected by soil type, so this factor was neglected. Alpha pinene, β-pinene, Limonene, carvacrol, Geranylacetate, β-caryophyllene and caryophyleene oxide increased at extension of irrigation to three weeks under biofertilizer and cell stabilizer treatments. For other components such as 1,8-cineole Linalool and campor the percentage increase was detected at the extension of irrigation to one and two weeks, then showed a slight drop at three weeks irrigation interval under different treatments compared to the control. Other components listed in Table 6 were not affected by biofertilizer nor cell stabilizer use under different irrigation treatments for both types of soils and showed a decrease in their contents such as Thymd, linalylacetate and β-terpineol and bromylacetate, which that of Terpinen 4-ol and borneol recorded a positive increase only at the irrigation interval every week in addition to cell stabilizer application.

Increasing levels of water stress reduce rosemary growth and yield due to reduction in photosynthesis and plant biomass. Under increasing water-stress levels photosynthesis was limited by low CO_2 availability due to reduced stomatal and mesophyll conducatance, while photochemical and biochemical processes were not affected. Defline *et al.*[3]. Also Mulas *et al.*[8], found a positive correlation between leaf width and shoot fresh weight, suggesting that the first character could be used to estimate leaf biomass. Rao *et al.*[9], suggested that the water requirement of some aromatic crops was found to be high, but it is possible to reduce the water use by appropriate timing and methods of irrigation biofertilization was effective in promoting plant production. Mona[10], recommended the application of compost at 10 tonnes/fed to the soil, to reduce water stress.

Application of cell stabilizer increased oil content in plants grown under water stress this may be attributed to the effect of cell stabilizer in increasing the radical scavenging activity, the rosmarinic and carnosic acids were found to be the best rosmary scavengers. Luis *et al.*[11].

On the constrary Munnu[12] proved that the content and quality of rosemary oil were not influenced by plant spacing, fertilizer or irrigation regime. For nutrient contents it was observed that K percentage increased with the increase in irrigation interval which was supported by Mostafa[13] who found that extensive irrigation decreased N and moderate irrigation increased P, while prolongation irrigation increased K percentage in herb and fruit of fennel plant. Also Rao *et al.*[9], proved that K deficiency affected the content and quality of some essential oil and they suggested that K deficiency may become a production constraint in future.

Santoyo *et al.*[14], identified 33 compounds of the rosemary plant essential oil. The main components of these fractions were alpha-pinene, 1-8-cineole, camphor, verbenone and borneol constituting 80% of the total oil. Also, Rao *et al.*[15], deduced that early distillate fractions contained most of the alpha-thujene, alpha-pinene, camphene, beta pineneand 1,8-cineole (eucalyptol), while later fractions contained most of the camphor and bornyl acetate.

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