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THE INFLUENCE OF SPRAYING WITH ACTIVE YEAST EXTRACT ON VEGETATIVE GROWTH AND VOLATILE OIL OF RIVER RED GUM PLANT (*Eucalyptus camaldulensis* Dehn.)

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ABSTRACT

The research work presented in this paper was carried out during the two growing seasons of 2005 and 2006 in order to study the influence of foliar application with different levels from active yeast extract (50, 100 and 200 ml/liter) on vegetative growth and volatile oil of River red gum (*Eucalyptus camaldulensis* Dehn.).

The obtained results indicated that the relatively low used concentration of 50 ml active yeast extract per liter showed no significant effect on vegetative growth characters of River red gum plant, aged nine months, in both studied seasons. On the other hand, the other used concentrations of active yeast extract (100 and 200 ml/L.) induced significant increase in plant height, diameter of the main stem, number of branches per plant, mean leaf area, fresh weight of stems per plant, fresh weight of leaves per plant, biomass of shoot per plant and dry weight of shoot per plant of River red gum in both studied seasons. The maximum increase in this respect was recorded at 200 ml YE/L.

As to the effect on volatile oil, data revealed that the percentage of volatile oil in leaves of River red gum was increased with increasing the concentration of active yeast extract reaching its maximum at 200 ml YE/L. At the same time, all sprayed concentrations of active yeast extract did not affect volatile oil components. However, the percentages of volatile oil components were slightly affected by the tested treatments.

Keywords: Active Yeast Extract, *Eucalyptus camaldulensis* Dehn., River Red Gum, Vegetative Growth, Volatile Oil.

INTRODUCTION

The family Myrtaceae consists of about 140 genera and 3000 or more species, trees or shrubs found in tropical and subtropical regions throughout the world, and also well developed in temperate Australia.

One of the largest and most important genera of Myrtaceae is *Eucalyptus* (500 species). Many species of *Eucalyptus* are cultivated for ornament, and used for commercial timber and as street – trees. *Eucalyptus* forms great forests under warm – temperate climatic conditions in southern Australia; some species attain a height of more than 100 m (Cronquist, 1981). In this respect, Jones and Luchsinger (1987) stated that *Eucalyptus* is the most important genus of the family Myrtaceae which used for timber, ornamentals, reforestation, and the source of eucalyptus oil commonly used as a flavouring, expectorant, and inhalant.

It is well known that various species of *Eucalyptus* have important medicinal value. One of the familiar species in this concern is *Eucalyptus camaldulensis* Dehn. (River red gum). Different plant parts of such species are used in the indigenous system of medicine for the treatment of various

human ailments such as diarrhea, chronic dysentery, malaria, infection of the upper respiratory tract, and certain skin diseases. Moreover, oil and some flavonoids of the plant possess antifungal and antibacterial activity (Siddiqui *et al*., 1997).

Eucalyptus camaldulensis Dehn. (the subject of the present investigation) is one of the most familiar species which introduced into Egypt and planted increasing as exotic tree for windbreaks and shelter belts (Shehata *et al.,* 2002). The wood is hard and durable and has been used for many purposes, including railway sleeper, flooring fencing, plywood, veneer, turnery and firewood. Moreover, the trees are good producers of pollens and nectar for honeybee.

Recently, a great attention has been focused on the possibility of using natural and safety substances in order to improve plant growth, flowering and fruit setting. In this connection, yeasts have been reported to be a rich source of phytohormones, vitamins, enzymes, amino acids and minerals (Barnett *et al*., 1990 and Mahmoud, 2001). It was reported about its stimulatory effects on cell division and enlargement, protein and nucleic acid synthesis and chlorophyll formation (Kraig and Huber, 1980 and Castelfranco and Beale, 1983). It participate a beneficial role during stress due to its cytokinins content (Barnett *et al*., 1990). Improving growth and fruiting of horticultural plants by application of active yeast extract were recorded by Bowe *et al*. (1989), Ahmed *et al*. (1997), Atawia and Desouky (1997), Hegab *et al*. (1997), El-Mogy *et al.* (1998), Abd El-Ghany *et al.* (2001) and Ismaeil *et al.* (2003).

Therefore, the present investigation was designed to disclose the influence of different levels from active yeast extract on vegetative growth and volatile oil of *Eucalyptus camaldulensis* Dehn. (River red gum).

MATERIALS AND METHODS

The research work presented in this paper was carried out at the wire green-house of Agricultural Botany Department, Faculty of Agriculture, Cairo University, Giza, Egypt during the two growing seasons of 2005 and 2006 in order to study the influence of different levels from active yeast extract on vegetative growth and volatile oil of *Eucalyptus camaldulensis* Dehn. (River red gum).

Preparation of yeast extract (YE):

The pure dry yeast powder was activated by using sources of carbon and nitrogen with the ratio of 6:1. This ratio is suitable to get the highest vegetative production of yeast, each ml of activated yeast contained about 12000 yeast cells (Barnett *et al.,* 1990). Such technique allowed yeast cells to be grown and multiplied efficiently during conductive aerobic and nutritional conditions. To produce *de novo* beneficial bioconstituents; i.e., phytohormones, carbohydrates, proteins, amino acids, fatty acids, vitamins, enzymes, minerals …. etc, hence allowed such constituents to release out of yeast tissues in readily form. Such technique for yeast preparation based on:

1- Nutritional media of glucose and casein as favourable sources of C, N and other essential elements (P, K, Ca, Mg, Fe, Mn, Cu, B and Mo as well as Na and Cl) in suitable balance (Barnett *et al.,* 1990). 2- Air pumping and adjusting incubation temperature. The media then subjected to two cycles of freezing and thawing for disruption of yeast tissues and releasing their bioconstituents directly before usage. Tween-20 was added as a spreading agent for tested treatments.

Source of seeds and procedure of the experiment:

Seeds of *Eucalyptus camaldulensis* were collected from authentic mother trees, 38 years old, grown in El-Kanater El-Khayria (20 km. North-West Cairo). Seeds were sown on 8th March, 2005 in the first season and replicated on 9th March, 2006 in the second one to provide the experimental plant materials. Seeds were sown in plastic trays, 40×60 cm, filled with peatmoss and clean sand at the ratio of 1:1 by volume. One month from sowing date, the emerged uniform seedlings were transplanted to plastic pots, one seedling per pot, (25 cm diameter) filled with about 7 kg of clay and clean sand at the ratio of 1:1 by weight. Each pot was received NPK at the recommended rates.

Three months from transplanting (four months from sowing date), plants were sprayed with the prepared active yeast extract at the rates of 50, 100 and 200 ml/L., beside the control treatment where plants were sprayed with tap water.

The experiment was made in a complete randomized design with four replicates. The replicate contained 40 pots, each 10 pots were assigned for one treatment.

Recording of data:

At the end of the experiment in each of the two growing seasons (eight months from transplanting), 20 plants from each treatment, five from each replicate, were lifted from pots for recording the data of vegetative growth. The recorded data were:

1-Plant height (cm).

2-Main stem diameter (mm), at its basal portion.

3-Number of developed branches/plant.

4-Mean leaf area (cm^2).

5-Fresh weight of stems (g)/plant.

6-Fresh weight of leaves (g)/plant.

7-Biomass of shoot (g)/plant.

8-Dry weigh of shoot (g)/plant.

Statistical analysis:

Data on vegetative growth characters of each growing season were subjected to conventional methods of analysis of variance according to Snedecor and Cochran (1982). The least significant difference (L. S. D.) at

0.05 level was calculated for each investigated character under different tested treatments.

Determination and analysis of the volatile oil:

A chemical analysis was carried out to gain information about the effect of yeast extract on volatile oil of *Eucalyptus camaldulensis* leaves. Duplicate water distillation of the volatile oil were conducted on samples from different treatments taken from plants at the end of the experiment of the second season using the following technique (Anonymous, 1980).

A hundred grammes of leaves from each of the investigated treatments were chopped and mixed with 1000 ml of water in a spherical two-liter flask. The receiver capacity was 5 ml with graduation of 1/20 ml accuracy. Distillation was carried out until there was no further increment in volume of the volatile oil. The time of distillation was 3 to 4 hours.

Percentage of the volatile oil was calculated on fresh weight basis according to the following formula:

$$\frac{\text{Volume of the volatile oil in the receiver}}{\text{Sample weight}} \times 100$$

The volatile oil was removed from the receiver using ether to aid its collection, and then placed in a sealed brown small specimen tube, which contained anhydrous sodium sulphate for 18 hours to be dried. The volatile oil obtained was stored in the dark at temperature of 0^{o}C until being required for analysis.

GLC (Gas Liquid Chromatography) technique was used to separate and detect the volatile oil constituents. Analysis was performed at Central Laboratory of Agricultural Biochemistry Department, Faculty of Agriculture, Cairo University, Giza, Egypt. Conditions used are as follows:
Instrument: Pye Unicam Progc; Detector: 300^{o}C (H_2) Flame ionization detector (FID); Column: V17 (Methyl phenyl silicone) 1.5 m × 4 mm; Temperature programming : Initial temp. : 70 ^{o}C; Initial time : 5 min.; Rate : 8 ^{o}C/min.; Upper temp. : 200 ^{o}C; Upper time : 30 min.; Injector: 250 ^{o}C (N_2); Carrier flow rate of gasses: N_2 : 30 ml/min.; H_2 : 33 ml/min.; Air : 300 ml/min.; Chart speed : 0.4 cm / min.

Identifications of different constituents were carried out by comparing the relative retention time of each peack with those of authentic samples. The percentage of individual constituents was computed according to their proportional peak area in the chromatogram.

RESULTS AND DISCUSSION

I- Vegetative growth characters:

Data on vegetative growth characters of River red gum as affected by spraying with various levels of active yeast extract in two growing seasons are presented in Table (1):

1-Plant height:

Data given in Table (1) clearly show that the relatively low used concentration of 50 ml YE/L. showed no significant effect on plant height of River red gum, aged nine months, in both studied seasons. Whereas, spraying active yeast extract at the relatively median used concentration of 100 ml/L. or at the relatively high used concentration of 200 ml/L. induced significant increase in height of River red gum plant in both studied seasons and the difference between these two assigned concentrations proved significant. The maximum increase in plant height was detected at 200 ml YE/L., being 37.6 and 34.9% more than the height of untreated plants in the first and second season; respectively.

Table (1): Vegetative growth characters of *Eucalyptus camaldulensis* Dehn. plants, at the age of nine months, as affected by different rates of active yeast extract in two growing seasons

Treatments	Vegetative growth characters							
	Plant height (cm)	Main stem diameter (mm)	No. of branches / plant	Mean leaf area (cm^2)	Fresh weight of stems (g)/plant	Fresh weight of leaves (g)/plant	Biomass of shoot (g)/plant	Dry weight of shoot (g) /plant
First season (2005)								
Control	79.5	8.4	5.7	6.9	38.5	22.4	60.9	20.28
50 ml YE/L.	76.3	8.7	5.5	7.4	37.2	24.6	61.8	20.73
100 ml YE/L.	94.8	10.2	7.3	9.8	45.9	30.2	76.1	25.62
200 ml YE/L.	109.4	10.6	7.4	10.0	52.4	31.7	84.1	28.23
L.S.D. (0.05)	10.7	1.1	0.68	1.23	4.9	3.6	8.2	3.27
Second season (2006)								
Control	74.8	9.1	6.2	8.4	42.7	25.2	67.9	22.85
50 ml YE/L.	78.4	8.9	6.3	8.6	44.5	26.8	71.3	23.74
100 ml YE/L.	90.5	10.8	7.7	11.5	51.3	32.6	83.9	27.97
200 ml YE/L.	100.9	11.3	8.1	11.6	57.0	34.5	91.5	30.51
L.S.D. (0.05)	9.2	1.3	0.72	1.19	5.3	3.8	8.5	3.42

2- Diameter of the main stem:

It is realized from Table (1) that all sprayed concentrations of active yeast extract increased significantly main stem diameter of River red gum plant in both studied seasons except that of plants sprayed with 50 ml YE/L. where the difference with the control plants proved insignificant in this respect. The maximum diameter was achieved when plants were sprayed with 200 ml YE/L., which in turn being indifferent with that recorded by plants sprayed with 100 ml YE/L. The maximum increase in main stem diameter was 26.2% more than the control in the first season and it was 24.2% more than the control in the second one.

3- Number of branches per plant:

Data pertaining to number of branches per plant of River red gum as affected by spraying with different rates of active yeast extract in two

successive seasons and the results of their statistical analysis are presented in Table (1).

It is obvious that the relatively low used concentration of 50 ml YE/L. had no statistical effect on number of branches developed per plant of River red gum in both studied seasons. By contrast, the other two sprayed concentrations of active yeast extract (100 and 200 ml/L.) increased significantly number of branches per plant of River red gum in both studied seasons without significant difference between them. The maximum number of branches per plant was recorded at 200 ml YE/L., being 29.8 and 30.6% more than the control in the first and second season; respectively.

4- Mean leaf area:

Results given in Table (1) indicate that all tested concentrations of active yeast extract increased mean leaf area of River red gum plant in both studied seasons. The significant increase in mean leaf area was detected when plants were sprayed with 100 or 200 ml YE/L. with no significant difference between these two concentrations. The maximum increase in mean leaf area was achieved at 200 ml YE/L., being 44.9 and 38.1 % more than mean leaf area of untreated plants in the first and second season; respectively.

5- Fresh weight of stems per plant:

It is clear from Table (1) that foliar application with active yeast extract at relatively low used concentration of 50 ml/L. showed no significant effect on fresh weight of stems per plant of River red gum, aged nine months, in both studied seasons. At the same time, the other two sprayed concentrations of active yeast extract (100 and 200 ml/L.) increased significantly fresh weight of stems per plant of River red gum in both studied seasons with significant difference between them. The maximum increase in stems fresh weight was recorded when plants of River red gum were sprayed with 200 ml active yeast extract per liter, being 36.1 and 33.5% more than fresh weight of stems per untreated plant in the first and second season; respectively.

6- Fresh weight of leaves per plant:

Data presented in Table (1) clearly show that all sprayed concentrations of active yeast extract increased fresh weight of leaves per plant of River red gum in both studied seasons. The significant increase in leaves fresh weight was observed at 100 or 200 ml YE/L. with no significant difference between these two concentrations. The maximum increase in fresh weight of leaves per plant was detected at 200 ml YE/L., being 41.5% more than the control in the first season and it was 36.9% more than the control in the second one.

7- Biomass of shoot per plant:

Data on shoot biomass per plant of River red gum as affected by spraying with various concentrations of active yeast extract in two growing seasons and the results of their statistical analysis are presented in Table (1).

It is realized that all tested concentrations of active yeast extract increased shoot biomass per plant of River red gum in both studied seasons. The significant increase in shoot biomass was detected at 100 or 200 ml YE/L. with no significant difference between these two concentrations. The

maximum increase in shoot biomass was recorded when plants of River red gum were sprayed with 200 ml YE/L., being 38.1% more than shoot biomass per untreated plant in the first season and it was 34.8% more than shoot biomass per untreated plant in the second season.

8- Dry weight of shoot per plant:

It is clear from Table (1) that foliar application with active yeast extract at relatively low used concentration of 50 ml/L. showed no significant effect on dry weight of shoot per plant of River red gum, aged nine months, in both studied seasons. On the other hand, the other two sprayed concentrations of active yeast extract (100 and 200 ml/L.) increased significantly dry weight of shoot per plant of River red gum in both studied seasons with no significant difference between these two concentrations. The maximum increase in dry weight of shoot per plant was detected at 200 ml YE/L., being 39.2 and 33.5% more than the control in the first and second season; respectively.

From the above mentioned results, it could be stated that the relatively low used concentration of 50 ml active yeast extract per liter showed no significant effect on vegetative growth characters of River red gum in both studied seasons. On the other hand, the other used concentrations of active yeast extract (100 and 200 ml/L.) induced significant increase in all vegetative growth characters under investigation in both studied seasons. The maximum increase in this respect was recorded at 200 ml YE/L.

In this connection, Bowe *et al.* (1989), Ahmed *et al.* (1997), Atawia and Desouky (1997), Hegab *et al.* (1997)., El-Mogy *et al.* (1998), Abd El-Ghany *et al.* (2001) and Ismaeil *et al.* (2003) recorded enhancement in vegetative growth of horticultural plants by foliar application of active yeast extract, being in harmony with the present findings.

II- Volatile oil:

1- Percentage of volatile oil:

Data on volatile oil percentages in leaves of River red gum plants, aged nine months, as affected by spraying with different levels of active yeast extract in the second season of 2006 are presented in Table (2).

Table (2): Volatile oil percentage in leaves of *Eucalyptus camaldulensis* Dehn. plants, aged nine months, as affected by spraying with different levels of active yeast extract in the second season of 2006

Treatments	% of volatile oil
Control	1.00
50 ml YE/L.	1.05
100 ml YE/L.	1.20
200 ml YE/L.	1.30

It is obvious from Table (2) that volatile oil percentage in leaves of River red gum plants, aged nine months, was increased gradually with increasing the concentration of active yeast extract reaching its maximum

(1.3%) when plants of River red gum were sprayed with 200 ml active yeast extract per liter, being 30% more than volatile oil percentage in leaves of untreated plants (1%).

2- Components of volatile oil :

Data presented in Table (3) reveal that all tested concentrations of active yeast extract had no effect on the components of volatile oil in leaves of River red gum aged nine months. At the same time, the percentages of volatile oil components were slightly affected by applied treatments. Data indicate that geraniol is the first main component of volatile oil which comprised 26.96% of volatile oil in control and from 27.39 to 28.37% in treated plants. The second main component is P-cymene which comprised 14.83% of volatile oil in control and from 13.96 to 14.22% in treated plants. The third main component is cuminal (11.39% in control and from 11.67 to 12.30% in treated plants) followed by citronellol (9.43% in control and from 8.9 to 9.72% in treated plants) and linalool which represented 7.93% in control and from 7.88 to 8.23% in treated plants. Other constituents were detected at percentages ranging from 1.48 to 5.03% of volatile oil such as camphene (4.7 to 5.03%), citronellal (3.87 to 4.21%), borneol (3.48 to 3.71%), citronellyl acetate (2.77 to 3.22%), limonene (2.28 to 2.48%), cineole (1.96 to 2.14%), Y-terpinene (1.97 to 2.07%) and terpineol (1.48 to 1.70%). In addition, some constituents were detected at the rate of less than 1%, such as β-pinene, β-phellandrene and α-pinene.

Table (3): Components and their percentages of volatile oil of *Eucalypltus camaldulensis* Dehn. as affected by spraying with different rates of active yeast extract

Components	Retention time (min)	Percentages			
		Control	50 ml YE/L.	100 ml YE/L.	200ml YE/L.
α - pinene	1.917	0.423	0.477	0.503	0.442
Camphene	2.260	4.826	5.032	4.948	4.699
β - pinene	3.933	0.793	0.816	0.831	0.784
Limonene	8.317	2.481	2.295	2.277	2.318
Cineole	9.697	2.136	1.964	2.054	1.959
Y- terpinene	13.417	2.074	1.979	2.028	1.973
P- cymene	14.683	14.831	14.216	13.962	14.014
β - phellandrene	16.083	0.605	0.558	0.627	0.538
Citronellal	17.650	4.119	3.872	4.205	3.967
Linalool	20.433	7.926	8.114	8.226	7.882
Terpineol	21.233	1.704	1.669	1.483	1.594
Citronellyl acetate	22.500	2.983	3.215	2.769	3.176
Borneol	26.683	3.714	3.587	3.484	3.492
Citronellol	27.383	9.426	9.522	8.899	9.718
Cuminal	30.350	11.385	11.719	11.665	12.295
Geraniol	30.933	26.962	27.826	27.392	28.374

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تأثير الرش بمستخلص الخميرة النشطة على النمو الخضرى والزيت الطيار لنبات الكافور البلدى

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أجرى هذا البحث خلال موسمى ٢٠٠٥ و ٢٠٠٦ بهدف دراسة تأثير الرش الورقى بمعدلات مختلفة من مستخلص الخميرة النشطة (٥٠، ١٠٠و ٢٠٠ مل مستخلص خميرة للتر) على النمو الخضرى والزيت الطيار للكافور البلدى.

أوضحت النتائج المتحصل عليها أن رش مستخلص الخميرة النشطة بالتركز المنخفض نسبيا (٥٠ مل/ لتر) لم يكن له أى تأثير معنوى على جميع صفات النمو الخضرى لنبات الكافور البلدى (عمر ٩ شهور) فى كلا موسمى الدراسة. وعلى العكس من ذلك أدى الرش بأى من التركيزين الآخرين من مستخلص الخميرة النشطة (١٠٠ أو ٢٠٠ مل /لتر) إلى حدوث زيادة معنوية فى جميع صفات النمو الخضرى لنبات الكافور البلدى تحت الدراسة (ارتفاع النبات ، قطر الساق الرئيسية ، عدد الافرع على النبات، متوسط مساحة الورقة، الوزن الرطب للساق والأفرع للنبات، الوزن الرطب لأوراق النبات، الكتلة الحية للمجموع الخضرى للنبات والوزن الجاف للمجموع الخضرى للنبات) فى كلا موسمى النمو، وقد تم الحصول على أقصى زيادة معنوية لصفات النمو الخضرى عندما رشت نباتات الكافور البلدى بالتركيز العالى نسبيا من مستخلص الخميرة النشطة (٢٠٠ مل / لتر).

بالنسبة للتأثير على الزيت الطيار فقد إزدادت نسبته فى أوراق الكافور البلدى بزيادة التركيز المستخدم فى الرش من مستخلص الخميرة النشطة، وكانت أقصى زيادة فى نسبة الزيت الطيار تم الحصول عليها عندما رشت النباتات بالتركيز العالى نسبيا من مستخلص الخميرة النشطة وهو ٢٠٠ مل/لتر. فى نفس الوقت لم يكن لأى من التركيزات المستخدمة من مستخلص الخميرة النشطة تأثيرا على مكونات الزيت الطيار ولكن كان هناك تأثير طفيف على نسب هذه المكونات.