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IN VITRO CULTURE AND STUDYING THE CHEMICAL COMPOSITION OF THE ESSENTIAL OILS EXTRACTED FROM THREE SAMPLES OF *ERIOCEPHALUS AFRICANUS* L. PLANT IN EGYPT

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ABSTRACT: The present study aimed to establish new protocol for propagation via tissue culture techniques to observe the effect of plant growth regulators especially cytokinins, gibberellic acid and auxins with different concentrations on *in vitro* growth of *Eriocephalus africanus* L. for improving the potentiality of regeneration and secondary metabolites production and identification of the main active constituents of volatile oil by GC/MS. The results showed that, the best sterilization treatment was the shoot tip explants rinsed in a solution of clorox at 15% for 15 min was gave the highest values for survival percentage and plant strength 100% and 4.58, respectively also B_5 medium at full strength gave the best results in the both growth measurements. BAP at 2.00 mg/l recorded the highest values in survival percentage (93.33%), shootlet number/cluster (16.50) and shootlet strength (4.50), respectively. Using the high level from GA_3 (4.00 mg/l) in medium was more effective in the elongation of shootlets. In rooting stage B_5 medium supplemented with 0.50 mg/l IBA and 0.15% active charcoal was more effective for increasing root number/explant to 8.67 and root length to 5.78 cm. The chemical analysis for the volatile oils extracted from three samples (field, *in vivo* and *in vitro*) of *Eriocephalus africanus* L. by using GC/MS confirmed that, the total number of the constituents identified ranged from 30-34 compounds representing 79.67-99.41% of the total oil contents. Artemisia ketone (17.10-30.62%), bicyclogermacrene (4.14-15.56%), globulol (2.17-8.30%), allo-aromadendrene epoxide (0.51-9.00%), caryophyllene oxide (2.85-6.76%) and α-pinene (3.10-4.25%) were the main compounds.

Key words: *Eriocephalus africanus L.*, B_5 medium, BAP, GA_3, IBA, Active Charcoal, Volatile Oils, GC-MS and Artemisia ketone.

INTRODUCTION

The genus *Eriocephalus*, native from South Africa and naturalized in the Mediterranean region, is a very large and diversified member of the family Asteraceae, comprises about 32 species (Njenga *et al*., 2005 and Verdeguer *et al*., 2009). *Eriocephalus africanus* L. is commonly known as wild or African rosemary (Merle *et al*., 2007). It is the only species that has been introduced and cultivated in Egypt as an ornamental and nice smelling shrub. It is a small fast growing evergreen shrub, with green-grey foliage and snow white flowers of a distinctive fragrance that give rise to cottony seeds (Merle *et al*., 2007 and Salie *et al*., 1996). Infusions of the plant are used as

diuretic and diaphoretic, as well as to treat gastrointestinal disorders, asthma, coughs, fever and painful conditions. Moreover, this plant has also been traditionally used as medicine for the treatment of inflammation and dermal complications (Njenga and Viljoen, 2006 & Salie *et al.*, 1996). Plant tissue culture has been used as a biotechnological tool for the conservation and rapid micropropagation of medicinal plants and also for providing a source of secondary metabolites as well as overcoming the limitations of extracting useful metabolites from limited natural resources, significant climatic variations, risks from pathogens and is independent of soil conditions (Nagesh *et al*., 2010 and André *et al*., 2015).

Five broad classes of plant growth regulators are important in plant tissue culture: the auxins, cytokinins, gibberellins, abscisic acid and thidiazuron (Olszewski *et al*., 2002 & Liu *et al*., 2003). Plant growth regulators are one of the most important factors affecting cell growth, differentiation and metabolite formation in plant cell and tissue cultures (Baiceanu *et al*., 2015). Microbial diseases rank as number one cause for almost half of the deaths in underdeveloped and tropical countries. The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immunocompromised patients in developed countries (Al-Bari *et al.,* 2006). Medicinal plants represent a rich source of antimicrobial agents whereas; plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava *et al*., 1996). The objective of the present study was to study the effect of B_5 medium alone or supplemented with different concentrations of cytokinin, gibberellic acid and auxins on *in vitro* growth and development of *Eriocephalus africanus* L. explants. This study also aimed to evaluation of essential oils contents for three samples of *Eriocephalus africanus* L. (field plants, *in vivo* grown plants and *in vitro* produced plantlets).

MATERIALS AND METHODS

This work was carried out in Applied Research Center of Medicinal Plants (Tissue Culture and Phytochemistry Lab.), National Organization for Drug Control and Research (NODCAR) Giza, Egypt, and tissue culture lab of Horticulture Department, Faculty of Agriculture, Cairo University, Giza, Egypt, during the period of 2015 – 2018.

All chemicals, solvents and reagents used were of analytical and pure grade. All standers were purchased from Sigma Chemical Co. (St. Lewis, USA) or El-Gomhouria (Cairo, Egypt).

Plant Material:

The plant was kindly identified by Dr. Mohamed EL-Jabali of Herbarium, Orman Botanical Garden, and Ministry of Agriculture. Giza, Egypt. A voucher herbarium specimen had been deposited in the herbarium of Applied Research for Medicinal Plant Center (NODCAR).

Terminal cuttings from mother stock plants (six months old) were taken from field of the Applied Research Center of Medicinal Plants (ARCMP) and planted in controlled greenhouse at 27±1°C during 2-3 months and then placed *in vivo* outside of greenhouse (mother plant) which it considered as *in vivo* sample as in Photo (1) and the well *in vitro* plants developed (shoots with roots) as in Photo (3).

Tissue culture preparation:

Shoot tip segments of 1-1.25 cm in length were used as start material obtained from mother plant. The explants were kept in an anti-oxidant solution containing 100 mg/l ascorbic acid + 100 mg/l citric acid + 100 mg/l poly vinyl pyrrolidone (PVP) for one hour and washed several times by tap-water, then rinsed with a small amount of liquid soap 5% for 5 minutes to remove the assuring of most external contamination, and rinsed again under running tap water for 30 minutes to remove all the remaining

Photo 1. ***In vivo*** **stock mother plants.**

Photo 2. Flowers of ***E. africanus*** **L.**

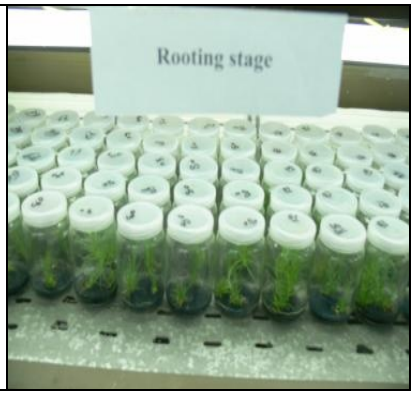

Photo 3. ***In vitro*** **plantlets of** ***E. africanus*** **L.**

detergent, after that the sterilization began under aseptic condition. This procedure and all the steps of the sterilization were done under complete aseptic condition in the laminar air flow. Explants were immersed in 95% ethanol for 2 sec, approximately 2 mm was removed from cut ends of the explants and they were thrice washed with a sterile distilled water for 10 min duration each. Explants were then kept for 10 min in 100 mg/l ascorbic acid+100 mg/l citric acid+100 mg/l poly vinyl pyrrolidone (PVP) solution. The sterile explants were planted in sterile jars of 350 ml containing 40 ml of B_5 (Gamborg *et al*., 1968) basal medium supplemented with 30 g/l sucrose and solidified by 5.0 g/l agar. The essential chemicals used for preparing the media were stock solution. The pH value was adjusted to 5.7-5.8 by adding suitable amount of 0.1 N HCI and 0.1 N KOH by using the pH meter prior to autoclaving at 1.3 kg/cm^2 for 20 minutes.

The work designing:

1. ***In vitro*** **micropropagation:**

a. Effect of clorox (sodium hypochlorite) concentrations and periods of sterilization on survival percentage and plant strength during surface sterilization of *Eriocephalus africanus* L. The shoot tip explants were cultured on B_5 medium at full salt strength.

b. Effect of B_5 medium salt strength without hormones on the growth of *Eriocephalus africanus* L. cultured *in vitro*. The shoots were obtained from shoot tip explants grown on B_5 medium at full salt strength were cut into stem nodes containing two axillary buds (0.50-0.75 cm) and recultured on B_5 full strength, $^3/_4$ B_5, $^1/_2$ B_5, $^1/_4$ B_5 and $^1/_8$ B_5.

c. Effect of cytokinin, benzyl amino purine (BAP) on the growth and development of shoots. The shoots were obtained from shoot tip explants which were grown on B_5 medium at full salt strength (in starting stage) and were cut into stem nodes containing two axillary buds (0.50-0.75 cm) and recultured on different concentrations of BAP by rates of 0.0, 0.25, 0.50, 1.0, 2.0 and 4.0 mg/l individually. Data were recorded after 6 weeks incubation period.

d. Effect of auxin, indole-3-butyric acid (IBA) on root formation of shoots *in vitro*. The plantlets were obtained from clusters grown on B_5 medium supplemented with the best concentration of GA_3 at last experiment (in multiplication stage) recultured on different concentrations of indole-3-butyric acid (IBA) at rates of 0.0, 0.25, 0.50, 1.0 and 2.0 mg/l individually and was added to B_5 basal medium+0.15% active charcoal. Data were recorded after 6 weeks incubation period.

Growth measurements:

Survival %, av. shoot number/explant, av. shoot length (cm), av. leaf number/explant, av. plant strength, rooting %, av. root number/explant and av. root length (cm). Plant strength was estimated (as score) and presented as follows according to the method described of Pattino, (1981), (a) negative growth result = 1 (b) below average growth = 2 (c) average growth = 3 (d) above average growth = 4 (e) excellent growth = 5.

2. Phytochemical analysis:

a. Plant material:

1. Samples of fresh aerial parts from mother stock plants cultivated in field Farm of Applied Research Center for Medicinal Plants (ARCMP), National Organization for Drug Control and Research (NODCAR) were taken before the flowering stage and washed with a tap-water.
2. The fresh aerial parts from *in vivo* grown plants (mother plants) at outside the greenhouse were collected before the flowering stage and washed with a running water and was considered as *in vivo* sample.
3. The fresh plantlets produced *in vitro* after roots formation were collected and washed with a running water to remove the media and was considered as *in vitro* sample.
4. The age of all the plant samples was eight months. Both of them (1, 2 and 3) were then transferred to air dried room until completely dry and then crushed with mortar. The test samples (1, 2 and 3) were taken between November and January.

b. Preparation of the volatile oil:

A sample of dry plant (100 g) was subjected to hydro distillation for 3 h using a Clevenger-type apparatus according to the method recommended by the Egyptian Pharmacopoeia (1984). The obtained essential oils were dried over anhydrous sodium sulphate and after filtration the oil percentage in *Eriocephalus africanus* L. samples were determined then the samples were kept in brown bottle and saved in refrigerator at 4 ℃ until analysis.

c. Chemical composition of the volatile oil constituents by using gas chromatography/mass spectrometer GC/MS:

The essential oil samples (field plants, *in vivo* grown plants and *in vitro* produced plantlets) were analyzed using GC/MS in gas chromatography/mass spectrometer laboratory, National Research Center. GC/MS analysis of the essential oils was performed using a thermo trace GC 2000 (Thermo Quest, TX, USA)/MS Finnigan mat SSQ 7000 system, with the following conditions:

1. Column: DB-5, 30 m x 0.25 mm i.d., 0.25 µm film
2. Carrier gas: helium (flow rate 1ml/min)
3. Detector temperature 270 ℃.
4. Injector temperatures are 220 ℃.
5. Oven temperature programmed:

a. Initial temperature program: 40 ℃ isothermal for 3 min, then gradually increasing to 160 ℃ at rate of 4 ℃/min, followed by 10 ℃/min to 280 ℃.

b. Ionization mode: EL; ion source; 70 eV; mass range: 40-550 amu.

After stabilizing the condition, the volatile oil of the *Eriocephalus africanus* samples was subjected to gas chromatography coupled with mass spectrometric analysis (GC-MS) for investigation of their chemical composition. Identification of the essential oil constituents was achieved by library searched data base Willey 275 LIB and by comparing the retention indexes and mass fragmentation patterns to those of published data (Adams, 2004). The percentage composition of volatile oil components was determined by computerized peak area measurements.

Statistical analysis:

Data of all experiments were statistically analyzed by one way randomized blocks of variance (ANOVA) using Costat 6311Win and the mean values were compared using the L.S.D method at 5% level of significance (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

1. *In vitro* micropropagation:

a. Effect of clorox (sodium hypochlorite) concentrations and periods of sterilization on survival percentage and plant strength of shoot tip explants:

The presented data in Table (1) showed that, using 15% clorox for 15 min seemed to be the most suitable treatment for survival percentage and plant strength of shoot tip explants (100% and 4.58, respectively) while the lowest survival percentage and plant strength were recorded for 20% clorox for 15 min (84.62% and 2.17, respectively).

These results were found to be in agreement with those of Yassien *et al*. (2016) who sterilized the explants shoot tips of *Artemisia abrotanum* L. with a 20% (w/v) sodium hypochlorite (NaOCl) for 15 min. Madzikane-Mlungwana *et al*. (2017) observed that the shoot tips of *Eriocephalus africanus* were surface decontaminated in a laminar flow bench using 70% ethanol (v/v) for 60 s followed by sodium hypochlorite (NaOCl; 2.00% or 3.50%) for either 10 or 20 min.

b. Effect of B_5 medium salt strength on the growth of *Eriocephalus africanus* L. stem nodes:

The effects of B_5 medium on growth of *Eriocephalus africanus* L. as shoot and root formation parameters as shown in Table (2) the highest significant values were recorded with B_5 full strength as follows; survival percentage 100%, shoot number 5.40, shoot length 3.00 cm, leaf number 8.66, shoot strength 4.50, rooting percentage 60%, root number 3.67 and root length 3.74 cm comparing with other strength medium. Whereas, one eighth strength of the medium recorded the lowest values of the parameters; survival percentage 57.14%, shoot number 1.00, shoot length 0.63 cm, leaf number 3.83 and shoot strength 2.27 also the same treatment had non-significant effect on rooting percentage, root number and root length.

The present results were in harmony with those of Soliman, (2011) who observed that the highest significant values were recorded with full B_5 and $^1/_2$ B_5 medium salt strength for all parameters when culturing the shoot tip explants of *Rosmarinus officinalis* L. var. ternifolius. Monfort *et al*. (2018) found that the B_5 medium full strength had significant effect on the growth and development for the shootlets produced from nodal segments of *Ocimum basilicum* where recorded the highest values of leaf number (20), shoot height (4.54 cm), root number (6) and root length (2.84 cm) after 40 days from culturing.

c. Effect of different concentrations of benzyl amino purine (BAP) on the growth and development of explants cultured on B_5 medium:

The presented data in Table (3) showed that, B_5 medium supplemented with the high concentrations from BAP (2.0 and 4.0 mg/l) gave the highest value of survival percentage (93.33%) for each, while B_5 medium (control) and B_5 medium supplemented with the low concentration from BAP at 0.25 mg/l gave the lowest value of 66.67%. Shootlet number/cluster and shootlet strength were significantly affected by the concentration of 2.0 mg/l BAP (16.50 and 4.50, respectively), whereas the B_5 medium (control) gave the smallest number (1.70) and the shootlet strength recorded the smallest value (3.00) with increase the BAP concentration from 0.25 to 1.0 mg/l. B_5 medium (control) without any growth regulators recorded the highest values for shootlet length and leaf number (1.94 cm and 9.22, respectively), while the high concentration of BAP (4.0 mg/l) recorded the lowest values (0.32 cm and 4.97, respectively).

Table 1. Effect of clorox (sodium hypochlorite) concentrations and periods of sterilization on survival percentage and plant strength of shoot tip explants after 6 weeks from culturing.

Time of sterilization	Concentrations of clorox (sodium hypochlorite)			
	15% clorox (0.79 % NaOCl)	20% clorox (1.00% NaOC)	15% clorox (0.79 % NaOC)	20% clorox (1.00% NaOC)
	Survival %		Av. plant strength	
10 min	85.71	92.31	3.92 B	2.42 C
15 min	100	84.62	4.58 A	2.17 D
L.S.D at 5%	-	-	0.2308	

Table 2. Effect of B_5 medium salt strength on the growth of *Eriocephalus africanus* L. stem nodes cultured *in vitro* after 6 weeks from culturing.

Treatment B_5 salt strength	Survival %	Av. shoot number	Av. shoot length (cm)	Av. leaf number	Av. shoot strength	Rooting %	Av. root number	Av. root length (cm)
Full	100	5.40 A	3.00 A	8.66 A	4.50 A	60.00	3.67 A	3.74 A
3/4	77.80	3.06 C	2.26 C	8.04 C	3.01 C	25.00	1.37 C	1.17 C
1/2	88.90	3.75 B	2.62 B	8.33 B	3.83 B	50.00	3.00 B	1.83 B
1/4	71.14	2.17 D	1.30 D	5.10 D	2.67 D	33.33	1.00 D	0.57 D
1/8	57.14	1.00 E	0.63 E	3.83 E	2.27 E	0.00	0.00 E	0.00 E
L.S.D at 5%	-	0.6447	0.3162	0.2694	0.3357	-	0.3457	0.5558

Table 3. Effect of different concentrations of benzyl amino purine (BAP) on the growth and development of explants cultured on B_5 medium.

Treatment (BAP mg/l)	Survival %	Av. shootlet number/cluster	Av. shootlet length (cm)	Av. leaf number	Av. shootlet strength
B_5 Control	66.67	1.70 F	1.94 A	9.22 A	3.40 C
0.25	66.67	6.70 E	1.23 B	8.30 B	3.00 D
0.50	80.00	9.33 D	0.90 C	6.42 D	3.00 D
1.00	83.33	9.83 C	0.53 E	5.96 E	3.00 D
2.00	93.33	16.50 A	0.74 D	7.11 C	4.50 A
4.00	93.33	14.00 B	0.32 F	4.97 F	4.00 B
L.S.D at 5%	-	0.4710	0.1970	0.4450	0.3510

These results were in agreement with those of El-Tarras *et al*. (2015) who demonstrated for *Ficus palmata* Forsk, that the maximum average number of multiplied shoots (3.25) was produced on 2.00 mg/l BAP and Abd EL-Hamied (2016) who found on the *in vitro* growth of *Artemisia abrotanum* L. that the shoots number was 35.65 shoots/explant at 2.00 mg/l BAP.

d. Effect of auxin indole-3-butyric acid (IBA) on rooting and growth of *Eriocephalus africanus* L. shoots *in vitro* cultured on B_5 medium:

For the effect of different concentrations of auxin (IBA), data are shown in Table (4). The best survival and rooting percentage (100%) were recorded with B_5 medium (control) without hormones but the

Table 4. Effect of different concentrations of auxin indole-3-butyric acid (IBA) on rooting and growth of *Eriocephalus africanus* L. shoots cultured *in vitro* after 6 weeks from culturing.

Treatment (IBA mg/l)	Survival %	Av. shoot length cm/explant	Rooting %	Av. root number/ explant	Av. root length cm/explant	Av. plant strength
B_5 Control	100	3.27	82.86	3.78	5.08	2.56
0.25	80	2.98	70	5.75	3.51	2.63
0.5	100	5.35	100	8.67	5.78	4.00
1.0	100	4.29	100	5.33	4.76	2.87
2.0	40	4.58	40	5.76	2.27	3.30
L.S.D at 5%	-	0.2750	-	0.4035	0.4601	0.2317

maximum concentration of IBA at 2.0 mg/l recorded the lowest values of each survival percentage (40%), rooting percentage (40%) and root length (2.27cm/explant). However, the concentration 0.50 mg/l IBA recorded the highest values of shoot length cm, root number, root length cm/explant and plant strength (4.58 cm, 8.67, 5.78cm and 4.0, respectively) compared with B_5 medium (control) without hormones and minimum concentration of IBA at 0.25 mg/l which recorded the lowest values of plant strength (2.56 and 2.63, respectively) as well as the lowest value of shoot number/explant (1.91) recorded with 0.5 mg/l IBA.

These results agreed with those of Gopinath *et al*. (2014) who found that, the well developed *in vitro* shoots of *Artemisia annua* L. were transferred on rooting media supplemented with various concentration of IBA (1.00 mg/l) showed efficient root induction and further development of healthy root and then acclimatized and successfully established in field with 85% of survival. Labade *et al*. (2016) studied the effect of different concentrations of IBA on the rooting of *Chrysanthemum morifolium* shoots raised from nodal segments. The result showed superiority of ½MS+0.20 mg/l IBA as compared to all the other treatments as it produced significantly maximum number of roots per explant (11.80±0.75) and largest roots (9±0.19 cm) and survival rate was 90% as IBA is considered as the most efficient auxins in root induction and development.

2. Phytochemical analysis: Chemical composition of the volatile oil constituents by using gas chromatography/mass spectrometer GC/MS:

A comparative study was carried out between three samples of *Eriocephalus africanus* L. (field plants, *in vivo* grown plants and *in vitro* produced plantlets). The samples were analyzed by GC-MS, the qualitative and quantitative composition of the volatile oils constituents are shown in Tables (5 & 6) and illustrated in Figures (1&2&3). Thirty to thirty-four compounds were identified, accounting for 79.67–99.41% of the total oil contents. Essential oils for the three samples were rich in oxygenated compounds, 9 oxygenated monoterpenes representing 26.53% at *in vitro* plantlets, 8 oxygenated monoterpenes with percentage of 40.31% at *in vivo* plants and 8 oxygenated monoterpenes representing 41.30% in field plants, and 11 oxygenated sesquiterpenes with percentage of 15.05% in field plants, 28.72% at *in vitro* plantlets and 35.36% at *in vivo* plants.

The highest percentage of the main constituents were Artemisia ketone 30.62% which is considered as the characteristic compound of *Eriocephalus africanus* L. and artemisia alcohol 4.61% in field plants and also 1,8-cineole 3.93%, globulol 8.30%, allo-

Table 5. Constituents of the essential oils from three samples of *Eriocephalus africanus* L. by GC-MS analysis.

Peak No.	Name of compound	KI	Percentage of compound %		
			Field plants	*In vivo* plants	*In vitro* plantlets
	Monoterpene hydrocarbons				
1	**α-Pinene**	939	4.25	3.64	3.10
2	**Camphene**	954	0.20	1.60	1.00
3	**β- Ppinene**	979	1.50	1.00	0.85
	Oxygenated monoterpenes				
4	**Yomogi alcohol**	999	0.83	0.87	0.66
5	**1,8-Cineole**	1031	0.33	3.93	1.60
6	**Artemisia ketone**	1062	30.62	30.31	17.10
7	**Artemesia alcohol**	1084	4.61	1.52	1.64
8	**Trans-pinocarveol**	1139	1.21	0.40	1.00
9	**Camphor**	1146	0.26	2.31	3.03
10	**Pinocarvone**	1165	1.50	0.64	0.90
11	**Myrtenol**	1196	0.74	0.33	0.60
	Sesquiterpene hydrocarbons				
12	**α-Cubebene**	1351	1.50	0.23	0.60
13	**α-Copaene**	1377	2.10	-	-
	Oxygenated monoterpenes				
14	**Geranyl acetate**	1381	1.20	-	-
	Sesquiterpene hydrocarbons				
15	**Caryophyllene <E>**	1419	2.50	0.31	0.40
16	**α-Humulene**	1455	1.01	-	-
17	**Alloaromadendrene**	1460	0.57	-	-
18	***cis*-β-*Guaiene***	1493	0.36	0.33	0.85
19	**α-Selinene**	1498	2.44	1.04	0.47
20	**β-Selinene**	1490	1.52	0.53	0.43
21	**Bicyclogermacrene**	1500	4.14	13.82	15.56
22	**Selina-3,7(11)-diene**	1547	2.32	0.34	0.36
	Oxygenated sesquiterpenes				
23	**Spathulenol**	1578	1.30	1.26	0.44
24	**Caryophyllene oxide**	1583	2.85	5.23	6.76
25	**Globulol**	1585	2.17	8.30	5.64
26	**β-Copaen-4α-ol**	1591	1.30	0.75	0.44
27	**Veridiflorol**	1593	0.80	1.06	1.36
28	**Guaiol**	1601	0.37	1.37	0.40
29	**10-epi- γ-Eudesmol**	1624	1.93	0.90	1.42
30	**Allo-Aromadendrene epoxide**	1641	0.51	9.00	7.00
31	**β- Eudesmol**	1651	1.52	5.97	2.36
32	**α-Cadinol**	1654	1.50	1.21	0.60
33	**Eudesm-7(11)-en-4-ol**	1700	0.80	0.31	2.30
	Oxygenated diterpenes				
34	**Phytol**	1943	0.35	0.90	0.80

KI: Kovats retention indices according to Adams (2004).

Table 6. Percentage of different group constituents of the essential oils of samples of *Eriocephalus africanus* L.

Group constituents	Percentage of group constituents		
	Field	***In vivo***	***In vitro***
Hydrocarbons			
Monoterpene hydrocarbons	5.95	6.24	4.95
Sesquiterpene hydrocarbons	18.46	16.60	18.67
Total hydrocarbons	24.41	22.84	23.62
Oxygenated compounds			
Oxygenated monoterpenes	41.30	40.31	26.53
Oxygenated sesquiterpenes	15.05	35.36	28.72
Oxygenated diterpenes	0.35	0.90	0.80
Total oxygenated compounds	56.70	76.57	56.05
Total oil composition %	81.11	99.41	79.67
Total identified compounds	34 compounds	30 compounds	30 compounds

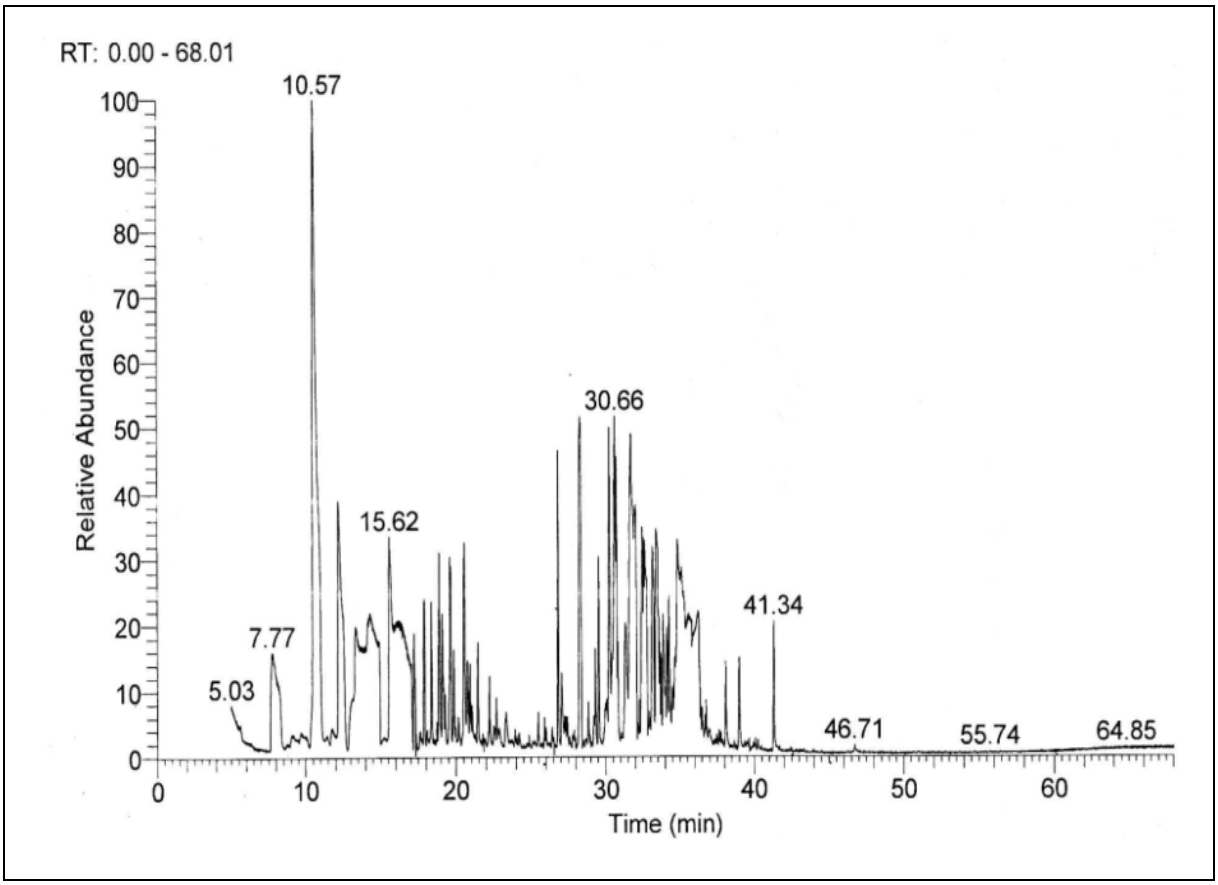

Figure 1. The chromatogram of GC/MS analysis of the essential oil of *Eriocephalus africanus* L. in field.

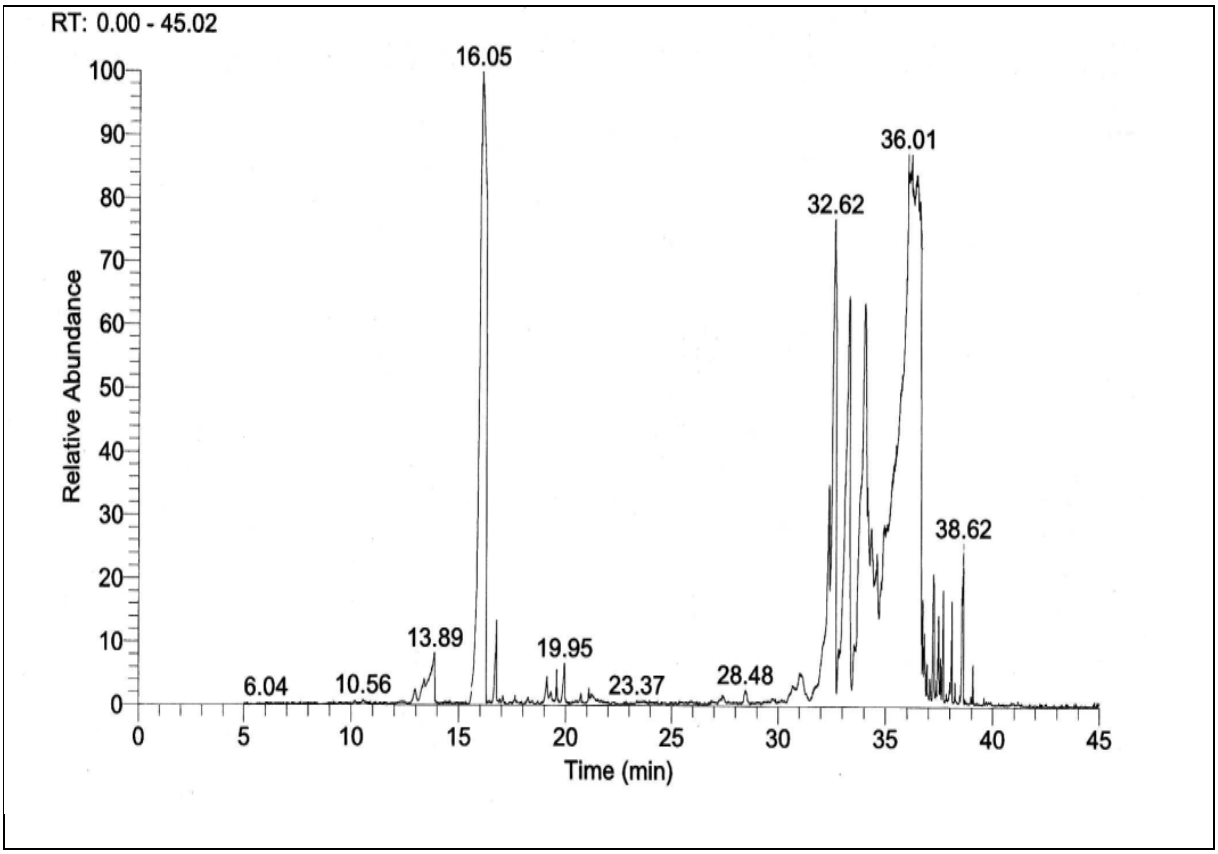

Figure 2. The chromatogram of GC/MS analysis of the essential oil of *Eriocephalus africanus* L. *in vivo*.

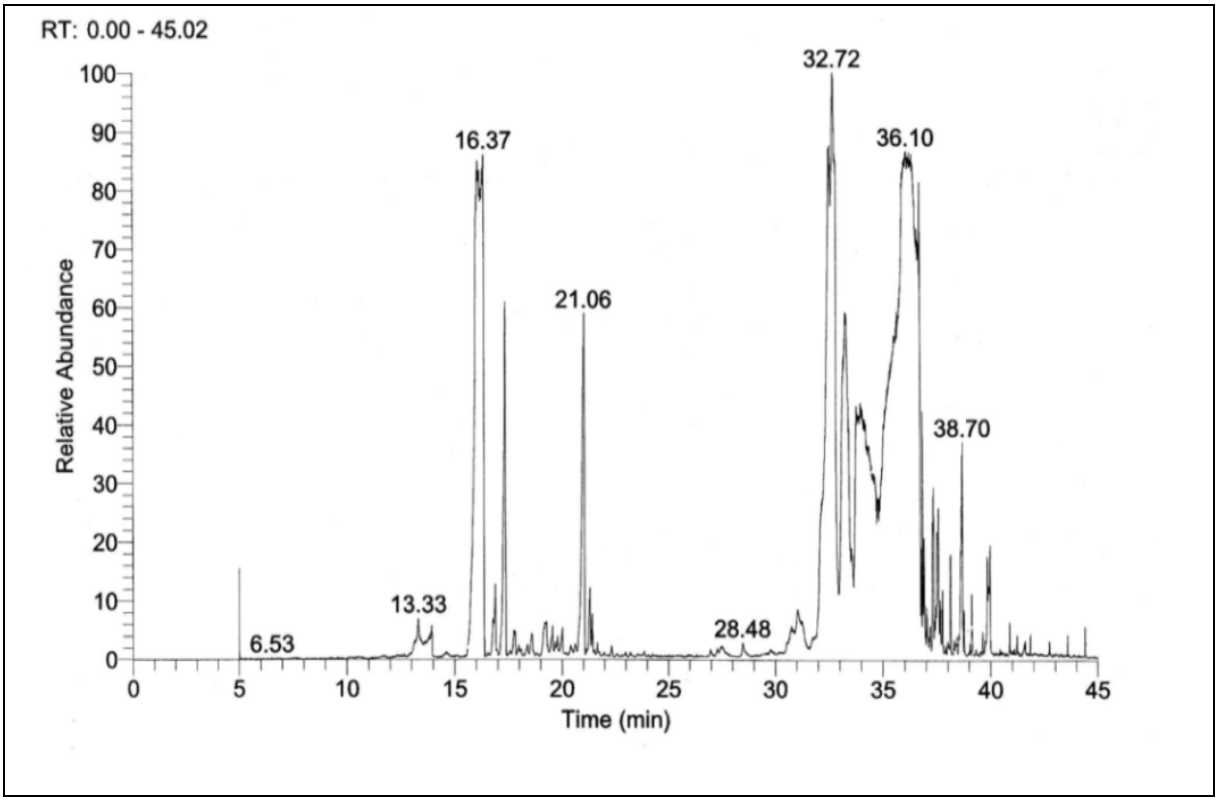

Figure 3. The chromatogram of GC/MS analysis of the essential oil of *Eriocephalus africanus* L. *in vitro*.

aromadendrene epoxide 9.00% and β-eudesmol 5.97% were at *in vivo* plants as well as camphor 3.03%, caryophyllene oxide 6.76% and eudesm-7(11)-en-4-ol 2.30% at *in vitro* plantlets. However, the lowest percentage of compounds were artemisia ketone 17.10% at *in vitro* plantlets and also artemisia alcohol 1.52% and eudesm-7(11)-en-4-ol 0.31% at *in vivo* plants as well as 1,8-cineole 0.33%, camphor 0.26%, caryophyllene oxide 2.85%, globulol 2.17%, allo-aromadendrene epoxide 0.51% and β-eudesmol 1.52 % in field plants.

The three monoterpene hydrocarbons were ranged between about 4.95 at *in vitro* plantlets, 5.95% in field plants and 6.24% at *in vivo* plants, and seven sesquiterpene hydrocarbons with percentage of 16.60% at *in vivo* plants, ten representing 18.46% in field plants and seven with percentage of 18.67% at *in vitro* plantlets.

The highest percentage of the main constituents were α-pinene 4.25%, caryophyllene <E> 2.50%, α-selinene 2.44% and selina-3,7(11)-diene 2.32% in field plants and also bicyclogermacrene 15.56% at *in vitro* plantlets. Whereas the lowest percentage of compounds were α-pinene 3.10% and α-selinene 0.47% at *in vitro* plantlets as well as caryophyllene <E> 0.31% and selina-3,7(11)-diene 0.34% at in *vivo* plants too bicyclogermacrene was 4.14% in field plants.

These results were in agreement with those of Jesionek *et al*. (2016) who observed that the essential oil content of the maternal plant, *in vitro* shoots and the regenerates of *Rhododendron tomentosum* Harmaja was determined by steam distillation and the obtained volatile fractions were analyzed by GC/MS. Some compounds like methyl everninate was present only in microshoots and regenerated plants' volatile fraction, while others were found solely in the ground material (for instance Ledol). Alloaromadendrene was the predominant constituent in the microshoots (4.50–9.20%), while γ-terpineol (8.80-15.0%), palustrol (11.50–15.70%) and ledol (9.60–12.10%) occurred in large quantities in maternal plants. Other compounds, like p-cymene was abundant in both *in vitro* cultures and maternal plants (4.60–5.20%). Kulpa *et al*. (2018) obtained the essential oils by hydro distillation in Deryng and Clevenger apparatus from *in vitro* shoot cultures of *Thymus vulgaris* L. The essential oils were analyzed using gas chromatography-mass spectrometry (GC-MS). Analysis revealed the presence of 54 components represented mainly by oxygenated monoterpenes (56.81-57.28%) and monoterpene hydrocarbons (31.90-33.72%). Among the identified constituents, the most abundant were thymol (33.37-34.05%), γ-terpinene (11.62-11.91%), p-cymene (9.81-10.07%), carvacrol (5.63-5.96%), carvacrol methyl ether (3.86-3.87%) and linalool (3.16-3.36%).

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الزراعة داخل المعمل ودراسة التركيب الكيميائى للزيوت الأساسية المستخلصة من ثلاث عينات لنبات إريوسيفالوس أفريكانوس فى مصر

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هدفت الدراسة الحالية إلى إنشاء بروتوكول جديد للاكثار عن طريق تقنيات زراعة الأنسجة لدراسة تأثير منظمات النمو النباتية وخاصة السيتوكينينات وحمض الجبريليك والأوكسينات ذات التركيزات المختلفة على النمو داخل المعمل للأريوسيفالوس أفريكانوس لتحسين إمكانيات التجديد وانتاج المركبات الثانوية وتحديد المكونات النشطة الرئيسية من الزيت الطيار بواسطة جهاز الفصل كروماتوجرافيا الغاز السائل المقترن بمطياف الكتلة. أوضحت النتائج أن أفضل معاملة تعقيم كانت غسل المنفصلات النباتية القمة النامية فى محلول كلوروكس ١٥٪ لمدة ١٥ دقيقة حيث أعطت أعلى القيم لنسبة النباتات الحية وقوة النبات ١٠٠٪ و ٤,٥٨ على التوالى كما أعطت بيئة جامبورج كاملة القوة أفضل النتائج فى كل قياسات النمو. ٢ ملليجرام/لتر بنزايل أمينو بيورين سجلت أعلى القيم فى نسبة النباتات الحية وعدد الأفرع/عنقود وقوة الفرع ٩٣,٣٣٪ و١٦,٥٠ و٤,٥٠ على التوالي. إستخدام مستوى عالي من حامض الجبرليك (٤ ملليجرام/لتر) فى البيئة كان أكثر تأثيراً فى أستطالة الأفرع. فى مرحلة التجذير بيئة جامبورج المضاف إليها ٠,٥٠ ملليجرام/لتر إندول حامض البيوتريك و٠,١٥٪ من الفحم النشط كانت أكثر تأثيراً فى زيادة عدد الجذور/منفصل نباتى وطول الجذر إلى ٨,٦٧ و٥,٧٨ سم.

أظهر التحليل الكيميائي باستخدام جهاز الفصل كروماتوجرافيا الغاز السائل المقترن بمطياف الكتلة للزيوت الطيارة المستخلصة من ثلاث عينات (الحقل وخارج المعمل وداخل المعمل) للإريوسيفالوس أفريكانوس أن إجمالي عدد المكونات التي تم تحديدها تراوح بين (٣٠-٣٤) مركب تمثل (٧٩,٦٧-٩٩,٤١٪) من إجمالي مكونات الزيت. الأرتيمزيا كيتون (١٧,١٠-٣٠,٦٢٪)، Bicyclogermacrene (٤,١٤-١٥,٥٦٪)، جلوبولول (٢,١٧-٨,٣٠٪)، allo-Aromadendrene epoxide (٠,٥١-٩,٠٠٪)، كاريوفيلين أوكسيد (٢,٨٥-٦,٧٦٪) و ألفا - بينين (٣,١٠-٤,٢٥٪) كانت المركبات الرئيسية.