In Vitro Volatile Oil Products of *Melaleuca ericifolia* Smith

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Abstract

The effect of gamma–irradiation and colchicine treatments on the yield and spectrum of volatile oil products of *Melaleuca ericifolia* explants in vitro were studied. Using a low dose of gamma-rays (10 Gy) resulted in the highest yield of volatile oil. Applying a high concentration of colchicine (400 ppm) or a low dose of gamma-rays (10 Gy) remarkably maximized the methyl eugenol content. Colchicine treatment at 200 ppm exhibited the maximum relative amounts of thujone, limonene and terpinyl acetate. In the case of a high dose of gamma-rays (40 Gy), the highest levels of *p*-cymene and α-terpineol were determined.

INTRODUCTION

Melaleuca ericifolia Smith (Myrtaceae) is an evergreen tree and from its leaves the volatile oil, which has antimicrobial and preservative properties, are widely applied for several medical and pharmaceutical purposes. It was affirmed that subjecting the explant tissues to various treatments of either irradiation or chemical mutagen compounds prior to culturing in vitro affected the yield and spectrum of the volatile oil produced (Bricout et al., 1978 and Cambecedes et al., 1992). Therefore, the current study was planned to investigate the effect of different doses of gamma-irradiation and various concentrations of colchicine on the quantity and quality of volatile oil products of *M. ericifolia* explants in vitro.

MATERIALS AND METHODS

In vitro Cultures

The study was conducted at the Tissue Culture Res. Lab., Hort. Res. Inst., Giza-Egypt, in two successive years: 1999 and 2000. Authenticated *Melaleuca ericifolia* (20-year-old) grown at the Hort. Res. Inst., Giza was used as the source of the vegetative materials for establishment of the in vitro shootlet cultures.

The basal MS-culture medium used was the formulation of Murashige and Skoog (1962) enriched with (per litre): casein hydrolysate (100 mg), D-calcium pantothenate (1 mg), kinetin (1 mg), IBA (0.5 mg), sucrose (25g), and Anachemia agar (7 g). The medium was adjusted to pH 5.7± 0.1, then poured in 50 mL capacity glass jars before autoclaving at 121°C and 1.2 Kg/cm^2 for 15 min.

The cultures were incubated in a growth chamber at 24±1 °C under 16 hr. photoperiod (day light fluorescent tubes, 5 K lux).

Gamma-Irradiation and Colchicine Treatments

For gamma-irradiation treatments, the in vitro shootlets (5.5± 0.5 cm long), grown on solidified MS-medium, were irradiated with gamma rays from a cesium-137 source. The used doses were 10, 20, and 40 Gy (1.25 Gy/min). One week after irradiation, the shootlets were aseptically sectioned into nodal explants and recultured on fresh solidified MS-medium.

As for colchicine treatments, the shootlets were aseptically immersed in 125 mL-capacity Erlenmeyer flasks containing 50 mL liquid MS-medium amended with colchicine at the concentrations: 100, 200 and 400 ppm. The treated cultures were subjected to continuous circular motion of 30 rpm on a platform shaker. After 48 hr of

colchicine exposure, the shootlets were rinsed, divided into nodal explants and recultured on fresh solidified MS-medium.

All the treatments tested, including three doses of gamma-irradiation and three concentrations of colchicines, in addition to the control, were triplicated. Ten weeks after treatment, the obtained shootlets were repeatedly subcultured into fresh solidified MS-medium four times at ten week intervals.

Volatile Oil Isolation, Identification and Determination

Air-dried samples (150 g) of the treated shootlets, in three replicates, were hydro-distillated for four hours using a Clavenger light oil apparatus to yield the volatile oil. The obtained oil was measured, calculated and expressed as a percentage of the dry weight (V/DW). The separated oil was dried over anhydrous sodium sulphate. Gas-Liquid Chromatographic (GLC) analysis was performed for the obtained volatile oil and the identification and determination of each component was achieved by comparing their retention times with seven authentic samples (methyl eugenol, cineol, thujone, limonene, terpinyl acetate, *p*-cymene and α-terpineol) under the following conditions: Apparatus: Hewlett Packard; GC 5890; Column: 2.5 m x 0.32 mm carbowax 20M; Programming temperature: from 70°C to 180°C at the rate 4°C/min for 20 min; Temperature: injection (220°C) and detector (270°C); Gas flow rates: for N_2, H_2 and air were 30, 30, and 300 mL/min, respectively.

Statistical Analysis

The statistical lay-out of the experiments was completed by randomised design. LSD was used for comparison of the mean values (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Volatile Oil Content

It is evident from the data in Table 1 that applying gamma-rays at 10 Gy yielded the highest percentage of volatile oil (3.62%). This value significantly fell about 2 and 5 fold as the doses of gamma-rays increased to 20 and 40 Gy, respectively. It obviously appears that a low dose (10 Gy) of gamma-irradiation had the most prominent effect on biosynthesis of the volatile oil in the greatest quantity and this might be due to such treatment having the ability to maximise volatile oil synthesis at higher rates.

Increasing the concentration of colchicine from 100 to 400 ppm augmented the volatile oil percentage 1.7 fold. The effect of colchicine, reviewed by Bricout et al. (1978), showed that the addition of colchicine to the culture medium of *Mentha piperita* produced a three-fold stimulation of the production of essential oil.

Volatile Oil Components

Data illustrated in Table 2 shows that the major volatile oil components were methyl eugenol and cineole, while the minor ones were thujone, limonene, terpinyl acetate, *p*-cymene, and α-terpineol. Gamma-rays at 20 Gy and colchicine at 100 or 400 ppm maximized the methyl eugenol anabolism in higher proportions (93.0, 94.5 and 97.6 %, respectively). Using a low dose of gamma-rays (10 Gy) or a medium concentration of colchicine (200 ppm) resulted in higher cineol amounts (37.8 and 55.9 %, respectively). Applying colchicine at 200 ppm was the most favourable treatment to produce higher fractions of thujone, limonene and terpinyl acetate (3.6, 3.8 and 8.6%, respectively). Treating with gamma-irradiation at a high dose (40 Gy) increased the formation of *p*-cymene and α-terpineol (0.06 and 0.48%, respectively). These results parallel those reviewed by Bricout et al. (1978) on *Mentha piperita*, who reported that the relative proportion of monoterpenes presented in the oil was influenced by colchicine treatment. In addition, Chavadej (1983) found in *Valeriana wallichii* that, in the case of colchicine treatment, the valepotriate content was 5-10 fold higher than in the control.

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Tables

Table 1. Effect of gamma-irradiation and colchicine treatments on volatile oil content of *Melaleuca ericifolia* shootlet tissues in vitro.

Treatments	**volatile oil (%)**
Control	0.86 cd
Gamma–ray (10 Gy)	3.62 a
Gamma–ray (20 Gy)	1.91 b
Gamma–ray (40 Gy)	0.69 d
Colchicine (100 ppm)	0.82 cd
Colchicine (200 ppm)	0.81 cd
Colchicine (400 ppm)	1.36 bc
LSD (5 %)	0.666
LSD (1 %)	0.927

a.b.c.d.:The means followed by different letters are significantly different at the 5 % level according to the LSD test.

Table 2. Effect of various gamma-irradiation and colchicine treatments on volatile components (%) of the oil of *Melaleuca ericifolia* shootlet tissues in vitro.

Volatile components (%) Treatments	Methyl eugenol	Cineole	Thujone	Limonene	Terpinyl acetate	*ρ*-cymene	α-terpineol
Control	91.66	5.69	0.28	0.51	0.47	-----*	0.09
Gamma–ray (10 Gy)	59.60	37.85	0.15	0.21	0.39	-----*	-------*
Gamma–ray (20 Gy)	93.10	5.70	0.10	0.03	0.22	0.03	0.04
Gamma–ray (40 Gy)	86.80	10.36	0.32	-------*	1.04	0.06	0.48
Colchicine (100 ppm)	94.55	2.02	0.19	-------*	0.97	-----*	0.21
Colchicine (200 ppm)	26.34	55.99	3.68	3.83	8.64	-----*	------*
Colchicine (400 ppm)	97.66	0.36	0.16	0.13	0.45	-----*	------*

*: Not identified