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Research Article

VOLATILE TERPENOID SPECTRUM OF ESSENTIAL OIL OF MICRO PROPAGATED AND NATURALLY GROWN PLANTS IN COTTON LAVENDER (*Santolina chamaecyparissus* L.)

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Abstract- *Santolina chamaecyparissus* L. is important herb known for its anti-helminitc, antispasmodic and emmenagogic properties. Metabolic profiling using GC and GC-MS was applied to determine chemical differences between conventionally seed propagated and micro-propagated plants. Oil yield range was showed that the essential oil isolated from foliage of regenerated plantlets was a complex mixture, 25 compounds were identified corresponding to 80% of the total oil content. The analysis of the identified constituents included mono-terpene hydrocarbon, oxygenated mono-terpene, sesqui-terpene hydrocarbons and oxygenated sesqui-terpene The major constituents were artemisia ketone, 1,8-cineole, myrcene, limonene, linalool, cis β-ocimene and β-caryophyllene oxide.

Keywords- *Santolina chamaecyparissus,* Compositae, Essential oils, Tissue culture micro-propagated plants, Terpenoids

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Introduction

Tissue culture of aromatic plants has gained considerable importance in recent years [1]. The Genus *Santolina* is a rich source of terpenoids, which are used in pharmaceuticals industries. Among the various species *Santolina chamaecyparissus* L. is the most common herb native to Mediterranean region. The infusion, essence, powder and oil from the foliage and to a lesser extent from the flower heads of this plant species, are widely used as folk and herbal medicine on account of its marked anti-helminitc, antispasmodic and emmenagogic properties. Beside, its use in the treatment of eye infections, in stimulating the proliferation of scar tissue and in relief of insect bites has also been reported [2].

Applications of *in vitro* methods have been considered as attractive alternative approaches for multiplications and production of essential oils in aromatic plants of commercial value. Recently we have reported biosynthetic potentialities of various culture lines of *Santolina chamaecyparissus* to produce volatile terpenes [3]. The present study reports field evaluations and metabolic profiling of *in-vitro* micro-propagated and naturally grown Cotton Lavender (*Santolina chamaecyparissus* L) plants.

Materials and Methods

Plant regeneration *in vitro*

The young shoots of *Santolina chamaecyparissus* collected from Green House grown plants at Jammu and kept under running tap water for two hours followed by soaking with 5 % Teepol for 10 min. After thorough washings the source tissue were surface sterilized using 70% EtOH for 1 min followed by 0.1% (w/v) $HgCl_2$ and rinsed three times with sterilized double distilled water. The shoot apices were dissected to appropriate size of 0.5 cm each and cultured onto Murashige and Skoog [4] agar solidified medium supplemented with 3% sucrose, BA, Kn, NAA, IAA and IBA alone or in diverse combinations using various concentrations (0.1-5.0 mg.l^{-1}). The pH of the medium adjusted to 5.8 prior to addition of 0.8% (m/v) agar. The cultures were incubated at 25 ± 2^0 C under 16-h day light photoperiod regimes. The *in-vitro* regenerated shoot lets obtained from such cultures were subjected to MS rooting medium supplemented with IAA or IBA in range of 0.5-1.0 mg.1^{-1}. The *in-vitro* regenerated plantlets with well-developed roots and shoots were removed carefully and washed with distilled water and transferred to pots in sand soil mixture and hardened under Green House conditions (25^0 ± 5^0 C; RH 70-80%). After one month the well-acclimatized hardened plants were transferred to the Experimental field at Jammu (320° 44' (N) Latitude and 74° 55' (E) longitude and 400 meters above the sea level with a temperature ranging 5°-45°C and total rainfall 506 mm) and grown up to the maturity. The foliage of *in vitro* raised plants and naturally grown plants (100g) of the same age was harvested at various growth stages and hydro distilled for recovery of essential oil using Clevenger-type apparatus at 60°C for 3 h.

Essential oil analysis

The oil was collected in benzene after hydro distillation, as the percentage was very low. Triplicate distillations were performed in succession for each sample. The oil samples were stored at 4^0 C until used further for analysis by the combination of GC and GC-MS. TLC was carried out on pre-coated Silica gel plates using benzene, Pet Ether- Ethyl acetate (90:10) as an eluant. The spots were detected by spraying with 2% vanillin–sulfuric acid as visualization agent. The oil analysis performed on NUCON Gas Chromatograph, using the conditions: Fused silica capillary column coated with FFAP; Helium as carrier gas. The compounds peaks were identified on the basis of relative retention times with those of known compounds run under similar conditions and by enrichment technique. GC-MS was performed on Shimadzu [Model QP-2000] fitted with fused Silica capillary column [30m x 0.30mm] coated with SE-30. The oven temperature

programming was: Initial temperature 90° C/2 min-220 °C with rise at the rate of 7 ° C/ min to 220° C. The helium as carrier gas was used with split ratio of 1:50. Mass spectral data were analysed *via* library search and comparison with published data Constituents were identified at different growth stages [Table-1 and 2].

Table-1 *Volatile terpenoid constituents (%)of naturally grown and tissue culture regenerated plants of Santolina chamaecyparissus*

S. No.	Constituents	A	B	C
1.	Essential oil content*	0.1	0.1	0.32
2.	Monoterpene hydrocarbon	32.0	19.0	43.0
3.	Oxygenated monoterpene	20.0	23.0	31.1
4.	Sesquiterpene hydrocarbon	4.0	1.0	7.4
5.	Oxygenated sesquiterpene	9.0	18.0	4.0

* =ml/100g

A=naturally cultivated plants [Garg, et. al. 2001]; B=plants cultivated at Jammu site (subtropical); C=tissue culture regenerated plants cultivated at Jammu site (Subtropical conditions).

Table-2 *Volatile terpenoids spectrum of essential oil of in vitro regenerated plantlets and naturally grown plants of S. chamaecyparissus*

S.No.	Constituents	Naturally field grown plants	*In vitro* regenerated plantlets
1.	Artemisia ketone	+	-
2.	Artemisia alcohol	+	-
3.	Yomogi alcohol	+	-
4.	Santolinat riene	+	+
5.	Myrcene	+	+
6.	β-Pinene	+	+
7.	Ar-curcumene	+	-
8.	β-Bisabolene	+	-
9.	α-Humulene	+	-
10.	i- Thujone	+	-
11.	p-Cymene	+	+
12.	β-Elemene	+	+
13.	χ-Cadinene	+	-
14.	α-Cubebene	+	-
15.	α-Copaene	+	-
16.	δ-Cadinene	+	-
17.	α-Ylangene	+	-
18.	Spathulenol	+	-
19.	Vulgarone	+	-
20.	t-Cadinol	+	-
21.	Allo-aromadendrene	+	-
22.	α-Murolene	+	-
23.	Elemol	+	-
24.	Cubenol	+	-
25.	Ledol	+	-
26.	β-Phellandrene	+	-
27.	trans-Chrysanthemyl alcohol	+	-
28.	Lavandulol	+	-
29.	α-Himachalene	-	-
30.	β-Himachalene	-	-
31.	Himachalol	-	-
32.	Ar-Himachalene	-	-
33.	α-Thujene	+	+
34.	α-Pinene	+	+
35.	3-Carene	+	+
36.	Limonene	+	+
37.	1,8-Cineole	+	+
38.	Cis-β-Ocimene	+	+
39.	Trans-β-Ocimene	+	+
40.	α-Terpinene	+	+
41.	Cryptone	+	+
42.	Linalool	+	+
43.	Terpinenol-4	+	+
44.	α-Terpineol	+	+
45.	Borneol	+	+
46.	β-Caryophyllene	+	+
47.	Humulene	+	+
48.	Gurjunene	+	+
49.	Globulol	+	+
50.	Oplopenone	+	+
51.	β-Caryophyllene oxide	+	+

+ compound present; - compound absent

Results and Discussion

Green shining callus was initiated from cultured shoot tips using MS medium supplemented with 0.5 mg.l-1l AA. The one-month-old stock callus conditioned under white fluorescent light showing sign of green color and shoot primordial was

selected and transferred to bud induction medium, containing 0.2mg. l^{-1} IAA for four weeks. The shoot buds thus differentiated were promoted into shoots on transfer to MS medium containing 1.0 mg.l^{-1} BAP under incubation of light. After three weeks about 4—5 cm elongated shoots were isolated and placed on root initiation medium containing 1.0 mg.l^{-1}l AA or IBA. After a total of four weeks sufficient roots had formed and plantlet ready to be transplanted. Before transplantation to a sterile mixture of 1:1 Sand-Soil, agar medium was carefully removed from the roots. The plantlets were acclimatized under very high humidity in Mist House and subsequently under Green House conditions. Well-hardened plants were finally transferred to field. The plants have reached to the height of 15-18 cm after three months of transfer [Plate-2].

Plate-1 Various stages for micro propagation of cotton Lavender (*Santolina chamaecyparissus L*) plants

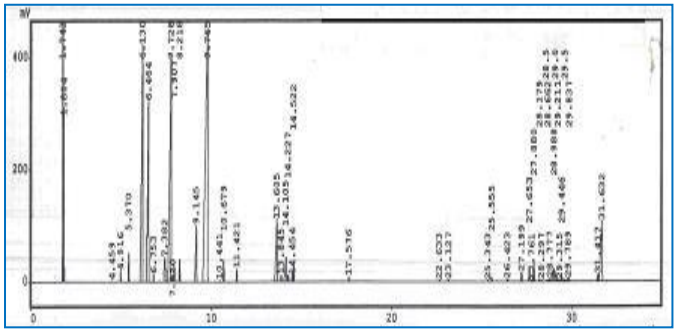

Plate-2 GC chromatograms of hexane and methanol extracts on Capillary column (30m x 0.30mm) coated with SE-30. The peaks were identified on the basis of Retention Time

The essential oil isolated from foliage of proliferating shoots and plantlets regenerated *in vitro* was a complex mixture of 49 components, 25 of which were identified corresponding to 80% of the total oil. The various constituents in total essential oil pool with their percentages are listed in [Table-1 and 2]. The detected constituents included mono-terpene hydrocarbon, oxygenated mono-terpenes, sesqui-terpenes hydrocarbon and oxygenated sesqui-terpenes The major identified peaks were myrcene, limonene, cis β-ocimene and linalool [Plate-2]. However, the other constituents identified *viz.* artemisia ketone, santolinatriene, myrcene, α-pinene, p-cymene, β- elemene, α-thujene, pinene, 3-carene, limonene, 1,8-cinole, cis and trans-ocimene, terpinene, cryptone, linalool, 4-terpinenol, α-terpineol, borneol, β-cryophyllene, humulene, gurjunene, globulol, oplopenone and β-caryophyllene oxide was detected in proliferating shoots and in *vitro* regenerated plants. These constituents have also been reported earlier from naturally grown plants of *S. chamaecyparissus* [2]. The absence of these constituents in callus cultures indicate that required degree of differentiation stage is required to initiate synthesis and subsequent accumulation of these constituents, which suggested biogenesis, is under the regulation of morphogenic event.

Investigations leading to organogenic response and potential to synthesize volatile terpenoids from multiple shoots and regenerated plants have been reported in number of aromatic species *viz. Lavandula angustifolia* [5], *Rosmarinus officinalis* [6] and *Mentha arvens* [7]. The degree of differentiation and state of morphogenesis is key factor influencing volatile terpenoids constituents. Thus, micro-propagated plants provide a good alternative approach to quick multiplication as uniform source for production of essential oils. Higher product yield, stability with regard to production and growth offers tissue culture raised plants attractive approach for production of volatile compounds [8-11]. Beside, providing genetically homogenous and stable material, i) they may be propagated at any time in large quantities and ii) *in-vitro* biomass is good system for feeding experiments with possible precursors of biosynthesis of oils under controlled conditions for getting enhanced product yield.

The present study extend possibility for *in-vitro* regeneration *via* axillary shoot proliferation leading to plant regeneration and production of essential oils from *Santolina chamaecyparissus.* Comparative account of naturally grown plants and *in-vitro* regenerated plants provide good alterative of regenerated micro-propagated plants as resource for production of essential oil. Micro-propagated material ensures uniformity in terms of biochemical constituents. As such the presently described approach offers a meaningful alternative procedure for commercial exploitation, alternatively to raise biomass if *S. chamaecyparissus* shoot cultures are cultivated in bioreactors. The possibility to cultivate plantlets using liquid submerged system has been demonstrated in recent years for number of medicinal and aromatic plants [8-11]. The same strategy could be applied for commercial exploitation using present standardized procedure for *S. chamaecyparissus,* which is an commercially important aromatic plant.

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Abbreviations: BA: 6-Benzyladenine, IAA: Indole-3-acetic acid, IBA: Indole-3-butyric acid, KN: Kinetin, MS: Murashige and Skoog medium, NAA: Naphthalene acetic acid, GC-MS: Liquid chromatography–mass spectrometry and TLC: Thin layer chromatography

Conflict of Interest: None declared

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