



***In vitro* Propagation and GC-MS Studies of *Ocimum basilicum* Linn. var. *pilosum* (Willd.) Benth**

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Authors' contributions

This work was carried out in collaboration by five authors. Author PRA suggested the study and extended overall guidance during the experimentation and made the final draft of the manuscript. Author GVG performed the experimental work, analyzed data and wrote the first draft of the manuscript. Author SRR designed GC-MS studies. Author VRT designed in vitro propagation protocol and author SR managed the literature searches and performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

An *in vitro* propagation method is outlined for *Ocimum basilicum* Linn. var. *pilosum* (Willd.) Benth., a wild aromatic plant belongs to Lamiaceae family. Shoot buds were used as source of explants on MS media supplemented with different concentrations of growth regulators for callus growth, induction of multiple shoots and roots respectively. MS media with 1.5 mg/L of kinetin and 0.5 mg/L of NAA showed 95.5% shooting, maximum number of shoots (7.33) and relatively better shoot lengths (4.15 cm). Excised shoots were carefully transferred to half-strength MS medium supplemented with 1.0 mg/L indole-3-butyric acid (IBA) for root induction and it yields 86.6% rooting. Whereas, average root length and number of roots observed were 1.73 cm and 3.31 respectively per explants. Rooted plantlets were hardened and successfully established in natural soil, where they grew and matured normally. GC-MS studies in methanolic leaf extract of naturally grown *Ocimum* species yielded 15 compounds.

Two compounds viz. cis-9-Hexadecenal (35.06%) and n-Hexa decanoic acid (21.6%) accounted for the major share (56.66%). On the other hand, 2-hydroxy-6-methylbenzaldehyde (10.99%) as well as 4H-1-Benzopyran-4-one and 5-Hydroxy-6, 7-Dimethoxy-2-(4-Methoxyphenyl) (7.75%) represented only 18.74%. Establishment of reliable *in vitro* propagation protocol, with phytochemical profile of hitherto unreported *Ocimum* species further widens the scope to evaluate its therapeutic properties.

Keywords: *Ocimum basilicum* Linn. var. *pilosum* (Willd.)-Benth.; Nodal segments; *In vitro* propagation; GC-MS analysis; 2, 4- Dichlorophenoxy acetic acid.

1. INTRODUCTION

Plants constitute the major source of raw materials for preparation of drugs. Due to wide spread toxicity and harmful side effects often caused by synthetic drugs and antibiotics, modern society increasingly prefers drugs of natural origin. Thus, about 75% of the world population still depends on medicinal plants for meeting primary health care needs [1]. The success of any healthcare programme depends on the accessibility of appropriate drugs on a sustainable basis.

The genus *Ocimum* contains about 150 species and almost all are aromatic in nature with a wide range of essential oils and many of which are extensively used in traditional medicine [2] and exhibit phytotherapeutic properties [3], antimicrobial [4], antifungal [5], as well as antioxidant and insect repellent activities [6, 7]. Various parts of *Ocimum* species are used in folk medicine for treatment of a wide range of health conditions [8]. Leaves are mainly used for the treatment of cough, bronchitis, skin disease, measles, abdominal pains and diarrhea [9]. Rats exposed to electromagnetic field of sweet basil showed decrease in immobility score and increase in forced swimming [10].

Species of *Ocimum* are usually propagated by seeds although, some exhibit poor viability and low germination. Moreover, seed-derived progenies are not true to type, due to cross-pollination. In addition, genetic diversity of these plants is threatened with extinction due to over exploitation, environment-unfriendly harvesting techniques, loss of growth habitats, uncontrolled trade and indiscriminate collection of economically important species by traditional ayurvedic practitioners. Furthermore, propagation of such plants by conventional techniques like rooting of cuttings and grafting is highly inadequate to meet the ever-growing demand. In view of this, it is important to develop suitable techniques for rapid mass propagation to meet the commercial need and also for prevention of genetic erosion. Plant tissue culture techniques offer one of the best options for conservation of rare, threatened or endangered medicinal plants.

Although reports on *in vitro* propagation studies in several *Ocimum* species are available, development of efficient micro propagation protocols for many *Ocimum* species is still in progress [11]. Rapid micro propagation protocols in young leaves, node, axillary shoot, shoot tip and inflorescence explants have been reported in *O. basilicum* [12,13], *O. sanctum* [14], *O. Kilimandscharicum* [15], *O. gratissimum* [16], *O. americanum*, *O. americanum* and *O.canum* [17]. In *Ocimum basilicum*, direct somatic embryogenesis from explants of leaf callus has also been reported [18].

The chemical composition and essential oil distribution in a vast number of *Ocimum* species have been studied using different extraction methods including Gas chromatography-Mass Spectrum [19,20], dry method and extraction pressure [21], steam and microwave distillation [22] and Super critical CO₂ extraction [23] in different plant parts. In another study, the influence of polyamines on essential oil composition is also reported in *Ocimum basilicum* [24]. Although extensive research has been conducted in several species in the genus *Ocimum*, *in vitro* propagation studies in *Ocimum basilicum* Linn. var. *pilosum* (Willd.) Benth. has not been studied due to its restricted availability. The present study made an attempt to corroborate the phytochemical composition of *Ocimum basilicum* Linn.var. *pilosum* species and also establish a reliable and efficient *in vitro* propagation protocol using nodal explants for large-scale production and germplasm conservation of this plant.

2. MATERIALS AND METHODS

2.1 Plant Materials

Healthy plants were collected from natural habitat of Kondapalli reserve forest (fly ash dumping site) which lies between 16°37' N latitude and 80°32' E longitude at a height of 168 meters above mean sea level, Vijayawada of Krishna district, Andhra Pradesh, India. Plants were successfully planted in the University botanical garden for further use. Identification of plant was done at Botanical Survey of India, Southern Regional Centre, Coimbatore and the voucher specimen is preserved in the Department of Biotechnology, KL University.

Shoot tips (nodal segments of 0.8-1.0 cm with dormant auxiliary buds of about 0.6 cm) were excised from plants and used for *in vitro* propagation studies. Selected nodal segments were immediately washed in tap water followed by washing with 10% teepol (10 min) and 1 % (w/v) Bavastin for 5 minutes. The explants were then sterilised for 20 seconds with 70% ethanol, followed by 0.1% (w/v) HgCl₂ for 2-3 minutes and rinsed 5-7 times in sterile distilled water in laminar air flow chamber. Thereafter, explants were trimmed at the cut ends and inoculated.

2.2 Culture Medium and Conditions

Shoot tips were cultured on modified MS basal medium [25] containing 3% (w/v) sucrose for callus initiation. The pH of the medium was adjusted to 5.7 with 1 N NaOH or 1 N HCl before gelling with 0.8% (w/v) agar. Vertically implanted explants in test tubes were maintained at 25 ± 2 °C temperature, continuous light with a 16 hours photoperiod at 50 μmol m⁻² s⁻¹ irradiance by cool white fluorescent tubes with 60-70% relative humidity.

2.2.1 Callus induction

Two week old sub cultured shoot buds were inoculated on modified MS medium supplemented with 2, 4 - D (0.1mg/L) and different concentrations of Kinetin (0.25–1mg/L) for optimization of callus.

2.2.2 Shoot bud initiation

Nodal segments of *O. basilicum* were inoculated on MS media supplemented with different concentrations of Indole-3-acetic acid, (IAA 0.1- 3.0 mg/L), Naphthalene acetic acid, (NAA

0.1- 3.0 mg/L and Kinetin (Kn 0.1-3.0 mg/L) either independently or in combination with other growth hormones.

2.2.3 Multiple shoot initiation

Fully matured shoots of around 3 - 4 cm in height were cut at their nodal segments and transferred to MS full strength medium (supplemented with Kn 1.0+NAA 0.5).

2.2.4 In Vitro rooting

For root induction, excised micro shoots with 3 - 4 fully expanded leaves from *in vitro* grown plants were transferred to half strength basal MS medium supplemented with different concentrations of IBA. The rooting results were taken 15 days after inoculation.

2.3 Acclimatization of Regenerated Plants

Fully rooted plantlets of nearly 5 to 6 cm in length were removed from the culture medium and washed under running sterile water to remove agar. The plantlets were transferred to poly trays containing sand, vermiculite and soil (1:1:2) and covered with transparent plastic bags to prevent loss of humidity. The set-up was maintained at $26 \pm 1^\circ\text{C}$, 80 – 85% relative humidity and at a light intensity of $50 \mu\text{mol m}^{-2} \text{s}^{-2}$ under a 16 h photoperiod in culture room conditions and acclimatized for a period of 3 weeks. After primary hardening the plantlets were transferred to a greenhouse with simulated habitat for improved survival.

2.4 Statistical Analysis

The experiments were performed using completely randomized design and replicated three times. 12 to 15 explants per replicate were used in each treatment. Data were analyzed by one way ANOVA and the mean values for treatments were compared by using Turkey's HSD test at $p \geq 0.05$ with SPSS ver.13.0. The results are expressed as means \pm SE of three experiments.

2.5 Isolation of Phytochemical Compounds

Fresh and fully expanded field grown *Ocimum* leaves from six week old plants were shade dried at room temperature, blended and made in to fine powder. 100 gm of leaf powder was mixed with 1000 ml (1:10) of methanol in a Schott Duran bottle and kept air tight for 48 hours on magnetic stirrer with continuous stirring for proper mixing of powdered samples. The solutions were distilled and the extracts were used for GC - MS studies.

2.6 Gas Chromatography - Mass Spectrometry Analysis

Phytochemicals were analyzed by GC-MS (SHIMADZU QP 2010) employing the electron impact (EI) mode at an ionizing potential of 70 eV with a $30 \text{ m} \times 0.32 \text{ mm}$, film thickness and $1.8 \mu\text{m}$ capillary column (Resteck-624 MS) packed with 5% phenyl dimethyl silicone at an ion source temperature of 200°C . For further analysis, GC/MS settings were as follows: the initial column temperature was set at 45°C and held for 4 min; the temperature was raised to 50°C and then increased up to 175°C at a rate of $10^\circ\text{C} / \text{min}$ for 2 minutes, and then finally programmed to 240°C at a rate of $25^\circ\text{C} / \text{min}$, and kept isothermal for 2 minutes. Helium was

used as carrier gas with a flow rate of 1.491 ml / min with a split ratio of 1:10. During sample analysis the column oven temperature was maintained at 280°C [19].

2.7 Identification of Compounds

The fraction composition of the sample was computed from the GC peak areas and compared with the spectra of compounds stored in the spectral database, NIST08s, WILEY8, and FAME libraries.

3. RESULTS AND DISCUSSION

3.1 Culture Response to Growth Regulators: Multiple Shooting

Ocimum basilicum Linn. var. *pilosum* (Willd.) Benth., plants were efficiently regenerated from nodal explants from field-grown young plants (Fig.1A), on MS medium supplemented by 0.5-3 mg/L kinetin with 0.5- 2.5 mg/L NAA or 0.5-3 mg/L of IAA for multiple shoot induction. The multiple shoot induction response with respect to the test concentrations of growth hormones is presented in Table 1. Of the two combinations (Kinetin with IAA / NAA) tested in the present study, Kinetin with NAA was found to exhibit highest shooting rate and better shoot length per explants (Fig. 1B). MS media supplemented with Kn 0.5 + IAA 1.5 mg/L and Kn 0.5 mg/L in combination with 1.5 mg/L of NAA gave 84.4% and 86.7% shoots respectively. On the other hand, MS media supplemented with IAA (Kn1.5 + IAA 0.5 mg/L) showed 71% shooting with less number of shoots (2.24) and shorter shoot lengths (1.97cm) per explants. MS media supplemented with Kn 0.5 and 1.5 mg/L of NAA generated 5.96 shoots per explants whereas multiple shoot production on MS media supplemented with 0.5 mg/L of kinetin with 1.5 mg/L of IAA showed 3.58 shoots. Average number of shoots on MS medium supplemented with NAA was found to be significantly higher ($P < 0.5$) when compared to the MS media supplemented with IAA. On the other hand, shoot lengths appeared to be almost similar in the two combinations of growth hormones tested in the present study. Overall, MS media supplemented with 1 mg/L of kinetin with 0.5 mg/L of NAA showed highest multiple shoots (95.5%), maximum number of shoots (7.33) and even relatively longer shoots (4.81 cm) per explants (four weeks after ideal cultural conditions). This response was significantly better ($P < 0.05$) than that from other combinations tested in the present investigation. From the results, it is evident that the higher proportion of kinetin with half the concentration of NAA holds good for attaining rapid multiple shoots under *in vitro* conditions. Kinetin with different root induction growth hormones (auxin types and concentrations) greatly influences auxillary shoot regeneration from nodal explants. Medium without growth regulator (control) gave no regeneration response and explants swelled and became necrotic two weeks after inoculation. Multiple shoot production, number of shoots and length of the multiple shoots per explants significantly reduced in all combinations of growth hormones tested either above or below optimized concentrations. Growth response (reduction in number of shoots per each node) at higher or lower than optimal concentration of cytokine has also been reported in several medicinal plants [26-28]. Other cytokinins like Benzyl adenine have been reported to overcome apical dominance, release of lateral buds from dormancy and promote shoot formation in dicot plants [29]. Effect of BA on multiple shoot formation of *in vitro* propagated *O. basilicum* has also been reported [11,12]

Table 1. Effect of different concentrations of Kinetin (Kn) in combination with Indole Acetic Acid (IAA) and Naphthalene acetic acid (NAA) for multiple shoot induction from nodal explants of *Ocimum basilicum* Linn. var. *pilosum* (Willd.) Benth.

S. No	Growth Regulators (mg/L)	Shooting Percentage	Number of Shoots / explant	Shoot length / explant (cm)
0	Control (basal medium)	0.00	0.00	0.00
1	MS + Kn 0.5 + IAA 0.5	57.7	1.20 ^b ± 0.1815	1.17 ^b ± 0.1978
2	MS + Kn 1.0 + IAA 0.5	64.4	1.36 ^b ± 0.1963	1.63 ^a ± 0.1975
3	MS + Kn 1.5 + IAA 0.5	71.1	2.24 ^a ± 0.2424	1.97 ^a ± 0.2068
4	MS + Kn 2.0 + IAA 0.5	66.6	1.98 ^a ± 0.2389	1.81 ^a ± 0.2037
5	MS + Kn 3.0 + IAA 0.5	60.0	1.60 ^b ± 0.2118	1.38 ^b ± 0.1764
6	MS + Kn 0.5 + IAA 1.0	63.3	2.60 ^b ± 0.2551	2.26 ^b ± 0.2191
7	MS + Kn 0.5 + IAA 1.5	84.4	3.58 ^a ± 0.2431	3.47 ^a ± 0.2305
8	MS + Kn 0.5 + IAA 2.0	77.7	3.29 ^a ± 0.2746	2.72 ^b ± 0.2124
9	MS + Kn 0.5 + IAA 3.0	68.8	2.71 ^b ± 0.2836	1.89 ^c ± 0.2077
10	MS + Kn 0.5 + NAA 0.5	82.2	3.78 ^c ± 0.2996	3.11 ^b ± 0.2435
11	MS + Kn 1.0 + NAA 0.5	95.5	7.33 ^a ± 0.2947	4.81 ^a ± 0.1736
12	MS + Kn 1.5 + NAA 0.5	88.8	5.58 ^b ± 0.3474	4.34 ^a ± 0.2456
13	MS + Kn 2.0 + NAA 0.5	75.5	2.76 ^c ± 0.2546	2.51 ± 0.2239
14	MS + Kn 3.0 + NAA 0.5	64.4	2.22 ^c ± 0.2713	1.67 ^c ± 0.1978 ^d
15	MS + Kn 0.5 + NAA 1.0	80.0	4.89 ^a ± 0.3882	2.97 ^b ± 0.2375
16	MS + Kn 0.5 + NAA 1.5	86.7	5.96 ^a ± 0.3651	3.96 ^a ± 0.2372
17	MS + Kn 0.5 + NAA 2.0	75.6	3.53 ^b ± 0.3476	2.42 ^b ± 0.2219
18	MS + Kn 0.5 + NAA 3.0	66.7	2.44 ^c ± 0.2905	1.89 ^c ± 0.2131

Values are means ± SE. (n = 15). Means followed by different letters in the same column found significant at 5% level, Means followed by same letters do not differ significant at 5% level. (Turkey's HSD test).

3.2 Rooting

It is a well established concept that *in vitro* root induction in many plant species at slightly higher proportions of auxin to cytokine ratio in MS media yields better results. Per cent root induction, number of roots and root lengths obtained in this study are shown in Table 2. Generally, MS media supplemented with 1 mg/L of IBA were found to be remarkable among all root induction hormones tested in the present study. The best response with optimum rooting (86.6%) was observed in MS media containing 1 mg/L of IBA which also gave the highest number of roots (3.31) per explants and an average of 1.73 cm of root length (Fig. 1C). On the other hand relatively poor rooting response was observed on MS media supplemented with 0.5 to 2 mg/L of NAA and / or IAA. Other studies reported that optimum rooting in shoots of *O. basilicum* was achieved on half strength MS medium supplemented with 1.0 mg/1 NAA and thus support the theoretical concept of root induction in media supplemented with either NAA or IBA. Turkey's HSD test confirms $P \leq 0.05$ significance among the three concentrations (0.5, 1 and 2 mg/L IBA) tested in the present investigation.

Table 2. Effect of different concentrations of Naphthalene Acetic Acid (NAA), Indole butyric Acid (IBA) and Indole Acetic acid (IAA) on root induction from nodal explants of *Ocimum basilicum* Linn. var. *pilosum* (Willd.) Benth

S. No	Growth Regulators (mg/L)	Rooting Percentage	Number of Roots / explant	Root length / explant (cm)
1	MS+ NAA 0.5	17.7	0.53 ^c ± 0.1786	0.36 ^b ± 0.1174
2	MS + NAA 1.0	37.7	1.16 ^b ± 0.2334	0.72 ^a ± 0.1469
3	MS + NAA 0.5	53.3	1.73 ^a ± 0.2589	0.97 ^a ± 0.1494
4	MS + NAA 2.0	44.4	1.38 ^a ± 0.2447	0.82 ^a ± 0.1486
5	MS + IBA 0.5	77.7	2.67 ^b ± 0.2441	1.46 ^{ab} ± 0.1347
6	MS + IBA 1.0	86.6	3.31 ^a ± 0.2221	1.73 ^a ± 0.1267
7	MS + IBA 1.5	73.3	2.40 ^b ± 0.2344	1.23 ^b ± 0.1267
8	MS + IBA 2.0	66.6	1.87 ^c ± 0.2146	1.10 ^{bc} ± 0.1287
9	MS + IAA 0.5	26.6	0.87 ^a ± 0.2216	0.49 ^b ± 0.1266
10	MS + IAA 1.0	33.3	0.98 ^a ± 0.2144	0.62 ^{ab} ± 0.1396
11	MS + IAA 1.5	42.2	1.24 ^a ± 0.2317	0.76 ^a ± 0.1436
12	MS + IAA 2.0	35.5	1.09 ^a ± 0.2309	0.69 ^{ab} ± 0.1459

Values are means ± SE. (n = 15). Means followed by same letters in the same column do not differ significantly at 5% level (Turkey's HSD test).

3.3 Callus Induction

Dedifferentiated nodal explants were transferred on to the callus induction medium supplemented with 0.1 mg/L of 2, 4-D and different concentrations (0.25 – 1 mg/L) of kinetin (Table 3). Growth of callus was measured in four week old cultures and expressed as dry biomass. Overall, highest callus dry biomass (64 mg) was recorded in MS medium supplemented with 0.1 mg/L of 2, 4-D and 0.5 mg/L of Kinetin (Fig. 1E). Other combinations tested in the present study were also found to be promising in callus growth. Generally, callus induction studies in many *in vitro* propagation protocols reveal that equal proportions of growth hormones in culture media play a crucial role in optimal callus induction. Contrary to this trend, in the present study it was found that at a relatively higher proportion of Cytokine to auxin, optimum callus biomass prevailed. Surprisingly, approximately equal proportions of auxin to cytokinin induce extensive rooting rather than callus formation in the present study. The altered rooting response in the highly aromatic *Ocimum* species is attributed to secondary metabolite production especially formation of tryptophan, the precursor for auxin synthesis (Data Unpublished).

Table 3. Effect of different concentrations of Kinetin on callus dry biomass from nodal explants of *Ocimum basilicum* Linn. var. *pilosum* (Willd.) Benth

S. No	Growth Medium	Concentration of growth regulators(mg/L)	Dry biomass of Callus (mg)
1	MS Basal medium	0.00	0.00 ^{NA}
2	MS Basal medium	2,4-D (0.1), Kinetin (0.25)	54.3 (±0.5786)
3	MS Basal medium	2,4-D (0.1), Kinetin (0.50)	64.0 (±0.3527)
4	MS Basal medium	2,4-D (0.1), Kinetin (0.75)	42.0 (±0.6794)
5	MS Basal medium	2,4-D (0.1), Kinetin (1.00)	33.9 (±0.4991)

^{NA} Without growth regulators; ^{NA} Callus growth not observed

Values are means of 10 replicated calli grown in three different culture bottles. ± SE

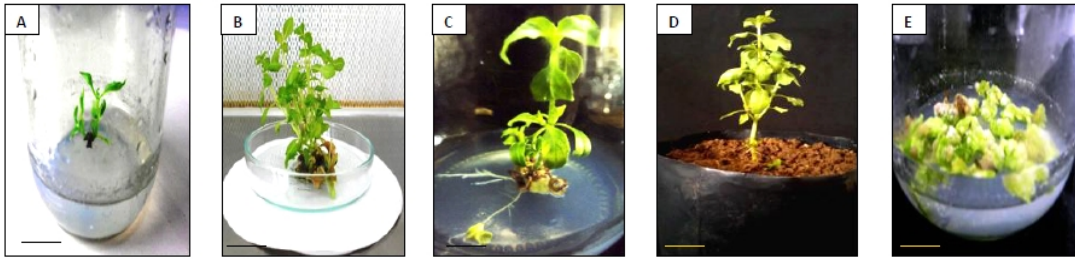


Fig. 1. *In vitro* clonal propagation of *Ocimum basilicum* Linn. var. *pilosum* (Willd.) Benth. – developmental stages (A-E).

A) Single nodal explants with induced growth of nodal buds on MS medium supplemented with 1 mg / L of kinetin (Kn) and 0.5 mg / L of Naphthalene acetic acid (NAA)-one week after inoculation. Bar = 0.5 cm. B) Shoot multiplication on MS medium supplemented with Kinetin 1.0 + NAA 0.5 mg / L 4 weeks of culture, Bar = 0.8 cm. C) Regenerated shoots with well developed adventitious roots cultured on ½ MS medium supplemented with 1 mg / L of BA, Bar = 0.7cm. D) Well developed hardened plant, Bar = 4 cm.) E) Proliferation of callus from nodal segments on MS medium supplemented with 0.1 mg / L of 2,4-D with 0.5 mg/L of Kinetin, Bar = 0.5 cm.

3.4 Chemical Composition of the Essential Oils

The essential oils in shade dried leaves of *Ocimum basilicum* Linn. var. *pilosum* (Willd.) Benth. extracted in methanol were analyzed by GC-MS. The qualitative and quantitative parameters for all the 15 compounds along with per cent distribution and molecular structures are shown in Fig. 2. The distribution of phyto constituents in this species differs from what was obtained in other *Ocimum* species reported so far. Major constituents the essential oil of *O. basilicum* Linn. var. *pilosum* (Willd.) Benth. were cis-9 -Hexadecenal (35.06%) and n - Hexadecanoic acid (21.6%). Others were, 2 - hydroxyl - 6-methylbenzaldehyde (10.99%), 4H-1-Benzopyran-4-one, 5-Hydroxy-6, 7-Dimethoxy-2-(4-Methoxyphenyl) (7.75%), Phytol (4.37%), Cycloisolongifolene,7-bromo (3.31%), Neophytadiene (2.75%), Benzoic acid (2.62%), Olealdehyde (2.4%), 1,2,3 - Propanetriol and Mono acetate(2.16%). Geranic acid, p -Methylbenzoic acid, All-trans-Squalene, 1, 2 - Benzenediol and Propylure were also present in relatively low proportions. The essential oil composition and antimicrobial activity of different *Ocimum* species have been investigated in sufficiently large number of species globally [12,19,30]. Although, essential oil composition in *O. basilicum* Linn. var. *pilosum* (Willd.) Benth. has been previously reported [31], the chemical constituents in the naturally available aromatic *Ocimum* species grown in fly ash dumping location typically exhibit novel oil composition and the results contradict the earlier reports in this species. Same species grown in different climatic conditions might alter the basic mechanism for production of essential oils in order to survive in the adverse environment and thus could play a significant role in changing the chemical composition of the species. Impact of climatic conditions on altered phytochemical constituents and its adaptability to the changing environment suggests that *Ocimum basilicum* Linn. var. *pilosum* (Willd.) Benth. could be a good source for development of new and improved traits, which in turn could be exploited in the fragrance industry.

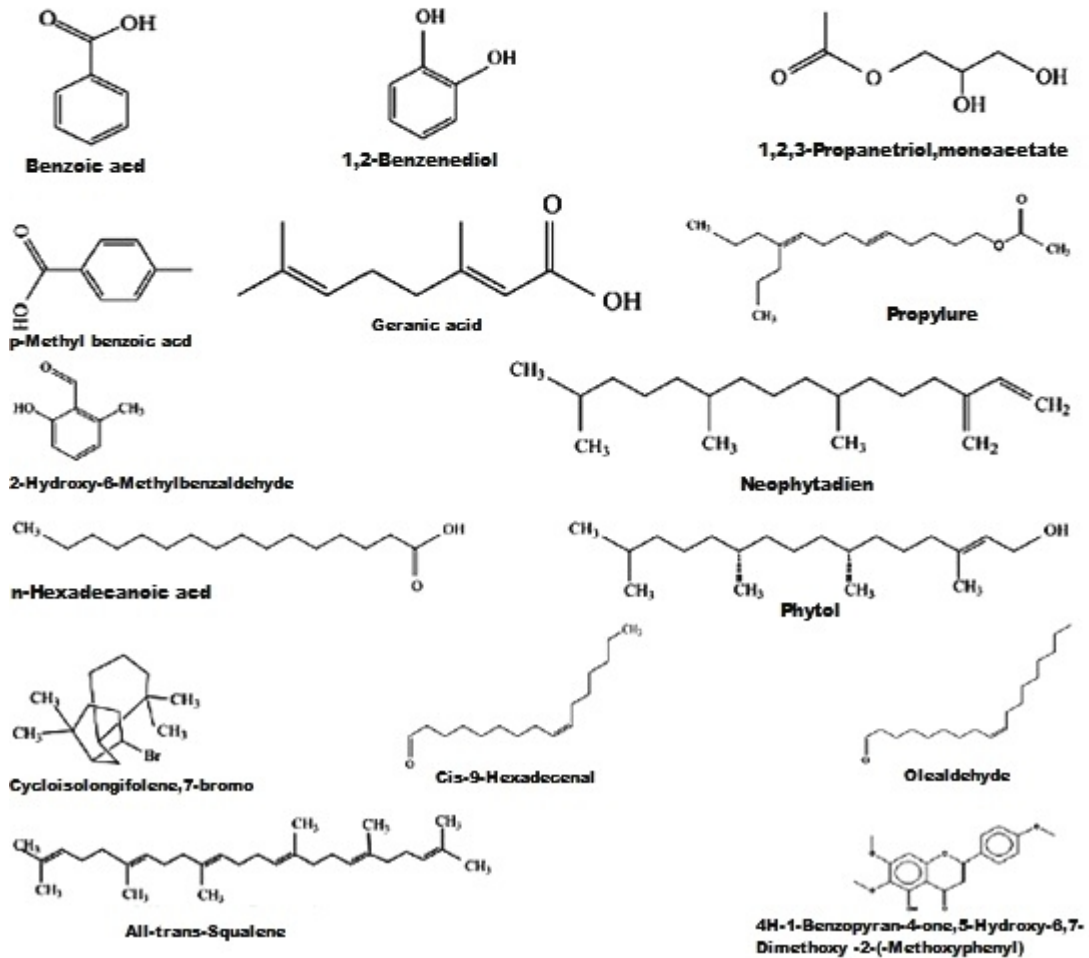
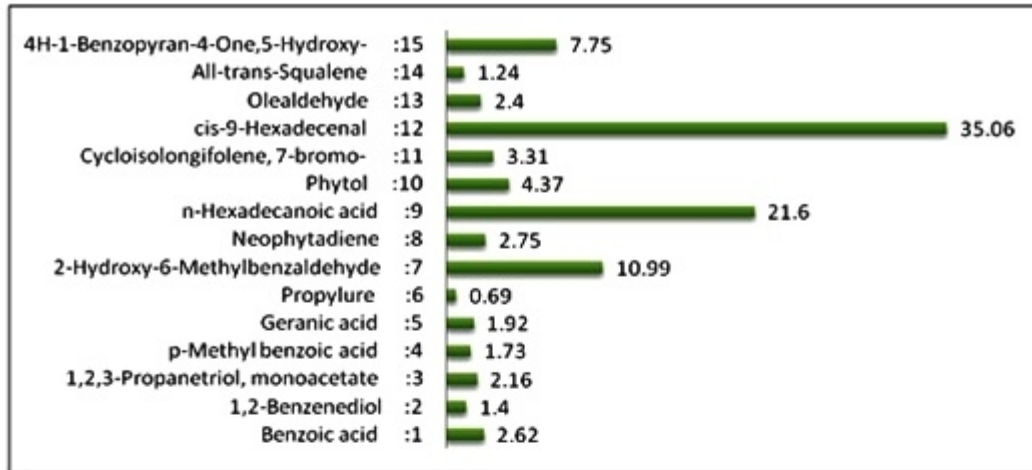


Fig.2. Per cent distribution and structures of phytochemicals in *Ocimum basilicum* Linn. var. *pilosum* (Willd.) Benth.

4. CONCLUSION

In the present study, a competent and reliable micropropagation protocol for *in vitro* regeneration of *Ocimum basilicum* Linn. var. *pilosum* (Willd.) Benth, from nodal ex-plant has been established. This ensures large scale supply of the targeted plants through micro propagation technique, which is important for sustainability and conservation of germplasm. Further, the study also demonstrated that areal parts of *O. basilicum* Linn. var. *pilosum* (Willd.)-Benth. possess a wide range of phytochemicals capable of generating aromatic compounds of commercial importance which can meet the needs of flavoring industries.

COMPETING INTERESTS

Authors declare that there are no competing interests between individuals that can affect the publication of this work.

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