In Vitro Culture-An Alternative Method for The Enhanced Production of Some Volatile Components of Ocimum citriodorum Vis.

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Abstract

Ocimum plants are highly valued for their effective antibacterial, antifungal, antioxidant and therapeutic activities. These properties are attributed to some active metabolites. In order to enhance and develop an alternative source for the production of such active components, a comparative study was carried out. Composition of volatile organic compounds emitted by in vitro shoots, calli derived from leaf and nodal explant of Ocimum citriodorum Vis. were studied using Gas Chromatography-Mass Spectroscopy (GC-MS) and compared to those emitted by mother plants. Solvent extraction method was used for obtaining essential oils from the samples. Total 18 volatile compounds from the leaf extract of mother plant, 14 volatile compounds from the leaf extract of in vitro regenerated plants, 5 volatile compounds from the calli derived from leaf and 10 volatile compounds from the calli derived from nodal explant were identified.The content of nerol (2.44%) and geranylacetate (1.53%) was greater in in vitro regenerated plantlets than in comparison to mother plant (nerol- 0.88% and geranylacetate- 0.32%). Seven new compounds (isocaryophyllene, β-farnesene, β-himachalene, globulol, geranylformate, β-citronellylacetate, citronellylisobutyrate) were identified from the in vitro regenerated plants while six and three new compounds were identified from the calli derived from nodal explant (geraniol, trans-β-bergamotene, β-longipinene, bergamotene, geranylformate and β-citronellyacetate) and leaf (β-curcumene, trans-β-bergamotene, β-citronellol) respectively. From the present study, it can be concluded that in vitro propagated plants as well as calli derived from leaf and nodal segment may be used as an alternate source for the increased production of bioactive compounds.

Keywords: Ocimum citriodorum Vis., GC-MS, Monoterpenes, Sesquiterpenes, Phenolic terpenes, Terpene esters

1. Introduction

Lemon basil (*O. citriodorum* Vis.) or maeng-lak in Thai, is rich in aromatic essential oils and valuable for its medicinal, volatile and culinary properties. It is considered to be a natural hybrid between basil (*Ocimum basilicum*) and African basil (*Ocimum americanum*) (Bodhipadma *et al.,* 2012; Janarthanam and Sumathi, 2012). It is one of the most economically important spices for its use in Arabian, Indonesian, Laotian, Persian and Thai cuisine (Janarthanam and Sumathi, 2012), effective essential oils against various pathogens, high antioxidant content and capability (Bodhipadma *et al.,* 2012). This herb is grown primarily in northeastern Africa and southern Asia, for its strong lemon fragrance, used in cooking and also in antioxidant tea bags (Janarthanam and Sumathi, 2012). Lemon basil grows upto a height of 20-40 cm. It has white flowers in late summer to early fall. The leaves are similar to basil but narrower. The fruits don't shatter; dried seeds can be collected directly from the plant.

Ocimum possesses essential oil, which contains biologically active constituents that are insecticidal, nematicidal, fungistatic, antimicrobial or antioxidant (Tripathi, 2011; Janarthanam and Sumathi, 2012; Tripathi *et al*., 2014). Greater escape response of two strains of *Aedes aegypti* mosquitoes, a vector of dengue, was observed from the essential oil of lemon basil (Sangvanph *et al.,* 2011). The plant extract is used in ayurvedic remedies for common colds, headaches, inflammation, stomach disorders, heart disease and malaria (Janarthanam and Sumathi, 2012). It is helpful against insects also (Kalkan, 2012). The essential oils of basil are used in the flavoring of food and in perfumery because of their aromatic properties (Prideevech *et al.,* 2010).

The volatile organic compounds (VOC) of *O. citriodorum* usually contain estragole as a major compound (Simon *et al.,* 1999; Liber *et al.,* 2011). Liber *et al.* (2011) grouped *Ocimum* plants into five chemotypes namely linalool/ eugenol chemotype, linalool chemotype, estragole/ linalool chemotype, (Z)-methylcinnamate chemotype and estragole chemotype. The composition of volatile

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organic compounds impacts the quality and bioactivity of aromatic herbs and essential oils (Zielinska *et al.*, 2011). Further, the therapeutic uses of essential oils have made the production and identification of its bioactive components essential. *In vitro* methodology provides a useful tool for investigating individual factors at work during volatile biosynthesis (Predieri and Rapparini 2007). However, the secondary metabolite profile of *in vitro* regenerated plants is usually altered in comparison to that of mother plants, although the detailed mechanism of this phenomenon is not fully understood (Morone-Fortunato and Avato, 2008; Nitnaware *et al.*, 2011; Sood and Chauhan 2010).

The aim of the present study was to identify the main volatile constituents of *in vitro* regenerated plants through GC-MS and compare with that of mother plant. The VOC profile of regenerants was compared with callus derived from leaf (lC) and nodal explant(nC) also. The influence of the source of explant (leaf and nodal explant for the present study) and organogenesis on the proportions of the volatile components was analyzed and discussed.

2. Materials and Method

2.1. Plant Material

Plants of *O. citriodorum* were cultivated in the Botanical Garden of University of Delhi, India. The VOC were identified from the leaves of the mother plants and *in vitro* regenerated plantlets as well as from the one month old calli derived from the leaf and nodal explants.

2.2. Establishment of Cultures

2.2.1. Plantlet Regeneration

In vitro plantlets were obtained from the nodal explant of *O. citriodorum.*The explants were inoculated on the MS basal medium (Murashige and Skoog, 1962) containing 3% (w/v) sucrose and 0.8% (w/v) agar supplemented with BAP (0.5 mg/l) for shoot regeneration. Multiple shoots were observed from the explants after 2 weeks of culture. These elongated micro shoots were separated and transferred to the ½ MS medium supplemented with NAA (1 mg/l) for rooting. Plantlets with four to six fully expanded leaves and well-developed roots were successfully acclimatized in the greenhouse, in pots containing sterile soil and humus (1:1) within 2 weeks and eventually established in the field with a survival frequency of 80%. The established plants were apparently uniform in morphology and did not show any detectable variation.

2.3. Callus Induction

In order to evaluate the influence of organogenesis on the essential oil producing capacity of explants, calli derived from leaf and nodal explants were used. Callus cultures were initiated on the MS medium containing sucrose (3%) as carbon source and agar (0.8%) as solidifying agent supplemented with NAA. It was observed that NAA at 1mg/l concentration proved to be the best for callus induction from the nodal explants while a higher concentration of NAA (2 mg/l) was essential in order to induce optimum calli from the leaf explants.

2.4. Extraction of Essential Oil

Essential oil was extracted from the leaves of mother plant (mP) and *in vitro* regenerated plants (ivP) as well as from the leaf (lC) and nodal explant (nC) derived callus, by solvent extraction method (Burbott and Loomis, 1967; Charles and Simon, 1990). Plant material was ground in a mortar containing hexane and anhydrous Na_2SO_4 and extracted four times with hexane to give a total volume of 10 ml of the extract. A small amount of activated charcoal was added to each extract and then removed by centrifugation at 5000 rpm for 10 min. The clear solutions were then concentrated under a stream of air at room temperature. Essential oil samples were stored in silica vials with teflon sealed caps at 4°C in the refrigerator until analyzed.

2.5. GC-MS of Essential Oil

The essential oil component identification was achieved by the GC-MS analysis carried out in CMS- OP2010 Plus GC-2010 (Shimadzu) mass selective detector coupled to an GC-2010 (Shimadzu) gas chromatograph. GC-MS was done using the following conditions: Column oven temperature 100°C; injection temperature 250 °C; splitless injection; linear velocity, pressure 95.1kPa, total flow 16.3 ml/min, column flow 1.21 ml/ min, linear velocity 40.9 cm/ sec, purge flow 3 ml/min, split ratio

10, oven temperature programmed from 100°C (3 min hold) to 200°C (2 min hold) and 280°C (17 min hold); ion source temperature 230 °C; interface temperature 270°C; start m/z 40; end m/z 650 and scan speed 1250 . The identification of chemical components was confirmed by comparison of retention times and with WILEY8.LIB (data base library).

3. Results

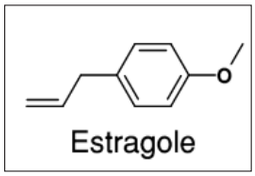
3.1. GC-MS Analysis

The GC-MS analysis of all the four samples (mP, ivP, lC and nC) ensured the presence of volatile contents with some other constituents (*viz.* alkanes and fatty acids). Linoleic acid is an essential fatty acid (EFA) which is not synthesized in human body because of the lack of appropriate enzymes (Zielinska *et al.,* 2014). Other polyunsaturated fatty acids can be synthesized only if EFA will be delivered with food and when no enzyme defect would occur in the metabolic pathway (Zielinska *et al.,* 2014). Its higher content was obtained from lC (44.3%) followed by ivP (8.53%), nC (2.43%) and mP (1.68%) (Table 1).

The oils were shown to be a complex mixture of several components, mainly monoterpenes and sesquiterpenes**.** Moreover, several phenolic terpenes and esters of terpenes were also identified. In mP, ivP, lC and nC the total volatile contents were 83.39%, 22.25%, 2.89% and 12.75% respectively (Table 1).Total 18 volatile compounds from the leaf extract of mother plant, 14 volatile compounds from the leaf extract of *in vitro* regenerated plants, 5 volatile compounds from calli derived from leaf and 10 volatile compounds from the calli derived from nodal explant were identified (Table 2). The volatile fractions of the samples (mP, ivP, lC and nC) were characterized mainly by oxygenated monoterpenes with hydrogenated sesquiterpenes. Terpene esters were also identified from all the four samples (Fig. 1). The GC-MS analysis showed that many volatile contents of mP were present in ivP also. However, mP showed the presence of a single hydrogenated monoterpene, pinene, which was not identified in the ivP. Similarly a phenolic terpene (eugenol) was obtained from the mP that was absent in the rest of the three samples (ivP, lC and nC).

The volatile constituents of mP were consisted of oxygenated monoterpenes like estragole (59.12%), linalool (1.86%), camphor (0.53%), nerol (0.88%), ocimenol (0.47%); hydrogenated sesquiterpenes like germacrene B (2.44%), β caryophyllene (1.22%), γ-murrolene (2.36%); oxygenated sesquiterpenes like cubenol (1.06%), geranylgeraniol (1.09%) (Table- 2). The volatile constituents of ivP were consisted of oxygenated monoterpenes like estragole (12.43%), nerol (2.44%); hydrogenated sesquiterpenes like germacerene B (0.35%), γ- murrolene (1.3%); oxygenated sesquiterpenes like geranylgeraniol (0.72%). The higher percentage of nerol (2.44%) and geranylacetate (1.53%) was obtained from the ivP in comparison to mP (Nerol- 0.88% and geranylacetate- 0.32%). Some new components were also identified from the ivP (isocaryophyllene-1.03%, β farnesene-0.20%, β- himachalene- 0.34%, globulol-0.37%, geranylformate- 0.57%, β citronellylacetate-0.28%, citronellylisobutyrate- 0.46%).

In lC total 5 volatile constituents were observed while from nC 10 volatile constituents were identified (Table 2). The main constituents of the extract of both the calli were sesquiterpenes. From lC and nC respectively 3 hydrogenated sesquiterpenes (β-curcumene- 0.18%, trans-α bergamotene- 0.33%, germacerene B- 0.50%) and 4 hydrogenated sesquiterpenes (germacerene B- 0.26%, trans-α bergamotene- 0.33%, β- longipinene- 0.46%, bergamotene- 0.25%) were identified. Six new volatile components were obtained from nC (geranylformate-0.43%, β- citronellyacetate- 0.31%, bergamotene- 0.25%, β- longipinene- 0.46%, trans-α-bergamotene- 0.33%, geraniol- 6%) while from lC only 3 new contents were identified (β citronellol- 0.99%, β curcumene- 0.18%, trans-α-bergamotene- 0.33%) (Table 2). These new compounds were absent in the mP. The content of terpene ester (geranylacetate- 2.83%, geranylformate- 0.43%, β- citronellylacetate- 0.31%) was higher in nC than in comparison to lC (geraniol acetate- 0.89%).



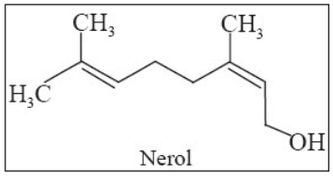


Table 1: Volatile and Other Contents of Extracts

Content	***O. citriodorum* (%)**			
	mP	*ivP*	*lC*	*nC*
Volatile content	83.39	22.25	2.89	12.75
Others	14.98	69.04	52.81	84.82

Content	*O. citriodorum* (%)			
	mP	*ivP*	*lC*	*nC*
Linoleic Acid	1.68	8.53	44.3	2.43
Total	100	100	100	100

Note: mP = mother plant, ivP = *in vitro* regenerated plant, lC = calli derived from leaf, nC = calli derived from nodal segment, PT = phenolic terpene, TE= terpene ester, SO = oxygenated sesquiterpene, SH = hydrogenated sesquiterpene, MO = oxygenated monoterpene, MH = Hydrogenated monoterpene

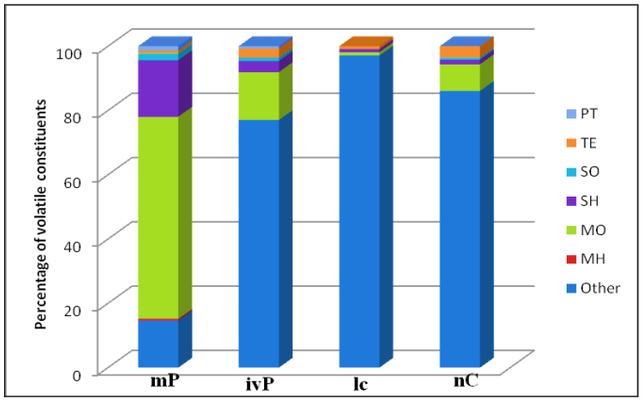


Fig. 1: Identification of Essential Oil (Terpene Composition) of Mother Plant (mP), *in Vitro* Regenerated Plants (ivP), Callus Derived from Leaf (lC) and Callus Derived from Nodal Explant (nC) of *O. Citriodorum* by using the GC-MS Technique

Note: mP = mother plant, ivP = *in vitro* regenerated plant, lc = calli derived from leaf, nC = calli derived from nodal segment, PT = phenolic terpene, TE= terpene ester, SO = oxygenated sesquiterpene, SH = hydrogenated sesquiterpene, MO = oxygenated monoterpene, MH = Hydrogenated monoterpene

Table 2: Main Volatile Constituents of *O. citriodorum* (mP, ivP, lC and nC) Identified by GC-MS

Compound	*Type*	*mP (%)*	*ivP (%)*	*lC (%)*	*nC (%)*
Pinene	MH	0.54	-	-	-
Linalool	MO	1.86	-	-	-
Camphor	MO	0.53	-	-	-
Ocimenol	MO	0.47	-	-	-
Estragole	MO	59.12	12.43	-	1.27
Nerol	MO	0.88	2.44	-	-
β- citronellol	MO	-	-	0.99	-
Geraniol	MO	-	-	-	6
Germacrene B	SH	2.44	0.35	0.5	0.26
β-caryphyllene	SH	1.22	-	-	-
Caryphyllene	SH	0.38	-	-	-
Germacrene D	SH	0.37	0.23	-	-
β-humulene	SH	0.95	-	-	-
γ-murrolene	SH	2.36	1.3	-	-
β-gurjunene	SH	7.91	-	-	-
Isocaryophyllene	SH	-	1.03	-	-
β-farnesene	SH	-	0.2	-	-
β-himachalene	SH	-	0.34	-	-
β-curcumene	SH	-	-	0.18	-
trans-α-bergamotene	SH	-	-	0.33	0.33
β-longipinene	SH	-	-	-	0.46
Bergamotene	SH	-	-	-	0.25
Cubenol	SO	1.06	-	-	-
Geranylgeraniol	SO	1.09	0.72	-	0.61
Globulol	SO	-	0.37	-	-
Eugenol	PT	1.39	-	-	-
Geranylacetate	TE	0.32	1.53	-	2.83
Geraniolacetate	TE	0.5	-	0.89	-
Geranylformate	TE	-	0.57	-	0.43
β-citronellylacetate	TE	-	0.28	-	0.31
Citronellylisobutyrate	TE	-	0.46	-	-

Note: mP = mother plant, ivP = *in vitro* regenerated plants, lc = calli derived from leaf, nC = calli derived from nodal explant, PT = phenolic terpene, TE= terpene ester, SO = oxygenated sesquiterpene, SH = hydrogenated sesquiterpene, MO = oxygenated monoterpene, MH = Hydrogenated monoterpene

4. Discussion

Essential oil extraction in the present study was performed by the solvent extraction method. The advantage of solvent extraction method is in single leaf analysis when plant material is very limited or where the major essential oil constituents, rather than essential oil content, is of primary interest. (Charles and Simon, 1990). The GC-MS analysis of samples (mP, ivP, lC and nC) ensured that lower percentage of volatiles were obtained from the ivP, lC and nC than in comparison to mP. It could be probably due to the fact that monoterpene synthesis in higher plants takes place through mevalonic acid-independent pathway (Lichtenthaler *et al.*, 1997). The greater development rate of the aerial part of plantlets in an active growth phase

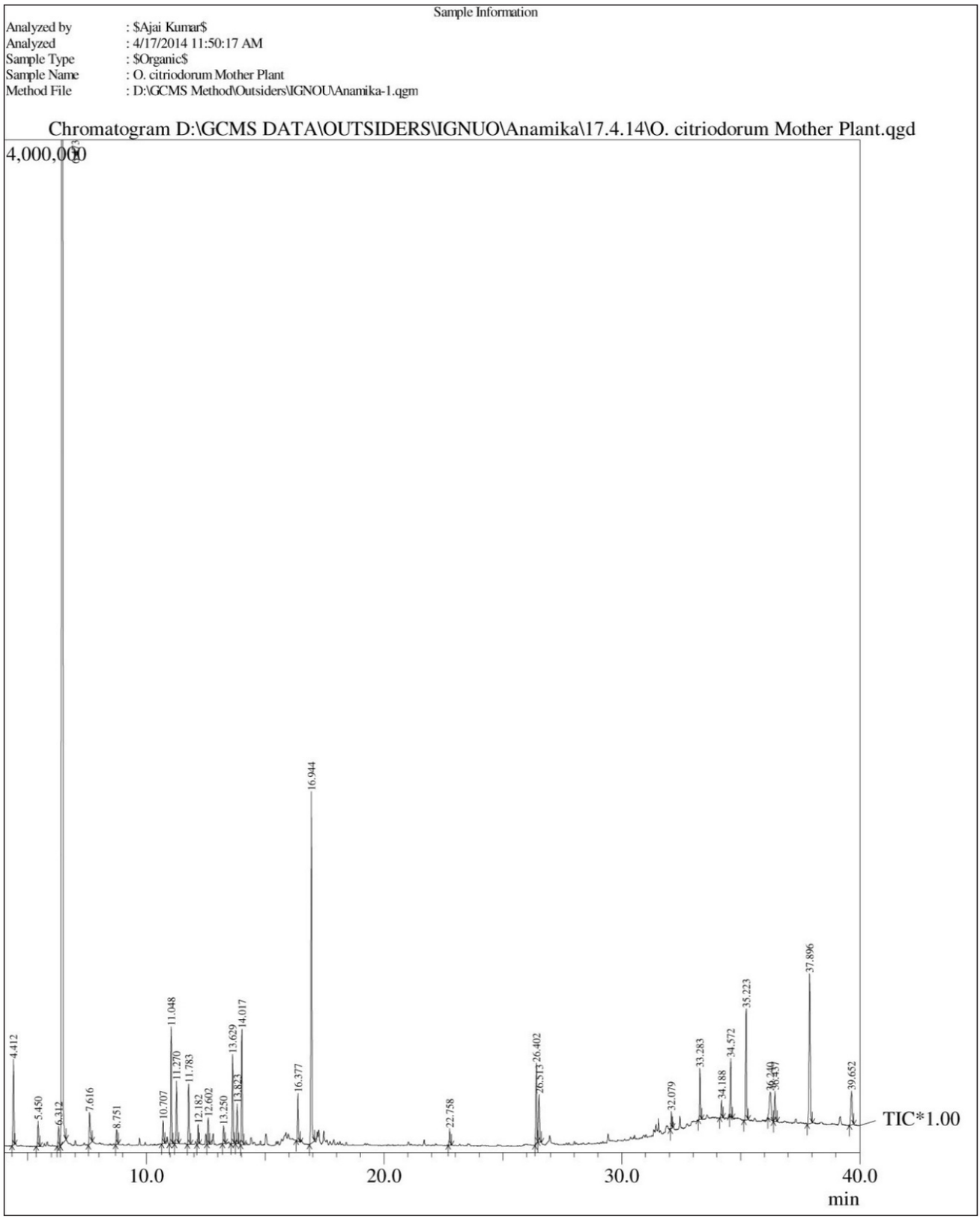


Fig. 2: Chromatogram of Mother Plant (mP) of *O. citriodorum*

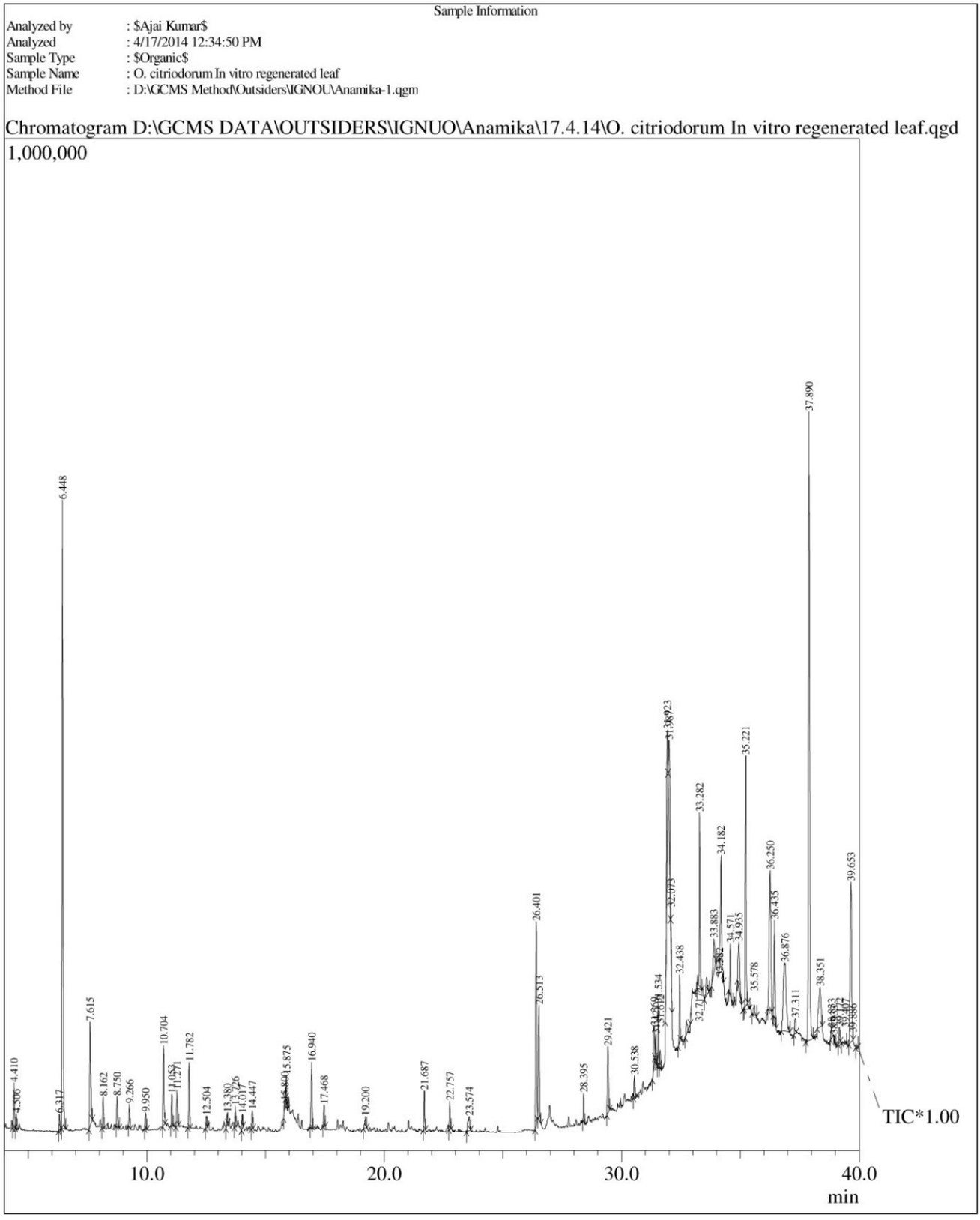


Fig. 3: Chromatogram of *in Vitro* Regenerated Plant (ivP) of *O. citriodorum*

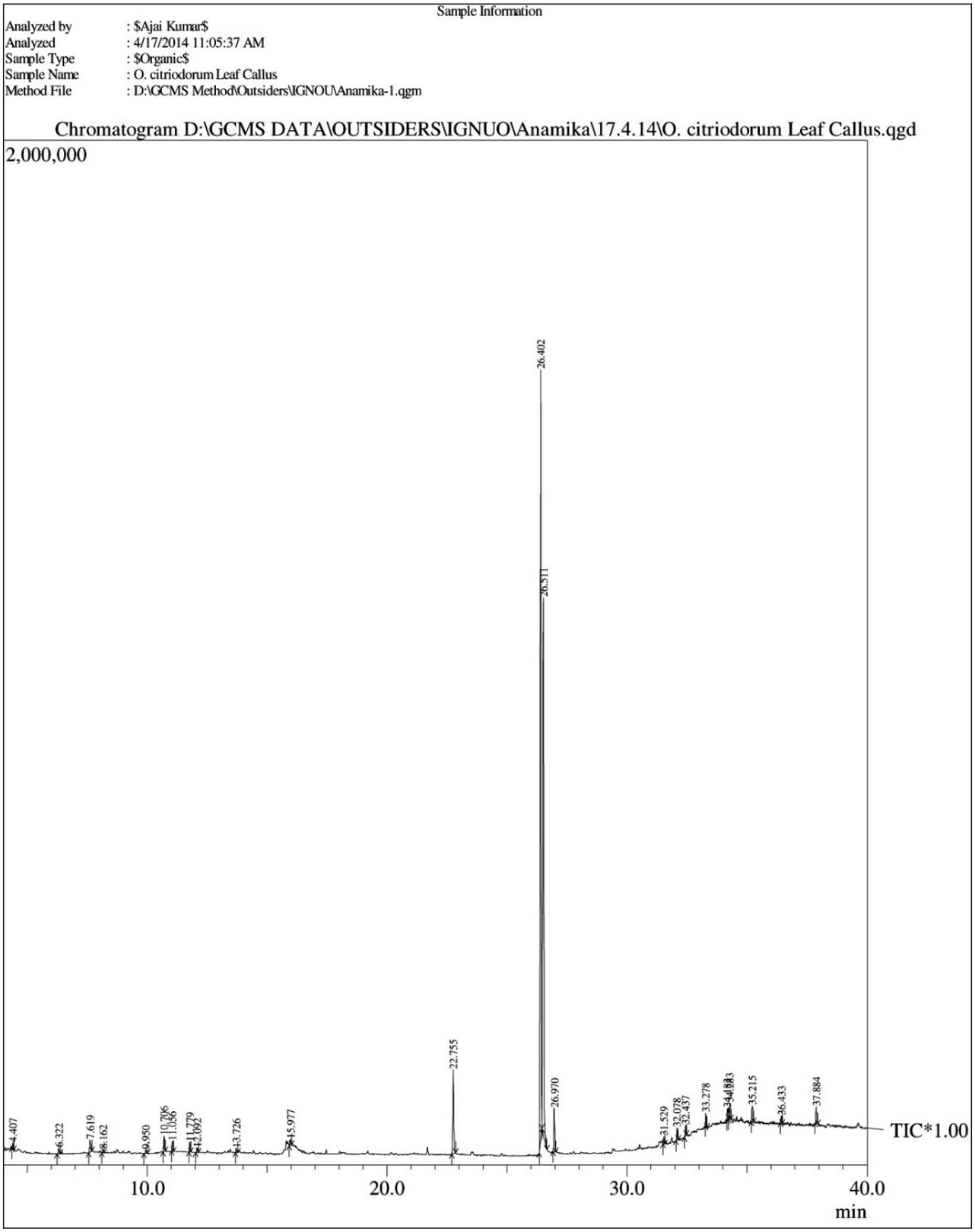


Fig. 4: Chromatogram of Calli Derived from Leaf (lC) of *O. citriodorum*

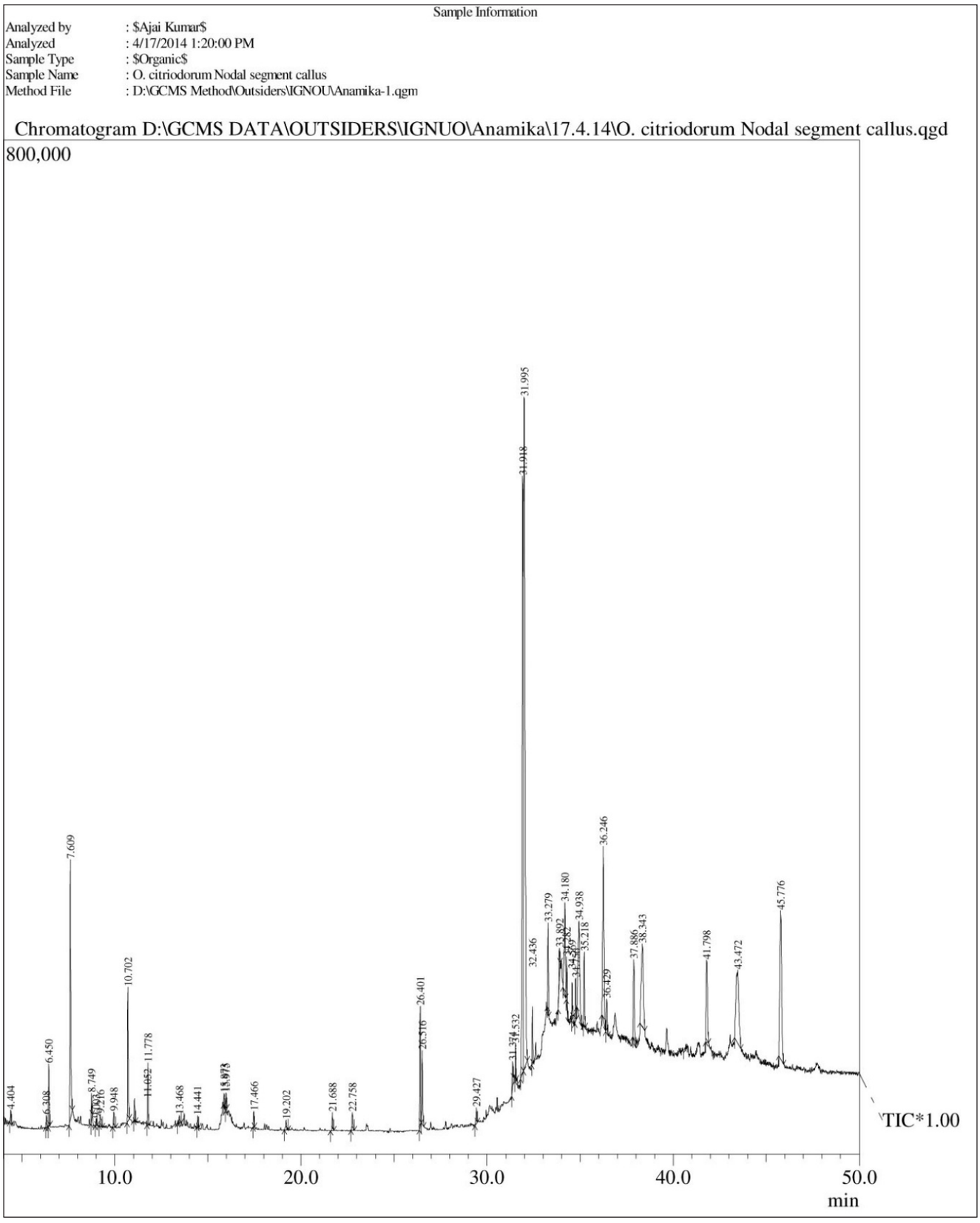


Fig. 5: Chromatogram of Calli Derived from Nodal Segment (nC) of *O. citriodorum*

would require more primary terpenoid metabolites. This would activate HMG (Hydroxymethylglutaryl) -CoA reductase enzyme which synthesizes mevalonic acid which is the first precursor in the biosynthesis of sesquiterpenes, triterpenes and triterpenoids (Brown and Charlwood, 1986; McCaskill and Corteau, 1999). Tusro *et al.,* (2010) also obtained lower percentage of essential oils from the *in vitro* regenerated plants of *L. vera.* Chan *et al.* (2005) also reported the lower level of essential oil from the *in vitro* plants than in comparison to mature plants.Thus our results were in similarity with the results obtained from the other researchers. However, contrary to this Paul *et al*. (2010) reported higher essential oil content from the *in vitro* derived plants (4%) of *Pogostemon cablin* than in comparison to *in vivo* plants (3.2%).

In the present study 5 volatile contents were identified from lC and 10 volatile contents from nC. Becker (1970) reported that no volatiles were accumulated in undifferentiated callus cultures of *Origanum vulgare, Rosamarinus officinalis* and *Salvia officinalis*. Likewise no monoterpenes could be detected in undifferentiated tissue cultures of *Lavandula angustifolia* (chWebb *et al.*, 1984) and *R. officinalis* (Webb *et al.*, 1984) grown under a variety of conditions. Thus, the results of the present study were in contrary with those obtained by other workers. However, the concentration of most of the volatile components was higher in mP and ivP than in comparison to lC and nC. It may be speculated that developmental processes can orchestrate the complex volatile biosynthesis in plants resulting in significant variations in their composition (Zielinska *et al.,* 2011). Further, the number of secretary glands also influences the production of essential oil. The secretary glands would be more in number on the surface of leaves of plants than in comparison to the callus.

The comparison of GC-MS identified volatiles of samples (mP, ivP and nC) of *O. citriodorum* suggested estragole as the main component. Estragole is a natural constituents of a number of plants and their essential oils have been widely used in foodstuffs as flavoring agents. Its percentage decreased in the ivP (12.43%) followed by nC (1.27%) in comparison to mP (59.12%) and in lC it was absent. The results of the present study are in accordance with another study (Shin *et al.* 2001) on volatiles (extracted with dichloromethane) from the tissue culture of *A. rugosa*, where completely altered profile from callus was observed in comparison to the mature plant and a low percentage (0.58% compared to over 50% in an extract from leaves) of estragole was reported. Zielinska *et al.* (2011) also reported lower percentage of estragole from *in vitro* plants than *in vivo* plants. This could be due to the fact that specific mechanisms are involved in differential production and/ or accumulation of specific metabolites in the tissues (Ma and Gang, 2006). Further, organogenesis is necessary for the induction of volatile substances comparable to those of intact plants.

Increased percentage of nerol (2.44%) and geranylacetate (1.53%) was obtained from the ivP in comparison to mP (Nerol- 0.88% and geranylacetate- 0.32%).The difference in the essential oil content between *in vitro* derived plants and *in vivo* plants could be attributed to the fate of glandular trichomes to which the synthesis of monoterpene is restricted (Arikat *et al.,* 2004). Under *in vitro* conditions, harvested shoots are young and thus chemical compounds that are secreted by the trichomes accumulate in the shoot and their amount is concentrated. Furthermore, the increased yield of essential oils in *in vitro* derived plants may be related to the marked influence of cytokinin in the culture medium. Benzyladenine (BA) in the medium was shown to have a positive effect on the capacity of *Lavandula dentata* plantlets to produce and/or accumulate essential oils, the amount was 150% more than that produced in the absence of BA (Sudria *et al.,* 1999). Thus results of the present study were in conformation with the previous findings.

5. Conclusions

From the present study, it can be concluded that *in vitro* propagation could be used for the production of essential oils from the *O. citriodorum* plants. It takes less time to produce the plantlets once the *in vitro* plantlet culture protocol is established. Moreover, the *in vitro* plantlet culture can ensure disease free condition. Metabolic engineering approaches may make it possible to increase the content of volatile compounds and to alter the constituents of oil, thereby providing new essential oils.

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References

1. Arikat, N. A., Jawad, F. M., Karam, N. S., and Shibli, R. A. (2004). Micropropagation and accumulation of essential oils in wild sage (*Salvia fruticosa*Mill.), Sci. Hortic., 100, 193-202.
2. Becker, H. (1970). Untersuchungen zur Frage der Bildung fluechtiger Soff wechset produckte in callus kultunen, Biochem. *Physiol. Pflanz,* 161, 425- 441.
3. Bodhipadma, K., Noichinda, S., Phromthara, T., Rutatip, S., and Nathalang, K. (2012). Determination of antioxidants from lemon basil callus during induction. *The Journal of Applied Science,* 11(2), 1-8.
4. Brown, J. T., and Charlwood, B. V. (1986). The accumulation of essential oils by tissue cultures of *Pelargonium fragrans* (Willd.). *Federation of European Biochemical Societies,* 204(1), 117- 120.
5. Burbott, A. J., and Loomis, W. D. (1967). Effects of light and temperature on the monoterpenes of peppermint. *Plant Physiol.,* 42, 20-28.
6. Chan, L. K., Dewi, P. R., and Boey P. L. (2005). Effect of plant growth regulators on regeneration of plantlets from bud cultures of *Cymbopogon nardus* L. and the detection of essential oils from the *in vitro* plantlets. *Journal of Plant Biology,* 48(1), 142- 146.
7. Charles, D. J., and Simon, J. E. (1990). Comparison of extraction methods for the rapid determination of essential oil content and composition of basil. *Journal of the American Society for Horticultural Science*, 115(3), 458-462,
8. Janarthanam, B., and Sumathi, E. (2012). Plantlet regeneration from nodal explants of *Ocimum citriodorum Vis.*. *Bangladesh Journal of Scientific and Industrial Research*, 47(4), 433-436.
9. Kalkan, E. (2012). Chemical profiling and extraction processing of basil (*Ocimum*) cultivars grown in Oklahoma, Ph.D. Thesis, Oklahoma State University.
10. Liber, Z., Stanko, K. C., Politeo, O., Strikic, F., Kolak, I., Milos, M., and Satovic, Z. (2011). Chemical characterization and genetic relationships among Ocimum basilicum L. cultivars. *Chemistry and Biodiversity,* 8, 978-1989.
11. Lichtenthaler, H. K., Schwender, J., Disch, A., and Rohmer, M. (1997). Biosynthesis of isoprenoids in higher plant chloroplasts proceeds *via* a mevalonate-independent pathway. *FEBS Lett.,* 400, 271-274.
12. Ma, X., and Gang, D. R. (2006). Metabolic profiling of turmeric (*Curcuma longa* L.) plants Derived from *in vitro* micropropagation and conventional greenhouse cultivation. *Journal of Agricultural and Food Chemistry,* 54 (25), 9573-9583.
13. McCaskill, D., and Croteau, R. (1999). Isopentenyl diphosphate is the terminal product of the deoxyxylulose-5-phosphate pathway for terpenoid biosynthesis in plants. *Tetrahedron Letter,* 40, 653-656,
14. Morone-Fortunato, I., and Avato, P. (2008). Plant development and synthesis of essential oils in micropropagated and mycorrhiza inoculated plants of *Origanum vulgare* L. ssp. hirtum (Link) Ietswaart. *Plant Cell Tissue and Organ Culture,* 93, 139-149.
15. Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant,* 15, 473-479.
16. Nitnaware, K. M., Naik, D. G., and Nikam, T. D. (2011). Thidiazuron-induced shoot organogenesis and production of hepatoprotective lignin phyllanthin and hypophyllanthin in *Phyllanthus amarus*, *Plant Cell Tissue and Organ Culture*, 104, 101-110.
17. Paul, A., Thapa, G., Basu, A., Mazumdar, P., Kalita, M. C., and Sahoo, L. (2010). Rapid plant regeneration, analysis of genetic fidelity and essential aromatic oil content of micropropagated plants of Patchouli, *Pogostemon cablin* (Blanco) Benth.- an industrially important aromatic plant. *Industrial Crops and Products,* 32, 366-374.
18. Prideevech, P., Chumpolsri, W., Suttiarporn, P., and Wongporanchai. (201). The chemical composition and antioxidant activities of basil from Thailand using retention indices and comprehensive two dimensional gas chromatography. *Journal of Serbian Chemical Society,* 75(11), 1503-1513.
19. Predieri, S., and Rapparini, F. (2007). Terpene emission in tissue culture. *Plant Cell, Tissue and Organ Culture,* 91(2), 87-95.
20. Sangvanphet, T., Chareonviriyaphap, T., and Naksathit, A. (Repellent function of essential oils from *Ocimum citriodorum* on *Aedes aegypti* (Diptera: Culicidae) mosquitoes, a vector of dengue, Science Exhibition 2011, BI- P- 28, Science University.
21. Shin, S. H., Kim, Y. S., and Kang, C. A. (2001). Production of volatile oil components by cell culture of *Agastache rugosa* O. Kuntze. *Natural Product Science*, 7, 120-123,
22. Simon, J. E., Morales, M. R., Phippen, W. B., Vieira, R. F., and Hao, Z. (1999). Basil: a source of aroma compounds and a popular culinary and ornamental herb, Reprinted from: *Perspectives on new crops and new uses, J. Janick* (ed.), ASHS Press,

Alexandria, VA,

23. Sood, H., and Chauhan, R. S. (2010). Biosynthesis and accumulation of a medicinal compound, Picroside-I, in cultures of *Picrorhiza kurroa* Royle ex Benth. *Plant Cell Tissue and Organ Culture,* 100, 113-117.
24. Sudria, C., Pinol, M. T., Palazon, J., Cusido, R. M., Vila, R., Morales, C., Bonfill, M., and Canigueral, S. (1999). Influence of plant growth regulators on the growth and essential oil content of cultured *Lavandula dentata*plantlets. *Plant Cell Tissue and Organ Culture,* 58, 177-184.
25. Tripathi, A., Abbas, N. S., and Nigam, A. (2014). Micropropagation of an endangered medicinal herb *O. citriodorum* Vis. *Journal of Plant Development Sciences*, 6(3), 365-374.
26. Tripathi, A. (2011). Anticarcinogenic potential of some medicinal plants. *The Botanica,* 59(61), 169-175.
27. Tusro, M., Inoue, M., and Kameoka, H. (2001). Variation in essential oil components in regenerated lavender (*Lavendula vera* DC) plants. *Scientia Horticulturae,* 88, 309-317.
28. Webb, J. K., Banthorpe, D. V., and Watson, D. G. (1984). Monoterpene synthesis in shoots regenerated from callus cultures. *Phytochemistry,* 23, 903-904.
29. Zielinska, S., Piatczak, E., Kalemba, D., and Matkowaski, A. (2011). Influence of plant growth regulators on volatiles produced by *in vitro* grown shoots of *Agastache rugosa* (Fischer and C.A. Meyer) O. Kuntze, *Plant Cell, Tissue and Organ Culture*, 107, 161- 167.
30. Zielinska, A., and Nowak, I. (2014). Fatty acids in vegetable oils and their importance in cosmetic industry. *Chemik,* 68(2), 103-110.