International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Fungi as a Biotransformation Tool in Patchouli (*Pogostemon patchouli* Pellet) Essential Oil

Ashwini Jayaram[1], Vasundhara M[2], Ananda S.[3]

[1]Division of Medicinal and Aromatic Plants, Department of Horticulture
University of Agricultural Sciences, GKVK Campus, Bengaluru- 560065, India

[2]Division of Medicinal and Aromatic Plants, Department of Horticulture,
University of Agricultural Sciences, GKVK Campus, Bengaluru- 560065, India

[3]Division of Biotechnology, Sapthagiri College of Engineering, Bangalore-560057, India

Abstract: ***Five and half month old crop was harvested, sprayed with fifteen combinations of five fungi isolated from the rhizosphere of patchouli and incubated for five days. The essential oil was extracted by hydrodistillation and analysed by Gas Chromatography. The components of patchouli oil were identified by Gas chromatography/Mass Spectrometry (GC/MS). Twenty compounds were identified with Patchouli alcohol as the major component followed by α bulnesene. The oil content was in the range 1-1.65 % (v/w) on fresh weight basis. Treatment T2 (Aspergillus terreus) was found to be statistically superior with high patchouli alcohol (58.72±0.39) and low amount of other sesquiterpenes (trans-caryophyllene (2.21±0.05), Guaiene (9.89±0.43), α-Patchoulene (2.56±0.04) and α-Bulnesene (8.43±0.8). This indicated an enzymatic bioconversion of precursor molecules to patchouli alcohol by the fungi.***

Keywords: Patchouli oil, fungi, Patchouli alcohol, sesquiterpene

1. Introduction

Biotransformation is the chemical modification of a compound by microorganisms to produce high value products with low cost precursors [1]. The ability of microorganisms to introduce functional groups into chemically inactive complex molecules has made microbial transformations an indispensable part of the manufacturing process of some molecules. Whole cell biocatalysts such as fungi, bacteria, and algae have been extensively applied in the flavor and fragrance industry over the last half a century [2]. They also provide an ecofriendly alternative for synthetic synthesis that are known to produce large amounts of harmful wastes [3], [4]. Biotransformation of different volatile isolates which attempts to reduce the market cost has extensively been reviewed [2]. *Aspergillus* species is known to have biotransformed menthol, isopinocampheol, flavones and 6-hydroxyflavone to give various hydroxylated and reduced products [5], [6], [7].

Patchouli (*Pogostemon patchouli* Pellet) is a perennial aromatic herb, belonging to the family Lamiaceae. It is a bushy plant about three feet tall, native to Philippines and Indonesia [8].Leaves constitute the economic part containing patchouli oil that is concentrated on the outer surface of leaves and in the internal tissues; some quantity is also found in the tender parts of the stem. Unlike other aromatic crops, volatile oil is distilled from dried leaves of patchouli by steam distillation. The oil yield is in the range of 2-4%w/w, which depends on quality and maturity of leaves [9]. Patchouli oil comprises of monoterpenes (7%), and largely sesquiterpenes (40-80%), of which patchouli alcohol is about 30-50%. The other major sesquiterpenes include Trans-Caryophyllene, α-Bulnesene, Nor-Patchoulenol and Patchouli alcohol. The oil is extensively used in perfumery due to its oriental notes [8] and strong fixative properties.

The global demand of Patchouli oil is 1600 tonnes per annum with a value of 240 crores. India imports 220 tonnes of Patchouli oil valued at 33 cores annually. Most of the demand is met from Indonesian imports which produces 80% of patchouli oil [10].Currently, India is producing a meagre quantity of patchouli oil and the main challenge is to produce good quality oil that can compete with the Indonesian oil .Research work on improving the quantity and quality of Patchouli oil with different package of practices is exhaustive [11], [12]& [13].

Biotechnological approaches of tissue culture have been tried to enhance the patchouli alcohol content [14]. In the present study the research work was focused on improvement of oil quality and quantity from fungi, isolated from rhizosphere of patchouli. Series of experiments with soil fungi were conducted on the harvested patchouli herbage and the quality was analyzed by Gas Chromatography technique and confirmed by GC-MS.

2. Material and Methods

Patchouli (*Pogostemon patchouli* var. CIM-SHRESTA) for the research work was cultivated at Sanjeevani Vatika, Department of Horticulture, University of Agricultural Sciences, Bangalore. The package of practice was followed [13]. Forty five day old rooted saplings of Patchouli were planted during July 2013 in 10 guntas of land with spacing of 45X45cm under open conditions. After five and a half months the herbage was harvested during December, 2013.

2.1 Isolation and Identification of microorganisms

Fungal species for this study were isolated from rhizosphere of Patchouli plants, grown in Sanjeevani Vatika. Isolation of fungi was done by pour plate technique. Single colonies were selected and pure cultured in Potato Dextrose Agar

(PDA) and bacterial growth was inhibited by adding streptomycin. Using poison food technique, the fungi were tested for resistance to patchouli oil (1%). Five fungi were found to be tolerant to patchouli oil and these were the biocatalysts for further studies. The five fungal cultures were sent to Microbial Type Collection Centre (MTCC), Chandigarh for identification. They were identified as *Penicillium citrinum* (MTCC 10849), *Botryosphaeria sp*. (MTCC10850), *Aspergillus terreus* (MTCC11026), *Phoma sp*. (MTCC 11027), *and Fusarium solani complex* (MTCC11028).

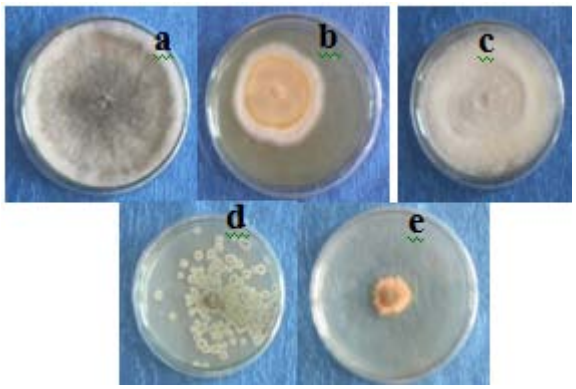

Figure 1: Fungal isolates obtained from rhizosphere patchouli growing area.a=*Botryosphaeria sp*. (MTCC10850), b=*Aspergillus terreus*(MTCC11026), c=*Phoma sp*. (MTCC 11027), d=*Penicillium citrinum* (MTCC 10849), e=*Fusarium solani complex* (MTCC11028).

2.2 Treatments and Scale Up

An experiment with fifteen treatments (T1-T15) and two replications were taken up to carry out biotransformation studies. Five microorganisms (*Botryosphaeria sp,, Aspergillus terreus, Phoma sp, Fusarium solani complex, Penicillium citrinum*) were grown in 15 different combinations (Table 1). The five organisms were initially grown in replications in 25ml of sterilized Potato Dextrose Broth (PDB) (Autoclaved at 121^{0}C for 15min) separately for 48 hours at room temperature. Further, 1ml of the culture (48hours) was inoculated into 200ml of sterilized Potato Dextrose Broth (Autoclaved at 121^{0}C for 15min) and incubated at room temperature for 5days. The mycelia was separated from the broth by filtration with Whattman No 1 filter paper. The separated mycelia was blended with sterile distilled water to break the clumps. This was the inoculum (biocatalyst) for the experiment.

2.3 Biotransformation Studies on Fresh herbage

The harvested fresh herbage was sprayed with fifteen treatments and shade dried for five days. The moisture loss (%) of the herb was recorded prior to distillation and the leaf: stem ratio was also recorded. Extraction of volatile oil was done by Clevenger's apparatus (Hydrodistillation). About 100g of dry leaves from each treatment was loaded in the Clevenger apparatus, the process was carried on for six hours for maximum extraction of essential oil. The oil content (%) was recorded and analyzed to understand the changes in oil.

Table 1: Fungal combinations for biotransformation studies

Treatment	*Combination*
T1	*Botryosphaeria sp*
T2	*Aspergillus terreus*
T3	*Phoma sp*
T4	*Fusarium solani complex*
T5	*Penicillium citrinum*
T6	*Botryosphaeria sp +Aspergillus terreus*
T7	*Botryosphaeria sp +Phoma sp*
T8	*Botryosphaeria sp +Fusarium solani complex*
T9	*Botryosphaeria sp +Penicillium citrinum*
T10	*Aspergillus terreus +Phoma sp*
T11	*Aspergillus terreus +Fusarium solani complex*
T12	*Aspergillus terreus +Penicillium citrinum*
T13	*Phoma sp + Fusarium solani complex*
T14	*Phoma sp +Penicillium citrinum*
T15	*Fusarium solani complex +Penicillium citrinum*
T16	Control (Untreated Herbage)

2.4 Gas chromatography analysis:

Patchouli oil subjected to biotransformtion was analysed by Gas Chromatographic. Analysis was done in Varian 450 GC, with CP-SIL capillary column (30Mx0.25mmx0.25µm film thickness) which is coated with poly dimethyl siloxane, Nitrogen, (flow rate 1ml/min) was used as the carrier gas, hydrogen and zero air as detector gases. An FID (Flame Ionization Detector) was used as the detector; Injector temperature was maintained at 250^{o}C and Detector temperature at 280^{o}C, oven temperature program: 0-100^{o}C for 1min; @3^{o}C/min - 200^{o}C for 10min.

2.5 Gas chromatography-Mass Spectrometry analysis (GC-MS):

GC-MS analysis was carried out in Agilent 7890A GC combined with 5975 inert MASS SELECTIVE DETECTOR using Split-Splitless inlet and data acquisition through MSD-Chemstation. Compounds were separated on a HP-5MS (30Mx0.25mmX0.25µm film thickness) which is coated with 5% phenyl methyl siloxane. Injector temperature was maintained at 250^{o}C. Oven temperature program was set at 80^{0}C for 1min, then @ 2^{0}C/min, the temperature was increased to 140^{0}C and held for 5min. then, at the rate of 1^{0}C/min, finally at 5^{0}C/min to 240^{0}C and held for 6min. 0.1µl neat sample with a split ratio of 50:1 was injected. Helium was used as a carrier gas. The spectrophotometer was operated in EI mode and the scan range was 35-650amu. The inlet ionization source temperature was 250^{0}C and 280^{0}C respectively.

2.6 Statistical Analysis

Analysis of variation in volatile oil content and composition as affected by different fungal treatments were analyzed using the SAS program. The experiment had fifteen treatments replicated twice and expressed as ±SEM. Duncan's multiple range test (DMRT) was applied to determine the significant difference at p≤0.05 for absolute quantities of each chemical compound.

3. Results and Discussion

Successful approach on improving patchouli oil quality has always posted a great challenge, as chemical synthesis of patchouli alcohol, the key ingredient in patchouli oil has not been successful on an industrial scale [15]. It is critical to find a new technique which is farmer friendly to help the patchouli oil production.

In this study an attempt was made for the first time to test the effect of soil fungi on patchouli oil content and quality. The soil fungi in fifteen different combinations were sprayed on patchouli herbage. After five days of drying the essential oil was extracted. The results are classified under the following sections:

a) Identification and Quantification of compounds
b) Essential oil content (%) and chemical composition of patchouli oil.

3.1 Chemical Characterization and Quantification of Compounds

The chemical components present in the volatile oil were identified by Gas Chromatography coupled with Mass Spectroscopy and Retention Index (Table-2). 95.8% of compounds were identified by GC-MS. Patchouli alcohol was found to be the major component [9] followed by α-guaiene and α-bulnesene.

3.2 Essential Oil Content (%) and Chemical Composition of Patchouli Oil

In the present study the data on essential oil content (%) obtained after different treatments are presented in table 3. All the treatments were found to be significant for the volatile oil content. The oil content was in the range of 0.9±0 to 1.65±0.05%. Similar results have been observed in different accessions of patchouli in the range of 1.56-1.84% [16] and 1.23-1.75% [17].

Of all the treatments, T13 (*Phoma sp + Fusarium solani complex*) recorded highest oil content of 1.65±0.05. This was statistically similar to treatments T15 (*Fusarium solani complex +Penicillium citrinum*), T12 (*Aspergillus terreus +Penicillium citrinum*), T5 *(Penicillium citrinum*).The increase in oil content maybe due to the production of pectinase enzyme which breaks down the cell wall and aid the release of oil [19].

Lowest oil content was recorded in treatment T4 0.9±0 (*Fusarium solani complex*) and T9 (*Botryosphaeria sp +Penicillium citrinum*). Low oil content was reported due to variation in leaf moisture [19]. Variables like time of harvest, drying conditions, distillation parameter and fermentation conditions of the fungal sprays may have also affected the oil content [9].

Table 2: Chemical composition of patchouli oil using GC-MS

RT	*Name*	*%Area*	*Identification*
15.23	β-Patchoulene	2.21	GC-RI
16.069	β-elemene	0.468	GC-MS
17.344	Thujapsene	0.647	GC-MS
18.32	Trans caryophyllene	3.895	GC-MS
20.416	α-guaiene	21.9	GC-MS
21.2	α-humulene	0.103	GC-RI
21.71	α-Patchoulene	5.47	GC-MS
22.05	γ-patchoulene	2.84	GC-RI
24.32	Germacrene D	0.22	GC-MS
26.14	Aciphylene	3.6	GC-RI
26.92	α-bulnesene	19.56	GC-MS
27.53	7-epi- alpha selenene	0.35	GC-RI
27.92	α-alaskene	0.06	GC-RI
30.27	Nor patcholenol	0.69	GC-RI
27.92	Delta elemene	0.066	GC-MS
32.78	Caryophyllene oxide	0.14	GC-MS
33.433	-Saputhenol	0.11	GC-MS
40.085	Patchouli alcohol	32.48	GC-MS
46.718	Pogostone	0.848	GC-MS
47.52	Farnesol	0.16	GC-MS
		95.80%	

The data on volatile components viz trans-caryophyllene, Guaiene, α-Patchoulene, α-Bulnesene, nor patchoulenol and Patchouli alcohol are presented in Table 3 and Fig 2. All the treatments in the fresh herbage studies were highly significant at 5% level of significance for the volatile components except nor patchoulenol.

The data (Table 3) revealed that in fresh herbage treatment, the content of major sesquiterpene patchouli alcohol varied from 44.83±1.12 to 58.72±0.39 and the content of other sesquiterepenes like trans-caryophyllene varied from 3.8±0.07 to 2.21±0.05, Guaiene (16.27±0.02 to 9.89±0.43), α-Patchoulene (3.78±0.41 to 2.56±0.04), and α-Bulnesene (14.07±0.05 to 8.43±0.8). Among the treatments, T2 (*Aspergillus terreus*) was found to be statistically superior as the amount of patchouli alcohol was 58.72±0.39 as compared to untreated control 45.67±1.13. Along with this it was observed that the amount of other sesquiterpenes was least (trans-caryophyllene 2.21±0.05, Guaiene 9.89±0.43, α-Patchoulene 2.56±0.04 and, α-Bulnesene 8.43±0.8), in treatment T2 (*Aspergillus terreus*) as compared to control (trans-caryophyllene 3.8±0.07, Guaiene 16.27±0.02, α-Patchoulene 3.78±0.41, α-Bulnesene14.07±0.05) [Fig 3a&b].

Treatments T5 (*Penicillium citrinum*) and T8 (*Botryosphaeria sp +Fusarium solani complex*) were in par with each other with patchouli alcohol of 55.76±0.11 and 56.05±1.97 respectively. Decrease in minor sesquiterpene was also observed in these treatments as compared to control. The amount of Trans-caryophyllene in treatments T5 and T8 was 2.78±0.09 and 2.28±0.7, similarly guaiene was 10.57±0.14 and 10.46±0.36, α-Patchoulene 3.05±0.05and 3.34±0.33, α-Bulnesene 8.76±0.08 and 9.52 respectively, as compared to control (trans-caryophyllene 3.8±0.07, Guaiene 16.27±0.02, α-Patchoulene 3.78±0.41, α-Bulnesene14.07±0.05).

In treatment T11 (*Aspergillus terreus +Fusarium solani complex*) and T9 (*Botryosphaeria sp +Penicillium citrinum*) was found to have least amount of patchouli alcohol (44.83±1.12 and 45.14±0.52 respectively) which was on par with control (45.67±1.13). The minor sesquiterpene were found to be statistically similar to control.

Patchouli alcohol indicates the quality of essential oil [16]. Increases in patchouli alcohol due to mycorrizal fungal treatments have also been reported [17]. The increase in patchouli alcohol may be due to fungal enzymes like alcohol dehydrogenases [19] that are known to enhance the production of alcohol. The pathway hypothesized by [20] suggests that the precursor molecule Germacradienyl cation which majorly produces patchouli alcohol, maybe influenced by fungal enzymes.

4. Conclusion

Patchouli plants are the only commercial source of patchouli alcohol and synthetic routes are yet to be developed commercially. The economic value of patchouli oil is associated with the patchouli alcohol concentrationin the current study biotransformation by soil fungi (biocatalyst) on fresh patchouli herbage was attempted. *Aspergillus terreus* treated herbage showed an increase in patchouli alcohol content and decrease in minor sesquiterpenes. It may be concluded that fungi like *Aspergillus terreus* may be used as effective biocatalyst to enhance the quality of Patchouli oil.

References

[1] H. Wolfgang and H. Dirk, "Fungal Bio-transformations in Pharmaceutical Sciences," in The Mycota- Industrial Applications, II edition, ed. by Karl Esser, Springer Publications, Germany, pp.293-315, 2010.
[2] Y. Gounaris, "Biotechnology for the production of essential oils, flavours and volatile isolates- A review," Flavour and Fragrance Journal, 25, pp. 367-386, 2010.
[3] W.A. Duetz, H. Bouwmeester, J.B. Beilen, and B. Witholt, "Biotransformation of limonene by bacteria, fungi, yeasts and plants," Applied Microbiology and Biotechnology, 61, pp. 269-277, 2003.
[4] M .Torshabi, M. Badiee , M. A. H. Faramarzi,H. Rastegar, H. Forootanfar, and E. Mohit, "Biotransformation of methyltestosterone, by the filamentous fungus Mucor. Chemistry of natural compounds," **47**(1), pp. 59-63, 2011.
[5] Y. Noma, Y. Asakawa, H.Takahashi and M. Toyota, "Biotranformation of Monoterpenoids(-)- and (+)-Menthols, Terpinolene and Carvotanancetone by *Aspergillus* Species, Phytochemistry," 30(12), pp. 3981-3987,1991.
[6] M. Miyazawa, Y. Suzuki and H. Kameoka, "Biotransformation of (-) - and (+) - Isopinocamphenol by three fungi," Phytochemistry," 45(5), pp.945-950, 1997.
[7] E. K. Suslow, J. D. Gladysz, A. Bialonska, Z. Ciunik, W Rymowicz "Microbial Transformation of Flavanao and 6-hydrozyflavanone by *Aspergillus niger* strains," Journal of Molecular Catalysis B: Enzymatic, 39, pp.18-23,2006.
[8] M. Ahmed, "Patchouli- an ideal aromatic crop of commercial importance," Naya Udyog Press, Kolkata, pp.71-73, 2002.
[9] Akhila and Tewari, "Chemistry of Patchouli oil," Current research on medicinal and aromatic plants, 6, pp.38-54, 1984.
[10] A.R. Rani, P. Chakrapani, K.. Venkatsh, C. S. Singh, B. A. Joythi, P. Kumar, P. Amareshwari, "Phytochemical, Pharmacological importance of Patchouli (*Pogostemon cablin*(Blanco) Benth an aromatic medicinal plant," International Journal of Pharmaceutical Sciences Review and Research, 21(2), pp.7-15,2013.
[11] S. Bhaskar, J. Vasanth Kumar, and J.C. Srivasthava, "Effect of growth regulators on production of herbage and oil in patchouli (*Pogostemon patchouli*)," Indian Perfumer, 41(3), pp.98-101, 1997.
[12] A. A. Farooqi, M. Vasundhara, K. N. Srinivasappa, "Patchouli cultivation as an intercrop in plantations," in National Seminar on Transfer of Technology of Medicinal and Aromatic Crops, pp. 182-188, 2001.
[13] M. Vasundhara, "Studies on Production potential of Patchouli (*Pogostemon cablin*) as an under crop, Nutrient management and processing," project report of ICAR-AP CESS FUND-ad-hoc No. 180, 2003.
[14] S. Bunrathep, B. Lockwood, and N. Ruangrungsi, "Chemical constituents from leaves and cell culture of *Pogostemon cablin* and use of precursor feeding to enhance Patchoulol content," Scienceasia, 32, pp. 293-296, 2006.
[15] E. Davis, "The sweet scent of success," Chemistry world, pp 40-44, 2009.
[16] A.F. Blank, T.C.P. Sant'ana, P.S. Santos, M.F. Arrigoni-Blank, A. P. N. Prata, H. R. Jesus and P .B. Alves, Chemical characterization of essential oil from patchouli accessions harvested over four seasons," Industrial crops and products, 34, pp.831-837, 2011.
[17] Parameswaran and B. Abdul Rahiman, "Symbiotic response of Patchouli (*Pogostemon cablin* (Blanco) Benth. to different Arbuscular mycorrhizal fungi, Advances in Environmental Biology, 2(1), pp.20-24, 2008.
[18] S.V.M. Krishna and H. Velankar, Process for increase patchoulol content in essential oil of *Pogostemon cablin* , United states: Patent US patent no: US2007/0298482 A1, 2007.
[19] N. Hussin, L. Mondello, R. Costa, N.I.N. Yousoff, M.A. Yarma, A. Wahab and M. Said," Quantitative and Physical Evaluation of Patchouli Essential oils obtained from different sources of *Pogostemon cablin*," Natural Products Communications, 7(7), pp.927-930, 2012.
[20] F. Deguerry, L. Patore, S. Wu, A. Clark, J. Chappel and M. Schalk, "The diverse sesquiterpene profile of patchouli, *Pogostemon cablin*, is correlated with a limited number of sesquiterpene synthases," Archives of Biochemistry and Biophysics(Science Direct), 454, pp.123-136, 2006.

Author Profile

Dr. Vasundhara is incharge of Medicinal and Aromatic Crops Section and has established Medicinal and Aromatic garden in 30 acres at UAS (B). She has developed a variety 'AISIRI' in medicinal *Coleus forskohlii* and Stevia (alternate sweetener). Completed as PI, 4

projects under State Plan Grants, ICAR-AP Cess Fund Project on Patchouli, ICAR-NATP, NAIP NHM & NMPB Projects. She is a major adviser to 29 M.Sc., 5 Ph.D. students and a member for 42 M.Sc. and 13Ph.D. students. She has 142 research papers, 24 popular scientific articles, 49 Bulletin/Books/Manual/Brouchers.At various national/international seminars, 82 research papers have been presented. She is also a recipient of National award of Meritorious Scientist by the EOAI, New Delhi, and Distinguished Woman Scientist from UAS (B) Alumni Association. She was a lead speaker in Indonesia, Nigeria and Mauritius. She is trained at Taiwan in Tissue culture and on Natural Fragrances in 2010 at Grasse, France. National Consultant for MADP under FAO, Rome, Nominated by ICAR as member, Institute Management Committee DMAPR, Gujarat and also Expert Committee Member for sourcing proprietary perfumery materials to KS & DL, GOK. Recipient of Best author award by KS&TA, GOK.

Mrs. Ashwini Jayaram is a Ph.D scholar in Microbiology from VTU, Belgaum.. She began her career as a Project Trainee (Nov2002- June 2003) at Indian Institute of Science (IISc), on Genetic transformation in pigeon pea with HN gene edible vaccine for PPRV via *Agrobacterium tumefaciens*. She has worked as a faculty at JIVAS and PES College, Bangalore. She is carrying out her doctoral research on Biotransformation of Patchouli oil and is currently working as a Research Assistant under Dr. M. Vasundhara, Dept. of Horticulture, UAS, GKVK, Bangalore. She was associated in establishing quality analytical laboratory under NAIP-Patchouli, 2009-10 at UAS, Bangalore. She is assisting in training Undergraduate and Post graduate Life Sciences students in conducting research projects with qualitative and quantitative estimation of phytochemicals and active ingredients through GC and HPLC, evaluating Antimicrobial and antioxidant properties of various Medicinal and Aromatic plants.

Dr. Ananda S Professor & Head of the Department of Biotechnology, Sapthagiri college of Engineering, Bangalore. He has done his Ph.D. from Kidwai Memorial Institute of Oncology, Bangalore, for his Ph. Degree he has worked on PTPN11 Gene Mutation in Child Acute Lymphoblastic leukemia between 2002-2009 and His graduation from University of Mysore from the Department of Biotechnology, Manasa Ganghotri Mysore in the year-2000.He is also did his P.G diploma in Intellectual Property Rights form NLSIU, Bangalore in the year-2007.He has received Young scientist award from Vision Group of Science and Technology, Government of Karnataka in the year 2009 under the scheme of Seed money for Young research Scientist. He is also a recipient Of Intellectual Ventures award in Spark'09 with the cash prize of Rs. 1Lakh for the innovative idea: Advance *Diagnostic kits Using Microbial Fuel Cell"*, the competition conducted across multiple Universities in India. He is supervising 6 students for their Ph.D.Program under Visvesvaraya technological University (VTU), Belgaum-INDIA. He is also a member BOE for Biotechnology and member BOS in nanotechnology VTU. He has 18 International and 6 national publications in his credit.

Table 3: Variation of essential oil content (%) and chemical composition of patchouli oil

Treatment	*Caryophyllene*	*Guainene*	*Patchoulene*	*Bulnesene*	*Nor Patchouleneol*	*Patchouli alcohol*	*Oil content*
T1	$3.31^{ab} \pm 0.1$	$14.37^{c} \pm 0.42$	$3.16^{cde} \pm 0.22$	$13.2^{abc} \pm 0.31$	$1.76^{a} \pm 0.03$	$49.92^{cd} \pm 0.54$	$1.11^{bcd} \pm 0.4$
T2	$2.21^{d} \pm 0.05$	$9.89^{f} \pm 0.43$	$2.56^{f} \pm 0.04$	$8.43^{g} \pm 0.8$	$1.51^{abc} \pm 0.32$	$58.72^{a} \pm 0.39$	$1^{bcd} \pm 0$
T3	$2.71^{bcd} \pm 0.06$	$11.73^{e} \pm 0.08$	$3.31^{abcde} \pm 0.15$	$11.16^{de} \pm 0.11$	$1.32^{c} \pm 0.05$	$47.46^{de} \pm 0.45$	$1.2^{cd} \pm 0.2$
T4	$3.07^{abc} \pm 0.15$	$12.2^{de} \pm 0.41$	$2.82^{ef} \pm 0.02$	$11.53^{de} \pm 0.4$	$1.69^{ab} \pm 0.07$	$51.77^{c} \pm 1.24$	$0.9^{d} \pm 0$
T5	$2.78^{bcd} \pm 0.09$	$10.57^{f} \pm 0.14$	$3.05^{de} \pm 0.05$	$8.76^{g} \pm 0.08$	$1.36^{bc} \pm 0.13$	$55.76^{b} \pm 0.11$	$1.4^{abc} \pm 0.1$
T6	$2.87^{bcd} \pm 0.16$	$15.21^{bc} \pm 0.41$	$2.99^{def} \pm 0.14$	$10.5^{ef} \pm 0.61$	$1.51^{abc} \pm 0.03$	$49.1^{cd} \pm 1.55$	$1.2^{abcd} \pm 0.1$
T7	$2.78^{bcd} \pm 0.06$	$12.76^{d} \pm 0.24$	$3.17^{cde} \pm 0.09$	$9.65^{gf} \pm 1.8$	$1.5^{abc} \pm 0.04$	$50.44^{c} \pm 0.12$	$1.3^{abcd} \pm 0.2$
T8	$2.28^{d} \pm 0.7$	$10.46^{f} \pm 0.36$	$3.34^{abcd} \pm 0.33$	$9.52^{gf} \pm 0.01$	$1.44^{abc} \pm 0.06$	$56.05^{b} \pm 1.97$	$1.25^{abcd} \pm 0.15$
T9	$3.22^{abc} \pm 0.19$	$14.84^{bc} \pm 0.15$	$3.73^{ab} \pm 0.07$	$11.52^{de} \pm 0.16$	$1.63^{abc} \pm 0.16$	$45.14^{e} \pm 0.52$	$0.9^{d} \pm O$
T10	$3.19^{abc} \pm 0.06$	$14.48^{c} \pm 0.4$	$3.51^{abcd} \pm 0.09$	$11.88^{cde} \pm 0.07$	$1.56^{abc} \pm 0.04$	$51.32^{c} \pm 0.09$	$1.3^{abcd} \pm 0.2$
T11	$3.41^{ab} \pm 0.29$	$16.33^{a} \pm 0.19$	$3.78^{a} \pm 0.41$	$13.88^{a} \pm 0.25$	$1.56^{abc} \pm 0.04$	$44.83^{e} \pm 1.12$	$1.0^{cd} \pm 0$
T12	$3.28^{ab} \pm 0.02$	$14.54^{c} \pm 0.46$	$3.34^{abcd} \pm 0.01$	$12.12^{bcd} \pm 0.45$	$1.55^{abc} \pm 0$	$47.48^{de} \pm 0.98$	$1.4^{abc} \pm 0$
T13	$3.24^{abc} \pm 0.02$	$14.5^{c} \pm 0.49$	$3.46^{abcd} \pm 0.09$	$13.43^{ab} \pm 0.06$	$1.41^{abc} \pm 0.04$	$47.46^{de} \pm 0.09$	$1.65^{a} \pm 0.05$
T14	$3.35^{ab} \pm 0.03$	$15.48^{ab} \pm 0.21$	$3.58^{abc} \pm 0.1$	$13.6^{ab} \pm 0.13$	$1.53^{abc} \pm 0.03$	$47.42^{de} \pm 0.12$	$1.30^{abcd} \pm 0$
T15	$2.5^{cd} \pm 0.3$	$12.9^{d} \pm 0.09$	$3.24^{bcde} \pm 0.08$	$11.47^{de} \pm 0.26$	$1.56^{abc} \pm 0.05$	$51.73^{c} \pm 0.41$	$1.50^{ab} \pm 0.1$
T16	$3.8^{a} \pm 0.07$	$16.27^{a} \pm 0.02$	$3.81^{a} \pm 0.01$	$14.07^{a} \pm 0.05$	$1.55^{abc} \pm 0.05$	$45.67^{e} \pm 1.13$	$1.05^{bcd} \pm 0.15$
Trt	0.37**	8.62**	0.24**	6.56**	0.025-NS	33.29**	0.26*
Rep	0.15	0.75	0.149	3.03	0.04	3.68	0.13

[Values are Mean±SEM of two independent experiments. Data points were significant (except Norpatchoulenol) $p \leq 0.05$ according to DMRT]

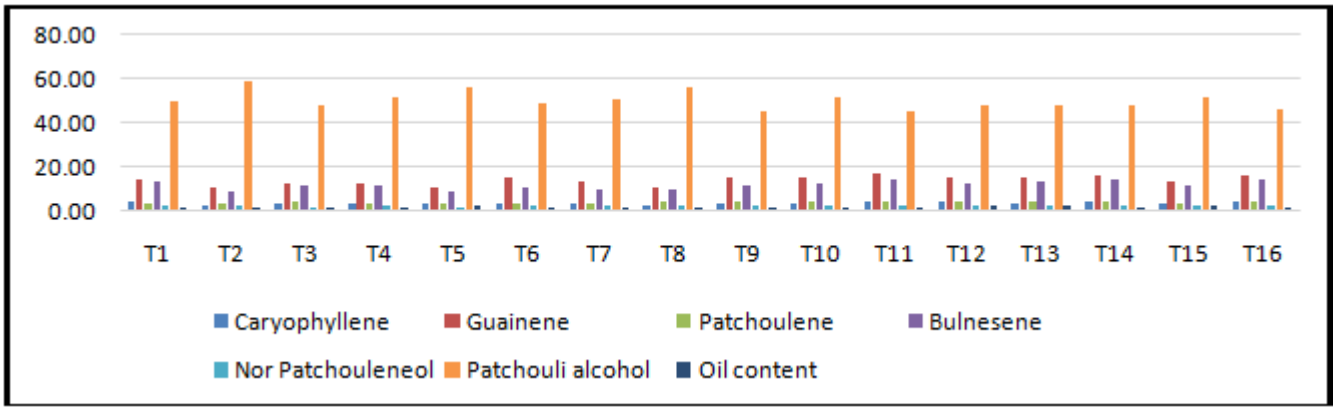

Figure 2: Variation of oil and sesquiterpene content

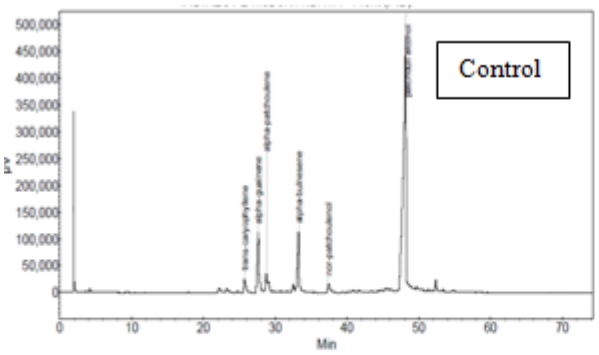

Figure 3a: Gas Chromatographic profile of Control (untreated)

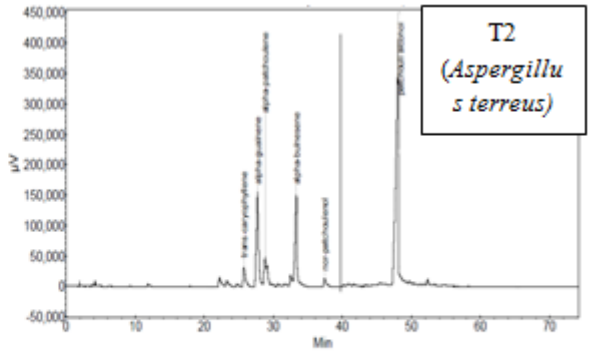

Figure 3b: Gas Chromatographic profile of T2 (*Aspergillus terreus*)