RESEARCH PAPER | International Journal of Agricultural Engineering | Volume 11 | Sp. Issue | April, 2018 | 53-64
ISSN–0974–2662 ■ Visit us : *www.researchjournal.co.in* ■ **DOI: 10.15740/HAS/IJAE/11.Sp. Issue/53-64**

Effect of biotransformation on patchouli oil using GC-MS

■ **Navneet Khare, Dharmendra Khokhar, Soumitra Tiwari, Yashwant Kumar and Yogesh Kumar**

Received : 10.04.2018; **Accepted :** 13.04.2018

See end of the Paper for authors' affiliation

Correspondence to :

Navneet Khare
Department of Agricultural Processing and Food Engineerng, S.V. College of Agricultural Engineering and Technology (Indira Gandhi Krishi Vishwavidyalaya), Raipur (C.G.) India
Email : navneet91001@gmail.com

■ **Abstract :** Biotransformation is the chemical modification made by a microorganism on small molecules as well as on macro-molecules of biological origin. This process results in increased patchouli alcohol content along with improvement in oil extraction. A series of experiments were carried out to understand the biotransformation effect of three selected microorganisms on the quantity and quality of patchouli oil. Dry herbage was the substrate treated with the microbial inoculants. Higher end analysis of the oil samples with GC-MS indicated the effect biotransformation efficiency of different microorganisms on the patchouli oil component. Patchouli alcohol, the active component of the oil is found 31.78, 33.73 and 35.56%, for the oils extracted after the incubation with *Aspergillus foetidus*, *Penicillium citrinum, Trichosporon asteroides*, respectively. While the oil extracted from the fresh and control samples contain 26.63 and 27.35% patchouli alcohol. Other components of the oil also affected by the fermentation/biotransformation process. From the above it can be suggested that fermentation/ bitransformation of patchouli is important for the oil recovery as well as patchouli alcohol percentage.

■ **Key words :** Biotransformation, Patchouli oil, Patchouli alcohol, Fungi

■ **How to cite this paper :** Khare, Navneet, Khokhar, Dharmendra, Tiwari, Soumitra, Kumar, Yashwant and Kumar, Yogesh (2018). Effect of biotransformation on patchouli oil using GC-MS. *Internat. J. Agric. Engg.*, **11**(Sp. Issue) : 53-64, **DOI: 10.15740/HAS/IJAE/11.Sp. Issue/53-64.**

Patchouli (*Pogostemon cablin* Pellet: Lamiaceae), native to South East Asia, produces oil of commercial importance, finds its extensive use in cosmetics, as a fixative and in aromatherapy. The main composition of patchouli oil is patchouli alcohol, nor-patchoulene, bulnesene and beta-patchoulene. Tenacity of odour is the virtue of patchouli oil and is one of the qualities for its versatile use (Reddy, 2012). In aromatherapy it is used for tiredness, tension, dandruff and oily skin or scalp. Patchouli essential oil is widely used in perfumes as one of the important natural essential oils used to give a base, lasting character and fixative ability to a fragrance. The oil is almost a perfume by itself. It is widely used in soap, cosmetic, tobacco and incense production and fancy product. In very low concentration, the oil is used to flavour foods, beverages, candy and bake products. These plant species are also sources of spices, plant based medicines, botanical pesticides, insect repellents, cosmetics, pharmaceuticals and herbal health drinks. The oil gives one of the finest attars when blended with sandalwood oil. It blends well with sandalwood, germanium, vetiver, cedar wood derivatives, clove oil, lavender, bergamot and many others. The oil possesses antibacterial activity and it is used as an ingredient in insect repellant preparations. The leaves and tops are added in bath for their anti-rheumatic action. It is also used as a masking agent for alcoholic breath. The therapeutic properties of patchouli oil are antidepressant, antiseptic, aphrodisiac, astringent, cicatrisant, cytophylactic, deodorant, diuretic, febrifuge,

fungicide, insecticide, sedative and tonic. It is used to sharpen intelligence and improve concentration (Shukor, 2008 and Ramya *et al*., 2013).

Biotransformation is the chemical modification made by a microorganism on a chemical compound. Whole cell biocatalysts using fungi, bacteria and algae have been extensively applied in the flavor and fragrance industry over the last half century. 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. Biocatalysis also provides an environmental friendly alternative for chemical synthesis methods that are known to produce large amounts of harmful wastes. A new strategy is to check the effect of microorganisms using the principle of biotransformation to enhance the quality of patchouli oil.

A series of experiments were carried out to understand the effect of biotransformation of five selected microorganisms on the quality of patchouli oil in terms of odor and chemical composition is been carried out. Dry as well as fresh herbage were the substrate treated with the microbial inoculants.

The world trade in patchouli oil is to the tune of Rs 200 crores. Consumption of this oil in the world is about 2000 tonnes per year. Indonesia meets 90 percent of the total world requirement. The country's demand for patchouli oil is around 220 tonnes of oil per annum and is valued at around rupees 33 crores (Vijayakumar, 2004).

Oil extraction can be enhanced using the pactinase producing microbes which can soften the oil bearing tissues. Patchouli alcohol is the principal active component of patchouli oil, percentage of patchouli alcohol can be increased by incubating the foliage with the culture having dehydrogenase activity.

The value addition in the oil will definitely enhance the quality as well as remuneration from it. This will also enhance the wider range of application in various places. Therefore, in the present need to provide the technology for processing of oil for improvement of percentage of oil and its quality.

METHODOLOGY

The experiment carried out at the department of Agricultural Processing and Food Engineering, faculty of Agricultural Engineering, in collaboration with the department of Plant Physiology, Agricultural Biochemistry, Medicinal and Aromatic Plants, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.).

Selection and procurement of microorganisms :

Preliminary screening was carried out by searching the database of National Repositories for the microbes having pactinase and dehydrogenase activity. Further, the cultures were selected on the basis of enzyme activity for pactinase and dehydrogenase enzymes reported in literatures by various researchers. On the basis of above information following four groups were selected namely *Aspergillus*, *Penicillium*, *Trichosporon* and *Bacillus*. Finally three fungal strains were selected and procured from Microbial Type Collection Centre (MTCC), Chandigarh.

The microbial cultures used in the study are given in Table A.

Table A : Culture procured for fermentation

Sr. No.	Name of the culture	Source
1.	*Aspergillus foetidus* MTCC 10559	MTCC, Chandigarh
2.	*Penicillium citrinum* MTCC 6590	MTCC, Chandigarh
3.	*Trichosporon asteroids* MTCC 7632	MTCC, Chandigarh

Fig. A : Culture procured for fermentation

Media preparation :

Media for the revival and maintenance of the procured fungi strains were MGYP solid media having composition: malt extract-0.3g, glucose-1.0g, yeast extract-0.3 g, peptone-0.3 g and agar-agar-2g per 100ml and CYP composition: Czapek concentrate–100ml, ($NaNO_3$-30g, KCl-5 g, $MgSO_4.7H_2O$-5g, $FeSO_4.7H_2O$ -0.1 g) stored without sterilization. Czapak concentrate -1ml, yeast extract-0.5g, K_2HPO_4-0.1 g, dextrose-3 g, agar-agar-1.5g per 100 ml. The pH was maintained at

7.0 using dilute HCl or NaOH and the media was autoclaved at 121°C and 15 psi for 20 minute for the sterilization purpose.

Maintenance of cultures :

Sterilization :

The nutrient media, solutions and glassware used during experimentations were plugged with cotton and steam sterilized in an autoclave at 121°C for 15-20 min.

Inoculations :

All inoculations were done over a flame in the laminar air flow chamber. The chamber was initially surface sterilized with rectified spirit and by UV irradiation.

Revival of cultures :

Cultures were supplied by MTCC in lyophilized form. These strains were revived separately on specified media in petri plates at 28±1°C in Remi make incubator. Further, single colony of the culture from the petri plate was inoculated in the 50 ml MGYP broth in 100 ml conical flask separately and incubated for 48 hours at 28±1°C. The 24hr grown cultures was used as mother culture/ pre-culture. Mother cultures were again inoculated in the broth to find out the log phase of the culture. Cultures were used as inoculums in all the experiment only from the specified time (log phase).

Fig. B : Autoclave

Treatment and scale up :

An experiment with all three procured cultures (*Aspergillus foetidus, Penicillium citrinum, Trichosporon asteroides)* was carried out for three different incubation period. All the experiments were done in triplicate. All three *(Aspergillus foetidus, Penicillium citrinum, Trichosporon asteroides)* strains were grown in 100 ml conical flasks at specified conditions in 50 ml of sterilized MGYP and CYP media. After optimum growth the mycelia were separated from broth by filtration with Whattman No. 1 filter paper. The separated mycelia were blended with sterile distilled water to break the

Fig. C : Laminar

Fig. D : Incubator

Fig. E : Growth mycelia

clumps; this was the inoculum (biocatalyst) for the experiment.

Biotrasformation of patchouli herbage :

The harvested dried herbage sample 200 g each was sprayed with the different cultures and kept for incubation at room temperature. Control (herbage sprayed with only distilled water) was also kept for comparison. Extraction of volatile oil was done at 0 day, 2nd day, 4th day, 6th and 8th day of incubation.

Fig. F : Fermentation process on patchouli

Fig. G : Fermented patchouli

■ RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Gas chromatography mass spectrometer (GC-MS) analysis :

The analysis of extracted oil was carried out with the Shimadzu GC-MS QP-2010 plus, with the condition described in material method.

Chemical characterization and quantification of compounds :

The chemical components present in the patchouli oil were analyzed by Gas Chromatography-Mass Spectroscopy and identified by mass spectra. The data are presented in table. Volatile oil was extracted from the samples after incubation with three different microbial culture *Aspergillus foetidus* 10559, *Penicillium citrinum* 6590 and *Trichosporon asteroides* 7632 after different intervals of time *viz.*, 2, 4, 6 and 8 days along with the fresh and control samples.

The major compounds identified are (α-pinene, β–pinene, Copaene, caryophyllene, Naphthalene, Azulene, Thujopsene, Benzene, Patchoulene, Caryophyllene oxide, α–Guaniene, Epiglobulol, longifolenaldehyde, α –humulene and Patchouli alcohol). The different treatments of compounds are detected in GC-MS analysis at different retention time.

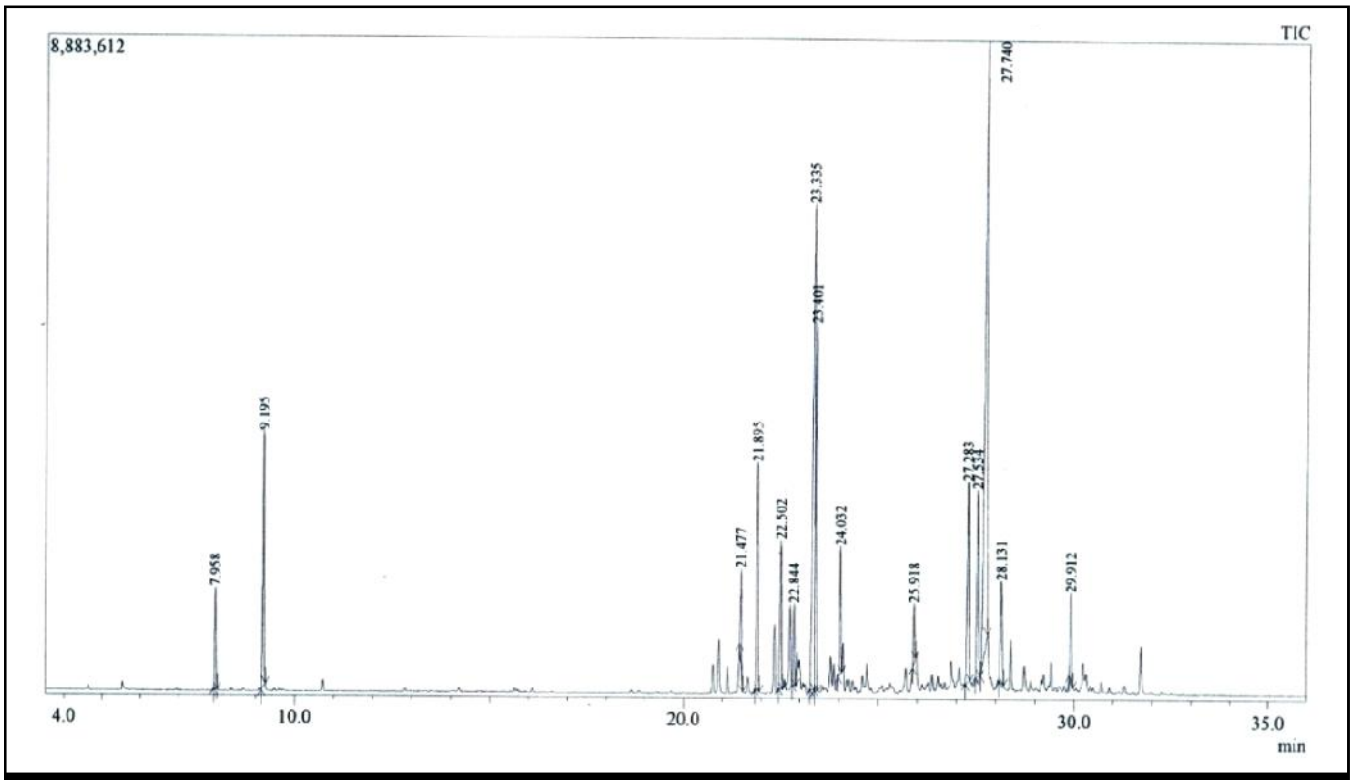

Fig. 1 : Chromatograph of patchouli oil extracted from fresh sample

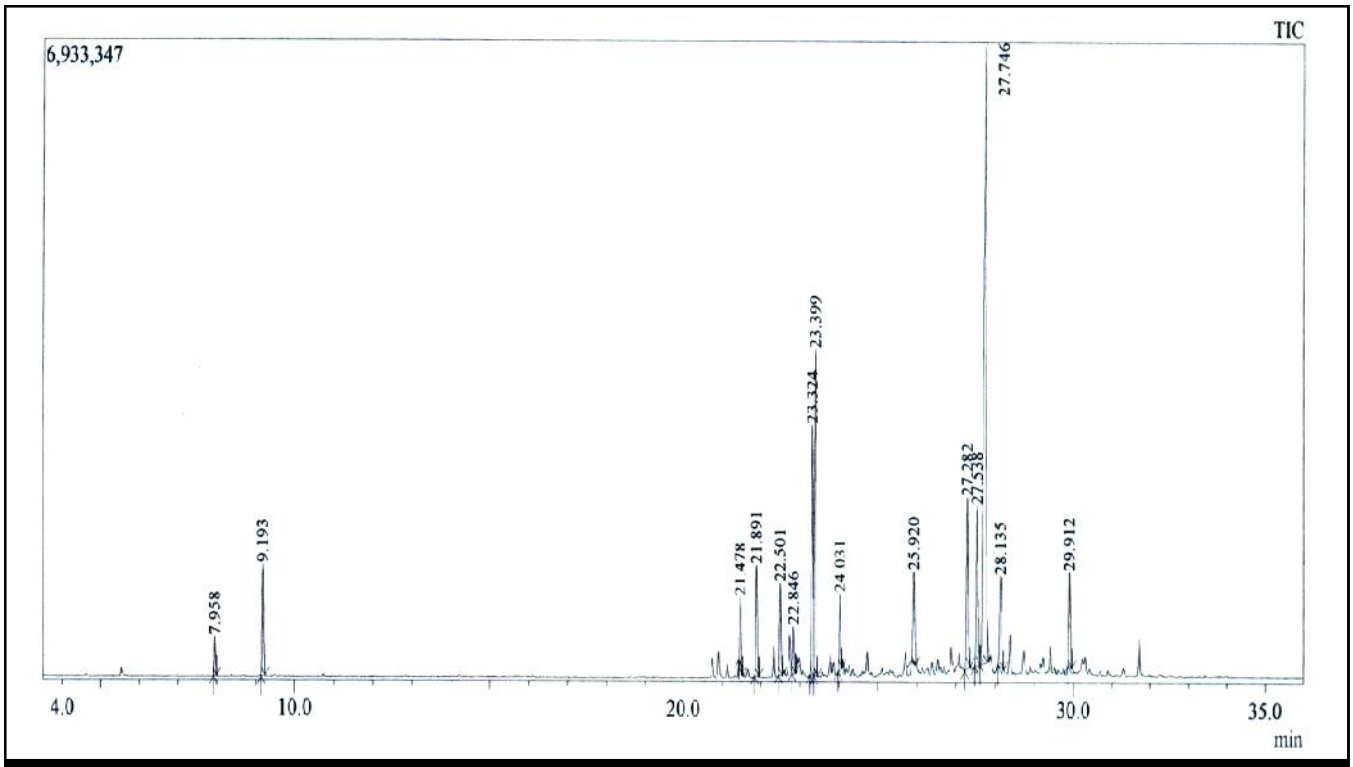

Fig. 2 : Chromatograph of patchouli oil extracted from control sample

Chemical composition of patchouli oils extracted after incubation with *Aspergillus foetidus* :

The data given in Table 1 and Fig. 3-5 presents the analysis of oil extracted from the fermented herbage (herbage incubated with culture *Aspergillus foetidus*) after different intervals of times. The major content of sesquiterpene, patchouli alcohol varied from 29.85, 30.64 and 31.78% in culture treated samples at 2, 4, and 6 days. Fresh and control samples showed 26.63 and 27.35% patchouli alcohol. The other component like α-pinene varied from 1.87 to 1.82%, β–pinene 5.42 to 5.35%, Copaene 3.06 to 2.90%, Caryophyllene 5.64 to 5.34%, Naphthalene 4 to 3.93%, Azulene 1.98 to 1.57%, Thujopsene 13.01 to 12.62%, Benzene 11.73 to 12.39, Patchoulene 2.98 to 2.86, Caryophyllene oxide 2.01 to 1.92%, α–Guaniene 6.1 to 5.84%, Epiglobulol 5.69 to 5.53%, longifolenaldehyde 3.8 to 3.4%, α–humulene 2.84 to 2.75%. Among the treatments, oil extracted after 6

Table 1 : GC-MS data showing the biotransformation effect of *Aspergillus foetidus* on patchouli oil

Sr. No.	RT(min)	Compound	Fresh	Control	Day2	Day4	Day6
1.	7.95	α –pinene	2.28	2.2	1.87	1.56	1.82
2.	9.19	β –pinene	6.39	6.19	5.42	4.68	5.35
3.	21.47	Copaene	2.23	3.82	3.06	2.58	2.90
4.	21.89	Caryophylliene	6.64	6.31	5.64	5.64	5.34
5.	22.5	Naphthalene	4.14	4.03	4.00	3.99	3.93
6.	22.84	Azulene	2.16	2.04	1.98	1.89	1.57
7.	23.33	Thujopsene	17.14	16.11	13.01	14.48	12.62
8.	23.4	Benzene	9.90	9.3	11.73	12.2	12.39
9.	24.03	Patchoulene	3.16	3.28	2.98	3.13	2.86
10.	25.91	Caryophyllene oxide	1.42	2.02	2.01	3.18	1.92
11.	27.28	α –Guaniene	6.08	5.78	6.1	5.83	5.84
12.	27.53	Epiglobulol	5.72	5.55	5.69	5.29	5.53
13.	27.74	Patchouli alcohol	26.63	27.35	29.85	30.64	31.78
14.	28.13	Longifolenaldehyde	3.73	3.6	3.8	3.46	3.4
15.	29.91	α –humulene	2.36	2.35	2.84	2.83	2.75

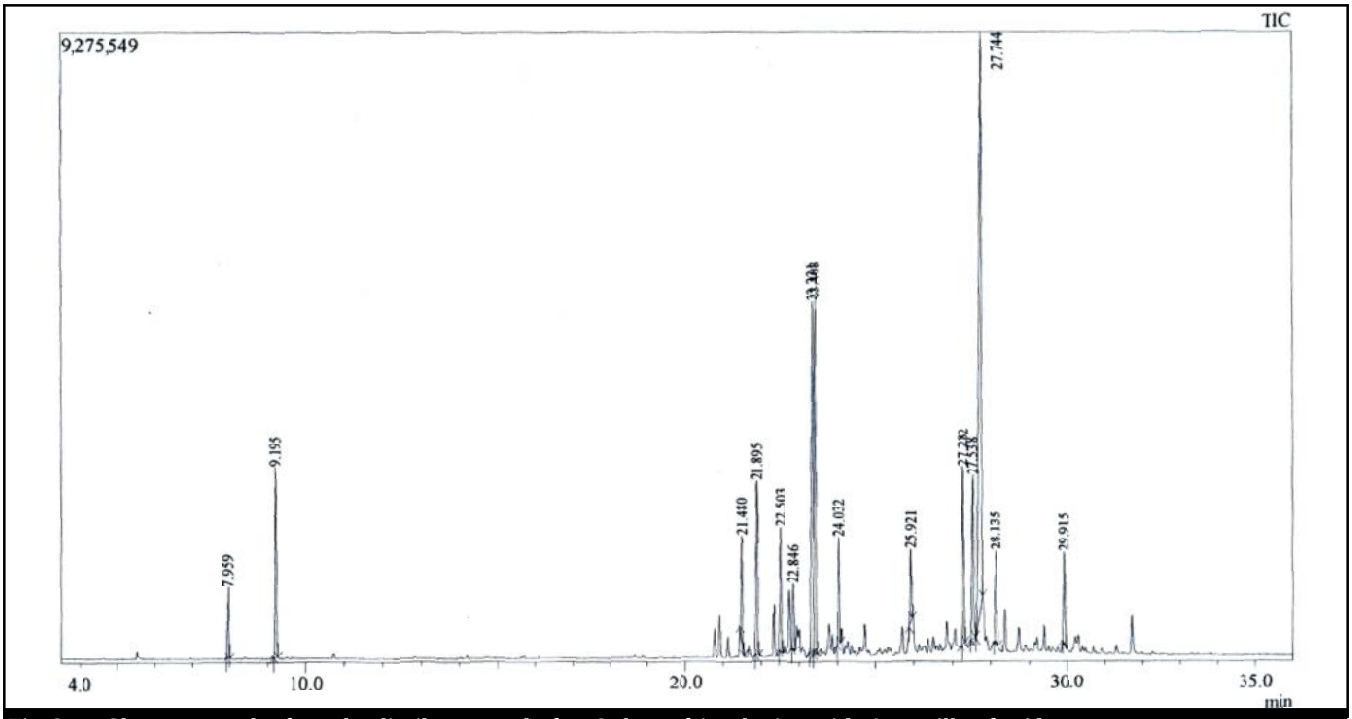

Fig. 3 : Chromatograph of patchouli oil extracted after 2 days of incubation with *Aspergillus foetidus*

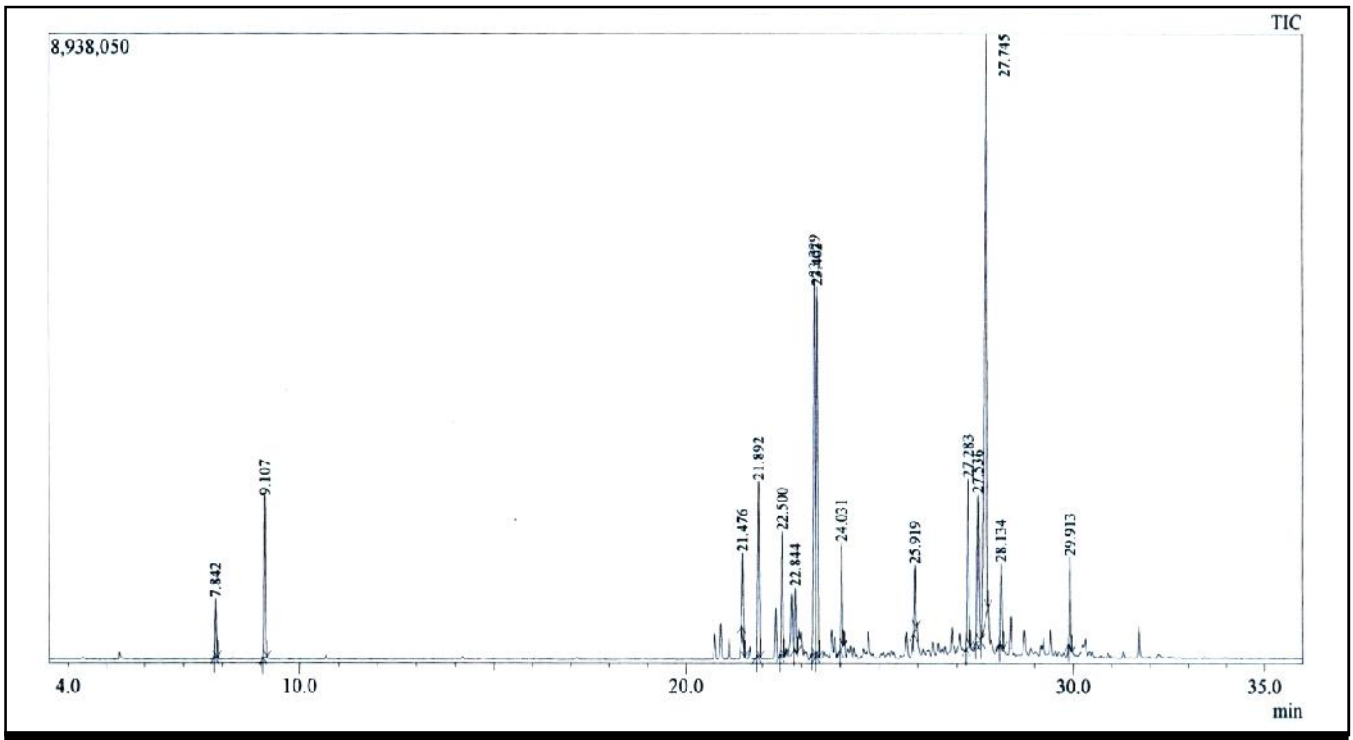

Fig. 4 : Chromatograph of patchouli oil extracted after 4 days of incubation with *Aspergillus foetidus*

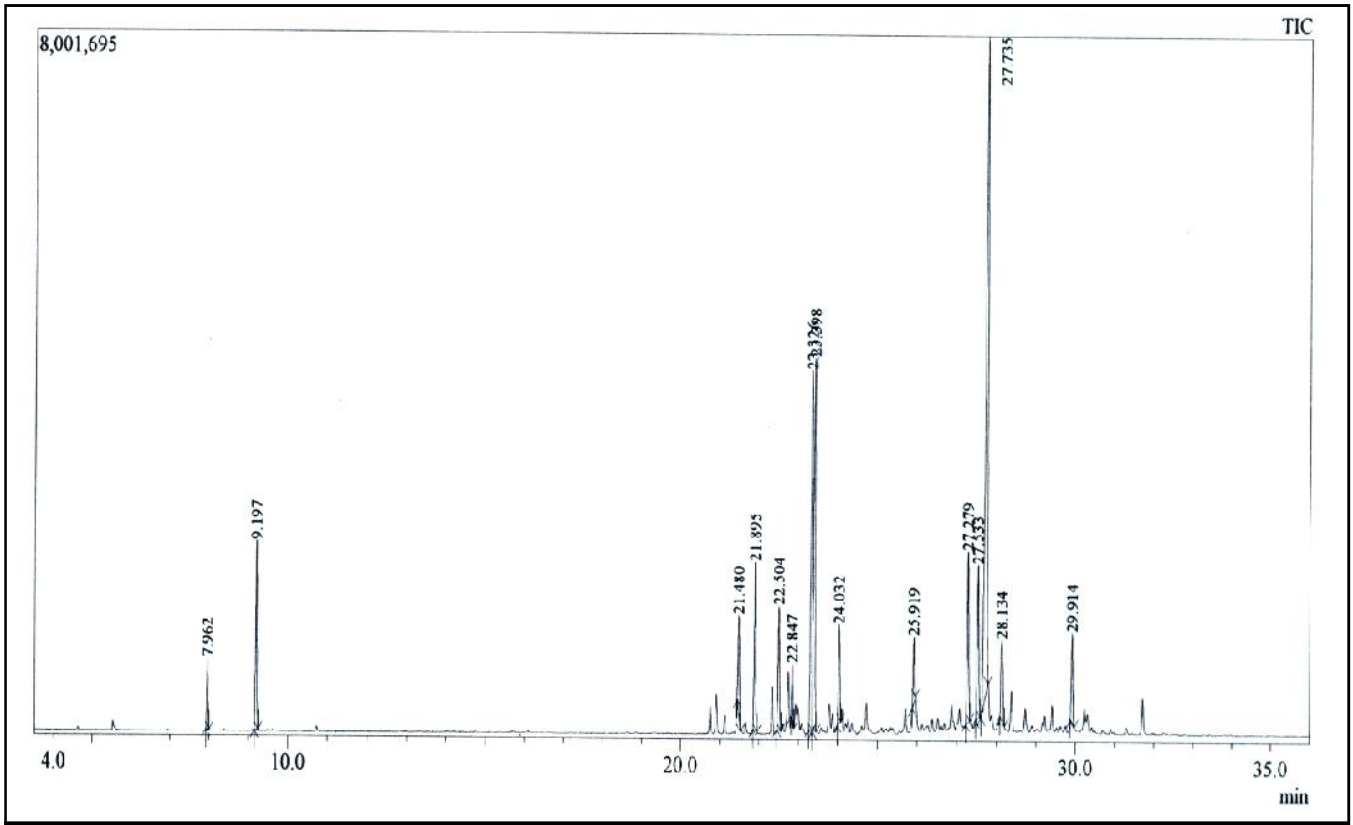

Fig. 5 : Chromatograph of patchouli oil extracted after 6 days of incubation with *Aspergillus foetidus*

days of incubation gives statistically superior oil quality in respect to patchouli alcohol, as the amount of patchouli alcohol was 31.78% as compared to untreated control 27.35% and fresh 26.63%.

Chemical composition of patchouli oils extracted after incubation with *penicillium citrinum* :

The data given in Table 2 and Fig. 6-8 presents the analysis of oil extracted from the fermented herbage (herbage incubated with culture *penicillium citrinum*) after different intervals of times. The major content of sesquiterpene, patchouli alcohol varied from 30.01, 31.21 and 33.73% in culture treated samples at 2, 4 and 6 days. Fresh and control samples showed 26.63 and 27.35% patchouli alcohol. The other component like α-pinene varied from 1.58 to 1.78%, β –pinene 4.79 to 5.27%, Copaene 2.5 to 2.85%, Caryophyllene 6.32 to 4.99%, Naphthalene 4.09 to 3.58%, Azulene 2.05 to 1.69%,

Table 2 : GC-MS data showing the biotransformation effect of *Penicillium citrinum* on patchouli oil

Sr. No.	RT(min)	Compound	Fresh	Control	Day2	Day4	Day6
1.	7.95	α –pinene	2.28	2.2	1.58	2.13	1.78
2.	9.19	β –pinene	6.39	6.19	4.79	4.78	5.27
3.	21.47	Copaene	2.23	3.82	2.5	3.37	2.85
4.	21.89	Caryophylliene	6.64	6.31	6.22	5.29	4.99
5.	22.5	Naphthalene	4.14	4.03	4.09	3.9	3.58
6.	22.84	Azulene	2.16	2.04	2.05	1.92	1.69
7.	23.33	Thujopsene	17.14	16.11	14.27	12.34	11.39
8.	23.4	Benzene	9.9	9.3	8.97	9.71	11.58
9.	24.03	Patchoulene	3.16	3.28	3.3	2.71	2.73
10.	25.91	Caryophyllene oxide	1.42	2.02	1.08	2.63	2.15
11.	27.28	α –Guaniene	6.08	5.78	6.71	6.29	6.03
12.	27.53	Epiglobulol	5.72	5.55	6.35	5.95	5.68
13.	27.74	Patchouli alcohol	26.63	27.35	30.01	31.21	33.73
14.	28.13	Longifolenaldehyde	3.73	3.6	4.48	4.05	3.79
15.	29.91	α –humulene	2.36	2.35	3	2.99	2.76

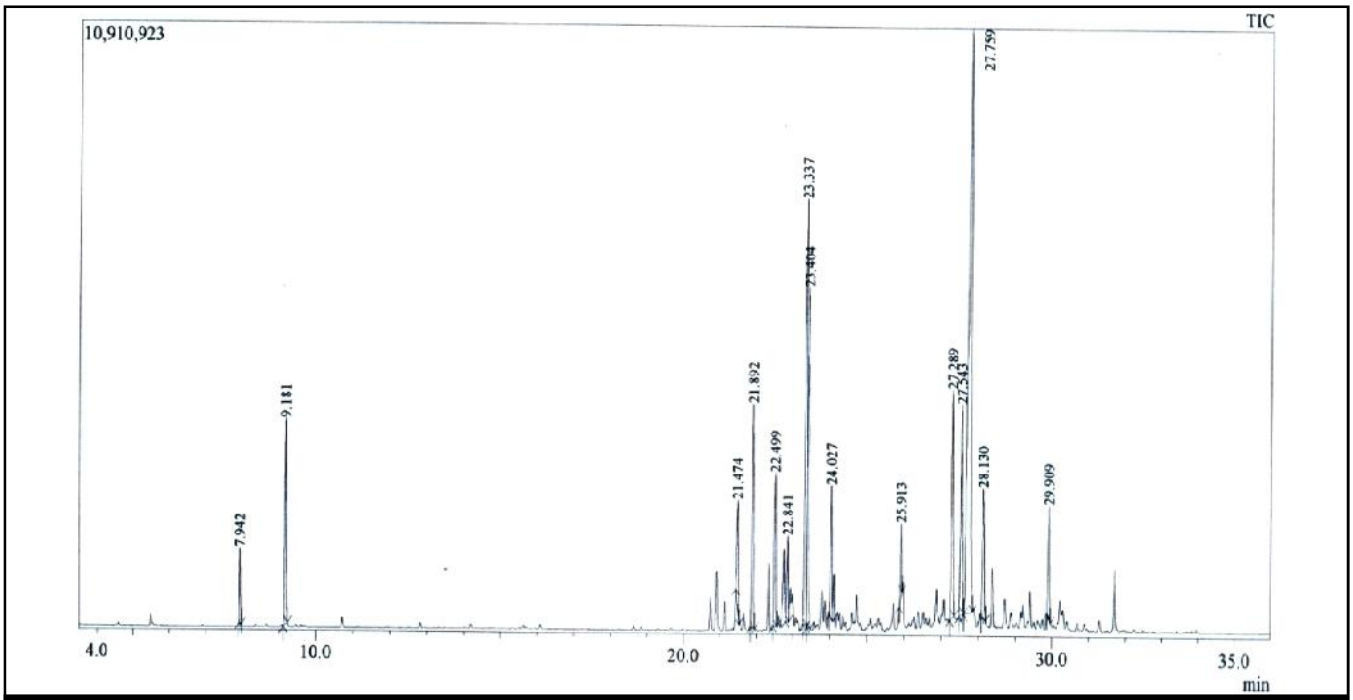

Fig. 6 : Chromatograph of patchouli oil extracted after 2 days of incubation with *Penicillium citrinum*

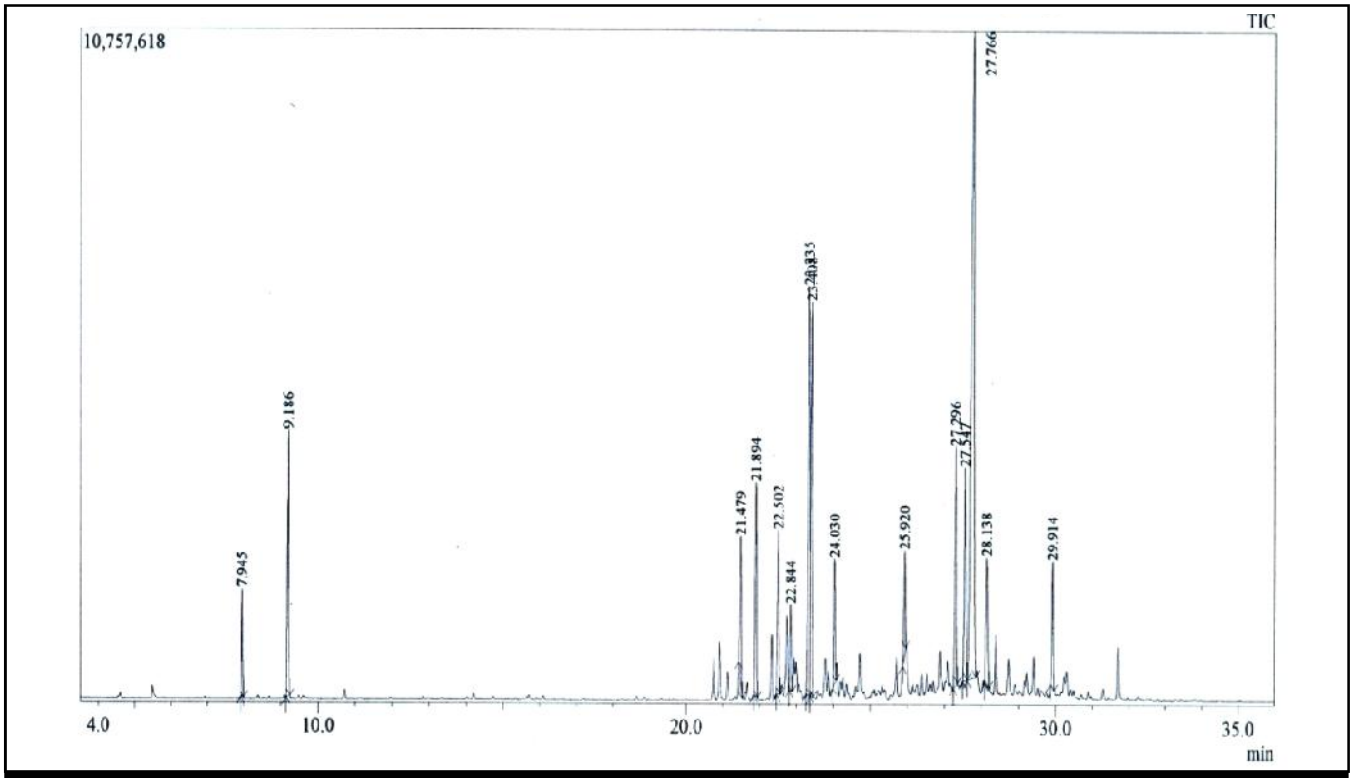

Fig. 7 : Chromatograph of patchouli oil extracted after 4 days of incubation with *Penicillium citrinum*

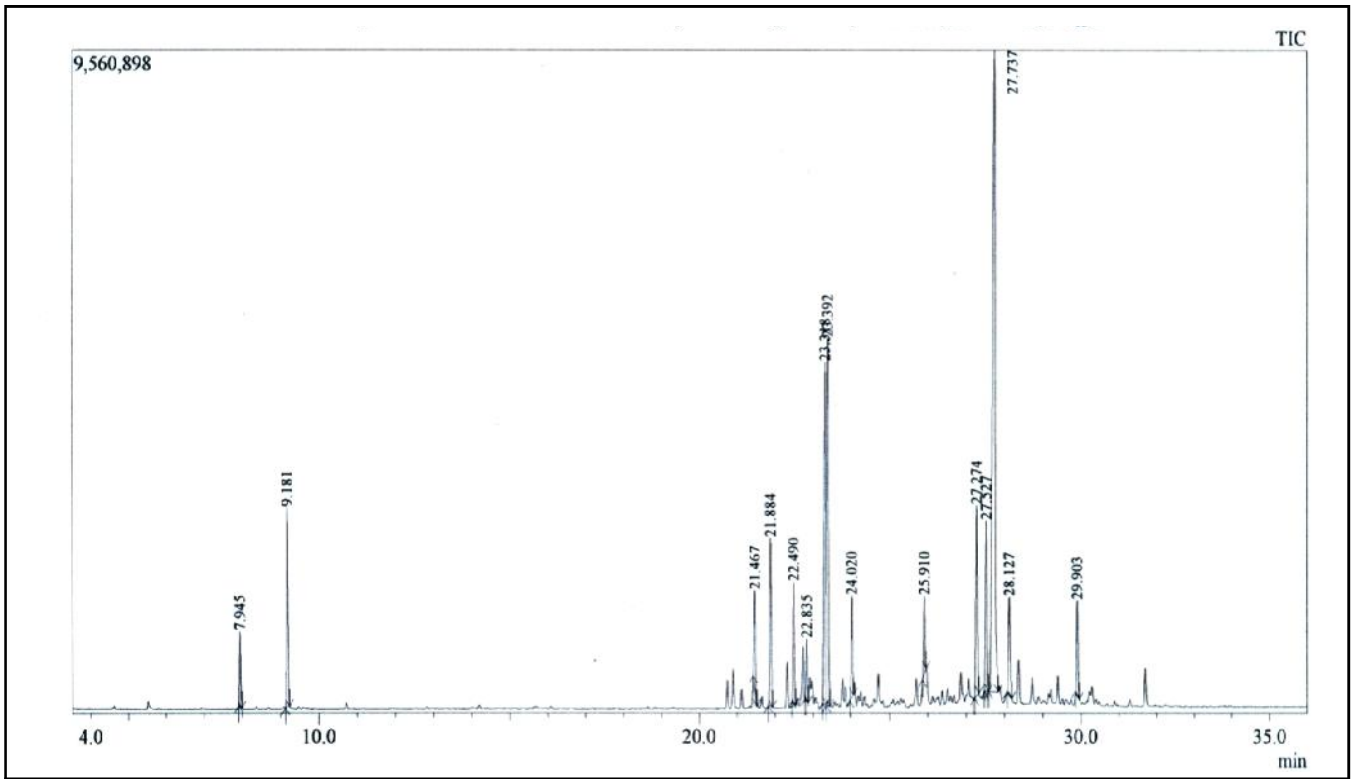

Fig. 8 : Chromatograph of patchouli oil extracted after 6 days of incubation with *Penicillium citrinum*

Thujopsene 14.27 to 11.39%, Benzene 8.97 to 11.58, Patchoulene 3.3 to 2.73%, Caryophyllene oxide 1.08 to 2.15%, α–Guaniene 6.71 to 6.03%, Epiglobulol 6.35 to 5.68%, longifolenaldehyde 4.48 to 3.79%, α–humulene 3 to 2.76%. Among the treatments, oil extracted after 6 days of incubation gives statistically superior oil quality in respect to patchouli alcohol, as the amount of patchouli alcohol was 33.73% as compared to untreated control 27.35% and fresh 26.63%.

Chemical composition of patchouli oils extracted after incubation with *Trichosporon asteroides* :

The data given in Table 3 and Fig. 9-11 presents the analysis of oil extracted from the fermented herbage

Table 3 : GC-MS data showing the biotransformation effect of *Trichosporon asteroides* on patchouli oil

Sr. No.	RT(min)	Compound	Fresh	Control	Day 2	Day 4	Day 6
1.	7.95	α –pinene	2.28	2.2	1.23	2.02	1.44
2.	9.19	β –pinene	6.39	6.19	4.44	5.75	4.95
3.	21.47	Copaene	2.23	3.82	2.39	3.06	2.96
4.	21.89	Caryophylliene	6.64	6.31	5.96	5.54	4.5
5.	22.5	Naphthalene	4.14	4.03	4.24	3.94	3.44
6.	22.84	Azulene	2.16	2.04	2.07	1.92	1.47
7.	23.33	Thujopsene	17.14	16.11	10.69	13.52	8.46
8.	23.4	Benzene	9.9	9.3	13.15	10.94	10.8
9.	24.03	Patchoulene	3.16	3.28	3.11	2.85	2.33
10.	25.91	Caryophyllene oxide	1.42	2.02	1.91	2.07	2.23
11.	27.28	α –Guaniene	6.08	5.78	6.77	5.68	7.15
12.	27.53	Epiglobulol	5.72	5.55	6.32	5.13	6.66
13.	27.74	Patchouli alcohol	26.63	27.35	31.08	31.55	35.56
14.	28.13	Longifolenaldehyde	3.73	3.6	3.9	3.45	4.41
15.	29.91	α –humulene	2.36	2.35	2.74	2.58	3.64

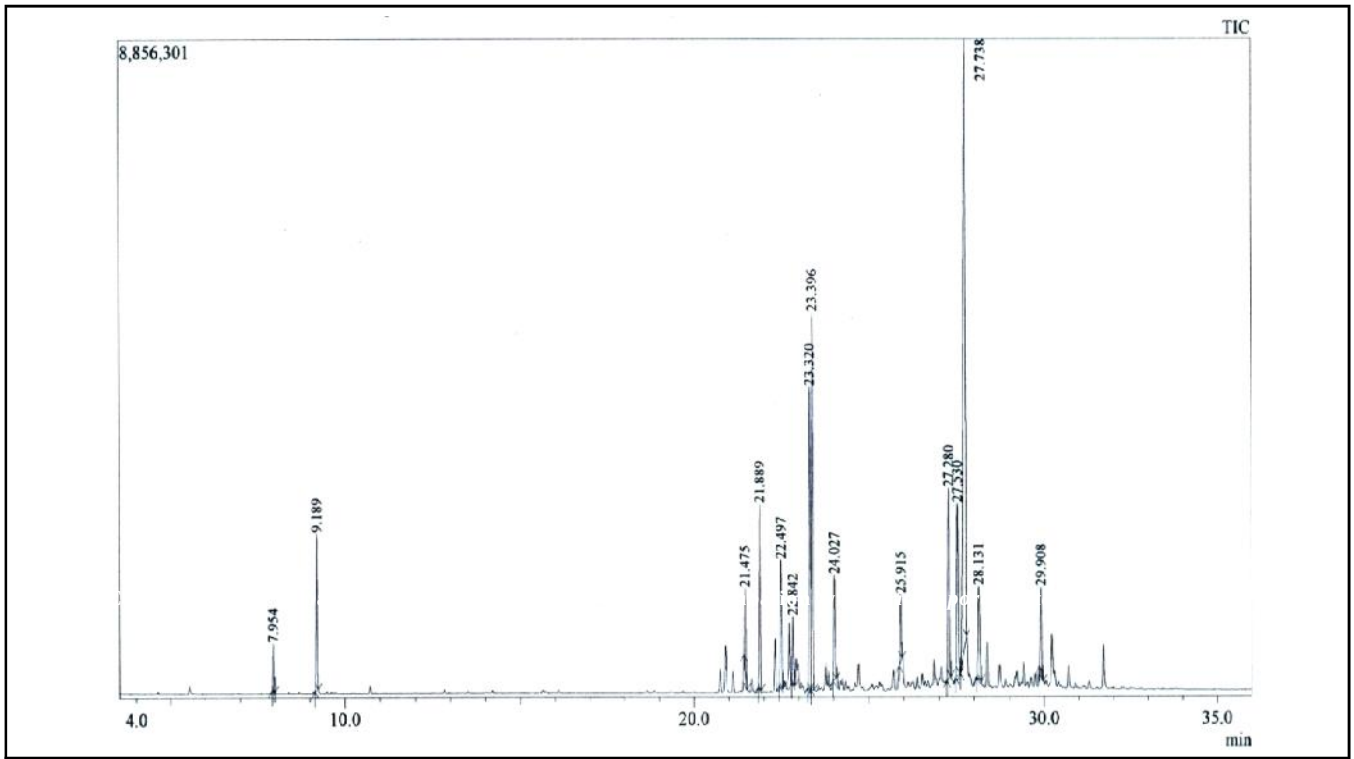

Fig. 9 : Chromatograph of patchouli oil extracted after 6 days of incubation with *Penicillium citrinum*

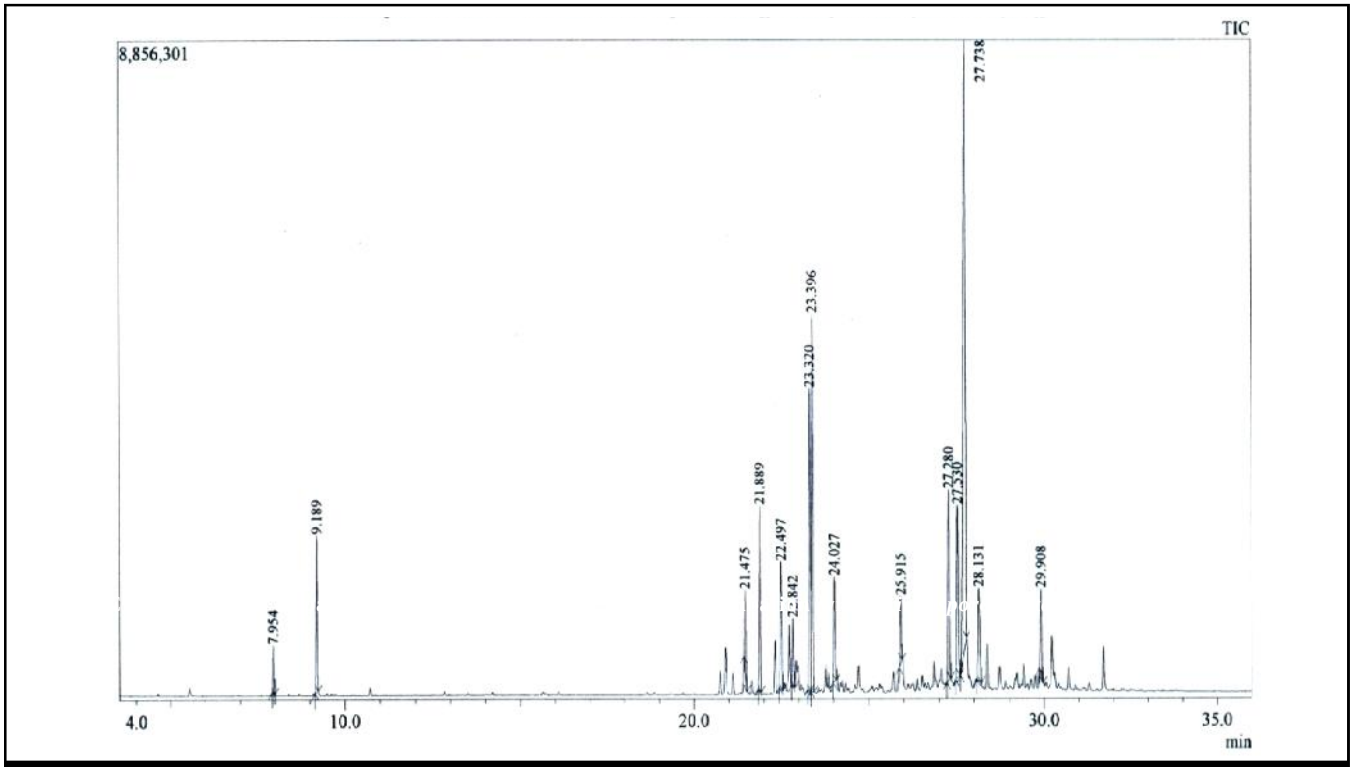

Fig. 10 : Chromatograph of patchouli oil extracted after 6 days of incubation with *Penicillium citrinum*

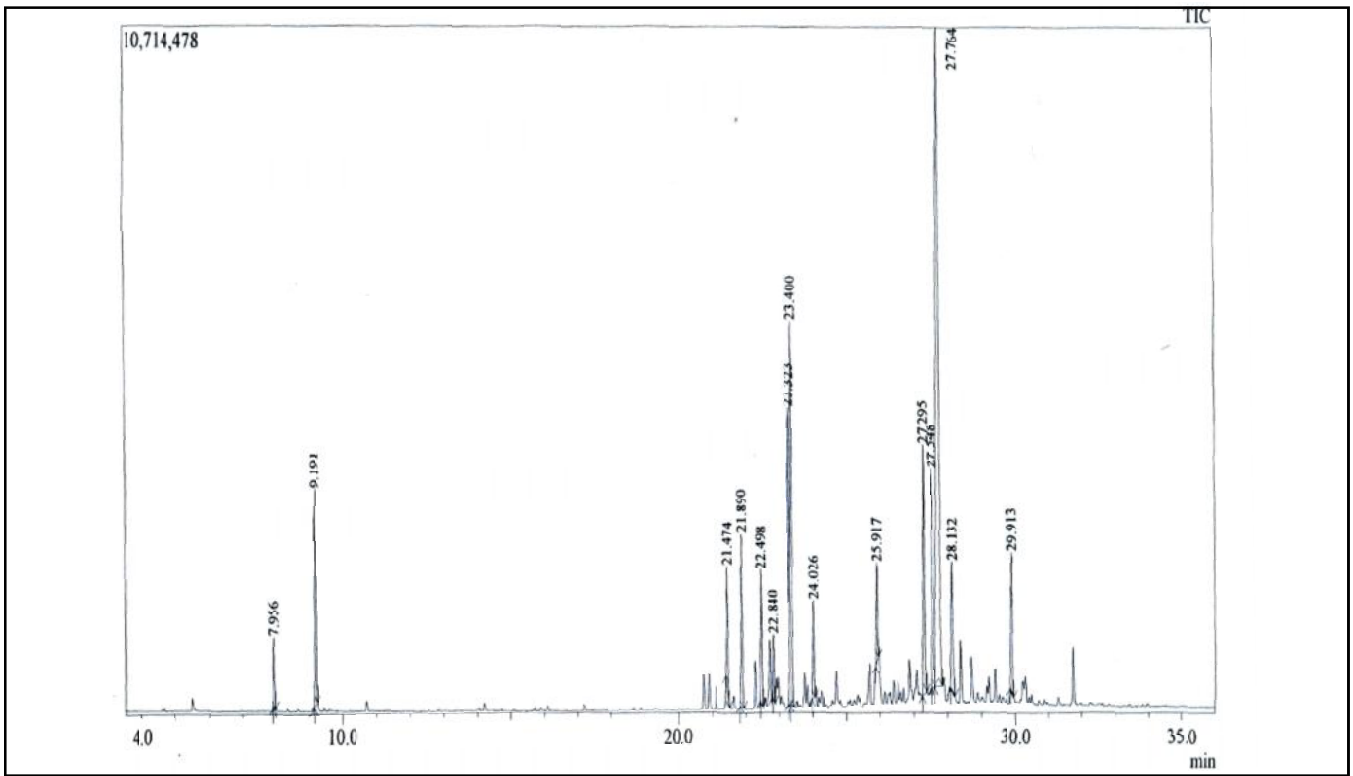

Fig. 11 : Chromatograph of patchouli oil extracted after 6 days of incubation with *Trichosporon asteroides*

(herbage incubated with culture *Trichosporon asteroides*) after different intervals of times. The major content of sesquiterpene, patchouli alcohol varied from 31.08, 31.55 and 35.56% in culture treated samples at 2, 4, 6 days. Fresh and control samples showed 26.63 and 27.35% patchouli alcohol. The other component like α-pinene varied from 1.23 to 1.44%, α–pinene 4.44 to 4.95%, Copaene 2.39 to 2.96%, Caryophyllene 5.96 to 4.5%, Naphthalene 4.24 to 3.44%, Azulene 2.07 to 1.47%, Thujopsene 10.69 to 8.46%, Benzene 13.15 to 10.8, Patchoulene 3.11 to 2.33%, Caryophyllene oxide 1.91 to 2.23%, α–Guaniene 6.77 to 7.15%, Epiglobulol 6.32 to 6.66%, longifolenaldehyde 3.9 to 4.41%, α–humulene 2.74 to 3.64% and patchouli alcohol 31.08 to 35.56%. Among the treatments, oil extracted after 6 days of incubation gives statistically superior oil quality in respect to patchouli alcohol, as the amount of patchouli alcohol was 35.56% as compared to untreated control 27.35% and fresh 26.63%.

Conclusion :

Biotransformation is the chemical modification made by a microorganism on chemical/biochemical molecules. 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. A new strategy is to check the effect of microorganisms using the principle of biotransformation to enhance the recovery as well as quality of patchouli oil. On the basis of the experiment and observation, the findings are as follows:

Patchouli alcohol, an important and major component of the oil increases with the incubation period with all three cultures.

The best results were obtained with *Trichosporon asteroides* in improving the recovery of patchouli oil and patchouli alcohol content upto 1.98% and 35.56%, respectively.

The studies revealed a definite pattern of decrease in monoterpenes and sesquiterpenes and increase in patchouli alcohol content in most treatments.

Biotransformation method can be utilized to improve oil recovery along with the oil quality.

Authors' affiliations:

Dharmendra Khokhar, Department of Agricultural Processing and Food Engineerng, S.V. College of Agricultural Engineering and Technology (IGKV), Raipur (C.G.) India

Soumitra Tiwari, Yashwant Kumar and Yogesh Kumar, Department of Food Processing and Technology, Bilaspur University, Bilaspur (C.G.) India

REFERENCES

Ramya, H.G., Palanimuthu V. and Rachna, S. (2013). An introduction to patchouli (*Pogostemon cablin* Benth.) – A medicinal and aromatic plant: It's importance to mankind. Agri. Engg. Int: *CIGR Journal*, **15**(2): 243-250.

Reddy, B.R.N. (2012). A value chain on enhanced productivity and profitability of Patchouli (*PogostemonPatchouli*). ICAR, Jewargi Agro Food Park Ltd., (JAFPL) Bangalore, Karnataka. p. 34-40.

Shukor, Muhd Zahiruddin Bin (2008). Extraction Of Essential Oils From Patchouli Leaves Using Ultrasonic-Assisted Solvent Extraction Method. Thesis, Universiti Malaysia Pahang.

Vijayakumar, K (2004). Patchouli and India–a great leap forward, National Seminar of Prospectus and Potentials of Medicinal and Aromatic Crops, 106–10.