PILOT SCALE STUDY FOR ENHANCEMENT OF PATCHOULI ESSENTIAL OIL QUANTITY & QUALITY USING BIOTRANSFORMATION PROCESS

M. Tech. (Agril. Engg.) Thesis

by

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DEPARTMENT OF AGRICULTURAL PROCESSING AND FOOD ENGINEERING

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FACULTY OF AGRICULTURAL ENGINEERING

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Thesis

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in

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CERTIFICATE-I

This is to certify that the thesis entitled **"Pilot scale study for enhancement of patchouli essential oil quantity & quality using biotransformation process"** submitted in partial fulfillment of the requirements for the degree of **Master of Technology in Agricultural Engineering** of the Indira Gandhi Krishi Vishwavidyalaya, Raipur, is a record of the bonafide research work carried out by **Bhoj Ram** under our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instructions.

No part of the thesis has been submitted for any other degree or diploma or has been published/published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by him.

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Date: 20.07.17

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Member	(Dr. R. Soni)	
Member	(Er. N. K. Mishra)	

CERTIFICATE – II

This is to certify that the thesis entitled **"Pilot scale study for enhancement of patchouli essential oil quantity & quality using biotransformation process"** submitted by **Bhoj Ram** to the Indira Gandhi Krishi Vishwavidyalaya, Raipur, in partial fulfillment of the requirements for the degree of **Master of Technology in Agricultural Engineering** in the Department of **Agricultural Processing and Food Engineering** has been approved by the external examiner and Student's Advisory Committee after oral examination.

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Place: Raipur

BRsinha

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LIST OF SYMBOLS

±	Plus/minus
%	Percent
@	At the rate
g	Gram
et al.	*et alibi*
ºC	Degree Celsius
h	hour
ml	milliliter
mg	milligram
µl	micro liter
α	Alpha
β	Beta
C	Culture
etc	Etcetera
viz.	Videlicet
i.e.	That is
L	Liter
mc	moisture content
wb	wet basis
db	dry basis
V	Volume
W	Weight
kg	kilogram

LIST OF ABBRAVATIONS

Fig.	Figure
GC-MS	Gas chromatography-Mass spectrometer
N	Normality
Agri.	Agriculture
ANOVA	Analysis of variance
M. W.	Molecular Weight
CD	Coefficient of Deviation
j.	Journal
M.Tech.	Master of Technology
Engg.	Engineering
SD	Standard Deviation
Temp.	Temperature
pH	Physical hydrolysis
B.P.	Boiling point
FAE	Faculty of Agricultural Engineering
IGKV	Indira Gandhi Krishi Vishwavidyalaya
C.G.	Chhattisgarh
Dept.	Department
SVCET&RS	Swami Vivekanand College of Agricultural and Technology & Research Station

THESIS ABSTRACT

Title of the Thesis	:	Pilot scale study for enhancement of patchouli essential oil quantity & quality using biotransformation process
Full Name of the Student	:	Bhoj Ram
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Name and Address of the Major Advisor	:	Dr. D. Khokhar Scientist, Department of Agricultural Processing and Food Engineering, SVCAET & RS, FAE, IGKV, Raipur
Degree to be Awarded	:	Master of Technology in Agricultural Engineering

Signature of the Student

Signature of Major Advisor

Signature of Head of the Department

Date: 20.07.17

ABSTRACT

Patchouli (Pogostemon cablin) is an aromatic crop, belonging to the family Lamiaceae and is commonly known as Patchouli. It is native to subtropical Himalayas, Southeast Asia and the Far East, and has been cultivated extensively in Indonesia, Malaysia, China, and Brazil for the essential oil namely "Patchouli Oil". The leaves constitute the economic part, which contain the oil glands. Currently, India is producing a meager quantity of patchouli oil and the main challenge is to produce good quality oil that can compete with the Indonesian oil.

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.

The aim of this study was to increase oil recovery as well as patchouli alcohol percent in patchouli oil by fungi biotrasformation. Drying of herbage before extraction was done to nearly 15% moisture content. The shade dried samples were kept at room temperature and

treated with microbial culture *Aspergillus foetidus, Penicillium citrinum* and *Trichosporon asteroides* for 2, 4, 6 and 8 days along with fresh and control samples. It was observed that the oil recovery increased by the treatment of all three cultures *viz. Aspergillus foetidus, Penicilliun citrinum* and *Trichosporon asteroides* from 1.17, 1.33, 1.42 and 1.43 % (w/w); 1.22, 1.35, 1.48 and 1.49% ; 1.35, 1.47, 1.60 and 1.62 % (w/w) respectively. Recovery of patchouli oil gradually increases with the time of incubation with the cultures, whereas remains constant in fresh sample and control samples. The oil recovery was highest in the herbage samples treated with the *Trichosporon asteroides.* Increase in the oil recovery may be due to the pactinase enzyme which is produced and extra cellularlly secreted by the microbial cultures. The difference in oil extracting efficiency largely may be due to difference in the activity/ secreted quantity of pactinase enzyme.

The patchouli oil samples extracted during the study were also analyzed for its physico-chemical properties. All the extracted oil samples have shown the values of physico-chemical parameter in the standard permissible range of patchouli oil except the oil extracted after 6 days of incubation with the culture *Aspergillus foetidus, Penicillium citrinum Trichosporon asteroides*.The acid value of the oil extracted after 6 days incubation of the samples with *Aspergillus foetidus, Penicillium citrinum, Trichosporon asteroides* increases beyond 4.0, which is not permissible range for the patchouli oil.

The essential oil was extracted by steam distillation and the components of patchouli oil were identified by Gas chromatography/Mass Spectrometry (GC/MS). Fifteen compounds were identified with Patchouli alcohol as the major component followed by benzene. The oil content was in the range 1-1.65 % on fresh weight basis. *Trichosporon asteroids* was found to be statistically superior with high patchouli alcohol (31.25%).

Higher end analysis of the oil samples with GC-MS indicated the effect of biotransformation effeciency of different microorganisms on the patchouli oil component. Patchouli alcohol, the active component of the oil is 30.27%, 28.11% and 31.25% for the oils extracted after the incubation with *Aspergillus foetidus, Penicillium citrinum, Trichosporon asteroides* respectively. While the oil extracted from the fresh samples contain 25.75% 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.

शोध प्रबंध

शोध प्रबंध का शीर्षक	:	जैव रूपांतरण प्रकिया का उपयोग करके पचौली आवश्यक तेल मात्रा और गुणवत्ता बढ़ाने के लिये प्रारम्भिक पैमाने पर अध्ययन
छात्र का पूरा नाम	:	भोज राम
प्रमुख विषय	:	कृषि प्रसंस्करण एवं खाद्य अभियांत्रिकी
मुख्य सलाहकार का नाम व पता	:	डॉ. धमेंद्र खोखर वैज्ञानिक,कृषि प्रसंस्करण एवं खाद्य अभियांत्रिकी विभाग, एस.वी.सी.ए.ई.टी. एवँ अनुसँधान केन्द्र, कृषि अभियाँत्रिकीय संकाय ,इंदिरा गांधी कृषि विश्वविद्यालय रायपुर (छ.ग.)
सम्मानित की जाने वाली उपाधि	:	प्रौद्योगिकी में स्नातकोत्तर

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दिनांक 20.07.17 :

सारांश

पचौली (पोगोस्टोमन कैब्लिन) एक सुगंधित फसल है जो कि लैमियासी परिवार से संबंधित है और सामान्यतः यह पचौली के नाम से जाना जाता है। यह उपोष्णकटिबंधीय हिमालय, दक्षिण पूर्व एशिया और सुदूर पूर्व में पाया जाता है और इंडोनेशिया, मलेशिया, चीन और ब्राजील में प्रचुर मात्रा में ''पचौली ऑयल'' नामक तेल के लिये खेती की जाती है। पत्तियों में आर्थिक हिस्सा होता है जिसमें तेल ग्रथिंयॉ पायी जाती है। वर्तमान में भारत कम मात्रा में पचौली तेल का उत्पादन कर रहा है और मुख्य चुनौती यह है कि अच्छी गुणवत्ता वाले तेल का उत्पादन करना जो कि इंडोनेशियाई तेल के साथ स्पर्धा कर सके।

प्रयोगों की एक श्रृंखला पचौली तेल की मात्रा और गुणवत्ता को जांचने के लिए तीन प्रकार के सूक्ष्मजीवों का उपयोग किया गया है। सूखी पचौली तृण को सूक्ष्म जीवाणुओं के साथ उपचारित किया गया।

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इस अध्ययन का उद्देश्य जैव रूपांतरण द्वारा पचौली तेल में तेल प्राप्ति के साथ ही पचौली अल्कोहल की प्राप्ति पर किया गया है। छाया में सुखाये गये ताजा और नियंत्रित नमूने के साथ–साथ 2, 4, 6 और 8 दिनों के लिए सूक्ष्म जीवाणु एस्पर्जिलस फोएटिड्स, पेनिसिलियम सिट्रिनम और ट्राइकोस्पोरोन एस्टेरॉइड्स के साथ उपचारित कर सभी तीन जीवाणुओं के उपचार से तेल निकासी में वृद्धि हुई, जो कि एस्पर्जिलस फोएटिड्स, पेनिसिलियम सिट्रिनम और ट्राइकोस्पोरोन एस्टेरॉइड्स क्रमशः 1.17, 1.33, 1.42 और 1.43 प्रतिशत (डब्ल्यू / डब्ल्यू); 1.22, 1.35, 1.48 और 1.49 प्रतिशत; 1.35, 1.47, 1.60 और 1.62 प्रतिशत (डब्ल्यू / डब्ल्यू)।

पचौली के तेल की निकासी में सभी जीवाणुओं से समय के साथ–साथ वृद्धि प्रदर्शित हुई, जबकि ताजा व नियंत्रित नमूनों में 2 दिन के बाद कोई वृद्धि नहीं हुई। ट्राइस्पोरोन एस्टेरॉइड्स के साथ उपचारित नमूनों में तेल प्राप्ति सबसे ज्यादा हुई। तेल प्राप्ति में वृद्धि का कारण पेक्टिनेज़ एन्जाइम हो सकता है, जो कि सूक्ष्म जीवाणुओं द्वारा उत्पादित व स्रावित किया जाता है। तेल निकालने की क्षमता में अन्तर काफी हद तक पेक्टिनेज़ एन्जाइम के संश्लेषण में अन्तर के कारण हो सकता है।

अध्ययन के दौरान निकाले गए पचौली तेल के नमूने की भौतिक व रासायनिक गुणवत्ता के लिए विश्लेषण किया गया। सभी निकाले नमूनों की भौतिक व रासायनिक गुणवत्ता एस्पर्जिलस फोएटिड्स, पेनिसिलियम सिट्रिनम और ट्राइकोस्पोरोन एस्टेरॉइड्स के साथ उपचारित किये गए नमूनों में 6 दिन बाद निकाले पचौली तेल को छोड़कर अच्छी श्रेणी में प्राप्त हुई, जबकि एस्पर्जिलस फोएटिड्स, पेनिसिलियम सिट्रिनम और ट्राइकोस्पोरोन एस्टेरॉइड्स उपचारित नमूनों के अम्लीय मूल्य में 6 दिन के बाद वृद्धि 4 से अधिक हो जाती है, जो कि मान्य नहीं है।

आवश्यक तेल भाप आसवन द्वारा निकाला गया था और पचौली तेल के घटकों को गैस क्रोमोटोग्राफी / मास स्पेक्ट्रोमेट्री (जीसी / एमएस) द्वारा पहचाने गये थे। 15 संयुग्मों में पचौली अल्कोहल प्रमुख घटक के बाद पहचान किया गया। ताजा वजन के आधार पर तेल सामग्री 1–1.65 प्रतिशत (वी / डब्ल्यू) पाया गया। ट्राइस्पोरोन में उच्च पचौली अल्कोहल (31 प्रतिशत) के साथ सांख्यिकीय रूप से बेहतर पाया गया था।

गैस क्रोमोटोग्राफी मास स्पेक्ट्रोमीटर के साथ तेल के नमूनों के उच्च अंतः विश्लेषण से पचौली तेल के घटकों पर जीवाणुओं द्वारा किये गये जैव रूपांतरण का पता चलता है। पचौली अल्कोहल जो कि पचौली का मुख्य घटक है। एस्पर्जिलस फोएटिड्स, पेनिसिलियम सिट्रिनम और ट्राइकोस्पोरोन एस्टेरॉइड्स के प्रभाव से 30.27, 28.11 और 31.25 प्रतिशत पाया गया है। ताजा नमूनों से निकाले गये तेल में 25.75 प्रतिशत पचौली अल्कोहल पाया गया है। तेल के अन्य घटक भी जैव रूपांतरण प्रक्रिया द्वारा प्रभावित हुए। अतः उपयुक्त परिणामों को देखते हुए यह कहा जा सकता है कि जैव रूपांतरण के निकासी के साथ पचौली अल्कोहल प्रतिशत में वृद्धि के लिये महत्वपूर्ण है।

CHAPTER-I
INTRODUCTION

Patchouli *(Pogostemon cablin)* is an aromatic crop, belonging to the family Lamiaceae and is commonly known as Patchouli. It is native to subtropical Himalayas, Southeast Asia and the Far East, and has been cultivated extensively in Indonesia, Malaysia, China, and Brazil for the essential oil namely "Patchouli Oil". The leaves constitute the economic part, which contain the oil glands. The commercial oil of patchouli is obtained by steam distillation of the shade dried leaves. The essential oil is used in food and perfumery industry (Akhila *et al.* 1984). There is no synthetic substitute for patchouli oil and hence it has a great demand in perfumery industries. The oil is used as a flavoring ingredient in major food products including alcoholic and non–alcoholic beverages, frozen dairy desserts, candy, baked foods, meat and meat products. Dry patchouli leaves are used to scent the wardrobes. In Chinese medicine, decoction from the leaves is used with other drugs to treat nausea, vomiting, diarrhea, cold and headache (Kader *et al.* 2006). It also has therapeutic properties, namely antidepressant, anti–inflammatory, antiseptic, aphrodisiac, astringent, carminative, diuretic, febrifuge, fungicide, insecticide, sedative and tonic.

Its oil is a viscous, pale to dark amber brown coloured liquid and is extensively used in perfumery, being its main components sesquiterpenes and sesquiterpenoid alcohols. It is used as a stimulant, relaxing, digestive, respiratory, nervous system, spleen, stomach, is not irritating and toxic (Holmes 1997). 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 (Akhila *et al.* 1984).

The leaves of patchouli plant contain 1.5-4% volatile oil composed mainly of patchouli alcohol and other sesquiterpenes such as pogostol, bulnesol, norpatchoulinol, a-guaiene, abulnesene and 13-patchoulene (Akhila *et al*. 1984).

Patchoulic alcohol is commonly used as an indicator for the quality assessment of dried *P. cablin*. However, the complexity of the herbal constituents makes it difficult for using conventional gas chromatography (GC) for analytical purpose (Hu *et al.* 2006). Other compounds found in the Patchouli oil include cycloseychellene, patchoulipyridine, epiguaipyridine, guaipyridine, benzaldehyde, cinnamaldehyde, limonene, camphene, a- pinene, 13-pinene, and eugenol (Avan *et al.* 1973). Patchouli alcohol and norpatchoulenol are mostly responsible for the odor of patchouli oil. In this study, the chemical composition, physico-chemical properties, antimicrobial activity against clinical isolates, and the bioactive property of patchouli oil are evaluated within a framework of standard pharmacological research.

Indonesia is the major producer of patchouli oil in the world (1100 tonnes per year), contributing more than 91.7% of the total world production (Lawrence 2009). Patchouli oil is an essential ingredient and used as a ‘base’ material in perfumery industry. There is no synthetic substitute for patchouli oil, which increases its value and demand in the perfumery market. Consumption of Patchouli oil in the world is about 2000 tonnes per annum. In India due to increase in chewing tobacco and pan masala industries, consumption has gone up to about 300 tonnes per annum while the production is below 50 tonnes per annum. Hence, the country mostly depends on import mainly from Indonesia and on reconstituted oil.

Biotransformation is the chemical modification of a compound by microorganisms to produce high value products with low cost precursors (Wolfgang *et al.* 2010). 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 (Gounaris 2010).

The global demand of Patchouli oil is 1600 tonnes per annum with a value of 240 crores. India imports 200 tonnes of Patchouli oil valued at 33 crores annually. Most of the demand is met from Indonesian an import which produces 80% of patchouli oil (Chakrapani *et al.* 2013).

Currently, India is producing a very less quantity of patchouli oil and thus is annually importing about 20 tonnes of pure patchouli oil and 100 tonnes of formulated oils (Ramya *et al.* 2013). Patchouli is now becoming popular in Chhattisgarh. In Chhattisgarh it is cultivated in various districts - Surguja, Raigarh, Kabirdham, Durg, Korba, Bilaspur and Jagdalpur (Raghu 2006).

India is producing a meager 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 (vasundhra 2003). Hence, keeping above points in mind the present research entitled, Pilot scale study for enhancement of patchouli essential oil quantity and quality using biotransformation process has been undertaken with the following objectives:

1. To standardize the condition for incubation of patchouli foliage with selected culture.
2. To determine the essential oil recovery after incubation of patchouli foliage.
3. To study the physico-chemical properties of extracted essential oil.

CHAPTER- II
REVIEW AND LITRATURES

The chapter covers information regarding recent research work done and cited in the scientific literature related to the production and processing of patchouli along with physico-chemical quality and chemical composition of patchouli oil. As per objectives of present investigation the available literature has been reviewed under the following heads:

2.1 Importance

Patchouli is an essential oil bearing aromatic herb plant. Patchouli is native to the Philippines and grows wild and also cultivated in Malaysia, Indonesia, Singapore, China and India. The dry leaves and stems of patchouli on steam distillation yield an essential oil called the oil of patchouli. Indonesia is the major producer of patchouli oil in the world, which produced more than 80 percent of the total. Production of patchouli oil in India is negligible as against the global production. Presently, India is importing the oil from Indonesia, Malaysia and Singapore.

Aromatic plants possess odorous volatile substances which occur as essential oil, gum exudate, balsam and oleoresin in one or more parts, namely, root, wood, bark, stem, foliage, flower and fruit. The characteristic aroma is due to a variety of complex chemical compounds. The term *essential oil* is concomitant to fragrance or perfumes because these fragrances are *oily* in nature and they represent the *essence* or the active constituents of the plants. They are called volatile or ethereal oils as evaporate when exposed to air at ordinary temperatures. Essential oils are highly concentrated, low volume, high value products (Joy *et al.* 2002). Rao and pandey (2007) reported that essential oils are highly concentrated substances extracted from flowers, leaves, stems, roots, seeds, barks, resins, or fruit rinds. These oils are often used for their flavour and their therapeutic or odoriferous properties, in a wide selection of products such as foods, medicines, and cosmetics. Essential oil is a concentrated liquid that contains various elements such as aromatic compounds, organic constituents, including

hormones, vitamin and other natural elements. These compounds are extracted from various parts of a plant and are highly volatile. In the plant, essential oils are produced inside the protoplasm of the cells and stored as micro droplets in the glands of the plant. The oils are rich in energy and chemically active. Essential oils are used for many different reasons and in different ways. Commercially, essential oils are mainly used extensively in three different industries which are food, pharmaceutical and fragrance industries. Modern scientific research leads to production of synthetic essential oil that creates the fragrances; however, they are dissimilar from natural fragrance oils or perfume (Shukor *2008*)

Kumar (2010) reported large number of herb materials contain essential oils with extensive bioactivities. Acknowledging the importance of plants and its medicinal value, extraction of essential oil had been done using steam distillation method. In this project steam distillation was used to extract oil from different plant materials like eucalyptus leaves, curry leaves, hibiscus leaves, lemon leaves, marigold flowers, rose flowers, orange peels etc. Research has confirmed centuries of practical use of essential oils, and the 'fragrant pharmacy' contains compounds with an extremely broad range of biochemical effects. Essential oils are so termed as they are believed to represent the very essence of odor and flavor.

2.1.1 Anti-microbial and insecticidal activity:

Zhu *et al.* (2003) studied on toxicity and repellency of patchouli oil and patchouli alcohol against formosan subterranean termites *Coptotermes formosans* shiraki. In these study, patchouli oil obtained from *Pogostemon cablin* (Blanco) Benth and its main constituent, patchouli alcohol, were tested for their repellency and toxicity against Formosan subterranean termites (*Coptotermes formosanus* shiraki). Found to be toxic and repellent. Unusual tissue destruction was noted inside the exoskeleton of the termite after patchouli alcohol was topically applied to the dorsum.

Zeng *et al.* (2006) studied on insecticidal activity and toxic component of essential oil from *Pogostemon cablin*. The insecticidal activity and toxic component of essential oil from *Pogostemon cablin* were studied through bioassays in laboratory. The results showed that essential oil and pogostone were very effective against *Pieris rapae* and *Plutella xylostella*. The essential oil and

pogostone possessed strong antifeeding effects on *pieris rapae* and *Plutella xylostella* larvae when feeding at a concentration of 2.5-40.0 mg/ml essential oil or 1-10 mg/m pogostone. The values of LC50 of essential oil and pogostone against the fourth instar larvae of *Pieris rapa*e were 104.28 and 32.20 micro g/ml, respectively. Pogostone, a component of essential oil, was proved to be one of insecticidal component of *Pogostemon cablin* by a series of bioassays. Microbiocides of patchouli oil was evaluated against several microorganisms viz. *Bacillussubstilis*, *Staphylococcus aureus, Streptococcus pyogenes, Enterobacter aerogenes, Pseudomonus aeruginosa , Escherichia coli, Klebsiella pneumoniae* and *Serratia marcescens* by agar diffusion technique. The minimum Inhibition Concentration (MIC) of the patchouli oil was appointed by the dilution method in the tube and the results revealed the concentration dependent ($p<0.001$) potential antimicrobial activity of both the oils by determined with zone of inhibition against standard ampicillin. It proved patchouli is a strong potential antimicrobial plant (Das *et al.* 2011).

2.2 Production

Singh *et al.* (2002) conducted field experiment to study the influence of irrigation, organic mulch and nitrogen application on its growth, herbage, oil yield and quality of patchouli (*Pogostemon cablin* (Blanco) Benth). Irrigation at 1.0 IW: CPE ratio (irrigation water: cumulative pan evaporation), 5 t/ha distilled waste material of palmaris, or 200 kg N/ha produced maximum herbage and oil yields. Organic mulch reduced weed biomass significantly. The oil content varied from 0.61 to 0.73%. The highest oil content was recorded with irrigation at 0.8 IW: CPE ratio, no mulch and 100 kg N/ha.

Puttanna *et al.* (2005) studied on the effect of shade and nitrogen on herb yield and longevity of patchouli (*Pogostemon cablin*). A preliminary study on the effect of shading (0, 50 and 75%) and nitrogen (0, 100 and 200 kg/ha/year) on the growth, productivity and nutrient uptake of patchouli (*P. cablin*) was conducted in Bangalore, Karnataka, to provide improved agro technology for the production of patchouli. Patchouli grew taller and greener, and recorded higher leaf area and moisture content when grown under 50 and 75% shading. Across treatments, N, P, K contents and uptake averaged 1.422, 0.248, 1.778% and 28.8, 5.03, 35.5 kg/ha,

respectively. Essential oil yields and oil content were similar regardless of the degree of shading. The parameters measured in patchouli increased with increasing rates of N up to 200 kg N/ha, suggesting that liberal N applications is crucial for high herb and oil production. The results indicate that patchouli can be grown either in shade as an intercrop in plantations or in open fields as a short term crop in an existing cropping system.

Ramana and Patil (2009) conducted an experiment on patchouli for its herbage yield and oil content in bettalands of Uttara Kannada district, Karnataka, India. Among the locations, highest fresh herbage yield was observed in normal bettaland at Terakanahalli (20.22 tonnes/year from 3-4 harvests). Lowest yield was recorded in degraded bettaland of Sugavi (6.25 tonnes/year from 3-4 harvests). On an average the oil recovery was in the range of 2.15-2.65% on dry weight basis. The economically important active principles of patchouli viz. patchouli alcohol and nor-patchoulinol are found to vary from location to location. Highest amount of patchouli alcohol (31.54%) was found in patchouli grown in normal bettaland in Terakanahalli area and lowest amount was found in degraded bettaland located at Sugavi (28.11%).

2.3 Processing

Patchouli is harvested after its optimum growth in the field to obtain higher yield of patchouli oil. Harvested patchouli herbage is dried before extraction of the oil.

Arpi *et al.* (2011) reported patchouli oil is one of important essential oils as a source of Indonesian foreign exchange. The treatments were the original area of raw material and the distillation method used in the original area of the raw material (BM1-4), and the length of distillation (5, 6 and 7 hours).The results indicated that the original area of raw material and distillation method (BM) had a significant effect on yield, refractive index, clarity, and acid number. The yield was 2.85%-4.5%, and patchouli oil from BM4 and BM2 gave higher yield but lower patchouli alcohol concentration, and clarity. However, it affected the lower alcohol solubility and clarity, the higher ester number, and the darker colour of the patchouli oil. The concentration of patchouli alcohol in this study ranges from 21.36% to 34.03%.

2.3.1 Drying

Suliaiman *et al.* (2008) reported that part of the plant used was the leaves and stick. Firstly, the raw material was exposed to 3 hours under direct sunlight and 3 days at room temperature. Dried patchouli plants were cut to 2 cm in size. Then, the leaves were stacked in the extraction vessel for oil extraction.

Kongkathip *et al.* (2009) reported that fresh patchouli leaves gave much lower yield than dried leaves. Dried leaves fermented for 77 days at room temperature produced the highest yield (2.48% dry wt), which was much higher than from drying in an oven at 50°C for 40 h (0.30% dry wt).

Dawn *et al.* (2013) studied on Conventionally Patchouli Herbage (*Pogostemon cablin*) is shade dried for extraction of aromatic oil. However, improper drying results in poor yield and quality of the oil. A study was undertaken to find the effect of drying on the yield of volatile oil of Patchouli. Patchouli herbage was dried under forced flow system of drying in a mechanical drier at 40°C for 5 hours and 45°C for 4 hours and also shade dried for 45 hours. The essential oil was obtained by steam distillation from each treatment. Statistical analysis showed significant differences in the essential oil content of leaves dried by different drying methods. The volatile oil content of sample dried at 40°C was found to be 2.46%. In the case of 45°C drying air temperature, the oil content was 2.60%. The volatile oil content of shade-dried sample was 2.40%.

2.3.2 Oil extraction

Extraction of essential oils is one of the most time and effort-consuming processes. The way in which oils are extracted from plants is important because some processes use solvents that can destroy the therapeutic properties. There are wide number of ways to extract the Essential oil but the quality never remains the same. Steam distillation method for extraction is the cheapest way for the extraction of oils from the different parts of the plants. The vapour allows passing through condenser and oil is collected in separating funnel and separated.

Joddy *et al.* (2007) studied molecular distillation in the vacuum pressure about 10-3 mbar, temperature in the range of 80°C –100°C, and wiper rate between 60 – 80 rpm was used for the separation and purification of patchouli oil.

Patchouli oil has a large composition of patchouli alcohol which is usually used as a fixative for perfumery, cosmetics and pharmaceuticals. Pursuant to condition above produced purification of patchouli alcohol due to the patchouli oil is about 73.37%.

Araujo *et al.* (2008) reported the Supercritical Fluid Extraction (SFE) of Patchouli [*Pogostemon cablin* (Blanco) Benth.] essential oil has been studied. The effects of harvest seasons about SFE yield. The patchouli leaves (raw material) was collected always in first hours of sunny days. Immediately, it was taken to the Process Control Laboratory, at EQA/UFSC, where it was dried at 30°C and 120 h. Then, the raw material was fractioned in a grounder and it was classified in a sieve shaker. One fixed bed extractor (length 50.0 cm, inner diameter 2.1 cm) was packed manually with 50g, approximately. The SFE was carried out at 100 bar and 32°C by 7 hours, with one hour of static period. The solvent used was CO_2 supercritical fluid. These conditions were performed according. The results were analysed in terms of yield (g essential oil/g patchouli), varying the raw material harvest seasons (winter 2006 and 2007, fall 2007). The yield values of SFE carried out were in 2006 winter (3.13%), in 2007 winter (5.94%) and 2007 fall (5.06%). Mass transfer by diffusion of CO_2 on patchouli is a slowly step and govern the first minutes of process. The amount of patchouli and its pack inside extractor control the time of extraction and the diffusion. However, information on the influence time of photo-period and harvest seasons are important to evaluate the process and its yield. The composition of patchouli essential oil was analyzed using GC-MS and FID systems which showed the presence of main components (sesquiterpenes and sesquiterpenoid alcohols)

Suliaiman *et al.* (2008) extracted patchouli oil by steam distillation method. In this study the key objectives were to vary the effect of different extraction time and sample mass on the yield. High pressured steam passed through the plant material from the bottom of the vessel. Hot steam will force open the pocket in which the essential oil of the patchouli was kept. Next, the steam which contains the essential oil passed through cooling system to condense the steam which would separate the essential oil from water. Pure oil was extracted with this method. For

this equipment with the range of 7 hours extraction time and 2 kg to 4 kg sample masses, the optimum extraction time was at 7 hours with 3 kg sample mass.

Mustakim (2008) studied on patchouli oil extraction by using hydro distillation. Effects of extraction time and particle size (grinded and non grinded leave) were studied on the yield of patchouli essential oil. The extraction process was performed by using hydro distillation method. Extraction time is varied at 1, 2, 3 and 4 hours and the extraction process was repeated several times on grinded and non grinded patchouli leave. The essential oil obtained, was analyzed by using GC-MS. From the result, the yield of patchouli essential oil is increased as extraction time increased for both grinded and non grinded leave. The highest yield of grinded and non grinded patchouli leave is at four hours in which 1.32% and 0.89% of yield was obtained respectively. Grinded patchouli leave produces more oil than non grinded patchouli leave.

Donelian *et al.* (2008) studied the extracted patchouli oil with supercritical carbon dioxide under different conditions of pressure (8.5 and 14 MPa) and temperature (40°C and 50°C) and also by steam distillation to compare the extraction methods. It was demonstrated that the extraction with supercritical carbon dioxide provided a higher yield and a better quality of patchouli oil.

Nasharudin *et al.* (2008) extracted patchouli oil by ultrasonic waves to determine optimum condition to produce higher yield of patchouli oil. It was observed that ultrasonic waves penetrated into the leave cells and extract the essential oil from the leaves. Water was used as the extraction medium in this process. Basically, there were two parameters have been studied which were extraction temperature and extraction time. In the first experiment, the extraction time was varies into four different times which were 1.5, 2.0, 2.5 and 3.0 hours. For the second experiment, the extraction temperature was varies into four different temperatures which were 50°C, 65°C, 80°C and 95°C. Based on these parameters, the best condition to obtain the best oil yield was determined at three hours extraction time and extraction temperature of 95ºC.

Kongkathip *et al.* (2009) worked on water-steam distillation and suggest that it is the best method for extraction of patchouli oil. Soxhlet extraction of dried patchouli leaves with hexane for 15 h provided crude hexane extract (4.97% dry

wt). The hexane extract was further separated and purified to obtain patchouli alcohol (0.05% dry wt), a mixture of β-sitosterol and stigmasterol (0.09% dry wt) and 7.3, 4-tri-*O*-methyleriodictyol (0.04% dry wt). Antibacterial activity assay showed that patchouli oil could inhibit *Staphylococcus aureus* and *Bacillus subtilis* better than the hexane extract.

The recovery of essential oil (the value added product) from the raw botanical starting material is very important since the quality of the oil is greatly influenced during this step. The distillation was conducted in Clevenger apparatus in which boiling, condensing and decantation was done (Kumar 2010). Analysis of essential oil was done using gas chromatography-mass spectrometer apparatus, which gives evaluates essential oil qualitatively and quantitatively.

Clara *et al.* (2010) analyzed the physico-chemical properties and vapour liquid equilibrium data for steam distilled lemon essential oil. Density and refractive index for steam distilled lemon essential oil were obtained at several temperatures and vapour pressure measurements over the pressure and temperature ranges of P (2.5 to 80.0) kPa and T (342.57 to 440.39) K, respectively. It was observed that density and refractive index lemon essential oil decreases as temperature increases.

Zainol (2010) extracted patchouli oil using hydro distillation. This research was conducted to study the effect of amount of patchouli leaves (raw material) on the extraction process and to find the optimum extraction time in the extraction of patchouli oil. In the first experiment, five different amounts of patchouli leaves, varying from 250 gram to 450 gram will be applied to identify the effect of amount of raw material in the extraction process. In the second experiment, different extraction times which were 3, 3.5, 4, 4.5 and 5 hour were used to find the optimum extraction time for extraction of oil. When the amount of patchouli leaves used was increase, the percentage of oil yield was also increased. 450gram patchouli leaves produced the highest yield which was 1.61%. When the extraction time was increased, the percentage of oil yield was also increased. Extraction time at 5 hour was the optimum extraction time because it produced the highest oil yield that was 2.467%. For both experiment, the temperature was set up at 100°C and the pressure was at 1atm.

Das *et al.* (2011) the present investigation was evaluated the potential antimicrobial activity of patchouli oil (procured from fresh and dried patchouli leaf extracts, cultivated in Indian acidic soil zone). Extraction of patchouli oil was carried out by hydrodistillation method using Clevenger apparatus. The content of patchouli alcohol was estimated by gas chromatography (GC) method.

Parganiha (2013) has studied on Patchouli (*Pogostemon cablin*) is fragrant herb produce essential oil, known as patchouli oil having high economic importance. Patchouli oil extraction is still new but has large market demand due to therapeutic and healing properties of oil, however, cost-effective route for its extraction is yet to be developed. Due to large gap between demand and supply, it is important to focus on the post harvest processing particularly on the extraction of its oil. Extraction of patchouli essential oil was done using Clevenger apparatus. Drying of herbage before extraction was done in oven at 50°C. Recovery of patchouli oil at different moisture content *viz* 30, 25, 20, 15 and 10% (wb) were 1.46, 1.49, 1.74, 1.95 and 1.89%, respectively. The highest recovery of oil was obtained at 15% of moisture content which gives oil to the tune of 1.95% when compared with the other samples. The patchouli oil samples extracted during the study were also analyzed for its physico-chemical quality. All the extracted oil samples have shown the values of physico-chemical parameter in the standard permissible range of patchouli oil.

Muyassaroh *et al.* (2016) conducted experiment to find out the appropriate distillation pressure to generate patchouli oil containing high patchouli alcohol and high yield. The method used was steam distillation which spent 6 hours long, with the operational pressure of 0,1; 0,2; 0,3; 0,4; 0,5 kg/cm^2, using patchouli leaves with three treatments are fresh leaves, aerated leaves and burned leaves in the oven. This study's results concluded that: the best result was obtained from the burned leaves in the oven where the pressure was 0.4 kg/cm^2, yield 2%, containing patchouli alcohol 40.06% and specific gravity was 0.961. The treatment to materials and stem pressure are not significantly affecting the specific gravity of essential oil. The organoleptic test results showed that it produced various colors from light yellow until tawny and all of them have the typical smell of patchouli oil.

Hamidi (2016) extracted the oil using microwave assisted hydro distillation (MAHD) and hydrodistillation (HD). The results were compared for their effectiveness in the extraction of essential oils from patchouli leaves. In present study the MAHD methods was operated with some levels of electrical power. The results showed that MAHD methods can reduce the extraction time and increase the yield. MAHD was also found to be a green technology since it required less energy than HD. The energy consumption of HD is 30% higher than MAHD. The results also indicated that power levels of MAHD have significant effect on ultimate extraction yield and time consumption. The higher power of MAHD can obtained higher ultimate yield.

2.3.3 Oil recovery

Oil recovery depends on the harvesting stage of herbage, drying of herbage and oil extraction process. As the oil has high market price, hence loss of oil after harvesting is a serious concern of the researchers. Efforts have been made to improve the oil recovery by physical or biological means.

Patchouli oil is synthesized in green part of the plant and is stored in oil glands of the plants. Extraction of oil from the oil gland can be achieved only after the disintegration of the oil gland. Disintegration of the gland/ plant tissue can be achieved by heating or by using biological activity/enzyme activity to degrade the bio-polymer (pactin), which play important role in integration of the plant tissue.

2.3.3.1 Physical

Sarma and Sarma (2003) studied oil recovery of patchouli (*Pogostemon cablin*), indicated that almost 100% oil was recovered within 3 hrs in case of fresh leaf (80-87% moisture). Semi-dried leaf (30-40% moisture) took about 5-6 hrs for recovery of 80-90% oil, while more than 90% oil was recovered within 9 hrs of distillation in case of dried leaf (10-20% moisture). Shade drying of leaf and storage up to 150 days seemed to be congenial condition for maximum recovery of oil. Ageing period exceeding 150 days for both shades and sun drying of leaf had negative effect on oil recovery. Patchouli alcohol was found to be maximum (42.37%) when dried leaves were distilled for 11 hrs.

Shukor (2008) investigated the effect of ultrasonic and type of solvent on extraction process. In this experiment three solvents viz. ethanol, hexane and acetone were used. Among the three solvents the best solvent used for solvent extraction was ethanol because it produced highest quality and most yields of patchouli oil.

Donelian *et al.* (2008), extracted patchouli oil with supercritical carbon dioxide under different conditions of pressure (8.5 and 14 MPa) and temperature (40°C and 50°C) and also by steam distillation to compare the extraction methods. It was demonstrated that the extraction with supercritical carbon dioxide provided a higher yield and a better quality of patchouli oil.

2.3.3.2 Biological

Espino *et al.* (2002) analyzed fungal pectinase on the extraction of essential oils from patchouli leaves *(Pogostemom cablin)*. Aqueous enzymatic extraction was investigated for the recovery of essential oil from patchouli *(Pogostemon cablin*). Using the optimized conditions, the highest percent oil yield was 2.09% for the enzyme treated sample and 0.45% for the control. Krishna and velankar (2011) incubated the patchouli leaves with microbial culture, and observed high patchouli content in the oil after processing along with enriched aroma.

Kumar *et al.* (2012) *pectinolytic* profile of some fungal strains, the present investigation was undertaken with an objective to select a promising pectinase producing fungal strain. Twelve *pectinolytic* strains of fungi were procured from culture collection centers in India and 5 strains were isolated from decaying fruits of coconut and *jamun*. The cultures were grown on a selective medium (pectin 1 %, yeast extract 0.1 %, pH 7.0 at 35°C) for production of *pectinolytic* enzymes under submerged fermentation. After 8 days of incubation, culture filtrates were analyzed for pectin methyl esterase (PME*), exo–and endo–polygalacturonase* (PG) enzymes and for parameters, such as reducing sugar, soluble protein and biomass. A fungal strain (MPUAT–2), isolated from coconut hull dipped in distilled water at 35 ± 2°C, was found a superior pectinase producer than others. The strain has been identified as *Aspergillus foetidus* (MTCC 10559) from Institute of Microbial Technology (IMTECH), Chandigarh.

Raharjo and Retnowati (2012) studies to obtain the increase yield of patchouli oil in patchouli leaf steam distillation of the fermentation of dewaxing drying dried process. Dewaxing process is a process of removing a layer of wax cuticle on the upper and bottom surface of the leaf patchouli. The fermentation process is a process of degradation of compounds the cell wall of patchouli leaf tissue through lysis, so that the oil patchouli oil gland cells are protected patchouli leaf tissue more easily isolated during the process of steam distillation. Yield of patchouli oil obtained by distillation of patchouli leaves dewaxing and increases the yield of the control fermentation, the ratio of stater in the media 1%, 2%, 3% and a long fermentation of 4 days, 6 days, 8 days.

Rulianah *et al.* (2015) reported that one of the factors that affect the fermentation process on the production of patchouli oil by *Phanerochaete chrysosporium* is the composition of media and fermentation time. In general, the type of media used to growth of *Phanerocheate chrysosporium* is the NLM that it contains pure $MgSO_4$. The aims of this research was to determine the effect of kieserite addition and fermentation time to yield, refractive index, and patchouli alcohol content of patchouli oil production. The final product of patchouli oil was analyzed yield, refractive index, fat content, and % patchouli alcohol to get of the best yield. The variables used in this research are fermentation time (7, 9, 11, 13, 15 days) and kieserite addition (0.5, 1, 1.5 g / L media). The best of the product on this research was result of variable fermentation time of 15 days and kieserite addition 0.5 g/L media which the best result was yield (5.32%), refractive index (1.509), fat (negative), and patchouli alcohol (34.3%).

2.3.3.3 Biotransformation process

Jayaram *et al.* (2012) experimented on five and half month old crop harvested and 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

of 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.

Khare (2016) experimented to increase oil recovery as well as patchouli alcohol percent in patchouli oil by fungi biotransformation. Drying of herbage before extraction was done at 15% moisture content. The shade dried samples were kept at room temperature as treated with microbial culture *Aspergillus foetidus, Penicilliun citrinum* and *Trichosporon asteroides* for 2, 4, 6 and 8 days along with fresh and control samples. It was observed that the oil recovery increased by the treatment of all three cultures *viz. Aspergillus foetidus, Penicilliun citrinum* and *Trichosporon asteroides* from 1.43 to 1.85%, 1.92% and 1.98% (w/w) respectively. Recovery of patchouli oil gradually increases with the time of incubation with the cultures, whereas remains constant from day 0 in fresh sample and from 2nd day in control samples. The oil recovery was highest in the herbage samples treated with *Trichosporon asteroides.* Increase in the oil recovery may be due to the pactinase enzyme which is produced and extra cellularlly secreted by the microbial cultures. The difference in oil extracting efficiency largely may be due to difference in the synthesis of pactinase enzyme.

2.4 Physico-chemical Quality

Espino *et al.* (2002) reported that physico-chemical properties of the enzyme treated patchouli oil were as follows: specific gravity = 0.9548; refractive index = 1.39; acid value = 3.30; and solubility in 90% ethanol. Extraction of essential oil from fresh patchouli leaves by fungal pectic enzyme treatment showed significant improvement in both oil recovery and quality over the traditional steam distillation process.

Bunrathep *et al.* (2006) studied on the chemical constituents of an essential oil of *Pogostemon cablin* was carried out by hydrodistillation of leaf explants and the oil analysed by Gas Chromatography Mass Spectrometry (GC/MS).The oil yield was found to be 0.30 % (v/w) of fresh weight. Twenty two compounds were

identified by GC/MS as eighteen sesquiterpenes and three oxygenated sesquiterpenes. Among these, patchouli alcohol (60.30%) was the major component, followed by germacrene A (11.73 %). In order to study the chemical constituents of the essential oil of plant cell cultures, leaves were surface sterilised and callus cultures initiated on MS media containing naphthaleneacetic acid (0.5 mg/l), and benzyladenine (1 mg/l), followed by incubation in suitable culture conditions. Cell suspension cultures were initiated by subculturing callus cultures into new liquid media and maintained in the same conditions. Chemical constituents of the essential oils produced by both callus and cell suspension cultures were extracted with dichloromethane and analysed by GC and GC/MS. The results showed that essential oil obtained from these cultures contained the same major constituents, namely patchouli alcohol, as in the intact plant, but the level was low, and also contained a small amount of minor constituents.

Yuliani *et al.* (2012) investigated fractional distillation of patchouli oils (*Pogostemon cablin*) to increase the level of patchouli alcohol in patchouli oils. The separation process is carried out by fractional distillation method applying four fractionation temperatures: 120°C, 125°C, 130°C, and 135°C. The compositions of each fraction were identified using gas chromatography–mass spectrometry (GC-MS). Patchouli oil A and B with initial patchouli alcohol content of 27.03% and 36.87% were successfully fractionated resulting four fractions of patchouli oils. The final levels of patchouli alcohol are increased to 35.35% and 43.62%, respectively as well as the densities of the four fractions.

Harunsyah and Yunus (2012) reported that patchouli oil is part of the essential oils obtained from patchouli plants by distillation. Patchouli oil is widely used in industry as provider of aroma and flavor. Quality of patchouli oil is determined by the its natural characteristics and foreign materials contain in the patchouli oil. The foreign materials contain in the patchouli oil can be damage the quality of patchouli oil. The main purpose of this research is to increase yield and the quality of patchouli oil by using of refinery equipment modification process to meet quality standards. In this research the former drum is replaced by stainless steel drum. Method of test quality and procedure of test quality same as standard method of SNI-06-2385-2006. The results showed that the using of refinery

equipment (stainless steel drum) able to increase the yield and oil quality, especially in terms of color, physicochemical properties and concentration of its main components and also meet the quality requirements of national standards.

Karimi (2014) studied the physico-chemical properties of Philippine patchouli oil, hydro-distilled from fresh leaves and young shoots of *Pogostemon cablin* were characterized and found to be within the specifications set by the United States Essential Oils Society. Philippine patchouli oil and commercial patchouli oil have the same major components as shown by GC-MS analyses: patchouli alcohol, α-guaiene, α-guaiene, α-patchoulene, seychellene, 3-patchoulene, and trans-caryophyllene, with slightly lower concentrations in the Philippine oil. Using the disk diffusion method.

Dharmadasa *et al.* (2014) reported *Pogostemon heyneanus* Benth. (Lamiaceae) is an aromatic, perfumery important, industrial crop widely cultivated in many Asian countries for its distinguished fragrance and other therapeutic purposes. However, commercial cultivation of *P. heyneanus* was hampered due to lack of high quality planting materials. Purpose of the present study is to explore superior quality *P. heyneanus* variety by means of physical (morphological), chemical (physico-chemical, phytochemical, essential oil content and composition) and biological [total antioxidant capacity (TAC)] parameters in order to establish commercial cultivation. Morphological, physico-chemical and phytochemical analysis were performed according to the methods described in WHO guidelines and other classical texts. The TAC was performed using Ferric Reducing Antioxidant Power (FRAP) assay. Essential oil was analyzed by gas chromatography Mass Spectrometry (GC/MS). Out of 26 morphological characters assessed, 5 characters were *i.e.* plant height, leaf margin, leaf apex, and leaf base and leaf shape polymorphic. All phytochemicals tested were identical to both varieties. However, presence of a prominent spots at Rf 0.12 (dark brown spot), 0.20 (rose colour spot), 0.45 (dark green spot) were characteristic for local variety. Significantly higher total ash content (12.32 %), oil content (0.52%), higher number of compounds in essential oil, patchouli alcohol content (57.0 %) and antioxidant capacity (108.53 ± 2.5 mg Trolox equivalent per g of extract) were reported in introduced variety. According to the results, introduced variety

possesses superior quality physical, chemical and biological properties and therefore, introduced variety could be recommended for establishment of commercial cultivation

Indeswari (2015) studies of the oil chemical properties on purification that include solubility in alcohol, total acid, total ester, Fe Content, Patchouli Alcohol Content, Alpha Copaene ($C_{15}H_{24}$) Content, and purity. The method of research used is complete randomized design (CRD) with factorial design. Acid –activated bentonite by number of bentonite given affects ethanol solubility, total acid, total ester, Fe content, alpha copaene content, and patchouli alcohol content. H_2SO_4 – Activated bentonite by 2% given was indicated as to best result by increasing percentage of transmittance from 69% to 81,550%, ethanol solubility 4,050%, total acid 2,120, total ester 7,975, alpha copaene content 0,04%, Patchouli alcohol content 25,02%, and Fe content 5,864 mg/kg.

Swamy and Sinniah (2015) reported that *pogostemon cablin* Benth. (Patchouli) is an important herb which possesses many therapeutic properties and is widely used in the fragrance industries.The main phytochemical compounds are patchouli alcohol, α-patchoulene, β-patchoulene, α-bulnesene, seychellene, norpatchoulenol, pogostone, eugenol and pogostol. Modern studies have revealed several biological activities such as antioxidant, analgesic, anti-inflammatory, antiplatelet, antithrombotic, aphrodisiac, antidepressant, and antimutagenic, antiemetic, fibrinolytic and cytotoxic activities. This information will provide a potential guide in exploring the use of main active compounds of patchouli in various medical fields.

2.5 Gas Chromatography Mass Spectrometer Analysis

Leclercq (1989) analyzed the essential oil of *Pogostemon cablin* (Blanco) Benth. Grown in Vietnam by capillary gas chromatography and mass spectrometry. Patchouli alcohol accounted for about 32-38% of the patchouli oil. Ten more compounds were identified, of which *α-bulnesene* and *α-guaiene* was the main components.

Boutekedjiret *et al.* (2003) extracted rosemary oil by both steam and hydrodistillations then analysed by gas chromatography and gas chromatography–

mass spectrometry. The effect of time of extraction enabled us to follow the evolution of the yield and oil composition obtained by both processes.

Bure and Sellier (2004) analyzed the essential oil of Indonesian patchouli (*Pogostemon cabin* Benth.*)* Patchouli oil from Indonesia was analyzed qualitatively and quantitatively by using GC (FID) and GC/MS (EI/CI).Using different ionization techniques in mass spectrometry (EI, NCI and PCI with ammonia and deuterated ammonia as reagent gases), 41 compounds were separated, 28 of which (92.9% of the total oil) were identified. Four new compounds were found in this oil: *γ-gurjunene* (2.2%), *germacrene* D (0.2%), *aciphyllen*e (3.4%) and 7-*epi-α- selinene* (0.2%).

Bunrathep *et al.* (2006) studied the chemical constituents of an essential oil of *Pogostemon cablin* was carried out by hydrodistillation of leaf explants and the oil analysed by gas chromatography mass spectrometry (GC/MS). The oil yield was found to be 0.30 % (v/w) of fresh weight. Twenty two compounds were identified by GC/MS as eighteen sesquiterpenes and three oxygenated sesquiterpenes. Among these, patchouli alcohol (60.30%) was the major component, followed by germacrene a (11.73 %). Chemical constituents of the essential oils produced by both callus and cell suspension cultures were extracted with dichloromethane and analysed by GC and GC/MS.

Masrur (2008) extracted patchouli oil by microwave assisted extraction using ethanol as solvent. It was a simple technique that provides a novel way of extracting soluble products into a fluid, from a wide range of materials, helped by microwave energy. From GC analysis, it is identified that the main component of patchouli essential oil is patchouli alcohol. Its existence was shown as a high peak in all of the analysis. Other components present in the essential oil are *Caryophyllene, β-Patchoulene α-guaiene* and *α-Bulnesene*. Microwave assisted extraction method to extract patchouli essential oil was feasible. It also cut the extraction time and solvent usage compared to other conventional methods.

Prakash *et al.* (2008) studied on sesquiterpenoid rich essential oil from the leaves of *Pogostemon* patchouli pellet grown organically under tarai conditions. The essential oil obtained by hydrodistillation from the dry leaves of patchouli [*P. cablin*] grown organically in Pantnagar, Uttaranchal, India, was analysed by GC

and GC-MS. The analysis revealed the presence of 19 compounds, 12 of which represented 98% of the oil. The oil had a high amount of sesquiterpenes. Patchouli alcohol (32.3%), *alpha-guaiene* (23.3%), *alpha-bulnesene* (21.4%), *gamma-patchoulene* (6.3%), *beta-caryophyllen*e (4.8%), *seychellene* (3.2%), *alpha-patchoulene* (2.5%) and *beta-patchoulene* (2.3%) were the major constituents. The oil had high amounts of hydrocarbons (64%) and patchouli alcohol (32.3%).

Sudaresan *et al.* (2009) done comparative study on the essential oil constituents of *Pogostemon cablin* (Blanco) Benth. (Patchouli) and *P. travancoricus* Bedd.var. *travancoricus* were investigated using GC and GC/MS analysis. Eleven compounds from *P. cablin* oil (Patchouli) and 13 from *P. travancoricus* var. *travancoricus* oil were identified. Both species sharedcompounds like α- and β-patchoulene, patchouli alcohol (patchoulol), β-caryophyllene, α-guaiene, seychellene andselinene, although quantitatively less in *P. travancoricus* var. *travancoricus*.

Derwich *et al.* (2010) analyzed the essential oils of leaves of *Mentha pulegium*, a traditional herbal medicine in Morocco, extracted by hydrodistillation. gas chromatography equipped with flame ionisation detector (GC-FID) and gas chromatography coupled to mass spectrometry (GC–MS), to determine the chemical composition of the volatile fraction and identify their chemotypes. Twenty eight constituents were identified. The essential oil yield and the percentage of identified compounds were 1.66% and 97.34% respectively. The major component was piperitone (35.56%), other predominant constituents were: piperitenone (21.18%), α-terpineol (10.89%), pulegone (6.452%), piperitone oxide (4.02%), menthol (3.28%), menthone (3.09%), neomenthol (2.80%), menthofuran (2.15%), isomenthone (1.56%), carvone (1.13%), geranyl acetate (1.06%), germacrene D (1.03%) and limonene (1.02%).

Murugan *et al.* (2010) extracted the volatile oil of the leaves of *Pogostemon heyneanus* Benth. (Lamiaceae) and analyzed by GC and GC-MS. Twenty-six components representing 96.0% of the oil were identified. The major components of the oil were acetophenone (51.0%), β-pinene (5.3%), (*E*)-nerolidol (5.4%), and patchouli alcohol (14.0%).Comparison of the compositions of the oils of *P. heyneanus* and *P. cablin* (Blanco) Benth. (Patchouli oil) showed wide

variation between them. Though 13 sesquiterpenes and oxygenated sesquiterpenes were detected in both oils, their concentrations in the oils differed widely. Acetophenone, benzoyl acetone and (*E*)-nerolidol present in the oil of *P. heyneanus* were not detected in patchouli oil.

CHAPTER – III

MATERIALS AND METHOD

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.).

3.1 Details of the Experimental Materials and Methods

3.1.1 Collection and drying of the patchouli herbage

Herbage of *Pogostemon cablin* was collected in February 2017, from the Instructional cum Research Herbal Garden, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G). It is a known fact that oil is present in all parts of the patchouli plant, however high oil content is found in the leaf portion of the plant. The leaves were dried for 8-10 days by laying it on cement surface in the shade. Proper care was taken to dry the leaves up to 15% moisture content (db) to prevent the mold formation and subsequent loss of oil.

Fig. 3.1 Fresh patchouli leaves

Fig. 3.2 Shade drying of herbage

3.1.2 Selection and procurement of microorganisms

On the basis of earlier work done (Khare, 2016) at our department on the biotransformation of patchouli, the following three cultures were selected & procured for pilot scale study.

Table: 3.1 Culture procured for fermentation

S. 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

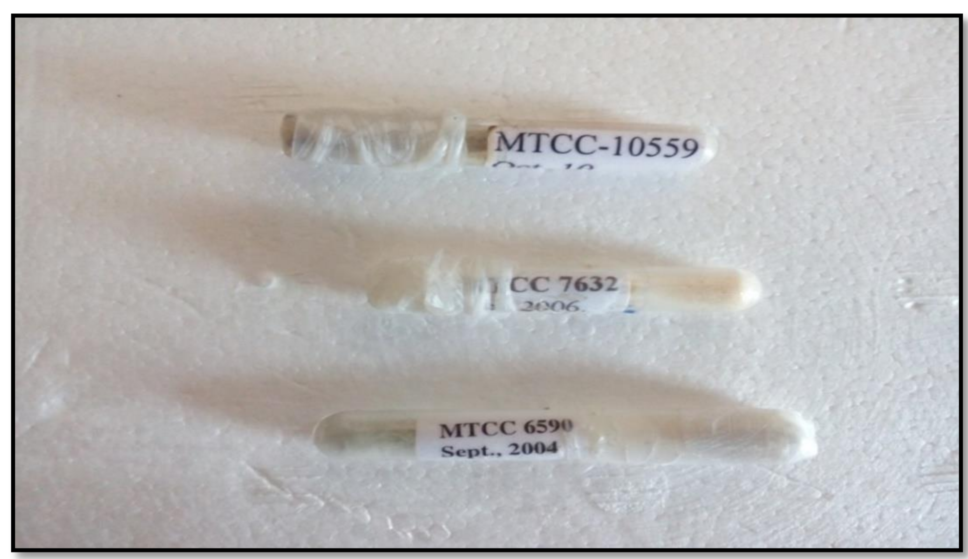


Fig. 3.3 Culture procured for fermentation

3.1.3 Media preparation

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

agar – 1.5gm 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.

3.1.4 Maintenance of cultures

3.1.4.1 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.

Fig. 3.4 Autoclave

3.1.4.2 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.

Fig. 3.5 Laminar flow

3.1.4.3 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 24 hr 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).

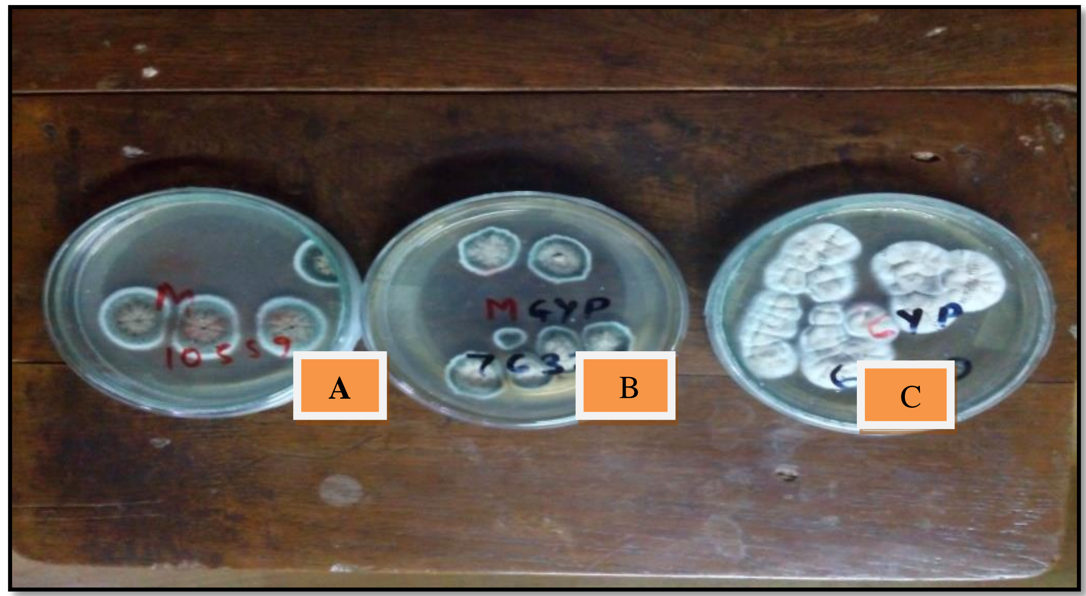


Fig. 3.6 Revival of fungal strain on media: A, *Aspergillus foetidus*(MTCC 10559; B, *Trichosporon asteroides* (MTCC 7632)); C, *Penicilium citrinum* (MTCC 6590)

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

Fig 3.7 Incubator **Fig 3.8 Growth of culture on broth media**

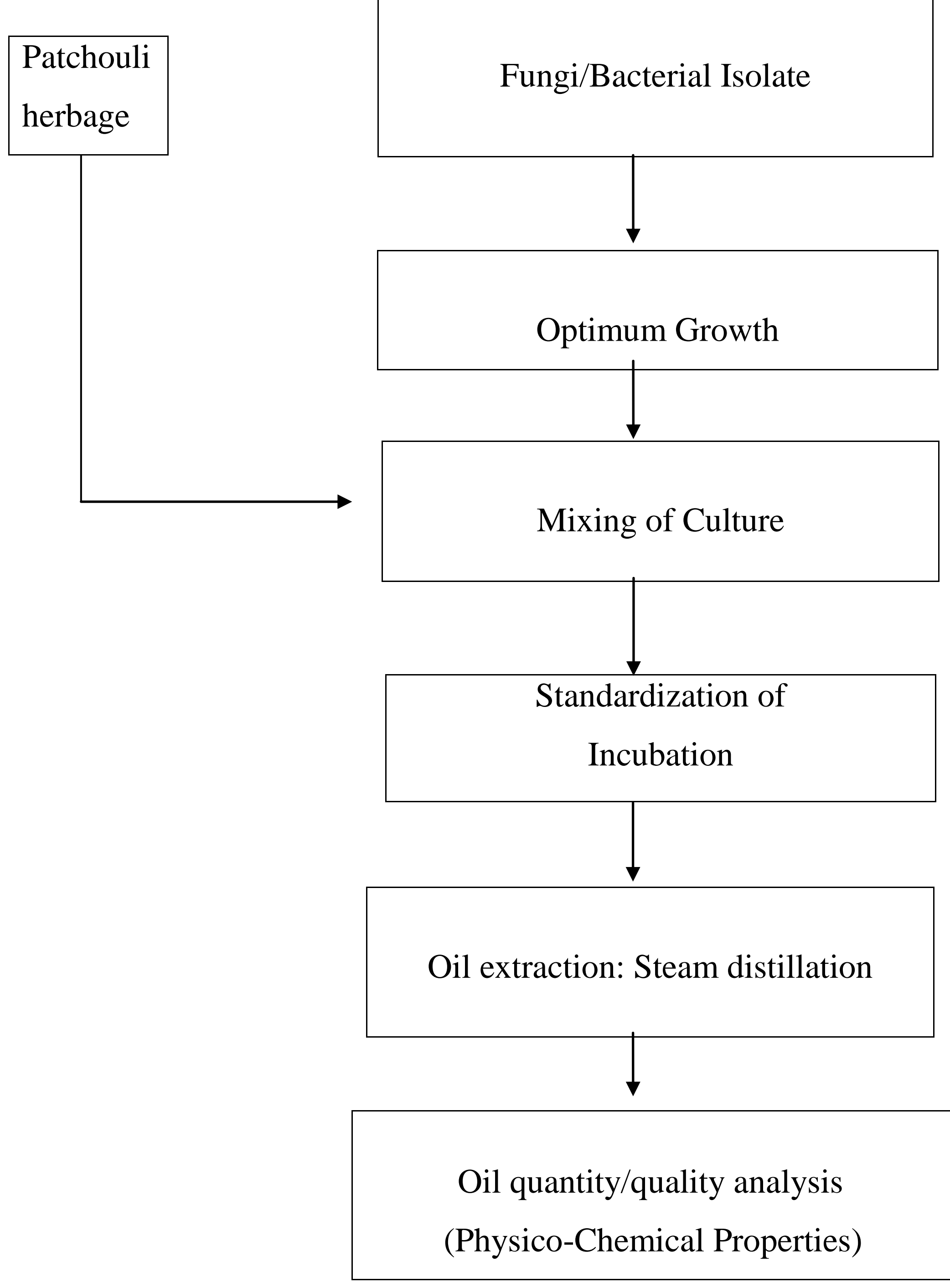


Fig. 3.9 Flow diagram for patchouli processing

3.1.6 Incubation of patchouli herbage with culture

The harvested dried herbage sample of 2 kg 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 0th day, 2nd day, 4th day, 6th and 8th day of incubation.

Fig. 3.10 Fermentation process on patchouli   Fig. 3.11 Fermented patchouli

3.1.7 Oil extraction

For oil extraction leaves from each treatment along with 2 kg fresh sample was loaded in the steam distillation tank (made of stainless steel). Distillation process was carried out for seven hours for maximum extraction of essential oil and the oil content (%) was recorded.

Shade dried leaves subjected to steam distillation for obtaining the oil of patchouli. The distillation unit consists of a boiler, distillation tank, condenser and separator. The distillation tank is made up of preferably food grade stainless steel. The vessel has a perforated metal sheet or net at the bottom to support the herb, which is loaded into the still for distillation. Steam is charged through perforated coils that uniformly pass through the material. This steam while passes through takes out the oils by rupturing the oil glands that moves to condenser along

with water vapour. The condenser, which cools the vapours received from the distillation tank, consists of mainly tubes made up of stainless steel and mounted inside a jacket. The condenser is provided with inlet and outlet for the circulation of cool water. The hot vapours consisting of steam and essential oil vapours are cooled in the condenser tubes and the condensate then flows out into the separator. The oil being lighter than water and insoluble flows on the top in the receiver and only the water gets drained out. The oil is drawn off separately at the end of distillation.

Firstly, the fresh (dried up to 15% mc) 2kg patchouli herbage was subjected for oil extraction. Recovery of oil was recorded up to time (7h).

Fig. 3.12 Steam distillation unit

3.1.8 Separation and dehydration

Oil separation and dehydration was done using chloroform, separating funnel and anhydrous sodium sulfate. To separate the essential oil from the water. The oil is insoluble in water and is soluble in non-polar solvents like chloroform. Chloroform is added to the oil-water mixture in a separating funnel. This funnel allows the two solvents to layer and subsequently drain one solvent layer away

from the other. After a few moments of shaking, the oil will partition into the chloroform layer. Draining the water layer from the chloroform removes the oil from the water. The partitioning is almost never complete, so an extraction is usually carried-out multiple times.

After that, for dehydration very small quantity of anhydrous sodium sulfate was mixed in the separated oil and kept it overnight. Sodium sulfate absorbs the moisture present in oil and pure patchouli essential oil was obtained. Oil was collected in test tubes and stored in cool place.

3.2 Quality Analysis

3.2.1 Density

Density of an essential oil is defined as the ratio of the weight of a given volume of oil to the volume of oil at constant temperature. This is usually reported at $20^{O}C$. A temperature correction of approximately 0.00045 per degree may be made either by subtracting or addition to bring it to $20^{O}C$ (Khare 2016).

The weight of the oil can be determined with the help of a thoroughly cleaned and dried pycnometer.

$$\text{Density } (20\,°\text{C}) = \frac{W_O}{V_O}$$

Where,

W_O= Weight of oil (gm),

V_O = Volume of oil (ml)

3.2.2 Refractive index

This can be determined with the help of a refractometer which gives the reading directly after calibration. The reading can be observed at room temperature with the help of ATC probe.

Density and refractive indices are very sensitive to temperature changes. The low temperatures tend to increase the values considerably while at higher temperatures the values are sufficiently lowered. (Khare 2016)

3.2.3 Acid value

Most of the essential oil contains small amounts of various free acids and therefore, the content is usually reported as acid number rather than its percentage. The acid number of oil is defined as the number of mg of potassium hydroxide required to neutralize the free acids in 1 g of oil. While determining the acid number the alkalis should be quite dilute (0.1 N) as strong alkalis may hydrolyze the esters even in cold conditions, thereby giving a higher acid number. In case, large excess of phenols are present, the indicator should be changed from phenolphthalein to phenol red. (Khare 2016)

3.2.3.1 Procedure

Weight accurately about 500 mg of the oil into 100 ml saponification flask. Add 10 ml of neutral alcohol and a drop of phenolphthalein. Titrate this against standard solution of 0.1 N KOH to the end point. This titration requires only a few drops of the alkali. Keep a blank reading also.

3.2.3.1.1 Calculation

Volume consume = $Y - X = Z$

Volume of alkali used = $P - Z = R$

Blank reading = P

Initial reading = X

Final reading = Y

$$A_V = \frac{56.1 \times R \times N}{W_O}$$

Where,

A_V= Acid value ;

N = Normality; and

W_O= Weight of oil (mg).

3.2.4 Ester value

Esters are normally calculated as ester number or value because they are usually mixtures of unknown esters. An ester number is defined as the number of mg of potassium hydroxide required to saponify the esters present in 1g of oil. For the esters of dibasic acids or dihydroxy alcohols, the ester number is divided by 2 (Khare 2016).

3.2.4 .1 Procedure

Weight accurately about 500 mg of the oil into a 100 ml saponification flask. Add about 10 ml of neutral alcohol and a drop of phenolphthalein. Titrate this against standard solution of 0.1 N KOH to the end point. This titration requires only a few drops of the alkali. Keep a blank reading also.

To the above flask add 10 ml of 0.5 N alcoholic KOH, attach the air condenser and reflux the contents on the water bath for 2 hr. Cool and titrate against 0.5 N HCl (Khare 2016).

3.2.4 .1 .1 Calculation

Volume consumed $= Y' - X' = Z'$

Amount used for saponification $= P' - Z' = R'$

Blank reading $= P'$

Initial reading $= X'$

Final reading $= Y'$

$$E_v = \frac{56.1 \times R' \times N}{Wo}$$

Where,

E_V=Ester value ;

N = Normality; and

W_O = Weight of oil in m

3.2.5 Gas chromatography-mass spectrometer analysis (GC-MS)

GC-MS analysis was carried out in Shimadzu QP2010 using Split inlet. Compounds were separated on Rtx-5MS (30MX0.25mmX0.25µm film thickness) which is coated with 5% diphenyl dimethyl polysiloxane. Injector temperature was maintained at 280ºC. Oven temperature program was set at 40ºC for 0.5min. The temperature was increased at the rate 5ºC/min to 240ºC and held for 8min. The 5 µl oil sample was diluted to 200 µl with GC grade acetone. Diluted 1 µl sample was injected using Hamilton syringe with a split ratio of 100:1. Helium was used as a carrier gas at the constant flow rate 36ml per min. The spectrophotometer was operated in EI mode and the mass range was 40-500amu. The ion source temperature and interface temperature was set at 250ºC and 220ºC respectively.

Fig. 3.13 Gas Chromatography-Mass Spectroscopy (GC-MS)

3.2.5.1 Analysis and identification of compounds

The compositional analysis of the oil was carried out using the software provided along with the GC-MS. The compound identification was done using the NIST library and retention time.

3.2.6 Statistical analysis

Analysis of variation in volatile oil content and composition as affected by different fungal treatments were analyzed using the CRD program.

CHAPTER- IV

RESULT AND DISCUSSION

Patchouli oil is an important ingredient in many fine fragrance products but the patchouli oil extraction is still new compared to other essential oil extraction. However, cost-effective route to produce the oil has yet to be developed. Furthermore, the price of patchouli oil increasing by years. Oil recovery depends on various factor i.e. distillation time, temperature, nature of components of oil. Distillation is done for long time in case of patchouli oil extraction; however the long time distillation leads to deterioration of the oil quality. The oil quality is analysed on the basis of physico-chemical properties along with the active ingredients. The market value of the patchouli oil depended on the patchouli alcohol percentage which is the major ingredient of the oil. In this study experiments was carried out to improve the oil recovery along with quality. The patchouli herbage was incubated with the microbes to improve the recovery as well as quality. The experimental results are presented and discussed in this chapter.

4.1 Oil Recovery

Dried (15% db) patchouli samples were subjected to extraction of oil using steam distillation. Dried patchouli was obtained by shade drying at room temperature. The oil obtained from different samples are presented in Table 4.1, 4.2 and 4.3. Recovery of oil presented in the table is on the volume basis, weight basis and percentage (dry and wet basis).

4.1.1 Patchouli oil recovery after incubation with *Aspergillus foetidus* at different intervals of time

Patchouli oil was extracted from the samples after treatments of microbial culture *Aspergillus foetidus* at 2, 4, 6 and 8 days as compare to fresh and control sample. The oil recoveries obtained from different samples are presented in Table

4.1. Data indicates that the recovery of patchouli oil is significantly affected by the treatments at different days by microbial inoculation.

The oil recovery obtained after completion of steam distillation process from the fresh patchouli herbage was 1.03% and control 1.07%. The oil obtained from the incubated samples were 1.17, 1.33, 1.42 and 1.43 % (w/w) respectively for the 2, 4, 6 and 8 days fermented samples.

The Fig. 4.1 shows the patchouli oil recovery (%) at different interval of days after treatment of microorganism *Aspergillus foetidus*. Figure indicates the gradual increase in recovery of oil as number of days increases. The recoveries of patchouli oil were determined as 1.03 and 1.07 % (w/w) from fresh and control remain similar for further incubation period up to 8 day. Oil recovery gradually increases in the samples incubated with *Aspergillus foetidus* up to 6 day (1.42%) and after 6th day the oil recovery do not increase significantly.

Table 4.1 Effect of *Aspergillus foetidus* in patchouli oil recovery

Weight of sample (g)	Treatment	Oil recovery (ml)	Oil recovery (g)	Recovery in (%)
2000	Fresh	21.26	20.62	1.03
	Control	22.07	21.45	1.07
	Day 2	24.11	23.46	1.17
	Day 4	27.28	26.62	1.33
	Day 6	29.07	28.49	1.42
	Day 8	29.12	28.57	1.43

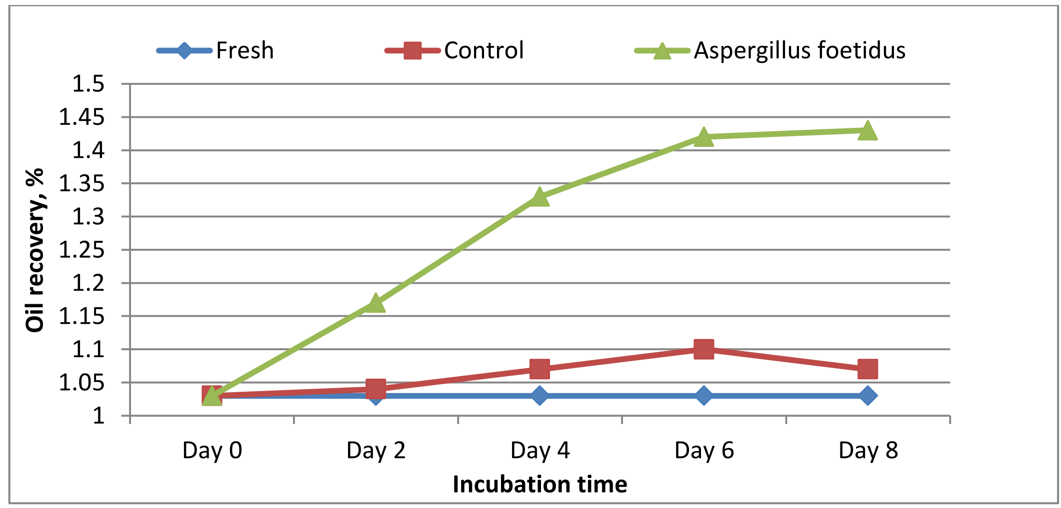


Fig 4.1 Oil recovery at different intervals of time

Data obtained during the course of investigation were subjected to statistical analysis to test the effect of culture incubation up to 8 days on the recovery of patchouli oil. Statistical analysis was done using Completely Randomized Design (CRD). The raw data of the experiment are given in Appendix A whereas the ANOVA table is given in Table 4.2. Statistical analysis of data clearly indicates that there is significant difference in recovery of patchouli oil in respect of intervals of days after treatment.

Table 4.2 ANOVA for effect of *Aspergillus foetidus* in patchouli oil recovery

Source	D F	M S	F Cal	S Em	CD (5%)	CV %
Treatment	5	36.940	33.308 **	0.608	1.894	
Error	12	1.109				4.235
Total	17					

(** Significant at 1% level)

4.1.2 Patchouli oil recovery after incubation with *Penicillium citrinum* at different intervals of time

Patchouli samples treated with *Penicillium citrinum* at 0, 2, 4, 6 and 8 days were distilled for extraction of incubated oil as mention in the material and method section 3.1.

Table 4.3 Effect of *Penicillium citrinum*in patchouli oil recovery

Weight of sample (g)	Treatment	Oil recovery (ml)	Oil recovery (g)	Recovery in (%)
2000	Fresh	21.26	20.62	1.03
	Control	22.07	21.45	1.07
	Day 2	25.12	24.47	1.22
	Day 4	27.75	27.11	1.35
	Day 6	30.35	29.77	1.48
	Day 8	30.34	29.85	1.49

The oil recoveries obtained from the different samples are presented in Table 4.3. It shows that there is gradual increase in recovery of oil as compare to fresh and control. The oil recovery obtained after completion of distillation process

from samples having different treatment of 2, 4, 6 and 8 were 1.22, 1.35, 1.48 and 1.49% respectively. Oil recovery gradually increases in the samples incubated with *Penicillium citrinum* up to 6 day (1.48%) and least increased at 8 day (1.49%) after the 6 day sample. The highest recovery of the oil was at 8 day, however the recovery at 6 day was at par with the 8 day sample. Data indicates that the recovery of patchouli oil have significantly affected by the incubation of patchouli samples with *Penicillium citrinum* culture.

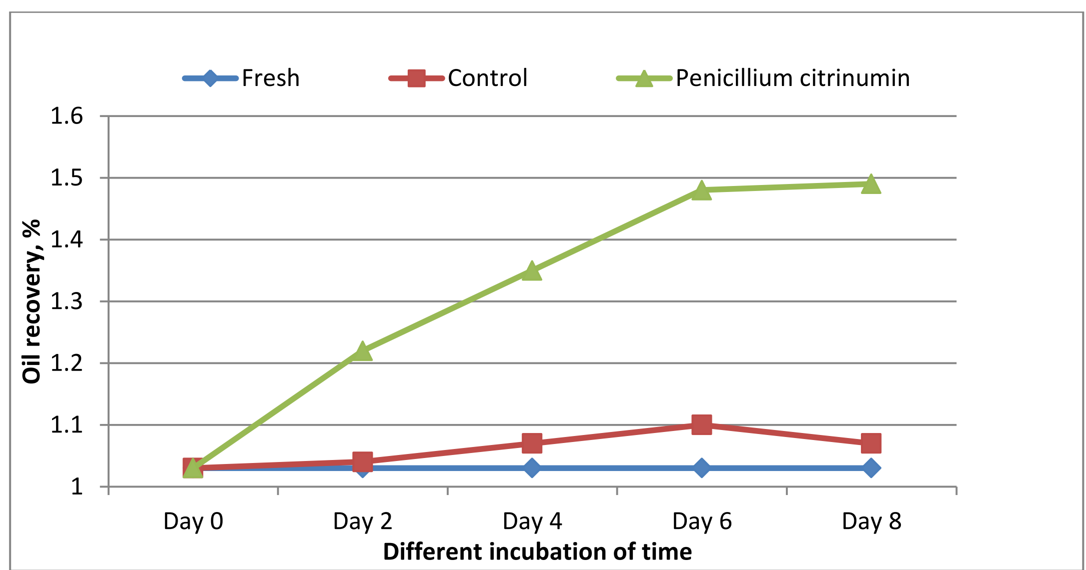


Fig 4.2 Oil recovery at different intervals of time

The Fig. 4.2 shows the patchouli oil recovery (%) at different interval of days after incubation with *Penicillium citrinum*. Figure indicates the gradual increase in recovery of oil as number of day's increases. Fresh and control samples gives least recovery of patchouli oil and remain nearly constant after 2 day.

The effect of different interval of days on the recovery of patchouli oil was analyzed statistically and the raw data of the experiment are given in Appendix A, where as the ANOVA table is given in Table 4.4. Statistical analysis clearly indicates that there is significant difference in recovery of patchouli oil in comparison to fresh and control sample.

Table 4.4 ANOVA for effect of *Penicillium citrinum* in patchouli oil recovery

Source	D F	M S	F Cal	S Em	CD (5%)	CV %
Treatment	5	48.608	40.617 **	0.632	1.968	
Error	12	1.197				4.282
Total	17					

(** Significant at 1% level)

4.1.3 Patchouli oil recovery after incubation with *Trichosporon asteroides* at different intervals of time

Table 4.5 Effect of *Trichosporon asteroides* in patchouli oil recovery

Weight of sample (g)	Treatment	Oil recovery (ml)	Oil recovery (g)	Recovery in (%)
2000	Fresh	21.26	20.62	1.03
	Control	22.07	21.45	1.07
	Day 2	27.74	27.13	1.35
	Day 4	30.17	29.59	1.47
	Day 6	32.49	32.04	1.60
	Day 8	32.83	32.47	1.62

Patchouli oil was extracted from the shade dried samples incubated with *Trichosporon asteroides* for different intervals of days along with the fresh and control samples. The oil recoveries obtained from the samples at different intervals of time are presented in Table 4.5.and Fig.4.3. Data indicates that the recovery of patchouli oil is significantly affected by the treatment of culture. The oil recovery from the samples increases with the time. However, oil recovery in fresh and control samples remains nearly constant. The oil recovery obtained after completion of distillation process from samples distilled at different intervals of incubation viz. 2, 4, 6 and 8 days were 1.35, 1.47, 1.60 and 1.62 % respectively. Oil recovery gradually increases in the samples incubated with of *Trichosporon asteroides* up to 6 day (1.60%) and least increased at 8 day (1.62%) after the 6 day sample.

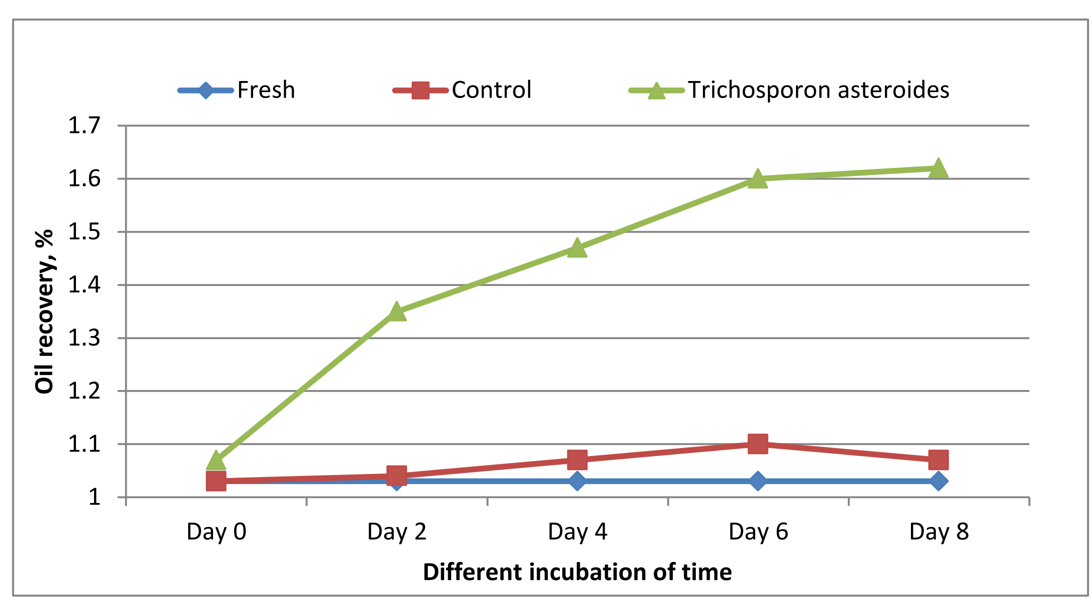


Fig 4.3 Oil recovery at different intervals of time

Table 4.6 ANOVA for effect of *Trichosporon asteroides* in patchouli oil recovery

Source	D F	M S	F Cal	S Em	CD (5%)	CV %
Treatment	5	79.975	42.316 **	0.794	2.473	
Error	12	1.890				5.051
Total	17					

(** Significant at 1% level)

4.2 Physico-chemical Quality of Patchouli Oil

The physico-chemical quality of patchouli essential oil extracted from different samples were analyzed by using standard methods as mention in the materials and methods section 3.2. Density, refractive index, acid value and ester value were analyzed for physico-chemical quality of patchouli oil extracted from different extracts. Data obtained are presented in Table 4.7, 4.8 and 4.9.

4.2.1 Physico-chemical quality of patchouli oil extracted after incubation with *Aspergillus foetidus*

The patchouli oil extracted at different intervals of days after fermentation was analyzed for density, refractive index, acid value and ester value. The data obtained are presented in Table 4.7. The given data indicates that the physico-chemical quality; density, refractive index, acid value and ester value of patchouli oil are significantly affected by the treatment of cultures with respect to days. Data indicates the increase in density however, decrease in refractive index, ester value and increase acid value with respect to increase in incubation time.

It is observed that the density of patchouli oil increases from 0.970 to 0.981 g/ml. The density of patchouli oil extracted from various incubated and control samples are 0.970, 0.972, 0.973, 0.976 0.980 and 0.981 g/ml for fresh control, 2, 4, 6 and 8 days treatment of microorganism. The oil extracted from fresh and control patchouli has lower value of density 0.970 and 0.972 g/ml, respectively. The oil extracted from after 6 days *Aspergillus foetidus* incubated sample gives highest value 0.981 g/ml compared to other samples. Increasingly heavy weight fraction contained in the oil, hence greater the density. Similar results have been reported by Joddy *et al.* (2007), Arpi *et al.* (2011) and Karimi (2014) on patchouli oil.

Table 4.7 Physico-chemical quality of patchouli oil extracted after incubation with *Aspergillus foetidus*

Treatment	Density (g/ml)	Refractive Index	Acid Value	Ester Value
Fresh	0.970	1.5098	2.65	9.76
Control	0.972	1.5070	3.07	8.77
Day 2	0.973	1.5029	3.17	7.71
Day 4	0.976	1.5024	3.65	6.14
Day 6	0.980	1.5008	3.77	3.75
Day 8	0.981	1.5002	4.40	3.06

Table 4.7. presents refractive index of patchouli oil samples. The refractive index of the extracted oil were 1.5098, 1.5070, 1.5029, 1.5024, 1.5008 and 1.5002 for fresh, control & 2, 4, 6 and 8 days *Aspergillus foetidus* treated extracts, respectively. The oil extracted from fresh patchouli gives highest refractive index 1.5098 and the oil

extracted from 8 days *Aspergillus foetidus* sample gives least value 1.5002 compared to other samples. High refractive index indicates the amount of turbidity and concentration substrate. The density of essential oil increases the light that comes will be more difficult to be refracted due to a greater refractive index of oils. Similar results have been reported by Noor *et al.* (2017), Joy *et al.* (2002), Joddy *et al.* (2007) and Rulianah *et al.* (2015) on essential oil from patchouli.

Acid value of different extracted patchouli oil were found 2.65, 3.07, 3.17, 3.65, 3.77 and 4.40 form fresh, control & 2, 4, 6 and 8 days respectively. The oil extracted from 8 days sample gives highest acid value 4.40 respectively. The oil extracted from fresh sample gives least acid value compared to other samples. The acid value of the oil increases with increase the incubation time. Noor *et al.* (2017) also observed that storage of oil for longer period or contact between patchouli oil with light or ambient air in the bottle increases the acid value.

Ester value of different extracted patchouli oil were found 9.76, 8.77, 7.71, 6.14, 3.75, and 3.06 from fresh, control & 2, 4, 6 and 8 days respectively. The oil extracted from fresh sample gives highest value 9.76 respectively. The oil extracted from 8 days sample gives least ester value compared to other samples. Joddy *et al.* (2007), Karimi *et al.* (2014), Joy *et al.* (2002), Harunsyah and Yunus (2012) reported similar results.

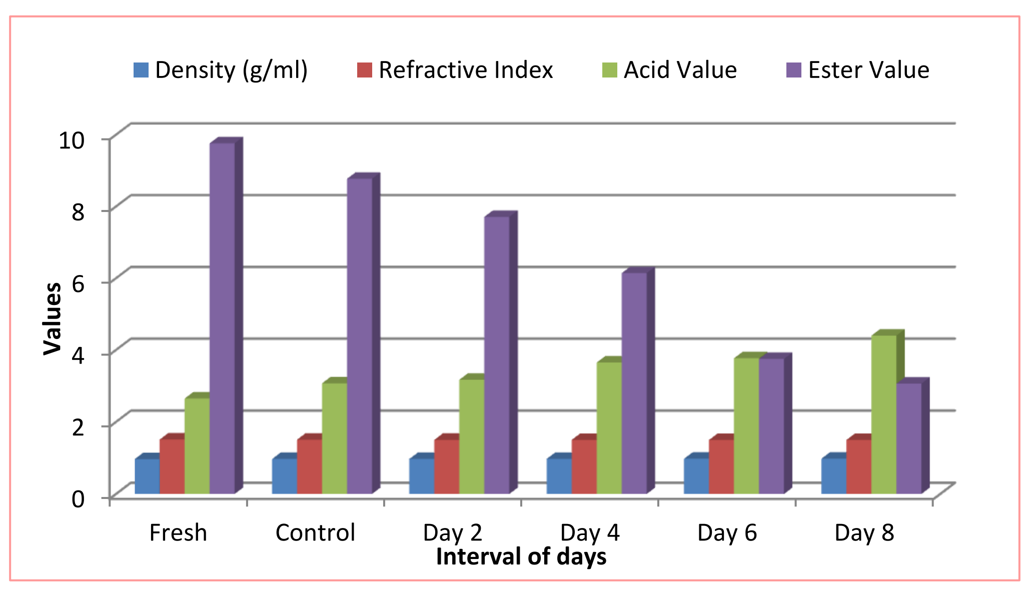


Fig 4.4 Physico-chemical quality of patchouli oil extracted after incubation with *Aspergillus foetidus*

4.2.2 Physico-chemical quality of patchouli oil extracted after incubation with *Penicillium citrinum*

The patchouli oil extracted at different intervals of days after fermentation was analyzed for density, refractive index, acid value and ester value. The data obtained are presented in Table 4.8. The given data indicates that the physico-chemical quality; density, refractive index, acid value and ester value of patchouli oil are significantly affected by the treatment of cultures with respect to days.

It is observed that the density of patchouli oil increases from 0.970 to 0.984 g/ml. The density of patchouli oil extracted from various incubated and control samples determined was 0.970, 0.972, 0.974, 0.977, 0.981 and 0.984 g/ml for fresh control, 2, 4, 6 and 8 days treatment. The oil extracted from fresh and control patchouli gives least value of density 0.970 and 0.972 g/ml. The oil extracted from *Penicillium citrinum* incubated sample after 8 days gives highest density 0.985 g/ml. compared to other samples. High refractive index indicates the amount of turbidity and concentration substrate. The density of essential oil increases the light that comes will be more difficult to be refracted due to a greater refractive index of oils. Similar results have been reported by Noor *et al.* (2017), Joy *et al.* (2002), Joddy *et al.* (2007) and Rulianah *et al.* (2015) on essential oil from patchouli.

Table 4.8 Physico-chemical quality of patchouli oil extracted after incubation with *Penicillium citrinum*

Treatment	Density (g/ml)	Refractive Index	Acid Value	Ester Value
Fresh	0.970	1.5098	2.65	9.76
Control	0.972	1.5070	3.07	8.77
Day 2	0.974	1.5024	3.12	4.88
Day 4	0.977	1.5014	3.67	4.28
Day 6	0.981	1.5013	3.70	3.71
Day 8	0.984	1.5008	4.95	3.11

From Table 4.8.Refractive index obtained was 1.5098, 1.5070, 1.5024, 1.5014, 1.5023 and 1.5008 for fresh, control 2, 4, 6 and 8 days sample incubate with *Penicillium citrinum* extracts. The oil extracted from fresh patchouli gives

highest value of refractive index 1.5098 and the oil extracted from sample treated with *Penicillium citrinum* after 8 days sample gives least value 1.5008 compared to other samples.High refractive index indicates the amount of turbidity and concentration substrate. The density of essential oil increases the light that comes will be more difficult to be refracted due to a greater refractive index of oils. Similar results have been reported by Noor *et al.* (2017), Joy *et al.* (2002), Joddy *et al.* (2007) and Rulianah *et al.* (2015) on essential oil from patchouli

Acid value of oil obtained after different treated and untreated samples were 2.65, 3.07, 3.12, 3.67, 3.70, 4.95 from fresh, control & 2, 4, 6 and 8 days respectively. The oil extracted from 8 days samples gives least value 3.12 as compared to other samples. The oil extracted from 8 days sample gives highest value 4.95 as compared to other samples. Further increase in acid value will lead to no market for the oil. The acid value higher than 4.0 indicates the poor quality of oil and is not acceptable in the market. The acid value of the oil increases with the incubation time. Noor *et al.* (2017) also observed that storage of oil for longer period or contact between patchouli oil with light or ambient air in the bottle increases the acid value.

Ester value of different extracted oil were 9.76, 8.77, 4.88, 3.28, 3.71 and 3.11 from fresh , control, & 2, 4, 6 and 8 days respectively, when the oil extracted from fresh patchouli gives highest ester value 9.76 and the oil extracted from 8 days *Penicillium citrinum* treated sample gives least value 3.11 as compared to other treatment.

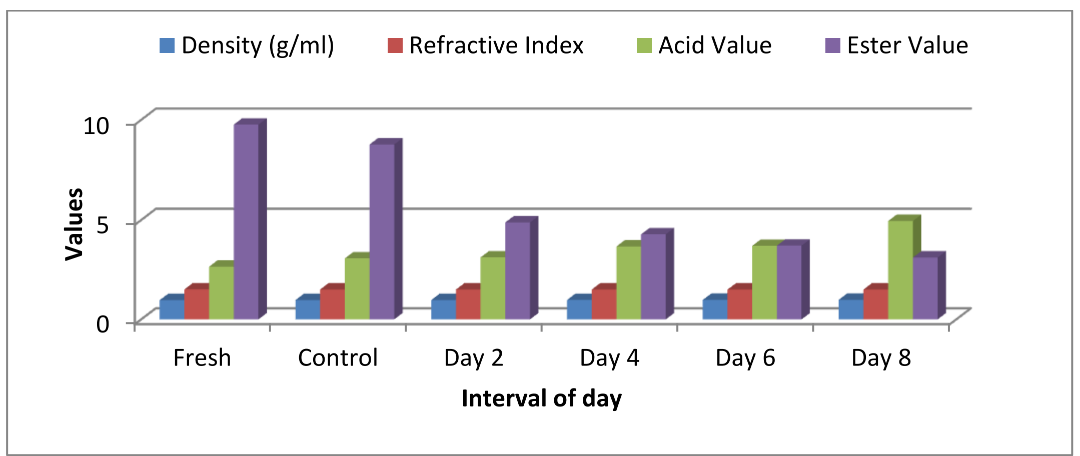


Fig 4.5 Physico-chemical quality of patchouli oil extracted after incubation with *Penicillium citrinum*

4.2.3 Physico-chemical quality of patchouli oil extracted after incubation with *Trichosporon asteroides*

The patchouli oil extracted at different intervals of days after fermentation was analyzed for density, refractive index, acid value and ester value. The data obtained are presented in Table 4.9. The given data indicates that the physico-chemical quality; density, refractive index, acid value and ester value of patchouli oil are significantly affected by the treatment of cultures with respect to days. Data indicates the increase in density however, decrease in refractive index, ester value and increase acid value with respect to increase in incubation time.

It is observed that the density of patchouli oil increases from 0.970 to 0.989 g/ml. The density of patchouli oil extracted from various incubated and control samples are 0.970, 0.972, 0.978, 0.981, 0.986 and 0.989 g/ml for fresh, control, 2, 4, 6 and 8 days treatment. The oil extracted from fresh and control patchouli gives least value of density 0.970 and 0.972 g/ml. The oil extracted from *Trichosporon asteroides* incubated sample after 8 days gives highest density 0.989 g/ml. compared to other samples. . Increasingly heavy weight fraction contained in the oil, the greater the density value. Similar results have been reported by Arpi *et al.* (2011) Indeswari *et al.* (2015), Joddy *et al.* (2007) and Karimi (2014) on patchoulioil.

Table 4.9 Physico-chemical quality of patchouli oil after incubation with *Trichosporon asteroides*

Treatment	Density (g/ml)	Refractive Index	Acid Value	Ester Value
Fresh	0.970	1.5098	2.65	9.76
Control	0.972	1.5070	3.07	8.77
Day 2	0.978	1.5029	3.14	7.46
Day 4	0.981	1.5018	3.40	6.11
Day 6	0.986	1.5014	3.66	5.61
Day 8	0.989	1.5014	4.25	5.60

From Table 4.9 Refractive index of patchouli oil obtained was 1.5098, 1.5070, 1.5029, 1.5018, 1.5014 and 1.5014 form fresh, control, 2, 4, 6 and 8 days sample incubated with *Trichosporon asteroides* extracts.

Fig.4.3. Data indicates that the recovery of patchouli oil is significantly affected by the treatment of culture. Oil recovery from the samples increases with the time. However, oil recovery in fresh and control samples after 2 day, remains constant. The oil recovery obtained after completion of distillation process from samples distilled at different intervals of incubation viz. 2, 4 and 6 days were 1.35, 1.47, 1.60and 1.62 % respectively. Highest oil recovery (1.62%) was recorded in the samples incubated up to 8 day with the *Trichosporon asteroides.* However, increase in the oil recovery is negligible after the 6 day of incubation.

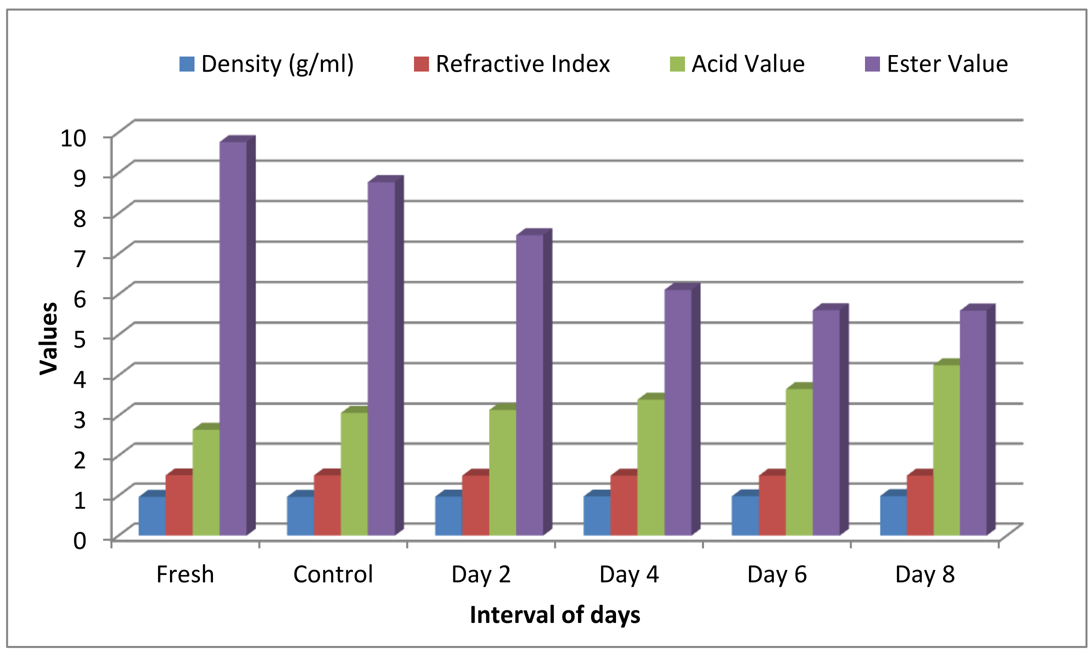


Fig 4.6 Physico-chemical quality of patchouli oil extracted after incubation with *Trichosporon asteroides*

The oil extracted from fresh patchouli gives highest value of refractive index 1.5098 and the oil extracted from sample treated with *Trichosporon asteroides* after 8 days sample gives least value 1.5014 compared to other samples. High refractive index indicates the amount of turbidity and concentration substrate.The essential oil of medium density will increase so that the light that comes will be more difficult to be refracted due to a greater refractive index oils Similar results have been reported by Noor *et al.* (2017), Arpi *et al.* (2011) Indeswari *et al.* (2015), Joy *et al.* (2001) and Joddy *et al.* (2007).

Acid value of oil obtained after different treated and untreated samples was 2.65, 3.07, 3.14, 3.40, 3.66 and 4.25 from fresh, control, 2, 4, 6 and 8 days samples. The oil extracted from fresh sample gives lower acid value 2.65 as compared to others sample, further increase in acid value will lead to no market for the oil. The acid value higher than 4.0 indicates the poor quality of oil and is not acceptable in the market. Oil storage too long or contact between patchouli oil with light or ambient air in the bottle while allowing an increase in acid number. Noor *et al.* (2017)

Ester values of different extracted oil were 9.76, 8.77, 7.46, 6.11, 5.61 and 5.60 from fresh, control, 2, 4, 6 and 8 days samples. When the oil extracted from fresh patchouli gives highest ester value 9.76 and the oil extracted from 8 days *Trichosporon asteroides* treated sample gives least value 5.60 as compared to other treatment. Similar results have been reported by Arpi *et al.* (2011) and Indeswari *et al.* (2015)

4.3 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.

4.3.1 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. Patchouli alcohol could be used as a marker for quality control. The combination GC-MS and the new optimum condition were used to determine the amount of patchouli alcohol in samples as shown in table Table 4.10, Table 4.11 and Table 4.12. Volatile oil was extracted from the samples after incubation with three different microbial culture *Aspergillus foetidus*, *Penicillium citrinum* and *Trichosporon asteroides* after different intervals of time *viz.* 2, 4, 6 and 8 days along with the fresh and control samples. The different compounds are detected in GC-MS analysis at different retention time.

The major compounds identified are (2-Pentanone, Copaene, Caryophyllene, Naphthalene, Azulene, Patchoulene, Thujopsene, Cyclohexane, p-

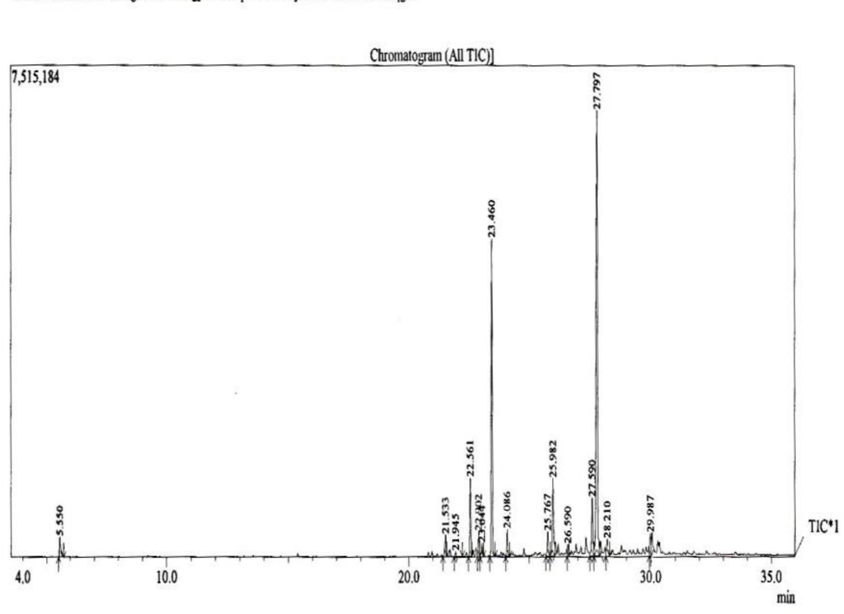
Menthane, Longifolenadehyde, Caryophyllene oxide, Epiglobulol, Patchouli alcohol, α –Bisabolol, β–humulene).

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

The data given in Table 4.10 and Fig. 4.7, 4.8, 4.9, 4.10 and 4.11 presents the analysis of oil extracted from the incubated herbage (herbage incubated with culture *Aspergillus foetidus*) after different intervals of times.The major content of sesquiterpene, patchouli alcohol obtained from 26.63%, 27.40%,29.31% and 30.27% in culture treated samples at 2, 4, 6 and 8 days. Fresh samples showed 25.75% patchouli alcohol. The other component varied like 2-Pentanone 0.75 to 0.90% Copaene 1.71to 1.12%, Caryophyllene 0.36 to 0.02%, Naphthalene 4.40 to 4.34%, Azulene 1.37 to 0.69%, Patchoulene 0.94 to 0.74%, Thujopsene 13.53 to 13.93%, Cyclohexane 1.34 to 0.55%,p-Menthane 1.59 to 1.90%, Longifolenadehyde 5.01 to 5.17%, Caryophyllene oxide 0.94 to 0.66%, Epiglobulol 3.81 to 3.29%, α –Bisabolol 1.19 to 0.74%, β–humulene 1.42 to 0.80%. Among the treatments, oil extracted after 8 days of incubation gives statistically superior oil quality in respect to patchouli alcohol, as the amount of patchouli alcohol was 30.27% as compared to fresh 25.75%.

Table 4.10 GC-MS data showing the biotransformation effect of *Aspergillus foetidus* on patchouli oil

S.No.	RT(min)	Compound	Fresh	Day2	Day4	Day6	Day8
1	5.54	2-Pentanone	1.32	0.75	0.95	0.91	0.90
2	21.53	Copaene	1.33	1.71	1.66	1.39	1.12
3	21.94	Caryophyllene	0.24	0.36	0.27	0.07	0.02
4	22.55	Naphthalene	3.68	4.40	4.23	4.18	4.34
5	22.90	Azulene	1.13	1.37	1.33	1.02	0.69
6	23.03	Patchoulene	0.78	0.94	0.92	0.79	0.74
7	23.46	Thujopsene	15.27	13.53	13.75	14.06	13.93
8	24.08	Cyclohexane	1.22	1.34	1.26	0.87	0.55
9	25.76	p-Menthane	1.42	1.59	1.48	1.63	1.90
10	25.98	Longifolenadehyde	4.72	5.01	5.13	5.05	5.17
11	26.58	Caryophyllene oxide	0.60	0.94	0.98	0.64	0.66
12	27.59	Epiglobulol	3.57	3.81	4.07	3.92	3.29
13	**27.79**	**Patchouli alcohol**	**25.75**	**26.63**	**27.40**	**29.31**	**30.27**
14	28.20	α –Bisabolol	1.27	1.19	1.43	1.03	0.74
15	29.98	β–humulene	1.39	1.42	1.39	1.12	0.80



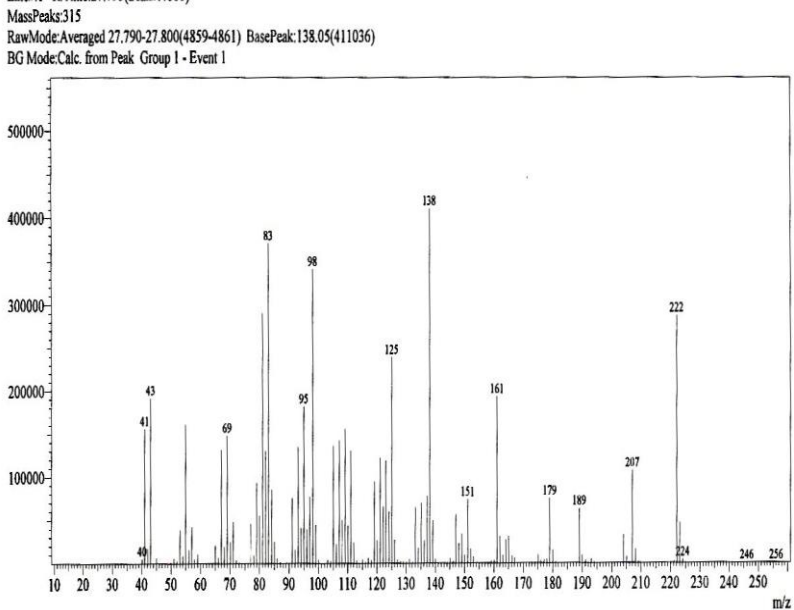
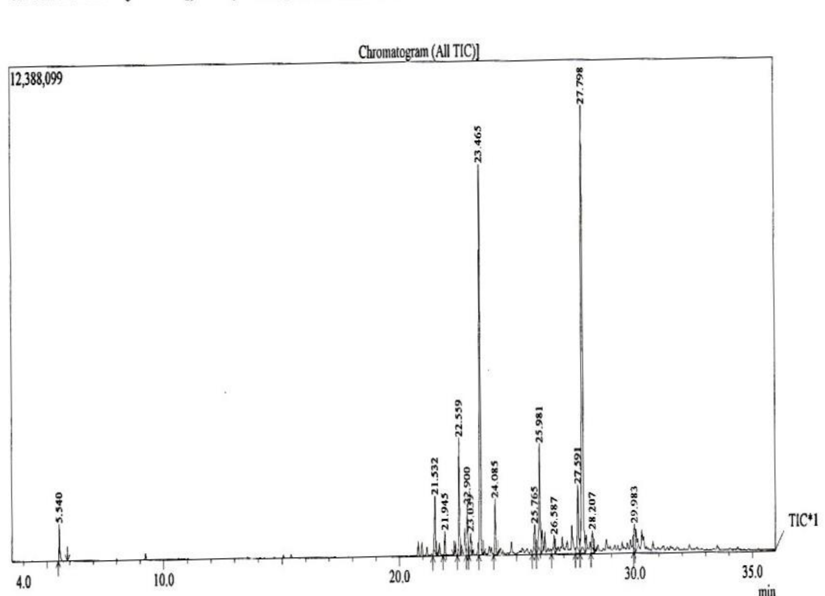


Fig. 4.7 Chromatograph of patchouli oil extracted from fresh sample



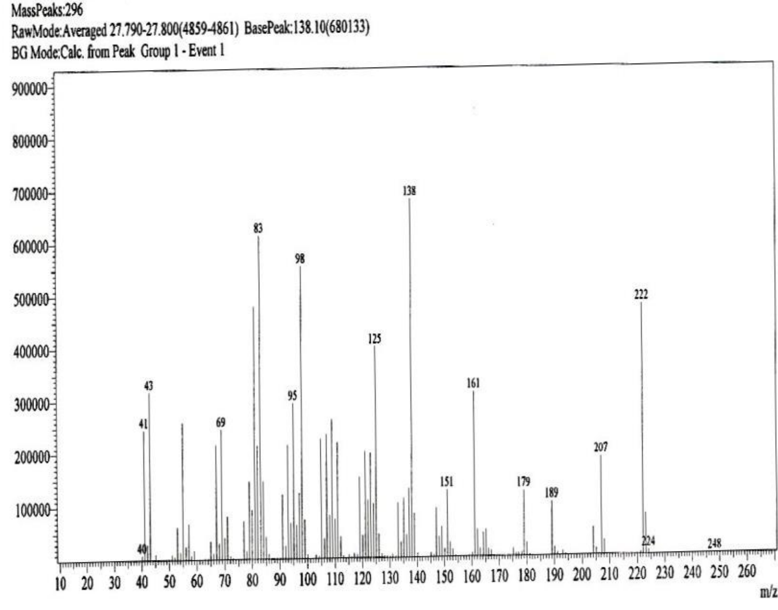
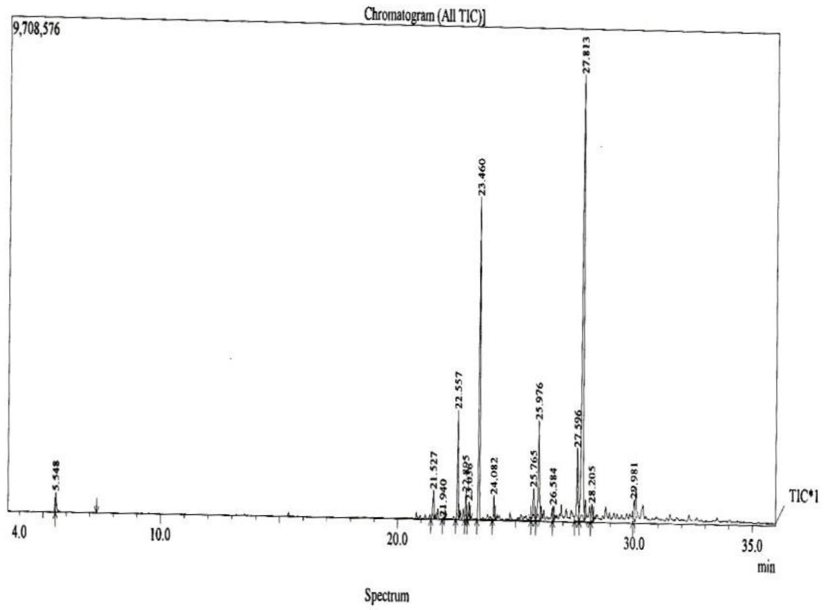


Fig. 4.8 Chromatograph of patchouli oil extracted from control sample



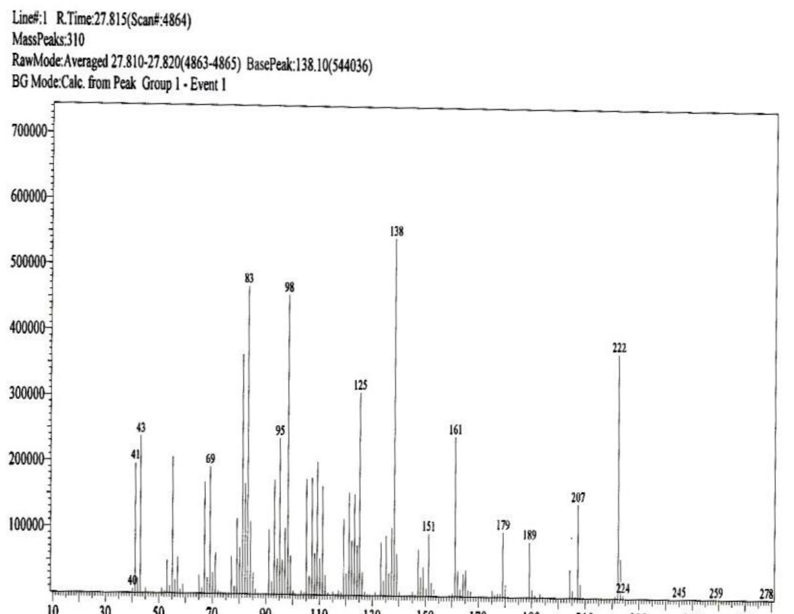
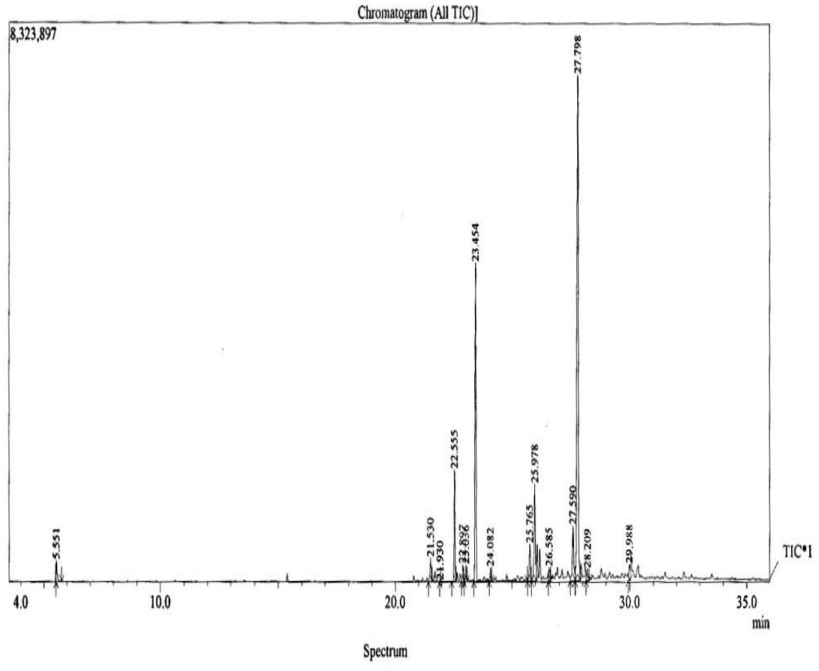


Fig. 4.9 Chromatograph of patchouli oil extracted after 2 days of incubation with *Aspergillus foetidus*



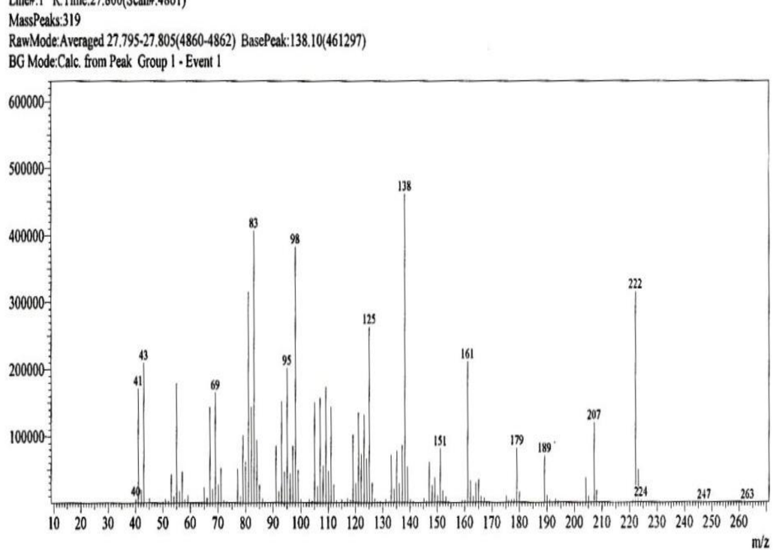


Fig. 4.10 Chromatograph of patchouli oil extracted after 4 days of incubation with *Aspergillus foetidus*

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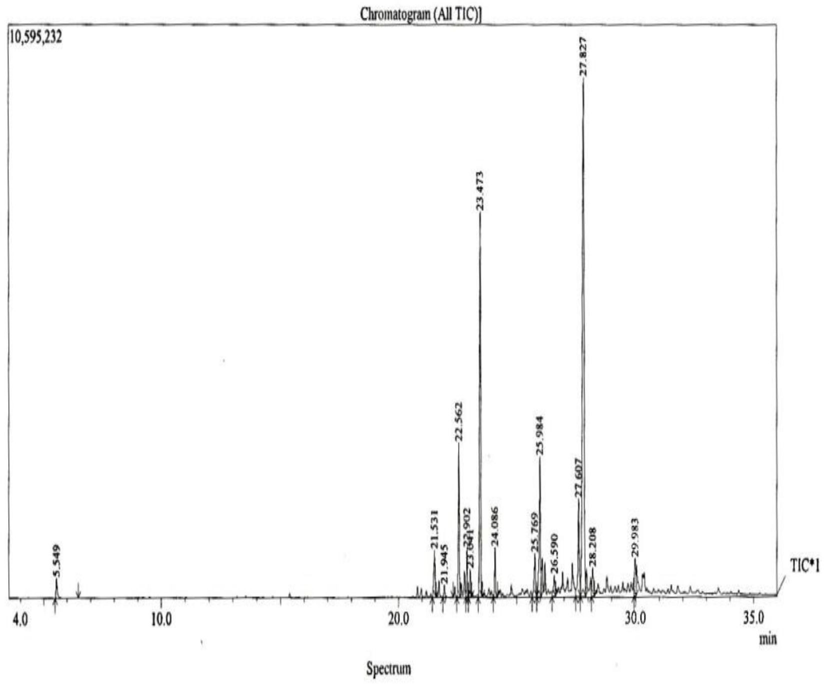


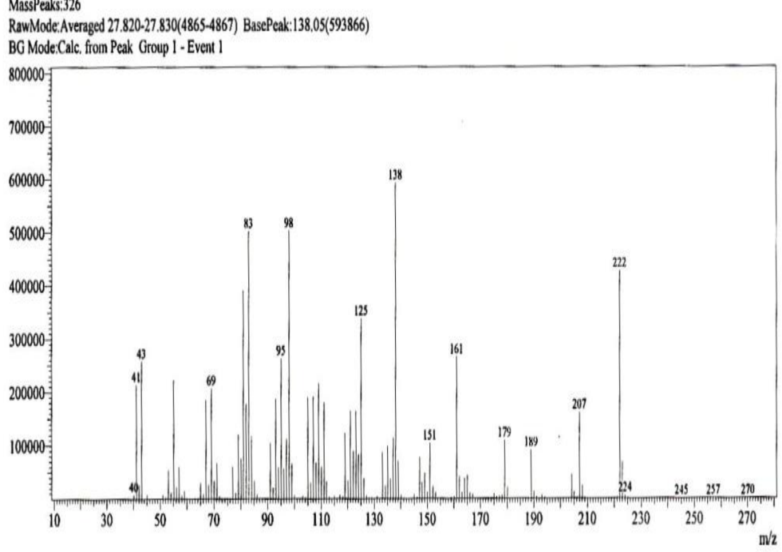
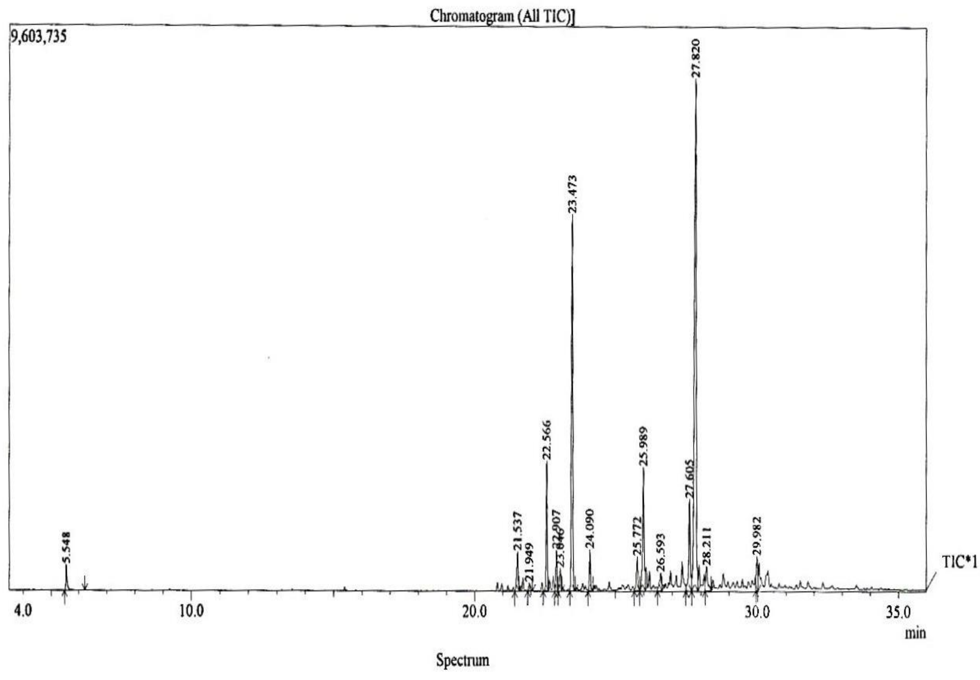


Fig. 4.11 Chromatograph of patchouli oil extracted after 6 days of incubation with *Aspergillus foetidus*



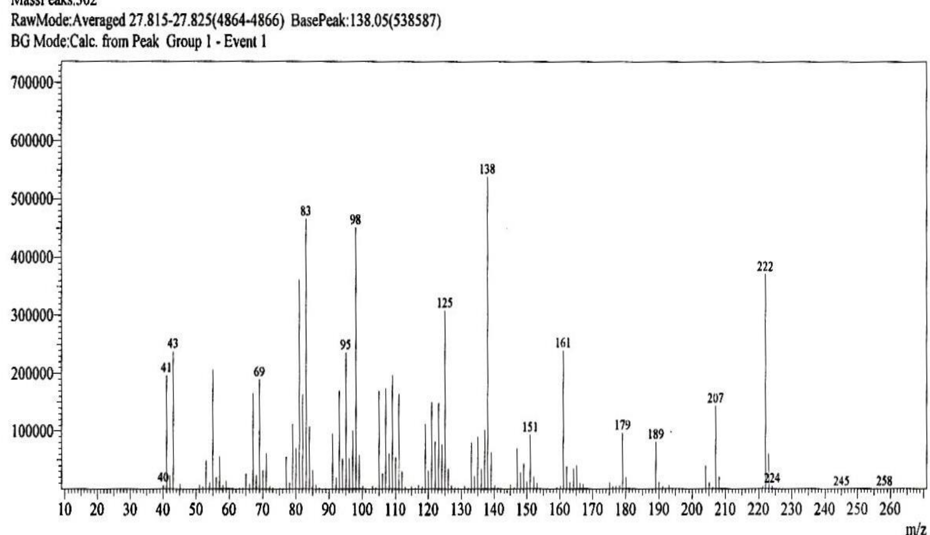


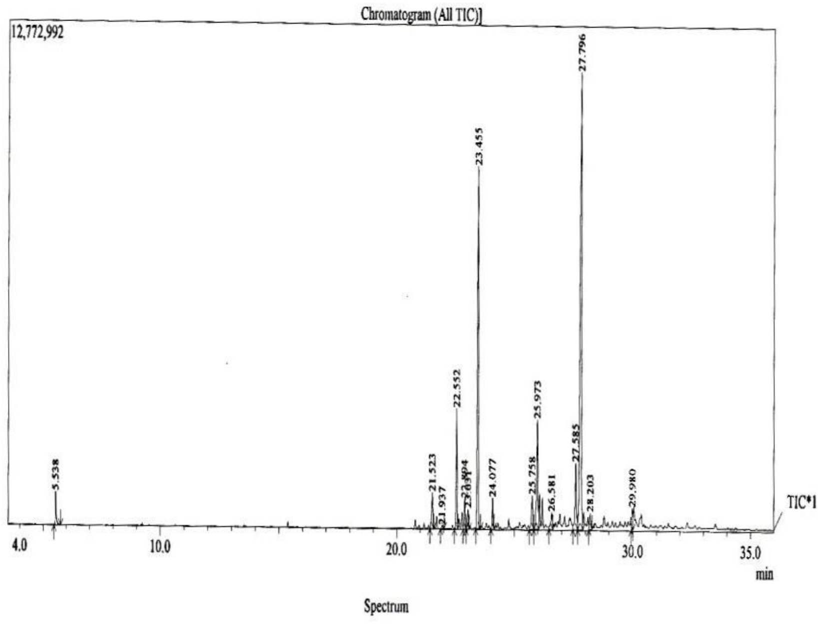
Fig. 4.12 Chromatograph of patchouli oil extracted after 8 days of incubation with *Aspergillus foetidus*

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

The data given in Table 4.11 and Fig 4.12, 4.13 and 4.14 presents the analysis of oil extracted from the incubated herbage (herbage incubated with culture *penicillium citrinum*) after different intervals of times. The major content of sesquiterpene, patchouli alcohol varied from 26.10%, 26.40%, 27.02% and 28.11% in culture treated samples at 2, 4 6 and 8 days. Fresh samples showed 25.75% patchouli alcohol. The other component like like 2-Pentanone 0.58 to 1.12% Copaene 1.64 to 1.68%, Caryophyllene 0.45 to 0.15%, Naphthalene 4.50 to 4.47%, Azulene 1.49 to 1.14%, Patchoulene 0.99 to 0.84%, Thujopsene 13.52 to 15.02%, Cyclohexane 1.51 to 1.09%,p-Menthane 1.67 to 1.69%, Longifolenadehyde 5.07 to 5.44%, Caryophyllene oxide 1.00 to 1.03%, Epiglobulol 3.71 to 3.42%, α –Bisabolol 1.39 to 0.99%, β–humulene 1.54 to 1.00%. Among the treatments, oil extracted 8 days of incubation gives statistically superior oil quality in respect to patchouli alcohol, as the amount of patchouli alcohol was 28.11% as compared to fresh 25.75%.

Table 4.11 GC-MS data showing the biotransformation effect of *penicillium citrinum* on patchouli oil

S.No.	RT(min)	Compound	Fresh	Day2	Day4	Day6	Day8
1	5.54	2-Pentanone	1.32	0.58	0.45	0.95	1.12
2	21.53	Copaene	1.33	1.64	1.69	1.67	1.68
3	21.94	Caryophyllene	0.24	0.45	0.33	0.10	0.15
4	22.55	Naphthalene	3.68	4.50	4.82	4.11	4.47
5	22.90	Azulene	1.13	1.49	1.47	1.01	1.14
6	23.03	Patchoulene	0.78	0.99	0.98	0.78	0.84
7	23.46	Thujopsene	15.27	13.52	14.21	13.51	15.02
8	24.08	Cyclohexane	1.22	1.51	1.41	0.97	1.09
9	25.76	p-Menthane	1.42	1.67	1.62	1.76	1.69
10	25.98	Longifolenadehyde	4.72	5.07	5.30	5.25	5.44
11	26.58	Caryophyllene oxide	0.60	1.00	1.02	1.06	1.03
12	27.59	Epiglobulol	3.57	3.71	3.58	3.71	3.42
13	**27.79**	**Patchouli alcohol**	**25.75**	**26.10**	**26.40**	**27.02**	**28.11**
14	28.20	α –Bisabolol	1.27	1.39	1.22	1.05	0.99
15	29.98	β–humulene	1.39	1.54	1.27	1.18	1.00



Spectrum

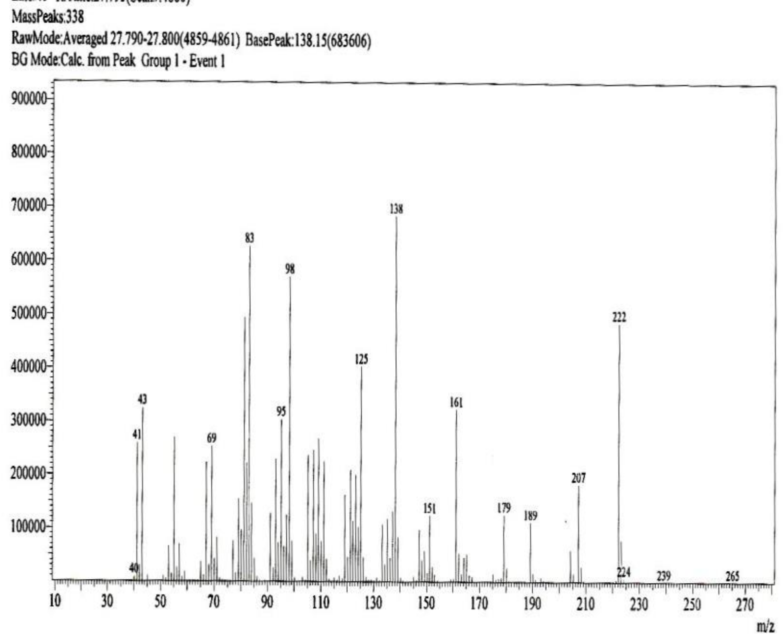
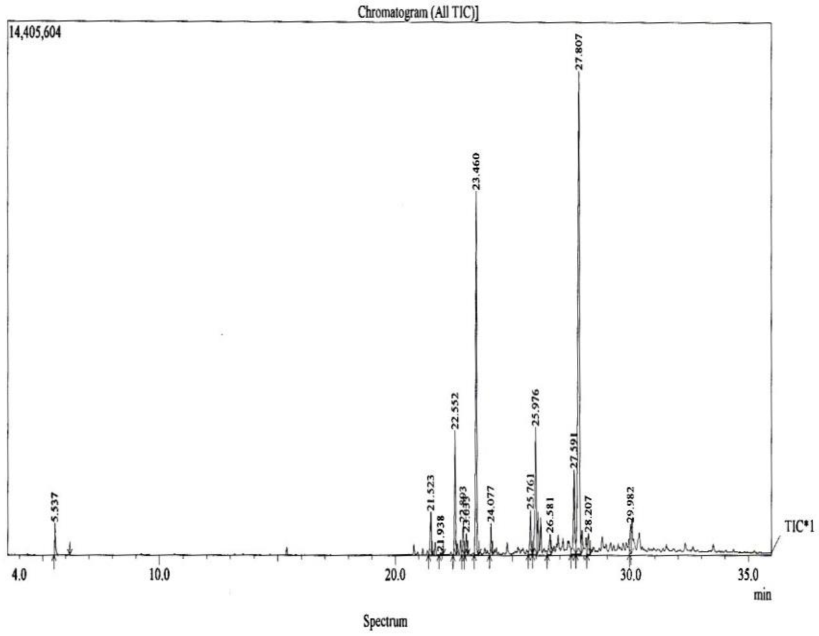


Fig. 4.13 Chromatograph of patchouli oil extracted after 2 days of incubation with *penicillium citrinum*



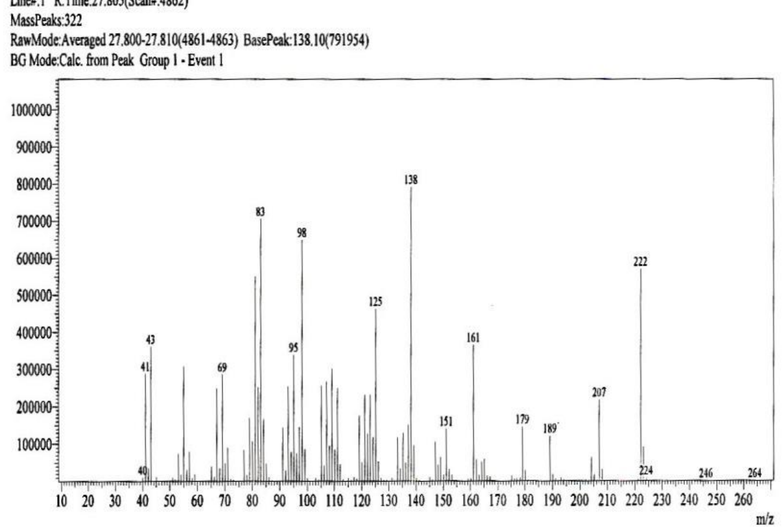


Fig. 4.14 Chromatograph of patchouli oil extracted after 4 days of incubation with *penicillium citrinum*

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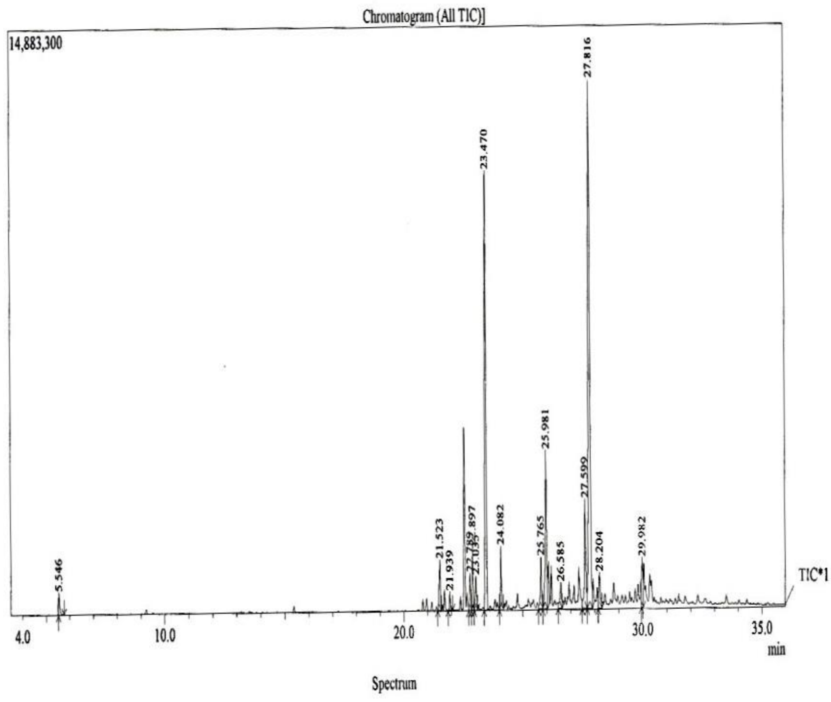


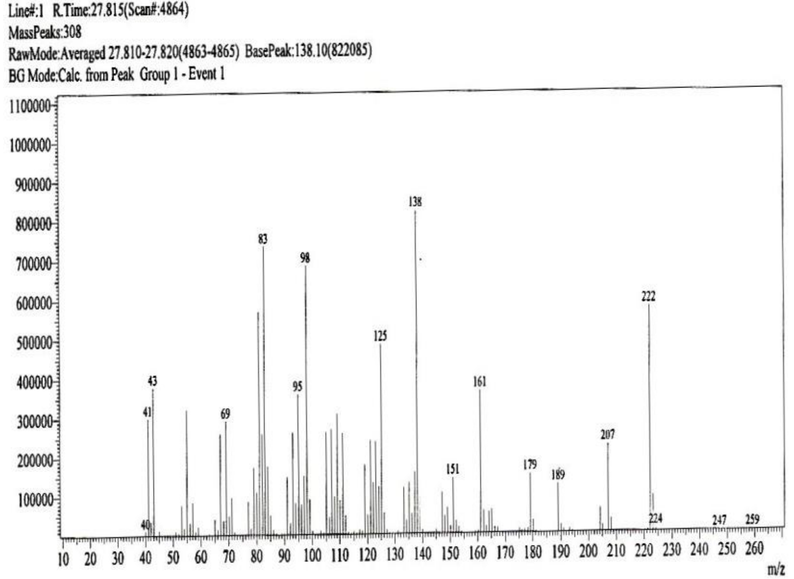
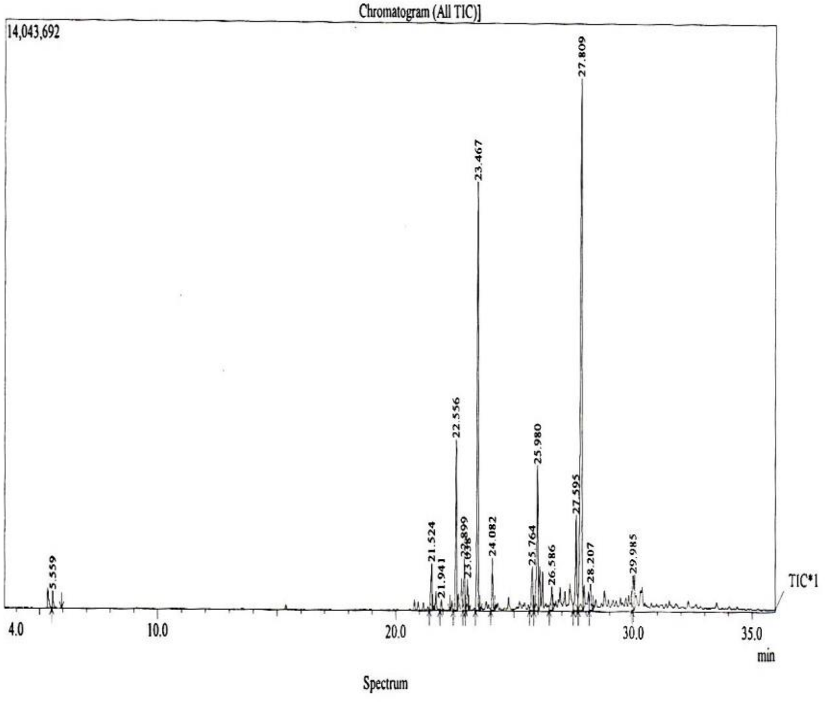


Fig. 4.15 Chromatograph of patchouli oil extracted after 6 days of incubation with *penicillium citrinum*



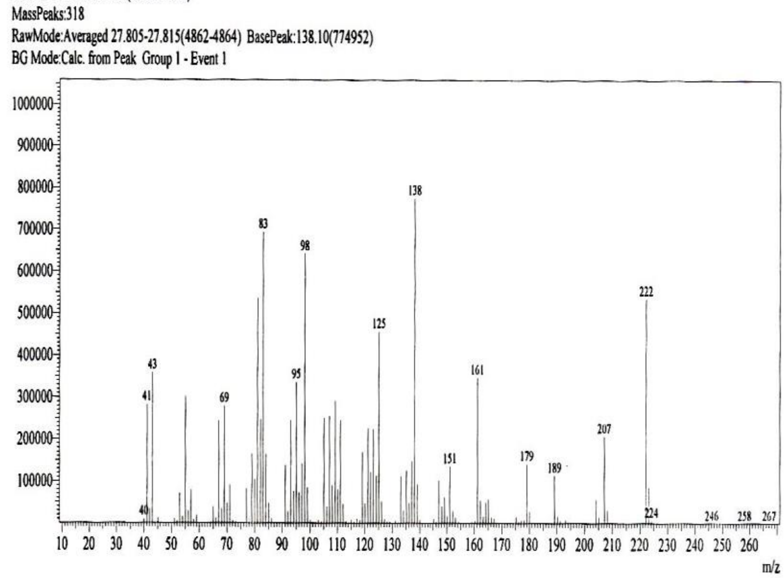


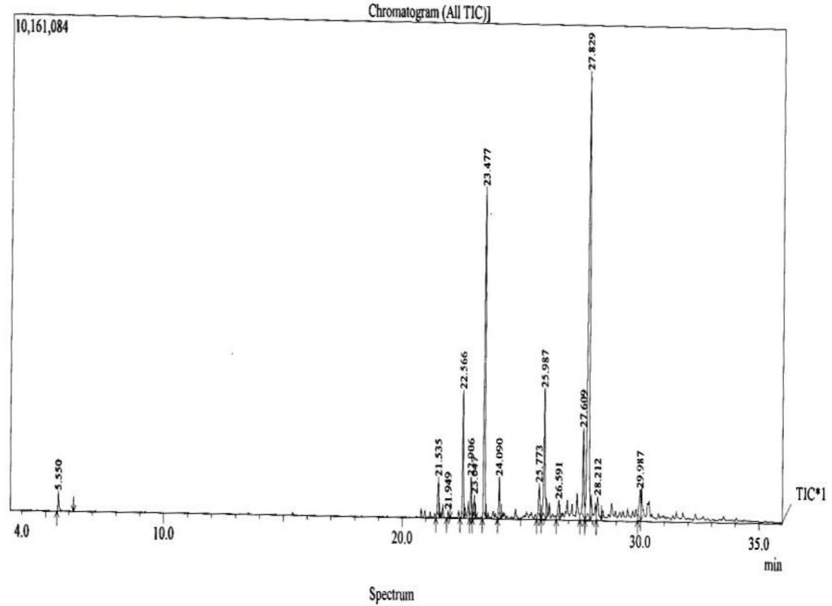
Fig. 4.16 Chromatograph of patchouli oil extracted after 8 days of incubation with *penicillium citrinum*

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

The data given in Table 4.12 & Fig. 4.15, 4.16, 4.17 presents the analysis of oil extracted from the incubated herbage (herbage incubated with culture *Trichosporon asteroides*) after different intervals of times. The major content of sesquiterpene, patchouli alcohol varied from 26.53%, 26.68%, 30.02% and 31.25% in culture treated samples at 2, 4, 6 days. Fresh samples showed 28.89% patchouli alcohol. The other component 2-Pentanone 0.81 to 1.07% Copaene 1.71 to 1.77%, Caryophyllene 0.24 to 0.28%, Naphthalene 4.22 to 3.92%, Azulene 1.33 to 1.32%, Patchoulene 0.92 to 0.92%, Thujopsene 13.02 to 14.53%, Cyclohexane 1.31 to 1.41%, p-Menthane 1.61 to 1.33%, Longifolenadehyde 5.57 to 5.39%, Caryophyllene oxide 1.11 to 0.64%, Epiglobulol 4.19 to 4.43%, α –Bisabolol 1.41 to 1.53%, β–humulene 1.78 to 1.77 %. Among the treatments, oil extracted after 8 days of incubation gives statistically superior oil quality in respect to patchouli alcohol, as the amount of patchouli alcohol was 31.25% as compared to fresh 25.75%.

Table 4.12 GC-MS data showing the biotransformation effect of *Trichosporon asteroides* on patchouli oil

S. No.	RT(min)	Compound	Fresh	Day2	Day4	Day6	Day8
1	5.54	2-Pentanone	1.32	0.81	0.80	1.29	1.07
2	21.53	Copaene	1.33	1.71	1.67	0.86	1.77
3	21.94	Caryophyllene	0.24	0.24	0.32	0.25	0.28
4	22.55	Naphthalene	3.68	4.47	4.06	2.65	3.92
5	22.90	Azulene	1.13	1.33	1.26	0.87	1.32
6	23.03	Patchoulene	0.78	0.92	0.90	0.61	0.96
7	23.46	Thujopsene	15.27	13.02	13.74	13.25	14.53
8	24.08	Cyclohexane	1.22	1.31	1.36	1.07	1.41
9	25.76	p-Menthane	1.42	1.61	1.60	1.60	1.33
10	25.98	Longifolenadehyde	4.72	5.57	5.34	4.80	5.39
11	26.58	Caryophyllene oxide	0.60	1.11	1.05	0.96	0.64
12	27.59	Epiglobulol	3.57	4.19	4.17	4.24	4.43
13	**27.79**	**Patchouli alcohol**	**25.75**	**26.53**	**26.68**	**30.02**	**31.25**
14	28.20	α –Bisabolol	1.27	1.41	1.42	1.13	1.53
15	29.98	β–humulene	1.39	1.78	1.30	1.23	1.77



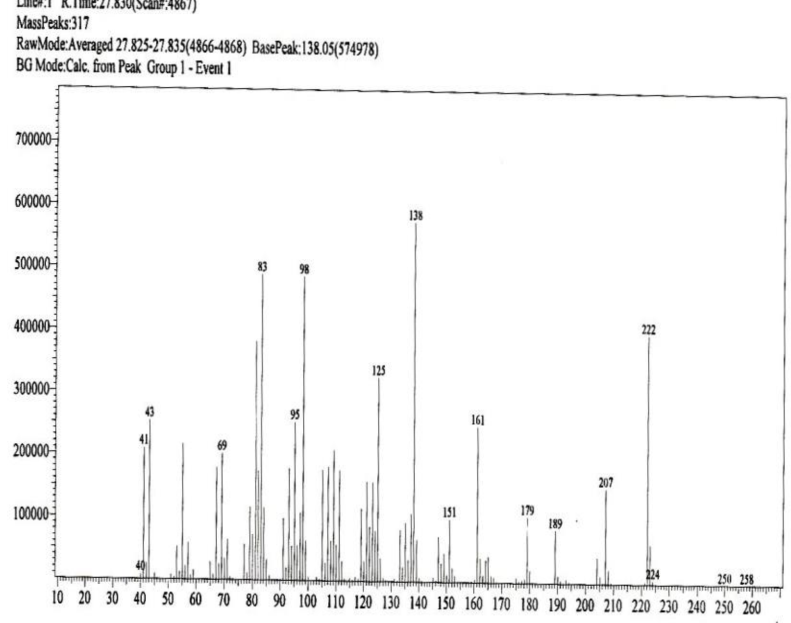
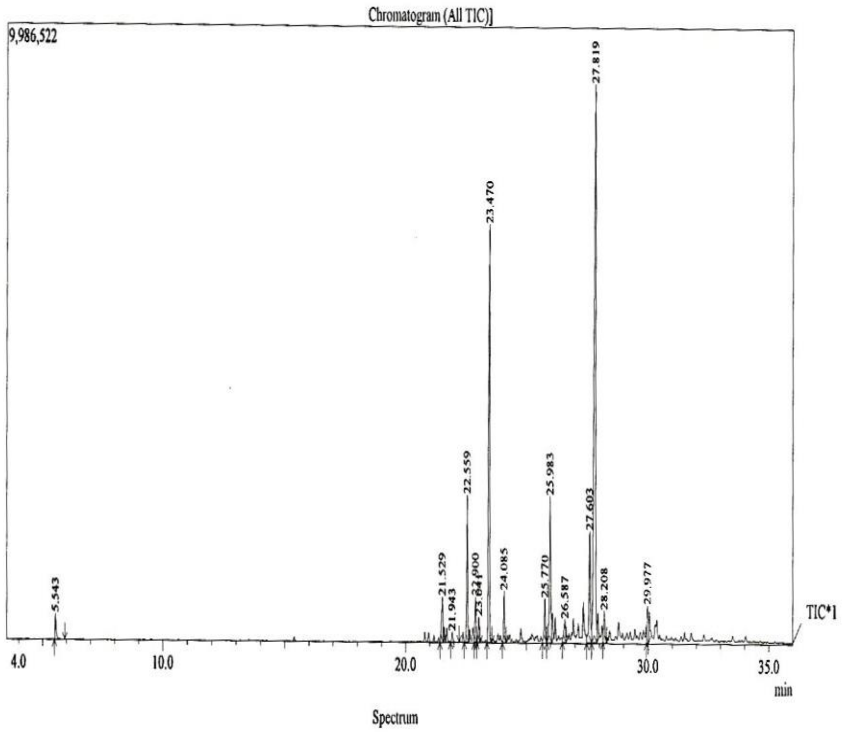


Fig. 4.17 Chromatograph of patchouli oil extracted after 2 days of incubation with *Trichosporon asteroids.*



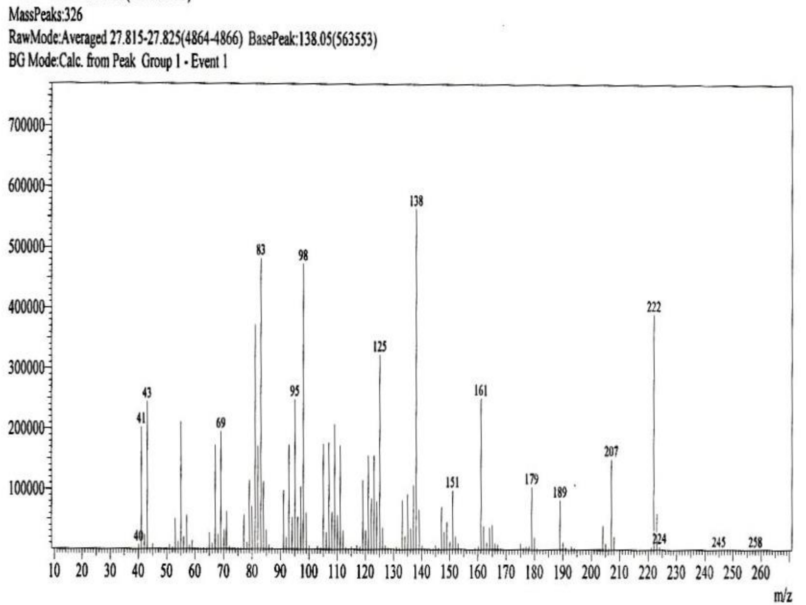
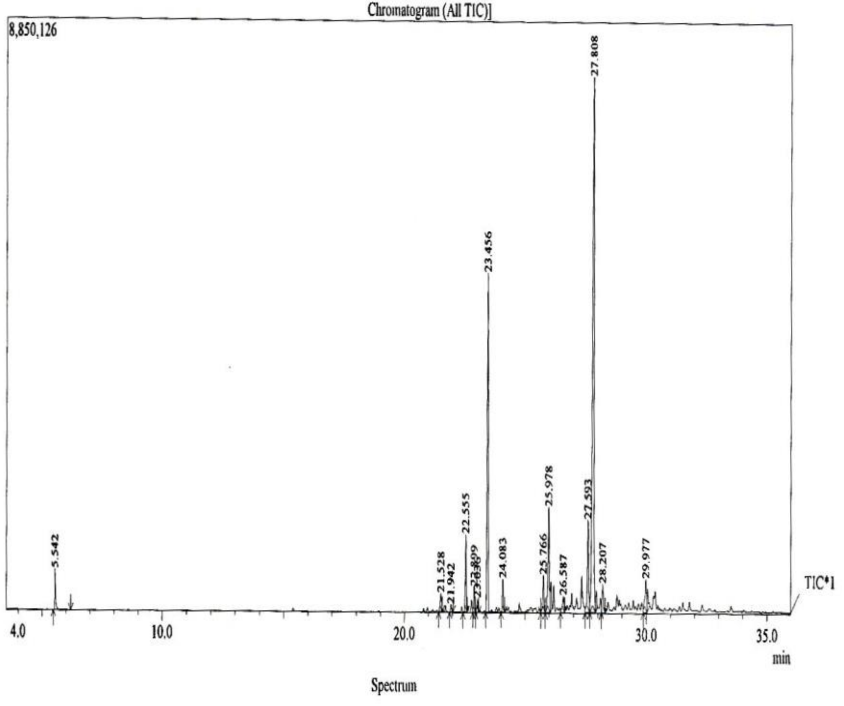


Fig. 4.18 Chromatograph of patchouli oil extracted after 4 days of incubation with *Trichosporon asteroides*



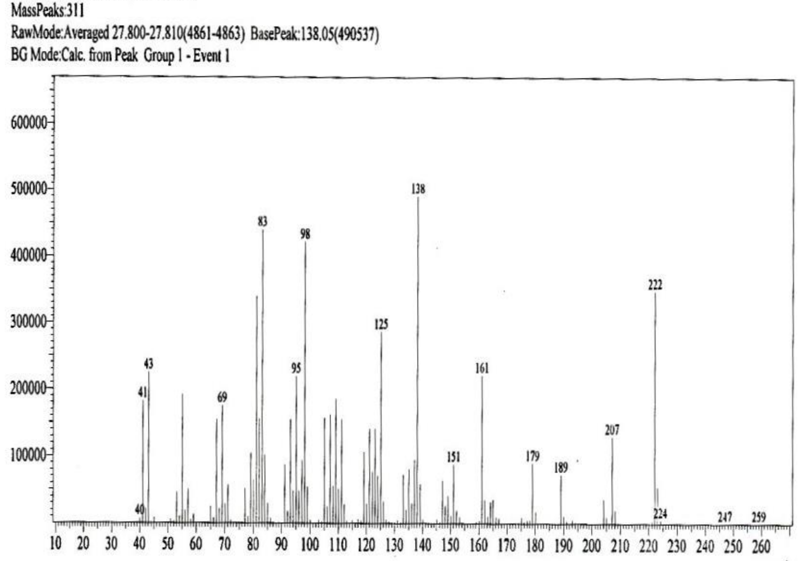
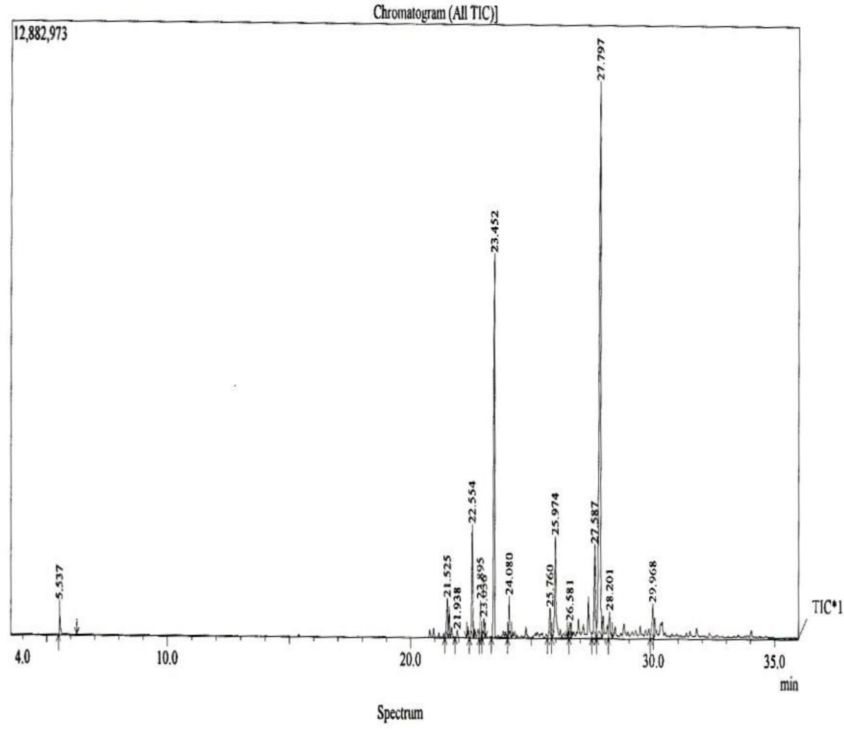


Fig. 4.19 Chromatograph of patchouli oil extracted after 6 days of incubation with *Trichosporon asteroides*



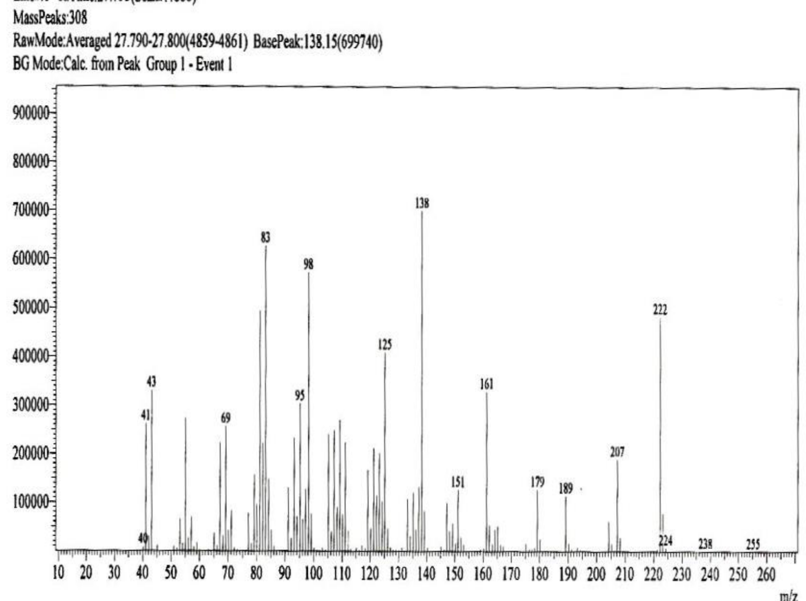


Fig. 4.20 Chromatograph of patchouli oil extracted after 8 days of incubation with *Trichosporon asteroides*.

CHAPTER – V
SUMMARY AND CONCLUSIONS

Patchouli oil is one of popular commodities in oil trading world. It is extracted from distilling the leaves and trees of patchouli plants *Pogostemon cablin* Benth. This oil is known as a fragrant aroma fixative and prevents evaporation of fragrant gases in perfume. In recent years, the patchouli oil produced by local farmers has very low grade, making the selling price relatively cheaper. The cause of low-grade quality is that the patchouli oil produced is still containing organic or inorganic colorants that makes the oil dark liquid. Therefore, it is necessary to develop an in expensive means to eliminate colorants in patchouli oil for better quality.

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.

The purpose of research was to determine the effect the growth of fungi *Aspergillus foetidus, Penicillium citrinum and Trichosporon asteroids patchouli herbage* and to determine the effect of incubation time on the yield and patchouli alcohol concentration in patchouli oil. Therefore, in the present need to provide the technology for oil extraction process to increasing the recovery percentage of patchouli oil by microorganisms and its quality the present research entitled "Pilot scale study for enhancement of patchouli essential oil quantity & quality using biotransformation process" has been undertaken. On the basis of the experiment and observation, the findings are as follows:

1. The recoveries of patchouli oil by treatment of *Aspergillus foetidus* increased up to 6 days (1.42%). After 6 days there was no significant increase in the oil quantity.
2. The recoveries of patchouli oil by treatment of *Penicillium citrinum* increased up

to 8 days (1.49%). However, increase in oil quantity after 6 days is negligible.

3. *Trichosporon asteroides* affected the oil recovery up to 8 days (1.62%). However, increase in oil quantity after 6 days is negligible.
4. The highest oil recovery was obtained with the *Trichosporon asteroids* in comparison to other two cultures.
5. The patchouli oil extracted after treatment with *Aspergillus foetidus* were analyzed for its physico-chemical quality. Density, refractive index, acid values and ester values for oil extracted from the different sample *viz.* fresh, control 2, 4, 6 and 8 days was found 0.970, 0.972, 0.973, 0.976 0.980 and 0.981 g/ml; 1.5098, 1.5070, 1.5029, 1.5024, 1.5008 and 1.5002; 3.65, 3.07, 3.17, 3.65, 3.77 and 4.40; 9.76, 8.77, 7.71, 6.14, 3.75, and 3.06 respectively.
6. The patchouli oil extracted after treatment with *Penicillium citrinum* were analyzed for its physico-chemical quality. Density, refractive index, acid values and ester values for oil extracted from the different sample *viz.* fresh, control 2, 4, 6 and 8 days was found 0.970, 0.972, 0.974, 0.977, 0.981 and 0.984 g/ml; 1.5098, 1.5070, 1.5024, 1.5014, 1.5023 and 1.5008; 2.65, 3.07, 3.12, 3.67, 3.70, and 4.95; 9.76, 8.77, 4.88, 3.28, 3.71 and 3.11 respectively.
7. The patchouli oil extracted after treatment with *Trichosporon asteroides* were analyzed for its physico-chemical quality. Density, refractive index, acid values and ester values for oil extracted from the different sample *viz.*fresh, control 2, 4, 6 and 8 days was found 0.970, 0.972, 0.978, 0.981, 0.986 and 0.989 g/ml; 1.5098, 1.5070, 1.5029, 1.5018, 1.5014 and 1.5014; 2.65, 3.07, 3.14, 3.40, 3.66 and 4.25; 9.76, 8.77, 7.46, 6.11, 5.61 and 5.60 respectively.
8. The best results were obtained with *Trichosporon asteroides* in improving the recovery of patchouli oil and patchouli alcohol content upto 1.62% and 31.25% respectively.

From the experiments conducted during the investigation and observation the following conclusion can be drown:

1. Oil recovery is improved with the use of microbial culture.
2. Quality of oil deteriorates, especially acid value increase with the increase in incubation period, which is not a good sign for oil quality.
3. The studies revealed a definite pattern of decrease in monoterpenes and sesquiterpenes and increase in patchouli alcohol content in most treatments.

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

Future scope:

1. Other new microorganisms may be screened or genetically engineered to improve the oil quality along with recovery.
2. Extraction of essential oils using steam distillation can be done at industrial scale to make various finished products.

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Raw data of recovery from patchouli herbage

1. Raw data for recovery of patchouli oil by *Aspergillus foetidus* at different intervals of time

Treatment	Oil recovery, g			
	R1	**R2**	**R3**	**Mean**
Fresh	19.25	21.90	20.71	20.62
Control	20.40	23.15	20.80	21.45
Day2	22.45	24.32	23.61	23.46
Day4	26.19	27.28	26.40	26.62
Day6	28.15	29.50	27.81	28.49
Day8	27.85	29.45	28.40	28.57

2. Raw data for recovery of patchouli oil by *Penicillium citrinum* at different intervals of time

Treatment	Oil recovery, g			
	R_1	**R_2**	**R_3**	**Mean**
Fresh	19.25	21.90	20.71	20.62
Control	20.40	23.15	20.80	21.45
Day2	23.78	24.60	25.03	24.47
Day4	26.57	27.86	26.90	27.11
Day6	28.92	31.25	29.14	29.77
Day8	29.54	30.80	29.21	29.85

3. Raw data for recovery of patchouli oil by *Trichosporon asteroides* at different intervals of time

Treatment	Oil recovery, g			
	R_1	**R_2**	**R_3**	**Mean**
Fresh	19.25	21.90	20.71	20.62
Control	20.40	23.15	20.80	21.45
Day2	26.98	27.31	27.10	27.13
Day4	28.21	31.55	29.02	29.59
Day6	30.02	33.85	32.24	32.04
Day8	31.68	33.24	32.50	32.47

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