## 3. Results and Discussion

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

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

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

### 3.1 Chemical Characterization and Quantification of Compounds

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

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

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

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

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

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

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

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

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

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

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

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

## 4. Conclusion

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

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## Author Profile

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

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

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

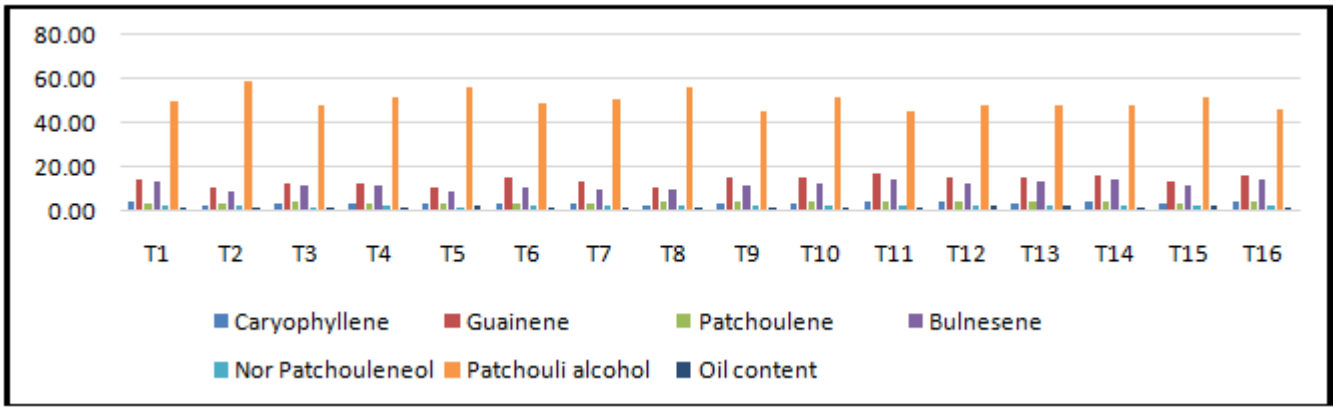


**Figure 2:** Variation of oil and sesquiterpene content

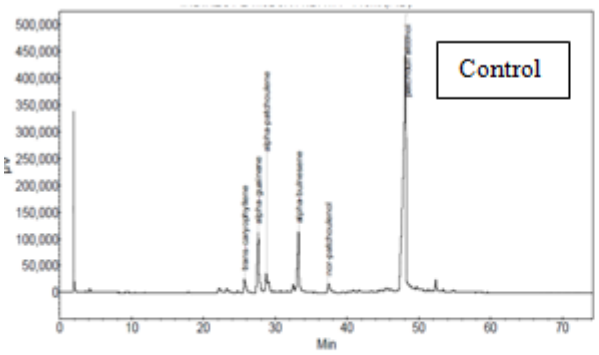


**Figure 3a:** Gas Chromatographic profile of Control (untreated)

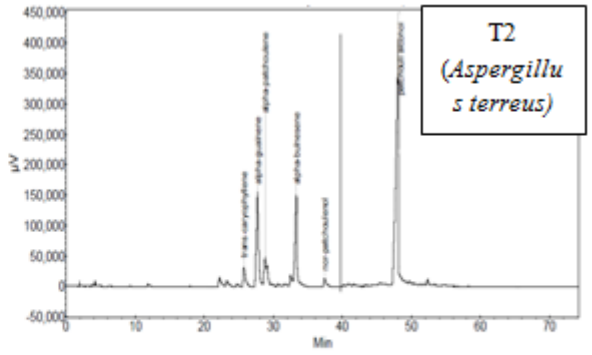


**Figure 3b:** Gas Chromatographic profile of T2 (*Aspergillus terreus)*