Introduction

Chemo-taxonomy is the approaches of taxonomy, it gives the close relationship between chemical constituents of plants and their taxonomical status. It will be the most useful guide to man in his search for new industrial and medicinal plants and chemical characteristics of plants will be most valuable to plant taxonomy in the future (De Candolle, 1816). Some possibilities of chemo-taxonomic study or chemical characters of the plants are as a guide for classification and give support to in unambiguous identifications of plants. However, many more aspects, which make the study of chemical characters at infra-specific and specific levels a very fascinating one. Besides, being helpful with the identification of plant specimens, it informs us about patterns of chemical variation within genera and cumulative species, it may ultimately express how one pattern of plant constituents evolved from a preceding one. A systematic knowledge in these fields is essential for a judgement of the overall taxonomic implications of the overwhelming multitude of phyto-chemical patterns. Therefore, it is easy to understand why chemotypic studies of the active constituents are playing a gradually more important role over and above chemical and analytical evaluations.

Several species of the family Lamiaceae which are rich in essential oils, of great importance as herbs and spices. As regards their chemical compositions and morphology, a significant proportion of these plants are polymorphic. Forty percent of the species of Lamiaceae family are thought to contain compounds that possess aromatic properties (Lawrence, 1992). In this context, complex research into the family Lamiaceae was begun some decades ago as a cooperative program in which several Hungarian research centers are engaged in the evaluation of medicinal plants. Many plants have various phenotypes that differ in their appearance, and both quantitative and qualitative diversity is often detected in the composition of essential oils obtained, e.g., in the cases of Oregano (Pasquier, 1997). Participating in this research, present study focused on *O. vulgare*, herb of diverse appearance.
and chemical constitution, in order to study their variability and constituent characteristics.

A number of studies have shown that *O. vulgare* subsp. *hirtum* (Link) Ietswaart is a very variable taxon in both morphological and chemical terms. In particular, it has been found that the essential oil content of this taxon in Greece ranges from 1.1 to 8.2%. Furthermore, it has been reported that the essential oil composition of the wild-growing plants is characterized either by the predominant presence of carvacrol or thymol. The monoterpenic hydrocarbons γ-terpinene and p-cymene were consistently present in all the essential oils analyzed, but always in lower amounts than those of the two phenols (Kokkini and Vokou, 1989; Kokkini *et al*., 1994; Kokkini *et al*., 1997 and Kokkini *et al*., 2004). The constituents γ-terpinene and p-cymene are biosynthetic precursors of the isomeric phenols carvacrol and thymol (Poulose and Croteau, 1978 and Nhu-Trang *et al*., 2006).

Many quantitative investigations of Oregano and its essential oils did not differentiate the numerous subspecies that exhibit slight morphological and chemical differences (Faleiro *et al*., 2005). The yield of essential oil is the only character that demonstrates relative stability, thus being useful for the identification of subspecies (Russo *et al*., 1998). The *Origanum* species vary in respect of their total amount of essential oils and their qualitative composition. On the basis of their essential oil content, the different taxa of the genus can be divided into three main groups:

1. Less essential oil content taxa (<0.5%), e.g. the Greece endemic *O. vulgare* subsp. *vulgare* and *O. laevigatum*.

2. Taxa with an essential oil content between 0.5 and 2%, e.g. the Cretan endemic *O. microphyllum* known as Cretan marjoram.


A number of studies have shown that variation in chemical features may occur within a single *Origanum* species. Furthermore, it has been found that the pattern of variation of a single species follows its geographical distribution or depends on the season of plant collection (Kokkini, 1997). The variation in the essential oil content of...
*O. vulgare* plants grown all over the Greece has been studied (Kokkini *et al*., 1991 and Kokkini *et al*., 1994). A survey of the literature reveals that *O. vulgare* subsp. *vulgare* has an extremely low essential oil yield and rich in acyclic compounds and sesquiterpenoids (Sezik *et al*., 1993 and Kaul *et al*., 1996). Its principal constituents are sabinene, Z-β-ocimene, β-caryophyllene and germacrene D, while the phenols thymol and carvacrol were absent (Russo *et al*., 1998).

On the basis of chemical diversity several chemotypes or chemical groups were separated *viz*., plant grown in an experimental field in Italy: 6 chemical groups, in which hydrocarbons predominated (Chalchat and Pasquier, 1998); Melegari *et al*. (1995) found 4 chemotypes: p-cymene, terpinen-4-ol, thymol and β-caryophyllene from the essential oils of Oregano inflorescences; in a study of Lithuanian samples (β-caryophyllene, 10.8–15.4%; germacrene D, 10.0–16.9%; sabinene, 6.4–14.2%; Z-β-ocimene, 6.2–11.0%; and E-β-ocimene, 7.0–11.5%) (Mockute *et al*., 2001). An Indian oil unusually contained γ-muurolene (62.2%) (Pande and Mathela, 2000). The chemical composition of the essential oil of *O. vulgare* L. varies with geographical location of collection site, climate and other ecological conditioning factors (Werker *et al*., 1985; Melegari *et al*., 1995; Kaul *et al*., 1996; Baratta *et al*., 1998; Chalchat and Pasquier, 1998; Pande and Mathela, 2000; D’Antuono *et al*., 2000; Mockute *et al*., 2001; Mockute *et al*., 2003; Kumar *et al*., 2007). Moreover, chemo-systematic investigation of mono and sesquiterpenoids in *Origanum* has been published (Skoula *et al*., 1999).

### 8.2 Material and Methods

The material and methods are discussed in detail in chapter number-3.

### 8.3 Results

#### 8.3.1 Essential Oil

Result shows that the total essential oil content was found significantly varies (at p<0.01 and p<0.05) between NT and FGB Conditions. Maximum total essential oil content on (GM) gross mean basis 0.88% found in NT condition and minimum 0.58% in FGB condition. Among all the germplasm samples collected from different locations was found significantly different at p<0.01 and maximum essential oil i.e.,
1.70% was found in NKO-68/IC589087 population followed by NKO-58/IC574520, NKO-77 and NKO-20/IC573219 with 1.60%, 1.30% and 1.25% respectively while, minimum (0.20% and 0.25%) value were recorded from the population of NKO-41/IC574505 and NKO-09/IC573209 respectively.

The variation in essential oil percentage was found significant in some accessions collected from natural conditions at p<0.05. The accession NKO-68/IC589087 extracted maximum essential oil content (2.07%) followed by NKO-20/IC 573219 (2.00%) and NKO-58/IC 574520 (1.70%) in NT condition, while minimum content 0.17% was recorded in NKO-09/IC573209 population (Table- 8.1). Among the 33 populations of NT condition, essential oil content was varied ranging from 0.17% to 2.07%.

8.3.2 Chemical compositions of *Origanum vulgare* essential oil

The results for the major chemical compounds of *Origanum vulgare* essential oil are presented in table (8.2 & 8.3).

8.3.2a p-cymene

The content of p-cymene in essential oil extracted ranging from 1.01% to 13.74%. The availability of p-cymene content shows significant at p<0.01 in NT and FGB conditions. In NT condition, it revealed maximum value (5.51%) of p-cymene and minimum 4.82% in FGB condition. The variation of p-cymene content was found significant difference at p<0.01 with higher value (13.74%) was found in NKO-15/IC573214 population followed by NKO-58/IC574520, NMJO-3077/IC589092 and NMJO-2993/IC582510 populations with 11.19%, 11.12% and 10.91% respectively. The lowest value (1.01%) was found in NKO-09/IC573209 population. Between NT and FGB conditions, maximum p-cymene value (17.31%) was found in FGB condition (NKO-15/IC573214) followed by 17.21% in NMJO-3015/IC582532 whereas 15.16% (NMJO-2993/IC582510) in NT condition, whereas the minimum value (0.35%) was found in NMJO-2983/IC582500 population in FGB condition (Table-8.2).

8.3.2b Zβ-Ocimene

The percentage of Zβ-ocimene in essential oil found significant (at p<0.01 & p<0.05) between NT and FGB condition. Maximum content (0.65%) was recorded in
FGB condition and minimum 0.61% in NT condition. The variation was found significant at $p<0.01$, varied from 0.08% to 2.57%. The highest content (2.57%) was found in NKO-72/IC589090 population followed by NMJO-3077/IC589092 and NMO-3015/IC582532 with 2.08% and 2.00% respectively whereas the lowest content (0.08% and 0.10%) was observed in NKO-77 and NKO-41/IC574505 respectively. Interaction between NT and FGB conditions, the maximum content (2.57%) was found in NMO-3015/IC582532 population in FGB condition followed by 2.60% in NKO-72/IC589090 population in NT condition, whereas the minimum content (0.03%) was recorded in NKO-16/IC 573215 population in FGB condition (Table 8.2).

8.3.2c Eβ-Ocimene

Result shows that Eβ-ocimene content of essential oil was found significant (at $p<0.01$) between NT & FGB conditions and revealed that Eβ-ocimene content was found 0.62% (maximum) in FGB condition and 0.50% (minimum) in NT condition. The variation was found significant at $p<0.01$ and the highest value (2.78%) was recorded from NMJO-3077/IC589092 population followed by NKO-20/IC573219 with 1.11%. The lowest content was recorded in NKO-77 population with 0.05%. Between NT & FGB conditions, maximum content (2.78%) was found in both NT & FGB condition in NMJO-3077/IC589092 population followed by, 1.56% (NKO-15/IC573214) in FGB condition whereas the minimum content (0.05%) was found from NKO-77 population in both NT & FGB conditions. Variation among NT & FGB condition was found significant at $p<0.01$ and ranging between 0.05% to 2.78% in essential oil (Table 8.2).

8.3.2d γ-Terpinene

The content of γ-terpinene in essential oil was found significant (at $p<0.01$) in both NT & FGB conditions. In FGB conditions the maximum content (8.15%) were recorded and minimum content (6.92%) was found in NT condition. The variation was found significant at $p<0.01$. The highest content (24.25%) was found in NMJO-3077/IC589092 population followed by NKO-64/IC589084 and NKO-21/IC573220 with 21.80% and 16.10% respectively. The lowest content (1.60%) was found in NKO-77 population. Interaction between conditions and germplasm was found significant variation at $p<0.05$. Population NMJO-2983/IC582500 shows maximum
γ-terpinene content (29.09%) in NT condition followed by NKO-64/IC589084 (25.40%) whereas the minimum content (0.14%) was also found in NT condition from NKO-25/IC573224, population. Among both condition, the content of γ terpinene was varied ranging between 0.14% to 29.09% (Table 8.2).

8.3.2e Linalool

Result shows that variation of linalool content in essential oil was signification (at p<0.01 & p<0.05) between NT & FGB conditions. The highest content (6.70%) was observed in FGB condition and the lowest content (5.46%) in NT condition. The data revealed a significant variation at p<0.05 and ranging between 0.33% to 20.77%. The highest content (20.77%) was found in NKO-41/IC574505 population followed by NKO-26/IC573225 (16.60%). The lowest content (0.33%, 0.34% and 0.39%) was found in NKO-68/IC589087, NKO-77 and NKO-65/IC589085 populations respectively. Among NT and FGB conditions, the upper limit (24.40%) was found in NMJO-2983/IC582500 in FGB condition afterward 20.77% was found in both NT and FGB condition in NKO-41/IC574505 population and NKO-72/IC589090 (16.96%) in FGB condition. The lowest content (0.16%) was found in NKO-65/IC589085 in FGB condition. The variation between NT and FGB condition was found significant at p<0.05 (Table 8.3).

8.3.2f Thymol

The quantitative content of thymol in essential oil represent significant (at p<0.01 & p<0.05) between NT and FGB conditions. Maximum content (23.27%) was recorded in FGB condition and minimum (22.47%) in NT condition. Over all it was found significant variation at p<0.01 & p<0.05, varied from 053% to 81.25%. The highest content (81.25%) was found in NKO-68/IC589087 followed by NKO-65/IC589085 (79.42%) populations whereas the lowest content (0.53% and 0.65%) was recorded in NKO-77 and NMJO-3077/589092 respectively. Between NT and FGB conditions, the highest content was found in NT condition in NKO-68/IC589087 and NKO-65/IC589085 population with 85.87% and 81.45% respectively while, the lowest content (0.30%) was recorded in NKO-18/IC573217 population in FGB condition and in NKO-25/IC573224 (0.32%) in NT condition. Variation among NT and FGB conditions was found significant at p< 0.01 & p< 0.05, ranged from 0.30% to 85.87% (Table 8.3).
8.3.2g Carvacrol

The variation of percentage carvacrol in essential oil was found significant (at p<0.01 & p<0.05) between NT and FGB conditions. Maximum content 13.85% was recorded in NT condition and minimum 11.78% in FGB condition. Over all the variation was found significant at p<0.01 & p<0.05, varied between 0.25% to 80.11%. The highest content (80.11%) was found in NKO-77 population followed by MMBO-3055/IC589079 and MMBO-3040/IC589077 with 57.38% and 48.53% respectively. The lowest content (0.25% and 0.27%) was recorded in NKO-45/IC574508 and NMJO-3077/IC589092 populations. Interaction between NT and FGB conditions, maximum content (81.01% and 79.21%) was found in both NT and FGB conditions respectively in NKO-77 population, followed by 63.60% in NT condition (MMBO-3055/IC589079). Minimum content (0.02%) was recorded in NMVMKO-14/IC573213 in NT condition. Variation among NT and FGB conditions was found significant at p<0.01 & p<0.05 and varied between 0.02% to 81.01% (Table 8.3).

8.3.3 Chemotypic Investigation

Aerial parts of all accessions collected from both condition (NT and FGB) were subjected to Clevenger Hydro-distillation and observed 0.17% to 2.07 % of essential oil. While scanning the analytical data it is evident that 13 of 33 accessions have an essential oil yield from 1.06% to 2.07% in NT condition and 5 accessions have an essential oil yield from 1.0% to 1.50% in FGB condition. This range considered as an extremely high as compared to reported value of 0.15 to 0.41% (Anonymous, 1966) on dry weight basis (DWB). Some of the genotypes were identified for high oil yield in both conditions, as it was three to five times greater (2.07%) in comparison to reported value (0.41%) such as NKO-56/IC574518 (1.21% & 1.25%), NKO-77 (1.30% & 1.29%), NKO-58/IC574520 (1.70% & 1.50%), NKO-20 (2.00% & 0.50%) and NKO-68/IC589087 (2.07% & 1.33%) (Table 8.1).

The obtained results derived from the analysis of 33 accessions, for extraction of essential oil yield and its percentage relative composition of major chemical constituents are summarized in Table 8.2 and 8.3. Gas chromatography samples of these essential oil have revealed presence of aroma constituents particularly major phenolic compounds (Thymol and Carvacrol), hydrocarbons and mono-terpenes.
(p-cymine, Zβ-Ocimene and Eβ-Ocimene), terpenes (γ-Terpinene) and alcohol oxides (Linalool) these can be used in perfumery, cosmetics, pharmaceuticals, food and beverage industry. On the basis of these seven major chemical constituents, following seven chemotypes were identified (Figure 8.3a & b, 8.4 and 8.5a & b).

8.3.3a Chemotype-I (Thymol rich type)

Two accessions were investigated as a high content of thymol content. Average thymol content 81.25% (85.87% in NT condition and 76.63% in FGB condition) was recorded in NKO-68/IC589087 from 3262 to 3300 m asl., in district Chamoli, Central Himalaya. The plant is having light pinkish colour flowers with purple colour bracts. Another accession NKO-65/IC589085, average thymol content 79.42% (81.45% in NT condition and 77.38% in FGB condition) from 3550 m asl., in district Rudrapryag, Central Himalaya. The plant is having light pinkish colour flowers with purple colour bracts. Plant population of these Origanum accession were observed in the other plant association ship of Allium auriculatum Kunth, Bistorta affinis D. Don, Geranium wallichianum D. Don, Impatiens sulcata Wall., Podophyllum hexandrum Royle, Potentilla fulgens Wall. ex Hook, Bistorta affinis D. Don, Erigeron multiradiatus (Lindl. ex DC.) Benth. & Hooker, Geranium wallichianum D. Don, Impatiens sulcata Wall., Potentilla fulgens Wall. ex Hook., Rumex acetosa L., etc. at high altitudes.

8.3.3b Chemotype-2 (Carvacrol rich type)

Carvacrol rich [average 57.38% (63.60% in NT condition and 51.15% in FGB condition)] found in MMBO-3055/IC589079 from 2258 to 2300 m asl. elevation, in district Bageshwar, Central Himalaya. The plant is having white flowers. Plant population of Origanum was observed in the other plant association ship of oak-rhododendron forest and Gonostegia hirta Miq., Micromeria biflora Benth., Swertia paniculata Wall., Trifolium repens L., Poa annua L., Valeriana jatamansi Jones., etc of mid hills. One more accession NKO-77, average carvacrol content 80.11% (81.01% in NT condition and 79.21% in FGB condition) from 2160 m asl. elevation, in district Nainital, Central Himalaya. The plant bears white flowers with green colour bracts. Plant population was observed in the other plant association ship of Calmantha umbrosa Fish & C. A. Mey, Dicliptera bupleuroides (Nees) Wall., Erigeron karvinskianus DC. etc. of mid temperate hills.
Table 8.1: Essential oil (%) content of *Origanum vulgare* L. collected from 33 localities of Uttarakhand Himalaya.

<table>
<thead>
<tr>
<th>Collector No.</th>
<th>IC No.</th>
<th>Alt. (m)</th>
<th>Essential Oil (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>NT</td>
</tr>
<tr>
<td>MMBO-3040</td>
<td>IC589077</td>
<td>1148</td>
<td>0.57</td>
</tr>
<tr>
<td>NKO-58</td>
<td>IC574520</td>
<td>1227</td>
<td>1.70</td>
</tr>
<tr>
<td>NKO-16</td>
<td>IC573215</td>
<td>1283</td>
<td>0.70</td>
</tr>
<tr>
<td>NKO-17</td>
<td>IC573216</td>
<td>1470</td>
<td>1.43</td>
</tr>
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<td>NKO-76</td>
<td>IC589099</td>
<td>1475</td>
<td>1.20</td>
</tr>
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<td>NKO-09</td>
<td>IC573209</td>
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<td>0.17</td>
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<td>NMJO-3077</td>
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<td>0.98</td>
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GM  0.88  0.58  0.73

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<tr>
<th>Condition</th>
<th>S Em</th>
<th>CD (5%)</th>
<th>CD (1%)</th>
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<tr>
<td></td>
<td>0.0015</td>
<td>0.0042</td>
<td>0.0055</td>
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<tr>
<td>Germplasm</td>
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<td>0.0234</td>
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<td>Condition x Germplasm</td>
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<td>0.0251</td>
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<td>CV (%)</td>
<td>2.222</td>
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Data are presented as the mean (n = 3); CD = Critical Difference; CV = Coefficient of Variation; *Significant at p < 0.01 (1%) & 0.05 (5%)*
Table 8.2: Major chemical constituents (%) in essential oil of *O. vulgare* L. (33 accessions) collected from Natural (NT) and Field Gene Bank (FGB) conditions.

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<tr>
<th>Collector No.</th>
<th>IC No.</th>
<th>Alt. (m)</th>
<th>p-Cymene NT</th>
<th>FGB</th>
<th>Mean</th>
<th>Zβ-Ocimene NT</th>
<th>FGB</th>
<th>Mean</th>
<th>Eβ-Ocimene NT</th>
<th>FGB</th>
<th>Mean</th>
<th>γ-Terpinene NT</th>
<th>FGB</th>
<th>Mean</th>
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<td>MMBO-3040</td>
<td>IC589077</td>
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<td>1.71</td>
<td>3.96</td>
<td>2.84</td>
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<td>0.59</td>
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Data are presented as the mean (n = 3); CD = Critical Difference; CV = Coefficient of Variation; Significant at p < 0.01 (1%) & 0.05 (5%).
Table 8.3: Major chemical (Linalool, Thymol and Carvacrol) contents (%) in essential oil of *O. vulgare* L. (33 accessions) collected from Natural (NT) and Field Gene Bank (FGB) conditions.

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<td>14.68</td>
<td>7.80</td>
</tr>
<tr>
<td>NKO-64</td>
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</tr>
<tr>
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<td>16.60</td>
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<tr>
<td>MMBO-3055</td>
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</tr>
<tr>
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<td>IC582510</td>
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<tr>
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</tr>
<tr>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NT</td>
<td>FGB</td>
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<td>6.08</td>
<td>22.47</td>
</tr>
<tr>
<td>Condition (NH)</td>
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<td>S Em</td>
<td>CD (5%)</td>
<td>CD (1%)</td>
<td>S Em</td>
</tr>
<tr>
<td>Germplasm (FGB)</td>
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<td>0.0358</td>
<td>0.0473</td>
<td>0.0356</td>
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<tr>
<td>Condition x Germplasm (NHxFGB)</td>
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<td>0.2004</td>
<td>0.1510</td>
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<td>0.2834</td>
<td>0.2136</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as the mean (n = 3); CD = Critical Difference; CV = Coefficient of Variation; Significant at p < 0.01 (1%) & 0.05 (5%).
8.3.3c Chemotype-3 (p-cymine rich type)

Accession NKO-15/IC573214 contains the highest content of p-cymine constituent among the 33 accessions and reported as p-cymine rich [average 13.47% (10.17% in NT condition and 17.31% in FGB condition)] chemotype, from 2140 to 2150 m asl. altitude, in district Pithoragarh, Central Himalaya. The plant bears white flowers with pale green colour bracts. Plant population was observed in the other plant associationship of *Calmantha umbrosa* Fish & C. A. Mey, *Conyza canadensis* L., *Fragaria nubicola* Lindl., *Poa annua* L., *Rumex acetosa* L., *Valeriana jatamansi* Jones etc. of mid hills.

8.3.3d Chemotype-4 (Zβ-Ocimene rich type)

The maximum content of Zβ-Ocimene [average 2.57% (2.60% in NT condition and 2.54% in FGB condition)] in essential oil of NKO-72/IC589090, this accession reported as Zβ-Ocimene rich type chemotype, collected from 2075 m asl. altitude, in district Chamoli, Central Himalaya. The plant bears white flowers with green colour bracts. Plant population was observed in the other plant associationship of *Blumea fistulosa* (Roxb.) Kurz, *Duchesnia indica* (Andrews) Focke, *Valeriana jatamansi* Jones., *Viola canescens* Wall, etc. of mid hills.

8.3.3e Chemotype-5 (Eβ-Ocimene rich type)

Eβ-Ocimene rich [average 2.78% (2.78% in both NT & FGB condition)], NMJO-3077/ IC589092 from 1540m asl. altitude, in district Pauri, Central Himalaya. The plant bears white flowers with light green colour bracts. Plant population was observed under Pinus forest and other plant association ship of *Ambrosia artemisiifolia* L., *Dicliptera bupleuroides* Nees in Wall., *Gallium aparina* L., *Stellaria media* (L.) Vill. etc. of lower hills.

8.3.3f Chemotype-6 (γ-Terpinene rich type)

Accession NMJO-3077/IC589092 was also reported as γ-Terpinene rich [average 24.25% (24.25% in both NT & FGB condition)] chemotype, from 1540m asl. altitude, in district Pauri, lower hills of Central Himalaya.
Figure 8.1: Dendrogram showing the different associations identified by the Ward’s Minimum Variance cluster analysis based on thymol content in 33 accessions of ‘Oregano’

Figure 8.2: Dendrogram showing the different associations identified by the Ward’s Minimum Variance cluster analysis based on carvacrol content in 33 accessions of ‘Oregano’
A) Chemotype-I, Thymol rich type (NKO-68/IC589087); B) Chemotype- I, Thymol rich type (NKO-65/IC589085); C & D) Chemotype-II, Carvacrol rich type (MMBO-3055/IC589079 and Origanum vulgare); E) Chemotype-III, p-cymine rich type (NKO-15/IC573214); F) Chemotype-IV, Zβ-Ocimene rich type (NKO-72/IC589090) in Natural habitats.

Figure: 8.3a. Elite chemotypes of Origanum vulgare L.
Figure: 8.3b. Elite chemotypes of *Origanum vulgare* L.

G) Chemotype-V and VI, Eβ-Ocimene rich type and γ-Terpinene rich type (NMJO-3077/IC589092); H) Chemotype- VII, Linalool rich type (NKO-41/IC574505) in Natural habitat.

Figure 8.4: A) Essential oil extraction of *Origanum vulgare* through hydro-distillation process; B) Variation in essential oil colour of Oregano; C) Clarus-500 Gas Chromatometer manufactured by Perkin Elmer used for GC.
Figure 8.5a: Chromatogram of thymol rich and carvacrol rich chemotypes:

(A & B) Thymol rich type (NKO-68/IC589087 and NKO-65/IC589085)
(C & D) Carvacrol rich type (MMBO-3055/IC589079 and NKO-77).
Figure 8.5b: Chromatogram of p-cymine rich, Zβ-Ocimene rich, Eβ-Ocimene rich and linalool rich type chemotypes: (E) p-cymine rich type (NKO-15/IC573214); (F) Zβ-Ocimene rich type (NKO-72/IC589090); (G) Eβ-Ocimene rich and γ-Terpinene rich type (NMJO-3077/IC589092); (H) Linalool rich type (NKO-41/IC574505).
8.3.3g Chemotype-7 (Linalool rich type)

The highest percentage of linalool content [average 20.77% (20.77% in both NT & FGB condition)] in the essential oil of NKO-41/IC574505 was reported. The chemotype was collected from 1700 m asl. elevation, in district Almora, Central Himalaya. The plant bears white flowers with green colour bracts. Plant population was observed under Pinus forest and other plant association ship of *Asparagus adscendens* Roxb., *Dicliptera bupleuroides* Nees in Wall., *Erigeron annuus* Pers., *Oxalis corniculata* L. etc of lower hills.

8.3.4 Cluster analysis

The hierarchical cluster analysis distinguished the observations into homogeneous and distinctive five groups/associations with percentage of thymol content among the 33 accessions (Figure 8.1). Thymol rich type chemotype (02 accessions) were grouped in group one (G1). Carvacrol based clustering partitions data sets into six groups (Figure 8.2), carvacrol rich type chemotype (04 accessions) came under group one (G1) while, other similar accessions have been grouped together with the similar proportion of thymol and carvacrol content, which collected from 33 different locations of Uttarakhand Himalaya.

8.4 Discussion

8.4.1 Essential oil Yield (% DWB)

The essential oils contents (% of dry plant materials) of 33 accessions of Oregano plants collected from different NT conditions and FGB are listed in Table 8.1. Among all the accessions the minimum and maximum essential oil contents were derived from the aerial parts of NKO-41/IC574505, NKO-09/IC573209 and NKO-68/IC589087, NKO-58/IC574520 respectively. Average greater essential oil yields (1.23% to 1.70%) in both conditions were acquired from five accessions viz., NKO-68/IC589087, NKO-58/IC574520, NKO-77, NKO-20/IC573219 and NKO-56/IC574518 (Figure 8.6 and Table 8.1). Among the 33 populations, the average essential oil content was varied from 0.20% to 1.70%. The variations in the contents of the essential oils with respect to different accessions were significant at p<0.01 and p<0.05. Our results are in agreement with those of Gurudatt et al. (2010), who
investigated the essential oil content of different seasons (0.2–1.3%) along with phenological stage. Variation in the essential oil content was found in different plant stages. Similar trend has also been reported in other members of the family Lamiaceae (Ebrahimi et al., 2008). Our results are in accordance with the finding of Vokou et al. (1993), who reported diverse essential oil yields (0.17% to >0.70%) among the twenty-three samples of *O. vulgare* ssp. *hirtum* collected from different localities of Greek territory. Veres (2007) screened the essential oil contents of individual plants of a population of *O. vulgare* and *O. vulgare* subsp. *hirtum* grown in Hungary as low essential oil content, ranging from 0.07 to 0.3% and had substantially higher essential oil content, ranging from 1.42% to 6.35%. Sahin et al. (2004) reported the oil yield from *Origanum vulgare* ssp. *vulgare* in the Eastern Anatolia region of Turkey to be 2.31%, which is considerably higher than the values determined in our present study. Little variations in the essential oil content of different Lamiaceae species across countries might be attributed to the varied agro-climatic conditions of the regions.

### 8.4.2 Chemotypic variations

Essential oils from the 33 accessions of *O. vulgare* L. collected from NT condition, contained predominance of the main components did not remain consistent, and the proportional quantities of the individual components did not meet the potential on several conditions (Figure 8.7, Table 8.2 & 8.3). This phenomenon may reflect the changes in the climatic conditions. Generally chemotypes form biochemical varieties or physiological form in particular genotypes, each of which with a specific enzymatic properties. These genotypes/ ecotypes are genetically codified and direct their biosynthesis to the preferential formation of a definite compound. An examination of Table 8.2 and 8.3 revealed definite chemotaxonomic similarities and differences among the collections from 33 locations. Seven chemotypes were identified on the basis of the major phenolic compounds (thymol and carvacrol), hydrocarbons and mono-terpenes (p-cymene, Zβ-ocimene and Eβ-ocimene), terpenes (γ-terpinene) and alcohol oxides (linalool), the first of these being the most common chemotype. A significant variability of composition was observed that possibly correlated with the individual genotypes (Russo et al., 1998). In both the condition (NT and FGB), the essential oils were dominant by their phenolic content fraction. The contents of this fraction or gross mean (GM) were 22.87% and 12.81%
Figure 8.6: Geographical variation in essential oil content of aerial part of *O. vulgare* L.

Figure 8.7: Compositions of the essential oils isolated from 33 selected samples of *O. vulgare* from natural condition.
respectively. The average thymol content of both conditions in the essential oil varied between 0.53% to 81.25% and carvacrol content ranged stuck between 0.25% to 80.11%, which is visually significant variation (Table 8.3). Oregano populations from high altitude NKO-68/IC589087 and NKO 65/IC589085, contained high percentage of thymol while, high carvacrol noticed in NKO-21/IC573220, MMBO-3055/IC589079 and NKO-77 population of MT region only. Same trends follow of these accessions, extracted and quantify from FGB condition. Present results are in accordance with the finding of Bisht et al. (2009), who reported thymol and carvacrol content were 29.2% to 82.0% and 1.1% to 27.4% respectively while, Gurudatt et al. (2010) screened the essential oil composition from one accession, extracted in different growth seasons and showed thymol content 0.37% to 0.70% and carvacrol content 35.02% to 62.89%. It seems that these contents are significantly lower than the values determined in our present study and identified elite chemotype I (thymol rich type) and chemotype II (carvacrol rich type). The major hydrocarbons and mono-terpenes detected from all accessions of essential oils were p-cymine, Zβ-ocimene and Eβ-ocimene, respectively. The contents of these fraction (GM value) were 5.16%, 0.63%, 0.56% and varied from 1.01% to 13.74%, 0.08% to 2.57% and 0.05% to 2.78% respectively. The terpenes (γ-terpinene) and alcohol oxides (linalool) fraction was 7.54% and 6.08% with ranged from 1.21% to 24.25% and 0.33% to 20.77% respectively (Table 8.2 and 8.3). These hydrocarbons/mono-terpenes, terpenes and alcohol oxides in the present study were found much higher as compared to earlier reports of (Bisht et al., 2009, Gurudatt et al., 2010, Pande et al., 2012 and Verma et al., 2012) from Uttarakhand Himalaya. Previously reported works on the essential oils of the genus *Origanum* were rich in thymol, carvacrol, p-cymene and α-terpinene (Daouk et al., 1995; Sokovic et al., 2002; Esen et al., 2007). However, some *Origanum* oils belonging to different genotypes have higher percentage of thymol than carvacrol (Daferera et al., 2003; Bendahou et al., 2008). For instance, the essential oil of *O. vulgare* growing in Greece was found to be rich in thymol (63.7%), p-cymene (13.0%) and carvacrol (8.6%) (Daferera et al., 2003). Ezzeddine et al. (2001) and Vagi et al. (2005) reported terpinen-4-ol, to be the main component in the marjoram essential oils. Other studies in the literature suggested that *O. vulgare* subsp. *hirtum* is more commonly rich in carvacrol and less commonly rich in thymol.
(Akgul and Bayrak, 1987; Baser et al., 1994; Skoula et al., 1999 and Figueredo et al., 2006). Therefore, on the basis of present observation and comparison with known literature data, our samples contained the highest essential oil composition and identified elite or superior chemotypes.

8.4.3 Co-relationship of major chemical constituent’s in essential oil of *O. vulgare* L. among NT and FGB conditions

Relationship between major chemical constituents in essential oil of *O. vulgare* among NT and FGB conditions represent a significant matrix (Table 8.4). The percentage of p-cymine and γ-terpinene of NT condition was non-significant positively correlated ($r = 0.19$ and $r = 0.32$ at $p<0.05$) with the percentage of FGB condition and significantly positive associated with Eβ-ocimene of FGB condition ($r = 0.39$ at $p<0.05$) and γ-terpinene of NT condition ($r = 0.37$ at $p<0.05$) while, Zβ-ocimene, linalool, thymol and carvacrol content are non-significant positively and negatively linked. In case of p-cymine of FGB condition was significant positively correlated ($r = 0.50$ at $p<0.01$) with Eβ-ocimene and Zβ-ocimene of FGB condition while, linalool, thymol and carvacrol contents are non-significant positively and negatively related. Zβ-ocimene of NT condition was significant positively correlated ($r = 0.59$ at $p<0.01$ and $r = 0.39$ at $p<0.05$) with Zβ-ocimene and Eβ-ocimene of FGB condition and Eβ-ocimene, γ-terpinene of NT condition ($r = 0.52$ at $p<0.01$ and $r = 0.35$ at $p<0.05$): Eβ-ocimene of NT condition was significant positively correlated ($r = 0.59$ at $p<0.01$ and $r = 0.37$ at $p<0.05$) with Eβ-ocimene and γ-terpinene of FGB condition and NT condition ($r = 0.64$ at $p<0.01$) while, Eβ-ocimene of FGB condition was also significantly positive correlated with γ-terpinene of FGB condition ($r = 0.61$ at $p<0.01$). Linalool, thymol and carvacrol contents of NT condition significantly positive correlated with FGB condition ($r = 0.50$, 0.82 and 0.78 at $p<0.01$ respectively), whereas, linalool content of NT condition was significant negatively correlated ($r = -0.46$ at $p<0.01$ and $r = -0.37$ at $p<0.05$) with thymol and carvacrol content of NT condition. In both conditions thymol and carvacrol content was non-significant negatively associated with each other (Table 8.4).

The results of this study indicate that the composition of essential oil concentrations in the NT condition populations of Oregano were highly and positively correlated to each other and were significantly variable across the studied populations.
Table 8.4: Co-relationship of major chemical constituents in essential oil of *O. vulgare* L. among NT and FGB conditions.

<table>
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<tr>
<th></th>
<th>p. cymine</th>
<th>Z β - Ocimene</th>
<th>E β - Ocimene</th>
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<th>Carvacrol</th>
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<td>0.59**</td>
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<td>-0.12</td>
<td>0.01</td>
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</table>

DF=32; r = 0.349 (at p= 0.05); r =0.449 (at p= 0.01)

* Significant at p < 0.05**; Significant at p < 0.01.
All populations retained a chemical proportion of their essential oil, when they were transplanted in FGB that they had evolved in different chemotype/ecotypes. The variations in the chemical compositions of the essential oils with respect to seasons might be attributed to the phenological status and environmental conditions can influence the regulation of the biosynthesis of essential oil, the two major environmental factors that appeared to influence the essential oil concentration/composition were the dryness and the thermal efficiency of the localities from where the populations were sampled (Vokou et al., 1993). This suggests that these environmental factors function as major selective agents for increased oil production. The effect of the thermal efficiency, soil moisture content and drought, especially during the blooming period, appears to be a simulative factor that increases the essential oil concentration in oregano cultivars (Azizi et al., 2009). Besides, being a genetic component to the variation in oil composition (between chemotype/ecotypes), the differences in essential oil concentration/composition in similar natural populations among FGB indicate that there is also an environmental component rendering genetically similar plants to produce different concentrations/composition of essential oil when grown in different environments.

### 8.4.4 Intra-specific variation in Essential Oil yield and composition

An assessment of table 8.1, 8.2, 8.3 and figure 8.8 revealed definite intra-specific variation on essential oil yield and its chemical composition among the accessions from 33 locations. Many studies on *Origanum vulgare* have pointed out the presence of intra-species variations in volatile composition, and the existence of some chemotypes has been proposed (Russo et al., 1998). Various factors that determine the chemical composition and yield of the essential oil. In some instances, it is difficult to separate out these factors from each other, since many are interdependent and influence one another. These variables may include geographical variation, seasonal and maturity variation, genetic variation, growth stages, part of plant utilized and post harvest drying and storage (Marotti et al., 1994; Hussain et al., 2008; Anwar et al., 2009). The influence of those factors on the accumulation of distinct secondary metabolites in taxon, defines its chemotypes. Three distinct oil compositions have been reported from India. The first is characterized by a high \( \alpha \)-muurolene proportion (Pande and Mathela, 2000), the second is characterized by \( \beta \)-
myrcene (Kaul et al., 1996) and the third is represented mainly by thymol (Kumar et al., 2007).

### 8.4.5 Geographical variation

There are several literatures showing the variation in the yield and chemical composition of the essential oil with respect to geographical regions (Kaul et al., 1996; Baratta et al., 1998; Chalchat and Pasquier, 1998; Pande and Mathela, 2000; D’Antuono et al., 2000; Mockute et al., 2003; Kumar et al., 2007; Celiktas et al., 2006). Altitude seems to be another important environmental factor influencing the essential oil content and chemical composition (Vokou et al., 1993). The yield and chemical composition of essential oils from *Origanum vulgare* ssp. *hirtum* essential oils from twenty three localities, scattered all over Greece were varied significantly (Vokou et al., 1993). Uribe-Hernandez et al., (1992) also reported that the essential oil yield and composition varied significantly, depending on the locations where the plants grew (Figure 8.6).

Relationship between altitude and major chemical constituents in essential oil, among 33 accessions of *O. vulgare* represents a diverse correlation matrix (Table 8.5). Altitude was significantly positive correlated with thymol content (r = 0.52 at p<0.01) while, p. cymine, Zβ-ocimene, Eβ-ocimene, γ-terpinene, linalool and carvacrol contents were not associated with altitudinal gradients. The content of p-cymine significantly positive correlated (r = 0.37 at p<0.05) with γ-terpinene while, non-significant positively correlated (r = 0.11, 0.34 and 0.13 at p<0.05) with Zβ-ocimene, Eβ-ocimene and thymol content but non-significant negatively correlated (r = -0.10 and -0.16 at p<0.05) with linalool and carvacrol. Zβ-ocimene was significant positively correlated (r = 0.52 at p<0.01 and r = 0.35 at p<0.05) with Eβ-ocimene and γ-terpinene but non-significant positively correlated (r = 0.23 at p<0.05) and non-significant negatively correlated (r = -0.32 at p<0.05) with linalool, thymol and carvacrol contents respectively. Eβ-ocimene content significantly positive correlated (r = 0.64 at p<0.01) with γ-terpinene but non-significant negatively correlated (r = -0.01, -0.18 and -0.12 at p<0.05) with linalool, thymol and carvacrol contents respectively while, γ-terpinene content non-significant negatively correlated (r = -0.24, -0.12 and -0.17 at p<0.05) with linalool, thymol and carvacrol contents.
respectively. The content of linalool significantly negative correlated \( r = -0.46 \) at \( p<0.01 \) and \(-0.37 \) at \( p<0.05 \) with thymol and carvacrol while, thymol content was non-significant negatively correlated \( r = -0.09 \), at \( p<0.05 \) with carvacrol content (Table 8.5).

**Table 8.5: Relationship between altitute and major chemical constituent’s in essential oil of **O. vulgare L.**

<table>
<thead>
<tr>
<th></th>
<th>Alt. (m)</th>
<th>p. cymine</th>
<th>Z β -Ocimene</th>
<th>E β –Ocimene</th>
<th>γ Terpinene</th>
<th>Linalool</th>
<th>Thymol</th>
<th>Carvarol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alt. (m)</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p. cymine</td>
<td>-0.02</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z β-Ocimene</td>
<td>0.01</td>
<td>0.11</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E β-Ocimene</td>
<td>-0.24</td>
<td>0.34</td>
<td>0.52**</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>γ Terpinene</td>
<td>-0.20</td>
<td>0.37*</td>
<td>0.35*</td>
<td>0.64**</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linalool</td>
<td>-0.19</td>
<td>-0.10</td>
<td>0.23</td>
<td>-0.01</td>
<td>-0.24</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thymol</td>
<td>0.52**</td>
<td>0.13</td>
<td>-0.32</td>
<td>-0.18</td>
<td>-0.12</td>
<td>-0.46**</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Carvarol</td>
<td>-0.11</td>
<td>-0.16</td>
<td>-0.32</td>
<td>-0.12</td>
<td>-0.17</td>
<td>-0.37*</td>
<td>-0.09</td>
<td>1.00</td>
</tr>
</tbody>
</table>

\[ DF=32; r = 0.349 \text{ (at } p= 0.05\text{); } r =0.449 \text{ (at } p= 0.01\text{)} \]

* Significant at \( p < 0.05 \); **Significant at \( p < 0.01 \).

The interrelationship on the basis of regression and correlation matrix between the altitude and thymol content revealed a positive relationship \( y = 0.0221x - 20.798 \) \( R^2 = 0.2741 \). It seems the percentage of thymol was found increasing towards higher elevations while, carvacrol content was not associated with altitude of the Central Himalaya (Figure 8.9 and 8.10). Climatic factors such as heat and drought were also related to the essential oil profiles. Moreover, the preference of the plant for these conditions suggest that genetic make-up of the plant, rather than the soil-type in which it is growing, should have a greater influence on the chemical profile of the oil produced (Graven *et al.*, 1990; Milos *et al.*, 2001).
Figure 8.8: Comparison of essential oil content between natural and field gene bank condition germplasm of *O. vulgare* L.

Figure 8.9: Relationship between altitude and thymol content in essential oil of 33 ‘Oregano’ accessions.
8.4.6 Seasonal and maturity variation

A visually significant variation in essential oil yield and its composition in
examined Table 8.1, 8.2 and 8.3 seem due to collection time, seasons and growth
stage. There are some more reports regarding the variation in the chemical
composition of essential oils from various Lamiaceae plants collected during different
seasons (Kofidis et al., 2003; Celiktas et al., 2006; Hussain et al., 2008); variation in
the yield and quality of essential oils with respect to maturity stages (Marotti et al.,
1994; Skoula and Harborne, 2002; Yildirim et al., 2004; Anwar et al., 2009).

8.4.7 Genetic variation

The genetic make-up of an organism is an important part of unevenness.
Variations in chemical composition from the same population and location,
demonstrating the presence of different chemotypes within the species (Catalan and
De Lampasona, 2002; Juliani et al., 2002; Wink, 2003; Ahmad et al., 2006). Our
Present results are in accordance with the finding of earlier reports (Table 8.1, 8.2, 8.3
and Fig. 8.4). Galambosi and Peura (1996) grew 24 wild and 19 cultivated caraway
populations under the same conditions and found significant differences between the
oil composition and yields of the wild and cultivated types. It seems that this factor significantly determined in our present study and identified elite chemotypes.

Many other factors which affect the oil yield and composition such as time of sowing (Galambosi and Peura, 1996), exposure of sunlight (Burbott and Loomis, 1957; Clark and Menary, 1979), accessibility of water (Galambosi and Peura, 1996), plant density (Graven et al., 1990; Clark and Menary, 1979), used plant part (Chalchat et al., 1995), diseases and insects (Graven et al., 1990) and post harvesting process (Skoula and Harborne, 2002). The oil composition and yield may also change as a result of the used harvesting methods (Bonnardeaux, 1992), moisture content of the plants at the time of harvest (Burbott and Loomis, 1957), Drying methods (Soysal and Oztekin, 2001) and employed isolation techniques (Charles and Simon, 1990; Moates and Reynolds, 1991).

The recent work of Ietswaart (1980), currently accepted as the taxonomic reference for this genus worldwide, distinguished six subspecies on the basis of morphological characters viz., gracile (Kock) Ietswaart, glandulosum (Desfontaines) Ietswaart, hirtum (Link) Ietswaart, vulgare L., virens (Hoffmannsegg et Link) Ietswaart and viride (Boissier) Hayek. Out of these six subspecies, hirtum has been more thoroughly investigated for oil composition (Kokkini, 1997; Putievsky et al., 1997) and the some studied accessions or populations of MT and UT/SA region resemble with ssp. hirtum. Therefore, we can say on the basis of chemotypic study, Indian populations were not homogenous and include two intermediate types containing thymol and carvacrol phenolic contents. To date, only three reports have been published on Origanum vulgare from Indian Himalaya. The oil analyzed by Pande and Mathela (2000) had no general similarity to the present study. However, the oils investigated by Kaul et al. (1996) and Kumar et al. (2007) resemble with our studies with respect to mono-terpenoids. Thus, conspicuous chemotaxonomic differences exist in Origanum vulgare populations. In conclusion, it has been stated that intermediate types are in common existence within this species in the natural habitat of Central Himalaya. Moreover, genetic and environmental factors both play a role in determining the composition of essential oils of the Origanum species studied.
The present work showed that the various populations of Oregano developed in various localities in Uttarakhand Himalaya, some of them were identified as superior or elite chemotype/ecotypes. A prime target for selection of these chemotype/ecotypes is likely to be useful for the production of essential oils to face the highly variable climate, topography and pharmaceutical demand. Wild populations of *O. vulgare* have been studied to contribute to the characterization of the geographical and biochemical variability of the taxon; to provide information on its essential oil composition in an area not previously explored and to characterize as an important source of spice and essential oil, in order to contribute to the conservation and exploitation of genetic resources in hill state of Uttarakhand, India.
GROWTH PATTERN, AGO-TECHNIQUES, CONSERVATION & DOMESTICATION STRATEGIES OF ELITE CHEMOTYPE