REVIEW OF LITERATURE
2. Review of literature

The word basil is taken from the Greek word *Basilicum*, meaning "king" or "royal". It is a fairly small annual herb that typically grows up to 3 feet (1 meter) in height. Basil is a thick-stemmed plant with larger lush leaves and small, delicate flowers that range in color from white to pink. The color of the Basil stems and leaves can also range in color from a vibrant, radiant green to a deep, dark purple (referred to Dark Opal Basil). This remarkable variation in color can easily be attributed to decades of cross pollination, from which an extremely large number of species, varieties and forms have been born. Today, over 50 species and more than 60 varieties of *Ocimum basilicum* L. currently exist. This range includes *Ocimum suave* known as tree basil, which grows in India and Africa and can reach heights of 6 to 9 feet (2 or 3 meters). Another common form of basil is *Ocimum citriodorum* Vis, which is more commonly referred to as lemon basil, because of its strong lemon scent. *Ocimum kilimandscharicum* is yet another basil. This basil is native to the USA and is commonly referred to as African blue basil or camphor basil, because of the easy to smell camphor present in the oil. Other types of basil are most easily differentiated by measuring their chemical constituents.

Basil oil is believed to possess biologically active constituents that may be insecticidal, anti-fungal and anti-bacterial. It is believed to assist with such things as Bronchitis, asthma, colds, coughs, exhaustion, flatulence, flu, gout, insect bites, insect repellent, muscle aches, constipation, nausea, rheumatism, sinus congestion, mental clarity, steady nerves, migraines, fevers, menstrual problems and ear infections. Researchers from the Landspitali University Hospital in Reykjavik, Iceland have completed a comprehensive study on the ability of basil oil to aid the body against ear infections. They were able to determine that vapors released from basil oil may actually diffuse through the eardrum, penetrating the eardrum allowing it to reach infected areas in the middle ear. The research accomplished testing basil oil against a placebo, on rats, and concluded that 81 percent of the animals achieved the desired results and the infection was no longer present.
In the world of perfume making, basil is considered a top (head) note. Top notes are often the first recognizable scent that evaporates in short time. These essential oils are typically refreshing and mentally stimulating. Basil offers a clean, clear, light, peppery and sweet aroma. Middle (heart) notes are described as the main or dominant scent. These essential oils are typically flowery or spicy scents. Bottom (base) notes are the scents that remain after all other scents have evaporated. These essential oils are typically sweet and earthy scents.

Basil oil contains numerous chemical compounds, including: 1,8-cineole, alpha-pinene; alpha-terpineol, β-pinene, camphene, camphor, cis-ocimene, citronellol, eugenol, geraniol, limonene, linalool, methyl chavicol, methyl cinnamate, myrcene, sesquiterpenes, terpinolene; terpinen-4-ol and γ-terpineol. Basil oils offering the greatest benefits often contain rich levels of methyl chavicol, eugenol linalool, camphor, and methyl cinnamate. Traditionally, the highest quality basil oil has been produced in Europe, specifically in Bulgaria and in the Mediterranean region. Basil oil harvested in Europe is considered to be superior, because it contains extremely high levels of linalool and methyl chavicol. Bulgarian oils are traditionally rich in methyl-cinnamate and eugenol respectively.

Sweet basil or holy basil (Ocimum sanctum L.) contains traditionally high levels of eugenol and is grown throughout Malaysia, Australia, India, and western Asia. Basil grown in Africa, the East Indies and Belgium (formerly Ocimum americanum L., currently Ocimum canum Sims.) is well known, because they also possess high methyl-cinnamate content. Ocimum gratissimum L. is often called tree basil, because of its clove like scent. It is native to Southeast Asia and contains a high content of eugenol.

The literature reviewed till date on the different aspects is listed under the following subheadings.

2.1. Morphological characterization of different accession of Ocimum spp.
2.2. To study the genetic diversity of accession of Ocimum spp using RAPD markers
2.3. Use of protein electrophoresis for differentiation of accession of Ocimum spp.
2.4. Quantitative and qualitative estimation of essential oil of different accession of
*Ocimum* spp.

2.1 Morphological characterization

(Darrah, 1980) classified the Most commercial basil cultivars available in the market belong to the species *O. basilicum* cultivars in seven types: (1) tall slender types, which include the sweet basil group; (2) large-leafed, robust types, including 'Lettuce Leaf' also called 'Italian' basil; (3) dwarf types, which are short and small leafed, such as 'Bush' basil; (4) compact types, also described, *O. basilicum var. thrysiflora*, commonly called 'Thai' basil; (5) *purpurascens*, the purple-colored basil types with traditional sweet basil flavor; (6) purple types such as 'Dark Opal', a possible hybrid between *O. basilicum* and *O. forskolei*, which has lobed-leaves, with a sweet basil plus clove-like aroma; and (7) *citriodorum* types, which includes lemon-flavored basils.

(Simon et al., 1990) observed the taxonomy of basil is complicated by the existence of numerous botanical varieties, cultivar names, and chemo types within the species that may not differ significantly in morphology.

(Paton, 1992) reported that the genus *Ocimum*, Lamiaceae, collectively called basil, has long been acclaimed for its diversity. *Ocimum* comprises more than 30 species of herbs and shrubs from the tropical and subtropical regions of Asia, Africa, and Central and South America, but the main center of diversity appears to be Africa.

(Simon, 1995) recorded the difficulty in classifying the more than 60 varieties of *Ocimum basilicum* L. has been attributed to the plant's polymorphic character and cross-pollination, resulting in large numbers of subspecies, varieties, and forms. The probable occurrence of seed from distinctive but erroneously named intergrades between *O. basilicum* and closely related species has been recognized and there is a need to taxonomically reclassify the genus. Many other *Ocimum* species are cultivated and utilized throughout the world, although none are as economically important as sweet basil. *O. sanctum* L. (holy basil), an annual native to Malaysia, Australia, India, and western Asia,
has a strong, pungent, clove-like odor because of its high eugenol content, and is used fresh and dried as a flavoring or spice. Holy basil, considered the most sacred plant in the Hindu religion, is grown extensively in India, and many cultivars have been recorded. A methyl-cinnamate-type basil oil is distilled from *O. canum* Sims., formerly *O. americanum* L., in Africa, the East Indies, and Belgium *O. citriodorum* Vis., or lemon basil, has a strong lemon scent, though whether this lemon basil constitutes a separate species is unclear. *O. kilimandscharicum* Guerke, types of which are called African Blue basil or camphor basil (due to a strong, pungent camphor odor) are used locally and have not entered into the commercial international market, but are becoming available as ornamental in US trade. *O. gratissimum* L. (sometimes called tree basil), is a perennial, woody basil grown in southeast Asia, reaches a height of 2 m and has a powerful clove scent because of the high phenol content of eugenol. Other chemotypes reported to be high in thymol and geraniol, respectively have been reported and could serve as natural sources for these chemicals. *O. suave* wild or tree basil is a densely woody shrub, reaching heights of 2 to 3 m and found in India and Africa. *O. crispum*, erect basil from Japan, is now correctly classified as *Perilla frutescens* cv. *crispum*. *O. minimum*, bush basil, used as a border plant, is considered by many to be a dwarf from of sweet basil, *O. basilicum* L. The spice basil grown by some American farmers and gardeners may actually be *O. sanctum* L. or a hybrid, rather than *O. basilicum*, as morphologically it is distinct from *O. basicilicum*.

(Grayer et al., 1996) observed the traditional types of basil, other *Ocimum* species have been introduced into the North American horticultural trade with new culinary and ornamental uses and may be potential sources of new aroma compounds. However, interspecific hybridization and polyploidy, common occurrences within this genus (Harley et al., 1992) have created taxonomic confusion making it difficult to understand the genetic relationship between many types of basil.

(Paton and Putievsky, 1992, 1996) proposed a system of standardized descriptors, which include volatile oil, by and this should permit easy communication and identification of the different forms of *O. basilicum*. Investigations to revise the genus are underway at the Royal Botanical Garden, Kew, London) and at Delaware State University. The cultivars displayed a wide diversity in growth habit, flower, leaf and stem colors, and
aromas. Many of the cultivars evaluated belong to the "Sweet" basil group, with 'Genovese', 'Italian large leaf', 'Mammoth', 'Napolitano', and 'Sweet' dominating the American fresh and dry culinary herb markets. Several others like 'Sweet Fine' appear similar to 'Sweet' basil though its leaves tend to be smaller.

(Lachowicz et al., 1997) observed differences in morphological features, growing characteristics and yields of essential oil produced per-unit area of land. He observed that anise basil was most production in terms of plant biomass, while cinnamon basil produced the most essential oil.

(Phippen and Simon, 1998) observed the lemon-scented cultivars ('Lemon' and 'Lemon Mrs. Burns') differed from each other in days to flower, and total oil content, but not in citral content. The 'Maengclak Thai Lemon' basil, which varied in appearance from the other lemon basils, is an attractive ornamental. Among the purple basils, 'Ocimum Purple' and 'Red Rubin Purple Leaf' were the most attractive and best retained their purple leaf color. Anthocyanins in purple basils are genetically unstable leading to an undesirable random green sectoring and reversion over the growing season. Several basils with dwarf growth habit were developed as ornamental border plants including 'Bush', 'Green Globe', 'Dwarf Bush', 'Spicy Globe', and 'Purple Bush'. Ocimum selloi Benth a native plant of Brazil, has medicinal uses as anti-diarrheic, antispasmodic and anti-inflammatory product. The yield of essential oils of the inflorescences, containing flowers and seeds, was 0.6%, and the yield of leaves, collected in two different seasons, was 0.25% (June, 2000) and 0.20% (January, 2001).

(Verma et al., 1998) observed high range of variation (99.2 to 415.1 g/ha) in eight genotypes belonging to seven Ocimum species and oil yield (43 to 160.4 lt./ha) in three cuts taken during the year. He recorded the highest herb and oil yield from O. basilicum (French basil), followed by O. citriodorum (EC 110586) yielding 394.2 quintal herb and 157.8 litre, oil yield/ha. Oil content on fresh weight basis varied from 0.21 to 0.51 per cent. It was highest for O. canum, followed O. carinoseum (0.48 %) and O. basilicum Indian basil (0.46 %). He recorded the high heritability (broad sense) and high genetic gain for oil yield, fresh herb yield and oil content in that order and indicated the
great possibility of further improvement for these characters through appropriate plant breeding methods.

(Karlovic et al., 2001) observed great variability of morphological characters among the basil genotypes. They recorded considerable variation for all agronomic traits except plant height and number of branches per plant. Dry leaf weight per plant varied from 6.56 gm. (O. basilicum cv. Dark Opal from Slovakia) to 17.86 gm. (O. basilicum var. Diforme from Germany).

(Vieira et al., 2001) studied morphological, chemical and genetic differences of 12 tree basil (O. gratissimum) genotypes to determine whether volatile oils and flavonoid can be used as taxonomic markers and to examine the relationship between random amplified polymorphic DNA and these chemical markers. Cluster analysis showed that there were three groups genetically distinct and highly correlated to volatile oil constituents. Morphological, chemical and genetic differences of 12 tree basil (Ocimum gratissimum L.) accessions were studied to determine whether volatile oils and flavonoids can be used as taxonomical markers and to examine the relationship between RAPDs to these chemical markers. Eugenol, thymol, and geraniol were the major volatile oil constituents found in Ocimum gratissimum. Xanthomicrol and cirsiramaritin were the major external flavones. The accessions morphologically described as O. gratissimum var. gratissimum contained eugenol as the major volatile oil constituent, and cirsiramaritin as the major flavone. Ocimum gratissimum var. macrophyllum accessions contained thymol as the major volatile oil constituent, and xanthomicrol as the major flavone. A distinct essential oil and flavone chemotype (producing geraniol and a mixture of the flavones cirsiramaritin, isothymusin, xanthomicrol, and luteolin) was found in an accession genetically more distant from the other two groups when analyzed by molecular markers. The accessions could be divided based on volatile oil constituents into six groups: (1) thymol: α-copaene (ot24, ot25, ot26, and ot28); (2) eugenol: spathulenol (ot17, ot63, and ot52); (3) thymol:p-cymene (ot65); (4) eugenol:γ-murolene (ot27 and ot29); (5) eugenol:thymol: spathulenol (ot85); and (6) geraniol (ot84). Cluster analysis of RAPD markers showed that there are three groups that are distinct genetically and highly correlated (r=0.814) to volatile oil constituents.
According to (Grayer, 2002) characterizations of each species in this genus (family Lamiaceae) are based on the leaves and the habitat. The shape of the leaves in Ocimum Sanctum and its close relatives varies in size of leaves, vein and petioles. The colours of the leaves vary from bright green to dark green and sometime almost black. Though the colours in the plants vary, but the reason behind it, especially in basils, are not being studied yet.

(Ahmad and Khaliq, 2002) experimented on the four genotypes collected from different localities of district Poonch and found large diversity among genotypes for leaf area, number of racemes per plant, number of flowers per raceme, plant height, 1000-seed weight and days to seed maturity.

(Singh et al., 2002) analyzed variations for morphology, phonology and essential oil composition in genotypes of sweet basil (O. basilicum L.) an important medicinal and aromatic crop. Significant variations were observed for morphological and essential oil traits.

(Tesi and Lenzi, 2002) described the behavior of some dwarf genotypes of basil. They also studies plant growth and seed production of basil genotypes. The dwarf genotypes showed a large decrease in the length of spike and seed production.

(Senanayake and Paton, 2002) described the horticulture characteristics, including quantitative and qualitative traits along with the chemical variation of phenolic acids of 23 accession of basil (Ocimum basilicum L.) from Iran were studied. Morphological studies of accession showed a high level of variability in recorded traits. Several authors recognize more than 60-150 species in the genus. Characterizations of each species in this genus (family Lamiaceae) are based on the leaves and habitat.

(Labra, 2004) describe the regular occurrence of the interspecific hybridization within the genus, have created taxonomic challenges, leaving very little publications on basil taxonomy which follows the International Code of Botanical nomenclature due to the complicacies in identifying the species.
(Massimo et al., 2004) has concluded that identification can be optimized by combined analysis of morphological traits, essential oil composition and molecular markers while studying.

(Sharma, 2005) genetic divergence observed significant differences among the genotypes for all the characters except days to seed maturity and essential oil content. Genetic variability was maximum for spikes per plant and minimum for essential oil content, as reflected by their genotypic coefficient of variations. The heritability estimates in broad sense were high for seed yield per plant, plant height, 10000-seed weight and spikes per plant, while moderate heritability were observed for primary branches per plant, lamina length and days to flower initiation. Days to seed maturity showed lowest heritability. Expected genetic advance as per cent of mean was high for seed yield per plant, plant height, spikes per plant. Medium value of expected genetic advance was obtained for 1000-seed weight, plant height and spikes per plant while high heritability coupled with medium value of genetic advance was observed for 1000-seed weight. Moderate heritability coupled with medium value of genetic advance was observed for primary branches per plant.

(Vani, 2009) observed that, distinct varieties of basil types in the genus *Ocimum* which makes them very special. Genus *Ocimum* is widespread over Asia, Africa and Central & Southern America. All basils are member of the *Lamiaceae* family. The colors of the leaves vary from bright green to purple-green and sometimes almost black. Fresh basil leaves have a strong and characteristic aroma, not comparable to any other spice, although there is a hint of clove traceable. *Ocimum sanctum*, also addressed as *Ocimum tenuiflorum* is a sacred plant in the Hindu culture and known as Tulasi in Tamil or Holy Basil in English. Mean-while *Ocimum basilicum*, known as Common or Sweet Basil has very dark green leaves.

### 2.2 RAPD Profile

(Portar and Smith, 1982) said that the more polymorphic and heritable the trait, the greater is its potential value for germplasm characterization.
(Sambrook et al., 1989) reported DNA markers are considered the best tools for determining genetic relationship / diversity, as they are unlimited in number, show high polymorphism and are independent of environmental interaction i.e., highly heritable.

(Reyes and Singh et al., 1998) reported the RAPD markers have been widely used for molecular characterization of plant species.

(Barbier and Ishihama 1990) studied on Molecular studies on evolution, I. Annual and perennial differentiation in wild rice Oryza rufipogon. A perennial and an annual ecotype have previously been identified in O. rufipogon. The nucleotide sequences of 3 introns in the phytochrome gene were determined by polymerase chain reaction amplification and dideoxy sequencing for 8 annual and perennial strains from India, Indonesia and Thailand. O. longistaminata was similarly analysed. The 2 species differed by about 0.042 substitutions per site while O. rufipogon strains differed by 0.0017 to 0.005 substitutions per site. These results suggested the 2 species diverged 5-6 million years ago while the O. rufipogon strains diverged only a few hundred thousand years ago. Sympatric strains were a little more closely related than allopatric ones. The data also indicated that the annual-perennial differentiation was more recent than the geographic differentiation.

(Paulo, 2003) reported RAPD technique has been used to study the genetic relations between the different species of coffee and to determine the relationship between hybrids.

(Ajay and Samresh et al., 2006) was observed genetic relationship among thirty germplasm accession belonging to five Ocimum species using RAPD markers. A very high degree of polymorphism (98.20 %) was observed and showed that RAPD technique is a sensitive, precise and efficient tools for genomic analysis in Ocimum species, that may be useful in further studies, by assigning new unclassified germplasm accession to specific taxonomic groups and reclassifying previously classified accessions to other Ocimum species by traditional criteria on a more objective basis.

(Siva Raju et al., 2008) studied molecular diversity in Indian tobacco types as revealed by randomly amplified DNA polymorphism. During the past five decades, a
large number of tobacco varieties have been developed for different end uses in India through pure line selection from local land races, mutation breeding, and hybridization involving local selections and exotic introductions followed by pedigree selection. No systematic effort has been made to understand the existing diversity pattern in these varieties, which is crucial to define future breeding strategy in this important commercial crop. We characterized 46 varieties belonging to 10 different manufacturing tobacco types cultivated under different agro-climatic conditions in India along with two wild species of Nicotiana using 40 arbitrary primers in RAPD. The level of polymorphism among the varieties of N. tabacum was 59.4%, which was more than double the level observed in the other cultivated species N. rustica (25.2%). A broader range (0.64 to 0.94) of pair wise similarity measures in N. tabacum than in N. rustica (0.83 to 0.92) reflected the more diversified breeding efforts in the major cultivated species. The two wild species namely, N. glutinosa and N. gossei clustered separately from the two cultivated species. Molecular classification of the varieties corresponded largely with their manufacturing trait and parentage. RAPD markers provided sufficient resolution to distinguish among closely related tobacco types. Nine RAPD markers were found conserved across all the varieties and species. The markers found specific to the varieties can be used in correct identification of the carrier genotypes in trade and commerce. This is the first report on the molecular diversity analysis of India.

(Sun et al., 2006) studied diversity and relationships among Elymus trachycaulus, E. subsecundus, E. virescens, E. violaceus, and E. hyperarcticus (Poaceae: Triticeae) as determined by amplified fragment length polymorphism. Morphological similarity among E. trachycaulus, E. virescens, E. violaceus, and E. hyperarcticus has often been noted. Taxonomists have tried to discriminate among these taxa using morphological characters and a number of different relationships among them have been suggested. However, the genetic relationships among these taxa are still unknown. AFLP analysis was used to characterize the molecular diversity of these taxa and to examine genetic relationships among them. A high degree of genetic identity was apparent among 7 accessions of E. virescens. The similarity values ranged from 0.90 to 0.99 with an average of 0.94. The mean similarity values among 3 E. hyperarcticus and among 5 E.
violaceous accessions were 0.84 (0.81-0.87) and 0.77 (0.66-0.90), respectively. The similarity values among 17 E. trachycaulus accessions ranged from 0.49 to 0.92 with an average of 0.73. The 5 accessions of E. subsecundus displayed high variation, with similarity values between 0.52 and 0.68 and a mean value of 0.59. Both maximum-parsimony (MP) and neighbour-joining (NJ) analyses showed that all 7 accessions of E. virescens formed a clade, indicating a monophyletic origin. On the other hand, Elymus trachycaulus, E. subsecundus, and E. violaceus were each paraphyletic and separated into different genetically distinct groups. Among these 5 taxa, E. virescens was genetically similar to E. trachycaulus, and E. violaceus was genetically similar to E.

(Shivapriya et al., 2006) worked on “Detection of genotype specific fingerprints and molecular diversity of selected Indian locals and landraces of rice (Oryza sativa L.)”. using DNA markers. A set of sixty-five local rice (Oryza sativa L.) accessions and three improved rice varieties were screened to identify the extent of genetic diversity present at the molecular level using RAPD markers. Sixteen primers generated 112 marker levels with 74.1% polymorphism. Dendrogram constructed based on molecular polymorphism unveiled considerable amount of diversity among the varieties. Genotype specific DNA bands were identified for selected lines. These distinct markers have the potential to be employed as genetic fingerprints for future varietal identification and classification. The use of primer duplexes resulted in generation of higher number of DNA bands compared to single primer. The study indicated that the RAPD markers provide an easy, rapid and simple technique for the preliminary assessment of genetic diversity among the local rices.

(Rudresh et al., 2005) revealed molecular diversity and identification of genotype specific markers in cowpea (Vigna unguiculata (L.) Walp) using RAPD. Genetic diversity among 8 cultivars of cowpea was analysed using 25 RAPD primers and a cluster dendrogram was constructed. The data generated from 15 primers showed polymorphism and revealed 3 clusters. Genotypes C-152, TVX-944-02E, KBC-2 and Lolita formed cluster I; V-130 and Pusa Phalgungi formed cluster II; and APC-412 and IITA Cowpea Black formed cluster III. APC-412 and V-130 were the most divergent, while C-152 and TVX-944-02E were the least divergent, among the 8 cultivars studied. The results
indicated that there is sufficient polymorphism among the 8 cowpea cultivars which may be exploited in hybridization programmes. The genetic variation observed in this experiment showed that the RAPD marker can be used as a potential tool for genetic diversity assessment in cowpea.

(Pojanagaroon et al., 2004) characterized Krachai-Dam (Kaempferia parviflora) cultivars using RAPD markers, morphological traits and chemical components of essential oil from rhizomes. Twelve collected Krachai-Dam (Kaempferia parviflora) cultivars from commercial cultivated area in Loei, Phitsanulok and Phetchabun were grown in 2003 at the Phurua Highland Agricultural Experiment Station, Phurua, Loei in Thailand. They were studied using random amplified polymorphic DNA (RAPD) markers. Sixty random decamer primers were screened and 31 of these produced 139 reproducible polymorphic amplification fragments. Results were analysed for a similarity among the cultivars, and an unweighted pair group method cluster analysis was performed. The analysis revealed that the cultivars could be divided into 2 main groups: 'Green leaves' and 'Red leaves', each main group could be also divided into 2 subgroups. The dendrogram showed good relationship between the banding patterns of Krachai-Dam cultivars and the morphological traits of leaves, petioles and rhizomes; especially the internal skin colour of rhizomes.

(Sangwan et al., 2001) studied molecular analysis of genetic diversity in elite Indian cultivars of essential oil trade types of aromatic grasses (Cymbopogon species). Eleven elite and popular Indian cultivars of Cymbopogon aromatic grasses of essential oil trade types (citronella (C. winterianus), palmarosa (C. martinii var. motia) and lemongrass (C. pendulus)) were characterized by means of random amplified polymorphic DNA (RAPDs) to discern the extent of diversity at the DNA level between and within the oil biotypes. Primary allelic variability and the genetic bases of the cultivated germplasm were computed through parameters of gene diversity, expected heterozygosity, allele number per locus, sum of effective number of alleles and Shannon’s information indices. The allelic diversity was found to be in the order: lemongrass > palmarosa > citronella. Lemongrasses displayed higher (1.89) allelic variability per locus than palmarosa (1.63) and citronella (1.40). Also, RAPDs of diagnostic and curatorial
importance were discerned as 'stand along' molecular descriptors. Principal component analysis (PCA) resolved the cultivars into four clusters: one each of citronella and palmarosa, and two of lemongrasses (one of C. flexuosus and another of C. pendulus and its hybrid with C. khasianus). Proximity of the two species-groups of lemongrasses was also revealed as they shared the same dimension in the three-dimensional PCA. The molecular distinctions are discussed in relation to oil-chemotypic variations.

(Patnaik et al., 2000) reported biotechnological strategies for improvement of Cymbopogon: status and prospects. Application of biotechnological methods for improvement of aromatic plants is lately gaining momentum. The present paper deals with the recent approaches towards qualitative and quantitative improvement in essential oil yielding plants of the important aromatic genus Cymbopogon. Mass propagation and qualitative improvement studies were carried out in aromatic grass. In palmarosa (Cymbopogon martini var. motia), successful standardization of high frequency somatic embryogenesis in callus and suspension cultures and subsequent plant regeneration were conducted. Successful encapsulation of somatic embryos in sodium alginate gel and their subsequent germination were achieved in vitro following low temperature storage. Superior somaclonal lines with respect to high herb and oil yield and high geraniol content in oil were screened and field evaluated for future varietal releases. Possibilities were explored for generating variability through in vitro mutagenesis with chemical mutagens. The spectrum of agronomically important variation generated by these chemical mutagens was not significantly broader than that obtained through somaclonal variation. To exploit the wasteland reclaiming potential of the moderately salinity-tolerant aromatic grass like palmarosa, in vitro selection methods were adopted. Screening and isolation of salinity tolerant callus lines and subsequent regeneration of salt tolerant plants was achieved. Such studies are now being carried out with other aromatic grasses like lemongrass and jamirosa. The potential application of some other recent biotechnological tools for future commercial exploitation of the Cymbopogon crops are discussed.

(Masi et al., 2005) worked on the agro-morphological characteristics, essential oil composition and randomly amplified polymorphic DNA (RAPD) markers were studied to
estimate the relationships among 12 basil (*Ocimum basilicum* L.) genotypes, belonging to nine known cultivars grown in Italy. The basil cultivars were distinguished on the basis of agro-morphological determinations and constituents of essential oil. Chemical compounds of essential oils were found variable in the various basil cultivars. As a consequence, the plants were classified into main phenotypes and chemotypes. RAPD markers were used in order to assess the genetic relatedness among the basil cultivars. On the basis of their genetic similarities, RAPD analysis allowed to group the samples into two main clusters. One of these included cultivars suitable for food industry, which were also correlated via agro-morphological features. However, the same cultivars produced distinct essential oil profiles, which did not match with results obtained by agronomic and genetic analysis. This fact, maybe due to a different genetic expression of the key enzymes involved in biosynthetic pathways that produce chemical compounds.

*(Carovic et al., 2006)* Phylogenetic relationships were determined by neighbor joining cluster analysis based on dice distance between accessions. A very strong correlation between dice distance matrices based on RAPD data were observed and the resulting neighbour-joining trees were congruent. High bootstrap support values for the branches separating *O. tenuiflorum* and *O. gratissimum* accessions, a cluster containing *O. americanum* and *O. × citriodorum* accessions, and a cluster containing *O. basilicum* and *O. minimum* accessions were observed in both cases giving a good representation of traditional taxonomic relationships. Within the *O. basilicum* cluster, similar accessions grouped together specifically *O. minimum*, *O. basilicum 'Dark Opal'* and *O. basilicum var. difforme* accessions. In addition to morphological, chemical and crossability data, RAPD analyses can be a useful tool for resolving existing problems in identification and classification of basil.

*(Smita et al., 2006)* was observed in eighteen phenotypically and biochemically distinct *Gymnema* accessions representing different geographical regions of Kerala were characterized using isozymes and RAPD markers. In the isozyme analysis, three enzyme systems *viz.*, malate dehydrogenase, esterase, and RUBISCO were studied. Five out of the eight resolved loci (62.5%) were polymorphic with the number of alleles expressed at the polymorphic loci being 10. RUBISCO activity was monomorphic across the
accessions. In the RAPD assay, 123 amplified products were generated using 15 selected random primers. Of these, 90 bands were polymorphic with the total frequency of polymorphic markers as high as 73.2%. Specific bands were obtained for five accessions. Jaccard’s coefficient ranged between 0.72 and 0.85 indicating a moderate level of variability. Cluster analysis of RAPD data using Unweighted Pair Group Method of Arithmetic Averages produced two major clusters and three sub-clusters. Overall, molecular fingerprinting revealed the existence of considerable genetic variations in the *Gymnema* germplasm collection from Kerala.

(Harisaranraj *et al.*, 2008) examined the genetic inter relationship of seven *Ocimum* species was estimated using Random Amplified Polymorphic DNA markers. The 15 selected RAPD primers out of 2 primers were amplified in all species. *O. basilicum* has very close similarity (89%) with *O. tenuiflorum* and another two species of *Ocimum*. Result suggested that genetic relationships in to conserve genetic resources of *Ocimum* species,

2.3 Protein electrophoresis

(Cherry, Ory, 1972 and Goodenough 1978) reported the gel electrophoresis has shown that many isoenzymes and polymorphic proteins are widely distributed in plants and that polymorphism signals the existence of allelism.

(Nei *et al.*, 1978) reported that seed protein electrophoretic patterns have provided the valied evidence for addressing genetic, taxonomic and evolutionary relationship in plants.

(Stegmann *et al.*,1980) studied *Ocimum* accessions based on the data obtained from the electrophorertic analysis supports the validity of the technique of seed protein electrophoresis as a tool for cultivar identification as well as studying genetic diversity and taxonomic relationship in *Ocimum* at both specific and intra-specific levels.

(Matta *et al.*, 1981) reported the electrophoretic techniques are used on large scales in protein and enzyme analysis to identify and characterize the genotype
differences among plant species and varieties. They studied *Vicia* legumin structure by polyacrylamide gel electrophoresis. Many authors used the electrophoretic tool to characterize the differences and similarities between plants. Among these authors are: Abdel-Tawab et al., (1982), Giannasi and Crawford (1986), Gamal El Din et al., (1988), Eweda (1989), Vries (1996) and Kamel and Hassan (2001).

**Vernon, 1983** characterized the glycoprotein in Soybean. Removing the pods from soybean (*Glycine max* [L.]) plants induces a change in leaf function which is characterized by a change in the leaf soluble protein pattern. The synthesis of at least four polypeptides (~27, 29, 54, and 80 kilodaltons) is enhanced, and these polypeptides accumulate to levels comprising over 50% of the soluble protein. Heat girdling the petiole also causes the accumulation of these polypeptides, suggesting that the signal for changing leaf function may be associated with inhibition of phloem transport. The 27 and 29 kilodalton polypeptides are glycosylated and have been purified to greater than 90% by (NH₄)₂SO₄ fractionation, concanavalin, a affinity, and gel filtration chromatography. These peptides appear to comprise a single protein. Mouse antiserum has been prepared against this glycoprotein and has been used to check for cross-reactivity with seed proteins and to quantitate changes with leaf development. No cross-reactivity was observed with seed soluble proteins from several stages of development. Quantitation showed the highest content in podded plants at, and shortly following, flowering, with levels subsequently declining in conjunction with seed growth. In depodded plants, the level of glycoprotein continued to increase following flowering and accounted for 45% of the soluble leaf protein by 4 weeks after depodding.

**(Purohit et al., 1998)** observed distinct seed polypeptides profiles in different morphological variant.

**(Hassan, 2001)** studied *Vicia* legumin structure by polyacrylamide gel electrophoresis. Many authors used the electrophoretic tool to characterize the differences and similarities between plants.
(Ahmad and kamal, 2002) observed the SDS-PAGE technique having more advantages in the classification of different genotypes of *Ocimum sanctum*. The phenotypic as well as genotypic studies revealed greater variation among the local genotypes with immense potential for future improvement using conventional breeding techniques. The general information revealed its potential to cultivate the plant directly without improvement under degraded soils of Azad Kashmir and elsewhere. However, in order to improve it’s nutritional, medicinal and nitrogen fixing qualities and to breed thornless varieties for smooth harvesting, some conventional and non-conventional plant improvement techniques would be more meaningful.

(Sangwan et al., 2003) reported that in SDS-PAGE analysis presence of unique polypeptide fragments (97.7 kDa to 31.6 k Da in varieties IW3124SE ‘RRL(B) 77, Tripui, Trishna. ‘PRC’ and var. sofia, generated as a diagnostic marker. In general, molecular distinctions associated with var. motia and var. sofia have been clearly noticed in *C. martinii*.

(Karihaloo et al., 2002) Studied seed protein profiles by SDS-PAGE were in 72 accessions of *Solanum melongena* L. and its related taxa in subgenus *Leptostemonum* (Dunal) Bitter. Comparisons based on Jaccard’s similarity and UPGMA clustering revealed interrelationships broadly in conformity with the conventional taxonomic treatments. Thus, *S. viarum* Dunal (section *Acanthophora* Dunal), *S. torvum* Swartz (section *Torva* Nees) and *S. sisymbriifolium* Lam. (section *Cryptocarpum* Dunal) were located on separate branches of the dendrogram while all taxa of section *Melongena* Dunal, except *S. macrocarpon* Dunal, were clustered closer together. *S. melongena*, “incanum” and “insanum”, all members of the eggplant complex, were revealed to bear very high similarity with each other. Most of their accessions had identical band patterns supporting the contention that these represent an interbreeding complex with limited genetic differentiation.

(Azeez, 2004) crude leaf proteins were extracted from them and characterized using polyacrylamide gel electrophoresis. Intercultivar qualitative as well as quantitative protein bands depict some degree of relationship among the *Lycopersicon* cultivars
The degree of variation in protein bands as a measure of genetic divergence between *L. esculentum* cultivars and *T. cucumerina*.

(Morakinya et al., 2004) were observed protein distribution patterns in three cultivars of *Lycopersicon* and one cultivar of *Trichosanthes* studied reveal distinct quantitative and qualitative intra and interspecific variations in terms of numbers, positions and band intensity. Bands common to two or more were observed.

(Mohamed et al., 2006) studied leaf Protein Electrophoretic Profiles and chromosome numbers of Some Araceae. Chromosome number of 19 species representing 14 genera of Araceae we recorded, of which 9 counts were novel. Different levels of polyploidy were evident in 5 species. The combination of chromosome numbers and the profiles of leaf proteins of the 19 species (as revealed by numerical analysis of SDS-PAGE results) highlighted some hitherto un-recognized relationships among the genera and species. While there was positive correlation between chromosome numbers and protein patterns in *Aglaonema* and *Anthurium* so that they seemed taxonomically homogeneous, such correlation was not evident among the species representing *Syngonium* and *Philodendron*. The electrophoretic analysis of proteins revealed a total of 57 protein bands in the leaves of the 19 species under investigation. The number of bands differed from a species to another, with the largest number (18) in *Aglaonema commutatum* and the lowest (5) in *Diffenbachia amoena*. For ease of comparison, the 57 protein bands were lumped together into 7 categories of molecular masses and the number of bands from each category was scored for every species. However, relationships between the species were determined numerically on the basis of the distribution of individual protein bands.

(Zaher et al., 2006) reported the seed protein or isozyme data clearly demonstrated the existence of genetic diversity among and within population of *ocimum* that might be related to natural hybridization and fluctuations in environmental condition. Seed protein and isozyme polymorphism exhibited validity for studying genetic diversity and taxonomic relationship in *ocimum* at both species and infra-specific levels. *Ocimum*
*Kilimandscharicum* exhibited higher levels of genetic variation and also higher number of unique alleles than *Ocimum basilicum*.

### 2.4 Quantitative and qualitative estimation of essential oil

The genus *Ocimum*, (Lamiaceae formerly Labiatae), collectively called basil has long been recognized as a diverse and rich source of essential oils. *Ocimum* contains between 50 to 150 species of herbs and shrubs from the tropical regions of Asia, Africa, and Central and South America (Bailey 1924, Hortus III 1976 Darrah, 1980). Plants have square stems, fragrant opposite leaves, and whorled flowers on spiked inflorescences (Darrah, 1980). Interspecific hybridization and polyploidy, common occurrences within the genus have created taxonomic confusion and challenges, yet very little has been published on basil taxonomy which follows the International Code of Botanical nomenclature (Tucker, 1986). The morphological diversity within basil species has been accentuated by centuries of cultivation with great variation in pigmentation, leaf shape and size, and pubescence. Taxonomy is further complicated by the existence of chemotypes or chemical races within the species that do not differ significantly in morphology.

(Kratz, 1963) analyzed the chemical composition of the essential oils was made using gas chromatography – mass spectrometry, (GC-MS, Shimadzu, QP 5000); operating at 70 eV, equipped with capillary column of fused silica DB-5 (J & Wiley Scientific, 30 m, 0.25mm,0.25μm), injector and detector at 240°C and 230°C, helium as carrier gas (1.7 mL/min) and the following temperature program: 50°C (5min) –280°C, 5°C/min. The retention indexes (RI) of the substances were obtained by co-injection of the essential oil with a standard mixture of hydrocarbons (C9-C40), using the Van den Dool and Kratz equation.

(Asta, 1968; Guenther, 1972; Heath, 1981; Sievers, 1928) reported, the essential oils are primarily composed of mono- and sesquiterpenes and aromatic polypropanoids synthesized via the mevalonic acid pathway for terpenes and the shikimic acid pathway for aromatic polypropanoids. The essential oils from aromatic plants are for the most part volatile and thus, lend themselves to several methods of extraction such as hydrodistillation,
water and steam distillation, direct steam distillation, and solvent extraction. The specific extraction method employed is dependent upon the plant material to be distilled and the desired end-product. The essential oils which impart the distinctive aromas are complex mixtures of organic constituents, some of which being less stable, may undergo chemical alterations when subjected to high temperatures. In this case, organic solvent extraction is required to ensure no decomposition or changes have occurred which would alter the aroma and fragrance of the end-product. Newer methods of essential oil extraction such as using supercritical CO₂ which yield very high quality oils are commercially used, but are less common and beyond the financial means of most processors.

The essential oils of basil extracted via steam distillation from the leaves and flavoring tops are used to flavor foods, dental and oral products, in fragrances, and in traditional rituals and medicines (Guenther, 1949; Simon et al., 1984). Extracted essential oils have also been shown to contain biologically-active constituents that are insecticidal (Deshpande and Tipnis, 1977; Chauvi and Nikam, 1982; Chogo and Crank, 1981), nematicidal (Chatterjee et al., 1982), fungistatic (Reuveni et al., 1984) or which have antimicrobial properties (Ntezurubanza et al., 1984). These properties can frequently be attributed to predominant essential oil constituents, such as methyl chavicol, eugenol, linalool, camphor, and methyl cinnamate. Two minor components of the essential oil of sweet basil, juvocimene I and II have been reported as potent juvenile hormone analogs (Nishida et al., 1984). Sweet basil (Ocimum basilicum L.), a common garden herb, is cultivated in the United States for culinary purposes as a fresh herb and as a dried spice. While there are many cultivars (Simon and Reiss-Bubenheim, 1988) little information is available on the essential oil compounds responsible for the plant's flavor and fragrance.

(Guenther, 1949; Simon et al., 1984) reported that several types of basil oil in international commerce, each derived principally from different cultivars or chemotypes of sweet basil. The oils of commerce are known as European French or Sweet Basil, Egyptian, Reunion or Comoro; and to a lesser extent Bulgarian and Java basil oils. The European type of basil oil considered to be the highest quality, and producing the finest odor, characteristically contains: linalool; methyl chavicol; and to a lesser extent 1,8-cineole,
alpha-pinene; B-pinene; myrcene; ocimene; terpinolene; camphor; terpinen-4-ol; alphaterpineol; eugenol; and sesquiterpenes.

(Fleischer, 1981) observed that Egyptian basil oil of commerce is similar to European basil oil except that the concentration of d-linalool is significantly lower while the concentration of methyl chavicol is significantly higher. In contrast, Reunion or Comoro basil oil contains little if any d-linalool and is a harsher, spicy oil due to the very high concentration of methyl chavicol, and to a lesser extent, 1, 8-cineole, borneol camphor and eugenol.

(Heath, 1981) reported that the recoveries of nonvolatile essential oils are also obtained by solvent extraction although the process is more difficult and complex than the recovery of the volatiles. This process yields an aromatic resinous product known as an oleoresin, which is more concentrated than an essential oil and which has wide application in the food industry. Lawrence et al., (1972) Simon et al., (1984) observed Bulgarian and Java basil oils are rich in methyl-cinematic and eugenol.

(Anonymous, 1982), found forty-five Compounds and Oils in Basil. The main constituents in volatile oil from basil are rosmarinic acid (a strong antioxidant), linalol, methylchavikol, methylcinnamat, 1, 7-dimethyl-1, 6-octadien-3-ol, and eugenol. Its medicinal effects are mostly due to rhymol, eugenol and camphor. The mucilage is composed of sugars; xylose and polysaccharides. The seeds contain oil composed of fatty acids and sitosterol. It is one of the healing herbs that, contains vitamin A and C, as well as antioxidants that help to prevent cell damage that can lead to cancerous conditions. In fact, another animal study shows that basil "stimulates production of disease fighting antibodies by up to 20%.

(Dey, 1983) constituents obtained from variation between chemical compositions, depending of location, seasonal variation and stages of development. Ocimum Sanctum grown in India, in the field, under natural conditions gave highest percentage of methyl eugenol in young leaves (5-10 days old). Since 1984; scientist had been characterizing the chemical diversity of Ocimum spp. to identify chemotypes of potential commercial interest.
Genetic and breeding studies have been initiated to increase the total essential oil content (concentration) of commercial basil chemotypes and to increase the content of specific oil constituents in other chemotypes such as those high in methyl chavicol and methyl cinnamate. A germplasm collection of basil (*Ocimum* spp.) consisting of more than 100 accessions from the USDA Plant Introduction Station which include *O. basilicum*, *O. canum*, *O. gratissimum*, *O. kilimandscharicum*, *O. citriodorum*, *O. micranthum*, *O. sanctum* plus other commercial and noncommercial seed sources were field grown in central Indiana and were initially screened organoleptically for aroma and flavor differences.

(Laskar and Majumdar, 1988) Methyl chavicol stimulates liver regeneration, shows hypothermic and DNA binding activities. Methyl eugenol also shows DNA binding activities, spasmolytic and gives muscle relaxant effects.

(Sheen et al., 1991) obtained oils from various parts (leaf, flower, stems, and whole plant and leaf flower) of *Ocimum basilicum*, which were categorized into five groups by preference ranking test of their aromas. High statistical correlations were found among the volatile components of the oil.

(Susan, 1990) reported the use of genetic information expressed in isozymic from has enhance our understanding of heritable variation within and among plant population. For over three decades now, protein electrophoresis coupled with histochemical staining has provided data on the concordance of observable feature in plants (morphology, cytoology, ecology adaptation) with isozyme phenotype.

(Sousa et al., 1991) reported the presence of trans-anethol as a major constituent in essential oils explains the odor similarity of this species as compared to anise (*Pimpinella anisum*), which presents aromatizing activity, and is stimulant of digestion and carminative.

(Rajeshwari, 1992) analyzed the essential oils extracted from the leaves of *Ocimum sanctum* L. has been found to inhibit in-vitro growth of *E. coli*, *B. anthracis* and *P. aeruginosa* showing its antibacterial activity. Tulsi also has anti-tubercular activity and inhibits in-vitro growth of *M. tuberculosis*. The essential oils extracted from Tulsi leaves also possess anti-fungal and anti-viral activity.
(Lawrence, 1992 and Grayer et al., 1996) observed that difference in the essential oil compositions in *O. basilicum* from different geographical localities led to the classification of basil into chemotypes on the basis of the prevalent chemical components or components having composition greater than 20 percent.

(Bonnardeoux, 1992) extracted essential oils by steam distillation from whole plant or from flowers only. Essential oil yield were higher from plants harvested when they were 3-11 cm in length. (Lawrence, 1993) analyzed that essential oils are composed primarily of monoterpenes and sesquiterpenes and have been the subject of extensive studies due to their economic importance. Rajeshwari observed that aerial parts (leaves, flowers & stem) of these plants contain essential oils with good percentage of eugenol. The leaves of *Ocimum sanctum L.* are chief source of essential oils followed by the inflorescence and stem; however, flowers contain more essential oils than leaves in *Ocimum basilicum*. The roots and fruits of these plants are almost completely devoid of any essential oil.

(Mori, 1993) reported that methyl chavicol and methyl eugenol, which are found abundantly in *Ocimum Basilicum* and *Ocimum Sanctum* volatile essential oil used extensively in various application including perfumes.

(Harborne, 1993) analyzed that methyl chavicol stimulates liver regeneration, shows hypothermic and DNA binding activities. Methyl eugenol also shows DNA binding activities, spasmylytic and gives muscle relaxant effects.

(Hopp, 1993) observed that essential oil's combined effect, which depends on the desired application, is more powerful than that of individual oil.

(Harborne and Baxter, 1993) said that essential oils are fragrant, highly concentrated essences of plants which are considered to exemplify the soul or life-force of the plant. Essential oils are approximately 75-100 times more concentrated than dried herbs.
(Hopp and Mori, 1993) Investigate the volatile essential oil used extensively in various application including perfumes, food seasoning and flavoring, aromatherapy and medicinal applications.

(Sino and coworkers, 1992) and Wossa and co-workers (2004) analyzed that hydrodistilled aerial parts of *O. basilicum*, *O. tacillum* and *O. canum* afforded pale yellow colored oils in 1.0, 0.4 and 0.01 percent yields respectively. GC/MS analysis of the oil indicated *O. basilicum* to be composed of 11 components; *O. tacillum* with 6 components and *O. canum* with 5 components. The major components of *O. basilicum* were geraniol (44.5 %) and neral (36.1 %). The other important components identified were linalool (6.0 %), cis-α-bisabolene (3.8 %) and nerol (3.3 %) whilst other monoterpenes made up the remainder. Estragole (96.6 %) was found to be the major component of *O. tacillum* whilst the major components in *O. canum* were eugenol (35.3 %), linalool (27.2 %) and 1,8-cineole (5.6 %). this composition is comparable to that as reported from the lemon grasses *Cymbopogon citratus* (Poaceae) oil from PNG by containing 68 and 91 percent citral composition respectively.

(Adams, 1995) identified the chemical constituents was effected by means of a comparative analysis of the mass spectrum of the substances with those of the database of the system GC-MS (Nist 62.lib) literature and retention index.

(Petropoulos, 1995) Essential oils were obtained by a Clevenger hydrodistillation apparatus from nine Greek populations of basil and analysed by GLC Chromatography. Major flavor components indentified were linalool, methyl chavicol, methyl cinnamate, eugenol, cineole and gerarioil. Large variations in the chemical composition of the essential oils among groups of these populations were noted that permit to classify these basil oils in three types, named “Kinos”, “Sgouros” and “Mauromytikos”. The chemical composition, commercial value and use of these three types are discussed. The comparison of these types with the four known international commercial types of basil oil named European, Réwnion, Methyl Cinnamate and Eugenol is discussed, too. The concusion is that the majority of greek basil oils belong to the sweet basil (European
Type) that is suitable for flavor purposes and less to Methyl Cinnamate Type that lends itself to perfume work.

(Mukherji, 1995) had investigated the composition of essential oils of Ocimum sanctum L. growing in different parts of India. The percent of eugenol in essential oil of Ocimum sanctum L. varies from 40% (in Jammu) to 71% (in Assam). Seasonal variation has also been observed in composition of essential oil extracted from Tulsi leaves.

(Pandey and Chowdhury, 1998) identified twenty four constituents representing 98.4% of the essential oil. Eugenol was the major component (84.84%), followed by linalool, limolene, methyl eugenol and β-salinene in O. gratissium.

(Laskar, 1998) isolated the essential oil from Ocimum sanctum but with absolute seasonal variation. Eugenol is the main component of Ocimum sanctum grown in Bangladesh, Germany, Cuba, Northeastern Brazil, methyl eugenol from India.

(Raju et al., 1999) observed the major constituents of the plant are volatile oils including euginol, carvacrol, methyl-euginol etc. Besides the volatile oils the plant is reported to contain alkaloids, flavonoids, glycosides, saponins, tannins, triterpene and ascorbic acid and carotene.

(Nakatsu et al., 2000) observed essential oils are generally extracted by distillation, expression, solvent extraction, cold pressing, maceration or supercritical carbon dioxide extraction. It has been reported that the quality and quantity of essential oil produced by plants depends on various factors such as seasonal variation.

(Harris, 2002) said that, this is commonly known as a synergistic blend. Essential oil exhibits many usages such as in medicinal application herbs, culinary and are used as perfume for herbal toiletries, aromatherapy treatment and also in perfume industry.

(Singh et al., 2002) found that eugenol (~21%) and β-caryophyllene (37%) constituted the major components in O. sanctum further, a number of sesquiterpenes and
monoterpenes viz., bornyl acetate, β elenene, methyleugenol, neral, β-pinene, camphene, α-pinene etc were also present in the essential oil of *O. sanctum*.

*(Javanmardi et al., 2002)* observed that quantification of phenolic acids was determined using liquid chromatography and showed drastic variations between accessions. Chemical studies revealed that rosmarinic acid is the predominant phenolic acid present in both flower and leaf tissues. Unusual basil accessions were identified that can serve as genetic sources of acids for crop improvement.

*(Ozcan et al., 2002)* isolated the essential oil by hydro distillation of the over ground parts of *Ocimum basilicum* L. and *Ocimum minimum* L. from turkey were examined by GC-MS. A total of 49 and 41 components, respectively, were identifying accounting for 88.1% and 74.4% of the oils of *O. basilicum* and *O. minimum*, respectively. The oil of *O. bacularicum* contained, as main components, methyl eugenol (78.02%), nerol (0.83%). Major components in the volatile oil of *O. minimum* were geranyl acetate (69.48%), terpine-4-ol (2.35%) and octan –3-yl acetate (0.72%). the essential oil of *O. baclicicum* was characterized by its high content of methyl eugenol (78.02%), whereas the most important essential oil constituent of *O minimum* was geranyl acetate (69.48%).

*(Barbieri, 2004)* reported, basil leaves were dried using either conventional hot air (50, 60 and 70 °C) and low pressure superheated steam (LPSS) dryers. The effect of the drying method on the retention of some volatile compounds was evaluated. The extraction from the fresh and dried products was performed by the simultaneous distillation–extraction technique. Identification and quantification was performed by capillary gas chromatography–mass spectrometry (GC–MS) and gas chromatography (GC) respectively. The identified compounds were 23, with 61% of monoterpenes, 26% of aromatic and 13% of aliphatic compounds, out of which those characteristic of this spice were detected (1,8-cineole, methyl chavicol, methyl cinnamate and linalool).

*(Smith et al., 2005)* Essential oils and herbal extracts have attracted a great deal of scientific research interest due to their potential as natural flavors. A scientifically based guide has been developed to assess the safety of naturally occurring mixtures based
on chemical composition, particularly essential oils, for their intended use as flavor
ingredients this research reports the seasonal variation of essential oil composition of
*Ocimum basilicum* and *Ocimum sanctum*.

(Bayram, 2006) studied, the composition of 18 Turkish basil essential oils was
investigated by GC and GC-MS. Variation of essential oils in the landraces was
subjected to cluster analysis, and seven different chemotypes were identified. They were
(1) linalool, (2) methyl cinnamate, (3) methyl cinnamate/linalool, (4) methyl eugenol, (5)
citral, (6) methyl chavicol (estragol), and (7) methyl chavicol/citral. Methyl chavicol with
high citral contents (methyl chavicol/citral) can be considered as a “new chemotype” in
the Turkish basils. Because methyl eugenol and methyl chavicol have structural
resemblance to carcinogenic phenylpropanoids, chemotypes having high linalool, methyl
cinnamate or citral contents and a mixture of these is suitable to cultivate for use in
industry.

(Mukherjee et al., 2006) reported that *Ocimum Sanctum* has been extensively
used in Ayurvedic system of medicine for various ailments including capability lowering
plasma glucose.

(Telci et al., 2006) presented by seven types of chemotypes also included methyl
chavicol (68.3%) for *Ocimum Basilicum* varieties grown in Turkey.

(Hussain, 2008) extracted the essential oil of *Ocimum Basilicum* high during the
winter season, giving high percentage of linalool (60.6%), 52.6 and 58.26% estragol
from leaves and flowers.

(Vani, 2009) observed that, the genus *Ocimum* is cultivated for its remarkable
essential oil which exhibits many usages such as in medicinal application, herbs,
culinary, perfume for herbal toiletries, aromatherapy treatment and as flavoring agent.
Due to varying essential oil profiles even within the same species, plants may often be
classified as a different species as a result of different scents. In the present study, volatile
constituents of *Ocimum sanctum* and *Ocimum basilicum* were extracted using various
solvents and their chemical constituents were identified and quantified by using GC-MS
in optimized conditions. The profiles of extract from both species were compared in an effort to investigate effects of seasonal variation on their chemical compositions. The predominant species in *Ocimum Sanctum* and *Ocimum Basilicum* was found to be methyl eugenol and methyl chavicol, respectively, during different months of analysis.