Review of Literature

2.1. A brief history of Ethnobotany

The word "*Ethno*" originated from the Greek word "*ethnokos*" meaning the human race and "botany" means the study of plant. Thus, "ethnobotany" can be defined as the relationship of human races with plants. The term "ethnobotany" was first coined by Harshberger during 1896. He defined "ethnobotany as the use of plants by aboriginal people dealing with the study of direct interactions between humans and plants". Later on, ethnobotany emerged as a distinct subject studied by several researchers. For example, ethnobotany has been termed as the interrelations of primitive man and plants (Jones, 1941), total relationship between man and his surrounding vegetation (Faulks, 1958), coexisting relationship between people of primitive society and their plant society (Schultes, 1962), total natural and traditional relationship and interactions between man and his surrounding plants wealth (Jain, 1987), utilization of useful plants for their commercial exploitation (Wickens, 1990), the mutual relationships between plants and traditional people (Cotton, 1996). Balick and Cox (1996) carried more in depth studies on ethnobotany where several aspects such as traditional knowledge of local people, utilization of plants for various medicinal and therapeutic purposes and their cultural and religious association with them have been recorded.

2.2. Indian history of Ethnobotany

The plants having medicinal values are being utilized by the human beings from historical past. The earliest literature (*Rig Veda*, 3500 – 1600 BC) portrays that plants are useful to mankind as medicine. Subsequently, the properties and therapeutic application of medicinal plants were researched in details. *Ayurveda* was a basic foundation of primitive medical knowledge in India. *Ayurveda* is the oldest medicare system with earliest times between 4500 – 1600 BC. The *Rig Veda* was followed by *Atharva Veda*, which threw light on the medico religious uses of plants in India. The Ayurvedic practice grows during *Charaka Samhita* (1000 – 800 BC), *Sushruta Samhita* (800 – 700 BC) and Vagbhata's *Ashtanga Hridaya* (500 – 600 BC).

In India, Dr. Janaki Ammal first took the initiative to start ethnobotanical works at Allahabad circle of BSI and then Dr. Sudhanshu Kumar Jain had taken the baton from Dr. Janaki Ammal and started the ethnobotanical field works in the tribal communities of central India. Dr. S. K. Jain is credited as the "Father of Indian Ethnobotany". He edited the book "Glimpses of Indian Ethno-botany" which is known as the first book of ethnobotany from India. This book described the uses of 1500 plants by the different tribal communities from different regions of the country. Bibliography of Ethnobotany (Jain *et al*., 1984), A Manual of Ethnobotany (Jain, 1987), Methods and Approaches in Ethnobotany (Jain, 1989), Dictionary of Indian Folk Medicine and Ethnobotany (Jain, 1991) etc. are some of his legendary works.

In West Bengal some of the remarkable works in the field of ethnobotany were less known plant food among the tribals of Purulia (Jain and De, 1964), medicinal plant-lore of Santals (Jain and Tarafder, 1970) etc. Later on, Chaudhuri and Pal (1976) made a preliminary study on ethnobotany of Medinipur district. Ghosh (1986) studied the ethnobotanical survey of Cooch Behar district; use of plants by Lodha tribe in Midnapur District (Pal and Jain, 1989); ethnobotanical study of Purulia district (Sur *et al*., 1992) etc.

2.3. Ethnobotany of Dakshin Dinajpur District

Dakshin Dinajpur is a small and diversified ethnic culture district of West Bengal. The tribal people of this district are still using plants for their medicinal purposes. However, few studies have been reported which were showed the ethnobotanical prospects and how the medicinal plants have been incorporated into the cultural tradition of local ethnic people of Dakshin Dinajpur district.

Sur along with his co-workers (1987) had studied ethno-economic importance of 73 plant species under 67 genera in the district Malda and West Dinajpur. Immediately after completion of the work again in the year 1990, they documented 52 plants under 48 genera for ethno-economic importance in the same study area. Both the ethno-economic works have been documented the methods of application of plants in treating certain diseases and their distribution. Similarly, Banerjee and Ghora (1996) documented some domestic uses of plants which were unreported from West Dinajpur district. In 1991 West Dinajpur district was subdivided into North Dinajpur and South Dinajpur districts. In this study total 29 common wild plants were documented highlighting their use as food and as vegetable-ingredients mostly during drought/flood by the common people. The study of Mitra and Mukherjee (2005a) showed that 16 grass taxa were found to have 27 ethnobotanical uses and *Vetiveria zizanioides* was the mostly used taxon. The study demands that the Santals are more ethnomedicinally more soundly than the other tribal communities such as the Mundas and Oraons. Mitra and Mukherjee (2005b) reported 107 less known uses of the root and rhizome drugs of 71 species of angiosperms belongings to 68 genera of 45 families grown in West Dinajpur district for the treatment of different common human diseases. Further, they identified 62 local plant species having medicinal importance to cure different types of gastro-intestinal problems like constipation, diarrhoea, dysentery, indigestion, flatulence, dyspepsia and inflammation of liver, stomachache, abdominal and intestinal worm mostly prevailed in the North Bengal area (Mitra and Mukherjee, 2010).

De Sarker *et al*. (2011) carried out a documentation work on medicinal plants, their uses and availability in Uttar Dinajpur, Dakshin Dinajpur and Malda district. In this study total 610 plant species were well documented. The documentation work recorded *Arum margaritiferum*, *Rauvolfia serpentina*, *Geodorum densiflorum*, *Gloriosa superba*, *Aristoloahia indica Tylophora indica* as endangered species. Chowdhury *et al*. (2011) documented the plants which were being used to treat diabetes by the ethnic tribal communities of Dinajpur (Uttar & Dakshin) and Malda District of West Bengal. The study recorded 31 plant species belonging to 21 families which were commonly used for the remedial of diabetes.

It is found from the study of Kundu and Bag (2012) that the Rajbanshis of Dakshin Dinajpur district use plants as a preventive and curative health measures. Talukdar and Talukdar (2012) had studied the floral diversity and its folk uses in the banks of Atreyee River at Balurghat, Dakshin Dinajpur. The study revealed that 39 plants used as herbal medicine by different ethnic communities, namely Santhals and Mundas residing along the basin of the River Atreyee. In addition to medicinal uses, plant resources were also utilized as forage, manure, fishing, sheltering, vegetable and religious purposes. Later, 62 plant species belonging to 34 families were identified by

Talukdar and Talukdar (2013) which also have similar kind of medicinal importance. According to this study leaves are the mostly used plant parts.

Recently, Chowdhury *et al*. (2014) documented some traditional uses of plants by the different communities of Dakshin Dinajpur District. Results revealed that maximum number of plants was used in gastrointestinal problems followed by gynecological problems. The study showed that among the various plant parts used, leaf was maximally used for medicinal preparation followed by root, whole plant, stem bark etc. Total 132 plant species belonging to 65 families under 120 genera were recorded in the aforementioned study. Though all the 132 plants have their medicinal values but these were unveiled before the study, and most importantly 56 plants are found to have medicinal importance which is not known before the study. According to Saha *et al.* (2014a) the native tribes of Malda district (W.B.) have age old knowledge on the consumption of uncultivated plant species for their healthcare. The study documented total 53 medicinal plants that were regularly used to cure 44 different categories of diseases. They recorded that herbs were most frequently used plant followed by shrubs, trees, climbers and parasites. The most useful plant parts are roots, though the other parts like leaves, seeds, bark etc are also occasionally used. The major diseases treated were dysentery, azoospermia, menstrual disorder, rheumatism, diabetes etc. Further, in the year 2015 they studied the ancient knowledge and socioeconomic significance of indigenous liquor prepared by the Oraon tribe in district of Malda, West Bengal. Ethno-medicinal investigation of local plants gives an indication that the plants can also be used to prepare a local variant of alcohol that directly and indirectly influence the socio-economic condition of the tribal communities (Saha *et al*., 2015).

2.4. Ethnobotany of the genus *Ocimum*

Ocimum tenuiflorum (“Tulsi”) is considered as the most sacred and auspicious plant in India. The name Tulsi is derived from the Sanskrit word which means matchless one (Ghosh, 1995). In Ayurveda, Tulsi is known as the incomparable one, mother medicine of nature, the queen of herbs and elixir of life (Singh *et al*., 2002a). Hindus worship this plant in the morning as well as in the evening by giving some “Prosad/Vog” with wet rice, flowers, vermillion and sweets. Many “Sadhus”, particularly those belonging to the *Bairagi/Baishnab* sector, put on a garland of tulsi around their necks. They used

'Japmala' (Chanting beads) to count or chant the names of Ram from tulsi. The Baishnab devotee offers his daily meals to Vishnu by putting a leaf of tulsi in his food (Upadhyaya, 1964). According to Hindu tradition, tulsi leaves are placed on the eyes of the dead body and planted at the funeral place and this plant is never burnt by Hindus (Kumar *et al*., 2006).

The traditional uses of *Ocimum* species are well documented in some Indian text by different authors (Chopra, 1953; Chopra *et al*., 1956; CSIR 1966; Kirtikar and Basu, 1975; Nadkararni and Nadkarni, 1976; Satyavati *et al*., 1976; Warrier *et al*., 1995). Tulsi is also a good home remedy for various diseases such as common cold and cough, asthma, headaches, bronchitis, liver diseases, fever, lumbago, hiccups, eye infections, ringworm, gastric disorders, diarrhoea, insomnia, arthritis, urinary disorders, skin diseases, sore throat, vomiting, antidote for snake bite and scorpion sting (Singh *et al*., 2002a; Prajapati *et al*., 2003; Das and Vasudevan, 2006; Ulbricht, 2010; Cohen, 2014). Another report showed the ethno-veterinary use of *Ocimum* (Galav *et al*., 3013). Naghibi *et al*. (2005) documented the folk medicinal uses of Labiatae family from Iran. They documented total 410 species and subspecies of 46 genera. 18% species of the family lamiaceae were used as medicinally. In this review four *Ocimum* species and their ethnobotanical uses are well documented.

Prakash and Gupta (2005) reviewed the therapeutic uses of *Ocimum sanctum* with a note on eugenol and its pharmacological property. In the Indian classical systems of medicine, various parts of *O. sanctum* has been prescribed for curing asthma, bronchitis, dysentery, diarrhoea, skin infections, joint inflammation, eye diseases, interminable fever, malaria, insect bite etc. However, eugenol has been found to be most important ingredient.

Mondal *et al*. (2009) reviewed the science behind sacredness of tulsi. In this review efforts had been made to sum up various aspects of scientific studies on this plant. Scientific evidences are available on different medicinal aspects like antimicrobial, antidiabetic, adaptogenic, hepato-protective, anti-inflammatory, radioprotective, anti-carcinogenic, immunomodulatory, cardio-protective, neuro-protective, mosquito repellent etc. Most of this information was proved *in-vitro* by different researchers and few studies are still in experimental condition. Singh and his associates (2011) reviewed the folk uses of *Ocimum sanctum*. The study clearly describes the symptoms of different ailments and various mode of administration of *Ocimum* for the management of healthcare system.

Prabhu *et al*. (2009) reviewed the chemical, pharmacological and ethno-medicinal properties of *O. gratissimum*. Folklore medicine claims its use in headache, fever, diarrhoea, pneumonia etc. Research has been carried out to support the biological activity of different *Ocimum* species by using different *in vitro* and *in vivo* techniques. This review nicely represented the ethno-botanical, natural product chemistry, pharmacological, clinical and toxicological data of the plant. Kashyap *et al*. (2011) reviewed the ethnomedicinal, phytochemical and pharmacological survey of *O. kilimandscharicum*. The plant has been used generally in Kenya against cold and cough, measles, diarrhoea, abdominal pain and mosquito. The terpenoids have been accountable for different pharmacological actions like antioxidant, wound healing, insecticidal, mosquito repellent, antimicrobial, against melanoma and radio-protection.

Agarwal *et al*. (2013a) studied the ethnomedicinal uses of *Ocimum* species from Rajasthan. The study showed that traditional healers of Rajasthan having commendable knowledge of the medicinal values of plants growing around them. Mamun-Or-Rashid and his associates (2013) reviewed the ethno-medicobotanical study on *Ocimum sanctum*. This study revealed the enormous diversity of its medicinal uses and wide range of common ailments curing like fever, malaria fever, asthma, bronchitis, colic pain, sore throat and hepatic diseases. Beside the ethobotanical uses they also listed the phytochemicals and various other important medicinal properties. Similar study has been conducted by Tiwari *et al*. (2014).

2.5. Taxonomy of the genus *Ocimum*

The taxonomy of the genus *Ocimum* is vast and more complex. The complexity is mainly due to high degree of variation within the genus and a number of biotic and abiotic factors. As of now, more than 160 species were identified in the genus *Ocimum*. The plant species within the genus of *Ocimum* mainly vary in their height, shape, size, leaf colour and flower colour etc. In view of this, it has been estimated that only 65 species of the genus *Ocimum* should be considered as true species and rest should be discarded as synonyms or false attribution (Paton *et al*., 1999). *Ocimum* belongs to the subfamily of Nepetoideae of the family Labiatae. The notable characteristics of this family are square stem, inverse and decussate leaves with many doted glands. The flowers are zygomorphic with two unequal lips. The important characteristics feature of the Nepetoideae subfamily is that the species under this subfamily are strongly aromatic due to terpenes in essential oils.

The genus *Ocimum* was first described by Linnaeus (1753) who listed five species under this genus. Further, Bentham (1832) classified *Ocimum* into 3 sections based on morphology of stamens like appendiculate posterior stamens (*Ocymodon* Benth.), posterior stamens with hairs at the base (*Hierocymum* Benth.) and posterior glabrous stamens (*Gymnocymum* Benth.). *O. basilicum* L., *O. gratissimum* L. and *O. kilimandscharicum* Guerke. were placed in the first section (*Ocymodon* Benth.) in which appendiculate posterior stamens were observed. *O. tenuiflorum* L. (Syn. *O. sanctum* L.) was placed in second section (*Hierocymum* Benth.) and *O. campechianum* Mill was placed in third section (*Gymnocymum* Benth.).

Bentham (1848) subdivided section *Ocimum* [*Ocymodon*] into three subsections namely, *Ocimum*, *Gratissima* and *Hiantia* based on calyx morphology. For example, the throat of the fruiting calyx is open and bearded incase of *Ocimum*. The throat is closed by the middle lobes of the lower lip in case of *Gratissima* and with truncate lateral calyx lobes incase of *Hiantia* Benth.

Later, Paton (1992) carried out an exhaustive classification of *Ocimum* incorporating the approaches of morphological pattern of calyx (Bentham, 1848; Harley *et al*., 1992). Pushpangadan and Bradu (1995) took a different approach of infrageneric classification where *Ocimum* has been divided into two groups *viz*., *'Basilicum'* and *'Sanctum'* group. The *'Basilicum'* group consisting of herbaceous annuals or occasionally perennials and seeds are black, ellipsoid, highly mucilaginous with haploid chromosome number, n=12, while the *'Sanctum'* group contains perennial shrubs with brown globose/subglobose non-mucilaginous or feebly mucilaginous seeds with haploid chromosome number, n=8.

However, the classification framework does not satisfactorily express the dissimilarity inside the genus and has some limitations (Paton and Putievsky, 1996). Further, the taxonomic problems in the genus of *Ocimum* were reviewed based on morphology and chromosome number by Carovic-Stanko *et al*. (2010a). They observed that nomenclature of *Ocimum* is generally complex due to the presence of several varieties, cultivars and chemotypes within the species whose morphology do not differs considerably.

2.6. Ecology of the genus *Ocimum*

The genus *Ocimum* is an aromatic, annual to perennial herb or shrub native to the tropics and subtropics of Asia, Africa and central South America (Darrah, 1980; Balyan and Pushpangadan, 1988; Gupta 1994; Paton *et al*., 1999). It is believed that the main centre of *Ocimum* diversity is in Africa (Hedge, 1992), even though several species are native to Asia (India) and South America (Brazil) (Vieira and Simon, 2000). The distribution of *Ocimum* species is very diverse and it can be grown in various agro-climatic environments. For example, it can be seen to grow in the countries such as India, Pakistan, Malaya, Australia, Philippines, Brazil, Arabia, Persia, Nepal, Indonesia, Egypt, Morocco, France, Greece, Hungary and the United States (Grieve, 1992; Bhattacharjee, 1998; Bahl *et al*., 2000). In India, it is mainly grown in the states like Assam, West Bengal, Bihar, Uttar Pradesh, Madhya Pradesh, Maharashtra and Jammu (Rao *et al*., 2007). Common species of *Ocimum* in India are *O. tenuiflorum* L. (Syn. *O. sanctum*), *O. gratissimum* L., *O. basilicum* L., *O.* x *africanum*, *O. americanum* L. and *O. kilimandscharium* Guerke.

The genus *Ocimum* is considered as a very hardy plant species and it can be grown in different ecological conditions up to 900 m altitude from the mean sea level. For example, it survives in the cold moist to tropical rain forest regions with temperature ranging from 6 °C to 24 °C and annual rainfall receiving 500–800 mm (Duke and Hurst, 1975). It grows well under long days with bright sunlight. The plant is moderately tolerant to drought and frost. However, high temperature and humidity have been found favorable for its luxuriant vegetative growth and high amount of oil production. Warmth, light and humidity are the fundamental environmental necessities for cultivation of *Ocimum* species (Halva, 1987; Nykanen, 1989). Nazim and his associates (2009) carried out a study to examine the growth potential of two species of *Ocimum viz*., *O. basilicum* and *O. sanctum* in sandy soil of Karachi. It was observed that both the species were effectively completed their life cycle (germination, vegetative/reproductive growth and seed production) and also formed higher quantity of viable seeds. They recommended that these imperative medicinal herbs could be cultivated effectively in sandy soil.

The quadrat study of plants habitat gives an idea about the species and its associated plant communities. Thus, phyto-sociological/phyto-association study is the study of plant communities in relation to their inter species relationship within a similar ecological condition (Sarah *et al*., 2015). A very few phyto-sociological works has been done in relation to *Ocimum* species but a number of phyto-sociological research works had been on different other plant species.

De Sarker and Kundu (1996) studied the effect of various gaseous and particulate pollutants emanating from brick kilns on the adjoining vegetation of West Dinajpur (West Bengal). The relative density of different herbs showed that there was distinct decrease in densities of herbs in the adjoining area of brick kilns as compared to control. However, some slant slike *Blumia lacera*, *Calotropis* and *Gnaphalium* sp. remain unaffected/less affected. The frequency of the plant community reveals that except *Blumia lacera* and *Calotropis procera* the occurrence of other plant species reduce towards the brick kilns.

Baig *et al.* (2012) studied the distribution pattern and current conservation status of six threatened medicinal plants in Daksum Kokernag, Kashmir Himalayas by random quadrate sampling in different habitat types to understand the distribution and conservation status of medicinal plants in their natural habitats, owing to their increased demand and value.

Manna and his associates (2013) had studied the phytoassociation of *Helminthostachys zeylanica* in the Danga forest, Raghunathpur, West Bengal to analyze its potential habitat and nature of association with co-existed plants. The study depicts some strong possibilities of good positive association between *Vetiveria zizanioides*, *Barringtonia acutangula* and A*ntidesma acidum* and also a strong negative relationship with *Eucalyptus globulus*. These findings describe the importance of presence of *V.*

zizanioides, *B. acutangula* in the nearby habitat for establishment and increase the population size of *H. zeylanica*. Further, Manna and Roy (2014) had studied the plant association and conservation of two rare species namely, *Ophioglossum nudicaule* and *Ophioglossum vulgatum*. Basic parameters of the community, for example, density, abundance, relative abundance, frequency, relative frequency and IVI of two types of *Ophioglossum* and their existing together plants were calculated based on a quadrat study of three tropical deciduous forests of the lateritic part of West Bengal.

Krishna and his associates (2014) studied the pattern of plant diversity analyzing the phytosociological parameters of grasslands in Rajasthan. Sinha and Sinha (2014) had carried out a study on diversity of the medicinal plants and their ecological status in Koria district, Chhattisgarh, India. The plant species showed maximum and minimum frequency of *Tribulus terrestris* and *Amomum subulatum* respectively. Population-wise *Cleome gynandra* and *Vicia sativa* exhibited comparatively higher density.

Sarah and his associates (2015) carried out phytosociological study on the flora and vegetation community of *Palaquium gutta (*Hook.f.) Baill. in compartment 13 of Ayer Hitam Forest Reserve, Selangor. The main objectives of this study were identifying the natural habitat and associated plants present in the community of *P. gutta* in Ayer Hitam Forest Reserve. Further, Sarah and Noorma (2015) studied the phytosociology on the floristic and vegetation communities of *Aquilaria malaccensis* in Sungai Udang Forest Reserve, Malacca, Malaysia. The main objectives of this study were identifying the natural habitat and associated plants present in the community of *A. malaccensis*. The study generates useful information to know the growth response of the mixed dipterocarp forest for proper forest management.

Ismail and Elawad (2015) had studied the phytosociological characteristics and the diversity patterns of herbaceous plants in Rashad and Alabassia localities. Fourteen vegetation sites were selected to conduct this study. Important Value Index was used to estimate the phytosociological characteristics. The phytosociological characteristics revealed that *Tetrapogon cenchriformis* dominated herbaceous species in all the studied sites followed by *Spermacoce pusilla*.

2.7. Variation in *Ocimum* species

The genus *Ocimum* is showing enormous variations in respect of morphological, chemical and genetic constituents.

2.7.1. Morphological

Ocimum species are well characterized in terms of morphological characters *viz.*, plant height, intermodal length, leaf area, leaf shape, leaf margin, leaf colour, inflorescence length, flower colour, seed colour etc. which were reflecting in several studies (Singh *et al.*, 2002b; Labra *et al.*, 2004; Abduelrahman *et al.*, 2009).

Plant height is an important morphological trait to distinguish *Ocimum* species. Based on plant height *O. basilicum* was classified as tall group (> 50 cm), medium group (40-50 cm) and short group (< 40 cm) height. Basil cultivars were also classified into four groups based on leaf size; large, medium, small and very small (Masi *et al.*, 2006). Similar kind of results was observed in 34 cultivars of basil (Svecova and Neugebauerov, 2010). The cultivars were grouped into bunches as indicated by leaf colour shades and leaf estimate (green little-leafed and purple-leafed). The green-leafed cultivars dominatingly had a white corolla and green calyx whereas, in purple-leafed cultivars the corolla and calyx were different purple shades and in many cultivars the calyx was pubescent.

Carovic-Stanko *et al*. (2010b, 2011a) reported five morphotypes of basil taxa on the basis of their morphology *viz.*, True basils, Small-leaf basils, Lettuce-leaf basils, Purple basils A (var. *purpurascens*) and Purple basils B (Dark Opal). Previously, Darrah (1980) classified the *O. basilicum* cultivars on the morphology into seven types *viz.*, dwarf types, compact types, tall slender types, large-leafed robust types, purpurascens, purple types with clove-like aroma and citriodorum types (lemon-flavoured basil). Erum and his associates (2011) had studied qualitative and quantitative trait of two *Ocimum* species namely, *O. basilicum* and *O. sanctum* collected from different agro-ecological zones of the world. The study showed that all the basil varieties significantly differed in germination, plant height, canopy, spikes/plant, florets/spike, leaf area, leaf thickness, thickness of stem and petiole length.

Sastry and his associates (2012) reported the morphological traits of the three species of *Ocimum viz*., *O. gratissimum*, *O. basilicum* and *O. tenuiflorum.* Among the three *Ocimum* species, *O. gratissimum* was showed highest plant height and *O. basilicum* showed wide range of plant height varied from 44.00-125.00 cm. Canopy spread of *O. gratissimum* was comparatively less than *O. basilicum* and *O. tenuiflorum* and both species were recorded high number of leaves/plant. *O. tenuiflorum* recorded higher number of inflorescences/plant, whereas *O. gratissimum* showed higher number of whorls/inflorescence (27-28). *O. gratissimum* had lengthy peduncle, while *O. basilicum* and *O. tenuiflorum* showed maximum petiole length. The flowers were relatively large in *O. basilicum* with large size bracts, sepals and petals. The stem colour was green or greenish purple in *O. basilicum*, whereas in *O. tenuiflorum* and *O. gratissimum* stem colour was greenish purple. Flower colour of *O. basilicum* was white to whitish purple. *O. tenuiflorum* had purple/purplish white flowers, while the colour of *O. gratissimum* flower was purplish white. Stamen colour of *O. basilicum* exhibited white or creamy white or whitish purple while in *O. tenuiflorum* and *O. gratissimum* the stamen colour were similar to flower colour. Seeds were black in *O. basilicum* and brown in *O. tenuiflorum* and *O. gratissimum.*

Singh (2012) studied the seedling morphology of four *Ocimum* species *viz*., *O. americanum*, *O. basilicum*, *O. gratissimum* and *O. tenuiflorum* were collected from various places of Varanasi district, Uttar Pradesh, India. Some morphological characters of seedlings *viz.*, secondary root surface, collet and number of leaf veins were found appropriate to differentiate the investigated species at their young phase. The author suggested that seedling morphology should be taken into consideration in a comprehensive way to distinguish the species and in solving taxonomic and phylogenetic implications.

Anyaoha (2013) studied the agro-morphological variability of *O. gratissimum* and three different accessions of basil from South-western Nigeria. Comparatively variation was high in characters like plant height, leaf length, petiole length, number of branches and inflorescence length. Variation was minimum in characters like 1000 seed weight, number of seeds/pod, leaf width and canopy cover. Leaf length and leaf width fluctuated from 3.9 to 20 cm and 1.7 to 10.95 cm correspondingly and furthermore a broad variation was recorded for qualitative traits, for example, flower, seed, leaf and stem colour and whole plant parts were glabrous.

Agarwal *et al*. (2013b) had made a comparative study of the sixteen different genotypes of *Ocimum* species to assess the variability of qualitative and quantitative morphological characters under same climatic condition and location. The overall analysis showed that location had considerable effect on all the six characters. The interaction between environment and genotype was also prominent for all the characters. Similar results were observed in some earlier studies (Verma *et al*., 1989; Tesi *et al*., 1991; Sarin *et al*., 1992; Szabo *et al*., 1996) in different *Ocimum* species.

Nurzynska-Wierdak (2013) had studied the morphological differences of seventeen sweet basil cultivars. Significant morphological contrasts were seen in the examined sweet basil qualities identifying with the plant's height, canopy, plant weight and also number of branches and inflorescences. Results of the previous and present investigations (Nurzynska-Wierdak, 2007a, b) proved that enormous morphological as well as developmental unevenness of sweet basil. Bernhardt *et al*. (2014) have described eight distinct *O. basilicum* gene bank accessions through morphological assessment, essential oils composition and RAPD molecular marker. Among the eight accessions, two accessions with anthocyanin colorations (‘Dark Opal’, ‘Piros’). From the cluster analysis of morphological data two groups were obtained.

Malav *et al*. (2015) collected forty-nine accessions of cultivated holy basil (*O. tenuiflorum*) from four phyto-geographical regions of India and analysed 18 qualitative and 14 quantitative characters utilizing minimal descriptors created by the NBPGR. Analysis showed high level of variation showing high variability in the populations from various phytogeographical regions and relatedness among the morphotypes. Principal component analysis showed that leaf length and width, plant height, petiole length, number of primary branches and leaf weight contributed maximum to the first principal component.

Patel *et al*. (2015a) had studied the morphological difference of five *Ocimum* species *viz*., *O. tenuiflorum*, *O. americanum*, *O. basilicum*, *O. gratissimum* and *O.* ×

citriodorum using their seed characteristics. The study showed that in case of *O. americanum*, *O. basilicum* and *O.* × *citriodorum* ellipsoid shape and black colour seed was observed. However, *O. gratissimum* and *O. tenuiflorum* had subglobose to broadly ellipsoid and brown to yellow colour seeds.

2.7.2. Chemical components of fixed oil

Fixed oils are generally non-volatile, mixture of natural animal or plant oils that contains esters of higher fatty acids, glycerin usually triglycerides. Fixed oil also called as fatty acid. Fixed oils do not evaporate at room temperature. Seeds of *Ocimum* species are good sources of fixed oils. It has been reported that various factors are responsible for chemical components of essential as well as fixed oil. These factors may include geographical origin, seasonal and maturity variation, developmental stages of plants, time of harvesting, genetic variation, plant parts utilized, storage and process of extraction method (Marotti *et al*., 1996; Anwar *et al*., 2005; Bassole *et al*., 2005; Hussain *et al*., 2008) and light (Rakic and Johnson, 2002; Fernandes *et al*., 2013).

Different parts of the *Ocimum* sp. were utilized as experimental tool to study the fatty acid components. The seed oil of *O. kilimandscharicum* contained linoleic, α-linolenic and oleic acid as the main compound (Henry and Grindley, 1944). Xaasan *et al*. (1980) reported presence of oleanolic and ursolic acids in the leaves and flowers of *O. canum*. It has also been reported that arachidonic acid was identified as unsaturated fatty acid from the leaves of *O. gratissimum* grown in Nigeria (Onajobi, 1986). Moreover, a relatively high concentration of protein, carbohydrates, vitamins A, C, rosmarinic acid and xanthomicrol were present in the dried leaves and flowers of *O. basilicum* (Leung and Foster, 1996).

Angers *et al*. (1996) studied the fatty acid variation in seed extracted oil of different *Ocimum* species (*O. basilicum, O. canum, O. gratissimum,* and *O. sanctum*). The result revealed that linolenic, linoleic, oleic and palmitic acid were identified as major fatty acids. It was also found that linolenic acid was maximum in *O. canum* and lowest in *O. sanctum*. Similar result was obtained from the seed oil of *O. basilicum* (Prakash and Gupta, 2000; Azhari *et al*., 2009). Linoleic and α-linolenic acid were major fatty acid obtained from the cold pressing seed of *O. basilicum* (Domokos *et al*., 1993). A previous report showed that instead of α-linolenic acid, linoleic acid has been found to be the major compound in the fixed seed oil of *O. canum* and *O. pilosum* (Khan *et al*., 1961).

Singh and Majumdar (1997) reported fixed oil of *O. sanctum* contains five fatty acids (stearic, palmitic, oleic, linoleic and linolenic acids). The fixed oil of *O. sanctum* seeds is associated with a high quantity of linoleic acid and linolenic acid. Palmitic acid, oleic acid and stearic acid also found as fatty acid (Malik *et al*., 1987; Sethy and Kaur, 2014). Recently, effort was made to extract and evaluate the fixed oils from the seed of *O. sanctum* and *basilicum* (Kadam *et al*., 2012). The study opens up a new perspective in using fixed oil as base in designing new oil base formulations.

There is a widespread variation in chemical content of basil essential oils as well as fixed oils within the same species and their morphotypes. It was found that monoterpenes and phenyl propanoids always predominate in essential oils of basil (Marotti *et al*., 1996). Several research findings showed the variety in the yield and substance constituents of the essential oil concerning geographical locations and farming practices (Javanmardi *et al*., 2002; Bowes and Zheljazkov, 2004; Anwar *et al*., 2005; Van Vuuren *et al*., 2007; Patel *et al*., 2015b).

Purkayastha and Nath (2006) have been reported camphor, limonene and β-selinene as the major components in *O. basilicum* essential oils from North East India. Recently, Ghasemi Pirbalouti (2014) studied the important constituents of essential oil in *O. basilicum.* Methyl chavicol/estragol and linalool were found as major components.

Eugenol is the major constituent of essential oil in *O. tenuiflorum* grown in various parts of the globe (Jorge *et al*., 1998; Brophy *et al*., 1993; Sharma *et al*., 2014). However, Raina *et al*. (2013) identified **e**ugenol, methyl eugenol, β-caryophyllene, β-elemene as major chemical compositions. Similar results also reported earlier (Maheshwari *et al*., 1987; Kothari *et al*., 2005; Padalia and Verma 2011). On the other hand, Joshi and Hoti (2014) reported methyl eugenol as major compound.

Rana and Blazquez (2015) studied the essential oils composition of five *Ocimum species* from Western parts of India. Results showed that highest amount of eugenol, germacrene D and *cis*-ocimene were found in *Ocimum viride*. In *O. tenuiflorum* green and *O. tenuiflorum* purple contain eugenol, β-elemene and β-caryophyllene as main compounds. In addition that methyleugenol was also found as major compound in *O. tenuiflorum* purple. Camphor, β-selinene, α-selinene, maaliol, β-caryophyllene, β-gurgunene were main compounds in *O. kalimanduscharicum*. Similarly, *O. basilicum* was found to contain methyl chavicol, *trans*-methyl cinnamaldehyde and linalool while *O. gratissimum* contains *trans*-methyl isoeugenol, *cis*-ocimene, germacrene-D and β-caryophyllene as major compounds. Most recently, Verma *et al*. (2016) reported characteristic composition of the essential oils in four *Ocimum* spp. from peninsular India. The results revealed that *O. gratissimum* contain higher amounts of eugenol, caryophyllene oxide and (*Z*)-*β*-ocimene. Major constituents of *O. tenuiflorum* were methyl eugenol, caryophyllene oxide and (*E*)-caryophyllene. In *O. americanum* camphor was predominating constituent.

In *Ocimum*, the composition of essential oil varied considerably as because of stages of harvesting and seasonal variation. Significant variations in essential oil compositions of *O.basilicum* were found to be influenced by the seasonal factors (Da-Silva *et al*., 2003). The most important constituents of essential oils i.e. eugenol and linalool varied considerably in four basil varieties (*O. basilicum* var. *odoratus*, *O. basilicum* var. *alba*, *O. basilicum* var. *thyrsiflorum* and *O. basilicum* var. *purpurascens*) cultivated under different seasons (Said-Al Ahl *et al*., 2015). More recently, Saharkhiz and his associates (2015) concentrated the variation in chemical composition of the essential oils of *O. sanctum* at various stages of harvesting. The study showed that the main compound of oils was eugenol at all the developmental stages but the concentration was high at floral budding and full flowering stages. Similarly, Sims *et al*. (2014) reported the amount of eugenol increase or decrease that depends on harvesting times.

The essential oil composition variation was found on plant parts used. Iwalokun *et al*. (2001) reported *O. gratissimum* seeds contain thymol and eugenol. Whereas, Keita *et al*. (2000) reported thymol, p-cymeme and γ terpene + trans-sabiene hydrate. Machado *et al*. (1999) reported **e**ugenol is the main volatile constituents of leaves and flowers of

O. tenuiflorum. Javanmardi *et al*. (2002) reported rosmarinic acid as the principal phenolic acid present in flowers and leaves of *O. basilicum.* Chalchat and Ozcan (2008) reported the chemical composition of leaves, stems and flowers of *O. basilicum.* The main constituents of leaves, stems and flowers oils were estragole, limonene and p-cymene. Lawal *et al*. (2014) had shown the chemical variation of different parts of *O. kilimandscharicum*. The major chemical components of the leaves of *O. kilimandscharicum* were methyl eugenol and γ-cadinene while flowers contained methyl eugenol, borneol and linalool.

Method of extraction and use of solvents also affect the chemical composition of *Ocimum*. Several workers previously reported the effects of different methods and solvent used for oil extraction (Vani *et al*., 2009; Khair-ul-Bariyah *et al*., 2012; Chenni *et al*., 2016). Chemical compositions of *O. basilicum* by using different solvent were studied by Dev *et al.* (2011). Result showed that 2-pentanone and caryophyllene oxide obtain from N-hexane; 1, 2-dimethoxy-4-(2-propynel)-benzene from ethyl acetate; 1, 2-benzene dicarboxylic acid from ethyl acetate and eugenol from chloroform. Eugenol and caryophyllene were identified as major compound in hydroalcoholic extract of *O. sanctum* (Devendran and Balasubramanian, 2011). Behera *et al*., (2012) reported flavonoids, terpenoids and reducing sugars from ethanol and water extracts of *O. canum* leaves. The results showed that tannins were present in the ethanolic extract but absent in the water extract. Similarly, Shobo *et al*. (2015) studied the phytochemical screening of methanol extract of *O. canum* leaves and showed the occurrence of alkaloids, cardiac glycosides, saponins, flavonoids and terpenoids.

A classification system of standardized descriptors based on volatile oil has proposed by Lawrence (1992) and Grayer *et al*. (1996). Lawrence (1988, 1989) classified four major chemotypes of basil based on essential oils *viz*., (1) methylchavicol-rich, (2) linalool-rich, (3) methyleugenol-rich and (4) methyl cinnamate-rich. Previously Vernin *et al*. (1984) also reported the same chemotypes. Grayer *et al*. (1996) described that the major components being geranial and neral in *O*. x *citriodorum* while linalool, methyl chavicol, eugenol, methyl eugenol and geraniol in *O. basilicum.* Recently, Carovic-Stanko *et al*. (2011b) found geranial/neral two distinct chemotypes in *O. africanum* and *O. americanum* accessions and estragol chemotype in *O. basilicum.*

Masi *et al*. (2006) grouped the basil cultivars into five chemotypes on the basis of relative predominance components present in essential oils, such as i) linalool > estragole > eugenol, ii) estragole > linalool > eugenol, iii) linalool > eugenol > estragole, iv) linalool > methyl cinamate > estragole and v) citral > linalool type. Telci and his associates (2006) identified seven distinct chemotypes in 18 basil landraces from Turkey. Each chemotype comprises the major volatile compound as linalool, methyl cinnamate, methyl cinnamate/linalool, methyl eugenol, citral, methyl chavicol (estragole) and methyl chavicol/citral. Similar results were also observed by Zheljazkov *et al*. (2008) and recently Verma *et al*. (2013) also describe seven chemotypes in 34 *Ocimum* taxa growing in foot and mid-hills of northern India**.** Linalool was found as dominant constituent of essential oils in nine basil accessions from Italy (Labra *et al*., 2004). Keita *et al*. (2000) reported that the oil of *O. basilicum* contained linalool, eugenol, (E)-α- bergamotene and thymol.

In North East India, three chemotypes were characterized by high content of camphor, methyl chavicol and linalool from *O. basilicum*. In addition to that, methyl cinnamate has also been reported from *O. basilicum* (Saikia and Nath, 2003; Purkayastha and Nath, 2006). These chemotypes were distinctive one from another according to epidermal cells and stomata (Barua and Nath, 2000).

Chemotypes described in *O. gratissimum* are eugenol, thymol (Jirovetz *et al*., 2003; Tchoumbougnang *et al*., 2006; Dambolena *et al*., 2010; Verma *et al*., 2013) and geraniol (Charles and Simon, 1992) types. Vieira *et al*. (2001) had divided *O. gratissimum* into six groups based on volatile oil constituents, (1) thymol, α-copaene, (2) eugenol, spathulenol, (3) thymol, p-cymene, (4) eugenol, γ-muurolene, (5) eugenol, thymol, spathulenol and (6) geraniol. However, chemotypes described by Singh *et al*. (2013) in his study variation in essential oil composition of *O. americanum* L. from North-Western Himalayan region and classified the species into six groups. I- methyl chavicol, 1, 8-cineole, (E)-γ-bisabolene, β-bisabolene and eugenol; II- (E)-γ-bisabolene, aliphatic hydrocarbons, eugenol, β-bisabolene and methyl chavicol; III- Eugenol and (E)-caryophyllene; IV- linalool with methyl chavicol; V- contained aliphatic hydrocarbons, eugenol, camphor and 1, 8-cineole and group VI- contain camphor and aliphatic hydrocarbons as major components. Previously chemotypes describe in *O. americanum* by Sarin *et al*. (1992).

More recently, Sims and his associates (2014) reported eugenol and β-caryophyllene rich two chemotypes of *O. tenuiflorum*. Chemotypes reported for *O. tenuiflorum* essential oils were eugenol (Mondello *et al*., 2002; Verma *et al*., 3013), methyl eugenol (Jirovetz *et al*., 2003; Kothari *et al*., 2005), methylchavicol (Brophy *et al*., 1993) and sesquiterpene (Simon *et al*., 1990; Verma *et al*., 3013) types.

2.7.3. Genetic variation

Now a days, the use of molecular markers to evaluate genetic variability has become an important tool in the study of genetic variation of different medicinal and aromatic plant species (Liu *et al*., 2006; Fracaro and Echeverrigaray, 2006; Agostini *et al*., 2008).

In general, three kinds of markers such as morphological, chemical and DNA markers are taken into account for the study of diversity. Apart from the construction of linkage maps, DNA markers have numerous applications in plant breeding such as assessing the level of genetic diversity within germplasm and identification of cultivars (Winter and Kahl, 1995; Baird *et al*., 1997; Henry, 1997). In recent years hybridization-based (RFLP), PCR-based (RAPD, AFLP, ISSR, SSR etc.) and sequencing based molecular techniques (ITS) are used for detection and proper identification of species or cultivars (Jones *et al*., 1997; Gupta *et al*., 1999; Joshi *et al*., 1999; Sharma *et al*., 2008). Most widely used molecular markers are RAPD (Random Amplified Polymorphic DNA), RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism), ISSR (Inter Simple Sequence Repeats), SSRs (Simple Sequence Repeats), VNTRs (Variable Number of Tandem Repeats) etc. which are not influenced by environment factors and stages of plant development (Winter and Kahl, 1995; Harisaranraj *et al*., 2008; Patel *et al*., 2015c).

Introduction of molecular technique has opened up a new avenues and opportunities in the diversified field of research and scientific investigations. Application of molecular markers especially RAPD is used to study the taxonomic and genetic diversity of various species. After the development of Polymerase Chain Reaction (PCR), a more economic class of markers namely RAPD was introduced (Williams *et al*., 1990). RAPD is the most extensively used technique for genetic diversity study in plants especially in medicinal and aromatic plants including the genus *Ocimum* due to low cost, time saving, easy to handle, sequence information not required, comparative analysis are rapid, cover large genome area and gives high level of polymorphism (Metz and Palumbi, 1996; Singh *et al*., 2004).

Vieira *et al*. (2001) used RAPD marker to characterize genetic diversity among 12 tree *O. gratissimum* accessions. Further, Vieira *et al*. (2003) used RAPD marker to evaluate the genetic diversity of different *Ocimum* species. The results shown that *O. minimum*, *O. basilicum* and *O. × citriodorum* had highest similarity indices within the species, while *O. americanum*, *O. gratissimum* and *O. kilimandscharicum* showed lowest similarity. RAPD results indicated that *O. minimum* should not be considered as a separate species but to a variety of *O. basilicum*. Genetic relationship was assessed among thirty germplasm accessions belonging to five *Ocimum* species using RAPD markers by Singh *et al*. (2004). The results grouped five *Ocimum* species into two major clusters. Cluster-I included *O. basilicum, O. americanum* and *O. kilimandscharicum*, while cluster-II included accessions belonging to *O. tenuiflorum* and *O. gratissimum.* The results supported the classical taxonomy of *Ocimum* species where *Ocimum* classified into two groups *viz*., Basilicum and Sanctum group.

Carovic-Stanko and his associates (2007) investigated the intra and interspecific genetic relationship of different *Ocimum* species and six varieties of *O. basilicum* in combination of RAPD and AFLP molecular markers. A very strong relationship was observed between dice distance matrices based on RAPD and AFLP data. High bootstrap support values for the branches separating *O. tenuiflorum* and *O. gratissimum* accessions. A cluster was containing *O. americanum* and *O. × citriodorum* and another one containing *O. basilicum* and *O. minimum.* The results of the both molecular markers were showed a very good representation of classical taxonomy. Within the *O. basilicum* cluster, similar accessions grouped together specifically *O. minimum*, *O. basilicum* 'Dark Opal' and *O. basilicum* var. *difforme* accessions.

RAPD markers were used to assess the inter-species relationships of different *Ocimum* species from India (Harisaranraj *et al*., 2008). Results showed that *O. tenuiflorum* has very closely similar (89%) with *O. basilicum* and other two species of *O. gratissimum* and *O. micranthum*. Saha *et al*. (2008) investigated the genetic diversity of three *Ocimum* species through RAPD markers. The constructed dendrogram showed two groups of cluster in the three *Ocimum* species on the basis of RAPD analysis. *O. canum* and *O. basilicum* species are grouped together, while *O*. *gratissimum* species on a separate grouped.

Sairkar *et al*. (2012) studied the genetic variability and phylogenetic relationship among eight *Ocimum* species through RAPD. The results showed high degree of genetic distinction among different *Ocimum* species. *O. gratissimum* has very close similarity with *O. sanctum* var. black whereas a least similarity was showed with *O. bassilicum* and *O. sanctum* var. green. The genetic diversity of nine accessions of *O. sanctum* (Krishna Tulsi) has been reported from different parts of Madhya Pradesh, India by using RAPD marker (Tilwari *et al*., 2013). Similarly, Chikkaswamy *et al*. (2013) studied the genetic relationships among six *Ocimum* species through RAPD marker.

Bernhardt *et al*. (2014) characterized eight *Ocimum basilicum* accessions using morphology, essential oil composition and RAPD molecular markers. Based on RAPD-PCR analysis the accessions formed two clusters. In group A the purple-leaved accessions (‘Dark Opal’, ‘Piros’) were situated and the remaining ones (‘Genovese’, ‘Arvada’, ‘Lengyel’, ‘Rit-Sat’, ‘Mittelgrobbla¨ ttriger Gru¨ nes’, ‘A-10) were in group B. According to Giachino *et al*. (2014) Turkish basil (*Ocimum basilicum* L.) may be grouped into two main clusters. Sundaram and his associates (2014) investigated the diversity of 20 accessions of three *Ocimum* species. (*O. gratissimum*, *O. basilicum* and *O. sanctum*) gathered from various parts of India. The study showed interesting findings and proved to be a bidirectional evolution in *Ocimum* species. They also suggested that RAPD markers may be utilized in the systematics of wild and cultivated taxa. Similarly, nine Omanian landraces of common basil (*Ocimum basilicum* L.) were assessed using RAPD to know the genetic diversity by Al-Maskri *et al*. (2013).

Patel and his associates (2015c) have made an attempt to characterize 17 *Ocimum* genotypes belonging to five species (*O. basilicum*, *O. americanum*, *O. sanctum*, *O.*

gratissimum and *O. Polystachyon*) through RAPD and ISSR markers. The results showed numerous distinct species specific alleles which were amplified by ISSR and RAPD markers. In both systems of marker, highest number of unique allele was observed in *O. sanctum.* The results of the investigation provided a valid guideline for collection, characterization and conservation of *Ocimum*.

2.8. Propagation and conservation of *Ocimum*

Ocimum species are grown naturally from a wide range of habitat and agro-climatic condition. Destruction of natural habit, rapid change and adverse effect of climatic conditions causes threat to the different *Ocimum* species. As a result, conservation is urgently needed to ensure their availability to the pharmaceutical/cosmetic industry as well as traditional system of medicine. If timely steps are not taken for their conservation by means of cultivation and mass propagation, they may be lost from the natural vegetation forever.

Conservation of any plant genetic resources involves mainly two basic strategies *viz.*, *in-situ* and *ex-situ*. The most commonly *in-situ* conservation methods are national parks, biosphere reserves, on-farm system, home gardens etc. Seed bank, gene bank, cryopreservation, botanical garden, *in vitro* (tissue culture) technology etc. are the means of *ex-situ* conservation. In India three national gene banks have been established for *ex-situ* conservation of medicinal and aromatic plants i.e. (i) National Bureau of Plants Genetic Resources (NBPGR), New Delhi, (ii) Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow and (iii) Tropical Botanical Gardens Research Institute (TBGRI), Thiruvananthapuram.

Similarly other medicinal and aromatic plants, *Ocimum* species may be propagated through seeds and cuttings for *ex-situ* conservation in herbal garden (Patel, 2015). Seed germination is season specific. Conservation of pure parental seed is very much essential to maintain the purity of cultivars which requires specific skills and also more time.

A research has been carried out to know the germination potential in different *Ocimum* species at various environmental conditions (Gupta and Shahi, 1998). It has been found that in *O. basilicum* the percentage of seed germination (86-90%) was optimum at 20-30 °C with70% – 80% relative humidity (Mijani *et al*., 2013), 25 °C (Kumar, 2012), 13 – 30 °C, > 80% after 4 days (Putievasky, 1983). 20 °C to 45 °C (Gupta and Shahi, 1998), and 15 °C, 25 °C and 35 °C (Ramin, 2006). It can be germinated up to 40.7 °C and optimum growth of seedlings was observed at 30 °C (Mijani *et al*., 2013). On the other hand 25 °C temperature was found to ideal for *Ocimum gratissimum* seed germination (Obaremi *et al*., 2002).

Seedlings cannot be raised throughout the year due to its dormancy. Several studies have been made related to breaking dormancy of *Ocimum* species. Different treatments are used for breaking the dormancy like scarification, hot water treatment, cold stratification and use of various growth regulators and chemical (KNO_3, Thiourea, GA3, KCl, H_2O_2 salts etc.) (Gupta, 2002, 2003).

Although, there are several biotechnological methods like plant tissue culture is being used as mass multiplication as well as conservational purposes (Pattnaik *et al*., 1995; Dode *et al*., 2003; Gopi *et al*., 2006; Saha *et al*., 2010; Janarthanam and Sumathi, 2012; Tripathi *et al*., 2014; Mishra, 2015; Chaturvedi and Patra, 2015), but still some reports are available on medicinal and aromatic plants conservation by means of vegetative propagation (Hartmann and Kester, 1983; Butola and Butola, 2007; Vashistha *et al*., 2009). Schopp-Guth and Fremuth (2001) and Hamilton (2004) reported sustainable uses and natural conservation of some important medicinal and aromatic plants.

Mass multiplication through stem cutting is a cost effective and very common method of propagation. Sulistiarine (1999) reported that *O. gratissimum* is conventionally propagated by the seed germination and stem cutting. The problem related with conventional method of propagation is that very poor germination percentage of seeds (<10%) and 28 days required for the rooting from stem cuttings (Saha *et al*., 2012, 2014b). On the other hand Pattnaik and Chand (1996) reported that *Ocimum* species cannot be propagating through vegetative. Acharjee *et al*. (2015) carried out a study on propagation through stem cuttings and effect of biofertilizers on growth of *Ocimum kilimandscharicum*. Similarly, a study has been done on stem cutting propagation for ex-situ conservation of *Stevia rebaudiana* in herbal garden (Patel, 2015).