Ethnobotany of Dakshin Dinajpur district with special reference to diversity and conservation of *Ocimum* species

Thesis submitted to the University of North Bengal for the Award of the degree of Doctor of Philosophy in Botany

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April 2017

In the memory
of my Mother

DECLARATION

I declare that the thesis entitled **"Ethnobotany of Dakshin Dinajpur district with special reference to diversity and conservation of *Ocimum* species"** has been prepared by me under the joint supervision of Dr. Dilip De Sarker, Associate Professor (Retired), Department of Botany, Raiganj College (University College) (Now Raiganj University) and Dr. Subhas Chandra Roy, Associate Professor, Department of Botany, University of North Bengal. No part of this thesis has formed the basis of any previously awarded degree or fellowship.

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Abstract

The thesis entitled "**Ethnobotany of Dakshin Dinajpur district with special reference to diversity and conservation of *Ocimum* species**" has been dealt with the available species and varieties of *Ocimum* L. in the district of Dakshin Dinajpur (Eight blocks) of the state of West Bengal. Geographically, this district is located in the eastern part of India, bordering Bangladesh and with even topography. Ethnography shows that the area under study is inhabited by a mostly poor people belonging to tribal and non-tribal communities and depends on agriculture.

The genus *Ocimum* L. is one of the most successful genus of the entire dicotyledons having worldwide distribution. Most interestingly, this genus includes culinary basil (*O. basilicum*) and holy basil (*O. tenuiflorum*, Syn. *O. sanctum*). Earliest mention of use of holy basil can be traced back to the time of Vedic people. There is distinct mention of medicinal uses of holy basil in *Charaka Samhita* (Ray and Gupta, 1965). Several species of the genus *Ocimum* have been shown to possess spectacular medicinal property for treatment against viruses, bacteria, fungi insect larvae and insects.

The ethnobotanical study adopted here was based on works of Jain (1987) and Jain and Mudgal (1999). A set of well thought questionnaires were prepared and following one to one interaction with the locally renowned Kabiraj/Baidaya, these sheets were filled up. The interaction focused on method of preparation of herbal formulations, mode of application and plant/plant parts used. Plants were collected from the primary sources, preserved and identified by appropriate authority.

A total of nine taxa of *Ocimum* were identified from the study area which included in the present study. These were *viz*., two morphotypes of *O. tenuiflorum* L. (purple and green type), two morphotypes of *O. basilicum* L. (Babu and Marua tulsi), two morphotypes of *O. gratissimum* L. (Ram and Ajowan tulsi) and one taxa each of *O.* × *africanum* Lour., *O. americanum* L. and *O. kilimandscharicum* Guerke. These

species/morphotypes were authenticated by the technical support at Acharya Jagadish Chandra Bose Indian Botanic Garden, Sibpur, Howrah, West Bengal.

Quadrat studies were applied to elucidate the detailed ecological status and micro-composition of the nine *Ocimum* taxa. Standard ecological parameters like density, relative density, frequency, relative frequency, abundance and value index were analysed adopting standard procedure (Cottom and Curtis, 1956).

Soil is the most important factor for predicting the compositional behaviour of the elements in ecosystem. Hence, soil parameters such as soil pH, electrical conductivity, organic carbon, available nitrogen, available phosphorus and available potassium were analysed following standard procedure (Jackson, 1967; Bower *et al*., 1952; Jackson, 1967; Subbiah and Asija, 1956; Watanbe and Olsen, 1965; Jackson, 1958).

Morphological investigations were carried out during reproductive period of the materials included in the study. A total of thirty five parameters were analysed based on the standard descriptor developed by NBPGR (Singh *et al*., 2003).

The genus *Ocimum* is renowned for presence of several volatile and non-volatile oils. To get insight knowledge on the available oils and their compositional variations were analysed applying gas chromatography/mass spectrometry (GC-MS) at Indian Institute of Science (IISc) Bangalore in conformity with standard laboratory procedure.

Easy crossability brought a difficult task for the taxonomist in describing a species in the genus *Ocimum*. Genetic variation study based on RAPD fingerprinting was included in the present study to get a detailed insight of genetic construction of the taxa included in the present study. Agarose gel electrophoresis of RAPD-PCR band profiling has been done and analysed.

Rapid urbanization and population density poses a threat on habitat vegetation. Hence, propagation and conservation methods and development of suitable techniques were included in the present study. For rapid propagation seed germination and stem cutting techniques were developed for future conservation of *Ocimum* species.

The present investigation clearly recorded the rich heritage of ethnobotanical knowledge in this district. There were 138 angiospermic species belonging to 121 genera and 65 families recorded to be used by the local people of various caste and religious communities. As regard to the mostly used plants families were Lamiaceae

followed by asteraceae, papilionaceae, euphorbiaceae, acanthaceae, asclepiadaceae, cucurbitaceae and verbenaceae. Other angiospermic families less commonly used were – anacardiaceae, convolvulaceae, liliaceae, malvaceae, mimosaceae, myrtaceae, orchidaceae, oxalidaceae, piperaceae, sterculiaceae and vitaceae. There were other 37 families where only one plant was used for at least different categories of health disorder and/or health enhancer. Almost all plant parts in conjunction with/or alone plant exudates have been found useful.

The quadrat study indicated that *Ocimum* species grow well in association with dicotyledons than monocotyledons. Association of growth was significant between *O. americanum* with *O.* × *africanum*. Interestingly, it was found that allergenic plant *Parthenium hysterophorus* an exotic species growth was very poor in the community of *Ocimum* species.

The physio-chemical study of soil showed that the pH varies between 5.7 to 6.3. The organic carbon (%) ranges from 0.52 to 0.82, the available nitrogen ranges from 223-353 (kg/ha) available phosphorus ranges from 60-70 (kg/ha) and available potassium ranges from 214-400 (kg/ha).

Qualitative traits study showed high level of variability with respect to almost all traits (in the present study) among the taxa studied. Johnson (1998) applied principal component analysis (PCA) to screen most correlation between the traits. The study revealed the presence of four distinct groups of species based on qualitative and quantitative analysis.

Chemical compositional analysis has been done from the ethanolic extracts of nine *Ocimum* taxa. Total seventy three compounds were identified of which 12 were aliphatic acids, 3 aliphatic alcohols, 7 amino acids, 2 aromatic compounds, one fused ring aromatic hydrocarbon, 23 carbohydrates, 5 phenolic compounds, one quinone, 3 steroids, 12 terpenoids, vitamin E and 3 unidentified compounds. The study also revealed that there were significant differences among the *Ocimum* taxa with respect to chemical component and their composition.

As regards to RAPD analysis, a total of 17 RAPD primers were used to delineate the inter and itraspecific genetic diversity among the *Ocimum* species and results were analyzed and a dendrogram was constructed. There were definitely two main clusters.

The first cluster contained two close taxa of *O. gratissimum*, two close taxa of *O. tenuiflorum* and separately *O. kilimandschericum*. The second cluster housed *O. americanum*, *O.* x *africanum* and two taxa of *O. basilicum.*

Studies relating to germination of seeds showed that *Ocimum* taxa germination was prolific on day three and on day five germination percentage score up to 92% during summer, vis-a-vis during winter it came to as low as 26%. It is suggested that propagation of *Ocimum* may be done through stem cutting during summer or winter. This low cost method of propagation can be exploited for conservation of the *Ocimum* taxa.

Overall contributions of this present work are

1. Documentation of 138 plants/plant parts for ethnomedicinal use especially popular among low economic marginal people of Dakshin Dinajpur. 57 ethnobotanical uses have been newly documented from this district.

2. Factor for optimum growth and plant association of *Ocimum* species was revealed.

3. Low cost technique for rapid propagation for *Ocimum* species was elucidated.

4. Chemical analysis discovered some un-identified compound and demands further study and this application in human welfare.

5. DNA analysis suggested the exact position of *O.* x *africanum* in the taxonomic hierarchy and recorded for the first time from this district as well as from West Bengal.

6. Nine taxa of *Ocimum* belonging to six species have been found in regular use by people of various economic groups as folk medicine. The uses include mosquito repellent, antidote to insect bite, in headache, in gonorrhoea, in rheumatism and in various infections. Thus this work opens the avenue of research and application in Ayurveda, Siddha, Unani and other related domain of human welfare.

Preface

The present work is an outcome of my five years of research experience that has been carried out from 2012, since I was enrolled as a Ph.D. scholar in the Plant Genetics and Molecular Breeding Laboratory, Department of Botany, University of North Bengal. The study has been carried out by the author in the Department of Botany and Sericulture, Raiganj University, Raiganj, Uttar Dinajpur and being submitted for the partial fulfilment of Ph. D. in Science (Botany) under University of North Bengal, Derjeeling, West Bengal, India. These years have been a challenging journey with ups and downs. It would not have been possible for me to write this thesis without the help and support of the kind people around me. The study clearly describes the ethonobotanical uses, natural habit and habitat and community study of all *Ocimum* taxa available in the district of Dakshin Dinajpur. The diversity analysis was carried out in terms of morphological, chemical and through RAPD. This huge diversity of *Ocimum* taxa motivated me to study the details along with ethnomedicinal study. The final aim of the present study is to find scientific ways for the conservation of native *Ocimum* taxa realising the present state of ecology.

I am deeply indebted to my Supervisor, Dr. Dilip De Sarker, Associate Professor (Retired), Department of Botany, Raiganj College (University College) (Now Raiganj University), and Co-Supervisor, Dr. Subhas Chandra Roy, Plant Genetics and Molecular Breeding Laboratory, Department of Botany, University of North Bengal (UNB) for their keen interest, valuable and unconditional help, constant guidance and wise counseling extended by them right from the selection of the topic to successful completion of my entire research. Their meticulous scientific knowledge, sincerity and genuine help guided me a lot despite of their busy academic and professional commitments. This thesis would not have been completed without their valuable input. I sincerely express my gratitude to the HOD (present and past), Department of Botany, UNB and all the faculty members/staffs of the department for providing me various helps during the course of my research. I express my deep sense of gratitude to respected Prof. Anil Bhuimali, Vice-Chancellor, Raiganj University for evincing keen interest in the subject and providing me necessary research facilities. During the years of my research work I had to take help from various other pioneer research institutions

of India namely Indian Institute of Science (IISc.), Bangaluru for GC-MS analyses; Botanical Survey of India (BSI), Kolkata for identification of *Ocimum* species and their morphotypes. My special thanks go to Professor P. D. Ghosh of the Cytogenetics and Plant Breeding Section of the Department of Botany, Kalyani University for molecular study. I thank Sri Subhasis Mali, WBAS (Research), Assistant Agricultural Chemist, Govt. of West Bengal for providing me lab facilities during soil analysis. The Additional Director of Agriculture, North Bengal Regional Office, Jalpaiguri, Govt. of West Bengal is sincerely acknowledged for providing necessary meteorological data.

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I do not have enough words to thank my parents and family, who are always in my emotions, and silently give me strength to overcome many difficulties in my life. I am glad to pay my words fail thanks to my beloved wife Deboshree for her endless sacrifice, which are the essence of my success. I apologize to my little angel Nishka for not being able to give her enough time and attention whenever she needed. Last but not the least, I seek blessing from my mother late Rikta Chowdhury for the successful completion of my research work and pray the providence to bestow the mental strength as to overcome all hindrance in future.

Tanmay Chowdhury

Abbreviation

ADA	Additional director of agriculture
AHC	Agglomerative hierarchical cluster
AMDIS	Automated mass spectral deconvolution and identification system
BSI	Botanical Survey of India
BSTFA	Bis (Trimethylsilyl) Trifluoroacetamide
CTAB	Cetyl trimethylammonium bromide
DNase	Deoxyribonuclease
dNTPs	Deoxynucleotide triphosphates
EC	Electrical conductivity
EDTA	Ethylene diamine tetra acetic acid
EtBr	Ethidium bromide
FAS	Ferrous ammonium sulphate
GC-MS	Gas Chromatography Mass Spectrometry
IVI	Importance Value Index
M	Molar
mL	Millilitre
mm	Millimetre
mM	Milli mole
MS	Mass spectra
MSD	Mass selective detector
NBPGR	National Bureau of Plant Genetic Resources
NIST	National Institute Standard and Technology
NTsys-PC	Numerical Taxonomy System for personal computer
PCA	Principal Component Analysis
PCoA	Principal Coordinates Analysis
PCR	Polymerase chain reaction
pH	Potential of hydrogen
PIC	Polymorphic information content
ppm	Parts per million
PVP	Poly vinyl- pyrollidone
RD	Relative Density
RF	Relative Frequency
RNase A	Ribonuclease A
rpm	Rotation per minute
SAHN	Sequential Agglomerative Hierarchical and Nested algorithm
SUs	Sampling units
TAE	Tris-acetate-EDTA
Taq DNA	Thermus aquaticus DNA polymerase
TE buffer	Tris-EDTA buffer
TMCS	Trimethylchlorosilane
Tris Hcl	Tris hydrochloric acid
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
µg	Microgram
µL	Microlitre
µm	Micrometer

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Introduction

The records of conscious use of plants other than food by human races have been obliterated in remote historical past. When the "Vedas" appeared in written form, the use of plants appeared to be the first documentary evidence as curative agent. The subject ethnobotany gain importance in the beginning of 20th century. In those days it was thought that the knowledge of plants of different ethnic people is the main bowl of ethnobotany. Those people had no written alphabet. It was further conceived that discourses about the uses of plants by these aborigines and ethnic people is the ethnobotany. But the area of ethnobotany is deeper and wider. The whole human races consisting of all sub races, castes groups and within direct or indirect uses of plants and their research investigation, inquiry and discussions is the subject of ethnobotany. These discussions may be aesthetic, utilitarian or historical.

In the year 1895, on 4th December, Dr. John William Harshberger, a professor of botany at Pennsylvania University used the term "Ethnobotany" while delivering a lecture on archaeology. In 1896, this paper was published in Philadelphia Evening Telegraph with a title "some new ideas". That was the point of time when ethnobotany came up to us as a branch of science.

Rural India relies on local traditional medical practitioner for various ailments. Such treatments are ill organised. Some of the local medical practitioners called as 'Kabiraj'/ 'Baidya' – who uses their experiences and inherited knowledge of medicinal plants from several sources – mostly from family knowledge. Tribal healers often use medical knowledge derived from earlier generations – which is quite different from Āyurvedic medical knowledge. Thus there is distinct knowledge – dichotomy between Āyurvedic knowledge and knowledge of medicinal plants and their uses by tribals of various categories. *Āyurveda* means – the knowledge (*Veda*) of the life span (*Ayus*). It teaches how one may utilize the span of life activity by nature. In other terms *Āyurveda*, an ancient science of life is deeply rooted in Indian culture. Most commonly *Āyurveda* has been applied related to medical matters and thus it is justified to say it as medicine

provided to the various ailments. Meulenbeld (1990) observed, "The classical treatises on *Āyurveda*" clearly states that this science can be applied to all living organism.

The *Charak Samhita* begins with the verse –

Athato dirghajiviyiyamadhyam vyakhyasyamah// (Charak Samhita, Su, 1.1)

The English translation is like this:-

"So then we shall explain the lesion about longevity". It transpires from this verse and etymologically *Āyurveda* signifies the knowledge of longevity. The Indian indigenous system of medical treatment has a long history of about 3000 years where "Vedic" people had expertise for utilizing local plants. The ancient system of treatment could be distinctly divided into medicine (*Charak Samhita* – 1000 BC-100 AD) and Surgery (*Sushrata Samhita* – 800-700 BC). However, this system of medical practice confronted with the arrival of colonial medicine. Today's medical treatments are almost the contribution of western medicine knowledge. Ancient medicine was not solely based on empiricism and this is evident from the fact that some medicinal plants which were used in ancient times still have their place in modern therapy (Das and Mondal, 2012). Herbal medicine is still the mainstay of about 70 – 80% of the Indian population and the major part of the traditional therapy involves the use of plant extract and their active constituents (Akerele, 1993). A significant number of wild plants and the purified extracts have shown beneficial therapeutic potentials owing to presence of phenolics, flavonoids, alkaloids, antioxidant compounds etc. There is extensive evidence to implicate free radicals in the development of degenerative diseases. Almost all organisms possess antioxidant defenses that protect against oxidative damage and numerous damage removal and repair enzymes to remove or repair damage molecules.

The genus *Ocimum* of the family Lamiaceae consists of aromatic perennial or annual herbs or undershrub. *Ocimum* is a successful genus of entire dicotyledonous having worldwide distribution. Most importantly this genus includes culinary basil (*O. basilicum*) and the holy basil (*O. tenuiflorum*, Syn. *O. sanctum*). Earliest reference to medicinal uses of holy basil (*O. tenuiflorum*) may be traced to ancient literature like Rigveda and Atharvaveda (Dash and Kashyap, 1987). Most distinctively medicinal uses have been mentioned in *Charaka Samhita* (Ray and Gupta, 1965) and since then holy basil is being worshiped throughout the Indian subcontinent.

Taxonomists have estimated between 63-150 species of *Ocimum*, which includes natural species, natural hybrids and artificial hybrids. Majority of taxonomists felt it is a difficult job to distinguish between some closely related species. This indistinctiveness difficulty arises due to easy hybridity and crossability between species to species rendering difficulty for reproductive isolation – a criterion for specific definition.

The species of *Ocimum* especially *O. tenuiflirum* is widely recognized for its therapeutic potential from time immemorial (Singh *et al*., 2002a). The members of the genus have shown spectacular uses including antiviral, antibacterial, antifungal, larvicidal and insect repellent properties (Chopra *et al*., 1956; Kirtikar and Basu, 1975; Simon *et al*., 1999; Pragadheesh 2013; Pattanayak and Dhal, 2015). Of late, *Ocimum* is used as an important plant bio-resource to shed light on plant association and their communities. It is seen to be grown in varied agro-climatic condition ranging from tropical to sub-tropical regions. India being the tropical country is one of the rich depositories of *Ocimum* species. It is naturally grown mostly in the uncultivated lands, wastelands and along the roadsides. However, the most preferable ecological condition for *Ocimum* species is slightly sloppy to plain, alluvial soils with comparatively high temperature and humidity.

The morphological variation observed within the species of *Ocimum* includes plant height, leaf shape, leaf colour, flower colour, seed shape, seed colour etc. These variations are not well documented and pose a taxonomic puzzle. Taxonomists often find it difficult to classify the species more accurately by their phenotypic characters only. The morphological variation in the genus *Ocimum* is greatly influenced by the environment, therefore, it is necessary to think in a broader perspective implying more sophisticated biological tools. In this context, Random Amplified Polymorphic DNA (RAPD) opens up a new avenue in studying the genetic variation in *Ocimum* species more precisely. Recently, RAPD emerged as accurate and trustworthy molecular technique to investigate the genetic diversity at the DNA level throwing light on plant evolution (Singh *et al*., 2004).

Linolenic, linoleic, oleic and palmitic acid were found as major fixed oil components in different *Ocimum* species. However, these components significantly vary intra

specifically and contribute as important chemical indicator for taxonomical classification (Azhari *et al*., 2009). Therefore, traditional and chemotaxonomy may be combined with molecular markers for characterization, classification and evaluation of different *Ocimum* species, sub-species and varieties.

Dakshin Dinajpur district came up in the year 1991 by the bifurcation of erstwhile West Dinajpur district of West Bengal. Presently Dakshin Dinajpur consisting of eight developmental blocks is a primarily agriculturally sustained district. The principal tribal communities are – Santal, Munda, Oraon along with Scheduled caste communities like Rajbanshis. This district has an old folk culture of using herbal medicines. However, the importance and such use of medicinal plants/plant parts are being lost due to rapid urbanization and deforestation. As a result many useful medicinal plants are becoming threatened and precious knowledge is lost.

Therefore, the present study has been undertaken with the following objectives–

- To document the traditional knowledge of local people in utilizing different wild plant species and their extracts for therapeutic purposes.
- To study the ethnobotanical perspectives of medicinal plants particularly concentrating on *Ocimum* in Dakshin Dinajpur district.
- To study the ecological factors responsible for optimum growth of *Ocimum* in natural habitat and their associations with other plant communities.
- To study the diversity in *Ocimum* adopting morphological, chemical and genetical approaches for characterization, classification and evolution of its genotypes.
- To find out a method for low cost technique for mass multiplication of *Ocimum* for its conservation.

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).

Materials and Methods

3.1. Ethnobotanical data collection, area of study

Field study was done covering eight blocks of Dakshin Dinajpur district of West Bengal. The district lies between 25° 10´ N - 25° 40´ N latitude and 88° 10´ E - 89° 00´ E longitude and covering an area of 2162 sq. Km. The district is situated between Bangladesh on the east and south, Uttar Dinajpur district on the North and West and some southern part lies adjacent to Malda district (Figure 3.1). The main source of income is based on agriculture. The district witness annual temperature of 20-30 °C, relative humidity of 64-90% with average rainfall of 120 mm (data compiled from the Meteorological Department, Office of the ADA, NB Regional Office, Jalpaiguri, GoWB). The topography of the study site is mostly even plain land.

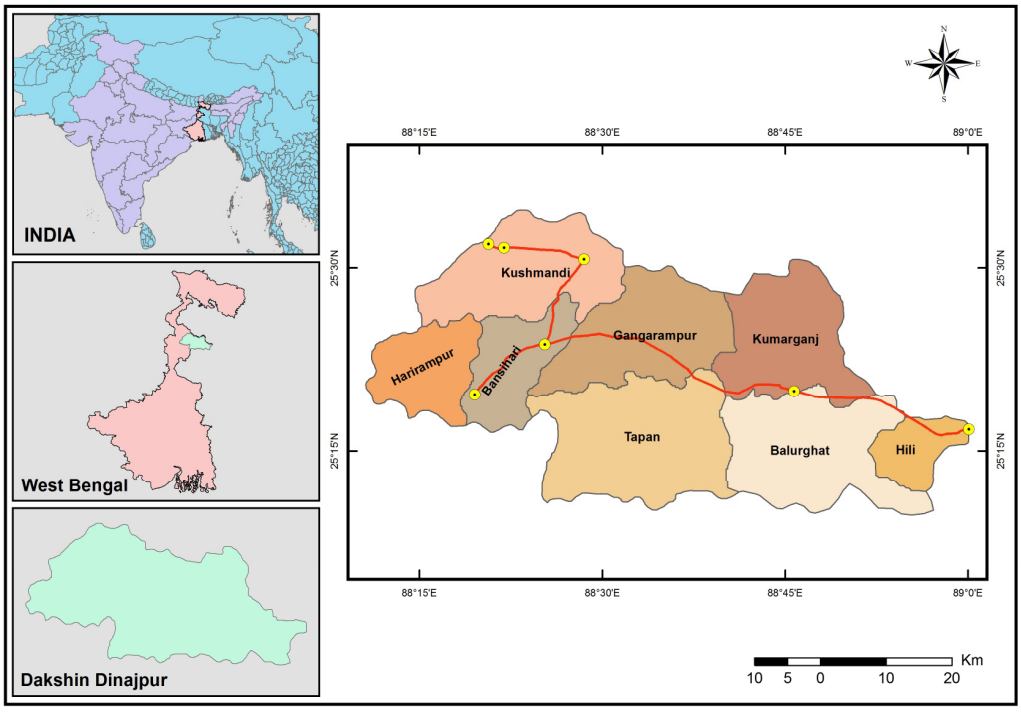


Figure 3.1: Map of the study area

Eight blocks of the study area are Hili, Balurghat, Kumarganj, Gangarampur, Tapan, Bangshihari, Harirampur and Kushmandi.

Various data collection techniques like personal interview, focused group discussion, semi-structured questionnaires following the method of participatory approaches were adopted in the present study (Jain, 1987; Jain and Mudgal, 1999). The structure of the questionnaire is given in Table 1 (Appendix A). Data were collected targeting mainly the knowledgeable tribal people locally known as Kabiraj/Baidya having traditional knowledge of using plants and their extracts for medicinal purposes. Total 160 persons were interviewed randomly taking 20 people from each block ensuring equal representation of the population in the sample targeted. The sample population targeted mostly belongs to the age group of 30 - 70 years.

Traverse walking was conducted during the initial phase interacting mainly with the old aged tribal people and Kabirajes/Baidyas. The main aim was to get an overall knowledge about the village and association of the tribal people in utilizing different locally available medicinal plants for curing various ailments. Several data were collected during the field study. For example, local name of the plant, plant part used for medicinal purpose, method of preparation, ingredients used and modes of administration etc. The duration of study covers two years from November 2013 to November 2015. The data collected were analysed employing appropriate statistical methods using Microsoft Excel version 2007 and represented as graphs, pie charts, tables etc.

3.2. Identification

The plants were collected at their reproductive stage, specimen herbarium prepared and preserved following the standard procedure of Jain and Rao (1977). The taxonomical study of the collected specimens and their characterization and identification was done following standard taxonomic literatures (Prain, 1903; Mukerjee, 1940; Hooker, 1973; Guha Bakshi, 1984; Mitra and Mukherjee, 2013). Nomenclature and correct author citation for all the species were thoroughly checked in the (The Plant List, 2013) database. Total 9 taxa of *Ocimum* were identified from the study area and included for the present study (Figure 3.2). Out of the 9 taxa, 2 morphotypes from *O. tenuiflorum* L. (Purple and Green type, commonly known as Krishna and Radha tulsi respectively), 2 morphotypes from *O. basilicum* L. (Babu and Marua tulsi), 2 morphotypes from *O. gratissimum* L. (Ram and Ajowan tulsi) and each from *O.* × *africanum* Lour. (Lebu

tulsi), *O. americanum* L. (Bon tulsi) and *O. kilimandscharicum* Guerke. (Karpur tulsi) species were considered for the present investigation (Table 3.1.). Their detailed characterization and classification were validated by the Botanical Survey of India (BSI), Howrah, West Bengal. The voucher specimens were deposited in the Department of Botany, University of North Bengal.

Table 3.1: *Ocimum* taxa collected from different places of the district Dakshin Dinajpur, West Bengal

Sl No.	Accession No.	*Ocimum* Taxa	Local name	Collection site	Longitude/ Latitude
1.	NBU-09801	*O. gratissimum* L.	Ram tulsi	Harirampur	88° 26´ 80´´ E 25° 37´ 73´´ N
2.	NBU-09802	*O. gratissimum* L.	Ajowan tulsi	Balurghat	88° 46´ 38´´ E 25° 13´ 16´´ N
3.	NBU-09795	*O. tenuiflorum* L.	Krishna tulsi	Gangarampur	88° 31´ 53´´ E 25° 23´ 58´´ N
4.	NBU-09796	*O. tenuiflorum* L.	Radha tulsi	Daulatpur	88° 19´ 36´´ E 25° 19´ 44´´ N
5.	NBU-09799	*O. basilicum* L.	Babu tulsi	Kushmandi	88° 22´ 06´´ E 25° 31´ 23´´ N
6.	NBU-09800	*O. basilicum* L.	Marua tulsi	Jordighi	88° 24´ 09´´ E 25° 26´ 28´´ N
7.	NBU-09797	*O. americanum* L.	Bon tulsi	Patiram	88° 76´ 14´´ E 25° 33´ 48´´ N
8.	NBU-09798	*O.* x *africanum* Lour.	Lebu tulsi	Bansihari	88° 24´ 04´´ E 25° 26´ 01´´ N
9.	NBU-09803	*O. kilimandscharicum* Guerke.	Karpur tulsi	Hili	89° 00´ 06´´ E 25° 16´ 39´´ N

*Altitudinal range 24 to 39 m

Figure 3.2: Nine *Ocimum* taxa used in the present study

3.3. Ecology of *Ocimum*

3.3.1. Quadrat studies

Sites were selected based on natural occurrence of *Ocimum* species targeting all the eight blocks. Natural occurrence of *Ocimum* was found abundantly along the road side. Three sites (*viz*., Site-1, Site-2 and Site-3) were identified for the present study (Table 3.2). Twenty five quadrat plots were selected randomly from each site totaling 75 quadrat plots. Each quadrat plot is designed as per the method of Misra (1968). Each plot measures 1x1 m^2 in size and contains at least one *Ocimum* species. The study was conducted in the month of August-November. Each quadrat plot is thoroughly assessed and number of different plant species belong to each plot was recorded. Quadrat studies were applied to elucidate the ecosystem of *Ocimum*. Following parameters like Density (D), Relative Density (RD), Frequency (F), Relative Frequency (RF), Abundance (A) and Importance Value Index (IVI) were developed for phyto-association studies of *Ocimum* with other plant communities adopting the standard procedure of Cottom and Curtis (1956).

Density: Density represents the numerical strength of a species in the unit area or volume. The number of individuals of the species in any unit area is its density. Density gives an idea of degree of competition. It is calculated as:

$$\text{Density} = \frac{\text{Total number of individuals of a species in all quadrats}}{\text{Total number of quadrats studied}}$$

Relative density: Relative density is used to express the contribution of individuals of one species in relation to the total number of individuals of all species.

$$\text{Relative density} = \frac{\text{Density of individuals of a species}}{\text{Total density of all species}} \times 100$$

Frequency: It is the measure of commonness and distribution of a species within a study area. Frequency is the number of sampling units (as %) which a particular species occurs. Thus frequency of each species calculated as follows:

$$\text{Frequency (\%)} = \frac{\text{Total number of quadrats in which the species occur}}{\text{Total number of quadrats studied}} \times 100$$

Relative frequency: The dispersion of species in relation to that of all the species is termed as relative frequency of a species.

$$\text{Relative frequency} = \frac{\text{Frequency of individuals of a species}}{\text{Total frequency of all species}} \times 100$$

Abundance: This is the number of individuals of any species per sampling unit of occurrence. It is related to density but is a qualitative estimate. It is calculated as follows:

$$\text{Abundance} = \frac{\text{Total number of individuals of a species in all quadrats}}{\text{Total number of quadrats in which the species occurred}}$$

Importance value Index (IVI): Important Value Index (IVI) is the sum of quantities of relative frequency and relative Density.

Importance Value Index (IVI) = Relative frequency + Relative density

Table 3.2: Details of the study sites

Sites	Location	Latitude	Longitude	Altitude
		Range	Range	Range
S-1	Hili to Patiram (SH-10)	89° 10´ E to 88° 45´ E	25° 10´ N to 25° 19´N	25-30 m
S-2	Bangshihari to Ushaharan via Mahipal road	88° 25´ E to 88° 28´ E	25° 23´ N to 25° 32´N	29-34 m
S-3	Patirum to Daulatpur (NH-512)	88° 45´ E to 88° 46´ E	25° 19´ N to 25° 13´N	30-37 m

3.3.2. Soil sampling and analysis

Five hundred gram soil (from the upper soil layer not below 15 cm in depth) was collected from each quadrat plot. Sampling was done from the centre and four corners of each quadrat plot. Samples collected were air-dried, sieved in 2 mm mesh for analysis.

3.3.2.1. Soil pH

The pH of soil was determined using pH meter (Digital pH meter Model Systronics No. 802) adopting the method of Jackson (1967). 20 g soil sample was mixed with 40 mL

distilled water (1: 2 ratio). The suspension was stirred occasionally with glass rod for 30 minutes and settled down for one hour. The electrode was inserted in the clear supernatant and pH was measured.

3.3.2.2. Electrical conductivity

The electrical conductivity of a soil sample was determined utilizing digital electrical conductivity meter (303 Systronics) as depicted by Bower *et al*. (1952). 20 g soil sample was mixed with 40 mL of distilled water. The mixture was stirred with a glass rod for 30 min and kept it for settled down. Conductivity cell was embedded in the supernatant and EC was recorded.

3.3.2.3. Organic carbon (%)

The amount of organic carbon of the soil was measured by technique for Walkley and Black (1934) portrayed by Jackson (1967). 1 g soil sample was taken into 500 mL conical flask, to which 10 mL of 1 N potassium dichromate and 20 mL concentrated H_2SO_4 was included. The mixture was shaken for a minute and permitted to set for 30 minutes. 200 mL distilled water, 10 mL concentrated orthophosphoric acid and 1 mL diphenylamine indicator was included. The solution was titrated against standard ferrous ammonium sulfate (FAS) till colour appears from blue violet to brilliant green. The clear titration (without soil sample) was done initially.

$$\% \text{ organic carbon} = (B - S) \times N \times 0.003 \times \frac{100}{\text{wt. of dry soil}}$$

Where,

B = mL of std. 0.5 N ferrous ammonium sulphate required for blank.

S = mL of std. 0.5 N ferrous ammonium sulphate required for soil sample.

N = Normality of std. ferrous ammonium sulphate (0.5N).

3.3.2.4. Available nitrogen (N)

Available N of soil sample was determined adopting the method of Subbiah and Asija (1956). 20 g soil sample and 20 mL distilled water were added in 1000 mL Kjeldahl flask. Then 100 mL potassium permanganate (0.32 %) and 100 mL NaOH (2.5%) solution were mixed thoroughly and attached it to Kjeldahl flask for boiling. The solutions were distilled in a kjeldahl and collected in the Erlenmeyer flask that contains boric acid with methyl red and bromocresol green indicator. After 30 minutes the

solution was titrated with 0.02 N H_2SO_4 until the colour appeared from green to pink. Blank (without soil) sample was run simultaneously. Available nitrogen was calculated from the following equation,

$$\% \text{ available N} = (S - T) \times (\text{N, of Acid}) \times 0.014 \times \frac{100}{W}$$

$$\text{Available N kg/ha} = \% \text{ of N} \times \frac{2240000}{100}$$

Where,

W = Weight of dry soil

S = Blank titration, mL standard NaOH required for 25 mL H_2SO_4, blank used for received the distillation of blank

T = Titration of sample, ml standard NaOH required for 25 mL H_2SO_4 receiving the sample

N = Normality of sulphuric acid

3.3.2.5. Available phosphorus (P)

Available phosphorus was measured by Olsen's technique modified by Watanbe and Olsen (1965). 2.5 g soil, 50 mL of Olsen's reagent (0.5M $NaHCO_3$ Solution, pH 8.5) and one teaspoonful of activated charcoal were mixed in a conical flask. The flask was shaken for 30 minutes and the solution was filtered through Whatman filter paper (No. 40). The filtrate (5 mL) was taken in a volumetric flask (50 mL) and neutralized with H_2SO_4. The volume was made up by adding distilled water. Blue colour developed when few drops of ascorbic acid-mixed solution was added to the solution. After 10 minutes optical density was read by spectrophotometer using 730-840 nm wave length. Blank (without soil) sample was run simultaneously. The measure of phosphorus was estimated by utilizing equation,

$$\text{P (ppm in soil)} = \text{ppm P in aliquot} \times \frac{\text{Total volume of extract}}{\text{Aliquot taken (ml)}} \times \frac{1}{\text{Wt.of soil (g)}} (R \times F)$$

$$\text{P (Kg/ha)} = \text{ppm P in soil} \times 2.24$$

$$P_2O_5 \text{ (Kg/ha)} = \text{P (kg/ha)} \times 2.24 \times 2.29$$

3.3.2.6. Available potassium (K)

The flame photometric method was utilized to estimate the available K present in a sample (Jackson, 1958). 5 g soil sample and 25 mL of 1 N ammonium acetate was added in 150 mL Erlenmeyer flask. The solutions were shaken for 30 minutes and

filtered through Whatman filter paper (No. 40). 5 mL of filtrate was diluted with 25 mL distilled water. Atomized the solution to flame photometer (Digital flame Photometer-130, Systronics) and recorded the reading. The amount of potassium was estimated by the following formula:

Available K (Kg/ha) = $\frac{x \times v \times 2.24}{w}$

Where,

x = ppm K_2O obtained from standard curve

v = volume of extractant [i.e. 1 N CH_3COONH_4 (pH 7.0) taken for extraction of K_2O from soil (mL)]

W = weight of the soil sample taken (g)

3.4. Morphological studies

Morphological study was carried out during the flowering period (Septembers to January) of different *Ocimum* collections. Total 35 morphological characters including both the qualitative and quantitative ones were recorded (Table 3.3) based on the standard descriptor developed by NBPGR (Singh *et al*., 2003) (Table 1 in Appendix B).

Table 3.3: List of qualitative/quantitative data recorded for morphological evaluation

Sl. No.	Qualitative character	Quantitative character
1	Habit	Plant height (cm)
2	Growth habit	Canopy (cm)
3	Mode of reproduction	Leaf length (cm)
4	Stem colour	Leaf width (cm)
5	Stem shape	Leaf area (cm^2)
6	Stem pubescence	Petiole length (cm)
7	Leaf colour	Inflorescence length (cm)
8	Leaf surface	No. of whorls/inflorescence
9	Leaf margin	Bract length (cm)
10	Leaf tip	Bract width (cm)
11	Leaf shape	Peduncle length (cm)
12	Inflorescence type	Sepals length (cm)
13	Inflorescence colour	Sepals width (cm)
14	Flower colour	Petal length (cm)
15	Anther colour	Petal width (cm)
16	Seed mucilage	Stamen length (cm)
17	Seed colour	Style length (cm)
18	Seed shape	- - - -

All the 18 qualitative characters were converted into quantitative ones adopting the numerical data matrix developed by Singh *et al*. (2003) for the purpose of statistical analysis (Table 2 in Appendix B). Principal Component Analysis (PCA) was carried out to measure the significant variation of morphological diversity among the *Ocimum* taxa. The identical ordinal variables detected and eliminated particularly for the qualitative characters (e.g. mode of reproduction and plant growth habit). Further, Agglomerative Hierarchical Cluster (AHC) was constructed using dissimilarities with Euclidean Distance by Ward's method. Data were analyzed using of XLSTAT software (2015).

3.5. Chemical components analysis through GC-MS

3.5.1. Preparation of ethanolic extracts

Leaves were collected from all the 9 taxa of *Ocimum* during inflorescence period. Laves collected were shade dried and ground to powder. 100 g from each sample was dissolved in 500 mL of ethanol and kept for 7 days at room temperature (Emamuzo *et al*., 2010). The extract was then filtered using Whatman filter paper (No. 41). The solvent was recovered using a rotary evaporator (Buchi Rotavapor R-3; Buchi Labortechnik AG, Flawil, Switzerland) at 40 °C. Finally, the yellow-greenish ethanolic extracts were lyophilized and kept in a sealed labeled vial at 4 °C in dark condition until tested and analyzed.

3.5.2. Gas chromatography/mass spectrometry (GC-MS)

The GC–MS was done at Indian Institute of Science (IISc.), Bangalore. A composition of the ethanolic extracts of each *Ocimum* taxa was assessed by GC–MS. 10 µL sample was diluted in 1 mL of ethanol (1:100 dilutions). From this 100 µL of the ample was completely dried using nitrogen. Sample was derivatised using 30 µL pyridine and 50 µL of BSTFA: TMCS (99:1) and incubated at 60 °C for 60 min. Derivatised samples were subjected to GC-MS. The GC analysis was done using an Agilent Technology 7890A equipped with a DB 5 MS capillary column (30 m L x 0.25 mm ID x 0.25 µm film thicknesses dimension). The carrier gas was helium with the flow rate of 1.0 mL/min. Initial column temperature was maintained at 70 °C with 2 min hold time. Then ramp the temperature to 150 °C at the rate of 5 °C/min and again to 280 °C at the rate of 3 °C/min with 2 min hold time and finally to 20 °C temperature at the rate of 10

°C with 3 min hold time. 1.0 µL of sample was subjected to GC-MS using the split mode (split ratio 10:1). The GC-MS analysis was done on the Agilent Technologies 5975CMSD (Mass selective detector). Ionization for MS was Electron Impact Ionization and mass analyzer was single quadrupole. Mass spectra scan range was from 30 m/z to 600 m/z with +ve polarity.

3.5.3. Identification and quantification of components

The compounds were identified based on the comparison of mass spectra (MS) obtained with those of the mass spectra from the library. The relative percentage of each component was calculated by the relative percentage of the total peak area in the chromatogram. AMDIS was used as a deconvolution tool and National Institute Standard and Technology (NIST 2011) was used to identify the compounds.

3.6. Genetic variation based on RAPD fingerprinting

3.6.1. DNA extraction and purification

DNA was isolated from tender fresh leaves of all the nine taxa of *Ocimum* adopting the Cetyl trimethyl ammonium bromide method as per Murray and Thompson (1980). Please see Table 1 in Appendix C for CTAB buffer preparation. Leaves were surface sterilized with teepol (Extran) for 5 minutes and rinsed with sterile double distilled water and wiped off with clean tissue paper to remove surface water completely. Isolation of DNA from the leaves was carried out with following procedures-

a. Crushed 1 g of fresh young surface sterilized leaf tissue in liquid nitrogen with the help of pre-chilled mortar and pestle.

b. Transferred immediately the paste leaf tissue to clean autoclaved 10 mL polypropylene tube containing 5 mL of extraction buffer and mixed well to form slurry (Table 2 in Appendix C for preparation of extraction buffer).

c. Incubate homogenate at 65 °C for 1 hour in a water bath with intermittent shaking.

d. The homogenate mixture was cooled at room temperature and added an equal volume of chloroform : isoamyl alcohol (24:1) and mix by inversion for about 10 minutes.
e. The tube was centrifuged at 6500 rpm for 10 minutes at 25 ℃. The upper aqueous layer was transferred to a fresh polypropylene tube and added 2/3 volume of ice cold Isopropanol by quick gentle inversion for about 2 minutes.
f. The tube was incubated overnight at -20 ℃.
g. After overnight incubation, the tube was centrifuged at 6500 rpm for 10 minutes at 4 ℃.
h. The supernatant was discarded gently and the pellet was washed with 70% chilled ethanol.
i. Again centrifuged at 10000 rpm for 5 minutes at 4 ℃. The step repeated about 3-4 times.
j. Air dried the pellet for about 30 minutes at room temperature followed by dissolving in 1 mL of TE buffer (pH-8.0).

Purification of extracted DNA

a. DNase free RNaseA (10 µL of 10 mg/mL) was added to the genomic DNA dissolved in 1 mL TE buffer (pH 8.0) and incubated at 37 ℃ for 1 hour in a dry water bath.
b. An equal volume of Phenol: Chloroform (1:1) was then mixed to each sample by gentle inversion for 2 min and centrifuged at 10000 rpm for 10 min at 4 ℃.
c. The aqueous phase was then transferred to a sterile micro centrifuge tube and repeats the extraction twice with Chloroform: Isoamyl alcohol (24:1) and centrifuged at 6000 rpm for 5 minutes at 4 ℃.
d. Each sample was mixed with 0.1 volume of 3M sodium acetate (pH 5.2) and double volume of isopropanol and stored overnight for DNA precipitation. It

was then centrifuged at 10000 rpm for 5 minutes at 4ºC. The supernatant was discarded and the pellet was washed with 1 mL of 70% chilled ethanol thrice and dried at room temperature.

e. Finally, the DNA pellet was dissolved in 100 µL TE buffer (pH 8.0) and stored at -20 °C for quantification.

3.6.2. Quantification of DNA

Reliable measurement of DNA concentration is important for applications in molecular biology including amplification of target DNA by polymerase chain reaction and complete digestion of DNA by restriction enzymes. DNA quantification is generally carried out by spectrophotometric measurements or by agarose gel analysis for DNA qualification. Both the methods were used in the present study.

i. Spectrophotometric measurement

The isolated DNA was measured using UV-Spectrophotometer (CECIL, CE 7200, Germany). 998 µL of TE buffer was taken in a quartz cuvette and 2 µL of extracted genomic DNA was added to it. The optical density (absorbance A) was taken at 260 nm (A_{260}) and 280 nm (A_{280}). For an ideal DNA preparation the A_{260}/A_{280} ratio should be ≥ 1.8 (Sambrook and Russed, 2001). DNA concentration was estimated by employing the following formula:

$$\text{Amount of DNA (ng/µL)} = \frac{\text{OD260} \times 50 \times \text{dilution factor}}{1000}$$

For double stranded genomic DNA, 1 O.D. corresponds to 50 µg/mL of DNA (Sambrook and Russed, 2001).

$$\text{Dilution factor} = \frac{998\ \mu\text{L TE buffer } 2\ \mu\text{L DNA}}{2\mu\text{L}} = 500$$

ii. Quality check through agarose gel

Qualification of isolated genomic DNA of nine genotypes was performed by 1 % agarose gel electrophoresis. 1 % agrose mixed with 1X TAE buffer for gel casting. 5 µL Ethidium bromide (Genei) was added and pour to the gel casting tray. 2 µL DNA of each genotype was mixed with 8 µL 1x TAE and 2 µL gel loading dye on a 0.2 mL tube and loaded onto each well for electrophoresis. The gel was allowed to run at constant voltage of 60v for about 1.5 hours till the tracking dye reaches end of the gel. Then the

gel was analysed under UV-translluminator to check the quality. Photographs were taken using Gel Doc system (Bio-Rad, Gel Doc-2000).

3.6.3. RAPD-PCR analysis

RAPD amplification was performed with extracted and purified genomic DNA from nine taxa of *Ocimum* using 10 RAPD primers (Genei) (Table 3.4). Primers were selected on the basis of previous works on *Ocimum* species (Saha et al., 2012; Singh, 2004). Each RAPD PCR mixture (25 µL) contained 25 ng genomic DNA as template, 2.5 µL PCR Assay buffer (1X), 2.5 µL of each dNTPs (final concentration of 200 µM each dNTP), 1µL $MgCl_2$ (final concentration of 1.0 mM $MgCl_2$), 0.5 µL Taq DNA polymerase (3U/µL) and 2.5 µL of each primers (final concentration of 1µM) (Table 3 in Appendix C for composition). The final volume of 25 µL was made up with PCR grade water (Genei, Pvt. Ltd., Bangalore, India).

The PCR were performed in a thermo-cycler (Perkin Elmer Gene Amp 2400 PCR system) programmed as follows.

Step 1: Initial denaturation at 94 °C for 4 min

Step 2: Denaturation at 94 °C for 40 sec
Annealing at 36 °C for 40 sec
Extension at 72 °C for 60 sec
} 35 cycles

Step 3: Final extension at 72 °C for 7 min

Step 4: 4 °C forever

Table 3.4: List of RAPD primers with their sequence

Sl. No	Primer code	Sequence (5´-3´)
1	BGM-1	TGCCGAGCTG
2	BGM-3	GTGACGTAGG
3	BGM-4	AGGTCTTGGG
4	BGM-5	GGTGCTGCGC
5	BGM-7	CTGGGCAACT
6	BGM-9	GAAACGGGTG
7	BGM-12	GGAACGGGTG

8	BGM-13	CATCCCGACA
9	BGM-15	GCACGCCGGA
10	BGM-17	CTATCGCCGC

Note: All the primers (oligos) RAPD primers were supplied as lyophilized powder form from Genei, Pvt. Ltd., Bangalore, India. The hydration was performed by adding 100 µL sterile PCR grade water (Genei) and incubation the vial at 65 °C for 10 minutes. After tapping and vortexing for a few seconds, the vials were stored at -20 °C.

3.6.4. Agarose gel electrophoresis of RAPD-PCR band profiling

The PCR amplified products were separated on horizontal gel electrophoresis using 1 % agarose gel containing 0.5 µg/mL ethidium bromide (EtBr). 7µL EtBr was mixed with warmed agarose before pouring in casting tray. Solidified gel was placed within the submerge gel and immersed with sufficient 1X TAE buffer (Table 4 in Appendix C for composition). 10 µL PCR products of each sample mixed with 2 µL gel loading dye and then loaded in the wells of the agarose gel. A low range DNA ladder (100-3000bp) (Genei) was used as known molecular weight marker and loaded separately. Gel electrophoresis was done at a constant voltage of 70V for 1.5 hours until the tracking dye reaches at the end of the gel. The gel was observed in a UV-transilluminator and photographed by Gel Doc System (Bio-Rad, Gel Doc-2000).

The clear and visible amplified bands from the photographic gels were considered for the analysis. The amplified bands were scored as 1 or 0 on the basis of present and absent of bands to generate a binary data matrix. Only reproducible bands were considered for the analysis. The binary data matrix was used to calculate Jaccard's similarity coefficient among 9 *Ocimum* taxa using the Simqual module of NTsys-PC (Numerical Taxonomy System version 2.1) (Rohlf, 2000). These distance coefficients were used to construct dendrogram using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) employing the Sequential Agglomerative Hierarchical and Nested (SAHN) algorithm for determining the genetic diversity and relationships among the genotypes. Principle coordinate analysis (PCA) was performed using the EIGEN and PROJ modules of NTsys-PC.

The polymorphic information content (PIC) is generally used in genetics as a measure of polymorphism for a marker locus utilizing linkage analysis. The PIC value was

computed using the formula- PIC= $1-\Sigma p_i^2$, where, p_i is the frequency of the i^{th} allele of the locus in the set of nine *Ocimum* taxa (Anderson *et al*., 1993).

3.7. Propagation and conservation of *Ocimum*

3.7.1. Germination of seeds

Seeds from all the 9 taxa of *Ocimum* were collected from the AASM garden of Raiganj University. The collected seeds were allowed to germinate on moistened cotton bed in petri dishes for 7 days. The bed was kept moistened with periodic addition of distilled water as required. Seed germination was carried out during summer and winter seasons at room temperature. Three replicates were maintained keeping 50 seeds in each replicate. Seed germination percentage was calculated using the following formula-

$$\text{Germination percentage } (\%) = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds tested}} \times 100$$

3.7.2. Multiplication through stem cutting

The healthy apical portion of each taxa of *Ocimum* was selected for preparing the cutting. Length of the cutting was kept 8-10 cm in height keeping at least 4-5 leaves at the top. The bottom portion of the cuttings were submersed in water and monitored regularly to record the root growth. Care was taken to maintain proper hygienic condition. The samples were kept at room temperature and 8 hours in day light. Three replicates were maintained for each taxa keeping 50 cuttings in each replicate. The rooting percentage was calculated using the following formula-

$$\text{Rooting percentage } (\%) = \frac{\text{Total number of cutting rooted}}{\text{Total number cutting planted}} \times 100$$

Results and Discussions

4.1. Ethnobotanical study

4.1.1. Indigenous ethnomebotanical practices

The present study reconfirms the rich heritage of ethnobotany in Dakshin Dinajpur and the local people still practice traditional ethnomedicinal knowledge for daily needs. The scientific name and voucher specimen number, vernacular name, family, parts used and medicinal uses of each species were provided (Table 3 and 4 in appendix A). The present investigation recorded 138 species of plant belonging to 121 genera and 65 families were being utilized by various tribal and non-tribal groups of this district for the treatment of various illness. The most frequently used plant family was Lamiaceae with 10 species followed by asteraceae with 8 species, papilionaceae with 7 species and euphorbiaceae with 6 species. The acanthaceae, asclepiadaceae, cucurbitaceae and verbenaceae were having 5 species each. Poaceae with 4 species and amaranthaceae, apocyanaceae, caesalpiniaceae, combretaceae, menispermaceae, rutaceae, solanaceae and zingiberaceae contributed 3 plant species in each family. Anacardiaceae, convolvulaceae, liliaceae, malvaceae, mimosaceae, myrtaceae, orchidaceae, oxalidaceae, piperaceae, sterculiaceae and vitaceae were with 2 species in each. The other 37 families were represented by one species each (Figure 4.1). Moreover, a single plant is utilized for curing more than one disease, for example, *Cocculus hirsutus* (gonorrhea, sex stimulant and physical weakness), *Kalanchoe pinnata* (cold and cough, acidity, flatulence and burns), *Litsea glutinosa* (dysentery and spermatorrhoea), *Mangifera indica* (stomachache, dysentery and diarrhoea), *Momordica charantia* (guinea worms, allergy and diabetes), *Vitex negundo* (fever, rheumatism and diabetes), *Amaranthus spinosus* (diphtheria, dysentery and indigestion), *Mimosa pudica* (blood dysentery and leucorrhea), *Tinospora cordifolia* (physical weakness, cholesterol and blood sugar), *Acacia nilotica* (acidity, indigestion, dysentery, diarrhoea, cough, sexual disease and diabetes), *Azadirachta indica* (skin diseases, curbunle and boil), *Abroma augustum* (sesual disease and physical weakness), *Abrus precatorius* (sexual disease and jaundice), *Vernonia cinerea* (piles, diarrhea and stomachache), *Ocimum americanum* (flatulence and sexual diseases), *Ocimum gratissimum* (dry cough,

gastrointestinal problems, boils and poisonous insect stings). The present study very importantly exhibits the uses of plants in the study area as new records of application of plants/plant parts. The new records involves the uses of such plants, *viz.*, *Acacia nilotica*, *Acmella oleracea*, *Alocasia macrorrhiza*, *Alstonia scholaris*, *Bambusa tulda*, *Basella alba*, *Borassus flabellifer*, *Canna indica*, *Chromolaena odorata*, *Cocculus hirsutus*, *Curcuma aromatic*, *Geodorum densiflorum*, *Gloriosa superb*, *Helminthostachys zeylanica*, *Molineria capitulate*, *Physalis peruviana*, *Trichosanthes dioica*, *Ocimum* × *africanum* (records indicated by asterisk in the appendix A, Table 3 and 4). A couple of medicinal plants were utilized to treat similar illness however method of formulation and mode of application was different from the earlier reports. However, previous similar investigations (Mitra and Mukherjee, 2005a, b, 2010; De Sarker *et al*., 2011; Kundu and Bag, 2012; Talukdar and Talukdar, 2013; Chowdhury *et al*., 2011, 2014) were made but newer discoveries are being fulfilled by the present investigator. The application of plants/plant parts vis-a-vis diseases have been presented in Figure 4.2. The figure interestingly showed that 22 plants were used to treat arthritis/rheumatism followed by 16 plants for dysentery, 12 plants for cold and cough, 11 plants for gonorrhoea, 10 plants for boils and carbuncle etc.

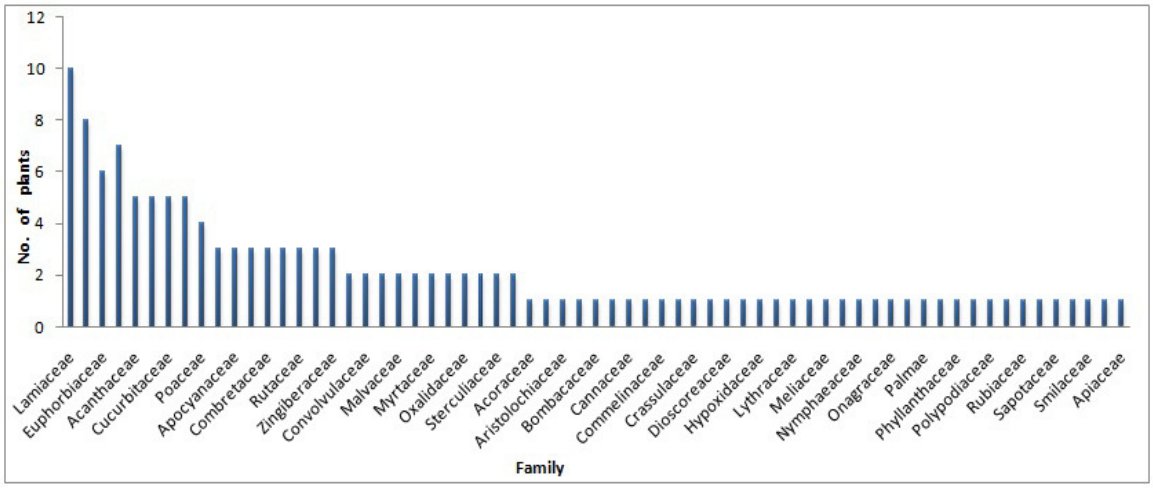


Figure 4.1: Distributions of ethnomedicinal plants (Family-wise) of Dakshin Dinajpur

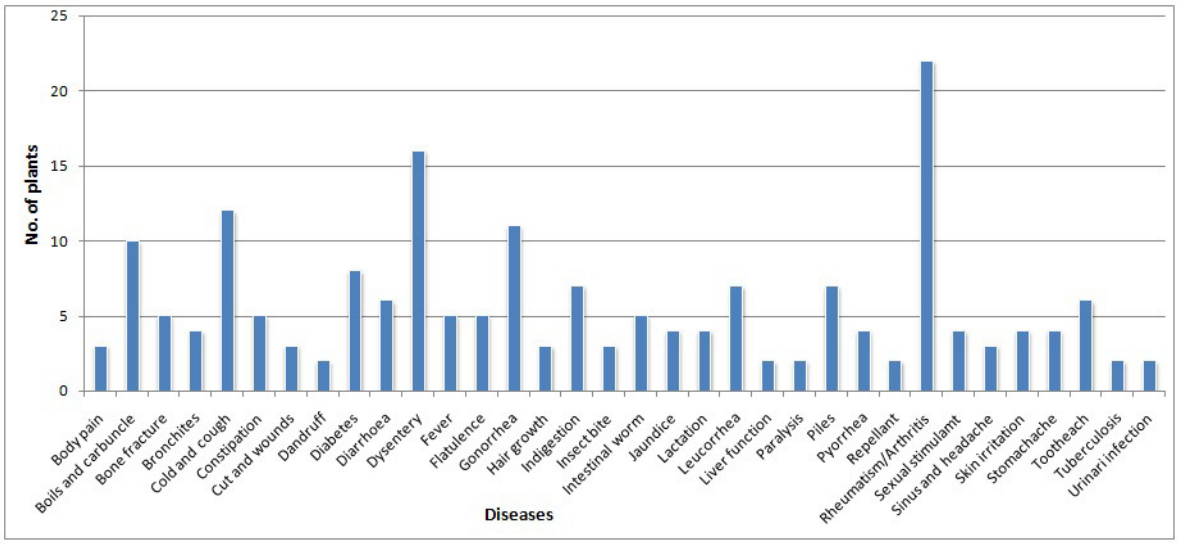


Figure 4.2: Number of plants used for treating various ailments

4.1.2. Ailment categories

The primary data obtained from the local "Kabirajes" were classified in to 15 categories *viz*., dental care, dermatological infections/diseases (DID), endocrinal disorders (ED), fever (Fvr), gastrointestinal problems (GP), hair problems, insomnia, liver problems (LP), orthopaedic/muscular problems, piles, poisonous bites (PB), repellents, respiratory problems, sexual and gynaecological problems and other diseases (Table 4.1).

Table 4.1: Ailments grouped by different ailment categories

Ailment categories	Biomedical terms	Local terms used
Dental care	Tootheach, pyorrhea	Danth batha, pairia
Dermatological infections/diseases (DID)	Boil, carbuncle, allergy, cuts and wound, skin irritation	Fora, Bish fora, Katha-chera, prodaha
Endocrinal disorders (ED)	Diabetes	Madhu meho
Fever	Fever, remittent fever	Jar, Chikan Jar
Gastrointestinal problems (GP)	Flatulence, indigestion, dairrhea, dysentery, constipation, stomachache, intestinal worm	Pet fapa, Patla paikhana, Rakto amasha, Bod hajom, Krimi, Kasha
Hair problems	Hair problems (Hair growth, hair fall, dandruff)	Chuler bridhi, Chul para, Khuski
Insomnia	Insomnia	Anidra
Liver problems (LP)	Jaundice, Liver function	Jandice, Pitta

Ailment categories	Biomedical terms	Local terms used
Orthopedic/muscular problems	Gout, joint pain, arthritis, rheumatism, body pain, bone fracture, paralysis	Bath, Git/gete batha, Ga batha, Har vanga, Paralysis
Others	Sinus and headache, earache	Matha batha, Kan batha
Piles	Piles	Arsho
Poisonous bites (PB)	Poisonous insect bites	Bishakto pokar kamor
Repellant	Mole and mosquito repellant	Chucha o masha bitarak
Respiratory problems	Bronchites, tuberculosis, cold and cough	Shardi kashi/Hooping, Tb kashi
Sexual and gynocological problems	Gonorrhea (kind of sexual disease), sexual stimulamt, leucorrhea, lactation, urinary infection	Meho/Dhatu durballo, Uttejok, Swet shrab, Dugdho kharan, Proshrab janito samossa

4.1.3. Life form and parts used

It has been observed that within the documented species, 64 plants were herb, 20 plants were shrub, 25 plants were tree and 29 were climber (Figure 4.3). The study indicated that herbs as the major contributor (46%) of medicinal plants followed by climbers (21%), trees (18%) and shrubs (15%). Some rare/threatened plants (*Rauvolfia serpentina*, *Geodorum densiflorum*, *Gloriosa superb* and *Helminthostachys zeylanica*) were also documented in the present study as medicinal plants. The plant parts utilized for ethnomedicinal purpose were whole plant, leaf, root/rhizome/tuber, fruits, stem or stem bark, flower, seed, latex, petiole and spine. The study showed that among the diverse plant parts utilized, leaves contributed (34%), trailed by root/ rhizome/ tuber (29%), stem or stem bark (10%), whole plant (9%), fruit and seed (6%) etc. for medicinal purposes (Figure 4.4). It was found that leaves or leaves mixed with other plant parts were used most commonly for the preparation of medicine to cure different ailments.

4.1.4. Method of preparation and mode of administration of plants

The preparation and uses of plant parts were categorized into 11 types (Figure 4.5). Out of these, the most frequently utilized technique for preparation was juice (31%), paste (19%), decoction (12%), mixture (9%), infusion (7%), raw (7%) etc. The paste was made by grinding/crushing the dried or fresh plant parts mixed with water. The dried

plant parts were soaked in water for 1-2 hours before grinding or crushing. The powder was made by grinding of shade dried chopped plant parts.

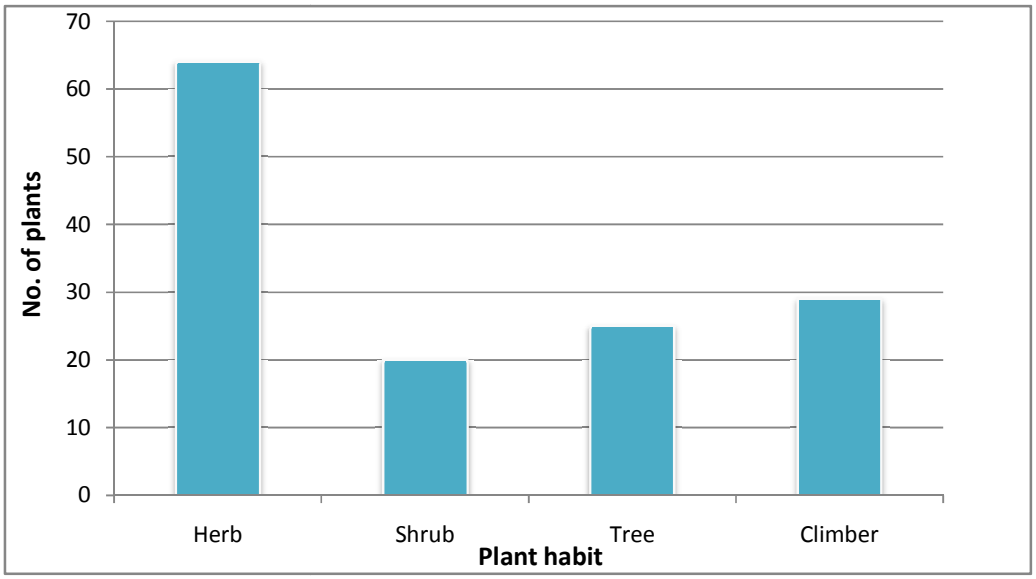


Figure 4.3: Analysis of habit with respect to number of plant species

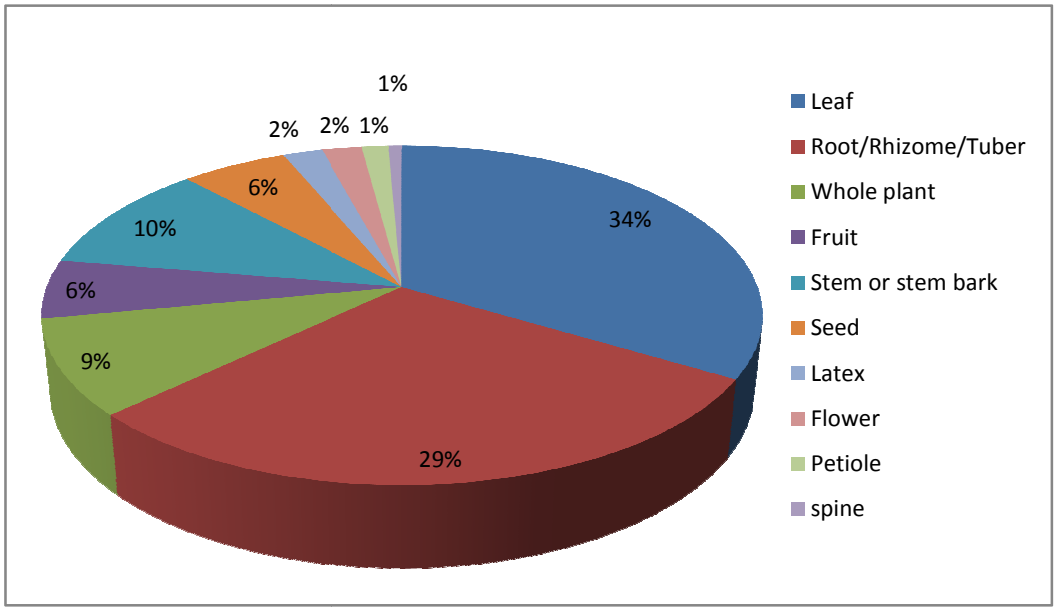


Figure 4.4: Statistics of plant parts used

The decoction was prepared by boiling up the dried or fresh plant parts in water until the amount of the water reduced to required amount. Mixture was prepared by adding two or more plant parts. Fomentation is the method by which a thin layered of oil/ghee is given on leaf before warming and applied as poultice. Medicated oil prepared by

boiling the fresh or dried plant parts in mustered oil and the filtrate used for massages. It has been observed that for oral application the plant parts were used as decoction, juice, mixture, infusion, powder and even in some cases in crude condition. On the other hand paste, medicated oil, fomentation etc. were used for external application. Oral administration (71%) was predominating over external uses (29%). In case of external use, direct application of paste or medicated oil or fomentation are the most commonly used method for the treatment of skin disorders, wounds, poison bites, heel cracks, rheumatism, body pain and headache. Occasionally decoction, juice and powder form of preparation is also used for external purposes. For instant, decoction of *Azadirachta indica* is used for boil, carbuncle and skin infections for quick healing, juice of roasted immature shoot of *Bambusa tulda* applied to cure earache and stem ash powder of dried *Basella alba* used as tooth paste to cure pyorrhoea. Most of the medicines were given orally. Similar kind of observations were made by Lee *et al.* (2008) and Poonam and Singh (2009).

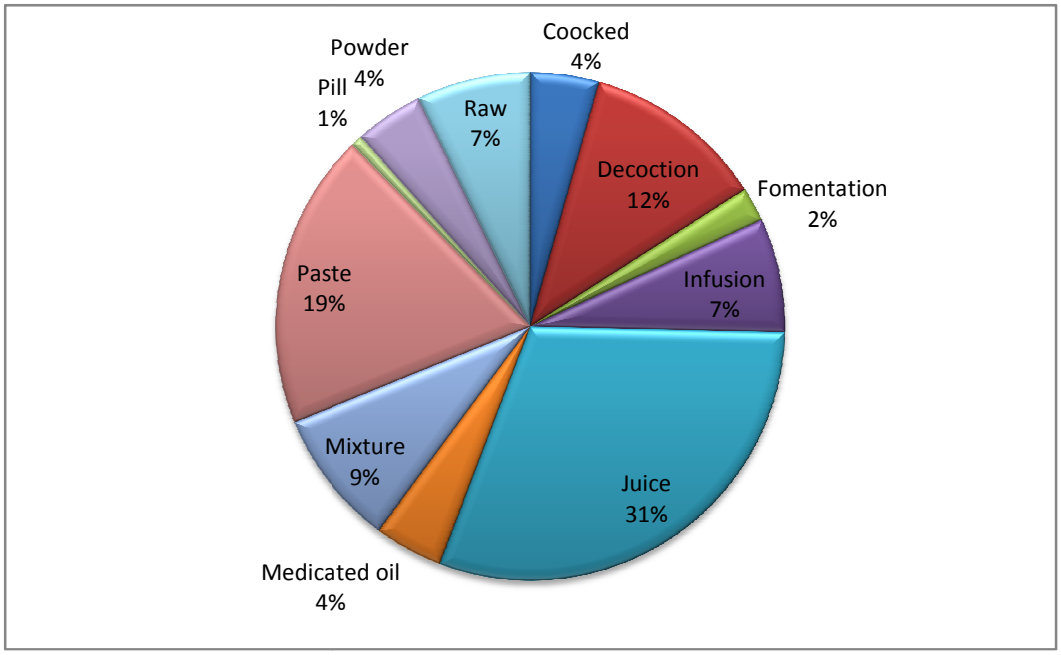


Figure 4.5: Various forms of medicinal preparation

4.1.5. Additional ingredients

Most often the tribals prepared the herbal medicine by adding one, two or more plants/plant parts in the mixture. The preparation methods varied in each case. It has been recorded that herbal preparation made from one plant in 120 formulations. There were 18 formulations where combination of plants was used. Similar observations were made in other reports (Ignacimuthu *et al*., 2008; Upadhyay *et al*., 2010). It has been also recorded that use of same plant in different illness. Mixture of several plant parts is more effective than single plant part as because of their synergistic impact (Giday *et al*., 2007; Teklehaymanot *et al*., 2007).

In some cases, "Kabirajes" of the study area frequently used some adjuvants such as a few quantities of salt, sugar candy, sugar, jaggery, milk, lime water, honey, black pepper, turmeric powder and cream to enhance the acceptability and medicinal property of certain remedies (Table 4.2). On the other hand, boiling, lukewarming, heating and roasting is also prescribed to improve the effectiveness of the remedies immediately or make it tastier for oral utilization. Different types of oils *viz*., mustard, coconut, castor, pongamia and cream or ghee were generally utilized for the preparation of medicated oil. Traditional healers especially "Kabirajes" were utilizing particular plant/plant parts in certain dosages and the dosage given to the patient relied on upon health conditions, physical status and age.

Table 4.2: Ingredients added for the preparation of ethnomedicine

Botanical name	Other plants added in medicinal preparation	Other ingredients added
Achyranthes aspera	*Zingiber officinale*	–
Alocasia macrorrhiza	–	Coconut oil
Ampelocissus latifolia	*Piper nigrum*	–
Averrhoa carambola	–	Sugar
Bauhinia acuminata	–	Mustard oil
Blumea lacera	*Zingiber officinale*	–
Borassus flabellifer	*Achyranthes aspera*	–
Calotropis gigantea	–	Ghee
Cassia sophera	*Zingiber officinale*	–
Cissus quadrangularis	–	Ghee
Cocculus hirsutus	–	Sugar
Corchorus olitorius	–	Salt and turmeric powder
Costus speciosus	*Zingiber officinale*	–
Cryptolepis buchananii	*Zingiber officinale*	Sugar, Milk

Botanical name	Other plants added in medicinal preparation	Other ingredients added
Curculigo orchioides	*Piper betel*	–
Curcuma aromatica	–	Honey
Deeringia amaranthoides	*Achyranthes aspera*	–
Dregea volubilis	*Piper nigrum*	–
Drynaria quercifolia	*Piper nigrum*	–
Euphorbia nerifolia	–	Mustard oil
Flacourtia indica	*Bombax ceiba*	–
Gloriosa superba	*Zingiber officinale, Desmodium triflorum*	–
Heliotropium indicum	–	Mustard oil
Hibiscus rosa-sinensis	–	Jaggery
Ipomoea mauritiana	–	Milk
Litsea glutinosa	–	Salt and turmeric powder
Mangifera indica	–	Lime water
Marsilea quadrifolia	–	Milk
Mucuna pruriens	–	Ghee
Nymphaea rubra	*Eleusine indica*	–
Ocimum americanum	–	Milk
Ocimum gratissimum	–	Honey
Ocimum tenuiflorum	–	Honey, Lemon juice, Salt
Physalis peruviana	–	Honey
Pterospermum acerifolium	–	Jaggery
Ricinus communis	–	Coconut oil
Salvinia auriculata	*Piper nigrum*	–
Solanum virginianum	*Piper nigrum*	–
Spondias pinnata	–	Salt
Syzygium cumini	–	Salt
Thunbergia laurifolia	*Cynodon dactylon*	–
Vangueria spinosa	–	Mustard oil
Vernonia anthelminctica	–	Cream
Vitex negundo	–	Mustard oil
Zingiber zerumbet	*Withania somnifera*	–

4.2. Ecology of *Ocimum*

Phyto-sociological parameters *viz*., density, relative density, frequency, relative frequency, abundance and importance value index (IVI) of each plant species along with *O. americanum*, *O.* × *africanum* and both morphotypes of *O. tenuiflorum* (Green and purple) were collected from each quadrat plots of different study sites (Table 4.3, 4.4 and 4.5). IVI was calculated to demonstrate the sociological structure of a particular species as a whole in its community (Figure 4.6).

4.2.1. Plant community study of Site-1 and site-2

The frequency of *O.* × *africanum* ranged from 48% (site-2) to 64% (site-1). The frequency gives an idea regarding the measure of distribution of every species in a particular study area. The density at site-1 was 12.52 plants/m^2 and 4.68 plants/m^2 at sites-2. However, the abundance was 19.56 and 9.75 correspondingly at site-1 and site-2. The IVI were 5.29 at site-1 and 2.94 at site-2.

The frequency of *O. americanum* was 52% and 72% respectively at site-1 and site-2. The recorded density was 16.84 plants/m^2 and 24.44 plants/m^2 correspondingly at site-1 and site-2. The abundance was varied from 32.38 at site-1 to 33 at site-2. The IVI values were 5.26 and 6.83 respectively at site-1 and site-2.

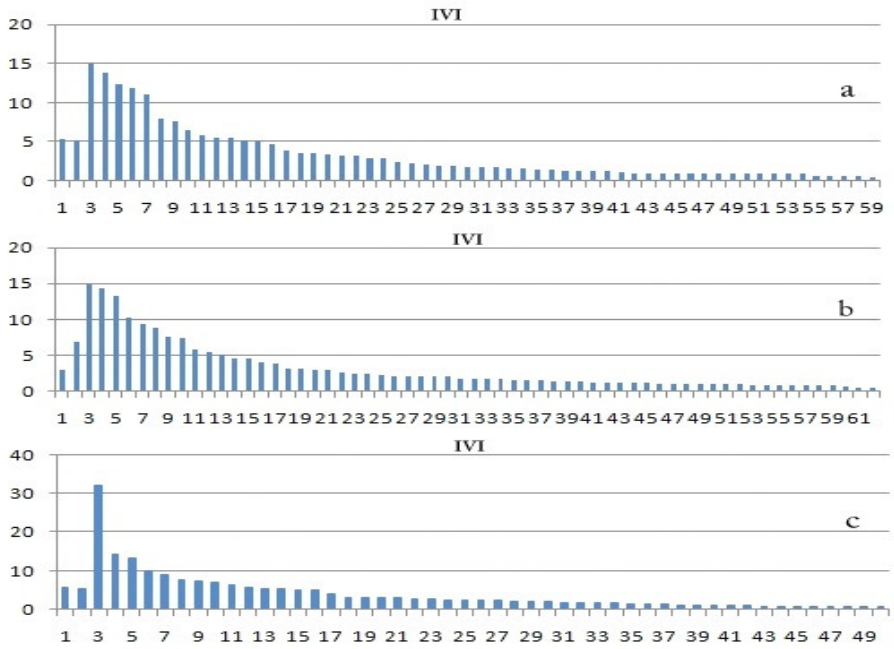


Figure 4.6: Importance Value Index of different species at three study sites, a-IVI values of site-1, b-IVI values of site-2 and c-IVI values of site-3

It has been observed that in site-1 (Hili to Patiram), *O. americanum* and *O.* × *africanum* were coexistant with 57 other plant species. They were mostly belonging to 51 genera and 22 families (18 families from dicotyledons and 4 families from monocotyledons). Out of 57 plant species 36 were from dicotyledons and 21 were from monocotyledons (Figure 4.7). The most dominanted family was Poaceae which comprises of 12 plant species followed by 6 species of Asteraceae and 6 species of Cyperaceae. On the other hand, in site-2 (Bangshihari to Ushaharan via Mahipal) a total 60 plant species

belonging to 23 families (19 families from dicotylednos and 4 families from monocotyledons) and 51 genera were found in *O. americanum* and *O.* × *africanum* community. Out of 60 plant species 37 were from dicotyledons and 23 were from monocotyledons (Figure 4.8). The largest family was Poaceae (consisting14 species) followed by Asteraceae (consisting 7 species) and Cyperaceae (consisting 5 species). The recorded species were mostly annual and herbaceous in nature of these two study sites (Site-1 and site-2).

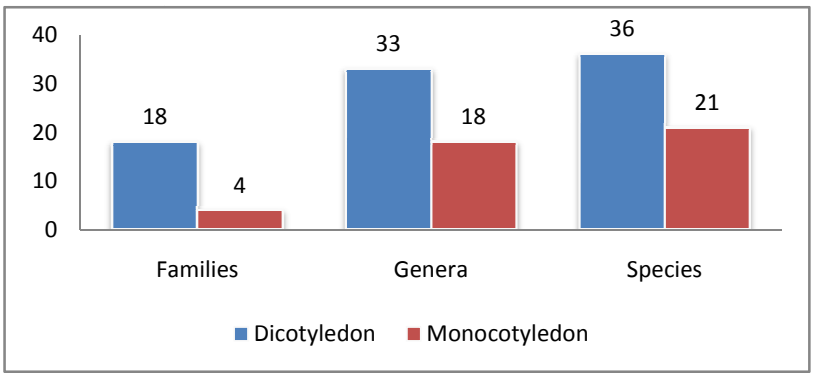


Figure 4.7: Graphical representation of species detailed in site-1

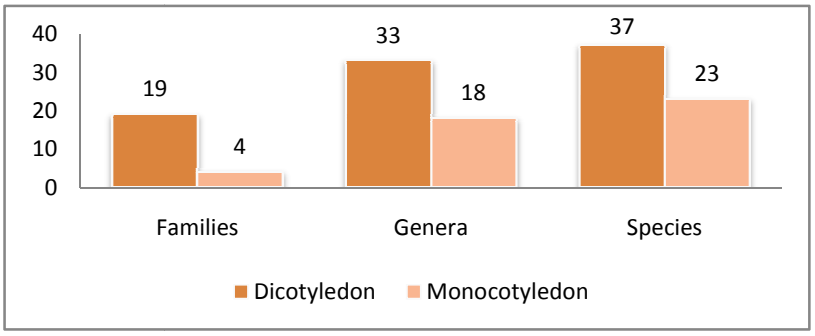


Figure 4.8: Graphical representation of species detailed in site-2

At site-1, the highest density was observed for *Cynodon dactylon*, *Axonopus compressus*, *Brachiaria paspaloides*, *Oplismenus burmannii* and *Chrysopogon aciculatus* were 82.12, 74.56, 64.48, 63.36 and 56.88 respectively. Maximum relative density was observed 11.88 for *Cynodon dactylon* followed by 10.78 (*Axonopus compressus*), 9.33 (*Brachiaria paspaloides*), 9.16 (*Oplismenus burmannii*) and 8.23 (*Chrysopogon aciculatus*) at site-1. Whereas, maximum density and relative density were recorded 87.92 and 12.24 for *Cynodon dactylon* followed by 78.36 and 10.91

(*Oplismenus burmannii*), 73.92, 10.29 (*Chrysopogon aciculatus*) and 54.36, 7.57 (*Axonopus compressus*) at site-2.

In site-1, the frequency of different plant species ranged from 8% to 72%. The maximum frequency was recorded 72% for *Senna tora*, 64% for *Ocimum* × *africanum*, 60% for *Oplismenus burmannii*, 56% for *Cynodon dactylon*, *Axonopus compressus* and *Clerodendrum viscosum*, 52% for *Chrysopogon aciculatus*, *Evolvulus nummularius*, *Ocimum americanum* and *Oldenlandia diffusa*. On the other hand, *Physalis peruviana* showed minimum (8%) frequency (Table 4.3). But in site-2, the frequency of the different plant species was varied from 12% to 80%. The maximum frequency (80%) was recorded for *Senna tora* followed by 72% for *Ocimum americanum*, *Kyllinga brevifolia*, *Evolvulus nummularius* and *Oplismenus burmannii*, 64% for *Clerodendrum viscosum*, *Murdannia nudiflora* and *Chrysopogon aciculatus*, 56% for *Axonopus compressus* and *Cynodon dactylon*, *Desmodium triflorum* and 52% for *Eragrostis tenella* and *Brachiaria villosa*. Whereas, the minimum frequency (12%) was recorded for *Sida cordifolia*, *Blumea lacera*, *Boerhavia diffusa* and *Solanum anguivi* (Table 4.4).

Abundance of *Cynodon dactylon* was observed maximum (146.64) as compare to *Brachiaria paspaloides* (134.33), *Axonopus compressus* (133.14), *Chrysopogon aciculatus* (109.38), *Oplismenus burmannii* (105.6), *Kyllinga brevifolia* (100.2) and *Rungia pectinata* (87.75) at site-1. At site-2, the highest abundance rate was observed for *Cynodon dactylon* (157) followed by *Chrysopogon aciculatus* (115.5), *Oplismenus burmannii* (108.83), *Axonopus compressus* (97.07), *Desmodium triflorum* (94.31) and *Brachiaria paspaloides* (76.75). Similar study has been reported by Chowdhury *et al*. (2016).

Dominance of a species was determined on the basis of their IVI values (Table 4.6). At site-1, *Cynodon dactylon* was the most dominanting species having highest IVI value of 14.92 followed by *Axonopus compressus* (13.83), *Oplismenus burmannii* (12.42), *Brachiaria paspaloides* (11.93) and *Chrysopogon aciculatus* (11.05). The lowest IVI values of *Physalis minima, Solanum anguivi* and *Physalis peruviana* showed that they are the rare species of that particular community. However, at site-2, *Cynodon dactylon* was most dominating species having highest (14.91) IVI value took after by

Oplismenus burmannii (14.34), *Chrysopogon aciculatus* (13.34), *Axonopus compressus* (10.24) and *Desmodium triflorum* (9.31). The least IVI values of *Achyranthes aspera*, *Spermacoce hispida*, *Amaranthus viridis*, *Heliotropium indicum*, *Scoparia dulcis*, *Physalis minima*, *Mikania micrantha*, *Blumea lacera*, *Boerhavia diffusa* and *Solanum anguivi* represent that they are the rare species of that particular community.

Table 4.3: Quantitative analysis of phytosociological data of site-1 (Hili to Patiram)

Sl No	Species	Family	D	RD	F (%)	RF	AB	IVI
1	*Ocimum × africanum*	Lamiaceae	12.52	1.81	64.00	3.48	19.56	5.29
2	*Ocimum americanum*	Lamiaceae	16.84	2.44	52.00	2.83	32.38	5.26
3	*Cynodon dactylon*	Poaceae	82.12	11.88	56.00	3.04	146.64	14.92
4	*Axonopus compressus*	Poaceae	74.56	10.78	56.00	3.04	133.14	13.83
5	*Oplismenus burmannii*	Poaceae	63.36	9.16	60.00	3.26	105.60	12.42
6	*Brachiaria paspaloides*	Poaceae	64.48	9.33	48.00	2.61	134.33	11.93
7	*Chrysopogon aciculatus*	Poaceae	56.88	8.23	52.00	2.83	109.38	11.05
8	*Kyllinga brevifolia*	Cyperaceae	40.08	5.80	40.00	2.17	100.20	7.97
9	*Desmodium triflorum*	Papilionaceae	36.24	5.24	44.00	2.39	82.36	7.63
10	*Digitaria longiflora*	Poaceae	28.80	4.17	44.00	2.39	65.45	6.56
11	*Rungia pectinata*	Acanthaceae	28.08	4.06	32.00	1.74	87.75	5.80
12	*Cyperus rotundus*	Cyperaceae	23.20	3.36	40.00	2.17	58.00	5.53
13	*Evolvulus nummularius*	Convolvulaceae	18.60	2.69	52.00	2.83	35.77	5.52
14	*Senna tora*	Caesalpiniaceae	9.28	1.34	72.00	3.91	12.89	5.26
15	*Oxalis corniculata*	Oxalidaceae	19.68	2.85	40.00	2.17	49.20	5.02
16	*Eragrostis tenella*	Poaceae	15.88	2.30	44.00	2.39	36.09	4.69
17	*Oldenlandia diffusa*	Rubiaceae	7.60	1.10	52.00	2.83	14.62	3.93
18	*Alternanthera sessilis*	Amaranthaceae	8.52	1.23	44.00	2.39	19.36	3.62
19	*Clerodendrum viscosum*	Verbenaceae	3.24	0.47	56.00	3.04	5.79	3.51
20	*Croton bonplandianum*	Euphorbiaceae	5.96	0.86	48.00	2.61	12.42	3.47
21	*Paspalum laeve*	Poaceae	10.16	1.47	32.00	1.74	31.75	3.21
22	*Cyperus compressus*	Cyperaceae	7.12	1.03	40.00	2.17	17.80	3.20
23	*Leucas aspera*	Lamiaceae	3.88	0.56	44.00	2.39	8.82	2.95
24	*Eleusine indica*	Poaceae	3.60	0.52	44.00	2.39	8.18	2.91
25	*Paspalidium flavidum*	Poaceae	6.40	0.93	28.00	1.52	22.86	2.45
26	*Fimbristylis dichotoma*	Cyperaceae	5.16	0.75	28.00	1.52	18.43	2.27
27	*Murdannia nudiflora*	Commelinaceae	3.56	0.51	28.00	1.52	12.71	2.04

Sl No	Species	Family	D	RD	F (%)	RF	AB	IVI
28	*Alysicarpus vaginalis*	Papilionaceae	1.84	0.27	32.00	1.74	5.75	2.01
29	*Lindernia crustacea*	Scrophulariaceae	2.68	0.39	28.00	1.52	9.57	1.91
30	*Lindernia pusilla*	Scrophulariaceae	2.00	0.29	28.00	1.52	7.14	1.81
31	*Dactyloctenium aegyptium*	Poaceae	1.84	0.27	28.00	1.52	6.57	1.79
32	*Fimbristylis Miliacea*	Cyperaceae	2.96	0.43	24.00	1.30	12.33	1.73
33	*Phyllanthus urinaria*	Euphorbiaceae	1.20	0.17	28.00	1.52	4.29	1.70
34	*Boerhavia diffusa*	Nyctaginaceae	1.20	0.17	28.00	1.52	4.29	1.70
35	*Commelina benghalensis*	Commelinaceae	1.36	0.20	24.00	1.30	5.67	1.50
36	*Amaranthus spinosus*	Amaranthaceae	0.76	0.11	24.00	1.30	3.17	1.41
37	*Senna occidentalis*	Caesalpiniaceae	0.44	0.06	24.00	1.30	1.83	1.37
38	*Colocasia esculenta*	Araceae	1.44	0.21	20.00	1.09	7.20	1.30
39	*Ageratum conyzoides*	Asteraceae	1.16	0.17	20.00	1.09	5.80	1.25
40	*Centella asiatica*	Apiaceae	2.44	0.35	16.00	0.87	15.25	1.22
41	*Eclipta prostrata*	Asteraceae	2.04	0.30	16.00	0.87	12.75	1.16
42	*Bulbostylis barbata*	Cyperaceae	2.76	0.40	12.00	0.65	23.00	1.05
43	*Amaranthus viridis*	Amaranthaceae	1.20	0.17	16.00	0.87	7.50	1.04
44	*Eragrostis unioloides*	Poaceae	1.08	0.16	16.00	0.87	6.75	1.03
45	*Acmella paniculata*	Asteraceae	1.00	0.14	16.00	0.87	6.25	1.01
46	*Lindernia ciliata*	Scrophulariaceae	0.92	0.13	16.00	0.87	5.75	1.00
47	*Parthenium hysterophorus*	Asteraceae	0.84	0.12	16.00	0.87	5.25	0.99
48	*Achyranthes aspera*	Amaranthaceae	0.72	0.10	16.00	0.87	4.50	0.97
49	*Euphorbia hirta*	Euphorbiaceae	0.64	0.09	16.00	0.87	4.00	0.96
50	*Tephrosia purpurea*	Papilionaceae	0.52	0.08	16.00	0.87	3.25	0.94
51	*Scoparia dulcis*	Scrophulariaceae	0.52	0.08	16.00	0.87	3.25	0.94
52	*Heliotropium indicum*	Boraginaceae	0.44	0.06	16.00	0.87	2.75	0.93
53	*Blumea lacera*	Asteraceae	0.36	0.05	16.00	0.87	2.25	0.92
54	*Cayratia trifolia*	Vitaceae	0.32	0.05	16.00	0.87	2.00	0.92
55	*Tridax procumbens*	Asteraceae	0.28	0.04	12.00	0.65	2.33	0.69
56	*Ludwigia perennis*	Onagraceae	0.24	0.03	12.00	0.65	2.00	0.69
57	*Physalis minima*	Solanaceae	0.16	0.02	12.00	0.65	1.33	0.68
58	*Solanum anguivi*	Solanaceae	0.16	0.02	12.00	0.65	1.33	0.68
59	*Physalis peruviana*	Solanaceae	0.08	0.01	8.00	0.43	1.00	0.45

(D=Density, RD=Relative Density, F=Frequency, RF=Relative Frequency, AB=Abundance, IVI=Important Value Index)

Table 4.4: Quantitative analysis of phytosociological data of site-2 (Bangshihari to Ushaharan via Mahipal)

Sl No.	Species	Family	D	RD	F (%)	RF	AB	IVI
1	*Ocimum × africanum*	Lamiaceae	4.68	0.65	48	2.29	9.75	2.94
2	*Ocimum americanum*	Lamiaceae	24.44	3.4	72	3.43	33.94	6.83
3	*Cynodon dactylon*	Poaceae	87.92	12.24	56	2.67	157	14.91
4	*Oplismenus burmannii*	Poaceae	78.36	10.91	72	3.43	108.83	14.34
5	*Chrysopogon aciculatus*	Poaceae	73.92	10.29	64	3.05	115.5	13.34
6	*Axonopus compressus*	Poaceae	54.36	7.57	56	2.67	97.07	10.24
7	*Desmodium triflorum*	Papilionaceae	49.04	6.83	52	2.48	94.31	9.31
8	*Kyllinga brevifolia*	Cyperaceae	38.76	5.4	72	3.43	53.83	8.83
9	*Evolvulus nummularius*	Convolvulaceae	29.92	4.17	72	3.43	41.56	7.6
10	*Brachiaria paspaloides*	Poaceae	36.52	5.09	48	2.29	76.08	7.37
11	*Brachiaria villosa*	Poaceae	24.2	3.37	52	2.48	46.54	5.85
12	*Senna tora*	Caesalpiniaceae	12.36	1.72	80	3.81	15.45	5.53
13	*Eragrostis tenella*	Poaceae	18.8	2.62	52	2.48	36.15	5.09
14	*Murdannia nudiflora*	Commelinaceae	11.44	1.59	64	3.05	17.88	4.64
15	*Cyperus rotundus*	Cyperaceae	17.88	2.49	44	2.1	40.64	4.59
16	*Digitaria longiflora*	Poaceae	17.72	2.47	32	1.52	55.38	3.99
17	*Clerodendrum viscosum*	Verbenaceae	6.72	0.94	64	3.05	10.5	3.98
18	*Oldenlandia diffusa*	Rubiaceae	6.52	0.91	48	2.29	13.58	3.19
19	*Eragrostis gangetica*	Poaceae	11.76	1.64	32	1.52	36.75	3.16
20	*Paspalidium flavidum*	Poaceae	11.16	1.55	32	1.52	34.88	3.08
21	*Eleusine indica*	Poaceae	5.52	0.77	48	2.29	11.5	3.05
22	*Fimbristylis Miliacea*	Cyperaceae	6.28	0.87	36	1.71	17.44	2.59
23	*Rungia pectinata*	Acanthaceae	10	1.39	24	1.14	41.67	2.54
24	*Fimbristylis dichotoma*	Cyperaceae	5.76	0.8	36	1.71	16	2.52
25	*Paspalum laeve*	Poaceae	8.8	1.23	24	1.14	36.67	2.37
26	*Alternanthera sessilis*	Amaranthaceae	4.8	0.67	32	1.52	15	2.19
27	*Commelina benghalensis*	Commelinaceae	3	0.42	36	1.71	8.33	2.13
28	*Ageratum conyzoides*	Asteraceae	4.24	0.59	32	1.52	13.25	2.11
29	*Centella asiatica*	Apiaceae	5.4	0.75	28	1.33	19.29	2.09
30	*Croton bonplandianum*	Euphorbiaceae	2.48	0.35	36	1.71	6.89	2.06
31	*Dactyloctenium aegyptium*	Poaceae	2.36	0.33	32	1.52	7.38	1.85
32	*Cyperus compressus*	Cyperaceae	3.72	0.52	28	1.33	13.29	1.85

Sl No.	Species	Family	D	RD	F (%)	RF	AB	IVI
33	*Lindernia pusilla*	Scrophulariaceae	2.12	0.3	32	1.52	6.63	1.82
34	*Lindernia crustacea*	Scrophulariaceae	2.76	0.38	28	1.33	9.86	1.72
35	*Senna occidentalis*	Caesalpiniaceae	0.68	0.09	32	1.52	2.13	1.62
36	*Alysicarpus vaginalis*	Papilionaceae	2	0.28	28	1.33	7.14	1.61
37	*Phyllanthus urinaria*	Euphorbiaceae	1.28	0.18	28	1.33	4.57	1.51
38	*Oxalis corniculata*	Oxalidaceae	5.04	0.7	16	0.76	31.5	1.46
39	*Lindernia ciliata*	Scrophulariaceae	1.72	0.24	24	1.14	7.17	1.38
40	*Eclipta prostrata*	Asteraceae	1.6	0.22	24	1.14	6.67	1.37
41	*Colocasia esculenta*	Araceae	1.28	0.18	24	1.14	5.33	1.32
42	*Leucas aspera*	Lamiaceae	1.08	0.15	24	1.14	4.5	1.29
43	*Acmella paniculata*	Asteraceae	2.4	0.33	20	0.95	12	1.29
44	*Tridax procumbens*	Asteraceae	1.92	0.27	20	0.95	9.6	1.22
45	*Eragrostis unioloides*	Poaceae	1.52	0.21	20	0.95	7.6	1.16
46	*Euphorbia hirta*	Euphorbiaceae	1.04	0.14	20	0.95	5.2	1.1
47	*Parthenium hysterophorus*	Asteraceae	2.28	0.32	16	0.76	14.25	1.08
48	*Ludwigia perennis*	Onagraceae	0.84	0.12	20	0.95	4.2	1.07
49	*Amaranthus spinosus*	Amaranthaceae	0.8	0.11	20	0.95	4	1.06
50	*Typhonium trilobatum*	Araceae	0.48	0.07	20	0.95	2.4	1.02
51	*Sida cordifolia*	Malvaceae	3.16	0.44	12	0.57	26.33	1.01
52	*Cayratia trifolia*	Vitaceae	0.32	0.04	20	0.95	1.6	1
53	*Amaranthus viridis*	Amaranthaceae	0.84	0.12	16	0.76	5.25	0.88
54	*Achyranthes aspera*	Amaranthaceae	0.8	0.11	16	0.76	5	0.87
55	*Spermacoce hispida*	Rubiaceae	0.72	0.1	16	0.76	4.5	0.86
56	*Scoparia dulcis*	Scrophulariaceae	0.56	0.08	16	0.76	3.5	0.84
57	*Heliotropium indicum*	Boraginaceae	0.48	0.07	16	0.76	3	0.83
58	*Physalis minima*	Solanaceae	0.44	0.06	16	0.76	2.75	0.82
59	*Mikania micrantha*	Asreraceae	0.28	0.04	16	0.76	1.75	0.8
60	*Blumea lacera*	Asteraceae	0.36	0.05	12	0.57	3	0.62
61	*Boerhavia diffusa*	Nyctaginaceae	0.24	0.03	12	0.57	2	0.6
62	*Solanum anguivi*	Solanaceae	0.16	0.02	12	0.57	1.33	0.59

(D=Density, RD=Relative Density, F=Frequency, RF=Relative Frequency, AB=Abundance, IVI=Important Value Index)

4.2.2. Plant community study of Site-3

At site-3 the frequency of *O. tenuiflorum* (Green) and *O. tenuiflorum* (Purple) were 48% and 50% respectively. Density of *O. tenuiflorum* (Green) and *O. tenuiflorum* (Purple) was 7.28 and 5.28 (plants/m^2). In case of the abundance of *O. tenuiflorum* (Green) and *O. tenuiflorum* (Purple) was 15.17 and 10.15. The IVI value of *O. tenuiflorum* (Green) was 5.67 and *O. tenuiflorum* (Purple) 5.42.

Two species of *Ocimum* were found to coexist with 48 other species belonging to 19 (16 dicots and 3 monocots) families and 46 genera. Out of 48 species 29 were from dicotyledons and 19 were from monocotyledons (Figure 4.9). Most of the recorded species were annual and herbaceous. The most abundant family was Poaceae (14 species) followed by Lamiaceae (5 species) and Papilionaceae (4 species).

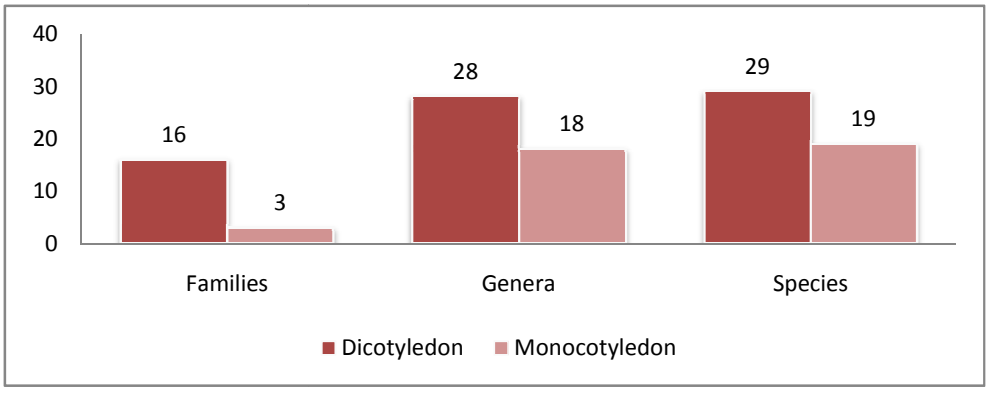


Figure 4.9: Graphical representation of species detailed in site-3

Coexistence of other species and their frequency with *O. tenuiflorum* (both green and purple type) ranged from 8% to 84% with maximum frequency was recorded for *Evolvulus nummularius* (84%) followed by *Cynodon dactylon* (72%), *Brachiaria paspaloides* and *Desmodium triflorum* (60%), *Eleusine indica* (48%) etc. and *Paspalidium flavidum*, *Euphorbia hirta*, *Tephrosia purpure*, *Leucas aspera*, *Alysicarpus vaginalis*, *Spermacoce hispida*, *Boerhavia diffusa*, *Anisomeles indica* and *Hyptis suaveolens* (8%) was recorded with minimum frequency (Table 4.5).

The maximum density and relative density was observed for *Cynodon dactylon* (97.2 and 26.65) followed by *Brachiaria paspaloides* (35.04 and 9.61), *Evolvulus*

nummularius (24.56 and 6.73), *Kyllinga brevifolia*, *Oplismenus burmannii* (24.16 and 6.62) and *Axonopus compressus* (19.6 and 5.37).

Highest abundance value (135) was observed for *Cynodon dactylon* followed by *Axonopus compressus* (98), *Brachiaria paspaloides* (58.4), *Brachiaria villosa* (55.38), *Kyllinga brevifolia* (54.91) and *Chrysopogon aciculatus* (47.56).

Cynodon dactylon was found to be the most prevailing species in study site-3 with IVI value 32.16 followed by *Brachiaria paspaloides* (14.2), *Evolvulus nummularius* (13.16), *Kyllinga brevifolia* (9.99) and *Desmodium triflorum* (8.86) (Table 4.6). The lowest IVI values of *Anisomeles indica* and *Hyptis suaveolens* (0.63) showed that they are the rare species of that particular community.

Table 4.5: Quantitative analysis of phytosociological data of site-3 (Patiram to Daulatpur)

Sl No.	Species	Family	D	RD	F (%)	RF	AB	IVI
1	*Ocimum tenuiflorum* (Green)	Lamiaceae	7.28	2	48	3.67	15.17	5.67
2	*Ocimum tenuiflorum* (Purple)	Lamiaceae	5.28	1.45	52	3.98	10.15	5.42
3	*Cynodon dactylon*	Poaceae	97.2	26.65	72	5.5	135	32.16
4	*Brachiaria paspaloides*	Poaceae	35.04	9.61	60	4.59	58.4	14.2
5	*Evolvulus nummularius*	Convolvulaceae	24.56	6.73	84	6.42	29.24	13.16
6	*Kyllinga brevifolia*	Cyperaceae	24.16	6.62	44	3.36	54.91	9.99
7	*Axonopus compressus*	Poaceae	19.6	5.37	20	1.53	98	6.9
8	*Brachiaria villosa*	Poaceae	17.72	4.86	32	2.45	55.38	7.31
9	*Chrysopogon aciculatus*	Poaceae	17.12	4.69	36	2.75	47.56	7.45
10	*Desmodium triflorum*	Papilionaceae	15.6	4.28	60	4.59	26	8.86
11	*Oplismenus burmannii*	Poaceae	13.64	3.74	32	2.45	42.63	6.19
12	*Chloris barbata*	Poaceae	10.84	2.97	28	2.14	38.71	5.11
13	*Cyperus rotundus*	Cyperaceae	10.68	2.93	36	2.75	29.67	5.68
14	*Rungia pectinata*	Acanthaceae	8.2	2.25	40	3.06	20.5	5.31
15	*Fimbristylis dichotoma*	Cyperaceae	6.44	1.77	16	1.22	40.25	2.99
16	*Eleusine indica*	Poaceae	5.32	1.46	48	3.67	11.08	5.13
17	*Parthenium hysterophorus*	Asteraceae	3.6	0.99	52	3.98	6.92	4.96
18	*Eragrostis tenella*	Poaceae	3.56	0.98	16	1.22	22.25	2.2
19	*Hemigraphis hirta*	Acanthaceae	3.28	0.9	40	3.06	8.2	3.96

Sl No.	Species	Family	D	RD	F (%)	RF	AB	IVI
20	*Senna tora*	Caesalpiniaceae	3.28	0.9	28	2.14	11.71	3.04
21	*Oldenlandia diffusa*	Rubiaceae	3	0.82	24	1.83	12.5	2.66
22	*Clerodendrum viscosum*	Verbenaceae	2.52	0.69	28	2.14	9	2.83
23	*Dactyloctenium aegyptium*	Poaceae	2.44	0.67	24	1.83	10.17	2.5
24	*Alternanthera sessilis*	Amaranthaceae	2.32	0.64	32	2.45	7.25	3.08
25	*Digitaria longiflora*	Poaceae	2.32	0.64	16	1.22	14.5	1.86
26	*Paspalum laeve*	Poaceae	2.32	0.64	12	0.92	19.33	1.55
27	Commelina sp.	Commelinaceae	2.08	0.57	24	1.83	8.67	2.41
28	*Murdannia nudiflora*	Commelinaceae	1.6	0.44	16	1.22	10	1.66
29	*Croton bonplandianum*	Euphorbiaceae	1.28	0.35	24	1.83	5.33	2.19
30	*Ageratum conyzoides*	Asteraceae	1.24	0.34	16	1.22	7.75	1.56
31	*Paspalidium flavidum*	Poaceae	1.24	0.34	8	0.61	15.5	0.95
32	*Achyranthes aspera*	Amaranthaceae	1.12	0.31	24	1.83	4.67	2.14
33	*Centella asiatica*	Apiaceae	1.08	0.3	12	0.92	9	1.21
34	*Eragrostis unioloides*	Poaceae	1.04	0.29	12	0.92	8.67	1.2
35	*Sida cordifolia*	Malvaceae	1	0.27	16	1.22	6.25	1.5
36	*Cayratia trifolia*	Vitaceae	0.96	0.26	28	2.14	3.43	2.4
37	*Amaranthus spinosus*	Amaranthaceae	0.92	0.25	12	0.92	7.67	1.17
38	*Phyllanthus urinaria*	Euphorbiaceae	0.56	0.15	12	0.92	4.67	1.07
39	*Senna occidentalis*	Caesalpiniaceae	0.4	0.11	24	1.83	1.67	1.94
40	*Scoparia dulcis*	Scrophulariaceae	0.4	0.11	12	0.92	3.33	1.03
41	*Euphorbia hirta*	Euphorbiaceae	0.4	0.11	8	0.61	5	0.72
42	*Tephrosia purpurea*	Papilionaceae	0.4	0.11	8	0.61	5	0.72
43	*Ludwigia perennis*	Onagraceae	0.28	0.08	12	0.92	2.33	0.99
44	*Leucas aspera*	Lamiaceae	0.28	0.08	8	0.61	3.5	0.69
45	*Alysicarpus vaginalis*	Papilionaceae	0.28	0.08	8	0.61	3.5	0.69
46	*Spermacoce hispida*	Rubiaceae	0.28	0.08	8	0.61	3.5	0.69
47	*Boerhavia diffusa*	Nyctaginaceae	0.2	0.05	8	0.61	2.5	0.67
48	*Crotalaria pallida*	Papilionaceae	0.16	0.04	12	0.92	1.33	0.96
49	*Anisomeles indica*	Lamiaceae	0.08	0.02	8	0.61	1	0.63
50	*Hyptis suaveolens*	Lamiaceae	0.08	0.02	8	0.61	1	0.63

(D=Density, RD=Relative Density, F=Frequency, RF=Relative Frequency, AB=Abundance, IVI=Important Value Index)

Phytosociological investigation revealed that dicotyledons species were observed most extreme in number when contrasted to monocotyledons in all quadrat plots of all the sites studied but monocotyledons were more dominant species. Most common and abundance species along with *O. americanum*, *O.* × *africanum* and both the morphotypes of *O. tenuiflorum* (purple type and green type) were *Axonopus compressus*, *Cynodon dactylon*, *Brachiaria paspaloides*, *Kyllinga brevifolia*, *Chrysopogon aciculatus*, *Oplismenus burmannii*, *Brachiaria paspaloides*, *Desmodium triflorum* etc. The composition of species and their distribution of the three sites were almost similar. It may be due to same or slightly different altitude, same ecological conditions and nutritional requirement for their normal growth. Abundance of the species composition also depends on their germination time/season. *Ocimum* species naturally germinate during rainy season (June to July) and the germination is season specific and may be true for other associated species.

It has been observed that *O. americanum* and *O.* × *africanum* sometimes grown separately or alongside with each other. But both morphotypes of *O. tenuiflorum* grow separately. The co-existance of *O.* × *africanum* and *O. americanum* indicate that there might be a communal association between them (Chowdhury *et al*., 2016). Beside its medicinal applications it was found that *Ocimum* species have an allelopathic effect on seed germination and seedling development on most unsafe weed like *Parthenium hysterophorus* (Knox *et al*., 2010). The study also revealed that where *Ocimum* species grown, the density and frequency of *P. hysterophorus* was very low. Thus, increase of natural vegetation of *Ocimum* species may be an excellent way to eradicate *Parthenium* species.

Nevertheless, this phytosociological study may be used for *in situ* and *ex situ* conservation of *O. americanum*, *O.* × *africanum* and *O. tenuiflorum* (purple and green) through maintaining moderate populations of *Axonopus compressus*, *Brachiaria paspaloides*, *Cynodon dactylon*, *Chrysopogon aciculatus*, *Oplismenus burmannii*, *Kyllinga brevifolia*, *Desmodium triflorum*, *Evolvulus nummularius* etc.

Table 4.6: Dominant and most frequent associated species with *O.* × *africanum*, *O. americanum*, *O. tenuiflorum* (Purple) and *O. tenuiflorum* (Green) in different study sites based on IVI and frequencies

***Ocimum* species**	**Sites**	**Dominant associates species**	**Most frequently observed species**
Ocimum × *africanum* *Ocimum americanum*	Site-1	*Cynodon dactylon* (14.92), *Axonopus compressus* (13.83), *Oplismenus burmannii* (12.42), *Brachiaria paspaloides* (11.93) and *Chrysopogon aciculatus* (11.05)	*Senna tora* (72%), *Oplismenus burmannii* (60%), *Cynodon dactylon* (56%), *Axonopus compressus* (56%), *Clerodendrum viscosum* (56%)
Ocimum × *africanum* *Ocimum americanum*	Site-2	*Cynodon dactylon* (14.91), *Oplismenus burmannii* (14.34), *Chrysopogon aciculatus* (13.34), *Axonopus compressus* (10.24) and *Desmodium triflorum* (9.31)	*Senna tora* (80%), *Kyllinga brevifolia*, *Oplismenus burmannii* and *Evolvulus nummularius* (72%), *Clerodendrum viscosum*, *Murdannia nudiflora* and *Chrysopogon aciculatus* (64%)
O. tenuiflorum (Green) *O. tenuiflorum* (Purple)	Site-3	*Cynodon dactylon* (32.16), *Brachiaria paspaloides* (14.2), *Evolvulus nummularius* (13.16), *Kyllinga brevifolia* (9.99) and *Desmodium triflorum* (8.86)	*Evolvulus nummularius* (84%), *Cynodon dactylon* (72%), *Brachiaria paspaloides* and *Desmodium triflorum* (60%)

4.2.3. Climatic and physicochemical properties of soil

The occurrences of all the species studied including *Ocimum* were grown naturally during rainy season (June-July). From the meteorological data it was clear that 15.2-33.1 ^{o}C temperature, 48-95 % relative humidity with average rainfall of 183.1 mm were most preferable for their luxuriant growth and development (Figure 4.10). Although *Ocimum* species were prefer to grow in clayey-loam or loamy soil but they can grow well in sandy to clayey soil. It was noted that *O. americanum*, *O.* × *africanum* and *O. tenuiflorun* (purple and green) preferred slightly acidic soil except water logged conditions for their natural growth and development. Soil pH ranged from 5.9 to 6.3 for site-1, 5.9- 6.5 for site-2 and 5.7- 6.3 for site-3 which indicated the slightly acidic nature soil and there was not much variation in the pH values at different sites. Electrical conductivity (EC) of site-3 was comparatively lower than site-1 and site-2. Organic carbon ware high in site-1 (0.77-0.82%) and site-2 (0.5-0.77%) and medium in

site-3 (0.66-0.72%). The available nitrogen was found to be higher in site-1 in comparison to other two sites under study. Available phosphorus ranged between 60 – 70 kg ha-^{1} for site-1; 60 – 80 kg ha-^{1} for site-2 and 65 – 70 kg ha-^{1} for site-3. The available potassium content of all the studied sites were recorded high (Table 4.7).

Table 4.7: Physico-chemical characteristics of soil samples from different study sites

Site	Soil texture	pH	EC (Mmhos/cm)	Organic C (%)	Available N (kg ha^{-1})	Available P (kg ha^{-1})	Available K (kg ha^{-1})
S-1	Sandy- clay loam	5.9- 6.3	0.16- 0.18	0.77- 0.82	331- 353	60 - 70	>400
S-2	Loamy - clay loam	5.9- 6.5	0.12- 0.18	0.52 - 0.77	223 - 331	60 - 80	>400
S-3	Sandy loam- Clayey	5.7- 6.3	0.07- 0.14	0.66 - 0.72	284 - 309	65 - 70	214- >400

EC- below 1=Normal, 1-2=CG (critical for germination), 2-3=CGS (critical for salt sensitive crops), >3=IC (injurious to most of the crops); **N**-280-450=Medium, <280=Low, > 450=High; **P**- 45-90=Medium, <45=Low, > 90=High; **K**-<200=Low, 200-350=Medium, > 350=High; **Organic carbon** - 0.5-0.75=Medium, < 0.5=Low, > 0.75=High.

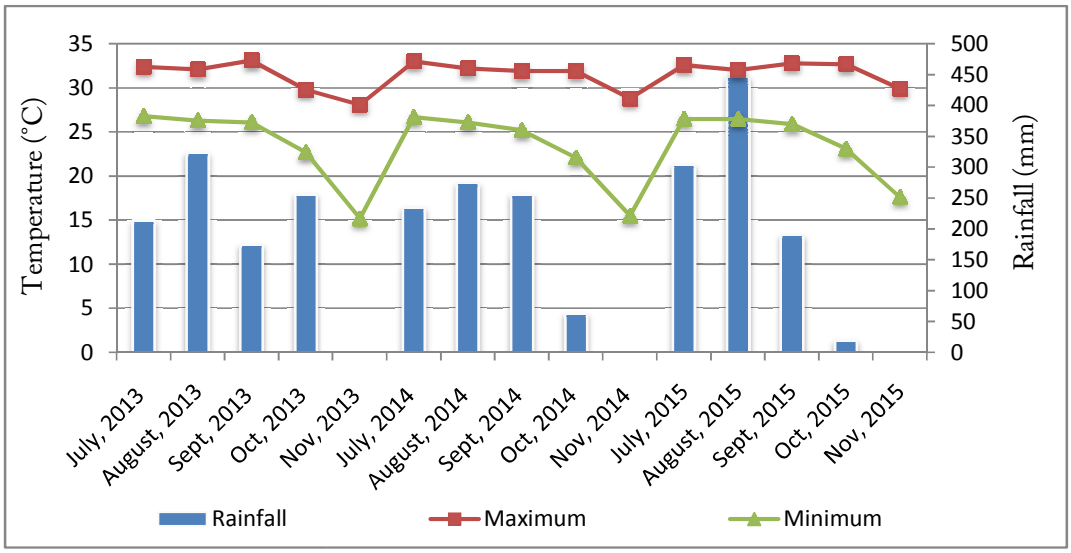


Figure 4.10: Meteorological data of the study area, based on the temperature (min. and max.) and rainfall during study period

4.3. Morphological variation

4.3.1. Qualitative traits

It is very important to understand the diversity of a plant species since morphological characters are related to numerous branches of biological sciences. A significant level of morphological variability was recorded in *Ocimum* species. For identification of *Ocimum* species morphological traits play the major role (Agarwal *et al*., 2013b). In the qualitative characters a significant variability was noticed on stem pubescence, stem colour, leaf surface, leaf margin, leaf tip, leaf shape, inflorescence type, flower colour, anther colour, seed shape and seed colour (Table 4.8). However, plant growth habit (erect) and mode of reproduction (sexual) were found to be monomorphic in nature.

Generally, three types of plant habit were found in *Ocimum* species *viz*., (i) annual, (ii) biannual and (iii) perennial. It was observed that both the two morphotypes of *O. gratissimum* (Ram and Ajowan tulsi) and *O. kilimandscharicum* were perennial. *O. basilicum* (Babui and Marua tulsi), *O.* × *africanum* and *O. americanum* were annual, on the other hand both type of *O. tenuiflorum* (Krishna and Radha tulsi) were found to be biannual. It was also observed that among the nine *Ocimum* taxa studied, all were erect type. Two types of stem shape were observed as quadrate and square among all the taxa. Six of them namely *O. gratissimum* (Ram tulsi), *O. gratissimum* (Ajowan), *O. basilicum* (Babu tulsi), *O. basilicum* (Marua tulsi), *O.* × *africanum* and *O. americanum* had quadrangular/square stem, while both *O. tenuiflorum* (Krishna and Radha tulsi) and *O. kilimandscharicum* had round type of stem (Figure 4.11).

Pubescence is a typical character of *Ocimum* species. Pubescence was recorded in various plant parts, for example, stem, leaf, inflorescence and flower. Sparse type of stem pubescent [*O. gratissimum* (Ajowan tulsi), *O. americanum*, *O. basilicum* (Babu tulsi) and *O.* × *africanum*], dense type of stem pubescent [*O. kilimandscharicum* and *O. tenuiflorum* (Krishna and Radha tulsi)] and glabrous stem [*O. basilicum* (Marua tulsi) and *O. gratissimum* (Ram tulsi)] have been observed in various *Ocimum* taxa studied (Figure 4.11). It has been observed that the stem colour greatly varied [Brownish- *O. gratissimum* (Ram and Ajowan tulsi); Light green- *O. kilimandscharicum*, *O. americanum* and *O.* × *africanum*; Purple green- *O. basilicum* (Babu tulsi) and Deep purple colour- *O. basilicum* (Marua tulsi) and *O. tenuiflorum* (Krishna tulsi)] within the *Ocimum* taxa (Figure 4.11).

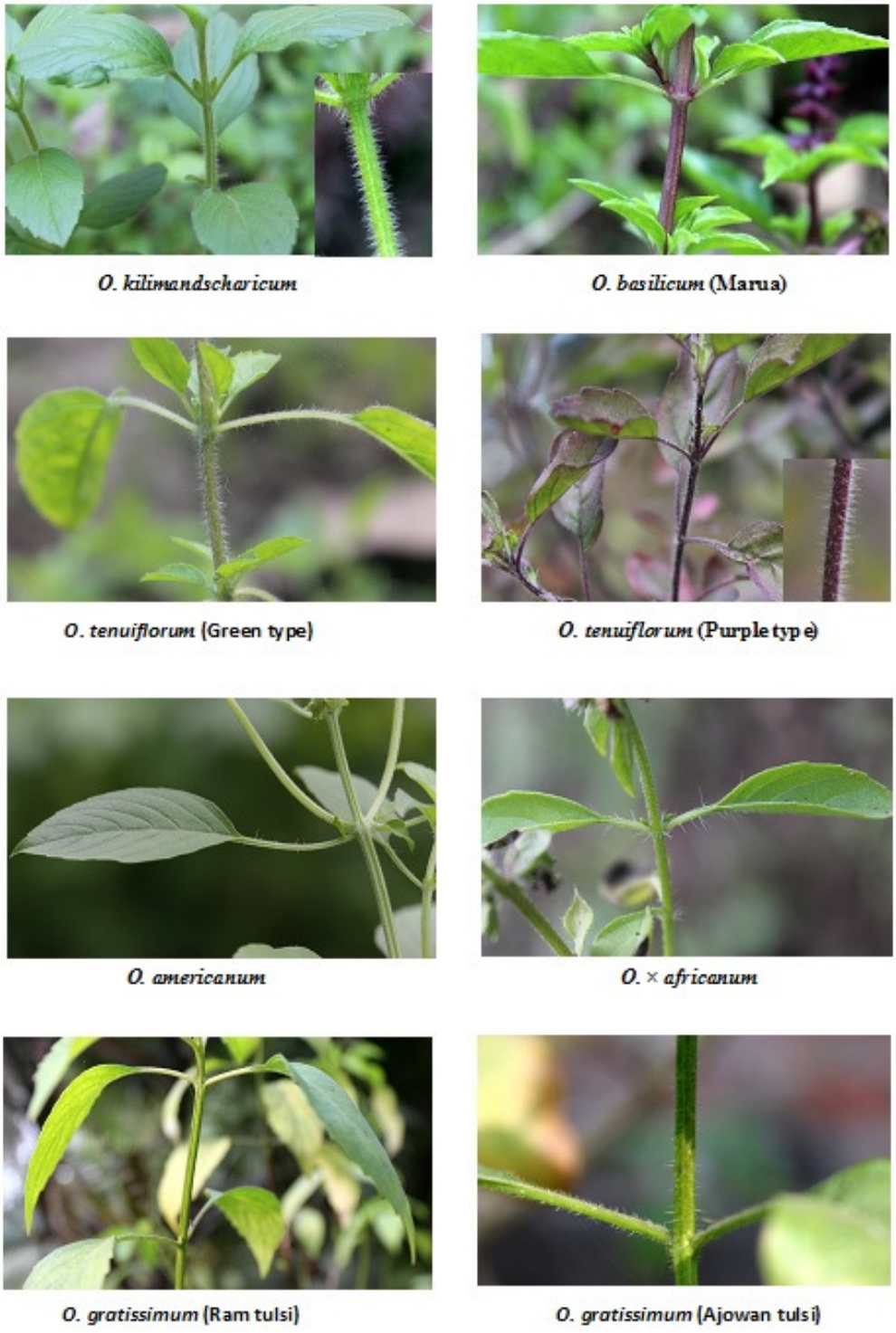


Figure 4.11: Stem shape, colour and pubescence of different *Ocimum* taxa

Leaf margin varied from serrate [*O. gratissimum* (Ajowan and Ram tulsi), *O. kilimandscharicum*, *O. americanum*, *O. basilicum* (Babu and Marua tulsi) and *O.* ×

africanum] to dented [*O. tenuiflorum* (Krishna and Radha tulsi)]. *O. gratissimum* (Ajowan and Ram tulsi) showed acute to acuminate leaf tip and broad ovate to lanceolate leaf shape. *O. kilimandscharicum*, *O. americanum* and *O. basilicum* (Babu and Marua tulsi) on the other hand showed acute leaf tip with elliptic leaf shape. But *O. tenuiflorum* (Krishna and Radha tulsi) have obtuse to acute leaf tip with ovate leaf shape (Figure 4.12). Similar kind of results was reported by Chowdhury *et al*. (2017).

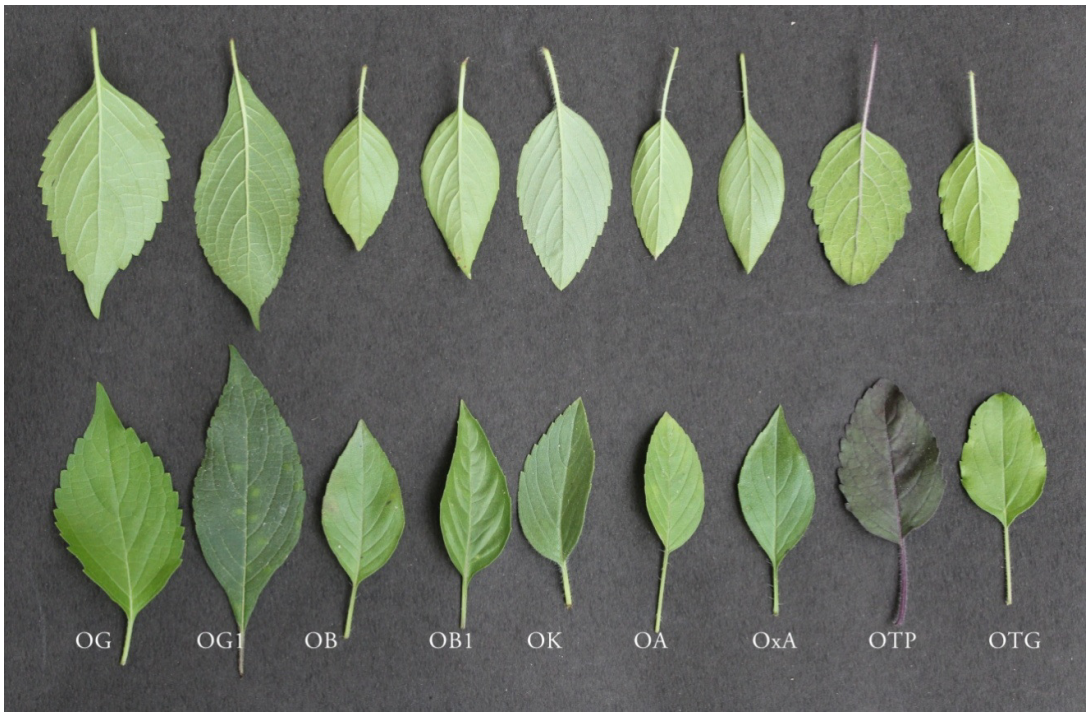


Figure 4.12: Leaf shape and colour variation of studied *Ocimum* taxa, **OG**- *O. gratissimum* (Ram tulsi); **OG1**- *O. gratissimum* (Ajowan tulsi); **OB**- *O. basilicum* (Babui tulsi); **OB1**- *O. basilicum* (Marua tulsi); **OK**- *O. kilimandscharicum* (Karpur tulsi); **OA**- *O. americanum* (Bon tulsi); **OxA**- *O.* × *africanum* (Lebu tulsi); **OTP**- *O. tenuiflorum* (Krishna tulsi); **OTG**- *O. tenuiflorum* (Radha tulsi)

Leaf surface showed significant level of variations *viz*., glabrous except hairy midrib, veinlets and margin [*O. basilicum* (Babu tulsi), *O.* × *africanum* and *O. americanum*], sparse and wavy or undulated [*O. gratissimum* (Ajowan tulsi)], patently hairy to clothed with soft spreading hairs [*O. tenuiflorum* (Purple and Green type) and *O. kilimandscharicum*], while *O. basilicum* (Marua tulsi) showed glabrous leaf surface. Especially, most of the *Ocimum* taxa showed light green leaf colour except *O. gratissimum* (Ajowan tulsi) (Deep green) and *O. tenuiflorum* (Krishna tulsi) (Purple colour). In the present study, two morphotypes of *O. tenuiflorum* showed purple and light green colour of leaf. Light green leaves colour tulsi recognized as "Radha tulsi"

and the other one with deep green or purple leaves usually known as ‘‘Krishna tulsi’’ (Figure 4.12). Three morphotypes of *O. tenuiflorum* (green, purple and purple-green) were reported earliar by Maheshwari *et al*. (1987) while, five morphotypes were reported later by Mondello *et al*. (2002).

Inflorescence arrangement composed of two opposite cymes known as verticillaster which is a characteristic feature of *Ocimum* species. Variation was also observed in inflorescence type (Figure 4.13). Out of nine taxa *O. gratissimum* (Rum and Ajowan tulsi), *O. tenuiflorum* (Krishna and Radha tulsi) and *O. kilimandscharicum* showed branched inflorescence and rest of the species showed unbranched or simple type of inflorescence. It has been observed that green colour inflorescence in *O. americanum*, *O. basilicum* (Babu tulsi), *O.* × *africanum* and *O. tenuiflorum* (Green), greenish purple in *O. gratissimum* (Ram tulsi), purple in *O. tenuiflorum* (Purple) and greenish gray in *O. kilimandscharicum*. Presence of pubescence on inflorescence was observed in all the *Ocimum* taxa but degree of pubescence varied greatly. For example, *O. tenuiflorum*, *O. gratissimum* and *O. basilicum* species had weak hairiness in the inflorescence while, *O. kilimandscharicum* and *O. americanum* species had medium or strong hairiness.

Floral characters play most important role in the identification of any plant system. Flowers, specifically colour of corolla showed a distinct variation among the *Ocimum* taxa (Figure 4.13). Four types of flower colour were observed in the studied *Ocimum* taxa. These were yellowish white [*O. gratissimum* (Ajowan and Ram tulsi)], white (*O. kilimandscharicum*, *O. americanum* and *O.* × *africanum*), whitish pink [*O. basilicum* (Babu and Marua tulsi)] and purple [*O. tenuiflorum* (Krishna and Radha tulsi)]. Pollen colour is an important character to distinguish *Ocimum* species. *O. gratissimum* (Ajowan and Ram tulsi) and *O. tenuiflorum* (Krishna and Radha tulsi) showed yellow coloured pollen while *O. americanum*, *O. basilicum* (Babui and Marua tulsi) and *O.* × *africanum* showed white coloured pollen. *O. kilimandscharicum* has brick red or gray coloured pollen which was entirely different from other taxa. Colour of pollen is therefore may serve as an important morphological traits to understand the diversity among *Ocimum* taxa.

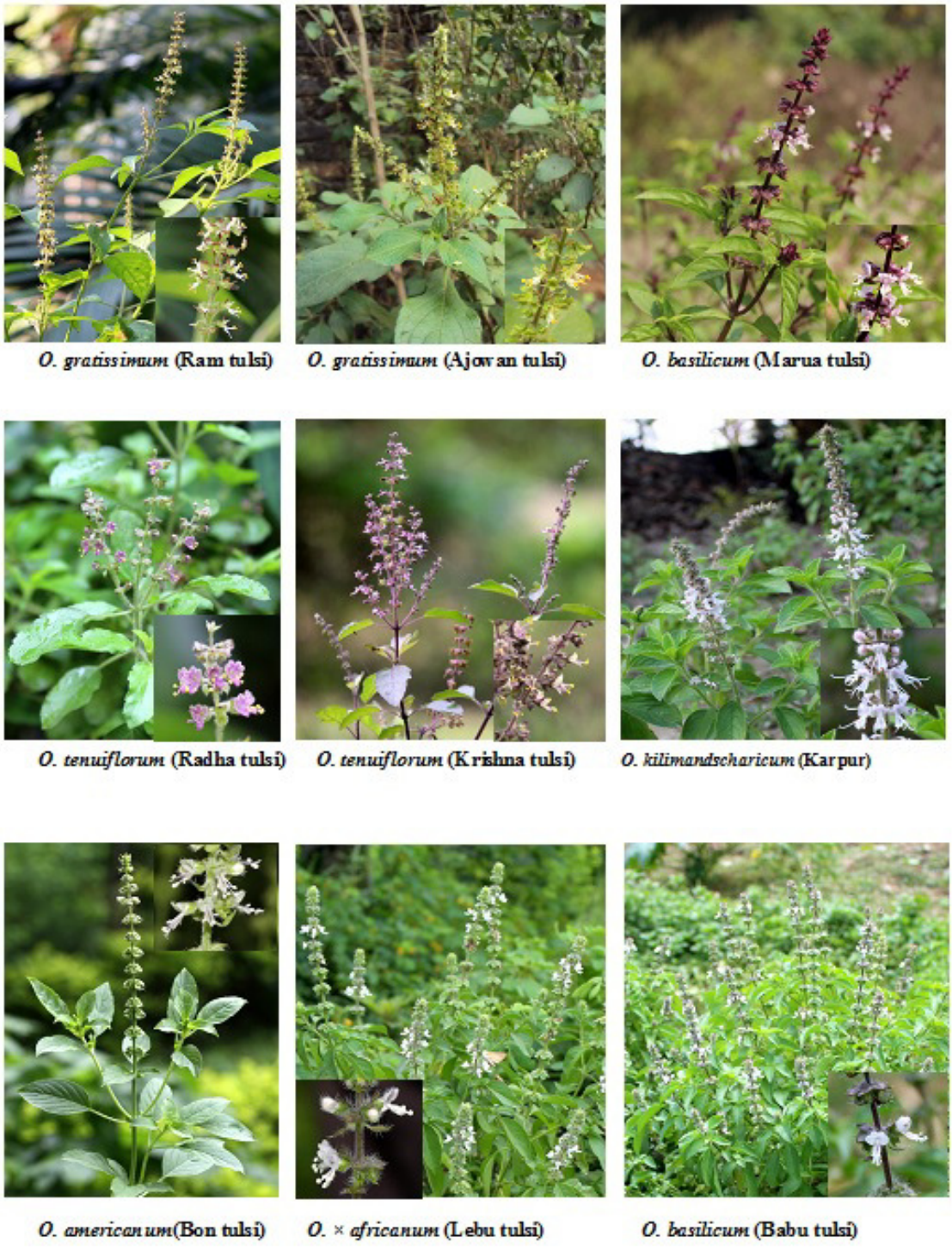


Figure 4.13: Inflorescence type and flower colour of different *Ocimum* taxa

Recently, few reports are available on the basis of their seed morphological characters to differentiate closely related *Ocimum* species (Patel *et al.*, 2015a). Seeds of all the *Ocimum* taxa vary from brown to black in colour. The seed coat of both *O. tenuiflorum*

(Krishna and Radha tulsi) and *O. gratissimum* (Ram and Ajowan tulsi) had brown or reddish brown in colour while *O. basilicum* (Babu and Marua tulsi), *O.* × *africanum*, *O. americanum* and *O. kilimandscharicum* were found to have black coloured (Figure 4.14). Seed shape also showed significant difference among the *Ocimum* taxa studied. The observed seed shape were subglobose to globose [*O. gratissimum* (Ajowan and Ram tulsi)], globose [*O. tenuiflorum* (Krishna and Radha tulsi)], small elliptic (*O. kilimandscharicum* and *O. americanum*) and broadly elliptic [*O. basilicum* (Marua and Babu tulsi) and *O.* × *africanum*]. It was observed that few seeds were mucilaginous [*O. basilicum* (Babu and Marua tulsi), *O.* × *africanum*, *O. americanum* and *O. kilimandscharicum*] and few were non-mucilaginous [*O. gratissimum* (Ajowan and Ram tulsi) and *O. tenuiflorum* (Krishna and Radha tulsi)] when moistened in water. These observations are in conformity with Patel *et al*. (2015a).

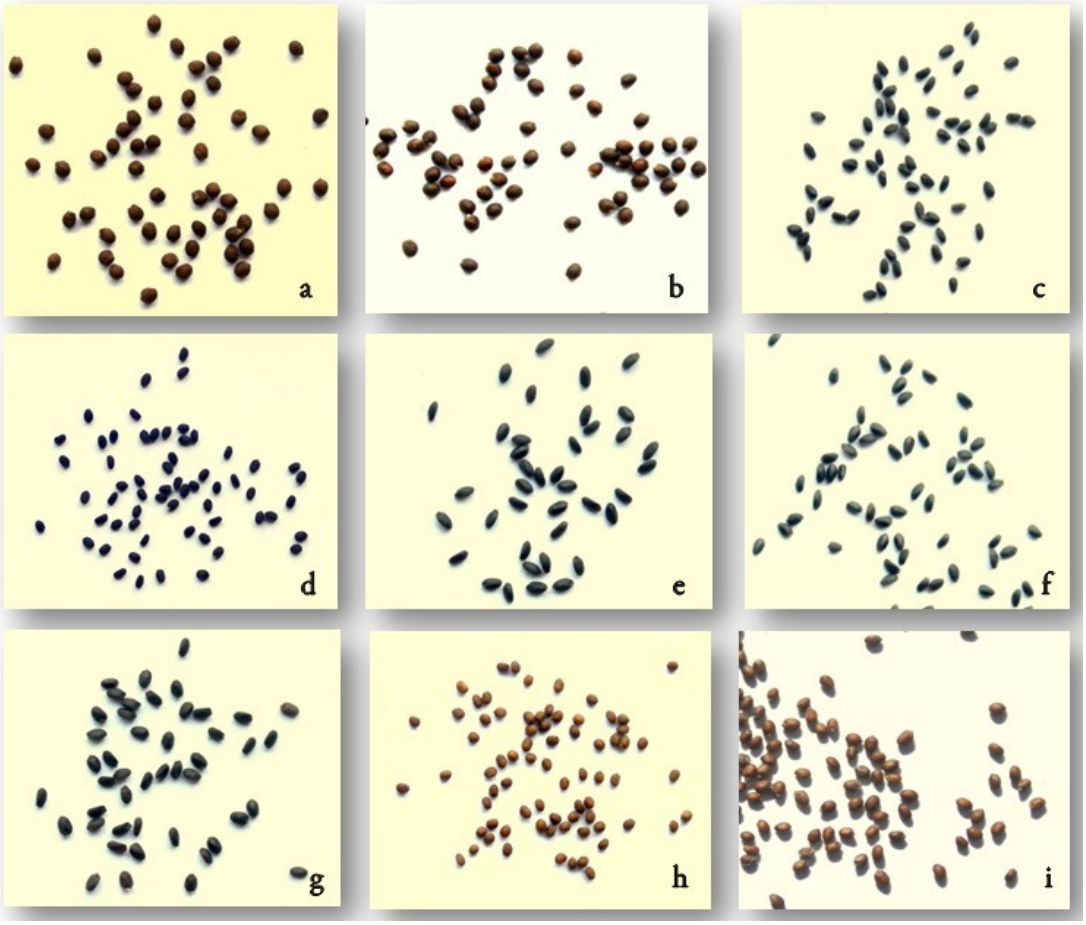


Figure 4.14: Seed shape variation of different *Ocimum* taxa, **a**- *O. gratissimum* (Ram tulsi), **b-** *O. gratissimum* (Ajowan tulsi), **c-** *O. americanum*, **d-** *O. kilimandscharicum*, **e-** *O.* × *africanum*, **f-** *O. basilicum* (Babu tulsi), **g-** *O. basilicum* (Marua tulsi), **h-** *O. tenuiflorum* (Purple type), **i-** *O. tenuiflorum* (Green type)

Table 4.8: Qualitative characters of nine *Ocimum* taxa

Characters	*O. gratissimum* (Ajowan tulsi)	*O. gratissimum* (Ram tulsi)	*O. kilimandscharicum*	*O. americanum*	*O. basilicum* (Babu tulsi)	*O.* × *africanum*	*O. basilicum* (Marua tulsi)	*O. tenuiflorum* (Purple type)	*O. tenuiflorum* (Green type)
Habit	Perennial	Perennial	Perennial	Annual	Annual	Annual	Annual	Biannual	Biannual
Growth habit	Erect	Erect	Erect	Erect	Erect	Erect	Erect	Erect	Erect
Mode of reproduction	Sexual	Sexual	Sexual	Sexual	Sexual	Sexual	Sexual	Sexual	Sexual
Stem colour	Brownish	Brownish	Light green	Light green	Purple-green	Light green	Purple-green	Purple	Light green
Stem shape	Quadrangular	Quadrangular	Quadrangular	Quadrangular	Quadrangular	Quadrangular	Quadrangular	Round	Round
Stem pubescence	Sparse	Glabrous	Dense	Sparse	Sparse	Sparse	Glabrous	Dense	Dense
Leaf colour	Dark green	Light green	Light green	Light green	Light green	Light green	Light green	Purplish-green	Light green
Leaf surface	Sparse	Glabrous	Sparse	Glabrous	Glabrous	Glabrous	Glabrous	Sparse	Sparse
Leaf margin	Serrate	Serrate	Serrate	Serrate	Serrate	Serrate	Serrate	Dented	Dented
Leaf tip	Acute-acuminate	Acute-acuminate	Acute	Acute	Acute	Acute	Acute	Obtuse to acute	Obtuse to acute
Leaf shape	Ovate-lanceolate	Ovate-lanceolate	Elliptic ovate	Elliptic	Elliptic	Elliptic	Elliptic	Ovate	Ovate
Inflorescence type	Branched	Branched	Branched	Simple	Simple	Simple	Simple	Branched	Branched
Inflorescence colour	Greenish	Greenish purple	Greenish grey	Green	Green	Green	Purple	Purple	Green
Flower clour	Yellowish white	Yellowish white	White	White	Pinkish-white	White	Pinkish-white	Purplish	Purplish
Anther colour	Yellow	Yellow	Brick red	White	White	White	White	Yellow	Yellow
Seed mucilage	Non-mucilaginous	Non-mucilaginous	Mucilaginous	Mucilaginous	Mucilaginous	Mucilaginous	Mucilaginous	Non-mucilaginous	Non-mucilaginous
Seed colour	Brown	Brown	Black	Black	Black	Black	Black	Brown	Brown
Seed shape	Subglobose	Subglobose	Small ellipsoid	Small ellipsoid	Ellipsoid	Ellipsoid	Ellipsoid	Globose	Globose

4.3.2. Quantitative traits

The descriptive analysis of nine *Ocimum* taxa in the present study showed significant variation in their quantitative traits. In addition to observed qualitative characters, *Ocimum* taxa were revealed considerable variation in quantitative characters such as plant height and canopy, petiole length, leaf length, leaf width, leaf area, inflorescence length and number of whorls/inflorescence, bract length and width, peduncle length, sepal length and width, petal length and width, stamen and style length (Table 4.9).

The average height of *Ocimum* plants varied from 35 to 204 cm. *O. americanum* was found shortest and *O. gratissimum* (Ajowan tulsi) was the tallest one. Both *O. basilicum* (Babu and Marua tulsi) and *O.* × *africanum* were found similar in plant height (73.25 - 74.25 cm) from the study site. The height of *O. tenuiflorum* varied significantly under different study sites. The differences of plant height may be due to the habitat as well as agro climatic conditions. Kritikar and Basu (1984) previously reported the plant height varied from 95 - 120 cm but few researchers reported different plant height. Both the morphotypes of *O. tenuiflorum* (green and purple types) showed 103.25 - 105.75 cm plant height. The results of the present study were similar with Kritikar and Basu, (1984). Interestingly, the two morphotypes of *O. gratissimum* (Ram and Ajowan tulsi) differ in their plant height. The mean plant height of *O. gratissimum* (Ajowan tulsi) was 125 - 260 cm and *O. gratissimum* (Ram tulsi) was found to 140 - 200 cm in the present study site. This difference in plant's height helps to classify the two morphotypes of *O. gratissimum*. However, on the contrary, Patel *et al*. (2015b) reported the variation of plants' height from 80.53 - 84.26 cm. The results of plant height of *O. gratissimum* are in agreement to that reported by Kritikar and Basu, (1984) and Sastry *et al*. (2012) earliar. Moreover, plant height of *O. basilicum* (Babu tulsi) was 45 - 100 cm and for *O. americanum* was 20 - 60 cm. some workers also found similar results earlier (Omer *et al*., 2008; Sastry *et al*., 2012; Verma *et al*., 2013).

Petiole length varied from 1.5 - 4.46 cm. The highest petiole length showed in *O. gratissimum* (Ram tulsi) and lowest in *O. basilicum* (Babu tulsi) while the range of petiole length was 1.5 - 2.6 cm in *O.* × *africanum*.

A great variation of leaf length was observed that ranged from 3.5 to 14.69 cm. leaf length differ from 3.95 to 4.03 cm in both the morphotypes of *O. tenuiflorum* (Krishna and

Radha tulsi). In case of *O. basilica* (Babu and Marua tulsi) this difference varied from 2.06 - 2.24 cm. whereas in *O. gratissimum* (Ram and Ajowan tulsi) the leaf length varied from 8.13 - 14.69 cm. The average leaf lenght of *O. kilimandscharicum*, *O.* × *africanum* and *O. americanum* were 4.65 cm, 4.73 cm and 3.5 cm correspondingly. The average leaf area of all the *Ocimum* taxa varied widely in the present study. The mean leaf area varied from 3.76 cm^2 (*O. americanum*) to 38.67 cm^2 [*O. gratissimum* (Ajowan tulsi)] (Table 4.9). In the present investigation, findings as observed in case of leaf area variations (3.76 to 57.3 cm^2) were in conformity with that of the study of Ahmad and Khaliq (2002).

In the present investigation there was a significant variation in inflorescence length (10.04-23.48 cm). *O. tenuiflorum* (Krishna tulsi) showed the lowest inflorescence length. On theother hand, *O. basilicum* (Babu tulsi) showed highest inflorescence length. The observed highest mean value of the inflorescence length at the interspecific level was in case of *O. basilicum* followed by *O.* × *africanum*, *O. americanum*, *O. kilimandscharicum, O. gratissimum* and *O. tenuiflorum.* The mean variation of the number of whorls/inflorescence was from 11.5 to 16.35. Number of whorls per inflorescence was observed maximum in *O. gratissimum* (Ram tulsi) and minimum in *O. tenuiflorum* (Krishna and Radha tulsi). Most interestingly to note that comparatively shortest inflorescence of *O. gratissimum* (Ajowan tulsi and Ram tulsi) showed maximum number of whorls per inflorescence (6 to 24) as compared to longest inflorescence of *O. basilicum* (Babu tulsi) (6 to 20) while, 7 to17 number of whorls/inflorescence was observed in *O.* × *africanum*.

In case of *O. tenuiflorum* (Krishna and Radha tulsi) the bract length was found as 0.28 cm and for *O. basilicum* (Marua tulsi) it was 0.91cm having green to purple colour correspondingly. Maximum bract wide (0.45 cm) observed in *O. basilicum* (Marua tulsi) and minimum (0.3 cm) in *O. tenuiflorum* (Krishna and Radha tulsi). Interestingly, *O.* × *africanum* and *O. basilicum* (Babu tulsi) is different species but the length (0.5 - 0.8 cm) and wide (0.3 - 0.5 cm) of bract was almost same.

In *Ocimum* species calyx/sepal characters are very important to identification. Length and width of sepal was varied from 0.4 to 0.8 cm and 0.2 to 0.4 cm respectively. The maximum length and width was observed in *O. basilicum* (Babu and Marua tulsi) while minimum in *O. tenuiflorum* (Krishna and Radha tulsi). A minor difference was found in

the mature calyx length of of *O.basilicum* and *O.* × *africanum*. The fruiting calyx length of *O.basilicum* was 0.4 to 0.8 cm while, in *O.* × *africanum* 0.4 to 0.7 cm long.

The petal length ranging from 0.39 cm to 1.0 cm in *O. gratissimum* (Ajowan and Ram tulsi) and *O. basilicum* (Marua tulsi) respectively. Length of stigma varied from 0.36 cm (*O. americanum*) to 0.76 cm (*O. kilimandscharicum*). On the other hand, length of the style ranged from 0.48 cm [*O. tenuiflorum* (Krishna and Radha)] to 1.15 cm (*O. kilimandscharicum*). Babui tulsi (*O. basilicum*) and Lebu tulsi (*O.* × *africanum*) have maximum similarities in their morphology except their aroma. Babu tulsi (*O. basilicum*) has the sweet odour and Lebu tulsi (*O.* × *africanum*) has lemon flavour aroma. The morphological variation in *Ocimum* species is very diverse as because of cross pollination between inter and intra specific hybridization.

Table 4.9: Quantitative traits of nine *Ocimum* taxa (mean and range)

Species/varieties	Plant height (cm)	Canopy (cm)	Leaf length (cm)	Leaf width (cm)	Leaf area (cm2)	Petiole length (cm)	Inflorescence length (cm)	No. of whorls / inflorescence	Bract length (cm)	Bract width (cm)	Peduncle length (cm)	Sepals length (cm)	Sepals width (cm)	Petal length (cm)	Petal width (cm)	Stamen length (cm)	Style length (cm)
O. gratissimum	204.75	154.75	14.69	6.14	38.67	3.95	10.51	14.50	0.46	0.32	0.26	0.45	0.38	0.39	0.32	0.39	0.60
(Ajowan tulsi)	125-260	90-245	6.8-19	4-7.8	25.7-57.3	1.3-6.5	6.3-16	6-24	0.4-0.6	0.3-0.4	0.2-0.3	0.3-0.5	0.3-0.4	0.3-0.4	0.2-0.4	0.3-0.5	0.6-0.7
O. gratissimum	178.25	151.50	8.13	5.17	34.04	4.46	16.27	16.35	0.44	0.31	0.26	0.49	0.38	0.39	0.32	0.39	0.60
(Ram tulsi)	140-200	110-180	4.1-10.6	3.8-6.7	19.5-52.3	2.8-6.4	10.5-19.5	10-24	0.4-0.5	0.2-0.4	0.2-0.3	0.3-0.6	0.3-0.4	0.3-0.4	0.2-0.4	0.3-0.5	0.6-0.7
O. kilimandscharicum	90.75	88.75	4.65	2.25	7.69	1.72	15.60	14.25	0.54	0.34	0.27	0.37	0.27	0.72	0.64	0.76	1.15
	60-120	60-110	2.9-6.1	1.3-3.1	4.12-14.5	0.9-2.6	11-20	11-20	0.5-0.6	0.3-0.4	0.2-0.3	0.3-0.4	0.2-0.3	0.6-0.8	0.6-0.7	0.7-0.8	1.1-1.2
O. americanum	35.50	41.00	3.50	2.07	3.76	2.96	12.19	13.85	0.49	0.31	0.24	0.38	0.32	0.39	0.30	0.36	0.50
	20-60	20-60	2.3-4.7	1.4-2.8	2.42-5.5	1.5-3.6	8-16.5	8-20	0.4-0.6	0.2-0.4	0.2-0.3	0.3-0.5	0.3-0.4	0.3-0.4	0.2-0.3	0.2-0.4	0.5-0.6
O. basilicum (Babu	73.50	62.25	4.34	2.06	6.52	1.50	23.48	13.60	0.66	0.38	0.29	0.61	0.37	0.75	0.57	0.50	0.75
tulsi)	45-100	45-85	3.5-6.2	1.5-3.1	3.87-12.6	1.1-2.6	9.5-32	6-20	0.5-0.8	0.3-0.5	0.2-0.3	0.4-0.8	0.3-0.4	0.6-0.8	0.5-0.6	0.5-0.6	0.7-0.8
O. × *africanum*	73.25	61.25	4.73	2.02	6.11	1.97	20.96	12.70	0.66	0.39	0.27	0.56	0.38	0.76	0.58	0.50	0.76
	45-105	45-90	3.9-5.2	1.7-2.3	4.42-7.5	1.5-2.6	13-30	7-17	0.5-0.8	0.3-0.5	0.2-0.3	0.4-0.7	0.3-0.4	0.7-0.8	0.5-0.6	0.4-0.6	0.7-0.8
O. basilicum	74.25	63.75	4.63	2.24	7.30	1.59	20.23	12.95	0.91	0.45	0.37	0.64	0.60	1.00	0.60	0.71	1.06
(Marua tulsi)	55-100	50-85	3.1-6.2	1.5-3	3.55-13.16	1.1-2.5	12.1-27	8-18	0.8-1.1	0.3-0.6	0.3-0.4	0.4-0.8	0.4-0.7	0.9-1.1	0.5-0.7	0.6-0.8	1-1.1
O. tenuiflorum	105.75	88.75	4.03	2.41	6.91	2.01	10.04	11.85	0.28	0.30	0.27	0.35	0.20	0.44	0.30	0.37	0.48
(Purple type)	70-150	65-110	2.2-5.7	1.1-3.3	2.5-14.5	1.2-3.2	7-15.8	7-18	0.2-0.4	0.2-0.4	0.2-0.3	0.2-0.4	0.2-0.3	0.4-0.5	0.3-0.4	0.3-0.4	0.4-0.5
O. tenuiflorum	103.25	90.50	3.95	2.32	6.48	2.15	10.88	11.50	0.28	0.30	0.27	0.35	0.20	0.44	0.30	0.37	0.48
(Green type)	70-160	60-120	2.4-6.2	1.2-4.1	4.12-14.5	1.2-2.9	6.5-15.5	6-18	0.2-0.4	0.2-0.4	0.2-0.3	0.2-0.4	0.2-0.3	0.4-0.5	0.3-0.4	0.3-0.4	0.4-0.5

4.3.3. Principal Component Analysis (PCA) and Agglomerative Hierarchical Clustering (AHC)

Principal component analysis has been widely used to screen multivariate data with significantly high correlation (Johnson, 1998). To find out the principal components, 18 qualitative and 17 quantitative morphological traits were analyzed. The first component (PC1) contributed 40.07 %, the first two components (PC1 and PC2) 66.79 % and three components together (PC1, PC2 and PC3) contributed 82.85 % of the total variability. In each principal component the maximum variability was contributed by the first principal component (40.07) followed by second PC (26.71) and third PC (16.05) (Figure 4.15). Bract length, seed colour, seed mucilage, bract width, petal length, petal width, inflorescence length and inflorescence type contributed as the first principal component. In second principal component the traits contributing to the total variability were leaf tip, leaf shape, leaf area, leaf width, number of whorls/Inflorescence, leaf length and petiole length. The third principal component was mostly influenced by the traits that were anther colour, inflorescence colour and leaf surface/texture (Table 4.10 and 4.11).

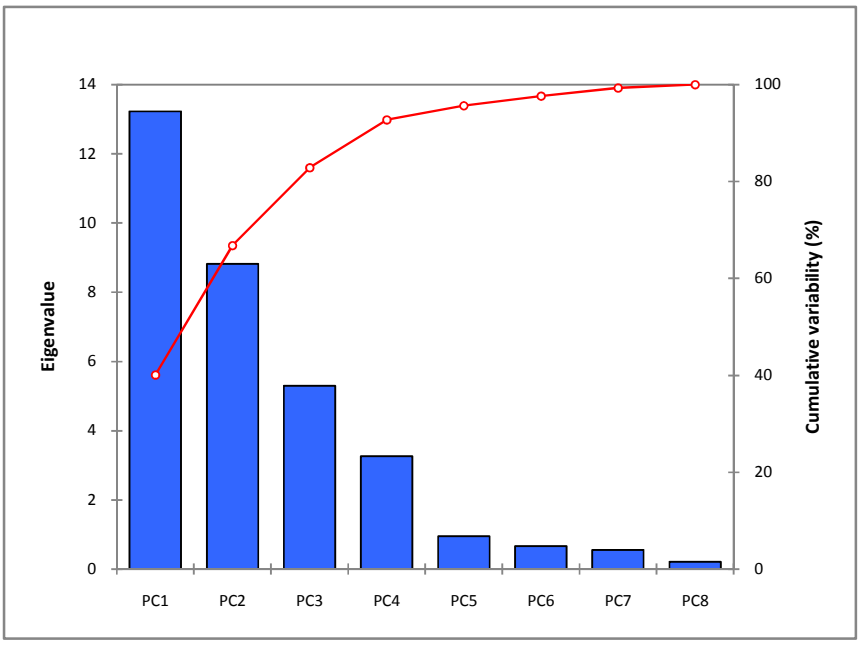


Figure 4.15: Eigen values and cumulative variability based on principal component analysis of the morphological characteristics in nine *Ocimum* taxa

Table 4.10: Eigen values, variability and cumulative variability among morphological traits (qualitative and quantitative) of nine *Ocimum* taxa based on principal component analysis

Component	Eigen value	Variability (%)	Cumulative %	Major traits contributing the variability
PC1	13.226	40.079	40.079	BL, SM, SC, BW, PL_2, PW, IL, IT
PC2	8.817	26.717	66.796	LT, LS_1, LA, LW, NW/I, LL, PL
PC3	5.299	16.059	82.855	AC, IC, LS

PC- Principal Component, BL-bract length, SM-seed mucilage, SC-seed colour,BW-bract width, PL_2-petal length, PW-petal width, IL-inflorescence length, IT- inflorescence type, LT-leaf tip, LS_1- leaf shape, LA-leaf area, LW-leaf width, NW/I- number of whorls/Inflorescence, LL- leaf length, PL- petiole length, AC-anther colour, IC-inflorescence colour and LS- leaf surface/texture

Table 4.11: Contribution of the variables (%) in each principal component

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
H	3.612	4.524	1.339	1.170	1.315	0.092	0.042	0.421
MOR	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGH	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SC	0.020	0.021	6.048	8.606	32.073	8.481	5.885	0.013
SP	2.992	3.911	3.857	0.263	0.053	5.129	1.882	0.620
SS	3.552	4.591	1.406	1.122	1.303	0.096	0.038	0.416
LC	2.723	0.127	0.923	3.379	42.432	0.703	8.243	7.299
LS	0.298	3.540	**9.935**	3.646	0.000	0.014	0.406	0.202
LM	3.646	2.231	4.722	0.052	0.526	1.369	1.869	20.538
LT	0.237	**10.427**	0.833	0.055	0.145	0.290	0.028	0.003
LS_1	0.237	**10.427**	0.833	0.055	0.145	0.290	0.028	0.003
IT	**4.789**	0.069	3.107	5.513	0.958	0.154	0.824	0.395
IC	0.246	0.606	**15.686**	2.243	0.568	0.068	0.512	0.324
FC	1.175	1.624	6.958	6.902	0.920	1.682	6.605	23.176
AC	0.208	0.573	**15.972**	2.072	0.415	0.092	0.326	0.678
SM	**6.255**	0.557	0.578	2.123	1.957	0.042	0.307	1.342
SC	**6.255**	0.557	0.578	2.123	1.957	0.042	0.307	1.342
SS_1	4.343	0.043	4.203	4.137	4.670	1.293	1.085	2.225
PH	2.356	5.581	0.107	5.140	0.920	0.511	1.862	0.024
C	2.518	5.174	0.573	4.782	1.652	1.117	0.010	0.400
LL	0.776	**7.797**	0.095	1.669	0.441	4.491	14.710	15.889
LW	1.558	**8.475**	0.071	1.142	0.001	0.399	0.228	0.825
LA	1.163	**8.566**	0.119	2.021	0.001	0.281	1.973	2.623
PL	1.939	**7.246**	0.097	1.491	0.209	0.472	4.747	8.878
IL	**5.486**	0.073	0.477	0.121	2.188	31.625	1.069	0.644
NW/I	0.041	**7.488**	2.947	0.282	0.629	8.867	17.994	1.783
BL	**6.847**	0.395	0.443	0.724	0.007	1.827	0.014	0.049

BW	**5.971**	0.008	1.309	3.228	0.683	1.078	2.853	2.358
PL_1	2.702	0.086	2.779	11.497	1.106	8.641	7.835	0.149
SL	4.237	1.363	3.729	0.641	1.761	6.628	5.714	3.767
SW	3.864	2.964	1.872	1.481	0.118	8.346	3.134	2.696
PL_2	**5.958**	0.451	0.110	4.692	0.554	0.003	1.181	0.547
PW	**5.601**	0.268	1.167	3.223	0.227	3.915	7.215	0.023
SL_1	4.073	0.229	3.372	7.593	0.030	1.333	0.863	0.086
SL_2	4.321	0.009	3.751	6.810	0.035	0.630	0.214	0.260

PC- Principal Component, H-habit, MOR-mode of reproduction, PGH-plant growth habit, SP-stem pubescent, SS-stem shape, LC-leaf colour, LS- leaf surface/texture, LM-leaf margin, LT-leaf tip, LT-leaf tip, LS_1-leaf shape, IT-inflorescence type, IC-inflorescence colour, FC-flower colour, AC-anther colour, SM-seed mucilage, SC_1-seed colour, SS_1-seed shape, PH-plant height, C-canopy, LL-leaf length, LW-leaf width, LA-leaf area, PL-petiole length, IL-inflorescence length, NW/I- Number of whorls/Inflorescence, BL-bract length, BW-bract width, PL_1-peduncle length, SL-sepal length, SW-sepal width, PL_2-petal length, PW-petal width, SL_1-stamen length, SL_2-style length

From the morphological traits (both qualitative and quantitative) four distinct group of species were plotted in the two dimensional plot of PCA. First (40.07 %) and second (26.71 %) principal component showed the relations among the qualitative and quantitative traits of nine *Ocimum* taxa which ware showed total 66.79 % variability (Figure 4.16).

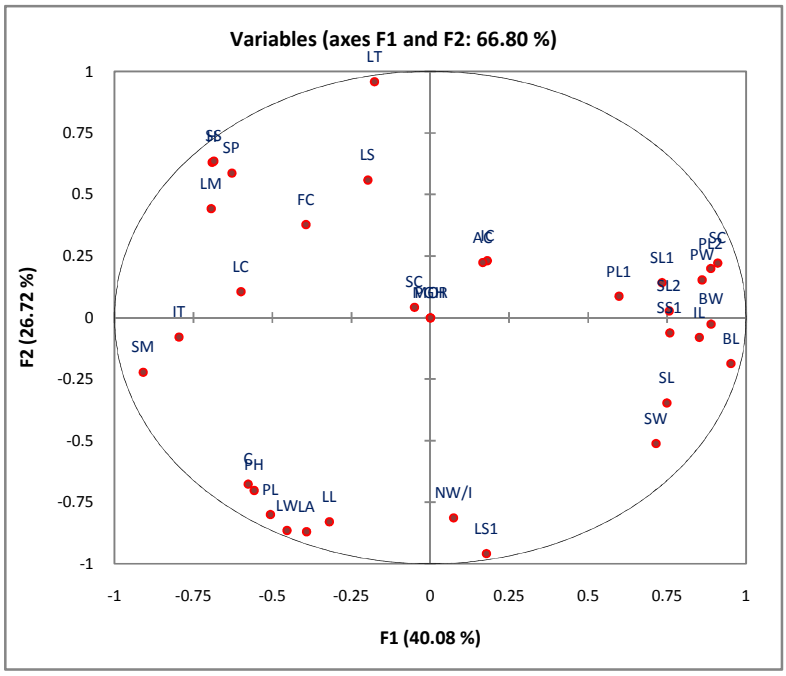


Figure 4.16: Relationships among the 35 morphological characters used for nine *Ocimum* taxa based on principal component analysis

The cluster analysis was performed to classify all the nine *Ocimum* taxa according to their most important components. From the Agglomerative hierarchical clustering (AHC) two clearly distinct groups were obtained on the basis of the morphological traits using Euclidean distance by Ward's method (Figure 4.17). In the first group *O. tenuiflorum* (Green type), *O. tenuiflorum* (Purple type) and in II cluster *O. gratissimum* (Ram tulsi) and *O. gratissimum* (Ajowan tulsi) are grouped together and they were close to each other. Cluster III constituted largest species including *O.* × *africanum*, *O. basilicum* (Babu tulsi), *O. basilicum* (Marua tulsi) and *O.* × *americanum*. However, cluster IV contained only *O. kilimandscharicum*. This study clearly differentiates all the studied *Ocimum* taxa as sanctum and basilicum groups which were earlier reported by Khosla, (1995). Four distinct groups of species were plotted in the two dimensional plot of PCA based on morphological traits (both qualitative and quantitative) and confirmed the dendrogram constructed by Ward's method (Figure 4.18).

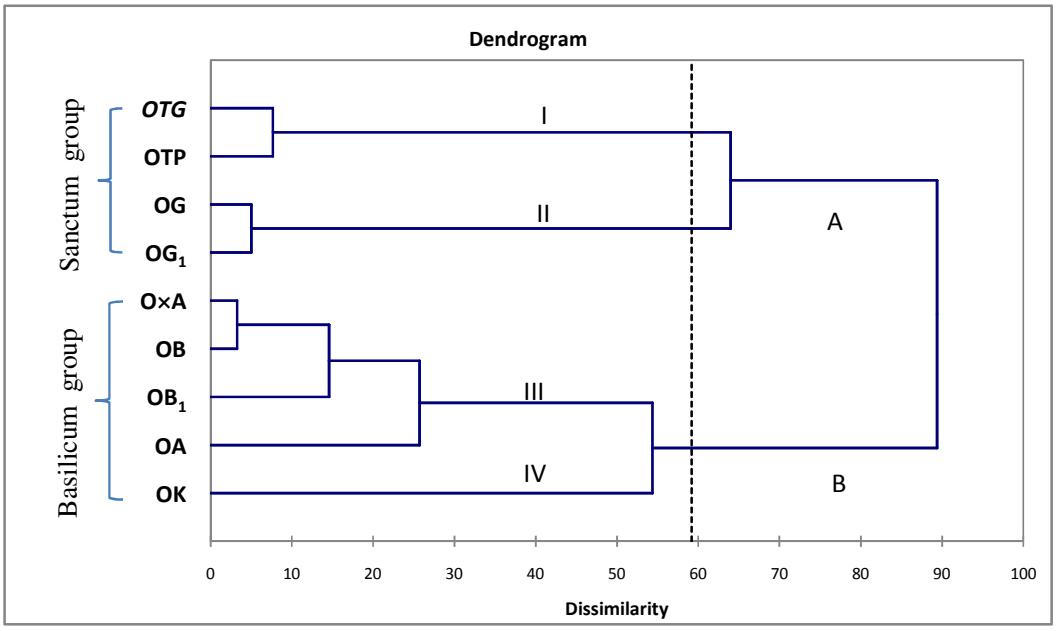


Figure 4.17: Dissimilarity dendrogram generated by Ward's method showing major clusters among nine *Ocimum* taxa based on their morphological traits, **OTG-** *O. tenuiflorum* L. green (Radha tulsi); **OTP-** *O. tenuiflorum* L. purple (Krishna tulsi); **OG-** *O. gratissimum* L. (Ram tulsi); **OG_1-** *O. gratissimum* L. (Ajowan tulsi); **O×A-** *O.* × *africanum* Lour. (Lebu tulsi); **OB-** *O. basilicum* L. (Babu/babui tulsi); **OB_1-** *O. basilicum* L. (Marua tulsi); **OA-** *O. americanum* L. (Bon tulsi); **OK-** *O. kilimandscharicum* Guerke (Karpur tulsi)

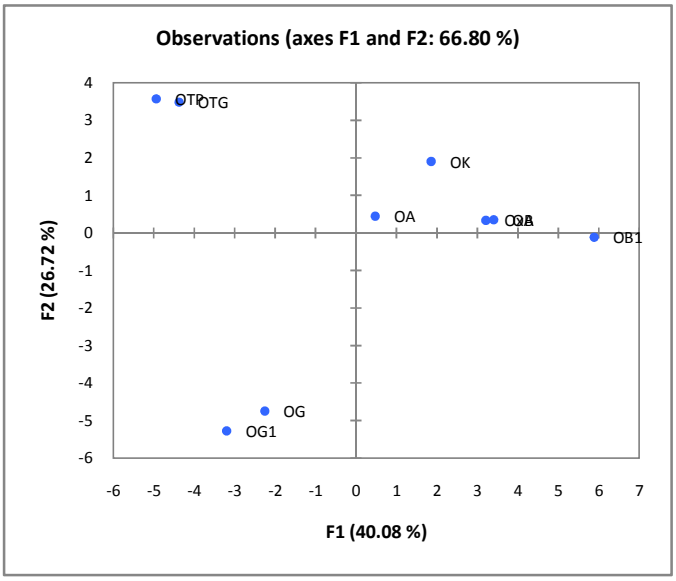


Figure 4.18: Two dimensional scatter plots based on the Principal Component Analysis of nine *Ocimum* taxa

4.4. Chemical compositional variation of fixed oil

Chemical compositions were determined by GC-MS analysis from the ethanolic extracts of nine *Ocimum* taxa (Table 4.12). In this analysis, total 73 compounds were identified of which twelve aliphatic acids, three aliphatic alcohols, seven amino acids, two aromatic compounds, one fused ring aromatic hydrocarbon, twenty three carbohydrates, five phenolic compounds, one quinone, three steroids, twelve terpenoids, vitamin E and three unidentified compounds. Similar results were reported by Chowdhury *et al.* (2017).

Table 4.12: Chemical composition of nine *Ocimum* taxa through GC-MS analysis

	Relative area percentage (peak area relative to the total peak area, expressed as percentage)								
Compounds	**OA**	**OB**	**OB_1**	**OG**	**OG_1**	**OK**	**OTP**	**OTG**	**OxA**
Aliphatic acid									
Acetic acid	-	-	0.74	-	-	-	-	-	-
Butanedioic acid	0.36	-	-	0.72	0.3	-	0.27	0.09	-
Butanoic acid	-	-	-	-	-	-	0.03	-	-
Dodecanoic acid	-	-	0.37	-	-	-	-	-	-
Eicosanoic acid	0.04	-	-	-	-	-	-	0.93	-
Hexadecanoic acid	0.39	29.64	13.46	10.37	6.87	0.69	0.34	15.01	6.8
n-Pentadecanoic acid	-	-	0.12	-	-	-	-	-	-
Octadecanoic acid	1.55	6.26	2.83	1.92	0.48	2.08	0.07	8.62	2.49

	Relative area percentage (peak area relative to the total peak area, expressed as percentage)								
Compounds	**OA**	**OB**	**OB_1**	**OG**	**OG_1**	**OK**	**OTP**	**OTG**	**OxA**
Propanedioic acid	-	-	-	-	-	-	0.11	-	-
Propanoic acid	1.05	1.93	0.02	0.04	1.77	1.42	0.5	0.05	1.77
Linoleic acid	-	2.14	2.39	-	0.13	-	0.45	-	2.56
α-Linolenic acid	2.94	14.47	12.64	3.80	0.94	7.05	7.78	-	12.63
Aliphatic alcohol									
Glycerol	7.36	15.42	7.17	0.48	0.07	53.29	5.45	21.84	10.87
Hexadecenol	3.87	12.76	4.62	7.1	-	6.3	-	9.94	10.06
Meso-Erythritol	-	5.27	0.48	-	-	5.13	-	0.14	0.74
Amino acid									
L-Alanine	-	-	-	-	-	-	0.25	-	-
L-Norleucine	-	-	-	-	-	-	0.01	-	-
L-Proline	0.74	-	-	-	-	-	1.23	-	-
L-Threonic acid	-	-	-	-	-	-	0.14	-	-
L-threonine	-	-	-	-	-	-	0.08	-	-
L-Valine	0.08	-	-	-	-	-	-	-	-
Pipecolic acid	-	-	-	-	-	-	-	0.05	-
Aromatic acid									
Benzoic acid	0.14	-	0.19	-	-	-	0.01	-	-
Cinnamic acid	-	-	-	0.21	-	-	-	-	-
Fused ring aromatic hydrocarbon									
Naphthalene	0.07	-	-	-	-	-	-	-	-
Carbohydrates									
Arabinitol	0.04	2.22	-	-	-	-	-	0.01	0.01
Fructose	3.65	-	4.2	5.08	3.47	-	27.24	-	3.56
D-(+)-Talofuranose	-	-	-	-	-	-	0.37	-	-
D-Allofuranose	-	-	-	-	-	-	0.41	-	-
D-Arabinopyranose	-	-	-	-	-	-	0.06	-	-
D-galactose	-	-	0.56	-	-	2.7	0.04	-	-
D-gluconic acid	0.2	-	-	-	-	-	0.36	-	-
D-Mannitol	0.49	-	-	-	-	1.53	-	-	-
D-Psicose	4.04	-	-	-	-	-	-	-	-
D-Sorbitol	-	-	-	-	-	-	0.55	-	-
Furanone	-	-	-	-	-	-	0.03	-	-
L-(-)-Arabitol	-	-	-	-	-	-	0.68	-	-
L-(-)-Sorbose	6.91	-	-	5.9	-	-	9.1	-	-
Myo-Inositol	0.3	-	0.18	0.01	-	-	1.16	-	-
Sucrose	-	-	1	-	-	-	5.65	-	-
α-D-(-)-Tagatose	-	-	4.95	-	3.46	-	19.02	-	-

	Relative area percentage (peak area relative to the total peak area, expressed as percentage)								
Compounds	**OA**	**OB**	**OB_1**	**OG**	**OG_1**	**OK**	**OTP**	**OTG**	**OxA**
α-D-(+)-Mannose	0.5	-	14	-	-	-	0.24	0.22	13.75
α-D-(+)-Talose	-	-	0.14	13.35	-	-	1.31	-	-
α-D-Glucose	55.32	-	-	-	-	-	22.15	0.02	17.23
β-D-allopyranose	0.24	-	-	-	-	-	-	-	-
β-D-glucose	4.29	-	17.76	26.98	16.14	2.78	2.65	0.73	4.23
L-Threitol	1.73	-	-	-	0.34	0.21	-	-	-
Xylitol	-	-	0.26	-	-	1.75	-	-	-
Phenolic compounds									
Caffeic acid	-	-	-	-	0.21	-	-	-	-
Catechol	-	-	-	-	-	-	0.08	-	-
Vanillin	-	-	-	0.71	-	-	-	-	-
Eugenol	-	-	-	12.46	-	-	-	8.61	-
Methyl eugenol	-	-	15.53	-	-	-	-	-	-
Quinone									
Tert-Butylhydroquinone	-	-	-	-	0.09	-	-	-	-
Steroid									
Ergosterol	-	-	0.65	-	-	-	-	1.26	0.88
Stigmasterol	-	-	0.79	0.88	2.29	-	-	3.59	1.51
β-Sitosterol	0.99	3.62	2.55	2.7	3.36	6.31	-	4.53	2.5
Terpenoids									
Carvacrol	-	-	-	-	0.54	-	-	-	-
Caryophyllene oxide	-	-	-	1.36	-	-	-	0.48	-
Germacrene D	-	-	-	-	-	-	-	0.01	-
Norpinene	-	-	0.01	-	-	-	-	-	-
Phytol	-	-	0.54	-	14.68	-	-	-	2.94
Squalene (Precursor)	-	-	-	1.69	4.78	-	-	-	-
Tau-Cadinol	-	-	0.36	-	-	-	-	-	-
Thymol	-	-	-	-	29.8	-	-	0.69	0.02
α-Amyrin	-	-	0.79	1.42	-	-	-	-	-
α-Selinene	-	-	-	-	-	-	-	0.43	-
β-Elemene	-	-	-	-	-	-	-	1.15	-
β-Selinene	-	-	-	-	-	-	-	0.64	-
Vitamin E									
α-Tocopherol	0.16	-	1.01	2.39	1.4	0.44	-	0.5	0.4
Unknown	2.55	7.83	0.91	3.16	2.91	7.14	0.43	5.28	3.79

OA- *O. americanum* (Bon tulsi), OB- *O. basilicum* (Babu tulsi), OB_1- *O. basilicum* (Marua tulsi), OG- *O. gratissimum* (Ram tulsi), OG_1- *O. gratissimum* (Ajowan tulsi), OK- *O. kilimandschericum* (Karpur tulsi), OTP- *O. tenuiflorum* (Krishna tulsi), OTG- *O. tenuiflorum* (Radha tulsi), OxA- *O.* x *africanum* (Lebu tulsi)

Results showed that in the ethanolic extracts, carbohydrates were the major components of *O. tenuiflorum* (Krishna tulsi), *O. americanum* (Bon tulsi), *O. gratissimum* (Ram tulsi), *O. basilicum* (Marua tulsi) and *O.* × *africanum* (Lebu tulsi) in 91.03%, 77.71%, 51.31%, 43.06% and 38.77% respectively. α-D-glucose was found to be the chief carbohydrate in *O. americanum* and *O.* × *africanum* with natural abundance of 55.32% and 17.23% respectively whereas in *O. tenuiflorum* (Krishna tulsi) fructose was the main carbohydrate with 27.24% natural abundance. However, *O. gratissimum* (Ram tulsi) and *O. basilicum* (Marua tulsi) have β-D-glucose as the main constituents (17.76% and 26.98% respectively) (Figure 4.19). The anomeric difference in the sugar content was at first sight remarkable. It may be due to the difference in biological structure space in the enzymes where from it was being synthesized. The higher percentage of carbohydrate describes its high food value.

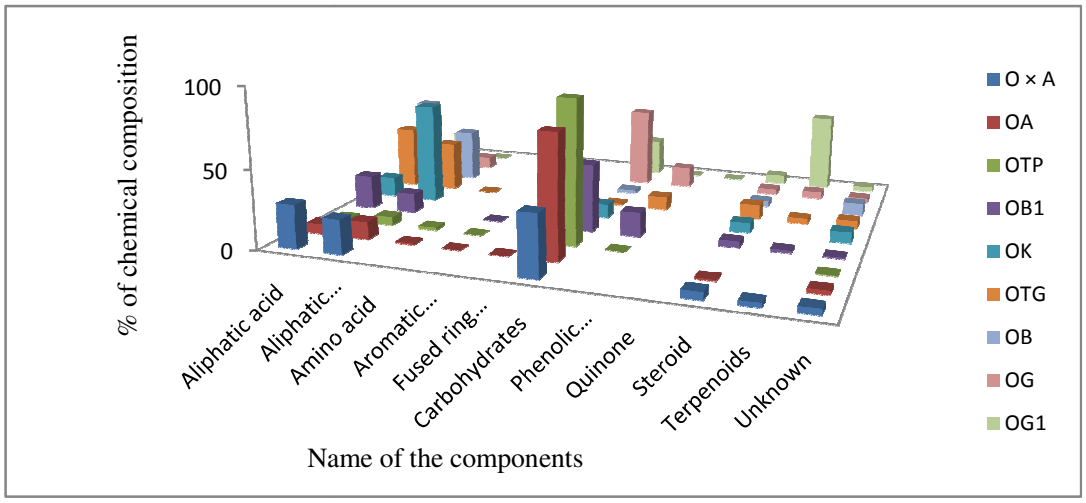


Figure 4.19: Abundance of different chemical components in the ethanolic extract of nine different *Ocimum* taxa, OxA- *O.* x *africanum* (Lebu tulsi), OA- *O. americanum* (Bon tulsi), OTP- *O. tenuiflorum* (Krishna tulsi), OB_1- *O. basilicum* (Marua tulsi), OK- *O. kilimandschericum* (Karpur tulsi), OTG- *O. tenuiflorum* (Radha tulsi), OB- *O. basilicum* (Babu tulsi), OG- *O. gratissimum* (Ram tulsi), OG_1- *O. gratissimum* (Ajowan tulsi)

The variation of the chemical constituents in the studied *Ocimum* taxa and their morphotypes were quite prominent. Nature and amount of chemical constituents varied from species to species. In *O. basilicum* (Babu tulsi) and *O. tenuiflorum* (Radha tulsi) the main constituents in the ethanolic extracts were aliphatic acids with a total of 52.87% and 39.9% respectively whereas in *O. kilimandschericum*, aliphatic alcohol was the main constituent having 64.72% natural abundance. Hexadecanoic acid and α-linolenic acid were the main aliphatic acids of *O. basilicum* (Babu tulsi) and *O.*

tenuiflorum (Radha tulsi) with natural abundance of 29.64% and 15.2% respectively. Similar results were reported by Domokos *et al*. (1993) and Chowdhury *et al*. (2017). Especially, α–linolenic acid and β-sitosterol were found to present in the ethanolic extract of *O. sanctum*. This finding was in accordance with that reported by Nadkarni and Patwardhan (1952), Singh *et al*. (1996) and Angers *et al*. (1996).

Hexadecanoic acid commonly known as the palmitic acid is a very good antioxidant and recently Harada *et al*. (2002) reported its selective antitumor activity. High content of palmitic acid in Babu tulsi (*O. basilicum*) indicate that this extracts may be used directly to suppress the growth of tumor cells. In *O.* × *africanum*, *O. americanum*, *O. kilimandschericum*, *O. tenuiflorum* (Radha tulsi) and *O. gratissimum* (Ajoyan) α-linolenic acid was the main aliphatic acids which exhibit maximum abundance of 16.61%, 2.94%, 8.23%, 15.2% and 7.05% respectively and in conformity with the study of Henry and Grindley (1944). α-linolenic acid has reported to have anti inflammatory effects (Singh and Majumdar, 1997; Singh, 1998). This finding attributes the local use of "Tulsi" leaves against inflammation. From the CG-MS analysis it was found that all the nine *Ocimum* extracts contains fatty acids in higher percentage. Presence of higher concentration of fatty acids indicated the ability to reduce blood cholesterol level and good sources of antioxidant (Suanarunsawa *et al*., 2009).

In the present investigation, interestingly found the presence of some amino acids like L-alanine, L-norleucine, L-proline, L-threonic acid, L-threonine, L-valine and pipecolic acid. *O. americanum* has 0.74% L-proline and 0.08% L-valine. *O. tenuiflorum* (Radha tulsi) contain only 0.05% pipecolic acid whereas in *O. tenuiflorum* (Krishna tulsi) 0.24% L-alanine, 0.01% L-norleucine, 1.23% L-proline, 0.14% L-threonic acid and 0.08% L-threonine were present as chief amino acids. Since amino acids are the building blocks of proteins, the occurrence of amino acids with higher yields indicates the presence of various proteins in the ethanolic extracts of these nine *Ocimum* taxa. "Tulsi" may, therefore, be a very good source of plant derived proteins that are friendly to us.

Sterols were also found in the ethanolic extracts of different *Ocimum* species/morphotypes. *O.* x *africanum* containes 0.88% ergosterol, 1.51% stigmasterol

and 2.50% β-sitosterol. But *O. americanum*, *O. kilimandschericum* and *O. basilicum* (Babu tulsi) contained only β-sitosterol as 0.99 %, 6.31% and 3.62% respectively. *O. basilicum* (Marua tulsi) and *O. tenuiflorum* (Radha tulsi) has ergosterol (0.65%, 1.26%), stigmasterol (0.79%, 3.59%) and β-sitosterol (2.55%, 4.53%). β-sitosterol (2.70%) and stigmasterol (0.88%) were the major compounds in *O. gratissimum* (Ram tulsi). *O. gratissimum* (Ajowan tulsi) contains stigmasterol (2.29%) and β-sitosterol (3.36%). All these sterols especially stigmasterol has anti-poisonous/anti-venom effects in literature (Gomes *et al*., 2007). The present investigation might give a scientific base to the traditional medicine culture of using leaves of Tulsi against toxic insects/strings and lowering the high fever.

Methyl eugenol (15.53%) was present as major component of *O. basilicum* (Marua tulsi) while in *O. basilicum* (Babu tulsi) the concentration of hexadecanoic acid/palmitic acidis (29.64%) was very high. Except methyl eugenol, however, there was no other volatile components (phenylpropanoids and monoterpenes) based on which it can make a clear distinction between the two morphotypes of *O. basilicum* (Chowdhury *et al*., 2017). Thus, chemical method of distinction is insufficient to identify the morphotypes at the intra-specific level.

Methyl eugenol has chemical attractant property mostly against common fruit flies, *Bactrocera dorsalis* (Vargas *et al*., 2000). The present investigation is also revealed similar kind of result (Figure 4.20). Thus, Marua tulsi (*O. basilicum*) extracts can be used in bio-based pest control management by exploiting its chemical attractant property.

Eugenol (12.46%) was the main phenolic compound in clove like flavour *O. gratissimum* (Ram tulsi) whereas in carom seed like spicy flavoured *O. gratissimum* (Ajowan tulsi) was rich with thymol (29.8%) and phytol (14.68%). The results of chemical analysis clearly indicates the existence of thymol and eugenol rich two different chemotypes of *O. gratissimum* (Jirovetz *et al*., 2003; Tchoumbougnang *et al*., 2006; Dambolena *et al*., 2010; Verma *et al*., 2013, Chowdhury *et al*., 2017). This variation of chemical constitutions may be the primary cause of difference in aroma. *O. tenuiflorum* (Radha tulsi) contained eugenol (8.61%), thymol (0.69%) and β-elemene (1.15%). But in the other morphotype of *O. tenuiflorum* (Krishna tulsi) eugenol, thymol

and β-elemene were not detected. Previously, Verma *et al.* (2013) reported the presence of β-elemene in *O. tenuiflorum*.

Figure 4.20: *Bactrocera dorsalis* (common fruit fly) is attracted by *O. basilicum* (Marua tulsi) due to the presence of Methyl eugenol

O. × *africanum* (Lebu tulsi) and *O. basilicum* (Babu tulsi) are morphologically very similar to each other but abruptly different in their chemical constituents. Ergosterol, stigmasterol, phytol and thymol were present in *O.* × *africanum* (Lebu tulsi), but all were absent in *O. basilicum* (Babu tulsi). Therefore, *O.* × *africanum* is chemically different other than *O. basilicum* (Babu tulsi). Hence, chemical method is another better way out over morphological methods to identify the intra specific level of diversity of *Ocimum* species. This difference was further verified by RAPD analysis.

4.5. Genetic variation through RAPD marker

4.5.1. DNA isolation, purification, quantification and quality check

The DNA extracted from plant tissue includes contaminants like RNA, protein, polysaccharides etc. which severely hampers the downstream process. Thus, purification of DNA is very essential. RNA was removed by treating the sample with RNase enzyme. Most of the proteins were eleminated from the DNA sample by extraction with phenol: chloroform followed by RNase treatment. CTAB buffer eliminated polysaccharides from DNA to a large extent.

Two different methods were followed for quantification and qualification of extracted DNA of nine *Ocimum* taxa. Spectrophotometric method was used for quantification and agarose gel electrophoresis was used for qualification. The DNA was quantified in a

UV spectrophotometer with 260 nm and 280 nm filters. Results were scored and the ratio of A260/A280 was calculated. Three or more replicates were maintained in each experimentation and the samples showed an average ratio of around 1.8 (Table 4.13). The intactness of the DNA was determined with the help of 1 % agarose gel electrophoresis using known molecular marker (100-3000 bp) (Figure 4.21). The quantity of DNA was estimated by comparing the sample DNA with the control by eye adjustment. The combination of the above three steps (extraction, purification and quantification) allowed the extraction of sufficient amount of pure DNA from the leaves for PCR amplification.

Table 4.13: The quality and quantity of the isolated genomic DNA

Ocimum taxa	Purity (A260/A280)	Concentration (ng/µl)
O. gratissimum L.	1.75	100
O. × *africanum* Lour.	1.80	50
O. basilicum L.	1.72	100
O. tenuiflorum L. (Purple)	1.79	150
O. basilicum L. (Marua)	1.80	100
O. americanum L.	1.75	100
O. tenuiflorum L. (Green)	1.80	50
O. gratissimum L. (Ajowan)	1.79	100
O. kilimandscharicum Guerke.	1.78	100

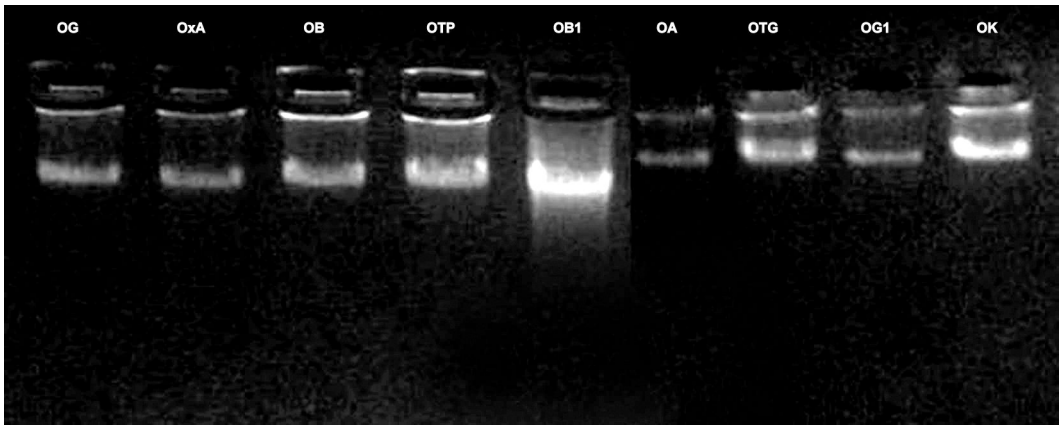


Figure 4.21: Genomic DNA extracted from leaves of nine *Ocimum* taxa (Murry and Thompson, 1980) and visualized on a 1% agarose gel, OG- *O. gratissimum*; OxA- *O.* × *africanum*; OB- *O. basilicum*; OTP- *O. tenuiflorum* (Purple type); OB1- *O. basilicum* (Marua tulsi); OA- *O. americanum*; OTG- *O. tenuiflorum* (Green type); OG1, *O. gratissimum* (Ajowan tulsi); OK- *O. kilimandscharicum*

4.5.2. RAPD analysis

In the present analysis a total 17 (BG 1-17) RAPD primers were used to detect inter and intra-specific diversity of *Ocimum* taxa found in Dakshin Dinajpur district. 10 primers produced clear scorable and reproducible bands out of 17 primers (Figure 4.22). From the 10 primers a total of 88 scorable and distinct amplified bands were obtained (Table 4.14). The number of bands of each primer was ranging from 5 (BGM-5 and BGM-13) to 13 (BGM-7) with an average 8.8 loci per primer. The number polymorphic amplicons varied from 5 (BGM-5 and BGM-13) to 12 (BGM-7) with a mean of 8.5 loci for each primer. Three primers namely BGM-1, BGM-7 and BGM-17 produced monomorphic band in each taxa. The highest number of bands (13) obtained from the primer (BGM-7) with 92.31% polymorphism while the lowest number of bands (5) obtained from the primers (BGM-5 and BGM-13) with 100% polymorphism respectively. Therefore, different primers showed different levels of polymorphism ranging from 83.33% (BGM-17) to 100% (BGM-3, BGM-4, BGM-5, BGM-9, BGM-12, BGM-13 and BGM-15) with an average of 96.56%. The amplicon size ranges with different primers were 200bp to 3000bp.

PIC values were calculated for each primer based on polymorphic band. The highest PIC value (0.470) was obtained from the primer BGM-15 whereas, the lowest PIC value (0.350) with an average of 4.00 was obtain from the primer BGM-1. The RAPD primers generated 5 highly informative polymorphic loci (PIC> 0.4) among 50 percent polymorphic fragments. However, the primer BGM-15 showed highest PIC value of 0.470 recommended for germplasm analysis. This may be due to the polyallelic nature of RAPD markers. Similar results were reported by Chowdhury *et al*. (2017).

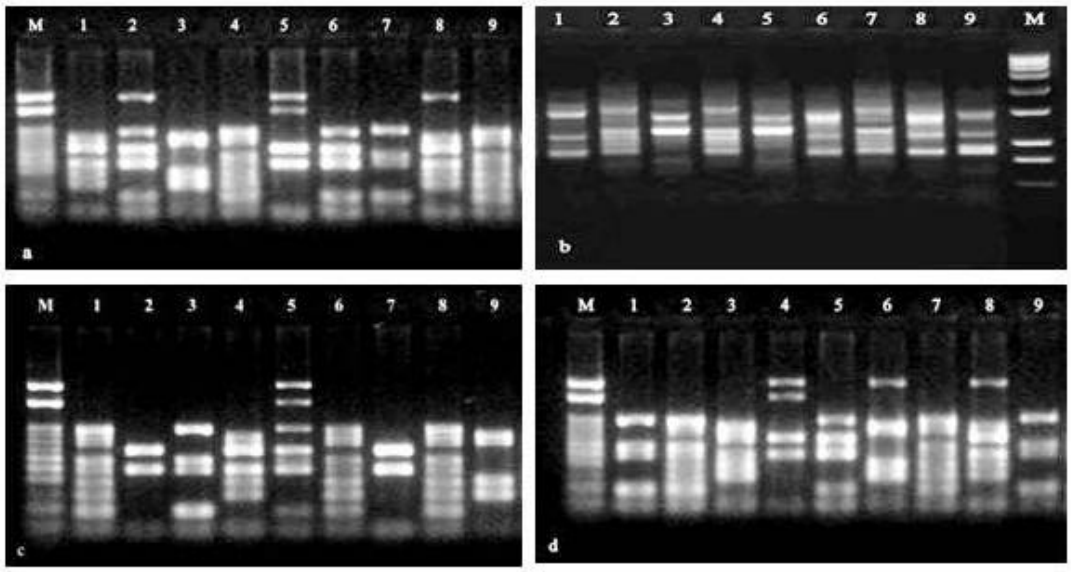


Figure 4.22: A representative of RAPD profile of nine taxa of *Ocimum* amplified with (a) BGM-1, (b) BGM-3, (c) BGM-4 and (d) BGM-7 primer. M-Marker (100-3000 bp DNA ladder); Lane 1-*O. gratissimum*; 2-*O.* × *africanum*; 3-*O. basilicum*; 4-*O. tenuiflorum* (Purple type); 5-*O. basilicum* (Marua tulsi); 6-*O. americanum*; 7-*O. tenuiflorum* (Green type); 8-*O. gratissimum* (Ajowan tulsi); 9-*O. kilimandscharicum*

Table 4.14: Analysis of polymorphism percentage among nine taxa of the genus *Ocimum* using RAPD primers

Primer code	Sequence (5´-3´)	Total no. of bands	No. of polymorphic bands	Polymorphism (%)	PIC	Fragment size range (bp)
BGM-1	TGCCGAGCTG	10	9	90	0.35	400-3000
BGM-3	GTGACGTAGG	8	8	100	0.437	500-800
BGM-4	AGGTCTTGGG	11	11	100	0.412	200-3000
BGM-5	GGTGCTGCGC	5	5	100	0.424	390-890
BGM-7	CTGGGCAACT	13	12	92.31	0.378	500-3000
BGM-9	GAAACGGGTG	10	10	100	0.353	400-700
BGM-12	GGAACGGGTG	9	9	100	0.416	600-1200
BGM-13	CATCCCGACA	5	5	100	0.375	260-700
BGM-15	GCACGCCGGA	11	11	100	0.47	390-880
BGM-17	CTATCGCCGC	6	5	83.33	0.386	530-1200
Total		**88**	**85**	**965.64**		
Average		**8.8**	**8.5**	**96.564**	**4**	

Using Jaccard's coefficient a similarity matrix has been obtained from the nine *Ocimum* taxa. Based on the similarity matrix, the genetic similarities of all the taxa of *Ocimum* varied from 0.215 to 0.620. The present analysis showed that *O. gratissimum* (Ram tulsi) and *O. gratissimum* (Ajowan tulsi) exibited maxmium (0.620) similarity. On the

other hand, *O. gratissimum* (Ram tulsi) and *O. basilicum* (Babui tulsi) showed minimum (0.215) genetic similarity (Table 4.15). Analysing the RAPD binary data matrix, the genetic similarity of nine *Ocimum* taxa of six *Ocimum* species under three morphotypes has been determined. These genetic similarities revealed varying degree of genetic relatedness among different *Ocimum* taxa belonging to diverse species.

Table 4.15: Genetic similarity matrix of nine *Ocimum* taxa based on RAPD data matrix

	OG	O×A	OB	OTP	OB_1	OA	OTG	OG_1	OK
OG	1								
O×A	0.303	1							
OB	0.215	0.413	1						
OTP	0.356	0.406	0.239	1					
OB_1	0.254	0.483	0.577	0.258	1				
OA	0.403	0.516	0.475	0.338	0.406	1			
OTG	0.304	0.456	0.226	0.604	0.288	0.313	1		
OG_1	0.620	0.264	0.313	0.393	0.273	0.438	0.322	1	
OK	0.373	0.358	0.273	0.441	0.254	0.415	0.368	0.344	1

OG- *O. gratissimum* (Ram tulsi); O×A- *O.* × *africanum* (Lebu tulsi); OB- *O. basilicum* (Babu tulsi); OTP- *O. tenuiflorum* (Krishna tulsi); OB_1- *O. basilicum* (Marua tulsi); OA- *O. americanum* (Bon tulsi); OTG- *O. tenuiflorum* (Radha tulsi); OG_1- *O. gratissimum* (Ajowan tulsi); OK- *O. kilimandscharicum* (Karpur tulsi)

Using UPGMA and SHAN clustering method a dendrogram was constructed from the genetic similarity data matrix (Figure 4.23). The dendrogram divided nine *Ocimum* taxa into two main clusters. Furthermore, within the clusters those *Ocimum* taxa very closely analogous to each other were sub clustered collectively. Cluster-I content three separate subclusters which include the nine *Ocimum* taxa belonging to *O. gratissimum* (Ajowan tulsi) and *O. gratissimum* (Ram tulsi) in subcluster-i. *O. tenuiflorum* (Green) and *O. tenuiflorum* (Purple) in subcluster-ii and separately *O. kilimandscharicum* belongs to subcluster-iii. Cluster-II furthermore was divided into two subclusters. *O. americanum* and *O.* × *africanum* was in subcluster-i whereas *O. basilicum* (Marua tulsi) and *O. basilicum* (Babu tulsi) in subcluster-ii.

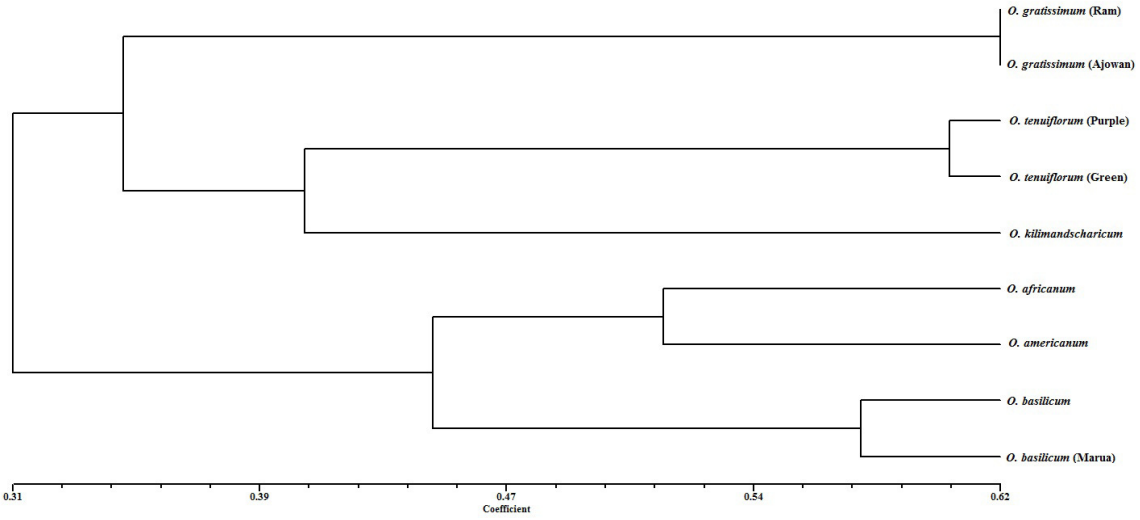


Figure 4.23: Dendrogram represented inter and intra specific relationship among nine *Ocimum* taxa based on RAPD similarity matrix

To find out the consistency of the differentiation amid nine *Ocimum* taxa defined by the cluster analyses the PCA (Principal Coordinate Analysis) was performed. The PCA indicated that the effect of individual amplification products on the overall variation observed was lesser, hence a total of ten RAPD products were required to explain 72.90% of the variation among the nine *Ocimum* taxa. The analysis indicated that, 60.10% of the total variation was accounted from the first two principal coordinates. The RAPD-based PCA revealed that the *Ocimum* taxa belonging to a specific cluster were assembled collectively in the PCA plot. Similar results were also observed in two and three dimensional representations (Figure 4.24).

For plant breeding programme evaluation of the genetic variation in intra-specific and inter-specific level is a prerequisite. It has a vital role in the conservation of plants' genetic resources. RAPD marker is a versatile method for assessment of genetic variability among different species, morphotypes and their cultivars. The marker is generally used for the purpose of selection of parents for any breeding programs. In this present investigation, RAPD genetic marker has been used for genetic diversity analysis of nine *Ocimum* taxa of six species. The findings indicated the occurrence of a wide genetic variability among the studied different *Ocimum* taxa. The polymorphism generally found in a specific population is often due to the presence of genetic variants

that has been defined by the total number of alleles at a particular locus and their ease of frequency of distribution in a particular population.

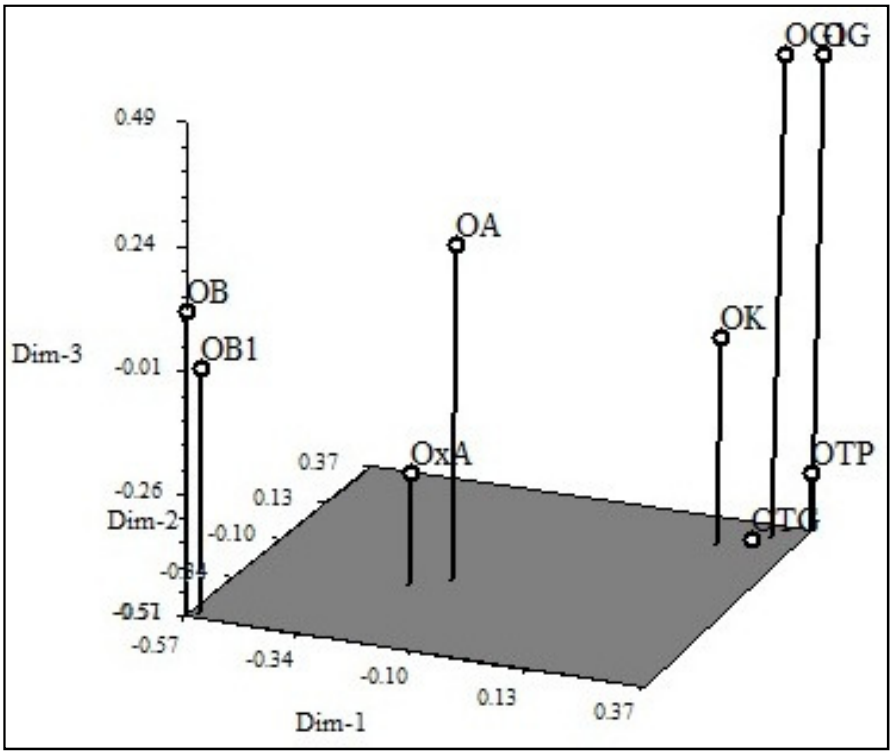


Figure 4.24: Three-dimensional plot by PCA (principal coordinate analysis) using a RAPD marker. **OG**-*O. gratissimum* (Ram tulsi); **OxA**-*O.* x *africanum* (Lebu tulsi); **OB**-*O. basilicum* (Babui tulsi); **OTP**- *O. tenuiflorum* (Krishna tulsi); **OB1**-*O. basilicum* (Marua tulsi); **OA**- *O. americanum* (Bon tulsi); **OTG**- *O. tenuiflorum* (Radha tulsi); **OG1**- *O. gratissimum* (Ajowan tulsi); **OK**- *O. kilimandscharicum* (Karpur tulsi).

According to RAPD genetic analysis, it has been found that *O.* × *africanum* (Lebu tulsi) was genetically similar to *O. americanum* (Bon tulsi) and two morphotypes of *O. basilicum* (Babu tulsi and Marua tulsi) (Chowdhury *et al*., 2017). This genetic similarity may be rationalized by the cross hybridization between *O. americanum* and *O. basilicum* (Babu tulsi) (Grayer *et al*., 1996; Satovic *et al*., 2002; Vieira *et al*., 2003; Carovic-Stanko, 2011b; Mishra *et al*., 2014). Although, taxonomic origin of *O.* x *africanum* (a lemon scented *Ocimum*) is very much confusing but still it is considered as a variety of *O. basilicum* var. *citriodorum* or even as a variety of a separate species, *O. americanum* var. *pilosum* (Pushpangadan and Sobti, 1982; Paton and Putievsky, 1996).

4.6. Propagation and conservation of *Ocimum*

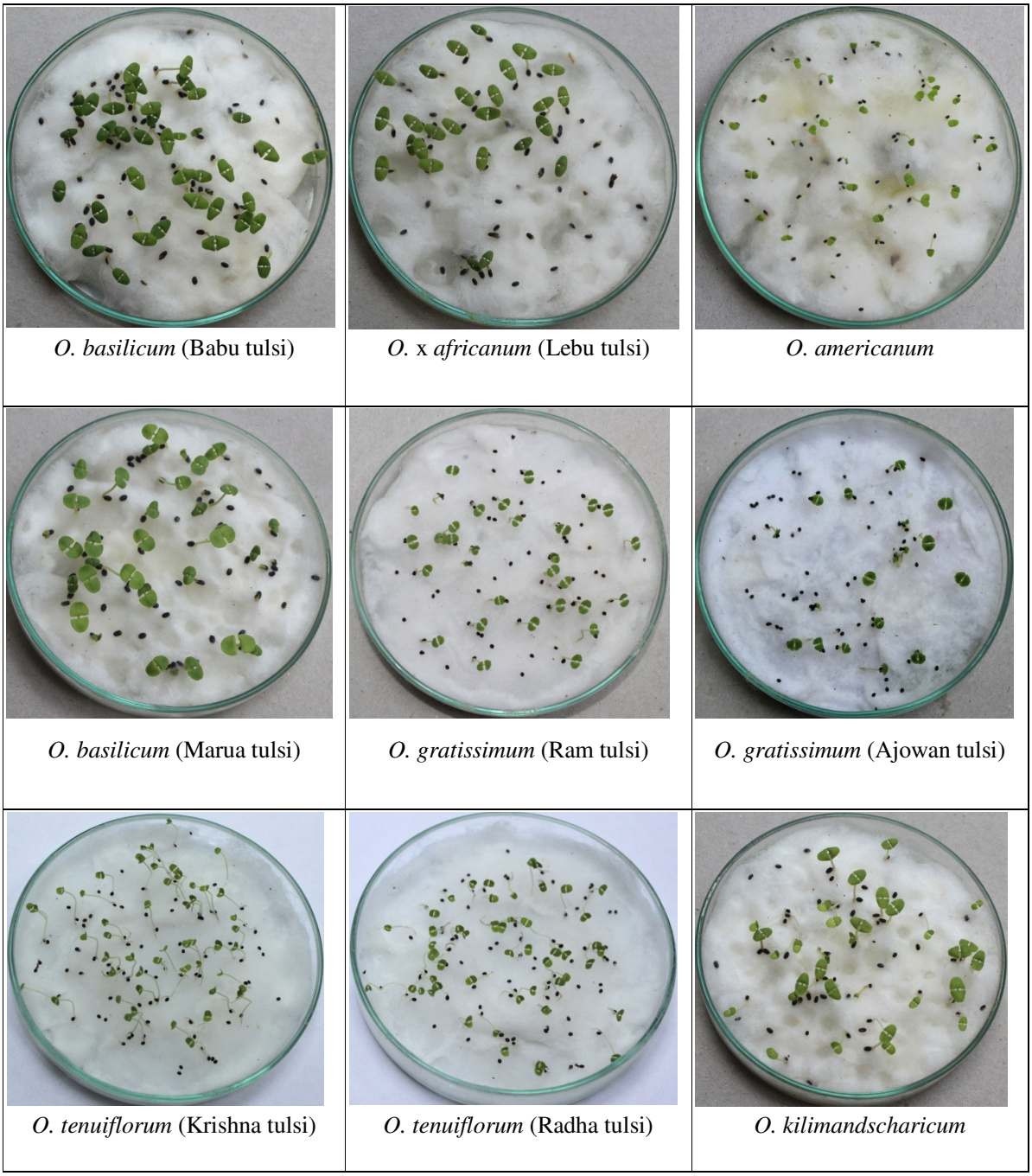
Temperature and humidity has a significant effect on germination of all the *Ocimum* species. Results showed that during summer season the highest percentage of germination (92%) was observed at 28 ± 5 ^{o}C temperature and 75 ± 10% relative humidity on 5th day. *O. tenuiflorum* (Krishna tulsi) showed maximum germination (92%) while, *O. gratissimum* showed only 60% seed germination. 90% seed germination was observed in *O. tenuiflorum* (green), *O. basilicum* (Babu tulsi), *O. basilicum* (Marua tulsi) and *O.* × *africanum*. In case of *O. americanum*, *O. gratissimum* (Ram tulsi) and *O. kilimandschericum* seed germination percentage were 88, 64 and 80 respectively (Table 4.16 and Figure 4.25).

Table 4.16: Mean germination percentage of nine *Ocimum* taxa after five days during summer season

Days		**OB**	**OA**	**O × A**	**OG_1**	**OG**	**OTP**	**OTG**	**OB_1**	**OK**
1st	Mean	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
2nd	Mean	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
3rd	Mean	46	64	66	30	44	68	74	52	48
	SE	0.33	0.33	0.33	0.58	0.33	0.33	0.33	0.33	0.33
4th	Mean	78	86	82	52	56	90	88	80	70
	SE	0.33	0.33	0.33	0.88	0.33	0.33	0.58	0.58	0.33
5th	Mean	**90**	**88**	**90**	**60**	**65**	**92**	**90**	**90**	**80**
	SE	0.33	0.33	0.58	0.33	0.58	0.33	0.33	0.58	0.33

SE= Standard error

During winter season seed germination was showed very low in respect of all the *Ocimum* species at 15 ± 5 ^{o}C temperature and 50 ± 10% relative humidity on 7th day. Lowest germination (26%) occurred in *O. gratissimum* (Ajowan tulsi) and maximum germination (46%) occurred in *O. tenuiflorum* (Green) (Table 4.17). Low temperature may be responsible for low germination percentage. Low temperature and critical high temperature would be expected to retard the metabolic rate to the point where pathways essential for the onset of germination would cease to operate (Roberts 1988; Verma *et al*., 2010). On the other hand, temperature can affect the percentage and rate of germination through its effect on the low rate of protein synthesis by the embryo due to non-availability of active mRNA as reported in *Zea mays* (Riley, 1981).



O. basilicum (Babu tulsi) | *O.* x *africanum* (Lebu tulsi) | *O. americanum*

O. basilicum (Marua tulsi) | *O. gratissimum* (Ram tulsi) | *O. gratissimum* (Ajowan tulsi)

O. tenuiflorum (Krishna tulsi) | *O. tenuiflorum* (Radha tulsi) | *O. kilimandscharicum*

Figure 4.25: Seed germination % of nine *Ocimum* taxa during summer season

Table 4.17: Mean germination percentage of different *Ocimum* taxa after seven days during winter season

Days		**OB**	**OA**	**O × A**	**OG_1**	**OG**	**OTP**	**OTG**	**OB_1**	**OK**
1st	Mean	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
2nd	Mean	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
3rd	Mean	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
4th	Mean	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
5th	Mean	22	30	22	12	14	22	24	26	24
	SE	0.33	0.33	0.33	0.33	0.33	0.58	0.33	0.33	0.33
6th	Mean	36	38	32	18	20	38	32	38	34
	SE	0.33	0.33	0.33	0.58	0.33	0.58	0.33	0.33	0.58
7th	Mean	**42**	**44**	**40**	**26**	**28**	**44**	**46**	**42**	**39**
	SE	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33

SE= Standard error

During summer, germination started from 3rd day onwards while in winter from 5th day onwards. Seed germination percentage of *Ocimum* species varies with temperature. Better germination was observed during summer at 28 ± 5 ^{o}C. Earlier workers also observed similar results in *O. basilicum* (Gupta and Shahi, 1998; Ramin, 2006; Mijani *et al*., 2013). The results of the present investigation indicate that the seeds have more germination potential during summer (June) at temperature of 28 ± 5 ^{o}C and relative humidity of 75 ± 10%. However, germination was found to be varied with days. It has been observed that during summer the seed germination starts on 3rd day while in winter season it takes 5th day. Seed germination of different *Ocimum* species with standard error (SE) at 5% level is depicted in Figure 4.26 and 4.27.

Mass multiplication through stem cutting is a low-cost and common method of propagation. Sulistiarine (1999) reported that *O. gratissimum* is conventionally propagated by the seed germination and stem cutting. Results showed that summer and winter were the most preferable season for mass multiplication of *Ocimum* species through cutting. Optimum root initiation to the highest level was found in both the seasons (Figure 4.28 and 4.29). Although, previously reported *Ocimum* species cannot be propagated through vegetative (Pattnaik and Chand, 1996). During summer (June) rooting started 3rd day onwards while in winter from 5th day. Root initiation was observed within 7-10 days in both the seasons (Figure 4.30). It was observed that in *O. gratissimum* (Ram and Ajowan tulsi) rooting initiation for both seasons required more

time as compared to other species. The rooted cuttings are then transferred to the herbal garden for their conservation (Figure 4.31). The advantage of this technique are very congenial, low cost, can be done throughout the year, not required expert.

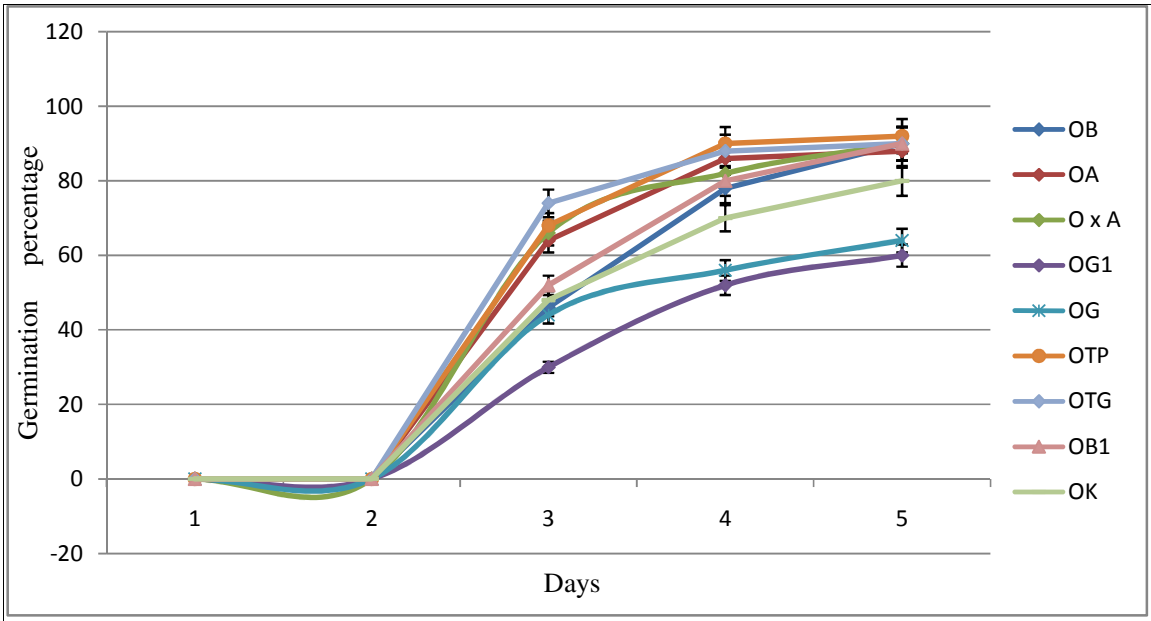


Figure 4.26: Seed germination % of different *Ocimum* taxa during summer season at 5% standard error.

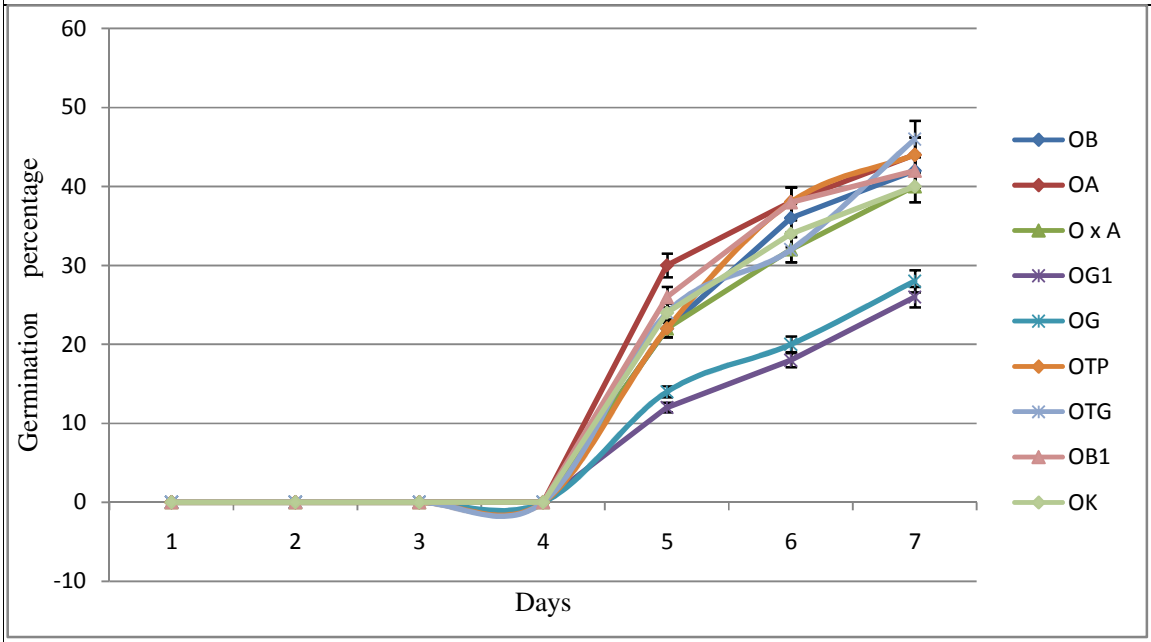


Figure 4.27: Seed germination % of different *Ocimum* taxa during winter season at 5% standard error

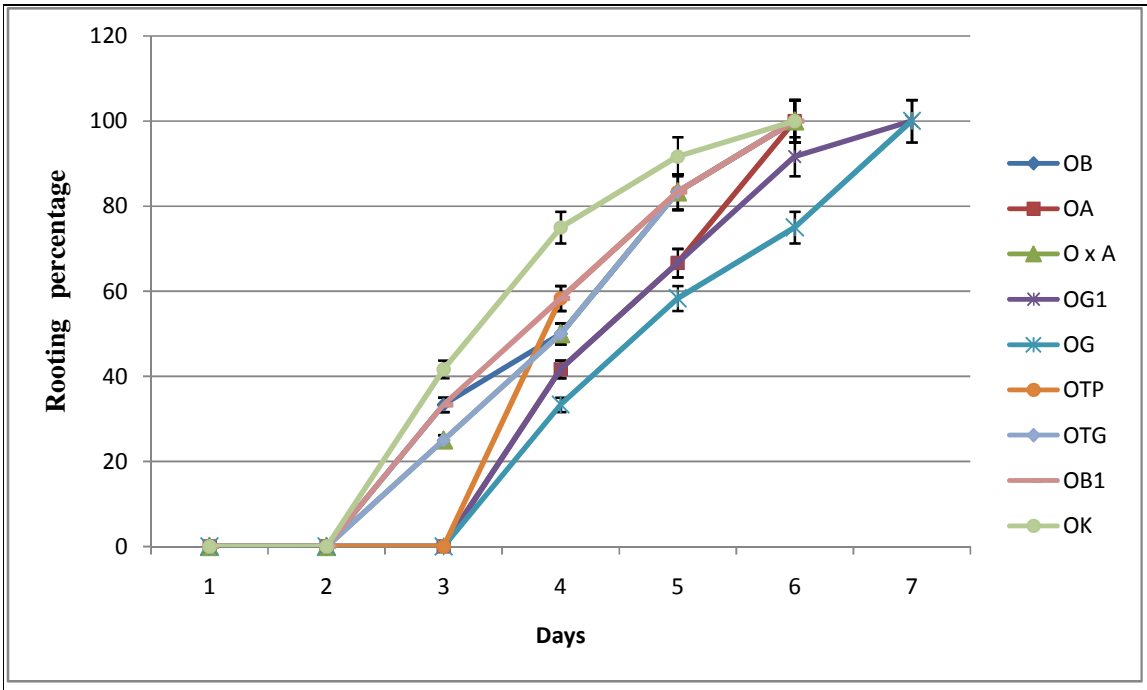


Figure 4.28: Rooting % through stem cutting of different *Ocimum* taxa during summer season at 5% standard error

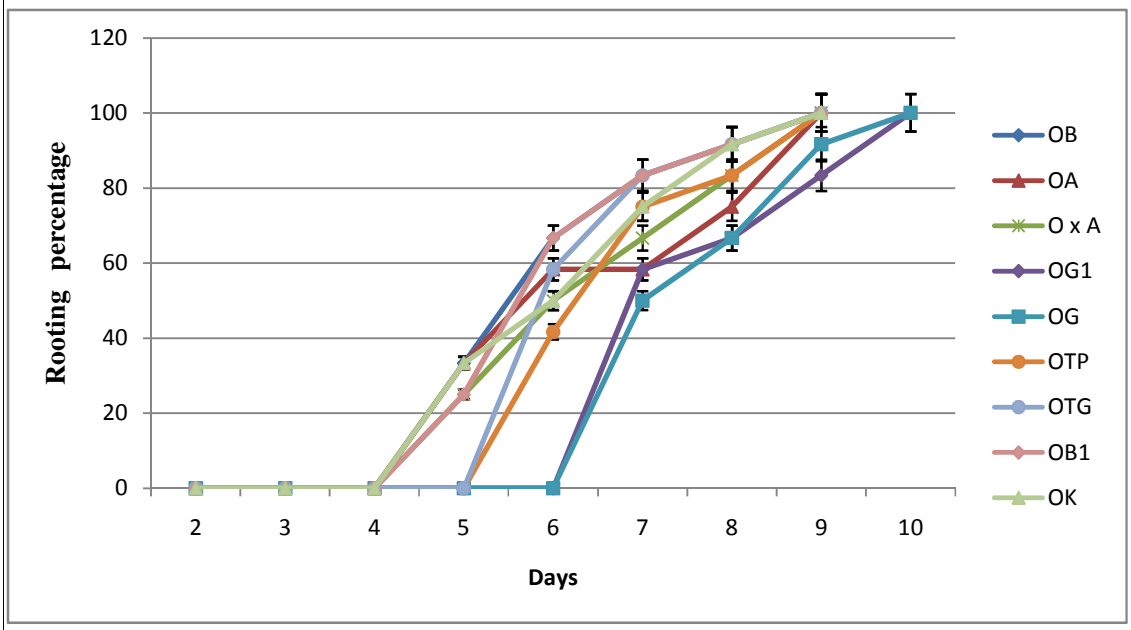
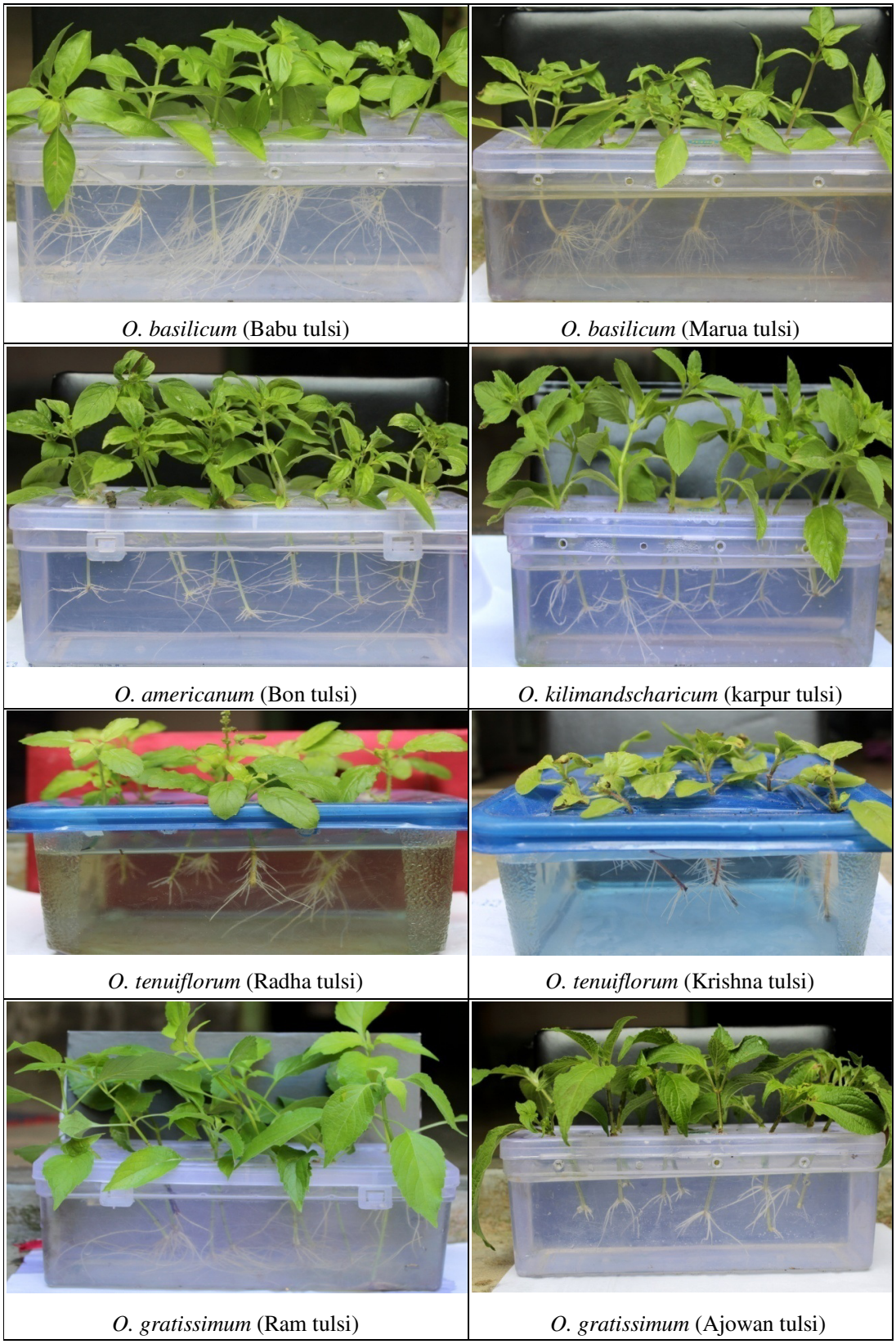


Figure 4.29: Rooting % through stem cutting of different *Ocimum* taxa during winter season at 5% standard error



O. basilicum (Babu tulsi) | *O. basilicum* (Marua tulsi)

O. americanum (Bon tulsi) | *O. kilimandscharicum* (karpur tulsi)

O. tenuiflorum (Radha tulsi) | *O. tenuiflorum* (Krishna tulsi)

O. gratissimum (Ram tulsi) | *O. gratissimum* (Ajowan tulsi)

Figure 4.30: Mass multiplications of different *Ocimum* taxa through stem cutting

Potted *O.* x *africanum* (Lebu tulsi)

Transplanted in the medicinal plants garden

Potted *O. tenuiflorum* (Radha tulsi)

Transplanted in the medicinal plants garden

Potted *O. americanum* (Bon tulsi)

Transplanted in the medicinal plants garden

Figure 4.31: Rooted cuttings of different *Ocimum* species were planted in the pot and medicinal plants garden for their conservation

Overgrazing, unconscious removal of economically important medicinal plants, biotic interference affecting the nature, structure and composition of plant communities are also the critical factors affecting the natural habitat of the *Ocimum* taxa and their existence. These secondary activities are accountable for changing natural vegetation to limited natural vegetation. Protection of the naturally growing plant species from overgrazing is necessary, particularly during seedling time. Protection is necessary to preserve the desired plants in high proportion, to evade invader plants and to restore the affected natural flora (Arshad *et al*., 2002). In addition, the study also suggests few conceivable outcomes for the conservation of naturally growing *Ocimum* species. First one is through the proper utilization of ecological principles—like protecting the remaining natural surroundings and reestablishing altered habitats to maintain wild population. A second way is to build up a more ecologically aware local people. *In situ* conservation through setting up botanical gardens in the surrounding areas of their natural habitat is the third alternative and finally cultivation of different *Ocimum* species would be another option for their conservation.

Conclusion

The present investigation reaffirms the rich heritage of ethnobotanical knowledge in the district of Daakshin Dinajpur, West Bengal. The repository of such knowledge is within the tribal and non-tribal communities. There are 138 plant species (angiosperm) belonging to 121 genera and 65 families have been found useful. As regard to the mostly used plants families were Lamiaceae followed by asteraceae, papilionaceae, euphorbiaceae, acanthaceae, asclepiadaceae, cucurbitaceae and verbenaceae. Other angiospermic families less commonly used were - anacardiaceae, convolvulaceae, liliaceae, malvaceae, mimosaceae, myrtaceae, orchidaceae, oxalidaceae, piperaceae, sterculiaceae and vitaceae. There were other 37 families where only one plant was used for at least different categories of health disorder and/or health enhancer. Almost all plant parts in conjunction with/or alone plant exudates have been found useful.

The holy basil (*O. tenuiflorum*) still occupies a sacred status in every household of almost all communities. There is wide gap of knowledge between sacred status and scientific use of holy basil. To fill up such gap of knowledge an in depth study is required.

Ecology of *Ocimum* was analysed using quadrat techniques. The quadrat study indicated that *Ocimum* species grow well in association with dicotyledons than monocotyledons. Association of growth was significant between *O. americanum* with *O.* × *africanum*. Interestingly, it was found that allergenic plant *Parthenium hysterophorus* an exotic species growth was very poor in the community of *Ocimum* species.

The physio-chemical study of soil showed that the pH varies between 5.7 to 6.3. The organic carbon (%) ranges from 0.52 to 0.82, the available nitrogen ranges from 223-353 (kg/ha) available phosphorus ranges from 60-70 (kg/ha) and available potassium ranges from 214-400 (kg/ha).

The constancy of characters among the variables of quantity and quality was analysed using Principal Component Analysis. Qualitative traits study showed high level of variability with respect to almost all traits (in the present study) among the taxa studied. Johnson (1998) applied principal component analysis (PCA) to screen most correlation among the traits. Principal component analysis (PCA) showed that bract length, seed colour, seed mucilage, bract width, petal length, petal width, inflorescence length and type of inflorescence contributed as first principal component, leaf tip, leaf shape, leaf area, leaf width, number of whorls/Inflorescence, leaf length and petiole length as second principal component and the third principal component was mostly influenced by the anther colour, inflorescence colour and leaf surface/texture. From the Agglomerative hierarchical clustering (AHC) two clearly distinct groups were obtained on the basis of the morphological traits using Euclidean distance by Ward's method**.** This study highlighted the separation among different *Ocimum* taxa as sanctum and the basilicum group.

Chemical compositional analysis has been done from the ethanolic extracts of nine *Ocimum* taxa. Total seventy three compounds were identified of which 12 were aliphatic acids, 3 aliphatic alcohols, 7 amino acids, 2 aromatic compounds, one fused ring aromatic hydrocarbon, 23 carbohydrates, 5 phenolic compounds, one quinone, 3 steroids, 12 terpenoids, vitamin E and 3 unidentified compounds. The study also revealed that there were significant differences among the *Ocimum* taxa with respect to chemical component and their composition. Methyl eugenol was present as chief compound of *O. basilicum* (Marua tulsi) whereas in *O. basilicum* (Babu tulsi) methyl eugenol was absent and concentration of hexadecanoic acid/palmitic acid is very high. The carom seed like spicy flavour of *O. gratissimum* (Ajowan tulsi) is due to the presence of terpenenoids, especially thymol in high content whereas clove like odour of *O. gratissimum* (Ram tulsi) may be due to the high content of eugenol. *O. tenuiflorum* (Radha tulsi) contained eugenol, thymol and β-elemene whereas eugenol, thymol and β-elemene were not detected in the other morphotypes of *O. tenuiflorum* (Krishna tulsi). *O.* × *africanum* (Lebu tulsi) and *O. basilicum* (Babu tulsi) are very close to their morphology but differ abruptly in their chemical constituent. Ergosterol, stigmasterol, phytol and thymol were present in *O.* × *africanum* (Lebu tulsi), but all were found to be absent in *O. basilicum* (Babu tulsi). *O.* × *africanum* is therefore a different species other than *O. basilicum* (Babu tulsi). This difference was further verified by RAPD analysis.

Methyl eugenol has chemical attractant property mostly against common fruit flies, *Bactrocera dorsalis* (Vargas *et al*., 2000). The present investigation also found similar result. Thus, Marua tulsi (*O. basilicum*) extracts can be used as bio-based pest control exploiting its chemical attractant property.

In this study, the diversity of nine *Ocimum* taxa grown naturally in the Dakshin Dinajpur district has been described from the perspective of its morological, chemical and RAPD analyses. These three analyses have clearly made distinction between *O. basilicum* (Babu tulsi) **-** *O. basilicum* (Marua tulsi), *O. gratissimum* (Ram tulsi) **-** *O. gratissimum* (Ajoyan tulsi) and *O. tenuiflorum* (Krishna tulsi) **-** *O. tenuiflorum* (Radha tulsi). Among nine *Ocimum* taxa six (*O. americanum*, *O.* × *africanum*, *O. basilicum*, *O. gratissimum*, *O. kilimandscharicum* and *O. tenuiflorum*) are different species of *Ocimum* and the rest are morphotypes. This study strongly recommended that both morpho-chemical and molecular assay could be used as complementary methods in describing the diversity of *Ocimum* taxa, their correct identification and taxonomic classification. Notably, it is possible to identify a local *Ocimum* taxa namely, Lebu tulsi as *O.* × *africanum* for the first time from this region as well as from West Bengal, India.

Conservation of *Ocimum* species and its propagation through seed germination is most consistent way to test its viability which is the most crucial part of seed conservation. The study revealed that summer season is the most favourable for seed germination of *Ocimum*. However, propagation through stem cutting can be practiced to conserve the *Ocimum* throughout the year. This type of vegetative reproduction may enhance clonal propagation of true type plant in a very short period most effectively and economically.

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Descriptor used in the present study morphological analysis

Table 1: Descriptor used in the present study

Sl. No.	Qualitative characters	Characters used for preparing descriptor			
1	Habit	Annual (1)	Perennial (2)	Others (99)	
2	Growth habit	Erect (1)	Semi-erect (2)	Others (99)	
3	Mode of reproduction	Sexual (1)	Asexual (2)	Others (99)	
4	Stem colour	Light green (1)	Brownish (2)	Purple-green (3)	Purple (4)
5	Stem shape	Quadrangular (1)	Round (2)	Others (99)	
6	Stem pubescence	Glabrous (0)	Sparse (3)	Dense (7)	
7	Leaf colour	Green (1)	Deep green (2)	Purple (3)	
8	Leaf surface	Glabrous (0)	Sparse (3)	Dense (7)	
9	Leaf margin	Entire (1)	Serrate (2)	Dentate (3)	
10	Leaf tip	Acute-acuminate (1)	Acute (2)	Obtuse to acute (3)	
11	Leaf shape	Ovate (1)	Elliptical (2)	Lanceolate (3)	
12	Inflorescence type	Simple (1)	Branched (2)		
13	Inflorescence colour	Green (1)	Greenish purple (2)	Purple (3)	Greenish grey (99)
14	Flower colour	Yellowish white (2)	White (1)	Pinkish-white (3)	Purplish (4)
15	Anther colour	White (1)	Yellow (2)	Brick red (99)	
16	Seed mucilage	Mucilaginous (1)	Non-mucilaginous (2)		
17	Seed colour	Brown (1)	Black (2)		
18	Seed shape	Globose (1)	Subglobose (2)	Ellipsoid (3)	Small ellipsoid (4)

Source: Minimal descriptors of agri-horticultural crops (Singh *et al*., 2003) and slightly modified by the author

Table 2: Transformation of qualitative traits into quantitative ones

	H	MOR	PGH	SC	SP	SS	LC	LS	LM	LT	LS_1	IT	IC	FC	AC	SM	SC_1	SS_1
OG	2	1	1	2	3	1	1	0	2	1	3	2	2	2	2	2	1	2
OG_1	2	1	1	2	3	1	2	0	2	1	3	2	1	2	2	2	1	2
OB	1	1	1	3	3	1	1	0	1	2	2	1	1	3	1	1	2	3
OB_1	1	1	1	3	0	1	1	0	1	2	2	1	3	3	1	1	2	3
O×A	1	1	1	1	3	1	1	0	2	2	2	1	1	1	1	1	2	3
OA	1	1	1	1	3	1	1	0	2	2	2	1	1	1	1	1	2	4
OK	2	1	1	1	7	1	1	7	3	2	2	2	99	1	99	1	2	4
OTP	99	1	1	4	7	2	3	3	3	3	1	2	3	4	2	2	1	1
OTG	99	1	1	1	7	2	1	3	3	3	1	2	1	4	2	2	1	1

OG- *O. gratissimum* (Ram tulsi); **OG1-** *Ocimum gratissimum* (Ajowan tulsi); **OB-** *O. basilicum* (Babui/Babu tulsi); **OB_1-** *O. basilicum* (Marua); **O×A-** *O.* x *africanum* (Lebu tulsi); **OA-** *O. americanum* (Bon tulsi); **OK-** *O. kilimandscharicum* (Karpur tulsi); **OTP-** *O. tenuiflorum* (Krishna tulsi); OTG- *O. tenuiflorum* (Radha tulsi); H-Habit, MOR-Mode of reproduction, PGH-Plant growth habit, SC-Stem colour, SP- Stem pubescence, SS-stem shape, LC-Leaf colour, LS-leaf surface, LM-Leaf margin, LT-leaf tip, LS_1-leaf shape, IT-Inflorescence type, IC-inflorescence colour, FC-Flower colour, AC-anther colour, SM- Seed mucilage, SC_1-Seed colour and SS_1-Seed shape.

Buffers and chemicals used for DNA fingerprinting study

Table 1: Stock preparation for CTAB

Stocks	Composition/preparation
CTAB DNA extraction buffer	CTAB (Cetyltrimethylammonium bromide) (Merck) extraction buffer was prepared taking 100 mM Tris-Cl (pH 8.0), 25 mM EDTA, 250 mM NaCl and CTAB (2%). CTAB extraction buffer (50 ml) was prepared by mixing the stock solution in desired amounts in a sterilized conical flask. **Note:** 0.2% β-mercaptoethanol (v/v) and 1 % Polyvinylpyrollidone, PVP (w/v) were added to the extraction buffer during extraction process.
100 ml 3 (M) Sodium Acetate (pH 5.2)	21.61 g sodium acetate was dissolved in 100 ml of double distilled water prior to autoclaving. The solution was autoclaved at 121ºC and 15 psi for 20 mins and stored at room temperature for further use. The pH of the solution was adjusted to 5.0 by addition of glacial acetic acid.

Table 2: Stock preparation for DNA extraction

Sl. No.	Buffer/Solution	Composition/preparation
1	Tris-HCl (1M, pH-8.0)	12.114 g Tris base was dissolved in 100 ml deionized water.
2	EDTA (0.5M, pH-8.0)	18.612 g EDTA was dissolved in 100 ml deionized water.
3	TE buffer	10 mM Tris-Cl (pH-8), 1mM EDTA (pH-8)1M NaCl (Merck). The extracted genomic DNA was diluted and stored in salt TE buffer.
4	TE Saturated Phenol	100 mL liquid phenol was taken and 99 mL Tris-Cl (pH 8.0) was added (for saturating 100 ml phenol) and the bottle stirred continuously for about 1h. The upper Tris-Cl layer was discarded and poured in a measuring cylinder. 50 ml double distilled water added into the bottle containing phenol and stirred for 30 min. The upper Tris-Cl layer was again transferred to the measuring cylinder and little mL double distilled water added and the procedure repeated to replace 50 mL double Distilled water. In another sterilized conical flask, 100 ml double distilled water, 1 mL (M) Tris-Cl and 200 µl 0.5 (M) EDTA was taken and transferred to an amber bottle containing phenol. A pinch of 8-Hydroxylquinoline (Merck) was added in this solution. The solution, then stirred continuously overnight. Next day the Tris-

		EDTA saturated phenol was collected and stored in an amber bottle at 40 °C.
5	NaCl (5M)	10 ml solution of 5 (M) NaCl stock was prepared taking 2.922 g NaCl (Merck) dissolved in 10 mL double distilled water.

Table 3: Chemicals required for PCR reactions

Chemicals	Composition/preparation
Assay buffer (10X)	Assay buffer containing 100 mM Tris-HCl (pH 9.0), 500 mM KCl and 0.01% gelatine (w/v), without $MgCl_2$ was diluted to 1X during PCR mix preparation (Genei).
dNTP Mix	Each dNTP was supplied as 10 mM stock. These were diluted to 2 mM as working standard with PCR grade water (Genei). 20 µl of each dNTP was added from 10 mM stock, to 20 µL sterile PCR grade water in a sterile vial to prepare dNTP mix of 100 µl with 2 mM of each dNTP (Genei).
$MgCl_2$	25 mM $MgCl_2$ solution was supplied (Genei).
Taq DNA polymerase	*Taq* DNA polymerase of 3U/µL strength without gelatine was used in all PCR reactions (Genei).

Table 4: Stock preparation and procedure for Gel electrophoresis

Stocks	Composition/preparation
Running buffer (1x TAE)	Tris- Cl (pH 8.0) (i.e. 10 mM) =0.6055gm and EDTA (pH 8.0) (i.e. 1 mM) =0.186 g were dissolved separately and finally mixed together and the final volume was made up to 1000 ml with double distilled water prior to autoclaving. The buffer was autoclaved at 121 ºC and 15 psi for 20 mins and stored at room temperature for further use.
Gel loading Buffer (6x)	25 mg Bromophenol blue dye (Sigma) was mixed with 25 mg xylene cyanol (Merck), 9.36 mL of 30 % glycerol (Merck) dissolved in water to prepare 25 mL of gel loading buffer (6x). Gel loading buffer was diluted to 1x with addition of 1x TAE during loading of DNA.
Gel staining dye (EtBr)	10 mg Ethydium bromide (Genei) dissolved in 1 mL sterile double distilled water. The solution was stored at room temperature, and used for staining the DNA gel at a working concentration of 0.5µg/ mL (Sambrook and Russed, 2001)

Chromatogram and mass spectra of the ethanolic extract of different *Ocimum* taxa

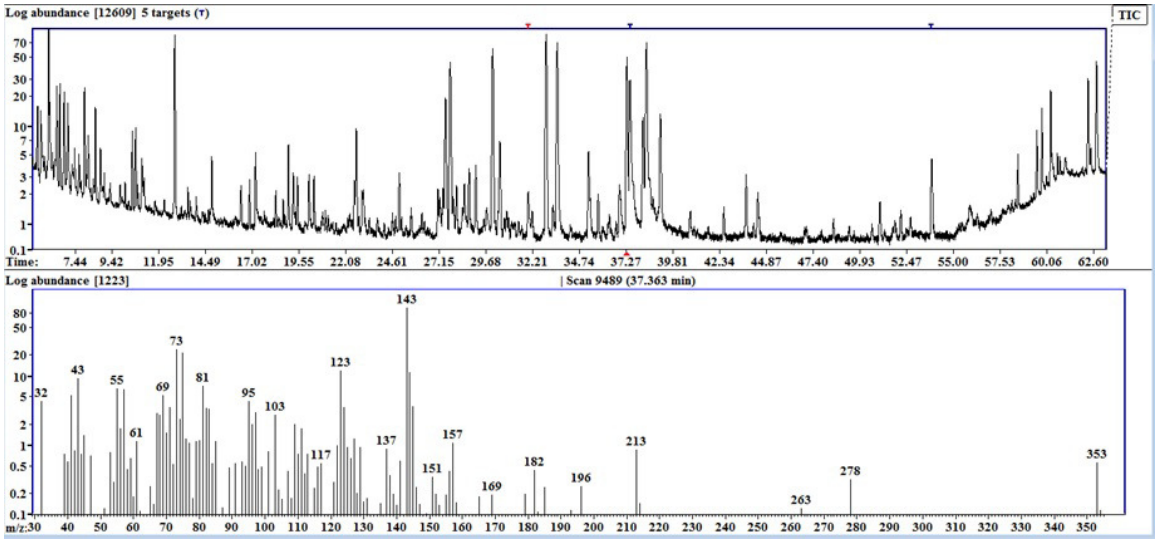

Figure 1. GC-MS Chromatogram and mass spectra of the ethanolic extract of *O.* x *africanum* (Lebu tulsi) leaves

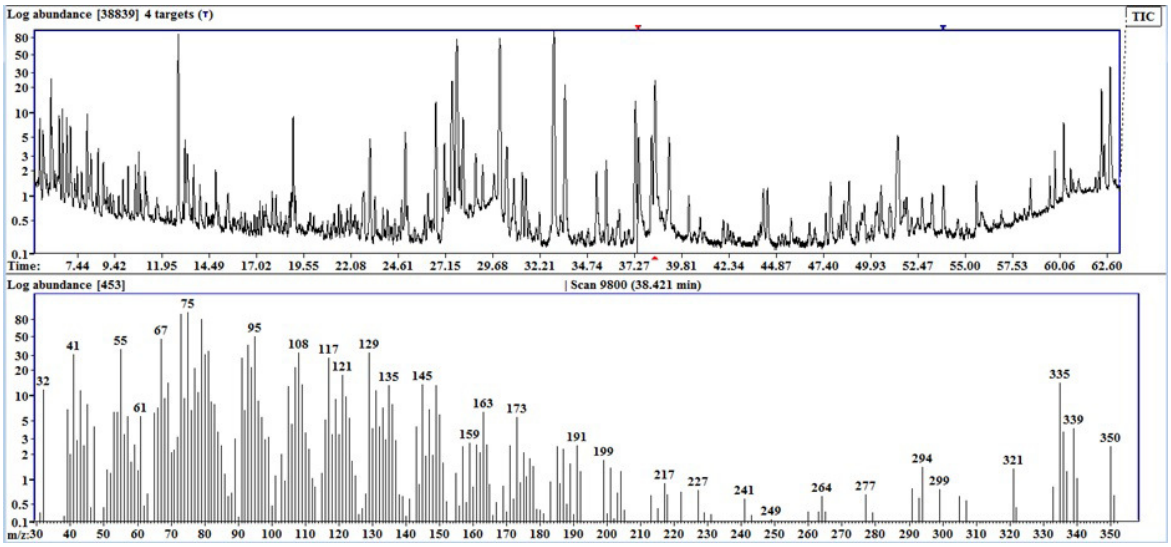

Figure 2. GC-MS Chromatogram and mass spectra of the ethanolic extract of *O. americanum* (Bon tulsi)

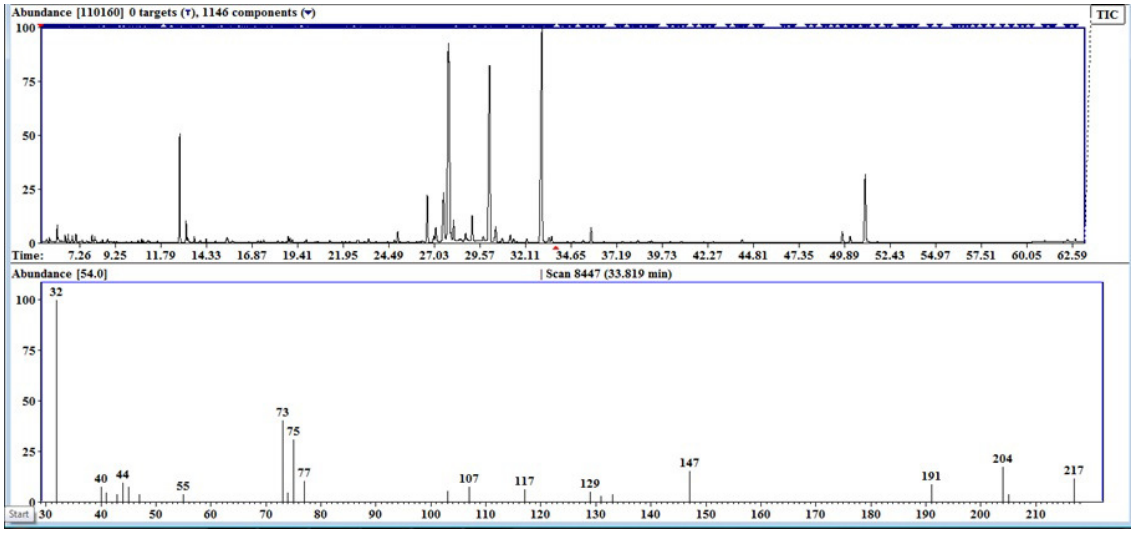

Figure 3. GC-MS Chromatogram and mass spectra of the ethanolic extract of *O. tenuiflorum* (Krishna tulsi)

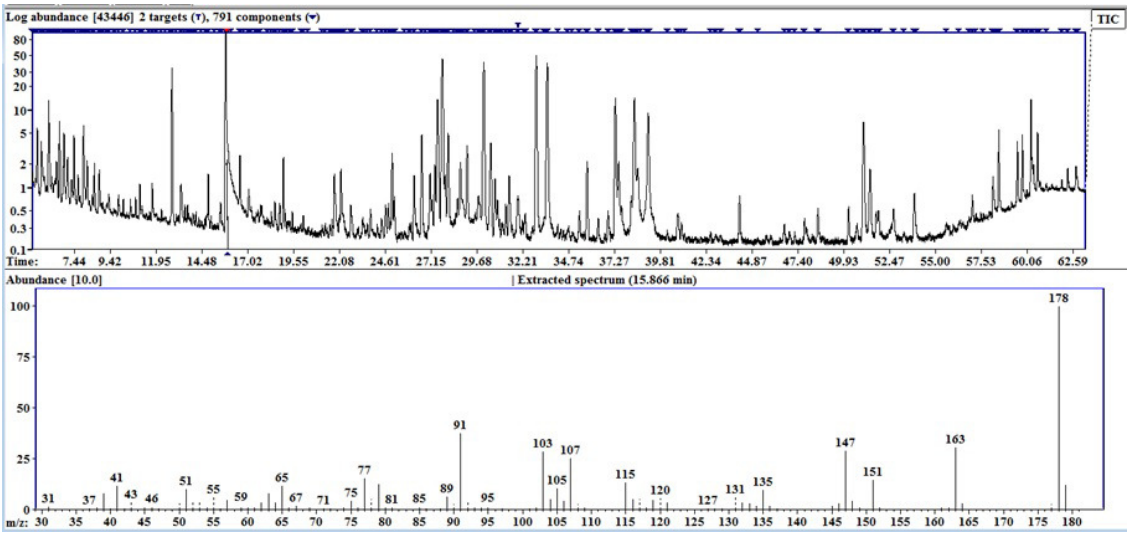

Figure 4. GC-MS Chromatogram and mass spectra of the ethanolic extract of *O. basilicum* (Marua tulsi)

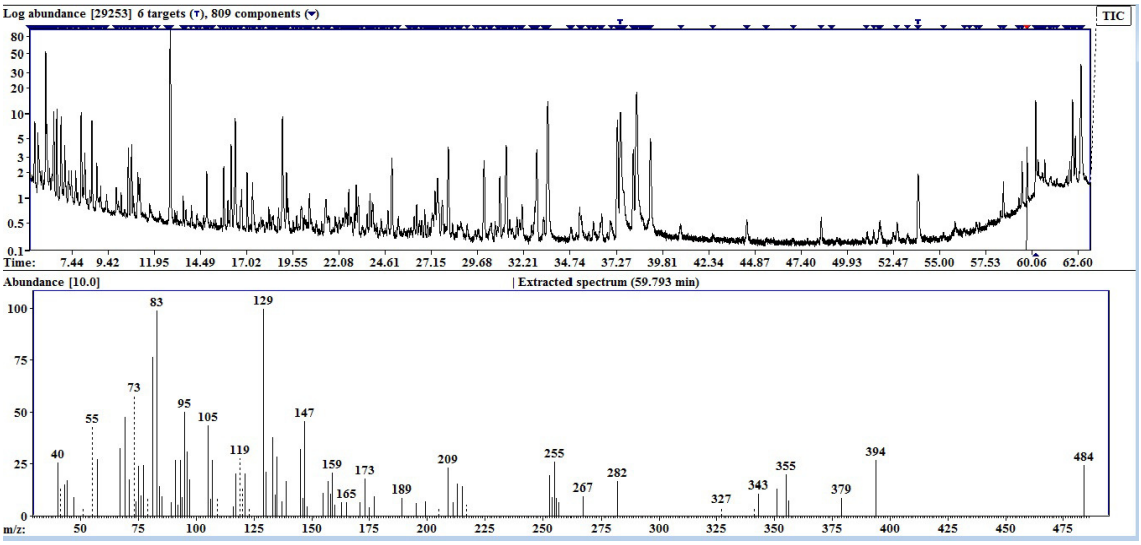

Figure 5. GC-MS Chromatogram and mass spectra of the ethanolic extract of *O. kilimandschericum* (Karpur tulsi)

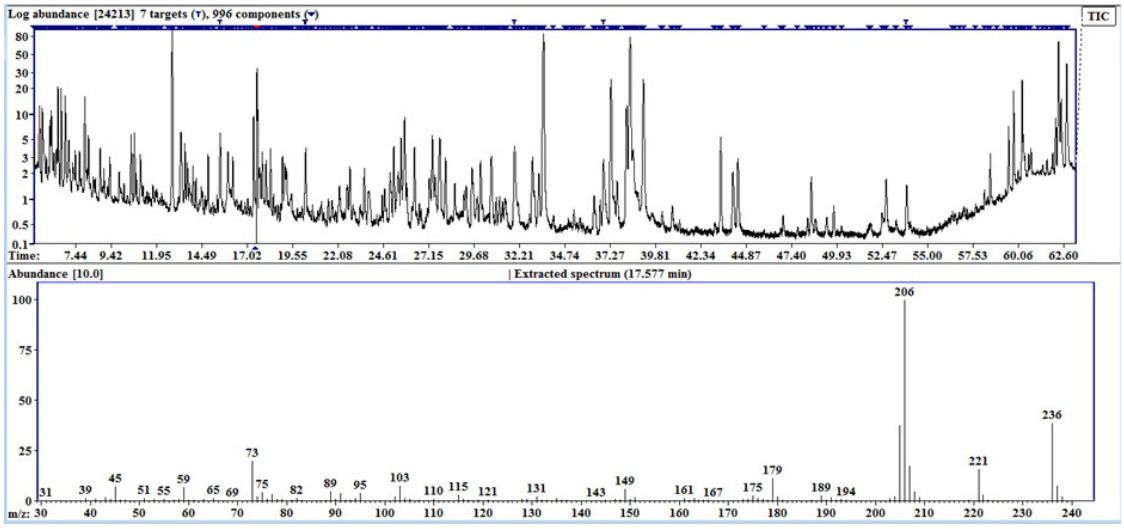

Figure 6. GC-MS Chromatogram and mass spectra of the ethanolic extract of *O. tenuiflorum* (Radha tlsi)

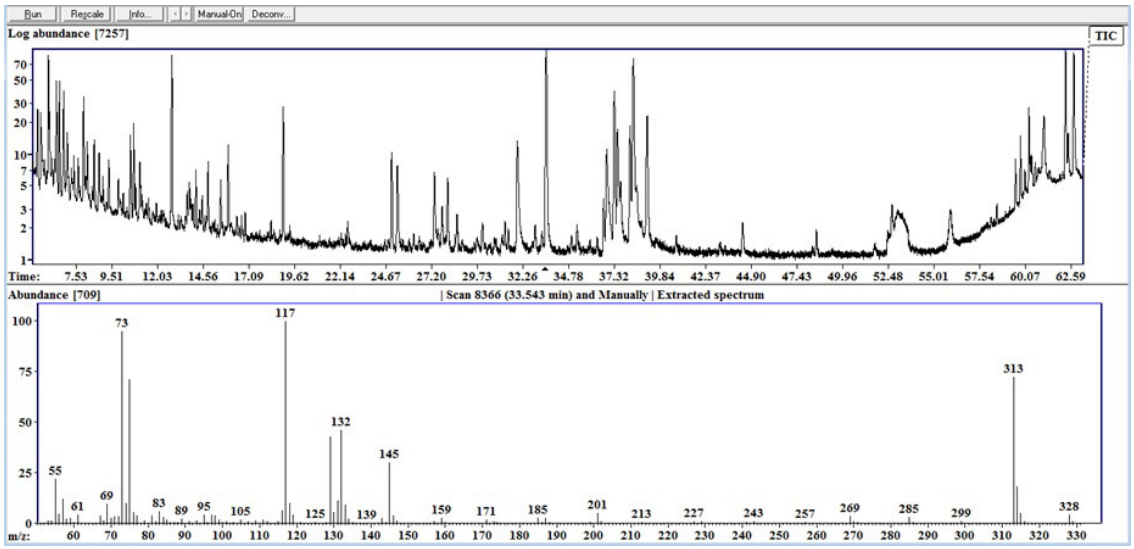

Figure 7. GC-MS Chromatogram and mass spectra of the ethanolic extract of *O. basilicum* (Babu tulsi)

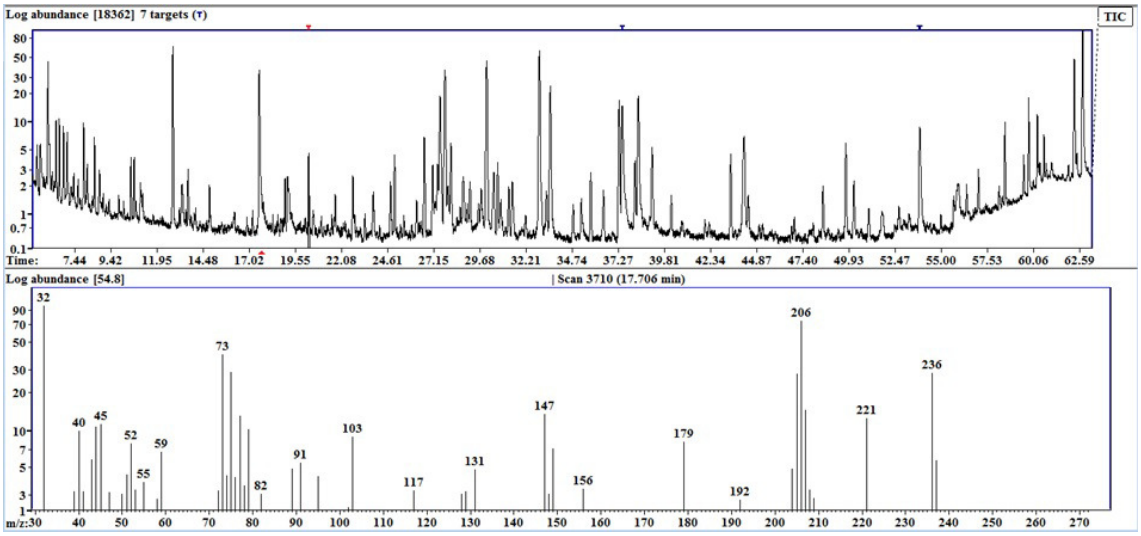

Figure 8. GC-MS Chromatogram and mass spectra of the ethanolic extract of *O. gratissimum* (Ram tulsi)

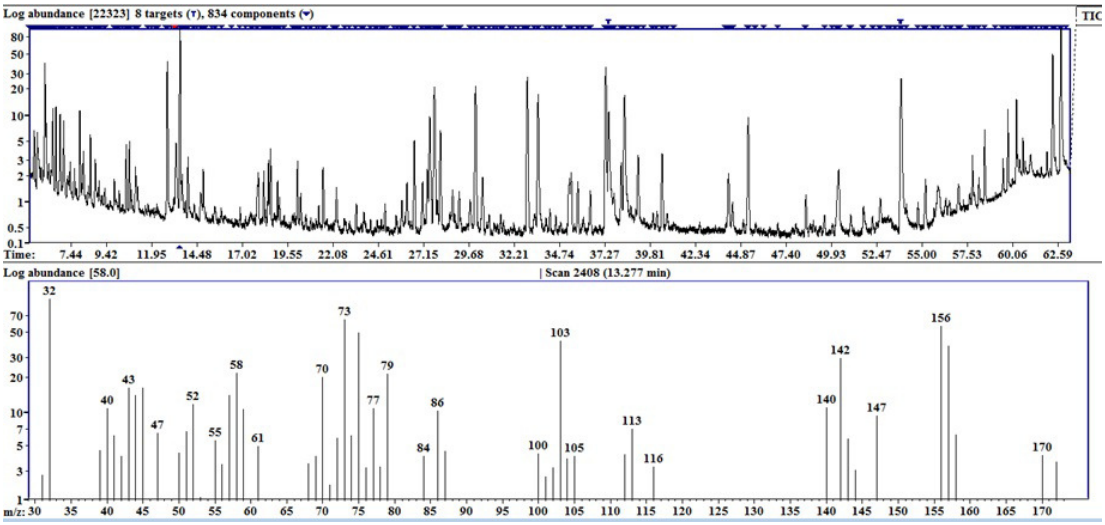

Figure 9. GC-MS Chromatogram and mass spectra of the ethanolic extract of *O. gratissimum* (Ajowan tulsi)

Thesis related publication till April, 2017

Chowdhury T, Mandal A, Roy SC, De Sarker D 2017. Diversity of the genus *Ocimum* (Lamiaceae) through Morpho-molecular (RAPD) and Chemical (GC-MS) analysis. *J Genet Eng Biotechnol*. DOI: 10.1016/j.jgeb.2016.12.004

Chowdhury T, Mandal A, Jana AK, Roy SC, De Sarker D 2016. Study of phyto-sociology and ecology of naturally growing *Ocimum* species with their conservational strategies in Dakshin Dinajpur district of West Bengal. *Acta Ecologica Sinica*, **36**, 483-491.

Chowdhury T, De Sarker D, Roy SC 2014. Local folk use of plants in Dakshin Dinajpur district of West Bengal, India. *Int. Res. J. Biological Sci*., **3** (5), 67-79.

Chowdhury T, De Sarker D, Saha M 2011. Survey of plants used for the treatment of diabetes in Dinajpur (Uttar & Dakshin) and Malda districts of Paschimbanga, In: Ghosh C and Das AP (ed.), Proceeding *Recent studies in biodiversity and traditional knowledge in India*, Gour Mahavidyalaya, Malda, pp 295-299.