Essential oils from different parts of Large Cardamom (*Amomum subulatum Roxb.)* and composting of available biomass

A Thesis Submitted

To

Sikkim University

In Partial Fulfilment of the Requirement for the

Degree of Doctor of Philosophy

By

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School of Life Sciences

February 2022

DECLARATION

I, Mr. **Sangay Gyampo Bhutia,** hereby declare that the thesis entitled **"Essential oils from different parts of Large Cardamom (*Amomum subulatum Roxb.*) and composting of available biomass"** was done by me and the contents of this thesis did not form basis of the award of any previous degree to me or, to the best of my knowledge, to anybody else and that the thesis has not been submitted by me for any other research degree in any other University/ institute.

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"Essential oils from different parts of Large Cardamom (*Amomum subulatum Roxb.*) and composting of available biomass"

Submitted by **Mr. Sangay Gyampo Bhutia** under the supervision of **Dr. Sujata Upadhyay, Department of Horticulture, Sikkim University, Gangtok, 737102, India.**

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All the assistance and help received during the course of the investigation have been duly acknowledged by him.

I recommend this thesis to be placed before the examiners for evaluation.

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Dated: 28/02/2022 **Sangay Gyampo Bhutia**

Place: Gangtok

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Abbreviations

>	**Greater than**
%	**Percentage**
~	**Approximately**
°C	**Degree centigrade**
µg	**Micro gram**
µl	**Microliter**
µM	**Micro mole**
BC	**Before Christ**
C18	**Column number 18**
cm	**Centimeter**
DAD	**Dode array detector**
DCM	**Dichloromethane**
DNA	**Deoxyribonucleic acid**
D	**Deoxyribonucleic acid**
E	**East**
et al.	**et. alli (Latin), others**
g	**Gram**
GC	**Gas Chromatography**
GC-MS	**Gas Chromatography-Mass Spectrometry**
h	**Hour**
ha	**Hectare**
HM	**Herbal medicament**
HPLC	**High performance liquid chromatography**
HPTLC	**High Performance Thin Layer Chromatograpy**

ICRI	**Indian Cardamom Research Institute**
kg	**Kilogram**
km^2	**Kilometer square**
l	**Litre**
LC	**Liquid chromatography**
m	**Meter**
M	**Molar**
mg	**Milligram**
mg/ml	**Miligram / Mililiter**
min	**Minute(s)**
ml	**Millilitre**
mm	**Millimeter**
mM	**Milli mole**
µM	**Micro mol**
MS	**Mass spectrometry, Murashige/Skoog (medium)**
msl	**Mean sea level**
MT	**Metric ton**
N	**North**
N_2	**Nitrogen gas**
nm	**Nano metre**
s	**Second(s)**
SD	**Standard deviation**
Sec	**Second**
TLC	**Thin Layer Chromatography**
U	**Unit**

U/ml	**Unit / Mililiter**
UV	**Ultra violet**
v/v	**Volume/volume**
v/w	**Volume/weight**
w	**Weight of the sample**
w/v	**Weight/volume**
w/w	**Weight/Weight**
W1	**Weight of the beaker**
W2	**Weight of the sample and beaker**
α	**Alpha**
β	**Beta**
γ	**Gamma**
µg	**Microgram**
µg/ml	**Microgram / Mililiter**
µl	**Microliter**
No.	**Number**
i.e.	**That is**
Fig.	**Figure**
e.g.	**For example**
CD	**Critical difference**
AMSL	**Above mean sea level**
ANOVA	**Analysis Of Variance**
CRD	**Completely Randomized Design**
Etc	**Et cetera**
GPS	**Global Positioning System**

pH	**Hydrogen ion concentration**
Sp.	**Species**
B	**Boron**
Ca	**Calcium**
Ce	**Cerium**
Co	**Cobalt**
Cu	**Copper**
Fe	**Iron**
ICPMS	**Inductively Coupled Plasma Mass Spectrometry**
K	**Potassium**
Mg	**Magnesium**
Mn	**Manganese**
Mo	**Molybdenium**
N	**Nitrogen**
Na	**Sodium**
ND	**Non detectable**
Ni	**Nickel**
P	**Phosphorus**
S	**Sulphur**
Zn	**Zinc**

Chapter 1

INTRODUCTION

Amomum subulatum Roxburg commonly known as large cardamom belonging to the family Zingiberaceae under the order Scitaminae is an important spice crop of India (Gautam *et al.,* 2016). It is also called as Greater Cardamom, Black cardamom in English, Bada ealichi in Bangla, Badi llayachi in Hindi, Sthulaila, Bhadraila in Sanskrit, alainchii in Nepali and lengee in Bhutia. It has been a well known spice since ancient time and has been valued for its acceptable taste, flavour and pleasant aromatic odour (Bisht *et al.,* 2011). It is native to Sikkim Himalayas and from there it has spread to sub- Himalayan areas like Darjeeling, Uttaranchal, Assam, Nagaland, Bhutan and Nepal (Vijayan *et al*., 2017 and Kumar *et al*., 2013). It is the major cash crop in Sikkim and it is the good source of income for the farmers (Sharma *et al.,* 2000). Sikkim is the largest producer of large cardamom and constitutes lion share of India and World market (Bisht *et al.,* 2012). About 3863 MT of large cardamom is being produced annually from 26,459 ha of Sikkim region which is emerging as India's organic hub (Bisht *et al.,* 2011). East district (1487.80 MT) had highest production from 6514 Area (Ha), South district (983.94 MT) from 3707.95 Area (Ha) was rank second, followed by West district (974.54 MT) from 3645.20 Area (Ha) and North district (939.00 MT) from 3868 Area (Ha) (Source: Anonymous 2009-2018, Spice Board, Govt. of India).

Large cardamom is a perennial, tall, evergreen, herbaceous monocot plant (Gopal *et al.,* 2012). Generally the height of the mature plants ranges from 1.5-3.0 m (Bisht *et al*., 2011). It contains subterranean rhizomes with shoots and stem is called

Plate No 1: Productive tiller with a mature spikes ready for flowering.

Plate No 2: Large cardamom plants at flowering stage.

Plate No 3: Mature yellowish colour flowers attracting bumble bees.

Plate No 4: Bumble bees pollinating large cardamom flowers.

Plate No 5: Post pollination plants at their fruiting stage.

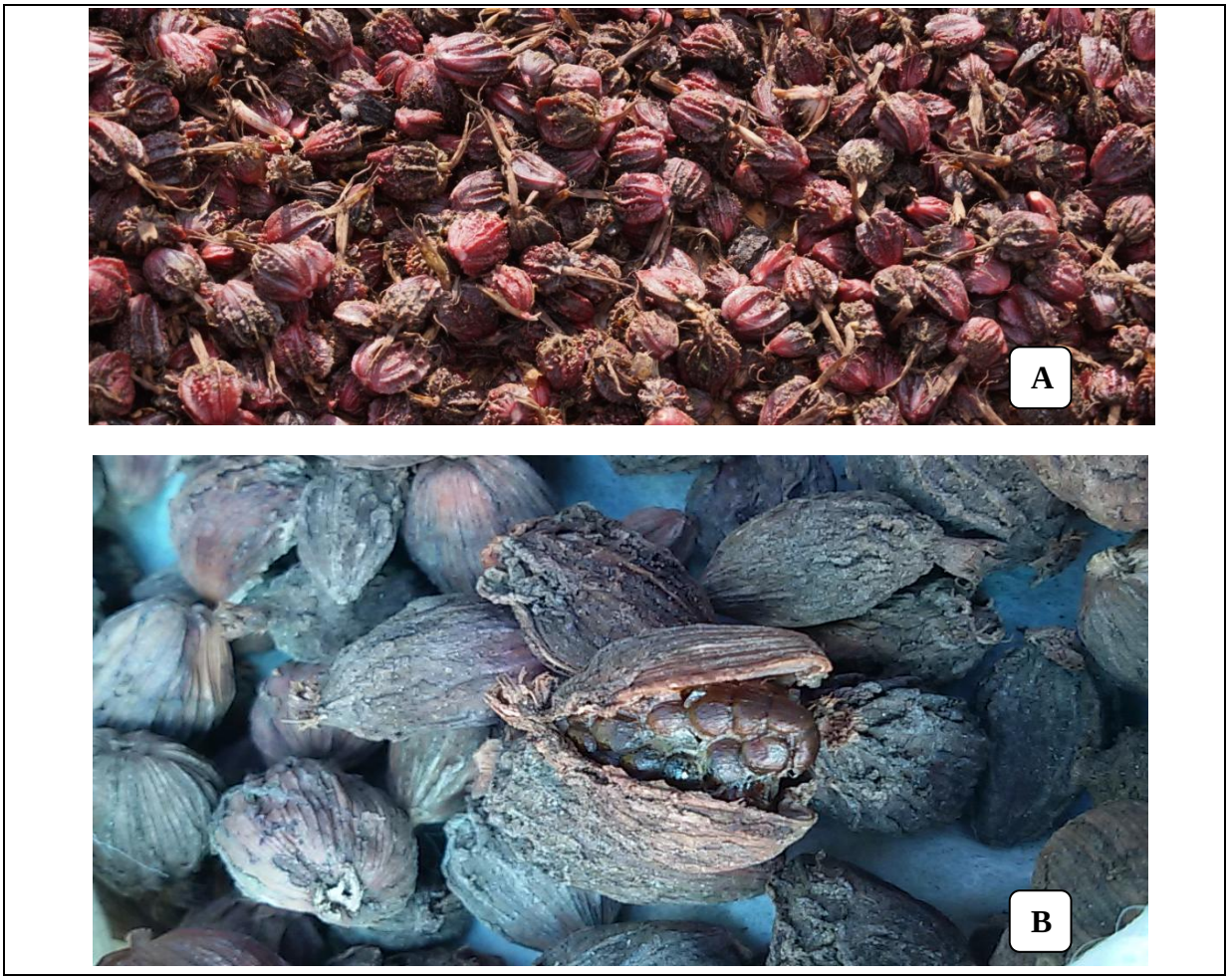


Plate No 6: A. Capsules separated from the spike before curing, B. Cured capsules.

tiller which is a pseud ostem. It is a shade loving plant and grown under mixed forest tree, preferably along the streams. Its inflorescence is called spike, 15 to 40 spike are observed in a clump and number of spike in a clump depends on varieties and plant age, around 35 to 55 flowers bloom in a spike (Kishore *et al*., 2012). Flowers are yellow in colour with modified corolla called labellum, bisexual and zygomorphic. Bumble bees are the major and most efficient pollinators of large cardamom. The fruit of the large cardamom is capsule with oval to round in shape, echinated, maroon in colour, trilocular with 40 to 50 seeds. It is whitish in immature stage and turn black in mature stage. Generally plants produce flower after the third year of the plantation. Whole spike are harvested using knives called as Elaichichhuri when top most capsules turns brown. The capsules are dried after separated from the spikes. Tail (Calyx) of the cured capsules are removed by rubbing it on wire mesh. Traditionally large cardamom capsules are cured in Bhatti (Plate No. 7). In this system the capsules are dried by direct heating with smoky smell. If capsules quantity is less then sun drying method is used for curing, it improves the capsule colour but takes more time (Plate No. 8).

There are mainly six varieties of large cardamom found in Sikkim viz. Sawney, Ramsey, Golsey, Ramla, Varlangey and Seremna. The details characteristics of different varieties of large cardamom have been given in Table 1.1.

Its fruit contains 2 to 3% of essential oil rich in cineole having powerful flavouring agent which is mainly used as spice in preparation of curries, soups, sausages and other meat preparations in India and around the globe.

Table 1.1: The details characteristics of different varieties of large cardamom.

Character/ Variety	Sawney	Ramsey	Golsey	Ramla	Varlangey	Seremna
Altitude	Medium to high altitude (975-1515 m MSL and >1515 m MSL)	High altitude (1515 m MSL)	Low to medium altitude (975m MSL)	High altitude	Medium to high altitude (>1515 m MSL)	Low altitude
Status	Tall, 1.5-2.0 meters, vigorous, bent downwards, leaves are ovate and broad	Tall, 1.5-2.0 meters, vigorous wide clump growth, narrow leaves	Plant height 1.0-1.5 meters, Less vigorous with erect leafy stem bearing stout upright leaves, clumps medium	Tall, 1.5-2 meters, vigorous like Ramsey, leaves are broad and long	Tall, robust type, plant height 1.5-2.5 meters, narrow leaves having wavy margins	Tall, 1.5-2.0 meters, leaves are mostly drooping type
Stem colour	Pinkish with dark colour	Maroonish colour	Greenish to marronish colour	Maroonish colour	Maroonish colour	Green colour
Flowers	Yellowish with pink tinge as base of corolla, Flowering starts from March-May	Yellowish and small, corolla tip with pink tinge at base, Flowering starts in May	Yellowish-orange, Flowering starts in March	Flowering starts in May	Flowering starts in May at medium altitude and June-July at high altitudes	On an average 2-3 spikes in each productive tillers
Capsules and harvesting	Big, bold, 35-50 seeds, Harvest	Smaller, 2.0-2.3 cm length, dark	Bold, big, pinkish in colour, 50-70 seeds,	Capsules are dark pinkish in colour, 2.0-	Bold with 50-70 seeds, harvesting	10 capsule in each spike, 65-70 seeds,

	begins in September-October, November in high altitude areas	pinkish colour, 25-40 seeds, harvesting starts from October-November	harvesting is done in September-October	2.3 cm length, 30-40 seeds, harvesting commences in October	is done up to the end of November in high altitude	this variety is known for its high yield potential

Source: Gautam *et al.,* (2016), Vijayan *et al.,* (2017)

It possesses medicinal properties of acting as stomachic, analgesic, carminative, diuretic, antimicrobial and an effective cardiac stimulant. It is also used as remedy for throat, respiratory troubles and the decoction of seeds is used as gargle in infection of teeth and gums. Its seeds are also considered as an antidote to snake venom or scorpion venom. In Ayurvedic and Unani medicine large cardamom seeds are used as preventive as well as a curative for throat trouble, congestion of lungs, inflammation of eyelids, digestive disorders and in the treatment of pulmonary tuberculosis (Gautam *et al.,* 2016). Therefore, large cardamom is highly valued spice and needs research on production, composition, different parts used and its by-products etc.

There has been no systematic study to find metabolic constituents of different plants parts of large cardamom. There have been few works done in some cultivars of large cardamom for knowing the different components present in the seed and pericarp (husk) but other parts have not been under focus which also has economic importance and also there is no comparative study between capsules with other parts of large cardamom. The present study was carried to carry out metabolic constituents by taking different plants parts such as leaves, tillers, spike remains and capsule, which will be helpful for knowing the components which are unknown and also compost will be prepared by using available biomass and it may add some more

Plate No 7: Curing fresh capsules in traditional bhatti.

Plate No 8: Sun drying methods using bamboo mat.

economic and medicinal value to the crop. Hence, keeping above mentioned facts in view the present study was conducted with following objectives:

OBJECTIVES:

1. To extract and quantify the essential oil from the different parts of large cardamom varieties.
2. To isolate and quantify the different metabolites present in the essential oil.
3. Standardization of the compost preparation from available biomass.
4. Nutrient and physicochemical analysis of compost.

SCOPE AND RELEVANCE OF THE PROPOSED WORK

Large cardamom leaves, tillers and spike remains are the waste remains for farmer after harvesting capsules. In the present work the oil was extracted from the different parts of large cardamom varieties to find their metabolic constituents which were helpful for knowing the components which are unknown and it may add some more economic and medicinal value to the crop. From the research, the following outcomes may be expected:-

1. Identification of large cardamom part which contain high amount of essential oil.
2. Identification of the different metabolites present in the essential oil.
3. Standardization of the compost preparation from available biomass.
4. Identification of different nutrients present in the compost of available biomass.

Chapter 2

REVIEW OF LITERATURE

Large cardamom is an aromatic and medicinal spice native to Eastern Himalayas and it is one of the major cash crops of Sikkim. It is used as flavouring and preservative to different types of coffee, liquors, confections, beverages and tobacco. Its powder, oleoresin and essential oils have many culinary and therapeutic uses. After harvesting large cardamom many parts of the plant are uneconomical for farmer and left in the fields as such. As there is essential oil in the parts left in the field like spike remains, leaves and tillers because of this reason it take a lots of time in decaying. The leftover plant parts create unsanitary in the cardamom field and also act as the source of disease inoculums such as fungal (Colletotrichum blight), viral (Chirke and Foorkey) and pests for example stem borer. Therefore, utilization of the plant waste like spike remains, leaves and tillers add additional income and maintain hygiene, which is most important for healthy cardamom field. The method for utilizing field waste can be extraction of essential oil, compost making, which is a basic needs for farming and also primary raw material for flavouring industry. Compost is nowadays the most efficient alternative of chemical fertilizers for the farmers. It is used as a natural and ecological beneficial means of improving crop production by enhancing soil fertility. The available relevant review of the several works on different aspects of present investigation had been undertaken, which are divided into different sub headings base on research parameters are given as follows:

1. The family Zingiberaceae.

2. Large cardamom.

3. Composition and uses of large cardamom.

4. Essential oil.

5. Cultivation of large cardamom.

6. Propagation of large cardamom.

7. Soil and climate of large cardamom.

8. Extraction and analysis of the essential oil of large cardamom (*Amomum subulatum* Roxb.) and GC-MS work in other related species.

9. Nutrient analysis and physicochemical analysis of compost.

2.1. The family Zingiberaceae

The Zingiberaceae is the largest family of Zingiberales and consists of the large number of medicinal plants. In India it is one of the ten largest monocotyledonous families. Its centre of distribution is in South East Asia. It is mostly distributed in tropical and subtropical areas worldwide. According to (Kress *et al*. 2002) the Zingiberaceae family consists of 53 genera and 1200 species. There are 9 genera and 70 species in South India (Sabu, 2006). As reported by (Habsah *et al*., 2005) the 50 genera with 1500 species are known around the world, from which almost 20 genera and 300 species are found in Malaysia only.

The Zingiberaceous plants are annual or perennial rhizomatous herbs with a pseudo stem. The stem is formed by clasping leaf sheaths called tillers. True aerial stem with nodes and internodes are present in genera such as *Alpinia, Amomum, Elettaria, Globba, Hedychium* and *Zingiber.* True stem is present in *Curcumorpha,*

Kaempferia, Scaphochlamys and *Stahilanthus* (Sabu, 2006).

The rhizome form a series by connecting known as sympodium meaning 'jointed feet' in Greek. The roots are developed from the rhizome which are long or short, fleshy or fibrous (Sabu, 2006).

The inflorescences are produced from the base of the psudo stem, which are terminal, lateral or both on the plant with different seasons. Bracts are arranged in spirals, it may be raceme or panicle. The fertile bract is the primary bract and bracteoles are the secondary bracts. The bracts are fused about their $^{1}/_{3}$ or ½ to form a pouch in *Curcuma*. In some species comma bracts are present at the tip of the inflorescence this are white or attractively coloured sterile bracts. There is only one flower present in the axil of each primary bract in *Kaempferia* (Nair, 1997; Sabu, 2006).

Flowers are epigynous, zygomorphic, trimerous, dichlamydeous, bisexual and irregular. The calyx is tubular form, apex with three lobed, occasionally one side are deeply splitted. The corolla tube is form with three petals and are fused at its base, mainly different size, with a hood in *Curcuma.* The colour of the corolla differs from purple or pink, white to yellow or orange. The size of the lip or labellum is big, it is clearly visible in the flower. In the majority of cases staminodes are fused to the corolla tube. Ovary is inferior, normally trilocular with axile placentation, it is occasionally unilocular or bilocular with parietal placentation. Style are long, filiform, commonly as long as the stamen and stigma is normally well expanded (Sabu, 2006).

Zhang *et al.* (2014) worked on identification of seven Zingiberaceous species based on comparative anatomy of microscopic Characteristics of seeds. He works

mainly in the seeds and fruits of *Alpinia galanga* (L.) Willd. *Alpinia katsumadai* Hayata, *Alpinia zerumbet* (Pers.) Burtt. & Smith, *Amomum kravanh* Pierre ex Gagnep. *Amomum subulatum* Roxb. *Amomum tsao-ko Crevost* et Lemaire, and *Elettaria cardamomum* (L.) Maton from Alpinia, Amomum, and Elettaria genera in the Zingiberaceae family which are difficult to identify and separate from each other. Main aims of the study were to identify the seeds based on comparative anatomy of microscopic characteristics of seven species from Zingiberaceae family. The morphological structures and macroscopic characteristics of the seed coat are described and compare in detail. In results seeds of the three genera could not be identified to the species level based on their macroscopic features. However, based on the anatomical features of the seed coat observed in transverse sections, a dichotomous key for these seven species was feasible.

Jafri *et al.* (2006) carried out study on gastroprotective effect of cardamom, *Elettaria cardamomum* Maton. fruits in rats. The fruits of *Elettaria cardamomum* Maton. specially known as "Heel khurd" is used in Unani system of medicine to treat gastrointestinal disorders. A crude methanolic extract (TM), essential oil (EO), petroleum ether soluble (PS) and insoluble (PI) fractions of methanolic extract, were studied in rats at doses of 100–500, 12.5–50, 12.5–150 and 450 mg/kg, respectively for their ability to inhibit the gastric lesions induced by asprin, ethanol and pylorous ligature. In addition their effects on wall mucus and gastric acid output were recorded. The best gastroprotective effect was found in the PS fraction, which inhibited lesions by nearly 100% at 12.5 mg/kg.

Gebreazgaabher *et al.* (2000) conducted experiment on influence of harvesting, drying structures and drying durations on physical quality characters of

korarima (*Aframomum corrorima*) capsules in Ethiopia. It is the indigenous spice of Ethiopia. Proper harvesting is needed for the superior quality of Korarima and reduces market challenges. The mature deep red capsules dried on wire mesh (for 10 days) give the better physical quality of the dried capsules.

2.2. Large cardamom (*Amomum subulatum* Roxburgh)

Amomum subulatum Roxb (Zingiberaceae), commonly known as large cardamom, is a perennial herbaceous plant with subterranean rhizomes which produces several leafy shoots and panicles. It is a native to Sikkim and from there it is spread to neighbouring areas like Darjeeling, Assam, Bhutan and Nepal. India is the largest producer of large cardamom with an annual production of 4000 MT, followed by Nepal (2500 MT) and Bhutan (1000 MT) (Berrig C, 1993). Sikkim state of India alone contributes 50% of the world's production of large cardamom (Sharma 2002). The fruit is a trilocular many-seeded capsule. It contains 1.95 to 3.23% of essential oil having typical characteristic flavour and possesses stimulant, stomachic, alexipharmic and astringent effects (Gupta PN. 1986). The fruits are prescribed to treat indigestion, vomiting, biliousness, abdominal pains, rectal diseases, throat troubles, congestion of the lungs, inflammation of the eyelids, digestive disorders, pulmonary tuberculosis, loss of appetite, gastric troubles, and liver complaints (Nadkarni AK. 1976; Jafri MA 2001 and Verma SK, 2010). Due to its pleasant aroma, it has been used as an essential ingredient in mixed spices. The major constituent of large cardamom essential oil is 1,8-cineole (65–80%) while the content of terpenyl acetate is low (traces to five per cent). The monoterpene hydrocarbon content is in the range of 5–17% of which limonene, sabinene, and the pinenes are significant components. The terpineols comprise approximately five to seven per cent of the oil. The high cineole and low

terpenyl acetate probably account for the very harsh aroma of this spice in comparison with that of true cardamom (Pruthi JS 1993).

2.2.1 Composition and uses of large cardamom

Isolation and identification of new phytoconstituents from the fruit extract of ***Amomum subulatum*** Roxb was carried out by Kumar *et al.* (2013) using spectral data analysis. Two well known compounds oleodilinolein and glyceryl trilinoleniate with other new four compounds geranilan-9-carboxy-a-L-arabi- nopyranoside, geranil-3(10)-en-9-carboxyl-P-D-arabinopyranoside, geranil-3(10)-en-9-olyl octadec-9-enoate and stigmast-5-en-3p-ol-3p-D-arabinopyranosyl-2'-(3'-methoxy) benzo- ate-3'-octadec-9 ,12 ,15 -trienoate were detected.

Similar study was conducted on *Amomum subulatum* Roxb known as 'Badi Elaichi' or Greater Cardamom. Mostly seed contain essential oil, carbohydrates, flavonoids, and fats where the aroma containing essential oil have medicinal properties and the pericarp is used for curing stomatitis and headache. It consist of volatile oil 2.80%, ash insoluble in acid 0.42%, alkalinity of water soluble ash 0.90%, water soluble ash 2.15%, volatile extract 2.8%, alcohol extract 7.02%, volatile ether extract 3.0%, non -volatile ether extract 2.31%, crude fiber 22.0%, starch 43.21% and protein 6.0% (Kumar *et al.* (2012).

Another experiment was conducted on evaluation of antioxidant activity of large cardamom leaves (*Amomum subulatum* Roxb) by Prakash *et al.* (2012) using different methods such as 1,1Diphenyl -2-picrylhydrazyle (DPPH) free radical scavenging activity, total phenolic contents and β-carotene bleaching assay from ethanolic and aqueous extracts. The result revealed BHA to be 50.3±0.6, ascorbic acid

was 2.0±0.14 µg/ml, IC_{50} of ethanolic extract (8.25±2.0 µg/ml), total phenolic content (11.04±0.2) and mean antioxidant activity (41.2±1.5%) in which ethanolic extract reported to have significant antioxidant activity.

Large Cardamom is a popular spice used in different dishes as flavouring agent native to the Eastern Himalayan region especially Nepal, Bhutan, and India and around 50% of the world's production is from Sikkim. The main essential oil in large cardamom is 1,8-cineole and it consist of 1.95 to 3.32% of essential oil having characteristic aroma and possesses medicinal properties. It is reported to have pharmacognostic properties due to the existence of terpinols (5 to 7% of the oil), monoterpene hydrocarbon in the range of 5 to 17% of which major compounds are found to be lamonene, sabeinene and pinenes. It also consist of 4.88% alcohol extract, 91.4% of total solid, 5% total ash value, 8.6% moisture, 4% non-volatile ether extract, 3.5% water soluble ash value and 1.5% ash insoluble in acid (Bisht *et al.* (2011).

2.2.2 Essential oil

A study was carried out by Bhandari *et al.* (2013) on large cardamom (*Amomum subulatum* Roxb.). Compounds such α-bisabolene (1.4%), v-terpinene (1.8%), β-pinene (2.12%), terpinen-4-ol (2.82%), α-pinene (2.9%), α-terpinyl acetate (3.33%), limonene (4.2%) and α-terpineol (4.23%) were detected. The oil from the dried capsules/seeds were extracted using hydro-distillation and further examined by GC-MS. Further 18 compounds were observed in which 99.21% of the total oil contents were identified by mass spectra and relative retention indices. 8-cineole (73.27%) was reported to be the major compound in large cardamom.

Another experiment was conducted on chemical composition of the volatile oil from the pericarp (husk) of large cardamom (*Amomum subulatum* Roxb.). Result show that main component was found to be 1,8-cineole (38.7%) which was less than 50% when compared with the seed oil followed by β -pinene (13.6%), α-terpineol (12.6%), spathulenol (8.3%), 4-terpineol (4.5%), germacrene-D (3.0%), α-pinene (2.8%) and β -selinene (2.7%). Further 0.18% volatile oil was obtained by Clevenger hydro distillation method and the volatile oil was analyzed by GC–MS in which 37 compounds were detected constituting > 98% of the total oil. Oil was determined for physical parameters such as refractive index (1.4733), specific gravity (0.9148) and optical rotation (−7.700).

Arora *et al.* (2013) studied *Amomum subulatum* leaves for the isolation and characterization of a novel chemical entity using chlorophyll free dried leaves with petroleum ether. By using Silica gel as absorbent the extract was concentrated further isolated the compounds column chromatography. The result revealed the detection of the isolated compound using elemental analysis, physicochemical data and spectral interpretation.

2.2.3 Cultivation of large cardamom

Introduction and advancement in cultivation of large cardamom (*Amomum subulatum* Roxb.) in Uttarakhand, India was carried out in different cultivars namely Dzongu Golsey (900-1200 m asl), Sawney (1200-1600m asl) and Varlangey (1500-1800 m asl) particularly with relation to germination and yield performance. The result revealed that as compared to other cultivars Sawney was found to be the best cultivars. For different cultivars the average seed germination was found to be 18.0-41.5% and for one year the seedlings were maintained thereafter transplanted in the

farmers' fields. About 1.46 lakh seedlings were used for planting for an area of 36.60ha and as a result after 3 years of plantation yield was recorded to be in the range of 248-429 kg/ha/year. Further the yield is estimated to increase by 20-25 percent for the upcoming year and remain persistent for 5-8 years (Bisht *et al.* (2010).

The cardamom based agro forestry system in the Himalayas is a traditional knowledge system in large cardamom farming supporting multiple functions and ecosystem services such as conserving soil and water, high rate of carbon sequestration than any other land use systems in the region and by maintaining soil fertility. The system plays a vital role in performing ecological function as well as providing ecotourism, cultural, educational and recreational values, socio-ecological sustainability and watershed function (Sharma *et al.* (2009).

2.2.4 Propagation of large cardamom

In a study Pradhan et al. (2014) performed in-vitro micropropagation of Amomum subulatum Roxb. (Zingiberaceae) using standardized micropropagation methods for *Ammomum subalatum* cv. Ramsey. They examined 52 different hormone concentrations of growth hormone/ cultured medium where modified MS medium with 4 % of sucrose with different concentration of hormone was found to be suitable medium. It was also observed that maximum number of shoots and roots were recorded on (MS+ sucrose 40 g + BAP 3mg/l+0.5 NAA+2mg/l) followed by (MS+ 40g sucrose + BAP 3.5mg/l+0.5 NAA + 2mg/l). Within 10-15 days after individual subcultured on same fresh medium, adventitious buds were initiated and within 50 days full plantlets developed which are further maintained for 18 months. Successful rate was shown with combination of cytokinins and auxins in the culture medium as it increase the growth of shoot and root.

Clone multiplication unit of large cardamom unit was developed and work by Bhattarai *et al.* (2013). National Agricultural Innovation Project (NAIP) Components-III project initiated in 2007 has been implemented in Dzongu, North District of Sikkim with the objective of development of improved farming systems in large cardamom plantation. The study revealed that product from North Sikkim are in high demand both National and International market as Mangan variety and total amount of large cardamom from the state of Sikkim was contributed from North Sikkim. In the area, ethnic tribe Lepcha's were the main occupants along with few Bhutia and Nepali speaking among them where farming was their main occupation. It was revealed that large cardamom was the major income for the farmers with the main cultivar Dzongu golsey and other crops like maize, millets and wheat are also cultivated. Reports says various diseases and factors like Colletotrichum blight, Chirke, foorkey, pest incidence, lack improper planting materials, lack of irrigation and phytosanitary measures leads to drastic reduction in production and productivity. Area expansion through gap filling and replantation on establishment of large cardamom sucker multiplication nursery was given importance to control the issue.

2.2.5 Soil and climate of large cardamom

Influence of stand age on nutrient and energy release through decomposition in alder-cardamom agroforestry systems of the eastern Himalayas was studied by Sharma *et al.* (2007). Comparison between N_2-fixing alder (*Alnus nepalensis*) and non-N_2-fixing large cardamom (*Amomum subulatum*) systems on the influence of stand age (5, 10, 15, 20, 30 and 40 years) was done. The study revealed that in younger stands (10- to 15-years) the decomposition rate was considerably high and decrease in older stands. Energy and nutrient release were found to be slow at a high

initial lignin-to-initial N ratio and C-to-N ratio and inverse relationship was observed between the k-value of ash-free-mass and N expressed as a function of the C-to-N ratio. In 15 years number of nutrient release and energy loss per unit area in 24 months of decomposition were highest and later drop with advancing age. C-to-N ratio, litter temperature and litter moisture was found to be firmly connected with relative loss rate of ash-free mass, nutrients and energy content. Further nutrient and energy releases in younger stands were observed to be rapid by the effect of *Alnus* that show accelerated nutrient cycling and energy dynamics and the intensity of the processes was substantially high in younger stands up to 20 years. Sustainability in 15–20 years was found to be suitable for management cycle of the *Alnus*- cardamom system.

2.2.6 Manuring and fertilizer of large cardamom

Sharma *et al.* (2009) reported that the 5-40 year old alder trees add 58-155 kg/ha of nitrogen to the soil through nitrogen fixation; with highest amount of nitrogen added by 10–15 year old trees. Other important shade tree species for large cardamom commonly found growing in Sikkim include *Albizia spp*. *Terminalia myriocarpa, Bucklendia populnea, Macaranga indica, Edgeworthia gardneri, Vibernum coriaceum, Maesa chisia, and Symplocos ramosissima.*

2.2.7 Extraction and analysis of the essential oil of large cardamom (*Amomum subulatum* Roxb.) and GC-MS work in other related species

Analysis of the essential oil of large cardamom (*Amomum subulatum* Roxb.) in Sikkim was studied by Rout *et al.* (2000). Oil from seeds of green, freshly dried and those accessible in the local market were extracted using hydro-distillation. The

study revealed that 1,8-cineole (81.5-86%) was the main component of oil and further 33 compounds were detected using GCMS.

Similar work was studied by Joshi *et al.* (2012) in different agro-climatic zones of Himachal Pradesh, India on essential oil of large cardamom (*Amomum subulatum* Roxb.) They observed that 1,8-Cineole, α-terpineol, DL-limonene, nerolidol, 4- terpineol, δ- terpineol, δ-3-carene, β-nyrcene, germacrene D, α- terpinene and longifolenaldehyde were the main compounds of oil. A total of 55 compounds were examined representing 98% of total oil using gas chromatography (GC), gas chromatography-mass spectrometer (GC-MS) and gas chromatography-olfactometry (GC-O).

Another study was conducted by Bhandari *et al.* (2013) on the 1,8-Cineole: A predominant component in the essential oil of large cardamom (*Amomum subulatum* Roxb)

in Uttarakhand. They found 18 compounds constitute 99.21% of the total oil and the predominant compounds of the oil were 1, 8-cineole (73.27%) follwed by α-terpineol (4.23%), limonene (4.2%), α-terpinyl acetate (3.33%), α-pinene (2,9%), terpinen-4-ol (2.82%), β-pinene (2.12%), v-terpinene (1.8%) and α-bisabolene (1.4%). Through GC- MS oil were extracted from dried capsules/seeds of large cardamom.

Testing Phenol Compounds in Spices were analysed in cardamom fruits and carnation buds using High-yield liquid chromatography (HPLC) by Evdokimova *et al.* (2013) indicated 12 phenol compounds in cardamom fruits namely : gallic acid, isoferulic acid, chlorogenic acid, epicatechin, chicory acid, caffeic acid, dehydroquercetin, ferulic acid, luteolin, quercetin, rutin, o-methoxycoumarin,

cinnamic acid, in carnation buds - 12 phenolic compounds: gallic acid, catechin, isoferulic acid, chicory acid, coffee acid, dihydroquercetin, ferulic acid, luteolin, quercetin, rutin, 0- methoxycoumarin, cinnamic acid. Permanganate titration was used and Tanning agent and found to be 4.11- 5.02 % and 0.02- 0.06 % in carnation buds and cardamom fruits respectively. Total amount of flavonoids varies from 3.55 % to 5.10% in carnation buds in terms of rutin using differential spectrophotometry.

A comparison between essential oil components of green and bleached cardamom were obtained through microwave-assisted hydro distillation and further analysed by GC-MS. A total of 31 and 46 compounds were analysed for green cardamom and bleached cardamom respectively which were extended to 55 and 70 components, respectively using multivariate curve resolution methods. 33 compounds are found familiar between the two types of cardamom despite different cultivation. Main compounds in green cardamom were 1,8-cineol (47.18%) , alpha-terpinyl acetate (14.33%) , linalool (6.28%) , terpineol (4.94%) l-4-terpineol (2.48%) and in bleached cardamom are 1,8-cineol (34.12%) ,alpha-terpineol (26.91%), alpha-terpinyl acetate (21.04%), linalool (6.39%) and l-4-terpineol (1.89%) (Jabbar e*t al.*, 2014).

Worked on extraction of cardamom oil by supercritical carbon dioxide and sub-critical propane through high-performance liquid chromatography (HPLC) and gas chromatography–mass spectroscopy (GC–MS) to analysed pigments (chlorophylls and carotenoids), volatile constituents, fatty acids and tocopherols was studied by Daood *et al.* (2007). The result shows that propane was observed to be more capable than CO_2 to recover seed oil at sub-critical conditions with lower ratio of solvent and greater quality. Moreover, fatty acid composition and content of volatiles in the recovered extracts were found to be affected by Supercritical fluid

extraction to some level. Through supercritical fluid CO_2 and sub-critical propane natural pigments and antioxidants were recorded maximum in extracts recovered. Adding ethanol to CO_2 up to 25 percent positively affected the natural pigment of the extract and negative effect on aroma profile but did not improve the extractability of fatty acids. At sub-critical conditions cardamom show easy-to-extract by fluid CO_2 with very cost-effective ratio of solvent/solid.

Study have been conducted on essential oil and oleoresins of cardamom (*amomum subulatum* roxb.) by Kapoor *et al.* (2009) where essential oil and oleoresins (methanol, acetone, isooctane and carbon tetrachloride) of large cardamom were used as natural food preservatives for juice of sweet orange (*Citrus sinensis*). Superior result was observed in cardamom oil and oleoresin (acetone) and essential oil and oleoresins show significant effect on shelf life of juice.

Bhattacharjee *et al.* (2013) conducted experiment on antimicrobial efficacy of essential oils extracted from some Zingiberaceae species and antifungal assay was done for the *Curcuma amada* Roxb, *Zingiber officinale* Rosc. *var.* Moran, *Z. officinale* Rosc. *Z. zerumbet* (L.) showed greater inhibitory effect over the microbial strains. Efficacy of essential oils obtained from 3 species of Zingiberaceae under genus *Curcuma* and *Zingiber* is experimented on certain pathogenic bacteria and fungi. The result shows they were active only under bacteriostatic condition but so sign of any bactericidal activity.

Processing parameters on the yield and 6-gingerol content of *Zingiber officinale* extract was done by Hasham *et al.* (2014) using hydro distillation extraction method and further analyzed using High Performance Liquid Chromatography (HPLC). The study reveal that the best parameter were examined based on its

maximum yield and 6-gingerol content from ginger extraction which were 7.02 % (w/w) and 35.3404 mg/L, respectively.

Essential oil from five species of Zingiberaceae namely ginger (*Zingiber officinale* Roscoe.), galanga (*Alpinia galanga* Sw.), turmeric (*Curcuma longa* L.), kaempferia (*Boesenbergia pandurata* Holtt.) and bastard cardamom (*Amomum xanthioides* Wall.) were extracted by hydrodistillation and two solvent petroleum ether and ethanol were used. By Disc diffusion assay their antibacterial effects towards *Escherichia coli*, *Staphylococcus aureus, Bacillus cereus* and *Listeria monocytogenes* were carried out. They found that the main constituent of ginger, galanga, turmeric, kaempferia, and bastard were zingiberene, methyl chavicol, turmerone, γ-terpinene, and methyl chavicol, respectively. Further it was also found that essential oil of ginger obtained from hydrodistillation show highest efficiency against three positive strains of bacteria (*S. aureus, B. cereus* and *L. monocytogenes*) with a minimum concentration to inhibit *B. cereus* and *L. monocytogenes* of 6.25 μg/ml. Moreover, essential oil of kaempferia and bastard cardamom extracted from hydrodistillation could inhibit growth of all tested bacteria. All the extracted volatile compounds were analyzed using gas chromatography-mass spectrometry (Orapin *et al.*, 2008)

Hastati *et al.* (2015) conducted an experiment on determination of the curcumin pigment extract *Curcuma domestica* using High Performance Liquid Chromatography HPLC, LC-10AT, column C-18 VP-ODS, acetonitril:acetic acid:aquabides as mobile phase, flow rate 1 ml/min and detection at 425nm. The study reveal that curcuminoid content from Extract *Curcuma domestica* contain highest curcumin (16.1%) following metoxycurcumin (3.2%) and

bisdemetoxycurcumin 2.8%.

An experiment has been conducted on isolation, purification and identification of curcuminoids from turmeric (*Curcuma longa* L.) by column chromatography following purity analysis using HPLC. Maximum yield of each curcuminoids were found in acetone out of different solvents used for each extraction and chloroform: methanol (5:2) at 5°C was done for crystallization of each compound .The study show that the isolated curcuminoids (C, DMC, and BDMC) record single peaks at retention times of 10.81, 12.79, 13.03 min respectively on HPLC. The study also show that different solvent at different polarity were pre-tested in TLC for separation of curcuminoids, chloroform: methanol at 95:5 showed better resolution of Rf value at 0.75, 0.55, 0.27, as Curcumin(C), Demethoxycurcumin (DMC), Bisdemethoxycurcumin (BDMC) respectively (Revathy *et al.*,2011).

Another experiment was conducted on preliminary phytochemical evaluation of the oil extracted from leaves of *Curcuma longa L.* and its application as biofuel (Mundle *et al.,* 2011). Hydro distillation method was used for extraction of oil from *Curcuma longa* leaves and test was done for physicochemical and phytochemical from the extracted oil. The oil obtained from leaves was used to run two stroke and four stroke engine**.** The presence of terpenes was found from oil obtained from the turmeric leaf test for phytochemical evaluation. Result shows that with turmeric oil engine eliminates less environment harmful product in comparison to petrol that lead to the conclusion that turmeric leaf oil can be used as alternative biofuel for petrol.

Comparative and quantitative amounts of turmeric oleoresin was studied on four varieties of turmeric BSR-01, BSR-02, CL-101 and CL-219 by Muhamed *et al.*,2015 using a simple column extraction method and the oil were obtained by

alcohol and acetone as a solvent. They found oleoresin rich turmeric variety from the analysis and high recovery of oil was observed.

Pandey *et al.* (2013) review on curcuminoid content in *curcuma* spp. Curcumin is a mixture of curcumin, demethoxy curcumin and bis demethoxy curcumin. with curcumin as the major compound important for turmeric biological actions. Turmeric belongs to Zingiberaceae family which is the common name for dried rhizome *Curcuma longa* L. The study reveal about the experiment for characterization, selection and evaluation of turmeric genotypes in the field. It is important to go for selection of genotype having good quality and higher yield with variation in content and quality characters.

A study on antioxidant effects of 28kda antioxidant protein from turmeric (*Curcuma longa* L) was done by Ramadas *et al.* (2011) where 28 kDa glyco proteins was isolated, purified and further characterized from boiling water of Turmeric extract and name as BGS-Haridrin. It was observed that as evidenced by agarose gel electrophoresis BGS-Haridrin prevents H2O2 (1mM) caused calf thymus DNA damage and further in human peripheral lymphocytes, BGS Haridrin effectively protects H2O2 (100µM) induced cell death. The study also record that when compared to BHA, Curcumin (400 µM) and α-tocopherol (400µM), BGS-Haridrin scavenged hydroxyl, DPPH radicals, superoxide radicals 76-82 percent about and inhibited lipid peroxidation about 78 percent at a maximum dosage of 0.9 nM concentration which conclude *in vitro* antioxidant effects of BGS-Haridrin antioxidant protein from Turmeric (*Curcuma longa L*).

Another study on the effect of factors like different ratios of 6-benzylaminopurine (6-BA), sucrose concentration, α-naphthalene acetic acid (NAA)

and light quality on the accumulation of curcumin and other curcuminoids in *Curcuma aromatica* were examined. The result show more curcumin and other curcuminoids were produced in comparison to other ratios of 6-BA and NAA, micro rhizomes induced on 3% sucrose media supplemented with 3.0 mg/L 6-BA and 0.5 mg/L NAA but the amount were less in micro rhizomes which are grown on 3percent sucrose. The amount of curcumin and curcuminoids increased and that exposure to red light also enhanced production with the used of 5 percent sucrose medium supplemented with 3.0 mg/L of 6-BA and 0.5 mg/L of NAA. Further concluded that more curcumin and other curcuminoids were produced in micro rhizomes grown on media containing 3% sucrose than those that are grown on higher concentrations (Zhang *et al.*2014).

Development of quercetin and curcumin in polyherbal churna and its validation was studied by Salunkhe *et al.* (2015) using UV spectrophotometric and HPLC. Curcumin and quercetin in *Madhujeevan churna (MJC)* was validated and developed by RP-HPLC method. Agilent LC-P-1120 with HiQ Sil C-18 column by isocratic elution in the ratio of 42.5: 42.5: 15 % v/v/v using methanol: acetonitrile: phosphate buffer (pH 5) as the mobile phase were used to determined Standard curcumin. For isobestic point, 265 nm was set as detection and 1.2 ml/ min as the flow rate. The result show the concentration of quercetin and curcumin found in raw material of *Madhujeevan churna* was observed as 0.1347%w/w and 0.208%w/w respectively in *Madhujeevan churna (MJC).* All the compounds showed good linearity (r = 0.98332) in a relatively wide concentration range and the method for the recovery was in the range of 98.50-99.40 %.

Jankasem *et al.* (2013) studied antidermatophytic properties of *Ar*-Turmerone,

turmeric Oil and *Curcuma longa b*y broth dilution technique where 6 and 10% w/w turmeric oil obtained from turmeric creams were used and further tested for their clinical strains of dermatophytes. They reported that compared to 3.90–7.81 g/mL of standard ketoconazole, with the MICs of 1.56–6.25 g/mL *Ar*-turmerone produced more effective antidermatophytic activity. For antidermatophytic preparation 6% w/w turmeric oil in the cream was noted to be suitable and less fungicidal concentrations of 6 and 10% w/w turmeric creams were recorded to be 312 g/mL.

Zhan *et al.* (2011) work on extraction of *Zingiber zerumbet* Smith oil using Soxhlet extarction method. Recent advances on supercritical fluid extraction of essential oils. Supercritical fluid extraction (SFE) is one of the most commonly used extraction techniques in the course of analysis or preparation. It is environmentally friendly and has some advantages over other conventional extraction methods. This review covers the recent developments of SFE in the extraction of essential oils from the plant materials during the period 2005 to 2011, in particular some factors influencing SFE extraction yield, its characteristics and applications.

Soxhlet extraction method was used to obtained oil from the rhizomes of *Zingiber zerumbet* (*L*) Smith using dichloromethane, benzene and methanol as solvent and to get concentrated oil the extracted oil were evaporated using rotary evaporator which were further examined through GCMS. Times for the extraction are 4 hr, 6 hr, 8 hr and 10 hr where the result show 6 hr to be the best time for extraction. In comparison to the other solvents used for oil extraction methanol was found to have higher yield. Compound zerumbone and α-caryophyllene are observed from the oil obtained by extraction by GCMS (Fadzil *et al.*, 2010).

Dried leaves of *Alpinia purpurata* (Vieill.) K. Schum were analysed to detect

flavonoids using high-performance liquid chromatography. Solvents ethyl acetate and butanol were used followed by thin-layer chromatography (TLC) and HPLC. Report show maximum flavonoid i.e. 94.3 percent in butanolic extract further at higher concentration in ethyl acetate and butanolic extracts, flavonoids kaempferol-3-*O*-glucuronide and rutin were observed.

By using gallic acid as standard total phenols was determined by Folin-Ciocalteau method and the result was 15.6 mg GAE g^{-1} (Victorio *et al.*, 2009).

Experiment was performed on antioxidant activities of hydroethanolic leaf extract of *Kaempferia galanga* Linn determining β- carotene linoleic acid, metal chelating, DPPH radical scavenging and anti-Lipid peroxidation activities using HPLC by Rao *et al.* (2015)**.** Phytochemical screening was done to test saponins, carbohydrates, terpenoids, flavonoids, tannins, phytosterols and phenols. Organisms namely *Klebsiella pneumonia, Escherichia coli, Pseudomonas aeroginosa, Bacillus cereus* and *Staphylococcus aureus* were used for the experiment. Using agar well diffusion method antibacterial activity was test and erythrocytes hemolytic assay was also conducted to examine the effect of extract on human. Result from the extract recorded antioxidant potential with IC50 values of 635µg/mL for metal chelating assay, 531µg/mL for anti-Lipid peroxidation assay and 32µg/mL for β carotene linoleic acid assay. The study also noted that Fourier Transform Infra Red (FT-IR) evaluation show identification of functional groups. Hemolytic activity against human erythrocytes was not observed by hydroethanolic extract. Inhibition zones of 19 mm, 14 mm and 12 mm diameter against *K. pneumonia*, *P. aeroginosa* and *S. aureus* shows moderate antimicrobial activity. Coumaric acid, Ferulic acid and Butein were found present analysed by HPLC thus concluded that *K. galanga* extract show good

antioxidant property and can be utilised for treatment for free radical associated human illness.

Victorio *et al.* (2009) carried out a study on flavonoid extraction from *Alpinia zerumbet* dried leaves extraction using distillated water and 70% ethanol as solvents and by various methods such as shaking maceration, ultrasonic, microwave and stirring. The study revealed that by ethanol extract relative concentration of rutin and kaempferol-3-*O*-glucuronide were observed to be higher using ultrasonic (1.5 and 5.62 mg g^{-1} dried leaves) and microwave (1.0 and 6.64 mg g^{-1} dried leaves) respectively. There was no significant yielding variation seen by 70% ethanol (11 to 14%) for ultrasonic, microwave and stirring methods. The study also revealed that flavonoids extraction from 70% ethanol was more efficient compare to water extract further rutin and kaempferol-3-*O*-glucuronide were identified using TLC and reversed-phase HPLC methods.

Another study was carried out for simultaneous estimation of isocurcumenol, ar-turmerone and α,β- turmerone in hexane soluble fraction and its formulations of *Curcuma* on improved HPLC method. Study reported that ar-turmerone was observed to be susceptible for oxidative, photolytic, oxidative and heat conditions. By calibration curve of ar-turmerone the quantification of α, β-turmerone was carried out as alternate equation and the technique was found efficient in forced degradation study of ar-turmerone, bulk HM oil, HM capsule formulation and stability testing. It was also noted that anti -stroke activity was observed to be superior in *C. longa* rhizomes (Herbal Medicament (HM) by standardized hexane soluble extract Dwivedi *et al.* (2014).

Antimicrobial activity and chemical composition from *Zingiber Officinale* of

the crude extracts by n-hexane and methanol as solvents was studied. Soxhlet method was carried out from ginger extraction using the solvents and was determined by HPLC. Furthermore by HPLC *in vitro* effects of micro-organisms in the plant were examined. The result found one fungus and seven kinds of bacteria strains detected antimicrobial activities of two crude extracts in different concentrations of plant using Agar well diffusion procedure. Methanol extract was noted to be higher as compare to hexane extract against the same tested micro-organisms and two extract also found antimicrobial activity. The study also record that using HPLC seven constituents were detected Hasan *et al.* (2012).

Nag *et al.* (2013) conducted an experiment on antioxidant activities and cytotoxicity of *Zingiber zerumbet* (L.) Smith rhizome using ethanol extract. Using different concentrations of ZZ (0, 15, 30, 60, 120, 300 and 600 µg/ml) human peripheral blood lymphocyte cells were incubated for 3 h at 37 °C. Result revealed that significant radical scavenging activities of ZZ were found using 2,2-di(4-tert-octylphenyl)-1- picryl-hydrazyl (DPPH) and hydroxyl radical scavenging assays. Result also show at higher concentration the rhizome extract was observed to be cytotoxic for human consumption and using trypan blue exclusion test cytotoxicity was evaluate *in vitro.* Further revealed that polyphenol and flavonoid from the extract are found to be abundant and HPLC examine show ZZ as rich source of kaempferol result in the conclusion that rhizome can be harmlessly utilised as a therapeutic antioxidant.

Another study was conducted on characterization of CurcuEmulsomes a nano formulation for enhanced solubility and delivery of curcumin by Ucisik *et al.* (2013) where curcumin is encapsulated inside the solid core of emulsomes. Polyphenolic

compound are found in *Curcuma longa* rhizome that exhibit intrinsic anti-cancer properties. Due to its bioavailability and very low water solubility its medical utilization remains confined that result drug delivery systems along with the nano particle technology become evident. Study reported that up taken by HepG2 human liver carcinoma cell line CurcuEmulsomes reveal notably prolonged biological activity and demonstrated therapeutic efficacy close to free curcumin against HepG2 *in vitro* with a delay in response, as evaluate by cell viability, cell cycle study and apoptosis. Study also observed that corresponding to a concentration of 0.11 mg/ml encapsulation enhances the bioavailability of curcumin up to 10,000 fold.

Alpinia nigra an anthelmintic medicinal plant of North-East India was studied by Roy *et al.* (2012). In various parts of North-East India *Alpinia nigra* is extensively utilised as one of the medicinal plant to cure intestinal helminth infection. Various species of Alpinia contain essential oils such as flavonoids, terpenoids and kavalactones which are practised to treat hypertension, inflammation, bacterial and helminth infection. People living in remote areas with weaker economic background depends on traditional method through folk medicine to treat parasitic infections.

Yob *et al.* (2011) survey ethno medicinal, chemical, and Pharmacological uses on *Zingiber zerumbet* (L.) Smith. Zerumbone is the main active pharmacological compound in *Z. zerumbet* rhizomes generally studied. It is a perennial herb found in many tropical countries as well as Malaysia known to the Malay as “Lempoyang”. In Malay traditional medicine the rhizomes extract were applied to treat different illness like inflammatory and pain mediated diseases, worm infestation and diarrhea and the rhizomes are usually utilised in many Malays cuisines as appetizer and food flavouring.

Bioguided fractionation and purification of natural bioactives obtained from *Alpinia conchigera* water extract with melanin inhibition activity was conducted by Ujang *et al.* (2013). The study revealed that the crude extract from ethanolic and super critical fluid extraction were found to be toxic to the melanocyte cells but significant melanin inhibition activity did not show when compared to the water extract. Further at a concentration of 500 µg/mL, melanin inhibition of 96.38 ± 1.60% and cell viability 109.90 ± 8.32% from aqueous extract was observed. Compound trans-cinnamaldehyde and chavicol glucopyranoside were identified revealing strong anti-melanogenesis activity. Study also found trans-cinnamaldehyde and chavicol glucopyranoside gave 85% inhibition of melanin formation *in vitro* with 77% and 97% cell viability respectively at t 4.9 µg/ml and at 100 µg/mL kojic skin lightening acid result in 90.0% inhibition. The study concluded that *Alpinia conchigera* can be utilized for reducing skin pigmentation in cosmetic as well as pharmaceutical applications.

A study was done on drying characteristics of large-cardamom by Rao *et al.* (2001). For drying of large-cardamom smoking method by traditional bhatti is still practised which is ineffective and ancient way of drying large cardamom that give poor charred and blackened quality product moreover fuel wood wastage. To have moisture less than 10 percent by curing, after harvest immediately it has to dry in order to get the fresh cardamom capsules with 80-85 percent and aroma for storing in longer period.

The pathogen that cause blight and serious destruction in large cardamom (*Amomum subulatum* Roxb.) in Sikkim and Darjeeling Hills was studied to identity colletotrichum infections by Saju *et al.* (2013). They isolated the pathogen and

identified as *gloeosporioides* (Penz.) Sacc. {perfect state *Glomerella cingulata* (Stoneman) Spauld. & Schrenk} further name as colletotrichum blight for the disease.

2.3 Nutrient analysis and physicochemical analysis of compost

Composting of rice straw was done with effective microorganisms (EM) along with goat manure and green waste for observing its influence on compost quality (Jusoh *et al.,* 2013) performed this research for 90 days with 3replications andtwo treatments *viz*, one pile was applied with EM and another pile without EM. The parameters for the temperature, pH, TOC and C/N ratio, show that decomposition of organic matter occurs during the 90-day period. The t-test conducted shows that the application of EM in compost increases the macro and micronutrient content as compared without treatment.

Work on composting of common organic wastes using microbial inoculants was carried out where 3 bacterial isolates namely RAT/5 as *Pseudomonas sp*., D3L/1 as *Bacillus subtilis* and 16S rDNA as B1U/1 were examine by Pan *et al*., (2012). Result shows that during decomposition process PO_4^{-3} ion concentration was high and the mature compost pH was 7.0 ± 0.2. After 120 days maturity of compost were shown by the reduction in NH_4^{+} and NO_3^{-} ion concentrations while within 120 days the C/N ratio of each substrate decrease to 25–30:1 and then remain constant. Similar work was done on composting of paddy straw with efficient microorganism (EM) consortium by Sharma *et al*., (2014) using Lactobacillus sp., *Streptomyces globisporous* (C3), *Phanerochaete chrysosporium* (VV18), *Candida tropicalis* (Y6) and photosynthetic bacterial inoculums for rapid composting of paddy straw. It was recorded that microbial activity as dehydrogenase (158.64 µg TPF/g/day) and hydrolytic enzyme carboxymethyl cellulase (CMCase) (0.43 IU/g) were found high

while Paddy straw with EM and CI within sixty days developed total humus content of 4.82 % and fasten the composting process by reducing C: N ratio to 15:1.

Another experiment was conducted on effect of compost derived from decomposed fruit wastes by effective microorganism (EM) technology on plant growth parameters of *Vigna mungo.* Compost treated plot shows higher soil heterotrophic microbial population, soil nutrients and phyllosphere by means of total organic carbon, humic acid, potassium, phosphorous and nitrogen in plant growth parameters. Lesser pest infestation and disease spots were observed and total foliage density/plant, total leaves and branches emerged in the plant, height of the plant, total chlorophyll, leaf surface area and shoot length were also recorded to increase in compost treated plants (Namasivayam and Bharani 2012)

Another study was studied on the role of effective microorganisms in the composting of banana (Musa ssp.) residues where non-EM (Effective microorganisms) treatments were compared to EM Bokashi (organic compost) produced with molasses (M) as an EM additive, water (W) and with sterilized EM (EMst). The study shows Nmic was maximum in EMst (615 µg g^{-1}), Cmic was found maximum in Bokashi produced with molasses (3892 µg g^{-1}) whereas both microbial biomass carbon (Cmic) and microbial biomass nitrogen (Nmic) were minimum in EM (Cmic = 3121 µg g^{-1}; Nmic = 449 µg g^{-1}). Further it was also recorded that Ergosterol concentration was lowest in EMst Bokashi (29 µg (g dry soil) $^{-1}$) and was highest in EM Bokashi (77 µg (g dry soil) $^{-1}$). Bokashi to young banana plants remarkably enhanced shoot growth while Bokashi with only EM Bokashi and molasses lower the number of root nematodes in greenhouse conditions as compared to plants in a control soil (Formowitz *et al*., 2007).

Another study was conducted on the status of N P K in vermicompost prepared from two common weed and two medicinal plants. The study revealed different attribute as NPK values of A*zadirachta indica*, *Parthenium hysterophorus*, *Argemone mexicana,* and *Vitex negundo* vermicompost. Further it was also observed that in vermicomposts organic carbon - nitrogen ratios (C/N) were found higher produced with plant except in *Argemone mexicana*. Compared to the cow dung compost all four vermicompost has significantly higher K contents and Potassium (K %) ranges 0.8% to 15.8%. Phosphorus (P %) is found higher in A*zadirachta indica* vermicompost and ranges 1.3% to 1.6%. Nitrogen (N) content (%) was reported to have maximum in other plant compost except A*zadirachta indica* and *Vitex negundo,* maximum in Parthenium vermicompost (1.8%) and low in cow dung compost (1.7%). The pH was in range of 7.5 – 8.1in the compost (Mistry *et al.,* 2015).

Different factors such as pH, oxygen level and temperature plays a vital role for decomposition of organic wastes and performance as these factors are important for sustainable waste management practices and result revealed increase agricultural production using high value compost prepared from improved techniques and utilized in soil recycling. Composting are determined by microbial population and balanced through their physiological activity by microbial decomposition process. The use of compost supports agricultural sustainability by various processes such as leaf manuring, green manuring, composting and mulching (Maheshwari *et al*., 2014).

A study on composting of water hyacinth was carried out using a Pilot Scale Rotary Drum Composter by Singh *et al,*. (2012). During the 20 days composting process, physico-chemical characteristics such as pH, moisture, temperature, nitrogen dynamics, total organic matter (OM) and nutrients were determined using four

different proportions namely hyacinth, mixed with cattle manure, rice husk and sawdust for composting. Further CO2 evolution rates and oxygen uptake rate (OUR) were also analyzed to have the stabilities of the composts. Result revealed that among the entire test, the best was found in composting mixture trial 1 (6 water hyacinth, 3 cattle manure, 1 rice husk) with the highest temperature profile and OM loss, lowest OUR and CO2 evolution rate.

The experiment conducted by Lim *et al*., (2015) the banana biofertilizer was found maximum content of potassium (3.932 g K/L) and for pH value watermelon biofertilizer was recorded maximum pH (5.15). Test was carried on plant samples of 5 weeks of age to examine the effectiveness of the biofertilizer and five different agro-wastes namely banana, orange, citrus, pineapple and papaya were utilized as biofertilizer. Good results on physical characteristics were also observed in plant samples treated with biofertilizer from banana, papaya and watermelon.

Emendu *et al*., (2021) conducted a study on analysis of micro and macro nutrient levels in compost and vermicompost fertilizer from agro-waste with earthworm (*Eisena fetilda*) earthworm with mixture of farm and poultry droppings in the ratio of 5:1. In all the samples of compost and vermicompost the result for macro nutrients and micro nutrients shows Mn (27.50mg/kg-34.71mg/kg), Zn (4.91mg/kg-11.20mg/kg), Cu (26.01mg/kg-39.15mg/kg), Fe (24.00mg/kg-33.41mg/kg), P (30.80mg/kg-38.51mg/kg), K (25.51mg/kg-32.01mg/kg), N (20.00mg/kg-29.41.00mg/kg), Ca (18.20mg/kg-25.51mg/kg) and Mg (21.51mg/kg-4.19mg/kg).

Similar work was conducted on physico-chemical and mineral analysis of composts fortified with NPK fertilizer, ammonium chloride and kaolin by Ogundare and Lajide (2013) using waste materials of cow dung, orange, banana, vegetables and

cassava fortified each with 100 g of kaolin, 100 g of NPK and 100 g of ammonium chloride. The result shows nitrate and sulfate contents are lower by the fortification with NPK, kaolin and ammonium chloride. Further mineral content was noted that fortification with kaolin boosted the calcium and magnesium content whereas the fortification with NPK and ammonium chloride increased the magnesium content of the compost sample. The result also revealed that the fortification with kaolin (56.31%), NPK (53.21%) and ammonium chloride (36.75%) also improve the phosphorus content over that of the unfortified. The fortification with kaolin (38.8 %,), NPK (56.23%) and ammonium chloride (71.17%) increased the percentage of nitrogen over that of the unfortified compost. As compared to NPK chemical fertilizer all the compost samples have little nitrogen. Ash content and phosphorus content was found maximum for physicochemical analysis in the kaolin fortified compost (KFC) while maximum organic matter was reported in the unfortified compost (UC).

Physico-chemical of soil plays a vital role as both physical and chemical properties are influenced by the soil productivity. It is based on different parameters like soil organic matter, temperature, moisture, texture, electrical conductivity, pH, available nitrogen, phosphorus and potassium which are also necessary for proper application of the other management practices (Tale and Ingole 2015).

An experiment on modelling the effects of moisture content in compost piles was carried out by Luangwilai *et al*., (2011) using one and two dimensional spatially dependent models and the heat release rate is modeled by Arrhenius kinetics. It was recorded that when the water content is very high, the reaction only commences whereas the reaction is almost insignificant when the water is very low. Further there is a probability of spontaneous combustion of the compost pile and biological reaction

is at its peak for an intermediate water content range. At high temperatures microorganisms die or become dormant and the heat release rate through biological activity is modeled that at high temperatures, functions of temperature is lower and at lower temperature, the function of temperature is higher. The model is based on four mass-balance equations viz. liquid water concentrations, vapour, energy and oxygen as moisture is an important factor in degradation process of compost.

Khali *et al.,* (2013) studied on evaluation of the composting process through the changes in physical, chemical, microbial and enzymatic parameters. The result shows that after 30 days xylanase activity rise and reached the peak thereafter reduced while CMCase activity also rises and reached the peak from 10 to 30 days and thereafter reduced. The study also revealed that during initiation α-amylase was high thereafter reduced whereas the enzymes activity was found maximum in the winter season as compared to other seasons and maximum activity was noted in El-Montaza plant. Light brown colour was recorded in the final composts while nearly dark brown was observed in El-Montaza plant in the winter season. The thermophilic fungi vanished at the initiation and developed after ten days thereafter vanished from 20 to 30 days whereas the mesophilic fungi developed at the initiation thereafter reduced and vanished from 10 to 20 days. With time the C/N, C/P and C/K reduced, moisture reduced and pH values changes.

Physicochemical changes during composting rice straw with chicken and donkey manure were examine by Karanja *et al.,* (2019) where electrical conductivity (EC), pH and temperature are maintained during composting process and a control of un-treated rice straw (T0), rice straw co-composted with chicken manure (T1) and rice straw co-composted with donkey manure (T2) were observed. They reported that

the composting process was remarkably enhanced by the application of chicken and donkey manure. They also observed that the 3 compost types are physicochemically different in cation exchange capacity, nitrogen, carbon, phosphorus content and the EC, pH as well as temperature were recorded to have notable variations at 5% level of confidence using Tukey's test.

A study on physical and chemical properties of compost from different materials such as sugar cane plants residues, herbal plants residues and cattle manure were carried out. The result reported that C/N ratio ranged from 14.22:1- 18.52:1, total organic matter ranged from 28.60 - 41.20 %, total organic carbon ranged from 16.6 - 23.89 %, total phosphorus ranged from 0.27 to 1.13 %, total potassium ranged from 0.27 - 2.11 %, total nitrogen values ranged from 0.95 - 1.68 %, EC ranged from 2.6 - 4.1 dS m^{-1}, pH ranged from 6.3 to 7.8, porosity ranged from 60.69 - 72.47 %, water holding capacity ranged from 3.50 - 4.40 g water/g dry, moisture content ranged from 23.50 - 32.10 % and bulk density ranged from 420 -655 kg m^{-3} (Khater, 2015). Similar experiment was carried out on physicochemical changes during vermicomposting of water hyacinth (*Eichhornia crassipes*) and grass clippings by Ansari and Rajpersaud (2012) using biodung and vermicomposting with 3 different treatments viz. grass (T1), water hyacinth (T2) and water hyacinth + grass (T3). The study revealed that vermicomposting in T3 produced maximum vermicompost resulting in very rich nutrients followed by T2 and T1. During the vermicomposting process temperature was recorded to be 28.26 ± 2.19◦C in T1 followed by 27.31 ± 0.80◦C in T2 and 26.94 ± 0.68◦C in T3.

Chapter 3

MATERIALS AND METHODS

The present investigation entitled **"Essential oils from different parts of Large Cardamom (*Amomum subulatum Roxb*.) and composting of available Biomass"** was carried out in the Department of Horticulture, Sikkim University, 6th Mile, Tadong, Sikkim, during the year 2016-2019. The materials utilized, the experimental approaches and the procedures adopted during the course of investigations are described as follows:

3.1. Experimental materials:

The experimental materials for the present study was different parts such as leaves, tillers, spike remains and capsule of large cardamom (*Amomum subulatum* Roxb.) varieties: 1. Sawney 2. Golsey 3. Ramsey 4. Saremna (Plate No.9, 10, 11, 12).

3.1.1. Collection of Samples

Samples of large cardamom was collected in the month of November–December, 2016 from the different farmers fields with the GPS coordinate details (Table 3.1). Cured capsule, leaves, spike remains and tillers of large cardamom varieties were collected from Sawney, Golsey, Ramsey and Saremna (Plate No. 13, 14, 15, 16). The cured capsule minimum five hundred grams from each variety was collected from different places of Sikkim in the month of October - November. Collected sample were dipped in liquid nitrogen immediately stored at -80°C until further analysis.

Plate No. 9: Sawney variety.

Plate No. 10: Golsey variety.

Plate No. 11: Ramsey variety.

Plate No. 12: Saremna variety.

Plate No. 13: Cured capsules of Sawney variety.

Plate No. 14: Cured capsules of Golsey variety.

Plate No. 15: Cured capsules of Ramsey variety.

Plate No. 16: Cured capsules of Saremna variety.

Table 3.1: GPS coordinates of different varieties cultivated

Varieties	Name of the place	Latitudes (N)	Longitude (E)	Altitude range (m from MSL)
Sawney	Kartok, East Sikkim	27°14.436"	088°35.048"	1624 M
		27°14.338"	088°35.091"	1612 M
		27°14.510"	088°34.911"	1599 M
		27°14.514"	088°35.317"	1512 M
Golsey	Thingling, West Sikkim	27°21.991"	088°12.294"	1302 M
		27°21.959"	088°12.272"	1319 M
		27°20.850"	088°12.298"	1262 M
		27°21.837"	088°12.235"	1315 M
Ramsey	Yuksam, West Sikkim	27°22.716"	088°13.129"	1743 M
		27°22.260"	088°13.200"	1747 M
		27°22.250"	088°13.193"	1751 M
		27°22.181"	088°13.111"	1751 M
Saremna	Yuksam, West Sikkim	27°22.370"	088°13.313"	1754 M
		27°22.362"	088°13.304"	1727 M
		27°22.159"	088°13.342"	1692 M
		27°22.386"	088°13.335"	1728 M

The samples of different parts such as leaves, tillers and spike remains of large cardamom were collected, minimum 500 gram from the different farmers field for the extraction of essential oil.

3.2. Extraction of essential oil

Extraction of essential oil was done by hydro distillation using a Clevenger apparatus.

3.2.1. Preparation of sample

Cured capsule, leaves, spike remains and tillers samples were collected from four major cultivars of large cardamom found in Sikkim i,e. Sawney, Golsey, Ramsey and Saremna (Plate No.17, 18, 19, 20). Collected samples except capsules were dried at shade places to reduce moisture. The sample were grinded by using Willey's mill grinder. The powder sample were weighed using digital weighing balance and then transferred to a round bottom flask (2000 ml) followed by adding water enough to cover the sample. The round bottom flask was installed on the heating mantle with Clevenger then inlet and outlet silica pipe was connected to a condenser and inlet pipe connected to tap water. After installing Clevenger the tape water was opened and on the heater with maintaining 40^0C temperature for 6 hours (Rout *et al.,* 2003). The oil yield was recorded then oil was collected in a culture tube with screw cap(Plate No.21, 22, 23). The water content in the essential oil was removed by adding anhydrous sodium sulphate. The essential oil was stored in refrigerator at 4^0C until further analysis (Plate No.24, 25).

3.2.2. Essential Oil (%)

The extracted essential oil (%) was calculated by using following equation

$$\text{Oil \%} = \frac{\text{Weight of the oil extracted}}{\text{Weight of the sample used}} \times 100$$

3.3. Gas chromatography-Mass spectrometry (GC-MS) analysis

The essential oil analysis was carried out on GC-MS (Shimadzu QP-2010 Ultra, Japan) (Plate No.26). The column oven temperature was maintained at 50^0C.

Plate No.17: Dried capsule of large cardamom

Plate No.18: Spike remains of large cardamom

Plate No.19: Leaves of large cardamom

Plate No.20: Tillers of large cardamom

Plate No.21: Hydro distillation by Clevenger apparatus

Plate No.22: Collecting oil from Clevenger apparatus

Plate No.23: Oil layer

Plate No.24: Essential oil in vials

Plate No.25: Storing essential oil extracted from large cardamom

Plate No.26: GC-MS (Shimadzu QP-2010 Plus)

Helium gas was used as a carrier at a flow rate of 1.5 mL/minute and ionization energy was maintained at 70 eV. Injection temperature was 250°C, and the pressure was maintained at 69 kPa, total flow was 125.2 mL minute^{-1}, column flow was 1.21 mL minute^{-1}, linear flow was maintained at 39.9 cm second^{-1}, purge flow was 3 mL minute^{-1} The iron source temperature was 230°C, interface temperature was 270°C (Vijayan *et al.,* 2017). GC-MS profile of essential oil constituents of the four major cultivars of large cardamom has been shown in Table No.4.1.

3.3.1. Compound Identification

The identification of the compounds was done by comparison of their mass spectra with the Wiley & NIST library and also with the published mass spectra (Adam *et al.,* 2001).

3.4. Compost preparation

The compost was prepared from waste biomass collected from cardamom field.

3.4.1. Collection of Samples

Leaves, spike remains and tillers samples were collected from four major cultivars of large cardamom found in Sikkim i,e. Sawney, Golsey, Ramsey and Saremna (Plate No.27, 28, 29, 30). The compost was prepared by using commercially available decomposer such as Madhyam and EM for decomposing available biomass.

Madhyam is a mixture of cultures containing microorganisms specifically developed for accelerated aerobic composting of organic waste. It contains cultures of bacteria, fungi and actinomycetes along with enzymes.

Plate No.27: Cardamom field with current season waste biomass

Plate No.28: Cardamom field with previous season waste biomass

Plate No.29: Cardamom field with current season waste biomass

Plate No.30: Cardamom field with previous season waste biomass

Effective microorganisms (EM) was developed by Dr. Teruo Hega, from Japan and the main microbes present in EM are Lactic acid bacteria (*Lactobacillus Casei*), Photosynthetic bacteria (*Rhodopseudomonas palustris*) and yeast (*Saccharomyces cerevisiae*).

To activate effective microorganisms (EM) one kg jaggery were dissolved in 20 litre of water than 1 litre EM.1 was added. Solution were mixed thoroughly with stick and kept in a cool place with air tight lid. The solution were stirred every day for 7 days and released accumulated gas. After 7 days 1 litre activated EM was diluted with 100 parts of water (1:100).

Available biomass such as leaves, spike remains and tillers was collected from the cardamom field and chopped into the small pieces (2-3cm) and mixed thoroughly(Plate No.31, 32). The different treatments combinations was designed for the composting are treatments combinations T1 (Available biomass), T2 (Available biomass + EM), T3 (Available biomass + Madhyam) and T4 (Available biomass + EM + Madhyam).

The pit was designed as above ground perforated pits (1 m^3 dimensions) using bamboo as done by Sharma *et al.*, 2014 with slight modification (Plate No.33). A 2.5 cm thick layer was made by mixed biomass which was drenched by using activated EM solution followed by madhyam broadcasted on the surface of the layer and this procedure was repeated until the height of the heap becomes about 1 meter. The process was repeated for each treatment with details of the treatments combinations. The mixtures were turned at fortnight intervals to manage porosity. The moisture content was maintained at 50-60% by often examine and it was managed by the addition of water over the active composting period.

Plate No.31: Available biomass chopped into the small pieces

Plate No.32: Biomass mixed thoroughly

Plate No.33: Above ground perforated pits (1 m^3 dimensions) using bamboo

4. Nutrient analysis in different varieties of large cardamom

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Agilent 7900 (Agilent Technologies, USA) was applied for nutrients analysis of the sample. Samples were dried in hot air oven. Further, it was digested by open air digestion method and transferred into narrow mouth bottle with details of the sample for further analysis. The value of the nutrient were presented as (mg/kg).

4.1. Digestion of compost

The dry compost was ground into a fine powder and each sample taken for digestion was 0.5 g. Samples were digested by using di-acid (nitric acid and perchloric acid in the ratio of 9:4) and the sample was kept on the hot plate till the clear white fumes were appeared and volume was made up to 50ml by using double distilled water and then transferred into narrow mouth bottle with details of the sample for further analysis (Plate No.34, 35).

4.2. Nutrient Estimation

The digested sample were subjected to ICP-MS (Inductively Coupled Plasma Mass Spectrometry) Agilent 7900 (Agilent Technologies, USA) for the ionic constitution using multi elements standards for Mg, Ca, Fe, Mn, B, Mo, Cu, Zn, Na, Ni and Co analytes.

Plate No.34: Open air digestion on hot plate

Plate No.35: Digested samples store in narrow mouth bottle with details.

5. Physicochemical analysis

5.1. Colour of compost

The colour of compost observed visually using maple colour chart in all the varieties and its treatments combinations in different stages.

5.2. Texture of compost

Texture was observed visually and coding techniques was used to determine the texture of compost in all the varieties and its treatments combinations in different stages. The scale given for the texture was (1-10) number scale, one number is for coarse textured and ten number for fine texture.

5.3. Moisture of compost

The moisture content was determined by gravimetric method. 2 g sample was placed on pre-dried and cleaned crucible. It was then placed at hot air oven at 100℃. Afterwards, the crucible was removed, cooled in desiccators and weighed (Jusoh *et al*., 2013).

The moisture was calculated by using the following formula:

$$\text{Moisture content \%} = \frac{\text{Weight of the residue}}{\text{Weight of the sample}} \times 100$$

5.4. pH of compost

The pH was determined by using a digital electrode pH meter. pH was measured by dissolving 1g of powdered compost sample into 10 ml of double distilled water and the pH was taken after one hour. The pH value was recorded directly from display.

5.5. Electrical conductivity (EC) of compost

The EC was determined by using a digital electrode EC meter.

5.6. Temperature

The temperature was determined by using a digital probe thermometer. The temperature was measured by inserting a metal probe into the centre of the compost then reading was recorded.

6. Statistical analysis

There were four large cardamom varieties taken in the research work and the experiment was done in quadruplicates. The experiment was performed using two factor Completely Randomized Design (CRD) ANOVA.

Chapter 4

RESULTS

The present study entitled **"Essential oils from different parts of Large Cardamom (*Amomum subulatum Roxb*.) and composting of available biomass"** was carried out in the Department of Horticulture, Sikkim University, 6th Mile, Tadong, Sikkim, during the year 2016-2019. Accordingly, the results obtained from analyses are presented in the following chapter.

4.1. Estimation of essential oil in four varieties of Large cardamom

4.1.1. Varieties

In terms of varieties highest oil yield content was found in Golsey with 0.85% (V2), whereas the lowest oil yield content was recorded in Sawney which is 0.63% (V1). However, it was found to be at par with Saremna (V4) which is 0.65% (Table No.1, Fig. No.2).

4.1.2. Plant parts used

The highest oil yield was found in capsules (2.18%). However, the lowest oil yield was found in tillers (0.03%) which was followed by spike remains (0.6%).

4.1.3. Interaction

In the interaction between varieties and plant parts used, variety Golsey showed the highest oil yield content in capsule (2.71%) which was followed by Ramsey (2.11%) in capsules. Whereas, the lowest oil yield was observed in Golsey and Ramsey variety with (0.02%) in tillers.

Fig. 1: Essential oil content (%) in different parts of large cardamom

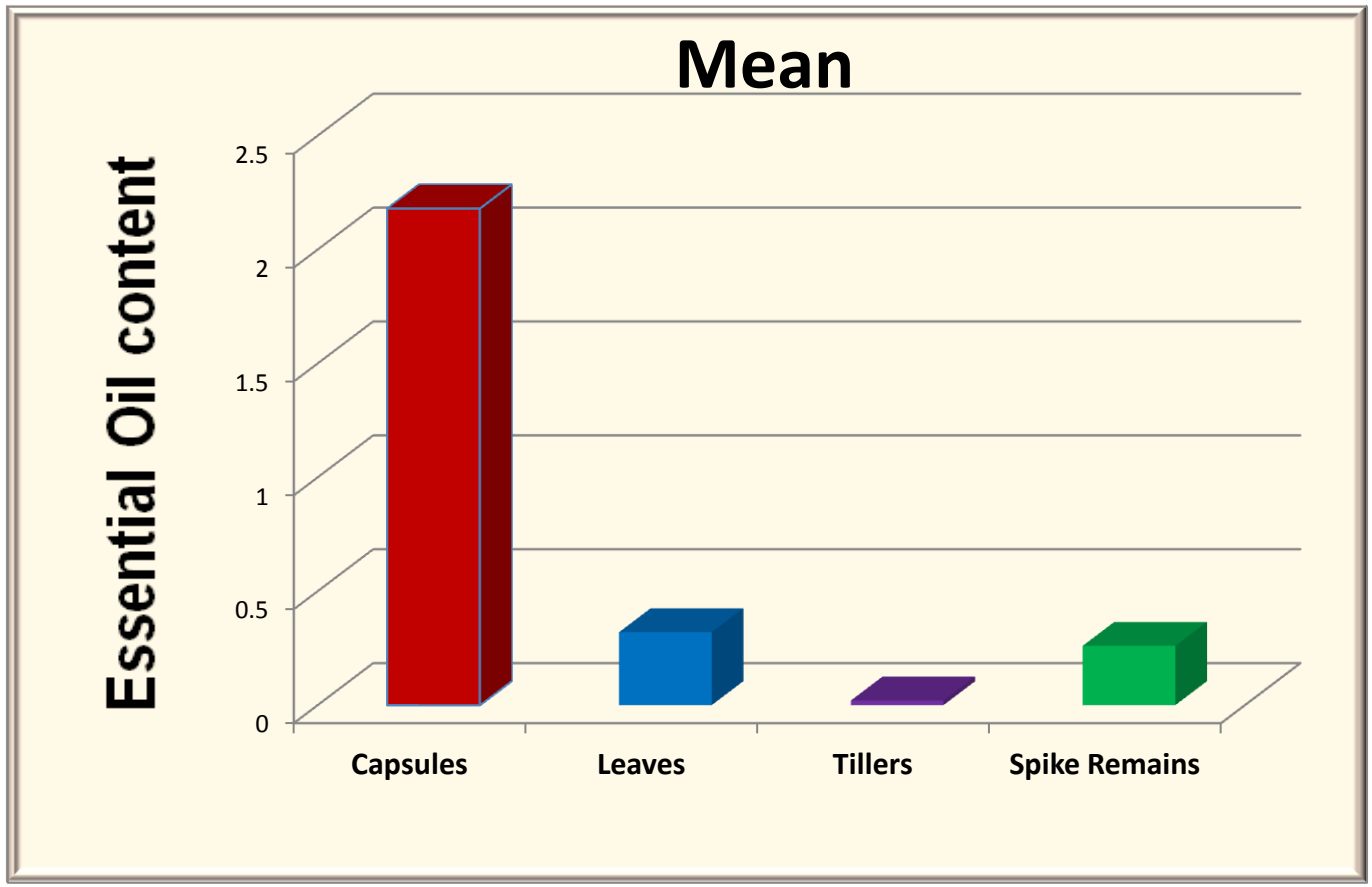


Fig.2: Essential oil content (%) in four major varieties of large cardamom

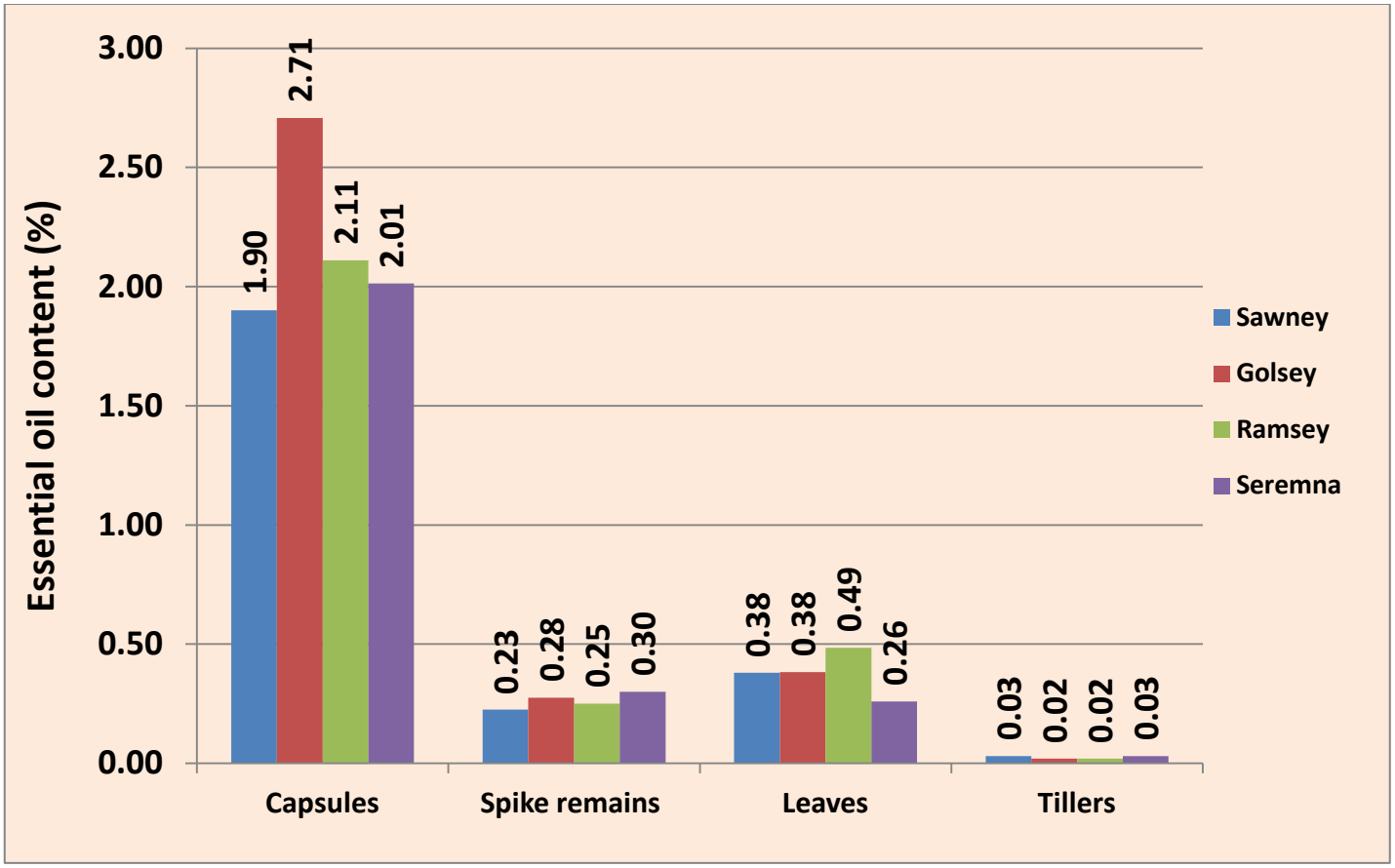


Table No. 4.1: Essential oil contain (%) in four varieties of large cardamom

Varieties	Capsules	Spike remains	Leaves	Tillers	Mean A
V1 (Sawney)	1.90	0.23	0.38	0.03	**0.63**
V2 (Golsey)	2.71	0.28	0.38	0.02	**0.85**
V3 (Ramsey)	2.11	0.25	0.49	0.02	**0.72**
V4 (Saremna)	2.01	0.30	0.26	0.03	**0.65**
Mean B	**2.18**	**0.26**	**0.38**	**0.03**	
Factors		**C.D (5%)**	**S.E(d)**	**SE(m)**	
Factor (A)		**0.051**	**0.025**	**0.018**	
Factor (B)		**0.051**	**0.025**	**0.018**	
Interaction (A X B)		**0.103**	**0.051**	**0.036**	

Note: A= Varieties and B= Plant parts used.

4.2. GC-MS analysis in different varieties of large cardamom

4.2.1. Plant parts used (Capsules)

In capsules of four varieties, 47 compounds were identified by GC-MS analysis which contributed 94.49 %-100.48 % of total oil. The major constituents of the essential oils from the four cultivars of large cardamom are 1,8-cineole, α-terpineol, β-pinene, α-pinene, limonene, δ-terpineol, myrcene, δ-terpinene and α-thujene, Nerolidol, terpinnen-4-ol, Germacrene D, Spathulenol, α-Cadinol and Globulol.

4.2.1.1. Sawney variety

In Sawney variety, 29 compounds were identified representing 95.50 % of the total oil. The highest percentage of compounds recorded were 1,8-cineole (54.78 %) followed by α-Terpineol (11.63 %), β-Pinene (5.91 %), Nerolidol (5.63 %), terpinnen-4-ol (4.39 %), α- pinene (2.24 %), δ-terpineol (1.89 %), Limonene (1.82 %), Germacrene D (1.32 %), Spathulenol (0.60 %), Germacrene B (0.55 %), α-Cadinol (0.54 %), δ-Terpinene (0.52 %), Myrcene (0.45 %), β-phellandrene (0.39 %), δ-Cadinene (0.36 %), α- terpinyal acetate (0.34 %), (E)-caryophyllene (0.28 %), Globulol (0.20 %), cis-p-menth-2-en-1-ol (0.18 %), α-Thujene (0.12 %), α- Terpinene (0.12 %), ϒ-Cadinene (0.12 %) and Isoascaridole (0.11 %).

4.2.1.2. Golsey variety

In Golsey variety, 30 compounds were identified representing 99.74 % of the total oil. The highest percentage of compounds recorded were 1,8-cineole (60.10 %) followed by α-Terpineol (10.36 %), β-Pinene (7.63 %), α- pinene (4.77 %), terpinnen-4-ol (4.20 %), Nerolidol (3.25 %), δ-terpineol (1.90 %), limonene (1.89 %), Linalool (0.08 %), Myrcene (0.73 %), δ-Terpinene (0.70 %), Germacrene D (0.45 %), β-phellandrene (0.37 %), α-Thujene (0.27 %), limonen oxide (0.25 %), Spathulenol (0.25 %), δ-Elemene (0.24 %), α- Terpinene (0.19 %), cis-p-menth-2-en-1-ol (0.18 %), muurolol (0.18 %), longifolene (0.14 %), Camphene (0.13 %), (E)-caryophyllene (0.13 %), δ-Carene (0.12 %), trans-p-menth-2-en-ol (0.10 %), cis-pinocarveol (0.09 %), δ-Cadinene (0.09 %), β-Fenchol (0.07 %), α- terpinyal acetate (0.06 %) and Pinocarvone (0.04 %) (Table No.4.2).

4.2.1.3. Ramsey variety

In Ramsey variety, 14 compounds were identified representing 100.48 % of the total oil. The highest percentage of compounds recorded were 1,8-cineole (86.09

%) followed by β-Pinene (4.43 %), α-Terpineol (2.46 %), α- pinene (1.93 %), terpinnen-4-ol (1.79 %), limonene (1.44 %), δ-Terpinene (0.47 %), δ-terpineol (0.47 %), Sabinene (0.34 %), (E)-caryophyllene (0.34 %), ϒ-Terpinene (0.26 %), α-Thujene (0.21 %), Myrcene (0.19 %) and cis-pinocarveol (0.06 %) (Table No.4.2).

4.2.1.4. Saremna variety

In Saremna variety, 28 compounds were identified representing 94.49 % of the total oil (Table No.4.2). The highest percentage of compounds recorded were 1,8-cineole (59.38 %) followed by β-Pinene (5.37 %), Nerolidol (4.89 %), 4-Thujanol (3.95 %), 4-Terpineol (3.95 %), α-Terpineol (3.95 %), limonene (2.99 %), α- pinene (2.72 %), δ-terpineol (2.37 %), Sabinene (0.79 %), Spathulenol (0.59 %), Pinocarvone (0.53 %), α- terpinyal acetate (0.48 %), Myrcene (0.44 %), Germacrene D (0.37 %), δ-Terpinene (0.30 %), trans-Sabinene hydrate (0.25 %), ϒ-Elemene (0.21 %), cis-pinocarveol (0.17 %), trans-p-menth-2-en-ol (0.13 %), (E)-caryophyllene (0.13 %), α-Thujene (0.11 %), δ-Cadinene (0.10 %), ϒ-Muurolene (0.08 %), Camphene (0.07 %), α-Terpinene (0.06 %), δ-Carene (0.06 %) and allo- Aromadendendrene (0.05 %).

4.2.2. Plant parts used (Spike Remains)

4.2.2.1. Sawney variety

In Sawney variety, 18 compounds were identified representing 60.53 % of the total oil (Table No.4.2). The highest percentage of compounds recorded were β-Pinene (26.13 %) followed by Spathulenol (18.38 %), α-pinene (4.34 %), 1,8-cineole (2.74 %), cis-pinocarveol (2.37 %), ϒ-Muurolene (1.36 %), allo- Aromadendendrene (1.13 %), 4-Terpineol (0.53 %), Globulol (0.53 %), α- Caryophyllen (0.44 %), p-cymene (0.42 %), α-Cadinol (0.42 %), β-elemene (0.41 %), Germacrene D (0.40 %),

Pinocarvone (0.27 %), Limonene (0.26 %), Camphene (0.25 %) and δ-Cadinene (0.15 %).

4.2.2.2. Golsey variety

In Golsey variety, 15 compounds were identified representing 43.65 % of the total oil. The highest percentage of compounds recorded were β-Pinene (18.01%) followed by Spathulenol (14.80 %), α-pinene (3.37 %), cis-pinocarveol (1.76%), allo-Aromadendendrene (1.22 %), β-elemene (0.80 %), 1,8-cineole (0.72 %), ϒ-Muurolene (0.71 %), δ-Cadinene (0.52 %), 4-Terpineol (0.51 %), α-Caryophyllen (0.31 %), Globulol (0.28 %), Limonene (0.23 %), α-Cadinol (0.21 %) and p-cymene (0.20 %) (Table No.4.2).

4.2.2.3. Ramsey variety

In Ramsey variety, 19 compounds were identified representing 46.54 % of the total oil. The highest percentage of compounds recorded were β-Pinene (18.41 %) followed by Spathuleno (17.06 %), α-pinene (2.72 %), allo- Aromadendendrene (1.46 %), ϒ-Muurolene (1.10 %), cis-pinocarveol (1.07 %), Germacrene D (0.64 %), δ-Cadinene (0.63 %), Globulol (0.49 %), 1,8-cineole (0.40 %), β-elemene (0.38 %), 4-Terpineol (0.36 %), α-Cadinol (0.36 %), ϒ-Cadinene (0.34 %), Sabinene (0.28 %), Pinocarvone (0.24 %), Limonene (0.23 %), p-cymene (0.21 %) and ϒ-Muurolene (0.16 %) (Table No.4.2).

4.2.2.4. Saremna variety

In Saremna variety, 17 compounds were identified representing 40.80 % of the total oil (Table No.4.2). The highest percentage of compounds recorded were

Spathulenol (17.05 %) followed by β-Pinene (14.32 %), α-pinene (2.60 %), β-elemene (1.30 %), cis-pinocarveol (1.29 %), allo- Aromadendendrene (1.14 %), Germacrene D (0.60 %), Globulol (0.57 %), δ-Cadinene (0.38 %), 4-Terpineol (0.33 %), Limonene (0.22 %), ϒ-Cadinene (0.22 %), Sabinene (0.20 %), α-Cadinol (0.19 %), Pinocarvone (0.16 %), p-cymene (0.12 %) and 1,8-cineole (0.11 %).

4.2.3. Plant parts used (Leaves)

4.2.3.1. Sawney variety

In Sawney variety, 12 compounds were identified representing 49.36 % of the total oil (Table No.4.2). The highest percentage of compounds recorded were Spathulenol (35.11 %) followed by Globulol (5.06 %), allo- Aromadendendrene (2.50 %), α-Cadinol (1.90 %), ϒ-Muurolene (0.99 %), ϒ-Cadinene (0.94 %), β-Pinene (0.90 %), α- Muurolene (0.69 %), δ-Cadinene (0.62 %), Germacrene D (0.33 %), β-elemene (0.26 %) and α- pinene (0.06 %).

4.2.3.2. Golsey variety

In Golsey variety, 6 compounds were identified representing 54.10 % of the total oil. The highest percentage of compounds recorded were Spathulenol (40.34 %) followed by Globulol (8.90 %), α-Cadinol (2.10 %), allo- Aromadendendrene (1.39 %), ϒ-Muurolene (1.00 %) and α- pinene (0.37 %) (Table No. 4.2).

4.2.3.3. Ramsey variety

In Ramsey variety, 19 compounds were identified representing 56.66 % of the total oil. The highest percentage of compounds recorded were Spathulenol (32.62 %) followed by α-Cadinol (8.89 %), allo- Aromadendendrene (3.44 %), Germacrene D

(2.17 %), β-Pinene (1.96 %), ϒ-Muurolene (1.74 %), ϒ-Cadinene (1.49 %), Globulol (1.43 %), α- Muurolene (1.24 %), δ-Cadinene (0.47 %), cis-pinocarveol (0.27 %), α- Caryophyllen (0.23 %), α- pinene (0.17 %), β-elemene (0.16 %), 4-Terpineol (0.13 %), Sabinene (0.12 %), Pinocarvone (0.05 %), p-cymene (0.04 %) and Limonene (0.04 %) (Table No.4.2).

4.2.3.4. Saremna variety

In Saremna variety, 5 compounds were identified representing 49.52 % of the total oil (Table No.4.2). The highest percentage of compounds recorded were Spathulenol (37.28 %) followed by Globulol (6.98 %), α-Cadinol (2.03 %), allo-Aromadendendrene (1.84 %) and ϒ-Muurolene (1.39 %).

4.2.4. Plant parts used (Tillers)

4.2.4.1. Sawney variety

In Sawney variety only three compounds were identified which representing 50.51 % of the total oil. The highest percentage of compounds was recorded 28.42 % in Spathulenol which was followed by α-Cadinol (15.26 %) and Globulol (6.83 %) (Table No.4.2).

4.2.4.2. Golsey variety

In Golsey variety, six compounds were identified which representing 37.05 % of the total oil (Table No.4.2). The highest percentage of compounds recorded were (E)-caryophyllene (27.97 %) followed by Spathulenol (6.25 %), ϒ-Muurolene (1.21 %), β-Pinene (1.11 %), allo- Aromadendendrene (0.38 %) and α- pinene (%).

Table No. 4.2: GC-MS profile of the area (%) of essential oil of four major cultivars of large cardamom

SL. No.	Compound name	Capsules				Spike Remains				Leaves				Tillers			
		Sawney	Golsey	Ramsey	Saremna	Sawney	Golsey	Ramsey	Saremna	Sawney	Golsey	Ramsey	Saremna	Sawney	Golsey	Ramsey	Saremna
1	α-Thujene	**0.12**	**0.27**	**0.21**	**0.11**	-	-	-	-	-	-	-	-	-	-	-	-
2	α- pinene	**2.24**	**4.77**	**1.93**	**2.72**	**4.34**	**3.37**	**2.72**	**2.6**	**0.06**	**0.37**	**0.17**	-	-	**0.13**	**0.17**	-
3	Camphene	**0.05**	**0.13**	-	**0.07**	**0.25**	-	-	-	-	-	-	-	-	-	-	-
4	Sabinene	-	-	**0.34**	**0.79**	-	-	**0.28**	**0.2**	-	-	**0.12**	-	-	-	-	-
5	β-phellandrene	**0.39**	**0.37**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	β-Pinene	**5.91**	**7.63**	**4.43**	**5.37**	**26.13**	**18.01**	**18.41**	**14.32**	**0.9**	-	**1.96**	-	-	**1.11**	**1.89**	-
7	Myrcene	**0.45**	**0.73**	**0.19**	**0.44**	-	-	-	-	-	-	-	-	-	-	-	-
8	α- Terpinene	**0.12**	**0.19**	-	**0.06**	-	-	-	-	-	-	-	-	-	-	-	-
9	p-cymene	**0.07**	-	-	-	**0.42**	**0.2**	**0.21**	**0.12**	-	-	**0.04**	-	-	-	-	-
10	Limonene	**1.82**	**1.89**	**1.44**	**2.99**	**0.26**	**0.23**	**0.23**	**0.22**	-	-	**0.04**	-	-	-	-	-
11	1, 8-Cineole	**54.78**	**60.1**	**86.09**	**59.38**	**2.74**	**0.72**	**0.4**	**0.11**	-	-	-	-	-	-	-	-
12	ϒ-Terpinene	-	-	**0.26**	-	-	-	-	-	-	-	-	-	-	-	-	-
13	δ-Terpinene	**0.52**	**0.7**	**0.47**	**0.3**	-	-	-	-	-	-	-	-	-	-	-	-
14	4-Thujanol	-	-	-	**3.95**	-	-	-	-	-	-	-	-	-	-	-	-
15	δ-Carene	**0.07**	**0.12**	-	**0.06**	-	-	-	-	-	-	-	-	-	-	-	-
16	trans-Sabinene hydrate	-	-	-	**0.25**	-	-	-	-	-	-	-	-	-	-	-	-
17	Linalool	**0.08**	**0.86**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	β-Fenchol	-	**0.07**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	cis-p-menth-2-en-1-ol	**0.18**	**0.18**	-	**0.17**	-	-	-	-	-	-	-	-	-	-	-	-
20	cis-pinocarveol	**0.15**	**0.09**	**0.06**	-	**2.37**	**1.76**	**1.07**	**1.29**	-	-	**0.27**	-	-	-	-	-
21	trans-p-menth-2-en-ol	**0.1**	**0.1**	-	**0.13**	-	-	-	-	-	-	-	-	-	-	-	-
22	Pinocarvone	**0.06**	**0.04**	-	**0.53**	**0.27**	-	**0.24**	**0.16**	-	-	**0.05**	-	-	-	-	-
23	δ-terpineol	**1.89**	**1.9**	**0.47**	**2.37**	-	-	-	-	-	-	-	-	-	-	-	-

SL.	Compound name	Capsules	Spike Remains	Leaves	Tillers

No.		Sawney	Golsey	Ramsey	Saremna	Sawney	Golsey	Ramsey	Saremna	Sawney	Golsey	Ramsey	Saremna	Sawney	Golsey	Ramsey	Saremna
24	4-Terpineol	-	-	-	3.95	0.53	0.51	0.36	0.33	-	-	0.13	-	-	-	-	-
25	terpinnen-4-ol	4.39	4.2	1.79	-	-	-	-	-	-	-	-	-	-	-	-	-
26	α-Terpineol	11.63	10.36	2.46	3.95	-	-	-	-	-	-	-	-	-	-	-	-
27	Isoascaridole	0.11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	α- terpinyal acetate	0.34	0.06	-	0.48	-	-	-	-	-	-	-	-	-	-	-	-
29	limonen oxide	-	0.25	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	β-elemene	0.08	-	-	-	0.41	0.8	0.38	1.3	0.26	-	0.16	-	-	-	-	-
31	(E)-caryophyllene	0.28	0.13	0.34	0.13	-	-	-	-	-	-	-	-	-	27.97	-	-
32	α- Caryophyllen	0.06	-	-	-	0.44	0.31	-	-	-	-	0.23	-	-	-	-	-
33	allo-Aromadendendrene	0.1	-	-	0.05	1.13	1.22	1.46	1.14	2.5	1.39	3.44	1.84	-	0.38	-	-
34	ϒ-Muurolene	-	-	-	0.08	1.36	0.71	1.1	-	0.99	1	1.74	1.39	-	1.21	-	-
35	Germacrene D	1.32	0.45	-	0.37	0.4	-	0.64	0.6	0.33	-	2.17	-	-	-	-	-
36	Germacrene B	0.55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	α- Muurolene	0.09	-	-	-	-	-	0.16	-	0.69	-	1.24	-	-	-	-	-
38	δ-Elemene	-	0.24	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	ϒ-Elemene	-	-	-	0.21	-	-	-	-	-	-	-	-	-	-	-	-
40	ϒ-Cadinene	0.12	-	-	-	-	-	0.34	0.22	0.94	-	1.49	-	-	-	-	-
41	δ-Cadinene	0.36	0.09	-	0.1	0.15	0.52	0.63	0.38	0.62	-	0.47	-	-	-	-	-
42	Nerolidol	5.63	3.25	-	4.89	-	-	-	-	-	-	-	-	-	-	-	-
43	Spathulenol	0.6	0.25	-	0.59	18.38	14.8	17.06	17.05	35.11	40.34	32.62	37.28	28.42	6.25	0.43	29.04
44	muurolol	-	0.18	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	longifolene	-	0.14	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	Globulol	0.2	-	-	-	0.53	0.28	0.49	0.57	5.06	8.9	1.43	6.98	6.83	-	-	8.19
47	α-Cadinol	0.54	-	-	-	0.42	0.21	0.36	0.19	1.9	2.1	8.89	2.03	15.26	-	-	16.16
Total		**95.40**	**99.74**	**100.48**	**94.49**	**60.53**	**43.65**	**46.54**	**40.80**	**49.36**	**54.10**	**56.66**	**49.52**	**50.51**	**37.05**	**2.49**	**53.39**

GC-MS chromatogram of essential oil of large cardamom

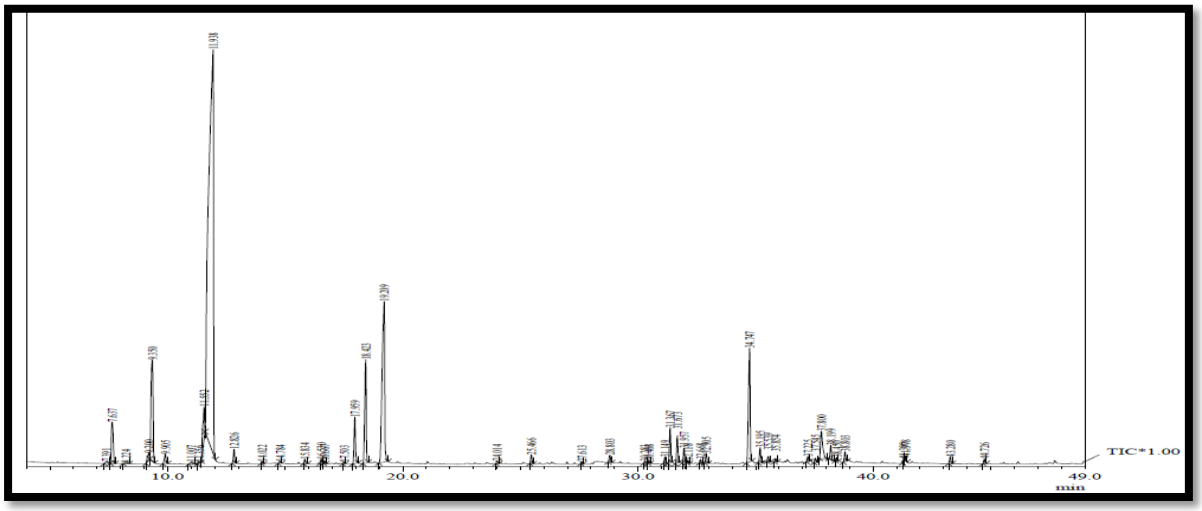


Fig. No.3: GC-MS chromatogram of essential oil of Sawney capsule

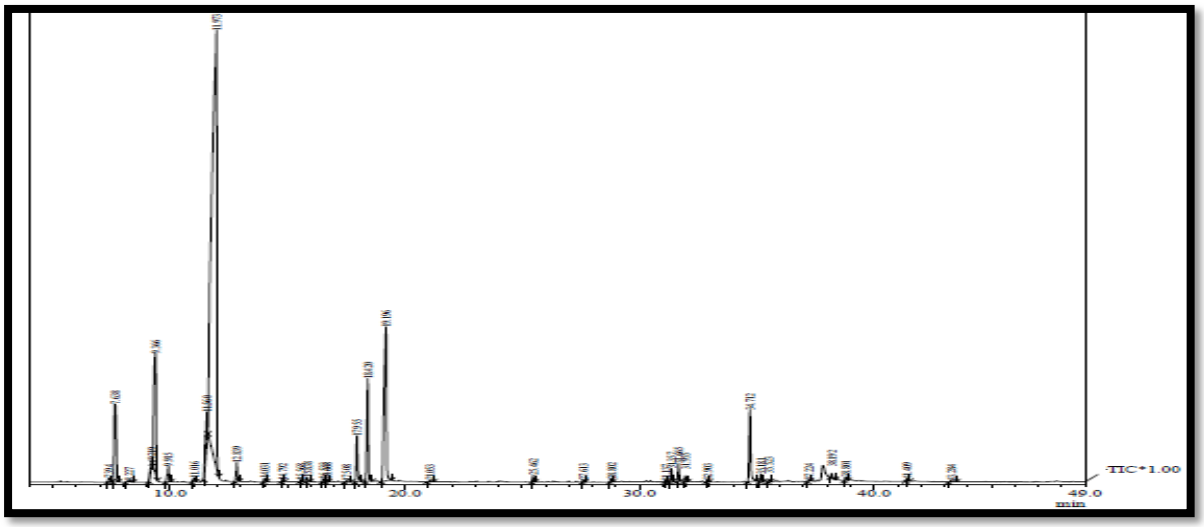


Fig.No.4: GC-MS chromatogram of essential oil of Golsey capsule

Fig. No.5: GC-MS chromatogram of essential oil of Ramsey capsule

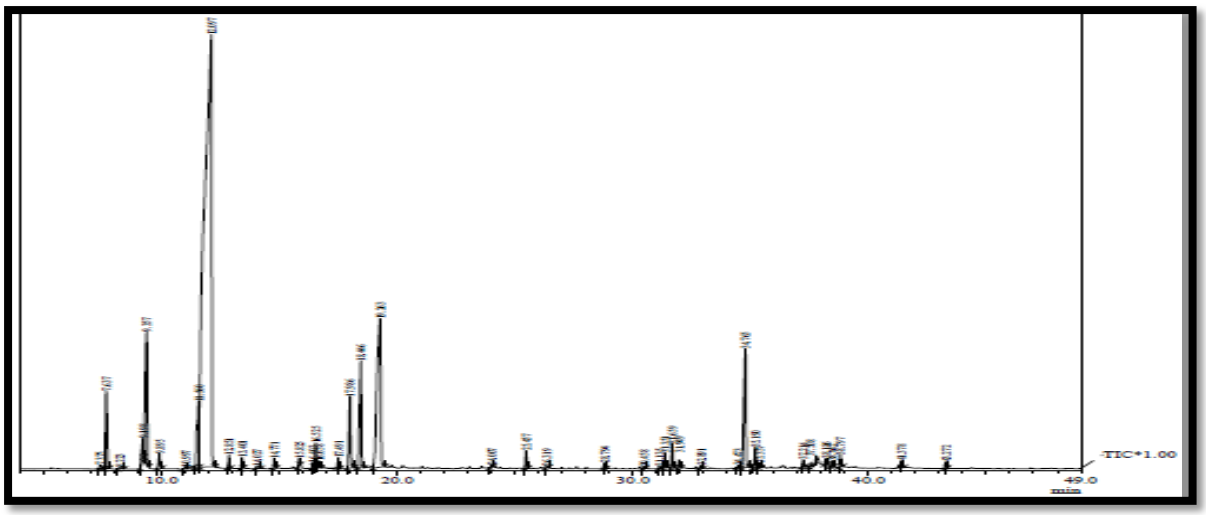


Fig. No.6: GC-MS chromatogram of essential oil of Saremna capsule

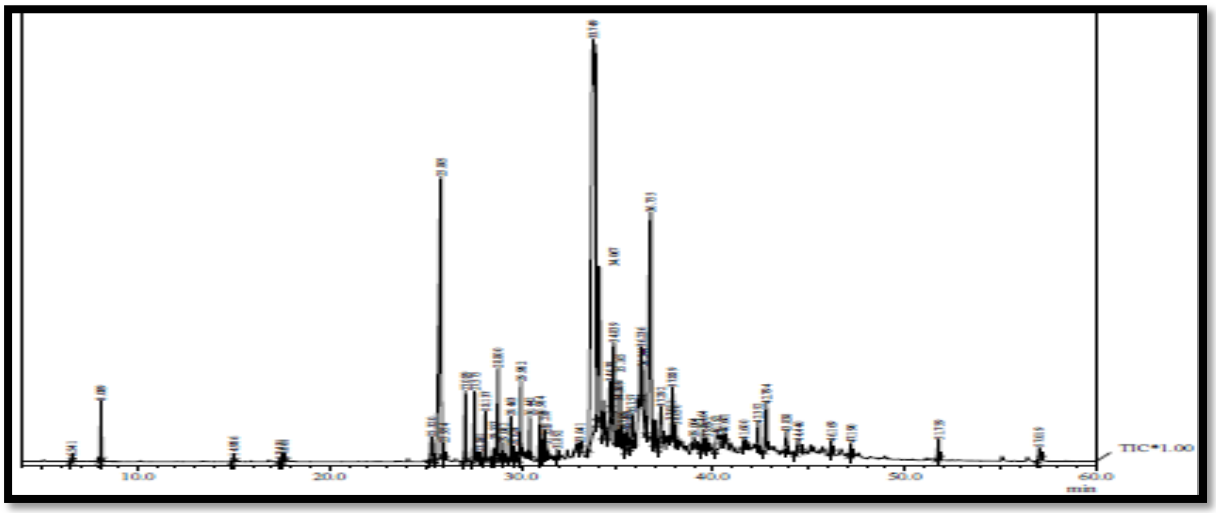


Fig. No.7: GC-MS chromatogram of essential oil of Sawney leaves

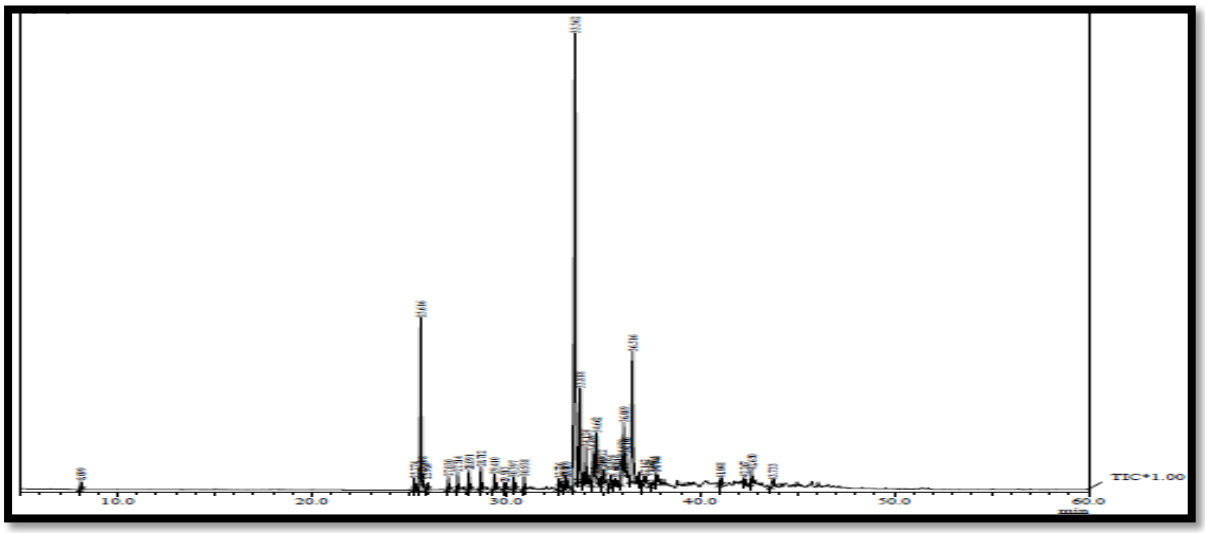


Fig. No.8: GC-MS chromatogram of essential oil of Golsey leaves

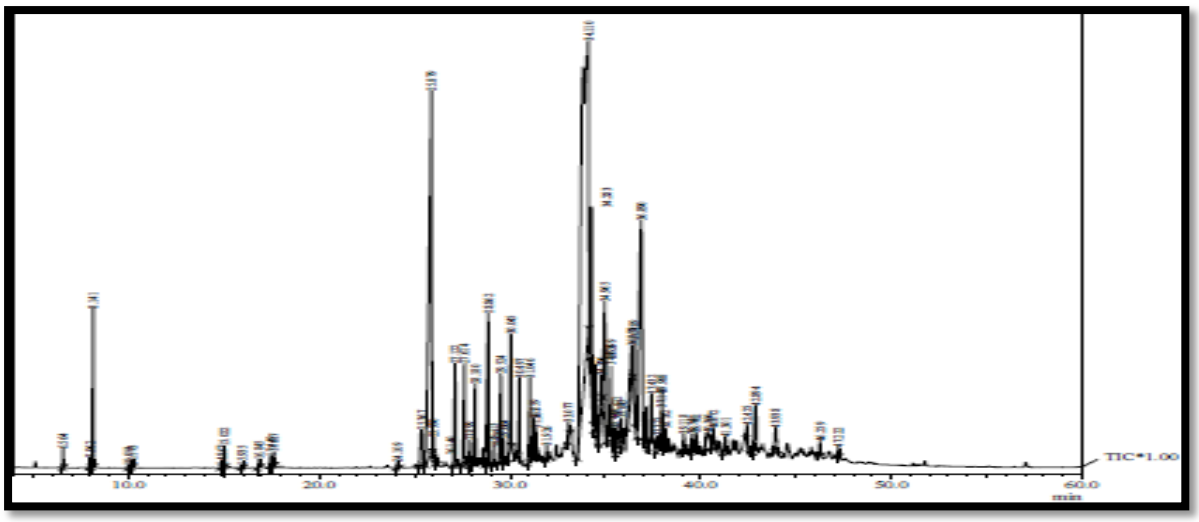


Fig. No.9: GC-MS chromatogram of essential oil of Ramsey leaves

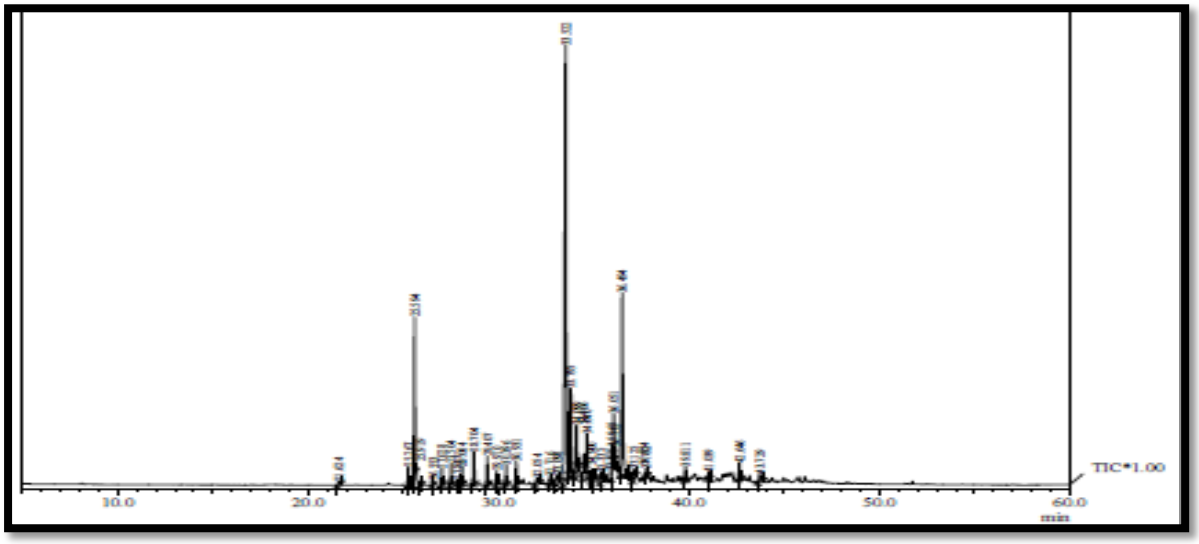


Fig. No.10: GC-MS chromatogram of essential oil of Saremna leaves

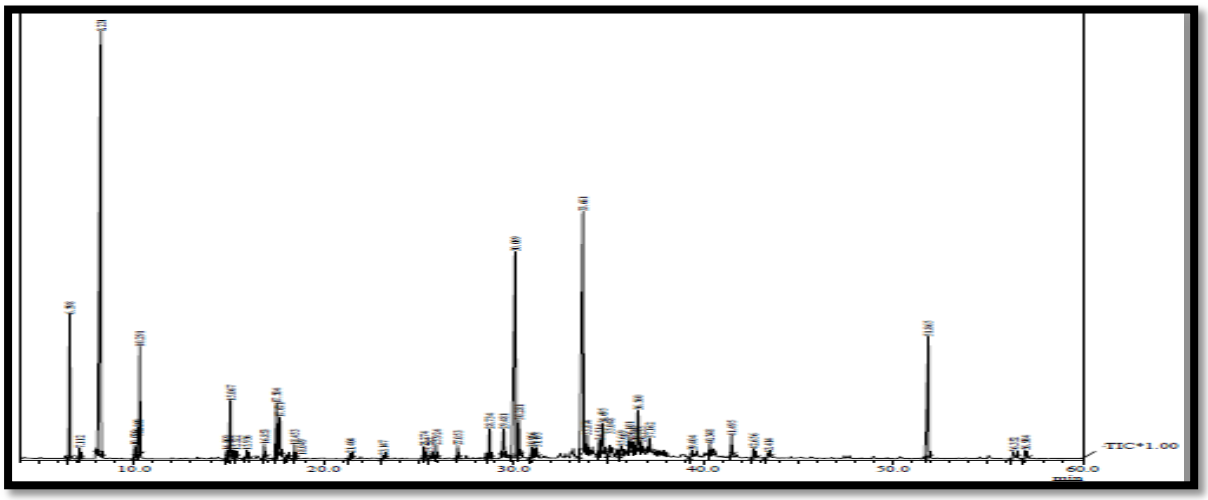


Fig. No.11: GC-MS chromatogram of essential oil of Sawney spike remains

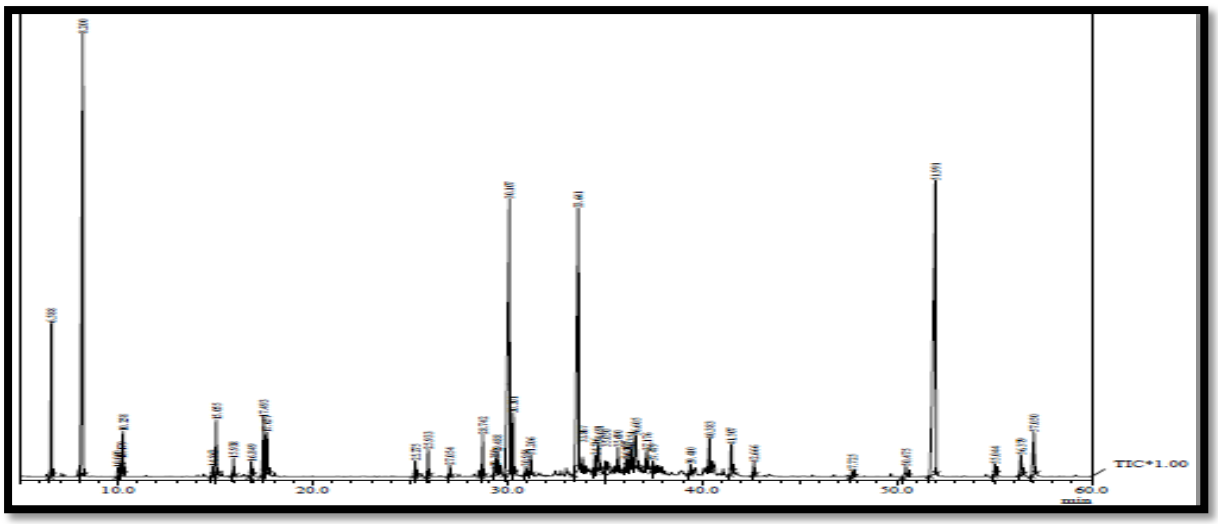


Fig. No.12: GC-MS chromatogram of essential oil of Golsey spike remains

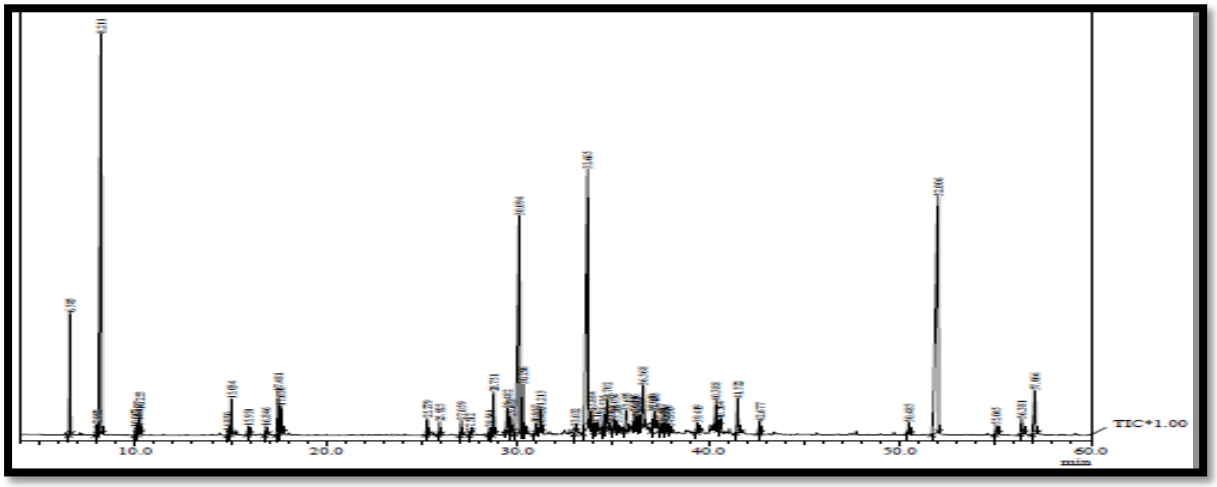


Fig. No.13: GC-MS chromatogram of essential oil of Ramsey spike remains

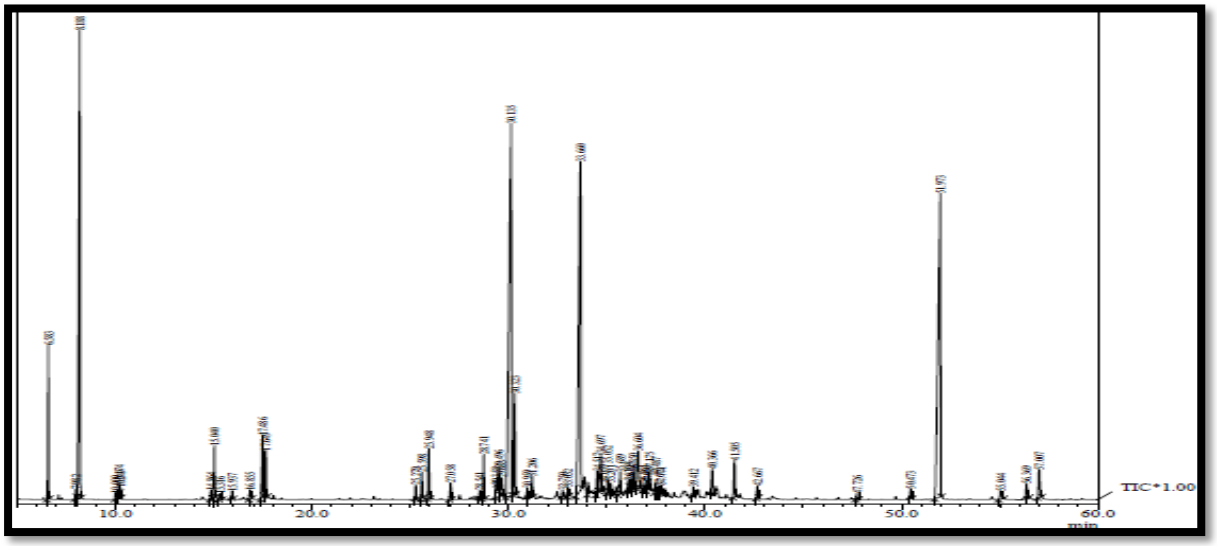


Fig. No.14: GC-MS chromatogram of essential oil of Saremna spike remains

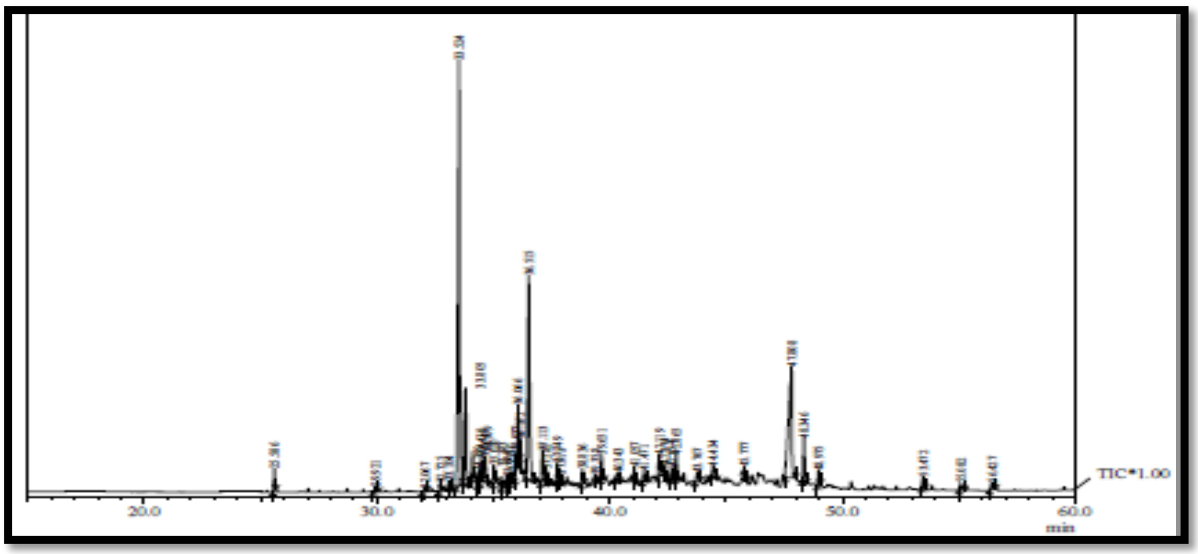


Fig. No.15: GC-MS chromatogram of essential oil of Sawney tillers

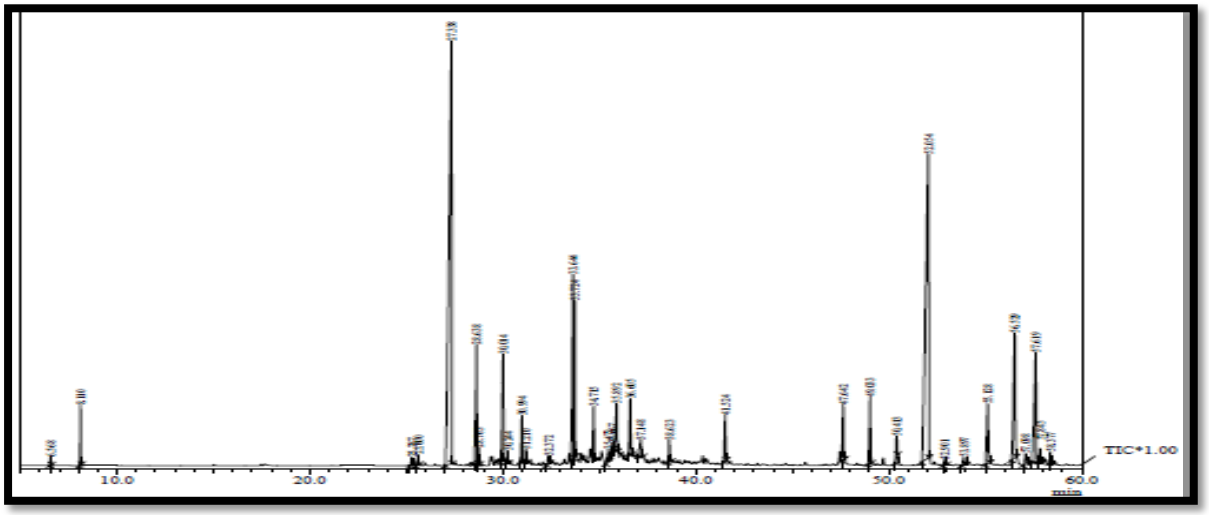


Fig. No.16: GC-MS chromatogram of essential oil of Golsey tillers

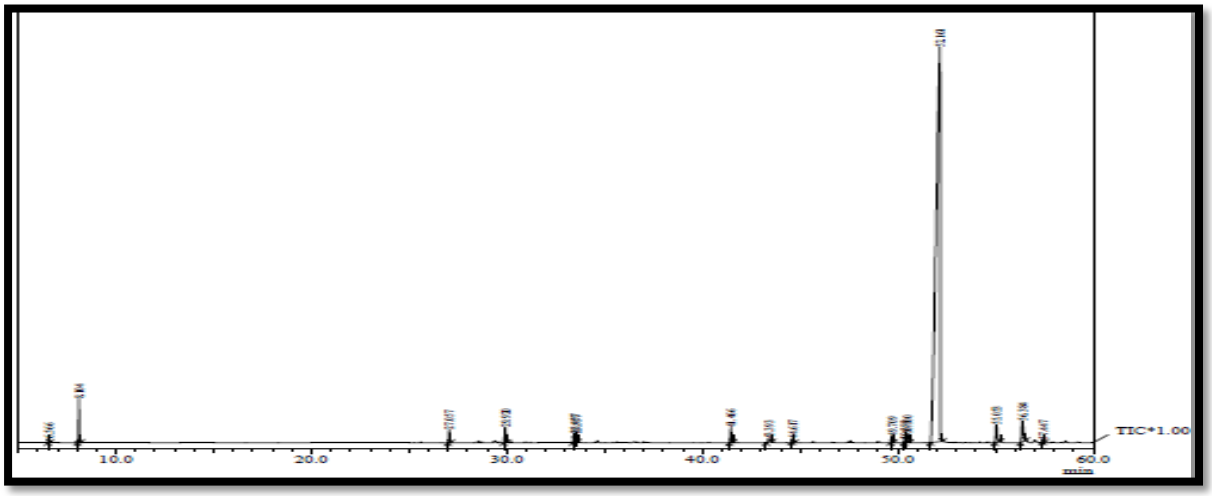


Fig. No.17: GC-MS chromatogram of essential oil of Ramsey tillers

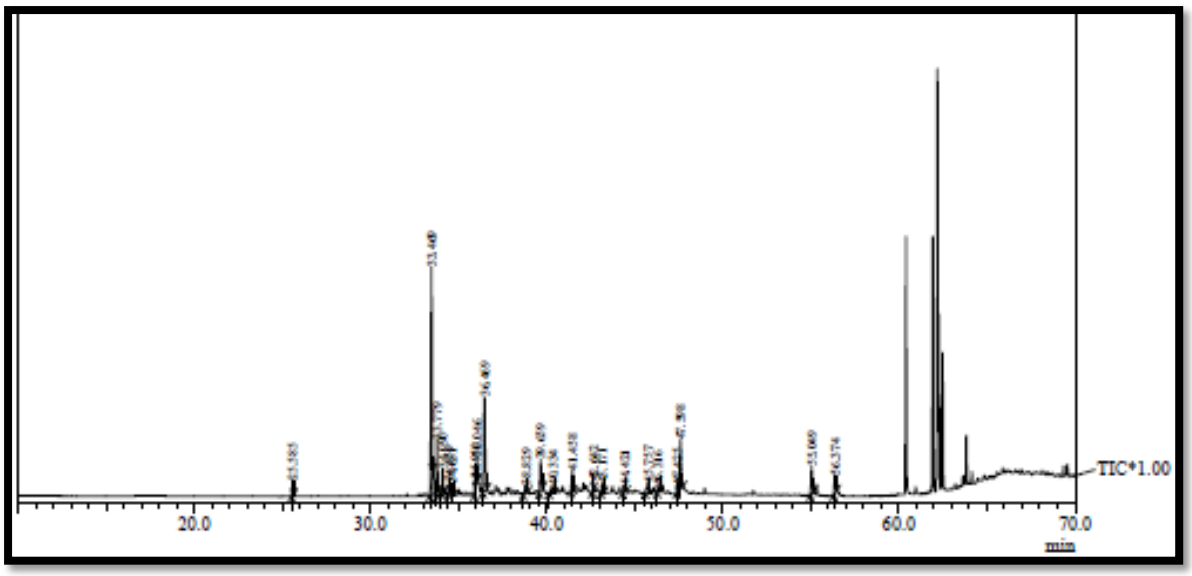


Fig. No.18: GC-MS chromatogram of essential oil of Saremna tillers

4.2.4.3. Ramsey variety

In Ramsey variety, three compounds were identified which representing 2.49 % of the total oil. The highest percentage of compounds recorded were β-Pinene

(1.89 %) followed by Spathulenol (0.43 %) and α- pinene (0.17 %) (Table No.4.2).

4.2.4.4. Saremna variety

In Saremna variety, three compounds were identified which representing 53.39 % of the total oil (Table No.4.2). The highest percentage of compounds recorded were Spathulenol (29.04 %) followed by α-Cadinol (16.16 %) and Globulol (8.19 %)

4.3. Nutrient analysis of different varieties of large cardamom

4.3.1. Nitrogen (N) %

4.3.1.1. Varieties

The nitrogen content of different varieties have been depicted in Table No.4.3. The result of the present investigation showed that the highest nitrogen content was observed in Ramsey variety (0.66 %) followed by Sawney variety (0.50 %) which was found to be at par with Golsey variety i.e. (0.49 %). Whereas, the lowest nitrogen content was recorded in Saremna variety (0.58 %).

4.3.1.2. Treatments combinations

Comparison among treatments showed that the combination T3 (available biomass + Madhyam) showed the highest nitrogen content (0.67 %) which was followed by treatments combination T4 (available biomass + EM + Madhyam) which

was (0.56 %). T4 was found to be at par with T2 (available biomass + EM) which is (0.52 %) and T1 (available biomass) i.e. (0.38 %).

4.3.1.3. Interactions

Among the different interaction Saremna variety in T3 (available biomass + Madhyam) showed the maximum nitrogen content that is (0.92 %).

Table No.4.3: Nitrogen content (%) in the final compost

Varieties	T1 (Available biomass)	T2 (Available biomass + EM)	T3 (Available biomass + Madhyam)	T4 (Available biomass + EM + Madhyam)	Mean A
V1 (Sawney)	0.31	0.56	0.69	0.46	**0.50**
V2 (Golsey)	0.27	0.54	0.45	0.71	**0.49**
V3 (Ramsey)	0.60	0.64	0.63	0.75	**0.66**
V4 (Saremna)	0.36	0.34	0.92	0.32	**0.48**
Mean B	**0.38**	**0.52**	**0.67**	**0.56**	
Factors		**C.D (5%)**	**SE(d)**	**SE(m)**	
Factor(A)		**0.10**	**0.05**	**0.03**	
Factor(B)		**0.10**	**0.05**	**0.03**	
Factor(A X B)		**0.20**	**0.10**	**0.07**	

Note: A= Varieties and B= Treatments combinations.

Likewise, Ramsey variety in T3 (available biomass + Madhyam) with (0.75 %) which was at par with Golsey variety in T4 (available biomass + EM + Madhyam) with (0.71 %), Sawney variety in T3 (available biomass + Madhyam) (0.69 %), Ramsey variety in T2 (available biomass + EM) (0.64 %), Ramsey variety in T3 (available biomass + Madhyam) (0.63 %), Ramsey variety in T1 (available biomass) (0.60 %) and Sawney variety in T2 (available biomass + EM) (0.56 %). Whereas the lowest

nitrogen content was found at Golsey variety in T2 (available biomass + EM) which was (0.27 %).

4.3.2. Phosphorus (P) %

4.3.2.1. Varieties

The phosphorous content of different varieties have been presented in the Table No.4.4. The highest amount of phosphorus content was observed in the Saremna variety (0.65 %) followed by Golsey and Ramsey variety (0.55 %) while, the phosphorous content was recorded lowest in Sawney variety (0.30 %).

4.3.2.2. Treatments combinations

Comparison among treatments showed that the combination T4 (available biomass + EM + Madhyam) showed the highest phosphorus content (0.57 %) which was followed by treatments combination T3 (available biomass + Madhyam) which was (0.51 %). It was found to be at par with T1 (available biomass) which was (0.50 %). Whereas the least phosphorus content was recorded in treatments combination T2 (available biomass + EM) with (0.45 %).

4.3.2.3. Interactions

In the interaction between varieties and treatments combinations Golsey variety in T3 (available biomass + Madhyam) showed highest phosphorus content (0.70 %) which was at par with Ramsey variety in T4 (available biomass + EM + Madhyam) that is (0.68 %). It was followed by Saremna variety in T4 (available biomass + EM + Madhyam) that is (0.67 %) furthermore it was at par with Saremna variety in T3 (available biomass + Madhyam) with (0.66 %) and Saremna variety in

T2 (available biomass + EM) with (0.65 %). Whereas the lowest phosphorus content was found at Sawney variety in T3 (available biomass + Madhyam) which was (0.13 %).

Table No.4.4: Phosphorus content (%) in the final compost

Varieties	T1 (Available biomass)	T2 (Available biomass + EM)	T3 (Available biomass + Madhyam)	T4 (Available biomass + EM + Madhyam)	Mean A
V1 (Sawney)	0.43	0.15	0.13	0.48	**0.30**
V2 (Golsey)	0.47	0.56	0.70	0.47	**0.55**
V3 (Ramsey)	0.52	0.44	0.56	0.68	**0.55**
V4 (Saremna)	0.60	0.65	0.66	0.67	**0.65**
Mean B	**0.50**	**0.45**	**0.51**	**0.57**	
Factors		**C.D (5%)**	**SE(d)**	**SE(m)**	
Factor(A)		**0.007**	**0.003**	**0.002**	
Factor(B)		**0.007**	**0.003**	**0.002**	
Factor(A X B)		**0.014**	**0.007**	**0.005**	

Note: A= Varieties and B= Treatments combinations.

4.3.3. Potassium (K) %

4.3.3.1. Varieties

The potassium content of different varieties have been shown in the Table No.4.5. and data revealed highest potassium content was found in Sawney variety (2.55 %) followed by Golsey variety (1.98 %), Ramsey (1.66 %). However, lowest potassium content was recorded in Saremna variety (1.48 %).

4.3.3.2. Treatments combinations

Comparison among treatment shows that combination T1 (available biomass) was observed highest potassium content (2.05 %) followed by T2 (available biomass

+ EM) with (2.02 %). Whereas lowest potassium content was obtained in T3 (available biomass + Madhyam) that is (1.79 %) which was at par with T4 (available biomass + EM + Madhyam) which was (1.80 %).

4.3.3.3. Interactions

Among the different interaction Sawney in T2 (available biomass + EM) showed the maximum potassium content that is (3.48 %) which was followed by Golsey variety in T1 (available biomass) with (3.37 %), Sawney variety in T4 (available biomass + EM + Madhyam) with (2.97 %). However Ramsey variety in T4 (available biomass + EM + Madhyam) content (1.54 %) which was at par with Sawney in T1 (available biomass) with (1.55 %). On the other hand, lowest potassium was observed in Saremna variety in T4 (available biomass + EM + Madhyam) that is (1.24 %).

Table No.4.5: Potassium content (%) in the final compost

Varieties	T1 (Available biomass)	T2 (Available biomass + EM)	T3 (Available biomass + Madhyam)	T4 (Available biomass + EM + Madhyam)	Mean A
V1 (Sawney)	1.55	3.48	2.19	2.97	**2.55**
V2 (Golsey)	3.37	1.51	1.58	1.45	**1.98**
V3 (Ramsey)	1.85	1.59	1.65	1.54	**1.66**
V4 (Saremna)	1.45	1.48	1.75	1.24	**1.48**
Mean B	**2.05**	**2.02**	**1.79**	**1.80**	
Factors		**C.D (5%)**	**SE(d)**	**SE(m)**	
Factor(A)		**0.004**	**0.002**	**0.001**	
Factor(B)		**0.004**	**0.002**	**0.001**	
Factor(A X B)		**0.008**	**0.004**	**0.003**	

Note: A= Varieties and B= Treatments combinations.

4.3.4. Magnesium (Mg) mg/kg

4.3.4.1. Varieties

The magnesium content of different varieties have been depicted in Table No.4.6. The result of the present investigation showed that the highest magnesium content was observed in Sawney variety (33.78 mg/kg) followed by Ramsey variety (31.51 mg/kg), Saremna variety (26.17 mg/kg). Whereas, the lowest amount of magnesium content was recorded in variety Golsey (23.03 mg/kg).

4.3.4.2. Treatments combinations

Comparison among treatments showed that the combination T2 (available biomass + EM) showed the highest magnesium content (31.69 mg/kg) which was followed by treatments combination T1 (available biomass) which was (30.95 mg/kg), T4 (available biomass + EM + Madhyam) which was (27.14 mg/kg). Whereas the lowest magnesium content was recorded in treatments combination T3 (available biomass + Madhyam) with (24.70 mg/kg).

4.3.4.3. Interactions

Among the different interaction between varieties and treatments combinations variety Sawney in T1 (available biomass) showed the maximum magnesium content that is (40.53 mg/kg) which was followed by Sawney variety in T2 (available biomass + EM) with (38.71 mg/kg), Saremna variety in T1 (available biomass) with (36.14 mg/kg). However lowest magnesium was observed in Golsey variety in T1 (available biomass) that is (11.07 mg/kg).

Table No.4.6: Magnesium content (mg/kg) in the final compost

Varieties	T1 (Available biomass)	T2 (Available biomass + EM)	T3 (Available biomass + Madhyam)	T4 (Available biomass + EM + Madhyam)	Mean A
V1 (Sawney)	40.53	38.71	20.77	35.11	**33.78**
V2 (Golsey)	11.07	32.06	26.51	22.46	**23.03**
V3 (Ramsey)	36.06	32.52	28.52	28.93	**31.51**
V4 (Saremna)	36.14	23.46	23.01	22.07	**26.17**
Mean B	**30.95**	**31.69**	**24.70**	**27.14**	
Factors		**C.D (5%)**	**SE(d)**	**SE(m)**	
Factor(A)		**0.002**	**0.001**	**0.001**	
Factor(B)		**0.002**	**0.001**	**0.001**	
Factor(A X B)		**0.004**	**0.002**	**0.002**	

Note: A= Varieties and B= Treatments combinations.

4.3.5. Calcium (Ca) mg/kg

4.3.5.1. Varieties

The presence maximum of calcium content was observed from the Sawney variety (1.08 mg/kg) followed by Golsey variety (0.95 mg/kg), Ramsey variety (0.78 mg/kg) whereas the presence of minimum calcium content was obtain in the Saremna variety (0.74 mg/kg).

4.3.5.2. Treatments combinations

The calcium content of different treatments have been shown in the Table No.4.7. and data revealed highest calcium content was recorded in T2 (available biomass + EM) (0.95 mg/kg) followed by T1 (available biomass) which is (0.92 mg/kg), T4 (available biomass + EM + Madhyam) with (1.66 mg/kg). However,

lowest calcium content was recorded in T3 (available biomass + Madhyam) that is (1.48 mg/kg).

4.3.5.3. Interactions

The highest calcium content was observed in the interaction of Sawney in T2 (available biomass + EM) with (1.21 mg/kg) followed by Sawney in T1 (available biomass) i.e. (1.20 mg/kg), Golsey in T3 (available biomass + Madhyam) with (1.13 mg/kg), Sawney in T4 (available biomass + EM + Madhyam) with (1.11 mg/kg), Golsey in T2 (available biomass + EM) (1.06 mg/kg). Further, Ramsey variety in T1 (available biomass) which is (0.89 mg/kg) which was at par with Golsey in T4 (available biomass + EM + Madhyam) and Ramsey variety in T2 (available biomass + EM) i.e. (0.88 mg/kg). In addition, lowest calcium content was observed in Golsey variety in T2 (available biomass + EM) with (0.06 mg/kg).

Table No.4.7: Calcium content (mg/kg) in the final compost

Varieties	T1 (Available biomass)	T2 (Available biomass + EM)	T3 (Available biomass + Madhyam)	T4 (Available biomass + EM + Madhyam)	Mean A
V1 (Sawney)	1.20	1.21	0.81	1.11	**1.08**
V2 (Golsey)	0.72	1.06	1.13	0.88	**0.95**
V3 (Ramsey)	0.89	0.82	0.65	0.76	**0.78**
V4 (Saremna)	0.88	0.72	0.68	0.66	**0.74**
Mean B	**0.92**	**0.95**	**0.82**	**0.85**	
Factors		**C.D (5%)**	**SE(d)**	**SE(m)**	
Factor(A)		**0.005**	**0.003**	**0.002**	
Factor(B)		**0.005**	**0.003**	**0.002**	
Factor(A X B)		**0.010**	**0.005**	**0.004**	

Note: A= Varieties and B= Treatments combinations.

4.3.6. Iron (Fe) mg/kg

4.3.6.1. Varieties

The iron content of different varieties have been shown in Table No.4.8. The result of the present investigation showed that the highest iron content was observed in Ramsey variety (10.30 mg/kg) followed by Golsey variety (2.08 mg/kg), Sawney variety (1.17 mg/kg). Whereas, the lowest amount of iron content was recorded in Saremna variety (0.65 mg/kg).

4.3.6.2. Treatments combinations

Comparison among treatments showed that the combination T4 (available biomass + EM + Madhyam) showed the highest iron content (4.98 mg/kg) which was followed by treatments combination T3 (available biomass + Madhyam) which was (4.10 mg/kg), T2 (available biomass + EM) which was (30.90 mg/kg). However, the least iron content was found in treatments combination T1 (available biomass) with (1.23 mg/kg).

4.3.6.3. Interactions

Among the different interaction Variety Ramsey in T4 (available biomass + EM + Madhyam) showed the maximum iron content that is (15.11 mg/kg) which was followed by Ramsey variety in T3 (available biomass + Madhyam) with (13.01 mg/kg), Ramsey variety in T2 (available biomass + EM) with (10.81 mg/kg). However Sawney variety in T4 (available biomass + EM + Madhyam) i.e. (0.04 mg/kg) which was at par with Saremna variety in T3 (available biomass + Madhyam) with (0.01 mg/kg) which was the lowest among all the treatments combinations.

Table No.4.8: Iron content (mg/kg) in the final compost

Varieties	T1 (Available biomass)	T2 (Available biomass + EM)	T3 (Available biomass + Madhyam)	T4 (Available biomass + EM + Madhyam)	Mean A
V1 (Sawney)	0.02	2.24	1.25	1.19	**1.17**
V2 (Golsey)	1.24	1.40	2.11	3.57	**2.08**
V3 (Ramsey)	2.28	10.81	13.01	15.11	**10.30**
V4 (Saremna)	1.37	1.17	0.01	0.04	**0.65**
Mean B	**1.23**	**3.90**	**4.10**	**4.98**	
Factors		**C.D (5%)**	**SE(d)**	**SE(m)**	
Factor(A)		**0.01**	**0.005**	**0.004**	
Factor(B)		**0.01**	**0.005**	**0.004**	
Factor(A X B)		**0.02**	**0.01**	**0.007**	

Note: A= Varieties and B= Treatments combinations.

4.3.7. Manganese (Mn) mg/kg

4.3.7.1. Varieties

The manganese content of different varieties have been depicted in Table No.4.9. The result of the present investigation showed that the highest manganese content was observed in Ramsey variety (18.711 mg/kg) followed by Sawney variety (16.981 mg/kg), Saremna variety (13.431 mg/kg) whereas, the lowest amount of manganese content was recorded in variety Golsey (2.381 mg/kg).

4.3.7.2. Treatments combinations

Comparison among treatments showed that the combination T2 (available biomass + EM) showed the highest manganese content (14.263 mg/kg) which was

followed by treatments combination T4 (available biomass + EM + Madhyam) which was (14.140 mg/kg) whereas the least manganese content was recorded in treatments combination T3 (available biomass + Madhyam) with (9.803 mg/kg).

4.3.7.3. Interactions

Among the different interaction variety Ramsey in T2 (available biomass + EM) with (21.560 mg/kg) showed the maximum manganese content that is (21.200 mg/kg) which was followed by variety Sawney in T4 (available biomass + EM + Madhyam) with (20.990 mg/kg), variety Saremna in T1 (available biomass) with (20.943 mg/kg), Sawney variety in T1 (available biomass) with (20.653 mg/kg). On the other hand, lowest manganese was observed in variety Golsey in T1 (available biomass) that is (0.013 mg/kg).

Table No.4.9: Manganese content (mg/kg) in the final compost

Varieties	T1 (Available biomass)	T2 (Available biomass + EM)	T3 (Available biomass + Madhyam)	T4 (Available biomass + EM + Madhyam)	Mean A
V1 (Sawney)	11.585	20.990	14.150	21.200	**16.981**
V2 (Golsey)	0.013	1.980	2.190	5.340	**2.381**
V3 (Ramsey)	20.653	21.520	14.310	18.360	**18.711**
V4 (Saremna)	20.943	12.560	8.560	11.660	**13.431**
Mean B	**13.298**	**14.263**	**9.803**	**14.140**	

Factors	C.D (5%)	SE(d)	SE(m)
Factor(A)	**0.001**	**0.000**	**0.000**
Factor(B)	**0.001**	**0.000**	**0.000**
Factor(A X B)	**0.002**	**0.001**	**0.001**

Note: A= Varieties and B= Treatments combinations.

4.3.8. Boron (B) mg/kg

4.3.8.1. Varieties

The boron content of different varieties have been depicted in Table No.4.10. The result of the present investigation showed that the highest boron content was observed in Ramsey variety (0.008 mg/kg) it was found to be at par with Golsey variety (0.005 mg/kg), Ramsey variety (0.005 mg/kg) and Sawney variety (0.004 mg/kg).

4.3.8.2. Treatments combinations

Comparison among treatments showed that the combination T4 (available biomass + EM + Madhyam) showed the highest boron content (0.006 mg/kg) it was found to be at par with T2 (available biomass + EM), T3 (available biomass + Madhyam) which is (0.005 mg/kg) and T1 (available biomass) i.e. (0.004 mg/kg). Whereas the least boron content was recorded in treatments combination T1.

4.3.8.3. Interactions

Among the different interaction variety Ramsey in T3 (available biomass + Madhyam) and Ramsey in T4 (available biomass + EM + Madhyam) showed the highest boron content (0.010 mg/kg) which was followed by variety Sawney in T1 (available biomass), variety Ramsey in T2 (available biomass + EM) with (0.008 mg/kg) which are at par. On the other hand, lowest boron was observed in variety Saremna in T1 (available biomass) that is (0.002 mg/kg).

Table No.4.10: Boron content (mg/kg) in the final compost

Varieties	T1 (Available biomass)	T2 (Available biomass + EM)	T3 (Available biomass + Madhyam)	T4 (Available biomass + EM + Madhyam)	Mean A
V1 (Sawney)	0.008	0.002	0.003	0.005	**0.004**
V2 (Golsey)	0.002	0.004	0.003	0.004	**0.003**
V3 (Ramsey)	0.003	0.008	0.010	0.010	**0.008**
V4 (Saremna)	0.002	0.006	0.005	0.005	**0.004**
Mean B	**0.004**	**0.005**	**0.005**	**0.006**	
Factors		**C.D (5%)**	**SE(d)**	**SE(m)**	
Factor(A)		**0.001**	**0.000**	**0.000**	
Factor(B)		**0.001**	**0.000**	**0.000**	
Factor(A X B)		**0.001**	**0.001**	**0.000**	

Note: A= Varieties and B= Treatments combinations.

6.9. Molybdenum (Mo) mg/kg

4.3.9.1. Varieties

The molybdenum content of different varieties have been depicted in Table No.4.11. The result of the present investigation showed that the highest molybdenum content was observed in Sawney variety (0.003 mg/kg) which was found to be at par with (0.002 mg/kg) whereas the lowest amount of molybdenum content was recorded in Golsey and Ramsey variety i.e. (0.001 mg/kg).

4.3.9.2. Treatments combinations

Comparison among treatments showed that the combination T4 (available biomass + EM + Madhyam) showed the highest molybdenum content (0.003 mg/kg) was found to be at par with T1 (available biomass), T2 (available biomass + EM), T3 (available biomass + Madhyam) that is (0.001 mg/kg).

4.3.9.3. Interactions

Among the different interaction variety Sawney in T4 (available biomass + EM + Madhyam) showed the maximum molybdenum content that is (0.009 mg/kg) which was followed by variety Golsey in T1 (available biomass), variety Golsey in T2 (available biomass + EM), variety Saremna in T1 (available biomass), variety Saremna in T2 (available biomass + EM) and variety Saremna in T3 (available biomass + Madhyam) i.e. (0.002 mg/kg) was found to be at par with variety Sawney in T1 (available biomass), variety Sawney in T2 (available biomass + EM), variety Sawney in T3 (available biomass + Madhyam), variety Golsey in T3 (available biomass + Madhyam), variety Golsey in T4 (available biomass + EM + Madhyam), variety Ramsey in T1 (available biomass), variety Ramsey in T2 (available biomass + EM), variety Ramsey in T4 (available biomass + EM + Madhyam) and variety Saremna in T4 (available biomass + EM + Madhyam) with (0.001 mg/kg).

Table No.4.11: Molybdenum content (mg/kg) in the final compost

Varieties	T1 (Available biomass)	T2 (Available biomass + EM)	T3 (Available biomass + Madhyam)	T4 (Available biomass + EM + Madhyam)	Mean A
V1 (Sawney)	0.001	0.001	0.001	0.009	**0.003**
V2 (Golsey)	0.002	0.002	0.001	0.001	**0.001**
V3 (Ramsey)	0.001	0.001	0.000	0.001	**0.001**
V4 (Saremna)	0.002	0.002	0.002	0.001	**0.002**
Mean B	**0.001**	**0.001**	**0.001**	**0.003**	
Factors		**C.D (5%)**	**SE(d)**	**SE(m)**	
Factor(A)		**0.001**	**0.000**	**0.000**	
Factor(B)		**0.001**	**0.000**	**0.000**	
Factor(A X B)		**0.001**	**0.001**	**0.000**	

Note: A= Varieties and B= Treatments combinations**.**

4.3.10. Copper (Cu) mg/kg

4.3.10.1. Varieties

The copper content of different varieties have been depicted in Table No.4.12. The result of the present investigation showed that the highest copper content was observed in Ramsey variety (0.010 mg/kg) followed by Sawney variety, Saremna variety that is (0.008 mg/kg). Whereas, the lowest amount of copper content was recorded in Golsey variety (0.005 mg/kg).

4.3.10.2. Treatments combinations

Comparison among treatments showed that the combination T1 (available biomass) showed the highest copper content (0.010 mg/kg) which was followed by

treatments combination T3 (available biomass + Madhyam), T4 (available biomass + EM + Madhyam) which was (0.008 mg/kg). Whereas the least copper content was recorded in treatments combination T2 (available biomass + EM) with (0.005 mg/kg).

4.3.10.3. Interactions

Among the different interaction Sawney variety in T4 (available biomass + EM + Madhyam) showed the maximum copper content that is (0.013 mg/kg) which was followed by Ramsey variety in T1 (available biomass) with (0.012 mg/kg) and Sawney variety in T1 (available biomass) with (0.011 mg/kg). On the other hand, lowest copper was observed in Sawney variety in T2 (available biomass + EM) and Golsey in T2 (available biomass + EM) with that is (0.001 mg/kg).

Table No.4.12: Copper content (mg/kg) in the final compost

Varieties	**T1 (Available biomass)**	**T2 (Available biomass + EM)**	**T3 (Available biomass + Madhyam)**	**T4 (Available biomass + EM + Madhyam)**	**Mean A**
V1 (Sawney)	0.011	0.001	0.008	0.013	**0.008**
V2 (Golsey)	0.010	0.001	0.006	0.006	**0.005**
V3 (Ramsey)	0.012	0.010	0.009	0.009	**0.010**
V4 (Saremna)	0.009	0.009	0.008	0.006	**0.008**
Mean B	**0.010**	**0.005**	**0.008**	**0.008**	
Factors		**C.D (5%)**	**SE(d)**	**SE(m)**	
Factor(A)		0.001	0.001	0.000	
Factor(B)		0.001	0.001	0.000	
Factor(A X B)		0.003	0.001	0.001	

Note: A= Varieties and B= Treatments combinations.

4.3.11. Zinc (Zn) mg/kg

4.3.11.1. Varieties

The zinc content of different varieties have been illustrated at Table No.4.13. The result of the present study showed that the highest zinc content was observed in Saremna variety (5.86 mg/kg) followed by Saremna variety (2.52 mg/kg), Ramsey variety (1.27 mg/kg). On the other hand, the lowest was recorded in Golsey variety (0.15 mg/kg).

4.3.11.2. Treatments combinations

Comparison among treatments combinations showed that the combination T3 (available biomass + Madhyam) showed the highest zinc content i.e. (4.34 mg/kg) which was followed by treatments combination T2 (available biomass + EM) which was (3.10 mg/kg), T4 (available biomass + EM + Madhyam) which was (2.13 mg/kg). Whereas the least zinc content was recorded in treatments combination T1 (available biomass) with (0.01 mg/kg).

4.3.11.3. Interactions

Among the different interaction Sawney variety in T3 (available biomass + Madhyam) showed the maximum zinc content that is (9.22 mg/kg) which was followed by Saremna variety in T2 (available biomass + EM) with (7.84 mg/kg), Saremna variety in T3 (available biomass + Madhyam) with (7.69 mg/kg). However lowest zinc content was recorded in Golsey variety in T1 (available biomass) which is (0.01 mg/kg).

Table No.4.13: Zinc content (mg/kg) in the final compost

Varieties	T1 (Available biomass)	T2 (Available biomass + EM)	T3 (Available biomass + Madhyam)	T4 (Available biomass + EM + Madhyam)	Mean A
V1 (Sawney)	0.31	0.22	9.22	0.34	**2.52**
V2 (Golsey)	0.01	0.20	0.18	0.22	**0.15**
V3 (Ramsey)	0.36	4.14	0.29	0.31	**1.27**
V4 (Saremna)	0.23	7.84	7.69	7.66	**5.86**
Mean B	**0.23**	**3.10**	**4.34**	**2.13**	
Factors		**C.D (5%)**	**SE(d)**	**SE(m)**	
Factor(A)		**0.007**	**0.004**	**0.003**	
Factor(B)		**0.007**	**0.004**	**0.003**	
Factor(A X B)		**0.015**	**0.007**	**0.005**	

Note: A= Varieties and B= Treatments combinations.

4.3.12. Sodium (Na) mg/kg

4.3.12.1. Varieties

The sodium content of different varieties have been depicted in Table No.4.14. The result of the present investigation showed that the highest sodium content was found in Saremna variety (4.469 mg/kg) followed by Saremna variety (1.426 mg/kg), Ramsey variety (1.277 mg/kg). Whereas, the lowest amount of sodium content was recorded in Golsey variety (1.170 mg/kg).

4.3.12.2. Treatments combinations

Comparison among treatments showed that the combination T2 (available biomass + EM) showed the highest sodium content (3.181 mg/kg) which was

followed by treatments combination T1 (available biomass) which was (1.885 mg/kg) and T4 (available biomass + EM + Madhyam) which was (1.855 mg/kg). Whereas the least sodium content was recorded in treatments combination T3 (available biomass + Madhyam) with (1.421 mg/kg).

4.3.12.3. Interactions

Among the different interaction Saremna variety in T2 (available biomass + EM) showed the maximum sodium content that is (8.616 mg/kg) which was followed by Saremna variety in T4 (available biomass + EM + Madhyam) with (3.750 mg/kg), Saremna variety in T1 (available biomass) with (3.682 mg/kg). On the other hand, lowest sodium was observed in Saremna variety in T3 (available biomass + Madhyam) that is (1.421 mg/kg).

Table No.4.14: Sodium content (mg/kg) in the final compost

Varieties	T1 (Available biomass)	T2 (Available biomass + EM)	T3 (Available biomass + Madhyam)	T4 (Available biomass + EM + Madhyam)	Mean A
V1 (Sawney)	1.723	1.627	0.927	1.428	**1.426**
V2 (Golsey)	0.582	1.489	1.775	0.833	**1.170**
V3 (Ramsey)	1.552	0.993	1.155	1.410	**1.277**
V4 (Saremna)	3.683	8.616	1.829	3.750	**4.469**
Mean B	**1.885**	**3.181**	**1.421**	**1.855**	
Factors		**C.D (5%)**	**SE(d)**	**SE(m)**	
Factor(A)		**0.001**	**0.000**	**0.000**	
Factor(B)		**0.001**	**0.000**	**0.000**	
Factor(A X B)		**0.002**	**0.001**	**0.001**	

Note: A= Varieties and B= Treatments combinations.

4.3.13. Nickel (Ni) mg/kg

4.3.13.1. Varieties

The nickel content of different varieties have been depicted in Table No.4.15. The result of the present investigation showed that the highest nickel content was observed in Sawney variety (0.006 mg/kg) followed by Ramsey variety (0.005 mg/kg). Whereas, the lowest amount of nickel content was recorded in Golsey and Saremna variety (0.004 mg/kg).

4.3.13.2. Treatments combinations

Comparison among treatments showed that highest nickel content was found in T1 (available biomass), T2 (available biomass + EM) and T4 (available biomass + EM + Madhyam) with (0.005 mg/kg). Whereas the least nickel content was recorded in treatments combination T3 (available biomass + Madhyam) with (0.004 mg/kg).

4.3.13.3. Interactions

Among the different interaction Sawney variety in T4 (available biomass + EM + Madhyam) and Ramsey variety in T1 (available biomass) showed the maximum nickel content that is (0.008 mg/kg) which was followed by Sawney variety in T1 (available biomass), Sawney variety in T2 (available biomass + EM) and Golsey variety in T2 (available biomass + EM) with (0.006 mg/kg). On the other hand, lowest nickel was observed in Saremna variety in T4 (available biomass + EM + Madhyam) that is (0.003 mg/kg).

Table No.4.15: Nickel content (mg/kg) in the final compost

Varieties	T1 (Available biomass)	T2 (Available biomass + EM)	T3 (Available biomass + Madhyam)	T4 (Available biomass + EM + Madhyam)	Mean A
V1 (Sawney)	0.006	0.006	0.004	0.008	**0.006**
V2 (Golsey)	0.000	0.006	0.004	0.005	**0.004**
V3 (Ramsey)	0.008	0.005	0.004	0.005	**0.005**
V4 (Saremna)	0.005	0.004	0.004	0.003	**0.004**
Mean B	**0.005**	**0.005**	**0.004**	**0.005**	
Factors		**C.D (5%)**	**SE(d)**	**SE(m)**	
Factor(A)		**0.001**	**0.000**	**0.000**	
Factor(B)		**0.001**	**0.000**	**0.000**	
Factor(A X B)		**0.001**	**0.001**	**0.000**	

Note: A= Varieties and B= Treatments combinations.

4.3.14. Cobalt (Co) mg/kg

4.3.14.1. Varieties

The cobalt content of different varieties have been depicted in Table No.4.16. The result of the present investigation showed that the highest cobalt content was observed in Sawney variety and Golsey variety i.e. (0.005 mg/kg). Whereas, the lowest amount of cobalt content was recorded in Saremna variety i.e. (0.002 mg/kg) which was at par with Ramsey variety i.e. (0.003 mg/kg).

4.3.14.2. Treatments combinations

Comparison among treatments showed that the combination T1 (available biomass) and T2 (available biomass + EM) showed the highest cobalt content (0.004 mg/kg). Whereas the least cobalt content was recorded in treatments combination T3 (available biomass + Madhyam) and T4 (available biomass + EM + Madhyam) with (0.003 mg/kg).

4.3.14.3. Interactions

Among the different interaction Golsey variety in T2 (available biomass + EM) showed the maximum cobalt content that is (0.006 mg/kg) which was followed by Sawney variety in T1 (available biomass), Sawney variety in T4 (available biomass + EM + Madhyam), Golsey variety in T4 (available biomass + EM + Madhyam) and Sawney variety in T3 (available biomass + Madhyam) with (0.005 mg/kg). On the other hand, lowest cobalt was observed in Saremna variety in T4 (available biomass + EM + Madhyam), Ramsey variety in T4 (available biomass + EM + Madhyam), Ramsey variety in T3 (available biomass + Madhyam) and Saremna variety in T3 (available biomass + Madhyam) that is (0.002 mg/kg).

Table No.4.16: Cobalt content (mg/kg) in the final compost

Varieties	T1 (Available biomass)	T2 (Available biomass + EM)	T3 (Available biomass + Madhyam)	T4 (Available biomass + EM + Madhyam)	Mean A
V1 (Sawney)	0.005	0.004	0.005	0.005	**0.005**
V2 (Golsey)	0.004	0.006	0.004	0.005	**0.005**
V3 (Ramsey)	0.003	0.004	0.002	0.002	**0.003**
V4 (Saremna)	0.003	0.003	0.002	0.002	**0.002**
Mean B	**0.004**	**0.004**	**0.003**	**0.003**	
Factors		**C.D (5%)**	**SE(d)**	**SE(m)**	
Factor(A)		**0.001**	**0.000**	**0.000**	
Factor(B)		**0.001**	**0.000**	**0.000**	
Factor(A X B)		**0.001**	**0.001**	**0.000**	

Note: A= Varieties and B= Treatments combinations.

4.3.15. Carbon (C) %

4.3.15.1. Varieties

The carbon content of different varieties have been depicted in Table No.4.17. The result of the present investigation showed that the highest carbon content was observed in Golsey variety (4.33 %) followed by Ramsey variety (4.26 %), Sawney variety (4.25 %). Whereas, the lowest amount of carbon content was recorded in variety Saremna (4.08 %).

4.3.15.2. Treatments combinations

Comparison among treatments showed that the combination T4 (available biomass + EM + Madhyam) showed the highest carbon content (4.26 %) which was at

par with T2 (available biomass + EM) which was (4.25 %). Whereas the least carbon content was recorded in treatments combination T1 (available biomass) i.e. (4.18 %).

4.3.15.3. Interactions

Among the different interaction Variety Ramsey in T3 (available biomass + Madhyam) showed the maximum carbon content that is (4.77 %) which was followed by Golsey variety in T1 (available biomass) with (4.65 %), Sawney variety in T2 (available biomass + EM) and Sawney variety in T4 (available biomass + EM + Madhyam) with (4.62 %). On the other hand, lowest carbon was observed in Sawney variety in T3 (available biomass + Madhyam) that is (3.78 %).

Table No.4.17: Carbon content (%) in the final compost

Varieties	T1 (Available biomass)	T2 (Available biomass + EM)	T3 (Available biomass + Madhyam)	T4 (Available biomass + EM + Madhyam)	Mean A
V1 (Sawney)	3.97	4.62	3.78	4.62	**4.25**
V2 (Golsey)	4.65	4.35	4.20	4.11	**4.33**
V3 (Ramsey)	3.99	4.02	4.77	4.26	**4.26**
V4 (Saremna)	4.11	4.02	4.14	4.05	**4.08**
Mean B	**4.18**	**4.25**	**4.22**	**4.26**	
Factors		**C.D (5%)**	**SE(d)**	**SE(m)**	
Factor(A)		**0.004**	**0.002**	**0.001**	
Factor(B)		**0.004**	**0.002**	**0.001**	
Factor(A X B)		**0.008**	**0.004**	**0.003**	

Note: A= Varieties and B= Treatments combinations.

4.4 Physicochemical analysis of compost

4.4.1. Colour of compost

The colour of compost observed from all the varieties and its treatments combinations in different stages were mentioned in Table No.4.18 and Plate No.36.

4.4.1.1. First stage (Day 1)

In the first stage brown sugar colour (Hex:#B37b50) was observed at different varieties and its treatments combinations i.e. Sawney variety in T1 (Available biomass), Sawney variety in T3 (Available biomass + Madhyam), Sawney variety in T4 (Available biomass + EM + Madhyam), Golsey variety in T1 (Available biomass), Golsey variety in T3 (Available biomass + Madhyam), Ramsey variety in T1 (Available biomass), Ramsey variety in T2 (Available biomass + EM), Ramsey variety in T3 (Available biomass + Madhyam), Ramsey variety in T4 (Available biomass + EM + Madhyam), Saremna variety in T2 (Available biomass + EM), Saremna variety in T3 (Available biomass + Madhyam) and Saremna variety in T4 (Available biomass + EM + Madhyam). Camel colour (Hex:#C19a6b) was found at Sawney variety in T2 (Available biomass + EM), deer colour (Hex:#Ba8052) was observed at Golsey variety in T2 (Available biomass + EM), Light Taupe colour (Hex:#B68e65) was observed at Golsey variety in T4 (Available biomass + EM + Madhyam) and coconut (Hex:#9f643c) was observed at Saremna variety in T1 (Available biomass).

4.4.1.2. Second stage (Day 45)

In the second stage cologne earth brown colour (Hex:#613F2E) was observed at different varieties and its treatments combinations i.e. Sawney variety in T1 (Available biomass), Sawney variety in T3 (Available biomass + Madhyam), Sawney variety in T4 (Available biomass + EM + Madhyam), Golsey variety in T2 (Available biomass + EM), Ramsey variety in T1 (Available biomass), Ramsey variety in T3 (Available biomass + Madhyam), Saremna variety in T2 (Available biomass + EM), Saremna variety in T3 (Available biomass + Madhyam), Saremna variety in T4 (Available biomass + EM + Madhyam). Garnet colour (Hex:#763C2C) was observed at Sawney variety in T2 (Available biomass + EM), Golsey variety in T3 (Available biomass + Madhyam), Saremna variety in T1 (Available biomass). Chestnut colour (Hex:#8F432B) was observed at Golsey variety in T4 (Available biomass + EM + Madhyam), Ramsey variety in T2 (Available biomass + EM) and Ramsey variety in T4 (Available biomass + EM + Madhyam).

4.4.1.3. Third stage (Day 90)

In third stage darker sienna (Hex:#431811) was observed at different varieties and its treatments combinations are Sawney variety in T1 (Available biomass), Sawney variety in T3 (Available biomass + Madhyam), Golsey variety in T1 (Available biomass), Golsey variety in T3 (Available biomass + Madhyam), Ramsey variety in T1 (Available biomass), Ramsey variety in T2 (Available biomass + EM), Saremna variety in T2 (Available biomass + EM) and Saremna variety in T4 (Available biomass + EM + Madhyam).

Table No.4.18: Colour changes take place during the time of composting

Varieties	Treatments combinations	Day 1	Day 45	Day 90
V1 (Sawney)	T1 (Available biomass)	Brown Sugar Hex:#B37b50	Cologne Earth Brown Hex:#613F2E	Darker Sienna Hex:#431811
	T2 (Available biomass + EM)	Camel Hex:#C19a6b	Garnet Hex:#763C2C	Chocolate Brown Hex:#340006
	T3 (Available biomass + Madhyam)	Brown Sugar Hex:#B37B50	Cologne Earth Brown Hex:#613F2E	Darker Sienna Hex:#431811
	T4 (Available biomass + EM + Madhyam)	Brown Sugar Hex:#B37B50	Cologne Earth Brown Hex:#613F2E	Royal Brown Hex:#56342A
V2 (Golsey)	T1 (Available biomass)	Brown Sugar Hex:#B37B50	Garnet Hex:#763C2C	Darker Sienna Hex:#431811
	T2 (Available biomass + EM)	Deer Hex:#Ba8052	Cologne Earth Brown Hex:#613F2E	Darker Cola Hex:#432D25
	T3 (Available biomass + Madhyam)	Brown Sugar Hex:#B37B50	Garnet Hex:#763C2C	Darker Sienna Hex:#431811
	T4 (Available biomass + EM + Madhyam)	Light Taupe Hex:#B68e65	Chestnut Hex:#8F432B	Chocolate Brown Hex:#340006
V3 (Ramsey)	T1 (Available biomass)	Brown Sugar Hex:#B37B50	Cologne Earth Brown Hex:#613F2E	Darker Sienna Hex:#431811
	T2 (Available biomass + EM)	Brown Sugar Hex:#B37B50	Chestnut Hex:#8F432B	Darker Sienna Hex:#431811
	T3 (Available biomass + Madhyam)	Brown Sugar Hex:#B37B50	Cologne Earth Brown Hex:#613F2E	Chocolate Brown Hex:#340006
	T4 (Available biomass + EM + Madhyam)	Brown Sugar Hex:#B37B50	Chestnut Hex:#8F432B	Royal Brown Hex:#56342A
V4 (Saremna)	T1 (Available biomass)	Coconut Hex:#9f643c	Garnet Hex:#763C2C	Royal Brown Hex:#56342A
	T2 (Available biomass + EM)	Brown Sugar Hex:#B37B50	Cologne Earth Brown Hex:#613F2E	Darker Sienna Hex:#431811
	T3 (Available biomass + Madhyam)	Brown Sugar Hex:#B37B50	Cologne Earth Brown Hex:#613F2E	Darker Cola Hex:#432D25
	T4 (Available biomass + EM + Madhyam)	Brown Sugar Hex:#B37B50	Cologne Earth Brown Hex:#613F2E	Darker Sienna Hex:#431811

Chocolate brown colour (Hex: #340006) was found at Sawney variety in T2 (Available biomass + EM), Golsey variety in T4 (Available biomass + EM + Madhyam) and Ramsey variety in T3 (Available biomass + Madhyam). Royal brown colour (Hex:#56342A) was observed at different varieties and its treatments combinations are Sawney variety in T4 (Available biomass + EM + Madhyam), Ramsey variety in T4 (Available biomass + EM + Madhyam) and Saremna variety in T1 (Available biomass). Darker colour (Cola:#432D25) was found at Golsey variety in T2 (Available biomass + EM) and Saremna variety in T3 (Available biomass + Madhyam).

4.4.2. Texture of compost

The texture of compost observed from all the varieties and its treatments combinations in different stages were depicted in Table No.4.19. Coding techniques was used to determine the texture of compost in all the varieties and its treatments combinations in different stages. In the first stage (Day 1) all the varieties and its treatments combinations was recorded 1.

The texture of compost observed in the second stage (Day 45) at Golsey in T4 (Available biomass + EM + Madhyam) and Saremna T2 (Available biomass + EM) was recorded 6 which was highest among all the varieties and its treatments combinations. The texture at Sawney in T2 (Available biomass + EM), T3 (Available biomass + Madhyam) and T4 (Available biomass + EM + Madhyam), Golsey in T1 (Available biomass), T2 (Available biomass + EM) and T3 (Available biomass + Madhyam), Ramsey in T2 (Available biomass + EM), T3 (Available biomass + Madhyam) and T4 (Available biomass + EM + Madhyam), Saremna in T1 (Available

biomass), T3 (Available biomass + Madhyam) and T4 (Available biomass + EM + Madhyam) was recorded 5.

Table No.4.19: Texture changes take place during the time of composting

Varieties	Treatments combinations	Day 1	Day 45	Day 90
V1 (Sawney)	**T1 (Available biomass)**	1	4	9
	T2 (Available biomass + EM)	1	5	10
	T3 (Available biomass + Madhyam)	1	5	10
	T4 (Available biomass + EM + Madhyam)	1	5	10
V2 (Golsey)	**T1 (Available biomass)**	1	5	10
	T2 (Available biomass + EM)	1	5	10
	T3 (Available biomass + Madhyam)	1	5	10
	T4 (Available biomass + EM + Madhyam)	1	6	10
V3 (Ramsey)	**T1 (Available biomass)**	1	4	10
	T2 (Available biomass + EM)	1	5	10
	T3 (Available biomass + Madhyam)	1	5	10
	T4 (Available biomass + EM + Madhyam)	1	5	10
V4 (Saremna)	**T1 (Available biomass)**	1	5	9
	T2 (Available biomass + EM)	1	6	10
	T3 (Available biomass + Madhyam)	1	5	10
	T4 (Available biomass + EM + Madhyam)	1	5	10

In the third stage (Day 90) highest texture content was found at Sawney in T2 (Available biomass + EM), T3 (Available biomass + Madhyam) and T4 (Available biomass + EM + Madhyam), Golsey in T1 (Available biomass), T2 (Available

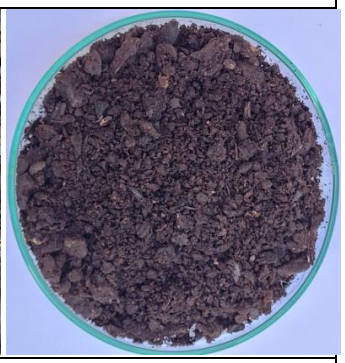
Treatments	Day 1	Day 45	Day 90
1. V1T1			
2. V1T2			
3. V1T3			
4. V1T4			

Plate No.36 : Colour and texture changes take place during the time of composting

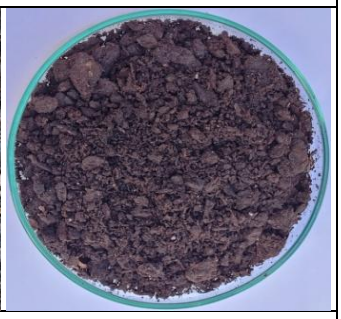
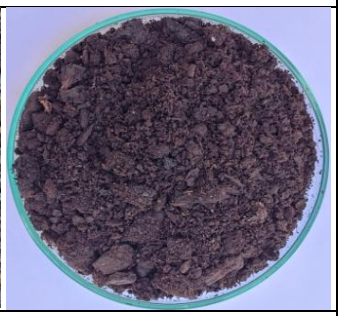
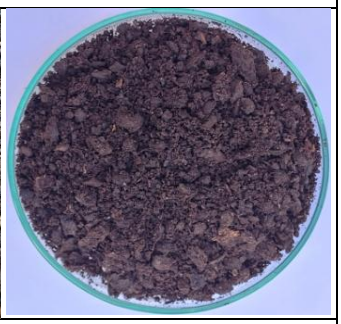
Treatments	Day 1	Day 45	Day 90
5. V2T1			
6. V2T2			
7. V2T3			
8. V2T4			
9. V3T1			

Plate No.36 : Colour and texture changes take place during the time of composting

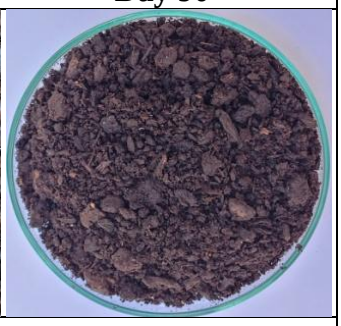
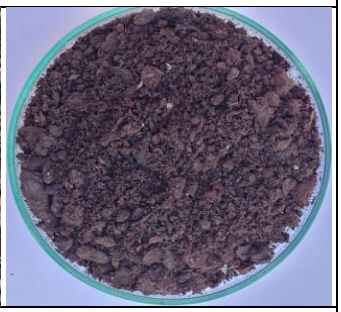
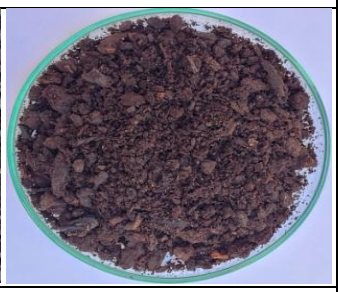
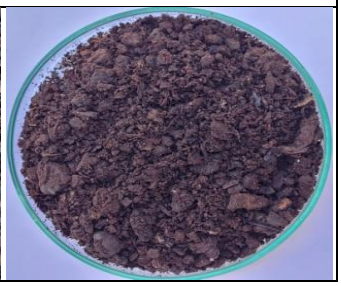
Treatments	Day 1	Day 45	Day 90
10. V3T2			
11. V3T3			
12. V3T4			
13. V4T1			
14. V4T2			

Plate No.36 : Colour and texture changes take place during the time of composting

Treatments	Day 1	Day 45	Day 90
15. V4T3			
16. V4T4			

Plate No.36 : Colour and texture changes take place during the time of composting

biomass + EM), T3 (Available biomass + Madhyam) and T4 (Available biomass + EM + Madhyam), Ramsey in T1 (Available biomass), T2 (Available biomass + EM), T3 (Available biomass + Madhyam) and T4 (Available biomass + EM + Madhyam), Saremna in T2 (Available biomass + EM), T3 (Available biomass + Madhyam) and T4 (Available biomass + EM + Madhyam) was recorded 10. On the other hand, lowest texture was observed at Sawney variety in T1 (Available biomass) and Saremna variety in T1 (Available biomass) that is 9.

4.4.3. Moisture of compost

4.4.3.1. Varieties

Highest moisture content was found in Golsey variety (48.033%) which was followed by Sawney (45.622%). Lowest moisture content was found in Saremna (41.263%) (Table No.4.20, Fig. No.19).

4.4.3.2. Treatments combinations

Comparison among treatments showed that the combination T2 (available biomass + EM) showed the highest moisture content (46.32%) which was followed by treatments combination T4 (available biomass + EM + Madhyam) which is (45.088%).

4.4.3.3. Interactions

The interactions between varieties and treatments combinations showed (49.695%) of moisture contain in variety Golsey with treatment combination of available biomass + EM which was found to be at par at Golsey variety in treatment combination available biomass + EM + Madhyam (49.663%).

Fig. No.19. Moisture content (%) in the compost

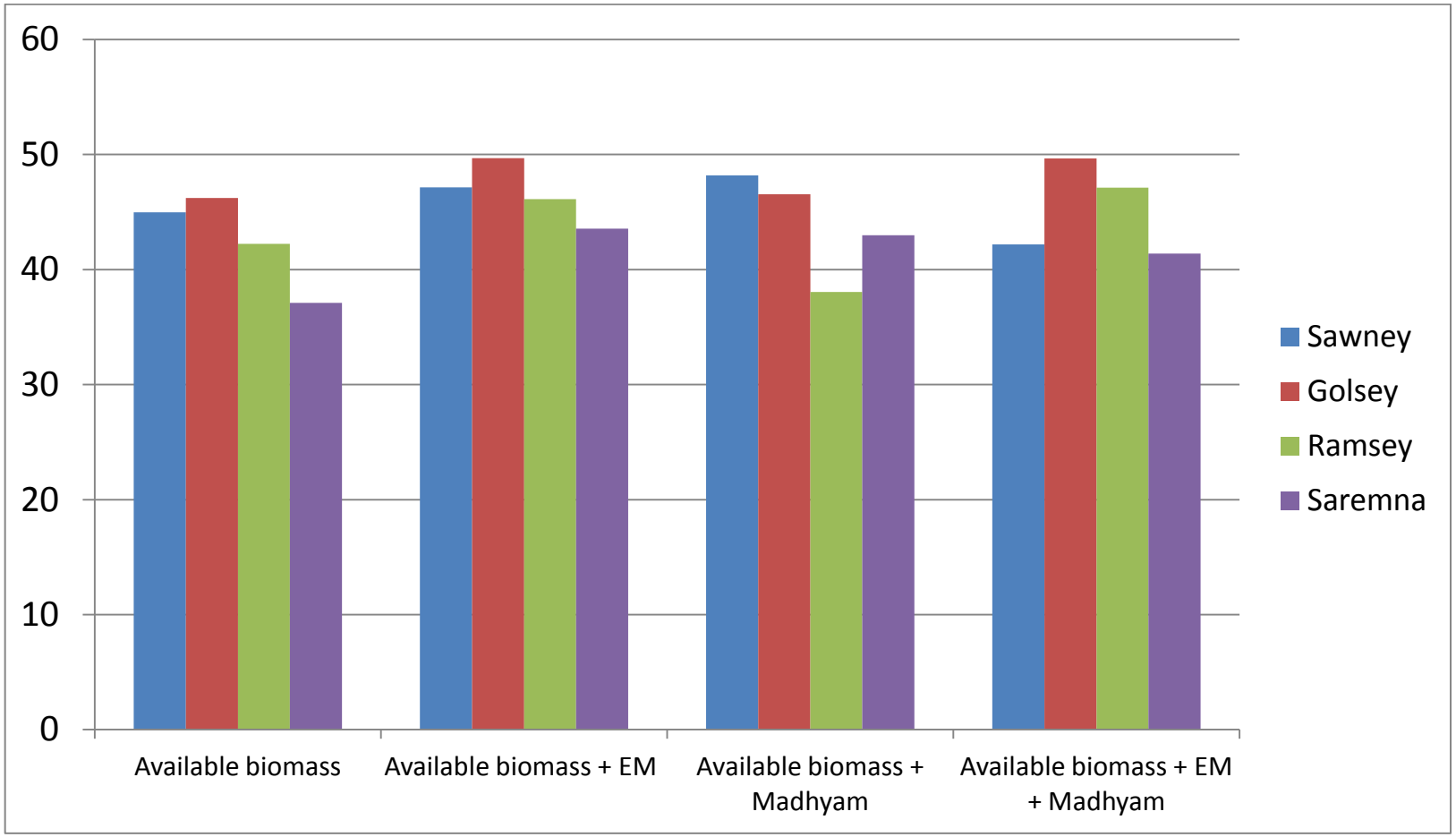


Table No.4.20: Moisture content in the compost (%)

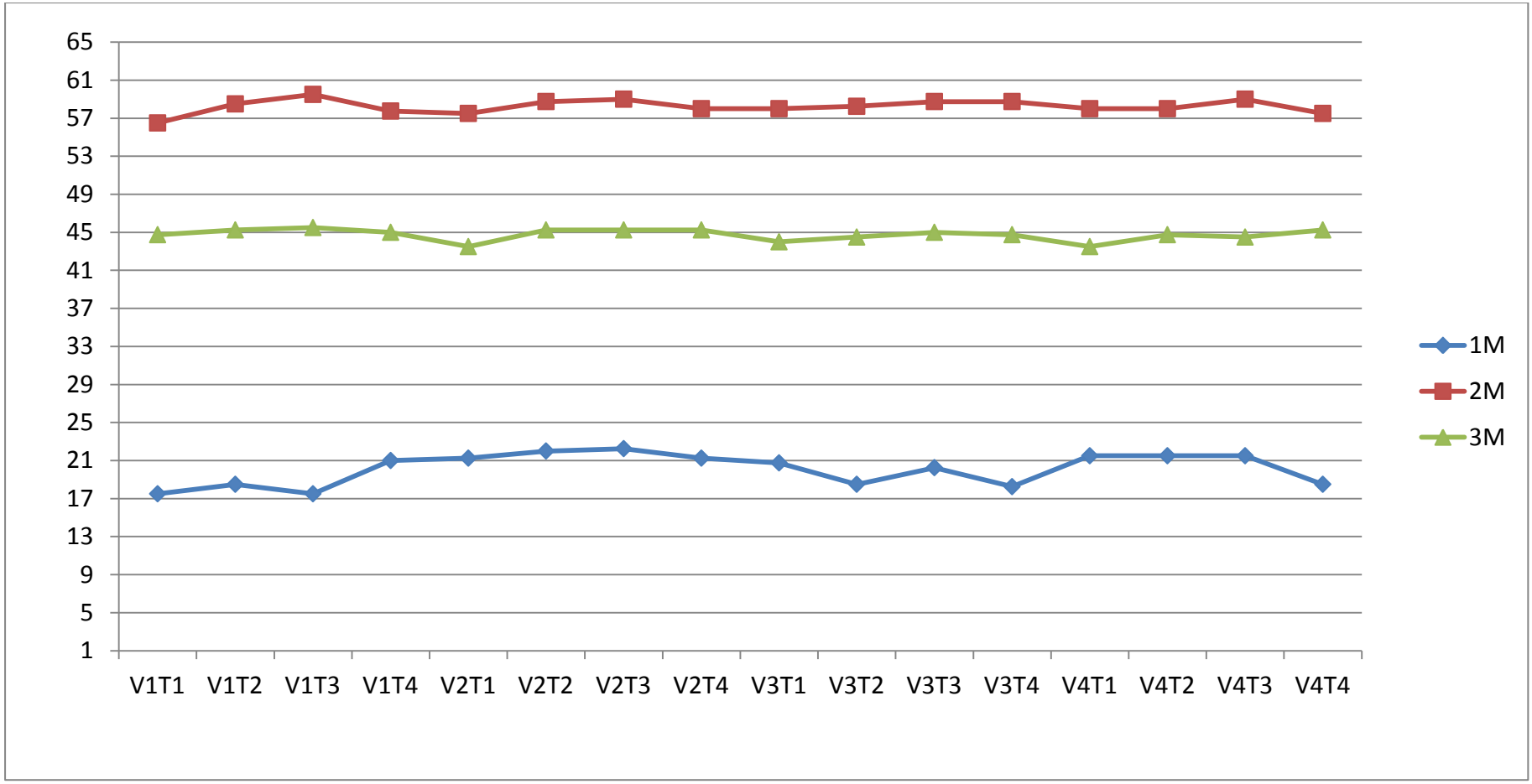
Varieties	T1 (Available biomass)	T2 (Available biomass + EM)	T3 (Available biomass + Madhyam)	T4 (Available biomass + EM + Madhyam)	Mean A
V1 (Sawney)	44.973	47.153	48.185	42.178	**45.622**
V2 (Golsey)	46.22	49.695	46.555	49.663	**48.033**
V3 (Ramsey)	42.24	46.12	38.053	47.113	**43.381**
V4 (Saremna)	37.108	43.56	42.985	41.398	**41.263**
Mean B	**42.635**	**46.632**	**43.944**	**45.088**	
Factors		**C.D (5%)**	**SE(d)**	**SE(m)**	
Factor (A)		**0.546**	**0.271**	**0.192**	
Factor (B)		**0.546**	**0.271**	**0.192**	
Factor (A X B)		**1.093**	**0.542**	**0.383**	

Note: A= Varieties and B= Treatments combinations.

4.4.4. Temperature

It was observed that the temperature at the beginning of compost preparation was around 20 ^{0}C in all treatments combinations. The temperature was increased to around 60 ^{0}C in the middle of the compost preparation (2nd month). However, towards the end of 3rd month at the time of final compost preparation the temperature decrease towards 45 ^{0}C on average in all treatments combinations (Fig. No.20).

Fig. No.20: Changes in temperature (^{0}C) during composting process



4.4.5. pH of compost

It was observed that the pH at the beginning of compost preparation was around 6 to7 in all treatments combinations. The pH was increased to around 7.5 in the middle of the compost preparation (2^{nd} month). However, towards the end of 3^{rd} month at the time of final compost preparation the temperature was 7 in all treatments combinations (Fig. No.21).

4.4.6. Electrical conductivity (EC) of compost

4.4.6.1. Varieties

Among the different varieties it was observed that Golsey (V2) showed the highest electrical conductivity (EC) that is 2.16 mS/cm which was followed by Saremna (V4) 1.88 mS/cm.

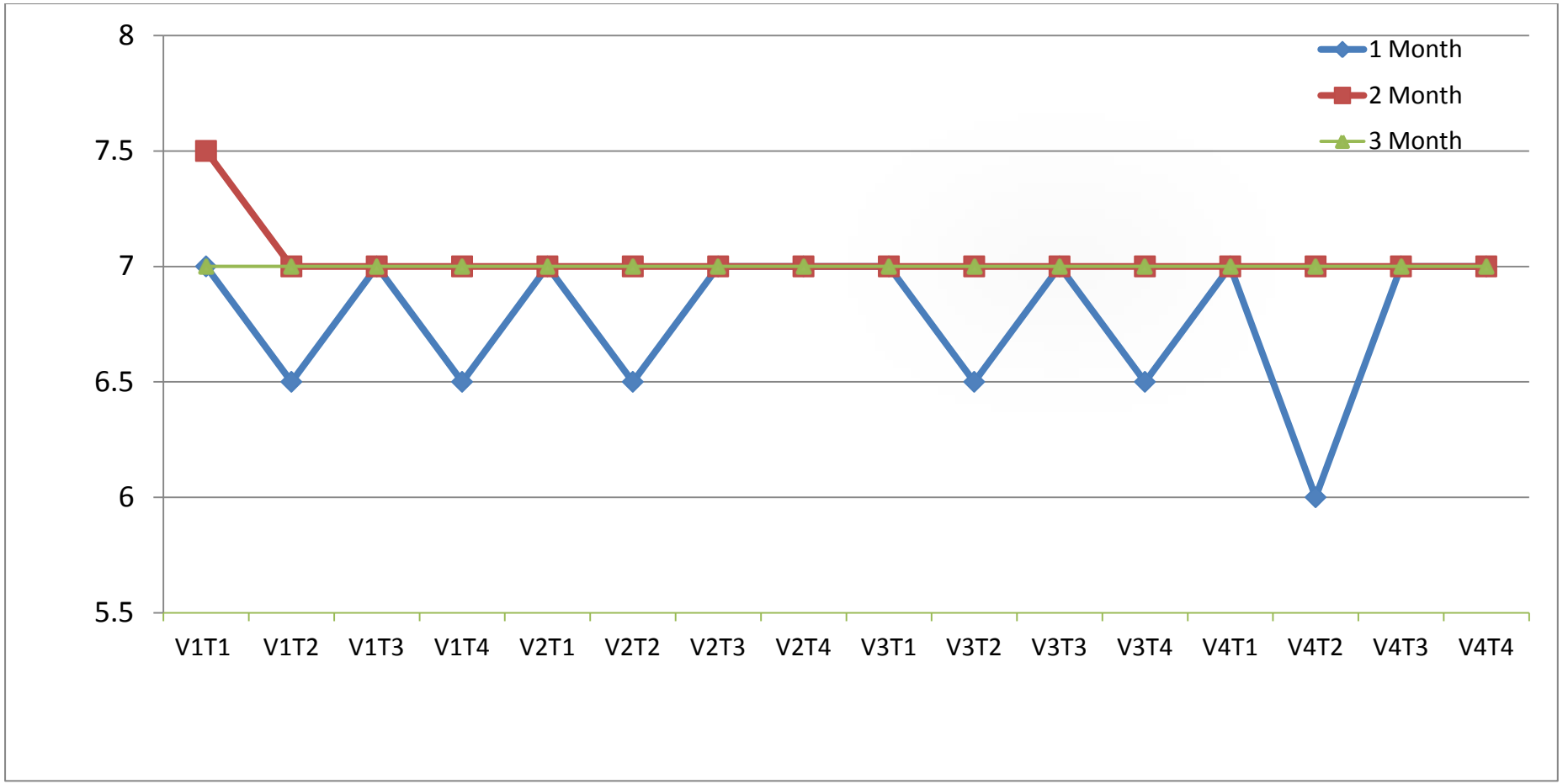
4.4.6.2. Treatments combinations

Treatments combination T4 (Available biomass + EM + Madhyam) that is 2.16 mS/cm being the highest EC followed by followed by T2 (Available biomass + EM) which is 1.90 mS/cm. On the other hand, lowest EC was recorded in T1 (Available biomass) with 1.53 mS/cm.

4.4.6.3. Interactions

Among the different interaction Golsey variety in T4 (Available biomass + EM + Madhyam) showed the highest EC content i.e. 2.55 mS/cm which was followed by Golsey variety in T3 (Available biomass + Madhyam) i.e. 2.29 mS/cm. In addition, the lowest EC content was detected in Sawney variety at T3 (Available

Fig. No.21: Changes in pH during composting process



biomass + Madhyam) which is 1.10 mS/cm. However, it was found to be at par with at Sawney variety at T3 (Available biomass + Madhyam) treatment combination which is 1.26 mS/cm (Table No.4.21.

Table No.4.21: Electrical conductivity (EC) (mS/cm) of the compost

Varieties	T1 (Available biomass)	T2 (Available biomass + EM)	T3 (Available biomass + Madhyam)	T4 (Available biomass + EM + Madhyam)	Mean A
V1 (Sawney)	1.22	1.82	1.10	1.59	**1.43**
V2 (Golsey)	1.57	2.25	2.29	2.55	**2.16**
V3 (Ramsey)	1.66	1.66	1.26	2.15	**1.68**
V4 (Saremna)	1.69	1.87	1.87	2.11	**1.88**
Mean B	**1.53**	**1.90**	**1.63**	**2.10**	
Factors		**C.D (5%)**	**SE(d)**	**SE(m)**	
Factor(A)		**0.10**	**0.05**	**0.04**	
Factor(B)		**0.10**	**0.05**	**0.04**	
Factor(A X B)		**0.20**	**0.10**	**0.07**	

Note: A= Varieties and B= Treatments combinations.

Chapter 5

DISCUSSION

The present investigation entitled **"Essential oils from different parts of Large Cardamom (*Amomum subulatum Roxb*.) and composting of available biomass"** was carried out in the Department of Horticulture, Sikkim University, 6th Mile, Tadong, Sikkim, during the year 2016-2019. Accordingly, the results obtained from the present investigation have been discussed with possible scientific causes along with the support of available literature under the following chapter.

5.1. Essential Oil

Large cardamom capsules contain 2-3% essential oil which is used as flavouring agent and spice (Gudade *et al*., 2013 and Vijayan *et al*., 2017). In the present study essential oil from capsules was recorded in a range of 2.71% to 1.90% (Table No. 4.1). The highest essential oil yield was observed in Golsey variety 2.71%. The essential oil yield was higher than Rout *et al*., 2003 reported 2.4% to 2.7% of essential oil and Gurudutt *et al*., (1996) obtained 2.4% essential oil. Essential oil can be utilised for pharmaceuticals, flavouring agent and cosmetic industries (Vijayan *et al.,* 2017). It was followed by Ramsey variety 2.11%, Saremna variety 2.01% whereas, lowest essential oil was recorded in Sawney variety 1.90. The essential oil yield was higher than reported by Vijayan *et al.*, (2017) in Sawney variety collected from Pangthang (Sikkim), Nalagand and Myanmar (Burma) i.e. 1.5%, 2.4% and 2.4% further in Ramsey variety from Upper Lingzey (East Sikkim) i.e. 2.8%. These variations in the essential oil yield may be due to different variety used, different geographical variations, i.e. climatic conditions, altitude etc. The essential oil content

in large cardamom possesses medicinal properties like stomachic, stimulant, astringent, alexipharmic and also has a typical characteristic flavour (Gautam *et al.,* 2016).

The highest essential oil from spike remains was recorded in Saremna variety 0.30% followed by Golsey variety 0.28%, Ramsey variety 0.25% whereas, lowest essential oil was recorded in Sawney 0.23%. Naik *et al.* (2003) reported 0.18% of essential oil yielded from large cardamom pericarp (husk) by Clevenger hydrodistillation methods. It is noticeable from the present investigation that essential oil yield from spike remains was higher than pericarp (husk). Spike remains is readily available as raw material for distilling essential oil and it is economically viable of oil production on the other hand, large cardamom pericarp is not easily available. Kapoor *et al.,* (2009) reported the essential oil and oleoresins of cardamom have a significant effect on shelf life of juice. However, large cardamom essential oil is nontoxic, safe for human health. Further, it can be used as alternative to chemical preservatives.

The variety Ramsey recorded highest essential oil from leaves 0.49% followed by Sawney and Golsey 0.38% further, least essential oil was obtained from Saremna variety 0.26%. Jaafar *et al.,* in 2007 reported 0.0735% essential oil from leaves of *Etlingera elatior* (Jack) R. M. Smith which belongs to the Zingiberaceae family. It is traditionally used as medicine and flavouring. likewise 0.09% essential oil was reported from the leaves of *Alpinia galanga* which is used in cosmetic products and applications in food flavouring (Jirovetz *et al.,* 2003). In the present study the yield of essential oil from large cardamom leaves was recorded significantly higher than the above mention species. The ethanolic extract of the large cardamom leaves showed significant antioxidant activity (Prakash *et al.,* 2012).

In the tillers highest essential oil was recorded in variety Sawney and Saremna 0.03% on the other hand, lowest essential oil was recorded in variety Golsey and Ramsey 0.02% (Table No.4.1, Fig. No.2). None of the earlier worker reported yield of essential oil from large cardamom tillers. Jirovetz *et al.,* 2003 reported similar kind of research in tillers of *Alpinia galanga* and found 0.1% essential oil. Further, earlier worker Jaafar *et al.,* in 2007 reported 0.0029% essential oil from the tillers of *Etlingera elatior* (Jack) R. M. Smith and monoterpenes hydrocarbons present as major compounds in the essential oils.

5.2. GC-MS analysis

Forty seven compounds were identified by GC-MS analysis in different parts of the large cardamom which contributed 2.49%-100.48% of total oil (Table No.4.2). In capsules (Sawney 35, Golsey 30, Ramsey 14 and Saremna 28) compounds, in spike remains (Sawney 18, Golsey 15, Ramsey 19 and Saremna 17) compounds were found. Further, the compounds present in the capsules and spike remains was higher than leaves and tillers. The number of compounds found in leaves were (Sawney 12, Golsey 6, Ramsey 19 and Saremna 5) whereas least compounds were found in tillers i.e. (Sawney 3, Golsey 6, Ramsey 3 and Saremna 3). The major constituents of the essential oils from the four cultivars of large cardamom are 1,8-cineole, α-terpineol, β-pinene, α-pinene, limonene, δ-terpineol, myrcene, δ-terpinene and α-thujene, Nerolidol, terpinnen-4-ol, Germacrene D, Spathulenol, α-Cadinol and Globulol.

The pungency in the large cardamom is due to the presence of high cineole content and terpenyl acetate contributes the pleasant aroma (Gautam *et al.*,2016). 1,8-cineole was a major compound content in the large cardamom oil which was reported by all the earlier workers (Bhandari, Joshi, Gurudutt, Gopal, Rout,

Gautam, Kumar and Vijayan etc.). The highest 1,8-cineole was found in the capsules of Ramsey variety (86.09%) and lowest was found in Sawney (54.7%). However, spike remains of Sawney variety contain highest 1,8-cineole (2.74%) and lowest in Saremna variety (0.11%) further, it was not found in the leaves and tillers. Mahmud *et al.,* (2008) analyzed essential oil of *Elettaria cardamomum* leaves by GC-MS and reported 25.74% 1,8-cineole. Whereas in the present study it was not found in the essential oil of large cardamom leaves. 1,8-cineole which has strong therapeutic properties, strong healing potential, anti-inflammatory, antibacterial, antioxidant and antiviral properties (Vijayan *et al.,* 2017). Bhandari *et al.,* (2013) analyzed essential oil of Sawney variety from Uttarakhand and reported 73.27% 1,8-cineole and Alam *et al.,* (2019) found 61.83% 1,8-cineole in Sawney variety, 72.5% 1,8-cineole in Saremna variety collected from Spice Board and ICAR, Tadong (Sikkim). Joshi *et al.,* (2012) also reported 50.55-60.46% 1,8-cineole in large cardamom oil collected from Himachal Pradesh. Kumar *et al.* (2012) collected large cardamom sample from Sikkim which was subjected to GC-MS analysis and reported 65.39% of 1,8-cineole. Vijayan *et al.,* (2017) reported 68.8% of 1,8-cineole in Sawney variety collected from Pangthang, Sikkim, 72.2 % 1,8-cineole in Sawney variety from Nagaland and 70.1% 1,8-cineole in Sawney variety from Myanmar (Burma). In the present study 54.7% 1,8-cineole was found in Sawney variety. Vijayan *et al.,* (2017) reported 69.5% of 1,8-cineole in Ramsey variety collected from Upper Lingzey (East Sikkim) where as in the present study it was found to be 86% (Ramsey variety) which was also highest among the other tested cultivars. However, the variations in the metabolite compounds may be attributed to different protocol used, different geographical variations, i.e. climatic conditions, altitude etc.

The highest α-terpineol was found in Sawney capsules (11.63%) and lowest was found in Ramsey capsules (2.46%). However it was not found in the other parts i.e. spike remains, leaves and tillers of large cardamom. Vijayan *et al.,* (2017) reported 6.5% of α-terpineol in Sawney variety collected from Pangthang, Sikkim, 6.2% α-terpineol in Sawney variety from Nagaland and 7.4% α-terpineol in Sawney variety from Myanmar (Burma). Kumar *et al.,* (2012) reported 10.15% α-terpineol, Alam *et al.,* (2019) found 7.16% α-terpineol in Sawney variety collected from Spice Board and ICAR, Tadong (Sikkim). In the present study 11.63% α-terpineol was found in Sawney variety which is higher than the all previous work. Further, 6.7% of α-terpineol in Ramsey variety collected from Upper Lingzey (East Sikkim) where as in the present study it was found to be 2.46% (Ramsey variety). The observed variations may be attributed to different protocol used, different geographical variations, i.e. climatic conditions, altitude etc. The chemical composition of oleoresins and the essential oil yield is affected by the nature of the solvent used for extraction and the production conditions (Singh *et al.,* 2008). α-terpineol present in essential oil of large cardamom showed antioxidant activity (Bisht *et al.,* 2011).

β-pinene was found highest in Sawney capsules (5.91%) and lowest in Ramsey capsules (4.43%). In spike remains highest β-pinene was found in Sawney (26.13%) and lowest was observed in Saremna (14.32%). The highest β-pinene was found in Ramsey leaves (1.96%) and lowest in Sawney leaves (1.96%). In tillers highest was found in Ramsey (1.89%) and lowest was found in Golsey (1.11%). Nike *et al.,* in 2004 found 13.6% α-pinene in the pericarp (husk) essential oil of large cardamom whereas it was in the same range as spike remains from the present study.

Nissen *et al.,* in 2010 reported α-pinene has broad-spectrum antibiotic activities and anti-inflammatory activities. 1,8-cineole, D-nerolidol, limonene, α-terpineol, β-pinene and α-pinene found in *A. subulatum* essential oil have antibacterial activity (Braca *et al.,* 2008, Nezhad *et al.,* 2009). The highest α-pinene was found in Golsey capsules (4.77%) and lowest was observed in Ramsey capsules (1.93%). In case of spike remains highest was observed in Sawney (4.43%) and lowest in Saremna (2.6%). In case of leaves highest was found in Golsey (0.37%) and lowest in Sawney (0.06%). In tillers highest was found in Ramsey (0.17%) and lowest in Golsey (0.13%) however it was not found in Sawney and Saremna variety. Vijayan *et al.,* (2017) found 4.7% of α-pinene in Sawney variety collected from Pangthang, Sikkim, 3.8% α-pinene in Sawney variety from Nagaland and 4.0% α-pinene in Sawney variety from Myanmar (Burma). However, in the present study the α-pinene content was found in a same range. Nike *et al.,* in 2004 reported 2.8% α-pinene in the pericarp (husk) essential oil of large cardamom which was in the same range with spike remains in the present investigation. 1.633% α-pinene was found in the essential oil of *Elettaria cardamomum* leaves which higher than the present study.

Bhandari *et al.,* (2013) seperated the components of the oil and found 4.2% limonene using GC-MS. whereas, Alam *et al.,* (2019) reported 2.58% limonene in Sawney Variety and 2.39% on Saremna variety. In the present investigation the highest limonene was found in the capsules of Saremna variety (2.99%) and lowest was found in Ramsey capsules (1.44%) Further, it was found in spike remains of Ramsey and Golsey varieties i.e (0.23%) followed by Sawney and Saremna varieties 0.26%, 0.22%. However it was not found in the other parts i.e. leaves and tillers of large cardamom. The variations in the limonene may be due to regional planting material and geographical variations.

There are some compounds which are found particularly in capsules oil of some verities such as ϒ-Terpinene 0.26% in Ramsey variety, 4-Thujanol 3.95% in Saremna variety, trans-Sabinene hydrate 0.25% in Saremna variety, β-Fenchol 0.07% in Golsey variety, Isoascaridole 0.11% in Sawney variety, limonen oxide 0.25% in Golsey variety, Germacrene B 0.55%, δ-Elemene 0.24% in Golsey variety, ϒ-Elemene 0.21% in Saremna variety, muurolol 0.18% in Golsey variety and longifolene 0.14% in Golsey variety.

5.3. Nutrient analysis

Fourteen elements were determined using IC-PMS from all the treatments viz. N, P, K, Mg, Ca, Fe, Mn, B, Mo, Cu, Zn, Na, Ni and Co.

Nitrogen is one of the essential macro elements required for successful plant growth and it is also important in the synthesis of some vitamins. It is part of the chlorophyll molecule which provide plants their green colouring matter (Emendu *et al.,* 2021). The nitrogen content in different treatments combinations was shows in the Table No.4.3. The present experiment data revealed that the nitrogen content ranged from 0.92% to 0.27%. The Saremna variety in T3 (available biomass + Madhyam) recorded the maximum nitrogen content that is (0.92 %) followed by Ramsey variety in T3 (available biomass + Madhyam) with (0.75 %), Golsey variety in T4 (available biomass + EM + Madhyam) with (0.71 %), Sawney variety in T3 (available biomass + Madhyam) (0.69 %), Ramsey variety in T2 (available biomass + EM) (0.64 %), Ramsey variety in T3 (available biomass + Madhyam) (0.63 %), Ramsey variety in T1 (available biomass) (0.60 %) and Sawney variety in T2 (available biomass + EM) (0.56 %) whereas the lowest nitrogen content was found at Golsey variety in T2 (available biomass + EM) which was (0.27 %). Khater in 2015 detected nitrogen

ranged from 0.95% to 1.68% which was prepared from cattle manure, herbal plants residues and sugar cane plants residues. Jusoh *et al.,* 2013 found nitrogen ranged from 0.64±0.07 in rice straw, 2.55±0.28 in goat manure and 2.90±0.35 in green manure. Sharma *et al.,* in 2014 reported nitrogen ranged from 2.01% to 0.03% whereas, the observed variation may be due to variation in raw material used for compost making, different microbial actions and other physicochemical factors.

The present experiment data revealed that the phosphorus content ranged from 0.70% to 0.13% are shows in the Table No.4.3. Phosphorus is one of the main three nutrients generally found in fertilizers. Plants required phosphorus to form the seedling stage through to maturity (Emendu *et al.*, 2021). The highest phosphorus content was observed from the Golsey variety in T3 (available biomass + Madhyam) 0.70% which was followed by Ramsey variety in T4 (available biomass + EM + Madhyam) that is (0.68 %), Saremna variety in T4 (available biomass + EM + Madhyam) that is (0.67 %), Saremna variety in T3 (available biomass + Madhyam) with (0.66 %) and Saremna variety in T2 (available biomass + EM) with (0.65 %). Whereas, the lowest phosphorus content was found at Sawney variety in T3 (available biomass + Madhyam) which was (0.13 %). Jusoh *et al.,* 2013 found phosphorus ranged from 0.21±0.07 in rice straw, 0.24±0.03 in goat manure and 0.18±0.04 in green manure. Khater in 2015 detected phosphorus ranged from 0.27% to 1.13% which was prepared from cattle manure, herbal plants residues and sugar cane plants residues. The increased in phosphorus was observed in the present study is probably due to mineralization and mobilization of phosphorus. Phosphorous is needed by plant to form the seedling stage to maturity stage and it is one of the main three nutrients which is most commonly found in fertilizers (Ansari 2012).

Potassium is one of the seventeen essential elements required by plants for reproduction and good growth. It improves crop quality, protein synthesis in plants and also enhances enzymes activation (Emendu *et al.,* 2021). Among the different interaction Sawney in T2 (available biomass + EM) showed the maximum potassium content that is (3.48 %) followed by Golsey variety in T1 (available biomass) with (3.37 %), Sawney variety in T4 (available biomass + EM + Madhyam) with (2.97 %), Ramsey variety in T4 (available biomass + EM + Madhyam) content (1.54 %) and Sawney in T1 (available biomass) with (1.55 %). Further, lowest potassium was observed in Saremna variety in T4 (available biomass + EM + Madhyam) that is (1.24 %) which is depicted in Table No.4.4. Emendu *et al.,* in 2021 reported potassium range 0.27% to 2.11% and Jusoh *et al.*,2013 found potassium range 1.12 ± 0.11 in rice straw, 1.81±0.15 in goat manure and 0.95±0.06 in green waste. In the present experiment potassium was found higher from Sawney in T2 (available biomass + EM) showed the maximum potassium content that is (3.48 %). However the variation in the potassium content may be due to microorganisms present in EM 1 and different raw materials used for making compost.

Magnesium is the central molecule in chlorophyll and it is the power house for photosynthesis in plants and also contributes to the plant's ionic balance (Emendu *et al.,* 2021). Among the different interaction between varieties and treatments combinations variety Sawney in T1 (available biomass) showed the maximum magnesium content that is (40.53 mg/kg) which was followed by Sawney variety in T2 (available biomass + EM) with (38.71 mg/kg), Saremna variety in T1 (available biomass) with (36.14 mg/kg). However lowest magnesium was observed in Golsey variety in T1 (available biomass) that is (11.07 mg/kg).

Calcium is a compound of plant cell walls which regulates cell wall construction. It is important for fruit and seed formulation in plants and cell elongation in both roots and shoots. It regulate nutrient transport and also support many enzyme functions (Emendu *et al.*,2021). The highest calcium content was observed in the interaction of Sawney in T2 (available biomass + EM) with (1.21 mg/kg) followed by Sawney in T1 (available biomass) i.e. (1.20 mg/kg), Golsey in T3 (available biomass + Madhyam) with (1.13 mg/kg), Sawney in T4 (available biomass + EM + Madhyam) with (1.11 mg/kg), Golsey in T2 (available biomass + EM) (1.06 mg/kg), Ramsey variety in T1 (available biomass) which is (0.89 mg/kg), Golsey in T4 (available biomass + EM + Madhyam) and Ramsey variety in T2 (available biomass + EM) i.e. (0.88 mg/kg). In addition, lowest calcium content was observed in Golsey variety in T2 (available biomass + EM) with (0.06 mg/kg).

Iron (Fe) is essential for chlorophyll synthesis and its deficiency results in chlorosis. Iron content ranged from 129.20±1.90 in rice straw, 1003.00±12.49 in goat manure and 145.20±1.70 in green manure (Jusoh *et al.*,2013). Among the different interaction variety Ramsey in T4 (available biomass + EM + Madhyam) showed the maximum iron content that is (15.11 mg/kg) followed by Ramsey variety in T3 (available biomass + Madhyam) with (13.01 mg/kg), Ramsey variety in T2 (available biomass + EM) with (10.81 mg/kg), Sawney variety in T4 (available biomass + EM + Madhyam) i.e. (0.04 mg/kg), Saremna variety in T3 (available biomass + Madhyam) with (0.01 mg/kg) which was the lowest among all the treatments combinations.

Manganese is an important plant micronutrient which activates some important enzymes involved in chlorophyll formation, also assist plants during respiration and nitrogen assimilation. Manganese deficient plants shows chlorosis

between the veins of its leaves (Emendu *et al.,* 2021). Ramsey in T2 (available biomass + EM) with (21.560 mg/kg) showed the maximum manganese content that is (21.200 mg/kg) which was followed by variety Sawney in T4 (available biomass + EM + Madhyam) with (20.990 mg/kg), variety Saremna in T1 (available biomass) with (20.943 mg/kg), Sawney variety in T1 (available biomass) with (20.653 mg/kg). On the other hand, lowest manganese was observed in variety Golsey in T1 (available biomass) that is (0.013 mg/kg).

Among the different treatments the variety Ramsey in T3 (available biomass + Madhyam) and Ramsey in T4 (available biomass + EM + Madhyam) showed the highest boron content (0.010 mg/kg) which was followed by variety Sawney in T1 (available biomass), variety Ramsey in T2 (available biomass + EM) with (0.008 mg/kg). On the other hand, lowest boron was observed in variety Saremna in T1 (available biomass) that is (0.002 mg/kg).

The present experiment data revealed that variety Sawney in T4 (available biomass + EM + Madhyam) showed the maximum molybdenum content that is (0.009 mg/kg) followed by variety Golsey in T1 (available biomass), variety Golsey in T2 (available biomass + EM), variety Saremna in T1 (available biomass), variety Saremna in T2 (available biomass + EM) and variety Saremna in T3 (available biomass + Madhyam) i.e. (0.002 mg/kg). However, lowest molybdenum content was observed at variety Saremna in T4 (available biomass + EM + Madhyam) with (0.001 mg/kg).

In the present study among the different interaction Sawney variety in T4 (available biomass + EM + Madhyam) showed the maximum copper content that is (0.013 mg/kg) which was followed by Ramsey variety in T1 (available biomass) with

(0.012 mg/kg) and Sawney variety in T1 (available biomass) with (0.011 mg/kg). On the other hand, lowest copper was observed in Sawney variety in T2 (available biomass + EM) and Golsey in T2 (available biomass + EM) with that is (0.001 mg/kg).

Zinc is an essential nutrient generally required by plant in lesser amount which helps in hormone production and internode elongation and also play role in controlling gene expression (Emendu *et al.*,2021). Jusoh *et al.,* in 2013 found zinc ranged from 38.40±2.20 in rice straw, 65.20±2.03 in goat manure and 64.30±0.51 in green manure. The present experiment data revealed that among the different interaction Sawney variety in T3 (available biomass + Madhyam) showed the maximum zinc content that is (9.22 mg/kg) followed by Saremna variety in T2 (available biomass + EM) with (7.84 mg/kg), Saremna variety in T3 (available biomass + Madhyam) with (7.69 mg/kg). However, lowest zinc content was recorded in Golsey variety in T1 (available biomass) which is (0.01 mg/kg).

Among the different interaction Saremna variety in T2 (available biomass + EM) showed the maximum sodium content that is (8.616 mg/kg) which was followed by Saremna variety in T4 (available biomass + EM + Madhyam) with (3.750 mg/kg), Saremna variety in T1 (available biomass) with (3.682 mg/kg). On the other hand, lowest sodium was observed in Saremna variety in T3 (available biomass + Madhyam) that is (1.421 mg/kg).

The present experiment data revealed that Sawney variety in T4 (available biomass + EM + Madhyam) and Ramsey variety in T1 (available biomass) showed the maximum nickel content that is (0.008 mg/kg) followed by Sawney variety in T1 (available biomass), Sawney variety in T2 (available biomass + EM) and Golsey

variety in T2 (available biomass + EM) with (0.006 mg/kg). Whereas, lowest nickel was observed in Saremna variety in T4 (available biomass + EM + Madhyam) that is (0.003 mg/kg).

The present experiment data revealed that Golsey variety in T2 (available biomass + EM) showed the maximum cobalt content that is (0.006 mg/kg) which was followed by Sawney variety in T1 (available biomass), Sawney variety in T4 (available biomass + EM + Madhyam), Golsey variety in T4 (available biomass + EM + Madhyam) and Sawney variety in T3 (available biomass + Madhyam) with (0.005 mg/kg). On the other hand, lowest cobalt was observed in Saremna variety in T4 (available biomass + EM + Madhyam), Ramsey variety in T4 (available biomass + EM + Madhyam), Ramsey variety in T3 (available biomass + Madhyam) and Saremna variety in T3 (available biomass + Madhyam) that is (0.002 mg/kg) which is depicted in Table No.4.16.

Carbon is a major component of organic molecules, these are the building blocks of all organisms further, it is the basic energy source for the composting process (Ansari *et al.,* 2012). The present experiment data revealed that variety Ramsey in T3 (available biomass + Madhyam) showed the maximum carbon content that is (4.77 %) which was followed by Golsey variety in T1 (available biomass) with (4.65 %), Sawney variety in T2 (available biomass + EM) and Sawney variety in T4 (available biomass + EM + Madhyam) with (4.62 %). On the other hand, lowest carbon was observed in Sawney variety in T3 (available biomass + Madhyam) that is (3.78 %).

5.4 Physicochemical analysis of compost

The colour of compost observed from all the varieties and its treatments combinations in different stages were mentioned in Table No.4.18 and Plate No.36. Karanja *et al.,* (2019) found much darker brown or black colours at 62nd day in all the composting materials with stable temperature and homogeneity of materials whereas in the present investigation found similar colours result. Gradual changes of the texture of the raw materials were observed after 30 days and the appearance of a black coloured humus-like substance observed during the final stage of composting. The optimum amount of decomposition was occurred during the final stage of composting process However, the moisture content was allowed to reduce after final stage. In the first stage brown sugar colour (Hex:#B37b50) was observed at different varieties and its treatments combinations i.e. Sawney variety in T1 (Available biomass), Sawney variety in T3 (Available biomass + Madhyam), Sawney variety in T4 (Available biomass + EM + Madhyam), Golsey variety in T1 (Available biomass), Golsey variety in T3 (Available biomass + Madhyam), Ramsey variety in T1 (Available biomass), Ramsey variety in T2 (Available biomass + EM), Ramsey variety in T3 (Available biomass + Madhyam), Ramsey variety in T4 (Available biomass + EM + Madhyam), Saremna variety in T2 (Available biomass + EM), Saremna variety in T3 (Available biomass + Madhyam) and Saremna variety in T4 (Available biomass + EM + Madhyam). Camel colour (Hex:#C19a6b) was found at Sawney variety in T2 (Available biomass + EM), deer colour (Hex:#Ba8052) was observed at Golsey variety in T2 (Available biomass + EM), Light Taupe colour (Hex:#B68e65) was observed at Golsey variety in T4 (Available biomass + EM + Madhyam) and coconut (Hex:#9f643c) was observed at Saremna variety in T1 (Available biomass).

In the second stage cologne earth brown colour (Hex:#613F2E) was observed at different varieties and its treatments combinations i.e. Sawney variety in T1 (Available biomass), Sawney variety in T3 (Available biomass + Madhyam), Sawney variety in T4 (Available biomass + EM + Madhyam), Golsey variety in T2 (Available biomass + EM), Ramsey variety in T1 (Available biomass), Ramsey variety in T3 (Available biomass + Madhyam), Saremna variety in T2 (Available biomass + EM), Saremna variety in T3 (Available biomass + Madhyam), Saremna variety in T4 (Available biomass + EM + Madhyam). Garnet colour (Hex:#763C2C) was observed at Sawney variety in T2 (Available biomass + EM), Golsey variety in T3 (Available biomass + Madhyam), Saremna variety in T1 (Available biomass). Chestnut colour (Hex:#8F432B) was observed at Golsey variety in T4 (Available biomass + EM + Madhyam), Ramsey variety in T2 (Available biomass + EM) and Ramsey variety in T4 (Available biomass + EM + Madhyam).

In third stage darker sienna (Hex:#431811) was observed at different varieties and its treatments combinations are Sawney variety in T1 (Available biomass), Sawney variety in T3 (Available biomass + Madhyam), Golsey variety in T1 (Available biomass), Golsey variety in T3 (Available biomass + Madhyam), Ramsey variety in T1 (Available biomass), Ramsey variety in T2 (Available biomass + EM), Saremna variety in T2 (Available biomass + EM) and Saremna variety in T4 (Available biomass + EM + Madhyam). Chocolate brown colour (Hex:#340006) was found at Sawney variety in T2 (Available biomass + EM), Golsey variety in T4 (Available biomass + EM + Madhyam) and Ramsey variety in T3 (Available biomass + Madhyam). Royal brown colour (Hex:#56342A) was observed at different varieties and its treatments combinations are Sawney variety in T4 (Available biomass + EM + Madhyam), Ramsey variety in T4 (Available biomass + EM + Madhyam) and

Saremna variety in T1 (Available biomass). Darker colour (Cola:#432D25) was found at Golsey variety in T2 (Available biomass + EM) and Saremna variety in T3 (Available biomass + Madhyam).

The texture of compost observed from all the varieties and its treatments combinations in different stages were depicted in Table No.4.19 and Plate No. 36. Coding techniques was used to determine the texture of compost in all the varieties and its treatments combinations in different stages. In the first stage (Day 1) all the varieties and its treatments combinations was recorded 1. The texture of compost observed in the second stage (Day 45) at Golsey in T4 (Available biomass + EM + Madhyam) and Saremna T2 (Available biomass + EM) was recorded 6 which was highest among all the varieties and its treatments combinations. The texture at Sawney in T2 (Available biomass + EM), T3 (Available biomass + Madhyam) and T4 (Available biomass + EM + Madhyam), Golsey in T1 (Available biomass), T2 (Available biomass + EM) and T3 (Available biomass + Madhyam), Ramsey in T2 (Available biomass + EM), T3 (Available biomass + Madhyam) and T4 (Available biomass + EM + Madhyam), Saremna in T1 (Available biomass), T3 (Available biomass + Madhyam) and T4 (Available biomass + EM + Madhyam) was recorded 5. In the third stage (Day 90) highest texture content was found at Sawney in T2 (Available biomass + EM), T3 (Available biomass + Madhyam) and T4 (Available biomass + EM + Madhyam), Golsey in T1 (Available biomass), T2 (Available biomass + EM), T3 (Available biomass + Madhyam) and T4 (Available biomass + EM + Madhyam), Ramsey in T1 (Available biomass), T2 (Available biomass + EM), T3 (Available biomass + Madhyam) and T4 (Available biomass + EM + Madhyam), Saremna in T2 (Available biomass + EM), T3 (Available biomass + Madhyam) and T4 (Available biomass + EM + Madhyam) was recorded 10. On the other hand,

lowest texture was observed at Sawney variety in T1 (Available biomass) and Saremna variety in T1 (Available biomass) that is 9. The texture in the final compost was found in the range 9 to 10 i.e. fine texture and much darker brown or black colours. Karanja *et al.*,(2019) found the fine texture at 62nd day in all the composting materials with stable temperature.

The interactions between varieties and treatments combinations showed (49.695%) of moisture contain in variety Golsey with treatment combination of available biomass + EM which was found to be at par at Golsey variety in treatment combination available biomass + EM + Madhyam (49.663%). Zakarya *et al.,* (2021) found moisture content in the range of 40% to 60% which was higher than the present study values.

Temperature is one of the best indicator to check the progress in composting process. Heat generated by composting destroys pathogenic organisms and weed seeds (Barker 1996). It was observed that the temperature at the beginning of compost preparation was around 20^0C in all treatments combinations. The temperature was increased to around 60^0C in the middle of the compost preparation (2^{nd} month). However, towards the end of 3^{rd} month at the time of final compost preparation the temperature decreases towards 45^0C on average in all treatments combinations (Fig. No.20). Karanja *et al.*,(2019) found the temperature range from 23^0C to 56^0C and 20^0C to 50^0C was reported by Zakarya *et al.,* (2021).

The optimal range of pH is from 5.2 to 7.3 (Khater 2015). It was observed that the pH at the beginning of compost preparation was around 6 to7 in all treatments combinations. The pH was increased to around 7.5 in the middle of the compost preparation (2^{nd} month). However, towards the end of 3^{rd} month at the time of final

compost preparation the pH was 7 in all treatments combinations (Fig. No.21). Khater in 2015 found the pH value ranged from 6.3 to 7.0, Ansari in 2012 reported pH in the range of 6.81 ± 0.18 which is in a similar range with the present study. Zakarya *et al.*, (2021) found pH in the range of 8 to 10 which was higher than the present study values. The alkaline condition can inhibit the growth of pathogen such as fungi that can live in acidic conditions and pH also determines the availability of manganese (Irvan *et al.,* 2018).

Electrical conductivity (EC) reflects the degree of salinity in the composting final product. Further it also reflects phototoxic, phyto-inhibitory effects on the growth of plants. Sharma *et al.*,(2014) found EC 2.56 mS/cm after 60 days of composting and the sample was Em-inoculated. The optimal range of EC values is <4mS/cm for mature compost. In the present study among the different treatments combinations Golsey variety in T4 (Available biomass + EM + Madhyam) showed the highest EC content i.e. 2.55 mS/cm which was followed by Golsey variety in T3 (Available biomass + Madhyam) i.e. 2.29 mS/cm. In addition, the lowest EC content was detected in Sawney variety at T3 (Available biomass + Madhyam) which is 1.10 mS/cm (Table No. 4.21). Khater in 2015 found the EC value ranged from 2.6 to 4.1 dS m-1 However, it was found in the same range. The EC range (2.0 to 4.0) is the optimum range for growing media. The EC range 2.55 to 1.10 mS/cm was found in the present study which comes under optimum range. However, the compost can be use as growing media.

Chapter 6

SUMMARY AND CONCLUSION

The present investigation entitled **"Essential oils from different parts of Large Cardamom (*Amomum subulatum Roxb*.) and composting of available biomass"** was carried out in the Department of Horticulture, Sikkim University, 6th Mile, Tadong, Gangtok, Sikkim, during the year 2016-2019. The observations were recorded with respect to assessing essential oil, quantification of different components present in essential oil from four major varieties of large cardamom, physicochemical analysis and nutrient estimate from the compost. The major outcome of the present investigation are being summarized as follows:

- This studies is indeed of much importance because all components of essential oil of four major varieties of large cardamom from Sikkim have been determined.
- The highest percentage of essential oil yield was obtained in the capsules of Golsey variety 2.71% followed by 0.30% from the Spike remains of Saremna variety, 0.49% from the leaves of Ramsey variety and 0.03% from the tillers of Sawney and Saremna varieties.
- As Sikkim is largest producer of large cardamom and as essential oil has great medicinal uses, the study has great importance regarding its industrial value.
- 47 compounds were identified by GC-MS analysis which contributed 94.49 %-100.48% of total oil from the four varieties of large cardamom.

- The major constituents of the essential oils from the four cultivars of large cardamom are 1,8-cineole, α-terpineol, β-pinene, α-pinene, limonene, δ-terpineol, myrcene, δ-terpinene and α-thujene, Nerolidol, terpinnen-4-ol, Germacrene D, Spathulenol, α-Cadinol and Globulol.

- Out of 47 components analysed it was found that 1,8-Cineole 86.09% in the capsules of Ramsey variety was the major component in the large cardamom oil.

- Among the varieties analyzed Golsey variety was found to be containing highest numbers of major metabolites.

- In the GC-MS analysis the number of components were highest in Sawney variety and the highest percentage of 1,8-cineole was found in Ramsey variety However, the present study may add some more economic and medicinal value to the crop.

- The comparative study among different varieties showed that Ramsey and Golsey varieties can be recommended to farmers for commercial cultivation as 1,8-cineole components, was highest in the essential oil which can be utilised for pharmaceuticals, flavouring agent and cosmetic industries.

- Fourteen elements were analyzed using IC-PMS from all the treatments viz. N, P, K, Mg, Ca, Fe, Mn, B, Mo, Cu, Zn, Na, Ni and Co.

- The prepared compost of all the varieties under study were observed to have nearly all macronutrients and micronutrients and it can be used for large cardamom field or any other crop or species as well.

- Beneficial nutrients like Nickel and Cobalt were also observed in the prepared compost.

- The texture in the final compost was found in the range 9 to 10 i.e. fine texture followed by the appearance of black colours.

- The optimal range of pH is from 5.2 to 7.3 (Khater 2015). It was observed that the pH at the beginning of compost preparation was around 6 to 7 in all treatments combinations. The pH was increased to around 7.5 in the middle of the compost preparation (2^{nd} month). However, towards the end of 3^{rd} month at the time of final compost preparation the pH was 7 in all treatments combinations.

- The EC range 2.55 to 1.10 mS/cm was found in the present study which comes under optimum range. However, the compost can be use as growing media.

- This study is very much beneficial for farming communities because instead of waste remains (leaves, tillers and spike remains) in large cardamom fields with their little efforts they would be get good compost which would be useful in further cropping.

Conclusion

Hence, based on the present major findings it can be concluded that the highest amount of essential oil content was obtained in the capsules of Golsey variety. Further, it was found to be highest than the essential oil estimated in any other variety of large cardamom so far. The essential oil content varied from 0.02% to 2.71% among different varieties and its parts used. The amount of essential oils extracted was higher than any earlier reporters. Hence, essential oil extractor could be

efficiently used for extracting essential oil from large cardamom. In addition, it may be concluded among all the collected varieties Golsey was superior in terms of essential oil. The study performed will largely benefit Sikkim farmers and compost prepared from large cardamom remains may be commercial also.

Chapter 7

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