MORPHOLOGICAL CHARACTERIZATION OF JAVA CITRONELLA VARIANTS AND ENCAPSULATION OF OIL FOR SLOW RELEASE

THESIS

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Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola
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By

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DECLARATION OF STUDENT

I hereby declare that the experimental work and its interpretation in the thesis entitled**"Morphological characterization of java citronella variants an encapsulation of oil for slow release"** or part thereof has neither been submitted for any other degree or diploma of any university, nor the data have been derived from any thesis / publication of any university or scientific organization. The source of material used and all assistance received during the course of investigation have been duly acknowledged.

Place: Akola **(Gangurde Anuja suklal)**

Date: / / 2017 **Enrolment No. NN-3166**

CERTIFICATE

This is to certify that thesis entitled **"MORPHOLOGICAL CHARACTERIZATION OF JAVA CITRONELLA VARIANTS AND ENCAPSULATION OF OIL FOR SLOW RELEASE"** Submitted in partial fulfillment of the requirement for the degree of **"Master of Science in Agriculture(Agricultural Biotechnology)"** of the Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola is a record of bonafide research work carried out by**Gangurde Anuja Suklal**under my guidance and supervision.

The subject of the thesis has been approved by the Students Advisory Committee

Place: Akola **(Dr. A. A.Akhare)**
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“Words provide wings to our ideas but not always can words express ourfeelings in words or languages”. No research work is possible alone, as it requiresmany minds and hands to beautify and to make it possible. Formal words cannotcarry the fragrance of emotions with them; still they are only available means ofexpressing emotions.

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(D) LIST OF ABBREVIATIONS

%	:	per cent
°C	:	Degree Celsius
µl	:	Micro liter
Cm	:	Centimeter
et al.	:	et alia (And others)
etc.	:	Etcetera
Fig.	:	Figure
G	:	Gram
G	:	gram
Ha	:	Hectare
Hr	:	Hour
i.e.	:	id est. (that is)
L	:	Litre
Mg	:	Miligram
Min		Minutes
Ml	:	Millilitre
N	:	Normal
NaOH	:	Sodium Hydroxide
v/v	:	Volume by volume
w/v	:	Weight by volume
в		beta
Φ	:	phi
ψ		psi
No	:	Number

F) THESIS ABSTRACT

a)	Title of the thesis	:	**MORPHOLOGICAL CHARACTERIZATION OF JAVA CITRONELLA VARINATS AND ENCAPSULATION OF OIL FOR SLOW RELEASE.**
b)	Full name of student	:	**Gangurde Anuja Suklal**
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ABSTRACT

The present investigation entitled “Morphological characterization of java citronella variants and encapsulation of oil for slow release” was carried out at Biotechnology Centre, Department of Agricultural Botany,

was studied at Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola in academic year 2016-2017.

The study was aimed to identify the high oil yielding *In vitro* mutagenized variants of java citronella along with their morphological description. The qualitative determination of oil constituents of each variant was carried out and oil profiling was done using TLC.The experiment was conducted using Randomize Block Design with three replication.

Morphological characterization of five selected variants of *in vitro* mutagenized plants along with 4 promising control genotypes of java citronella i.e Bio-13, jalpallavi, medini and mandakini was done. On the basis of various qualitative character such as plant height(cm), number of tillers, leaf width (cm), leaf blade length (cm), yield parameter such as oil yield (100g/ml), biomass (g), morphological characters such as steam colour characterization was done. All the five variants of *in vitro* mutagenized plants have performed well for characters like plant height, number of tillers, leaf blade length and biomass but the variant 4 and 5 performed best over control genotypes for the character such as for plant height variant 4 (118.40) cm and variant 5 (113.60) cm, number of tillers (59.40),(61.93), leaf blade length (88.86) cm, (90.73) cm, Biomass (4204.47)g, (4526.69)g. While for characters like leaf width the variants were small as compared to control Bio-13 (2.0) cm, jalpallavi (1.78)cm High oil yield was recorded in variant 4, 5 (1.38),(1.40), Bio-13 (1.45), jalpallavi (1.42) 100g/ml. steam colour had shown variation from as green, light green, green brown, dark brown in variants and control genotypes respectively.

Qualitative determination of oil constituents by thin layer chromatography was done by using different solvent system but among that Hexane : Ethyl acetate (80:20 v/v) has given best separation and identification of components .The components identified using this solvent system were geraniol with Rf value (0.77), (-)- citronellal (0.98), β-citronellol (0.80), (-)-β citronellol (0.81) withGreenish blue, Dark violet, Faint blue colour were obtained.

The Oil in water(o/w) type chitosan encapsulated volatile Citronella Oil microcapsules were prepared with various concentration of chitosan ranging from (0.5 percent to 1.5 percent) and NaOH concentration ranging from (0.5 percent to 1.5 percent). The significant formlulation of microcapsules were obtained at 1.5 percent chitosan and 1 percent NaOH.The encapsulation effeciency of 95.61 percent was observed with storage stability upto 25 days.

Chapter I

INTRODUCTION

1.1 Background Information

Java Citronella (*Cymbopogan winterianus)* is a grass belonging to Pocacea family which gives essential oil on steam distillation. The name *Cymbopogon* is derived from theGreek words “kymbe” (boat) and “pogon”(beard), referring to the specific flower spikearrangement. The java citronella is originated from Shri Lanka and in local language it is called as Mahapengeri. It is classified in trade into ceylon type obtained from *cympobogan nardus*(inferior type) and java type obtained from *cymbopogan wintreanus*(superior type).The Java citronella *(C. winterianus* Jowitt) is grown mainly in Java, Haiti, Honduras, Taiwan,Guatemala, and China, and is highly priced in comparison to the Ceylon typebecause its oil contains higher percentages of monoterpene alcohols and their esters.Citronella was first introduced in India in 1959 from Indoneasia (from java island hence name java citronella).India has been leading producer of essential oils including oil of citronella. In India, first successful cultivation of java citronella was done by Central Institute of Medicinal and Aromatic Plants (CIMAP) regional centre at Banglore in 1961,later on the crop was introduced in north-easterrn part of the country,now cultivation is done on large scale in the state of Assam, Meghalaya, Karnataka, Tamil naidu,Maharashtra, Uttar pradesh and various parts of India. Currently, Assam accounts for more than 80% of essential oil production in India. Areas receiving good and distributed rainfall throughout the year are suitable for cultivtion of citronella. Like palmrosa, this crop is hardy and adapts to a range of adverse soil conditions including moisture strees conditions.Cymbopogans are highly stress-tolerant plants that survive diverse edapho-climatic conditions prevailing in tropical and sub-tropical areas of Asia, Africa and America(Sangwan et al.2001;and Shasany et al.2000).

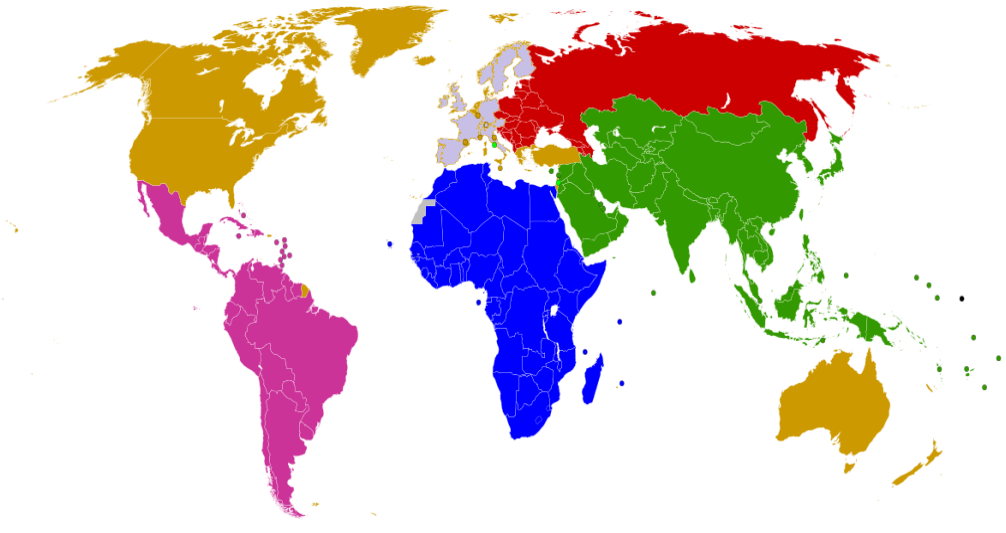
Although citronella resembles in appearance to lemon grass and palmrosa its clumps are more dense and compact. Citronella is an aromatic

perennial herb with fibrous roots. It is erect about 2m tall with smooth and shiny leaves.The leaf blades are linear and gradually tapering, long, membranous,acuminate and upto 1m long and 1.5 cm wide at two third of its length.The leaf sheath is smooth, yellow or purpulish red in rebranched axis.The plants are found to grow well under varying soil conditions but sandy loam soil with abundant organic matter is most suitable. Citronella thrieves well in a range of soil P^H ranging from 5.8-8.0. Heavy clay and sandy soils are not siutable as they do not support good growth.The crop is very sensetive to water-logging which shouldbe avoided to get optimum yields. It requires abundunt moisture and sun shine for its growth, High atmospheric humidity appears to influence the plant growth, yield and quality of oil favourably. The growth of citronella is reported to get restricted when grown on higher altitude above 400mresulting in lower yields(Chandra,1973).Addition of FYM and spentwash to the sodiac soil significantly increases herb and oil yield of java citronella (Prased and Patra,2004).

Viable seeds are not formed because of their irregularities in meosis and therefore these species can be propagated only by vegetative means for this, one year old or even older clumps are dug out and are split into slips, each containing 2 to 3 tillers. The Fibrous roots and leaves should be trimmed off the slips before planting.These slips are planted with onset of monsoon to ensure better establishment even without irrigation. However, July-August and February-March, have been found ideal time for planting.These slips are planted 8-12 cm deep into the soil maintaining a distance of 60x60 cm apart. Even a close spacing of 30x30 cm has been found beneficial in keeping the weed growth under check(Rao et al.1993).However, the most effective level of phosphorus has beenfound, to be 60kg/ha. The oil quantity also improved due to the applcation of fertilizer(Munsi and Mukherjee,1988).Citronella requires sufficient moisture for good growth and yields of leaves in the areas where humidity is high.There are two important diseases of citronella,which affect the growth of plant and oil production, these are leaf blight and anthracnose.The crop gets ready for first harvest after 9 months of planting.The crop is harvested

in the month of March, June and September.The crop flowers during October-November and the flowering stalks should be nipped off to discourage flowering.If the flowering stalks are allowed to grow, the plants will tend to age very soon and their life span may be reduced. The oil content of the leaves is affected by various factors, such as soil,climate,age of the plantation and method of efficiency of distillation. On an average, however, the oil content is about 1% on the basis of fresh weight of leaves.The yield of leaves may range from 15-20 tonnes per hectare. The yield of oil obtained during the 1st year is about 100-150kg per ha and in subsequent is years about 200 kg per ha.

1.2 Global citronella oil Producers: Region wise outlook



Source: Transparency market research and forecast 2016

Fig1: Map showing major contributing countries in citronella oil production.

The global citronella oil market is divided into seven regions namely North America, Latin America, Asia Pacific, Western Europe, Eastern Europe, Japan, and Middle east and Africa. Asia pacific holds the major share in the global citronella oil market owning the high production of essential oil for frangance and flavour industry and also share major

contribution in essential oil export.The major countries such as Shri-lanka, India, China, Java, Indoneasia, Taiwan tops the citronella oil market in terms of production in Asia pacific region followed by asia pacific is north america and europe and are expected to grow moderately high CAGR (compound annual growth rate) during the forecast period. Latin america is anticipated to project stable growyth rates in terms of value during forecast period.

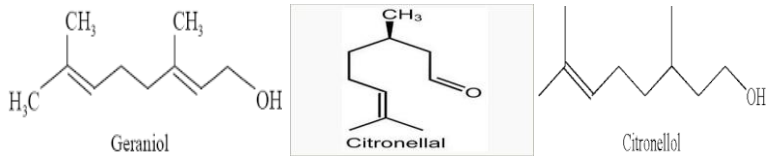
1.3 Citronella Oil Constituents

The industrial interest in essential oil is due to their applications as fragrances in perfumes, as flavour additives in food products or as pharmaceutical products and desirable repellent characteristics against mosquitoes (Katz et al .2008; Silva et al. 2011).

Citronella oil is rich in citronellal, geraniol and citronellol (Katiyar et al, 2011) but also have of other constituents like citronellyl acetate, L-limonene, ellemol and other sesquiterpene alcohols. It also consist of monoterpene constituents like citral, citronellol, citronellal, linallool, elemol, 1,8-cineole, limonene, geraniol, b-carophyllene, methyl hepteonene, geranyl acetate and geranyl formate.

In thetrade, citronella oil is classified into two chemotypes: Ceylon-type citronella oil and Java-type citronellaoils. Ceylon-type citronella oil is extracted from *C. nardus* Rendle, and Java-type citronella oilis obtained from *C. winterianus* Jowitt (Torres and Tio 2003). The main difference between the Ceylon and Java types of oil is the relative proportion of geranioland citronellal. Java-type citronella oil is characterized by a high proportion of geraniol (11%–13%)and citronellal (32%–45%), making it an important source of derivatives such as citronellol andhydroxycitronellal, which are extensively used in compounding high-grade perfumes.Ceylon-type citronella oil contains a relatively low proportion of geraniol (18%–20%) and citronellal(5%–15%), and is mainly used as such in lower-grade products. Citronella oil (both Ceylon and Java types) is also a renowned plant-based insect repellent, andhas been registered for use in the United States since 1948 as “McKesson’s Oil of Citronella” forhuman application to repel and mosquitoes. The U.S. Environment Protection Agency

(EPA)considers oil of citronella as a biopesticide with a nontoxic mode of action (Anonymous 1997, 2001,2004, 2006, 2007).



(a) (b) (c)

Fig. 2 Chemical structures ofmajor chemical components in citronella oil from Java citronella (a) geraniol (b) citronellal (c) citronellol.

1.4 Uses of citronella oil

Aromatic plants possess aromatic compounds used mainly as flavors (e.g. in spices and herbs) and fragrances(e.g. in perfumery, cosmetics, soaps) (Chomchalow et al., 2000).Citronella oil is commonly known for its natural insect repellent properties.It can be used as massage oil for aching joints and muscles.The oil can be effectively use in nebulizing or humidifying diffuser for its insect repellent properties. Chemicals like the geraniol, hydroxy citronellol and citronellal are some components of the java citronella used in the perfumery industry. Geraniol and citronellol function as antiseptics and are used in disinfectants. It is also used in soaps, perfumes, detergents and other household productsCitronella oil had been used as flavouring agent for food and beverages in very low quantities (Katz et al 2008). Citronella oil confined to be used as mosquito repellent,antiparasitic, nematicidal, antifungal and anti-bacterial agents. The repellent efficiciency of 38 essential oils against mosquito bites, including the species *Aedes aegypti* were found. Citronella oil was the most effective among other essential oils which provided 2 hours of repellency (Trongktokit et al 2005)

1.5 Microencapsulation and its role in slow release of essential oil

It can be defined as a process in which, small solid particles, liquid components or gaseous materials such as bubbles are coated by or entrapped within another inert shell material which isolates and protects the core material from environmental factors.The main advantages of

microencapsulation are protecting the susceptible and unstable materials from environmental factors, enhancing the processibility of them, controlling the release mechanism of the core materials by providing targetted and timed release, masking the undesired odor or taste, making it easier to handle the active compound by modifying the physical characteristics of it, providing a desired dilution or separating components from each other within a mixture (Kuang, Oliveira, & Crean, 2010; Ghosh, 2006; Desai & Park, 2005).

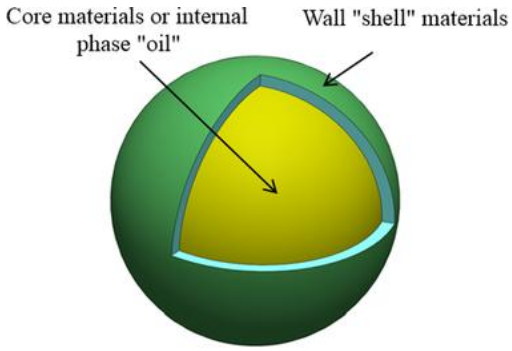


Fig 3 Composition of an oil microcapsule in simplified form.

Control release has been defined as a method by which one or more active ingredients or ingredients are made availale at a desired site and time at a specific rate(Pothakamury and Barbosa-Canovas 1995). Controlled release technology is used to deliver various comounds such as drugs, pestiscides, fragrances or Flavour at recommende rates, todether with improved effeciency and safety (Martins et al 2014a).

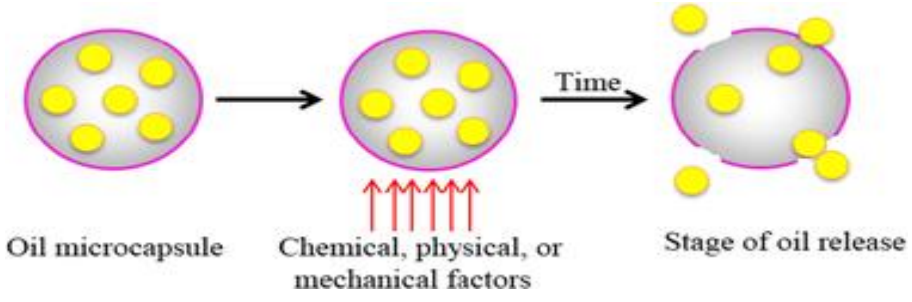


Fig 4 Schematic representation on control release mechanism

Various essential oils have insecticide, pesticide and/orpest repellent actions. The use of essential oils wasenhanced due to their eco-friendly and biodegradablenature. But their effect is limited due to their short-

termaction. The activity of the essential oils was enhanced byprolonging their release via microencapsulation techniques.The growing consumer demand for eco-friendly, highquality, and long-shelf life food products has made theresearchers to develop alternative methods of foodpackaging such as antioxidant, antimicrobial, and ediblefilms. Since essential oils are rich in these properties,bioactive films have been developed by encapsulatingvarious essential oils. Preservation of flavor for longer periods of time hasbeen a growing concern in food industries. Evaporation/spoilage of the active ingredients is the major factorfor the loss of flavor in foods. Microencapsulation isthe natural choice to preserve the flavor. The additional advantage with encapsulation is thatthe release of the active ingredients can be controlled. Though microencapsulation has been widely used forthe controlled release of flavor, the same principle canalso be applied to mask the flavor.

1.6 Importance of study

The demand for essential oil of citronella is increasing in the international market for its use in perfumery and cosmetic industry. Citronella is also focused as a crop for tribles which could gain them good returns. Government has provided subsidies for citronella cultivation and setting of oil production units. The involvement of large numbers of tribles, marginal and poor farmer in cultivation of citronella can give them good returns and will help in economic upliftment. But the percentage of oil yield level of the present genotypes is making this task difficult. Development of superior lines in terms of oil and biomass production in crops is a major thrust area of aromatic crops. *Cymbopogon winterianus* is the source of the high quality java citronella essential oil. The constituent of essential oil varies greatly in quantity from plant to plant in wild populations. The species of citronella are naturally cross pollinated and they are propagated vegetatively by means of slips. Therefore the population of citronella is highly heterozygous. Intra-clonal variations have also been reported in field grown populations (Nayak et al., 1996).

The present study provides availability of scientific information on its morphology and other characters that give quality to produce the lack of

improved varieties, determination of rapid technique for qualtitative analysis of oil components while use of microencapsulation technology in controlling the volatility and release properties of citronella oil.

1.7 Objectives of study

1. Morphological characterization of selected citronella variants.

2. Oil profiling with TLC of selected citronella variants.

3. Encapsulation of oil for slow release.

1.8 Hypothesis

Morphological characterization of java citronella will provide a set of morphological descriptors which can be used to identify the cultivar, while it wouldalso provide a basic information needed for crop improvementthrough various biotechnological approaches such as use of *in vitro* mutagenesis to create novel genotypes with desirable traits such high oil yields,screening and selection of agronomically usefulsomaclonal variants with high oil yieldand desirable quality, as well as detection of gross genetic changes can be done.

Citronella oil posses various components which are utilized by various flavour and fragrance, perfume industry thus thin layer chromatography can be utilized as easy and rapid technique for preliminary identification,qualitative analysis of oil components.

Citronella oil is volatile in nature thus micro-encapsulation technology shows great potential to deliver various compounds such as drugs, pestiscides, fragrance and flavours at recommended rates thus this technology can be utilize to decrease the volatility of oil and can be utilize as a functionalize control release mechanism.

1.9 Scope and Limitations

Citronella oil is one of the major essential oils.It has a rose like odour and bitter taste. It is used extensively as source of perfumery, soap,cosmetic and flavouring industry through the world.citronella oil is a raw material for production of geranial, citronellal, hydroxy-citronellal and other similiar high value perfumery bases. It is also widely used as starting

material for polishes, mosquito repellent, deodrants and sprays.The essential oil containing plants are playing a major role in commercial production of aromatic oils making india a major partner in the world scenario. It has become a more profitable business in agriculture produce and post harvest processing industry. Presently, India's position in world market is at top in the production of mints, grasses, spices, exotic flower, roots, and woody oil etc. However India is now facing stiff competition from other developing countries, in several commodities in quality and price.At present 300 to 500 tons of oil is produced in india for last 6 to 8 years in the state of Assam, Karnataka, Maharashtra, Tamil Naidu, West Bengal and Uttar Pradesh. According to FFDC (Fragrance and Flavour of Development Centre,Govt of India) the demand of citronella oil is 620 tonnes per year but the production of oil is 480 tonnes per year. India is facing deficiet of 140 tonnes oil per year (Verma et al 2015).

This study has now opened up a new direction for the study and exploration of citronella oil as major essential oil.

CHAPTER II

REVIEW OF LITERATURE

Java citronella (C.winterianus) is a perrinial, multiharvest aromatic grass, the shoot biomass of which, on steamdistillation, yields an essential oil extensively used in fragrance and flavour industries. Java citronella oil is one of the most important essential oils because of the high content of citronellal, which is converted into citronellol. Citronellol is further converted into citronellol esters, hydroxy citronellal and synthetic menthol (Dev kumar et al, 1977). C.winterianus oil finds use in providing scents and good smell tolow coast products such as soaps, sprays, disinfectants, polish and all kinds of technical preparation. Several products and formulation have been prepared using citronella oil (Chiopharma 1970; Kichiyoshi et al, 1981) for preventing thinner sniffing and slowing the relase of the rapidly evaporating substances into the atmosphere.

2.1 Morphological Characterization

Singh and Badolai (1980) studied response of Java citronella cultivars to nitrogen under Jorhat conditions. Their results revealed that the three citronella [Cymbopogon sp.] cvs Jorlab C-1, Jorlab C-2, Jorlab C-3 were examined. They received 0, 50, 100, 150, 200, 250 or 300 kg N/ha in each of two successive years. Herb and essential oil yields of Jorlab C-2 were somewhat higher than those of Bangalore and much higher than those of Jorhat. Yield and growth increased with N rates up to 200 kg.

Singh and Gupta (1983) studied genetic variability in clonal population of java citronella (*C. winterianus*). Results revealed that genotype variance varied from 0.02 for oil content to 519.17g for green yield and high heritability values were assosiated with high genetic advance.

Kole and Sen (1986) in *Cymbopogn winterianus* found that all the characters under study exhibited significant variation except leaf number per tiller. Morphological and genetic estimates of three parameters revealed leaf breadth to be the most promising character in terms of expected genetic gain in selection.It possessed that the highest value for

H(0.98) and G% of mean (55.47) coupled with high GCV(27.13). leaf length exhibited H(0.84), tiller number showed high GCV (21.88) while the highest GCV(30.76) and high genetic advance %of mean (51.44) were realized for oil yield.

Alam and janadhanan et al.,(1994) reported collor rot and wilt disease of java citronella. Their results revealed that causal organism for collar rot and wilt was *Fusarium moniliforme*, anamorph of *Gibberella fujikuroi.*Isolates of the pathogen originating from Lucknow and Pantnagar differed in their pathogenicity on the host plant under glasshouse conditions. There were also differences in growth rates, pigment production and sporulation between isolates.

Singh and Singh (1999) undertook the morphological characterization of 12 genotypes of lemongrass and observed considerable variability among the genotypes for all the morphological characters, except plant height. The maximum range of variability was observed for fresh weightof herb followed by oil yield and plant height and was minimum for oil content.

Sarma et al.,(2001) found thatJava citronella cultivated at an altitude of 60 m showed elevated biomass production (24.5 t/ha)and volatile oil content (1.3%). An increase in citronellal content and a decrease in citronellol andgeraniol content were observed at this altitude.

Sharma (2002) found variation in citronella oil and its major constituents due to seasonal change. Results revealed that rainfall, temperature, sunshine and relative humidity had cumulative effect on oil yield and its major constituents namely citronellal, citronellol, geraniol. Post monsoon months were seemed to be favourable, contributing highest oil yield. Citronellal content was higher during September (44.3%) and October (45.7%). They also observed that light rainfall (100 to 200 mm), moderate temperature (20-30^{0}C), sunshine hours of 5 to 6 hours and high humidity (90-95%) were favourable meterological parameters for higher oil yield and citronellal content in citronella oil. Growing period or stage of crop growth also had profound effect on the oil yield and citronellal content. Older crop with high matured leaves yielded higher oil and less citronellal.

Sharma et al., (2002) analyzed morphological characters of lemongrass duringdifferent growth phases. The study revealed that plant height, number of leaves per plant,number of tillers per plant, biomass production and oil yield per plant were found optimal atflowering stage. The morphological characters plant height, number of leaves per plant andnumber of tillers per plant were found to be correlated to essential oil yield.

Rocha et al., (2002) observed maximum production of citronella between 900 t/ha and 1100 t/ha. The least limonene content (1.17%) was observed in fresh leaves,. However, the highest limonene yield and content was achieved in plants harvestedduring winter. The content of citronellol in fresh biomass significantly decreased at 1500 t/ha during winter.

Rajeswara et al.,(2004) found that yellowing and crinkling disease influenced theessential oil yield and composition of citronella oil. The disease decreased biomassyield inthe first and second years of harvesting by 62.80 t/ha and 82.70 t/ha, respectively. Thecorrespondingdecrease in essential oil yield per plant were 62.80% and 79.00%.

Sarma et al.,(2005) studied morphological characters of lemongrass accessions andfound them to be different from each other. The leaf area of accession RLJ-TC-11 showedminimum difference inthe length of ligulae, auricle, awn and number of tillers per bush from each other.

Sakai et al., (2006) studied the variation of essential oil content in leaf and inflorescence of java citronella. Maximum essential oil accumulation was recorded at a crop age of 69 days during summer.

Gill et al.,(2007) standardized the harvest intervals for citronella grass Results revealed thatthe first cutting yielded 46.0, 44.6, and 28.5 L/ha of citronella oil at 75, 100 and125 days of harvesting intervals.

Blank et al.,(2007) found that the grass is cut at all times of the year, but the yield varies with season; it is highest during thehot period, and low during the wet season and flowering period. The highest fresh biomass yieldwas obtained during summer (9326 kg/ha), fall (8174 kg/ha), and spring (8352 kg/ha),and the lowest yield were recorded during winter

(3788 kg/ha). Seasons had significant effect on dry herbage biomass.Higher dry biomass production was achieved during fall and summer (5363 and 4897 kg/ha,respectively). A lower dry biomass production was obtained during winter and spring (1625 and3189 kg/ha, respectively); however, proportionally higher moisture was observed as61.82% and57.11%, respectively.

Reddy et al.,(2007) evaluated 842 accessions of proso millet germplasmconserved at the International Crops Research Institute for the Semi-Arid Tropics. Data wererecorded on various morphoagronomic traits such as time to 50% flowering, plant height,growth habit, culm branching, sheath pubescence, ligule pubescence, leaf pubescence, andinflorescence traits like inflorescence length. The characterization data revealed that dwarf plant height accessions were from Mexico and tall plant height accessions werefrom Sri Lanka. Good exsertion accessions were from Australia and China, and shorterpanicle accessions occurred in former USSR, while the longest was found in Nepal.

Eltahir and Abuereish,(2010) reported that *Cymbopogon citratus* is native to Srilanka and South India. The plant is widely cultivated in Tropical Asia and America. The plant is a tall (up to 1.8 m) rhizomatous, perennial Asian grass. Leaves are strap-like up to 2.5 cm wide, 90 cm long, bright bluish-green and glabrous. Inflorescence is 30-60 cm long and spikelets are subtended by spathes

Wanay et al., (2013) found chemical composition resulted in several other benefits apart from development of analytical methods for quality assessment. They found that immature grass had higher content of terpene hydocarbons than the mature ones and the wilting process was also necessary for the production of good quality oil. They also studied the therapeutic use of of citronella oil as an antifungal agent, antiparasitic agent, a potent mosquito repellent and antibacterial agent in addition they also gave the techniques led to develop the discovery of several possible varieties of citronella which consistently gave oils of composition different to either ceylon or java type.

Tewari and Mohan (2014) observed that cultivation of citronella grass is commercially done for its aromatic oil. They found that scientific method of cultivation give good oil yield, and the appropriate time of harvest gives good quality of oil. Aromatic oil obtained by method of steam distillation is useful in cosmetic industry, perfume, soaps, and detergents.

Verma et al (2015) studied effect of cropping pattern on growth, yield attribute and system productivity of citronella when intrcropped with mustard in central U.P. Their results revealed that on an average significantly higher herbage yield (87.08q/ha) was recorded in citronella sole cropping followed by 1:2 ratio of citronella+mustard crop. The higher B:C ratio was recorded under citronella sole (12.63), intercropping system citronella:mustard 2:2 row ratio (3.00) than other cropping system.

2.2 Thin layer chromatographic Analysis

Nigam and Levi (1964) had performed thin layer chromatography which has been applied to the analysis of essential oils and their constituents. R_f-data are given for terpene alcohols, esters and ketones. Vanillinsulfuric acid (5% w/v.) is used as spray reagent and sensitivity limits are reported for the compounds examined. Characteristic colorations observed were also recorded. Typical thin layer chromatograms are shown to illustrate the detection of sophistication of a commercial rose oil, the distinction between genuine palmarosa and gingergrass oils, and the recognition of botanical and/or geographical origins of authenticated spearmint and citronella oils.

Nigam and Levi (1965) studied detection and estimation of menthofuran in *Mentha arvensis* and other mint species bythin-layer chromatography. They found that the occurrence of means of coupled gas-liquid—thin-layer chromatography in genuine essential oilsmenthofuran in *Mentha arvensis*was reported for the first time. From 0.01 to 0.04 per cent of the heterocycle was determined by, the classical distinction between *Mentha piperita* (peppermint) and *M. arvensis* (mint), ostensibly based on the presence or absence of menthofuran, is therefore no longer justifiable. *Mentha spicata* L. cultivar common or native American spearmint, and *Mentha viridis*), pennyroyal (*Mentha pulegium* L. and *Hedeoma pulegioides*

(L.) Pers.), bergamot mint (*Mentha citrata* Ehrh.), *Mentha sylvestris* L., and *Mentha rotundifolia*, contained trace amounts of menthofuran.

Zschoke et al. (1998) used thin layer and high performance liquid chromatographic methods for a rapid and clear differentiation of morphologically similar umbelliferous drugs, namely *Angelica sinensis* (Oliv.) Diels, *A. pubescens* Maxim. *f. biserrata.* Their results revealed that A. pubescens, characterized by osthol and various angelol type coumarins, and *A. dahurica*, containing mainly linear furanocoumarins, are very easy to distinguish from *A. sinensis*, which contains *Z*-ligustilide as a major compound. On the other hand, very similar phthalides and phthalide dimers, as well as falcarindiol and coniferyl ferulate have been found in *A. sinensis, Ligusticum chuanxiong, L. porteri* and *Levisticum officinale*.

Joseph Sherma (1999) reviewed thin-layer chromatography in food and agricultural analysis. Various different techniques and its applications for a wide range of analyte and sample matrix types are covered, withspecification of the particular layers, mobile phases, detection methods and quantification.

Shrithran et al. (2010) carried out thin layer chromatography of essential oil from leaf, stem-bark and root-bark of the genus *Cinnamomum*.Wild *Cinnamomum* species such as *Cinnamomum capparucoronde*, *Cinnamomum citriodarum*, *Cinnamomum dubium*, *Cinnamomum litseaefolium*, *Cinnamomum ovalifolium*, *Cinnamomum rivulorm* and *Cinnamomum sinharajanse* were investigated, together with the cultivated cinnamonto identify their major chemical constituents.Results revealed that Cinnamaldehyde, eugenol, linalool, a-terpineol, acetyleugenol and cineole were primarily detected. There were some variations among the species in the main components of leaf and stem-bark oils. Major constituents in stem-bark oil were either cinnamaldehyde, linalool, α-terpineol, or eugenol. In oil extracted from the leaf, the principal constituents were either eugenol, linalool, terpineoland citronellal. The presence of citronellal in cinnamomum species was reported for the first time. All species examined had camphor as the major component in their root-bark oils.

Antony et al.(2012) studiedessential oilsunique ability to act as antioxidants. Antioxidant activities of 423 essential oils of 48 different botanical families were evaluated for their antioxidant activities as free radical scavenging agents using the 1,1-diphenyl-2-picrylhydrazyl method. Seventy-three oils showed 50% or more inhibition at a concentration of 1.25 mg/mL. The 73 most active oil samples were further evaluated for their scavenging activities using series of dilutions to estimate their EC_{50}. The EC_{50} of the 73 most active oils ranged from 4 to 2000 µg/mL. Oils having an EC_{50} of less than 300 µg/mL (20 selected samples) were subjected to β-carotene bleaching antioxidant activity test and more detailed analysis including thin layer chromatography Results revealed that Essential oils of the botanical families Lamiaceae and Myrtaceae were the most effective antioxidants. Thymol and carvacrol were the major constituents in most of the essential oils of the family Lamiaceae and eugenol was the major terpene in all of the essential oils of the family Myrtaceae.

Karpagam et al.(2016) studied bioactivity of lemongrass oil The purposeof their investigation was to examine the bioactivity of lemongrass oil with various enzymatic inhibition assays. Theirstudy targets where potentiality of lemongrass oil which could be the alternative approach for the treatmentof chronic disease such as diabetes etc. The total phenolic content of the essential oil was analyzed qualitativelyby thin layer chromatography and quantitatively by total phenolic content and ABTS assay.

Yadav and Paudel (2016) studied physico-chemical parameters andchemical composition of essential oil of Citronella. TLC was performed in total,fifteen compounds were identified by GCMS analysis and some of these major compounds were Nerol, Neral, Nerol acetate, Citronellol, Citronellal.

Rout et al. (2012) carried out an improved TLC separation and quantitative estimation of ursolic acid in different plant parts of various*Ocimum* species. Excellent separation of the components was achieved on high-performance precoatedTLC plates by using optimized

ternary mobile phase consisting of toluene: acetone:formic acid (7.8 : 2.2 : 0.15, v/v). Quantification anddensitometric determination were performed after derivatization of the plate with methanol-sulphuric acid reagent in reflection/absorptionmode at 540 nm.Ursolic acid was efficiently separated from the other components bythe proposed method which was very simple, rapid, precise, sensitive and accurate for the quantification of ursolic acid in different plantparts of *Ocimum* species. The maximum ursolic acid content was found in the stem.

Mohsen.A.EL Hassady (2014) foundTLC screening of volatile oil obtained from the aerial parts of *Rosemarimus officinalis*mixture.Investigation included comparison between thenumber of spots [number of the main volatilecompounds] , the colour after spraying with specific reagent [indicating the intensity of each volatile compound] and the retardation factor [Rf values] foreach colored spot in the chromatogram both in day and under UV light for each treatment. This study revealedthat most examined samples showed six main volatile components having nearly the same Rf values.

Igbokwe etal., (2015) studied physicochemical properties of the volatile oils from the aerial parts of *Lantana camara, Hyptis suaveolens,*and *Cleome viscose*by Thin layer chromatography. Their results showed the presence of such compounds as γ-Ionone, Piperitone, andCitronellal.

Ibrahim et al., (2015)performed TLCof clove volatile oilwith standard sample compression, (Rf) of clove volatile oil reached to 0.59, these resultswere equal to the Rf of standard compound.

2.3 Encapsulation of Oil for Slow Release

Wong etal., (2005) found citronella to be effective in deterring the infestation of cartons containing muesli and wheat germ by red flour beetles, citronella-treated cartons (0.2 g/m^2 of carton board). Results revealed reduced beetle infestation to approximately 50% of the level observed in control cartons. Evidence was provided to indicate that an insect repellent effect persists for at least 16 weeks. Additional work on the

controlled release of the insect repellent would be required to prolong the effect.

Hsieh et al., (2006) studied to prepare the O/W type chitosan encapsulated volatile citronella oil microcapsules. The results suggest that the forming of microcapsules should be processed under the fundamental conditions of: (1) the concentration of chitosan is at least 0.2 wt%, (2) NaOH is greater than 0.1 wt%, and (3) with the additive of coconut oil as natural surfactant, so that we could obtain final product of microcapsules with better formation and dispersion. The changes in concentration of chitosan will affect the encapsulation efficiency of the volatile citronella Oil. When the concentrations of chitosan were 0.5%, 1.0% and 1.5%, the encapsulation efficiencies were 98.2%, 95.8% and 94.7%, respectively.

Baranauskienėetal., (2007) evaluated the effect of different commercial modified food starch carrier materials on the flavor retention of the essential oil (EO) of peppermint (*Mentha piperita* L.) during spray drying and storageresults revealed that the emulsification and encapsulation efficiencies of peppermint EO were higher for all *n*-octenyl succinic anhydride (OSAN)-modified starches as compared to those of hydrolyzed starches (dextrins). Their results revealed that compositions of pure, emulsified, and encapsulated peppermint EOs in different matrices were quite similar; however, some changes in the percentages of some individual compounds were observed. Larger differences in the compositions of surface oils from various encapsulation products were obtained.

Sakulkuetal., (2009) tried to reduce the rate of evaporation of the oil via microencapsulation. The effects of three variables, stirring rate, oil loading and the amount of cross-linking agent, on encapsulation efficiency (EE, %) were studied response surface methodology was employed to optimize the EE. The results showed that the MCs under the optimized conditions provided EE of 60%. The optimized MCs were observed to have a sustained *in vitro* release profile (70% of the content was released at the 10th hour of the study) with minimum initial burst effect. Microencapsulation decreased membrane permeation of the CO by at least 50%. The amount

of CO permeated was dependent on the type of ointment base used. PEG base exhibited the highest degree of release. Therefore, microencapsulation reduces membrane permeation of CO while maintaining a constant supply of the oil.

Leimann et al., (2009) studiedmicroencapsulation by simple coacervation. Depending on the experimental conditions, microcapsules in the range of 10 μm to 250 μm were obtained. Microcapsules presenting no agglomeration were obtained when SDS at 0.03 wt% were used. The composition and the antimicrobial properties of the encapsulated oil were determined, demonstrating that the process of microencapsulation did not deteriorate the encapsulated essential oil.

Specoset al., (2010) prepared microcapsules containing citronella essential oilprepared by complex coacervation and applied to cotton textiles in order to study the repellent efficacy of the obtained fabrics. Their results suggest thatfabrics treated with microencapsulated citronella presented a higher and longer lasting protection from insects compared to fabrics sprayed with an ethanol solution of the essential oil, assuring a repellent effect higher than 90% for three weeks.

Zhiyi etal., (2010) prepared the microcapsules with cores of ethylenediamine tetraacetic acid tetrasodium salt (Na_4-EDTA) and walls of polyurea were synthesized *via* an interfacial polycondensation reaction with 2,4-tolylene diisocyanate as an oil-soluble monomer and diethyl triamine as a water-soluble monomer results revealed that the mean diameter of optimal microspheres was approximately 6 μm, and microcapsules were spherical, the Na_4-EDTA release profiles were biphasic with a burst release followed by a gradual release phase. The optimal synthesis conditions for the microcapsules with stable, good morphology and good controlled-release properties were as follows: emulsifier Span-80 10% (by mass), agitation speed 900 $r \cdot min^{-1}$, stirring time 30 min, and the ratio of the wall materials to core materials 0.15.

Matalanis et al., (2011) has given excellent review on various number of different approaches that can be used to create structured delivery systems based on biopolymers, including molecular complexation,

coacervation, thermodynamic incompatibility, moulding, and extrusion methods for food-grade ingredients .

Songkroetal., (2012) investigated inclusion complexes of citronella oil, citronellal and citronellol with β-cyclodextrin for mosquito repellent.the SEM technique revealed drastic changes in the shapes and morphologies of the particles for the inclusion complexes. The highest mosquito repellent activity was observed in the formulation which contained citronella oil–β-cyclodextrin inclusion complex at weight ratio of 1:1.

Lopezetal., (2012) studied controlled-release of linalool which was entrapped into microcapsules, inclusion complexes, and beads, obtained by different methods, inverse gelation (IG1, IG2, IG3, IG4, and IG5), oil-emulsion-entrapment (OEE), interfacial coacervation (INCO), and chemical precipitation (Cyc5 and Cyc10). Results revealed that in controlled-release, OEE followed by INCO presented a long time necessary for releasing as a result of the presence of glycerol or chitosan.

Zhu etal., (2012) in his study prepared microcapsules with three methods (1) a cross-linking method with glutaraldehyde, (2) an interaction method with alginate, and (3) a drying-in-liquid method with aqueous sodium hydroxide medium detailed investigations showed that the average diameters of the chitosan microspheres were controlled in the range of submicrometer to 10 μm .SPG membranes were used for preparing chitosan emulsions by method 1, alginate emulsions by method 2, or chitosan emulsions by method 3.

Chungetal., (2013) investigated the general characteristics and release behavior of microcapsules, and their repellent effect against insects. Results revealed that the highest loading efficiency appeared in microcapsules using SLS, had good thermal resistance and smooth surface. The release rate of thyme oil from microcapsules were not only dependent on the storage temperature but also emulsifier type microcapsules showed the sustained release properties for a long time. Diets, which were mixed with encapsulated thyme oil, expressed high insect repellent efficacy over 90% for 4 weeks.

Huangetal., (2013) prepared oil-chitosan composite sphereschitosan droplets, dropping into NaOH solution and in situ solidification the diameters of the prepared spheres were 2.48 ± 0.11 mm (pure chitosan spheres), 2.31 ± 0.08 mm (oil-chitosan composites), 1.49 ± 0.15 mm (iron-oxide embedded oil-chitosan composites), and 1.69 ± 0.1 mm (epirubicin and iron oxide encapsulated oil-chitosan composites), respectively .They developed a novel approach to an *in situ* process for fabricating oil-chitosan composite spheres with dual encapsulation properties, which are potential multifunctional drug carriers.

Nuisinetal., (2013)prepared an oil-in-water (o/w) emulsion using the Shirasu Porous Glass (SPG) membrane emulsification technique and high-speed dispersion technique for preparing a mixed o/w emulsion. Their results revealed that the average diameter of emulsion droplets of 28.3 μm and the crosslinked microcapsule size and size distribution of mixed emulsion droplets decreased with the increasing crosslinking time. The menthol release was a diffusion control which depended on the proportion of amino group in chitosan-to-tripolyphosphate molar ratio and crosslinking time.

Cerempei et al., (2014) studied flavouring effect that allowed the controlled release of biologically active constituentsThe results suggest that the forming of emulsions should be processed under the following conditions: a concentration of chitosan of 0.250% (w/v), geranium essential oil 0.450% (w/v), Tween 80 1% (w/v) and glycerine 2% (w/v), so that we could obtain biomaterials with the best controlled release of biologically active compounds.

Martinset al.,(2014) found microencapsulation as an important tool in cosmetic and pharmaceutical industry, enabling protection and controlled release of several active agents. The encapsulation of essential oils in core–shell or matrix particles has been investigated for various reasons, e.g., protection from oxidative decomposition and evaporation, odour masking or merely to act as support to ensure controlled release.

Souza etal., (2014) prepared chitosan microcapsules containing limonene essential oil as active ingredient.Three different concentrations of

NaOH (0.50, 1.00, 1.45 wt%) and fixed concentrations of chitosan and surfactant of 0.50 wt%. The concentration of 1.00 and 1.45 wt% clearly show the best results in terms of dimension and shape of the microcapsules as well as in the volatility results.

Xiaoetal., (2014) provided an excellent review on the recent advances in complex coacervation methods, the preparation and application of flavour and essential oils microcapsules based on complex coacervation technologythe different coating materials and their application.Proteins extracted from animal-derived products (gelatin, whey proteins, silk fibroin) and from vegetables (soy proteins, pea proteins), and polysaccharides such as gum Arabic, pectin, chitosan, agar, alginate, carrageenan and sodium carboxymethyl cellulose, flavour and essential oils, microcapsules, their practical applications in food, textiles, agriculturals and pharmaceutical, the information obtained allows criteria to be established for selecting a method for the preparation of microcapsules according to their advantages, limitations and behaviours as carriers of flavours and essential oils.

Prata et al., (2015) studied a complex coacervation between gelatin and chitosan structure and propertiesthat would facilitate the control for the eventual release of the core material. Results revealed that Spherical particles formed had an average diameter ($D_{3,2}$) of 30 μm and were prepared with 89.7% efficiency.

Bakry et al., (2016) have given excellent review on microencapsulation of marine, vegetable, essential oils and various methods including emulsification, spray-drying, coaxial electrospray system, freeze-drying, coacervation, *in situ* polymerization, melt-extrusion, supercritical fluid technology, and fluidized-bed-coating.Spray-drying and coacervation to enhance the oxidative stability, thermostability, shelf-life, and biological activity of oils. In addition, it can also be helpful in controlling the volatility and release properties of essential oils.

Chapter III

MATERIALS AND METHODS

Present investigation entitled “Morphological characterization of java citronella variants and encapsulation of oil for its slow release” was conducted during 2015-17 at Biotechnology center, Department of Agriculture Botany, Dr Panjabrao Deshmukh Krishi Vidyapeeth, Akola. The details of the materials used and methods adopted for the present investigation are given in this chapter.

3.1 Material Required

3.1.1 Plant Material

Rooted slips of *in vitro* mutagenized variants of java citronella were collected from the mother block of citronella from Centre of excellence in plant biotechnology, Dr panjabrao Deshmukh Krishi Vidyapeeth, Akola and the rooted slips of other genotypes i.e Bio-13, Jallpallavi, Mandakini, Medini were collected from Mahatma Phule Krishi Vidyapeeth,Rahuri.

3.2 Methods

3.2.1 Morphological characterization of java citronella variants

3.2.1.1 Experimental Details

A field trial was conducted at Centre of Excellence in Plant Biotechnology, Dr.Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra during 2015-17.

The experiment was laid out in Randomized Block Design(RBD)with ninetreatments and three replications. Block size for each entry was 0.60 m x3.00 m with the plant to plant spacing of60 x 60 cm.

3.2.1.2 Sowing and transplanting material

The healthy slips of *invitro* mutagenized varaints and the slips of promising genotypes (Bio-13, Jallpallavi, Mandakini and Medini) were

transplanted. The transplanting of this healthy rooted slip was done at the distance of 60 x 60 cm of the experimental plot on18 October 2015.

3.2.1.3 Thinning and gap filling

Thinning and gap filling was carried out where everrequired to maintain a single healthy plant. The field waskept clean from weeds, the crop was irrigated whenever as required.

3.2.1.4 Fertilizer application

Half dose of nitrogen (50 kg ha-1) in the form of urea and remaininghalf dose of nitrogen was given by top dressing during 3 month aftertransplanting.

3.3 Observation Recorded

Five plants were tagged randomly for recording observations for each entry in eachreplication. Mean of five plants for each entry in each replication was worked out for eachcharacter and used for statistical analysis. Observations on the followingcharacters were recorded at after 4 months of transplanting as specified below.

3.3.1 Growth Studies

(i) Plant Height (cm)

Height of the plant from the base of culm above ground level to the growing tip wasrecorded as plant height in cm.

(ii) Type of Growth

Growth habitat was recorded as erect, semi-erect or spreading of five selected plants were recorded.

(iii) Number of Tillers per plant

Tiller number was counted from each tagged plant and the mean of five plants wascomputed as tillers per plant.

(iv) Leaf width (cm)

Leaf width of first and second fully opened leaves in cm was recorded and the mean was computed.

(v) Leaf blade length (cm)

Leaf blade length of first and second fully opened leaves from tip was recorded in cm and mean was computed from five observations.

3.3.2 Yield Attributes

(i) Oil yield (g/ml)

After distilling known quantity (100g) of herbage for known period of time (two hours) in Clevenger's apparatus theevaporated oil was collected in the condenser and measured. The oil yield was expressed as 100 g per ml of shade dried herbage (24 hours shade drying)

(ii) Four Month Biomass(g)

On the basis of fresh weight the whole plant was cut from groundlevel and weight was recorded as the biomass in (g)

3.4 Oil extraction

Leaves (24 hours shade dried), plastic bags, weighing balance, polyproplyne plastic tubes, clevengers appratus were used for oil extraction.

3.4.1 Procedure for oil extraction by using Clevenger's appratus

1. The selected variant plants were collected and oil was extracted by using cleavengers appratus.
2. The leaves were cut into small pieces (2-3 cm) and shade dried (24 hours).
3. The shade dried leaves were weighed to take 100 g of sample.
4. The dried sample was placed in the 1000 ml round bottom flask.
5. 250 ml distilled water was added to the flask.
6. The temperature was set at 100^0 C the herb material at this condition softing of the cells and allowed the essential oil to escape in vapour form.
7. The essential oil formed a film on the surface of water in the condensor. The water was discarded and the oil was collected in plactic tubes. These oil sample were further used for TLC analysis.

3.5 Oil profiling by TLC (Thin layer Chromatography)

a) Instruments : Hot air oven, CAMAG solvent chamber

b) Glasswares: Beakers, measuring cylinder, glass plates etc. were purchase from Riviera. All the chemicals, solvents used were of analytical grade. Various chemical used for analysis are listed below.

Table 3.1 List of chemical and reagent used in Thin layer chromatography

Sr.no	Chemical	Molecular formula	Purity (%)
1	Silica gel (TLC grade)	-	-
2	Vaccum grease Silicone	-	-
3	Vanillin	$C_5H_8O_3$	99
4	Methanol	CH_3OH	99
5	Ethanol	C_2H_5OH	99
6	sulfuric Acid	H_2SO_4	95
7	Hexane	C_6H_{12}	84.16
8	Benzene	C_6H_5	99.7
9	Ethyl-acetate	$C_4H_8O_2$	99.8

Table 3.2 List of TLC Standard used in present study

Sr.no	Name	Purity(%)
1	Geraniol	96
2	(-)-Citronellal	96
3	β-citronellol	92
4	(-)-β citronellol	98

3.4 Procedure for Oil profiling by TLC (thin layer chromatography)

The oil components present in the selected *invitro* mutagenized variants of java citronella and other promising cultivar plant were analyzed by TLC.

3.4.1 Preparation of TLC plates (chromatoplates)

1. The glass plates (20x20 cm) were precoated with silica gel.
2. Silica gel usually which is in powder form was approximately 15g was weighed and dissolved in 30 ml of distilled water to produce a slurry.
3. The slurry of the silica gel was spread uniformly (usually of 1.5mm thickness) on the glass plate.
4. The TLC plates were air dried and prepared one day prior before use.

3.4.2 Preparation of TLC plates for sample Application

1. The plates were marked with the help of pencil and ruler at about 1.5 cm bottom line and a distance of 2cm distance was maintained between which the sample was applied.
2. At each 2 cm distance the sample and the standard (usually of 1 µl) were applied.

3.4.3 Preparation of Solvent System

1. The various solvent system were made by combining the solvents with different ratio.
2. The solvents were presaturated prior to use.
3. The various solvent system used for study are as follows

Table 3.3 Solvent systems used for Thin Layer Chromatography

Sr no	Solvent System	Ratio
1	Hexane:ethyl acetate	60:40
2	Benzene:Methanol	40:50
3	Hexane:ethyl acetate	80:20

4	Hexane:ethyl acetate	50:40

4. The plates were kept in the presaturated solvent chamber containing various solvent system.
5. The plate was kept in saturated chamber till the 80% solvent traveled vertical distance on the plate.

3.4.4 Preparation of vanillin reagent

1) 6 g vanillin was weighed and added in the beaker followed by addition of 95 ml of ethanol.
2) A clear colourless solution was formed.
3) 1.5 ml concentrated H_2SO_4 was carefully added to the solution.
4) Final product formed was clear colourles solution.
5) The plate was sprayed vanillin reagent.
6) The plate was heated at 50^0C for 5 min in Hot air oven.

3.4.5 Identification and Calculation of Retardation Factor (Rf) value.

1) The spots developed were analyzed by their colour with reference to the standard.
2) The Rf value was calculated by the given formula as follows -

   Rf = distance traveled by solute / distance travelled by solvent.

3) The Rf value was calculated and the component was identified.

3.5 Encapsulation of Oil

Dessicator**,** magnetic stirrer, weighing balance, distilled water Glasswares (beakers, measuring cylinder, petridish) were used for the present study. The chemical used for the encapsulation are listed below.

Table 3.4 List of Chemical used for encapsulation

Sr no	Chemical
1	Chitosan (80%deactylated)
2	Sodium hydrooxide pellets
3	Yellow oil dye (Auramine C.I Basic Yellow)

4	Glacial acetic acid

3.5 Procedure for prepartion of microcapsules

3.5.1 Preparation of coating Material

1) All the coating materials were prepared one day prior to emulsification process.

2) Chitosan solutions were prepared in three different concentration (0.5%, 1.0, 1.5% weight/ volume).

3) The required quantity of chitosan was dissolved in 0.1 M glacial acetic acid with three hours continouse magnetic stirring at 800 rpm.

4) 100 ml of glacial acetic acid stock was prepared by dissolving 0.6 ml glacial acetic acid in 99.4 ml of distilled water

3.5.2 preparation of emulsion

1) 0.5% wt yellow oil dye was weighed and added in 20 ml citronella oil.

2) From the above 20 ml of yellow citronella oil 2 ml was added in 20 ml of solution containing (0.5%, 1.0%, 1.5%) of chitosan.

3) This solution were stirred for 30 min, at 700-1000 rpm speed using Magnetic stirrer for 1 hour.

3.5.3 Formation of wall membrane

1) Required quantity of NaOH solutions (0.1%, 0.5%, 1.0% 1.5%) were prepared by dissolving in distilled water.

2) The emulsion were dripped in 0.1%, 0.5%, 1.0%, 1.5% NaOH and continous stirring at 800 rpm was done for 1 hour which lead to formation of wall membrane.

3) The microcapsules formed were washed with distilled water.

3.5.4 storage of microcapsules

1) The prepared capsules were placed in the 5% wt natural coconut oil for 10 days.

2) The microcapsules were vaccum dried in vaccum oven.

3) The microcapsules prepared from 0.5%, 1.0%, 1.5% chitosan and 0.5%, 1.0%,1.5% NaOH solution were stored in dessicator for 25 days.

4) The sampleweight was measured and defined as*W*m.

3.5.5 control release and data analysis

1)Three sets ofmicrocapsules observations were recorded per replication for each treatment. Every treatment was replicated thirce.The data of present investigation was analyzed and ANOVA was carried out by using completely randomized design. The mean and standard error, critical difference were calculated as per the procedure given by Panse and Sukhatme (1988).

2)The mean observations were recorded and furthur oil release and encapsulation effeciency of microcapsules prepared at various chitosan concentration were recorded for interval of 2 hrs and 50 min for 5, 10 15, 20, 25 days was calculated by the given formula:

3) The oil release content were analyzed by the formula as below:

$$\Psi(\%) = \frac{[W_m - Wm\ (t)]}{[W_m - W_0]} x100$$

3) where W_0 denotes the weight of microcapsules measured aftercomplete evaporation of Citronella Oil.

4) Encapsulation effeciency was calculated by following formula

$$\phi\ (\%) = \frac{W_m - W_0}{W_m}\ x100$$

Chapter IV

RESULTS AND DISSCUSSION

The results for the present investigation entitled “Morphological characterization of java citronella variants and encapsulation of oil for slow release” carried out during 2015-17, at Dr P.D.K.V, Akola are presented in following sections.

4.1 Morphological Characterization of java citronella

Morphological description have traditional significance and have been adoptedas classical taxonomical approach for identification of crop varieties. The average mean performance of java citronella variants and control genotypes over three replications was recorded for the following characters as per methodology as mentioned in chapter three.

4.1.1 Plant height (cm)

Plant height was recorded after 4 months of transplanting in centimeter. The average mean performance for the plant height is presented in table 4.1. The table 4.1 revealed that the five selected variants of *in vitro* mutagenized slips were significantly differ in the plant height from the control genotypes except Bio-13.

All the five variants recorded higher plant height as compared to Jallpallavi (74.06) cm , medini (82.60) cm and Mandakini (87.33) cm, where as variant 1, 2 and 3 are at par with the control Bio-13 and variant 4 (118.40) cm and variant 5 (113.60) cm are significantly higher as compared to all four control including Bio-13 (100.3) cm as shown in (Fig 5).

Bardolai (1982) has decribed *C.winterianus* as a stoloniferous perrinial that may grow up 1m high thus young shoots, growing from the axillary leaves of the mother plant, develop into largeclumps with leaves bending outward.

Since plant height contributed the total biomass of crop, the variants 4 and 5 were recorded as best performing variants for plants height.

Plate no 1 Experimental plot
Location: Centre of Excellence in plant biotechnology,Dr P.D.K.V,Akola

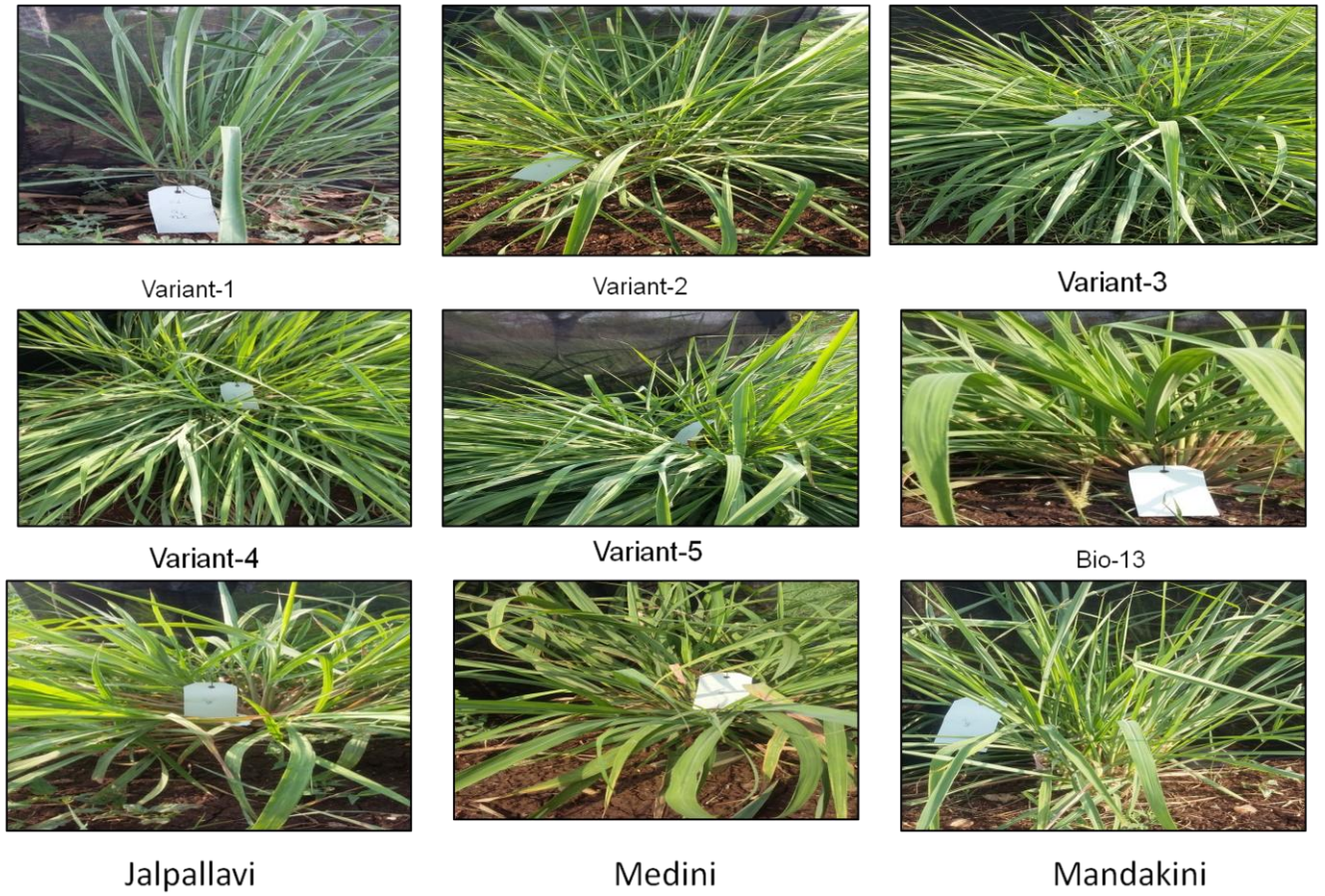


Plate no 2 Selected variants and control genotypes of java citronella

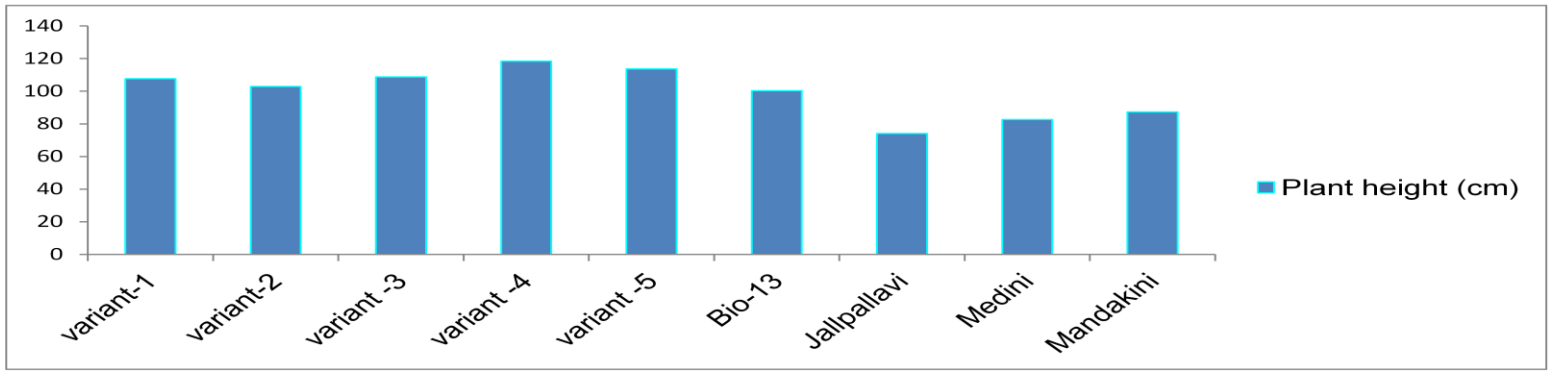


Fig 5 Average mean performance of selected variants and control genotypes for plant height .

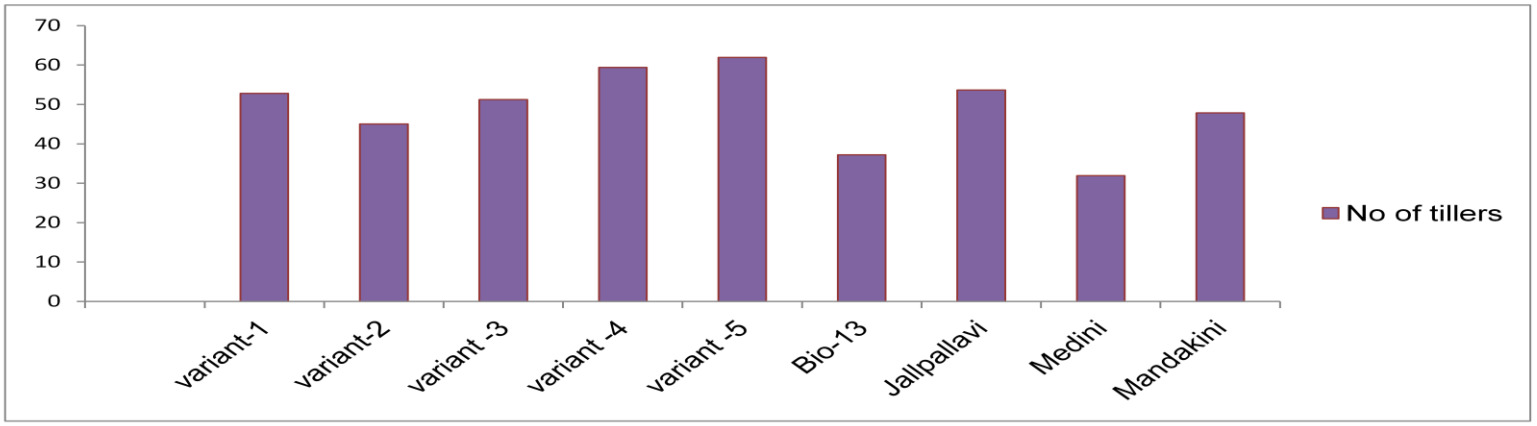


Fig 6 Average mean performance of selected variants and control genotypes for number of Tillers .

4.1.2 Number of Tillers

Tillers are the lateral shoot from the base of the steam of the plant and in java citronella leaves are considered as most important oil producing plant part, thus number of tillers contribute to produce large number of leaves in crop.

Number of tillers were recorded after 4 months of transplanting. The average mean performance for the number of tillers is presented 4.1. The table 4.1 revealed that the five selected variants of *in vitro* mutagenized slips had shown significant difference for number of tilers from the control genotypes except Jalpallavi.

All the five variants recorded higher number of tillers as compared to Bio-13 (37.20), Jalpallavi (53.66) and Medini (31.93), where as variant 1, 2 and 3 are at par with the control Jalpallavi and variant 4 (59.40) and variant 5 (61.93) as shown in (Fig 6).

Anumol T. (2010) reported stability parameters with respect to number of tillers in various genotypes of lemongrass. He found highest number of tiller (93.79) in CPK-25 genotype and lowest in Parman (29.06).

Hence number of tiller contributed the large production to leaves formation of crop, the variant 4 and 5 had shown the best performance for number of tillers.

4.1.3 Leaf width (cm)

Leaf width can be described as the widest portion of leaf, this character can be utilized to distinguish the genotypes from one another. Leaf width was recorded after 4 months of transplanting and measured in centimeter. The average mean performance for the leaf width is prsented in table 4.1. The table 4.1 revealed that the five selected variants of *in vitro* mutagenized plants and control genotype Mandakini have shown significant difference for leaf width than Bio13, Jalpallavi, Medini.

All the variants 1 (1.4) cm, 2 (1.2) cm, 3 (1.3) cm, 4 (1.5) cm, 5 (1.5) cm and control genotype Mandakini(1.3) cm were recorded and had shown lower leaf width as compared to Bio-13 (2.0) cm, Jalpallavi (1.7) cm, Medini (1.8) cm as shown in (Fig 7)

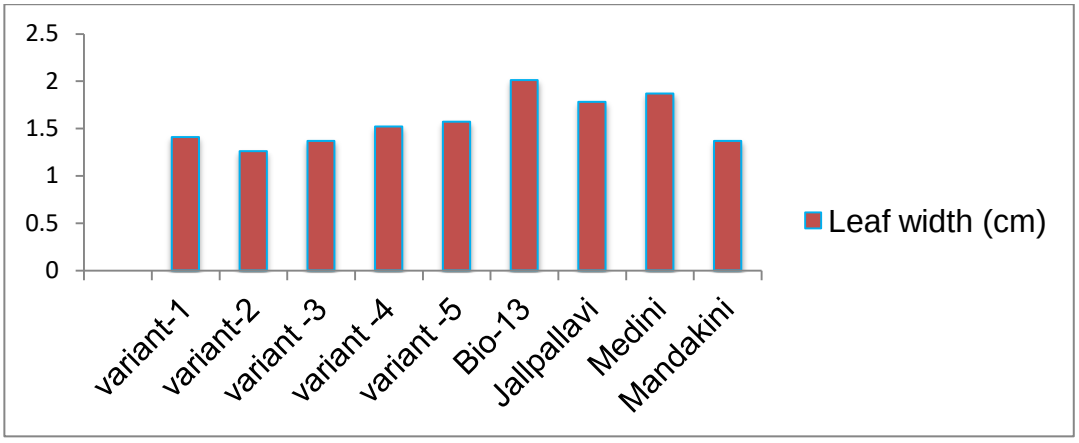


Fig 7 Average mean performance of selected variants and contol genotypes for leaf width.

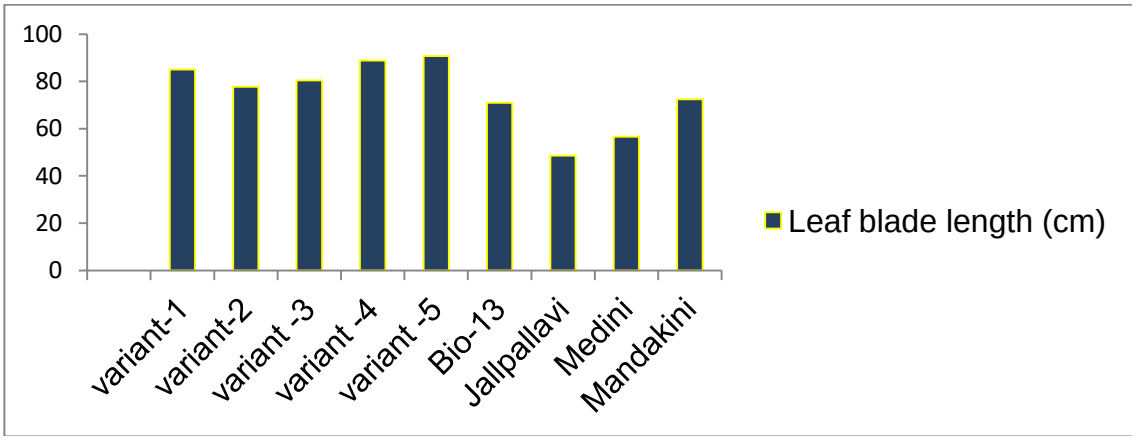


Fig 8 Average mean performance of selected variants and control genotypes for Leaf blade length.

Rao etal., (1998) reported the superiority in lemongrass genotype krishna for leaf width over other genotypes in this genotype the leaf width (1.4) cm were recorded.

Since it can be utilized as a useful morphological character to distinguish all the variants and control genotypes mandakini from other control genotypes Bio-13, jalpallavi, medini.

4.1.4 Leaf Blade length (cm)

In general leaf blade is a wide flattened area of leaf for concentrating sunlight on photosynthetic cells. But in Cymbopogan species it can be consider as important morphological character to distinguish the closely related species.

While measuring the plant height the leaf blade length was also recorded in centimeters . The average mean performance for the plant height is presented in table 4.1. The table 4.1 revealed that the five selected variants of *in vitro* mutagenized slips were significantly higher for leaf blade length from the control genotypes except Mandakini.

All the five variants recorded higher leaf blade length as compared to Bio-13 (71.00) cm, Jalpallavi (48.52) cm, Medini (56.60) cm, where as variant 1, 2 and 3 are at par with the control Mandakini and variant 4 (88.86) cm and variant 5 (90.73) cm are significantly higher as compared to all four control including Mandakini (72.46) cm as shown in (Fig 8).

Lawrence (1991) has distinguished the two closely related species of cymbopogan which are quite similiar in various aspects i.e *C.winterianus* and *C.nardus* ,thus on the basis of morphology of the shape and length of their leaves these two species were distinguished.

While leaf blade length contributed the height of the crop and reveal the growth pattern of genotype to distinguish from each other as well, thus variant 4 and 5 were recorded as best performing variants for leaf blade length.

4.1.5 four month Biomass (g)

All the morphological characters mentioned above get interlinked and contribute to produce a major morphological character known as biomass, thus it can be considered as the one of the important yield parameter in java citronella.

The biomass was recorded after 4 months of transplanting and expressed in terms of grams. The average mean performance for the biomass is presented in table 4.1. The table 4.1 revealed that all selected variants of *in vitro* mutagenized slips were significantly higher for biomass from the control genotypes except jalpallavi.

All the five variants recorded higher biomass as compared to Bio-13 (1132.28) g and Mandakini (3702.32) g, whereas variant 4 (4204.87) g and variant 5 (4526.69)g as shown in (Fig 9)

Shasany et al. (2000) conducted a comparative analysis of yields of herb, oil percentage and oil constituents for eight prevalent*C.winterianus* cultivars comparing them between themselves as well as against an accession of *C. nardus*.

4.1.6 Oil yield (100g/ml)

It is consider as the one of the important yield parameter next to biomass as it provides information related to oil production in plants in various environmental conditions.

Oil yield was recorded after 4 months of transplanting and expressed in grams per mililiter .The average mean performance for the oil yield is presented in table 4.1. The table 4.1 revealed that five selected variants of *in vitro* mutagenized slips recorded higher oil yield as except Bio-13 and jalpallavi.

All the five variants recorded high oil yield as compared to Medini (1.26) 100g/ml and mandakini (1.24) 100g/ml.,where as Bio-13 (1.45) 100g/ml and jalpallavi (1.42) 100g/ml were signigicantly higher as compared to variant 1, 2, 3,4,5.

Padalia et al., (2011) reported the average oil content is about 1% on the basis of fresh leaves within 2-3 hours of distillation, whereas the oil yield is about 1.2% (v/w) in Bio-13, jalpallavi and medini.

Since oil yield is considered as the important parameter for the crop. Variant 4 (1.38) 100g/ml and 5(1.40)100g/ml had given best performance.

4.1.7 Type of growth

On the basis of angle of emergence of stem nodes the growth pattern is differentiated as erect, semi-erect, spreading in grasses, thus it can be used as one of the useful agronomical character to distinguish the genotypes.

Type of growth pattern was recorded 4 months after transplanting on the basis of their gowing pattern all the five selected variants are presented in table 4.1. The table 4.1 reveal that morphologically the variants of *in vitro* mutagenized slips and the control genotypes had shown different type of growth pattern. Variant 3,4 and control genotype Jalpallavi,Medini, Mandakini were semi-erect type, whereas variant 1 and 2 were erect type and Bio -13 had shown spreading type of growth pattern.

Sharma and Sharma (2005) reported that morphologically the five accessions of *Cymbopogon flexuosus viz:* RLJ-TC-1, RLJ-TC-4, RLJ-TC-5, RLJ-TC-9 and RLJ-TC-10 had shown different type of growth pattern , All the accessions were straightin habit, however, RLJ-TC-4 and RLJ-TC-5 showed semi pendant leaves

4.1.8 Stem colour

From the taxonomical point of the view the steam colour may be used to identify the crop varities. Stem colour was recorded 4 months after transplanting on the basis of their visual appearence the colour of steam was described.

All the five selected variants are presented in table 4.1. The table 4.1 reveal that morphologically the variants of *in vitro* mutagenized slips and the control genotypes had shown different steam colour.

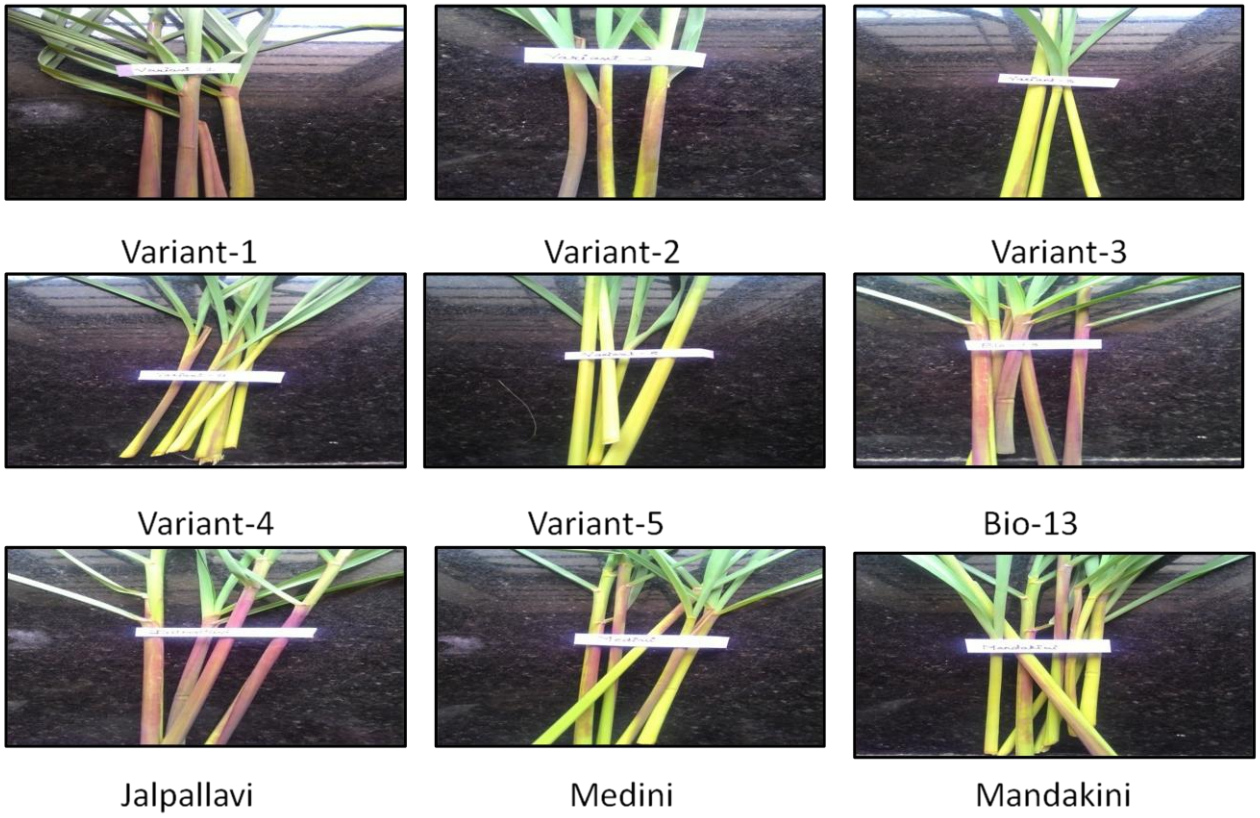


Plate no 3 Morphological variation in steam colour of selected variants and control genotypes of java citronella.

Variation in variants of *in vitro* mutagenized slips and control genotypes was for steam colour was observed. Variant 1 and 4 were faint green brown in colour, variant 2 and 5 were green and variant 1, Bio-13 and jalpallavi were dark brown respectively.

Since, it can be used as one of important character for easy determination of crop varities.

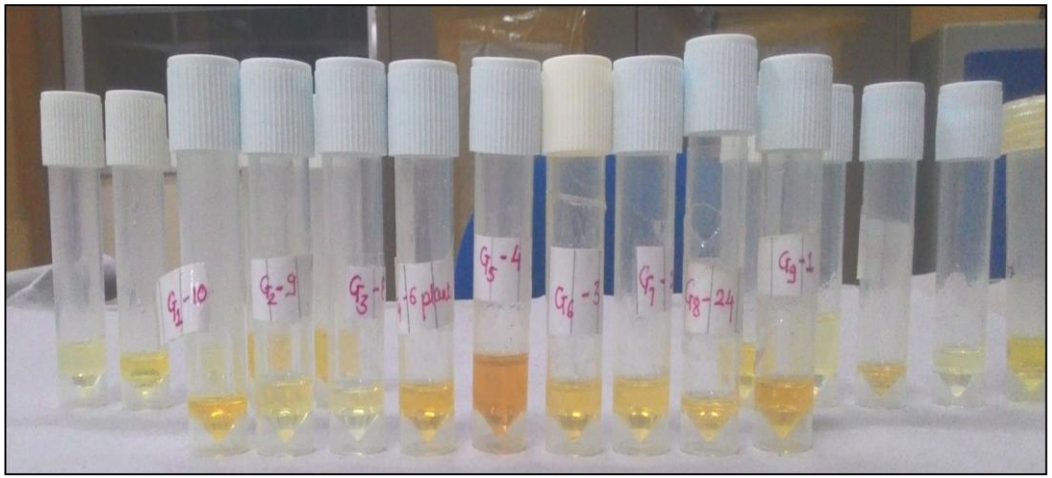
4.1.9 Total Estimated Oil Yield (g/ ml)

Total estimated yield was recorded on the basis of biomass production .The suspected oil yield on the basis biomass production is presented in table 4.1. The table 4.1 revealed that the five selected variants of *in vitro* mutagenized slips were significantly differ in the biomass from the control genotypes except jalpallavi.

All the five variants recorded higher biomass as compared to Bio-13 (1132.58) g and mandakini (3702.32) g, whereas variant 4 (4204.47) g and variant 5 (4526.69)g .

The total estimated oil yield was (6286.98) g/ml for variant 5 followed by (526.86) for variant 4. The lowest oil yield was obtained in medini (1339.52).

From the above result it can be concluded that biomass is one of the most promisingmorphological character to obtain high oil yields in java citronella.



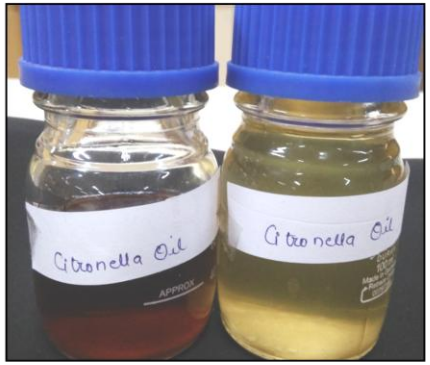


Plate no 4 citronella oil extracted from selected variants and control genotypes of java citronella

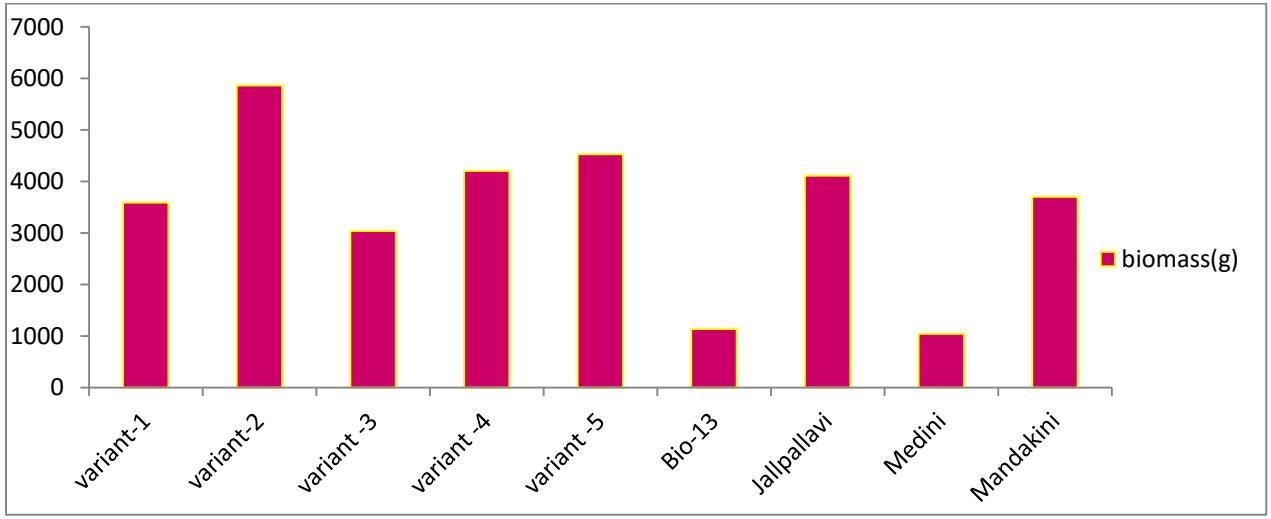


Fig 9 Average mean performance of selecte variants of java citronella variants and control genotypes for Biomass.

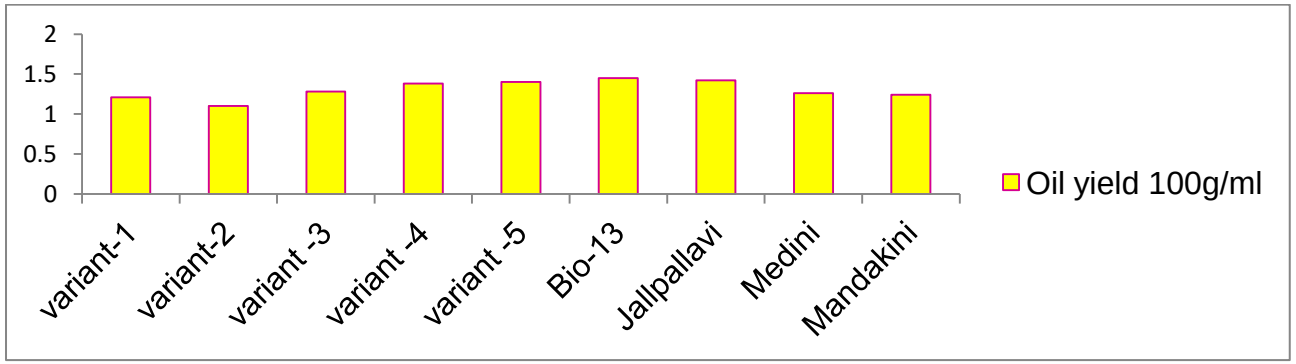


Fig 10 Average mean performance of selected variants of java citronella and control genotypes for oil yield.

Table 4.1 Average mean performance of java citronella variants and control genotype

Variants	Plant height (cm)	No of tillers	Leaf width (cm)	Leaf blade length (cm)	4 month Biomass(g)	Oil yield 100g/ml	Type of growth	Stem colour	Total estimated oil yield (Biomass/ml)
variant-1	107.66	52.80	1.41	85.00	3591.08	1.21	Erect	Dark-brown	4261.62
variant-2	102.93	45.06	1.26	77.73	5862.57	1.10	Erect	Faint green brown	3438.82
variant -3	108.73	51.26	1.37	80.46	3039.307	1.28	Semi-erect	Green	4521.08
variant -4	118.40	59.40	1.52	88.86	4204.47	1.38	Erect	Faint green brown	5726.86
variant -5	113.60	61.93	1.57	90.73	4526.69	1.40	Semi-erect	Green	6286.98
Control									
Bio-13	100.33	37.20	2.01	71.00	1132.58	1.45	spreading	Dark brown	1807.42
Jallpallavi	74.06	53.66	1.78	48.53	4109.41	1.42	Semi-erect	Dark brown	5686.10
Medini	82.60	31.93	1.87	56.60	1042.4	1.26	Semi-erect	Faint green brown	1339.506
Mandakini	87.33	47.86	1.37	72.46	3702.323	1.24	Semi-erect	Faint green brown	4311.976
SE (M)	2.933742	2.546336	0.107045	4.594414	12.30	0.020443	_	_	-
CD at 5%	8.245	7.629	0.140	13.761	36.88	0.061	_	_	-
CV	4.786	8.992	5.134	10.656	0.678	2.713			-

4.2 Oil profiling with TLC

4.2.1 Number of components detected with various concentration

Within a short period of time thin layer chromatography has come a most important technique for idenification, characterization and determination of chemical compounds as well as complex mixtures.

In the present study various concentration of solvents systems were tried to determine good solvent system which gives good separation and identification of component as subjected below.

Table 4.2 Various solvent systems tried in the present study

Sr no	Solvent systems	Ratio (v/v)
1	Benzene : Hexane	20:80
2	Ethyl-acetate : Benzene	80:20
3	Hexane : Methanol	40:60
4	Benzene: Tolune	10:1
5	Methanol: Tolune	20:1
6	Ethyl acetate: Hexane	90:10
7	Hexane:ethyl acetate	40:60
8	Hexane:ethy acetate	50:40
9	Hexane:ethyl acetate	80:20
10	Benzene:methanol	80:20

4.2.1.1 Component detected at Hexane: ethyl acetae (50:40 v/v)

Out of various solvent systems as mentioned in table 4.2 the best component identification was observed in the solvent systems as per the methodology mentioned in chapter three and the various components detected concentration wise are presented in table 4.2.

Component analysis was done from the oil extracted after 4 months of transplanting and their presence and absence with their reference standards was done .As the table 4.3 revealed that the

concentration of (50:40 v/v) Hexane : ethyl acetate resolved oil component in all the five variants and control .

Geraniol had recorded Rf value at (0.77) , (-) – citronellal (0.98), β- citronellol (0.80) and (-) – β citronellol (0.81). After heating the plate for about 5 min characteristic colouration were observed for each particular component such as greenish blue for geraniol, dark violet zone for (-) – citronellal, faint blue for β- citronellol and faint blue for (-) - β- citronellol as shown plate 5 (a)

At this concentration all the variant had showed the good separation of all the oil component.

4.2.1.2 Component detection at Hexane:ethyl acetate (40:60 v/v)

The lower concentration of hexane with higher concentration ofethyl acetate usually 40:60 (v/v) gave the clear separation of for the β- citronellol. The table 4.3 revealed that their was absence of β- citronellol in all the five selected variants plant oil except Bio-13 Jalpallavi, Medini.

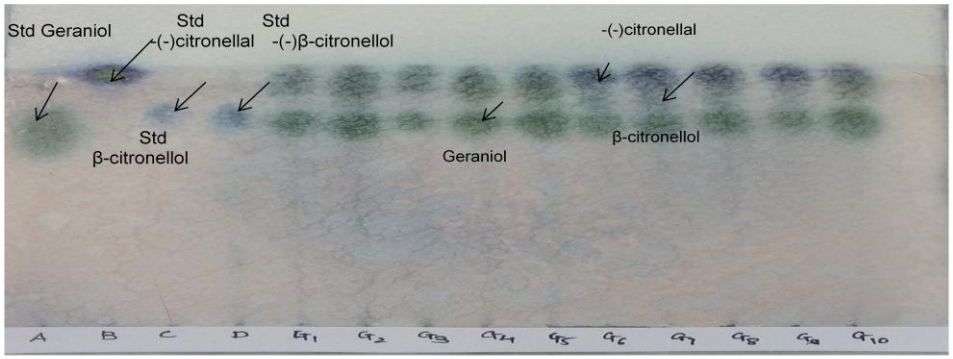
The β- citronellol was not clearly visualized in the five selected variants at this concentration but this component was clearely identified in control Bio-13, Jalpallavi and Medini with Rf value at (0.80) and faint blue colur was observed as shown in Plate 5(b).

At this concentration control Bio-13, Jalpallavi and Medini performed best.

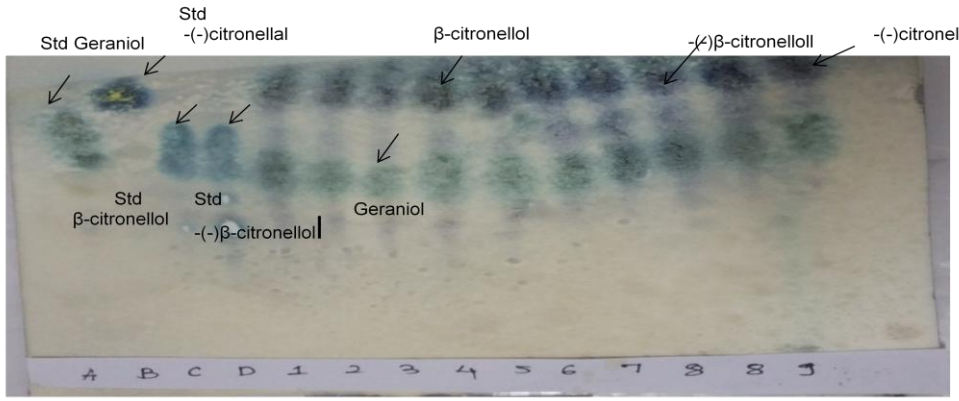
4.2.1.3 Component detected at Hexane:ethyl acetate (80:20 v/v)

With the higher concentration of hexane and lower concentration of ethyl acetate the separation and component identification was done.

At this concentration a very good separation of all the components was done and gave clear identification of components. The table 4.3 revealed at the concentration of (80:20 v/v) Hexane : ethyl acetate all the five variants had showed presence of all the component, at this concentration one unknown component was identified as well which was strongly present in five selected variants plant oil.



A.



B.

Plate no 5: (A)Oil profiling with Hexane: Ethyl acetate (40:50v/v) (B)Oil profiling with Hexane:Ethyl acetate (60:40 v/v)

Table 4.3. Oil profiling with various Hexane : ethyl acetate solvent systems with various concentration

Name of the sample	Sample Id	Hexane : ethyl acetate(v/v)												
		50:40				40:60				80:20				
		Geraniol Rf0.77	(-)-citronellal Rf0.98	β-citronellol Rf0.80	(-) –citronellol Rf0.81	Geraniol Rf0.77	(-)-citronellal Rf0.98	β -citronellol Rf0.80	(-) –citronellol Rf0.81	Geraniol Rf0.77	(-)-citronellal Rf0.98	β -citronellol Rf0.80	Unknown Rf 0.87	(-) –citronellol Rf0.81
Variant-1	G1	√	√	√	√	√	√	-	√	√	√	√	√	√
Variant-2	G2	√	√	√	√	√	√	-	√	√	√	√	√	√
Variant-3	G3	√	√	√	√	√	√	-	√	√	√	√	√	√
Variant-4	G4	√	√	√	√	√	√	-	√	√	√	√	√	√
Variant-5	G5	√	√	√	√	√	√		√	√	√	√	√	√
Bio-13	G6	√	√	√	√	√	√	√	√	√	√	√	-	√
Jalpallavi	G7	√	√	√	√	√	√	√	√	√	√	√	-	√
Medini	G8	√	√	√	√	√	√	√	√	√	√	√	-	√
Mandakini	G9	√	√	√	√	√	√	-	√	√	√	√	-	√

√ Refers presence, - Refers absence

Geraniol at Rf value (0.77), (-) – citronellal (0.98), β- citronellol (0.80) and (-) – β citronellol (0.81) were identified an unkown component with Rf value (0.87) was identified. After heating the plate for 5 min characteristic colouration were observed for each particular component such as greenish blue for geraniol, dark violet zone for (-) citronellal, faint blue for β- citronellol and faint blue for (-) - β- citronellol as shown plate 6 (c)

Thus this concentration gave good separtion of the oil component in the variants and proved to be the best solvent system.

Wany et al., (2014) reported that hexane: ethyl acetate(3:2 v/v) was the good solvent system for separtion of oil component from crude extracted citronella oil such as geraniol with Rf value 0.82 and citronellol with Rf value 0.72-0.78, with characteristic colouration weak violet zone for geraniol and blue zone for citronellol.

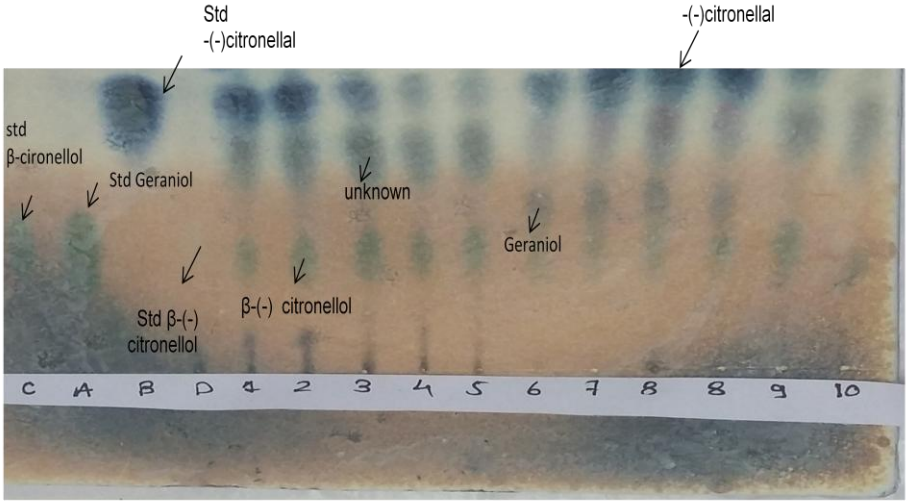
Geraniol had Rf value (0.77) , (-) – citronellal (0.98), β- citronellol (0.80) and (-) – β citronellol (0.81). and the unkown component had Rf (0.87) . After heating the plate about 5 min characteristic colouration were observed for each particular component such as greenish blue for geraniol, dark violet zone for (-) – citronellal, faint blue for β- citronellol and faint blue for (-) - β- citronellol as shown plate 6 (c)

Since this concentration gave good separtion of the oil component in the variants and proved to be the best solvent system.

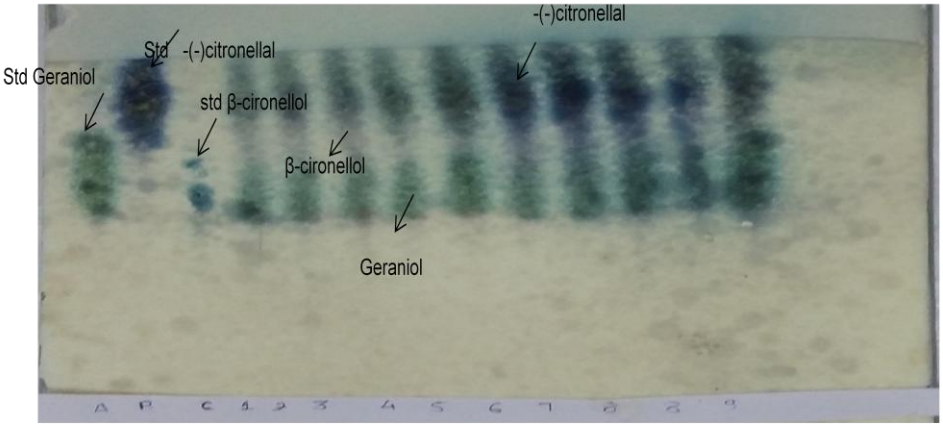
Wany et al., (2014) reported that hexane: ethyl acetate(3:2 v/v) was the good solvent system for separtion of oil component from crude extracted citronella oil such as geraniol with Rf value 0.82 and citronellol with Rf value 0.72-0.78, with characteristic colouration weak violet zone for geraniol and blue zone for citronellol.

4.2.1.4 Component detected at Benzene : methanol (50:40)

With the higher concentration of benzene and lower concentration of methanol the separation and component identification was done.



C.



D.

Plate no 6: (C)Oil profiling with Hexane: Ethyl acetate (80:20v/v) (D)Oil profiling with Benzne:Methanol (50:40 v/v)

At this concentration a very good separation of all the components was done and gave clear identification of components. The table 4.3 revealed at the concentration of (50:40 v/v) benzen:methanol all the five variants had showed presence of all the component.

Table 4.4 Oil profiling with Benzene : methanol solvent system.

Name of the sample	Sample Id	Benzene :methanol (v/v)50:40		
		Geraniol Rf. 0.80	(-)-citronellol Rf. 0.98	β-citronellol Rf. 0.75
Variant-1	G1	√	√	√
Variant-2	G2	√	√	√
Variant-3	G3	√	√	√
Variant-4	G4	√	√	√
Variant-5	G5	√	√	√
Bio-13	G6	√	√	√
Jalpallavi	G7	√	√	√
Medini	G8	√	√	√
Mandakini	G9	√	√	√

√ Refers presence, - Refers absence

Geraniol had Rf value at (0.80) , (-) – citronellal (0.98),and β-citronellol (0.75) cm . After heating the plate about 5 min characteristic colouration were observed for each particular component such as greenish blue for geraniol, dark violet zone for (-) – citronellal, faint blue for β-citronellol and faint blue for (-) - β- citronellol as shown plate 6 (d)

Levi etal.,(1965) carried out on essential oil of palmrosa, mint to determine the nature of constituents. Benzene: methanol (10.2) was used to determine alcohols, terpenoids, where as terpenoids displayed characteristic colouration when sprayed with the vanillin in conentrated sulphuric acid spray agent.

4.3 The formation of microcapsules and its encapaulation effeciency.

4.3.1 Formation of microcapsules

Essential oil are volatile at room temperature and some of these essential oil inherently possess antibacterial, antifungal and antiviral properties. These properties tends to change if the oils gets oxidized and gets rancid.In this regard these properties of oil can be preserved by by

formulating different formulation and one of these is formulation of chitosan coated micrcapsules.

In the present study a protocol was standardize for formation of microcpsules and analysis for the slow releas effect was studied. Nine combination were tried for preparation of microcapsules, among those only three combination had shown slow release effect and only one combination had shown the storage stability upto 25 days.

Microcapsules with varying concentration of chitosan at(0.5 wt%, 1.0 wt%, and 1.5 wt%) and NaOH (0.5 wt%, 1.0 wt%, 1.5 wt%) were tried out of nine combinations were tried in all nine combination with varied effeciency formation of microcapsules were obtained. Formation of microcapsules were obtained at 0.5wt% chitosan,1.0wt% chitosan,1.5 wt% chitosan as shown in plate 6. Among these nine combinations 1% chitosan+1%NaOH, 1.5% chitosan+0.5%, 1.5wt% chitosan+1% NaoH shown the best manufacturing condition as shown in plate 7.

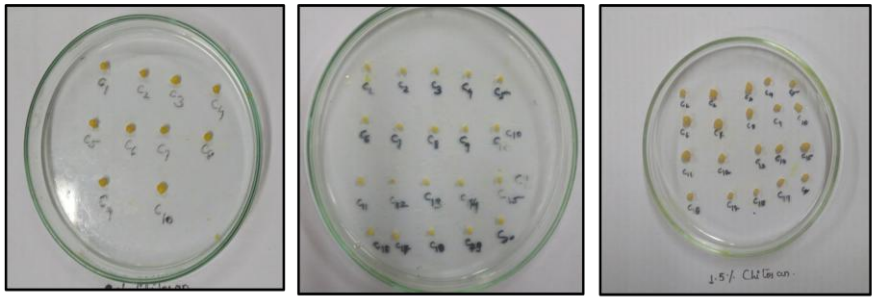
In the present study formation of good microcapsules was obtained using chitosan as a coating material.

Anal et al., 2006 reported that chitosan forms hydrogel particles from large to nanometer scale under relatively mild gelation conditionsand thus can be used for encapsulation of bioactive compounds.

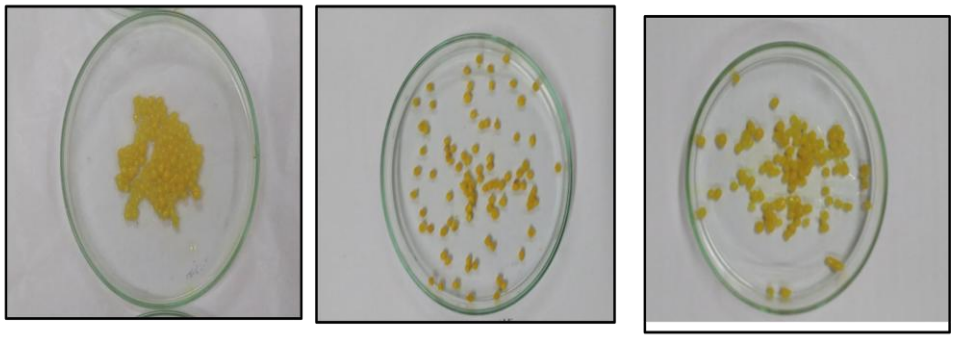
Hseih et al.,(2006) reported the condition of 0.5 wt% Chitosan, 0.5 wt% NaOH, and with0.5 . Underthis manufacturing condition, good formation and dispersion ofmicrocapsules can be obtained.

4.3.1 Encapsulation effeciency of microcapsules

Generally the success of preparation of microcapsules depended on how much quantity of oil the chitosan was able to encapsulate and for how much of time this was expressed as encapsulation effeciency. Thus the encapsulation effeciency of microcapsules was calculated by formula as mentioned in chapter three and the encapsulation effeciency at various concentration of chitosan and NaOH is mentioned in table 4.5.



A. a. 0.5% chitosan b. 1% chitosan c. 1.5% chitosan



B. a. 1% chitosan+1%NaoH b.1.5%chitosan+0.5%NaoH c. 1.5%chitosan+1%NaoH

Plate No 7 A) Microcapsules produced with different concentration of chitosan and 1% NaoH

B) Good combination to manufacture microcapsules

Table 4.5 Encapsulation Effeciency of microcapsules

Sr no	Concentration	Encapsulation effeciency(%)
1	0.5% chitosan+0.5% NaoH	73.30
2	0.5% chitosan+1.0%NaoH	79.31
3	0.5%chitosan+1.5%NaoH	78.86
4	1.0%chitosan+0.5%NaoH	81.97
5	1.0%chitosan+1.0%NaoH	83.74
6	1.0%chitosan+1.5%NaoH	83.38
7	1.5%chitosan+0.5%NaoH	91.96
8	1.5%chitosan+1.0%NaoH	95.61
9	1.5%chitosan+1.5%NaoH	90.63

According to table 4.5 microcapsules were prepared with 9 combination of 0.5wt%, 1.0wt%, 1.5wt% chitosan and 0.5wt%, 1.0wt%, 1.5wt% NaoH. The various combination showed encapsulation effeciency ranging from 73.30% to 95.61%. Among the nine combination the highest encapsulation effeciency was found in 1.5wt chitosan %+1wt % NaoH with 95.61% effeciency, followed by 1.5 chitosan wt% + 0.5 NaOH % with 91.96% effeciency and the lowest encapsulation effeciency was found within 0.5wt% chitosan+0.5%NaoH with 73.30% effeciency (fig 10)

4.4 Control oil release and data analysis

4.4.1 Release effect of chitosan concentration on wall membrane

The microcapsules prepared with chitosan wall membrane in 0.5 wt%, 1.0 wt%, 1.5 wt% and their influence of release effect to encapsulated volatile oil within 120 min interval of time has been shown in table 4.6.

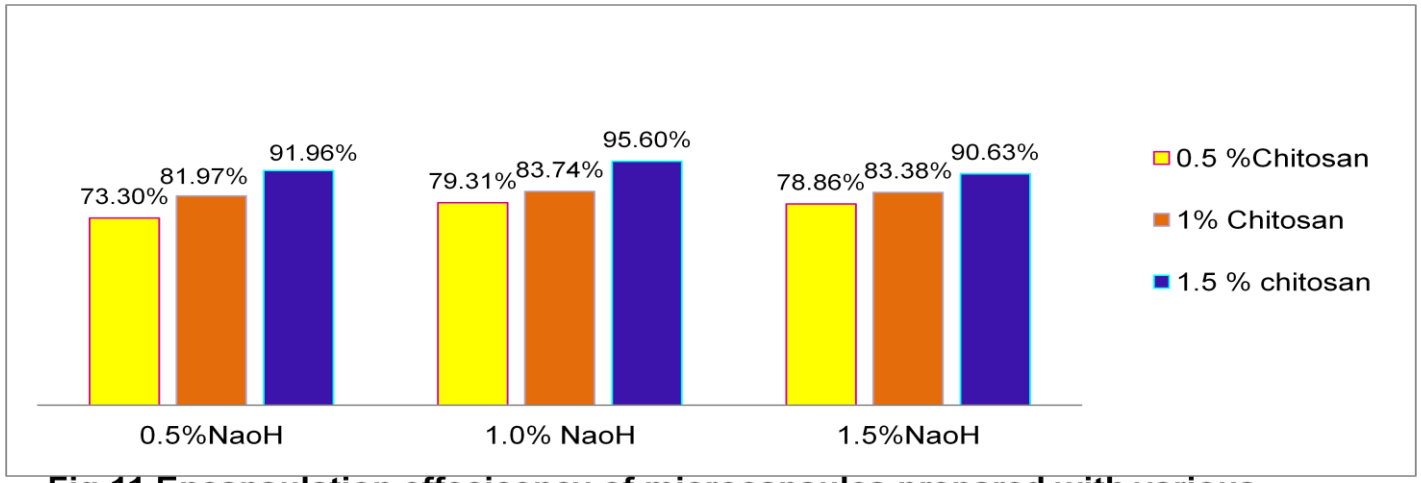


Fig 11 Encapsulation effecicency of microcapsules prepared with various combination of chitosan and NaOH

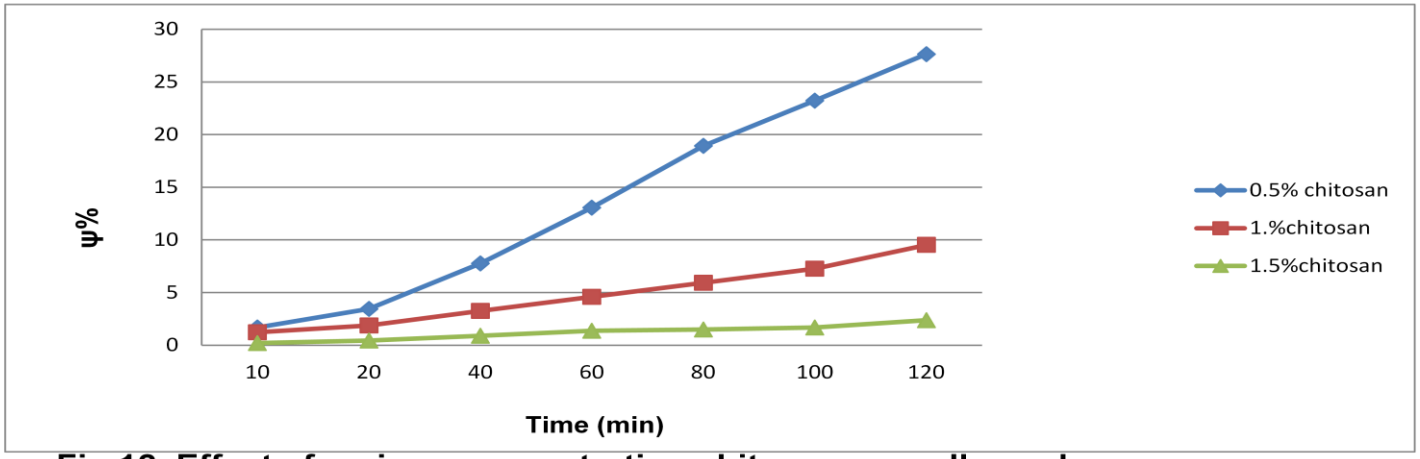


Fig 12 Effect of various concentration chitosan on wall membrane

Table 4.6 Percentage of oil release and effect of chitosan on wall membrane

Time(Min)	0.5wt% chitosan	1.0wt% chitosan	1.5wt% chitosan
10	1.68	1.24	0.21
20	3.44	1.87	0.45
40	7.77	3.26	0.89
60	13.07	4.59	1.38
80	18.92	5.93	1.50
100	23.22	7.26	1.68
120	27.63	9.52	2.38
SE (M)	0.043	0.051	0.049
CD at 1%	0.185	0.077	0.053

According to table 4.6 control oil release was tested for three combinations1.0%chitosan+1.0%NaoH(T1), 1.5%chitosan+0.5%NaoH (T2) and 1.5%chitosan+1%NaoH (T3) as shown in as shown in (Fig 11) and the percent of oil release was calculated by the oil release formula as mentioned in chapter three.

Microcapsules prepared with 0.5 wt % chitosan had shown highest release of oil over the period of 120 min counted with the interval of 20 min over the concentration of chitosan.

At 120 min 27.63 % oil was released from the microcapsules prepared with 0.5 wt % chitosan.The percentage of oil release was low in 1% wt chitosan that is 9.52% where as the oil release further decreases at 1.5 wt% chitosan concentration.

The data indicates that morever thicker the chitosan concentration,slower is the release rate of citronella oil, thus 1.5% chitosan proved to be the best concentration for the encapsulation of oil.

Heish et al. (2006) reported the release rate depends on the thickness of the coating material , the rate ofemployed chitosan has

influence, because thicker the Chitosan concentration, the slowerthe release rate.

4.4.2 Oil Release and storage stability

One of the most important parameters in microencapsulation of volatile oils is the capability of the wall material to retain the volatile compounds during storage.

The microcapsules formed in treatment T1(1.0% chitosan+1% NaOH), T2(1.5% chitosan+0.5% NaOH), and T3(1.5% chitosan+ 1% NaOH) were subjected to the oil release upto 25 days. Observations were recorded at the interval of 5 days.

The data shown in table 4.7 revealed that upto 5 days 12.59% oil was release from microcapsules prepared from 1% chitosan +1%NaoH, while 6.43% oil release from 1.5% chitosan + 0.5% and 4.47% oil release from 1.5% chitosan +1% NaoH microcapsules was observed.

While till the 10^{th} day 19.71% oil was release from the microcapsules prepared from 1%chitosan+1%NaoH, where as 7.63%, followed by 6.03 % of oil release from the microcapsules prepared from 1.5%chitosan +1%NaoH was observed.

At 15^{th} day 40.82%, 15.53%, 11.95% oil was release from 1% chitosan+1% NaoH, 1.5% chitosan + 0.5% NaoH, 1.5% chitosan + 1.5% NaoH.

And upto 20th day the microcapsules prepared from 1.5 % chitosan with 0.5% and 1% NaoH were not shrink and the oil release was upto 35.46% and 27.67% resspectively.

Till 25^{th} day the microcapsules prepared from 1.5% chitosan+ 1% NaoH had shown 91.67% oil release. The microcapsules manufactured at the concentration of 1.5% chitosan+1% NaoH showed the slow oil release and had best storage stability.(fig 12).

Similiar findings were observed that there aredifferent volatility values for microcapsules produced withdifferent NaOH concentrations.The microcapsulessynthesized with 0.5 wt% of NaOH showed higher volatilityof

the encapsulated active agent, whereas the microcapsulessynthesized with 1.45 wt% of NaOH had a lower release rate.The results showed that by adjusting the NaOH concentrationit is possible tosignificantly influence the controlled releaserate of the limonene on the chitosan microcapsules Jefferson et al.(2014).

Table4.7 Oil release and storage stability of microcapsules prepared at various concentration upto 25 days

Sr No	Storage days	Oil release% (ψ)		
		1% Chitosan + 1% NaoH	1.5% Chitosan + 0.5% NaoH	1.5% Chitosan + 1% NaoH
1	5	12.59	6.43	4.47
2	10	19.71	7.63	6.03
3	15	40.82	15.53	11.96
4	20	***	35.46	27.22
5	25	***	***	91.67

***signifies that the capsules were shrink and no more available for further study

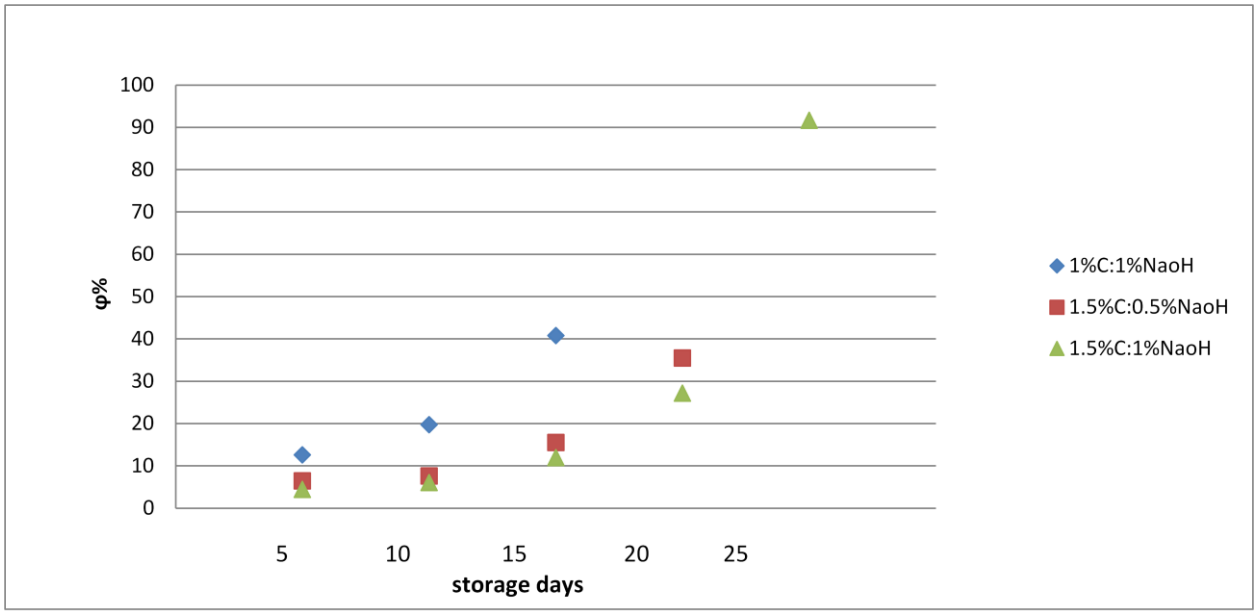


Fig 13 Oil release and storage stability of microcapsules

Chapter V

SUMMARY AND CONCLUSION

The present study was conducted to characterize the selected variants and promising genotypes based on plant morphological characters.Observations on four quantitative trait and two qualitative trait including yield and yield components wererecorded .Further the oil component analysis which has economic value in manufacturing of soaps, household product, perfume and flavour industry.Were analyzed by rapid and simple technique thin layer chromatography, the citronella oil which is volatile in nature to decrease its volatilty the attempt for microencapsulation and study for its slow release was conducted. The results obtained in the present study are summarized below.

Among the four quantitative traits which are biomass, oil yield, plant height,leaf width no of tillers and two qualitative trait which are stem colour, type of growth characterization using different plant morphological characters showed that there iswide variability among the genotypes tested and these characters can be used forvarietal identification.The highest biomass was recorded in variant 5 with (4526.69) g and variant 4 with (4204.47) g and the lowest biomass was found in Medini wit(1042.4)g/plant. For oil yield the Bio-13 shown the average yield upto 1.45%, followed by Jalpallavi with 1.40% whereas the variant 4 and 5 also shown the oil yied ranging from 1.38% and 1.40%. Plant height was higher in all the variants1 with (5 118.40 cm) and lower in Jalpallavi with (74.06cm). The leaf width was usually greater in Bio-13 with (2.01 cm) and lower in variant (1.26cm). Tillers were highest in variant 1 (61.93) per plant and lower in Medini with (31.93cm).Based on the coiour of stem the variants and the genotypes were grouped into three colours as green,faint green brown, dark green brown and on the basis of growth pattern the variants and the genotypes were grouped as erect, semi-erect, spreading.

After a several trial experiments with different concentration of hexane:ethy acetate . Hexane:ethy acetate with (80:20v/v) as solvent system gave good separation of oil components, comet shape with

different colour components were observed on the silica plate. All the variant 1, 2, 3, 4, 5 have shown the presence of geraniol, (-)- citronellal, (-)-β citronellol and β-citronellol and genotypes Bio-13, Jalpallavi,Medini, mandakini shown the above mention components as well.

Microcapsules of citronella oil with various concentration of chitosan ranging from (0.5%, 1.0% ,1.5%) and NaOH concentration (0.5%, 1.0%, 1.5%) were made preliminary 9 different combinations of chitosan and NaoH were tried. Among these combinations1.0%chitosan+1.0%NaoH,combination1.5%chitosan+0.5%NaoH and combination1.5%chitosan+1.0%NaoH shown highest encapsulation effecieny with 83.74%, 91.96% and 95.61%. Among these combination microcapsules prepared from 1.5% chitosan +1%NaoH shown the slow release and sorage stability upto 25 days. Thus from the above experiment it can be concluded that

1) Morphological characterization of java citronella would be useful in varietal identification in accordance to taxomonic point of view. Further the variants plants of 4 and 5 of which have showed morphologically higher biomass, plant height, higher oil yields can be released as new cultivar.
2) Thin layer chromatography helped in analysis of oil component analysis at (80:20) v/v hexane:ethyl acetae solvent system the all component resolved clearly and one unknown component was detected at this concentration which was strongly present in all five variants.
3) Microencapsulation proved one of the most important technique to decrease the volatility of citronella oil upto 91.60% volatility was decrease by microcapsules prepared with 1.5% chitosan + 1 % NaOH and the release effect were slow.

CHAPTER VI

IMPLICATIONS

Morphological description have traditional significance and can be adoptedas classical taxonomical approach for identification of crop varietiesat primary level. The oil possesses mosquito repellentand germicidail properties it provides opportunity for development of essential oil-based insecticides, fungicides and herbicides for agricultural and industrial applications and for the consumer market as well asitcould be used in development of value added products such as scenting of soaps, masking the odourofgermicides, mosquito repellent creams etc. capturing essential oils in capsules, to achieve the delayed release effect, Controlled slow release with protecting the active components until release or using oil-in-water micro emulsions as a pesticide delivery system to replace the traditional emulsifiable concentrates (oil), in order to reduce the use of organic solvent and increase the dispersity, wettability and penetration properties of the droplets.

Chapter VII

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