

QUALITY OF LEMONGRASS (*Cymbopogon flexuosus*) OILS UNDER DIFFERENT STORAGE CONDITIONS AND THEIR NEMATICIDAL ACTIVITY

BY

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(2004 BS146 M)

*Thesis submitted to the Chaudhary Charan Singh Haryana
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requirements for the degree of*

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In
CHEMISTRY



**COLLEGE OF BASIC SCIENCES AND HUMANITIES
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2006

**Dedicated
To My Didi.....**

CERTIFICATE - I

This is to certify that this thesis entitled, “**Quality of Lemongrass (*Cymbopogon flexuosus*) oils under different storage conditions and their nematocidal activity**”, submitted in partial fulfillment for the degree of **Master of Science** in the subject of **Chemistry**, to the Chaudhary Charan Singh Haryana Agricultural University, Hisar, is a bonafide research work carried out by **Mr. Yogesh Kumar Singh (2004 BS146 M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigations have been fully acknowledged.

(Dr. V. K. Madan)
Major Advisor

CERTIFICATE - II

This is to certify that the thesis entitled, “**Quality of Lemongrass (*Cymbopogon flexuosus*) oils under different Storage conditions and their nematocidal activity**”, submitted by **Mr. Yogesh Kumar Singh (2004 BS146 M)** to the CCS Haryana Agricultural University, Hisar in partial fulfillment of the requirements for the degree of **Master of Science** in the subject of **Chemistry**, has been approved by the Student’s Advisory Committee after an oral examination on the same.

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CHAPTER I

INTRODUCTION

The Indian subcontinent abounds in aromatic vegetation and essential oils extracted from these are popular throughout the world, but the pride of the place among the essential oils earning foreign exchange package mainly goes to the oil of Lemongrass, because it is the chief source of citral which finds innumerable use in perfumery, cosmetics, flavouring, medicines and in the synthesis of Vitamin A, geraniol and ionone.

Lemongrass belongs to the genus *Cymbopogon* Spreng (family Gramineae syn. Poaceae), consists of about 140 species (Chase and Niles, 1962; Patra *et al.*, 1999). Among the 140 species recognized so far more than 52 are found in Africa, 45 in Indian subcontinent, 6 each in America and Australia, 4 in Europe and about 24 species are reported in India (Kak and Kaul, 1997). However, their identification has been a problem as they hybridize easily and also because some of them do not flower at all.

There are two main types of Lemongrass namely, the East Indian Lemongrass (*Cymbopogon flexuosus*) and the West Indian Lemongrass (*Cymbopogon citratus*) (Kulkarni, 2000). The East Indian Lemongrass is indigenous to India and mainly grown in Western Ghats comprising Kerala, Karnataka and Tamil Nadu, Maharastra and parts of Uttar Pradesh and Haryana. It is also known locally as Cochin or Malabar grass which produces the genuine oil for commercial use (Hussian, 1988). The West Indian Lemongrass (*Cymbopogon citratus*) is native of Madagascar, the Comoro islands, Guatemala, Brazil and Haiti, West Indies, Thailand and Bangladesh. A small quantity of oil is also obtained from *Cymbopogon pendulus* popularly known as Jammu Lemongrass or North Indian Lemongrass, since it is grown mainly in Jammu and other North Indian States. Many other

Cymbopogon species also contain volatile oils having important chemical constituents (Rizk, 1983; Manzur-i-Khuda & Rahman, 1984; Nigam, 1987; Mathela & Pant, 1988; Mathela, 1990; Boelens, 1994).

The oils of East Indian and West Indian Lemongrass are dark-yellow to reddish-brown liquids having a characteristic sharp, pungent, lemon-like odour. Both contain aldehydes from 75 to 85 per cent, but the oil of East Indian Lemongrass differs slightly with West Indian product which is usually less soluble in 70 per cent alcohol. The lower solubility of the West Indian Lemongrass (*C. citratus*) oil particularly noticeable after storage of the freshly distilled oil, is due to the presence of myrcene, an olefinic terpene, which on exposure to air and light readily polymerizes. Although the two species can almost be used interchangeably, *C. citratus* is more relevant for cooking. There are two varieties of *C. flexuosus* which are recognized; the white stemmed known as ówella pooluô (*C. flexuosus* Stapf var. *albesecens*) and the red-stemmed known as óchoomanna pooluô (*C. flexuosus* Stapf). The latter produces genuine East Indian oil and is the type commercial cultivated (Hussian, 1988). The oil from the former is of poor quality due to lesser aldehyde contents.

The essential oils of the Lemongrass are a rich source of monoterpenoids. Due to presence of important chemical constituents, Lemongrass oil has commercial value. In the good quality Lemongrass oil (Guenther, 1977), contents of aldehydes i.e. citrali a (geranial) 40-48% and citrali b (neral) 30-37% should be maximum and geraniol (2.9%), citronellal (1.1%), methyl heptenone (1.9%), geranyl acetate (6.7%), betaï elemene (0.075%) and betaï caryophyllene (0.32%) should be minimum. Citral is the name given to a natural mixture of two isomeric acyclic monoterpene aldehydes: geranial (*trans*-citral, citrali a) and neral (*cis*-citral, citrali b). Normally one isomer does not occur without the other. Hence, *C. flexuosus* oil is the most important source of citral, which is a starting material for the production of important chemical compounds viz., ionone, geraniol and Vitamin A (Wright, 1999). The

essential oil is also used in perfumery, flavouring, cosmetics and laundry products due to strong lemon like odour of the oil (Harbone and Baxter, 2001). Lemongrass oil is also used in traditional medicines. It is used in treatment of cough, fever, leprosy and stomach disorders (Kirtikar and Basu, 1935; Maheshwari and Singh, 1965). The tea made from the leaves of Lemongrass is a stomachic tonic, diaphoretic, diuretic and refrigerant. A paste of the leaves made with buttermilk is applied to eradicate ringworm. According to Chawla (1982) the spent marc of *Cymbopogon* sp. can be used for the manufacture of fibre boards, straw boards and paper pulp. Since, good quality lemongrass oil has its importance in chemical preparation, in various industries and in medicines, hence, some research workers have studied the effect of storage and containers in which it is kept, on quality of lemongrass oil (Bhattacharya *et al.*, 1998; Raina *et al.*, 1998).

In spite of numerous uses of lemongrass oil in various industries, it also has wide applications in agriculture. The use of essential oil in pest control has been emphasized by Singh (1997). The oils of certain species of lemongrass have been found effective as germicide and bactericide (Dayal and Purohit, 1971). Several research workers have reported the insecticidal (Rao *et al.*, 2000), nematocidal (Sangwan *et al.*, 1985; Tiyaagi *et al.*, 1986, 1990; Pandey *et al.*, 2001 and Pandey, 2002) and fungicidal (Dikshit & Hussain, 1984; Saksena & Tripathi, 1985; Shadab *et al.*, 1992) activities of lemongrass oils.

Nematodes are a serious threat to the cultivation of agriculture crops all over the world. Root-knot nematodes (*Meloidogyne* spp.) are the most wide spread nematode pests parasitizing almost all the crops, grown all over the world (Saka and Carter, 1987). It has been known to cause the diseases of vegetable crops since 1855. However, in India, its incidence for the first time was noticed in 1901 from tea plants from Southern parts of India (Barber, 1901). The *Rotylenchulus reniformis* is another important nematode which attacks over 140 species of more than 115 plants genera in 46 families. *R. reniformis* is a sedentary,

semi endoparasitic pest of wide range of food, fibre, oilseed, fruit, and vegetable and plantation crops in subtropical and tropical regions of the world. The synthetic chemicals are effective in controlling of nematodes problem but their effect on environment is hazardous due to less degradation. Thus, there is a necessity to search newer and cheaper as well as eco-friendly compounds for nematodes control.

Farmers which grow the aromatic crops do not get proper price in the market due to unorganized market and no minimum support price from any Government/private agencies. Hence, they store the essential oils of the aromatic crops in different manners. Different storage methods affect the quality of lemongrass oil due to its exposure to air and sunlight. The survey of the literature reveals that very little work has been done on effect of storage methods on quality of lemongrass oils. Hence, it was thought desirable to study the effect of storage methods on the quality of Lemongrass oil and also to study its nematocidal activity being wide applications in the agriculture. The present research work is, therefore, undertaken with the following objectives:

1. To select a good storage condition of Lemongrass (*Cymbopogon flexuosus*) oil on the basis of its qualitative parameters.
2. To study the nematocidal activity of essential oils of Lemongrass (*Cymbopogon flexuosus*) for the control of nematodes under laboratory conditions.

CHAPTER II

REVIEW OF LITERATURE

The essential oils of aromatic grasses are used in perfumery, cosmetics and pharmaceuticals. In India, ample researches have been conducted on various aspects of *Cymbopogon* genus such as breeding, agronomy, cytogenetics, and hybridization (Chandra, 1969, 1970, 1975; Gupta, 1972; Hazarika *et al.*, 1981; Kak and Kaul, 1997; Kanzilal *et al.*, 1981; Rao, 1997; Rao *et al.*, 1992; Virmani *et al.*, 1977). However, in comparison to these aspects very little information is available on the qualitative studies and nematocidal activity of the *Cymbopogon flexuosus*. The scientific and economic potential of this grass is so important that any amount of research done would be insufficient to illuminate the complete spectrum of properties of this genus. This chapter deals with review of phytochemicals studies and uses of some *Cymbopogon* grasses including *Cymbopogon flexuosus* in agriculture under the following heads:-

- 2.1 Chemical constituents of essential oils of *Cymbopogon* genus
- 2.2 Effect of various storage methods/techniques on the quality of essential oils
- 2.3 Applications of essential oils in Agriculture

2.1 Chemical constituents of essential oils of *Cymbopogon* genus:

Despite the continuing invention of synthetic chemicals, the essential oils of aromatic plants still remain the most important part of the fine fragrance. Many essential oils are now a days used in perfumery, cosmetics, flavour and pharmaceutical industries. Therefore, quality of essential oils and their isolates is most important. Essential oils from the chemical point of view are often extremely complex mixtures whose components in case of any particular oil are relatively constant when viewed qualitatively or quantitatively. The

qualitative and quantitative makeup of an essential oil is a characteristic which nevertheless varies between certain limits. Variation in oil composition may be due to genetic effects, climatic influence, type of plant organs from which oils are isolated, cultured conditions of the plants and development of plant and plant parts. The quality of the essential oils is assessed by olfactory evaluation and also by the chemical compositions of the oils. The chemical compositions of various *Cymbopogon* grasses are discussed under the following headings:-

2.1.1 *C. flexuosus* (East Indian Lemongrass) oil

2.1.2 *C. citratus* (West Indian Lemongrass) oil

2.1.3 *C. winterianus* & *C. nardus* (Citronella) oil

2.1.4 *C. martinii* var. *motia* (Palmarosa) & *C. martinii* var. *sofia* (Gingergrass) oils

2.1.1 *C. flexuosus* (East Indian Lemongrass) oil

The oil obtained from the *C. flexuosus* is called the East Indian lemongrass oil. The oil of *C. flexuosus* is mobile, yellow to reddish brown in colour, having a characteristic sharp pungent, lemon like odour. Oil is extracted from grass either through hydro-distillation or steam distillation. Masada (1976) reported the following chemical constituents of *C. flexuosus* oil: citralⁱ a and citralⁱ b (65-85%), myrcene (10-20%) geraniol (2i 3%), methyl heptenol (1i 2%), sesquiterpenes (1%) and methyl heptenone (0.2-0.3%). According to Guenther (1977) the important chemical constituents of *C. flexuosus* are myrcene, methyl heptenone, limonene, dipentene, methyl heptenol, linalool, citronellal, nⁱ decylaldehyde, citralⁱ a, citralⁱ b, nerol, geraniol and farnesol. Good quality lemongrass oil contains a minimum of 75 per cent of total citral (both citralⁱ a and citralⁱ b) and other components should be minimum.

The Gas Chromatography (GC) of volatile oil of *C. flexuosus* showed about 45 peaks and some of the identified components viz., new sesquiterpene alcohol (50.56%);

juniper camphor (9.23%); elemicin (7.22%); β -phellendrene (5.78%); β -eudesmol (4.0%); β -selinene (3.84%); β -caryophyllene (3.72%); β -elemene (2.36%); citronellal (1.2%); α -cadinene (1.18%); β -pinene (1.01%); farnesol (0.97%); methyl eugenol (0.92%); limonene (0.84%); myrcene (0.66%); γ -elemene (0.66%); elemol (0.49%); β -caryophyllene oxide (0.48%); geraniol (0.46%); methyl heptenone (0.41%); geranyl acetate (0.20%); α -terpinene (0.18%); p -cymene (0.18%); β -bisabolene (0.13%); eugenol (0.12%); α -curcumene (0.11%); α -pinene (0.10%); α -carene (0.10%); ocimene (0.09%); β -bisabolene (0.08%); terpinolene (0.07%); α -elemene (0.05%); γ -cadinene (0.05%); (Thappa *et al.*, 1979).

Mohammad *et al.* (1981) reported that the hydro distillation of *C. flexuosus* selections *viz.*, SDi 68 and ODi 19 resulted in essential oil content of 0.49% and 0.43%, respectively, on a Fresh Weight Basis (FWB). The oils were examined by Gas Liquid Chromatography (GLC). The main components were geranial, neral and farnesol. Both contained traces of terpinen-4-ol, β -terpineol, α -terpinyl acetate and borneol and SDi 68 also contained nerolidol. No limonene was found in both the selections.

The main components of *C. flexuosus* reported by Formacek and Kubeczka (1982) were geranial (51.19%); neral (30.06%); whereas other minor constituents were geraniol (3.80%); limonene (2.42%); geranyl acetate (1.95%); methyl heptenone (1.43%); linalool (1.34%); myrcene (0.46%); citronellol (0.44%); nerol (0.39%); α -terpineol (0.38%); citronellal (0.37%); caryophyllene (0.32%); α -pinene (0.24%); 2-nonanone (0.07%); *trans*- β -ocimene (0.07%); *cis*- β -ocimene (0.06%); terpinolene (0.05%) and α -thujene (0.03%).

Quality data of *C. flexuosus* oil as reported by Taskinen *et al.* (1983) revealed 60% total citral (neral and geranial) being the principle component. The other minor components reported were: 1,8-cineole; 3,7-dimethyl-7-octen-1-ol; 6-methyl-5-hepten-2-one;

perillene; menthone; linalool; linalyl acetate; neral; neryl acetate; piperitone; geranial; nerol; *p*-cymenol; geraniol; methyl eugenol; elemicin; sesquiterpene alcohol. Manzoori & Khuda *et al.* (1986) reported that *C. flexuosus* of Bangladesh contains methyl eugenol (23.0%), methyl isoeugenol (20%) and limonene (16.5%).

GLC analysis of oil produced from variety OD 19 showed the following constituents: citral a (46.60%); citral b (27.70%); farnesol (12.80%); farnesal (3.00%); alpha terpineol (2.25%); borneol (1.90%); geraniol + nerol (1.50%); terpinyl acetate (0.90%); terpinenol (0.60%); beta terpineol (0.40%); p-cymene (0.04%) and myrcene (0.02%) and the rest being unidentified compounds (Thomas, 1995).

Maheshwari (1995) recorded GC composition of two commercially produced lemon grass oils of *C. flexuosus* and a hybrid CKP 25 (*C. khasianus* × *C. pendulus*). *C. flexuosus* contains: citral a (48.15%); citral b (28.19%); low boilers (6.01%); geranyl acetate (4.63%); geraniol (4.11%); beta caryophyllene (2.76%); methyl heptenone (1.94%); citronellal (1.75%); linalool (1.08%); higher boilers (0.45%); beta elemene (0.42%) and neryl acetate (0.18%). While CKP 25 contains: citral a (44.72%); citral b (32.61%); geranyl acetate (6.71%); low boilers (5.42%); geraniol (2.86%); methyl heptenone (1.94%); linalool (1.10%); citronellal (1.09%); neryl acetate (0.62%); beta caryophyllene (0.32%); higher boilers (0.14%) and beta elemene (0.08%). Composition of these oils are very close except geranyl acetate is higher (6.71%); while geraniol, beta elemene and beta caryophyllene are in much lesser amounts in CKP 25 developed by Regional Research Laboratory, Jammu.

A natural variant of *C. flexuosus* with a citronella-like odour was isolated from cycle 4 population of a recurrent selection program in lemongrass for its essential oil composition (Kulkarni *et al.*, 1997). The main components of the oil were geranyl acetate (25.9%), geraniol (13.1%) and citronellyl acetate (11.2%), while geranial (5.8%) and neral

(3.7%) were minor constituents.

Sarma *et al.* (1999) investigated the chemical composition of the oil of a phenotypic variant of *C. flexuosus*, growing in North East India by Gas Chromatography (GC) and Gas Chromatography coupled with Mass Spectroscope (GC/MS) and identified fifty compounds representing 98.3% of the oil. The major constituents were elemicin (53.0%), limonene (11.6%), betaï ocimenes (7.6%), camphene (4.1%) and lï epï cubenol (3.9%).

The main constituent of *C. flexuosus* essential oil reported by Shiva *et al.* (2002) was citral (75.9%). The other minor constituents were linalool, geraniol, citronellol, nerol, 1,8ï cineole, citronellal, linalyl acetate, geranyl acetate, alphaï pinene, limonene, caryophyllene, ocimene, terpinolene, methyl heptanone, 2ï nonanone and alphaï terpineol.

A Gas Liquid Chromatography (GLC) analysis of the essential oil of *C. flexuosus* showed the presence of the citralï a (46.6%); citralï b (27.7%); farnesol (12.80%) as major constituents and farnesal (3.0%); alpha terpineol (2.25%); borneol (1.90%); geraniol + nerol (1.50%); triphenyl acetate (0.90%); terpinene (0.5%) and Beta terpineol (0.40%) as minor constituents (Farooqi & Sreeramu, 2004).

Bhattacharya (2005) reported the oil content varies from 0.25 to 0.37 per cent. Good quality oil is assessed on the basis of its aldehyde content, which ranges from 70 to 85 per cent. The chemical constituents in the oil are methyl heptenone, myrcene, limonene, terpene, methyl heptenol, linalool, citronellal, nï decylaldehyde, citral, nerol, nerylacetate, geranylacetate, betaï elemene, betaï caryophyllene, geraniol, and farnesol.

2.1.2 *C. citratus* (West Indian Lemongrass) oil

The essential oil obtained from *C. citratus* is called West Indian Lemongrass oil. It contains aldehydes mainly citral from 75 to 85 per cent, but the oil differs slightly from the East Indian oil in that the West Indian oil is usually less soluble in 70 per cent alcohol than the East Indian oil. The lower solubility of the West Indian oil, particularly noticeable after

storage of the freshly distilled oil, is due to the presence of myrcene, an olefinic terpene, which on exposure to air and light readily polymerizes (Guenther, 1977).

The complete investigation of the West Indian type of lemongrass oil revealed the presence of following compounds: isovaleraldehyde, furfural, myrcene, dipentene, methyl heptenone, citronellal, *n*-decylaldehyde, citral *a* (geranial), citral *b* (neral), farnesol, δ -dihydropseudoionone, methyl heptenol, *l*-linalool, α -terpineol, isopulegol, geraniol, nerol, citronellol, farnesol, a bicyclic sesquiterpene, β -camphorene, isovaleric acid, caprylic acid, capric acid, citronellic acid and neric acid (Guenther, 1977).

Formacak and Kubeczka (1982) and Sarer *et al.* (1983) reported the following major components of *C. citratus*: geranial (45.2–55.9%); neral (25–28%); myrcene (8.2–19.2%) and minor constituents *viz.*, *m*-methylhept-5-en-2-one (0.5–2.3%); linalool (0.8–1.1%); geranyl acetate (1.0%); nonanal (0.2–0.7%); geraniol (0.5–0.6%); undecan-2-one (0.4–0.6%); nerol (0.3–0.4%); borneol (0.1–0.4%); α -terpineol (0.2–0.3%); *trans*- β -ocimene (0.1–0.2%); 1,8-cineole (0.2%); *cis*- β -ocimene (0.2%); citronellol (0.1%); α -thujene (trace–0.1%); α -thujone (0.1%); citronellal (0.1%) and limonene (trace).

Abegaz *et al.*, (1983) reported the following constituents in essential oil of Ethiopian *C. citratus*: α -oxo-bisabolene (12.0%); borneol (5.0%); neral (3.3%); fenchone (0.3%); camphor (0.2%); menthone (0.2%); *c*-3-ene (0.1%) and terpinolene (tr.). The essential oil of *C. citratus* was investigated by Ekundayo (1985) and isolated various esters as major components. Some of the reported compounds were α -pinene (1.3%); β -pinene (1.5%); camphene (0.90%); citronellol acetate (1.3%); farnesol (2.4%); geranial acetate (9.9%); hept-5-en-2-one, 6-methyl (1.10%); heptan-2-one, 3-methyl; limonene, linalool oxide (1.0%); menthol (0.6%); nerol acetate (7.5%) .

Recently, Rauber (2005) developed a High Performance Liquid Chromatography (HPLC) method for quantitative determination of citral in *C. citratus* volatile oil. The

concentration of citral in *C. citratus* volatile oil obtained with this assay was 75 per cent. The essential oils obtained by hydroi distillation of fresh leaves of *C. citratus* in Cameroon were analyzed by GC and GC/MS. The main constituents of the oil of *C. citratus* were geranial (32.8%), neral (29.0%), myrcene (16.2%) and beta-pinene (10.5%) (Tchoumboungang *et al.*, 2005).

2.1.3 *C. winterianus* & *C. nardus* (Citronella) Oil

The citronella oil is obtained almost exclusively from *C. nardus* and *C. winterianus* Jowitt. *C. nardus* is commercially cultivated in Sri Lanka only but *C. winterianus* is cultivated in Indonesia, Guatemala, China, India and Vietnam. The oil obtained from *C. nardus* is called Ceylon citronella oil whereas the oil from *C. winterianus* is called Java citronella oil. Ceylon citronella oil generally contains 55-60% geraniol and 7-15% aldehydes whereas Java citronella oil contains minimum 35% of both geraniol and aldehydes (Weiss, 1997). An interspecific hybrid *C. confertiflorus* × *C. jwarancusa*, jamrosa yields an oil containing geraniol 75-80% and geranyl acetate 20-25% (Krishnamoorthy, 1989).

Guenther (1977) reported the following components of Ceylon citronella oil: camphene, limonene, dipentene, methyl heptenone, di-citronellol, l- borneol, thujyl alcohol, nerol, geraniol, geranyl acetate, citronellyl acetate, citronellyl n- butyrate, methyl eugenol, sesquicitronellene, a sesquiterpene and farnesol. The constituents of Java citronella oil were isobutyl alcohol, isoamyl alcohol, 3-hexenol, l- 3-methyl-1-pentanol, n-1-hexanol, methyl heptenone, isovaleraldehyde, furfural, benzaldehyde, di- l- methyl-3-cyclohexanone, 2-hexenol, l- 3-methyl-1-pentanal, l- limonene, dipentene, citronellal, di-citronellol, geraniol, citral, chavicol, methyl eugenol, elemol, cadinol, cymbopol, gamma-cadinene, sesquicitronellene, dicitronelloxide and vanillin.

Farooqi & Sreeramu (2004) reported that *C. winterianus* (Java type) oil has higher

alcohol content (90-95%) than the *C. nardus* (Ceylon type) oil. Citronella oil contains geranyl acetate, citronellyl acetate, limonene, elemol & other sesquiterpene alcohols, beta-elemene, gamma-cadinene and traces of cubebene, calamenene, bourbonene, bisabolene, eugenol, isopulegol, nerol, linalool, methyl heptenone, myrcene, and alpha pinene. Average oil content is about 1.0% on Fresh Weight Basis (FWB) of leaves.

2.1.4 *C. martinii* var. *motia* (Palmarosa) & *C. martinii* var. *sofia* (Gingergrass) oils

Palmarosa oil, commonly known as East Indian geranium oil and gingergrass oil, are obtained from two varieties of *C. martinii*, known in India as rosha or russa grass. Although morphologically almost identical, the two varieties have different growth forms, favour different environments and yield distinct oils. Palmarosa oil is obtained from *C. martinii* (var. *motia*) and gingergrass oil from *C. martinii* (var. *sofia*). Palmarosa oil (also known as Rusa oil and East Indian geranium oil) is pale yellow to olive with a sweet floral odour. Over 100 compounds have been found in the oil major components are geraniol, limonene, linalool, beta-caryophyllene, neral and farnesol (Surburg, 1988; Boelens, 1994). The chemical components of palmarosa reported by Guenther (1977) were formaldehyde, isovaleraldehyde, methyl heptenone, dipentene, citronellal, citral, geraniol (75-95%), geranyl acetate, geranyl n-caproate and farnesol, while that of gingergrass oil were d-limonene, dipentene, di alpha phellandrene, an aldehyde (C₁₀H₁₆O).

Several workers have contributed to the identification of different aroma chemicals in the oils of palmarosa. Saxena & Maheshwari (1980) isolated geranyl butyrate and Mohamad Faiz *et al.* (1981) have brought out the presence of beta cymene, nananol, citronellol, beta terpeneol, beta humulene and alpha terpeneol. Analysis of Madagascar oil showed 4.75% hydrocarbons, consisting of 46% monoterpenes, 52% sesquiterpenes, 1.6% alkanes and 0.4% unknowns (Gaydou and Randriamiharisoa, 1987). Thomas (1995) reported the following major components in the palmarosa oil: geraniol (70-80%) and

geranyl acetate (5i 20%).

2.2 Effect of various Storage methods/techniques on quality of Essential oils:

Storage of essential oils is an important aspect in essential oil industry. Hence, procurement of appropriate storage vessels and ensurement against contamination and adulteration must get prior consideration. All kinds of essential oils must be free from moisture and metallic impurities. Fresh volatile oils on long standing are affected in colour and quality by light, heat and air. Spoilage process is activated by oxygen, moisture and light. Oxidation and resinification of essential oils cause darkening of colour. Apart from this polymerization, hydrolysis of esters and interactions of functional groups also cause spoilage. Essential oils with high terpene contents are prone to spoilage by oxidation and resinification. Oils containing high percentage of esters turn acidic due to partial hydrolysis of esters under improper storage conditions.

2.2.1 Effect of storage methods on quality of *C. flexuosus* (East Indian Lemongrass) oil:

Oils correctly stored i.e. protected from air, heat and sunlight remain stable for long periods, with little or no loss of citral. To reduce the adverse effect of storage on citral content of *C. flexuosus* oil in India, several indigenous antioxidants were tested; an extract of betel leaves (*Piper betle*) proved most successful, maintaining citral content at 83% compared to 72% in oil which was not treated (Kurain & Nair, 1984).

Bhattacharya *et al.* (1998) compared the quality of freshly distilled and one year stored (in aluminium containers with a head space kept under ambient conditions in semi arid tropical climate) essential oils of Lemongrass [*C. flexuosus* (nees) ex. Steud Wats]. The oil quality deteriorated on prolonged storage. The freshly distilled oil contained higher concentration of neral (33.21%), geranial (53.11%) and caryophyllene oxide (1.30%). The stored oil recorded greater contents of 6i methylhepti 5i eni one (3.79%), limonene (1.26%), gammai terpinene (1.19%), linalool (1.47%), citronella (4.71%), isopulegol (1.43%),

borneol (1.15%), geraniol (3.49%), geranyl acetate (1.68%), and betaï caryophyllene (1.15%). The neral and geranial percentages decreased by 22.82% and 27.66% in the stored oil in comparison to freshly distilled oil. Therefore, prolonged storage of lemongrass oil should be avoided and the oil should be marketed as quickly as possible.

The effect of container materials and long storage periods on the quality of aromatic grass oils was studied by Raina *et al.* (1998). The freshly distilled light yellow coloured oils of palmarosa (*C. martinii*), citronella (*C. nardus*) and lemongrass (*C. flexuosus*) were stored separately in 1 litre size containers made of amber glass, aluminium, iron and high density polyethylene. The containers were placed in a dark room at room temperature in completely randomized design with five replications. The oil samples were drawn from the storage containers periodically and analyzed for the color and percent composition of the major constituents of the oil. The oil colour changed only when stored in iron containers. There was little change in the percentage composition of major constituents in the oils stored in the three containers made of widely different materials.

In an experiment on *Cymbopogon flexuosus* oil carried out at Puerto Rico, it was reported that contents of citral decreases gradually on storage and aging of the oil. This decrease is usually accompanied by an increase in specific gravity of the oil. Oil with citral content of 78% and specific gravity of 0.889, after two years of storage, exhibited a citral content of only 60% and a specific gravity of 0.895. In a sample, which contained moisture, the citral content decreased from 79 to 68% within three months, whereas another sample, which had been carefully dried, under the same conditions, exhibited a decrease, in citral content, from 79 to 74% only (Guenther, 1977).

2.2.2

Effect of storage methods on quality of *C. citratus* (West Indian Lemongrass) oil:

Brandares, *et al.* (1987) conducted storage studies of essential oils from some

Philippine plants. *C. citratus* (D.C.) Stapf. lemongrass oil was extracted from fresh; air dried and wilted leaves of *C. citratus* by water distillation. The effects of temperature, light and storage period on the quality of oil were examined. Evaporation by exposure to sunlight was three times faster than at room temperature and 46 times faster than when stored in refrigerator. The citral content decreased after a year of exposure under the same temperature. There was deterioration in the quality of oil after exposure to light. The most stable of the three oil samples was the one extracted from fresh leaves.

Experimenting with the West Indian type of lemongrass oil, the Puerto Rico Experiment Station, United States Department of Agriculture, found that samples of lemongrass oil exposed to air and light suffered the most physical and chemical changes (Guenther, 1977). Their solubility was lowered considerably, the citral content decreased (from 86.07 to 78.81%, after eleven months), and the specific gravity increased (from 0.8971 to 0.9055) during this period. The freshly distilled oils were soluble in 3 volumes of 70% alcohol; after eleven months they were soluble in 3.7 volumes of 80% alcohol. At the end of the experiments (eleven months) the oil was viscous and resinous. Therefore, in the storage of lemongrass oil, it is important to exclude air, light, and moisture before it is filled into the storage tanks. Handling (filtering, etc.) of oil in open air, should be avoided, as this may affect the citral content.

2.3 Applications of essential oils in Agriculture:

Agriculture production is affected by a number of pest organisms such as insects, mites, fungi, bacteria and nematodes. Increased resistance developed by insects and pathogens to chemical pesticides and the public concern about the chemicals in general initiated a re-evaluation of pesticide use. The developed and developing nations are looking towards the use of natural products i.e. plant origin materials as pest control agents which have negligible effect on natural beneficial insects, low environmental impact because of

their biodegradability. Chances of development of resistance in insects to the plant extracts are unlikely because extracts have more than one chemical for biological activity. Moreover plant extracts are cheaper than synthetic pesticides. Here, review of applications of essential oils/extracts of *Cymbopogon* genus in controlling nematodes and other pathogens are described.

2.3.1 Application of essential oils/extracts of *Cymbopogon* genus in controlling nematodes:

Plant parasitic nematodes, which cause damage to crop not only by feeding on plants but also interacting with other organisms, are called phytonematodes. For economic crop production it is essential to control nematodes in fields. The high cost of synthetic nematicides poses a serious problem in their wider application especially in undeveloped and developing countries. However, botanicals have been found promising against several nematode pests.

Sangwan, *et al.* (1985) reported the nematicidal activity of essential oils of *Cymbopogon* grasses. The essential oils of three *Cymbopogon* grasses (*C. martinii* var. *motia*, *C. flexuosus* and *C. winterianus*) were found to be toxic to varying extents against *Anguina tritica*, *Tylenchulus semipenetrans*, *Meloidogyne javanica* and *Heterodera avenae*. The leaf extract prepared from *C. flexuosus* was highly toxic to *Meloidogyne incognita*, *Rotylenchulus reniformis*, *Tylenchorhynchus brassicae*, *Hololaimus indicus* and *Helicotylenchus indicus* (Tiyagi *et al.*, 1990). The toxicity was related to concentration of leaf extract and to the exposure period. *Meloidogyne incognita* was the most affected with 100% mortality in standard solution at 12 h, while *H. indicus* was least affected with 20% mortality. The extract also significantly inhibited egg hatch by *Meloidogyne incognita*. The inhibition of the juvenile hatching over control was 100, 85 and 76%, respectively in standard, 10 and 100 times dilution.

Pandey (2002) reported that the dried powder of *C. flexuosus*, *C. winterianus*,

Spilenthesis acmella, *Costus speciosus* and neem cake suppressed the root knot nematode *Meloidogyne javanica* in *Ammi majus*, which is a newer and cheaper as well as eco-friendly for nematode control. Pandey, *et al.* (2001) studied the nematicidal activities of the flower extracts of 20 plants at 1:10 (w/v) concentration against root knot nematode (*Meloidogyne incognita*) under laboratory conditions. Extracts of *Moringa oleifera*, *Tagetes erecta*, *Alstonia scholaris*, *Caesalpinia pulcherrima*, *Argemone mexicana*, *Brassica campestris* were highly toxic. Mild toxicity was recorded by *Lippia alba* and *C. flexuosus* whereas the least activity was recorded by *Calliandra* sp., *Hibiscus rosasinensis*, *Vetiveria zizanioides*, *Cyperus rotundus*, *Tabernaemontana divaricata*, *Thevetia nerifolia* and *A. moschatus*.

Goel & Madan (2004) evaluated the aqueous extracts of various aromatic plants *in vitro* for their nematicidal activity against root-knot nematode, *Meloidogyne javanica*. The aromatic plants screened included oils of *C. flexuosus* (Lemon grass), *C. winterianus* (Java-citronella) and *C. martinii* (Palmarosa). Oils of *C. flexuosus* and *C. martinii* were found highly effective against root knot nematode.

2.3.2 Applications of essential oils/extracts of *Cymbopogon* spp. in controlling pathogens, fungal and bacterial diseases:

Various plant origin materials have been in use in agriculture for the management of crop diseases caused by various types of pathogens, fungal and bacterial diseases. Some of the applications of essential oils/extracts of *Cymbopogon* spp. are as follows:

Handique and Singh (1990) reported the antifungal action of lemongrass oil (*C. citratus*) on plant pathogens viz., *Rhizoctonia solani*, *Sclerotium rolfsii* and *Sclerotinia sclerotiorum*. Maximum inhibition was observed in the case of *Rhizoctonia solani*, followed by *Sclerotinia sclerotiorum* and *Sclerotium rolfsii*. The essential oils of lemongrass (*C. citratus*), rosha grass or palmarosa (*C. martinii* var. *motia*) and khavi grass (*C. jawarancusa*) showed antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Shigella flexneri* and *Salmonella typhi*. The activity of the oil might be attributed to the components

citral, geraniol and piperitone (Syed *et al.*, 1990). Gangradel *et al.* (1990 & 1991) studied *in vitro* antimicrobial effect of the essential oils of *C. martinii* var. *motia* against *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*, *Penicillium* sps, *Staphylococcus aureus*, *Staphylococcus pyogenes*, *E. coli* and *Corynebacterium oviswas*. The inhibitory effect of lemongrass (*C. flexuosus*) essential oil (citral content 70–80%) against *Monila sitophilia*, *Penicillium digitatum*, *Aspergillus parasiticus*, *Aspergillus niger* and *Aspergillus fungus* was reported by Shadab *et al.* (1992).

The aqueous extracts of leaves of *C. pendulus*, was found effective against *Collectotrichum camelliae*, *Alternaria solani*, *Curvularia lunata*, *Microsporum gypseum* and *Trichophyton mentagrophytes* (Chakraborty *et al.*, 1991; Pandey *et al.*, 1996). Mehmood *et al.* (1997) reported the antifungal properties of essential oils of Lemongrass (*C. flexuosus*), Palmarosa (*C. martinii*) against *Aspergillus*. Citral, geraniol and eugenol showed considerable antifungal activity while citronellol and cinnamaldehyde were inactive. Palmarosa (*C. martinii* var. *motia*) oil was evaluated against chilli fruit rot caused by *Colletotrichum capsici* at different concentrations *in vitro* following poisoned food technique and spore germination assay. It exerted 100% inhibition on mycelial growth and spore formation even at 0.025% concentration (Jeyalakshmi and Seetharaman, 1997). The essential oils of *C. citratus* have shown strong antifungal activity against *Aspergillus flavus*, the dominant storage fungus causing biodeterioration of some food stuffs (Dubey *et al.*, 1999 & 2000). Saikia *et al.*, (1999) reported antimicrobial activity of essential oils from *C. martinii* (Palmarosa), *C. flexuosus* (Lemongrass) and *C. nardus* (Citronella) against *Aspergillus niger*, *Candida albicans*, *Sporothrix schenckii*, *Microsporum gypseum*, *Aspergillus flavus*, *Staphylococcus aureus*, *Enterococcus faecalis* and *S. epidermidis*. In the bacterial inhibition assay, Lemongrass and Palmarosa oils were found to be superior to citronella oil. While linalool, linalyl acetate and citronellal did not show activity against both

bacterial and fungal strains, geraniol and citronellol has conspicuous antimicrobial activity.

The antibacterial activity of Lemongrass (*C. flexuosus*) inflorescence oil against *Klebsiella pneumoniae*, *Bacillus subtilis*, *Proteus vulgaris* and *Pseudomonas aeruginosa* was reported by Sarma (2003) while Prashar *et al.* (2003) reported antimicrobial properties of palmarosa (*C. martinii*) oil against cells of *Saccharomyces cerevisiae*. Bassole *et al.* (2003) reported the ovicidal and larvicidal activity of essential oils of *C. proximus* against *Aedes aegypti* and *Anopheles gambiae*. Chandrashekar and Joshi (2004) studied antimicrobial efficacy of essential oils from the leaves of *C. citratus*, *C. flexuosus* (citril chemotype), *C. flexuosus* (geraniol chemotype) and *C. nardus* against four organisms viz. *Porphyromonas gingivalis*, *Prevotella intermedia*, *Beta-phi-lotomicron* and *Bacteroides fragilis* causing inflammation of gums. The oils showed good to moderate efficacy against *Porphyromonas gingivalis* and *Prevotella intermedia*.

Recently, Yonzon *et al.* (2005) showed the antimicrobial activities of the essential oil of palmarosa (*C. martinii* var. *martinii*) against *Staphylococcus aureus*, *Corynebacterium amycolactum*, *Escherichia coli*, *Candida albicans* and *Aspergillus Ochraceus*. The antifungal efficacy of essential oils of five plants including *C. flexuosus* against *Candida albicans* have been reported by Tampieri *et al.* (2005). Wannissorn *et al.* (2005) reported the antimicrobial activity of thirty two essential oils including *C. citratus* against *Salmonella* spp., *E. coli*, *Camphylobactor jejunii* and *Clostridium perferingens*. The essential oils of *C. martinii* and *C. winterianus* were found effective against *Candida albicans* (Duarte *et al.*, 2005). The powder and essential oil from leaves of *C. citratus* were found effective against *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus tamaris* and *Penicillium citrinum* (Bankole *et al.* 2005). Tchoumbougna *et al.* (2005) reported that the essential oil of *C. citratus* showed significant antimalarial activities on mice.

CHAPTER III

MATERIALS AND METHODS

3.1 Procurement of Lemongrass materials

The materials of six genotypes/varieties of *C. flexuosus* grasses were obtained from the Research Farms of Medicinal, Aromatic and Under Utilized Plants Section, Department of Plant Breeding, CCS HAU, Hisar. The six genotypes/varieties were as follows: genotypes HLi 2, HLi 3, HLi 11 & NLGi 118 and varieties ODi 19 & CKPi 25. The crops were harvested during November 2005.

3.2 Isolation of Essential oils

The essential oils of these six genotypes/varieties were extracted by hydro distillation of freshly harvested grasses. The weighed samples (one kg each) of the freshly harvested grasses were filled in the distillation flasks fitted with the Clevenger apparatus. The apparatus consists of specially designed oil traps and a small condensor of the ñcold fingerò type. The distillation unit was heated for 4 to 5 hrs so that all the essential oil distills out from the grass. After separation of essential oil from water, anhydrous sodium sulphate in small quantity was added in essential oils to remove the traces of water. The per cent essential oil content on Fresh Weight Basis (FWB) was calculated as per the following formula:

$$\text{Oil content (\%)} = \frac{\text{Oil content (ml)} \times 100}{1000 \text{ (g)}}$$

The essential oils were stored in the glass vials and kept in refrigerator at 4°C.

3.3 Quality of Essential oils

The essential oils were analyzed by Gas Liquid Chromatography (GLC) using

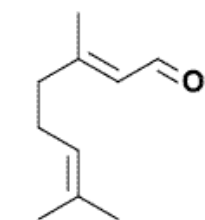
Shimadzu Gas Chromatograph (Model GC 17A) fitted with Flame Ionization Detector (FID) and a capillary column (30 m $\times$ 0.32 mm I.D. $\times$ 0.25 μ m film thickness) with thermo TR-WAX (Polyethylene glycol) as the stationary phase. The GLC conditions are given in following Table-1.

Table 1: GLC parameters for estimation of citral-a (geranial), citral-b (neral), geraniol and citronellal in essential oil of the lemongrass

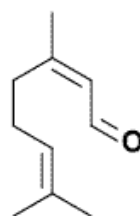
Oven temperature		
70°C (2 min.)	@ 4°C / min	90° C (0 min.)
	→	@ 10° C/min.
		200°C (5 min.)
Injector Port Temperature	:	240°C
Detector Port Temperature	:	260°C
Total Run time	:	23 minutes
Gas Flow		
N ₂ (Through column)	:	1.3 ml/min
N ₂ (Makeup gas)	:	54 ml/min
Split ratio	:	1:40
Velocity	:	24
Gas Pressures		
Zero Air	:	50 kpa
H ₂	:	60 kpa
N ₂ (Auxillary gas)	:	60 kpa

Constant flows of N₂, H₂ and Zero air gases were maintained through constant pressure of each gases for constant retention time and sensitivity of each constituents analysed. 0.5 μ l of each essential oil was injected in the injector port of GLC and retention time and area of peaks were recorded. The components citrali a (geranial), citrali b (neral), geraniol and citronellal were identified by comparing their retention times with those of standards recorded under the same operating parameters. The retention times observed for

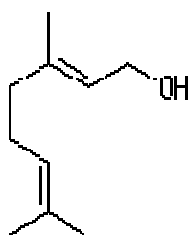
citrali a (geranial), citrali b (neral), geraniol and citronellal were 16.42, 15.53, 17.84 and 12.35 minutes, respectively. The structures of citrali a, citrali b, geraniol and citronellal are given below:



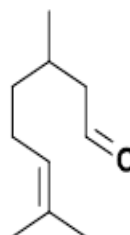
Citral-a (Geranial)



Citral-b (Neral)



Geraniol



Citronellal

3.4 Isolation of Essential oil of CKPi 25 for Storage Studies

On the basis of per cent oil content data and quality analysis, explained in section 3.3, CKPi 25 variety was selected for procurement of large quantity of essential oil for studying storage effect on quality of lemongrass oil. About 300 ml of essential oil of CKPi 25 was extracted using Clevenger apparatus. For the collection of the 300 ml essential oil, four Clevenger apparatus were used daily for ten days, as one set gives about 7.5 ml of essential oil with one kg of fresh weight sample. At the end, essential oil from all the sets was pooled together, its quality analysis was done before storage and stored in different vials in the laboratory as per the method given in the next section 3.5.

3.5 Storage of Essential oil of CKP-25

The essential oil of CKPi 25 isolated as per section in section 3.4 was stored as per the following storage methods:

- (i) T₁ Storage of oil in closed glass (Borosil made) vials.
- (ii) T₂ Storage of oil in closed metal (Aluminium) vials.
- (iii) T₃ Storage of oil in closed plastic (High density polyethylene) vials.
- (iv) T₄ Storage of oil in open glass (Borosil made) vials.
- (v) T₅ Storage of oil in open metal (Aluminium) vials.
- (vi) T₆ Storage of oil in open plastic (High density polyethylene) vials.

Fifteen ml of essential oil was kept in each vial as per the above storage methods. Each treatment was replicated thrice. The quality analysis of these samples was done initially at 0 month i.e. just before keeping oil in different vials for storage and after 2, 4 and 6 months of storage. The following chemical constituents were estimated from the oils of different vials at different storage periods.

- (i) Citral a (geranial)
- (ii) Citral b (neral)
- (iii) Citronellal
- (iv) Geraniol

3.6 Nematicidal activity of aqueous extracts of Essential oil of lemongrass genotypes/varieties

Nematicidal activity of essential oils of six genotypes/varieties of *C. flexuosus* were performed on three prominent nematodes species, chosen on the basis of their geographical distribution and of their economic importance. These are

- i) *Meloidogyne javanica*
- ii) *Meloidogyne incognita*
- iii) *Rotylenchulus reniformis*

3.6.1 Preparation of Stock solution

For evaluating the nematicidal activity stock solution of essential oils were prepared by making an emulsion of 0.5 ml of oil, 7 drops of exalin (as surfactant) and few drops of

water. The final solution (1%, v/v) was made 50 ml by adding distilled water. The 1% solution was taken as Stock solution. Seven drops of exalin were just sufficient to make an emulsion of essential oil and water.

3.6.2 Preparation of control solution

For control solution only exalin (7 drops) was dissolved in 50 ml of distilled water.

3.6.3 Root-knot nematodes (*Meloidogyne javanica* and *Meloidogyne incognita*)

Culture preparation, Experiment design, Data observation and Per cent mortality was done as per the following methods:

3.6.3.1 Culture

Identification of root-knot nematodes, *Meloidogyne javanica* and *Meloidogyne incognita* was done prior to its propagation in pure culture. For the purpose of identification, galled brinjal roots were collected from root-knot infested fields/pots and were brought to the laboratory. The nematodes infecting brinjal roots were identified as *Meloidogyne* sp. on the basis of perineal patterns of adult females (Taylor *et al.*, 1955). The egg masses from the infected roots were used as inoculum to propagate the pure culture of *M. javanica*/*M. incognita* in earthen pots (30 cm). Four week old brinjal seedlings (cv. Pusa Purple Long) raised in steam sterilized soil, were transplanted into these pots and inoculated with egg masses of *M. javanica*/ *M. incognita*. Four months after inoculation, the potted plants with heavy galling were re-examined for nematode species. Perineal pattern of 20 randomly picked females were prepared and the species confirmed to be *M. javanica*/*M. incognita*. The culture was periodically sub cultured for multiplication and purity.

3.6.3.2 Experiment:

Measured quantity of larval suspension, containing approximately 100 to 200 second stage larvae of *nematodes* were taken in 5 cm diameter Petri dishes. The following concentrations were taken:

- i) 1:5 (1 ml of stock solution + 5 ml of nematode suspension)
- ii) 1:10 (0.5 ml of stock solution + 5 ml of nematode suspension)
- iii) 1:20 (0.25 ml of stock solution + 5 ml of nematode suspension)
- iv) 1:40 (0.125 ml of stock solution + 5 ml of nematode suspension)
- v) 1:80 (0.0625 ml of stock solution + 5 ml of nematode suspension).
- vi) Control (1 ml of control solution + 5 ml nematode suspension)

Each treatment was replicated three times. The Petri dishes were kept in BOD incubator for 48h at $28 \pm 1^{\circ}\text{C}$.

3.6.3.3 Data observation

Larval mortality after 48 hours of exposure to different concentrations of extracts was recorded by counting living and dead second stage larvae under stereoscopic binocular microscope.

3.6.3.4 Per cent Mortality

Per cent larval mortality was calculated and the data analyzed statistically by applying CRD factorial design. Percent Mortality was calculated using the following formula:

$$\text{Per cent mortality} = \frac{\text{Number of Dead larvae} \times 100}{\text{Total number of larvae}}$$

3.6.4 *Rotylenchulus reniformis*

Culture preparation, Experiment design, Data observation and Per cent mortality was done as per the following methods:

3.6.4.1 Culture

The species identification of *Rotylenchulus reniformis* was confirmed on the basis of morphological characters of immature females, males and juveniles as given by Dasgupta *et al.*, (1968). For the preparation of pure culture of *R. reniformis*, the egg masses were

recovered from the infected roots of jasmine plants. Soil was steam sterilized and 30 cm diameter clay pots were filled and castor seeds were sown. After germination, plants were inoculated with single egg mass. Periodic attention was given to nematode development and purity of the culture. The desired numbers of egg masses collected from infected castor plants were kept over assembly of double fold tissue paper, wirenet and Petri plate (10 cm diameter). Sufficient amount of water was added to the plates to keep the egg masses just submerged. The juveniles collected were kept in water for a week so that all juveniles may reach to immature stage. The aliquot was examined for immature females and males of *R. reniformis* under binocular stereoscopic microscope in counting dish. One ml of the suspension was counted thrice and accordingly the nematodes (immature females and males) present in total volume of suspension were calculated. Various stages of females were compared with the description of *R. reniformis* described by Dasgupta *et al.* (1968) so as to confirm the species.

The Experiment, Data observation and Per cent mortality were as per section 3.6.3.2; 3.6.3.3 and 3.6.3.4, respectively.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Standardization of GLC conditions

For estimation of citrali a (geranial), citrali b (neral), geraniol and citronellal in the essential oils of lemongrass (*C. flexuosus*) genotypes/varieties various GLC conditions were tried for optimum area and better resolution of peaks of citrali a (geranial), citrali b (neral), geraniol and citronellal. The standardized GLC conditions for estimation of citrali a, citrali b, geraniol and citronellal in the essential oil of lemongrass are given in Tablei 1 in Materials and Methods. The retention times observed for citrali a, citrali b, geraniol and citronellal were 16.42, 15.53, 17.84 and 12.35 minutes, respectively.

4.2 Qualitative analysis of essential oils of Lemongrass:

The data of oil content on Fresh Weight Basis (FWB) in six genotypes/varieties of lemongrass is given in Tablei 2. The data showed that oil content ranged from 0.24 to 0.74 per cent. The highest oil content was recorded in case of CKPi 25 (0.74%) followed by HLi 11 & NLGi 118 (both 0.36%) and HLi 2 (0.27%).

The qualitative and quantitative data of citrali a, citrali b, geraniol and citronellal in the essential oils of lemongrass genotypes/varieties is presented in Tablei 2. The citrali a (geranial) content ranged from 47.1 to 52.4 per cent. The citrali a (geranial) content was maximum in HLi 2 (52.4%) followed by CKPi 25 (49.6%), ODi 19 (48.5%) and HLi 11 (48.1%). The citrali b (neral) content ranged from 30.3 to 37.5 per cent. The maximum neral content was recorded in NLGi 118 (37.5%) followed by HLi 3 (36.0%), ODi 19 (35.7%) and HLi 11 (35.1%). The total citral content ranged from 82.7 to 84.8 per cent. The total citral content was maximum (84.8%) in NLGi 118 followed by ODi 19 (84.2%) and CKPi 25 (83.5%).

Table 2: Oil content (%), Citral-a, Citral-b, Geraniol and Citronellal contents (%) in Essential oils of 6 promising genotypes/varieties of Lemongrass (*Cymbopogon flexuosus*)

Sr. No.	Genotypes /Varieties	Oil content (% FWB)	Citral content (%)			Geraniol (%)	Citronellal (%)
			Citral-a (geranial)	Citral-b (neral)	Total Citral (%)		
1.	HL-2	0.27	52.4	30.3	82.7	2.8	0.9
2.	HL-3	0.25	47.1	36.0	83.1	2.5	0.8
3.	HL-11	0.36	48.1	35.1	83.2	2.7	1.1
4.	NLG-118	0.36	47.8	37.5	84.8	2.8	0.9
5.	OD-19	0.24	48.5	35.7	84.2	2.7	0.9
6.	CKP-25	0.74	49.6	33.9	83.5	2.8	1.0
Range		0.24-0.74	47.1-52.4	30.3-37.5	82.7-84.8	2.5-2.8	0.8-1.1

The geraniol content ranged from 2.5 to 2.8 per cent (Tablei 2). The essential oil of HLi 2, NLGi 118 and CKPi 25 recorded the maximum geraniol content (2.8%) followed by HLi 11 and ODi 19 (both containing 2.7%). Citronellal content ranged from 0.8 to 1.1 per cent. Maximum citronellal content was recorded in HLi 11 (1.1%) followed by CKPi 25 (1.0%) and NLGi 118, HLi 2 and ODi 19 (all 0.9%).

4.3 Effect of storage on qualitative parameters of essential oil of CKP-25

To elucidate variations in citrali a, citrali b, geraniol and citronellal in oil of CKPi 25 during storage, the GLC analysis of the freshly distilled essential oil of CKPi 25, stored at room temperature upto six months in different vials viz., glass, metal (aluminium) and plastic in both closed and open conditions was carried out. The data of citrali a, citrali b, geraniol and citronellal is presented in Tables 3i 12.

4.3.1 Citral-a (geranial) content

The data (Tables 3i 4) showed that the average citrali a content was 47.0% initially (at zero month) just before storage which decreases during storage in closed as well as in open vials containing essential oils. In the essential oil kept in closed glass vials, average citrali a content was (Tablei 4) 44.2%, 42.2% and 41.5% after 2, 4 and 6 months of storage, respectively. In the essential oil kept in closed metal vials, average citrali a content was 47.0% at initial stage and 41.3%, 38.0% and 35.4% after 2, 4 and 6 months of storage, respectively. In the essential oil kept in closed plastic vials, average citrali a content was 47.0% at initial stage (zero month) and 42.5%, 39.7% and 38.0% after 2, 4 and 6 months of storage, respectively.

In the essential oil kept in open glass vials, citrali a content was 47.0% at initial stage and 43.5%, 41.1% and 39.0% after 2, 4 and 6 months of storage, respectively (Tablei 4). In the essential oil kept in open metal (aluminium) vials, citrali a content was 47.0% at initial stage and 40.8%, 36.3% and 33.8% after 2, 4 and 6 months of storage, respectively.

Table 3: Citral-a content (%) of Essential oil of Lemongrass variety CKP-25 stored in different conditions at different storage periods

Sr. No.	Treatments	Vials ↓	Citral-a content (%)															
		Storage → months	0 Month				2 Months				4 Months				6 Months			
			R ₁	R ₂	R ₃	Mean	R ₁	R ₂	R ₃	Mean	R ₁	R ₂	R ₃	Mean	R ₁	R ₂	R ₃	Mean
1.	Closed	Glass	45.4	46.5	49.1	47.0	41.3	44.9	46.4	44.2	38.4	42.7	45.5	42.2	37.6	42.0	44.9	41.5
2.		Metal	45.4	46.5	49.1	47.0	44.3	40.9	38.7	41.3	38.2	35.7	40.6	38.0	32.7	36.3	37.2	35.4
3.		Plastic	45.4	46.5	49.1	47.0	45.7	40.1	41.7	42.5	36.4	40.9	41.8	39.7	37.9	40.3	35.8	38.0
Mean			45.4	46.5	49.1		43.8	42.0	42.3		36.7	39.8	42.6		36.1	39.5	39.3	
4.	Open	Glass	45.4	46.5	49.1	47.0	42.0	43.4	45.1	43.5	39.6	41.2	42.5	41.1	36.9	40.2	39.9	39.0
5.		Metal	45.4	46.5	49.1	47.0	38.1	44.4	39.7	40.8	33.9	40.1	34.9	36.3	30.5	37.4	33.5	33.8
6.		Plastic	45.4	46.5	49.1	47.0	40.4	39.8	45.2	41.8	37.4	36.4	41.6	38.5	33.3	34.6	39.2	35.7
Mean			45.4	46.5	49.1		40.2	42.5	43.3		40.0	39.2	39.7		33.6	37.4	37.5	

Table 4: Mean Citral-a content (%) data of Essential oil of Lemongrass variety CKP-25 stored in different conditions at different storage periods

Sr. No.	Treatments↓		Citral-a content (%) [*]				
	Storage Months→		0 Month	2 Months	4 Months	6 Months	Mean
1.	Closed	Glass	47.0	44.2	42.2	41.5	43.7
2.		Metal	47.0	41.3	38.0	35.4	40.4
3.		Plastic	47.0	42.5	39.7	38.0	41.8
Mean			47.0	42.7	40.0	38.3	
4.	Open	Glass	47.0	43.5	41.1	39.0	42.7
5.		Metal	47.0	40.8	36.3	33.8	39.5
6.		Plastic	47.0	41.8	38.5	35.7	40.8
Mean			47.0	42.0	38.6	36.2	
Overall Mean			47.0	42.4	39.3	37.3	

* Average of Three Replicates

CD at 5%

Factor A (Closed/ Open)	=	N.S.
Factor B (Glass/Metal/Plastic)	=	1.516
Factor C (Storage Period)	=	N.S.
Factor A × B	=	2.144
Factor A × C	=	N.S.
Factor B × C	=	N.S.
Factor A × B × C	=	N.S.

In the essential oil kept in open plastic vials, citral α content was 47.0% initially (zero month) and 41.8%, 38.5% and 35.7% after 2, 4 and 6 months of storage, respectively.

The overall mean (Table 4) of citral- α content was maximum in the essential oils kept in closed glass vials (43.7%) followed by plastic vials (41.8%) and metal (aluminium) vials (40.4%). Similar trend (glass, 42.7%; plastic, 40.8%; and aluminium, 39.5%) was also observed in the essential oil kept in open vials. However, the statistical analysis of citral data (Table 4) showed that the effect of closed and open vials was non significant whereas the effect of glass, metal and plastic vials was found significant. The effect of storage period on citral- α content was non significant.

4.3.2 Citral-b (neral) content:

The data (Tables 5 & 6) showed that the citral β content of essential oil of CKPi 25 was 36.5% initially (at zero month) just before storage which decreases during storage in closed vials as well as in open vials containing essential oils. In the essential oil kept in closed glass vials, average citral β content (Table 6) was 35.1%, 33.3% and 32.5% after 2, 4 and 6 months of storage, respectively. In the essential oil kept in closed metal vials, citral β content was 36.5% at initial stage and 32.5%, 29.9% and 28.9% after 2, 4 and 6 months of storage, respectively. In the essential oil kept in closed plastic vials, citral β content was 36.5% at initial stage (zero month) and 33.1%, 30.7% and 30.3% after 2, 4 and 6 months of storage, respectively.

In the essential oil kept in open glass vials, citral β content was 36.5% at initial stage and 34.0%, 32.1% and 31.3% after 2, 4 and 6 months of storage, respectively (Table 6). In the essential oil kept in open metal vials, citral β content was 36.5% at initial stage and 31.0%, 29.4% and 28.0% after 2, 4 and 6 months of storage, respectively. In the essential oil kept in open plastic vials, citral β content was 36.5% initially (zero month) and 32.1%, 30.3% and 29.7% after 2, 4 and 6 months of storage, respectively.

Table 5: Citral–b content (%) of Essential oil of Lemongrass variety CKP-25 stored in different conditions at different storage periods

Sr. No.	Treatments	Vials ↓	Citral–b content (%)															
		Storage → months	0 Month				2 Months				4 Months				6 Months			
			R ₁	R ₂	R ₃	Mean	R ₁	R ₂	R ₃	Mean	R ₁	R ₂	R ₃	Mean	R ₁	R ₂	R ₃	Mean
1.	Closed	Glass	36.9	35.2	37.4	36.5	35.6	34.7	35.5	35.1	34.1	31.6	34.2	33.3	33.7	30.9	32.9	32.5
2.		Metal	36.9	35.2	37.4	36.5	32.3	30.7	34.5	32.5	30.0	28.4	31.3	29.9	29.1	27.5	30.1	28.9
3.		Plastic	36.9	35.2	37.4	36.5	31.9	32.6	34.8	33.1	28.2	31.3	32.6	30.7	27.8	30.8	32.1	30.3
Mean			36.9	35.2	37.4		33.3	32.7	34.8		30.8	30.4	32.7		30.2	29.7	31.7	
4.	Open	Glass	36.9	35.2	37.4	36.5	33.7	32.5	35.8	34.0	31.3	31.5	33.5	32.1	29.7	30.1	34.1	31.3
5.		Metal	36.9	35.2	37.4	36.5	30.7	29.3	33.0	31.0	29.3	27.2	31.7	29.4	27.4	25.8	30.8	28.0
6.		Plastic	36.9	35.2	37.4	36.5	31.6	30.5	34.2	32.1	29.4	28.8	32.7	30.3	28.9	28.1	32.1	29.7
Mean			36.9	35.2	37.4		32.0	30.8	34.3		30.0	29.2	32.6		28.7	28.0	32.3	

Table 6: Mean Citral-b content (%) data of Essential oil of Lemongrass variety CKP-25 stored in different conditions at different storage periods

Sr. No.	Treatments↓		Citral–b content (%) [*]				
	Storage Months→		0 Month	2 Months	4 Months	6 Months	Mean
1.	Closed	Glass	36.5	35.1	33.3	32.5	34.4
2.		Metal	36.5	32.5	29.9	28.9	32.0
3.		Plastic	36.5	33.1	30.7	30.3	32.7
Mean			36.5	33.6	33.6	30.6	
4.	Open	Glass	36.5	34.0	32.1	31.3	33.5
5.		Metal	36.5	31.0	29.4	28.0	31.2
6.		Plastic	36.5	32.1	30.3	29.7	32.2
Mean			36.5	32.4	30.5	29.7	
Overall Mean			36.5	33.0	30.9	30.2	

* Average of Three Replicates

CD at 5%

Factor A (Closed/Open) = N.S.

Factor B (Glass/Metal/Plastic) = 0.991

Factor C (Storage Period) = N.S.

Factor A × B = 1.401

Factor A × C = N.S.

Factor B × C = N.S.

Factor A × B × C = N.S.

The overall mean of (Tablei 6) citrali b content was maximum in the essential oils kept in closed glass vials (34.4%) followed by plastic vials (32.7%) and metal (aluminium) vials (32.0%). Similar trend (glass, 33.5%; plastic, 32.2%; and aluminium, 31.2%) was also observed in the essential oil kept in open vials. However, the statistical analysis of citrali b data (Tablei 6) showed that the effect of closed and open vials was non significant whereas the effect of glass, metal and plastic vials was found to be significant. The effect of storage period on citrali b content was non significant.

4.3.3 Total Citral (geranial + neral) content

The data (Tables 7i 8) showed that the total citral content was 83.5% initially (at zero month) just before storage which decreases during storage in closed as well as in open vials containing essential oil of CKPi 25. In the essential oil kept in closed glass vials, average total citral content (Tablei 8) was 79.3%, 75.5% and 74.0% after 2, 4 and 6 months of storage, respectively. In the essential oil kept in closed metal vials, total citral content was 83.5% at initial stage and decreased to 73.8%, 67.9% and 64.3% after 2, 4 and 6 months of storage, respectively. In the essential oil kept in closed plastic vials, total citral content was 83.5% initially and 75.6%, 70.4% and 68.3% after 2, 4 and 6 months of storage, respectively.

In the essential oil kept in open glass vials, total citral content was 83.5% at initial stage and 77.5%, 73.2% and 70.2% after 2, 4 and 6 months of storage, respectively (Tablei 8). In the essential oil kept in open metal (aluminium) vials, total citral content was 83.5% at initial stage and 71.8%, 65.7% and 61.8% after 2, 4 and 6 months of storage, respectively. In the essential oil kept in open plastic vials, total citral content was 83.5% at initial stage and 73.9%, 68.8% and 65.4% after 2, 4 and 6 months of storage, respectively.

The overall mean of total citral content was maximum in the essential oils kept in closed glass vials (78.1%) followed by closed plastic vials (74.5%) and metal (aluminium)

Table 7: Total Citral content (%) of Essential oil of Lemongrass variety CKP-25 stored in different conditions at different storage periods

Sr. No.	Treatment	Vials ↓	Total Citral content (%)															
		Storage → months	0 Month				2 Months				4 Months				6 Months			
			R ₁	R ₂	R ₃	Mean	R ₁	R ₂	R ₃	Mean	R ₁	R ₂	R ₃	Mean	R ₁	R ₂	R ₃	Mean
1.	Closed	Glass	85.3	82.4	82.8	83.5	77.6	80.4	77.1	79.3	71.0	78.1	77.4	75.5	73.5	72.8	75.7	74.0
2.		Metal	85.3	82.4	82.8	83.5	74.6	71.5	75.3	73.8	70.5	65.7	67.5	67.9	68.5	61.9	62.5	64.3
3.		Plastic	85.3	82.4	82.8	83.5	76.2	76.7	73.9	75.6	66.9	70.4	74.1	70.4	65.3	67.8	71.8	68.3
Mean			85.3	82.4	82.8		76.1	76.2	75.4		69.5	71.3	73.0		69.1	67.5	70.0	
4.	Open	Glass	85.3	82.4	82.8	83.5	82.3	74.5	75.7	77.5	76.8	74.4	68.4	73.2	69.8	74.5	66.6	70.3
5.		Metal	85.3	82.4	82.8	83.5	66.3	73.5	75.6	71.8	62.3	70.4	64.4	65.7	65.3	59.1	61.0	61.8
6.		Plastic	85.3	82.4	82.8	83.5	70.2	79.1	72.4	73.9	73.6	63.2	69.6	68.8	60.8	67.2	68.2	65.4
Mean			85.3	82.4	82.8		72.9	75.7	74.6		70.9	69.3	67.5		65.3	66.9	65.3	

Table 8: Mean Total Citral content (%) data of Essential oil of Lemongrass variety CKP-25 stored in different conditions at different storage periods

Sr. No.	Treatments↓		Total Citral content (%) [*]				
	Storage Months→		0 Month	2 Months	4 Months	6 Months	Mean
1.	Closed	Glass	83.5	79.3	75.5	74.0	78.1
2.		Metal	83.5	73.8	67.9	64.3	72.4
3.		Plastic	83.5	75.6	70.4	68.3	74.5
Mean			83.5	76.2	71.3	68.9	
4.	Open	Glass	83.5	77.5	73.2	70.2	76.2
5.		Metal	83.5	71.8	65.7	61.8	70.7
6.		Plastic	83.5	73.9	68.8	65.4	72.9
Mean			83.5	74.4	69.2	65.8	
Overall Mean			83.5	75.3	70.2	67.4	

* Average of Three Replicates

CD at 5%

Factor A (Closed/Open) = 1.541

Factor B (Glass/Metal/Plastic) = 1.887

Factor C (Storage Period) = N.S.

Factor A × B = 2.669

Factor A × C = N.S.

Factor B × C = N.S.

Factor A × B × C = N.S.

vials (72.4%) (Tablei 8). Similar trend (glass, 76.2%; plastic, 72.9%; and metal, 70.7%) was also observed in the essential oil kept in open vials.

The statistical analysis of total citral data (Tablei 8) showed that effect of closed and open vials was significant. Similarly, the effect of glass, metal and plastic vials was also significant, whereas the effect of storage period on total citral content was non significant.

4.3.4 Geraniol content

The perusal of data (Tables 9i 10) showed that the geraniol content of essential oil of CKPi 25 was 2.8% initially (at zero month) which decreases during storage in the closed as well as open vials containing essential oils. In the essential oil kept in closed glass vials, average geraniol content (Tablei 10) was 2.5%, 2.3% and 2.1% after 2, 4 and 6 months of storage respectively. In the essential oil kept in closed metal (aluminium) vials, geraniol content was 2.8% initially and 2.2%, 2.0% and 1.8% after 2, 4 and 6 months of storage, respectively. In the essential oil kept in closed plastic vials, geraniol was 2.8% at zero month and 2.4%, 2.1% and 1.9% after 2, 4 and 6 months of storage, respectively.

In the essential oil kept in open glass vials, average geraniol content was 2.8% at initial stage and 2.4%, 2.1% and 1.8% after 2, 4 and 6 months of storage, respectively (Tablei 10). In the essential oil kept in open metal vials, geraniol content was 2.8% initially and 2.0%, 1.7% and 1.5% after 2, 4 and 6 months of storage, respectively. In the essential oil kept in open plastic vials, geraniol content was 2.8% at initial stage and 2.2%, 1.9% and 1.7% after 2, 4 and 6 months of storage, respectively.

The overall mean data (Tablei 10) of geraniol content was maximum in the essential oil kept in closed glass vials (2.4%) followed by closed plastic vials (2.3%) and aluminium vials (2.2%). Similar trend (glass 2.3%, plastic 2.2%, metal 2.0%) was also observed in the essential oil kept in open vials. However, the statistical analysis of geraniol data (Tablei 10) showed the effect of closed and open vials was significant. The effect of glass, metal and

Table 9: Geraniol content (%) of Essential oil of Lemongrass variety CKP-25 stored in different conditions at different storage periods

Sr. No.	Treatments	Vials ↓	Geraniol content (%)															
		Storage → months	0 Month				2 Months				4 Months				6 Months			
			R ₁	R ₂	R ₃	Mean	R ₁	R ₂	R ₃	Mean	R ₁	R ₂	R ₃	Mean	R ₁	R ₂	R ₃	Mean
1.	Closed	Glass	2.6	2.8	2.9	2.8	2.5	2.6	2.4	2.5	2.5	2.4	2.1	2.3	2.4	2.0	1.9	2.1
2.		Metal	2.6	2.8	2.9	2.8	2.0	2.2	2.4	2.2	1.7	2.1	2.2	2.0	1.4	1.9	2.0	1.8
3.		Plastic	2.6	2.8	2.9	2.8	2.2	2.6	2.5	2.4	1.9	2.2	2.3	2.1	1.6	2.0	2.1	1.9
Mean			2.6	2.8	2.9		2.2	2.5	2.4		2.0	2.2	2.2		1.8	2.0	2.0	
4.	Open	Glass	2.6	2.8	2.9	2.8	2.2	2.5	2.5	2.4	1.9	2.3	2.0	2.1	1.6	2.0	1.9	1.8
5.		Metal	2.6	2.8	2.9	2.8	1.7	2.1	2.2	2.0	1.5	1.8	1.9	1.7	1.3	1.5	1.7	1.5
6.		Plastic	2.6	2.8	2.9	2.8	2.0	2.4	2.3	2.2	1.8	2.2	1.7	1.9	1.4	2.0	1.6	1.7
Mean			2.6	2.8	2.9		2.0	2.3	2.3		1.7	2.1	1.9		1.4	1.8	1.7	

Table 10: Mean Geraniol content (%) data of Essential oil of Lemongrass variety CKP-25 stored in different conditions at different storage periods

Sr. No.	Treatments↓		Geraniol content (%) [*]				
	Storage Months→		0 Month	2 Months	4 Months	6 Months	Mean
1.	Closed	Glass	2.8	2.5	2.3	2.1	2.4
2.		Metal	2.8	2.2	2.0	1.8	2.2
3.		Plastic	2.8	2.4	2.1	1.9	2.3
Mean			2.8	2.4	2.1	1.9	
4.	Open	Glass	2.8	2.4	2.1	1.8	2.3
5.		Metal	2.8	2.0	1.7	1.5	2.0
6.		Plastic	2.8	2.2	1.9	1.7	2.2
Mean			2.8	2.2	1.9	1.7	
Overall Mean			2.8	2.3	2.0	1.8	

*** Average of Three Replicates**

CD at 5%

Factor A (Closed/Open) = 0.102

Factor B (Glass/Metal/Plastic) = 0.125

Factor C (Storage Period) = N.S.

Factor A × B = 0.177

Factor A × C = N.S.

Factor B × C = N.S.

Factor A × B × C = N.S.

plastic vials was also significant whereas the effect of storage period on the geraniol content was found to be non significant.

4.3.5 Citronellal content

The perusal of data (Tables 11i 12) showed that the citronellal content of essential oil of CKPi 25 was 1.1% initially (at zero month) which decreases during storage in the closed as well as open vials containing essential oils. In the essential oil kept in closed glass vials, average citronellal content (Tablei 12) was 0.9%, 0.8% and 0.7% after 2, 4 and 6 months of storage, respectively. In the essential oil kept in closed metal (aluminium) vials, citronellal content was 1.1% initially and 0.7%, 0.6% and 0.5% after 2, 4 and 6 months of storage, respectively. In the essential oil kept in closed plastic vials, citronellal was 1.1% at zero month and 0.8%, 0.7% and 0.6% after 2, 4 and 6 months of storage, respectively.

In the essential oil kept in open glass vials citronellal content was 1.1% at initial stage and 0.8%, 0.6% and 0.5% after 2, 4 and 6 months of storage, respectively (Tablei 12). In the essential oil kept in open aluminium vials, citronellal content was 1.1% initially and 0.6%, 0.4% and 0.3% after 2, 4 and 6 months of storage, respectively. In the essential oil kept in open plastic vials, citronellal content was 1.1% at initial stage and 0.7%, 0.5% and 0.4% after 2, 4 and 6 months of storage, respectively.

The overall mean data (Tablei 12) of citronellal content was maximum in the essential oil kept in closed glass vials (0.9%) followed by closed plastic vials (0.8%) and aluminium vials (0.7%). Similar trend (glass, 0.8%; plastic 0.7%; metal 0.6%) was also observed in the essential oil kept in open vials. However, the statistical analysis of citronellal data (Tablei 12) showed the effect of closed and open vials was significant. The effect of glass, metal and plastic vials as well as effect of storage period was non significant.

The overall summary of the results of storage effects on the quality of essential oil kept in different vials under closed and open conditions are given on the pagei 44.

Table 11: Citronellal content (%) of Essential oil of Lemongrass variety CKP-25 stored in different conditions at different storage periods

Sr. No.	Treatments	Vials ↓	Citronellal content (%)															
		Storage → months	0 Month				2 Months				4 Months				6 Months			
			R ₁	R ₂	R ₃	Mean	R ₁	R ₂	R ₃	Mean	R ₁	R ₂	R ₃	Mean	R ₁	R ₂	R ₃	Mean
1.	Closed	Glass	0.9	1.3	1.0	1.1	0.8	1.1	0.8	0.9	0.6	1.0	0.7	0.8	0.5	0.9	0.6	0.7
2.		Metal	0.9	1.3	1.0	1.1	0.9	0.7	0.6	0.7	0.8	0.5	0.6	0.6	0.6	0.3	0.5	0.5
3.		Plastic	0.9	1.3	1.0	1.1	0.8	0.6	1.0	0.8	0.7	0.9	0.4	0.7	0.3	0.8	0.6	0.6
Mean			0.9	1.3	1.0		0.8	0.8	0.8		0.7	0.8	0.6		0.5	0.7	0.6	
4.	Open	Glass	0.9	1.3	1.0	1.1	0.6	1.0	0.8	0.8	0.6	0.4	0.8	0.6	0.7	0.3	0.4	0.5
5.		Metal	0.9	1.3	1.0	1.1	0.8	0.6	0.5	0.6	0.3	0.6	0.4	0.4	0.1	0.4	0.3	0.3
6.		Plastic	0.9	1.3	1.0	1.1	0.7	0.9	0.6	0.7	0.4	0.5	0.6	0.5	0.3	0.5	0.4	0.4
Mean			0.9	1.3	1.0		0.7	0.8	0.6		0.4	0.5	0.6		0.4	0.4	0.4	

Table 12: Mean Citronellal content (%) data of Essential oil of Lemongrass variety CKP-25 stored in different conditions at different storage periods

Sr. No.	Treatments↓		Citronellal content (%)*				
	Storage Months→		0 Month	2 Months	4 Months	6 Months	Mean
1.	Closed	Glass	1.1	0.9	0.8	0.7	0.9
2.		Metal	1.1	0.7	0.6	0.5	0.7
3.		Plastic	1.1	0.8	0.7	0.6	0.8
Mean			1.1	0.8	0.7	0.6	
4.	Open	Glass	1.1	0.8	0.6	0.5	0.8
5.		Metal	1.1	0.6	0.4	0.3	0.6
6.		Plastic	1.1	0.7	0.5	0.4	0.7
Mean			1.1	0.7	0.5	0.4	
Overall Mean			1.1	0.8	0.6	0.5	

* Average of Three Replicates

CD at 5%

Factor A (Closed/Open) = 0.089

Factor B (Glass/Metal/Plastic) = N.S.

Factor C (Storage Period) = N.S.

Factor A × B = 0.155

Factor A × C = N.S.

Factor B × C = N.S.

Factor A × B × C = N.S.

1. Citrali a (geranial), citrali b (neral), total citral, geraniol and citronellal contents in the essential oils decreased during storage period.
 - i) Citrali a (geranial) was initially 47.0% before storage which reached to 42.4%, 39.3% and 37.3% after 2, 4 and 6 months of storage, respectively (Tablei 4).
 - ii) Citrali b (neral) was initially 36.5% before storage which reached to 33.0%, 30.9% and 30.2% after 2, 4 and 6 months of storage, respectively (Tablei 6).
 - iii) Total citral was 83.5% at initial stage (zero month) which reached to 75.3%, 70.2% and 67.4% after 2, 4 and 6 months of storage, respectively (Tablei 8).
 - iv) Geraniol was initially 2.8% before storage which reached to 2.3%, 2.0% and 1.8% after 2, 4 and 6 months of storage, respectively (Tablei 10).
 - v) Citronellal was initially 1.1% before storage which reached to 0.8%, 0.6% and 0.5% after 2, 4 and 6 months of storage, respectively (Tablei 12).
2. The decrease in citrali a (geranial), citrali b (neral), total citral, geraniol and citronellal contents during storage was minimum in the essential oil kept in glass vials followed by plastic vials and aluminium vials. Similar trend was observed in closed as well as open vials.
3. The decrease in citrali a, citrali b, total citral, geraniol and citronellal contents was less in essential oil kept in closed vials in comparison to open vials.

Very few research workers have studied the effect of storage/containers on the quality of essential oils. Some of them have reported the reasons for the change in qualitative parameters. Bhattacharya *et al.*, (1998) reported that the quality of *C. flexuosus* essential oil stored in aluminium containers deteriorated during storage. The concentrations of neral (33.21%) and geranial (53.11%) decreased to 22.82% and 27.66%, respectively after one year of storage. Raina *et al.*, (1998) also reported change in colour and the

percentage composition of major constituents of essential oils viz., citrali a (geranial), citrali b (neral), geraniol and citronellal stored in amber glass, plain glass, aluminium, iron and high density poly ethylene (HDPE) containers. Orav *et al.*, (2004) reported that the concentrations of major constituents of the essential oil of *Piper nigrum* fruits decreases during storage from 47.1 to 10.6%, the content of sesquiterpenes increased to 77.3% and the share of oxygenated increased from 1.4 to 7.9 in oil of black pepper. They also reported that evaporation of more volatile compounds, oxidation and other processes of chemical changes could take place during storage of pepper, which affects the quality of essential oil.

Kaul *et al.*, (1997) studied the changes in chemical composition of rose-scented geranium oil during storage and reported that under storage conditions, the concentrations of menimone, citronellal and citronellyl formate increased and the concentrations of linalool, geraniol, geranyl formate and lo-epi-gamma-eudermol decreased. They also reported that polymerisation was observed in essential oil irrespective of storage conditions. Sakamura (1987) reported that during storage of essential oil of fresh rhizomes of *Zingiber officinale*, the content of neral and geranial in the rhizome increased to about 60% of the essential oil, while the content of geraniol and geranyl acetate decreased to an undetectable amount. They reported that this may be due to hydrolysis of geranyl acetate into geraniol and oxidation of geraniol to geranial and neral during storage of the rhizome. Mazza (1987) reported that the reasons for chemical changes in essential oils are various but one of the most important reactions involved is certainly the oxidation reaction.

In our present studies, the decrease in citrali a (geranial), citrali b (neral), geraniol and citronellal content was more in open vials as compared to closed vials, which may be due to more oxidation of citrali a (geranial), citrali b (neral), geraniol and citronellal into their derivatives resulting in less concentrations of these constituents in open vials.

The quality of oil kept in metal (aluminium) vials was affected more in comparison

to plastic and glass vials, which may be due to certain reactions between metal (aluminium) and major constituents of oils leading to decrease in these contents. Since, glass surface generally has no interaction with the chemicals and hence, the quality of the oil kept in glass vials was least affected.

4.4 Nematicidal activity of aqueous extracts of essential oils of lemongrass against *Meloidogyne javanica*, *Meloidogyne incognita* and *Rotylenchulus reniformis*:

The per cent mortality data of aqueous extracts of six genotypes/varieties against *Meloidogyne javanica*, *Meloidogyne incognita* and *Rotylenchulus reniformis* are presented in Tables 13 to 33.

4.4.1 Nematicidal activity of aqueous extracts of essential oils of lemongrass against *Meloidogyne javanica*:

The per cent mortality data of aqueous extracts of six Lemongrass genotypes against root knot nematode *Meloidogyne javanica* under laboratory conditions is presented in Tables 13 to 18 and their summary table is presented in Table 19. The average percent mortality data (Table 13) of aqueous extract of essential oil of HL 2 genotype was found to be 100.0% at 1:5 concentration against *M. javanica* followed by 98.2% at 1:10; 65.5% at 1:20; 31.9% at 1:40 and 10.6% at 1:80 concentrations. A sharp decrease in per cent mortality was found from 1:10 to 1:80 concentrations. The average percent mortality data (Table 14) of aqueous extract of essential oil of HL 3 genotype was found to be 90.2% at 1:5 concentration against *M. javanica* followed by 56.4% at 1:10; 39.2% at 1:20; 21.1% at 1:40 and 1.8% at 1:80 concentrations. A sharp decrease in per cent mortality was found on dilution. The average percent mortality data (Table 15) of aqueous extract of essential oil of HL 11 genotype was found to be 76.5 % at 1:5 concentration against *M. javanica* followed by 46.3% at 1:10; 34.0% at 1:20; 10.9% at 1:40 and 3.4% at 1:80 concentrations.

Table 13: Per cent mortality data of aqueous extract of Essential oil of HL-2 against *Meloidogyne javanica*

Concentrations	Per cent Mortality			
	R ₁	R ₂	R ₃	Mean
1:5	100.0	100.0	100.0	100.0
1:10	98.2	98.7	97.7	98.2
1:20	64.9	68.2	63.3	65.5
1:40	32.6	30.2	32.9	31.9
1:80	10.6	11.8	9.5	10.6
Mean	61.3	61.8	60.7	
SEm±	0.817			
CD at 5%	2.607			

Table 14: Per cent mortality data of aqueous extract of Essential oil of HL-3 against *Meloidogyne javanica*

Concentrations	Per cent Mortality			
	R ₁	R ₂	R ₃	Mean
1:5	90.1	92.8	87.7	90.2
1:10	56.9	55.9	56.3	56.4
1:20	38.9	40.3	38.3	39.2
1:40	20.6	23.9	18.9	21.1
1:80	1.8	1.6	1.9	1.8
Mean	41.7	42.9	40.6	
SEm±	0.977			
CD at 5%	3.117			

Table 15: Per cent mortality data of aqueous extract of Essential oil of HL-11 against *Meloidogyne javanica*

Concentrations	Per cent Mortality			
	R ₁	R ₂	R ₃	Mean
1:5	78.0	71.6	79.8	76.5
1:10	46.9	45.4	46.6	46.3
1:20	35.5	31.1	35.5	34.0
1:40	11.4	10.0	11.2	10.9
1:80	3.2	4.1	2.9	3.4
Mean	35.0	32.4	35.2	
SEm±	1.332			
CD at 5%	4.252			

The average percent mortality data (Tablei 16) of aqueous extract of essential oil of NLGi 118 genotype was found to be 98.4 % at 1:5 concentration against *M. javanica* followed by 84.4 % at 1:10; 49.8 % at 1:20; 20.5 % at 1:40 and 8.7 % at 1:80 concentrations. The average percent mortality data (Tablei 17) of aqueous extract of essential oil of ODİ 19 genotype was found to be 97.0 % at 1:5 concentration against *M. javanica* followed by 58.2% at 1:10; 34.3% at 1:20; 14.8% at 1:40 and 1.9% at 1:80 concentrations. The average percent mortality data (Tablei 18) of aqueous extract of essential oil of CKPi 25 genotype was found to be 79.2 % at 1:5 concentration against *M. javanica* followed by 67.6% at 1:10; 53.6% at 1:20; 38.5% at 1:40 and 7.8% at 1:80 concentrations.

The mean data of per cent mortality of aqueous extracts of six essential oils at various concentrations viz., 1:5, 1:10, 1:20, 1:40 and 1:80 against root knot nematode *M. javanica* is presented in Tablei 19. Mean per cent mortality of six aqueous extracts was 90.2% at 1:5 concentration followed by 68.5 at 1:10, 46.1% at 1:20, 23.0% at 1:40 and 5.7% at 1:80 concentrations. Among the aqueous extracts of essential oil of six genotypes, HLi 2 recorded highest mean mortality of 61.2% followed by NLGi 118 (52.4%), CKPi 25 (49.3%), HLi 3 (41.8%), ODİ 19 (41.2%) and HLi 11 (34.3%).

4.4.2 Nematicidal activity of aqueous extracts of essential oils of lemongrass against *Meloidogyne incognita*:

The per cent mortality data of aqueous extracts of six lemongrass genotypes against root knot nematode *Meloidogyne incognita* under laboratory conditions is presented in Tables 20i 25 and their summary table is presented in Tablei 26. The average percent mortality data (Tablei 20) of aqueous extract of essential oil of HLi 2 genotype was found to be 100.0% at 1:5 and 1:10 concentrations against *M. incognita* followed by 90.4% at 1:20; 80.6% at 1:40 and 71.6% at 1:80 concentrations. The average percent mortality data (Tablei 21) of aqueous extract of essential oil of HLi 3 genotype was found to be 100.0% at 1:5,

Table 16: Per cent mortality data of aqueous extract of Essential oil of NLG-118 against *Meloidogyne javanica*

Concentrations	Per cent Mortality			
	R ₁	R ₂	R ₃	Mean
1:5	99.3	97.6	98.4	98.4
1:10	85.2	86.4	81.7	84.4
1:20	53.1	49.3	47.1	49.8
1:40	21.0	22.9	17.7	20.5
1:80	6.9	10.0	9.3	8.7
Mean	53.1	53.2	50.8	
SEm±	1.303			
CD at 5%	4.159			

Table 17: Per cent mortality data of aqueous extract of Essential oil of OD-19 against *Meloidogyne javanica*

Concentrations	Per cent Mortality			
	R ₁	R ₂	R ₃	Mean
1:5	95.1	98.2	97.8	97.0
1:10	57.9	59.1	57.6	58.2
1:20	33.9	32.1	36.8	34.3
1:40	16.8	14.7	12.9	14.8
1:80	2.1	2.2	1.6	1.9
Mean	41.2	41.3	41.3	
SEm±	0.931			
CD at 5%	2.973			

Table 18: Per cent mortality data of aqueous extract of Essential oil of CKP-25 against *Meloidogyne javanica*

Concentrations	Per cent Mortality			
	R ₁	R ₂	R ₃	Mean
1:5	79.6	79.6	78.3	79.2
1:10	65.7	67.1	70.1	67.6
1:20	52.5	53.9	54.3	53.6
1:40	40.5	35.7	39.3	38.5
1:80	10.3	7.3	5.9	7.8
Mean	49.7	48.7	49.6	
SEm±	1.089			
CD at 5%	3.477			

Table-19: Per cent mortality data of aqueous extracts of Essential oils of 6 genotypes/varieties of Lemongrass against *Meloidogyne javanica*

Concentrations	Genotypes/varieties of Lemongrass (<i>Cymbopogon flexuosus</i>)						
	HL-2	HL-3	HL-11	NLG-118	OD-19	CKP-25	Mean
1:5	100.0	90.2	76.5	98.4	97.0	79.2	90.2
1:10	98.2	56.4	46.3	84.4	58.2	67.6	68.5
1:20	65.4	39.4	34.0	49.8	34.3	53.6	46.1
1:40	31.9	21.1	10.9	20.5	14.8	38.5	23.0
1:80	10.6	1.8	3.4	8.7	1.9	7.8	5.7
Mean	61.2	41.8	34.3	52.4	41.2	49.3	
SEm±	0.817	0.977	1.332	1.303	0.931	1.089	
CD at 5%	2.607	3.117	4.252	4.159	2.973	3.477	

Table 20: Per cent mortality data of aqueous extract of Essential oil of HL-2 against *Meloidogyne incognita*

Concentrations	Per cent Mortality			
	R ₁	R ₂	R ₃	Mean
1:5	100.0	100.0	100.0	100.0
1:10	100.0	100.0	100.0	100.0
1:20	91.5	91.9	87.8	90.4
1:40	81.8	78.6	81.3	80.6
1:80	68.1	71.6	75.0	71.6
Mean	88.3	88.4	88.8	
SEm±	1.154			
CD at 5%	3.683			

Table 21: Per cent mortality data of aqueous extract of Essential oil of HL-3 against *Meloidogyne incognita*

Concentrations	Per cent Mortality			
	R ₁	R ₂	R ₃	Mean
1:5	100.0	100.0	100.0	100.0
1:10	100.0	100.0	100.0	100.0
1:20	100.0	100.0	100.0	100.0
1:40	86.2	82.6	86.9	85.2
1:80	59.3	60.4	63.0	60.9
Mean	89.1	88.6	89.9	
SEm±	0.772			
CD at 5%	2.463			

1:10 and 1:20 concentrations against *M. incognita* followed by 85.2% at 1:40 and 60.9% at 1:80 concentrations. The average percent mortality data (Tableï 22) of aqueous extract of essential oil of HLi 11 genotype was found to be 100.0% at 1:5 and 1:10 concentrations against *M. incognita* followed by 96.9% at 1:20; 82.0% at 1:40 and 51.5% at 1:80 concentrations.

The average percent mortality data (Tableï 23) of aqueous extract of essential oil of NLGi 118 genotype was found to be 100.0% at 1:5 and 1:10 concentrations against *M. incognita* followed by 95.7% at 1:20; 91.2% at 1:40 and 80.8% at 1:80 concentrations. The average percent mortality data (Tableï 24) of aqueous extract of essential oil of ODi 19 genotype was found to be 100.0% at 1:5 and 1:10 concentrations against *M. incognita* followed by 94.3% at 1:20; 64.2% at 1:40 and 43.3% at 1:80 concentrations. The average percent mortality data (Tableï 25) of aqueous extract of essential oil of CKPi 25 variety was found to be 100.0% at 1:5 and 1:10 concentrations against *M. incognita* followed by 95.2% at 1:20; 54.2% at 1:40 and 24.1% at 1:80 concentrations.

The mean data of per cent mortality of aqueous extracts of six essential oils at various concentrations viz., 1:5, 1:10, 1:20, 1:40 and 1:80 against root knot nematode *M. javanica* is presented in Tableï 26. Mean per cent mortality (Tableï 26) of six aqueous extracts of essential oils was 100% upto 1:5 and 1:10 concentrations followed by 95.3% at 1:20, 76.2% at 1:40 and 55.4% at 1:80 concentrations. Among the aqueous extracts of essential oil of six genotypes, NLGi 118 recorded highest mean mortality of 93.6% followed by HLi 3 (89.2%), HLi 2 (88.5%), HLi 11 (86.1%), ODi 19 (80.2%) and CKPi 25 (74.7%).

4.4.3 Nematicidal activity of aqueous extracts of essential oil of lemongrass against *Rotylenchulus reniformis*:

The per cent mortality data of aqueous extracts of six lemongrass genotypes against *Rotylenchulus reniformis* under laboratory conditions is presented in Tables 27i 32 and their summary table is presented in Tableï 33.

Table 22: Per cent mortality data of aqueous extract of Essential oil of HL-11 against *Meloidogyne incognita*

Concentrations	Per cent Mortality			
	R ₁	R ₂	R ₃	Mean
1:5	100.0	100.0	100.0	100.0
1:10	100.0	100.0	100.0	100.0
1:20	96.0	96.6	98.0	96.9
1:40	80.4	84.1	81.6	82.0
1:80	53.3	50.6	50.7	51.5
Mean	85.9	86.3	86.1	
SEm±	0.681			
CD at 5%	2.174			

Table 23: Per cent mortality data of aqueous extract of Essential oil of NLG-118 against *Meloidogyne incognita*

Concentrations	Per cent Mortality			
	R ₁	R ₂	R ₃	Mean
1:5	100.0	100.0	100.0	100.0
1:10	100.0	100.0	100.0	100.0
1:20	95.2	96.4	95.6	95.7
1:40	90.4	92.8	90.4	91.2
1:80	81.1	80.8	80.6	80.8
Mean	93.3	94.0	93.3	
SEm±	0.396			
CD at 5%	1.265			

Table 24: Per cent Mortality data of aqueous extract of Essential oil of OD-19 against *Meloidogyne incognita*

Concentrations	Per cent Mortality			
	R ₁	R ₂	R ₃	Mean
1:5	100.0	100.0	100.0	100.0
1:10	100.0	100.0	100.0	100.0
1:20	94.2	94.9	93.8	94.3
1:40	61.4	65.3	65.8	64.2
1:80	42.9	43.6	43.4	43.3
Mean	79.7	80.4	80.6	
SEm±	0.645			
CD at 5%	2.059			

Table 25: Per cent Mortality data of aqueous extract of Essential oil of CKP-25 against *Meloidogyne incognita*

Concentrations	Per cent Mortality			
	R ₁	R ₂	R ₃	Mean
1:5	100.0	100.0	100.0	100.0
1:10	100.0	100.0	100.0	100.0
1:20	94.6	95.7	95.3	95.2
1:40	53.3	56.8	52.5	54.2
1:80	25.8	22.5	24.1	24.1
Mean	74.7	75.0	74.4	
SEm±	0.742			
CD at 5%	2.369			

Table 26: Per cent mortality data of aqueous extracts of Essential oils of 6 genotypes /varieties of Lemongrass against *Meloidogyne incognita*

Concentrations	Genotypes/varieties of Lemongrass (<i>Cymbopogon flexuosus</i>)						
	HL-2	HL-3	HL-11	NLG-118	OD-19	CKP-25	Mean
1:5	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1:10	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1:20	90.4	100.0	96.9	95.7	93.6	95.2	95.3
1:40	80.6	85.2	82.0	91.2	64.2	54.2	76.2
1:80	71.5	60.9	51.5	80.9	43.3	24.1	55.4
Mean	88.5	89.2	86.1	93.6	80.2	74.7	
SEm±	1.154	0.772	0.681	0.396	0.645	0.742	
CD at 5%	3.683	2.463	2.174	1.265	2.059	2.369	

The average percent mortality data (Tablei 27) of aqueous extract of essential oil of HLi 2 genotype was found to be 64.5% at 1:5 concentration against *Rotylenchulus reniformis* followed by 48.4% at 1:10; 31.0% at 1:20; 19.0% at 1:40 and 6.6% at 1:80 concentrations. The average percent mortality data (Tablei 28) of aqueous extract of essential oil of HLi 3 genotype was found to be 52.0% at 1:5 concentration against *Rotylenchulus reniformis* followed by 32.0% at 1:10; 16.8% at 1:20; 2.3% at 1:40 and 1.9% at 1:80 concentrations. The average percent mortality data (Tablei 29) of aqueous extract of essential oil of HLi 11 genotype was found to be 81.3% at 1:5 concentration against *Rotylenchulus reniformis* followed by 74.6% at 1:10; 63.1% at 1:20; 45.3% at 1:40 and 32.8% at 1:80 concentrations.

The average percent mortality data (Tablei 30) of aqueous extract of essential oil of NLGi 118 genotype was found to be 98.9% at 1:5 concentration against *Rotylenchulus reniformis* followed by 79.8% at 1:10; 46.9% at 1:20; 20.7% at 1:40 and 11.0% at 1:80 concentrations. The average percent mortality data (Tablei 31) of aqueous extract of essential oil of ODi 19 genotype was found to be 81.7% at 1:5 concentration against *Rotylenchulus reniformis* followed by 63.0% at 1:10; 40.7% at 1:20; 16.9% at 1:40 and 8.9% at 1:80 concentrations. The average percent mortality data (Tablei 32) of aqueous extract of essential oil of CKPi 25 genotype was found to be 93.2% at 1:5 concentration against *Rotylenchulus reniformis* followed by 83.6% at 1:10; 71.1% at 1:20; 65.0% at 1:40 and 42.4% at 1:80 concentrations.

The mean data of per cent mortality of aqueous extracts of six essential oils at various concentrations viz., 1:5, 1:10, 1:20, 1:40 and 1:80 against root knot nematode *Rotylenchulus reniformis* is presented in Tablei 33. Mean per cent mortality (Tablei 33) of six aqueous extracts of essential oils was 78.6% at 1:5 concentration followed by 63.6 at 1:10, 44.9% at 1:20, 28.2% at 1:40 and 17.4% at 1:80 concentrations. Among the aqueous

Table 27: Per cent mortality data of aqueous extract of Essential oil of HL-2 against *Rotylenchulus reniformis*

Concentrations	Per cent Mortality			
	R ₁	R ₂	R ₃	Mean
1:5	61.1	68.7	63.6	64.5
1:10	48.0	48.3	48.9	48.4
1:20	32.7	32.5	27.5	31.0
1:40	18.4	17.6	20.9	19.0
1:80	9.9	5.8	4.1	6.6
Mean	34.0	34.6	33.0	
SEm±	1.544			
CD at 5%	4.927			

Table 28: Per cent mortality data of aqueous extract of Essential oil of HL-3 against *Rotylenchulus reniformis*

Concentrations	Per cent Mortality			
	R ₁	R ₂	R ₃	Mean
1:5	51.6	51.9	52.3	52.0
1:10	31.4	34.8	29.9	32.0
1:20	17.3	18.4	14.8	16.8
1:40	3.9	1.8	1.1	2.3
1:80	2.2	1.2	2.3	1.9
Mean	21.3	21.6	20.1	
SEm±	0.906			
CD at 5%	2.893			

Table 29: Per cent mortality data of aqueous extract of Essential oil of HL-11 against *Rotylenchulus reniformis*

Concentrations	Per cent Mortality			
	R ₁	R ₂	R ₃	Mean
1:5	79.9	79.1	84.9	81.3
1:10	72.8	74.7	76.4	74.6
1:20	62.4	66.5	60.5	63.1
1:40	46.1	44.3	45.4	45.3
1:80	31.6	34.2	32.5	32.8
Mean	58.6	59.8	60.0	
SEm±	1.293			
CD at 5%	4.128			

Table 30: Per cent mortality data of aqueous extract of Essential oil of NLG-118 against *Rotylenchulus reniformis*

Concentrations	Per cent Mortality			
	R ₁	R ₂	R ₃	Mean
1:5	98.7	99.3	98.8	98.9
1:10	81.7	77.3	80.5	79.8
1:20	47.3	47.1	46.2	46.9
1:40	23.0	18.1	20.9	20.7
1:80	12.3	11.7	9.1	11.0
Mean	52.6	50.7	51.1	
SEm±	0.985			
CD at 5%	3.144			

Table 31: Per cent mortality data of aqueous extract of Essential oil of OD-19 against *Rotylenchulus reniformis*

Concentrations	Per cent Mortality			
	R ₁	R ₂	R ₃	Mean
1:5	82.6	79.2	83.3	81.7
1:10	66.8	61.9	60.2	63.0
1:20	39.5	43.2	39.3	40.7
1:40	17.7	18.6	14.4	16.9
1:80	6.8	11.5	8.5	8.9
Mean	42.7	42.9	41.1	
SEm±	1.459			
CD at 5%	4.657			

Table 32: Per cent mortality data of aqueous extract of Essential oil of CKP-25 against *Rotylenchulus reniformis*

Concentrations	Per cent Mortality			
	R ₁	R ₂	R ₃	Mean
1:5	93.6	92.9	93.2	93.2
1:10	86.6	80.4	83.8	83.6
1:20	68.3	70.5	74.4	71.1
1:40	66.5	62.4	66.2	65.0
1:80	42.0	44.4	40.7	42.4
Mean	71.4	70.1	71.7	
SEm±	1.368			
CD at 5%	4.365			

Table 33: Per cent mortality data of aqueous extracts of Essential oils of 6 genotypes/varieties of Lemongrass against *Rotylenchulus reniformis*

Concentrations	Genotypes/varieties of Lemongrass (<i>Cymbopogon flexuosus</i>)						
	HL-2	HL-3	HL-11	NLG-118	OD-19	CKP-25	Mean
1:5	64.5	52.0	81.3	98.9	81.7	93.2	78.6
1:10	48.4	32.0	74.6	79.8	63.0	83.6	63.6
1:20	31.0	16.8	63.2	46.8	40.7	71.1	44.9
1:40	19.0	2.3	45.3	20.7	16.9	65.0	28.2
1:80	6.6	1.9	32.8	11.7	8.9	42.4	17.4
Mean	34.0	21.0	59.5	51.5	42.2	71.1	
SEm±	1.544	0.906	1.293	0.985	1.459	1.368	
CD at 5%	4.927	2.893	4.128	3.144	4.657	4.365	

extracts of essential oil of six genotypes, CKPi 25 recorded highest mean mortality of (71.1%) followed by HLi 11 (59.5%), NLGi 118 (51.5%), ODi 19 (42.2%), HLi 2 (34.0%) and HLi 3 (21.0%).

The overall summary of the nematicidal activity of aqueous extracts of six essential oils of lemongrass is as follows:

- a) On the basis of mean per cent mortality data of six genotypes/varieties of lemongrass, HL-2 recorded highest mean mortality of 61.2% against *Meloidogyne javanica* followed by NLG-118 (52.4%), CKP-25 (49.3%), HL-3 (41.8%), OD-19 (41.2%) and HL-11 (34.3%).
- b) On the basis of mean per cent mortality data of six genotypes/varieties of lemongrass, NLG-118 recorded highest mean mortality of 93.6% against *Meloidogyne incognita* followed by HL-3 (89.2%), HL-2 (88.5%), HL-11 (86.1%), OD-19 (80.2%) and CKP-25 (74.7%).
- c) On the basis of mean per cent mortality data of six genotypes/varieties of lemongrass, CKP-25 recorded highest mean mortality of 71.1% against *Rotylenchulus reniformis* followed by HL-11 (59.5%), NLG-118 (51.5%), OD-19 (42.2%), HL-2 (34.0%) and HL-3 (21.0%).

Many research workers have studied the nematicidal activity of extracts of various essential oils & plants and reported various reasons of Nematicidal activity. Sangwan *et al.* (1985) reported that the nematicidal activity of essential oils of *Cymbopogon* grasses *C. martinii* var. *motia*, *C. flexuosus* and *C. winterianus*) was due to presence of monoterpenes and their derivatives. Therefore, though lemongrass oil was effective against all four nematodes tested, citral, its principal constituent, was effective only against three namely seed-gall nematode (*Anguina tritici*), citrus nematode (*Tylenchulus semipenetrans*) and cereal cyst nematode (*Heterodera avenae*). The alcoholic constituents, geraniol and

citronellol were effective against *M. javanica* while aldehydic constituents, citral and citronellal were much less effective. The unsaturated analogues (geraniol and citral) were more effective than saturated analogues (citronellol and citronellal) at lower concentration. They also reported that these compounds differed in their nematocidal activity to each nematode due to differential susceptibilities of the nematodes to these compounds.

The relationship between the bactericidal action and the chemical constitution of the compounds was studied by Subrahmanyam & Bose (1956). The aldehyde group was found to be more active than the alcoholic group. The presence of an α - β -double bond in terpene aldehydes and alcohols was found to enhance the efficiency of the active group. According to them lemongrass oil was found to be susceptible to autoxidation and the loss of citral and germicidal property during the storage. It was demonstrated that the fall of citral and bactericidal potency was principally due to the autoxidation of the oil. Srivastava *et al.* (1990) tested the toxicity of leaves of 12 plant species against *Fusarium oxysporum* f. sp. *udum*, and reported that *C. martinii* exhibited the strongest toxicity (100%) inhibiting the mycelial growth of the test pathogens completely. They also reported that the antifungal property was because of the presence of aldehydes in the oil. Pandey *et al.*, (1982) reported fungitoxicity of essential oil of *Callistemon lanceolatus* D.C. against *Fusarium oxysporum* f. sp. *udum*, due to phenolic nature of essential oil.

In our present studies aqueous extracts of essential oils of six lemongrass (*Cymbopogon flexuosus*) genotypes/varieties were effective in varying amount of mortality against all the three nematodes viz., *M. javanica*, *M. incognita* and *Rotylenchulus reniformis*. The lemongrass oil contains aldehyde group in citral α (40-47%), citral β (30-37%) as major constituents and in citronellal ($\approx$ 1.1%) as minor constituents. Alcohol group i.e. geraniol ($\approx$ 2.9%) alongwith other chemical constituents are also present as minor constituents. The probable reason of nematocidal activity of aqueous extracts of lemongrass

essential oils may be due to the presence of aldehyde group in major constituents [citrali a (geranial), citrali b (neral) and citronellal] and alcoholic group (geraniol) as minor constituents. The results are in agreement with those reported by Sangwan *et al.*, (1985) who has reported that mixture of monoterpenes and their detrivatives are mainly responsible for nematacidal activity of *Cymbopogon* grasses. Further, the nematacidal activity of these essential oils against three nematodes was highest against *M. incognita* followed by *M. javanica*, and *Rotylenchulus reniformis*. This may be probably due to differential susceptibility of these nematodes towards major constituents [citrali a (geranial), citrali b (neral)] and minor constituents (geraniol and citronellal) which constitute about 85 to 90% of these essential oils. Sangwan *et al.*, (1985) also reported that *Cymbopogon* grasses (*C. martinii* var. *motia*, *C. flexuosus* and *C. winterianus*) differed in their nematicidal activity against *Tylenchulus semipenetrans*, *Meloidegyne javanica*, and *Heterodera avenae* due to their differential susceptibility of nematodes towards various compounds.

CHAPTER V

SUMMARY AND CONCLUSIONS

Farmers which grow the aromatic crops do not get proper price in the market due to unorganized market and no minimum support price from the any Government/private agencies. Hence, they store the essential oils of aromatic plants in different manners. Different storage methods may affect the quality of the lemongrass oil due to its exposure to air and light. Hence, present research studies were carried out under laboratory conditions to study the effect of different storage methods on the quality of the essential oils of lemongrass during storage period. Since, the essential oils play an important role in controlling nematodes, hence nematicidal activity of aqueous extracts of essential oils of six genotypes/varieties of lemongrass were also tested against the nematodes *M. javanica*, *M. incognita* and *Rotylenchulus reniformis*.

For the standardization of GLC conditions different conditions were tried for optimum area and better resolution of peaks of citrali a (geranial), citrali b (neral), geraniol and citronellal. In a good quality lemongrass oil, the contents of aldehydes i.e. citrali a (geranial) 40-48%, citrali b (neral) 30-37% should be maximum and geraniol (2.9%) and citronellal (1.1%) should be minimum.

During the storage studies, the essential oil of lemongrass variety CKP-25 was kept in three different vials viz., glass (Borosil type), metal (Aluminium) and plastic (High density polyethylene). The samples of the essential oils were kept in open as well as close vials for six months. Each treatment was replicated thrice. The essential oils kept in different vials were analysed on Gas Liquid Chromatograph (GLC) initially at before storage i.e. zero month and subsequently after 2, 4 and 6 months of storage for citrali a

(geranial), citrali b (neral), geraniol and citronellal contents.

The retention times observed for citrali a, citrali b, geraniol and citronellal were 16.42, 15.53, 17.84 and 12.35 minutes. The components of citrali a, citrali b, geraniol and citronellal were identified by comparing their retention times with those of standards recorded under the same operating parameters.

Nematicidal activity of essential oils of six genotypes/varieties of *C. flexuosus* were performed on three prominent nematodes species viz., *M. javanica*, *M. incognita* and *Rotylenchulus reniformis*, chosen on the basis of their geographical distribution and of their economic importance.

The findings of qualitative parameters, investigation on storage methods and storage period and nematicidal activity are summarized below:

2) Qualitative analysis of essential oils of lemongrass

Oil content was in the range from 0.24 to 0.74%. Citrali a, citrali b and total citral contents, which should be maximum in the lemongrass oil, ranged from 47.1 to 52.4%, 30.3 to 37.5% and 82.7 to 84.8%, respectively. Geraniol content and citronellal content, which should be minimum in the lemongrass oil, were in the range from 2.5 to 2.8% and 0.8 to 1.1%, respectively. NLG 118 recorded highest total citral content (84.8%) followed by ODi 19 (84.2%) and CKPi 25 (83.5%).

3) Effect of storage on qualitative parameters of essential oil of CKP-25

- a) Citrali a (geranial), citrali b (neral), total citral, geraniol and citronellal contents in the essential oils decreased during storage period.
 - i) Citrali a (geranial) was initially 47.0% before storage which reached to 42.4%, 39.3% and 37.3% after 2, 4 and 6 months of storage, respectively (Table 4).

- ii) Citrali b (neral) was initially 36.5% before storage which reached to 33.0%, 30.9% and 30.2% after 2, 4 and 6 months of storage, respectively (Tablei 6).
 - iii) Total citral was 83.5% at initial stage (zero month) which reached to 75.3%, 70.2% and 67.4% after 2, 4 and 6 months of storage, respectively (Tablei 8).
 - iv) Geraniol was initially 2.8% before storage which reached to 2.3%, 2.0% and 1.8% after 2, 4 and 6 months of storage, respectively (Tablei 10).
 - v) Citronellal was initially 1.1% before storage which reached to 0.8%, 0.6% and 0.5% after 2, 4 and 6 months of storage, respectively (Tablei 12).
- b) The decrease in citrali a (geranial), citrali b (neral), total citral, geraniol and citronellal contents during storage was minimum in the essential oil kept in glass vials followed by plastic vials and aluminium vials. Similar trend was observed in closed as well as open vials.
- c) The decrease in citrali a, citrali b, total citral, geraniol and citronellal contents was less in essential oil kept in closed vials in comparison to open vials.
- 4) Nematicidal activity of aqueous extracts of essential oils of lemongrass against *Meloidogyne javanica*, *Meloidogyne incognita* and *Rotylenchulus reniformis*:**
- a) On the basis of mean per cent mortality data of six genotypes/varieties of lemongrass, HL-2 recorded highest mean mortality of 61.2% against *Meloidogyne javanica* followed by NLG-118 (52.4%), CKP-25 (49.3%), HL-3 (41.8%), OD-19 (41.2%) and HL-11 (34.3%).
 - b) On the basis of mean per cent mortality data of six genotypes/varieties of lemongrass, NLG-118 recorded highest mean mortality of 93.6% against *Meloidogyne incognita* followed by HL-3 (89.2%), HL-2 (88.5%), HL-11 (86.1%), OD-19 (80.2%) and CKP-25 (74.7%).
 - c) On the basis of mean per cent mortality data of six genotypes/varieties of lemongrass, CKP-25 recorded highest mean mortality of 71.1% against

Rotylenchulus reniformis followed by HL-11 (59.5%), NLG-118 (51.5%), OD-19 (42.2%), HL-2 (34.0%) and HL-3 (21.0%).

CONCLUSIONS:

1. NLGİ 118 recorded highest total citral content (84.8%) followed by ODİ 19 (84.2%) and CKPİ 25 (83.5%).
2. Citralİ a (geranial), citralİ b (neral), total citral, geraniol and citronellal contents in the essential oils of lemongrass decreased during storage period.
3. The decrease in citralİ a (geranial), citralİ b (neral), total citral, geraniol and citronellal contents during storage was minimum in the essential oils kept in glass vials followed by plastic vials and metal (aluminium) vials.
4. The decrease in citralİ a, citralİ b, total citral, geraniol and citronellal contents was less in the essential oils kept in closed vials in comparison to open vials.
5. The per cent mortality data of aqueous extracts of essential oils of six genotypes/varieties of lemongrass was highest against the nematode *M. incognita* followed by *M. javanica*, and *Rotylenchulus reniformis*.

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ABSTRACT

- a Title of Thesis : **Quality of Lemongrass (*Cymbopogon flexuosus*) oils under different storage conditions and their nematicidal activity**
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Keywords: *Cymbopogon flexuosus*, lemongrass, essential oil quality, storage effect, nematicidal activity, *Meloidogyne javanica*, *Meloidogyne incognita* and *Rotylenchulus reniformis*

Laboratory studies were conducted for qualitative and quantitative estimation of essential oils of lemongrass (*Cymbopogon flexuosus*) genotypes/varieties, effect of various storage methods on quality of essential oil of variety CKP 25 and nematicidal activity of aqueous extracts of essential oils of six genotypes/varieties of lemongrass against nematodes *Meloidogyne javanica*, *Meloidogyne incognita* and *Rotylenchulus reniformis*. The essential oils were analysed by GLC techniques employing Flame Ionization Detector (FID) equipped with Capillary Column Thermo TR Wax (Polyethylene glycol). For storage studies the essential oil was kept for six months in glass, metal and plastic vials, replicated thrice under closed as well as open conditions. The quality of oils kept in different vials was estimated just before storage and subsequently after 2, 4 and 6 months of storage. The retention times for citral a (geranial), citral b (neral), geraniol and citronellal were 16.42, 15.53, 17.84 and 12.35 minutes, respectively. The oil content on Fresh Weight Basis (FWB) was in the range from 0.24 to 0.74%. The range of percent composition of various major and minor constituents were as follows: citral a (47.1 to 52.4%), citral b (30.3 to 37.5%), total citral (82.7 to 84.8%), geraniol (2.5 to 2.8%) and citronellal (0.8 to 1.1%). Citral a (geranial), citral b (neral), total citral, geraniol and citronellal contents in the essential oils decreased during storage period. The decrease in citral a (geranial), citral b (neral), total citral, geraniol and citronellal contents during storage was minimum in glass vials followed by plastic vials and metal (aluminium) vials. Between closed and open vials, the decrease in citral a, citral b, total citral, geraniol and citronellal contents was less in closed vials in comparison to open vials. The probable reason for degradation may be due to oxidation of citral a, citral b, geraniol and citronellal into their derivatives resulting in less concentration of these constituents in the essential oil. The nematicidal activity of aqueous extracts of essential oils of lemongrass was highest against the nematodes *M. incognita* followed by *M. javanica* and *Rotylenchulus reniformis*. The probable reason for nematicidal activity of aqueous extracts of lemongrass essential oils may be due to the presence of aldehydic group in major constituents (citral a, citral b) & minor constituents (citronellal) and alcoholic group (geraniol) in minor constituents. Keeping in view the storage studies under laboratory conditions, it can be inferred that essential oils of lemongrass can be kept in closed glass vials for less degradation in quality of essential oil and also these essential oils are effective in controlling the nematodes under laboratory conditions.

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