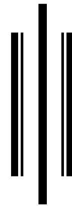
ANTIFUNGAL ACTIVITY OF ESSENTIAL OIL OF SOME MEDICINAL PLANTS AGAINST (DRY ROT OF POTATO) *FUSARIUM OXYSPORUM* Schlecht



A Dissertation
Submitted to the Central Department of Botany
for the Partial Fulfilment as the Requirement of M.Sc. in Botany

Submitted By

Rita Poudel
Batch Number: 2060/062
Symbol Number: 1830
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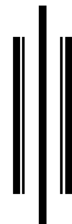

Central Department of Botany
Tribhuvan University, Kirtipur
Kathmandu, Nepal

May, 2009

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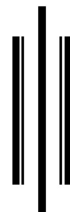

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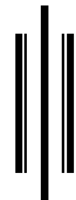

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INSTITUTE OF SCIENCE AND TECHNOLOGY
CENTRAL DEPARTMENT OF BOTANY
OFFICE OF THE HEAD OF DEPARTMENT

Ref. No:

Kirtipur
Kathmandu, Nepal

CERTIFICATE

This is to certify that the dissertation work entitled **"Antifungal Activity of Essential Oil of Some Medicinal Plants against (Dry Rot of Potato) *Fusarium oxysporum* Schlecht"** was conducted by Ms. Rita Poudel under my supervision. The result of this work has not been submitted for any other degree.

I, therefore, recommend this dissertation to be accepted for the partial fulfillment as the requirement of Master's Degree in Botany with Plant Pathology specialization in the Tribhuvan University, Nepal

---

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TRIBHUVAN UNIVERSITY
INSTITUTE OF SCIENCE AND TECHNOLOGY
CENTRAL DEPARTMENT OF BOTANY
OFFICE OF THE HEAD OF DEPARTMENT

Ref. No. Kirtipur, Kathmadu, Nepal

LETTER OF APPROVAL

This dissertation work entitled "**Antifungal Activity of Essential Oil of Some Medicinal Plants against (Dry Rot of Potato) *Fusarium oxysporum* Schlecht"** submitted by Ms. Rita Poudel has been accepted for partial fulfillment of Master's Degree in Botany with Plant Pathology specialization in the Tribhuvan University, Nepal.

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May, 2009 Rita Poudel

ABSTRACT

Potato samples were collected from the Kalimati Vegetable Markets of Kathmandu. The fungus, isolated from the infected potato tubers was identified as ***Fusarium oxysporum*** Schlecht, which causes dry rot disease of potato tubers in storage.

Pathogenecity test was carried out for the conformation of disease. It was confirmed by inoculating the pathogen in to the healthy tubers. *Cinnamomum tamala, Nardostachys grandiflora, Eucalyptus citriodora* and *Cymbopogon citratus* were hydro-distilled in laboratory for the extraction of essential oils. The other two essential oils of *Mentha arvensis* and *Acorus calamus* were obtained from the HPPCL. Each essential oil was diluted to the different concentrations of 40.0µlml^{-1}, 20.0µlml^{-1}, 10.0µlml^{-1}, 5.0µlml^{-1}, 2.5µlml^{-1}, 1.25µlml^{-1} and 0.625µlml^{-1} in 80% acetone. Fungitoxicity was assessed by poisoned food technique. Essential oil of *Eucalyptus citriodora* showed the highest fungitoxicity (100%) at the concentration of 20.0µlml^{-1}, while essential oil of *Cymbopogon citratus, Nardostachys grandiflora* and *Acorus calamus* showed the highest fungitoxicity (100%) at the concentration of 40.0µlml$^{-1.}$ Similarly, essential oils of *Cinnamomum tamala* and *Mentha arvensis* showed the highest fungitoxicity (90.59% and 67.60% respectively) at the concentration of 40.0µlml^{-1}.

The Minimum Inhibitory Concentration (MIC) of different essential oils was obtained. The MIC of *Eucalyptus citriodora* was 20.0µlml^{-1} i.e. the complete inhibition of colony growth of *Fusarium oxysporum* was seen at 20.0µlml^{-1} concentration. Similarly, the MIC of *Cymbopogon citratus, Nardostachys grandiflora* and *Acorus calamus* was found 40.0µlml^{-1} against the mycelial growth of *Fusarium oxysporum.* Thus, comparative antifungal activities of 6 different essential oils were observed against *Fusarium oxysporum* (the causal agent of dry rot disease of potato tubers in storage).

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ACRONYMS AND ABBREVIATIONS

cm : centimeter
et al. : and others (from the Latin *et all*)
etc. : and so forth (from the Latin *et cetera*)
FAO : Food and Agricultural Association of United Nations
g : gram
GoN : Government of Nepal
kg : kilogram
kcal : kilocalorie
m : meter
masl : meter above sea level
mg : milligram
mm : millimeter
ºC : degree Celsius
ºF : degree Fahrenheit
% : per cent
TU : Tribhuvan University
viz. : namely (from the Latin v*idelicet*)
CDB : Central Department of Botany
E. oil : Essential oil
ft : feet
GC : Gas Chromatography
HPPCL : Herb Production and Processing Co. Ltd
MAPS : Medicinal and Aromatic Plants
MIC : Minimum Inhibitory Concentration
μlml^{-1} : microliter per milliliter
µm : micrometer
NTFP : Non-Timber Forest Product
PDA : Potato Dextrose Agar
ppm : parts per million
TLC : Thin Layer Chromatography
Ref. No. : Reference Number
DPR : Department of Plant Resources

CHAPTER-ONE

1 INTRODUCTION

1.1 Background

Nepal is an agricultural country having peculiar diverse fauna & flora, where more than 80% of the economically active population is involved in agriculture (Chapagain, 2001). It has been regarded as 'The Natural Showroom of Biodiversity' because of its geological, ecological and climatic variations (Rajbhandary, 2001). The diverse geographical terrains and climatic conditions of the country range from tropical to alpine zones after tremendous possibilities for the introduction of many more medicinal and other plants of economic value (Malla, 1991).

About 5,160 species of higher plants have been enumerated by Hara & Williams (1979) and Hara et al. (1978) and 5,833 by Koba et al. (1994). Among these plant species, Medicinal and Aromatic Plants (MAPS) are the most popular and rich resources of the country. DMP (1970, 1984) accounted 571 species of MAP in Nepal. DPR (2001) enumerated the total number of flowering plants to be 6,501 (5,636 species, 206 sub-species, 599 varieties and 60 forma)**,** in which about 15% (i.e. about 1600 species) of plant species are considered to be medicinally used in Nepal (Shrestha et al., 2000).

Medicinal plants are simply defined as any plant or plant parts that shows curative properties. Medicinal plants have not only the healing substances for Nepalese people but also have important sources of cash income (Shrestha,T.B.,1994). Plants diversity serves the human kind as renewable natural resources for a variety of biological active chemicals. Most of the plants possess medicinal properties viz. antibacterial, antifungal, antiviral, antihelminthatic, anticancer, sedative, laxative, cardiotonic, diuretic and others (Parajuli et al., 1998). Active chemicals of the medicinal and aromatic plants have been found to be less phytotoxic, more systemic (Fawcett & Spencer, 1970) easily biodegradable (Beye, 1978) and also as host metabolism stimulatory pesticides (Tripathi et al., 1980).

Oils found in plants are classified as either volatile or fixed oil. The volatile fraction from the fresh leaves can be isolated in the form of essential oils through hydro-distillation by Clevenger's apparatus. Volatile oil contains the mixture of hydrocarbons

containing 10-15 carbon atoms while fixed oils contain ester of glycerol's and long chain aliphatic acids (Manandhar, 2004).

Plants are composed of cell materials such as chlorophyll, starch, sugar, proteins, lipids vitamins etc. The medicinal property of plants is due to the presence of active components. The main groups of active components are alkaloids, glycosides, saponins, essential oils, mucilage, tannins, quinines and phenols etc. (Kruger, 1992).

The active components are extractable with different solvents. The active compounds capable of inhibiting or killing micro-organism are called antimicrobial and those capable of being against fungal species are called antifungal agents. Plant disease is a complex phenomenon and is an interaction among the host, the parasite and the environment. The crop protection against pathogen pest and weeds is a major necessity in plant pathology. Antimicrobial chemicals viz. benzimidazoles, aromatic hydrocarbons and sterol biosynthesis inhibitors are often used in the control of plant disease in agriculture. However, there is a series of problems against the effective use of these chemicals in area where the fungi have resistance (Brent & Hollomon, 1998). In order to overcome these problems, higher concentration of these chemicals was used but this increased the risk of high-level toxic residues in the products which were not beneficial for human beings and environment (Cakir et al., 2004). Crop protection is a principal of crop production. Pathogens, pests and weeds are natural enemies of our agriculture and forestry. The chemicals are non- selective and may also damage the non target parts of the plants. Thus, there has been a growing interest on the research of the possible use of the plant essential oils in the disease control, which is less damaging to the human health and environment (Costa et al., 2000; Dura et al., 2003).

Various plant-based oils are capable of controlling several plant diseases. Oils obtained from seeds of several plants such as sunflower, olive, corn and soybeans give excellent control of some plant disease (Mehrotra et al., 2003). Essential oils are even more effective and have more antimicrobial effect. They sometime serve as antiseptic and stimulants as ingredient in medicines, as a lab reagent, as solvents on the paints industry, as insecticides and as a component of plastics, polishes, pastes, ink and glue (Schery, 1972). The presence of antifungal compounds in higher plants has long been recognized as an important factor in disease resistance (Thapaliya & Nene, 1967).

Essential oils are mainly derived from aromatic plants. Essential oils are also known as essences or volatile oils, complex mixture of volatile substances biosynthesized by living organism. They are extracted from the host organism by distillation or extraction with the suitable solvent. They are used in flavor, fragrance food, cosmetic, toiletry and pharmaceuticals industries (Rawal & Pradhan, 2001).

Essential oils are rich source of terpenoids which impart them antifungal and antibacterial properties. They are formed within plants on various locations such as flowers, leaves, roots, wood, barks, seeds, resin etc. (Picman and Towers, 1984).

Harvested fruits and vegetables are vulnerable to attack by microorganisms because of their high moisture content and rich nutrients. During harvesting, packing and transportation, injuries of various kinds are caused which facilitates the entry of certain pathogens. The severity of other disease infection increased as the level of damage increased. Scuffs, where the skin is broken and some bruising occurs, splits and skin grazes were all shown to be entry points for rots. Uninjured potatoes do not develop post harvest rots. A single infected fruit or vegetable can be the source of the infection to the others. Such diseases, which are noticed after the harvest, gradually reduce the nutrients and commercial value of the products.

There are two main post harvest rots of potatoes (Jenny Jobling, 2000), one is bacterial soft rot and another is *Fusarium* rot. Bacterial soft rot tissue is characteristically creamy at first and later black. *Fusarium* rot is commonly called dry rot. The symptoms of this rot are that the rot forms in the cavities in the flesh, which are lined with a pale covered mould and skin, tend to wrinkle as it loses water. The surface dries out over the infection and becomes brown or black. (Source:www.postharvest.com.au)

1.2 Potato and its production

1.2.1 Classification

Class: Magnoliopsida

Subclass: Asteridae

Order: Solanales

Family: Solanaceae

Genus: *Solanum*

Species: *tuberosum*

Potato (*Solanum tuberosum* L.) is one of the most important food plants and staple in the world. It is characteristically a crop of cool temperate region. It requires cool night and well drained sufficient moisture and is propagated through tubers.

The first unambiguous evidence of potato cultivation in Nepal dates to 1793, in records by a British Colonel Kirkpatrick. The potato remained a relatively minor and unrecognized crop in Nepal for over many years, until the first official attempt to improve potato production in Nepal occurred in 1962 under a program sponsored jointly by Nepal and India. In 1972 the National Potato Development Programmed was founded by the Government of Nepal, focused on the production of higher quality potato seed tubers (Akius et al., 1990).

Over the past few decades, potato has become the fastest growing staple crop in Nepal. The great agro-ecological diversity of Nepal allows for potato cultivation to occur somewhere at any times of year. Potato is a winter crop in the terai and low-hills, a spring and autumn crop in the mid-hills and summer crop in the high hills and mountains. The duration of the crop is variable by variety, but longer at high altitudes. The generalized cropping calendar is summarized in **Annex I** and potato production trend in Nepal in **Annex II**.

General recommendations of potato crop spacing by the Department of Agriculture are to plant 25-50g tubers, 60-75cm row to row and 20-30cm plant to plant for an overall seed rate of one to two tons per hectare. According to place and varieties of potato 15-30 tons per hectares were produced. In Nepal produced merely 233000 tons of potato in the years 1961/63 which increase 1440000 tons in the year 2001/03, 1738841 metric tons in the years 2004/05 and total production in the years 2006/07 is 2054817 metric tons. The area of potato cultivation was 41000 hectares in 1961/63, 135000 hectares in 2001/03, 146789 hectares in2004/05 and 156737 hectares in 2006/07. Estimated area and production of potato crops cultivation by the Development Regions of Nepal, 2006/07 is given in **Annex III**.

Population density is particularly high in the valleys and lowlands where the vast majority of the population of Nepal is concentrated. Potato, as a crop capable of high productivity relative to land and time, is likely to remain important to Nepal's food security and agricultural economy. According to the FAO Data of 1998, worldwide

potato production stands at 293 million tons and covers more than 18 million hectares. These include China with 6.2% average annual rate of growth in potato production over the last 10 years; Indonesia 10.6%; Nepal 8.8%; India 4.6% and Pakistan 6.0% respectively (FAOSTAT, September, 1998).

1.2.2 Nutrition Value of Potato

Potatoes are good sources of carbohydrates and vitamin C. They are good for hyperacidity patients and also can be given to infants since they are easy to digest. Potassium and magnesium contents are also rich in potatoes. Magnesium prevents calcification of tissue like kidney and in overcoming formation of stones in the bladder. Because of high nutritional property, it helps in keeping blood pressure low. The nutrition value of potato is summarized in **Annex IV.**

1.3 Potato Diseases

Potato disease is an interaction among a host (potato), pathogen (bacterium fungus, virus, mycoplasma and nematode) and the environment that impairs productivity or usefulness of the crop. The diseases of the potato are listed in Table 1.

Table 1: Potato Diseases

S.N.	Diseases	Pathogens
1	*Fusarium* dry rot of tuber	*Fusarium oxysporum*
2	Wilt	*Fusarium* species
3	Ring rot	*Corynebacterium sepedonicum*
4	Black leg	*Erwinia atroseptica*
5	Common scab	*Streptomyces scabies*
6	Early blight	*Alternaria solani*
7	Late blight	*Phythophthora infestans*
8	Leak	*Phythium species*
9	Pink rot	*Phythophthora erythoseptica*
10	Powdery scab	*Sponspora subterranean*
11	*Rhizoctonia* disease	*Rhizoctonia solani*
12	Silver scurf	*Helmonthosporium solani*
13	*Verticillum* wilt	*Verticillium albo-atrum*
14	PLRV	*Potato leaf roll virus*
15	Virus diseases	*Virus x, y, M, S & A*

Source : www.1.maldonado@cgiar.org.

For the experiments in this study, *Fusarium oxysporum* Schlecht, the causal agent of dry rot of potato tuber was taken as a test fungus.

1.4 Test Fungus

1.4.1 Classification

Class: Deuteromycetes

Order: Moniliales

Family: Tuberculariaceae

Genus: *Fusarium*

Species: *F. oxysporum.*

Fusarium dry rot is one of the most important post harvest diseases of potatoes worldwide. It can reduce crop establishment by killing developing potato sprouts, and crop losses can be up to 25%, while more than 60% of tubers can be infected in storage. Losses appear to be increasing because *Fusarium oxysporum* has become resistance to thiabenzimidazole fungicides that are commonly used to control it (Rosemary, 1983).

1.4.2 Symptoms

Fusarium oxysporum Schlecht, appear as slightly sunken, small brown patch on the tuber surface especially on wound. The sunken lesions enlarge and wrinkle sometimes in concentric rings as the dead tissues dry out. The internal appearance of the infected tuber changes from moist, soft, brown-colored lesion to a cavity which is bordered by hard, leathery or chalky textured rotted tissue. From the rotted tissue white mycelial tufts and fruiting structures appear. This disease affects planted seed tubers, mature tubers, and tubers in storage. It affects tubers about 1 month of harvest (Smith & Swingle 1904). Most varieties do not have resistance to dry rot (Secor, G. A. and et al. 1992).

1.4.3 Pathogen

Fusarium oxysporum Schlecht, f.sp. tuberosi Synder & Hansen was reported in 1940 as causing dry rot of potatoes. It is the most frequently encountered and is most aggressive pathogen (Stachewicz, 1974). When cultured in PDA medium, the pigmentation shows cream to light brown. Aerial mycelium is sparse to dense and floccose, appears as cottony white colonies with innumerous conidia production. Conidiophores are short and bear macro- and micro-conidia. Micro conidia develop after 2-3 days. These are simple phialides arising laterally from short sparsely branched conidiophores. Micro-conidia are oval-ellipsoid cylindrical, straight to curved, aseptate or singly septate and

measure 5.94-13.86 µm×2.64-4.62µm. Macro-conidia develop after 4-7 days which are borne on more elaborately branched conidiophores. They are thin walled, generally 3-5 septate, mostly 3 septate, sickle shaped, pointed at both ends and measure 26.4-47.5µm×3.3-5.28µm. Chlamydospores develop after 7-14 days and they are smooth walled.

1.4.4 Disease Cycle

The fungus can survive for several years in the field soil, but the primary inoculum is generally borne on seed tuber surface. Infected tubers when used as seed in field help in the spread of the disease. Dry rot develops most rapidly in high humidity and at the temperature 15-20ºC (Booth, 1971 and www.doctorfungus.org).

1.4.5 Common Management Practices for Dry Rot Disease

- Most techniques for managing dry rot are aimed at preventing injury to the tubers, either seed or the harvested crop. Preventing bruises will greatly aid in avoiding infection. Practicing the following procedures will help to prevent dry rot.
- Seed should be inspected preferably during the last month of storage.
- Warm seed tubers at least 50ºF before handing and cutting to minimized injury and promote rapid growth. Cold tubers are very prone to shatter bruising.
- Clean and disinfect the seed storage area before receiving seeds.
- Protect the seeds from wind and sunlight. It is best to cut seeds only as much as can be planted within in 24 hours.
- There should be good air circulation and temperature 55ºF. (Rosemary,1983).

1.4.6 Control Measures of Dry Rot Disease

- Cultural practices
- Sanitation
- Chemical measures
- Use of benzimidazole and thiobenzimidazole fungicides

1.5 Test Plant Species

Six plant species, listed in Table 2, were taken for the assessments of fungitoxicity.

Table 2: Test Plant Species

S.N.	Scientific Name	Local Name	Family	Parts Used	Place of Collection
1	*Mentha arvensis*	Pudina	Labiatae	Leaves	HPPCL
2	*Cinnamomum tamala*	Tejpat	Lauraceae	Leaves and Bark	Kaski Lekhnath N.P
3	*Cymbopogon citrates*	Lemongrass	Poaceae	Leaves	CDB garden
4	*Acorus calamus*	Bojho	Araceae	Rhizomes and roots	HPPCL
5	*Eucalyptus citriodora*	Masala	Myrtaceae	Leaves	CDB garden
6	*Nardostachys Grandiflora*	Jatamansi	Valerianaceae	Rhizomes	Pyuthan Okharkoat V.D.C

These above given medicinal and aromatic plants are available in Nepal. From the study, it was found that these above given plant species are good source of essential oils and contain different chemicals that have antifungal properties. Detail description of these test plant species are given in **Annex V.**

CHAPTER - TWO

2 OBJECTIVES OF THE STUDY

The study was carried out with the following objectives.

i) To isolate the pathogen from infected potato tuber and test it's pathogenicity.
ii) To test the antifungal activity of essential oil of 6 medicinal plants against the test fungus.
iii) To know the Minimum Inhibitory Concentration (MIC) of the test plant oils against the test fungus.
iv) To compare the antifungal properties of the essential oils of 6 test plant species.

2.1 Justifications of the Study

Potato (*Solanum tuberosum*) is an economically important food plant, rich in carbohydrate, vitamins and minerals. The tubers are eaten as boiled, fried or cooked. *Fusarium oxysporum* causal agent of dry rot disease of potato tubers cause serious damage to the vegetable and may adversely affect to our food security. Therefore, there should be the provision of the protection of tubers against the disease during storage.

Tuber protection by using the commercially available chemicals brings a series of problems in human health and environment. Essential oils are the plant components that are less phytotoxic, more systemic, easily biodegradable and environmentally non-pollutive (Parajuli et al., 2005). All the consumers will be directly benefited because health exposure can be minimized. The utilization of naturally available plants extracts for the control of various types of crop diseases will be cheaper than the expensive chemicals. The study of post harvest disease of potato is lacking far behind in Nepal. Control of dry rot disease of potato in storage by essential oils treatment in vitro has not been done yet in Nepal.

2.2 Limitations of the Study

- Essential oils of 6 plants were used for the experiment.
- Four selected plants were used for extraction and two essential oils were taken from the HPPCL.
- TLC and GC could not be carried out for screening essential oil.
- Time constraint

CHAPTER - THREE

3 LITERATURE REVIEW

Shrivastav, Sudha (1970) first reported *Fusarium* wilt in India from (1964-1966), incidence reaching 60-70% *Fusarium oxysporum F.sp.tuberosi* and *Fusarium solani*. She found positive pathogenecity response on potato.

Strachewicz, H. (1974) found that *Fusarium spp.* were responsible for 80% of tuber rot which caused several losses in storage in 1971/73.

Moshe Shimoni et al. (1993). Essential oils were extracted from *Majorana syriaca, Satureja thymbra, Micromeria fruticosa*, and *Salvia triloba*, and their volatile fractions were tested for their antifungal activity against the soil-borne pathogens *Fusarium oxysporum* and *Macrophomina phaseolina* and the foliar pathogens *Botrytis cinerea* and *Exserohilum turcicum*. Results showed a fungitoxic effect of 1, 2.5, and 5µl of various essential oils on fungal mycelium growth. The most significant effect was exerted by essential oils extracted from *M. syriaca*, which inhibited the growth of *B. cinerea* by 44% and of all the other fungi tested by 100%.

Shrestha & Sharma (1988) studied antimicrobial activities of some plant products viz. *Mentha arvensis, Acorus calamus, Zanthoxylum oxyphyllum* and turpentine oil against some fungi and bacteria.

Mishra et al. (1990) found that *Eucalyptus* oil inhibited absolute mycotoxicity against *Microsporium gypseum* and *Trihophyton mentagrophytes* at its minimum inhibitory concentration of 400ppm. It showed broad mycotoxic spectrum inhibitory 5 out of 10 human as well animal pathogenic fungi tested as higher concentration.

Mishra & Tiwari (1990) screened ethanol extracts of leaves of *Claotropis procera, Azadirachta indica* and *Datura stramonium* to evaluate their toxicity against *Pyricularia oryzae, Rhozoctonia solani, Fusarium moniliforme* and *Aspergillus niger*. Plants tested showed toxic principles against one or other tested pathogens.

Gourinath & Manoharacharya (1991) tested the antifungal activities of 20 medicinal plants against the pathogenic fungi namely *Curularia lunata, Cylindrocarpon* sp,

Fusarium sp. and *Myrothecium leucotrichum*. Extracts of all the test plant species were fungitoxic and showed inhibitory effect on the test fungi.

Shrestha et al. (1994). Two hundred and nineteen species of aromatic and medicinal plants belonging to sixty plant families were collected from different part of the country. The essential oil content of these plants were determined by hydro-distillation method. Chemical constituents of some of the oils were determined by GC.

Isman (2000) studied the utility of essential oils of aromatic plants and analyzed their possible herbicidal activities. He mentioned that these essential oils could be the good herbicides since they do not persist in soil or contaminated ground water and cause little or no mammalian toxicity.

Chhetri (1999). Ecological study of *Nardostachys grandiflora* was conducted in three different sites and essential oils were collected by hydro-distillation method. Essential oil content varied from 0.4 to 1.66% depending up on the geographical location and habitat.

Dubey et al. (1983) collected E. oils from the volatile antifungal fraction of the leaves of *Melaleuca leucadendron* by hydro-distillation in Clavenger's apparatus and evaluated for its activity against *Rhizoctonia solani*.

Snowdon (1991) reported *Fusarium* rot of tomatoes, pepper and egg plants caused by *F. solani, F. oxysporum, F. moniliforme, F. equiseti*. According to him, the fungus may be seed borne and spraying the crop with palm kernel oil controls the *Fusarium* rot of pepper and a post harvest dip in such oil can protect the peppers during storage at ambient temperatures in the tropics.

Mehood et al. (1997) studied the antifungal properties of *Cinnamomum* sp. using well agar diffusion method and filter paper disc diffusion method.

Parajuli et al. (2005) tested the fungitoxicity of E. oils of *Xanthoxylum armatum, Nardostachys grandiflora, Juniperus recorva, Artemisia dubia A. gmelinii* and *Thymus linearis* against *Alternaria brassicicola*, the causal agent of leaf spot disease of cabbage by poisoned food technique. The fungitoxicity was found to be variable with different oils.

Rai et al. (2002) reported that extracts of *Thuja*, *Vinea*, lower dosage of *Cinnamomum* oil and higher dosage of *clove* oil exhibited the inhibition of colonization of *Fusarium moniliforme.*

Bonsignore et al. (1990) screened the essential oils of five Sardinian plant viz. *Juniperus oxycedrus* sub sp. *oxycedrus, Spartium junceum, Helichysum italicum* sub sp. *microphyllum, Inula viscose* and *Asphodelus microcarpus* showed antifungal activity against some species of blastomycetes.

Fandohan et al. (2004) from their analysis reported that several compounds were present in the essential oil of which monoterpenes were predominant and also found that oil from *Cymbopogon citratus* contained citral (neral and geranial) 47% and myrcene 28% where as oil from *Eucalyptus citriodora* contained mainly citronellal 66% along with a small amount of the alcohol citronnellol 12%.

Singh et al. (1980) found that essential oil from *Cymbopogon martini*, *C. oliveri* and *Trachyspermum ammi* exhibited strong antifungal activity against *Helminthosporium oryzae*.

Pattnaik et al. (1996) had taken essential oils of different plants such as lemon grass, eucalyptus, orange, geranium, palmorosa etc and tested for antibacterial activity against 22 bacteria and 12 fungi. They found out the Minimum Inhibitory Concentration (MIC) of eucalyptus, lemon grass, palmorosa and peppermint oils ranged from 0.16 to 20$\mu l ml^{-1}$ for 18 bacteria and from 0.25 to10$\mu l ml^{-1}$ for 12 fungi.

Fiori et al. (2000) studied antifungal activity of leaf extracts and essential oils of some medicinal plants against *Didymella bryoniae*. They reported that the crude extracts of *Eucalyptus* and *Ageratum* species showed inhibitory effect on mycelial growth and germination of spore where as essential oil of *Cymbopogon citratus, Ageretum conizoids* and *Eucalyptus citriodora* provided 100% inhibition of the mycelia growth and germination of spores of *Didymella bryoniae*.

Bankole et al. (2002) studied the potentiality of powder and essential oil from dried ground leaves of *Cymbopogon citratus* (lemon grass) to control storage deterioration and aflatoxin contamination of melon seeds. Four mould species: *Aspergillus flavus*, *A. niger*, *A. tamarii* and *Penicillium citrinum* were inoculated in the form of conidia

suspension into shelled melon seeds. The essential oil at 0.1 and 0.25ml/100g seeds and ground leaves at 10g/100g seeds significantly reduced deterioration and aflatoxin production in shelled melon seeds treated inoculated with toxigenic *A. flavus.* At higher dosage (0.5ml and 1.0ml/100g seeds), the essential oil completely prevented aflatoxin production.

Nguefack et al. (2004) investigated the effect of essential oils extracted from *Cymbopogon citratus*, *Monodora myristica, Ocimum gratissimum, Thymus vulgaris* and *Zinziber officinale* against three food spoilage and mycotoxin producing fungi *Fusarium moniliforme, Aspergillus flavus* and *Aspergillus fumigatus*. They reported that the essential oils from *Ocimum gratissimum*, *Thymus vulgaris, Cymbopogon citratus* had effectively prevented conidial germination and the growth of all three fungi on corn meal agar at 800, 1000 and 1200ppm respectively.

Manandhar A. (2005) investigated antifungal activities of essential oils from six plants against *Bipolaris sorokiniana.* she reported appreciable inhibitory effects towards the growth of colony of fungi. Among six plants, essential oil of *Thymus linearis* was found to be the most effective in its fungitoxic properties.

Kuwar & Tripathi (2002). The essential oil of *Cinnamomum tamala* in 500ppm and 1000ppm can inhibit 30% and 56.6% of mycelial growth of *Aspergillus flavus* respectively. Similarly, the oil can inhibit mycelial growth of *Aspergillus niger* less than *Aspergillus flavus* i.e. 23% at 500ppm and 50% at 1000ppm.

According to Gulati & Suri (1982), essential oils are considered as luxurious items because of their traditional use of flavoring agent in perfumes, cosmetics, beverage, food and confectionery. Few people, who are actively associated with commerce or research of essential oils are aware of their inherent antibacterial, antifungal, insecticidal and antihelminthetic efficiency which makes them highly valuable.

Ascensao et al. (2003) found the essential oil of *Thymus pectinatus* extracted in menthol exhibit strong antimicrobial activity against all microorganisms tested.

Tabak et al. (1999) studied the effect of ethanol and methylene chloride extract of *Cinnamomum* sp. on *Helicobacter pylori* growth and found methylene chloride to inhibit growth of *Helicobacter pylori*.

Ahmed et al. (2000) analyzed the water-distilled E. oil of *Cinnamomum tamala* by GC-MS. 63 compounds were identified representing β–caryophyllene (25.3%), linalool (13.4%) and caryophyllene oxide (10.3%) were the main constituents of the oil obtained in 0.03% yield.

Dwivedi et al. (1990) found that the essential oil of *Lippia alba* exhibited absolute inhibition of mycelial growth of *Macrophomina phaseolina* at 2000ppm following poisoned food technique. The oil was found to be non phytotoxic in nature.

Renu et al. (1980) found that the MIC of the *Cestrum diurnum* against *Helminthosporium oryzae, Helminthosporium carbonum, Helminthosporium graminium, Helminthosporium maydis, and Helminthosporium turcicum* was at 0.7%.

Pawar, V.C. & Thaker, V.S. (2007) evaluated the inhibitory effects of essential oils extracted from 10 Indian plants against five fungi. The plants used for extraction of essential oils were six species of the genus *Eucalyptus* and *Ocimum basilicum, Prosopis cineraria* and *Derris indica*. The fungi used in the experiments were *Fusarium solani*, *F. oxysporum*, *F. pallidoroseum*, *F. acuminatum* and *F. chlamydosporum*. The susceptibility of the *Fusarium* species was tested by the paper disc method and the serial dilution technique. The results were compared with the inhibitory effects of miconazole on the fungi. The essential oils extracted from the *Eucalyptus* species markedly inhibited fungal growth. *Prosopis cineraria* did not show inhibiting properties. Among the fungi, *F. oxysporum* proved to be the most resistant species.

Pandey et al. (1983). Leaves of 25 plant species were screened for their toxicity against *Fusarium lateritium f.*sp.*ajani*. Out of those species, *Aegle marmelos, Citrus aurantifolia* and *Mentha arvensis* exhibited strong toxicity inhibiting the mycelia growth completely. The volatile constituents from each plant were isolated in the form of essential oil and the fungitoxicity of each oil was tested separately.

CHAPTER - FOUR

4 MATERIALS AND METHODS

4.1 Materials

Along with plants and extracts, different equipments, glassware and chemicals were used in performing the research work. All the materials used are listed in the **Appendix**.

4.2 Methods

4.2.1 Collection of Diseased Potato Tubers

For the investigation, the diseased samples of potato tubers were collected from the local vegetable market of Kathmandu (Kalimati), Nepal.

4.2.2 Isolation of *Fusarium oxysporum* (Test fungus)

Infected tubers were collected from Kalimati vegetable market, Kathmandu. The infected pieces were surface sterilized. Surface sterilization was done with 0.1% mercuric chloride followed by washing with sterilized water. With the help of sterilized needles and forceps, some piece of fungal colony was transferred aseptically into Petri plate containing PDA media. It was incubated in an inverted position in an incubator at aseptic condition at 25±2°C for a week. Then, the mycelial growth of the test fungus was observed.

4.2.3 Conidial Measurement of the Test Fungus

For the conidial measurement, ocular micrometer was placed inside the eyepiece of a compound microscope and calibrated by superimposing the graduation of stage micrometer. The number of divisions of ocular micrometer coinciding with the number of divisions in stage micrometer was noted down and calculated the calibration factor applying the formula:

$$\text{One ocular division} = \frac{\text{No. of divisions on stage micrometer}}{\text{No. of divisions on ocular micrometer}} \times 10 \bullet \text{m}$$

Then, the stage micrometer was replaced by a fine slide of *Fusarium oxysporum* mounted in lacto phenol. Size of conidia were measured by determining the no. of ocular micrometer (**Annex VI**). In each measurement readings were taken.

Measurement was carried out to find the range of size of the different parts of the fungus.

4.2.4 Media Preparation

Detail of ingredients for the preparation of 1 liter PDA medium is given in the Table 3.

Table 3: Ingredients for the PDA medium

S.N.	Ingredients	Quantity
1	Potato	200g
2	Agar	20g
3	Dextrose	20g
4	Distilled water	1000ml

Glassware were cleaned thoroughly in cleaning mixture and finally with distilled water. The glassware were dried and than wrapped with paper and sterilized in hot air oven for 2 hours at 160°C. Potato Dextrose Agar (PDA) media was prepared and poured into petriplates and culture tubes aseptically **(Annex VII).**

4.2.5 Pathogenecity Test

For carrying out the pathogenecity test, at first, the infected tubers were collected and characteristic symptoms were noted down. Then from infected potato tuber, *Fusarium oxysporum* was isolated in PDA as pure culture. Inoculum from the pure culture was transferred to the healthy tubers. When these tubers were kept at the temperature of 25±2°C, the characteristic symptoms were produced after 7 days, which were found to be similar with the symptoms on tubers previously collected from the field. The fungus was re-isolated and its characters were compared with the previously isolated fungus.

4.2.6 Identification of Fungus

From the pathogenecity test, the disease was proved to be caused by *Fusarium oxysporum*. The characteristic features of the fungus were identified with the help of book “The Genus Fusarium” (G. Booth, 1971).

4.2.7 Maintenance of Pure Culture

The pure culture of the test fungus was maintained after every one week by sub-culturing test fungus by in sterilized petriplates and slants containing PDA media

4.2.8 Inoculum Disc

Inoculum discs of 5mm in diameter were prepared from one-week-old culture for the inoculation in to the media for further experiment.

4.2.9 Collection of Test Plant Species

Among different plants species *Cinnamomum tamala* were collected from Kaski district, *Eucalyptus citriodora* and *Cymbopogon citratus* were collected from the CDB, TU Kirtipur and *Nardostachys grandiflora* were collected from Pyuthan district, and two oil samples *Mentha arvensis* and *Acorus calamus* were taken from the HPPCL, Kathmandu, Nepal.

4.2.10 Processing of Samples

The collected plant species were processed for the hydro-distillation of essential oils by the following steps.

Shade Drying: The collected plants were cut in to smaller pieces and spread under the shade at room temperature and turned up and down to hasten drying about 5 days.

Storage of Samples: The completely dried samples were packed in waterproof bags. Then the samples were taken for hydro-distillation turn by turn to extract essential oil.

Extraction of Essential Oils: 50g of shade-dried sample of plant species was surface sterilizes with 0.1% mercuric chloride solution followed through washing with distilled water. The sample was then pulverized in distilled water and subjected to hydro distillation for 6-8 hours in Clevenger's apparatus (Cleavenger,1928). The volatile fractions condensed after hydro-distillation exhibited two distinct layers an upper aromatic layer of essential oil and a lower colorless aqueous layer. The aromatic layer was collected and dried over anhydrous sodium sulphate and stored at low temperature (10°C) following Rao & Srivastava (1994).

4.2.11 Dilution of Essential Oil

Essential oil was diluted in to different concentrations with 80% acetone (Rao & Srivastava, 1994). The oil was diluted in to 40.0µlml^{-1}, 20.0µlml^{-1}, 10.0µlml^{-1}, 5.0 µlml^{-1}, 2.5 µlml^{-1}, 1.25µlml^{-1}, and 0.625µlml^{-1} for assaying fungitoxicity. First, 0.4ml of essential oil was added to 9.6ml acetone to make 40.0µlml^{-1} concentration. Six sterilized plastic containers were taken and poured to 5ml acetone in each container to prepare the oils of different concentrations ranging from 20 to 0.625µlml^{-1}. 5ml of diluted essential oil from 40.0µlml^{-1} was poured to 5ml acetone to make 20.0µlml^{-1} concentration. Similarly, 5ml diluted essential oil from each 20.0µlml^{-1}, 10.0µlml^{-1}, 5.0µlml^{-1}, 2.5µlml^{-1} and 1.25µlml^{-1} was poured separately to 5ml acetone to make 10.0µlml^{-1}, 5.0µlml^{-1}, 2.5µlml^{-1} 1.25µlml^{-1} and 0.625µlml^{-1} concentration respectively. Further, 5ml acetone was taken in a separate container and that was regarded as 0µlml^{-1} essential concentration. The oils in plastic containers were labeled and stored at 10°C.

4.2.12 Experiments on Antifungal Assay

The Antifungal activity of the oil was assessed by using the poisoned food technique (Grover & Moore, 1962) in whom the antifungal efficacy of oil was tested by poisoning the media with the oil.

The mycelial growth of *Fusarium* discs (from its one week old culture) on PDA media poisoned with 7 different concentrations (with1 control group) of 6 essential oils ranging from 40.0-0.625µlml^{-1} were assessed making three replicas for each concentration. That is for each oil 24 petriplates were made.

0.5ml from every concentration of oil was taken in a pre sterilized and cooled Petri plate. 9.5ml melted culture medium was poured on that with gently swirling to mix the contents thoroughly. The essential oil was replaced by equal volume of acetone in control set. After the medium solidified inoculum's disc, 5mm in diameter, of the test fungus was aseptically inoculated upside down on the medium in the centre of each plate. The plates were than incubated at 25±2°C for a week following Rao & Srivastava (1994).

4.2.13 Calculation of Inhibition of Mycelial Growth

After the one week of incubation, the average diameter of the colonies of the test fungus in each and every concentration was measured.

Antifungal activity was assessed in triplicates in each concentration of essential oils. It was assessed in terms of percentage inhibition of mycelial growth of the test fungus Rao & Srivastava, 1994).

$$\% \text{ inhibition of mycelial growth } = \frac{\text{gc - gt}}{\text{gc}} \times 100$$

Where, g_c - growth of mycelial colony after incubation period in control set = diameter of colony minus diameter of inoculum disc

g_t - growth of mycelial colony after incubation period in treatment set = diameter of colony minus diameter of inoculum disc

4.2.14 Photography

Infected potato tubers, the pure culture, the conidia and the inhibition of mycelial growth of the test fungus by the different plant essential oils at different concentrations have been illustrated in the **Plates**.

CHAPTER - FIVE

5 OBSERVATIONS AND RESULTS

5.1 Assessment of Antifungal Activity of E. Oils

Antifungal activity of each essential oil was assessed at different concentrations against *Fusarium oxysporum* by poisoned food technique. All essential oils were diluted into similar degree of concentration. The antifungal activity was assessed in terms of the percentage of mycelial growth inhibition. The percentage of mycelial growth of the test fungus was found to be different with different concentrations of the essential oils used. Mycelial growth and growth inhibition percentage of *Fusarium oxysporum* by using different essential oil viz. *Mentha arvensis, Cinnamomum tamala, Cymbopogon citratus, Acorus calamus, Eucalyptus citriodora* and *Nardostachys grandiflora* are given in the Tables 4, 5, 6, 7, 8, and 9.

Graphical representations of the growth inhibition percentage are shown in the Figures 1, 2, 3, 4, 5, 6 and 7 respectively.

5.1.1 Antifungal Activity of Essential Oil of *Mentha arvensis*

Inoculum size: 5mm in diameter							
Concentration of Essential Oil ($\mu l ml^{-1}$)		**Size of Colony (Diameter in mm)**				**Mycelial Growth (mm)**	**Growth Inhibition %**
		Obs. 1	**Obs. 2**	**Obs. 3**	**Mean**		
Control Set		85	90	90	88.33	83.33	0.00
Treatment Set	0.625	70	75	70	71.67	66.67	20.00
	1.25	65	67	65	65.67	60.67	27.20
	2.5	60	60	60	60.00	55.00	34.00
	5.0	55	52	54	53.67	48.67	41.60
	10.0	50	51	49	50.00	45.00	46.00
	20.0	40	40	40	40.00	35.00	58.00
	40.0	32	33	31	32.00	27.00	67.60

Table 4: Antifungal activity of Essential Oil of *Mentha arvensis* against *Fusarium oxysporum*

Essential oil of *Mentha arvensis* showed mycelial growth inhibition as 67.60%, 58.00%, 46.00%, 41.60%, 34.00%, 27.20% and 20.00% at concentration 40.0$\mu l ml^{-1}$, 20.0$\mu l ml^{-1}$, 10.0$\mu l ml^{-1}$, 5.0$\mu l ml^{-1}$, 2.5$\mu l ml^{-1}$, 1.25$\mu l ml^{-1}$ and 0.625$\mu l ml^{-1}$ against *Fusarium oxysporum* respectively.

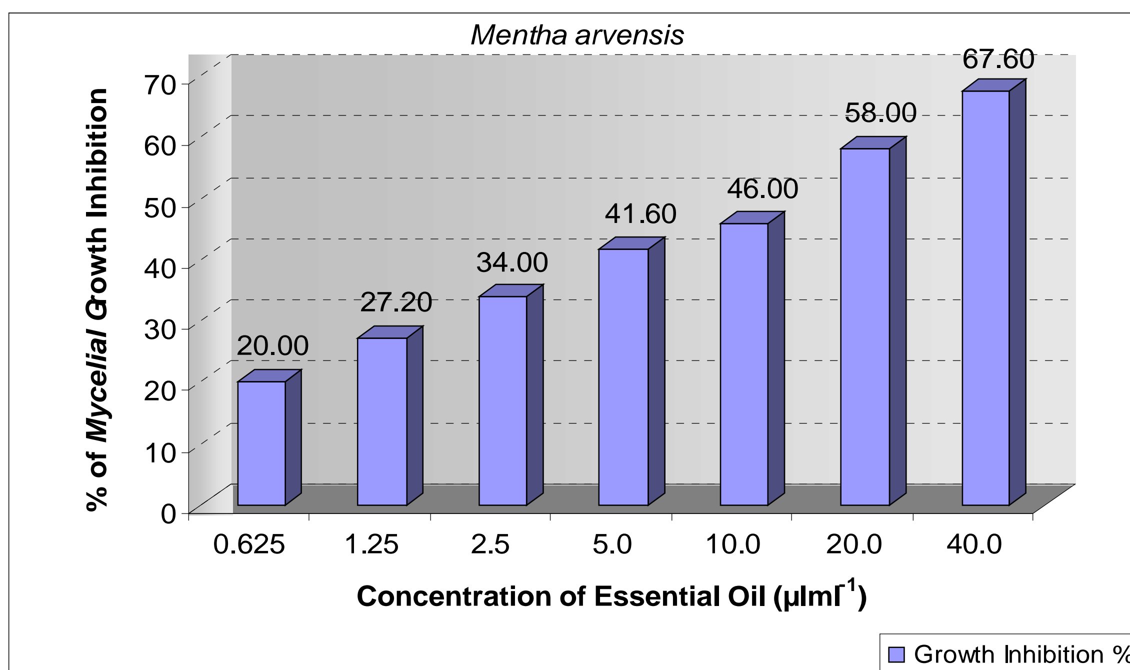

Figure 1: Graphical representation of growth inhibition percentage of *Fusarium oxysporum* by essential oil of *Mentha arvensis*

5.1.2 Antifungal Activity of Essential Oil of *Cinnamomum tamala*

Inoculum size: 5mm in diameter

Concentration of Essential Oil (µlml^{-1})		Size of Colony (Diameter in mm)				Mycelial Growth (mm)	Growth Inhibition %
		Obs. 1	Obs. 2	Obs. 3	Mean		
Control Set		90	90	90	90.00	85.00	0.00
Treatment Set	0.625	77	80	83	80.00	75.00	11.76
	1.25	72	72	70	71.33	66.33	21.96
	2.5	68	66	66	66.67	61.67	27.45
	5.0	61	60	60	60.33	55.33	34.90
	10.0	55	52	50	52.33	47.33	44.31
	20.0	35	30	30	31.67	26.67	68.63
	40.0	12	15	12	13.00	8.00	90.59

Table 5: Antifungal activity of essential oil of *Cinnamomum tamala* against *Fusarium oxysporum*

Essential oil of *Cinnamomum tamala* showed mycelial growth inhibition as 90.59%, 68.63%, 44.31%, 34.90%, 27.45%, 21.96%, and 11.76% at concentrations 40.0µlml^{-1}, 20.0µlml^{-1}, 10.0µlml^{-1}, 5.0µlml^{-1}, 2.5µlml^{-1}, 1.25µlml^{-1} and 0.625µlml^{-1} against *Fusarium oxysporum* respectively.

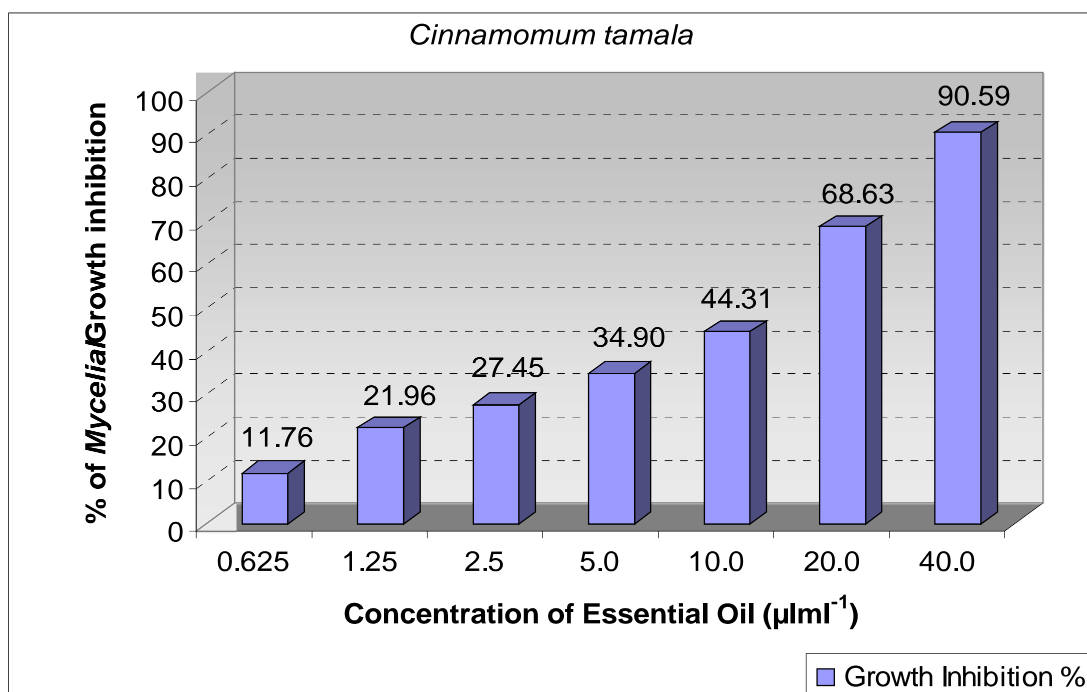

Figure 2. Graphical representation of growth inhibition percentage of *Fusarium oxysporum* by essential oil of *Cinnamomum tamala*

5.1.3 Antifungal Activity of Essential Oil of *Cymbopogon citratus*

Inoculum size: 5mm in diameter							
Concentration of Essential Oil (μlml^{-1})		Size of Colony (Diameter in mm)				Mycelial Growth (mm)	Growth Inhibition %
		Obs. 1	Obs. 2	Obs. 3	Mean		
Control Set		90	90	90	90.00	85.00	0.00
Treatment Set	0.625	81	80	82	81.00	76.00	10.59
	1.25	78	77	77	77.33	72.33	14.90
	2.5	74	75	72	73.67	68.67	19.22
	5.0	70	70	70	70.00	65.00	23.53
	10.0	27	30	25	27.33	22.33	73.73
	20.0	13	13	13	13.00	8.00	90.59
	40.0	0	0	0	0.00	0.00	100.00

Table 6: Antifungal activity of essential oil of *Cymbopogon citratus* against *Fusarium oxysporum*

Essential oil of *Cymbopogon citratus* showed mycelial growth inhibition as 100%, 90.59%, 73.73%, 23.53%, 19.22%, 14.90% & 10.59% at concentrations 40.0μlml^{-1}, 20.0μlml^{-1}, 10.0μlml^{-1}, 5.0μlml^{-1}, 2.5μlml^{-1}, 1.25μlml^{-1} and 0.625μlml^{-1} against *Fusarium oxysporum* respectively.

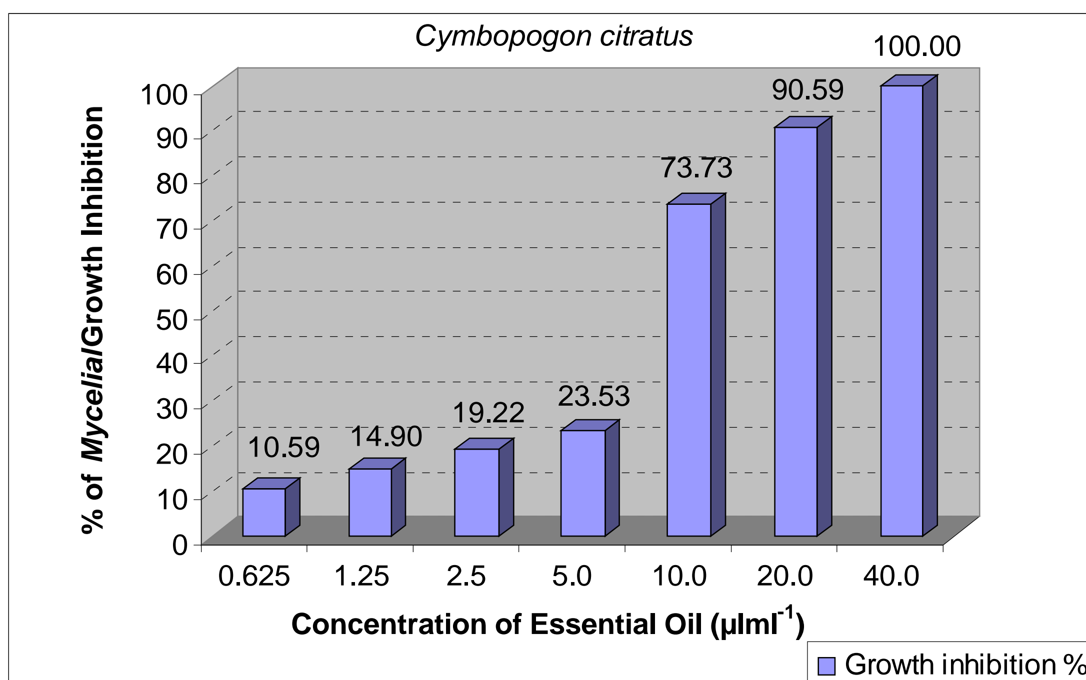

Figure 3. Graphical representation of growth inhibition percentage of *Fusarium oxysporum* by essential oil of *Cymbopogon citratus*

5.1.4 Antifungal Activity of Essential Oil of *Acorus calamus*

Inoculum size: 5mm in diameter							
Concentration of Essential Oil ($\mu l m l^{-1}$)		**Size of Colony (Diameter in mm)**				**Mycelial Growth (mm)**	**Growth Inhibition %**
		Obs. 1	**Obs. 2**	**Obs. 3**	**Mean**		
Control Set		90	90	90	90.00	85.00	0.00
Treatment Set	0.625	80	85	81	82.00	77.00	9.41
	1.25	76	70	71	72.33	67.33	20.78
	2.5	62	65	60	62.33	57.33	32.55
	5.0	50	45	47	47.33	42.33	50.20
	10.0	40	35	35	36.67	31.67	62.75
	20.0	16	18	15	16.33	11.33	86.67
	40.0	0	0	0	0.00	0.00	100.00

Table 7: Antifungal activity of essential oil of *Acorus calamus* against *Fusarium oxysporum*

Essential oil of *Acorus calamus* showed mycelial growth inhibition as 100%, 86.67%, 62.75%, 50.20%, 32.55%, 20.78% and 9.41% at concentrations 40.0$\mu l m l^{-1}$, 20.0$\mu l m l^{-1}$, 10.0$\mu l m l^{-1}$, 5.0$\mu l m l^{-1}$, 2.5$\mu l m l^{-1}$, 1.25$\mu l m l^{-1}$ and 0.625$\mu l m l^{-1}$ against *Fusarium oxysporum* respectively.

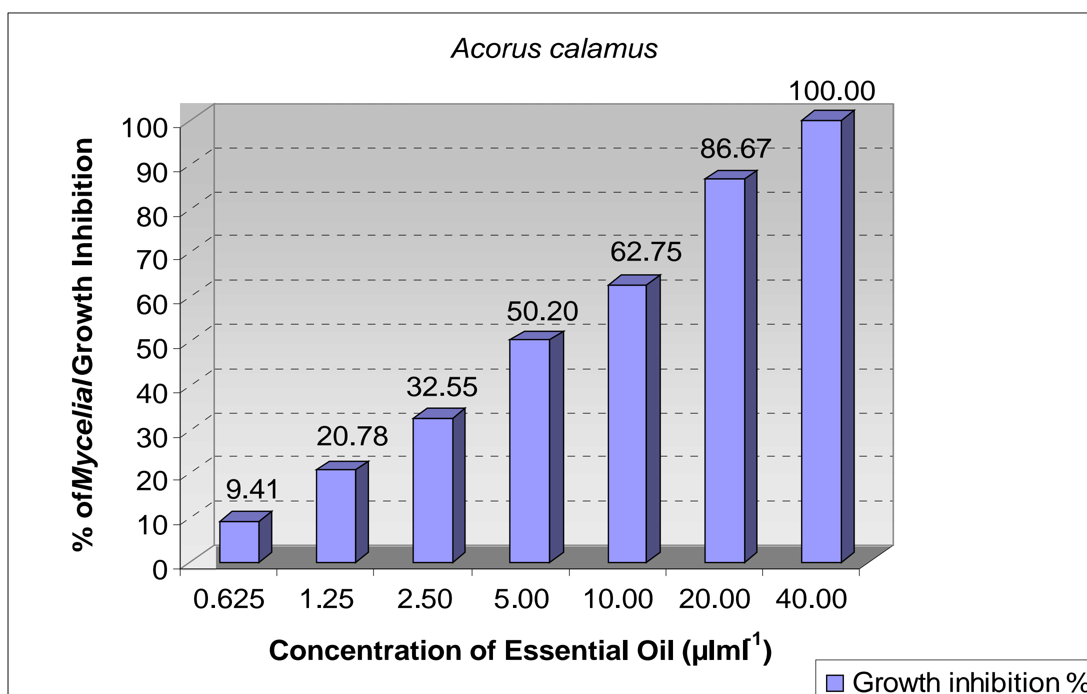

Figure 4. Graphical representation of growth inhibition percentage of *Fusarium oxysporum* by essential oil of *Acorus calamus*

5.1.5 Antifungal Activity of Essential Oil of *Eucalyptus citriodora*

Concentration of Essential Oil (μlml^{-1})		Size of Colony (Diameter in mm)				Mycelial Growth (mm)	Growth Inhibition %
		Obs. 1	Obs. 2	Obs. 3	Mean		
Control Set		90	90	90	90.00	85.00	0.00
Treatment Set	0.625	44	42	44	43.33	38.33	54.90
	1.25	41	41	40	40.67	35.67	58.04
	2.5	33	35	34	34.00	29.00	65.88
	5.0	21	20	22	21.00	16.00	81.18
	10.0	7	7	8	7.33	2.33	97.25
	20.0	0	0	0	0.00	0.00	100.00
	40.0	0	0	0	0.00	0.00	100.00

Inoculum size: 5mm in diameter

Table 8: Antifungal activity of essential oil of *Eucalyptus citriodora* against *Fusarium oxysporum.*

Essential oil of *Eucalyptus citriodora* showed mycelial growth inhibition as 100%, 100%, 97.25%, 81.18%, 65.88%, 58.04% and 54.90% at concentrations 40.0μlml^{-1}, 20.0μlml^{-1}, 10.0μlml^{-1}, 5.0μlml^{-1}, 2.5μlml^{-1}, 1.25μlml^{-1} and 0.625μlml^{-1} against *Fusarium oxysporum* respectively.

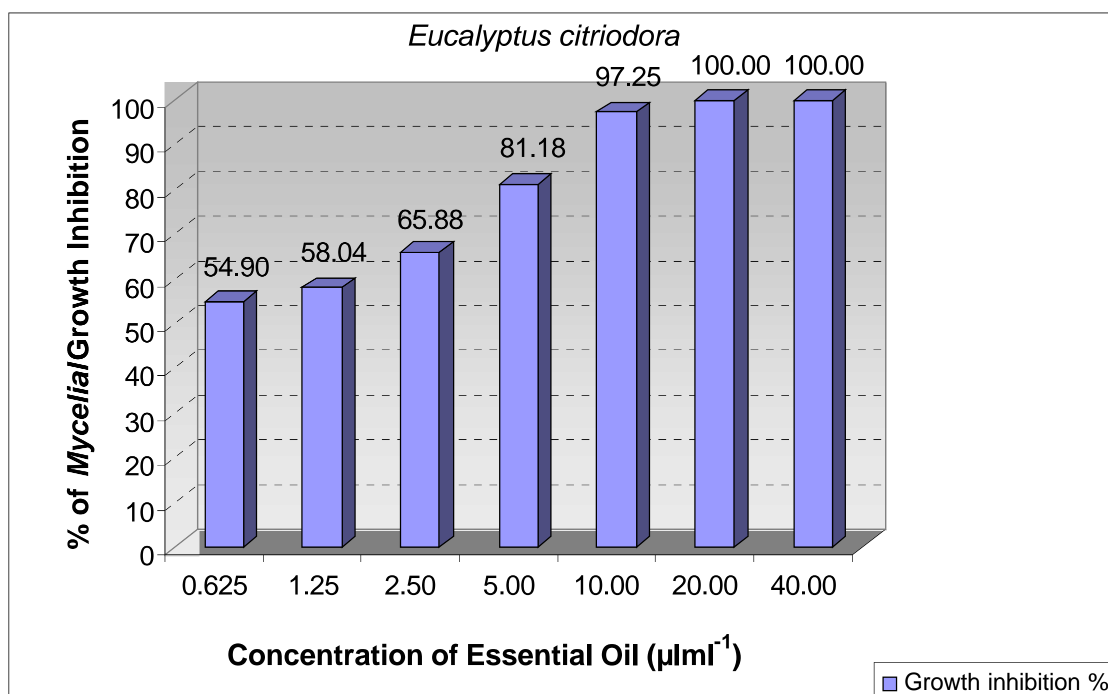

Figure 5. Graphical representation of growth inhibition percentage of *Fusarium oxysporum* by essential oil of *Eucalyptus citriodora*

5.1.6 Antifungal Activity of Essential Oil of *Nardostachys grandiflora*

Inoculum size: 5mm in diameter

Concentration of Essential Oil (µlml^{-1})		Size of Colony (Diameter in mm)				Mycelial Growth (mm)	Growth Inhibition %
		Obs. 1	Obs. 2	Obs. 3	Mean		
Control Set		90	90	90	90.00	85.00	0.00
Treatment Set	0.625	56	55	54	55.00	50.00	41.18
	1.25	35	35	32	34.00	29.00	65.88
	2.5	30	29	30	29.67	24.67	70.98
	5.0	27	25	25	25.67	20.67	75.69
	10.0	23	22	20	21.67	16.67	80.39
	20.0	15	13	14	14.00	9.00	89.41
	40.0	0	0	0	0.00	0.00	100.00

Table 9: Antifungal activity of essential oil of *Nardostachys grandiflora* against *Fusarium oxysporum*

Essential oil of Nardostachys grandiflora showed mycelial growth inhibition as 100%, 80.39%, 75.69%, 70.98%, 65.88% and 41.18% at concentrations 40.0µlml^{-1}, 20.0µlml^{-1}, 10.0µlml^{-1}, 5.0µlml^{-1}, 2.5µlml^{-1}, 1.25µlml^{-1} and 0.625µlml^{-1} against *Fusarium oxysporum* respectively.

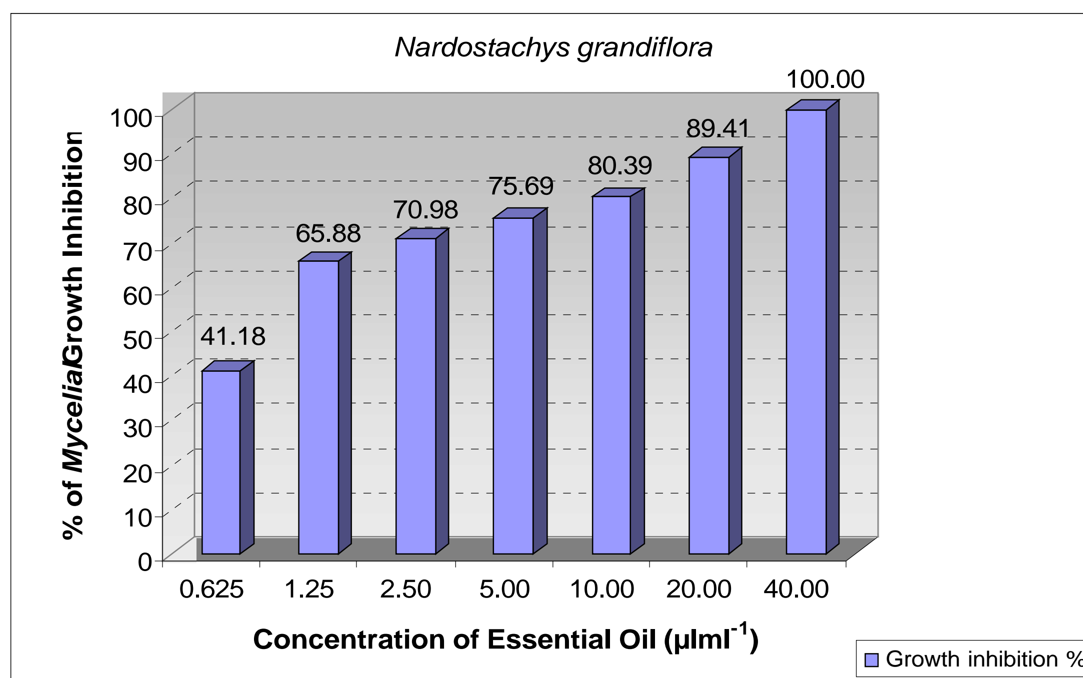

Figure 6. Graphical representation of growth inhibition percentage of *Fusarium oxysporum* by essential oil of *Nardostachys grandiflora*

5.2 Summary of Result

Fusarium oxysporum (test fungus) was isolated from infected tuber of potato. Pathogenecity test was carried out to confirm the pathogen. All the essential oils were assessed for their antifungal activities against *Fusarium oxysporum* and calculate the percentage of inhibition of mycelial growth.

The growth of mycelium was inhibited 20.00%, 27.20%, 34.00%, 41.61%, 46.00%, 58.00% and 67.60% by *Mentha arvensis*; 11.76%, 21.96%, 27.45%, 34.9%, 44.31% 68.63% and 90.59% by *Cinnamomum tamala*; 10.59%, 14.90%, 19.22%, 23.53%, 73.73%, 90.59% and 100% by *Cymbopogon citratus*; 9.41%, 20.78%, 32.55%, 50.20%, 62.75%, 86.68% and 100% by *Acorus calamus*; 54.90%, 58.04%, 65.88%, 81.18%, 97.25%, 100% and 100% by *Eucalyptus citriodora*; 41.18%, 65.88%, 70.98%, 75.69% 80.39%, 89.41% and 100% by *Nardostachys grandiflora* at 0.625µlml^{-1}, 1.25µlml^{-1}, 2.5µlml^{-1}, 5.0µlml^{-1}, 10.0µlml^{-1}, 20.0µlml^{-1} and 40.0µlml^{-1} essential oil concentrations respectively.

All the essential oils showed different antifungal activity. It varied with different concentrations of essential oils. The mycelial growth of the test fungus was completely checked by the essential oils of *Eucalyptus citriodora, Cymbopogon citratus, Acorus calamus* and *Nardostachys grandiflora*. Among them, the essential oil of *Eucalyptus citriodora* was found to be more effective inhibiting the mycelial growth completely at the concentration of 20.0µlml^{-1}. The essential oils of *Cymbopogon citratus, Acorus calamus* and *Nardostachys grandiflora* completely inhibited the mycelial growth at 40.0µlml^{-1} concentration. In addition, the percentage of mycelial growth inhibition of the test fungus by the essential oils of *Mentha arvensis* and *Cinnamomum tamala* at 40.0µlml^{-1} concentration were found to be 67.60% and 90.59% respectively.

Minimum Inhibitory Concentration (MIC) of essential oil was found in four species of plants. The MIC of *Eucalyptus citriodora* is 20.0µlml^{-1} i.e. there is complete inhibition of colony growth of *Fusarium oxysporum* at 20.0µlml^{-1} concentration. However, the MIC of essential oils was found 40.0µlml^{-1} for *Cymbopogon citratus*, *Acorus calamus* and *Nardostachys grandiflora.*

CHAPTER - SIX

6 DISCUSSION

The present study has been carried out to isolate the causal agent of dry rot of potato (*Fusarium oxysporum*) and test it's pathogenecity and then control of the fungus with essential oils of *Mentha arvensis, Cinnamomum tamala, Cymbopogon citratus, Acorus calamus, Eucalyptus citriodora and Nardostachys grandiflora*. The main objective of the study is to evaluate the antifungal activity of essential oils of test medicinal plants, to evaluate the comparative antifungal activities of the essential oils of the test plant species against *Fusarium oxysporum* and to know the minimum inhibitory concentration (MIC) of test plant oil.

Potato is one of the most important vegetables of the world. The disease caused by the fungus, bacteria, viruses, insects and adverse conditions create a considerable damage to the potato crops and the questions to our food security may arise. Plant pathology deals with the study of these diseases and formulates the control measures. The chemicals used to increase the productivity through plant disease control have been found to display many side effects in the form of carcinogenic, tertogenicity, pollutive effects and other residual toxicities (Cakir et al., 2004). Thus the control of the plant diseases through the essential oils has been increased tremendously which are considered to be less harmful to the animal kind and the environment (Duru et al., 2003).

Antifungal activity was found to be variable with regard to different concentrations of different plant essential oil used. Among the test plants taken for the study, *Eucalyptus citriodora, Cymbopogon citratus, Nardostachys grandiflora* and *Acorus calamus* were able to inhibit the growth of the mycelium of the test fungus completely. The inhibition percentage of the mycelial growth of the test fungus by the essential oil of *Cinnamomum tamala* was found to be more than 90 per cent. The essential oil of *Mentha arvensis* was found to be comparatively less effective to control the disease.

6.1 Comparison of Antifungal Activity of E. Oils at Different Concentrations

Eucalyptus citriodora, Cymbopogon citratus, Nardostachys grandiflora and *Acorus calamus* showed the highest growth inhibition (100%) followed by *Cinnamomum tamala* (90.50%) and *Mentha arvensis* (67.60%) at 40.0µlml^{-1} essential oil concentration respectively.

Eucalyptus citriodora showed the highest growth inhibition (100%), followed by *Cymbopogon citratus* (90.59%), *Nardostachys grandiflora* (89.41%), *Acorus calamus* (86.67%), *Cinnamomum tamala* (68.63%) and *Mentha arvensis* (58.00%) at 20.0µlml^{-1} essential oil concentration respectively.

Eucalyptus citriodora showed the highest growth inhibition (97.25%), followed by *Nardostachys grandiflora* (80.39%), *Cymbopogon citratus* (73.73%), *Acorus calamus* (62.75%), *Mentha arvensis* (46.00%) and *Cinnamomum tamala* (44.31%) at 10.0µlml^{-1} essential oil concentration respectively.

Eucalyptus citriodora showed the highest growth inhibition (81.18%), followed by *Nardostachys grandiflora* (75.69%), *Acorus calamus* (50.20%), *Mentha arvensis* (41.61%), *Cinnamomum tamala* (43.90%) and *Cymbopogon citratus* (23.53%) at 5.0• lml^{-1} essential oil concentration respectively.

Nardostachys grandiflora showed the highest growth inhibition (70.98%), followed by *Eucalyptus citriodora* (65.88%), *Mentha arvensis* (34.00%), *Acorus calamus* (32.55%), *Cinnamomum tamala* (27.45%) & *Cymbopogon citratus* (19.22%) at 2.5µlml^{-1} essential oil concentration respectively.

Nardostachys grandiflora showed the highest growth inhibition (65.88%), followed by *Eucalyptus citriodora* (58.04%), *Mentha arvensis* (27.20%), *Cinnamomum tamala* (21.96%), *Acorus calamus* (20.78%) and *Cymbopogon citratus* (14.90%) at 1.25µlml^{-1} essential oil concentration respectively.

Eucalyptus citriodora showed the highest growth inhibition (54.90%), followed by *Nardostachys grandiflora* (41.18%), *Mentha arvensis* (20.00%), *Cinnamomum tamala* (11.76%), *Cymbopogon citratus* (10.59%) and *Acorus calamus* (9.41%) at 0.625µlml^{-1} essential oil concentration respectively.

Essential oil in different concentrations showed different level of antifungal activities. Essential oil of each plant species was diluted in 80% acetone into 7 different concentrations viz. 40.0µlml^{-1}, 20.0µlml^{-1}, 10.0µlml^{-1}, 5.0µlml^{-1}, 2.5µlml^{-1}, 1.25µlml^{-1} and 0.625µlml^{-1}.

The difference in antifungal activity at the same concentration in different essential oils may be due to different chemical composition of the oils Rao & Srivastava (1994).

The result obtained from the study may not be true forever because the concentration of various ingredients in essential oil of the same plant species varies due to several factors such as growth stage, ecological factors etc. as mentioned in Rao & Srivastava (1994).

An abstract of comparison of antifungal activity of all essential oils at various concentrations is given in Table 10 and the result is presented in Figure 7.

Table 10: Antifungal activity of essential oils at different concentrations

S.N.	Concentration of Essential Oil (μlml^{-1})	Mycelial Growth Inhibition (%)					
		Mentha arvensis	*Cinnamomum tamala*	*Cymbopogon citrates*	*Acorus calamus*	*Eucalyptus citriodora*	*Nardostachys grandiflora*
1	0.625	20.00	11.76	10.59	9.41	54.90	41.18
2	1.25	27.20	21.96	14.90	20.78	58.04	65.88
3	2.5	34.00	27.45	19.22	32.55	65.88	70.98
4	5.0	41.60	34.90	23.53	50.20	81.18	75.69
5	10.0	46.00	44.31	73.73	62.75	97.25	80.39
6	20.0	58.00	68.63	90.59	86.67	100.00	89.41
7	40.0	67.60	90.59	100.00	100.00	100.00	100.00

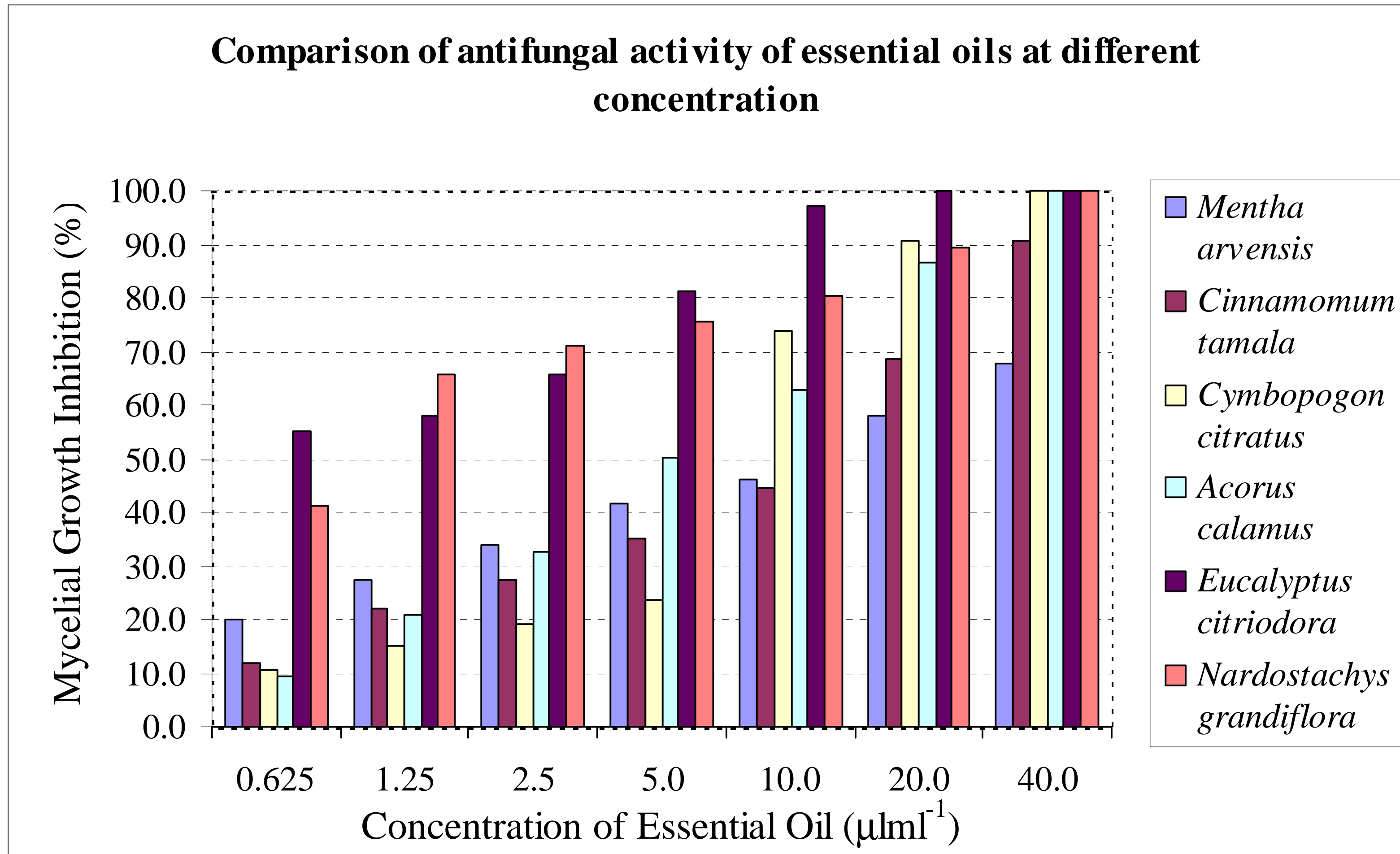

Figure 7: Comparison of antifungal activity of essential oils at different concentrations

CHAPTER - SEVEN

7 CONCLUSION AND RECOMMENDATION

7.1 Conclusion

From the study, it could be concluded that most of the test plants contain chemicals that have antifungal properties. The antifungal activity was found to be variable with regard to different concentrations of the different essential oils used. In all cases, with increase in concentration of essential oils there was decrease in colony size of the test fungus in laboratory conditions which indicated fungicidal characteristics of the used essential oils. Out of 6 test plant essential oils, *Eucalyptus citriodora* was found to be most effective followed by *Nardostachys grandiflora, Cymbopogon citratus* and *Acorus calamus* against the test fungus. These essential oils could be recommended for the potato tuber protection against *Fusarium oxysporum.* Besides, the essential oil of *Cinnamomum tamala* could also be recommended as this gave the inhibition percentage more than 90%. The essential oils of these plants could give the best result in the inhibition of the mycelial growth of the test fungus.

Minimum Inhibitory Concentration (MIC) of essential oil of 4 plant species was found out. A perusal literature showed that determination of the MIC of essential oil against *Fusarium oxysporum* has not yet been done in Nepal. Thus, this is the first experiment to study fungitoxicity and find the MIC of oils against *Fusarium oxysporum*. The present study is to be extended further involving more test plants, test fungus and analytical method to yield result in economic front.

7.2 Recommendation

- This investigation was carried out strictly under the controlled conditions in laboratory. So, the field trial experiments of these essential oils as bio fungicide are emphasized to carry out.
- The researches in the line of chemical characterization of used essential oils and their possible role as biological fungicides along with the mechanism of inhibition is speculated to justify the present findings.

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APPENDIX

Materials:

Glassware, test tubes, culture tubes, funnel, pipettes, conical flasks, glass rods, Petri dishes, inoculating needles, ocular micrometer, stage micrometer, slides, inoculating lop, forceps, scissors, burner, beakers, cover slip, muslin cloth, cotton, plastic containers etc.

Instruments and Appliances:

Sprit lamp, laminar airflow, microscope, hot air oven, incubator, refrigerator, Clevenger's apparatus, autoclave, photographic camera, balances etc.

The Test Plant Species:

Mentha arvensis, Cinnamomum tamala, Acorus calamus, Cymbopogon citratus, Eucalyptus citriodora and *Nardostachys grandiflora.*

The Test Fungus:

Fusarium oxysporum isolated from infected potato tuber.

Required Chemicals:

Distilled water, 80% acetone, mercuric chloride, rectified spirit, cotton blue, lacto phenol, potatoes, dextrose, agar, ice, etc.

ANNEXES

ANNEX I: Cropping Calendar by Altitudinal Zones in Nepal (Dhital, 2000)

Zone	Altitude (masl)	Planting months	Harvesting Months
Terai	Up to 350	October-November	January –February
Low hills	350-1000	September-December	Dec-March
Mid hills	1000-1800	January-February August-September	April-June November-December
High hills	1800-2200 2200-3000	February-March March-April	July-August July-September
Mountains	3000-4000	Late April-Early May	September-October

ANNEX II: Potato Production Trend in Nepal

Description	1961-63	2001-03	Comparison
Total Area Cultivated ('000 hectares)	41	135	329%
Per Capita Area Cultivated (m^2)	39.5	54.9	139%
Average Yields (tons per hectare)	5.6	10.7	191%
Total Production ('000 tons)	233	1,440	618%
Per Capita Production (kilograms)	22.5	58.5	260%

- Data are three-year averages, comparison 2001-03 to 1961-63.
- Population estimates for per capita data: 1961-63 = 10,369,000; 2001-03 = 24,611,000 (Source: FAOSTAT)

ANNEX III: Potato Crops Cultivation by Development Regions

Regions	Area (hectare)	Production (metric ton)
Eastern Development	59208	719865
Central Development	48306	726138
Western Development	20930	259044
Mid western Development	19296	242991
Far western Development	8997	106779
Total	156737	2054817

Source:•Statistcal information on Nepalese agriculture 2006/07 Agri-Business Promotion• Statistics Division MOAC.

ANNEX IV: Nutrition Value of Potato

Contents	Per 100g of Potato	Contents	Per 100g of Potato
Energy	97kcal	Phosphorous	40mg
Protein	1.6g	Iron	0.48mg
Carbohydrates	22.6g	Magnesium	30mg
Fats	0.1g	Sodium	11mg
Vitamin C	17mg	Potassium	247mg
Vitamin B1	0.1mg	Calcium	10mg
Vitamin B2	0.01mg	Fiber	0.6g

Source: http://www.pnas.org/cgi/content/ull/102/41/14694.

ANNEX V: Test Plant Species

Six following plants species were taken for the assessments of fungitoxicity.

Annex V.1: *Mentha arvensis* L.

Family: Labiatae

Local name: Pudina

Distribution: It is common perennially growing herbs found through out the Nepal but especially found in the Northern regions.

Description: Aromatic perennial herbs strongly scented, erect or diffuse growing 10-60cm tall, root stock creeping. Leaves are in opposite pairs, nearly sessile, 2-6.5cm long and 1-2cm broad, lanceolate ovate and pubescent with a coarsely serrated margin. Flowers small, pale purple in large wholes crowed in axially and terminal, cylindrical, tapering spikes, calyx hairy, bell shaped, acutely 5-toothed, corolla-tube in the calyx limb, erect, 4-lobes nearly equal, stamens 4, protruding, filament naked.

Uses: The oil obtained from the leaves has extensive used in various pharmaceutical preparations, flavoring food, perfumery and ornamental (Stainton, 1984). It is used as stimulant, carminative and for removing nausea sickness and vomiting.

Source: en.wikipedia.org/wiki/menthe_arensis

Annex V.2: ***Cinnamomum tamala*** **(Buchanan-Hammilton) Nees and Eberm**

Family: Lauraceae

Local name: Tejpat

Distribution: It is found on Tropical and sub tropical Himalayas at 500-2000 m altitude.

Description: Evergreen tree about 15m high. Bark dark brown wrinkled. Leaves stalked alternate or sub opposite 7.5-20cm long, 3.5-6.5 cm wide ovate to oblong long pointed three veined entire, glabrous, shiny above, pink when young. Flowers stalked yellowish. Fruit-a drupe ovoid succulent, supported by the thickened peduncle, black when ripe.

Uses: Leaf and bark are used to treat colic and diarrhea (Manandhar, 2002).

Annex V.3: ***Cymbopogon citratus*** **(DC) Stapf.**

Family: Poaceae

Local name: Lemon grass

Distribution: Widely distributed all over the tropics of the hemisphere.

Description: The plant is perennial with short rhizome. It forms dense clumps up to 1.8m tall and 1.2m wide. The roots are fibrous and leaves are graminaceous with parallel venation. It posses silica lining and is rough in touch. The leaves are tapered at both ends and are up to 1m long by 1.2cm wide with scabrous margins.

Uses: Lemongrass is used as a fragrance and flavoring and in folk medicine as an antiseptic, anticonvulsant, hypertensive, antirheumatic and as treatment for nervous conditions, oral disorders, fevers and indigestion.

Source: www.drugs.com/npp/lemongrass.htm/

Annex V.4: ***Acorus calamus*** **L.**

Family: Acoraceae

Local name: Bojho

Distribution: Semi-aquatic plant found 100-2300m; West-East Nepal.

Description: Hardy perennial herb of marshy places .It has a branched and aromatic root or rhizome (underground horizontal stem of a plant that produces roots) from which rise its long erect leaves. In late spring, green flowers appear in 2 to 4 long spadices. The flowers eventually give way to small berries. The sheathing leaves of this perennial are from 2 to 6 feet in height and about 1 inch in width. They are sharp pointed and have a ridged midrib running their entire length.

Uses: *calamus* is an important herb and is valued as a rejuvenator for the brain and nervous system and as a remedy for digestive disorders. It helps distended and uncomfortable stomachs, and headaches associated with weak digestion such as gas, bloating, colic and poor digestive function.

Acorus calamus extract is anti-rheumatic and analgesic. It is very much useful in case of asthma, bronchitis and cough and also anesthetic for toothache and headaches.

Source: www.efloras.org/florataxon.asp, www.a1b2c3.com/drugs/Var002.htm.

Annex V.5: *Eucalyptus citriodora* Hook.

Family: Myrtaceae

Local name: Masala

Distribution: Morang District and adjoining areas of Nepal.

Description: The plant is an evergreen tree 24–40m high with tall straight trunk 0.6–1.3m in diameter, and thin, graceful crown of drooping foliage. Bark smooth, gray, peeling off in thin irregular scales or patches and becoming mottled, exposing whitish or faintly bluish inner layer with powdery surfaces appearing dimpled. Twigs slender, slightly flattened, light green, tinged with brown. Leaves alternate, narrowly lance-shaped, 10–20cm long, 1–2.5cm wide, apically acuminate, basally acute, entire, glabrous, thin, light green on both surfaces, with many fine parallel straight veins and with vein inside edge. Corymbs terminal and at leaf base 5 to 6cm long, branched. Flowers many, 3–5 on equal short stalks (umbels) from ovoid buds 8–12mm long, 5–8mm wide. Stamens many, thread likes, white, 6mm long, spreading approximately 12mm across, anthers with long gland. Pistil inferior 3-celled ovary and long, stout style. Capsules few, urn-shaped or ovoid, narrowed into short neck, 10–12mm long, 8–10mm

wide, brown with scattered raised dots. Seeds few, irregularly ellipsoid, 4–5mm long, shiny black (Little, 1983).

Uses: *Eucalyptus* oil obtained from the leaves. It is used in the treatment of throat and nose disorders, malaria and various cold afflictions.

Source: journals.sfu.ca/Nepal/index.php/ON/article/view/797/766-3k.

Annex V.6: *Nardostachys grandiflora* D. C.

Family: Valerianaceae

Local name: Jatamansi

Distribution: It is one of the important medicinal plants of Nepal. It is found mostly at an altitude of 1200-3000m from the sea level (Press *et. al* 2000).

Description: It is perennial herb growing in moist and shady places with creeping rhizome. The height of plant is up to 45cm tall with thick, horizontal, nodular and aromatic rootstock and tufted stem. Leaves are both cauline and radically. Radical leaves are more or less opposite, petiolate, heart shaped, ovate, glaborous and 3.5-5cm by 2.5-3cm in size. Flower is liliac and small white in color. Flowering season is March to April. The ordinary soil well supplied with moisture and farmyard manure is best preferred by the plant. Under the forest act (1993) this plant has been banned for export. However, by obtaining permission from government, its processed material can be exported (Joshi and Joshi 2000).

Uses: The rhizome is considered to as tonic, stimulant, antispasmodic, diuretic, deobstruent, emmenagogue, stomachic and laxative. It is also used in epilepsy, hysteria, cholera, etc (Anonymous, 1993).

ANNEX VI: Preparation of Potato Dextrose Agar (PDA) Media

Composition

Peeled potatoes- 200g

Dextrose- 20g

Agar- 20g

Distilled water-1000ml

Peeled and sliced 200g potatoes were boiled in 500ml of clean water for 15 minutes. The cooked potato chips were filtered by muslin cloth squeezing out all liquid. 500ml water was heated in another flask and added 20g agar bit by bit to dissolve on well. 20g dextrose was added to potato extract. Then, both the contents of two different containers were mixed and well shaken. The flasks were cotton plugged and sterilized by autoclaving for 20-30 minutes in 15lb pressure at 121^0C .Then media was poured in different sterilized culture tubes and Petri plates in aseptic conditions.

ANNEX VII: Micrometry

In micrometer, 100 divisions = 1000µm Or, 1 divisions = 1000 /100µm =10µm

15 divisions of ocular micrometer coincided with 10 divisions of stage micrometer.

i.e. 15 divisions of ocular micrometer = 10 divisions of stage micrometer

1 divisions of ocular micrometer = 10 /15 divisions of stage micrometer

Or 1 divisions of ocular micrometer = 10/15 ×10µm=6.66µm

Measurement of Conidia

S.N	Micro-conidia		Macro-conidia	
	Length (µm)	Breadth(µm)	Length (µm)	Breadth(µm)
1	7.92	2.64	42.90	5.28
2	10.56	4.62	38.94	4.62
3	12.54	3.96	33.3	5.28
4	11.88	4.62	27.3	4.62
5	11.22	3.96	47.5	4.62
6	6.6	3.3	44.98	5.28
7	5.94	0.64	37.62	4.62
8	8.58	3.3	26.64	3.30
9	13.86	3.96	33.3	4.62
10	13.2	4.62	29.30	3.30

Hence, the size of the micro-conidia and macro-conidia ranges from 5.94-13.86µm × 2.64-4.62µm and 26.64-47.5µm × 3.3-5.28µm respectively.

DATA ANALYSIS BY SPSS

- Concentrations of each essential oils ranges from 0.625 to 40.00µlml^{-1}.
- The experimental data of concentrations of each plant essential oil and mean colony size of mycelial growth of test fungus were taken from observations (Chapter-Five) and correlations between them were analyzed using Statistical Package for the Social Sciences (**SPSS**). Followings are the statistical results.

Correlations:

1. ***Mentha arvensis***

Table 1: Correlation between concentrations of essential oil of *Mentha arvensis* and mean colony size of *Fusarium oxysporum*

		Concentration of E. Oil ((µlml^{-1})	**Mean Colony Size**
Concentration of E. Oil (µlml^{-1})	Pearson Correlation	1	-0.834(*)
	Sig. (2-tailed)		.010
	N	8	8
Mean Colony Size	Pearson Correlation	-0.834(*)	1
	Sig. (2-tailed)	.010	
	N	8	8

*Correlation is significant at the 0.05 level (2-tailed).

2. ***Cinnamomum tamala***

Table 2: Correlation between concentrations of essential oil of *Cinnamomum tamala* and mean colony size of *Fusarium oxysporum*

		Concentration of E. Oil ((µlml^{-1})	**Mean Colony Size**
Concentration of E. Oil (µlml^{-1})	Pearson Correlation	1	-0.946(**)
	Sig. (2-tailed)		.000
	N	8	8
Mean Colony Size	Pearson Correlation	-0.946(**)	1
	Sig. (2-tailed)	.000	
	N	8	8

** Correlation is significant at the 0.01 level (2-tailed)

3. ***Cymbopogon citratus***

Table 3: Correlation between concentrations of essential oil of *Cymbopogon citratus* and mean colony size of *Fusarium oxysporum*

		Concentration of E. Oil ((µlml^{-1})	**Mean Colony Size**
Concentration of E. Oil (µlml^{-1})	Pearson Correlation	1	1.000(**)
	Sig. (2-tailed)		.000
	N	8	8
Mean Colony Size	Pearson Correlation	1.000(**)	1
	Sig. (2-tailed)	.000	
	N	8	8

** Correlation is significant at the 0.01 level (2-tailed).

4. ***Acorus calamus***

Table 4: Correlation between concentrations of essential oil of *Acorus calamus* and mean colony size of *Fusarium oxysporum*

		Concentration of E. Oil ((µlml^{-1})	**Mean Colony Size**
Concentration of E. Oil (µlml^{-1})	Pearson Correlation	1	-0.910(**)
	Sig. (2-tailed)		0.002
	N	8	8
Mean Colony Size	Pearson Correlation	-0.910(**)	1
	Sig. (2-tailed)	.002	
	N	8	8

** Correlation is significant at the 0.01 level (2-tailed).

5. ***Eucalyptus citriodora***

Table 5: Correlation between concentrations of essential oil of *Eucalyptus citriodora* and mean colony size of *Fusarium oxysporum*

		Concentration of E. Oil ((µlml^{-1})	**Mean Colony Size**
Concentration of E. Oil (µlml^{-1})	Pearson Correlation	1	-0.688(**)
	Sig. (2-tailed)		0.059
	N	8	8
Mean Colony Size	Pearson Correlation	-0.688(**)	1
	Sig. (2-tailed)	0.059	
	N	8	8

**Correlation is significant at the 0.01 level (2-tailed).

6. ***Nardostachys grandiflora***

Table 6: Correlation between concentrations of essential oil of *Nardostachys grandiflora* and mean colony size of *Fusarium oxysporum*

		Concentration of E. Oil ((µlml^{-1})	**Mean Colony Size**
Concentration of E. Oil (µlml^{-1})	Pearson Correlation	1	-0.708(**)
	Sig. (2-tailed)		0.050
	N	8	8
Mean Colony Size	Pearson Correlation	-0.708(**)	1
	Sig. (2-tailed)	.050	
	N	8	8

**Correlation is significant at the 0.01 level (2-tailed).

Table 7. Correlation Test

Correlation Between	**Correlation Coefficient**
Concentration of essential oil of *Mentha arvensis* and mean colony size	-0.834
Concentration of essential oil of *Cinnamomum tamala* and mean colony size	-0.946
Concentration of essential oil of *Cymbopogon citratus* and mean colony size	1.000
Concentration of essential oil of *Acorus calamus* and mean colony size	-0.921
Concentration of essential oil of *Eucalyptus citriodora* and mean colony size	-0.688
Concentration of essential oil of *Nardostachys grandiflora* and mean colony size	-0.708

The data analyses from the experiments are given in table1 to table 6 and correlation coefficients are summarized in the table 7. All correlation coefficients are highly negative except for *Cymbopogon citratus* (table 3). It suggest that the concentration has negative relation with mean colony size i.e. higher the concentration produces lower the mean colony sizes where as these variables, in the table 3, are linearly correlated with value 1. It means that as the concentration increases colony size decreases linearly for *Cymbopogon citratus.*

PLATES

Plate 1. Antifungal Activity of Essential Oils

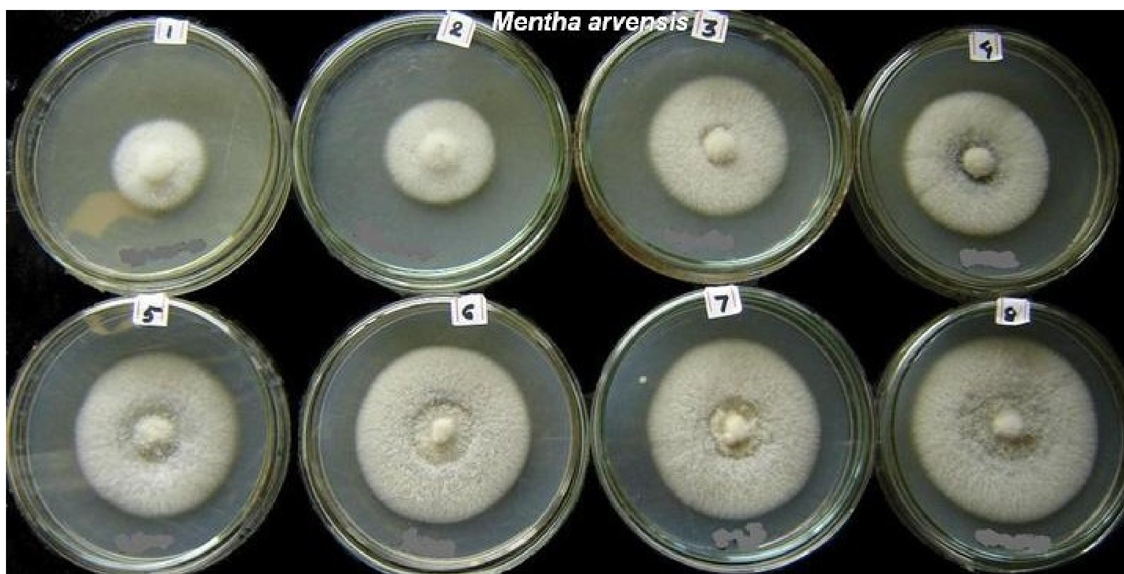

Plate 1.A: Antifungal activity of *Mentha arvensis* against *Fusarium oxysporum*
1= 40.00µlml^{-1}, 2 = 20.00µlml^{-1}, 3= 10.00µlml^{-1}, 4= 5.00µlml^{-1}, 5= 2.50µlml^{-1},
6= 1.25µlml^{-1}, 7= 0.625µlml^{-1}, 8= Control

Plate 1.B: Antifungal activity of *Cinnamomum tamala* against *Fusarium oxysporum*
1= 40.00µlml^{-1}, 2 = 20.00µlml^{-1}, 3= 10.00µlml^{-1}, 4= 5.00µlml^{-1}, 5= 2.50µlml^{-1},
6= 1.25µlml^{-1}, 7= 0.625µlml^{-1}, 8= Control

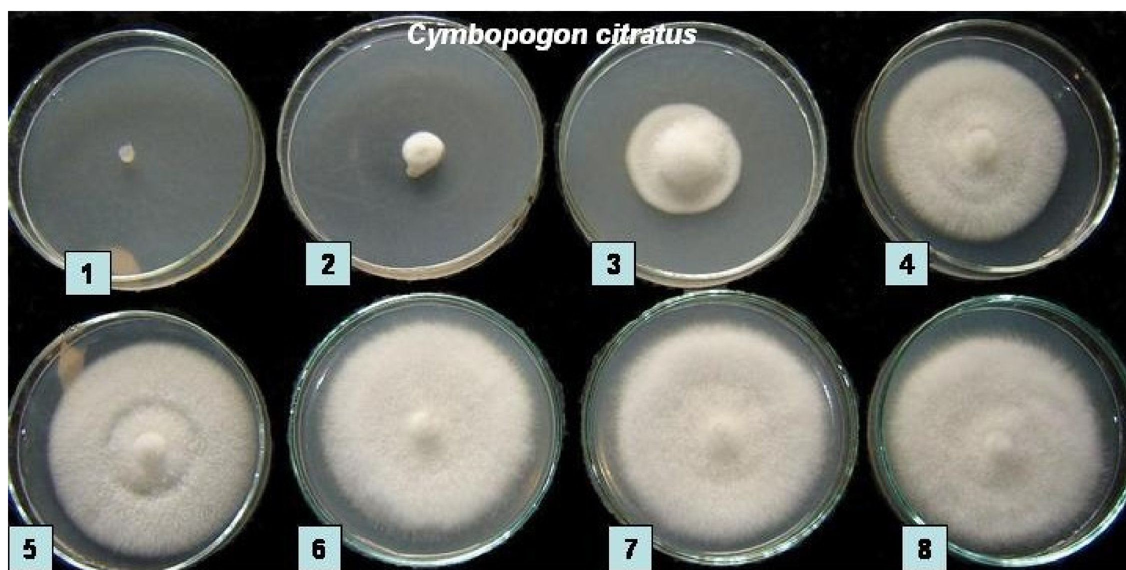

Plate 1.C: Antifungal activity of *Cymbopogon citratus* against *Fusarium oxysporum*
1= 40.00µlml^{-1}, 2 = 20.00µlml^{-1}, 3= 10.00µlml^{-1}, 4= 5.00µlml^{-1}, 5= 2.50µlml^{-1},
6= 1.25µlml^{-1}, 7= 0.625µlml^{-1}, 8= Control

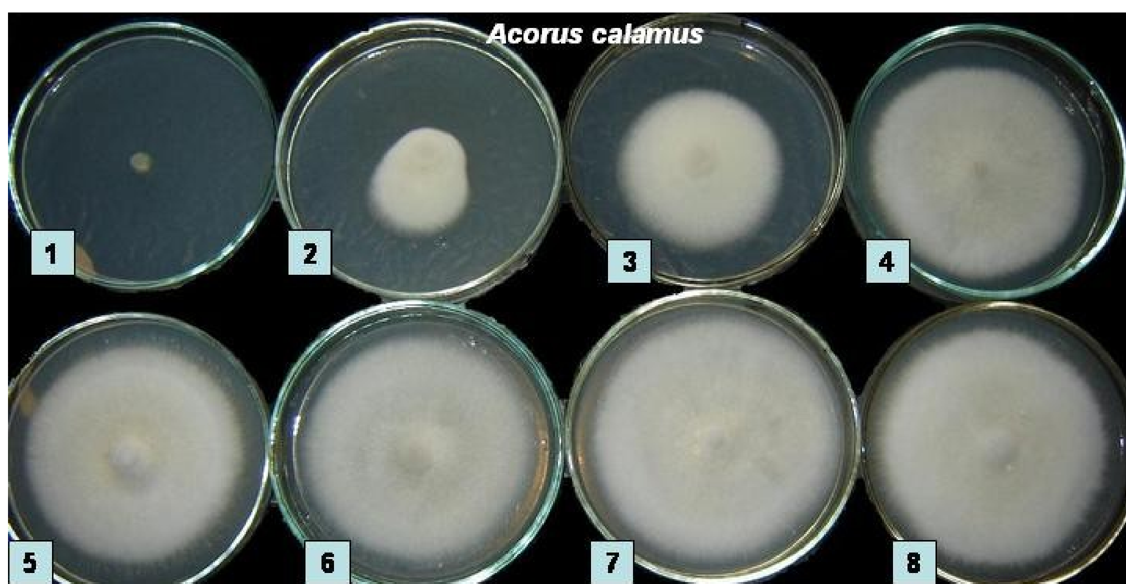

Plate 1.D: Antifungal activity of *Acorus calamus* against *Fusarium oxysporum*
1= 40.00µlml^{-1}, 2 = 20.00µlml^{-1}, 3= 10.00µlml^{-1}, 4= 5.00µlml^{-1}, 5= 2.50µlml^{-1},
6= 1.25µlml^{-1}, 7= 0.625µlml^{-1}, 8= Control

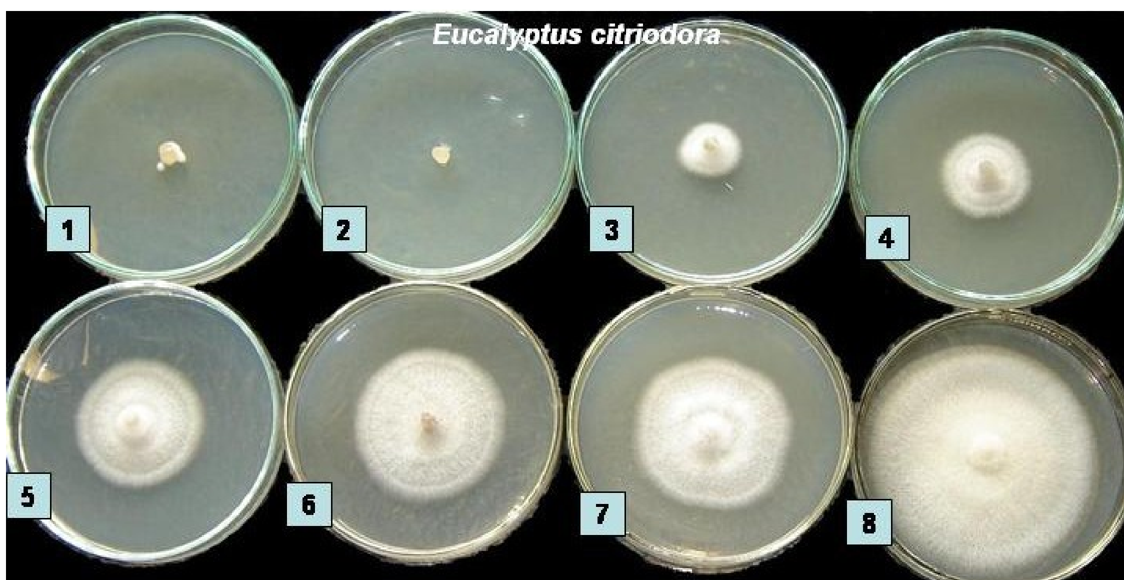

Plate 1.E: Antifungal activity of *Eucalyptus citriodora* against *Fusarium oxysporum*
1= 40.00µlml^{-1}, 2 = 20.00µlml^{-1}, 3= 10.00µlml^{-1}, 4= 5.00µlml^{-1}, 5= 2.50µlml^{-1},
6= 1.25µlml^{-1}, 7= 0.625µlml^{-1}, 8= Control

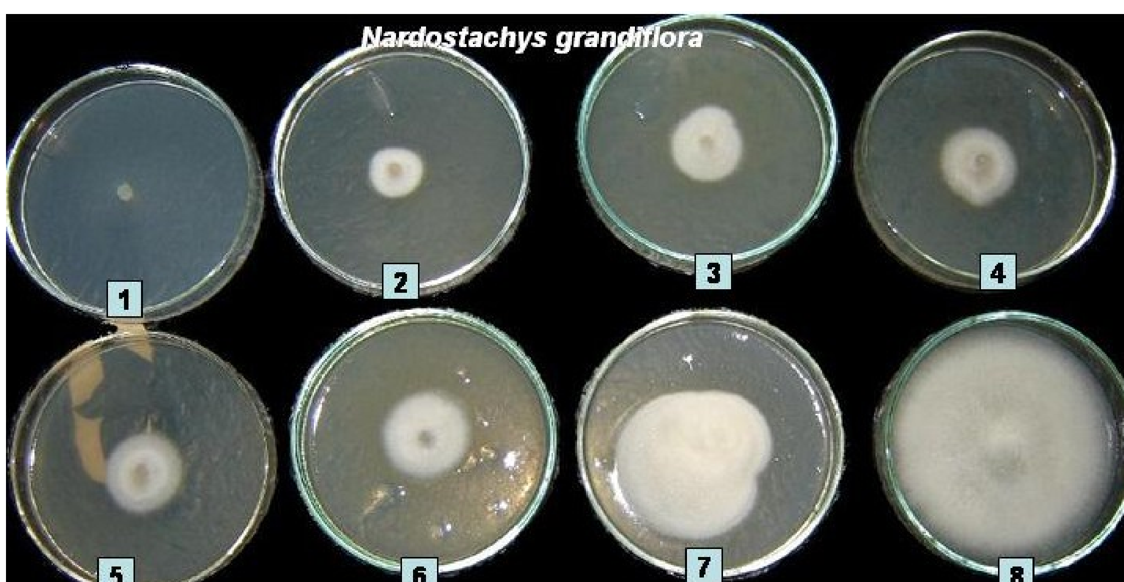

Plate 1.F: Antifungal activity of *Nardostachys grandiflora* against *Fusarium oxysporum*
1= 40.00µlml^{-1}, 2 = 20.00µlml^{-1}, 3= 10.00µlml^{-1}, 4= 5.00µlml^{-1}, 5= 2.50µlml^{-1},
6= 1.25µlml^{-1}, 7= 0.625µlml^{-1}, 8= Control

Plate 2. Diseased Potato Samples

Plate 2.A: Diseased Samples of Potato Tubers

Plate 2.B: Pathogenecity Test

Plate 3. Mycelium, Micro and Macro Conidia

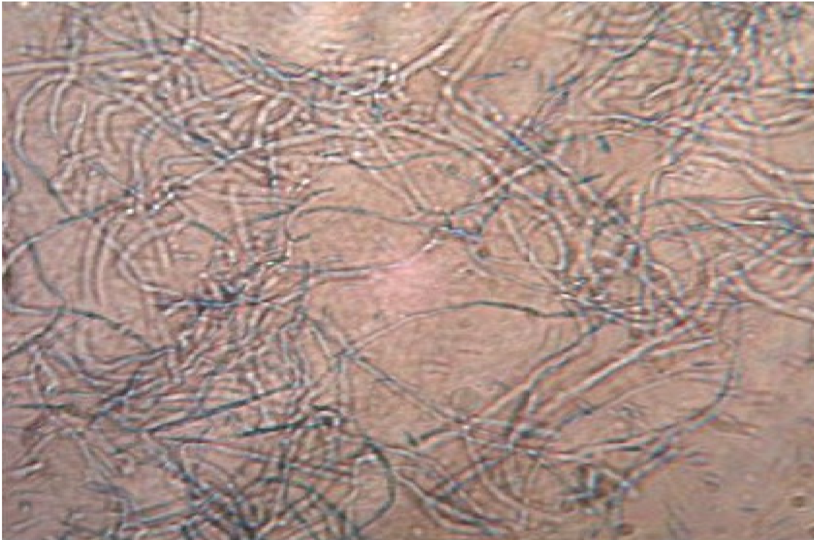

Plate 3.A: Microscopic view of Mycelium with Micro and Macro-conidia

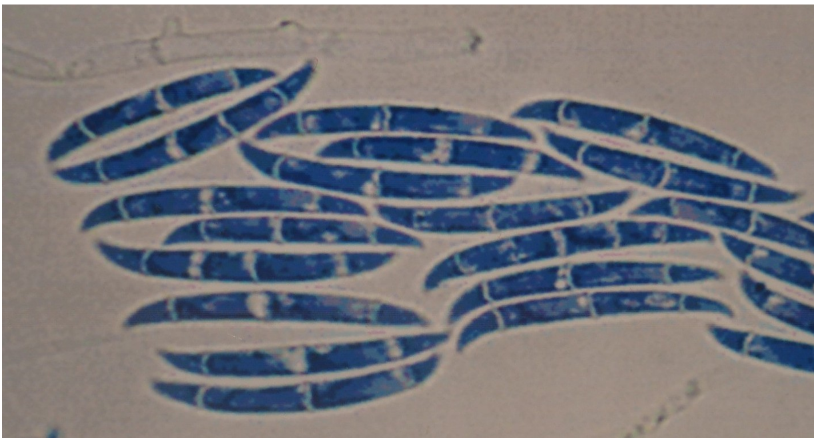

Plate 3.B: Macro-conidia

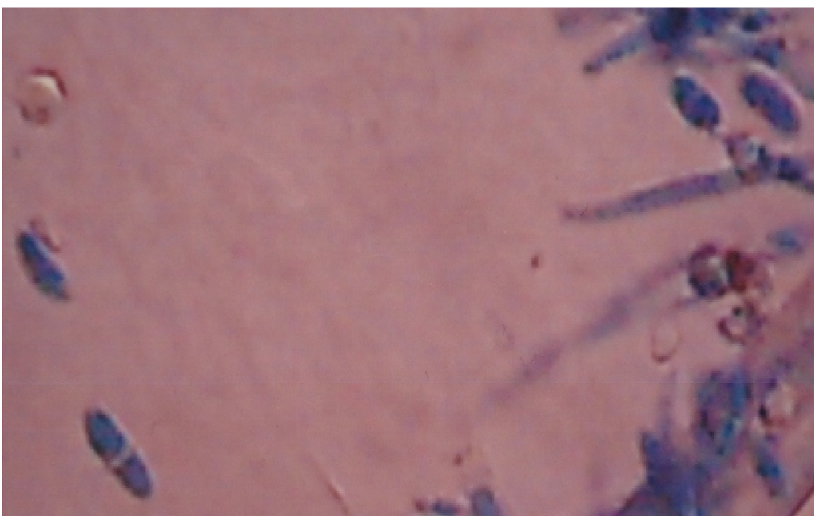

Plate 3.C: Micro-conidia