CHAPTER - ONE

1. INTRODUCTION

1.1 General Background

Nepal has been regarded as the "Nature's paradise" for its high range of biological diversity. It is primarily an agricultural country and nearly 95% of its population depends on agriculture for their livelihood. His Majesty's Government of Nepal has given a top priority to agricultural sector. Almost all every kinds of cereal, fruits and vegetables can be grown here.

In Nepal about 200 species of vegetables are found. About 29% of the total area of Nepal is covered with forest similarly area of vegetable has also increase from 0.14 million hectare to 0.157 hectare. Parwar (Pointed gourd) is one of the important vegetable growing in Nepal. The MoAC is also launching parwar farming in eleven districts along with the program targeted to uplift under privileged group in Terai. In India Parwar (Pointed gourd) covered 1.1 millions hectare with annual production of 48000 metric tons. Export of Parwar has declined because of the loss of competitiveness due to high cost.

A brief survey of the present situation in crop production indicates that the prevalence of soil borne, seed borne and other disease.Post harvest damage is also the major factors contributing to the yield loss.

To minimize the loss of yield various control measure like treatment of disease by biological method, improved cultivation practices, using improved cultivars and proper handling of crop after harvesting are only the ways for the increasing of food with no side effects and also reduce the health hazard of user.

Most of the aromatic plants possess medicinal properties such as antibacterial, antifungal, antiviral, anthelmintic, anticancer, sedative, laxative, cadiotonic, diuretic and others (Parajuli *et al*, 1998).

Plant extracts and oils as biological pesticides:

Fungi cause great hazards to plant in field, transit and storage; although a large number of synthetic chemicals have been recommened to control these losses most of these have proved to be pollutive, carcinogenic, tetragenic. Besides some of these have no effect upon the organism due to the development of new pathogenic races with great resistance power.

Certain plants contain products such as alkaloids, tannins, quinines, coumarines, phenolic compounds in their extracts and exudates and they are known for antifungal activities. The presence of antifungal compounds in plant tissues has long been recognized as an important factor of disease resistance (Fawcett and Spencer, 1966, 1970).

Angiospermic plants are reservoir of the chemotherapeutants and provide inexhaustible sources of useful pesticides (Swaminathan, 1978).Various plant based oils are capable of controlling several plant disease, oils found in plants are classified as either volatile or fixed oil. Volatile oil contain the mixture of hydrocarbon containing 10-15 carbon atoms while fixed oils contain ester of glycerol and long chain aliphatic acids (Manandhar, 2004). Oils obtained from seed of several plants such as Sunflower, Olive, Corn and Soyabeans gives excellent control of some plant disease (Mehrotra *et al*, 2003). Essential oils are derived from aromatic plants, and also known as essences or volatile oils, complex mixture of volatile substances are biosynthesized by living organism. They are extracted with the suitable solvents. They are used in flavor, fragrance food, cosmetics, toiletry and pharmaceutical industries (Rawal, 2001). Essential oils are even more effective and have more antimicrobial effect. They sometime serve as antiseptic and stimulant, as ingredient in medicines, as a laboratory reagent, as solvent in the paint industry, as insecticides and as a component of plastics, polishes, pastes, ink, glue (Schery, 1972).

The medicinal property of a plant is due to the presence of active components or secondary metabolites. The main groups of active components are alkaloids, glycosides, saponins, essential oils, mucilage, tannins and bitter principles etc (Kruger, 1992).

Essential oils usually occur as mixture of many oils and evaporate in the contact with air which can readily be removed from plant tissues without any change in composition. Their structures depend upon crude separation the essence in commonly fixed with smaller quantities of various acids, base, phenols, ketones and aldehydes, the presence of which may affect the delicate nuance of fragrance so desired in certain oils. Frequently they are considered hydrolyte products of complex of glycodes. They occour in some 60 families and are particularly characteristic of Lamiaceae, Myrtaceae, Composite, Lauraceae and Umbelliferae (Schery, 1972).

Essential oils are rich source of terpenoids which impart them antifungal and anti bacterial properties.They are formed in the plant itself on various locations such as flowers, roots, wood, bark, seed, resin etc (Pieman and Towers, 1984).

Postharvest Damage:

The importance of post harvest damage:

Economic losses caused by post harvest diseases are considerably more then realized because fruits and vegetable increase manifold in unit value while passing from the field at harvest to the consumer. According to U.S department of agricultural research survey in 1965 the annual loss of fresh fruits and vegetables in developing countries have been estimated to be in the range of 5-20% or more of the harvest (Mehrotra and Aggarwal, 2003).

The principal causes of damage:

All fruits, vegetable and root crops are living plant parts containing 65-95 percent water and they continue their living processes after harvest. Their post harvest life depends on the rate at which they used up their stored food reserves and their rate of water loss. When food and water reserves are exhausted, they produce dies and decays. Anything that increases the rate of this process may make the produce inedible before it can be used, in the marketing of fresh produces they all interact, and the effects of all are

influenced by external condition such as temperature and relative humidity (John Burden *et al*, 1989).

Damages from mechanical injury:

The high moisture content and soft texture of fruit, vegetable and crops made them susceptible to mechanical injury, which can occurs in any state from production to retail marketing because of:

- Poor harvesting practices, unsuitable field or marketing containers and crates, which may have wood, sharp edges, poor nailing or stapling.
- Over packing
- Careless handling,
- Transport or marketing causes internal brushing, which results in abnormal physical damage or splitting and skin breaks thus the skin provides the sites for infection by disease organism causing decay (John burden *et al*, 1989).

Post harvest disease also can be spread by:

- Field boxes contaminated by soil or decaying produce both.
- Contaminated water used to wash produce before packing.

1.2 Introduction of Pointed Gourd

Trichosanthes dioica Roxb. is also known as Pointed gourd, Paraval / Parwal (from Nepali) Parwal, Parval, Palwal (from Hindi), colloquially in India it is often called green Potato, Potol (from Bengali), Ye she gua (from Chinese) Patol (from Germany).

Distribution:

It is tropical vegetable crop with origin in the Indian sub continent, widely cultivated in the eastern part of India, particularly in Orrissa, Bengal, Bihar Germany, China, Nepal etc. In Nepal it is commonly found in Makwanpur, Biratnagar, Morang, Kanchapur, and

Ilam etc. Currently, most of the Parwal in the United States is imported and thus making it a very expensive vegetable costing as high as $ 7.00.

It is good source of nutritions as carbohydrates, vitamin A and C also contain major nutrients and trace elements (Magnesium, Potassium, Copper, Sulphur and Chlorine) which are needed in small quantities, for playing essential roles in human physiology.

Description:

It is a perennial fruit, similar to cucumber and squash. It is a dioecious (male and female plants) vine (creeper) plant with heart-shaped leaves (cordate) and is grown on a trellis. The fruits are green with white or no stripes. Size can vary from small and round to thick and long 2 to 6 inches (5 to 15 cm). It thrives well under a hot to moderately warm and humid climate. The plant remains dormant during the winter season and prefers a fertile, well-drained sandy loam soil due to its susceptibility to water-logging.

Parts used:

Fruits, Root

Use:

It is used as ingredients of soup, stew, curry, sweet, eaten fried or combination with other vegetable or meats. Parwal soup is traditionally prepared for sick people in Nepal. It is also purposed that pointed gourd posses the medicinal property of lowering total cholestral and blood sugar, also used for constipation, fever skin infection.

Disease of Pointed Gourd:

Various disease found in Pointed gourd are Black rot caused by *Didymella bryoniae,* Leaf spot or grey spot by *Cercospora sp.,* Downy mildews by *Pseudoperenospora cubensis,* Powdery mildews by *Erysiphe cichoracearum,* Foot, root, stem, Vine and fruit rot by *Pythium cucurbitacearum , Phytophthora melonis* and *Fusarium solani* .

1.3 Fruit rot of cucurbits caused by *Fusarium* species

Fruit rot caused by various *Fusarium* species are some of the most common pre and post harvest diseases of cucurbits fruit. *Fusarium* rot have been reported on cucumber, melon, honeydew, watermelon, squash, and pumpkin. Immediate interest to New York growers are the reports of fruit infection of melon by *Fusarium oxysporum* f. sp. *melonis*, infection of pumpkin by *Fusarium solani* f. sp. *cucurbitae* other discussed earlier, infection of pumpkin by various species of *Fusarium* (*F. acuminatum*, *F. graminearum*, *F. equiseti*, and *F. avenaceum)* in Arkansas and Connecticut and infection of butternut squash by *Fusarium* species. In the case of *Fusarium oxysporum* f. sp. *melonis* infection of melon, *Fusarium* rarely directly penetrates the epidermis; rather, it invades the fruit through the stem end. As post harvest breakdown proceeds, other secondary fungi like the dark mycelial growth of *Alternaria* rot (*Alternaria alternata*) may also be found, wounding also does not seem to play a role in the infection of pumpkin by *F. solani* f. sp. *cucurbitae* and other *Fusarium* species.

1.4 Introduction of *Fusarium*

The genus *Fusarium* was ercected by Link (1809) for species with fusiform, nonseptate spores borne on a stroma and was based on *Fusarium roseum*. Species of *Fusarium* are among the commonest fungi, as well as being of great economic importance, *Fusarium* species are widely distributed in soil and on organic substances. Its true parasitic capabilities are not always clear. It is often found associated with wound or with localized infections caused by species of *Pythium*, *Phytophtora*, *Rhizoctonia* and other species of *Fusarium*. It is also associated with the damping off of seedling, including tree seedling and in the destruction of spawn in beds of cultivated mushrooms (*Agaricus*).

The serious wilts such as panama disease of banana caused by *Fusarium oxysporum* are amongst the most devasting plant disease in the world. *Fusarium moniliformae*, *Fusarium graminearum*, *Fusarium avanaceum* and *Fusarium culmorum* are serious pathogen of gramineae, strains of *Fusarium solani* are also of world-wide occurrence as root rots, may also caused cankers of hardwood trees, Fruit, Vine and Stem, rot.

Fusarium solani (Mart.) Sacc, Synder and Hansen has been reported from the plants of different habitat are Cucurbits species, *Dalbergia sissoo, Solanum tuberosum, Lycoperson esculentum Vicia faba, Morus* species, *Phaseolus* species, *Piper nigrum, Pisum sativum* etc (C. Booth, 1977).

1.5 Rot of Pointed Gourd Caused by *Fusarium solani*

1.5.1 Systematic position of *Fusarium solani* (Mart.) Sacc, Synder and Hansen

Class: Deuteromycetes
Order: Moniliales
Family: Tuberculariaceae
Genus: *Fusarium*
Species: *F. solani*

1.5.2 Symptoms

During summer season, the disease forms a luxuriant wooly mycelium weft on the affected fruit which appears as if wrapped in absorbent cotton, the fruits in intimate contact with the soil suffer most. Before the cottony growth of fungus appear, the skin of the fruits shows soft, dark green, water soaked lesion which gradually develop in to a watery soft rot, and on this rotting portion the cottony mycelium develops, abundantly in a humid atmosphere. The tissue in the interior of the fruit becomes watery and soft and the decaying matter emits a bad odour. *Fusarium* fruit rot is usually firm and dry although secondary organisms may invade and cause a wet rot. The disease is common in the field during and after the rains. Most of the fruits lying on the soil or hanging near the ground level are attacked. The disease spread among the fruit during the storage and transit.Such types of symptoms are also found on other types of cucurbits are, bottle gourd (*Lagenaria vulgaris*), Snake gourd (*Trichosanthes anguina*), Cucumber (*cucumis sativus*) etc. Some times Muskmelon and Watermelon undergo rotting after rains due to this disease.

1.5.3 Causal Organism

Fusarium fruit rot of *Trichosanthes dioica* is caused by *Fusarium solani*. The fungal colony appear cottony white, the mycelium is hyaline, branched, both macro and micro conidia are present, microconidiophores elongate, widely branched , microconidia are oval, single or absent of septa and size of microconidia 5.76-11.52 x 1.92-3.84 µm and macroconidia are widest in upper half, sickle shaped, commonly 3-5 septa are found , size of macroconidia is 15.36-38.4 x 3.07-5.3 µm, Terminal and intercalary chlamydospores also develop after 7-14 days, they are globose to oval, occasionally do they form in chains size of chlamydospore is 5.76-11.53 x 3.84-9.61 µm.

1.5.4 Disease Cycle

This fungus is soil-borne but it can also be carried by the seed. Seed transmission rates of 100% are possible. The fungus can survive up to three years in the soil or the seed. *Fusarium solani* occurs in the form of chlamydospores in naturally infested soil, and stated that chlamydospores were the resting stage of *Fusarium solani* in the soil. Spores of the fungus are produced on the infected plant material and can be transported by rain splash, running water, cultivation, and any other practice which transports soil around the field. The heaviest concentrations tend to be where infected plants were previously grown. Secondary infection by means of conidia dispersal by wind, rain, birds, insect etc.

1.5.5 Control

Different technique for the control of the *Fusarium solani*:

- The Disease is soil borne so the disease plant and plant parts should be collected and burnt after harvesting the crop.
- Cultivation of high yielding Resistant/ tolerant varieties is economical and durable means of controlling the disease.
- Three year rotation is probably sufficient to control the disease but a four year rotation may be advisable where problems have been severe.
- Disease control by the use of rules of Qurantine regulation

- Since the disease is seed borne so, the treatment of seed by the use of Organomercurial fungicides like Ceresan, Agrosan GN, and Certosan.
- Hot water treatment of the seeds at 55°C for half an hour.
- Liming the soil to pH 6-7, as well as Reduces the use of Nitrogenous manures so that the incidence of disease can be checked.
- Biological control by the use of *Trichoderma* sp. by dual culture technique.

1.6 Objectives of the Study

- To isolate the pathogen from infected Pointed gourd and test its pathogenicity.
- To study and compare the antifungal activities of essential oils and extracts of different aromatic plants (*Zanthoxylum armatum, Mentha arvensis, Amomum subulatum, Valeriana jatamansi* and *Cymbopogon flexuosus*) against the test fungus *Fusarium solani.*
- To determine the minimum inhibitory concentration (MIC) and percentage of inhibition of mycelial growth of the essential oils and extract against the test fungus *Fusarium solani.*

1.7 Justification of the study

- Pointed gourd is one of the important edible and commercial vegetable of Nepal.
- *Fusarium* rot of Pointed gourd caused by *Fusarium solani* mainly effect in storage.
- Control of disease through use of plant extract and oil to *Fusarium* rot could be one of the cheapest and effective methods of disease management which helps the economy of farmers and country as whole.

- Plant extracts and oil are less toxic, more systemic (Fawcett and Spencer, 1970) easily bio-degradable (Host metabolism stimulatory fungicide (Dixit, 1978) and non toxic to flora and fauna.
- Cheaper than any chemical fungicide and has no any side effects,from which all the consumer will be directly benefited because health hazard will be minimized.

1.8 Limitation of the study

Due to time and economic factor the study suffers from following limitations:

- The study was carried out in limited time for one year only.
- Only single pathogen *Fusarium solani* causing *Fusarium* rot of Pointed gourd was selected for the experiment.
- Essential oil and crude extract of five plant species were used for assessing their antifungal activities against the test fungus by poison food technique.
- The antifungal activity of essential oil was conducted in lab scale (*in vitro*) only.
- T.L. C and G.C could not be carried out for screening composition of essential oils and extracts.

CHAPTER- TWO

2. LITERATURE REVIEW

All available literature, which seems to meet the requirement of present work, had been reviewed as much as possible from various related papers and cited:

Detection of *Fusarium solani* from the pods of *Dalbergia sissoo* by using Standard blotter method, and its control measured has been undertaken by hot water treatment and biological control using *Trichoderma* species. *Fusarium solani* was completely eliminated, when seeds have been controlled with hot water at 50-55^{o}c for forty minutes. For biological control the three different species of *Trichoderma* (*T.aureoviride, T.harzianum, and T.viride*) had been used in dual culture technique against *Fusarium solani*. The maximum inhibitory effect on *Fusarium solani* under the antagonistic effect of *Trichoderma harzianum was* 3.15 cm. and only *Trichoderma harzianum* had overgrown the colony of *Fusarium solani* in 9-10 days (Bajracharya, 2006). The causal agent responsible for the dieback of *Dalbergia sissoo* in the western terai district of Nepal (Rupandehi and Kapilbastu) was the fungus (*Fusarium solani, Ganoderma lucidum*) and insect borers (Adhikari, 2006).Eleven fungi including *Fusarium solani* was recorded from sissoo seeds, and concluded that *Fusarium solani* was transmitted through pods, also recommended cold water treatment and Indophil M-45 for seed treatment (Khanal, 2005). *Fusarium solani* along with *Fusarium oxysporum* were demonostrated *from* the seeds of both healthy and diseased sissoo trees from Dharan BSO, Saptari, Morang and Koshi Tappu (Lamgade, 2004). *Fusarium solani* to be the causal agent of dieback of *Dalbergia sissoo* were found to differ in their virulence indicating probable existence of genetically different strains (Lakhey, 2004). Isolation of four *Fusarium* species from the soil and roots of dieback-affected trees from different BSOs, but percentage of *Fusarium solani* was found highest (Subedi, 2003). *Fusariumsolani, Fusarium oxysporum, Fusarium moniliforme, Fusarium* species from soil, Among these fungi, only *Fusarium solani* was consistently isolated from the roots of dieback-affected trees from different BSOs, but not from those of healthy ones.Disease manifestation was affected by pH of

the soil and soil texture (Shrestha, 2003) Report on dieback disease of sissoo and its control has suspected *Fusarium* species complex to be the cause of vascular wilt. Twelve fungal species were identified from different parts of the tree. Insects were supposed to have some association in the development of fungus at the damage part of the tree (DFRS, 2000). *Fusarium* species and *Fusarium solani* was the most common pathogen associated with sissoo trees suffering from dieback disease. The effect of dieback was less in places where quality seed and planting materials, along with proper silviculture practices, had been used, for preventing sissoo dieback (Karki *et al*, 2000). On the basis of preliminary investigations on dieback of sissoo in eastern terai distrists (Siraha, Sunsari, Morang and Jhapa) the symptoms exhibited by the dieback affected trees as the dark red to reddish-brown sap from infected trees and top dying, half dying and entirely dead crown as a result of progressive death starting from tip.*Ganoderma lucidum, Polyporus Spongiosum* and *Fusarium* species are the main fungal element responsible for the death of sissoo (Parajuli *et al*, 1999). Seeds of *Dalbergia sissoo* to be attacked by *Fusarium* species .In Sagarnath, sissoo was infected by *Fusarium oxysporum* (Karki 1992).

Four seed dresser fungicide, two bioagent (*Trichoderma harzianum* and *T.viride*) and two phyto-extract for Fungitoxicity against *Fusarium solani* causing wilt in Cumin was evulated both *in vitro* as well as *in vivo* conditions, except a fungicide Bavistin, all fungicide and bioagent gave higher seed germination and vigor index and minimum Pre and Post emergence mortality and least number of seedling showing wilt symptoms. (Ghasolia and Jain, 2003). *Trichoderma harzianum* was a better antagonist agent against *Fusarium solani* for the biocontrol of Rhizome rot of Ginger (Mev and Meena, 2003). Pathogenic effects of some *Fusarium* species and prioritized on *Trichoderma harzianum* due to its greater rhizosphere competence and parasitic effect against pathogenic fungi (Naik, 2003).

Biological control by using *Gliocladium virens*, *Trichoderma viride* and *Burkholderia cepacia*, all had antagonistic effects against *Pythium* and *Fusarium* spp. this causes damping off in Corn. These antagonists were found to increase the plant height and Fresh weight besides decreasing the severity of the disease (Mao *et al*, 1997). In vitro studies

with *Trichoderma* species demonstrated the efficiency of the fungal antagonists in inhibiting the growth and sporulation of several soil- borne plant pathogenic fungi, some volatile and non volatile metabolites produced by these antagonists inhibiting the fungal growth against *Fusarium solani,* the causal agent of Chickpea black root rot (Okhovvat, 1997).

Inhibitory activity of some plant essential oils and extracts:

Inhibitory activity of some plant essential oils of *Thymus linearis, Zanthoxylum armatum, Mentha arvensis, Juniperus recurva, Artemisia indica* were assessed against the fungus *Glomerella cingulata*, the causal agents of bitter rot of apple by poisoned food technique. The fungioxicity was found to be variable with different plant essential oils; *Thymus linearis* and *Mentha arvensis* were found more effective in its fungitoxic properties and showed the highest fungitoxicity (100%) at 5000 ppm. (Kuinkel, 2007). Fungitoxicity of essential oils of Inhibitory activity of some plant essential oils of *Thymus linearis, Zanthoxylum armatum, Nardosyostachys grandiflora, Juniperus recurva, Artemisia dubia,*and *Artemisia gemelinii* were assessed against *Alternaria brassicicola*, the causal agents of leaf spot disease of cabbage,by poisoned food technique (Parajuli, 2005). Post harvest disease (sour rot) of Tomato and its control by some local plant extracts of *Allium sativum, Azadirachta indica, Artemisia dubia, Capsicum annuum, Eupatorium adenophorum*, the extract of *Allium sativum* 66.66%, *Artemisia dubia* 83.3% and *Eupatorium adenophorum* 50% has shown the highest antifungal activities (Shrestha *et al*, 2005). Assessment for antifungal activities of plant essential oils of *Thymus linearis, Tanacetum gracile, Clinopodium umbrosum* and *Elsholtzia eriostachya , Zanthoxylum armatum* and *Cinnamomum tamala* were used against *Bipolaris sorokiniana.*The essential oils of *Thymus linearis* showed highest fungitoxicity (100%) at 10000 ppm (Manandhar, 2005). A commonly available lemon plant leaves extract in aqueous or ethanolic extract preparation and essential oil displayed fungitoxic effect against three destructive fungal pathogen viz, *Pyricularia grisea, Aspergillus niger* and *Aspergilus flavus* (Tiwari *et al*, 2004).

Essential oil product have a powerful smell because they have highly volatileand many of them vaporize quickly even at room temperature. Fumigants are also volatile, a property that allows them quickly evaporate, leaving no residue (US Researchers 2004). Antifungal activity of the steam distilled essential oil fraction of *Artemisia douglasiana* was detected by bioautography on silica gel TLC plates against three *Colletotrichum sp.* (Meepagala *et al,* 2003).

The distilled oil was found to be more enriched in monoterpenoid hydrocarbons (Chatopoulou *et al,* 2002). Essential oil and several pure sulfur compounds isolated from *Rodopholeus zenkeri* were tested for antifungal activity using a paper disc method, the poisoned food techniques, a micro atmosphere technique measurement of cellular ATP present (Koukam *et al,* 2002). Thirty two essential oils were extracted from higher plants and tested against *Aspergillus flavus, Aspergillus niger, Alternaria alternata* etc. The oil showed 100% inhibition in mycelial growth of *Alternaria alternata* at 600 ppm oil concentration (Kuwar and Tripathi, 2002). The essential oil of *Cinnamomum tamala* in 500 ppm and 1000 ppm 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 500 ppm and 50% at 1000 ppm (Kuwar and Tripathi, 2002).

The extracts of *Thuja, Vinea,* lower dosage of *Cinnamomum* oil and higher dosage of *clove* oil exhibited the inhibition of *Fusarium moniliforme* (Rai *et al,* 2002). Aqueous extract effects of 64 weed species on growth and development of *Alternaria solani, Helminthosporium sativum* and *Rhizoctonia solani* were studied in vitro. Extracts varied in the strength and persistence of their antifungal effects against the three fungal species (Qusem and Abu-blan, 1996). On screening the extracts of different parts of 122 higher plants for their volatile antifungal activity against *Aspergillus niger* and *Curvularia ovoidea*, the flower bud extract of *Syzygium aromaticum* exhibited absolute toxicity against both the test fungi inhibiting the mycelial growth completely (Khan and Tripathi, 1994).

Antifungal activities of 20 medicinal plants were tested against four pathogenic fungi namely *Curvularia lunata, Cylindrocarpon sp, Fusarium solani* and *Myrothecium leucotrichum.* Extracts of all the plant species were fungitoxic and showed inhibitory effect on the test fungi (Gourinath and Manoharacharya, 1991). In a preliminary microbiological screening, the essential oils of five Sardian plant viz. *Juniperus oxycedrus* subsp. *oxycedrus, Spartium junceum, Helichysum italicum* subsp. *microphyllum, Inula viscose* and *Asphodelus microcarpus* showed activity against some species of blastomycetes (Bonsignore *et al,* 1990). The essential oil of *Lippia alba* exhibited absolute inhibition of mycelial growth of *Macrophomina phaseolina* at 2000 ppm following poisoned food technique. The oil was found to be non phytotoxic in nature (Dwivedi *et al,* 1990).

Eucalyptus oil exhibited absolute mycotoxicity against *Microspornum roseum* and *Trichophyton mentagrophytes* at its minimum inhibitory concentration of 400 ppm against *Pyricularia oryzae* and *Aspergillus niger* (Mishra *et al,* 1990). Essential oil from fresh leaves of nine plants was tested against the fungus *Pyriculana grisea, Aspergillus niger* and *Aspergillus flavus.* Essential oil from *Amomum subulatum* exhibited complete inhibition of the fungus at 3000 ppm. Other plants as *Ageratum naustonianum, Alpinia galangal, Artemisia indica, Curcuma longa, Elettana cardanomomm, Lippia alba* and *Salvia pleneiacamomum* were also tested against the fungus (Mishra and Tiwari, 1990).

The yield of essential oil of *Eupatorium triplinerve* by hydrodistillation was found to be 6 percent. The essential oil showed antifungal and antibacterial activity (Yadava and Saini, 1990).

Antimicrobial activities of some plant products viz. *Mentha arvensis, Acorus calamus, and Zanthoxylum oxyphyllum* and turpentine oil were studied against some fungi and bacteria (Shrestha and Sharma, 1988). Leaf extract of 30 species were tested against *Aspergillus flavus,* only the leaf extract of *Anisomeles ovata* showed absolute toxicity (Upadhaya *et al,1987).* Essential oil extracted from the leaves of *Aegle marmelos* inhibited the mycelial growth of the test fungus *Rhizoctonia solani* completely at its

minimum inhibitory concentration of 3000 ppm (Renu *et al,* 1985). The volatile antifungal action of the leaves of *Melaleuca leucodendeon* was collected as an essential oil by hydrodistillation in Clevenger's apparatus and evaluated for its activity against *Rhizoctonia solani* (Dubey *et al,* 1983).

Leaves of 25 plant species were screened for their volatile toxicity against the test pathogen *Fusarium lateritium* f. *cajani*. Out of the test plant species *Aegle marmelos, Citrus aurantifolia* and *Mentha arvensis var piperascens* exhibited strong toxicity inhibiting the mycelial 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 (Pandey *et al,* 1983).

The essential oil from the seed kernels of *Putranjiva roxburghii* Wall was found toxic against *Helminthosporium oryzae*. It was found that *Alternaria brassicae, Alternaria alternata, Alternaria solani* and *Alternaria raphani* were inhibited by 100% at 500 ppm essential oil concentration (Saxena *et al,* 1983). Distribution of fungitoxicity was studied at five different growth stages of *Iberis amara* against *Helminthosporium oryzae* by spore germination, poisoned food and modified paper techniques (Tripathi *et al,* 1983). The essential oil of the rhizome of *Alpinia galanga* exhibited the highest toxicity to the mycelial growth of *Helminthosporium oryzae.* The volatile oil was fungistatic at MIC of 0.4% in the medium.The oil controlled brown spot disease on detached paddy leaves and had no adverse effect on seed germination and growth of paddy seedling (Tripathi *et al,* 1983). Volatile constituents from the root wood of *Cedrus deodara* exhibited strong fungitoxicity against the fungus *Helminthosporium oryzae.* The oil inhibited the mycelial growth of *Alternaria brassicae, Alternaria raphani* and *Alternaria solani* by 100% at 3000ppm concentration (Dikshit and Dixit *etal,* 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 anthelmintic efficiency which makes them highly valuable (Gulati and Suri, 1982). Hot water dip at 50°c for 5 minutes controlled various post harvest rots caused by

Trichothecium rosuem, Minilinia laxa, Glomerella cingulata, Penicillium expansum (Kaul and Munjal, 1980). The minimum inhibitory concentration (MIC) of the *Cestrum diurnum* against *Helminthosporium oryzae, Helminthosporium carbonum, Helminthosporium graminium, Helminthosporium maydis, and Helminthosporium turcicum* was found at 0.7% (Renu *et al*, 1980).

CHAPTER – THREE

3. MATERIALS AND METHODS

3.1 Materials

Causal organism (*Fusarium solani)* isolated from infected Pointed gourds (Parwar) collected from local markets of Kathmandu valley was considered as the material for this research. Also the different scientific equipments, glassware's and chemicals were used in performing the research work. All materials used are listed in the Appendix I.

3. 2 Methods

3.2.1 Collection of Host and Isolation of test Fungus

During research period, the infected pointed gourd was collected from Market and fungus (*Fusarium solani*) was isolated in pathology laboratory.

3.2.2 Collection, Processing, Drying and Storage of Aromatic plants

During this research period, plant samples of *Valeriana jatamansi* Jones brought from Dolpa district and *Amomum subulatum* Roxb from market of Ktm valley was processed for the extraction of essential oil on laboratory of plant pathology of CDB. Remaining three samples of essential oils of *Zanthoxylum armatum, Mentha arvensis* and *Cymbopogon flexuosus* were taken from HPPCL. Similarly the crude extracts of aromatic plants were made by grinding on mortar with pistal on the pathology labrotary of CDB. The oils and crude extracts were tested against the fungus *Fusarium solani*. The preliminary antifungal action of oils and extracts was then determined. In case of positive activity, MIC (Minimum inhibitory concentration) of all essential oils and extracts was determined against the test fungus.

The collected plant species (*Valeriana jatamansi* and *Amomum subulatum*) were processed for the hydrodistillation of essential oils in the following steps. The collected

plants were cleaned, removal of grasses, mud etc were done. The plants were then spread under the shade at the room temperature and turned up and down to hasten drying. The collected plants were cut into smaller pieces and packed in water proof bags. Then the samples were taken in hydro distillation turn by turn for extraction of essential oils.

3.2.3 Extraction of essential oils and extracts

50 gm of shade dried sample of plant species were surface sterilized with 0.1% of mercuric chloride solution followed by through washing with distilled water. The sample was then pulverized. The hydro distillation of plant samples was carried out for 6-8 hours in Clevenger's apparatus in 500 ml water.

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 dehydrated over anhydrous sodium sulphate and stored at (<10°C) low temperature (Rao and Srivastav, 1994).

Ten gram of fresh plant sample were surface sterilized with 0.1% of mercuric chloride solution followed by through washing with distilled water. Then it was crushed in mortar with pistal.Solution of crushed material was filtered through muslin cloth and kept in centrifuging machine for three minutes. These extract were treated as pure solution considering as a hundred percent. In this way volume of extracts were obtained.

3.2. 4 Determination of yield of essential oils

Hydro distillation of fifty gram of each dried plant sample was done for extraction of essential oil. The volume of oil extracted was noted down. The yield of the essential oil was calculated in terms of % using following formula.

$$\% \text{ of essential oil} = \frac{\text{Volume of essential oil}}{\text{Weight of sample}} \times 100$$

(a) *Valeriana jatamansi*

$$\text{Yield of essential oil} = \frac{\text{Volume of essential oil}}{\text{Weight of sample}} \times 100$$

$$= 0.8/50 \times 100\ \%$$

$$= 1.6\ \%$$

In the same way, the essential oil content *of Amomum subulatum* is found to be 1.6 %.

3.2.5 Dilution of essential oils and crudes extract

Essential oils were diluted into different concentration with 80 % acetone. The oils were diluted into 0.625, 1.25, 2.5, 5.0 and 10.0 $\mu l ml^{-1}$. Each crude extracts were diluted into different concentrations of into 0.625, 1.25, 2.5, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0 and 100.0 $\mu l ml^{-1}$ with distilled water. Then different concentration of each oil and extract was labeled and stored at low temperature (<10°c).

3.2.6 Media preparation

Potato Dextrose Agar (PDA) medium was used for the experiment. For preparing 1000 ml of PDA media, the following ingredients are required in following amount.

Potato- 200 gm

Dextrose-20 gm

Agar-20 gm

200 gm of Peeled potato was washed with clean water. Then cut into small pieces. These pieces were boiled in about 500 ml of water till the pieces were soft to touch. The juice of boiled potato was filtered by the help of muslin cloth on conical flask, and added required amount of Agar and Dextrose and shaked the solution, finally the volume in total was made to 1000 ml, by adding distilled water, then PDA was autoclaved, to get sterilized.

3.2.7 Isolation and Identification of the test fungus (*Fusarium solani*)

Infected Pointed gourds were collected from the local markets of Kathmandu valley, by the help of sterilized needles and forceps some pieces of fungal colony from the infected Pointed gourd was transferred aseptically on a Petri plate containing PDA media then it was incubated in inverted position in an incubator at 25±2°C for one week. After one week the growth of fungal colony were observed in petriplate and colony of the culture was observed under the compound microscope and studied the characteristics of the pathogen. The characteristics features of the fungus which were resemble with the *Fusarium solani* were identified with the help of standard literature (C. Booth, 1977) and also by observing the features of Macro and Micro conidia from the Stereo-Microscope at NARC.

3.2.8 Maintenance of the pure culture

The pure culture of *Fusarium solani* was preserved by sub-culturing in several slants and plates containing PDA media.

3.2.9 Preparation of one week old culture

For testing the antifungal activity of the essential oils and extracts, inoculum disc from one week old culture is required. For preparation of one week culture, the fungus from pure culture was inoculated into PDA and after seven days the inoculum disc was taken from the culture for further experiment.

3.2.10 Assessment of toxicity of oil and extract against fungal pathogen

The toxicity of the oil and extract was assessed by using the poisoned food technique (Grover and Moore, 1962).

Poisoned food technique

It is the way of testing the antifungal efficacy of oil and extract by poisoning the media with the oil and extract.

In this technique, 0.5 ml of each concentration of oil and extract was taken in pre sterilized cooled petriplate and 9.5 ml of PDA media was poured on that with gently swirling to mix the contents thoroughly. In control set the essential oil was replaced by equal volume of Acetone (80%) and the control set of crude extract was replaced by equal volume of distilled water. The inoculums disc (4mm diameter) taken from the periphery of 7 days old culture of the test pathogen, was placed aseptically in the centre of each plate and turned upside down in its position. The plates were then incubated at 25±2°C for 7 days. All experiments were revised thrice. Diameter of fungal colony of treatment and control sets were measured in the mutually perpendicular directions on the 7 days. The percentage inhibition of mycelial growth of test fungus was calculated separately.

3.2.11 Calculation of percentage of mycelial growth inhibition

Fungitoxicity was assessed in triplicates in each concentration of essential oils and extracts Fungitoxicity was assessed in terms of percentage inhibition of mycelial growth of test fungus (Rao and Srivastava, 1994).

$$\% \text{ of inhibition of mycelial growth} = \frac{g_c - g_t}{g_c} \times 100$$

Where,

g_c = growth of mycelial colony after incubation in control set i.e. diameter of colony in control set - diameter of inoculum disc

g_t = growth of mycelial colony after incubation period in treatment set i.e. diameter of the colony in treatment set - diameter of inoculum disc.

3.2.12 Determination of minimum inhibitory concentration (MIC) of essential oil and extract

The minimum inhibitory concentration (MIC) is the concentration of any substance in the external medium which just inhibits cell division of a normal cell.

MIC has been expressed as the minimum dose of the essential oil and extract required for complete (100%) inhibition of mycelial growth of the test fungus (Rao and Srivastav, 1994).

The MIC of different essential oils and extract were obtained against the test fungus by the poisoned food technique employing different doses of oil and extract till the minimum dose of the oil and extract was achieved for complete inhibition of mycelial growth of the test fungus.

3.2.13 Pathogenicity Test

For carrying out the pathogenicity test, the infected fruits of Pointed gourd were collected and symptoms were noted down, then *Fusarium solani* was isolated in PDA media as pure culture. Inoculum from the pure culture was transferred to the healthy fruits. When incubated at 25±2°C for 7 days, the characteristics symptoms were produced, which were found to be similar with the symptoms on fruit previously collected. The fungus was isolated and its character was compared with the previously isolated fungus.

3.2.14 Measurement of conidia size

Ocular micrometer was placed inside the eye piece of a compound microscope and calibrated by superimposing the gradiations of stage micrometer.The number of division of ocular micrometer coinciding with the number of divisions in stage micrometer was noted down and calculated the calibration factor applying the formula.

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

Then, the stage micrometer was replaced by a fine slide of *Fusarium solani* mounted on lactophenol. Size of the Macro and Micro conidia were measured by determining the number of divisions of ocular micrometer (Appendix-II). In each measurement reading were taken, measurement was carried out to find the range of size of the conidia.

3.2.15 Photography

Fresh and infected fruit of Pointed gourd, Test plants (*Zanthoxylum armatum, Mentha arvensis, Amomum subulatum, Valeriana jatamansi,* and *Cymbopogon flexuosus*), Fungal structures (Macro and Micro conidia,Single phailade of *Fusarium solani*), Clevenger's apparatus, Working on Laminar air flow in CDB, Working at NARC with Stereo–microscope, Different types of essential oil were taken. Besides these inhibitions of mycelial colony in different concentration by different essential oils and extracts were also subjected to photography.

CHAPTER – FOUR

4. OBSERVATION AND RESULT

4.1 Assessment of fungitoxicity of essential oils and extracts

Fungitoxicity of each essential oils and extracts was assessed in different concentrations against *Fusarium solani* by poisoned food technique. Essential oils and extracts of different plant species showed different efficacies in inhibition the mycelial growth.

Antifungal activity of different oils and extracts against test fungus

Table 1: Percentage of inhibition of *Fusarium solani* mycelial growth using essential oils of *Cymbopogon flexuosus*.

S.N	Concentration of oil $\mu l ml^{-1}$	Inoculum Size (mm)	Colony size (mm)			Mean colony size (mm)	Mycelial growth (mm)	% of inhibition of mycelial growth
			I	II	III			
1	0	4	42	42	42	42.00	38.00	0.00
2	0.625	4	19	18	19	18.67	14.67	61.40
3	1.25	4	12	11	12	11.67	7.67	79.82
4	2.5	4	8	7	8	7.67	3.67	90.35
5	5	4	4	4	4	4.00	0.00	100.00
6	10	4	4	4	4	4.00	0.00	100.00

Minimum inhibitory concentration (MIC) =5 $\mu l ml^{-1}$

Essential oil of *Cymbopogon flexuosus* showed mycelial inhibition as 0%, 61.40%, 79.82%, 90.35%, 100% and 100% at 0, 0.625, 1.25, 2.5, 5.0 and 10.0 $\mu l ml^{-1}$ oil concentrations against *Fusarium solani* respectively.

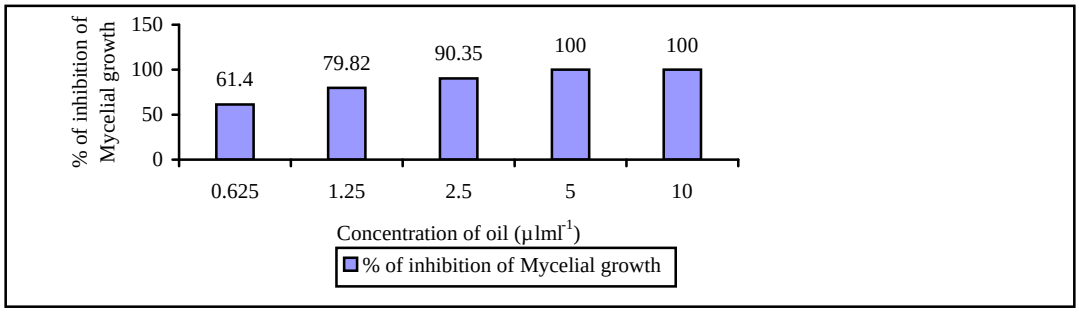

Fig 1: Percentage of antifungal activity of *Cymbopogon flexuosus* oil against *Fusarium solani.*

Table 2 Percentage of inhibition of *Fusarium solani* mycelial growth using extract of *Cymbopogon flexuosus*.

S.N	Concent-ration of extract µlml^{-1}	Inoculum Size (mm)	Colony size (mm)			Mean colony size (mm)	Mycelial growth (mm)	% of inhibition of mycelial growth
			I	II	III			
1	0	4	55	55	55	55.00	51.00	0.00
2	0.625	4	50	50	51	50.33	46.33	9.15
3	1.25	4	23	23	22	22.67	18.67	63.40
4	2.5	4	18	18	18.5	18.17	14.17	72.22
5	5	4	10	10	9.5	9.83	5.83	88.56
6	10	4	9	9	9	9.00	5.00	90.20
7	20	4	7	7	6.5	6.83	2.83	94.44
8	30	4	6	5	5.5	5.50	1.50	97.06
9	40	4	5	5.5	5	5.17	1.17	97.71
10	50	4	4	4	4	4.00	0.00	100.00
11	100	4	4	4	4	4.00	0.00	100.00

Minimum inhibitory concentration (MIC) =50 µlml^{-1}

Extract of *Cymbopogon flexuosus* showed mycelial inhibition as 0%, 9.15%, 63.40%, 72.22%, 88.56%, 90.20%, 94.44%, 97.06%, 97.71%, 100% and 100% at 0, 0.625, 1.25, 2.5, 5.0, 10.0, 20, 30, 40, 50 and 100 µlml^{-1} oil concentrations against *Fusarium solani* respectively.

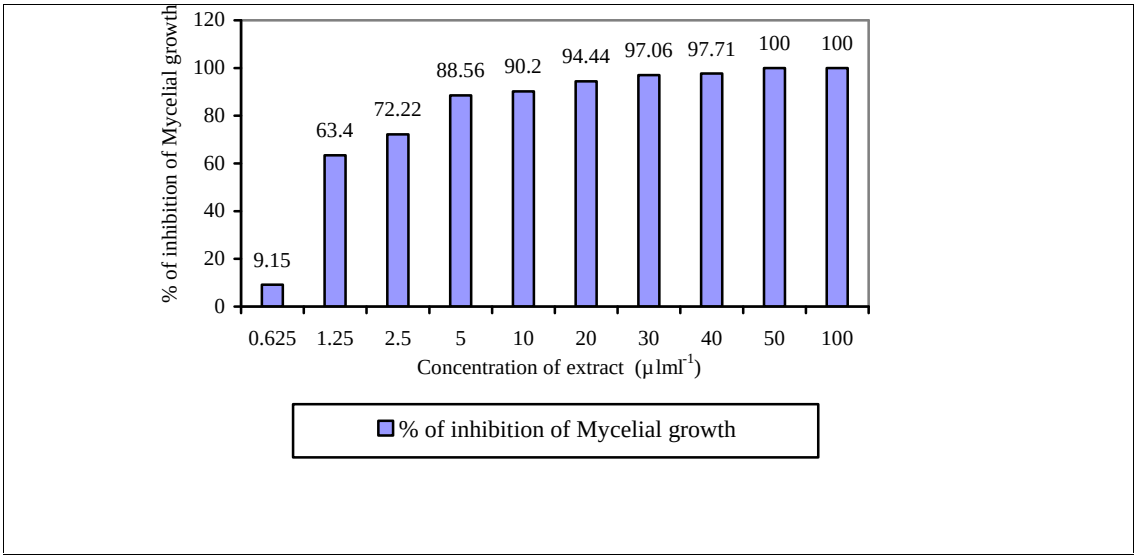

Fig 2: Percentage of antifungal activity of *Cymbopogon flexuosus* extract against *Fusarium solani*

Table 3: Percentage of inhibition of *Fusarium solani* mycelial growth using essential oils of *Zanthoxylum armatum.*

S.N	Concent-ration of oil ($\mu l ml^{-1}$)	Inoculum Size (mm)	Colony size (mm)			Mean colony size (mm)	Mycelial growth (mm)	% of inhibition of mycelial growth
			I	II	III			
1	0	4	42	42	42	42.00	38.00	0.00
2	0.625	4	20	19	20	19.67	15.67	58.77
3	1.25	4	15	14	15	14.67	10.67	71.93
4	2.5	4	10	10.5	10	10.17	6.17	83.77
5	5	4	5	5	5	5.00	1.00	97.37
6	10	4	4	4	4	4.00	0.00	100.00

Minimum inhibitory concentration (MIC) =10 $\mu l ml^{-1}$

Essential oil of *Zanthoxylum armatum* showed mycelial inhibition as 0%, 58.77%, 71.93%, 83.77%, 97.37% and 100% at 0, 0.625, 1.25, 2.5, 5.0 and 10.0 $\mu l ml^{-1}$ oil concentration against *Fusarium solani* respectively.

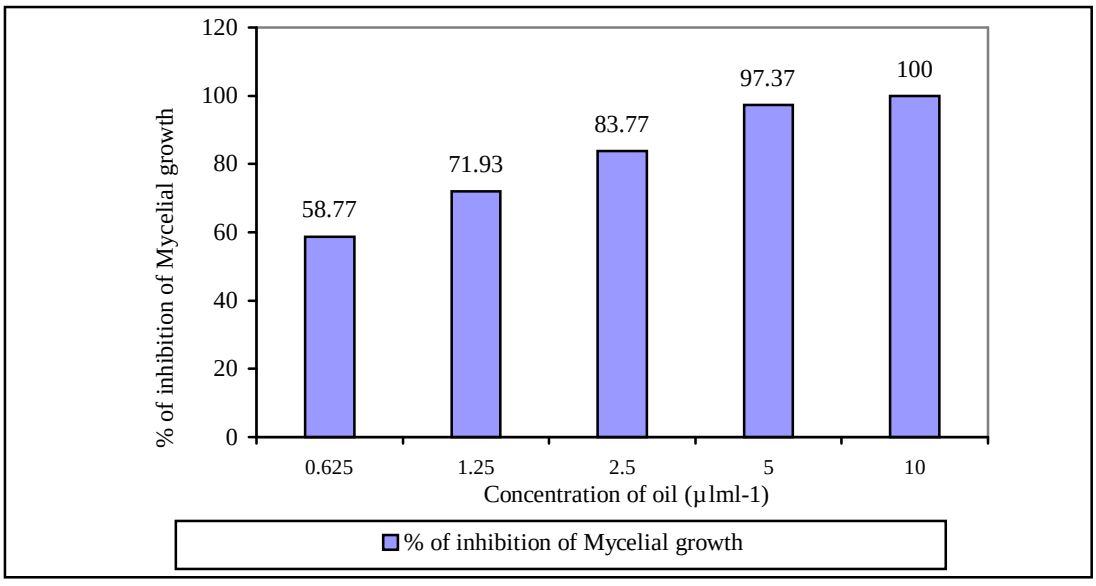

Fig 3: Percentage of antifungal activity of *Zanthoxylum armatum* oil against *Fusarium solani*

Table 4: Percentage of inhibition of *Fusarium solani* mycelial growth using extract of *Zanthoxylum armatum*.

S.N	Concent-ration of extract µlml-1	Inoculum Size (mm)	Colony size (mm)			Mean colony size (mm)	Mycelial growth (mm)	% of inhibition of mycelial growth
			I	II	III			
1	0	4	55	55	55	55.00	51.00	0.00
2	0.625	4	42	41	41.5	41.50	37.50	26.47
3	1.25	4	32	32	33	32.33	28.33	44.44
4	2.5	4	15	16	15.5	15.50	11.50	77.45
5	5	4	12	11.5	12	11.83	7.83	80.64
6	10	4	11	10.5	11.5	11.00	7.00	86..27
7	20	4	10	9	8	9.00	5.00	90..20
8	30	4	9	8.5	9	8.83	4.83	90..52
9	40	4	6	6	6	6.00	2.00	96.08
10	50	4	5	4	4.5	4.50	0.50	99.02
11	100	4	4	4	4	4.00	0.00	100.00

Minimum inhibitory concentration (MIC) =100 μlml^{-1}

Extract of *Zanthoxylum armatum* showed mycelial inhibition as 0%, 26.47%, 44.44%, 77.45%, 80.64%, 86.27%, 90.20%, 90.52%, 96.08%, 99.02% and 100% at 0, 0.625, 1.25, 2.5, 5.0, 10.0, 20, 30, 40, 50 and 100 oil concentrations against *Fusarium solani* respectively.

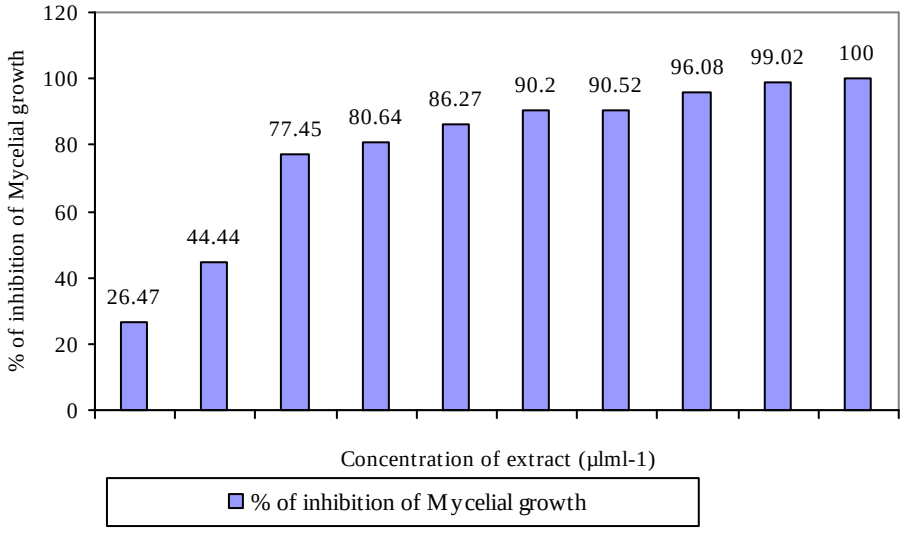

Fig 4: Percentage of antifungal activity of *Zanthoxylum armatum* extract against *Fusarium solani*

Table 5: Percentage of inhibition of *Fusarium solani* mycelial growth using essential oils of *Mentha arvensis.*

S.N	Concent-ration of oil µlml^{-1}	Inocul-um Size (mm)	Colony size (mm)			Mean colony size (mm)	Mycelial growth (mm)	% of inhibition of mycelial growth
			I	II	III			
1	0	4	42	42	42	42.00	38.00	0.00
2	0.625	4	27	27	26	26.67	22.67	40.35
3	1.25	4	22	21	22	21.67	17.67	53.51
4	2.5	4	12	11	11.5	11.50	7.50	80.26
5	5	4	7	6	7	6.67	2.67	92.98
6	10	4	4	4	4	4.00	0.00	100.00

Minimum inhibitory concentration (MIC) =10 µlml^{-1}

Essential oil of *Mentha arvensis* showed mycelial inhibition as 0%, 40.35%, 53.51%, 80.26%, 92.98% and 100% at 0, 0.625, 1.25, 2.5, 5.0 and 10.0 µlml^{-1} oil concentrations against *Fusarium solani* respectively.

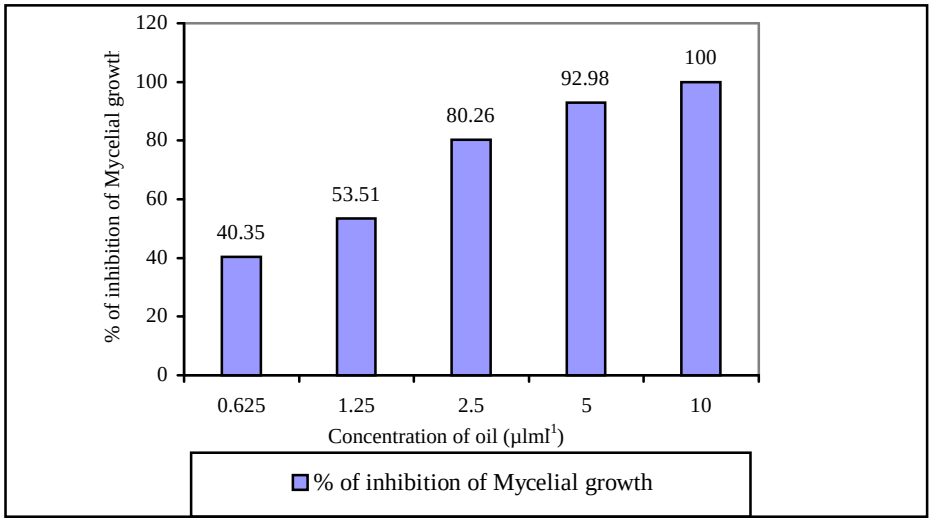

Fig 5: Percentage of antifungal activity of *Mentha arvensis* oil against *Fusarium solani*

Table 6: Percentage of inhibition of *Fusarium solani* mycelial growth using extract of *Mentha arvensis*.

S.N	Concent-ration of extract µlml^{-1}	Inoculum Size (mm)	Colony size (mm)			Mean colony size (mm)	Mycelial growth (mm)	% of inhibition of mycelial growth
			I	II	III			
1	0	4	55	55	55	55.00	51.00	0.00
2	0.625	4	48	48	47	47.67	43.67	14.38
3	1.25	4	45	44	44	44.33	40.33	20.92
4	2.5	4	42	41	41	41.33	37.33	26.80
5	5	4	25	24	25	24.67	20.67	59.48
6	10	4	20	20	19	19.67	15.67	68.28
7	20	4	18	17	17.5	17.50	13.50	73.53
8	30	4	16	15	15	15.33	11.33	77.78
9	40	4	13	12	12.5	12.50	8.50	83.33
10	50	4	9	9.5	8	8.83	4.83	90.52
11	100	4	4	4	4	4.00	0.00	100.00

Minimum inhibitory concentration (MIC) =100 µlml^{-1}

Extract of *Mentha arvensis* showed mycelial inhibition as 0%, 14.38%, 20.92%, 26.80%, 59.48%, 69.28%, 73.53%, 77.78%, 83.33%, 90.52% and 100% at 0, 0.625, 1.25, 2.5, 5.0, 10.0, 20, 30, 40, 50 and 10.0 µlml^{-11} oil concentrations against *Fusarium solani* respectively.

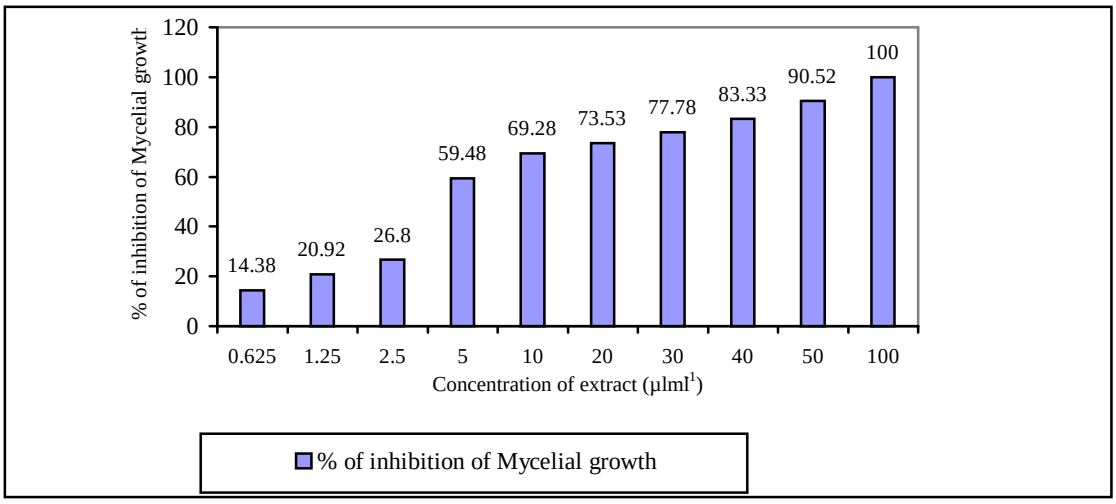

Fig 6: Percentage of antifungal activity of *Mentha arvensis* extract against *Fusarium solani*

Table 7: Percentage of inhibition of *Fusarium solani* mycelial growth using essential oils of *Amomum subulatum*.

S.N	Concent-ration of oil $\mu l ml^{-1}$	Inoculum Size (mm)	Colony size (mm)			Mean colony size (mm)	Mycelial growth (mm)	% of inhibition of mycelial growth
			I	II	III			
1	0	4	45	45	45	45.00	41.00	0.00
2	0.625	4	36	35	36	35.67	31.67	22.76
3	1.25	4	30	30	31	30.33	26.33	35.77
4	2.5	4	27	26	26	26.33	22.33	45.53
5	5	4	5	5	5	5.00	1.00	97.56
6	10	4	4	4	4	4.00	0.00	100.00

Minimum inhibitory concentration (MIC) =10 $\mu l ml^{-1}$

Essential oil of *Amomum subulatum* showed mycelial inhibition as 0%, 22.76%, 35.77%, 45.53%, 97.56% and 100% at 0, 0.625, 1.25, 2.5, 5.0 and 10.0 $\mu l ml^{-1}$ oil concentrations against *Fusarium solani* respectively.

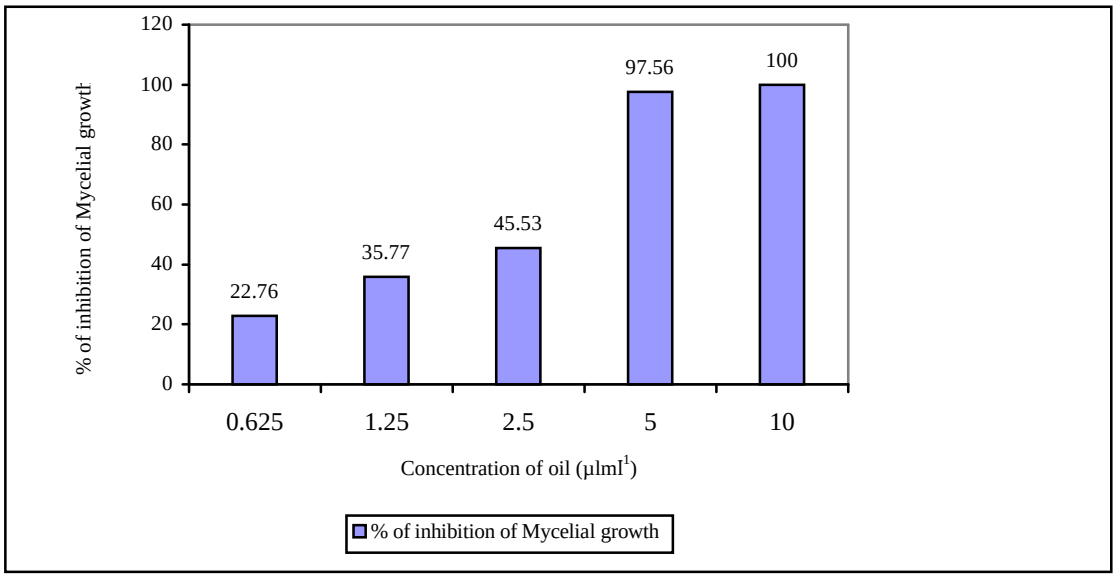

Fig 7: Percentage of antifungal activity of *Amomum subulatum* oil against *Fusarium solani*

Table 8: Percentage of inhibition of *Fusarium solani* mycelial growth using extract of *Amomum subulatum*.

S.N	Concent-ration of extract µlml-1	Inoculum Size (mm)	Colony size (mm)			Mean colony size (mm)	Mycelial growth (mm)	% of inhibition of mycelial growth
			I	II	III			
1	0	4	55	55	55	55.00	51.00	0.00
2	0.625	4	40	40	38	39.33	35.33	30.72
3	1.25	4	35	34	34.5	34.50	30.50	40.22
4	2.5	4	12	11	12	11.67	7.67	84.97
5	5	4	10	10	10.5	10.17	6.17	87.91
6	10	4	9	9.5	9	9.17	5.17	89.87
7	20	4	8	8	8.5	8.17	4.17	91.83
8	30	4	7	6	7	6.67	2.67	94.77
9	40	4	6	5	6	5.67	1.67	96.73
10	50	4	5	5	4.5	4.83	0.83	98.37
11	100	4	4	4	4	4.00	0.00	100.00

Minimum inhibitory concentration (MIC) =100 μlml^{-1}

Extract of *Amomum subulatum* showed mycelial inhibition as 0%, 30.72%, 40.20%, 84.97%, 87.91%, 89.87%, 91.83%, 94.77%, 96.73%, 98.37% and 100% at 0, 0.625, 1.25, 2.5, 5.0, 10.0, 20, 30, 40, 50 and 10.0 μlml^{-1}oil concentrations against *Fusarium solani* respectively.

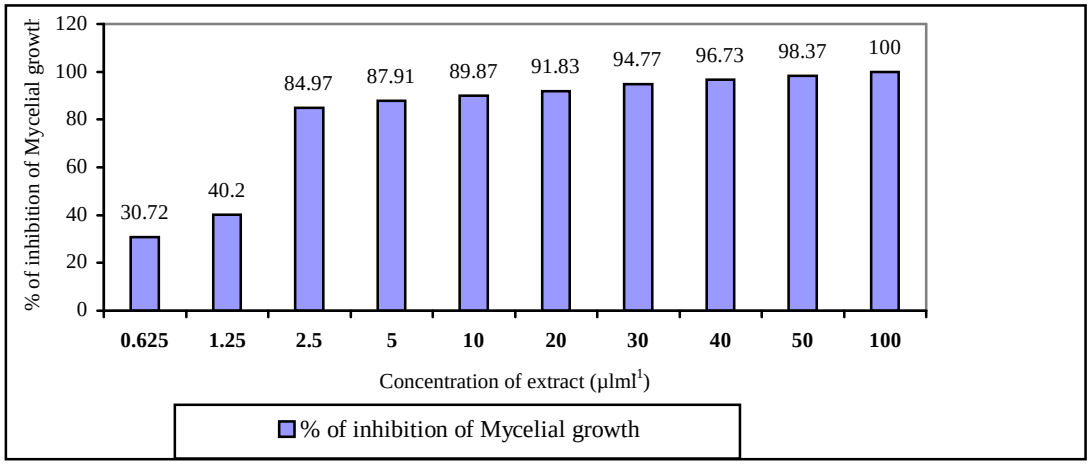

Fig 8: Percentage of antifungal activity of *Amomum subulatum* extract against *Fusarium solani*

Table 9: Percentage of inhibition of *Fusarium solani* mycelial growth using essential oils of *Valeriana jatamansi*.

S.N	Concent-ration of oil µlml-1	Inoculum Size (mm)	Colony size(mm)			Mean colony size(mm)	Mycelial growth (mm)	% of inhibition of mycelial growth
			I	II	III			
1	0	4	40	40	40	40.00	36.00	0.00
2	0.625	4	23	22	22.5	22.50	18.50	48.61
3	1.25	4	15	15	14	14.67	10.67	70.37
4	2.5	4	12	11	12	11.67	7.67	78.70
5	5	4	10	9	9	9.33	5.33	85.9
6	10	4	4	4	4	4.00	0.00	100.00

Minimum inhibitory concentration (MIC) =10 μlml^{-1}

Essential oil of *Valeriana jatamansi* showed mycelial inhibition as 0%, 48.61%, 70.37%, 78.70%, 85.9% and 100% at 0, 0.625, 1.25, 2.5, 5.0 and 10.0 μlml^{-1} oil concentrations against *Fusarium solani* respectively.

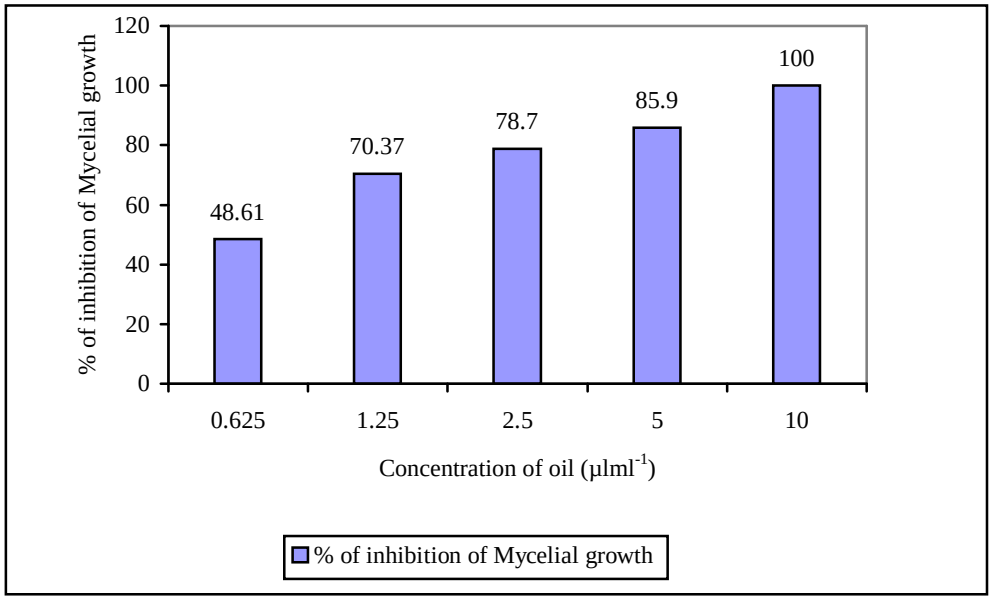

Fig 9: Percentage of antifungal activity of *Valeriana jatamansi* oil against *Fusarium solani*

Table 10: Percentage of inhibition of *Fusarium solani* mycelial growth using extract of *Valeriana jatamansi*.

S.N	Concent-ration of extract µlml^{-1}	Inoculum Size (mm)	Colony size (mm)			Mean colony size (mm)	Mycelial growth (mm)	% of inhibition of mycelial growth
			I	II	III			
1	0	4	55	55	55	55.00	51.00	0.00
2	0.625	4	45	46	45	45.33	41.33	18.95
3	1.25	4	30	30.5	30	30.17	26.17	48.69
4	2.5	4	28	28	27	27.67	23.67	53.59
5	5	4	25	25	26	25.33	21.33	58.17
6	10	4	23	22	24	23.00	19.00	62.75
7	20	4	15	15	16	15.33	11.33	77.78
8	30	4	12	11	12	11.67	7.67	84.97
9	40	4	11	10	10	10.33	6.33	87.58
10	50	4	5	4.5	5	4.83	0.83	98.37
11	100	4	4	4	4	4.00	0.00	100.00

Minimum inhibitory concentration (MIC) =100 µlml^{-1}

Extract of *Valeriana jatamansi* showed mycelial inhibition as 0%, 18.95%, 48.69%, 53.59%, 58.17%, 62.75%, 77.78%, 84.97%, 87.58%, 98.37% and 100% at 0, 0.625, 1.25, 2.5, 5.0, 10.0, 20, 30, 40, 50 and 10.0 µlml^{-11} oil concentrations against *Fusarium solani* respectively.

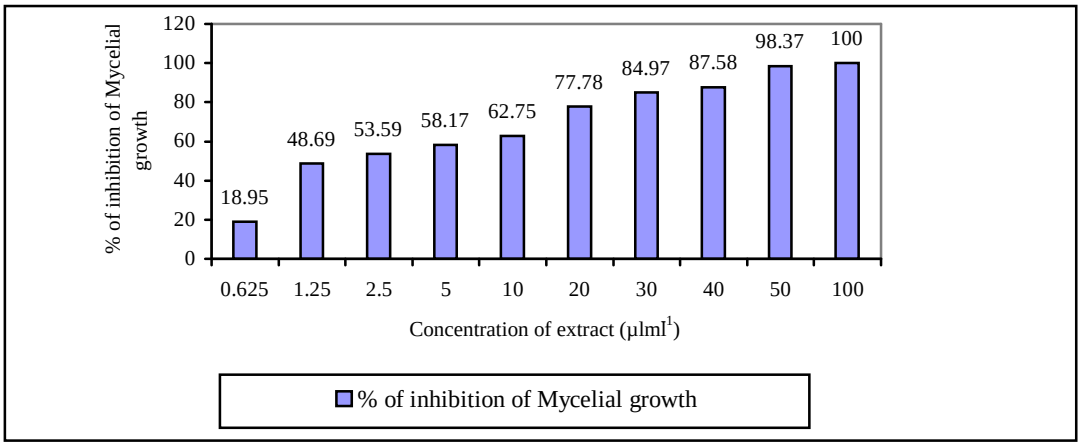

Fig 10: Percentage of antifungal activity of *Valeriana jatamansi* extract against *Fusarium solani*

4.2 RESULT

*Fusarium solan*i causing fruit rot of Pointed gourd was taken as test fungus. The fungus was isolated from the infected fruits of Pointed gourd in Pathology laboratory of CDB and identified from NARC and pathogenicity test was carried out by transfering the inoculum from infected to healthy Pointed gourd.

Minimum Inhibitory Concentration (MIC) of different essential oils and extracts

MIC value of Aromatic plants oils and extracts were achieved through poisoned food technique.

Table 11: MIC of essential oils and extracts.

S.N	Aromatic Plants	MIC($\mu l ml^{-1}$)	
		oil	Extract
1	*Cymbopogon flexuosus*	5	50
2	*Zanthoxylum armatum*	10	100
3	*Amomum subulatum*	10	100
4	*Valeriana jatamansi*	10	100
5	*Mentha arvensis*	10	100

All the five oils and extracts obtained from five Aromatic plants showed positive results in inhibiting the mycelial growth of *Fusarium solani.* All the essential oils and extracts showed their different potential of fungitoxicity.

Comparative fungitoxicities of different essential oils and extracts:

Table 12: Comparative fungitoxicities of different essential oils in different concentrations

S.N	Concent-ration of oil µlml^{-1}	Mycelial growth inhibition %				
		Cymbopogon flexuosus	*Zanthoxylum armatum*	*Valeriana jatamansi*	*Mentha arvensis*	*Amomum subulatum*
1	0	0	0	0	0	0
2	0.625	61.4	58.77	48.61	40.35	22.76
3	1.25	79.82	71.93	70.37	53.51	35.77
4	2.5	90.35	87.77	78.7	80.26	45.53
5	5	100	97.37	85.9	92.98	97.56
6	10	100	100.00	100	100	100

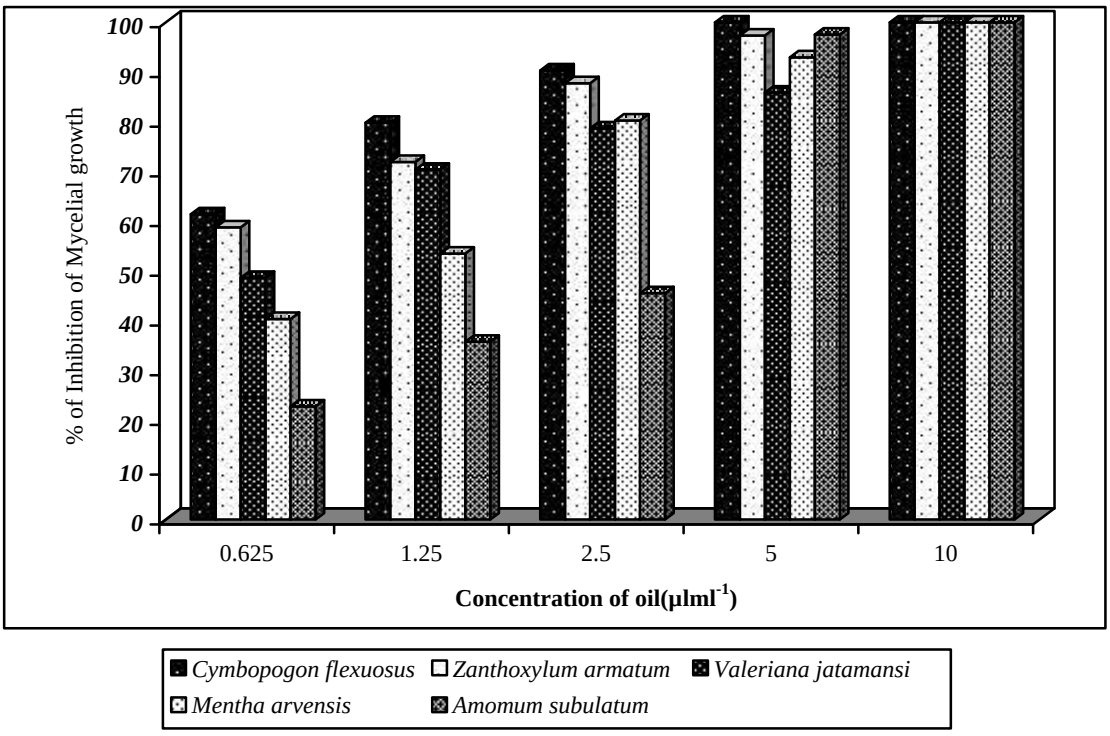

Fig 11: Fungitoxicities of different essential oils in different concentrations.

Table 13:Comparative fungitoxicities of different extracts in different concentrations

S.N	Concent-ration of extract $\mu l ml^{-1}$	Mycelial growth inhibition %				
		Amomum subulatum	*Zanthoxylum armatum*	*Valeriana jatamansi*	*Mentha arvensis*	*Cymbopogon flexuosus*
1	0	0	0	0	0	0
2	0.625	30.72	26.47	18.95	14.38	9.15
3	1.25	40.22	44.44	48.69	20.92	63.4
4	2.5	84.97	77.45	53.59	26.80.	72.22
5	5	87.91	80.64	58.17	59.48	88.56
6	10	89.87	86.27	62.75	68.28	90.2
7	20	91.83	90.20	77.78	73.53	94.44
8	30	94.77	90.52	84.97	77.78	97.06
9	40	96.73	96.08	87.58	83.33	97.71
10	50	98.37	99.02	98.37	90.52	100
11	100	100	100.00	100	100	100

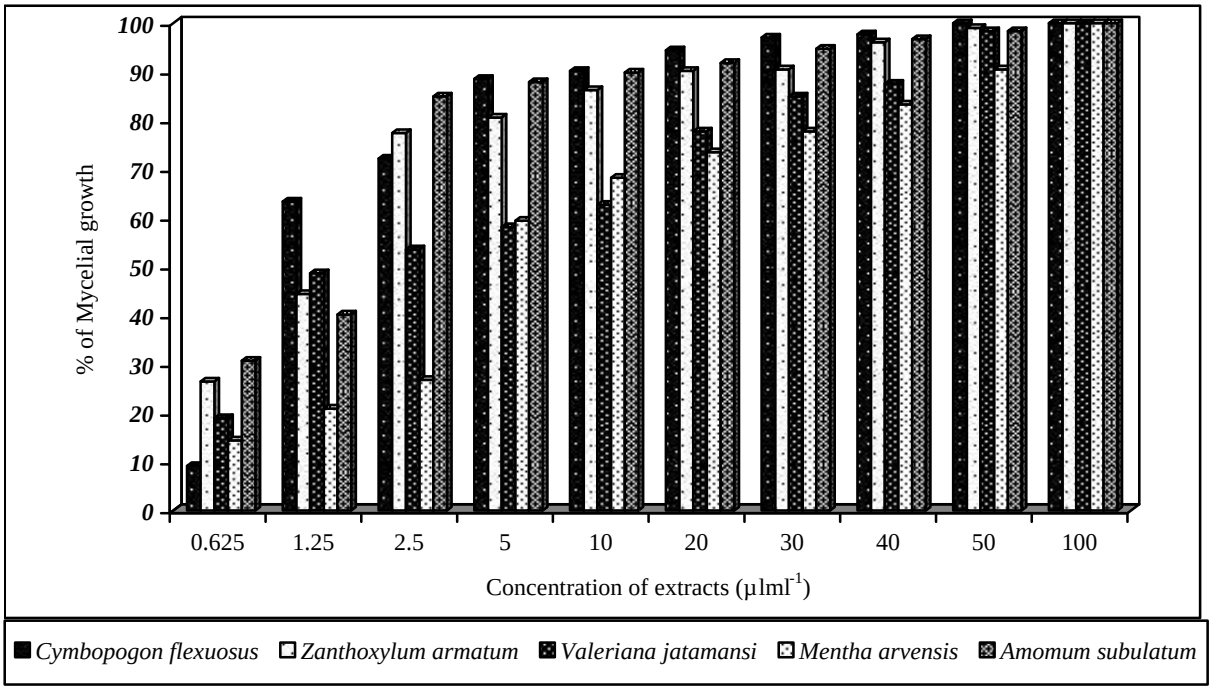

Fig 12: Fungitoxicities of different extracts in different concentrations.

CHAPTER -FIVE

5. DISCUSSION

In the present dissertation, attempts was made to study about the plant pathogenic fungi associated with the post harvest Pointed gourds found around the local markets of Kathmandu valley and ascertain the effect of pathogen on their storage and losses of Pointed gourds.

Present study has been emphasized on fruit rot disease of Pointed gourd caused by *Fusarium solani.* Pathogenicity test was carried out by transfering the inoculum from infected to healthy Pointed gourd. The isolated fungus was found to be *Fusarium solani,* which was identified by the characteristic features of the fungus by the standard literatures (C. Booth, 1977). The comparative study of the effectiveness of some plant essential oils and extracts like *Cymbopogon flexuosus, Mentha arvensis, Valeriana jatamansi, Amomum subulatum* and *Zanthoxylum armatum* to control the *Fusarium solani* of Pointed gourds were also made which was carried out *in vitro.*

The post harvest damages of Pointed gourds are found to be caused by different factors; they were damages from mechanical injuries, temperature effects, by diseases and pests, through the injuries caused by careless handling. This damages fall into two main catories- loss in quality and loss in quantity of Pointed gourd.

Qusem and Abulan (1996) worked for the in vitro treatment of aqueous extract of 64 weed species against *Helminthosporium sativum* and found the effect of oil in controlling the growth of fungus.

Several other researchers have worked for the control of *Fusarium solani.* Shrestha (2003) and subedi (2003) isolated *Fusarium solani* from soil, on inoculation with pathogenic organisms; the healthy plants did not develop typical dieback symptoms. However, the inoculated plants were stunted in comparison to controlled ones. Disease manifestation was affected by pH of the soil and soil texture. Lamgade (2004) and Khanal (2005) had detected *Fusarium solani* from the sissoo pods and their study was undertaken to control *Fusarium solani* by seed and soil treatment. Khanal (2005) recommened cold-water treatment and Indophil M-45 for seed treatment.

The experiment for the control of *Fusarium solani* has been undertaken by Hot water treatment and Biological control using *Trichoderma* species. *Fusarium solani* was completely eliminated, when seeds has been controlled with hot water at 50-55^{o}c for forty minutes and also the three species of *Trichoderma* (*T.aureoviride, T.harzianum, T. viride*) has been used in dual culture technique against *Fusarium solani on* sterilized PDA, the maximum inhibitory effect on *Fusarium solani* under the antagonistic effect of *Trichoderma harzianum* was 3.15 cm. and only *Trichoderma harzianum* had overgrown the colony of *Fusarium solani* has been done in Nepal (Bajracharya et al, 2006).

In context of Nepal, no any special control measure was found to be applied by farmers for controlling the fruit rot disease of Pointed gourd. The aim of this study is also to collect proof for an antifungal efficacy of essential oils and extract of aromatic plants. Since the fruit rot is an important disease of pointed gourd, it should be protected from fungal pathogen (*Fusarium solani*) by biological method with an aim to control with no side effects.

The difference in fungitoxicity at same concentration in different essential oil and extracts may be due to different chemical composition of the oils and extracts Singh *et al,* (1983); Maheshwari (1985), Philip and Damodaran (1985) cited in Rao and Srivastava (1994).

The result obtained from the study may not be true forever because the concentration of various ingredients in essential oil and extract of the same plant species varies due to several factors such as growth stage, ecological factors etc. As mentioned by Nilov and Pant (1939); Gulati (1980), Pareek *et al*. (1980) cited in Rao and Srivastava (1994).

CHAPTER - SIX

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The fungus, isolated from the infected Pointed gourds was identified as *Fusarium solani*, which was responsible for the fruit rot disease of Pointed gourd. Pathogenicity test was carried out by transfering the inoculum from infected to healthy Pointed gourd.

For the control, different aromatic plants (*Cymbopogon flexuosus, Mentha arvensis, Valeriana jatamansi, Amomum subulatum* and *Zanthoxylum armatum)* essential oils and extracts were used. To find out the MIC and also evaluate the comparative fungitoxicity of essential oils and extracts against the fungus *Fusarium solani*, the toxicity of the essential oils and extracts was assessed by using poisoned food technique (Grover and Moore, 1962).

From the research it can be concluded that the test plants harbored the fungitoxic properties due to presence of antifungal compounds, from this fungitoxic principle that inhibits the mycelial growth of *Fusarium solani*. Out of five test plants, essential oil and extract of *Cymbopogon flexuosus* was found more effective in its fungitoxic properties and showed 100 % inhibition of mycelial growth of the test fungus at MIC of 5.0 μlml^{-1} and 50 μlml^{-1}. Similarly *Zanthoxylum armatum, Valeriana jatamansi, Mentha arvensis, Amomum subulatum* were found effective and showed 100% mycelial growth inhibition at 10 μlml^{-1} and 100 μlml^{-1} respectively. Similarly the highest percentages of mycelial growth inhibition were found to be oils of *Cymbopogon flexuosus* followed by *Amomum subulatum, Zanthoxylum armatum, Mentha arvensis,* and *Valeriana jatamansi* respectively. Similarly extracts of *Cymbopogon flexuosus* followed by *Zanthoxylum armatum, Amomum subulatum, Valeriana jatamansi* and *Mentha* arvensis respectively.

A perusal literature showed that the similar experiment for the control of *Fusarium solani* using essential oils and extract has not yet been done in Nepal. So this is the first study to assess fungitoxicities of essential oils and extract against *Fusarium solani*.

6.2 Recommendations

- This research work has been strictly done under controlled environment (*in vitro*) so the result found might not correspond with those when performed in commercial field under the influence of a number of physical factors like temperature, pH, photolysis of constituents, buffer, solubility etc. Hence a rigorous study in the commercial field is recommended.
- I should recommended the Government policy makers to manage or control the plant disease by using the rules of Quarantine regulations, by establishing Quarantine station at Airport, Railway station and Buspark areas.
- At last I want to request all the farmers to control the plant disease through the use of Biological control method, it may reduce the cost, less toxic and has no any side effect as comparision to the use of chemical Fertilizer.

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APPENDIX-1

Materials Used For the Study

Apparatus and Equipment:

Test tubes

Culture tubes

Funnel

Pipettes

Conical flasks

Glass rod

Petri dishes

Inoculating needles

Ocular micrometer

Stage micrometer

Glass Slides

Inoculating loop

Forceps

Scissors

Burner

Beakers

Cover slip

Muslin cloth

Cotton

Measuring cylinder

Borers

Plastic containers

Sprit lamp

Laminar airflow

Microscope

Hot air oven

Incubator

Refrigerator

Autoclave

Photographic camera

Balances

Aromatic Plant Samples:

a) ***Zanthoxylum armatum*** DC.

Family: Rutaceae

Nepali name: Timur

Description:

Spinous shrub or small tree with corky bark, up to 70 m. High, with pinnately compound leaves, leaflets elliptic lanceolate with small yellow flower in short branched lateral cluster (Rajbhandari, 2001). Flowers commonly 1mm. unisexed, calyx with 6-8 acute lobes, petal absent, stamen 6-8, much larger than calyx in male flower. Leaf stalk narrowly winged, leaf 2-6 pairs, about 8 cm, toothed, sparsely gland dotted ripe capsule, 3-4 mm globular red wrinkled aromatic seed, shining black (Polunin and Stainton, 1984).

Distribution:

Western, Central and Eastern Nepal, altitude 1100-2500 m. Himalaya (Kashmir to Bhutan), North India to China, Taiwan, Phillpines (Press et al, 2000).

Parts used:

Fruit

Uses:

Decoction of fruit is used in cold treat constipation and as anthelmintic for cattles. Seeds are extremely used in the preparation of tooth powder. Seeds are used as a spice and treat stomach disorder.Fruits are taken to cure in digestion. Powdered fruit is taken internally as stigmachic and also used curry as well as antimicrobial spice in mushroom cooking. Bark is used as fish poison (Acharya, 1996; Bhattrai, 1990; Parajuli, 2000).

b) ***Mentha arvensis*** L**.**

Family: Labiatae

Nepali name: Pudina

Description:

Aromatic herbs, strongly scented, erect or diffuse herb, root stock creeping. Leaves nearly sessile, lanceolate, ovate and pubscent.

Flower small, in large whorls crowed in axillalary and terminal, cylindrical, tapering spikes, calyx, hairy, bell shaped, actually 5 toothed, corolla-tube in the calyx limb, erect, 4 lobes, lobes nearly equal, stamen 4, protruding, filament nacked.

Distribution:

Central Nepal, alt. 1200-2000 m. Europe, west to China, North America, Artic to Himalayas, South East Asia to Hawaii and Australia, throughout Africa, Cosmopolitan (Press et.al, 2000).

Parts used:

Leaves

Uses:

Flavoring of food, essential oil used in perfumery pharmacy, and ornamental (Stainton, 1984).

c) ***Amomum subulatum*** Roxb.

Family: Zingiberaceae

Nepali name: Nepal Cardamom, Alaichi

Description:

An aromatic herbs with leafy stem up to 90-100 cm. in height, leaves ablong, lanceolate, bright green, glabrous on both surface, leaf 30-60 cm long and 7.5-15 cm broad, flower white in colour, globose, shortly peduncled spike bracts redish brown, anther crest small,

truncate, entire fruit reddish brown, densely echinate globose capsules, seed may held together by viscid sugary pulps, flowering at April/May, fruiting in July /August.

Distribution:

Found in 600-2000 m. altitude in Nepal. Also found in Indonesia, Thiland, West Africa, India and Srilanka.

Parts used:

Seed

Uses:

The seed are acrid bitter, aromatic, thermogenic, appetizer, digestive, stomachic, vulnerary cardiac and liver tonic, diuretic. They are useful in vomiting vitiated condition of diarrohoea, dysentery, skin disease, wound ulcer, fever and gonorrhoea. Used in spices in different dishes. Making different cosmetics also.

d) *Valeriana jatamansi* Jones

Family: Valerianaceae

Nepali name: Samayo, Sugandawal

Description:

Hairy perennial herb, up to 45 cm high, leaf arises from basal portion of stem. Petiole is long, leaf heart shaped, or oval, serate, leaf 7.5 cm long, 4.5 cm broad, bisexual, flower white or pink, roots are indense in the field, roots are brown and 2-4 cm long, 1-2 cm width, flowering at march, fruiting at September.

Distribution:

Western, Central and Eastern Nepal, altitude 1500-3300 m. Also found in China, Bhurma and Afganithan.

Parts used:

Roots

Uses:

Used to cure in eye and headache treatment, energetic, as tonic in heart disease, in mouth diease, diarrohea. Used as Ayurvedic medicine as Sudarshan Churna. Also used to make perfume and dhup. When mixed with the paste of sugar to cure urine disease treatment.

e) *Cymbopogon flexuosus* Steud.

Family: Graminae

Nepali name: Kagati ghans

Description:

Aromatic herb,odour same as lemon so called lemon grass, average 3 m in height, bushy, leaf 125 cm long and 1.7 cm broad, serate, small hairy, thin long.

Distribution:

Found in 150-1500 m. altitude in Nepal. Also found in West Indies, India, China, Bengal etc.

Parts used:

Including leaves all the parts of above ground.

Use:

It is used as grasses. For making herbal tea. For making soap, sweets, perfume.For killing pests.To cure eye disease and also oil can be obtained after distillation process from leaf. Leaf contains 80% citrol.

The Test Fungus:

Fusarium solani isolated from infected Pointed gourd.

Chemicals and Reagent:

Distilled Water

80% Acetone

Cotton blue

Lacto phenol

Potatoes

Dextrose

Agar

Sprit

Ice

0.1% Mercuric Chloride

Anhydrous Sodium Sulphate etc.

Apparatus Used In Oil and Extract Extraction:

For Oil:

Clevenger's apparatus

Round Bottom Flask

Condenser

Ice

Bucket

For Extract:

Mortar

Pistal

Test tubes

Centrifuge

Muslin cloth

APPENDIX-2

Micrometry:

In stage micrometer, 100 divisions = 1000µm.

$$\text{or, 1 divisions} = \frac{1000}{100} = 10\ \mu m.$$

13 divisions of occular micrometer coincided with 5 divisions of stage micrometer.

i.e. 13 divisions of ocular micrometer = 5 divisions of stage micrometer.

1 division of ocular micrometer = 5 / 1 3 division of stage micrometer.

$$\text{or, 1 division of O. M} = \frac{5}{13} \times 10$$

$$= 3.84\ \mu m$$

Measurement of Macroconidia

S.N.	Length (µm)	Breadth (µm)
1	19.2	3.072
2	23.04	4.608
3	19.968	3.456
4	23.424	3.456
5	15.36	3.072
6	17.28	3.072
7	21.12	3.456
8	38.4	5.376
9	18.816	3.072
10	34.56	3.84

Measurement of Microconidia

S.N	Length (µm)	Breadth (µm)
1	11.52	3.84
2	7.68	2.304
3	5.76	1.92
4	10.752	3.072
5	11.52	3.84
6	11.136	3.456
7	6.912	2.304
8	9.6	2.688
9	7.296	1.92
10	9.984	2.688

LIST OF PLATES

Plate 1: Pure Culture of *Fusarium solani*

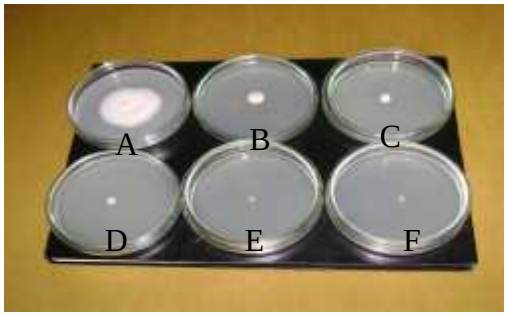

Plate 2: Antifungal efficacy of essential oil of *Cymbopogon flexuosus* against *Fusarium solani.*

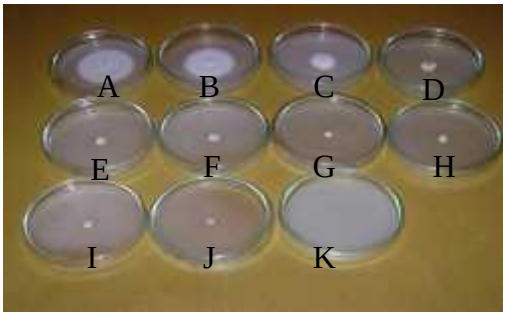

Plate 3: Antifungal efficacy of extract of *Cymbopogon flexuosus* against *Fusarium solani*

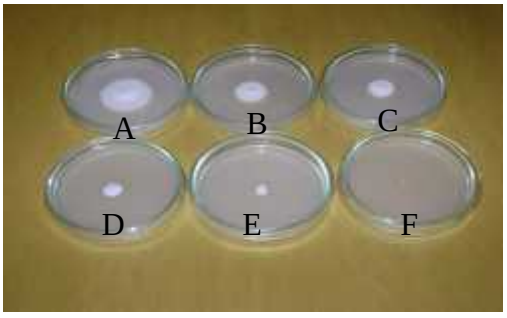

Plate 4: Antifungal efficacy of essential oil of *Zanthoxylum armatum* against *Fusarium solani*.

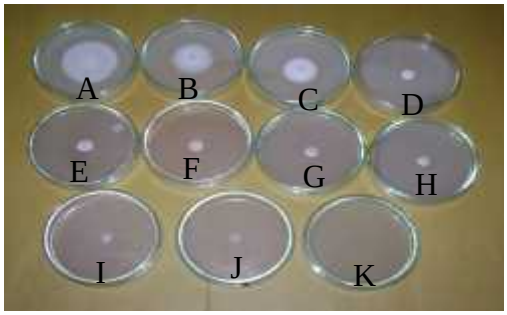

Plate 5: Antifungal efficacy of extract of *Zanthoxylum armatum* against *Fusarium solani*

Note:- A= Control, B= 0.625 $\mu l ml^{-1}$, C=1.25 $\mu l ml^{-1}$, D=2.5 $\mu l ml^{-1}$, E=5.0 $\mu l ml^{-1}$, F=10.0 $\mu l ml^{-1}$, G=20.0 $\mu l ml^{-1}$, H=30.0 $\mu l ml^{-1}$, I=40.0 $\mu l ml^{-1}$, J=50.0 $\mu l ml^{-1}$, K=100.0 $\mu l ml^{-1}$.

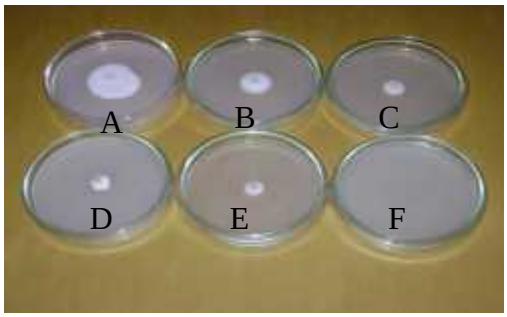

Plate 6: Antifungal efficacy of essential oil of *Mentha arvensis* against *Fusarium solani* .

Plate 7: Antifungal efficacy of extract of *Mentha arvensis* against *Fusarium solani*

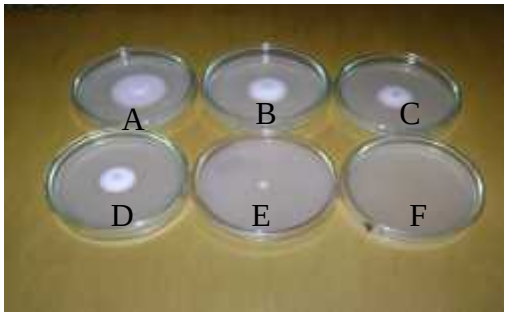

Plate 8: Antifungal efficacy of essential oil of *Amomum subulatum* against *Fusarium solani*.

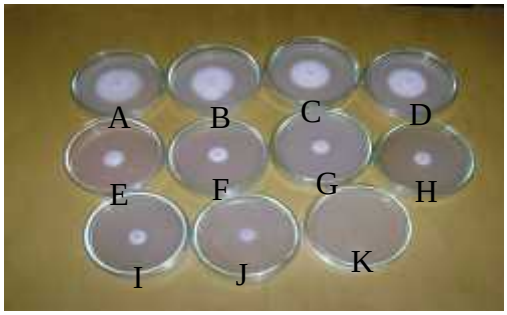

Plate 9: Antifungal efficacy of extract of *Amomum subulatum* against *Fusarium solani* .

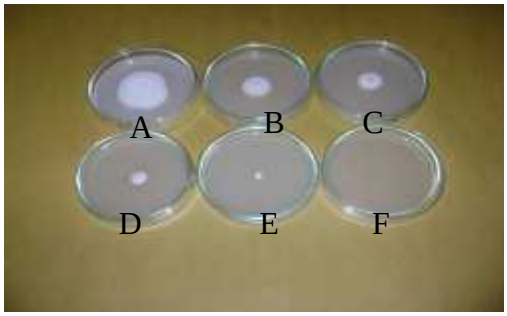

Plate 10: Antifungal efficacy of essential oil of *Valeriana jatamansi* against *Fusarium solani* .

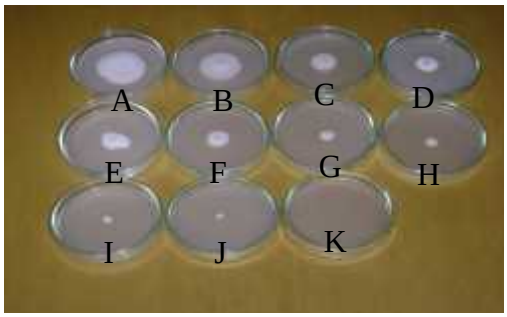

Plate 11: Antifungal efficacy of extract of *Valeriana jatamansi* against *Fusarium solani* .

Note:- A= Control, B= 0.625µlml^{-1} , C=1.25µlml^{-1}, D=2.5µlml^{-1}, E=5.0µlml^{-1}, F=10.0 µlml^{-1}, G=20.0µlml^{-1} , H=30.0µlml^{-1}, I=40.0µlml^{-1}, J=50.0µlml^{-1}, K=100.0µlml^{-1}.

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Photo 2: *Zanthoxylum armatum*

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Photo 6: *Cymbopogon flexuosus*

Photo 7**:** Clevenger's Apparatus

Photo 8: Observation of Slides from Stereo-microscope at NARC.

Photo 9: Working on Laminar Air Flow of Laboratory of CDB

Photo 10: Macro and Micro Conidia of *Fusarium solani* by Stereo Microscope.

Photo 11: Single Phailade showing Conidia of *Fusarium solani* by Stereo Microscope.

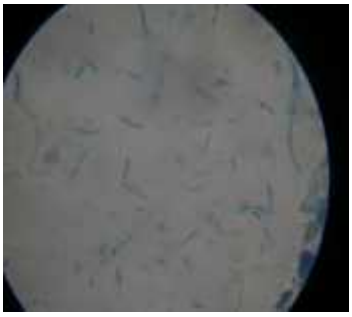

Photo 12: Macro and Micro Conidia of *Fusarium solani* by Compound Microscope.

Photo 14: Different types of essential oil.