CHAPTER- 1

1. INTRODUCTION

1.1 General Background

Nepal is rich in biodiversity owing to the variation in physiographic structures and climate. Nepal, a basically an agricultural country boost about 80 percent of economically active population involved in agriculture. However, agricultural production has remained almost stagnant since the last decade and is rather low. The country is also facing the challenges of the overpopulation. The land is limited and is almost occupied for the human settlement but to feed all the mouths of the people, it becomes a need to increase the cultivation of the crops by the proper management of land, plant diseases and pests.

Area, production and yield of vegetables in Nepal (2006/07)

Region	Area (Ha.)	Production (Mt.)	Yield (Kg/Ha.)
Eastern	46,088	5,13,995	11,152
Central	81,418	10,21,203	12,543
Western	29,564	3,48,505	28,847
Mid-western	24,668	2,85,609	11,578
Far- western	10,184	1,29,377	12,704

Statistical information on Nepalese Agriculture 2006/07, Agribusiness promotion and statistics division MOAC, Nepal.

Medicinal plants can be known as any plant or plant parts that contain curative properties no matter to which source or country it belongs. Several species of medicinal plants are used for treatment of various diseases. The medicinal properties such as antifungal, antibacterial, antiviral, anticancer and others are present in medicinal plants (Parajuli *et al*., 1998). The plant parts, their constituents such as essential oils are to be found less phytotoxic, more systemic and easily bio-degradable (Beye, 1978). The active components or secondary metabolites like alkaloids,

saponins, essential oils, tannins etc are the main reasons for the medicinal properties (Kruger, 1992).

1.2 Plant, its cultivation and diseases

Plant disease can be known as a serious ill which causes the destructions of plants in a great deal. In other way, it is an interaction between plants, pathogens and environment. The hazardous chemicals are being used to control several plant diseases. However, the use of these chemicals is not an intelligent way to control the plant diseases as they increase the animal health hazards when consumed. They are even responsible for environmental pollutions. Moreover, it may affect the non target or non diseased plant parts when applied.

In the recent years, the plant extracts or essential oils have got more interest in the control of the agricultural crops as they provide inexhaustible sources for disease control in plants (Swaminathan, M.S. 1978). They are less toxic and do not cause environmental pollutions. They may also serve as antiseptic, as a lab reagent, as insecticides etc. (Schery, R.W. 1972).

1.2.1 *Brassica oleracea* L.

Cauliflower (*Brassica oleracea* L.) is one of the important vegetable species of the family Crucifereae. It is an annual plant that reproduces by seed. Typically, only the head (the white curd) is eaten while the stalk and surrounding thick, green leaves are discarded.

The cultivation of cauliflower is preferred in well drained, moist soil which has significant organic matter with a pH range of about 6 to 7. Cauliflower is typically started indoors six to eight weeks prior to setting out in the garden and is typically ready to harvest two months after transplanting. The seeds germinate best with a soil temperature of 25 ± 1^{o}C. The vegetables require a cool and moist climate.

Different hybrid varieties of cauliflower is being cultivated in Nepal. An annual report from NARC,2006/07 suggests 11 hybrid varieties of cauliflowers *viz.* Snow crown, Snow mastique, Silver cup, Ohlsens Enke, Sweta, Snow King, Snow Grace, White Flash, Gospel, Green Stone and Gianty $F_{1.}$

It is highly nutritious and may be eaten cooked, raw or pickled. Cauliflower is low in fat, high in dietary fiber, folate, water and vitamin C, possessing a very high nutritive density. It also contains other glucosinolates besides sulfurophane substances which improve the liver's ability to detoxify carcinogenic substances. A high intake of cauliflower has been found to reduce the risk of aggressive prostate cancer (USDA Nutrient database, 2005).

The nutritional facts of cauliflower can be tabulated as follows:

Cauliflower, raw
Nutritional value per 100 g

Carbohydrates	5 g
- Sugars	2.4 g
- Dietary fiber	2.5 g
Fat	0 g
Protein	2 g
Thiamin (Vit. B1) 0.057 mg	4%
Riboflavin (Vit. B2) 0.063 mg	4%
Niacin (Vit. B3) 0.53 mg	4%
Pantothenic acid (B5) 0.65 mg	13%
Vitamin B6 0.22 mg	17%
Folate (Vit. B9) 57 µg	14%
Vitamin C 46 mg	77%
Calcium 22 mg	2%
Iron 0.44 mg	4%
Magnesium 15 mg	4%
Phosphorus 44 mg	6%
Potassium 300 mg	6%
Zinc 0.28 mg	3%

Source: USDA Nutrient database, 2005

1.2.2 Cultivation

Soil and site

All cauliflowers need a neutral or slightly alkaline soil to do well. If the soil is too acidic, the plants will be unable to obtain all the trace elements they need, and may develop whiptails. On the other hand, soils which are too limey or chalky can lead to stunted and discolored cauliflower. Cauliflowers will definitely suffer if they are grown on the same plot for two or more years in a row.

Cauliflower requires a fairly fertile, well drained soil with a good water-holding capacity and a pH range of 6 to 7. Working the soil to break up any hardpan, and incorporating manure or compost to a depth of 6–8 inches the soil must be treated for root knot nematode.

Planting

Cauliflower can be transplanted or seeded directly into the garden. If transplants are to be used, sow seed in seedling flats, beds, or pots. Seeds will germinate in 3–5 days and can be ready for transplanting in 3–6 weeks. Water the seedlings regularly, preferably in the morning to allow the plants to dry as soon as possible. Seedlings that are overcrowded and kept wet will often become infested with diseases. Seedlings that are stunted or checked in growth will usually form small, button-sized heads prematurely after transplanting. Transplant seedlings 2–3 ft. apart between rows and 12–15 inches apart within the rows, depending upon the variety grown.

Cultivate the garden frequently to control weeds and break up surface crusting to improve water penetration. Cultivation should be shallow to prevent root injury. Irrigate regularly, or whenever needed to prevent plants from wilting. If allowed to wilt, the crop will be delayed in maturing and the size of heads will be reduced.

Fertilizers

A general garden fertilizer, such as 10-30-10, can be applied at the rate of 2–3 pounds per 100 square feet. Apply one-half of the complete fertilizer at the time of transplanting or soon thereafter, and the second half 3– 4 weeks later. An additional

side-dressing of about 1 / 2 pound of sulfate of ammonia per 100 square feet at the stage of curd formation will help increase the size of heads.

Harvesting and after care

A cauliflower is ready for cutting when the upper surface of the curd is fully exposed and the inner leaves no longer cover it. Unfortunately, cauliflowers tend to mature all at once. If the weather is warm and leaves the cauliflowers in the ground once they have matured, the heads expand and they become discolored and less appealing. To avoid this lift some early, they will be quite edible. Alternatively, gather up the leaves and tie them together over the curd so that they cover it, using garden twine, an elastic band or raffia. It will also protect the winter ones from the frost.

To keep them for two or three weeks once they are mature, lift the whole plant, including roots and hang them upside down in a cool shed and syringe them daily. Cauliflowers freeze well and you can deal with an over abundant crop by freezing the surplus for later use. When harvesting, cut in the early morning when the plant is freshest, ideally with dew on it. During frosty weather however, it is better to wait till the warmest part of the day. Cut through the stalk with a sharp knife, leaving enough leaves around the curd to protect it.

1.2.3 Diseases and pests

Cauliflowers are prone to several diseases under adverse conditions. Of the 11 hybrid cauliflower verities, variety Gianty F_1 shows highest disease incidence (Annual Report 2006/07, NARC). The report also suggests that Tistung-palung, Kavre and Panauti areas have to suffer much from lots of problematic diseases on cauliflowers. One of the most common diseases is Leaf spot and blight caused by *Alternaria brassicae* and *Curvularia lunata*. Besides, white rust caused by *Albugo candida* is also the destructive disease. The bacteria *Xathomonas compestris* causing black rot also causes great loss in the productivity. The Club root disease caused by *Plasmodiophora brassica* is also the destructive disease. Others are *Perenospora parasitica* causing downy mildew and *Fusarium oxysporum* causing wilt (Rangaswami, 1999).

1.3 *Curvularia lunata* Wakker

Curvularia is a dematiaceous filamentous fungus. Among the species, *Curularia lunata* is the most prevalent cause of disease in plants, human and animals.

1.3.1 Symptoms

The early symptoms are in to form of small, yellowish brown spots on the leaves which enlarge in size and become rounded. Entire lamina, petiole and stem are badly damaged in severe infection. The brown spots change into black colored at the late stage. The symptoms mostly appear in the late stage of the life cycle of the host**.**

1.3.2 Pathogen

Hyphae are septate and brown. Conidiophores are brown, simple or branched and are bent at the points where the conidia originate. This bending pattern is called sympodial geniculate growth. Conidia are straight or pyriform, brown and multiseptate. The outer edges of the condia look dark black and the middle part is light colored with 3-4 septa. These septa are transverse and divide each conidium in to multiple cells. The number of septa ranges from 3-4. The size of conidia ranges (20-32x9-15) µm.

1.3.3 Disease cycle

The pathogen is mostly air borne. The collateral hosts such as rice and sorghum also play a significant role in the perpetuation and dissemination of the fungus.

1.3.4 Taxonomic rank

Saprophytic fungi, multicellular, septate mycelium, perfect stage unknown

- Deuteromycotina

With the characteristic of the sub-division

- Deutermycetes

True mycelium, conidia produced in special conidiogenus hyphae (conidiophores) arising in various ways other than in Picnidia and Acervuli.

- Hyphomycetidae

Hyphomycetious fungi producing conidia, Plant pathogenic, no cleistothecia

- Moniliales

Dark conidia, no organized fruiting body and plant parasite

- Dematiaceae

Large phragmospores, curved conidia, end cells are pale than pother cells

- *Curvularia*

Crescent shaped conidia with 3-4 septa, end cells blunt

- *C. lunata*

(Alexopoulos and Mims, 1953 " *Introductory Mycology"* 3rd. ed.)

1.4 Description of Plant species used in the experiment.

Extracts of five aromatic plants were used for the experimentation to asses the effects of those extracts on the mycelial growth of the test fungus. Following are the details of the used plants:

1.4.1 *Allium sativum* L.

- Family: Liliaceae
- Local name: Lasun
- Description: Bulbous herb, bullets enclosed in a white or purple envelope. Leaves are all basal, flat. Flowers are white or pink in lax umbels on a long terete scape exceeding the leaves. (Manandhar, 2002).
- Chemical components: Four major compounds of *Allium sativum* are known by gas chromatography. The compounds were Diallyl trisulfide, Diallyl disulfide, Diallyl sulfide and Caryophyllene.
- Uses: Cloves are used as a condiment. Cloves are carminative and stimulating. They possess antiseptic and antibacterial properties (Manandhar, 2002).

1.4.2 *Tagetus patula*

- Family: Compositae
- Local name: Sayapatri
- Description: Hairless, annual herb with branchuing stem to 50cm, leaves stalkless, pinnately cut into usually 5-7 pairs of oblong lanceolate sharply toothed lobes bearing scattered glands. Flower heads 3-4cm which are swollen at below the head. Involucre blue green cylindric with 5 short triangular teeth. Ray florets, rounded, notched at apex. Disc- floret with style arms exerted (Manandhar, 2002).
- Chemical components: It contains β- caryophyllene, limonene, methyleugenol, piperetone.
- Uses: In addition to colouring foods, yellow dye from the flowers is also used to color textiles. The whole plant is harvested when in flower and distilled for its essential oil. The oil is used in perfumery; it is blended with sandalwood oil to produce 'attar genda' perfume. The oil is also being investigated for antifungal activity, including treatment of candidiasis and treating fungal infections in plants.

1.4.3 *Ocimum sanctum* L.

- Family: Labiatae
- Local name: Tulsi
- Description: Much branched herb, 30-60cm high, leaves 2.5-6.5cm long, oblong or elliptic or acute entire or sub serrate. Petioles 1.2-2.5cm long. Flowers in raceme and purplish pink corolla. It is used in the treatment of viral encephalitis and tropical pulmonary eosionphalia in children, asthma, bronchitis etc. (Manandhar, 2002).
- Chemical components: The oils bear eugenol, eugenal. Carvacrol, methyl-chavicol, limatrol and caryophylline.

- Uses: Tulasi's extracts are used in ayurvedic remedies for common colds, headaches, stomach disorders, inflammation, heart disease, various forms of poisoning, and malaria. Traditionally, tulasi is taken in many forms such as herbal tea, dried powder, fresh leaf, or mixed with ghee. Essential oil extracted from Karpoora Tulsi is mostly used for medicinal purposes and in herbal cosmetics. Widely used in skin preparations for its anti-bacterial activity. From centuries, the dried leaves of Tulasi have been mixed with stored grains to repel insects.

1.4.4 *Azadirachta indica* A.Juss

- Family: Meliaceae
- Local name: Neem
- Description: Large tree 12-15m high, leaves compound, leaflets 2.5-7.5cm long, lanceolate, opposite toothed, long pointed flowers axillary, white honey scented and 4.5cm long. It is a popular medicine of leprosy and skin diseases. The leaves are used as antiseptic, anthelmintci purposes. (Manandhar, 2002).
- Chemical components: The oils contain azadirachtin, salannin, meliantriol and nimbin.
- Uses: Neem is deemed very effective in the treatment of scabies although only preliminary scientific proof exists which still has to be corroborated, and is recommended for those who are sensitive to permethrin, a known insecticide which might be an irritant. Also, the scabies mite has yet to become resistant to neem, so in persistent cases neem has been shown to be very effective. There is also anecdotal evidence of its effectiveness in treating infestations of head lice in humans. A tea made of boiled neem leaves, sometimes combined with other herbs such as ginger, can be ingested to fight intestinal worms

1.4.5. *Lantana camara* L.

- Family: Verbanaceae
- Local name: Masino kanda, Sitaji phool
- Description: Straggling shrub, about 2m high with recurved spines on the branches. Leaves stalked, opposite,1.5-8.5cm long,0.8-6cm wide, ovate to oblong, acuminate, serrate, base more or less cordate. Flowers stalked orange, crimson and purple. Fruit a drupe, black when ripen, 1- seeded. About 2 teaspoons of leaf juice is given three times a day to treat malarial fever. A decoction of root is used for influenza, cough, and mumps. Flowers are diaphoretic and are used for cough, colds, fever and jaundice (Manandhar, 2002).
- Chemical components: The oils of flowers contain β-caryophyllene, limonene, piperretone.
- Uses: About 2 teaspoons of leaf juice is given three times a day to treat malarial fever. A decoction of root is used for influenza, cough, and mumps. Flowers are diaphoretic and are used for cough, colds, fever and jaundice (Manandhar, 2002).

1.5 Objectives of the study

I. To identify the leaf spot of cauliflower

II. To test the inhibitory effect of the extracts of five aromatic plants at different concentrations against *Curvularia lunata*.

III. To asses the MIC (Minimum inhibitory concentration) value of the plant extracts used.

IV. To compare the efficacies of the plant extracts.

1.6 Significance of the study

Cauliflower is the important vegetable and is rich in different types of nutrients which are listed above. Cauliflower is low in fat, high in dietary fiber, folate, water and vitamin C, possessing a very high nutritional density.

As a member of the Cruciferae family, cauliflower shares several phytochemicals similarities with broccoli and cabbage which are beneficial to human health, including sulfurophane, an anti-cancer compound released when cauliflower is chopped or chewed Source: (USDA Nutrient database, 2005).The compound indole-3-carbinol, which appears to work as an anti-estrogen, appears to slow or prevent the growth of tumors of the breast and prostate. Cauliflower also contains other glucosinolates besides sulfurophane, substances which may improve the liver's ability to detoxify carcinogenic substances. A high intake of cauliflower has been found to reduce the risk of aggressive prostate cancer.

Above nutrients data shows that cauliflower is one of the most important and income generating vegetables for the farmers of developing countries like Nepal. Different diseases of the crops cause considerable loss in productivity and income. Therefore, the control of the plant diseases should be emphasized.

The control of the plant diseases with the different sorts of chemicals cause harmful effects in human health and it may also lead to pollution. These harmful chemicals can be overcome by the use of the plant extracts. There are many benefits of plant extracts over commercially used harmful chemicals. The extracts are free of pollution and are not or less harmful in comparison to the chemicals (Costa *et al*., 2000).

Since Nepal is an agricultural country, the use of the plant extracts in the control of the agricultural products may be beneficial. The control of the plant diseases by plant extracts treatment has been practiced in Nepal.

1.7 Limitations of the study

Due to time and economic factors, the study suffered from following limitations.

- The study was carried out only for 12 months under limited laboratory resources of Central Department of Botany.
- The whole research was conducted in laboratory and field application was not trialed.
- Necessary chemical characterization of plant extracts through GC – MS or any other way (s) could not be accomplished.

CHAPTER-2

2. REVIEW OF THE LITERATURE

2.1 Works on *Curvularia lunata*

Chakraborty *et al*., (2005) tested the leaf extracts of *Azadirachta indica* was found to be effective against *Curvularia pallescens*.

Gourinath & Manoharachary, (1991) tested the antifungal activities of 20 medicinal plants against the pathogenic fungi namely *Curvularia lunata, Cylindrocarpon sp., Fusarium solani* and *Myrothecium leucotrichum*. Extracts of all the test plant species were fungitoxic and showed inhibitory effects on the test fungi.

Khan & Tripathi, (1994) screened 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 tested fungi inhibiting the mycelial growth completely.

2.2 Works on plant extracts

Adhikari, (1998) investigated *Allium sativum, Artemeisa vulgaris, Hydrocotyl asiatica and Melia azadirachta* for their antifungal activity against some filamentous and non filamentous fungi and bacteria. These plants were reported to be inhibitory for the growth of many fungal species.

Meepagala *et al*., (2003) studied antifungal activity of the steam distilled essential oil fraction of *Artemisia douglasiana* which was detected by bioautography on silica gel TLC plates against three *Colletotrichum spp*. The yield of essential oil from *Artemisia douglasiana* was about 0.6-0.8% by weight of the dry material including plant stems.

Miah *et al*., (1990) tested the efficacies of 16 plant extracts against the pathogens of five major rice diseases namely sheath blight, sheath rot, blast, bakane and leaf scald. Out of those tested species, *Sapium indicum, Tagestes erecta, Polyalthia longifolia, Luecaena leucocephala* exhibited more than 50 percent antifungal activity.

Mishra *et al*., (1990) found that Eucalyptus oil inhibited absolute mycotoxicity against *Microsporium gypsum* and *Tricho-phyton mentagrophytes* at its minimum inhibitory

concentration of 400 ppm. It showed broad mycotoxic spectrum inhibitory 5 out of 10 human as well as animal pathogenic fungi tested at higher concentration.

Parajuli *et al*., (2005) tested the fugitoxicity of essential oils of *Xanthoxyllum armatum, Nardostachs 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.

Qusem & Abublan, (1996) studied the aqueous extracts effects of 64 weed species on growth and development of *Alternaria solani, Helminthosporium sativum* and *Rhizoctonia solani*. Plant pathogenic fungi were studied in-vitro. Extracts varied in the strength and persistence of their antifungal effects against three fungal species.

Shrestha and Tiwari, (2006) tested the plant extracts of *Allium sativum, lantana camara, zingiber officinale* and *Phyllathus emblica against Fusarium solani* and found that the most of the extracts were found to be effective to control the pathogen.

Singh *et al*., (1983) screened fresh rhizomes of 125 species of *Zingiber officinale* and only few were found to be fungitoxic against the test organism *Alternaria solani* showing the broad spectrum of activity.

Taylor *et al*., (1995) while screening selected medicinal plants of Nepal for antifungal activities found that the extracts of twenty one species of plants showed activity against at least two bacterial species and twenty two showed activity against at least two fungal species and three were active against gram negative bacteria.

Tiwari *et al*., (2000) found that a commonly available lemon plant leaves extract in aqueous or ethanolic extract preparation and essential oil displayed fungitoxic effect against three destructive fungal pathogens viz. *Pyricularia grisea, Aspergillus flavus* and *A.niger*.

Upadhaya *et al*., (1987) tested leaf extracts of 30 plant species against *Aspergillus flavus*. Only the leaf extracts of *Anisomeles ovata* showed absolute toxicity. Its essential oil inhibited the growth of mycelium of *Alternaria alternata, A.solani and A. Tenuis* by 0.2% concentration.

CHAPTER - 3

3. MATERIALS AND METHODS

3.1 Materials

Different equipments, chemicals and glass-wares were used in the research works which are listed in Annex-I.

3.2 Test fungus isolation

The diseased leaf samples of the cauliflower were collected from the local market of kirtipur and the test fungus was isolated. A small part from the infected leaf was transformed aseptically on a pre-sterilized petriplate containing Potato Dextrose Agar (PDA) medium. Then it was incubated in an inverted position at 25 ± 2^0c for one week. After a week, the mycelial growth of the test fungus was observed.

3.3 PDA media

For the preparation of 500ml of PDA media

Ingredients	Quantity
Potato:	100gm
Agar:	10gm
Dextrose:	10gm
Distilled water:	500ml

The potato dextrose agar media was used in the experiment for the growth of the test fungus. Firstly, the potatoes were peeled and 100gm of the potatoes were cut into small pieces and boiled in a beaker containing 500ml water. After the potato pieces were soft to touch, the extract of the boiled potato pieces was filtered through muslin cloth in a conical flask. 10gm Agar and Dextrose were added and stirred thoroughly and final volume of 500ml was made. It was then autoclaved at 15 lb. pressure for 30 min. at 121^oC. In this way, 500ml of PDA media was made.

3.4 Test fungus Identification

The fungus which was initially supposed to be the *Alternaria* sp. later on was identified as *Curvularia* with the help of different standard texts. Further the species *C. lunata* was identified taking the help of the standard website www.doctorfungus.com.np. The symptoms were in the form of small, rounded, yellowish brown spots on the leaves. The brown spots change into black colored at the late stage.

3.5 Pathogenecity test

The diseased leaf samples were collected and the symptoms were noted. The fungus was isolated and cultured in PDA medium. The healthy leaf was inoculated with the pathogen using one week old culture. The symptoms produced in the inoculated leaf were compared with the symptoms firstly noted. The fungus was re-isolated and studied. Its characteristics were found to be similar which proves that the fungus in both the cases was *Curvularia lunata* Wakker.

3.6 Fungus culture

The pure culture of the test fungus was maintained after every week. It was done by sub culturing the test fungus in sterilized petriplates and slants containing PDA media and incubating at $25 \pm 2^{o}C$ for a week. The obtained pure cultures were used to test the antifungal effect of the test plants. During the experiment, inoculums discs of 5 mm diameter were prepared from one week old pure culture using a cork borer of same diameter. The entire inoculums disc should be of same diameter i.e. 5 mm. These discs were used in the testing of antifungal activity of the plant extracts against the test fungus.

3.7 Experiments

The antifungal effect of the test plants was tested on the test fungus using different concentrations of the plant extracts. Two gm of the required parts of each plant were firstly surface sterilized with the help of alcohol (90%) and was shredded and grinded in pre-sterilized mortar - pestle adding 8ml distilled water.

The grinded parts were filtered using clean and sterilized muslin cloth and the upper clear solution (filtrate) was separated. This volume was used to make five different concentrations *viz.* 20%, 40%, 60%, 80% and 100% in separate sterilized test tubes. All these steps were carried out inside the laminar air flow to maintain the aseptic environment. While working, the hands are disinfected using the spirit and certain precautions should be taken to avoid contaminations.

3.8 Poisoned food technique

The effect of the extracts of test plants was assessed using poisoned food technique (Grover & Moore, 1962).

A volume of 0.5ml of each concentration was aseptically poured into three pre-sterilized petriplates and 9.5ml of melted PDA was poured. The petriplates were swirled gently to allow thorough mixing of the contents.

In the control set, no extract was used. After solidification of the media, one inoculum disc of 5mm in diameter was aseptically inoculated upside down at the centre of each petriplate which was then incubated at 25 ± 2^{o}c for 7 days. All the experiments were carried thrice and the mean value was taken. The diameters of the fungal colonies were measured on the 7^{th} day of incubation.

3.9 Mycelial growth inhibition

According to Rao & Srivastava, 1994, the antifungal effect of the different concentrations of different plant extract was calculated in terms of percentage of mycelial growth inhibition

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

Where, g_c = growth of mycelial colony after incubation period in control set.

g_t = growth of mycelial colony after incubation period in treatment set.

3.10 Conidial measurement

Ocular micrometer was placed inside the eyepiece of a compound microscope and was calibrated by superimposing the graduations of stage micrometer. The number of divisions of ocular micrometer coinciding with the number of divisions in stage micrometer was noted down and the calibration factor was calculated by the help of the formula.

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

Then after, the stage micrometer was replaced by a fine slide of the *Curvularia lunata*. Size of the conidia was found to be (20-32x9-15) μ m. and it was calculated by determining the number of division of ocular micrometer which is listed in Annex-II.

3.11 Statistical test

The statistical test was applied wherever necessary. Correlation analysis was done using SPSS programme (Windows version 11.5) for calculation of correlation factors.

3.12 Photographs

The photographs of all the test plants, test fungus and the results of the antifungal activity of those plants on the 7^{th} day of incubation were taken. Some important ones are given at the Photoplates – I and II.

CHAPTER- 4

4. RESULTS

The colony size of the test fungus at different concentrations of plant extracts was measured on the 7th day of incubation and the inhibitory effects of the test plants were calculated. The measurement of the diameter of the control set and the treatment sets was done by using the ruler and finally the inhibition percentages were calculated. For each concentration, the diameters of triplicates were measured and the mean is calculated. The inhibition percentages were finally calculated in reference to the colony diameter in control set. The number of concentrations in each experimental set is six (ie. n=6).Following are the tables for results of the experiment.

Table -1: Colony size of test fungus on7th. day of incubation at varying concentration of *Lantana camara.*

S.N.	Inoculum size (mm)	Conc.n of extract (%)	Colony size (mm)			Mean colony size (mm)	Inhibition of mycelial growth (%)
			I	II	III		
1	5	0	59	59	62	60	---
2	5	20	28	34	31	31	48.3
3	5	40	25	28	25	26	56.65
4	5	60	20	15	16	17	71.65
5	5	80	11	9	13	11	81.65
6	5	100	11	8	8	9	85

Table -2: Colony size of test fungus on7$^{th.}$ day of incubation at varying concentration of *Azadirachta indica*

S.N.	Inoculum size (mm)	Conc.^{n} of extract (%)	Colony size (mm)			Mean colony size (mm)	Inhibition of mycelial growth (%)
			I	II	III		
1	5	0	59	59	62	60	---
2	5	20	43	40	40	41	31.66
3	5	40	30	31	31	31	48.33
4	5	60	28	28.5	27.5	28	53.33
5	5	80	20	23	26	23	61.66
6	5	100	8	8	8	8	86.66

Table -3: Colony size of test fungus on7$^{th.}$ day of incubation at varying concentration of *Allium sativum*

S.N.	Inoculum size (mm)	Conc.^{n} of extract (%)	Colony size (mm)			Mean colony size (mm)	Inhibition of mycelial growth (%)
			I	II	III		
1	5	0	59	59.5	61.5	60	---
2	5	20	35	37	33	35	25
3	5	40	30	31	35	32	46.5
4	5	60	28	28.5	27.5	28	53.33
5	5	80	22	23	21	22	63.3
6	5	100	22	19	16	19	68.3

Table - 4: Colony size of test fungus on7$^{th.}$ day of incubation at varying concentration of *Ocimum sanctum*

S.N.	Inoculum size (mm)	Conc.^{n} of extract (%)	Colony size (mm)			Mean colony size (mm)	Inhibition of mycelial growth (%)
			I	II	III		
1	5	0	59	59.5	61.5	60	---
2	5	20	45	41	43	43	28.33
3	5	40	36	38	34	36	40
4	5	60	26	24	25	25	58.33
5	5	80	8	7	9	8	86.66
6	5	100	6	6	6	6	90

Table - 5: Colony size of test fungus on7$^{th.}$ day of incubation at varying concentration of *Tagetus patula*

S.N.	Inoculum size (mm)	Conc.^{n} of extract (%)	Colony size (mm)			Mean colony size (mm)	Inhibition of mycelial growth (%)
			I	II	III		
1	5	0	58	59	63	60	---
2	5	20	33	31	32	32	46.5
3	5	40	28	34	31	31	48.3
4	5	60	28	25	22	25	58.3
5	5	80	23	23	23	23	61.65
6	5	100	20	25	18	21	65

The effect of these plant extracts on mean colony diameter of the test fungus reported from the experiment was plotted on the graph given in figure- 1. The graph clearly shows the comparative efeect of all plant extracts on the mycelial growth of test fungus.

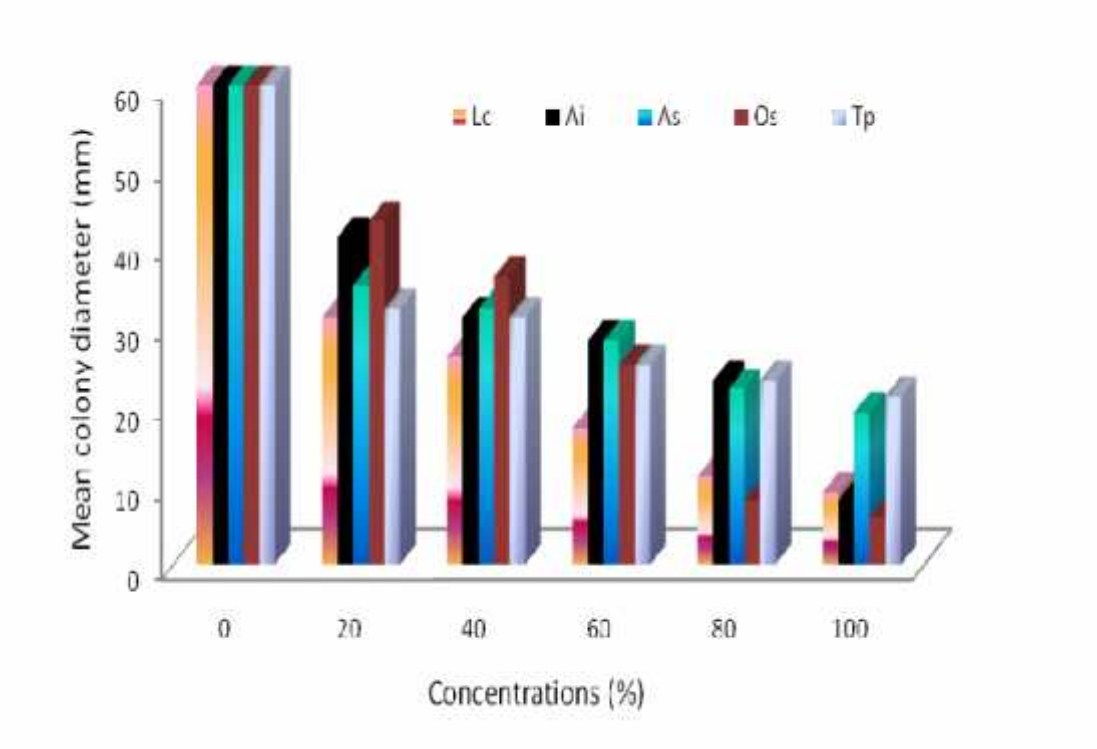

Fig.- 1: The comparative graph for the effect of all plant extracts at their varying concentrations on mean colony diameter of test fungus.

The extract of *Ocimum sanctum* was found most effective in the control of *Curvularia lunata*. The inhibition on the mycelial growth of the test fungus on 100% extract from *Ocimum sanctum* was reported up to 90% which clearly indicates its high degree of effectiveness in controlling the fungus in *in vitro* condition.

The extract of *Azadirachta indica* is also the effective one which inhibited the fungus growth by 86.66% at its 100% concentration (Fig-2). The hyphae of the fungus were found to be destroyed by the extract used after second week of incubation which also prove its long term effect over the mycelial growth of the test fungus.

The extract of *Lantana camara* was also found to be best in controlling the pathogen. Inhibition percentage was found reaching to 85.4 at its 100% concentration (Fig-2).

At this concentration also, the mycelial growth was arrested completely and lead to the destruction of the test fungus at the periphery of the small colony.

The extracts of *Allium sativum* and *Tagetus patula* were less effective in the control of the fungus in comparison to others which gave the inhibition effect percentage by 68.3 and 65 respectively (Fig-2).

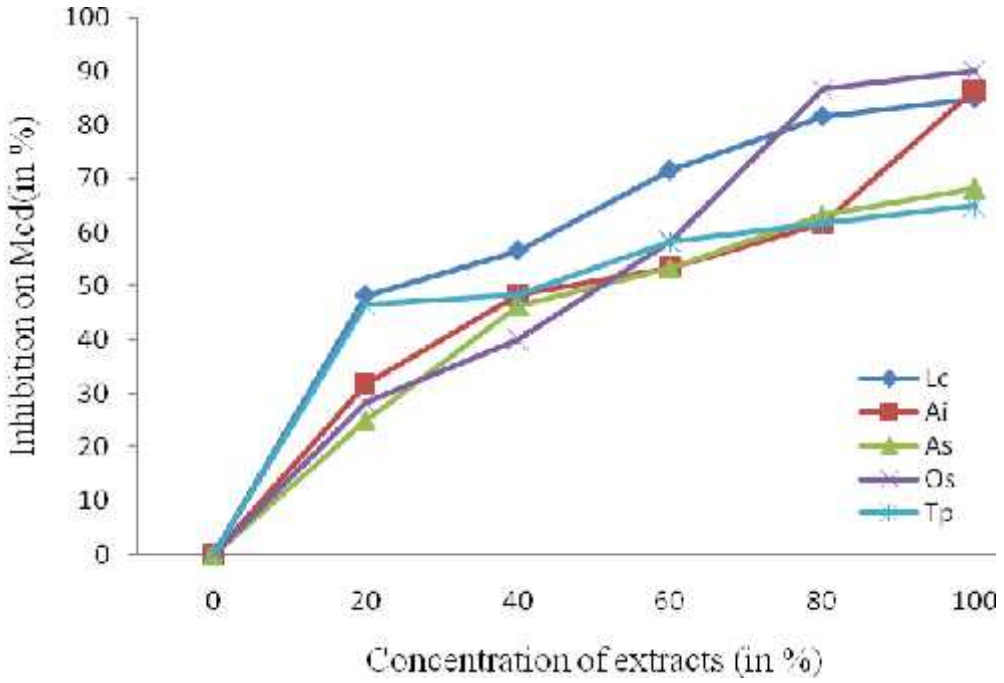

Fig.- 2: The comparative graph for inhibitory action of all plant extracts at their varying concentrations on the mean colony diameter of test fungus.

The correlation analysis also revealed the negative effects of these plant extracts on the mycelial growth of the test fungus. All the correlation factors for those plant extracts against mean colony diameter of the test fungus were found negative with 1% level of significance in case of *Lantana camara, Azadirachta indica* and *Ocimum sanctum.* Furthermore, the values for the same were found significant at 5% level in case of *Allium sativum* and *Tagetus patula.* The table for the respective correlation factors of all test materials is given below.

Table- 6: Correlation factors of different plant extracts against mean colony diameter of test fungus

Test plants	Correlation factor	Significance level
Ocimum sanctum	- 0.986**	1%
Azadirachta indica	- 0.966**	1%
Lantana camara	- 0.919**	1%
Allium sativum	- 0.904*	5%
Tagetus patula	- 0.847*	5%

The calculations were done by using SPSS 11.5 Windows Version.

The table above shows higher negative correlation factor for *Ocimum sanctum* (-0.986) which clearly indicates its highest degree of negative impact on the mean colony diameter of the test fungus *i.e.* highest inhibition in mycelial growth of the test fungus than others. Similarly, the least effective plant on the basis of same correlation analysis was found to be *Tagetus patula* (C*f* = -0.847) laying others in between them.

Thus, the order of the plant extracts on the basis of their effectiveness in arresting the mycelial growth of test fungus was found as:

$$Os > > Ai \geq Lc >> As > Tp.$$

CHAPTER- 5

5. DISCUSSION

Plant extracts containing some percentage of essential oils are considered as antibacterial, antifungal, insecticides and inhibitory agent to the biological systems. Also these extracts are popularly known to have antifungal property which might be due to the presence of some characteristic compounds of the group mono and di-terpenes, sesquiterpenes etc. Therefore, different plants may have different chemical components and that may account to produce different results. In this experiment also different plant extracts showed varying degree of inhibition at varying concentrations.

In this experiment, the extract of *Ocimum sanctum* was the most effective in the control of the fungus. The reason for its high degree of inhibitory effect on the mycelial growth of the test fungus might be due to the presence of higher concentration of active inhibitory chemicals on the plant extract that could sufficiently suppress the physiological development of the fungus. The similar finding by Lemos *et al*. (2005) who tested the antifungal activity from *Ocimum sanctum* towards *Cryptococcus neoformans* and found high degree of inhibitory action of the plant over the fungus also supports the present finding of this study.

Likewise, the extract of *Azadirachta indica* was also found to be effective in the control of *Curvularia lunata.* The result is found to be corresponding to the work of Zhang *et al*. (1999). They had analysed the antimicrobial activity of oil of *Azadirachta indica* and found that the oil was effective against standard test cultures of *E.coli* (Gram negative) and *Stephylococcus aureus* (Gram positive).

Lantana camara shows the inhibition percentage by 85% which show its effectiveness in the inhibition of the growth of fungus. This finding matches with the investigation of Saxena and Sharma (2000). They studied the antimicrobial activity of the essential oil of *Lantana camara* against some fungi and found that the oil was effective at 1000 ppm. Presence of some active inhibitory chemicals like β-caryophyllene, limonene, methyleugenol, piperetone. on their extracts might be the cause of this fruitful finding.

Shrestha and Tiwari (2006) tested the antifungal activity of *Lantana camara* and *Allium sativum* against *Fusarium solani* (Mart.) Sacc. And they found that the extract of *L. camara* was found to be less effective in the control of the test pathogen while the extract of *A. sativum* was most effective. But in this study the extract of *A. sativum* was found to be less effective to check the colony growth of *Curvularia lunata* in comparision to *Lantana camara.* The difference in the variety of *Allium sativum* used in the experiment might be one of the causes of such antagonistic results. Furthermore, the environmental influence during the growth and development of the test plant species also accounts for such divergence in the results since the concentration of concerned effective chemicals on those materials varies as according to the environmental change. Besides, some sort of experimental difficulties, time of incubation, incubation temperatures etc. also could play a key role in the mycelia growth of the test fungus.

The least effectiveness of the extracts of *Tagetus patula* might be due to the low concentration of inhibitory chemicals like methyleugenol, piperetone on its plant parts.

Possible roles of these extracts used in the experiment in regulating hydrolytic enzymes *de novo* synthesized in growing fungus, can not be overruled. Works in the line of further chemical characterization of these extracts and their function in growth and development of fungi are speculated to justify the finding of the present investigation.

CHAPER- 6

6. CONCLUSION

The experimental results show that most of the extracts of the test plants were effective in the disease control. Among them, the most effective plant species is *Ocimum sanctum* followed by *Azadirachta indica* and *Lantana camara*.

The other two plant species such as *Tagatus patula* and *Allium sativum* were not so effective in the disease control in comparison to others.

The extracts of the effective plants in this experiment can be used in the control of the disease as the plant extracts are less poisonous in comparison to the commercially applied chemicals which are toxic to plant parts as well as animals.

The unscientific use of the chemical fungicides and pesticides may lead to the pollution of the air and water sources. The farmers of Nepal are illiterate and they don't know the exact method of using the chemicals in the agricultural fields. This may finally lead to the loss of fertility power of soil. Such problems can be overcome by the use of easily available plant extracts. The use of such extracts may also lead to decrease the economic burden of the poor farmers.

CHAPTER- 7

7. RECOMMENDATIONS

The study on *Curvularia lunata* is lacking in the plant pathology unit of Central Department of Botany. So, it is requested to the upcoming students of the plant pathology unit for further extensive study on this pathogen.

The entire experiments were inhabited within the lab. of Central Department of Botany with controlled conditions of light and temperature etc. So the result may or may not hold true when applied in the field. So, the study is recommended for the field experiment.

Gas chromatography and Mass spectroscopy can be carried out for chemical characterizations of used extracts.

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ANNEX- I

Equipments used in Experiment

Hot air oven, Laminar air flow, Incubator, Autoclave, Spirit lamp, Microscope, Slides, Cover slips, Needles, Inoculating loop, Camera, Forceps, Ocular micrometer, Stage micrometer etc.

Chemicals used

90% alcohol, Agar, Dextrose, Lacto-phenol, Cotton blue, Potato, Distilled water etc.

Glasswares Used

Test tubes, Pipette, Funnel, Culture tube, Conical flasks, Glass rod, Petridishes etc.

ANNEX- II

PDA MEDIA

Ingredients:

Potato:	100gm
Agar:	10gm
Dextrose:	10gm
Distilled water:	500ml

Process

Firstly, the potatoes were peeled and 100gm of the potatoes were cut into small pieces and boiled in a beaker containing 500ml water. After the potato pieces were soft to touch, the extract of the boiled potato pieces was filtered through muslin cloth in a conical flask. 10gm Agar and Dextrose were added and stirred thoroughly and final volume of 500ml was made. It was then autoclaved at 15 lb. pressure for 30 min. at 121^{o}C. In this way, 500ml of PDA media was made. This PDA media was used in the experiment.

ANNEX-III

Micrometry

In micrometer, 100 div. = 1000µm

1 div. = 1000/100µm=10µm

64 div. of ocular micrometer coincided with 13 div. of stage micrometer.

1 div. of ocular micrometer coincided with 13/64 div. of stage micrometer

=13/64x10 µ

= 2.03 µm

S.N	Length (µm)	Width (µm)
1	22	12
2	20	15
3	28	9
4	26	10
5	32	12
6	30	15
7	25	12
8	23	9

The size of conidia ranges from (20-32x9-15) µm.