CHAPTER I

1. INTRODUCTION

Most of the plants possess one or more of the chemical properties such as antimicrobial, antifungal, antiviral, antihelminthic, anticancer, sedative, laxative, cardio tonic and diuretic (Parajuli et al., 1998). Different parts of plants can be successfully used for controlling various insect pests. The main group of active component in plants is alkaloids, glycosides, saponins, tannin, essential oils, etc. These active components of plants which inhibit or kill the microorganisms are called antimicrobials. These antimicrobials are extractable with different kinds of solvents (Kruger, 1992).

Potato is one of the world's most nutritious crops and can meet the food needs of people in a substantial manner (Chadha and Grewal, 1993). Potato is a wholesome food. It is nutritionally superior to most of the crops in providing high quality protein per unit area and time by providing most of the essential amino acids needed in requisite amount. Hence, the crop has immense potential for providing the nutritional food to the malnourished population (Ross, 1986). The crop is worldwide in distribution. Now it is one of the most important tuber crops in Nepal (NPRP, 2005/06).

The number of diseases affecting the potato plant is indeed large. Individually or collectively they take a heavy toll of the crop every year. The symptoms caused by different diseases vary markedly. Some hamper germination, others kill the foliage and still others cause heavy rottage of tubers in the soil or in storage. Many organisms create difficulties during more than one phase of the plant's life cycle (Pushkarnath, 1976).

To meet the requirements of food for ever increasing populations, most of the world's food crops are being produced using agrochemicals. However, the development of sustainable strategies for crop protection does not rely only on the use of broad-spectrum pesticides (Kerry, 1995). In 1990, the total world pesticide market was estimated as US $24.7 billions, of which the United States shared 21.9%, Western Europe 26.7%, Eastern Europe 7.7%, South and South East Asia 26.7%, Africa and

West Asia 5.7% and Latin America 2.8% (Raheja, 1995). During 2000, the world pesticide trade was worth US$22 billions of which the USA share's was US$1.97 billion and Nepal accounted the import of pesticides worth value of US$778 thousands (FAO, 2002). The continuous use of pesticides has resulted in highly resistant target pests, unintentional mortality of beneficial organisms, environmental pollution, human health hazards and unacceptable levels of pesticide residues in produce. Concerns about pesticide residues on fresh vegetables are growing on many countries in the Asian region (Berke, 2002). In Nepal, 10 to 20% of all pesticides are used in vegetables and 90% of fresh vegetables were reported with pesticide residues higher than the maximum safe level (Thapa, 2002).

The global concern of the negative impact of pesticides on human health, environment and the development of resistance by the pests is due to the injudicious and indiscriminate use of pesticides to increase agricultural production. It is extremely difficult to quantify losses due to disease over a large region, but some authorities estimate losses due to pathogens, weeds and insects to be about 30% of the total worldwide food production (Fry, 1982).

The 500 insect species, 200 plant pathogens and 273 nematodes have been reported to have developed resistant to pesticides (Pedigo, 2002). This may be the main reason that biological control is of increasing interest and a promising steps to solve such problems. In developing countries, land holdings are very small (<1ha), and intensive farming without crop rotation is common practice. Cheap and sustainable pest control measures are important to benefit the farmers (Brader, 1979).

The botanical pesticides are natural products and far low toxic to non target organisms. The botanical products can even be used in their crude form. Many of the potential botanical pesticidal plants are available in Nepal and many of the farmers who are still following their traditional farming systems are unaware about such plants. Such plants which have multiple uses (as fuel-wood, timber, fodder and other domestic uses) can be easily grown in the farms (Neupane, 2003).

CHAPTER II

2. OBJECTIVES

2.1 GENERAL OBJECTIVE

- To evaluate antifungal activity of plant extracts on fungal pathogens of potato plant.

2.2 SPECIFIC OBJECTIVES

- To extract crude antimicrobial compounds from selected plant materials with ethanol as solvent.
- To isolate and identify the pathogens causing fungal diseases of potato plant.
- To evaluate antifungal activity of crude extracts against the isolated fungi.
- To determine the MIC and MFC of crude extracts on the isolated fungi.

CHAPTER III

3. LITERATURE REVIEW

3.1 POTATO (*Solanum tuberosum* L.)

3.1.1 Origin and distribution

Potato (*Solanum tuberosum* L.) is a crop of New World Origin. The available archaeological, historical and linguistic evidences reveal that the crop originated in the Peru- Bolivian region of South America and it diffused rapidly throughout the high Andes of Peru and Bolivia after its domestication. The crop was already under cultivation at the time of the discovery of South America (Hawkes, 1992). In Nepal, potato was started cultivating from the end of the 18th century (Khairgoli, 2037).

3.1.2 Biosystematics

Potato is an herbaceous plant. All cultivated potatoes belong to the genus Solanum of the family Solanaceae. The genus Solanum consists of more than 1000 species out of which about 230 are wild potatoes, 7 are cultivated potatoes and the remaining are non tuberiferous species, spiny herbs and shrubs (Hawkes, 1992).

3.1.3 Importance of the potato

Potato is one of the most important food crops in the world. The crop is worldwide in distribution and ranks the fourth position in world food production after rice, maize and wheat. The per unit production of potato (16.4t/ha) is 4 to 6 times higher than those of wheat (2.7 t/ha), rice (3.9 t/ha) and maize (4.3t/ha). Such high productivity of potatoes clearly shows a significant contribution of potato in food supply and its potential to fulfill the food requirement of the ever-growing world population (FAO, 2001).

The potato is a good source of dietary energy and some micronutrients, and its protein content is very high in comparison with other roots and tubers. Boiling potatoes in their skins prevents loss of nutrients.

Table 1. Nutrient content of potatoes

SN	Constituents in Potato	Amounts
1	Carbohydrates	20.13 gm
2	Fibre	1.8 gm
3	Fat	0.1 gm
4	Protein	1.87 gm
5	Potasium	379 mg
6	Calcium	5 mg
7	Phosphorus	44 mg
8	Iron	0.31 mg
9	Vitamin C	13 mg
10	Riboflavin	0.02 mg
11	Thiamin	0.106 mg
12	Niacin	1.44 mg

(Per 100 g, after boiling in skin and peeling before consumption) Source: United States Department of Agriculture, National Nutrient Database

Potato occupies the fifth position in area coverage and second in total production and first in productivity among the food crops grown in Nepal. The area under potato is about 150864 ha and total production 1974755 Mt with an average productivity of 13.09t/ha (NPRP, 2005/06).

Ministry of Agriculture and Cooperative considered potato as one of the important cash crop of Nepal. However, potato serves as staple food in the high hills and plays a vital role in the food security in the country. Therefore potato could be compared with other cereal crops. Out of the total area under potato production, 18% is in the high hills and mountains, 42% in the mid-hills and 40% in terai (ABPS, 2005).

The great agro-ecological diversity of Nepal allows for potato cultivation to occur somewhere at any times of year. The generalized cropping calendar is summarized as

Table2. Cropping calendar by altitudinal zones in Nepal

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

Source: Dhital, 2000

3.1.4 Major constraints for potato production

Various biotic and abiotic factors are responsible for a low production and productivity of potato in the world. Since the crop is grown from the tropics to temperate zones, the production constraints may be different for different environment and their relative importance may be different. In the tropical highlands, potato production is limited by frost incidence, moisture stress, physiological ageing of seed potatoes and the associated pests and diseases (Midmore, 1992).

Major potato production constraints in Nepal are the low yield potential of local varieties, losses due to pests, traditional cultivation practices, use of poor quality seed, limited area under cultivation with high yielding varieties, imbalance of soil nutrients and lack of irrigation (Anonymous, 1997). Potato disease is an interaction between a host (potato), pathogen (bacteria, fungi, virus, mycoplasma and nematode) and the environment that impairs productivity or usefulness of the crop. Diseases such as late blight, bacterial wilt, wart and viruses have been identified as major biotic constraints to potato production in Nepal (NPRP, 2005/06).

Table 3. The diseases of potato are listed below:

SN	Disease	Pathogen
	Fungal Diseases	
1	Late blight	*Phytophthora infestans*
2	Early blight	*Alternaria solani*
3	Black scurf	*Rhizoctonia solani*
4	Charcoal rot	*Rhizoctonia bataticola*
5	Leaf blotch	*Cercospora concors*
6	Wart	*Synchytrium endobioticum*
7	Powdery scab	*Spongospora subternanea*
8	Rot	*Sclerotium rolfsii*
9	Wilt	*Fusarium oxysporum*
	Bacterial Diseases and Actinomycetes	
10	Common scab	*Streptomyces scabies*
11	Bacterial wilt	*Pseudomonas solanacearum*
12	Bacterial soft rot	*Ralstonia solanacearum*
13	Black leg	*Erwinia caraoivora*
	Mycoplasmal Disease	
14	Purple top poll	Mycoplasma (PTR)
15	Marginal flavescence	Mycoplasma (MF)
16	Witch's broom	Mycoplasma (WB)
17	Potato phyllody	Mycoplasma (PP)
18	Purple top wilt	Mycoplasma (PTW)
19	Potato stolbur	Mycoplasma (Stolbur)
	Viral Diseases	
20	Faint mosaic (latent)	Potato virus X (PVX)
		Potato virus S (PVS)
21	Mild mosaic	Potato virus A(PVA)
		Potato virus M (PVM)
22	Severe mosaic	Potato virus Y (PVY)
		Acuba mosaic
		Calico mosaic
		Crinkle and rugose
23	Leaf roll	Potato virus leaf roll (PVLR)

Source: Pandey, 2002

3.2 LATE BLIGHT OF POTATO

3.2.1 Origin, distribution and economic importance

Late blight caused by *Phytophthora infestans*, is the most important biotic constraints of potato production in the world (Hooker, 1981). Central Mexico is believed to be the center of origin of the *P. infestans*- Solanaceae pathosystem (Niederhauser, 1991). The disease is endemic in many potato and tomato growing regions and reaches epidemic and pandemic levels under favorable environmental conditions for the pathogen. The epidemics of potato late blight during 1845-51 in Ireland resulted in the death of millions of people and nearly 2 million were forced to migrate to different parts of the world (Agrios, 2008). Before and after this catastrophic blight epidemic there have been many other incidences, but none of them reached this level of devastation mainly due to the diversification of food crops grown in those areas. Though extensive efforts are directed at the control of late blight disease it is still causing serious crop losses. One of the best example is the over $200 million loss from late blight disease in potatoes and tomatoes in 1994 in the USA (Fry and Goodwin, 1995). In Nepal the yield loss caused by this disease ranges from 50- 90% annually in the epidemic years (NPRP, 2005/06).

3.2.2 Symptoms

The disease attacks the crop at any stage of crop development. It usually starts from the foliage preferably from the leaf tip and leaf margins. Initial infection is expressed as a chlorotic area in the leaf, followed by water soaked lesion and finally as dark brown to black necrotic patches. The disease subsequently appears in other parts of the leaf, petiole and stem. Under cool and moist environmental conditions a white mass of sporangia can be seen on the lower surface of the infected leaves. Favorable environmental conditions lead to a rapid build up of the disease and a complete damage of the crop in 2-3 days. The infection of leaves and other aerial parts is usually followed by tuber infection. Generally the tuber becomes infected in the later part of the season when rainwater washes the sporangia from foliage to the tubers and/or the harvested tubers come in contact with the diseased foliage. Infected tubers show slightly brown or

purple blotches on the skin. Under moist soil conditions the progression of disease takes place rapidly and the tubers rot before or after harvest. In drier conditions the discoloration of the tuber occurs very slowly and they may serve as the primary inoculum for the next crop (Erwin and Ribeiro, 1996)

3.2.3 Pathogen Biology

P. infestans is a member of oomycetes and is also called water mold fungus because it produces spores and causes infection only when free water is present on the plants. The mycelium is white and coenocytic and the nuclei are diploid. (Alexopoulus and Mims, 1979).

3.2.4 Biosystematics

P. infestans is one of the most important members of the family Pythiaceae in the class Oomycetes. The organism in this group are characterized by the absence of chitin in the cell walls (true fungi contain chitin), presence of zoospores with heterokont flagella borne in sporangia, diploid nuclei in vegetative cells and sexual reproduction via antheridia and oogonia. *P. infestans* in endophytic, consisting of hyaline, much branched, coenocytic mycelium (Agrios, 2008). Mycelium grows intercellularlly with single or double club shaped haustoria and haustoroid hyphae. Sporangiophores are compound, sympodial with a small characteristic swelling just below the sporangium. Sporangiophores are slender, hyaline, branched and indeterminate. Sporangia are ovoid, ellipsoid to limoniform tapering at the base, caduceus and semi papillate. Sporangia are multi nucleate (7-30 nuclei) with an average size ranging from 36 ⎪22⇑m to 29 ⎪19⇑m. Sporangia germinate indirectly, producing zoospores under low temperature and directly producing germ tubes under high temperature (Waterhouse, 1983).

3.2.5 Asexual reproduction

Under favourable weather conditions, *P. infestans* produces mycelium, which is the vegetative growth of the fungus. The mycelium then produces sporangiophores with sporangia. The sporangiophores are indeterminate (continue to grow and produce sporangia). Sporangia may be blown to neighboring fields, but do not survive long

distance dispersion due to exposure to solar radiation. In warmer conditions, sporangia may germinate directly by the formation of germ tubes. In the cool and wet conditions, the sporangia may produce a number of zoospores, which are small and biflagellate. These zoospores may swim for sometime in the free water on the surface of plants and start infection on the plants (Andrivon, 1995).

3.2.6 Sexual reproduction

P. infestans is heterothallic which recognized A1 and A2 mating types. Two different mating types should be present adjacently for sexual reproduction. The male and female organs are called antheridium and oogonium (gametangia) respectively. The female hypha grows through the young antheridium and develops into a globose oogonium above the anteridium. The fertilized oogonium develops into a thick walled and hardy oospore. The oospore germinates by producing a germ tube and the germ tube often produces sporangia. The rate of soil borne oospores as a source of primary inoculum in the field is still controversial. However, the role of oospores in the infected tuber as a source of primary inoculum has been reported (Andrivon, 1995).

3.2.7 Life cycle

P. infestans mycelium over-winters in the infected potato tubers. The over wintered mycelium in the tubers spreads with in the tissues of the tuber and finally reaches the shoots produced from these infected tubers. The mycelium spreads upwards through the stem most rapidly in the cortical region and later on it grows mostly between the pith cells of the stem. Once the mycelium reaches the aerial part of the plant it produces sporangiophores. The sporangiophores produce sporangia, which are detached and drift off or are distressed by rain and wind. Sporangia coming in contact with a potato or tomato leaf or stem germinate and penetrate the cuticle or enter through the stomata under favorable environmental conditions. The fungus grows inter- cellularly producing curled haustoria. The invaded cells die and decay and the fungal mycelium spreads peripherally into fresh tissues. After a few days of infection sporangiophores emerge from the stomata and produce numerous sporangia (about 300000 per lesion). Under

favorable weather conditions the period from infection to sporangia formation may be as short as four days. Tuber infection in the field occurs under wet weather when sporangia produced on aerial parts of plant are washed down into the soil. The emerged zoospores attack the tubers through lenticels or wounds (Rowe et al., 2002).

3.2.8 Epidemiology

Temperature and relative humidity play important roles in the development of late blight. Relative humidity of above 90% and temperature of 12-24⌈C favour the development of the disease. The pathogen can sporulate from 4-6 days old lesions when the weather is favorable (leaf wetness for 10-12 hours and temperature of 15-18⌈C) (Rowe et al., 2002). Growth and reproduction of the pathogen is favored by moderate temperature (15-25⌈C) and wet conditions. The pathogen may develop in very warm dry temperature (35⌈C) if conditions are extremely wet and night temperature is moderate (15-25⌈C) (Johnes et al., 1991).

Epidemics can be rapid and devastating because of the high reproductive potential of this pathogen. The individual lesions can produce 100000 to 300000 sporangia per day. Thus, rapid reproduction of the pathogen and its destructive nature can defoliate potatoes and completely destroy healthy fields in a short time. The blight cycle may complete itself in less than 5 days. However, it will be late to manage the disease by this time (Fry, 2002).

3.2.9 Disease diagnosis

Late blight is generally identified by visual symptoms in the leaf, stem or fruit. The characteristic diagnostic sign is cottony growth of the fungus underneath infected lesions of leaves that are visible. This symptom can be confirmed by microscope. Detection of latent infections can easily be done with molecular diagnostic technique that helps in the control of the reemerging late blight disease (Martin et al., 2000)

3.2.10 Disease management measures

Integrated use of disease resistant varieties and strategic application of fungicides is normal practice of late blight disease management. Disease free seed potatoes should be used for planting and potato dumps, cull piles and self sown potatoes should be destroyed to avoid the possible source of primary inoculum. The use of protective fungicides prior to the infection has been one of the common approaches against late blight disease (Schwinn and Margot, 1991). Contact fungicides such as Bordeaux mixture, pthalimides and dithiocarbamates were in common use until the late 1970s (Rich, 1983). The development of systemic fungicides and their greater effectiveness over contact fungicides have increased the use of these systemic fungicides (such as metalaxyl) in recent days (Schwinn and Morton, 1990). Despite the effectiveness of phenylamide fungicides such as metalaxyl against late blight, the development of resistance within *P. infestans* population has been a problem. Resistance to metalaxyl has been reported from various countries in the world (Gisi and Cohen, 1996). Therefore the use of mixed formulations of systemic and contact fungicides is common practice in slowing down the rate of resistance build up (Erwin and Ribeiro, 1996). The use of short duration varieties, balanced use of chemical fertilizers and crop rotations are useful in reducing late blight incidence (O Brien and Rich, 1976).

3.3 EARLY BLIGHT OF POTATO

The disease is common wherever potato is grown and under favorable conditions may cause serious damage to the crop. In those areas where both late and early blight diseases occur, it is the early blight that comes in the season earlier than the late blight. While late blight epidemics are in cooler areas, the early blight is free from inhibitions caused by weather conditions and occurs in cool as well as warm areas. The disease also occurs on tomato, chilies and related wild hosts. Among the fungal diseases, early blight is most destructive (Mehrotra and Aggarwal, 2006).

3.3.1 Origin, distribution and economic importance

The disease seems to have originated in the USA. Now the disease is worldwide in distribution (Pandey, 2003). In Nepal, the disease occurs in hilly and Terai regions. In hilly region, it usually appears along with Cercospora and late blight in June-July and in Terai it appears in November-December (Khairgoli, 2037). The primary damage caused from early blight is due to premature defoliation of the plant. Photosynthesis rates increase and respiration rates decrease in apparently healthy tissues (Visser, 1999). Although it occurs annually to some degree in most production areas, the timing of appearance and rate of disease progress help to determine the impact on the potato crop. Heavy infection early in the growing season can cause yield losses of 20 – 50% (Denner and Theron, 1999).

3.3.2 Symptoms

The disease first becomes visible as small, isolated, scattered, pale brown spots on the leaflets. These spots become covered with a deep growth of fungus. The lowest leaves are attacked first and the disease progresses upwards. In the necrotic spots, concentric rings appear on the older leaves and darkened areas on the stem. When the leaf lesions involve larger veins, chlorosis commonly extends well beyond the necrotic spots. If we study these spots with the help of hand lens, they look like 'target boards', these symptoms of the disease are called the 'target board effect'. This is due to the toxin alternaric acid produced by fungus that is translocated through the veins. In dry weather, the spots become hard and the leaves curl. In humid weather, the affected areas increase and large rotting patches may appear (Mehrotra and Aggarwal, 2006).

In severe conditions of the disease, the other parts of the plant such as petiole, stems and tubers are also affected. The skin of infected tuber becomes dark brown and the irregular or rounded spots develop on it. These spots are slightly sunken and vary in size up to 2 cm in diameter. The pulp of the tuber just below the infected skin becomes rusty or brown in appearance. Fissures may develop in mature lesions (Pandey, 2003).

3.3.3 Pathogen Biology

The causal organism for the early blight of potatoes is *Alternaria solani*. The pathogen was first described by Ellis and Martin in 1882 (Pandey, 2003). The mycelium is endophytic, branched, septate, inter and intracellular, geniculate, light brown and without haustoria. One can get the mycelium by teasing the infected leaf. The conidia are beaked, dark colored, transversely and longitudinally septate (Pandey, 1997).

3.3.4 Biosystematics

A. solani is one of the most important members of the family Dematiaceae in the class Deuteromycetes. The mycelium consists of septate, branched, light brown hyphae which become darker with age. The hyphae are at first intercellular, later penetrating into the cells of the invaded tissues. Conidiophores emerge through the stomata from the dead center of the spots. These are 50-90 |9⇑m, dark colored and borne singly. In culture, they form chains of conidia (the chains are of 2-3 conidia only); conidia are beaked, muriform, dark colored and 120-296 |12-20⇑m in size. There are both transverse and longitudinal septa in the mature conidia. *A. solani* produces a non specific toxin called as alternaric acid. The role of this acid in disease is doubtful. The causal agent of the early blight of potato usually does not sporulate in pure culture. It is demonstrated that if cultures are exposed to sunlight, sporulation is induced (Padhi and Rath, 1973).

3.3.5 Reproduction

The fungus reproduces only by asexual method. The asexual bodies are conidia, developing on the terminal ends of the conidiophores. Each conidiophore bears a single conidium at its terminal end. The conidiophores are aerial and septate. Each conidium is elongated and 5-10 times septate. The septation takes place in both transverse and longitudinal directions. The apex of the conidium is slender, elongated and beak like. Sometimes the germ tubes may also be seen coming out from the cells of the conidium under microscope (Pandey, 1997).

Sporulation is possible over a wide range of temperature; it develops freely and rapidly at about 24°C. High humidity generally favours sporulation (Pushkarnath, 1976). The optimum temperature for germination of conidia is 28-30°c. Heavy dews with frequent rains are essential for abundant production of conidia. In moist weather conidia germinate readily (Pandey, 2003).

3.3.6 Life cycle

The pathogen is mainly soil-borne. The primary infection may be through tubers. The mycelium of the fungus remains dormant in dry infected leaves for a year or more. Conidia have been found to retain viability for 17 months at room temperature. Mycelium and conidia thus survive in the soil in diseased plant debris to cause primary infection in the next year's crop. Collateral hosts such as tomato play an important role in the perpetuation and dissemination of the pathogen. The climate and soil exert a considerable influence on the development of the disease. It becomes serious when the season begins with abundant moisture followed by low temperature. Hence the disease is readily perpetuated from season to season (Mehrotra and Aggarwal, 2006).

Many cycles of early blight spore production and lesion formation occur within a single growing season once primary infection is initiated. Secondary spread of the pathogen begins when spores are produced on foliar lesions and carried to neighboring leaves and plants. Early blight is largely a disease of older plant tissues and is more prevalent on senescing tissues on plants that have been subjected to stresses induced by injury, poor nutrition, insect damage or other types of stress. Early in the growing season the disease develops first on fully expanded leaves near the soil surface and progresses slowly on juvenile tissues near the growing point. The rate of disease spread increases after flowering and can be quite rapid later in the season during the bulking period and during periods of plant stress (Mehrotra and Aggarwal, 2006).

In potato tubers, germinated spores penetrate the tuber epidermis through lenticels and mechanical injuries to the skin. Tuber often becomes contaminated with *A. solani* spores during harvest. In storage individual lesions may continue to develop but

secondary spread does not occur. Early blight lesions on tubers, unlike late blight lesions, are usually not sites of secondary infection by other decay organisms (Mehrotra and Aggarwal, 2006).

3.3.7 Epidemiology

The disease occurs over a wide range of climatic conditions and depends in a large part on the frequency of foliage wetting from rainfall, fog, dew or irrigation on the nutritional status of foliage and cultivar susceptibility. The incipient infection foci develop rather rapidly within 2-3 days, if the prevalent temperature ranges between 21 and 24°c. The disease becomes serious when the season begins with abundant moisture followed by high temperature. Overwintering spores and mycelia of *A. solani* can withstand a wide range of environmental conditions including exposure to sunlight and repeated cycles of drying, freezing and thawing. Alternating wet and dry periods with temperatures in this range favor spore production. Few spores are produced on plant tissues that are continuously wet or dry (Pushkarnath, 1976).

3.3.8 Disease diagnosis

Early blight is generally identified by visual symptoms in the leaf, stem or fruit. The diagnostic sign is small, isolated, scattered and concentric pale brown spots on the infected lesions of leaves that are usually covered with a deep growth of fungus. The lowest leaves are attacked first and the disease progress upwards. Early in the growing season, lesions on young, fully expanded succulent leaves may be large upto half an inch in diameter and may, due to their size, be confused with late blight lesions. Leaf lesions are relatively easy to identify in the field because lesion development is characterized by a series of dark concentric rings. Elongated superficial brown or black lesions may also form on stems and petioles. These symptoms can be confirmed by microscope (Mehrotra and Aggarwal, 2006).

3.3.9 Disease management measures

Integrated use of disease resistant varieties and strategic application of fungicides is normal practice of early blight disease management. The disease is controlled primarily

through the use of cultural practices, resistant cultivars and foliar fungicides (Pushkarnath, 1976).

Since the disease is soil borne, crop rotation is helpful. Dead haulms should be raked together and burnt. Copper oxychloride sprays have been recommended for the control of early blight of potato (Mehrotra and Aggarwal, 2006). Fungicidal sprays, preferably with copper fungicides or Zineb given at 15 day interval effectively control the disease. Since the same spray schedule controls late blight also, it has become a regular practice among potato growers in many tracts to spray the crop with copper fungicides at least 3 or 4 times, starting from about 6 weeks after planting (Rangaswami and Mahadevan, 1999; Singh et al, 1989). Weekly spraying of Bordeaux mixture (5:5:50) has also been proved quite effective. The spraying is done throughout the growing period of the crop (Pandey, 2003).

A simple and cost effective method of control has been derived i.e. optimum nitrogen application at planting followed by supplemental doses through sprays at 60-70 days at crop growth (Shekhawat et al, 1999; Singh et al, 1989). Cultivars with good levels of field resistance are available, however no immunity to early blight has been found in commercial potato cultivars or in their wild parents (Pandey, 2002). Field resistance to foliage infection is associated with plant maturity. Thus late maturing cultivars are usually more resistant than early maturing cultivars and therefore, one should avoid planting early and late cultivars in the same or adjacent fields. Also the soil nutrient condition should be maintained (Khairgoli, 2037). Some of the moderately resistant varieties of potato against early blight are Kufri Naveen, Kufri Sindhuri and Kufri Jeevan (Mehrotra and Aggarwal, 2006).

3.4 FUNGAL WILT OF POTATO

This disease is caused by the wide spread soil fungus *Fusarium oxysporum*. The hosts of *F. oxysporum* include: potato, sugarcane, garden bean, cowpea, Prickly pear, cultivated zinnia, pansy, Assam rattlebox, Baby's breath, and *Musa* sp. (Raabe et al.,

1981). It can attack any part of the potato plant and produce a wilting and death of the plant with or without rotting of roots, stems or tubers (Heald, 1963).

3.4.1 Distribution and economic importance

Overall, the distribution of *F. oxysporum* is known to be cosmopolitan. However, the different special forms (f.sp.) of *F. oxysporum* often have varying degrees of distribution. Several *Fusarium* species cause wilt, root-, stem- and ear-rot, resulting in severe reductions in crop yield, often estimated at between 10% and 40% (Antonio and Giancarlo, 2002).

3.4.2 Symptoms

F. oxysporum and its various formae speciales have been characterized as causing the following symptoms: vascular wilt, yellows, corm rot, root rot, and damping-off. The most important of these is vascular wilt. Of the vascular wilt-causing Fusaria, *F. oxysporum* is the most important species (Agrios, 1988; Smith et al., 1988).

In general, fusarium wilts first appear as slight vein clearing on the outer portion of the younger leaves, followed by epinasty (downward drooping) of the older leaves. At the seedling stage, plants infected by *F. oxysporum* may wilt and die soon after symptoms appear. In older plants, vein clearing and leaf epinasty are often followed by stunting, yellowing of the lower leaves, formation of adventitious roots, wilting of leaves and young stems, defoliation, marginal necrosis of remaining leaves, and finally death of the entire plant (Agrios, 1988). Browning of the vascular tissue is strong evidence of fusarium wilt. Further, on older plants, symptoms generally become more apparent during the period between blossoming and fruit maturation (Jones et al., 1982; Smith et al., 1988).

3.4.3 Pathogen Biology

In solid media culture, such as potato dextrose agar (PDA), the different special forms of *F. oxysporum* can have varying appearances. In general, the aerial mycelium first appears white, and then may change to a variety of colors - ranging from violet to dark

purple - according to the strain (or special form) of *F. oxysporum.* If sporodochia are abundant, the culture may appear cream or orange in color (Smith et al., 1988). *F. oxysporum* is an abundant and active saprophyte in soil and organic matter, with some specific forms that are plant pathogenic (Smith et al., 1988). Its saprophytic ability enables it to survive in the soil between crop cycles in infected plant debris. The fungus can survive either as mycelium, or as any of its three different spore types (Agrios, 1988).

3.4.4 Biosystematics

Fusarium belongs to subdivision Deuteromycotina; order: Moniliales; Family: Tuberculariaceae. *F. oxysporum* produces three types of asexual spores: microconidia, macroconidia, and chlamydospores. Microconidia are oval to kidney shaped, 5-12×2.2-3.5 µm, one or two celled, and are the type of spore most abundantly and freqeuntly produced by the fungus under all conditions. It is also the type of spore most frequently produced within the vessels of infected plants. Macroconidia are three to five celled, 27-66×3-5 µm, gradually pointed and curved toward the ends. These spores are commonly found on the surface of plants killed by this pathogen as well as in sporodochialike groups. Chlamydospores are round, thick-walled spores, produced either terminally or intercalary on older mycelium or in macroconidia. These spores are either one or two celled (Agrios, 1988; Hyun et al., 2004).

3.4.5 Life cycle

Healthy plants can become infected by *F. oxysporum* if the soil in which they are growing is contaminated with the fungus. The fungus can invade a plant either with its sporangial germ tube or mycelium by invading the plant's roots. The roots can be infected directly through the root tips, through wounds in the roots, or at the formation point of lateral roots. Once inside the plant, the mycelium grows through the root cortex intercellulary. When the mycelium reaches the xylem, it invades the vessels through the xylem's pits. At this point, the mycelium remains in the vessels, where it usually advances upwards toward the stem and crown of the plant. As it grows the mycelium

branches and produces microconidia, which are carried upward within the vessel by way of the plant's sap stream. When the microconidia germinate, the mycelium can penetrate the upper wall of the xylem vessel, enabling more microconidia to be produced in the next vessel. The fungus can also advance laterally as the mycelium penetrates the adjacent xylem vessels through the xylem pits (Agrios, 1988).

Due to the growth of the fungus within the plant's vascular tissue, the plant's water supply is greatly affected. This lack of water induces the leaves' stomata to close, the leaves wilt, and the plant eventually dies. It is at this point that the fungus invades the plant's parenchymatous tissue, until it finally reaches the surface of the dead tissue, where it sporulates abundantly. The resulting spores can then be used as new inoculum for further spread of the fungus (Agrios, 1988).

3.4.6 Epidemiology

F. oxysporum is a soil borne fungus, primarily spread over short distances by irrigation water and contaminated farm equipment. The fungus can also be spread over long distances either in infected transplants or in soil. Although the fungus can sometimes infect the fruit and contaminate its seed, the spread of the fungus by way of the seed is very rare. It is also possible that the spores are spread by wind. This disease is prevalent in hot, dry seasons when plants are under water stress (Agrios, 1988).

3.4.7 Disease diagnosis

Neither the vascular discoloration in the stem nor the stem end browning of tubers is a diagnostic character of the disease, since the symptom may be absent in wilt or may develop as a result of drought and high temperatures (Heald, 1963).

3.4.8 Disease management measures

Control of the disease cannot be accomplished by cutting off the discolored stem ends of seed tubers, but such discolored tubers are objectionable for seed purposes, as they are likely to produce weak plants (Heald, 1963). In general, some effective means of controlling *F. oxysporum* include: disinfestation of the soil and planting material with

fungicidal chemicals, crop rotation with non-hosts of the fungus, or by using resistant cultivars (Jones et al., 1982; Agrios, 1988; Smith et al., 1988).

3.5 CHEMICAL FUNGICIDES AND PLANT EXTRACTS

Chemical fertilizer was introduced to Nepal in the early 1960s. However, large amount of foreign exchange are required to import it. In general, chemical fertilizers are not available in most of the hills and mountains when needed due to transportation difficulties. It is estimated that only 5% of the fertilizer imported is being used in hilly regions, which represent 64% of the total cultivated area (Ferchak and Ribeiro, 2005). The use of chemical pesticides is increasing daily. In some places, it is being used unnecessarily resulting in the negative impacts on human health. In some foodstuffs (those which are produced around city areas such as vegetables, fruits, maize, rice, wheat, etc.) the residual amount of these chemicals have been found above limits. Use of these chemicals by illiterate farmers, no actual knowledge about these chemicals to distributors and users results in the death or ill of hundreds of people annually. Similarly, there is problem for the maintenance of the unused chemicals (Dahal, 1995).

In recent years, there has been a growing movement in the world to reduce the amount of pesticides being applied to the environment. According to a study, only 1% of the total pesticide applied is effective in controlling pests, remaining 99% goes into various environmental systems (Dhaliwal and Arora, 2001). The persistent nature and accumulation of pesticides over a period of time, especially in the soil and the aquatic ecosystem, may cause health hazards to plants, animals and human beings. This may be the main reason that biological control is of increasing interest and a promising steps to solve such problems. In future, biological control may become an important component of plant disease management practices and can be an alternative to chemical control.

Some botanical plant materials were used for the control of storage pests and appeared to be most effective. Nepal is rich in natural resources of botanical origin and some of them have pesticidal properties; others have deterrent and repellent properties.

Traditionally, compost has been used in the hills but it is not sufficient for the improvement of production. Therefore, it is essential for Nepal to develop alternatives (Ferchak and Ribeiro, 2005).

More then 2000 species of plants are thought to have potential for use in natural control and 324 plant species are available in Nepal so far having some kind of pesticidal properties (Neupane, 2003). This number includes 23 (out of 42 species) as most potential botanicals for Asian farming systems (Grainge and Ahmed, 1988).

Antimicrobial properties of plant components were first documented in the late nineteenth century (Zaika, 1975). Many plants have been used as antimicrobial agents due to the secondary metabolites. These products are commonly known for the active substances, for example, the phenolic compounds which are part of the essential oils (Jansen et al., 1987) as well as tannin, alkaloids, terpenoides, flavonoids (Saxena et al., 1994). One of the factors for plants having antimicrobial activities is because different types of antimicrobial compounds play a role in plant defense, poliphenolic compounds being known to have multiple functions. Flavonoids such as naringenin, flavone and flavonol including kaemferol, morin and quercetin constitute a large group of secondary plant metabolites that have been reported to have antimicrobial activities (Rauha et al., 2000).

Table 4. Plants examined for fungicidal and pesticidal activities in Nepal

Family	**Scientific name**	**English name**	**Nepali name**
Acanthaceae	*Justicia adhotoda*	Adhotoda, Malaba	Asuro
Amaryllidaceae	*Agave Americana*	Century plant	Ketuke/ Hatibar
	Allium sativum	Garlic	Lasun
Annonaceae	*Annona reticulate*	Custard apple	Ramfal
Apiaceae	*Ferula assa-foetida*	Asafetida	Hing
Araceae	*Acorus calamus*	Sweet flag	Bojho
Asclepiadaceae	*Calotropis gigantean*	Crown plant	Aank
Asteraceae	*Ageratum conyzoides*	Ageratum	Gandhe jhar
	Artemisia vulgaris	Mug-wort	Titepati
	Chrysantherum cinerariaefolium	Pyrethrum	Paridrum
	Chrysantherum morifolium		
	Eupatorium adenophorum	Crofton weed	Banamara

Brassicaceae	*Brassica campestris*	Rapeseed	Tori
Carprifoliaceae	*Sambucus spp*	Elderberry	
Euphorbiaceae	*Ricinus communis*	Castor bean	Aandir
	Sapium insigne	Chirata	Khirro
Gentianaceae	*Swertia chirata*		Chiraito
Lamiaceae	*Mentha arvensis*	Field mint	Pudina
	Mentha spicata	Spearmint	Babari
	Mentha sylvestris		
	Ociumum tenuiflorum	Loly basil	Tulasi
	Ociumum gratissimum		
Lauraceae	*Cinnamomum sp*		
	Cinnamomum camphora	Camphor tree	Kapur
	Cinnamomum verum	Cinnamon	Dalchini
	Lindera neesiana		Siltimur
	Litsea cubeba		
	Litsea monopetala		
Meliaceae	*Azadirachta indica*	Neem	Neem
	Melia azedarach	Chinaberry	Bakaino
Moraceae	*Cannabis sativa.*	Hemp, marijuanas	Gaanja
Myrtaceae	*Eucalyptus citridora*	Eucalyptus	
	Eucalyptus sp.	Eucalyptus	
Polygonaceae	*Polygonum hydropiper*	Marshpepper	Pirejhar
Rutaceae	*Citrus limon*	lemon	Nibuwa
	Evodia fraxinifolia	Babis	
	Zanthoxylum acanthopodium	Prickly ash	Boketimur
	Zanthoxylum armatum	Prickly ash	Boketimbur
Sapindaceae	*Sapindus marginatus*	Florida soapberry	Rittho
Solanaceae	*Capsicum frutescens*	Chilli	Khorsani
	Nicotiana sp.	Tobacco	Surti
Urticaceae	*Urtica dioica*	Stinging nettle	Sisnu
Verbenaceae	*Lantana camara*	Lantana	Dhungri phool
	Vitex negundo	Indian prived	Simali

Source: Neupane (1998)

3.6 PLANTS SELECTED IN THE STUDY

3.6.1 *Brassica nigra*

The common name of the plant is black or true mustard and it is called Kaaltori or Raayo in Nepali. It is cultivated in Europe, North Africa, West Asia and Nepal. It belongs to the family Brassicaceae. It is an herb. The leaves are petioled. Flowers are

yellow colored and are in long racemes. The seeds are round, dark grey colored and 1mm in diameter.

The leaves are used as vegetable, which is laxative. Seeds are powerful stimulant, stomachic, used in neuralgic and rheumatic affections. Seed oil when applied to skin produce almost instant vesication. It is recommended to persons for use in daily diet for chronic constipation. It is also used to promote hair growth (Baral and Kurmi, 2005).

The liquid obtained by grinding the seeds and soaking in water and methanol is effective in killing *Aedes aegypti*. The plant is also effective against worms and fungi that cause disease on the other plants (Grainage and Ahmed, 1988). The biological active compound in the seeds is the allyl isothiocyanate (CSIR, 1976).

3.6.2 *Cinnamomum camphora*

The common name of the plant is camphor and it is called kapur in Nepali. It is a native plant of central China, Japan and Formosa and is widely cultivated elsewhere. It belongs to the family Lauraceae. It is an evergreen tree. The leaves are in alternate arrangement and are penni-nerved. Flowers are small.

The essential oil is sedative, anodyne, antispasmodic, diaphoretic, anthelmintic, and stimulant. It is of great value in all inflammatory affections, fevers, dyspsia, myalgia, cardiac, debility, cough, asthma, convulsion and hysterical complaints, also beneficial in gout, rheumatic pain and neuralgia and is highly valued in all irritations of the sexual organs, chronic pulmonary inflammation (Baral and Kurmi, 2005). It contains camphor, safrol, linalool, sesquiterpens, caryophyllene, cineol, etc. (CSIR, 1976). The odorous oil is obtained from the bark and leaf of the tree onto which camphor lies. Camphor contains pesticidal properties (Grainage and Ahmed, 1988).

3.6.3 *Eupatorium adenophorum*

The common name of the plant is crofton weed and it is called banmara in Nepali. It belongs to the family Compositae. It is a tall diffused and often decumbent herb. The leaves are 2-2.5cm long and 1.5-5cm broad, opposite, petioled, rhomboid ovate, base

acute, margin coarsely serrate, sparingly puberculous beneath, petioles up to 3cm long, glandular pubescent.

The whole part of the plant can be used for the application. The paste of leaves is applied in boils. Similarly the paste of buds, leaf juice is applied in cut wounds to prevent bleeding. Also the root juice is taken in fever (Baral and Kurmi, 2005).From this plant a cadinene compound is obtained (Harmatha and Nawrot, 1984).

3.6.4 *Lantana camara*

The common name of the plant is lantana weed and it is called ban phaanda in Nepali. It belongs to the family Verbenaceae. It is naturalized in many parts of Nepal as a troublesome prickly robust weed. It is a rambling rough hairy evergreen shrub with four sided branches. The stems are 1.25-2.25cm long with ovate toothed leaves. The leaves are opposite, shortly stalked with 2.5-7.5cm lengths. Flowers are in a long stalked rounded heads 2-3cm across, numerous white, pale purple or commonly orange or yellow. Fruits are in a stalked cylindrical cluster.

The plant is vulnerary, diaphoretic, carminative, antispasmodic and tonic. It is useful in tetanus, malaria, epilepsy and gastropathy. Root decoction is a good gargle for odontalgia. Powdered leaves are used for cuts, wounds, ulcers and swellings. Leaf infusion is good for bilious fever, eczema and eruptions. Fruits are edible and useful in fistula, pustules, tumors and rheumatism. Plant is of great economic, domestic and medicinal values (Baral and Kurmi, 2005). It contains pesticidal properties. It contains lantanine named alkaloid that is pesticidal (Grainge and Ahmed, 1988).

3.6.5 *Melia azedarach*

The common name of the plant is chinaberry, bead tree or umbrella tree and it is called bakaaino or mahaanim in Nepali. It belongs to the family Meliaceae. It is distributed in tropical and subtropical regions. Also it is in cultivation. The tree has got large leaves with 20-45cm lengths. Flowers are liliac, honey scented and 6.8cm long.

The root is acrid, bitter, astringent to the bowels, anthelmintic, emetic, blood purifier, relieves headache, cures fever, urinary discharges. Leaves are bitter, astringent, expectorant, vermicidal, antilithic, and diuretic; its decoction is stomachic. They are useful in hysteria, leprosy, scrofula, cardiac diseases, urolithiasis, verminosis, scabies, etc. Gum is useful in spleen enlargement. Flowers and leaves are diuretic, emmenagogue. Poultice of flower is applied to interruptive skin. Seeds are bitter, anthelmintic and aphrodisiac and are useful in typhoid fever, pain in the pelvic region. Seed oil is considered as brain tonic, laxative. Fruits are poisonous, induce vomiting and develop symptoms of paralysis when eaten (Baral and Kurmi, 2005).

The pesticidal properties are present in the leaf, fruit and oil. So, it is used for the crop protection in various countries. It contains pesticidal properties (contact and systemic), and growth inhibitory substances. It mainly contains meliantriol onto which several terpenoidal compounds are present. The pesticidal compounds present in chinaberry easily dissolve in alcohol and rarely in water (Lovie et al., 1967).

3.7 RESEARCH ON ANTIMICROBIAL ACTIVITIES

Renu *et al*. (1980) screened 25 plants for antifungal activity and from the study new potent fungitoxic compounds were obtained. Out of them, *Cestrum diurnum* exhibited strong activity against *Alternaria solani*, *A. alternata*, *A. brassicae*, *A. rumphii*, *A. tenuis* and other fungal species.

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

Meena and Mariappan (1993) carried out invitro tests for the efficacy of plant products on seed borne mycoflora. The leaf extracts of *Azadirachta indica*, *Mentha arvensis*, *Aegle marmelos*, *Catharanthus roseus*, *Lantana camara*, *Pongamia pinnata*, *Vitex negundo* and *Nerium odorum* and flower extracts of *C. roseus* inhibited mycelial growth and spore germination of the seedborne mycoflora of sorghum including *Alternaria*

alternate, *Aspergillus flavus*, *Curvularia lunata*, *Fusarium moniliforme* and *Rhizopus stolonifer*. The extracts of *A. indica*, *C. roseus* and *L. camara* were more effective than the other plant extracts tested.

Bowers and Locke (2000) investigated botanical extracts and essential oils as possible alternatives to soil fumigation for control of Fusarium wilt diseases. Soil infested with *Fusarium oxysporum* f. sp. *chrysanthemi* was treated with 1, 5, and 10% aqueous emulsions of formulated extracts of clove (70% clove oil), neem (90% neem oil), pepper/mustard (chili pepper extract and essential oil of mustard), cassia (extract of cassia tree), and Banrot (a standard fungicide applied at different labeled rates) in separate experiments. Treatment of the soil with 5 and 10% aqueous emulsions resulted in significant ($P < 0.05$) differences among treatment means at each assay date. After 3 days, pepper/ mustard, cassia, and clove extracts added as 10% aqueous emulsions reduced the population density of *F. oxysporum* f. sp. *chrysanthemi* 99.9, 96.1, and 97.5%, respectively, compared with the untreated control. Neem oil extract increased the population density of *F. oxysporum* f. sp. *chrysanthemi* at all concentrations tested. Banrot did not reduce the population density of *F. oxysporum* f. sp. *chrysanthemi* in any experiment.

Bhat and Shukla (2001) reported the effect of leaf extracts of *Azadirachta indica*, *Lantana camara*, *Pinus roxburghii* and *Tagetes erecta* on germination, growth of mycelium and sporulation of *Drechslera graminea*. The results indicated that diluted neem and marigold extracts may be used effectively for the control of this strip disease of barley.

Shrestha (2003) carried out an invitro test in the laboratory using Krilaxyl (0.75g/l), Dithane M-45 (1.5g/l), neem (5% extract/l), mugwort (5% extract/l) and Trichoderma (2.5g/l) to determine their effects on the development of mycelium of Phytophthora infestans Jharrrasi B isolate from Nepal. All were found to be significantly effective over the control.

Timila (2004) studied on the Phytophthora blight of pepper. Under greenhouse or screenhouse conditions, significant reductions in disease incidence and severity were found in 7 and 8 week seedlings compared to younger seedlings. *Trichoderma harzianum* and *Gliocladium virens* also reduced disease significantly compared to control. Under screenhouse conditions among organic amendments, only mustard meal reduced disease incidence significantly.

Wang et al. (2004) extracted oily, water-insoluble pastes from the leaves of *Inula viscosa* on acetone and n-hexane. The pastes, either dissolved in acetone or emulsified in water, effectively controlled downy mildew of cucumber, late blight of potato or tomato, powdery mildew of wheat, and rust of sunflower. Mean effective dose (concentration) required for 90% inhibition of disease values for acetone solutions and water emulsions ranged from 0.68 to 1.02% and 0.65 to 1.00% (wt/vol), respectively. Dry matter content in fresh leaves, paste-extract yield in dry leaves, and disease control efficacy of paste extracts were similar in leaves of *I. viscose* collected during May to October, suggesting that, for practical use, harvests can be conducted during most of the growing season.

Ghorbani et al. (2005) observed the effects of the antagonistic microorganisms and the compost tea extracted at different developmental stages by detached leaf bioassay method. Twelve of the antagonists showed between 10 to 50 % reduction in blight development compared to control plants. And the different compost tea extracts showed the variation in the reduction of the blight disease with about 30 to 40%.

Laura and Bhardwaj (2005) performed lab bioassays to determine the antimicrobial activity of 100 plant extracts of 90 plant species against 5 plant pathogenic bacteria (*Erwinia cootovora*, *Pseudomonas syringae*, *Rathayibacter tritici*, *Rhizobium radiobacter* and *Xanthomonas campestris*) and 7 plant pathogenic fungi (*Alternaria brassicae*, *Aspergilus oryzae*, *Chaetomium globosum*, *Coriolus versicolor*, *Curvularia lunata*, *Fusarium moniliforme* and *Fusarium solani*). Most of the plant part extracts have shown very good activity against these phytopathogenic bacteria and fungi. More

than 80% growth inhibition was observed in case of fungus and a inhibition zone of 20-32mm in case of the bacterial strains.

Shrestha (2005) tested the 5 plants namely *Allium sativum*, *Artemisia dubia*, *Eupatorium adenophorum*, *Capsicum annuum* and *Azadirachta indica* against *Geotrichum candidum*. The crude extract of *A. dubia*, *A. sativum* and *E. adenophorum* has shown the highest antifungal activity with the mycelial growth inhibition of 83.3%, 66.66% and 50% respectively.

Guleria and Kumar (2006) investigated lipophilic (dichloromethane) leaf extract of medicinal plants used by Himalayan people in antifungal tests. The screening of the zone of inhibition of fungal growth was determined by the measure of ZOI. *Alternaria alternarata* and *Curvularia lunata* were used as test organism in bioautography. The results revealed that 5 plant species among the 12 investigated showed antifungal activity. Clear inhibition zones were observed for lipophilic extracts of *Vitex negundo*, *Zanthoxylum alatum*, *Ipomea carnea*, *Thuja orientalis* and *Cinnamomum camphora*. The best antifungal activity was shown by lipophilic leaf extract of *T. orientalis*.

Balasubramanian et al. (2007) screened the antiviral activity of Indian medicinal plants against white spot syndrome virus in shrimp. The 20 species selected for the study were *Aegel marmelos*, *Allium sativum*, *Aristolochia indica*, *Azadirachta indica*, *Cassia fitula*, *Catharanthus roseus*, *Curcuma longa*, *Cynodon dactylon*, *Lantana camara*, *Melia azedarach*, *Mimosa pudica*, *Momordica charantia*, *Morus alba*, *Mcimum americana*, *Phyllantus amarus*, *Phyllantus emblica*, *Psidium guava*, *Solanum nigrum*, *Tridax procumban* and *Tylophora indica*. These plants were used to extract antiviral substances with petroleum ether, benzene, diethyl ether, chloroform, ethyl acetate, methanol, ethanol and distilled water separately using a soxhlet apparatus. Each extract was tested for its activity against WSSV in marine shrimp and fresh water crabs. Only 5 plants among 20 (*A. marrmelos*, *C. dactylon*, *L. camara*, *M. charantia* and *P. amarus*) showed antiviral activity.

Khair and Wafaa (2007) evaluated aqueous extracts of sun – dried samples of nine Egyptian medicinal plant species, i.e. basil leaves (*Ocinum bacilicum*), chilli fruits (*Capsicum frutescens*), eucalyptus leaves *(Eucalyptus globulus*), garlic bulbs (*Allium sativum*), lemon grass leaves (*Cymbopogon citratus*), marjoram leaves (*Majorana hortensis*), onion seeds (*Allium cepa*) and peppermint leaves (*Mentha piperita*) against *P. infestans* and *A. solani* in vitro and in field. Plant extracts reduced mycelial growth and inhibited spore germination of both fungal species. The extracts reduced the disease infection with both fungal species compared to control in detached leaves technique. In winter growing season, the extracts of all medicinal plants reduced the disease severity of late blight. Lemon grass leaves and/or chilli fruit extracts gave the most reduction in late blight disease severity compared to control. Results also showed that all tested medicinal plant extracts lowered the disease severity of early blight in summer growing season, especially the extracts of lemon grass leaves, garlic bulbs, basil leaves and marjoram leaves, respectively. The aqueous extract of lemon grass leaves was the best one in controlling both late and early blights. Also, data indicated that the increasing in some vegetative growth characters (i,e. average stem height and average leaves number per plant) and tubers yield of potato were corresponded with the reduction of disease severity.

Sharma (2007) conducted a field experiment to compare the efficacy of 2 plant extracts and 2 species of *Trichoderma* along with Krinoxyl (Metalaxyl 8% + Mancozeb 64%), a fungicide against the late blight of potato during spring and autumn seasons in 2002-2003 at Hattiban farm, Khumultar, Lalitpur. 3 sprays of Krinoxyl showed highest remained green leaf area upto 80 days after planting (46.2%) and increased 28.6% tuber yield followed by 2 sprays and single spray of the same fungicide. The antagonist fungus *Trichoderma viridae* and extracts of *Artemisia indica* Willd could not control potato late blight. Despite little efficacy to disease control, application of EM extracts of *Justicia adhatoda* against *Phytophthora infestans* on seed tuber treatment along with 3 foliar sprays increased the tuber yield significantly by 20.4%. Seed tuber and soil

treatment with *T. harzianum* was found to be effective in increasing tuber yield by 14.3%.

Doughari and Obidah (2008) carried out the antifungal activity of the stem bark extracts of *Leptadenia lancifolia* using various solvents (water, acetone and methanol). Methanol extracts demonstrated the highest activity against *Cryptococcus neoformans* (30mm zone diameter of inhibition, MIC 0.5mg/ml, MFC 1.0mg/ml), and *Candida albicans* (28mm zone diameter of inhibition, MIC 0.5mg/ml, MFC 1.5mg/ml), followed by acetone extracts against (28mm zone diameter of inhibition) against *Phytophthora infestans*, at 50 mg/ml.

Marasini (2008) tested the antimicrobial activities of several medicinal plants against some fungi and antibiotic resistant bacteria. The extraction of the plants was carried out in 90% ethanol by soxhlet apparatus. The yield of *Cinnamomum camphora* and *Eupatorium adenophorum* were 29.7% and 26.9% respectively. The extract of *C. camphora* with 50mg/ml concentration showed the zone of inhibition of 8mm against *Alternaria spp* but *E. adenophorum* was found ineffective.

Mishra (2008) studied the antifungal efficacy of some plant extracts against *Colletotrichum falcatum* Went. The plants used in the study were *Lantana camara* L., *Cymbopogan citrates(DC)* Stap.f, *Allium cepa* L., *Allium sativum* L., *Zingiber officinale* Rosc, *Ocimum tenuiflorum* L., *Aloe vera* (L.) Burm.f., *Artemisia indica* Willd and *Eupatorium adenophorum* Spreng. The plant extract of *A. sativum* L. showed the highest fungitoxicity (100%) at 20% concentration.

Pant et al. (2008) studied the two different concentrations (50% and 100%) of compost and vermicompost teas against 3 major phyptopathogens viz. *Fusarium oxysporum*, *Fusarium moniliformi* and *Helminthosporium maydis* by agar well diffusion method. The vermicompost tea of 100% was found most effective to control growth of the tested organisms followed by 50% vermicompost tea, 100% compost tea and 50% compost tea. The zone of inhibition of 100% vermicompost tea against *F. oxysporum*, *F. moniliformi* and *H. maydis* was found 12mm, 16.6mm and 13.2mm respectively.

Timila and Ashley (2008) tested the organic amendments such as rice hull, chicken manure, compost and mustard meal for the management of Phytophthora blight disease of Pepper. The significant reduction in incidence of the disease was observed only with mustard meal (0.5% amended). The mean disease incidence was significantly lower in mustard meal treatment (19.2% incidence) compared to control (82.5% incidence).

CHAPTER IV

4. METHODOLOGY

4.1 REQUIREMENTS

All the requirements used to complete this study are listed in the appendix I.

4.2 METHODS

Fig.1 shows the methods followed in this study.

4.2.1 Collection and drying of the fungicidal plant materials

All the selected fungicidal plant materials are listed in appendix II. The weight of the freshly collected plant materials was taken. The collected plant materials except cake of *Brassica nigra* were washed with water to remove the dirt particles. These were left to dry over the clean newspaper in the shade. The weight of the dried plant materials was taken till constant weight appeared. To find out the moisture content in the selected plant materials, the weight of the dried materials was substracted from the weight of the freshly collected materials.

Moisture content (%) = (weight of the material before drying – weight of the material after drying) × 100

The completely dried plant materials were chopped and grinded in a grinder to make fine powder. The powdered materials were kept in an air tight container until extraction process.

4.2.2 Extraction of the fungicidal plant materials

Shade dried fine powder of fungicidal plant materials were subjected to continuous soxhlet extraction to obtain crude extracts (Tiwari et al., 1992). The extraction was carried out in the solvent ethanol (100%). Known weight (100gm) of dried powdered plant materials was packed in sterile filter paper and it was loaded in a clean and dried thimble of Soxhlet extractor. It was then fitted with clean and pre-dried round bottom flask. Then the ethanol was slowly poured from the upper mouth of the soxhlet

extractor. The upper mouth part was fitted with condenser having cold water running through tap. The flask was constantly heated with electric heating mantle with controlled temperature. The solvent vaporized and condensed by condenser through the plants powder. The solvent soluble compounds were eluted in the conical flask through siphon tube. The process was continued until the colorless solvent appeared from plant materials in the siphon.

The round bottom flask containing extract was fitted with rotary vacuum evaporator under reduced pressure. The flask was constantly heated in rotating condition by using water bath at around 60°C. Solvent was completely removed and was collected in separate round bottom flask of evaporator which was stored in sterile bottle. The extract obtained in round bottom flask was then weighed till constant weight appeared. To find out the extract yield, the weight of pre-weighed round bottom flask was subtracted from the weight of round bottom flask with extract. The crude extract obtained was transferred in a sterile bottle with the help of sterile spatula and was labeled and stored in refrigerator until use. The yield of respective extract was calculated by the formula;

Percentage yield (%) = (dry weight of extract / dry weight of plant part) × 100

4.2.3 Preparation of working solution

The different concentration (0.5%, 1%, 3%, 5%, and 10%) of the crude extract was made in Dimethyl sulfo oxide (DMSO). After making working solution, the test tubes were capped, sealed and stored in refrigerator until use.

4.2.4 Collection of the leaf samples of infected potato plant

A total of 35 leaf samples of infected potato plant were collected in clean plastic bags from the fields of 7 localities and labeled properly. The symptoms observed in the collected leaf samples were noted.

4.2.5 Preparation of the media

The media used in the study were prepared according to the manufacturer's recommendation. The detailed procedure for the preparation of media is given in the appendix III.

4.2.6 Isolation of the pathogenic organisms from the collected samples

The phytopathogenic fungal organisms were isolated from the infected potato leaf samples. Several small sections of 5 to 10 mm square were cut from the margin of the infected lesions so that they contain both the diseased and healthy looking tissue. These were placed in the surface sterilizing agent 70% ethanol solution making sure that the surfaces get wet. After about 10 to 15 seconds, the sections were taken out aseptically one by one. The sections were then washed in three changes of sterile water and were finally placed onto the 2 types of nutrient medium i.e. Rye A agar and Potato Dextrose Agar (PDA) (Aneja, 2001). The inoculated Rye A agar medium plate was incubated at 18°C in the dark for 7 days (Sato and Kato, 1993) while the inoculated PDA plate was incubated at 27°C for 7 days (Aneja, 2001). The types of the fungal colonies isolated in both the media plates were noted.

4.2.7 Identification of the pathogenic fungal organisms

The cultural characteristics and microscopic observation of the isolated fungal organisms were done for their identification (Aneja, 2001). The microscopic study of the fungal organisms was done by Cellophane tape method. In this method a drop of lactophenol cotton blue was placed at the centre of a clean slide. The centre of the sticky side of the tape was pressed firmly onto the surface of the fungus colony and was placed on the drop of the lactophenol cotton blue. It was then observed under the microscope.

The identification of the fungal species was done by the measurement of the size of the spores or conidia. For this the microscope was firstly calibrated with the ocular and the stage micrometer. The slide of the fungal organisms was prepared and the size of the spores or conidia of the organisms was measured.

4.2.8 Preparation of standard culture inoculums

For antifungal assay, the standard inoculum of the fungal organisms was prepared (Aberkane et al., 2002). A loopful of isolated fungal organisms were placed in Potato Dextrose Broth (PDB) media and incubated at 18°C for 7 days for *Phytophthora infestans* and at 27°C for 7 days for *Alternaria solani* and *Fusarium oxysporum*. The inoculum size was adjusted to a range of 1×10^6 – 5×10^6 spores/ml by microscopic enumeration with a cell counting Haemocytometer.

4.2.9 Screening and evaluation of antifungal activity

Agar well diffusion method and two fold broth dilution method were used in the study for screening and evaluation of antifungal activity of the crude plant extracts.

The antifungal activity of crude extracts of fungicidal plants materials was screened against the tested organisms by agar well diffusion method (Dingle et al., 1953). In agar well diffusion method, the diameter of zone of inhibition (ZOI) produced by plant extract on particular phytopathogenic fungal organism was measured for the estimation of antifungal activity of the crude plant extract.

Sterile PDA plates were prepared. Before using the plates, they were dried in hot air oven at 40°C for 5 min to remove excess of moisture from the surface of the media. Sterile cotton swab was dipped into the prepared inoculums and excess of inoculums was removed by pressing and rotating against the upper inside wall of the tube above the liquid level and seeded carefully all over the plate. The plate was rotated through an angle of 60°C after each swabbing. Finally the swab was passed round the angles of the agar surface. The inoculated plates were left to dry for few minutes at room temperature with lid closed (WHO, 1991).

The wells were made in the inoculated media plates with the help of sterile cork borer (diameter of 6mm) and labeled properly. Then 50µl of the working solution of the plant extract were loaded into the respective wells with the help of micropipette. The solvent itself was tested for its activity as a control at the same time in a separate well. The plates were then left for half an hour with the lid closed so that the extracts diffused to

the media. Then the plates were incubated at the respective temperatures i.e. 18ºC for *P. infestans* and at 27ºC for *A. solani* and *F. oxysporum* for 7 days. The plates were then observed for the zone of inhibition which was suggested by the clean area without growth around the well.

The crude extract of the plants which showed antimicrobial activity were subjected to two fold serial dilution method (Baron et al., 1994) to determine MIC and MFC.

In this method, a set of screw capped 12 test tubes containing 2ml PDB for each fungus were prepared. The test tubes were labeled as positive growth control, no. 1 to 10 and negative growth control. In case of negative growth control the PDB was discarded. Then 2ml of the crude extract from particular plants were added aseptically to each tube labeled as negative growth control and no.1 labeled test tube. The first tube now contains 2ml of broth and 2ml of extract. After complete homogenization 2ml of its content was transferred aseptically to second tube. Similarly, after complete homogenization of the content in the second tube, 2ml of it was transferred to third tube. In the same way, two fold dilutions were prepared up to the tenth tube. From the tenth tube, 2ml of the content was discarded. Hence all the tubes from negative control to no.10 contained equal volume i.e. 2ml with gradually decreasing concentration. No plant extract was added to the tube labeled as positive control. Now, with the help of micropipette 20 µl of inoculums was added to all the tubes except the one which was labeled as negative control i.e. negative control contains only extract but no broth and no organisms, positive control contains broth and organisms but not plant extract while tube 1 to 10 contains PDB, test organism and plant extract. The tubes were incubated at the respective temperatures i.e. 18ºC for *P. infestans* and at 27ºC for *A. solani* and *F. oxysporum* for 7 days.

After incubation of a series of test tubes containing the serially diluted crude extracts of plants and the test fungal organisms, the results were compared with positive and negative control tubes. The results were interpreted on the basis of the fact that growth occurs on the positive control and any other tube in which the concentration of the extract is not sufficient to inhibit the growth. The lowest concentration of antimicrobial

agent that inhibits the growth of organisms as detected by lack of visible turbidity or growth is the MIC. All the tubes without growth were subcultured on PDA plates containing no crude extracts with proper label and incubated at the respective temperature for 7 days. Then the plates were examined for the growth of the organism. The tube with minimum concentration of extract in which the growth was completely checked was also clearly notified. Then, MFC was determined. The lowest concentration of the antimicrobial agents that completely checks out the growth of the fungal organism is the MFC.

4.2.10 Statistical analysis

Statistical analysis was performed to test the effectiveness of various levels of concentration of different extracts in the inhibition of the fungal organisms scientifically. In this experiment the ANOVA was analyzed (Appendix V).

CHAPTER V

5. RESULTS

5.1 MOISTURE CONTENT IN THE FUNGICIDAL PLANT MATERIALS

The five different fungicidal plant materials namely *Brassica nigra* (cake), *Cinnamomum camphora* (fruits), *Eupatorium adenophorum* (twigs), *Lantana camara* (twigs) and *Melia azedarach* (fruits) were collected from Kirtipur. These were weighed, shade dried and powdered.

Moisture content (%) = (weight of the material before drying – weight of the material after drying) × 100

Table 5: Moisture content in the fungicidal plant materials.

SN	Name of the plants	Weight before drying (gm)	Weight after drying(gm)	Moisture content (%)
1	*Brassica nigra* (Cake)	100	95	5
2	*Cinnamomum camphora* (Fruits)	200	120.69	39.65
3	*Eupatorium adenophorum* (Twigs)	500	174.21	65.16
4	*Lantana camara* (Twigs)	500	104.53	79.09
5	*Melia azedarach* (Fruits)	200	133.23	33.38

The moisture content in the different plant materials is shown in table 5. *L. camara* contained the highest moisture (79.07%) while least moisture content was obtained in the cake of *B. nigra* (5%).

5.2 PERCENTAGE YIELD OF CRUDE EXTRACTS OF FUNGICIDAL PLANT MATERIALS

The powdered plant materials were continuously extracted in soxhlet apparatus using ethanol as solvent to obtain the crude extracts.

Percentage yield (%) = (dry weight of extract / dry weight of plant part) × 100

Table 6: Yield percentage of the ethanolic extract of the plant materials.

SN	Name of the plants	Yield of crude extracts on dry weight basis (%)
1	*Brassica nigra* (Cake)	18.38
2	*Cinnamomum camphora* (Fruits)	70
3	*Eupatorium adenophorum* (Twigs)	32.03
4	*Lantana camara* (Twigs)	30.29
5	*Melia azedarach* (Fruits)	9.75

The ethanolic extracts of the 5 different plant materials obtained by using soxhlet apparatus is given in Table 6. Highest percentage yield was obtained from *C. camphora* (70%) and the lowest yield was obtained from *M. azedarach* (9.75%).

5.3 INFECTED LEAF SAMPLES COLLECTED FROM FIELDS

A total of 35 leaf samples of infected potato plant were collected from the 7 sites of Kathmandu valley.

Table 7: Collection of samples from different sites.

Sample No.	Site	Symptoms on leaves
1	Tokha	Black patches on the leaves
2	Tokha	Black patches on the leaves
3	Tokha	Black patches on the leaves
4	Tokha	Wilting of the leaves
5	Tokha	Yellowing of the leaves
6	Mulpani	Black patches on the leaves
7	Mulpani	Black patches on the leaves
8	Mulpani	Black patches on the leaves
9	Mulpani	Black patches on the leaves
10	Mulpani	Black patches on the leaves
11	Lubhu	Black patches on the leaves
12	Lubhu	Black patches on the leaves
13	Lubhu	Black patches on the leaves
14	Lubhu	Black patches on the leaves
15	Lubhu	Yellowing of the leaves
16	Thecho	Black patches on the leaves
17	Thecho	Black patches on the leaves
18	Thecho	Black patches on the leaves
19	Thecho	Black patches on the leaves
20	Thecho	Black patches on the leaves
21	Chyamasingh	Black patches on the leaves
22	Chyamasingh	Black patches on the leaves
23	Chyamasingh	Black patches on the leaves
24	Chyamasingh	Black patches on the leaves
25	Chyamasingh	Black patches on the leaves
26	Bode	Black patches on the leaves
27	Bode	Black patches on the leaves
28	Bode	Black patches on the leaves
29	Bode	Black patches on the leaves
30	Bode	Black patches on the leaves
31	Kirtipur	Black patches on the leaves
32	Kirtipur	Black patches on the leaves
33	Kirtipur	Black patches on the leaves
34	Kirtipur	Black patches on the leaves
35	Kirtipur	Black patches on the leaves and white mycelial growth on underside

5.4 ISOLATION OF THE PATHOGENIC FUNGAL ORGANISMS

From the leaf samples of infected potato plant, the pathogenic fungal organisms were isolated on different media.

Table 8. Fungal colonies isolated on Rye A agar medium

Sample No.	Types of fungal colonies isolated	*Phytophthora infestans*	*Alternaria solani*	*Fusarium oxysporum*
1	1	-	-	-
2	1	-	-	-
3	1	-	-	-
4	-	-	-	-
5	-	-	-	-
6	1	-	-	-
7	2	-	-	-
8	1	-	-	-
9	-	-	-	-
10	1	-	-	-
11	1	-	-	-
12	1	-	-	-
13	-	-	-	-
14	1	-	-	-
15	-	-	-	-
16	1	-	-	-
17	1	-	-	-
18	1	-	-	-
19	-	-	-	-
20	-	-	-	-
21	1	-	-	-
22	-	-	-	-
23	-	-	-	-
24	-	-	-	-
25	1	-	-	-
26	1	-	-	-
27	-	-	-	-
28	1	-	-	-
29	-	-	-	-
30	-	-	-	-
31	1	-	-	-
32	1	-	-	-
33	2	+	-	-
34	1	-	-	-
35	1	+	-	-

Note: + : presence - : absence

Table 9. Fungal colonies isolated on PDA medium

Sample No.	Types of fungal colonies isolated	*Phytophthora infestans*	*Alternaria solani*	*Fusarium oxysporum*
1	1	-	-	-
2	1	-	-	+
3	2	-	+	-
4	1	-	+	-
5	1	-	-	+
6	3	-	-	-
7	1	-	-	-
8	1	-	-	-
9	2	-	+	-
10	-	-	-	-
11	2	-	-	-
12	1	-	-	-
13	1	-	+	-
14	2	-	+	-
15	1	-	-	-
16	-	-	-	-
17	2	-	+	-
18	2	-	+	-
19	1	-	+	-
20	2	-	-	-
21	-	-	-	-
22	1	-	-	-
23	3	-	-	-
24	2	-	+	-
25	-	-	-	-
26	-	-	-	-
27	1	-	-	-
28	2	-	+	-
29	2	-	+	-
30	-	-	-	-
31	1	-	-	-
32	1	-	+	-
33	2	-	+	-
34	-	-	-	-
35	1	-	-	-

Note: + : presence

- : absence

5.5 IDENTIFICATION OF THE PATHOGENIC FUNGAL ORGANISMS

The isolated fungal organisms were identified on the basis of the cultural characteristics and the microscopic observation. The identification of the species was done by the measurement of the size of the fungal spore or conidia.

Table 10. Identification of the fungal organisms

SN	**Media used**	**Colony characteristics**	**Microscopic observation**	**Size of fungal spore/ conidia (appendix IV)**	**Inference**
1	Rye A agar	Orange colored at the centre and white at the periphery	Aseptate mycelium with a small characteristic swelling just below the sporangium on the sporangiophores. Sporangia are semipapillate.	32×20µm	*Phytophthora infestans*
2	PDA	Brown colored	Mycelium septate, branched with light brown hyphae. Beaked, dark colored conidia are formed in chains. Both transverse and longitudinal septa in the mature conidia.	129×15µm	*Alternaria solani*
3	PDA	White colored that changed to pink.	Presence of aseptate, oval to kidney shaped microconidia and 3-5 septate, sickle shaped macroconidia.	Microconidia (6×3µm) Macroconidia (52×5µm)	*Fusarium oxysporum*

5.6 EVALUATION OF ANTIFUNGAL ACTIVITY

The diameter of zone of inhibition given by the extracts against the test fungal organisms was measured for the estimation of the potency of the particular extract. In order to evaluate the validity of the test scientifically, the data was analyzed using ANOVA (Appendix V).

Table 11: Antifungal activity of different concentration of plant extracts against *Phytophthora infestans.*

SN	Extract of plant	Zone of inhibition (mm) (Diameter of well: 6mm)				
		0.5%	1%	3%	5%	10%
1	*Brassica nigra* (cake)	-	8	9	11	12
2	*Cinnamomum camphora* (fruits)	-	-	7	8	9
3	*Eupatorium adenophorum* (twigs)	-	7	8	9	11
4	*Lantana camara* (twigs)	-	8	8	9	10
5	*Melia azedarach* (fruits)	-	7	8	10	11
6	Relaxin*(control)	10	15	20	24	26

Note: (-) indicates no ZOI

*Fungicide

The in vitro antifungal activity of the different concentration of the 5 different plant extracts and the fungicide against *P. infestans* is given in Table 11. None of the extracts at the 0.5% concentration showed the inhibitory activity against *P. infestans*. Among the plant extracts, *B. nigra* showed the higher antifungal activity while *C. camphora* was found least effective. The different types of extracts with different concentration significantly ($P < 0.05$) inhibited the growth of *P. infestans* (Appendix V).

Table 12: Antifungal activity of different concentration of plant extracts against *Alternaria solani*

SN	Extract of plant	Zone of inhibition (mm) (Diameter of well: 6mm)				
		0.5%	**1%**	**3%**	**5%**	**10%**
1	*Brassica nigra* (cake)	-	7	9	11	13
2	*Cinnamomum camphora* (fruits)	-	-	7	9	11
3	*Eupatorium adenophorum* (twigs)	8	9	10	13	15
4	*Lantana camara* (twigs)	7	9	10	12	14
5	*Melia azedarach* (fruits)	8	9	11	16	20
6	Relaxin* (control)	7	9	11	14	19

Note: (-) indicates no ZOI

*Fungicide

The in vitro antifungal activity of the different concentration of the 5 different plant extracts and the fungicide against *A. solani* is given in Table 12. Among the plant extracts, *M. azedarach* showed the higher inhibitory activity against *A. solani,* while *C. camphora* was found least effective. The different types of extracts with different concentration significantly ($P<0.05$) inhibited the growth of *A. solani* (Appendix V).

Table 13: Antifungal activity of different concentration of plant extracts against *Fusarium oxysporum*

SN	Extract of plant	Zone of inhibition (mm) (Diameter of well: 6mm)				
		0.5%	**1%**	**3%**	**5%**	**10%**
1	*Brassica nigra* (cake)	-	7	8	10	14
2	*Cinnamomum camphora* (fruits)	8	10	11	12	13
3	*Eupatorium adenophorum* (twigs)	-	8	9	11	15
4	*Lantana camara* (twigs)	7	9	10	15	17
5	*Melia azedarach* (fruits)	-	8	9	10	14
6	Relaxin* (control)	30	31	32	35	40

Note: (-) indicates no ZOI

*Fungicide

The in vitro antifungal activity of the different concentration of the 5 different plant extracts and the fungicide against *F. oxysporum* is given in Table 13. Among the plant extracts, *C. camphora* and *L. camara* showed the inhibitory activity against F. *oxysporum* at all the tested concentration while the others were ineffective at the 0.5% concentration. The different types of extracts with different concentration significantly ($P<0.05$) inhibited the growth of *F. oxysporum* (Appendix V).

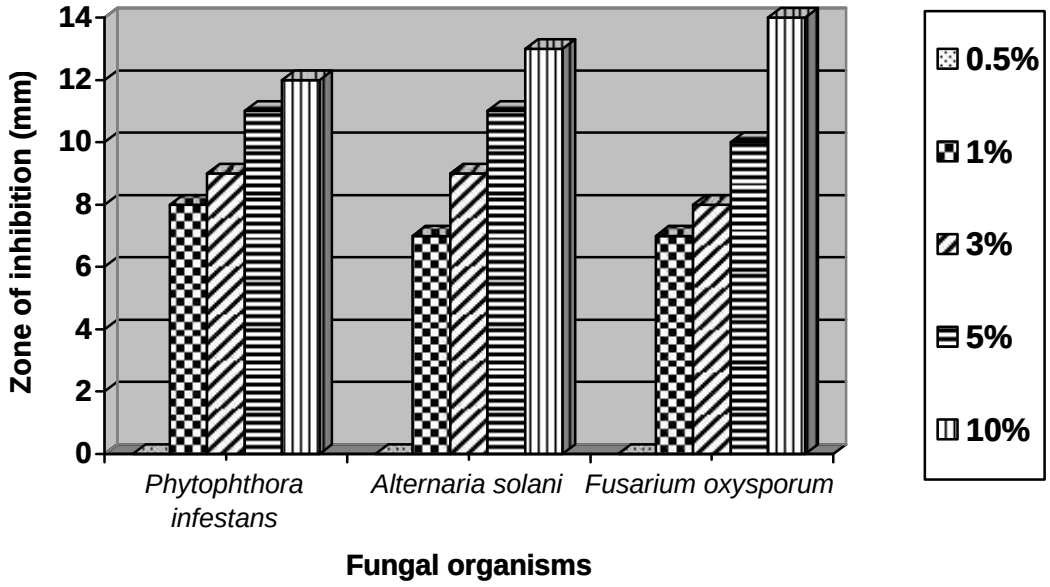

Fig.2 Zone of inhibition given by the different concentration of the ethanolic extract of *Brassica nigra* against the tested fungal organisms.

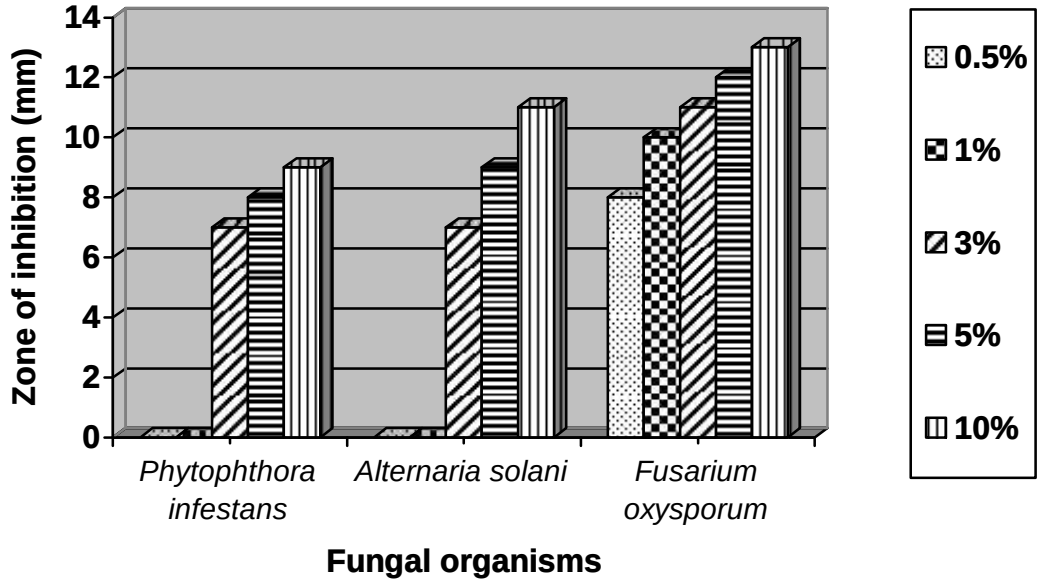

Fig.3 Zone of inhibition given by the different concentration of the ethanolic extract of *Cinnamomum camphora* against the tested fungal organisms.

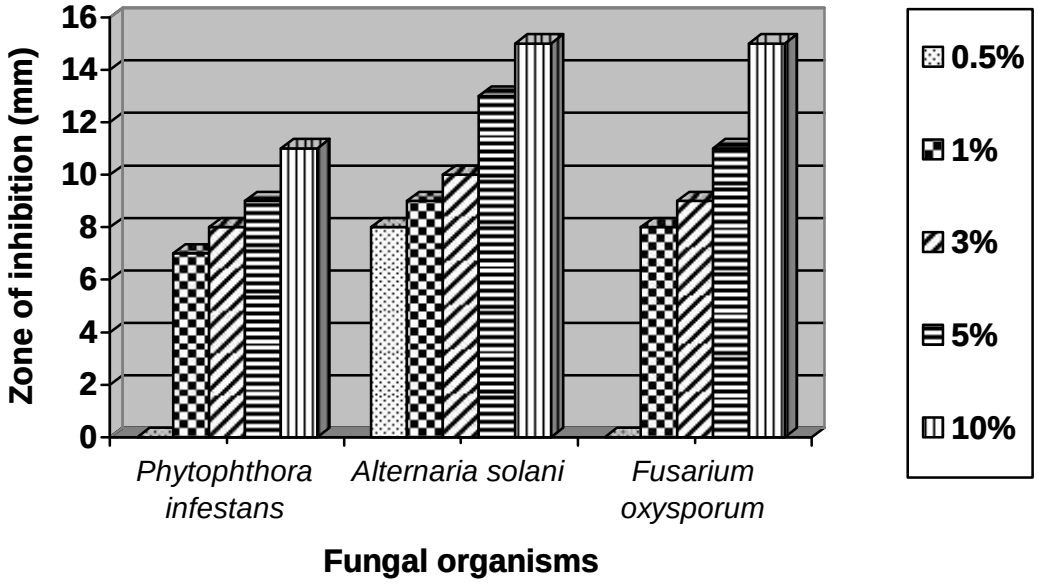

Fig.4 Zone of inhibition given by the different concentration of the ethanolic extract of *Eupatorium adenophorum* against the tested fungal organisms.

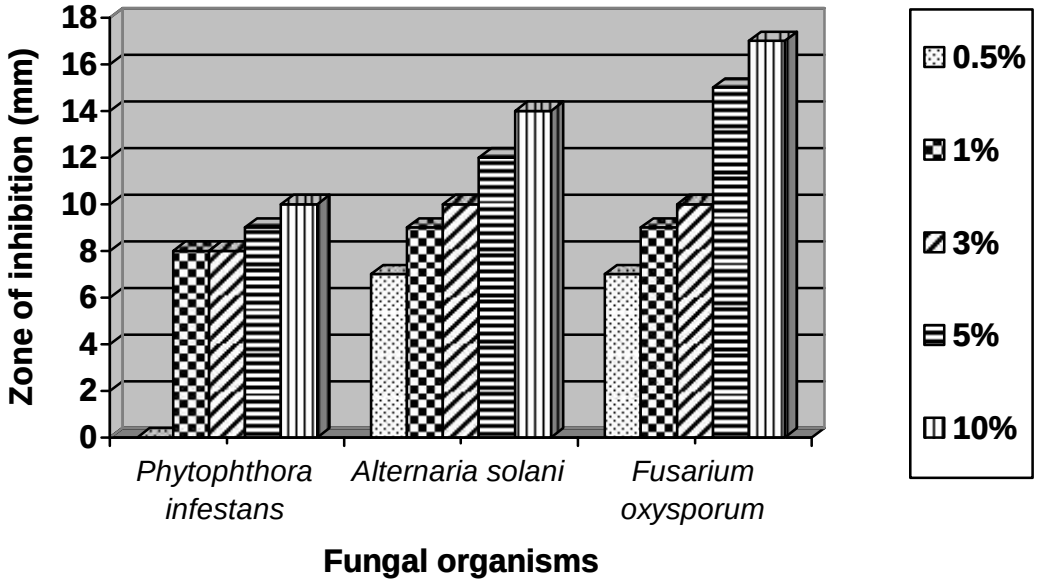

Fig.5 Zone of inhibition given by the different concentration of the ethanolic extract of *Lantana camara* against the tested fungal organisms.

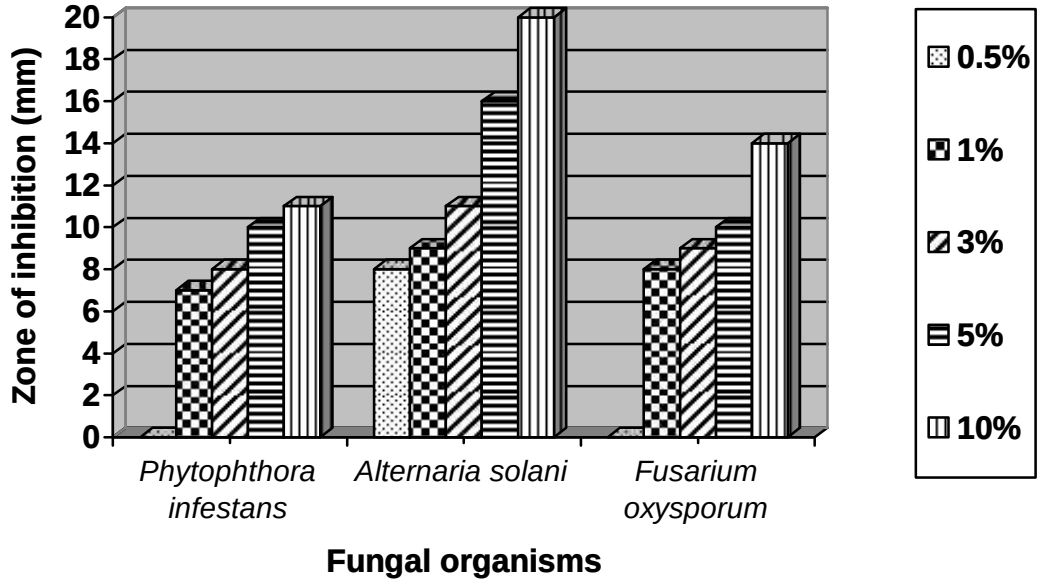

Fig.6 Zone of inhibition given by the different concentration of the ethanolic extract of *Melia azedarach* against the tested fungal organisms.

Table 14: MIC and MFC of different plant extracts on *Phytophthora infestans*.

SN	Plant extract	MIC (mg/ml)	MFC (mg/ml)
1	*Brassica nigra* (cake)	6.25	6.25
2	*Cinnamomum camphora* (fruits)	12.5	25
3	*Eupatorium adenophorum* (twigs)	6.25	12.5
4	*Lantana camara* (twigs extract)	6.25	6.25
5	*Melia azedarach* (fruits)	6.25	12.5

The MIC and MFC value of the plant extracts against *P. infestans* is given in the Table 14. The MIC value of *B. nigra*, *E. adenophorum*, *L. camara* and *M. azedarach* was found to be 6.25 mg/ml and of *C. camphora* 12.5 mg/ml. But the MFC value of the plant extracts ranged from 6.25 mg/ml to 25 mg/ml.

Table 15: MIC and MFC of different plant extracts on *Alternaria solani*

SN	Plant extract	MIC (mg/ml)	MFC (mg/ml)
1	*Brassica nigra* (cake)	6.25	12.5
2	*Cinnamomum camphora* (fruits)	25	25
3	*Eupatorium adenophorum* (twigs)	3.125	6.25
4	*Lantana camara* (twigs)	3.125	6.25
5	*Melia azedarach* (fruits)	3.125	3.125

The MIC and MFC value of the plant extracts against *A. solani* is given in the Table 15. The MIC value of *E. adenophorum, L. camara* and *M. azedarach* was found to be 3.125 mg/ml and that of *B. nigra* and *C. camphora* 6.25 mg/ml and 25 mg/ml respectively. And the MFC value of the plant extracts ranged from 3.125 mg/ml to 25 mg/ml.

Table 16: MIC and MFC of different plant extracts on *Fusarium oxysporum*

SN	Plant extract	MIC (mg/ml)	MFC (mg/ml)
1	*Brassica nigra* (cake)	6.25	12.5
2	*Cinnamomum camphora* (fruits)	3.125	3.125
3	*Eupatorium adenophorum* (twigs)	6.25	12.5
4	*Lantana camara* (twigs)	3.125	6.25
5	*Melia azedarach* (fruits)	6.25	12.5

The MIC and MFC value of the plant extracts against *F. oxysporum* is given in the Table 16. The MIC value of *C. camphora* and *L. camara* was found to be 3.125 mg/ml and that of *B. nigra, E. adenophorum* and *M. azedarach* was found to be 6.25 mg/ml and 25 mg/ml. And the MFC value of the plant extracts ranged from 3.125 mg/ml to 12.5 mg/ml.

CHAPTER VI

6. DISCUSSION AND CONCLUSION

6.1 DISCUSSION

The study was conducted from July 2008 to July 2009 at Natural Products Research Laboratory of Nepal Academy of Science and Technology, Khumultar for the purpose of studying the effect of ethanolic extracts of plant materials on *P. infestans*, *A. solani* and *F.oxysporum*.

In the present study, the five different fungicidal plant materials namely mustard oil cake, camphor (fruit), china berry (fruit), crofton weed (twigs) and lantana weed (twigs) were selected randomly and collected from Kirtipur. The collected plant materials were shade dried. Among the plant materials collected, the moisture content was obtained highest in the twigs of *L. camara* i.e.79.09% and lowest in the cake of *B. nigra* i.e.5%. Large plant materials were chopped, grinded and then subjected to extraction process to facilitate complete and easy extraction of the active compounds.

Water, the universal solvent, found to be used by the local farmers was not used in the antifungal tests as it can't dissolve non polar organic compounds and also the removal of water from the solution needs high temperature or complex equipment. In a study carried out by Rabe and Van (1997), the antimicrobial activity was shown higher by the alcoholic fraction rather than aqueous extract. So the ethanol was chosen as solvent for the extraction.

Upon going through various literatures, it was found that there is no specific process for the extraction of compounds from the plants. Among percolation and soxhlet extraction process, the mostly preferred one is the soxhlet extraction process for the easy and better extraction of compounds from plants. In this study, the ethanolic extracts of the plant materials was obtained by the soxhlet extraction process.

In the extraction, the percentage of extract yield on dry weight basis of *E. adenophorum* and *C. camphora* in 100% ethanol was 32.03% and 70% respectively which was significant. However in the previous result obtained by Marasani (2008), the extract yield of C. *camphora* and *E. adenophorum* in 90% ethanol were 29.7% and 26.9% respectively. The yield percentage of *E. adenophorum* was slightly higher but that of *C. camphora* was much higher in 100% ethanol in comparison to 90% ethanol.

There was a distinct difference in the percentage yield of extracts from different plant materials. The difference in yield might be due to the various factors such as time of extraction, type and part of plant materials, fineness of powder and extent of dryness, etc. Similarly, incomplete extraction results in lesser yield.

The pathogenic fungal organisms were isolated from the infected potato leaf samples using suitable culture media and were identified on the basis of cultural characteristics and microscopic observation. The identification of the fungal species was done by the measurement of the size of their spores or conidia. *A. solani* and *F. oxysporum* were isolated on the PDA medium. *P. infestans* does not grow luxuriantly on some of the usual media used for the cultivation of fungi such as cornmeal, PDA or oatmeal agar. The fungus grows slowly and is easily overgrown by other fungi or bacteria (Ingram and Williams, 1991). Hence the antibiotic amended rye A agar medium was used for the cultivation of the fungi and was subcultured on PDA media (Sato and Kato, 1993).

The crude extracts of the plant materials obtained after removal of solvent were dissolved in 100% DMSO to make different concentration of the extracts. These extracts were tested for antimicrobial activity against the isolated fungal organisms by agar well diffusion method of Dingle et al. (1953). Qualitative screening of antimicrobial substances does not indicate the extent of the potency of a sample. A substance may demonstrate a wide spectrum of activity but may have minuscule potency. Such substance has no utility practically and development of such product if not economically feasible. So, the antimicrobial activity must also be quantitatively assayed. For this, two fold serial dilution method (Baron et al., 1994) was employed.

In this study, the higher concentration of the extracts was required to inhibit the growth of *P. infestans*. The extracts of *B. nigra*, *E. adenophorum*, *L. camara* and *M. azedarach* inhibited the pathogen from 1% concentration, *B. nigra* being the most effective. *C. camphora* showed the inhibitory activity only from the 3% concentration. Similar result was observed by Timila and Ashley (2008) in which mustard meal (0.5% amended) significantly reduced the incidence (19.2% incidence) of Phytophthora blight disease of Pepper compared to control (82.5% incidence). Timila (2004) also found that only mustard meal reduced the disease incidence of Phytophthora blight disease of Pepper compared to control. Shrestha (2003) found that neem (5% extract/l), mugwort (5% extract/l) and Trichoderma (2.5g/l) were significantly effective over control on the development of mycelium of *P. infestans*. Similarly, Wang et al. (2004) observed the pastes from the leaves of *Inula viscosa* on acetone and n-hexane effectively controlled late blight of potato. Khair and Wafaa (2007) reported that the aqueous extract of lemon grass leaves was most effective in lowering the disease severities of late blight. In the study conducted by Sharma (2007) the antagonist fungus *Trichoderma viridae* and extracts of *Artemisia indica* Willd were ineffective against *P. infestans* while the extract of *Justicia adhatoda* was little effective. Doughari and Obidah (2008) found that the acetone extracts of the stem bark of *Leptadenia lancifolia* was effective against *P. infestans* at 50 mg/ml. Also in the study carried out by Ghorbani et al. (2005) the late blight infection of potato was reduced to some extent with the use of antagonists and the compost extracts in comparison to the control.

During the study, *M. azedarach* showed the higher inhibitory activity against *A. solani*. The extract of *E. adenophorum* and *L. camara* were effective even at 0.5% concentration while the extract of *B. nigra* and *C. camphora* were effective at higher concentration. In the study conducted by Renu et al. (1980) *A. solani* was strongly inhibited by *Cestrum diurnum* extract. Khair and Wafaa (2007) reported that all the aqueous extracts of basil leaves, chilli fruits, eucalyptus leaves, garlic bulbs, lemon grass leaves, marjoram leaves, onion seed and peppermint leaves lowered the disease severity of early blight and among those lemon grass leaves was most effective.

In this study, *C. camphora* and *L. camara* were most effective against *F. oxysporum* while *B. nigra*, *E. adenophorum* and *M. azedarach* were effective only at the concentration of 1% and more. According to Bowers and Locke (2000) 1, 5 and 10% aqueous emulsions of formulated extracts of clove oil, neem oil, mustard essential oil, chili pepper extract, cassia tree extracts reduced the population density of *F. oxysporum* but Banrot (a standard fungicide applied at different labeled rates) did not reduce the population density of *F. oxysporum*. Pant et al. (2008) found that among the compost and vermicompost teas at 50% and 100% concentration, the vermicompost tea at 100% was found most effective to control growth of *F. oxysporum* followed by vermicompost tea 50%, compost tea 100% and compost tea 50%.

In agar well diffusion method, the material under the test diffuses from well into the surrounding agar in a concentric circle and inhibits or kills the microorganisms that are susceptible. This effect manifested by a clear zone around the well is measured as ZOI.

The diameter of ZOI largely depends upon the diffusibility of the antimicrobial substance. Hence some times the ZOI demonstrated by the antimicrobial substance does not compensate the efficacy of the substance. This necessitates the determination of MIC and MFC to give correct picture of antimicrobial potency of the compound.

The extracts from the plant materials showing large ZOI and small MFC value may contain those compounds, which are able to inhibit or kill the microbial population of the tested organism. Conversely, the extracts from the plant materials showing large ZOI and large MFC value may contain those compounds which diffuse through the medium readily and inhibit the growth but could not kill the organism at the same time.

The extract of *B. nigra* was most effective against *P. infestans* as shown in fig.2 with both MIC and MFC value 6.25 mg/ml. Similarly, Timila and Ashley (2008) and Timila (2004) found the mustard meal most effective in the reduction of disease incidence of Phytophthora blight of Pepper. The MIC and MFC value of *B. nigra* was 6.25 mg/ml and 12.5 mg/ml for *A. solani* and *F. oxysporum* respectively.

C. camphora was found effective against *P. infestans* and *A. solani* at the concentration of 3% and more while it was most effective against *F. oxysporum* as shown in fig.3. Both the MIC and MFC value of *C. camphora* against *F. oxysporum* was 3.125 mg/ml and for *A. solani* was 25 mg/ml. Similarly, the MIC and MFC value of *C. camphora* against *P. infestans* was 12.5 mg/ml and 25 mg/ml respectively.

E. adenophorum was effective against *A. solani* even at the concentration of 0.5% while the ZOI against *P. infestans* and *F. oxysporum* was observed only from the concentration of 1% as shown in fig.4. The MIC and MFC value of *E. adenophorum* against *A. solani* were 3.125 mg/ml and 6.25 mg/ml respectively. Similarly, the MIC and MFC value of *E. adenophorum* were 6.25 mg/ml and 12.5 mg/ml respectively for both *P. infestans* and *F. oxysporum*.

L. camara showed ZOI against *A. solani* and *F. oxysporum* even at the concentration of 0.5% while the ZOI against *P. infestans* was observed only from the 1% concentration as shown in fig.5. Both the MIC and MFC value of *L. camara* against *P. infestans* was 6.25 mg/ml. Similarly, the MIC and MFC value of *L. camara* were 3.125 mg/ml and 6.25 mg/ml respectively for both *A. solani* and *F. oxysporum*.

M. azedarach was most effective against *A. solani* as shown in fig.6 with both MIC and MFC value 3.125 mg/ml. *M. azedarach* inhibited *P. infestans* and *F. oxysporum* only from the concentration of 1%. The MIC and MFC value of *M. azedarach* were 6.25 mg/ml and 12.5 mg/ml respectively for both *P. infestans* and *F. oxysporum*.

P. infestans, *A. solani* and *F. oxysporum* cause late blight, early blight and fusarial wilt of the potato respectively. Pathogens while infecting plants, in the course of their obtaining food for themselves, depending on the kind of pathogen and on the plant tissue they infect, pathogens interfere with the different physiological functions of the plant and lead to the development of different symptoms. The infection of the leaves of the plant results in the decrease in the photosynthesis rate. In plant diseases in which the pathogen infects the leaves, the transpiration is usually increased due to the increase in the permeability of leaf cells and the dysfunction of stomata. Also, the rate of

respiration is increased such that affected tissues use up their reserve carbohydrates faster than healthy tissues. Plant pathogens may interfere with the movement of organic nutrients from the leaf cells to the phloem, with their translocation through the phloem elements, or, possibly with their movement from the phloem into the cells that will utilize those (Agrios, 2008).

The plant extracts showed the different antifungal activity. One of the reasons for different fungitoxicity activity of various plant extracts may be due to their different chemical composition (Rao and Srivastava, 1994). The composition of plant oils and extracts is known to vary according to local climatic and environmental conditions (Jansen et al., 1987). However, the result obtained in controlling plant diseases by using different plant extracts may differ as many factors vary between assays. These may include difference in microbial growth, exposure of organisms to plant extract, the solubility of components and the use and quantity of an emulsifier (Reynolds, 1996).

6.2 CONCLUSION

From this study we can conclude that the selected fungicidal plant materials under this study had antifungal activity against the fungal pathogens isolated from the diseased potato leaves. Although all the ethanolic extracts showed the antifungal activity at the higher concentration, the crude extracts of mustard meal, *M. azedarach* and *C. camphora* were found most effective against *P. infestans*, *A. solani* and *F. oxysporum* respectively.

CHAPTER VII

7. SUMMARY AND RECOMMENDATIONS

7.1 SUMMARY

1. Altogether five fungicidal plant materials belonging to five different families were selected in this study.

2. Among the selected plant materials, the moisture content was found highest in the twigs of *L. camara* (79.09%) and lowest in the cake of *B. nigra* (5%).

3. The Soxhlet extraction of these materials was carried out using absolute ethanol as solvent to obtain the crude extracts.

4. On dry weight basis, the highest percentage yield was obtained from *C. camphora* (70%) and the lowest from *M. azedarach* (9.75%).

5. The three fungal pathogenic organisms namely *P. infestans*, *A. solani* and *F. oxysporum* were isolated from the infected potato leaf samples. The identification of the fungal organisms was done on the basis of their cultural characteristics and the microscopic observation. The fungal species was identified by the measurement of size of their spores or conidia.

6. The agar well diffusion method and two fold serial dilution method were used to evaluate the antifungal activity of the crude extracts obtained.

7. All the crude extracts obtained were found to be ineffective against *P. infestans* at the 0.5% concentration. The extracts of *B. nigra* showed the higher antifungal activity against *P. infestans* while *C. camphora* was found least effective. The different types of plant extracts of different concentration significantly at ($P<0.05$) inhibited the growth of *P. infestans*.

8. In the invitro antifungal assay, the crude extracts of *M. azedarach* showed the higher inhibitory activity against *A. solani,* while *C. camphora* was found least effective. The

different types of plant extracts of different concentration significantly ($P<0.05$) inhibited the growth of *A. solani*.

9. Among the crude extracts obtained, *C. camphora* and *L. camara* showed the inhibitory activity against *F. oxysporum* at all the tested concentration while the others were ineffective at the 0.5% concentration. The different types of plant extracts with different concentration significantly ($P<0.05$) inhibited the growth of *F. oxysporum*.

10. The MIC value of *B. nigra*, *E. adenophorum*, *L. camara* and *M. azedarach* was found to be 6.25 mg/ml and of *C. camphora* 12.5 mg/ml for *P. infestans*. But the MFC value of the plant extracts ranged from 6.25 mg/ml to 25 mg/ml.

11. The MIC value of *E. adenophorum*, *L. camara* and *M. azedarach* was found to be 3.125 mg/ml and that of *B. nigra* and *C. camphora* was 6.25 mg/ml and 25 mg/ml respectively for *A. solani*. And the MFC value of the plant extracts ranged from 3.125 mg/ml to 25 mg/ml.

12. The MIC value of *C. camphora* and *L. camara* was found to be 3.125 mg/ml and that of *B. nigra*, *E. adenophorum* and *M. azedarach* was found to be 6.25 mg/ml for *F. oxysporum*. And the MFC value of the plant extracts ranged from 3.125 mg/ml to 12.5 mg/ml.

7.2 RECOMMENDATIONS

Based on the findings of the study, the recommendations are made as follows:

1. Due to the time and other factor, only five plant extracts have been evaluated. So, further research on other plants should be conducted.

2. Only ethanolic extract has been used in this study. There are various bioactive compounds extractable in different solvents. So, other solvents should also be tried for extraction.

3. Few fungi were selected for the study. There are other organisms (bacteria, fungi, virus, parasites, pests, etc.) of concern that cause disease of potato which should be considered.

4. Plants which exhibited substantial antifungal effect during evaluation should be analyzed in detail to identify the bioactive compounds.

5. This result was based on the in vitro study. There are many parameters that interfere with antifungal agents in the environment. So, the field trial should be conducted to generalize the results obtained.

CHAPTER VIII

8. RFERENCES

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APPENDIX I

LIST OF REQUIREMENTS

1. Equipments

i. Autoclave (Vesta)

ii. Hot air oven (Universal)

iii. Incubator (Universal)

iv. Laminar flow (Toshiba)

v. Microscope (Olympus)

vi. Refrigerator (Samsung)

vii. Rotary Vacuum Evaporator (Eyela)

viii. Soxhlet Extractor (Ogawa)

ix. Weighing balance (Ohaus)

2. Microbiological Media

i. Potato Dextrose Agar

ii. Potato Dextrose Broth

iii. Rye A agar

3. Glass wares

i. Beakers

ii. Conical flasks

iii. Culture tubes

iv. Measuring cylinder

v. Microscopic slides

vi. Petriplates

vii. Pipettes

viii. Test tubes

4. Chemicals/ Reagents

i. Ethanol

ii. Dimethyl sulpho oxide (DMSO)

iii. Cotton blue

iv. Lactophenol

v. Dextrose

vi. Sucrose

5. Miscellaneous

i. Sealed plastic bag

ii. Cellophane tape

iii. Innoculating loop

iv. Bacto agar

v. Spatula

vi. Rye grain

vii. Forceps

viii. Innoculating wire

ix. Micropipette

x. Cork borer

xi. Ocular micrometer

xii. Stage micrometer

xiii.Haemocytometer

APPENDIX II

List of fungicidal plant materials used in the evaluation of antifungal activities

Botanical Name	**Family**	**English Name**	**Local Name**	**Parts Used**	**Place Of Collection**
Brassica nigra	Brassicaceae	Black or true mustard	Kaaltori or raayo	cake	Kirtipur
Cinnamomum camphora	Lauraceae	Camphor	Kapur	fruits	Kirtipur
Eupatorium adenophorum	Compositae	Crofton weed	Banmara	twigs	Kirtipur
Lantana camara	Verbenaceae	Lantana weed	Ban phaanda	twigs	Kirtipur
Melia azedarach	Meliaceae	Chinaberry or umbrella tree	Bakaaino or mahaanim	fruits	Kirtipur

APPENDIX III

A. COMPOSITION AND PREPARATION OF MEDIA

1. Rye A agar amended media

Ingredients	Gram/litre
Rye grain	60
Sucrose	20
Bacto agar	15

The 60 gm of rye grain was soaked in distilled water for 24 hours at room temperature. This was done in a small plastic tray so that water just covered grain. Tray was covered tightly with aluminum foil. Next day, supernatant was poured off and put aside from germinated grain that was macerated for 5 minutes and cooked in a water bath for 1 hour at 68°C. Cooked rye was filtered through 2 layers of cheesecloth squeezing gently to remove residual liquid. Filtrate was combined with original supernatant and 20 g sucrose and 15 g Bacto agar were added. Final volume of media was adjusted to 1 lt. with water and autoclaved at 15 psi for 20 minutes. The media was cooled to about 60°C and was amended with antibiotics Ampicillin (200 ppm), Nystatin (100 ppm) and Refampicin (100 ppm). The media was shaked such that the antibiotics dissolve properly and the media was then plated.

2. Potato Dextrose Agar

Ingredients	Gram/litre
Potato	200
Dextrose	20
Agar	20

The 27 gm of the medium was dissolved in 1000 ml distilled water and autoclaved at 121°c for 15 min.

3. Potato Dextrose Broth

Ingredients	Gram/litre
Potato	200
Dextrose	20

The 200 gm of peeled potato was weighed and cut into small pieces. It was boiled for few minutes and squeezed as much of pulp as possible through a layer of muslin cheese cloth. 20 gm dextrose and distilled water was added to make the final volume 1 lt. It was autoclaved at 121°C for 15 min.

B. COMPOSITION AND PREPARATION OF REAGENTS

1. Lactophenol

Ingredients	Amount
Phenol pure crystal	100gm
Lactic acid	100ml
Glycerol	20ml
Distilled water	10ml

The lacto phenol was prepared by warming pure crystal phenol with distilled water until the crystals were dissolved. The lactic acid and glycerol were added.

2. Cotton blue

One gram of cotton blue was dissolved in 100ml of water to make 1% of aqueous solution.

APPENDIX IV

Measurement of the spore size of *Phytophthora spp.*

SN	Length of spore (µm)	Breadth of spore (µm)
1	33	22
2	32	21
3	31	19
4	33	22
5	32	20
6	33	21
7	31	19
8	30	19
9	32	20
10	31	20
Total	318	203
Mean	31.8(~32)	20.3 (~20)

Measurement of the size of conidia of *Alternaria spp.*

SN	Length of spore (µm)	Breadth of spore (µm)

1	128	13
2	130	14
3	128	14
4	129	14
5	130	15
6	131	16
7	131	16
8	130	15
9	129	14
10	129	14
Total	1295	145
Mean	129.5 (~129)	14.5 (~14)

Measurement of the size of microconidia of *Fusarium spp.*

SN	**Length of spore (µm)**	**Breadth of spore (µm)**

1	6	3
2	6	3
3	5	2
4	5	2
5	5	3
6	6	3
7	6	3
8	6	3
9	6	3
10	6	3
Total	57	28
Mean	5.7 (~6)	2.8 (~3)

Measurement of the size of macroconidia of *Fusarium spp.*

SN	**Length of spore (µm)**	**Breadth of spore (µm)**
1	52	5
2	51	4
3	51	4

4	53	5
5	53	5
6	52	5
7	52	4
8	53	5
9	53	5
10	52	5
Total	522	47
Mean	52.2 (~52)	4.7 (~5)

APPENDIX V

Statistical Analysis

ANOVA table for *Phytophthora infestans*

Source of variation	**Sum of squares (SS)**	**Degree of freedom (d.f.)**	**Mean sum of squares (MS)**	**Calculated F value (F)**	**P value**	**Tabulated F value (F at 5%)**

Due to extract (A)	SSA=28.4	4	MSA=7.1	3.786667	<0.05	3.006917
Due to concentration (B)	SSB=347.6	4	MSB=86.9	46.34667	<0.05	3.006917
Error	30	16	MSE=1.875			
Total	406	24				

Null hypothesis (H_o): There is no statistical difference in the zone of inhibition given by the different types of plant extracts at different concentration against *P. infestans.*

Alternate hypothesis (H_1): There is statistical difference in the zone of inhibition given by the different types of plant extracts at different concentration against *P. infestans.*

The calculated F value was greater than the tabulated F value. Hence, the alternate hypothesis was accepted.

ANOVA table for *Alternaria solani*

Source of variation	**Sum of squares (SS)**	**Degree of freedom (d.f.)**	**Mean sum of squares (MS)**	**Calculated F value (F)**	**P value**	**Tabulated F value (F at 5%)**
Due to extract (A)	SSA=165.04	4	MSA=41.26	13.70764	<0.05	3.006917

Due to concentration (B)	SSB=323.04	4	MSB=80.76	26.83056	<0.05	3.006917
Error	48.16	16	MSE=3.01			
Total	536.24	24				

Null hypothesis (H_o): There is no statistical difference in the zone of inhibition given by the different types of plant extracts at different concentration against *A. solani.*

Alternate hypothesis (H_1): There is statistical difference in the zone of inhibition given by the different types of plant extracts at different concentration against *A. solani.*

The calculated F value was greater than the tabulated F value. Hence, the alternate hypothesis was accepted.

ANOVA table for *Fusarium oxysporum*

Source of variation	**Sum of squares (SS)**	**Degree of freedom (d.f.)**	**Mean sum of squares (MS)**	**Calculated F value (F)**	**P value**	**Tabulated F value (F at 5%)**
Due to extract (A)	SSA= 57.2	4	MSA=14.3	4.806723	<0.05	3.006917

Due to concentration (B)	SSB=369.2	4	MSB=92.3	31.02521	<0.05	3.006917
Error	47.6	16	MSE=2.975			
Total	474	24				

Null hypothesis (H_o): There is no statistical difference in the zone of inhibition given by the different types of plant extracts at different concentration against *F. oxysporum.*

Alternate hypothesis (H_1): There is statistical difference in the zone of inhibition given by the different types of plant extracts at different concentration against *F. oxysporum.*

The calculated F value was greater than the tabulated F value. Hence, the alternate hypothesis was accepted.