INTEGRATED MANAGEMENT OF STALK ROT DISEASE (*Sclerotinia sclerotiorum)* OF CAULIFLOWER IN THE EASTERN HILLS OF NEPAL

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I certify that this work has not been accepted in substance for any degree, and is not concurrently submitted for any degree other than that of Doctor of Philosophy (PhD) of the University of Greenwich. I also declare that this work is the result of my own investigations except where otherwise stated.

Supervisor (Dr. Robert. Black)....

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Abstract

Cauliflower is a high value cash crop for the resource-poor farmer of the eastern hills of Nepal. Non-governmental organisations are enabling resource-poor farming communities to gain a better share in economic development by facilitating input supply and helping to explore market facilities. Previous work indicated that stalk rot (*Sclerotinia sclerotiorum)* is an important disease of cauliflower. Currently, no single management technique provides a satisfactory level of control of stalk rot disease; therefore the management of stalk rot had to be approached in several ways in order to develop the basis for an integrated management strategy.

Surveys of farmers' experience and the results of investigative work indicated that stalk rot (*S. sclerotiorum*) and damping-off *(S. sclerotiorum*, *Alternaria* spp., *Pythium* sp., *Rhizoctonia* spp. and *Fusarium* spp.) are major constraints for satisfactory cauliflower production. Stalk rot is problematic for all stages of the crops. Seeds used by the farmers did not meet an acceptable standard of germination. Experimental data indicated that *S. sclerotiorum* and *Fusarium* spp. are major fungal pathogens associated with poor germination and seedling mortality after emergence. In the case of *S. sclerotiorum,* it is likely that sclerotial contamination of seed is the source of these problems.

Farmers are generally aware of cultural practices to manage disease problems, but lack awareness of correct methods of disposal and alternative use of diseased debris. Studies on the effect of weeds on cauliflower production did not support farmers' perceptions that weeds reduce the disease incidence in the fields. Weeds did reduce curd size and yield presumably due to competition with crop plants. Some fungal antagonists of *S. sclerotiorum,* particularly a *Trichoderma harzianum*, showed promising activity but further work is necessary to translate the results on detached curds into a practical technique. Source of resistance to *S. sclerotiorum* were found in cv. Kathmandu Local that could be exploited in breeding varieties of cauliflower acceptable to farmers. The results are used to develop a strategy for integrated management of cauliflower diseases that could be adopted by agricultural scientists, extension workers, NGOs and farmers.

Abbreviations and glossary

%	Per cent
µm	Micrometer
°C	Degree Celsius
@	At the rate of
$	The US Dollar
*	Probability at 5% level significantly different
**	Probability at 1% level significantly different
AIC	Agricultural Inputs Corporation
Altitude	Metre above sea level
APP	The Agricultural Prospective Plan
ARS	Agricultural Research Station
Bari-land	Rain-fed land
Categories A	Food sufficient for more than 12 months
Categories B	Food sufficient for 12 months
Categories C	Food sufficient for 6 to 9 months
Categories D	Food sufficient for less than 6 months
CEAPRED	Centre for Environmental and Agricultural Policy Research, Extension and Development
cfu	Colony formation unit
cm	Centimetre(s)
cv.	Cultivar
d.f.	Degree of freedom
DFID	Department for International Development
e.g.	For example
EC	Emulsifiable concentration
Farmers categories	Category of farmer(s) based on food sufficiency from on-farm production for the last crop year
g	Gram(s)
GO	Governmental organization
ha	Hectare
Hat / Bazaar	Local market

High altitude	altitude higher than 1700 meters
HMG/N	His Majesty's Government of Nepal
hr	Hour(s)
IDM	Integrated disease management
i.e.	That is
IPM	Integrated pest management
K	Potash
Kg	Kilogram(s)
Khet-land	Irrigated land
KOSEPAN	Koshi hills Seed Entrepreneurs Association
l	Litre
L1	Top growing leaf
L2	Second leaf from the top
L3	Third leaf
L4	Second leaf from bottom
L5	Lowest leaf
LARC	Lumle Agricultural Regional Centre
Low altitude	altitude lower than 1100 meters
m	Metre(s)
m^2	Square metre
Mid altitude	altitude range in between 1100 to 1700 meters
ml	Millilitre
mm	Millimetre
N	Nitrogen
NARC	Nepal Agricultural Research Council
NGO	Non-governmental organization
NRI	Natural Resources Institute
NRs	Nepalese rupees
NS	Not significantly different
ODA	Overseas Development Administration
P	Phosphorus
PDA	Potato Dextrose Agar

PAC	Pakhribas Agricultural Centre
SSSP	Seed Sector Support Project
Terai	The flat land of the Gangetic plain
VDC	Village Development Committee
VDD	Vegetable Development Division
YDC	Yeast Dextrose Chalk

TABLE OF CONTENTS

CHAPTER 3

CHAPTER 4

CHAPTER 5

CHAPTER 6

CHAPTER 7

CHAPTER 10

LIST OF TABLES

LIST OF FIGURES

APPENDICES

CHAPTER 1

1.1 STALK ROT DISEASE IN CAULIFLOWER PRODUCTION IN NEPAL

Nepal is an agriculture-based, land-locked country that remains one of the poorest countries in the world although strongly supported by many international donors. In Nepal, 36% of the population consume less than the estimated minimum calorie requirements and about 29% of the population exhibit second or third degree malnutrition, while 6.5% suffer from some degree of mental retardation associated with nutritional deficiencies (Anonymous, 1996). Hence the important role that vegetables can play in dietary improvement.

Broadly, Nepal is divided into two geographic regions, i.e. Terai and Hills. The Terai is a part of the Gangetic plain and constitutes the southern border area of Nepal. It is low lying (altitude range from 60 to 310 m) and flat, and includes most of the fertile land. It occupies about 14% of the total land area; the climate is warm, subtropical. The hills, occupying the remaining 86% of total land area, rise abruptly from the northern edge of the Terai to the highest peak of the world, Mount Everest (altitude range from 310 to 8848 m), the climate varying from subtropical to cool temperate. Farming is performed up to 4000 m (field and horticultural crops). Hill farming is subsistence-based but land is intensively cropped. About 95% of the people living in poverty are concentrated in the hills. The hill farmers are particularly characterized by low purchasing power, low farm income, inadequate education and poor health.

1.2 NEPALESE HILL FARMING

Hill farming should play a major role in the economic development of Nepal. Hill farming alone contributes a 52% share of the agricultural gross domestic product of the country.

Nepalese hills are gifted with an abundance of good soil and varied geographical and agro-climatic conditions. Land is intensively cropped in two main categories: Khet-land (irrigated land) and Bari-land (rainfed). Three distinct agro-ecological zones are described: high altitude (higher than 1700 m), mid altitude (1100 to 1700 m) and low altitude (lower than 1100 m). Variation in climatic conditions is a very prominent feature of these agro-ecological zones. Rainfall is extremely variable (500 mm to >5000 mm per annum). Almost all types of subtropical and temperate crops may be grown throughout the year in one or more agro-ecological zones. Staple food crops are maize, potatoes, rice, wheat, finger millet and vegetables including radish, broad-leaf mustard, cabbage and cauliflower. Depending upon the market facilities subtropical and temperate types of fruits are also grown. Cash crops include pulses, oilseeds, fresh vegetables, vegetable seeds, cardamom, ginger, orange, pear and apple.

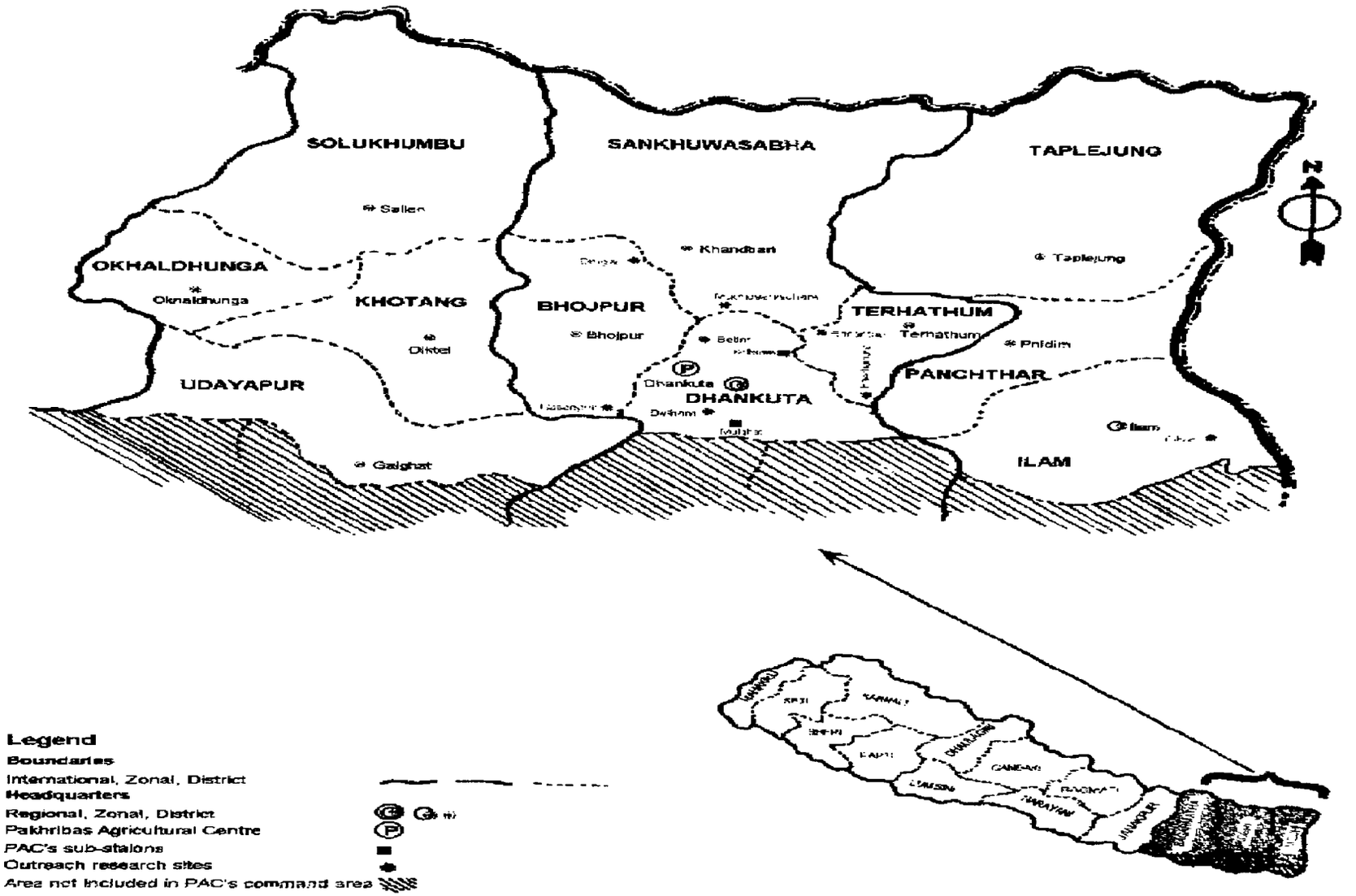
In the hills of Nepal, most of the crops are grown by resource-poor farmers and techniques for disease control are not available or are not appropriate for their circumstances. Women play an important role in vegetable production in the hills, but they lack much involvement in the decision-making processes, being mainly a

source of labour. Hence, women know even less than men about disease management.

1.3 THE EASTERN HILLS OF NEPAL

The Nepal Agricultural Research Council (NARC), the coordinating body of national (publicly funded) agricultural research in Nepal, has established one agricultural research station in each region of Nepal. Agricultural Research Station (ARS) Pakhribas is one of them (the old name of ARS Pakhribas was Pakhribas Agricultural Centre, PAC). The ARS Pakhribas command area is the eastern hills of Nepal comprising eleven Hill Districts (Figure 1.1). Eastern hill farmers face acute difficulties in transport, access to markets and access to the most basic agricultural inputs including those required for crop protection. As a result, all the necessary agricultural technical specialisations had to be developed at ARS Pakhribas in order to help development and promotion of technologies.

The eastern hills consist of strongly dissected relief ranging in altitude from 300 meters in the Arun and Tamor river valleys to land rising steeply to the main ridges at 3000 meters. Most agricultural farming occurs between these altitudes. The climate is monsoonal with 70% of the rain falling between June and September. Annual rainfall varies between 900 mm and 2000 mm. Climate varies from hot, dry subtropical through warm, moist temperate to alpine. The command area of ARS Pakhribas includes about 350,000 farm households. The main ethnic groups in the eastern hills are Brahimin, Chhetri, Limbu, Rai, Gurung, Magar, Tamang, Sherpa and Bhote. There is only one major road, so most of the goods are carried in and out of the area by porters. Land holdings are generally small with 43% of the



Figuure 1.1 Map of Nepal showing ARS Pakhribas command areas.

population having holdings of less than 0.5 ha. Moreover, small-holder farmers usually have access only to Bari-land which is less productive than Khet-land. However, despite the many problems with which eastern hill farmers have had to contend, there has been a gradual diversification and intensification of the farming system to meet the increasing demand of the urban population. The result is that cash cropping has expanded.

Due to various combinations of climate, topography, altitude and social organization, a wide variety of farming systems have arisen. Vegetables are considered an important crop in the ARS Pakhribas command area. Commercial and semi-commercial production of vegetables is expanding to meet the growing needs of the Kathmandu valley, the Terai markets and the export trade through the adjoining borders of India. As well as government involvement through agricultural research stations and extension programme (Pakhribas, Paripatle and District Agriculture Development Offices), the expansion and intensification of vegetable production in the eastern hills of Nepal has been associated with the establishment of non-governmental organizations (NGOs) such as Koshi Hills Seed Entrepreneurs Association (KOSEPAN), Seed Sector Support Project (SSSP), and the Centre for Environmental and Agricultural Policy Research, Extension and Development (CEAPRED). These NGOs are facilitating the formation of community-based farmer co-operatives, based mostly on technology generated at ARS Pakhribas and ARS Paripatle. One of their objectives is to improve the market potential for local farmers, particularly with respect to markets across the borders in India and China. However with the establishment of these NGOs, there has been a significant

increase in pesticide application leading to concerns about environmental and health hazards (Dahal, 1995). Nepal has to depend on imported pesticides. Dahal (1995) reported that the annual business of pesticides in Nepal is worth US $1.5 million. In the business of supplying pesticides to farmers, there is a lack of proper handling techniques and no regulations for the proper management of their disposal. Considerable quantities of expired pesticides are present, most of which are stored in densely populated areas. In some places, pesticides are stored in living accommodation. According to Klarman (1987) "this situation is a time-bomb that can only lead to a major catastrophe if not corrected". These problems were recognised in the Agricultural Perspective Plan (APP) (Anonymous, 1995a) which includes integrated pest management (IPM) as one of the priority areas relating to increased productivity.

1.4 CAULIFLOWER IN NEPAL

Vegetables are an integral component of hill farming systems of Nepal (Shrestha and Ghimire, 1996), being one of the high value commodities and given high priority in the APP (Anonymous, 1995a). Among vegetables, cauliflower (*Brassica oleracea* L. *var. botrytis*) is one of the most widely cultivated commercial crops. Cauliflower can be successfully grown in the high, mid and low hills of Nepal. In the past, cauliflower was mostly imported from India (Koirala *et al.*, 1995), but during recent years, with the establishment of the NGOs and the formation of community-based farmer co-operatives, the off-season production of cauliflower in the hills of Nepal has increased considerably. Nowadays cauliflower is actually exported to India. In Nepal, the area under cauliflower production is 19,267 ha (i.e.

13.7% of the total area under vegetables) with average productivity of 7.8 tonnes per ha (Anonymous, 1995b).

Cauliflower cultivation in the hills is a highly labour-demanding enterprise, helping to generate employment opportunities and improving living standards. Secondly, it may reduce the migration of farmers from the hills to densely populated areas (Terai). Finally, sufficient cauliflower in the hill farmers' diet may help alleviate nutritional deficiencies because it is a good source of minerals, vitamins and fibre.

1.5 IMPORTANCE OF *SCLEROTINIA* STALK ROT IN NEPAL

Cauliflower is attacked by various diseases in Nepal (Table 1.1).

Table 1.1 Cauliflower diseases recorded in the eastern hills of Nepal.

Disease	Pathogens
Stalk rot/ Watery soft rot	*Sclerotinia sclerotiorum* (Lib) de Bary
Damping-off	*Pythium aphanidermatum* *Rhizoctonia* spp. *S. sclerotiorum* *Phytophthora* spp. *Fusarium* spp.
Downy mildew	*Peronospora parasitica*
Alternaria leaf spot	*Alternaria brassicicola* (Schw) Wiltshire *Alternaria brassicae* (Berk) Sacc
Black rot	*Xanthomonas campestris* pv. *campestris* (Pammel) Dowson
Soft rot	*Erwinia carotovora* subsp. *carotovora*

(Source: Thapa *et al.*, 1995; ARS Pakhribas disease identification logbook, 1998, unpublished.)

Among the diseases of cauliflower, *Sclerotinia* stalk rot is reported to be one of the most important (Thapa *et al.*, 1995). In Nepal, this disease has been reported from all the important commercial cauliflower growing areas including Rukum, Palpa, Dhankuta and Kathmandu (Bhurtayal, 1985). According to Shrestha and Timila (1990) this disease was first reported from Dhankuta district in 1978 and thereafter it was reported from Musicot, Palpa and Khumaltar in 1979, 1981, and 1982 respectively (Shrestha, 1990). In Pokhra, the disease was reported in 1988 (Shrestha, 1985b; Shrestha *et al.*, 1989).

Sclerotinia disease is well documented to have a major effect on cauliflower seed production. Up to 30-40% of the yield of cauliflower seed crops is estimated to be lost by infection every year in Nepal (Bhurtayal, 1985). In 1983, this disease caused almost total failure of seed production, causing for example, 83% loss in a farm in Dhankuta (eastern hills). Similarly, it was reported by the Vegetables Development Division (1982) that this disease reduced the seed yield in Khumaltar from the expected 150 kg to 45 kg. A preliminary study at ARS Pakhribas was performed in 1991/92 in a micro-plot seed multiplication block. The results indicated that the disease caused 17.3% loss of cauliflower seed under the natural epiphytotic conditions occurring (Duwadi *et al.*, 1993). Similarly, in Dhankuta only 53 kg per ha cauliflower seed could be obtained instead of the expected 300 - 400 kg due to *S. sclerotiorum* disease (Rekhi, 1983). **The biology of *S. sclerotiorum* is considered in detail in Chapter 2. The pathogens associated with damping-off and curd rot are considered in Chapter 5.**

Plant disease in the context of the cropping system

Productivity of farming is decreasing each year due to a number of factors, among which insect pests and diseases are considered to be the most important. A significant proportion (20 to 25%) of agricultural produce in Nepal is lost each year as a result of insect pests (Anonymous, 1990); another 10 to 20% of yield loss is estimated to be due to plant diseases (Joshi *et al.*, 1991).

According to the experiences of scientists at ARS Pakhribas, indigenous or local cultivars are still popular among the farmers and the majority of hill farmers believe in cultural practices for reducing pest attack rather than in the use of pesticides. They are still using traditional organic practices of pest control by applying wood ash, cow urine, inter-cropping and indigenous plant products, or fail to practise pest control at all (Shrestha *et al.*, 1996). These practices are associated with the fact that pest control inputs are scarcely available in the hills, despite considerable losses from plant diseases.

Experience has shown that very little available technology was relevant to farmers growing cauliflower. Most cauliflower growers are poor and `control inputs are expensive and not easily available to them. The first need in the hills and mountain areas of Nepal are more effective technologies appropriate to the hill farmers and their agricultural systems. Secondly, farmers must be in a position to adopt the technology.

Women are involved in almost all aspects of vegetable production or utilization. As well as working directly in the vegetable fields they are also responsible for compost making and irrigation and they cook meals for their families based on vegetables. Overall the roles women play in the production of vegetables are critical to the economics of hill farming. The author's experience at ARS Pakhribas indicates that integrating gender issues into technology generation and dissemination, particularly with reference to an integrated approach in cauliflower production, will help faster adoption of technologies.

A survey was, therefore, carried out to define the technology gaps and the constraints limiting the adoption of technology by the hill farmers of eastern Nepal (Chapter 3).

1.6 INTEGRATED DISEASE MANAGEMENT

There is growing concern world-wide about environmental degradation. Out of several factors that contribute to the problem, increased use of pesticides in crop production is considered to be an important one.

"Integrated Pest Management (IPM) is a sustainable approach to managing pests by combining biological, cultural, physical and chemical tools in a way that minimizes economic, health and environmental risk" (Anonymous, 1994). According to Dickinson and Lucas (1977) the concept of integrated disease management (IDM), derived from IPM, involves the creation of systems which utilise all available methods in a compatible a manner as possible, so as to maintain a pathogen

population at levels below that causing economic loss. Some would argue that IPM/ IDM should only involve "natural" methods to reduce and maintain pest damage to an acceptable level. However, to be realistic, IDM should include regulatory inspection for healthy seeds or nursery crop production, cultural practices (e.g. pruning, crop rotation), encouragement of biological control (e.g. parasites, antagonists), resistant varieties and appropriate chemical control.

It has been reported in the Agriculture Perspective Plan (Anonymous, 1995a, chapter 3, page 199) that:

> "Agriculture that requires high input brings two problems: liberal use of pesticide and poor practices with resultant pollution. In general, such practices are uneconomic but are pursued because farmers are unaware of the underlying cost and possible alternative technologies".

Thus, His Majesty's Government of Nepal (HMG/N) has already endorsed Integrated Pest Management (IPM) as an official policy to achieve sustainable agricultural development in Nepal. This is reflected in the approved long-term Agricultural Perspective Plan 1995-2015 (1995) and the present HMG/N Eighth Five Year Plan (1992-1997) (Anonymous, 1992; Anonymous, 1995a). Integrated methods of pest and disease management are therefore officially endorsed as being economic, viable and justifiable in the hills of Nepal.

1.7 BREEDING FOR RESISTANCE

Use of resistant crop varieties is potentially one of the most effective and economic methods of disease control particularly in areas where pathogens are soil-borne. It has been reported that screening for partial resistance against *S. sclerotiorum* in cauliflower has been successfully achieved. The output of past experiments carried out in Nepal and India was encouraging (Kapoor, 1986; Sharma and Kapoor, 1995; Duwadi and Paneru, 1997). Sharma *et al.* (1984) studied the reaction of eight genotypes of cauliflower and found two genotypes which showed least infection. Similarly, Kapoor (1986) reported that out of 79 exotic and indigenous lines, four lines showed a high degree of resistance in two consecutive generations of screening. However, screening successive generations requires long periods of time. The screening technology explored by Kapoor *et al.* (1985) and Kapoor (1986), namely artificial inoculation of seedlings using ascospores on partially colonized mustard petals, helped to reduce screening time.

There are numerous examples in other situations where resistant varieties have contributed significantly to increased productivity. However, farmers in the hills of Nepal continue to grow genotypes which are susceptible to stalk rot disease such as Kibogiant, Terai-3, and Kathmandu Local. Probably, this is because resistant genotypes are unavailable or the chosen variety has other popular or suitable characteristics such as marketable size, taste or more leaves for animal feed. **Therefore, screening of cauliflower genotypes was carried out to identify sources of resistance or tolerance of cauliflower varieties currently used by, or recommended to, farmers (Chapter 7).**

1.8 CULTURAL PRACTICES

Sitepu and Wallace (1984) reported that fungicides have not controlled successfully *S. sclerotiorum* in vegetable crops in South Austarlia. Similarly Trutmann *et al.* (1980) reported that chemical control is too expensive or the level of control achieved is poor in Victoria (Australia). Under controlled conditions, soil fumigation with methyl bromide or steam sterilization can eliminate the disease (McQuilken and Whipps, 1995). Moreover, the number of effective fungicides available is gradually decreasing because of health concerns, soil fumigants are not environmentally friendly, and steam sterlization is very expensive. Therefore there is a need for additional methods to control *S. sclerotiorum*.

Research findings indicated that *Sclerotinia* disease of cauliflower and lettuce could be reduced through the addition of organic matter such as composted sewage, sunflower inflorescence residues and pine needles into soil (Millner *et al.*, 1982; Lumsden *et al.*, 1983; Lumsden *et al.*, 1986; Sharma *et al.*, 1986). According to Asirifi *et al.* (1994) incidence of *Sclerotinia* rot of lettuce increased with a run down of soil organic matter. As women in the hills are responsible for compost making, their education in the cultivation and multiplication of antagonists in compost or farmyard manure (see later) could probably contribute significantly to *Sclerotinia* disease management.

Crop rotation is a disease management option that has often been recommended for control of *S. sclerotiorum* (Shrestha, 1990). However, crop rotation may not be a

practical option for many farmers because *Sclerotinia* has a wide range of hosts and the pathogen can survive several years in the soil in the absence of host plants.

Flooding a field continuously for 23-45 days or a cycle of alternate flooding and drying led to destruction of sclerotia of *S. sclerotiorum* (Moore, 1949) in south Florida of the United States. But flooding in Bari-land (rainfed upland where most cauliflower is grown) in Nepal is impracticable.

Competition between crop plants and weeds is an important factor in growing vegetable crops. Weeds are the major problem contributing to low yields in cauliflower farming. Certain annual weeds (*Ageratum comyzoides* and *Galinsoga parviflora)* have more than more one flush of seedlings emerging during one cycle of cauliflower. Two to three sequential weedings at an interval of three weeks may be necessary to maximize the control of such weeds. Hand weeding by using a spade is a common practice. Weeding requires more than 50% of the total labour in cauliflower production and may be a significant factor in disease management strategies if weeds or weeding affect *Sclerotinia*.

Hill farmers' knowledge and perception of the effects of cultural practices on stalk rot was investigated (Chapter 3). The effect of weeds on cauliflower production is reported in Chapter 4.

1.9 POTENTIAL FOR BIOLOGICAL CONTROL OF *S. SCLEROTIORUM*

In recent years, the biological control of plant disease has been studied intensively in order to generate alternatives to chemical control. Biological control may be defined as the use of predators, parasites or pathogens to maintain an organism's population density at a lower average than would otherwise occur. The degree of population reduction achieved through use of a biological control agent (BCA) may often be enhanced through manipulation of the environment by various means to favour the BCA. Such strategies may be part of a package of measures e.g. solarization, drought and flooding, crop rotation and possibly genetic manipulation of the crop.

Biological control of *S. sclerotiorum* using micro-organisms has been explored by various researchers. The antagonist *Coniothyrium minitans* applied to soils as spore suspensions killed 85 to 99% sclerotia of *S. trifoliorum* within 11 weeks in a pot experiment in UK (Tribe, 1957). Promising biological control of *S. sclerotiorum* in some crops including cauliflower has been obtained by incorporating the mycoparasitic fungi *C. minitans, Gliocladium roseum*, G. *virens*, *Sporidesmium sclerotivorum* and *Trichoderma viride* in *Sclerotinia* infected soil (Agrios, 1988).

The fungal antagonist *C. minitans* is well documented as a mycoparasite of *S. sclerotiorum* (Turner and Tribe, 1976; Huang, 1980; Budge and Whipps, 1995; El-naggar and Vajna, 1995; Gerlagh *et al.*, 1995; Luth, 1995; McQuilken and Whipps, 1995; Sesan and Csep, 1995). *C. minitans* has the capacity to kill a large proportion of the population of field sclerotia of *S. sclerotiorum* within one month provided that moist conditions and moderate temperatures prevail (Trutmann *et al.*, 1980). *C.*

minitans also has the ability to kill hyphae of *S. sclerotiorum.* It produces cell wall degrading enzymes which ultimately cause the disintegration of the cell wall, death of hyphae and sclerotial decay (Huang and Hoes, 1976). *Sporidesmium sclerotivorum* under field tests has also shown potential for biological control of *Sclerotinia* lettuce drop (Adams and Ayers, 1982). *Teratosperma oligocladum* in a natural soil had high potential as an applied biological control agent for reducing the inoculum density of sclerotia-forming plant pathogens (Ayers and Adams, 1981). It has been reported that under greenhouse condition *C. minitans, Gliocladium catenulatum* and *T. viride* effectively destroyed the sclerotia of *S. sclerotiorum* (Huang, 1980).

Successful biological control techniques must involve delivery of viable biological control agents capable of mycelial growth and sporulation in soil after application. Inocula of antagonists have been applied in soil, on growing plants, on crop debris, and on seed. Three different formulations have been used most frequently for the field and glasshouse application of antagonists: liquid suspensions, alginate pellets and powders (Fravel *et al.*, 1985; Lewis and Papaviza, 1987).

Methods of incorporation of spores or hyphal biomass into alginate pellets, with or without nutrient source or other additive, in organic matter or in clay have been described (Fravel *et al.*, 1985; Lewis and Papavizas, 1987; Papavizas *et al.*, 1987). These application methods preserve high viability and long shelf life of the formulated agents (Fravel *et al.*, 1985; Lewis and Papavizas, 1987). *G. virens* and

T. harzianum have been produced in liquid culture and formulated as alginate pellets (Lumsden and Lewis, 1989; Knudsen *et al.*, 1991).

Biocontrol of *Sclerotinia* stem rot (*S. minor*) in sunflower by seed treatment with *G. virens* has been explored by Burgess and Hepworth (1996). Seeds were soaked in a conidial suspension of *G. virens* for two hours before planting. Results were effective in field soil as well as in pasturized potting medium. Increasing concern with soil conditions in the Nepalese hills means that biological control of soil-borne fungi by the use of indigenous micro-organisms is highly desirable. **A search for effective biocontrol agents against *S. sclerotiorum* was initiated (Chapter 8).**

1.10 OVERALL APPROACHES TO STALK ROT DISEASE MANAGEMENT

Stalk rot disease of cauliflower in the hills of Nepal is a most common and destructive disease and it has been causing considerable losses to the hill farmers. The disease is well established and can survive under drought conditions. Being soil-borne, and considering the hill situations, chemical control is not available. Crop rotation has limited application but attention should be given to the effect of other cultural practices, such as weeding, on *Sclerotinia* disease. Understanding the life cycle of the *S. sclerotiorum,* crop management practices and ecology of the biological control agents and their application methods are possibly the most important factors for stalk rot disease management in the hills of Nepal.

Objectives of this research

The long-term aim of this project is to achieve better management of stalk rot disease of cauliflower and increase the long-term productivity of cauliflower in the eastern hills of Nepal.

Specific objectives of the project were

- to explore farmers' perceptions of cauliflower diseases and the losses they cause;
- to explore the practices farmers use to manage diseases;
- to investigate potential components of integrated stalk rot disease management (screening for resistance, cultural practices);
- to identify potential biocontrol agents;
- to develop strategies for stalk rot management.

If a control programme for stalk rot disease of cauliflower were to be developed, it would improve the long-term productivity of both commercial and subsistence farmers across the hills of Nepal without adverse effects on the environment. By bringing together the results of survey activities, breeding for resistance, and experiments in biological control and cultural practices it was intended to formulate recommendations for integrated stalk rot management.

CHAPTER 2

2. INTRDUCTION TO *SCLEROTINIA SCLEROTIORUM*

2.1. LIFE CYCLE OF *S. SCLEROTIORUM*

The life cycle of *Sclerotinia sclerotiorum* is shown in Figure 2.1. The fungus forms rounded, resting structures termed "sclerotia". Sclerotia consist of compact masses of intertwined hyaline hyphae (pseudoparenchymatous tissue) enveloped by a layer of black pigmented cells (Jones and Watson, 1969). The tuberoid sclerotium is characteristic of *S. sclerotiorum*. Sclerotia of *S. sclerotiorum* are nutrient rich, the main cytoplasmic reserves present in sclerotia being glycogen, protein, polyphosphate and lipids (Willetts and Bullock, 1992). Sclerotia can remain dormant or quiescent when the environment is unfavourable and germinate to perpetuate the fungus life cycle when conditions become favourable.

Infection of susceptible host plants can occur from mycelium either through the eruptive germination of sclerotia in soil or from germinating ascospores (Purdy, 1979; Kapoor, 1986). Mycelium from either origin can cause infection of healthy plants. After infection a white mycelium develops when the environmental conditions are favourable; this mycelium kills the affected parts of the host plant. The life cycle is completed as sclerotia are produced externally and internally in the stem, pith or fruit cavity or between bark and xylem.

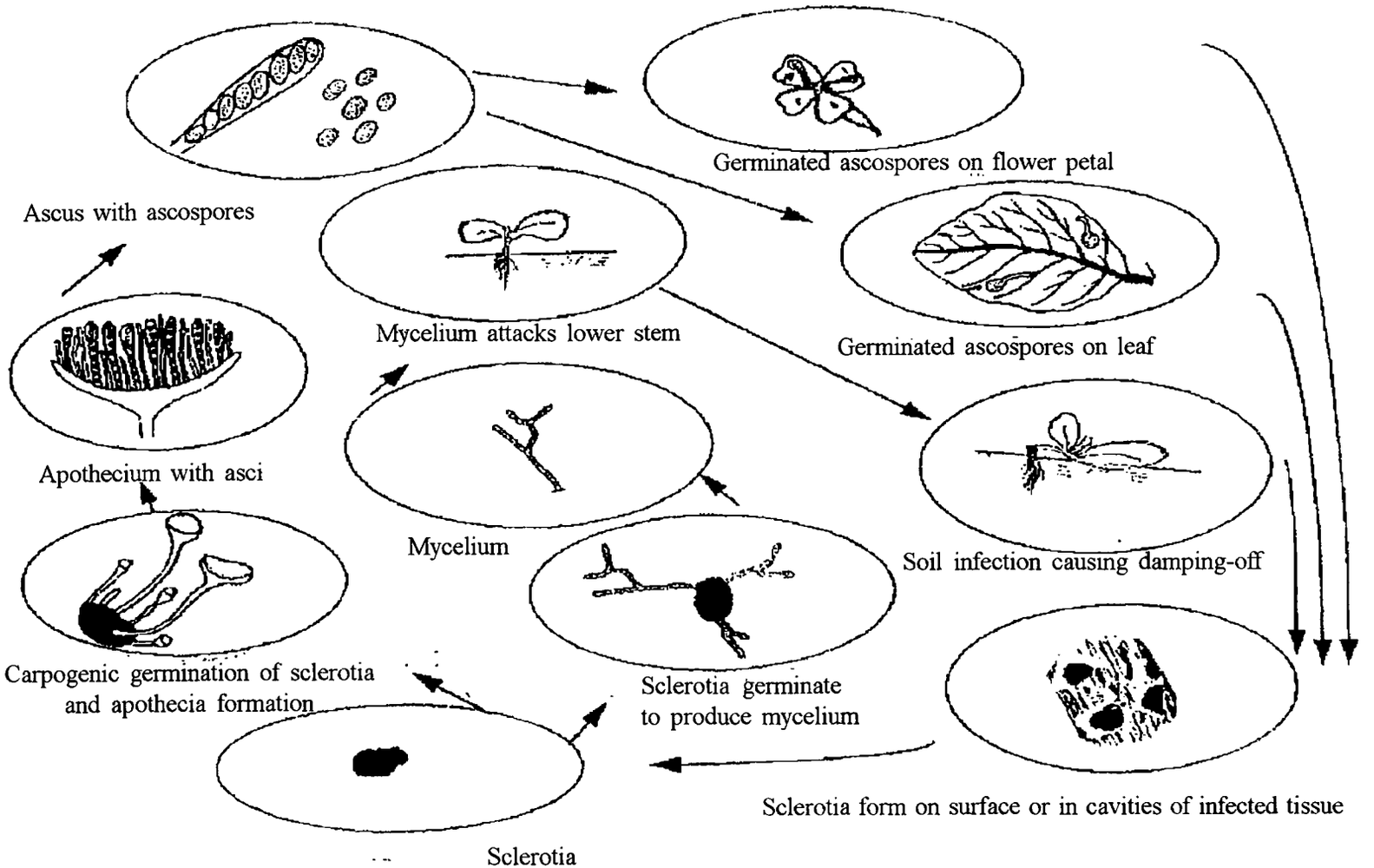


Figure 2.1 Development and symptoms of cauliflower disease caused by *S. sclerotiorum.*

(Source: Agrios, 1988; Sesan and Csep, 1995; Singh *et al.*, 1987.)

Under cool temperature and high moisture conditions, sclerotia may germinate to form long-stalked apothecia. These produce air-borne ascospores which, when released, are the main source of inoculum for plant infection (Jones and Watson, 1969; Huang, 1980). Ascospores may be produced over range of temperatures (0-25 °C), but are produced optimally at 15-20 °C (Smith *et al.*, 1988). Carpogenic germination of sclerotia has been obtained experimentally by placing the sclerotia on a substrate low in nutrients such as moist sand, cotton or polyurethane, water agar, or water (Tourneau, 1979; Mylchreest and Wheeler, 1987; Kapoor *et al.*, 1987; Singh *et al.*, 1995).

Sclerotia are of paramount importance in survival and epidemiology of the pathogen (Hoes and Huang, 1975), serving as the main over-wintering propagules (Huang, 1980). The optimum temperature for sclerotial formation in axenic culture is 22 - 25 °C. In more complex media e.g. PDA this may be higher (25- 27 °C) (Marukawa *et al.*, 1974). Approximately 90% of the life cycle of *Sclerotinia* species is spent in soil as sclerotia (Adam and Ayers, 1979). After three months in the soil, sclerotia are "conditioned" and produce apothecia (Purdy, 1979; Kapoor, 1986). There are a number of reports of sclerotia surviving for long periods, for example Kapoor (1986) quoted Brown and Butler (1936) as saying that sclerotia under favourable dry conditions can remain viable for at least 10 years. Sclerotia held under dry storage for seven year, germinated readily and produced viable ascospores (Hungerford and Pitts, 1953). Similarly, Coley-Smith *et al.* (1990) reported that sclerotia of *S. cepivorum* persisted 20 years in the absence of host plants. However, under certain conditions sclerotia may only survive for 30 weeks (Merriman, 1976).

Certainly, the survival of sclerotia in soils is very variable and depends on environmental conditions, soil moisture, soil temperature, nutrient status of the soil (particularly its organic matter content) and the presence of soil micro-organisms such as hyperparasites (Moore, 1949; Coley-Smith and Cooke, 1971; Hoes and Huang, 1975; Turner and Tribe, 1976). Soil moisture plays a vital role in the survival of sclerotia. All sclerotia were killed when soil was flooded with water for 26 to 31 days (Moore, 1949). Similarly, soil temperature is another important factor in the survival of sclerotia. According to Adams (1975) soil temperatures from 10° to 30°C did not adversely affect the survival of sclerotia, but a constant 35°C soil temperature for three weeks or more reduced their survival. Biotic factors are also important for survival of sclerotia in soil, especially antagonistic micro-organisms such as *Trichoderma* spp. and *Coniothyrum minitans* (Campbell, 1947; Tribe, 1957; Jones and Watson, 1969).

Dissemination mechanisms of *S. sclerotiorum*

The fungus is air-borne, soil-borne and seed-borne. Ascospores of *Sclerotinia* spp. are spread from field to field and from one geographical area to another by wind. Inocula in the form of mycelium or sclerotia are spread from one area to another by soil adhering to seedlings, farm equipment, animals or humans, or as mycelium in infected host tissue (Adams and Ayers, 1979; Shrestha 1985a). Adams and Ayers (1979) quoted Dillon-Weston *et al.* (1946) as showing that on farms where diseased plant tissue is used as cattle feed or bedding, the spreading of manure onto fields may be a source of infection. However, less than 2% of sclerotia of *S. sclerotiorum*

passing through the digestive tract of sheep remained in a viable condition (Brown, 1937). Irrigation water is also a source of sclerotial dissemination (Steadman *et al.*,1975). However, the greatest potential for long-distance dissemination of *S. sclerotiorum* in cauliflower appears to be as seed-borne inoculum, either by seed infected with mycelium or by seed contaminated with sclerotia (Neergaard, 1958).

Mode of infection

The infection process of *S. sclerotiorum* was first investigated by de Bary (1887). The sclerotia germinate by production of mycelia or apothecia. Mycelial infections are initiated at the soil line resulting in damping-off or basal stem rot, or by root contact with mycelium germinating from sclerotia (Huang and Dueck, 1980; Purdy, 1979). Buried seeds infected with mycelium may not germinate and those that do often fail to reach the soil surface (Hungerford and Pitts, 1953). *S. sclerotiorum* overwintering on the soil surface or in the seed as mycelium in may not initially appear to be an. Transmission is usually indirect. Sclerotia in the soil (which may originate from the debris of previous crops or as contaminants associated with the seed) germinate to produce ascocarps from ascospores are discharge and subsequently infect growing crops (Maude,1996). Where sclerotia are in contact with the stem bases of plants, mycelial transmission may occur (Scott and Evans,1984) Once in the soil, *S. sclerotiorum* mycelium will colonise seed and seedlings resulting ultimately in the production of more sclerotia (Blodgett, 1946).

Infections above ground are initiated by ascospores (Abawi and Grogan, 1979; Boland and Hall, 1987). *S. sclerotiorum* ascospores normally infect susceptible

plants only if a saprophytic food base is available in the infection court, typically during flowering by fallen petals lodged in the leaf axis (Morrall and Dueck, 1982). Previous studies had indicated that a suitable nutrient-rich medium such as dead petals was necessary for ascospore germination and subsequent penetration into host tissues (Abawi and Gorgan, 1975; Abawi *et al.*, 1975; Cooke *et al.*, 1975; Purdy, 1979). If conditions are not suitable for germination, the ascospores may remain viable for a short time and germinate when conditions again become favourable (Purdy, 1979).

Inglis and Boland (1992) reported that viable inoculum of *S. sclerotiorum* could be isolated from field-collected petals of rape seed (*Brassica napus*) and faba beans (*Vicia faba*). This findings was supported by an experiment carried out by Jamaux *et al.* (1995), in which ascospores were inoculated in leaf discs of rape seed, rape seed petals and petals placed on leaf discs. The results indicated that ascospores failed to germinate after three hours on the leaf surface, but ascospores on petals germinated, giving rise to one short germ tube each. Similarly, ascospores that had landed naturally on the petal surface germinated and penetrated (Purdy, 1979).

2.2 TAXONOMY AND NOMENCLATURE

Sclerotinia sclerotiorum the causal fungus of cauliflower stalk rot disease

Sclerotinia sclerotiorum (Lib.) de Bary belongs to the Ascomycota order Leotiales family Sclerotiniaceae (Hawksworth *et al.*, 1995). The synonyms of the fungus are *Peziza sclerotiorum* Libert (1837), *S. libertinia* Fuckel (Wakefield, 1924), *Whetzelinia sclerotiorum* (Lib.) Korf and Dumont (Korf and Dumont, 1972), *S.*

minor Jaggar (Jaggar, 1920) and *S. trifoliorum* Erikss. (Eriksson, 1880). *Sclerotinia sclerotiorum* was first described as *Peziza sclerotiorum* by Madame M A Libert in 1837 (Libert, 1837). Fuckel (1870) created and described the genus *Sclerotinia* by renaming *Peziza sclerotiorum* with a newly coined binomial *Sclerotinia libertinia*. However, this binomial was inconsistent with the International Code of Botanical Nomenclature. Replacing *S. libertinia* with *S. sclerotiorum*, Wakefield (1924) cited G E Massee as the proper authority (*S. sclerotiorum* (Lib.) Massee) as he had used that binomial as early as 1895. However, the same binomial was used as early as 1884 by de Bary (de Bary, 1884). This should be cited as the proper authority for this fungus (Wakefield, 1924). *S. sclerotiorum* (Lib.) de Bary was confirmed as the type species of *Sclerotinia* in the reappraisal of the genus and related fungi by Whetzel (1945), by Korf and Dumont (1972) and by Buchwald and Neergaard (1973; 1976). The names *Sclerotinia minor* Jaggar and *Sclerotinia trifoliorum* Erikss. are also commonly found in the taxonomic literature. The former was described by Jaggar (1920) for isolates with small sclerotia from lettuce, celery and other crops in New York State. The latter was used by Eriksson (1880) for the pathogen of clover stem rot. However, both the pathogens *S. minor* Jaggar and *S. trifoliorum* Erikss. are considered as identical to *S. libertinia* (*S. sclerotiorum*) (Smith, 1900). Irrespective of taxonomy, the three species, *S. sclerotiorum, S. trifoliorum,* and *S. minor* have been studied extensively due to their economic importance (Willetts and Wong, 1980).

Measurements of asci and ascospores are quite variable for different isolates of *S. sclerotiorum*. According to Saccardo (1889), Libert gave ascus and ascospore

measurements of 130-135 x 8-10 μm and 9-13 x 4-6.5 μm, respectively. Purdy (1955) quoted work of Ramsey (1925) and stated that twenty-eight isolates were identified as *S. sclerotiorum* primarily on sclerotium size, but the ascus and ascospore ranged far beyond the limit set forth in the original description. The length of asci varied from 81.0 to 199.4 μm and width from 4.3 to 12.4 μm. Similarly, the length of ascospores varied from 6.0 to 17.0 μm and width from 2.0 to 8.4 μm (Purdy, 1955). However, all isolates were tentatively identified by Purdy (1955) as *S. sclerotiorum* on the basis of sclerotium size and host association. Almost all isolates so identified were of the large sclerotium type (3-10 x 3-7 mm). The overall size range of sclerotia of *S. sclerotiorum* is 1 to 30 mm (Davis, 1925). The size of sclerotia formed is influenced by temperature; smaller sclerotia are produced at low temperatures (c. 10 °C), larger ones at higer temperatures (c. 25°C). All species retained in *Sclerotinia* show a positive reaction (turning blue) of the ascus pore channel wall in Melzer's Iodine (Khon, 1979).

2.3 HOSTS RANGE

S. sclerotiorum has been reported on a remarkable 75 plant families, 278 genera and 408 species. Except for *Rumohra adiantiformis* in the Pteridophyta, all hosts of *S. sclerotiorum* occurred within the plant division Spermatophyta. Within the Spermatophyta hosts are recorded from the classes Gymnospermae and Angiospermae. Families that contain large number of hosts are reported to include Asteraceae, Fabaceae, Brassicae, Solanaceae, Apiaceae and Ranunculaceae, in decreasing order of host number (Boland and Hall, 1994). On the other hand, Willetts and Wong (1980) reported that the main hosts occur in the families

Solanaceae, Cruciferae, Umbelliferae, Compositae, Chenopodiaceae and Leguminosae. Amongst crops, the fungus attacks mostly vegetables and oilseed crops. Partyka and Mai (1962) referring to a thesis by Dickson (1930), indicated that 172 species from 118 genera in 37 plant families were known to be susceptible hosts, while in a review by Purdy (1979) *S. sclerotiorum* occurred on 225 genera, 361 species and 22 other taxa (cultivars) for a total of 383 species and other categories distributed in 64 plant families. Farr *et al.* (1989) listed 148 genera of plants that are susceptible to *S. sclerotiorum*. Whatever the exact numbers of hosts, it is clear that *S. sclerotiorum* is a most successful pathogen in term of host range. Equally *S. sclerotiorum* is found in a wide range of climatic conditions.

2.4 GEOGRAPHIC DISTRIBUTION AND ECONOMIC IMPORTANCE

S. sclerotiorum has been reported from many countries in almost all continents (Purdy, 1979). Most typically it occurs in the relatively cool and moist areas of the world, but it can be found in both hot and dry areas (Purdy, 1979; Jones and Watson, 1969) and in temperate and subtropical climates (Zazzerini and Tosi, 1985). As with most other plant pathogenic fungi *S. sclerotiorum* is much less active when the temperature approaches freezing point (0 °C) or when a temperature of more than 32°C prevails (Purdy, 1979). Spores landing on potential hosts need water for germination. Germination of spores are possible from 0 to 25 °C, with an optimal temperature at 15 to 20°C (Smith *et al.*, 1988).

In India, the disease was first reported in 1973 in cauliflower in the Saproon valley (Anonymous, 1974). The rate of incidence of the disease has increased year on year

and the disease has reduced seed yields by 80 to 90% in affected fields (Sharma, 1979). Likewise, in Nagaland the pathogen caused a yield loss up to 50% of khol rabi (knol-khol) while in other cole crops such as cabbage and cauliflower reduction of up to 100% of seed yield occurred (Singh *et al.*, 1987). Sharma and Kunwar (1989) reported that white rot (*Sclerotinia* rot) can cause up to 50.8% reduction in the green-pod yield of the garden pea under hill conditions.

2.5 DISEASES AND SYMPTOMATOLOGY

The symptom expression in diseases caused by *S. sclerotiorum* vary according to host and host part affected as presented in Table 2.1.

Table 2.1 Diseases caused by *Sclerotinia* on different hosts and host parts

Host	Name of disease
Bean, cabbage, carrot, eggplant, citrus, peanut, potato, stock and tobacco	Cottony rot, white mould or watery soft rot
Cucumber, squash, bean, artichoke, asparagus, chrysanthemum, dahlia, delphinium, peony, potato, tomato, soyabean and sweet potato	Stem rot and tuber rot
Lettuce, broad bean, beet and cabbage	Drop
Celery and lettuce	Damping off
Columbine and snapdragon	Crown rot or wilt
Narcissus and Camellia	Blossom blight
Pepper	Pink joint of red pepper
Hollyhock	Stem canker
Clover	Root and crown rot
Fruits, apricot and fig	Root rot, basal stem canker, head rot and wilt, fruit rot, green fruit rot of apricot, limb blight of fig

Source: Agrios, 1988; Huang, 1980; Purdy, 1979.

Symptoms caused by *Sclerotinia* rot in cauliflower vary according to the host part affected such as damping-off or basal stem rot in the seedling stage, dark brown to necrotic lesions on leaves, dark brown to black rotting on petioles, brown to dark brown soft rot in curd (Purdy, 1979; Huang and Dueck, 1980; Gupta and Dohroo, 1993). One can conclude that *S. sclerotiorum* can infect any part or any stage of the cauliflower plant and cause the expression of many symptoms.

CHAPTER 3
SOCIO-ECONOMIC SURVEYS OF FARMERS' PERCEPTION OF CAULIFLOWER DISEASE MANAGEMENT

3.1 Background

In the eastern hills of Nepal the farming systems are diverse and mostly semi-subsistence. Cropping patterns vary both within and across physical environments, depending on the needs of the family and resources available e.g. irrigation, market facilities, seeds and other inputs.

The main vegetable crops grown on the eastern hills are cabbage, cauliflower, broadleaf mustard, radish, peas and beans. According to the author's unpublished survey work at ARS Pakhribas since 1988 cauliflower cultivation during the last 10 years has increased tremendously. This was mainly due to a market with good prices, the availability of production technology and suitable varieties of seeds. Newly established non-governmental organizations (NGOs) are facilitating the formation of community-based co-operatives, increasing the market potential of local farmers' produce. As a result of expansion of the cultivated area, introduction of different varieties and continuous cultivation of cauliflower, disease problems have increased. Preliminary survey results carried out by the ARS Pakhribas indicated that a large number of pests and diseases cause crop losses (Duwadi *et al.*, 1997).

In such a situation, management strategies targeting a single disease may not be sufficient to minimise crop losses. Furthermore, farmers are ultimately the real

managers of pests, and farmers within different socio-economic groups and altitude zones perceive and tackle disease problems differently. Gender issues are also important in the generation and verification of integrated pest management technology. In order to focus on farmers' needs, socio-economic surveys of farmers' perceptions of cauliflower disease management were carried out with the following objectives:-

- to estimate farmers' perceptions of losses caused by diseases
- to determine the impact of farmers' practices on stalk rot management

A market price study was also undertaken to determine the reasons for price change and its implications for cauliflower production and disease management.

3.2 Materials and methods

In the villages of the eastern hills, only farm households that were involved in growing cauliflower were included in the surveys. A participatory methodology was adopted to collect the data whereby farmers played important roles in almost all the survey activities. A total of 72 individual and groups interviews were conducted during 1997 and 1998. The survey was based on draft interview techniques originally developed by a team of NRI natural and social scientists during 1995 and subsequently modified. The actual methods used are described in detail below and in Appendix 1.

Review of preliminary data and information

Before starting the collection of primary data from farmer interviews, secondary data from a number of sources on various aspects of cauliflower production, disease

management and farmers' perceptions of cauliflower diseases were collected and reviewed including:-

- outreach site profiles of the Outreach Division, ARS Pakhribas,
- annual reports, research reports and records of ARS Paripatle, Dhankuta,
- reports from the Koshi Hills Seed Entrepreneurs Association (KOSEPAN), Seed Sector Support Project (SSSP), Centre for Environmental and Agricultural Policy Research Extension and Development (CEAPRED) and Sindhuwa community-based farmers co-operative,
- a review of similar types of survey performed by the researcher during his earlier work at ARS Pakhribas.

Survey sites

The surveys involved interviews and field observations during normal and off-season fresh and seed cauliflower production. The surveys sites were villages from Village Development Committees (VDC) of Terhathum and Dhankuta districts of the eastern hills of Nepal. These are major commercial and semi-commercial cauliflower production districts, and because of outbreaks of *Sclerotinia*, yield losses in these districts are very high. The VDC is the smallest unit of local government in Nepal. It has a population of between 2,000 and 10,000 and has nine constituent wards. VDC as a political boundary may cover a wide range of topography and different villages in the hills. Only nine villages in the VDCs of these two districts were selected for the survey, but farmers interviewed and fields observed are representative of almost all potential areas where cauliflower is grown either for

fresh vegetable or for seed production in the eastern hills of Nepal. The location of the survey districts are indicated in Figure 3.1.

Most of the survey sites are not accessible by roads. The nearest sites of Dhankuta district are accessible by about two hours walking from the road head. The farthest survey sites of the Terhathum district are about 10 hours normal walking (with light loads) from the road heads. The VDCs of the survey sites in these two districts are presented in Table 3.1.

Table 3.1 Surveyed VDC of Dhankuta and Terhathum Districts

District	Village development committee (VDC)
Terhathum	Low, mid and high altitudes of Terhathum district: Oyakjung and Atharai Sakranti VDCs
Dhankuta	Low, mid and high altitudes of Dhankuta district: Phalate, Parewadin, Murtidhunga, Chumbang, Guthitar and Belhara VDCs and Dhankuta municipality.

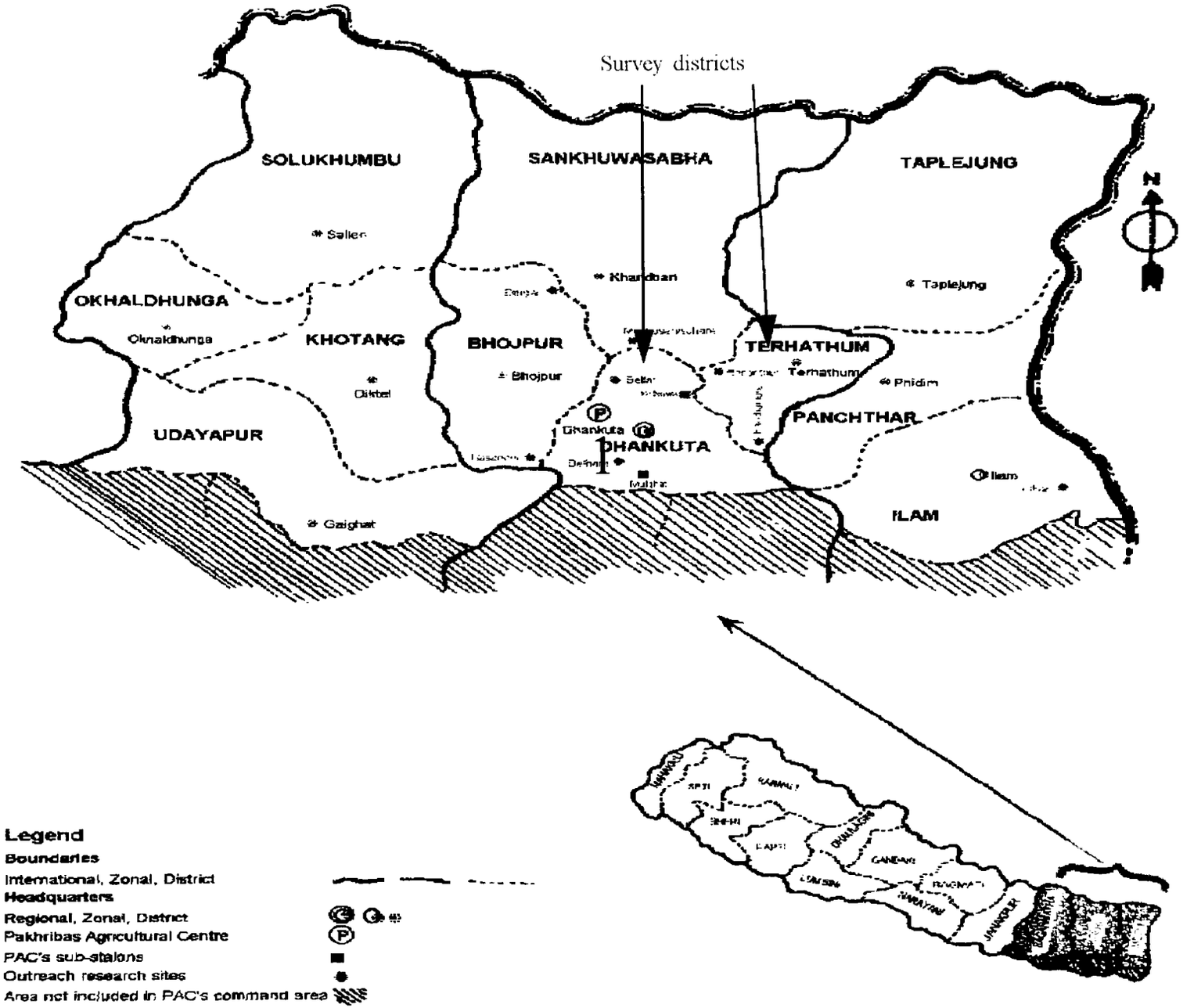


Figure 3.1 Map of ARS Pakhribas command areas showing survey districts.

Survey timing

Surveys were conducted in three different cropping seasons as given below. Surveys were not made in June and July because this is the peak rainy season in the eastern hills and most of the farmers are involved in priority work such as potato harvesting and rice planting. The first survey season was April to May, during which period seed crops are harvested. It is easy to observe and validate diseases while a seed crop is standing in the field. The second season was August to November; during this season most farmers grow cauliflower for off-season production to catch the early market (high prices); seedlings are also raised for the normal season December to March during which season farmers grow cauliflower as a seasonal crop for fresh vegetable production. In the latter season, seed crops are at flowering to bolting stage and seedlings are raised for year-round production.

Questionnaire Development

In 1995, the Overseas Development Administration (ODA), now Department for International Development, was asked by the ARS Pakhribas to provide expert advice on conducting a survey of farmers' perceptions of crop pests and diseases in the eastern hills. Two different teams comprising natural and social scientists from Natural Resources Institute (NRI) visited ARS Pakhribas in February, and June - July 1995 respectively. They helped to develop draft guidelines for a survey to prioritize crop pests and diseases in the ARS Pakhribas command area (with the help of counterpart staff of ARS Pakhribas and NARC). The draft guidelines and semi-structured interview check lists developed by the team were then modified for the survey reported here (Appendices 1, 2 and 3).

Conduct of Interviews

Farmers were selected (Appendices 1 and 5) and interviewed (Appendices 2 and 3) in the low, mid and high altitude sites of Terhathum and Dhankuta districts where cauliflower crops were grown extensively. Surveys sites were visited and key informants contacted to locate cauliflower growers.

The interviews were conducted in two stages: (a) informal interviews leading to prioritization of problems, and (b) completion of a check list on stalk rot disease of cauliflower. The questionnaire used to prioritize problems of cauliflower provided information on:

- Cropping system
- Major diseases of cauliflower
- Prioritization of these diseases
- Disease occurrence pattern
- Farmers' knowledge and practices of disease management
- Farmers perceptions regarding the introduction of new varieties and disease management practices.

The checklist on *Sclerotinia* stalk rot disease of cauliflower provided information on:

- Variety differences
- Seed sources
- Disposal methods of diseased crop debris
- Use of compost
- Methods used for stalk rot disease management.

At the beginning of the surveys photographs of diseased cauliflower were taken and used for further work. Photographs of several diseases were shown to the farmers to assess their knowledge of different diseases and to differentiate between types of rot caused by *Sclerotinia*, bacteria, and downy mildew (Figure 3.2). Another photograph (Figure 3.3) illustrating different symptoms produced by *Sclerotinia* was shown during the specific interviews about *Sclerotinia* disease. Farmers' interviews were followed by field visits.

For the interviews, farmers were categorised according to food sufficiency from on-farm production for the last crop year (Appendix 4). For ease of analysis, data from interviews with farmers from categories A+B and C+D were combined.

A total of 34 individual male, 15 individual female farmers, 12 male groups and 11 female groups were interviewed. Details of sample size and their domains are presented in Appendix 5. The EPI INFO software package was used to design the questionnaire and checklists and for recordings and analysis of the qualitative and quantitative data obtained. Data could be easily transferred from EPI INFO to spreadsheet or statistical packages for further analysis.

Bacterial rot

Downy mildew rot

Sclerotinia rot

Figure 3.2. Three different types of rot of cauliflower.

Figure 3.3 Stalk rot (*Sclerotinia sclerotiorum*) symptoms at different stages of cauliflower development (arrows indicate the sites of infection)

Co-ordinated assessment of incidence of disease and crop loss

Based on preliminary surveys it was noted that farmers were able to assess economic loss by semi-quantitative methods. Farmers at the survey sites were familiar with the concept of incidence, perceiving that the proportion of diseased plants can be recorded as a percentage figure. Furthermore farmers perceived that each unit of plant infection (1%) was equivalent to 1% reduction in economic value. This method of economic analysis of loss may be applicable in the case of curd rotting and the number of curds harvested but in the case of seed production or yield of curds in terms of weight, the above methodology is inaccurate as it ignores variation in curd or seed weight. Also there may be the possibility of compensation in a crop with internal competition. Methods used by farmers and researchers to assess incidence of the various target diseases in the field are presented in Table 3.2.

Table 3.2 Methods used to assess disease incidence (% plants infected) in the field

Disease	**Method of assessment of incidence, % of plants or seedlings**
Stalk rot (*Sclerotinia sclerotiorum)*	showing curd rotting, browning/whitening of branches with their siliqua (pods) at the time of seed maturity.
Downy mildew (*Peronospora parasitica*)	showing disease symptoms such as light leaf spot, browning of curds.
Soft rot (*Erwinia carotovora* subsp. *carotovora*	producing the symptoms of soft rot with bad odour.
Black rot (*Xanthomonas campestris* subsp. *campestris)*	producing the symptoms of black rot, no bad odour.
Alternaria leaf spot	having more than 50% of leaves showing dark leaf spot.
Damping-off	in the nursery with girdling / toppling down.

Data on disease incidence recorded during interviews were confirmed by field visits and sometimes samples were taken to laboratory for further investigation (refer to Chapter 5).

3.4 Survey results and discussion

Varietal selection

Overall the Kathmandu Local variety of cauliflower was the most popular grown in the eastern hills. Results revealed that 62.5% of the respondent farmers grew Kathmandu Local (Table 3.3). Snowcrown was also a popular variety, but only at the high altitude zone. A total of 20.8% of all the respondents and 50% of the high altitude farmers grew Snowcrown. Although the seed of Snowcrown is very expensive, the variety is suitable for off-season production. The variety Kathmandu Local has a number of desired characters, such as marketable size and preferred taste superior to newly introduced hybrid varieties such as Snowcrown or Snowmistique. Kathmandu Local is also slightly earlier (15 days) than the cultivar Snowball-16 in seed crop maturity. Generally, the monsoon rains start one month earlier in the eastern region than in the west. As a result, the seed crop of Kathmandu Local in the eastern hills can be harvested before onset of the monsoons. Despite Kathmandu Local being a very good cultivar for normal season production, farmers are looking for an alternate variety suitable for off-season production (Figure 3.4), so they can harvest early and sell their produce at a good price when there is a scarcity of cauliflower in the Terai and adjoining areas of India. Consequently, farmers of the survey sites are concerned about time of harvest in addition to productivity.

Table 3.3 Frequencies of cauliflower variety grown by the 72 respondent farmers at three different altitudes

Variety	Number of respondents			Total %
	High altitude	Mid altitude	Low altitude	
Kathmandu Local	11	18	16	62.5
Kibogiant	1	6	1	11.1
Snowcrown	12	0	3	20.8
Pusadeepali	0	0	1	1.4
Terai-3	0	0	3	4.2

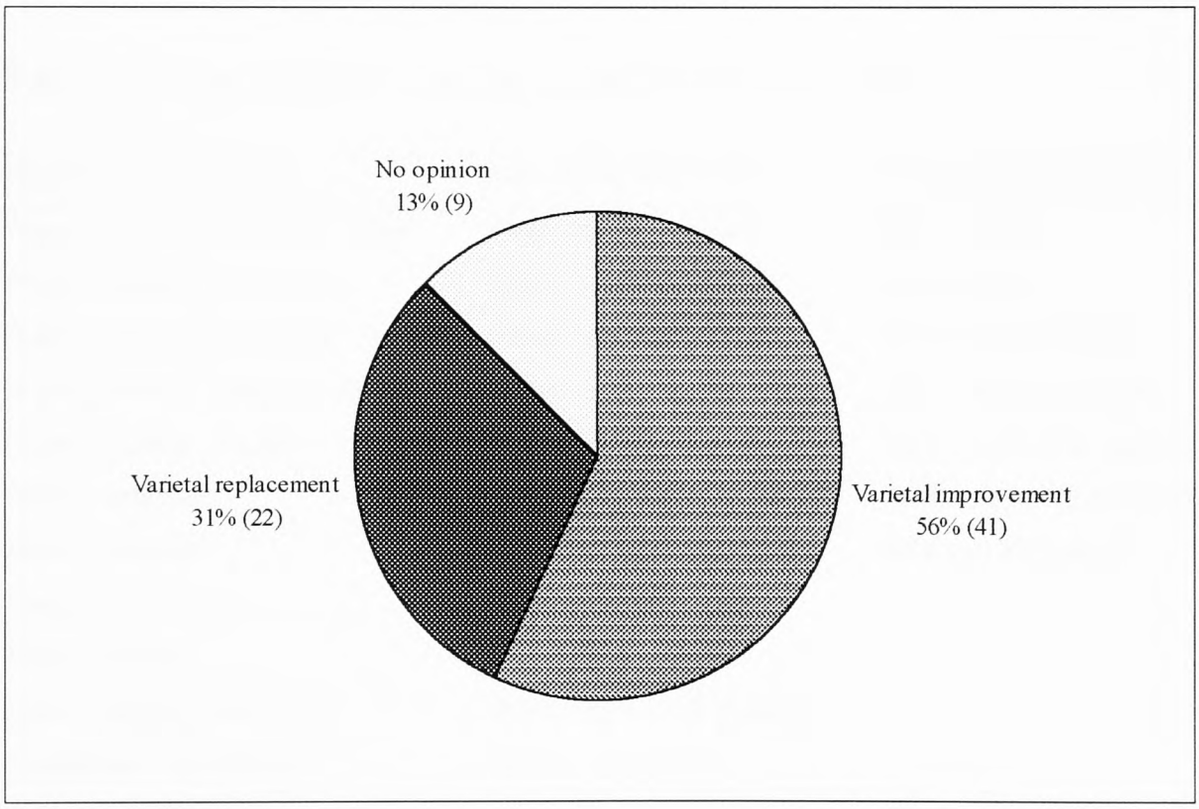


Figure 3.4 Farmers' choices for the improvement of cauliflower production in the eastern hills of Nepal (n=72).

Crop rotation and cropping systems

The survey of cropping patterns in the eastern hills summarized in Table 3.4, showed that usually maize is inter-cropped with potato followed by vegetables, cauliflower followed by cauliflower and cabbage followed by cabbage in the high altitude Bari-land. However, continuous cropping of cole crops is also practised. In the mid altitude Bari-land maize - vegetable cropping systems also predominate. By contrast in the mid altitude Khet-land farmers grow rice followed by wheat, maize, vegetables or fallow. In the high altitude zone cabbage and cauliflower are the most important vegetable crops, followed by potato. The continuous cropping of cole crops is likely to lead to inoculum build-up in the fields.

Table 3.4 Crop rotation or cropping sequences at the survey sites

High altitude Bari-lands	Mid altitude Bari-lands	Mid altitude Khet-lands
Potato + maize / soyabean - fallow	Maize / millet - fallow	Rice - wheat
Potato + maize - pea - fallow	Maize + soyabean - fallow	Rice - fallow
Potato + maize / cauliflower - fallow	Maize - mustard - fallow	Rice - maize -fallow
Potato + maize / cabbage - fallow	Maize - winter potato - fallow	Rice - mustard - fallow
Potato + maize - mustard	Maize / millet / niger	Rice - cauliflower seed and
Potato - cabbage	Maize + local pulse - fallow	fresh vegetable production
Maize - mustard	Maize - cauliflower - fallow	Rice - winter potato
Maize - pea	Maize - cabbage - fallow	
Maize - wheat	Maize - pea - fallow	
Maize / millet - fallow	Maize - broad leaf mustard	
Cauliflower - cauliflower	Maize - vegetables	
Cabbage - cabbage		

Seed sources

Because of the potential for year-round production of cauliflower in the hills, various traders and seed entrepreneurs (Table 3.5) are importing different varieties of

cauliflower seeds according to farmers' demand and their business interests without considering quarantine regulations. The statistical distribution of the different sources of seed was analysed by the Chi-square (X^2) test. The significant variation in seed sources suggests there may be consequent variation in quality and health of seed available to farmers (Table 3.5). On this basis a separate study was set-up to determine the effect of seed quality on cauliflower production (see Chapter 6).

Table 3.5 Source of cauliflower seeds obtained from 72 eastern hill farmers analysed by X^2 test

Seed sources	Frequency
Agricultural Inputs Corporation	3
Farmer to farmer	5
Co-operative/ group organiser	38
Local Hat Bazaar	11
Koshi Hills Seed Programme	1
Pakhribas Agricultural Centre	7
Paripatle Horticultural Research Station	7
Chi-square (X^2)	**

** = significantly different at 1% level.

Farmers' general perceptions of cauliflower diseases

Qualitative information on cauliflower diseases obtained during the field surveys are grouped together and summarized below. Farmers at the survey sites had no clear ideas about the pattern of disease occurrence in cauliflower. Generally, however high and mid altitude farmers perceived that:

- Diseases of cauliflower appear in patches in the field and cause considerable losses every year.
- Soil moisture plays an important role in enhancing cauliflower disease. Wet and

moist condition when there is fog in the environment causes severe attacks of *Sclerotinia* stalk rot disease in the field and damping-off disease in the nursery.

- Growing of cauliflower followed by cabbage or vice versa in the same field leads to severe disease in the following crop.
- Weeding or manipulating the soils around plants also increases disease in cauliflower and cabbage.
- Use of locally available poor quality seeds are another source of diseases.

Low altitude hill farmers perceived that:

- Crop variety is one of the important factors affecting disease in cauliflower. For example cauliflower variety Terai-3 and Kibogiant are suitable for seed production in the low hills but are highly susceptible to stalk rot disease. If these varieties are grown in a multi-cropping sequence of cauliflower - cereals - cauliflower, there will be severe attacks of disease in cauliflower crops. In other words, a simple crop rotation is not effective.
- Seeds or seedlings are a primary source of disease.

Farmers were less concerned about diseased debris left in the field after harvesting the crop and in manure prepared from diseased plants. Organic manure (farm yard manure and compost) from animal husbandry was used in almost 100% of the holdings in the survey sites, almost all farmers using sufficient quantities of organic manure to fertilize their cauliflower fields.

Farmers' prioritization of cauliflower diseases

The prioritization was made by farmers considering all diseases in their cauliflower fields over the previous five years. Ranking was in order of impact, starting with diseases with the most impact.

Overall analysis of prioritization data (Table 3.6) indicated that stalk rot (61.2%) followed by damping-off (37.5%) and then downy mildew (1.3%) were accorded top priority. For second priority disease problems the order was damping-off (40.7%), stalk rot (27.8%), *Alternaria* leaf spot (22.2%), downy mildew (7.4%) and bacterial rot (1.9%). For third priority disease problems the order was *Alternaria* leaf spot (40%), stalk rot (33.3%), damping-off (13.3%) and downy mildew (13.3%).

Farmers say crop losses caused by stalk rot disease were much higher in seed crops than in fresh vegetable crops. Damping-off disease also seemed to be one of the main problems in seed beds / nurseries.

Table 3.6 First, second and third priority diseases of cauliflower based on responses from 72 farmers at three different altitudes

Disease	First priority disease		Second priority disease		Third priority disease	
	Responses		Responses		Responses	
	number	%	number	%	number	%
Alternaria leaf spot disease	0	0	12	22.2	6	40
Bacterial rot disease	0	0	1	1.9	0	0
Downy mildew disease	1	1.3	4	7.4	2	13.3
Damping off disease	27	37.5	22	40.7	2	13.3
Stalk rot disease	44	61.2	15	27.8	5	33.3

Overall stalk rot was ranked as the top priority disease (61.2% of the respondents) with the most impact followed by damping-off (37.5% respondents). Damping-off was also ranked as the most frequently mentioned second priority disease (40.7% respondents) followed by stalk rot (27.8% respondents). This therefore indicates that both stalk rot and damping-off are important diseases.

Farmers' practice of disposal methods and alternate use of diseased plants

Various methods of destructive disposal or alternative use of diseased plants were found. Analysis with the Chi-square test indicated that there are significant differences between destructive disposal methods alternative uses of diseased plants (Table 3.7).

Survey results indicated that the majority of the farmers are neither familiar with appropriate disposal methods nor the proper alternative use of diseased plants.

The majority of the farmers build up disease inoculum by disposing of diseased plants in compost pits and by dumping farm refuse and diseased plant tissue, used as cattle feed and bedding materials, in their compost heap or pit. Generally, farmers do not wait for complete decomposition of the farm refuse in a compost pit or heap. They seem to aim for a large quantity of compost at planting time rather than high quality, properly decomposed compost.

Similarly, inocula are also built up by feeding diseased plants to farm animals (sheep, goat, cattle and buffaloes) and then using their faeces as manure. It was reported by Brown (1937) that less than 2% of sclerotia of *S. sclerotiorum* passing through the digestive tract of sheep were recovered in a viable condition, but given the number of sclerotia, even this level is potentially serious for disease multiplication.

Table 3.7 Destructive disposal methods and alternative uses of diseased plants by 72 eastern hill farmers

	Frequency of methods reported	
	Destructive disposal	Alternate uses
Animal feed	0	64
Burning	18	1
Composting	46	6
Human consumption	0	1
Left in the field	8	0
Chi-square (X^2)	**	**

** = significantly different at 1% level.

Farmers' valuation of economic losses

Analysis of the maximum economic losses (%) of cauliflower crops grown for both seed and curd production due to first priority diseases as reported by the farmers and collateral field assessment indicated maximum losses of 71-80%, 71-80%, and 41-50% in high, mid, and low altitudes respectively (Figure 3.5). However, the modal classes of maximum economic loss due to first priority diseases in high, mid and low altitudes were 41-50%, 21-30% and 21-30% respectively. Particularly, stalk rot and damping-off diseases cause economic losses up to 71-80% (Figure 3.6).

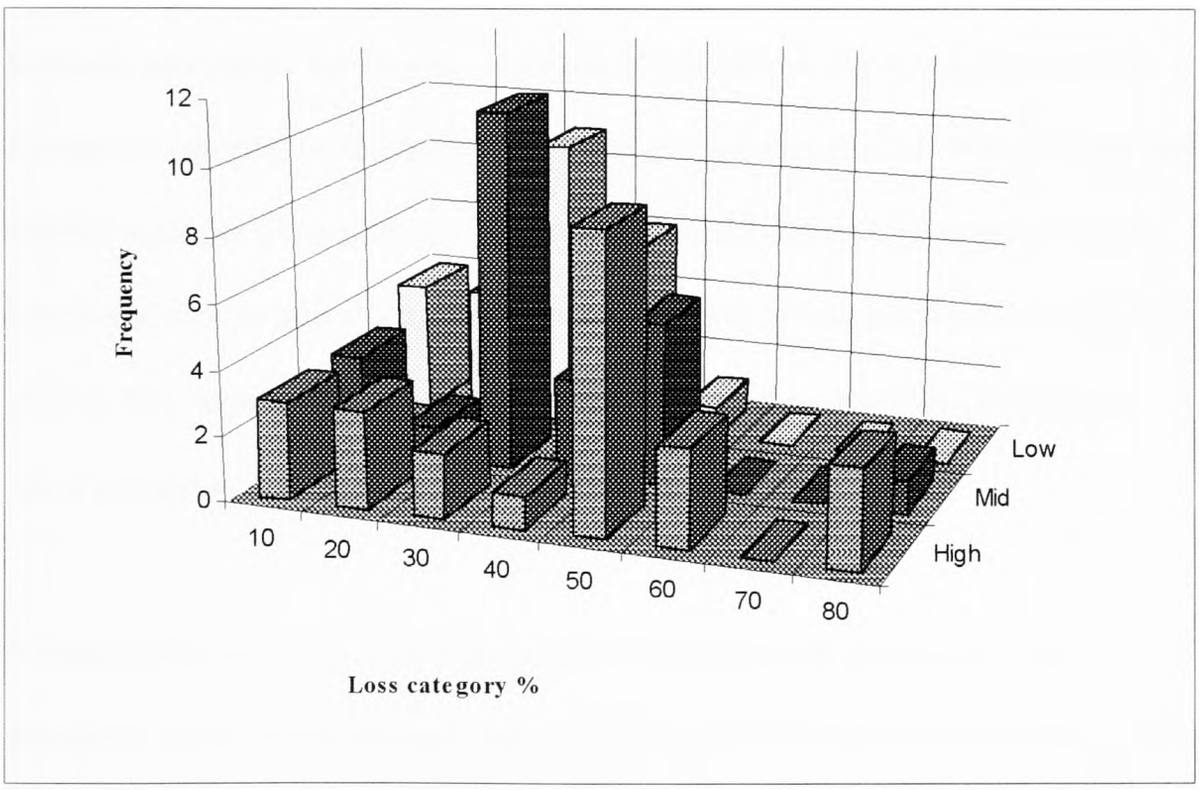


Figure 3.5 Frequency of the maximum economic loss % caused by first priority diseases in cauliflower based on 72 farmers' perceptions at three different altitudes.

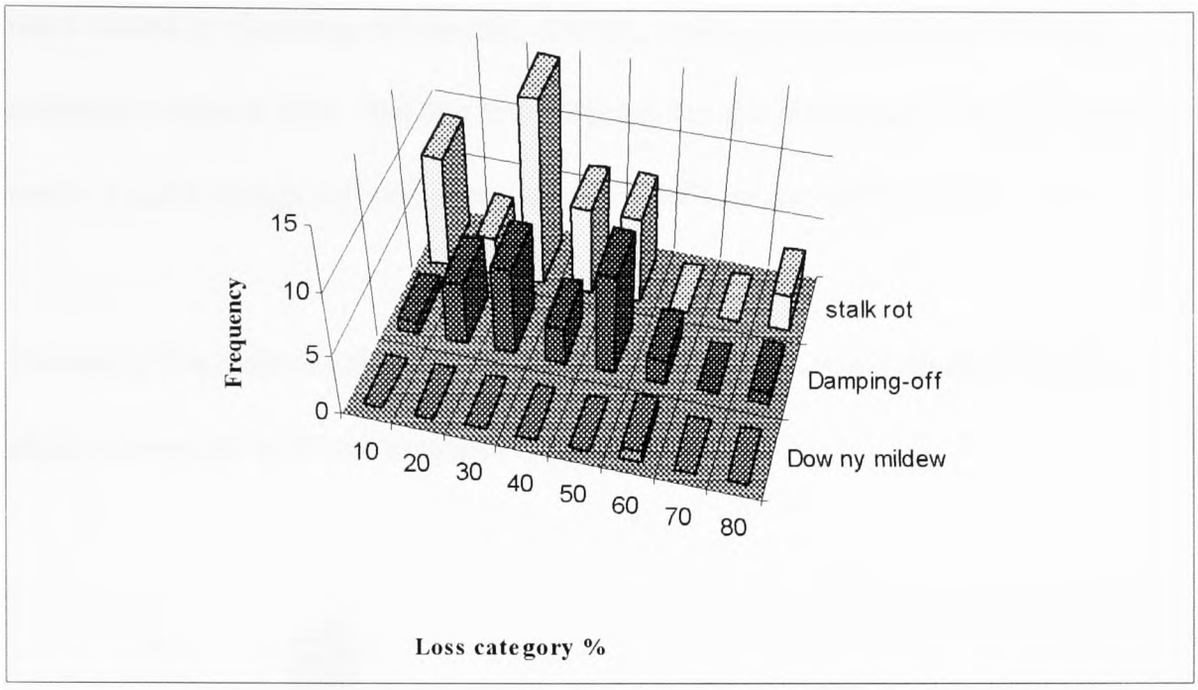


Figure 3.6 Frequency of the maximum economic loss % caused by first priority diseases in cauliflower based on 72 farmers' perceptions.

Similarly, analysis of the average economic losses (%) of crop due to first priority diseases as reported by the farmers indicated average losses of 31-40%, in high, mid, and low altitudes (Appendix 6). However, the modal classes of average economic loss due to first priority diseases in high, mid and low altitudes were 0-10%, 0-10% and 11-20% respectively. Damping-off and stalk rot diseases were estimated to cause average economic losses up to 40% (Appendix 7).

A comparision (Figure 3.7) of the maximum and the average economic loss frequency reported indicates that the maximum economic loss caused by first priority disease lies in between 21 to 30% whereas the average economic loss is lies in between 1 and 10%.

Overall, maximum economic losses of 21 to 30% were caused by stalk rot disease in mid and low altitudes. But in high altitude maximum economic losses of 41-50%

were caused by damping-off disease. Mostly, stalk rot disease caused average economic losses in high, mid and low altitudes but the magnitude of economic losses were 11-20% in high and mid altitudes and 0-10% in low altitude.

Therefore this indicates that damping-off is the most important disease in high altitude zone and stalk rot in mid and low altitudes.

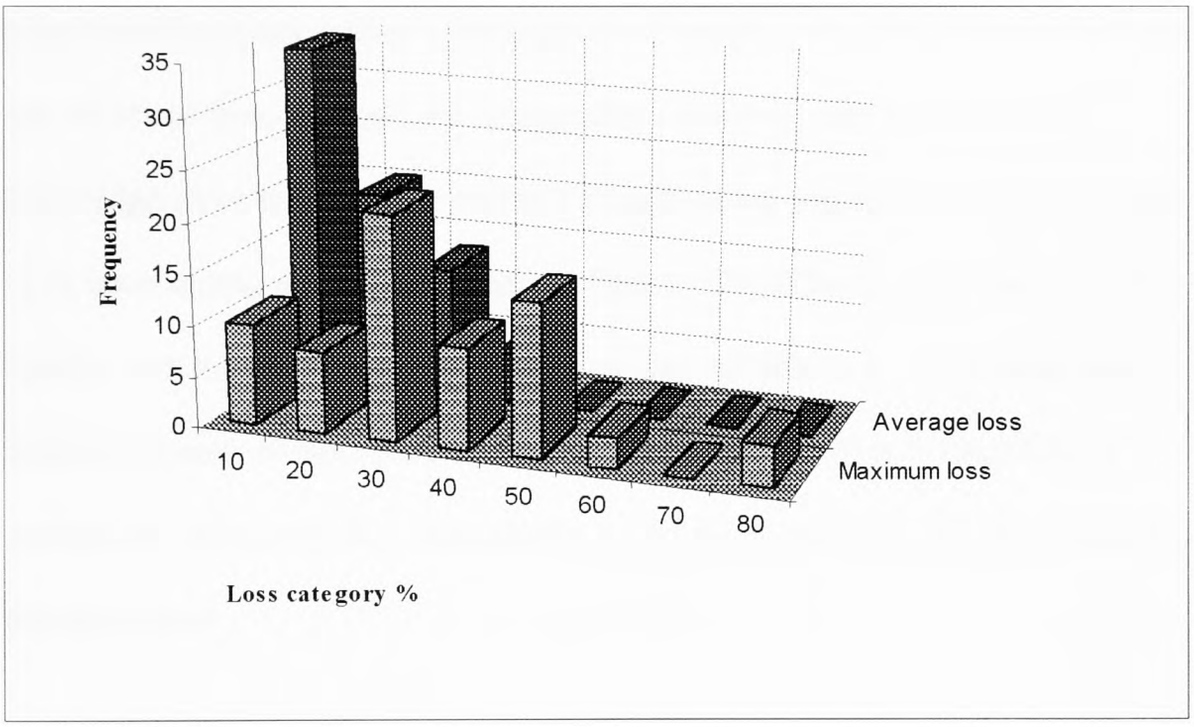


Figure 3.7 Frequency of maximum and average economic loss caused by first priority diseases in cauliflower based on 72 farmers' perceptions.

Farmers knowledge and practice of disease management

Overall analysis of farmers' disease management practices (Figure 3.8) show that most of the eastern hill farmers believe in the efficacy of cultural operations like timely hoeing and exposing their fields during summer by ploughing to reduce disease and pest problems. A total of 98.7% of respondents adopted cultural methods of disease control. Similarly, most of the farmers surveyed (86.1%) were

familiar with a few indigenous methods of pest control (for example use of cattle urine against powdery mildew disease, or use of wood ash for soil treatment). But they were not confident about doses and appropriate methods of application. In general, almost all the farmers who grew cole crops (either for fresh vegetable or for seed production) use pesticides. Pesticides were available to almost all the farmers surveyed / interviewed from newly established NGOs like CEAPRED and farmers' co-operatives. A total of 94.5% of farmers surveyed were familiar with the prescribed fungicides such as carbendazim and maneb against the *Sclerotinia* disease and 89.5% of them had used these fungicides. However, they had very little knowledge about timing, dose, methods of application, and precautionary measures to be taken during pesticide application. Only 33.3% of the surveyed farmers were familiar with the use of pest and disease resistant varieties as a crop management method but none of them were actually using resistant varieties through lack of availability. Moreover, few respondents (1.3%) were aware of biological methods of disease control.

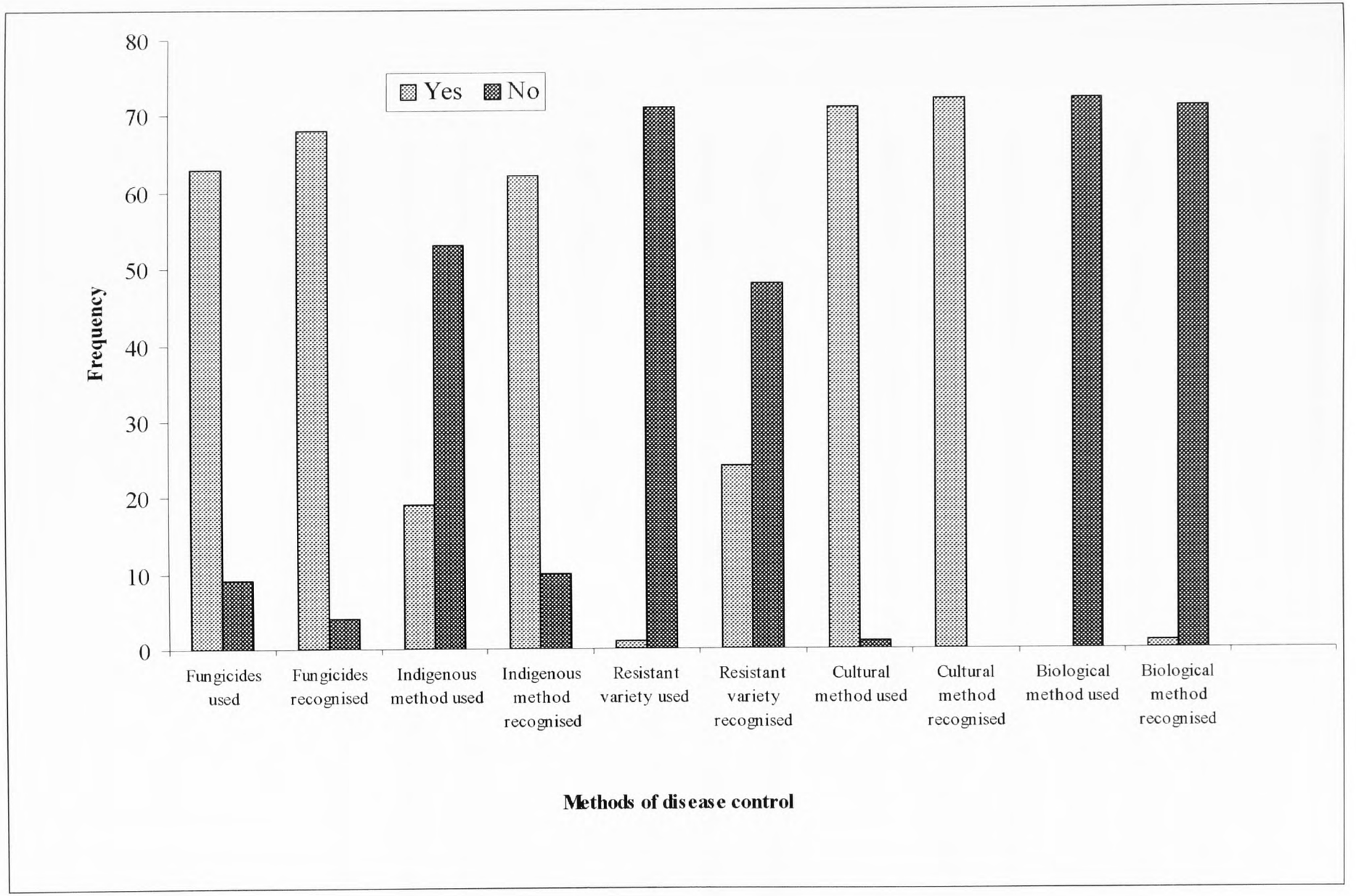


Figure 3.8 Knowledge about five different methods of disease control among the 72 surveyed farmers.

3.5 A note on market prices of cauliflower at Sindhuwa bazaar and in the ARS Pakhribas neighbouring areas.

Market price reflects the exchange values as determined by the demand of, and supply to, consumers in a particular period of time. For instance, the rapid expansion of a production area may be due to availability of new production technology or availability of a market with good prices. In the long run, market prices might be an important indicator in assessing the relative values and significance of a particular crop in the overall economy.

Vegetable marketing in the eastern hills of Nepal is largely centred on the local bazaars (market). The opening of the Dharan to Basantpur road has played a significant role in the marketing of vegetable products from the hills to the Terai and even to neighbouring regions of India.

This note compiles the market prices of cauliflower from 1977 to 1986 and from 1993 to 1998. Market price data was first recorded by ARS Pakhribas staff at the Hile and Budhabare local (Hat) market. Retail price data for normal season and off-seasons are presented in Tables 3.8 and 3.9 respectively.

Table 3.8 Monthly market price in rupees per kilogram of cauliflower in the two Hat bazaars of Hile and Budhabare during the normal season from 1977 to 1986

Year	Dec	January	Feb	March	April	Average	Maximum	Minimum
1977	3.24	3.25	3.83			3.44	3.83	3.24
1978	3.50	2.50	2.50	2.50		2.75	3.50	2.50
1979		3.50			3.00	3.25	3.50	3.00
1980								
1981								
1982								
1983	3.33	3.50	4.33			3.72	4.33	3.33
1984		3.67	4.00	4.00		3.89	4.00	3.67
1985	8.00			10.00		9.00	10.00	8.00
1986	5.50	5.00	6.00	7.00		5.87	7.00	5.00

Rows with blank spaces indicate no information available.

Source: Rai *et al.*, 1990.

Table 3.9 Monthly market price in rupees per kilogram of cauliflower in the two Hat bazaars of Hile and Budhabare during the off-season from 1977 to 1986

Year	May	June	July	August	Sept	Oct	Nov	Average	Maximum	Minimum
1977										
1978				6.00				6.00	6.00	6.00
1979										
1980										
1981										
1982							4.00	4.00	4.00	4.00
1983							8.00	8.00	8.00	8.00
1984										
1985							9.00	9.00	9.00	9.00
1986						10.00	6.00	8.00	10.00	6.00

Rows with blank spaces indicate no information available.
Source: Rai *et al.*, 1990.

Market prices collected by the author from the Sindhuwa Co-operative Centre covering the period from 1993 to 1998 for the normal and off-season crop are presented in Tables 3.10 and 3.11 respectively. These primary data were collected by making a monthly visit to Sindhuwa Co-operative fresh vegetables collection centre. Sindhuwa co-operative is situated in Dhankuta district on the Dhankuta and

Basantapur (Terhathum district) road, which is the most extensively used road and links the eastern hills with the East West highway of Nepal. Sindhuwa Bazaar is one of the biggest vegetable collection centres in the eastern hills. Wholesale prices of cauliflower in each month were collected from the logbook of Sindhuwa community-based farmers co-operative.

Table 3.10 Monthly market price in rupees per kilogram of cauliflower in a Sindhuwa Co-operative during the normal season from 1993 to 1998

Year	Dec	January	Feb	March	April	Average	Maximum	Minimum
1993	9.75					9.75	9.75	9.75
1994	5.30	6.00	6.00	7.00	8.50	6.56	8.50	5.30
1995	5.60	9.00	9.00		9.30	8.22	9.30	5.60
1996	4.20	3.70	3.70		7.90	4.87	7.90	3.70
1997	5.00	5.00	5.00	5.00	6.49	5.29	6.49	5.00
1998	9.17	5.80	5.80	7.50	6.00	6.85	9.17	5.80

Rows with blank spaces indicates no cauliflower was available in the Sindhuwa Co-operative.

Table 3.11 Monthly market price in rupees per kilogram of cauliflower in a Sindhuwa Co-operative during the off-season from 1993 to 1998

Year	May	June	July	August	Sept	Oct	Nov	Average	Maximum	Minimum
1993			22.90	23.00	20.90	13.50	10.00	18.06	23.00	10.00
1994	13.00	12.50	9.00	17.40	12.80	14.40	7.60	12.38	17.40	7.60
1995	10.40	6.90	19.60	19.80	12.30	15.70	10.20	13.55	19.80	6.90
1996	10.40	12.40	10.90	10.00	13.20	13.40	4.90	10.74	13.40	4.90
1997	8.96	9.60	9.40	11.80	14.70	14.30	4.40	10.45	14.70	4.40
1998	6.77	7.40	8.05	12.18	17.38	18.98	20.21	12.99	20.21	6.77

Rows with blank spaces indicates no cauliflower was available in the Sindhuwa Co-operative.

The price changes taking place on a monthly and yearly basis during the period of 1977 to 1998 were analysed in order to identify the reasons for price fluctuations

and their implications for cauliflower production. There are three limitations of the data:

- Recorded market prices are not linked to varieties, qualities, size and taste of cauliflower,
- The volume of trade data were not available,
- Market prices of cauliflower during 1987 to 1992 were not available for compilation.

Analysis of results for the main season price (Table 3.8) for 1977 to 1985 indicates that there is no particular year-to-year trend of average, minimum, and maximum prices. However, the price increased over the period.

The lack of data from most of the off-season 1977 to 1985 (Table 3.9) indicates that cauliflower was not available in the Hat market from May to November. The reason for non-availability was that farmers were not aware of off-season production technology. When cauliflower was produced, the price was much higher than in the main season.

The main season price data (Table 3.10) for 1993 to 1998 includes data for each month. During the period 1994 to 1998, the average price varies from 4.87 to 9.75 NRs/kg and the maximum price varies from 6.49 to 9.75 NRs/kg. For the same years during the off-season the average price varied from 10.45 to 18.06 NRs/kg while the maximum price varied from 13.40 to 23.00 NRs/kg. However, there was no clear trend of market price.

Overall analysis by the t-test of the price data of normal and off-season average minimum, and maximum price for the period of 1993 to 1998 indicates that there are significant differences in the average and the maximum prices data during normal and off-season (Appendices 8 and 9). But there are no significant differences between the minimum price data (Appendix 10). A 95% confidence interval for the average market price for the true difference between off-season and normal season was 3.10 to 9.11 NRs/kg. Similarly, the 95% confidence interval for the maximum price difference was 6.10 to 13.03 NRs/kg. These results indicate that both the average and maximum prices were significantly higher at the 5% level in the off-season for 1993 to 1998.

Favourable prices offered during off-season will tend to increase production. It seems clear that the availability of a market with good prices during the off-season has encouraged cauliflower production in the hills. As a result of this, year-round production or continuous cultivation of cauliflower on limited land or in the same field has increased tremendously. This is one of the primary factors behind the build-up of disease inoculum in the fields. Therefore, market price and marketing situations are coherent parts of the disease management strategy.

Discussion

The survey information of from the eastern foot-hills showed that the "normal" season in which cauliflowers are grown in from October to March. However, the crop is increasingly being produced in the off-season i.e. April to November. The off-season production of cauliflower in high and mid hills (altitude range in between

1100 m to 2500 m) has increased tremendously mainly due to a market with good prices through out the year, but in the off-season in particular. As a consequence, farmers are looking for alternative varieties which are agronomically suitable for off-season production with minimum economic losses by diseases. Present agronomic practices (continuous cropping of cole crops in the same field and disposing of diseased plants in their compost pits and then using incompletely decomposed compost on fields) may lead to disease inoculum build-up in the fields. A significant proportion of cauliflower are lost each year as a result of diseases particularly stalk rot and damping-off. Farmers perceived that, as a result of diseases, maximum economic loss of cauliflower at high, mid and low altitudes, varied from 41 to 50%, 21 to 30% and 21 to 30% respectively.

CHAPTER 4

EFFECT OF WEEDS ON CAULIFLOWER PRODUCTION

4.1 Background

A weed may be defined as "a plant growing where it is not wanted". However, it is said that allowing weeds to grow on cultivated land may protect the soil structures and prevent soil erosion, thereby acting as a cover crop. Besides, weeds may provide habitats for predators and antagonists of crop pests and thus act as companion plants. Competition between crop plants and weeds is an important factor in growing vegetable crops. Vegetable crops may form a canopy over the soil and thus retard weed growth..

During a field survey in September 1997, it was observed that most of the cauliflower fields in the Sindhuwa area were not weeded. The author was told by farmers that weeds have a positive effect on cauliflower production by reducing disease incidence. On this assumption farmers are not weeding their cabbage and cauliflower in the Sindhuwa area of Dhankuta district (high altitude), where cauliflower is one of the priority semi-commercial vegetable crops. It is also perceived by the farmers that the cauliflower crop is protected from scorching by sunlight and from insect pests and diseases with the help of growing weeds. "No weeding" is therefore a practice recently adopted in cauliflower and cabbage farming. To test the validity of the farmers' perceptions, a study was proposed to explore the effects of weeding in production and disease management in cauliflower.

4.2 Materials and methods

The study was carried out during two consecutive cropping seasons. The first season was a normal production season, October 1997 to April 1998, and the second was an off-season, August 1998 to December 1998. The study was performed at an altitude of 1750 m equivalent to high altitude in the Dhanbari block of ARS Pakhribas. In both seasons, the experiments were performed on the same area of land of the same terrace known to be a *Sclerotinia* sick plot. Snowcrown, a hybrid variety of cauliflower was included in both seasons, this being one of the most commonly grown varieties of cauliflower under non-weeded conditions. For the study, seeds were planted in plastic trays on 22 October 1997 and 7 August in 1998 for the normal season and off-season respectively. The trays were filled with a mixture of compost and forest soil. The experiments were carried out in a completely randomised design with six replications. Thirty-five and 28 day old seedlings were transplanted in both seasons directly into the sick plot. Plot size was 6.5 m^2 (2 m x 3.25 m) and net harvest area was maintained at 3.6 m^2 (1.5 m x 2.4 m) by excluding border rows. Spacing was maintained at 40 cm plant-to-plant and 50 cm row-to-row. The treatments were: (1) no weeding, (2) two weedings (40 days interval), (3) four weedings (15 days intervals) and (4) six weedings (10 days intervals), respectively for the normal season study. Similar treatments (1, 2, 3 and 4) were no weeding, two weedings (30 days interval), four weedings (15 days intervals) and six weedings (10 days intervals), respectively for the off-season study. Manure and fertilisers were applied at the rate of 10 t/ha compost and 60: 100: 60: kg per ha NPK (farmers' practice). In both seasons, the crops were top dressed with nitrogenous fertiliser (urea 46% N) at the rate of 1.7 g per plant 76 days after

transplanting. The field was irrigated on the basis of farmers' experience, the water requirement of the crop plants and soil moisture conditions of the field.

In both seasons there were severe attacks by red ants in the field trial. Red ant (*Dorylus* spp.) is one of the established pests in the eastern hills; cauliflower is their most preferred host; they damage the crop by feeding on roots and the cortex of the stem. It was therefore necessary to treat with insecticide. Seventy-six days after transplanting, soil around the cauliflower plants was drenched with endosulfan (Thiodane 35 EC) @ 2 ml /litre water in normal season. In the off-season, 116 days after transplanting soil around the cauliflower plants were drenched with chloropyrifos at 0.05% active ingredient in water. Two different types pesticides were applied for two different seasons because at the time of application the same pesticide was not available.

A total of six plants were marked at random by using random numbers from each plot to measure curd diameter, curd weight and curd yield. However, the number of diseased plants was recorded from whole plots. Fresh weights of the weed species of individual plots were recorded immediately after weeding and analysed by analysis of variance. Weeding time and cost per plot were calculated by allowing two labourers independently to perform weeding jobs. The time involved for weeding per plot was recorded by using a stop watch.

4.3 Results and discussion

A total of 15 weed species (1 to 15 in Table 4.1) were observed in the normal season

crop whereas only 9 species (1 to 6, and 12 to 14 in Table 4.1) of weeds were observed during the off-season study. The most important weeds, according to their fresh biomass yield, were *Ageratum comyzoides* L., *Galinsoga parviflora* Cav., *Oxalis* sp. and *Stellaria media.* In the control plot, only the total number of weed species was recorded 26 days after cauliflower transplanting. Weed species recorded from the experimental plots were representative weeds of almost all potential areas of high altitude where cauliflower is grown as a commercial fresh vegetable. The intensity of weeds such as *Oxalis* sp., *A. comyzoides, G. parviflora, S. media*, and *Digitaria adscendes* were very high in both seasons. Other weeds such as *Graphalium luteo album* Roxb., *Raphanus sativus, Trifolium* sp., *Setaria* sp. did not appear in the off-season field trial but appeared in the normal season field trial.

Table 4.1 Weeds recorded and identified in the cauliflower weeding field trial during the normal season 1997/98 and off-season 1998/99

Vernacular name of the weeds	Latin name of the weeds	Number assigned see Tables 4.6 and 4.7
Pigweed	*Oxalis* sp.	1
Remai	*Ageratum comyzoides* L.	2
Ratnaulo	*Galinsoga parviflora* Cav.	3
Armale	*Stellaria media* Night	4
Banso	*Digitaria adscendes* (Kuath.) Hear	5
Bermudagrass	*Cynodon dactylon* (L.)	6
Bukifool	*Graphalium luteo album* Roxb.	7
Radish	*Raphanus sativus*	8
Berseem	*Trifolium* sp.	9
Setaria (Fox tail)	*Setaria* sp.	10
Layure	Unidentified	11
Ghungrin	Unidentified	12
Rayo	*Brassica* sp.	13
Panijhar	Unidentified	14
Kodejhar	*Eleusine* sp.	15

Photographs showing the growth of weeds in normal season and off-season field trials are presented in Figure 4.1 and 4.2 respectively.

Figure 4.1 Weed situation in normal season cauliflower field trial.

Figure 4.2 Weed situation in off-season cauliflower field trial.

During the normal season a total of 16 plants out of 432 died due to fungal diseases, combined attack of fungus and nematodes, red ant and rodent (Table 4.2).

Table 4.2 Plant death caused by disease, pest and rodents in the cauliflower weeding field trial during the normal season 1997/98

Cause of plant death	Number of plants	Treatments
S. sclerotiorum	3	6 weedings
Fusarium spp. & nematodes	1	6 weedings
S. sclerotiorum	2	4 weedings
S. sclerotiorum	3	2 weedings
Rodent	3	2 weedings
Fusarium spp. & nematodes	1	0 weeding
S. sclerotiorum	2	0 weeding
Red ants	1	0 weeding

During the off-season a total of 18 plants out of 432 died due to fungal diseases, combined attack of fungus and nematodes, nematodes, bacteria and the combined attack of bacteria and nematodes (Table 4.3).

Table 4.3 Plant death caused by disease in the cauliflower weeding field trial during the off-season 1998/99

Cause of plant death	Number of plants	Treatments
Fusarium sp.	1	6 weedings
Pythium spp. & nematodes	1	6 weedings
Erwinia sp. & nematodes	1	6 weedings
Erwinia sp.	2	4 weedings
Nematodes	6	4 weedings
Erwinia sp.	1	2 weedings
Pythium spp.	1	2 weedings
S. sclerotiorum	1	2 weedings
Pythium spp. & nematodes	2	0 weeding
Pythium spp.	1	0 weeding
Nematodes	1	0 weeding

Analyses of yield data for the normal season field trials are presented in Tables 4.4 and 4.5. Results indicate that curd diameter, curd weight, curd yield and number of diseased plants were not significantly different from the control treatment, indicating that there were no effects of weeding on disease incidence or other factors measured.

Table 4.4 Mean curd diameter, curd weight, curd yield, number of diseased plants and weed biomass yield recorded in the cauliflower weeding trial plots during the normal season 1997/98

Treatments	Curd diameter (cm)	Curd weight (g)	Curd yield (g)	Diseased plants (no.)	Weed biomass yield (g)
No weeding	12.33	162	928	1.17	-
Two weedings	13.92	215	1285	0.67	784
Four weedings	14.93	232	1389	0.83	433
Six weedings	15.18	267.	1584	1.00	426

- Observation not recorded.

Table 4.5 Analysis of variance of curd diameter, curd weight, curd yield and number of diseased plants recorded in the cauliflower weeding trial plots during the normal season 1997/98

Source	d. f.	Curd diameter	Curd weight	Yield	Number of diseased plants
		F Probability	F Probability	F Probability	F Probability
Treatments	3	0.439 NS	0.342 NS	0.294 NS	0.698 NS
Error	20				
Total	23				

NS = Not significantly different.

Weed biomass data recorded during the normal season field trials are presented in Table 4.4. Weed biomass values in the different weeding treatments were significantly different at $P<0.05$.

Analysis of variance of curd diameter, curd weight, and number of diseased plants for the off-season field trial (Tables 4.6 and 4.7) indicated that curd diameters, curd weight and curd yield were significantly different from the control plots, but the number of diseased plants were not significantly different. The results also show that there was no benefit to be gained from more than two weedings.

Table 4.6 Mean curd diameter, curd weight, curd yield, number of diseased plants and weed biomass yield recorded in the cauliflower weeding trial plots during the off-season 1998/99

Treatments	Curd diameter (cm)	Curd weight (g)	Curd yield (g)	Diseased plants (no.)	Weed biomass yield (g)
No weeding	6.33	41.0	246	0.67	-
Two weedings	10.94	123.3	740	0.50	47868
Four weedings	10.69	119.9	710	1.33	28931
Six weedings	10.08	100.3	602	0.5	8142

- Observation not recorded.

Table 4.7 Analysis of variance of curd diameter, curd weight, curd yield and number of diseased plants recorded in the cauliflower weeding trial plots during the off-season 1998/99

Source	d. f.	Curd diameter	Curd weight	Curd yield	Number. of diseased plants
		F Probability	F Probability	F Probability	F Probability
Treatments	3	<.001**	<.001**	<.001**	0.952 NS
Error	20				
Total	23				

** = Significantly different at 1% level.
NS = Not significantly different.

Weed biomass recorded from the off-season field trials are presented in Table 4.6. Weed biomass values in the differently weeded treatments were significantly different at $P<0.05$ (Appendix 11).

Overall, combined analysis of variance showed that the weed biomass of two seasons was significantly different (Appendix 12). Unlike weed biomass the number of diseased plants were not significantly different (Appendix 13).

Differences in weed biomass between the normal (October to January) and off-season (August to November) were probably due to climatic factors. Eighty seven per cent of the rainfall was concentrated during the period May to September (Appendix 14). The highest rainfall is expected to occur in July and lowest in November at the ARS Pakhribas. There was very little rain during winter. Soil temperature down to 10 cm depth is lower from November to March (Gurung and KC, 1993). During the normal crop season there was unusually low rainfall, only 176.4 mm rain during October to March. The maximum temperature was 18.8 °C or below except in October (Appendices 15 and 16). As a result the low residual soil moisture and low temperature helped to suppress weed growth for the normal season cauliflower crop. During the normal season the crop covers the soil canopy before emergence of weeds and retards the growth of early-germinated weeds (Figure 4.1). Therefore, the lowest total fresh biomass yield of weeds was recorded (426 g) from the six weedings and the highest total fresh biomass weed yield was recorded (784 g) from the two weedings from the normal season cauliflower trial.

Unlike the normal season, during the off-season soil temperature and soil moisture were very high. The maximum temperature was above 20.3 °C except in December and a total of 500.2 mm of rain fell from August to December (Appendices 15 and 16). As a result, growth of weeds was very high. The lowest fresh weed biomass

was recorded (8142 g) from the six weedings and the highest was (47868 g) from the two weedings from the off-season field trial.

The time requirement for hand weeding was recorded at 5.5 to 6.5 minutes per plot (3.6 m^2), which is equivalent to 38 labourers per ha. Labour required for weeding depends on weed intensity, age of the seedlings, soil type and soil moisture. The cost of hiring labourers plays an important role in the net return from the harvest. Farmers' perception that weeds reduces disease incidence is not supported by analysis of the results from either season. However, weeds played a significant role in yield reduction during the off-season due to competition with the cauliflower crop. Therefore, farmers may indirectly have perceived that not weeding is economical by thinking that weeding increases disease incidence in cauliflower fields.

Discussion

The recently adopted crop management regime of zero weeding of cauliflower fields at high altitude sites led to farmers perceiving that the presence of weeds reduces disease incidence. It was hypothesised that zero weeding might help to minimise the contact of pathogen with host and reduce the chance of contact of contaminated soil with hosts. This hypothesis was not supported by experimental results carried out during two consecutive seasons. Although, competition associated with weeds reduced the yield and size of the curds, it had no effect on disease incidence. The results also show that there was no benefit to be gained from more than two weedings for the off-season crop.

CHAPTER 5

PATHOGENS ASSOCIATED WITH DAMPING-OFF DISEASE AND CURD ROT OF CAULIFLOWER IN THE EASTERN HILLS OF NEPAL

5.1 Background

Damping-off is a destructive disease of cauliflower at the seedling stage. Damping-off caused by *Phytophthora* spp. collapses young seedlings especially under moist and crowded conditions (Jones, 1987). Damping-off caused by *Alternaria brassicae* and *Rhizoctonia solani* prevents seed emergence and causes toppling of infected seedlings (Singh *et al.*, 1990; Yang *et al.*, 1996). It has also been reported that damping-off caused by *Pythium* spp. is a severe constraint for satisfactory cauliflower seedling production in the eastern hill districts (Gautam *et al.*, 1989). In the field, damping-off is most severe when soil moisture is medium to high and the temperature is comparatively high. The disease has been reported from ARS Pakhribas and its command areas of the eastern hills (Duwadi *et al.*, 1996) and is reported to be more problematic when seedlings are raised during the rainy season, July - August (Gautam *et al.*, 1990), than for early, October-November, and main season (November - December) production of cauliflower.

Similarly, *Alternaria brassicae* causes severe damage to cauliflower from germination until harvest by causing symptoms of damping-off and curd rot (Singh *et al.*, 1990). Curd rot is a complex and important disease of cauliflower in the eastern hills of Nepal. Rotten curds are neither suitable for cooking nor for seed production. In India, curd rot is caused by five different pathogens *S. sclerotiorum, Peronospora parasitica, Alternaria* spp., *Xanthomonas campestris* and *Erwinia carotovora*

(Kapoor and Thakur, 1997). In India, complex curd rotting causes as much as 30% reduction in yield (Kapoor and Thakur, 1997). However, the causes and extent of losses caused by this disease complex in the eastern hills had yet to be determined.

At the beginning of a field survey in September 1997, it was observed that many seedlings were dying in cauliflower nursery beds due to damping-off disease and the author was told by the farmers that damping-off and curd rot diseases were major constraints for cauliflower production in the eastern hills. Therefore, this study was set-up to determine the:-

- organisms associated with damping-off disease of cauliflower seedlings in the eastern hills of Nepal, and
- curd rot diseases of cauliflower in the eastern hills of Nepal and the role of *Sclerotinia* in causing curd rot.

5.2 Materials and methods

Monitoring of damping-off disease in cauliflower nurseries was carried out during two consecutive seasons. The first season was January to February, 1998, and the second season was September to October, 1998. Visits were made to cauliflower growing areas of Dhankuta district, where seedlings were raised under arch-shaped polythene tunnels. A total of 95 damped-off seedlings from five different high-altitude nurseries were collected for the first season study (Table 5.1). For the second season study 345 diseased seedlings were collected from 75 different nurseries of the high, mid and low-altitude zones (Table 5.2).

Table 5.1 The number of damped-off seedlings sampled and tested from high altitude sites during the off-season, 1998

Cultivar	The total number of damped-off seedlings
Snowcrown	66
Kibogiant	29
Total	95

Table 5.2 The number of damped-off seedlings sampled and tested from high, mid and low-altitudes during the normal season, 1998

Cultivar	The total number of damped-off seedlings
Snowcrown	132
Kathmandu Local	169
Kibogiant	18
Snowball-16	6
Pusadeepali	3
Terai-3	17
Total	345

The age of the seedlings for both seasons varied from 25 to 35 days. After sampling, soil was removed from the roots and seedlings were wrapped with blotting paper, placed separately into envelopes with an identification tag. Finally, they were brought to the laboratory for disease diagnosis.

The damped-off seedlings were washed in running tap water. Thin slices of the advancing edge of the disease were cut with surgical blades (surface sterilised with 70% alcohol and finally rinsed with sterile water). A blotter test was performed by placing the cut pieces in Petri dishes on three layers of moistened filter paper. After

incubation in a temperature controlled room for a period of 5 days at 20°C under light, isolates were examined microscopically.

A study of pathogens associated with curd rot of cauliflower was also carried out during two consecutive normal crop growing seasons under natural epiphytotic conditions. Cauliflower fields were visited and samples of three different types of rotten curds, as described below, were collected as available. A total of 70 and 107 rotten curd samples in the first and second years, respectively of three different types of rot "A", "B" and "C" (Table 5.3) from three different high, mid and low altitude zones were collected. Diseased curds of four different cultivars namely Terai-3, Kibogiant, Kathmandu Local and Snowcrown (hybrid) were sampled in the first and second years respectively (Table 5.4.)

Table 5.3 Categorisation of curd rot according to symptoms

Rot type	Symptoms category
A	Brownish irregular shape and dry rotting of curd
B	Creamy white soft rot with bad smell
C	Soft rot with fluffy white mycelium

Table 5.4 The number of rotten curds sampled and tested from high, mid and low altitudes during two consecutive normal crop growing seasons in 1998 and 1999

Cultivar	Number of rotten curd samples collected during normal season	
	1998	1999
Snowcrown	25	23
Kathmandu Local	25	62
Terai-3	13	13
Kibogiant	7	9
Total	70	107

Samples of rotten curd were wrapped in a blotting paper, placed into a large sized envelope with an identification tag and brought to the laboratory for diagnosis of curd rot.

The rotten curds were washed in running tap water. Thin slices of advancing edge of the rotten tissue were cut with surgical blades (surface sterilised with 70% alcohol and finally rinsed with sterile distilled water). Blotter tests were performed by placing the cut pieces in Petri dishes, on three layers of moistened filter paper. After incubation in a temperature controlled room for a period of 7 days at 20°C under fluorescent light, isolates were examined under the microscope.

Isolations were made from rots apparently caused by bacteria after making suspensions of rotted tissue and dilution cultures. Bacteria were identified by colony character when streaked on YDC (yeast dextrose carbonate) media and by the KOH (potassium hydroxide) solubility test (for Gram reaction). Bacteria showing white colonies when streaked on YDC media and found to be Gram negative by the KOH

solubility test were further tested by inoculating a potato slice to reconfirm soft rot (*Erwinia carotovora* subsp. *carotovora)*. Bacteria showing yellow colonies when streaked on YDC media and found to be Gram negative by the KOH solubility test could be *Xanthomonas campestris* pv. *campestris,* a known cause of curd rot (Kapoor and Thakur, 1997) but media and tests for further identification were not available.

5.3 Results and discussion

The identity of pathogens isolated from damped-off cauliflower seedlings during the off-season and normal seasons are presented in Figure 5.1, and from damping-off seedlings during normal season at three different altitudes in Figure 5.2. Details of pathogens isolated from damped-off seedlings according to varieties for off-season are presented in Appendix 17 with analysis according to altitude and variety in Appendices 18 and 19. The results indicate that damping-off is a well established disease in the high, mid and low altitudes and associated were various pathogens known to cause damping-off, namely *Pythium aphanidermatum, Rhizoctonia* spp., *S. sclerotiorum, Phytophthora* spp., *Fusarium* spp., and *Alternaria* spp. *Rhizoctonia* spp. followed by *P. aphanidermatum* and *S. sclerotiorum* were major pathogens associated with damping-off in the off-season and *P. aphanidermatum* followed by *Fusarium* spp., *Rhizoctonia* spp., *Alternaria* spp. and *S. sclerotiorum* and then nematodes were major pathogens in the normal season. Furthermore, the results indicate that there were no differences in pathogens associated with damped-off seedlings in the two different cultivars, Snowcrown and Kibogiant, at high altitude during the off-season (Appendix 17). Similarly, in the normal season,

pathogens associated with damped-off seedlings in six different cultivars such as Snowcrown, Kathmandu Local, Kibogiant, Pusadeepali, Snowball-16 and Terai-3 were also the same (Appendix 18). Therefore, this indicates that pathogens associated with damped-off seedlings are not specific to a particular cultivar. However, according to altitude zones *P. aphanidermatum* is frequently associated with damping-off at all altitudes but *Fusarium* spp. is more important at mid and low altitudes (Figure 5.2.).

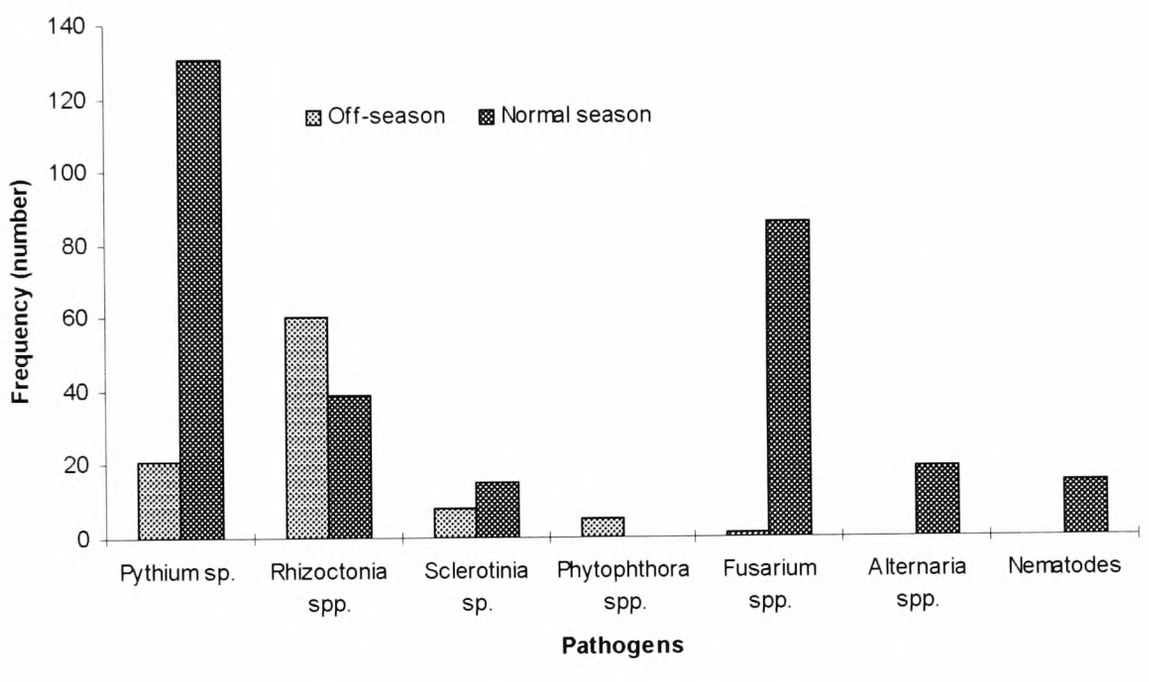


Figure 5.1 Frequency occurrence of major pathogens associated with damping-off disease during off-season 1998 and normal season 1999.

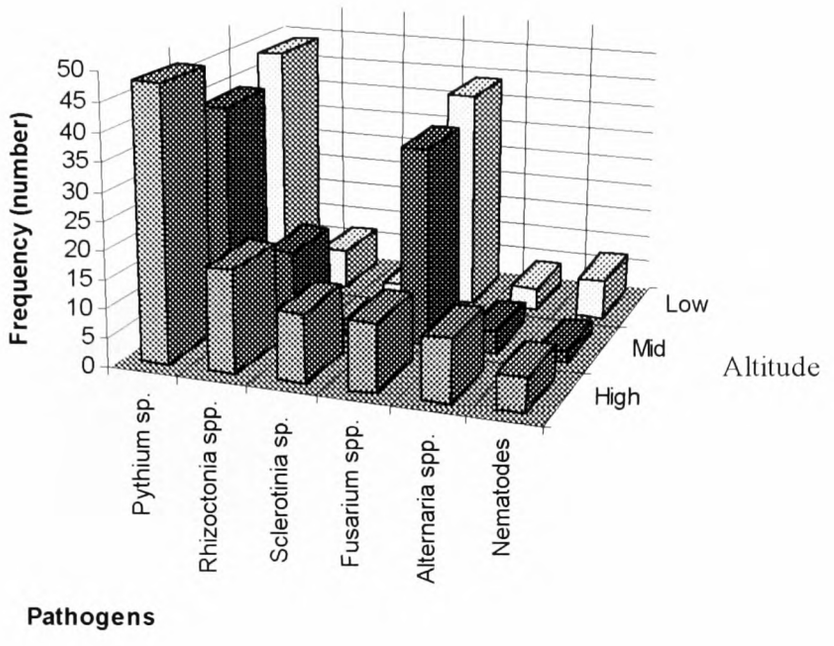


Figure 5.2. Pathogens causing damping-off in cauliflower seedlings during the normal season at three different altitudes.

Full details of pathogens associated with curd rot of cauliflower on the basis of rot symptoms and altitudes are presented in Appendix 19 for both years. Details of the pathogen associated with curd rot according to rot type and pathogens associated with curd rot according to variety for both years are presented in Appendices 20, 21, 22 and 23 respectively. Overall, the results indicate that curd rot of cauliflower is a disease complex (Figure 5.3). Curd rot of cauliflower in the eastern hills is associated with pathogens *P. parasitica, S. sclerotiorum, Pythium* spp., *Fusarium* spp., *Alternaria* spp., *Erwinia carotovora* subsp. *carotovora* and Gram negative, yellow colony-forming bacteria. Except for *Fusarium* spp., *Pythium* spp. and the yellow Gram negative bacteria, all the organisms isolated were known to cause curd rot (Kapoor and Thakur, 1997).

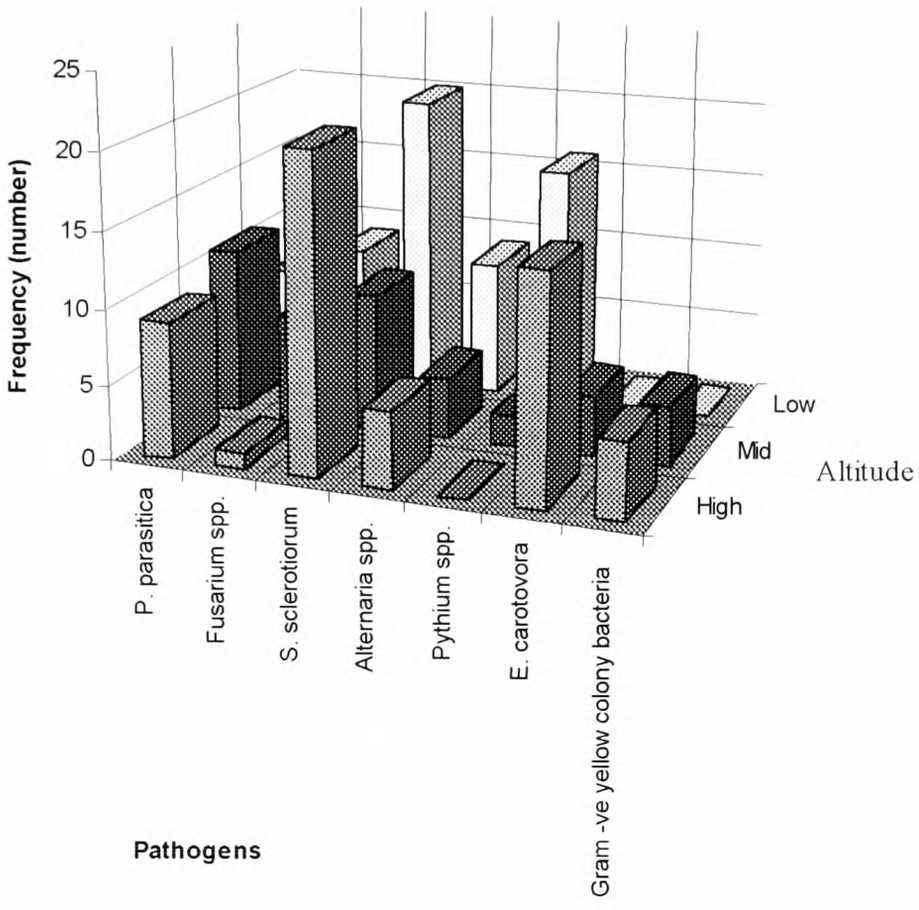


Figure 5.3 Major pathogens associated with curd rot in three different altitudes in two consecutive seasons in the eastern hills of Nepal.

Curd rot samples tested during 1998 (Table 5.5) indicate that majority of "A" type of curd rots were associated with *P. parasitica* followed by *Alternaria* spp. and *Fusarium* spp. and most of the "B" type of rots were associated with *Erwinia carotovora* and Gram negative, yellow colony-forming bacteria followed by *S. sclerotiorum.* On the other hand the "C" type of rot was associated almost exclusively with *S. sclerotiorum*. In 1999, "A" type of rots were associated with *Fusarium* spp. followed by *Pythium* spp. and "B" type of rots were associated with *Erwinia carotovora* and Gram negative yellow colony-forming bacteria followed by *S. sclerotiorum*; pathogens associated with "C" type of rots were *S. sclerotiorum*

followed by bacteria (Table 5.6). In general, more putative pathogens were observed in 1999 than 1998. However, type "B" and "C" were consistent in terms of major pathogens, but organisms associated with type "A" were not consistent mainly due to more samples being collected from municipality areas (low altitude sites) that were not fresh. Hence, the lack of records of *P. parasitica* (obligate pathogen).

Table 5.5 Pathogens associated with three different types of rotten curd samples of cauliflower during the normal crop growing season 1998

Rot type*	Number of diseased sample		Pathogen associated with curd rot
	tested	infected	
A	41	26	*P. parasitica*
		10	*Alternaria* spp.
		1	*Fusarium* spp.
		4	No pathogen
B	11	3	*Erwinia carotovora*
		2	Gram negative, yellow colony-forming bacteria
		1	Bacteria + *P. parasitica*
		5	*S. sclerotiorum*
C	18	17	*S. sclerotiorum*
		1	*Fusarium* spp.+ nematodes

* See Table 5.3

Table 5.6 Pathogens associated with three different types of rotten curd samples of cauliflower during the normal crop growing season 1999

Rot type*	Nnumber of diseased sample		Pathogen associated with curd rot
	tested	infected	
A	46	14	*Fusarium* spp.
		13	*Pythium* spp.
		5	*Alternaria* spp.
		3	*Erwinia carotovora*
		1	Gram negative, yellow colony-forming bacteria
		1	*Fusarium* spp. + bacteria
		2	*S. sclerotiorum*
		1	*P. parasitica*
		2	Nematodes
		1	*Rhizoctonia* spp.
		1	*Fusarium* spp. + *Pythium* spp.
		2	*Penicillium* spp.
B	20	12	*Erwinia carotovora*
		3	Gram negative, yellow colony-forming bacteria
		1	*Erwinia* sp. +*Fusarium* spp.
		2	*S. sclerotiorum*
		1	*S. sclerotiorum* + *Alternaria* spp.
		1	*Fusarium* spp.
C	41	24	*S. sclerotiorum*
		1	*S. sclerotiorum* + bacteria
		1	*S. sclerotiorum* + *Alternaria* spp.
		3	Gram negative, yellow colony-forming bacteria
		1	*Erwinia carotovora*
		5	*Pythium* spp.
		3	*Alternaria* spp.
		1	*Fusarium* spp.
		2	Nematodes

* See Table 5.3

Overall in both years (1998 and 1999) the results indicate that "A" type of rots were associated with *P. parasitica* followed by *Alternaria* spp. and *Pythium* spp. and then *Erwinia carotovora*; "B" type of rots were associated with *Erwinia carotovora* and Gram negative, yellow colony-forming bacteria followed by *S. sclerotiorum*; whereas "C" type of rot was associated with *S. sclerotiorum.* Minor pathogens associated with "A" type of rots were nematode, *Rhizoctonia* spp. and *Penicillium* spp. and with "C" type of rot it was nematodes.

The results also indicate that all pathogens appear in all cultivars. However, bacterial infection was not recorded in the low altitude cultivar Terai-3 in either year (Appendices 22 and 23); nematode infection (minor occurrence) was recorded only at low altitude. Based on the frequency of observation in different altitude zones, *S. sclerotiorum* followed by *Erwinia carotovora* subsp. *carotovora* at high altitude, *P. parasitica* followed by *S. sclerotiorum* at mid altitude and *S. sclerotiorum* followed by *Pythium* spp. at low altitude are the major pathogens associated with curd rot complex disease (Figure 5.3). Overall *S. sclerotiorum* was the fungus most frequently associated with curd rot.

Discussion

A diagnostic investigation of diseased cauliflower in farmers' field in the eastern hills indicated that stalk rot, damping-off, downy mildew, *Alternaria* leaf spot and bacterial rots (the latter caused by *Erwinia carotovora* subsp. *carotovora* and other bacteria) were present on crops grown in the field. The isolations from the material collected enabled their identification as *P. aphanidermatum* and *S. sclerotiorum*

associated with damping-off during off-season seedling production in high altitude zones; *P. aphanidermatum*, *Fusarium* spp., *Rhizoctonia* spp. and *S. sclerotiorum* were the major pathogens associated with damping-off during normal season seedling production in high, mid and low altitudes. Furthermore, the results indicate that pathogens associated with damped-off seedlings are not specific to a particular variety.

Similar diagnostic studies indicated that *P. parasitica*, *S. sclerotiorum*, *Fusarium* spp., *Alternaria* spp., *Erwinia carotovora* and Gram negative yellow colony-forming bacterium were associated with the curd rot complex. The Gram negative yellow colony-forming could be *Xanthomonas campestris*, a known cause of curd rot (Kapoor and Thakur, 1997) but media and tests for further identification were not available. However, minor pathogens such as nematodes, *Rhizoctonia* spp. and *Penicillium* were also associated with the curd rot complex. Overall, the results indicate that *S. sclerotiorum* was most frequently associated with curd rot and it is problematic to all stages of the crop such as seedling, curd for vegetable production and seed crops.

CHAPTER 6

EFFECT OF SEED SOURCE AND QUALITY ON STALK ROT DISEASE AND CAULIFLOWER PRODUCTION

6.1 Background

It has been reported that about 90% of all food crops in the world are propagated by seeds (Schwinn, 1994) and seeds are passive carriers of pathogens which are transmitted when the seed hosts are planted and emerge under favourable environmental conditions (Noble, 1971).

Most of the imported hybrid seeds of cabbage and cauliflower that are supplied to the eastern hill farmers by local seed suppliers do not pass through a quarantine or seed health laboratory. It is reported that the increasing movement of seed as germplasm around the world provides an opportunity for the dissemination of all crop pathogens (Neergaard, 1977). The eastern hill farmers are less concerned about seed health than timely availability of seeds. The Seed Act of Nepal is responsible for quarantine and quality control of seed both in field and laboratory. However, the agricultural authorities are not in a position to enforce the existing Seed Act because of poor technical and administrative infrastructure. As a result, vegetable seedlings are either not emerging properly or appearing with damping-off symptoms after emergence.

In general, the major pathogens isolated from damped-off vegetable seedlings are *Pythium* spp., *Alternaria* spp. and *S. sclerotiorum* and these are also known to be associated with seed (Chapter 5). Healthy seeds not only ensure better seedling

production but a vigorous plant growth leads ultimately to higher yield. Introduction of new varieties may introduce additional diseases to the areas, because the quarantine rules are not properly followed. Overall, therefore it was important to determine the quality of seed available from different sources in Nepal.

6.2 Materials and methods

A total of 15 different seed lots of six different cultivars of cauliflower namely Terai-3, Snow Mystique (hybrid), Kathmandu Local, Snowball-16, Kibogiant, and Snowcrown were collected from different sources such as ARS Pakhribas, ARS Paripatle, VDD, KOSEPAN, CEAPRED, and farmers. Each seed lot was divided into two parts to perform a germination test in the laboratory (blotter test) and an emergence test in a sterile soil.

Before the seed germination test in the laboratory and seedling emergence test in sterile soil all seed lots were directly examined for pathogen contamination by placing the seed lots in a seed testing purity board.

6.2.1 Seed test in the laboratory (blotter test)

In this test, there were four replications of 100 seeds each of 15 lots covering six cultivars. The seeds were incubated on moist blotting paper in a humid atmosphere for 12 hr at 20 °C alternating with 12 hr at 30 °C. The first count of germination was done at the 5 days and the second count 10 days after seeding. At each count the seeds were examined under a stereoscopic microscope for growth of different fungi which were identified to genus level. Data such as the number of germinated

seeds, number of diseased seeds and number of seeds infected with various pathogens were recorded.

6.2.2 Emergence test in a sterile soil

Seeds from the 15 different seed lots of six different cultivars were sown in plastic trays. The size of the tray was 40 cm long, 28 cm wide and 6 cm deep. The trays were filled with a sterilised forest soil and 100 seeds of each lot were sown in each tray. Spacing was maintained 10 cm row-to-row and seed-to-seed continuously. The soil was watered with sterile water using a 1 litre plastic sprayer on the basis of the water requirement of the seedlings and soil moisture conditions of the trays. Seedling status was regularly monitored up to 50 days after sowing. Dead seedlings were uprooted and the pathogens associated with them identified after further incubation in a blotter method as described in Chapter 5. Similar to the seed test, the total number of emerged seedlings, seedling mortality after emergence and number of dead seedlings with various pathogens were recorded.

6.2.3 Monitoring of sclerotial germination

Seedling emergence tests in a sterile soil (6.3.2) indicated that poor seed health was mainly due to the fungi *S. sclerotiorum* and *Alternaria* spp.; seedling mortality after emergence was associated with *S. sclerotiorum*. Therefore, the study described below was performed with the objective of monitoring the relationship between environmental factors and sclerotial germination in relation to seed germination.

Monitoring of sclerotial germination was carried out (a) in a *S. sclerotiorum* sick plot and (b) in earthenware pots at the ARS Pakhribas (1750 m). In the sick plot regular monitoring of germination of sclerotia *in situ* was performed from April 1997 to February 1999 within a marked area of 1 m x 2 m having approximately in total over one hundred sclerotia. For the earthenware pot study matured sclerotia of *S. sclerotiorum* were collected from the naturally-infected seed crop of cauliflower at ARS Pakhribas north farm during May to June 1997. Collected sclerotia were washed in sterile water, air dried at room temperature for 7 days and stored in a sealed glass bottle. Soil from an apparently healthy plot was collected and used to fill nine different earthenware pots (25 cm in diameter and 24 cm deep). A total of 10 air-dried, morphologically similar sclerotia were planted into each earthenware pot on 25th January 1998, there being one earthenware pot for each planting depth (1, 2, 3, 4, 5, 6, 7, 8 and 9 cm). The earthenware pots were then buried in the soil leaving only the upper surface of the soil exposed. Monitoring of seclerotial germination in the earthenware pot was done from January 1998 to February 1999.

6.3 Results and discussion

6.3.1 Seed test in the laboratory

Qualitative examination of seed lots indicated that two lots were contaminated with sclerotia of *S. sclerotiorum*. Seed germination test results (Table 6.3.1.1) indicated that representative seed lots available to the eastern hill farmers varied from 0 to 94% in germination. Fifty-three per cent of the seed lots obtained through different sources had shown satisfactory germination (>76%) but the remaining 47% had less than 76% of germination. Fungi associated with poor germination were *Fusarium* spp., *S. sclerotiorum*, *Alternaria* spp., *Phoma* spp., and *Rhizopus* spp. The results

indicate that different sources of the same cultivars differed significantly from each other with respect to the number of diseased seeds and germination percentage (Appendix 24).

Table 6.3.1.1 Overall seed infection and breakdown according to individual fungi associated with fifteen different seed lots of cauliflower collected from various parts of Nepal (blotter tests)

Cultivar	Seed sources	Number of seed tested	Germination %	% of diseased seed recorded & examined	% of seed infected with				
					Fusarium	*Sclerotinia*	*Alternaria*	Phoma	Rhizopus
Terai-3	PAC	400	0	18	13	1.8	1	2.2	0
Kibogiant	CEAPRED	400	20	4.5	1.8	0.2	0.3	2.2	0
Snow Mystique	CEAPRED	400	94	10	5.3	1	2.2	1.5	0
Snowcrown	CEAPRED	400	94	3	0.3	0	2.7	0	0
Kathmandu Local	PAC 97	400	76	10.5	1	8	1.5	0	0
Kathmandu Local	VDD 96	400	91	17	5.5	2	8.5	1	0
Kathmandu Local	VDD 97	400	86	20.5	8	4.5	7.5	0.5	0
Kathmandu Local	PAC 96	400	87	15.5	3.5	5	5	2	0
Kathmandu Local	Bhaktapur 96	400	89	25.5	12	2.5	11	0	0
Kathmandu Local	Paripatle 97	400	92	24	10	2	9.5	2.5	0
Kathmandu Local	Paripatle 98	400	45	41	31.5	1	8	0.5	0
Kibogiant	Paripatle 98	400	87	23	12	1.8	7.5	1.7	0
Kathmandu Local	KOSEPAN	400	15	44.5	39.5	1	3.5	0.5	0
Kathmandu Local	Pakhribas Farmer	400	7	30	20.5	6.5	0	1	8
Snowball-16	KOSEPAN	400	26	12.5	8	1.5	2	1	0

Correlation analysis between different factors are presented in Table 6.3.1.2. The correlation analysis result revealed that:

- Germination per cent versus seed infected with *Fusarium* spp. and *Alternaria* spp. were significant at the 5% level.

- The correlation between seed germination percentage and seed infected with *Fusarium* spp. was significant at the 5% level with r value of -0.501. The negative correlation was explained by a small number of seed lots heavily infected by *Fusarium* spp. showing poor germination. Otherwise the % of seed infected with *Fusarium* spp. is quite uniform across a range of germination (Figure 6.3.1.1).

- The correlation between seed germination percentage and seed infected with *Alternaria* spp. was positive with r value is 0.608 with the r^2 value 0.36. This indicates that the presence of *Alternaria* is associated with improved germination. However, far fewer seeds were infected with *Alternaria* spp. than with *Fusarium* spp. (Figure 6.3.1.1) so this result may not be very meaningful.

- There was a strong correlation (r = 0.942) between percentage of diseased seed and the percentage of seed infected with *Fusarium* spp., significant at the 1% level (Figure 6.3.1.2). The r^2 value 0.8873 indicate that 88% of the variation in the percentage of diseased seed was explained by seed infected with *Fusarium* spp. in different seed lots.

Table 6.3.1.2 Correlation analysis between different factors associated with fifteen different seed lots of cauliflower (blotter test)

	Versus	r value	r^2 value
Germination percentage	% of diseased seed	-0.302	0.0912
	% of seed infected with *Fusarium*	-0.501 *	0.2510
	% of seed infected with *Sclerotinia*	0.052	0.0027
	% of seed infected with *Alternaria*	0.608 *	0.3696
	% of seed infected with *Phoma*	-0.161	0.0259
	% of seed infected with *Rhizopus*	-0.403	0.1624
% of diseased seed	% of seed infected with *Fusarium*	0.942 **	0.8873
	% of seed infected with *Sclerotinia*	0.022	0.0004
	% of seed infected with *Alternaria*	0.373	0.1391
	% of seed infected with *Phoma*	-0.168	0.0282
	% of seed infected with *Rhizopus*	0.231	0.0533

* Correlation value is significantly different at 5% level.

** Correlation value is significantly different at 1% level.

Seed test results indicated that 47% of available seeds lots were below the standard of germination and poor germination of seeds was caused by poor seed health i.e. seeds were infected with *Fusarium* spp.

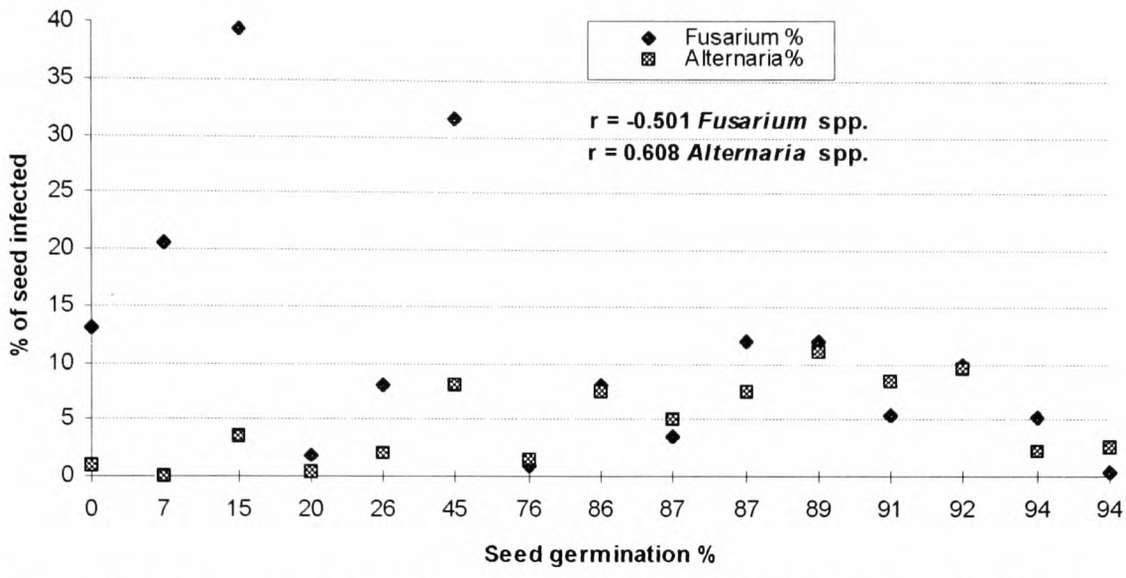


Figure 6.3.1.1 A correlation plot between seed germination % and % of seed infected with *Fusarium* spp. and *Alternaria* spp. in 15 different seed lots of cauliflower.

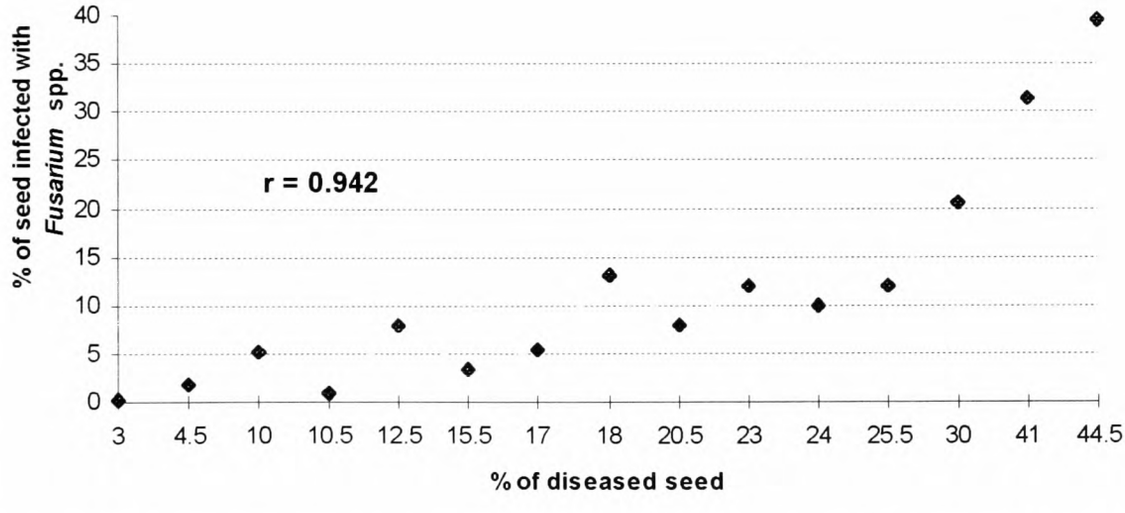


Figure 6.3.1.2 A correlation plot between % of diseased seed and % seed infected with *Fusarium* spp. in 15 different seed lots of cauliflower.

6.3.2 Seedling emergence test in sterile soil

The results of seedling emergence test in sterilised soil (Table 6.3.2.1) indicate similar trends to germination in the blotter test. Forty-seven per cent of seed lots obtained from the different sources have more than 76% field emergence but the remaining 53% did not meet this standard. Emergence percentage varied from 0 to 91%. Cause of poor emergence could be diseased seed as reported in the blotter test. By continuing observations up to 50 days after sowing, the highest post-emergence mortality was recorded at 37%.

Analysis of variance showed that emerged number of seedlings, seedling mortality after emergence, number of dead seedlings with *Pythium* spp., *S sclerotiorum*, and *Alternaria* spp. were significantly different between the difference sources of seed lots (Appendix 25). Seedling death and fungal pathogens associated with nine different seed lots of cauliflower collected from various sources are presented in Table 6.3.2.2. Correlation analysis between different factors are presented in Table 6.3.2.3.

Correlation analysis results revealed that:

- The correlation between seedling mortality after emergence and presence of *S. sclerotiorum* was significant at the 1% level, r = 0.937 and r^2 = 0.877 (Figure 6.3.2.1).
- The correlation between seedling mortality after emergence and presence of *Alternaria* spp. was significant at the 5% level, r = 0.653 and r^2 = 0.426 (Figure 6.3.2.1).
- There was no correlation between the emerged number of seedlings and seedling

mortality after emergence associated with any of the different fungi. This contrasts with the results of the blotter test (Table 6.3.1.2).

- There was a strong correlation between seed emergence and the blotter test germination results. The r value is 0.969 with the r^2 value 0.9406. Note that there are two distinct phases of scatter plot in Figure 6.3.2.2, one where seed germinated and seedling emergence are both rising and the second where germination has levelled off.

The seedling emergence test results indicated that 53% of the available seed lots were below the emergence standard. The cause of poor emergence was poor seed health. Poor seed health was mainly due to the fungi *S. sclerotiorum*, and *Alternaria* spp.

Overall, *Fusarium* spp. was apparently pathogenic in the blotter test. *S. sclerotiorum* and *Alternaria* spp. were apparently pathogenic in the seedling emergence test i.e. in soil. There was a positive correlation between germination % (blotter test) and seed infected with *Alternaria* spp. This indicate that *Alternaria* spp. are probably not pathogenic in the blotter test and that their presence might help to exclude the growth of other pathogenic fungi. However, the low level of seed contamination by *Alternaria* (9.5% as maximum) suggests that this result should be treated with caution.

Table 6.3.2.1 Overall seedling infection and breakdown according to individual fungi associated with fifteen different seed lots of cauliflower collected from various parts of Nepal

Cultivar	Seed source	Seedling emergence mean		Seedling mortality after emergence		Number of dead seedlings with			
		number	%	number	%	*Pythium*	*Sclerotinia*	*Alternaria*	*Alternaria+ Sclerotinia*
Terai-3	PAC	0	0	0	0	0	0	0	0
Kibogiant	CEAPRED	27	27	0	0	0	0	0	0
Snow Mystique	CEAPRED	91	91	0	0	0	0	0	0
Snowcrown	CEAPRED	94	94	0	0	0	0	0	0
Kathmandu Local	PAC 97	81	81	4	4.9	3	1	0	0
Kathmandu Local	VDD 96	87	87	1	1.1	0	0	0	1
Kathmandu Local	VDD 97	91	91	20	22.0	2	7	8	3
Kathmandu Local	PAC 96	84	84	13	15.5	0	5	7	1
Kathmandu Local	Bhaktapur 96	82	82	30	36.6	0	14	16	0
Kathmandu Local	Paripatle 97	75	75	3	4.0	0	2	1	0
Kathmandu Local	Paripatle 98	60	60	2	3.3	1	0	1	0
Kibogiant	Paripatle 98	75	75	0	0.0	0	0	0	0
Kathmandu Local	KOSEPAN	36	36	0	0.0	0	0	0	0
Kathmandu Local	PAC farmer	9	9	3	33.3	1	2	0	0
Snow ball- 16	KOSEPAN	28	28	8	28.6	0	6	2	0

Table 6.3.2.2 Overall seedling death and breakdown of mortality according to fungal pathogen associated with nine different seed lots of cauliflower collected from various parts of Nepal (seeds germinated in sterile soil)

Cultivar and seed sources	Seedling mortality % after emergence	% of dead seedlings with			
		Pythium	*Sclerotinia*	*Alternaria*	*Alternaria* & *Sclerotinia*
Kathmandu Local (PAC 97)	4.9	3.7	1.2	0	0
Kathmandu Local (VDD 96)	1.1	0	0	0	1.1
Kathmandu Local (VDD 97)	22	2.2	7.7	8.8	3.3
Kathmandu Local (PAC 96)	15.5	0	5.9	8.3	1.1
Kathmandu Local (Bhaktapur)	36.6	0	17.1	19.5	0
Kathmandu Local (Paripatle 97)	4	0	2.7	1.3	0
Kathmandu Local (Paripatle 98)	3.3	1.7	0	1.7	0
Kathmandu Local (PAC farmer)	33.3	11.1	22.2	0	0
Snow ball-16 (KOSEPAN)	28.6	0	21.4	7.2	0

Table 6.3.2.3 Correlation analysis between different factors associated with nine different seed lots of cauliflower (seedling emerged in sterile soil)

	Versus	r value	r^2 value
Seed germination %	Seedling emergence %	0.969	0.940
Seedling emergence %	Dead seedlings with *Pythium* spp.	0.097	0.009
	Dead seedlings with *S. sclerotiorum*	0.125	0.001
	Dead seedlings with *Alternaria* spp.	0.297	0.088
	Dead seedlings with *Alternaria* spp. and *S. sclerotiorum* (both fungi occurring together)	0.369	0.136
Seedling mortality after emergence	% of dead seedlings with *Pythium* spp.	0.323	0.1043
	% of dead seedlings with *S. sclerotiorum*	0.937**	0.8779
	% of dead seedlings with *Alternaria* spp.	0.653*	0.4264
	% of dead seedlings with *Alternaria* spp. & *S. sclerotiorum* (both fungi occurring together)	-0.003	0.0000

* Correlation value is significantly different at 5% level.
** Correlation value is significantly different at 1% level.

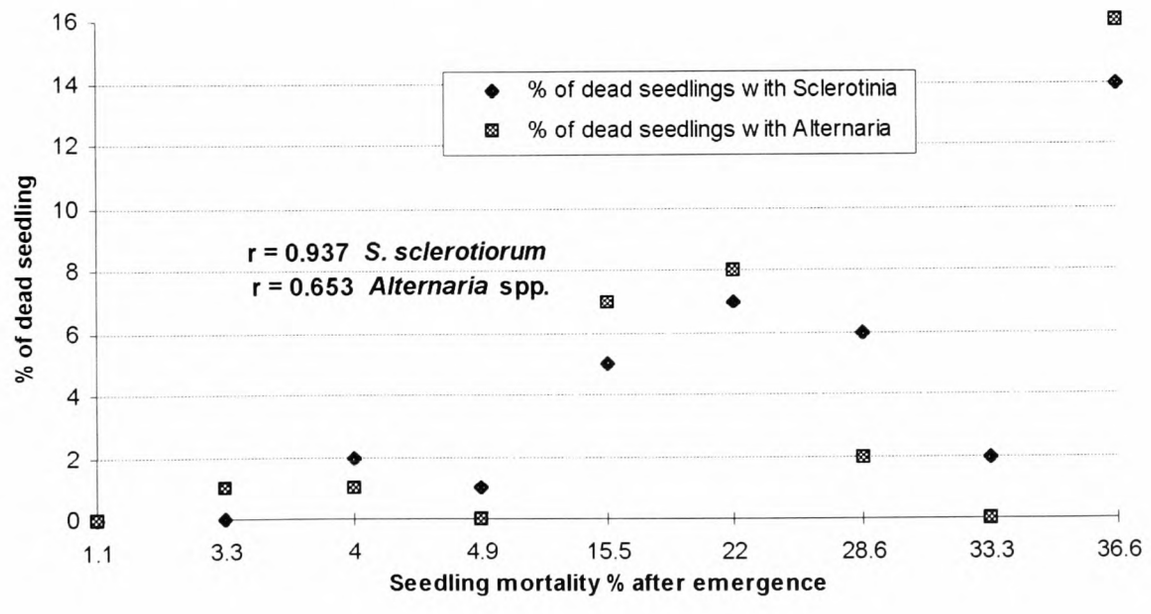


Figure 6.3.2.1 A correlation plot between seedling mortality % after emergence and % of dead seedlings with *S. sclerotiorum* and *Alternaria* spp. in nine different seed lots of cauliflower.

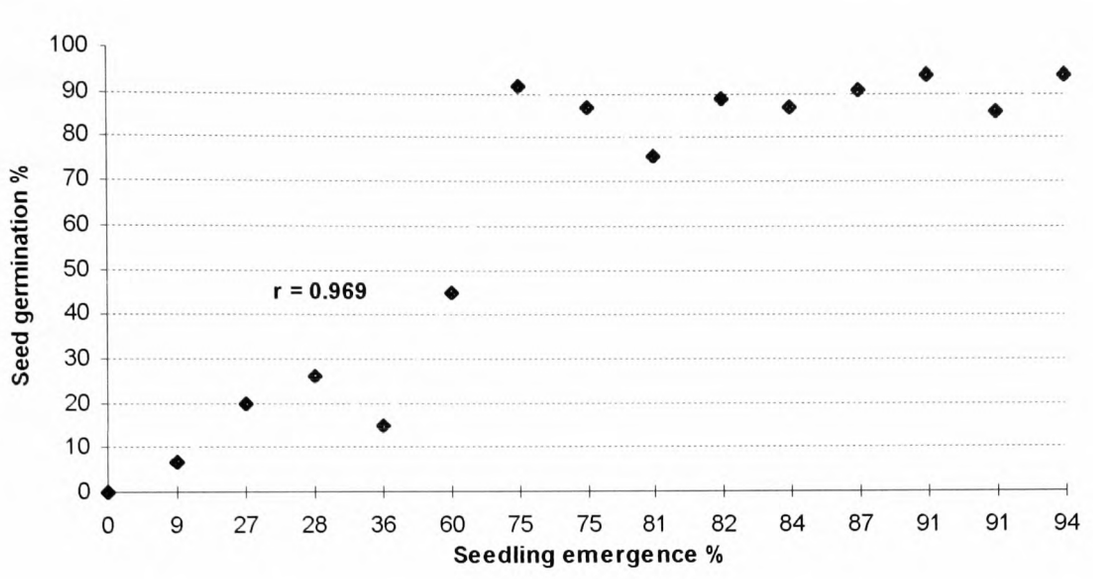


Figure 6.3.2.2 A correlation plot between germination % and % of emerged seedlings in 15 different seed lots of cauliflower.

6.3.3 Monitoring of sclerotial germination

(a) General

It is to be expected that the principal seed-borne inoculum of *Fusarium* spp. is conidia, whereas with *S. sclerotiorum* (no conidia) the inoculum could be mycelium or sclerotia. Examination of the seed lots used in this study revealed the presence of sclerotia in at least two lots. Sclerotial contamination of cauliflower seed is widespread (Shrestha, 1990).

Monitoring was continued daily except during the weekend up to 25 February 1999. Results are presented in Table 6.3.3.1 with corresponding meteorological data. The results indicate, first, that germinated sclerotia that exhibited long stipes bore multiple apothecia from August 1997 in the sick plot and from August 1998 in the earthen pots. Secondly in sick soil, sclerotia continued to germinate and produced apothecia in August to October 1997, July to September and November 1998 and

January 1999 whereas in the earthenware pots in August and October 1998 and February 1999. Ascus cup producing sclerotia were uprooted and discarded after counting.

(b) Sick plot study

Analysing germination with the corresponding meteorological data (Table 6.3.3.1) indicated that sclerotia germinated when soil temperature at 5 cm depth was between 10.4°C and 24.9°C. The maximum germination and production of apothecia were recorded when the soil temperature at 5 cm depth was around 23 to 24°C.

(c) Earthenware pot study

Analysing germination with the corresponding meteorological data (Table 6.3.3.1) indicated that sclerotia germinated when soil temperature at 5 cm depth was between 10.2°C and 23.1°C. The maximum germination and production of apothecia were recorded when the soil temperature at 5 cm depth was around 18°C. Sclerotia planted at 2 to 3 cm depth took 6 months to germinate and to produce apothecia in the earthenware, but sclerotia planted at 4 to 5 cm depth germinated only after 14 months.

Overall the indications were that sclerotia can germinate and produce apothecia when soil temperatures at 5 cm depth were between 10.2°C and 24.9°C. The results also indicate that sclerotia planted at shallow depth germinate earlier than those planted more deeply. The seedling emergence test in the sterile soil was performed in September to November 1998; during that period soil temperature at 5 cm depth

was between 17.5°C and 22.9°C (Appendix 16). This would allow sclerotial germination and infection of seedlings.

Table 6.3.3.1 Number of sclerotia germinating in the sick plots and in the earthenware pot at ARS Pakhribas from August 1997 to February 1999

Date	No. of sclerotia germinated			Soil temperature (°C)	Air Temp(°C)	
	Sick soil	Earthenware	Depth of germination	at 5 cm depth	Max.	Min
25-Aug-97	2	0		25.1	24.5	18.5
04-Sep-97	2	0		23.3	22.5	16.5
05-Sep-97	9	0		23.6	22.5	17
07-Sep-97	2	0		24.9	25	17.5
19-Sep-97	1	0		23.3	22.5	17
05-Oct-97	3	0		22.4	22	12.5
14-Jul-98	21	0		23.2	23	18.5
02-Aug-98	1	2	2 cm	11.4	15.5	6.0
10-Aug-98	8	1	2 cm	10.4	12.5	2.0
14-Aug-98	0	2	3 cm	10.2	15.0	4.5
16-Aug-98	5	3	3 cm	10.8	15.5	6.5
22-Aug-98	0	3	2 cm = 2, 3 cm = 1	11.4	16.5	6.5
23-Aug-98	2	2	3 cm	11.7	16.5	6.5
24-Aug-98	0	4	2 cm = 2, 3 cm = 2	12.2	16.5	7.0
28-Aug-98	0	1	2 cm	13.3	18.0	7.5
07-Sep-98	58	0		23.3	24.5	17.0
08-Oct-98	0	2	2 cm	23.1	24.0	16.5
03-Nov-98	4	0		20.1	23.0	12.5
05-Nov-98	2	0		18.8	22.5	11.5
11-Nov-98	3	0		18.7	20.0	11.0
13-Nov-98	3	0		17.1	21.5	11.5
16-Nov-98	1	0		17.7	21.5	12.0
21-Jan-99	2	0		11.9	17.0	7.5
25-Feb-99	0	18	4 cm = 8, 5 cm = 10	17.9	20.5	10.5

Discussion

Variable levels of germination and seedling health have resulted in concern on quality of seeds for cauliflower crop production. Qualitative examination of 15 different seed lots indicated that two lots were contaminated with sclerotia of *S. sclerotiorum*. The poor germination of seed lots tested was associated with *Fusarium* spp. and *S. sclerotiorum*, while poor emergence in soil-based test was associated with *Pythium* spp., *S. sclerotiorum* and *Alternaria* spp. With the exception of one of the accession (Terai-3) which was non-viable, poor emergence of seed planted in sterile soil was observed to be correlated with seed-borne fungi identified when showing seed testing blotter methods.

Overall, it would appears that eastern hill farmers may be using seed of poor quality and that the seed supply systems in the hills are unreliable. This is probably because imported seeds are not passed through a quarantine or seed health laboratory and the hill farmers are less concerned about seed health than timely availability of seeds.

CHAPTER 7

ASSESSMENT OF THE RANGE OF STALK ROT (*S. SCLEROTIORUM*) RESISTANCE IN CAULIFLOWER IN NEPAL

7.1 Background

Commercial farming of cauliflower, both fresh and seed crops, in the eastern hills of Nepal has been set back by stalk rot and damping-off diseases caused by *S. sclerotiorum* and other pathogens (Chapters 5 and 6). Use of resistant varieties would be one of the most effective and economical methods of disease control. Resistant varieties are particularly important in areas where pathogens are soil-borne because soil sterilisation treatment is expensive or impractical. In India, Singh and Kalda (1995) studied the varietal reaction of sixty-nine genotypes of cauliflower at the seedling stage and found four with low infection (less than 50% of the leaf surface infected). Lower infection (so called resistance) was recorded in winter cauliflower only. On the premise that there might be sources of heritable resistance in cauliflower germplasm available in Nepal, this study was carried out with the following objectives:

- To compare leaf and curd susceptibility to *S. sclerotiorum*
- To investigate the effect of leaf age on infection development
- To attempt selection for resistance in cauliflower cv. Kathmandu Local using the above methodology

7.2 Materials and methods

7.2.1 Leaf and curd susceptibility tests

(a) Susceptibility of seedlings

Seeds of thirteen different cultivars of cauliflower, namely Pusadeepali, Snowball-16, Terai-3, Kibogiant, Hindselected Kuwari, Hind First Crop, Chinolate, White summer, White Top, Nozaki Wase, Kathmandu Local, Indam Early (hybrid), and Snowcrown (hybrid) were collected from different sources. Seedlings were raised in plastic trays filled with a mixture of compost and forest soil with three replications during the normal growing season (22 October 1997). Including Kathmandu Local, only six lots of seed produced the required number of seedlings for the study. (The seeds of cv. Kathmandu Local used in this study were from earlier work on inheritance of stalk rot resistance; seeds had been harvested from apparently disease resistant plants.) Forty-day old seedlings were inoculated at two different places on the third lower leaf from the top using a mustard petal that had been partially colonised by *Sclerotinia* mycelium for the previous 24 hr (Kapoor, 1985; Kapoor, 1986) and by discs of mycelium from the growing edge 48 hr old colonies on potato dextrose agar (PDA) plate cultures. Air temperature and relative humidity were maintained at 19 to 24 °C and > 81% respectively by making an arch-shaped plastic tunnel and placing wet jute sacks inside. A visual assessment of disease development was made seven days after inoculation. The disease scale and lesion categories used are presented in Table 7.2.1.1.

Table 7.2.1.1. Disease scale and categories used to assess the disease reaction in leaves

Disease reaction	Scale	Category of lesion
Resistant	0	No browning
Moderately resistant	1	Some browning, lesion < 2 mm wide
Intermediate reaction	2	Browning extended all around the inoculation point, lesion size 2 - 3 mm wide
Moderately susceptible	3	Lesion size 3 - 5 mm size
Susceptible	4	Lesion ring 5 - 6 mm
Highly susceptible	5	Lesion size > 6 mm

After monitoring disease development, inoculated leaves were detached and the same seedlings were transplanted in a soil apparently free of *S. sclerotiorum*. After curd formation, i.e. when the curd size attained > 10 cm in diameter, the curd surface was inoculated with partially colonized petals and mycelial discs (2 mm) after making a small hole with a cork borer. The field was irrigated using a hose pipe to create humid conditions. The disease scale and rotting categories used to assess the disease reaction on the curd are presented in Table 7.2.1.2.

Table 7.2.1.2 Disease scale and rotting areas used to assess the disease reaction on curds

Disease reaction	Scale	Rotting categories
Resistant	0	Spread of lesion restricted to the site of inoculation
Moderately resistant	1	Up to 25% of the curd rotting
Intermediate type	2	26 to 50% of the curd rotting
Moderately susceptible	3	51 to 75% of the curd rotting
Susceptible	4	76 to 100% of the curd rotting
Highly susceptible	5	Rotting spread down the stalk of the plant

(b) Curd susceptibility of apparently resistant seedlings

Over one hundred seedlings of six different cultivars (Kathmandu Local, Pusadeepali, Snowball-16, Kibogiant, Nozaki Wase and Snowcrown) were screened by partially colonized petal and mycelial disc inoculation on leaves. Seeds of Kathmandu Local used in this study were from the same source as above. Ten seedlings from each cv. which appeared resistant after both partially colonized petal and mycelial disc inoculation were transplanted for curd inoculation into an uncontaminated soil on 7 December 1997. After curd formation (when the curds size attained > 10 cm diameter) the curd surface was inoculated as above. A visual assessment was made 30 days after inoculation to assess disease level on the curd surface on the basis of the rotting categories described in Table 7.2.1.2.

7.2.2 Effect of leaf age on susceptibility

(a) Detached leaf susceptibility test on cultivars Kathmandu Local and Snowcrown (hybrid)

Six, 50 day old (five-leaf stage) seedlings of cauliflower cv. Kathmandu Local and another six, 46 day old (five-leaf stage) seedlings of cv. Snowcrown were selected from the nursery bed. Leaves of the selected seedlings were detached with a 1 cm long petiole. Immediately after detaching the leaves from the seedlings the leaf petiole was wrapped with moist tissue paper. These leaves were placed into a 5-ounce plastic sandwich box on two layers of moist tissue paper. Leaves were inoculated at four places on the upper surface after making wounds with an inoculation needle: two inoculations were made on one side of the mid-rib of the

leaf with partially colonized petal; the lamina on the other side of the mid-rib was inoculated twice with mycelial discs (Figure 7.3.2.5). Effect of leaf age on lesion development was assessed under controlled conditions, where temperature and light intensity were maintained 20 °C and 3000 Lux respectively. Disease progress in Kathmandu Local on each leaf L1, L2, L3, L4 and L5 (described in Table 7.2.2.1) was measured for two different methods of inoculation using a new piece of transparent graph paper at 20, 24, 42, 46 and 63 hr after inoculation. With cv. Snowcrown, disease was assessed at 27, 48, 54, 72 and 78 hr after inoculation but otherwise as Kathmandu Local.

Table 7.2.2.1 Description of leaf position

Symbol assigned	Leaf position
L1	Top growing leaf
L2	Second leaf from the top
L3	Third leaf
L4	Second leaf from bottom
L5	Lowest leaf (excluding cotyledon leaf)

(b) Susceptibility test on attached leaves of cultivars Kathmandu Local and Snowcrown (hybrid)

A total of 30 seedlings of cv. Kathmandu Local were raised in polythene pots. Two seedlings were damaged while handling so twenty-five seedlings, each 62 days old, were selected for the study. For the cv. Snowcrown 30 seedlings were selected. All seedlings were raised in the nursery. Thirty-five day old seedlings were planted into polythene pots when the seedlings had established and produced five leaves. The study was performed in a randomised block design with five replications for Kathmandu Local and six replications for Snowcrown. Seedlings were inoculated with partially colonized petals and mycelial discs as described in the detached leaf tests. After inoculation leaves were sprayed with sterile water and plants were allowed to grow under natural conditions. Lesion progress was measured daily from 6 to 20 days after inoculation for Kathmandu Local and 5 to 14 days for cv. Snowcrown with a piece of graph paper.

7.2.3 Selection for stalk rot resistance level in cauliflower cv. Kathmandu Local

Seed of cv. Kathmandu Local from nine different sources were collected in 1995 from different agricultural stations and farmers' fields. These seed lots were tested for inheritance of stalk rot resistance starting in 1996 and continuing for two years. Seeds were harvested in June 1997 in bulk from plants showing a resistant reaction only after partially colonized petal inoculation at seedling, curd and flowering stages.

Including the bulk seed from the above a total of ten different seed lots were included for screening work. These were grown in nurseries in sterile soil during normal growing seasons (September-October 1997). From the reference of 7.3.2 (b) the third lower leaf from the top of 28 day old seedlings was inoculated in two places on the upper surface of the leaf with both petal and mycelial disc as in 7.2.1 (a). Air temperature and relative humidity of the nurseries were maintained as described in the leaf and curd susceptibility tests. A visual assessment was made six days after inoculation to assess the disease level of inoculated seedlings as described earlier.

Seedlings showing a resistant reaction at the nursery stage were then planted in the main field and a second inoculation was done seven days after transplanting with partially colonized petal and mycelial disc. The second inoculation was done to avoid the chances of inoculum failure during seedling screen at the nursery stage giving an apparently resistant reaction. Seedlings showing a susceptible reaction (stalk rot symptoms) 30 days after inoculation were uprooted and the rest were allowed to grow to the curd stage for further screening as described above in the leaf and curd susceptibility tests.

Plants showing resistant reactions were caged with a pollination net after bolting and subjected to manual self-pollination. Seeds harvested from the disease resistant plants during 1997 were further tested during 1998. A summary of the screening methodology is presented in Figure 7.2.3.1.

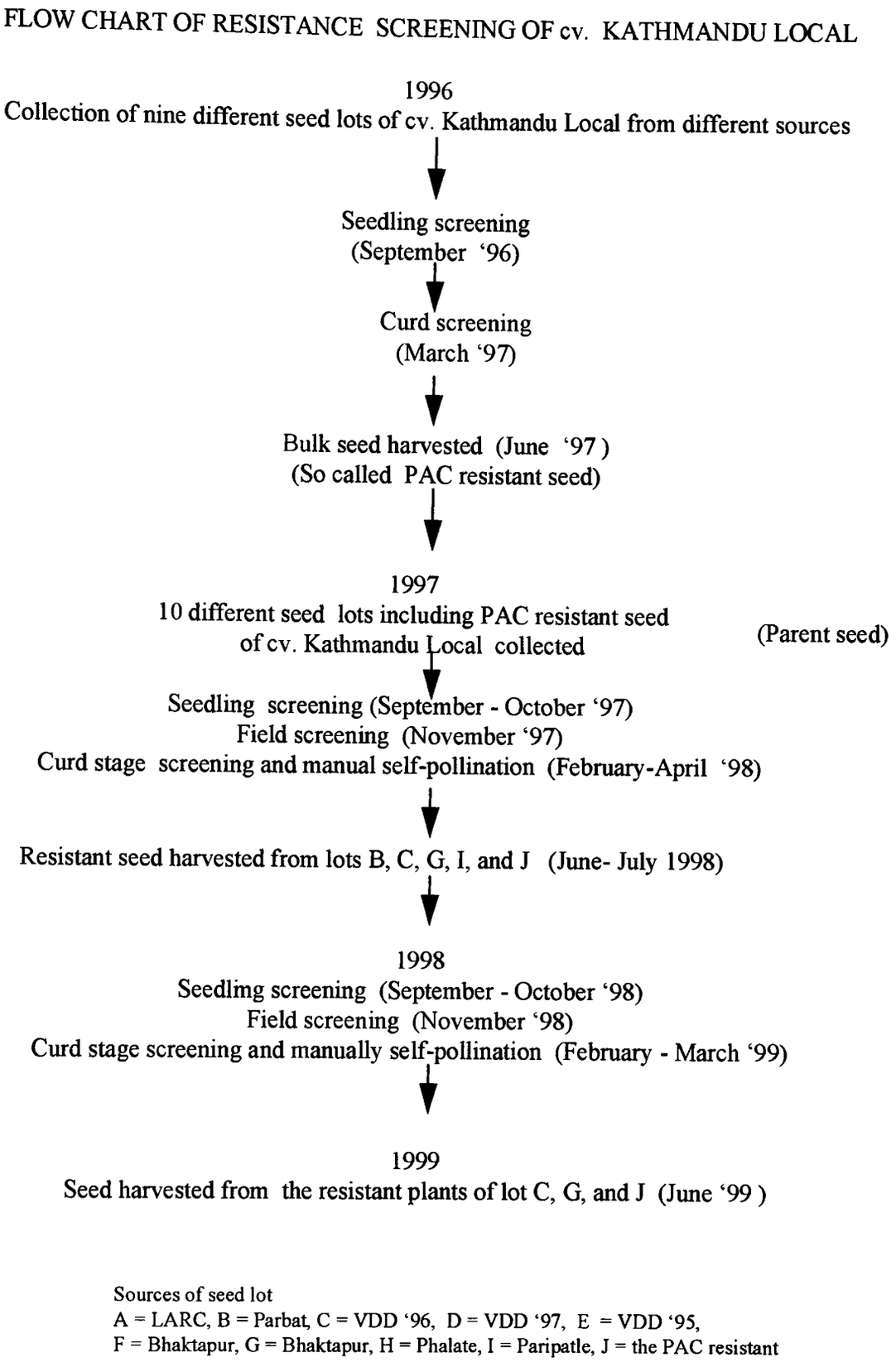


Figure 7.2.3.1 Summary of screening methodology.

7.3 Results and discussion

7.3.1 Leaf and curd susceptiblity tests

(a) Susceptibility of seedlings

The six different cultivars showed wide variation in disease reactions in the nursery. Considerable differences were found between results of inoculation by partially colonized petals and mycelial discs (Table 7.3.1.1). For example cultivars Kathmandu Local and Snowcrown had scored less than 2 on the scale by petal inoculation whereas the same leaf scored greater than 2 by mycelial disc inoculation. Only the cultivar Nozaki Wase showed similar trends of disease reaction with both methods.

Curd test results indicated that, except for Kathmandu Local, all cultivars were highly susceptible (Table 7.3.1.2). The results also indicated that there was no overall correlation between leaf and curd susceptibility. However, in cv. Kathmandu Local there was some degree of correlation between the two assessments. This was expected as the seeds of cv. Kathmandu Local used in this study was selected from earlier work on inheritance of resistance of stalk rot disease and there appeared to be some resistance in the plants under test.

Table 7.3.1.1 Levels of disease shown by the six different cultivars of cauliflower during nursery evaluation after partially colonised petal and mycelial disc inoculation (conducted at ARS Pakhribas during October-December 1997)

Plant no.	Pusadeepali		Snowball-16		Kibogiant		Nozaki Wase		Kathmandu Local		Snowcrown	
	Disease scale rating by pre colonised mustard petal and mycelial disc inoculation methods											
	Petal	Mycelial	Petal	Mycelial	Petal	Mycelial	Petal	Mycelial	Petal	Mycelial	Petal	Mycelial
1	5	3	3	5	3	5	5	5	1	5	1	4
2	0	3	1	3	1	5	5	5	1	5	1	4
3	0	2	1	3	1	5	5	5	1	5	1	3
4	1	1	2	3	2	5	5	5	1	4	1	3
5	2	0	2	0	2	1	1	4	0	4	0	2
6	5	3	1	3	1	5	5	5	0	5	1	5
7	4	1	1	3	1	5	5	5	1	5	2	5
8	3	1	3	3	3	1	1	5	1	5	0	5
9	0	1	2	3	2	1	1	5	0	5	1	3
10	0	0	1	1	1	2	2	4	1	3	1	3

Table 7.3.1.2 Disease reaction shown by the six different cultivars of cauliflower planted in an uncontaminated soil and inoculated during curd stage with partially colonised petals and mycelial discs (conducted at the ARS Pakhribas March to June 1998)

Plant no.	Pusadeepali		Snowball-16		Kibogiant		Nozaki Wase		Kathmandu Local		Snowcrown	
	Disease scale rating by pre colonised mustard petal and mycelial disc inoculation methods											
	Petal	Mycelial	Petal	Mycelial	Petal	Mycelial	Petal	Mycelial	Petal	Mycelial	Petal	Mycelial
1	HS	HS	HS	HS	HS	HS	HS	HS	M	M	HS	HS
2	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS
3	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS
4	HS	HS	HS	HS	HS	HS	HS	HS	M	M	HS	HS
5	HS	HS	HS	HS	HS	HS	HS	HS	M	M	HS	HS
6	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS
7	HS	HS	HS	HS	HS	HS	HS	HS	M	R	HS	HS
8	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS
9	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS
10	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS

HS = Highly susceptible.
M = Intermediate type of disease reaction.
R = Resistant.

Note: Seeds were harvested only from plants showing intermediate reaction.

(b) Curd susceptibility of apparently resistant seedlings

The results of tests of curd susceptibility of apparently resistant seedlings (Table 7.3.1.3) indicate similar trends to those observed in the leaf and curd susceptibility tests. Except for Kathmandu Local all five cultivars were highly susceptible. The results also indicate that there was no overall correlation between the resistance of seedling leaves and curds except perhaps in cv. Kathmandu Local. Overall, results of this study indicate that resistance to stalk rot expressed by disease resistance reactions is not a consistent trait as plants apparently resistant as seedlings when transplanted in the field were often susceptible at the curd and flowering stages.

Table 7.3.1.3. Disease reaction to petal and mycelial inoculation in curds of cauliflower seedlings selected as disease free at the seedling stage

Plant no	Pusadeepali		Snow Ball-16		Kibogiant		Nozaki Wase		Kathmandu Local		Snowcrown	
	Disease scale rating by pre colonised mustard petal and mycelial disc inoculation methods											
	Petal	Mycelial	Petal	Mycelial	Petal	Mycelial	Petal	Mycelial	Petal	Mycelial	Petal	Mycelial
1	HS	HS	HS	HS	HS	HS	HS	HS	M	M	HS	HS
2	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS
3	HS	HS	HS	HS	HS	HS	HS	HS	M	R	HS	HS
4	HS	HS	HS	HS	HS	HS	HS	HS	M	M	HS	HS
5	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS
6	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS
7	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS
8	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS
9	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS
10	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS

HS = Highly susceptible.
M = Intermediate type of disease reaction.
R = Resistant.
Note: Results of this study indicate that only three plants of cv. Kathmandu Local with an intermediate type of disease reaction produced seed.

7.3.2 Effect of leaf age on susceptibility

(a) Detached leaf susceptibility test on cultivars Kathmandu Local and Snowcrown (hybrid)

Mean disease ratings on cv. Kathmandu Local are presented in Table 7.3.2.1 Analysis of variance showed that there were no significant differences between disease progress at different leaf positions when inoculation was by mycelial disc (Table 7.3.2.1). However, with the petal method of inoculation there were significant differences at 24, 42 and 46 hr after inoculation with differently positioned leaves (Table 7.3.2.1). Two-way analysis of variance confirmed the effect of inoculation methods (Table 7.3.2.1). The conclusion is that with petal inoculation, susceptibility of different leaves can be differentiated. Disease progress curves on different age groups of leaves inoculated by the two different methods are presented in Figures 7.3.2.1 and 7.3.2.2.

Similarly, mean disease ratings on leaf cv. Snowcrown are presented in Table 7.3.2.2. Analysis of variance showed that there were no significant differences between disease progress at different positions when inoculated by either mycelial disc or partially colonized petal (Table 7.3.2.2). Two-way analysis of variance confirmed the lack of effect of inoculation methods at 54, 72 and 78 hr after inoculation (Table 7.3.2.2). Disease progress curves on different age groups of leaves inoculated by the two different methods are presented in Figures 7.3.2.3 and 7.3.2.4.

To conclude, there was a significant difference between methods of inoculation.

Detached susceptibility tests on cv. Snowcrown indicated that there were no significant differences between disease progress on differently positioned leaves when inoculated by either partially colonized petal or mycelial disc. However, partially colonized petal inoculation showed up differences in Kathmandu Local probably because there appeared to be some background level of resistance in contrast to Snowcrown (see 7.3.1). Disease progress on differently positioned leaves by partially colonized petal was slower in cv. Kathmandu Local up to 46 hours of inoculation than with mycelial disc. Up to 78 hr of inoculation similar trends were observed in cv. Snowcrown. In addition to that, disease progress trends in cv. Snowcrown was slower by either method of inoculation compared to Kathmandu Local. Typical lesion development on a detached leaf after mycelial disc and petal inoculation is illustrated in Figure 7.3.2.5.

Table 7.3.2.1 Mean lesion diameter on cv. Kathmandu Local after inoculation with partially colonized petals and mycelial discs

Leaf position	Disease progress (cm) after hr of petal and mycelial disc methods of inoculations									
	20 hr		24 hr		42 hr		46 hr		63 hr	
	Petal	Mycelial	Petal	Mycelial	Petal	Mycelial	Petal	Mycelial	Petal	Mycelial
Top growing leaf	0.158	0.383	0.333	0.675	0.825	1.49	1.200	1.95	2.08	2.61
Second lower leaf	0.450	0.475	0.383	0.833	1.042	1.56	1.383	1.77	2.33	2.43
Third leaf	0.358	0.417	0.258	0.683	0.850	1.78	1.350	2.03	2.53	2.89
Second leaf from bottom	0.333	0.358	0.583	0.767	1.600	1.79	1.925	2.27	2.83	2.74
Lowest leaf	0.403	0.533	0.558	1.067	1.475	2.14	2.042	2.67	3.04	2.96
Leaf position	NS	NS	*	NS	**	NS	*	NS	NS	NS
Methods	NS		**		**		**		NS	
Leaf position	NS		NS		NS		NS		NS	
Method & leaf position	NS		NS		NS		NS		NS	

* = Significantly different at 5% level.

** = Significantly different at 1% level.

NS = Not significantly different.

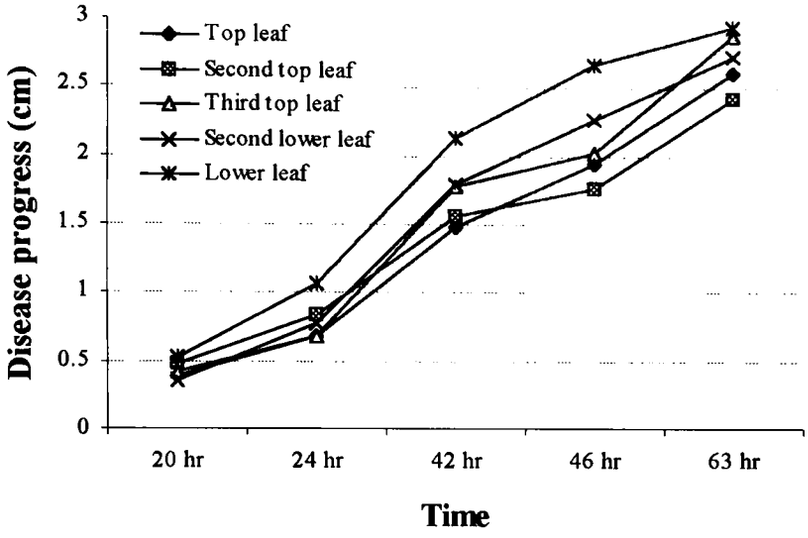


Figure 7.3.2.1 Disease progress on five different age groups of detached leaves of cauliflower (cv. Kathmandu Local): mycelial disc inoculation.
LSD of means of lesion diameter in five different age groups after 20, 24, 42, 46 and 63 hr of inoculation are 0.443, 0.576, 1.128, 1.185 and 1.057 respectively.

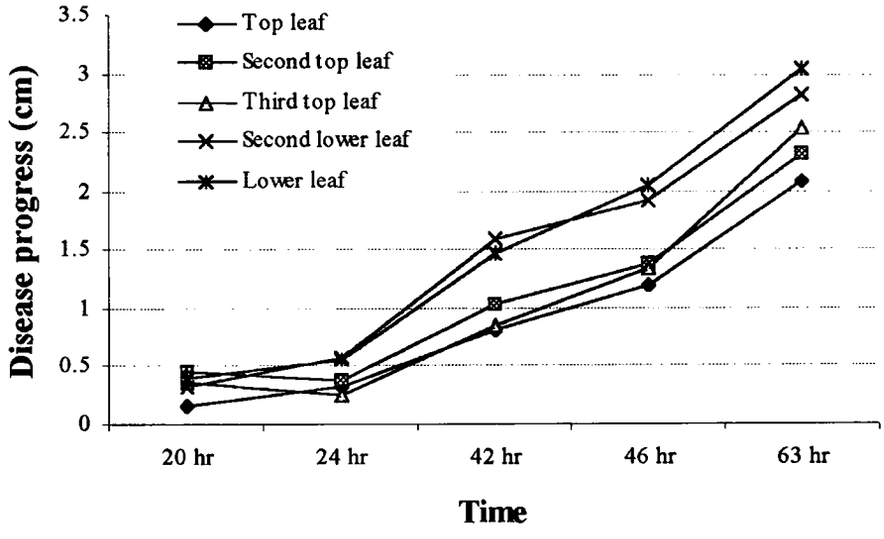


Figure 7.3.2.2 Disease progress on five different age groups of detached leaves of cauliflower (cv. Kathmandu Local): partially colonized petal inoculation.
LSD of means of lesion diameter in five different age groups after 20, 24, 42, 46 and 63 hr of inoculation are 0.428, 0.235, 0.509 and 0.834 respectively.

Table 7.3.2.2 Mean lesion diameter on cv. Snowcrown after inoculation with partially colonized petals and mycelial discs

Leaf position	Disease progress (cm) after hr of petal and mycelial disc methods of inoculation									
	27 hr		48 hr		54 hr		72 hr		78 hr	
	Petal	Mycelial	Petal	Mycelial	Petal	Mycelial	Petal	Mycelial	Petal	Mycelial
Top growing leaf	0.417	0.317	0.97	0.87	1.02	1.12	1.23	1.68	1.43	2.03
Second lower leaf	0.267	0.350	0.88	1.12	1.03	1.35	1.50	1.88	1.53	2.23
Third leaf	0.267	0.467	0.68	1.18	0.78	1.38	1.40	1.92	1.38	2.07
Second leaf from bottom	0.250	0.400	0.85	1.15	1.03	1.43	1.95	2.18	2.05	2.42
Lowest leaf	0.200	0.417	0.57	1.13	0.58	1.37	1.00	1.98	1.28	2.20
Leaf position	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Methods	NS		NS		*		*		*	
Leaf position	NS		NS		NS		NS		NS	
Method & leaf position	NS		NS		NS		NS		NS	

* = Significantly different at 5% level.
NS = Not significantly different.

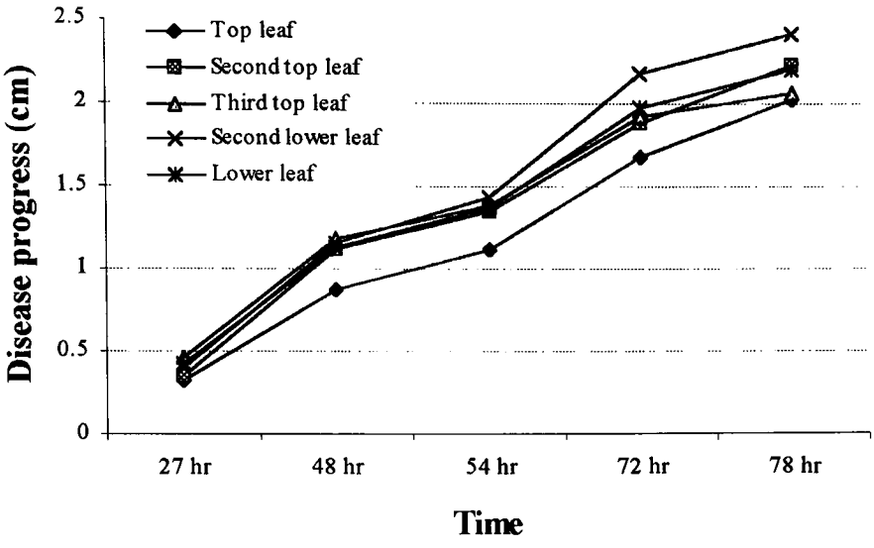


Figure 7.3.2.3 Disease progress on five different age groups of detached leaves of cauliflower (cv. Snowcrown): mycelial disc of inoculation.
LSD of means of lesion diameter in five different age groups after 27, 48, 54, 72 and 78 hr of inoculation are 0.276, 0.696, 0.805, 0.964 and 0.861 respectively.

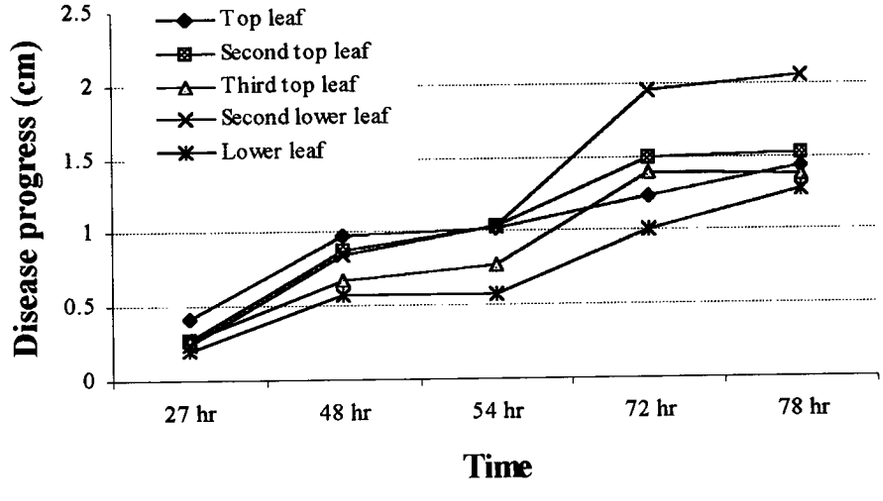


Figure 7.3.2.4 Disease progress on five different age groups of detached leaves of cauliflower (cv. Snowcrown): partially colonized petal inoculation.
LSD of means of lesion diameter in five different age groups after 27, 48, 54, 72 and 78 hr of inoculation are 0.394, 0.853, 0.930, 1.273 and 1.285 respectively.

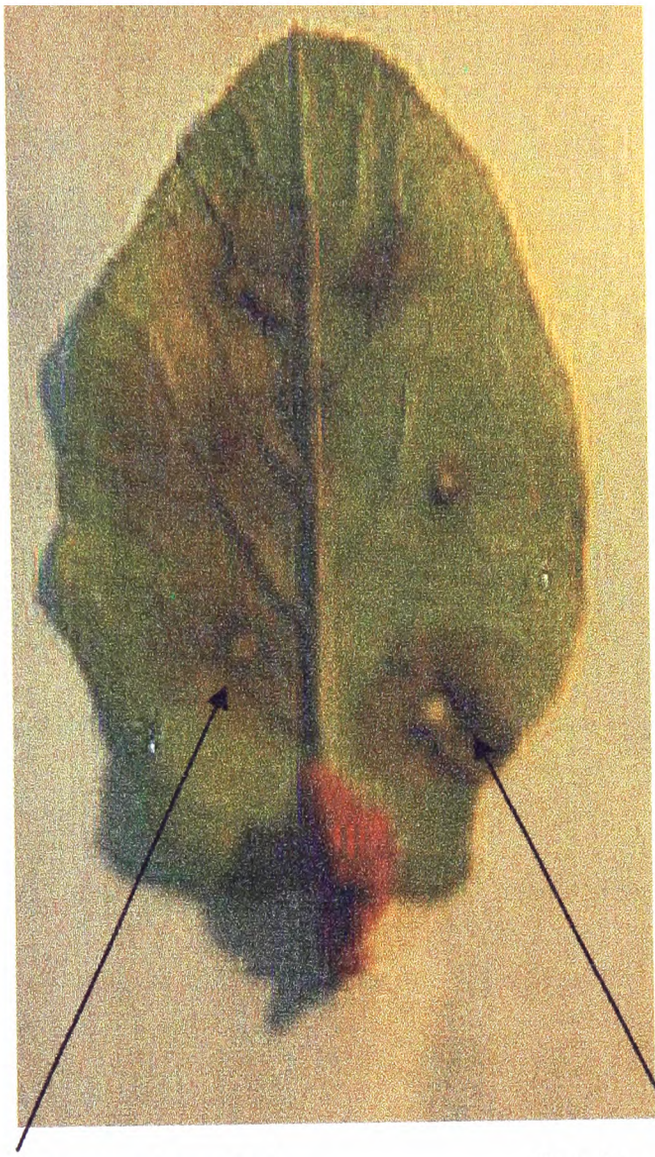


Figure 7.3.2.5 A typical lesion development on a detached leaf.

(b) Susceptibility tests on attached leaves of cultivars Kathmandu Local and Snowcrown (hybrid)

Mean lesion diameters on leaves 6 to 20 days after inoculation in cv. Kathmandu Local are presented in Tables 7.3.2.3 and 7.3.2.4. Analysis of variance showed that there were no significant differences in disease progress between differently positioned leaves when inoculated by the partially colonized petal methods (Appendix 26). However, with the mycelial disc method of inoculation there were significant differences between differently positioned leaves, 13 to 18 days after inoculation (Appendix 26). Two-way analysis of variance confirmed the effect of inoculation methods (Appendix 26). The conclusion is that with mycelial disc inoculation susceptibility of different leaves can be differentiated. Disease progress curves on different age groups of leaves inoculated by the two different methods are presented in Figure 7.3.2.6 and 7.3.2.7. Figures 7.3.2.6 and 7.3.2.7 show considerable differences in disease progress on differently positioned leaves, but because of variability in replications there were no significant differences.

Similarly, lesion development on leaves of cv. Snowcrown are presented in Tables 7.3.2.5 and 7.3.2.6. Analysis of variance showed that there were no significant differences between differently positioned leaves when inoculated by either mycelial disc or partially colonized petal (Appendix 27). Two-way analysis of variance confirmed the effect of inoculation methods 5 to 12 days after inoculation but not for 13 and 14 days (Appendix 27). Disease progress curves on different age groups of leaves inoculated by two different methods are presented in Figure 7.3.2.8 and 7.3.2.9.

Disease progress on differently positioned growing leaves of cv. Kathmandu Local was slower than Snowcrown using both methods of inoculation. The differences between the detached leaf and growing leaf tests were that disease progress were slower in Snowcrown with former while the reverse was found for the latter. Overall results demonstrate lack of consistency in relation to either method of inoculation or leaf position. For further work both methods of inoculation and the third leaf from the top were considered for further seedling screening.

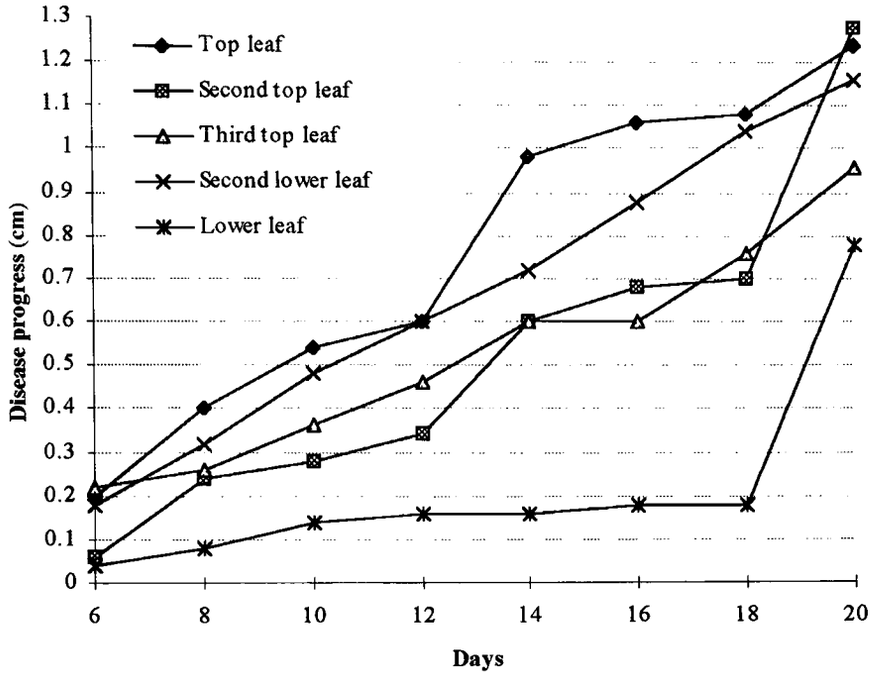


Figure 7.3.2.6 Disease progress on five different age groups of growing leaves of cauliflower (cv. Kathmandu Local): mycelial disc inoculation.
LSD of means of lesion diameter in five different age groups after 6, 8, 10, 12, 14, 16, 18 and 20 days of inoculation are 0.190, 0.389, 0.462, 0.484, 0.457, 0.419, 0.537 and 0.680 respectively.

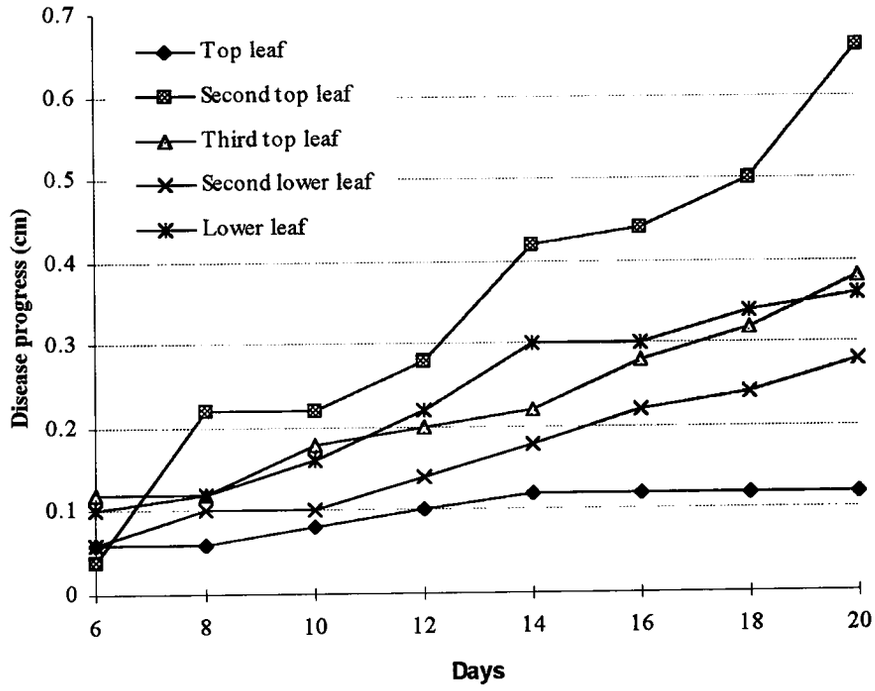


Figure 7.3.2.7 Disease progress on five different age groups growing leaves of cauliflower (cv. Kathmandu Local): partially colonized petal inoculation.
LSD of means of lesion diameter in five different age groups after 6, 8, 10, 12, 14, 16, 18 and 20 days of inoculation are 0.154, 0.325, 0.347, 0.408, 0.524, 0.545, 0.629 and 0.834 respectively.

Table 7.3.2.3 Mean lesion diameter on cv. Kathmandu Local after inoculation with partially colonized petals

Leaf position	Diseased progress (cm) following days after partially colonized petal inoculation														
	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days	14 days	15 days	16 days	17 days	18 days	19 days	20 days
Top growing leaf	0.060	0.060	0.060	0.080	0.080	0.080	0.100	0.120	0.120	0.120	0.120	0.120	0.120	0.12	0.12
Second leaf from top	0.040	0.120	0.220	0.220	0.220	0.220	0.280	0.400	0.420	0.420	0.440	0.440	0.500	0.64	0.66
Third leaf	0.120	0.120	0.120	0.160	0.180	0.180	0.200	0.220	0.220	0.240	0.280	0.300	0.320	0.34	0.38
Second leaf from bottom	0.060	0.080	0.100	0.100	0.100	0.100	0.140	0.180	0.180	0.180	0.220	0.240	0.240	0.28	0.28
Lowest leaf	0.100	0.100	0.120	0.160	0.160	0.160	0.220	0.300	0.300	0.300	0.300	0.300	0.340	0.36	0.36
Probability	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS = Not significantly different.

Table 7.3.2.4 Mean lesion diameter on cv. Kathmandu Local after inoculation with mycelial discs

Leaf position	Diseased progress (cm) following days after mycelial disc inoculation														
	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days	14 days	15 days	16 days	17 days	18 day	19 days	20 days
Top growing leaf	0.200	0.280	0.400	0.480	0.540	0.540	0.600	0.940	0.980	0.980	1.060	1.080	1.080	1.10	1.240
Second leaf from top	0.060	0.060	0.240	0.260	0.280	0.320	0.340	0.600	0.600	0.600	0.680	0.660	0.700	0.96	1.280
Third leaf	0.220	0.220	0.260	0.340	0.360	0.360	0.460	0.600	0.600	0.600	0.600	0.760	0.760	0.80	0.960
Second leaf from bottom	0.180	0.300	0.320	0.480	0.480	0.500	0.600	0.720	0.720	0.720	0.880	0.940	1.040	1.12	1.160
Lowest leaf	0.040	0.080	0.080	0.140	0.140	0.140	0.160	0.160	0.160	0.160	0.180	0.180	0.180	0.28	0.780
Probability	NS	NS	NS	NS	NS	NS	NS	*	*	*	*	*	*	NS	NS

* = Significantly different at 5% level.

NS = Not significantly different.

Table 7.3.2.5 Mean lesion diameter on cv. Snowcrown after inoculation with mycelial discs

	Disease progress (cm) at following days after mycelial disc inoculation									
Leaf position	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days	14 days
Top growing leaf	0.433	1.02	1.17	1.28	1.43	1.57	1.65	1.70	1.80	1.87
Second leaf from top	0.217	1.23	1.38	1.52	1.68	1.78	1.85	1.97	2.03	2.07
Third leaf	0.433	1.00	1.08	1.18	1.42	1.62	1.72	1.78	1.83	1.92
Second leaf from bottom	0.483	0.93	1.05	1.15	1.32	1.37	1.50	1.57	1.65	1.73
Lowest leaf	0.617	0.87	1.03	1.20	1.40	1.53	1.65	1.70	1.80	1.87
Probability	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS = Not significantly different.

Table 7.3.2.6 Mean lesion diameter on cv. Snowcrown after inoculation with partially colonized petals

	Disease progress (cm) at following days of petal inoculation									
Leaf position	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days	14 days
Top growing leaf	0.117	0.200	0.317	0.483	0.783	0.967	1.183	1.317	1.483	1.700
Second leaf from top	0.100	0.233	0.333	0.533	0.817	1.100	1.250	1.383	1.600	1.900
Third leaf	0.183	0.300	0.417	0.600	0.933	1.233	1.417	1.533	1.633	1.883
Second leaf from bottom	0.100	0.167	0.267	0.400	0.717	1.033	1.267	1.400	1.533	1.750
Lowest leaf	0.117	0.200	0.250	0.417	0.617	0.917	1.150	1.317	1.567	1.867
Probability	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS = Not significantly different.

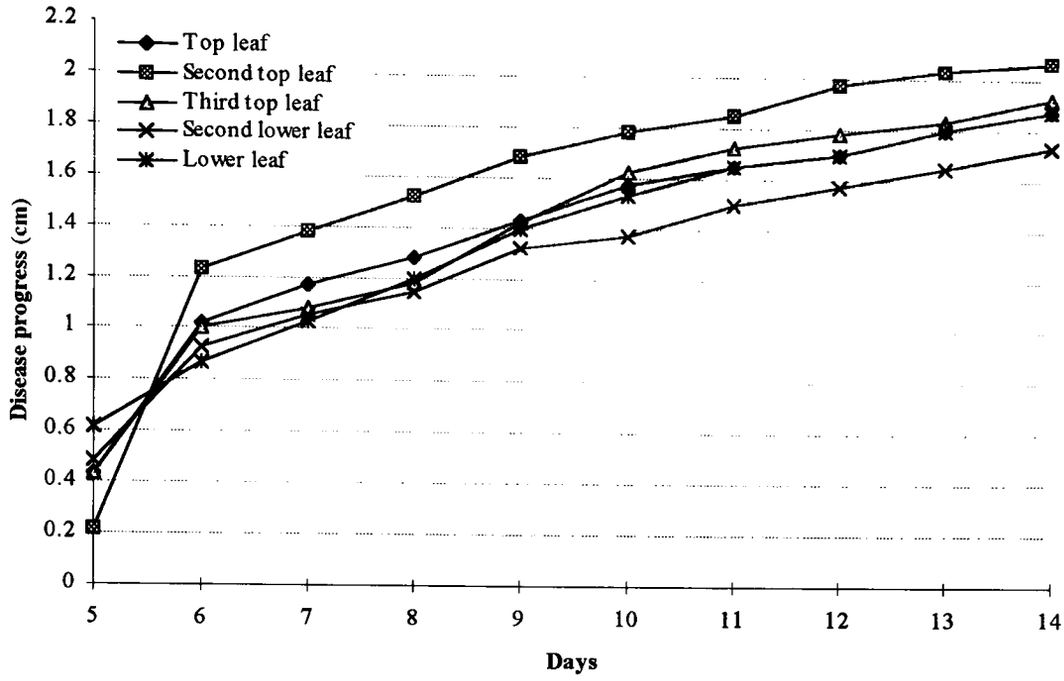


Figure 7.3.2.8 Disease progress on five different age groups of growing leaves of cauliflower (cv. Snowcrown): mycelial disc inoculation.
LSD of means of lesion diameter in five different age groups after 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14 days of inoculation are 0.583, 1.147, 1.160, 1.159, 1.103, 0.979, 0.911, 0.861, 0.818, and 0.818 respectively.

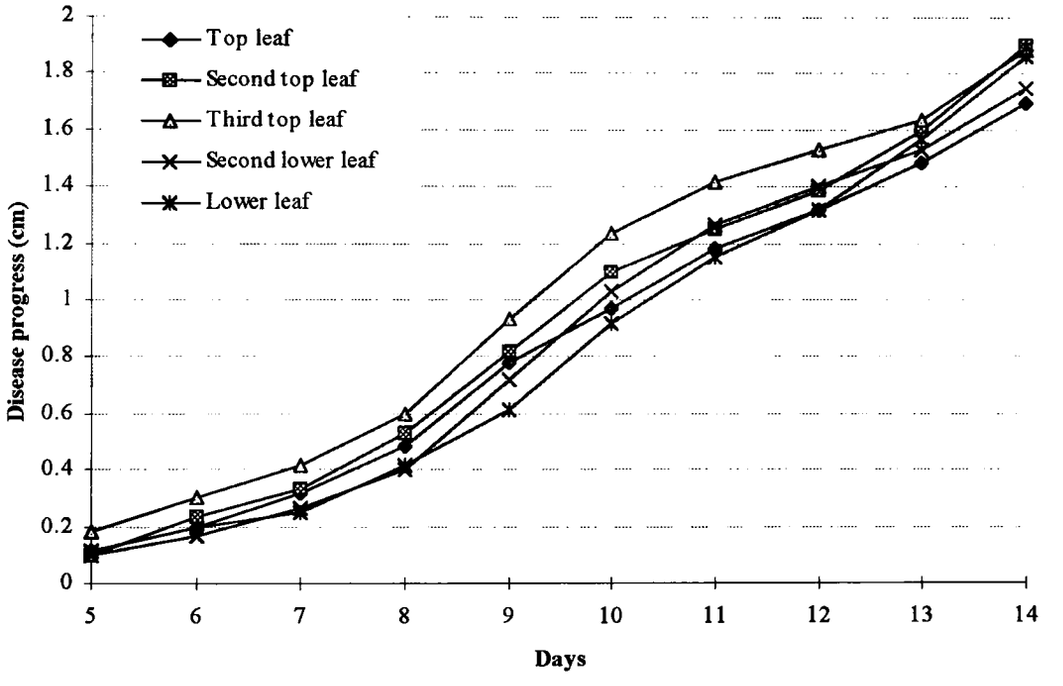


Figure 7.3.2.9 Disease progress on five different age groups of growing leaves of cauliflower (cv. Snowcrown): partially colonized petal inoculation.
LSD of means of lesion diameter in five different age groups after 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14 days of inoculation are 0.777, 0.102, 0.146, 0.219, 0.271, 0.318, 0.287, 0.241, 0.282, and 0.330 respectively.

7.3.3 Selection for stalk rot resistance level in cauliflower cv. Kathmandu Local

The results obtained from seedling, mature plant and curd stage screening during 1997 and 1998 are presented in Tables 7.3.3.1 and 7.3.3.2 respectively. The results indicate that there is a wide variation within the lines of cultivar Kathmandu Local (from different seed sources). Furthermore, except for the PAC resistant source of Kathmandu Local, resistance to stalk rot was not a reproducible trait as apparently resistant seedlings transplanted in the field were all susceptible at curd and flowering stages (Table 7.3.3.1).

A total of 109 seedlings (11%) out of 990 from ten different seed sources (parent seed) showed a resistant reaction in the nursery. When these seedlings were transplanted into the main field and a second inoculation was done, as many as 98 plants (89.9%) showed a resistant reaction, but only 5 plants (5.7%) showed a resistant reaction at curd and flowering stages. These results indicate that there is resistance in cv. Kathmandu Local, but that it is more apparent at the seedling stage of growth, and it is not a guide to resistance after transplanting.

The results obtained in 1998 from the seed harvested from resistant plants in 1997 (Table 7.3.3.2) indicated similar trends to those observed in during the 1997 season. A total of 155 seedlings (18.9%) out of 817 from the five different resistant seed lots showed resistant reactions in the nursery. However, 121 plants (78%) showed resistant reaction after seedling establishment, but only 9 plants

(8%) showed a disease resistant reaction at curd and flowering stages. As in the 1997 trial, apparent resistance at the seedling stage was not a guide to resistance after transplanting. The number of resistant plants screened from the seedling stage, established plant and curd stage in each year were analysed after arc-sin transformation by the t-test for paired sample means: there were no significant differences between resistance at these stages over the two years (Table 7.3.3.3).

Screening in 1997 was carried out with high disease pressure in the three different stages; a total of 5 lines of the ten original survived for the June - July 1998 seed harvest (Figure 7.2.3.1). In further screening from these seeds all five lines survived the three stages of screening, but only three lines showed apparent resistance. Looking at the results from successive years, the different genotypes did not maintain the same relative level of resistance (ranking varied from year to year). Given that climatic conditions varied (Appendices 15 and 16) it may be that the variation in apparent resistance was at least partly due to differences in plant adaptation to these conditions and hence to differences in plant vigour. The contribution of inherited resistance and the mechanism of any such resistance remains to be tested by crossing the apparently resistant selection with eliminated susceptible lines and other genotypes.

The highest degree of stalk rot resistance in the lines of cv. Kathmandu Local during two years of consecutive screening was exhibited by the PAC resistant seed followed by Vegetable Development Division (VDD) and farmers' seeds

collected from Bhaktapur. In any case, resistance was far greater at the establishment phase than at the curd stage so that this resistance may, unfortunately, be of limited potential.

Discussion

Commercial farming of cauliflower in the eastern hills of Nepal has probably been set back by stalk rot and damping-off disease caused by *S. sclerotiorum* and other pathogens. Due to small size of land holdings, farmers have to grow the crop on the same piece of land season after season and farmers are not familiar with an appropriate disposal method for diseased debris. Alternative methods to reduce stalk rot disease, such as deployment of disease resistant germplasm therefore need to be explored. Use of resistant varieties could potentially be one of the most applicable, low-cost and attractive technologies for the eastern hill farmers. In order to screen cauliflower lines for resistance to *S. sclerotiorum*, two different methods of inoculation were tested. This involved either petal or mycelial disc methods of inoculation. The former was found to be most appropriate for detached leaves whereas the latter was more suitable for inoculation of leaves of growing plants.

Six varieties of cauliflower were screened using both methods of inoculation at seedling and curd stage. No resistance was found in any of these cultivars except Kathmandu Local. This is perhaps not surprising because cultivars other than Kathmandu Local have been adopted in the eastern hill for a much shorter period. Certain lines of Kathmandu Local did show resistance to stalk rot in the seedling stage. The same seedlings at the curd stage showed very little resistance.

Table 7.3.3.1 Reaction from nursery, field and curd stages evaluation after partially colonised petal and mycelial disc inoculation techniques during September 1997 to June 1998 at ARS Pakhribas.

Treatment/ Seed sources	Total number of seedlings at nursery stage (September - October 1997)			Total number of seedlings after transplanting at main field [2] (November 1997)			Total number of plants at curd stage[3] (February-July 1998)		
	inoculated	susceptible	disease free[1]	inoculated	susceptible	disease free[1]	inoculated	susceptible	intermediate disease reaction[1]
A. LARC	111	106	5	5	0	5	5	5	0
B. Parbat	88	75	13	13	3	10	10	9	1
C. VDD96	112	97	15	15	2	13	13	12	1
D. VDD97	110	104	6	6	2	4	4	4	0
E. VDD95	89	82	7	7	1	6	6	6	0
F. Tulsi	103	80	23	23	0	23	12	12	0
G. Radhe	51	45	6	6	1	5	5	4	1
H. Phalate	112	105	7	7	1	6	6	6	0
I. Paripatle	111	92	19	19	1	18	18	17	1
J. PAC	103	95	8	8	0	8	8	7	1
Total	990	881	109 (11%)	109	11	98 (89.9%)	87	82	5 (5.7)

Figure in parenthesis are the percentage of resistant.

[1] Seedlings that are disease free in nursery.

[2] Plants disease free on second inoculation.

[3] Plants that are intermediate disease resistant in curd and flowering stages.

Table 7.3.3.2 Reaction from nursery, field and curd stages evaluation after partially colonised petal and mycelial disc inoculation techniques during September 1998 to June 1999 at ARS Pakhribas.

Treatment/ Seed sources	Total number of seedlings at nursery stage (September - October 1998)			Total number of seedlings after transplanting at main field[2] (November 1998)			Total number of plants at curd stage[3] (February-July 1999)		
	inoculated	susceptible	disease free[1]	inoculated	susceptible	disease free[1]	inoculated	susceptible	intermediate disease reaction[1]
B. Parbat	258	229	29	29	9	20	20	20	0
C. VDD96	110	77	33	33	0	33	25	23	2
G. Radhe	220	191	29	29	2	27	25	23	2
I. Paripatle	172	81	48	48	19	29	29	29	0
J. PAC	57	41	16	16	4	12	8	3	5
Total	817	619	155 (18.9%)	155	34	121 (78%)	107	98	9 (8%)

Figure in parenthesis are the percentage of resistant.

[1] Seedlings that are disease free in nursery.

[2] Plants disease free on second inoculation.

[3] Plants that are intermediate disease resistant in curd and flowering stages.

Table 7.3.3.3 Comparative results of two years consecutive screening

Year	Seed source	Seedling			Established plant			Curd stage		
		inoculated (no.)	% of resistant [1]	arc-sin transformed data	inoculated (no.)	% of resistant [2]	arc-sin transformed data	inoculated (no.)	% of resistant [3]	arc-sin transformed data
1997	B	88	14.8	22.63	13	76.9	61.27	1	10.0	18.44
	C	112	13.4	22.73	15	86.7	68.61	1	7.7	16.11
	G	51	11.8	20.96	6	83.3	65.88	1	20.0	26.56
	I	111	17.1	24.43	19	94.7	76.69	1	5.6	13.69
	J	103	7.8	16.22	8	100.0	90.00	1	12.5	20.70
1998	B	258	11.2	19.55	29	69.0	56.17	0	0.0	0
	C	110	30	33.21	33	100.0	90.00	2	8.0	16.43
	G	220	13.2	21.30	29	93.1	74.77	2	8.0	16.43
	I	172	27.9	31.88	48	60.4	51.00	0	0.0	0
	J	57	28.1	32.01	16	75.0	60.00	5	62.5	52.24
t testat 4 df				NS			NS			NS

[1] Seedlings that are disease free in nursery.

[2] Plants disease free on second inoculation.

[3] Plants that are intermediate disease resistant in curd and flowering stages.

NS = Not significantly different.

CHAPTER 8

BIOLOGICAL CONTROL BY FUNGAL ANTAGONISTS OF STALK ROT DISEASE OF CAULIFLOWER

8.1 Background

More than 30 species of fungi and bacteria antagonistic to *S. sclerotiorum* have been reported (Adams and Ayers 1979) but *C. minitans*, *Gliocladium* sp., *Sporidesminum sclerotiovorum* and *Trichoderma* spp. have been reported most frequently as effective biocontrol agents in soil (Ayers and Adams, 1979; Huang, 1980; Adams and Ayers, 1982; Budge and Whipps, 1995). Biological control of stalk rot disease would be an attractive component of an integrated management strategy as various soil micro-organisms have shown a possibility to do this. Therefore, this study was performed to isolate antagonistic mycoparasites from sclerotia of *S. sclerotiorum* and to test their antagonistic activities under laboratory and field conditions.

8.2 Materials and methods

8.2.1 Screening of biocontrol agents

Four lots of soil from *S. sclerotiorum* sick plots were collected from Lumle Agricultural Regional Centre's farm, the Vegetable Development Division's farm, the north farm of ARS Pakhribas and a farmer's field. Isolation of mycoparasites was done by a baiting technique. About 500 g of the sick soils were collected from different sources into 5-ounce plastic sandwich boxes. Twenty-five sclerotia of *S. sclerotiorum* which were collected from work on the inheritance of stalk rot resistance in June 1997 were planted into each of the boxes and incubated at 20° C for three months. After three months planting, rotten sclerotia were recovered from

the soil, washed in running tap water and surface sterilized with 70% alcohol, before the mycoflora was isolated on PDA culture plates for seven days. Various colonies formed from the rotten sclerotia were purified by subculture. Suspected mycoparasites were maintained on PDA culture slopes for further tests. Selected isolates were sent to CABI Bioscience, UK, for confirmation of identification.

Antagonistic activity of the isolated fungi was tested by dual culture on PDA at pH 5.5. Forty-eight hr old growing edge PDA plate cultures of suspected mycoparasites and *S. sclerotiorum* were used for screening in 90 mm Petri dishes at 20°C with alternating 12 hr dark and 12 hr light. The inhibition zones of mycelial growth of *Sclerotinia* was measured with a piece of graph paper. The study was continued with two series of experiments with four replications.

8.2.2 Test of antagonistic activities on detached curds

Inocula of mycoparasites and *S. sclerotiorum*

Suspensions of each potential biocontrol agent was made by adding 10 ml of sterile distilled water to 10 day-old PDA petri dish cultures and rubbing with a glass spreader. Spore concentration of *Trichoderma harzianum* suspensions were counted in a haemocytometer and adjusted to give approximately 10^8 cfu spores per ml. (Other mycoparasites did not produced spores).

Inocula of *S. sclerotiorum* (2 mm diameter) were taken from the growing edge of mycelial cultures on 48 hr PDA plates incubated at 20° C.

Inoculation of curd

Two experiments were carried out simultaneously to test the efficacy of biocontrol agents on detached curds. For the first experiment, 40 freshly harvested curds of cv. Snowcrown approximately 7.5 cm diameter were used. The curds were washed with running tap water followed by surface sterilisation with 70% alcohol, and finally washed with sterile water. These curds were placed into an air tight alcohol sterile plastic sandwich box on two layers of moist tissue paper. The experiment was designed with 10 treatments (Table 8.3.1) including water controls (no inoculum of *Sclerotinia* with each antagonist) and 4 replications.

For the first experiment 2 mm growing mycelial discs of *S. sclerotiorum* followed by two drops of biocontrol spore suspension were inoculated on the curd surface at the same time by making a small hole with a cork borer; the curds were then incubated at 20 °C up to 11 days with alternating 12 hr light and 12 hr dark. Rotting areas, i.e. diameter of rot on curds, were measured from 2 to 11 days after inoculation to assess the antagonistic activities. For the second experiment, the same procedure was followed except biocontrol agents were inoculated 2 hr after inoculation with *S. sclerotiorum.*

8.2.3 Antagonistic activities test on plants

Assuming that sterile soil had not enough nutrients to maintain the plants; the study was performed using two different types of soil (a) sterile forest soil (b) non-sterile forest soil. Due to lack of sufficient seedlings of Kathmandu Local at the time of

trial establishment, cv. Kathmandu Local was planted in the sterile soils whereas cv. Snowcrown was in the non-sterile soils.

Inocula of biocontrol agents were prepared in corn sand meal. The meal (1 kg) was prepared by thoroughly mixing crushed maize in sand at a proportion of 2:1 (weight ratio) and 20g PDA. It was placed in a flask and plugged with non-absorbent cotton wool and sterilised at 121 °C for 20 min. Sterilised media (100 ml) were poured into a 5-ounce sterilised plastic sandwich box and biocontrol agents were allowed to multiply in it for seven days at 20° C.

Sclerotia collected from experiments on the inheritance of stalk rot resistant in June 1998 were washed thoroughly with sterile water and planted into a sterile soil for conditioning on 27 September 1998. Twenty-five sclerotia (50 and 57 day conditioned) were planted 2 to 3 cm deep in polythene pots (24 cm in diameter, 20 cm deep and 12 cm in bottom diameter) having sterile and non-sterile soils respectively. Each pot was filled with 1.5 kg soil (pH 4.7) after thoroughly mixing with 100 ml of seven day-old cultures of biocontrol agents. Thirty-five and 26 day-old seedlings of cauliflower cv. Kathmandu Local and Snowcrown were planted on 16 and 23 November 1998 in sterile and non-sterile soil experiments respectively. The pots were irrigated as and when required with sterile water for sterile soils and tap water for non-sterile soil. Plants were supplied four times with 1% (150 ml) urea solution 28, 43, 52 and 65 days after planting for Kathmandu Local and 21, 36, 45 and 58 days after planting for Snowcrown. Monitoring of seedlings was continued up to 115 days after planting.

8.3 Results and discussion

8.3.1 Screening of biocontrol agents

The results obtained from the dual culture tests are presented in Tables 8.3.1.1 and 8.3.1.2 respectively. Maximum inhibition of mycelial growth of *S. sclerotiorum* was recorded with *Trichoderma harzianum* (W6115), *Pestalotiopsis* sp., *Fusarium solani* (W6112), and *Fusarium solani* (W6113). In the first series of dual culture tests, mycoparasites *T. harzianum*, and *Pestalotiopsis* sp. showed significant differences from the others whereas in the second series of test *Trichoderma, Pestalotiopsis* and *Fusarium* (W6112) also showed significant differences from the others. However, because of the large inhibition zone it caused, *F. solani* (W6113), was also included for further tests. Overall all these antagonists were considered as potential biocontrol agents for further study.

Table 8.3.1.1 *Sclerotinia* mycelial inhibition in the dual culture plates by six different mycoparasitic fungi after a 21 days period

Suspected mycoparasites [1]	Inhibition zone (mm) of *S. sclerotiorum* days after inoculation in dual culture test						
	3	6	9	12	15	18	21
F. solani (W6112)	32.2[a]	52.5[a]	53.2[a]	51.7[a]	48.5[a]	44.5[a]	43.75[a]
T. harzianum (W6115)	31.5[a]	62.0[b]	64.0[b]	62.7[b]	57.7[b]	54.0[a]	50.75[b]
Pestalotiopsis sp.	38.2[a]	69.0[b]	69.5[b]	68.7[b]	57.5[b]	54.7[a]	49.75[b]
D (Unidentified)	26.0[a]	50.5[a]	52.5[a]	51.5[a]	46.7[a]	46.2[a]	43.75[a]
E (Unidentified red fungus)	26.0[a]	49.7[a]	52.0[a]	50.7[a]	45.7[a]	44.2[a]	41.00[a]
F. solani (W6113)	22.0[a]	52.0[a]	52.5[a]	52.0[a]	53.2[a]	47.0[a]	45.75[a]
F Probability	NS	**	**	**	**	NS	NS

[1] = Character and figure in parenthesis are the identification accession number provided by CABI Bioscience.

** = Significantly different at 1% level, NS = Not significantly different.

Value of inhibition zone with the same superscripts are not significantly different at P=0.05 (Duncan multiple range test).

Table 8.3.1.2 *Sclerotinia* mycelial inhibition in the dual culture plates by seven different mycoparasitic fungi after a 21 days period

Suspected mycoparasites[1]	Inhibition zone (mm) of *S. sclerotiorum* days after inoculation in dual culture test					
	3	6	9	12	15	18
Unidentified A	8.5[a]	17.0[a]	25.3[a]	29.3[a]	32.2[a]	35.0[a]
Unidentified B	17.5[a]	18.5[a]	24.5[a]	27.3[a]	32.3[a]	36.0[a]
Pestalotiopsis sp.	24.5[a]	30.5[b]	33.7[a]	46.5[b]	52.2[b]	53.7[a]
Fusarium solani (W6112)	28.3[a]	25.0[a]	33.2[a]	37.2[b]	38.2[a]	42.2[a]
T. harzianum (W6115)	20.5[a]	30.3[b]	35.7[a]	39.5[b]	44.7[b]	46.0[a]
Fusarium solani (W6113)	22.5[a]	25.5[a]	28.5[a]	33.2[a]	34.5[a]	36.2[a]
Unidentified red fungus	13.3[a]	17.5[a]	23.0[a]	25.3[a]	27.8[a]	30.8[a]
F Probability	NS	*	NS	**	**	**

[1] = Character and figure in parenthesis are the identification accession number provided by CABI Bioscience.

* = Significantly different at 5% level, ** = Significantly different at 1% level, NS = Not significantly different.

Value of inhibition zone with the same superscripts are not significantly different at P=0.05 (Duncan multiple range test).

8.3.2 Test of antagonistic activities on detached curds

The results obtained from the first experiment indicated that *Pestalotiopsis* sp. and *T. harzianum* were significantly effective up to 48 hr inoculation on detached curds. However, in later stages none of the biocontrol agents was effective (Table 8.3.2.1). The results from the second experiment indicated that only the *T. harzianum* was significantly effective up to 120 hr of inoculation (Table 8.3.2.2).

In both experiments *Trichoderma* was effective in reducing development of curd rot for a limited period with the indication that delaying inoculation by *Trichoderma* increased its effectiveness. *Pestalotiopsis* on the other hand was only effective (up to 48 hr) when inoculated together with *S. sclerotiorum*.

Table 8.3.2.1 Effect of antagonistic fungi on *Sclerotinia* curd rot (cm) at 8 different time intervals when pathogen and antagonists were inoculated at the same time

Treatments	Curd rot diameter (cm) at an interval of hr of inoculation							
	48 hr	72 hr	120 hr	144 hr	187 hr	211 hr	235 hr	264 hr
Sclerotinia only (control)	1.205	1.260	1.397	1.448	1.508	1.530	1.560	1.560
F. solani (W6112) and *Sclerotinia*	1.151	1.278	1.497	1.509	1.526	1.556	1.593	1.600
F. solani (W6113) *Sclerotinia*	0.807	0.895	0.991	1.030	1.482	1.510	1.515	1.515
Pestalotiopsis sp. and *Sclerotinia*	0.595*	0.939	1.037	1.065	1.316	1.554	1.644	1.680
T. harzianum (W6115) and *Sclerotinia*	0.602*	0.899	1.110	1.157	1.588	1.559	1.614	1.629
F Probability	0.001	0.001	0.001	0.001	0.001	0.000	0.001	0.001
L.S.D. value	0.5182	0.4933	0.5741	0.5914	0.2237	0.3593	0.1287	0.1214

* = Indicates significant difference from *Sclerotinia* only at 95%.

Without *Sclerotinia* (water control) there was no curd rot. None of the antagonists produce curd rot in the absence of *Sclerotinia.*

Table 8.3.2.2 Effect of antagonistic fungi on *Sclerotinia* curd rot (cm) at 8 different time intervals when antagonists inoculated 2 hr after pathogen establishment

Treatment	Curd rot diameter (cm) at an interval of hr of inoculation of antagonists							
	48 hr	72 hr	120 hr	144 hr	187 hr	211 hr	235 hr	264 hr
Sclerotinia only (control)	1.116	1.200	1.343	1.399	1.418	1.460	1.488	1.523
F. solani (W6112) and *Sclerotinia*	1.119	1.189	1.278	1.323	1.331	1.377	1.457	1.535
F. solani (W6113) and *Sclerotinia*	1.227	1.307	1.458	1.544	1.556	1.595	1.632	1.624
Pestalotiopsis sp. and *Sclerotinia*	0.873	1.023	1.536	1.572	1.618	1.639	1.656	1.644
T. harzianum (W6115)and *Sclerotinia*	0.226*	0.600*	0.665*	0.862	1.582	1.638	1.661	1.659
F Probability	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001
L.S.D. value	0.3568	0.536	0.4843	0.5854	0.4350	0.4332	0.4270	0.4236

* = Indicates significant difference from *Sclerotinia* only at 95%.

Without *Sclerotinia* (water control) there was no curd rot. None of the antagonists produce curd rot in the absence of *Sclerotinia.*

8.3.3 Antagonistic activities test on plants

As a result of mycelial growth produced by the sclerotia, seedlings started dying from 57 and 78 days after planting in sterile and non-sterile soil experiments respectively. Data were analysed by the two-way Chi-square (X^2) for the number of survived plants in non-contaminated soil and sick soil. Analysis of results from the both experiments indicated that there were no significant differences between survival of plants in non-contaminated soil and sick soil due to the effect of treatments (Table 8.3.3.1).

Table 8.3.3.1 Antagonistic activities record in sterile and non-sterile soils

Treatment	Sterile soil		Forest soil	
	un-contaminated	sick soil	un-contaminated	sick soil
	number of surviving plant	number of surviving plant	number of surviving plant	number of surviving plant
T. harzianum (W6115)	4	0	4	1
Pestalotiopsis sp.	4	1	3	1
F. solani. (W6112)	4	1	4	0
F. solani (W6113)	3	2	3	1
Control	4	2	3	1
Chi-square value at 4 d f	2.34 NS		2.07 NS	

NS = Not significantly different.

Biocontrol agents isolated locally from parasitized sclerotia were partially effective in the detached curd test but were not effective when applied in soil with growing plants. This may be due to small number of plants (sample size) in the experiments. Overall, *Trichoderma* was most effective on detached curds either inoculated at the same time or two hours after *Sclerotinia* establishment.

Discussion

Only few respondents (1.3%) at survey sites were aware of biological methods of disease control. Potential antagonists such as *Trichoderma harzianum* and *Pestalotiopsis* sp. isolated locally by bating techniques from parasitized sclerotia of *S. sclerotiorum* showed potential for controlling *S. sclerotiorum* in dual culture. Similarly effective control was achieved when these agents were inoculated on to diseased curds. Therefore, it would appear that there is potential for use of local antagonistic fungi for the biological control of *S. sclerotiorum*. However, when the study was extended and the biocontrol agents were applied to sclerotia infected soil in potted cauliflowers, successful control was not achieved. This could be because the inoculum pressure of *S. sclerotiorum* was not matched by a sufficient concentration of antagonists i e. 25 conditioned sclerotia per pot. Additionally, the size of polythene pots used for the study was small and probably the soil inside the pot was not enough to grow the plants. Overall, there is potential for use of antagonistic fungi but further testing is required with proper concentration of antagonists, pot size and replications.

CHAPTER 9

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

9.1 Background

Cauliflower farming in the eastern hills of Nepal has greatly intensified over the last 10 years in order to meet the increasing demands of the urban population. Cauliflower is important in the horticultural economy of Nepal: it is a high value cash crop with potential for small enterprises, and involves women to a high degree. These attributes benefit the resource-poor, eastern hill farmers. Cauliflower could make a significant contribution towards the improvement of farm incomes. The potential production of this crop has been improved as a consequence of support to farmers by NGOs. This has included facilitating the formation of community-based co-operatives and increasing the market potential of local farmers' produce.

Stalk rot and damping-off diseases were formerly thought to be major constraints for satisfactory production of cauliflower in the eastern hills. However, farmers are the ultimate managers of pests and their cropping systems vary greatly within the eastern hills; the farmers in different localities and altitudes may perceive and tackle pest problems in different ways. Considering the complexity of eastern hill vegetable production systems and the problems faced by farmers, activities of this study were undertaken on six areas. The outcomes from these are presented and discussed below.

9.2 Socio-economic surveys of farmers' perceptions

The information presented in Chapter 3 showed that farmers at the survey sites

perceived that diseases are causing considerable losses in cauliflower for both seed and fresh vegetable crops every year. They are aware that disease mostly appears in patches both in the field and in the nursery and that the extent of disease problems varies from year to year. Poor quality seed and wet weather are seen to be responsible for diseases (particularly damping-off and stalk rot); but lack of resistant crop varieties also plays an important role e.g. farmers perceived Kibogiant to be very susceptible to stalk rot.

Farmers indicated that stalk rot followed by damping-off and then downy mildew are the most important diseases, in their experience. However, *Alternaria* leaf spot followed by bacterial rots are their second priority diseases (Chapter 3 Section 3.4).

According to farmers, the average economic loss caused by various diseases at high and mid altitudes is 20% whereas at low altitudes it is 10%. Similarly, analysis of the maximum economic losses of crops grown for both seed and fresh vegetable production due to first priority diseases as reported by the farmers and field assessment indicated that, in the high hills, losses of up to 50% are caused by damping-off at the nursery stage. In the mid and low hills, 30% losses occur due to *Sclerotinia* stalk rot disease. During the surveys, it was not possible to ask farmers to differentiate economic loss caused by disease in the fresh cauliflower crop and seed crops because most of the farmers grow these together. However, earlier work of other scientists including the author of this report have shown that stalk rot disease reduced the seed yield of cauliflower by 17 to 40% every year in Nepal (Bhurtayal, 1985; Duwadi *et al.*, 1993).

The Surveys reported in Chapter 3 indicate that farmers are aware of the value of indigenous practices of disease management, especially crop rotation (Figure 3.8). However due to the small size of land holdings, farmers have to grow the crop on the same piece of land season after season and, as a result, inoculum builds up in the soil (Table 3.4). The literature surveyed (Chapter 2) indicated that disease transmission is usually indirect. Sclerotia germinate to produce ascocarps from which ascospores are discharged to infect plants (Maude, 1996). However, where sclerotia are in contact with the stem bases of plants, mycelial transmission may occur (Scott and Evans, 1984). Thus increase of *S. sclerotiorum* in the growing crop is mainly dependant on those factors which encourage ascospore production and subsequent infection by these propagules. Persistence of inoculum from one season to the next would appear to be as sclerotia. The important and significant means by which disease will enter a new area would appear to be through sclerotia mixed in with the seed. Hence the need for alternative methods to reducing inoculum of *S. sclerotiorum* (particularly sclerotia) both in the field and in seed stocks from which crops are grown.

The studies reported in Chapter 6 on monitoring of sclerotial germination at different depths indicated that sclerotia planted at 2 to 5 cm depth could germinate 6 to 14 months after planting. Thus confirming that *S. sclerotiorum* sclerotia can easily remain viable between cauliflower cropping season and thus act as a source of inoculum as described above. As survival of sclerotia decreases with time and depth of burial, movement of sclerotia to depths greater than 10 cm will prevent infection (Imolehin and Grogan, 1980; Grogan *et al.*, 1980). Therefore deep ploughing of

fields could reduce the inoculum potential. This is compatible with findings of Subbarao (1998) that deep ploughing may reduce the air-borne phase of *Sclerotinia.* Once buried sufficiently deep, the successful development of ascospores from sclerotia does not occur due to a variety of factors (one of which may be mycoparasitism of the sclerotia which is discussed below). Therefore, farmers having a limited piece of land and without options for crop rotation, should plough their field deeply before planting the cauliflower crop.

Based on literature reviews, solarization reduces the populations of sclerotia of *S. sclerotiorum* in soil and the ability of surviving sclerotia to develop apothecia. The greatest reduction was reported in the top 5 cm layers of soil but significant effects were observed at 5 and 10 cm depth (Phillips, 1990). In the present hill context, polythene rolls are one of the commonly accessible farm supplies and almost all farmers use them for various purposes. The conclusion is that farmers may have an option to solarize their fields during summer.

Survey results indicated that farmers were neither familiar with an appropriate method for disposal of diseased debris nor with the proper alternative use of diseased plants (Table 3.7). Most of the farmers disposed of diseased plants of poor quality into a compost pit, but then farmers use incompletely decomposed compost on their fields (Chapter 3). However, it is reported that addition of organic matter such as well composted sewage in the field could reduce the *Sclerotinia* disease of cauliflower and lettuce (Millner *et al.*, 1982). Either farmers should wait for complete decomposition of diseased debris or alternatively they should burn the

disease debris since burning of crop residue reduces the *Sclerotinia* inoculum level (Gilbert, 1991). The survey indicated that 25% of the respondent farmers already burn the crop residue after harvesting (Table 3.7). This practice could be disseminated from farmer to farmer. Combining effective organic amendments with solarisation has also been suggested as a method of improving control (Katan, 1981).

Sclerotinia in the field was reduced ten fold and disease incidence was reduced by 50% in succeeding crops by roguing (Patterson and Grogan, 1985). Similarly, flooding a field continuously more than 23-45 days or a cycle of alternate flooding and drying, or planting rice led to destruction of sclerotia (Moore, 1949; Shrestha and Timila, 1990). However, flooding is only practical in Khet-land and only few farmers grow cauliflower in mid altitude Khet-land.

With the establishment of NGOs there has been a significant increase in pesticide application, as a part of the effort to increase production by timely supply of inputs. Sometimes pest control methods advocated by NGOs rely heavily on pesticides. However, fungicides used by farmers vary from location to location depending on (a) what is stocked by the local dealers / Co-operatives / Agricultural Inputs Corporation and (b) the local requirements for the cauliflower. The fungicides Benlate (benomyl) and MBC (carbendazim) are reported to be best to reduce disease incidence and improve seed yield; but benomyl was the most cost effective fungicide (Sharma and Sharma, 1984). The survey reported in Chapter 3 revealed that the highest usage of pesticides on vegetables is on cole crops (94.5% of total use),

applied within the prescribed pre-harvest interval. To reduce the dependence on chemicals, use of pesticide should be integrated with environmentally safe alternatives. Farmers must be made aware of the risk to consumers and their families of eating vegetables contaminated with pesticides when the safe period after pesticide application is not observed. Further research should be carried out on the use of indigenous plants with pesticide properties as alternatives to the exclusive use of chemical pesticides; the use of traditional methods of disease control such as use of wood-ash in the nursery bed should be reviewed and promoted.

9.3 Effect of weeds on cauliflower production

The recently adopted practice of zero weeding of cauliflower fields at high altitude sites led to farmers perceiving that presence of weeds reduces disease incidence. This was not supported by the experimental data (Chapter 4). However, weeds played a significant role in yield reduction presumably by competition with the cauliflower crop. Furthermore, the off-season field trial results indicated that there was no significant difference between two weedings or more. Therefore, two timely weedings are recommended for a good crop.

9.4 Investigation of pathogens of cauliflower

A diagnostic investigation of diseased cauliflower in farmers' fields in the eastern hills indicated that stalk rot, damping-off, downy mildew, *Alternaria* leaf spot, and other bacterial rots (the latter caused by *Erwinia carotovora* subsp. *carotovora* and other bacteria) were present on crops grown in the field. The isolations from the material collected enabled their identification as *P. aphanidermatum* and *S.*

sclerotiorum associated with damping-off during off-season seedling production in high altitude zones; *P. aphanidermatum*, *Fusarium* spp., *Rhizoctonia* spp., *Alternaria* spp. and *S. sclerotiorum*, were the major pathogens associated with damping-off during normal season seedling production in high, mid and low altitudes (Chapter 5). Furthermore the results indicate that pathogens associated with damped-off seedlings are not specific to particular cauliflower varieties.

The curd rot diagnostic studies indicated that the curd rot complex disease in cauliflower was associated with *P. parasitica*, *S. sclerotiorum*, *Fusarium* spp., *Alternaria* spp. *Erwinia carotovora* and a Gram negative yellow colony-forming bacterium (Chapter 5). The latter might be *Xanthomonas campestris*, a known cause of curd rot (Kapoor and Thakur, 1997), but media and tests for further identification were not available.

S. sclerotiorum followed by *E. carotovora* at high altitude, *P. parasitica* followed by *S. sclerotiorum* at mid altitude and *S. sclerotiorum* followed by *Pythium* spp. were the major pathogens associated with the curd rot disease complex. However, minor pathogens such as nematodes, *Rhizoctonia* spp. and *Penicillium* were also associated with curd rot; bacterial rot was not recorded in the low altitude cv. Terai-3.

Overall, *S. sclerotiorum* was the fungus most frequently associated with curd rot and damping-off. This finding was also supported by the farmers' prioritisation of cauliflower disease (Chapter 3). The results support the initial assumption that stalk

rot is not only a disease of seed crops. It is problematic at all stages of the crop such as seedling, curd for vegetable production and seed crops in the eastern hills of Nepal.

9.5 Seed health and quality

Farmers obtain cauliflower seed from many different sources (Chapter 3). This diversity has led to concern that there was significant variation in seed health and seed quality (Chapter 6). Quality examination of 15 different seed lots indicated that two lots were contaminated with sclerotia of *S. sclerotiorum* which are potentially very important in establishment of disease epidemics.

Experiments reported in Chapter 6 indicated that poor germination (in a standard blotter test) of the seed lots tested was due to *Fusarium* spp. and *S. sclerotiorum*, while poor emergence in a soil-based test was due to *Pythium* spp., *S. sclerotiorum*, and *Alternaria* spp. With the exception of one of the accession (Terai-3) which was non-viable, poor emergence of seed planted in sterile soil was observed to be correlated with the seed-borne fungi identified when showing seed testing blotter method. The contaminated seeds were recorded from the purity tests of limited number of seed samples.

It has been reported that in a large number of seed samples, a maximum of five fragments of sclerotia in 70 g sample is the permitted contamination level (Maude, 1996), as determined by sieving in a standard test. Given the use of such tests elsewhere and the apparent significance of sclerotial inoculum discussed here, it

would appear likely that differential sieving of larger samples of cauliflower seed in Nepal would be a next step to determining the significance of this inoculum source.

Overall, it would appear that hill farmers may be using seed of poor quality and that the seed supply systems in the eastern hills could be improved. The reasons are that:

1. farmers are less concerned about seed health than the timely availability of seed.
2. imported seeds are (probably) not passed through a quarantine or seed health laboratory.

Therefore to improve the seed supply system and to improve seed quality, it is suggested that the following are necessary:

1. Locally produced seed must pass through a seed health laboratory before selling or distribution to the farmers, and imported seed must pass through quarantine inspection before distribution to retail outlets.
2. Seed treatment is also necessary before distribution of seed by the seed traders. (Gupta and Dohroo (1993) reported that benomyl and dichlozoline caused at least 60% inhibition of mycelial growth and the fungicides completely inhibited ascospore germination. During the survey in the eastern hills, it was reported that the most commonly used fungicides for field use are carbendazim and maneb. In general, farmers have very little knowledge about dose, methods of application and precautionary measures concerning pesticides. They need to be aware of the consequence of using fungicide treated seed.)
3. Education of public and private seed traders / entrepreneurs regarding the importance of quality seed via training, enhancement of extension services and

education of farmers using local media (posters and broadcasting from local radio stations) is required.

9.6 Screening for disease resistance

Work presented in Chapter 3 showed that although Kathmandu Local is the most popular cultivar of cauliflower (62.5%); cv. Snowcrown has become more popular (20.8%) in road head areas of high altitude sites in the eastern hills because it is a suitable cultivar for off-season production. The road head farmers are concerned about the time of harvest of cauliflower so that they can sell their product at a good price when there is scarcity of product in urban markets (Chapter 3). With favourable market prices, particularly for the off-season produce, year-round cauliflower production has increased tremendously, causing a build-up of inoculum in the soil.

Use of resistant varieties of cauliflower could potentially be one of the most applicable, low cost and attractive technologies for the eastern hill farmers. Survey results indicated that currently no resistant cultivars are available. Farmers continue to grow genotypes which are susceptible to stalk rot disease. In order to screen cauliflower lines for resistance to *S. sclerotiorum*, two different methods of inoculation were tested (Chapter 7). This involved either petal or mycelial disc methods of inoculation. The former was found to be most appropriate for detached leaves whereas the latter was more suitable for inoculation of leaves of growing plants. As the detached leaf tests are generally performed in the laboratory, there is less chance of petal inoculum falling off before the host is infected than under field

conditions. The mycelial disc provides sufficient food to establish the pathogen on the host surface before penetration and it is somewhat more adhesive than petals.

Six varieties of cauliflower were screened using both methods of inoculation at seedling and curd stage. Some degree of seedling resistance was found in each variety. Curd resistance was encountered only in Kathmandu Local. This is perhaps not surprising because cultivars other than Kathmandu Local have been adopted in the eastern hills for a much shorter period. Certain lines of Kathmandu Local did show resistance to stalk rot in the seedling stage. Although there is little resistance in the curd and flowering stages, the resistance expressed during seedling screening needs to be exploited as such effects may usefully slow epidemic progress, particularly as part of an integrated strategy.

9.7 Biological control of stalk rot disease

Survey results indicate that farmers were not familiar with biological (antagonistic) methods of disease control. Potential antagonists such as *Trichoderma harzianum* and *Pestalotiopsis* sp. isolated locally by baiting techniques from parasitized sclerotia of *S. sclerotiorum* showed potential for controlling *S. sclerotiorum* in dual culture (Chapter 8). Similarly effective control was achieved when these agents were inoculated on to diseased curds. Therefore, it would appear that there is potential for use of local antagonistic fungi for the biological control of *S. sclerotiorum*. However, they were not effective when applied to sclerotia infected soil in potted cauliflowers. This could be because the inoculum pressure of *S. sclerotiorum* was not matched by a sufficient concentration of antagonists. Additionally, the size of

polythene pots used for the study was small and probably the soil inside the pot was not enough to grow the plants. Overall, there is potential for use of antagonistic fungi, but further testing is required with proper concentration of antagonists, pot size and replications. The antagonists found to be most effective in this study are already being used in research at ARS Pakhribas by NARC scientists.

Work is now under way at ARS Pakhribas to produce inoculum in various types of farmyard manure. Women are the major source of labour and their role in vegetable production is critical. Women must therefore be mobilised in the decision-making processes by increasing their participation in the various vegetable production activities such as training programmes run by the Department of Agriculture and the NGOs. Compost production provides a route to improve disease control and is one of the activities typically carried out by women.

9.8 Overviews

An IPM strategy should recognise that there is need for varieties suitable for the off-season production for road head farmers; a market with good prices has been established by farmers' co-operatives.

Stalk rot and damping-off disease are the major constraints for satisfactory production of cauliflower in the eastern hills of Nepal. According to farmers, the maximum economic losses up to 50% is caused by damping-off at nursery stage and 30% in mid and low hills by *Sclerotinia* stalk rot.

It would appear that eastern hill farmers may be using seed of poor quality and that the seed supply system in the hills are unreliable. This is probably because imported seeds are not passed through a quarantine or seed health laboratory and the hill farmers are less concerned about seed health than timely availability of seeds. Therefore the following areas should be considered as the main components of potential integrated management of stalk rot disease in the eastern hills of Nepal:

- Seed quality
- Seed health

  The quality seed distribution system could be improved by issuing a quality test certificate with seed packed by proper authorities and treating the seeds with prescribed fungicides.

  Training on quality aspects of seed to farmers and seed retailers should be incorporated into regular farmers' training programmes run by the Department of Agriculture.

  The technical and administrative infrastructure should be developed to make the Seed Act and quarantine regulations more functional by proper authorities. For example, providing proper training to the personnel involved in quality control and quarantine.

However, this will not be possible without a co-ordinated effort by the agricultural authorities and foreign technical assistance.

There are various NGOs, GOs and donor agencies involved in increasing farm productivity in the hills of Nepal. Among them the Seed Sector Support Project (SSSP), the Netherlands Volunteer Service (SNV) and Hill Agriculture Research

Project (HARP) are supported by the national agricultural system through extension, training and research programmes to improve quality seed production and distribution. The proper agriculture authorities should develop a seed programme in collaboration with donors' technical assistance programmes. This will not only benefit the agricultural authorities but also help to expedite the donors' objectives. Furthermore, expert advice and in-country or foreign training provided by these donors should be targeted towards improvement of seed quality. These donors could be convinced of the importance of developing infrastructure such as quarantine and quality control laboratories.

The Agricultural Inputs Corporation (AIC), an HMG corporation, is responsible to the agricultural input supply system in Nepal. There should be a very close link between the Plant Protection Division and AIC to maintain a timely supply of recommended pesticides targeted to seed treatment and crop protection.

9.9 Conclusions

The research presented in this thesis has shown the potential value of various strategies for the integrated control *S. sclerotiorum* on cauliflowers in the eastern hills of Nepal.

- the importance of crop rotation and its practical value,

As a consequences of present agronomic practices i.e. continuous cropping of cole crops in the same field may lead to disease inoculum build-up in the fields. Therefore a practical value of crop rotation is worth explaining to growers.

- disposal techniques and decomposition of debris in compost pits,

Lack of awareness of correct methods of disposal and alternate use of disease debris is another factor leading to inoculation build-up in the fields. This source of inoculum could be minimised either by suggesting farmers wait for complete decomposition of disease debris in compost pits or alternatively they should burn cauliflower debris, as it is a normal practices in the eastern hills. Burning of crop residue decreases the *Sclerotinia* inoculum level (Gilbert, 1991).

- weeding necessary for a good crop of cauliflower,

Perception of high hill farmers that weeds help to reduce disease incidence was not supported by experiments results. Therefore, there is an urgent need to explain to farmers that competition associated with weeds reduces the yield and size of the curds, it had no effect on disease incidence.

- use of fungicide seed treatment,

Most of the respondent farmers use pesticides in cole crops. Use of fungicides on the standing crops could be minimising by targeting seed treatments. Seed treatment is not only cost effective, but also avoids the excessive use of pesticides in the environment. However, there is need to test the efficacy of fungicides used by the farmers.

- non-chemical control treatment,

Potential antagonists such as *Trichoderma harzianum* and *Pestalotiopsis* sp. isolated locally showed potential for controlling *S. sclerotiorum* in dual culture test and on

diseased curds. However when the biocontrol agents were applied to sclerotia infected soil in potted cauliflower, successful control was not achieved. This could be because the inoculum pressure of *S. sclerotiorum* was not matched by a sufficient concentration of antagonists. There are prospects for use of antagonists fungi, but further testing is required.

With the appropriate resources, there are prospects for much improved management of *S. sclerotiorum* disease of cauliflower. Overall, the technology developed / identified to control stalk rot disease could be applied to farming systems elsewhere, as cauliflower is grown all over the world and *Sclerotinia* is a world-wide problem. However, potential users might wait to see the outcome of a package successfully applied first in Nepal.

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Appendix 1. Semi-structured interviews

i. Differences in cropping pattern and altitude zones were used for the selection of the sites in which the survey was to be conducted, to ensure that they are representative of the ARS Pakhribas command area as a whole (particularly with regards to the diseases complexes which occur in cauliflower).

ii. Interviews or discussions were conducted either with individuals or with small groups of farmers (3-4 to reduce heterogeneity within the groups). Interviews with women were held separately from men to achieve a 50% female participation rate in interviews (Appendix 4). To allow analysis by socio-economic status, interviews were with farmers selected from two food sufficiency categories using the ARS Pakhribas farmer categorisation method (Appendix 5) combined as A+B and C+D. After collection of half the data, the socio-economic status was then ignored.

iii. The two farmer categories (A-B, and C-D) were equally represented in the sample (participation of land-less farmer was not included) to address the perceived priorities of all groups, including the lower category farmers. For obvious logistic reasons, interview groups comprised of farmers living in the same area (at least within easy walking distance). Thus, randomisation from farmer lists was not practicable.

iv. Farmers from within the same altitude zone (Low, medium and high) were used to form groups so as to test whether differences in priorities were related to zone (Appendix 4).

v. The survey team adopt a pragmatic approach to sampling, by seeking assistance of outreach (OR) Junior Technicians, KOSEPAN Koshi hills seed producer groups association, Agricultural Inputs Corporation, District Agriculture Development Office, Agricultural Services Centre's support to identify representative groups for interview (if the survey site belonged to their command areas), complemented by limited random sampling of individual households as a cross-check.

vi. Interviews were based on a set of guidelines (Appendices 2 and 3) to elicit information on diseases priorities (with careful probing required to identify correctly the diseases involved), to rank perceived pest importance and to try to ascertain the level of yield losses incurred over time. Disease ranking by card sorting was a part of this process, and other informal techniques were also used, for example, diagrammatically representing of yield variations or assigning weights through the division of a pile of grains.

vii. Interviews were conducted by the author with assistance of Junior Technicians to provide complementary insights and to facilitate the interview/discussion process. Note-taking/recording was done during the interview by the author to minimize effects the quality and flow of information. To retain the interest of farmers, and not to keep them away from their work too long (particularly during the cropping season), the discussion was scheduled for about one hour (although it could last longer if farmers were willing). Appointments for group interviews were fixed in advance, and timed to suit farmers' wishes.

viii. A small group interview approach was preferred so as to stimulate discussion within the group, increase the number of individuals consulted and to try to arrive at a consensus of opinion on disease priorities. When a group consensus was not achieved, the different perceptions of individuals were recorded, as well as the reasons why they differed.

(Source: O'Reilly and Black, 1995: O'Reilly and Russell-Smith, 1995)

Diseases problems occurring on cauliflower were recorded as the farmers explained the symptoms. To facilitate analysis the symptoms were grouped into five namely, stalk rot, downy mildew, bacterial rot, *Alternaria*, and damping-off.

Appendix 2. Informal interview format for survey of farmers' perception regarding the introduction of new varieties and disease management practices

Date of interview; Name of interviewer

Interview location: District VDC Ward Village

Altitude zone:

1. General information

Number of interviewees: Male, Female, and names

Relation with ARS Pakhribas: none/knowledge/direct contact

2. Cropping system information

Principal crops grown by group members:

AGRONOMIC

HORTICULTURAL

CASH

Rank the main sources of on-farm cash income, if any:

3. List of main disease of cauliflower

Over the past 5 years, what have been the main diseases affecting your cauliflower crop in your farms?

List names. For each, note whether farmer- or researcher-identified. If ambiguities in disease identification, note local name/description/symptoms.

4. Disease prioritization through card sorting

Considering all diseases on your cauliflower crop over the past 5 year period, what are the diseases which have caused the greatest losses to your household's food supply and/or cash income? Rank them in order of impact, starting with the biggest.

Using up to 5 priority diseases identified above, conduct a card sorting exercise with the whole groups.

Does it limit to early/ or late season production?

What are the main targets for the grower early production for high price or maximum yield?

5. First disease priority

For the 1st priority disease:

Which crop/crops does it attack *(if not already noted)*?

Why do you consider this the biggest problem/first priority?

Over the past 5 years, how many years has attack occurred?

Relative seriousness of attack (low-level, moderate, severe) in each year?

Was it a problem previously? If not, any explanation for this?

At what stage in crop development or time of year does the attack occur?

Does it occur in all fields or only some?

Any explanation for why the attack occurs? *(e.g. related to weather, soil type, planting dates)*

What is a "normal" (expected) yield for this crop?

When did you last get this yield?

How much of total crop yield is lost due to this pest ?

- in year of average attack

- in year of severe attack

(absolute amount or proportion of 'normal' yield lost - note both quantity and quality losses

How can you tell that this yield loss is due to this disease? (Are other disease also affecting the crop?)

6. Second disease priority

For the second ranked disease, ask questions as at 5. above

7. Third disease priority

As above

8. Farmer knowledge and practice of disease management

What, if any, measures are used to limit the damage by any of these diseases?

If none, are you aware of any possible means of reducing pest damage? Which?

If aware, what prevents you from applying these measures?

For each measure,

- for which disease?

- source of information?

- is adequate control achieved?

- If not, on which diseases?

For pesticides, what type, who applies, success?

Does the farmer know of alternative (non-pesticide, biological, resistant, indigenous) measures?

Why does s/he not use these?

9. Farmers perception regarding the introduction of new varieties and disease management?

10. Socio-economic category of farmers at the survey year.

Category of farmer(s) based on food sufficiency from on-farm production for the last crop year (number in each category)

A more than 12 months

B 12 months

C 6-12 months

D <6 months

Was this a typical year? If not, then ask for "usual" food sufficiency status.

(Source: O'Reilly and Black, 1995: O'Reilly and Russell-Smith, 1995)

Appendix 3. Checklists

1. What are the main vegetable crop enterprises on your farm?

2. What do you consider as the main crop on the farm?

3. Do you plant cauliflower and when do you plant the crop?

4. What is the cropping sequence?

5. How do you raise seedlings for planting?

6. What varieties of cauliflower do you plant?

7. Are there any particular varieties you prefer and why?

8. Where do you obtain the seeds?

9. Do you have any problem with disease on your farm? List the disease and their importance?

10. Is stalk rot an important problem on your farm? (farmers to rank with their other problems)

11. How much of your produce ends up as rotten? (estimate)

12. How are you trying to solve the problem of stalk rot on the farm?

13. Are there particular practices you think when applied can alleviate the stalk rot problem?

14. How are you solving the stalk rot problem on your farm?

15. Do you observe any differences between varieties?

16. Where do you take the diseased plants?

17. What do you use rotten curds for?

18. Where do you take the stover after harvesting the cauliflower? (burn, left to rot on the farm, remove and stored for animal feed).

19. Compost use?

Appendix 4. Farmers' categorization based on food sufficiency

Farmers' categories	Food sufficiency from on farm production for the last year crop
A	Category A farmer is one having food for more than 12 months
B	Category B farmer is one having food for 12 months
C	Category C farmer is one having food for six to nine months
D	Category D farmer is one having food for less than 6 months
E	Land-less

Appendix 5. Sample size for survey

The following sampling framework was used for interviews of farmers' perception to determine the yield loss and disease management practices (n = 72).

Altitude zone	Sex	Farmers categories	Group interviews		Individual interviews	
			Target number	Actual number	Target number	Actual number
High altitude (>1700m)	Men	AB	4	2	4	5
	Men	CD	4	3	4	4
	Women	AB	4	4	4	2
	Women	CD	4	2	4	3
Mid altitude (1100-1700m)	Men	AB	4	2	4	3
	Men	CD	4	0	4	7
	Women	AB	4	2	4	2
	Women	CD	4	3	4	4
Low Altitudes (<1100m)	Men	AB	4	4	4	6
	Men	CD	4	1	4	9
	Women	AB	4	0	4	2
	Women	CD	4	0	4	2

Appendix 6. Frequencies of the average economic loss percentage by first priority diseases in cauliflower based on 72 farmers' perception at three different altitudes

	Losses %			
Altitude	1-10	11-20	21-30	31-40
High	16	0	7	1
Mid	12	9	2	1
Low	6	11	4	3

Appendix 7. Frequencies of the average economic loss percentage by first priority diseases in cauliflower based on 72 farmers' perception

	Losses %			
Diseases	1-10	11-20	21-30	31-40
Downy mildew	1	0	0	0
Damping-off	4	8	12	3
Stalk rot	29	12	1	2

Appendix 8 Analysis of variance of the average price data for normal and the off-season crops for the period of 1993 to 1998

Source of variation	d.f.	s.s.	m.s.	variance ratio	F probability
Seasons	1	111.813	111.813	20.48	0.001
Residual	10	54.589	5.459		
Total	11	166.402			

Appendix 9 Analysis of variance of the maximum price data for normal and the off-season crops for the period of 1993 to 1998

Source of variation	d.f.	s.s.	m.s.	variance ratio	F probability
Seasons	1	274.563	274.563	37.85	<.001
Residual	10	72.540	7.254		
Total	11	347.104			

Appendix 10 Analysis of variance of the minimum price data for normal and the off-season crops for the period of 1993 to 1998

Source of variation	d.f.	s.s.	m.s.	variance ratio	F probability
Seasons	1	2.448	2.448	0.59	0.458
Residual	10	41.174	4.117		
Total	11	43.622			

Appendix 11 Analysis of variance of weed biomass for the off-season 1998/99

Source of variation	d.f.	s.s.	m.s.	Variance ratio	F Probability
Treatment	2	9.297E+07	4.648E+07	4.23	0.035
Residual	15	1.650E+08	1.100E+07		
Total	17	2.580E+08			

Appendix 12 Combine analysis of variance of weed biomass for the normal and off-season 1997/98 and 1998/99

Source of variation	d.f.	s.s.	m.s.	Variance ratio	F Probability
Season	1	2.236E+08	2.236E+08	29.46	<.001
Residual	34	2.580E+08	7.589E+06		
Total	35	4.816E+08			

Appendix 13 Combine analysis of variance of number of diseased plants for the normal and off-season 1997/98 and 1998/99

Source of variation	d.f.	s.s.	m.s.	Variance ratio	F Probability
Season	1	0.05429	0.05429	1.13	0.293
Residual	46	2.21032	0.04805		
Total	47	2.26461			

Appendix 14 METEOROLOGICAL MONTHLY MEAN LONG TERM MEAN DATA OF (1976 TO 1997) 22 YEARS

ALTITUDE : 1741 MASL LONGITUDE: 87'17'

RECORDING TIME : 0845, 1315 & 1745 LATITUDE :27'17'

Month	Air Temp.(°C)		Grass Temp. (°C)	No. of rainy day	RH %	Rainfall Total (mm)	Evapor-ation (mm/day)	Wind speed (Km/h)	Sun shine (h/day)	Soil Temperature (°C)			
	Max.	Min.								5 cm	10 cm	30 cm	50 cm
Jan	14.1	4.8	1.0	2.1	73.7	14.9	1.5	3.4	6.7	8.6	9.0	10.9	11.8
Feb	15.8	6.1	3.2	2.1	72.9	17.4	2.2	3.1	7.0	10.5	10.9	12.6	13.1
Mar	20.3	9.9	5.9	4.0	65.9	26.5	3.3	4.4	7.8	15.4	15.4	16.5	16.7
Apr	23.7	12.5	9.7	6.5	67.6	55.3	3.9	4.7	7.6	19.4	18.3	19.7	20.2
May	24.0	14.8	12.8	13.0	79.7	131.7	3.6	4.7	6.6	21.5	21.1	21.5	21.8
Jun	23.8	16.9	15.7	20.8	86.2	260.6	2.7	4.2	3.2	22.3	21.9	22.3	22.6
Jul	23.1	17.4	16.7	25.1	91.4	410.1	2.0	3.0	1.7	22.1	22.0	22.1	22.3
Aug	23.5	17.4	16.6	22.9	89.1	359.8	2.0	3.2	2.4	22.1	22.0	22.3	22.4
Sep	22.9	16.2	15.0	17.0	88.9	214.8	1.9	2.7	2.9	22.1	21.1	21.6	22.0
Oct	21.7	12.8	10.2	5.0	82.0	54.4	2.2	2.9	6.0	18.5	18.7	20.0	20.7
Nov	19.0	9.5	5.6	1.0	76.2	17.6	2.0	4.1	7.4	14.4	15.0	16.4	17.4
Dec	15.8	6.3	1.4	1.2	70.9	15.4	1.8	3.5	7.5	10.3	11.1	13.0	14.2

Appendix 15 METEOROLOGICAL MONTHLY MEAN DATA JANUARY - DECEMBER 1997

ALTITUDE : 1741 MASL

LONGITUDE: 87'17'

RECORDING TIME : 0845, 1315 & 1745

LATITUDE :27'17'

Month	Air Temp.(°C)		Grass Temp. (°C)	No. of rainy day	RH %	Rainfall Total (mm)	Evapor-ation (mm/day)	Wind speed (Km/h)	Sun shine (h/day)	Soil Temperature (°C)			
	Max.	Min.								5 cm	10 cm	30 cm	50 cm
Jan	13.6	4.1	-2.2	4.0	73.6	27.6	1.7	2.3	6.6	10.9	11.3	11.3	12.3
Feb	14.0	4.7	-1.0	4.0	72.9	9.0	2.0	2.2	6.1	12.2	12.1	11.5	12.0
Mar	20.8	9.7	3.7	3.0	70.8	13.0	3.5	2.7	7.8	20.1	19.3	17.5	17.2
Apr	20.9	11.0	6.6	16.0	80.6	163.6	3.7	3.4	6.2	19.7	19.3	17.9	17.8
May	24.3	14.8	9.3	12.0	77.4	35.4	3.9	3.0	7.8	25.4	24.2	22.5	21.5
Jun	23.8	16.8	14.5	17.0	88.3	199.4	3.0	2.8	4.3	24.2	24.0	22.7	22.2
Jul	23.7	17.9	16.7	28.0	93.3	296.0	2.6	2.8	2.1	24.1	23.9	22.6	22.3
Aug	23.7	17.6	16.5	21.0	92.2	333.7	2.3	4.1	3.0	23.7	23.7	22.7	22.6
Sep	22.4	16.0	14.8	18.0	93.1	275.9	2.1	1.4	2.2	22.8	22.6	22.0	21.9
Oct	21.1	11.6	8.3	6.0	80.9	39.6	2.9	1.6	7.6	20.9	20.5	19.9	20.3
Nov	18.8	9.4	4.6	0.0	79.6	0.0	2.1	1.3	7.7	17.5	17.6	17.2	17.7
Dec	14.9	6.3	1.6	5.0	75.0	77.0	1.8	2.5	7.6	12.0	12.3	12.4	13.4

Appendix 16 METEOROLOGICAL MONTHLY MEAN DATA JANUARY - DECEMBER 1998 AND JAN -FEB 1999

ALTITUDE : 1741 MASL
RECORDING TIME : 0845, & 1745

LONGITUDE: 87'17'
LATITUDE :27'17'

Month	Air Temp.(°C)		Grass	RH	Rainfall	Evapor-	Wind	Sun	Soil Temperature (°C)			
	Max.	Min.	Temp. (°C)	%	Total (mm)	ation (mm/day)	speed (Km/h)	shine (h/day)	5 cm	10 cm	30 cm	50 cm
Jan	14.2	4.5	-1	76.5	0,0	1.7	1.6	7.6	11.5	11.7	11.1	11.9
Feb	17.2	6.9	1.5	71.9	7.7	2.4	2.1	7.5	15.4	14.9	13.7	13.9
Mar	19.2	8.4	3.6	76.5	52.1	2.8	2.8	6.6	16.6	17.3	16.6	16.5
Apr	23	13.1	9.4	77.9	112.4	3.6	1.8	6.7	20.5	20.7	19.8	19.1
May	24.8	16	14.2	86.5	159.2	2.9	1.6	5.9	24	24.1	22.7	22
Jun	24.4	18.4	17.2	93.2	245.2	2.1	0.8	2.5	24.9	25.1	24	23.4
Jul	23.1	18.3	17.9	94.7	300.5	1.8	1.4	1.3	23.6	24	23.2	22.9
Aug	22.6	18.0	17.8	95.2	328.8	1.6	0.7	1.6	23.4	23.9	23.1	22.9
Sep	23.4	16.9	15.2	91.4	126.3	2.3	1.5	3.8	22.9	23.5	22.8	22.6
Oct	22.6	15.0	13.0	90.4	7.6	2.2	0.6	5.4	21.8	22.6	22.2	22.1
Nov	20.3	11.3	7.5	83.5	37.5	2.4	1.1	7.2	17.5	18.5	18.7	18.6
Dec	17.1	7.6	2.2	83.1	0.0	2.1	0.4	8.6	12.8	14.2	14.5	15.4
Jan '99	15.7	6.1	1.2	75.9	0.0	2.3	1.0	8.8	11.4	12.8	16.1	13.6
Feb '99	20.9	10.1	3.8	77.5	0.0	2.9	0.6	7.9	16.4	17.2	16.1	15.8

Appendix 17 Pathogens associated with damped-off of cauliflower seedlings in five different nurseries at high altitude (>1700 m) during January-February 1998

Nursery	Variety	Total number of diseased seedlings		Pathogen associated with diseased seedlings
		tested	infected	
1	Snowcrown	22	4	*Pythium aphanidermatum*
			15	*Rhizoctonia* spp.
			2	*S. sclerotiorum*
			1	*Phytophthora* spp.
2	Snowcrown	22	1	*P. aphanidermatum*
			21	*Rhizoctonia* spp.
3	Snowcrown	22	7	*P. aphanidermatum*
			10	*Rhizoctonia* spp.
			4	*S. sclerotiorum*
			1	*Phytophthora* spp.
4	Kibogiant	22	7	*P. aphanidermatum*
			11	*Rhizoctonia* spp.
			1	*S. sclerotiorum*
			2	*Phytophthora* spp.
			1	*Fusarium* spp.
5	Kibogiant	7	2	*P. aphanidermatum*
			3	*Rhizoctonia* spp.
			1	*S. sclerotiorum*
			1	*Phytophthora* spp.

Appendix 18 On the basis of variety pathogens associated with damped-off of cauliflower seedlings in seventy-five different nurseries at low, mid and high altitudes during September–October 1998

Cultivar	Altitudes	Total number of diseased			Pathogens associated with diseased seedlings
		nursery	seedlings tested	seedlings infected	
Snowcrown	High	25	121	12	*Fusarium* spp.
				48	*P. aphanidermatum*
				10	*Alternaria* spp.
				18	*Rhizoctonia* spp.
				6	Nematodes
				3	*P. aphanidermatum* & Nematodes
				1	*P. aphanidermatum* & *Alternaria* spp.
				1	*Fusarium* spp. & Nematodes
				1	*Fusarium* spp. & *Rhizoctonia* spp.
				2	*P. aphanidermatum* & *Fusarium* spp.
				9	*S. sclerotiorum*
				10	*S. sclerotiorum* & *Erwinia* sp.
Kathmandu Local	High	5	8	1	*Alternaria* spp.
				2	*S. sclerotiorum*
				3	*S. sclerotiorum* & *Erwinia* sp.
				2	*S. sclerotiorum* & *Alternaria* spp.
Kibogiant	High	1	3	1	*S. sclerotiorum*
				2	*S. sclerotiorum* & *Erwinia* sp.
Snowcrown	Mid	3	11	1	*Fusarium* spp.
				4	*P. aphanidermatum*
				2	*Rhizoctonia* spp.
				1	Nematodes
				1	*P. aphanidermatum* & Nematodes
				1	*S. sclerotiorum*
				1	*S. sclerotiorum* & *Erwinia* sp.
Kathmandu Local	Mid	17	88	33	*Fusarium* spp.
				30	*P. aphanidermatum*
				4	*Alternaria* spp.
				10	*Rhizoctonia* spp.
				1	Nematodes
				2	*P. aphanidermatum* & Nematodes
				4	*Fusarium* spp. & Nematodes
				2	*Fusarium* spp. & *Rhizoctonia* spp.
				2	*P. aphanidermatum* & *Fusarium* spp.
Kibogiant	Mid	2	9	3	*Fusarium* spp.
				5	*P. aphanidermatum*
				2	*Rhizoctonia* spp.
				1	*P. aphanidermatum* & *Fusarium* spp.
Kathmandu Local	Low	16	73	27	*Fusarium* spp.
				34	*P. aphanidermatum*
				3	*Alternaria* spp.
				4	*Rhizoctonia* spp.
				2	Nematodes
				2	*S. sclerotiorum*
				1	*P. aphanidermatum* & *Fusarium* spp.
Pusadeepali	Low	1	3	2	*Fusarium* spp.
				1	*P. aphanidermatum*
Kibogiant	Low	1	6	3	*Fusarium* spp.
				1	*P. aphanidermatum*
				2	*Rhizoctonia* spp.
Snoball-16	Low	1	6	3	*Fusarium* spp.
				1	*P. aphanidermatum*
				1	*Alternaria* spp.
				1	*Rhizoctonia* spp.
Terai-3	Low	3	17	4	*Fusarium* spp.
				7	*P. aphanidermatum*
				5	Nematodes
				1	*P. aphanidermatum* & *Fusarium* spp.

Appendix 19 Pathogens associated with rotten curd samples of cauliflower in three different types of rot at three different altitudes during normal crop growing season 1998 and 1999

Altitude	Total number of rotten curd samples in 1998		Total number of rotten curd samples in 1999		Pathogens associated with rotten curds
	tested	infected	tested	infected	
High	29	9	34	0	*Peronospora. parasitica*
		1		0	*Fusarium* spp
		9		12	*S. sclerotiorum*
		3		2	*Alternaria* spp.
		3		12	*Erwinia carotovora*
		1		0	*Fusarium* spp. & nematodes
		3		0	No pathogens
		0		5	Gram negative yellow colony forming bacteria
		0		1	*S. sclerotiorum* & bacteria
		0		2	*S. sclerotiorum* & *Alternaria* spp.
Mid	19	10	25	1	*P. parasitica*
		5		4	*S. sclerotiorum*
		2		2	Gram negative yellow colony forming bacteria
		1		0	*P. parasitica* & bacteria
		1		3	*Alternaria* spp.
		0		4	*Erwinia carotovora*
		0		7	*Fusarium* spp.
		0		2	*Fusarium* spp. & bacteria
		0		2	*Pythium* spp.
Low	22	7	48	0	*P. parasitica*
		6		3	*Alternaria* spp.
		8		12	*S. sclerotiorum*
		1		0	No pathogens
		0		4	Nematodes
		0		16	*Pythium* spp.
		0		9	*Fusarium* spp.
		0		2	*Penicillium* spp.
		0		1	*Rhizoctonia* spp.
		0		1	*Fusarium* spp. & *Pythium* spp.

Appendix 20 Pathogens associated with rotten curd samples of cauliflower in three different types of rot at three different altitudes during normal crop growing season 1998

Rot type	Altitude	Total number of rotten curd samples		Pathogen associated with rotten curd	Remarks
		tested	infected		
A	High	16	9	*Peronospora parasitica,*	
			1	*Fusarium* spp.	
			3	*Alternaria* spp.	
			3	No pathogen	
	Mid	11	10	*P. parasitica,*	
			1	*Alternaria* spp.	
	Low	14	7	*P. parasitica,*	
			6	*Alternaria* spp.	
			1	No pathogen	
B	High	5	3	*Erwinia* sp.	
			2	*S. sclerotiorum*	
	Mid	3	1	*P. parasitica* and bacteria	mixed
			2	Gram negative yellow colony forming bacteria	
	Low	3	3	*S. sclerotiorum*	
C	High	8	7	*S. sclerotiorum*	
			1	*Fusarium* spp. and Nematodes	mixed
	Mid	5	5	*S. sclerotiorum*	
	Low	5	5	*S. sclerotiorum*	
Total		70	70		

Appendix 21 Pathogens associated with rotten curd samples of cauliflower in three different types of rot at three different altitudes during normal crop growing season 1999

Rot type	Altitude	Total number of rotten curd samples		Pathogen associated with rotten curd	Remarks
		tested	infected		
A	High	1	1	*Alternaria* spp.	
	Mid	13	3	*Erwinia* sp.	
			1	Gram negative yellow colony forming bacteria	
			6	*Fusarium* spp	
			1	*Alternaria* spp.	
			1	*P. parasitica*	
			1	*Fusarium* spp & Bacteria	
	Low	32	2	Nematodes	
			13	*Pythium* sp.	
			2	*S. sclerotiorum*	
			8	*Fusarium* spp.	
			3	*Alternaria* spp.	
			2	*Penicillium* sp.	
			1	*Rhizoctonia* sp.	
			1	*Fusarium* spp & *Pythium* sp.	
B	High	17	12	*Erwinia* sp.	
			2	*S. sclerotiorum.*	
			2	Gram negative yellow colony forming bacteria	mixed
			1	*Alternaria* spp. & *S. sclerotiorum*	
		3			mixed
	Mid		1	*Fusarium* spp. & *Erwinia* sp.	
			1	Gram negative yellow colony forming bacteria	
			1	*Fusarium* spp.	
	Low	0	0		
C	High	16	1	*Alternaria* spp.	
			10	*S. sclerotiorum*	
			1	*S. sclerotiorum* & bacterai.	
			3	Gram negative yellow colony forming bacteria.	
			1	*Alternaria* spp. & *S. sclerotiorum*	
	Mid	9	1	*Erwinia* sp.	
			2	*Alternaria* spp.	
			4	*S. sclerotiorum*	
			2	*Pythium* sp.	
	Low	16	2	Nematodes	
			3	*Pythium* sp.	
			10	*S. sclerotiorum*	
			1	*Fusarium* spp	
Total		107			

Appendix 22 Pathogens associated with curd rot according to varieties during normal crop growing season 1998

Variety	Rotten curd		Remarks
	number	infected with	
Snowcrown	11	*P. parasitica,*	
	1	*Fusarium* spp.	
	2	*Alternaria* spp.	
	1	*Erwinia* sp.	
	6	*S. sclerotiorum*	
	4	No pathogen	
Kathmandu Local	6	*P. parasitica,*	
	1	*P. parasitica,* and Bacteria	mixed
	1	*Fusarium* spp and Nematodes.	mixed
	12	*S. sclerotiorum*	
	1	*Alternaria* spp.	
	2	*Erwinia* sp.	
	2	Gram negative yellow colony forming bacteria.	
Terai-3	5	*P. parasitica,*	
	6	*Alternaria* spp.	
	2	*S. sclerotiorum*	
Kibogiant	4	*P. parasitica,*	
	2	*S. sclerotiorum*	
	1	*Alternaria* spp.	

Appendix 23 Pathogens associated with curd rot according to varieties during normal crop growing season 1999

Variety	Rotten curd sample			Remarks
	number	infected number	infected with pathogen	
Snowcrown	23	10	*S. sclerotiorum*	
		8	*Erwinia* sp.	
		3	Gram negative yellow colony forming bacteria	mixed
		1	*S. sclerotiorum* & bacteria.	
		1	*Alternaria* spp.	
Kathmandu Local	62	2	Nematodes	
		14	*Pythium* sp.	
		9	*S. sclerotiorum*	
		14	*Fusarium* spp.	
		6	*Alternaria* spp.	
		2	*Penicillium* sp.	
		1	*Rhizoctonia* spp.	
		1	*Fusarium* spp. & *Pythium* sp.	mixed
		5	*Erwinia* sp.	
		4	Gram negative yellow colony forming bacteria	mixed
		1	*Fusarium* spp. & *Erwinia* sp.	mixed
		2	*Alternaria* spp. & *S. sclerotiorum*	mixed
		1	*S. sclerotiorum* & bacteria	
Terai-3	13	2	Nematodes	
		2	*Pythium* sp.	
		7	*S. sclerotiorum*	
		1	*Fusarium* spp.	
		1	*Alternaria* spp.	
Kibogiant	9	2	*Pythium* sp.	
		2	*S. sclerotiorum*	
		1	*Fusarium* spp.	
		1	*P. parasitica*	
		3	*Erwinia* sp.	
Total	107			

Appendix 24 Log transformation means of diseased number of seed, number of germinated seed, seed infected with *Fusarium* spp., *S. sclerotiorum*, *Alternaria* spp., *Phoma* and *Rhizopus*

Cultivar and seed source	Number of seed		Seed infected with				
	diseased	germinated	*Fusarium*	*Sclerotinia*	*Alternaria*	*Phoma*	*Rhizopus*
Terai-3 (PAC)	1.220	0.0000	1.037	0.369	0.175	0.445	0.000
Kibogiant (CEAPRED)	0.724	1.3173	0.389	0.075	0.075	0.445	0.000
Snow Mystique (CEAPRED)	1.021	1.9787	0.784	0.239	0.433	0.301	0.000
Snowcrown (CEAPRED)	0.595	1.9777	0.075	0.000	0.564	0.000	0.000
Kathmandu Local (PAC 97)	1.047	1.8829	0.175	0.941	0.358	0.000	0.000
Kathmandu Local (VDD 96)	1.255	1.9637	0.806	0.464	0.977	0.270	0.000
Kathmandu Local (VDD 97)	1.329	1.9368	0.936	0.739	0.926	0.151	0.000
Kathmandu Local (PAC 96)	1.215	1.9417	0.639	0.765	0.762	0.464	0.000
Kathmandu Local (Bhaktapur)	1.418	1.9542	1.096	0.520	1.075	0.000	0.000
Kathmandu Local (Paripatle 97)	1.396	1.9637	1.018	0.401	1.013	0.540	0.000
Kathmandu Local (Paripatle 98)	1.623	1.6574	1.512	0.270	0.953	0.151	0.000
Kibogiant (Paripatle 98)	1.380	1.9444	1.113	0.325	0.926	0.433	0.000
Kathmandu Local (KOSEPAN)	1.654	1.1901	1.603	0.301	0.651	0.000	0.000
Kathmandu Local (PAC farmer)	1.487	0.8997	1.332	0.822	0.000	0.301	0.401
Snowball-16 (KOSEPAN)	1.130	1.4232	0.940	0.345	0.496	0.301	0.000
Grand mean	1.233	1.6021	0.897	0.438	0.625	0.253	0.027
F Probability	<.001	<.001	<.001	<.001	<.001	<.001	<.001

Appendix 25 Log transformation means of emerged number of seedling, number of dead seedlings, seedling number infected with *Pythium* spp., *S. sclerotiorum*, *Alternaria* spp. and combined with *Alternaria* spp. and *S. sclerotiorum*

Cultivar and seed source	Number of seedlings		Number of dead seedlings with			
	emerged	dead	*Pythium*	*Sclerotinia*	*Alternaria*	*Alternaria & Sclerotinia*
Kibogiant (CEAPRED)	1.4469	0.000	0.000	0.000	0.000	0.000
Snow Mystique (CEAPRED)	1.9638	0.000	0.000	0.000	0.000	0.000
Snowcrown (CEAPRED)	1.9777	0.000	0.000	0.000	0.000	0.000
Kathmandu Local (PAC 97)	1.9138	0.542	0.389	0.151	0.000	0.000
Kathmandu Local (VDD 96)	1.9444	0.151	0.000	0.000	0.000	0.151
Kathmandu Local (VDD 97)	1.9637	1.034	0.301	0.628	0.699	0.389
Kathmandu Local (PAC 96)	1.9294	0.874	0.000	0.540	0.651	0.151
Kathmandu Local (Bhaktapur)	1.9190	1.203	0.000	0.900	0.954	0.000
Kathmandu Local (Paripatle 97)	1.8807	0.389	0.000	0.301	0.151	0.000
Kathmandu Local (Paripatle 98)	1.7853	0.301	0.151	0.000	0.151	0.000
Kibogiant (Paripatle 98)	1.8808	0.000	0.000	0.000	0.000	0.000
Kathmandu Local (KOSEPAN)	1.5682	0.000	0.000	0.000	0.000	0.000
Kathmandu Local (PAC farmer)	0.9978	0.389	0.151	0.239	0.000	0.000
Snow ball-16 (KOSEPAN)	1.1761	0.690	0.000	0.602	0.239	0.000
Grand mean	1.7391	0.392	0.071	0.240	0.203	0.049
F Probability	<.001	<.001	0.005	<.001	<.001	0.017

Appendix 26 Combine analysis of variance of mean lesion diameter (cm) on cv. Kathmandu Local following days after partially colonized petals and mycelial discs inoculation

Day	Top growing leaf		Second leaf from top		Third leaf		Second leaf from bottom		Lowest leaf		F probability		
	Petal	disc	Petal	disc	Petal	disc	Petal	disc	Petal	disc	Method	Leaf position	Method* position
6	0.06	0.2	0.04	0.06	0.12	0.22	0.06	0.18	0.1	0.04	NS	NS	NS
7	0.06	0.28	0.12	0.06	0.12	0.22	0.08	0.3	0.1	0.08	NS	NS	NS
8	0.06	0.4	0.22	0.24	0.12	0.26	0.1	0.32	0.12	0.08	NS	NS	NS
9	0.08	0.48	0.22	0.26	0.16	0.34	0.1	0.48	0.16	0.14	*	NS	NS
10	0.08	0.54	0.22	0.28	0.18	0.36	0.1	0.48	0.16	0.14	*	NS	NS
11	0.08	0.54	0.22	0.32	0.18	0.36	0.1	0.5	0.16	0.14	*	NS	NS
12	0.1	0.6	0.28	0.34	0.2	0.46	0.14	0.6	0.22	0.16	*	NS	NS
13	0.12	0.94	0.4	0.6	0.22	0.6	0.18	0.72	0.3	0.16	**	NS	NS
14	0.12	0.98	0.42	0.6	0.22	0.6	0.18	0.72	0.3	0.16	**	NS	NS
15	0.12	0.98	0.42	0.6	0.24	0.6	0.18	0.72	0.3	0.16	**	NS	NS
16	0.12	1.06	0.44	0.68	0.28	0.6	0.22	0.88	0.3	0.18	**	NS	NS
17	0.12	1.08	0.44	0.66	0.3	0.76	0.24	0.94	0.3	0.18	**	NS	NS
18	0.12	1.08	0.5	0.7	0.32	0.76	0.24	1.04	0.34	0.18	**	NS	NS
18	0.12	1.1	0.64	0.96	0.34	0.8	0.28	1.12	0.36	0.28	**	NS	NS
20	0.12	1.24	0.66	1.28	0.38	0.96	0.28	1.16	0.36	0.78	**	NS	NS

* = Significantly different at 5% level.
** = Significantly different at 1% level.
NS = Not significantly different.

Appendix 27 Combine analysis of variance of mean lesion diameter (cm) on cv. Snowcrown following days after partially colonized petals and mycelial discs inoculation

Day	Top growing leaf		Second leaf from top		Third leaf		Second leaf from bottom		Lowest leaf			F probability	
	disc	Petal	disc	Petal	disc	Petal	disc	Petal	disc	Petal	Method	Leaf position	Method* position
5	0.433	0.117	0.217	0.1	0.433	0.183	0.483	0.1	0.617	0.117	**	NS	NS
6	1.02	0.20	1.23	0.233	1.0	0.30	0.93	0.167	0.87	0.20	**	NS	NS
7	1.17	0.317	1.38	0.333	1.08	0.417	1.05	0.267	1.03	0.25	**	NS	NS
8	1.28	0.483	1.52	0.533	1.18	0.60	1.15	0.40	1.20	0.417	**	NS	NS
9	1.43	0.783	1.68	0.817	1.42	0.933	1.32	0.717	1.40	0.617	**	NS	NS
10	1.57	0.967	1.78	1.10	1.62	1.233	1.37	1.033	1.53	0.917	**	NS	NS
11	1.65	1.183	1.85	1.25	1.72	1.417	1.50	1.267	1.65	1.15	*	NS	NS
12	1.70	1.317	1.97	1.383	1.78	1.533	1.57	1.40	1.70	1.317	*	NS	NS
13	1.80	1.483	2.03	1.60	1.83	1.633	1.65	1.533	1.80	1.567	*	NS	NS
14	1.87	1.70	2.07	1.90	1.92	1.883	1.73	1.75	1.87	1.867	NS	NS	NS

* = Significantly different at 5% level.
** = Significantly different at 1% level.
NS = Not significantly different.