STUDIES ON SCLEROTINIA ROT OF CAULIFLOWER AND ITS MANAGEMENT THROUGH ECO-FRIENDLY APPROACHES

Thesis

by

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CERTIFICATE-I

This is to certify that the thesis entitled, "**Studies on Sclerotinia rot of cauliflower and its management through eco-friendly approaches**", submitted in partial fulfilment of the requirements for the award of degree of **MASTER OF SCIENCE** in **MYCOLOGY AND PLANT PATHOLOGY** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) is a record of bonafide research work carried out by **Mr. Nguyen Duc Cuong (H-2003-29-M)** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigations have been fully acknowledged.

Place : Nauni, Solan
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This is to certify that the thesis entitled, "**Studies on Sclerotinia rot of cauliflower and its management through eco-friendly approaches,** submitted by **Mr. Nguyen Duc Cuong (H-2003-29-M)** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.), in partial fulfilment of the requirements for the award of degree of **MASTER OF SCIENCE** in **MYCOLOGY AND PLANT PATHOLOGY** has been approved by the Student's Advisory Committee after an oral examination of the same in collaboration with the external examiner.

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(Nguyen Duc Cuong)

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INTRODUCTION

Cauliflower (*Brassica oleracea* var. *botrytis* L.) belonging to family *Cruciferae* is one of the most important cole crops of Europe, Australia and India (Mitra *et al*., 1990). It attains important place in vegetable crops because of its delicious taste, flavour and nutritive value. The popularity of the crop has been constantly increasing and now it occupies more area than any other vegetable crop in certain parts of the world (Pathania, 2003). Although, there is still a confusion on the exact origin of this crop, as cauliflower evolved from sprouting broccoli (Swiader *et al*., 1994), which originated from the Mediterranean region and Asia Minor (MacGillivray, 1961). Secondary centre of origin of cauliflower were central to northwest Europe, including Germany, Belgium, Denmark, and the Maritime regions of northwest Europe, including parts of France, Britain and the Netherlands (Fuller, 1982).

Cauliflower was introduced in India in 1822. The initial introduction was the Cornish types from England and later on other European types were also introduced. The typical Indian cauliflowers were the result of intercrossing between the Cornish and other European types. Thus, a new development took place in India resulting in tropical cauliflower resistant to hot weather and high rainfall (Bose and Som, 1986). These cultivars formed curd at 20°C or high temperatures. Plants of these cultivars are short, leaves loosely arranged, broadly waxy, curds flat, somewhat loose, yellow to creamy and highly flavoured. In plains of India, its cultivation starts from May-June to January followed by the only temperate cauliflower known as Snowball.

At a global level, it is grown in an area of 892,216 ha with annual production of 16,383,583 MT and yield of 11, 769,543 (Hg/ha) where as in India, cauliflower occupies an area of 280,000 ha with a production of 4,800,000 MT and yield of 171,429 (Hg/ha) (Anonymous, 2004). The seed production has mainly confined to hilly areas of Himachal Pradesh, Uttar Pradesh, West Bengal, and Jammu and Kashmir (Sandhu, 1992). Himachal Pradesh is endowed with varied agro-climatic conditions, which are congenial for cauliflower seed production. It is grown as an off-season crop in H.P. with an area of 1370 ha and annual production of 24,980 MT (Anonymous, 2003).

Cauliflower is a sensitive crop and the varieties are very specific to seasons. Broadly these can be grouped into four seasons; early varieties, early and mid season varieties, main season varieties and late varieties. Among the Indian cauliflowers, 4 varieties (Pusa Deepali, Early Kanwari, Pant Gobhi, PG-26, PG-35, Hisar-1, IIHR-101, IIHR-105, Pusa Shubra and Pusa Himjyoti), 3 Synthetics (Pusa Early Synthetic, Pusa and Pant Shubra) and F_I hybrid (Pusa hybrid-2) have been recommended at regional and national levels for growing in different agro-climatic zones (Kohli and Kanwar, 2000) whereas the snowball group has attained special significance in the state because of seeds production and meeting the seed requirement of the country.

Cauliflower seedlings are used for salad, and the curd is used in curries, soups, pakoras and pickles. Leaves are also used as cattle feed and also for animals and birds. Cauliflower contains 91.7 per cent water and the food values per 100 g of edible portion are energy 31 calories; protein 2.4 g; calcium 22 g; vitamin A 40 I.U.; ascorbic acid 0.57 mg. In addition, it is known for its rich source of vitamin A and C; minerals like potassium, sodium, iron, copper, phosphorus, magnesium, carbohydrates and amino acids such as arginine, histidine, lysine, tryptophan, tyrosine, cystine, methionine, threonine, leucine and valine.

Though, cauliflower is an important crop of Himachal Pradesh yet the future of cauliflower seed industry in the state has been threatened with the serious recurrence of Sclerotinia rot, a destructive disease caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. The fungus was described by Madame Libert as *Peziza sclerotiorum* in 1837 and later Fuckel 1870 changed the name to *S. libertiana*. In India, the disease was first observed in Saproon valley in the year 1973 (Sharma *et al.*, 1983). Low temperature and high humidity conditions favour the disease development to an epidemic form and the crop is badly damaged at curd formation and flowering stages. The disease is a serious menace and has been reported to reduce the potential seed yields by 89-90 per cent (Sharma, 1979). It is now feared that it may become a limiting factor in seed production of cauliflower in the region. The plants are vulnerable to attack of the pathogen at all stages of its growth depending upon the favourable temperature and moisture conditions.

S. sclerotiorum has a wide host range and can survive in soil under adverse climatic conditions. Several fungicides are effective against this pathogen but their residue on curd leads to many health problems besides contaminating the soil and ground water. Breeding for disease resistance is difficult partly because of low correlation between field and laboratory test of resistance (Boland and Hall, 1987). Crop rotation is also unrealistic due to the long survival ability of sclerotia in the soil and the vast host range of this pathogen (Nelson, 1998). Since cauliflower is the main dietary constituent of food in India and public concern today is to use pesticide free vegetables, therefore, it is imperative to investigate ecofriendly disease management strategy after investigating occurrence of the disease in different cauliflower growing areas of Himachal Pradesh. The present study was undertaken with the following objectives:

i) to study the occurrence of the disease in cauliflower growing areas of Solan and Sirmour districts of H. P.,

ii) to establish identity as well as pathogenic ability of causal organism,

iii) to work out morphological characters, cultural and physiological requirements of the fungus,

iv) to study the role of microclimatic conditions favouring disease development,

v) to devise eco-friendly disease management strategy through antagonists, botanicals and plant derived commercial product and host resistance.

REVIEW OF LITERATURE

Chapter-2

REVIEW OF LITERATURE

2.1 *Sclerotinia sclerotiorum*

2.1.1 Nomenclature

Sclerotinia sclerotiorum is the causal organism of Sclerotinia rot of cauliflower. The nomenclature of *S. sclerotiorum* has undergone several revisions since the species was first described by M. A. Libert as *Peziza sclerotiorum* in 1837 and later the name was changed to *S. libertiana* by Fuckel 1869-70, citing *Peziza sclerotiorum* as a synonym (Wakefield, 1924; Purdy, 1979). This binomial was accepted until it was demonstrated as consistent with the International Rules of Botanical Nomenclature. Accordingly, the name *S. libertiana* was changed to *Sclerotinia sclerotiorum* (Lib.) Massee (Wakefield, 1924). Since *S. sclerotiorum* consisted of the perfect stage of many of the imperfect fungi, Korf and Dumont (1972) devised the genus on the basis of the imperfect stages and pathogenic abilities thus changing the name to *Whetzelinia sclerotiorum* where sclerotia did not digest host tissue. However, it was later found that de Bary had used this Latin name earlier, so the proper name was established as *Sclerotinia sclerotiorum* (Lib.) de Bary (Purdy, 1979).

2.1.2 Taxonomy

Sclerotinia sclerotiorum is classified within the genus *Sclerotinia* of the *Sclerotiniaceae,* an important family of *Discomycetes* of the class *Ascomycetes* (Kora *et al.*, 2003). The early taxonomy of the species was based on the size and general characteristics of the *sclerotiorum*, host range, and dimension of ascospores and asci (Willetts and Wong, 1980). However, several studies (Purdy 1955; Grogan, 1979) showed that this system was inadequate. The taxonomy of the *Sclerotiniaceae* has been a source of controversy for years and continues to be under investigation (Kora *et al.*, 2003). The taxonomic position of the genus *Sclerotinia* and characterization of *S. sclerotiorum* as a distinct species of genus *Sclerotinia* are substantiated by several lines of evidence, including genetic markers and nucleotide sequence homologies (Kohn *et al.*, 1988).

2.1.3 Host range

S. sclerotiorum is among the most nonspecific, omnivorous, and successful of plant pathogens. Plants susceptible to this pathogen encompass 64 families, 225 genera, and 361 species (Purdy, 1979). The fungus was recorded for the first time in England in the year 1837 on vegetable and thereafter was reported on the field carrots in Belgium by M. E. Coemans in 1860 (Kora *et al.*, 2003), on cauliflower in California (Snyder and Baker, 1945), on cabbage seed plants from Washington (McLean, 1949), on peas from Kincardineshire in Britain (Gray and Findlater, 1957), on groundnuts in U.S.A. (Porter and Beute, 1974), on cabbage and cauliflower in Denmark during 1953-1958 (Neergaard, 1958), on cauliflower and tomato in New South Wales in 1971 (Letham *et al.*, 1976), on bean in north central region of the United States during 1992-1994 (Yang *et al.*, 1997), on grape in Chile (Latorre and Guerrero, 2001), on carrot in Northeast of Scotland (Couper *et al.*, 2001) and Canada (Kora *et al.*, 2003).

2.1.4 Yield losses

S. sclerotiorum caused huge losses from time to time in different crops. Losses on many crops caused by *S. sclerotiorum* in North American, Europe and Asia have been reported (Willetts and Wong, 1980). It caused 50-80 per cent losses of celery in Florida (Tisdale and Kelbert, 1936); 25 per cent of potato in Ireland (Eddins, 1937); 100 per cent of beans in South Florida (Moore, 1948); 100 per cent of cabbage in experimental fields in Washington (McLean, 1949); and over 50 per cent of peanuts in North Carolina and Virginia in U.S.A. (Beute *et al.*, 1975); 30 per cent of lettuce in Israel (Ben-Yephet *et al.*, 1986); 30 to 50 per cent of carrot in Canada (Finlayson *et al.*, 1989); 170-335 kg per ha on soybean in Iowa of U.S.A (Yang *et al.*, 1999); 50 per cent loss in lettuce in England (Young *et al.*, 2004).

The losses in seed yield of cauliflower in India due to plant mortality caused by Sclerotinia curd rots, at different locations ranged from 2 to 208 kg per ha, while the corresponding monetary losses ranged from Rs. 300-42000/ ha (Shyam *et al.*, 1994). Sharma (1979) also reported the reduction in seed yields up to 80-90 per cent in disease affected field. Similarly, the seed yield losses in cauliflower have been recorded up to the tune of 50 per cent in various parts of India (Sharma and Sharma, 1984).

2.1.5 Symptomatology

The most obvious and typical early symptom of Sclerotinia rot is the appearance on the infected plant of a white fluffy mycelial growth in which soon afterwards develop large, compact resting bodies or sclerotia (Agrios, 1988). Affected plants lose their turgid during the day, but regain turgidity during night or early morning. Petioles of the lower leaves touching the soil show dark brown to back soft rot and are covered in most cases with fluffy growth of extramatrical fungus under cool and humid weather. Stem pith also rots giving way to large cavities lined inside with fluffy mycelium and sclerotia of the causal fungus. With progress of the disease, curd is also affected and shows brown to dark brown rotting. During bolting and seed setting, the disease progresses fast and affect inflorescence, which dries up. The affected branches dry out and bear shrivelled seeds (Sharma *et al.*, 1983).

Besides cauliflower, workers have reported different types of symptoms on different hosts. Agrios (1988) reported that *S. sclerotiorum* diseases are under variety of names such as cottony rot, white mold, or watery soft rot of bean, cabbage, carrot, peanut, potato; stem rot of cucumber, bean, potato, tomato, soybean; drop of lettuce, broad bean, beet, cabbage; damping off of celery and lettuce. Singh (1985) recorded Sclerotinia blight infection at any part of the foliage, mainly the stem or branches in brinjal. At the point of infection a dry, discoloured spot develops. It gradually girdles the entire stem and also progresses up and down. Similarly, in white mold of soybean infected petioles and leaves first show brown water soaked lesions, which spread rapidly to the stem and branches. Later, the superficial cottony growth of white mold occurs on the infected petioles, stems, and pods (Tu, 1989). Kora *et al.* (2003) described Sclerotinia rot of carrot which appeared first as water soaked, dark olive green lesions associated with necrotic tissues. Sclerotinia root rot of radish revealed excessive shrinking of affected shoots with bleached appearance. The bleached portion extended 5-25 cm longitudinally with a slimy water soaked areas distally.

Infected plants succumbed to the attack prematurely without producing any seed (Kapoor *et al.*, 1987).

2.1.6 Morphology

A developing sclerotium appears as a white mass of interwomen mycelial strands covered with numerous droplets of liquid, each surrounded by membranous material (Colotelo, 1974). Mycelium of the fungus is hyaline, much branched, consisting of large, closely septate hyphae, which are intercellular and intracellular and invade all the tissues of the host (Sharma, 1979; Singh, 1985). The hyphae are 9 to 18 μm broad and filled with dense protoplasm. Sclerotia vary in size and shape according to environment and location. It measured 2.36±1.07 mm in width, 3.33±1.11 mm in length (Yang *et al.*, 1997), 2-10 mm in diameter (Agrios, 1988) and 6.8±3.7 mg in weight, with an average germination rate of 88 per cent after 8 months of production (Yang *et al.*, 1997). Ben-Yephet *et al.* (1993) classified sclerotia by weight in to four-groups 14-40, 7-17, and 1-3 mg. These sclerotia after germination gave rise to several columnar structures called stipes with 2.5-24 mm in length (Eddins, 1937).

The apothecial cups were well expanded and measured from 1.5 to 10 mm in diameter (Eddins, 1937). Ascospores are discharged in abundance from these cups. The ascospores are always eight in each ascus, which has an apical pore through which spore discharge occurs with violence (Singh, 1985). Coe (1944) reported that average size of ascospores and asci were 12.61 x 6.63 μm and 135.99 x 8.06 μm. While, Purdy (1955) confirmed measurements of Madame Libert which were 130-135 x 8-10 μm and 9-13 x 4-6.5 μm. Mehta *et al.* (1946) found that the asci contained in these bodies measured 108-153 x 4.5-8.1 (average 122.9 x 5.9) μm, and ascospores 7.2-11.7 x 3.6-5.4 (8.9 x 3.9) μm. Finally, Purdy (1955) showed that the asci varied from 81.0 to 199.4 μm in length and 4.3 to 12.4 μm in width and the ascospores from 6.0 to 17.0 μm in length by 2.0 to 8.4 μm in width.

Microconidia (spermatia) are produced on sclerotia, on the discs of overmature apothecia, and in culture. These spermatia are formed in chains at the tips of short lateral branches of the vegetative mycelium. The spermatia germinate very sparsely in water and culture media and apparently do not serve as source of infection or dissemination of the fungus (Singh, 1985).

2.1.7 Life cycle of *S. sclerotiorum*

During its life cycle, *S. sclerotiorum* progresses through three stages of development that include dormancy, saprophytism and parasitism (**Kora *et al.*, 2003).**

2.1.7.1 Dormancy

S. sclerotiorum spends about 90 per cent of its life cycle in soil as dormant sclerotia which develop primarily from mycelium on disease tissues (Adams and Ayers, 1979). Mature sclerotia can survive in soil for 1 to 15 years, depending on the interaction of various physical and biological factors (Cook *et al.*, 1975). Physical factors in the soil environment, including prolonged periods of high temperatures, flooding, sequential drying and wetting, deep burial below the soil surface, and exposure to solar radiation, may reduce the viability of sclerotia. However, microbial degradation remains the most significant factor affecting populations of sclerotia in natural ecosystems (Adams and Ayers, 1979). Structural malformations or breakage of melanized rind may also contribute to reduced longevity of sclerotia by microbial degradation (Coley-Smith and Cooke, 1971).

2.1.7.2 Saprophytism

2.1.7.2.1 Germination

During suitable environmental conditions, mature sclerotia can germinate myceliogenically to form mycelium or carpogenically to form apothecia (Adams and Ayers, 1979). During myceliogenic germination, sclerotia produce masses of mycelia, which are capable of direct penetration of the host cuticle but require an exogenous nutrient source to be infective (Abawi and Grogan, 1975; Lumsden, 1979). Mycelia originating from sclerotia of *S. sclerotiorum* posses limited competitive saprophytic ability and, in soil, were unable to infect plants located more than 2 cm from the source (Newton and Sequeira, 1972).

Carpogenic germination requires functionally mature and preconditioned sclerotia located in the upper 2 to 3 cm layer of soil (Abawi and Grogan, 1979). The dormancy period required before sclerotia can germinate carpogenically varies from 13 to 208 days depending on environmental and physiological factors (Willetts and

Wong, 1980). Typically, constitutive dormancy can be relieved by conditioning sclerotia for prolonged periods in cool, moist conditions, or by over wintering in soil (Abawi and Grogan, 1979). In the laboratory, full hydration of sclerotia occurred within 4 hrs of immersion in water, while release of soil moisture from fully hydrated to fully desiccated sclerotia required up to 25 hr. in field conditions. Development of apothecia is stimulated by extended periods of high soil moisture potentials (0 to -0.3 bars), cool (4 to 20°C) soil temperatures, and a dense plant canopy over soil (Grogan and Abawi, 1975).

Carpogenic germination leading to formation of phototrophic stipes and development of mature apothecia is optimal in soil temperatures of 11 to 15°C (Abawi and Grogan, 1975). However, differentiation and full expansion of the apothecia disc and ascospore production occur only at temperatures 15 to 20°C (Coley-Smith and Cooke, 1971 and Le Tourneau, 1979). Mature ascospores are forcibly discharged to a distance of more than 1 cm above the upper surface of the apothecium when subjected to decrease in moisture tension in the surrounding air (Abawi and Grogan, 1979). Apothecia can remain functional for 5 to 10 days in field conditions and can produce up to 3×10^7 ascospores (Steadman, 1983).

2.1.7.2.2 Aerobiology

According to Hudyncia *et al.* (2000), a diurnal distribution of ascospores characterized by consistent peaks of spore deposition occurring between 900 to 1300 hr. in several geographic regions. Seasonal patterns of ascospore distribution varied among geographic regions because of differences in climates and cropping seasons. Aerial dispersal of ascospores could reach up to several kilometers (Abawi and Grogan, 1979). Ungerminated ascospores survived for up to 12 days in the crop depending on their position in the canopy and environmental conditions. Ascospore mortality increased with increasing temperatures above 21°C and exposure to ultraviolet radiation (Caesar and Pearson, 1983).

2.1.7.3 Parasitism

2.1.7.3.1 Histopathology

Germinating ascospores of *S. sclerotiorum* initiate infection through the production of simple, single appressoria that penetrate directly through the host cuticle while penetration by mycelium, is achieved by complex, multicelled appressorial masses. Penetration is mainly by mechanical pressure added by the activity of fungal enzymes involved in the modification of the cuticle (Lumsden, 1979). After penetration, an inflated, granular vesicle develops that gives rise to infection hyphae. Mycelial growth produces from these infective hyphae and colonizes host tissues by inter-' and intracellular penetration. After extensive colonization of the interior tissues, ramifying hyphae emerge through the cuticle, forming mycelial tufts on the surface. Later, mature sclerotia form on diseased tissues and are released onto the soil along with plant debris (Lumsden, 1979).

2.1.7.3.2 Pathogenesis

S. sclerotiorum possesses a nonselective mechanism of attack that can successfully invade a host plant before it can respond (Kora *et al.*, 2003), but subsequent colonization occurs mainly by enzymic tissue dissolution (Willetts and Wong, 1980). Pathogenesis by *S. sclerotiorum* is attributed to the secretion of oxalic acid (Maxwell and Lumsden, 1970) and a series of cell wall degrading and cell membrane modifying enzymes, including pectolases (Lumsden, 1979), celluloses (Lumsden, 1969), hemicellulases (Hancock, 1967), and phosphatidases (Lumsden, 1969) that degrade and kill plant cells in advance of invading hyphae. Oxalate enhances the activity of lytic enzymes by reducing the pH of the infected tissue, weakens the plant cell by sequestering cell wall Ca^{2+}, and suppresses host defense mechanisms by inhibiting polyphenol oxidases (Maxwell and Lumsden, 1970). It appeared to act synergistically with pectic and cellulolytic enzymes for destruction of host tissues (Maxwell and Lumsden, 1970). The accumulation of oxalic acid offers a lower pH environment, which is a better condition for the activities of other enzymes (Dutton and Evans, 1996).

2.1.8 Epidemiology

S. sclerotiorum is a soil inhabiting fungus and sclerotia in the soil are primary source of the infection, which remain viable for longer periods in infected fields at 5-20 cm depth. Apothecia produced on these sclerotia release ascospores, which are the potential and primary source of infection in cauliflower (Sharma and Sharma, 1986a). Wind dispersal of air borne ascospores and transportation of sclerotia through farming operations including tillage, irrigation, and manure fertilizations are important means for long distance distribution of the pathogen (Adams and Ayers, 1979). These release wind-borne spores that travel up to 1 km or several miles (Venette, 1998). Necrotic tissues resulting from injury or pathogenesis are likewise ideal locations for infection. A free moisture period of 48-72 hours is required for establishment of infection and lesion expansion (Tu, 1989).

Mycelium initiated from sclerotia can also account for primary infection to some extent under favourable environmental conditions (Sharma and Sharma, 1986a). However, the mycelium directly originating from sclerotia lying close to the seedling rarely succeed to initiate infection (Kapoor *et al.*, 1990). This type of germination is associated with small sclerotia types. Hyphal germination of sclerotia causes infection by first invading nonliving organic matter and forming a mycelium, which is an intermediate necessity for mycelial infection (Purdy, 1979).

Secondary infections are achieved by natural contact of plant parts with diseased ones. The factors such as frequency and duration of rain and dew, wind, aeration, row spacing and plant morphology have a profound effect on disease incidence as well as severity (Tu, 1989). Sclerotia contaminated seeds and seeds infected with mycelium are also potential forms of pathogen introduction (Schwartz and Steadman, 1978).

2.1.9 Environmental factors

2.1.9.1 Temperature

Sclerotinia rot is favoured by cool temperatures and prolonged periods of precipitation. In dry weather, disease progression may be slow, but extended periods of plant wetness favour fungal growth (Venette, 1998). Tankrikut and Vaughan (1951) observed that *S. sclerotiorum* has varied temperature requirements. They found best vegetative growth at 15-25°C and sclerotial production between 0-30°C. Sharma (1979) reported that growth of mycelium was observed between temperature ranges of 2-30°C with an optimum of 20-25°C. Temperature may exert an indirect influence on survival by virtue of a direct effect on sclerotial germination. Low or fluctuating temperature activation of some carpogenic forms may result in the formation of fruit bodies that consume considerable quantities of the sclerotial reserves and hence hasten decay (Coley-Smith and Cooke, 1971). Cook *et al.* (1975) reported that, sclerotial survival was not always correlated with carpogenic germination, but adversely affected by high soil temperature and soil moisture. However, there was no clearly defined optimum temperature for infection of sclerotia. After incubation for 3 weeks at 35°C, survival of sclerotia declined to zero per cent and reduction in survival of sclerotia occurred at 15°C (Phillips, 1986).

Henson (1940) reported that constant moisture, a temperature of 14°C, and sufficient time of exposure were important factors in the initiation of stipes of *S. sclerotiorum*. Abawi and Grogan (1975) confirmed that, ascospore germination was not drastically affected by temperature, but 25°C was optimum for germ tube growth. Mycelial growth, sclerotial production, and lesion initiation and development were optimum at 20-25°C. The temperature range for apothecial production was 10 to 25°C, with an optimum of about 10°C. Similarly, Clarkson *et al.* (2004) reported that carpogenic germination of sclerotia occurred between 5 and 25°C but optimum at temperatures of 15 to 25°C, little or no germination occurred at 26 or 29°C.

Sharma and Sharma (1985) reported temperature of 20-25°C and RH 95-100 per cent, were congenial for disease development in 1977-78 and 1978-79 cropping seasons and 73 per cent of the disease was controlled by these factors.

2.1.9.2 Soil moisture and Relative Humidity

Moisture is also a contributing factor to the development of disease in case of *Sclerotinia* spp. Grogan and Abawi (1975) found that mycelial growth and lesion expansion of *S. sclerotiorum* (Lib.) de Bary on agar amended with soluble salts and on moistened bean stems increased as water potential and moisture levels increased, respectively. With the exception of saturation, high soil moisture tends to promote sclerotial germination. Willetts and Wong (1980) reported that, when sclerotia were dried in the field and subsequently placed under optimal conditions for carpogenic germination, including free water, apothecia failed to develop, although the sclerotia still retained their viability. Ferraz *et al.* (1999) added that, carpogenic germination was increased by high soil organic matter content. Grogan and Abawi (1975) confirmed that sclerotia of *S. sclerotiorum* could germinate directly at soil moisture levels between 0 and -0.6 MPa. However, Abawi *et al.* (1980) reported that germination of sclerotia occurs when soil matric potential is between -0.03 MPa and -1.5 MPa, with an optimum at -0.03 MPa, whereas, optimum apothecia occurs in a soil matric potential of -0.25 bars (Steadman, 1983). Miller and Burke (1986) reported that infection from Sclerotinia wilt increased with increasing irrigation. These results are similar to those observed in irrigation studies concerning white mold of edible dry beans caused by *S. sclerotiorum* (Weiss *et al.*, 1980). Rainfall has also been associated with Sclerotinia outbreaks. Disease spread was associated with the amount and frequency of rainfall, and therefore, moisture records can be used to predict disease initiation and subsequent spread with reasonable success in fields infested with the white mold fungus (Tu, 1989). Phillips (1986) reported that germination of conidia and infection by sclerotia took place only at 100 per cent RH.

2.1.9.3 Effect of pH

Sharma (1979) studied the growth of fungus at different pH levels ranging from 3.0 to 9.0 with an optimum at pH 5.0. Phillips (1986) reported that germination of conidia was rapid at pH 4.6-5.0 but was reduced at higher pH levels. However, it was not possible to determine per cent germination of conidia at pH 4.0-5.6 due to excessive growth of germ tubes and mycelium. Infection of sclerotia occurred over the pH range 4.0-8.0 and was optimum at around pH 5.0. Khan (1976) confirmed that pH of 4.6 and 4.5 were optimum for mycelium growth under *in vitro* condition. Willetts and Wong (1980) reported that *Sclerotinia* spp. generally have pH optima below 5.0, therefore, their activities are enhanced under acidic condition. The pH of the medium may be changed during growth by the production of organic acids (Le Tourneau, 1979).

2.1.9.4 Effect of nutrition

S. sclerotiorum has varied nutrient requirements, as indicated by its wide host range and ability to subsist as a saprophyte for long periods under certain conditions. Tankrikut and Vaughan (1951) proved that fungus was capable of growing on almost all the substrates tested, with or without mineral or organic nutrient. However, in the absence of nitrogen, phosphorus or carbohydrates, growth was scanty and sclerotia were few or lacking. Studies with synthetic media have shown that *Sclerotinia* spp. utilize many organic compounds as carbon source for growth and sclerotia production (Le Tourneau, 1979). Willis (1968) studied the growth responses of *S. sclerotiorum* and *S. trifoliorum* to 23 different nitrogen sources and found that good sources of nitrogen were casein hydrolysate, L-proline, L-arginine, L-glutamic acid, *etc.* Wang and Le Tourneau (1972) observed that amino acids containing sulphur were generally poor nitrogen sources. Aspartic and glutamic acids and their amides, alanine and serine, closely related to the tricarboxylic acid cycle compounds were good nitrogen sources for growth and sclerotium formation. Khan (1976) reported asparatic and potassium nitrate to be good nitrogen sources and maltose and sucrose as carbon sources required well by the fungus. For vitamins, 10-20 ppm concentration of riboflavin was found to be the only one giving good vegetative growth. When the fungus grew in a liquid medium containing purified chemicals in plastic flasks, less dry weight was produced and three of four isolates did not produce sclerotia in the absence of Zn^{++} (Vega and Le Tourneau, 1974).

2.2 Management of Disease

2.2.1 Disease forecasting

The disease forecasting is mainly based on the weather parameters and petal test that is available by using commercial test kit. The petal test was developed to predict the disease based on the significant relationship between the disease incidence and the level of infestation of rapeseed petals by *S. sclerotiorum* ascospores at early bloom stage (Turkington *et al.*, 1991a). This method suggests that petals should be sampled in the afternoon, due to diurnal variation of the petal infestation level (Turkington *et al.*, 1991b). In addition, Clarkson *et al.* (2004) developed forecasting system for carpogenic germination of *S. sclerotiorum*.

2.2.2 Cultural practices

2.2.2.1 Crop rotation

Crop rotation is a disease control recommendation and often has been advocated for control of Sclerotinia diseases (Steadman, 1979). The crop rotation results tried by Gupta *et al.* (1987) showed that cauliflower-paddy-cauliflower was most effective in reducing disease incidence by more than 51 per cent. Similarly, results in a two years experiment showed that a crop rotation with paddy was highly effective in reducing the disease by more than 60 per cent (Sharma *et al.*, 1983). In addition, crop rotation and no tillage of soybean was the most useful combination treatment that reduced the primary inoculum of *S. sclerotiorum* in infested soybean fields (Gracia-Garza *et al.*, 2002). However, 3 and 4 years rotation with non-host crops have not been effective in decreasing the frequency of sclerotia in soil (Schwartz *et al.*, 1978). This is because, tillage operations generally make the sclerotia ineffective at or near the soil surface (Cook *et al.*, 1975) and the long survival ability of sclerotia in the soil as well the vast host range of this pathogen (Nelson, 1998). He showed that even when the sclerotia population is as low as one sclerotia per 800 cm^3 of soil, 5 to 6 years rotation was needed and even such a long rotation did not guarantee the elimination of *S. sclerotiorum*. The fungus has an extremely wide host range and attacks crop such as bean, pea, sweet clover, sunflower, mustard and potato, therefore, limits the crops that can be used in a rotation to manage the disease.

2.2.2.2 Irrigation

Moore (1948) reported that flooding a field continuously for 25-45 days or cycles of alternate flooding and drying led to destruction of sclerotia of *S. sclerotiorum*. However, this technique would have only limited usefulness in most nonirrigated areas. Subbarao (1998) studied the effect of furrow and subsurface drip irrigation on diseases and yield of lettuce. He showed that subsurface drip irrigation revealed a significant reduction in the incidence of lettuce drop and increased yield. Optimum soil moisture and grass mulching may reduce the production of apothecia of *S. sclerotiorum* even in soils rich in organic matter (Ferraz *et al.*, 1999). Grau and Radke (1984) reported that yields were improved 10-22 per cent in soybean by reducing disease severity through reduced irrigation. Reduction in the number of irrigations, especially those at the end of the season, can reduce disease in absence of rainfall (Steadman, 1979).

2.2.2.3 Seed treatment

Seed treatment is advocated to control Sclerotinia root rot of sunflower and to eliminate sclerotia from infested rapeseed (Steadman, 1979). Tu (1988) demonstrated that *S. sclerotiorum* could survive in infected seeds as dormant mycelia in testae and cotyledons for 3 years or longer. When infected seeds were sown in soil or sand, 88-100 per cent failed to germinate, depending on severity of the infection (Tu, 1989). Inbar *et al*. (1996) reported that coating seeds with *T. harzianum* conidia reduced the pre-emergence and post-emergence effect of *S. sclerotiorum* in cucumber by 69 and 80 per cent, respectively, and in lettuce by 46 and 72 per cent, respectively. In the green house, the disease caused by *S. sclerotiorum* in lettuce was reduced by treating seedling with a peat-bran preparation of *T. harzianum*. The dormant mycelia in the infected seeds play an important role not only in dissemination of the fungus but also in epidemiology of the disease. Only disease free seed should be planted in fields that are free from white mold infestation (Tu, 1989).

2.2.2.4 Tillage, ploughing and soil solarization

Tillage can be regarded as an effective method of reducing the disease by burying the sclerotia. Deep ploughing also has been recommended for control of white mold of bean, but ploughing to a depth of 25 cm did not affect disease servirity (Cook *et al.*, 1975). Soil solarization can effectively reduce the population of sclerotia in soil, prevent their germination, or decrease disease in several crops. Soil solarization was first developed as a control for root diseases in Israel and California (Katan *et al*., 1976). Porter and Merriman (1985) studied the effect of soil solarization on the viability of plant pathogens and diseases, showed that solarization of artificially inoculated soils reduced inoculum levels to at least a depth of 10 cm and effectively controlled diseases caused by *S. minor* and *S. sclerotiorum*, on lettuce.

2.2.2.5 Sanitation, site selection and microclimate modification

Any method that reduces sclerotia could contribute to a control program. Sanitation methods such as steam sterilization of soil for at least 10 minutes resulted in up to 85 per cent mortality of sclerotia buried at 2 cm in the field. The efficacy of steam sterilization was possitively related to the moisture content of the soil and number of sclerotia and inversely related to the depth at which sclerotia were buried (Kora *et al.*, 2003). In addition, sclerotia sometimes are harvested along with several seeds like sunflower, pea, bean, or other seeds. The use of certified seed will reduce chances of introduction of the pathogens into clean fields (Steadman, 1979). The degree of field infestation by *S. sclerotiorum* varies greatly. Such variation contributes in part to the difference in disease incidence in the fields ranging from 0 to 85 per cent. Fields with a previous history of severe white mold should be planted with resistant crops (Tu, 1997). An association between plant canopy development and Sclerotinia disease incidence has been observed in various crops (Steadman, 1979). Manipulation of environment to reduced leaf and flower wetness should help to check white mould of soybean caused by *S. sclerotiorum* (Munshi and Sokhi, 2000) and Sclerotinia diseases in different crops. Under less and favourable conditions for disease, some reduction of infection was obtained in the blend, but no concomitant yield response was observed (Steadman, 1979).

2.2.2.6 Soil amendments with organic matter

Soil amendments with cakes of sunflower seed and rape seed as well as artificial defoliation of infected leaves at weekly intervals proved effective in reducing the incidence of Sclerotinia stalk rot and increasing the seed yield of cauliflower (Sharma and Sharma, 1986b). Soil mulch with pine needles or sunflower inflorescence residue was also found effective and was more or less at par with each other in reducing disease incidence by more than half and increasing seed yields by more than 50 per cent (Sharma *et al.*, 1983). The disease incidence of Sclerotinia soft rot of lettuce and survival rate of sclerotia of *S. sclerotiorum* was reduced by the addition of organic amendments to field plots. Of six materials tested, stable manure, fowl manure, and lucerne hay were the best, and all except brown coal significantly reduced disease compared with control (Asirifi *et al.*, 1994).

Singhaujla *et al.* (2001) reported that amongst different soil amendments, mustard cake, FYM and gypsum recorded the lowest disease intensity. Gupta *et al.* (1986) reported that application of different amendments to soil decreased disease incidence of Sclerotinia rot and increased seed yields of cauliflower. Out of the ten different soil amendments tested sunflower cake, mustard cake, gypsum and bean straw were found effective in managing the disease. Farm yard manure, neemax, sheep manure and horse stable manure were effective in the management of white rot disease and reduced disease intensity of 7.43, 6.44, 8.45 and 8.00 per cent in comparison with 12.27 per cent disease intensity of control (Handoro *et al.*, 2001).

Similarly, application of FYM (10-40 ton per acre) affectively controlled this disease and reduced the population of sclerotia (Sandhu, 1992).

2.2.3 Biological control

2.2.3.1 Fungi - *S. sclerotiorum* interaction

2.2.3.1.1 *In vitro* test

Parasitic fungi have been widely studied as biocontrol agents for *S. sclerotiorum* such as *Coniothyrium minitans* (Huang and Hoes, 1976; Zazzerini and Tosi, 1985; Whipps and Budge, 1990), *Trichoderma* spp. (Zazzerini and Tosi, 1985), *Alternaria alternata* and *Epicoccum purpurascens* (Mercier and Reeleder, 1987), and *Bradysia coprophila* (Gracia-Garza *et al.*, 1997). Zazzerini and Tosi (1985) reported that *Trichoderma* spp., *Coniothyrium minitans* and species of *Fusarium* and *Penicillium* showed strong antagonistic activity against *S. sclerotiorum* under *in vitro* conditions. Bhardwaj *et al.* (1990) showed that *G. virens* and isolates of *T. viride* and *T. harzianum* could be used in establishing the better insight of the nature of the antagonism under *in vitro* and subsequently could be tried for the management of Sclerotinia rot of cauliflower. In addition, Singh (1998) confirmed that *T. harzianum* showed strong mycoparasitism and covered 100 per cent colony growth of the pathogen (*S. sclerotiorum*), whereas *T. viride* showed strong antibiosis and formed 2-3 mm zone of inhibition after 6 days of incubation in dual culture. This is because, hyphal and sclerotial parasitism by means of coiling, penetration, and lytic degradation were probably the dominant mechanism by which *T. harzianum* controlled S. *sclerotiorum* (Inbar *et al.*, 1996). Mueller *et al.* (1985) tested 2 isolates of *Gliocladium roseum* i.e., *G. roseum*-12 and *G. roseum*-14 against *S. sclerotiorum* and results showed that a distinct zone of inhibition was formed between the two colonies. Mercier and Reeleder (1987) using *in vitro* technique to determine effect of selected fungi viz., *Trichoderma viride*, *Alternaria alternata*, and *Epicoccum purpurascens* significantly reduce on ascospore germ-tube elongation by 34.2, 60.6 and 38.3 per cent, respectively. For management of stalk rot of cauliflower, 14 fungi isolated from the rhizosphere of cauliflower plants inhibited the growth of *S. sclerotiorum*, while maximum antagonism was exhibited by *Trichoderma viride* followed by *Aspergillus terreus*, *Rhizopus arrhizus* and *Fusarium solani* (Gupta and Agarwala, 1988). Turhan and Grossmann (1989) confirmed that *Acrophialophora* *levis* is a strong antifungal antagonist having a wide spectrum of effectiveness against various soil-borne pathogens belonging to different subdivisions of the fungal kingdom. Twenty five isolates of *Coniothyrium minitans* were screened for antagonism to *S. sclerotiorum* in Petri dish biossay using tomato stem segments on sterile sand. The antagonistic activity of 23 isolates was quite uniform and only two less antagonistic isolates were identified (Gerlagh *et al.*, 1996). Whipps and Budge (1990) confirmed that *Gliocladium virens* and *G. minitans*, both originally isolated from sclerotia of *S. sclerotiorum* decreased viability of sclerotia than the other mycoparasites.

2.2.3.1.2 Green house experiments on biological control

The biocontrol agents applied to the soil inhibited the sclerotia and their carpogenic germination. Phillips (1986) reported that *Gliocladium virens* parasitises and decayed sclerotia of *S. sclerotiorum*, *S. minor*, *Botrytis cinerea*, *S. rolfsii* and *Macrophomina phaseolina* on laboratory media and caused a reduction in survival of sclerotia of *S. sclerotiorum* in soil. Gupta and Agarwala (1988) studied biocontrol in pot tests for management of stalk rot of cauliflower with 4 fungi and found *F. solani* and *A. terreus* effective in controlling the disease under green house conditions. *Coniothyrium minitans*, *T. harzianum* (HH3) and *Trichoderma* sp. (B1) were tested for ability to control disease caused by *S. sclerotiorum* in a sequence of celery and two lettuce crops in glass house condition. The results showed that *C. minitans* treatment in the first lettuce crop decreased disease and increased marketable yield (Budge and Whipps, 1991). McLaren *et al.* (1996) studied the effect of application of *C. minitans* to soil at seedling stage and found that it reduced apothecia production of sclerotia under the canopies of bean, canola, wheat and barley. Conidia of *C. minitans* were applied to infected sunflower seeds and sclerotia of *S. sclerotiorum* by polymer film coating. In glass house and field pot bioassays with sclerotia buried in soil for 20 and 34 weeks, *C. minitans* completely suppressed apothecial production and no sclerotia were recorded from the *C. minitans* treatment (McQuilken *et al.*, 1997).

2.2.3.1.3 Field application of biocontrol agents

Knudsen *et al.* (1991) confirmed that colonization of *S. sclerotiorum* by *T. harzianum* reduced the pathogen's inoculum producing ability. However, proliferation of *T. harzianum* (strain ThzID1) in soil was not observed, so it was likely that colonization of sclerotia in the field resulted mainly from initial hyphal growth from pellets. Application of *T. flavus* and *C. minitans* to soil at seedling stage reduced disease incidence and subsequently seed yield (McLaren *et al.*, 1994). *C. minitans* was applied to soil as a maize meal perlite preparation in order to determine its effect on sclerotial survival and apothecial production of *S. sclerotiorum*. The mycoparasite infected sclerotia decreased sclerotial survival, carpogenic germination and production of apothecia (McQuilken *et al.*, 1995). Gerlagh *et al.* (1999) applied *C. minitans* and *Trichoderma* spp. in soil against *S. sclerotiorum* diseases of bean, potato, carrot and chicory and results showed that *Trichoderma* spp. did not suppress sclerotia, but *C. minitans* infected at least 90 per cent of *S. sclerotiorum* sclerotia on treated crops by the end of each season and reduced disease incidence in the bean crop by 50 per cent during the fifth year of the trial. Del Rio *et al.* (2002) studied the effectiveness of *Sporidesmium sclerotivorum* to control Sclerotinia stem rot of soybean at Ames, Humboldt, and Kanawha between 1996 and 1998 under two crops rotation and results showed that, Sclerotinia stem rot was completely suppressed in all plots at Humboldt, white the commercial field surrounding the experimental plots had 17 per cent disease incidence.

2.2.3.2 Bacteria - *S. sclerotiorum* interaction

Little information is available about the effect of bacteria on the survival of sclerotia. However, damaged sclerotia appear more susceptible to bacterial invasion. Bacteria are consistently found in rotted sclerotia as in many other saprophytic fungi. Bin *et al.* (1991) reported that high population levels of antagonistic bacteria in bulk soil suppressed a fungal biocontrol agent. Similarly, Phookan and Chaliha (2000) reported that mycelial and sclerotial growth of *S. sclerotiorum* was significantly suppressed by *Bacillus subtilis*, *G. virens* and *T. viride* under *in vitro* test. Huang *et al.* (1993) studied *in vitro* effect of 2 strains of *B. cereus* against *S. sclerotiorum*. It revealed that strain of *B. cereus* differed in their antagonistic activities against *S. sclerotiorum*. Vegetative growth and ascospore germination of *S. sclerotiorum* were inhibited by diffusible metabolites induced by *B. cereus* strain alfa-87A, but unaffected by strain B43. Expert and Digat (1995) reported that, seed bacterization with *P. fluorescens* and *P. putida* (1×10^6 bacteria per seed) under *in situ* tests and field trials resulted in significant protection of sunflower from Sclerotinia wilt.

2.2.3.3 Botanicals and microorganism-derived products

Turhan and Grossmann (1986) studied antibiotic efficacy of 300 isolates of actinomycetes obtained from soil samples from Turkey against *Rhizoctonia solani*, *Alternaria alternata*, *Pythium debaryanum*, *Cochliobolus sativus* and *Macrophomina phaseolina*, and results showed that *S. sclerotiorum* was completely inhibited by more than 90 per cent by the tested isolates. Chattopadhyay *et al.* (2004) studied inhibition effect of bulb extract of *Allium sativum* as seed treatment integrated with its foliar spray against Sclerotinia rot and a combination including seed treatment with *A. sativum* bulb extract (1% w/v) or *T. viride*, significantly reduced the disease incidence and improved seed yield, plant height and initial plant stand over control. Singh *et al.* (1980) studied effect of aqueous extracts and neem oil on soil borne fungi (*S. sclerotiorum*, *S. rolfsii*). The results showed that growth of the pathogens in liquid medium was inhibited by extracts of leaf, trunk bark, fruit pulp and oil of neem. Zewain *et al.* (2005) reported that application of Bavistin (1.5 g/l), Sailaxyl-MZ (5.25 g/l), mancozeb (8.34 g/l) and neem extract (6 ml/l) gave maximum reduction in stalk rot disease of cauliflower. In addition, six neem based biopesticides were evaluated at two concentrations (0.5 and 1%) against *S. sclerotiorum* and results revealed Wanis as the most effective botanical followed by Neemgold (Kapil and Kapoor, 2005).

2.2.4 Integrated disease management

Zhou and Reeleder (1989) reported that application of *E. purpurascens* in malt extract (1%) and iprodione to snap bean during flowering significantly reduced disease incidence, disease index and percentage of disease pods. Similarly, Budge and Whipps (2001) indicated that disease caused by *S. sclerotiorum* in lettuce was significantly reduced by soil applications of *C. minitans* and a single application of iprodione. Sharma and Basandrai (1997) studied the effect of the biocontrol agents with fungicides and crude leaf extracts of neem (*Azadirachta indica*), *Lantana camara* and *Cannabis sativa* on the viability of sclerotia of *S. sclerotiorum* isolated from pea (*Pisum sativum*), chickpea (*Cicer arietinum*), cauliflower (*Brassica oleracea* var. *botrytis*) and cabbage (*B. oleracea* var. *capitata*) and found that all the treatments were effective in reducing sclerotial germination irrespective of the crop from which the sclerotia were collected. *T. harzianum*, carbendazim, triademefon and *A. indica* extract were highly effective in reducing sclerotial viability. Zewain *et al.* (2005) studied various aspects of integrated disease management and showed that seed treatment with *T. harzianum*, *A. niger*, Kalisena, *T. harzianum* + *A. niger*, Booty + Kalisena and Booty increased seed production.

2.2.5 Varietal reaction

Genetic resistance to *S. sclerotiorum* was observed first by Anton de Bary in 1887 (Steadman, 1979). The resistance to *S. sclerotiorum* has been reported in carrot, field pea, lettuce, swede, tomato and *Phaseolus* sp. by many workers. However, in cauliflower polygenic recessive gene control of resistance to stalk rot was first noticed (Baswana *et al.*, 1991). These genes were reported to have additive effects. Sharma *et al.* (1982) screened eight genotypes of cauliflower and observed that Master Osena and Avans (F1 hybrids) were comparatively less susceptible than others. Similarly seventy-nine lines were screened by Kapoor (1986) at the seedling stage at Katrain and reported that EC-103576, EC-131192, Janavon and KN-81 (heading broccoli) were resistant. Dohroo and Korla (1988) reported that EC-191203 was resistant while EC-191030, EC-191177 and EC-191021 were moderately resistant out of 40 collections screened under Solan conditions in Himachal Pradesh. Whereas, Janavon as resistant source and Early Winter Adam White as moderately resistant source to stalk rot were observed by Baswana *et al.* (1990). Singh and Kalda (1995) screened 69 lines of cauliflower at seedling stage and reported that 4 winter cultivars, Janavon, EC103576, EWAW and EC177283 as resistant. Sharma *et al.* (1995) reported that among 75 lines tested for resistance, Early Winter Adam White Head was highly resistant to Sclerotinia rot and downy mildew, whereas EC162587 was highly resistant to Sclerotinia rot and black rot while RSK 1301 and MRS-1 were moderately resistant. Sharma *et al.* (2000) evaluated thirty four genotypes of cauliflower against stalk rot under artificial epiphytotic conditions and showed lines SN-445, RSK, RSK-1301, BR, KN-81, KM-1, KM-1xKJ-38, RSK-1301xKJ-38 to be resistant. Thakur *et al.* (2001) screened the available germplasm of late group cauliflower for resistance against stalk rot (*S. sclerotiorum*) and revealed that Janavon and RSK-1301 were resistant to stalk rot.

MATERIALS AND METHODS

Chapter-3

MATERIALS AND METHODS

The present studies were carried out in the Department of Mycology and Plant Pathology of Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) during 2004-05, the area is situated at an elevation of 1250 meters above mean sea level between 30°50'45" North latitude and 70°08'50" East longitude. During research work, *in vitro* experiments were conducted in the laboratories of Department of Mycology and Plant Pathology and Vegetable Crops, while field experiments were laid out at experimental farm of Department of Vegetable Crops. For survey of the disease, different locations in districts of Solan and Sirmaur of Himachal Pradesh were surveyed to record Sclerotinia rot incidence on cauliflower.

Glassware

Petri plates 90 mm diameter, test tubes (20 x 145 mm), microscopic glass slides (7 x 2 mm), Erlenmeyer flasks of 100, 250, 500 ml; beakers of 250, 500 ml and pipettes of 1, 2, 5, and 10 ml capacity were used during the course of research work. The glassware used were Borosil mark.

Equipments, apparatus and other materials

Weighing of chemicals was done on a single pan electrical balance. Different materials tested against the pathogen were incubated in BOD incubators. Mixer and grinder were used for grinding of different plant materials. Vertical laminar airflow chamber was used for aseptic culturing of test fungus, bacterium, plant extracts, etc. Autoclave was used for sterilization of liquid and solid media. Hot air oven was used for sterilization of glassware. Earthen pots of 12 cm and 25 cm diameter were used for raising the crop. Dial type digital thermometer was used to record soil temperature. Plant botanical materials used as extracts against the pathogen were procured locally either from herbal garden of the university or brought from the market. Antagonistic microorganisms used in various experiments were isolated from infested soil and sclerotia of *Sclerotinia sclerotiorum*. The culture of these microorganisms were also collected from other sources i.e. Division of Microbiology, IARI and Vegetable Pathology Laboratory, Department of Mycology and Plant Pathology, UHF, Nauni. Other materials such as tray, chamber, sterilized sand and soil were also used for the study of sclerotial germination.

Sterilization

The sterilization of various liquid and solid culture media and water were done by autoclaving at 1.05 Kg cm^{-2} pressure for 20 minutes. Hot air oven was used for sterilization of glassware at 180°C for two hours and drying soil samples at 105°C for 24 hours. All the growth media like corn sand meal; wheat bran-saw dust as well as soil and sand were sterilized for one hour for 2 consecutive days. Disease samples used for inoculation on PDA medium were sterilized by mercuric chloride (0.1%) for 15 seconds and then washed thoroughly three times with sterilized distilled water. Soil used in the present studies was sterilized by 40 per cent formaldehyde diluted with water in 1: 7 ratio and covered with polyethylene sheet for 7 days, followed by 10 to 12 days exposure to atmosphere for eliminating the fumes of formaldehyde. All *in vitro* experiments were carried out under aseptic conditions using laminar air flow chamber and flame for sterilizing inoculating needle.

The studies using different methodologies and techniques adopted during the course of the research work have been categorized under the following heads:

3.1 Survey and surveillance

3.2 Isolation and identification

3.3 Morphological, cultural and physiological studies

3.4 Role of microclimatic conditions

3.5 Eco-friendly disease management

3.1 Survey and Surveillance

Cauliflower in different localities of Himachal Pradesh, i.e. Nauni, Saproon, Chambaghat, Kandaghat in Solan district and Rajgarh, Matnali in Sirmour district were surveyed in the months of December to February during the year 2004-05.

During the course of survey, the diseased plants were collected and stored in alkathene bags in laboratory at room temperature for immediate use and were stored in a refrigerator at 5°C for later use. Similarly, incidence of Sclerotinia rot and symptoms of the disease o.: various parts of the cauliflower plants were also recorded. The observations were recorded on per cent mortality basis as follows:

$$\text{Disease Incidence (\%)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

3.2 Isolation and Identification

3.2.1 Isolation and identification

Isolations were taken from diseased leaves and stalks of cauliflower collected during the season on potato dextrose agar (PDA) medium. Small bits of 2-3 mm size were cut from the junction of diseased and healthy tissues with the help of a sterilized scalpel. The bits were surface sterilized by dipping in 0.1 per cent mercuric chloride solution for 15 seconds and then washed 3 times in sterilized distilled water. These bits were then placed on a sterilized filter paper to remove excess of moisture. Finally, the bits were transferred to potato dextrose agar slants in culture tubes and also in Petri plates under aseptic conditions, and incubated at 25±1°C. Cultures of the fungus were also obtained from cut pieces of sclerotia sticking to diseased cauliflower plants by wiping out their surface with rectified spirit. Cultures so obtained were purified by single hyphal tip method, identified and maintained on potato dextrose agar medium at 4±1°C. Stock culture was subcultured at intervals of 20-25 days.

3.2.2 Proving Koch's postulates

An experiment was conducted to prove the association of the isolated fungus with the disease. The pathogen was subjected to pathogenicity tests in trays on moist blotters and by making the soil sick to the pathogen.

3.2.2.1 Pathogenicity tests in moisture chamber

Pathogenicity tests were conducted under moisture chamber. Healthy leaves, petioles, stalks and curds of cauliflower Pusa Snow Ball K-1 were thoroughly sterilized with alcohol (95%) and placed in a chamber containing blotting paper, which was already moistened with sterilized distilled water. These plant parts were inoculated by placing a small piece (2-3 mm) of 5 days old culture of *S. sclerotiorum*. The leaves, petioles, stalks and curds without inoculation served as control. The chamber was then covered with polythene sheet and regularly moistened with sterilized distilled water (20 ml for one irrigation) to maintain 90 per cent humidity inside. The leaves, petioles, stalks and curds were observed everyday for the development of symptoms of the disease after inoculation and Koch's postulates were established by re-isolating the fungus from such diseased leaves, petioles, stalks and curds and confirmed it with the original culture.

3.2.2.2 Pathogenicity tests by in-soil method

Pathogenicity was conducted under pot experiments by making the soil sick. Healthy seedlings of cauliflower Pusa Snowball K-1 were raised from seeds sterilized with 0.1 per cent mercuric chloride solution and then sown in seedbed. After one and half month when plants were 10 to 12 cm high above ground level, the seedlings were planted in earthen pots (25 cm diameter) containing 3 kg sterilized soil. Three plants were maintained in each pot and three pots were kept for each treatment. When the seedlings were well established, these were inoculated by making the soil sick with the pathogen. The inoculation was done with 10 days old culture of *S. sclerotiorum* multiplied on corn sand meal (CSM) medium as suggested by Dohroo (1988). The soil was inoculated by adding culture of the pathogen 1 cm deep in soil at the collar region and 1 cm away from the seedlings. The quantities of mass culture inoculated were 50, 125, 250, and 500 mg per seedling. To maintain high soil moisture, sterilized dried organic matter was kept above soil surface and irrigations were given after every 2 days. The control pots were also simultaneously maintained without having the test fungus. Each treatment was replicated thrice. Observations on disease incidence after inoculation were recorded at weekly intervals till five weeks. Koch's postulates were established by re-isolating the fungus from such diseased plants and confirmed it with the original culture.

3.3 Morphological, Cultural and Physiological Studies

3.3.1 Morphological study

Observations on colour and size of the mycelium and conidia were recorded by growing fungus *in vitro* on potato dextrose agar medium and sclerotial germination on sand for apothecia, asci and ascospores.

3.3.1.1 Myceliogenic study

Morphological characters (shape and size) of mycelium and conidia of the pathogen were studied while growing it on PDA medium. The PDA was prepared and sterilized at 1.05 kg cm^{-2} for 20 minutes in an autoclave, cooled and was poured in 90 mm Petri plates (30 ml for each plate) under aseptic conditions. The poured Petri plates were inoculated with a uniform disc (5 mm diameter) of 5 days old culture of the pathogen at the centre of Petri plates. The inoculated Petri plates were incubated at 25±1°C and observed for morphological characters.

3.3.1.2 Carpogenic study

To see the carpogenic germination of sclerotia in moist sand in Petri plates, clean sand was sterilized for one hour in autoclave at 1.05 kg /cm^{-2} for two consecutive days. Sixty gram of sterilized sand was spread in each Petri plate (9 cm diameter by 1.5 cm depth). Five sclerotia per Petri plate were buried in sand and moistened with sterilized distilled water regularly and kept for carpogenic germination at room temperature (10±5°C). Observations on mycelial growth from sclerotia and apothecia formation were made regularly.

Carpogenic germination of sclerotia was also studied in sterilized trays (12 cm x 48 cm size) filled with 500 g sterilized sand. One hundred sclerotia were placed in sand and pressed to embed the sclerotia. The sand was moistened with sterilized distilled water and kept at room temperature (10±5°C). Observations on sclerotia forming apothecia, asci and ascospores were made and the size of each was also measured using micro-scale.

3.3.2 Cultural studies

Different solid and liquid media were tested to find out the medium sustaining maximum growth of the fungus.

3.3.2.1 Solid media

Six solid media i.e., potato dextrose agar, corn meal agar, French bean seed agar, oat meal agar, pea seed agar, lettuce leaf agar were prepared (Appendix I), filtered through two layers of muslin cloth and autoclaved at 1.05 kg cm^{-2} pressure for twenty minutes. The pH of each medium was adjusted at pH 5.0 prior to autoclaving. Uniform quantities (30 ml) of each medium were poured in 90 mm Petri plates. Each Petri plate was inoculated separately with uniform culture bits (5 mm) cut from young (5 days) vigorously growing culture and incubated at 25±1°C. Each treatment was replicated four times. Observations on radial growth of mycelium, number of days taken by mycelium to fill Petri plate and average number of sclerotia formed per Petri plate were recorded.

3.3.2.1 Liquid media

Five liquid media i.e., Richard solution, Asthana and Hawker medium, Czapek sucrose nitrate solution, Leonian solution, Corn liquid medium were prepared (Appendix II). The pH in each case was also adjusted at 5.0 before autoclaving. Each medium (30 ml) was poured separately in 150 ml Erlemeyer flasks, plugged with non-absorbent cotton and sterilized in an autoclave at 1.05 kg cm^{-2} for 20 minutes. Each flask was inoculated separately with uniform quantity of homogenous culture suspension (1 ml) prepared by triturating mycelial mat of one flask grown on Richard solution. The inoculated flasks were incubated at 25±1°C for 15 days. Thereafter, the mycelial contents were filtered out through already weighed Whatman No 1 filter papers and constant dry weight of the mycelial mat from each flask was recorded after drying at 75°C in a hot air oven for 24 hours. Each treatment was replicated four times and data were recorded on dry weight of mycelium and sclerotia as well as average number of sclerotia formed per flask.

3.3.3 Physiological Studies

3.3.3.1 Effect of temperature on growth of *S. sclerotiorum*

Petri plates containing uniform quantities (30 ml) of sterilized PDA medium were inoculated with 5-day old uniform culture bits (5 mm diameter) of the pathogen (*S. sclerotiorum*) and incubated at different temperatures i.e., 5, 10, 15, 20, 25 and 30°C. Each treatment was replicated four times and data were recorded on radial growth of mycelium, number of days taken by mycelium to fill Petri plate and average number of sclerotia formed per Petri plate.

3.3.3.2 Effect of pH on growth of *S. sclerotiorum*

Richard medium was used as a basal medium for finding out the pH requirement of the fungus. Different pH levels i.e., 4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 were maintained. For ino^ulation, the same procedure was done as in case of liquid media. The experiment was designed in a CRD with eight treatments, which were replicated four times. Data on dry weight of mycelium and sclerotia as well as average number of sclerotia formed per flask were recorded.

3.4 Role of Microclimatic Conditions

3.4.1 Disease incidence

In order to find out the role of soil hydrothermal regimes on the disease development, incidence of Sclerotinia rot of cauliflower was recorded at seven days interval at experimental block of Department of Vegetable Crops, Nauni during crop season 2004-05 starting from 20 days after transplanting till harvesting of the crop. Simultaneously, soil hydrothermal regimes i.e., soil temperature and soil moisture were also recorded for the intervening period.

3.4.2 Soil moisture

Soil moisture was determined by collecting twenty random soil samples from the rhizosphere (10 cm depth) of cauliflower in the field. These soil samples were carefully placed in containers (aluminum boxes) to avoid evaporation. The fresh weights of soil samples were taken with the help of electronic balance. Soil moisture was determined by the differences between the fresh weights of soil with dry weights of soil after drying in hot air oven at 105°C for 24 h by the following formula:

$$\text{Per cent soil moisture} = \frac{\text{Fresh weight of soil - Dry weight of soil}}{\text{Dry weight of soil}} \times 100$$

The mean of per cent moisture content of twenty soil samples was worked out to represent the average soil moisture in the field.

3.4.3 Soil temperature

Soil temperature of rhizosphere was recorded after every seven days intervals with dial type digital thermometer at 10 cm depths to study effect of soil temperature on disease incidence. Three soil thermometers were inserted at root zone depth in the field at suitable sites for recording soil temperature. Sixty readings were taken and the mean of these readings was worked out which represented the average of soil temperature in the cauliflower field.

3.4.4 Data analysis

The data obtained on disease incidence of Sclerotinia rot, soil moisture, soil temperature were subjected to statistical analysis to find out the simple, partial and multiple correlations, if any, between Sclerotinia rot and two independent factors viz., soil moisture and soil temperature (Gomez and Gomez, 1983).

3.5 Disease Management

3.5.1 Isolation of antagonists/ mycoparasites from sclerotia and infested soil

3.5.1.1 Isolation from sclerotia

Sclerotia of *S. sclerotiorum* were collected from six locations i.e. Nauni, Saproon, Chambaghat and Kandaghat in district Solan and Rajgarh and Matnali in district Sirmour of Himachal Pradesh. These were washed thoroughly with sterilized water and plated on Potato Dextrose Agar (PDA) medium. Data on sclerotia contaminated or infected with antagonists/ other microflora as well as non-viable sclerotia were recorded after 5 days of incubation at 25±1°C. Pure cultures of the isolated microflora were established and identified wherever possible as per Gilman (1957).

3.5.1.2 Isolation from infested soil

Soil samples were collected from *S. sclerotiorum* infested cauliflower fields from five locations in Solan and Sirmaur districts. Isolation of microflora was done by soil dilution method given by Johnson (1957).

One gram of soil from each sample was sieved (2 mm mesh) and was added in 9 ml of sterilized distilled water. The suspension was homogenously mixed with the help of magnetic stirrer and was diluted to 10^{-4} by subsequent dilutions. Rose Bengal Agar (Martin media) was used as the plating medium. Antibiotic streptomycin was added to the medium (3 µg/l) after autoclaving and just before pouring. Approximately 30 ml of the media was poured into each Petri plate. The suspensions (0.1ml) of soil sample were dropped on poured plates using micropipette and carefully spread on the solidified surface with the help of sterilized L-shape glass rod. Three replications were maintained for each soil sample. The inoculated plates were later incubated in BOD incubator at 25±1°C and observations of any growth of the fungus were made after every 24 hours. The cultures so obtained were maintained in slants of PDA medium and kept in refrigerator. Pure cultures were established from the isolated microflora and identified as per Gilman (1957).

3.5.2 *In vitro* screening of antagonists against the test pathogen

3.5.2.1 Dual culture method

In order to evaluate the antagonistic activity of isolated soil microflora against *S. sclerotiorum* (test pathogen), small culture discs (5 mm diameter) of the potential antagonists (*Trichoderma viride, T. harzianum, T. hamatum, T. polysporum, Torula* sp., *Penicillium* sp., *T. virens, Paecilomyces lilacinus, Aspergillus niger, Fusarium* sp., *Laetisaria arvalis, T. longibrachiatum, Bacillus subtilis, Pseudomonas fluorescens,* unidentified actinomycetes) and the test pathogen (*S. sclerotiorum*) were taken from the margins of vigorously growing colonies and transferred to 90 mm PDA culture plates at two opposite sides. The pathogen being slow growing was inoculated two days earlier to the antagonist in PDA plates (Singh, 1998) and distance between the inoculation points of *S. sclerotiorum* and the antagonist was about 5 cm (Huang and Hoes, 1976). The disc containing culture of the pathogen alone served as control. The cultures were then incubated at 25±1°C in BOD incubator and each treatment was replicated four times. Radial growths of the potential antagonists and the test pathogen (*S. sclerotiorum*) were recorded after 24 hour intervals. The growth inhibition of the test pathogen over control was calculated according to Vincent (1947). Subsequently, plates were also examined for the formation of inhibition zone, if any.

To see the antagonistic activity of the bacteria, streak plate method given by (Huang *et al.*, 1993) was adopted. The Petri plates containing sterilized PDA were streaked with 48 hours old colonies of bacteria with the help of bacterial loop and mycelial bit (5 mm diameter) of the test pathogen was placed on opposite sides at a distance approximately 3.5 cm from the streak. Petri plates without bacterial streak served as control for comparison. Each treatment was replicated four times and the Petri plates were incubated at 25±1°C. Radial growth of the test pathogen (*S. sclerotiorum*) was recorded after 24 hours and per cent inhibition was calculated according to Vincent (1947).

3.5.2.2 Well method

The antagonistic activity of isolated microflora was also tested using the well method (Singh and Webster, 1973). Liquid cultures of isolated microflora were raised separately in 150 ml Erlenmeyer flasks, each containing 30 ml of potato dextrose broth, while the actinomycetes culture was raised on KenKnight medium. These cultures were incubated at 25±1°C for 5 and 7 days, respectively. The culture filtrates were obtained by filtration through Whatman No 1 filter paper. The filtrates were then sterilized by autoclaving at 1.05 kg cm^{-2} for 20 minutes and then tested for their antagonistic activity against *S. sclerotiorum*. For this purpose, four wells, 6 mm diameter, were made in the sterilized PDA layer in Petri plates (90 mm diameter), at four corners with the help of a sterilized cork borer. The approximate thickness of medium was kept 5 mm. The culture filtrate (0.5 ml per wells) of respective fungus and actinomycetes were poured in the wells with the help of a sterilized pipette. The checks having only uninoculated sterilized liquid medium in the wells were also maintained. Finally, a culture disc, 6 mm diameter of the test pathogen (*S. sclerotiorum*) taken from the margin of a vigorously growing culture was placed over the well of each prepared plate to cover the well. Each treatment was replicated four times. Observations on the radial growth of the test pathogen were recorded regularly after 24 hours.

3.5.3 *In vitro* evaluation of botanicals and biopesticide against *S. sclerotiorum*

3.5.3.1 Plants used for botanical extracts

Extracts of different plant species viz., agave (*Agave americana*), broccoli (*Brassica oleracea* var. *italica*), eucalyptus (*Eucalyptus globulus*), tulsi (*Ocimum* *sanctum*), neem (*Azadirachta indica*), pine (*Pinus roxburghii*), ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), chilli (*Capsicum frutescens*), sarson (*Brassica campestris*) and garlic (*Allium sativum*) belonging to different families were tested for their antifungal activity against *S. sclerotiorum* by poisoned food technique as per method given by Verma and Dohroo (2003).

3.5.3.2 Preparation of plant extracts

The extracts of different plant parts as mentioned above were prepared by macerating their tissues in distilled water, on weight/volume ratio. In all the cases, 200 g of the plant material was washed under the tap water, macerated in mortar with a pestle for 5 min by adding small quantity of sterilized distilled water and homogenized in an electric blender, with known volume (100 ml) of the distilled water. The homogenate was filtered out through double layers of muslin cloth and later by Whatman No 1 filter paper. The resultant extract were considered as 200 per cent concentration and kept in a refrigerator till further use.

3.5.3.3 Evaluation of botanical extracts and biopesticide

The plant extracts were diluted to requisite concentrations with sterilized distilled water and were evaluated against the test pathogen by poisoned food technique. For this purpose, the botanical extracts were first tested at 100 and 50 per cent concentration and later the promising ones were further evaluated at lower concentrations *i.e.* 10, 5, 2.50, 1.50, 0.75 and 0.25 per cent. To evaluate extracts at 100 per cent concentration, 50 ml of extracted botanical (200%) were mixed with 50 ml double strength potato dextrose agar (PDA) medium (ratio of 1:1) in conical flasks before autoclaving and then sterilized by autoclaving at 1.05 kg cm^{-2} for 20 minutes. Autoclaved poisoned food was poured in sterilized Petri plates (90 cm) and inoculated with 5 mm disc of 5 days old culture. The Petri plates were incubated at $25\pm1^{\circ}C$ to study the inhibitory effect of botanicals on the mycelial growth of *S. sclerotiorum*. Medium without plant extract served as checks. Each treatment was replicated thrice. The data on radial growth (mm) of the fungus were recorded by measuring the colony diameter at 24 hr interval and per cent inhibition was also calculated (Vincent, 1947).

$$\text{Inhibition (\%)} = \frac{C - T}{T} \times 100$$

Where C: mycelium growth (mm) in control

T: mycelium growth (mm) in botanical treatment

3.5.4 Field Experiment

3.5.4.1 Preparation of field trial

Field experiment was laid out to study the effect of potential biocontrol agents on incidence of Sclerotinia rot, plant frame, stalk length, number of leaves, gross curd weight, net curd weight and curd colour. The field study was carried at the farm of Department of Vegetable Crops, showing high incidence of the disease during the previous years with Cauliflower Pusa Snowball K-1. The 40-day old seedlings were transplanted in plots (4 x 5 m size) with a proper spacing (60 x 45 cm). The experiment was designed in RBD with five treatments including the check replicated four times.

3.5.4.2 Preparation of mass culture of antagonists

3.5.4.2.1 Preparation of mass culture of fungal antagonists

Wheat bran and saw dust medium was prepared after thoroughly mixing of wheat bran in saw dust (3:1 w/w) and 2 per cent of sucrose. It was put in autoclavable polypropylene bags (200 g each bag) and plugged with non-absorbent cotton. These bags were sterilized at 1.05 kg cm^{-2} for one hour for two consecutive days. Autoclaved bags were kept in room temperature for 1-2 days in order to reduce moisture inside and then inoculated with small cut pieces (2 mm size) of potential antagonists (*T. viride* and *T. harzianum*). After inoculation, these bags were incubated at 25±1°C for 10 days.

3.5.4.2.2 Preparation of mass culture of actinomycetes

A culture of unidentified actinomycetes was prepared in KenKnight medium. A loop (3 mm diameter) of 5 days old culture of actinomycetes was added to 100 ml sterilized KenKnight medium in 250 ml conical flask under aseptic conditions. These flasks were then incubated at 25±1°C in BOD incubator for four days. After five days of incubation, the flasks were kept in orbital shaker-cum incubator at 25±1°C for 36 hours. The culture was then carried out for field application (10^8 c.f.u. /ml).

3.5.4.3 Field application of biocontrol agents

In a Randomized Block Design experiment with four replications, biocontrol agents and neem formulation were applied as root dip treatment (15 minutes) before transplanting of seedlings and soil application after 30 days of transplanting. 10 ml culture of unidentified actinomycetes was added to 1000 ml of water and the suspension was used for root dip treatment. The roots of cauliflower seedlings were dipped upto collar portion. The same concentration was applied for soil drench (50 ml suspension per plant). Similarly, 0.75 per cent concentration of neem treatment was also used for root dip and soil drench. For fungal antagonist application, 10 g of each antagonist (*T. viride* and *T. harzianum*) was added in 1000 ml of water for root dip treatment (1%) of cauliflower seedlings for 15 minutes before transplanting. These antagonists were also used for soil application (5 g per plant).

3.5.4.4 Data observation

Observations were made on disease incidence, plant frame, stalk length, number of leaves, gross curd weight, net curd weight and curd colour and data were recorded.

3.5.5 Varietal screening

Reaction of five varieties of cauliflower such as Cauliflower II VRMC-12, Cauliflower DC-76, Cauliflower KT-22, Cauliflower KT-08, Cauliflower PSBK-25 under artificial epiphytotic conditions was studied to record the source of resistance, if any. Screening of the germplasm was done by artificially inoculating the plants with the pathogen. The inoculation was done with 10-day old culture of *S. sclerotiorum* multiplied on corn sand meal (CSM) medium as suggested by Dohroo (1988).

3.5.5.1 Growing of seedlings for inoculation

The seedlings of different varieties were first raised in the seedbed on sterilized soil, under optimum conditions of water and nutrient supply. After one and half month, when plants were ten to twelve cm high above ground level, three seedlings were planted in each earthen pot (12 cm diameter), containing 250 g of sterilized soil. Before transplantation, seedlings were dipped into 0.1% solution of mercuric chloride. After a week of transplantation when the seedlings were well maintained, were inoculated with 10-day old culture of *S. sclerotiorum* multiplied on corn sand meal (CSM) medium.

3.5.5.2 Preparation of mass culture of pathogen

The pathogen was mass cultured on corn sand meal (CSM) medium. The crushed maize grains were boiled just to get it soft. Excess of water was drained out and corn grains were air-dried. After that, thoroughly mixing of ground boiled corn in sand was made (1:2 w/w) along with 2 per cent sucrose. The medium thus prepared was put in heat resistant propylene bags (200 g each bag) and plugged with non-absorbent cotton. These bags were sterilized at 1.05 kg cm^{-2} for one hour for two consecutive days. Autoclaved bags were kept at room temperature for 1-2 days in order to reduced moisture inside. The sterilized growth medium was inoculated under aseptic conditions with five days old culture of *S. sclerotiorum*. Three bits of 2 mm size of *S. sclerotiorum* were placed in different sides of each bag. The inoculated bags were incubated at 25±1°C for 10 days and used for various experiments.

3.5.5.3 Inoculation of mass culture pathogen

Five varieties of cauliflower were inoculated by mixing the culture of *S. sclerotiorum* in soil at 1 cm depth and 1 cm away from the plant. The culture was inoculated @ 250 gm per seedling. To maintain high soil moisture, irrigations were provided after every two days. The observations on disease symptoms and disease incidence were recorded after 7, 14, and 21 days of inoculation.

3.5.5.4 Disease reaction

The final disease incidence figures were worked out after taking the mean of all the readings and the genotypes were grouped into four categories (Dohroo, 1988; Thakur *et al.*, 2001).

- Immune = No infection
- Resistant =< 10 % infection
- Moderately Resistant = 11 – 20 % infection
- Susceptible = 21 – 40 % infection
- Highly susceptible => 40 % infection

3.6 Data management and analysis

The data recorded from various laboratory and field experiments were subjected to statistical analysis, wherever, necessary. The differences exhibited by treatments in various experiments were tested for their significance, using standard procedure as described by Gomez and Gomez (1983).

EXPERIMENTAL RESULTS

Chapter-4

EXPERIMENTAL RESULTS

4.1 Survey and Surveillance

A general survey was conducted in the months of December, January and February 2004-05 in order to assess the incidence as well as symptoms of Sclerotinia rot of cauliflower in the field in various parts of Himachal Pradesh. The disease incidence was calculated by taking 100 plants at random in a field and counting the number of diseased plants.

4.1.1 Symptomatology

The symptoms of disease (Sclerotinia rot) on different parts of cauliflower were recorded and divided into three categories as follows:

Symptoms on leaves

The symptom of disease was observed initially on leaves in the month of December, which continued upto the end of January or beginning of February. First of all, leaves of the infected plants lost their turgor during the day and drooped down to the ground, but regained turgidity during night or early mornings. The bright green sheen of such affected leaves was replaced with dull whitish green colour, ultimately becoming pale yellow. The yellowing started from the tip of older leaves downwards till the whole leaf was involved; such affected leaves were prematurely shed and started falling out from February onwards. In most of the cases, mid-rib of the leaves at a point touching the soil showed dark brown to black rotting which was covered in most of the cases with growth of extra-matrical fungus under cool and humid weather.

Symptoms on the stalk

Petioles of the basal leaves started rotting as a result of infection by the fungus. Affected portions were dark brown to black, watery with white cottony tuft of mycelium on the surface. With the advancement of the fungus towards the stalk, dark brown to black spots were produced on the stalk, which enlarged in size girdling whole of the stem at the ground level. Thereafter, the rot progressed towards the curd and occasionally whole of the pith upto the point of attachment of the curd was completely found rotten. In later stages, the pith gave way to large cavities lined inside with fluffy mycelium of the causal fungus. In wet weather, the mycelium of the fungus could also be seen in the affected portions of the plant. Sclerotia were found sticking both inside the hollow portion of the pith as well as in fluffy mycelium outside the affected portion of the plant.

Symptoms on the curd

With the progress of the disease, curds were also found affected. The affected curds showed brown to dark brown rotting, which started from any portion of the curd, but generally from the peduncle. The rot progressed fast under cool humid weather engulfing whole of the curd in most cases. The tissue became soft as well as mushy and sclerotia were found sticking in between them.

4.1.2 Incidence of Sclerotinia rot

Incidence of Sclerotinia rot of cauliflower was recorded during the cropping season of 2004-2005 in different cauliflower growing areas of two districts of Himachal Pradesh (Table 4.1).

Table 4.1 Disease incidence of Sclerotinia rot of cauliflower in Himachal Pradesh

District	Locality	Disease incidence (%)	Average (%)
Solan	Chambaghat	70.30	55.36
	Kandaghat	29.54	
	Nauni	64.39	
	Saproon	57.23	
Sirmour	Matnali	65.37	69.32
	Rajgarh	73.26	

The disease was found to be serious in all the locations surveyed. Maximum incidence (73.26%) of the disease was recorded in Rajgarh followed by Matnali (65.37%) in district Sirmour, while the incidence was least (29.54%) at Kandaghat in district Solan. The data also indicate that the locations surveyed in the district of Sirmour had higher incidence of the Sclerotinia rot ranging from 65.37 to 73.26, with average of 69.32 per cent in comparison to Solan district with average disease incidence of 55.36 per cent. Over all, the incidence of disease ranged between 29.54 to 73.26 per cent in different locations that was detrimental for the production of quality curds and seed of the crop. This necessitated the need for exploring new innovative approaches for the management of the disease.

4.2 Isolation and Identification

4.2.1 Isolation of the pathogen

Isolations of the pathogen associated with Sclerotinia rot disease were made from infected plant parts like stalks, curds and leaves, on potato dextrose agar (PDA) medium using standard technique (3.2.1). The pathogen was cultured on the PDA by taking the smaller bits of the host tissues from the peripheral regions of the affected plant parts, where infected area joined with the healthy tissues. In majority of the bits cultured from stalks, curds and leaves, the fungus protended out of the infected tissues into the medium. The isolated fungus was then purified by hyphal tip method on the plates containing PDA. Purity and virulence of the isolated fungus was maintained by sub-culturing the fungus after every 20 to 25 days.

4.2.2 Identification of the pathogen

Diseased samples (3.1), comprising of rotten plant parts of cauliflower were examined by microscopic examination of sections of infected tissues. The culture of the Sclerotinia rot pathogen (3.2.1) was examined for morphological and cultural characteristics as well as sclerotial formation on PDA medium (4.3) after 24 hours interval on the basis of morphological characters documented in taxonomic keys (Willetts and Wong, 1980) and the identity of the fungus was confirmed as *Sclerotinia sclerotiorum* (Lib.) de Bary.

The fungus produced aerial mycelium, which was hyaline, well developed and appeared cottony. The fungus took 4-5 days to fully occupy the Petri plate. The sclerotia were formed after 5 days of inoculation at the periphery or in the middle of the colony forming concentric rings. Sclerotia were first white in colour and then turned black with round or oval to irregular in shape and black in colour.

4.2.3 Proving Koch's postulates

In order to prove the Koch's postulates, the fungus was subjected to inoculation directly on leaves, petioles, stems and curds of cauliflower and incubated in moist chamber under room temperature (20±5°C) and by making the soil sick. The disease symptoms which appeared after inoculation were recorded. The fungus was then re-isolated from such infected plant parts and was found to be identical in all respects to the original fungus inoculated i.e. *S. sclerotiorum*.

4.2.3.1 Pathogenicity test in-moisture chamber

Different parts of cauliflower plants i.e. leaves, petioles, stems and curds were inoculated with *S. sclerotiorum* and incubated in moist chamber to evaluate parts of cauliflower susceptible to Sclerotinia rot and establish Koch's postulates. The incubation period in inoculated leaves, petioles and stems were found to be 48 hours, except curds and old leaves. Young leaves were found most susceptible with 100 per cent infection on inoculated leaves. White cottony growth of the fungus appeared on infected surface after 5 days of inoculation followed by complete rotting after 8 days of inoculation with lesion size of 0.9-12.00 cm. Petioles were found next susceptible to the fungus with 100 per cent infection and symptoms appeared as water soaked lesions which were brown to black in colour. White cottony growth also appeared on infected portion after 6 days of inoculation with lesion size of 0.4-8.50 cm on injured and 0.3-2.50 cm on un-injured petioles. Symptoms on curds appeared as water soaked areas 0.75-6.70 cm resulting in rot with white cottony mycelium on infected surface. Similar symptoms were formed on stem with lesion size of 0.4-4.5 cm on injured and 0.55-1.3 cm on un-injured part. There was delay in symptom development on un-injured plant parts. Old leaves were, however, least susceptible to Sclerotinia rot, with minimum lesion size (0.28-4.50 cm). The infected plant parts of cauliflower were also subjected to re-isolation and confirmed with original culture to establish Koch's postulates.

4.2.3.2 Pathogenicity test by in-soil method

To prove Koch's postulates by making the soil sick, healthy seedlings of cauliflower Pusa Snowball K-1 were transplanted in pots and inoculated by making the soil sick with culture of the pathogen (3.5.5.2) at 1 cm depth surrounding the

A

B

C

D

E

F

Plate 1: Proving Koch's Postulates

(A) Young leaf inoculation (B) Petiole inoculation
(C) Curd inoculation (D) Stem inoculation
(E & F) Inoculation by in-soil method

collar region i.e. 1 cm away from the seedlings. The quantities of the culture inoculated were 50, 125, 250, and 500 mg per seedling. The control treatments were also simultaneously maintained without having the pathogen. The inoculated plants were watered regularly with sterilized water and were kept under gcreen house conditions. After 14 days of inoculation, symptoms began to appear when older leaves lost their sheen and bright green colour and started yellowing from tip downwards. On the stem of such plants, water soaked lesions near the ground level appeared which gradually girdled the whole stem. Cottony white hyphal strands were also observed growing over the affected area of the stem. The data on disease incidence after different dose inoculation showed that, application of 250 and 500 mg of mass culture of the pathogen gave maximum diseased incidence (100%) at 21 and 28 days after inoculation, whereas, application of 50 mg gave only (66.66%) disease incidence after 28 days of inoculation. The infected plants in different treatments were also subjected to re-isolation and confirmed with original culture to establish Koch's postulates.

Table 4.3 Effect of different inoculation doses of the mass culture pathogen on disease incidence of Sclerotinia rot of cauliflower

Dose (mg)	Disease incidence (%) after days		
	14	21	28
50	11.11	44.44	66.66
125	44.44	77.77	88.88
250	66.66	100	100
500	88.88	100	100
Control	0.00	0.00	0.00

4.3 Morphological, Cultural and Physiological Studies

4.3.1 Morphological

The fungus was grown on potato dextrose agar medium to record the shape and size of the mycelium, conidia and sclerotia while apothecial studies were made by sclerotial germination on moist sand.

4.3.1.1 Myceliogenic and sporogenic characters

Morphological characters (shape and size) of mycelium and conidia of the pathogen were studied by growing of the fungus on PDA medium. The observations

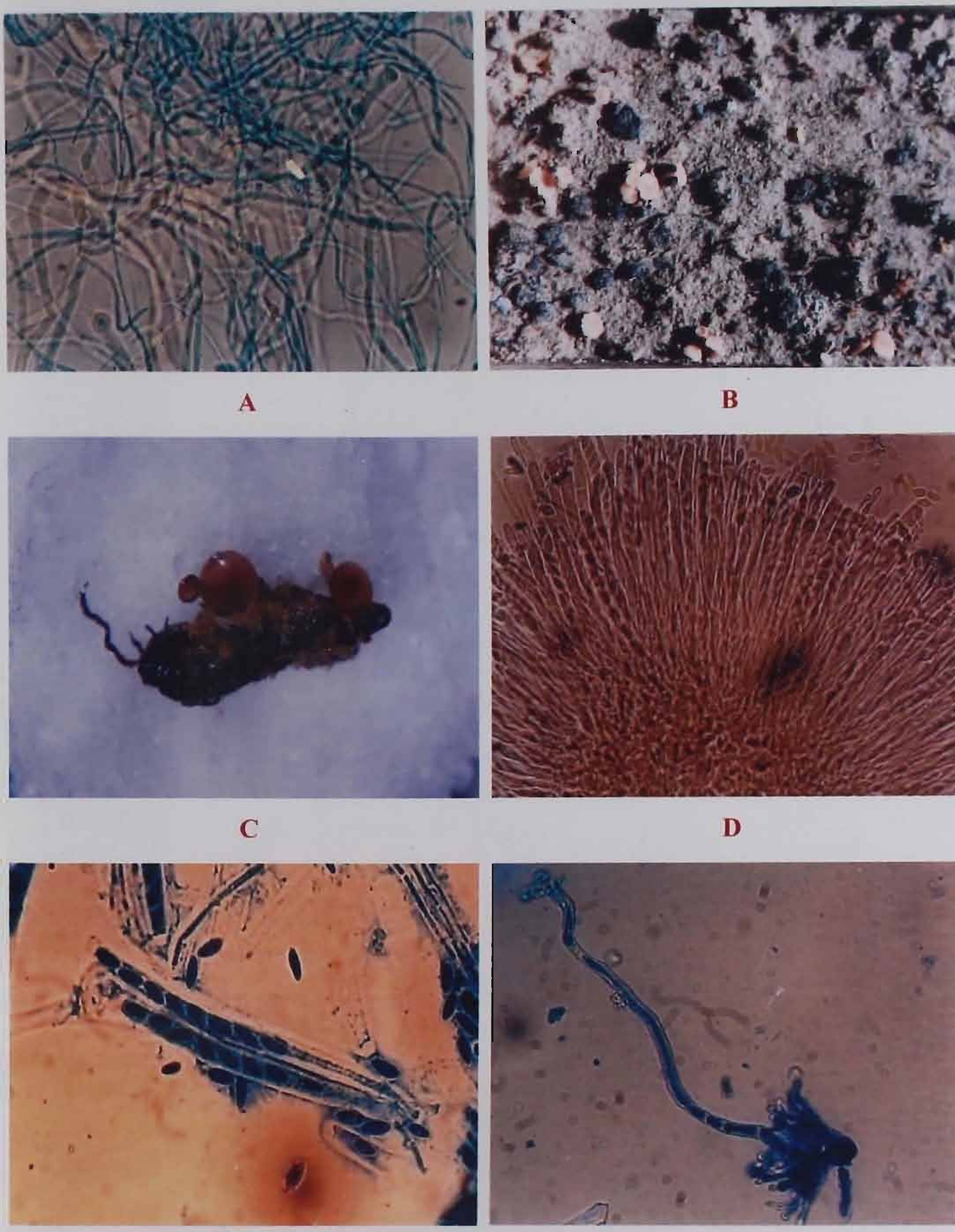


Plate 2: Morphological characters of *Sclerotinia sclerotiorum*

(A) Hyphae (B) Sclerotia germination in moist sand

(C) Sclerotium bearing apothecia (D) Apothecium bearing numerous asci

(E) Asci and released ascospores (F) Micro-conidial stage on myceliogenic and sporogenic characters were presented in Table 4.4. The fungus produced aerial mycelium, which was hyaline, branched, well developed and appeared cottony, consisting of closely septate hyphae which were both inter- and intracellular. The hyphae were 2.0-9.4 µm in width and contained dense granular protoplasm. The culture of the fungus was white to slight greyish in colour on PDA and took 4 days to fully occupy the Petri plate. The fungus does not produce true conidia or macroconidia, however, as the available food supply declined, micro-conidia were found to be produced on sclerotia. The micro-conidia were produced on conidiophores of the vegetative mycelium and measured 1.5-3.5 µm in culture.

4.3.1.2 Carpogenic characters

When the food supply was exhausted and the vegetative growth ceased, the hyphae with thick granular protoplasm and short cells collected in small dense masses, which gradually became sclerotia. The sclerotia were round to irregular in shape in culture and measured from 1.5-7 mm in width and 2-15 mm in length. Their number varied from 22-45 per Petri plate. In the month of November, when room temperatures went down (10-16°C), sclerotia started germination and gave rise to

Table 4.4 Myceliogenic, sporogenic and carpogenic characters of *S. sclerotiorum*

Fungal part	Colour	Number per unit	Shape	Size	
				Width/ diameter	Length
Hyphae	Hyaline	--	Branched, cottony, closely septate	2.0-9.4 µm*	Indeterminate
Micro-conidia	Hyaline	--	Globose	1.5-3.5 µm**	--
Sclerotia	White to black	22-45 (Petri plate)	Round or irregular	1.5-7 mm*	2-15 mm
Apothecia	Brown	1-9 (Sclerotia)	Round or globate	2-9 mm**	5-21mm
Asci	Hyaline	Numerous (Apothecium)	Cylindrical	4.9-8.5 µm*	91-165 µm
Ascospore	Hyaline	8 (Ascus)	Elliptical	4.2-6.6 µm*	6.5-13 µm

* Width; ** Diameter; -- Nil/ Not recorded

several columnar structures (stipes). The stipes later developed funnel shaped cup (apothecium) at the tip. Apothecia were brown in colour and were round or globate type. The length of apothecia measured from 5-21 mm, whereas diameter of the apothecial discs ranged from 2-9 mm with number ranging from 1-9 per sclerotia. The asci were hyaline and cylindrical in shape, which measured 91-165 x 4.9-8.5 µm in size. Each asci contained eight ascospores which were found to be released in clouds. Ascospore formation took place after 48 hours of apothecia formation which were found to be released in smoke upto a height of 6-9 cm from the base. Ascospores were elliptical and ranged from 6.5-13 x 4.2-6.6 µm in size.

4.3.2 Cultural studies

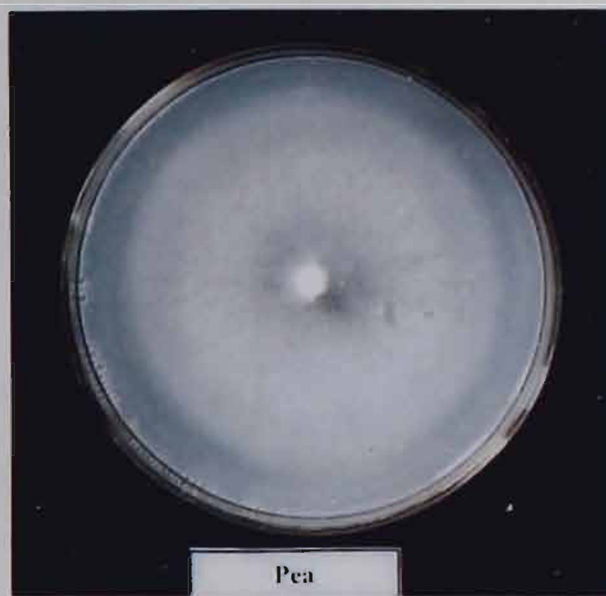
4.3.2.1 Growth of *S. sclerotiorum* on solid media

With a view to select out the best solid medium for growth of the fungus, six solid media were studied. Data regarding the radial growth of mycelium, number of days taken by mycelium to fill Petri plates and average number of sclerotia formed per Petri plate are presented in Table 4.5.

Table 4.5 Mycelial growth of *S. sclerotiorum* and sclerotial development on different solid media

Solid medium	Average radial growth of mycelium after 4 days (mm)	Days to fill the Petri plate	No. of sclerotia formed/ Petri plate (90 mm)
Potato dextrose agar	52.47	4	30.75
Pea seed agar	50.92	4	18.00
French bean seed agar	48.33	4	11.50
Oat meal agar	44.05	5	15.25
Corn meal agar	34.66	6	4.75
Lettuce leaf agar	30.65	7	1.50
SE ±	1.39		1.40
$CD_{(0.05)}$	2.92		2.94

A study of Table 4.5 revealed that growth of the fungus was best achieved on potato dextrose agar (52.47 mm) and pea seed agar (50.92 mm), which were statistically at par with each other. It was followed by French bean seed agar (48.33



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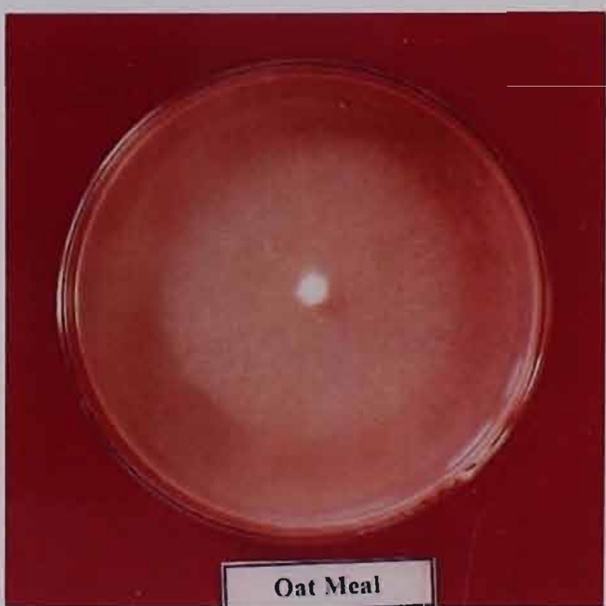

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D

Plate 3: Mycelial growth of *S. sclerotiorum* on solid media (pH 5.0)

(A) PDA medium

(B) Pea seed medium

(C) Oat meal agar medium

(D) Lettuce leaf medium

several *columnar structures* (stipes). The stipes later developed funnel shaped cup (apothecium) at the tip. Apothecia were brown in colour and were round or globate type. The length of apothecia measured from 5-21 mm, whereas diameter of the apothecial discs ranged from 2-9 mm with number ranging from 1-9 per sclerotia. The asci were hyaline and cylindrical in shape, which measured 91-165 x 4.9-8.5 µm in size. Each asci contained eight ascospores which were found to be released in clouds. Ascospore formation took place after 48 hours of apothecia formation which were found to be released *in smoke upto a height of 6-9 cm* from the base. Ascospores were elliptical and ranged from 6.5-13 x 4.2-6.6 µm in size.

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A study of Table 4.5 revealed that growth of the fungus was best achieved on potato dextrose agar (52.47 mm) and pea seed agar (50.92 mm), which were statistically at par with each other. It was followed by French bean seed agar (48.33

mm). Lettuce leaf agar gave the least growth of the fungus, which required 7 days for full growth in the Petri plates, with average radial growth (30.65 mm) after 4 days of inoculation. Similarly, potato dextrose agar was again the best for formation of maximum number of sclerotia (30.75) which started on 5th day in the middle of the culture, followed by pea seed agar (18.00) and oat meal agar (15.33). Lettuce leaf agar again gave rise to very less number of sclerotia, with average number of sclerotia 1.5 per Petri plate.

4.3.2.2 Growth of *S. sclerotiorum* on liquid media

In order to select out the best liquid medium for the growth of *S. sclerotiorum*, five liquid media viz. Richard solution, Czapek sucrose nitrate solution, Leonian solution, Coon liquid medium and Asthana and Hawker medium were tried. The inoculated flasks were incubated at 25±1°C and mycelium mat was filtered out after 15 days of growth to record mycelial dry weight in each case. The results obtained are presented in Table 4.6.

Table 4.6 Mycelial growth of *S. sclerotiorum* and sclerotial development on different liquid media

Liquid medium	Dry weight of mycelium (mg)	No. of sclerotia formed	Dry weight of sclerotia (mg)
Richard solution	242.85	15.00	122.63
Czapek sucrose nitrate solution	222.58	14.00	93.14
Leonian solution	122.15	11.25	76.40
Coon liquid medium	103.10	6.50	34.60
Asthana and Hawker medium	71.90	0.75	0.90
SE ±	16.74	1.76	16.07
$CD_{(0.05)}$	35.68	3.74	34.26

As is evident from Table 4.6, the fungus gave maximum growth on Richard solution (242.85 mg) and Czapek sucrose nitrate solution (222.58 mg) and were non-significant. The growth of fungus was very poor (71.90 mg) on Asthana and Hawker medium. Data on sclerotial formation and dry weight were found to be correlated with mycelium growth and were recorded best in case of Richard solution, followed by

Czapek sucrose nitrate solution. There was very poor sclerotial formation in case of Asthana and Hawker medium.

4.3.3 Physiological studies

4.3.3.1 Effect of temperature on growth of *S. sclerotiorum*

With a aim to find out the effect of different temperatures on the growth of *S. sclerotiorum,* 90 mm Petri plates containimg 30 ml of PDA were inoculated with 5 days old culture of *S. sclerotiorum* and incubated at different temperatures viz. 5, 10, 15, 20, 25, and 30°C. Data on colony diameter, time taken by the fungus to fill Peti plates and sclerotia formation were recorded and presented in Table 4.7.

Table 4.7 Effect of temperature on mycelial growth of *S. sclerotiorum* and sclerotial development

Temperature (°C)	Radial growth of mycelium (mm)	Days to fill the Petri plate	No. of sclerotia formed
5	5.88	15	5.00
10	20.93	10	19.75
15	29.23	7	27.25
20	43.99	5	28.50
25	50.28	4	30.00
30	3.17	--	0.00
SE ±	2.28		1.55
$CD_{(0.05)}$	4.80		3.26

From a perusal of the data presented in Table 4.7 it can be observed that the fungus tolerated low temperature much better for its growth than high temperature. Growth occurred between temperature ranges of 5-30°C. The optimum temperature for the growth of the fungus ranged between 20-25°C. The fungus took only four days to fill the Petri plate at 25°C and five days at 20°C with radial diameter of 50.28 mm and 43.99 mm, respectively. Growth of the fungus was very poor at 30°C with average diameter of 3.17 mm after four days of inoculation, while the growth completely ceased after 15 days of inoculation. It was further observed that sclerotial formation was directly correlated with growth of the fungus requiring again a temperature range of 20-25°C for its optimum production i.e., 28.5 and 30 per Petri plate, respectively. While, the fungus incubated at 5°C was very poor in production of sclerotia (5.0 per Petri plate). There was no production of sclerotia at 30°C.

4.3.3.2 Effect of different hydrogen ion concentrations on growth of *S. sclerotiorum*

In order to find out the effect of different hydrogen ion concentrations on the growth of *S. sclerotiorum*, Richard liquid medium was used as a basal medium for the experiment. Culture of *S. sclerotiorum* was inoculated on 30 ml Richard liquid medium at different pH levels i.e. 4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0. The results obtained are presented in Table 4.8.

Table 4.8 Growth of *S. sclerotiorum* at different pH levels

pH level	Dry weight of mycelium (mg)	No. of sclerotia formed	Dry weight of sclerotia (mg)
4.0	227.29	14.00	122.21
5.0	241.65	17.25	133.59
5.5	239.06	14.75	132.83
6.0	204.25	13.50	110.61
6.5	157.48	11.00	65.65
7.0	108.23	5.25	30.05
7.5	65.54	0.00	0.00
8.0	38.05	0.00	0.00
SE ±	10.60	1.60	10.46
$CD_{(0.05)}$	21.88	3.31	21.59

The data in respect of growth of *S. sclerotiorum* were statistically analysed and the difference among the treatments was found to be highly significant among each other. The best growth of the fungus occurred between a pH range of 5.0 and 5.5, followed by pH levels of 4.0 and 6.0. However, pH levels of 7.5 and 8.0 did not favour good growth. pH levels ranging from 4.0 to 5.5 favoured maximum dry mycelial weight of *S. sclerotiorum* (227.29 to 241.65) and were non-significant among each other. However, sclerotial formation was best at pH levels 5.0 to 5.5 and were at par with each other. Sclerotial formation was found to be directly correlated with growth of mycelium, optimum pH level for sclerotial formation and dry weight was 5.0 followed by 5.5 and 4.0. There was, however, no sclerotial formation at pH levels of 7.0 and 8.0.

4.4 Influence of soil hydrothermal regimes on Sclerotinia rot under field conditions

The studies on the effect of soil hydrothermal regimes on Sclerotinia rot were conducted under field conditions at the experimental farm of Department of Vegetable Crops during crop season 2004-05 starting from twenty days after transplanting till the harvesting of the crop. The observations on soil temperature, soil moisture and disease incidence of Sclerotinia rot were recorded at seven days interval and are presented in Table 4.9. The data showed that the incidence of Sclerotinia rot appeared on last week of October 2004. The disease was maximum in second week of December 2004 and first week of January 2005. Afterwards, the disease incidence increased at decreasing rate. Since the edaphic factors viz., soil temperature and soil moisture influenced the disease development, correlations were therefore, worked out to establish the contribution of these factors individually or jointly the progress of the disease among the host population.

Table 4.9 Incidence of Sclerotinia rot of cauliflower in relation to soil hydrothermal regimes

Date of observation		Mean soil temperature (°C)	Mean soil moisture (%)	Increase in disease incidence (%)
October, 2004	23	19.40	16.05	0.00
	30	19.80	16.95	4.82
November, 2004	06	18.91	17.97	2.03
	13	17.38	18.08	1.95
	20	17.54	19.05	2.60
	27	16.75	19.88	3.05
December, 2004	04	16.40	20.07	4.03
	11	16.85	22.57	6.00
	18	15.15	20.85	3.54
	25	14.86	22.00	4.50
January, 2005	01	14.78	25.00	7.35
	08	13.50	20.15	2.85
	15	12.25	21.06	2.08
	22	11.56	21.65	1.65
	29	11.00	19.82	0.50

4.4.1 Simple correlation

The data from the Table 4.10 showed that soil temperature and soil moisture were positively correlated with the Sclerotinia rot. Simple correlation of Sclerotinia rot with soil moisture (0.64) was found to be stastically significant at 1 per cent level of significance, whereas, simple correlation of Sclerotinia rot with soil temperature (0.13) was found non-significant, thereby indicating the dependence of Sclerotinia rot on soil moisture during crop season.

4.4.2 Partial correlation

Change in one parameter is often accompanied by simultaneous changes in several other parameters of a disease epidemic. Therefore, partial correlation coefficients were worked out between combinations of disease incidence with soil moisture and soil temperature and presented in Table 4.10.

Table 4.10 Correlation coefficients of disease incidence with edaphic factors

Variables	Year	Simple correlation	Partial correlation
Disease incidence x soil temperature	2004-05	0.13	0.82*
Disease incidence x soil moisture	2004-05	0.64*	0.89*

* Significant at 1 per cent level of significance

The data (Table 4.10) indicated that partial correlation coefficient between disease incidence and soil temperature removing the effect of soil moisture increased to significant level as compared to simple correlation coefficient. Thus, when the variation in soil moisture was kept constant, the relationship between Sclerotinia rot and soil temperature becomes positively correlated and highly significant (0.82). Similarly, relationship between disease incidence and soil moisture removing the effect of soil temperature was highly significant (0.89) and positively correlated.

It was inferred that the disease incidence and independent factors showed less positive relationship in presence of each other but when the variation in one independent factor was kept constant, the net correlation between the disease incidence and independent factor became very high and significant.

4.4.3 Multiple correlation

The coefficient of multiple correlation (R^2) was calculated to measure the contribution of linear function of independent variables such as soil temperature (X1) and soil moisture (X2) on dependent variable (Y). The data obtained are presented in Table 4.11.

The multiple correlation between disease incidence of Sclerotinia rot and a pair of independent variables i.e., soil temperature and soil moisture was found to be 0.8088 which indicated that 80.88 per cent changes in disease incidence of Sclerotinia rot was caused collectively by soil temperature and soil moisture whereas the remaining variation was due to unexplainable factors of variation which were not included in investigation. The soil temperature and soil moisture were found significant to contribute in development of the disease incidence.

4.4.4 Regression equation

The multiple regression equations Table 4.11 showed that soil temperature and soil moisture were found to be correlated positively and significantly with disease incidence and a unit change in soil temperature and soil moisture could alter the disease incidence upto 0.55 and 0.94 units, respectively.

Table 4.11 Multiple correlation and regression equation between disease incidence and edaphic factors

R^2	Coefficient of multiple determination R^2 (%)	Regression equation
0.8088	80.88	$Y = -24.66^* + 0.55\ X_1^* + 0.94\ X_2^*$ (3.99) (0.11) (0.13)

* Significant at 1 per cent level of significance

Where Y = Disease incidence (%)
X1 = Mean soil temperature (%)
X2 = Mean soil moisture (°C)

4.5 Disease Management

4.5.1 Isolation of antagonistic micro organisms

Different microorganisms isolated from *S. sclerotiorum* (3.5.1.2) infested soil and sclerotia of the pathogen (3.5.1.1) as well as collected from other sources were further tested for their antagonistic activity under *in vitro* conditions and field experiment. Among the fungal species, which were isolated from infested soil, majority of the isolation results yielded *Fusarium* sp., *Penicillium* sp., *Trichoderma polysporum* and *T. longibrachiatum*. Similarly, microflora was also isolated from the sclerotia of the pathogen viz., unidentified actinomycetes, *Torula* sp. and *Aspergillus niger*. *Pseudomonas fluorescens* and *Bacillus subtilis* were collected from Division of Microbiology, IARI, New Delhi and *T. harzianum*, *T. viride*, *T. virens*, *Paecilomyces lilacinus* and *Laetisaria arvalis* were collected from Vegetable Pathology Laboratory of Department of Mycology and Plant Pathology, Dr. Y.S. Parmar University of Horticulture and Forestry.

4.5.2 *In vitro* evaluation of antagonists against *S. sclerotiorum*

4.5.2.1 Dual culture method

In the present study, efforts were made to determine the antagonistic activity of the native microorganisms against the Sclerotinia rot (*S. sclerotiorum*) so that the effective antagonists were identified and could be tested for management of the disease.

All the microbial antagonists used against the pathogen, inhibited the growth of the Sclerotinia rot pathogen ranging from 27.54 to 63.26 per cent (Table 4.12). Out of six native species of *Trichoderma* tested against the stalk rot pathogen, *T. harzianum* was found most effective (63.26%) in inhibiting the pathogen followed by *T. viride* (60.94%), *T. hamatum* (60.69%) and *T. polysporum* (59.78%). *T. longibrachiatum* (57.76%) was the next best antagonist. *T. virens* was found least effective among the test fungi with 29.81 per cent inhibition in mycelial growth of the pathogen. Among the other antagonists, unidentified actinomycetes was found most effective (59.99%), whereas, *Bacillus subtilis* and *Pseudomonas fluorescens* were least effective with 27.54 and 28.92 per cent inhibition, respectively. The observations on inhibition zone formation revealed that, unidentified actinomycetes formed a good inhibition zone with the pathogen (6.33 mm), followed by *T. harzianum* (4.17 mm), *T. viride* (3.83 mm), *T. hamatum* (3.25 mm) and *Penicillium* sp. (2.17 mm). From the data (Table 4.12) on percentage inhibition of mycelium growth and inhibition zone formed, it is evident that two fungal antagonists (*T. harzianum* and *T. viride*) and an unidentified actinomycetes were found most effective and non-significant, which were later, tested under green house as well as field conditions against the disease.

Table 4.12 *In vitro* efficacy of antagonists against *S. sclerotiorum*

Antagonist	Inhibition in mycelial growth (%)	Inhibition zone formed (mm)
Trichoderma viride	60.94	3.83
T. harzianum	63.26	4.17
T. polysporum	59.78	1.17
T. hamatum	60.69	3.25
T. longibrachiatum	57.76	0.00
T. virens	29.81	1.77
Torula sp.	34.79	0.00
Penicillium sp.	33.88	2.17
Paecilomyces lilacinus	32.39	0.00
Laetisaria arvalis	45.95	0.00
Aspergillus niger	39.44	0.00
Fusarium sp.	32.02	0.00
Unidentified actinomycetes	59.99	6.33
Pseudomonas fluorescens	28.92	0.00
Bacillus subtilis	27.54	0.00
SE ±	2.31	
$CD_{(0.05)}$	4.65	

4.5.2.2 Well method

The test antagonists, which either gave maximum inhibition in mycelial growth of the pathogen or inhibition zone formed by dual culture method, were further screened for their antagonism against *Sclerotinia* by well method (3.5.2.2). The observations recorded on the growth inhibition of the test fungi are tabulated in Table 4.13. A perusal of data revealed that the autoclaved culture filtrates of test fungi did not exhibit much growth inhibition of test pathogen in either case. The maximum growth inhibition was obtained when test pathogen was grown after using autoclaved culture filtrate of unidentified actinomycetes (8.94%) followed by *T. harzianum* (8.29%), *T. viride* (7.96%) and *T. hamatum* (7.67%). The growth inhibition of test pathogen by *Paecilomyces lilacinus* was found least effective (1.84%).

Table 4.13 Effect of culture filtrates of different antagonistic fungi on mycelial growth inhibition of *S. sclerotiorum*

Antagonist	Inhibition in mycelial growth (%)
Trichoderma viride	7.96 (2.81)
T. harzianum	8.29 (2.88)
T. polysporum	6.36 (2.51)
T. hamatum	7.67 (2.77)
T. longibrachiatum	6.30 (2.51)
T. virens	6.42 (2.53)
Torula sp.	2.09 (1.4)
Penicillium sp.	7.17 (2.67)
Paecilomyces lilacinus	1.84 (1.34)
Laetisaria arvalis	5.72 (2.38)
Aspergillus niger	3.22 (1.78)
Unidentified actinomycetes	8.94 (2.99)

Figures in parentheses are square root transformed values

SE ±	0.14
$CD_{(0.05)}$	0.28

4.5.3 *In vitro* evaluation of botanicals and bio-pesticide against *S. sclerotiorum*

4.5.3.1 Botanicals

Extracts of different plant species viz., agave, broccoli, eucalyptus, tulsi, neem, pine; ginger rhizome, turmeric rhizome, chilli fruits, sarson seed and garlic cloves were evaluated under *in vitro* conditions against the *S. sclerotiorum* pathogen at 100 and 50 per cent concentrations (3.5.3.3) by poison food technique.

It is evident from the data (Table 4.14) that all the plant extracts inhibited the mycelial growth of the pathogen in comparison to control. Extract (100%) of garlic cloves was found most effective with 100 per cent inhibition of the Sclerotinia rot pathogen followed by agave (76.88%), chilli (51.55%), tulsi (49.39%), and turmeric rhizome with 39.24 per cent mycelial inhibition. Extracts (100%) of sarson and pine were found least effective with mycelial inhibition of 14.74 and 17.84 per cent, respectively. Similar results were obtained at 50 per cent concentration i.e., extract (50%) of garlic was most effective with (100%) inhibition, followed by agave (65.11%), chilli (38.18%), tulsi (35.83%), and turmeric (23.44%). Again, extract (50%) of sarson and pine were found least effective with mycelial inhibition of 9.84 and 13.26 per cent, respectively. It was also noticed that as the concentration increased (50 to 100 per cent), there was corresponding increase in per cent mycelial inhibition of the pathogen.

Table 4.14 *In vitro* evaluation of botanicals against *S. sclerotiorum*

Plant	Botanical name	Growth inhibition (%)	
		100	50
Garlic (C)	*Allium sativum*	100 (90.00)	100 (90.00)
Ginger (R)	*Zingiber officinale*	35.15 (36.33)	21.28 (27.37)
Chilli (F)	*Capsicum frutescens*	51.55 (45.89)	38.18 (38.11)
Tulsi (L)	*Ocimum sanctum*	49.39 (44.65)	35.83 (36.75)
Turmeric (R)	*Curcuma longa*	39.24 (38.79)	23.44 (28.93)
Eucalyptus (L)	*Eucalyptus globulus*	22.16 (28.08)	18.54 (25.48)
Neem (L)	Azadirachta indica	37.45 (37.73)	27.49 (31.62)
Sarson (S)	*Brassica campestris*	14.74 (22.57)	9.84 (18.25)
Agave (L)	*Agave americana*	76.88 (61.27)	65.11 (53.80)
Pine (N)	*Pinus roxburghii*	17.84 (24.98)	13.26 (21.31)
Broccoli (L)	*Brassica oleracea*	20.95 (27.22)	15.69 (23.33)

(L) Leaves; (S) Seed; (F) Fruit; (C) Cloves; (R) Rhizome; (N) Needles
Figures in parentheses are arcsine transformed values

SE±	1.23	1.80
$CD_{(0.05)}$	2.56	3.74

4.5.3.2 *In vitro* efficacy of Nemazal and *Allium* extract against *S. sclerotiorum*

Neemazal and *Allium* extract were evaluated under *in vitro* conditions against Sclerotinia rot pathogen at different concentrations. The growth of the pathogen was found to be inhibited at all the test concentrations in comparison to control (Table 4.15). In general, at 10 per cent concentration, the per cent inhibition in the mycelial growth of the pathogen resulted by Nemazal and *Allium* were 100 and 97.16 per cent,

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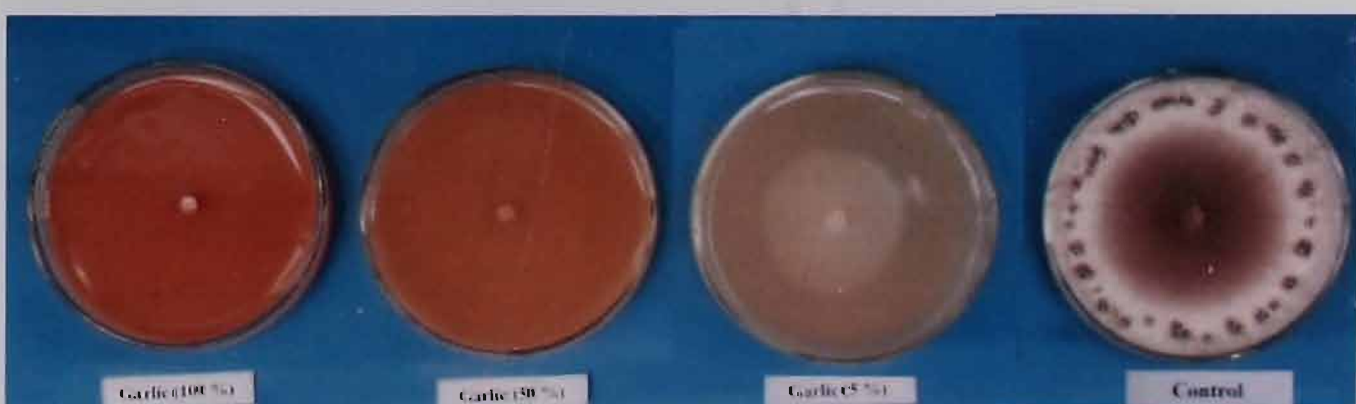

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Plate 4: *In vitro* screening of antagonists and botanicals against *S. sclerotiorum*

(A) *T. harzianum*, *T. viride*, unidentified actinomycetes and *S. sclerotiorum*

(B) Nemazal (5, 2.5, 1.5, 0.75 and 0.25%) and *S. sclerotiorum*

(C) Garlic (100, 50 and 5%) and *S. sclerotiorum* respectively and significantly superior over all other test concentrations. Whereas, application at 0.25 per cent were found least effective i.e., 32.05 per cent in case of Nemazal and 3.83 per cent in *Allium* extract. It was also observed that as the concentration of formulation. increased, per cent inhibition in the mycelial growth of the pathogen also increased with maximum inhibition at 10 per cent concentrations, which was statistically superior over all other concentrations. Lowest concentration of 0.25 per cent was found least effective in mycelium growth of the test pathogen.

Table 4.15 *In vitro* evaluation of botanicals against *S. sclerotiorum*

Concentration (%)	Inhibition in mycelial growth (%)	
	Nemazal	*Allium sativum*
0.25	32.05 (34.48)	3.83 (11.25)
0.75	50.22 (45.13)	25.60 (30.39)
1.5	73.37 (59.26)	42.87 (40.98)
2.5	81.35 (64.42)	59.53 (50.51)
5.0	90.01 (71.67)	78.27 (62.23)
10	100 (90.00)	97.16 (84.35)

Figures in parentheses are arcsine transformed values

SE±	2.46	3.46
$CD_{(0.05)}$	5.35	7.55

4.5.4 Field experiment

Two fungal antagonists viz. *T. harzianum* and *T. viride*, an unidentified actinomycetes and a neem formulation (Nemazal) were applied as root dip and soil application against Sclerotinia rot of cauliflower in replicated field experiment during crop season of 2004-05. The data on disease incidence, plant frame, stalk length, number of leaves, gross curd weight, net curd weight and curd colour were recorded and presented in Table 4.16.

The incidence of the Sclerotinia rot was calculated by recording the number of diseased and healthy plants in each plot. In general, application of Nemazal at 0.75 per cent concentration as rot dip and soil drench had a significant effect on reduction of disease incidence (30.49%) in comparison to 55.77 per cent in the control

Table 4.16 Effect of biocontrol agents on incidence of Sclerotinia rot and horticulture parameters of cauliflower

Treatment	Application (dose)	Disease incidence (%)	No. of leaves	Plant height (cm)	Stalk length (cm)	Frame size (cm)	Curd size (cm)	Curd weight (kg/plot)	Curd colour
Trichoderma harzianum	Root dip (1%) & soil amendment (5g/plant)	40.62	14.93	52.00	3.65	127.88	54.45	12.60	White
Trichoderma viride	Root dip (1%) & soil amendment (5g/plant)	46.92	14.49	51.00	3.48	124.17	51.52	11.76	White
Unidentified actinomycetes	Root dip & soil drenching (1%)	48.16	14.68	51.39	3.51	126.35	53.83	12.00	White
Nemazal	Root dip & soil drenching (0.75%)	30.49	15.03	53.75	3.71	131.08	55.90	14.28	White
Control	--	55.77	14.37	48.42	3.18	123.33	51.39	11.28	White
SE ±		4.42	0.48	2.36	0.27	5.29	2.44	1.49	
CD$_{(0.05)}$		9.63	1.05	5.14	0.60	11.53	5.32	3.26	

treatment. Similarly, *T. harzianum* treatment as root dip and soil application was also effective and reduced the incidence to 40.62 per cent in comparison with control. Treatments of *T. viride* and unidentified actinomyces were less effective wherein incidences of Sclerotinia rot were reduced only to 48.16 and 46.92 per cent, respectively and was at par in comparison with control.

The application of antagonists as well as neem formulation gave higher number of leaves per plant, plant height, stalk length, frame size, curd weight and curd size, however, these were non-significant except for plant height in Nemazal as compared to control treatment.

4.5.5 Screening of cultivars for resistance to *S. sclerotiorum*

In order to ascertain the sources of resistance, if any, a collection of five varieties of cauliflower, viz. Cauliflower PSBK-25, Cauliflower KT-08, Cauliflower KT-22, Cauliflower DC-76, Cauliflower II VRMC-12 available in the Department of Vegetable Crops, Nauni, were screened against *S. sclerotiorum* (Sclerotinia rot pathogen) under artificial epiphytotic conditions in the Department of Mycology and Plant Pathology during 2004 according to the procedures described (3.5.5) and observations in respect of disease reaction under artificial epiphytotic conditions (Table 4.17) were recorded after inoculation of the pathogen. The results showed that, almost all the varieties were susceptible to the attack of the pathogen (*S. sclerotiorum*) except cauliflower DC-76. However, these were found to vary in their degree of susceptibility. Varieties of Cauliflower PSBK-25, Cauliflower KT-22, and Cauliflower II VRMC-12 were highly susceptible, while Cauliflower KT-08 was susceptible and cauliflower DC-76 showed resistant reaction.

Table 4.17 Reaction of cauliflower varieties to Sclerotinia rot under artificial epiphytotic conditions

Variety	Disease incidence (%)	Disease Reaction
Cauliflower DC-76	20	Resistant
Cauliflower KT-08	40	Susceptible
Cauliflower PSBK-25	60	Highly susceptible
Cauliflower KT-22	60	Highly susceptible
Cauliflower II VRMC-12	60	Highly susceptible

A

B

Plate 5: Reaction of cauliflower varieties against *S. sclerotiorum*

(A) Cauliflower DC-76 (Resistant)

(B) Cauliflower II VRMC-12 (Highly susceptible)

DISCUSSION

Chapter-5

DISCUSSION

Sclerotinia sclerotiorum, one of the most destructive soil borne pathogens has been reported to affect a wide range of wild and cultivated host plants and causes considerable damage to the host under congenial environments. The pathogen has been reported to hamper the cauliflower cultivation by causing stalk rot in different cauliflower growing areas such as United States during 1942-1943 (Snyder and Baker, 1945) and particularly in India in Himachal Pradesh in 1973 (Sharma *et al.*, 1983). Since then it has caused serious losses to the seed crop in different localities of the state. However, with expanding strides in cauliflower seed production in the region, the ravages from the disease also increased and caused serious losses to the seed crop.

The existing control measures of this disease by application of fungicide are often cost prohibitive and impractical (Sharma, 1979 and Gupta, 1985) because of the long survival ability of sclerotia in the soil and the vast host range of the pathogen (Nelson, 1998). However, Sclerotinia rot of cauliflower is an important disease in Himachal Pradesh. Since, very little information is available on its eco-friendly management, the present studies were, therefore, undertaken on various aspects of the pathogen with a view to understand the occurrence of the disease at different locations of the state; symptomatology, causal organism, its pathogenicity, morphological and physiological studies, correlation with edaphic factors and to devise eco-friendly disease management strategy.

The symptoms of Sclerotinia rot on leaves, stems as well as curds were studied in detail. On the basis of symptoms observed during the crop season, these were categorized into three different types i.e. the leaves of the affected plant turned pale and dropped-off, dark brown lesions appeared on the stalk; stems of the affected plants became hollow and in later stages were filled with numerous black sclerotia. Curd rot was characterized by initiation of rot from the stem of curd and progressing upwards. The affected stems turned dark brown and watery with white cottony tuft of mycelium on the surface and rot ultimately invaded the peduncles. The symptoms of the disease studies were in conformity to earlier workers (McLean, 1949; Khan, 1976; Kaith, 1977; Sharma, 1979; Sharma *et al.*, 1983; Gupta, 1985 and Sandhu, 1986).

In the present study, an extensive field survey was carried out in different cauliflower growing areas of Himachal Pradesh. Disease was found to be prevalent in all the locations of surveyed. Sclerotinia rot was more severe in sub-mountainous regions with maximum incidence at Matnali (65.37%) and Rajgarh (73.26%) falling in district Sirmour. The low incidence at Kandagath in district of Solan (29.54%) might be attributed to low inoculum level for progress of the disease. Sharma (1995) reported that disease incidence of Sclerotinia rot on gram in Sirmour district (46.7%) was higher in comparison with Solan district (21.25%).

The cause of the Sclerotinia rot was ascertained and the fungus associated with the infected leaves, stems and curds was isolated in pure culture. The fungus produced aerial mycelium, which was hyaline, well developed and appeared cottony. Sclerotia were produced at growing margins or centre of colony at 5 days of incubation and formed concentric rings, became pigmented and were black in colour with age. Their number varied from 22 to 45 per Petri plate. The fungus was identified as *Sclerotinia sclerotiorum* (Lib.) de Bary and the characters were in accordance with the keys given by earlier workers (Colotelo, 1974; Khan, 1976; Willetts and Wong, 1980; Singh, 1985 and Agrios, 1988).

Different parts of cauliflower viz. leaves, petioles, stems and curds were inoculated with culture bits of *S. sclerotiorum* under moist chamber condition to evaluate susceptible parts of cauliflower and to establish Koch's postulates. The incubation period on plant parts inoculated leaves, petioles and stems was found to be 48 hours, while it was different in case of curds (72 hours) and old leaves (96 hours) which were not tender. The difference could be attributed to type of cellulose present in curds and old leaves. While, among the inoculated plant parts, young leaves were found most susceptible with 100 per cent disease incidence and 0.9-12 cm lesion size on mid rib and 0.8-9.8 cm lesion size on laminar part of the plant. Petioles were found next susceptible with 100 per cent disease incidence with lesion size of 0.4-8.5 cm on injured and 0.3-2.5 cm on un-injured part. On curds, 75 per cent disease incidence was recorded with lesion size of 0.75-6.70 cm. Stems and old leaves on inoculation were found less susceptible with 50 per cent disease incidence. The disease symptoms observed were similar to those described by Kaith (1977), Sharma (1979) and Sharma *et al.* (1995). The variation in incubation period might be due to the method of pathogen inoculation and plant part, inoculated. Observations on inoculum load showed that, application of 250 and 500 mg of growth culture of the pathogen gave almost maximum diseased incidence (100%) after 28 days of inoculation, whereas, 50 mg gave minimum (66.66%) disease incidence. The studies conducted by Dohroo (1988) also revealed similar results.

The fungus produced aerial mycelium, which was hyaline, branched, well developed and appeared cottony, consisting of closely septate hyphae which were both inter- and intracellular. In addition, Colotelo (1974) reported that, subsurface mycelial cells swelled, became pigmented and formed the dark bulbous rind cells; which darkened with age. The hyphae were 2.0-9.4 μm in width and contained dense granular protoplasm. The micro-conidia were produced on conidiophores of the vegetative mycelium and measured 1.5-3.5 μm in culture. This observation is in agreement to Sharma (1979) who reported micro-conidia to be 1.5-3.5 μm while Khan (1976) reported that hyphae were 6-16 μm in width. The sclerotia were round to irregular in shape in culture and measured 1.5-7 mm in width and 2-15 mm in length. Singh (1985) reported that, sclerotia varied in shape and size according to environment and location. Sclerotia formed on host surface were usually loaf shaped or globose while those formed in the pith of the stem were elongated, which could be due to the space available for growth. Sharma (1979) confirmed that, sclerotia produced in culture were similar to those produced on the host in all morphological characters. Sclerotia started germination and giving rise to several columnar structures (stipes). The stipes later developed funnel shaped cup (apothecium) at the tip. Apothecia were brown in colour and were round or globate type. The length of apothecia measured from 5-21 mm, whereas diameter of the discs ranged from 2-9 mm with number ranging from 1-9 per sclerotia. The asci were hyaline and cylindrical in shape, which measured 91-165 x 4.9-8.5 μm in size. Each asci contained eight ascospores which were found to be released in clouds. Ascospores were elliptical and ranged from 6.5-13 x 4.2-6.6 μm in size. The morphological characters of the fungus were in accordance with the taxonomic keys given by Willetts and Wong (1980). These also tallied closely to those reported by earlier workers (Eddins, 1937; Coe, 1944; Purdy, 1955; Coloteo, 1974; Porter and Beute, 1974; Sharma, 1979 and Singh, 1985) thus confirming the identity of the fungus as *Sclerotinia sclerotiorum* (Lib.) de Bary.

Cultural studies with six solid media showed that potato dextrose agar medium was the best supporting media for mycelial growth (52.47 mm) of the fungus and produced maximum number (30.75) of sclerotia. These results are in accordance with the findings of Khan (1976) and Sharma (1979) who also found potato dextrose agar medium suitable for growth of the fungus and production of maximum number of sclerotia. While, lettuce leaf agar was not found suitable for growth of the fungus, which required 7 days for full growth in the Petri plates, with average radial growth of 30.65 mm and 1.5 average number of sclerotia per Petri plate. The medium was tried for the first time and there was no conformity with previous study though, the fungus infected lettuce plants as per earlier reports. However, Khan (1976) and Kaith (1977) also reported poor growth on some other natural media such as sunflower seed extract and rape seed extract, which might be due to presence of some growth inhibitory substances in their extract.

Among liquid media studies, Richard solution was found the best for vegetative growth (242.85 mg) as well as sclerotial formation. The results are in agreement with those of Khan (1976) and Sharma (1979) who also found Richard solution suitable for the growth of the fungus and thus used this medium in their physiological studies. While, Asthana and Hawker medium was not found suitable for vegetative growth (71.90 mg) as well as sclerotial formation. The result was in close agreement with finding of Sharma (1979) who also reported similar results for Asthana and Hawker medium. Khan (1976) reported that Czapek medium was not suitable for mycelium growth as well as sclerotial formation while Kaith (1977) did not found sarson seed extract and oat seed extract suitable for the mycelium growth.

In temperature studies, growth of the fungus was observed between 5-30°C. The result indicated that, the fungus tolerated low temperature much better for its growth than high temperature. The optimum temperature for the growth of the fungus ranged between 20-25°C with average colony diameter of 43.99 and 50.28 mm after four days of inoculation. However, optimum temperature for production of sclerotia was 15-20°C. Growth of the fungus was very poor at 30°C with average colony diameter 3.17 mm after four days of inoculation with no sclerotia formation. Similar results were also reported by Bedi (1962), Khan (1976) and Sharma (1979). Abawi and Grogan (1975) reported that mycelial growth and sclerotial production were optimum at 20-25°C. Kapoor (1994) multiplied *S. sclerotiorum* at 24±1°C cn PDA medium. Coe (1944) reported best growth of *S. sclerotiorum* at 19-20°C on PDA medium while Purdy (1956) found that temperature effected size of sclerotia with the largest size occurring at 25°C. Willetts and Wong (1980) reported that growth of fungus occurred over a wide range of 0-35°C. While Bilgrami and Verma (1981) confirmed that optimum temperature ranged for many fungi between 15-30°C.

Under pH studies, suitable growth of *S. sclerotiorum* was obtained on Richard liquid medium at different pH levels ranging from 4.0-8.0. The results indicated that the best growth of the fungus occurred at pH 5.0 with mycelium dry weight of 241.65 mg followed by pH 5.5 with mycelium dry weight of 239.06 mg. While, pH levels of 7.5 and 8.0 did not favour good growth of the fungus. The results are also in close agreement with Sharma (1979) who found pH 5.0 suitable for vegetative growth of the fungus. However, Willetts and Wong (1980) reported that pH below 5.0 was optimum, whereas, Khan (1976) confirmed optimum pH of 4.6 and 4.5 for best growth of the fungus. Sclerotial formation was found to be directly correlated with vegetative growth of mycelium with optimum pH levels of 5.0 and 5.5. Le Tourneau (1979) explained that numerous sclerotia were formed by the fungus growing on a suitable medium and supported good growth of the fungus. There was no sclerotia formation at pH levels of 7.0 and 8.0. Khan (1976), Kaith (1977) and Sharma (1979) found that pH 9.0 was not suitable for vegetative growth as well as sclerotia formation of *S. sclerotiorum*.

The results on the influence of soil hydrothermal regimes on the disease development under field conditions revealed that the disease initiated in the last week of October during the crop season 2004-05 with soil temperature (19.80°C) and soil moisture (16.95%). The disease was found to increase with the increase in soil moisture and decrease in soil temperature which was maximum in first week of

January when soil moisture was 25 per cent and soil temperature was 14.78°C. Miller and Burke (1986) reported that infection from Sclerotinia wilt increased with increasing irrigation. Clarkson *et al.* (2004) reported that carpogenic germination of sclerotia occurred between 5 and 25°C but optimum at temperatures of 15 to 25°C. A positive correlation between disease incidence with soil moisture and soil temperature was observed. Soil temperature and disease incidence were found non-significant in simple correlation (0.13), however, were highly significant in partial correlation (0.82). Whereas, soil moisture and disease incidence were found significant in both simple (0.64) and partial correlation (0.89). The results, therefore, suggested the prominent role of soil temperature and soil moisture in disease development. The result is also in agreement with Verma (2001) who reported that soil temperature and soil moisture had highly significant correlation with disease incidence of Fusarium wilt. While Khan (1976), Kaith (1977) and Sharma (1979) reported a high correlation between air temperature, relative humidity and rainfall with disease incidence of cauliflower. Multiple correlation coefficients between disease incidence and edaphic factors suggested 80.88 per cent Sclerotinia rot incidence was due to these factors. Thus, moderate soil temperature (14.78°C) coupled with moderate soil moisture (25%) were found to favour the disease.

The screening of fifteen microbial species against *S. sclerotiorum* revealed that, *T. harzianum* was found most effective (63.26%) in inhibiting the test pathogen, followed by *T. viride* and *T. hamatum* with mycelium growth inhibition of 60.94 and 60.69 per, respectively, followed by unidentified actinomycetes and *T. polysporum* with inhibition of 59.99 and 59.78 per cent, respectively. Among the test antagonists, *T. harzianum*, *T. viride* and unidentified actinomycetes formed inhibition zone of 4.17, 3.83 and 6.33 mm, respectively. Further studies were conducted by taking *T. harzianum*, *T. viride* and unidentified actinomycetes for the management programme. These fungi were also reported by Sharma (1989) to be inhibitory for the pathogen. Singh (1998) reported that *T. harzianum* had strong mycoparasitism and inhibited the pathogen, whereas *T. viride* showed strong antibiosis and formed 2-3 mm zone of inhibition in dual culture. Gupta and Agarwala (1988) tested isolates of 14 fungi against *S. sclerotiorum* and found inhibition of the pathogen by *T. viride* followed by *Aspergillus terreus*, *Rhizopus arrhizus* and *Fusarium solani*. Kansal (1988) reported that maximum growth inhibition of the test pathogen was obtained by *T. viride*, followed by *T. harzianum*, *G. virens* and *Aspergillus niger*. Biocontrol agents such as *Trichoderma* spp., *Gliocladium* spp., *Penicillium* spp. have been reported by various workers for their antagonistic activity against *S. sclerotiorum* (Huang and Hoes, 1976; Mueller *et al.*, 1985; Zazzerini and Tosi, 1985; Mercier and Reeleder, 1987; Whipps and Budge, 1990; Gerlagh *et al.*, 1996; Bhardwaj *et al.*, 1990; Inbar *et al.*, 1996).

The test microorganisms, which either formed inhibition zone, or gave higher percentage of inhibition by dual culture method, were further screened for their antagonism against the pathogen by well method with a aim to find out the most effective antagonistic microbe for field application in management study. However, unlike dual culture method, autoclaved culture filtrates of test microorganisms did not exhibit much growth inhibition of test pathogen in either case. The unidentified actinomycetes gave the most effective inhibition (8.94%), followed by *T. harzianum* (8.29%) and *T. viride* (7.96%), *T. hamatum* (7.67%), *Penicillium* sp. (7.17%). The results, thereby indicated that the growth inhibiting substances of culture filtrates were thermolabile. These finding are in agreement with Kansal (1988) and Sharma (1989) who also found that the autoclaved filtrates did not cause growth inhibition of *S. sclerotiorum*.

Extracts of different plant species viz., agave, broccoli, eucalyptus, tulsi, neem, pine, ginger rhizome, turmeric rhizome, chilli fruits, sarson seed and garlic cloves were evaluated under *in vitro* conditions against the *S. sclerotiorum* pathogen at 100 and 50 per cent concentrations. The results indicated that all the extracts inhibited the mycelial growth of the pathogen in comparison to control. Extract (100%) of garlic cloves was found most effective with 100 per cent inhibition of the Sclerotinia rot pathogen followed by agave (76.88%), chilli (51.55%), tulsi (49.39%), and turmeric rhizome with 39.24 per cent mycelial inhibition. Extract (100%) of sarson and pine were found least effective against the pathogen. Similarly results were also found at 50 per cent concentration. The antimicrobial and biocidal properties of garlic have been reported by Amolkar and Banerji (1971). Verma and Dohroo (2003) also found garlic cloves extract (25%) inhibitory towards *Fusarium oxysporum*. Chattopadhyay *et al.* (2004) studied the effect of bulb extract of *A. sativum* as seed treatment integrated with its foliar spray against the pathogen and found highest reduction of Sclerotinia rot. Singh *et al.* (1980) studied effect of aqueous extracts of

January when soil moisture was 25 per cent and soil temperature was 14.78°C. Miller and Burke (1986) reported that infection from Sclerotinia wilt increased with increasing irrigation. Clarkson *et al.* (2004) reported that carpogenic germination of sclerotia occurred between 5 and 25°C but optimum at temperatures of 15 to 25°C. A positive correlation between disease incidence with soil moisture and soil temperature was observed. Soil temperature and disease incidence were found non-significant in simple correlation (0.13), however, were highly significant in partial correlation (0.82). Whereas, soil moisture`and disease incidence were found significant in both simple (0.64) and partial correlation (0.89). The results, therefore, suggested the prominent role of soil temperature and soil moisture in disease development. The result is also in agreement with Verma (2001) who reported that soil temperature and soil moisture had highly significant correlation with disease incidence of Fusarium wilt. While Khan (1976), Kaith (1977) and Sharma (1979) reported a high correlation between air temperature, relative humidity and rainfall with disease incidence of cauliflower. Multiple correlation coefficients between disease incidence and edaphic factors suggested 80.88 per cent Sclerotinia rot incidence was due to these factors. Thus, moderate soil temperature (14.78°C) coupled with moderate soil moisture (25%) were found to favour the disease.

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Commercial formulations of neem and garlic clove extracts were tested under *in vitro* conditions with an aim to find the effective treatments which could be evaluated under field conditions for management of the disease. In general, Nemazal was found more effective in comparison with garlic. Neemazal (10%) was found most effective with complete inhibition of the fungus followed by its different concentrations (5, 2.5 and 1.5%). Murti *et al.* (1940) studied various antimicrobial substances like acids and sulphur containing compounds from neem oil. Dubey and Kumar (2003) reported that neem oil application (30-40 ppm) completely inhibited mycelium growth of *Macrophomina phaseolina*. Singh *et al.* (1980) studied the effect of neem oil on the survival of *Rhizoctonia solani*, *S. rolfsii* and *S. sclerotiorum*. The growth of these fungi was reported to be completely inhibited by neem oil (100-5000 ppm). Garlic (10%) was also found most effective with 97.16 per cent mycelial growth inhibition followed by its different concentrations (5, 2.5 and 1.5%). Nemazal and garlic were found least effective when tested at 0.25 per cent concentration. The results are also in agreement with Shinde and Patel (2004) who also found garlic extract (10%) suitable for complete inhibition of mycelium growth and sclerotia formation of *R. solani*. Narasimhan *et al.* (1995) reported bulb extract of garlic (*Allium sativum*), leaf extract of vilvam (*Aegle marmelos*) and flower extract of red periwinkle (*Catheranthus roseus*) at 10 per cent concentration to be most effective in inhibiting the spore germination and mycelium growth of *Alternaria solani*. Kurucheve and Padmavathi (1997) recorded the minimum growth of *Pythium aphanidermatum* with extracts of *Allium sativum* bulbs (10%) followed by *Lawsonia inermis* leaf extract.

Two fungus antagonists viz. *T. harzianum*, *T. viride*, unidentified actinomycetes and neem formulation (nemazal) were applied as root dip to seedling and soil application against Sclerotinia rot of cauliflower in replicated field experiment during crop season of 2004-05. The results indicated that application of Nemazal (0.75%) had a significant effect on the average disease incidence (30.49%) in comparison to the control treatment (55.77%). The effectiveness of neem oil in reducing the fungal disease was reported by Mohan *et al.* (1995) who found that application of neem oil and neem seed extract as sprays (0.4%) reduced blast disease of garlic and Phyllosticta leaf spot of ginger. Neem oil was also reported effective against *F. oxysporum* causing wilt of tomato (Eswaramurthy *et al.*, 1989). *T. harzianum* application was also effective in reducing the disease incidence and significant in comparision with control. Treatments of *T. viride* and unidentified actinomycetes were least effective and reduced Sclerotinia rot incidence only to 46.92 and 48.16 per cent. *T. harzianum* have been earlier used by many workers for the control of soil borne diseases. Sharma (1995) reported that application of biocontrol agents at growing time was effective in the control of stem rot of gram. He found that minimum disease incidence occurred in *T. harzianum*, *T. viride* and *Absidia cylindrospora*. Sharma (1989) reported that *T. harzianum*, *T. viride* and *G. roseum* soil application reduced disease incidence of white rot of pea. Knudsen *et al.* (1991) confirmed that colonization of *S. sclerotiorum* by *T. harzianum* reduced the pathogen inoculum producing ability. Gerlagh *et al.* (1999) applied *C. minitans* and *Trichoderma* spp. in soil against *S. sclerotiorum* diseases of bean, potato, carrot and chicory. Researchers from different parts of the world have reported the effectiveness of *T. harzianum* against soil borne fungi causing diseases in different crops (Kraft and Papavizas, 1983; Mathew and Gupta, 1998; Chaudhary and Kumar, 1999; Kumar and Dubey; 2001).

The application of antagonist as well as neem formulation gave higher number of leaves per plant, plant height, stalk length, frame size, curd weight and curd size, however, these were non-significant except for plant height in Nemazal as compared to control treatment. Anith and Manomohandas (2001) confirmed that number of leaves per plant was non-significant when application of *T. harzianum* was made in soil for control of nursery rot disease of black pepper. Jeyalakshmi and Seetharaman (1998) reported that application of neem oil (3%) was effective in the control of fruit rot and die back of chilli. Kurma and Dubey (2001) reported that seed treatment application of *T. harzianum* against collar rot of pea increased green pod yield of pea. Pant and Mukhopadhyay (2001) also reported that soybean yield was increased by seed treatment application of *G. virens* and *T. harzianum* against seed and seedling rot complex caused by *R. solani*, *S. rolfsii*, *Fusarium* spp. Anahosur (2001) reported that field application of *T. harzianum*, *T. viride*, *G. virens* and *P. fluorescens* was effective in the control of Sclerotium wilt of potato which also increased plant stand and yield of potato.

The screening of five varieties of cauliflower against *S. sclerotiorum* (*Sclerotinia* rot pathogen) under artificial epiphytotic conditions indicated that almost all the varieties were susceptible to the attack of the pathogen. However, there were found to vary in their degree of susceptibility. Varieties of Cauliflower PSBK-25, Cauliflower KT-22, and Cauliflower II VRMC-12 were highly susceptible, while Cauliflower KT-08 was susceptible. A number of workers have evaluated cvs./ lines in the laboratory as well as field for their resistance or susceptibility and found few cvs./ lines as resistant to this disease (Sharma *et al.*, 1982; Dohroo, 1988; Thakur *et al.*, 2001). Among the varieties screened, Cauliflower DC-76 showed resistant reaction. There was no conformity about Cauliflower DC-76; however, different workers have reported resistance in different varieties/ germplasm lines. Kapoor (1986) screened seventy-nine lines of cauliflower at the seedling stage at Katrain and showed that EC-103576, EC-131192, Janavon and KN-81 were resistant. Dohroo and Korla (1988) reported that EC-191203 was resistant while EC-191030, EC-191177 and EC-191021 were moderately resistant out of 40 collections screened under Solan conditions. Janavon as resistant source and Early Winter Adam White as moderately resistant source to stalk rot were observed by Baswana *et al.* (1990). Singh and Kalda (1995) screened 69 lines of cauliflower at seedling stage and reported that 4 winter cultivars, Janavon, EC103576, EWAW and EC177283 as resistant. Sharma *et al.* (1995) reported that among 75 lines tested for resistance, Early Winter Adam's White and EC162587 were highly resistant, whereas RSK 1301 and MRS 1 were moderately resistant. Sharma *et al.* (2000) evaluated thirty four genotypes of cauliflower against stalk rot under field condition by artificial inoculation of the pathogen. The results indicated that lines SN-445, RSK, RSK-1301, BR, KN-81, KM-1, KM-1xKJ-38, RSK-1301xKJ-38 were resistant and had less than 10 per cent disease incidence.

SUMMARY

Chapter-6

SUMMARY

Studies on stalk rot of cauliflower were conducted during the years 2004-2005. Different localities of Solan and Sirmour districts were surveyed for recording the progress of disease incidence. The samples of diseased plants were collected for isolation and identification as well as morphological and physiological studies. Edaphic factors such as soil moisture and soil temperature were studied for their correlation with disease development. Disease management study was undertaken by screening of biopesticide and antagonists for their antagonistic activities against *S. sclerotiorum* and for working out effective ones which could be used in eco-friendly disease management programme. Screening of different varieties of cauliflower was made for their resistance against Sclerotinia rot pathogen.

A general survey was conducted in different cauliflower growing areas of the state and the incidence of the disease was recorded which varied from place to place. Matnali in district Sirmour had the highest incidence (73.26%), while Kandaghat in district Solan had the minimum disease incidence (29.54%) during the cropping season of 2004-05. The data also indicated that the locations surveyed in the district of Sirmour had higher incidence of the Sclerotinia rot, with average of 69.32 per cent in comparison to Solan district with average disease incidence of 55.36 per cent. The disease was more severe in sub-mountainous region of the state.

The symptoms of the disease were characterized by yellowing of the leaves from tip downward till the whole leaf became pale and started falling out. The base of the petioles started rotting, the infected portions became soft and turned brown to black, with white cottony mycelial growth on the surface. The collar portion of the stem was later affected, showing a dark brown to black girding. The pith portion of such affected stalks also showed rotting, which later became hollow and filled with white mycelial growth and black sclerotia. Later, curds were found infected and covered with white mycelial growth. Sclerotia were found sticking in between the rotted portion of the curd.

The fungus associated with the Sclerotinia rot pathogen was isolated in pure culture and was identified as *Sclerotinia sclerotiorum* (Lib.) de Bary on the basis of morphological characters *i.e.* the fungus produced aerial mycelium, which was hyaline, well developed and appeared cottony. Sclerotia were found to be produced at growing margins and middle of colony, formed in concentric rings with round or oval to irregular in shape and black in colour.

The fungus was subjected to inoculation directly on leaves, petioles, stems and curds of cauliflower and incubated in moist chamber under room temperature (20±5°C) and by making the soil sick. Disease appeared after 2-4 days under moist chamber incubation in which young leaves, petioles and curds were most sensitive to Sclerotinia rot pathogen whereas by making the soil sick with the pathogen (250-500 mg), the disease appeared after 2 weeks of inoculation. The symptoms appearing on plants in growth chamber were similar to those described under field conditions. The fungus was then re-isolated from such infected plant parts and was found to be identical in all respects to the original fungus inoculated.

The fungus produced aerial mycelium, which was hyaline, branched, well developed and appeared cottony, consisting of closely septate hyphae which was both inter- and intracellular. The hyphae were 2.0-9.4 µm and contained dense granular protoplasm. The micro-conidia were produced on conidiophores of the vegetative mycelium and measured 1.5-3.5 µm. The sclerotia were first white in colour and then turned black. They were round to irregular in shape in culture and measured from 1.5-7 mm in width and 2-15 mm in length. Sclerotia after germination gave rise to several columnar structures (stipes) which later developed funnel shaped cup (apothecium) at the tip. Apothecia were yellow to brown in colour and were round or globate type. The length of apothecia measured from 5-21 mm and diameter of the discs ranged from 2-9 mm. The asci were hyaline and cylindrical in shape, which measured 91-165 x 4.9-8.5 µm in size. Each asci contained eight ascospores which were found to be released in clouds. Ascospores were elliptical and ranged from 6.5-13 x 4.2-6.6 µm.

The fungus was found to grow best on potato dextrose agar medium followed by pea seed agar, which were statistically at par with each other. Whereas, lettuce leaf agar gave the least growth of the fungus. In liquid media, the fungus gave maximum growth on Richard solution; followed by Czapek sucrose nitrate solution and were non-significant. The growth of fungus was very poor on Asthana and Hawker medium.

The fungus tolerated low temperature ranges much better for its growth than high temperatures. Growth was observed between temperature ranges of 5-30°C. However, the optimum temperature for the growth of the fungus ranged between 20-25°C. The fungus was found to grow at all the pH levels (4.0-8.0) with an optimum at 5.0. Sclerotial formation was found to be directly correlated with its temperature and pH level requirements.

The edaphic factors viz., soil temperature and soil moisture influenced the disease development. Simple correlation coefficients between disease incidence and soil temperature were found to be nonsignificant and positive (0.13) while soil moisture (0.64) were found to be significant and positive. The partial correlation coefficient between disease incidence and soil temperature (0.82) was highly significant and positive, similarly correlation coefficient of soil moisture (0.89) was found to be positive and significant. Multiple correlation coefficients between disease incidence and edaphic factors suggested 80.88 per cent Sclerotinia rot incidence was due to these factors. Thus, moderate soil temperature (14.78°C) coupled with moderate soil moisture (25%) were found to favour the disease.

Among different antagonists, *T. harzianum* resulted in maximum inhibition (63.26%) of the pathogen followed by *T. viride* (60.94%) and *T. hamatum* (60.69%). However, inhibition zone was found most prominent with unidentified actinomycetes (6.33 mm) followed by *T. harzianum* (4.17 mm) and *T. viride* (3.83 mm).

Under *in vitro* conditions, garlic extracts (100 and 50%) were found to completely inhibit the growth of pathogen, while the seed extract of sarson was least effective. Among different concentrations of neem and garlic tested under *in vitro* conditions, Neemazal and garlic were found most effective with complete mycelial growth inhibition of the pathogen at 10 per cent concentration, while the garlic was found least effective at 0.25 per cent concentration.

Two fungal antagonists viz. *T. harzianum* and *T. viride*, unidentified actinomycetes and Nemazal were applied against Sclerotinia rot pathogen under a field experiment during the crop season of 2004-05. The results showed that application of Nemazal and *T. harzianum* as root dip and soil application had a significant effect on the average disease incidence to 30.49 and 40.62 per cent, respectively in comparison to the control treatment (55.77%). *T. viride* and unidentified actinomycetes were found least effective and at par in comparison with control. The application of antagonists as well as Nemazal gave higher number of leaves per plant, plant height, stalk length, frame size, curd weight and curd size, however, these were non-significant except for plant height in Nemazal as compared to control treatment.

The screening of five varieties of cauliflower against *S. sclerotiorum* (Sclerotinia rot pathogen) under artificial epiphytotic conditions indicated that almost all the varieties were susceptible to the attack of the pathogen except cauliflower DC-76 which showed resistant reaction.

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Chapter-7

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ABSTRACT

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Title of thesis	:	Studies on Sclerotinia rot of cauliflower and its management through eco-friendly approaches
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Admission Number	:	H-2003-29-M
Major Advisor	:	Dr N P Dohroo
Major Field	:	Mycology and Plant Pathology
Minor Fields	:	1) Vegetable Crops 2) Soil Science and Water Management
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ABSTRACT

The present studies were conducted in the Department of Mycology and Plant Pathology, Dr. Y.S. Parmar University of Horticulture and Forestry, during the years 2004-2005. The field survey during crop season of 2004-05 revealed that the disease was more severe in sub-mountainous region of the state. Young leaves of cauliflower were found most susceptible to Sclerotinia rot. The inoculum dose of 250 mg growth media/ plant in soil was found most suitable for disease development. The fungus produced aerial mycelium, which was hyaline consisting of closely septate hyphae and measured 2.0-9.4 µm. Micro-conidia were produced on conidiophores of the vegetative mycelium and measured 1.5-3.5 µm. Sclerotia were black in colour, round to irregular and measured 2-15x1.5-7 mm. Apothecia were round or globate type with diameter ranging from 2-9 mm. The asci were hyaline and cylindrical and measured 91-165x4.9-8.5 µm. Ascospores were elliptical and ranged from 6.5-13x4.2-6.6 µm. The fungus was found to grow best on potato dextrose agar medium and Richard solution, respectively. Optimum temperature and pH for the growth of the fungus were 20-25°C and pH 5.0, respectively. A positive correlation between edaphic factors viz., soil temperature and soil moisture with disease development was observed. High soil moisture (25%) and low soil temperature (14.78°C) were found to favour the disease development. Among botanical extracts and plant-derived product tested against the Sclerotinia pathogen under *in vitro* conditions, garlic extract and Nemazal were found to completely inhibit the growth of pathogen. *Trichoderma harzianum* gave the maximum inhibition (63.26%) of mycelial growth of the pathogen with prominent inhibition zone (4.17 mm). Under field condition, application of Nemazal and *T. harzianum* had a significant effect on reduction in disease incidence to 30.49 and 40.62 per cent, respectively in comparison to the control (55.77%). The screening of varieties of cauliflower against *S. sclerotiorum* under artificial epiphytotic conditions indicated that cauliflower DC-76 was disease resistant.

Signature of Major Advisor **Signature of the student**

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APPENDICES

APPENDIX-I

COMPOSITION OF SOLID MEDIA

Potato dextrose agar

Potato peeled	250 g
Dextrose	20 g
Agar	15 g
Distilled water	1000 ml

Corn meal agar

Crushed corn	20 g
Agar	15 g
Distilled water	1000 ml

French bean seed agar

French bean seed crushed	40 g
Agar	15 g
Distilled water	1000 ml

Oat meal agar

Oat meal	20 g
Agar	15 g
Distilled water	1000 ml

Pea seed agar

Pea seed crushed	40 g
Agar	15 g
Distilled water	1000 ml

Lettuce leaf agar

Lettuce leaf	40 g
Agar	15 g
Distilled water	1000 ml

Rose Bengal agar

Glucose	10 g
Peptone	5 g
KH_2PO_4	1 g
$MgSO_4$	0.5 g
Rose Bengal	0.03 g
Agar	15 g
Distilled water	1000 ml

APPENDIX-II

COMPOSITION OF LIQUID MEDIA

Richard solution

Potassium nitrate	10.0 g
Potassium monobasic phosphate	5.00 g
Magnesium sulphate	2.50 g
Ferric chloride	0.02 g
Sucrose	50.00 g
Distilled water	1000 ml

Asthana and Hawker medium

Glucose	5 g
Potassium nitrate	3.50 g
Potassium phosphate	1.75 g
Magnesium sulphate	0.75 g
Distilled water	1000 ml

Czapek sucrose nitrate solution

Sodium nitrate	2.00 g
Potassium dibasic phosphate	1.00 g
Potassium chloride	0.50 g
Magnesium sulphate	0.50 g
Ferrous sulphate	0.01 g
Sucrose	30.00 g
Distilled water	1000 ml

Leonian solution

Potassium monobasic phosphate	1.20 g
Magnesium sulphate	0.50 g
Peptone	0.60 g
Maltose	6.25 g
Distilled water	1000 ml

Corn liquid medium

Potassium nitrate	2.00 g
Magnesium sulphate	1.20 g
Potassium phosphate	2.70 g
Maltose	7.20 g
Distilled water	1000 ml

KenKnight medium

Glucose	1 g
KH_2PO_4	0.1 g
$NaNO_3$	0.1 g
KCl	0.1 g
$MgSO_4$	0.1 g
Distilled water	1000 ml

APPENDIX-III

ANOVA TABLES

ANOVA 1: Analysis of variance for radial growth of mycelium in different solid media (Table **4.5**)

Sources of variance	DF	SS	MS	F
Treatment	5	1610.2	322.05	83.24
Treatment x Replication	18	69.642	3.87	
Total	23	1679.9		

ANOVA 2: Analysis of variance for number of sclerotia in different solid media (Table **4.5**)

Sources of variance	DF	SS	MS	F
Treatment	5	2181.4	436.27	111.79
Treatment x Replication	18	70.25	3.90	
Total	23	2251.6		

ANOVA 3: Analysis of variance for dry weight of mycelium in different liquid media (Table **4.6**)

Sources of variance	DF	SS	MS	F
Treatment	4	91724	22931	40.91
Treatment x Replication	15	8408.2	560.55	
Total	19	10013		

ANOVA 4: Analysis of variance for number of sclerotia in different liquid media (Table **4.6**)

Sources of variance	DF	SS	MS	F
Treatment	4	556.50	139.12	22.56
Treatment x Replication	15	92.50	6.16	
Total	19	649.00		

ANOVA 5: Analysis of variance for dry weight of sclerotia in different liquid media (Table **4.6**)

Sources of variance	DF	SS	MS	F
Treatment	4	37095	9273.9	17.95
Treatment x Replication	15	7749.8	516.66	
Total	19	44845		

ANOVA 6: Analysis of variance for radial growth of mycelium at different temperatures (Table **4.7**)

Sources of variance	DF	SS	MS	F
Treatment	5	7498.3	1499.7	143.68
Treatment x Replication	18	187.87	10.44	
Total	23	7686.2		

ANOVA 7: Analysis of variance for number of sclerotia formed at different temperatures (Table **4.7**)

Sources of variance	DF	SS	MS	F
Treatment	5	3339.3	667.87	138.98
Treatment x Replication	18	86.5	4.81	
Total	23	3425.8		

ANOVA 8: Analysis of variance for mycelium dry weight at different pH levels (Table **4.8**)

Sources of variance	DF	SS	MS	F
Treatment	7	18354	26219	116.63
Treatment x Replication	24	5395.2	224.80	
Total	31	18893		

ANOVA 9: Analysis of variance for number of sclerotia formed at different pH levels (Table **4.8**)

Sources of variance	DF	SS	MS	F
Treatment	7	1298.7	185.53	36.13
Treatment x Replication	24	123.25	5.14	
Total	31	1422.0		

ANOVA 10: Analysis of variance for sclerotia dry weight at different pH levels (Table **4.8**)

Sources of variance	DF	SS	MS	F
Treatment	7	'94514	13502	61.69
Treatment x Replication	24	5252.6	218.86	
Total	31	99767		

ANOVA 11: Analysis of variance for per cent inhibition of mycelial growth **(Table 4.12)**

Sources of variance	DF	SS	MS	F
Treatment	14	11266	804.69	75.60
Treatment x Replication	45	479.01	10.65	
Total	59	11745		

ANOVA 12: Analysis of variance for per cent inhibition of mycelial growth (Table **4.13**)

Sources of variance	DF	SS	MS	F
Treatment	11	13.60	1.24	31.37
Treatment x Replication	36	14.42	0.039	
Total	47	15.02		

ANOVA 13: Analysis of variance for per cent inhibition of mycelium growth (Table **4.14**)

Sources of variance	DF	SS	MS	F
Treatment	10	11508	1150.8	504.20
Treatment x Replication	22	50.21	2.28	
Total	32	11558		

ANOVA 14: Analysis of variance for per cent inhibition of mycelium growth (Table **4.14**)

Sources of variance	DF	SS	MS	F
Treatment	10	12551	1255.1	257.07
Treatment x Replication	22	107.41	4.88	
Total	32	12658		

ANOVA 15: Analysis of variance for per cent inhibition of mycelium growth (Table **4.15**)

Sources of variance	DF	SS	MS	F
Treatment	5	5775.5	1155.1	127.56
Treatment x Replication	12	108.66	9.06	
Total	17	5884.2		

ANOVA 16: Analysis of variance for per cent inhibition of mycelium growth (Table **4.15**)

Sources of variance	DF	SS	MS	F
Treatment	5	9684.9	1937.0	107.69
Treatment x Replication	12	215.84	17.99	
Total	17	9900.7		

ANOVA 17: Analysis of variance for disease incidence (Table **4.16**)

Sources of variance	DF	SS	MS	F
Treatment	4	1429.3	357.33	9.13
Replication	3	398.33	132.78	3.39
Treatment x Replication	12	469.66	39.14	
Total	19	2297.3		

ANOVA 18: Analysis of variance for number of leaves (Table **4.16**)

Sources of variance	DF	SS	MS	F
Treatment	4	1.24	0.31	0.67
Replication	3	0.25	0.075	0.16
Treatment x Replication	12	5.53	0.46	
Total	19	6.99		

ANOVA 19: Analysis of variance for plant height (Table **4.16**)

Sources of variance	DF	SS	MS	F
Treatment	4	59.65	14.91	1.34
Replication	3	12.17	4.10	0.36
Treatment x Replication	12	133.68	11.14	
Total	19	205.50		

ANOVA 20: Analysis of variance for stalk length (Table **4.16**)

Sources of variance	DF	SS	MS	F
Treatment	4	0.67	0.17	1.10
Replication	3	1.24	0.41	2.69
Treatment x Replication	12	1.84	0.15	
Total	19	3.75		

ANOVA 21: Analysis of variance for frame size (Table **4.16**)

Sources of variance	DF	SS	MS	F
Treatment	4	153.43	38.36	0.69
Replication	3	332.07	110.69	1.98
Treatment x Replication	12	671.54	55.96	
Total	19	1157.0		

ANOVA 22: Analysis of variance for curd size (Table **4.16**)

Sources of variance	DF	SS	MS	F
Treatment	4	60.44	15.11	1.27
Replication	3	51.23	17.08	1.43
Treatment x Replication	12	143.23	11.94	
Total	19	254.90		

ANOVA 23: Analysis of variance for curd weight (Table **4.16**)

Sources of variance	DF	SS	MS	F
Treatment	4	21.59	5.40	1.20
Replication	3	6.22	2.07	0.46
Treatment x Replication	12	53.88	4.49	
Total	19	81.68		

CURRICULUM VITAE

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Title of thesis	:	Studies on Sclerotinia rot of cauliflower and its management through eco-friendly approaches
Name of the Student	:	Nguyen Duc Cuong
Admission Number	:	H-2003-29-M
Major Advisor	:	Dr N P Dohroo
Major Field	:	Mycology and Plant Pathology
Minor Fields	:	1) Vegetable Crops 2) Soil Science and Water Management
Degree Awarded	:	M.Sc. (Mycology and Plant Pathology)
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ABSTRACT

The present studies were conducted in the Department of Mycology and Plant Pathology, Dr. Y.S. Parmar University of Horticulture and Forestry, during the years 2004-2005. The field survey during crop season of 2004-05 revealed that the disease was more severe in sub-mountainous region of the state. Young leaves of cauliflower were found most susceptible to Sclerotinia rot. The inoculum dose of 250 mg growth media/ plant in soil was found most suitable for disease development. The fungus produced aerial mycelium, which was hyaline consisting of closely septate hyphae and measured 2.0-9.4 µm. Micro-conidia were produced on conidiophores of the vegetative mycelium and measured 1.5-3.5 µm. Sclerotia were black in colour, round to irregular and measured 2-15x1.5-7 mm. Apothecia were round or globate type with diameter ranging from 2-9 mm. The asci were hyaline and cylindrical and measured 91-165x4.9-8.5 µm. Ascospores were elliptical and ranged from 6.5-13x4.2-6.6 µm. The fungus was found to grow best on potato dextrose agar medium and Richard solution, respectively. Optimum temperature and pH for the growth of the fungus were 20-25°C and pH 5.0, respectively. A positive correlation between edaphic factors viz., soil temperature and soil moisture with disease development was observed. High soil moisture (25%) and low soil temperature (14.78°C) were found to favour the disease development. Among botanical extracts and plant-derived product tested against the Sclerotinia pathogen under *in vitro* conditions, garlic extract and Nemazal were found to completely inhibit the growth of pathogen. *Trichoderma harzianum* gave the maximum inhibition (63.26%) of mycelial growth of the pathogen with prominent inhibition zone (4.17 mm). Under field condition, application of Nemazal and *T. harzianum* had a significant effect on reduction in disease incidence to 30.49 and 40.62 per cent, respectively in comparison to the control (55.77%). The screening of varieties of cauliflower against *S. sclerotiorum* under artificial epiphytotic conditions indicated that cauliflower DC-76 was disease resistant.

Signature of Major Advisor **Signature of the student**

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