Perpetuation and Management of *Alternaria solani* (Ellis and Martin) Jones and Grout Causing Early Blight of Potato in Kashmir

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(2007-193-D)

Division of Plant Pathology

Faculty of Postgraduate Studies

Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir

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Perpetuation and Management of *Alternaria solani* (Ellis and Martin) Jones and Grout Causing Early Blight of Potato in Kashmir

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Thesis

Submitted to

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in partial fulfilment of requirement for the award of the degree of

Doctor of Philosophy in Agriculture
(Plant Pathology)

2012


Dedicated
to my beloved Family

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Division of Plant Pathology, Shalimar Campus, Srinagar
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CERTIFICATE – I

This is to certify that the thesis entitled **"Perpetuation and Management of *Alternaria solani* (Ellis and Martin) Jones and Grout Causing Early Blight of Potato in Kashmir"** submitted in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy in Agriculture (Plant Pathology)**, to the Faculty of Postgraduate Studies, Sher-e-Kahsmir University of Agricultural Sciences and Technology of Kashmir, is a record of bonafide research work carried out by **Mr. Shabeer Ahmad Ganie (Regd. No. 2007-193-D)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that information received during the course of investigation has duly been acknowledged.

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CERTIFICATE – II

We, the members of the Advisory committee of **Mr. Shabeer Ahmad Ganie (Regd. No. 2007-193-D)**, a candidate for the degree of **Doctor of Philosophy in Agriculture (Plant Pathology)**, have gone through the manuscript of the thesis entitled, **"Perpetuation and Management of *Alternaria solani* (Ellis and Martin) Jones and Grout Causing Early Blight of Potato in Kashmir"** and recommend that it may be submitted by the student in partial fulfilment of the requirements for the award of degree.

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Title of the Thesis : **"Perpetuation and Management of *Alternaria solani* (Ellis and Martin) Jones and Grout Causing Early Blight of Potato in Kashmir"**

ABSTRACT

The present study on perpetuation and management of *Alternaria solani*, causing early blight of potato in Kashmir was conducted during 2008 to 2009. An extensive survey conducted in four districts of Kashmir Valley viz., Budgam, Baramulla, Srinagar and Shopian revealed prevalence of early blight of potato in all the localities surveyed. Mean disease incidence and intensity of 24.54 and 13.84 per cent were recorded in the year 2008 and that of 28.23 and 15.98 per cent during the year 2009, respectively. The disease was characterised by small irregular to circular dark brown spots on the lower leaves measuring approximately 0.5 mm in size. The spots on coalescing formed irregular patches, which later on resulted in complete blighting of leaves. The causal fungus was isolated and identified as *Alternaria solani* (Ellis & Martin) Jones and Grout. The maximum apparent infection rate of 0.155 and 0.165 unit/day was recorded during the second fortnight of June in 2008 and 2009, respectively. Weather factors viz., temperature, relative humidity and rainfall were positively correlated with disease intensity (66.50 per cent contribution). The pathogen perpetuated as mycelium and

conidia throughout winter on diseased leaves left on the ground surface and on diseased potatoes kept in ambient store. The number of spores cm^{-2} leaf area and the viability of spores decreased with increase in depth of placement in soil. Maximum spores production on overwintered leaves and potatoes were observed during first fortnight of June. Disease tolerance of varying degree was observed under natural conditions in the available potato germplasm. Cultivar SM/92-338 proved tolerant to *A. solani* while as, cultivars Kufri himami, SM/96-127 and SM/94-44 showed moderate tolerance to the disease compared to rest of the 21 test cultivars. Evaluation of bioagents revealed that all the test bioagents were significantly effective in inhibiting the mycelial growth of *A. solani* under dual culture method. *Trichoderma harzianm* exhibited maximum mycelial growth inhibition of 71.85 per cent followed by *T. viride* (65.93%) and *Trichoderma virens* (58.65%). Evaluation of plant extracts revealed that all the test extracts were significantly superior in inhibiting the mycelial growth of *A. solani* at various test concentrations. *Datura stramonium* exhibited maximum mycelial growth inhibition of 61.12 per cent followed by *Artimesia absinthium* (58.54%), *Juglans regia* (38.31%), *Mentha spicata* (38.02%) and *Uretica dioica* (37.34%). Systemic fungitoxicants and non-systemics were tested *in vitro* for mycelial growth inhibition at (100-350 ppm and 1000-3500 ppm, respectively). Among the systemic fungitoxicants hexaconazole (0.03 %) followed by fenconazole (0.03%) proved significantly superior at all tested concentration, whileas mancozeb (0.2%) followed by propineb (0.2%) proved significantly superior in inhibiting mycelial growth the individual and combined effect of most effective *in vitro* fungitoxicant, bioagent and botanicals was also evaluated for the management of potato early blight in field. Among various treatment combinations seed treatment with mencozeb (0.2%) + foliar spray with hexaconazole (0.03%) + foliar spray with datura (50%) + foliar spray with *T. harzianum* (1×10^7 spores ml^{-1}) proved significantly superior.

Key words : *Alternaria solani*, management, early blight, perpetuation, bioagents, plant extracts, fungitoxicants

Signature of Student

Dated : _____

Signature of Major Advisor

Dated: _____

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"Success is the journey not the destination in synthesis"

"Nothing worth is ever achieved without deep thought and hard work"

"Give me a point of support, I will rotate the entire world" This point of support is almighty Allah who showered his best of zeal, enthusiasm, dedication, perseverance, vigour and wit with a thick positive outcome. So it is the time to bow down my head in his praise and laud "He who wishes to fulfil his mission in the world must be a man of one idea that is of one great overmastering purpose, overshadowing all his aims and guiding and controlling his entire life"

All is possible to him that believeth, who a definite goal has backed by the determination to achieve it, no matter what the odds or obstacles. The world will stand aside and let pass the man who knows where he is going and who strides majestically with steps firm faith undertaken. Where there is will there is always a way and an optimistic and enthusiastic heart will definitely find that way or make it.

Diligence and moderation are best steps to climb to excellence and reach the top. The heaven do not send their rains in floods but in tiny drops. An individual is neither wise, nor rich nor great at once. But by softly treading the path and firmly planting his steps his climb will be certain and advance sure. It is wisely said that the climb to the top is not a walk over, but a walk up.

Research is an evolving concept. Any endeavour, in this regard is challenging as well as exhilarating. It implies the testing of our nerves. It brings to light our patience, vigour and dedication. Every result arrived at is a modest beginning for a higher goal and no work can be termed as a one-man show. It needs the close cooperation and guidance of experts in the field to achieve something worthwhile and substantial.

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Shabeer Ahmad Ganie

Place : Shalimar, Srinagar

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Chapter – 1

INTRODUCTION

Potato, also known as white or Irish potato, is the most important and useful member of the family Solanaceae and is grown in tropics as well as sub-tropics during the cool as well as dry seasons. It belongs to the genus *Solanum*. The genus comprises of about 2000 species, of which 235 are tuber bearing. The cultivated potato (*Solanum tuberosum* L.) originated from Andean highlands of South America, was disseminated to other continents by Europeans. It is believed to have been introduced in India towards the beginning of seventeenth century most probably by the Portuguese traders or by British missionaries (Pushkarnath, 1976). It is a major world food crop ranking fourth after rice, wheat and maize (Shekhawat, 2001; Bowen, 2003). Potato is cultivated in 0.5 per cent area in the country and contributes approximately 2 per cent of the total output of agriculture. In India, 29.2 million tones of potato is produced from an area of 1.50 million hectares with productivity of 19.5 t/ha (Anonymous, 2006a). Potato produces more food per unit area and production. Today, India ranks fourth in the area and fifth in the production of potato in the world (Shailbala and Pathak, 2008). The year 2008 was celebrated as "International Potato Year" by United Nations Organization dated 18th October, 2007 (Shailbala and Pathak, 2008).

Potato is considered 'The King' of staple foods. It is the only non cereal nutritious food crop which commands a significant position in the world for possessing all the attributes to be a potential food crop. It can solve the problem of malnutrition and under nutrition, if adopted as a major food crop. It has been recognized as a wholesome food and richest source of energy in most countries of the world where it forms important part of the human diet. Potato contains significant levels of phenolic compounds and vitamin C as potent antioxidants (Brown, 2005). These compounds reportedly inactivate reactive oxygen species, reduce oxidative damage, lead to improved immune functions and reduce risk of

cardiovascular diseases, cancer, cataract, diabetes and aging (Kour *et al.*, 2004). It is one of the efficient source of starch producing plants and yield more carbohydrates per unit area and time, is rich in protein, minerals, vitamins and high quality dietary fibre. Potato protein is superior to that of the cereals being rich in "lysine", an essential amino acid. Potatoes are also used as feed for livestock and in the industry for the manufacture of starch and alcohol.

Potato is highly remunerative and nutritive crop in Jammu and Kashmir, particularly in high altitude cold and cold arid areas, where it serves as a staple food. The area under potato has increased from 5363 hectares in 1996-97 to 7045 hectares during 2005-2006 with a concomitant increase in production from 75472 to 105127 tones during the same period. In Kashmir, potato is grown over an area of 1.7 thousand hectares with a production of about 22.1 thousand tones (Anonymous, 2006b). Although it is being grown in Kashmir for many years but only few varieties are in cultivation which are either poor yielders, low in quality/and or susceptible to various pests and diseases.

The intensive and extensive cultivation, even under the most favourable environmental conditions for potato crop production in the state, failed to provide significant strides in potato yields. Amongst the various production constraints, Frequent occurrence of may fungal diseases viz., Early blight (*Alternaria solani*), Late blight (*Phytophthora infestans*), Powdery scab (*Spongospora subterranean*), Wart (*Synchytrium endobioticum*), Leaf black (*Cercospora concors*), Fusarium Wilt (*Fusarium solani* f.sp. *radicicola*), Black scurf (*Rhizoctonia solani*) and Charcoal rot (*Macrophomina phaseolina*) are note worthy and have been taking heavy toll of the produce.

Early blight of potato, caused by *A. solani* (Ellis and Martin) Jones and Grout has been recognized as a threat since its first occurrence in Wisconsin in 1892 (Jones, 1893 and Rands, 1917). In India, *A. solani* on leaves of potato was first reported from Farukhabad (U.P) by Butler in 1903 (Butler and Bisby, 1931). In Kashmir it was first reported from Srinagar in 1957 (Koul, 1957).

Early blight of potato is one of the most important foliar disease of potato worldwide (Christ, 1990; Pelletier and Fry, 1990; Shtienberg *et al.* 1990; Vander-Walls *et al.* 2001). In recent years, the extent of disease has been reported from various potato growing areas (Vloutoglou and Kalogerakis, 2000). Primary damage by early blight is mainly attributed to premature defoliation of the potato plants, resulting in reduction of tuber yield. Yield loss estimates resulting from foliar damage incited by *A. solani* on potato vary by location, cropping season, cultivar, and the stage of potato maturity. In general, yield reductions of 5-40 per cent have been reported in Israel (Rotem and Feldman, 1965) and 20-30 per cent in the USA (Christ and Maczuga, 1989; Shtienberg *et al.* 1990). The pathogen can also attack potato tubers and produce shallow, dry and corky rot, reducing both the quantity and quality of marketable tubers (Nnodu *et al.,* 1982). Environmental factors such as temperature, wetness duration and relative humidity (moisture) affect the spore germination, infection and development of early blight on potatoes (Harrison *et al.* 1965; Adams and Stevenson, 1990; Vloutoglou and Kalogerakis, 2000). Early blight is also enhanced through continuous potato cultivation (Olanya *et al.* 2009). The young plants of potato show high resistance to *A. solani* as compared to older ones (Bambawale, 1978). Within the same plant, the lower leaves which are physiologically different from middle and top ones (Dowley *et al.,* 1975) are more susceptible to certain pathogens with resistance increasing in an aeropetal direction. The symptoms first occur on lower older leaves, which become chloratic and abscise prematurely. Excessive defoliation may lead to death of the plant and consequent yield loss.

Early blight of potato overwinters and survives as conidia or mycelia on buried host debris and potato tubers, particularly in fields with poor cultural practices such as continuous cropping of tomatoes or potatoes. Survival for several years was reported for *A. solani* from potato (about 10 years, under some conditions) (Rotem, 1968).

Under Kashmir conditions, early blight of potato is posing a great threat for its cultivation. The systematic study on potato early blight has not been conducted so far under Kashmir conditions. Therefore keeping in view the devastating nature of disease a detailed investigation about the causal fungus/disease and its management was undertaken with the following objectives:

- To assess the status of the disease in the major potato growing areas of the Kashmir valley.
- To study the perpetuation of the causal organism.
- To study the role of meteorological parameters on disease development and
- To devise the management programme of the disease.

Chapter – 2

REVIEW OF LITERATURE

2.1 History

Early blight of tomato was first described by Ellis and Martin (1882) from potato leaves and the pathogen was named as *Macrosporium solani* Ellis and Martin. Later, Jones and Grout, 1897 transferred it to genus *Alternaria* because they found spores in chains of two in some of the cultures. Rands (1917) reported that the pathogen was responsible for inciting leaf and stem diseases both on potato and tomato. Later, Tisdale (1932) noticed that *Alternaria solani* attacked petioles, peduncle and fruits of tomato. This was probably the first record of the fungus attacking on the fruits. Early blight was first recorded in the epidemic form on tomatoes from Southern England in 1944 and later form Bulgaria in 1958 (Glasscock and Ware, 1944, Elenkov, 1958). In India, *A. solani* (Ellis and Mart.) Jones and Grout on leaves of potato (*Solanum tuberosum* L.) was first reported from Farukhabad (U. P) by Butler in 1903 (Butler and Bisby, 1931) whileas on tomato, it was recorded by Chona *et al.* (1958). The pathogen *A. solani* attacks both potato and tomato but the potato isolates produce less severe symptoms on tomato (Jones and Darling, 1953).

Apart from *A. solani* as cause of early blight there are many reports in the literature regarding the association of *Alternaria alternata* with the disease. Marcinkowaska (1982) isolated *A. alternata* more often than *A. solani* from tomatoes infected with early blight in Central Poland. Martinez *et al.* (2004) using AFLP analysis characterized various isolates of *Alternaria* associated with early blight of potato and tomato as *A. solani, A. porri* and *A. alternata.* Kuczynska (1992) predominantly recorded association of *A. alternata* from blight affected leaves and stems of potato. Both *A. alternata* and *A. solani* have also been reported to be cause of early blight of potato in Germany (Hausladen and Basselers, 2004). Likewise, association of *A. solani* and *A. alternata* has been

reported to be the cause of early blight of tomato in Himachal Pradesh (Sahi and Shyam, 1993; Sood and Sharma, 2004) and in Kumaon hills of Uttar Pradesh in India (Bhatt *et al.* 2000).

2.2 Disease status

Extensive surveys were conducted in Haryana, India from 1993-94 to 2002-03 to investigate the health status of potato crops in the region. Late blight (*Phytophthora infestans*) and early blight (*A. solani*) infections were recorded at crop maturity. The disease intensity or incidence of early blight and late blight varied in the range 2.2 – 8.6 and 1.8 – 61.2 respectively (Lakra, 2004). In 3 years field studies the severity of early blight (*A. porri* f. sp. *solani*) infection in 9 tomato cvs was reliably assessed by counting the leaves with over 75% necrotic area. The small areas with lesions on the remaining leaves seldom exceeded 6% of the total foliage surface (Basu, 1974) Wu (1979) reported results of surveys of onion, radish, cabbage, Chinese cabbage and black salsify. *A. porri* and *Stemphylium botryosum* reduced germination in onion. *A. brassicicola* was the commonest pathogen of cruciferous plants and their seeds. Hosagoudar *et al.* (2008) conducted survey in areas of Dharwad, Haveii, Belgaum, Bagalkot, Gadag, Bellary, Raichur and Gulbarga districts of North Karnataka to assess the incidence of diseases in farmers fields and Agricultural Research Stations. A survey for senna plantation and prevalence of leaf blight infestation caused by *A. alternata* was conducted in Jaipur, Nagpur, Bikaner, Jodhpur and Jaisalmer, Rajasthan, India, during the 2004 and 2005 wet seasons. The maximum mean disease intensity was recorded in Jaipur followed by Nagaur and Jodhpur(Tetarwal and Rai (2007). Survey conducted in carnation growing areas revealed that leaf spot and wilt were the most prevalent diseases of carnation in Kashmir. The extent of the two diseases varied from 2.1 to 26.9 and 3.7 to 14.9% respectively, in various locations (Qazi *et al.* 2006). Khan *et al.* (2007) conducted a field survey to monitor and assess the occurrence, incidence and intensity of *Alternaria* blight (caused by *A. brassicae*) on rapeseed during vegetative and podding stages in 8

locations in Aligarh Uttar Pradesh, India. Maximum occurrence was noted at University farm (80%), followed by Asadur (60%). Maximum incidence was also noted at University farm (93.3%), followed by Panjipur (86.7%). However disease severity was recorded to its maximum in Panjipur (73.35), followed by University farm (62.5%). Gorawar *et al.* (2006) conducted a survey of foliar diseases of turmeric in northern Karnataka during 2002-03. The incidence of leaf spot (caused by *Colletotrichum capsici*) was higher than that of leaf blight (caused by *A. alternata*). The incidence of leaf blight was greatest in Rannabelagali (66.60%) of Bagalkot, while the incidence of leaf spot was greatest in Salebiranhalli (50%) in Gulbarga district. A mycological survey carried out a Entre Rios province, Argentina on sorghum grain, maize, rice, soyabean seeds and on freshly harvested and stored wheat suggested that *A. alternata* was the major fungal species isolated from sorghum, rice, soyabean seeds and on freshly harvested wheat (Broggi *et al.* 2007). Shazia and Iftikhar (2005) conducted a survey for assessment of foliar blight of wheat in main rice-wheat cropping areas of Punjab, Pakistan. Prevalence of foliar blight was 100% in the four surveyed districts. An intensive roving survey was conducted during Kharif 2000 in the districts of Raichur, Gulbarga and Dharwad in Karnataka, India, to assess the incidence and severity of early *Alternaria* blight disease of tomato. The highest disease severity was recorded in Raichur (52.55%), followed by Dharwad (47.87%) and Gulbarga (39.39%) (Prasad and Naik 2004). A survey was conducted in experimental and commercial onion (*Allium cepa* L.) fields in the southern region of Puerto rico to isolate and identify fungi associated with this crop. In onion foliage, *Alternaria* was the most common genus, followed by *Stemphylium* and *Nigrospora* (Velez *et al.* 2004). Deara *et al.* (2004) observed a disease incidence of 49.5 per cent of tomato early blight of tomatoes caused by *A. solani* during 2000 and 2001 in Punjab. Mandal *et al.* (2002) reported the occurrence of severe blight in Kalazira growing belts. The pathogen, identified as *A. brunsii* resulted in substantial yield losses.

2.3 Morphology

Early blight of potato was first described by Ellis and Martin (1882) from potato leaves and the pathogen was named as *Macrosporium solani* Ell and Mart. Later Jones and Grout (1897) transferred it to genus *Alternaria* because they found spores in chains of two in some of the cultures. Cells of *A. solani* are multinucleate, but different organs vary in the number of nuclei. Heterokaryosis is suspected (Stall, 1958). Hyphal branching originates from the tip cells, from which the branches also receive nuclei (King and Alexander, 1969). According to M.B. Ellis (1971), the solitary and beaked conidia have 9 to 11 transverse septa and a few or no longitudinal or oblique septa, and the dimensions of the spore (width at the broadest part by length of the body and beak) range from 15 to 19 μm by 150 to 300 μm. The morphological, physiological and pathogenic variability of *A. solani* has given rise to claims of the existence of races. Some researchers defined races according to cultural characteristics of various isolates, dimensions of spores, sporulation capacity and virulence (Bonde, 1929; Neergaard, 1945; Rowell, 1953). Cultural characteristics (color, growth, sporulation etc.) also differ in various isolates, making it possible to find almost as many races as the number of isolates tested (Rotem, 1966). Virender Kumar *et al*. (2008) reported that pigmentation varied from yellow, brown, black, brownish to greenish black in isolates of *A. solani* on potato dextrose agar medium. Most of the isolates showed smooth mycelial growth with circular and irregular margin and without concentric zonation. Ellis and Gibson (1975) reported that isolates of *A. solani* grow well on nutrient media. Colonies are dense and have been described as spreading, hairy or possessing a texture similar to cotton, felt or velvet. Some isolates produce a water soluble yellow to red pigment on nutrient media. Neergaard (1945) disregarded morphological differences between *A. porri*, *A. solani* and *A. dauci* and distinguished all three from *A. brassicae* by the absence of chains.

3.4 Meteorological factors

A number of weather factors have been reported to favour the development of early blight on potato (Rands, 1917; Gratz, 1930; Wager, 1945). Contradicting evidences of weather dependence are also available. Harrison *et al.* (1965) observed that the severity of the disease on potato was same in cool and dry weather as it was under warm and moist conditions. Bambawale and Bedi (1982) found that moderate mean temperature (13.6 – 23.6^{o}C), inadequate rains but longer periods of RH > 80%, sufficient moisture in the form of dew and shorter photoperiods under the Punjab conditions led to faster development of the disease. Holley *et al.* (1985) reported negative correlation of air temperature with the early blight intensity and identified duration of leaf wetness as an important contributing work, average dew (mm) had not significant influence on disease development. A minimum period of leaf wetness/saturated atmosphere is essential for successful infection by the pathogen and futher development of the disease. A minimum period of 4 hr of high humidity is required for inducing disease in tomato plants and disease development increased but not proportionally, as the hours of humidity was increased (Moore, 1942). Similarly, Pelletier and Fry (1989) reported that the incidence in leaf wetness duration from 6 to 24 hr lesions caused by *A. solani* on potato plants increased in a linear manner. Dragomir (1995) reported that relative humidity of more than 90 per cent and presence of free moisture on tomato leaves for more than 2 hr per day favoured the disease. Vloutoglou and Kalogerakis (2000) reported that 4 hr leaf wetness after inoculation was sufficient to initiate disease on the plants. As leaf wetness duration increased upto 24 hr, there was an increase in the percentage of leaf area infected and per cent defoliation, but thereafter there was no significant increase in either parameter. Leiminger *et al.* (2005) reported symptoms and damage to potato plants by *A. solani* and *A. alternata.* Plants were treated with maneb fungicide. The effects of weather (especially rain and temperature) on fungal spores showed greater spread during humid warm weather. For instance, the high

temperatures claimed to favour epidemics of *A. solani* in winter tomatoes in Morocco (Berger, 1937) refer to temperatures higher than those regularly prevailing in the winter; in this system the epidemics were also supported by high humidity. A summary of all the records suggests that in the majority of cases the most severe epidemics were associated with a daily maximum of 28-32^{o}C, but wet conditions were always necessary for the epidemics to occur. A correlation between potato early blight and relatively low temperatures was also found in Ontario, where there was an inverse correlation between temperature and wetness; cooler locations had longer period of moisture (Holley *et al.*, 1985), which facilitated epidemics. Free moisture facilitates epidemics under all temperature conditions, but the higher the temperature, the quicker the spread of the epidemics. Epidemics caused by *A. melongenae* (probably *A. solani*) on egg plants in India are affected by moisture more than by temperature, but they develop best in the hot monsoon season (Rangaswami and Sambandam, 1961). An analysis of epidemics caused by *A. alternata* and *A. macrospora* on cotton in China showed that although years with high temperature were most conducive to disease (Ling and Yang, 1941) the years with blight attacks and those with none differed in humidity conditions rather than in seasonal fluctuations of temperature (Ling, 1944). Rain was claimed to be the main moisture factor in disease development in dew-deficient areas or season, as in *A. solani* on potatoes in Punjab, where dew is absent during most of spring (Bambawale and Bedi, 1982). The best known example of the effect of rain on *Alternaria* disease is potato early blight in Bermuda, which destroyed the crop within 3-4 weeks (Whetzel, 1923). Norse (1973) emphasized the importance of morning and evening showers that prolong the wetting caused by dew and denied the importance of infection. Of the various methods of irrigation, sprinkling is the most conducive to disease, because it wets the foliage and splash disperses spores (Rotem and Palti, 1969).

In an analysis based on measurements of weather factors in the field, the apparent infection rate of *A. solani* on potatoes in Canada was related to the

duration of leaf wetness, temperature, and the susceptibility of cultivars. The dominant factors were cultivar susceptibility and the duration of wetness, rather than temperature (Holley *et al.* 1985). Epidemics caused by *A. solani* and *A. alternata* in the Negev Desert are supported by sand storms, which maximize spore dispersal, wound the host; increase host susceptibility, and are regularly succeeded by periods of heavy dewfall (Rotem, 1965, 1981). In many other habitats in which sandstorms do not occur, a combination of other environmental factors leads to the spread of epidemics. Thus, tomato early blight in Hungary (Hodosy, 1968) and potato early blight in South Africa (Wager, 1945) and Belgium (Roth, 1936) have been associated with dry and hot days and dewy nights. In cooler Byelorussia, the effect of dew was supported by heat and rain (Dorozhkin and Ivanyuk, 1979).

2.5 Perpetuation of Pathogen

A. solani overwinters and survives as conidia or mycelia on buried host debris and potato tubers, particularly in fields with poor cultural practices such as continuous cropping of tomatoes or potatoes (Rotem, 1968). Survival for several years was reported for *A. solani* from potato (about 10 years, under some conditions) (Rotem, 1968). In *A. solani*, conidia required desiccation as a pre-requisite to chlamydospore transformation of cells. In contrast, chlamydospore production in hyphal cells was inhibited by desiccation (Patterson, 1991), a phenomenon that should be retested in view of the better survival of desiccated mycelium, compared to turgid mycelium. Patterson reported that infective chlamydospores persisted in soil for 10 months and are a factor in the long term survival of this pathogen (Patterson, 1991). The conidia of *A. solani* are capable of surviving freezing weather on the soil surface or when buried to a depth of 5-20 cm (Rands, 1917). The first infections of the new crop are produced from overwintering inoculums. Short rotations and continuous cropping intensify the amount of initial inoculums and contribute to the increasing importance of early blight (Manzer and Merriam, 1974). In Israel, oversummering of *A. solani* in

potato and tomato debris and overwintering of *A. macrospora* in cotton were much more successful (lasting upto 8 months) in debris deposited on the soil surface than in debris buried in the soil (Rotem, 1968, 1990). Other *Alternaria* have also been reported to over season better in debris present on the soil surface than that buried underground. For instance, in Punjab, *A. porri* survived 8 months in debris left on the soil surface but only 2 months in debris buried in the soil (Pandotra, 1965). Similar conclusions pertain to the over seasoning of *A. dauci* (Netzer and Kenneth, 1969) and *A. radicina* (Kuprashvili, 1973) on carrots. Oversummering of *A. tenuissima* on pigeon pea debris on the soil surface in India was successful despite soil surface temperatures of upto 45^{o}C (Singh and Fazili, 1988).

The differences between survival on the soil surface and survival under the soil surface derives from differences in environmental and biotic conditions in the two habitats. In particular, the soil surface is drier than the soil below and less microbial activity occur at the soil surface. These and other effects were studied in the oversummering of *A. solani* in different plot covering the site of a diseased winter tomato field (with sandy sand) in the rainless Negev Desert (Rotem, 1968). Mycelium of this fungus was more resistant than spores and survived under dry conditions at temperatures higher than those permitting growth. For example, mycelium survived for 16 months at 40^{o}C, under the same conditions spores survived for only 10 months (Rotem, 1968). In the hot and humid summer season in India, the longest periods of survival of *A. triticina* in debris and in wheat seeds were 4 and 10 months respectively (Vijaya Kumar and Rao, 1979a). In the cooler climate of Yugoslavia, debris rather than seeds supported overwintering of *A. helianthi* on sunflower (Islam and Maric, 1980). In the United Kingdom, improved application of fungicides to flowering brassicae and a fungicide seed dressing reduced the incidence of infection by seed borne *A. brassicae* and *A. brassicicola*, and infested debris became the main source of inoculums of these pathogens (Humpherson-Jones, 1989).

2.6 Disease management

2.6.1 Screening

Plants with little foliage and high yield are highly susceptible, where as plants with abundant and lower yield are relatively resistant. For the same reason, late-maturing potatoes and delayed fruiting tomatoes are more resistant than early maturing plants (Horsfall nad Houberger, 1942; Douglas and Pavek, 1992). Pelletier and Fry (1989), 1990) identified the components of resistance in three potato cultivars as length of the incubation period, the lesion expansion rate, spore production and the receptivity of the tissue to infection. Some of these components were found to be age dependent. Caligari and Nachmias (1988) based the suitability of potato lines for breeding on the association between sensitivity to disease and the period of yield bulking. Holley *et al*. (1983) evaluated resistance in potato according to apparent infection rate calculated from logit-transformed data on percent disease. Comparing several assessment methods, Christ (1991) concluded that disease severity in the middle third of the potato plant canopy provides the best information about resistance. Ivanyuk (1986) evaluated resistance according to lesion diameter and sporulation intensity on various cultivars. Hanneman (1989) selected a series of resistant accessions from the collection of the Inter Regional Potato Introduction Project (IR-1), in Winconsin. Individuals with a relatively high level of field resistance were found among wild diploid potatoes in the collection of the International Potato Center (IPC) in Lima, Peru. Breeding these plants with tetrapoloid species resulted in a few early maturing but resistant progenies (Jhompson and Mendoza, 1984; Mendoza, 1989). Johanson and Thurston (1990) through that lines with varying maturity are variety of the same genotype and pointed to the difficulty of selecting cultivars that are early maturing as well as resistant. In India, Saharan and Kadian (1983) measured resistance to *A. brassicae* in eight rapeseed and mustard cultivars according to the frequency and size of stomata, number and size of lesions, number of spores produced, and length of the incubation and latent periods. Demir and Levent

(2002) reported that reaction of some potato cultivars with different maturity features against early blight disease, caused by *A. solani*. Early maturity (Resy), middle early maturity (Marfona, Pasinler 92 and Granola), middle late maturity (Agria) and late maturity (Caspar) cultivars of potato were chosen to determine their reaction to *A. solani*. The middle early maturity cultivars were determined as more susceptible than the middle late maturity cultivar. In a study of 60 varieties, Suevia and Fidello showed field resistance to *P. infestans* and *A. solani*. Korneliya, Priekul, silk urozhainyl (Priekuli productive) Przdownik (leader), Fina and Charivnytsya (Enchantress) showed field resistance to one or other of the fungal diseases and relative resistance to virus diseases (Aleksandrov, 1973). Components of early blight resistance were quantified in leaves of different ages in four potato cultivars. The components of resistance : incubation period (IP), lesion number (LN), early bight severity, lesion expansion rate (LER), latent period and spore production by lesion area (SPLA) were evaluated separately in the lower, middle and upper leaves of four potato cultivars. Analysis according to plant part suggest that evaluations on leaves of the middle third part are most suitable for screening for early blight resistance in potato (Rodriguez *et al*., 2006).

In tests of 131 clones, early clones were susceptible to foliar infection by *A. solani* and quite resistant to tuber infection, but the opposite was true for late clones, which apparently possessed true genetic resistance (Douglas and Pavek, 1972). Moderate resistance to *A. solani* and *P. infestans* in the lab was found in EXA/645, EXA/667, EXA/742 and EXA/762 of the andigena group, which were also resistant to phoma spp. (Prasad and Nagaich, 1983). Rodriguez *et al*. (2007) observed that of the 45 lines selected and subsequently evaluated under conditions of natural infection in the green house six showed lesser degrees of early blight infection than the cv. Desiree control. Khan *et al*. (2001) observed that of the twenty five potato cultivars/advanced lines screened against the early blight disease, none of the commercially grown varieties was resistant to the disease. Sixteen cultivars/lines were susceptible. Six cultivars/lines exhibited highly

susceptible response. Naik *et al.* (1999) reported that among thirty potato genotypes from the all India coordinated research project on potato screened for reaction to early blight (*A. solani*), four genotypes (H222, JV62, KX123 and JX214) were highly resistant across all three years.

2.6.2 Bio-management of leaf spot of potato

Biological control is the reduction of the amount of inoculum or disease producing activities of a pathogen or parasite in its active or dormant stage by one or more organisms accomplished naturally or through manipulation of host, environment or antagonist or by mass introduction of one or more antagonists (Baker and Cook, 1974). Cook (1987) defined biological control as the use of natural or modified organisms, genes or gene products to reduce the effect of pests and diseases. At present biological control is an important component of plant disease management practices. The demand for alternatives to chemical control of plant pathogens has become stronger owing to the concerns about the safety and environmental impacts of chemicals (Ooijkaas *et al.* 1998). Several workers have reviewed the various developments in biological control of plant pathogens (Papavizas, 1985; Cook, 1993; Mukhopadhyay, 1994; Jayraj and Ramabadron, 1996; Dube, 2001; Ashwani *et al.,* 2004; Senhilvel, *et al.,* 2005; Shalini and Dohroo, 2005; Harman, 2006).

Several fungi have been reported as good biocontrol agents of plant pathogenic fungi such as *Chaetomium globosum, Caniothyrium minitans, Trichoderma spp, Peniophora gigantean, Pythium olegandrum, Aspergillus niger, Penicillium spp, Gliocladium virens* etc. Among these fungi *Trichoderma* species have greater attention with regard to their role in controlling root rots (Elad, 2000).

Various strains of *Trichoderma* species have been found to be strong opportunistic invaders, fast growers and prolific producers of spores and antiobiotics (Whipps and Lumsden, 2001). These properties make them

ecologically most suitable biocontrol agents and these strains have been found in agricultural, native prairie, forest, salt marsh and desert soils of all climatic zones (Monte, 2001). Different species/strains of *Trichorderma* when incorporated into the soil and/or applied to seeds have been found more effective against *Fusarium oxysporum* causing wilt of different forest plants (James, 1985; Le Bihan *et al.* 1997).

2.6.2.1 Mass multiplication of biocontrol agents

Kousalya and Jeyarajan (1990) tested several substrates for mass multiplication of antagonistic fungi. They found that well decomposed farmyard manure, tapioca rind, wheat bran and groundnut shell were superior for mass multiplication of *T. viride* and *T. harzianum*. Several workers have reported the effective substrates for mass multiplication of antagonists which include wheat-bran saw dust medium for *T. harzianum* and *T. viride* (Hadar *et al.,* 1979; Sharma, 1994), farmyard manure and groundnut shell for *Trichoderma* spp (Sangeetha and Jeyarajan, 1993) and wheat bran, farm yard manure and dung for *T. viride* (Jahangirdar *et al.,* 1998).

2.6.2.2 Mechanism of biocontrol

Trichoderma is known for its ability to act as biocontrol agents against plant pathogens. Since 1920's until recently the principal mechanisms for biocontrol are assumed to be mycoparasitism, antibiosis and competition for resources and space. However, recent advances demonstrate that *Trichoderma* influence the host plants and induce systemic or localized resistance (Harman, 2006). A new set of models of mechanism for *Trichoderma* action includes the inhibition of enzymes necessary for pathogens to penetrate plant surfaces (Zimand *et al.,* 1991) as well as competition for nutrients, necessary for the germination of pathogenic propagules present in the vicinity of planted seeds (Howell, 2003). *Trichoderma* apparently always produce low levels of an extracellular exochitinase enzyme. Diffusion of this enzyme catalyses to cell wall fragments

action of target fungi and this, in turn, induces the expression of fungitoxic cell wall degrading enzymes, that also diffuse and initiates the attack on target fungi before establishment of any contact (Zeilinger *et al.*, 1999, Viterbo *et al.*, 2002). Evidence reveal that *Trichorderma* virens produces chitinolytic enzymes (Dipietro *et al.*, 1993), antibiotics (Hawell *et al.*, 1993; Wilhite *et al.*, 1994) and various toxic metabolites (Howell and Stipanovic, 1984) which inhibit the growth and sporulation of the various pathogens. Lin *et al.* (1994) found 'tricholin', a ribsome inactivating protein from the culture broth of *T. viride* to be antagonistic to *Rhizoctonia solani*. The antifungal antibiotics 'gliotoxin' and 'gliovirin' produced by *Gliocladium virens* have been found to be associated with the efficacy of *G. virens* as biocontrol agent against seedling disease of cotton, incited by *R. solani* (Howell and Stipanovic, 1995*). T. harzianum* excreted β-1, 3-glucanase and chitinase into the medium when grown on either laminarin and chitin respectively, or cell walls of the pathogen *Sclerotium rolfsii* as sole carbon source (Elad *et al.*, 1982). *Trichoderma* spp. are aggressive mycoparasites of the hyphae and resting structures of many plant pathogens (Howell, 1982). Mycoparasitism in *T. virens* has attributed to its capacity to synthesize extracellular chitnase (Back *et al.*, 1999). *Trichoderma* spp form several loops and coil over the hyphae of *Fusarium* and *Rhizoctonia,* followed by rupture, twisting and leakage of hyphal protoplasm, air budding inside the cytoplasm and ultimately lead to severe vacuolation at later phase of interaction (Bunker and Mathur, 2001, Pandey *et al.*, 2005).

2.6.3 Botanical extracts

Several plant extracts are known to possess antimicrobial properties and are therefore, being exploited to achieve control over various plant ailments (Mishra and Dixit, 1977). Skinner (1955) suggested that the presence of some antibiotic constituents or some unknown substances contribute to the inhibitory activity of the plant extracts. Looking into deleterious effects of synthetic pesticides on life supporting systems and other associated problems such as pest resistance and detrimental effects on non-target organisms, there is an urgent need

to use alternative agents for pest control using the sources that are harmless. It is well known fact that higher plants and their products provide less phytotoxic, more systematic, easily degradable, non-pollutant and host metabolism stimulatory approach to plant disease management. With the growing awareness of harmful effect of synthetic pesticides world over, the use of plant products in the plant disease management is gaining importance in recent years (Singh, 1997).

Plant extracts have assured special significance in the present day strategy of developing ecologically safe methods for the management of plant diseases. Several commercial formulations of plant extracts are being investigated as possible alternatives for the management of fusarium wilt disease of different crop plants. Recently, plant extracts drawn from the various parts of certain plant species have been successfully tried to demonstrate their antifungal activities (Raja and Kurucheve, 1998; Datar, 1999; Ranjana *et al.*, 1999; Singh and Navi, 2000; Bowers and Locke, 2000). The antifungal activities of different plant extracts against many plant pathogenic fungi have been well documented (Dubey and Dwivedi, 1991; Biswas *et al.,* 1995).

Flori *et al.* (2000) suggested that leaf extracts and essential oils of certain plants could be used to suppress the growth of *Didymela bryoniae* while noticing 100 per cent inhibition of the mycelial growth and germination of spores of *Didymela bryoniae* by essential oils of *Cymbopogan citrates* and *Ageratum conyzoides.* Lakshman (1990) reported that the extracts of *Allium sativum*, *Ocimum tenuiflorum* and *Tridex procumbeus* suppressed the mycelial growth of *Cornyspora lassicola,* however, *A. sativum* exhibited the maximum inhibition of 95.8 per cent. Dubey and Dwivedi (1991) found that the bulb extract of *Allium sativum* was more effective than its leaf extract at 0.1 per cent concentration, inhibiting the mycelial growth of *macrophomina phaseolina* by 52.2 per cent besides, reducing the sclerotia formation. Ethanol extracts (1:1) of leaf and seeds of *Datura stramonium* completely inhibited the spore germination and mycelial growth of *A. macrospora* (Bambawale, 1996).

Shivpuri *et al.* (1997) reported that the ethanol extracts of all the ten plant species bioassayed against *A. brassicicola* significantly inhibited the mycelial growth as compared to check. The leaf extracts of *Azadirachta indica, Datura stramonium, Ocimum sanctum, Polyalthia longifolia* and *Vinia rosea* proved more fungitoxic than rest of the test extracts. They further reported that mycelial growth inhibition of test fungus in leaf extracts of *Allium cepa, A. sativum* and *Withamia somnifera* were significantly more at 100 µg/ml than 500 µg/ml. Zaman *et al.* (1997) reported that *A., Fusarium, Asperigillus, Penicillium, Rhizopus, Chaetomium* and *Curvularia* were associated with mustard seeds and out of test extracts only garlic and neem leaf extracts were found more effective. Dubey *et al.* (1998) observed that out of seven plant extracts tested against *A. tenuissima* on Tegetus sp., bulb extract of garlic was most effective followed by mint and datura. Three sprays of carbendazim (0.05%) and *A. sativum* (20%) were found statistically at par in controlling the leaf spot and blight of brinjal (Yadav *et al.*, 1998). *A. cepa, A. sativum* and *D. stramonium* at 5, 10, 15 and 20 per cent concentration significantly inhibited the mycelial growth of *A. alternata* and effectiveness of the extracts increased with increase in their concentration (Singh and Majumdar, 2001). While studying *in vitro* efficacy of 16 plant extract in controlling leaf spot in Ginger caused by *Phyllosticta zingiberi*, Ruchi *et al.* (2003) observed that Alove and tulsi *(Ocimum tenuiflorum)* extracts resulted in 100 per cent growth inhibition of the pathogen.

Balbipena *et al*. (2006) reported the *in vitro* fungicidal activity of turmeric extracts and curcumin against *A. solani* at 4 different concentrations. The concentrations of 10 and 15 per cent of non-autoclaved turmeric extracts inhibited the mycelial growth by 38.2 and 23.2 per cent repectively and the fungal sporulation by 71.7 and 87 per cent respectively. Anju-Sharma *et al.* (2007) reported the antifungal effect of neem (*Azadirachta indica*) leaf extract at 0.1 and 0.01 per cent in a potato dextrose agar medium on *A. solani, Fusarium oxysporum* and *Colletorichum gloeosporioides* (*Glomerella cingulata*). The radial growth of

these fungal pathogens was significantly effected by application of the extract at 0.1 per cent. Hassan and Dasem (1998) reported mucotoxic properties of some medicinal plants against *A. solani* and *Fusarium oxysporum* f. sp. *lycopersici.* Leaf and stem extracts of *Mentha viridis* and *Rosmarinus afficinalis* extract reduced growth of *Alternaira solani*. Aqsa *et al*. (2010) reported the efficacy of 5 indigenous medicinal plant extracts against 3 pathogens attacking commercial crops viz., *A. solani, R. solani* and *M. phaseolina*. Overall, *Dodonaea viscosa* appeared significantly the most effective and suppressed the radial mycelial growth of the *A. solani* and *R. solani*. Among 5 concentrations of plant diffusates, the highest inhibition in radial mycelial growth of all three pathogens was observed at 100 and 200 g/l, respectively, as compared to control. Suleiman (2010) reported *in vitro* fungitoxic activity of crude extracts of neem (*Azadirachta indica* (A.) Juss) and pawpaw (*Carica papaya* (L.) on *A. solani* isolated from rotting yam tubers. The highest mycelial growth inhibition was shown by pawpaw leaf extracts at various concentrations tested.

2.6.4 Management through fungicides

Our increasing knowledge of the host pathogen disease triangle has enabled us to apply certain principles to reduce the loss from the epidemic diseases by timely application of control measures. Still fungicides continue to serve an important tool for the management of plant diseases. Control of early blight disease by the use of fungicides has been reported by a number of workers in the past (Strong, 1948; Parviflora, 1955; Von Schmeling, 1962). Lodha and Prasad (1975) obtained the best control of *A. solani* by the application of Dithane Z-78 followed by crystalline actinione in both pot and *in vitro* tests. Khan *et al.* (1995) reported that mycelial growth of *A. alternata* on Richard's Agar Medium was completely inhibited by propiconazole at 30 ppm followed by Ridomil (mancozeb + metalaxyl), Daconil (chlorothalonil), and Antracol (propineb). Pavlova *et al.* (1999) tested the bio-efficacy of different triazoles viz. propiconazole, difenconazole, and tebuconazole under *in vitro* conditions against

A. alternata and reported low sensitivity of the pathogen towards all the fungicides. Dubey *et al.* (2000) reported that among different fungicides contaf (hexaconazole) proved most effective and caused complete inhibition in the mycelial growth of the pathogen at 100 µg/ml concentration. Recently, Singh and Singh (2006) also studied the efficacy of different contact and systemic fungicides under *in vitro* conditions and reported that hexaconazole was most effective as it caused 100 per cent growth inhibition even at lowest concentration of 250 ppm. The other fungicides like mancozeb, copper oxychloride, copper hydroxide, chlorothalonil, azoxystrobin and propineb were also effective and caused significant reduction in mycelial growth but at a much higher concentration. Under *in vitro* conditions low efficacy of mancozeb to *A. solani* even at a high concentration of 2500 ppm has already been reported (Choulwar and Datar, 1994).

Choulwar and Datar (1988) reported that six early sprays of mancozeb (0.2%) followed by six late and five early sprays reduced the disease of early blight of tomato and increased the yield. In general, early sprays were more effective than equal number of late sprays. Okasha *et al.* (1989) reported that propineb caused significant reduction in disease and increased the yield of tomato. Maheshwari *et al.* (1991) evaluated six fungitoxicants in field trials and observed that most effective control was provided by copper oxychloride (64.7%), followed by mancozeb (61.7%). High efficacy of mancozeb (Dithane M-45) in controlling early blight of tomato and increasing yield has also been reported by other workers (Sinha and Prasad, 1991, Bhardwaj *et al.* 1995). Deora *et al.* (2004) tested nine fungicides and observed that maximum disease control (> 50%) was obtained by Dithane M-45 followed by Kavach, and Tilt was less effective as compared to these fungicides to control the early blight disease. Likewise, in early blight of potato caused by *A. solani* the effectiveness of Dithane M-45 in controlling the disease has been well demonstrated under field conditions (Mohit *et al.* 1997, Dhabir *et al.* 2002). Ponce *et al.* (1992) reported that among different

fungicides applied as protective spray best control of *A. solani* on tomato was obtained with chlorothalonil used at the rate of 1.5 – 3.5 litre/ha followed by mancozeb (Dithane M-45). Dillard *et al.* (1997) tested chlorothalonil at 2.5 kg/ha and mancozeb at 1.68 kg/ha at 7, 10 and 14 days interval against early blight of tomato and observed that yield and financial benefit were consistently the highest in plots where chlorothalonil was applied at 7 day interval. Efficacy of chlorothalonil in controlling leaf blight of wheat caused by *A. triticina* has also been demonstrated (Follas *et al.* 1992; Diazo and Rodriguez, 1998) tested some new fungicides against early blight of tomato and reported that difenconazole at the rate of 125 g/ha at 14 days interval proved most effective in checking the disease. Efficacy of strobilurin compound azoxystrobin in checking early blight disease of tomato has also been demonstrated and treated plants produced less damaged fruits, increased the fruit weight and the fruit ripened slightly earlier (Siviero *et al.* 2001). The efficacy of 7 different fungicides i. e., zoxamide + mancozeb (Unikat 75 WG), Fluazinam (Altima 500 SC), Propineb (Antracol 70 WG), Cymoxanil + fomoxate (Tanos 50 WG), Fenamidon + mancozeb (Pyton 60 WG), Metalaxyl-M + mancozeb (Ridomil Gold MZ 68 WG) and Iprowalikarb + mancozeb (Melody Med 69 WG) were compared. The results showed that all the tested fungicides significantly reduced the mycelial growth. The lowest effectiveness was observed with the mixture cymoxanil + famoxate (Tanos 50 G) (Osowski, 2007). The efficacy of different fungicides in controlling early blight caused by *A. alternata* and *A. solani* was evaluated in field and laboratory experiments in Poland. The results showed that all tested fungicides significantly reduced early blight development in the field. In the laboratory experiments, only mancozeb and fenamidon reduced the development of *A. alternata* fungus. (Osowski, 2004). Osowski (2003) evaluated the efficacy of plant production products in control of early blight (*A. solani*) in Poland in two series of field experiments. In the series I propineb (Antracol 70 WP) showed the greatest efficacy in early blight control while in the series II mancozeb (Dithane M-45, 80 WP) showed the highest efficacy. Patel *et al.* (2004) reported that fungicide

treatment with mancozeb at 0.2% was significantly effective in reducing early blight of potato. Abdel-Rehman (1977) evaluated some fungicides against *A. solani,* using 7 cvs of different susceptibility and found that chlorothanlonil gave the best control followed by captafol, zinc ion-maneb, and triphenyltin hydroxide. Thind and Jhooty (1982) reported application of 3 sprays of 0.5% Dithane M-45 (mancozeb) sol at 200 l/ac gave effective control of *A. solani* infection on potatoes and significantly increased the tuber yield. Blitox (copper oxychloride) also controlled the infection but was less effective than Dithane M-45. Sangupta and De (1987) reported that Dithane M-45 (mancozeb), Daconil 2787 (chlorothalonil), Difolatan 80 W (captafol) and Blitox gave good control of early blight of potato. Dithane M-45 at 2 kg/ha was the most effective. Raj Kumar *et al.* (1990) reported that of the 10 fungicides tested, 3 sprays at intervals of 20 d of 1% thiophenate methyl (as Topsin-M), 0.1% carbendazim (as Bavistin), 0.2% mancozeb (as Dithane M-45) or 0.1 bittertanol (as Baycor) gave good control. Guddewar *et al.* (1992) reported that the best control of early blight of potato and highest yield were obtained with mancozeb (2 kg/ha), followed by captan (2.5 kg) and copperoxychloride (2.5 kg). Mohit Singh *et al.* (1997) tested the efficacy of 7 fungicide treatments for the control of early blight of potato caused by *A. solani.* Emisan-6 (2 methoxyethyl mercury chloride) was applied as seed treatement before planting. Indofil M-45 (mancozeb and thiophenate methyl), Indofil Z-78, Topsin-M (thiophenate methyl), Kavach (chlorothalonil), Blue-copper-50 (copper oxychloride) and Ziram were applied as sprays 40 days after sowing followed by 3 more sprays at 10 days interval. All the fungicide treatments significantly reduced disease incidence. Chaudhari *et al.* (2002) reported that three sprays of metalaxyl MZ 0.2% recorded maximum control of early blight of potato followed by propineb, mancozeb and chlorothalonil at 0.2% in increasing order. De and Chattopadhyay (1984) reported that the incidence of early blight of potato was lower in West Bengal when fungicides were used. Of 5 tested Dithane M-45 (mancozeb) performed best. Bagchi and Das (1976) reported that in lab. experiments the pathogen of early blight of potato tolerated increasing concs.

(1000-8000 ppm) of blitox-50, zineb and brestan-60, but not captan 83. Shteinberg (1994) reported that spraying the systemic fungicide tebuconazole in bi-weekly intervals (at rates of 0.187 or 0.250 kg a.i/ha) was more effective than weekly spraying mancozeb (at a rate of 2 kg a.i/ha). Maheshwari and Mehta (1993) reported that the most effective control of early blight of potato was given by Dithane M-45 (mancozeb), with an increase in yield of 91.87 per cent.

Andrew-Horsfield *et al.* (2010) conducted a series of experiments to evaluate fungicide use for the control of early blight (*A. solani*) of potatoes in Australia. The most effective spray programs included those with boscalid + metiram in the first two applications and resulted in significant increases in early blight control and tuber yields of over 20 %.

2.6.5 Integrated disease management

Early blight of potato incited by *A. solani* (Ellis and Martin) Jones and Grout is a serious disease attacking foliage. Although satisfactory control of the disease by using various chemicals has been documented in the literature (Mohit *et al.* 1997, Dhanbir *et al.* 2002), the continuous use of these agrochemicals for controlling the disease may pose several problems like toxicity to non-target organisms, development of resistance among the population of pathogen and environmental pollution. Biopesticides in integration with fungitoxicants offer a more reliable approach to effectively manage plant diseases (Tu, 1986; Patibanda *et al.*, 2002). As per the available literature meager work has been done on the integrated management of early blight disease of potato or tomato.

Phalisteen *et al.* (2008) evaluated the effect of medicinal plant (leaf) extracts and their combination with fungicides (carbendazim) against radial growth of *A. solani.* On the basis of results, the medicinal plant Neem (3%), Nerium (3%) added with carbendazim (1%) showed maximum inhibition of fungus (*A. solani*). Henis *et al.* (1978) observed additive effect of PCNB (pentachloro-nitrobenzene) with *T. harzianum* when applied simultaneously

against damping off (*R. solani*) of cotton and radish. Besides, they found synergistic effect of above treatments on the fungal population in soil. Verma and Gandhi (2007) evaluated some antagonists and plant extracts against *A. solani,* the causal agent of early blight of tomato. Foliar spray of *Clerodendrum aculeatum* leaf extracts (15%) immediately after appearance of symptoms or foliar spray of *T. viride* (10^7 cfu/ml) 24 hr before challenge inoculation with the test fungus was found effective in reducing the disease severity under screen house conditions. Several plant extracts from various plant species and some fungicides were tested for their fungitoxic effects on the mycelial growth and spore germination of *A. solani,* the causal agent of tomato leaf blight disease. Among them 10 per cent extracts of Acacia were effective followed by *Allium sativum, Anona* and Neem product. The fungicides were superior to all the plant products. The efficacy of plant products and fungicides on tomato early blight (*A. solani*) was investigated on cv. Dhanshri. The maximum radial mycelial growth inhinition was observed under Dithane M-45 treatment (89.98%) (Mate and Deshmukh, 2005). Abdul-Hafeez *et al.* (2001) reported the efficacy of bioagents and plant extracts against *A. solani.* Among bioagents *Verticillium sp.* was the most effective antagonist in reducing *A. solani* mycelial growth followed by *Beauveria bassiana, Bacillus subtilis, T. harzianum* and *Arachniotus sp*. None of the plant extracts proved effective in retarding the mycelial growth of *A. solani*. Kamlesh and Gujar (2002) reported that *T. viride*, *T. harzianum* and two isolates of *Fusarium solani* isolated from rhizosphere of chilli significantly inhibited the mycelial growth and sclerotial production of *R. solani* causing stem rot of chilli. Maximum inhibition of mycelial growth (68.6 and 61.0 mm) and sclerotial production (nil) were recorded with local isolates of *T. viride* and *T. harzianum* respectively. Out of 23 plant extracts *Plantago ovate, Allium sativum,* and *Trigonella foenum graecum* inhibited maximum mycelial growth in in vitro conditions. Yadav and Majumdar (2005) reported the efficacy of plant extracts, biological agents and fungicides against *Lasiodiplodia theobramae* causing die back of guava. Among plant extracts *Aloe barbadensis* was the best. Fungicidal control of the pathogen reveals

that carbendazim *(bavistin)* and mancozeb (Dithane M-45) completely inhibited the mycelial growth of the pathogen. Among antagonist maximum inhibition of mycelial growth was observed with *G. virens* (39 mm) followed by *T. viride* (35 mm) and *T. harzianum* (18 mm). Monica Sharma and Gupta (2003) reported that integration of efficacious seed treatment with biopesticides and biocontrol agents alone or in combination was effective in the management of root rot of French bean. Shahid Ahmad *et al.* (2000) reported the activity of various plant products along with various antagonistic microorganisms against dry root rot (*Rhizoctonia bataticola*) in chickpea cv. C235 under pot conditions. All treatments gave some control. Soil inoculation plus seed treatment was the most effective application method. Hoda *et al.* (2000) reported that garlic and onion aqueous crude extracts *in vitro* showed toxic effects on the mycelial growth of *R. solani, M. phaseolina* and *F. oxysporum* f sp. *vasinfectum*, the causal pathogens of root rots and wilt diseases of cotton. Garlic extract was more effective than onion extract. *T. harzianum, T. viride* and *B. subtillis* inhibited the mycelial growth of all fungi tested *in vitro. T. harzianum* was the most effective one followed by *T. viride* and *B. subtilis.* Aghnoon *et al.* (1999) reported effect of 6 antagonistic fungal isolates and 4 fungicides on Fusarium *in vitro*. Benomyl, iprodione + carbendazim, carboxin + thiram and captan reduced Fusarium mycelial growth. Under green house conditions, seed dressing with *T. harzianum* lowered disease incidence by 65.4 per cent and was more effective than seed treatment with fungicides. The comparative performance of chemical, biological and integrated control of wilt of pigeon pea caused by *Fusarium udum* was experimented in glass house. Among chemicals Bavistin proved efficient and among bioagents, *T. viride* and *T. harzianum* were best, and in integration approach bioagents with thiram reduced wilt incidence (Pandey and Upadhyay, 1999). Sharma and Bansandrai (1997) reported the effect of biocontrol agents, *T. harzianum* and *G. virens*, the fungicides carbendazim, triademdfon and triademefon and crude leaf extracts of neem (*Azadirachta indica*), *Lantana camara* and *Canabis sativa* on the viability of *sclerotia* of *S. sclerotiorum* isolated form pea (*Pisum sativum*), chick pea

(*Cicer arietinum*), Cauliflower (*Brassica oleracea var. botrytis*) and cabbage. *T. harzianum,* Carbendazim, Triademefon and *A. indica* extract were highly effective in reducing sclerotial viability. *T. harzianum* and carbendazim caused the highest inhibition of sclerotial germination from cauliflower where as *T. harzianum,* Triademefon and *A. indica* were most effective in reducing sclerotial viability in peas. Mina Koche *et al.* (2009) reported the efficacy of five fungicides and three botanicals against foliar diseases of turmeric. Amongst fungicides propiconazole (91.98%) hexaconazole (87.55%), penconazole (85.62%) and carbendazim (84.02%) were effective to inhibit the growth of *Colletotrichum dematium. Azadirachta indica* seed extract was effective amongst botanicals to check the mycelial growth of *C. dematium* (74.69%) as compared to control. Watve *et al.* (2009) reported that maximum per cent inhibition in colony diameter of the test fungus (*Colletotrichum gloeosporiodes*) was achieved due to *T. harzianum* (83.33%) followed by *T. viride* (77.78%) and *B. subtillis* (77.78%) when the test fungus was placed at centre. In the, *in vivo* assay, *T. harzianum* recorded the lowest leaf spot intensity (24.74 PDI) with per cent disease reduction 21.45, while *T. viride* recorded the leaf spot intensity (25.68 PDI) with per cent disease reduction 18.48 as compared to carbendazim (0.1%) which recorded the highest per cent (76.65%) of leaf spot intensity (7.38 PDI).

Chapter – 3

MATERIALS AND METHODS

The present investigations on the perpetuation and management of early blight of potato were conducted in the Division of Plant Pathology, SKUAST-K, Shalimar and Experimental Field of Division of Vegetable Science. The details of the methods adopted are described as under:

3.1 Survey

Survey for assessment of potato early blight disease was conducted in the major potato growing areas of the valley viz., Baramulla, Budgam, Srinagar and Shopian districts during the month of June-July 2008 and 2009. Each district was represented by three locations and each location by three sites. Five plants were randomly selected from four corners and centre of the plot representing each site. All the leaves were examined for recording incidence and intensity of disease. Symptoms are described in Plate-1.

3.1.1 Disease incidence

Per cent disease incidence was worked out as per the following formula given by James (1974):

$$\text{Per cent disease incidence} = \frac{\text{No. of diseased leaves}}{\text{Total No. of leaves examined}} \times 100$$

3.1.2 Disease intensity

For assessment of disease intensity, diseased leaves were categorized as per the scale (Plate-2) given by Pandey and Pandey (2002) with a little modification as under:

Plate-2 : **Scale (0-5) for assessment of *Alternaria* leaf spot intensity**

Category	Grade/Numerical value	Leaf area infected (%)
I	0	Disease free
II	1	1-10
III	2	11-25
IV	3	26-50
V	4	51-75
VI	5	>76

Per cent disease intensity (PDI) was calculated as per the following formula given by FAO (Anonymous, 1967):

$$PDI = \frac{\sum(n \times v)}{N \times S} \times 100$$

Where,

$\sum$ = Summation

N = No. of leaves in each category

V = Numerical value of leaves observed

S = Maximum numerical value/grade

3.2 Isolation, purification and maintenance of culture

3.2.1 Isolation of the pathogen

Potato leaves showing typical disease symptoms, collected from susceptible cultivar “Kufri Jyoti”, during the course of survey, were repeatedly used for isolation of the pathogen.

The diseased leaves were first examined for associated fungus by teasing

the diseased portion with the help of a teasing needle and observed under microscope at the margins of the diseased spots (10x X 10x). For the isolation of fungus, small segments of diseased tissue along with some healthy leaf portion (5 x 5 mm^2) were cut with a sterilized razor blade at the margins of the diseased spots on the leaves and surface sterilized in 0.1 per cent mercuric chloride solution for 30 seconds (Jhonston and Booth, 1983). The leaf segments were then rinsed thrice in distilled sterilized water to remove the last trace of mercuric chloride solution, blotted dry and placed on acidified potato dextrose agar medium (PDA) in sterilized Petriplates. Three pieces of sterilized specimen were placed in each Petriplate and incubated for 7 days at 25±2^oC. One set of PDA plates was seeded with bits without mercuric chloride treatment. The composition of potato dextrose agar medium used was:

Peeled potato	:	200 g
Dextrose	:	20 g
Agar	:	20 g
NaCl	:	1 g

Distilled water to make the volume 1000 ml

3.2.3 Purification and maintenance of pathogen

The culture was purified by hyphal tip method (Pathak, 1972) and single spore technique (Jhonston and Booth, 1983). As soon as the mycelial growth was observed in petriplates (3.2.1), advancing hyphal tips growing out of tissue segments were cut off with sterilized inoculation needle and transferred to potato dextrose agar slants for further growth.

In case of single spore technique 2-3 drops from spore suspension prepared from 10-day old culture and teased leaf tissue smear in autoclaved distilled water were used to spread on the surface of plain agar medium in petriplates and incubated at 25±2^oC for 24 hours. The plates were observed for

germinating spores under stereoscopic microscope and finally germinating spores were lifted by inoculation needle and transferred aseptically to potato dextrose agar slants for further growth. The pure cultures thus obtained, were maintained by repeated sub-culturing at an interval of 30 days for further studies. The stock culture in PDA slants was stored at 4^{o}C in refrigerator. To retain the vigour of the fungus, it was isolated repeatedly from naturally infected leaves and purified by the method described above.

3.3 Identification of the pathogen

The pathogen was identified on the basis of colony characters, viz., colour, growth, pigmentation etc. and morphological characters of its mycelium and conidia produced on host and in culture.

3.4 Pathogenicity test

The pathogenicity of the isolated fungal pathogen was conducted on detached leaves of "Kufri Jyoti" variety. Apparently healthy leaves were removed from plants, washed with sterilized distilled water and placed in sterilized petriplates, with their petiole inserted in moist cotton. Spore suspension was made from 15 days old culture with sterilized distilled water and diluted to get a concentration of 2×10^4 conidia/ml. The spore suspension was inoculated (sprayed) at different places on the upper leaf surface. Control was maintained by spraying only sterilized distilled water on the leaf surface. The leaves where then placed in humidity chambers, where humidity was maintained by keeping moist cotton in chambers. The chambers where placed in diffused sunlight on laboratory benches at room temperature (19-20^{o}C) till appearance of typical disease symptoms compared to those of in nature. Re-isolations of pathogen from artificially inoculated leaves where carried out and resultant cultures compared with the original culture to satisfy Koch's postulates.

3.5 Morphological and cultural characters

The morphological characteristics of the causal organism was studied on culture in the laboratory *(in-vitro)*. The important characters studied were as under:

Colony	:	Colour, shape, margins and pigmentation
Mycelium	:	Colour, shape, septation, branching
Conidia	:	Colour, shape, size and septation

3.6 Symptomatology and disease development

The symptomatology of the disease was studied on five randomly selected plants of susceptible cultivars "Kufri Jyoti". The selected plants were marked for continuous monitoring of disease development. Plants were kept unsprayed throughout the growing seasons (2008 and 2009) to study the symptoms of early blight leaf spot under natural epiphytotic conditions. First observation was taken as soon as the disease appeared. Periodic observations were recorded besides size, shape, coalescing and colour of the lesion on leaves. Size of the lesions was recorded in terms of average lesion size in mm.

3.7 Effect of meteorological factors on the disease

The role of various meteorological factors on disease intensity and infection rate (unit/day) on early blight of potato was assessed during the year 2008 and 2009 at Shalimar campus. The weather variables were temperature, relative humidity and precipitation. The field experiment was conducted in randomized block design with five replications. The plants were kept unsprayed to record the disease development under natural epiphytotic conditions. Development of the disease in terms of intensity was recorded periodically at seven day intervals starting from the first appearance of the disease. Disease intensity was recorded on 100 leaves randomly selected from each replication and using 0-5 rating scale as describe in section 3.1.2. Weekly means of

temperature, relative humidity and precipitation that prevailed during each disease scoring date were recorded and correlated with disease development. Linear multiple regression was calculated to determine the effect of weather factors on disease development. Growth of disease development in terms of apparent infection rate (unit/day) was calculated as per formula of Vanderplank (1963).

$$r = \frac{2.3}{t_2 - t_1} \quad x \log x \quad \frac{X_2 (1-X_1)}{X_1 (1-X_2)}$$

Where,

r = apparent infection rate (unit/day)

X_1 and X_2 = disease intensity at time t_1 and t_2, respectively

3.8 Survival of pathogen in over-wintered infected leaves and tubers

The survival of pathogen in fallen diseased leaves and tubers of susceptible potato cultivar "Kufri Jyoti" was studied during the year 2008 and 2009 at Shalimar campus of SKUAST-K.

3.8.1 Survival in over-wintered diseased leaves

Diseased leaves of potato were collected on 15th of August in the year 2008 and 2009. The leaves were kept in mesh wire bags, each bag containing equal number of leaves and divided into two sets. One set of such leaves were kept on soil surface (Plate-3a) and the other at 20 cm depth (Plate-3b) in the field. The diseased leaves, in the mesh wire bags were removed at fortnightly intervals beginning from first week of March onwards. Thirty leaf bits of 1 cm^2 area were randomly taken from one randomly selected bag and examined for the presence of conidia. These leaf discs were then crushed separately in 40 ml of sterilized distilled water and strained through a double layer cheese cloth. Twenty milliliter of the filtrate was centrifuged at 6000 rpm for 15 minutes. After centrifugation, 18 ml of the suspension was drawn off with a pipette. The pipette was re-suspended in 2 ml sterilized water and the number of conidia were counted with the help of

a) Overwintering of diseased potato leaves on ground surface

b) Overwintering of diseased potato leaves at 20 cm depth

Plate-3 : Perpetuation studies of the fungus

haemocytometer. To estimate the per cent viability of conidia in over-wintered diseased leaves, spore germination method was used. Two drop of 50 µl from each processed sample were placed on a glass slide and incubated in a moist chamber at 23±2^{o}C. The number of spores observed and the number of spores that germinated were recorded after 24 hour incubation for calculating the per cent viability of conidia.

3.8.2 Survival on tubers

The tubers collected in autumn at the time of harvest from the diseased crop were kept in ambient store and were periodically assessed for the presence/viability of fungal propagules. Ten randomly selected tubers were taken, ground/crushed separately in 80 ml of sterilized distilled water and strained through a double layered cheese cloth. The rest of the procedure was same as that of leaf sample. The per cent viability of spores was estimated as per the method describe in 3.8.1.

3.9 Disease Management

3.91 Screening

Potato germplasm available with the Division of Vegetable Science, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Shalimar, were screened against the early blight disease under natural conditions during 2008 and 2009 spring season. From each genotype, 10 plants selected randomly and kept unsprayed throughout the season, were tagged for the assessment of the disease. All the leaves of ten plants were counted and then grouped as healthy and diseased. The disease incidence and intensity was assessed in the month of July as in 3.1.1 and 3.1.2. The potato germplasm screened included varieties viz., Kufri Girdari, Kufri Jyoti, Kufri Himani, K. Shailja, K. Giriraj, K. Chandramukhi, SM/88-991, SM/45-43, SM/92-725, SM/92-338, SM/91-1515, SM/98-239, SM/93-237, SM/90-68, SM/87-55, SM/96-127, SM/90-45, SM/94-44, HB/18-36, HB/87-185, HB/82-18, HB/50-45, TPS-C_3,

Shalimar Potato-1 and Gulmarg Special.

The various cultivars were arbitrarily categorized into five different reaction groups on the basis of per cent disease intensity (PDI) as under:

Reaction category	PDI
Resistant (R)	0-5
Moderately resistant (MR)	5.1-10
Moderately susceptible (MS)	10.1-25
Susceptible (S)	25.1-50
Highly susceptible (HS)	50.1 and above

3.9.2 Management through botanicals

3.9.2.1 *In-vitro* evaluation

The ethanol extracts of the botanicals tested against leaf spot disease of potato were:

Common name	Local name	Botanical name	Plant part used
Datura	Datur	*Datura stramonium* L.	Leaves
Artimesia	Tethvan	*Artimesia indica*	Leaves
Nettle	Soi	*Urtica dioeca* L.	Leaves
Mint	Podina	*Mentha spicata* L.	Leaves
Walnut	Doon	*Jugalans regia* L.	Bark

The plant extracts were evaluated *in vitro* through poisoned food technique (Carpenter, 1942; Nene and Thapliyal, 1993). For obtaining ethanol extracts fresh plant materials were collected washed with sterilized distilled water and dried at room temperature, crushed and suspended in 80 per cent ethanol and filtered after one hour through Whatman No. 1 filter paper. These where evaporated to dryness on a water bath (40±2^{o}C), on cooling, their aqueous suspensions were prepared in the ratio of 1:1 (W/v) by adding sterilized distilled water.

The desired concentration of the test extracts was added to potato dextrose broth in sterilized conical flasks. The medium without extract served as check. Each flask was inoculated with 3 mm diameter mycelial disc taken from 10-day old culture with the help of cork borer, raised on potato dextrose agar medium (PDA). The inoculated flasks were incubated at 25±2^{o}C for 15 days. After incubation, the medium containing the mycelial growth of the fungus was filtered through previously weighed Whatman filter paper No. 41. The mycelial mat on filter paper was over dried at 60^{o}C and weighed. The dry mycelial weight was calculated by subtracting weight of previously weighed filter paper from weight of filter paper with mycelial mat. Per cent inhibition of mycelial growth was calculated using the formula of Vincent (1947).

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where,

C = Weight of fungal colony in control (mg)

T = Weight of fungal colony in treatment (mg)

3.9.3 *In vitro* studies of biocontrol agents

3.9.3.1 Radial growth inhibition in dual culture

Antagonistic activity of various fungal antagonists against *Alternaria solani* was assayed by dual culture technique (Utkhade and Rahe, 1983). Five mm discs of seven day old cultures of test pathogen as well as biocontrol agents were

taken with the help of a cork borer. Two discs, one each of pathogen and biocontrol agent, were placed equidistantly (60 mm) apart in each 90 mm petriplate containing PDA under aseptic conditions. The petriplates containing PDA without biocontrol agent served as control. Each treatment was replicated four times. The experiment was laid out in a completely randomized design. The petriplates were incubated at 25±2^{o}C. Observations on the growth of biocontrol agent and pathogen were recorded after 10 days of incubation and per cent mycelial inhibition calculated according to Vincent (1947). Based on the growth and mycoparasitic nature, biocontrol agents were grouped into various categories as per the scale given by (Bell *et al*. 1982) with slight modifications (Munshi and Dar, 2004).

I. **Strong antagonist:** Growth of biocontrol agent very fast, it covered the entire medium surface and completely overgrew the pathogen.

II. **Antagonistic:** Growth of biocontrol agent very fast, it covered at least 2/3rd medium surface but without showing mycoparasitic action.

III. **Moderate antagonist:** Growth of biocontrol agent very slow but rapidly it overgrew the pathogen once in contact.

IV. **Slow antagonist:** Growth of biocontrol agent and pathogen similar in magnitude, none appeared to be dominant to other.

V. **Poor antagonist:** Growth of the pathogen fast, it colonized at least 2/3rd of the surface and appeared to withstand encroachment by the biocontrol agent.

VI. **Non antagonist:** Growth of the pathogen very fast it completely overgrew the biocontrol agent and covered the entire medium surface.

Pure culture of biocontrol agents viz., *Trichoderma viride, T. harzianum* and *T. virens* were obtained from Division of Plant Pathology SKUAST-K, Shalimar. These biocontrol agents were maintained on PDA by periodic sub-

culture at monthly intervals. Mass culture of fungal biocontrol agents was made on wheat bran in 500 ml Erlenmeyer flasks.

3.9.3.2 *In vitro* evaluation of fungitoxicants

Five non-systemic fungitoxicants (chlorothalonil 50 WP, mancozeb 75 WP, captan 50 WP, propineb 70 WP and copper oxychloride 50 WP at six concentrations (1000, 1500, 2000, 2500, 3000 and 3500 ppm) each and five systemic fungitoxicants (thiophanate methyl 70 WP, carbendazim 50 WP, hexaconazole 5 EC, fenarimol 12 EC and difenconazole 25 EC) at six concentrations (100, 150, 200, 250, 300 and 350 ppm) each were evaluated in vitro against the test pathogen by poisoned food method (Nene and Thapliyal, 1993).

Fifty milliliter of basal medium (potato dextrose broth) was poured in 150 ml conical flasks, plugged with non-absorbent cotton and autoclaved at 15 lb pressure for 15 minutes. After cooling the medium, a known quantity of fungitoxicant as per treatment was incorporated into each flask, except control. Each treatment was replicated thrice in Complete Randomized Design (CRD). The flasks were then inoculated with 5 mm dia mycelial discs cut from actively growing fungus culture. The flasks were incubated at 25±2^{o}C for 15 days. The relative efficacy of fungitoxicants was ascertained by taking the dry mycelial weight of the fungus as per the method described in 3.7.

3.9.3.3 *In vivo* evaluation of biocontrol agents, botanical extracts and fungitoxicants

The fungitoxicants, biocontrol agents and botanicals which proved most effective under *in vitro* experiments were also evaluated *in-vivo* against the test pathogen. In case of biocontrol agents, a spore suspension of 1 x 10^7 cfu was used while botanicals were used at 50 per cent concentrations.

The experiment was laid out in randomized block design and each treatment replicated three times. The treatments consists of:

Treatment	Treatment detail
T_1	Hexaconazole (F.S.)
T_2	Mancozeb (F.S.)
T_3	Datura (F.S.)
T_4	*Trichoderma harzianum* (F.S.)
T_5	Mancozeb (S.T.) + hexaconazole (F.S.) + datura (F.S.) + *T. harzianum* (F.S.)
T_6	Mancozeb (S.T.) + hexaconazole (F.S.) + *T. harzianum* (F.S.)
T_7	Mancozeb (S.T.) + hexaconazole (F.S.) + datura (F.S.)
T_8	*T. harzianum* (S.T.) + datura (F.S.)
T_9	*T. harzianum* (S.T.) + mancozeb (F.S.)
T_{10}	*T. harzianum* (S.T.) + hexacnazole (F.S.)
T_{11}	Control

F.S. = Foliar spray; S.T. = Seed treatment

The first spray was given at the initiation of disease symptoms and second 15 days later. Data on disease intensity were recorded 15 days after the last spray as given in 3.1.2. In case of check, plants were sprayed with water.

3.10 Statistical analysis

The data generated on various aspects of research were subjected to statistical analysis as per the methods described by Panse and Sukhatme (1985). The software used for analysis was ‘Minitab’.

Chapter – 4

EXPERIMENTAL FINDINGS

The experimental findings of research work on perpetuation and management of early blight of potato in Kashmir Valley are presented under different heads as under :

4.1 Survey for disease status

To find out the status of early blight disease of potato in Kashmir valley, various potato growing areas in district Budgam, Baramulla, Srinagar and Shopian were surveyed during two consecutive years of 2008 and 2009 in the month of June. The data on disease incidence and intensity recorded during the year 2008 and 2009 are presented in Table 1 and 2 (Fig. 1 and 2).

4.1.1 Disease incidence

Perusal of data (Table 1; Fig. 1) revealed that early blight was present in all the locations surveyed during both the years of 2008 and 2009.

It was observed that district-wise mean disease incidence was maximum in Budgam (39.09%) followed by Baramulla (27.36%), Srinagar (22.53%) and Shopian (14.89%).

Comparison of year-wise data revealed more disease incidence (28.23%) during 2009 as compared to 2008 (24.54%). During the year 2008 maximum disease incidence was recorded at site Kawoosa (44.17%) followed by Mazhama (43.47%), Tarzoo (41.27%), Kuthipora (40.54%), Haigam (40.18%) and Wager (39.02%). Minimum disease incidence (10.22%) was recorded at site Warpora while during the year 2009 maximum disease incidence was recorded at Kawoosa (49.20%) followed by Wagar (49.01%), Mazhama (47.40%), Tarzoo (45.49%), Kuthipora (44.92%) and Haigam (43.66%). Minimum disease incidence (12.68%) was recorded at site Warpora. Mean disease incidence at the locations surveyed varied from 13.06 to 44.95 per cent with overall mean incidence of 25.96 per cent.

Table-1 : Incidence of early blight of potato in various districts of Kashmir Valley during 2008 and 2009

District	Location	Site	2008	2009	Pooled mean
Budgam	Khansahib	Kremshore	37.24	42.33	**39.78**
		Wager	39.02	49.01	**41.51**
		Khansahib	37.26	42.13	**36.69**
		Mean	**37.84**	**44.49**	**39.32**
	Chadoora	Nowbough	27.52	28.08	**27.03**
		Bugam	30.77	35.06	**32.91**
		Kaisarmulla	37.28	40.08	**39.04**
		Mean	**31.85**	**34.40**	**32.99**
	Mazhama	Mazhama	43.47	47.40	**45.44**
		Kuthipora	40.54	44.92	**42.73**
		Kawoosa	44.17	49.20	**46.68**
		Mean	**42.72**	**47.17**	**44.95**
		District Mean	**37.47**	**42.02**	**39.09**
Baramulla	Pattan	Pattan	24.19	27.27	**25.72**
		Ganiepora	31.65	34.29	**32.97**
		Ganjipora	31.56	33.79	**32.67**
		Mean	**29.13**	**31.78**	**30.45**
	Sopore	Haigam	40.18	43.66	**33.41**
		Tarzoo	41.27	45.49	**43.35**
		Krankshavan	28.66	31.88	**30.26**
		Mean	**36.70**	**40.34**	**35.67**
	Tangmarg	Yarikhah	19.01	20.76	**19.88**
		Zeeran	15.50	17.77	**16.63**
		Warpora	10.22	12.68	**11.36**
		Mean	**14.91**	**17.07**	**15.95**
		District Mean	**26.91**	**29.73**	**27.36**

Contd…..

Table-1 (Contd…)

District	Location	Site	2008	2009	Pooled mean
Srinagar	Noorbagh	Noorbagh	15.55	20.63	**18.09**
		Palpora	17.23	23.71	**20.47**
		Waganpora	19.55	24.16	**21.85**
		Mean	**17.44**	**22.83**	**20.13**
	HMT	Maloora	21.67	24.26	**22.97**
		Mujgund	23.57	26.67	**25.12**
		Zainkot	25.53	30.39	**27.76**
		Mean	**23.59**	**27.10**	**25.28**
	Harwan	Theed	25.19	28.15	**26.67**
		Darbagh	18.73	20.17	**19.45**
		Saidapora	19.59	21.30	**20.87**
		Mean	**21.17**	**23.20**	**22.33**
		District Mean	**20.73**	**24.38**	**22.53**
Shopian	Sedew	Sedew	14.77	18.18	**16.47**
		Chetipora	13.35	17.03	**14.86**
		Check	14.28	18.09	**16.18**
		Mean	**14.13**	**17.76**	**15.83**
	Herpur	Bohir hela	11.02	14.03	**12.52**
		Padapawan	12.04	15.38	**13.71**
		Herpur	11.41	14.54	**12.97**
		Mean	**11.49**	**14.65**	**13.06**
	Chogam	Chogam	13.02	18.35	**15.68**
		Kanipora	15.68	19.28	**17.48**
		Khudpora	12.08	16.27	**14.17**
		Mean	**13.59**	**17.96**	**15.77**
		District Mean	**13.07**	**16.79**	**14.89**
		Overall Mean	**24.54**	**28.23**	**25.96**

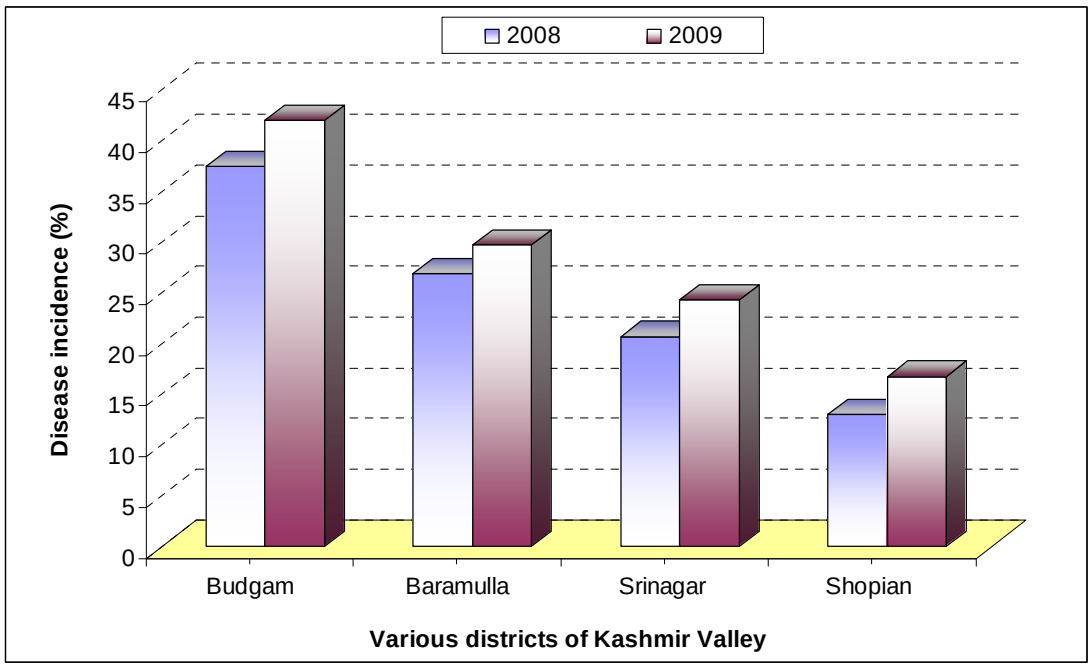


Fig. 1 : Incidence of early blight of potato in various districts of Kashmir Valley during 2008 and 2009

Maximum disease incidence of 44.95 per cent was noticed at Mazhama (Budgam) followed by Khansahib (39.32%), Sopore (35.67%), Chadoora (32.99%) and Pattan (30.45%).

On an overall comparison amongst different localities surveyed, the highest disease incidence of 44.95 per cent was recorded at Mazhama which was statistically at par with that of Khansahib (39.32%). The least disease incidence of 13.06 per cent was recorded at Herpur (Shopian).

4.1.2 Disease intensity

Perusal of data (Table 2; Fig. 2) revealed that mean disease intensity at the locations surveyed varied from 6.93 to 26.19 per cent with overall mean intensity of 14.84 per cent. Among sites, maximum disease intensity was recorded at Kawoosa (27.33%) followed by Mazhama (26.33%), Tarzoo (25.37%), Kuthipora (24.91%), Haigam (24.39%) and Wager (24.00%). While among locations maximum disease intensity was recorded at Mazhama (26.19%) followed by Khansahib (23.25%), Sopore (22.62%), Chadoora (18.19%), Pattan (17.10%) and HMT (14.66%).

Comparison of year-wise data revealed more disease intensity (14.98%) during 2009 as compared to 2008 (13.84%). District-wise mean disease intensity was maximum in Budgam (22.54%) followed by Baramulla (16.25%), Srinagar (12.53%) and Shopian (8.05%).

On an overall comparison amongst different localities surveyed, the highest disease intensity of 26.19 per cent was recorded at Mazhama followed by Khansahib (23.25%). The least disease intensity of 6.93 per cent was recorded at Herpur (Shopian).

Table-2 : Intensity of early blight of potato in various districts of Kashmir Valley during 2008 and 2009

District	Location	Site	2008	2009	Pooled mean
Budgam	Khansahib	Kremshore	21.35	24.82	**23.08**
		Wager	22.43	25.58	**24.00**
		Khansahib	21.02	24.33	**22.67**
		Mean	**21.60**	**24.91**	**23.25**
	Chadoora	Nowbough	19.80	16.08	**15.44**
		Bugam	16.29	18.96	**17.62**
		Kaisarmulla	20.58	22.48	**21.53**
		Mean	**18.89**	**19.17**	**18.19**
	Mazhama	Mazhama	25.37	27.28	**26.33**
		Kuthipora	23.76	26.06	**24.91**
		Kawoosa	26.04	28.61	**27.33**
		Mean	**25.05**	**27.31**	**26.19**
		District Mean	**21.84**	**23.80**	**22.54**
Baramulla	Pattan	Pattan	13.47	15.51	**14.49**
		Ganiepora	18.03	20.02	**19.03**
		Ganjipora	16.41	19.17	**17.79**
		Mean	**15.97**	**18.23**	**17.10**
	Sopore	Haigam	23.39	25.40	**24.39**
		Tarzoo	23.80	26.95	**25.37**
		Krankshavan	16.56	19.45	**18.10**
		Mean	**21.25**	**23.93**	**22.62**
	Tangmarg	Yarikhah	10.05	12.86	**11.45**
		Zeeran	8.31	10.91	**9.61**
		Warpora	5.63	6.52	**6.07**
		Mean	**7.99**	**10.09**	**9.04**
		District Mean	**15.07**	**17.42**	**16.25**

Contd…..

Table-2 (Contd…)

District	Location	Site	2008	2009	Pooled mean
Srinagar	Noorbagh	Noorbagh	7.83	10.63	**9.23**
		Palpora	8.89	11.66	**10.27**
		Waganpora	10.55	12.95	**11.75**
		Mean	**9.09**	**11.74**	**10.41**
	HMT	Maloora	12.75	14.05	**13.40**
		Mujgund	13.64	15.24	**14.44**
		Zainkot	14.35	17.94	**16.14**
		Mean	**13.58**	**15.74**	**14.66**
	Harwan	Theed	13.88	15.77	**14.82**
		Darbagh	10.00	11.59	**10.80**
		Saidapora	11.04	12.87	**11.95**
		Mean	**11.64**	**13.41**	**12.52**
		District Mean	**11.43**	**13.63**	**12.53**
Shopian	Sedew	Sedew	7.83	10.46	**9.14**
		Chetipora	7.11	10.18	**8.64**
		Check	7.50	9.42	**8.46**
		Mean	**7.48**	**10.02**	**8.74**
	Herpur	Bohir hela	5.68	7.64	**6.66**
		Padapawan	6.77	8.47	**7.62**
		Herpur	5.57	7.49	**6.53**
		Mean	**6.00**	**7.86**	**6.93**
	Chogam	Chogam	**6.94**	**9.88**	**8.41**
		Kanipora	8.59	10.48	**9.54**
		Khudpora	7.21	7.82	**7.51**
		Mean	**7.58**	**9.39**	**8.48**
		District Mean	**7.02**	**9.09**	**8.05**
		Overall Mean	**13.84**	**15.98**	**14.84**

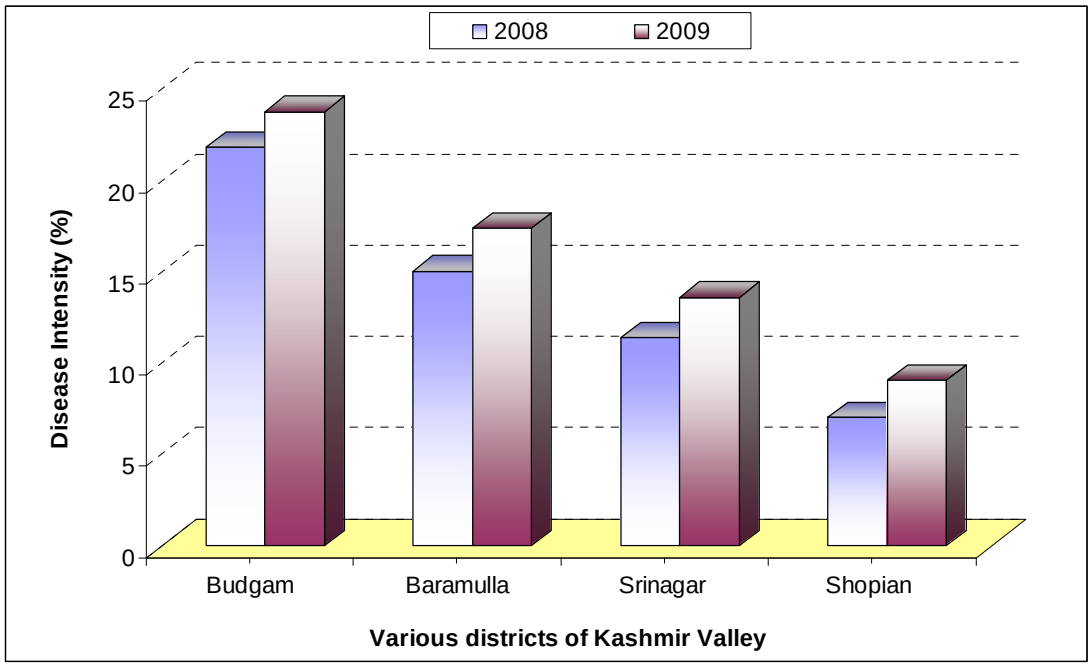


Fig. 2 : Intensity of early blight of potato in various districts of Kashmir Valley during 2008 and 2009

The present investigations indicated variable disease incidence as well as disease intensity at different places. It may be associated with prevalent environmental and/or pathogen factors. Changes in weather variables and amount of initial inoculum of *Alternaria solani* may be responsible for varying disease intensities at different locations (Vander-Walls *et al*., 2001).

4.2 Symptomatology

Symptoms of the early blight disease of potato under natural conditions of infection in field were studied on leaves of unsprayed susceptible potato genotype 'Kufri Jyoti' during the year 2008 and 2009.

The periodic (weekly) observations were recorded from first week of May. The initial disease symptoms were recorded in the second week of May. The first symptoms appeared as small irregular to circular dark brown spots on the lower leaves (Plate-4) measuring 0.5 mm in size. Apparently the leaves looked healthy but the lesions were visible only if the leaves were kept against the source of light. Periodic changes in size, shape and colour of the lesions were observed and the results are summarised in Table-3, Fig. 3.

The lesion progression was slow upto first week of June (0.5-0.6 mm/week), after which it showed a curvilinear behaviour upto third week of July, beyond which the lesion enlargement declined sharply. The maximum lesion size of 7.4 mm was recorded in the second week of August. Upto fourth week of June concentric rings form as a result of irregular growth patterns by the organism in the leaf tissue giving the lesion a characteristic 'target spot' or 'bulls eye' appearance. There was often a narrow, yellow halo around each spot and lesions were usually bordered by veins. Beyond second week of July, due to coalescing of 3-5 spots, small irregular patches were formed. The spots covered large area of the leaf in the last week of July. Severely infected leaves eventually wither and die but usually remain attached to the plant.

Table-3 : Symptomatology of potato early blight caused by *Alternaria solani* on genotype 'Kufri Jyoti' during the year 2008 and 2009

Time of observation		Symptoms	Lesion size (mm)
Month	**Week**		
May	I	No symptoms appeared	-
	II	Small irregular to circular dark brown spots on the lower leaves	0.5
	III	-do-	1.1
	IV	Spots enlarge and are surrounded by a border of yellow host tissue	1.8
June	I	-do-	2.4
	II	-do-	4.2
	III	Concentric rings giving "target spot" or "bulls eye" appearance	4.9
	IV	-do-	5.3
July	I	Narrow yellow halo around each spot	5.6
	II	Irregular lesions due to coalescing of 3-5 spots	6.2
	III	Irregular patches due to coalescing of spots	6.7
	IV	Irregular blighted patches	6.9
August	I	Severely infected leaves eventually wither and die	7.2
	II	-do-	7.4

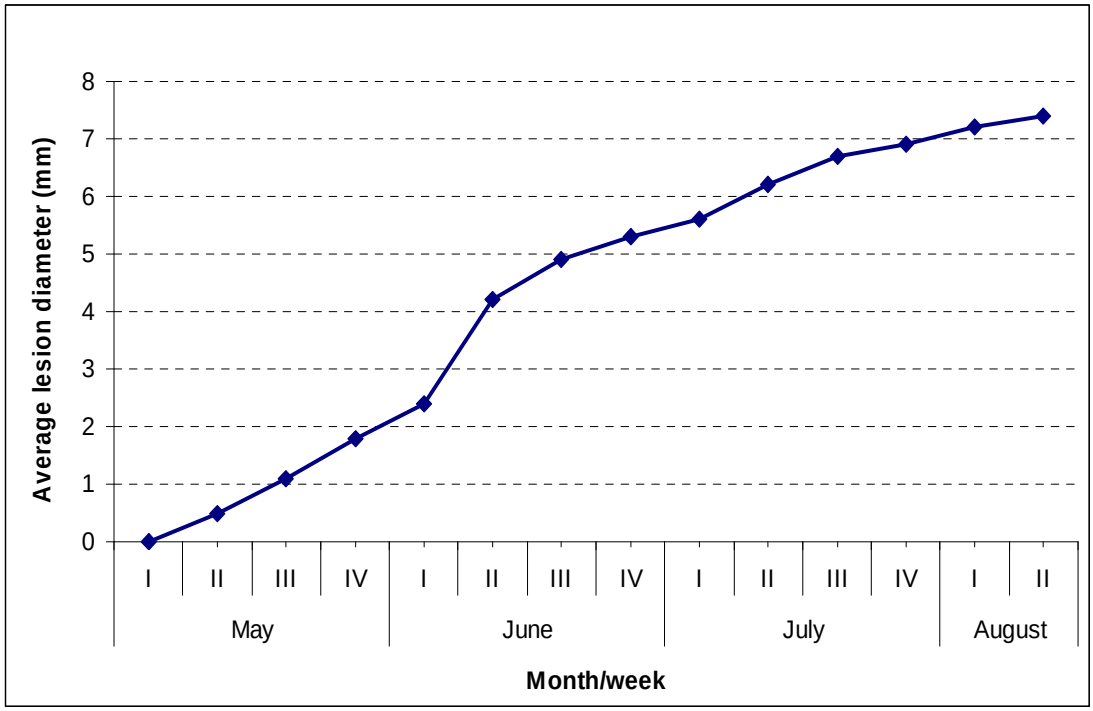
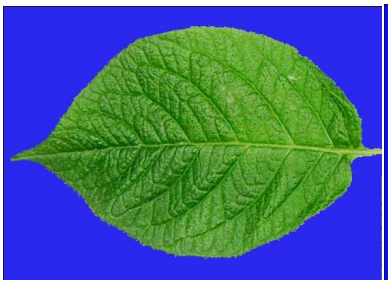
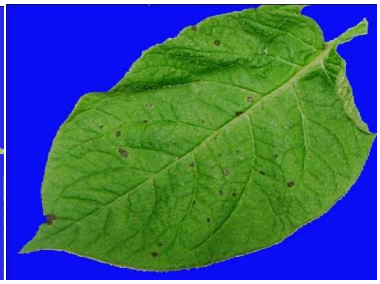


Fig. 3 : Periodical lesion progression of potato early blight caused by *Alternaria solani* on cultivar 'Kufri Jyoti'

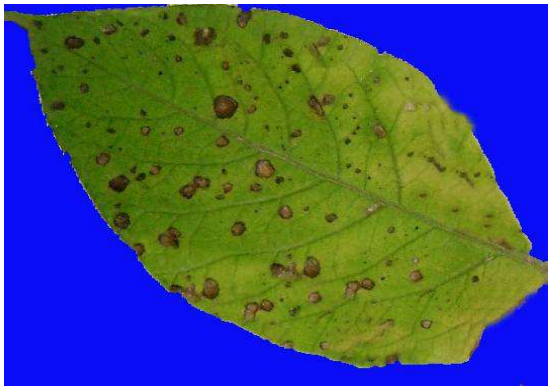


No symptoms appeared

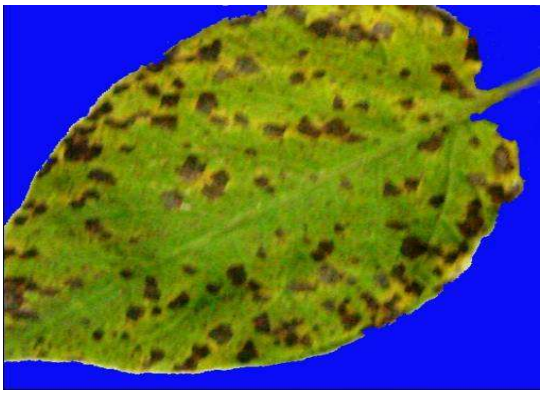


Small irregular to circular dark brown spots

Spots enlarged



Concentric rings giving “target spot” or “bulls eye” appearance



Narrow yellow halo around each spot

Irregular lesions due to coalescing of 3-5 spots

Severely infected Leaf

Plate-4 : Symptomatological development of *Alternaria* leaf spot in field

4.3 Isolation, purification and maintenance of pure culture

Isolations were made from diseased leaf tissues of potato genotype 'Kufri Jyoti'. After 72 hours of incubation at 25±2^{o}C, dark ranging from grey to black with tints of olive or brown mycelial growth started emerging from the diseased leaf tissues, inoculated on potato dextrose agar medium. The culture so obtained was purified by the hyphal tip and single spore isolation methods. The pure culture was maintained by subculturing at monthly intervals and stored in a refrigerator for further studies.

4.4 Pathogencity test

Observations regarding the pathogencity of the test fungus revealed the initiation of typical symptoms of the disease after 10 days of inoculation on injured detached leaves of potato (Plate-5). However, in field plantation, symptoms appeared on injured leaves 14 days after inoculation. In case of uninjured leaves the disease symptoms does not appear. Reisolations from infected leaves yielded typical cultures of the fungus thus satisfied the Koch's postulates.

4.5 Morphology of the fungus

The morphological characters of the pathogen were studied in culture (*in vitro*) are presented in Table-4; Plate-6. The morphological characters of fungus isolated from potato leaves, were studied on potato dextrose agar medium. The various morphological characters of the pathogen observed in culture are as under:

4.5.1 Macroscopic characters

The fungus at first produced a mycelium which was dark, ranging from grey to black with tints of olive or brown. Colonies were spreading hairy and grey brown to black, possessing a texture similar to cotton, felt or velvet.

Table-4 : Morphological characters of *Alternaria solani* causing early blight disease of potato

Structure	Characters	Size
Colony	Spreading, hairy and gray-brown to black in colour	-
Mycelium	Branched, septate, dark coloured with tints of olive brown	-
Conidiophores	Septate, short, simple, straight or flexuous dark coloured	50-90 x 9 µm (Av. 67 x 7 µm)
Conidia	Long beaked, muriform, dark coloured, borne singly, both longitudinal and transverse septa in mature conidia	15-19 x 150-300 µm (Av. 17 x 163 µm)

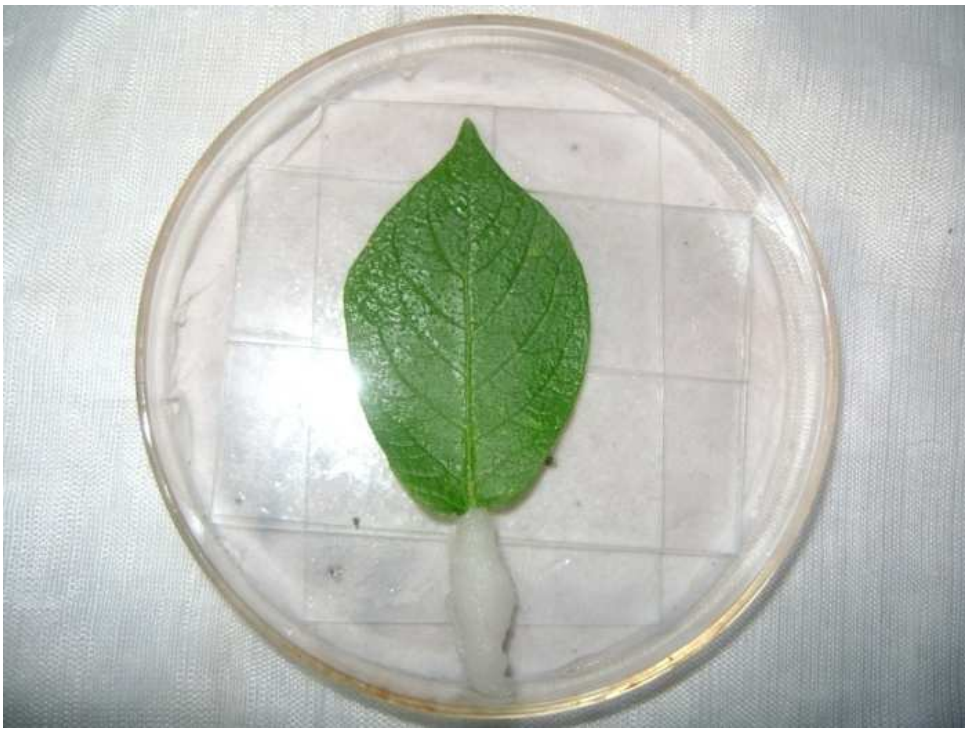
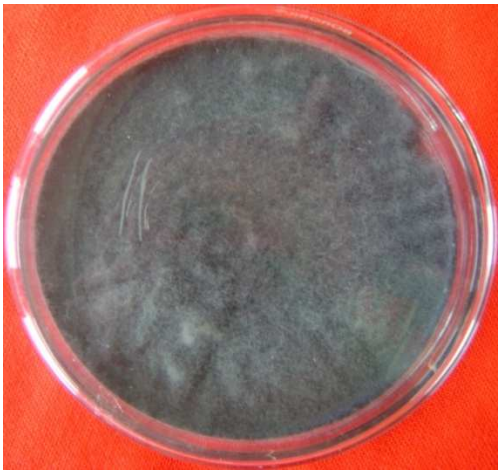
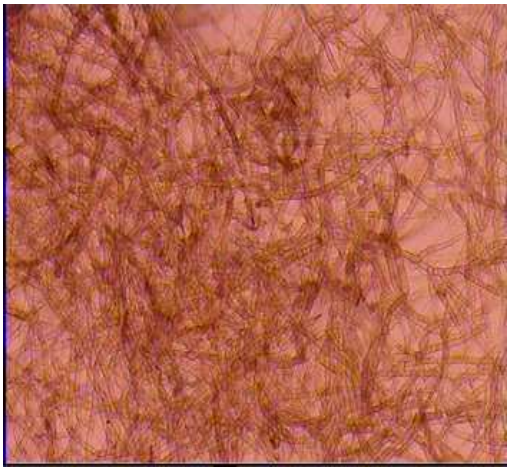


Plate-5 : Pathogenicity of *Alternaria solani* on potato



Culture of *Alternaria solani*



Mycelium of Alternaria solani

Mycelium and conidiophore

Conidium

Plate-6 : Morphological characters of *Alternaria solani*

4.5.2 Microscopic characters

The pathogen in culture produced septate, dark coloured, ranging from grey to black with tints of olive or brown mycelium. The conidiophores were septate, short, simple, erect, flexuous pale to olive brown in colour. They measured 50-90 x 9 μm with an average size of 60 x 7 μm. The conidia were dark coloured and muriform, with 9 to 11 transverse septa and 2-3 longitudinal septa. They were ellipsoid to oblong with a long beak which was occasionally branched. They measured 15-19 x 150-300 μm with an average size of 17 x 163 μm. The beak was flexuous pale and 2.0-5.0 μm wide.

4.5.3 Identification of the pathogen

On the basis of morphological characters, pathogencity and comparison with the authentic description, the fungus was identified as *Alternaria solani* (Ellis and Mart) Jones and Grout. Further its identity was confirmed by Dr. P.N. Chudhary, Principal Mycologist, National Centre of Fungal Taxonomy, New Delhi, under NCFT No. 4372.11.

4.6 Role of meteorological factors in disease development

In order to ascertain the role of various meteorological factors in disease development, an attempt was made to correlate the periodic disease intensity and apparent infection rate with prevailing temperature, relative humidity (RH) and precipitation during the year 2008 and 2009. Weekly data on mean temperature, relative humidity, precipitation and per cent disease intensity as well as apparent infection rate (unit/day) recorded are presented in Table-5 and 6; Fig. 4 and 5.

Table-5 : Influence of weather factors on development of early blight of potato during the year 2008

Time of observation		Disease intensity	Infection rate (unit/day)	*Max. Mean temperature (^{o}C)	*Max. Mean RH (%)	*Mean rainfall (mm)
Month/Year	Week					
May, 2008	I	0.00	-	24.57	67	0.68
	II	0.25	-	24.07	70	1.14
	III	0.55	0.115	26.65	69	1.61
	IV	1.17	0.109	24.70	75	2.91
June, 2008	I	2.69	0.121	28.78	86	3.48
	II	7.60	0.155	26.47	92	8.68
	III	11.43	0.064	26.64	84	5.82
	IV	12.57	0.015	27.43	70	1.76
July, 2008	I	15.36	0.033	28.21	82	4.78
	II	20.84	0.053	30.57	85	5.31
	III	23.39	0.021	29.76	80	2.72
	IV	24.52	0.008	30.64	79	0.84
August, 2008	I	28.66	0.030	31.57	82	3.51
	II	28.66	0.000	28.21	86	4.44

*Mean of seven days

Table-6 : Influence of weather factors on development of early blight of potato during the year 2009

Time of observation		Disease intensity	Infection rate (unit/day)	*Max. Mean temperature (^{o}C)	*Max. Mean RH (%)	*Mean rainfall (mm)
Month/Year	Week					
May, 2009	I	0.00	-	21.07	68	1.07
	II	0.30	-	23.92	71	0.71
	III	0.70	0.123	27.31	72	1.25
	IV	1.61	0.121	26.83	73	3.25
June, 2009	I	3.37	0.150	26.42	86	3.74
	II	10.01	0.165	27.32	91	6.42
	III	15.89	0.075	28.21	85	4.88
	IV	17.84	0.019	27.97	71	2.21
July, 2009	I	20.87	0.027	26.71	84	3.51
	II	25.91	0.040	28.52	91	6.68
	III	26.52	0.004	32.40	78	0.14
	IV	28.16	0.011	29.37	86	2.02
August, 2009	I	30.82	0.018	32.97	87	3.18
	II	30.82	0.000	34.07	88	3.44

*Mean of seven days

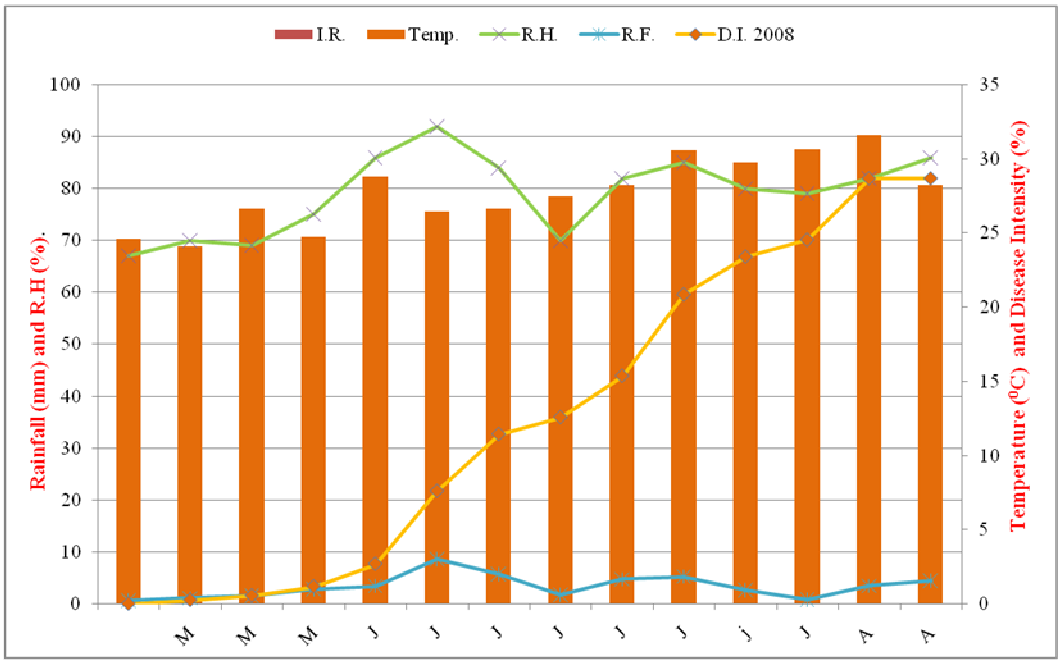


Fig. 4 : Influence of weather factors on development of early blight of potato during the year 2008

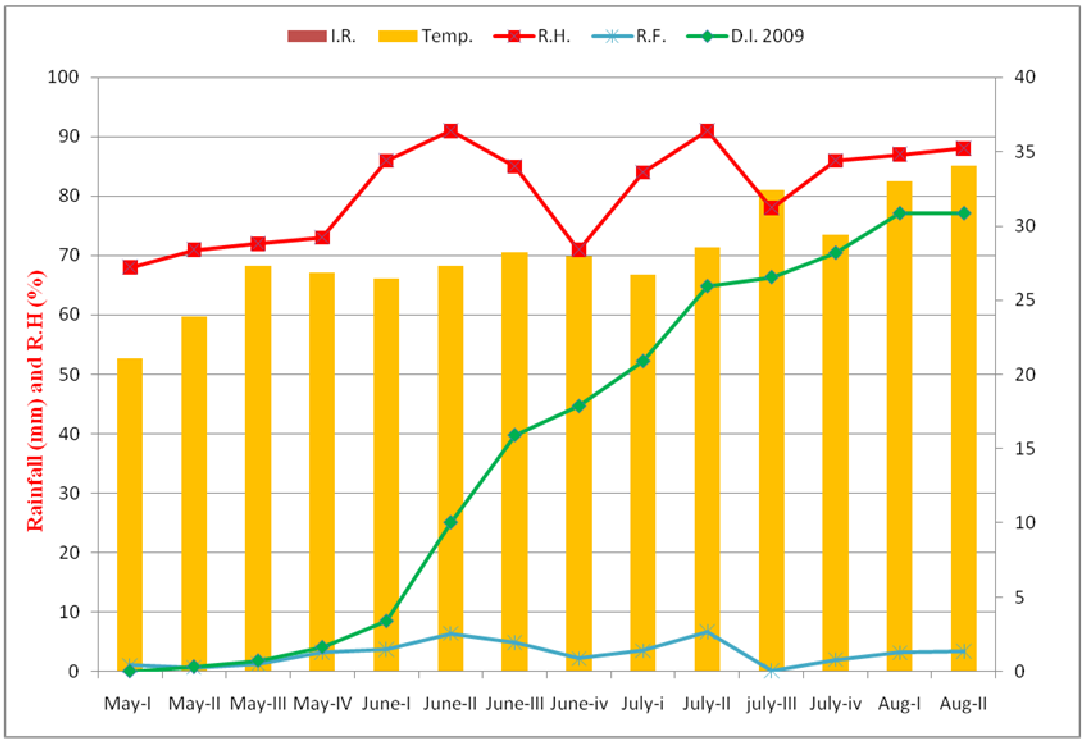


Fig. 5 : Influence of weather factors on development of early blight of potato during the year 2009

Perusal of data revealed that the disease appeared during the second week of May in both the years and became conspicuous during the month of July. The disease intensity reached its peak in the second week of August, beyond which disease severity could not be assessed, because of defoliation. Maximum apparent infection rate of 0.155 (unit/day) and 0.165 (unit/day) were observed during second week of June in both the years i.e. 2008 and 2009, respectively. The temperature 26.47 and 27.32 ^{o}C, relative humidity 92 and 91 per cent and precipitation 8.68 and 6.42 mm during 2008 and 2009, respectively, apparently favoured the maximum disease development during these periods.

The data was subjected to statistical analysis for finding the correlation between various meteorological factors and disease intensity. The correlation matrix (Table-7) indicated that temperature showed a significant and positive correlation with disease intensity, whereas, relative humidity and precipitation showed a positive but non-significant correlation with disease intensity. Multiple regression analysis was also worked out to know the extent of correlation which is presented in Table-8. Results of multiple regression analysis revealed that weather factors accounted for 66.5 per cent variation.

4.7 Perpetuation studies

The perpetuation of the fungus *Alternaria solani* was studied in diseased leaves by placing the leaves on ground surface and at 20 cm depth, as well as in diseased potatoes kept in ambient store during the year 2008 and 2009.

4.7.1 Survival on over-wintered diseased leaves

Diseased leaves in mesh wire bags were placed on ground surface as well as buried at 20 cm depth. Observations regarding the spore production and their viability were recorded at fortnightly intervals and are presented in Table-9 and 10.

Table-7 : Coefficients of simple correlation of meteorological factors with disease intensity of *Alternaria solani*

Weather factor	Disease intensity (%)	
	2008	2009
Mean temperature (^{o}C)	0.81*	0.83*
Mean relative humidity (%)	0.47	0.61*
Mean rainfall (mm)	0.18	0.20

* Significant at (p = 0.05)

Table-8: Multiple regression equation indicating the relationship of meteorological factors with disease intensity

Year	Linear equation	R^2 (%)
2008-09	Disease intensity (y) = 96.0 + 2.61 x1 + 0.50 x2 – 0.92 x3	66.5

Where,

x1 = Temperature,

x2 = Relative humidity

x3 = Rainfall

Table-9 : **Production and viability of *Alternaria solani* conidia on infected potato leaves kept on ground surface and 20 cm depth during the year 2008**

Month	Fortnight	Number of conidia (cm^{-2} leaf area)*		Viability (%)	
		Ground surface	20 cm	Ground surface	20 cm
March	I	20	-	27.0	NA
	II	63	-	29.4	NA
April	I	86	-	30.8	NA
	II	123	-	32.3	NA
May	I	173	-	37.0	NA
	II	203	-	40.6	NA
June	I	294	-	44.3	NA
	II	263	-	38.4	NA
July	I	160	-	31.7	NA
	II	-	-	-	NA

* Mean of three replications each comprising of 30 leaf discs of 1 cm^2 surface area

\- = Material perished

NA = Spores not available

Table-10 : Production and viability of *Alternaria solani* conidia on infected potato leaves kept on ground surface and 20 cm depth during the year 2009

Month	Fortnight	Number of conidia (cm^{-2} leaf area)*		Viability per cent	
		Ground surface	20 cm	Ground surface	20 cm
March	I	42	-	28.3	NA
	II	83	-	31.6	NA
April	I	110	-	32.0	NA
	II	138	-	34.4	NA
May	I	206	-	42.0	NA
	II	240	-	45.6	NA
June	I	323	-	49.3	NA
	II	308	-	40.5	NA
July	I	220	-	32.4	NA
	II	-	-	-	NA

*Mean of three replications each comprising of 30 leaf discs of 1 cm^2 surface area

- = Material perished

NA = Spores not available

Perusal of data revealed that the spore production in over-wintered diseased leaves continued for comparatively longer period, upto first fortnight of June, in both the years (2008 and 2009) of experimentation, when kept at ground surface. The average number of spores cm^{-2} diseased leaf area increased upto first fortnight of June in the year 2008 and 2009, with maximum number of 294 and 323 spores, respectively. However, by the first fortnight of July, the number gradually declined to 160 and 220 spores, respectively. In diseased leaves kept at 20 cm depth in soil, the spores were altogether absent throughout the observation period, because the leaves were decomposed.

The highest spore viability of 44.3 and 49.3 per cent in leaves on ground surface was recorded in the first fortnight of June, 2008 and 2009, respectively. However, in leaves buried at 20 cm depth the spores were altogether absent because the leaves were decomposed.

4.7.2 Survival on diseased potatoes kept at ambient store

Perpetuation of pathogen (*A. solani*) in diseased potatoes was studied by keeping them in ambient store and the production and viability of spores at fortnightly intervals commencing from the first fortnight of March was recorded. The data recorded is presented in Table-11 and 12.

The perusal of data revealed that the diseased potatoes continuously produced spores during entire period of study in both the years (2008 and 2009). The average number of spores increased upto first fortnight of June, both in 2008 and 2009, with a maximum number of 430 and 508 spores, respectively. The number then gradually declined to 216 and 263 spores, respectively, till last observation recorded in the second fortnight of July.

Initial viability of spores from diseased potatoes in first week of March was 24.5 and 29.0 per cent during 2008 and 2009, respectively. The viability of spores gradually increased with time till first fortnight of June during the year 2008 and 2009, respectively, which then showed a gradual decrease to 23.5 and 27.6 per cent till last observation.

Table-11 : Production and viability of *Alternaria solani* conidia on infected potato tubers kept in ambient store during the year 2008

Month	Fortnight	Number of conidia (cm^{-2} slice area)*	Viability (%)
March	I	30	24.5
	II	69	30.2
April	I	130	33.6
	II	200	38.2
May	I	305	42.3
	II	363	49.7
June	I	430	52.5
	II	385	46.3
July	I	308	35.0
	II	216	23.5

* Mean of three replications each comprising of 30 slice discs of 1 cm^2 surface area

Table-12 : Production and viability of *Alternaria solani* conidia on infected potato tubers kept in ambient store during the year 2009

Month	Fortnight	Number of conidia (cm^{-2} slice area)*	Viability (%)
March	I	52	29.0
	II	91	32.5
April	I	141	37.3
	II	225	42.5
May	I	313	45.6
	II	456	50.5
June	I	508	55.7
	II	436	51.3
July	I	370	40.2
	II	263	27.6

* Mean of three replications each comprising of 30 slice discs of 1 cm^2 surface area

4.8 Disease management

4.8.1 Screening of potato genotypes against early blight

The evaluation study of 25 potato genotypes conducted in the year 2008 and 2009 under natural epiphytotic conditions against early blight indicated that disease occurred on all the test genotypes during both the years (Table-13 and 14). However, analysis of data showed a differential response among the genotypes with regard to incidence as well as intensity.

4.8.1.1 Disease incidence

The disease incidence was significantly different for two years with 46.41 per cent recorded in the year 2009 and 40.52 per cent in 2008. The disease incidence among genotypes ranged between 13.96 to 63.20 per cent during the year 2008 as against 18.62 to 70.38 per cent in 2009 (Table-13).

Analysis of pooled data for two years indicated that most of the genotypes evaluated were susceptible to the disease but there existed a significant difference in disease incidence among different genotypes.

Maximum disease incidence was recorded in genotype SM/91-1515 which was statistically at par with Gulmarg special with average incidence of 66.92 and 64.31 per cent, respectively. The disease incidence of 61.58 per cent was recorded in SM/45-43 which was statistically at par with HB/18-36 (59.72%), SM/88 -991 (56.28%), SM/90-68 (55.41%) and HB/87-185 (55.36%). Kufri Jyoti with disease incidence of 51.51 per cent was statistically at par with SM/92-725 (48.911%), Shalimar Potato-1 (48.64%), Kufri Giriraj (47.76), SM/87-55 (46.78%) and TPS/C3 (45.45%). SM/98-239 with disease incidence of 41.85 per cent was statistically at par with Kufri Chandramukhi (41.69%), SM/93-237 (40.97%) and HB/82-18 (40.97%). The lowest disease incidence was recorded in genotype SM/92-338 which was statistically at par with Kufri Himami with average incidence of 16.29 and 17.60 per cent, respectively.

Table-13 : Evaluation of different potato genotypes against early blight caused by *Alternaria salani*

S. No.	Genotype	Disease incidence (%)		
		2008	2009	Pooled Mean
1.	Kufri Girdari	27.17 (31.38)	30.19 (33.29)	**28.68 (32.85)**
2.	Kufri Jyoti	46.71 (43.11)	56.28 (48.59)	**51.51 (45.86)**
3.	K. himami	14.71 (22.53)	20.50 (26.88)	**17.60 (24.80)**
4.	K. shalija	28.92 (32.49)	31.13 (33.82)	**30.02 (33.18)**
5.	K. Giriraj	44.17 (41.63)	51.36 (45.76)	**47.76 (43.71)**
6.	K.C.M.	39.04 (38.64)	44.35 (41.73)	**41.69 (40.21)**
7.	Sm/88-991	50.61 (45.33)	61.96 (51.91)	**56.28 (48.61)**
8.	SM/45-43	58.15 (49.68)	65.01 (53.72)	**61.58 (52.71)**
9.	Sm/92-725	44.84 (42.02)	52.98 (46.69)	**48.91 (44.37)**
10.	SM/92-338	13.96 (21.91)	18.62 (25.52)	**16.29 (23.78)**
11.	SM/91-1515	63.20 (52.65)	70.38 (57.19)	**66.92 (54.89)**
12.	SM/98-239	39.72 (39.04)	43.99 (41.52)	**41.85 (40.31)**
13.	SM/93-237	38.68 (38.43)	43.27 (41.11)	**40.97 (39.80)**
14.	SM/90-68	52.36 (46.33)	58.47(49.85)	**55.41 (48.10)**
15.	SM/87-55	43.81 (41.41)	49.76 (44.84)	**46.78 (43.15)**
16.	SM/96-127	23.90 (29.47)	28.81 (32.43)	**26.52 (30.99)**
17.	SM/90-45	33.03 (35.01)	36.93 (37.38)	**34.97 (36.24)**
18.	SM/94-44	25.66 (30.39)	26.12 (30.71)	**25.89 (30.57)**
19.	HB/18-36	57.36 (49.21)	62.08 (51.99)	**59.72 (50.61)**
20.	HB/87-185	50.71 (45.49)	60.02 (50.76)	**55.36 (48.09)**
21.	HB/82-18	37.82 (37.92)	44.13 (41.61)	**40.97 (39.80)**
22.	HB/50-45	32.62 (34.81)	35.53 (36.55)	**34.07 (35.70)**
23.	TPS/C3	39.70 (40.48)	48.73 (44.24)	**45.45 (42.39)**
24.	Shalimar Potato-I	45.30 (42.28)	51.99 (46.12)	**48.64 (44.22)**
25.	Gulmarg Special	60.91 (51.28)	67.85 (55.44)	**64.38 (33.35)**
	Overall mean	**40.52 (39.31)**	**46.41 (42.78)**	**43.52 (41.09)**
	CD (p=0.05)	**3.18**	**3.80**	**2.67**

*Mean of three replications

**Figures in parenthesis are arc sine transformed values

4.8.1.2 Disease intensity

The disease intensity was significantly different for two years with 24.30 per cent recorded in the year 2009 and 21.59 per cent in 2008. The disease intensity among genotypes ranged between 3.04 to 36.70 per cent during the year 2008 as against 4.72 to 40.88 per cent in 2009 (Table-14).

Analysis of the data (pooled) indicated that most of the genotypes evaluated were susceptible to the disease but there existed a significant difference in disease intensity among different genotypes.

Maximum disease intensity was recorded in genotype SM/91-1515 which was statistically at par with Gulmarg special and SM/45-43 with average intensity of 38.79, 37.96 and 36.19 per cent, respectively. The disease intensity of 34.83 per cent was recorded in HB/18-36 which was statistically at par with SM/88-991 (32.74%) and SM/90-68 (31.38%). HB/87-185 with disease intensity of 29.24 per cent was statistically at par with Kufri Jyoti (28.87%). Kufri Giriraj with disease intensity of 26.99 per cent was statistically at par with SM/92-725 (26.80%), SM/87-55 (25.88%) and TPS/C3 (25.45%). The least disease intensity was recorded in SM/92-338 which was statistically at par with Kufri Himami with average intensity of 3.88 and 5.38 per cent, respectively.

Of the potato genotypes screened (Table-15) only SM/92-338 was resistant (rating between 0-5 % PDI) and three genotypes viz., Kufri himami, SM/96-127 and SM/94-44 were rated as moderately resistant (5.1-10%). Nine genotypes viz., Kufri girdari, Kufri shailja, Kufri chandramukhi, SM/98-239, SM/93-237, SM/90-45, HB/82-18, HB/50-45 and Shalimar potato-1 were rated as moderately susceptible (10.1-25%). The remaining genotypes were susceptible in reaction.

Table-14 : Evaluation of different potato genotypes against early blight caused by *Alternaria salani*

S. No.	Genotype	Disease intensity (%)		
		2008	**2009**	**Pooled Mean**
1.	Kufri Girdari	14.38 (23.06)	15.46 (23.92)	**14.94 (23.49)**
2.	Kufri Jyoti	27.23 (32.55)	30.52 (34.67)	**28.87 (33.61)**
3.	K. Himami	4.34 (12.43)	6.43 (15.16)	**5.38 (13.79)**
4.	K. Shalija	15.23 (23.72)	17.15 (25.20)	**18.66 (24.46)**
5.	K. Giriraj	25.24 (31.20)	28.75 (33.53)	**26.99 (32.36)**
6.	K.C.M.	21.93 (28.80)	24.14 (30.45)	**23.03 (29.62)**
7.	SM/88-991	30.54 (34.70)	34.95 (37.48)	**32.74 (36.09)**
8.	SM/45-43	34.41 (37.15)	37.97 (39.35)	**36.19 (38.25)**
9.	SM/92-725	24.67 (30.82)	28.94 (33.65)	**26.80 (32.23)**
10.	SM/92-338	3.04 (10.38)	4.72 (12.84)	**3.88 (11.61)**
11.	SM/91-1515	36.70 (38.57)	40.88 (41.12)	**38.79 (39.84)**
12.	SM/98-239	20.60 (27.93)	22.02 (28.96)	**21.30 (28.44)**
13.	SM/93-237	13.05 (21.89)	16.31 (24.64)	**14.68 (23.26)**
14.	SM/90-68	29.90 (34.30)	32.87 (36.20)	**31.38 (35.25)**
15.	SM/87-55	23.91(30.29)	27.86 (32.94)	**25.88 (31.61)**
16.	SM/96-127	7.13 (16.00)	9.70 (18.74)	**8.41(17.37)**
17.	SM/90-45	18.43 (26.26)	19.60 (27.15)	**19.01 (26.70)**
18.	SM/94-44	7.88 (16.86)	9.35 (18.42)	**8.61 (17.64)**
19.	HB/18-36	33.36 (36.50)	36.30 (38.33)	**34.83(37.41)**
20.	HB/87-185	27.63 (32.76)	30.86 (34.90)	**29.24 (33.83)**
21.	HB/82-18	19.86 (27.38)	22.43 (29.23)	**21.16 (28.30)**
22.	HB/50-45	17.82 (25.83)	19.40 (27.02)	**18.60 (26.42)**
23.	TPS/C3	24.89 (30.96)	26.01 (31.72)	**25.45 (31.34)**
24.	Shalimar Potato-I	21.34 (28.47)	25.35 (31.26)	**23.34 (29.86)**
25.	Gulmarg Special	36.29 (38.33)	39.64 (40.38)	**37.96 (39.35)**
Overall mean		**21.59 (27.88)**	**24.30 (29.89)**	**23.04 (28.88)**
CD (p=0.05)		**2.54**	**3.08**	**2.95**

*Mean of three replications

**Figures in parenthesis are arc sine transformed values

Table-15 : Grouping of various potato genotypes based on per cent intensity of early blight

Reaction category	Per cent disease intensity (PDI)	Genotype
Resistant	0-5	SM/92-338
Moderately resistant	5.1-10	Kufri himami, SM/96-127 SM/94-44
Moderately susceptible	10.1-25	Kufri girdari, k. shailja, KCM, SM/98-239, SM/93-237, SM/90-45, HB/82-18, HB/50-45, Shalimar potato-1
Susceptible	25.1-50	Gulmarg special, TPS/C_3 HB/87-185, HB/18-36, SM/87-55, SM/90-68, SM-91-1515, SM/92-725, SM/45-43, SM/88-991, K. Giriraj, K. Jyoti
Highly susceptible	50.1 and above	-

4.9 Biocontrol agents

Three biocontrol agents viz., *Trichoderma harzianum, T. viride* and *T. virens* were screened *in vitro* against *Alternaria solani* by dual culture technique on potato dextrose agar medium. Data on radial mycelial growth, zone of inhibition and degree of antagonism recorded 10 days after incubation at 25±2^{o}C is presented in Table-16.

All the test biocontrol agents significantly inhibited the mycelial growth of test pathogen when compared to check (Table-16; Plate-7). *T. harzianum* was superior to all other test isolates by exhibiting the maximum mycelial growth inhibition of 71.85 per cent. *T. harzianum* did not show any zone of inhibition but completely covered the mycelial growth of test pathogen in 10 days and thus was highly antagonistic in nature. The next best biocontrol agent *T. viride* exhibited radial mycelial growth inhibition of 65.93 per cent. It was observed that *T. viride* mycoparasitized *A. solani* in dual culture within 11 days of incubation. It also showed zone of inhibition. Among biocontrol agents *T. virens* was least effective and inhibited 58.65 per cent mycelial growth of *A. solani. T. virens* neither colonized the fungal mycelium of the pathogen nor mycoparasitized it and was accordingly categorized as moderate antagonist.

4.10 Management through botanicals

The efficacy of different botanicals against *A. solani* was evaluated *in vitro* by poisoned food technique.

4.10.1 Effect on mycelial growth

The data on *in vitro* efficacy of test botanicals in inhibiting the mycelial growth of *A. solani* is presented in Table-17; Fig. 6 and Plate-8.

An insight into the data revealed that all the botanicals significantly inhibited mycelial growth of *A. solani* at all concentrations tested. *Datura stramonium* proved significantly superior to all other botanicals exhibiting 61.12 per cent of mycelial growth inhibition of the test fungus. This was followed by *Artimesia absinthium* (58.54%). The other botanicals in order of their efficacy

Table-16 : ***In vitro*** **effect of various antagonists on mycelial growth of** ***Alternaria solani*** **in dual culture**

Antagonist	Growth inhibition (%)	Zone of inhibition*
Trichoderma harzianum	71.85 (30.19)	-
Trichoderma viride	65.93 (33.62)	+
Trichoderma virens	58.65 (37.85)	-
CD (p = 0.05)	**2.88**	

* + inhibition zone present; - inhibition zone absent

Figures in parenthesis are arc sine transformed values

Table-17 : ***In vitro*** **efficacy of ethanol extracts of various botanicals in inhibiting the mycelial growth of *Alternaria solani***

Botanical \ Conc. (%)	Per cent inhibition of mycelial growth over control						
	10	**20**	**30**	**40**	**50**	**60**	**Mean**
Artimesia (*Artimesia absinthium)*	44.77* (6.69)	52.12 (7.21)	59.31 (7.70)	61.44 (7.83)	65.19 (8.07)	68.41 (8.27)	**58.54 (7.65)**
Datura (*Datura strmonium*)	46.40 (6.81)	56.16 (7.49)	61.81 (7.86)	63.87 (7.99)	66.95 (8.18)	71.57 (8.45)	**61.12 (7.81)**
Nettle (*Urtica dioica*)	27.65 (5.25)	30.50 (5.52)	33.15 (5.75)	40.07 (6.33)	44.50 (6.67)	48.22 (6.94)	**37.34 (6.11)**
Walnut (*Juglans regia)*	28.49 (5.33)	33.87 (5.81)	38.35 (6.19)	40.50 (6.36)	43.01 (6.55)	45.69 (6.75)	**38.31 (6.18)**
Mint (*Mentha spicata*)	21.72 (4.66)	30.84 (5.55)	35.01 (5.91)	41.11 (6.41)	46.49 (6.81)	52.96 (7.27)	**38.02 (6.16)**
Mean	**33.68 (5.80)**	**40.69 (6.37)**	**45.52 (6.74)**	**49.39 (7.02)**	**53.22 (7.29)**	**57.37 (7.57)**	

$CD_{(p=0.05)}$

Treatment	:	0.17
Concentration	:	0.19
Treatment x concentration	:	0.43

*Mean of three replications

*Figures in parentheses are square root transformed values

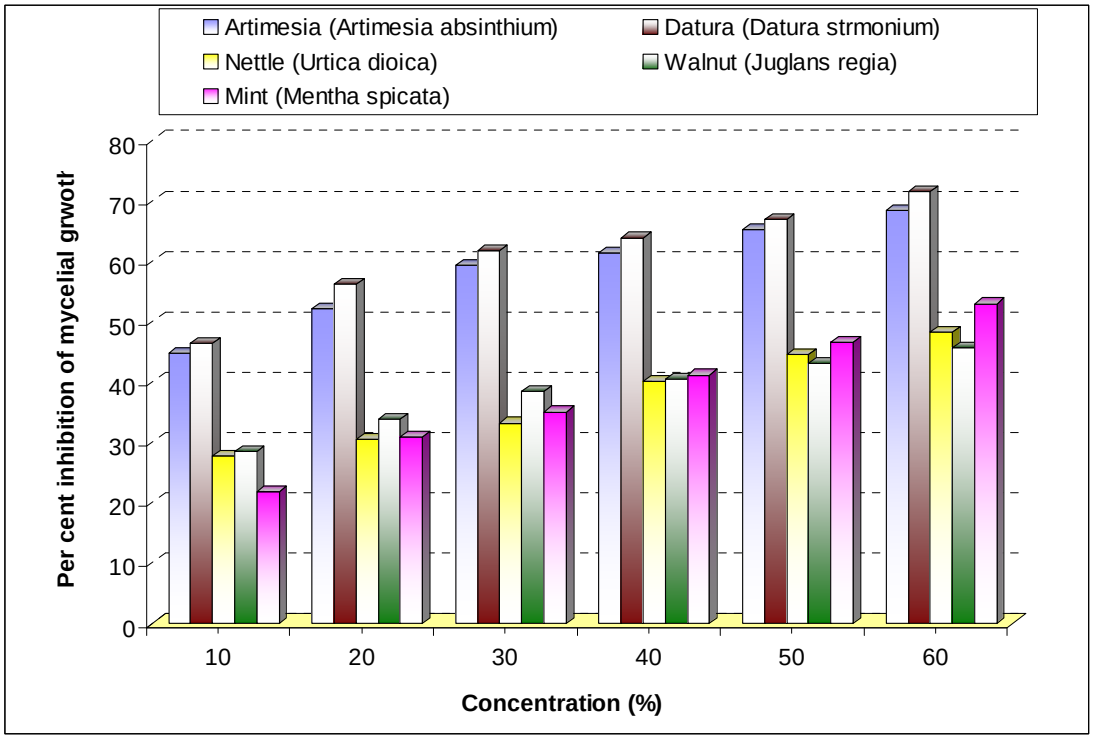
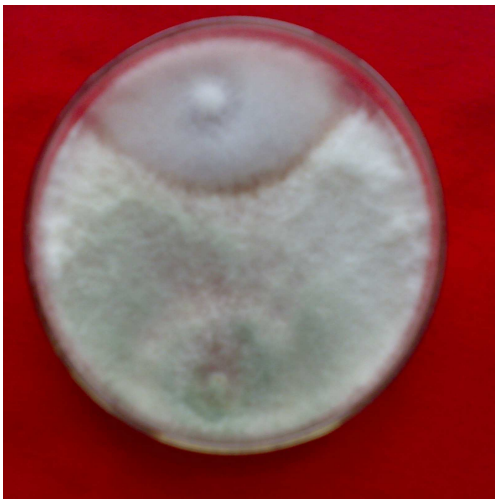


Fig. 6 : ***In vitro*** **efficacy of ethanol extracts of various botanicals in inhibiting the mycelial growth of** ***Alternaria solani***

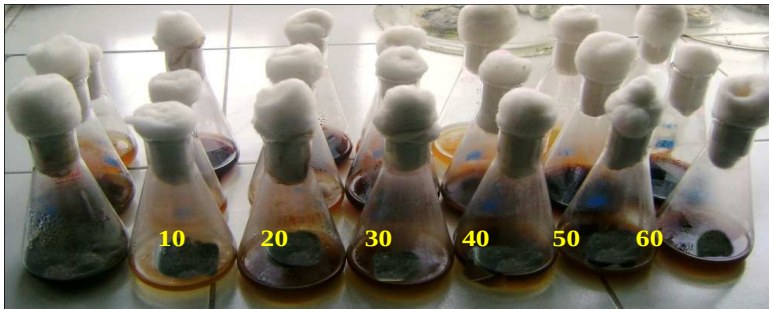
Trichoderma harzianum and *Alternaria solani*



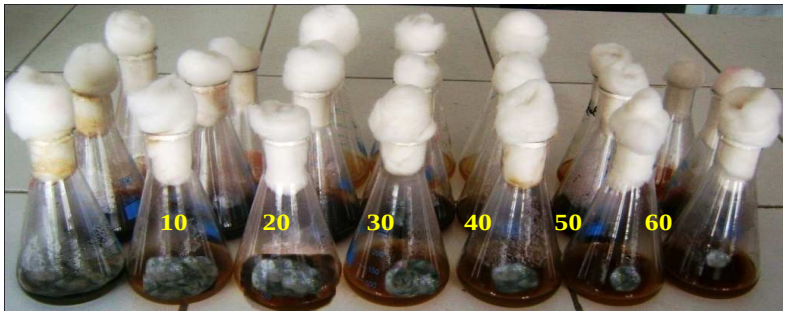
Trichoderma viride and *Alternaria solani*

Gliocladium virens* and *Alternaria solani

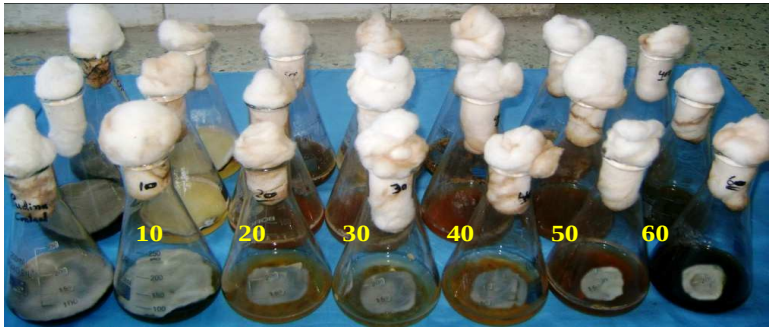
Plate-7 : ***In vitro* efficacy of bioagents in dual culture against *Alternaria solani***



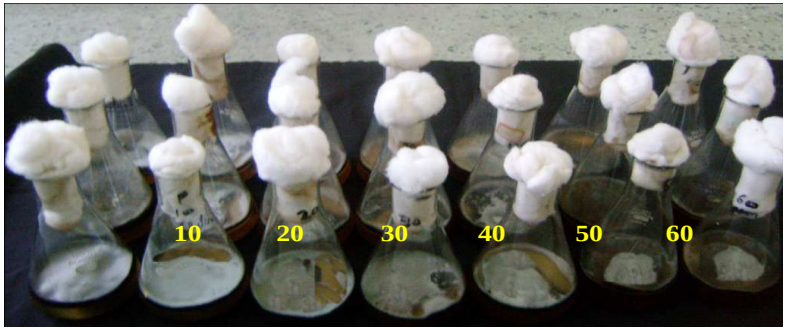
Artimesia



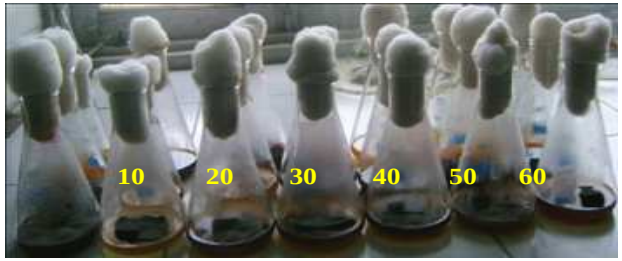
Datura



Mint



Nettle



Walnut hull

Plate-8 : ***In vitro*** **efficacy of ethanol extracts of various botanicals in inhibiting the mycelial growth of** ***Alternaria solani***

were *Juglans regia* (38.31%)>*Mentha spicata* (38.02%)>*Urtica dioica* (37.34%). On an overall basis, the extent of mycelial growth inhibition by the test botanicals increased with increase in their concentration with a minimum inhibition of 33.68 per cent at 10 per cent and increased gradually to 57.37 per cent at 60 per cent concentration.

Data further revealed a significant interaction between botanical and concentration. All the botanicals showed fifty per cent mycelial growth inhibition at 60 per cent concentration or beyond except *Urtica dioica* and *Juglans regia* which exhibited only 48.22 and 45.69 per cent inhibition, respectively.

Among the botanicals tested at 50 and 60 per cent concentration, *D. stramonim* proved superior with mycelial growth inhibition of 66.95 and 71.57 per cent over check. This was closely followed by *A. absinthium* 50 and 60 per cent concentration with mycelial growth inhibition of 65.19 and 68.41 per cent, respectively, over check. At lowest concentration of 10 and 20 per cent *D. stramonium* and *A. absinthium* were superior to other test botanicals. Similarly, at 30 per cent concentration, *D. stramonium* and *A. absinthium* proved effective over check. *U. dioica, M. spicata* and *J. regia* exhibited relatively least inhibition over check at all test concentrations.

4.11 Efficacy of fungitoxicants *in vitro*

The efficacy of different systemic and non-systemic fungitoxicant against *A. solani* was evaluated *in vitro* by poisoned food techniques.

4.11.1 Effect of non-systemic fungitoxicants on mycelial growth

The data on *in vitro* efficacy of test fungitoxicants in inhibiting the mycelial growth of *A. solani* is presented in Table-18; Fig. 7 and Plate-9.

The test fungus *A. solani* was allowed to grow on fungicide-poisoned potato dextrose broth of each treatment concentration. After 15 days incubation at 25±2^{o}C dry mycelial weight was recorded. An insight into the data revealed that

Table-18 : ***In vitro*** **efficacy of various contact fungitoxicants in inhibiting the mycelial growth of *Alternaria solani***

Fungitoxicant	Per cent growth inhibition at different concentrations (ppm)*						
	1000	**1500**	**2000**	**2500**	**3000**	**3500**	**Mean**
Mancozeb 75 WP	53.30 (7.30)	58.70 (7.66)	68.61 (8.28)	80.16 (8.95)	92.03 (9.59)	100.00 (10.00)	**75.46 (8.68)**
Captan 50 WP	36.57 (6.04)	41.44 (6.43)	54.33 (7.37)	76.17 (8.72)	87.91 (9.37)	100.00 (10.00)	**66.07 (8.12)**
Copper oxychloride 50 WP	24.24 (4.92)	36.54 (6.04)	48.66 (6.97)	63.81 (7.98)	78.43 (8.85)	95.18 (9.75)	**57.81 (7.60)**
Propineb 70 WP	37.91 (6.15)	49.63 (7.04)	65.75 (8.10)	78.75 (8.87)	82.78 (9.09)	93.77 (9.68)	**68.09 (8.25)**
Chlorothalonil 75 WP	24.29 (4.92)	38.82 (6.23)	49.29 (7.02)	66.13 (8.13)	77.65 (8.81)	97.16 (9.85)	**58.89 (7.67)**
Mean	**35.26 (6.02)**	**45.02 (6.70)**	**57.32 (7.57)**	**73.00 (8.54)**	**83.76 (9.15)**	**97.22 (9.86)**	

$CD_{(p=0.05)}$

Fungitoxicant	:	0.16
Concentration	:	0.18
Fungitoxicant x concentration	:	0.40

*Mean of three replications

*Figures in parentheses are square root transformed values

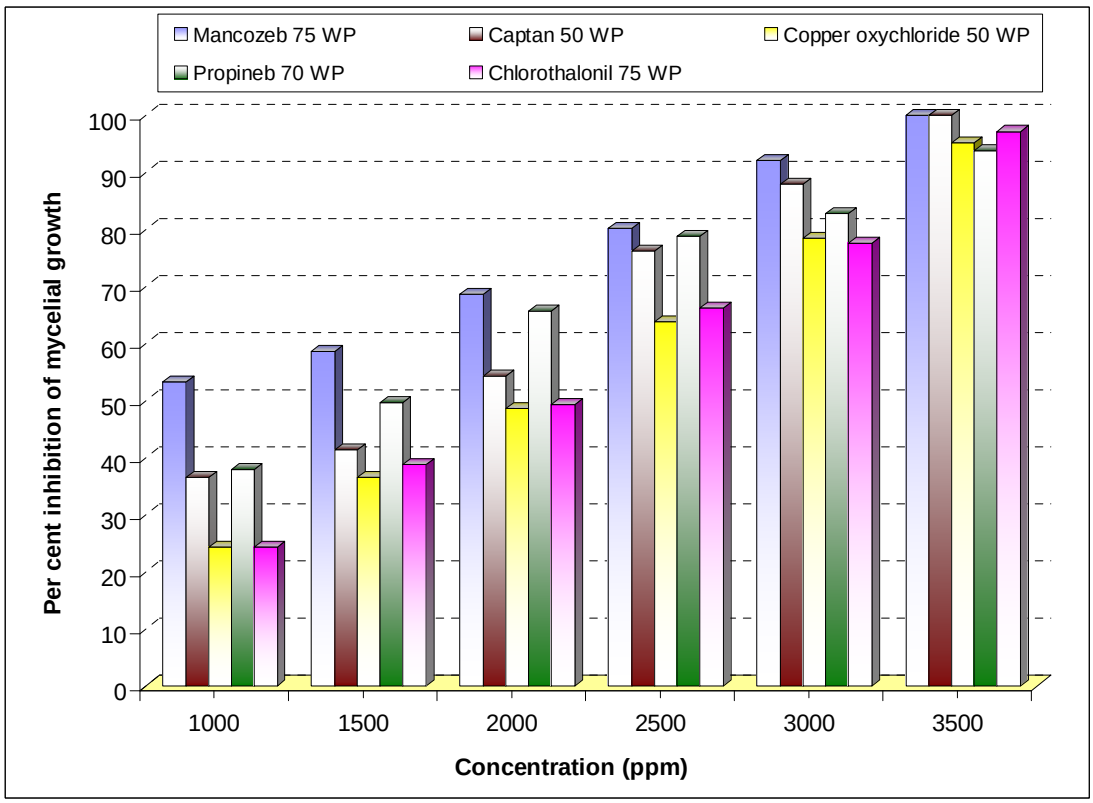
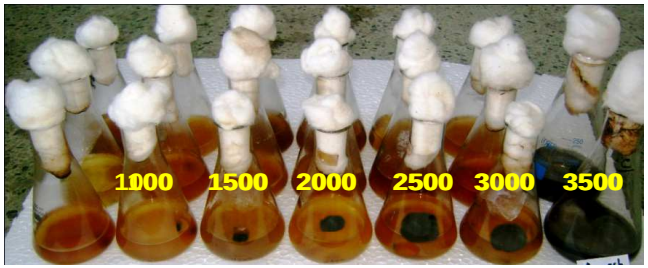
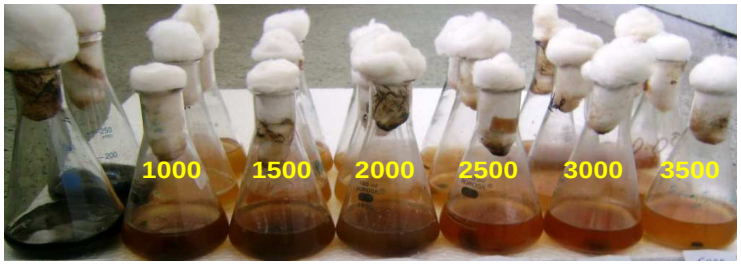


Fig. 7 : ***In vitro*** **efficacy of various contact fungitoxicants in inhibiting the mycelial growth of *Alternaria solani***



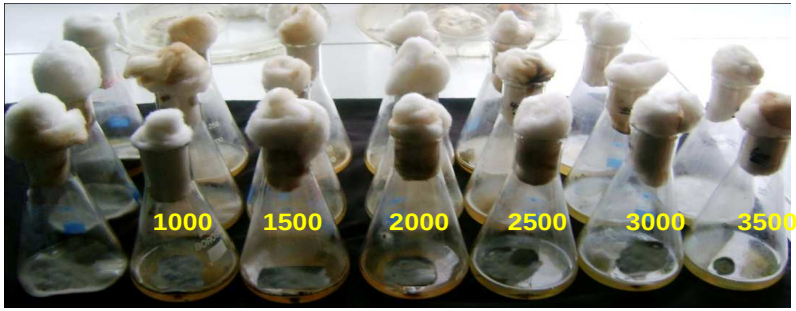
mancozeb



captan

Copper oxychloride

Propineb



Chlorothalonil

Plate-9 : ***In vitro*** **efficacy of various contact fungitoxicants in inhibiting the mycelial growth of** ***Alternaria solani***

all the test fungitoxicants at all the concentrations tested significantly inhibited the mycelial growth of *A. solani* in comparison to check. Mancozeb 75 WP, irrespective of concentration was most effective and exhibited a maximum mean mycelial growth inhibition of 75.46 per cent over check. This was followed by propineb 70 WP, captan 50 WP, chlorothalonil 75 WP and copper oxychloride 50 WP with mycelial growth inhibition of 68.09, 66.07, 58.89 and 57.81 per cent, respectively. It was observed that with the increase in the concentration of each fungitoxicant there was significant decrease in the mycelial growth and accordingly maximum inhibition was observed at highest concentration (3500 ppm) than at lowest concentrations.

Data further revealed a significant interaction between fungitoxicant and concentration. Two fungitoxicants (mancozeb and captan) showed complete mycelial growth inhibition at 3500 ppm concentration. Copper oxychloride, propineb and chlorothalonil which exhibited only 95.18, 93.77 and 97.16 per cent inhibition, respectively.

Among the fungitoxicants tested at 1000 and 1500 ppm concentrations, mancozeb proved superior to all other test fungitoxicants with mycelial growth inhibition of 53.30 and 58.70 per cent, respectively, over check. At lowest concentration of 1000 ppm, mancozeb, propineb and captan were superior to other test fungitoxicants exhibiting 53.30, 37.91 and 36.57 per cent inhibition, respectively.

However, copper oxychloride and chlorithalonil were statistically at par with each other at all concentrations tested over check.

4.11.2 Efficacy of systemic fungitoxicants on mycelial growth

The data on *in vitro* efficacy of systemic fungitoxicants in inhibiting the mycelial growth of *A. solani* is presented in Table-19; Fig. 8.

An insight into the data revealed that all the test fungitoxicants at all the concentrations tested significantly inhibited the mycelial growth of *A. solani* in comparison to check. Hexaconazole 5 EC, irrespective of concentrations was most effective and exhibited a maximum mean mycelial growth inhibition of 84.19 per cent over check. This was followed by difenconazole 25 EC which showed mean mycelial growth inhibition of 79.06 per cent followed by fenarimol 12 EC (76.97%) and carbendazim 50 WP (65.05%). Thiaphenate methyl 70 WP was least effective and inhibited meycelial growth by 53.15 per cent. It was observed that with the increase in the concentration of each fungitoxicant there was a significant decrease in the respective mycelial growth and accordingly maximum inhibition was observed at highest concentration (350 ppm) than at lower concentrations (Plate-10).

Data further revealed a significant interaction between fungitoxicant and concentration. Four fungitoxicants (fenarimol, hexaconazole, difenconazole and carbendazim) showed complete mycelial growth inhibition at 350 ppm concentration, except thiophenate methyl 70 WP which exhibited only 93.92 per cent inhibition. At lowest concentration of 100 ppm hexaconazole and difinconazole were superior to other test fungitoxicants, exhibiting 56.51 and 50.18 per cent inhibition, respectively. However, fenarimol and difenconazole were statistically at par with each other, at all the concentrations tested over check. Lodha and Prasad (1975) reported that among different fungicides mancozeb was highly effective to check the colony growth of *A. solani* under *in vitro* conditions. Similar results regarding high efficacy of mancozeb against *A. solani* were also reported by other workers (Choulwar and Datar, 1994). On the other hand, systemic fungicides were highly effective at much lower concentrations. Hexaconazole proved to be highly effective at much lower concentration. Hexaconazole proved highly effective as it caused complete inhibition in fungal growth at 300 ppm concentration. High efficacy of

Table-19 : ***In vitro*** **efficacy of various systemic fungitoxicants in inhibiting the mycelial growth of *Alternaria solani***

Fungitoxicant	Per cent growth inhibition at different concentrations (ppm)*						
	100	**150**	**200**	**250**	**300**	**350**	**Mean**
Fenarimol 12 EC	48.91 (6.99)	60.90 (7.80)	74.14 (8.61)	82.55 (9.08)	95.32 (9.76)	100.00 (10.00)	**76.97 (8.77)**
Hexaconazole 5 EC	56.51 (7.51)	74.43 (8.62)	84.24 (9.17)	90.00 (9.48)	100.00 (10.00)	100.00 (10.00)	**84.19 (9.17)**
Difenconazole 25 EC	50.18 (7.08)	60.75 (7.79)	76.88 (8.76)	88.92 (9.42)	97.67 (9.88)	100.00 (10.00)	**79.06 (8.89)**
Carbendazim 50 WP	24.91 (4.99)	44.88 (6.69)	55.78 (7.46)	72.63 (8.52)	92.10 (9.59)	100.00 (10.00)	**65.05 (8.06)**
Thiophenate methyl 70 WP	20.66 (4.54)	35.41 (5.95)	43.92 (6.62)	55.55 (7.45)	69.44 (8.33)	93.92 (9.69)	**53.15 (7.29)**
Mean	**40.23 (6.34)**	**55.27 (7.43)**	**66.99 (8.18)**	**77.93 (8.82)**	**90.90 (9.53)**	**98.78 (9.93)**	

$CD_{(p=0.05)}$

Fungitoxicant	:	0.13
Concentration	:	0.15
Fungitoxicant x concentration	:	0.34

*Mean of three replications

*Figures in parentheses are square root transformed values

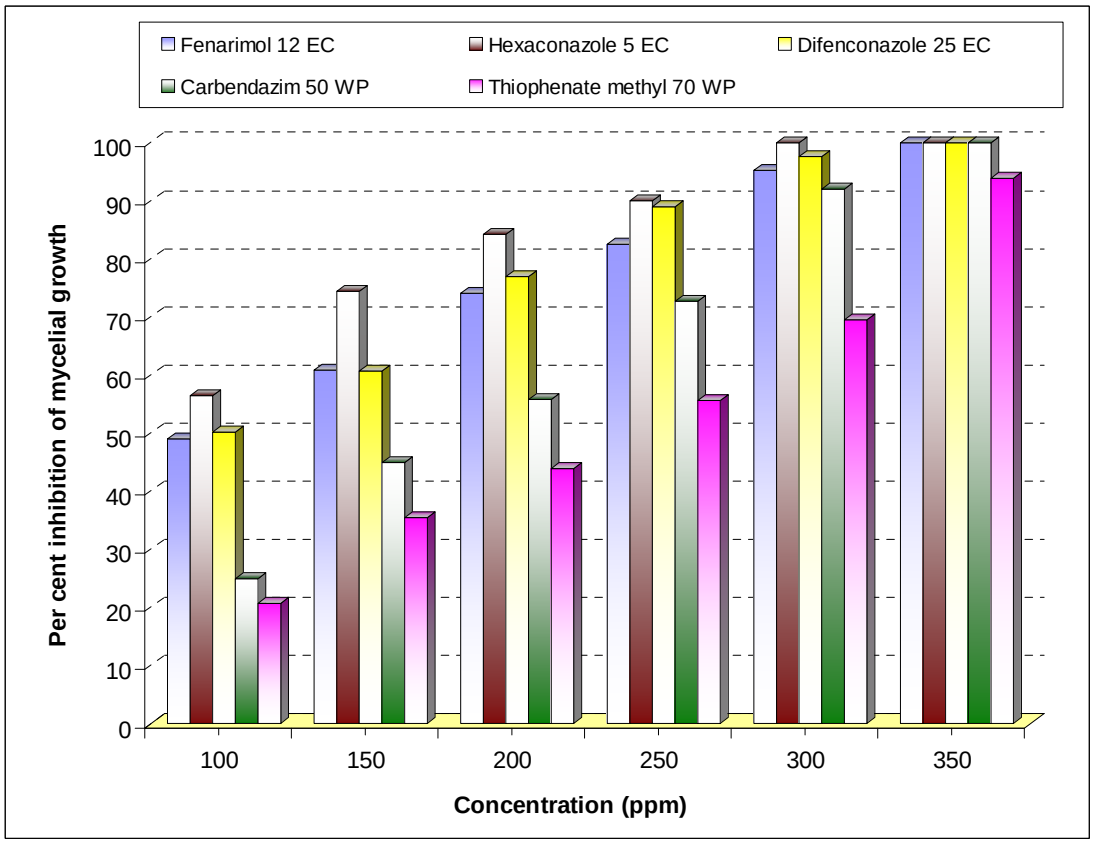
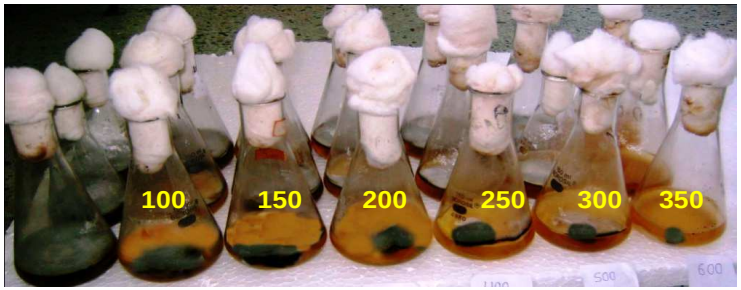
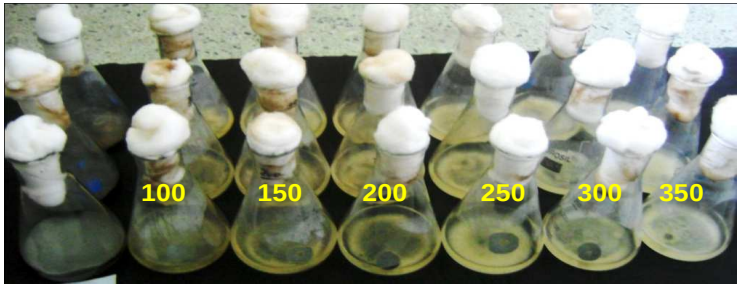


Fig. 8 : ***In vitro*** **efficacy of various systemic fungitoxicants in inhibiting the mycelial growth of *Alternaria solani***

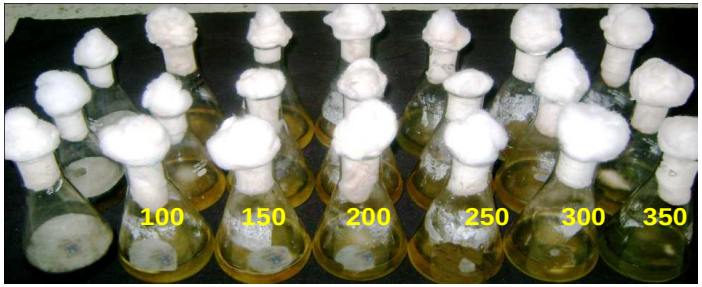


Fenarimol

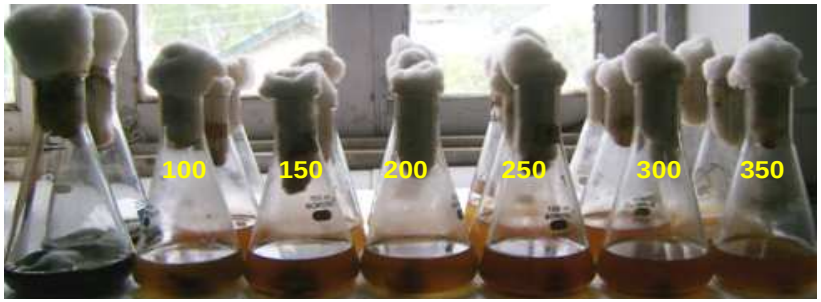
Hexaconazole



Difenconazole



Carbendazim



Thiophenate methyl

Plate-10 : ***In vitro*** **efficacy of various systemic fungitoxicants in inhibiting the mycelial growth of** ***Alternaria solani***

hexaconazole in causing complete inhibition in growth of *A. alternata*, has already been demonstrated (Dubey *et al*., 2000; Singh and Singh, 2006).

4.12 Integrated disease management under field conditions

The antagonists, botanicals and fungitoxicants which proved most effective in *in vitro* screening against *A. solani* were further assessed under field conditions against early blight disease of potato. The treatments comprised of *Trichoderma harzianum* @1 x 10^7 spore/ml, datura ethanol extract @50 per cent and two fungitoxicants (mancozeb 75 WP @ 0.3 % and hexaconazole 5 EC @ 0.1%). Observations on disease intensity were recorded 10 days after last spray.

It is evident from the data presented in Table-20, that during the year 2008, all the treatments significantly reduced the disease compared to control. However, the magnitude of reduction varied from treatment to treatment. The range of disease intensity in treatments varied from 4.91 to 18.84 per cent in comparison to 28.00 per cent recorded in check. Minimum disease intensity of 4.91 per cent was observed in plants treated with mancozeb 75 WP (seed treatment) + hexaconazole 5 EC (foliar spray) + Datura extract (foliar spray) + *T. harzianum* (foliar spray). This was followed by treatment mancozeb 75 WP (seed treatment) + hexaconazole 5 EC (foliar spray) + *T. harzianum* (foliar spray) with disease intensity of 8.70 per cent. However, treatments T_7 mancozeb 75 WP (S.T) + hexaconazole 5 EC (FS) + datura (FS) and treatment T_{10} (*T. harzianum* (ST) + hexaconazole 5 EC (FS)] were statistically at par showing disease intensity of 10.86 and 11.54 per cent, respectively. Treatments T_1 (hexaconazole 5 EC two foliar sprays) and T_9 (*T. harzianum* (ST) + hexaconazole 5 EC (FS)] with disease intensity of 12.06 and 12.18 per cent were statistically at par with each other. Ethanol extract of datura @ 50 per cent (two sprays) was least effective in comparison to all other treatments and was statistically at par with *T. harzianum* @ 1 x 10^7 spore/ml (two sprays) with disease intensity of 18.84 and 17.85 per cent respectively over check (28.00%).

During the year 2009 the experiment was repeated and results (Table-20) revealed that the treatments again proved effective in reducing the disease intensity and were significantly superior over check. Least disease intensity of 6.37 per cent was observed in treatment T_5 followed by treatment T_6 (9.70%) which were statistically at par with each other. Among other treatments tested, T_7 and T_{10} did not show significant difference and were statistically at par with each other with disease intensity of 12.30 and 12.77 per cent, respectively. Treatment T_3 (2 foliar sprays of datura ethanol extract @ 50 per cent) proved least effective in comparison to all other treatments but was significantly superior over check.

Two years pooled data (2008 and 2009) presented in Table-20 revealed significantly higher disease intensity of 16.54 per cent during the year 2009, in comparison to 14.01 per cent during the year 2008. All the treatments were significantly superior over control in reducing the disease intensity. Among the treatments disease intensity ranged from 5.64 to 19.59 per cent as against 31.19 per cent in check. The combined treatments viz., chemical + biological + extract were significantly superior over individual treatments.

Table-20 : Integrated management of early blight of potato under field conditions

	Treatment	Disease intensity (%)		Pooled mean
		2008	2009	
T_1	Hexaconazole (F.S.) 0.03% (2 sprays)	12.06 (19.41)	14.37 (22.23)	**13.21 (20.82)**
T_2	Mancozeb (F.S.) 0.2% (2 sprays)	14.02 (21.97)	17.76 (24.91)	**15.89 (23.44)**
T_3	Datura (F.S.) 50% (2 sprays)	18.84 (25.70)	20.34 (26.79)	**19.59 (26.24)**
T_4	*Trichoderma harzianum* (F.S.) 1 x 10^7 spores/ml (2 sprays)	17.85 (24.98)	19.56 (26.23)	**18.70 (25.60)**
T_5	Mancozeb (S.T.) + Hexaconazole (F.S.) + Datura (F.S.) + *T. harzianum* (F.S.)	4.91 (12.79)	6.37 (14.60)	**5.64 (13.69)**
T_6	Mancozeb (S.T.) + Hexaconazole (F.S.) + *T. harzianum* (F.S.)	8.70 (17.15)	9.70 (18.11)	**9.20 (17.63)**
T_7	Mancozeb (S.T.) + Hexaconazole (F.S.) + Datura (F.S.)	10.86 (19.23)	12.30 (20.50)	**11.58 (19.86)**
T_8	*T. harzianum* (S.T.) + Datura (F.S.)	15.17 (22.90)	18.76 (25.64)	**16.96 (24.27)**
T_9	*T. harzianum* (S.T.) + Mancozeb (F.S.)	12.18 (20.37)	15.45 (23.12)	**13.81 (21.74)**
T_{10}	*T. harzianum* (S.T.) + Hexaconazole (F.S.)	11.54 (19.83)	12.97 (21.08)	**12.25 (20.45)**
T_{11}	Control	28.00 (31.94)	34.38 (33.74)	**15.27 (22.41)**
	Overall mean	**14.01 (21.47)**	**16.54 (23.35)**	**13.82 (21.46)**
	CD (p = 0.05)	**1.88**	**3.22**	**1.53**

* Mean of three replications

Figures in parenthesis are arc sine transformed values

FS = Foliar spray; ST = Seed treatment

Chapter – 5

DISCUSSION

Potato (*Solanum tuberosum* L.) is one of the most valuable non-cereal food crops, grown in most of the temperate and subtropical regions of world. It provides highest amount of dry matter, protein and other nutrients per unit area and time. Potato is a unique crop which can supplement the food needs of the country in a substantial manner. The climatic conditions of Kashmir are most favourable for the cultivation of potato. However intensive and extensive cultivation of potato even failed to provide significant strides in potato yields, because of a number of production constraints. These include non availability of high yielding genotypes and occurrence of many fungal diseases. Among the major pathogens, *Alternaria solani* (Ellis and Martin) Jones and Grout causes leaf spot that posses a serious threat to the crop inflicting both qualitative and quantitative reduction in potato production. In Kashmir valley, the disease was previously considered of minor significance but has attained the status of a major disease during past few years. Assessment of status of the disease is one of the pre-requisites for devising suitable disease management strategies. Therefore, survey of various potato growing areas in four districts of Kashmir valley viz., Budgam, Baramulla, Srinagar and Shopian was undertaken during the year 2008 and 2009.

Early blight in potato was prevalent in all the locations surveyed. The disease incidence varied from location to location. The disease incidence and intensity was higher in the year 2009 than that of 2008 which may be attributed to variation in environmental conditions particularly during the early stages of crop. The variation in environmental factors such as temperature, wetness duration and relative humidity (moisture) has also been reported to affect the development of early blight in potatoes (Harrison *al*., 1965; Adams and Stevenson, 1990 *et*; Vloutoglou and Kalogerakis, 2000).

Water in the form of high relative humidity, rainfall or dew accumulation can increase conidial germination and pathogen infection (Rotem, 2004). Alternating low and high humidity conditions have also been shown to favour disease development (Van der Walls *et al*., 2001).

Amongst the various districts surveyed, Budgam recorded highest (39.09%) disease incidence followed by Baramulla (27.36%), Srinagar (22.53%) and Shopian (14.89%). The disease incidence at various locations ranged from 11.49 to 47.17 per cent during the two years with an average incidence of 24.54 to 28.23 per cent during the year 2008 and 2009, respectively. The disease intensity varied from 6.00 to 27.31 per cent during the two years with an average intensity of 13.84 to 15.98 per cent, respectively. The high disease severity during 2009 may be due to prevalence of environmental and/or pathogen factors. Changes in weather variables and amount of initial inoculum of *Alternaria solani* may be responsible for varying disease intensities at different locations (Van der Walls *et al*., 2003).

Of the locations surveyed, the highest disease incidence (44.95%) and intensity (26.19%) was recorded at Mazhama locality followed by Khansahab, Sopore, Chadoora, Pattan and HMT exhibiting a disease incidence of 39.32, 35.67, 33.99, 30.45 and 25.28 per cent and intensity of 23.25, 22.62, 18.19, 17.10 and 14.16 per cent, respectively. Higher disease status in these localities could probably be due to closer spacing from plant to plant and adoption of faulty cultural practices.

Observations recorded in disease development revealed that disease symptoms on leaves first appeared in the second week of May as small irregular to circular dark brown spots on the lower leaves measuring 0.5 mm in size which gradually increased and attained maximum size (7.4 mm) in second week of August. Upto fourth week of June concentric rings from as a result of irregular growth patterns by the organism in the leaf tissue giving the lesion a characteristic “target spot” or “bulls eye” appearance. Beyond second week of July due to

coalescing of 3-5 spots small irregular patches were formed. Severely infected leaves eventually wither and die. Such symptoms were also observed by Datar and Mayee (1981a). They also reported die back type of symptoms due to early blight. However during present studies no such symptoms were observed. The pathogen also attack potato tubers and produce shallow, dry, corky rot. Such symptoms were also observed by Falson and Bonde (1925), O'Brien and Rich (1976).

The causal organism was isolated from diseased leaves of potato cv. Kufri Jyoti and was cultured on potato dextrose agar (PDA) medium for further studies.

The pathogenic behaviour of the isolated causal organism was established following Koch's postulates on healthy leaves of potato cv. Kufri Jyoti. The typical symptoms of the disease in injured leaves were produced by the pathogen after 10 days and 14 days of inoculation under *in vitro* and *in vivo* conditions, respectively. However, pathogen failed to produce disease symptoms on uninjured leaves. Similar observations were also recorded by Foolad *et al*. (2000) on detached leaves of tomato.

The morphological characters of the pathogen were studied on culture grown on PDA medium. The fungal colonies were dense, sprcading, possessing a texture similar to cotton or velvet and were grey brown to black in colour. The mycelium was septate, branched usually dark, ranging from grey to black in colour. Conidiophores were septate short, simple and erect that bear single and branched chains of conidia in acropetal chains. Conidiophores were 50-90 x 9 μm in size. Conidia were dark coloured and muriform, with 9-11 transverse septa and 2-3 longitudinal septa. They were ellipsoid to oblong measuring 15-19 x 150-300 μm. The beak was long, floxuous measuring 210 μm in length and 2-5 μm wide. The morphological descriptions of the pathogen almost corroborate with descriptions giving by Neergard (1945). According to M.B. Ellis (1971), the solitary and beaked conidia have 9-11 transverse septa and a few or no longitudinal or oblique septa. Cultural characteristics (colour, growth, sporulation

etc.) also differ in various isolates, making it possible to find almost as many races as the number of isolates tested (Rotem, 1966). Virender Kumar *et al*. (2008) reported that pigmentation varied from yellow, brown, black, brownish to green black in isolates of *A. solani* on potato dextrose agar medium.

On the basis of morphological characters, pathogencity and comparison to the authentic description, the pathogen was identified as *Alternaria solani* (Ellis and Martin) Jones and Grout and its identity was confirmed by Dr. P.N. Chowdhry, Principal Mycologist, National Centre of Fungal Taxonomy, New Delhi under NCFT No. 4372.11.

The correlation studies of disease development with meterological factors revealed that the period from III week of May to III week of June and from III week of May to II week of July during 2008 and 2009, respectively, favoured the maximum disease development in terms of infection rate (unit/day) as during these periods maximum infection rate of 0.155 and 0.165, respectively was observed. A temperature of 26.47 and 27.32^{o}C, 92 and 91 per cent relative humidity and 8.68 and 6.42 mm precipitation favoured the maximum disease development during these periods, respectively. These studies indicated the maximum disease development during the periods of highest temperature, humidity and rainfall of both 2008 and 2009 cropping seasons and thus suggest the relationship between environmental factors and disease development. The disease development in terms of infection rate (unit/day) was found positively correlated with temperature, but non-significant with relative humidity and rainfall. The multiple regression analysis showed that the weather factors accounted for 66.5 per cent variation of disease development. These observations are supported by findings of Rands (1917), Gratz (1930) and Wager (1945). Dragomir (1995) reported that relative humidity of more than 90 per cent and presence of free moisture on tomato leaves for more than 2 hr. per day favoured the disease. Leiminger *et al*. (2005) reported symptoms and damage to potato plants by *A. solani* and *A. alternata*. The effects of weather (especially rain and

temperature) on fungal spores showed greater spread during humid warm weather. For instance, the high temperatures claimed to favour epidemics of *A. solan* in winter tomatoes in Morocco (Berger, 1937). Ling and Yong (1941) reported that epidemics caused by *A. alternata* and *A. macrospora* on cotton in China was mostly due to favourable temperature. Tomato early blight in Hungary (Hodosy, 1968) and potato early blight in South Africa (Wager, 1945) and Belgium (Roth, 1936) have been associated with dry and hot days and dewy nights.

Perpetuation of pathogen from one season to the next in the absence of a living host is pre-requisite for successful establishment of any plant disease. Present studies conducted on the perpetuation of the pathogen revealed that *A. solani* overwintered on diseased leaves left on the ground surface and on diseased potatoes in the form of conidia and mycelium throughout the winter. These findings are supported by Rotem (1968) who reported that *A. solani* overwinters and survives as conidia and mycelia on buried host debris and potato tubers, particularly in fields with poor cultural practices such as continuous cropping of tomatoes or potatoes. Rands (1917) reported that conidia of *A. solani* are capable of surviving freezing weather on the soil surface.

Further, diseased leaves left on the ground surface were observed to be the most important source of primary infection of early blight of potato. These findings are supported by Manzer and Merrium (1974) who also reported that the first infections of the new crop are produced from overwintering inoculums. However, the leaves buried in soil at 20 cm depth, the spores were altogether absent throughout the observation period during both the years of experimentation. The leaves buried at 20 cm depth, decomposed earlier than those at ground surface. This could be attributed to greater aerobic respiration, which favoured quick decomposition of leaves. The proportion of spores and their viability decreased with the increase in depth of placement in soil. The decrease in viability of conidia with increasing depth may be attributed to more microbial action on nutrient coating of conidia which has been reportedly observed in

Chlamydospore of *Fusarium solani* (Mayers and Cooks, 1972). Rotem (1968, 1990) reported that oversummering of *A. solani* in potato and tomato debris and overwintering of *A. macrospora* in cotton were much more successful (lasting upto 8 months) in debris deposited on the soil surface than in debris buried in soil. These findings were also supported by Pandotra (1965) who reported that in Punjab, *Alternaria* survived 8 months in debris left on the soil surface but only 2 months in debris buried in the soil.

The differences between survival on the soil surface and survival under the soil surface derives from differences in environmental and biotic conditions in the two habitats. In particular, the soil surface is drier than the soil below and less microbial activity occur at the soil surface. These and other effects were studied in the oversummering of *A. solani* in different plot covering the site of a diseased winter tomato field in the rainless Negev Desert (Rotem, 1968).

Perpetuation of pathogen (*A. solani*) in diseased potatoes kept in ambient store revealed that the diseased potatoes continuously produced spores during entire period of study in both the years (2008 and 2009). The average number of spores increased upto first fortnight of June both in 2008 and 2009, with a maximum number of 430 and 508 spores, respectively. The number then declined to 216 and 263 spores respectively, till last observation recorded in the second fortnight of July. Similar observations were reported by Vijaya Kumar and Rao (1979a) who reported that the longest periods of survival of *A. triticina* in debris and in wheat seeds were 4 and 10 months, respectively.

The production of viable spores in overwintered diseased leaves and potatoes increased from the month of April to June. This suggests that in spring under favourable weather conditions, lesions on overwintered leaves and potatoes kept in ambient store resume spore formation and the inoculum may build upto the levels sufficient to initiate primary infection. The environmental conditions in the early summer thus determine the extent of epidemic development. The information generated about the possible means of perpetuation of the fungus

during winter and production of conidia as a source of primary inoculum during the early spring highlights the importance of removal of plant debris as one of the strategies for disease management.

Inherent resistance or tolerance of plants to infection by pathogen is the most economic and ecofriendly disease management venture. Attempts made to identify the potato genotypes showing tolerance to *A. solani* under natural conditions indicated that only one genotype i.e. SM/92-338 exhibited tolerant reaction. The three other genotypes i.e. Kufri himami, SM/96-27 and SM/94-44 were moderately tolerant. Nine genotypes i.e. Kufri girdari, Kufri shailja, Kufri chandramukhi, SM/98-239, SM/93-237, SM/90-45, HB/82-18, HB/50-45 and Shalimar potato-1 were moderately susceptible. The remaining genotypes were either susceptible or highly susceptible in reaction. The present studies are in conformity with those of Horsfall and Heuberger (1942); Douglas and Pavek (1972). Khan *et al*. (2001) reported that of the twenty five potato genotypes screened against the early blight disease, none of the commercially grown varieties was resistant to the disease. Naik *et al*. (1999) reported that among thirty potato genotypes from the All India Coordinated Research Project on potato screened for reaction to early blight (*A. solani*), four genotypes (H222, JV62, JX123 and Jx214) were highly resistant across all three years. Pinto *et al*. (2002) reported that potato clones with good levels of resistance to early blight were identified as having tuber yields superior to the controls. Potato germplasm has been evaluated for resistance to early blight by a number of other researchers (Le, Clerg, 1946; O'Brien and Akeley, 1971; Douglas and Pavek, 1972; Bussey and Stevension, 1991; Christ, 1991). Platt and Reddin (1994) have identified some genotypes and advanced breeding selections with moderate resistance. In an extensive evaluation of 934 potato clones from breeding programmes around the world Boituex *et al*. (1995) found a strong correlation between early blight resistance and late maturity. The development of early blight resistance genotypes can be expected to increase profitability by reducing the amount of fungicides

used to produce a crop (Christ, 1990; Stevenson, 1994).

The genotypes with tolerant and moderately disease resistant reaction can be used in hybridisation programme to evolve genotypes possessing desirable traits besides resistance to early blight of potato pathogen.

The *in vitro* bioassay of biocontrol agents revealed that all the three biocontrol agents tested significantly inhibited the radial mycelial growth of *A. solani*. Among the antagonists, *T. harzianum* caused maximum mycelial growth inhibition of 71.85 per cent in dual culture, followed by *T. viride* (65.93%) on *A. solani*. *T. harzianum* and *G. virens* showed strong mycoparasitic activity and completely overgrew the host mycelium once in contact with it. Amongst above biocontrol agents only *T. viride* developed zone of inhibition against the pathogen. These findings are in conformity with Rudresh *et al*. (2005) who noticed 72.1 and 77.0 per cent growth inhibition of *R. solani* and *F. oxysporum* respectively by *T. harzianum*. Earlier rapid growth of *T. harzianum* covering the entire colonies of *S. sclerotiorum* and strong antagonism of *Trichoderma* spp. against *S. sclerotiorum* have been reported by many workers (Lee and Wu, 1979; Singh, 1998). The formation of inhibition zone by *T. viride* against *A. solani* in the present study suggests the involvement of strong antibiosis, possibly due to production of some diffusible substances. Various volatile metabolites viz., derivatives of lactones, alcohols and terpens etc., produced by *T. viride* (Zeppa *et al*., 1990), and chemicals like gliotoxin and gliovirin etc. produced by *Gliocladium* sp. are reported to be responsible for the formation of inhibition zone (Whhith *et al*., 1994; Howell and Stipanovic, 1995). Similar observations with regard to *Trichoderma* spp. and *G. virens* have been made by Elad *et al*. (1980); Tu (1980) and Tu and Vartaja (1981). The present findings are also in conformity with Munshi (1998) and Munshi and Dar (2004) who noticed the formation of inhibition zone by *Gliocladium* sp. against *Fusarium pallidoroseum*. Prasad and Rao (1990) have found effective inhibition of mycelial growth and sclerotial production of *Thanatophorus cucumeris* by *G. virens. T. viride* and *T. harzianum*

have also been reported to be effective fungal antagonists against *S. rolfsii* (Alice *et al*., 1998) and *R. solani* (Roy, 1977; Elad *et al*., 1980; Gokulapalan and Nair, 1984).

Currently efforts are being made to manage plant diseases through the use of different plant extracts or their products. A number of plants have been reported to possess antifungal activity (Shekhawat and Prasada, 1971; Dixit and Tripathi, 1975). These include bulb extracts of garlic and and leaf extracts of datura and mentha (Shivpuri *et al*., 1997; Shivpuri and Gupta, 2001; Chattopadhyay *et al*., 2002; Singh *et al*., 2003).

In the present study, efforts were made to explore the possibility of using extracts of locally available plants for the management of *Alternaria* leaf spot of potato. *In vitro* evaluation revealed that all the five test extracts at various concentration were significantly effective in inhibiting the mycelial growth of *A. solani*. It was observed that ethanol extract of *D. stramonium* irrespective of concentrations, exhibited maximum average mycelial growth inhibition of 61.12 per cent. This was followed by *A. absinthium* (58.54%). The least mycelial inhibition was exhibited by *U. dioica* (37.34%). It was further noticed that test botanicals increased the inhibition of mycelial growth with increase in their concentration. *D. stramonium* (66.95 and 71.57 %) and *A. absinthium* (65.19 and 68.41 %) proved significantly superior at 50 and 60 per cent concentration. At lower concentration of 10 and 20 per cent also, *D. stramonium* (46.40 and 56.16%) and *A. absinthium* (44.77 and 52.12%) were significantly superior to rest of the test botanicals, respectively. The presence of antibiotic constituent in the form of phenolic, resinous, gummy and non-volatile substances of unknown nature in different botanicals is reported by Skinner (1955). Amonkar and Banergi (1971) have attributed such antimicrobial properties to the presence of diallyl-disulphide and diallyl-tri-sulphide in *A. sativum* and fixed oils in *D. stramonium*. Similar results on the efficacy of different plant extracts against *Alternaria* spp. have been reported by Shivpuri *et al*. (1997). Several workers while studying the

in vitro effect of different plant extracts on *Fusarium solani* and other *Fusarium* spp. have reported almost similar findings (Vimala *et al*., 1993; Arya *et al*., 1995; Lolpuri, 2002). The presence of essential oils in garlic (*A. sativum*) and tulsi (*Ocimum sanctum*) fixed oils in datura (*D. alba*) and canabinol in cannabis (*Canabis sativa*) are also considered responsible for such inhibitions (Anonymous, 1972). Duru and Onyedineke (2010) revealed the presence of some bioactive compounds, alkaloids, anthranoids, anthraquinane, cardiac glycosides, phenols, phlobatanins, starch and tannins in ethnolic extracts of *Voacanga africana* seeds. Vanitha (2010) reported that EC formulation of winter green oil exhibited 100 per cent inhibition of mycelial growth of *A. chlamydospora.* Similar results on the efficacy of different plant extracts against *A. alternata* have also been reported by Wu Feng and Zheng (2007); Raghavendra *et al*. (2009) and Zaker and Mosallanejad (2010).

In *in vitro* studies, ten fungitoxicants, five non-systemic viz., mancozeb 75 WP, captan 50 WP, copper oxychloride 50 WP, propineb 70 WP and chlorothalonil 75 WP and five systemic fungitoxicants viz, hexaconazole 5 EC, carbondazim 50 WP, thiophenate methyl 70 WP, difenconazole 25 EC and Fenarimol 12 EC were evaluated at different concentrations against *A. solani* by poisoned food technique. It was observed that all the fungitoxicants at all the concentrations tested significantly inhibited the mycelial growth as compared to check. The systemic fungitoxicants were more effective as compared to non-systemic fungitoxicants. Among systemic fungitoxicants hexaconazole 5 EC was most effective in inhibiting the mycelial growth of *A. solani* at all the concentrations tested. This was followed by difenconazole 25 EC, fenarimol 12 EC, carbendazim 50 WP and thiophenate methyl 70 WP. Among non-systemic fungicides mancozeb 75 WP, irrespective of concentration was most effective and exhibited a maximum mean mycelial growth inhibition of 75.46 per cent over check. This was followed by propineb 70 WP, captan 50 WP, chlorothalonil 75 WP and copper oxychloride 50 WP with mycelial growth inhibition of 68.09,

66.07, 58.89 and 57.81 per cent, respectively. All the fungitoxicants were comparatively more effective at higher concentration than at lower concentrations. Present findings are in conformity with those of Shahzad (2003) who also reported that ESBI's (Penconazole 10 EC, hexaconazole 5 EC and Fenarimol 12 EC) at 100 ppm concentration inhibited maximum mycelial growth and spore germination of *A. mali* causing leaf blotch of apple. Lodha and Prasad (1975) also reported that among different fungicides mancozeb was highly effective to check the colony growth of *A. solani* under *in vitro* conditions. Similar results regarding high efficacy of mancozeb against *A. solani* were also reported by other workers (Choulwar and Datar, 1994). However non-systemic fungicides possessed low fungal activity and they were effective at higher concentrations. Hexaconazole proved to be highly effective at much lower concentration. High efficacy of hexaconazole in causing complete inhibition in growth of *A. alternata*, has already been demonstrated (Dubey *et al*., 2000; Singh and Singh, 2006). Akbari and Parakhia (2007) also reported that systemic fungicides completely inhibited the mycelial growth of *A. alternata* even at a minimum concentration of 50 ppm. While non-systemic fungicides thiram and mancozeb gave cent per cent inhibition of *A. alternata* at a minimum concentration of 500 ppm. Similar observations were also reported by Patel *et al*. (2005).

The *in vivo* studies of antagonists, botanicals and fungitoxicants revealed that all treatments significantly reduced the disease intensity as compared to check. However the magnitude of reduction varied from treatment to treatment. The present study revealed that seed treatment and foliar sprays with fungitoxicants to be significantly superior treatment over their individual treatments in suppressing disease severity. It seems that seed treatment with bioagent or plant extract plus foliar sprays of hexaconazole 5 EC or mancozeb 50 WP have exerted synergistic effect in reducing the disease severity. Patibanda *et al*. (2002) have reported synergistic effect of soil application of antagonist plus seed treatment with thiram in reducing the *Sclerotium* wilt of groundnut. Vyas

(1994) has reported the simultaneous seed application of *T. viride* or *T. harzianum* with carbendazim to be effective in reducing dry root rot of soyabean. In the present study seed treatment with mancozeb 75 WP + foliar spray with hexaconazole 5 EC (0.1%) + foliar spray with datura (50%) + foliar spray with *T. harzianum* (1 x 10^7 spores/ml) were highly effective in controlling the disease severity as compared to control. Similar observations were also reported by Patil *et al*. (2003) and verma and Gandhi (2007); Phalisteen *et al*. (2008); Verma *et al*. (2008). Anand and Bhaskaran (2009) also reported that leaf extracts and antagonistic organisms ranked next to the fungicide (Carbendazim 0.1%) in reducing fruit rot of chilli (*A. alternata*).

Chapter – 6

SUMMARY AND CONCLUSION

The investigations of "Perpetuation and management of early blight of potato in Kashmir valley" were carried out at SKUAST-K, during 2008 and 2009. The results are summarized hereunder :

Survey of potato growing areas conducted in four districts viz., Budgam, Baramulla, Srinagar and Shopian, revealed the prevalence of Alternaria leaf spot in all the localities with overall disease incidence and intensity of 11.49 to 42.72 and 60.00 to 25.05 per cent, during the year 2008 and 14.65 to 47.17 and 7.86 to 27.31 per cent during 2009, respectively. The highest disease incidence (44.95%) and intensity (26.19%) was observed in Mazhama locality (Budgam) and least (13.06%) and (6.93%) in Herpur locality (Shopian).

Under natural conditions, disease symptoms on leaves first appeared in the second week of May as small irregular to circular dark brown spots on the lower leaves measuring 0.5 mm in size. Concentric rings formed as a result of irregular growth patterns by the organism in the leaf tissue, giving the lesion a characteristics "target spot" or "bulls eye" appearance. Finally lesions covered large area of the leaf lamina and diseased leaves got blighted and eventually wither and die.

The causal fungus of early blight of potato was isolated from infected potato leaves on potato dextrose agar medium incubated at $25\pm2^{o}C$. The pathogencity of the isolated fungus was established by proving Koch's postulates. The culture of the pathogen produced characteristic disease symptoms after 10 days of inoculation on injured detached leaves and after 14 days of inoculation of intact injured leaves under field conditions. However, leaf inoculation without any injury did not produce symptoms.

The fungus on potato dextrose agar medium formed septate dark coloured

mycelium, ranging from grey to black with tints of olive or brown. Conidophores were short simple, erect, flexuous pale to olive brown in colour measuring 50-90 x 9 µm in size under *in vitro* conditions. Conidia were dark coloured and muriform, with 9 to 11 transverse septa and 2-3 longitudinal septa. They were ellipsoid to oblong with a long beak which was occasionally branched and measured 15-9 x 150-300 µm under *in vitro* conditions.

On the basis of morphological characters, pathogencity and comparison to the authentic descriptions, the pathogen was identified as *Alternaria solani* (Ellis and Martin) Jones and Grout, and the identification was confirmed by National Centre of Fungal Taxonomy, New Delhi under NCFT No. 4372.11.

The correlation of various meteorological factors with disease development in terms of infection rate (unit/day) indicated that temperature was significantly and positively correlated with infection rate and rainfall and relative humidity were also positively correlated but non significant. Weather factors had an impact of 66.5 per cent on the disease. Maximum apparent infection rate of 0.155 (unit/day) and 0.165 (unit/day) was recorded during second week of June in 2008 and 2009, respectively.

The studies on perpetuation through plant debris indicated that leaf bits kept at ground surface exhibited the presence of viable (conidia) spores upto first fortnight of July. However, spores were altogether absent throughout the observation period on leaf bits buried at 20 cm deep in soil. However, the diseased potatoes kept in ambient storage conditions produced spores during entire period of study in both the years. The viability of spores gradually increased with time till first fortnight of June during both years, which then showed a gradual decrease.

Among the 25 potato genotypes screened for tolerance to *A. solani*, under natural epiphytotic conditions, only SM/92-338 genotype showed tolerant reaction. Genotypes Kufri himami, SM/96-127 and SM/94-44 proved to be

moderately tolerant and genotypes Kufri girdari, K. shailja, K. chandramukhi, SM/98-239, SM/93-237, SM/90-45, HB/82-18, HB/50-45 and Shalimar potato-1 showed moderately susceptible reaction. Rest of the genotypes proved to be susceptible to *A. solani.*

Three bioagents were screened *in vitro* against *A. solani* by dual culture technique on potato dextrose agar medium. Among the bioagents, *T. harzianum* proved highly effective by exhibiting the maximum mycelial growth inhibition of 71.85 per cent. The other bioagents in decreasing order of their efficacy were *T. viride* and *G. virens.*

Five botanicals were screened at concentration 10, 20, 30, 40, 50 and 60 per cent for their *in vitro* efficacy against *A. solani* by poisoned food techniques. Among the botanicals *Datura stramonium* and *Artimesia absinthum* proved highly effective in inhibiting the mycelial growth of the fungus. The other botanicals in decreasing order of their efficacy were *Jugalens regia*, *Mentha spicata* and *Uretica dioica*. Least inhibition was exhibited by *Mentha spicata* and *Uretica dioica*.

Ten fungitoxicants (5 non-systemic and 5 systemic) were evaluated at various concentrations for their *in vitro* efficacy against *A. solani.* Among non-systemic fungitoxicants, mancozeb 75 WP and propineb 50 WP proved highly fungitoxic in inhibiting the mycelial growth. The other fungitoxicants in order of their efficacy were captan 50 WP>chlorthalonil 75 WP>copper oxychloride 50 WP. However, manoczeb 75WP and captan 50 WP at their 3500 ppm concentration completely inhibited the mycelial growth of pathogen.

Among systemic fungitoxicants, hexaconazole 5 EC, difenconazole 25 EC and Fenarimol 12 EC proved highly effective in inhibiting the mycelial growth of *A. solani.* These fungicides were followed by carbendazim 50 WP and thiophenate methyl 70 WP. Hexaconazole 5 EC at 300 ppm concentration and

difenconazole 25 EC, fenarimol 12 EC, and carbendazim 50 WP (each at 350 ppm concentration) completely inhibited the mycelial growth of test pathogen.

Field evaluation of bioagents, botanical extracts and fungitoxicants applied either as seed treatment or as spray suspension at 15 days interval during two cropping seasons of 2008 and 2009 revealed significant disease control by all these treatments. Among all treatment combinations, seed treatment with mancozeb 75 WP + foliar spray with hexaconazole 5 EC + foliar spray with datura extract + foliar spray with *T. harzianum* was significantly superior by restricting disease intensity upto 5.64 per cent in comparison to 31.19 per cent recorded in unsprayed check.

The conclusion drawn from the present investigations is presented as fallows :

- Early blight of potato (caused by *A. solani*) was prevalent in all the potato growing areas of Kashmir valley.
- Disease appeared in the 2nd week of May and inoculum levels were high on older leaves.
- The disease incitant was identified as *Alternaria solani* (Ellis and Martin) Jones and Grout.
- Environmental factors viz., temperature, relative humidity and rainfall favoured the disease development.
- The fungus perpetuates through conidia on overwintered leaves and in potato seeds.
- Tolerance of varying degree existed amongst the available germplasm. Genotype (SM/92-338, Kufri himami, SM/96-127 and SM/94-44) could be exploited in future for disease resistance programmes.

- The biocontrol agent *Trichoderma harzianum* proved effective against the test pathogen both in laboratory and under field condition.
- The plant extracts of *Datura stramonium* and *Artimesia absinthium* were effective against the test pathogen.
- Hexaconazole 5 EC @ 0.1% and mancozeb 75 WP @ 0.3% were effective againt the test pathogen both in laboratory and under field conditions.
- Integrated management with bioagents plus plant extracts + fungitoxicants proved to be highly effective against the test pathogen under field conditions.

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***Original not seen**

APPENDIX – I

Meteorological data of 2008 and 2009

Month	Temperature (^{o}C)	Rainfall (mm)	Relative humidity (%)
2008			
March	19.19	0.25	73.32
April	19.41	3.60	77.56
May	24.99	1.58	70.25
June	27.33	4.93	83.00
July	29.79	3.41	81.50
August	29.73	3.30	83.95
September	17.95	1.08	68.23
October	22.24	1.10	90.51
Mean	**23.82**	**2.40**	**78.54**
2009			
March	15.64	2.55	81.93
April	19.91	2.71	79.30
May	24.78	1.57	71.00
June	27.48	4.31	83.25
July	29.25	3.08	84.75
August	30.55	2.92	76.41
September	28.94	0.86	83.76
October	22.70	2.06	86.16
Mean	**24.90**	**2.50**	**80.82**

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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner Dr. P.S. Sekhon, Principal Scientist (Plant Pathology), Division of Plant Breeding Genetics & Biotechnology, Punjab Agricultural University, Ludhiana during Viva-Voce examination held on 8th of May, 2012 have been incorporated in the manuscript entitled **"Perpetuation and Management of *Alternaria solani* (Ellis and Martin) Jones and Grout Causing Early Blight of Potato in Kashmir"** submitted by **Mr. Shabeer Ahmad Ganie (Regd. No. 2007-193-D)**.

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