

**Studies on early blight of potato (*Alternaria solani*
Jones & Grout.)**



THESIS

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by

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CERTIFICATE - I

*This is to certify that the thesis entitled “**Studies on early blight of potato (Alternaria solani Jones & Grout.)**” submitted in partial fulfillment of the Degree of **MASTER OF SCIENCE IN AGRICULTURE (Plant Pathology)** of Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior is a record of the bonafide research work carried out by **Mr. HARISHANKAR DHAKAD** under my guidance and supervision. The subject of the thesis has been approved by the Student’s Advisory Committee and the Director of Instruction.*

No part of the thesis has been submitted for any other degree or diploma or has been published. All the assistance and help received during the course of these investigations has been acknowledged by the scholar.

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Place: Indore

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ABBREVIATIONS

S.N.	Legends	Description
1	S.N.	Serial number
2	CD	Critical difference
3	r /unit/day	rate of infection of disease per unit per day
4	Cm	Centimeter
5	Df	Degrees of freedom
6	<i>et al.</i>	Allied (and other)
7	G	Gram
8	i.e.	That is (in reference to)
9	Kg	Kilogram
10	mm.	Millimeter
11	sp. & sp.	Species
12	Viz.	Wide list
13	^o C	Degrees Celsius
14	%	Per cent
15	No.	Number
16	Fig.	Figure
17	PDA	Potato Dextrose Agar
18	MSW	Meteorological standard week
19	ppm	Parts Per Million
20	g ⁻¹	grams/ litre
21	EC	Emulsifiable Concentrate
22	SL	Suspendable liquid
23	WG	Wettable granules
24	WS	Water Soluble
25	MSS	Mean Sum of Square
26	SE (m)±	Standard error of mean
27	S.S.	Sum of Square
28	S.V.	Source of variance
29	CRD	Completely Randomised Design
30	C.V.	Coefficient of variation
31	ANOVA	Analysis of variance
32	EMS	Error means sum of square
33	cal.	Calculate
34	tab.	Tabulated

Chapter-1

Introduction

Potato (*Solanum tuberosum* L.) is a very important crop throughout the world and as such 3 billion people depend on it as staple food source (Anonymous, 2014 a). Globally, more than 300 m ha of land is under potato cultivation. Total world potato production was estimated 364 m tonnes in 2012 (Anonymous, 2014 b). China is now the biggest potato producer, and almost third of entire potatoes is harvested in China and India. India is the second largest producer of potato after China, with 45.3 m. tones annual production.

Potato is considered as 'The King' in food staples and hardly any domestic kitchen is available which does not use it in one or the other form. Potato is the only non cereal food crop to command such a high position in the world since being nutritious it can solve the problem of poor malnutrition if adopted as a major food crop.

Potato is a popular source of carbohydrate, and can be used both for table consumption as well as in processed products. Fresh potato tubers contain around 80% water and 20% dry matter. More than 75% of the dry matter is starch but it also contains protein, fibers and small amount of fatty acids (Prokop and Albert, 2008). It is also rich in minerals such as potassium, phosphorus, magnesium and vitamins like B1, B3 and B6 (Camire *et al.*, 2009). Potato contains high level of vitamin C (Prokop and Albert, 2008).

Late blight of potato has been a disease of historical importance as it resulted in Irish famine in 1845 with death of 1.5 million people. To ensure adequate yield security, a potato farmer has to contend with several pest and pathogens, one of which is early blight. Early blight is noteworthy disease in potato production because if left uncontrolled, can solely or in combination with another destructive disease like late blight wipe off the entire potato plantation (Leiminger and Hausladen, 2012).

Early blight of potato is caused by two pathogens viz. *Alternaria solani* (Jones & Grout) and *A. alternata* (Fr.) Keissler, but in some areas only *A. solani* is

considered as the causative organism of this disease. With their [spores] in abundance in the atmosphere (Iglesias et al., 2007) and in the soil, they are always a threat when conditions become conducive for infection, and thus represents a serious threat in potato production (Leiminger and Hausladen, 2012).

The name early blight was ascribed to the disease by Jones and Grout (1897) because of the severe attack on early maturing cultivars than medium or late maturing cultivars. The pathogens generally infect [old or aged] foliage of potato, and have therefore been referred to as disease of senescence (Sector and Gudmestad, 1999).

A dark and sunken concentric ring-like lesion is usually a characteristic feature of an early blight infection. Some reports indicate that in the absence of late blight, early blight is mostly the predominant cause of defoliation in potato plantation (Leiminger and Hausladen, 2012). The spores remain on the soil surface and on the leaves, and can penetrate into the potato tubers during harvest (Kemmitt, 2002; Wharton and Kirk, 2013), mainly through wounds made during harvest.

Pelletier and Fry (1990) indicated the wide spread use of susceptible cultivars and the reliance on fungicides which only control late blight, but not early blight are a few reasons which could explain why early blight is gaining importance in the recent years. (Leiminger and Hausladen, 2012).

Presently, fungicides control the early blight due to lack of resistant variety for farmers to use (Kemmitt, 2002). Curative and preventive fungicides have been extensively used in the control of early blight in most part of the world with success. Fungicides like Sigmun (Boscalid+Pyraclostrobin, BASF), Amistar (Azoxystrobin, Syngenta), Revus Top (Mandipropamid+Difenoconazole, Syngenta), Mancozeb (Ethylene(bis)dithiocarbamate, DuPont), etc have been used with success in controlling the disease. Due to destructive nature of the pathogen and the prolific sporulation of the fungus, farmers typically go for repeated-spraying from the early stages in the growing season till maturity.

Studies on early blight (*Alternaria solani*) of potato have therefore, been carried out with the following objectives:-

1. Survey for incidence of early blight in and around Indore.

2. Epidemiology of disease dynamics.
3. Cultural and morphological variability of isolates of *Alternaria solani* *in vitro*.
4. Management of early blight of potato through fungicidal chemicals.

CHAPTER – II

REVIEW OF LITERATURE

Potato (*Solanum tuberosum* L.) is considered as one of the most important global food crops which is grown in more than 100 countries with different climatic conditions including temperate, subtropical and tropical zones. Its origin is in the Andean region of Peru in South America. It is also a promising source for non-food industry. Potato is the most economic subtropical vegetable in India. It belongs to family Solanaceae. The farmers face yield losses both in quality and quantity of produce due to various diseases, one of the most significant being early blight, caused by the fungus *Alternaria solani*. List of important diseases attacking potato is given below.

Table 2.1: List of major disease of potato

Disease	Causal agent
Early blight	<i>Alternaria solani</i> Jones & Grout
Late blight	<i>Phytophthora infestans</i> (Mont.) de Bary
Black scurf, Rhizoctonia canker	<i>Rhizoctonia solani</i> Kuhn <i>Thanatephorus cucumeris</i> [teleomorph]
Wart	<i>Synchytricum endobioticum</i> (Schilb.)
Black heart	Oxygen starvation in storage
Charcoal rot	<i>Macrophomina phaseolina</i> (Tassi) Goid
Black leg and soft rot	<i>Erwinia carotovora</i> subsp. <i>Atroseptica</i>
Bacterial wilt = brown rot	<i>Ralstonia solanacearum</i> (Smith) <i>Pseudomonas solanacearum</i>
Scab	<i>Streptomyces scabies</i> Lambert and Loria

Cercospora leaf blotch	<i>Mycosphaerella concors</i> <i>Cercospora concors</i> (Casp.) Sacc. <i>Cercospora solani</i> <i>Cercospora solani-tuberosi</i>
Gray mold	<i>Botrytis cinerea</i> <i>Botryotinia fuckeliana</i> (de Bary) Whetzel
Potato cyst nematode	<i>Globodera rostochiensis</i> (Skarbilovich) <i>Globodera pallida</i>
Root knot nematode	<i>Meloidogyne</i> spp. Goeldi <i>Meloidogyne hapla</i> <i>Meloidogyne incognita</i> <i>Meloidogyne javanica</i> <i>Meloidogyne chitwoodi</i>

Source: "Plant Pathology" eleventh edition by B. P. Singh.

The *Alternaria* spp. cause diseases in considerably large number of crops. It is one of the eight genera of hyphomycetes. The taxonomy of the genus *Alternaria* has undergone several refinements since it was established by Nees (1817) (Cited in Rao, 1971) with *A. tenuis* as a type species.

The fungi *A. solani* and *A. alternata* are classified under *Eurkoyota* domain, Kingdom *Fungi*, phylum *Deuteromycota* (formerly) or *Ascomycota* (present), class *Hyphomycetes*, order *Hyphales* (Waals et al., 2001). These are classified as fungi imperfecti or Deuteromycetes because their sexual phase has not been discovered yet or if discovered lie in Ascomycotina.

2.1 Symptomatology

Datar and Mayee (1981) showed that *A. solani* attacks tuber in the ripe stages at the stem end, growth causes cracks and other wounds. Early blight on potato was characterized by the appearance of brown to dark brown colour necrotic

spots. Appearance of concentric rings inside the spot produced target broad effect later.

Waals *et al.* (2001) studied early blight of potato, resulting in major yield losses. Leaf symptoms are characteristic dark brown to black lesions with concentric rings. Morphological, physiological and molecular characters of the pathogen as well as the disease cycle, epidemiology and control of the disease have been reviewed.

Kemmitt (2002) and Zhang (2004) described that symptoms of early blight have been characterized by dark concentric-ring like lesions. This cycle continues until there is no healthy tissue to infect or the weather prevents further cycles of the disease. Stems may also be infected. Which show dark brown to black sunken and lens shaped lesions with a light center, and have the typical concentric rings.

2.2 Disease incidence and losses

Vander waals *et al.* (2003) conducted a survey of control practices and growers perceptions of early blight in South Africa. The predominant control method for early blight was the use of fungicides with mancozeb or chlorothalonil as active ingredient. Most respondent commencing spraying at flowering and spray at 7–10 day intervals.

Hossain *et al.* (2010) has conducted a survey during 2006 to 2008. The average higher leaf infection in early blight of potato and tuber infection in soft rot of potato were recorded 37% and 39%, respectively.

Ganie *et al.* (2013 b) reported that the *Alternaria* leaf blight is one of the most important diseases of potato (*Solanum tuberosum* L.) worldwide. The disease was prevalent in all the potato growing areas of Kashmir valley surveyed during 2009 and 2010. The overall mean disease incidence and intensity ranged from 24.54 to 28.23% and 13.84 to 15.98%, respectively. Up to fourth week of June concentric rings form as a result of irregular growth patterns by the fungus in the leaf tissue giving the lesion a characteristic 'target spot' or 'bulls eye' appearance. The maximum lesion size 7.4 mm was recorded in the second week of August.

Rani *et al.* (2015) conducted field surveys during 2011 and 2012 to diagnose the symptoms of early blight of tomato (*Lycopersicon esculentum* L.) in Jammu

Division of Jammu and Kashmir. The disease intensity and incidence varied from 21.66 to 34.13% and 10.48 to 18.56%, respectively. Weather factors (temperature, humidity and rainfall) were found to play a significant role in its development.

2.3 Effect of weather factors on the disease development

Basu (1971) reported that the overwintering conidia and mycelia are melanized, and thus are able to withstand varying kinds of environmental conditions including freezing and high temperatures. Chlamyospores have also been reported as survival structure of *A. solani*.

Pelletier (1988) and Foolad *et al.* (2008) reported that *A. solani* and *A. alternata* are polycyclic pathogens. Overwintering inoculums in debris, tubers, and on other Solanaceous crops and weeds like hairy night shade (*S. sarrachoides*) are the cause of primary infection on new crop. The pathogen(s) overwinter as conidia or mycelia in infested debris, soil, infected tubers, or on an alternative host (mainly plants in the family *Solanaceae*).

Hatzipapas *et al.* (2002) reported the infection process of *A. solani* and *A. alternata* begins with attachment of spores onto the leaves of the host plant. Subsequent to the attachment, spores germinate to produce germ tube under favourable environmental conditions.

Mehboob *et al.* (2013) reported that the epidemiological and biochemical factors play a significant role in early blight development in potatoes. For this 29 test cultivars/ lines, were screened, 15 lines including Cardinal and Desiree were highly susceptible. At varietal level, among 29 test varieties/ advanced lines, all the varieties showed highly significant correlation with minimum temperature and negative correlation with maximum temperature and no correlation with relative humidity and wind speed. Maximum disease severity was recorded at 17-20°C maximum temperature, 6-9°C of minimum air temperature and 1.9-2.4 mm pan evaporation. Relative humidity and wind speed almost had no significant effect on disease severity. On varietal level early blight disease severity had significant correlation with phenolic production both in leaves and tubers.

Ganie *et al.* (2015) showed the effect of weather factors on development of early blight of potato studied during the year 2009 and 2010 and indicated maximum apparent infection rate of 0.155 /unit/day and 0.165 /unit/day respectively. Temperature showed a significant and positive correlation with disease intensity, whereas relative humidity and precipitation showed positive but non significant correlation with disease intensity. Multiple regression analysis revealed that weather factors accounted for 65.5 per cent variation.

2.4 Cultural and morphological variability

Gherbawy (2005) studied of nine different species of *Alternaria* represented by 27 isolates were collected from several Egyptian crops by RAPD-PCR for genetic diversity using four different RAPD primers. Results indicated considerable genetic variability among *Alternaria* isolates, even within the same species group.

Rodrigues *et al.* (2010) reported that sporulation of *Alternaria solani* can be scarce and is often reduced when the fungus is cultivated *in vitro*. A series of experiments were conducted to assess the effects of moisture, mycelial wounding, light quality and photoperiod, and culture media on conidial production. A procedure to induce sporulation based on mycelial wounding and dehydration was adapted and validated. The effect of subculturing on *A. solani* sporulation was also assessed after six subcultures, every seven days, there was no reduction in sporulation of colonies.

Goyal *et al.* (2011) studied variation in morphology and cultural characteristics among 13 representative Indian geographical isolates from 219 collections of *Alternaria brassicae*, the causal agent of *Alternaria* blight of rapeseed-mustard. All the isolates showed high level of variability *in vitro* in respect of conidia length, width, beak length and number of septa.

Charlton (2014) evaluated a method for the production of spores from a strain of *Alternaria solani*, using ultra-violet light. Sporulation was also induced, but to a lesser degree, by oxidizing agents, in particular by hydrogen peroxide and ozone.

Kumar *et al.* (2015) studied *Alternaria solani* for various parameters of cultural characters. The optimum temperature was recorded 25 °C with pH 7.5 for the pathogen. Pigmentation varied from brown to black color on these media, light

yellow to black color at different temperatures and gray to brown color on different pH ranges. The sporulation was not found any tested media.

Chouhan *et al.* (2015) studied *A. solani* isolate collected from tunnel grown under vegetable area, Bahauddin Zakariya University. On PDA medium the pigmentation varied from creamy yellow, brown black to olivaceous brown while on HLEA medium it was light brown.

Verma *et al.* (2015) reported that *A. solani* is a shy sporulator.

Bhajbhujje (2015) reported the inhibitory effect of five plant growth regulators including indol-3-acetic acid; indol-3-butyric acid; naphthalene acetic acid; 2,4-dichlorophenoxy acetic acid; and phenyl acetic acid *in vitro* against the leaf blight pathogen, *Alternaria solani*. The results of the study revealed the possibility of usage of plant growth regulators in inducing phytoalexin compound in susceptible plant cultivars.

2.4.1 Effect of Temperature on the growth of *A. solani*

The favourable temperature required for the growth of *A. solani* was found in the range of 5-35°C (Bonde, 1929; Verma, 1970, Gemawat and Ghosh, 1980).

Kaul and Saxena (1988) reported that the maximum growth of five isolates of *A. solani* was at 25 °C followed by that at 20°C. *A. solani* germinated most rapidly in darkness when ambient temperature was near 25°C.

2.4.2 Effect of light on the growth of *A. solani*

According to Lukens (1963) the conidia of *A. solani* normally formed after incubation for 6 hours in the dark.

Prasad and Dutt (1971) found maximum sporulation in six day old culture with 24 hours of exposure to sunlight, than culture exposed to incandescent electric light or infrared light. Growth and sporulation of *A. solani* was sparse in Czapek's dox synthetic medium but the same was high in case semi-synthetic and natural carrot leaf media when exposed to light (Fencelli and Kimati, 1990).

Douglas (1972) reported that the sporulation of *Alternaria solani* was significantly affected by both fluorescent illumination and temperature. As temperature was reduced the light became lesser of a factor. However, cultures of *A. solani* of darkness produced the greatest number of spores.

2.4.3 Effect of culture medium on the growth of *A. solani*

Sidlauskiene *et al.* (2003) carried out studies on environmental conditions on the development of genus *Alternaria*. Micromycetes of *Alternaria* grew most intensively on malt extract agar and potato-glucose media. At 14 days of incubation, the average colony diameter was: 6.8-7.4 cm on malt extract medium, and 6.7-7.2 cm on potato-glucose medium.

Kumar *et al.* (2007) studied eleven isolates of *A. solani* designated as So, Dh, Sh, Va-5, Ka, Ma, Hy, Ba-1, My, Va-3 and Mi collected from different agroclimatic conditions and these isolates were characterized for cultural, morphological, pathogenic and molecular variations. The pigmentation varied from yellow, brown, black, brownish to greenish black in there isolates on potato dextrose agar medium.

Singh *et al.* (2014) found that radial growth was not significantly different for most of the isolates. Isolates of *Alternaria solani* depicted high variability on pigment production on PDA medium.

Nikam *et al.* (2015) conducted the studies for the pathogenic, cultural, morphological and molecular variability among the isolates of *A. solani*. The result revealed that all the eight isolates of *A. solani* were pathogenic to Tomato and showed variability among them. The test isolates could grow better on the basic culture medium PDA. These eight isolates exhibited a wide range of variability in respect of their mycelial and conidial dimensions and septation.

Koley and Mahapatra (2015) studied the growth of the fungus in culture in twelve different liquid and solid media and compared with each other. Potato dextrose agar and oat meal agar among solid media and Richard's broth and Sabouraud's broth among liquid media appeared to be better than other media for growth of tomato early blight pathogen.

2.5 Management of early blight of potato

Effect of fungicides

Choulwar and Datar (1988) reported the least disease intensity both at pre and post harvest stages with increased yield where six early sprays of mancozeb at 0.2% followed by six late and five early sprays were given for early blight of tomato. In general early sprays were most effective than equal number of late sprays.

Christ and Maczuga (1989) evaluated spray schedule of fungicide for their effect on controlling potato early blight under Pennsylvania growing condition. Disease severity and lesion number were lowest when fungicide sprays were initiated before flowering of the potato plant.

Sinha and Prasad (1991) reported that the best control of disease caused by *Alternaria solani* was achieved by Dithane M-45 (mancozeb) at 0.2% among 7 fungicides tested in the field over three seasons. The treatment was also the most cost effective and gave the highest yields.

Brammatta (1993) reported that the application of chlorothalonil (0.2%) decreased early blight severity. However, application did not affect the yield of any cultivar, nor were fungicide cultivar interactions detected. Cultivar differed in the extent of defoliation resulting from early blight.

Nagia *et al.* (1994) during a field trial in Haryana, in 1992-93 reported that oxydemeton methyl 25EC at 200 g.a.i./ha and Dimethoate 30 EC at 200 g.a.i./ha either alone or in combination with Mancozeb 75 WP at 1500 g.a.i./ha. and Ziram 27 SC at 1600 g.a.i./ha were effective against *Aphis gossypii* and *Myzus persicae* on potato. *Alternaria solani* was also effectively controlled.

Monaco *et al.* (2001) evaluated *in vitro* effect of fungicides to investigate possible use of chemical and biological approaches to control the early blight pathogen *Alternaria solani*. Six fungal antagonists of *A. solani* to the fungicides was determined *in vitro* by calculating ED50 values through linear regression analysis. Out of these *Chaetomium globosum* in combination with Dithane-45 was found to be most effective.

Khan *et al.* (2003) tested four fungicides against the early blight on twenty potato varieties/lines. All the fungicides reduced the disease severity compared to untreated check. However, the effect of Metalaxyl + Mancozeb (72 WP), Ridomil Gold (68WP), Score 250 and Banko 500 SC was more pronounced on tolerant and moderately resistant varieties compared to moderately susceptible or susceptible varieties.

Tofoli *et al.* (2003) conducted *in vitro* test for action of fungicides against *A. solani* causing early blight of tomato. Tests were carried out with the aim of evaluating the inhibitory action of 16 fungicides on the mycelial growth and conidial germination. The fungicides were tested at the concentration of 0, 10 and 100 µg. ml⁻¹ using the fungicides dilution technique and cellophane technique. The fungicides Iprodione, Cyprodinil, Procymidone, fluezinga and Pyrimethanil presented elevated inhibition level for both the criteria.

Ganeshan and Chethna (2009) evaluated pyraclostrobin 25%EC at., 50, 75 and 100 g.a.i./ha, for two seasons against early blight of tomato cultivar NS2535. The efficacy of pyraclostrobin was compared with commonly used chemicals viz., captan, mancozeb and an untreated control. Phytotoxicity effect of pyraclostrobin was evaluated using a single spray, at concentrations of 100, 200 and 400gm a.i./ha. Pyraclostrobin at all concentrations was found significantly effective in reducing the disease and in increasing the yield as compared to captan, mancozeb and control. Pyraclostrobin at 100, 200 and 400g a.i./ha did not cause any phytotoxicity symptoms in terms of chlorosis, necrosis, wilting, scorching, hyponasty and epinasty.

Hossain and Hossain (2009) evaluated the efficacy of seven fungicides, Dencozeb 80 WP (Mancozeb), Evamil 72 WP (Mancozeb 64% + Metalaxil 8%), Mosum 80 WP (Mancozeb), Companion 72 WP (Mancozeb 60% + Carbendazim 12%), Mancovit 70 WP (Mancozeb), Baimyl 72 WP (Mancozeb 64 % + Metalaxyl 8%), Pesterozeb 50 WP (Mancozeb) for their effectiveness against early blight (*A. solani*) of tomato. In an *in vitro* study all the test fungicides at 250 ppm significantly inhibited the mycelial growth of *A. solani* over control and Baimyl, Mancovit and Evamil gave complete inhibition of mycelium growth.

Arunakumara *et al.* (2010) conducted field studies of fungicidal management of early blight of tomato caused by *A. solani*. The treatments comprised control, 0.2% chlorothalonil, 0.2% Kasugamycin, 0.1 % Amistar, 0.1 % Propacanozol, 0.2 % Pyroclostrobin, 0.1 % Perfect, 0.2% Metalaxyl ,0.25% Mancozeb, and) 0.2% Benomyl applied as four sequential spray at 15 days intervals. Results showed that maximum disease control with 28.5 PDI was recorded in the propacanoazole – treated plots, followed by 35.33 % in the Pyroclostrobin treatment.

Horsfield *et al.* (2010) conducted a series of experiments to evaluate fungicide use strategies for the control of early blight. The protective and curative activity of fungicides was evaluated in glasshouse and field studies. Boscalid, azoxystrobin and difenoconazole were highly effective in the control of early blight when applied up to three days before or three days after inoculation.

Chourasiya *et al.* (2013) evaluated the effect of fungicides, namely mancozeb (0.2%), zineb (0.2%) and carbendazim(0.05%), botanicals i.e. *Azadirachta indica* (neem) leaf extract (20%), *Eucalyptus chamadulonsis* (eucalyptus) leaf extract (5%) and *Allium sativum* (garlic) extract (5%) in a field experiment at Allahabad, during 2012-13 *rabi* seasons for the management of early blight of tomato. The lowest percent disease incidence (PDI) was observed in mancozeb (18.36) followed by carbendazim (25.62) and zineb (27.84) treatments. The highest cost benefit ratio was obtained with mancozeb treatment (1:4.15) followed by carbendazim(1:3.73).

Ganie *et al.* (2013 a) evaluated five non-systemic fungitoxicants viz., 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 viz., thiophenate 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 *in vitro* against *Alternaria solani* through poisoned food technique. Among non-systemic fungitoxicants mancozeb 75 WP, irrespective of concentration was most effective followed by propineb 70 WP. Among systemic fungitoxicants hexaconazole 5 EC was most effective. Under *in vivo* conditions seed treatment with mancozeb 75WP (0.3 %) + foliar spray with hexaconazole 5 EC (0.1%) + foliar spray with datura

(50%) + foliar spray with *Trichoderma harzianum* (1×10^7 spore/ml) were highly effective in controlling the disease severity as compared to control.

Sahu et al.(2013) tested the efficacy of some newer molecules like pyraclostrobin, boscalid,and their combination maccani, pristine along with commonly used chemicals viz., mancozeb, copper oxychloride and chlorothalonil against early blight of tomato cultivar Pusa Ruby. results revealed that Pristine at both concentrations (64+126g a.i./ha and 76.8+151.2g a.i./ha) was found significantly effective in reducing the disease and increasing the fruit yield over control.

2.6 Effect on yield of potato

Arana et al. (2007) evaluated the influence of an early blight epidemic on potato tuber yield in three field experiments during 2000 to 2002 crop seasons. Through a disease severity gradient method, used four different dosages of fungicide chloratalonil were obtained four epidemic levels with severity range of D1 (maximum), plot without fungicide treatment, to D4 (minimum), plot with fully dosage fungicide commercial recommended, were obtained, which allowed for comparison of the epidemic effect on plant growth and tuber yield, The data revealed that green leaf area and tuber yield reduction caused by the early blight is dependent upon the disease severity.

Tsedaley. (2014) reported that the disease can damage both potato foliage and tubers and can cause yield losses of 5 - 50%. Early blight is a poly cyclic disease that can cause more than one disease epidemics within a single cropping season. It is difficult to control because of its capacity to produce huge amounts of secondary inoculums. Using good cultural practices and applying chemical fungicides are important in reducing as well as managing of early blight disease of potato. Even though there is no well developed biological control of early blight, it is very important to develop such management strategies. Biological control measures are specific, efficient and environmentally safe.

CHAPTER III

MATERIALS AND METHODS

The present studies on early blight of potato [(*Alternaria solani*) Jones & Grout] were undertaken for the survey to assess disease incidence and estimation of losses, development of disease in relation to weather parameters, cultural and morphological characters of the pathogen *in vitro* and management of disease by application of fungicides under field condition in the Department of Plant Pathology, College of Agriculture Indore, M.P. The details of materials used, experimental procedures followed have been described in this chapter.

3.1 Materials

The materials required to conduct the experiment's included nylon rope and bamboo stick, lime powder, one furrow opener, chemical for culture media, fungicides, glass wares, equipment's and few miscellaneous articles.

3.1.1 Diseased leaf samples:

Collection of the diseased plants exhibiting typical symptoms of early blight of potato was done from the research fields of College of Agriculture, Indore (M.P.) and cultivators fields in the vicinity of Indore.

3.1.2 Chemicals

(a) Cleaning solution

Cleaning solution contained potassium dichromate 80 g, distilled water 300 ml and concentrated sulphuric acid 400 ml. This was used to clean the glass wares.

(b) Mercuric chloride solution

A 1:1000 solution of HgCl_2 was prepared and used for surface sterilization of samples.

(c) Mounting medium

Lacto phenol and cotton blue with the following composition were used as the staining liquid for studying the characteristics of the pathogen.

(i) Lactophenol with the following composition were used as the working medium for studying the characteristics of the pathogenic fungus. Phenol (pure crystals, liquefied by 20 ml water gentle heating on a water bath)

Lactic acid	20 ml
Glycerol	40 ml
Distilled water	20 ml

(ii) Cotton blue

Anhydrous lacto phenol	67 ml
Distilled water	20 ml
Cotton blue	0.1 g

3.1.3 Media:

Ingredients of the culture media used in the study are given below:

Potato dextrose agar medium

Potato dextrose agar (PDA) medium having the following ingredients was used for a few physiological studies (Dhingra and Sinclair, 1993). PDA medium was used during all the physiological studies.

Peeled and sliced potato (extract)	200 g
Dextrose	20 g
Agar -agar	18 g
Distilled water	1 lit.
pH (adjusted to)	6.5

3.1.4. Glasswares

Standard "Borosil" make glassware's like Petri dishes, beakers, *funnels*, pipettes, Erlenmeyer flasks, culture tubes, measuring cylinder etc. were used during the course of study.

3.1.5. Equipments and miscellaneous articles

Equipments used during course of investigation included research Binocular microscope, refrigerator, autoclave, hot air oven, BOD incubator, Laminar, air flow, weighing balance, LPG gas burner and hot plate. Small instruments like inoculation needle, scalper, razor, glass cavity slides, glass rod, cork borer, wash bottle, forceps,

scissor, knife, polypropylene bags, desiccators, glass marking pencils, cover slips, brush, dropper, match box, plastic pots etc.

3.2 Methods

3.2.1 Survey for disease incidence of early blight and collection of infected leaves.

A field survey was carried out to assess the incidence of early blight in potato fields in villages in the vicinity of Indore starting from sowing till harvesting stage during *rabi* 2015-16. The early blight incidence was assessed by recording the observation on 100 random plants. In each village, one field were selected and from each field 100 plants were examined randomly and scored for disease incidence in 0-5 scale (Horsefall and Barette, 1945).

Table 3.1: Description of disease scale (0-5)

Category	Description of the symptoms
0	Leaves free from infection.
1	Small irregular spots covering <5% leaf area.
2	Small irregular brown spots with concentric rings covering 5.1-10% leaf area.
3	Lesions enlarging, irregular brown with concentric rings covering 10.1-25% leaf area.
4	Lesions coalesce to form irregular and appears as a typical blight symptom covering 25.1-50% leaf area.
5	Lesions coalesce to form irregular and appears as a typical blight symptom covering >50% leaf area.

Percent disease index (PDI) was calculated using formula of Mckinney (1923) as given here:

$$PDI = \frac{\sum \text{All numerical ratings} \times 100}{\text{Total numbers of leaves observed} \times \text{maximum disease rating (5)}}$$

3.2.2 Collection of diseased samples

Diseased plant samples were collected from the different potato growing field of villages and properly marked and packed in polythene bags and brought in laboratory for further studies. Each sample was kept in polythene bag and tied with a rubber band and labeled immediately. Information pertaining to the locality, crop history, etc. was also obtained about the samples.

3.2.3 Cleaning and sterilization of glasswares

The glassware were cleaned by dipping them in cleaning solution for 5 minutes and finally rinsed with running tap water for 30 minutes. The Petri dishes were sterilized in a hot air oven at $180 \pm 2^{\circ}\text{C}$ for 1.5 to

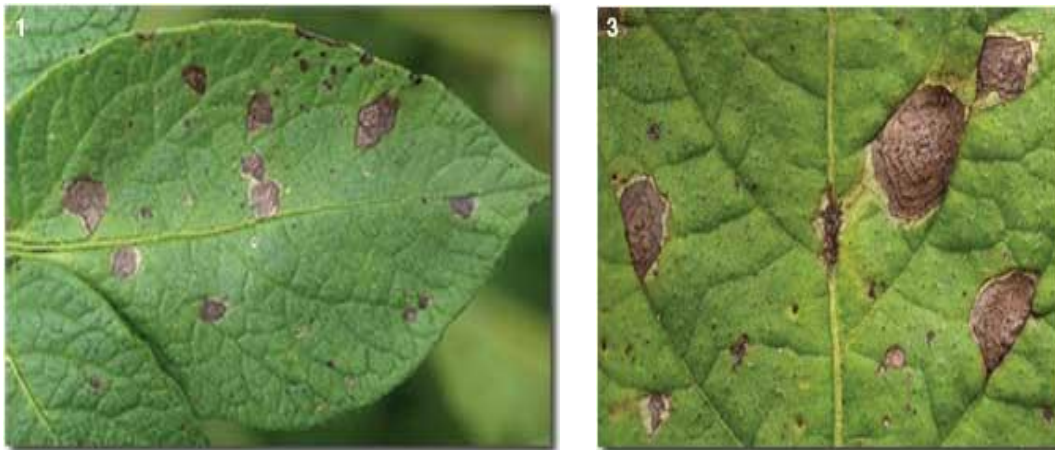


Plate 1 a : Early blight symptoms on leaves



Plate 1 b : Early blight symptoms on tubers

2 hours. The inoculation needle, cork borer and other metallic instruments were sterilized by dipping them in alcohol and heating red hot over flame of the spirit lamp/Bunsen burner.

3.2.4 Symptomatology

The symptoms of the disease were examined critically on leaves of potato plants of different ages at 15 day intervals to elucidate the course of development of the disease in the field.

3.2.5 Isolation of the pathogens

Standard tissue isolation technique was employed for isolation of *Alternaria solani* from plants showing typical symptoms of early blight. The infected plant parts (from leaves region) were washed thoroughly in running tap water repeatedly, till they were free of adhering soil and dust. The infected parts were cut into bits of five to six mm size and surface sterilized in 0.1 per cent mercuric chloride for a minute. This was followed by repeated washings in sterile water. The bits were transferred aseptically onto sterilized Potato Dextrose Agar (PDA) slants. The slants were incubated at room temperature ($27\pm 1^{\circ}$ C) for 72 hours. Pure culture of *Alternaria*

solani obtained and maintained on PDA medium. After ten days old cultures were used for further experiments.

3.2.6 Purification of the pathogen

The isolated pathogen was purified by single spore isolation and maintained on PDA slants at $25\pm 1^{\circ}\text{C}$. the cultures were revived every month by fresh transfers of culture on the PDA slants.

3.2.7 Identification of pathogen

The morphological studies were carried out. The observations were recorded from the 7 day old culture for the characters of colony, mycelium, conidiophores and conidia and their comparison was made.

3.2.8 Culturing of different isolates of *Alternaria solani* and their maintenance.

The pathogen *Alternaria solani* was isolated from leaves, stem and tubers of diseased plants collected from different regions and later purified by single spore isolation **method and maintained on potato dextrose slants.** The affected portions of diseased plants were collected, cut with the help of a sharp razor and rinsed with sterile water to remove traces of dirt. These were surface sterilized by dipping in 1:1000 mercuric chloride solutions for one minute and washed twice with sterile water. These pieces were transformed aseptically on to the sterilized Petri dishes containing solidified PDA in a laminar air flow. The Petri dishes were incubated at $28 \pm 2^{\circ}\text{C}$. The races were purified by single spore form developing colonies.

Cultural variability among isolates of *A. solani*

The cultural characters were recorded after 7th day of inoculation of all isolates of *A. solani* characters like mycelium growth diameter and growth characters, pigmentation on medium were recorded by direct observation of culture grown in petri plates on PDA medium. All the four isolates were tested for their cultural and morphological variation on PDA.

Morphological variability of different isolates of *A. solani*

Slides were prepared from culture of all isolates of *A. solani* separately in 9-day-old cultures on PDA and examined under the light microscope to record the

width of the conidiogenous hyphae. The calibration was done with the help of ocular and stage micrometer.

3.2.9 Development of early blight in relation to weather parameters

Observations of early blight were recorded at fortnight intervals starting from 1st November to 29th February 2015-16. Weather data for minimum and maximum temperature (°C), relative humidity (%), rainfall (mm), and disease incidence were recorded, later the correlation of disease development with different weather factors was worked.

Table 3.2: Meteorological data recorded at Indore (M.P.) during 2015-16

Month	Fortnight	Temperature (°C)		Relative Humidity (%)	Rainfall (mm)
		Max.	Min.		
November	I	31.2	20.6	78	0
	II	29.6	18.5	71.26	0
December	I	27.7	15.3	72.8	0
	II	23.9	9.5	57.33	0
January	I	28.9	11.6	74.6	0
	II	25.6	9.3	63.66	0
February	I	29.3	13.4	67.66	0
	II	31	18.7	69	0

Source: Meteorological observatory, AICRP on Dryland Agriculture, College of Agriculture, Indore (M.P.)

The coefficient of correlation (r) was calculated between variables X₁, X₂, X₃, X₄ With Y (X₁ - X₄ indicate weather variables and Y as disease incidence) using the formula:

$$r = \frac{\Sigma(XY)}{\sqrt{\Sigma X^2 \Sigma Y^2}}$$

X_1 = Maximum temperature (°C)

X_2 = Minimum temperature (°C)

X_3 = Relative humidity (%)

The value of R always remains less than 1 and between -1 to +1, employing the test of significance.

3.2.10 Management of early blight with chemical fungicides:

Location

The experiment was conducted in the Research Field, College of Agriculture, Indore (M.P.)

Experimental details:

Crop	: Potato
Variety	: Lavkar
Year	: 2015-16
Date of sowing	: 01 st November 2015
Design	: RBD
Treatments	: 8
Replications	: 3
Plot size	: 1.5x2 m ²

The field experiment for the evaluation of fungicides was conducted at the College of Agriculture, Indore (M.P.) during *Rabi* 2015-16. The experiment was laid out in randomized complete block design with three replications using variety Lavkar. All recommended agronomic practices were followed.

Five new fungicidal chemicals and their combine molecules *i.e.* Ametoctradin 300 g/l + Dimethomorph 225 g/l SC, Zampro 525 SC, Dimethomorph 50% WP, Cymoxanil 8% +Mancozeb 64% WP and their different concentration were evaluated against early blight disease and compared with untreated control.

Fungicide application was done by Knapsack sprayer. Two sprays of fungicides were given at 15 days interval. Data on the disease severity was recorded after every fifteen days interval. First observation on disease severity was recorded before the beginning of first spray and subsequent observations after first spray and before second spray and finally disease severity was recorded.

Table 3.3: Treatments combination

Symbols	Treatments	GAI	Conc. (%)
T ₁	Ametoctradin 300 g/l + Dimethomorph 225 g/l SC	420	0.16
T ₂	Ametoctradin 300 g/l + Dimethomorph 225 g/l SC	525	0.20
T ₃	Zampro 525 SC	420	0.16
T ₄	Zampro 525 SC	525	0.20
T ₅	Dimethomorph 50% WP	500	0.20
T ₆	Cymoxanil 8% +Mancozeb 64% WP	1080	0.30
T ₇	Ametoctradin 300 g/l + Dimethomorph 225 g/l SC	1050	0.40
T ₈	Untreated control		

3.2.11 Percentage disease control:

The percentage disease control was calculated by the following formula (Wheeler, 1969).

$$\text{Percent disease control} = \frac{\text{PDI in control} - \text{PDI in treatment} \times 100}{\text{PDI in control}}$$

3.2.12 Evaluation of fungicides against *Alternaria solani* in vitro.

The given five new fungicidal chemicals and their combine molecules *i.e.* Ametoctradin 300 g/l + Dimethomorph 225 g/l SC, Zampro 525 SC, Dimethomorph 50% WP, Cymoxanil 8% +Mancozeb 64% WP and their different concentration

tested against the tomato early blight pathogen *in vitro* by poisoned food technique (Falck, 1907).

The required quantity of the fungicide was added separately in Erlenmeyer flask containing molten sterilized PDA and cooled to 55°C so as to give the desired concentration and poured in sterilized Petri plates. Three replications were maintained for each treatment. After solidification of the medium each plate was inoculated by transferring a 6 mm. disc of 8 days old cultures of the pathogen containing spores and mycelium grown on PDA. Suitable controls without the addition of fungicides were also maintained. The plates were incubated at 28± 2°C. The data with respect to the radial growth and mycelium character were recorded at seven days after inoculation or full expansion of colony growth in control. Control treatments were maintained without adding any fungicide to the medium.

Inhibition of mycelium growth

The inhibition percent was calculated by formula (Vincent, 1927).

$$I = \frac{C-T}{C}$$

Where,

I = percent inhibition

C = Radial growth in control

T = Radial growth in treatment

3.2.13 Yield loss assessment

After crop maturity, tubers were dug up either by hand or with a tractor-mounted small-plot potato lifter. Tubers from each plot were collected and graded before weighing. Tubers were weighed to determine total yield. The tuber yield per hectare was calculated and the percentage difference between fungicide treated and non-treated plots was determined by:

Percentage yield increase over control % =

$$\text{Treated plot yield} - \text{untreated plot yield} / \text{untreated plot yield}$$

3.2.14 Apparent infection rate of disease development (r/unit/day):

The apparent infection rate of disease development /unit/day was determined in different fungicidal treatment according to Vander Plank (1963).

$$r = \frac{2.303}{t_2 - t_1} \left[\log_{10} \frac{x_2}{1 - x_2} - \log_{10} \frac{x_1}{1 - x_1} \right]$$

Where r = rate of disease development/unit/day

t₁ = date of first observation

t₂ = date of second observation

X₁ = disease severity at time t₁

X₂ = disease severity at time t₂

3.2.15 Cost benefit ratio

The cost benefit ratio was calculated to see the total benefit gained by using the fungicides i.e. score one application, score two application.

Cost benefit ratio was computed by using the formula

$$\text{B:C ratio} = \frac{\text{Gross income}}{\text{total cost}}$$

3.3 Statistical analysis

Randomized Block Design (RBD) (for survey on cultivators fields, wilt incidence and yield) and Completely randomized design (CRD) and factorial CRD (for in vitro experiments) were employed to analyses the data. The critical differences were worked out at 5% probability level.

The experiments were conducted in the PG laboratory, Department of Plant Pathology, College of Agriculture, Indore (M.P.).

CHAPTER – IV

EXPERIMENTAL RESULTS

Early blight of potato caused by *Alternaria solani* Jones & Grout is the historic disease of economic importance. Presently a field survey was conducted to gather information on the severity of disease incidence in different villages in vicinity of Indore. Study on the early blight development on potato in relation to weather parameters, cultural and morphological variability of isolates *in vitro*, evaluation of fungicides against pathogen *in vitro* were undertaken during 2015-16 in the Department of Plant pathology, College of Agriculture, Indore. Field evaluation of fungicides, for control of early blight was done in the Research field, College of Agriculture, Indore. The results on these aspects on early blight of potato are presented here in.

4.1 Survey for incidence of early blight of potato.

A field survey was carried out in different localities around Indore to assess the incidence of early blight of potato during *Rabi 2015-16*. Total eight locations were surveyed. The village wise disease incidence has been presented in table 4.1, during the survey it was revealed that early blight of potato appeared in almost all the villages and the disease incidence ranged from 35.50 to 42.25 percent (Avg. 39.48 %). The highest disease incidence (42.25) was observed in field of Dudhia, followed by Palda (41.50), Joshiguradia (41.30), Pipliahana (40.23) and Research field, College of Agriculture, Indore (38%). Where as minimum percent disease incidence was observed in Bicholi (35.50%) followed by Bijalpur (36.46%).

The data are based on the observations of 100 randomly selected plants / locality and represent the average. The disease appeared in the form of small, scattered dark colored areas which revied brown to black spots, up to 1 cm. diameter. Concentric rings may also be formed in the lesions. Later these spots increased in size and coalesced covering larger leaf area. The symptoms of the disease were of blight.

Table 4.1: Incidence of early blight of potato at different locations in the vicinity of Indore.

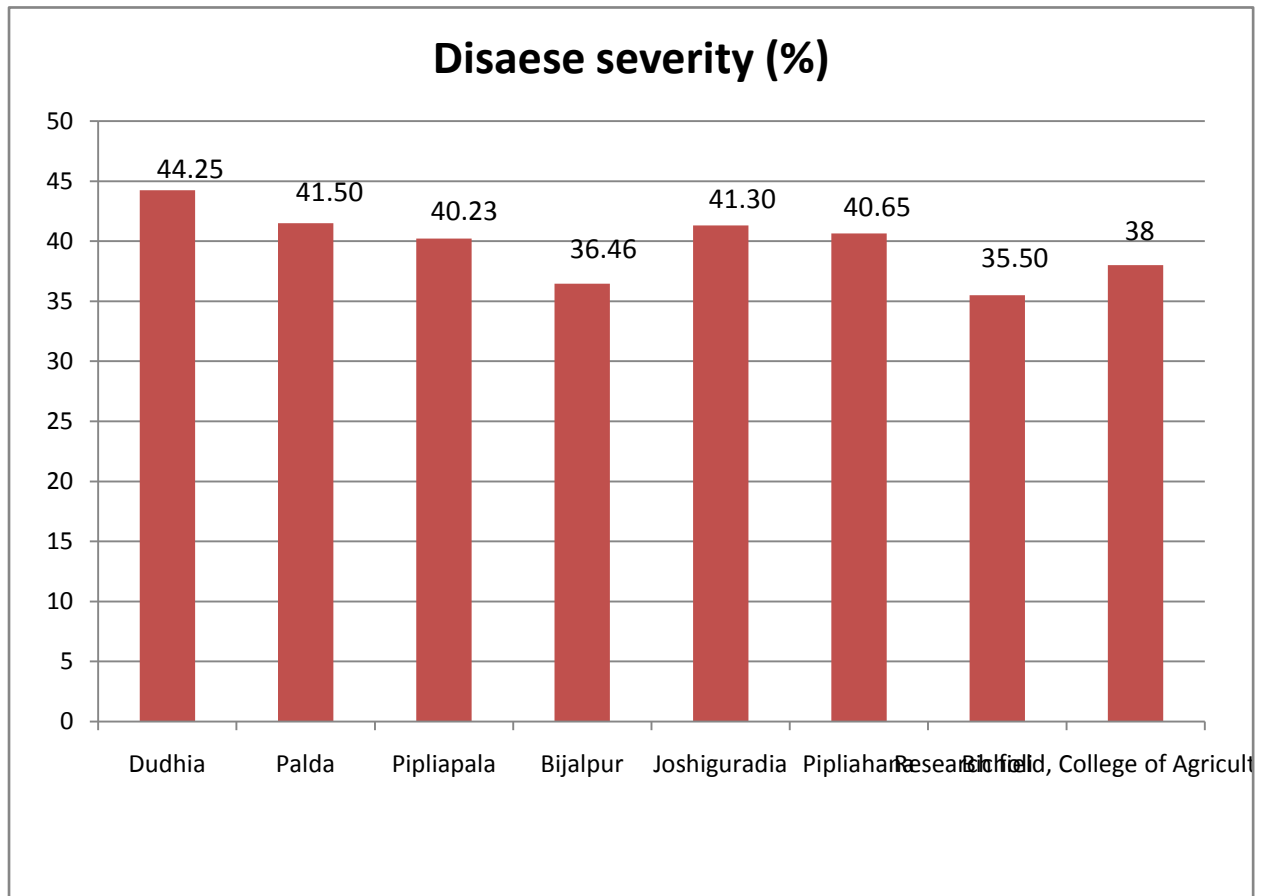
S. No.	Location	Disease severity (%)
1	Dudhia	44.25
2	Palda	41.50
3	Pipliapala	40.23
4	Bijalpur	36.46
5	Joshiguradia	41.30
6	Pipliahana	40.65
7	Bicholi	35.50
8	Research field, College of Agriculture, Indore	38
	Average	39.48
	Range	35.50 – 42.25

4.2 Isolation, purification and characterization of the pathogen

4.2.1 Isolation of the pathogen

Isolation of the pathogen was made from potato leaves showing typical symptoms of the disease. Standard tissue and single spore isolation method were followed after surface sterilization. The pure culture of the pathogen was obtained after eight days of inoculation which showed brownish colour. Such pure cultures were maintained in the refrigerator at 5°C for further studies. Characterization of the pathogen was done by watching the character of mycelium, conidiophores and conidia.

Fig 4.1 Incidence of early blight of potato at different locations in the vicinity of Indore.



4.2.2 Identification

The description of the pathogen isolated is as follows:

Mycelium: In culture the fungal colony was initially whitish / olive, gradually spreading and hairy growth with dark-brown colour, with little or limited aerial growth. Aged culture appeared completely black with no aerial mycelium.

Conidiophores: The conidiophores were dark-brown, thick-walled, straight to slightly bent, mostly solitary, rarely in groups of 2-3.

Conidia: Conidia of *A. solani* are basically ovoid, but long, narrow and broad forms. Conidia with long beaks are relatively long-ovoid or ellipsoid or muriform, whereas conidia with 2-3 beaks are short. Conidia with one beak reached a size of 109-115 × 18-26 µm plus beak of 80-118 µm. The conidia had 1-5 longitudinal and 5-11 transverse septa.

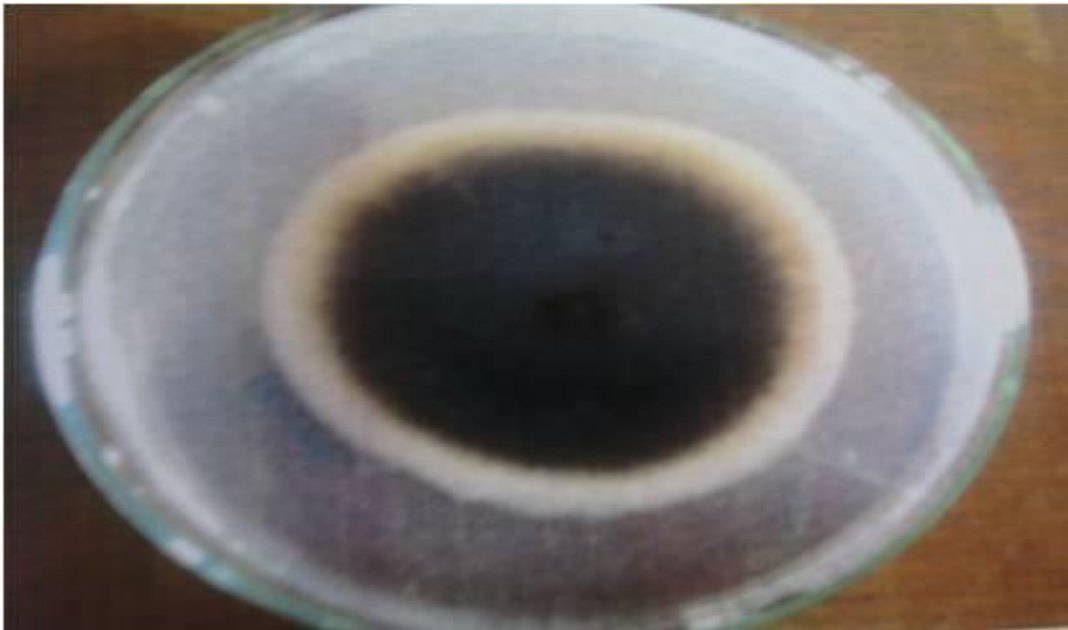


Plate 2 a: Pure culture of *Alternaria solani*

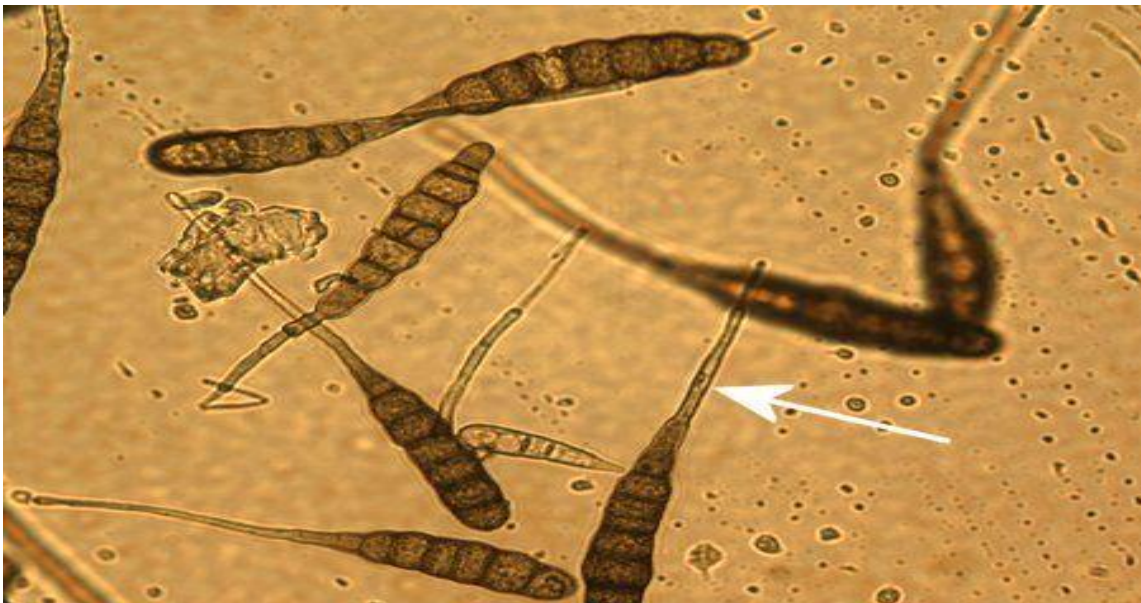


Plate 2 b: Conidia of *Alternaria so*

4.3 Cultural and morphological characters of different isolates of *A. solani* on PDA.

Radial growth of isolates presented in table 4.2 were significantly not different for most of the isolates with highest radial growth was observed in isolates IS-4 with 54.8mm and lowest radial growth was observed in Isolates IS-2 with 50.17 mm.

During the studies of cultural variability amongst isolates of *Alternaria solani* most of the isolates of *Alternaria solani* were not significantly different in radial growth but some of the isolates depicted variation in the growth on PDA.

Mycelial growth character:

Mycelia growth pattern was observed on PDA, where IS-2 and IS-3 grew with circular margin with smooth surface colony and IS-1 and IS-4 isolates were growing with irregular margin and rough surface.

Morphological character:

The single-spore cultures of *A. solani* showed morphological variability in respect of conidia length, width, and number of septa. Average conidial length, which varied from 16.5 to 22.30 μm was highest in IS – 1 and lowest in IS- 4. Average conidial width, which varied from 1.17 to 4.8 μm was highest in IS – 3 and lowest in IS- 4 1.17 μm . The average number of transverse septa, which varied from 4.0 to 7.2 (range: 3–8), was highest in IS- 2 and IS -3 isolates, (7.2 and 6.6) and lowest in IS- 4 (4.0). The average number of longitudinal septa varied from 0.2 to 0.4 (range: 0–2). Finally it was revealed that the smallest size of conidia and lowest number of septa was in isolate -4.

4.4 Symptomatology

The disease symptoms appeared on all the plant parts viz., leaf, stem, petioles, and tubers. First symptoms appeared on older leaves as minute dark brown, usually round necrotic spots of one to two mm. diameter later, the spots enlarged with characteristics concentric rings in the center to produce a target board effect and the colour of the spots changed from brown to dark brown. The adjacent spots eventually coalesced to form large irregular

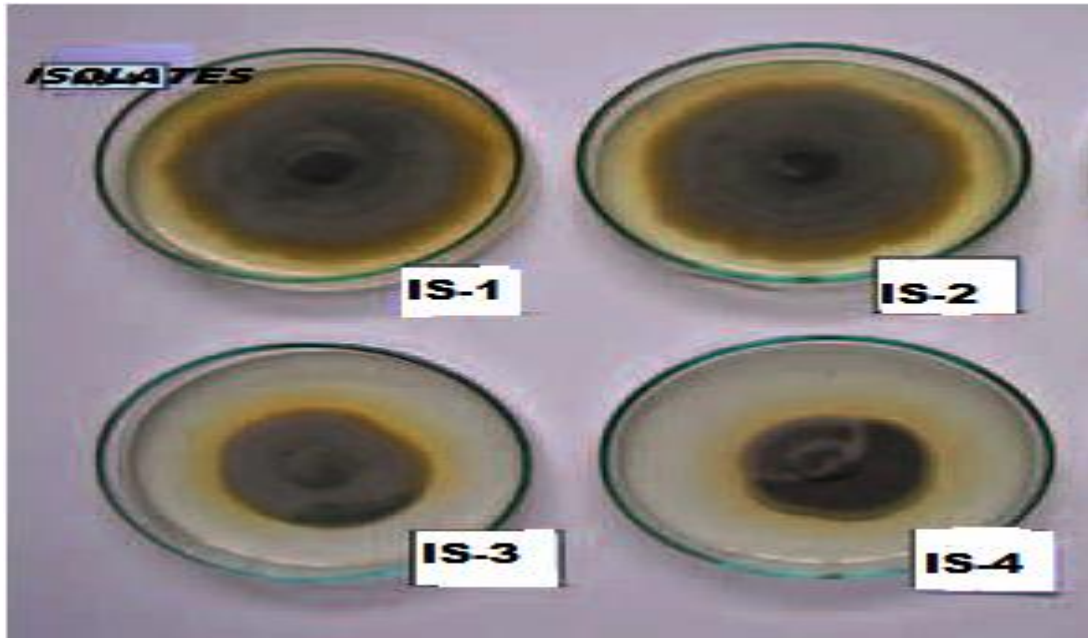


Plate 3: Cultural and morphological characters of different isolates of *A. solani* on PDA

spots leading to drying and defoliation. When plants were 60 -90 days old, symptoms also appeared on stem and petioles as brown to dark brown elongated cankerous target broad spots. These spots enlarged and covered the entire stem and petioles leading to withering of the plants. The symptoms on tuber show lesions which are usually sunken with raised borders, shallow and that is separated from healthy tissue by a purplish-brown metallic-hued cork layer.

4.5 Disease Development of early blight in relation to weather parameters

4.5.1 Progress of disease development (PDI and apparent infection rate)

The effect of weather factors on disease development of early blight was studied on the crop sown in November, the variety Lavkar was sown for this purpose. Observations on incidence of early blight starting from 1st November 2015 to 29th February 2016 were recorded at 15 days interval and presented in table 4.3. Early blight symptoms appeared 25 days after planting as minute brown to black usually round necrotic spots on older leaves.

The data indicated that disease incidence gradually increased from 15th November to 30th January. In the second fortnight of December and first fortnight of January the disease increased at faster rate (13.62% to 23.86%) when the maximum temperature was 23.9 °C and 28.9 °C and minimum temperature was 9.5 °C and 11.6 °C the relative humidity was 74.6 %. In second fortnight of January and February the disease incidence increased at slower rate. It was observed that the disease incidence was maximum during December – January when maximum temperature was 23.9 °C – 28.9 °C, minimum temperature was 9.3°C - 15.3 °C and average relative humidity ranged between 57.33% to 74.6% which was favourable for early blight development.

4.5.2 Correlation of PDI with weather parameters

The PDI recorded at fortnight intervals were correlated with weather parameters prevailed during the crop growth. The correlation coefficients are presented below. The results revealed that during 2015 -16, maximum temperature ($r = -0.025$) exhibited significantly negative correlated with PDI, minimum temperature ($r = -0.388$) was significantly negatively correlation with PDI and mean

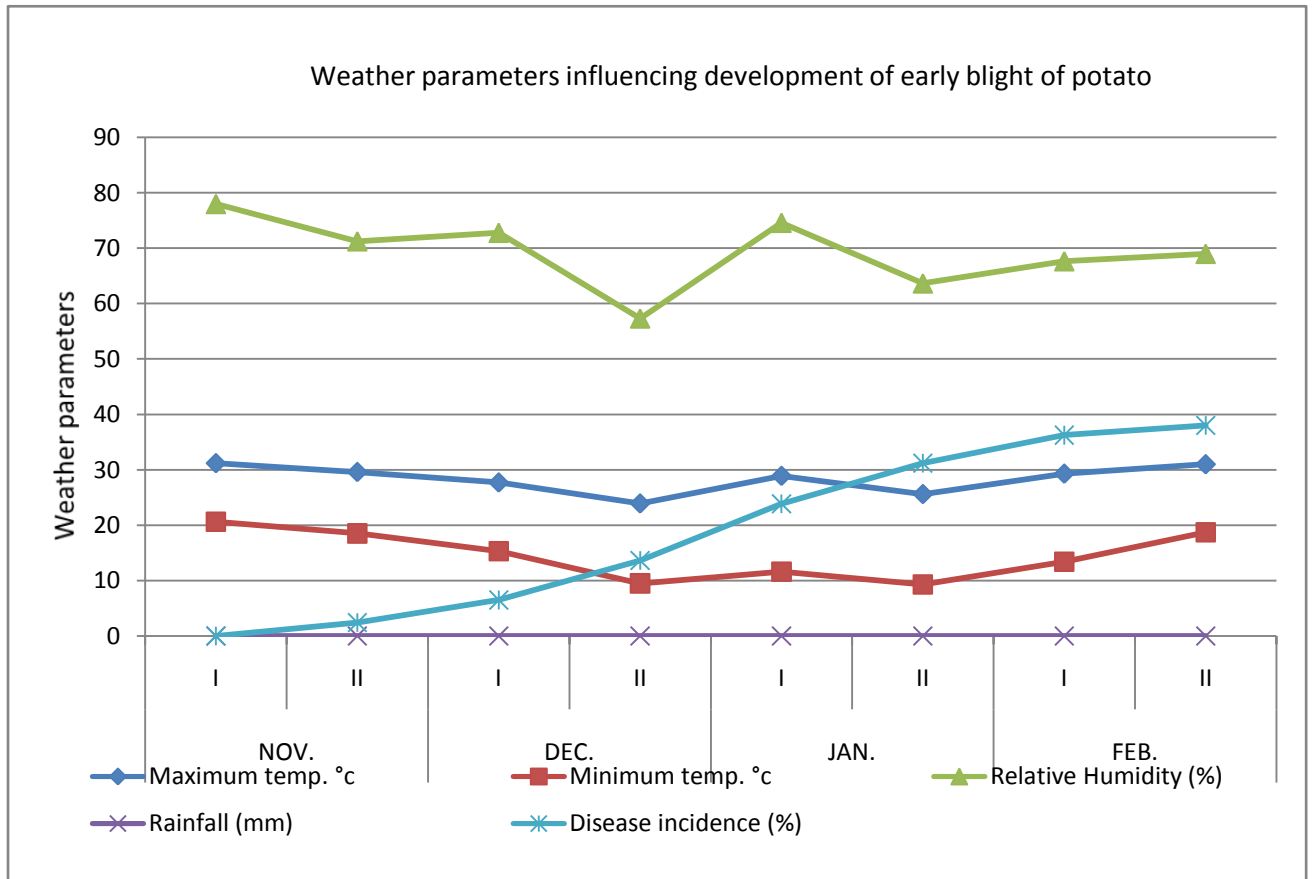
relative humidity ($r = -0.365$) was significantly negatively correlated with PDI. There was no rainfall during the crop season.

Thus lower minimum temperature, lower maximum temperature and lower R.H. favour the development of early blight.

Table: 4.3: Influence of weather factors on development of early blight of potato and Coefficients of correlation (r) between incidences of early blight with environmental factors.

Month	Fortnight	Temperature (°C)		Relative Humidity (%)	Rainfall (mm)	Disease incidence (%)
		Max.	Min.			
NOV.	I	31.2	20.6	78	0	0.00
	II	29.6	18.5	71.26	0	2.40
DEC.	I	27.7	15.3	72.8	0	6.50
	II	23.9	9.5	57.33	0	13.62
JAN.	I	28.9	11.6	74.6	0	23.86
	II	25.6	9.3	63.66	0	31.20
FEB.	I	29.3	13.4	67.66	0	36.25
	II	31	18.7	69	0	38.00
Coefficients of correlation (r)		-0.025	-0.388	-0.362	—	—

Fig 4.2: Influence of weather factors on development of early blight on potato



4.6 Evaluation of fungicides against *Alternaria solani* in vitro and for control of the disease in the field.

4.6.1 Influence of different fungicides on the growth of *A. solani* in vitro.

Five new fungicidal chemicals and their combines molecules *i.e.* Ametoctradin 300 g/l + Dimethomorph 225 g/l SC, Zampro 525 SC, Dimethomorph 50% WP, Cymoxanil 8% +Mancozeb 64% WP and their different concentration were screened for their fungitoxicity *in vitro* by poisoned food technique to assess their efficacy towards *A. solani* on potato dextrose agar amended with their required concentrations and incubated at $25 \pm 1^\circ\text{C}$ and the data on radial growth of mycelium (mm) and inhibition over control (%) are presented in Table 4.4 and fig 4.3.

The fungus grew in its characteristics manner in all the treatments. The data indicates that all the seven treatments were significantly inhibitory in reducing radial growth of *A. solani* over control.

The mean radial growth in different fungicides varied from 2.3 mm to 21.8 mm as against 39 mm in control. The radial growth in all the treatments was significantly less as compared to control. The radial growth was minimum in Ametoctradin 300 g/l + Dimethomorph 225 g/l SC at 0.4% concentration (2.3mm). It was followed by Cymoxanil 8% + Mancozeb 64 % WP (6.4 mm) at 0.3% and Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (10.5mm) at 0.2%. Among the treated culture, maximum radial growth of pathogen was recorded in Zampro 525 SC (21.8mm) at 0.16 % followed by Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (19.1mm) at 0.16% and Zampro 525 SC (18.4mm) at 0.2%. The highest growth of pathogen was recorded in control (39mm).

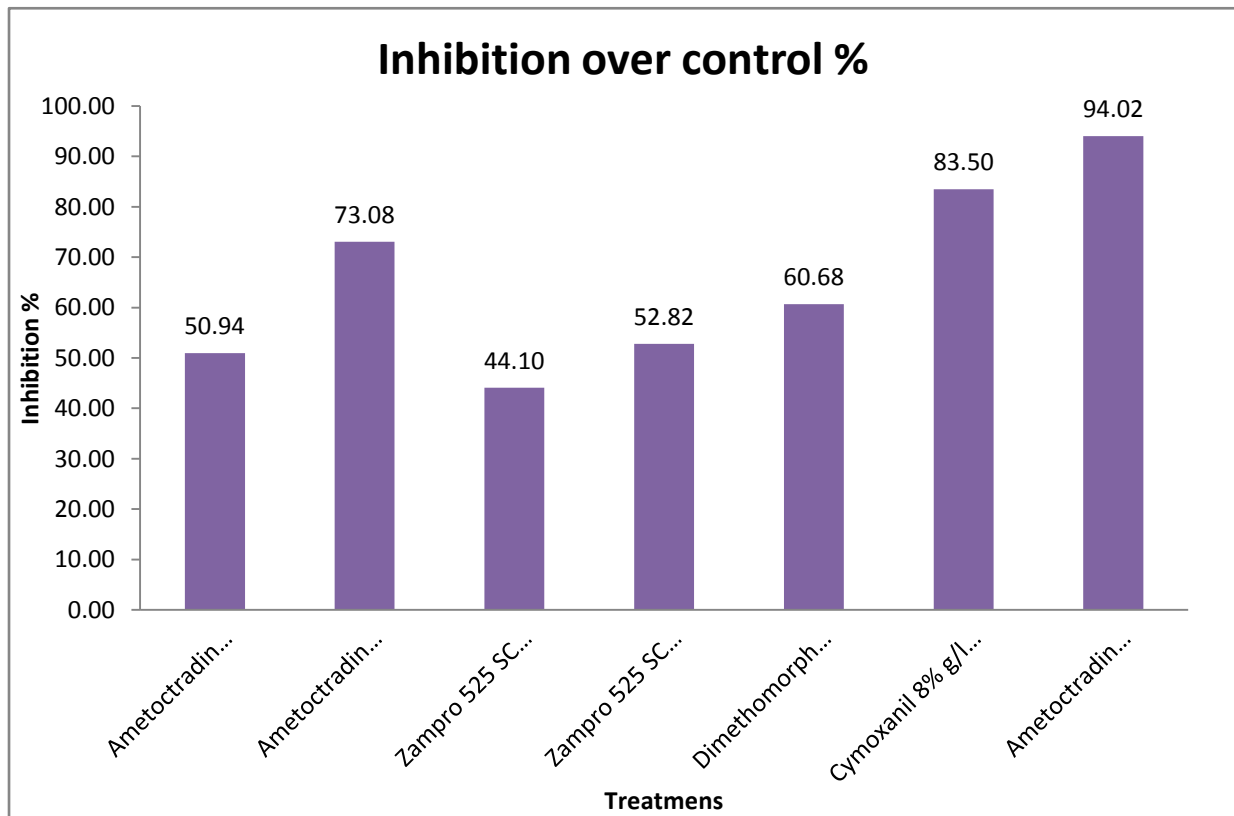
Table 4.4: Effect of different fungicides on radial growth of *A. solani*.

S. No.	Treatments	Diameter of mycelium (mm.)	Inhibition over control %
1	Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (420)	19.1	50.94
2	Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (525)	10.5	73.08
3	Zampro 525 SC (420)	21.8	44.10
4	Zampro 525 SC (525)	18.4	52.82
5	Dimethomorph 50 % WP (500)	15.3	60.68
6	Cymoxanil 8% + Mancozeb 64 % WP (1080)	6.4	83.50
7	Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (1050)	2.3	94.02
8	Untreated Control	39.0	-
SEm ±		0.47	
CD at (P=0.5)		1.01	
CV %		13.03	

4.6.2 Incidence of early blight on potato as influenced by different fungicides in the field.

Two foliar sprays of eight fungicidal treatments along with a check were given at 15 days interval to control of early blight of potato in the field. The data on percent disease incidence were recorded and disease control (%) was computed and has been presented in table 4.5.

Fig 4.3: Inhibition of growth of *A. solani* in vitro as influenced by fungicides.



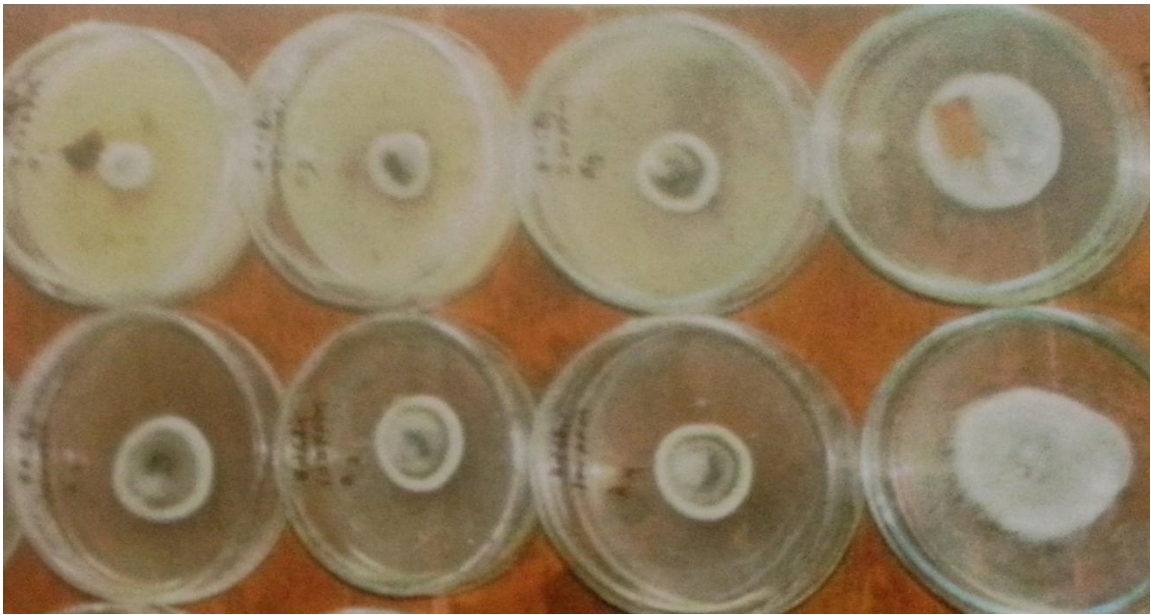


Plate 4: Effect of different fungicide and their concentration on radial growth of *Alternaria solani*

Disease incidence:

The data indicate that all the treatments were significantly effective in reducing the percent disease incidence as compared to check (water spray). The disease incidence (15 days after second spray) in all the treatments ranged from 15.80 to 24 percent irrespective of the concentration. Ametoctradine 300g/l + Dimethomorph 225 g/l SC (1050) when used as foliar spray showed significantly minimum percent disease incidence (15.80 %) which was at par with Cymoxanil 8% + Mancozeb 64 % WP (1080) (17.67 %) and Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (525) (18.83 %) which were at par. Whereas Zampro 525 SC (420) was least effective treatment observed maximum incidence (24 %). The highest percent incidence (38 %) was recorded in control (water spray).

In percent disease control Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (1050) showed maximum percent disease control (58.42 %) followed by Cymoxanil 8% + Mancozeb 64 % WP (1080) (53.50 %) and Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (525) (50.44%). Whereas, Zampro 525 SC (420) was showed least percent disease control (36.84 %) among all the treatments.

Table 4.5: Incidence of early blight on potato under the influence of different fungicides in the field.

S. No.	Treatments	Disease incidence (%)		Disease control (%)
		Before I spray	After II Spray	
1	Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (420)	11.00 (19.58)	23.25 (27.80)	38.81
2	Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (525)	11.75 (20.69)	18.83 (26.82)	50.44
3	Zampro 525 SC (420)	10.50 (19.98)	24.00 (30.14)	36.84
4	Zampro 525 SC (525)	12.25 (19.93)	22.75 (27.70)	40.13
5	Dimethomorph 50 % WP (500)	13.00 (20.58)	22.08 (25.48)	41.89
6	Cymoxanil 8% + Mancozeb 64 % WP (1080)	12.25 (20.08)	17.67 (31.12)	53.50

7	Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (1050)	11.20 (20.02)	15.80 (28.81)	58.42
8	Untreated Control	12.80 (20.11)	38.00 (28.72)	
S. Em ±		0.512	2.89	
CD at (P=0.5)		NS	3.14	
CV %		4.409	17.69	

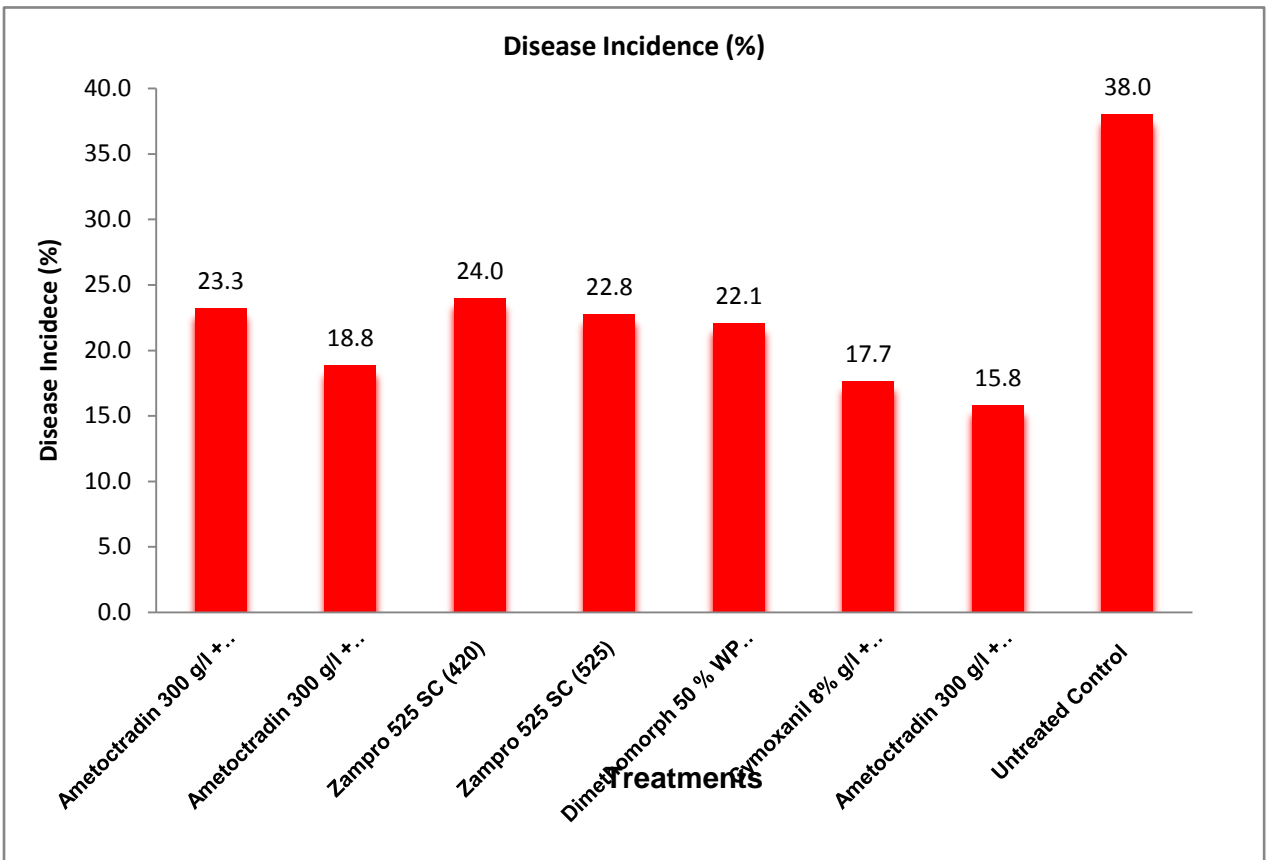
- Figures in parentheses indicate angular transformed values

F value (2 ,7)= 3.25

4.6.3 Yield of potato as influenced by different fungicidal applications.

The data on the effect of treatments on yield of potato indicated that all the treatments were significantly effective to increase the yield as compared to check (water spray). The highest yield (203.5 q/ha) was recorded by Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (1050) followed by Cymoxanil 8% + Mancozeb 64 % WP (1080) (195.7 q/ha) which were at par. Zampro 525 SC (420) recorded the least potato yield (173.3 q/ha) among the

Fig 4.4: Incidence of early blight on potato under the influence of different fungicides in the field.



treatments. Significantly minimum potato yield was recorded in check plot (158.9 q/ha).

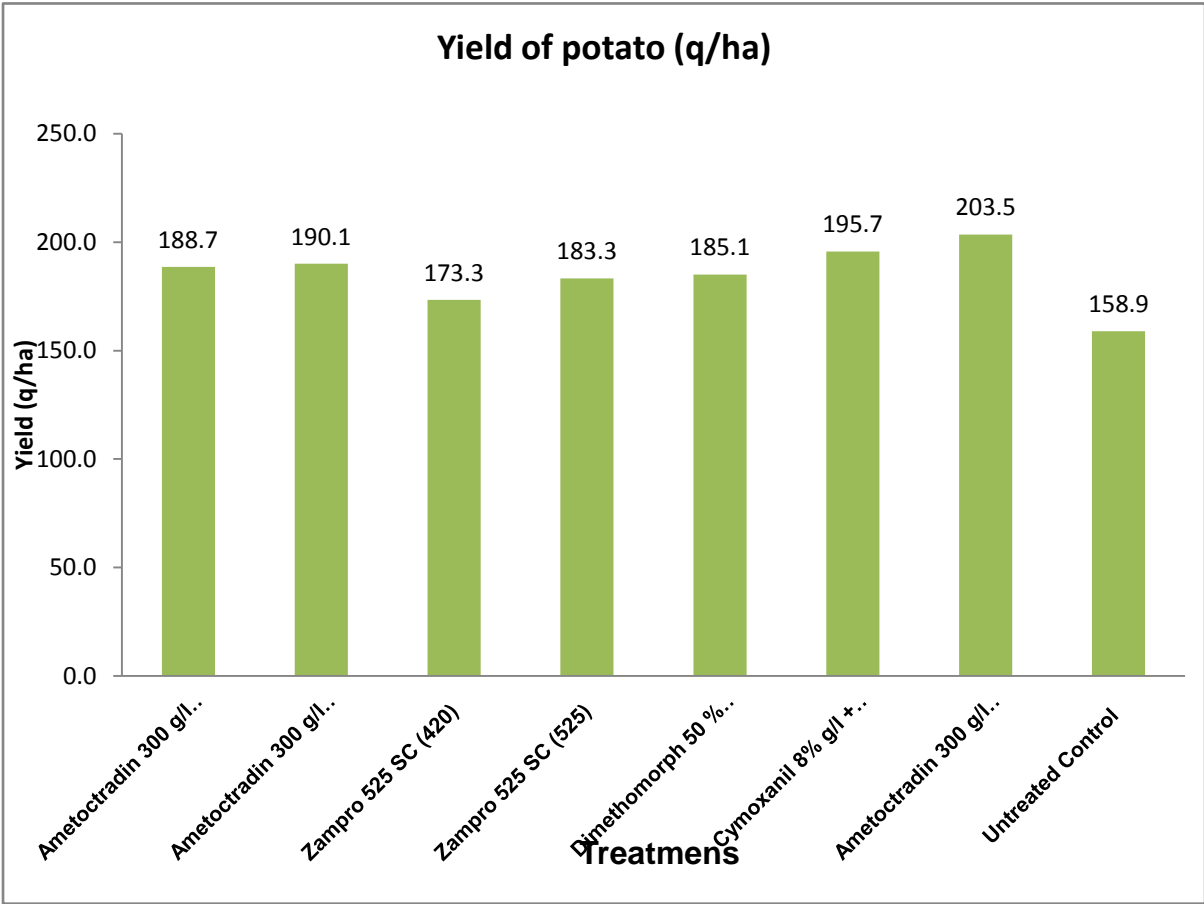
In all the treatments potato yield Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (1050) showed maximum increase in yield (28.06 %) followed by Cymoxanil 8% + Mancozeb 64 % WP (1080) (23.15%) and Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (525) (19.63%) whereas, Zampro 525 SC (420) was showed minimum increase in yield (9.06%) over the control.

The data revealed that in experiments there was highest yield recorded in Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (1050) followed by Cymoxanil 8% + Mancozeb 64 % WP (1080) and Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (525).

Table 4.6: Influence of different fungicides applied to control early blight on yield.

S. No.	Treatments	Yield (q ha⁻¹)	Increase in yield over control %
1	Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (420)	188.7	18.75
2	Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (525)	190.1	19.63
3	Zampro 525 SC (420)	173.3	9.06
4	Zampro 525 SC (525)	183.3	15.35
5	Dimethomorph 50 % WP (500)	185.1	16.48
6	Cymoxanil 8% + Mancozeb 64 % WP (1080)	195.7	23.15
7	Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (1050)	203.5	28.06
8	Untreated Control	158.9	
S. Em±		5.03	
CD at (P=0.5)		10.81	
CV %		13.03	

Fig.4.5: Yield q ha⁻¹ of potato as influenced by different fungicides applied to control the disease in the field.



4.6.4 Apparent infection rate (r/unit/day) of early blight developed as influenced by different fungicides.

The rate of disease development r/unit/day as evident from Table 4.7 ranged between 0.0049 – 0.0144 before and after first spray than in 0.0049 – 0.0363 in before and after second spray. The average rate of disease development was in between 0.0069 – 0.0254. Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (1050) observed average rate of disease development was minimum 0.0069 unit/day it was followed by Cymoxanil 8% + Mancozeb 64 % WP (1080) (0.0078/unit/day) and Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (525) (0.0088 /unit/day). Whereas average rate of disease development/ unit/ day was maximum in control plot(0.0254/unit/day).

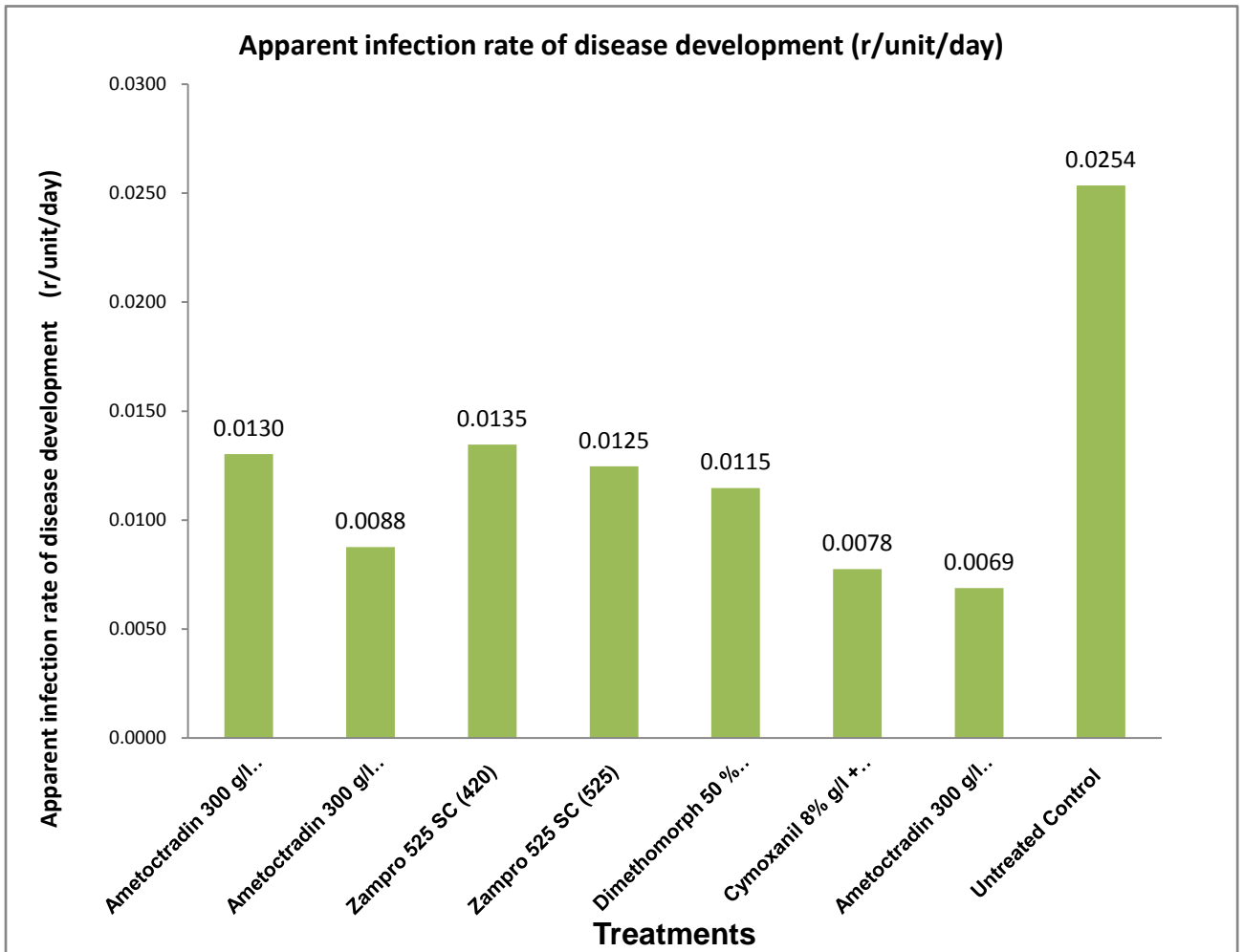
The data revealed that in experiment there was minimum rate of disease development (r/unit/day) recorded in Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (1050) followed by Cymoxanil 8% g/l + Mancozeb 64 % WP (1080) and Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (525).

Table 4.7 Apparent infection rate of development of early blight on potato (r /unit/day) under the influence of different fungicides.

S. No.	Treatments	Rate of disease development (r /unit/day)		
		Before & after I st spray	Before & after II nd spray	Average
1	Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (420)	0.0103	0.0157	0.0130
2	Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (525)	0.0066	0.0109	0.0088
3	Zampro 525 SC (420)	0.0107	0.0162	0.0135
4	Zampro 525 SC (525)	0.0110	0.0140	0.0125
5	Dimethomorph 50 % WP (500)	0.0133	0.0097	0.0115
6	Cymoxanil 8% + Mancozeb 64 % WP (1080)	0.0049	0.0106	0.0078
7	Ametoctradin 300 g/l + Dimethomorph 225 g/l SC	0.0089	0.0049	0.0069

	(1050)			
8	Untreated Control	0.0144	0.0363	0.0254
	Range	0.0049-0.0144	0.0049-0.0363	

Fig. 4.6: Apparent infection rate of development of early blight on potato (r/unit/day) as influenced by different fungicidal applications.



4.6.5 The cost benefit ratio of different fungicidal treatments

The highest yield of potato i.e.203.5 q/ha was obtained in the plots treated with Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (1050) whereas the lowest yield i.e. 158.9 q/ha was recorded in the control plots. Total cost of cultivation of potato was highest i.e. Rs. 52300/ha. In the plots treated with Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (1050) whereas the cost of other cultural practices remained the same for all other treatments.

Table 4.8 Computation of B:C ratio for application of fungicides to manage the disease

Treatments	Yield (q/ha)	Gross income (Rs.)	Total cost(Rs.)	Net return (Rs./ha)	Cost benefit ratio
Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (420)	188.7	150960	51950	99010	1.91
Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (525)	190.1	152080	52035	100045	1.92
Zampro 525 SC (420)	173.3	138640	51800	86840	1.68
Zampro 525 SC (525)	183.3	146640	51885	94755	1.83
Dimethomorph 50 % WP (500)	185.1	148080	51705	96375	1.86
Cymoxanil 8% g/l + Mancozeb 64 % WP (1080)	195.7	156560	52150	104410	2.00
Ametoctradin 300 + Dimethomorph 225 g/l SC (1050)	203.5	162800	52300	110500	2.11
Untreated Control	158.9	127120	48950	78170	1.60

The different as in cost of cultivation with different treatments were due to the different in cost price of fungicides. The gross income of potato was highest as Rs.110500 with the treatments of Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (1050) and the lowest gross income was Rs. 78170 per ha in control plot. The cost benefit ratio of potato was maximum 2.11 with the treatment of Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (1050) and lowest was 1.60 in control plot.

Chapter – V

DISCUSSION

Potato (*Solanum tuberosum* L.) is one of the most popular and widely grown vegetables in the world by virtue of being highly remunerative to the farmers and the processing industry. The crop suffers from many diseases and environmental disorders which cause reduction in yield and quality of the produce. Among the foliar diseases, early blight caused by *Alternaria solani* is quite destructive in the field condition. It causes defoliation of plant resulting in drastic reduction in tuber yield in the field, while tuber infection in the field and after harvest, reduced the quality and in turn the market value. Therefore, the present investigation was carried out on the aspects viz, survey for disease incidence in vicinity of Indore, symptoms and characterization of isolates of *A. solani*, development of the disease in relation to weather parameters, evaluation of fungicides for their efficiency against the pathogen *in vitro* and for management of the disease in the field. The results obtained on these aspects have been discussed herein,

5.1 Survey to assess the incidence of the disease

A survey was undertaken during Rabi 2015-16 to assess the distribution, incidence and spread of early blight on potato at different locations in the vicinity of Indore. Early blight on potato appeared in almost all the villages and its incidence ranged from 35.50 to 42.25 percent (avg. 39.38 %) in different villages surveyed. The highest disease incidence (42.25) was observed in field of Dudhia, followed by Palda (41.50), Joshiguradia (41.30), Pipliahana (40.23) and Research field, College of Agriculture, Indore. Whereas minimum percent disease incidence was observed in Bicholi (35.50%) followed by Bijalpur (36.46%). Similar findings have been reported by many workers like Vander walls *et al.* (2003), Hossain *et al.* (2010), Ganie *et al.* (2013 b) recorded 24.54 to 28.23 % and 13.84 to 15.98 % incidence of early blight on potato during survey while Ranie *et al.* (2015) recorded the disease incidence varied from 21.66 to 34.13 % and 10.48 to 18.56 % during 2011 and 2012. These findings support the present observation and indicate the moderate to high incidence of disease in several potato areas.

5.2 Symptomatology

The symptoms on the potato plants in the field were first noticed on older leaves as minute brown to black necrotic spots measuring 1–2 mm in diameter. These spots enlarged with characteristic concentric rings in the center to produce a target broad effect and the colour of the spots changed from brown to dark brown. Identical description of symptoms has been made by Datar and Mayee (1981), Vander walls *et al.* (2003). Ganie *et al.* (2013 b) recorded small irregular to circular dark brown spots on lower leaves, measuring 0.5 mm in size In early stages of disease development.

5.3 Characterization of the pathogen

The pathogen was identified on the basis of morphological characters such as shape, size, septation of conidiophores and conidia in the culture medium. The *Alternaria sp.* obtained on culture media showed conidiophores, formed singly or in groups. The conidiophores were straight or flexuous and brown to olivaceous brown. The conidia were solitary straight or slightly flexuous, oblong or ellipsoidal tapering to a beak, pale or olivaceous brown, smooth, 150-300 μm . in length 13-20 μm . thick in the broadest part with 8-10 transverse septa and none or few (1- 4) longitudinal septa. The beaks were flexuous, pale and sometime branched. The description of the fungus agreed with the description given for *A. solani* (Fries) Keisser, by Common Wealth Mycological Institute, Kew, Surrey, England (Ellis, 1971). In the present study also, *A. solani* was consistently isolated from the diseased potato leaves is in conformity with the original description.

5.4 Cultural and morphological characters of different isolates of *A. solani* on PDA.

Growth of isolates did not differ significantly but maximum radial growth was observed in isolates IS-4 (54.8mm) and lowest radial growth was observed in Isolates IS-2 (50.17 mm). Mycelia of IS-2 and IS-3 grew with circular margin with smooth surface colony and IS-1 and IS-4 isolates were seen with irregular margin and rough surface. The single-spore cultures of *A. solani* showed morphological variability in respect of conidia width, and number of septa. Average conidial width, which varied from 1.17 to 4.8 μm was highest in IS – 3 and lowest in IS- 4 1.17 μm .

The average number of transverse septa, which varied from 4.0 to 7.2 (range: 3–8), was highest in IS- 2 and IS -3 isolates, (7.2 and 6.6) and lowest in IS- 4 (4.0). The average number of longitudinal septa varied from 0.2 to 0.4 (range: 0–2). Finally it was revealed that the smallest size of conidia and lowest number of septa was in isolate IS- 4. Nikam *et al.* (2015) conducted studies for pathogenic, cultural, morphological and molecular variability of eight isolates could grow better on the basic PDA medium and exhibited a wide range of variability in respect of their mycelial and conidial dimensions and septation. Singh *et al.* (2014) found that radial growth was not significantly different for most of the isolates. Isolates of *Alternaria solani* depicted high variability for pigmentation on PDA medium.

5.5 Correlation of disease development with different weather parameters

The effect of weather factors on disease development was studied on the crop sown in November on variety Lavkar. Statistically significant correlations were observed between weather factors *viz.* minimum, maximum temperature (°C), relative humidity (%) and rainfall (mm) with disease incidence. In the second fortnight of December and first fortnight of January the disease increased quite fast (13.62% to 23.86%), when the maximum temperature was 23.9 - 28.9 °C and minimum temperature was 9.5 - 11.6 °C and relative humidity was 74.6%. The result revealed that maximum temperature ($r = -0.025$) was negatively correlated with PDI, minimum temperature ($r = -0.388$) was negatively correlated with PDI and mean relative humidity ($r = -0.365$) was also negatively correlated with PDI. The result obtained is in conformity with the result obtained by earlier workers. Bonde, (1929), Verma (1970) and Gemawat and Ghosh (1980), who observed *Alternaria solani* develops in the range of 5-35°C. Mehboob *et al.* (2013) also recorded maximum disease severity at 17 to 20 °C maximum temperature and 6 to 9 °C of minimum temperature.

5.6 Efficiency of fungicides against the pathogen *in vitro* and in controlling the disease in the field.

5.6.1 Influence of different fungicides on the growth of *A. solani in vitro*.

The use of fungicides has become an inevitable in the management of diseases particularly in potato in the absence of resistant cultivars to early blight. Five new fungicidal chemicals and their combine molecules *i.e.* Ametoctradin 300 g/l + Dimethomorph 225 g/l SC, Zampro 525 SC, Dimethomorph 50% WP, Cymoxanil

8% +Mancozeb 64% WP at different concentrations were screened for their fungitoxicity *in vitro* by poisoned food technique. The result revealed that mean radial growth recorded was minimum in Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (2.3mm) followed by Cymoxanil 8% + Mancozeb 64 % WP (6.4 mm) and Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (10.5mm). Ganie et al. (2013 a) recorded that non-systemic fungitoxicants mancozeb 75 WP, irrespective of concentration was most effective *in vitro* against *A. solani* followed by propineb 70 WP. Hossain and Hossain (2009) recorded efficacy of seven fungicides Dencozeb 80 WP (Mencozeb), Evamil 72 WP (Mencozeb 64% + Metalaxil 8%), Mosum 80 WP (Mancozeb), Companion 72 WP (Mancozeb 60% + Crbendazim 12%), Mancovit 70 WP (Mancozeb), Baimyl 72 WP (Mancozeb 64 % + Matalaxyl 8%) and Pesterozeb 50 WP (Mancozeb) at 250 ppm significantly inhibited the mycelia growth of *A. solani* over control.

5.6.2 Effectiveness of different fungicides on control of early blight in the field.

Two foliar sprays of eight treatments along with a check *viz.* Ametoctradin 300 g/l + Dimethomorph 225 g/l SC, Zampro 525 SC, Dimethomorph 50% WP, Cymoxanil 8% +Mancozeb 64% WP were evaluated for their efficiency in reducing the incidence of early blight. The data on yield are also recorded. Ametocradine 300g/l + Dimethomorph 225 g/l SC (1050) when used as foliar spray showed significantly minimum percent disease incidence (15.80 %), maximum disease control (58.42 %) and maximum increase in potato yield (28.06 %) followed by Cymoxanil 8% + Mancozeb 64 % WP (1080) (17.67 % disease incidence and 23.15 % increase in potato yield) and Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (525) (18.83 % disease incidence and 19.63 % increase in potato yield).

For computing the rate of disease development (r/unit/day) the average rate of disease development was minimum in spray of Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (1050) 0.0069 unit/day it was followed by Cymoxanil 8% + Mancozeb 64 % WP (1080) (0.0078/unit/day) and Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (525) (0.0088 /unit/day). Sinha and Prasad (1991) reported best control of disease by Dithane M-45 at 0.2% and also gave the highest yields. Brammatta (1993) observed that Chlorothalonil (0.2%) decreased early blight severity. Horsfield *et al.* (2010) found Boscalid, Azoxystrobin and Difenoconazole to be highly effective in the control of early blight when applied up to three days before

or three days after inoculation. Ganeshan and Chethna (2009) evaluated Pyroclostrobin 25% EC at 50, 75 and 100 gai /ha. against early blight all concentration significantly effective in reducing the disease and increasing the yield. All the earlier findings thus are in conformity with the present observations.

CHAPTER –VI

SUMMARY, CONCLUSION AND SUGGESTIONS FOR FURTHER WORK

6.1 Summary:

Investigations on the early blight of potato caused by *A. solani* were carried involving survey for disease incidence in different villages in vicinity of Indore, early blight development in relation to weather parameters, cultural and morphological variability of isolates *in vitro*, evaluation of fungicides against the pathogen *in vitro* and for management of the disease in the field. The results obtained on the above aspects have been summarized here under.

1. Early blight of potato appeared in all the villages surveyed during 2015-16 and the disease incidence ranged from 35.50 to 42.25 percent (Avg. 39.48%) recorded. The highest disease incidence (42.25) was recorded in field of Dudhia, followed by Palda (41.50), Joshiguradia (41.30), Pipliahana (40.23) and Research field of College of Agriculture, Indore (38%). Whereas minimum percent disease incidence was in Bicholi (35.50%) followed by Bijalpur (36.46%).

2. The colony of *A. solani* was initially whitish / olive, gradually spreading and hairy growth with dark-brown colour, with little or limited aerial growth. Aged culture appeared completely black with no aerial mycelium. The conidiophores were dark-brown, thick-walled, straight to slightly bent, mostly solitary, rarely in groups of 2-3. Conidia of *A. solani* are basically ovoid, but long, narrow and broad forms. Conidia with long beaks are relatively long-ovoid or ellipsoid or muriform, whereas conidia with 2-3 beaks are short. Conidia with one beak reached a size of 109-115 × 18-26 µm plus beak of 80-118 µm. The conidia bear 1-5 longitudinal and 5-11 transverse septa.

3. Isolates of *Alternaria solani* showed variability on PDA. Mycelia growth pattern were observed on PDA, wherein IS-2 and IS-3 grew with circular margin with smooth surface colony and IS-1 and IS-4 isolates were growing with irregular margin and rough surface. The single-spore cultures of *A. solani* showed morphological variability in respect of conidia width, and number of septa.

4. First symptoms appeared on older leaves as minute dark brown, usually round necrotic spots of one to two mm. diameters later, the spots enlarged with characteristics concentric rings in the center to produce a target board effect and the colour of the spots changed from brown to dark brown. The adjacent spot eventually coalesced to form large irregular spots leading to drying and defoliation. The symptoms on tuber showed lesions which are usually sunken with raised borders, shallow and that is separated from healthy tissue by a purplish-brown metallic-hued cork layer.

5. Effect of weather factors on disease development on variety Lavkar revealed that the incidence increased from 1st November 2015 to 29th February 2016. In the second fortnight of December and first fortnight of January the disease increased at faster rate (13.62% to 23.86%) when the maximum temperature was 23.9 °C and 28.9 °C and minimum temperature was 9.5 °C and 11.6 °C the relative humidity was 74.6 % was favourable for early blight development.

6. Maximum temperature ($r = -0.025$) exhibited negative correlation with PDI, and also the minimum temperature ($r = -0.388$) was negatively correlated with PDI and relative humidity ($r = -0.365$) were negatively correlated with PDI.

7. Among five new fungicidal chemicals combinations the mean radial growth was minimum in Ametoctradin 300 g/l + Dimethomorph 225 g/l SC at 0.4% concentration (2.3mm). It was followed by Cymoxanil 8% + Mancozeb 64 % WP (6.4 mm) at 0.3% and Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (10.5mm) at 0.2%.

8. Ametocradine 300g/l + Dimethomorph 225 g/l SC (1050) when used as foliar spray resulted in minimum percent disease incidence (15.80 %), maximum disease control (58.42 %) and maximum increase in potato yield (28.06 %) followed by Cymoxanil 8% + Mancozeb 64 % WP (1080) (17.67 % disease incidence and 23.15 % increase in potato yield) and Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (525) (18.83 % disease incidence and 19.63 % increase in potato yield).

9. computation of the rate of disease development (r/unit/day) in sprayed and unsprayed plot revealed that the average rate of disease development was minimum in spray of Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (1050) 0.0069 unit/day it was followed by Cymoxanil 8% + Mancozeb 64 % WP (1080) (0.0078/unit/day) and Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (525) (0.0088 /unit/day).

6.2 Conclusion:

1. The disease was prevalent in all potato growing areas in villages in the vicinity of Indore.
2. Isolates of *A. solani* showed cultural and morphological variability in respect of mean radial growth and conidia length, width, and number of septa.
3. The disease development was favoured by lower minimum temperature, lower R.H. and lower maximum temperature between 23.9 °C and 28.9 °C.
4. Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (1050 g a i) was found as the most effective against *Alternaria solani in vitro* and in the field.

6.3 Suggestions for Further work:

1. Further work on epidemiology of *Alternaria solani* in relation to different environment factors be taken up to develop a system for forecast.
2. Work on the variability in pathogen may be taken up.
3. Work on evaluation of fungicides for the control of early blight may be continued possibility of their integration need to be studied for management.

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APPENDIES

ANOVA for table 4.2

S.V.	DF	SS	MSS	F. Cal.	F. Tab.	SIG/NS	S.Em.	CD5 %
Treatment	3	38.86	4.32	5.20	4.07	SIG	0.53	2.10
Error	8	6.64	0.83					
Total	11	45.50	5.15					

ANOVA for table 4.3

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	F prob.
Regression	4	1379.861	344.9653	6.848146	0.072938
Residual	4	268.6588	67.1647		
Total	8	1648.52			

ANOVA for table 4.4

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Treatment	7	2652.51	378.93	1142.50	2.7
Error	16	5.31	0.33		
Total	23	2,657.81			

ANOVA for table 4.5 (A)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	F prob.
Replication	2	0.071			
Treatment	7	2.698	0.385	0.489	0.82709
Error	14	11.026	0.788		
Total	23	13.795	Non-Significant		

ANOVA for table 4.5 (B)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	F prob.
Replication	2	0.706	0.353		
Treatment	7	167.454	23.92	0.952	0.89688
Error	14	351.684	25.12		
Total	23	419.843	Significant		

ANOVA for table 4.6

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Replication	2	36.35	18.17	0.42	
Treatment	7	3949.78	564.25	13.02**	2.7
Error	14	606.53	43.32		
Total	23	4,556.31	** Significant		

ANOVA for table 4.8

	fungicides	Rent of land (Rs.)	Land preparation (Rs.)	Seed (Rs.)	Sowing (Rs.)	Fertilizer (Rs.)	Weeding (Rs.)	Harvesting (Rs.)	Earthing (Rs.)	Transport charge	Chemical (Rs.)+labour for 2 spray
T1	Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (420)	1500	3250	27000	3200	3500	2000	4000	1000	3500	3000
T2	Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (525)	1500	3250	27000	3200	3500	2000	4000	1000	3500	3085
T3	Zampro 525 SC (420)	1500	3250	27000	3200	3500	2000	4000	1000	3500	2850
T4	Zampro 525 SC (525)	1500	3250	27000	3200	3500	2000	4000	1000	3500	2935
T5	Dimethomorph 50 % WP (500)	1500	3250	27000	3200	3500	2000	4000	1000	3500	2755
T6	Cymoxanil 8% g/l + Mancozeb 64 % WP (1080)	1500	3250	27000	3200	3500	2000	4000	1000	3500	3200
T7	Ametoctradin 300 g/l + Dimethomorph 225 g/l SC (1050)	1500	3250	27000	3200	3500	2000	4000	1000	3500	3350
T8	Untreated Control	1500	3250	27000	3200	3500	2000	4000	1000	3500	0

VITA

The author of this thesis Mr. Harishankar Dhakad s/o Shri Nathulal Dhakad was born on 02 June 1991 Mandsaur (M.P). He passed high school examination in the year 2006 with **69.8** percent marks from School of Excellence, Mandsaur (M.P.) and higher secondary examination in the year 2009 with **75.4** percent marks from Dashpur Vidhya Mandir H. S. S., Mandsaur (M.P.).

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He has passed all required courses in M.Sc. (Ag.), Plant Pathology by obtaining **7.65** OGPA.

Now he is going to complete his master degree requirement by submission of this thesis.

Harishankar Dhakad