

**INVESTIGATIONS ON EARLY BLIGHT OF POTATO
CAUSED BY *Alternaria solani* (Ellis and Martin) Jones and
Grout**

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INTRODUCTION

Potato (*Solanum tuberosum* L.) is an important food crop of the world. It is used as vegetable, stock feed and in industries for manufacturing starch, alcoholic beverages and other processed products. Potato is the world's fourth-largest food crop, after maize, wheat and rice.

Potato, an important temperate crop, has adapted well for cultivation under sub tropical conditions. The tuber bearing *Solanum* occurs naturally over a wide area in South and Central America ranging in distribution from 30° N to 145° S latitude. The two main centers of diversity of the group are the high lands of Central Mexico and the Andes of Southern Peru, Bolivia and north-western Argentina. The cultivated potato *Solanum tuberosum*, originated around lake Titicaca in Bolivia and in southern Chile.

Potato belongs to genus *Solanum* in the family Solanaceae. The commercially cultivated potato is botanically known as *Solanum tuberosum* L. Potato is an annual herbaceous plant, mainly reproduced vegetatively by means of tubers and sometimes by botanical seeds i.e. True Potato seeds. The tubers are underground stems and from them new shoots are produced. The stem is erect in the early stage and becomes spreading and prostrate later on. The leaves are compound and alternate, irregularly odd pinnate. Buds formed in the axil of the leaves produce rhizomes which elongate rapidly and develop tubers at their extremities.

Potato is a highly nutritious food. It provides carbohydrates, proteins, minerals, Vitamin C, a number of B group vitamins and a high quality dietary fiber. Potatoes yield about 97 kilo calories per 100 g fresh weight, which is much less than cereals. The net protein utilization or biological value of potato protein (about 71% that of whole egg), is better than that of wheat (53%), maize (54%), peas (48%), beans (46%) and is comparable to cow's milk (75%).

Potato is the important vegetable crop of India cultivated in an area 1.86 m ha with production of 42.34 million tons with an average yield of 22.72 t/ha. during 2010-11 (Anon.,2012). India ranks second in terms of production while ranks third as per area under potato cultivation. Out of 10 agro climatic zones, agro climatic zone 4 has got 72000 ha. Uttar Pradesh, Himachal Pradesh, Punjab, West Bengal, Chhattisgarh, Bihar ,Karnataka and Assam are the major potato growing states.

In Karnataka, potato is grown over an area of 556.83 thousand hectares with a production of 7553.50 thousand tonnes. It is grown as rainfed crop in Karnataka. More than 40% of the area is located in Hassan district. It is cultivated to greater extent in Kolar and some districts of northern Karnataka like Dharwad, Belagavi etc.

Potato crop is susceptible to many diseases, some of which are widespread and others are localized. The causal agents of these diseases include fungi, bacteria, viruses, Phytoplasmas, viroids and nematodes. An other group disorders, called non infectious diseases, include those due to unfavourable environment, faulty nutrition or other abiotic factors (Kehr *et al.*, 1964)

The intensive and extensive cultivation under the most favorable environmental conditions for potato crop production in the state failed to provide significant strides in potato yields, because of a number of production constraints. Among all these diseases early blight caused by *Alternaria solani* (Ellis and Martin) Jones and Grout is one of the very important, old and well known diseases of potatoes. It occurs almost everywhere where the potatoes are grown. This disease has been underrated contrast to the more spectacular late blight disease. However, in many areas the average annual loss from this disease exceeds the losses from late blight. Yield loss is upto 6-40%. In India it is first reported in Farukabad (U.P) in 1903. In recent years, increase in *A. solani* disease on potato foliage has been reported in various potato growing areas (Vloutoglou and Kalogerakis, 2000). In India *A. solani* (Ellis and Martin) Jones and Grout on leaves of potato (*Solanum tuberosum* L.) was reported from Farukhabad (U.P) by Butler in 1903 (Butler and Bisby, 1931).

Primary damage by early blight is attributed to premature defoliation of the potato plants, resulting in tuber yield reduction. Yield loss estimates resulting from foliar damage incited by early blight on potato vary by location, cropping pattern. etc.

Early blight is one of the most widely spread than any other diseases of potato in northern Karnataka. So survey of the disease to know the severity of disease is essential. Environmental factors such as temperature, wetness duration and relative humidity affect the development of early blight of potato. So studying the relationship between the weather factors and disease development becomes important. Management of potato early blight completely has always been a challenge for

both the scientists and growers. Using of host resistance is primary pillar in an integrated approach to early blight management. There is need for finding a new and effective fungicides against the early blight.

So keeping in view of above facts and importance of those aspects related to damage caused by early blight disease, the present study was undertaken with the following objectives.

1. Survey for the incidence of early blight in major potato growing areas of northern Karnataka and proving the pathogenicity.
2. Collection of different isolates of *Alternaria solani* and studying the pathogen character.
3. To study the disease development in relation to weather factors.
4. a) *In vitro* and *in vivo* evaluation of fungicides.
b) Screening of potato varieties for early blight incidence

REVIEW OF LITERATURE

Early blight of potato caused by *Alternaria solani* is an important and widely distributed foliar disease wherever the potatoes are grown. So far many works have been done on various aspects like survey for the early blight of potato, studies on different isolates of the pathogen, *in vitro* and *in vivo* evaluation of fungicides and also screening of resistant genotypes against early blight of potato which are relevant to present objectives of investigation. Accordingly, the literature pertaining to the above aspects is presented here.

2.1 Symptomatology

According to Ellis (1971) early blight of potatoes affects all parts the above ground. On the leaves it causes round, oval or irregular, brown or dark brown, often concentrically ridged target spots. Under favorable conditions leaf spot enlarge rapidly and may eventually involve as much as half a leaf.

Singh (1987) reported that the spots were oval to angular in shape measuring up to 0.3-0.4 cm in diameter and usually with chlorotic zone around the spot. Older leaves were affected first and progressed upward finally the leaves dried up and fall off. According to Mayee and Datar (1986) the early blight disease of potato was characterized by the appearance of brown to dark brown colour necrotic spots. Appearance of concentric rings inside the spots produced target board effect.

Infection on the stem and tubers are rare .Tuber lesions are usually sunken with raised borders. They are shallow and distinctly set off from healthy tissue by a purplish-brown metallic-hued cork layer (Miller and Pollard, 1976).

Disease symptoms are characteristic dark brown to black lesions with concentric rings which produce a target spot effect. Symptoms are initially observed on older, senescing leaves. Enlarging lesions are often surrounded by a narrow chlorotic halo due to toxin produced by the pathogen, which move ahead into uninfected epidermal cells. (Vanderwaals *et al.*, 2001)

Ganie *et al.* (2013) reported that in early stages of disease development, small irregular to circular dark brown spots on lower leaves appear, measuring 0.5 mm in size. After 4 weeks 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 bull eye appearance.

2.1.1 Causal organism

Early blight of potato caused by *Alternaria solani* was first recorded in 1882 in New Jersey, USA (Bose and Som, 1986). The mycelium consisted of septate, branched, light brown hypahe, which turned darker with the age. The conidiophores were short, 50 to 90µm and dark coloured. Conidia are 120-296 x 12-20 µm in size, beaked, muriform, dark coloured and borne singly. According to Singh (1987) the conidia contained 5-10 transverse septa and 1-5 longitudinal septa.

The conidiophores arise singly or in small groups, straight or flexuous, septate, rather pale brown or olivaceous brown, upto 110µ long, 6-10µ thick. Conidia usually solitary, straight or slightly flexuous, obclavate or with the body of the conidium oblong or ellipsoidal tapering to a beak which is commonly the same length as or rather longer than the body, pale or mid pale golden or olivaceous brown, smooth, overall length usually 150-300µ, 15-19µ thick in the broadest part, with 9-11 transverse and 0 or a few longitudinal or oblique septa, beak flexuous, pale, sometimes branched, 2.5-5µ thick tapering gradually (Ellis, 1971)

According to Ganie *et al.* (2013) mycelium is branched, septate, dark coloured with tints of olive brown. Conidiophores were septate, short, simple, straight or flexous, dark coloured measuring 50-90 x 9 µm long. Conidia are long beaked, muriform, dark coloured, borne singly, both longitudinal and transverse septa are present in mature conidia. Size of the conidia ranges from 15-19 x 150-300µm.

2.2 Survey for the incidence of early blight in major potato growing areas of northern Karnataka

During August 1999 Bains *et al.* (2000) conducted survey in 104 potato fields from the northern, central and southern potato growing regions of Alberta. They were examined for evidence of brown lesions with concentric rings characteristic of early blight, caused by *Alternaria solani* . Isolates

of *A. solani* recovered from diseased leaves from each site confirmed visual observations. Fields with a few small lesions on isolated plants were categorized as having low, fields with large numbers of lesions on many plants as having moderate, and fields where whole leaves and stems were completely necrotic as having high levels of the disease.

Hossain *et al.* (2010) conducted a survey during October 2006 to June 2008 to observe disease prevalence of vegetable and fruit crops in Chittagong region. Through the survey, 24 diseases with their incidence and severity were recorded. The average higher leaf infection in early blight of potato and fruit infection in soft rot of potato were recorded 37 and 39 per cent, respectively.

Kulkarni (1998) conducted survey on early blight incidence of potato in and around Bangalore during both *kharif* and *rabi* seasons of 1997. He reported that the per cent disease index was ranged from 8.66 to 16.86 in *kharif* and from 17.6 to 36.22 per cent during *rabi*.

An extensive survey conducted by Ganie *et al.* (2013) 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.

Prasad (2002) conducted a field survey on early blight of tomato in northern districts of Karnataka viz. Raichur, Gulbarga and Dharwad during *Kharif* 2001 and recorded a per cent disease index of 28.60 to 65.36.

Abhinandan *et al.* (2004) conducted a survey for early blight of tomato in Punjab, India during 2001 and reported that maximum disease intensity of 49.5 per cent was observed at the Tapa district and minimum disease intensity of 8.2 per cent was observed at the Babakala district.

Roopa (2012) conducted survey for early blight of tomato in northern districts of Karnataka during 2011, the results revealed that, disease severity was found more in Haveri (31.03%) and least in Dharwad (20.16%) districts.

2.2.1 Pathogenicity

Dhiman *et al.* (1980) used suspension containing 20,000 spores/ml distilled water for proving the pathogenicity of early blight of potato caused by *Alternaria solani*. Further, they automated the culture suspension on three leaf stage seedlings at the rate of 30 ml per seedlings for successful inoculation.

Tipreswamy *et al.* (2010) confirmed the pathogenicity of early blight of tomato by spraying 104 conidial suspension of *A. solani* to one month seedlings (30 days), before flowering (60 days) and after flowering (90 days).

Roopa (2012) carried out pathogenicity test by inoculating with spore suspension and homogenized mycelial bits (2×10^4 spores/ml) of *A. solani* on foliage of 30 days old PKM-1 variety of tomato and proved the Koch's postulates.

Ganie *et al.* (2013) proved the pathogenicity test on Kufri Jyothi variety. Spore suspension was prepared from 15 days old culture to get a concentration of 2×10^4 conidia/ml. Observations revealed the initiation of typical symptoms of the disease appeared after 10 days of inoculation on injured detached leaves of potato.

2.3 Collection of different isolates of *A. solani* and studying the pathogen character

2.3.1 Growth phase studies of the pathogen on liquid media.

Sandhya (1996) reported that *A. alternata* attained maximum growth after 16 days of incubation in Czapeck's Dox medium

Kulkarni (1998) reported that *A. solani* attained maximum growth after 9th and 7th days of incubation in Richards and Potato dextrose agar medium respectively.

Arunkumar (2006) recorded maximum growth of *Alternaria solani* after nine days of incubation in potato dextrose broth.

Roopa (2012) recorded maximum dry mycelia weight (249.27 mg) of *Alternaria solani* after 9th day of inoculation in Potato dextrose broth.

2.3.2 Morphological and physiological variability among the isolates of *Alternaria solani*

Samuel and Govindaswamy (1972) demonstrated that good mycelial growth and sporulation of *A. solani* was between pH 4.0 to 8.0 and pH 5.0 was the best for mycelial growth and pH 7.0 for sporulation.

Kaul and Saxena (1988) reported that the maximum growth of five isolates of *A. solani* was at 25°C followed by 20, 15, 10 and 5°C with least growth at 35°C.

Bruce (2006) measured the morphophysiological variables like septation, length, width, shape, colour of conidia and number of beak, colony texture, colour, mycelial growth of the potato and tomato isolates. Conidia of isolates from both potato and tomato were dark brown and long cone shaped. Colony texture was in general cottony and aerial mycelium was reddish, yellow or predominantly, greenish yellow in colour.

Varma *et al.* (2007) analyzed the variability among isolates of *Alternaria solani* the causal agent of early leaf blight of tomato, collected from northern and southern parts of India was determined based on conidial morphology, pathogenicity tests and random amplified polymorphic DNA (RAPD) technique.

Naik *et al.* (2010) studied morphological, physiological, pathogenic and molecular variability among isolates of *A. solani* from tomato. Maximum growth and sporulation was recorded in Sabouraud's agar followed by PDA supplemented with CaCO₃ by ASB₂ isolate. Temperature of 25°C was optimum with ASB₂ and ASG₃ recording higher growth. ASG₃ produced higher growth and sporulation at 100% RH, where as ASB₂ sporulated even at 65% RH. Maximum growth and sporulation was obtained at 12h of alternate light and darkness followed by continuous darkness. The pathogenic reaction indicated ASB₂ as more virulent than others. The PCR analysis indicated maximum similarity (73.78%) in the isolates of northern Karnataka region. The isolates ASB₂ from southern Karnataka shared only 45% similarity indicating distinct polymorphism.

Yadav and Pathak (2011) studied the variability among isolates of *Alternaria solani*, the causal agent of early blight of potato, from four district of Uttar Pradesh. Variability was determined based on cultural, morphological and physiological variability tests. The isolates varied with respect to size of conidia, conidiophore, number of septa, conidial germination pattern, effect of different pH, temperature and media on radial growth variabilities were observed among different isolates of *A. solani*. Isolate S₄ (V) i.e. Varanasi isolate showed maximum variability.

Alhussaen (2012) studied the morphological and physiological characteristics of *A. solani* for identification and variability. The optimum PH levels for *A. solani* growth under *in vitro* were 6-7 and the optimum growing temperature of the isolates recovery in this study was 25°C and 30°C. The mycelial width between 0.8-1.5 µm and the conidia were 35-75 µm in length and 10-20 µm in width and 2-7 transverse septa and 1-4 longitudinal septa.

2.3.3 Cultural variability of isolates on different media.

Martinez and Solano (1995) reported that variability in four isolates of *A. solani* causing early blight of tomato with respect to morphological characters like colony growth, colony diameter, mycelial colour, colony texture, pigmentation and conidia size on medium, differed between isolates.

Kaul and Saxena (1988) noted the cultural variability of *A. solani* isolates on PDA and classified in to 4 distinct cultural groups based on types of growth, colony colour, colour of the substrate and growth rate. *A. solani* culture on PDA medium produced hyphae, which were grey white or grey brown in colour and even yellow pigment was secreted by some isolates.

Prasad (2002) reported that out of four non-synthetic media and three synthetic media tested for the growth of *A. solani* after nine days, PDA supplemented with CaCO₃ and Sabouraud's agar were found to be the best media.

Arunkumar (2006) observed the morphological variability in colour of colony, substrate colour, margin of colony, topography of colony, colony growth, sporulation among six isolates of *A. solani* from tomato. Two isolates namely AS 1 (Arabhavi) and AS 3 (Amminabavi) produced good sporulation in culture media.

Kumar *et al.* (2008) collected eleven isolates of *A. solani* on tomato designated as So, Dh, Sh, Va-5, Ka, Ma, Hy, Ba-1, My, Va-3 and Mi 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 isolates of *A. solani* on Potato dextrose agar medium. The fastest radial growth was recorded in the So isolate and slowest in the Ka isolate on PDA, while isolates Dh, Ba-1 and Va-3 were recorded to be faster in growth on ASM, V-8 juice agar and V-8 juice agar (synthetic) medium. The thickness of conidiogenous hyphae was maximum in the Va-5 and Ma isolates. Most of the isolates showed smooth mycelial growth with circular and irregular margin and without concentric zonation. Sporulation was not found in any of the isolates on four different nutrient media, whereas conidiogenous hyphal length was observed in V-8 juice agar medium only. Based on the pathogenicity, isolates were grouped into less virulent, avirulent and virulent

Somappa *et al.* (2013) tested different solid and liquid media under *in vitro* conditions to find out a suitable media for the mycelial growth and sporulation of *A. solani* causing early blight of tomato. Among the four different solid media under study maximum radial growth was recorded on Czapeck's agar medium (50mm) followed by Potato dextrose agar (37mm) and Martins medium (7.3mm). PDA was found best with sporulation (13.20×10^6 spores/ml), followed by Czapeck's agar medium (11.10×10^6 spores/ml). Among the liquid medium maximum growth was recorded on Czapeck's broth medium (76.77mg) followed by Potato dextrose broth medium (34.1 mg). PDB medium was best for sporulation (16.90×10^6 spores/ml) followed by Czapeck's broth medium (14.83×10^6 spores/ml).

2.4 To study the disease development in relation to weather factors

Chattopadhyay and De (1982) studied for two consecutive cropping seasons on prevalence of air borne conidia of *Alternaria solani* in relation to weather conditions. Counts of conidia trapped on slides on aeroscopes exposed for 12 hours were made twice daily once at 5 p.m. and another at 7 a.m. Daily records of maximum and minimum temperature, maximum and minimum relative humidity, wind velocity, dew deposition and sunshine hours were kept. Highly significant positive correlation was found between maximum and minimum temperature, maximum relative humidity, wind velocity and dew deposition. Partial regression showed maximum temperature to play the major role and wind velocity to some extent.

Kulkarni (1998) reported that intermittent rainfall was found to be more favourable for early blight development in potato. While, maximum and minimum temperatures were negatively correlated and RH-1 and RH-2 were positively correlated with PDI.

Kaspa (2004) reported that, under Polish climatic conditions early blight occurred at different level of incidence on over 90.6 per cent of surveyed fields. Time of disease appearance in different regions was closely related to climatic conditions. On average, early blight appeared on potato plants 59.3 days after planting, earlier than late blight. It seems that rainfall mostly affected the occurrence of early blight of potato.

Vanderwaals *et al.* (2003) monitored trends in weather variables and concentrations of airborne conidia of *Alternaria solani* in a potato field in South Africa during three potato-growing seasons in 2001 and 2002. Distinct seasonal variation was noted, with a drop in spore numbers during winter. Peaks in spore concentration coincided with periods favorable for spore formation and dispersal. Most notable was the effect of interrupted wetting periods. Diurnal periodicity of spore dispersal was also observed, with the peak of spore concentrations between 9 hours and 18 hours. Few spores were sampled at night, when wind velocity and temperature are lowest and relative humidity is highest. Increased numbers of spores were sampled during days of harvesting or when other ground-operated farm equipment was used.

Treikale *et al.* (2008) assessed the development of *A. solani* with 10 days interval in field trials on the variety "cultivated for industrial purposes. The intensity of potato early blight was affected by the weather conditions. The highest severity was observed under elevated air temperature and sufficient moisture in July, the lowest under conditions of a long dry period.

Champawat and Sharma (2009) studied the influence of environmental factors such as temperature, relative humidity and rainfall on the development of *Alternaria* blight of tomato from Rajasthan. The multiple regression analysis revealed that weather parameters contribute 77 per cent towards disease incidence. Maximum and minimum temperature has positive while maximum and minimum RH have negative significant correlations with appearance of *Alternaria* blight of tomato. The linear regression coefficients for temperature were positively significant while both the RH were negatively significant.

Escuredo and Seijo (2010) observed that over the 3 years of study, highest concentration of *Alternaria* spores were registered during last stage of crop. The parameter that showed the most significant correlation with spore concentrations was minimum temperature.

Ganie (2012) observed that weather factors viz. temperature, relative humidity and rainfall were positively correlated with disease intensity (66.50 per cent contribution.) of early blight of potato in Kashmir.

Mehboob *et al.* (2013) recorded maximum early blight severity was at 17-20°C, minimum air temperature of 6-9°C and 1.9-2.4mm pan evaporation. Relative humidity and wind speed almost had no significant effect on early blight severity of potato.

Roopa (2012) revealed that per cent disease index was progressing at linear rate throughout the plant growth and it was negatively correlated with minimum temperature, relative humidity (morning and evening) and rainfall. While, positively correlated with maximum temperature for early blight of tomato.

2.5 *In vitro* and *in vivo* evaluation of fungicides

Kulkarni (1998) tested seven fungicides under *in vitro* conditions. Iprodione at all concentrations and mancozeb at 2000 and 2500ppm gave total inhibition of fungal growth. In field evaluation Iprodione at 0.1% and mancozeb at 0.2% were most effective in reducing disease severity and increasing the tuber yield

Four fungicides were tested against the early blight of potato by Khan *et al.* (2003) The effect of Metalaxyl + Mancozeb (72 WP), Ridomil Gold (68 WP), Score 250 and Banko 500 SC were more pronounced on tolerant and moderately resistant varieties compared to moderately susceptible or susceptible varieties. The genetic potential of moderately resistant varieties/lines were greatly exploited by the application of fungicides and early blight disease was effectively controlled.

Abhinandan *et al.* (2004) tested the efficacy of commercial fungicides Dithane M-45 (mancozeb) at 0.25%, Kavach (chlorothalonil) at 0.25%, Rovral (iprodione) at 0.20%, Blitox (copper oxychloride) at 0.25%, Syllit (dodine) at 0.3%, Antracol (propineb) at 0.15% and Topaz (penconazole) at 0.05% in controlling the *Alternaria* leaf blight in tomato. Dithane M-45, followed by Kavach were found to be very effective in controlling the disease with >59 per cent disease control compared to the control treatment.

Sali *et al.* (2010) reported that two sprays of mancozeb (0.3%) or propiconazole (0.05%) at an interval of 15 days are effective for reducing the early blight of tomato disease from 54.32%(control) to 35.27% and 35.32% respectively.

Singh *et al.* (2001) noticed the best control of leaf blight disease of tomato caused by *A. solani* by 3 foliar sprays of Dithane M-45(0.2%) at 15 days interval. The crop sprayed with mancozeb produced significantly higher yield of tomato

Kaspa and Osowaki (2003) conducted study during 1997-2001 to estimate the efficacy of fungicides (Mancozeb and Chlorothalonil) and mixtures of plant protection products with a contact mode of action (Zoxamide + mancozeb) against early blight of potato. Spraying with fungicides limited the development of the disease and increased tuber yield ranging from 21.9 to 60.9 per cent for bonin and from 13.0 to 101.9 per cent for stare olesno surveys. The mixture of zoxamide with mancozeb showed the greatest efficacy.

Among non systemic fungicides Iprodione and Mancozeb and systemic fungicides thiophanate methyl was found to be effective under *in vitro* conditions as observed by Prasad and Naik (2003).

Singh and Singh (2006) tested the efficacy of seven fungicides viz. Chlorothalonil, Copper oxy chloride, Azoxystrobin, Propineb, Copper hydroxide, Mancozeb at 2500, 2000, 1000, 500 and 250 ppm and hexaconazole at 1000, 500, 200, 100 and 50 ppm against *Alternaria solani* and observed that all the fungicides significantly reduced the radial growth of the fungus. However, hexaconazole was very effective as it caused 100% growth inhibition.

Arunkumar (2006) tested ten fungicides under *in vitro* condition against *A. solani*, among the fungicides tested propiconazole (0.1%), metalaxyl MZ (0.2%), perfekt (0.3%) gave maximum inhibition. Under field conditions propiconazole (0.1%), pyrachlostrobin (0.2%) were

found to be most effective in reducing the severity of the disease and increasing fruit yield over control.

Issiakhem and Bouznad (2010) tested difenconazole and chlorothalonil on conidial germination and mycelial growth of *Alternaria solani* under *in vitro* conditions. The results revealed that difenconazole had a better effectiveness than chlorothalonil in inhibition of mycelial growth and conidial germination. Difenoconazole had a strong inhibition effect on *A. solani* (89%) with a concentration of 0.97 ppm (or 7.81 µl i.a. /l) and strongly reduced the conidial germination of *A. solani* and reached 92% at 1.95 ppm (7.81µl i.a. /l).

Ganie (2012) recorded that mancozeb treatment gave the highest cost benefit ratio of 1:11.4. Among the systemic fungitoxicants hexaconazole (0.03 %) followed by penconazole (0.03%) proved significantly superior at all tested concentration. Among various treatment combinations seed treatment with mancozeb (0.2%) + foliar spray with hexaconazole (0.03%) + foliar spray with datura (50%) + foliar spray with *T. harzianum* (1 x 10⁷ spores ml/l) proved significantly superior in addition to reducing the disease incidence of early blight of potato.

Roopa (2012) tested twelve different fungicides under *in vitro* conditions against *Alternaria solani* of tomato. Zineb 68% + Hexaconazole 4% (0.2%) and hexaconazole (0.1%) gave the maximum inhibition of mycelial growth of *A. solani*. In case of field evaluation of fungicides, Zineb 68% + Hexaconazole 4% at 0.2 per cent effectively controlled the disease which recorded very less per cent disease index (16.23) with maximum B:C ratio (5:10) followed by hexaconazole (0.1%) and mancozeb (0.2%).

Gondal *et al.* (2012) tested different doses of mancozeb (4 g/L, 8g/L, 12g/L. and 16g/L of water) were applied at 7 days interval. The highest reduction in the disease was achieved by applying mancozeb 12g/l water at an interval of 7, 14, 21 and 28 days. Overall results revealed that weekly sprays of mancozeb at 12g/l of water were cost effective and ecofriendly for the management of *Alternaria* blight of tomato.

Chourasiya *et al.* (2013) reported that lowest percent disease index of early blight of tomato was observed in mancozeb (18.36%) followed by carbendazim (25.62%) and zineb (27.84%) when sprayed at 3 times. Maximum yield of 246.30q/ha recorded from mancozeb at 0.02 per cent was on par with carbendazim at 0.05 per cent (220.83q/ha) as against the yield in control plots of just 80q/ha. Three sprays of mancozeb not only reduced the disease intensity but also gave the higher cost benefit ratio (4.15) followed by 0.05 per cent carbendazim (3.73) treated plot.

Sahu *et al.* (2013) tested some new molecules against early blight of tomato. Pristine 38% WG @ 64 + 126g a.i. /ha (31.88%) significantly reduced the disease followed by maccani 16%WG @ 60+180g a.i./ha (33.31%) and increase the yield from 33.50 tonnes/ha (Pristine 38%WG). Overall results revealed that Pristine at both concentrations (64+126 g a.i./ha and 76.8+151.2 g a.i./ha) was found significantly effective in reducing the disease and increasing the fruit yield over control.

2.6 Screening of potato varieties for early blight incidence

Alam (1985) screened nine *Solanum* spp. for resistance against *Alternaria solani* under artificial conditions of weekly inoculations. All individuals of *Solanum commersonii* remained uninfected after eight inoculations and manifested the highest degree of resistance. Plants of *Solanum chacoense* developed few small necrotic lesions after 5-7 inoculations and exhibited a tolerant/resistant reaction, other *Solanum* spp. did not survive after two inoculations.

Christ (1991) observed significant difference among several potato cultivars for disease reaction to early blight caused by *Alternaria solani* in field trails and also in greenhouse. The late maturing cultivars Katahdin and Kennebec were more resistant to *Alternaria solani* than the early maturing cultivars norland and superior but not necessarily more resistant than the midseason maturing cultivars Atlantic and Chieftain.

Rodriguez *et al.* (2006) quantified components of early blight resistance in leaves of different ages in four potato cultivars. The components of resistance: incubation period (IP), lesion number (LN), early blight severity, lesion expansion rate (LER), latent period (LP) and spore production by lesion area (SPLA), were evaluated separately in the lower, middle and upper leaves of four potato cultivars. Plants of cultivar Aracy (resistant), Delta (moderately resistant), Desirée (susceptible) and Bintje (susceptible) were inoculated with an *Alternaria solani* isolate at the beginning of the flowering stage. In all cultivars, regardless of resistance, the smallest values of LN, and severity were recorded on the upper leaves, suggesting that young tissues are less susceptible. In cultivar Aracy, the IP was

long, with small values of LN and LER and consequently, low values of early blight severity in all leaf positions were recorded. Although IP was long in cultivar Aracy, no differences between the moderately resistant cultivar Delta and the susceptible cultivars Bintje and Desirée could be detected for this component. The IP was only influenced by leaf position in cultivar Aracy. Clear differences in resistance levels among cultivars could be detected regarding LN, severity and LER. 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.* (2007) used reaction to the culture filtrate of *Alternaria solani* (Sorauer) was as an indicator in an *in vitro* screening test to select lines with decreased field susceptibility to early blight from a population of 1000 putative mutants. Pallets of cv. 'Desirée derived from irradiated callus of potato were inoculated *in vitro* with a culture filtrate of *A. solani* (Sorauer). Of the 45 lines selected and subsequently evaluated under conditions of natural infection in the greenhouse six showed lesser degrees of early blight infection than the cv. Desirée control. The six lines selected in the greenhouse retained lower degrees of infection during 2 years of field trials.

Ganie and Ghani (2013) screened twenty five potato genotypes for early blight of potato. Under natural conditions only one genotype SM/92-338 exhibited tolerant reaction. The three other genotypes viz. Kufri Himan, SM/96-27 and SM/94-44 were moderately tolerant. Nine genotypes viz. Kufri Girdari, Kufri Shailaja, Kufri Chandramkhi, 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.

Mehboob *et al.* (2013) screened 29 test lines against early blight of potato. Fifteen lines including Cardinal and Desire were found to be very highly susceptible. Eight lines including Diamant, FSD-white and TPS 9813 showed highly susceptible response. Two lines that is 9802 and 396266-33 were susceptible. FD-18 was found to be resistant. While two lines such as F3-39 and FD-48-41 were shown moderately resistant response.

MATERIAL AND METHODS

The present investigations on early blight disease of potato was conducted during 2013-2014 at the Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad. The field experiments were carried out during *kharif* season of 2013 in a farmer's field at Narendra village of Dharwad taluk in Dharwad district. Dharwad is situated in northern transitional zone (Zone 8) of Karnataka state at 15°15'N latitude, 75°7'N longitude and at an altitude of 774.0 m above mean sea level. The material used and methodology adopted during the course of investigations are presented in this chapter.

3.1 General laboratory procedures

3.1.1 Glassware cleaning

For all laboratory experimental studies, Corning and Borosil glassware were used. Wherever required, they were kept in the cleaning solution containing 60 g potassium dichromate ($K_2Cr_2O_7$) and 60 ml of concentrated sulphuric acid (H_2SO_4) in one liter of water for a day. Then, they were cleaned by washing with detergent followed by rinsing several times in tap water and finally in distilled water.

3.1.2 Sterilization

All glassware, solid and liquid media were subjected to sterilization by autoclaving at 1.1 kg per cm^2 ($121^\circ C$) for 20 minutes. The plant tissues were surface sterilized in 0.2% sodium hypochlorite solution for 30 seconds followed by three changes in sterile water. All cultural studies were conducted in aseptic condition under laminar flow. The tips of inoculation needle, forceps and cork borers were sterilized under flame.

3.2 Survey for the incidence of early blight disease in major potato growing areas of northern Karnataka and proving the pathogenicity

3.2.1 Survey for disease incidence of early blight of potato.

Roving survey was conducted in potato fields of different area along the survey route. Different potato growing areas of Dharwad, Belagavi districts of North Karnataka were covered during *Kharif/Rabi* season in the year 2013-14. In each village, five fields were selected and in each field ten plants were examined randomly and scored by using 0-9 scale (Mayee and Datar, 1986). These values were converted into percentage by using the formula Percent Disease Index (PDI) given by Wheeler (1969). The details of scales are as shown below.

Disease scale

Numerical rating	Description
0	No symptoms on leaf
1	Small, irregular brown spots covering 1% or less of the leaf area
3	Small, irregular, brown spots with concentric rings covering 1-10% of the leaf area.
5	Lesions enlarging, irregular, brown with concentric rings covering 11-25% of the leaf area.
7	Lesions coalesce to form irregular brown patches with concentric rings covering 26-50% of the leaf area. Lesions also on stem and petioles.
9	Lesions coalescing to form irregular, dark brown patches with concentric rings covering 51% or more of the leaf area. Lesions seen on the stem and petiole.

Percent disease index was calculated by using the following formula (Wheeler, 1969).

$$\text{PDI} = \frac{\text{Sum of numerical ratings}}{\text{Total number of leaves observed} \times \text{Maximum disease score}} \times 100$$

3.2.2 Isolation, identification and proving the pathogenicity of the fungus

3.2.2.1 Collection and isolation of the pathogen

The leaves of potato (*Solanum tuberosum*) showing the typical symptoms of the disease were collected from farmer's field of Narendra village where the experiments were carried out in the year 2013. The standard tissue isolation procedure was followed to isolate the pathogen. The infected leaf bits were surface sterilized with 0.2% sodium hypochlorite solution for 30 seconds and repeatedly washed separately in sterilized distilled water to remove the traces of sodium hypochlorite. Then transferred to sterilized Petriplates (1-2 leaf bits per Petri dish) containing Potato Dextrose Agar (PDA).

The Petriplates were incubated at room temperature ($27 \pm 1^{\circ}\text{C}$) and observed periodically for the growth. Bit of fungal growth developed from the infected tissue was transferred to PDA slants and incubated at $27^{\circ} \pm 1^{\circ}\text{C}$ for 12 days. Then such slants with pure culture were used for further studies.

3.2.2.2 Identification of the pathogen

The study was undertaken to confirm the identity of the isolated pathogen. Identification of the pathogen was made after examining one hundred conidia under microscope (under 100x) from mature pure culture of the fungus obtained from infected leaves of potato. Stage and ocular micrometers were used to measure its length, breadth and beak length of the fungus. The average length and breadth of the conidial body, beak and septal numbers were recorded. The morphological characters of the fungus such as conidiophores, conidia and mycelial characters were studied under microscope. These observations were compared with those of the standard measurements given by Ellis (1971) to identify the pathogen. The pathogen isolate of *Alternaria solani* was used for further studies.

3.2.2.3 Single spore isolation

Ten ml of clear, filtered two per cent water agar was poured into sterile Petriplates and allowed to solidify. Dilute spore suspension was prepared in sterilized distilled water from 15 days old culture. One ml of such suspension was spread uniformly on agar plate. These plates were incubated at $27 \pm 1^{\circ}\text{C}$ for 12 hr. Then such plates were examined under microscope to locate single isolated and germinated conidium and marked with ink on the surface of the plates.

The growing hyphal tip portion was transferred to PDA slants with the help of cork borer under aseptic conditions and incubated at $27 \pm 1^{\circ}\text{C}$. Such culture tubes were used for further studies.

3.2.2.4 Maintenance of the cultures

The fungus was sub-cultured on Potato dextrose agar (PDA) slants and allowed to grow at $27 \pm 1^{\circ}\text{C}$ for 16 days, such slants were preserved in refrigerator at 5°C and maintained.

Sub-culturing was done once in a month, such cultures were used throughout the study, virulence of the fungus was maintained by passing through the host after every three months.

3.2.2.5 Proving the pathogenicity

Potato was grown in the earthen pots filled with sterilized soil. Pathogenic culture of *A. solani* was grown on PDA for 8 days then with sterilized glass rod, spores and mycelial growth was harvested in small quantity of sterile distilled water. Then this suspension was sprayed on the potato crops raised in the earthen pots with the help of atomizer. The control was maintained by spraying the plant with sterile distilled water. All plants were covered with polythene bags for 48 hours to maintain humidity. After 48 hours of incubation, polythene bags were removed and the plants were kept in greenhouse. Observations were made for symptom development periodically. Reisolation was made from the spots which are showing typical symptoms on inoculated plants. The isolated culture was compared with the original culture to confirm the pathogen.

3.3 Collection of different isolates, and studying the pathogen character

3.3.1 Morphological characters of different isolates of the *Alternaria solani*

Isolates were collected from five different places viz. Dharwad, Hubballi, Belagavi, Bengaluru and Kolar. The isolates were named as follows.

Isolates	Location
AS 1	Dharwad
AS 2	Hubballi
AS 3	Belagavi
AS 4	Kolar
AS 5	Bengaluru

The spore character of the different isolates were studied by the length, width, beak length, beak width, colour and number of horizontal and vertical septation of conidia by using stage and ocular micrometer and drawings were made by using camera lucida.

These isolates were grown on different media, Potato dextrose agar, Host leaf extract agar, Potato carrot agar, Corn meal agar, Czapeck's dox agar, Sabouraud's agar and Richards agar and their colony characters, topography, sporulation and radial growth in (mm) were observed. The composition and preparation of above mentioned media were obtained from Ainsworth and Bisby's 'Dictionary of the fungi' by Hakswoth *et al.* (1983).

The composition and preparation of different media are given below

1. Potato Dextrose Agar (PDA)

Potato	- 200g
Dextrose	- 20g
Agar	- 20g
Distilled water	- 1000 ml

Potato slices were boiled in 500 ml distilled water for 20 minutes and filtered through muslin cloth. Agar was melted in another 500 ml distilled water, both were mixed and the volume made up to 1000 ml and autoclaved at 121 °C at 15 psi for 20 minutes.

2. Host leaf extract agar (HLEA)

Healthy potato plant leaves	200 g
Agar-agar	20 g
Distilled water	1000 ml

Potato plant leaves were boiled in 500 ml water for 30 min. Extract was collected by filtering through muslin cloth. The agar agar was melted in 500 ml water, both the solutions were mixed and the volume was made up to 1000 ml and was sterilized

3. Richards Agar

Potassium nitrate (KNO ₃)	- 10 g
Potassium monobasic phosphate (KH ₂ PO ₄)	- 5 g
Magnesium sulphate (Mg SO ₄ 7H ₂ O)	- 2.5 g
Ferric chloride (FeCl ₃ 6H ₂ O)	- 0.02 g
Sucrose (C ₁₂ H ₂₂ O ₁₁)	- 50 g
Agar agar	- 15 g
Distilled water	- 1000 ml

Agar was melted in 500 ml distilled water and rest of the ingredients were thoroughly dissolved in another 500 ml distilled water. Both the preparations were mixed and the final volume was made up to 1000 ml and then autoclaved.

4. Czapek's Dox agar

Sodium nitrate (NaNO ₃)	- 3 g
Potassium dihydrogen phosphate (K ₂ HPO ₄)	- 1 g
Magnesium sulphate (MgSO ₄ . 7H ₂ O)	- 0.5 g
Ferrous sulphate (FeSO ₄ . 7 H ₂ O)	- 0.19 g
Sucrose (C ₁₂ H ₂₂ O ₁₁)	- 30 g
Agar agar	- 15 g
Distilled water	- 1000 ml

Agar was melted in 500 ml distilled water and rest of the ingredients were thoroughly dissolved in another 500 ml distilled water. Both the preparations were mixed and the final volume was made up to 1000 ml and then autoclaved.

5) Corn meal agar

Corn meal	60 g
Agar agar	15 g
Distilled water	1000 ml

Sixty grams of powdered corn meal was placed in clean muslin bag and tied. The bag was steamed in 500 ml of distilled water in a beaker for one hour. Agar was melted separately in 450 ml of distilled water. The boiled corn meal was then strained into the melted agar and the volume was made to 1000 ml and then sterilized.

6) Sabouraud's agar

Dextrose	40 g
Peptone	10 g
Agar-agar	20 g
Distilled water	1000 ml

All the ingredients were dissolved one by one in 400 ml distilled water and agar was dissolved separately in 500 ml distilled water and mixed with the above solution and the volume was made upto one litre before sterilization.

7) Potato carrot agar

Grated potato	20 g
Grated carrot	20 g
Agar agar	20 g
Distilled water	1000 ml

Boil grated vegetables for 1 hr. in the tap water. Strain through fine sieve, add agar. Boil over water bath till agar dissolves, sterilize at 15 p.s.i. for 20 min.

3.3.2 Studies on the growth phase of the pathogen

The growth phase study was conducted on potato dextrose broth (PDB). 30 ml of broth was pipetted out in to each of 100 ml flasks. Those flasks were sterilized at 121 °C for 20 minutes at 15 lb psi pressure and then inoculated with 5 mm disc of *A. solani* culture. The inoculated flasks were incubated at 28±1 °C. A set of three flasks were harvested starting from first day up to 14thday after inoculation. The culture was filtered through Whatman No.1 filter. Before filtering the filter papers were dried to a constant weight by drying in hot air oven at 50°C. The mycelial mat on the filter paper was thoroughly washed with distilled water and dried in hot air oven at 50°C. The filter paper with mycelial

mat was weighed in a digital electronic balance. The weight of dry mycelial mat was recorded and the data were statistically analysed and maximum growth period was determined.

3.3.3 Effect of pH on growth of *A. solani*

Potato dextrose broth was used as a basal medium to study the effect of pH on the growth of *A. solani*. The pH of the medium was adjusted to various levels namely 5.0, 6.0, 7.0, 8.0 and 9.0 by adding 0.1 N sodium hydroxide and 0.1 N hydrochloric acid and it was determined by electronic pH meter. 30 ml of the medium with known pH was added to 100 ml conical flasks and then the flasks were sterilized. 5 mm discs taken from 9 days old 5 isolates of *A. solani* were inoculated and incubated at $27\pm 1^\circ\text{C}$ for 14 days. Three replications were maintained for each treatment. The dry mycelial weight was recorded and the data were analysed statistically.

3.3.4 Effect of temperature on growth of *A. solani*

Potato dextrose broth was used as a basal medium to study the effect of temperature on the growth of *A. solani*. 30 ml of the medium was added to 100 ml conical flasks and then the flasks were sterilized. 5 mm discs taken from 9 days old 5 isolates of *A. solani* were inoculated and incubated at different temperature viz. 15, 20, 25, 30, 35°C for 14 days. Three replications were maintained for each treatment. The dry mycelial weights were recorded and the data were analysed statistically.

3.4 Role of weather factors on the incidence of the disease

The effect of weather factors like temperature (maximum and minimum), relative humidity (morning and evening in per cent) rainfall (mm) and number of rainy days on the incidence and development of early blight will be studied in the farmer's field near UAS Dharwad campus. The meteorological observations at Main Agriculture Research station, UAS Dharwad was used for this experiment. This study was undertaken during *kharif* 2013. The observations will be made on disease incidence and severity starting from first day of its appearance and till the end of the crop. It was correlated with weather parameters by simple correlation.

In the field *i.e.* in control where no fungicides sprays were taken up, 20 plants were examined randomly and scored for disease severity by following 0-9 scale and percent disease index was recorded.

3.5 *In vitro* evaluation of fungicides

The efficacy of systemic, non systemic and combi products were assessed by poison food technique. The pathogen *A. solani* was grown on PDA medium in Petri plates for ten days prior to setting the experiment. The details are mentioned below.

Fungicide suspension was prepared in PDA by adding required quantity of fungicide to obtain the desired concentration on the basis of active ingredient and whole product present in the chemical. Twenty ml of poisoned medium was poured in each of the sterilized Petriplates. Mycelial disc of 0.5 cm was taken from the periphery of ten day old culture and will be placed in the center and incubated at $25\pm 1^\circ\text{C}$ till growth of the fungus touched the periphery in control plate. Suitable checks also maintained without addition of any fungicide, three replications were maintained for each treatment. The diameter of the colony was measured in two directions and average was worked out. The per cent inhibition of growth was calculated by using the formula given by Vincent (1947).

$$I = \frac{(C - T)}{C} \times 100$$

Where

I= Per cent inhibition of mycelium

C= Growth of mycelium in control

T= Growth of mycelium in treatment

The per cent values were converted to arc sin transformations, the data were analysed statistically.

The followings fungicides were tested

Non systemic fungicides	Trade name	Concentrations (%)		
		0.1	0.2	0.25
Mancozeb 75% WP	Indofil M- 45	0.1	0.2	0.25
Chlorothalonil 75% WP	Kavach	0.1	0.2	0.25
Zineb 75% WP	Dithane Z-78	0.1	0.2	0.25
Propineb 70% WP	Antracol	0.1	0.2	0.25

Systemic fungicides	Trade name	Concentrations (%)		
		0.05	0.1	0.15
Difenconazole 25% EC	Score	0.05	0.1	0.15
Hexaconazole 5% EC	Contaf	0.05	0.1	0.15
Tebuconazole 25% EC	Folicur	0.05	0.1	0.15
Propiconazole 25% EC	Tilt	0.05	0.1	0.15
Penconazole 25% EC	Topaz	0.05	0.1	0.15

Combi products	Trade name	Concentrations (%)		
		0.1	0.2	0.25
Mancozeb 63%+carbendazim 12% WP	Saaf	0.1	0.2	0.25
Captan 70%+Hexaconazole 5% WP	Taqat	0.1	0.2	0.25
Zineb 68%+Hexaconazole 4% WP	Avatar	0.1	0.2	0.25

3.6 *In vivo* evaluation of fungicides

A field experiment on potato crop was conducted at Narendra village, Dharwad district during *kharif* 2013 to know the efficacy of all the above mentioned fungicides. The experiment was laid out in Randomized Block Design (RCBD) with three replications (Fig. 1). Potato seeds were sown on 7th July 2013 and harvesting was done on 23rd September 2013. Three sprays of the fungicides were given at 15 days interval. The first spray was given immediately after the first appearance of early blight symptoms *i.e.* 35 days after planting. 2nd spray was given at 50 days after planting and 3rd spray 65 days after planting. Five plants in each subplot were scored for disease and data were converted into per cent disease index (PDI) as explained earlier. Finally yield data was taken after harvest and cost benefit ratio was calculated.

Non systemic fungicides	Trade name	Concentration (%)
Mancozeb 75% WP	Indofil M- 45	0.2
Chlorothalonil 75% WP	Kavach	0.2
Zineb 75% WP	Dithane Z-78	0.2
Propineb 70% WP	Antracol	0.2
Systemic fungicides		
Difenconazole 25% EC	Score	0.1
Hexaconazole 5% EC	Contaf	0.1
Tebuconazole 25% EC	Folicur	0.1
Propiconazole 25% EC	Tilt	0.1
Penconazole 25% EC	Topaz	0.1
Combiproducs		
Mancozeb 63% + carbendazim 12% WP	Saaf	0.2
Captan 70% + Hexaconazole 5% WP	Taqat	0.2
Zineb 68% + Hexaconazole 4% WP	Avatar	0.2

LEGEND

T₁ - Mancozeb 75% WP

T₂ - Chlorothalonil 75% WP

T₃ - Zineb 70% WP

T₄ - Propineb 70% WP

T₅ - Difenconazole 25% EC

T₆ - Hexaconazole 5% EC

T₇ - Tebuconazole 25% EC

T₈ - Propiconazole 25% EC

T₉ - Penconazole 10% EC

T₁₀ - Mancozeb 63% + Carbendazim 12% WP

T₁₁ - Captan 70% + Hexaconazole 5% WP

T₁₂ - Zineb 68% + Hexaconazole 4% WP

Legend

T ₁	: Mancozeb 75%WP
T ₂	: Chlorothalonil 75%WP
T ₃	: Zineb 75% WP
T ₄	: Propineb 70% WP
T ₅	: Difenconazole 25% EC
T ₆	: Hexaconzole 5%EC
T ₇	: Tebuconazole 25%EC
T ₈	: Propiconazole 25%EC
T ₉	: Penconazole 10%EC
T ₁₀	: Mancozeb 63% + Carbendazim 12% WP
T ₁₁	: Captan 70% + Hexaconazole 5% WP
T ₁₂	: Zineb 68% + Hexaconazole 4% WP
T ₁₃	: Control

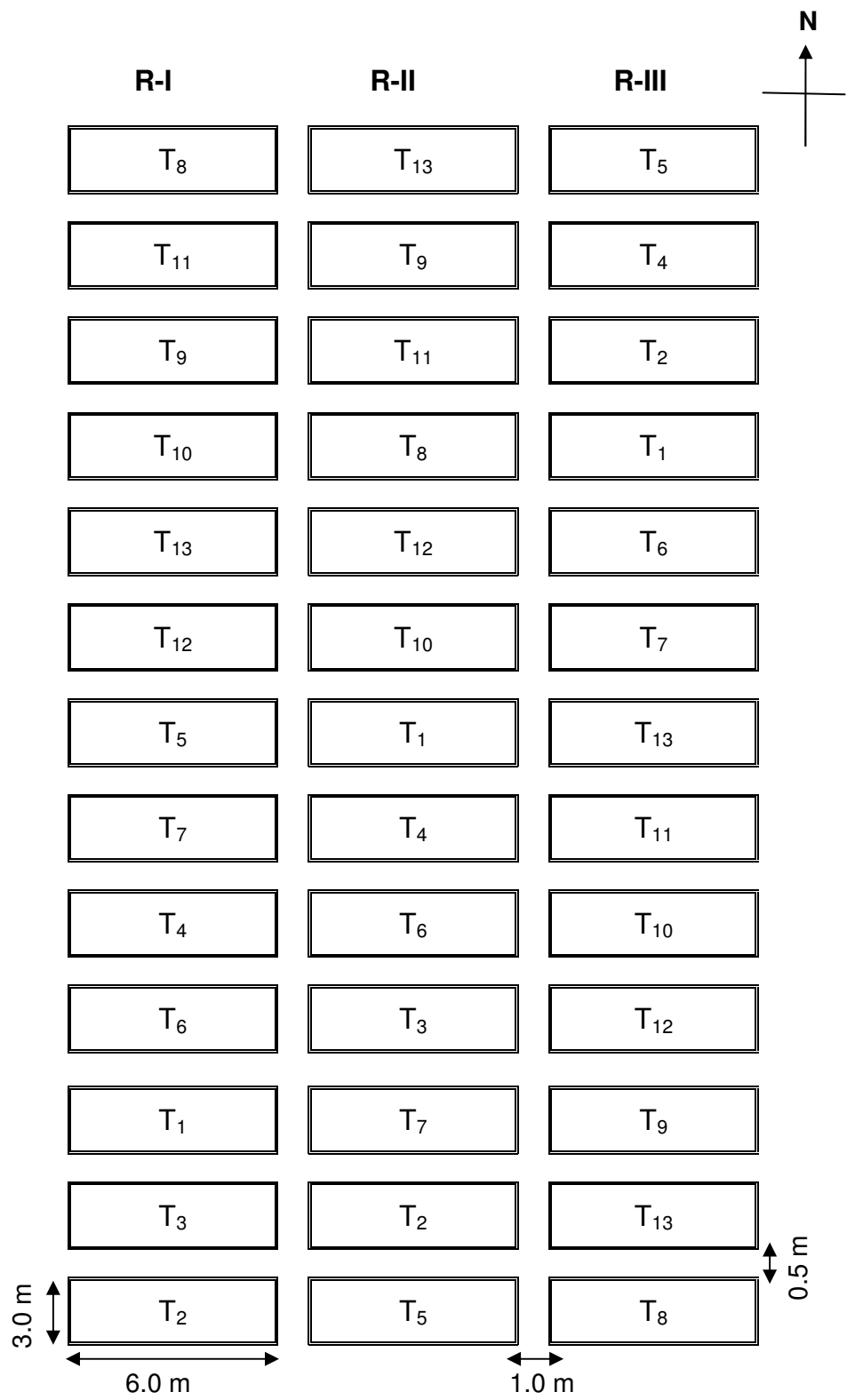


Fig. 1: Plan of layout

3.7 Screening of potato varieties for early blight incidence.

This experiment was carried out at All India Co-ordinated Research Project on potato at Saidapur farm. The natural incidence of early blight was recorded on different potato varieties grown in field conditions during the year 2013. The experiment was conducted with randomized block design with 4 replications. 45 potato genotypes obtained from All India Co-ordinated Research Project on potato, Dharwad were screened against *Alternaria solani*. Observations were recorded after the appearance of disease at an interval of 15 days by following 0 to 9 scale given by Mayee and Datar (1986).

The various genotypes were arbitrarily categorized into six different reaction group as follows.

Category	Reactions
0	Immune
1	Resistant
3	Moderately resistant
5	Moderately susceptible
7	Susceptible
9	Highly susceptible

EXPERIMENTAL RESULTS

Among all the foliar diseases of potato, early blight is one of the major, more common and devastating disease of potato which leads to loss in yield even up to 80 per cent if the conditions are favourable for the disease development. Hence, the present investigations were carried out with field studies and also the laboratory studies. Collection of different isolates and studying the variability among them, *in vitro* evaluation of fungicides, proving the pathogenicity of the fungus was conducted in laboratory during 2013 in the Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad.

Field evaluation of some of the fungicides against early blight of potato and early blight disease development in relation to weather parameters were studied in the farmer's field of Narendra village, Dharwad taluk. Screening of some of the potato genotypes against early blight of potato were done in AICRP on Potato, Dharwad. Survey was conducted to know severity of early blight of potato in Dharwad and Belagavi districts during *Kharif* 2013. The result thus obtained are presented here under.

4.1 Survey for Early blight of potato

Roving survey was undertaken during *Kharif* 2013 to estimate the severity of early blight of potato in major potato growing districts of northern Karnataka *viz.* Dharwad and Belagavi. The severity of early blight was recorded by using 0-9 disease scale given by Mayee and Datar (1986) and shown in Plate 1. The survey was conducted as explained in "Materials and Methods" and results are presented in Table 1 and Fig 2.

Data pertaining to survey conducted during 2013 as presented in Table 1 revealed that, early blight of potato was almost equally similar in both Dharwad and Belagavi districts and disease severity ranged between 12.33 to 38.33 per cent in both the districts. The highest severity of early blight was noticed in fields of Narendra village (38.33%) of Dharwad district. Where as least severity was observed in Kadoli village (12.33%) of Belagavi district.

Among the talukas surveyed, maximum PDI was recorded in Hubballi taluk (31.89%) of Dharwad district and Hukkeri taluk (31.89%) of Belagavi district. Least severity was observed in Belagavi taluk (21.31%). The highest district average disease severity was recorded in Dharwad (26.93%) followed by Belagavi (25.52%).

In Dharwad district, the highest disease severity of early blight was observed in Narendra village (38.33%) of Dharwad taluk followed by Neeralakatti village (38%) of Dharwad taluk. Least severity was observed in Hosatti village (14%) of Dharwad taluk followed by Garag village (15.67%) of Dharwad taluk. In Belagavi district, highest severity was observed in Yamakanamaradi (35%) of Hukkeri taluk followed by Belavadi (34.67%) of Bailahongala taluk and the least severity was observed in Kadoli village (12.33%) of Belagavi taluk followed by Kakathi village (13.56%) of Belagavi taluk.

4.1.2 Symptomatology

The symptoms of early blight of potato were mainly observed on the leaves. The disease appeared as characteristic dark brown to black lesions with concentric rings which produce a target spot effect. Symptoms are initially observed on older, senescing leaves. Enlarging lesions are often surrounded by a narrow chlorotic halo. Later these spots increased in size and coalesced covering larger leaf area leading to blighted appearance, under severe conditions brownish lesion can also be seen on the stem and tuber. Infection on the tuber is rare. Tuber lesions are usually sunken with raised borders. The symptoms of the disease in field during survey were presented in Plate 2.

4.2 Isolation, identification and proving the pathogenicity of the fungus

4.2.1 Isolation

Isolations were made from diseased leaf tissues of potato cultivar which are typically showing early blight symptoms. After 72 h of incubation at 25±2°C, dark ranging from grey to black with tints of olive or brown mycelial growth started emerging from the medium. The culture so obtained was purified by the hyphal tip and single spore isolation methods as explained in "Materials and Methods". The pure culture was maintained by subculturing at monthly intervals and stored in a refrigerator for

Table 1: Severity of early blight of potato in major potato growing areas of northern Karnataka during *Kharif* 2013

District	Taluk	Village	Variety	Stage of the crop (DAS)	Type of the soil	PDI	
Dharwad	Dharwad	Narendra	Kufri Jyothi	50	Black	38.33	
		Kamalapura	Kufri Jyothi	52	Black	33.00	
		Marewada	Kufri Jyothi	52	Black	25.67	
		Lakamapura	Kufri Chandramuki	55	Black	24.00	
		Yadavada	Kufri Jyothi	52	Black	26.67	
		Lokur	Kufri Chandramuki	58	Black	25.33	
		Garag	Kufri Jyothi	60	Black	15.67	
		Hosatti	Kufri Jyothi	65	Black	14.00	
		Madanabhavi	Kufri Jyothi	65	Black	28.00	
		Neeralakatti	Kufri Chandramuki	55	Black	38.00	
		Tadakoda	Kufri Jyothi	60	Black	26.33	
		Hangariki	Kufri Jyothi	53	Black	19.66	
		Dasanakoppa	Kufri Chandramuki	55	Black	27.00	
		Hebballi	Kufri Jyothi	60	Black	22.33	
		Chandanamatti	Kufri Jyothi	60	Black	26.00	
	Amminabavi	Kufri Jyothi	65	Black	27.00		
		Taluk mean					26.06
		Hubballi	Amargola	Kufri Jyothi	70	Black	32.67
			Rayanala	Kufri Chandramuki	56	Black	35.00
			Parasapura	Kufri Jyothi	55	Black	28.00
		Taluk mean					31.89
		Kundagol	Ramanakoppa	Kufri Chandramuki	70	Black	25.67
			Jigalur	Kufri Chandramuki	66	Black	27.00
			Malali	Kufri Jyothi	60	Black	26.33
		Taluk mean					26.33
	District mean						26.93

Contd...

Belagavi	Belagavi	Kadoli	Kufri Chandramuki	65	Black	12.33	
		Kakathi	Kufri Chandramuki	70	Red	13.56	
		Ambevadi	Kufri Jyothi	73	Red	25.77	
		Handignooru	Kufri Jyothi	65	Red	26.00	
		Halaga	Kufri Chandramuki	65	Black	24.50	
		Bastavada	Kufri Chandramuki	68	Loamy	26.32	
		Honaga	Kufri Jyothi	63	Loamy	15.33	
		Kendoor	Kufri Jyothi	65	Black	26.67	
		Taluk mean					21.31
	Bailahongala	Bagevadi	Kufri Chandramuki	65	Red	27.67	
		Chikkabagevadi	Kufri Jyothi	60	Black	32.00	
		Sampagavi	Kufri Jyothi	70	Loamy	22.33	
		Belavadi	Kufri Chandramuki	72	Loamy	34.67	
		Taluk mean					29.16
	Hukkeri	Yamakanamaradi	Kufri Jyothi	65	Red	35.00	
		Hattaragi	Kufri Chandramuki	65	Black	32.67	
		Halemastihole	Kufri Chandramuki	72	Black	28.00	
		Taluk mean					31.89
	District mean						25.52

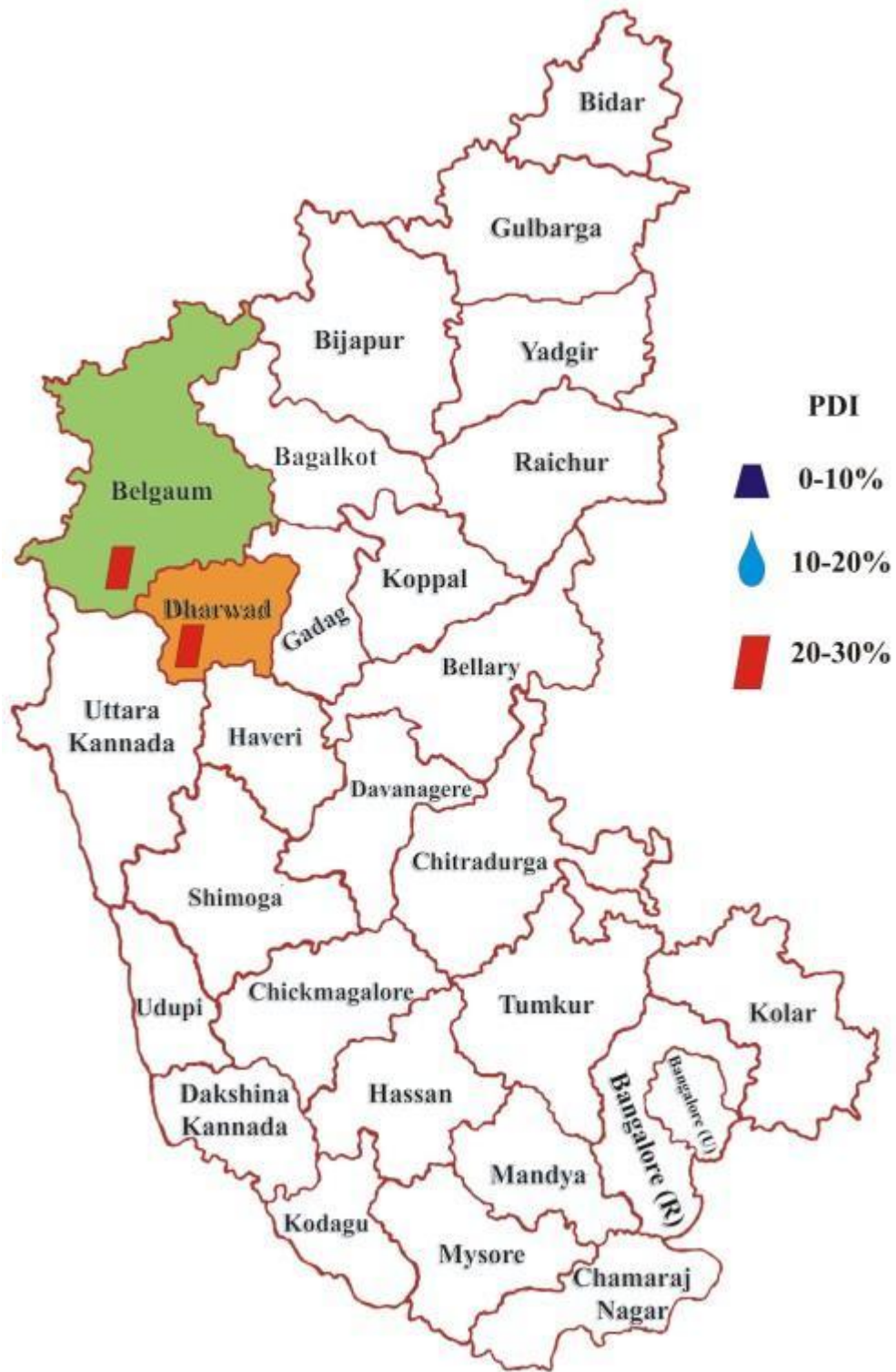


Fig 2. Severity of early blight of potato in major growing areas of northern Karnataka during *kharif* 2013

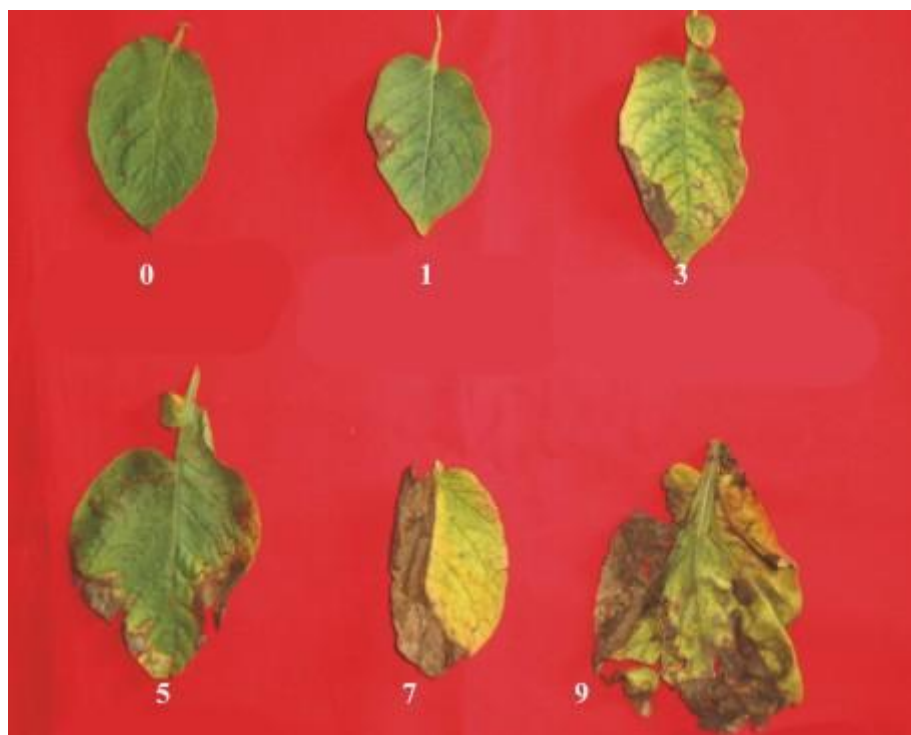


Plate 1. Disease scale (0-9) of early blight of potato



a. General view of survey field



b. Symptoms on foliage



c. Symptoms near the ground level



d. Concentric zonation



e. Sample collection during survey

Plate 2. Field view during survey

further studies. The pure culture of the fungus was obtained after ten days of inoculation which showed whitish growth at initial stage turning later to ash- gray to black colour. Photograph of pure culture is presented in Plate 3.

4.2.2 Pathogenicity test

For proving the pathogenicity on the potato plants, the pathogen was artificially inoculated on the leaves of potato plants under glasshouse conditions as described in "Materials and Methods". After 10 days of inoculation, the leaves exhibited symptom of infection. The symptoms on the leaves appeared as brown, round to oval spots with concentric zonations (Plate 3a).

The pathogen was again reisolated from the infected leaves. Purified culture from the artificially infected leaves were similar to that of original culture thus proving the Koch's postulates. So by this pathogenicity test it is confirmed that the causal organism of early blight of potato is *Alternaria solani* (Ellis and Martin) Jones and Grout.

4.2.3 Identification of the fungus

Identification of the fungus was carried out based on the morphological characters of the isolated fungus. The morphological characters of the fungus isolated from potato leaves were studied on Potato dextrose agar medium. The conidiophores arises singly or in small groups, straight or flexuous, septate, rather pale brown or olivaceous brown. Conidia usually solitary, straight or slightly flexuous, obclavate or with the body of the conidium oblong or ellipsoidal tapering to a beak which is commonly the same length as or rather longer than the body. Colour of the conidia is pale or olivaceous brown, overall length usually 140-230 μ , 19-36 μ thick in the broadest part, with 7-12 transverse and 3 to 6 longitudinal septa.

The description of this fungus agreed with the description given by Ellis (1971) from Common Wealth Mycological Institute, Kew, Surrey, England. Thus, on the basis of pathogenicity and authentic description of the fungus, the pathogen which causes early blight of potato was identified as *Alternaria solani* (Ellis and Martin) Jones and Grout.

4.3 Collection of different isolates and studying the pathogen character

The five isolates were collected from different districts and named them as follows. AS 1 (Dharwad), AS 2 (Hubballi), AS 3 (Belagavi), AS 4 (Kolar) and AS 5 (Bengaluru). Various studies have been conducted to know the cultural and morphological variability among them. The results of the experiments are presented below.

4.3.1 Growth phase studies of the fungus.

The fungus was grown on potato dextrose broth in 100ml conical flask containing 30 ml of the medium in order to study the growth pattern of *Alternaria solani*. Pure culture of the standard isolate has been taken and inoculated as described in "Material and Methods".

The mycelium was harvested from inoculated flasks for every two days interval starting from first day and upto 30 days. The dry mycelial weight was recorded and results obtained are presented in Table 2.

The maximum mycelial weight was obtained fourteenth day (288.66 mg) after inoculation. The mycelial growth was gradually increased from the second day onwards after inoculation and reached peak on 14th day and afterwards gradually decreased. Subsequent reduction in the mycelial weight was observed after fourteen days.

4.3.2 Conidia measurement of different isolates

The conidia were measured based on the length, breadth, beak length, beak width, vertical septation and horizontal septation. Average of 25 conidial measurements were taken from each isolate and which are presented in Table 3 and Plate 4. All the isolates ranged in its size from 140 to 285 μ m. with an average length of 157.43 to 254 μ m. Width of conidia ranges from 15-36 μ m with an average width of 18.49 to 30.30 μ m. Beak length is almost equal or more than the length of the conidial body. Beak length ranges from 60 to 180 μ m with an average length of 74.12 to 170.90 μ m. Beak width ranges from 6-14.8 μ m with a average width of 7.41 to 12.26 μ m. Number of horizontal

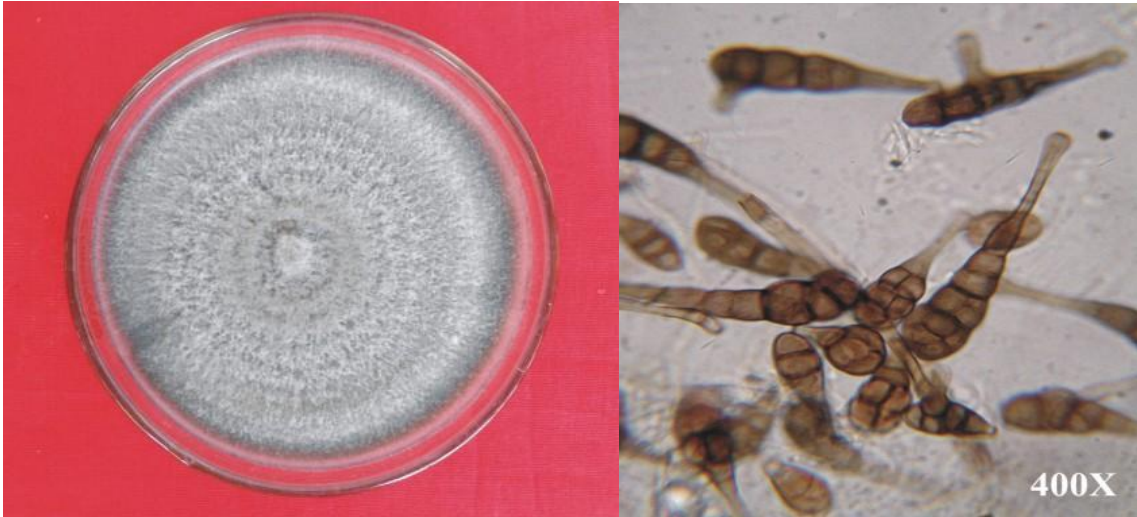


Plate 3. Photograph showing pure culture and conidia of *Alternaria solani*



Plate 3a. Proving the pathogenicity

Table 2: Growth phase studies of *Alternaria solani*

Interval (days)	Dry mycelial weight (mg)
2	37.33
4	77.66
6	94.33
8	172.66
10	196.66
12	228.66
14	288.66
16	275.33
18	274.00
20	267.33
22	262.67
24	259.67
26	237.00
28	209.33
30	199.67
S.Em._±	1.55
CD at 1%	6.03

Table 3 : Conidial morphology of different isolates of *Alternaria solani*

Isolates	Size of conidia (length x breadth) (μm)		Beak length (μm)		Beak width (μm)		Number of horizontal septa		Number of vertical septa	
	Range	Average	Range	average	Range	Average	Range	average	Range	Average
AS 1	140-230 X 19- 36	218.46 X 27.90	70 - 120	86.06	8 - 11.6	9.67	7-12	8	3-5	5
AS 2	200-260 X 19-25	214.84 X 19.98	60 - 105	74.12	6 - 8.5	7.41	5-10	7	2-5	3
AS 3	225-260 X 18- 28	222.12 X 27.94	102 -153	117.00	9 - 14.8	10.68	3-8	6	0-3	1
AS 4	235-285 X 29-32	254.00 X 30.30	138 -180	170 .90	7 - 10.6	10.85	2-7	4	0-2	1
AS 5	150-185 X 15-25	157.43 X 18.49	76 -130	85.80	9 - 14.0	12.26	3-6	4	1-3	2



a. AS 1 (Dharwad)



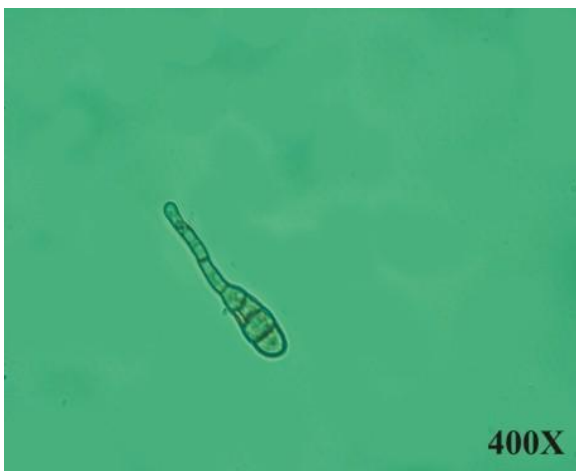
b. AS 2 (Hubballi)



c. AS 3 (Belagavi)



d. AS 4 (Kolar)



e. AS 5 (Bengaluru)

Plate 4. Conidial morphology of different isolates of *Alternaria solani*

septation ranges from 2 to 12 with a average of 4 to 8 and number of vertical septa lies between 0-5 with a average of 1-5.

Maximum length and thickness of conidia was observed in isolate AS 4 (235-285 X 29-32 μm) with an average size of 254.00 X 30.30 μm followed by AS 3 (225-260 X 18- 28 μm) and average size of 222.12 X 27.94 μm and minimum length and breadth of conidia were observed in AS 5 (150-185 X 15-25 μm) with a average of 157.43 X 18.49 μm . Longest beak was observed in isolate AS 4 which ranges between 138 -180 μm with a average of 170.90 μm and minimum length of beak was observed in isolate AS 5 (76 -130 μm) with a average length of 85.80 μm . Beak width was more in AS 5 (9 - 14 μm) with a average of 12.26 μm and it was least in AS 2 (6 - 8.5) with average width of 7.41 μm . Maximum number of horizontal septa was found in isolate AS 1 (7-12) with average of 8. Least number of horizontal septa were observed in AS 4 (2-7) and AS 5 (3- 6) with avearge of 4 and higher number of vertical septa was found in AS 1 (3-5) with average of 5 and less number of vertical septa was recorded in AS 3 (0-3) and AS 4 (0-2) with a average number of 1.

4.3.3 Colony characters of isolates on different solid media.

All 5 isolates were cultured on different solid media viz. Potato dextrose agar, Host leaf extract agar, Potato carrot agar, Corn meal agar, Czapeck's Dox agar, Richards agar and Sabouraud's agar to know the difference in the cultural characters. Sporulation and measurement of the colony were also recorded. The results are presented in Table 4 and Plate 5.

The colony colour of all the isolates varies from white to grey in all the medium except on Corn meal agar. It is black in colour on Corn meal agar. On PDA sectoring is present in isolate AS 4 and AS 5. Concentric rings were present in isolate AS 1 and AS 3. Isolates AS 1, AS 2 and AS 3 were flat in growth and isolates AS 4 and AS 5 were raised in growth. On PCA sectoring is present in AS 1 and AS 3. Concentric rings are present in AS 2 and AS 5. AS 1 and AS 4 were raised in growth. Distorted mycelia with irregular margin is present in all the isolates on corn meal agar. Raised and cottony mycelia was observed in all the isolates on Richards agar. Isolate AS 5 is flat and concentric rings were present in Sabouraud's agar.

The growth of the mycelium is very fast in Potato Carrot Agar followed by Czapeck's dox agar and Potato dextrose agar. Medium growth was found in Host leaf extract agar and Richards agar. The growth is very slow in Corn meal agar and Sabouraud's agar for all the isolates. Sporulation was excellent on Potato carrot agar followed by Potato dextrose agar, Host leaf extract agar and Czapeck's dox agar. Sporulation was very low in Sabouraud's agar followed by Richards agar in all the isolates. The isolate AS 5 showed slightly less growth on Corn meal agar, Czapeck's dox agar and Richards agar compared to other isolates.

The colony diameter of different isolates were also measured. Maximum colony diameter in isolate AS 3 on PDA, (90 mm), isolate AS 1, AS 2 and AS 4 on PCA, (90 mm), Isolate AS 1 on Czapeck's dox agar (90 mm) and isolate AS 4 on Richards agar (90 mm). Least measurement is obtained on corn meal agar (60.33 mm) in isolate AS 2 in all the isolates.

4.3.4 Effect of temperature levels on isolates of *Alternaria solani*.

The isolates of *Alternaria solani* were studied at five different temperature levels viz. 15°C, 20°C, 25°C, 30°C and 35°C on Potato dextrose broth as described in "Materials and Methods". The results are furnished in Table 5 and Plate 6 (a). The isolates, temperature and interaction effect were found to be significant with respect to dry mycelial weight. From the results it is observed that, isolates AS 1 and AS 2 showed good growth at 30° C and AS 3, AS 4 and AS 5 showed good growth at 25°C. The maximum dry mycelial weight is obtained in isolate AS 4 at 25°C (401.75 mg) followed by isolate AS 4 at 30°C (370mg) and isolate AS 5 at 25°C (369.25 mg). Least dry mycelial weight was obtained in isolate AS 5 at 15°C. All the isolates showed significantly less growth at 15° C temperature.

4.3.5 Effect of pH levels on isolates of *Alternaria solani*

The isolates of *Alternaria solani* were studied at five different pH levels viz. 5, 6, 7, 8 and 9 on PDB broth as explained in "Materials and Methods". The results are presented in Table 6 and Plate 6 (b). The results are found to be significant with respect to interaction effect of isolates and pH levels. From the table it is observed that isolates AS 1, AS 2 and AS 3 having maximum dry mycelial weight at pH 7 and isolates AS 4 and AS 5 are having good growth at pH 8. The maximum dry mycelium weight was found in isolate AS 2 (313 mg) at pH 7 and AS 5 (313 mg) at pH 8 which is on par with

Table 4: Cultural characters and growth (mm) of isolates of *Alternaria solani* on different solid media

Isolates	Media						
	Potato dextrose agar	Host leaf extract agar	Potato carrot agar	Corn meal agar	Czapeck's Dox agar	Richards agar	Sabouraud's agar
AS 1	Grey, flat, smooth and regular margin, concentric zonation, fast growth ++++	Pale grey, Flat, light grey regular margin, medium growth +++	Light grey, raised, grey colour regular margin, sectoring, fast growth ++++	Black colour, distorted mycelia, irregular margin, slow growth +++	Dark grey, raised growth, white centre, regular margin, fast growth +++	Dirty white, raised, cottony mycelia, white regular margin, medium growth ++	Cream to cement colour, cottony, raised, regular margin, slow growth +
	90	87.66	90.00	69.00	90.00	86.66	77.66
AS 2	Light grey, flat, dark grey smooth and regular margin, concentric zonation, fast growth +++	Pale grey, flat, Light grey regular margin, medium growth +++	grey colour, dark grey in the centre, flat, regular margin, concentric rings, fast growth ++++	Black colour, distorted mycelia, irregular margin, slow growth +++	Greyish to black colour, flat growth, regular margin, fast growth +++	White colour, raised cottony mycelia, regular white margin, medium growth ++	Cream to cement colour, cottony, raised, irregular margin, slow growth +
	90	70.66	90.00	60.33	76.66	89.00	68.33
AS 3	Greyish green, flat, white smooth and regular margin, concentric zonation fast growth ++++	Grey, flat, regular margin, concentric rings, medium growth +++	White to grayish colour, flat, regular margin, sectoring, fast growth ++++	Black colour, distorted mycelia, irregular margin, slow growth +++	Grey, raised, smooth regular margin, fast growth +++	White colour, raised cottony mycelia, regular white margin, medium growth ++	Cream to cement colour, cottony, raised, regular margin, slow growth +
	79.00	90.00	86.00	65.66	89	88.66	86.00

Contd...

AS 4	Dirty white, Raised, smooth and regular white margin sectoring is present, , fast growth +++	Pale grey, flat, regular margin, medium growth +++	Pale grey, raised, regular margin, fast growth ++++	Black colour, distorted mycelia, irregular margin, slow growth +++	Black centre, white regular margin. Raised, fast growth +++	White colour, raised cottony mycelia, regular white margin, medium growth ++	Cream to cement colour, cottony, raised, regular margin, slow growth +
	86.00	86.00	90.00	64.00	88.33	90.00	84.00
AS 5	Dirty white, Raised, smooth and regular whitemargin sectoring is present. , fast growth +++ 85.5	Dark grey , concentric, flat, regular margin, medium growth +++ 89.00	Pale grey, concentric, flat, regular margin, fast growth +++ 89.00	Black colour, distorted mycelia, irregular margin, slow growth +++ 64.00	Grayish to black, raised, irregular rough margin, medium growth +++ 88.33	White colour, raised cottony mycelia, regular white margin, , slow growth + 87.66	Cream, flat, concentric, regular margin, slow growth ++ 84.00

AS 1 = Dharwad

AS 2 = Hubballi

AS 3 = Belagavi

AS 4 = Kolar

AS 5 = Bengaluru

Colour of colony= White to dark grey

Topography = convex to flat

Circumference of margin = Regular to irregular

Sporulation:

Conidia/ microscopic field (100x)

++++

>30

+++

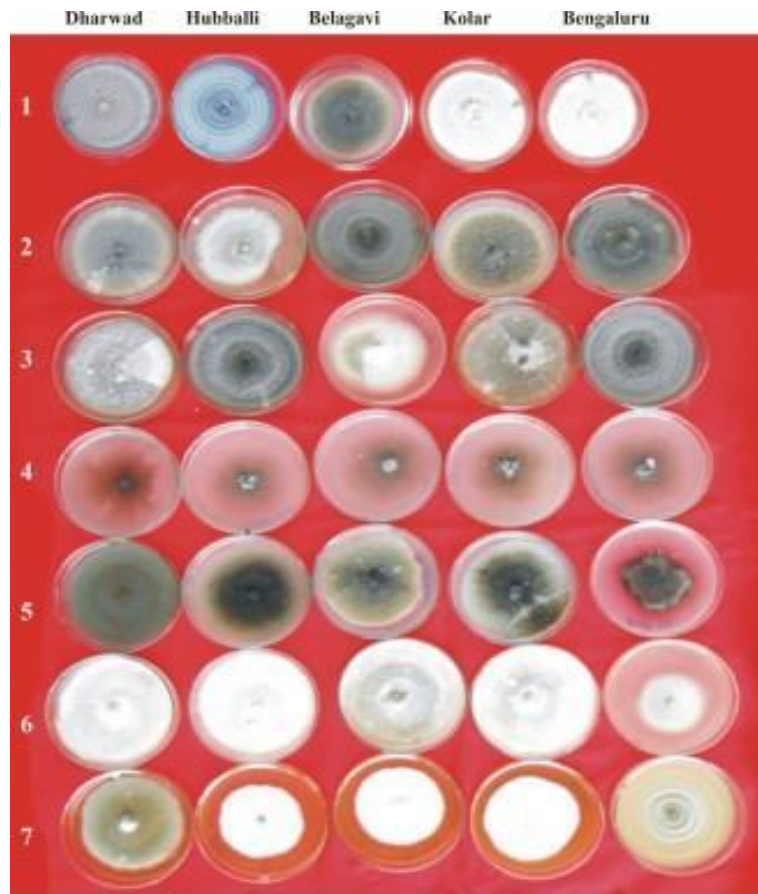
20-30

++

10-20

+

1-10



- | | |
|--------------------------|----------------------------|
| 1 - Potato Dextrose Agar | 2 - Host Leaf Extract Agar |
| 3 - Potato Carrot Agar | 4 - Corn Meal Agar |
| 5 - Czapeck's Dox Agar | 6 - Richards Agar |
| 7 - Sabourad's Agar | |

Plate 5. Cultural studies of different isolates of *A. solani*

Table 5: Effect of temperature levels on isolates of *Alternaria solani*

Temperature (°C)	Dry mycelial weight (mg)					Mean
	AS 1	AS 2	AS 3	AS 4	AS 5	
15	80.00	93.75	68.50	64.75	48.00	71.00
20	170.00	192.50	201.25	227.25	150.00	188.2
25	250.00	270.25	361.50	401.75	369.25	330.55
30	327.25	310.50	291.25	370.00	302.75	320.35
35	155.75	198.50	207.25	265.25	256.25	216.6
Mean	196.60	213.10	225.95	265.90	225.25	
	S.Em.±		CD at 1%			
Isolates (I)	0.94		3.77			
Temperature level (T)	0.83		3.58			
I X T	2.11		8.44			

isolate AS 4 (310.66 mg) at pH 8 and isolate AS 4 (306.66 mg) at pH 7. Least dry mycelial weight was recorded isolate AS 1 (90.33 mg) at pH 5. All the isolates showed significantly less growth at pH 5.

4.4 Disease development in relation to weather parameters

4.4.1 Disease development (PDI)

In the present investigation, disease development in relation to weather parameters were studied as described in 'Materials and Methods'. This study clearly depicts the relationship between the weather factors like temperature, relative humidity and rainfall with the development of the early blight of potato. The planting was taken on the 7th June 2013. Observations were taken from 27th standard week at weekly intervals. The data presented in Table 7 and field view of epidemiological plot is shown in Plate 7.

The PDI was lowest during 27th standard week (5.67) and increased throughout the cropping period. It was peak during last stage that is 34th and 35th standard week (72.66 and 78.33) respectively.

During cropping period maximum temperature ranged from 23.9°C (30th standard week) to 29.4°C (35th standard week), minimum temperature from 19.3°C (35th standard week) to 20.7°C (29th standard week), relative humidity (morning) from 91 per cent (35th standard week) to 95 per cent (27th – 31st standard week) and relative humidity (evening) from 63 per cent (35th standard week) to 88 per cent (30th standard week). Cumulative rainfall was 276 mm during the crop growth period.

4.4.2 Correlation of PDI with weather parameters

The PDI obtained at different stages of crop growth were correlated with weather parameters recorded during the respective stage. The correlation coefficients are presented in Table 8 and 8a. The results in Table reveals that during 2013, maximum temperature ($r = 0.45$) was non significantly positively correlated with PDI, minimum temperature ($r = -0.73$) was significantly negatively correlated with PDI. Morning relative humidity ($r = -0.71$) was significantly negatively correlated with PDI and evening relative humidity ($r = -0.36$) was non significantly negatively correlated with PDI. While, cumulative rainfall (0.96) was significantly positively correlated with PDI.

The data are again subjected to multiple linear regression analysis. The regression coefficient for PDI is given in the Table 9.

The regression equation is

$$Y = 753.41 - 5.88 X_1 + 28.71 X_2 - 11.82 X_3 - 0.78 X_4 + 0.24 X_5$$

Out of five weather variables selected for the study, only cumulative rainfall was found to be contributing significantly negative impact on PDI, while all other variables showed non significant. Including variables maximum and minimum temperature and relative humidity (morning and evening) and rainfall with $R^2 = 0.98$. According to these models, the observed and predicted disease severities of early blight of potato during *kharif* 2013 are given in Table 10. The results in Table gave actual and predicted PDI value which were in close resemblance to each other.

4.5 *In vitro* evaluation of fungicides

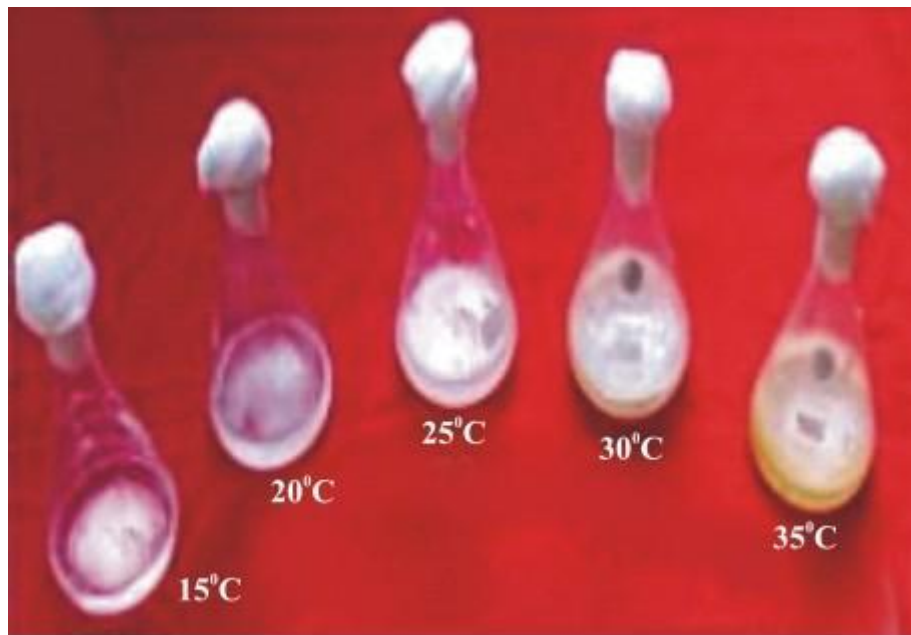
Four non systemic fungicides, five systemic fungicides and three combi products were tested against *Alternaria solani* by using poison food technique under laboratory conditions. The results on effect of contact fungicides and combi products were presented in table 11 and results on effect systemic fungicides on *Alternaria solani* were presented in Table 11a and Plate 8.

The results revealed that all the fungicides tested were significantly superior over the control in inhibiting the mycelium. Hexaconazole, tebuconazole, propiconazole, penconazole, Zineb 68% + Hexaconazole 4% and captan 68% + hexaconazole 5% are equally effective and significantly superior with 100% inhibition at all the concentration which are on par with difenconazole (100%) at 0.1 and 0.15 per cent concentration, mancozeb (99.33%) at 0.25% concentration, propineb (99.11%) at 0.25% concentration.

Least effective chemical was found to be chlorothalonil at all the concentration tested. At higher concentration (0.25%) of chlorothalonil the inhibition recorded was 44.73% followed by zineb (46.55%) at 0.1 per cent concentration.

Table 6: Effect of pH levels on isolates of *Alternaria solani*

pH	Dry mycelial weight (mg)				
	AS 1	AS 2	AS 3	AS 4	AS 5
5	90.33	181.66	125.66	161.33	108.33
6	177.33	291.00	233.66	212.66	147.66
7	290.33	313.00	304.33	306.66	285.33
8	269.66	299.00	296.66	310.66	313.00
9	202.66	178.00	238.00	198.33	253.66
Mean	205.26	252.66	239.66	237.93	221.60
	S.Em.±		CD at 1%		
Isolates (I)	0.99		3.96		
pH level (P)	0.61		3.32		
I X P	2.15		8.66		



a. Temperature



b. PH

Plate 6. Physiological studies of AS 1 isolate of *A. solani*

Table 7: Effect of weather parameters on Per cent Disease Index (PDI) of early blight of potato during *Kharif* 2013

Standard week No	Month and Date	Age of the crop (Days)	PDI	Temperature(°C)		Relative humidity (%)		Cumulative Rainfall (mm)
				Maximum	Minimum	Morning	Evening	
27	July 2-8	25	5.67	26.7	20.6	95.0	74.0	16.2
28	July 9-15	32	14.00	25.5	20.3	95.0	81.0	44.0
29	July 16-22	39	27.33	25.7	20.7	95.0	84.0	80.8
30	July 23-29	46	38.67	23.9	20.1	95.0	88.0	166.4
31	July 30-Aug 5	53	46.00	25.0	20.1	95.0	85.0	226.0
32	Aug 6-12	60	53.66	27.2	20.0	94.0	78.0	244.4
33	Aug 13-19	67	67.00	26.3	20.5	95.0	79.0	256.4
34	Aug 20-26	74	72.66	26.3	19.5	92.0	79.0	268.0
35	Aug 27-Sept 2	81	78.33	29.4	19.3	91.0	63.0	276.0



Plate 7. General view of experimental plot of epidemiological studies

Table 8: Correlation coefficient(r) for early blight of potato with weather parameters during 2013

Weather parameters	Correlation coefficient(r)
Maximum temperature (⁰ C)	0.45
Minimum temperature(⁰ C)	-0.73*
Relative humidity (morning) (%)	-0.71*
Relative humidity (evening) (%)	-0.36
Cumulative rainfall (mm)	0.96*

* = Significant at P=0.05

Table 8a: Correlation between PDI of early blight of potato in relation to weather parameters during 2013

Parameters	Y	X ₁	X ₂	X ₃	X ₄	X ₅
Y PDI	1.000					
X ₁ Maximum temperature (⁰ C)	0.459	1.000				
X ₂ Minimum temperature (⁰ C)	-0.737*	-0.487	1.000			
X ₃ Relative humidity (morning) (%)	-0.719*	-0.733	0.902	1.000		
X ₄ Relative humidity (evening) (%)	-0.360	-0.958	0.490	0.729	1.000	
X ₅ Cumulative rainfall (mm)	0.963*	0.335	-0.695	-0.592	-0.234	1.000

* = Significant at P=0.05

Table 9: Multiple linear regression between PDI of early blight of potato in relation to weather parameters during 2013

Parameter	X ₁ Maximum temperature (°C)	X ₂ Minimum temperature (°C)	X ₃ Relative humidity (morning) (%)	X ₄ Relative humidity (evening) (%)	X ₅ Cumulative rainfall (mm)
β- Value(RC)	-5.88	28.71	-11.82	-0.78	0.24
SE of β(r)	4.60	10.91	3.73	0.90	0.02
Intercept	753.41				
R ² value	0.98				
Multiple linear regression equation $Y=a+\beta_1X_1 +\beta_2X_2 +\beta_3X_3 +\beta_4X_4 + \beta_5X_5$					
$Y= 753.41 - 5.88 X_1 + 28.71 X_2 -11.82 X_3 -0.78 X_4 + 0.24X_5$					

Where

Y= PDI

X₁ =Maximum temperature (°C)

X₂ = Minimum temperature (°C)

X₃ =Relative humidity (morning) (%)

X₄= Relative humidity (Evening) (%)

X₅ =Rainfall (mm)

Table 10: Observed and predicted PDI of early blight of potato during *kharif* 2013

Time interval (week)	Per cent disease index(PDI)	
	Observed	Predicted
1	5.67	13.58
2	14.00	13.19
3	27.33	31.38
4	38.67	41.17
5	46.00	52.50
6	53.66	56.74
7	67.00	63.93
8	72.66	76.74
9	78.33	75.05

Table 11: *In vitro* evaluation of contact fungicides and combi -products against mycelial growth of *Alternaria solani*

Fungicides	Inhibition (%)			
	Concentrations (%)			Mean
	0.1	0.2	0.25	
Contact fungicides				
Mancozeb 75%WP	57.22 (48.91)*	65.23 (53.88)	99.33 (87.29)	73.92 (63.36)
Chlorothalonil 75%WP	15.23 (22.96)	34.31 (35.85)	44.73 (41.98)	31.42 (33.60)
Zineb 75% WP	46.55 (43.02)	62.77 (52.40)	52.33 (46.45)	53.88 (47.29)
Propineb 70%WP	47.28 (43.44)	57.31 (49.20)	99.11 (86.87)	67.9 (59.84)
Combiproducs				
Carbendazim 12% + Mancozeb 63% WP.	55.20 (47.99)	66.39 (54.57)	74.24 (59.51)	65.27 (54.02)
Captan 70% + Hexaconazole 5% WP	100 (90)	100 (90)	100 (90)	100 (90)
Zineb 68% + Hexaconazole4% WP	100 (90)	100 (90)	100 (90)	100 (90)
	S.Em. ±			CD at 1%
Fungicides (F)	0.42			1.58
Concentration (C)	0.22			1.03
F x C	1.36			2.75

*=Arcsine values

Table 11a: *In vitro* evaluation of systemic fungicides against mycelial growth of *A. solani*

Fungicides	Inhibition (%)			
	Concentration (%)			
	0.05	0.1	0.15	Mean
Difenconazole 25%EC	91.11 (72.67)*	100 (90.00)	100 (90.00)	97.03 (84.22)
Hexaconazole 5%EC	100 (90)	100 (90)	100 (90)	100 (90)
Tebuconazole 25%EC	100 (90)	100 (90)	100 (90)	100 (90)
Propiconazole 25%EC	100 (90)	100 (90)	100 (90)	100 (90)
Penconazole 10% EC	100 (90)	100 (90)	100 (90)	100 (90)
	S.Em. ±		CD at 1%	
Fungicides (F)	0.06		0.25	
Concentration (C)	0.05		0.19	
F x C	0.21		0.43	

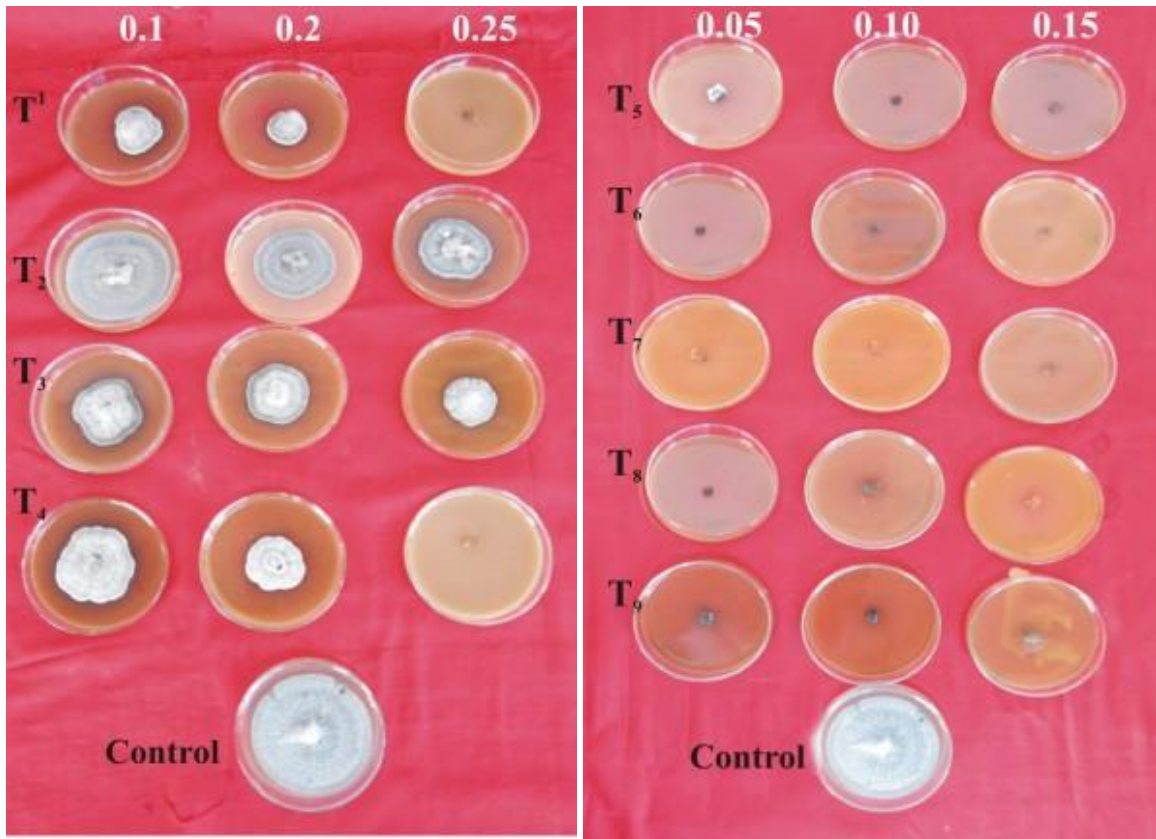
*=Arcsine values

LEGEND

- T₁ - Mancozeb 75% WP
- T₂ - Chlorothalonil 75% WP
- T₃ - Zineb 70% WP
- T₄ - Propineb 70% WP
- T₅ - Difenconazole 25% EC
- T₆ - Hexaconazole 5% EC
- T₇ - Tebuconazole 25% EC
- T₈ - Propiconazole 25% EC
- T₉ - Penconazole 10% EC
- T₁₀ - Mancozeb 63% + Carbendazim 12% WP
- T₁₁ - Captan 70% + Hexaconazole 5% WP
- T₁₂ - Zineb 68% + Hexaconazole 4% WP

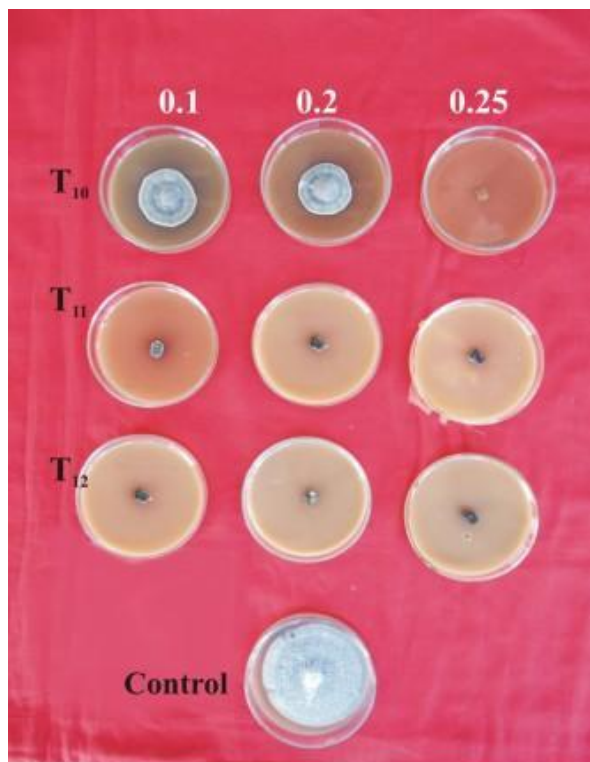
Legend

T ₁	: Mancozeb 75%WP
T ₂	: Chlorothalonil 75%WP
T ₃	: Zineb 75% WP
T ₄	: Propineb 70% WP
T ₅	: Difenconazole 25% EC
T ₆	: Hexaconazole 5%EC
T ₇	: Tebuconazole 25%EC
T ₈	: Propiconazole 25%EC
T ₉	: Penconazole 10%EC
T ₁₀	: Mancozeb 63% + Carbendazim 12% WP
T ₁₁	: Captan 70% + Hexaconazole 5% WP
T ₁₂	: Zineb 68% + Hexaconazole 4% WP
T ₁₃	: Control



a. Contact fungicides

b. Systemic fungicides



c. Combiproduts

Plate 8. *In vitro* evaluation of fungicides against *A. solani*

4.6 Field evaluation of fungicides

4.6.1 Field efficacy of fungicides

This study was undertaken to evaluate the efficacy of some of the fungicides against early blight of potato caused by *Alternaria solani*. Totally twelve fungicides were tested in farmers's field of Narendra village near UAS Dharwad. Three sprays were given in fifteen days interval starting from the initiation of the disease and control plot was maintained to know the difference between treated and the untreated plants. The observations were recorded before each spray. Further, these observations were taken by following 0-9 disease scale and converted into per cent disease index (PDI) using the formula given by the Wheeler(1969). The observations were presented in Table 12 and Plate 9.

Observations on disease severity was recorded before the first spray, which is non significant. There is no significant difference between the treated and the untreated checks. But later it is observed that at 50, 65 and 75 DAS the treatment differ significantly over the unprotected check. Maximum per cent disease index was noticed in control 72.66% followed by mancozeb (52%) at 75 DAS. The least PDI (21.50%) was noticed in Zineb 63% + Hexaconazole 4% which is on par with tebuconazole 25% EC (22%) and Captan 70% + Hexaconazole 5% (24.66%). The next best treatment was found to be Mancozeb 63% + Carbendazim 12% (27%) followed by hexaconazole 5% EC (28.66%) at 75 DAS.

4.6.2 Economic analysis of fungicides with early blight of potato.

The yield data of all the treatments has been taken and economics of cost benefit ratio has been calculated for all the fungicides tested which is presented in Table 13. The highest yield (18.83 tonnes/ha) was obtained in Zineb 63% + Hexaconazole 4% with cost benefit ratio of 4.55 which is on par with tebuconazole 25% EC (18 t/ha) with cost benefit ratio of 4.30 and captan 68% + hexaconazole 5% (17.83 t/ha) with B:C ratio of 4.29. Yield was least in control (8.83 t /ha) and cost benefit ratio was 1.54.

4.7 Screening of potato varieties for early blight incidence

In order to identify the resistant sources against early blight of potato forty five genotypes were screened by using 0-9 scale in *Khariif* 2013 at All India Coordinated Research Project on Potato at Regional Horticulture Research Centre, Saidapur farm , UHS Dharwad campus. The result pertaining to the field screening of genotypes were presented in Table 14 and Plate 10.

Among the forty five genotypes none of the genotypes showed immune and resistant reaction. Six of the genotypes were moderately resistant viz. C-13, Kufri Pukhraj, P-3, AICRP-SH-1, SH-1 and C-17. Nine genotypes were moderately susceptible viz. C-1, C-10, C-34, C-11, Kufri Ashoka, Kufri Surya, SH2, C-4 and P-4. Where as twenty three genotypes showed susceptible reaction viz, C-16, C-26, C-28, C-31, C-13, AICRP-SH-2, CH-3, P-1, MM-12, PH-3, PH-1, Kufri Jyothi, P-9, Kufri Sadabahar, Kufri Pushkar, Kufri Khyati, Kufri Himasona, C-24, C-13, C-22, LB-24 and LB-3 and seven genotypes viz, C-18 ,C-20, PH-2, P-2, EM-1, LB-4, LB-5 were highly susceptible.

Table 12 : Field efficacy of fungicides for the management of early blight of potato

Treatments	Conc (%)	Percent Disease Index (PDI)			
		Before 1 st spray (35DAS)	Before 2 nd Spray (50DAS)	Before 3 rd Spray (65 DAS)	10 days after last spray (75DAS)
Mancozeb 75%WP	0.2	14.13 (22.08)*	32.00 (34.41)	40.67 (39.61)	52.00 (46.15)
Chlorothalonil 75%WP	0.2	14.00 (21.96)	30.33 (33.41)	39.33 (38.77)	47.00 (43.28)
Zineb 75% WP	0.2	13.26 (21.36)	18.33 (25.34)	27.33 (31.48)	37.00 (37.46)
Propineb 70% WP	0.2	13.5 (21.55)	24.00 (29.32)	30.33 (33.41)	40.16 (39.33)
Difenconazole 25% EC	0.1	12.30 (20.53)	21.00 (27.22)	25.67 (30.34)	33.16 (35.15)
Hexaconazole 5%EC	0.1	11.96 (20.23)	18.50 (25.46)	20.33 (26.76)	28.66 (32.29)
Tebuconazole 25%EC	0.1	15.00 (22.78)	11.83 (20.12)	16.67 (23.98)	22.00 (27.97)
Propiconazole 25%EC	0.1	13.66 (21.68)	16.00 (23.57)	25.00 (29.84)	31.83 (34.34)
Penconazole 10%EC	0.1	14.00 (21.94)	23.33 (28.28)	32.67 (34.84)	39.00 (38.64)
Mancozeb 63% + Carbendazim 12% WP	0.2	14.66 (22.49)	14.33 (22.17)	18.00 (25.10)	27.00 (31.16)
Captan 70% + Hexaconazole 5% WP	0.2	15.33 (23.04)	9.67 (18.08)	17.00 (24.33)	24.66 (29.70)
Zineb 68% + Hexaconazole 4% WP	0.2	14.06 (22.02)	9.00 (17.30)	15.67 (23.29)	21.50 (27.61)
Control	-	14.00 (21.96)	37.33 (39.54)	53.66 (47.11)	72.66 (58.57)
Mean		13.83	20.43	27.87	36.66
S.Em. \pm		0.59	1.33	1.73	1.48
CD at 5%		NS	3.89	5.04	4.33

*=Arcsine values

Table 13: Economic analysis of management of early blight of potato

Treatments	PDI	Yield (t/ha)	B:C ratio
Mancozeb 75%WP	52.00 (46.15)*	11.50	2.41
Chlorothalonil 75%WP	47.00 (43.28)	12.67	2.73
Zineb 70% WP	37.00 (37.46)	14.17	3.20
Propineb 70% WP	40.16 (39.33)	15.33	3.53
Difenconazole 25% EC	33.16 (35.15)	16.83	3.88
Hexaconazole 5%EC	28.66 (32.29)	17.17	4.03
Tebuconazole 25%EC	22.00 (27.97)	18.00	4.30
Propiconazole 25%EC	31.83 (34.34)	16.50	3.87
Penconazole 10%EC	39.00 (38.64)	16.30	3.77
Mancozeb 63% + Carbendazim 12% WP	27.00 (31.16)	16.83	3.97
Captan 70% + Hexaconazole 5% WP	24.66 (29.70)	17.83	4.29
Zineb 68% + Hexaconazole 4% WP	21.50 (27.61)	18.83	4.55
Control	72.66 (58.57)	8.50	1.54
S.Em. ±	1.48	1.38	
CD at 5%	4.33	4.04	

*=Arcsine values



a. Field view of experimental plot for fungicide evaluation



b. Zineb 63% + Hexaconazole 4%



c. Control

Plate 9. Field evaluation of fungicides against early blight of potato

Table 14: Field evaluation of promising potato genotypes for disease resistance

Grade	Disease reaction	Name of the genotypes	No of genotypes
0	Immune	-	0
1	Resistant	-	0
3	Moderately resistant	C-13,Kufri Pukhraj, P-3, AICRP-SH-1, SH-1,C-17	6
5	Moderately susceptible	C-1, C-10, C-34, C-11, Kufri Ashoka, Kufri Surya, SH-2, C-4, P-4	9
7	Susceptible	C-16, C-26, C-28, C-31, C-13, AICRP-SH-2, CH-3, P-1, MM-12, PH-3, PH-1, Kufri Jyothi, P-9, Kufri Sadabahar, Kufri Pushkar, Kufri Khyati, Kufri Himasona, C-24, C-13, C-22, LB-24, LB-3	23
9	Highly susceptible	C-18, C-20, PH-2, P-2, EM-1, LB-4, LB-5	7



a. Overall view of screening field



b. Moderately resistant (kufri Pukhraj)



c. Highly susceptible (C-18)

Plate 10. Field screening of potato genotypes against early blight of potato

DISCUSSION

Potato is considered as 'The King' in food staples and hardly any domestic kitchen is available which does not use it one or the other form as it possesses all the attributes to be a potential food crop. 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 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).

Among all the fungal diseases of potato early blight of potato caused by *Alternaria solani* (Ellis and Martin) Jones and Groot has assumed a very serious problem all over the world. During the last few years early blight has been occurring almost every year primarily due to the local overwintering/oversummering of inoculum, cultivation of susceptible varieties and favourable environmental conditions. Foliar symptoms are most common, with tuber symptoms occurring infrequently, particularly when the foliar phase of the disease is well managed.

The first reference to the fungus as a parasite and its association with potato leaf blight was by Galloway (1891) in Australia. In the USA, Chester (1892) noted the disease on potatoes and other cultivated plants of the Solanaceae, particularly tomato (*Lycopersicon esculentum* Mill.) and eggplant (*Solanum melongena* L.). He described the symptoms and observed that the progress of early blight was slower than that of late blight, caused by *Phytophthora infestans* (Mont.) deBary. He also noticed that potato plants severely affected by early blight had lower yields and produced smaller tubers.

Potato is highly remunerative and nutritive crop in parts of northern Karnataka where it serves as a staple food. Its cultivation is carried in large tracts in major potato growing districts like Dharwad and Belagavi. So survey of the early blight disease has been carried out in these areas. Weather is main limiting factor for early blight disease development since it is beyond our control. So disease development in relation to weather factors has been studied. The ultimate control of pathogen is by the cultivation of resistant varieties. However, farmers in pursuance of high yield are inclined to cultivate some varieties which may be less resistant to disease. On such varieties fungicide application can reduce the disease severity and yield reduction due to disease can be minimized. In fact timely application of fungicides is the best method to control early blight as reported by several research workers (Mathur *et al.*, 1971; Singh, 1971; Dahmen & Staub, 1992.).

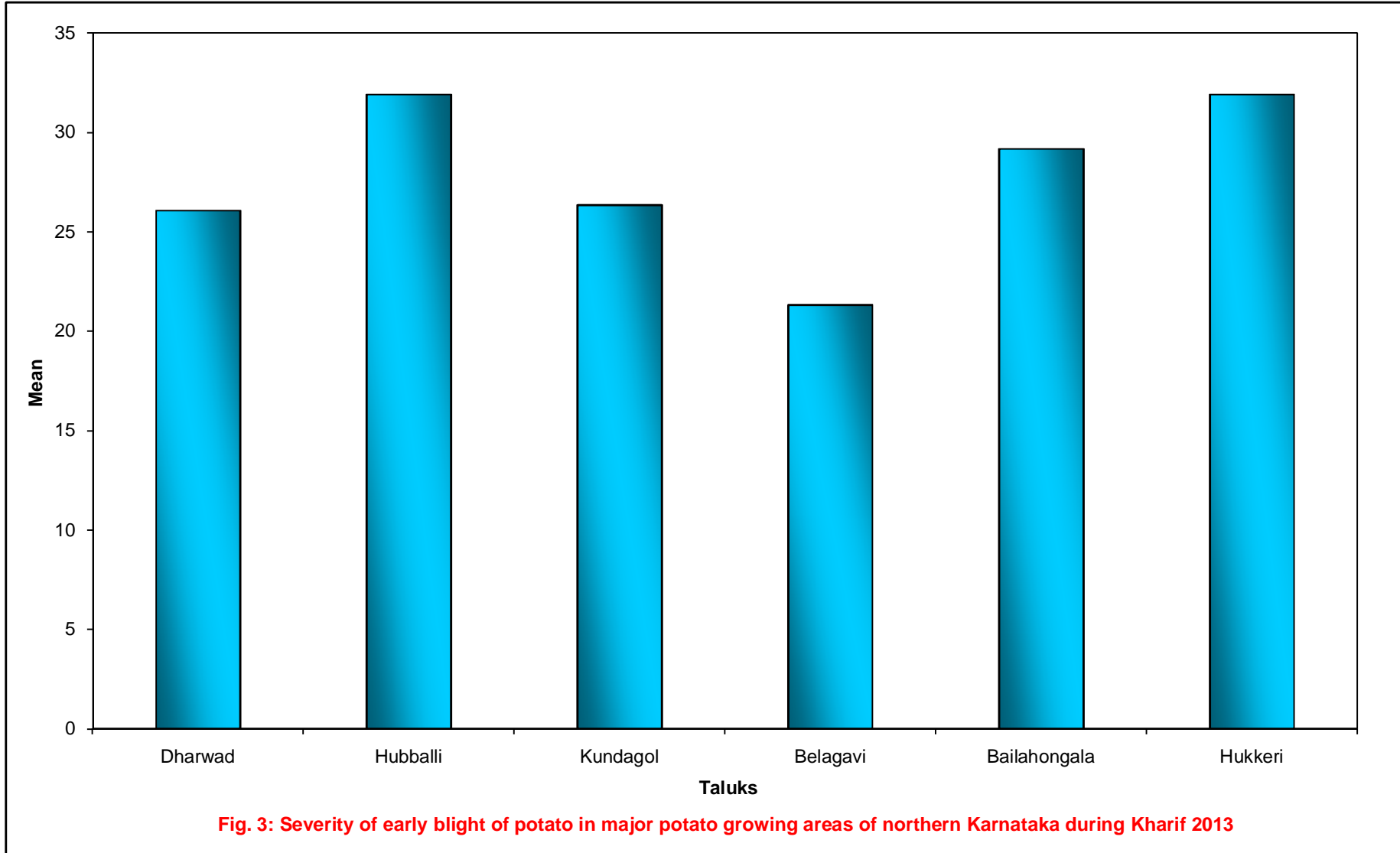
Some of the laboratory studies also given importance like isolation and proving the pathogenicity of the fungus, *in vitro* evaluation of fungicides and studying the characters of pathogen among different isolates collected during the survey. So considering the significance and prevalence of the disease, the above mentioned studies has been carried out and the results so obtained are discussed here under.

5.1 Survey for early blight of potato

A detailed roving survey was conducted during the *Kharif/rabi* 2013 in Dharwad and Belagavi districts of northern Karnataka to gather the information on the incidence, distribution and spread of *Alternaria* leaf blight in different localities. The data were depicted in Fig 3. The study is very helpful to know the severity of early blight in northern Karnataka where extensively potato has been cultivated over the years.

The early blight severity ranged between 12.33 to 38.33% in both the districts. The highest district average disease incidence was recorded in Dharwad (26.93%) followed by Belagavi. (25.52%). Among the talukas surveyed, maximum PDI was recorded in Hubballi taluk (31.89%) of Dharwad district and Hukkeri taluk (31.89%) of Belagavi district. Least severity was observed in Belagavi taluk (21.31%). Among the villages, the highest severity of early blight was noticed in fields of Narendra village (38.33%) of Dharwad district. Where as least severity was observed in Kadoli village, (12.33%) of Belagavi district.

The present investigations indicated variable disease incidence as well as disease intensity at different places. It may be associated with prevalent environmental and pathogen factors. Changes in weather variables and amount of initial inoculum of *A. solani* may be responsible for varying disease intensities at different locations (VanderWalls *et al.*, 2003). 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). These observations are also supported by the findings of Duhan and Suhag (1989), Hilal and Kamal (1990), Fazal *et al.* (1994), Rotem (1994), Anastasiam *et al.* (1998) and Ghosh *et al.* (2009). Lower disease at some locations could be attributed to balanced dose of fertilizers, wider spacing besides rapid disposal of debris. These observations are in accordance with the findings of Humpherson (1983).

5.1.2 Symptomatology

The symptoms on potato plants in the field were noticed on the older leaves as minute brown to black necrotic spots measuring one to two mm in diameter. These spots often enlarged with concentric rings to produce a characteristic target spot effect. Later upward progress of the disease was observed and leaves dried up and drooped down. Similar description of symptoms on tomato and potato were made by Walker (1952) and Singh (1987). Singh (1987) reported that the spots were oval to angular in shape measuring upto 0.3-0.4 cm in diameter and usually with chlorotic zone around the spot. Older leaves were affected first and progressed upward finally the leaves dried up and fell off. According to Mayee and Datar (1986), the early blight disease of potato was characterized by the appearance of brown to dark brown colour necrotic spots. Appearance of concentric rings inside the spots produced target spot effect.

5.2 Isolation, identification and proving the pathogenicity of the fungus

5.2.1 Isolation

In the present study, *Alternaria solani* was isolated from the leaves of potato showing typical symptoms of early blight using tissue isolation and purified using single spore isolation method. The pure culture of the fungus obtained from these two methods was used in various laboratory studies. Dhiman and Chadha (1986) obtained pure culture of the fungus using tissue isolation method and described it as a new technique for inoculum preparation.

5.2.2 Pathogenicity test

The pathogenicity of the fungus was established by artificial inoculation of spore suspension on potato plants under glasshouse conditions. First symptoms appeared on older leaves ten days after inoculation as brown necrotic spots with concentric rings at the center. A similar technique was followed by Dhiman *et al.* (1980) to prove the pathogenicity of *A. solani* on tomato. Similar observations were also recorded by Ganie *et al.* (2013) on detached leaves of potato. The reisolated pathogen exhibited similar characters as in the originally isolated culture of *Alternaria solani*.

5.2.3 Identification of the fungus

The fungus *Alternaria solani* was identified based on the morphological characters. The conidiophores arise singly or in small groups, straight or flexuous, septate, rather pale brown or olivaceous brown. Conidia are muriform usually solitary, straight or slightly flexuous, obclavate or with the body of the conidium oblong or ellipsoidal tapering to a beak which is commonly the same length as or rather longer than the body. Colour of the conidia is pale or olivaceous brown, overall length usually 140-230 μ , 19-36 μ thick in the broadest part, with 7-12 transverse and 3 to 6 longitudinal septa.

The description of the fungus was found to be in agreement with description given by Ellis (1971). On the basis of morphological characters, pathogenicity and comparison with the authentic description, the fungus was identified as *Alternaria solani* (Ellis and Martin) Jones and Grout. The morphological descriptions of the pathogen almost corroborate with descriptions given by Neergard (1945). Ganie *et al.* (2013) reported that conidia of *A. solani* are long beaked, muriform, dark coloured, borne singly, both longitudinal and transverse septa are present in mature conidia. Size of the conidia ranges from 15-19 x 150-300 μ m.

5.3 Collection of different isolates and studying the pathogen character

Five isolates were collected from different localities during the survey. The isolates were named as AS 1 (Dharwad), AS 2 (Hubballi), AS 3 (Belagavi), AS 4 (Kolar) and AS 5 (Bengaluru). The results of cultural and morphological variability among the different isolates are discussed hereunder.

5.3.1 Growth phase studies of the fungus

The growth pattern of the *Alternaria solani* was studied on Potato dextrose broth. The maximum mycelial weight was obtained fourteenth day (288.66 mg) after inoculation and it is considered as a optimum period for the growth of the fungus. Subsequent reduction in the mycelial weight was observed after fourteen days and data were presented in Fig 4.

The increase in the mycelial dry weight up to 14 days is attributed due to presence of nutrients in the medium and fungus showed gradual decrease in the weight by utilizing them to the medium extent. The decrease in the dry mycelial weight from 15 days onwards may probably be due to autolysis of the mycelium and exhaustion of nutrients in the medium. Sandya (1996) attained maximum dry mycelial weight of *Alternaria alternata* in Czapeck's dox medium sixteen days after incubation and Padmanabhan and Naryanswamy (1977) recorded maximum dry mycelial weight of *Alternaria macrospora* fourteen days after inoculation. Thus the results obtained in the experiment are found in conformity with earlier reports.

5.3.2 Conidia measurement of different isolates

The conidia of different isolates were measured based on length, breadth, beak length and beak width, number of horizontal and vertical septa. Since it act as a important tool for identification of the fungus. Variation was observed among the isolates of *A. solani*. Among the five isolates maximum length and thickness of conidia was observed in isolate AS 4 (235-285 X 29- 32 μm) and minimum length and breadth was recorded in AS 5 (150-185 X 15-25 μm). Longest beak was observed in isolate AS 4 (138 -180 μm) and minimum length of beak was observed in isolate AS 5 (138 -180 μm). Beak width was more in AS 3 (9 - 14.8 μm) and it was least in AS 2 (6- 8.5 μm). Maximum number of horizontal septa was found in isolate AS 1. (7-12) Least number of horizontal septa were observed in AS 4 and AS 5 (2-7 and 3-6) respectively and higher number of vertical septa was found in AS 1 (3-6) and less number of vertical septa was recorded in AS 4. (1-2). All the isolates were compared with standard description given by the Ellis (1971) and it is found in agreement with his description. So the identity of the causal organism of all the isolates was confirmed as *Alternaria solani*.

5.3.3 Colony characters of isolates on different solid media

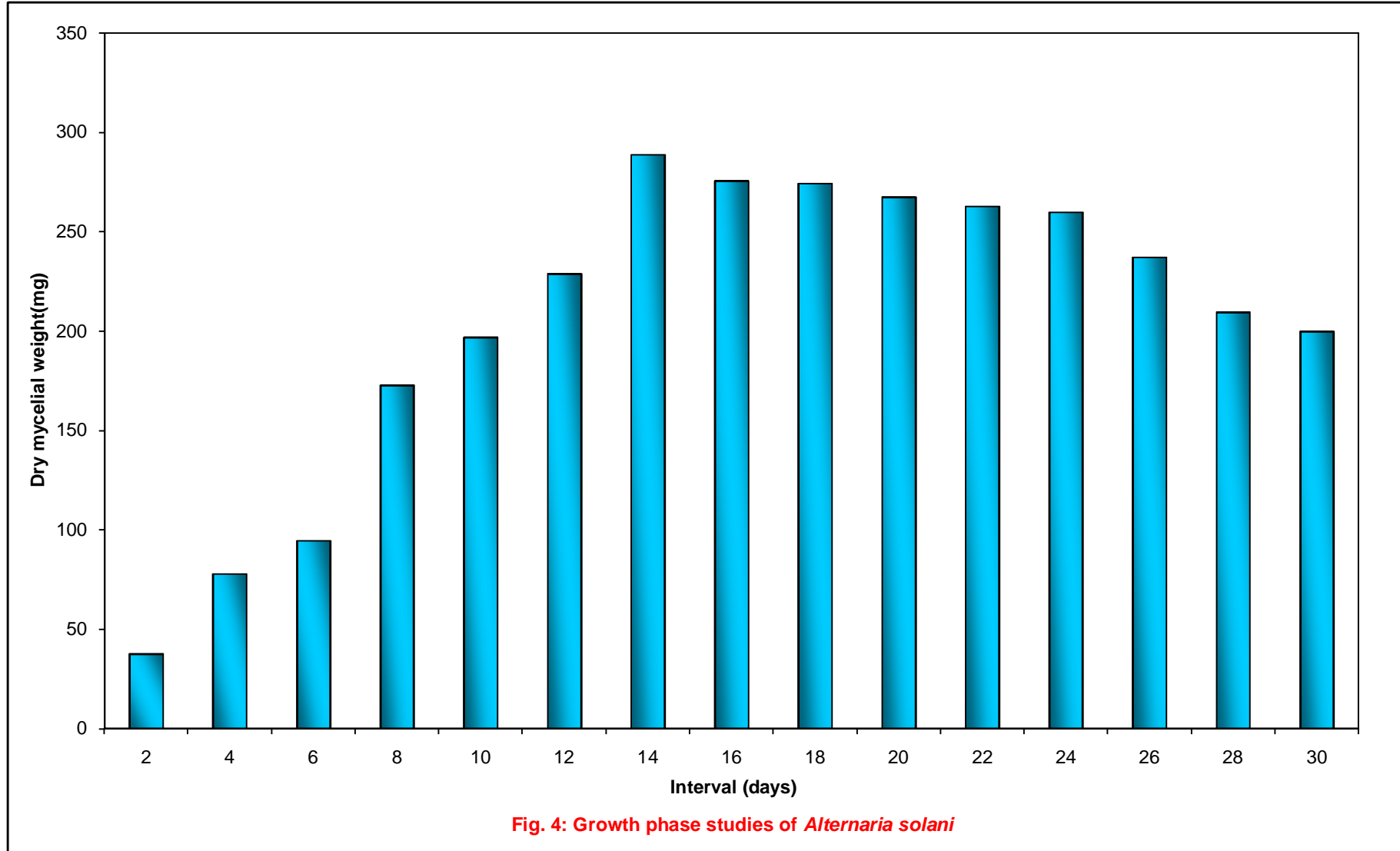
All the five isolates were studied on different synthetic and non synthetic media to know the morphological difference among them like colony colour, type of margin, topography of the isolates, sporulation. Colony diameter was also measured. The growth of the mycelium is very fast in Potato carrot agar followed by Czepeck's dox agar and Potato dextrose agar. The growth was found to be slow in Corn meal agar and Sabouraud's agar for all the isolates .The results are found in accordance with reports of Somappa *et al.* (2013) who observed that maximum radial growth was recorded on Czapeck's dox agar medium (50 mm) followed by Potato dextrose agar (37mm). The growth is very slow in corn meal agar and Sabouraud's agar for all the isolates. The isolate AS 5 showed slightly less growth on corn meal agar, Czapeck's dox agar and Richards agar compared to other isolates.

Maximum colony diameter is present in isolate AS 3 on PDA,(90 mm), isolate AS 1, AS 2 and AS 4 on PCA,(90 mm), Isolate AS 1 on Czapeck's dox agar (90 mm) and isolate AS 4 on Richards agar (90 mm). Least measurement is obtained on Corn meal agar (60.33 mm) in isolate AS 2. Sporulation was excellent in Potato carrot agar followed by Potato dextrose agar, Host leaf extract agar. Least sporulation was found in Sabouraud's agar. Bonde (1929), Neergaard (1945), Mazzonetto *et al.* (1996) and Arunkumar (2006) observed Potato dextrose agar as the best medium for *Alternaria* spp.

There was a considerable variation among the isolates of *A. solani* with respect to cultural, morphological and physiological characters indicating their wider adaptability and more virulence. The characters of pathogen found to vary with the difference in the geographical conditions. Morphological, cultural, physiological and molecular characters of different isolates of *Alternaria solani* were studied by Kumar *et al.* (2008). Similar attempts were made by Bruce (2006), Varma *et al.* (2007) and Naik *et al.*, (2010).

5.3.4 Effect of temperature levels on isolates of *Alternaria solani*

A diverse temperature requirement of five isolates of *A. solani* for their growth was observed and data were represented in Fig 5. The maximum dry mycelial weight is obtained in isolate AS 4 at 25°C (401.75 mg). Least dry mycelial weight (48 mg) was obtained in isolate AS 5 at 15°C. All the isolates showed significantly less growth at 15° C temperature. So from the experiment it is concluded



that optimum temperature required for the growth of *A. solani* is 25-30° C. The results of the experiment were found in accordance with results reported by Arunkumar (2006) and Alhussaen (2012) who observed that 25-30°C is optimum for the growth of *Alternaria solani*.

5.3.5 Effect of pH levels on isolates of *Alternaria solani*

Hydrogen ion concentration of the medium has a profound effect upon the rate and amount of growth and many other life processes of the fungus (Lilly and Barnett, 1951). All the isolates were studied at different pH levels and data were represented in Fig. 6. The maximum dry mycelium weight was found in isolate AS 2 (313 mg) at pH 7 and AS 5 (313 mg) at pH 8 which is on par with isolate AS 4 (310.66 mg) at pH 8 and isolate AS 4 (306.66 mg) at pH 7. Least dry mycelial weight (90.33 mg) was recorded isolate AS 1 at pH 5. All the isolates showed significantly less growth at pH 5. In the present investigation, neutral to slightly alkaline pH (7 to 8) was preferred by all the isolates. These results are in conformity with the reports of Gemawat and Ghosh (1980) who observed that *A. solani* was capable of growing on wide range of pH (4.0 to 9.5) and maximum growth and sporulation were observed at pH of 6.3.

5.4 Disease development in relation to weather parameters

The present investigation of early leaf blight of potato was undertaken at Narendra village of Dharwad taluk and represented in Fig 7. The total rainfall at Narendra was 276 mm during the cropping period. The mean minimum and maximum temperature during this period ranged from 19.3 to 29.4° C. The mean relative humidity ranging from 63 to 95 per cent. All these weather factors were favourable for the disease development during *kharif* 2013. Early blight develops more rapidly during periods when environmental conditions alternate between humidity and drought. The attacks cause serious economic losses in potato crops (Bashi and Rotem 1975). Many scientists have developed epidemiological models, in order to predict when the disease will occur and take up the control measures (Díaz *et al.* 1998, Fry 1998, Johnson *et al.* 1998, Shtienberg *et al.* 1989). Almost all these models are based on the use of meteorological parameters, especially relative humidity, temperature and rainfall.

In the weather studies the per cent disease index at weekly interval were also calculated. This was progressing at linear rate as the age of the plant was increasing. Older leaves were more susceptible than the younger leaves of the plants. This observation was in accordance with Roopa (2012) who reported progress of the disease with age of the plant on early blight of tomato. The formation of conidiophores and the spore content in the atmosphere are influenced by changes in atmospheric conditions. All weather parameters were correlated with PDI and data were depicted in Fig. 8 and 9. The maximum temperature was positively correlated with the disease. The spore content in the atmosphere increases when the mean, maximum and minimum temperatures increases, making *Alternaria* a temperature-dependent fungus. *Alternaria* is a saprophytic fungus with an optimal development shown to occur in the temperature ranges of 22–28°C (Hjelmroos 1993). The relative humidity (morning an evening) had negative correlation with PDI. Relative humidity may not play a major role in early blight disease development. The results found in conformity with Sabariego *et al.* (2000) who reported that negative correlation was observed with humidity. Mehboob *et al.* (2013) also reported relative humidity and wind speed almost had no significant effect on early blight severity of potato.

In the present investigations rainfall had significant positive effect on PDI. The rainfall during the experimental period might have favoured conidial germination, multiplication and disease development. Kulkarni (1998) reported that intermittent rainfall was found to be more favorable for early blight development in potato. The multiple linear regression equation developed for *kharif* 2013 was $Y = 753.41 - 5.88 X_1 + 28.71 X_2 - 11.82 X_3 - 0.78 X_4 + 0.24 X_5$. The weather factor put together influence PDI to the extent of 78.33%. The R^2 value was 0.98. This indicates that the disease was highly related with weather factors. The increase in severity of the disease was highly dependent on weather parameters. By using this equation the observed and predicted values were tabulated and presented in Fig. 10. There was not much difference observed. Similar model was developed by Arunkumar (2008) for *Alternaria* blight of chrysanthemum. Ravichandran (2012) reported that maximum temperature was significantly correlated with PDI on purple blotch of onion.

5.5 *In vitro* evaluation of fungicides

The use of fungicides is most effective method for the management of early blight of potato. Currently, there are good fungicide options available for potato early blight control. But careful product

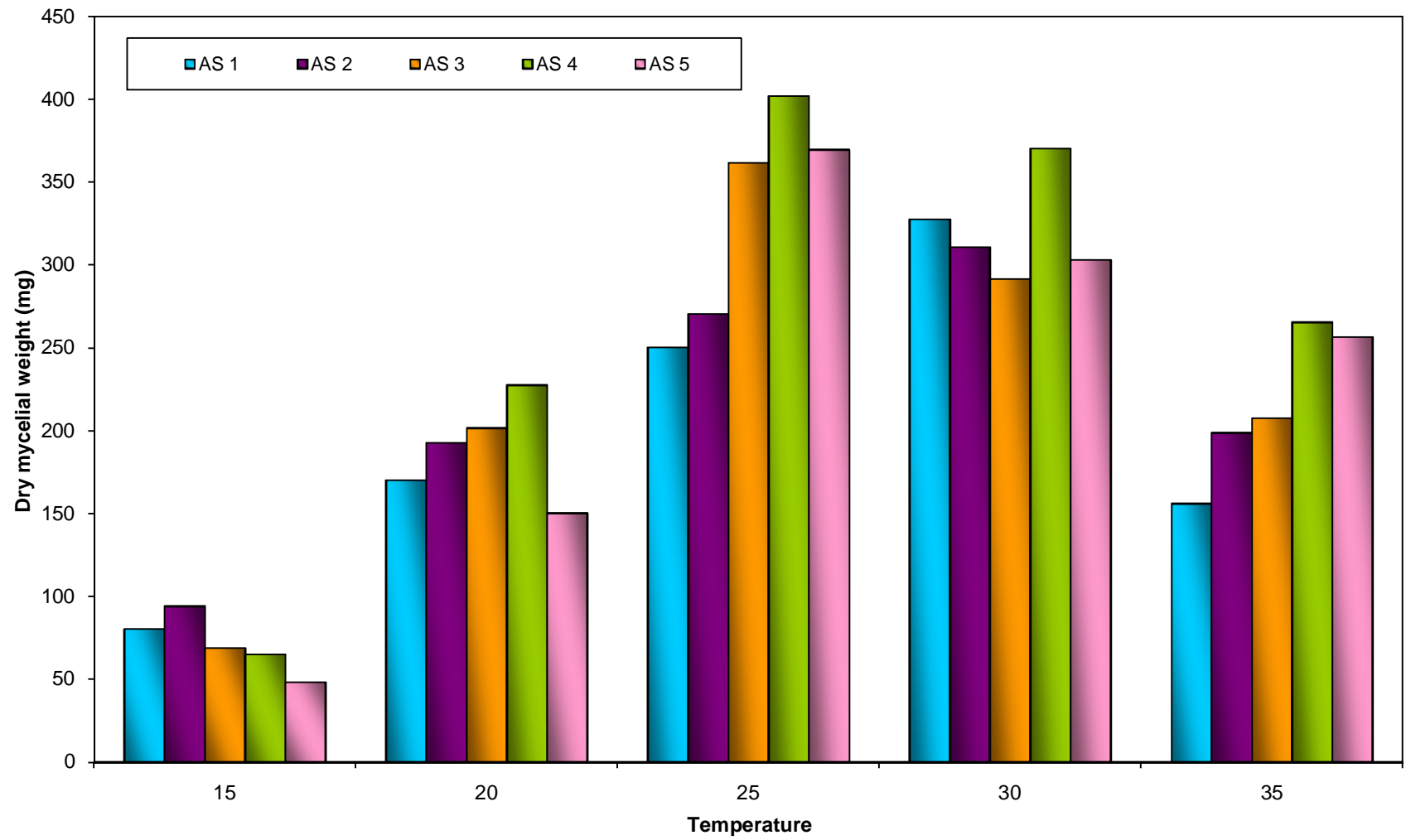
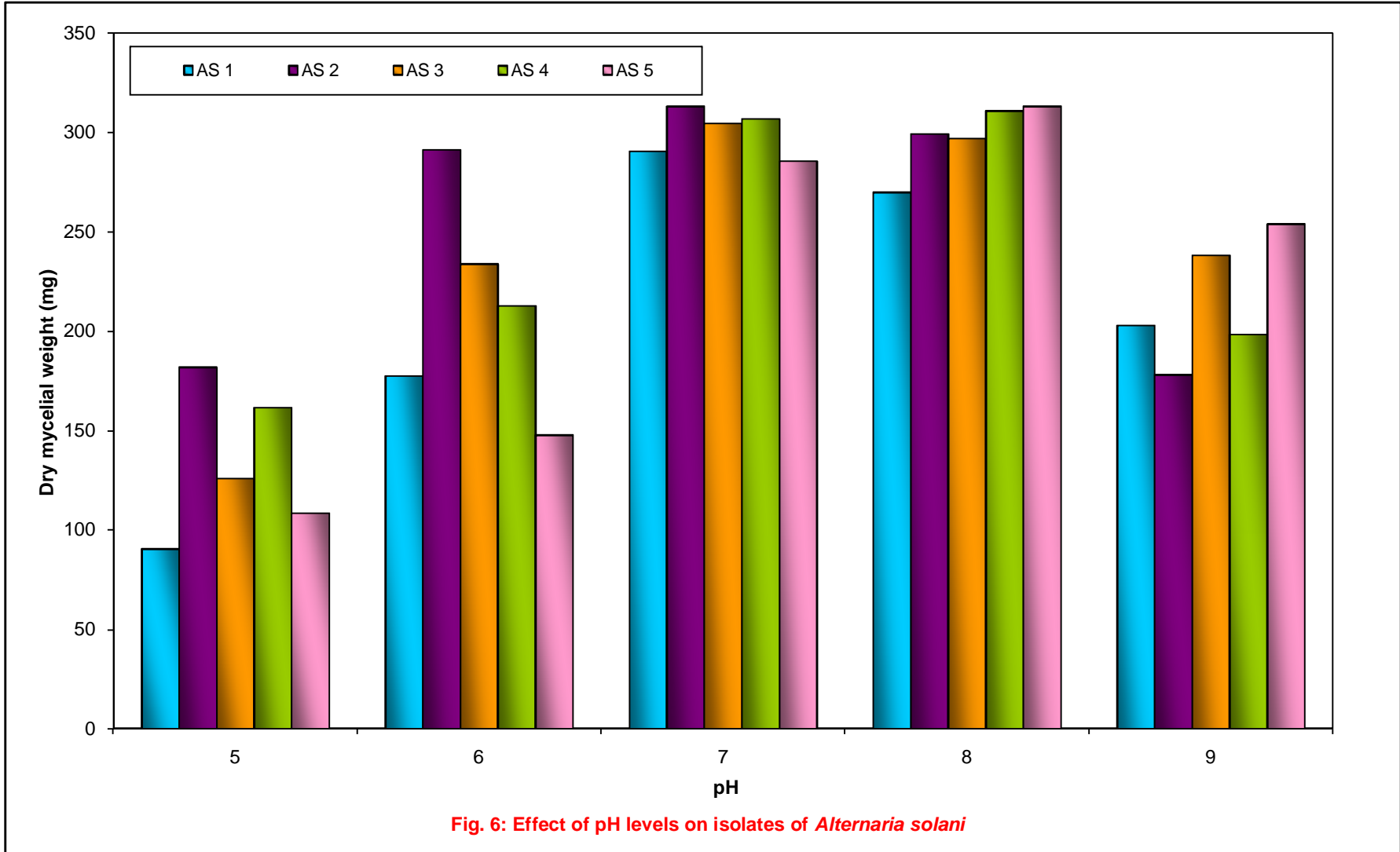
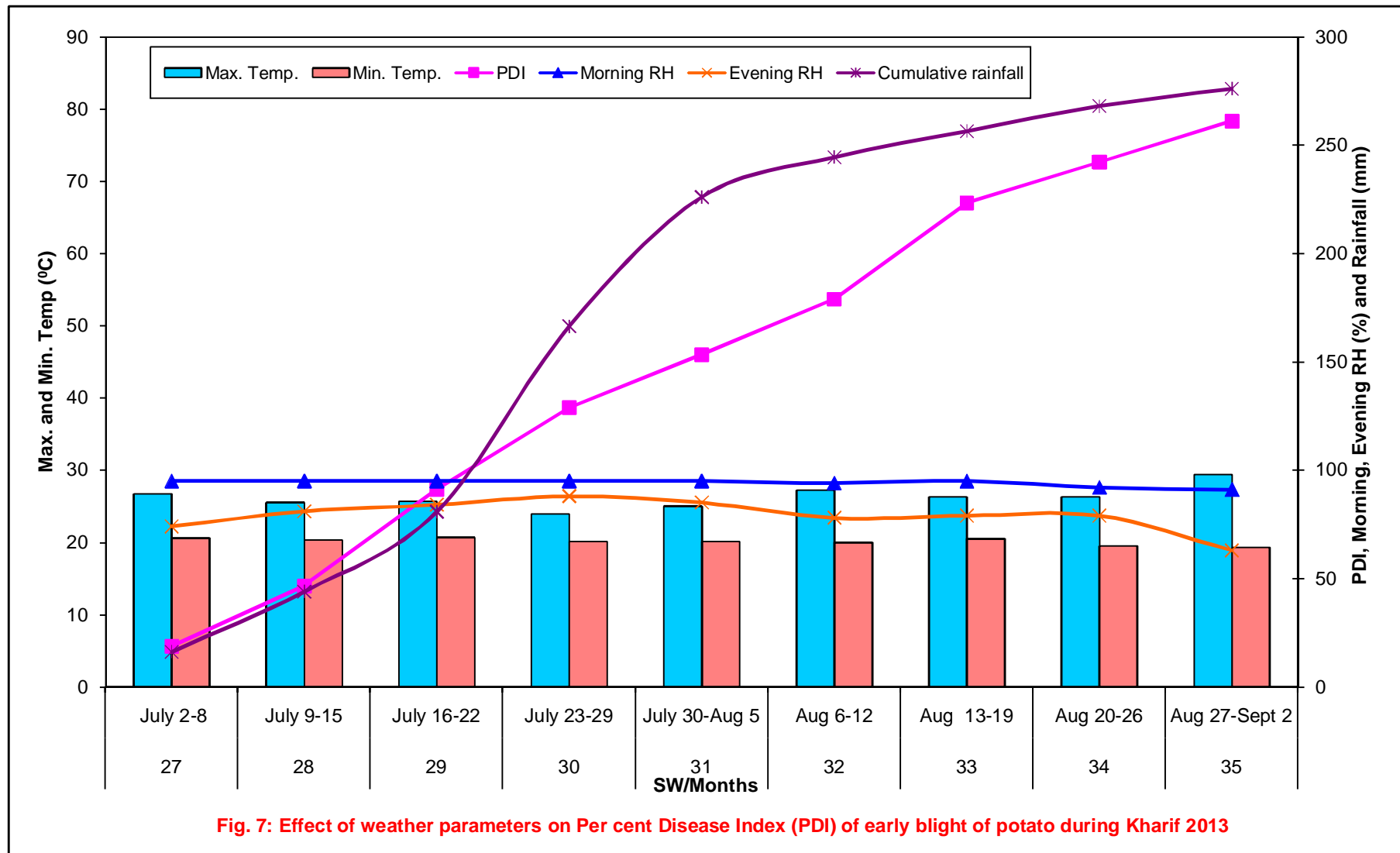
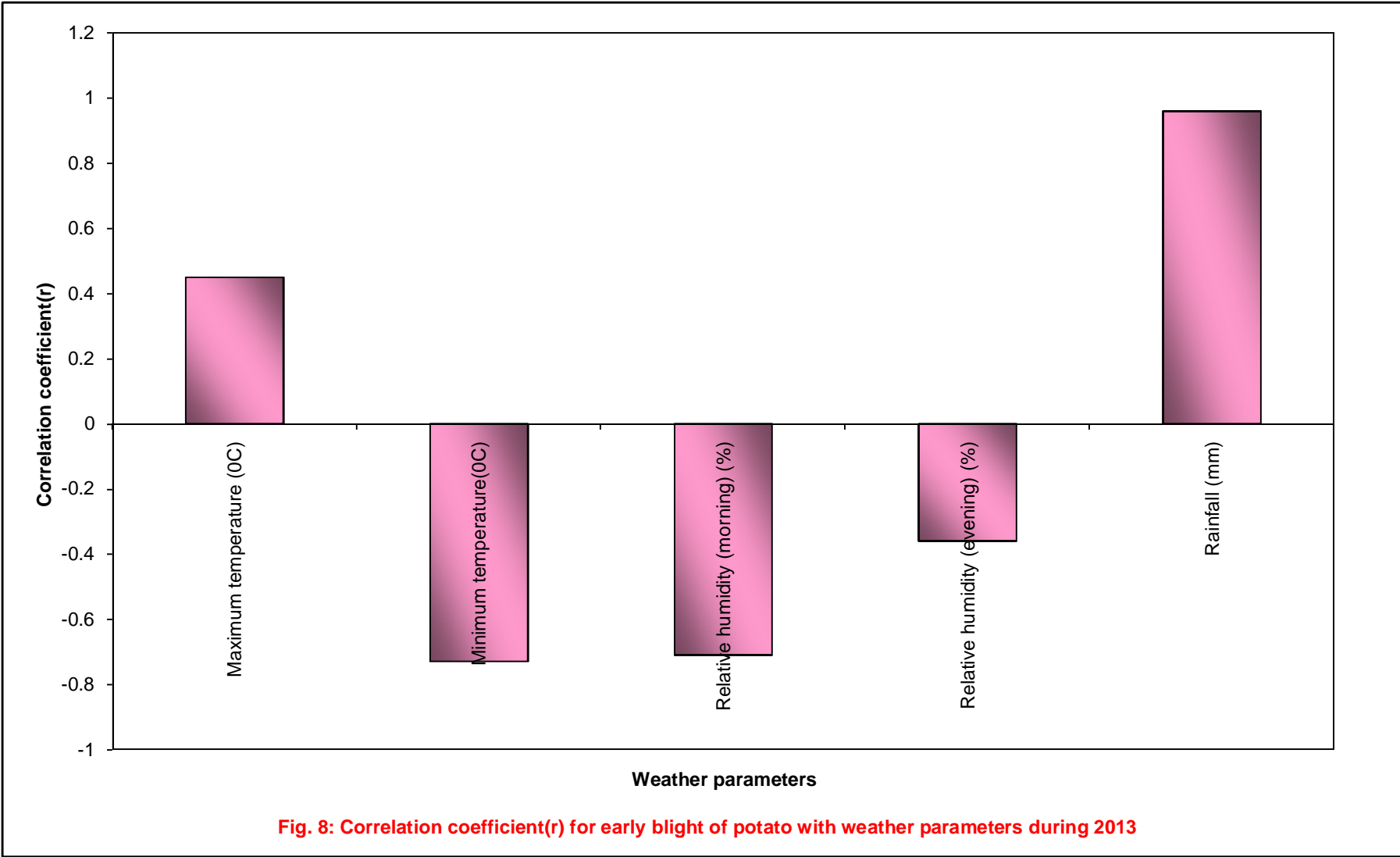
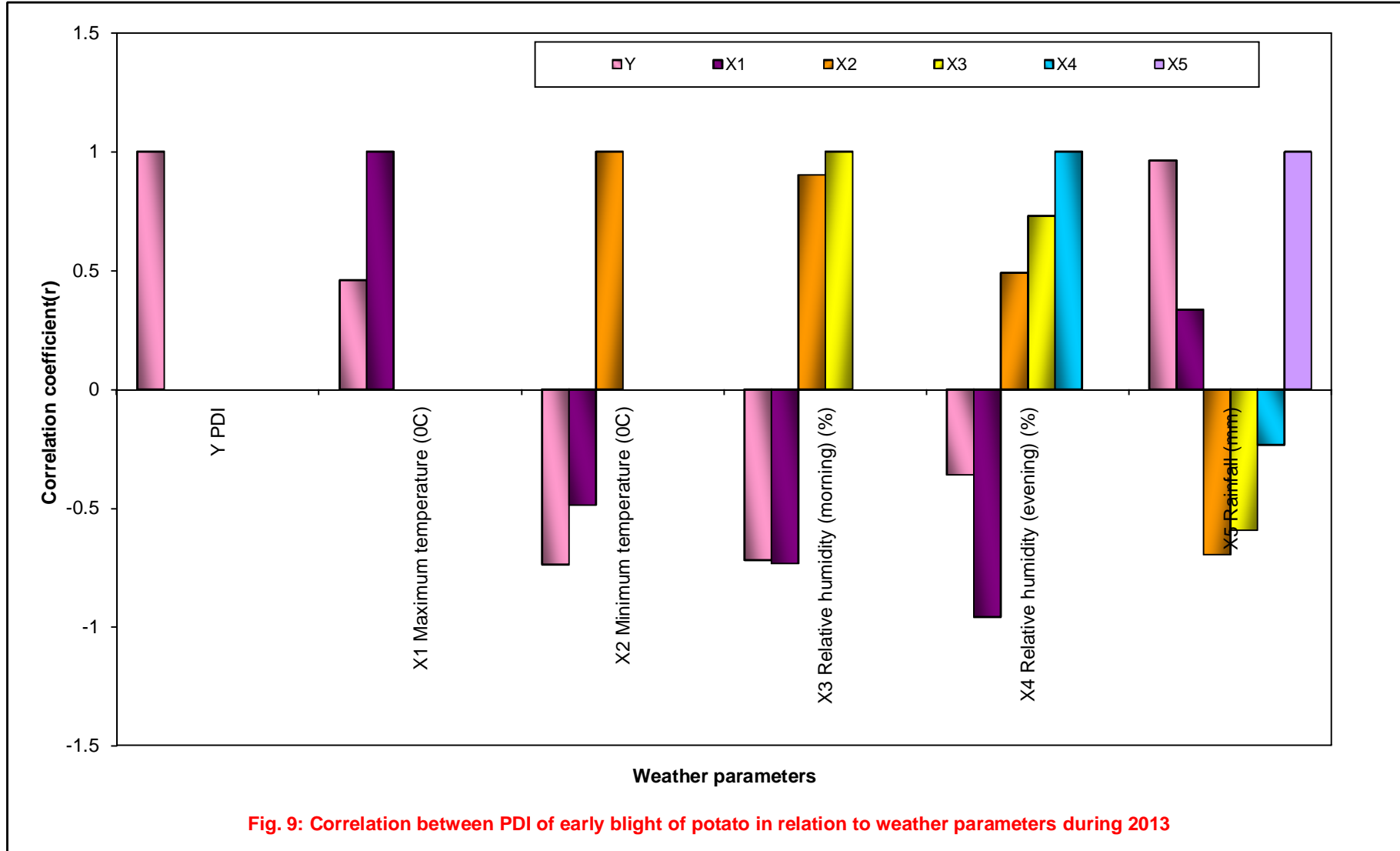


Fig. 5: Effect of temperature levels on isolates of *Alternaria solani*









selection and timing is essential to achieve control and maintain efficacy of site-specific fungicides. Some of the newer molecules were evaluated under *in vitro* conditions and data were represented in Fig. 11 and 12.

Out of twelve fungicides tested, four were non systemic, five were systemic and three were combi products. These were evaluated by means of poisoned food technique. Hexaconazole, tebuconazole, propiconazole, penconazole at 0.05, 0.1 and 0.15 per cent concentration. Zineb 68% + Hexaconazole 4% and captan 68% + hexaconazole 5% at 0.1, 0.2 and 0.25% concentration are equally effective and significantly superior with 100% inhibition which are on par with difenconazole (100%) at 0.1 and 0.15 per cent concentration, mancozeb (99.33%) at 0.25 per cent concentration, propineb (99.11%) at 0.25 per cent concentration.

Least effective chemical was found to be chlorothalonil at all the concentration tested. At higher concentration of chlorothalonil the inhibition recorded was 44.73% followed by zineb (46.55%) at 0.1 per cent concentration. The results obtained are in confirmation with the work of Singh and Singh (2006) who reported that hexaconazole was effective with 100% inhibition. Arunkumar (2006) reported propiconazole best at 0.1% concentration. Ganie (2012) who reported that among the systemic fungitoxicants hexaconazole (0.03 %) followed by penconazole (0.03%) proved significantly superior at all tested concentration.

5.6 Field evaluation of fungicides

Some of the site-specific fungicides with broad-spectrum protectants, combi products aid in resistance management as well as provide broader protection against a range of foliar pathogens. Good coverage, particularly on lower canopy and oldest leaves will enhance early season control – leading to overall reduction in field disease pressure throughout the season. So some of the effective, conventional fungicides were evaluated against early blight of potato under field conditions. Totally twelve fungicides were tested in farmers's field of Narendra village near UAS Dharwad and data were represented in Fig. 13. Three sprays were given in fifteen days interval starting from the initiation of the disease.

Observations on disease severity was recorded before the first spray, which is non significant. But later it is observed that at 50, 65 and 75 DAS the treatment differ significantly over the unprotected check. Maximum per cent disease index was noticed in control 72.66% followed by mancozeb (52%) at 75 DAS. The least PDI was noticed in Zineb 63% + Hexaconazole 4% (21.50%) which is on par with tebuconazole 25% EC (22%) and Captan 70%+ Hexaconazole 5% (24.66%). The next best treatment was found to be Mancozeb 63% + Carbendazim 12% (27%) followed by hexaconazole 5% EC (28.66%) at 75 DAS.

Similar results were obtained by Roopa (2012) who reported that Zineb 68% + Hexaconazole 4% at 0.2% effectively controlled the disease followed by hexaconazole (0.1%) on early blight of tomato. Rao (2006) and Tofali *et al.*, (2003) used combi products for the management of *Alternaria* leaf spot of sunflower and early blight of tomato respectively. Some of workers reported the efficacy of triazoles for management of early blight Abhinandan *et al.*, (2004), Ilhe *et al.*, (2008), Sali *et al.*, (2010) and Patel and Choudhary, (2010).

From the farmers point of view the economics of disease management is essential. Using of fungicides not only helps in reducing the disease severity but also helps in increasing the tuber yield of potato giving high benefit to the farmers. So the fungicides should be cost effective. So keeping this points in view the economic analysis for the management of early blight of potato has been done. The highest yield (18.83 tonnes/ha) was obtained in zineb 68% + Hexaconazole 4% with cost benefit ratio of 4.55 which is on par with tebuconazole 25% EC (18 t/ha) with cost benefit ratio of 4.30 and captan 68% + Hexaconazole 5% (17.83 t/ha) with B:C ratio of 4.29. Yield was least in control (8.83 t /ha) and cost benefit ratio was 1.54. So Zineb 68% + Hexaconazole 4% (0.2%), tebuconazole (0.1%) and Captan 68% + Hexaconazole 5% (0.25%) can be recommended to the farmers for early blight management.

5.7 Screening of potato varieties for early blight incidence

Planting cultivars that are less susceptible to early blight may reduce the severity of early blight of potato. It is the cheapest and economical method of controlling disease. It takes the major role in the integrated management of the any disease. So in order to identify the resistant sources against early blight of potato forty five genotypes were screened by using 0-9 scale in *kharif* 2013 at AICRP on Potato at Regional Horticulture Research Centre, Saidapur farm, UHS Dharwad campus.

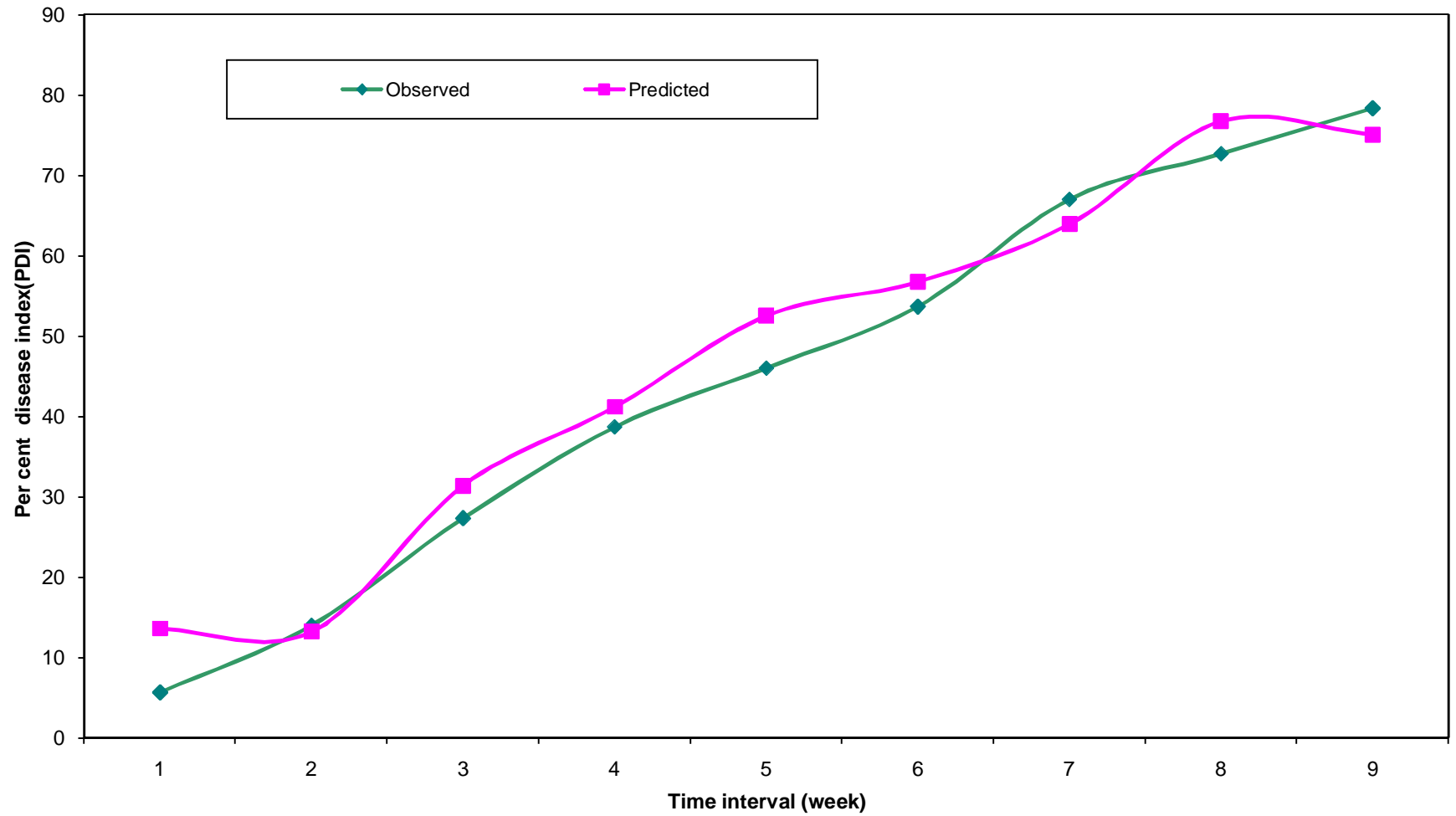


Fig. 10: Observed and predicted PDI of early blight of potato during kharif 2013

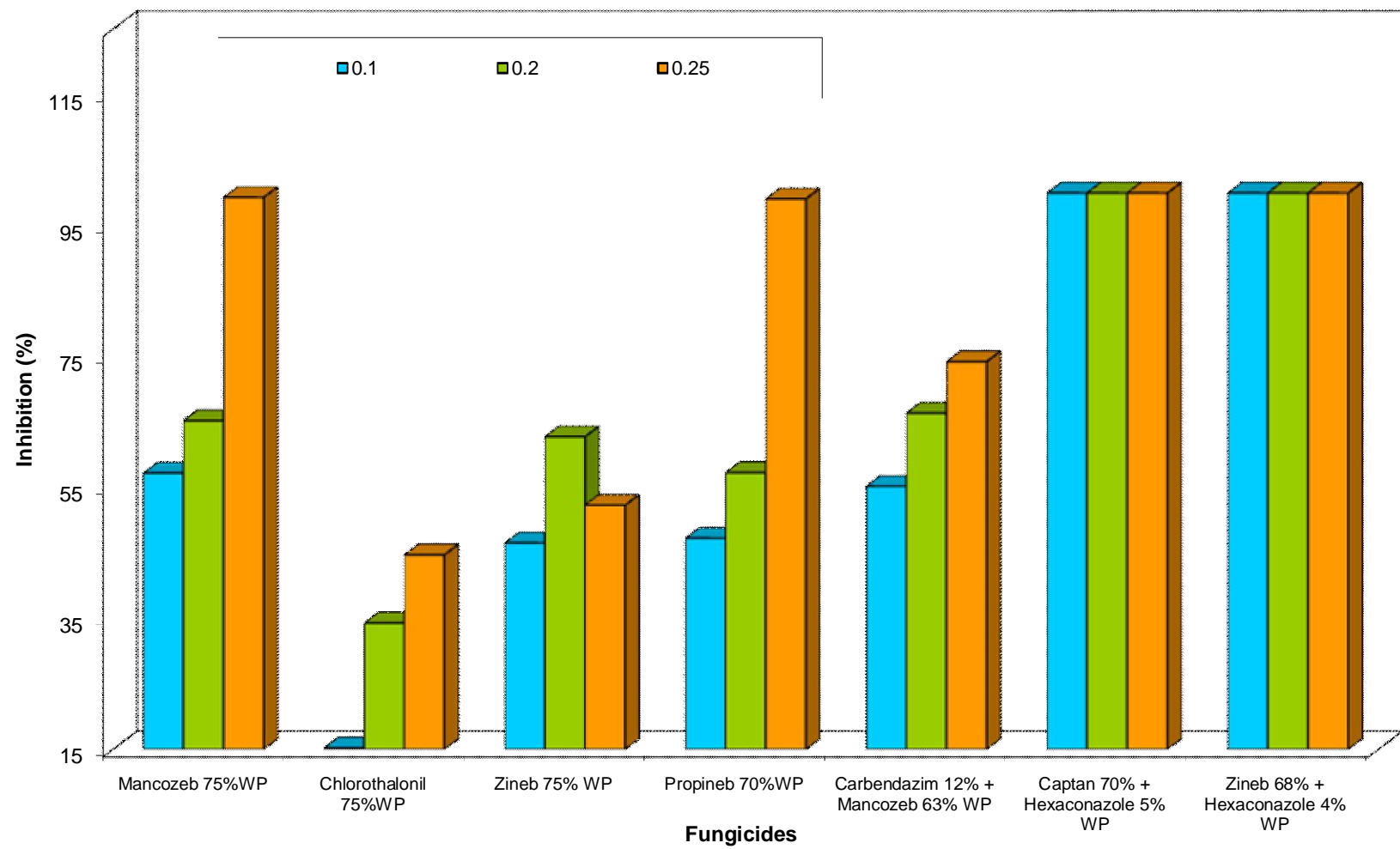


Fig. 11: *In vitro* evaluation of contact fungicides and combi-products against mycelial growth of *Alternaria solani*

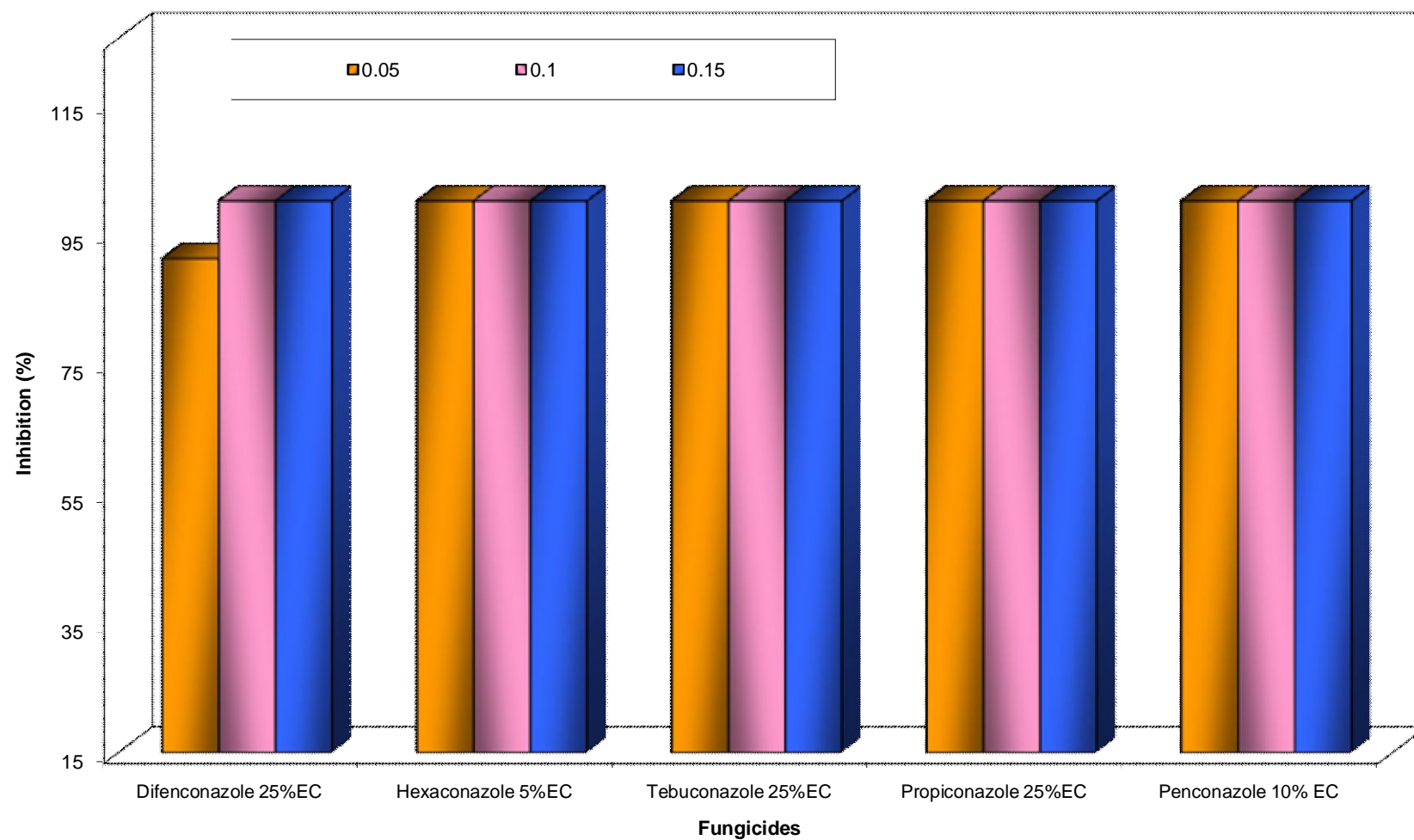
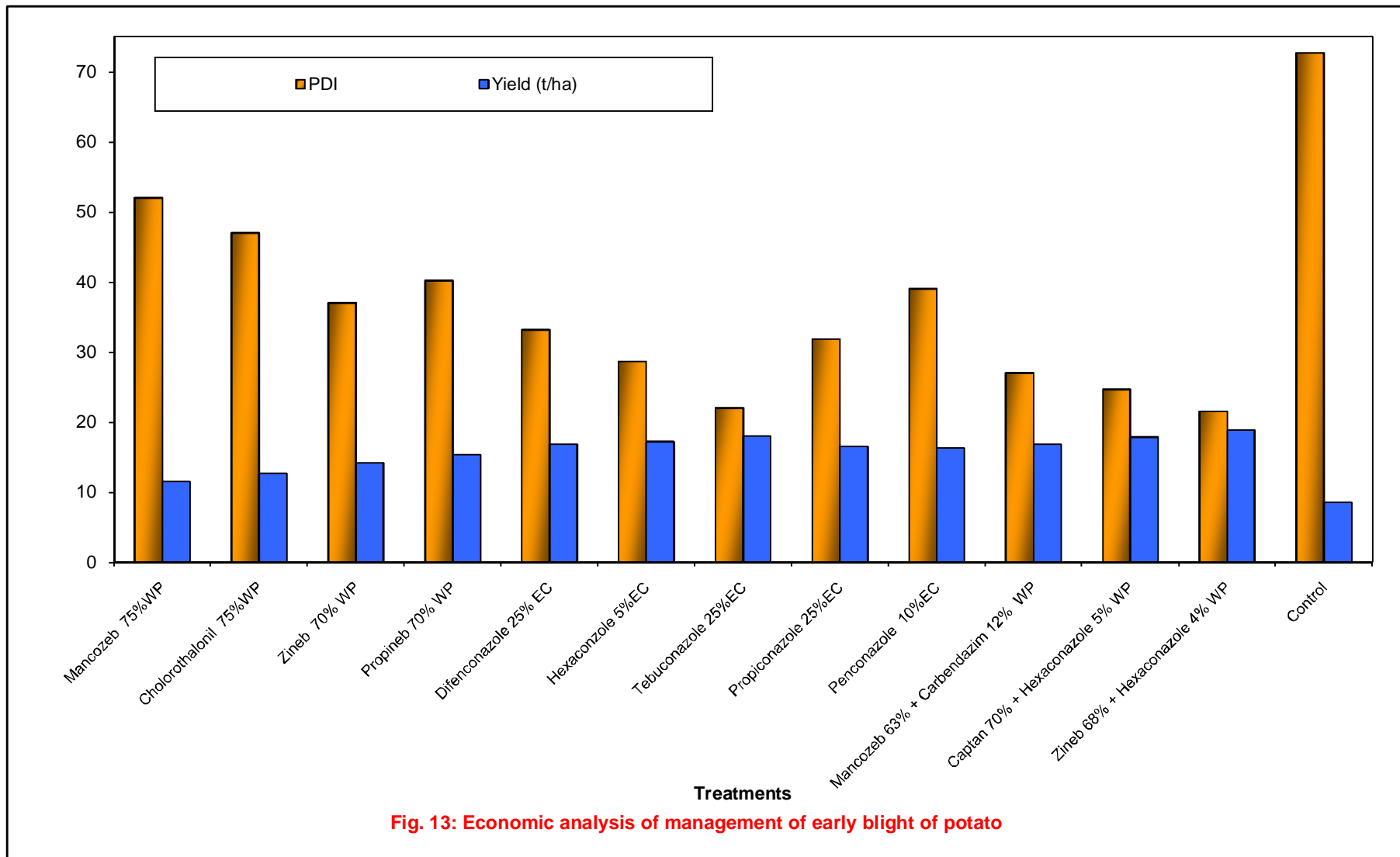


Fig. 12: *In vitro* evaluation of systemic fungicides against mycelia growth of *A. solani*



Significant difference were observed among several potato cultivars were observed for disease reaction to early blight. Among the forty five genotypes none of the genotypes showed immune and resistant reaction. Six of the genotypes were moderately resistant viz. C-13, Kufri Pukhraj , P-3, AICRP-SH-1, SH-1 and C-17. Nine genotypes were moderately susceptible viz, C-1, C-10, C-34, C-11, Kufri Ashoka, Kufri Surya, SH2, C-4 and P-4. Whereas twenty three genotypes showed susceptible reaction viz, C-16, C-26, C-28, C-31, C-13, AICRP-SH-2, CH-3, P-1, MM-12, PH-3, PH-1, Kufri Jyothi, P-9, Kufri Sadabahar, Kufri Pushkar, Kufri Khyati, Kufri Himasona, C-24, C-13, C-22, LB-24 and LB-3 and seven genotypes viz, C-18 ,C-20, PH-2, P-2, EM-1, LB-4 and LB-5 were highly susceptible.

Hence some of the moderately resistant genotypes can be recommended to the growers for early blight management. Similar attempts were made by Ghani and Ganie (2013) who reported that, three genotypes viz. Kufri Himan, SM/96-27 and SM/94-44 were moderately tolerant. Nine genotypes viz. Kufri Girdari, Kufri Shailaja, Kufri Chandramkhi, SM/98-239, SM/93-237, SM/90-45, HB/82-18, HB/50-45 and Shalimar potato-1 were moderately susceptible. Mehboob *et al.* (2013) reported that FD-18 was found to be resistant. While two lines such as F3-39 and FD-48-41 were shown moderately resistant response

Future line of work

1. A survey of the disease has to be undertaken all over Karnataka.
2. There is a need for studies on molecular variability of different isolates of *Alternaria solani*
3. Epidemiological studies may be continued for some more years so that suitable disease prediction model can be developed for early blight of potato.
4. Field screening of more number of fungicides, botanicals and biorationals, so that integrated management strategy against early blight of potato can be developed.

SUMMARY AND CONCLUSIONS

Potato (*Solanum tuberosum* L.) is an important food crop of the world. Early blight caused by *Alternaria solani* (Ellis and Martin) Jones and Grout is one of the major fungal foliar disease of potato. Early blight is one of the most widely present than any other diseases of potato in northern Karnataka and act as a major limiting factor for successful cultivation of potato. Hence survey of the disease, collection of isolates and studying the pathogen character, disease development in relation to weather parameters, *in vitro* and *in vivo* evaluation of fungicides and screening of resistant genotypes against early blight of potato has been carried out and results thus obtained are summarized here under.

Roving survey was undertaken during *kharif* 2013 to estimate the severity of early blight of potato in major potato growing districts of northern Karnataka *viz.* Dharwad and Belagavi. Severity ranged between 12.33 to 38.33% in both the districts. The highest severity of early blight was noticed in fields of Narendra village (38.33%) of Dharwad district. Where as least severity was observed in Kadoli village (12.33%), Belagavi district. Among the talukas surveyed, maximum PDI was recorded in Hubballi taluk (31.89%) of Dharwad district and Hukkeri taluk (31.89%) of Belagavi district. The highest district average disease severity was recorded in Dharwad (26.93%) followed by Belagavi (25.52%).

The pathogen *A. solani* was isolated from potato leaves showing typical symptoms. The fungus grown on PDA produced dark brown mycelium, brown to olivaceous brown conidiophores, with conidia having typical transverse and longitudinal septa with long beak. Pathogenicity of the fungus on potato was proved following Koch's postulates by inoculating spore suspension.

The five isolates were collected from different districts and named them as AS 1 (Dharwad), AS 2 (Hubballi), AS 3 (Belagavi), AS 4 (Kolar) and AS 5 (Bengaluru). Various studies have been conducted to know the cultural and morphological variability among the different isolates.

The fungus was grown on potato dextrose broth in order to study the growth pattern of *Alternaria solani*. The maximum dry mycelial weight was obtained fourteenth day (288.66 mg) after inoculation. Variability was observed among the isolates of *A. solani*. Maximum length and thickness of conidia was observed in isolate AS 4 (235-285 x 29-32 μ m) and minimum length and breadth was observed in AS 5 (150-185 X 15-25 μ m).

The isolates were studied on different solid media. The growth of the mycelium is very fast in potato carrot agar and least growth was found in corn meal agar in all the isolates. Sporulation was excellent on potato carrot agar in all the isolates. The isolates were studied at different temperature and pH levels to know the variability among them. The results revealed that the maximum dry mycelial weight is obtained in isolate AS 4 at 25°C (401.75 mg) and least dry mycelial weight was obtained in isolate AS 5 at 15°C (48 mg). All the isolates showed significantly less growth at 15°C temperature. The maximum dry mycelium weight was found in isolate AS 2 (313 mg) at pH 7 and AS 5 (313 mg) at pH 8 which is on par with isolate AS 4 (310.66 mg) at pH 8 and isolate AS 4 (306.66 mg) at pH 7. Least dry mycelial weight was recorded isolate AS 1 (90.33 mg) at pH 5. All the isolates showed significantly less growth at pH 5.

The epidemiological studies revealed that per cent disease index (PDI) was progressing at linear rate throughout the pl growth and it was positively correlated with maximum temperature and rainfall and negatively correlated with minimum temperature, morning and evening relative humidity. The multiple regression model developed for PDI is $Y = 753.41 - 5.88 X_1 + 28.71 X_2 - 11.82 X_3 - 0.78 X_4 + 0.24 X_5$ with R^2 value of 0.98. The observed and predicted value were in close resemblance to each other.

Out of twelve different fungicides tested *in vitro*, hexaconazole, tebuconazole, propiconazole, penconazole, Zineb 68% + Hexaconazole 4% and captan 68% + Hexaconazole 5% are equally effective and significantly superior with 100% inhibition at all the concentration which are on par with difenconazole (100%) at 0.1 and 0.15 per cent concentration, mancozeb (99.33%) at 0.25% concentration, propineb (99.11%) at 0.25% concentration.

In case of field evaluation the best fungicides was found to be Zineb 68% + Hexaconazole 4% (21.50%) which is on par with tebuconazole 25% EC (22%) and Captan 70%+ Hexaconazole 5% (24.66%). The highest yield (18.83 tonnes/ha) was obtained in Zineb 68% + Hexaconazole 4% with cost benefit ratio of 4.55 which is on par with tebuconazole 25% EC (18 t/ha) with incremental cost benefit ratio of 4.30 and captan 68% + Hexaconazole 5% (17.83 t/ha) with B:C ratio of 4.2. Hence these fungicides can be recommended for the farmers for efficient management of early blight of potato.

Forty five genotypes were screened to find out the resistant source against early blight of potato. Among the forty five genotypes none of the genotypes showed immune and resistant reaction. Six of the genotypes were moderately resistant viz., C-13, Kufri Pukhraj, P-3, AICRP-SH-1, SH-1 and C-17. Nine genotypes were moderately susceptible. Whereas twenty three genotypes showed susceptible reaction and seven genotypes were highly susceptible.

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INVESTIGATIONS ON EARLY BLIGHT OF POTATO CAUSED BY *Alternaria solani* (Ellis and Martin) Jones and Grout

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ABSTRACT

Early blight caused by *Alternaria solani* (Ellis and Martin) Jones and Grout is one of the major fungal foliar disease of potato (*Solanum tuberosum* L.). The maximum disease severity was recorded in Dharwad (26.93%) followed by Belagavi (25.52%). Pathogenicity was proved by inoculating the spore suspension.

The maximum dry mycelial weight was obtained on fourteenth day (288.66 mg) after inoculation. Maximum length and breadth of conidia was observed in AS 4 isolate (235-285 x 29-32 μ m). The growth of the mycelium is very fast in Potato Carrot Agar and least was found in Corn Meal Agar. Sporulation was excellent on PCA in all the isolates. Maximum dry mycelial weight is obtained in isolate AS 4 at 25°C (401.75 mg). AS 2 isolate showed good growth at pH 7 (313 mg).

PDI is positively correlated with maximum temperature, rainfall and negatively correlated with minimum temperature, morning and evening relative humidity. The multiple regression model developed for PDI is $Y = 753.41 - 5.88 X_1 + 28.71 X_2 - 11.82 X_3 - 0.78 X_4 + 0.24 X_5$ with R^2 value of 0.98. The observed and predicted values were in close resemblance to each other.

Hexaconazole, tebuconazole, propiconazole, penconazole, combi products of Zineb 68% + Hexaconazole 4% and captan 68% + Hexaconazole 5% were equally effective and significantly superior with 100% inhibition at all the concentrations tested under *in vitro*. In field evaluation, the best fungicide was found to be Zineb 68% + Hexaconazole 4% (21.50%) with highest yield (18.83 t/ha) and B:C ratio of 4.55.

Among the forty five genotypes screened, none of the genotypes showed immune and resistant reaction. Six of the genotypes were moderately resistant viz. C-13, Kufri Pukhraj, P-3, AICRP-SH-1, SH-1 and C-17, nine genotypes were moderately susceptible, twenty three genotypes showed susceptible reaction and seven genotypes were highly susceptible.