Detection and Management of Early Blight of Potato Caused by *Alternaria alternata*

अल्टरनेरिया अल्टरनेटा द्वारा जनित आलू की अगेती अगंमारी रोग का अनुसन्धान एवं प्रबंधन

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THESIS

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By

Priyanka Kumari Meena

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This is to certify that **Miss. Priyanka Kumari Meena** student of **Masters of Science in Agriculture (Plant Pathology)** had successfully completed the comprehensive examination held on 15-05-2020 as required under the regulation for Post-Graduate Studies.

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This is to certify that the thesis entitled **"Detection and Management of Potato Caused by *Alternaria alternata*."** submitted for the degree of **Masters of Science in Agriculture (Plant Pathology)** embodies bonafide research work carried out by **Miss. Priyanka Kumari Meena** under my guidance and supervision and that no part of this thesis has been submitted to any other degree. The assistance and help received during the course of investigation have been fully acknowledged. The draft of the thesis was also approved by the advisory committee on 24-06-2020

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This is to certify that **Miss. Priyanka Kumari Meena** student of Master of Science in Department of Agronomy, College of Agriculture, Ummedganj, Kota has made all corrections/modifications in the thesis entitled **"Detection and Management of Early Blight of Potato Caused by *Alternaria alternata*."** which were suggested by the external examiner and the advisory committee in the oral examination held on…….. The final copies of the thesis duly bound and corrected were submitted on…….

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Place: Kota
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Chapter 1

INTRODUCTION

The potato (*Solanum tuberosum*) is an important food crop in world as well as in India and important vegetable crops, belonging to family solanaceae. Potato originated from the hills of Andes and Bolivia in South America. It was introduced in Europe by Spaniards in the second half of sixteenth century; from there it spreads throughout Europe and rest of the world in the mid-seventeenth to mid-eighteenth century. It was introduced in India by *Portuguese* in the seventeenth century (Chakraborty, 2012). Potato is grown in more than 100 countries, under temperate, subtropical and tropical conditions. It is essentially a "cool weather crop", with temperature being the main limiting factor on production of tuber growth is sharply inhibited in temperatures below 10°C (50°F) and above 30°C (86°F), while optimum yields are obtained where mean daily temperatures are in the 18 to 20°C (64 to 68°F) range (FAO, 2008). India ranks as second world's largest potato producing nation after China. The major potato producing states are Uttar Pradesh, West Bengal, Bihar, Gujarat, Madhya Pradesh, Punjab, Haryana, Assam, Jharkhand and Chhattisgarh.

In Rajasthan total area of vegetables 168.55 ha and production of 1767.53 MT. In potato 13819 ha and production 279.54 MT and productivity 201.6 q/ha. The major potato producing districts are Dholpur, Bharatpur, Hanumangarh, Kota, Sirohi, Srigangangar and Jalore (State Department of Horticulture and Agriculture, 2018-19). In India total area of vegetables 10436 ha and production 187474 MT. (NHB 2018-19). Whereas total area in potato 2151000 ha and production 48529000 MT and productivity 225611 kg/ha (FAO 2018-19). The potato can be grown almost in any type of soil, except saline and alkaline soils. Naturally loose soils which offer the least resistance to enlargement of the tubers are preferred loamy and sandy loam soils that are rich in organic matter with good drainage and aeration are the most suitable. Soil with a pH range of 5.2-6.4 is considered ideal for its cultivation (FAO, 2008). Potato is a valuable source of carbohydrate and can be used both for table consumption as well as processed products. Fresh potato tubers contain around 80% water and 20% dry matter. More than 75% of dry matter is starch but it also contains protein, fibre and small amount of fatty acids (Prokop and Albert, 2008). It is also rich in minerals such as potassium, phosphorus, magnesium and vitamins like B_1, B_3 and B_6 (Camire *et al*., 2009). It is one of mankind's most valuable food crops (FAO,

2004). Potatoes are rich in protein, calcium and vitamin C and have an especially good amino acid balance. A single medium-sized potato contains about half the daily adult requirement of vitamin C other staples such as rice and wheat have none. Boiled potato has more protein than maize, and nearly twice the calcium (Umadevi *et al*., 2013). 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, of which may be due to frequent occurrence of fungi, bacteria and viruses (Khurana, 2004). Losses can occur when crops are growing, at lifting and when tubers are stored. Some diseases do not destroy tubers, but the surface blemishes they cause decrease marketable value. It is therefore, much emphasis should be given on the production and use of high quality and disease free seed. Though the list of pathogens infecting potato has remained almost unchanged over many decades, there has been steady expansion in the area under potato and improvement in tuber yield/productivity (CIP, 1996). Disease control is a prerequisite for improving and maintaining yield and quality of the potato crop and since the potato became widely grown serious outbreaks of disease and crop failures and consequent social and economic effects have repeatedly provided incentive for improvement. Early blight is one of the most important foliar diseases of potato (Christ, 1989; Pelletier and Fry, 1989; Steinberg *et al*., 1989 and Vanderwaals *et al*., 2001). It is caused by two species of genus *Alternaria*, i.e. *Alternaria solani* and *Alternaria alternata* worldwide on potato crops (Gudmestad and Pasche, 2007). *Alternaria solani* and *Alternaria alternata* both are more risk important pathogens on potato crops and significant impact on the tuber yield (Hausladen and Leiminger, 2007 and Kapsa, 2007). The symptoms on the foliage can appear at any stage of the growth and causes characteristic leaf spots and blight. Normally the disease symptoms become apparent during tuber bulking stage and develop leading to the harvest. The early blight is first observed on the plants as small, black lesions mostly on the older foliage. Spots enlarge, and by the time they are one-fourth inch in diameter or larger and concentric rings in a bull's eye pattern can be seen in the center of the diseased area. Tissue surrounding the spots may turn yellow. If high temperature and humidity occur at this time, much of the foliage is killed. Lesions on the stems are similar to those on leaves, sometimes girdling the plant if they occur near the soil line (Kemmitt, 2002). Due to this disease, heavy losses on crop productivity and tuber quality recorded. 5 to 40% of yield losses have

been reported in Israel (Rotem and Feldman, 1965) and 20 to 30% in the USA (Christ and Maczuga, 1989; Shtienberg *et al*., 1989). The average annual yield losses due to early blight vary enormously from 5-78% but it depends on weather conditions and type of variety grown (Waals *et al*., 2004 and Pasche *et al*., 2004, 2005). Young plants are relatively resistant, but the susceptibility increase gradually and continuously from the initiation of tuber formation so that mature plants are most susceptible to the disease (Campo *et al*., 2001). Reducing both the quantity and quality of marketable tubers (Nnodu *et al.,* 1982). Environmental factors such as temperature, wetness duration and relative humidity affect the development of early blight on potatoes (Harrison *et al*., 1965; Adams and Stevenson, 1990; Vloutoglou and Kalogerakis, 2000). Temperature increases *Alternaria solani* infection and sporulation (Vloutoglou and Kalogerakis, 2000). Water in the form of high relative humidity, rainfall or dew accumulation can increase conidia germination and pathogen infection (Rotem, 2004). Alternating low and high humidity conditions have also been shown to favour disease development (Van der Waals *et al.,* 2001). Potato is a source of both food and income in the growing countries of the world which able to change greatly the food security of countries because of its high productivity per unit area and time compared to other crops. The potato chips and wafers are popular processed food items that give considerable value addition to potato. Incidence of early blight has been observed in considerable form in potato growing areas of South Eastern Rajasthan during *Rabi* season. In view of the seriousness of the disease, the studies were planned to undertake on the following aspects which could help in the management of the disease.

i. To study the symptoms, isolation, purification, pathogenicity and identification of pathogen.
ii. To study the efficacy of bio-agents and media against the pathogen *in vitro* condition.
iii. Screening the genotypes against the disease.
iv. To study the bio-efficacy of fungicide against the pathogen *in vitro* and *in vivo* condition.

Chapter 2

REVIEW OF LITERATURE

2.1 To study the symptoms, isolation, purification, identification and pathogenicity of pathogen

Ellis (1971) described symptoms round, oval or irregular, brown or dark brown, often concentrically ridged target spots and under microscopy observed solidity and beaked conidia 9 to 11 transverse septa and a few or no longitudinal or oblique septa.

Pathak (1972) the culture of pathogen was purified by hyphal tip method.

Johnston and Booth (1983) studied isolation of pathogen with segments of diseased tissue along with some healthy leaf portion (5×5 mm^2) were cut with a sterilized razor blade at the margins of the diseased spots on the leaves and surface sterilized in 0.1% mercuric chloride solution for 30 second and single spore technique was used for purification. The leaf symptoms are characteristic dark brown reddish, yellow or mainly and greenish yellow in colour.

Foolad *et al*. (2008) reported that symptoms of disease small, dark and circular lesions flattering distinctly zonate as they expand and stem lesions occur as roughly circular, sunken, dark.

Kumar *et al.* (2008) found that isolation of *Alternaria* spp. showed pigmentation varied from yellow, brown, black, brownish to green black on potato dextrose agar medium.

Schultz and French (2009) observed that infected stems show sunken, elongated spots that may also show the typical concentric rings and lesions in tubers appear as slightly sunken dark irregular spots with raised borders.

Verma and Verma (2010) described the genus is characterized by the formation of polymorphous conidia either singly or in short or longer chains and provided with cross, longitudinal, as well as oblique septa and having longer or short beaks.

Roopa (2012) tested pathogenicity by inoculating with spore suspension and homogenized mycelial bits (2×10^4 spores/ml) of *Alternaria solani* on foliage of 30 days old and proved the Koch's postulates.

Ganie *et al*. (2013) proved the pathogenicity by using spore suspension from 15 days old culture with concentration of 2×10^4 conidia/ml on Kufri Jyoti variety of

potato and revealed that the initiation of typical symptoms of the disease appeared after 10 days of inoculation on injured detached leaves of potato. They also revealed that symptoms 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.

Mamgain *et al.* (2013) reported that spore wasattached in chains, multi–celled and pigmented. The symptoms appear as small, circular or irregular, dark- brown to black spots on the older (lower) leaves. These spots enlarge up to $^{3}/_{8th}$ inch in diameter and gradually may become angular-shaped. Initial lesions on young, fully expanded leaves may confuse with brown spot lesions. These first lesions appear about two to three days after infection observed by Bauske (2018).

Robinson (2018) reported that chlorotic symptoms may develop on infected leaves through time as lesions coalesce and clusters of infection form. Elongated, brown to black lesions may develop on the stems and petioles of infected plants. The infected tubers appear as dark-coloured, sunken lesions on the tuber surface and tuber lesions may be circular or irregular in shape and often are accompanied by a large, raised dark-brown border.

2.2 To study the efficacy of bio-agent and media against the pathogen *in vitro* condition

2.2.1 Efficacy of media study

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

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

Somappa *et al.* (2013) observed maximum radial growth on Czapeck'sdox agar medium (50 mm) followed by Potato dextrose agar (37 mm).

Koley and Mahapatra (2015) tested the growth of pathogen in twelve liquid and solid media and revealed that potato dextrose agar and oat meal agar among solid media and Richard's broth and Sabouraud's broth among liquid media seemed to be better for growth of *Alternaria solani.* Dry weight of *Alternaria solani* was maximum in potato dextrose broth medium (289.33 mg) followed by oat meal broth medium (281.33 mg) and Czapek's Dox broth (240.67 mg). The colour of the colony

Alternaria solani was having dark brown in case of potato dextrose agar medium, Oat meal agar medium imparted grey colour of the colony, Czapek's agar medium whitish colony with greenish border and substrate light colour. Maximum sporulation was observed on oat meal agar followed by potato dextrose agar medium. However, moderate sporulation was observed on Czapek's dox agar.

Kumar *et al.* (2018c) recorded highest radial growth of *Alternaria solani* on potato dextrose agar (63.00 mm) followed by oat meal agar (42.33 mm) and Czapek's dox agar (27.73 mm).

2.2.2 Efficacy of bio-agent

Yadav and Pathak (2011) observed that *Trichoderma viride* @ 0.5% was efficacious in controlling early blight of potato.

Sharma *et al*. (2014) reported that *Trichoderma harzianum* and *Trichoderma viride* are the widely used species and have been exploited on about 87 different crops and about 70 soils borne as well as 18 foliar pathogens, respectively. *Trichoderma* as a potential bio-control agent against *Alternaria* observed by Kushwaha and Verma (2014).

Murmu *et al.* (2015) found *Trichoderma viride* highly effective in percent disease reduction (52.39%) against early blight of potato with highest tuber yield (25.51 t/ha) as compared to control (19.53 t/ha).

Mahantesh *et al.* (2017) reported that *Trichoderma harzianum* showed the maximum percent inhibition (80.36%) followed by *Trichoderma viride* (75.86%) against *Alternaria solani* at UAHS, shivamogga.

Yadav *et al.* (2018) observed that *Trichoderma harzianum* at 3000 ppm concentration significantly inhibit the radial growth (4.88 mm) of *Alternaria solani* as compare to control (15.03 mm) *in vitro* condition.

Kumar *et al.* (2018b) recorded that maximum percent inhibition (78.97%) of the *Alternaria solani* by *Trichoderma viride* followed by *Trichoderma harzianum* inhibition percent (75.33%) as compare to control (87.16 mm).

Sarfraz *et al.* (2018) observed that *Trichoderma harzianum*at (48, 96, 144 and 172 hrs) growth inhibition (7.50, 12.90, 29.44 and 43.00%) followed by *Trichoderma viride* growth inhibition (4.40, 9.10, 29.10 and 37.29%) against *Alternaria solani* causing by early blight of potato.

Sreenivasunu *et al.* (2019) recorded that the radial growth (32.5 mm) and percent inhibition (47.29%) of *Alternaria solani* by using *Trichoderma viride.*

2.3 Screening the genotypes against the disease

Dey and Chakraborty (2012) evaluated ten varieties like K. Chandramukhi, K. Pukhraj, K. Chipsona-1, K. Jawahar, K. Anand, K. Giriraj, K. Surya and K. Baharand maximum incidence and intensity percentwere observed in the variety K. Giriraj (43.68 and 27.73%) followed by K. Anand (40.45 and 25.87%), K. Jawahar (33.67 and 23.87%), K. Pukhraj (27.08 and 22.27%), and K. Surya (18.95 and 6.27%) and K. Chipsona - l (15.31 and 7.33%) 21 days after first appearance of the symptomsat Adisaptagram block seed farm.

Mehboob *et al.* (2013) screened 29 test lines against early blight of potato. 15 lines including cardinal and desire were found to be very highly susceptible. 8 lines including diamante FST-white and PPS 9813 showed highly susceptible response. 2 lines that are 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 so shown moderately resistant response.

Ganie and Ghani (2013) screened twenty five potato genotypes for early blight of potato under natural conditions. Out of twenty five, one genotype SM/92-338 showed tolerant reaction while 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.

Kumar *et al.* (2015) evaluated genotypes against early blight of potato at main experiment station (MES) of Vegetable Science in Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad, Uttar Pradesh, Out of them, Kufri Pukhraj and Kufri Pushkar were found moderately resistant, remaining K. Bahar and Sindhuri found susceptible and K. Sutlej found highly susceptible.

Prabha and Nanda (2017) were screened 18 clonal bulk population, 43 F_1C_2 and 90 F_1C_3 clonal progenies of potato for resistance against *Alternaria solani* under natural and artificial conditions. All the genotypes of clonal bulks, F_1C_2 and F_1C_3 progenies showed moderate resistance reactions against blight incidence and most of the genotypes of clonal bulks, F_1C_2 and F_1C_3 clonal progenies showed moderate resistance against blight severity. Among artificial inoculated genotypes of clonal bulks and progenies, the genotype CIP 398201 of clonal bulk, four genotypes

(CIP 302024-11-2, CIP 398201-19-1, CIP 398201-19-2 and CIP 398201-20-1) of F_1C_2 progenies and five genotypes (CIP 398201-7-2-1, CIP 398201-15-4-1, CIP 398201-5-3-1, CIP 398201-11-6-1 and CIP 398201-2-2-1) of F_1C_3 progenies were found moderately resistant against blight incidence, while remaining genotypes showed moderately susceptible reaction.

2.4 To study the bio-efficacy of fungicide against the pathogen *in vitro* and *in vivo* condition

Arunkumar (2006) recorded maximum inhibition of Propiconazole (0.1%), Metalaxyl MZ (0.2%) and Perfekt (0.3%) against *Alternaria solani* under *in vitro* condition, while Propiconazole (0.1%), Pyrachlostrobin (0.2%) were found to be most effective in reducing the severity of the disease and increasing yield over control under field conditions.

Singh and Singh (2006) tested the efficacy of seven fungicides *viz.* Chlorothalonil, Copper Oxychloride, 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,* respectively and observed that all the fungicides significantly reduced the radial growth of the *Alternaria solani*. However, Hexaconazole was give 100% growth inhibition.

Kumar *et al.* (2007) recorded that Hexaconazole (0.05%) and Azoxystrobin (0.2%) was very effective in controlling early blight. Pyraclostrobin significantly reduced the early blight and increased the yield in tomato and potato reported by Ganesham and Chethana (2009).

Issiakhem and Bouznad (2010) tested Difenconazole and Cholrothalonil 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.

Horsfield *et al.* (2010) evaluated protective and curative activity of fungicides in glasshouse and field for the control of early blight. Boscalid, Azoxystrobin and Difenoconazole were highly effective in the control of early blight when applied up to three days before or three days after inoculation.

Jambhulkar *et al*. (2012) reported that Azoxystrobin 23% SC reducing early blight disease 38.9% as compare to control in tomato crop.

Genie (2012) evaluated different systematic and non-systematic fungicide against early blight of potato crop and reported that Mancozeb (17.76%) and

Hexaconazole (14.37%) found effective in reducing disease severity as compare to control (34.38%).

Sahu *et al*. (2013) reported that Mancozeb 75% WP (42.36%) and Pyraclostrobin 38% WG (35.73%) significantly reduced percent disease intensity with increased the yield (29.75 and 31.81 t/ha) as compared to control (60.80%) and yield (21.15 t/ha) in early blight disease in tomato.

Herle *et al.* (2014) reported that different fungicides tested against mycelial growth of *Alternaria solani* were Mancozeb 75% WP at (0.1, 0.2 and 0.25%) concentrations, percent inhibition (57.22, 65.23 and 99.33%) and Hexaconazole 5% EC and Propiconazole 25% EC at (0.05, 0.1 and 0.15%) concentrations are equally effective and significantly superior with 100% inhibition, which are on par with Difenconazole 25% EC (100% inhibition) at 0.1 and 0.25% and (91.11%) at 0.05% concentration in laboratory condition. Under field conditions,the minimum percent disease incidence by using different fungicides such as Mancozeb 75% WP at (35, 50, 65, 75 DAS) percent inhibition (14.13, 32.00, 40.67 and 52.00%), Difenconazole 25% EC (12.30, 21.00, 25.67 and 33.16%), Hexaconazole 5% EC (11.96, 18.50, 20.33 and 28.66%) and Propiconazole 25% EC (13.66, 16.00, 25.00 and 31.83%) maximum percent disease incidence was noticed in Mancozeb 75% WP (52%) at 72 days after sowing.

Stepanovic *et al.* (2015) observed that the tested *Alternaria solani* isolates were also highly sensitive to Difenconazole. All isolates were able to grow well at 0.001ug/ml Difenconazole concentration (<10% growth inhibition), but they were partially inhibited by the next two higher concentrations (0.01 and 0.1 ug/ml) and significantly inhibited by the concentration of 10 ug/ml (>95% growth inhibition). The values for Difenconazole ranged between 0.018 and 0.037 ug/ml. The growth inhibition of over 82% was achieved by Pyraclostrobin concentration of 10 ug/ml. Concentrations which inhibited mycelia growth by 50% (EC_{50}) ranged from 0.0014 to 0.0041 ug/ml.

More *et al.* (2016) conducted field experiments on efficacy of sequential sprays of different fungicides against early blight variety Kufri Pukhraj in potato for consecutive three years and revealed that spray of Hexaconazole 5 EC (0.05%) @ 0.5 ml/litre of water lower the disease intensity at 10 days after first spraying (10.33%), 20 DAS (13.05%) and 30 DAS (16.27%) with tuber yield (15.00 t/ha) as

compare to control 10 DAS (16.23%), 20 DAS (25.20%) and 30 DAS (30.87%) with tuber yield (11.96 t/ha).

More *et al.* (2016) observed minimum disease incidence and intensity of early blight Mancozeb 75% WP (20.83 and 16.16.26%) with increased the yield (16.13 t/ha) and Hexaconazole 5% EC (21.63 and 16.27%) with increased the yield (15.00 t/ha) as compared to control (45.06 and 30.87%) yield (11.96 t/ha) in potato crop (at 10 days after first spray (14.54%), 20 DAS (17.27%) and 30 DAS (21.63%) in potato crop.

Mahantesh *et al.* (2017) observed that Propiconazole @ 100, 250, 500, 1000 ppm showed 80.08, 83.48, 86.42 and 90.58% inhibition followed by Hexaconazole @ 100, 250, 500, and 1000 ppm and inhibition 80.75, 83.18, 85.12, 87.80% and then Difenconazole @ 100, 250, 500, and 1000 ppm with 78.24, 81.20, 84.63 and 88.07% inhibition and Azoxystrobin @ 100, 250, 500 and 1000 ppm with 75.36, 78.39, 81.86 and 83.53% inhibition and then Mancozeb 75 WP @ 100, 250, 500 and 1000 ppm and per cent inhibition of 72.69, 76.75, 81.30 and 88.42, respectively against *Alternaria solani* on potato dextrose agar medium using poison food technique.

Yadav *et al.* (2018) screened various fungicides and found that Hexaconazole @ 3000 ppm showed minimum radial growth (3.00 mm) of *Alternaria solani*, causing early blight of potato to evaluate the effect of different fungicide such as significantly at 3000 ppm concentration followed by Mancozeb (3.16 mm) as compare to control (15.03 mm).

Yadav *et al.* (2018) a study was carry out in the department of plant pathology, college of horticulture, Bharsar. Throughout *kharif* season 2016 to control *Alternaria solani* early blight of potato to evaluate the effect of different fungicide such as Hexaconazole and Mancozeb significantly lower the disease intensity (4.81, 10.51 and 18.26%) at 45, 60, 75 DAS and maximum tuber yield (446.95 g/plant) followed by Mancozeb (6.50, 13.70 and 22.00%) at 45, 60, 75 DAS and tuber yield (428.03 g/plant) as compare to control (20.00, 38.60 and 55.25%) at 45, 60, 75 DAS and (307.12 g/plant) *in vivo* condition.

Bansode *et al.* (2018) revealed that spray of Hexaconazole 5 EC @ 0.05% and then Mancozeb 75 WP @ 0.25% at 10 days interval was found significantly superior in controlling the early blight disease of potato with highest yield (22.40 t/ha) as compared to other treatments.

Kumar *et al.* (2018a) recorded that Propiconazole 25% EC (83.62%) gave maximum inhibition of the mycelial growth of *Alternaria solani* followed by Mancozeb 75% WP (56.85%) at 200 ppm concentration in early blight of potato in *vitro* condition.

Kumar *et al.* (2018a) a field experiment was conducted during *Rabi* season 2013 to control early blight of potato in Kufri Bahar variety to evaluate the Propiconazole 25% EC was most effective followed by Mancozeb 75% WP and also economical in reducing severity of the early blight and increasing yield over control.

Sharma *et al.* (2018) Evaluate the lowest per cent disease intensity (PDI) through the Difenconazole 25% EC @ 0.025% (20.59%) followed by Propiconazole 25% EC @ 0.025% (21.52%) and Mancozeb 75% WP @ 0.2% (24.72%) treatments as compare to untreated control on the early blight of potato.

Ahmad *et al.* (2019) revealed that Mancozeb 75 WP @ 2000 ppm gave lowest mycelial growth and highest inhibition percentage (3.65 mm and 85.53%) of *Alternaria solani* in potato.

Sreenivasunu *et al.* (2019) tested fungicides against *Alternaria solani* by using poison food technique and found that Propiconazole 25% EC at 0.1% gave 90.26% inhibition followed by Mancozeb 75% WP at 0.25% (87.57% inhibition). Similar trend of fungicide observed under field conditions, spray of Propiconazole 25% EC @ 0.1% gave highest yield (213.97 q/ha) followed by Mancozeb 75% WP at 0.25% concentration gave yield (178.20 q/ha).While, lowest yield (107.90 q/ha) in untreated control.

Chapter 3

MATERIAL METHOD

Present investigation on **"Detection and management of early blight of potato caused by *Alternaria alternata*"** was carried out at the field of Agricultural Research Station, Ummedganj and in laboratory of college of Agriculture, Agriculture University-Kota during the year 2019-20. The materials used and methods adopted during these studies are described here in this chapter:

3.1 General

3.1.1 Experimental site

The laboratory experiments were conducted at Laboratory of Plant Pathology, College of Agriculture, ARS, Kota and field experiments were conducted at the experimental site Agricultural Research Station, Ummedganj-Kota. Kota is situated at latitude 25.21‟ N, longitude of 75.86‟ E and altitude of 271 meters above MSL (mean sea level). The region falls under semi-arid eastern plain (Agro Climatic Zone- V) of Rajasthan.

3.1.2 Climate and Weather Condition

The maximum mean daily temperature ranges from 24.5^{o}C in the month of January and 42.6^{o}C in May and minimum 10^{o}C in January and 19.7^{o}C in month of May.

3.1.3 Soil

The soil of the University service area ranges from clay loam to clay. The major soils are Vertisols (71.7%), Alfisols (12.7%),) and Inceptisols (4.8%),). The average normal rainfall of the university jurisdiction varies 732-1005 mm and major source of irrigation are canal through rivers *viz.*, Chambal, Kalisindh and Parvanetc and tube wells.

3.2 Raising of crop

The field was prepared before sowing by cross ploughing with tractor drawn disc harrow and planked. The field experiments were conducted on variety Kufri Bahar of potato in *Rabi* season 2019-20 except screening of genotypes. The crop was raised in plots of 3.0 × 2.4 m^2 keeping P × R distance of 60 × 20 cm^2. The irrigation was applied as per the recommendations for the crop in this zone. One weeding and hoeing was done as per recommendation.

3.3 General laboratory procedure

3.3.1 Glassware cleaning

For all the laboratory experimental studies, Corning and Borosil glassware's were used. The glassware's were kept in cleansing solution containing 60.0 g of potassium dichromate ($K_2Cr_2O_7$) 60.0 ml of concentrated sulphuric acid (H_2SO_4) in 1000 ml of water for 24 hours. They were washed with detergent powder followed by washing in running tap water and then rinsed in distilled water before use.

3.3.2 Sterilization

All glass wares, solid and liquid media were exposed to sterilization by autoclaving at 1.1 kg/cm^2 (121.6°C) for 15 min. The seeds and plant tissues were surface sterilized in 1 per cent sodium hypochlorite solution for one minute followed by three changes in sterile water. All cultural studies were conducted under barren conditions in laminar flow. The tip of inoculated needle and forceps were disinfected over flame.

3.4 Preparation of media

3.4.1 Potato dextrose agar medium

For all the laboratory experimental studies, standard potato dextrose agar (PDA) medium was used for culturing the *Alternaria alternata.*

The composition of PDA used is given below.

Peeled potato	:	200 g
Dextrose	:	20 g
Agar-agar	:	20 g
Distilled water	:	1000 ml

Two hundred grams of peeled potatoes were cut into pieces. These pieces were boiled in water and the extract was collected by filtering through muslin cloth. Each of 20 g of dextrose and agar-agar were dissolved in potato extract and the final volume was made up to 1000 ml by adding distilled water. A known quantity of such medium was dispensed into number of conical flasks and plugged with nasty cotton and finally wrapped with paper. The flasks containing provided medium were sterilized at a pressure of 1.1 kg/cm^2 for 15 minutes.

3.5 Symptoms and isolation of the pathogen

Early blight symptoms of potato were isolated from infested leaf sample of potato plants showing typical symptoms on Kufri Bahar variety. The diseased leaves were examined for associated fungus by teasing the diseased portion with the help of a teasing needle and was observed under microscope at the margins of the diseased spots (10x × 10x). For the isolation of fungus, small segments of diseased tissue along with some healthy leaf portion (5 × 5 mm^2) was cut with a sterilized razor blade at the margins of the diseased spots on the leaves and surface sterilized in 0.1% mercuric chloride solution for 30 second. The leaf segments were rinsed thrice in distilled sterilized water to remove the last trace of mercuric chloride solution, blotted dry and placed on acidified potato dextrose agar medium (PDA) in sterilized Petri plates. Three pieces of sterilized specimen were placed in each Petri plate and incubated for 7 days at 25 ± 1°C. One set of PDA plates was seeded with bits without mercuric chloride treatment.

3.6 Purification of the pathogen

The isolated pathogen was purified by single spore isolation and maintained on PDA slants. The mycelial growth was observed in Petri plates, advancing hyphal tips growing out of tissue segments were cut off with sterilized inoculation needle and transferred to potato dextrose agar slants for further growth in Petri plates and incubated at 25 ± 1°C for 24 h. The pure cultures thus obtained, was maintained by repeated sub-culturing at an interval of 30 days for further studies. The stock culture in PDA slants was stored at 4°C in refrigerator. The culture was purified and maintained for further studies.

3.7 Pathogenicity of pathogen

To prove the ability of the isolated fungus to cause early blight disease in potato, were inoculated by spraying with conidial suspension of *Alternaria alternata.* The conidial suspension was prepared in sterilized distilled water by blending 7 days old fungal culture in grinder and mortar and filtered through cheese cloth. Conidia per ml were counted with the help of haemocytometer and were diluted with sterilized distilled water to have 1 × 10^5 conidia per ml of water. 15 days after transplanting potato plants were inoculated by spraying the conidial suspension with the help of an atomizer. The control was also maintained by spraying sterilized distilled water only. Inoculated and control plants were covered with plastic bags and incubated in a cage

house for 72 hours at 25°C, then uncovered and kept wet until symptoms appeared. One week after inoculation, small dark spots were observed, that rapidly developed into necrotic areas on the potato leaves. Re-isolation was done prepared from artificially inoculated plants and resulting culture was compared with the original one with admiration to colony characters, colony color, conidial morphology and septation.

3.8 Identification of pathogen

The morphological study was carried out. The observations were recorded from the 7 days old culture for the characters of colony, mycelium and conidia. The pathogen was identified on the basis of colony characters, *viz*., color, growth, pigmentation etc. The culture was sent for identification at species level at Indian Type Culture Collection, IARI for confirmation.

3.9 Media study

A modified method suggested by Koley and Mahapatra (2015) was followed to find favorable medium. Various leaf decoction media from plant species as well as synthetic and semi synthetic media as listed below were prepared and evaluated. Mycelial growth and dry mycelial weight was recorded. The broth media of below mentioned media prepared and dry mycelium weight was calculated (mg).

Experimental Details

Design :CRD Treatments :8 Replications :4

Treatment details

Sr. No	Name of media
1.	V-8 juice agar
2.	Czapek's agar
3.	Potato dextrose agar
4.	Oat meal agar
5.	Potato stem agar
6.	Potato leaf agar
7.	Potato carrot agar
8.	Pea agar

The composition and preparation of the above mentioned synthetic and non-synthetic/semi-synthetic media were obtained from Ainsworth and Bisby‟s "Dictionary of the Fungi" by Hawksworth *et al*. (1983).

The composition and preparation of the media is as follows:-

1. V-8 juice agar

V-8	:	200 ml
$CaCO_3$	:	3.0 g
Agar- Agar	:	20 g
Distilled water	:	1000 ml

Suspend 44.3 g in 1000 ml of distilled water and boiling. Sterilize by autoclaving at 15 lbs pressure 121°C for 15 minutes. We made a solution of 4.43 g in 100 ml distilled water.

2. Czapek's agar

Sucrose	:	30.0 g
Sodium nitrate ($NaNO_2$)	:	2.00 g
Potassium dihydrogen phosphate (KH_2PO_4)	:	1.00 g
Magnesium sulphate ($MgSO_2.\ 7H_2O$)	:	0.50 g
Ferric chloride ($FeCl_3.\ 6H_2O$)	:	0.01 g
Potassium chloride (KCl)	:	0.50 g
Agar - agar	:	20.00 g
Distilled water	:	1000 ml (volume to make up)

Agar-agar was melted in 500 ml distilled water. The other ingredients were dissolved in remaining 500 ml of distilled water. The two solutions were mixed thoroughly and the volume was made up to one litre. The medium was sterilized at 1.1 kg/cm^2 pressure for 15 minutes.

3. Potato dextrose agar (PDA)

Peeled potato	:	200.00 g
Dextrose	:	20.00 g
Agar-agar	:	20.00 g
Distilled water	:	Distilled water

200 gram of peeled potato was cut into small bits and boiled in distilled water and extract was collected by filtering through muslin cloth. Dextrose 20 g and Agar- agar powder 20 g each were dissolved in potato extract and the final volume was made up to 1000 ml with distilled water. Later, it was sterilized at 1.1 kg/cm^2 pressure for 15 minutes and preserved for future use. We made a solution of 3.9 g in 100 ml water.

4. Oat meal agar

Oat meal powder	:	40.00 g
Agar - agar	:	20.00 g
Distilled water	:	1000 ml (volume to make up)

The oat meal powder was dissolved in 500 ml of distil water and agar- agar was melted in 500 ml of distilled water separately. Both the solutions were mixed thoroughly. Then the volume was made up to one litre and sterilized.

5. Potato stem agar

Healthy potato plant stem	:	20.00 g
Agar-agar	:	2.00 g
Distilled water	:	100 ml

Potato plant stem were boiled in 50 ml water for 30 minutes. Extract was collected by filtering through muslin cloth. The agar-agar was melted in 50 ml water, both the solutions were mixed and the volume was made up to 100 ml and was sterilized.

6. Potato leaf Agar

Healthy potato plant leaves	:	20.0 g
Agar-agar	:	2.00 g
Distilled water	:	100 ml

Potato plant leaves were boiled in 50 ml water for 30 minutes. Extract was collected by filtering through muslin cloth. The agar-agar was melted in 50 ml water, both the solutions were mixed and the volume was made up to 100 ml and was sterilized.

7. Potato carrot agar

Carrot	:	200 g
Potato	:	250.00 g
Agar-agar	:	15.00 g
Distilled water	:	1000 ml

Carrots were boiled for 10-15 minutes in 400 ml distilled water; the extract was squeezed and filtered through muslin cloth. The extract was collected in a beaker, remaining ingredients were added to same and volume was made to one litre before sterilization.

8. Pea agar

Pea	:	20.00 g
Agar-agar	:	2.00 g
Distilled water	:	100 ml

Pea (Green) was boiled in 100 ml water for 30 minutes. Extract was collected by filtering through muslin cloth. The agar-agar was melted in 100 ml water and the volume was made up to 100 ml and was sterilized.

The sporulation of the spore suspension was estimated as per (Krishna *et al.*, 2018)

Number of spore per microscopic field	**Designation**
0	- (nil)
1-10	+ (poor)
11-20	++ (moderate)
21-30	+++ (good)
31-40	++++ (excellent)

3.10 Efficacy of bio-agent against the pathogen *in-vitro* condition

Various known bio-agent *Trichoderma* species *viz., Trichoderma virens, Trichoderma azospirillum, Trichoderma harzianum, Trichoderma viride* were collected from Department of Plant Pathology and screened for their effectiveness against *Alternaria alternata* by using CRD for their effectiveness using dual culture technique.

Experimental Details

Design : CRD

Treatment : 5

Replications : 4

Treatment details of bio-control agents

S. No.	**Name**
1	*Trichoderma virens*
2	*Trichoderma azospirillum*
3	*Trichoderma harzianum*
4	*Trichoderma viride*
5	Control

Dual culture technique

Bio-control agents were evaluated amount for their efficacy against *Alternaria alternata* using dual culture technique. Twenty ml of potato dextrose agar was poured into 90 mm diameter Petri dishes and permit to solidify. Mycelial disc of (6 mm) from seven days old actively growing culture of the bio-agents and the test pathogen were cut separately with the help of sterilized cork borer and placed on solidified PDA

approximately, 4 cm away from each other. Each treatment was replicated five times and incubated at 25 ± 1°C. The activity of antagonistic organisms were recorded by measuring the colony diameter of *A. alternata* in each treatment and compared with control. The per cent inhibition of growth of the pathogen was calculated by using the formula suggested by Vincent (1947).

$$I = \frac{C-T}{C} X100$$

Where

I= Per cent inhibition of mycelium

C= Growth of mycelium in control

T= Growth of mycelium in treatment

3.11 Screening the genotypes against the disease

Potato genotypes were screened against the early blight disease under natural conditions. From each genotype, 10 plants were selected randomly and kept unsprayed throughout the season and tagged for the assessment of the disease. Twenty eight genotypes as listed below were evaluated for their reaction to early blight disease of potato. Disease rating was done on a 0-5 arbitrary scale (Horsefall and Barette, 1945).

Description of the symptoms and disease reaction

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

HR= highly resistant, R= resistant, MS= moderately susceptible, S= susceptible, HS= highly susceptible.

Name of genotypes/varieties

Sr. No.	Name of genotypes/varieties
1.	NJ-1501
2.	MP-97
3.	MP/94-322
4.	NJ-44
5.	NJ-85
6.	NJ-34
7.	MP-97-1606
8.	NJ-1530
9.	PS/RI-135
10.	CP-3021
11.	J-92-164
12.	JI-93-77
13.	MS/94-118
14.	JN-1177
15.	MS/92-2105
16.	J/93-4
17.	NJ-1
18.	MS/85-1663
19.	Kufri Sindhuri
20.	Kufri Bahar
21.	Kufri Pukhraj
22.	Kufri Khayati
23.	Kufri Pushkar
24.	3153
25.	Atlantic
26.	Chip- 3
27.	Jx-161
28.	Ms/78-62

The disease incidence was calculated by using the following formula

Per cent disease incidence = $\frac{\text{No.of diseased leaves}}{\text{Total No.of leaves examined}} X100$

3.12 *In vitro* evaluation of fungicides

Various fungicides as listed below at different concentrations were evaluated for their effectiveness against pathogen by poisoned food technique as suggested by Nene and Thapliyal (1979).

The efficacies of systemic, non-systemic and products were assessed by poison food technique. The pathogen *Alternaria alternata* was cultured on PDA medium in Petri plates for ten days before setting the experiment. The details are mentioned below:-

3.12.1 Poisoned food technique

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 Petri plates. Mycelial disc of 0.5 cm was taken from the outer side of ten days old culture and was placed in the center and incubated at 25 ± 1°C till growth of the fungus touched the periphery in control plate. Suitable checks also maintained without addition of any fungicide. Four replications were maintained for each treatment. The diameter of the colony was measured bidirectional and average was worked out. The per cent inhibition of growth was calculated by using the formula given by Vincent (1947).

$$I = \frac{C-T}{C} X100$$

Where

I= Per cent inhibition of mycelium

C= Growth of mycelium in control

T= Growth of mycelium in treatment

Experimental Details

Design : CRD Replications : 4 Treatments : 8

Treatment of fungicides and concentration

Sr. No.	Name of fungicides	Concentration (ppm)
1.	Difenoconazole 25% EC	10, 20, 50
2.	Propiconazole 25% EC	10, 20, 50
3.	Hexaconazole 5% EC	10, 20, 50
4.	Azoxystrobin 23% SC	20, 50, 100
5.	Picoxystrobin 22.52 SC	20, 50, 100
6.	Pyraclostrobin 20% WG	20, 50, 100
7.	Mancozeb 75% WP	500, 1000, 1500
8.	Control	

4.13 *In vivo* evaluation of fungicides

A field experiment on potato crop was conducted at Agricultural Research Station, Kota district during *Rabi* 2019-20 to know the efficacy of all the above mentioned fungicides. The experiment was laid out in Randomized Block Design (RBD) with three replications. Potato tubers were sown on 7th November 2019 and harvested on 29 February 2020. Two 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. 45 days after planting. Second spray was given at 60 days after planting. Five plants in each subplot were considered for disease and data were converted into per cent disease intensity (PDI) and per cent disease incidence as explained earlier. Finally yield data was taken after harvest.

Treatment details of fungicides with dose (g) a.i./ha

S. No.	Name of fungicides	Dosage (ml/g)/ha		
		a.i.	**Formulation(ml)**	**Water (L)**
1.	Difenoconazole 25% EC	50 ml	250	500
2.	Propiconazole 25% EC	125 ml	500	500
3.	Hexaconazole 5% EC	25 ml	500	500
4.	Azoxystrobin 23% SC	125 ml	500	500
5.	Picoxystrobin 22.52 SC	100 ml	400	500
6.	Pyraclostrobin 20% WG	100 g	500	500
7.	Mancozeb 75% WP	2250 g	1500	500
8.	Control (only water spray)	-	-	-

The disease intensity was calculated by using the following formula

$$\text{Per cent Disease Intensity} = \frac{\text{Sum of Individual rating}}{\text{Number of Plant Observed X Maximum Disease rating}} X100$$

The per cent disease control was calculated by using the following formula

$$\text{Per cent disease control} = \frac{\text{Disease in control} - \text{Disease in treatment}}{\text{Disease in control}} X100$$

The disease incidence was calculated by using the following formula

$$\text{Per cent disease Incidence} = \frac{\text{No.of diseased leaf}}{\text{Total number of leaves examined}} X100$$

The yield increase over control was calculated by using the following formula

$$\text{Yield increase over control} = \frac{T-C}{C} X100$$

Where,

T = Yield of respective treatment (kg/ha)

C = Yield of control (kg/ha)

3.14 Statistical analysis

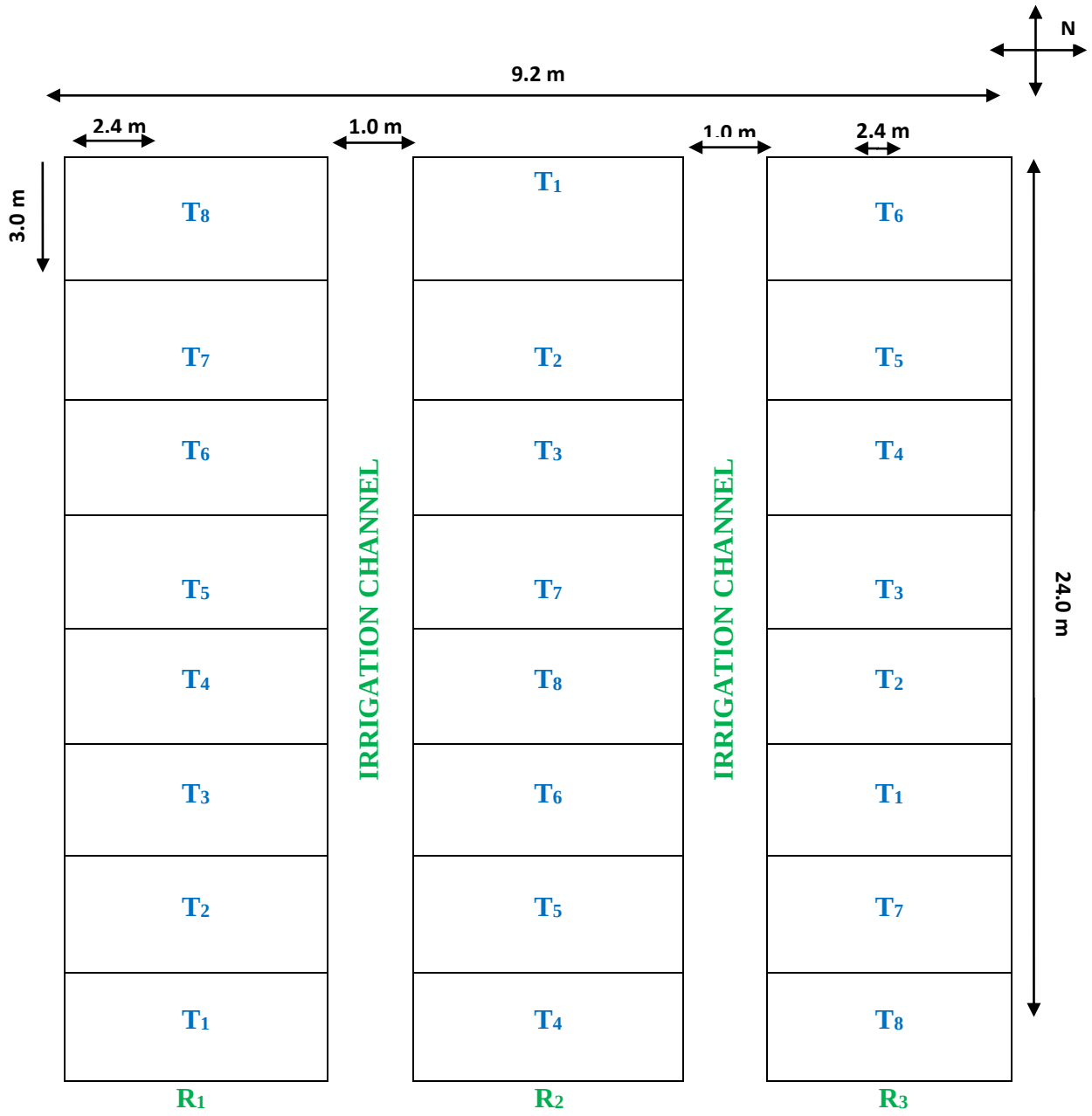
Randomized Block Design (RBD) (for cultivation of crop early blight incidence and yield) and Completely Randomized Design (CRD) (for *in vitro* experiments) were employed to analyse the data. The critical differences were worked out at 5% probability level. The experiments were conducted in the PG laboratory, Department of Plant Pathology, College of Agriculture, Ummedganj-Kota. Agriculture Research Station Ummedganj-Kota.

3.15 Cost benefit ratio

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

Cost benefit ratio was computed by using the formula

$$\text{B: C} = \frac{\text{Gross Income}}{\text{Net income}}$$



Treatment details		Other experimental details	
T_1-	Difenoconazole 25% EC	i.	Design - RBD
T_2-	Propiconazole 25% EC	ii.	Treatment - 8
T_3-	Hexaconazole 5% EC	iii.	Replication – 3
T_4-	Azoxystrobin 23% SC	iv.	Plot size – 3 m x 2.4 m
T_5-	Picoxystrobin 22.52 SC	v.	Spacing- 60 cm x 20 cm
T_6-	Pyraclostrobin 20% WG	vi.	Spacing between replication - 1.0 m
T_7-	Mancozeb 75% WP	vii.	Date of sowing- 7.11.2019
T_8-	Control	viii.	Date of harvesting- 29.02.2020

Chapter 4

EXPERIMENTAL RESULTS

The present research entitled **"Detection and management of early blight of potato caused by *Alternaria alternata*"** was conducted at the field of Agriculture Research Station, Ummedganj and in laboratory of college of Agriculture, Agriculture University-Kota, Rajasthan. The data recorded on different aspects and the results interpreted are presented in this chapter as under:

4.1 To study the symptoms, isolation, purification, pathogenicity and identification of pathogen

4.1.1 Symptomatology

Symptomatology study aids in providing information about symptoms produced by early blight disease and helps for diagnosis of disease in field conditions. Symptoms of early blight disease vary with potato variety, environmental conditions and stage of plant growth at the time of infection. The disease plants under field were observed weekly starting from germination to harvesting of crops. During the course of study disease symptoms become visible on all plant parts *viz.*, leaf, stem, petioles and tubers. The first symptom of the disease was seen on older leaves as small dark brown, frequently round necrotic spots of 1-2 mm diameters later, the spots enlarged with characteristics in center to produce a target board effect and the colour of spots altered from brown to dark brown. The contiguous spots eventually coalesce to form large irregular spots primary to drying and defoliation. When plants were 60-90 days mature, symptoms also appeared on stem and petioles as brown extended cankerous target broad spots. These enlarged and covered whole stem and petioles leading to withering of plants. The diseased plants produced less number of tubers and small sized tuber as compared to healthy plant. (Plate 4.1)

4.1.2 Isolation and purification

The associated pathogen was isolated by single spore isolation technique from infected potato leaves and pure culture of the fungus was obtained on potato dextrose agar medium. The pure culture of the fungus was obtained after eight days of inoculation which showed brownish colour. Purity and virulence of isolated fungus was regularly maintained and monitored by sub-culturing after 30 days and the culture

was maintained at 5^{o}C. For further studies, the original culture was revived once in 30 days (Plate 4.2).

4.1.3 Pathogenicity of the fungus

Pure culture was obtained by single spore isolation method and such culture was used for pathogenicity test. Inoculation with spore suspension and homogenized mycelial piece of *A. alternata* on foliage of 55 days old potato plants. The result revealed that the primary symptoms of disease occur on light brown spots seven days after inoculation as small chlorotic, disintegrate and light brown spots. Re-isolated and purified culture from these artificially infected leaves was similar to that of original culture in provided environmental conditions during study. The plants which were not inoculated with the fungal spore suspension did not show any symptoms of the disease (Plate 4.3 and 4.4).

4.1.4 Identification of the pathogen

An associated fungus was identified on the basis of its morphological and colony characters. The mycelium was greyish brown to black producing conidiophores moreover singly or in small groups which were straight or flexuous brown to olivaceous brown. The conidia were solitary straight or muriform or ellipsoidal narrowing to beak, pale or olivaceous brown, length 36.15-26.85 μm and 18.65-9.92 μm thick at the broadest piece with 3-4 transverse and 1-3 longitudinal septa. The beaks were flexuous, pale and occasionally branched. The identification of *Alternaria alternata* was further confirmed from Indian Type Culture Collection, Division of Plant Pathology, IARI, New Delhi-110012 with code number 295012.

4.2 To study the efficacy of bio-agents and media study against the pathogen *in vitro* conditions

4.2.1 Efficacy of media study

To find out the best medium for the mycelial growth and dry mycelial weight of *Alternaria alternata*, eight different solid and broth media were selected. The results presented in Table 4.1, Fig. - 4.1, and Plate 4.5 revealed that pathogen grew well on all the media tried. Perusal of data revealed that potato leaf agar medium was

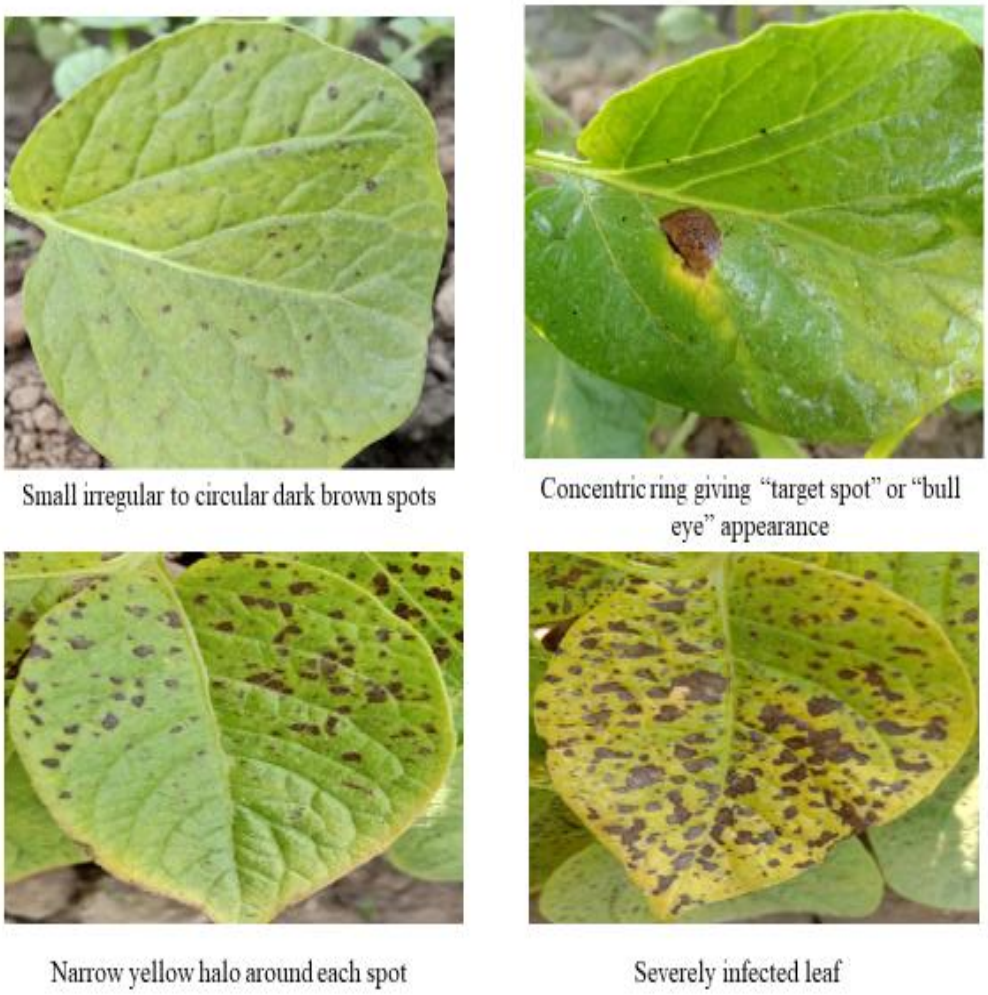


Plate – 4.1: Symptoms showing infected plants leaves

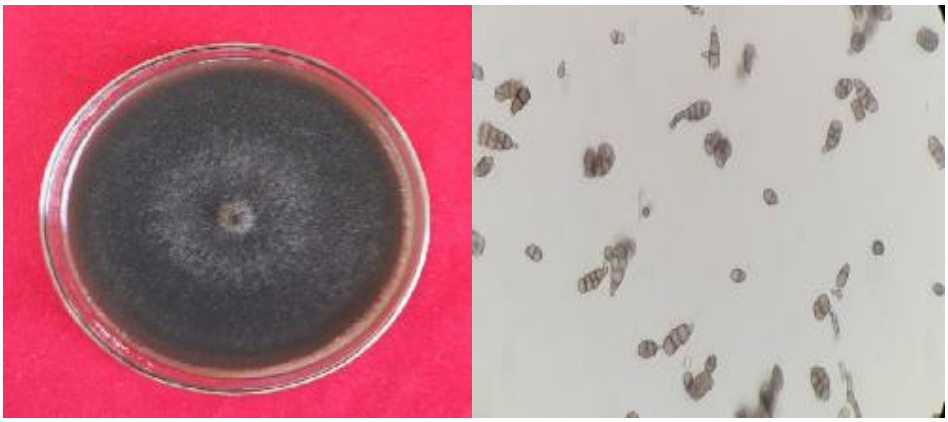


Plate – 4.2: Pure culture and spores of *A. alternata*

Plate – 4.3: Spraying of conidial suspention on potato plants for pathogenecity

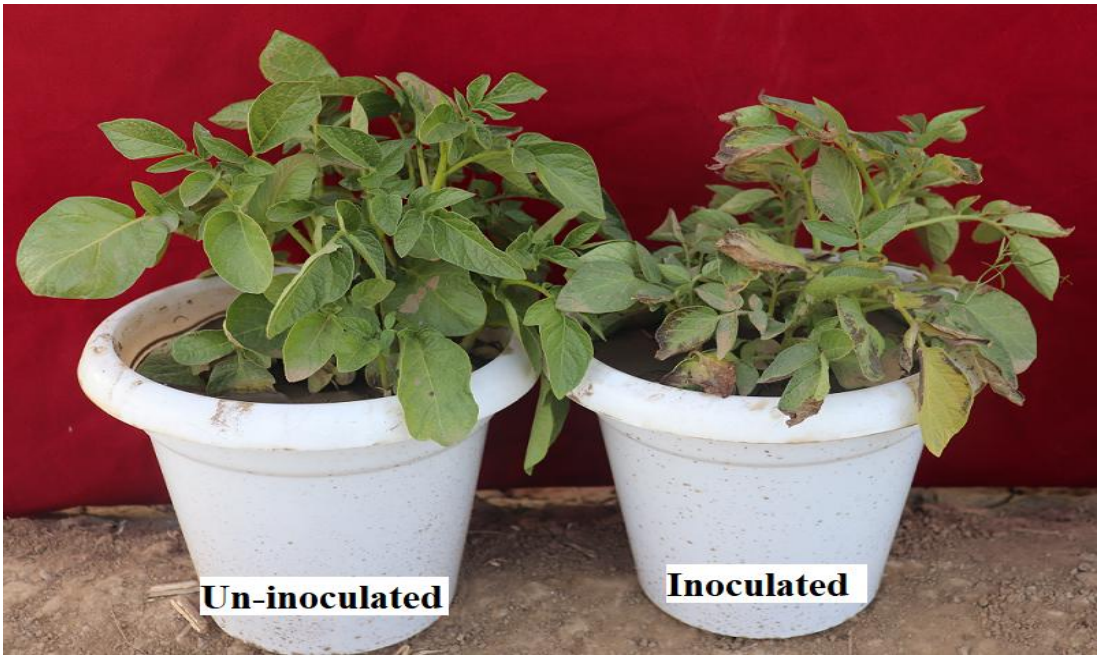


Plate – 4.4: Proving the pathogencity of early blight of potato in pots

significantly superior in supporting maximum mycelial growth at 5 days (70.50 mm) and 7 days (80.25 mm) of after inoculation the pathogen. This was followed by potato dextrose agar medium at 5 days with mycelial growth (64.75 mm) and 7 days (78.25 mm) over all other media used.

The next best media for mycelial growth in order of merit were potato stem agar medium, V-8 juice agar medium and pea agar medium with growth diameter at 5 days and 7 days (59.00, 57.50 and 58.00 mm), (67.25, 64.50, and 63.25 mm) respectively, three being statistically at par with each other. However, oat meal agar medium and potato carrot agar medium producing growth at 5 days and 7 days (50.00 and 46.50 mm), (58.25 and 53.75 mm), respectively and these both media were statistically at par with the each other. While, the czapek's agar medium supported least growth diameter of the fungus at 5 days and 7 days (37.50 and 42.75 mm).

Maximum dry mycelium weight i.e. 584.75 mg was recorded on potato leaf broth medium which was significantly superior as compare to other media used in study. Next best dry mycelium weight was recorded on potato dextrose broth 570.25 mg. The next in order of merit were potato stem broth 315.50 mg, V-8 juice broth 285.00 mg, pea broth medium 268.50 mg, oat meal broth 244.25 mg, potato carrot broth 212.25 mg while, and minimum in czapek's broth 176.50 mg (Plate – 4.6 and Fig.– 4.2).

Among the media tested, the potato leaf agar was significantly superior and most suitable medium for density of mycelial as well as dry mycelial weight followed by PDA.

The test culture media exhibited a wide range of sporulation. However, potato leaf agar exhibited excellent (++++) sporulation. It was good (+++) on potato dextrose agar and potato stem agar; moderate (++) on V-8 juice agar and potato carrot agar; whereas, it was poor (+) on Czapek's agar, Oat meal agar and pea agar medium. Whereas, the potato stem agar, V-8 juice agar, pea agar, oat meal agar, potato agar and czapek's agar medium were least effective in mycelial density.

Table 4.1 *In-vitro* evaluation of different media for mycelial growth of *A. alternata*

T. No.	Treatment name	Mycelial growth at 5 days (mm)	Mycelial growth at 7 days (mm)	Dry mycelium weight in broth media (mg)	Sporulation
1	V-8 juice agar	57.50 (49.31)*	64.50 (53.44)	285.00	++
2	Czapek's agar	37.50 (37.31)	42.75 (40.83)	176.50	+
3	Potato dextrose agar	64.75 (53.60)	78.25 (62.21)	570.25	+++
4	Oat meal agar	50.00 (45.00)	58.25 (49.76)	244.25	+
5	Potato stem agar	59.00 (50.19)	67.25 (55.10)	315.50	+++
6	Potato leaf agar	70.50 (57.12)	80.25 (63.63)	584.75	++++
7	Potato carrot agar	46.50 (42.99)	53.75 (47.16)	212.25	++
8	Pea agar	58.00 (49.62)	63.25 (52.69)	269.50	+
S. Em. ±		(0.86)	(0.89)	2.73	
CD at 5%		(2.50)	(2.60)	7.96	

+ = Poor, ++ = Moderate, +++ = Good, ++++ = Excellent

***Figures in parentheses are arcsine transformed values

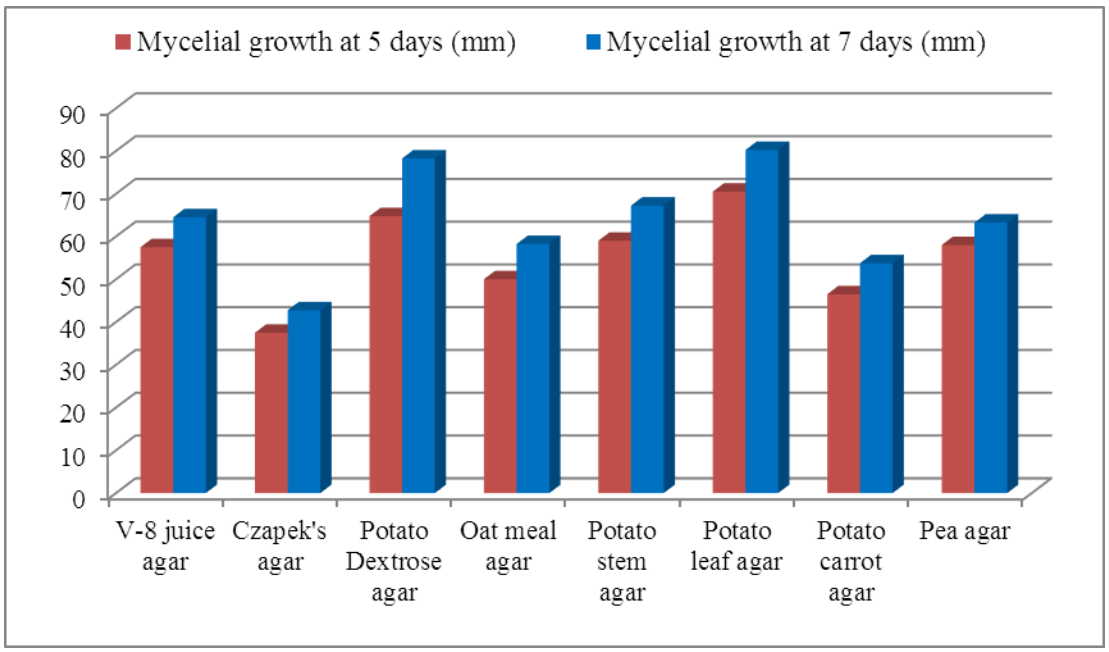


Fig. – 4.1: Evaluation of different solid media for mycelial growth of *A. alternata in vitro*

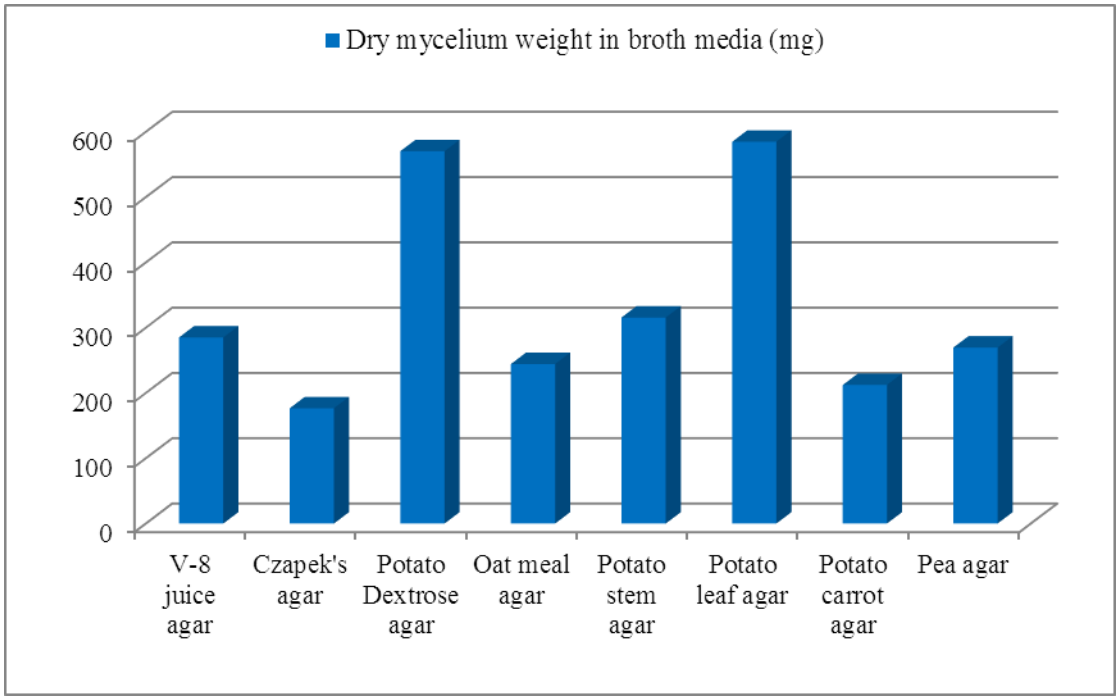


Fig. – 4.2: Evaluation of different broth media for dry mycelium weight of *A. alternata in vitro*

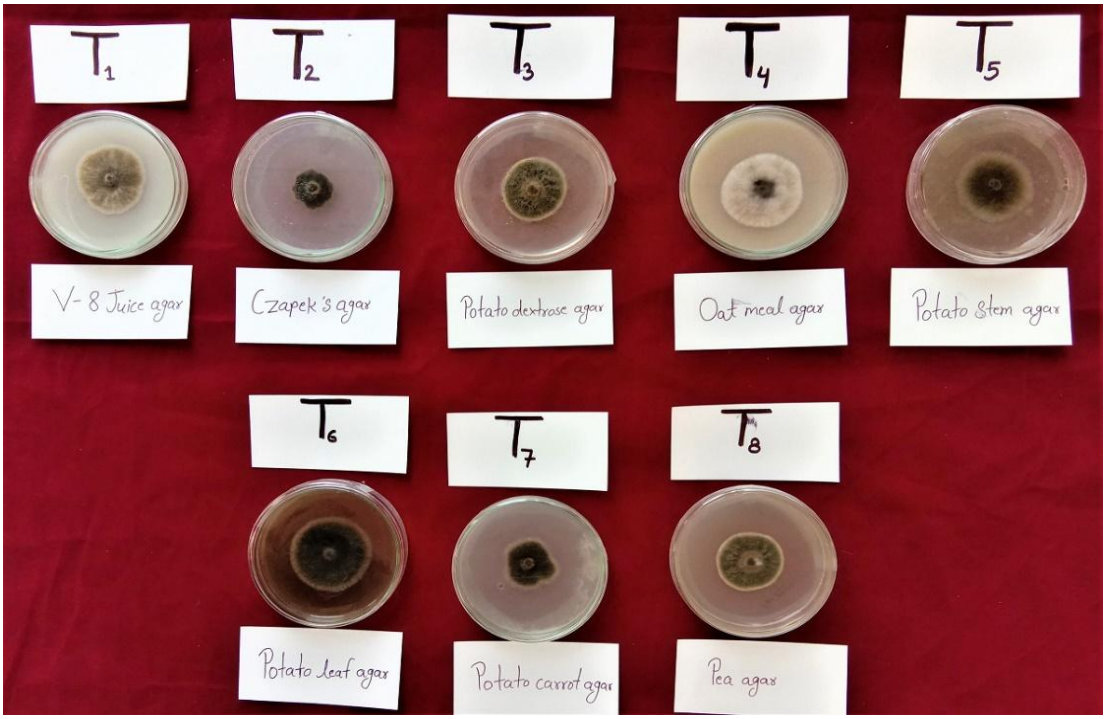


Plate – 4.5: Efficacy of different culture media against *A. Alternata*

T_1- V-8 Juice agar, T_2- Czapek's agar, T_3- Potato dextrose agar, T_4- Oat meal agar, T_5- Potato stem agar, T_6- Potato leaf agar, T_7- Potato carrot agar, T_8- Pea agar

Plate – 4.6: Dry mycelium weight of *A. alternata* on broth media

4.2.2 Efficacy of bio-agents

Four bioagents of *Trichoderma* species *viz.*, *Trichoderma virens*, *Trichoderma azospirillum*, *Trichoderma harzianum* and *Trichoderma viride* were evaluated for their antagonistic activity against *A. alternata* by dual culture technique. Based on the observations of radial growth of antagonist and fungus, per cent inhibition was calculated.

The results pertaining to antagonistic activity are presented in Table 4.2 revealed that all the four *Trichoderma* species significantly reduced the mycelial growth of the pathogen over the control. Significantly maximum inhibition of mycelial growth of the pathogen after 48 hours of incubation was obtained with *T. viride* (57.08%) followed by *T. azospirillum* (50.00%), *T. harzianum* (43.26%) and *T. virens* (37.08%) (Plate- 4.7 and Fig. - 4.3).

Table 4.2 Effect of bio-agents against *A. alternata in vitro* by dual culture

T. No	Treatment name	Mycelial growth At 48 hrs. (mm)	Per cent growth Inhibition
1	*Trichoderma virens*	28.00 (31.94)*	37.08
2	*Trichoderma azospirillum*	22.25 (28.14)	50.00
3	*Trichoderma harzianum*	25.25 (30.16)	43.26
4	*Trichoderma viride*	19.02 (26.02)	57.08
5	Control	44.50 (41.84)	--
S. Em. ±		(0.47)	
CD at 5%		(1.41)	

*****Figures in parentheses are arcsine transformed values

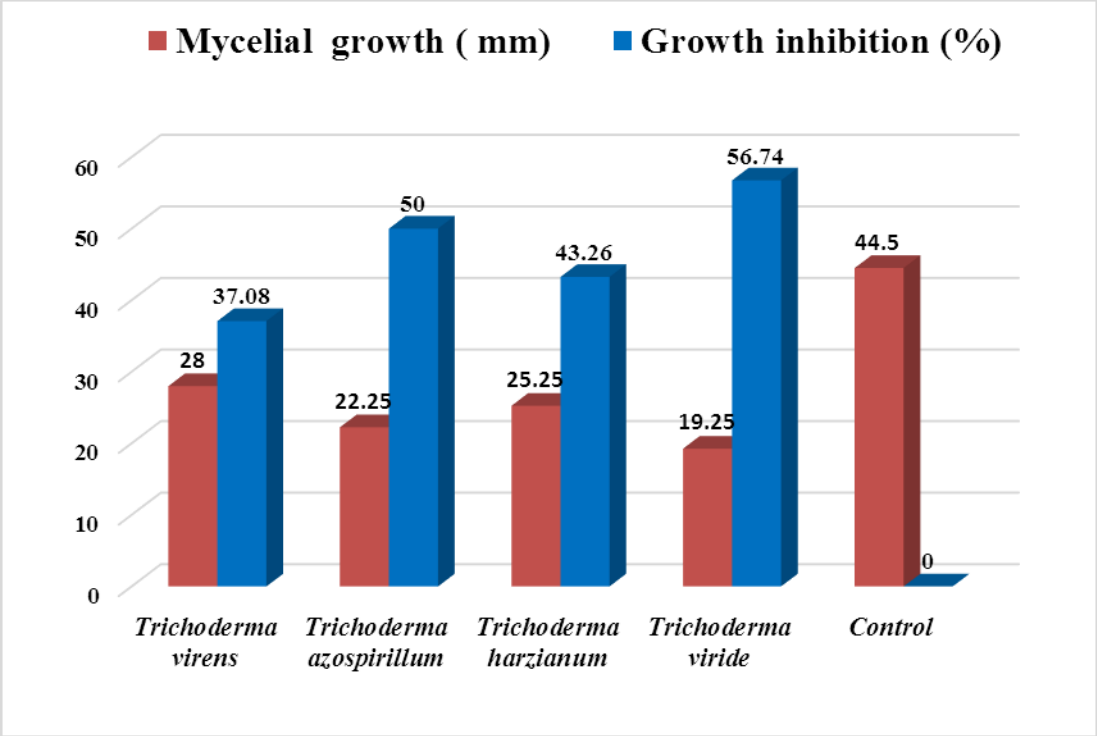


Fig. – 4.3: Effect of bio-agents against *A. alternata in vitro* condition

Plate – 4.7: *In vitro* evaluation of bio-agents against *A. alternata*

4.3 Screening the genotypes against the disease

Twenty-eight genotypes/varieties of potato were screened for resistance to early blight under natural field condition during *Rabi* 2019-20. The per cent germination as well as per cent disease incidence was calculated. The observation of early blight incidence was recorded at 60 and 75 days after sowing. Result in Table 4.3 and 4.4 revealed that different varieties were showed good germination percentage, which showed uniform growth of the plants (Plate - 4.8 and Fig. - 4.4).

Out of twenty eight genotypes/varieties, six genotypes *viz*., CP-3021, 3153, Atlantic, Chip-3, Jx-161 and MS/78-62 showed resistant reaction, while thirteen genotypes i.e., NJ-1501, MP-97, NJ-44, NJ-34, MP-97-1606, PS/RI-135, JI-93-77, MS/94-118, JN-1177, Kufri Sindhuri, Kufri Pukhraj, Kufri Khayati and Kufri Pushkar were moderately resistant. However, six genotypes *viz.*, NJ-85, NJ-1530, J-92-164, MS/92-2105, J/93-4 and Kufri Bahar were susceptible while, three genotypes i.e., MP/94-322, NJ-1 and MS/85-1663 were highly susceptible.

Table 4.3 Result summary of genotypes

Categories	**No.**	**Genotypes/varieties**
Resistant	6	CP-3021, 3153, Atlantic, Chip-3, Jx-161 and MS/78-62
Moderate resistant	13	NJ-1501, MP-97, NJ-44, NJ-34, MP-97-1606, PS/RI-135, JI-93-77, MS/94-118, JN-1177, Kufri Sindhuri, Kufri Pukhraj, Kufri Khayati and Kufri Pushkar
Susceptible	6	NJ-85, NJ-1530, J-92-164, MS/92-2105, J/93-4 and Kufri Bahar
Highly susceptible	3	MP/94-322, NJ-1 and MS/85-1663

Table 4.4 Screening of different genotypes/varieties against early blight of potato

S. No.	Name of genotypes /varieties	Germination (%)	Disease incidence (%)	
			At 60 DAS	At 75 DAS
1	NJ-1501	75	8	17
2	MP-97	66	6	15
3	MP/94-322	87	38	52
4	NJ-44	80	6	13
5	NJ-85	86	25	38
6	NJ-34	80	10	18
7	MP-97-1606	83	7	16
8	NJ-1530	70	24	37
9	PS/RI-135	92	5	14
10	CP-3021	85	2	4
11	J-92-164	82	26	39
12	JI-93-77	73	6	17
13	MS/94-118	68	5	14
14	JN-1177	65	7	16
15	MS/92-2105	90	25	36
16	J/93-4	80	27	39
17	NJ-1	76	40	54
18	MS/85-1663	80	39	55
19	Kufri Sindhuri	86	9	18
20	Kufri Bahar	88	29	38
21	Kufri Pukhraj	80	8	15
22	Kufri Khayati	70	5	13
23	Kufri Pushkar	73	5	12
24	3153	82	1	3
25	Atlantic	87	3	5
26	Chip-3	85	1	2
27	Jx-161	79	2	3
28	MS/78-62	90	2	4

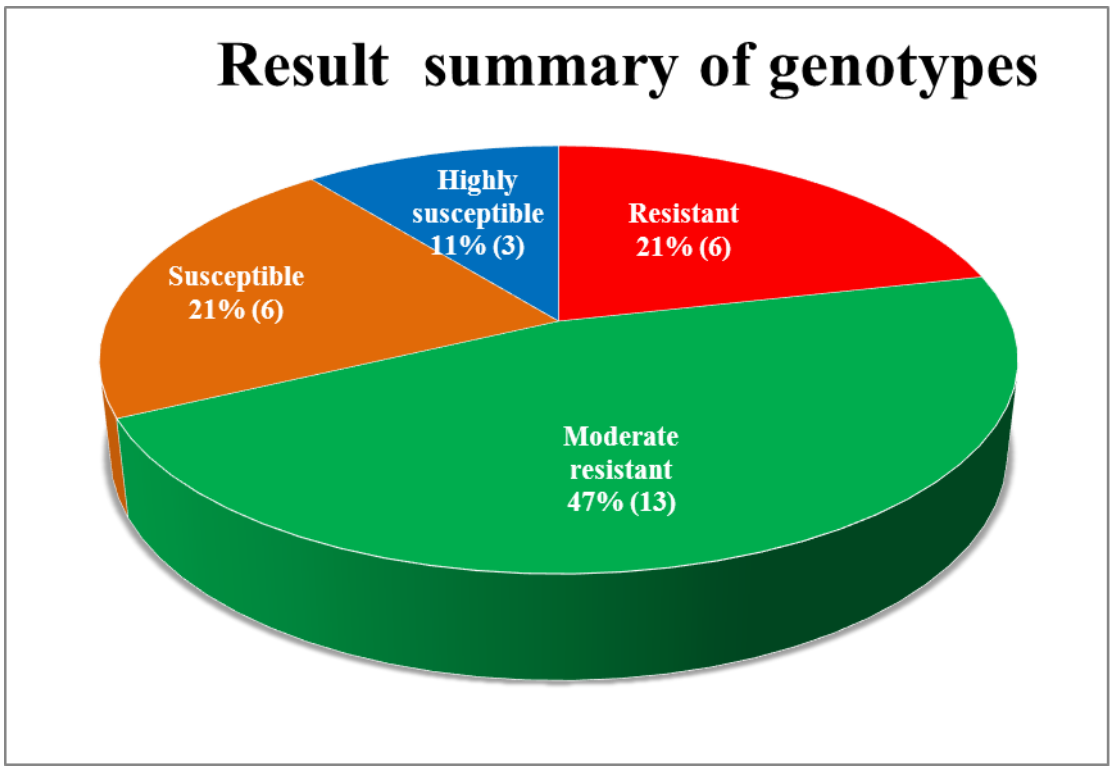


Fig. – 4.4: Result summary of genotypes under field condition

Plate – 4.8: Field view of potato genotypes

4.4 To study the bio-efficacy of fungicide against the pathogen *in vitro* and *in vivo* conditions

4.4.1 Bio-efficacy of fungicides against the pathogen *in vitro*

The efficacy of various systemic as well as non-systemic fungicides *viz.*, Difenconazole, Propiconazole, Hexaconazole, Azoxystrobin, Picoxystrobin, Pyraclostrobin and Mancozeb were evaluated for their efficacy against *A. alternata in vitro* by poisoned food technique.

The results presented in (Table 4.5 and Plate 4.9) indicated that different fungicides have different efficacy against the growth of *Alternaria alternata* and all the fungicides significantly reduced the growth of the *A. alternata* as compare to control. Propiconazole 25% EC and Hexaconazole 5% EC both at all the three concentrations as well as Mancozeb 75% WP at 1000 and 1500 ppm and Difenconazole 25% EC at 50 ppm were found significantly statistically at par with 100 percent inhibition as compared to rest of the fungicides.

Next best treatments in order of merit were Difenconazole 25% EC at 10 and 20 ppm, Mancozeb 75% WP at 500 ppm and Azoxystrobin 23% SC at all the three concentrations significantly recorded minimum mycelium growth and maximum inhibition compared to control. However, Picoxystrobin 22.52% SC and Pyraclostrobin 20% WG both at all the three concentrations gave significantly maximum mycelium growth and minimum percent growth inhibition.

Present study revealed that Triazole group fungicide *viz.*, Propiconazole 25% EC, Hexaconazole 5% EC and Defenconazole 25% EC were significantly superior and proved the most effective in inhibiting mycelial growth @ 10, 20 and 50 ppm as compared to strobilin group *viz.*, Azoxystrobin 23% SC, Picoxytstrobin 22.52% SC and Pyraclostrobin 20% WG at 20, 50, 100 ppm against the *Alternaria alternata in vitro* condition by poison food technique. While contact fungicide Mancozeb 75% WP showed maximum inhibition at higher dose 1000 and 1500 ppm. (Fig. - 4.5a, 4.5b and 4.5c).

Table 4.5 Effect of different fungicides and their concentrations on growth of *A. alternata in vitro* at 25 ± 1°C

T. No.	Treatment	Concentration (ppm)	Mycelial growth (mm)	Percent inhibition
1	Difenconazole 25% EC	10	13.00 (21.11)*	85.56
		20	7.75 (16.14)	97.73
		50	0.00 (0.00)	100
2	Propiconazole 25% EC	10	0.00 (0.00)	100
		20	0.00 (0.00)	100
		50	0.00 (0.00)	100
3	Hexaconazole 5% EC	10	0.00 (0.00)	100
		20	0.00 (0.00)	100
		50	0.00 (0.00)	100
4	Azoxystrobin 23% SC	20	23.75 (29.16)	73.61
		50	14.50 (22.37)	83.89
		100	13.00 (21.12)	85.56
5	Picoxystrobin 22.52% SC	20	36.00 (36.87)	60
		50	32.25 (34.60)	64.17
		100	11.50 (19.80)	87.22
6	Pyraclostrobin 20% WG	20	41.50 (40.10)	53.89
		50	34.75 (36.12)	61.39
		100	12.50 (20.69)	86.11
7	Mancozeb 75% WP	500	11.50 (19.80)	87.22
		1000	0.00 (0.00)	100
		1500	0.00 (0.00)	100
8	Control		90.00 (71.56)	--
	S. Em. ±		(0.39)	--
	CD at 5 %		(1.11)	--

*****Figures in parentheses are arcsine transformed values

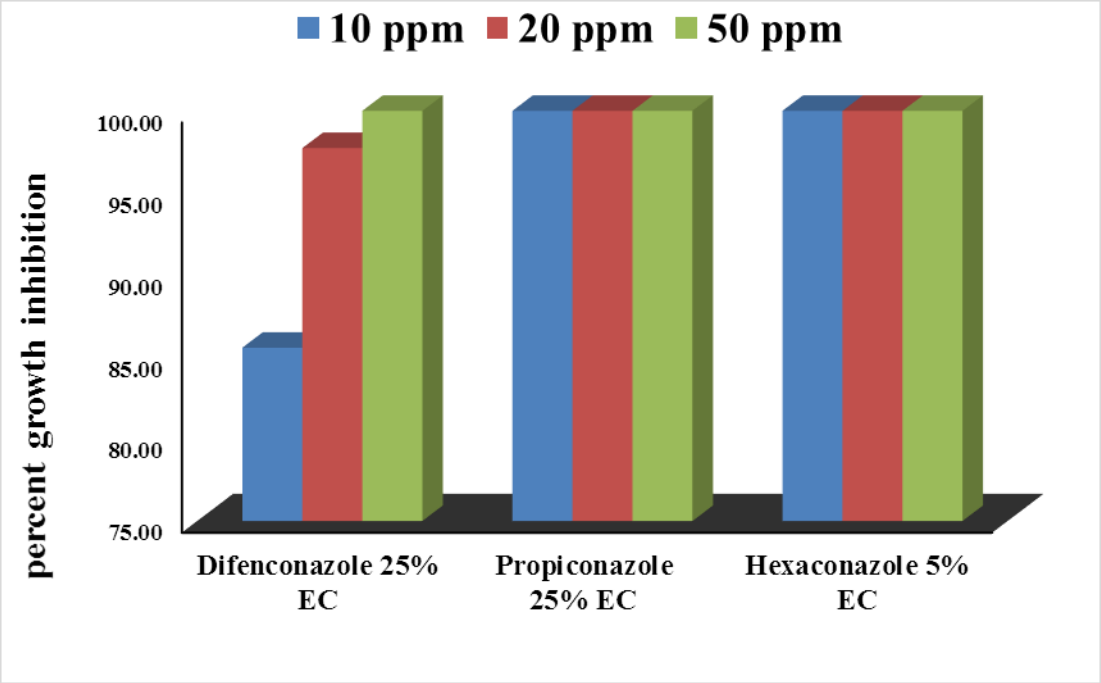


Fig. – 4.5a: *In vitro* bio-efficacy of trizole fungicides on percent growth inhibition of *A. alternata* by poisoned food technique at 10, 20 and 50 ppm concentrations.

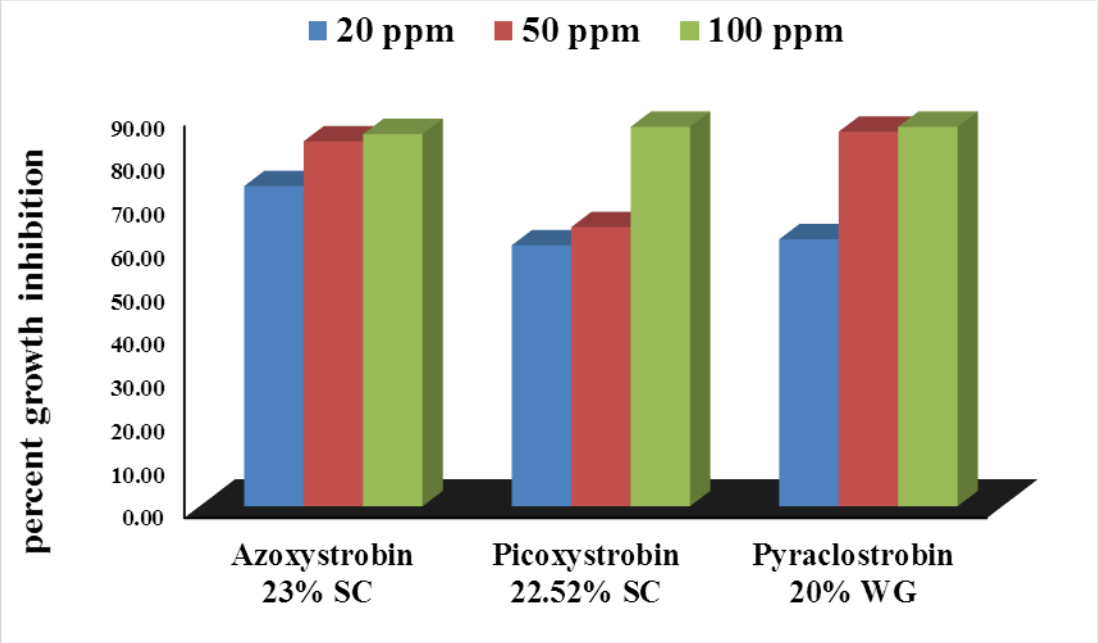


Fig. – 4.5b: *In vitro* bio-efficacy of strobilurin fungicides on percent growth inhibition of *A. alternata* by poisoned food technique at 20, 50 and 100 ppm concentrations.

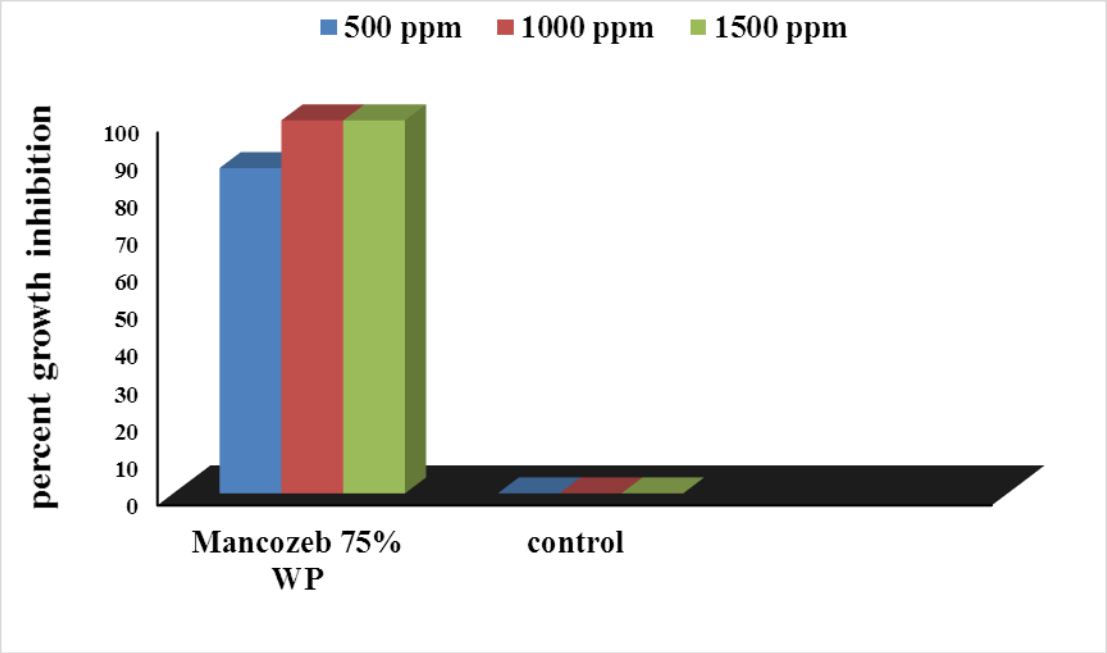


Fig. – 4.5c: *In vitro* bio-efficacy of contact fungicides on percent growth inhibition of *A. alternata* by poisoned food technique at 500, 1000 and 1500 ppm concentrations.

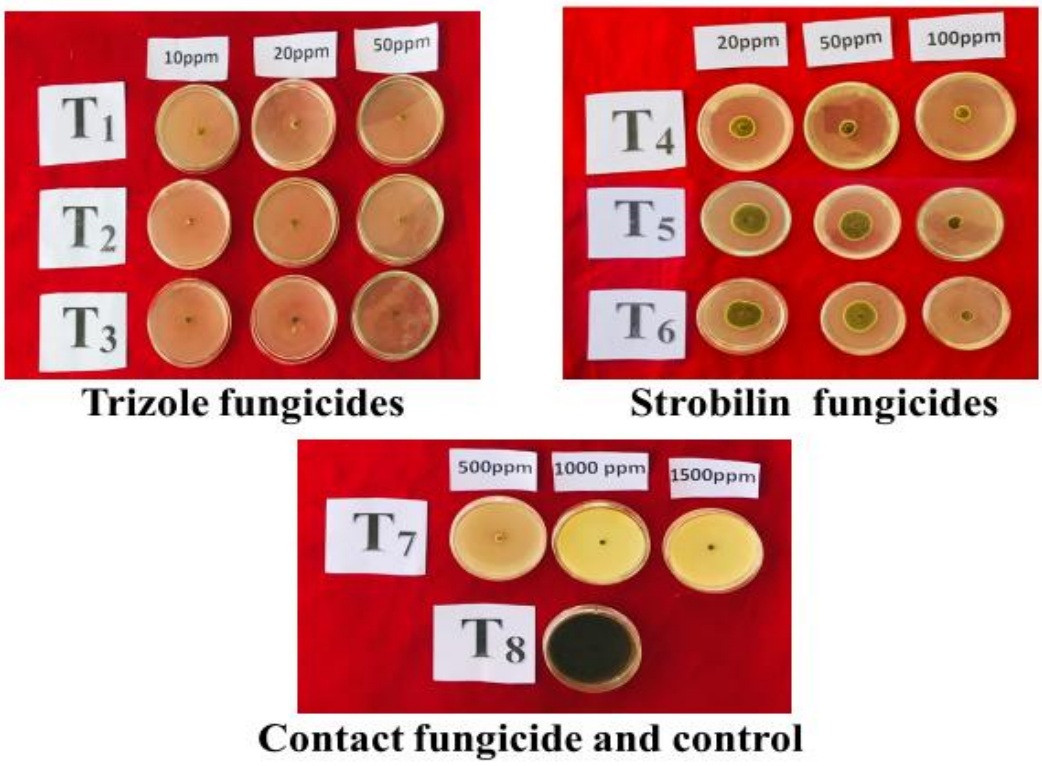


Plate – 4.9: Bio- efficacy of different fungicides against *A. alternata in vitro* condition

4.4.2 The bio-efficacy of fungicides

4.4.2.1 Per cent disease intensity

Fungicides found promising *in vitro* were further evaluated *in vivo* against early blight of potato. The germination was found non-significant in experiment. The first spray of the fungicide was given at initiation of early blight disease. All the tested systemic as well as non-systemic fungicides were found effective as foliar sprays against early blight of potato under field conditions and reduced the present disease intensity and gave significant reduction in disease over control. Significantly, minimum disease intensity was recorded in Dinfenconazole 5% EC @ 250 ml/ha after first (PDI 4.5%) and second (8.0%) spray at 14 days followed by Propiconazole 25% EC @ 500 ml/ha (PDI 4.7% and 8.3%), Azoxystrobin 23% SC @ 500 ml/ha (PDI 4.8% and 8.5 %). However, Picoxystrobin 22.52% SC @ 400 ml/ha showed PDI 5.3% and 9.0 % and PDC 68.2 % which was on par with Pyraclostrobin 20% WG @ 500 ml/ha (PDI 5.7% and 9.4 %) and PDC (66.3%) after 14 days of each spray, while, Hexaconazole 5% EC @ 500 ml/ha (PDI 6.3% and 10.1%) and PDC (63.6%) after 14 days of each spray and Mancozeb 75% WP @ 1500 gm/ha showed (6.4% and 12.6%) and PDC (57.4%) 14 days after first and second spray. Whereas, mean per cent disease intensity of control was recorded (19.48%) during study period. In all the treatments was significantly produced lower per cent disease intensity as compared to control. The highest per cent reduction over control i.e. 72.3% was recorded in i.e. Dinfenconazole 5% EC @ 250 ml/ha followed by spraying of Propiconazole 25% EC @ 500 ml/ha (71.3%) and Azoxystrobin 23% SC @ 500 ml/ha 70.2 % at 28 days of spray (Table 4.6) and (Fig.- 4.6a and 4.6b).

Table 4.6 Evaluation of fungicides as foliar spray under field conditions

T. No.	Treatment name	Doses per ha.	Germination (%) at 30 DAS	Percent disease intensity				Mean PDI	Percent disease control
				After 1st spray		After 2nd spray			
				7 days	14 days	7 days	14 days		
1	Difenconazole 25% EC	250 ml	94	3.0 (9.95)*	4.5 (12.23)	6.1 (14.28)	8.0 (16.43)	5.4	72.3
2	Propiconazole 25% EC	500 ml	90	3.2 (10.29)	4.7 (12.55)	6.4 (14.65)	8.3 (16.74)	5.6	71.3
3	Hexaconazole 5% EC	500 ml	95	4.1 (11.72)	6.3 (14.53)	7.73 (16.14)	10.1 (18.58)	7.1	63.6
4	Azoxystrobin 23% SC	500 ml	91	3.3 (10.43)	4.8 (12.70)	6.63 (14.92)	8.5 (17.02)	5.8	70.2
5	Picoxystrobin 22.52% SC	400 ml	93	3.5 (10.80)	5.3 (13.30)	6.97 (15.30)	9.0 (17.52)	6.2	68.2
6	Pyraclostrobin 20% WG	500 g	91	3.7 (11.18)	5.7 (13.89)	7.13 (15.49)	9.4 (17.85)	6.5	66.6
7	Mancozeb 75% WP	1500 g	92	4.2 (11.91)	6.4 (14.73)	9.7 (18.15)	12.6 (20.79)	8.3	57.4
8	Control		90	11.4 (19.79)	16.1 (23.70)	22.1 (28.05)	28.1 (32.05)	19.4	0.0
S. Em. ±			1.56	(0.31)	(0.35)	(0.31)	(0.31)		---
CD at 5 %			N/S	(0.94)	(1.06)	(0.96)	(0.94)		---

*****Figures in parentheses are arcsine transformed values

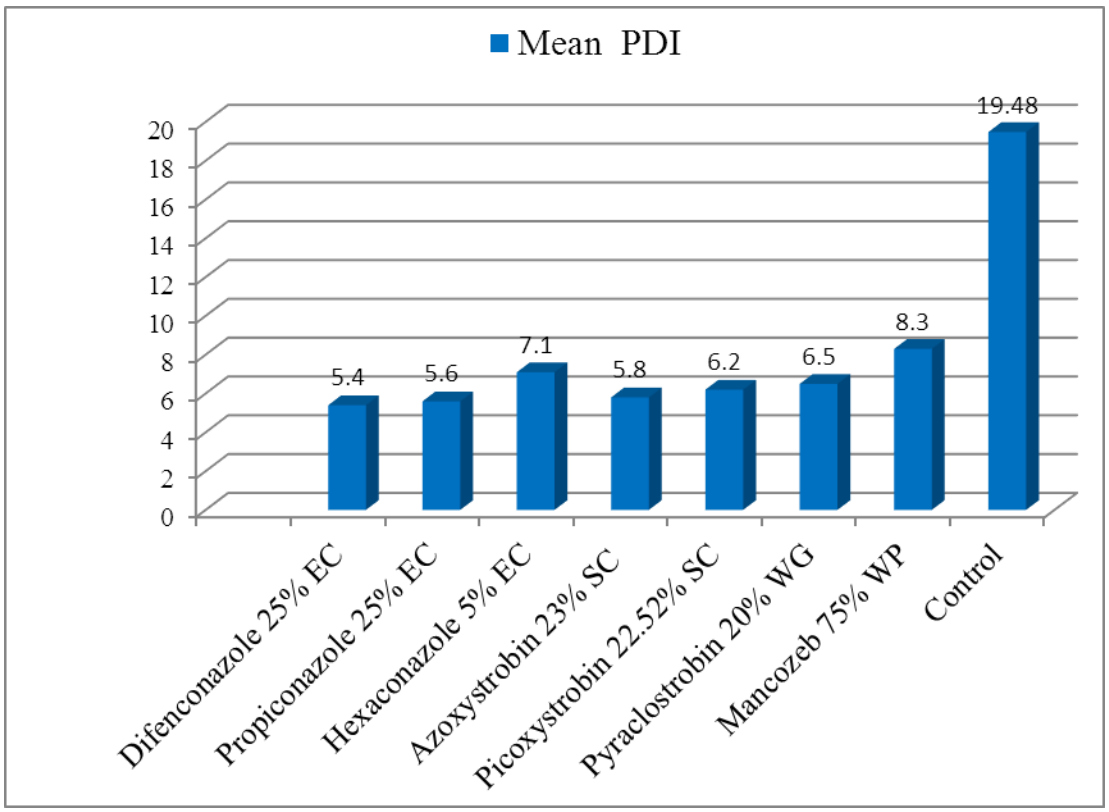


Fig. – 4.6a: Effect of different fungicides on percent disease intensity (*in vivo*)

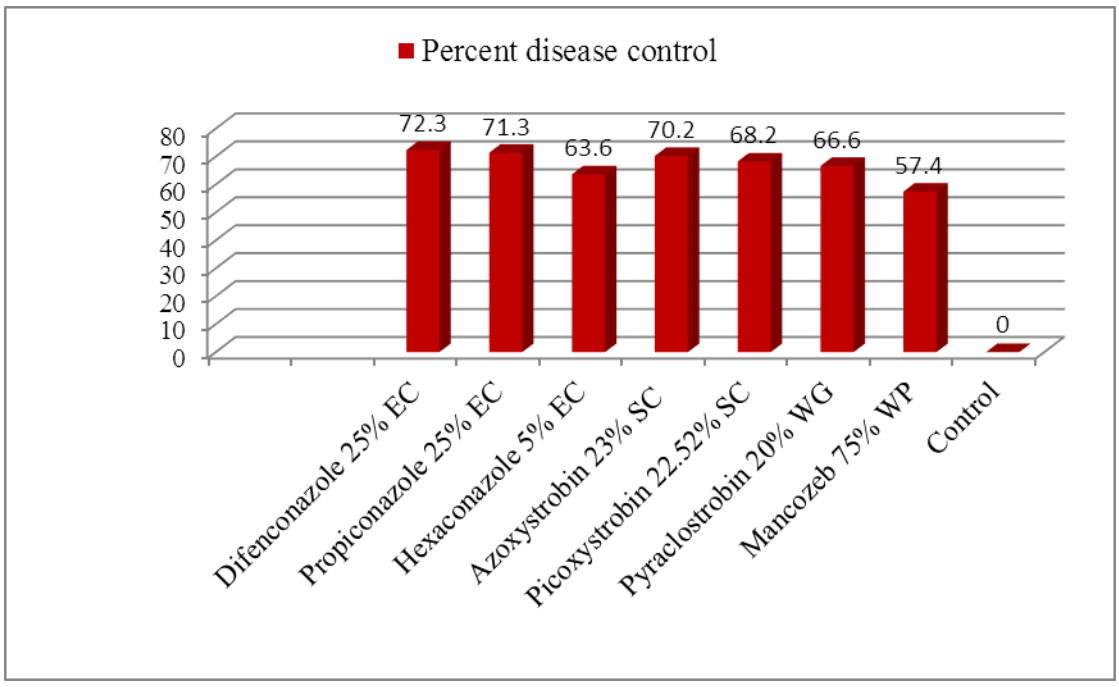


Fig. – 4.6b: Effect of different fungicides on percent disease control (*in vivo*)

4.4.2.2 Disease incidence

The result presented in table 4.7 indicates that the early blight incidence in potato was significantly reduced in fungicidal treatments. Minimum per cent disease incidence were recorded i.e. 14.03% in Difenconazole 25% EC. However, Propiconazole 25% EC showed 15.47% which was on par with Hexaconazole 5% EC (15.83%). Whereas, Azoxystrobin 23% SC (17.37%), Picoxystrobin 22.52% SC (18.73%) and Pyraclostrobin 20% WG (19.07%). while per cent disease incidence i.e. 20.80% was recorded in treatment of Mancozeb 75% WP as compared to control 34.57% (Fig.- 4.7a).

4.4.2.3 Dry matter

In all the fungicides were significantly showed better per cent dry matter as compared to control. Significantly maximum per cent dry matter was recorded by foliar spray of Azoxystrobin 23% SC @ 500 ml/ha (25.00 %). Next best treatments in order of merit were Pyraclostrobin 20% WG (24.03%), Picoxystrobin 22.52% SC (23.87%) and Hexaconazole 5% EC (23.07%), Mancozeb 75% WP (22.23%) and Difenconazole 25% EC (22.00%). Whereas, 21.20% was recorded in Propiconazole 25% EC and 20.13% in control.

In experiment revealed that Triazole group fungicide *viz.*, Propiconazole 25% EC, Hexaconazole 5% EC and Defenconazole 25% EC were significantly inferior compared to strobilin group *viz*., Azoxystrobin 23% SC, Picoxytstrobin 22.52% SC and Pyraclostrobin 20% WG for per cent dry matter of potato tuber (Fig.- 4.7b).

Table 4.7 Evaluation of fungicides as foliar spray under field conditions (disease incidence)

T. No.	Treatment name	Doses per ha	Disease incidence (%)	Dry matter (%)
1	Difenconazole 25%EC	250 ml	14.03 (22.00)*	22.00
2	Propiconazole 25%EC	500 ml	15.47 (23.16)	21.20
3	Hexaconazole 5% EC	500 ml	15.83 (23.45)	23.07
4	Azoxystrobin 23% SC	500 ml	17.37 (24.63)	25.00
5	Picoxystrobin 22.52% SC	400 ml	18.73 (25.65)	23.87
6	Pyraclostrobin 20% WG	500 g	19.07 (25.89)	24.03
7	Mancozeb 75% WP	1500 g	20.8 (27.13)	22.23
8	Control		34.57 (36.01)	20.13
S. Em. ±			(0.15)	0.17
CD at 5 %			(0.45)	0.53

*****Figures in parentheses are arcsine transformed values

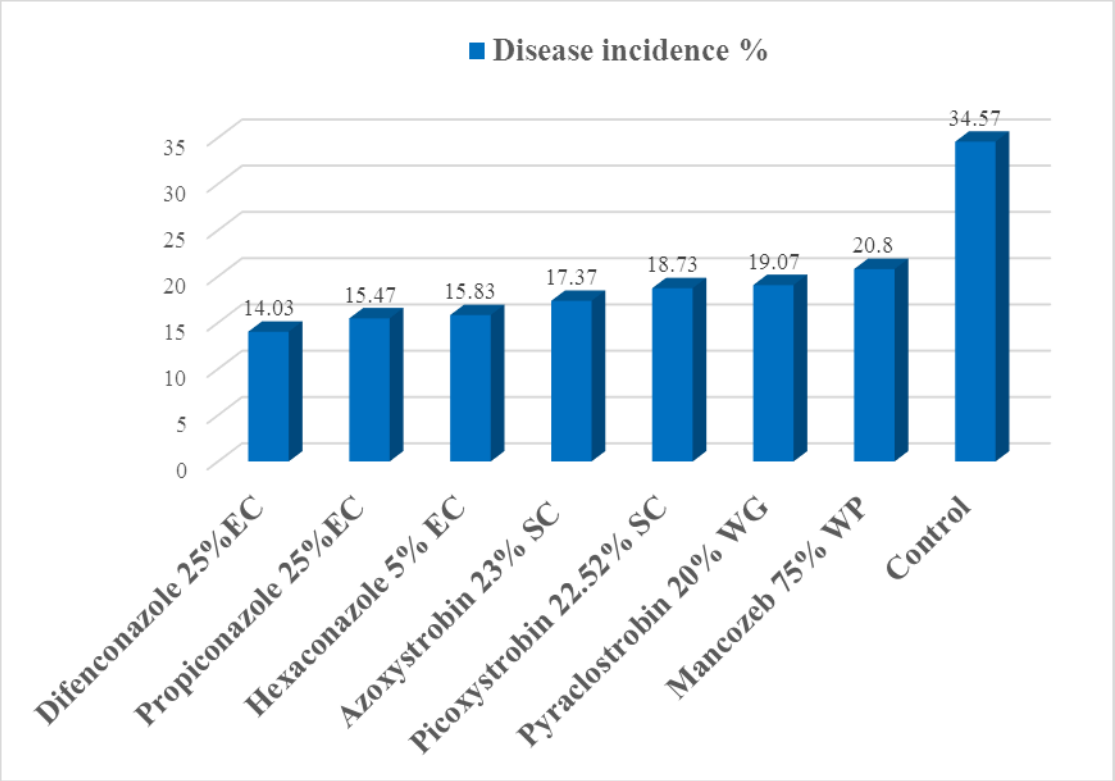


Fig. – 4.7a: Effect of different fungicides against *A. alternata* on disease incidence

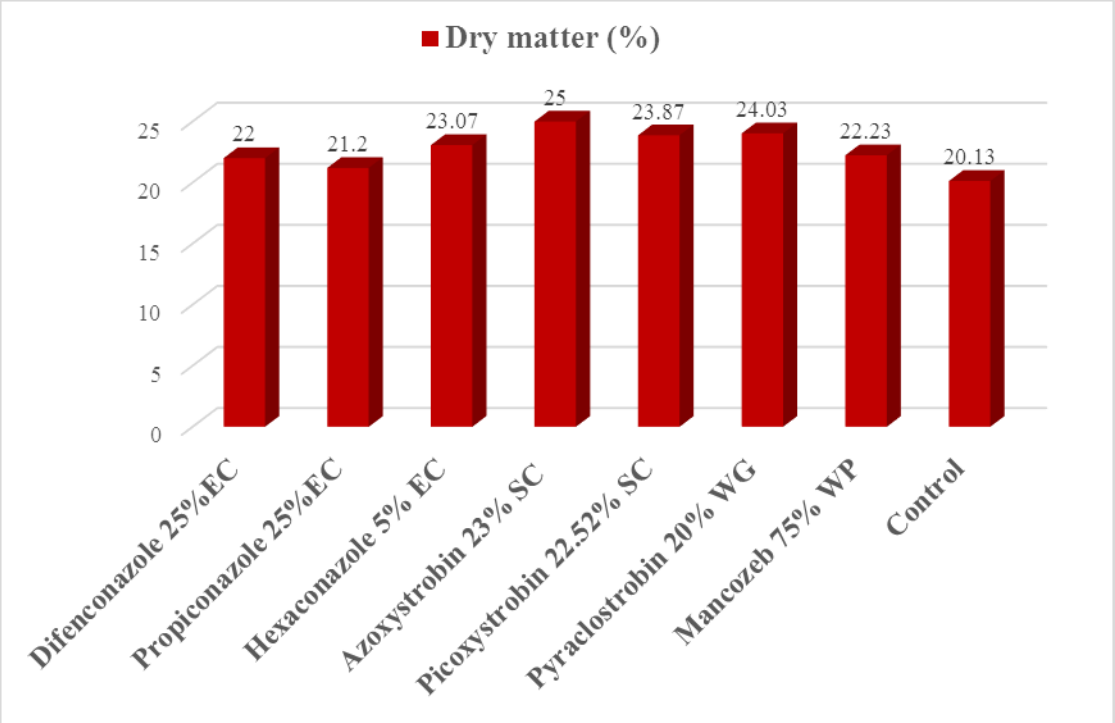


Fig. – 4.7b: Effect of different fungicides against *A. alternata* on dry matter of potato

4.4.2.4 Dry weight of dehalming cutting and number of shoots and tuber per plant and yield

4.4.2.4.1 Dry weight of dehalming cutting

Maximum dry weight of dehalming cutting i.e. 1.90 kg was recorded in Azoxystrobin 23% SC @ 500 ml/ha. However, Next best treatments in order of merit were Picoxystrobin 22.52% SC (1.74 kg), Pyraclostrobin 20% WG (1.64 kg), Difenconazole 25% EC (1.60 kg), Propiconazole 25% EC (1.55 kg), Mancozeb 75% WP (1.53 kg) and Hexaconazole 5% EC (1.51 kg) as compared to control (0.98 kg). The study revealed that strobilin group fungicides *viz.*, Azoxystrobin 23% SC, Picoxytstrobin 22.52% SC and Pyraclostrobin 20% WG were showed maximum dry weight of dehalming cutting (kg) as compared to Triazole group fungicides *viz.*, Propiconazole 25% EC, Hexaconazole 5% EC and Defenconazole 25% EC (Table- 4.8).

4.4.2.4.2 Number of shoots per plant

Observations in Table 4.8 revealed that the different fungicides were non-significant for number of shoots per plant as compared to control.

4.4.2.4.3 Total number of tubers per plot

All the treatments gave significantly maximum potato tuber as compared to untreated check. The highest (269) total number of tubers per plot were observed in i.e. Azoxystrobin 23% EC @ 500 ml/ha followed by i.e. Picoxystrobin 22.52% SC (260), Pyraclostrobin 20% WG (258), Difenconazole 25% EC (257) and Propiconazole 25% EC (247) which were at par with each other. However, in Mancozeb 75% WP (238) number of tubers were recorded while it was least in untreated check i.e. (198) tubers (Fig. - 4.8a).

Table 4.8 Evaluations of fungicides as foliar spray under field condition

T. No.	Treatment name	Doses per ha	Dry weight of dehalming cutting (kg)	Number of shoots per plant	Total tuber number per plot	Total tuber yield/plot	Percent yield increase over control
1	Difenconazole 25% EC	250 ml	1.60 (7.27)*	5.4 (13.43)	257.33	14.93	29.2
2	Propiconazole 25% EC	500 ml	1.55 (7.14)	5.2 (13.17)	247.67	13.83	23.6
3	Hexaconazole 5% EC	500 ml	1.51 (7.04)	5.6 (13.68)	235.33	12.93	18.3
4	Azoxystrobin 23% SC	500 ml	1.9 (7.92)	5.33 (13.35)	269.67	16.17	34.6
5	Picoxystrobin 22.52% SC	400 ml	1.74 (7.59)	5.53 (13.60)	260.67	15.8	33.1
6	Pyraclostrobin 20% WG	500 g	1.64 (7.35)	5.4 (13.43)	258.33	15.4	31.4
7	Mancozeb 75% WP	1500 g	1.53 (7.09)	5.33 (13.35)	238.67	14.63	27.8
8	Control		0.98 (5.68)	5.13 (13.09)	198.67	10.57	0.0
	S. Em. ±		(0.15)	(0.19)	8.41	0.80	---
	CD at 5 %		(0.47)	N/S	25.52	2.43	---

*****Figures in parentheses are arcsine transformed values

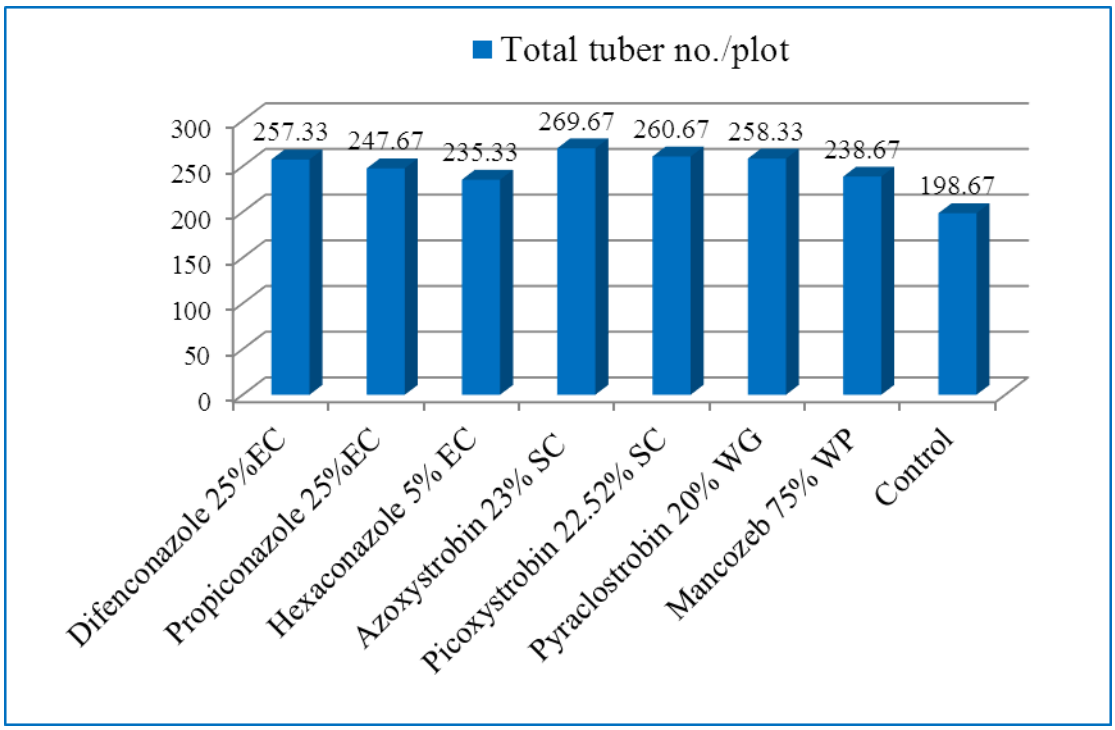


Fig. – 4.8a: Effect of different fungicides on total tuber number of potato

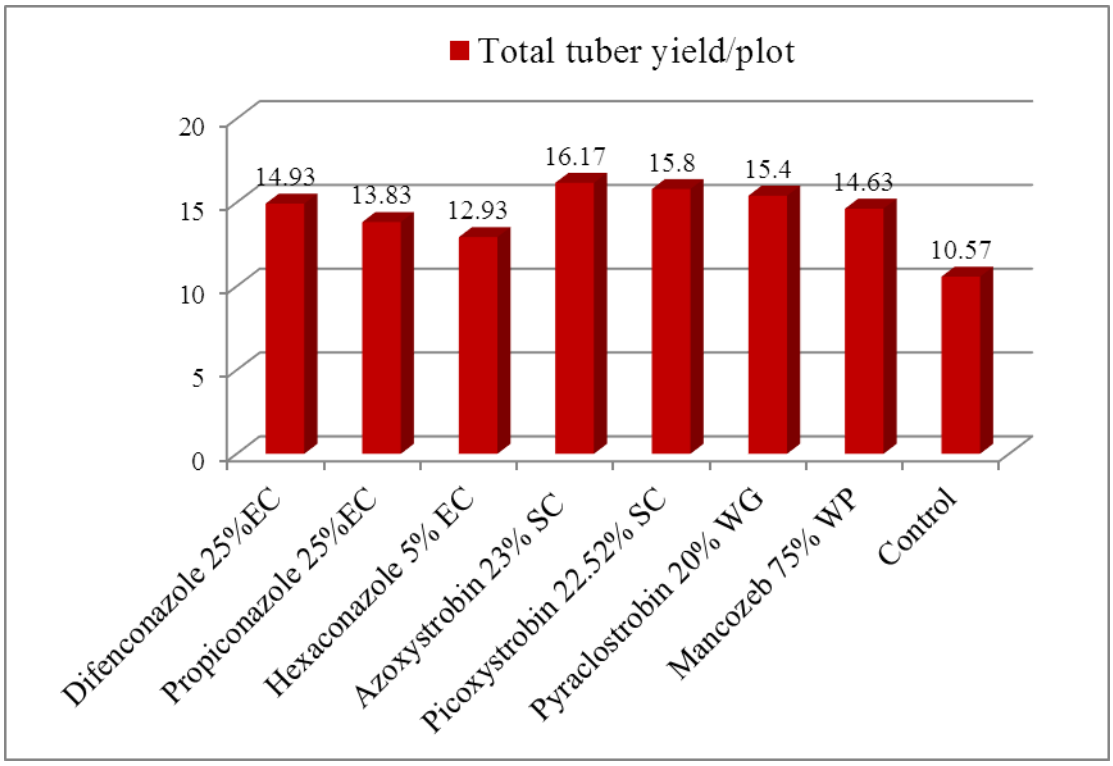


Fig. – 4.8b: Effect of different fungicides on potato tuber yield

4.4.2.4.4 Total tuber yield per plot and per cent yield increase over to control

A perusal of data (Table 4.8) indicated the significant increase in tuber yield in all the treatment as compared to control. The highest 16.17 kg total tuber yield per plot was recorded in Azoxystrobin 23% SC @ 500 ml/ha with maximum per cent yield increase over to control (34.6%) followed by Picoxystrobin 22.52% SC (15.8 kg) and yield increase (33.1%), Pyraclostrobin 20% WG (15.4 kg) and yield increase (31.4%), Difenconazole 5% EC (14.93 kg) and yield increase (29.2%) and Propiconazole 25% EC (13.83 kg) and yield increase (23.6%), Mancozeb 75% WP (14.63 kg) and yield increase (27.8%), and, and least tuber yield in Hexaconazole 5% EC (12.93 kg) and yield increase (18.3%) as compared to control (10.57 kg) (Plate- 4.9 and Fig.- 4.8b).

4.5 Economics of treatments

The yield data of all the treatments were recorded and economics of cost benefit ratio was calculated for all the fungicides tested which are presented in table-4.9. The highest yield (224.54 q/ha) was obtained in Azoxystrobin 23% SC with cost benefit ratio of 1.70 which is on par with Picoxystrobin 20% SC (219.44 q/ha) and Pyraclostrobin 20% WG (213.89 q/ha) with B: C ratio of 1.68 followed by Difenconazole 5% EC (207.41 q/ha) with B: C ratio of 1.63, Mancozeb 75% WP (203.24 q/ha) with B: C ratio of 1.61, Propiconazole 25% SC (192.13 q/ha) with B: C ratio of 1.48 and Hexaconazole 5% EC (179.63 q/ha) with B: C ratio of 1.36. Yield was least in control (146.76 q/ha) and cost benefit ratio was 1.54 (Table- 4.9).


Difenoconazole 25%EC
@ 0.5ml/lit.
T1


Propiconazole25%EC
@ 1ml/lit.
T2


Hexaconazole5%EC
@ 1ml/lit.
T3


Azoxystrobin23%SC
@ 1ml/lit.
T4


Picoxystrobin22.52%C
@ 0.8ml/lit.
T5


Pyraclostrobin20%WG
@ 1ml/lit.
T6

Plate – 4.10: Bio-efficacy of different fungicides (T_1 to T_8) against early blight of potato under field condition

Table 4.9 Economics of treatments

T. No.	Name of fungicides	Doses	Treatment cost	Basic cost	Cost of cultivation(₹/ha)	Yield q/ha	Gross return (₹/ha)	Net return (₹/ha)	B:C Ratio
1	Difenoconazole 25% EC	250	2250	45000	47250	207.41	124444.44	77194.44	1.63
2	Propiconazole 25% EC	500	1400	45000	46400	192.13	115277.78	68877.78	1.48
3	Hexaconazole 5% EC	500	650	45000	45650	179.63	107777.78	62127.78	1.36
4	Azoxystrobin 23% SC	500	4850	45000	49850	224.54	134722.22	84872.22	1.70
5	Picoxystrobin 22.52 SC	400	3900	45000	48900	219.44	131666.67	82766.67	1.69
6	Pyraclostrobin 20% WG	500	2960	45000	47960	213.89	128333.33	80373.33	1.68
7	Mancozeb 75% WP	1500	1650	45000	46650	203.24	121944.44	75294.44	1.61
8	Control (only water spray)	0	0	45000	45000	146.76	88055.56	43055.56	0.96

Plate – 4.11Experiment view of treatments

Plate – 4.12: Experiment presented to Director Education Dr. J. M. Dhakar, AU, Kota and Major Advisor Dr. D. L. Yadav, Asstt. Prof. Pl. Patho., ARS, Kota

Chapter 5

DISCUSSION

Early blight of potato caused by *Alternaria alternata* is one of the major diseases causing consideration yield lose. Hence, the present investigations on "Detection and management of early blight of potato caused by *Alternaria alternata*." was carried out in the laboratory as well as in the field during *Rabi* 2019-20 at the Agriculture Research Station and College of Agriculture, Ummedganj, Kota and Department of Plant Pathology, Agriculture University, Kota, Rajasthan. The discussion was on hand under following chapter.

5.1 To study the symptoms, isolation, purification, pathogenicity and identification of pathogen

5.1.1 Symptomatology

Symptomatology study aids in providing information about symptoms produced by early blight which was helpful for management of disease in field conditions. The first symptom of disease was seen on older leaves as small dark brown, frequently round necrotic spots of 1-2 mm diameters later, the spots enlarged with characteristics in center to produce a target board effect and the color of spots altered from brown to dark brown. Symptoms of disease observed on leaf, stem, petioles and tubers during present investigation are in accordance with symptoms described by earlier workers (Datar and Mayee, 1981; Gleason and Edmonds, 2006; Momel and Pemezny, 2006). Early blight may also cause dry rot of tubers, reducing both the quantity and quality of marketable tubers (Nnodu *et al*., 1982). Sukrutha *et al*. (2014) also observed that early blight develop more rapidly during periods when environmental conditions alternate between humidity and drought. Ganie *et al*. (2013) found that the first symptoms appeared as small irregular to circular dark brown spots on the lower leaves measuring 0.5 mm in size.

5.1.2 Isolation and purification

The associated pathogen was successfully isolated by single spore isolation technique from infected potato leaves and pure culture of fungus was obtained on potato dextrose agar medium. The pure culture of pathogen was obtained after eight days of inoculation which showed brownish colour. Purity and virulence of isolated fungus was regularly maintained and monitored by sub-culturing after 30 days and the culture was maintained at 5^{o}C. Isolation of pathogen *Alternaria* spp. has been reported

by other workers too. Kumar *et al.* (2008) found that isolation of *Alternaria* spp. showed pigmentation varied from yellow, brown, black, brownish to green black on potato dextrose agar medium. Pathak (1972) reported that culture of pathogen was purified by hyphal tip method.

5.1.3 Pathogenicity of the fungus

Pure culture was obtained by single spore isolation method and such culture was used for pathogenicity test. Inoculating with spore suspension and homogenized mycelial Piece of *A. alternata* on foliage of 55 days old plants of potato. The result revealed that the primary symptoms of disease occur on light brown spots seven days after inoculation as small chlorotic, disintegrate and light brown spots. Re-isolated and purified culture from these artificially infected leaves was similar to that of original culture in provided environmental condition during study. The plants which were not inoculated with fungal spore suspension did not show any symptoms of the disease. Pathogenicity tests of early blight of potato have been conducted by various workers (Foolad *et al*. 2000, Adams and Stevenson, 1990; Vloutoglou and Kalogerakis, 2000). Roopa (2012) tested pathogenicity by inoculating with spore suspension and homogenized mycelial bits (2 x 10^4 spores/ml) of *Alternaria solani* on foliage of 30 days old and proved the Koch's postulates.

5.1.4 Identification of the pathogen

An associated fungus was identified on the basis of its morphological and colony characters. The mycelium was greyish brown to black producing conidiophores moreover singly or in small groups which were straight or flexuous brown to olivaceous brown. The conidia were solitary straight or muriform or ellipsoidal narrowing to beak, pale or olivaceous brown, length 36.15-26.85 μm and 18.65-9.92 μm thick at the broadest piece with 3-4 transverse and 1-3 longitudinal septa. The beaks were flexuous, pale and occasionally branched. The studies on the cultural and morphological characters of the isolated pathogen showed its close identity with *Alternaria alternata* which was described by earlier workers. 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 15-19×150-300 um. The observations on culture and morphological characters revealed that culture produced characteristics of *Alternaria alternata*. For further, identification of early blight culture was sent to Indian Type

Culture Collection (ITCC), Division of Plant Pathology, IARI, New Delhi-110012 and identified *Alternaria alternata* with code number 295012.

5.2 To study the efficacy of bio-agents and media study against the pathogen *in vitro* condition

5.2.1 Efficacy of media study

Suitable medium is first step of pathogen for growth as well as sporulation for pathological research work. The results on the above studies indicated that potato leaf agar medium was significantly superior in supporting maximum mycelial growth at 5 days (70.50 mm) and 7 days (80.25 mm) of the fungus followed by Potato dextrose agar medium at 5 days (64.75 mm) and 7 days (78.25 mm). Potato stem agar medium v-8 juice agar medium and pea agar medium were the third group of the media with growth diameter at 5 days and and 7 days (59.00, 57.50 and 58.00 mm), (67.25, 64.50, and 63.25 mm) respectively, three being statistically at par with each other. However, oat meal agar medium and potato carrot agar medium producing growth at 5 days and 7 days (50.00 and 46.50 mm), (58.25 and 53.75 mm), respectively and these both media were statistically at par with the each other. While, the czapek's agar medium supported least growth diameter of the fungus at 5 days and 7 days (37.50 and 42.75 mm).

Maximum dry mycelium weight 584.75 mg was recorded on potato leaf broth medium was significantly superior. Next best dry mycelium weight was recorded on potato dextrose broth 570.25 mg followed by potato stem broth 315.50 mg, V-8 juice broth 285.00 mg, pea broth medium 268.50 mg, oat meal broth 244.25 mg, potato carrot broth 212.25 mg and czapek's broth 176.50 mg, while potato leaf broth was good for sporulation.

In present study, maximum mycelial growth was observed on potato leaf agar medium followed by potato dextrose agar. Potato leaf agar as host media was found suitable for best mycelial growth of *Alternaria alternata*. These results corroborate the findings of former literature such as Chand and Chandra (2014) recorded maximum vegetative growth of *Alternaria brassicicola* on host media. Plant Pathogenic fungi often used to induce sporulation on the source host tissues Photita*et al.* (2001). Potato dextrose agar was reported as most suitable culture medium for growth and sporulation of *Alternaria* spp., earlier by several workers Kumar *et al.* (2018) who observed that highest radial growth of *Alternaria solani* on potato

dextrose agar (63.00 mm) followed by oat meal agar (42.33 mm) and Czapek'sdox agar (27.73 mm), while, oat meal agar showed good sporulation of *Alternaria* spp. Roopa (2012) reported that maximum dry mycelia weight (249.27 mg) of *Alternaria solani* after 9th day of inoculation in potato dextrose broth. However, maximum dry matter was recorded by potato leaf agar medium followed by potato dextrose agar. Other culture media reported suitable for better growth and good sporulation of *Alternaria* spp. in present study Potato stem agar medium,V-8 juice agar medium, pea agar medium oat meal agar medium and potato carrot agar medium, while czapek's agar medium was observed poor medium against pathogen.

5.2.2 Efficacy of bio-agents

The result revealed that all the bio-control agents were significantly superior in inhibiting the mycelial growth of test fungus over the control. The mycelial growth of *Alternaria alternata* was least in presence of *T. viride* (19.25 mm) followed by *T. azospirillum* (22.25 mm), *T. harzianum* (25.25 mm) and *T. Virens* (28.00 mm).

In present study, fungal antagonist *T. Viride* exhibited 57.08 per cent inhibition of mycelial growth, while *T. azospirillum* (50.00%), *T. Harzianum* (43.26%) and *T. Virens* (37.08%) showed poor antagonism. The result is in conformity with the results of Kumar *et al.* (2018) they reported that maximum per cent inhibition (78.97%) of the *Alternaria solani* by *Trichoderma viride* followed by *Trichoderma harzianum* inhibition per cent (75.33%). Murmu *et al.* (2015) also founded *T. Viride* was highly effective with 52.39 per cent diseases reduction over control. While, Sarfraz *et al.* (2018) observed that *Trichoderma harzianum* at (48, 96, 144 and 172 hrs) growth inhibition (7.50, 12.90, 29.44 and 43.00%) followed by *Trichoderma viride* growth inhibition (4.40, 9.10, 29.10 and 37.29%) against *Alternaria solani* causing by early blight of potato.

5.3 Screening the genotypes against the disease

The resistant genotypes are considered as the best method of plant disease management. Screened the twenty eight potato genotypes/varieties against early blight of potato under natural conditions. Out of 28, observed the early blight incidence at 60 and 75 days after sowing. In present study, Six genotypes *viz.*, CP-3021, 3153, Atlantic, Chip-3, Jx-161 and MS/78-62 showed resistant reaction. Whereas, thirteen genotypes i.e., NJ-1501, MP-97, NJ-44, NJ-34, MP-97-1606, PS/RI-135, JI-93-77, MS/94-118, JN-1177, Kufri Sindhuri, Kufri Pukhraj, Kufri Khayati and Kufri Pushkar

were moderately resistant. Six genotypes *viz.*, NJ-85, NJ-1530, J-92-164, MS/92-2105, J/93-4 and Kufri Bahar were susceptible and three genotypes i.e., MP/94-322, NJ-1 and MS/85-1663 were highly susceptible.

Less information regarding screening of potato genotypes in field against early blight of potato has been generated. In present study, varieties *viz.*, Kufri Pukhraj, Kufri Khayati and Kufri Pushkar were found moderately resistant and Kufri Bahar as susceptible. Similar result was observed by Kumar *et al.* (2015) they reported Kufri Pukhraj and Kufri Pushkar as moderately resistant, remaining Kufri Bahar and Kufri Sindhuri susceptible and Kufri Sutlej highly susceptible but, in our study Kufri Sindhuri was found moderately resistant. Ganie and Ghani (2013) also screened twenty five potato genotypes for early blight of potato under natural conditions. Out of twenty five, one genotype SM/92-338 showed tolerant reaction while 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. Therefore, CP-3021, 3153, Atlantic, Chip-3, Jx-161 and MS/78-62 genotypes could be used for cultivation and breeding for resistance against early blight of potato.

5.4 Study the bio-efficacy of fungicide against the pathogen *in vitro* and *in vivo* conditions

5.4.1 Bio-efficacy of fungicides against the pathogen *in vitro*

Result of present study revealed that Propiconazole 25% EC and Hexaconazole 5% EC both at all the three concentrations as well as Mancozeb 75% WP at 1000 and 1500 ppm and Difenconazole 25% EC at 50 ppm significantly recorded no mycelial growth with 100 percent inhibition as compared to rest of the fungicides.

Next best treatments in order of merit were Difenconazole 25% EC at 10 and 20 ppm, Mancozeb 75% WP at 500 ppm and Azoxystrobin 23% SC at all the three concentrations significantly recorded minimum mycelium growth and maximum inhibition compared to control. However, Picoxystrobin 22.52% SC and Pyraclostrobin 20% WG both at all the three concentrations gave significantly maximum mycelium growth and minimum per cent growth inhibition.

Which were in conformity with the results of Arunkumar (2006) who reported that maximum inhibition of Propiconazole (0.1%), Metalaxyl MZ (0.2%) and Perfekt

(0.3%) against *Alternaria solani* under *in vitro* condition. Kumar *et al.* (2007) also recorded that Hexaconazole (0.05%) and Azoxystrobin (0.2%) was very effective in controlling early blight.

Similar results were also obtained by several workers Herle *et al.* (2014) reported that different fungicides tested against mycelial growth of *Alternaria solani* were Mancozeb 75% WP at (0.1, 0.2 and 0.25%) concentrations, per cent inhibition (57.22, 65.23 and 99.33%) and Hexaconazole 5% EC and Propiconazole 25% EC at (0.05, 0.1 and 0.15%) concentrations are equally effective and significantly superior with 100% inhibition, which are on par with Difenconazole 25% EC (100% inhibition) at 0.1 and 0.25% and (91.11%) at 0.05% concentration in laboratory conditions.

The above results thus ultimately indicate that Triazole group fungicide *viz*., Propiconazole 25% EC, Hexaconazole 5% EC and Defenconazole 25% EC were significantly superior and proved the most effective in inhibiting mycelial growth @ 10, 20 and 50 ppm as compared to strobilurin group *viz.*, Azoxystrobin 23% SC, Picoxytstrobin 22.52% SC and Pyraclostrobin 20% WG at 20, 50, 100 ppm against the *A. alternata in vitro* condition by poison food technique, while, contact fungicide Mancozeb 75% WP showed maximum inhibition at higher dose 1000 and 1500 ppm.

5.4.2 The bio-efficacy of fungicides

Fungicides found promising *in vitro* were further evaluated *in vivo* against early blight of potato. Significantly, minimum disease intensity was recorded in Dinfenconazole 5% EC @ 250 ml/ha after first (4.5%) and second (8.0%) spray at 14 days followed by Propiconazole 25% EC @ 500 ml/ha (PDI 4.7% and 8.3%), Azoxystrobin 23% SC @ 500 ml/ha (PDI4.8% and 8.5%).

However, Picoxystrobin 22.52% SC @ 400 ml/ha showed PDI 5.3% and 9.0% and PDC 68.2% which was on par with Pyraclostrobin 20% WG @ 500 ml/ha (PDI 5.7% and 9.4%) and PDC (66.3%) after 14 days of each spray, while, Hexaconazole 5% EC @ 500 ml/ha (PDI 6.3% and 10.1%) and PDC (63.6%) after 14 days of each spray and Mancozeb 75% WP @ 1500 gm/ha showed (6.4% and 12.6%) and PDC (57.4%) 14 days after first and second spray. Whereas, mean per cent disease intensity of control was recorded (19.48%) during study period. In all the treatments was significantly produced lower per cent disease intensity as compared to control. The highest per cent reduction over control i.e. 72.3 was recorded in i.e. Dinfenconazole

5% EC @ 250 ml/ha followed by spraying of Propiconazole 25% EC @ 500 ml/ha (71.3%) and Azoxystrobin 23% SC @ 500 ml/ha 70.2% at 28 days of spray.

In present study, minimum per cent disease incidence were recorded i.e. 14.03 in Difenconazole 25% EC. However, Propiconazole 25% EC showed 15.47% which was on par with Hexaconazole 5% EC (15.83%). Whereas, Azoxystrobin 23% SC (17.37%), Picoxystrobin 22.52% SC (18.73%) and Pyraclostrobin 20% WG (19.07%). while per cent disease incidence i.e. 20.80 was recorded in treatment of Mancozeb 75% WP as compared to control 34.57%.

These results are in conformity with Sharma *et al.* (2018) who reported lowest per cent disease intensity (PDI) through the Difenconazole 25% EC @ 0.025% (20.59) followed by Propiconazole 25% EC @ 0.025% (21.52) and Mancozeb 75% WP @ 0.2% (24.72) treatments as compare to untreated control on the early blight of potato. While, Kumar *et al.* (2018) reported Propiconazole 25% EC was most effective followed by Mancozeb 75% WP to control early blight of potato in Kufri Bahar variety.

In the present investigation, all the fungicides were significantly showed better per cent dry matter as compared to control. Significantly maximum per cent dry matter was recorded by foliar spray of Azoxystrobin 23% SC @ 500 ml/ha (25.00%). Next best treatments in order of merit were Pyraclostrobin 20% WG (24.03%), Picoxystrobin 22.52% SC (23.87%) and Hexaconazole 5% EC (23.07%), Mancozeb 75% WP (22.23%) and Difenconazole 25% EC (22.00%). Whereas, 21.20% was recorded by Propiconazole 25% EC and 20.13% in control.

Our results are in agreement with the findings of Horsfield *et al.* (2010) reported that Azoxystrobin and Difenoconazole were highly effective in the control of early blight when applied up to three days before or three days after inoculation, while, More *et al.* (2016) observed minimum disease incidence of early blight by Mancozeb 75% WP (20.83%) with increased the yield (16.13) and Hexaconazole 5% EC (21.63%) with increased the yield (15.00 t/ha) as compared to control (45.06%) yield (11.96 t/ha) in potato crop. The above results thus finally show that Triazole group fungicide *viz.*, Propiconazole 25% EC, Hexaconazole 5% EC and Defenconazole 25% EC were significantly minimum compared to strobilin group *viz.,* Azoxystrobin 23% SC, Picoxytstrobin 22.52% SC and Pyraclostrobin 20% WG for per cent dry matter of potato tuber.

In the present investigation, maximum dry weight of dehalming cutting i.e. 1.90 kg was recorded in Azoxystrobin 23% SC @ 500 ml/ha. However, Next best treatments in order of merit were Picoxystrobin 22.52% SC (1.74 kg), Pyraclostrobin 20% WG (1.64 kg), Difenconazole 25% EC (1.60 kg), Propiconazole 25% EC (1.55 kg), Mancozeb 75% WP (1.53 kg) and Hexaconazole 5% EC (1.51 kg) as compared to control (0.98 kg). The study revealed that strobilin group fungicides *viz.*, Azoxystrobin 23% SC, Picoxytstrobin 22.52% SC and Pyraclostrobin 20% WG were showed maximum dry weight of dehalming cutting (kg) as compared to Triazole group fungicides *viz.*, Propiconazole 25% EC, Hexaconazole 5% EC and Defenconazole 25% EC.

In the present study, all the treatments gave significantly maximum potato tuber as compared to untreated check. The highest (269) total tuber number per plot was observed in i.e. Azoxystrobin 23% EC @ 500 ml/ha followed by i.e. Picoxystrobin 22.52% SC (260), i.e. Pyraclostrobin 20% WG (258), i.e. Difenconazole 25% EC (257) and i.e. Propiconazole 25% EC (247) which were at par with each other. However, i.e. Mancozeb 75% WP (238), while minimum was recorded in untreated check i.e. (198) tubers.

The highest 16.17 kg total tuber yield per plot was recorded in Azoxystrobin 23% SC @ 500 ml /ha with maximum per cent yield increase over to control (34.6%) followed by Picoxystrobin 22.52% SC (15.8 kg) and yield increase (33.1%), Pyraclostrobin 20% WG (15.4 kg) and yield increase (31.4%), Difenconazole 5% EC (14.93 kg) and yield increase (29.2%) and Propiconazole 25% EC (13.83 kg) and yield increase (23.6%), Mancozeb 75% WP (14.63 kg) and yield increase (27.8%), and, and least tuber yield in Hexaconazole 5% EC (12.93 kg) and yield increase (18.3%) as compared to control (10.57 kg).

Findings of present study indicate that, Triazole group fungicide *viz.*, Propiconazole 25% EC, Hexaconazole 5% EC and Defenconazole 25% EC gave minimum per cent disease intensity, while, strobilurin group fungicides *viz.*, Azoxystrobin 23% SC, Picoxytstrobin 22.52% SC and Pyraclostrobin 20% WG gave maximum number of tuber per plot, yield kg/plot and per cent dry matter. These results corroborate the findings of former literature such as Strobilurin derivatives, such as Kresoxim-methyl and Azoxystrobin, have demonstrated enhanced greening of treated foliage, which has often translated into increases in yield potential (Wu and von Tiedemann, 2001). It is possible that these beneficial effects may have

contributed to the enhanced yield and quality of tubers in the absence of disease pressure. Strobilurin group fungicides modify the plant physiology, through changes in metabolism and growth, which increases crop yield (Amaro *et. al.*, 2020). The fungicide Azoxystrobin moves translaminarly as well as systemically. However, triazole compound may act in a slightly different part of the biochemical sterol-producing pathway, while the results are similar in various fungi abnormal fungal growth and death there are great differences in the activity spectra of these fungicides (Mueller and Daren S. 2006).

5.5 Economics of treatments

From the farmers point of view the economics of disease management is essential. Using of fungicides not only help 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 point of view the economics analysis for the management of early blight of potato has been done. The highest yield (224.54 q/ha) was obtained in Azoxystrobin 23% SC with cost benefit ratio of 1.70 which is on par with Picoxystrobin 20% SC (219.44 q/ha) and Pyraclostrobin 20% WG (213.89 q/ha) with B: C ratio of 1.68 followed by Difenconazole 5% EC (207.41 q/ha) with B: C ratio of 1.63, Mancozeb 75% WP (203.24 q/ha) with B: C ratio of 1.61, Propiconazole 25% SC (192.13 q/ha) with B: C ratio of 1.48 and Hexaconazole 5% EC (179.63 q/ha) with B: C ratio of 1.36. Yield was least in control (146.76 q/ha) and cost benefit ratio was 1.54. So Azoxystrobin 23% SC, Picoxystrobin 20% SC and Pyraclostrobin 20% WG can be recommended to the farmers for early blight disease management

Chapter-6

SUMMARY AND CONCLUSION

Early blight of potato caused by *Alternaria alternata* is one of the major diseases causing considerable yield loss. Hence, the present investigation on "Detection and management of early blight of potato caused by *Alternaria alternata*" was carried out in laboratory as well as in the field during *Rabi* 2019-20 at the Agriculture Research Station, College of Agriculture Ummedganj and Department of Plant Pathology, Agriculture University, Kota, Rajasthan. The summary and conclusion of finding of the present investigation are given below.

Symptomatological study revealed first symptom of disease was seen on older leaves as small dark brown, frequently round necrotic spots of 1-2 mm diameters later, the spots enlarged with characteristics concentric rings in centre to produce a target board effect and colour of spots altered from brown to dark brown. The disease symptoms perceptibly seen on all plant parts *viz.*, leaf, stem and petioles. The contiguous spots eventually coalesced to form large irregular spots primary to drying and defoliation. When plant was 60-90 days mature, symptoms also appeared on stem and petioles as brown protracted cankerous target broad spots. These expanded and protected whole stem and petioles leading to crushing of plants. The diseased plants formed less tuber and size of tuber as compared to healthy plants.

In our studies, the pathogen was isolated by single spore isolation technique from infected potato leaves and pure culture of fungus was obtained on potato dextrose agar medium. The pure culture of pathogen was obtained after eight days of inoculation which showed brownish colour.

Pure culture was obtained by single spore isolation method and culture was used for pathogenicity test. Inoculating with spore suspension and homogenized mycelial Piece of *A. alternata* on foliage of 55 days old plants of potato and successfully produced primary symptoms of the disease occur on light brown spots seven days after inoculation as small chlorotic, disintegrate and light brown spots. The plants which were not inoculated with the fungal spore suspension did not show any symptoms of the disease.

The studies on the morphological and colony characters of isolated pathogen showed its close identify with *Alternaria alternata.* The mycelium was greyish brown

to black producing conidiophores moreover singly or in small groups which were straight or flexuous brown to olivaceous brown. The conidia were solitary straight or muriform or ellipsoidal narrowing to beak, pale or olivaceous brown, length 36.15-26.85 µm and 18.65-9.92 µm thick at the broadest piece with 3-4 transverse and 1-3 longitudinal septa. The beaks were flexuous, pale and occasionally branched. For further, identification of pathogen was sent to Indian Type Culture Collection (ITCC), Division of Plant Pathology, IARI, New Delhi-110012. They identified culture as *Alternaria alternata* with code number 295012.

In vitro efficacy of leaf extract media from host as well as synthetic and semi synthetic media were used for the optimum mycelial growth and sporulation of the *Alternaria* spp. Among the media tested, the potato leaf agar and potato broth medium was significantly superior and most effective medium for growth as well as dry mycelial weight followed by PDA. However, potato leaf agar medium was also superior for sporulation of *A. alternata*. Whereas, the potato stem agar, V-8 juice agar, pea agar, oat meal agar and czapek's agar medium were least effective in mycelia growth. In present study, potato leaf agar was found superior for the growth of pathogen as well as sporulation.

Efficacy of bio-agents was studied and revealed the all four *Trichoderma* species significantly reduced the mycelial growth of the pathogen over the control by dual culture method. Significantly maximum inhibition of mycelial growth was obtained with *T. viride* (57.08%) followed by *T. azospirillum* (50.00%), *T. harzianum* (43.26%) and *T. virens* (37.08%) of the pathogen after 48hours of incubation. In present study, *T. viride* was observed inhibitorier against *A. alternata*.

Twenty eight genotypes/varieties of potato were screened under field conditions to early blight of potato. Out of twenty eight genotypes/varieties, Six genotypes *viz.*, CP-3021, 3153, Atlantic, Chip-3, Jx-161 and MS/78-62 were showed resistant reaction, while thirteen genotypes i.e., NJ-1501, MP-97, NJ-44, NJ-34, MP-97-1606, PS/RI-135, JI-93-77, MS/94-118, JN-1177, Kufri Sindhuri, Kufri Pukhraj, Kufri Khayati and Kufri Pushkar were moderately resistant. However, six genotypes *viz.*, NJ-85, NJ-1530, J-92-164, MS/92-2105, J/93-4 and Kufri Bahar were susceptible while, three genotypes i.e., MP/94-322, NJ-1 and MS/85-1663 were highly susceptible.

In present investigation various fungicides were tested against *Alternaria* spp. by poison food techniques and revealed that Propiconazole 25% EC and

Hexaconazole 5% EC @ 10, 20 and 50 ppm concentrations and Mancozeb 75% WP @ 1000 and 1500 ppm and Difenconazole 25% EC @ 50 ppm significantly were recorded 100 percent inhibition as compared to rest of the fungicides.

Next best treatments in order of merit were Difenconazole 25% EC @ 10 and 20 ppm, Mancozeb 75% WP @ 500 ppm and Azoxystrobin 23% SC @ 10, 50 and 100 ppm, Picoxystrobin 22.52% SC and Pyraclostrobin 20% WG @ 10, 50 and 100 ppm concentrations against the *Alternaria* spp.

Results of present study also showed that Triazole group fungicide *viz.,* Propiconazole 25% EC, Hexaconazole 5% EC and Defenconazole 25% EC @ 10, 20 and 50 ppm concentrations were higher inhibitory mycelial growth as compared to strobilurin group *viz.,* Azoxystrobin 23% SC, Picoxytstrobin 22.52% SC and Pyraclostrobin 20% WG @ 20, 50 and 100 ppm against the *Alternaria* spp. *in vitro* condition by poison food technique.

In present study, the highest per cent reduction over control i.e. 72.3 was recorded in i.e. Dinfenconazole 5% EC @ 250 ml/ha followed by spraying of Propiconazole 25% EC @ 500 ml/ha (71.3%) and Azoxystrobin 23% SC @ 500 ml/ha 70.2% after first and second spray. Minimum per cent disease incidence was recorded in Difenconazole 25% EC (14.03%) and Praopiconazole 25% EC (15.47%) and Hexaconazole 5% EC (15.83%) both are significantly at par. Next best treatments in order of merit were Azoxystrobin 23% SC (17.37%), Picoxystrobin 22.52% SC (18.73%) and Pyraclostrobin 20% WG (19.07%) and Mancozeb 75% WP (20.8%) significantly reduced the per cent disease incidence as compared to control (34.57%).

In this study also evaluated the effect of fungicide on dry weight of potato and found that all the fungicides were significantly showed better per cent dry matter as compared to control. Significantly maximum per cent dry matter was recorded by foliar spray of Azoxystrobin 23% SC @ 500 ml/ha (25.00%).

Next best treatments in order of merit were Pyraclostrobin 20% WG (24.03%), Picoxystrobin 22.52% SC (23.87%) and Hexaconazole 5% EC (23.07%), Mancozeb 75% WP (22.23%) and Difenconazole 25% EC (22.00%). Whereas, 21.20% was recorded by Propiconazole 25% EC and 20.13% in control. In present result, it was observed that Triazole group fungicide *viz.,* Propiconazole 25% EC, Hexaconazole 5% EC and Defenconazole 25% EC were found inferior compared to strobilin group

viz., Azoxystrobin 23% SC, Picoxytstrobin 22.52% SC and Pyraclostrobin 20% WG for per cent dry matter of potato tuber and dry weight of dehalming cutting (kg).

In present investigation, it was revealed that all the treatments gave significantly maximum potato tuber as compared to untreated check. The highest (269) total tuber number per plot was observed in i.e. Azoxystrobin 23% EC @ 500 ml/ha followed by. Picoxystrobin 22.52% SC (260), Pyraclostrobin 20% WG (258), i.e. Difenconazole 25% EC (257) and Propiconazole 25% EC (247) which were at par with each other. However, in Mancozeb 75% WP (238) number of tubers were recorded while least number of tubers were recorded in untreated check i.e. (198) tubers. The present study, highest 16.17 kg total tuber yield per plot was recorded in Azoxystrobin 23% SC @ 500 ml/ha with maximum per cent yield increase over to control (34.6%) followed by Picoxystrobin 22.52% SC (15.8 kg)and yield increase (33.1%), Pyraclostrobin 20% WG (15.4 kg) and yield increase (31.4%), Difenconazole 5% EC (14.93 kg) and yield increase (29.2%) and Propiconazole 25% EC (13.83 kg) and yield increase (23.6%), Mancozeb 75% WP (14.63 kg) and yield increase (27.8%), and least tuber yield in Hexaconazole 5% EC (12.93 kg) and yield increase (18.3%) as compared to control (10.57 kg).

In present study the efficacy of fungicides against early blight and its effect on tuber yield, it was revealed that Triazole group fungicide *viz.*, Propiconazole 25% EC, Hexaconazole 5% EC and Defenconazole 25% EC were strongly reduced early blight, whereas maximum tuber yield was observed in strobilurin group fungicides *viz.*, Azoxystrobin 23% SC, Picoxytstrobin 22.52% SC and Pyraclostrobin 20% WG.

In present investigation, it was revealed that all the treatments gave significantly maximum cost benefit ratio as compared to untreated check. The highest yield (224.54 q/ha) was obtained in Azoxystrobin 23% SC with cost benefit ratio of 1.70 which is on par with Picoxystrobin 20% SC (219.44 q/ha) and Pyraclostrobin 20% WG (213.89 q/ha) with B: C ratio of 1.68 followed by Difenconazole 5% EC (207.41 q/ha) with B: C ratio of 1.63, Mancozeb 75% WP (203.24 q/ha) with B: C ratio of 1.61, Propiconazole 25% SC (192.13 q/ha) with B: C ratio of 1.48 and Hexaconazole 5% EC (179.63 q/ha) with B: C ratio of 1.36. Yield was least in control (146.76 q/ha) and cost benefit ratio was 1.54.

Conclusion

- In the light of finding of present investigation, some conclusions are drawn here:
- The disease was prevalent in all potato growing areas in the vicinity of Kota.
- The pathogen isolated from infected potato plants of Kota district of Rajasthan was identified as *Alternaria alternata.*
- Out of four bio-control agents tested for their antagonistic activity against *A. alternata* significant maximum inhibition of mycelial growth of the pathogen after 48 hours of incubation was obtained with *Trichoderma viride* followed by *T. azospirillum*.
- Among the media tested, the potato leaf agar and broth medium were significantly superior in supporting maximum mycelial growth as well as dry mycelia weight at 5 and 7 days of the fungus. This was followed by Potato dextrose agar medium.
- Out of twenty eight genotypes/varieties, six genotypes *viz.*, CP-3021, 3153, Atlantic, Chip-3, Jx-161 and MS/78-62 showed resistant reaction. While thirteen genotypes were moderately resistant. However, six genotypes were susceptible while, three genotypes were highly susceptible.
- Present study revealed that Triazole group fungicide *viz.*, Propiconazole 25% EC, Hexaconazole 5% EC and Defenconazole 25% EC were significantly superior and proved the most effective in inhibiting mycelial growth @ 10, 20 and 50 ppm as compared to strobilin group *viz*., Azoxystrobin 23% SC, Picoxytstrobin 22.52% SC and Pyraclostrobin 20% WG at 20, 50, 100 ppm against the *A. alternata in vitro* condition by poison food technique. While contact fungicide Mancozeb 75% WP showed maximum inhibition at higher dose 1000 and 1500 ppm.
- All the treatments were significantly produced lower percent disease intensity as compared to control as well as per cent disease incidence. The highest per cent reduction over control was recorded in i.e. Dinfenconazole 5% EC @ 250 ml/ha followed by spraying of Propiconazole 25% EC @ 500 ml/ha and Azoxystrobin 23% SC @ 500 ml/ha.
- The Triazole group fungicides *viz.*, Propiconazole 25% EC, Hexaconazole 5% EC and Defenconazole 25% EC were significantly inferior compared to strobilin group *viz.*, Azoxystrobin 23% SC, Picoxytstrobin 22.52% SC and Pyraclostrobin 20% WG for per cent dry matter of potato tuber.

- The study revealed that strobilin group fungicides *viz*., Azoxystrobin 23% SC, Picoxytstrobin 22.52% SC and Pyraclostrobin 20% WG were showed maximum dry weight of dehalming cutting (kg) as compared to Triazole group fungicides *viz*., Propiconazole 25% EC, Hexaconazole 5% EC and Defenconazole 25% EC.

Chapter-7

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Detection and Management of Early Blight of Potato Caused by *Alternaria alternata*.

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ABSTRACT

Potato is the King of vegetables and third most important food commodity in terms of human consumption after rice and wheat. Potato has immense potential for food and nutritional security of the world. India is the second largest producer of potato in the world. Diseases are major constraint resulting in loss in tuber yield of potato.Early blight is one of most important foliar diseases of potato and reported 24 to 54% disease severity in South Eastern Rajasthan. Consequently, the present studies have been undertaken to overcome crop loss by finding suitable disease management. The present investigation on "Detection and Management of Early blight of potato caused by *Alternaria alternata*." was carried out at Agricultural Research Station, Agriculture University, Kota during *Rabi* 2019-20 under laboratory as well as field condition.

First symptom of the disease was seen on older leaves as small dark brown, frequently round necrotic spots, the spots enlarged with characteristics in the centre to produce a target board effect and the colour of the spots altered from brown to dark brown. The disease symptoms perceptibly seen on all plant parts *viz.*, leaf, stem and petioles. The contiguous spots eventually coalesced to form large irregular spots primary to drying and defoliation. The diseased plants formed less tuber and size of tuber as compared to healthy plant. Pathogen was isolated by single spore isolation technique from infected potato leaves and pure culture of the fungus was obtained on potato dextrose agar medium after eight days of inoculation which showed brownish colour. The studies on the morphological and colony characters of the isolated pathogen showed its close identify with *Alternaria alternata.* The mycelium was greyish brown to black producing conidiophores moreover singly or in small groups which were straight or flexuous brown to olivaceous brown. The conidia were solitary straight or muriform or ellipsoidal narrowing to beak, pale or olivaceous brown. For further, identification of pathogen was sent to Indian Type Culture Collection (ITCC), Division of Plant Pathology, IARI, New Delhi-110012. They identified culture as *Alternaria alternata* with code number 295012.

Significantly maximum inhibition of mycelial growth was obtained with *Trichoderma viride* (57.08%) followed by *T. azospirillum* (50.00%), *T. harzianum* (43.26%) and *T. virens* (37.08%) of the pathogen after 48hours of incubationby dual culture technique.

Among the media tested the potato leaf agar and was significantly superior and most effective medium for sporulation as well as potato leaf broth medium for dry mycelial weight followed by PDA. However potato leaf agar was also superior for sporulation of *A. alternata*. Whereas, the potato stem agar, V-8 juice agar, pea agar, oat meal agar and czapek's agar medium were least effective in mycelial growth.

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Screening of twenty eight genotypes/varieties of potatofor resistance to early blightdisease revealed six genotypes *viz.*, CP-3021, 3153, Atlantic, Chip-3, Jx-161 and MS/78-62 as resistant reaction. While thirteen genotypes i.e., NJ-1501, MP-97, NJ-44, NJ-34, MP-97-1606, PS/RI-135, JI-93-77, MS/94-118, JN-1177, Kufri Sindhuri, Kufri Pukhraj, Kufri Khayati and Kufri Pushkar moderately resistant. However, six genotypes *viz.*, NJ-85, NJ-1530, J-92-164, MS/92-2105, J/93-4 and Kufri Bahar susceptible, while three genotypes i.e., MP/94-322, NJ-1 and MS/85-1663 as highly susceptible.

Fungicides *viz.*, Propiconazole 25% EC and Hexaconazole 5% EC @ 10, 20 and 50 ppm concentrations and Mancozeb 75% WP @ 1000 and 1500 ppm and Difenconazole 25% EC @ 50 ppm significantly were recorded 100 per cent inhibition as compared to rest of the fungicides.Next best treatments in order of merit were Difenconazole 25% EC @ 10 and 20 ppm, Mancozeb 75% WP @ 500 ppm and Azoxystrobin 23% SC @ 10, 50 and 100 ppm, Picoxystrobin 22.52% SC andPyraclostrobin 20% WG @ 10, 50 and 100 ppm concentrations against the *Alternaria* spp.

The studies on foliar spray of fungicides against early blight under field condition revealed that the highest per cent reduction over control i.e. 72.3% was recorded in Dinfenconazole 5% EC @ 250 ml/ha followed by spraying of Propiconazole 25% EC @ 500 ml/ha (71.3%) and Azoxystrobin 23% SC @ 500 ml/ha 70.2% after first and second spray. Next best treatments in order of merit were Picoxystrobin 22.52% SC @ 400 ml/ha i.e. 68.2% which was on par with Pyraclostrobin 20% WG @ 500 ml/ha 66.3% disease control after 14 days of each spray, while Hexaconazole 5% EC @ 500 ml/ha gave 63.6% and Mancozeb 75% WP @ 1500 g/ha showed 57.4% disease control 14 days after first and second spray.

Significantly maximum percent dry matter was recorded by foliar spray of Azoxystrobin 23% SC @ 500 ml/ha (25.00%). Next best treatments in order of merit were Pyraclostrobin 20% WG (24.03%), Picoxystrobin 22.52% SC (23.87%) and Hexaconazole 5% EC (23.07%), Mancozeb 75% WP (22.23%) and Difenconazole 25% EC (22.00%). Whereas, 21.20% was recorded by Propiconazole 25% EC and 20.13% in control. The highest (269) total tuber no. per plot was observed in Azoxystrobin 23% EC @ 500 ml/ha followed by Picoxystrobin 22.52% SC (260), Pyraclostrobin 20% WG (258), Difenconazole 25% EC (257) and Propiconazole 25% EC (247) which were at par with each other. However, Mancozeb 75% WP (238), while minimum was recorded in untreated checks i.e. (198) tubers.The studies on the effect of foliar sprays of various fungicides on tuber yield revealed that Azoxystrobin 23% SC @ 500 ml /ha gave highest 16.17kg total tuber yield per plot followed by Picoxystrobin 22.52% SC (15.8 kg), Pyraclostrobin 20% WG (15.4 kg), Difenconazole 5% EC (14.93 kg) and Propiconazole 25% EC (13.83 kg), Mancozeb 75% WP (14.63 kg) and least tuber yield in Hexaconazole 5% EC (12.93 kg) as compared to control (10.57 kg).

The highest cost benefit ratio was obtained in strobolin group fungicides followed by Mancozeb 75% WP and trizole group fungicides. Cost benefit ratio was least in control.

अल्टरनेरिया अल्टरनेटा द्वारा जनित आलू की अगेती अगंमारी रोग का अनुसन्धान एवं प्रबंधन

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सारांश

आलू विश्व की एक प्रमुख खाद्य फसल है। आलू को सब्जियों का राजा भी कहा जाता है वर्तमान में आलू के उत्पादन मे भारत का दूसरा स्थान है। भारत मे आलू के उत्पादन के उत्तर प्रदेश अग्रणी राज्य है। आलू की फसल मे लगने वाले रोगो से आलू के उत्पादन व गुणवत्ता पर प्रभाव पड़ता है। अगेती अंगमारी आलू की मुख्य बीमारी है। जिसके कारण दक्षिण पूर्वी राजस्थान मे इससे 24 से 54 प्रतिशत नुकसान देखा गया है। शोध के लक्षणों की जाचं मे पाया गया कि रोग के कारण पत्तियों पर छोटे छोटे भूरे रगं के धब्बे बनते है। रोग के लक्षण स्पष्ट रुप से पौधो के सभी हिस्सो, पत्ती एवं तत्वों पर देखे जाते है। जब इन धब्बों को देखा जाए तो ऐसा लगता है जैसे इनका केन्द्र एक है लेकिन उनके गोलों का आकार अलग अलग है। इसे सकेन्द्रिक वलय कहॉ जाता है। सूखे वातावरण म धब्बे कठोर हो जाते है व नम वातावरण मे धब्बे आपस मे मिलकर बड़ा क्षेत्र बना लेते है। रोग के उग्र होने पर पत्तियॉ झूलसकर गिर जाती है। अध्ययन मे यह भी पाया गया कि रोग ग्रस्त पौधो मे स्वस्थ पौधो की तुलना मे कम कंद और कमं कदों के आकार का गठन हुआ जिससे पैदावार में नुकसान हुआ। रोग ग्रसित आलू की पत्तियों से रोग को कोच की अवधारणा द्वारा रोग जनक को पृथककरण करके शुद्विकरण किया। जिससे पाया की कवक की मायसिलियम भूरे रंग से काले रगं के कॉनिडीयोफोर छोटे छोटे समूह म दिखाई दी, कॉनिडीया एकान्त सीधे या भूरभूरे रगं या भूरे रगं का पीलापन लिए हुये सकंरी या दीर्घवृताकार होते है। रोगजनक की पहचान *अल्टरनेरिया अल्टरनाटा* के रुप मे हुई जिसको पुनः पुष्टिकरण क लिए इंडियन टाइप कल्चर कलेक्शन; पादप रोग विज्ञान विभाग नई दिल्ली द्वारा *अल्टरनेरिया अल्टरनेटा* कोड की सख्ंया 295012 के रुप मे पहचान हुई।

ट्राइकोड्रमा की 4 प्रजातियॉ के अध्ययन मे पाया की *ट्राइकोड्रमा* विरिडी ने सबसे ज्यादा काबू पाया जो (57.08 प्रतिशत) था तथा उसके बाद *ट्राइकोड्रमा* एजोस्पाइरिलिम (50 प्रतिशत), ट्राइकोड्रमा हैरजियनम (43.26 प्रतिशत) एवं *ट्राइकोड्रमा* वाइरन्स (37.08 प्रतिशत) था जो कि सबसे कम था । *अल्टनेरिया अल्टरनाटा* कवक को विभिन्न मिडिया मे रखा गया जिसमे सबसे उपर्युक्त पोटेटो लिफ अगार पाया गया, जिसके बाद पोटेटो डेक्सट्रोज अगार पाया गया।

आलू की 28 विभिन्न प्रजातियां को खेत मे रोग के प्रति जांचा गया तथा पाया कि प्रजातियॉ प्रतिरोधी तेरह मध्यम प्रतिरोधी छः संवेदनशील तीन अति संवेदनशील के रुप मे पाई गई थी। डाइफेनकानाजोल 25 ई.सी., 100 पी.पी.एम, प्रोपीकोनाजोल 25 प्रतिशत ई.सी. 10, 20 एवं 50 पी.पी.एम तथा मेन्कोजेब 75 प्रतिशत डब्लयू.पी. 1000 व 1500 पी.पी.एम 100 प्रतिशत रोग कारक सिद्व हुई। कवकनाशियों की लेबोरेटरी के अध्ययन मे पाया गया कि कवक नाशियों के छिड़काव से किए गये अध्ययन से पता चला कि डाइफेनकानाजोल 25 ई. सी. 250 मिली लीटर प्रति हैक्टेयर से 72.3 प्रतिशत राग की उग्रता मे कमी आई इसके बाद प्रोपिकोनाजोल 25 प्रतिशत ई.सी. 500 मि. लीटर/हैक्टेयर से 71.3 प्रतिशत और एजेक्सिस्ट्रोबिन 23 प्रतिशत एस.सी. 500 मि. लीटर/हैक्टेयर से 70.02 प्रतिशत नियत्रंण पाया। अध्ययन मे यह भी पाया की अधिकतम उपज व शुष्क घटक एजोक्सिस्ट्रोबिन 23 प्रतिशत एस.सी. के छिड़काव से प्राप्त हुई। उपरोक्त अध्ययन मे पाया गया कि सबसे अच्छी

उपज व शुद्व लाभ एजाक्सिस्ट्रोबिन 23 प्रतिशत एस.सी. द्वारा प्राप्त हुआ जो किसानों को रोग नियंत्रक के लिये सिफारिया किया जा सकता है।

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**सहायक प्रोफेसर पादप रोग विज्ञान विभाग'' कृषि अनुसंधान केन्द्र, उम्मेदगंज, कृषि विश्व विद्यालय, कोटा