EPIDEMIOLOGY AND MANAGEMENT OF ALTERNARIA BLIGHT ON SEED CROP OF CARROT (*Daucus carota*)

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE
in
PLANT PATHOLOGY
(Minor Subject: Entomology)

By

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(L-2010-A-85-M)

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CERTIFICATE I

This is to certify that the thesis entitled, **"Epidemiology and management of Alternaria blight on seed crop of carrot (*Daucus carota*)"** submitted for the degree of **M.Sc.,** in the subject of **Plant Pathology** (Minor subject: **Entomology)** of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Mr. Gurpreet Singh (L-2010-A-85-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

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

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CERTIFICATE II

This is to certify that the thesis entitled **"Epidemiology and management of Alternaria blight on seed crop of carrot (*Daucus carota*)"** submitted by **Mr. Gurpreet Singh (L-2010-A-85-M)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **M.Sc.** in the subject of **Plant Pathology** (Minor subject: **Entomology**) has been approved by Student's Advisory Committee along with Head of the Department after an oral examination on the same.

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(Dr Pushpinder Paul Singh) **(Dr Amarjit Singh)**

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ABSTRACT

Surveys conducted during 2011 and 2012 revealed that Alternaria blight of carrot was observed at all the locations on all the cultivars. The disease incidence and severity was the highest on Local varieties as compared to PAU recommended varieties. The highest disease severity was observed on local variety grown at village Bohan in district Hoshiarpur, while the lowest disease severity on Selection-21 grown at PAU Ludhiana. *Alternaria radicina* and *Alternaria dauci* were found to be associated with the disease. *Alternaria radicina* has highest frequency than *Alternaria dauci* on carrot plants. Maximum colony growth and sporulation of *Alternaria radicina* was observed on carrot leaf agar followed by unmbel extract agar medium. Lowest colony growth and sporulation was observed on Czapek's Dox agar medium. Maximum colony growth and sporulation was observed at 25°C temperature followed by 30°C, whereas no colony growth was observed at 35°C. Most suitable range of temperature was found to be 25 to 30°C. Maximum colony growth of *Alternaria radicina* was observed at pH 6, whereas maximum sporulation was observed at pH 7. The disease severity increase with the increase in the age of plants and a positive correlation between plant age and disease severity was observed. Similarly disease severity increased with the increase in the leaf wetness period. A positive correlation was found between leaf wetness period and disease severity. A minimum 8 hr of leaf wetness was required to cause infection on carrot plants. All three non systemic fungicides proved less effective than systemic fungicides. Among systemic fungicides ED_{50} values for difenconazole and propiconazole was less than 1µg/ml, while for hexaconazole and azoxystrobin was 1.0 and 5.9 µg/ml respectively. Among non systemic fungicides ED_{50} value was 17 for chlorothalonil, whereas it was 44 µg/ml for mancozeb. Under field conditions all systemic fungicides proved highly effective in controlling the disease as compared to non systemic fungicides. Azoxystrobin + Difenconazole @ 0.1 per cent proved most effective in checking the disease than other tested fungicides.

Keywords: Alternaria blight of carrot, *Alternaria radicina*, *Alternaria dauci*, medium, temperature, pH, plant age, leaf wetness, fungicides

Signature of the Major Advisor **Signature of the Student**

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ਸਾਰ-ਅੰਸ਼

ਸਾਲ 2011 ਅਤੇ 2012 ਦੌਰਾਨ ਆਯੋਜਿਤ ਕੀਤੇ ਗਏ ਸਰਵੇਖਣਾਂ ਤੋਂ ਪਤਾ ਲੱਗਾ ਕਿ ਗਾਜਰਾਂ ਦਾ ਅਲਟਰਨੇਰੀਆ ਬਲਾਈਟ ਰੋਗ ਦੇਖੇ ਗਏ ਸਾਰੇ ਖੇਤਾਂ ਅਤੇ ਸਭ ਕਿਸਮਾਂ ਤੇ ਪਾਇਆ ਗਿਆ ਸੀ। ਪੰਜਾਬ ਖੇਤੀਬਾੜੀ ਯੂਨੀਵਰਸਿਟੀ ਦੀਆਂ ਸਿਫਾਰਿਸ਼ ਗਾਜਰਾਂ ਦੀਆਂ ਕਿਸਮਾਂ ਦੇ ਮੁਕਾਬਲੇ ਰੋਗ ਦੀ ਤੀਬਰਤਾ ਸਭ ਤੋਂ ਵੱਧ ਸਥਾਨਕ ਕਿਸਮਾਂ ਤੇ ਸੀ। ਪਿੰਡ ਬੋਹਣ ਜਿਲ੍ਹਾ ਹੋਸ਼ਿਆਰਪੁਰ ਵਿਖੇ ਸਥਾਨਿਕ ਕਿਸਮਾਂ ਤੇ ਬਿਮਾਰੀ ਦੀ ਸਭ ਤੋਂ ਵੱਧ ਤੀਬਰਤਾ ਪਾਈ ਗਈ, ਜਦੋਂ ਕਿ ਪੀ.ਏ.ਯੂ. ਲੁਧਿਆਣਾ ਵਿਖੇ ਸੀਲੈਕਸ਼ਨ-21 ਤੇ ਘੱਟੋ-ਘੱਟ ਰੋਗ ਦੀ ਤੀਬਰਤਾ ਸੀ। ਇਸ ਬਿਮਾਰੀ ਲੱਗਣ ਦਾ ਮੁੱਖ ਕਾਰਣ *ਅਲਟਰਨੇਰੀਆ ਰੇਡੀਸਿਨਾ* ਅਤੇ *ਅਲਟਰਨੇਰੀਆ ਡਾਉਸੀ* ਪਾਇਆ ਗਿਆ। ਲੈਬਾਰਟਰੀ ਅੰਦਰ ਗਾਜਰ ਦੇ ਪੱਤੇ ਦੇ ਅਰਕ ਦੇ ਮੀਡੀਅਮ ਵਿੱਚ ਉੱਲੀ ਦਾ ਵਾਧਾ ਅਤੇ ਸਪੋਰੂਲੇਸ਼ਨ ਸਭ ਤੋਂ ਵੱਧ ਪਾਇਆ ਗਿਆ। ਤਾਪਮਾਨ ਦੇ ਅਸਰ ਨੂੰ ਜਦੋਂ ਉੱਲੀ ਦੇ ਵਾਧੇ ਤੇ ਸਪੋਰੂਲੇਸ਼ਨ ਲਈ ਖੋਜਿਆ ਗਿਆ ਤਾਂ ਇਹ ਪਾਇਆ ਗਿਆ ਕਿ 25-30 ਡਿਗਰੀ ਸੈਲਸੀਅਸ ਤਾਪਮਾਨ ਸਭ ਤੋਂ ਵੱਧ ਅਨੁਕੂਲ ਸੀ, ਜਦੋਂ ਕਿ 35 ਡਿਗਰੀ ਸੈਲਸੀਅਸ ਤਾਪਮਾਨ ਉੱਲੀ ਦੇ ਵਾਧੇ ਅਤੇ ਸਪੋਰੂਲੇਸ਼ਨ ਨੂੰ ਬਿਲਕੁਲ ਰੋਕ ਦਿੰਦਾ ਹੈ। ਇਸੇ ਤਰ੍ਹਾਂ ਹੀ 6 ਪੀ ਐੱਚ ਉੱਲੀ ਦੇ ਵਾਧੇ ਲਈ ਅਤੇ 7 ਪੀ ਐੱਚ ਸਪੋਰੂਲੇਸ਼ਨ ਲਈ ਸਭ ਤੋਂ ਵੱਧ ਅਨੁਕੂਲ ਪਾਈ ਗਈ। ਬੂਟਿਆਂ ਦੇ ਉਮਰ ਦੇ ਵਾਧੇ ਨਾਲ ਬਿਮਾਰੀ ਦੀ ਤੀਬਰਤਾ ਵੀ ਵੱਧ ਹੋਈ ਅਤੇ ਇਹਨਾਂ ਦਾ ਆਪਸੀ ਅੰਤਰ-ਸਬੰਧ ਹਾਂ ਪੱਖੀ ਵੇਖਿਆ ਗਿਆ। ਇਸ ਤਰ੍ਹਾਂ ਹੀ ਪੱਤਿਆਂ ਦੇ ਗਿੱਲੇਪਣ ਦੀ ਮਿਆਦ ਵਧਣ ਨਾਲ ਬਿਮਾਰੀ ਵੀ ਵੱਧ ਵੇਖੀ ਗਈ। ਪੱਤਿਆਂ ਦੇ ਗਿੱਲੇਪਣ ਅਤੇ ਬਿਮਾਰੀ ਦੇ ਵਾਧੇ ਵਿੱਚ ਹਾਂ ਪੱਖੀ ਅੰਤਰ-ਸਬੰਧ ਵੇਖਿਆ ਗਿਆ। ਬਿਮਾਰੀ ਸ਼ੁਰੂ ਕਰਨ ਲਈ ਘੱਟੋ ਤੋਂ ਘੱਟ 8 ਘੰਟਿਆਂ ਦੇ ਪੱਤਿਆਂ ਦਾ ਗਿੱਲਾਪਣ ਲੋੜੀਂਦਾ ਹੈ। ਵਿਚਾਰ ਅਧੀਨ ਤਿੰਨੇ ਨਾਨ ਸਿਸਟੈਂਮਿਕ ਉੱਲੀ ਨਾਸ਼ਕ, ਸਿਸਟੈਂਮਿਕ ਉੱਲੀ ਨਾਸ਼ਕਾਂ ਨਾਲੋਂ ਘੱਟ ਅਸਰਦਾਰ ਪਾਏ ਗਏ। ਸਿਸਟੈਂਮਿਕ ਉੱਲੀ ਨਾਸ਼ਕ ਜਿਵੇਂ ਡਾਈਫੈਨਕੋਨਾਜ਼ੋਲ ਅਤੇ ਪ੍ਰੋਪੀਕੋਨਾਜ਼ੋਲ ਦੀ ਈ.ਡੀ$_{50}$ 1 ਮਾਈਕ੍ਰੋਗ੍ਰਾਮ ਪ੍ਰਤੀ ਮਿਲੀਲੀਟਰ ਤੋਂ ਘੱਟ ਸੀ, ਜਦੋਂ ਕਿ ਹੈਕਸਾਕੋਨਾਜ਼ੋਲ ਅਤੇ ਐਜ਼ੋਕਸੀਸਟ੍ਰੋਬਿਨ ਦੀ ਈ.ਡੀ$_{50}$ ਕ੍ਰਮਵਾਰ 1.0 ਅਤੇ 5.9 ਮਾਈਕ੍ਰੋਗ੍ਰਾਮ ਪ੍ਰਤੀ ਮਿਲੀਲੀਟਰ ਸੀ। ਨਾਨ-ਸਿਸਟੈਂਮਿਕ ਉੱਲੀ ਨਾਸ਼ਕਾਂ ਜਿਵੇਂ ਕਲੋਰੋਥੈਲੋਨਿਲ, ਮੈਨਕੋਜ਼ੇਬ ਦੀ ਈ.ਡੀ$_{50}$ ਕ੍ਰਮਵਾਰ 17 ਅਤੇ 44 ਮਾਈਕ੍ਰੋਗ੍ਰਾਮ ਪ੍ਰਤੀ ਮਿਲੀਲੀਟਰ ਪਾਈ ਗਈ। ਖੇਤਾਂ ਵਿੱਚ ਕੀਤੇ ਗਏ ਤਜਰਬਿਆਂ ਦੌਰਾਨ ਇਹ ਦੇਖਿਆ ਕਿ ਸਿਸਟੈਂਮਿਕ ਉੱਲੀ ਨਾਸ਼ਕ ਬਿਮਾਰੀ ਦੀ ਰੋਕਥਾਮ ਲਈ ਵੱਧ ਅਸਰਦਾਰ ਸਾਬਤ ਹੋਏ। ਮਿਸ਼ਰਤ ਉੱਲੀ ਨਾਸ਼ਕ ਐਜ਼ੋਕਸੀਸਟ੍ਰੋਬਿਨ+ ਡਾਈਫੈਨਕੋਨਾਜ਼ੋਲ 0.1 ਪ੍ਰਤੀਸ਼ਤ ਸਭ ਤੋਂ ਵੱਧ ਅਸਰਦਾਰ ਸਾਬਤ ਹੋਇਆ।

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ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖ਼ਰ **ਵਿਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖ਼ਰ**

CONTENTS

CHAPTER I

INTRODUCTION

Carrot (*Daucus carota*) a member of family Umbliferae, is one of the important root crops cultivated throughout the world for its fleshy edible roots. It is grown in spring, summer and autumn in temperate countries and during winter in tropical and subtropical regions. Carrot roots are used as vegetable, soups, stews, curries and pies, pickles and for canning. Carrot juice is rich source of carotene and is sometimes used for colouring butter and other food articles. Black carrot is used for the preparation of beverage called *Kangi* which is considered to be a good appetizer. Carrot possesses many medicinal properties and is also used in Ayurvedic medicines. According to World Health Organization, vitamin A deficiency partially or totally blinds nearly 350,000 children from more than 75 countries every year. Carrot has pigmented flesh and is a rich source of carotene, the precursor of vitamin A (Pantastica 1975). It also contains appreciable amount of thiamine and riboflavin (Gopalan *et al* 1985). Carrot a native of Afghanistan (Banga 1976), is produced by many countries like China, USSR, USA, Poland, Japan, UK, France, Italy, Germany and Canada. It is also grown in tropics including India at high altitudes or during the cool season. Total world production is about 13 million tonnes (Gupta and Thind 2006). The major carrot growing states in India are Punjab, Uttar Pradesh, Karnataka, Tamilnadu and Andra Pradesh. In Punjab total area under different root crops is 16,504 hectares with annual production of 2, 90,794 metric tonnes (Anonymous 2011). Out of this, 50 per cent is under carrot alone. Still, there is a good scope for increasing area and production of this crop as the produce fetches good market price.

Carrot is attacked by a large number of fungal pathogens besides a few bacterial and some physiological disorders. As carrot is propagated by seed, there are more than ten pathogens which are seed borne in nature (Richardson 1990). These cause various diseases such as Alternaria blight, Cavity spot, Cercospora leaf spot, Powdery mildew, Stemphyllium root rot, Watery soft rot and Bacterial soft rot. Along with all the diseases, Alternaria blight caused by *Alternaria radicina* (Kuhn) Groves and Skolko, the pathogen was also named *Stemphyllum radicinum* (Meier *et al* 1922). This is important disease on seed of carrot and is a limiting factor for its cultivation (Pryor *et al* 1994, Farrar *et al* 2004). Alternaria blight of carrot was first reported in 1855 from Germany (Chupp and Sherf 1960) and later from many countries of temperate and Mediterranean regions of the world like Belgium, Holland, USA, Denmark, Israel, France and India (Farrar *et al* 2004, Jensen *et al* 2004). The pathogen attacks both root and seed crop but losses are more in case of seed crop. The disease appears as brownish water soaked lesions on the margins and tips of older leaflets which gradually extend to become deep brown and blighted. As the spot increase in number, the interveinal tissue dies until the entire leaflet is killed. In moist weather disease spread rapidly. On leaf petioles,

elongated dark spots appeared and the entire leaf dies without spots on the foliage. It also causes damping- off of seedlings, blight of seed stalk and black decay of roots. Under heavy infection conditions leaves are entirely destroyed and harvesting becomes difficult which results in 40-60 per cent yield loss (Vintal *et al* 1999, Farrar *et al* 2004).

A lot of information on biology and role of different weather factors influencing the infection and disease development is available in Belgium, Holland, USA, Denmark, Israel and France (Chupp and Sherf 1960, Maude 1966, Zimmer and Mckeen 1968, Strandberg 1987, Farrar *et al* 2004). Several workers have also investigated the potential of conventional as well as newly introduced fungicides in different countries of the world for the management of Alternaria leaf blight of carrot. However, a perusal of literature revealed that very little work has been done on this disease in India and practically no attempt has been made so far from Punjab to study the epidemiological parameters influencing the disease and its management. It was therefore, proposed to take-up detailed investigations on this disease with the following objectives.

Objectives

(i) To study the optimum conditions for the growth of the pathogen and development of the disease.

(ii) To manage disease with different fungicides.

CHAPTER II

REVIEW OF LITERATURE

2.1 History

Fungi of *Alternaria* genus are widely spread as pathogens on field, vegetables, ornamental and orchard plants and cause substantial yield losses in a broad range of host crop species (Strandberg 1992, Farrar *et al* 2004). Alternaria blight of carrot caused by *Alternaria radicina*, is one of the most common and destructive foliar disease of carrot, which was first reported in 1855 from Germany (Chupp and Sherf 1960, Pryor *et al* 1994). It was reported to cause sporadic disease losses in several Northern European countries. Subsequently, it has also been reported from many countries of temperate and Mediterranean regions of the world like Belgium, Holland, USA, Denmark, Israel and France (Farrar *et al* 2004, Jensen *et al* 2004) and has become one of the most destructive diseases of carrot (Farrar *et al* 2004). In India it was first recorded in Shillong during 1964 (Roy 1969).

2.2 Symptomatology

Maude (1966) reported that two *Alternaria* species cause damping-off of carrot seedlings but when carrots were grown at high densities, *Alternaria dauci* and *Alternaria radicina* caused severe foliar infection. The pathogen attacks all parts of the plant including leaves, stems, flowers and seedling (Soteros 1979, Mitakkakis *et al* 2001). *Alternaria radicina*, an internal as well as external seed-borne fungus (Soteros 1979, Shakir *et al* 2000) becomes active after the seed begins to germinate and causes seed decay (Strandberg 1992). Histopathological studies revealed that mycelium of the pathogen was found in the inner layer of the pericarp and occasionally in the testa, but it was not detected in the endosperm or embryo (Pryor 2002). More recently Kim and Mathur (2006) detected the pathogen in the pericarp and in the fused seed coat/endosperm. As the seed germinates, propagules of *A. radicina* infect the young carrot seedlings and produced black necrosis on the connective regions of the root and shoot, which resulted in deformation or death of the developing seedlings (Murtaza *et al* 1988). *Alternaria radicina* caused pre-and post-emergence damping-off losses in the field (Nowicki 1995). Leaf lesions produced on leaf and petiole were generally dark- brown to black with chlorosis of surrounding tissues. Gradually the spots increase in size and became confluent, finally the whole leaf became grayish- black, while the leaflets became curly and convolute. The older leaves were more heavily infected than the young ones. On stem, dark brown to black colour lesions were formed, as the necrotic areas expanded and coalesce which affects the photosynthetic activity and ultimately yield (Strandberg 1987, Tylkowsa 1992). *Alternaria radicina* also infects roots through wounded or non-wounded tissue but the infection was slower in non-wounded tissue (Merfield 2009). The root infection initially resulted in a black ring at the point of attachment

between the petiole and root. It also caused dark, longitudinal spots on flower- stalks and umbels. Severely infected umbels failed to produce any seed (Farrar *et al* 2004).

Alternaria dauci attack carrot leaves, generally at the margins, forming small, dark, irregular, necrotic lesions, which were often surrounded by a chlorotic halo. Under optimal conditions, these could coalesce and cover the entire leaf. Older leaves were infected at a higher rate than younger leaves (Strandberg 1983). The pathogen also cause damping off of carrot seedlings, producing a continuous constricted lesion, which is tan brown in colour, extending upwards from soil level and sometimes reaching the cotyledons (Maude 1966).

2.3 Morphology and taxonomy of the pathogen

The morphology of the fungus *Alternaria radicina* isolated from carrot was described by Meier *et al* (1922), Ellis and Holliday (1972). It produces septate hyphae 2.5 to 7.0 µm wide with clear constrictions at the septa which form a grayish bluish black mycelium. Conidia produced at the tip of conidiophores that arise from hyphae, were 200 µm long × 3-9 µm wide, rarely branched, being mostly unbranched, septate, and straight, flexuous and coloured pale to olivaceous brown (Ellis and Holiday 1972). Conidia being produced singly or in a short chain, light to dark olive brown in colour, having a clavate, ellipsoid, obovoid or turbinate shape, measuring 34-51×10-22 µm, having 3-8 transverse and one or more longitudinal septa, that divide one or all segments, but not the apical or basal cell, and always with constriction at the septa (Meier *et al* 1922).

Ellis and Holliday (1972) reported slightly different measurements of conidia having 3-7 transverse septa and one or several longitudinal septa, with a length of 27-57 (average 38) µm and a width of 9-27 (average 19) µm. Recently Saude and Hausbeck (2006) recorded measurements of the mature conidia as 35-45×15-18 µm, with 3-8 transverse and 1-4 longitudinal septa.

Alternaria dauci produced colourless pale to dark brown smooth walled hyphae, which were 15-30 x 25 µm in size and were not constricted at septa (Groves and Skolko 1944). Conidiophores were arising singly or in small group, straight or flexuous, sometimes geniculate, septate, pale or mid olivaceous brown up to 80 x 6-10 µm. Conidia were solitary or occasionally in chains of 2-3. At juvenile stage conidia were pale olivaceous brown and later on turned dark brown at maturity, smooth walled, straight curved, obclavate with 7-11 transverse septa and several longitudinal or oblique septa. It had a size of 100-450 x 16-25 µm included a flexuous, colourless beak up to 3 times the length of main spore body.

2.4 Cultural studies of the fungus

Alternaria radicina produces blackish brown to black colonies on potato dextrose agar medium (Ellis and Holliday 1972). A colony of the fungus grew slowly with an irregular margin, and releases a yellow coloured pigment into the medium. It also forms dendetritic crystals visible on the underside of the petri dish and growth never covers the entire Petri dish (Pryor and Gilberton 2002). However, on *A. radicina* selective agar (ARSA) medium it produces distinctive black colonies that grow down into the agar, with little aerial growth (Pryor *et al* 1998). *Alternaria dauci* produces carpet like mycellial mat with reddish brown coloration colony particularly on potato dextrose medium (Soylu *et al* 2005).

Zimmer and McKeen (1968) observed the sporulation in *Alternaria dauci* at temperature from 15 to 27^{o}C and various photoperiods of cool white fluorescent and long wave ultraviolet radiation. The long wave ultraviolet region of the electromagnetic spectrum stimulates conidiophores formation. Under continuous irradiation in which wavelengths of 370-510 nanometers were present and the temperature is 24°C or above, only conidiophores formation occurs. Conidia did not form unless the irradiation exposure was followed by a sufficient period of darkness or the fungus was subjected to radiation of wavelength 510 nm or above. Conidial formation in the dark occurred three times faster at 18°C than at 25°C. If temperature was 23°C or below then the formation of the conidiophores and conidia would occur under continuous irradiation. *Alternaria dauci* did not sporulate in continuous darkness.

Pryor *et al* (1998) reported that growth and sporulation of *Alternaria radicina* on carrot leaf agar media was affected by temperature, pH and exposure to light and darkness. Growth and sporulation occurred at 15-30^{o}C, which was optimum at 25^{o}C. Mycelial growth was maximum at pH 6.5 but occurred at all the tested pH values. An increase or decrease in pH from 6.5 gradually suppressed the growth of the pathogen. Sporulation occurred at all pH levels except 2.9 and 9.2. Alternate light and dark was better for growth than continuous light or complete darkness. *Alternaria radicina* produced good aerial mycelium on carrot leaf agar and profusely sporulate on carrot leaf agar media with optimum pH level of 6.5 and 7.0. The growth was recorded to be optimum at 25°C and ceased beyond 35°C (Shakir *et al* 2000).

Roy (1969) reported that growth of *Alternaria dauci* was best at pH value of 6 and and it was poor at pH 9 and 10. Shahin and Shepard (1979) reported that sporulation in *Alternaria* spp (*Alternaria dauci*, *Alternaria radicina* and *Alternaria alternata*) on corn meal agar was considerably less than potato dextrose agar media. Strandberg (1987) reported that on agar media, a pH from 6.0 to 6.5 was optimum for mycelial production of *Alternaria radicina*,

whereas pH near 7 was optimum for conidial production of *Alternaria radicina*. Growth rate of *Alternaria radicina* was proportional to temperature from 12°C to 28°C. A significant growth was observed at 25°C. Minimum day length of 4 hour was required for abundant production of conidia. In further study the author developed an *Alternaria dauci-radicina* selective medium (ADRSM) to detect *Alternaria dauci* and *Altenaria radicina* from seed, plant debris and other substrates (Strandberg 2002). The selective medium was based upon carrot leaf extract and promoted profuse growth and sporulation of both the pathogens, which could be identified from their morphological characters. Soylu *et al* (2005) reported that *Alternaria dauci* produced a carpet like mycelia mat and abundant conidia on Potato carrot agar (PCA) amended with streptomycin sulphate maintained at 26°C with less than 12 hour near ultraviolet photoperiod.

2.5 Pathogenicity tests

Roy (1969) tested the pathogenicity of *Alternaria dauci* on 2 to 2.5 inches grown plants in earthen pots as well as detached plants in conical flasks filled with water. Blight infection appeared after three days of inoculation with both 6 and 60 days old cultures on the older basal leaves, which was considerable after five days. The intensity of infection was maximum on the leaves when the atmosphere was kept completely saturated for 72 hours and gradually decreased in 48 and 24 hours. On young leaves, lesions could be produced as small necrotic spots after six days only with wounds. Strandberg (1987) reported that older leaves are more prone to disease than younger ones. Carrot plants of 5-8 week old developed more disease than 4 week old plants.

Strandberg (1988) sprayed the carrot plants at the 4 to 6 leaf stage with conidia of *Alternaria dauci* and found that temperature in the range 16°-28°C coupled with 100 per cent relative humidity plus leaf wetness for 24 hours is required for infection. During night hours more than 12°C temperature plus wetted leaves were necessary for disease development. Soylu *et al* (2005) tested the pathogenicity of *Alternaria dauci* on carrot seedling (cv Nanco) at the six leaf stage by spraying the lower leaves with conidial suspension ($1x10^4$ spores /ml). Inoculated plants were covered with polythene bag, incubated at 26°C for 2 days and subsequently transferred to a growth chamber at 26°C with 16 hour photoperiod. Symptoms of disease developed within 10 days of incubation.

2.5.1 Epidemiology

Despite advances in agricultural technology, adverse weather may still cause significant decrease in crop yields (Harrison 1992). Weather and climate directly affect the plants. Weather includes temperature, preciptation, relative humidity, radiation, wind speed and direction, cloud cover and atomspheric pressure. Weather has been corelated with

outbreak of disease by several workers (Pathak and Singh 1988, Raposo *et al* 1993, Johar *et al* 1997, Sunseri and Johnson 1999).

Madden *et al* (1978) included temperature and wetness as meteorological factors in a simulator fast to predict the severity of Alternaria blight of tomato crop. Strandberg (1988) reported that temperature range of 20-28°C with optimum 24°C alongwith prolonged hours of leaf wetness are highly conducive for the development of Alternaria blight of carrot under field conditions. Strandberg (1988) sprayed the carrot plants at the 4 to 6 leaf stage with conidia of *Alternaria dauci* and found that temperature in the range 16-28°C coupled with 100 per cent relative humidity plus leaf wetness for 24 hours was required for infection. During night hours more than 12°C temperature plus wetted leaves were necessary for disease development.

The fungus is both seed and soil borne. Seed infection with pathogens could play a role of disseminating the pathogen to a new place being a primary inoculum source in the field (Maude 1966, Netzer and Kenneth 1969, Scott and Wenham 1973, Soteros 1979, Standberg 1983). Seed infection was observed more often in wet and cold seasons as compared to a dry and warm season (Tylkowska 1992).

Rottem (1994) reported that wetness period and temperature were the most important environmental factors affecting the severity of early blight epidemic caused by *Alternaria* species. Hong and Fitt (1996) reported that the maximum disease incidence increased as wetness period after inoculation increased from 4 to 24 hours and as temperature increased to 20°C. The optimum temperature for growth and infection was 27°C, whereas the minimum and the maximum temperatures for infections are 14°C and 35°C, respectively (Rubatzky *et al* 1999). The spread of fungal spores took place by running water, splashing rains or in the contaminated soil. Infection took place rather slowly except in most favourable weather. When wounds were present incubation period is reduced appreciably (Westerveld *et al* 2002).

Farrar *et al* (2004) reported that conidiophores and conidia were produced at temperature from 8 to 28ºC and in presence of 96 to 100 per cent relative humidity or free water on plant surface. Conidia deposited on leaf surfaces will germinate and infect under appropriate temperature and leaf wetness conditions. At 24ºC damage generally increases with increasing hours of leaf wetness from 8 to 56 hour. Saude and Hausbeck (2006) reported that a temperature of 20ºC and more than 92 per cent relative humidity were favourable conditions for *A. radicina* infection.

Several workers have also demonstrated the effect of different durations of light/darkness on conidial production in other *Alternaria* species affecting different hosts. Optimum weather conditions required for infection and disease development have also be

demonstrated. Luckens (1960) reported that conidiophores of *Alternaria solani* were formed primarily in alternate light and darkness conditions. Further intensity of light and duration of exposure are the important factors determining the number and size of spores. Gupta and Nikhanj (1972) reported that light stimulated the spore germination at pH 6 while it has no effect on spore germination at pH 7 and a temperature of 22.5°C was best for germination of spores of *Alternaria solani*. However, Waggoner and Parlang (1975) observed that *Alternaria solani* spores germinated with equally rapidity at a temperature range of 16^0 to 35°C. Manjunath *et al* (2010) tested the different pH levels, temperature, light intensity and media against the growth of *Alternaria alternata* under *in vitro* conditions. The results of experiment indicated that the growth of *A. alternata* was maximum in pH range of 6.00-6.50 and temperature range of 25° to 30°C. The exposure of the fungus to alternate cycles of 12 hour light and 12 hour darkness resulted in the maximum mycelial growth of *A. alternata* compared to continuous light and dark on potato dextrose agar media.

Bashi and Rotem (1975) reported that wetting of potato leaves for 24 hours with 12 hour light and 12 hour dark regimes produced more spores but none or few spores were produced under 24 hours continuous dark or 12 hour dark followed by 12 hour light conditions. For maximum infection and disease development in mustard, a minimum period of 4 hour leaf wetness is essential. Longer period of leaf wetness at 25°C increased infection frequency on the leaves (Kadian and Saharan 1984). Pelletier and Fry (1989) reported that with increase in leaf wetness durations from 6 to 24 hour lesions caused by *Alternaria solani* on potato plants increased in linear manner. Vloutoglou and Kalogerakis (2000) reported effects of inoculums concentration, wetness duration and plant age on the development of tomato early leaf blight in relation to host susceptibility under controlled environment conditions. The main effect of early blight was premature defoliation, which was linearly related to percentage of leaf area showing symptoms. As inoculums concentration (conidia/ml) increased from 6.2 to 11.5, the percentages of leaf area affected and of defoliation increased linearly. Vloutoglou and Kalogerakis (1999) reported that 4 hour to 6 hour leaf wetness after inoculations was sufficient to initiate disease on plants. As leaf wetness duration increased up to 24 hour, there was an increase in the percentage of leaf area infected and per cent defoliation, but thereafter there was no significant increase in either parameter. Shrestha *et al* (2005) reported that 80-90 per cent RH, maximum temperature ranging from 18-25°C, minimum from $10\text{-}14^0$ C and 14-15 hr wetness period daily during the month of December and January favour the development of pathogen on tomato crop.

2.5.2 Survival

Pryor (2002) reported that *Alternaria radicina* survives in the infected seed, diseased plant debris in the soil and on weed hosts. Westerveld *et al* (2002) also reported that

Alternaria radicina and *Alternaria dauci* were seed and soil borne and disease debris play important role for their perenation. Maude and Shuring (1971) reported that *Alternaria radicina* survived on crop debris at least for 12 months under dry conditions and the survival in the form of hyphae and conidia of *Alternaria radicina* as reported by Rottem (1994) and Pryor *et al* (1998).

Some workers reported that the pathogen *Alternaria radicina* survive on alternate hosts which mostly belong to family Umbelliferae (Ellis and Holliday 1972, Tahvonen 1978). Infected seeds of carrot retained infection of *Alternaria radicina* for long time which also played a crucial role as reservoir of primary inoculums of the pathogen (Farrar *et al* 2004).

2.6 Host

Alternative hosts for *A. radicina* include celery (*Apium graveolens* L. var. *dulce* (P. Mill.) D.C.), parsnip (*Pastinaca sativa* L.), parsley (*Petroselinum crispum* (Miller) A.W. Hill), fennel (*Anethum graveolens* L.), dill (*Anethum graveolens* L.), celeriac (*Apium graveolens* L. var. *rapaceum* (Miller) Gaudin), *Fumaria muralis* and caraway (*Carum carvi* L.), (Neergaard 1945, Ellis and Holliday 1972, Gindrat 1979, Soteros 1979, Coles 2003). Richardson (1979) and Coles (2003) also reported that *Apium graveolens* and *Anethum graveolens* are susceptible to *Alternaria radicina* and thus serve as alternate hosts of the pathogen.

2.7 Varietal response

Fry (1982) reported that less susceptible cultivar could delay the onset of disease symptoms, reduce the rate of pathogen spread and slow the progress of an epidemic. Gugino *et al* (2007) screened different carrot varieties in New York namely Ithaca, Fullback, Kamaran, Napa, Nevis, White Fontana, Carson and Neal. He concluded that Ithaca and Fullback were less susceptible to *Alternaria radicina*, whereas Kamaran, Napa and Nevis were susceptible. White Fontana was most susceptible than other varieties.

Simon *et al* (1998) studied relative resistance/susceptibility of three varieties of carrot against Alternaria blight of carrot. Presto was highly susceptible variety, whereas Bolero and B-5280 were partially resistant to Alternaria leaf blight.

Roy (1969) tested eleven varieties namely Early Nantes, Nantes, Amsterdam Forcing, Champion Scarlet Horn, Long Orange, Early Gem, Danvers, Fyazabad, Golden Heart, Imperator and Tender Sweet. All the tested varieties were found to be susceptible to the disease with varying degrees of intensity.

2.8 Toxin production

Alternaria radicina was known to produce non-host specific toxins. Production of radicinin had been known for a long time, however radicinol and epi-radicinol were recently reported by Solfrizzo *et al* (2004). He found radicinin and radicinol toxins from the naturally diseased parts of carrots and suggested that they had a role in pathogenicity. Other authors

had also reported that *A. radicina* produced various toxic metabolites on carrot roots, primarily radicinin (Tylkowska *et al* 2003), *epi*-radicinol (Tylkowska *et al* 2005) and radicinol (Solfrizzo *et al* 2005). The effect of a high concentration (250 µg/ml) of radicinin and *epi*-radicinol were recently reported by Tykowska *et al* (2008) and demonstrated ultrastructural changes in various components of parenchyma cells of carrot roots, although the integrity of the membrane was not disturbed. These metabolites were phytotoxic and reduce the marketable value of carrot, but they had not been shown to cause any harmful effects to humans or animals (Solfrizzo *et al* 2005).

2.9 Management of the disease

Alternaria diseases can be controlled through integrated use of clean seed, sanitation, crop rotation, cultivar selection and fungicides (Soteros 1979). Hardison (1976) reported that mycelial growth of *Alternaria radicina* on PDA medium was completely inhibited by propiconazole at 30 ppm followed by Ridomil MZ (mancozeb+metalaxyl), Daconil (chlorothalonil) and Antracol (propineb). Bollen *et al* (1983) reported that among different fungicides Contaf (hexaconazole) proved most effective and caused complete inhibition in the mycelial growth of the pathogen at 1000 µg/ml concentration. Kumar *et al* (2006) tested seventeen fungitoxicants of various groups against *Alternaria brassicae*, incitant of Alternaria blight of radish. Out of which Chlorothalonil, propineb proven to be most effective as they inhibited growth of fungus completely at 0.2 per cent concentration. Singh and Singh (2006) studied the efficacy of different contact and systemic fungicides under *in vitro* conditions against *Alternaria alternata* and reported that hexaconazole was most effective as it caused 100 per cent growth inhibition even at lowest concentration of 250 ppm. The other fungicides like mancozeb, copper oxychloride, copper hydroxide, chlorothalonil, azoxystrobin and propineb were also effective and caused significant reduction in mycelial growth but at a much higher concentration. Under *in vitro* conditions low efficacy of mancozeb to *Alternaria solani* even at a high concentration of 250 ppm has earlier been reported (Choulwar and Datar 1994). Rogers *et al* (2010) studied response of three fungicides (azoxystrobin, chlorothalonil and boscalid) commonly used for Alternaria blight control of carrot. Inhibtion of conidial germination ranged from 0.01 to 0.37 µg/ml for azoxystrobin, 0.08 to 0.09 µg/ml for chlorothalonil and 0.09 to 0.59 µg/ml for boscalid. On average isolates were more sensitive to chlorothalonil than to azoxystrobin and bascalid.

In most of the carrot producing areas, routine application of fungicides is necessary to control Alternaria leaf blight. Under high disease pressure, no single control measure is sufficient to manage the disease adequately. Multiple applications of fungicides are required to achieve economic yield and acceptable quality in infected crop (Farrar *et al* 2004).

The disease can be kept under check by collection and destruction of infected plant debris, using healthy seed produced in disease free areas, well drained soils, long crop rotations and deep cultivation to place infected debris below 20 cm depth in the soil. Seed borne inoculum could be reduced by treating the infected seed by hot water at 50°C for 20 to 30 minutes without significant loss in germination (Nega *et al* 2003). Strandberg and White (1989) also reported that dipping of seed in hot water at 45-55°C for 4-20 minutes reduced the *Alternaria dauci/Alternaria radicina* population and germination was not affected. Seed treatment with fungicides like Vitavax (carboxin) + Thiram and Voronit @2g /kg seed is effective against Alternaria blight of carrot (Mirkove 1979).

Fallas *et al* (1992) reported that the disease can be managed in field conditions by using Blitox (0.25%) or Dithane M-45 (0.25%). Difenoconazole, an EBI fungicide @ 125 g/ha when applied at 21 days intervals provided excellent control of the Alternaria blight of carrot in comparison to Mancozeb. Poissonnier *et al* (1995) recommended sprays of iprodione and hexaconazole for the control of Alternaria blight of carrot in France. Resende *et al* (1996) reported that the efficacy of procymidone at 500 and 750g/ha, triflumizole @ 300 and 450g/ha, thiophanate methyl + chlorothalonil @ 500+1250g/ha, chlorothalonil @ 1500g/ha, iprodione @ 750g/ha, copper oxychloride + chlorothalonil @ 600 + 500g/ha and chlorothalonil + sulphur @ 1125+1300 g/ha in controlling of *Alternaria dauci* on carrot. Iprodione and procymidone gave the best disease control and yield. Mazur *et al* (2005) concluded that Amistar 250SC (azoxystrobin) showed higher efficacy in controlling Alternaria blight of carrot when the disease had already established under field conditions.

Gillepsie and Sutton (1979) developed a predictive scheme for timing fungicide applications to control Alternaria blight in carrots. When fungicide sprays applied according to the scheme over 4 growing seasons blight was effectively controlled and 1-5 fungicide applications were reduced as compared to regular schedules. Post-infection applications of triademefon also controlled blight effectively.

Schwarz *et al* (1998) tested the efficacy of five fungicides viz., difenoconazole, azoxystrobin, iprodione, chlorothalonil and macozeb against *Alternaria radicina* after the appearance of first symptoms in field. A good curative control was obtained with 3 sprays at 12- day intervals. It is suggested that chemicals should be applied immediately after onset of symptoms. It was also suggested that alternating chemicals would be more suitable than using single chemical.

Recently available fungicides like azoxystrobin and difenconazole provided better control of this disease as compared to traditional fungicides like copper oxychloride and sulphur (Ben-Noon *et al* 2001). Foliar application of fungicides like difenoconazole, chlorothalonil, iprodione (Farrar *et al* 2004, Saude and Hausbeck 2006), azoxystrobin (Farrar

et al 2004) have been reported to be effective against *A. radicina*. Ben noon *et al* (2001) conducted an experiment under which the efficacy of nine fungicides was determined in two field experiments. All fungicides reduced disease severity, but there was significant difference in efficacy among them. The most effective were difenconazole and chlorothalonil, less effective were copper hydroxide, tebuconazole, trifloxystrobin and mancozeb, the least effective were flutrifol, propineb and iprodione for the management of Alternaria blight under field conditions. Alkseeva (2009) conducted field trials in Russia and concluded that Score (difenconazole) was quite effective for management of Alternaria blight of carrot on appearance of first disease symptoms.

Sidlauskiene (2001) has demonstrated the efficacy of Amistar 250 SC, a strobilurin fungicide in reducing disease prevalence (90.8%) and intensity (88.8%) of Alternaria blight of tomato. Jat and Jain (1988) tested seven fungicides viz, Difolatan 80 WP, Dithane M- 45, Bavistin 75 WP, Blitox- 50, Topsin M and Sulfex 80 WP against *Alternaria alternata* under field conditions. Four sprays of topsin M (thiophenate- methyl) at 0.1 per cent at an interval of 20 days was found to be most effective followed by Dithane M-45 @ 0.3 per cent and Blitox- 50 @ 0.3 per cent. In early blight of potato caused by *Alternaria solani* the effectiveness of Dithane M-45 in controlling the disease has been well demonstrated under field conditions (Mohit *et al* 1997, Dhanbir *et al* 2002). Singh and Singh (2006) reported that carbendazim and mancozeb to be most effective against *Alternaria* species. Mixture of Carbendazim + mancozeb was also found to be equally effective against the *Alternaria solani*. Prasad and Naik (2002) reported Indofil M- 45 to be most effective against *Alternaria solani* causing alternaria blight on tomato.

Jensen *et al* (2004) has investigated the role of bio agents in managing Alternaria blight of carrot. Highly infected carrot seed with *A. dauci* / *A. radicina when* bioprimed with *Clonostachys rosea* strain IK 726, reduced the incidence of the pathogen to 4.8 per cent with a lower infection rate.

CHAPTER III

MATERIALS AND METHODS

3.1 Disease Survey and collection of diseased samples

Carrot growing areas in districts of Hoshiarpur and Ludhiana were surveyed during February-March 2011 and 2012. The observations were recorded on time of appearance, symptomatology, disease incidence and severity of disease on carrot plants in the field. The data on disease incidence were recorded on 50 plants selected at random in the field. Disease severity was recorded on 0 -9 rating scale (Gaube *et al* 2004) as follows:

Class rating		Extent of infection
0	_	Apparently no infection
1	_	<5% of leaf area infected
3	_	5-20% of leaf area infected
5	_	20-40% of leaf area infected
7	_	40-60% of leaf area infected
9	_	more than 60 per cent of leaf area infected

The per cent disease index (PDI) was calculated using the following formula

$$PDI = \frac{\text{Sum of numerical rating}}{\text{Total number of plants/leaves examined x maximum class rating}} \times 100$$

The infected leaves, stems and umbels showing typical symptoms of Alternaria blight were collected in polythene bags and brought to the laboratory for further use. These samples were dried in folds of filter papers for the isolation of pathogen.

3.2 Isolation and purification of the pathogen culture

Isolations of the pathogen were made from the diseased carrot leaf samples collected from Ludhiana and Hoshiarpur. The leaves were thoroughly washed under running tap water and dried under shade. Small bits along with some healthy portion were cut with the help of blade and surface sterilized with 0.1 per cent mercuric chloride ($HgCl_2$) solution for 30 seconds. To remove all traces of surface disinfectant solution from the surface, the carrot leaf bits were thoroughly washed thrice with sterilized distilled water. These bits were then transferred to Carrot leaf agar (CLA) slants under sterile conditions in a laminar flow and incubated at 25±1°C in a BOD incubator.

The culture of the fungus was purified by single spore isolation technique. Ten ml of 2 per cent water agar medium was poured in sterilized petriplates. The spore suspension was prepared in sterilized distilled water containing 5-10 conidia per microscopic field (10x). It was poured uniformly on solidified medium and incubated at 25±1°C for few hours. The plates were examined under the microscope for locating the geminating spores, which were then marked with glass marker on the glass surface of the plate. The marked agar areas were cut with cork borer and transferred to CLA slants with the help of sterilized inoculation needle and incubated at 25±1°C. Ten such single spore cultures were raised and compared their growth characteristics. The isolates were found to be identical and hence one of them was multiplied for further studies. The pure culture of the pathogen was maintained on CLA for further studies.

3.3 Morphological studies and confirmation of the identity of the pathogen

The cultures of the pathogen growing in the test tubes as well as in the petriplates were examined regularly for studying the initiation and pattern of conidia. The microscope used throughout the study was calibrated for taking measurements of the size of spores. The size of conidia was measured using ocular and stage micrometer which were calibrated using the formula given below.

$$1 \text{ ocular division} = \frac{\text{Coinciding division of the stage}}{\text{Coinciding division of the ocular}} \times 10\mu m$$

The conidia were lifted from culture, washed in tap water in watch glasses and examined carefully with hand lens to record their shape, texture and colour. The shape, colour, size and septation of conidia were measured from 15 days old culture. The size of spores formed on infected leaves was also measured based on 50 observations to confirm the identity of the pathogen through comparison with standard relevant literature.

Five mycelial discs (5 mm size) obtained from 15 days old cultures were eluted in 5 ml of distilled water to obtain spore suspension. A drop of spore suspension supported on glass slide was covered with cover slip and observation on spore length and breadth, number of horizontal and vertical septa, beak length, septation were taken under the compound microscope.

3.4 Cultural studies of the pathogen

All the glassware used during cultural studies was of ‘Borosil’ brand and the chemicals ‘AR’ grade. The petriplates used throughout the studies were 90 mm diameter in size. The Erlenmeyer flasks used were of 250 ml capacity. The flasks were first cleaned with cleaning solution (conc. Sulphuric acid 460 ml, Pottasium dichromate 60 g and water 300 ml) then washed in running tap water followed by rinsing with double distilled water.

3.4.1 Effect of different substrates on the radial growth of *Alternaria radicina*

A number of natural, semi-synthetic media were tested with a view to find out the most suitable medium which favours the best growth and sporulation of the pathogen. The following media were included in the study:

(i) Potato dextrose agar (PDA) medium

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

Preparation: The peeled potatoes were cut into small pieces and boiled in water. The extract was taken by filtering through double layer muslin cloth. The dextrose and agar were added and the total volume was made up to one litre.

(ii) Carrot leaf agar (CLA) medium

Dried carrot leaves	-	10 g
Agar agar	-	15 g
Distilled water	-	1000 ml

Preparation: The dried carrot leaves were cut into small pieces and boiled in one litre water for 25 minutes. The extract was taken by filtering through double layer of muslin cloth. The agar and water were added to bring the volume to one litre (Strandberg 2002).

(iii) Umbel extract agar medium

Umbels	-	10 g
Agar agar	-	15 g
Distilled water	-	1000 ml

Preparation: The carrot umbels were cut into small pieces and boiled in one litre water for 25 minutes. The extract was taken by filtering through double layer muslin cloth. The agar and water were added to bring final volume to one litre (Strandberg 2002).

(iv) Oat meal agar (OMA) medium

Oatmeal	-	30 g
Agar agar	-	20 g
Distilled water	-	1000 ml

Preparation: Oat flax were boiled in water and filtered through muslin cloth. The agar was added and final volume was made up to one litre.

(v) Corn meal agar medium

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

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

(vi) Czapek'sDox agar medium

Sodium nitrate ($NaNo_3$)	-	2 g
Dipotassium hydrogen phosphate (K_2HPO_4)	-	1 g
Magnesium sulphate ($MgSO_4$. $7H_20$)	-	0.50 g
Ferrous sulphate ($FeSO_4$.7 H_2O)	-	0.01 g
Sucrose (C_{12} $H_{22}O_{11}$)	-	30 g
Agar agar	-	20 g
Distilled water	-	1000 ml

(vii) Richard's agar medium

Potassium nitrate (KNO_3)	-	10 g
Potassium monobasic phosphate (KH_2PO_4)	-	5 g
Magnesium sulphate (Mg SO_4 $7H_2O$)	-	2.5 g
Ferric chloride ($FeCl_3$ $6H_2O$)	-	0.02 g
Sucrose ($C_{12}H_{22}O_{11}$)	-	50 g
Agar agar	-	15 g
Distilled water	-	1000 ml

Preparation: Sucrose was steam sterilized in an autoclave for three consecutive days. The agar along with other constituents was dissolved in water and autoclaved separately. The sterilized sugar solution was added to it aseptically.

Twenty ml of each medium was poured into the sterilized petriplates under aseptic conditions in a laminar flow. The petriplates were inoculated with 5mm discs of the mycelium cut with the help of sterilized cork borer from 15 days old culture of *Alternaria radicina* raised on Carrot leaf agar. Three replications were kept in each treatment. Inoculated petriplates were incubated in BOD incubator at 25± 1°C for 10 days. The observations were recorded in terms of daily radial growth of fungus in millimeter.

3.4.2 Effect of different substrates on the extent of sporulation of *Alternaria radicina*

The culture of *Alternaria radicina* grown on different media described in section 3.4.1 was used to see the extent of sporulation after 15 days of incubation at 25± 1°C. The sporulation was measured with the help of haemocytometer. Spore suspension was obtained by eluting five mycelial discs (5mm size) in 5ml of FAA (formaldehyde: absolute alchol:glacial acetic acid: water 2:10: 1:7) contained in capped glass vials. Three vials were used for each replication. The vials were thoroughly shaken for two to three minutes and eluted conidia were counted with the help of the haemocytometer.

3.4.3 Effect of temperature regimes on radial growth and sporulation

To determine the minimum, optimum and maximum range of temperature for the linear growth of the *Alternaria radicina*, 20 ml of the basal medium (CLA) was poured in the petriplates, inoculated and incubated at different regimes of temperature *viz*, 10, 15, 20, 25 and 35°C maintained in different BOD incubators. Each treatment was replicated thrice. The linear growth of the fungus was measured at 48 hr intervals up to 10 days of incubation at different temperatures.

The sporulation was measured with the help of haemocytometer. Spore suspension was obtained by eluting five mycelial discs (5mm size) in 5ml of FAA (formaldehyde: absolute alchol:glacial acetic acid: water 2:10: 1:7) contained in capped glass vials. Three vials were used for each replication. The vials were thoroughly shaken for two to three minutes and eluted conidia were counted with the help of the haemocytometer.

3.4.4 Effect of pH on radial growth and sporulation

To determine the optimum level of pH for growth of *Alternaria radicina* 100 ml of the medium (CLA) was dispensed in five Erlenmeyer flasks of 250 ml capacity, and the different pH levels of 5, 6, 7, 8 and 9 were adjusted per flask with help of digital (ELTOP 3030) pH meter by adding N/10 HCL or NaOH and plugged with non-absorbent cotton .The flasks were sterilized in an autoclave at 15psi pressure for 20 minutes. After autoclaving, when the media was lukewarm, 20 ml of the media was poured into each petriplates under aseptic conditions in the laminar air flow. Three replications were maintained for each pH level. When the media got solidified, each petriplate was inoculated with 5 mm mycelia discs of the pathogen cut with the help of sterilized cork borer from 15 days old culture of *Alternaria radicina* raised on CLA. Then the petriplates were incubated at 25±1°C in BOD

incubator. The linear growth of the fungus was measured at 48 hr intervals up to 10 days of incubation at different pH levels. The sporulation was measured with the help of haemocytometer.

3.5 Pathological studies

Carrot plants of cultivar PC- 34 were raised in 15 cm earthern pots in screen house of the Department of Plant Pathology, PAU Ludhiana following standard agronomic practices. In order to test the pathogenicity of the pathogen a large number of artificial inoculations were carried out with *Alternaria radicina*, on leaves (both young and old) and stem of young seedlings grown in pots and umbels of carrot cultivar PC-34. The observations in terms of per cent leaf area damaged as well as time required for development of symptoms (incubation period) of Alternaria blight was recorded. Spore suspension ($1x10^4$conidia/ml) was prepared in distilled water with the help of haemocytometer from 15 days old culture. Thirty days old plants were inoculated by spraying spore suspension of *Alternaria radicina.* The plants after inoculations were covered with moist polythene bags for 48 hours to facilitate infection. The observations on incubation period and final disease severity were recorded after 15 days of inoculation following 0-9 rating scale (Gaube *et al* 2004).

3.5.1 Raising of carrot plants

Seeds of carrot cultivar PC-34 were obtained from department of Vegetable Science, PAU, Ludhiana. The carrot seeds were sown in the field as per standard agronomic practices and steckling were transplanted in the field as per treatment for raising plants of different age groups i.e. 10, 20, 30, 40, 50 and 60 days old. Required fertilizers and other operations were performed as per Package of Practices for Vegetable Crops, PAU, Ludhiana. Alternatively, carrot plants of different age groups were also raised in 9" size earthen pots for experiments.

3.5.2 Effect of plant age on disease

To determine effect of plant age on disease, carrot plants of 10, 20, 30, 40, 50 and 60 days after transplanting of steckling were inoculated with spore suspension ($1x10^4$/ml). All treatments were replicated thrice keeping three plants per replicate. The inoculated plants were covered for 48 hr and kept in growth room at 25±1°C with 12 hr light durations for development of disease. The data on incubation period (first visible symptoms), per cent leaf area damaged was recorded after 10 days of inoculations.

3.5.3 Effect of duration of leaf wetness on disease development

Thirty days old stecklings of carrot cultivar PC-34, were artificially inoculated as described in 3.5.2. The plants after inoculations were covered with moist polythene bags for different durations 4, 8, 12, 16, 24, 48 hr of leaf wetness. These plants were kept in growth room at 25±1^{o}C temperature with 12 hr light durations for development of disease. For each treatment three replications were used, keeping three plants per replicate. The data on

incubation period (first visible symptoms) and per cent leaf damaged were recorded after 10 days of inoculation.

3.6 Evaluation of fungicides

3.6.1 *In vitro* evaluation of the fungicides

Three non-systemic fungicides *viz* Indofil M-45 (mancozeb), Antracol 70WP (propineb), Kavach 75WP (chlorothalonil) and six systemic fungicides *viz* Score 25EC (difenconazole), Folicur 25EC (tebuconazole), Tilt 25EC (propiconazole), Contaf 5EC (hexaconazole), Amistar 250SC (azoxystrobin) and Amistar top 325SC (azoxysrobin+ difenconazole) were evaluated in the laboratory against *Alternaria radicina* following growth inhibition technique. The commercial name, common name and chemical name of fungicides as well as their manufacturers are given in the table below:

Chemical name	Common name	Chemical name	Manufacturer
Indofil M-45	Mancozeb	Manganese ethylene bis-dithiocarbamate+2% Zinc ion	Indofil Chemicals
Antracol 70 WP	Propineb	Zinc-propylene-bis-dithiocarbamate	Bayer Crop Science
Kavach 75 WP	Chlorothalonil	2,4,5,6-tetrachloroisophthalonitrile	Syngenta India Ltd
Contaf 5EC	Hexaconazole	(RS)-2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl)Hexan-2-ol	Rallis India Ltd
Folicur 25 EC	Tebuconazole	(RS)-1-(4-chlorophenyl)-4,4-dimethyl 1-3-(1H-1,2,4-triazol-1yl methyl) pentan-3-ol	Bayer Crop Science
Score 25 EC	Difenconazole	-[(4-chlorophenoxy)-4chloro-2phenyl]-2(4-methyl dioxilanne-1,3yl-2	Syngenta India Ltd
Tilt 25 EC	Propiconazole	(-1-[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-ylmethyl]-1H-1,2,4-triazole	Syngenta India Ltd
Amistar 250 SC	Azoxystrobin	Methyl (E)-2-{2-6-(2-cyanophenoxy) pyrimidin-4-yloxy] phenyl}-3-methoxyacrylate	Syngenta India Ltd
Amistar top 325 SC	Azoxystrobin+ Difenconazole	Cis, trans-3-chloro-4-[4-methyl-2-(1H-1,2,4-triazol-1-ymethyl)-1,3-dioxolan-2-yl] phenyl4-chlorophenylether	Syngenta India Ltd

In case of non-systemic fungicides, stock solution prepared in sterilized distilled water was added under aseptic conditions in 250 ml flask containing CLA medium so as to

have the final concentrations (on a.i. basis) of 10, 25, 50, 100, 200, 500, 1000 µg/ml. While in case of systemic fungicides, the concentrations of the fungicides used in the medium were 1, 10, 15, 50, 100 and 200 µg/ml. The fungicides amended medium was poured in sterilized petri plates, allowed to cool down and inoculated in the centre with 5 mm mycelial disc of actively growing culture of *Alternaria radicina* separately. The petri dishes containing unamended medium serve as control. Each treatment was replicated thrice using one Petri dish in each replicate. The inoculated Petri dishes were wrapped with cellophane film to minimize the chances of contamination and incubated at 25±1°C temperature. The observations on colony diameter were recorded after 10 days of incubation. The per cent inhibition in mycelial growth was worked out by using the formula devised by Vincent (1947).

$$Pi = \frac{C-T}{C} \times 100$$

Where

Pi = Percent inhibition in mycelia growth

C = Radial growth in control

T = Radial growth in treatment

ED_{50} and ED_{90} values of different fungicides were calculated by plotting per cent inhibition in colony growth against concentrations of different fungicides.

3.6.2 Field evaluation of Fungicides

Efficacy of different non-systemic fungicides *viz* Indofil M-45 (macozeb), Antracol 70WP (propineb), Kavach 75 WP (chlorothalonil) each at the rate of 0.25 per cent and systemic fungicides *viz* Score 25EC (difenconazole), Folicur 250SC (teuconazole), Tilt 25EC (propiconazole),Contaf 5EC (hexaconazole), Amistar 250SC (azoxystrobin) and Amistar Top 325SC (azoxystrobin + difenconazole) each at the rate of 0.1 per cent were tested against Alternaria blight of carrot under artificial inoculated conditions in the field. Carrot plants of cultivar PC-34 were raised on ridges during the month of September. During January carrot steckling were transplanted in plots of 2x3 m size in the field area of Department of Plant Pathology following standard agronomic practices (Anonymous 2011). Each treatment was replicated thrice in randomized block design. First protective application of test fungicide was given in March 2011, 24 hr before inoculations. Inoculations were done with spore suspension (1x 10^4/ml) of *Alternaria radicina* during evening to ensure uniform development of disease in the field. Two fungicide sprays were given at 15 days interval. The observations on disease severity were recorded 10 days after last spray.

3.7 Statistical Analysis

The data of laboratory studies and field experiments were statistically analyzed as per completely randomized design and randomized block design respectively using computer programme CPCS 1 (Cheema and Singh, 1990) and the significance of differences was tested at 5 per cent level.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Disease Survey

Surveys were conducted in February –March for two consecutive years i.e. 2011and 2012, to record the incidence and disease severity of Alternaria blight of carrot as well as for the collection of disease samples from carrot growing areas of Hoshiarpur and Ludhiana districts of the Punjab state. Different carrot cultivars *viz* PC-34, Selection 21, PC-43 and PC-35 were found to be cultivated in all the carrot growing areas of the state. The data on prevalence (incidence and disease severity) of Alternaria leaf blight of carrot at different locations were recorded and is presented in Table 1.

The perusal of the data revealed that disease was observed in all the locations and on all the cultivars in both the years, 2011 and 2012. The disease incidence and disease severity on different cultivars varied from 28 to 80 per cent and 11 to 45 per cent, respectively, during 2011 crop season at different locations. During 2012 crop season, disease incidence and disease severity varied from 32 to 85 and 10 to 55 per cent, respectively. The highest disease incidence of 80 and 85 per cent was recorded from Bohan on Local variety with disease severity of 45 and 55 per cent, during 2011 and 2012, respectively. The incidence and severity of disease on Selection-21, PC-43 was low as compared to other cultivars of carrot. There was only 28 and 32 per cent disease incidence on Selection 21 with disease severity of 12 and 15 per cent during 2011 and 2012 crop season, respectively. On cultivars PC-43 the disease incidence was 35 and 40 per cent with disease severity of 11 and 18 per cent at Research farm of Department of Vegetable Science PAU, Ludhiana during 2011 and 2012 crop seasons, respectively. The disease incidence was comparatively high in the Hoshiarpur district, where it varied from 35 to 85 per cent as compared to Ludhiana district, where it varied from 28 to 45 per cent. It was concluded that Alternaria blight incidence was high on Local varieties as compared to PAU recommended varieties of carrot and disease was most severe in Hoshiarpur.

The present investigations indicated variable disease incidence as well as disease severity at different places on different cultivars. It may be associated with prevalent environment and/or pathogen factors. Pryor *et al* (1994) recorded 62 per cent disease incidence of Alternaria blight in California. Soylu *et al* (2005*)* recorded 65-90 per cent disease incidence of Alternaria blight in Turkey. Roy (1969) tested eleven varieties against Alternaria blight and reported that the tested varieties were found to be susceptible to disease with varying degree of intensity.

Table 1: Prevalence of Alternaria blight on seed crop of carrot in Hoshiarpur and Ludhiana districts of Punjab state during 2011 and 2012

District	Location	Variety	Crop Season				Average After pooling data	
			2011		2012			
			Incidence (%)	Severity (%)	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)
Hoshairpur (Block Hoshiarpur-11)	Mallmajara	Local variety	62	26	75	35	68.5	30.5
	Jattpura	Local variety	40	15	50	18	45	16.5
	Kodla	Local variety	68	25	60	22	64	23.5
	Chabbewal	Local variety	50	35	65	42	57.5	38.5
	Bohan	Local variety	80	45	85	55	82.5	50
	Bichoi	Local variety	55	28	68	35	61.5	31.5
	Chaggaran	Local variety	65	23	60	20	62.5	21.5
	Hariyan Wellan	Local variety	60	18	65	20	62.5	19
	Bohan Patti	Local variety	75	30	85	40	80	35
	Mallmajara	Local variety	60	22	70	35	65	28.5
	Nasran	Local variety	35	15	45	20	40	17.5
Ludhiana	Research Farm of Department of Vegetable Science	PC-34	35	15	40	17	37.5	16
		Selection- 21	28	12	32	15	30	13.5
		PC-43	35	11	40	18	37.5	14.5
		PC-35	45	20	33	10	39	15
	Field area of Department of Plant Pathology	PC-34	42	18	45	25	43.5	21.5

4.2 Symptoms

Symptoms were observed on leaves, stems and umbels (Plate I). Under field conditions, on leaves symptoms appeared as dark brown to black lesions on lower leaves starting from margins and becoming irregular in shape with advancement of the disease. Initially yellow halo surrounding the lesions was observed. Such lesions also develop on petioles leading to death of the entire leaflets, whereas on stem, black coloured necrotic lesions were formed. On umbels, symptoms were observed as large black lesions which start developing near the basal end of the inflorescence. These lesions generally increase in size under favorable environmental conditions and ultimately affect the seed quality and yield. Such symptoms were also observed by Maude (1966), Strandberg (1992), Coles and Wicks (2003).

4.3 Isolation of the causal pathogen

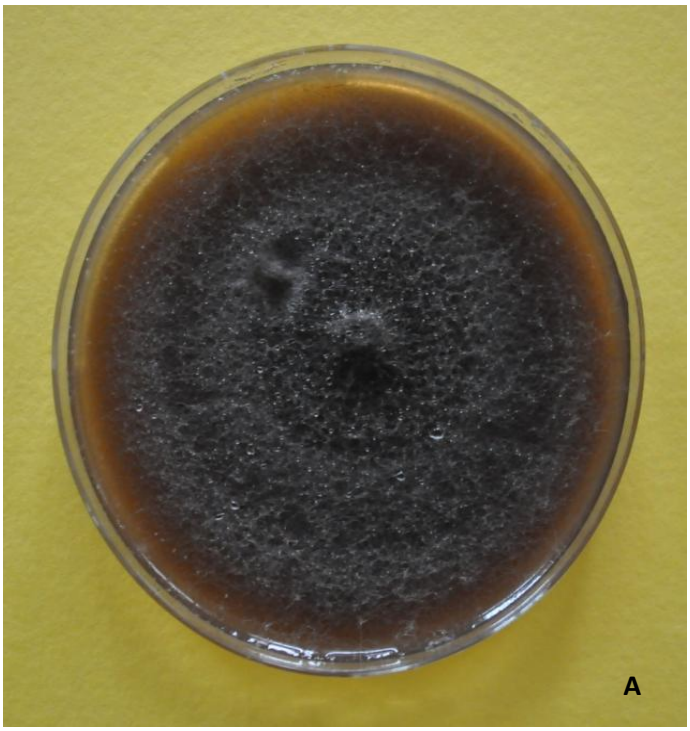
Isolations made from diseased leaves, stems and umbels invariably yielded cultures of *Alternaria radicina* (Plate II). However, at some locations isolations made from diseased parts also yielded cultures of *Alternaria dauci*. The data on the frequency of occurrence of *Alternaria radicina* and *Alternaria dauci* isolated from diseased samples collected from different locations are presented in Table 2. The frequency of occurrence of *Alternaria radicina* varied from 80 to 100 per cent while for *Alternaria dauci* it varied from 0 to 20 per cent. *Alternaria radicina* was predominantly isolated from all the samples collected from Hoshiarpur and Ludhiana districts. The frequency of occurrence of *Alternaria radicina* from leaves varied from 80 to 94 per cent, from carrot stem it varied from 92 to 100 per cent while from umbels it varied from 90 to 100 per cent. Likewise, frequency of occurrence of *Alternaria dauci* from leaves varied from 6 to 20 per cent and from stems it varied from 0 to 8 per cent while from umbels it varied from 0 to 10 per cent (Table 2). The frequency of occurrence of *Alternaria dauci* in terms of per cent was very low than the *Alternaria radicina* from Ludhiana and Hoshiarpur districts of Punjab. *Alternaria dauci* was mainly isolated from diseased leaves. However, it was completely absent in isolations made from umbels except at Chaggaran and Chabbewal in Hoshairpur district, where 6 per cent and 10 per cent of the isolations yielded cultures of *Alternaria dauci* respectively. The highest frequency of occurrence of *Alternaria dauci* was observed from leaf samples collected from Mallmjara (20%) followed by Chaggaran (16%) and Bohan (12%).

4.3 (a) Identification of the causal organism

It produces septate hyphae 2.0 to 6.9 µm wide with clear constrictions at the septa which form a grayish bluish black mycelium. Conidia produced at the tip of conidiophores that arise from hyphae, were 200 µm long × 3-9 µm wide, rarely branched, being mostly unbranched, septate, and straight, flexuous and coloured pale to olivaceous brown. Conidia

Plate I: Typical symptoms of Alternaria blight of carrot

A. **On leaves**
B. **On stems**
C. **Umbels**



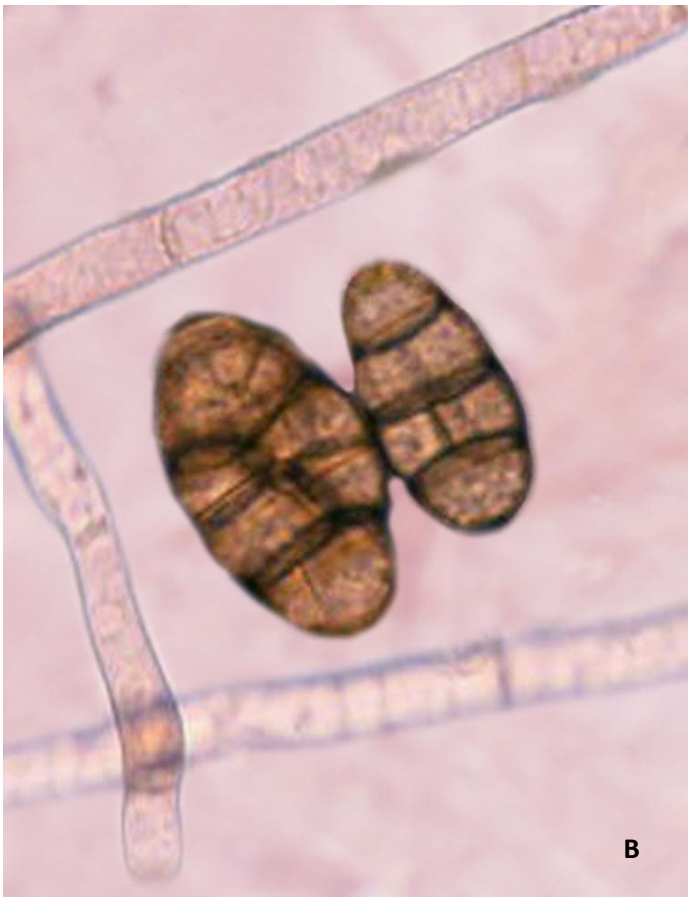


Plate II: ***Alternaria radicina***

A. **Culture of** ***Alternaria radicina*** **on Carrot Leaf Agar medium**
B. **Conidia of** ***Alternaria radicina***

Table 2: Frequency of occurrence of *Alternaria radicina* and *Alternaria dauci* from diseased samples collected at different locations of Hoshiarpur and Ludhiana districts of Punjab state

District	Location	Isolations from	Frequency of occurrence (%)	
			Alternaria dauci	*Alternaria radicina*
Hoshairpur Block (Hoshiarpur -II)	Bohan	Leaves	12	88
		Stems	2	98
		Umbels	0	100
	Jattpura	Leaves	8	92
		Stems	4	96
		Umbels	0	100
	Chaggaran	Leaves	16	84
		Stems	0	100
		Umbels	6	94
	Kodla	Leaves	6	94
		Stems	0	100
		Umbels	0	100
	Chabbewal	Leaves	12	88
		Stems	6	94
		Umbels	10	90
	Bichoi	Leaves	18	82
		Stems	5	95
		Umbels	0	100
	Mallmjara	Leaves	20	80
		Stems	6	94
		Umbels	0	100
Ludhiana	Research farm of department of Vegetable crop science	Leaves	15	85
		Stems	6	96
		Umbels	0	100
	Field area of department of Plant Pathology	Leaves	17	83
		Stems	8	92
		Umbels	0	100

being produced singly or in a short chain, light to dark olive brown in colour, having a clavate, ellipsoid, obovoid or turbinate shape, measuring 34-51×10-22 µm, having 3-8 transverse and one or more longitudinal septa, that divide one or all segments, but not the apical or basal cell, and always with constriction at the septa.

The conidia having 3-7 transverse septa and one or several longitudinal septa, with a length of 27-57 (average 38) µm and a width of 9-27 (average 19) µm. Recently Saude and Hausbeck (2006) recorded measurements of the mature conidia as 35-45×15-18 µm, with 3-8 transverse and 1-4 longitudinal septa.

The present studies indicated for the first time association of both *Alternaria radicina* and *Alternaria dauci* with the Alternaria blight of carrot at different locations in Hoshiarpur and Ludhiana districts, but *Alternaria radicina* is predominant pathogen under Punjab conditions. Similar observations were recorded by Farrar *et al* (2004) and Pryor *et al* (1994). They also reported the association of *Alternaria radicina* along with *Alternaria dauci* for causing Alternaria blight of carrot. Roy (1969) reported Alternaria blight of carrot caused by *Alternaria dauci*, first time in Shillong during May, 1964 in India. Pryor *et al* (1994) recorded 62 per cent disease incidence of Alternaria blight on carrot in California due to *Alternaria radicina.*

The average frequency of occurrence of *Alternaria radicina* and *Alternaria dauci* from different host parts is given in the Table 3. The data revelaed that the frequency of occurrence of *Alternaria radicina* on leaf was 86.2 per cent, on stem 96.2 per cent and on umbel 97.7 per cent, whereas in case of *Alternaria dauci* it was 13.8 per cent on leaf, 3.8 per cent on stem and 2.3 per cent on umbel (Table 3). The present study revealed that *Alternaria radicina* has high frequency of occurrence on all parts of host plant than *Alternaria dauci*. Coles and Wicks (2003) reported that *Alternaria radicina* attacked carrots at all stages and affects leaves, petioles and maturing carrots. Its frequency of occurrence was 47 per cent from seedlings and 88 per cent from mature plants. Gyu and Mathur (2006) reported that *Alternaria radicina* had 25.8-70.5 per cent frequency of occurrence on seed samples, whereas *Alternaria dauci* had 0.5- 7.5 per cent only.

Table 3: Average frequency of occurrence of *Alternaria radicina* and *Alternaria dauci* from different host parts

Host Part	Frequency of occurrence (%)	
	Alternaria dauci	***Alternaria radicina***
Leaf	13.8	86.2
Stem	3.8	96.2
Umbel	2.3	97.7

The pathogenicity of *Alternaria radicina* was proved by inoculating 30 days old plants of cultivar PC-34 under laboratory conditions. The symptoms developed after 7 days of inoculation at 25±1°C.

4.4 Cultural studies on the pathogen

Since *Alternaria radicina* was the major pathogen associated with diseased carrot plants at all the locations so further studies were conducted using *A. radicina* only.

4.4.1 Effect of different substrates on the radial growth of *Alternaria radicina*

Alternaria radicina was grown on different solid media viz, potato dextrose agar, carrot leaf agar, umbel extract agar, Czapeck's dox agar, oat meal agar, Richard agar and corn meal agar to observe the best solid medium for the maintenance of culture and further studies. The data in terms of radial growth recorded after 48 hours is presented in Table 4 and Fig. 1.

The data presented in Table 4 revealed that the mean radial growth of the pathogen was found to be significantly different on all the media. The maximum radial growth after 240 hours of inoculation was observed on (Plate III) carrot leaf agar (80.2 mm), followed by umbel extract agar (58.8 mm), PDA (53.3 mm), Richard agar (51.8 mm), corn meal agar (48.0 mm), oat meal agar (39.6 mm) and Czapeck's dox agar (38.2 mm) Fig 1. Carrot leaf agar was found to be the best medium for the growth of the *Alternaria radicina* fungus. Strandberg (2002) reported that carrot leaf agar was the best medium for *Alternaria radicina* colony growth. Shahin and Shepard (1979) reported carrot leaf agar as best medium for the *Alternaria dauci* growth. Roy (1969) reported that Vitamin Rich media supported the maximum colony growth of *Alternaria dauci* followed by PDA.

4.4.2 Effect of different substrates on the extent of sporulation

To determine the extent of sporulation of *Alternaria radicina*, the data obtained with the help of haemocytometer in terms of spores/ml is presented in the Table 4.

Table 4: Effect of different media/substrate on colony growth and sporulation of *Alternaria radicina*

Media	Colony growth (mm) after incubation (hrs)					Mean	Sporulation ($\times 10^6$spores/ml)
	48	96	144	192	240		
Carrot leaf agar	15.6	25.5	40.2	58.3	80.2	43.9	4.6 (15.34)
Umbel extract agar	13.8	23.1	33.1	47.6	58.8	35.3	4.0 (15.21)
Potato dextrose agar	12.6	20.3	30.6	44.6	53.3	32.3	3.3 (15.00)
Oat meal agar	10.8	15.8	22.1	28.3	39.6	23.3	2.5 (14.72)
Corn meal agar	10.6	17.1	24.1	35.1	48.0	27.0	1.6 (14.30)
Richard agar	8.6	16.6	26.0	38.3	51.8	28.3	1.3 (14.09)
Czapeck's dox agar	6.7	12.8	18.6	25.8	38.2	20.44	1.0 (13.84)
Mean	11.2	18.7	27.8	39.7	52.8		

Values in parenthesis are sq. root transformed values
CD (p = 0.05)
Media = 3.4
Hours = 2.9
Media x Hours = 2.1
Sporulation =0.15

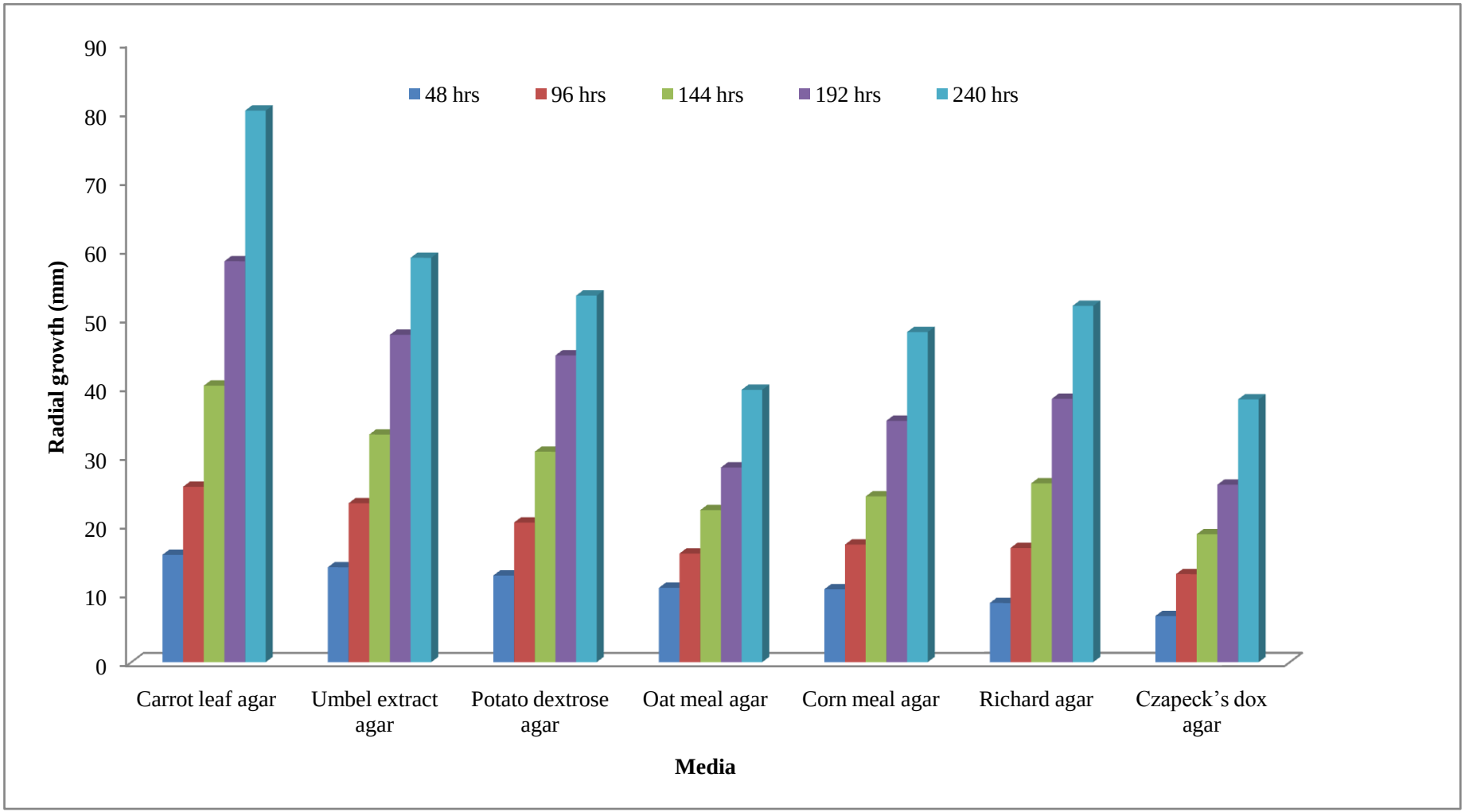


Fig.1: Effect of different media/substrate on colony growth of *Alternaria radicina*

The pathogen produces various extents of sporulation on different substrates. The highest sporulation was observed on carrot leaf agar (4.6 x 10^6 spores/ml) followed by umbel extract agar (4.0 x10^6 spores/ml), Potato dextrose agar (3.3 x 10^6 spores/ml), oat meal agar (2.5 x10^6 spores/ml), corn meal agar (1.6 x10^6 spores/ml), Richard agar (1.3 x10^6 spores/ml). The minimum sporulation was observed on Czapeck's dox agar (1.0 x10^6 spores/ml). From the data it is concluded that carrot leaf agar supported maximum sporulation as compared to other media (Table 4).

Strandberg (2002) reported that carrot leaf agar was best media for maximum sporulation of *Alternaria radicina*. Shahin and Shepard (1979) reported that sporulation in *Alternaria* spp on corn meal agar was considerably less than potato dextrose agar media. Strandberg (1987) reported carrot leaf agar is the best media for the growth and sporulation of *Alternaria dauci*. Carrot leaf agar medium provided good growth and abundant conidia, because this media supply factors that reduced the frequency of atypical conidia. This media also supplied factors that make requirements for light and temperature less stringent.

Since, Carrot leaf extract agar medium supported maximum radial growth and sporulation of the fungus, so it was selected as the basal medium for further cultural studies.

4.4.3 Effect of different temperatures on colony growth of *Alternaria radicina*

Petri plates lined with carrot leaf agar medium were inoculated with cultures of *Alternaria radicina* as described earlier. The inoculated petri plates were incubated separately at different temperatures of 10°, 15°, 20°, 25°, 30° and 35°C in BOD incubators.

The observations recorded on colony diameter after every 48 hours are presented in Table 5 and Fig. 2. It was observed that colony diameter remained higher during all the observations at 25°C (Plate IV). The colony diameter was maximum (80.6 mm) after 240 hours at 25°C and it was closely followed by colony growth of 72.3 mm at 30°C. On other hand the minimum colony diameter of 19.7 mm was recorded at 10^0 C after 240 hours. At temperature 35°C the pathogen did not produce any colony growth. The present findings showed maximum colony growth of the pathogen at 25°C, which was closely followed by at 30°C. Strandberg (1987) and Pryor *et al* (1998) also reported that maximum colony growth of *Alternaria radicina* was at 25°C on carrot leaf agar medium.

4.4.4 Effect of different temperatures on the extent of sporulation

To determine the extent of sporulation of *Alternaria radicina*, the data obtained with the help of haemocytometer in terms of spores/ml is presented in the Table 5. It is evident that the pathogen produced various extents of sporulation on Carrot leaf agar. The highest sporulation was observed at a temperature of 25°C (5.5 x 10^6) followed by 30°C (3.6 x 10^6) and 20°C (3.5 x 10^6). Minimum sporulation of 2.4 x 10^6 was observed at temperature 15°C. No sporulation was observed at temperature 10°C and 35°C.

Table 5: Effect of different Temperatures on colony growth and sporulation of *Alternaria radicina*

Temp (°C)	Colony growth (mm) after incubation (hrs)					Mean	Sporulation (×10^6spores/ml
	48	96	144	192	240		
10	6.8	10.8	14.8	17.5	19.7	13.9	0 (0)
15	11.0	15.8	24.6	30.1	36.1	23.5	2.4 (14.70)
20	14.8	25.0	42.0	52.0	61.8	39.1	3.5 (15.07)
25	18.8	32.0	49.1	64.8	80.6	49.1	5.5 (15.52)
30	15.8	28.3	44.8	58.6	72.3	44.0	3.6 (15.10)
35	0	0	0	0	0	0	0 (0)
Mean	11.5	18.8	29.0	37.0	45.0		

Values in parenthesis are sq. root transformed values
CD (p = 0.05)
Temp = 1.10
Hour = 1.01
Temp x Hour = 2.47
Sporulation = 0.62

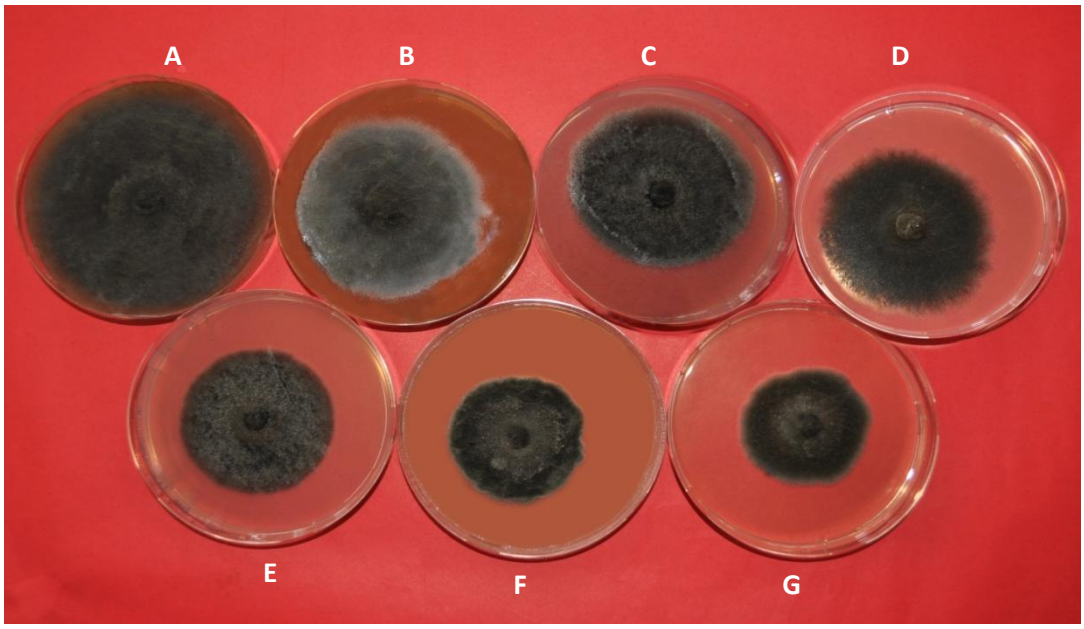


Plate III: Effect of different media/substrate on colony growth

A = Carrot leaf extract agar
B= Umbel extract agar
C= Potato dextrose agar
D = Richard agar
E = Corn meak agar
F = Oat meal agar
G = Czapeck's dox agar

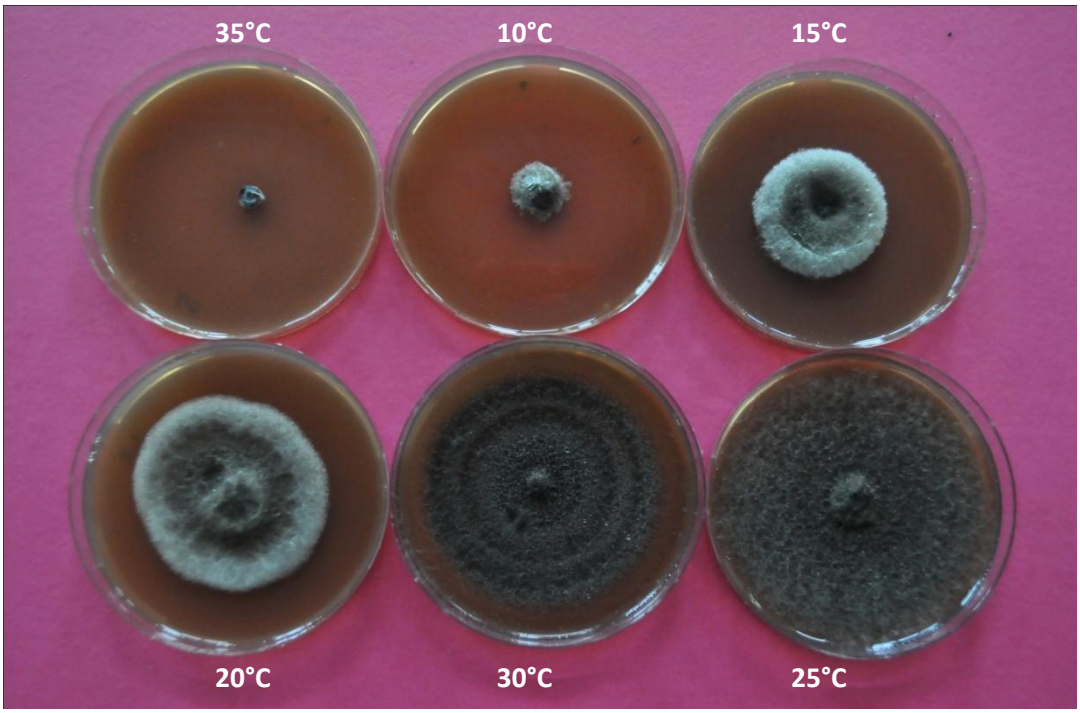


Plate IV: Effect of different temperature regimes on colony growth

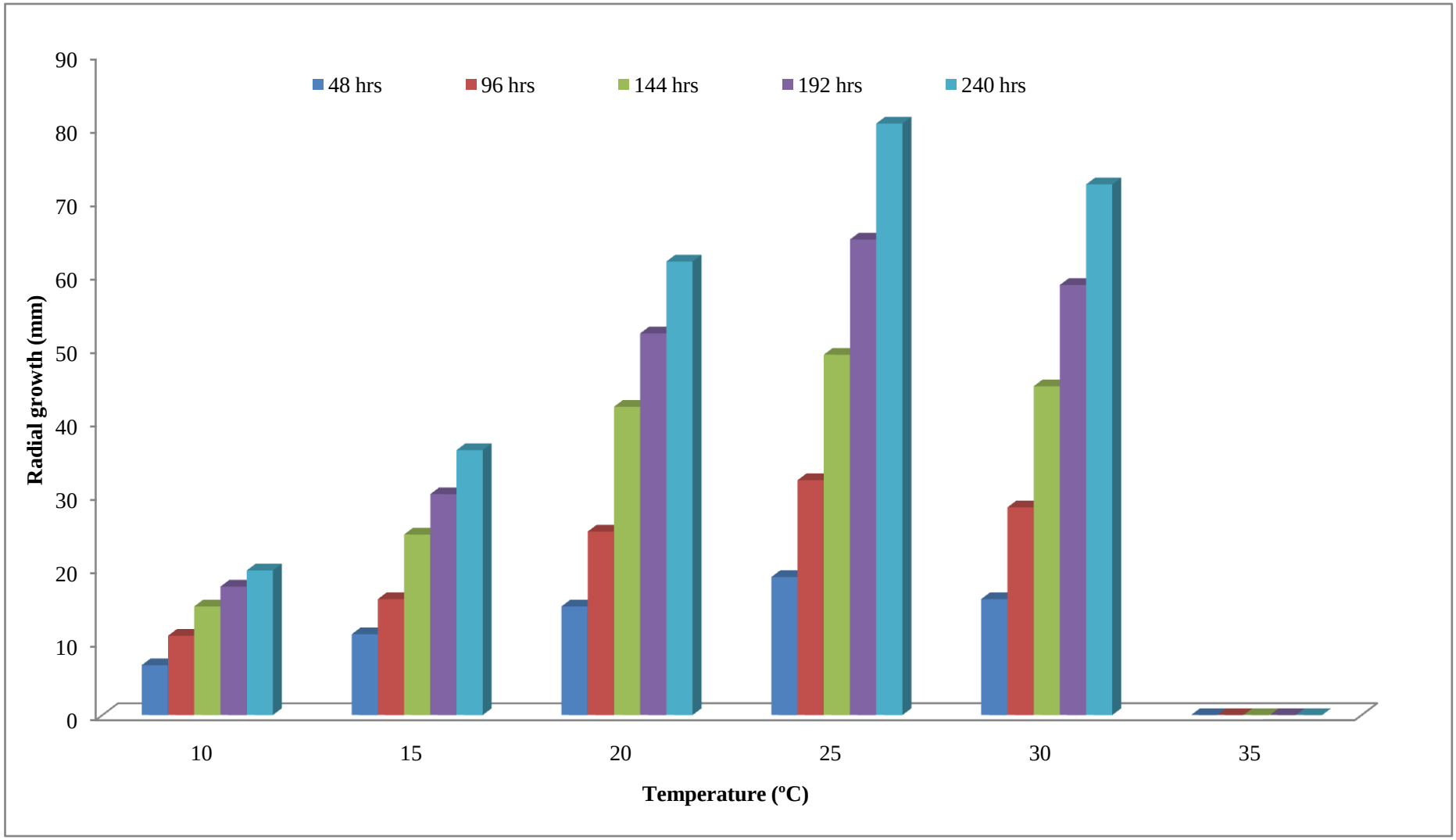


Fig.2: Effect of different Temperatures on colony growth of *Alternaria radicina*

The present finding showed that maximum sporulation of the pathogen occurred at temperature of 25°C and pathogen failed to sporulate at 10 and 35°C. Similarly, Strandberg (1987) and Pryor *et al* (1998) reported that maximum sporulation of *Alternaria radicina* was at 25°C temperature

4.4.5 Effect of different pH levels on colony growth

The pH value of a medium has a marked effect on growth of the pathogen. Five different pH levels viz., 5, 6, 7, 8 and 9 were maintained to study their effect on the growth of the pathogen. The data on the average radial growth of the *Alternaria radicina* was recorded after 10 days of inoculation and is presented in the Table 6 and Fig 3.

It is evident from the Table 6 that the fungus showed a distinct increasing trend in growth from pH 5 to 6 and thereafter it decreased significantly. The pathogen grew best at pH 6 with maximum radial growth of 80.3mm after 240 hours of incubation. The second best pH level was 7 (Plate V) where the pathogen show colony growth of 70.4 mm. All the pH levels exhibited significantly different radial growth of *Alternaria radicina.* In general, the pH level from 6 to 7 was found more favouable for the growth of the pathogen. Similar observations made by Roy (1969) and Strandberg (2002) corroborates the present finding where colony growth of *Alternaria radicina* was maximum at pH of 6.5.

4.4.6 Effect of different pH levels on extent of sporulation

To determine the extent of sporulation of *Alternaria radicina*, the data obtained with the help of haemocytometer in terms of spores/ml is presented in the Table 6. The sporulation of the pathogen varies from 0.91 to 8.3 $x10^6$ /ml at different pH levels on Carrot leaf agar. The significantly highest sporulation was recorded at pH 7 (8.3 $x10^6$). The second best sporulation (6.2 x 10^6) was recorded at pH 6 which was significantly better than the rest of treatments. On other hand lowest sporulation was recorded at pH 5 ($0.91x10^6$).

The present finding showed that maximum sporulation of the pathogen occurred at pH 7. Strandberg (1987 and 2002) reported that maximum sporulation of *Alternaria radicina* was at a pH range of 6.7to 7.0. Likewise, maximum sporulation of *Alternaria alternata* has also been reported at pH 7 (Manjunath *et al* 2010).

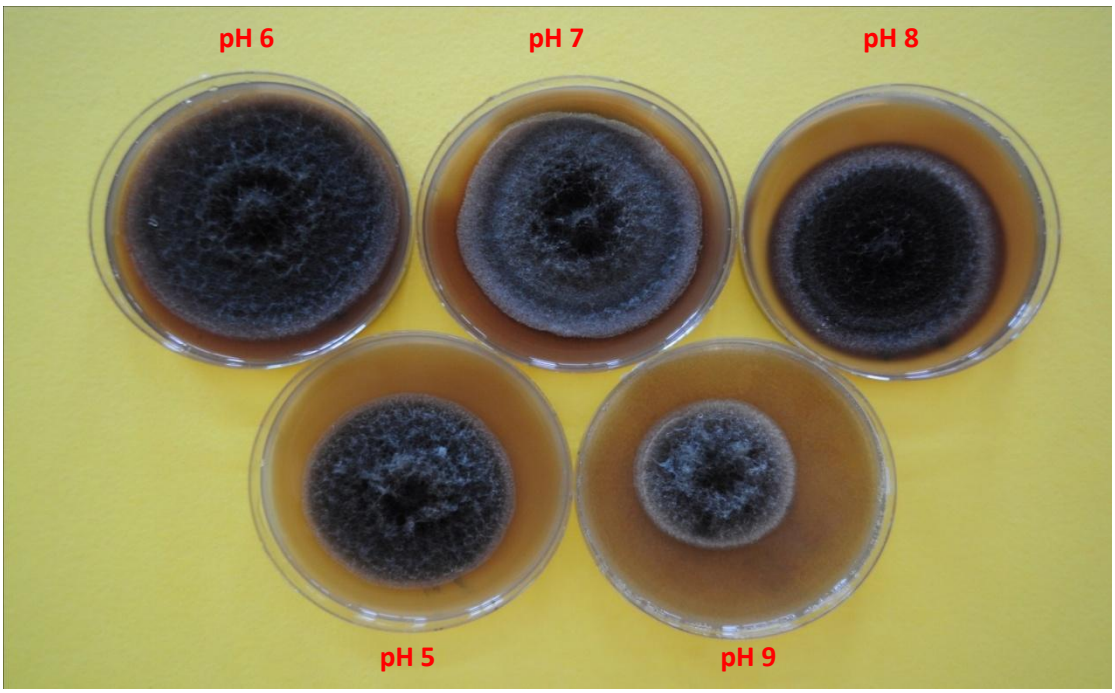


Plate V: Effect of different pH levels on mycelial growth of *Alternaria radicina*

Table 6: Effect of different pH levels on mycelial growth and sporulation of *Alternaria radicina*

pH	Colony growth (mm) after incubation (hrs)					Mean	Sporulation ($\times 10^6$)
	48	96	144	192	240		
5	11.0	21.3	28.8	39.3	46.3	29.3	0.91 (13.72)
6	18.0	32.1	48.1	63.4	80.3	48.4	6.2 (15.64)
7	14.5	29.0	39.8	57.2	70.4	42.2	8.3 (15.93)
8	11.8	27.3	37.0	47.0	61.7	36.9	3.6 (15.09)
9	6.4	13.0	16.5	23.6	27.5	17.4	3.1 (14.94)
Mean	12.3	24.5	34.0	46.1	57.2		

Values in parenthesis are sq. root transformed values
CD (p = 0.05)
pH = 1.02
Hour = 1.02
pH x Hour = 2.29
Sporulation == 0.18

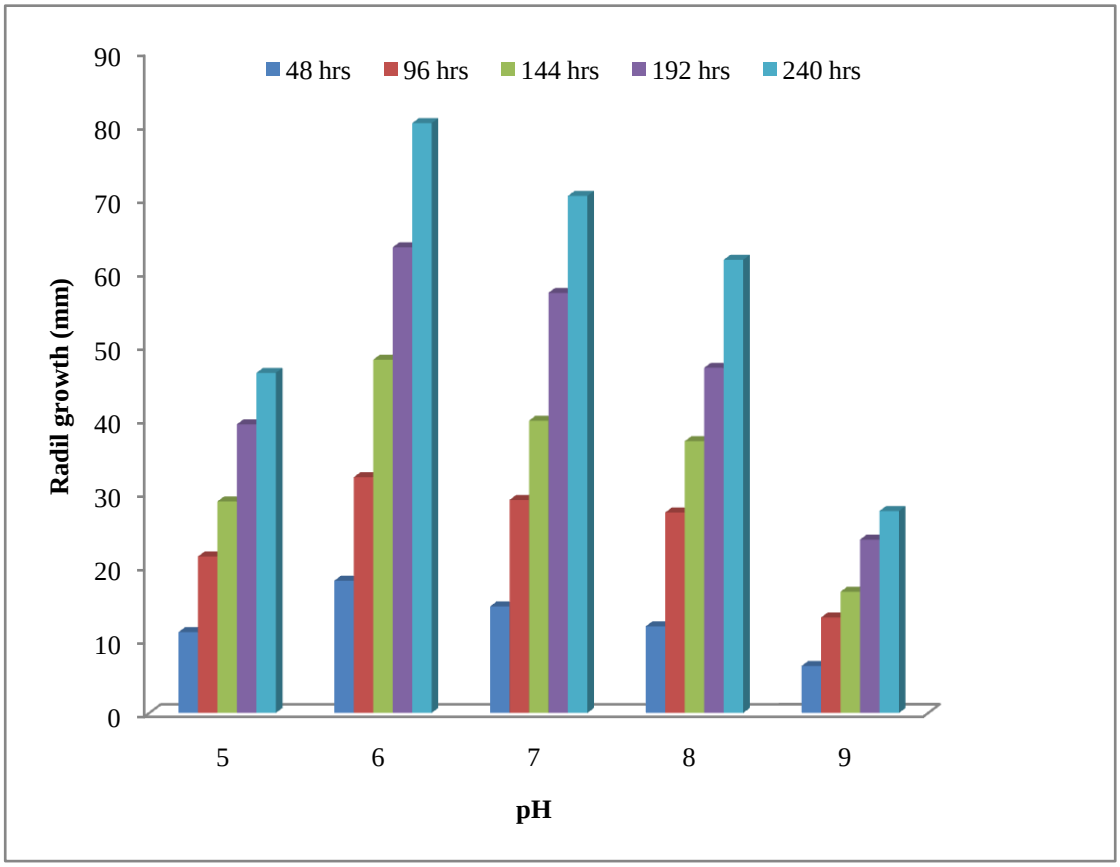


Fig.3: Effect of different pH levels on mycelial growth of *Alternaria radicina*

4.5 Pathological studies

4.5.1 Effect of leaf wetness duration on Alternaria blight of carrot

In order to observe the effect of duration of leaf wetness on incubation period and disease severity, 30 days old carrot steckling of cultivar PC-34 were provided with different leaf wetness durations of 0 to 48 hr after inoculations with mycelial and spore suspension of *Alternaria radicina* (Materials and Methods). The data presented in Table 7 exhibited that as the wetness duration increased disease severity also increased. No disease was recorded on carrot plants which were given leaf wetness durations of 0 and 4 hr after inoculations. A minimum of 8 hr of leaf wetness is required for infection and symptoms appeared within 7 days of incubation with disease severity of 2.1 per cent. Thereafter, the incubation period decreased while disease severity increased significantly with increase in leaf wetness from 8 to 48 hr and a positive correlation (r = 0.92) was observed between leaf wetness duration and disease severity. Strandberg (1988) reported that 4 to 6 hr of leaf wetness after inoculations was sufficient to initiate the disease and as the wetness duration increased up to 24 hr there was increase in disease index.

Table 7: Effect of leaf wetness period on incubation period and severity of Alternaria blight caused by *Alternaria radicina*

Leaf wetness (hrs)	Incubation period	Percent disease index (PDI)
0	-	0
4	-	0
8	7	2.1 (8.39)
12	7	3.4 (10.71)
16	7	6.3 (14.53)
18	6	10.7 (19.14)
24	6	15.2 (22.93)
48	6	18.23 (25.67)
CD(0.05)		0.41

Figures in parentheses are arc sine- transformed values
Correlation coefficient (r)
Leaf wetness and incubation period = (-0.49)
Leaf wetness and per cent disease Index = (0.92)

In the present investigations, it was found that a minimum 8 hr period of leaf wetness after inoculation was required to cause infection and with the increase in leaf wetness period up to 48 hr, there was increase in disease index.

4.5.2 Effect of Plant Age on Alternaria blight of Carrot

Plant age determines/influences the susceptibility or resistance of the plants to diseases. Therefore, an effort was made to study the effect of plant age on disease severity. The carrot plants of different age groups from 10 to 60 days old (after transplanting of steckling) were inoculated with mycelial and spore suspension of *Alternaria radicina* as described in materials and methods. The data on incubation period (initial symptoms) and disease severity was recorded after 10 days of inoculation are summarized in Table 8.

Table 8: Effect of plant age on severity of Alternaria blight caused by *Alternaria radicina*

Plant age (Days)	Incubation period (Days)	Percent disease index (PDI)
10	7	2.6 (7.6)
20	7	6.0 (14.6)
30	7	14.1 (22.4)
40	6	21.3 (27.4)
50	6	30.6 (33.6)
60	6	36.0 (36.8)
CD (p = 0.05)		3.9

Figures in parentheses are arc sine- transformed values
Correlation coefficient (r)
Plant age and incubation period = (-0.88)
Plant age and percent disease Index = (0.79)

The perusal of the data revealed that as the age of the plants increased, per cent disease severity also increased. There was significant positive correlation of r = 0.79 between plant age and disease severity, However, there was negative correlation between age and incubation period where significant negative correlation of r = -0.88 was observed. The lowest disease severity of 2.6 per cent was observed on 10 days old plants, while it was highest (36 per cent) on 60 days old plants. The incubation period was 7 days up to 30 days (after transplanting of steckling) old plants which decreased to 6 days thereafter.

Strandberg (1988) reported that carrot plants at all age were susceptible to Alternaria blight. However, the severity of infection increased with age of the host (Moore and Thomas 1943). Younger leaves possessed higher levels of tannin (gallotanic acid), whereas older leaves did not contain tannin which is mainly responsible for resistance of pathogen on young leaves (Newton and Yarwood 1930). Likewise, incubation period decreased with increase in age of the plant and a negative correlation between plant growth stage and length of incubation period is reported (Castellanos *et al* 1989). In the present investigations also, it was found that plant disease index increased with increase in plant age and a positive correlation of 0.79 was recorded whereas a negative correlation of -0.88 was recorded between plant age and incubation period.

4.6 Evaluation of Fungicides

4.6.1 *In vitro*

Efficacy of three non-systemic and six systemic fungicides (given in Tables 9 and 10) was determined against *Alternaria radicina* following growth inhibition technique. Carrot leaf extract agar medium amended with test concentrations (on a.i. basis) of the fungicide was poured in the Petri plates and inoculated in the center with 5 mm mycelial disc of actively growing cultures of *Alternaria radicina.* The data on per cent inhibition in colony growth calculated on the basis of colony diameter observed after 10 days of incubation at 25±1°C is presented in the tables 9, Fig IV and 10, Fig V. The ED_{50} and ED_{90} values for each fungicide were depicted in the Table 11.

Among non-systemic fungicides chlorothalonil 75 WP (Kavach) , propineb 70 WP (Antracol) and macozeb (Indofil M-45) completely inhibited colony growth at 100µg/ml, whereas chlorothalonil 75 WP proved most effective than other non-systemic fungicides and it recorded 72.40 per cent inhibition in colony growth at 50 µg/ml (Plate VI). Colony growth inhibition at 50 µg/ml of propineb was 65.85 per cent. Mancozeb proved to be less effective with 56.90 per cent inhibition in colony growth at 50 µg/ml. All the three fungicides completely inhibited the colony growth at 100 µg/ml.

Among systemic fungicides difenconazole 25 EC (Score), propiconazole 25 EC (Tilt), tebuconazole 25 EC (Folicur) and azoxystrobin + difenconazole 325 SC (Amistar top) completely inhibited the colony growth at 25µg/ml. Difenconazole 25 EC proved the most effective as it inhibited 90.30 per cent colony growth at a concentration of 10 µg/ml (Plate VII). It was closely followed by propiconazole 25 EC where 85.48 per cent colony growth inhibition was recorded. In case of other fungicides colony growth inhibition varied from 57.51 to 77.60 per cent at 10µg/ml. At 25 µg/ml concentration difenconazole, propiconazole, tebuconazole and azoxystrobin+difenconazole completely inhibited the colony growth whereas in case hexaconazole and azoxystrobin, per cent colony growth was 89.8 and 74.4

per cent respectively (Table 10). Azoxystrobin proved less effective as it completely check the colony at higher concentration of 100 µg/ml.

ED_{50} and ED_{90} values of different fungicides determined after plotting per cent growth inhibition against fungicide concentrations revealed that ED_{50} values for difenconazole 25 EC and propiconazole 25 EC were less than 1µg/ml while for hexaconazole 5 EC and tebuconazole 25 EC it was 1.0 and 1.1 µg/ml respectively (Table11). Among non-systemic fungicides, ED_{50} values varied from 17µg/ml to 44 µg/ml. Among all the systemic and non-systemic fungicides ED_{90} value was the lowest (9.0 µg/ml) for difenconazole and it highest (97.0 µg/ml) for mancozeb.

The present investigations revealed that difenconazole followed by proiconazole were most effective and completely inhibited the growth of the pathogen at lower concentration ranging from 9.0 to 11.1 µg/ml. The efficacy of propiconazole (at 30 µg/ml) is completely inhibiting the colony growth of fungus has also been reported by Hardison (1976). Among non-systemic fungicides chlorothalonil and propineb has been found to be highly effective. Similar observations have also been reported by Kumar *et al* (2006).

Rogers and Stevenson (2010) studied *in vitro* response of three fungicides (azoxystrobin, chlorothalonil and boscalid) commonly used for Alternaria blight control of carrot. Inhibtion of conidial germination ranged from 0.01 to 0.37 µg/ml for azoxystrobin, 0.08 to 0.09 µg/ml for chlorothalonil and 0.09 to 0.59 µg/ml for boscalid. On average *Alternaria* species were more sensitive to chlorothalonil than to azoxystrobin and bascalid.

Table 9: *In vitro* evaluation of different contact fungicides against *Alternaria radicina*

Fungicide	**Per cent inhibition in colony growth at different concentration (µg/ml)**						
	10	**25**	**50**	**100**	**200**	**500**	**1000**
Mancozeb	10.89 (19.23)	26.54 (30.99)	56.90 (48.94)	100 (89.96)	100 (89.96)	100 (89.96)	100 (89.96)
Propineb 70 WP	26.00 (30.64)	54.54 (47.56)	65.85 (54.22)	100 (89.96)	100 (89.96)	100 (89.96)	100 (89.96)
Chlorothalonil 75 WP	40.64 (39.59)	60.36 (50.96)	72.40 (58.28)	100 (89.96)	100 (89.96)	100 (89.96)	100 (89.96)

Figures in Parentheses represent per cent growth inhibition in arc sine transformed valu**e**

C D (p = 0.05)

Fungicide	=	0.269
Concentration	=	0.411
Fungicide x Concentration	=	0.713

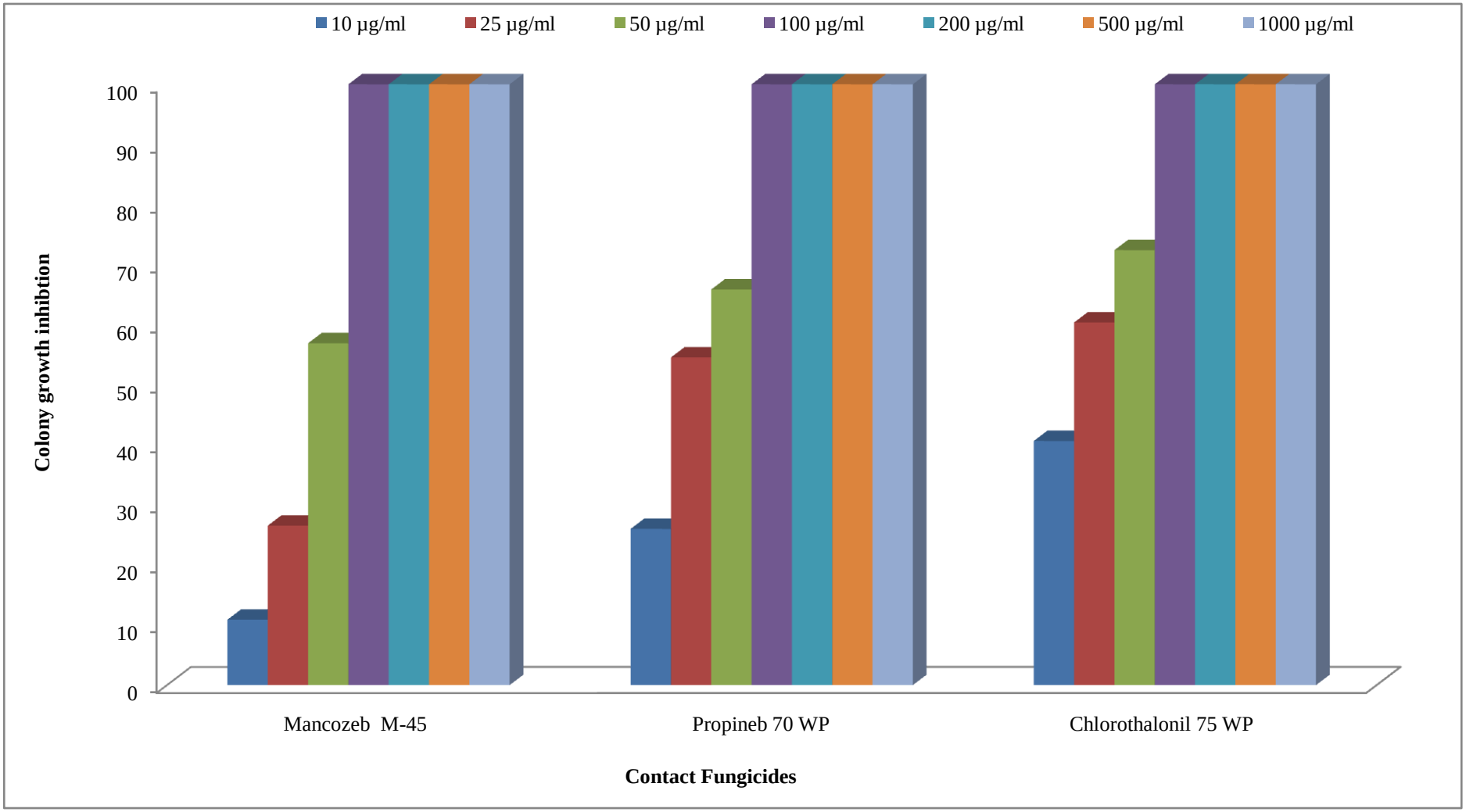


Fig.4: *In vitro* evaluation of different contact fungicides against *Alternaria radicina*

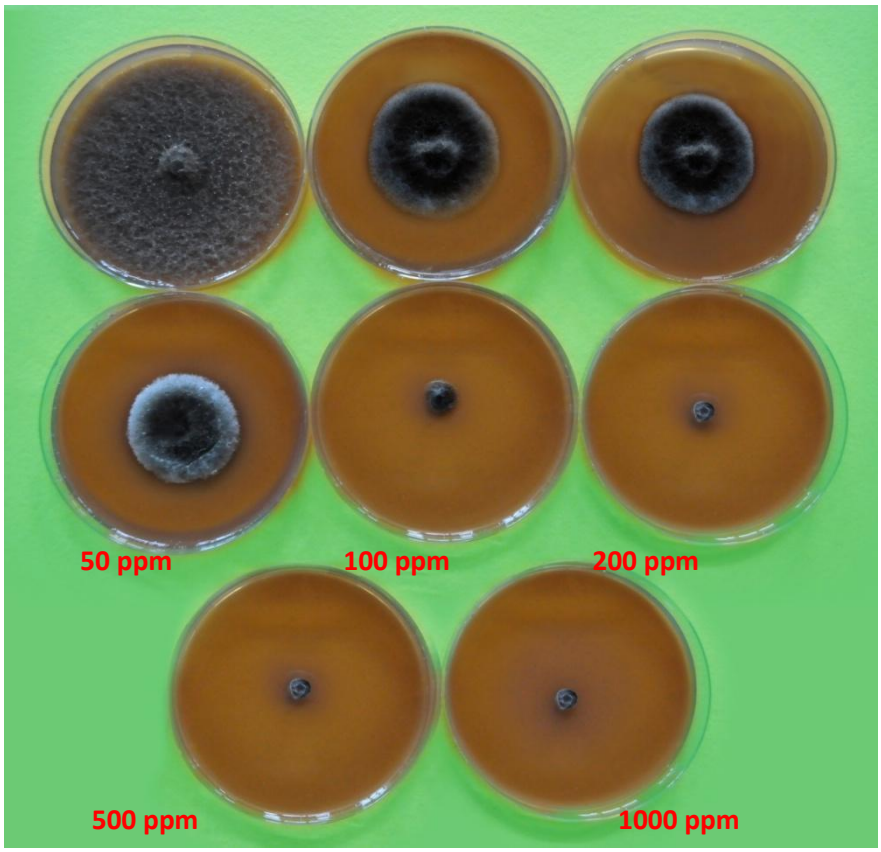


Plate VI: ***In vitro*** **evaluation of Chlorothalonil (Kavach) against** ***Alternaria radicina*** **at different concentrations**

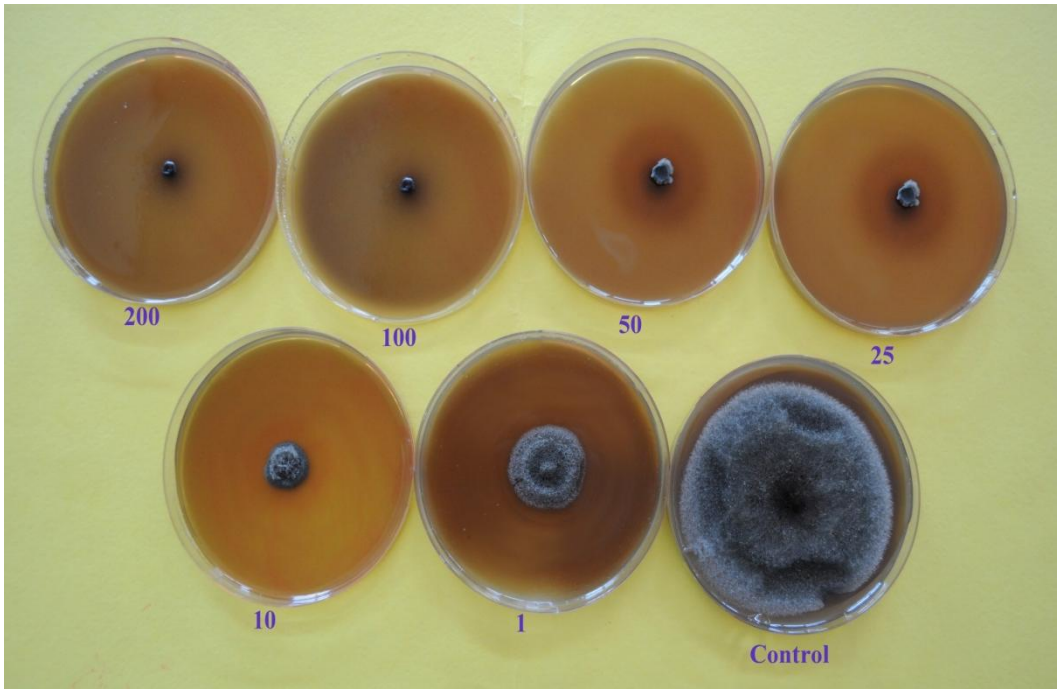


Plate VII: ***In vitro*** **evaluation of Difenconazole (Score 25EC) against** ***Alternaria radicina*** **at different concentrations**

Table 10: *In vitro* evaluation of different systemic fungicides against *Alternaria radicina*

Fungicide	Per cent inhibition in colony growth at different conc (µg/ml)					
	1	**10**	**25**	**50**	**100**	**200**
Difenconazole 25 EC	67.63 (55.30)	90.30 (71.85)	100 (89.96)	100 (89.96)	100 (89.96)	100 (89.96)
Propiconazole 25 EC	63.86 (53.02)	85.48 (61.46)	100 (89.96)	100 (89.96)	100 (89.96)	100 (89.96)
Hexaconazole 5 EC	59.83 (50.65)	77.60 (61.72)	89.83 (71.38)	100 (89.96)	100 (89.96)	100 (89.96)
Tebuconazole 25 EC	58.20 (49.69)	70.15 (57.09)	100 (89.96)	100 (89.96)	100 (89.96)	100 (89.96)
Azoxystrobin + Difenconazole 325 SC	56.09 (48.47)	72.11 (58.12)	100 (89.96)	100 (89.96)	100 (89.96)	100 (89.96)
Azoxystrobin 250 SC	40.24 (39.35)	57.51 (49.29)	74.43 (59.60)	87.23 (69.03)	100 (89.96)	100 (89.96)

Figures in parentheses represent growth inhibition in are sine transformed value
C D (p = 0.05)
Fungicide = 0.206
Concentration = 0.206
Fungicide x Concentration = 0.506

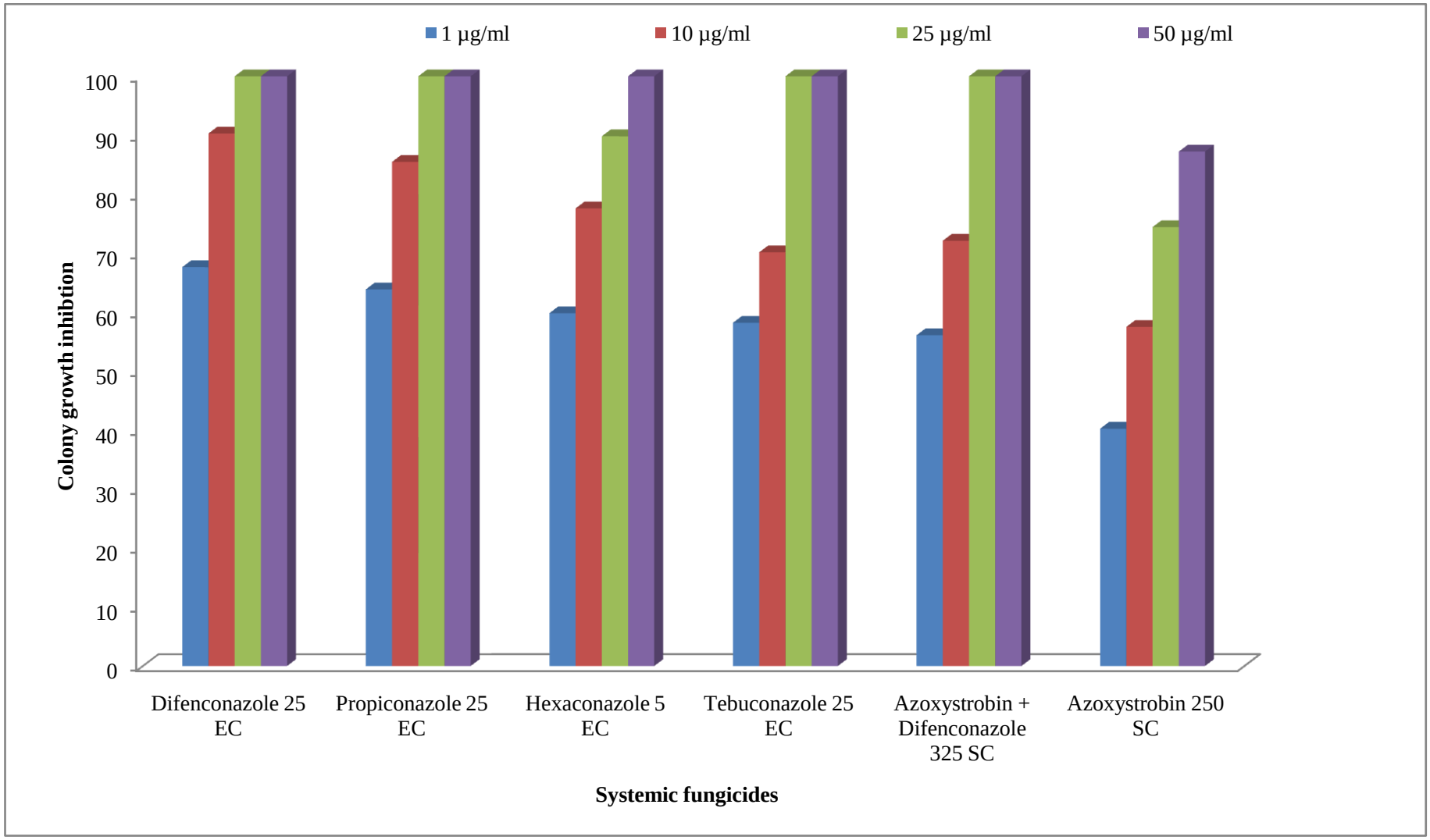


Fig.5: *In vitro* evaluation of different systemic fungicides against *Alternaria radicina*

Table 11: ED_{50} and ED_{90} values of different fungicides against *Alternaria radicina* causing Alternaria blight of carrot

Fungicide	**ED_{50}**	**ED_{90}**
Mancozeb	44	97
Propineb 70 WP	23	87
Chlorothalonil 75 WP	17	77
Difenconazole 25 EC	0.3	9
Propiconazole 25 EC	0.4	11.1
Hexaconazole 5 EC	1.0	11.4
Tebuconazole 250 EC	1.1	12
Azoxystrobin + Difenconazole 325 SC	2.3	18
Azoxystrobin 250SC	5.9	30.8

4.6.2 *In vivo*

Field performance of fungicides was tested on carrot cultivar PC-34 at the research farm of Department of Plant Pathology, PAU, Ludhiana during 2011-12 crop season. Nine fungicides at different concentrations were tested against Alternaria blight of carrot. First protective spray of fungicides was given 24 hours before inoculations with subsequent 2 sprays at 15 days interval. The data on per cent disease severity of Alternaria blight recorded 10 days after each spray is presented in Tables 12 and 13.

The perusal of the data presented in the Table 12 and 13 indicated that all the fungicide treatments were significantly superior to untreated control in reducing Alternaria blight and increasing the seed yield during 2011 and 2012 crop season. Systemic fungicides were more effective as compared to non-systemic fungicides.

During both the years azoxystrobin + difenconazole 325 SC @ 0.1 per cent was found to be more effective in reducing the disease severity (4.05 and 4.78 per cent) which was followed by azoxystrobin 250 SC @ 0.1 per cent (4.60 and 5.40 per cent) and tebuconazole 25 EC @ 0.1 per cent (6.05 and 6.99 per cent) compared with disease severity of 23.09 and 24.93 per cent in untreated control during 2011 and 2012 crop season. Highest disease severity of 14.42 and 15.61 per cent was recorded in case of mancozeb @ 0.25 per cent. It was followed by disease severity of 12.95 and 14.12 per cent in propineb 70 WP (Tables 12 and 13). All the treatments significantly improved the seed yield in comparison to control. Maximum yield of 4.34 and 4.20 q/hac was recorded in azoxystrobin + difenconazole 325 SC @0.1 per cent followed by 4.17 and 3.90 q/hac in Azoxystrobin 250 SC @ 0.1 per cent as compared to untreated control where only 1.18 and 1.00 q/ha seed yield was recorded during 2011 and 2012 crop season, respectively.

Table 12: Efficacy of different fungicides against Alternaria blight of carrot under field conditions during 2010-2011 crop season

S. No.	Treatments	Conc (%)	Average disease severity (%)			Seed yield q/ ha
			After Ist spray	After IInd spray	Mean	
1	Azoxystrobin + Difenconazole 325 SC	0.1	3.34	4.77	4.05	4.34
2.	Azoxystrobin 250 SC	0.1	4.13	5.07	4.60	4.17
3	Difenconazole 25 EC	0.1	6.19	7.91	7.05	3.10
4	Tebuconazole 250 EC	0.1	5.30	6.80	6.05	2.96
5	Propiconazole 25 EC	0.1	6.50	8.32	7.41	2.83
6	Hexaconazole 5 EC	0.1	7.23	8.16	7.69	2.64
7	Chlorothalonil 75 WP	0.25	9.00	13.22	11.11	2.21
8	Propineb 70 WP	0.25	10.33	15.58	12.95	1.97
9	Mancozeb M-45	0.25	11.23	17.62	14.42	1.68
10	Control		17.01	29.17	23.09	1.18
CD (p=0.05)			0.23	0.21	0.31	0.17

Table 13: Efficacy of different fungicides against Alternaria blight of carrot under field conditions during 2011-2012 crop season

S. No.	Treatments	Conc (%)	Average disease severity (%)			Seed yield q/ ha
			After Ist spray	After IInd spray	Mean	
1	Azoxystrobin + Difenconazole 325 SC	0.1	4.44	4.77	4.78	4.20
2	Azoxystrobin 250 SC	0.1	4.88	5.07	5.40	3.90
3	Difenconazole 25 EC	0.1	6.71	7.91	7.39	2.92
4	Tebuconazole 250 EC	0.1	6.09	6.80	6.99	2.81
5	Propiconazole 25 EC	0.1	7.64	8.32	8.64	2.70
6	Hexaconazole 5 EC	0.1	8.26	8.16	9.77	2.39
7	Chlorothalonil 75 WP	0.25	10.15	13.22	12.22	2.10
8	Propineb 70 WP	0.25	11.50	15.58	14.12	1.70
9	Mancozeb	0.25	12.63	19.20	15.61	1.52
10	Control		18.03	29.17	24.93	1.00
CD (p=0.05)			0.23	0.25	0.41	0.24

Fallas *et al* (1992) reported that the disease can be managed under field conditions by conventional fungicides like Blitox (0.25%) or Dithane M-45 (0.25%). However, difenoconazole, an Ergosterol Biosynthesis Inhibtors @ 125 g/ha when applied at 21 days intervals provided excellent control of the Alternaria blight of carrot in comparison to mancozeb. The present investigations also revealed high efficacy of systemic fungicides like difenconazole, azoxystrobin (alone or in combination), tebuconazole and propiconazole as compared to non-systemic fungicides like mancozeb, propineb and chlorothalonil. Ben-Noon *et al* (2001) also reported that azoxystrobin and difenconazole provided better control of this disease as compared to traditional fungicides like copper oxychloride and sulphur. Copper hydroxide, tebuconazole, trifloxystrobin and mancozeb were less effective whereas flutrifol, propineb and iprodione were least effective. Elsewhere foliar application of fungicides like difenoconazole, chlorothalonil, iprodione (Farrar *et al* 2004, Saude and Hausbeck 2006), azoxystrobin (Farrar *et al* 2004) have been reported to be effective against *A. radicina*. High efficacy of score (difenconazole) in managing Alternaria blight of carrot has also been demonstrated in Russia (Alkseeva 2009). In case of Alternaria blight of tomato Amistar 250 SC (azoxystrobin) has been reported to be highly effective in reducing the disease severity (Sidlauskiene 2001).

CHAPTER V

SUMMARY

Fungi of *Alternaria* genus are widely spread as pathogens on field, vegetables, ornamental and orchard plants and cause substantial yield losses in a broad range of host crop species (Strandberg 1992, Farrar *et al* 2004). Alternaria blight of carrot caused by *Alternaria radicina* and *Alternaria dauci* is one of the most common and destructive foliar disease of carrot. It causes 40-60 per cent yield losses. The pathogen attacks leaves, stems and umbels and produces dark brown to black irregularly shaped lesions on leaf blades. In present investigation epidemiology and management of *Alternaria radicina* has been studied.

Surveys conducted during February-March 2011 and 2012 revealed that disease was observed at all the locations (Hosiarpur and Ludhiana) on all the cultivars. Disease incidence and severity was highest on local variety grown in Hoshiarpur and lowest on Selection-21. Isolations made from diseased samples yielded cultures of *Alternaria radicina* and *Alternaria dauci* and their frequency of occurrence varied from 80 to 100 per cent and 0 to 20 per cent respectively. *Altrenaria radicina* isolated from all the diseased parts of the plant while *Alternaria dauci* was generally isolated from leaves and stems mainly. It has very low frequency of occurrence on umbels as compared to *Alternaria radicina*

Maximum colony growth and sporulation of *Alternaria radicina* was observed on carrot leaf extract agar medium and followed by umbel extract agar medium. Colony growth and sporulation was found to be maximum at 25±1°C temperature and closely followed by 30°C temperature. Minimum colony growth and no sporulation was observed at 10°C, whereas no colony growth and sporulation was observed at 35°C. Maximum colony growth was observed at pH 6, whereas maximum sporulation was observed at pH 7. Minimum colony growth was observed at pH 9, whereas minimum sporulation was observed at pH 5.

Carrot plants (after transplanting of steckling) at all age groups from 10 to 60 days old were susceptible to *Alternaria radicina.* The disease severity increased with increase in age of plants. There was positive correlation between plant age and disease severity while it was negative between plant age and incubation period. A minimum of 8 hr of leaf wetness period was required to cause infection and produce disease on carrot plants and thereafter disease severity increased with increase in the wetness period. A positive correlation was observed between leaf wetness duration and disease severity. There was significant increase in the disease severity as the leaf wetness duration increased from 24 to 48 hr.

All the three non-systemic fungicides proved less effective as compared to the systemic triazole and strobilurin fungicides. ED_{50} and ED_{90} value of different fungicides determined and it was revealed that ED_{50} value for difenconazole 25 EC and propiconazole 25

EC was less than 1µg/ml while for hexaconazole 5 EC, tebuconazole 250 EC, azoxystrobin + difenconazole 325 SC and azoxystrobin250SC was 1.0, 1.1, 2.3 and 5.9 respectively.

Among the non-systemic fungicides ED_{50} value for chlorothalonil 75 WP was 17, whereas for propineb 70 WP and mancozeb it was 23 and 44 respectively. ED_{90} value for difenconazole was 9, while it was 30.8 for azoxystrobin. ED_{90} value for chlorothalonil 75 WP was 77 whereas for propineb 70 WP and mancozeb it was 87 and 97 respectively.

The data on disease severity recorded after 10 days of the last spray indicated that all the fungicide treatments proved effective in controlling the disease to varying extents as compared to control in the field. All the systemic fungicides proved highly effective as compared to non-systemic fungicides. Azoxystrobin+difenconazole 325 SC proved to be most effective fungicide at 0.1 per cent concentration followed by Azoxystrobin 250 SC.

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