RVSKVV/Thesis/M.Sc. /No. 1655

Studies on anthracnose of chilli cause by *Colletotrichum capsici*

THESIS

Submitted to the

Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior

In partial fulfillment of the requirements for the Degree of

MASTER OF SCIENCE

In

AGRICULTURE

(PLANT PATHOLOGY)

By

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2018

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CERTIFICATE - I

This is to certify that the thesis entitled **"Studies on anthracnose of chilli cause by *Colletotrichum Capsici*"** submitted in partial fulfillment of the Degree of **MASTER OF SCIENCE IN AGRICULTURE (Plant Pathology)** of Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior is a record of the bonafide research work carried out by **Miss. BABITA RAIKWAL** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instruction.

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

july, 2018

Signature
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Chairman of the Advisory Committee

MEMBER OF STUDENT'S ADVISORY COMMITTEE

1. Chairman (Shri Ashish Bobade) : ----------------------------
2. Member (Dr. P. P. Shastry) : ----------------------------
3. Member (Shri S. K. Parsai) : ----------------------------

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CERTIFICATE – II

This is to certify that the thesis entitled **"Studies on anthracnose of chilli cause by *Colletotrichum Capsici*"** submitted by **Miss. BABITA RAIKWAL** to the **Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior**, in partial fulfillment of the requirements for the degree of **Master of Science in AGRICULTURE** in the **Department of Plant Pathology** has been accepted after evaluation by the external examiner and approved by the Student's Advisory Committee after oral examination.

Signature

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Chairman of Advisory Committee

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ACKNOWLEDGEMENT

I praise the God for enabling me to accomplish this great task of dissertation .I find no words to express my sincere feelings of gratitude towards my most esteemed guide and chairman of my advisory committee, ***Shri Ashish Bobade,*** *Assistant Professor Department of Plant Pathology, B. M. College of Agriculture, Khandwa, who deserves my most sincere thanks and respect for inspiring and excellent guidance, contact, encouragement, unceasing interest, constructive criticism and helping attitude throughout the investigation and preparation of the manuscript.*

I extend my sincere gratitude to other members of my advisory committee ***Dr. P. P. Shastry,*** *Professor Department of Plant Pathology and Dean of* B. M. *College of Agriculture, Khandwa and* ***Shri S. K. Parsai*** *(Principal Scientist) Department of Entomology, B. M. College of Agriculture, Khandwa, for their constant help ,suggestions and guidance during the research work.*

With profound respect, I wish to express my sincere gratitude to **Dr. S. K. Rao,** honorable Vice Chancellor RVSKVV Gwalior, **Dr. R .L.Rajput,** Director Instructions RVSKVV Gwalior, **Dr. B. S. Baghel,** Director Research Services RVSKVV and **Dr. P. P. Shastry** Dean, B. M. College of Agriculture, Khandwa, (M.P) for providing necessary facilities during the experiment.

I am also extremely thankful to **Shri Pravin Dhaske,** Lab technician, Department of Plant Pathology, *academic section and college Librarian* ***Smt. Arpana Modiraj*** for extending the help during my research work.

I thank to all my seniors and friends, P. Patidar, A. Ahirwar, Nirmla Mourya, V. jadhav, Divya Rai K. Mewada, Priyanka Uikey and and other, who helped me directly or indirectly during course of investigation.

I find no rhetorical gems from the ocean of word to express my pro-found feeling to most venerable mother Mrs. Sita bai, father Mr. Laljiram Raikwal and my brothers Manoj and Deepak who were always present in all of my good and the bad times. I also want to express my profound feelings to my loving sisters and family who has piloted me up to this stage and whose love, devotion, blessing and care throughout my life enabled me to achieve this seemingly invincible goal.

Place: Khandwa

Date: **(*Babita Raikwal)***

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LIST OF SYMBOL & ABBREVIATION

Symbol	Abbreviation	Stand for
@		At the rate of
%		Per cent
±		Plus or minus
%		Percentage
^{0}C		Degree centigrade
µ		Micron
	µm	Micrometer
	Avg.	Average
	WP	Wettable powder
	W / W	Weight by weight
	Spp.	Species
	Conc.	Concentration
	Sg	*Sclerospora graminicola*
	i.e.	That is
	viz.,	Namely
	et al.	Co- workers
	Fig.	Figure
	CD	Critical difference
	Cm	Centimeter
	Ha	Hectare
	Hrs	Hours
	Mt	Million tones
	Ml	Milliliter
	Mm	Millimeter
	G	Gram
	Mg	Milligram
	NS	Non significant
	SEm±	Standard error of mean
	Psi	Pound square inch
	Ppm	Parts per million
	Temp	Temperature

CHAPTER-I

INTRODUCTION

Chilli *(Capsicum annum L.)* is an important economic crop worldwide (Poulos, 1992) and *is* cultivated mainly in tropical and sub – tropical countries of the world. The red color in chilli is due to "Capsanthin". The pungency in chillies is due to "Capsaicin", an alkaloid which is extracted from chillies and is used for medicinal purpose. It belongs to the family solanaceae. Green chillies are rich source of vitamin A, C, B1 and B2 (Saimbhi *et al*., 1977; Sayed *and* Bhagvandas, 1980) and is also rich in vitamin P (rutin), which is of immense pharmaceutical importance.

Chilli was originated in the American tropics and has been propagated throughout the world including the tropics, subtropics, and temperate regions (Pickersgill, 1997). Chilli contains approximately 20-27 species, out of which five are domesticated namely *C. annum, C. baccatum, C. chinense, C. frutescens, and C. pubescens,* are cultivated in different parts of the world. Among the five species of cultivated *Capsicum, C. annum* is one of the most common cultivated crops worldwide (Tong and Bosland, 1999) followed by *C. frutescens* (Bosland and Votava, 2003).

India is the largest producer of chilli in the world accounting for over 45% of the total area under cultivation. Andhra Pradesh, Maharashtra, Karnataka, Orissa and Tamil Nadu account about 75% of the total area as well as production. In Madhya Pradesh chilli is grown in about 0.54mh area with 0.93mt annual production. It is an important cash crop of Nimar region of Madhya Pradesh. Madhya Pradesh ranks fourth in terms of chili production with the output making upto 9% of the country's production. Chilli is grown on 4,298 ha area with 22,349 ton annual production in Khandwa district (Anonymous, 2015).

Among the various fungal diseases of chilli, anthracnose is one of serious disease on chilli to cause the yield loss and to reduce the quality of marketable fruits (Manandhar *et al*., 1995). Disease incidence is recorded from 20 to 80% on fruits of *Capsicum annum* in the field conditions and post harvest losses of fruit quality ranges from 21 to 47% (Rajapakse *et al*., 2007).

Severely infected crop by anthracnose which may cause yield losses of up to 50% (Pakdeevaraporn *et al*., 2005).

Anthracnose derived from a Greek word “coal‘, which is common name for plant diseases characterized by very dark, sunken lesions,containing spores. Anthracnose of chilli was first reported from New Jersey, USA, by Halsted (1890) who described the causal agents as *Gloeopsorium piperatum* and *Colletotrichum nigrum.* Anthracnose disease can occur on leaves, stems, and both pre- and post-harvest fruits (Isaac, 1992). The management of this serious disease is one of the important tasks behind the farmers and researchers whereas little work is done by previous investigators but it is still required to search some more new molecules to manage the disease hence the present proposal of research under the objectives -

Objectives:

1. Survey of anthracnose disease of chilli in Burhanpur district
2. Isolation and purification of pathogen from collected samples.
3. To test the pathogenicity of collected isolated.
4. *In vitro* test in association with different fungicides
5. *In vivo* evaluation of agrochemicals against *Colletotrichum capsici*

CHAPTER – II
REVIEW OF LITERATURE

Study conducted under the title of "Studies on anthracnose of chilli cause by *Colletotrichum capsici"* at B. M. College of Agriculture, Khandwa (M. P.). the review of literature are present here under

The widespread occurrence and destructive nature of the pathogen caught attention of several scientists throughout the country. The disease has been studied and reports have been made from Andhra Pradesh (Rao and Rao, 1956). Doidge (1952) has recorded C*olletotrichum* on the genus *Cyamopsis* from South Africa has made no determination of species. In Papua, New Guinea, anthracnose infection rate ranged upto 17.2 per cent (Pearson *et al.,* 1984).

The seed-borne nature of *Colletotrichum capsici* has been demonstrated and reported by several workers [Kulshrestha *et al.* 1976, Grover and Bansal (1970), Suryanarana and Bhombe (1961), Adiver *et al.* (1987), Agrawal and Sinclair (1987), Kharkova *et al*. (1974), Sangchote and Junbhanich (1984) and Dhyani *et al.* (1990)].

Devi and Singh (1999) observed In November 1998, a severe anthracnose occurrence of chilli (*C. annuum*) at an experimental farm in Imphal, Manipur, India, causing 80% seedling mortality. The causal agent was identified as *C. gloeosporioides* [*Glomerella cingulata*] and its pathogenicity was confirmed.

A survey to assess the incidence of anthracnose of chilli in five locations in Rewa Province. The percentage incidence of anthracnose affected fruits under field conditions was more on green fruits which ranged from 55.53 to 71.10%. It was revealed the predominance presence of the anthracnose disease is the major constraints to profitable cultivation of chilli in Rewa region (Anamika *et al*. 2012).

Mathew *et al.* (1995) surveyed *C. annuum* in Vellanikkara, Trichur, Kerala, India, showed that Alternaria leaf blight ,die back and fruit rot (caused by *C. capsici*), were serious problems during the rainy season.

Ekbote (2002) conducted a survey of the prevalent diseases of chilli (*C. annuum*) in 6 talukas (Byadagi, Hirekerur, Haveri, Ranebennur, Savanur and

Shiggo) in the Haveri district of Karnataka, India was conducted from 1998-2000. Fruit rot caused by *C. capsici* was the most prevalent disease (36.4%) of chilli, followed by the Murda complex (34.56%), powdery mildew caused by *Levellula taurica* (17.54%) and leaf spot caused by *Cercospora capsici* (12.11%). Among the taluks surveyed, Ranebennur recorded the highest mean incidence of Murda complex (56.25%) and powdery mildew (24.50%), whereas Savanur recorded the highest mean incidence of fruit rot (42.00%) and leaf spot (15.00%).

Sharma *et al.* (2011) surveyed In 2007 and 2008, chilli and sweet pepper growing areas in Himachal Pradesh (India) for the prevalence of fruit rot/anthracnose disease caused by a complex of *Colletotrichum* species. Diseased fields were randomly sampled and 4 samples from each location were collected. Disease incidence ranged from 12.5-45.0% based on total plants assessed in the field. Disease symptoms included light brown, sunken lesions containing salmon-coloured masses of conidia and microsclerotia on the fruits. The causal pathogen was identified as *Colletotrichum coccodes* based on morphological, cultural, genetic (ITS) and pathogenicity tests. This is thought to be the first report of *C. coccodes* on *Capsicum* spp. in India.

Chigoziri and Ekefan (2013) conducted field survey in Gboko and Ohimini Local Government areas of Benue State where pepper is extensively cultivated to investigate the seed borne diseases of two Chilli pepper (*Capsicum frutescens*) types namely: 'Sombo' and Birdeye. Naturally infected pepper seeds were extracted from pepper fruits collected from farmers' fields between August and October, 2011 from the two local Government areas (LGAs). Using agar plate method a total of 20 genera and 36 species from three classes of fungi were isolated and identified from 800 seed samples of pepper. *Colletothrichum capsici, Aspergillus niger* and *A. flavus* were the most frequently isolated fungi with 54.75%, 44.00% and 29.75% occurrence respectively. *C. capsici and A. niger* interaction had the highest occurrence of 3% followed by *A. flavus and A. niger* interaction with 1.25%.

C. capsici (Sydo) Butler and Bisby causing anthracnose, die-back and fruit rot of chilli (*C. annum L*.). *C. capsici* was identified from various parts of Odisha according to their cultural, morphological characters and pathogenicity test and squash mount study under microscope (Ranasingh *et al*. 2013).

2.2 Taxonomy

Genus *Colletotrichum* was described by Corda in 1831. The conidia which are one celled, hyaline, ovoid to oblong are developed in acervuli on conidiophores with setae. In 1849, a closely related genus *Gloeosporium* having similar conidia in acervuli but devoid of setae was described by *Desmazieres* and *Montagne*. However, Shear and Wood (1907) showed by comparing cultures of *Gloeosporium musarum* Cooke and Massee, *Colletotrichum lagenarium* (Pass.) Ellis and Halst and *Colletotrichum atramenatrium* (Berk and Br.) Taub, that they are indistinguishable morphologically and that setae may be formed in some acervuli and absent in others in the same isolate. The genus was previously confused with *Vermicularia* established by Fries, for the fungi characterized with erumpent, setose, pulvinate stroma whose upper-most portion was differentiated as a palisade like layer of conidiophores bearing septate conidia. Duke (1928) studied the history of both the genera and examined Frie‘s material. He concluded that the two genera are the same, suggesting the retention of the name *Colletotrichum* because it is more widely known among plant pathologists as compared to *Vermicularia*. *Colletotrichum* and *Vermicularia* were merged in one by Duke (1928) and he regarded setae as the transitional to *Gloeosporium*. However, Ramkrishnan (1947) separated the two genera on the basis of setae formation and its essentialities. Marchionatto (1947) suggested the transfer of all species of *Vermicularia* and *Colletotrichum* with sub-epidermal or erumpent acervuli, fusiod conidia, *Colletotrichopsis* defending that Saccardo reserved *Colletotrichum* for those fungi having subepidermal acervulus with oblong, straight and blunt tipped conidia. He further added that fusoid, curved tipped conidia also disqualified to be included under *Vermicularia* and thus they could be referred to *Ellisiella* but *Ellisiella mutacae* of Saccardo has conidiophores with 2-3 acrogenous conidia with apices prolonged to filiform appendages and therefore *Vermicularia*, *Ellisiella* and *Colletotrichum* with fusiod, curved conidia should be referred to *Colletotrichopsis*. In 1918, Butler studied the saltation in *Colletotrichum capsici* and suggested it as polymorphic species constantly throwing new forms and to induce morphologically similar *Colletotrichum* under the same species. Butler and Bisby (1931) changed the name *V. capsici* to *Colletotrichum capsici.*

Arx (1957b) has made critical revision of *Colletotrichum.* He included the species of *Gloeosporium* in his studies and proposed a key for the identification of 23 species of *Colletotrichum* based on conidial morphology, presence or absence of chlamydospores, shape of acervuli, formation of appresoria on the host and in pure culture and host range (Arx, 1957 a, b).

In 1976, Kulshrestha and his co-workers have presented a critical identification concept of *Colletotrichum* species associated with seeds of 15 crops received from 12 countries in 95 samples, including *Capsicum annum*. Tiffany and Gilman (1954) studied the species of *Colletotrichum* attacking legumes and suggested identification of species and races on the basis of the shape of conidia. They divided the species into two groups, one with straight and another with curved conidia. According to them, specification should be based on cultural characters and host specificity. Prasad *et al.* (1967) made use of the two broad groups of Tiffany and Gilman (1954) and divided all the species of the genus into two master species that is *Colletotrichum dematium* (Pers, ex Fr.) Groove and *Colletotrichum gleosporium* Penz. The former includes species having curved conidia while the later has straight conidia. Further classification of these species into formae could be made on the basis of selective parasitism such as *Colletotrichum dematium* f.sp. *cyamopsicola* and *Colletotrichum gloeosporioides* f.sp. *dioscorea* attacking *Cyamopsis tetragonoloba* and *Dioscorea alata* L., respectively.

At least 19 generic names were proposed for anthracnose fungi during 1790-1960. Arx (1957a) reduced these species to 11 and Sutton (1973) accepted 51 species under two groups – straight and falcate spores. Morphological criteria dominated in delimitation of genera.

Later on, some excellent studies have been carried out in search of other stable criteria for the sub specific taxa. One of the most important criteria used to spore type is shape of appresorium and parasitism of the species with its host specificity (Sutton 1968, 1973).

2.3 Host Range

Gautam (2014) reported various plant diseases caused by *Colletotrichum* in India with special reference to present century (i.e. 2000-

2012). About 25 plant diseases were caused by different species of *Colletotrichum* namely, *C. gloeosporioides*, *C. capsici*, *C. falcatum*, *C. truncatum*, *C. sansevieriae*, *C. acutatum* and *C. coccodes*. The study showed that even a single species of *Colletotrichum* can affect multiple hosts.

Diseased fruit acts as a source of inoculum, allowing the disease to spread from plant to plant within the field (Roberts *et al*., 2001). Secondary cycles of anthracnose development during the growing season arise from spores produced on diseased fruit or leaves. Water splash or wind driven rain is required for dispersal of fungus spores or micro-sclerotia on soil particles .Wounds in fruit are not required for infection but wetness is essentially required for spore germination and infection (Ray, 2004). Alternate host include other solanaceous crop such as tomato, potato, egg plant (Roberts *et al*., 2001). Pring *et al*. (1995) studied the pathogenicity, host range and infection process of three isolates of *C. capsici* on cowpea (*Vigna ungiculata*), bean (*Phaseolus vulgaris*) and bettle vine (*Piper bettle*).

2.4 Symptomatology

Singh (1978) observed that the disease affected only ripe fruits turning red and small black circular spots appeared on the skin of the fruit which spreads in the direction of the long axis, becoming more or less elliptical. The disease is characterized by sunken spots of various colours on leaves, stems, fruits or flowers. These spots often enlarge, and lead to wilting, withering, and dying of infected plant tissues (Hiremath *et al.,* 1993). The typical anthracnose symptoms on chilli fruits include necrotic tissues, with concentric rings of acervuli. Fruits showing blemishes have reduced marketability (Manandhar *et al*., 1995). The disease appears as small, circular spots that coalesce to form large elliptical spots on fruits and leaves. Under severe conditions, defoliation of affected plant occurs (Kim *et al*., 2004).

The fungus, *C. capsici* infect the leaves, branches, green as well as ripe fruit. On the leaves small circular spots appear. Severely infected leaves fall off leading to defoliation. The infection of growing tips leads necrosis of branches which progress back ward on the diseased branches, a large no. of dots represent the presence of acervuli. The die back symptom is severe and it may kill the whole plant. (Kumar and Bhaskaran, 2007).

Fungal isolates from chilli (*Capsicum* spp.) fruits in Thailand that showed typical anthracnose symptoms were identified as *C. acutatum*, *C*.*capsici* and *C* .*gloeosporioides (*Than *et al*. 2008a). A mat of fungal hyphae covers the seeds. Such seeds turn rusty in colour. Affected fruits are deformed, white in colour and loose their pungency. Ultimately, the diseased fruit shrivels and dry up (Than *et al*., 2008b).

Colletotrichum is one of the major plant pathogenic genera responsible to anthracnose causes a plant disease on a variety of hosts from trees to grasses (Dean *et al.,* 2012).

2.5 Epidemiology

Conidia often do not germinate in situ because of the presence of germination inhibitors in the spore matrix, but will germinate after being washed or rain-splash disseminated (Manandhar *et al*., 1995). Under normal conditions, conidia dispersed by rainfall may remain on a plant surface and retain potential to cause disease for periods of over 7 days (Estrada *et al*., 1993). During wet periods, appressoria have been reported to produce secondary conidia, which may be involved in secondary spread to pepper fruits (Manandhar *et al*. 1995).

The disease is more likely to develop on mature fruits, although it can occur on immature fruits as well (Roberts *et al*., 2001). Pratibha *et al*. (2004) observed maximum disease intensity of fruit rot occurred when leaf surface during the morning was wetted with dew deposition and when the maximum temperature was 32.60C and minimum 190C. Severe losses occur during rainy weather because the spores are washed or splashed to other fruit resulting in more infections. Overhead irrigation will favour development of anthracnose because of increased relative humidity and increased during dew periods (Ray, 2004).

The pathogen requires warm and humid conditions to infect different plant hosts, including gymnosperm, angiosperms, ornamental and fruit plants, vegetables, crops or even grasses. As the primary inoculum is disseminated by wind or rain, the pathogen is cosmopolitan in distribution (Farr *et al.,* 2006). Most crops grown throughout the world are susceptible to one or multiple species of *Colletotrichum* (Weir *et al.,* 2012). The fungus prefers warm humid environment for spreading the anthracnose disease uniformly and effectively (Farr *et al.,* 2006).

The effect of relative humidity levels on conidial germination of *C. capsici* and *Leveillula taurica* and disease development in chilli (var. Parbhani Tejas). Results indicated that conidia of *C. capsici* and *L. taurica* could not germinate at 10% RH up to 48 hours of incubation. Maximum conidial germination of both these species took place at 100% RH followed by 75, 50 and 25% RH. Symptoms of *C capsici* on leaves were not observed at 10% RH and on fruits at 10 and 25% RH up to a fortnight. Incubation period was minimum at 100% RH and steadily increased as humidity levels decreased. Powdery mildew symptoms on leaves up to a fortnight were not observed at 10, 25 and 100% RH. These developed within a week's period at 50 and 75% RH (Hingole *et al*., 2011).

Darvin (2012) investigated the effect of environmental factors on chilli fruit rot infection, the surface sterilized, healthy chilli fruits artificially inoculated with conidial suspension (concentration of 106 conidia/ml) of *Colletotrichum capsici* and incubated at different temperatures (includes 15, 20, 25, 30, 35 and 40^{0}C), relative humidity levels (includes 75, 80, 85, 90, 95 and 100%) and light regimes (includes continuous light, continuous darkness, 18 h darkness followed by 6 h light period and18 h light followed by 6 h darkness period), respectively. Ten days after inoculation recorded the data on lesion size and per cent disease index (PDI). The highest lesion size (15.10 mm) and PDI (38.50) were recorded when the inoculated chilli fruits were incubated at temperature of 25^{0}C. Temperature beyond and below 25^{0}C caused significant reduction in both lesion size and per cent disease index (PDI). Among the four light regimes, 18 h light followed by 6 h dark period was optimum (recorded the highest lesion size (8.96 mm) and PDI (61.05)) for chilli fruit rot development. The inoculated chilli fruits incubated at relative humidity of 95 per cent reported the highest infection (lesion size (21.00 mm) and PDI (59.90)). When the relative humidity was decreased to 75%, the lesion size and per cent disease index showed a sharp decrease.

Singh (2013) reported that shortest period of 36 hours with temperature 25-30°C was most favorable for successful infection at red fruit wetness stage of susceptible chilli variety Pusa Jwala.

Kumar et al. (2014) determined the effect of weather parameters (maximum and minimum temperature, morning and evening relative humidity) on the development of fruit rot (*Colletotrichum capsici*) of chill cv. Pusa Jwala. There was a steep increase in the disease severity (6.86- 17.71%) during 16-28 September. The maximum number of spores (24/day) was trapped at temperature ranging from 22 to 31 ^{0}C with higher relative humidity. From the recorded data, it has been inferred that maximum temperature (30.6-35.9 ^{0}C), minimum temperature (21.7-26.5 ^{0}C), morning relative humidity (84.3-97.00%) and evening relative humidity (54.3-58.3%) were congenial for the disease development and spore production in the field.

Colletotrichum capsici was grown at nine different temperatures and 12 pH levels ranging from 5 to 40^{0}C and 3.5 to 10.0 pH, respectively. The optimum temperature and pH for growth of fungus were found to 28°C and 6.0, whereas, the minimum growth of fungus was recorded on the temperature of 5^{o}C and pH 10. Excellent sporulation was observed at 25-30^{o}C and pH level of 6.0-7.0 (Tripathi *et al.,* 2016).

Kommula *et al.* (2017) reported that temperature is most important physical, environmental factor in regulating the growth and reproduction of fungi. However, maximum growth and sporulation of fungus was recorded at 25^{o}C temperature and 6.5 pH.

2.6 Survey of anthracnose of chilli

Choudhury (1957) reported that this disease was quite serious and wide spread in Assam. The disease has been recorded from the state wherever chilli was grown resulting in a loss of 12 -30 per cent of the fruits.

Bansal and Grover (1969) during their studies on *Capsicum frutescens* L. reported that crop losses due to anthracnose disease ranged from 10-35 per cent in 1966 and 20-60 per cent during 1967 in six districts of Punjab and Haryana.

Thind and Jhooty (1985) reported that *C. capsici* was a predominant fungus in causing fruit rot of chilli. Its incidence varied between losses 66-84 per cent.

Paul and Behl (1990) reported that, the chilli suffers considerable losses due to fruit rot/dieback/anthracnose caused by *Colletotrichum capsici* in tropical and subtropical areas.

Howard *et al.* (1992) reported losses greater than 30 per cent in plant production in United Stated due to anthracnose.

Kannan *et al.* (1998) reported under suitable weather condition dieback and fruit rot caused by *Colletotrichum capsici* cause yield loss upto 12 to 15 per cent.

Verma and Sharma (1999) reported that fruit rot of chilli caused by *Colletotrichum capsici* (syd.) Buter and Bisby, was an important disease in field, transit, transport and storage.

Pandey and Pandey (2003) reported that yield losses due to anthracnose varied from 10–60% in different parts of India.

Bagri *et al.* (2004) reported that several diseases, particularly of fungal origin, attack the chilli crop. Among these, fruit rot, which caused 10-15 per cent losses to mature fruits during transit and storage in Udaipur.

Singh and Jameel Akhatar (2007) reported that chilli growing areas in Tarai belt of Uttaranchal Tarai (Sitarganj, Pantnagar,and Bilaspur) were surveyed during the crop season (2001-2002) and diseased plant parts showing anthracnose symptoms, particularly dieback and fruit rot were collected.

Poonpolgul and Kumphai (2007) reported that Thailand had encountered severe yield losses up to 80 per cent due to anthracnose (*Colletotrichum* spp.)

Sharma *et al.* (2011) surveyed In 2007 and 2008, chilli and sweet pepper growing areas in Himachal Pradesh(India) for the prevalence of fruit rot/anthracnose disease caused by a complex of Colletotrichum species. Diseased fields were randomly sampled and 4 samples from each location were collected. Disease incidence ranged from 12.5-45.0% based on total plants assessed in the field.

Chigoziri and Ekefan (2013) conducted field survey in Gboko and Ohimini Local Government areas of Benue State where pepper is extensively cultivated to investigate the seed borne diseases of two Chilli pepper (Capsicum frutescens) types namely: 'Sombo' and Birdeye. Naturally infected pepper seeds were extracted from pepper fruits collected from farmers' fields between August and October, 2011 from the two local Government areas (LGAs). Using agar plate method a total of 20 genera and 36 species from three classes of fungi were isolated and identified from 800 seed samples of pepper. Colletothrichum capsici, Aspergillus niger and A. flavus were the most frequently isolated fungi with 54.75%, 44.00% and 29.75% occurrence respectively. C. capsici and A. niger interaction had the highest occurrence of 3% followed by A. flavus and A. niger interaction with 1.25%.

Sattar *et al.* (2016) conducted field survey in five major chilli growing districts of Punjab province viz., Rawalpindi, Kasur, Vehari, Okara, Multan and Bahawal Nagar to access the disease incidence and severity. The mean disease incidence was highest in Kasur district (85.1%) followed by 81.83% in Vehari district. Of the five districts, minimum mean disease incidence was observed in Rawalpindi district (37%). The maximum disease severity (74.6%), measured in terms of fruit area infected from Kasur district followed by 72.83% from Vehari district and minimum severity was observed 35% in Rawalpindi district.

Koppad and Mesta (2017) conducted a roving survey to know the severity of *Colletotrichum capsici* in Belagavi, Dharwad, Gadag and Haveri districts during kharif /rabi 2013-14. The overall disease severity was ranged from 19.21 to 59.14 percent. The highest severity (59.14percent) of fruit rot was noticed in fields of Sankeshwar village in Belagavi district, while, least (19.21percent) incidence of the disease was recorded at Hulkoti village in Gadag district.

Yadav (2017) conducted a roving survey in last week of October from each visited location in Jaipur district and result revealed that overall 60.33 per cent fruit were found infected at surveyed four location of Jaipur district during 2015. Maximum per cent infected fruit (66.70%) was recorded from Chomu followed by Begus village (64.72%) and Kotputli (58.13%), while minimum per cent infected fruits (51.75%) was reported from Lalpura village.

2.7 Isolation and proving pathogenicity

Ramakrishna (1954) reported the pathogenicity of two isolates of *C. capsici* obtained from chilli fruits.

Rai and Chohan (1966) conducted pathogenicity test in chilli plants bearing 20 fruits by spraying with spore suspension of different isolates. The inoculated plants were placed in humid chamber. Observation on the per cent fruit rot caused by different isolates, number of days to produce symptoms, and intensity of spotting on the leaves were recorded.

Bansal and Grover (1969) obtained seven isolates from different localities of Punjab and Haryana and proved their pathogenicity on chilli verities in green house.

Kenchaiah (1975) proved the pathogenicity of the two isolates using both ripe and unripe fruits of *C. annum* and *C. fruitescens* and were pathogenic to their respective hosts and were cross inoculable.

Singh *et al.* (1977) isolated *C. capsici* from a severely infected chilli fruit and confirmed its pathogenicity experimentally.

Khaleeque and Khan (1991) proved pathogenicity of *C*. *capsici* in chilli fruits. Thind and Jhooty (1990) conducted the pathogenicity test on chilli by detached fruit method to and proved pathogenicity of *C*. *Capsici*.

Gomathi and Kannabiran (2000) reported that fruit samples of chillies showing typical fruit rot symptoms were collected from chilli growing fields around Pondicherry. Cultures were identified as *Gloeosporium piperatum* E11 and Ev and *C. capsici* (syd.) Buter and Bisby and tested for their pathogenicity.

Suthin Raj *et al.* (2006) as isolated pathogen from infected chilli fruits collected from the Chidambasam. The fungus was purified and identified as *C*. *capsici* by standard method. Chilli plants of 105 days old were used for inoculation. The plants kept in glass house were sprayed with sterile water followed by conidial suspension using atomizer in the late evening. The control plants sprayed with sterile distilled water. Periodical observations on fruit rot incidence were taken.

Intana *et al*. (2007) for pathogenicity test, each of eight isolates of *C. capsici* were cultured on PDA for 3 days. Then 0.7 cm agar plug contained with mycelia of *C. capsici* was placed on pierced area on chilli fruit (*Capsicum anuum* L. var. annuum) obtained from chilli plantations at Pak Phanang, Nakhon Si Thammarat province. All inoculated fruits were incubated in moist plastic chamber, kept at room temperature (27+1^{0}C). Disease severity of anthracnose infection was recorded at 5 days after incubation by measuring size of diseased lesion on chilli fruit.

Singh *et al.* (2007) collected plant part showing anthracnose symptoms, particularly dieback and fruit rot were collected and brought to laboratory for microscopic examination and isolation of pathogen. Purified cultures were tagged and tentitatively based on location of sample and proved *in vitro* pathogenicity on detached fruits of chilli cultivar ‘’Kandhari’’.

Roat *et al.* (2009) collected the infected fruits isolated the pathogen and identified. Fresh chilli fruit samples were surface sterilized and incubated for seven days and confirmed pathogenicity.

The isolates are used in the inoculation of chilli seedlings and fruits by the detached leaf assay procedure. (i) Conidial suspension (1 x 106conidia per ml) of twelve day old PDA grown cultures was sprayed on one-month-old chilli plants. (ii) The inoculated plants are covered with plastic bags for two days to maintain humidity. (iii) The plants were assayed for disease seven days after inoculation and continued to be so for up to 20 days; (iv) the presence of the pathogen was further confirmed by incubating the leaves in moist chambers for 5-7 days at 22 ± 1ºC and observed for the development of fungal growth.(Chandra Nayak *et al*., 2009)

Thirty-four isolates of *Colletotrichum* spp. including 2 species, *C. gloeosporioides* and *C. capsici*, from anthracnose on Bell pepper, pathogenicity tests divided pathogenic potential into low, medium and high virulence groups. It is clearly revealed that *C. capsici* from the three tested hosts expressed the highest virulent isolates. Cross-inoculation of three high virulent isolates of *C. capsici* in accordance with three chilli varieties showed that all isolates could produce anthracnose symptom in the same lesions. All tested isolates developed lesions after co-inoculation of all hosts (Kanchalika *et al.* 2010).

The pathogenicity of these isolates through different inoculation methods on detached chilli fruits variety Pusa Jawala revealed that *C. capsici* (Is2) was most virulent with an average lesion size of 10.95 mm. Evaluation of genotypes/varieties against *C. capsici* revealed that none was resistant, however, DC-4, Anka lohit, LCA-235, LCA-333, LCA-301 exhibited moderately resistant reaction under both field and pot culture conditions and minimum lesion size was also observed under detached method in these genotypes (Parey, 2013).

Rajamanickam and Sethuraman (2014) collected *Colletotrichum capsici* from different part of Tamil Nadu and assayed for their virulence, age of susceptibility of fruits and method of inoculation. The pathogen *C. capsici* isolate 1 caused the maximum fruit rot intensity of 72.27 per cent, while *C. capsici* isolate 9 showed least intensity of 2.93 per cent. The four methods of inoculation were tested under *in vitro* condition to assess the effectiveness of infection. Among the various methods of inoculation, Spray spore suspension after pinpricking the fruits method was enabled the maximum infection. Chilli fruits at six different ages *viz.*, 5, 10, 15, 20, 25 and 30 days were used to found the stage of susceptibility by the inoculation with virulent isolate of *C. capsici* under *in vitro*. Twenty five days old fruits were the most susceptible to the disease and ideal for artificial inoculation, while 5, 10, 15 and 20 days old green fruits were not infected by the pathogens.

Aiello *et al.* (2015) fulfilled Koch's postulates by pathogenicity tests carried out on fruit of ‘Tarocco Scirè’ and ‘Tarocco Nucellare’ with representative isolates of C. gloeosporioides and C. karstii. Field surveys and pathogenicity tests revealed significant differences in fruit susceptibility between ‘Tarocco Scirè’ and ‘Tarocco Nucellare’ and in virulence between the fungal species. To our knowledge, this is the first report on the emergence of Colletotrichum spp. causing anthracnose in preharvest conditions.

Moe *et al.* (2017) used forty cultivars to investigate the pathogenicity and to identify the possible source of resistance. The result revealed that all of chilli cultivars used in this studied susceptible to *C. scovillei*.

Sharma *et al.* (2017) investigated pathogenicity test and the result revealed that all *Colletotrichum* isolates from this study caused 100% disease incidence. *Colletotrichum aenigma* proved to be the most virulent pathogen of avocado in Israel with 92.6 ± 7.7% disease severity. The next two virulent pathogens were *C. alienum* and *C. theobromicola* with 90.10±6.70 and 88.90±3.70 %disease severity scores for other isolates were *C. siamense* (85.9 ± 4.3%), *C. fructicola* (85.2 ± 4.3%), *C. gloeosporioides* (82.7 ± 5.0%), *C. perseae* sp nov. (80.20±2.70%), *C. karstii* (67.90±6.50%) and *C. nupharicola* (63.00±14.70%).

2.8 Evaluation of fungicides on anthracnose of chilli under *in vitro* and *in vivo* condition

In vitro condition

Yadav *et al.,* (2014) tested the efficacy of Propiconazole (0.1%), Captan, (0.2%), Carbendazim (0.2%), Carboxin 37.5 per cent + Thiram 37.5 per cent (0.1%) and Thiram (0.2%) against anthracnose of chilli under *in vitro* condition. Among the fungicides, Propiconazole (100 %) gave maximum inhibition of the mycelial growth of pathogen followed by Captan (100%) and Thiram (93.45%). Least inhibition of mycelia growth was observed in Carbendazim (74.70 %).

Linu *et al.* (2017) evaluated three fungicides namely carbendazim (0.05%), mancozeb (0.2%) and azoxystrobin (0.1%) under *in vitro* by using poisoned food technique against *Colletotrichum capsici*. Among the three fungicides mancozeb (0.2 %) recorded 73.47 % inhibition followed by carbendazim (0.05%) which recorded 64.12% inhibition. The least inhibition (62.21%) was recorded in azoxystrobin (0.1%) treatment.

Gupta *et al.* (2018) evaluated Six fungicides against *C. capsici* under *in vitro* condition by adoption of Poisoned Food Technique with different concentration viz. 20, 50, 100, 250, 500 and 1000 µg/ml, respectively. Findings concluded that the minimum mycelial growth was recorded in 1000 µg/ml compared to rest of concentrations. Thiophanate methyl and Copper oxychloride were the best toxic fungicides inhibiting the mycelial growth *in vitro* at all the six concentrations. In fungicides, Thiophanate methyl inhibited highest mycelial growth, which was at par with Copper oxychloride.

In vivo condition

Kumawat (1997) reported the use of fungicides such as mancozeb, thiophanate methyl, Ziram, carbendazim for the control of anthracnose of chilli. Three sprays of mancozeb (0.25%) at 15 day intervals gave the highest reduction in disease incidence, followed by thiophanate methyl, ziram and carbendazim were reported by Malraja and Narayanswami (1988).

Yadav *et al.* (2014) conducted field experiment to evaluate the efficacy of Propiconazole (0.1%), Captan, (0.2%), Carbendazim (0.2%), Carboxin 37.5 per cent + Thiram 37.5 per cent (0.1%) and Thiram (0.2%) against anthracnose of chilli. Propiconazole was found most effective at 0.1 per cent concentration showing least percentage disease index of 20.32 per cent as against 62.15 per cent in control, followed by Vitavax power and Captan.

Jadon et al. (2015) tested hexazonazole, tebuconazole, propiconazole, difenoconazole, vitavax, carbendazim along with captan and mancozeb and various combinations as seed treated against diseases of groundnut in Gujarat at recommended doses. Among these, tebuconazole, mancozeb, propiconazole and carbendazim + mancozeb were found effective in managing soil borne diseases with apparent yield advantage over check.

Machenahalli et al. (2016) conducted an experiment on fruit rot and die back of chilli in Karnataka, India. There results indicated that adoptive module including seed treatment with Carboxin + Thiram (0.2%), seedling deep in Pseudomonas fluorescens (1%), spray with neem oil (1%), hexaconazole, propiconazole (0.1%) and carbendazim + mancozeb (0.2%) showed least seedling infection (7.06%), die-back incidence (1.21%) and severity (9.30 PDI) , fruit rot incidence (4.47%), severity (2.68 PDI) with high dry chilli yield (8.92 q/ha) and B:C ration (2.44)

Yadav (2017) investigated six fungicides were used to control the disease through foliar application in field conditions. All fungicides were able to reduce the disease intensity over control. Results of the study revealed that maximum reduction in disease (85.27%) with minimum per cent disease (7.93) and maximum fruit yield (8.91 q/ ha) were obtained with the application of propiconazole (0.1%) as foliar spray. This is followed by the application of hexaconazole (0.1%).

CHAPTER – III
MATERIAL AND METHODS

Present investigation was undertaken as study on anthracnose of chilli cause by *Colletotrichum Capsici.* The materials used and methods followed are described below:-

(A) Materials

1. General

The Borosil make glasswares were used throughout the course of study. Glasswares were cleaned with chromic acid solution followed by washing with detergent and finally rinsed with tap water. After drying, glasswares were sterilized in hot air oven at 180^{o}C for 2 hours. The metallic equipments like forceps, needle and cork borer were sterilized by dipping in alcohol and heating to red hot over flame of a spirit lamp.

Surface sterilization of plant parts and diseased materials were done by dipping then in 0.1% mercuric chloride solution for 1-2 minutes and washed in sterilized water for 3 times.

The culture media were sterilized in autoclave at 15 Ibs pressure per square inch (1.05 kg/cm^2) for 20 minutes. The soil and sand were sterilized at 30 Ibs pressure per square inch (3.1 kg/cm^2) for two hours.

1.1 Media

Following media were used in the course of investigation with following ingredients:

1.1.1 Potato Dextrose Agar (Riker and Riker, 1939)

Peeled and Sliced Potato	200 g
Dextrose	20 g
Agar	20 g
Distilled water	1000 ml

1.2 Source of seed and other materials

Chilli seeds and Fungicides were procured from Department of Plant Pathology, B. M. College of Agriculture, Khandwa (M.P.).

1.3 Survey

Survey of anthracnose disease was done at Burhanpur district of two block from each block five field were randomly selected. Diseased plant parts were collected in paper envelops and brought to the laboratory for isolation. From each selected field the observation on incidence of the disease was recorded.

1.4 Experimental site:

All the field experiments were conducted at the experimental field of sector of Plant Pathology, B. M. College of Agriculture, Khandwa during *kharif* season 2017-18.

(B) METHODS

2.1 Collection of diseased plant samples and symptomatology

Diseased chilli plants were collected from different locations of Burhanpur district of Madhya Pradesh. Samples were brought in the laboratory then symptoms were recorded in detail. The tissues of affected plants showing the symptom of anthracnose were examined under the microscope for the presence of the causal agent. The samples were dried and store in separate envalopes.

2.2 Isolation of pathogen from diseased samples

The diseased fruits of chilli showing the anthracnose symptoms were washed thoroughly with tap water and small pieces from infected fruit were cut with the help of sterilized blade. These pieces were surface sterilized with 0.1% mercuric chlorides ($HgCl_2$) solution for one minute followed by three changes in sterilized distilled water to remove trace of $HgCl_2$. The pieces of surface diseased fruits were transferred aseptically to petri plates containing PDA. Inoculated Petri plates were incubated at 25 ± 2^{0}C for seven days and examined at frequently intervals to see the growth of the fungus developing from different pieces.

2.3 Purification of pathogen

The fungus was further purified by single hyphal tip method. They are grown by inoculating in the centre of a plain agar plate. The fungus will spread out with its hyphal stands in search of nutrients. These hyphal strands could be located under low power of the microscope, and the isolated hyphal tips marked. These tips are carefully transferred to potato dextrose agar slants. The bacteria could also be more easily eliminated by making the agar medium acidic to about PH 4 to 5.

2.4 Identification of pathogen

The cultures of *Colletotrichum capsici* were purified by sub-culturing the single hyphal tip method and maintained by mass transfer on potato dextrose agar medium at room temperature. After purification these isolates of *Colletotrichum capsici* were identified by observing the colony against light with the naked eyes and later confirmed with the help of microscope.

2.5 Pathogenicity assay

Pathogenicity test were conducted during the course which methods described by Montri *et al.* (2009) and Mongkolporn *et al.* (2010). Healthy, ripe red and green chilli fruits were surface sterilised in 1 % NaClO for 5 min separately and washed twice with sterile-distilled water, then air dried on sterile filter paper. Each fruit was inoculated with 1 µL of a conidial suspension (1 × 106 conidial/mL), which was injected onto the non-wounded fruit surface using a microsyringe. Control fruits were treated with 1 µL of distilled water. Each isolate was inoculated to five replicate fruits. The inoculated fruits were kept under high humidity by blotter papers. They were kept at room temperature and observed frequently for development of symptoms.

Leaves of chilli selected for inoculation, were placed inside each petri plate. Leaves were sprayed with culture suspension of *C. capsici* with the help of atomizer. Petri plates with inoculated leaves were kept under high humidity by blotter papers. They were kept at room temperature and observed frequently for development of symptoms.

2.6 *In vitro* evaluation of fungicides against *Colletotrichum capsici*

Six fungicides were evaluated in laboratory against *C. capsici* by poisoned food techniques (Nene and thapliyal, 1979). The details of the fungicides used in the present study are summarized in Table-1.

Table-1: Fungicide with their coined, trade and chemical name

S.No	Common name	Trade name	Formulation	Chemical name
1	Mancozeb	**Dithane M-45**	75%WP	[[1,2-thanediylbis[carbamodithioato]] (2-)]manganese mixture with [[1,2-ethanediylbis [carbamodithioato]] (2-)] zinc
2	Copper oxychloride	**Blue copper 50**	50%WP	Dicopper chloride trihydroxide
3	Propiconazole	**Bumper**	25%EC	1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1,2,4-triazole
4	Metalaxyl	**Apron**	35 WS	2-[(2,6-dimethylphenyl)-(2-methoxy-1-oxoethyl) amino] propanoic acid methyl ester
5	Hexaconazole	**Trigger**	5%SC	2-(2,4-dichlorophenyl)-1-(1H-1,2,4-trizol-1-yl)hexan-2-ol
6	Tricyclazole	**Logik**	75%WP	5-methyl-1,2,4-triazolo[3,4-b][1,3]benzothiazole

Three replications were kept for recommended dose. Required quantity of the fungicide was added in PDA and thoroughly mixed at the time of pouring. Twenty ml of medium was poured in each pre-sterilized 9.0 cm diameter petri plate. A five mm disc of eight days old culture of *C. capsici* was placed on the medium in upside down position to maintain a continuous contact of fungus with poisoned medium. The medium without fungicide served as control. The radial growth of the colony was measured after seven days growth under different fungicides and control was calculated.

2.7 *In vivo* evaluation of agrochemicals against *Colletotrichum capsici*

A field trial was conducted to find out more effective and suitable chemical treatment to manage the disease with seven treatments, which was replicated three times and evaluated with RBD design. The observations were made on disease intensity and infection rate.

Details of experiment:

Crop - Chilli

Design - RBD

Replications - 3

Treatments - 7

Observations to be recorded: 1. Disease intensity

2. Infection rate

Treatments:

Symbols	Treatment	Formulation
T1	Mancozeb	75%WP
T2	Copper Oxychloride	50%WP
T3	Propiconazole	25%EC
T4	Metalaxyl	35%WS
T5	Hexaconazole	5%SC
T6	Tricyclazole	75%WP
T7	Control	

Statistical analysis

The data were subjected to statistical analysis after arc sin transformation. The data converted into percentage were transformed to angular values. The difference between the two means was computed by critical difference at 5% probability level. The CRD and RBD design were apply for the analysis of the experimental data.

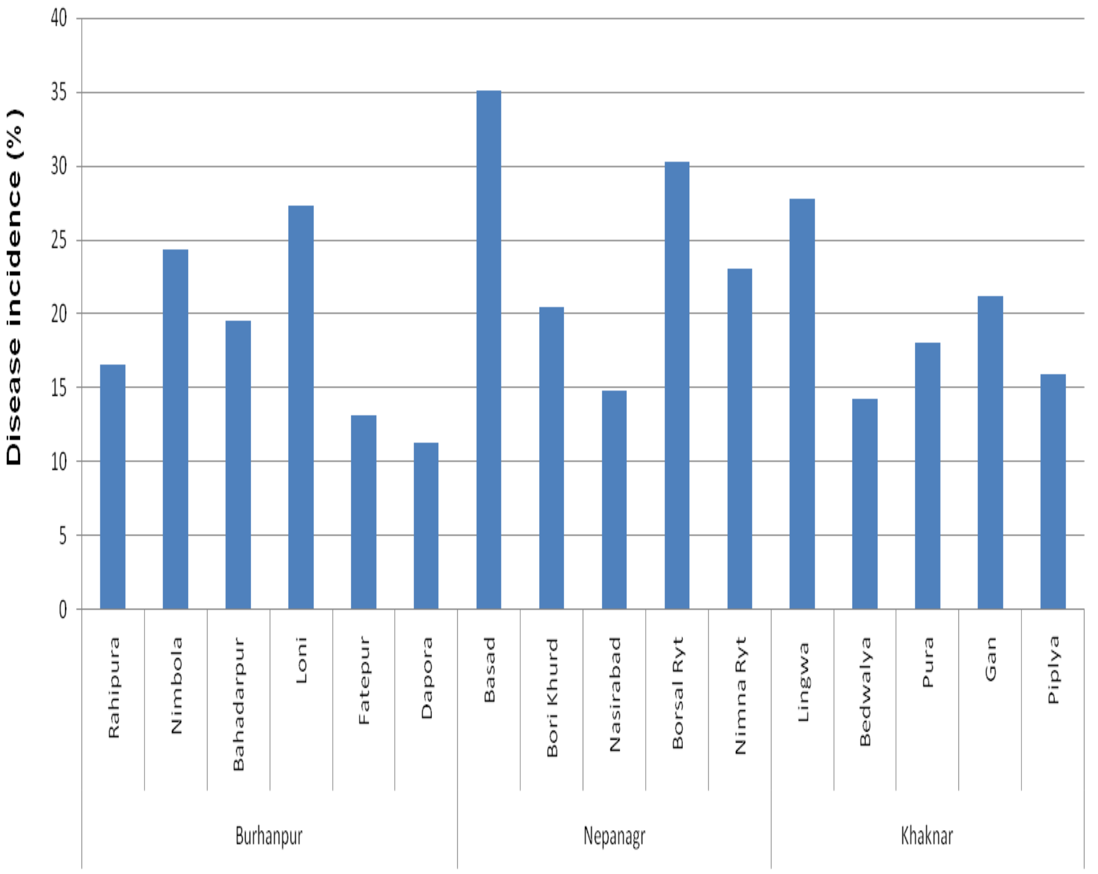


Fig.-1:Incidence of Anthracnose in chilli from three blocks of Burhanpur district

CHAPTER IV

EXPERIMENTAL FINDINGS

The findings of studies on anthracnose of chilli caused by *Colletotrichum capsici* were structured under the several parts such as survey Burhanpur on Anthracnose of chilli, pathogenicity and *in vivo and in vitro* evaluation of agrochemicals against *Colletotrichum capsici* is presented here under.

4.1 Survey, isolation and proving pathogenicity

4.1.1 Survey

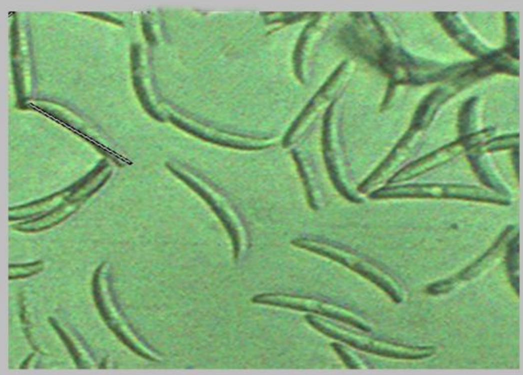
Roving survey was undertaken during *kharif* 2017 to assess the severity of anthracnose of chilli around the Burhanpur district. The survey of chilli crop was conducted at the three blocks of Burhanpur *viz.,* Burhanpur, Nepanagar and Khaknar in 16 locations. Percent disease index (PDI) was recorded and presented in table-2 and fig.-1.

Table-2: Incidence of Anthracnose in chilli from three blocks of Burhanpur district

S.No.	Block	Village	PDI (%)
1	**Burhanpur**	Rahipura	16.59
2		Nimbola	24.34
3		Bahadarpur	19.50
4		Loni	27.36
5		Fatepur	13.17
6		Dapora	11.34
		Mean PDI (%)	**18.72**
7	**Nepanagr**	Basad	35.08
8		Bori Khurd	20.49
9		Nasirabad	14.78
10		Borsal Ryt	30.24
11		Nimna Ryt	23.10
		Mean PDI (%)	**24.74**
12	**Khaknar**	Lingwa	27.80
13		Bedwalya	14.22
14		Pura	18.07
15		Gan	21.16
16		Piplya	15.91
		Mean PDI (%)	**19.43**

Radial growth

Acervuli



Sickle shaped conidia

Plate-1: Cultural and morphological characterestics of *Colletotrichum capsici*

The overall disease incidence was ranged from 11.34 to 35.08 per cent. The highest severity (35.08%) of anthracnose was noticed at Basad village of Nepanagar block. The least (11.34%) incidence was recorded in Dapora village of Burhanpur block. The highest mean disease incidence among the blocks were recorded in Nepanagar (24.74%) followed by Khaknar (19.43%). The least incidence was noticed in Burhanpur block (18.72%) (Table-2 and Fig.-1).

Out of six villages of Burhanpur block, the highest incidence was recorded in Loni (27.36%) followed by Nimbola (24.34%), Bahadarpur (19.50%), Rahipura (16.59%) and Fatepur (13.17%). The lowest incidence was noticed in Dapora village (11.34%).

Five villages of Nepanagar block *viz.,* Basad, Bori Khurd, Nasirabad, Borsal Ryt and Nimna Ryt were surveyed. Highest per cent disease index (PDI) was observed in Basad (35.08%) followed by Borsal Ryt, Nimna Ryt and Bori Khurd village (30.24, 23.10 and 20.49%, respectively).

In Khaknar block, out of five villages, only one village Lingwa (34.50%) showed disease incidence above 25%. Per cent disease incidence was lower than 20% in Pura (18.07%), Piplya (15.91%) and Bedwalya (14.22%).

4.1.2 Isolation and Identification of associated fungi

Infected fruit tissues of chilli collected from Burhanpur district were used for the isolation of the fungus. The pathogenic fungus was isolated on potato dextrose agar medium and purified by single spore isolation method. After inoculation and incubation as described in the material and methods, the white to light brown mycelia growth emerged from diseased leaf tissues on PDA medium in Petri plates. The fungus got isolated by single spore technique and transferring them to fresh slants containing potato dextrose agar medium. Pure culture of the fungus was obtained by several such transfers and kept viable by sub-culturing at the interval of 30 days. The pure culture thus obtained was maintained in the refrigerator at 4^0C for further studies.

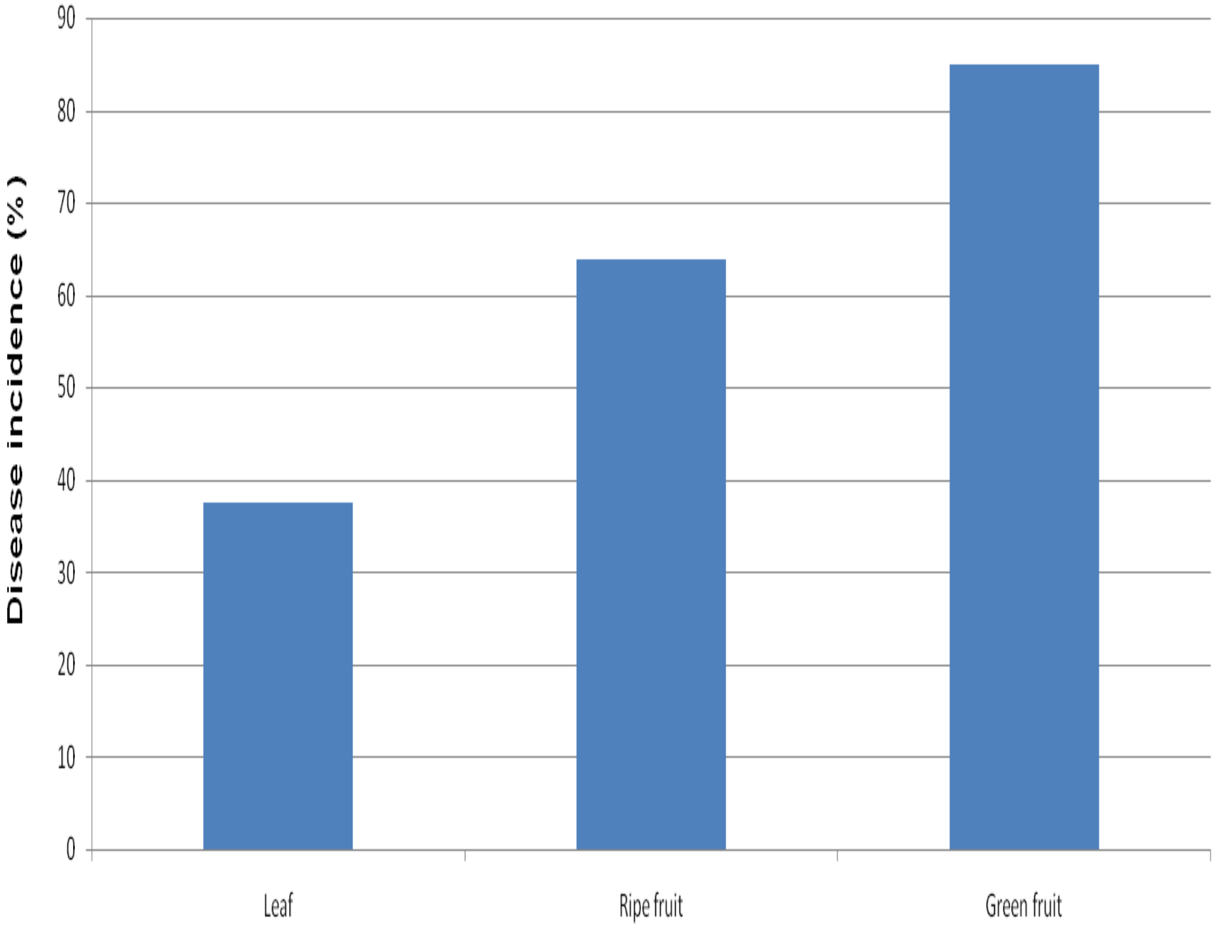


Fig.-2: Disease incidence during the pathogenicity test of *Colletotrichum capsici*

Microscopic examination of fungus revealed that the mycelium was septate with aseptate unbranched conidiophores. Conidia were sickle shaped, single celled, hyline, smooth walled with a central oil globule. The fungus started growing within 24 hours of inoculation. Hyphae were hyaline, septate, filled with several oil globules, producing initially cottony white, raised growth which later on turned grey to olive brown. The fungus produced submerged black dot like structures of pinhead size near the periphery of Petri-plates in circular ring fashion. Later on these acervuli ruptured and exuded orange pigmentation of spore mass in circular ring fashion (Plate-1). The fungus was identified as *Colletotrichum capsici* (Butler and Bisby) on the basis of morphological characters when compared with standard literature (Smith and Black, 1990).

4.1.3 Pathogenicity

The pathogenicity of the isolates of *Colletotrichum capsici* causing anthracnose of chilli was proved by Koch's postulate under *in vitro* condition. Healthy chilli fruits and leaves collected from the fields were washed with tap water and then surface sterilized with 70% ethyl alcohol. *C. capsici* was cultured on PDA for 10 days. 5 mm agar plug of 10 days old pure culture of pathogen was placed on pierced area on chili leaves and fruits. Leaves and fruits were inoculated with sterilized water served as control. Inoculated fruits were kept in moistened Petri plates to maintain humidity and incubated at 28 $\pm 2^0$C and observed daily for the disease symptoms. Pathogen was re isolated from the infected fruits and compared with the original culture (Plate-2).

The fungus produced initial symptoms of the disease between 5 to 7 days on infected leaves and fruits. The data presented in table-3 revealed that green fruits exhibited 85 per cent disease incidence followed by ripe fruits (63.80 % disease incidence). However, least disease incidence (37.60 %) was observed in infected leaves (Fig.-2).

Inoculated on	
Leaf	
Green fruit	
Ripe fruit	

Plate-2: The pathogenicity test of*Colletotrichum capsici*

Table-3: Disease incidence during the pathogenicity test of *Colletotrichum capsici*

Inoculated on	Disease incidence (%)*
Leaf	37.60 (37.71)**
Ripe fruit	63.80 (53.03)
Green fruit	85.00 (67.33)
SEm±	2.92
C. D. at 5%	6.35

* Mean of three replications

**Data in parenthesis are √per cent arc sin transformed values

4.2 *In vitro* test in association with different fungicides

4.2.1 At seven days after inoculation

In vitro evaluation of fungicides was carried out by poison food technique. Three concentration (100, 200 and 1000 ppm) of each fungicides was assayed against *C. capsici* on PDA. Observations on radial growth of *C. capsici* were recorded after seven days of inoculation (Table-4 and Fig.-3).

Data in table-4 shows that inhibition of mycelial growth of *C. capsici* increased with increase in concentration of fungicides. Significantly minimum growth was noticed ay 1000 ppm concentration followed by 200 ppm and 100 ppm. In absence of fungicide *C. capsici* showed the radial growth of 73.33 mm. Among the fungicides minimum growth (0.00 mm) were obtained in treatment Hexaconazole and Tricyclazole followed by Propiconazole (12.50), Mancozeb (13.34mm), Copper oxychloride (17.67mm) and Metalaxyl (24.67 mm) which were significantly over other treatments and control (73.33 mm).

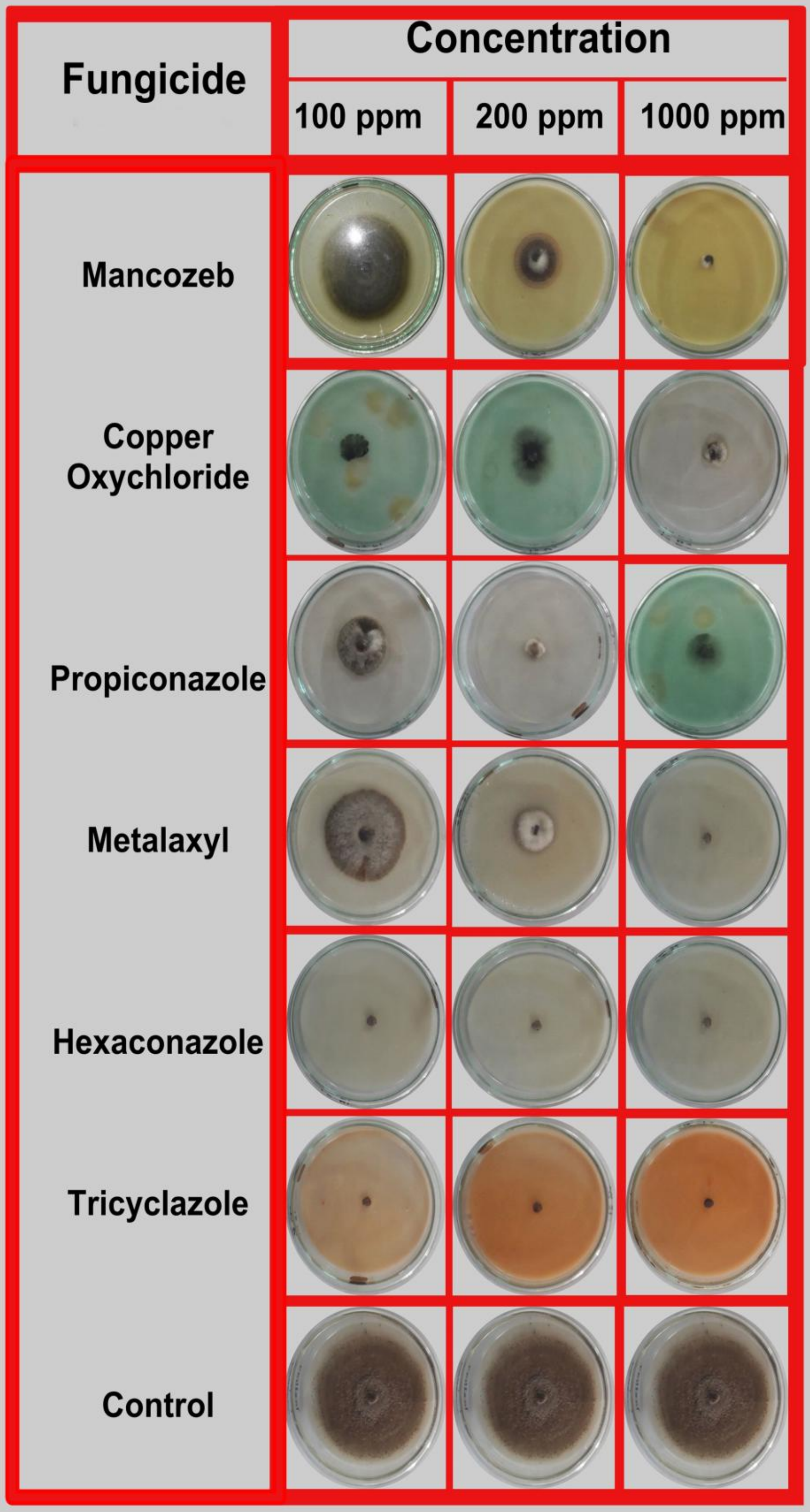


Plate-3: Radial growth of *Colletotrichum capsici* in fungicide amended medium at fourteen days after inoculation

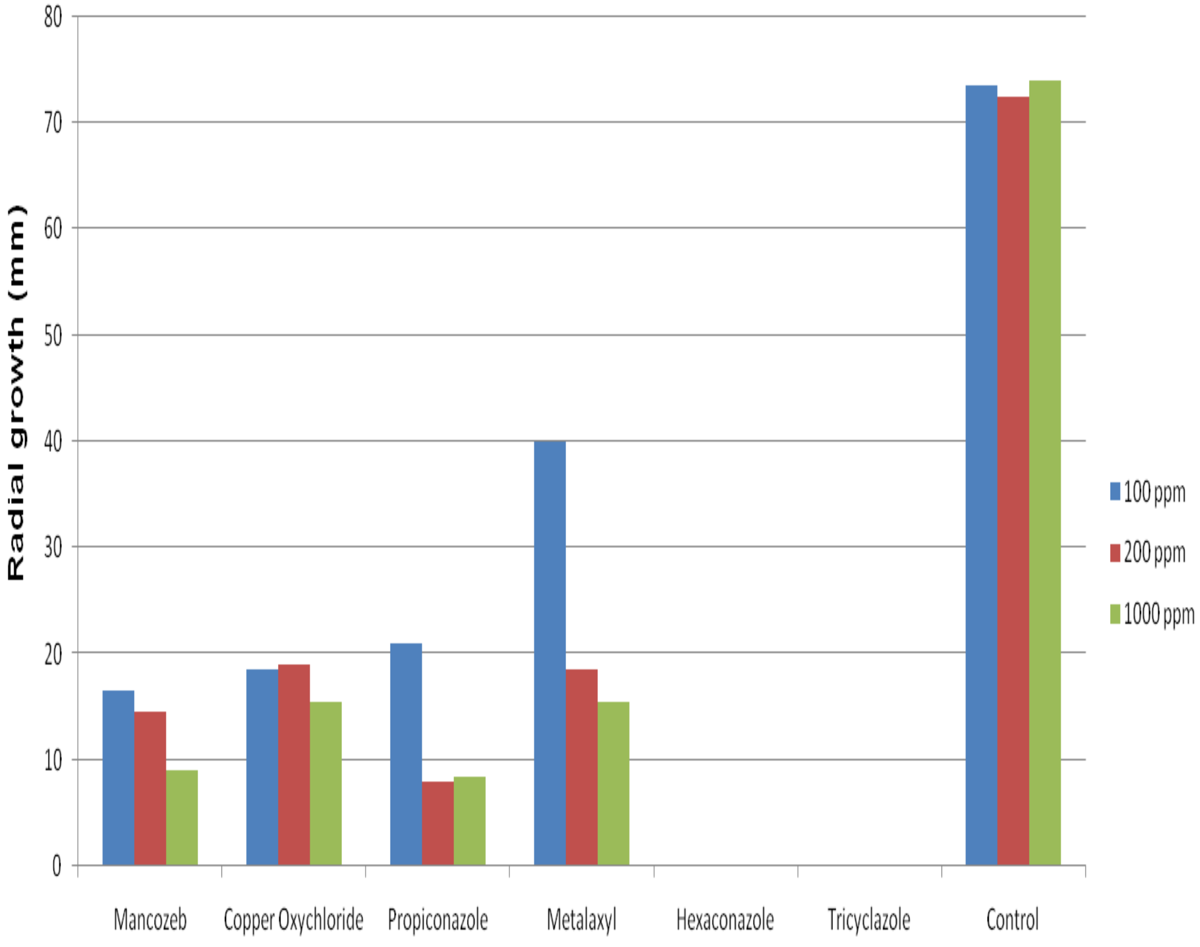


Fig.-3: Radial growth of *Colletotrichum capsici* in fungicide amended medium at seven days after inoculation

Fungicides Hexaconazole and Tricyclazole could check the growth of fungus completely at all concentrations.

Table-4: Radial growth of *Colletotrichum capsici* in fungicide amended medium at seven days after inoculation

Fungicides	Mean radial growth (mm)*			
	100 ppm	**200 ppm**	**1000 ppm**	**MEAN B**
Mancozeb	16.50	14.50	9.00	13.34
Copper Oxychloride	18.50	19.00	15.50	17.67
Propiconazole	21.00	8.00	8.50	12.50
Metalaxyl	40.00	18.50	15.50	24.67
Hexaconazole	0.00	0.00	0.00	0.00
Tricyclazole	0.00	0.00	0.00	0.00
Control	73.50	72.50	74.00	73.33
MEAN A	24.22	18.93	17.50	
Factors	**SE(m) ±1**		**C.D. at 5 %**	
Concentration (C)	1.12		3.27	
Fungicide (F)	1.71		5.00	
Inter (C×F)	2.97		8.66	

* Mean of three replications

4.2.2 At fourteen days after inoculation

Six fungicides were evaluated in laboratory for their toxicity against C. c*apsici* at different concentration *viz.* 100, 200 and 1000 ppm by poison food technique.

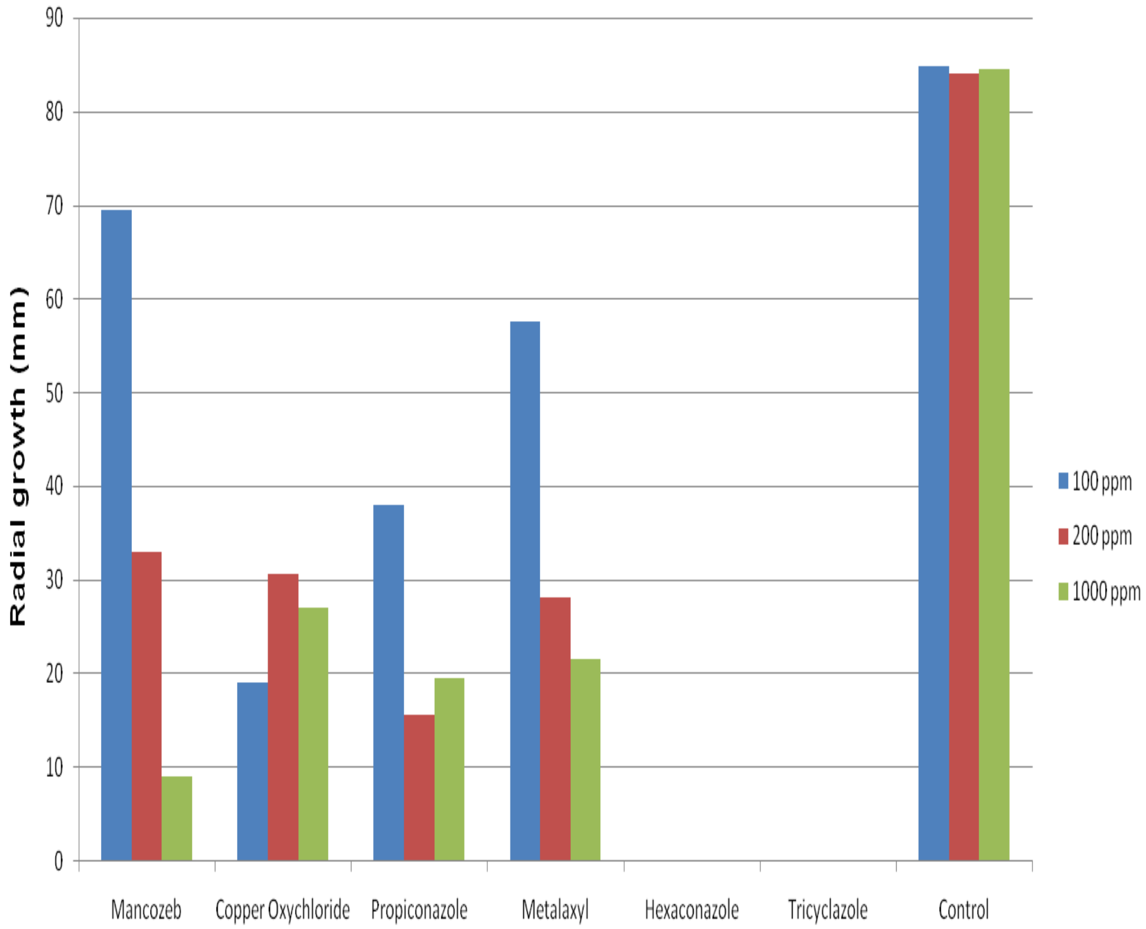


Fig.-4: Radial growth of *Colletotrichum capsici* in fungicide amended medium at fourteen days after inoculation

Significant variation in the fungicidal toxicity at all concentrations was observed against *C. capsici* (Table-4.4 and Plate-3). Significantly minimum growth was noticed ay 1000 ppm concentration followed by 200 ppm and 100 ppm. In absence of fungicide *C. capsici* showed the radial growth of 84.43 mm. Among the fungicides minimum growth (0.00 mm) were obtained in treatment Hexaconazole and Tricyclazole followed by Propiconazole (24.34), Copper oxychloride (25.50mm), Metalaxyl (35.67mm) and Mancozeb (37.17mm) which were significantly over other treatments and control (84.43 mm) (Fig.-4).

Table-5: Radial growth of *Colletotrichum capsici* in fungicide amended medium at fourteen days after inoculation

Fungicides	**Mean radial growth (mm)***			
	100 ppm	**200 ppm**	**1000 ppm**	**MEAN B**
Mancozeb	69.50	33.00	9.00	37.17
Copper Oxychloride	19.00	30.50	27.00	25.50
Propiconazole	38.00	15.50	19.50	24.34
Metalaxyl	57.50	28.00	21.50	35.67
Hexaconazole	0.00	0.00	0.00	0.00
Tricyclazole	0.00	0.00	0.00	0.00
Control	84.78	84.00	84.50	84.43
MEAN A	38.40	27.29	23.07	
Factors	**SE(m) ±1**		**C.D. at 5 %**	
Concentration (C)	1.37		3.99	
Fungicide (F)	2.09		6.10	
Inter (C×F)	3.62		10.57	

* Mean of three replications

Fungicides Hexaconazole and Tricyclazole could check the growth of fungus completely at all concentrations.

4.3 *In vivo* evaluation of agrochemicals against *Colletotrichum capsici*

The data on effect of agrochemicals on disease intensity of anthracnose of chilli in *C. capsici* infested soil are presented in table-6 and fig.-5.

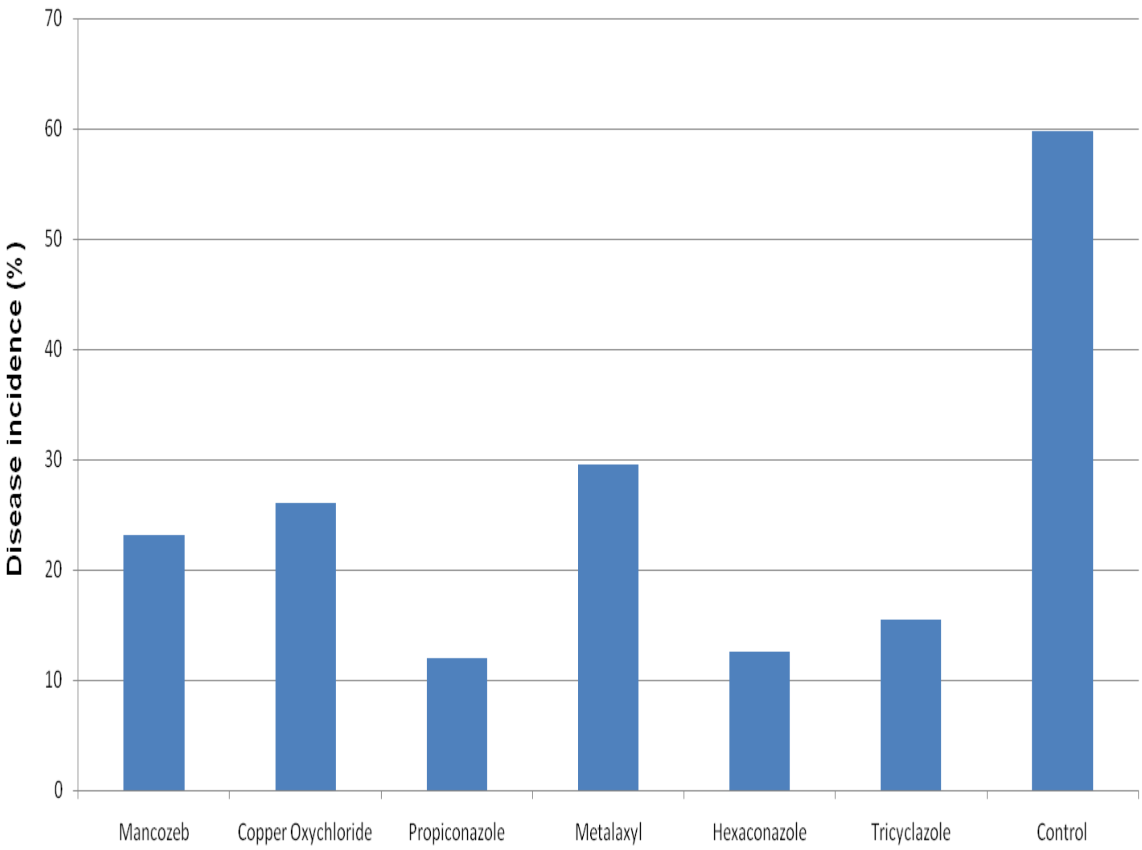


Fig.-5: ***In vivo*** **disease incidence of Anthracnose of chilli against agrochemicals**

Table-6: *In vivo* disease incidence of Anthracnose of chilli against agrochemicals

Treatments	Disease incidence (%)*
Mancozeb	23.17 (28.56)**
Copper Oxychloride	26.10 (30.07)
Propiconazole	12.00 (20.11)
Metalaxyl	29.50 (32.82)
Hexaconazole	12.60 (20.53)
Tricyclazole	15.48 (23.06)
Control	59.82 (50.81)
SE(m)±	3.73
C.D. at 5%	7.85

* Mean of three replications

****** Data in parenthesis are √per cent arc sin transformed values

Significant differences occurred with respect to disease intensity per cent in agrochemicals treated treatments.

The data in table-6 shows that disease incidence percentage of all agrochemical treated treatments were significantly lower than untreated control. The disease intensity ranged between 12.00 to 59.82 %. Significantly lowest intensity per cent was recorded in Propiconazole (12.00%) which was at par with Hexaconazole (12.60%) and Tricyclazole (15.48%) and differed significantly with rest of the treatments followed by Mancozeb, Copper oxychloride and Metalaxyl (23.17, 26.10 and 29.50%, respectively) as compared to control (59.82%).

CHAPTER-V

DISCUSSION

Results which was work out under the investigations on "Studies on anthracnose of chilli causes by *Colletotrichum capsici"* have been examined critically in chapter IV is discussed here with interpretations.

Survey of Anthracnose of chilli in Burhanpur. The overall disease severity was ranged from 11.34 to 35.08 per cent. The highest severity of anthracnose was noticed in fields of Basad village of Nepanagar block. The least incidence of the disease was recorded in Dapora village of Burhanpur block. With respect to the block means, highest mean disease incidence was recorded in Nepanagar followed by Khaknar. The least incidence was noticed in Burhanpur block. Koppad and Mesta (2017) conducted a roving survey to know the severity of *Colletotrichum capsici* in Belagavi, Dharwad, Gadag and Haveri districts during kharif /rabi 2013-14.

Isolation, identification and purification of pathogen, the pathogen was isolated from diseased fruits of chilli successfully during the isolation procedure standers precautions were followed and pathogen was purified by single spore isolation method and then was maintained on PDA slants.

Pathogenicity test of isolated pathogen of Anthracnose disease occurred on leaves, stems, and both pre- and post-harvest fruits. The disease attacked seedling first causing the necrotic erumpent, brittle black circular spots on the cotyledons and primary leaves. The pathogen was recorded as necrotic brown, black spots on leaves surrounded by dark margin. Typical fruit symptoms are circular or angular sunken lesions, with concentric rings of acervuli that are often wet and produce pink to orange conidial masses. Under severe disease pressure, lesions may coalesce. Conidial masses may also occur scatteredly or in concentric rings on the lesions. Similar types of symptoms due to anthracnose pathogen in chilli have been reported by Lakshmi sahitya *et al.,* (2014).

The fungus produced initial symptoms of the disease between 5 to 7 days on infected leaves and fruits. The result revealed that green fruits exhibited maximum disease incidence followed by ripe fruits. However, least disease incidence was observed in infected leaves. Earlier, Rajamanickam and Sethuraman (2014) were tested the four methods of inoculation under *in vitro* condition to assess the effectiveness of infection.

In vitro evaluation with different fungicides in the present study, inhibition of mycelial growth of *C*. *capsici* increased with increase in concentration of fungicides at seven days after inoculation. Significantly fungicides Hexaconazole and Tricyclazole could check the growth of fungus completely at all concentrations. In absence of fungicide *C*. *capsici* showed the highest radial growth. Among the fungicides minimum growth were obtained in treatment Hexaconazole and Tricyclazole followed by Propiconazole, Mancozeb, Copper oxychloride and Metalaxyl which were significantly over other treatments and control.

At fourteen days after inoculation, significantly minimum growth was noticed ay 1000 ppm concentration followed by 200 ppm and 100 ppm. Among the fungicides, no growth was obtained in treatment Hexaconazole and Tricyclazole followed by Propiconazole, Copper oxychloride, Metalaxyl and Mancozeb which were significantly over other treatments and control. Gupta *et al.* (2018) evaluated six fungicides at different concentrations and the result showed that Thiophanate methyl and Copper oxychloride were the best toxic fungicides inhibiting the mycelial growth *in vitro* at all the six concentrations. In fungicides, Thiophanate methyl inhibited highest mycelial growth, which was at par with Copper oxychloride.

Significant differences occurred with respect to disease intensity per cent in agrochemicals treated treatments. The result revealed that disease intensity percentage of all agrochemicals treated treatments were significantly lower than untreated control. The disease intensity ranged between 12.00 to 59.82 %. Significantly lowest intensity per cent was recorded in Propiconazole which was at par with Hexaconazole and Tricyclazole and differed significantly with rest of the treatments followed by Mancozeb, Copper oxychloride and Metalaxyl as compared to control. Similarly, Yadav *et al.* (2014) reported Propiconazole was found most effective fungicide against anthracnose of chilli.

CHAPTER-VI
SUMMARY, CONCLUSION AND SUGESTION FOR FURTHER WORK

❖ Summary

The present investigation on anthracnose of chilli cause by *Colletotrichum Capsici* was carried out during *Kharif* 2017-18, at Department of Plant Pathology, B. M. College of Agriculture, Khandwa (M.P.), R.V.S.K.V.V. Gwalior (M.P.)

- ✓ A roving survey was undertaken during *kharif* 2017 to assess the severity of chilli anthracnose at the three blocks of Burhanpur in 16 locations. The overall disease severity was ranged from 11.34 to 35.08 per cent. The highest incidence of anthracnose was noticed in Nepanagar block followed by Khaknar and the least incidence was noticed in Burhanpur block.
- ✓ Anthracnose disease can occur on leaves, stems, and both pre- and post-harvest fruits. The disease attacked seedling first causing the necrotic erumpent, brittle black circular spots on the cotyledons and primary leaves. The pathogen was recorded as necrotic brown, black spots on leaves surrounded by dark margin. Typical fruit symptoms are circular or angular sunken lesions, with concentric rings of acervuli that are often wet and produce pink to orange conidial masses. Under severe disease pressure, lesions may coalesce. Conidial masses may also occur scatteredly or in concentric rings on the lesions.
- ✓ The fungus produced initial symptoms of the disease between 5 to 7 days on infected leaves and fruits. The result revealed that green fruits exhibited maximum disease incidence followed by ripe fruits. However, least disease incidence was observed in infected leaves.
- ✓ At seven and fourteen days after inoculation, significantly fungicides Hexaconazole and Tricyclazole could check the growth of fungus completely at all concentrations. In absence of fungicide *C. capsici* showed the highest radial growth. Among the fungicides minimum growth were obtained in treatment Hexaconazole and Tricyclazole followed by Propiconazole, Mancozeb, Copper oxychloride and Metalaxyl which were significantly over other treatments and control.

- ✓ Significant differences occurred with respect to disease intensity per cent in agrochemicals treated treatments. The result revealed that disease intensity percentage of all agrochemicals treated treatments were significantly lower than untreated control. The disease intensity ranged between 12.00 to 59.82 %. Significantly lowest intensity per cent was recorded in Propiconazole which was at par with Hexaconazole and Tricyclazole and differed significantly with rest of the treatments followed by Mancozeb, Copper oxychloride and Metalaxyl as compared to control.

❖ Conclusion

- ✓ The highest severity of anthracnose was noticed in fields of Basad village of Nepanagar block. The least incidence of the disease was recorded in Dapora village of Burhanpur block. Among the blocks, highest mean disease incidence was recorded in Nepanagar followed by Khaknar. The least incidence was noticed in Burhanpur block.
- ✓ The fungus produced initial symptoms of the disease between 5 to 7 days on infected leaves and fruits. The result revealed that green fruits exhibited maximum disease incidence followed by ripe fruits. However, least disease incidence was observed in infected leaves.
- ✓ At seven and fourteen days after inoculation, significantly fungicides Hexaconazole and Tricyclazole could check the growth of fungus completely at all concentrations. Among the fungicides minimum growth were obtained in treatment Hexaconazole and Tricyclazole followed by Propiconazole, Mancozeb, Copper oxychloride and Metalaxyl which were significantly over other treatments and control.
- ✓ Six fungicides were tested *in vitro* and found that Propiconazole was highly toxic against Anthracnose disease of chilli which was at par with Hexaconazole and Tricyclazole. Rest of the treatments followed by Mancozeb, Copper oxychloride and Metalaxyl were less effective.

❖ Suggestion for further work

- ➢ Survey and collection of isolates of *C. capsici* should be done to find out the morphological variability on the basis of cultural, morphological and pathogenicity characters like, colony outline, shape, colour and texture. The variability among isolates should be confirmed by molecular tools.

- Confirmation of genetic potential of resistant lines of chilli should be repeated in upcoming season by insuring optimum inoculum road.
- Further authentification of inoculation technique for *in vitro* evaluation of different inoculation techniques.

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APPENDICES

Analysis of Variance Table – 4.2

SV	D.F.	S.S.	M.S.S.	Fcal	Ftab
Treatment	2	2194.31	1097.16	86.02	3.63
Error	12	153.06	12.76		
Total	14	2347.37			

Analysis of Variance Table – 4.3

SV	**DF**	**SS**	**MSS**	**Fcal**	**Ftab**
CONC. (C)	2	278.06	139.03	5.26	3.22
FUNGI	6	21878.58	3646.43	138.05	2.32
INTER(C*T)	12	30997.37	2583.11	97.80	1.99
ERROR	42	1109.36	26.41		
TOTAL	62	54263.36	6394.984		

Analysis of Variance Table – 4.4

SV	**DF**	**SS**	**MSS**	**Fcal**	**Ftab**
CONC. (C)	2	1057.47	528.74	13.45	3.22
FUNGI	6	29126.87	4854.48	123.46	2.32
INTER(C*T)	12	53294.36	4441.20	112.95	1.99
ERROR	42	1651.40	39.32		
TOTAL	62	85130.1	9863.73		

Analysis of Variance Table – 4.5

SV	DF	SS	MSS	Fcal	Ftab
REP	2	34.49	17.25	0.41	3.55
TREAT	6	2029.13	338.19	8.08	2.34
ERROR	12	502.12	41.84		
TOTAL	20	2565.75			

VITA

The author of this thesis **Miss. BABITA RAIKWAL** D/O Shri Lalgiram Raikwal was born on 05 April, 1993 at District Sehore in Madhya Pradesh. She has passed High School Examination from H. S. School, Maina (M.P.) in the year of 2009 with 51.00 per cent marks and passed Higher Secondary School Examination from H. S. School, Maina Dist. Sehore (M.P.) in the year of 2011 with 74.40 per cent marks.

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Date:

Place: Khandwa