“Studies on Anthracnose of Pomegranate Caused by *Colletotrichum gloeosporioides* (Penz and Sacc.) and Its Management”

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(PLANT PATHOLOGY)

By

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CERTIFICATE - I

This is to certify that the thesis entitled **"Studies on anthracnose of pomegranate caused by *Colletotrichum gloeosporioides* (Penz and Sacc.) andits management"** 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 bona fide research work carried out by **Mr. BHAGWAN SAHAY BIJARNIYA id No 19131802** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instruction.

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

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Date (**Dr. D.R. Saxena**)

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CERTIFICATE – II

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This is to certify that the thesis entitled **"Studies on anthracnose of pomegranate caused by *Colletotrichum gloeosporioides* (Penz and Sacc.) and its management"** submitted by **Mr. BHAGWAN SAHAY BIJARNIYA ID No 19131802** 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 of the same.

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Date: / / 2022 Chairman of Advisory Committee

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Place: Sehore

Date: **(Bhagwan Sahay Bijarniya)**

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ABBREVIATIONS

S.N.	Legends	Description
1	@	At the rate of
2	%	Per cent
3	^0C	Degrees Celsius
4	ANOVA	Analysis of variance
5	C.V.	Coefficient of variation
6	cal.	Calculated
7	CD	Critical difference
8	Cfu	Colony forming unit
9	Cm	Centimeter
10	CRD	Completely Randomized Design
11	DAT	Day after transplanting
12	DF	Degrees of freedom
13	EC	Emulsifiable Concentrate
14	EMS	Error means sum of square
15	*et al.*	*Et alia* (and others)
16	Fig.	Figure
17	G	Gram
18	gl^{-1}	grams/ liter
19	i.e.	That is (in reference to)
20	Kg	Kilogram
21	mm.	Millimeter
22	MSS	Mean Sum of Square
23	No.	Number
24	PDA	Potato Dextrose Agar
25	PPM	Parts Per Million
26	Q	Quintal
27	S.N.	Serial number
28	S.S.	Sum of Square
29	S.V.	Source of variance
30	SE (m)±	Standard error of mean
31	SL	Suspendable liquid
32	spp.& sp.	Species(plural)& Species (singular)
33	tab.	Tabulated
34	t/ha	Tones/hectare
35	*Viz.,*	Videlicet (namely)
36	WG	Wettable granule
37	WS	Water Soluble

CHAPTER-I

INTRODUCTION

Punica is a genus of deciduous shrubs or trees which bears fruits. From its known species, Pomegranate (*Punica granatum L.*) is the most historic fruit tree, belonging to family Punicaceae and is locally known as *Anar*. Pomegranate is known for its edible fruits also consumed in many processed forms such as beverages (wines and juices), as food products (jam, jellies, oil) and as extract in dietary supplements (Kaur *et al.,* 2018).

Pomegranate is commercially cultivated mainly in Maharashtra. In India, Pomegranate cultivation occupies an area of 2.83 lakh ha with an annual production of 31.86 lakh MT in 2019-20 (Anonymous, 2021). Madhya Pradesh occupies 9.68 thousand ha with an annual production 114.27 thousand tones, which is 4 percent of total production of country (Tewari and Avinashilingam, 2020).

Amongst the various fungal diseases, anthracnose caused by *Colletotrichum gloeosporioides* is one of the most serious diseases of pomegranate worldwide (Munhuweyi *et al*., 2016). In India, anthracnose is reported in the range of 15-25% in Northern Karnataka (Benagi *et al*., 2011).

Colletotrichum spp. can infect pomegranate leaves and fruits, causing anthracnose leaf blight and fruit rot, respectively (Day and Wilkins 2011; Munhuweyi *et al*. 2016). Latent infection may occur invisibly in healthy plants causing extensive crop losses due to anthracnose disease related symptoms.

Cultural practices are often not efficient and may also be very costly. The integration of different pest management strategies has been developed but the search for effective botanical fungicides has not yet been done. Chemical pesticides are widely used since they are effective, convenient, and readily available in markets; however, the abusive use of these synthetic pesticides posed harmful effects to living organisms and environment. Moreover, the improper application of chemical fungicides, especially by those people with limited knowledge and equipment, has made pathogens acquire more resistance. This will then require higher concentrations of fungicide application to control them in the long run (Fu *et al.,* 2007).

According to Soylu *et al.* (2010), and Hemant *et al.* (2014), increasing concern over the decreasing efficacy of synthetic fungicides, agricultural and forestry crop productivity, safety of the environment, and public health has brought about the need for the development of new and natural control alternatives such as naturally occurring pesticides. Plants produce secondary metabolites such as flavonoids, saponins, alkaloids, tannins, and phenols that are important for their adaptation and existence (Baker *et al.,* 2010). Extensive exploration of some biochemical constituents has led to discovery of different plant products as natural commercial bio-pesticides that can be alternative to currently used synthetic pesticides (Isman 2000; Burt 2004; Tegegne *et al.,* 2007; Haouala *etal.,* 2008).

Hence, this study was conducted to achieve the different goals mentioned below.

Objectives of investigation: -

1. To study epidemiology of anthracnose of pomegranate.
2. *In-vitro* evaluation of fungicides and botanicals against *C. gloeosporioides*.
3. Evaluation of efficacy of fungicides against anthracnose under field conditions.

CHAPTER-II
REVIEW OF LITERATURE

Anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. is the major pre and post-harvest disease of pomegranate. Presently, very little literature is available in India on *C. gloeosporioides* causing anthracnose of pomegranate fruit (Bose *et al*., 1973). Hence, the available literature on anthracnose of pomegranate and other fruit crops is being reviewed here.

Isolation, purification and Identification

Akthar (2000) reported that the fresh potato extract was the best source for routine isolation and growth of *C. gloeosporioides* causing mango anthracnose.

Sudhakar (2000) purified the *C. gloeosporioides* culture isolated from the infected tissues of mango and stylosanthes respectively by single spore isolation.

Reddy (2000) reported that potato dextrose agar was the best medium for fast growth and good sporulation of *C. gloeosporioides* of annona, ber, citrus, pomegranate and strawberry.

Saxena (2002) isolated *C. gloeosporioides* from Pomegranate put forth better growth on Potato dextrose agar, whereas, Richard's broth was suitable among the different liquid media used.

Zakaria (2000) isolated thirteen *Colletotrichum* isolates from several forest nurseries in Peninsular Malaysia and pure cultures were maintained on Mathur's medium.

Venkataravanappa (2002) purified the culture by single spore isolation after isolating *C. gloeosporioides* from infected tissues of mango and pomegranate using two per cent water agar media.

Muniz *et al.* (2003) isolated *C. gloeosporioides* from post-harvest disease affected orange fruits.

Murthy and Sandhya Rani (2004) found abundant mycelial growth as well as sporulation of *C. gloeosporioides* causing anthracnose of cashew on Martins rose Bengal agar. They were characterized according to their myceli growth and sporulation in potato dextrose agar (PDA). Colonies were white grey to pink in PDA.

Prashanth (2007) purified the culture by single spore isolation after isolating *C. gloeosporioides* from infected tissues of pomegranate using two percent water agar media.

Sangeeta and Rawal (2008) isolated *C. gloeosporioides* from infected mango leaves.

Vinod and Benagi (2009) worked on papaya anthracnose caused by *C. gloeosporioides* and reported that among different solid media Richards' agar showed good growth and sporulation. The fungus in liquid media recorded good growth up to 10 days of incubation and decreased after eleventh day.

Zakaria *et al.* (2009) isolated thirteen *Colletotrichum* isolates from different banana cultivars (*Musa* spp.) with symptoms of anthracnose lesions on the surface of the fruit or from pieces of infected tissues. Single spore isolates were prepared for each isolate for morphological and molecular characterization.

Awa *et al.* (2012) collected 96 isolates of anthracnose in mango caused by *C. gloeosporioides* in South-western Nigeria and isolated the fungal colonies, then sub- cultured into fresh plates until pure cultures were obtained. Pure cultures obtained were identified by visual examinations and viewing under stereo and compound electronic microscopes.

Pandey *et al.* (2012) isolated *C. gloeosporioides* from the mango anthracnose samples collected from Chittoor (Andhra Pradesh), Rewa (Madhya Pradesh), Lucknow (Uttar Pradesh), Muzaffarpur (Bihar) and Dapoli (Maharashtra).

Devanshu and Somasekhara (2018) isolated *C. gloeosporioides* from the pomegranate leaves and fruits showing typical anthracnose symptoms by standard tissue isolation method and further purified by single spore isolation method on Potato Dextrose Agar (PDA) medium.

Golakiya *et al.* (2020) collected pomegranate leaves and fruits with anthracnose symptoms from the farmer's field. On isolation, they yielded species of *C. gloeosporioides* with typical cultural and morphological characters on potato dextrose agar media and pure culture was obtained by hyphal tip method, such culture was used for pathogenicity test of Koch'spostulates.

Pathogenicity

Venkataravanappa (2002) proved pathogenicity of *C. gloeosporioides* by spraying the spore suspension (10^6 spores/ml) of fungus on five month old mango seedlings and reported that infection occurred twelve days after inoculation.

In pathogenicity studies, the conidial suspension of *C. gloeosporioides* was sprayed on healthy fruits and typical symptoms were noticed, seven days after incubation. Similar results were recorded while proving pathogenicity by Bhat (1991) and Ekbote (1994) on pomegranate and mango respectively. Kota (2003) proved the pathogenicity of *C. gloeosporioides* on mango and banana.

Prashnath *et al.* (2008) proved pathogenicity by artificial inoculation (pin prick method) of fungus on pomegranate fruit.

Masyahit *et al.* (2009) carried out pathogenicity *in vitro* on both healthy stem and fruit of Anthracnose disease caused by *C. gloeosporioides* (Penz.) Penz. & Sacc. on Dragon fruit (*Hylocereus* spp.) in Peninsular Malaysia.

Ratanacherdchai *et al.* (2010) isolated thirty four isolates of *Colletotrichum* spp. including two species, *C. gloeosporioides* and *C. capsici* from anthracnose on bell pepper, long cayenne pepper and bird's eye chilliand proved their pathogenicity by fruit inoculations.

Pathogenicity of anthracnose of mango caused by *C. gloeosporioides* in South- western Nigeria was proved *in vitro* condition by different methods such as whole fruit technique, detached leaf technique and detached panicle technique Awa *et al.* (2012).

Parey *et al.* (2013) tested pathogenicity of isolated fungi, *C. capsici causing* chilli anthracnose by four different inoculation methods under *in vitro* conditions, on detached semi-ripe fruits (turning red) of chilli. The methods included pin-prick, spore suspension spray, spore suspension injection and spore suspension dip.

The acervuli of *Colletotrichum capsici* were disc shaped, waxy, sub epidermal, typically with dark needle like septate setae, conidiophore short, simple, conidia hyaline, single celled, ovoid or oblong Gupta *et al.* (2017).

Pathogen

Taxonomy

The genus *C. gloeosporioides.* belongs taxonomically to the sub division of Deuteromycotina (fungi imperfecti), Class *Coelomycetes*, Order *Melanconiales*. The genus *Colletotrichum* was established by Corda (1831), for fungi characterized by hyaline, curved, fusiform conidia and setose acervuli. After that, followed a period of uncertainty as to the distinction between *Colletotrichum* and other genera such as *Vermicularia* and *Gloeosporium* (Jeffries *et al.,* 1990). Previously recognized genus *Gloeosporium Desm*. et Mont, was revised by Arx (1970). In few species, a perfect state was detected and described in the genus *Glomerella* (Stoneman) H. Schrenk et Spauld in the division of Ascomycota (Hindorf, 2000). The most well known generative and vegetative combination was represented with *G. cingulata* and *C. gloeoesporioides.*

Kingdom : Fungi

Division : Ascomycota

Class : Sordariomycetes

Order : Phyllachorales

Family : Phyllachoraceae

Genus *: Colletotrichum*

Species : *gloeosporioides*

Scientific Name: *C. gloeosporioides* (Penz.) Penz. & Sacc. Teleomorph: *Glomerella cingulata* (Stoneman) Spauld. & H.Schrenk.

Symptomatology

Arauz (2000) observed that leaf anthracnose on mango appeared as irregular shaped black necrotic spots on both the sides of the mango leaf which often coalesced and formed large necrotic areas.

Gaikwd (2002) reported that *C. gloeosporiodes* infected all above ground parts *viz*., flower buds, fruits, fruit stalks, petioles, leaf veins, young succulent short (Twigs).

Potphode (2004) reported symptoms of anthracnose on Jasmine as minute, pin head sized brownish necrotic spots on upper part of leaf lamina. Later, the spots increased in size and became circular in shape.

Prashanth (2007) described typical symptoms on inflorescence, leaves and fruits. Black to brown necrotic depressed spots appeared on the leaves with circular margin, in advanced stage, spots coalesced and resulted in bigger patches, lead to defoliation. On fruits, brown spherical depressed spots occurred in scattered form on the pericarp. In advanced stage, spots coalesced to form necrotic rotten patches. However, the anthracnose spots caused by *C. gloeosporioides* were also associated with various fungi.

Anamika *et al.* (2012) reported anthracnose in chilli with PDI ranged from 55.53 to 71.10% in Rewa region.

Pujari *et al*. (2013) observed the initial symptoms of anthracnose as characterized by small water soaked brownish spots in large numbers.

Gautam (2014) described the anthracnose symptoms as the sunken, water soaked spots, rapidly expand on infected plant tissue that become soft on full expansion and show a range of colours from red-brown to tan to black. However, a great variation in the symptoms produced by *C. gloeosporioides* was also recorded from host to host.

Gupta *et al.* (2017) reported anthracnose in different plant parts. 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.

Cara *et al.* (2020) observed an anthracnose-like disease on fruit in pomegranate orchards of cv. Wonderful in the Fier region of Albania. Disease prevalence in the assayed area ranged from 8 to 10%. Symptoms appeared as light to dark brown circular, sunken lesions with increasing diameter, whichthen merged.

Effect of environmental factors on disease

Pandey (2011) observed that temperature and moisture requirements for infection have also been used to build forecasting systems for mango anthracnose a vital component for the disease management

Sharma and Kulshrestha (2015) reported that environmental conditions favored the pathogen growth were temperature, 25-28°C being optimum, pH range of 5.8 to 6.5 and high humidity. Activity of this pathogen depend upon weather, *Colletotrichum* was inactive in dry season.

Amrutha and Vijayaraghavan (2020) reported that disease severity of leaf spot caused by *C. gloeosporioides* was negatively correlated with humidity (-0.868) and rainfall (-0.919) in Wayanad and positive correlation of 0.501 and 0.541 in Malappuram.

Pandit (2020) found mean temperature and relative humidity to be positively correlated; therefore it is evident that disease severity progressed significantly with rise in mean temperature and mean relative humidity. Rainfall and bright sunshine hours were found to influence disease insignificantly.

Management

Evaluation of fungicides

Jahagirdar *et al.* (2000) found that three sprays with kitazin at the rate of 0.2 per cent was found to be effective in checking the incidence of anthracnose of pomegranate (5.5%) compared to farmers method (16.3%).

Chhata and Kumawat (2001) studied the management of bacterial and fungal fruit spot of pomegranate two years old plants on farmer field and reported that four sprays of bavistin (0.1%) + streptocycline (0.04%) at an interval of 15 days reduced the PDI to 10.42 where as untreated control recorded 71.21 PDI.

Venkataravanappa and Nagrand (2002) reported tricyclazole as very effective under laboratory condition against *C. gloeosporioides* among all tested fungicides.

Alexander and Waldenmaier (2002) evaluated strobilurin fungicides azoxystrobin (Quadris), trifloxystrobin (Flint), and pyraclostrobin (Cabrio) and labelled for the control of anthracnose of chilli (0.1 %), and mancozeb (0.1%).

Saxena (2002) studied the efficacy of eight applications of fungicides like carbendazim, thiophanate methyl and hexaconazole at fortnightly intervals starting with the onset of the disease gave the best control of anthracnose of pomegranate caused by *C. gloeosporioides*.

Raghuwanshi *et al.* (2004) tested eight fungicides along with check (unsprayed) against leaf and fruit spot of pomegranate caused by *C. gloeosporioides* and found that carbendazim at 0.1 per cent was found to be effective reducing the per cent disease index (4.83) as compared to other fungicides tested.

Sundravadana *et al.* (2007) studied on the efficacy of azoxystrobin, one of the strobilurin class fungicides, was evaluated both *in vitro* and *in vivo* conditions. In in-vitro tests, azoxystrobin completely inhibited mycelial growth of *C. gloeosporioides*. In field experiment, azoxystrobin at 1, 2 and 4 ml/l significantly suppressed the development of both panicle and leaf anthracnose. The reduction of anthracnose incidence and yield increased curve obtained.

Filoda (2008) tested the efficacy of some fungicides (Sarfun 500 SC, Amistar 250 SC and Gwarant 500 SC) on mycelium growth of *C. gloeosporioides* the results showed that Sarfun 500 SC even at the twice reduced dose (0.05 %) concentration was most effective against this pathogen.

Singh (2013) evaluated the fungicides against anthracnose of mango and reported that foliar spray with carbendazim + mancozeb 0.2% found significantly superior over rest of the treatments in both years of evaluation followed by tricyclazole (0.1%). The efficacy of other fungicides viz., propineb (0.2%), mancozeb (0.2%), 23 chlorothalonil (0.2%), carbendazim (0.1%) and thiophanate methyl (0.1%) were also statistically on par over the control.

Kolase *et al.* (2014) observed among all systemic fungicides, carbendazim was found the most effective, while non-systemic fungicide, mancozeb showed the best inhibition of *C. gloeosporioides*, causing anthracnose of mango.

Jayalakshmi *et al.* (2015) studied management under field conditions and revealed that the treatments with foliar sprays of carbendazim + mancozeb (0.3 %) and propiconazole (0.1 %) reduced the disease drastically with high yield and good quality fruits.

Jagtap (2015) evaluated new synthetic fungicides against *C. gloeosporioides*. Among the non-systemic fungicides at 0.3 per cent concentration carbendazim + mancozeb showed 82.10 per cent inhibition of mycelial growth of the fungus followed by chlorothalonil with 75.80 per cent and least inhibition of mycelial growth was recorded in captan 63.48 per cent.

The systemic fungicides were evaluated against the pathogen at 0.05, 0.1, 0.15 per cent concentration. Among these concentrations all fungicides were significantly found superior at 0.15 per cent concentration compared to 0.1 and 0.05 per cent concentration. The maximum per cent inhibition of growth of *C. gloeosporioides* was observed in propiconazole (74.86%) followed by benomyl (68.17%), iprodione +carbendazim (67.67%), thiophanate methyl (64.97%). The least per cent inhibition of fungus was recorded in bittertanol (44.12%) and hexaconazole (32.62%).

Devanshu and Narendrappa (2016) screened fungicides against *C. gloeosporioides* in pomegranate and reported that combination product hexaconazole + zineb, Trifloxystrobin + Tebuconazole and a nonsystemic fungicide captan showed per cent inhibition after fungus at 100, 250, 500 and 1000 ppm concentrations. Similarly, systemic fungicides Hexaconazole, propiconazole, Penconazole, tebuconazole and carbendazim showed cent per cent mycelial inhibition at 500, 1000 and 2000 ppm concentrations.

Kumari *et al.* (2017) observed carbendazim was most effective *in vitro* and *in vivo*, for the control of *C. gloeosporioides* in mango.

Ranjitha *et al.* (2019) investigated the efficacy of 14 different fungicides in inhibiting the growth of the *C. gloeosporioides* under *In vitro* conditions. The result of the study indicated that, out of the 14 fungicides tested, a combi product Flurilazole + Carbendizim 37.5% WP and systemic fungicides Propiconazole 25% WP showed significantly superior inhibition over other fungicides with complete 100 per cent mycelial growth inhibition and absence of sporulation in all the three concentration *i.e,* 0.1%, 0.2%, 0.3% tested. Myclobutanil 10% WP showed inhibition of 97.2% followed by Tebuconazole + Trifloxystrobin 75% WG with 94.86% inhibition. Systemic fungicides Difenconazole 25% EC with 85.50% inhibition was at par with combi product (Zineb 68% +Hexaconazole 100% WP) with 85.06% inhibition.

Mahesh *et al.* (2020) conducted a study to screen the different chemicals under *In vitro* for the management of *C. gloeosporioides.* The bioefficacy of chemicals five each of contact (Copper oxy chloride, Captan, Mancozeb, Chlorothalonil and Zineb), systemic (Azoxystrobin, Carbendazim, Hexaconazole, Thiophanate methyl and Difenconazole) and combi fungicides (SAAF, Matco, Sectin, Melody Duo and Curzate) were evaluated under *In vitro* conditions against *C*. *gloeosporioides* for inhibition of radial growth on the PDA using poisoned food technique. The systemic fungicides were evaluated at 500 ppm, 1000 ppm and 1500 ppm concentration, whereas contact and combi fungicides were tried at 1000 ppm, 2000 ppm and 3000 ppm concentrations Among different systemic fungicides evaluated, Difenoconazole 25 EC was completely inhibited (100%) mycelial growth of the pathogen at all the three different concentrations tested. Among contact fungicides, Captan 50 WP was found effective in inhibition of mycelial growth to an extent of 89.26 (70.93) per cent followed by Chlorothalonil with mean mycelial inhibition of 86.17 (68.23) per cent. Similarly under combi fungicides evaluated, Sectin (Fenamidone 10 + mancozeb 50 WP) and SAAF were found effective in inhibiting mean mycelial growth of 91.23 (73.14) and 88.40 (73.63) per cent, respectively.

Mimrot *et al.* (2020) observed that azoxystrobin 8.3% + mancozeb 66.7% WG at 4 per cent at 10 per cent concentration was most effective in reducing the percent disease index of anthracnose of pomegranate under field conditions (*in vivo*).

GolakiyaIn *et al.* (2020) screened different fungicides against *C. gloeosporioides* and found that tebuconazole 25.9% EC and hexaconazole 5% EC effectively inhibited the cent per cent radial growth among systemic group of fungicides, while in non-systemic group of fungicides, captan 50% WP was quite effective and in case of ready mix fungicides azoxystrobin 11% + tebuconazole 18.30% SC, epoxiconazole 50g/l + pyraclostrobin 133g/l, tebuconazole 50% + trifloxystrobin 25% WG, zinab 68% + hexaconazole 4% WP were significantly inhibited the growth of test fungus under *in vitro*.

Evaluation of botanicals

Mukherjee *et al*. (2011) tested some plants extracts (tobacco leaf, keora seed, keora, mahogoni, gaint indian milky weed, garlic and ginger) at different concentrations (30%, 40%, 50%, 60% and 70%) for anti fungal efficacy against mycelial growth of *C*. *gloeosporioides*, causal agent of anthracnose of mango. The results revealed that the growth inhibition increased with the increase of concentration of all the plant extracts. Highest mycelial growth inhibition (74.35%) was observed in case of garlic extracts at 70% concentration. Garlic extract at 50% and 60% concentration were also effective than other treatments.

Jayalakshmi *et al.* (2013) conducted a study to evaluate the efficacy of plant extracts *viz.,* datura leaf, garlic bulb, ginger rhizome, eucalyptus leaf, tulasi leaf and neem leaf. Among these plant extracts, extract from leaves of datura and garlic bulbs at 30 per cent concentration gave higher level of inhibition of *C. gloeosporioides*.

Koshale *et al.* (2015) evaluated extracts of twelve plant species at three different levels of concentrations (5, 10 and 15%) for their antifungal activity under *in vitro* condition against *C. gloeosporioides*. The results from all experiments proved that extracts of all plants possessed fungistatic or fungicidal properties on growth of *C. gloeosporioides* which increased at higher extract concentrations. In general, better antifungal effect was observed with leaf extracts of *Mentha cordifolia* which had strong growth inhibition of *C. gloeosporioides* (29.33% and 42.78%) at 5 and 10 per cent concentration respectively and *Eucalyptus spp.* showed highest growth inhibition (63.48%) at 15 per cent concentration. Leaf extract of *Piper betle, Datura stramunium* and *M. cordifolia* exhibited a moderate action at 5, 10 and 15 per cent concentration respectively. The leaf extract of Fenugreek (*Trigonella foenumgraeum*) recorded lowest mean per cent growth inhibition for all the concentration (4.41, 10.74 and 11.52 per cent).

Asalkar *et al.* (2019) evaluated extracts of nine botanicals (each @ aqueous conc. of 10 %) under *In vitro* conditions against *C. gloeosporioides* and the results obtained on its mycelial growth and inhibitions revealed that all the nine botanicals evaluated were found fungistatic against *C. gloeosporioides* and recorded significantly reduced mycelial growth of the test pathogen over untreated control. At 10 per cent, mycelial growth inhibition of *C. gloeosporioides* ranged from 24.08 % (*Cathuranus roseus*) to 82.74 % (*Allium sativum*) per cent. However, significantly maximum mycelial growth inhibition was recorded with *A. sativum* (82.74 %). This was followed by the plant extracts *viz*., *Azadirachta indica* (80.77 %), *Ocimum sanctum*

(77.10 %), *Gliricidia maculate* L. (57.32 %), *Bougainvillea spectabilis* (54.75 %) *Allium cepa* (49.62 %), *Sapindus marginatus* (44.29 %) *Eucalyptus spp*. (38.83 %) and *C. roseus* (24.08 %).

Ranjitha *et al*. (2019b) conducted a study to evaluate the efficacy of aqueous extract and dry extracts of 12 different plant species against *C. gloeosporioides* the causal agent of mango anthracnose under natural disease epidemics. Significant difference was observed among the extracts in their effect on suppressing the mycelial growth of the pathogen under *In vitro* conditions. None of the extracts was able to completely prevent the development of the pathogen. However, most of the extracts significantly reduced disease development over the control. Among the fresh plant extracts, at 7.5% concentration, maximum inhibition was noticed in the *Simarouba glauca* extract with upto 59.41% mycelial inhibition followed by 50.03% by *Lawsonia inermis* and *Azadirachta indica* with 58.84 % mycelial inhibition. Among the dry powder extracts, at 7.5% concentration, *Moringa oleifera* showed a maximum inhibition of 40.81% followed by *S. glauca* with 37.64% inhibition and 32.25% inhibition with *Geranium sanguineum.*

De Alvindia and Mangoba (2020) conducted a study on bioactivities of *Allium longicuspis* against *C. gloeosporioides.* The study focused on the effect of *A. longicuspis* extracts (ALE) against anthracnose of mango fruit. *In vitro* tests (mycelial growth and conidial germination) showed that, ALE concentrated from 0.75 to 2.5 g L^{-1} completely inhibited the growth of *C. gloesporioides*. Cytoplasmic discharge, mycelial and conidial blasts were clearly observed when applied with ALE.

Nidiry and Lokesha (2020) conducted a study of the fungitoxic activity of extract of *Catharanthus roseus* (L.). The fungitoxic property of the extract of leaves and roots of *C. roseus* (L.) to the mycelial growth of *C. gloeosporioides* by poisoned food technique and spore germination of *Cladosporium cucumerinum* by TLC bioautography was evaluated.

CHAPTER –III

MATERIALS AND METHODS

The present study entitled "Studies on anthracnose of pomegranate caused by *Colletotrichum gloeosporioides* (Penz and Sacc.) and its management" was carried out at the Department of Plant Pathology, R.A.K. College of Agriculture, Sehore (M.P.) The materials used and methods followed during these studies are mentioned and described below.

Experimental site

The present studies were conducted at Research Farm of Fruit Research Station, Intkhedi, Bhopal (M.P.) under the jurisdiction of R.A.K. College of Agriculture, Sehore and at Department of Plant Pathology at the college during 2021.

Equipments and apparatus

The equipments and apparatus which have been used in the study are Laminar air flow, BOD incubator, Refrigerator, Autoclave, Glassware, Microscope, Hot air oven, pH meter, Electronic balance, Forceps, Inoculation Needle, Cork borer, Blade etc.

Cleaning and sterilization of equipments

Corning make glasswares were used during the period of investigation. All the glasswares were cleaned with chronic acid, followed by thorough washing with detergent powder and then rinsing tap water before use. The sterilization of media was done at 15lbs, pressure for 20 min. Petriplates were sterilized in hot air sterilizer at 180°C for 2 hrs. The plastic petriplates used in bio control study, were sterilized by alcohol. The isolation chamber was sterilized by alcohol, followed by ultraviolet exposure for 20 min. The other equipments used in isolation chamber like forceps, inoculation needle, cork borer, blade, etc. were sterilized by dipping them in alcohol, followed by heating on flame.

Sterilization procedure

A. Sterilization of glass wares

Glass wares were washed in liquid detergent under running tap water, and rinsed with distilled water 2-3 times. These were air-dried and then kept in oven for sterilization at 180 ^{0}C for at least 2 hrs. Plastic wares were autoclaved at 121 ^{0}C, 15 psi for 15 min.

B. Sterilization of inoculating needles, forceps, cork borer and workingtable

Clean inoculating needle was sterilized by dipping the loop of needle in spirit and heating over the flame until red hot. The process was repeated 2-3 times. Forceps and cork borer were also sterilized in the way of needle. The working table of laminar air flow was disinfected by sweeping with cotton soaked in absolute alcohol and exposing it to UV light for 30 minutes.

C. Sterilization of media and distilled water

Sterilized glassware and plastic wares were used for dispensing media and distilled water. All media were autoclaved at 121 ^{0}C, 15 psi pressure for 15-30 min.

D. Sterilization of laminar air flow

Prior to the day of inoculation of fungus sample, the laminar air flow was saturated with alcohol vapors. At the time of inoculation the laminar air flow chamber was wiped with 70% alcohol or general spirit. Then only required instruments were kept in the chamber and exposed to UV rays for 15-20 min. All the operation *viz*., transfer, inoculation etc. were done over a gas burner flame.

E. Culture media

All the solid media were sterilized in an autoclave at 15 lbs pressure (p.s.i) for 15 min. Liquid media were sterilized at 10 lbsp.s.i. for 10 min. and process was repeated after 24 hrs.

Preparation of culture medium

For isolation of target pathogen *in vitro* condition, potato dextrose agar (PDA) medium was used. For preparation of PDA, 200 g peeled potatoes were cut into slices and boiled in 500ml of distilled water in conical flask. The extract was strained through a piece of muslin cloth and 20 g dextrose was added in it. 20 g agar-agar was melted in 500ml of distilled water separately and was mixed in potato dextrose solution and the volume was made up to 1000 ml by adding distilled water. PDA was poured in flasks, plugged with non-absorbent cotton plugs and sterilized in an autoclave.

Isolation and purification of the pathogen

Pomegranate fruits and leaves infected with anthracnose were collected from experimental site and used for isolation of the fungus *in vitro*. The isolation of the fungus was made by using standard tissue isolation technique as described below.

Small pieces of infected leaves 1-2 mm dimension from the advancing margin of the spot, adjacent to healthy portions were cut with blade, washed well in distilled water to remove dust adhered to the infected pieces. Pieces were dipped in 0.1 per cent mercuric chloride solution for 30 seconds and finally washed well in three changes of sterilized distilled water. The bits were then transferred to PDA slants with the help of inoculating needle under aseptic condition and incubated at 28±1ºc. After 72 hrs, fragments of hyphal growth from the growing tips were transferred to fresh PDA slants. Pure culture was made, following repeated hyphal tip transfer. Pure culture was maintained on PDA slants by sub culturing it at 30 days intervals. For preservation of cultures the plugged end of the culture tubes were dipped in melted wax and stored in a refrigerator at 5±1ºC.

Pathogenicity

To test the pathogenicity of *C. gloeosporioides* detached leaf technique was used. Healthy leaves of pomegranate selected for inoculation, were cut along with twig and brought to the laboratory. The twigs were cut with the sterilized sharp razor blade. Single leaf was placed inside each petri plate. Leaves were sprayed with culture suspension of *C. gloeosporioides* with the help of atomizer. Petri plates with inoculated leaves were kept under high humidity with blotter papers. They were kept at room temperature and observed frequently for development of symptoms.

Periodic observation an anthracnose development and their relationship with weather parameters

Influence of rainfall, maximum and minimum temperature and relative humidity was examined on the development of anthracnose in the field. The size of plot was 5 × 5 m.

The percent disease index recorded by using the formula given by Narasimhudu (2007). Observations of percent disease index were scored by using 0-5 scale which was recommended by Wheeler (1969) as follows:

Rating	Leaf area infection (%)
0	0
1	1-10
2	11-20
3	21-30
4	31-50
5	50<

The percent disease index (PDI) was calculated by applying the following formula

$$PDI = \frac{\text{Sum of individual rating}}{\text{No. of leaves examined}} X \frac{100}{\text{Max. disease rating}}$$

The absolute disease intensity was calculated as the difference between two successive observations of PDI and correlation coefficient (‘r’) was computed between the absolute disease intensity and different meteorological factors like maximum and minimum temperature, relative humidity, and rainfall. The significance of correlation coefficient was judged by ‘t’ test as per standard statistical methods.

The data on disease intensity were plotted against meteorological parameters to predict the correlation between disease and environment. Regressions equations were also computed and scatter diagram were drawn.

Data on meteorological observations during the period was obtained from Meteorological Department, Fruit Research Station, Intkhedi on temperature, relative humidity, and rainfall (Table-3.1).

Table 3.1: Weekly meteorological data from July to October, 2021

Month	Standard Week	Week	Temperature (^{0}C)		Humidity (%)	Rainfall (mm)
			Max.	Min.		
July	27	2-8	36.0	24.9	70.0	0.0
	28	9-15	35.9	26.2	71.6	13.0
	29	16-22	35.4	25.2	69.1	0.0
	30	23-29	27.8	22.5	99.0	197.6
	31	30-05 Aug	27.3	24.1	99.0	89.6
August	32	06-12	32.3	23.9	98.5	81.5
	33	13-19	32.6	26.7	85.6	13.9
	34	20-26	29.4	24.5	86.2	53.5
	35	27-02 Sep	27.8	25.0	86.5	64.5
September	36	03-09	34.4	26.1	90.7	68.5
	37	10-16	35.2	25.6	91.3	74.3
	38	17-23	32.2	24.2	92.0	35.1
	39	24-30	31.3	25.7	91.8	30.8
October	40	01-07	33.9	25.8	87.1	34.0
	41	08-14	34.0	22.5	70.9	0.0
	42	15-20	31.2	21.4	85.8	62.5
	43	21-27	30.7	19.3	72.8	0.0

These were plotted against various degrees of disease intensities. On the basis of week to week observations, environmental conditions prevailed during the course of disease development were examined.

In vitro* efficacy of fungicides on mycelial growth of *Colletotrichum gloeosporioides

The selected fungicides listed below were evaluated at 500, 1000 and 1500 ppm against the pathogen for radial growth of mycelial under *in vitro* condition by “Poisoned Food Technique” (Grover and Moore, 1961).

The fungicides were weighed as per the desired concentrations and mixed thoroughly in the 250 ml conical flasks containing 60 ml sterilized and warm PDA under aseptic conditions. The uniform mixture was then poured in the sterilized Petri plates @ 20 ml per plate and allowed to solidify. Mycelial disc of five mm diameter was cut from 7 days old culture of pathogen and was inoculated in the centre of each plate. The inoculated plates were incubated at 28±10 ^{0}C till the pathogen completely occupied the control plate. Three replications were maintained for each fungicide, while the plates without fungicides were taken as control. The observations on radial growth (colony diameter) were recorded 3rd, 5th and 7th day after incubation. The per cent inhibition over control was calculated according to formula given by Vincent (1947). Percent inhibition of mycelial growth was calculated by the following formula,

Inhibition % = C–T/C × 100

Whereas

C = Diameter of fungus colony (mm) in control plate T = Diameter of fungus colony (mm) in treated plate

Experimental details

Design	:	Factorial CRD
Replications	:	3
Treatments	:	9

Symbol	Treatment
T_1	Difenoconazole 25% EC 0.1%
T_2	Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC) 0.05%
T_3	Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC) 0.075%
T_4	Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC) 0.1 %
T_5	Metiram 70% WP 0.1%
T_6	Propineb 70%WP 0.1%
T_7	Fluxapyroxad 300g/l SC 0.03%
T_8	Metiram 55%+pyraclostrobin 5% WG 0.35%
T_9	Control

Field evaluation of fungicides against anthracnose of pomegranate

The fungicides against anthracnose further evaluated in field against the disease. The details of experiments are as follows:

Experiment details:

Location		Experimental area of Fruit Research Station (FRS), Intkhedi, under R.A.K. College of Agriculture, Sehore, RVSKVV,Gwalior
Variety		Bhagwa
Treatments		09
Replications		03
Year		2021-22
Age of Plant		3 years
Design	:	RBD
Plot size	:	5 m x 5 m

Treatment details

S. No.	Treatment	Dose
T_1	Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC)	0.5ml/l
T_2	Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC)	0.75 ml/l
T_3	Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC)	1.0 ml/l
T_4	Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC)	1.25 ml/l
T_5	Difenoconazole25% EC	1.0 ml/l
T_6	Fluxapyroxad 300g/l SC	0.3 ml/l
T_7	Metiram 55% + pyraclostrobin 5% WG	3.5 g/l
T_8	Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC)	2.5 ml/l
T_9	Control	-

The percent disease index recorded by using the formula given by Narasimhudu (2007). Observations of percent disease index were scored by using 0-5 scale as follows:

Rating	Leaf area infection (%)
0	0
1	1-10
2	11-20
3	21-30
4	31-50
5	50<

The percent disease index (PDI) was calculated by applying the following formula.

$$PDI = \frac{\text{Sum of individual rating}}{\text{No. of leaves examined}} \times \frac{100}{\text{Max. disease rating}}$$

In-vitro evaluation of botanicals against _Colletotrichumgloeosporioides_

The effect of extract of seven botanicals *viz.,* Sadabahar leaf extract, Eucalyptus leaf extract, Turmeric rhizome extract, Onion bulb extract, Tulsi leaf extract, Kaner leaf extract and Karanj leaf extract on the growth of *C. gloeosporioides* was studied at 10 and 20 per cent. The medium without botanicals served as control. The pathogen was inoculated at the centre of the plate by placing a 5-days-old 5 mm culture disc of the pathogen. The plates were kept in an incubator at 25±1 ^{0}C and three replications were maintained for each medium and the radial growth of the fungus was measured seven days after inoculation (DAI).

Experimental details

Design : CRD Factorial

Replications : 3

Treatments: (a) Main treatments : 8

(b) Sub treatments : 2 (10% and 20%)

Treatments details

S. No.	Treatment
T_1	Sadabahar leaf extract
T_2	Eucalyptus leaf extract
T_3	Turmeric rhizome extract
T_4	Onion bulb extract
T_5	Tulsi leaf extract
T_6	Kaner leaf extract
T_7	Karanj leaf extract
T_8	Control

CHAPTER- IV

RESULTS

In 2021, studies were undertaken on several aspects as isolation, identification, pathogenicity, and management of anthracnose caused by *C. gloeosporioides* in pomegranates. Investigations were performed under controlled settings for pathogen isolation, identification, and testing pathogenicity. Studies with fungicides and their combinations were undertaken under both *in vivo* and *in vitro* conditions whereas, botanicals were tested *in vitro*.

Isolation and purification

The pathogen isolation was carried out by Standard tissue isolation technique from pomegranate fruits showing typical anthracnose symptoms. Repeated isolation yielded an isolate of C*olletotrichum gloeosporioides*.

Identification

Identification of the pathogen was carried out based on the morphological characters. The fungus in the present study produced septate mycelium. Later it produced conidiophores arising singly or closely packed together in rows. Conidiophores were single celled, hyaline and aseptate with one or several conidial scars. The conidia were oblong or cylindrical or slightly dumbel, hyaline, aseptate with rounded ends, one to two oil globules were observed in the conidium (Plate 1a).

Symptomatology

The typical major anthracnose symptoms were observed on leaves and fruits.

Symptoms on leaves

Pinhead size of black to brown water soaked spots appeared on the leaves with circular margin. In advanced stage, these spots enlarged, amalgamate and resulted in larger patches. In severe case, leaves dried up and drooped down (Plate 1b).

Symptoms on fruits

On fruits, brown spherical depressed spots occurred in scattered kind on the pericarp. In advanced stage, these spots amalgamated to make necrotic patches over the surface of the fruit. Once such unhealthy fruits were cut open the decay symptoms were ascertained. Brown to dark brown coloured seeds was seen in infected fruits. In advanced stage will observe the minute dark coloured acervulli were ascertained on depressed spots. Looking to those symptoms, the sickness is referred as “anthracnose” (Plate 1c).

Pathogenicity

Pure culture of *C. gloeosporioides* was used for test of pathogenicity of pathogen by using detached leaf technique. Leaves inoculated with culture of the pathogen produced characteristic anthracnose symptoms (Plate 2). However, leaves inoculated on upper surface tissue, showed the lesion development start after five days of inoculation. Minute pin head black to brown water soaked spots appear after five days of inoculation and spots turned slightly brown surrounded by chlorotic halo, while on tenth day these spots enlarged and showed dark brown colour surrounded by slight chlorotic halo. After ten days the central portion of spots showed minute dark coloured acervulli, later these spots coalesced. On re-isolation, the culture of *C. gloeosporioides* was obtained confirming the Koch’s postulates.

Periodic observation an anthracnose development and their relationship with weather parameters

The development of anthracnose intensity was studied in relation to various climatic parameters. The absolute anthracnose intensity recorded every week and correlated with mean maximum and minimum temperature, relative humidity and rainfall (mm) of the corresponding meteorological week and correlation coefficient (r) value was computed for each parameter given in Table 4.1.

To quantity the effect of various parameters the linear regression equation was also worked out (Table 4.1a).

Maximum temperature

Maximum temperature was found to have a significant positive correlation with anthracnose of pomegranate (r=0.57).The regression equation showed that anthracnose of pomegranate intensity increased by 0.158% with the increase of 1^0C maximum temperature (Table 4.1 and Fig. 4.1).

Minimum temperature

Minimum temperature was found to have a significant positive correlation with anthracnose of pomegranate (r=0.62). The regression equation showed that anthracnose intensity increased by 0.075% with increase of 1^0C minimum temperature (Table 4.1 and Fig. 4.2).

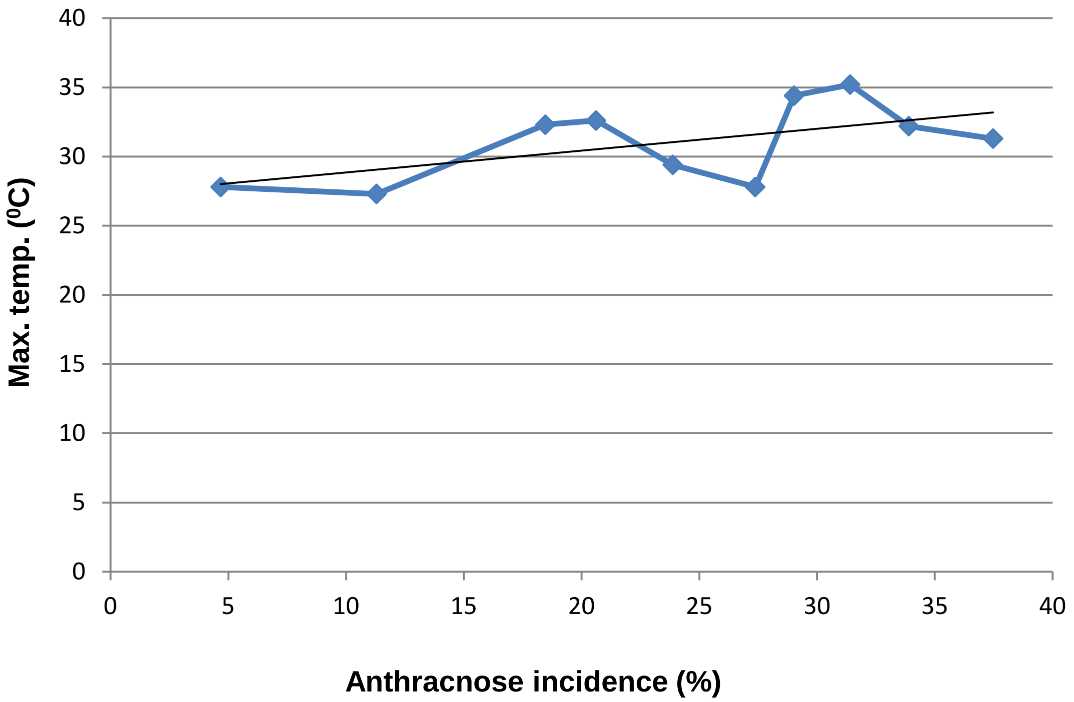


Fig.1: Regression line between anthracnose incidence and maximum temprature

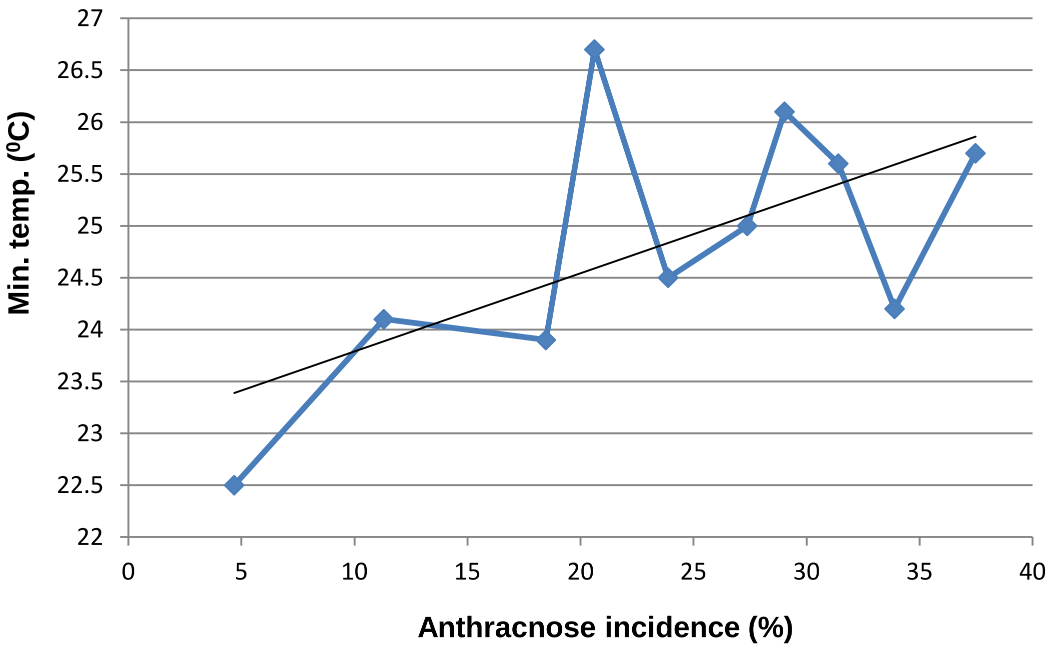


Fig.2: Regression line between anthracnose incidence and minimum temprature

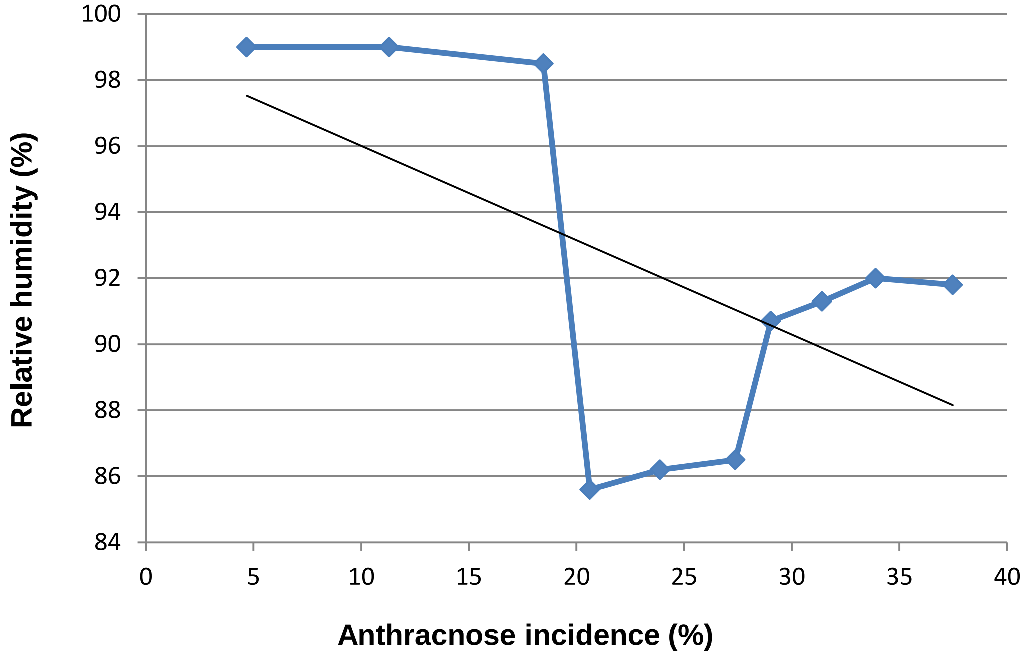


Fig.3: Regression line between anthracnose incidence and relative humidity

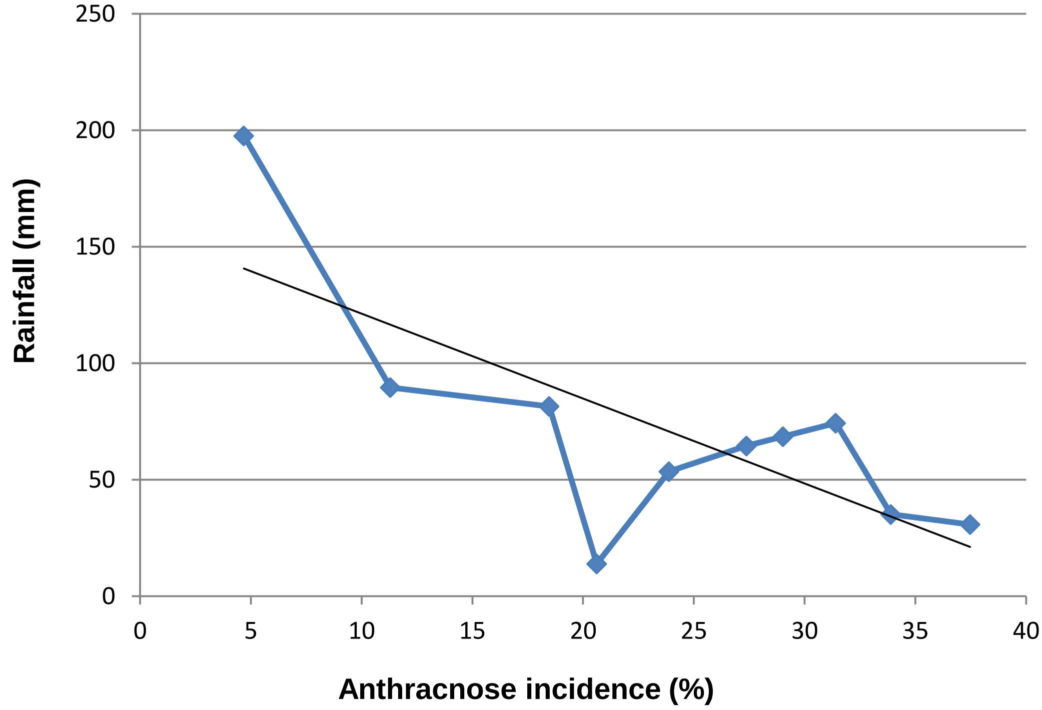


Fig.4: Regression line between anthracnose incidence and rainfall (mm)

Table 4.1: Correlation coefficient (r) values between intensity of anthracnose of pomegranate and various weather parameters

Standard Week	Meteorological duration	Anthracnose incidence (%)	(Max.) Temp. (^{0}C)	(Min.) Temp. (^{0}C)	Relative humidity (%)	Total rainfall (mm)
30	23 July-29 July	4.68	27.8	22.5	99.0	197.6
31	30 July-05 Aug.	11.30	27.3	24.1	99.0	89.6
32	06 Aug.-12 Aug.	18.47	32.3	23.9	98.5	81.5
33	13 Aug.-19 Aug.	20.62	32.6	26.7	85.6	13.9
34	20 Aug.-26 Aug.	23.88	29.4	24.5	86.2	53.5
35	27 Aug.-02 Sep.	27.38	27.8	25.0	86.5	64.5
36	03 Sep.-09 Sep.	29.03	34.4	26.1	90.7	68.5
37	10 Sep.-16 Sep.	31.41	35.2	25.6	91.3	74.3
38	17 Sep.-23 Sep.	33.9	32.2	24.2	92.0	35.1
39	24 Sep.-30 Sep.	37.48	31.3	25.7	91.8	30.8
Coefficient of correlation (*r*)			0.57	0.62	-0.56	-0.74
Test of significance (t)			1.99	2.25	-1.91	-3.11

Table 4.1a: Regression equation between intensity of anthracnoseof pomegranate and various weather parameters

Weather parameter	Regression equation
Maximum temperature	Y= 0.158x + 27.26
Minimum temperature	Y= 0.075x + 23.03
Relative humidity	Y= -0.285x + 98.86
Rainfall	Y= -3.643x + 157.7

Relative humidity

Maximum humidity was found to have a significant negative correlation with anthracnose intensity (r=-0.56). The regression equation indicated that with the every 1 per cent increase in relative humidity the disease intensity decreased by 0.285 per cent (Table 4.1 and Fig. 4.3).

Rainfall

Rainfall (mm) was found to have a significant negative correlation with anthracnose of pomegranate (r=-0.74). The regression equation showed that anthracnose intensity decrease by 3.643% with increase of one mm of rainfall (Table 4.1 and Fig. 4.4).

In-vitro* evaluation of new fungicides against *C. gloeosporioides

At 500 PPM

In vitro evaluation of fungicides was investigated by poison food technique at 500 PPM. Observations on radial growth of *C. gloeosporioides* were noticed after third, fifth and seventh day of incubation (Table 4.2 and Plate 3).

Table 4.2:Radial growth of *Colletotrichum gloeosporioides* in fungicides amended medium at 500 PPM

S.No.	Treatment	Radial growth (mm)*			
		3rd day after incubation	5th day after incubation	7th day after incubation	Mean
1	Difenoconazole 25% EC (0.1%)	7.00	13.00	18.00	12.67
2	Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.05%)	0.00	0.00	0.00	0.00
3	Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.075%)	0.00	0.00	0.00	0.00
4	Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.1%)	0.00	0.00	0.00	0.00
5	Metiram 70% WP (0.1%)	9.33	16.67	21.67	15.89
6	Propineb 70%WP (0.1%)	0.00	0.00	0.00	0.00
7	Fluxapyroxad 300g/l SC (0.03%)	9.33	11.67	22.00	14.33
8	Metiram 55%+ pyraclostrobin 5% WG (0.35%)	8.67	17.00	22.00	15.89
9	Control	14.00	51.33	86.67	50.67
Mean		5.37	12.19	18.93	
		SEm(±)		**C.D. at 5%**	
Day (D)		0.29		0.81	
Treatment (T)		0.50		1.40	
Interaction (D×T)		0.86		2.43	

*Mean of three replications

The efficacy of different fungicides against *C. gloeosporioides* are depicted in table 4.2. According to the investigation, all the treatments were significantly superior over control in decreasing mycelial growth of *C. gloeosporioides*. No radial mycelial growth was observed in Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.05%), Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.075%), Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.1%) and Propineb 70%WP (0.1%). It was significantly superior over other fungicides used followed by Difenoconazole 25% EC (0.1%) (12.67 mm), Fluxapyroxad 300g/l SC (0.03%) (14.33 mm), Metiram 70% WP (0.1%) (15.89 mm) and Metiram 55%+Pyraclostrobin 5% WG (0.35%) (15.89 mm) as compared to untreated control (50.67 mm). All these treatments showed significant differences excepted Metiram 70% WP (0.1%) and Metiram 55%+Pyraclostrobin 5% WG. Among the different period of intervals, 5.37 mm mycelial growth noticed after third day of incubation, 12.19 mm mycelial growth noticed after fifth day of incubation and 18.93 mm mycelial growth noticed after seventh day of incubation. The interaction between fungicides and time period was also significant.

At 1000 PPM

In vitro evaluation of fungicides was investigated by poison food technique at 1000 PPM. Observations on radial growth of *C. gloeosporioides* were noticed after third, fifth and seventh day of incubation (Table 4.3 and Plate 4).

The efficacy of different fungicides against *C. gloeosporioides* are depicted in table 4.3. According to the investigation, all treatments were significantly superior over control in decreasing mycelial growth. No radial mycelial growth was observed in Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.05%), Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.075%), Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.1%) and Propineb 70%WP (0.1%) followed by Difenoconazole 25% EC (0.1%) (9.67 mm), Fluxapyroxad 300g/l SC (0.03%) (10.56 mm), Metiram 70% WP (0.1%) (10.89 mm) and Metiram 55%+pyraclostrobin 5% WG (0.35%) (11.67 mm) as compared to untreated control (52.00 mm). In All of these followed treatments, between Diphenoconazole 25% EC (0.1%) and Fluxapyroxad 300g/l SC (0.03%), between Fluxapyroxad 300g/l SC (0.03%) and Metiram 70% WP (0.1%) as well as between Metiram Between 70% WP (0.1%) and Metiram 55%+pyraclostrobin 5% WG (0.35%) were not seen significant difference to each other. Among the different period of intervals, 4.93 mm mycelial growth noticed after third day of incubation, 10.85 mm mycelial growth noticed after fifth day of incubation and 15.81 mm mycelial growth noticed after seventh day of incubation. The interaction between fungicides and time period was also significant.

Table 4.3:Radial growth of *Colletotrichum gloeosporioides* in fungicides amended medium at 1000 PPM

S.No.	Treatment	Radial growth (mm)*			
		3rd day after incubation	5th day after incubation	7th day after incubation	Mean
1	Difenoconazole 25% EC (0.1%)	7.00	10.00	12.00	9.67
2	Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.05%)	0.00	0.00	0.00	0.00
3	Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.075%)	0.00	0.00	0.00	0.00
4	Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.1%)	0.00	0.00	0.00	0.00
5	Metiram 70% WP (0.1%)	7.00	11.33	14.33	10.89
6	Propineb 70%WP (0.1%)	0.00	0.00	0.00	0.00
7	Fluxapyroxad 300g/l SC (0.03%)	7.33	10.67	13.67	10.56
8	Metiram 55%+ pyraclostrobin 5% WG (0.35%)	6.67	13.33	15.00	11.67
9	Control	16.33	52.33	87.33	52.00
Mean		4.93	10.85	15.81	
		SEm(±)		**C.D. at 5%**	
Day (D)		0.21		0.59	
Treatment (T)		0.36		1.02	
Interaction (D×T)		0.62		1.76	

*Mean of three replications

At 1500 PPM

In vitro evaluation of fungicides was investigated by poison food technique at 1500 PPM. Observations on radial growth of *C. gloeosporioides* were noticed after third, fifth and seventh day of incubation (Table 4.4 and Plate 5).

The efficacy of different fungicides against *C. gloeosporioides* are depicted in table 4.4. According to the investigation, all treatments were significantly superior over control in decreasing mycelial growth *C. gloeosporioides*. No radial mycelial growth was observed in Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.05%), Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.075%), Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.1%) and Propineb 70%WP (0.1%) followed by Difenoconazole 25% EC (0.1%) (7.67 mm), Fluxapyroxad 300g/l SC (0.03%) (8.00 mm),

Table 4.4:Radial growth of *Colletotrichum gloeosporioides* in fungicides amended medium at 1500 PPM

S.No.	Treatment	Radial growth (mm)*			
		3rd day after incubation	5th day after incubation	7th day after incubation	Mean
1	Difenoconazole 25% EC (0.1%)	5.00	7.00	10.67	7.56
2	Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.05%)	0.00	0.00	0.00	0.00
3	Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.075%)	0.00	0.00	0.00	0.00
4	Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.1%)	0.00	0.00	0.00	0.00
5	Metiram 70% WP (0.1%)	6.00	8.67	10.67	8.44
6	Propineb 70%WP (0.1%)	0.00	0.00	0.00	0.00
7	Fluxapyroxad 300g/l SC (0.03%)	5.33	8.00	10.67	8.00
8	Metiram 55%+ pyraclostrobin 5% WG (0.35%)	5.67	8.67	11.33	8.56
9	Control	16.00	51.00	86.67	51.22
Mean		4.22	9.26	14.44	
		SEm(±)		**C.D. at 5%**	
Day (D)		0.25		0.71	
Treatment (T)		0.44		1.23	
Interaction (D×T)		0.75		2.14	

*Mean of three replications

Metiram 70% WP (0.1%) (8.44 mm) and Metiram 55%+pyraclostrobin 5% WG (0.35%) (8.56 mm) as compared to untreated control (51.22 mm). All the followed treatments were at par with one another and showed no significant difference. Among the different period of intervals, 4.22 mm mycelial growth noticed after third day of incubation, 9.26 mm mycelial growth noticed after fifth day of incubation and 14.44 mm mycelial growth noticed after seventh day of incubation. The interaction between fungicides and time period was also significant.

Field evaluation of fungicides against anthracnose of pomegranate

The experiment was conducted during 2021, with eight treatments and one untreated control. Two sprays were given at the interval of 15 days. The observation on the anthracnose development was recorded at seven days interval after spray. The data are presented in Table 4.5.

Pre treatment

Before any spray of fungicides, anthracnose intensity was at par in all the treatments.

After First spray

Observations were taken at the interval of seven days after first spray. All the treatments showed significant effect in reducing growth of *C. gloeosporioides*. Minimum percent disease index was recorded in Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC) 1.25ml/l water which was at par with Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC) 1.0 ml/l water (15.80) and Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC) 2.5ml/l water (16.47%) and was followed by Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC) 0.75ml/l water (18.10%), Metiram 55%+pyraclostrobin 5% WG 3.5 ml/l water (18.17%), Difenoconazole 25% EC 1.0 ml/l water (18.77%) and Fluxapyroxad 300g/l SC 0.3 ml/l water (19.13%).

Maximum percent disease index was recorded in control (24.53%). Apart from control maximum percent disease index was recorded in Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC) 0.5ml/l water (20.13%).

After second spray

Observations were taken at the interval of seven days after second spray. All the treatments showed significant effect in reducing growth of *C. gloeosporioides*. Minimum per cent disease index (8.47%) was recorded in Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC) 1.25ml/l water which was at par with Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC) 1.0 ml/l water (12.07%), Metiram 55%+pyraclostrobin 5% WG 3.5 ml/l water (12.37%), Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC) 2.5ml/l water (13.00%), Difenoconazole 25% EC 1.0 ml/l water (13.37%), Fluxapyroxad 300g/l SC 0.3 ml/l water (13.83%) and Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC) 0.75 ml/l water (13.87%).Maximum percent disease index was recorded in control (26.40%). Apart from control maximum percent disease index was recorded from Fluxapyroxad 75g/l+Difenoconazole 50g/l (SC) 0.5ml/l water (16.53%).

Table 4.5: *In vivo* evaluation of fungicides against *Colletotrichum gloeosporioides* of pomegranate

S.No.	Treatment	Percent disease index*			
		Pre - treatment	After first spray	After second spray	Mean
1	Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC) 0.5ml/l water	22.37 (28.22)	20.13 (26.66)	16.53 (23.99)	19.68 (26.29)
2	Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC) 0.75ml/l water	20.83 (27.16)	18.10 (25.17)	13.87 (21.85)	17.60 (24.72)
3	Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC) 1.0 ml/l water	18.87 (25.74)	15.80 (23.41)	12.07 (20.31)	15.58 (23.15)
4	Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC) 1.25 ml/l water	17.53 (24.75)	15.53 (23.21)	8.47 (15.72)	13.84 (21.23)
5	Difenoconazole 25% EC 1.0 ml/l water	21.37 (27.50)	18.77 (25.66)	13.37 (21.41)	17.83 (24.86)
6	Fluxapyroxad 300g/l SC 0.3 ml/l water	20.90 (27.20)	19.13 (25.93)	13.83 (21.79)	17.96 (24.98)
7	Metiram 55%+ pyraclostrobin 5% WG 3.5 ml/l water	20.73 (27.08)	18.17 (25.20)	12.37 (20.57)	17.09 (24.28)
8	Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC) 2.5 ml/l water	19.67 (26.32)	16.47 (23.90)	13.00 (21.01)	16.38 (23.75)
9	Control	23.7 (29.04)	24.53 (29.69)	26.40 (30.91)	24.83 (29.88)
	Mean	20.65 (26.99)	18.51 (25.42)	14.43 (21.95)	
	SEm(±)	0.85	0.66	1.81	
	C.D. at 5%	NS	1.97	5.41	

*Mean of three replications

() Data in parentheses are arc sine transformed values

4.7 *In-vitro* efficacy of botanicals against *C. gloeosporioides*

C. gloeosporioides was tested under *in vitro* condition with seven botanicals, including Sadabahar leaf extract, Eucalyptus leaf extract, Turmeric rhizome extract, Onion bulb extract, Tulsi leaf extract, Kaner leaf extract and Karanj leaf extract. After the seventh day of incubation, observations on *C. gloeosporioides* radial growth were taken (Plate 6). The data presented in Table 4.6.

Significantly minimum growth of *C. gloeosporioides* (41.53 mm) was noticed at 20 per cent concentration followed by 10 per cent (45.80 mm). In absence of botanical *C. gloeosporioides* showed the radial growth of 89.20 mm. At 10 per cent concentration, among the botanicals minimum growth (31.50 mm) were obtained in treatment Turmeric rhizome extract followed by Karanj leaf extract (36.00 mm), Tulsi leaf extract (37.80 mm), Sadabahar leaf extract (40.50 mm), Eucalyptus leaf extract (41.40 mm), Kaner leaf extract (43.20 mm) and Onion bulb extract (46.80 mm) as compared to control (89.20 mm) (Plate 6).

Table 4.6: Radial mycelial growth of *Colletotrichum gloeosporioides* in botanicals amended medium at 10 and 20 per cent

S.No.	Treatment	Radial growth (mm)*			
		10 per cent	Reduction over control (%)	20 per cent	Reduction over control (%)
1	Sadabahar leaf extract	40.50	54.60	36.00	59.64
2	Eucalyptus leaf extract	41.40	53.59	37.80	57.62
3	Turmeric rhizome extract	31.50	64.69	23.40	73.77
4	Onion bulb extract	46.80	47.53	44.10	50.56
5	Tulsi leaf extract	37.80	57.62	32.40	63.67
6	Kaner leaf extract	43.20	51.56	40.50	54.60
7	Karanj leaf extract	36.00	59.64	28.80	67.71
8	Control	89.20	-	89.20	-
		SEm(±)		**C.D. at 5%**	
Concentration (C)		0.40		1.15	
Treatment (T)		0.69		2.00	
Interaction (C×T)		1.20		3.46	

*Mean of three replications

At 20 per cent concentration, minimum growth (23.40 mm) were obtained in treatment Turmeric rhizome extract followed by Karanj leaf extract (28.80 mm), Tulsi leaf extract (32.40 mm), Sadabahar leaf extract (36.00 mm) and Eucalyptus leaf extract (37.80 mm). Mycelial growth of less than 40 mm was observed in all these botanical amended mediums whereas, while the remaining mediums *viz*., Kaner leaf extract (40.50 mm) and Onion bulb extract (44.10 mm) showed higher growth.

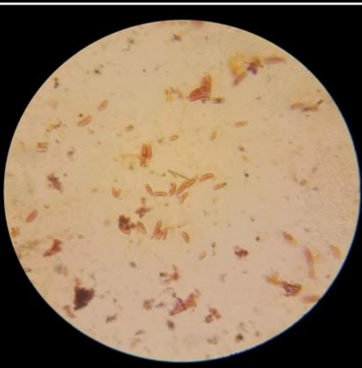
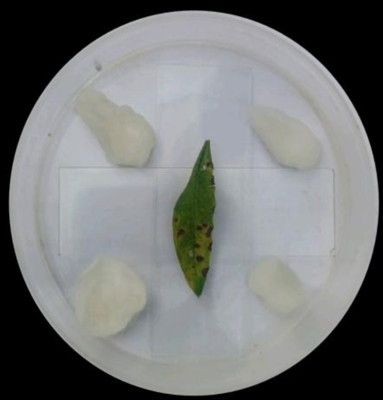


Plate-1a: Conidia of isolate of *C. gloeosporioides*

Plate-1b: Symptoms of anthracnose on pomegranate leaves

Plate-1c: Symptoms of anthracnose on pomegranate fruit

Healthy leaf



Diseased leaf

Plate-2: Pathogenicity of pathogen by using detached leaf technique

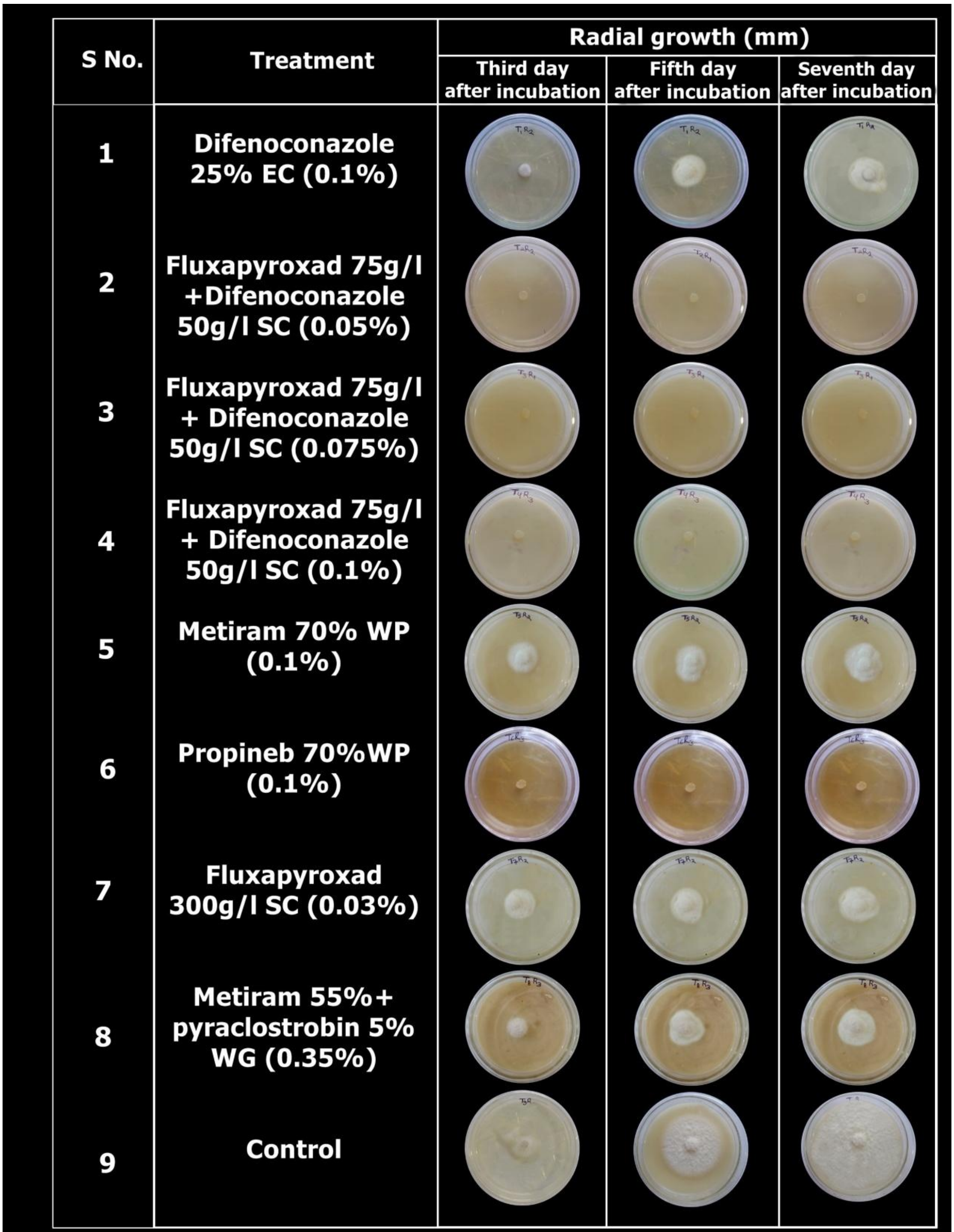


Plate-3: Radial growth of *Colletotrichum gloeosporioides* in fungicides amended medium at 500 PPM

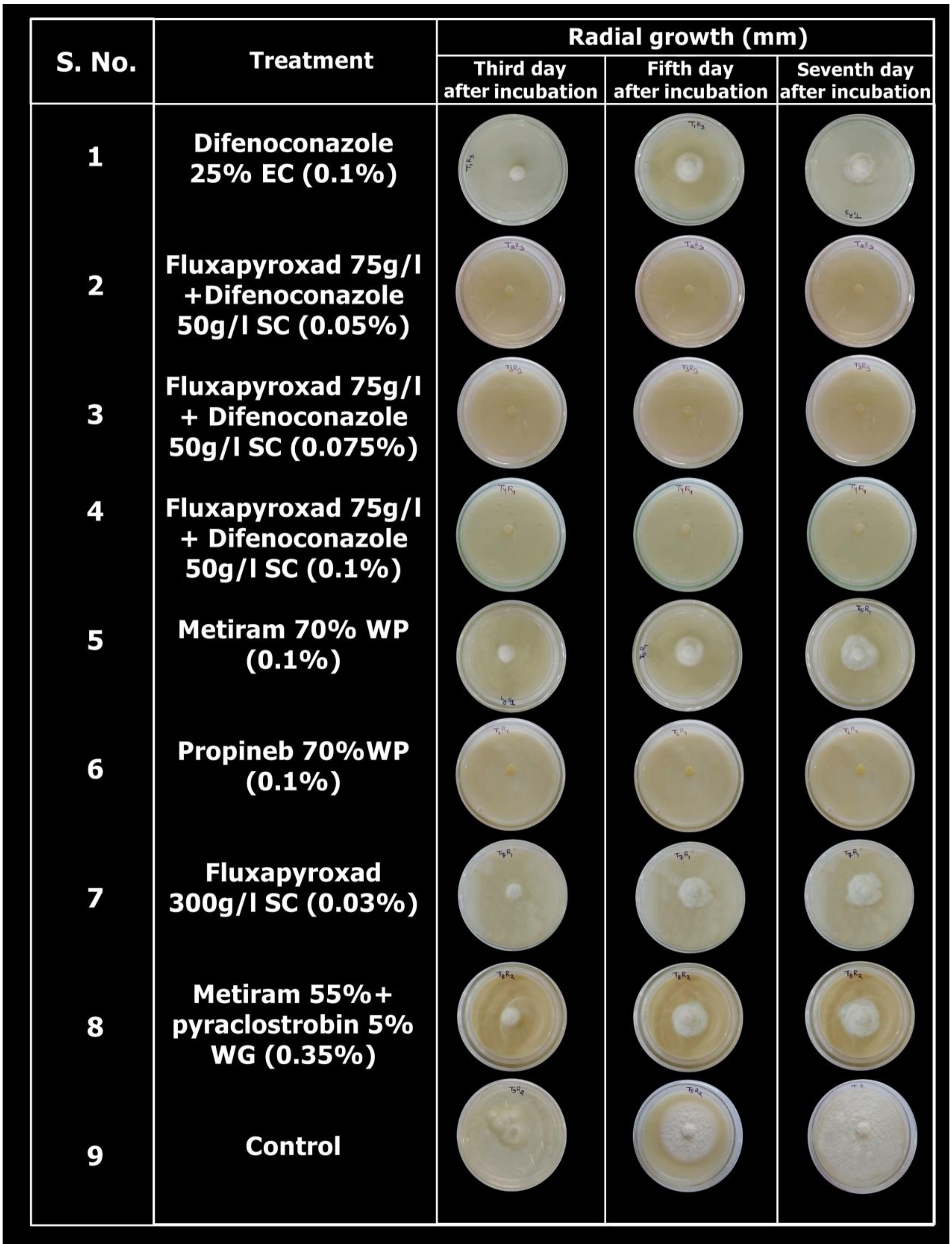


Plate-4: Radial growth of *Colletotrichum gloeosporioides* in fungicides amended medium at 1000 PPM

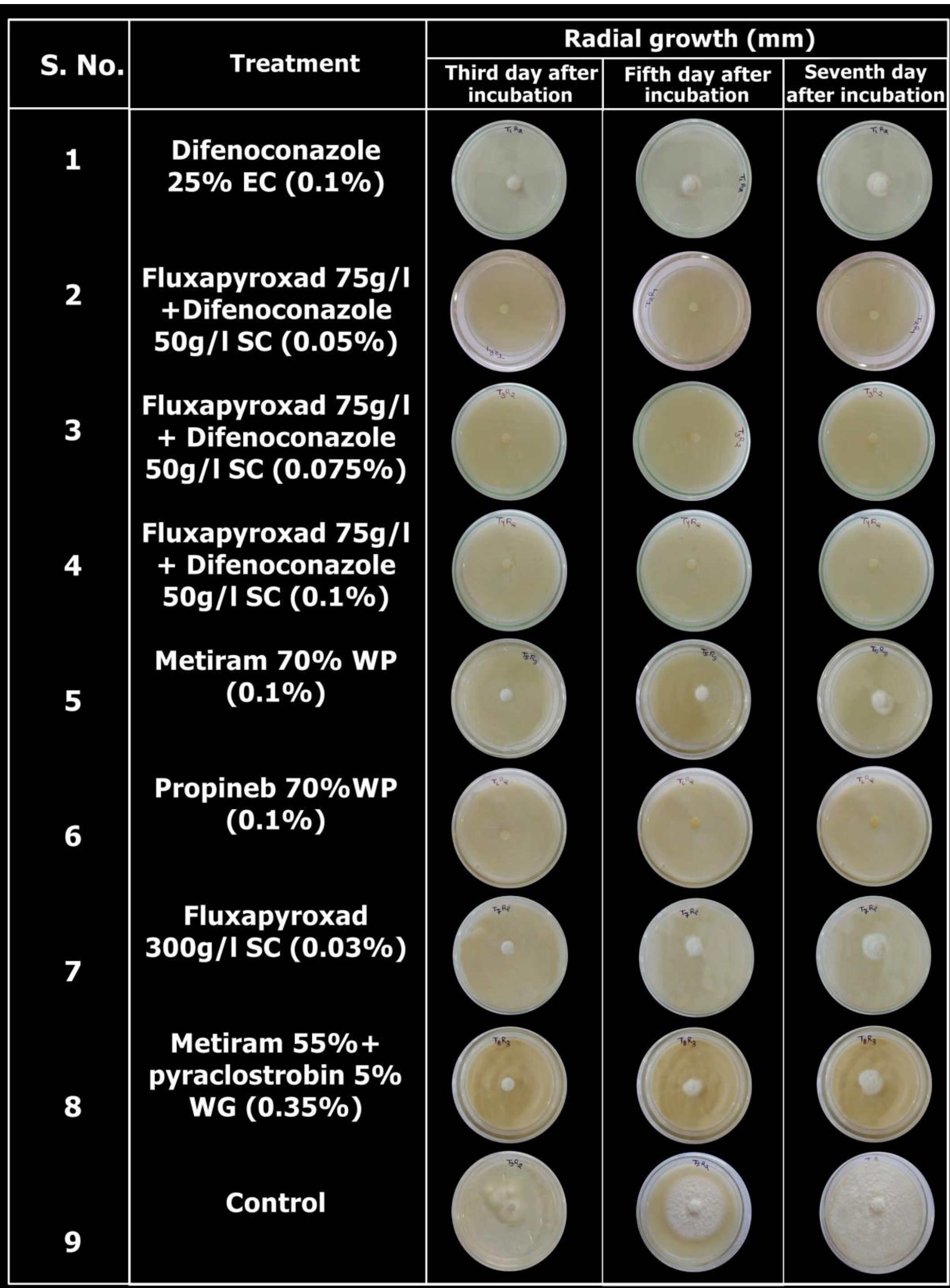


Plate-5: Radial growth of *Colletotrichum gloeosporioides* in fungicides amended medium at 1500 PPM

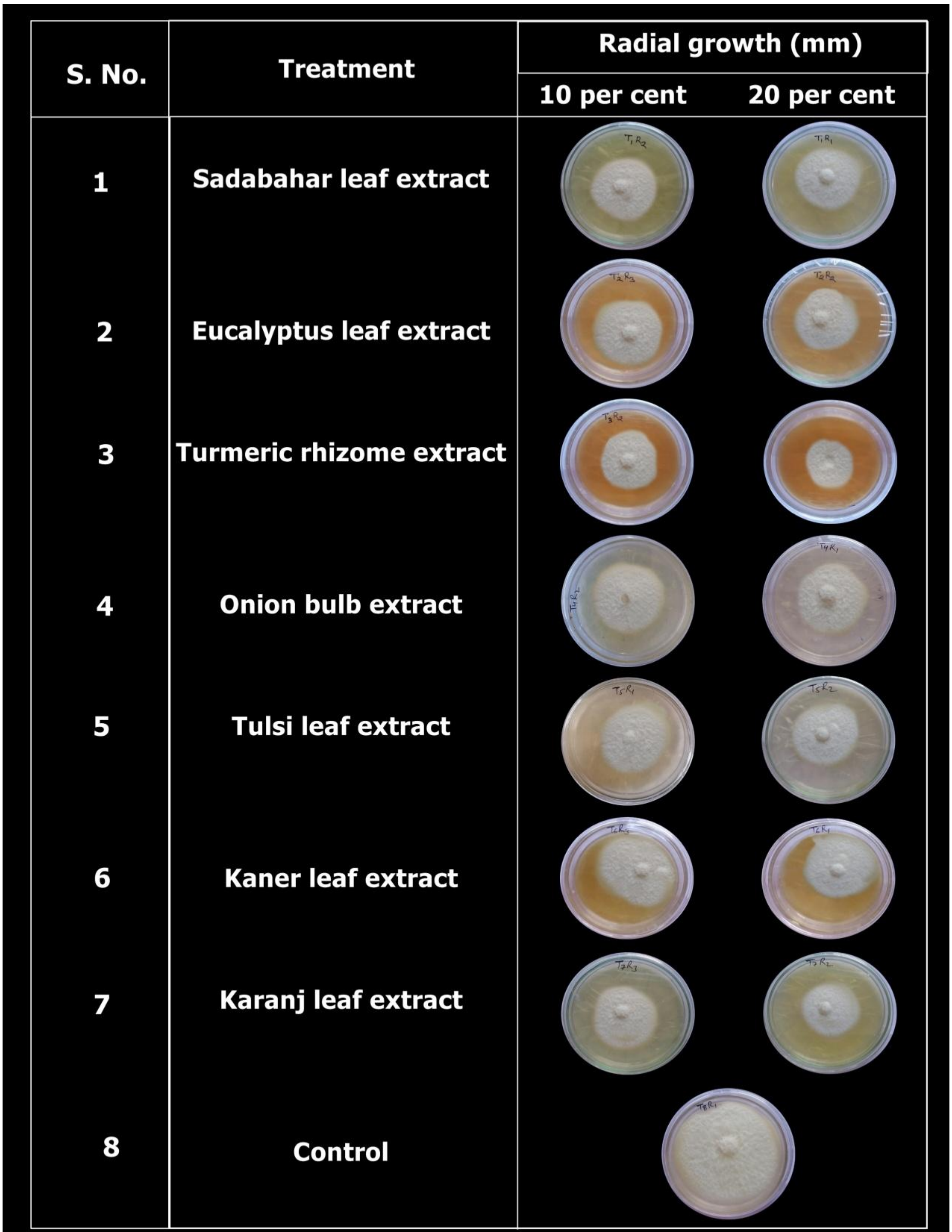


Plate-6 Radial growth of *Colletotrichum gloeosporioides* in botanicals amended medium at 10 and 20 per cent

CHAPTER- V

DISCUSSION

In 2021, studies were undertaken on several aspects like isolation, identification, pathogenicity, and management of anthracnose caused by *C. gloeosporioides* in pomegranates. Investigations were performed under controlled settings for pathogen isolation, identification, and testing pathogenicity. Studies with fungicides and their combinations were undertaken under *in vivo* and *in vitro* conditions whereas, botanicals were evaluated under *in vitro* condition against *C. gloeosporioides*. The findings are discussed in this chapter.

Isolation, purification and identification of pathogen

Infected specimens with characteristic anthracnose symptoms on leaves and fruits were collected throughout the experiment. The pathogen from the study area was obtained into pure culture after tissue isolation. The pathogen was characterized as *C. gloeosporioides* (Penz.) Penz.and Sacc. based on marphological aspects, as described by Ekbote (1994); Sudhakar (2000) and Prashanth (2007).

Identification of the pathogen was carried out based on the morphological characters. The fungus in the present study produced septate mycelium. Later it produced conidiophores arising singly or closely packed together in rows. Conidiophores were single celled, hyaline and aseptate with one or several conidial scars. The conidia were oblong or cylindrical or slightly dumbel, hyaline, aseptate with rounded ends, one to two oil globules were observed in the conidium. The results are consistent with those of previous researchers (Ekbote.,1994; Sudhakar 2000 and Prashanth, 2007).

Symptomatology

The typical major anthracnose symptoms were observed on leaves and fruits of pomegranate.

Pinhead size of black to brown water soaked spots appeared on the leaves with circular margin. In advanced stage, these spots enlarged, amalgamate and resulted in larger patches. In severe case, leaves dried up and drooped down. On fruits, brown spherical depressed spots occurred in scattered on the pericarp. In advanced stage, these spots amalgamated to make necrotic patches over the surface of the fruit. Once such unhealthy fruits were cut open, the decay symptoms were ascertained. Brown to dark brown coloured seeds were seen in the infected fruits. At advanced stage minute dark coloured acervulli were seen on depressed spots. Looking to these symptoms, the sickness is referred as “anthracnose”. In their study, Gupta *et al.* (2017) and Kara *et al.* (2020) also noticed similar symptoms associated with anthracnose of pomegranate.

Pathogenicity

Pure culture of *C. gloeosporioides* was used for testing pathogenicity of the pathogen by using detached leaf technique. Leaves inoculated with culture of the pathogen produced characteristic anthracnose symptoms. However, leaves inoculated on upper surface tissue, the lesion development started after five days of inoculation. Minute pin head black to brown water soaked spots appeared after five days of inoculation and spot turned slightly brown surrounded by chlorotic halo, while on tenth day these spots enlarged and showed dark brown colour surrounded by slight chlorotic halo. After ten days the central portion of spot showed minute dark coloured acervulli, later these spots coalesced. On re-isolation, the culture of *C. gloeosporioides* was obtained confirming the Koch’s postulates. Awa *et al.* (2012) concurred with the description stated. They conducted pathogenicity tests by the whole fruit technique, detached leaf technique, and detached panicle technique.

Periodic observation an anthracnose development and their relationship with weather parameters

The development of anthracnose intensity was studied in relation to various climatic parameters. Maximum and minimum temperature was found to have a significant positive correlation with anthracnose of pomegranate (r=0.57 and 0.62) and other parameter like relative humidity and rainfall (mm) were found to have a significant negative correlation with anthracnose of pomegranate (r= -0.56 and -0.74 respectively). Pandit (2020) found a positive correlation with mean temperature and relative humidity, indicating that disease severity increased in parallel with increasing mean temperature and relative humidity. Rainfall and bright sunshine hours were shown to have little effect on disease.

In-vitro evaluation of new fungicides against *C. gloeosporioides*

In vitro evaluation of fungicides was done by poison food technique at 500, 1000 and 1500 ppm concentrations. Observations on radial growth of *C. gloeosporioides* were recorded after third, fifth and seventh day of incubation. According to the findings, all treatments were significantly superior over control in decreasing the mycelial growth. Minimum mean mycelial growth was observed in Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.05%), Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.075%), Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.1%) and Propineb 70%WP (0.1%) with 0.00 mm mean radial growth followed by Difenoconazole 25% EC (0.1%), Fluxapyroxad 300g/l SC (0.03%), Metiram 70% WP (0.1%) and Metiram 55%+pyraclostrobin 5% WG (0.35%) as compared to untreated control. Among the different period of intervals, 5.37 mm, 4.93 mm and 4.22 mm mycelial growth was noticed after third day of incubation and 12.19 mm, 10.85 mm and 9.26 mm mycelial growth noticed after fifth day of incubation while 18.93 mm, 15.81 mm and 14.44 mm mycelial growth was noticed after seventh day of incubation at 500, 1000 and 1500 ppm, respectively.

GolakiyaIn *et al.* (2020) screened different fungicides against *C. gloeosporioides* and found that tebuconazole 25.9% EC and hexaconazole 5% EC effectively inhibited the cent per cent radial growth among systemic group of fungicides, while in non-systemic group of fungicides, captan 50% WP was quite effective and in case of ready mix fungicides azoxystrobin 11% + tebuconazole 18.30% SC, epoxiconazole 50g/l + pyraclostrobin 133g/l, tebuconazole 50% + trifloxystrobin 25% WG, zinab 68% + hexaconazole 4% WP significantly inhibited the growth of the test fungus under *in vitro* conditions.

Field evaluation of fungicides against anthracnose of pomegranate

The experiment was conducted during 2021, with eight treatments and one untreated control. Two sprays were given at the interval of fifteen days. The observation on pomegranate anthracnose was recorded at seven days interval after spray. Before any spray of fungicides, anthracnose intensity was at par in all the treatments. All the treatments showed significant effect on reducing anthracnose intensity. Minimum percent disease index was recorded in Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC) 1.25ml/l water, at par with

Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC) 1.0 ml/l water and Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC) 2.5ml/l water after first and second sprays. Mimrot *et al.* (2020) observed that azoxystrobin 8.3% + mancozeb 66.7% WG at 4 per cent and 10 per cent concentration was most effective in reducing the percent disease index of anthracnose of pomegranate under field conditions.

In-vitro **efficacy of botanicals against** ***C. gloeosporioides***

The efficacy of seven botanicals, including Sadabahar leaf extract, Eucalyptus leaf extract, Turmeric rhizome extract, Onion bulb extract, Tulsi leaf extract, Kaner leaf extract and Karanj leaf extract was tested against *C. gloeosporioides* under *in vitro* condition. After the seventh day of incubation, observations on *C. gloeosporioides* radial growth was taken. Significantly minimum growth (41.53 mm) was noticed at 20 per cent concentration followed by 10 per cent. In absence of botanical *C. gloeosporioides* showed the highest radial growth (89.20 mm). Among the botanicals minimum growth (27.45 mm) was obtained in Turmeric rhizome extract followed by Karanj leaf extract, Tulsi leaf extract, Sadabahar leaf extract and Eucalyptus leaf extract, Kaner leaf extract and Onion bulb extract as compared to control.

Nidiry and Lokesha (2020) investigated the fungitoxic action of *Catharanthus roseus* (L.) extracts against *C. gloeosporioides* and discovered that the extract of leaves and roots of *C. roseus* inhibited the mycelial growth.

CHAPTER-VI
SUMMARY, CONCLUSION AND SUGGESTIONS FORFURTHER WORK

Pomegranates are widely grown in the Mediterranean region as well as other regions of the world, including India. Pomegranate production has been successful in recent years, although it has been plagued by pests and illnesses. Anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc., is one of the most destructive diseases of pomegranates, remaining latent in initial stages of fruit formation and significantly lowering fruit quality.

The present Investigations were carried out at Fruit Research Station (FRS), Intkhedi, Bhopal (M.P.) under the jurisdiction of R.A.K. College of Agriculture, Sehore at Department of Plant Pathology during 2021.

Present studies were undertaken on several aspects like isolation, identification, pathogenicity, and management treatment of anthracnose caused by *C. gloeosporioides* in pomegranate. Investigations were performed under controlled settings for pathogen isolation, identification, and testing pathogenicity. Studies with fungicides and their combinations were undertaken under *in vivo* and *in vitro* whereas, botanicals were tested under *in vitro* against *C. gloeosporioides*. The following are the key results of the studies:

Summary

- The pathogen from the infected specimens with characteristic anthracnose symptoms on leaves and fruits was obtained into pure culture after tissue isolation and identified as *C. gloeosporioides* based on morphological characteristics.
- The disease mostly affected the fruits and leaves, causing symptoms such as a brown to dark brown circular spot with a diameter of 3-5 mm in the early stages and subsequently round, depressed patches on the peel of fully formed immature fruits during the rainy to post-rainy season. The damaged part of the fruit was harder than the healthy part.On fully developed ripe fruits, the disease spread quickly.
- The development of anthracnose intensity was studied in relation to various weather parameters. Maximum and minimum temperature was found to

have a significant positive correlation with anthracnose of pomegranate (r=0.57 and 0.62) and other parameter like relative humidity and rainfall (mm) were found to have significant negative correlation with anthracnose of pomegranate (r= -0.56 and -0.74 respectively) during the study.

- *In vitro* evaluation of fungicides was investigated by poison food technique at 500, 1000 and 1500 ppm. Among them, Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.05%), Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.075%), Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.1%) and Propineb 70%WP (0.1%) completely inhibited the growth of pathogen with 100 per cent growth inhibition at all the concentrations.
- The management of anthracnose of pomegranate using different fungicides under *in vivo* condition indicated that the spraying of Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC) 1.25ml/l water was significantly most effective and recorded minimum anthracnose intensity whereas, Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC)0.5ml/l water was found to be least effective.
- Among the botanicals, Turmeric rhizome extract was found most effective in reducing radial growth of *C. gloeosporioides* under *in vitro* condition at 10 and 20 per cent concentrations.

Conclusion

- Maximum and minimum temperature was found to have a significant positive correlation with anthracnose of pomegranate and other parameter like relative humidity and rainfall (mm) were found to have a significant negative correlation with anthracnose of pomegranate.
- *In vitro* studies on effect of fungicides suggested that Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.05%), Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.075%), Fluxapyroxad 75g/l + Difenoconazole 50g/l SC (0.1%) and Propineb 70%WP (0.1%)completely inhibited the growth of pathogen with 100 per cent growth inhibition at all the concentrations.
- The management of anthracnose of pomegranate using different fungicides under *in vivo* condition indicated that the spraying of Fluxapyroxad 75g/l + Difenoconazole 50g/l (SC) 1.25ml/l water was significantly most effective and recorded minimum anthracnose intensity.
- Among the botanicals, Turmeric rhizome extract was found most effective in reducing radial growth of *C. gloeosporioides* under *in vitro* condition at

10 and 20 per cent concentrations.

Suggestion for further work

- Regular epidemiological investigations on anthracnose of pomegranate for three to four years at more locations are required in order to develop model for accurate disease forecasting.
- New molecules need to be evaluated for disease management and resistance induction.
- Additional plant extracts need to be tested for their efficacy in inhibiting *C. gloeosporioides* and in managing anthracnose of pomegranate under field conditions.

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APPENDICES

Analysis of variance of Table-4.2

SV	DF	SS	MSS	Fcal.	Ftab.
Days (D)	2	2480.69	1240.35	561.27	3.17
Treatment (T)	8	30941.22	3867.65	1750.17	2.12
Inter(D*T)	16	6399.75	399.98	181.00	1.83
Error	54	119.33	2.21		
Total	80	39941.00			

Analysis of variance of Table-4.3

SV	DF	SS	MSS	Fcal.	Ftab.
Days (D)	2	1604.84	802.42	691.45	3.17
Treatment (T)	8	28471.89	3558.99	3066.79	2.12
Inter(D*T)	16	6253.60	390.85	336.80	1.83
Error	54	62.67	1.16		
Total	80	36393.00			

Analysis of variance of Table-4.4

SV	DF	SS	MSS	Fcal.	Ftab.
Days (D)	2	1410.77	705.38	414.03	3.17
Treatment (T)	8	26003.78	3250.47	1907.89	2.12
Inter(D*T)	16	6253.46	390.84	229.41	1.83
Error	54	92.00	1.70		
Total	80	33760.00			

Analysis of variance of Table-4.5 (Pre-treatment)

SV	DF	SS	MSS	Fcal	Ftab
Replication	2	4.25	2.125674	0.99	3.63
Treatment	8	39.24	4.904407	2.27	2.59
Error	16	34.50	2.156162		
Total	26	77.99			

Analysis of variance of Table-4.5 (After first spray)

SV	DF	SS	MSS	Fcal	Ftab
Replication	2	5.11	2.555404	1.97	3.63
Treatment	8	94.24	11.77974	9.07	2.59
Error	16	20.78	1.298531		
Total	26	120.13			

Analysis of variance of Table-4.5 (After first spray)

SV	DF	SS	MSS	Fcal	Ftab
Replication	2	8.36	4.179351	0.43	3.63
Treatment	8	387.15	48.3943	4.95	2.59
Error	16	156.57	9.785722		
Total	26	552.08			

Analysis of variance of Table-4.6

SV	DF	SS	MSS	Fcal.	FTAB
Concentration (C)	1	219.31	219.31	45.49	4.15
Treatment (T)	7	107041.56	15291.65	3171.72	2.31
Inter(C*T)	7	72.29	10.33	2.14	2.31
Error	32	154.28	4.82		
Total	47	107487.44			

VITA

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