STUDIES ON LEAF AND FRUIT SPOT OF POMEGRANATE [*Punica granatum* L.] UNDER NORTH GUJARAT CONDITION

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BY

THAKKAR BHUMIBEN AMRUTBHAI
B.Sc. (Hons.) Agriculture

DEPARTMENT OF PLANT PATHOLOGY
CHIMANBHAI PATEL COLLEGE OF AGRICULTURE
SARDARKRUSHINAGAR DANTIWADA AGRICULTURAL UNIVERSITY
SARDARKRUSHINAGAR - 385 506

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ABSTRACT

STUDIES ON LEAF AND FRUIT SPOT OF POMEGRANATE [*Punica granatum* L.] UNDER NORTH GUJARAT CONDITION

Name of Student	**Major Advisor**
THAKKAR BHUMIBEN A.	**Dr. R. F. CHAUDHARY**

DEPARTMENT OF PLANT PATHOLOGY
CHIMANBHAI PATEL COLLEGE OF AGRICULTURE
SARDARKRUSHINAGAR DANTIWADA AGRICULTURAL UNIVERSITY
SARDARKRUSHINAGAR-385 506

ABSTRACT

Pomegranate is one of the important commercial fruit crop of India. India ranks first in pomegranate production in world. Pomegranate is an important fruit crop of semi-arid and arid regions of the world. Botanical name of pomegranate *Punica granatum* L., belongs to family *Lythraceae* with the chromosome number 2n=16. Pomegranate is vital cash crop of India, commercially grown to many states *viz.*, Maharashtra, Karnataka, Gujarat, Andhra Pradesh, Madhya Pradesh, Tamil Nadu and Rajasthan. During 2021-22, pomegranate was cultivated over 2.82 lakh ha with an annual production of 32.16 lakh tonnes and productivity of 11.40 tonnes/ha in India.

Leaf and fruit spot is major disease of pomegranate caused by *Alternaria alternata.* The pathogen is airborne. Investigation were carried out for isolation, identification, pathogenicity test of *A. alternata, in vitro* bio-efficacy of bioagents, phytoextracts, fungicides and *in vivo* screening of germplasms/varieties of pomegranate against leaf and fruit spots.

The microscopic study of *A. alternata* revealed that the mycelial colour ranged from raised ashy white to flat ashy green to raised blackish green with all having black reverse. Conidia was typically muriform, dark brown, thick walled, in long branched chains (9 to 15). Hypha septated were brown in colour. Conidia was brown with length range from 31.5 to 62 µm and 9.4-14.6 µm wide.

Pathogenicity test revealed that the test isolates *A. alternata* was able to infect the inoculated pomegranate leaves, plant and fruit. Typical symptoms developed on leaves, plant and fruit were found to be similar to that of naturally infected pomegranate plant and were found identical and similar to that of original one.

The growth inhibtion of *A. alternata* was tested against nine bio-agents by dual culture method. Of them, *T. viride* (Anand) was proved as the most inhibitory with maximum growth inhibition (81.48 %) of *A. alternata* than with rest of the bio-agents. The next best bio-agent in order of merit was *B. subtilis* (Sardarkrushinagar) resulting in growth inhibition of (76.78 %). The least growth inhibition was recorded by *P. fluorescence* (Sardarkrushinagar) (40.74 %).

Bio-eeficacy of Phytoextracts was evaluated against *A. alternata* by poisoned food technique. In present investigation, ten different phytoextracts were tested and the highest mean inhibition of mycelial was obtained by garlic clove (58.09 %) followed by neem leaves extract (50.00%). Dhatura leaves extracts (39.01 %) were found less effective for inhibiting mycelial growth of the pathogen.

The *in vitro* bio-efficacy of six systemic fungicides were tested for their inhibitory role, the highest mean growth inhibition of mycelial was obtained with, propiconazole 25 EC (93.81 %) followed by difenoconazole 25 EC (91.68 %) which was at par with hexaconazole 5 EC (89.71 %). Carbendazim 50 WP recorded least effective (42.82 %) which was followed by azoxystrobin (48.96 %).

Among six non-systematic fungicides, the highest growth inhibition of mycelial was obtained by, mancozeb 75 WP with 70.53 per cent followed by copper oxychloride 50 WP with 67.63 per cent tested against *A. alternata in vitro* condition. Chlorothalonil 75 WP recorded least inhibition of only 53.38 per cent mycelial growth inhibition of the *A. alternata.*

Further, the *in vitro* bio-efficacy of six combined fungicides were tested against *A. alternata*. Among them, azoxystrobin 18.2+difenoconazole11.4 SC was found significantly higher than the rest of the combined fungicides with the cent per cent growth inhibition followed by carbendazim 25 + iprodione 25 WP with 89.10 per cent. Carbendazim 12 + mancozeb 63 WP recorded the least inhibition of only 56.81 per cent mycelial growth of *A. alternata.*

Eighteen varieties/genotypes were screened to identify the sources of resistance against *A. alternata.* Among them, fifteen varieties/genotypes were susceptible to the leaf and fruit spot of pomegranate whereas, less than 20 per cent of disease intensity of leaf and fruit spot observed in Bhagwa and Super Bhagwa. These two varieties found moderately susceptible to the disease. Boshlinski were recorded as highly susceptible to the leaf and fruit spot of pomegranate.

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CHIMANBHAI PATEL COLLEGE OF AGRICULTURE
SARDARKRUSHINAGAR-385 506

CERTIFICATE – I

Date : 07/09/2022

This is to certify that the thesis entitled, **"STUDIES ON LEAF AND FRUIT SPOT OF POMEGRANATE [*Punica granatum* L.] UNDER NORTH GUJARAT CONDITION"** submitted for the degree of **MASTER OF SCIENCE (Agriculture)** in the subject of **PLANT PATHOLOGY** is a record of bonafide research work carried out by **THAKKAR BHUMIBEN AMRUTBHAI** under my guidance and supervision and that no part of this thesis has been submitted for any other degree, diploma, associateship, fellowship or other similar titles. The assistance and help received during the course of investigation have been fully acknowledged.

(R. F. CHAUDHARY)
Major Advisor

(F. K CHAUDHARY)
Minor Advisor

(A. CHATTOPADHYAY)
Advisor

(JYOTIKA PUROHIT)
Advisor

(G. K. CHAUDHARY)
Advisor

SARDARKRUSHINAGAR DANTIWADA AGRICULTURAL UNIVERSITY
CHIMANBHAI PATEL COLLEGE OF AGRICULTURE
SARDARKRUSHINAGAR-385 506

CERTIFICATE – II

Date : 07/09/2022

This is to certify that **THAKKAR BHUMIBEN AMRUTBHAI,** Department of Plant Pathology, Chimanbhai Patel College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar has made all corrections/modifications in the thesis entitled, **"STUDIES ON LEAF AND FRUIT SPOT OF POMEGRANATE [*Punica granatum* L.] UNDER NORTH GUJARAT CONDITION"** as suggested by the External Examiner and the Advisory Committee in the oral examination held on **30/08/2022.**

(R. F. CHAUDHARY)
Major Advisor
Associate Research Scientist
Agroforestry Research Station,
S. D. Agricultural University,
Sardarkrushinagar-385 506.

(R. S. JAIMAN)
Professor and Head
Department of Plant Pathology,
C. P. College of Agriculture,
S. D. Agricultural University,
Sardarkrushinagar-385 506

(S. D. SOLANKI)
Principal and Dean
C. P. College of Agriculture,
S. D. Agricultural University,
Sardarkrushinagar-385 506

APPROVED (Seal and Date)

12/09

( )
Director of Research and
Dean, Post-Graduate Studies,
S. D. Agricultural University,
Sardarkrushinagar-385 506.

Director of Research, S.D. Agri. Uni., S.K. Nagar

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(Thakkar Bhumi A.)

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LIST OF ABBREVIATIONS AND SYMBOLS

%	:	Per cent
°C	:	Degree celsius
@	:	At the rate
µm	:	Micrometer
=	:	Equal to
×	:	In to
/	:	Per
>	:	Greater than
<	:	Less than
Anon.	:	Anonymous
C. D.	:	Critical Difference
cm	:	Centimeter
C. V.	:	Coefficient of Variance
et al.	:	et allii; and others
etc.	:	Etcetra
Fig.	:	Figure
Sr. no.	:	Serial number
Tr. No.	:	Treatment Number
WP	:	Wettable powder
EC	:	Emulsifiable concentrat
SC	:	Suspension concentrate
WG	:	Wettable granules
ml	:	Milli litre
No.	:	Number
PDA	:	Potato Dextrose Agar
spp.	:	Species
viz.,	:	Namely
i.e.	:	That is
v/v	:	Volume by volume
NaOH	;	Sodium hydroxide
SDAU	:	Sardarkrushinagar Dantiwada Agricultural University
Conc.	:	Concentraction
hrs.	:	hours
PDI	:	Per cent disease index
PGI	:	Per cent growth index
ppm	:	Parts per million
S.Em	:	Standard error of mean

INTRODUCTION

I. INTRODUCTION

Pomegranate is an important fruit crop of semi-arid and arid regions of the world. It has both cultivated (*Punica granatum* L.) and wild types (*Punica protopunica*). The chromosome number varies among different cultivars of pomegranate; 2n=16 or 18. Pomegranate is fruit bearing shrubs or trees comes under small genus of *Punica*. It was earlier placed in monogeneric family *Punicaceae*, now it has been shifted to family *Lythraceae*, recent morphological (Graham and Graham, 2014) and molecular (Berger *et al.,*2016) evidence, as well as the new classification in the APG IV system (Byng *et al.,*2016), suggests that it is instead a member of *Lythraceae*. Pomegranate is a native of Iran. Pomegranate cultivation is done in the countries like China, USA, India, Spain, Morocco, Egypt, Iran, Afghanistan, Baluchistan and Myanmar.

Pomegranate is one of the important commercial fruit crop of India. India ranks first in pomegranate production in world. It is regarded as a "vital cash crop", commercially grown to many states of India *viz.*, Maharashtra, Karnataka, Gujarat, Andhra Pradesh, Madhya Pradesh, Tamil Nadu and Rajasthan. During 2021-22, pomegranate was cultivated over 2.82 lakh ha with an annual production of 32.16 lakh tonnes and productivity of 11.40 tonnes/ha in India (Anon.,2022[a]). Maharashtra considered as pomegranate basket of India. In current scenario, Maharashtra with an area of 166.20 thousand ha is the leading state in acreage and accounts for 68.7 per cent of the total area under pomegranate in the country followed by Gujarat (44.57 thousand ha), Karnataka (28.20 thousand ha), Andhra Pradesh (18.90 thousand ha), Rajasthan (10.60 thousand ha) and Telangana (1.23 thousand ha) these are the leading states cultivating pomegranate commercially on a large scale. In Gujarat pomegranate is cultivated in region of Kutch, Banaskantha, Mahesana, Sabarkantha and Bhavnagar. Production of pomegranate in Gujarat is 6.84 lakh tonnes and productivity 15.34 tonnes/ha (Anon.,2022[b]). In recent years, pomegranate cultivation has also been started in Rajasthan, Orissa, Chhattisgarh, Uttarakhand and Madhya Pradesh at small scale.

Pomegranate is an important fruit crop popularly known as "Fruit of Paradise". Pomegranate fruit is highly nutritious, high in fibre, vitamins, protein and minerals. It is a good source of vitamin C, thiamin, niacin and riboflavin. It is rich in minerals like potassium, phosphorous, calcium, sodium and magnesium. The edible portion of pomegranate fruit is aril which is nearly 68 per cent of the total fruit containing 78 per cent of moisture, 0.7 per cent mineral matter, 1.6 per cent protein, 0.1 per cent fat, 14.5 per cent carbohydrate and 5.1 per cent fiber. The ellagic acid which is particularly plentiful in pomegranate prevents carcinogenic oxidation of cellular membranes (Lal *et al*., 2011).

Pomegranate are rich in antioxidant which support overall health and helps in preventing disease in human. The fruits of pomegranate are known to possess pharmaceutical and therapeutic properties. In Ayurvedic and Unani medicines system use of pomegranate is reported for treating human illness. It has medicinal values such as anticancer properties, anti-inflammatory properties, anti-microbial properties, reduce the blood pressure, prevent heart and kidney diseases (Bose, 1985). The flower buds are very useful in Ayurveda for managing bronchitis. The bark and pericarp of fruit is used for slimming, control of dysentery, diarrhoea and killing tapeworms. Extract of pomegranate fruit has antiviral activity (Konowalchuk and Spetis, 1976).

The ongoing global demand for a healthier and nutritional diet has led to rise in fresh food produce with less chemical additives and preservatives. There is high scope for processing industry to produce value added product from pomegranate and get good profit out of it. The conventional utilization of fruit aril is constituting the product like anardana and anardana powder. High sugar products such as Jam and jelly can made out from fresh arils of pomegranate. The arils of fruits are also consumed in juice and processed form like concentrated syrup and candy. The by-product of fruit is used in cosmeceutical industries. The bark of the stem and root contains number of alkaloids belonging to pyridine group. Rind of the fruit contains tannins, which are used as dyeing material for cloth and leather.

In international market there is high demand of fresh fruits of pomegranate. India is one of the main suppliers of pomegranate in international market. India produces finest varieties of pomegranate having soft seeds, less acids and attractive in colour. The popular varieties Bhagwa (also called Kesar), Ganesh, Mridula, Arakta and Ruby *etc*. Bhagwa variety has high acceptance in european market.

Pomegranate cultivation is under threat because of number of factors including biotic and abiotic factors. Among abiotic factors such as drought, salinity, combined drought and salinity stress, evapo-transpiration, root water uptake under salt stress. The biotic factors which is major threat and cause losses through the diseases like bacterial blight, wilts due *to Fusarium* spp., anthracnose and Alternaria leaf spot and fruit rot/spot. Non-availability of leading varieties which are resistant to biotic and abiotic factors have brought pomegranate cultivation on decline. Though bacterial blight infection on pomegranate due to *Xanthomonas axonopodis* pv. *punicae* has attracted attention by researchers, growers and policy makers alike. Nevertheless, the infections due to fungal species which cause diseases such as Anthracnose (*Colletotrichum gloeosporioides*), Leaf spot and severe fruit spot/rot by *Alternaria alternata, Cercospora* spp., *Drechslera* spp. and *Sphaceloma* spp. etc. are more or less equally important and harmful in some orchards. The fruit spots not only reduce the fruit yield but also reduce market value. Among these; severe spotting and fruit rotting due to *Alternaria alternata*; remains hitherto unexplored but potentially dangerous pathogen on pomegranate and considered to be an emerging disease. Madhukar and Reddy (1976) in India reported for the first time a leaf spot due to *A. alternata* on pomegranate however, fruit rot was not included in this report. Farr *et al.* (2007) reported the fruit rot caused by *Alternaria* spp. in USA, Mexico, while it's infection as a postharvest disease in Greece was reported by Pantidou (1973).

Among fungal foliar diseases, leaf and fruit spot pomegranate incited by *A. alternata* (Fr.) Keissler is the most common, widespread and destructive disease of pomegranate in India. It can cause losses in yield when become severe. It is prevalent in almost all pomegranate growing area of Gujarat. The spots of *A. alternata* on leaves of pomegranate are isolated, irregular to round blackish-brown and enlarge to cover large area. The affected leaves turn yellow, dry up and fall down. The spots on fruits cover large area; mostly mature fruits are attacked. The plants of pomegranate become more susceptible when it is under any kind of stress like drought, salinity, nutritional and environmental stress (Anon, 2009). During September and October 2005 and 2006, a fruit rot due to *A. alternata* in pomegranate orchards was continuously present in Larissa region of Central Greece, causing significant yield losses approximately to the tune of 40-50 per cent especially on 'Kapmaditika' cultivar. The internal symptoms consisted of a black rot of the fruit core beginning from the calyx area, while the hard, leathery rind appeared apparently healthy and fruits remained (Tziros *et al.,* 2008).

Isolation, identification and pathogenicity of *A. alternata* is a basic pre-requisite to understood expression of resistance. Pathogenicity testing can provide data on the resistance of crop to the fungal taxon and is useful in plant breeding programme. It is also important for integrated disease management programmes because the use of resistant plant varieties can reduce the negative effect of chemical use on the environment.

Screening for disease resistance is essential to identify resistant varieties /germplasms. Use of resistant variety is one of the best ways in reducing losses due to disease. Many of the commercially cultivated genotypes exhibit susceptibility against *A. alternata*. Therefore, there is a need to screen the pomegranate varieties/germplasms for identification of stable resistant sources against *A. alternata.*

There is only a little information available on the management of fungal leaf and fruit spot of pomegranate, but now a days there are large number of chemicals available in the market and their bio-efficacy and suitability needs to be verified by *in vitro* studies. Evaluation of fungicides to manage leaf and fruit spot disease is most essential within the reasonable limit of fungicidal residues permitted by the importing countries, so as to incorporate the effective ones in the management package. Hence *in vitro* studies on bio efficacy of fungicides need to be conduct. Keeping in view of the economic importance of the fruit and destructive nature of leaf and fruit spot diseases caused by *A. alternata* and extent of losses caused to pomegranate in the state, the present study is being proposed to be undertaken with the following objectives:

1. Isolation, purification and identification of pathogen associated with leaf and fruit spot
2. Pathogenicity test of pathogen associated with leaf and fruit spot disease
3. *In vitro* bio-efficacy of different bioagents against *A. alternata*
4. *In vitro* bio-efficacy of different phytoextracts against *A. alternata*
5. *In vitro* bio-efficacy of different fungicides against *A. alternata*
6. Screening of varieties /germplasms of pomegranate against leaf and fruit spot caused by *A. alternata* under field condition.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

The literature pertaining to leaf and fruit spot of pomegranate caused by *A. alternata* is available in scanty. However, plenty of information is available on leaf and fruit spot disease of pomegranate other crops like niger, soybean, cotton, aloe vera, safflower, groundnut, castor, pigeon pea *etc.* Hence, the available literature on leaf and fruit spot of pomegranate and other crops caused by *A. alternata* are reviewed here under.

2.1 Isolation, purification and identification of pathogen associated with leaf and fruit spot disease

Keissler (1912) described the morphology of *A. alternata* and found black or olivaceous black and sometimes grey conidiophores produced singly or in small groups, simple or branched, straight or flexuous, sometimes geniculate, pale to mid olivaceous or golden brown, smooth, measuring 50 µm long, 3-6 µm thick, with one or several conidial scars. Conidia formed in long often branched chains, obclavate, pyriform, ovoid or ellipsoidal often with short conical or cylindrical beak sometimes up to but not more than one third the length of the conidium, pale to mild golden brown, smooth or verrucose with to eight transverse and usually several longitudinal or oblique septa. The overall length varied from 20-63 µm and 9-18 µm thick in the broadest part, with pale beak and 2-5 µm in thickness.

Mirkova and Konstantinova (2003) observed that the conidia of *A. alternata* isolated from gerbera were catenated in long, sometimes branched chains of 5-12 spores, variable in size and shape, usually ovoid to ellipsoid or obclavate with usually pale long oval conidia, pale brown with 3-6 transverse and 0-2 longitudinal or oblique septa and measured 25-35 × 5-10 µm.

Chaudhary *et al.* (2005) studied morphological characters of *A. alternata* on PDA and they observed olivaceous in colour when young, later on turned dark brown to black colonies. Mycelium was irregularly brached at acute angle, septate and light brown in colour. Conidia were light brown to dark brown, muriform with 1-5 transverse and 0-3 longitudinal septa. They were obclavate to oval in shape with short cylindrical beak with measures 14.00-63.50 × 7.50-14.00 µm (Average 37.92 × 12.60 µm).

Tziros *et al.* (2008) reported that cultures of *A. alternata* isolated from infected tissue of fruit rot of pomegranate and grow rapidly on PDA and they were initially white turning to grey later. Conidiophores were short, septate, branched or unbranched and green to brown. The conidia were obpyriform with conical or cylindrical beak, ovoid or ellipsoidal and produced in long, single but most often branched chains. Conidia ranged from 10 to 21 µm, with length of 11 µm and width of 4 to 10 µm at the broadest point (average 17 × 6 µm).

Bhardwaj and Mathur (2010) reported leaf spot disease of aloe-vera caused *A. alternata* and observed that the *A. alternata* produced golden-brown mycelium, effused, olivaceous black colonies with dark olive-green margins and abundantly branched septa. The conidiophores were branched, straight, golden brown, smooth-walled, measuring up to 60 µm long and 3 µm wide with one conidial scar. The conidia were obpyriform, golden-brown, smooth-walled, produced in long branched chains, with a short pale beak.

Fang *et al.* (2010) isolated *A. alternata* pathogen from gerbera leaf spot disease. The pathogen was identified on the basis of pathogenic test as well as morphological characters and molecular identification. Twenty-two of possible isolates *A. alternata* were isolated from leaves of gerbera plants in Beijing.

Hubballi *et al.* (2010) isolated *A. alternata* by tissue segment method from infected tissues of noni and fungus was purified by single spore isolation technique using water agar media.

Ramjegathesh and Ebenezar (2012) studied 10 isolates of *A. alternata* causing leaf blight of onion collected from 10 different conventional onion growing areas of Tamil nadu. They reported that all isolates produced muriform, light brown conidia with average length of 30.99 to 42.47 µm, beak length of 11.90 to 17.37 µm, 2-9 horizontal septa and 3-8 vertical septations and days to sporulation varied from 13-16 days.

Mamta *et al*. (2013) isolated *A. alternata* from pigeon pea observed abundant branched, brownish septate mycelia. Conidiophores were simple, septate, olive-brown and varying in length with terminal conidia being either in short chains or solitary. Fully developed conidia measures from 10-30 by 5-12 µm with low coned beak or beakless. Conidia had 3-7 transverse septa, 1-5 longitudinal septa and in chains of 5-15 conidia.

Nagrale *et al.* (2013) observed the mycelium of *A. alternata* was hyaline that turned to grey-brownish, multicelled, septate and irregularly branched. Conidiophores arised singly or in clusters, usually 2-6 and were long or short. They were found pale olivaceous to olivaceous brown, straight or curved, geniculate, slightly swollen at apex having terminal scars indicating the point of attachment of conidia. The conidiophores measured 42.26 µm (27.30-112 µm) in length and 4.29 µm (3.12-8.43 µm) in width. Conidia were born in chains upto 10 or more on conidiophores. They were light olivaceous to dark brown in colour, varied in shape from obclavate to mostly ellipsoidal, muriform having tapered apex with 1 to 3 longitudinal and 2-10 transverse septa. The muriform conidia inclusive of beak measured 47.16 µm (21.82-96.40 mm) × 13.49 mm (8.26-16.52 µm). The beak measured 27.12 µm (22.62-58.69 µm) in length. The chlamydospores were formed in the old culture of *A. alternata.*

Hashem *et al*. (2014) found pure culture of the *A. alternata* and reported that colony appeared to be grayish white at first and became black later on. The fungus produced abundant conidia having 3-8 transverse septa and 1-2 longitudinal septa. Conidia were solitary, or in short chains, mostly ovoid with a short conical or cylindrical apical beak and smooth walled. Hyphae were branched, septate, brownish with simple olive-brown, septate conidiophores that were variable in length.

Bihon *et al.* (2015) purified the culture of *A. alternat*a through hyphal tip method and single spore isolation technique after isolating the fungus from infected tissues of solanaceous crops and onion, transferred the pure culture into PSA and PDA medium, respectively.

Czajka *et al.* (2015) isolated and identified as *Alternaria* sp. which produce dark gray to black colonies developed in all the petri dishes and based on the dark, globose and ovate shape with short beaks, contained four to eight transverse and zero to two longitudinal septa and were 30.95 to 43.69 µm long, 11.00 to 12.81 µm in the widest area and 3.00 to 3.82 µm in the narrowest area.

Devappa and Thejakumar (2016) studied culture of the *A. alternat*a and they observed initially white, cottony with profuse aerial mycelium which gradually turned grey colour. Aged culture appeared completely greyish with aerial mycelium and distinct concentric rings was formed on medium. Conidiophores were short to long, simple or branched arising singly. Conidiophores were golden to brown coloured with 2-9 transverse and 0-2 longitudinal septa. Conidia were borne in long chains (6-11) on conidiophores, they were thick walled, beaked and brown in colour.

Chethana *et al.* (2018) studied 6 isolates of *A. alternata* causing purple blotch disease of onion and reported that the mycelial colour ranged from raised ashy white to flat ashy green to raised blackish green with all having black reverse. Isolates varied in sporulation ranging from 0.5×10^5 conidia/ml for low sporulating isolate to 4.5×10^5 conidia/ml for highly sporulating isolate which were similar though with slight variations with results of the current study. The conidial shape was obclavate with colour being golden brown or light brown. The conidial length ranged between 26.89 µm for the shortest to 76.15 µm for the longest and the width ranging from 9.50 to 23.82 µm.

Abdessemed *et al.* (2019) isolated *A. alternata* from leaves of sow thistle (*Sonchus oleraceus* L.) and field bindweed (*Convolvulus arvensis* L.) on PDA media and identified as *A. alternata* on morphological and molecular basis (genetic marker, internal transcribed spacer, ITS of rDNA) further, pathogenicity test also confirmed.

Aslam *et al.* (2019) isolated *A. alternata* from symptomatic leaf samples of spinach and found that the cultures were olive green and turned gray to dark black. Conidia were in branched chains, pale brown to dark brown with a short beak at the tip and measured 27.8 to 61.2 µm long × 10.4 to 15.6 µm wide with 3 to 8 transverse septa and 1 to 2 longitudinal septa. Conidiophores were septate, branched or simple, light brown to olive brown, 23 to 56 µm long and 2 to 4 µm wide.

Kirareia *et al*. (2019) observed that *A. alternat*a isolates had grayish white compact aerial mycelium with black center, other isolates had dense raised grayish white aerial mycelium with small dark concentric rings and circular margins. The conidia varied in shape from mostly muriform to ellipsoidal having smooth walls and 1-3 longitudinal and 2 to 10 transverse septations with cylindrical curved beaks. The conidial length ranged between 24.76 µm for the shortest to 75.14 µm for the longest and the width ranged from 6.82 µm to 14.78 µm. The conidiophores were unbranched, arising singly or in clusters, long or short. Conidiophores were olivaceous to olivaceous brown with majority being straight and a few curved.

Nivedha *et al*. (2019) studied the *A. alternata* of *Jasminum grandiflorum* causing leaf blight was isolated on the PDA media and showed abundant of brownish septate mycelium and conidia were olive brown, produced in chains having both vertical and horizontal septations with dark coloured lesions.

Kant *et al.* (2020) studied that the leaf spot and blight disease caused on leaf of *Alianthus excelsa* in India. The leaf was collected and small portion was used in ITS and translation elongation factor 1-alpha and they confirmed that it was *A. alternata*. Koch's postulate was fulfilled by re-isolating *A. alternata* from the inoculated leaves.

This work was the first to confirm that *A. alternata* was associated with leaf spot and blight disease.

Nira *et al.* (2022) isolated and identified *A. alternata* causing *A.* leaf spot of broccoli. They described the morphology of *A. alternata* colonies which were surrounded by white young tip hyphae at the initial growth stage that later turned greenish black in colour. The conidia of the isolates were dark, cylindrical, obclavate and muriform. The conidiophore were olivaceous, septate and branched. The dimension of the conidia of the isolates ranged from 56.6-92.4 × 10-20 µm with 2-6 transverse and 0-3 longitudinal septa.

2.2 Pathogenicity test of pathogen associated with leaf and fruit spot disease

Mangala *et al*. (2006) proved the pathogenicity of *A. alternata* on chilli cultivars and they revealed that the fungus isolated from diseased chilli leaves produced typical leaf symptoms upon inoculation to healthy chilli plant that were similar to those recorded on naturally infected plants. Upon artificial inoculation, small necrotic spots were appeared on many plant species such as tomato, red gram, black gram, green gram, groundnut, cabbage and mustard.

Tziros *et al.* (2008) conducted pathogenicity tests on fruits of pomegranate. PDA-plugs, 5 mm diameter, with actively grown mycelium of *A. alternata* were transferred into the fruit calyx as well as on wounds made by a scalpel on previously sterilized fruit surfaces. Following inoculation, the fruits were placed in plastic bags and kept at 23 ºC for 10 days. Fruits inoculated in the same way using PDA disks were kept as control. Although there were no external symptoms on any of the fruits, decay of the core was observed when inoculated fruits were cut vertically.

Zakir and Sharma (2009) proved the pathogenicity of *A. alternata* on pomegranate fruit by pin-prick the fruits to a 1 mm depth with a sterile needle. The fungus isolated from diseased pomegranate fruit cultured in laboratory. The seven days old culture was inoculated on the fruit. On third day after inoculation symptoms were started to develop on the pomegranate fruit.

Ezra *et al.* (2010) made investigations on the pathogenicity of *A. alternata* in laboratory by detached leaf and fruit inoculation method. Drops of spore suspension of a 10^6 conidia/mL from 50 different isolates were inoculated on approximately 2 weeks old leaves and 2 months old fruit and placed in moist chamber at 25 ºC. Symptoms appeared 48-72 hrs. later. Plant of pomegranate (3-6 month old) sprayed or drop inoculated with a 10^6 conidia/mL and covered with plastic bag. Black spot was appeared on leaves one week after inoculation.

Arain *et al.* (2012) proved the pathogenicity of *A. alternata* in mature spore suspension. Pure culture of *A. alternata* grown in Petri plate was thoroughly mixed in 500 ml sterilized distilled water and then the suspension was sprayed on okra plant. The symptoms appeared on okra after 15 days of spray.

Ramjegathesh and Ebenezar (2012) proved pathogenicity of *A. alternata* causing leaf blight disease of onion. They reported that after twelve days of inoculation, first symptoms appeared in the form of small whitish flecks on the leaf tip. These increased in size and become sunken lesion. The lesion gradually exhibited a greyish tint at the center surrounded by a yellow halo.

Farhood and Hadian (2012) proved the pathogenicity of *A. alternata* on gerbera plants in a greenhouse. The gerbera plants were cultivated at regular in pots with sterile soil mixture and they were spray inoculated with 100 ml of the spore suspension per pot. Inoculated plants were placed in a humid chamber with 100 per cent relative humidity at 25 °C for 24 hrs. The plants were grown in a greenhouse (25-30 °C) for two weeks for symptom development. After two weeks typical symptoms were produced on inoculated leaves. The pathogen from the infected leaves was re-isolated on PDA medium. The morphological and cultural characteristics of the reisolated organism were compared with the original pathogen and they were same.

Gat *et al*. (2012) obtained 50 isolates of *A. alternata* from infected pomegranate plants that were classified based on pathogenicity using detached leaves, flower and fruit. The isolates were classified in four groups based on variability to the severity of symptoms in leaves. Pathogenicity tests were also done on flower and fruit and response were different than on leaves.

Meena *et al*. (2013) proved pathogenicity and investigation of host range of *A. alternata* causing Alternaria blight of isabgul on different plants namely ashwagandha, barley, cabbage, cauliflower, chilli, coriander, cumin, mustard, tomato and wheat by using artificial inoculation technique and results revealed that *A. alternata* could produce visible symptoms on all tested plants except ashwagandha. The symptoms expression took longer time 10 to 12 days in mustard, 7 to 9 days in coriander, cumin, cauliflower, chilli and tomato as compared to 5 to 6 days in isabgul.

Ammar and El-Naggar (2014) proved the pathogenicity of *A. alternata* on pomegranate fruit by pin-pricking the fruits to a 1 mm depth with a sterile needle then inoculating seven days old actively grown mycelium in to the skin wounds by pressing slightly into the fruits. Appearance of black spot-on fruit ranging from single lesion to lesions that coved more than 50 per cent of fruit surface found after 5 days of incubation.

Devappa and Thejakumar (2016) proved pathogenicity by inoculating the spore suspension of the *A. alternata* and control was maintained without inoculation. The symptoms of Alternaria leaf spot were recorded seven to nine days after inoculation on the inoculated leaves with a small, circular necrotic spot. These spots started to increase with irregular margin and it remained brown in colour surrounded by yellow hallow. Re-isolation of the fungus confirmed identity with the original culture.

Hosseinnia and Mohammadi (2018) proved pathogenicity of *A. alternata* isolates on *Lonicera japonica* leaf in greenhouse conditions and they were spraying 10^6 suspensions of fungal spores from seven days old colonies in PDA on healthy plant leaves. Symptoms observed three to five days after the spore spraying on the leaves and chlorosis was observed at the leaf surface. The results were evaluated after one week and from leaves with leaf spot symptoms and the fungus was recovered.

Aslam *et al.* (2019) tested pathogenicity of *A. alternata* on leaves of spinach by spraying spore suspension (10^6 conidia/ml) on 1 to 2 week old spinach plants (*cv.* Local Sindhi) grown in pots. Plants were covered for 24 h with plastic bags to maintain relative humidity and kept at 25±1 °C in a greenhouse. After 7 to 10 days, inoculated leaves showed symptoms similar to those observed in the field. The plants that were grown as the control were sprayed with sterile distilled water and did not express any symptoms. The pathogen was reisolated from the leaf lesions of artificially inoculated plants. Re-isolated pathogen was compared with the original one and was identical, completing Koch’s postulates.

Mohamed *et al.* (2019) conducted experiment for the pathogenicity of two purified isolates of *A. alternata* from cotton and were tested on Bt-cotton variety Seenil (*G. hirsutum*) and Barakat (*G. barbadense*) and confirmed by Koch's postulates.

Hamed *et al*. (2020) tested pathogenicity on three months old date palm seedlings using spore suspension per inoculation site. *Alternaria* spp. proved to be pathogenic on date palm leaves.

Shafique *et al.* (2021) proved pathogenicity of *A. alternata* on eggplant. They taken 6-week old healthy potted eggplants (*cv.* Bemisaal) were sprayed at the true leaf stage with conidial suspensions of *A. alternata* (10^6 conidia/ml obtained from 1-week old cultures) amended with 0.1% Tween 20 until runoff (1.5 to 2 ml/plant) using anatomizer in the greenhouse. Three plants were inoculated with each of the two isolates (JLUAF1 and JLUAF2) and three control plants were sprayed with sterile distilled water amended with 0.1% Tween 20. The plants were incubated at 25±2 °C in a greenhouse and the experiment was conducted twice. After ten days of inoculation, each isolate induced leaf spots similar to typical spots observed in the field, whereas control plants remained symptomless.

2.3. *In vitro* bio-efficacy of different bioagents against *A. alternata*

Kota (2003) reported that *Trichoderma harzianum* and *Trichoderma virens* were highly superior in inhibiting the growth of *A. alternata* under *in vitro* conditions.

Kumar *et al.* (2008) tested six biocontrol agents against *A. alternata* (Fr.) Keissler under laboratory condition by dual culture inoculation technique *T. harzianum* recorded highest inhibition of radial growth. *Trichoderma koningii, Trichoderma viride* and *Trichoderma virens* were next in order.

Mane (2008) evaluated *in vitro* efficacy of bio-agents *viz., T. viride, T harzianum, T. koningii* and *Gliocladium virens* against *A. alternata* causing leaf blight of chilli. He reported that maximum growth inhibition of the test fungus was achieved with *T. harzianum* (86.11 %). This was followed by *T. viride,* G. *virens* and *T. koningii* which recorded 81.33, 80.00 and 79.66 per cent inhibition, respectively.

Anand and Ramanujan (2009) reported that *T. viride* and *Pseudomonas fluorescens* were effective in inhibiting mycelial growth of *A. alternata* causing fruit rot of chilli.

Hudge *et al.* (2009) studied the antagonistic potential of *Trichoderma* spp. *viz., T viride, T. harzianum, T. hamatum, T. koningii, T. lignorum* against *A. alternata,* causing leaf spot of jatropha. They reported that all the *Trichoderma* species except *T. lignorum* reduced mycelial growth of the test fungus. The maximum reduction was observed with *T. hamatum* followed by *T. harzianum.*

Mandhare and Suryawanshi (2009) conducted laboratory experiments to determine the effect of airborne antagonist *Bacillus thermophillus* on *A. porri* (Purple blotch of onion) and *A. alternata* (leaf spot of pomegranate) where conidial germination and mycelial growth of both the fungi were inhibited by *Bacillus thermophillus* at 2.2×10^9 and 2.2×10^8 cfu/ml, respectively.

Joshi *et al.* (2010) tested eleven antagonists by dual culture method *in vitro* against *A. alternata* causing leaf spot and fruit rot of papaya and they reported *T. viride* (Sardarkrushinagar) significantly maximum per cent growth inhibition (91.95 %) and grow over the *A. alternata* colonies, followed by *T. viride* (Navsari) as 88.72 per cent growth inhibition. Minimum growth inhibition was recorded in *T. longibrachiatum* (Navsari) and *Bacillus subtilis* (Sardarkrushinagar) with 26.93 and 32.36 per cent growth inhibtion, respectively.

Thaware *et al.* (2010) studied antagonistic effect of the fungal bioagents against *A. alternata* and reported that *T. harzianum* and *T. viride* significantly inhibited the mycelial growth of *A. alternata* causing leaf blight of cow pea.

Gohel and Solanky (2011[a]) evaluated eight bioagents *in vitro* against *A. alternata* causing fruit rot of chilli by dual culture techniques, *i.e* by placing pathogen at periphery and pathogen at centre. They reported that the test antagonists significantly checked mycelial growth of the pathogen in both methods. However, *T. longibrachiatum* showed maximum mycelial inhibition (73.21 %), followed by *T. harzianum* (49.46 %) in pathogen at periphery method; whereas, *A. flavus* (77.00 %) showed maximum mycelial inhibition in pathogen at centre method. Overall, *Trichoderma* spp*., A. flavus, B. subtilis* were found effective against *A. alternata.*

Taj and Kumar (2012) studied the *in vitro* screening using the dual culture technique was undertaken to assess the potential of interaction of six native isolates of *T. harzianum* against gerbera leaf spot pathogen (*Alternaria* spp.). The result revealed that the native strains of *T. harzianum* use were able to inhibit the mycelial growth of the pathogen significantly.

Maheshwari and Krishna (2013) tested six bioagents *viz.*, *T. viride*, *T. harzanium*, *T. virens*, *G. roseum*, *G. virens* and *P. fluorescens in vitro* against *A. alternata* causing leaf spot of mung bean. They observed the highest fungal growth inhibition in *T. viride* (51.70 %), followed by the *T. harzanium* (45.30 %), *T. virens* (41.40 %), *G. roseum* (28.71 %), *G. virens* (26.56 %) and *P. fluorescens* (19.50 %).

Rajput *et al.* (2013) tested eight biocontrol agents against *A. alternata* causing leaf spot of brinjal by three different methods *viz.,* dual culture, pathogen at the center and periphery. They reported maximum mycelial growth inhibition with *T. viride* IARI isolate (74.77 %, 69.04 % and 79.45 %), *T. viride* Navsari isolate, (74.14 %, 66.08 % and 76.99 %), *T. harzianum* Junagadh isolate (71.25 %, 59.96 % and 74.78 %) in dual culture, pathogen at periphery and pathogen at the centre methods, respectively.

Apet *et al.* (2014) evaluated six bio agents against *A. alternata* causing leaf spot of gerbera and founded significantly highest mycelial growth inhibition with *T. viride* (86.67 %) followed by *T. hamatum* (78.34 %), *T. koningii* (76.67 %), *T. lingorum* (68.15 %), *T. harzianum* (53.16 %) and *P. fluorescens* (50.38 %).

Tagaram *et al*. (2015) studied antagonistic activity of *T. harzianum* and *T. viride* isolated *in vitro* conditions against *A. alternata* causing leaf spot disease on cassia. They revealed that the per cent growth inhibition of *A. alternata* by *T. viride* (80.10 %) and *T. harzianum* was (72.20 %) by dual culture method.

Tapwal *et al.* (2015) evaluated *in vitro* effect of *T. viride* and *T. harzianum* by dual culture, non-volatile metabolites and volatile compounds on growth of seed borne pathogen *A. alternata.* They observed that *T. harzianum* and *T. viride* found maximum mycelial growth inhibition of 34.20 and 34.27 per cent, respectively by dual culture method. After two weeks of incubation, the non-volatile and volatile compounds released by *T. viride* and *T. harzianum* also inhibited mycelial growth of the test pathogen. Maximum mycelial growth inhibition of 36.00 and 20.00 per cent was observed with *T. viride* produced by non-volatile and volatile compounds, respectively, whereas, it was comparatively minimum with *T. harzianum* produced by non-volatile (11.76 %) and volatile (11.11%) compounds.

Hans and Sharma (2017) tested five bioagents *in vitro* condition against *A. alternata* causing mouldy core, core rot of apple by dual culture technique. Among the tested bioagents *T. hamatum* was most effective and exhibited 55.13 % mycelial growth inhibition followed by *T. harzianum* (46.15 %), *P. fluorescens* (45.35 %), *B. subtilis* (42.28 %) and *T. viridi* (40.15 %) over the control.

Ajayabhai *et al.* (2018) evaluated antagonistic actions of the five known bio-agents against *A. alternata* causing Alternaria leaf blight of groundnut by dual culture technique. They observed maximum mycelial growth inhibition in *T. viride* (84.55 %) followed by *T. harzianum* and *P. fluorescens* restricting growth by 80.90 and 37.64 per cent, respectively.

Kadam *et al.* (2018) tested nine different bioagent against *A. alternata* causing leaf spot of pomegranate. They recorded highest mycelium growth inhibition of *A. alternata* with *Trichoderma* spp. followed by *B. subtilis* and *P. florescence*.

Suhas and Simon (2019) evaluated different antagonist *viz*., *T. viride* and *P. fluorescens* against *A. alternata* causing Alternaria blight of chickpea by dual culture technique. The result revealed that the maximum growth inhibition of *A. alternata* of chickpea were observed in *T. viride* (49.80 %) followed by *P. fluorescens* (41.84 %) over control.

Praveek *et al.* (2020) investigation on of the bio-efficacy of fungal and bacterial bio-agents against *A. alternata* inciting little millet leaf blight. Among different bio-agents, *Bacillus velezensis* (P42 strain) gave the best result with 84.75 per cent inhibition over control against *A. alternata* followed by *B. velezensis.*

Rahman *et al.* (2020) tested five *Trichoderma* strains significantly exhibited antibiotic potential against *A. alternata* causing leaf blight of ashwagandha and found *T. virens*, *T. pseudokoningii*, *T. harzianum* IMI-392432, *T. harzianum* IMI-392433 and *T. harzianum* IMI-392434 strains attained inhibition percentages of 43.62, 36.6, 53.83, 54.89 and 48.94, respectively. Strains *T. harzianum* IMI-392433 showed the highest per cent inhibition growth value of 54.89 %, while the least inhibition percentage was observed with the isolate *T. pseudokoningii* (36.60 %). This indicates that *T. harzianum* IMI-392433 strain had maximum antifungal activity against *A. alternata* compared to the other *Trichoderma* strains.

Shingne *et al.* (2020) assayed the efficiency of five bioagents against *A. alternata* causing leaf spot disease of niger *in vitro*. They reported that *Trichoderma reesei* gave the better effect against *A. alternata* forming maximum per cent mycelial inhibition (75.00 %), *P. fluorescence* was next best recorded (65.36 %) inhibition while the least mycelial inhibition was observed in *B. subtilis* (60.36 %) and *T. asperellum* (58.88 %).

Khillare *et al.* (2022) tested seven antagonists *in vitro* against *A. alternata* causing Alternaria blight of pigeon pea by dual culture technique and result revealed that highest mycelial growth inhibition was with *T. hamatum* (85.88 %), followed by *A. niger* (77.00 %), *T. asperellum* (74.77 %), *T. harzianum* (74.00 %), *T. koningii* (54.21 %), *T. longibrachatum* (44.80 %) and *P. fluorescens* (43.44 %) over control.

Ghosh *et al.* (2022) tested *Trichoderma* species against *A. alternata* causing leaf blight of *Bacopa monnieri* (L.) *in vitro*. They obsereved that highest per cent growth inhibition exerted by *T. asperellum* (77.22 %) followed by *T. harzianum* (75.55 %).

2.4 *In vitro* bio-efficacy of different phytoextracts against *A. alternata*

Chaudhary *et al.* (2003) screened ten indigenous species of plant extracts against *A. alternata* causing early blight of potato. They reported the extract of garlic at 10 per cent concentration showed significantly maximum growth inhibition (68.58 %) of *A. alternata* followed by datura (65.43 %) and lantana (63.43 %). The next effective phytoextracts in order of inhibition and different significantly were extracts of onion, bhoyringni, eucalyptus, ginger and neem.

Joshi *et al.* (2010) evaluated ten different phytoextracts having medicinal values through poisoned food technique *in vitro* against *A. alternata* causing leaf spot and fruit rot of papaya. They found garlic bulb extract at 15 per cent concentration recorded maximum growth inhibition (88.91 %) of *A. alternata,* which was at par with lantana leaves (88.36 %) and datura leaves (87.51 %) extract.

Waghunde *et al.* (2010) recorded lowest mycelial growth in garlic clove extracts (25.25 mm) giving 62.45 per cent inhibition over control *in vitro* against *A. alternata* causing Alternaria fruit rot of aonla.

Balai and Ahir (2011) studied *in vitro* efficacy of five plant extracts each @ 5, 10 and 15 % against *A. alternata* causing leaf spot of brinjal. They reported garlic clove extract with maximum inhibiting of mycelial growth (73.70 %) of the test fungus followed by NSKE (70.20 %), while bel extract was least effective. Higher concentration (15%) of plant extracts caused maximum inhibition of mycelial growth. The maximum inhibition of mycelial growth (86.00 %) was recorded at 15 per cent concentration with garlic clove extract followed by NSKE (80.00 %).

Anamika and Simon (2011) studied *in vitro* inhibitory effects of botanical extracts each @ 5 and 10 per cent against *A. alternata* of aloe vera. They reported that neem leaf extract gave 58.60 per cent inhibition of mycelial growth and 56.50 per cent inhibition of spore germination at 10 per cent concentration followed by *Ocimum sanctum* which gave 54.71 per cent inhibition of mycelial growth and 50.42 per cent inhibition of spore germination.

Ahire *et al.* (2012) evaluated 15 botanicals each @ 10 and 20 per cent against *A. alternata* causing blight of marigold *in vitro* and they reported that custard apple (*A. squamosa*) as most effective significantly highest mycelial growth inhibition (75.91 %) followed by garlic (*A. sativum*) (69.53 %) and *D. metal* (68.88 %). *P. longifera* and *T. procumbens* were found least effective with mycelial growth inhibition of (8.04 %) and (8.32 %), respectively.

Bavaji *et al.* (2012) studied the efficacy of eight plant extracts each @ 1000, 3000 and 5000 ppm were tested *in vitro* against *A. alternata* causing leaf blight of sesame. They reported the extracts of *Boswellia ovalifoliolata* @ 5000 ppm resulted in highest mycelial growth inhibition (66.60 %). The extracts of *Euphorbia tirucalli, Cassia lora, Carthamus roseus, Eclipta alba, Cleome viscose, Hiptis saveolenis* and *Almanda carhartica* (each @ 5000 ppm) caused mycelial growth inhibition of 63.30, 61.50, 52.20, 51.20, 49.70, 44.80 and 42.50 per cent, respectively.

Mishra and Gupta (2012) tested seven plant extracts *in vitro viz., A. cepa, A. sativum, A. indica, L. camara, Pongamia spices, O. sanctum* and *A. vera* against *A. porri* causing purple blotch of onion. They reported that garlic showed highest mycelia inhibition (58.05 %) followed by *A. vera* (53.50 %), ginger (29.87 %), *O. sanctum* (28.00 %), neem (27.87 %), pongamia leaves (26.00 %), *L. camara* (25.20 %) and *A. cepa* (23.00 %).

Waghmare (2012) studied the efficacy of aqueous and alcoholic leaf extracts (each at 25, 50, 75 and 100 per cent) of *Hyptis suvavealns, Tridax procumbens, Polyanlhia longifolia, Swietenia macrophylla, Parthenium hysterophonus* and *Vitex negundo* against *A. alternata* causing leaf spot of gerbera. They reported the alcoholic leaf extract of all the tested plant species used against *A. alternata* showed cent per cent control efficacy of at 25 per cent concentration. The aqueous leaf extracts of all the tested plants decreased the growth of the pathogen as the concentration of the extracts increased.

Apet *et al.* (2014) evaluated 13 plant extracts *in vitro* each @ 10 and 20 per cent against *A. alternata* causing leaf spot of gerbera and found significantly highest average mycelial growth inhibition with *A. sativum* (74.45 %) followed by *C. longa* (63.99 %), *D. metal* (53.06 %), *C. gigantica* (48.99 %) and *P. hysterophorus* (48.90 %).

Bhosale *et al.* (2014) evaluated different angiospermic plant extract against *A. alternata in vitro* and result revealed that dip treatment in different concentration of angiospermic plant extracts (10%, 15% and 20%) *Allium sativum* and *Allium cepa* brought about significant reduction in diseases intensity caused by *A. alternata* leaf spot on the soybean. The highest mean per cent inhibition were observed in *A. sativum* (83.72 %) followed by *A. cepa* (64.46 %), ginger (57.73 %), neem (51.59 %), parthenium (49.47 %), turmeric (44.24 %), ashwagandha (43.01 %), lantana

(41.33 %), eucalyptus (41.18 %), dhatura (39.58 %), mehndi (41.72 %) and beshram (40.10 %).

Kantwa *et al.* (2014) was conducted experiment on plants extract against *A. alternata* causing leaf blight of groundnut. Among all, garlic clove extract found most effective in inhibiting the mycelial growth and sporulation of *A. alternata* infecting the groundnut, followed by neem and datura leaf extracts. Babul leaf extract was not inhibiting the mycelial growth and sporulation of the fungus in poisoned food technique.

Barman *et al.* (2016) evaluated *in vitro* anti-fungal property of five plant extracts (aqueous, ethanolic and ethyl acetate) against *A. alternata* causing leaf blight of tomato. They observed that ethyl acetate leaf extract of *A. sativum* showed cent per cent mycelial growth inhibition. Ethyl acetate extracts of *X. strumarium, C. viscosum. P. longifolia* and *D. stramonium* also showed mycelial growth inhibition by 57.70, 75.50, 73.30 and 62.20 per cent, respectively. Aqueous leaf extracts of *A. sativum, C. viscosum. X. strumarium. D. stramonium and P. longifolia* recorded mycelial growth inhibition by (73.30 %), (51.10 %), (40.00 %), (28.80 %) and (13.30 %), respectively. While, ethanolic extracts of *A. sativum, C. viscosum, D. stramonium. P. longifolia* and *X. strumarium* were more effective with mycelial growth inhibition by 82.20, 60.00, 60.00, 48.80 and 46.60 per cent, respectively.

Hans and Sharma (2017) tested seven phytoextracts at four different concentrations *viz.* 10, 25, 50 and 100 per cent *in vitro* condition against *A. alternata* causing mouldy core, core rot of apple by poison food technique. They found *Dodonia viscosa* phytoextract was most effective against *A. alternata* resulting in significant inhibition by (66.55 %) followed by *A. indica* (33.34 %), *M. azaderach* (11.46 %), *V. negundo* (9.55 %) and *L. camara* (9.26 %).

Jakatimath *et al.* (2017) tested seven different botanical extracts against *A. alternata* causing fruit rot of brinjal *in vitro* conditions at concentration 5 and 10 per cent. They revealed that the highest inhibition was recorded in garlic and onion bulb extract (100 %) at both @ 5 and 10 per cent concentration followed by neem seed kernel extract (75.18 %) at 10 per cent concentration whereas at 5 per cent concentration neem seed kernel extract show least inhibition of 38.14 per cent.

Kadam *et al.* (2018) evaluated eleven phytoextracts *in vitro* against *A. alternata* causing leaf and fruit spot in pomegranate. Among tested phytoextracts average mycelial growth inhibition of the test pathogen was ranged from 19.81 to 75.56 per cent. However, significantly highest average mycelial growth inhibition was recorded with *A. sativum* (75.56 %), followed by *Z. officinale* (73.64 %), *A. indica* (71.17 %), *E. globulus* (66.36 %), *C. longa* (60.19 %), *D. metal* (56.36 %), *V. negundo* (54.20 %) and *L. camera* (50.68 %) whereas *P. pinnata*, *P.hysterophorus* and *Bougainveilliea spp*. recorded comparatively least mycelial growth of 40.37, 33.33 and 19.81 per cent, respectively.

Rajput and Chaudhari (2018) evaluated eleven phytoextract *in vitro* for their inhibitory effect on the mycelial growth and spore formation by *A. alternata*. They found rhizome extract of turmeric (54.42 %) was found significantly superior in inhibiting mycelial growth over the rest. The next best in order of merit was garlic (50.67 %) and neem (41.27 %) followed by dhatura (37.08 %), onion (35.40 %), nilgiri (33.52 %), kadvi mehandi (29.52 %) and barmasi (23.65 %). Lantana (20.71 %), jetropha (17.68 %) and vilayati baval (13.66 %) were comparatively less effective.

Rani *et al.* (2018) evaluated different botanicals against *A. alternata* infecting pigeon pea *in vitro* condition. They found that garlic clove extract at 10 % was most effective with 84.31 per cent inhibition of growth of *A. alternata* followed by onion bulb extract and neem extract.

Meena *et al.* (2020) studied the efficacy of three plant extracts against *A. alternata* at a concentration of 5 per cent by poison food technique under *in vitro* condition. They reported that the neem leaf extract was effective in suppressing the mycelium growth of *A. alternata* by (59.60 %) followed by *P. pinnata* (52.43 %) and *M. alliacea* (51.36 %).

Nagaraju *et al.* (2020) tested four botanical extracts *i.e.,* turmeric, tulsi, neem and aloe vera against *A. alternata* at three concentrations (2.5, 5 and 7.5%) by poison food technique under *in vitro* condition. Among all, tulsi extract found the maximum growth inhibition (51.90 %), (44.42 %) and (38.09 %) at 7.5, 5 and 2.5 per cent concentration, respectively.

Shingne *et al.* (2020) assayed the efficiency of five phytoextracts against *A. alternata* causing leaf spot disease of niger at 10 per cent concentration *in vitro* condition and reported that turmeric rhizome extract @ 10 per cent was maximum inhibition (54.92 %) of mycelial growth of test fungus and significantly superior to rest of the treatments followed by neem leaf extract and garlic bulb extract recorded (35.83 %) and (26.38 %) mycelial growth inhibition of *A. alternata,* respectively.

Rest of the plant extracts *viz*., tulsi leaf extract and eucalyptus leaf extract recorded (18.47 %) and (14.72 %) mycelial growth inhibition which was least effective against test pathogen.

Chaudhary and Singh (2021) evaluated different botanicals against *A. alternata* causing Alternaria leaf spot of ber by poison food technique and they observed highest average mycelium growth inhibition was obtained by garlic (74.88 %) followed by ginger (73.51 %) and neem (69.25 %), while minimum inhibition was found by parthenium (34.64 %) followed by dhatura (53.37 %).

Sharma *et al*. (2021) tested seven phytoextracts *in vitro* at three concentrations *viz.,* 5, 10 and 15 per cent against *A. alternata* and found extract of *A. sativum* was most effective in inhibiting mycelial growth (90.11, 100 and 100 %) of *A. alternata* at 5, 10 and 15 per cent, respectively, followed by *A. indica* leaves extract (79.45, 83.60 and 88.22 %). Extract of *Alstonia scholaris* leaves was found least effective in inhibiting mycelial growth of *Alternaria* with 36.22, 40.33 and 47.77 per cent at 5, 10 and 15 per cent concentration, respectively. All the concentrations (5, 10 and 15%) of *A. sativum* clove extract were found significantly superior over other treatments.

2.5. *In vitro* bio-efficacy of different fungicides against *A. alternata*

Phapale *et al*. (2010) tested ten fungicides from systemic and non-systemic groups at three different concentrations by poisoned food technique *in vitro* against *A. alternata*. They found, cent per cent inhibition *of A. alternata* was recorded with propiconazole, difenoconazole and hexaconazole at all three concentrations (250, 500, 1000 ppm). Copper oxychloride at all three concentrations (1500, 2000, 2500 ppm) while mancozeb at 2500 ppm resulted cent per cent inhibition. The next best in order of efficacy were mancozeb at 2000 (89.96 %) and 1500 ppm (88.94 %). Chlorothalonil was moderately effective while carbendazim and a product carbendazim + mancozeb were found least effective in growth inhibition as compared to other fungicides at all the three concentrations tried.

Thaware *et al.* (2010) evaluated different fungicide against *A. alternata* (Fr.) Keissler causing leaf blight of cow pea under laboratory condition and they reported that mancozeb (0.2 %) and propiconazole (0.05 %) completely inhibited the growth of the test fungus *A. alternata.*

Chaudhary *et at*. (2011) tested different fungicides *in vitro* by using poisoned food technique against early blight (*A. alternata*) of tomato and reported that difenoconazole at 250, 500 and 1000 ppm showed significant maximum growth inhibition of pathogen over carbendazim and thiophanate methyl. Among combination of systemic and non-systemic fungicides, maximum growth inhibition was recorded in metalaxyl + mancozeb at 1000, 1500 and 2000 ppm concentration.

Chowdary *et al.* (2011) tested different fungicides against the mycelial growth and conidial germination of *A. alternata* causing leaf blight disease of mulberry assessed at 1000 and 2000 ppm concentrations. They reported that, ferrous sulphate and copper oxychloride at 1000 and 2000 ppm were found effective against *A. alternata* under *in vitro* conditions.

Gohel and Solanky (2011[b]) performed *in vitro* screening of different fungicides by poisoned food technique at three different concentrations showed that propiconazole, difenoconazole, hexaconazole, copper oxychloride, mancozeb and propineb were highly fungitoxic to *A. alternata*.

Archana (2012) evaluated different fungicides *in vitro* against Alternaria leaf spot and fruit spot/rot of pomegranate and found highest inhibition by copper oxychloride (75.95 %) which was significantly superior over chlorothalonil (60.16 %) and zineb (51.22 %). Mancozeb (20.71 %) was found least effective in inhibiting the growth of *A. alternata.* Among five different systemic fungicides tested, significantly highest inhibition was obtained by propiconazole (100 %) which was superior over hexaconazole (92.85 %) and difenoconazole (84.34 %). carbendazim (48.81 %) was least effective in inhibiting the mycelial growth of *A. alternata*.

Ahire *et al.* (2012) assayed eleven fungicides each @ 500, 1000 and 1500 ppm against *A. alternata* causing blight of marigold *in vitro* condition. They reported that mancozeb and cyamoxanil + mancozeb gave cent per cent mycelial inhibition at all concentrations tested followed by hexaconazole and propineb with growth inhibition of 92.09 and 89.28 per cent, respectively.

Bavaji *et al.* (2012) studied *in vitro* efficacy of different fungicides against *A. alternata* causing blight of sesame and they reported that the fungicides tilt @ 250g/ml, blitox-50 @ 500 g/ml, monoceren @ 1000 g/ml completely inhibited mycelial growth. These were followed by ridomil MZ, dithane Z-78, chlorothalonil, thiram, defolatan, captan and aureofungin which inhibited mycelial growth by 71.10, 66.60, 63.33, 63.33, 61.10, 55.50 and 52.20 per cent tested each @ 1000 g/ml concentration, respectively.

Pareek *et al*. (2012) revealed that mancozeb found most effective followed by copper oxychloride, carbendazim + mancozeb and least effective was thiophanate methyl against mycelial growth inhibition and sporulation of *A. alternata* causing leaf spot of cucumber.

Rajani and Rakholia (2012) evaluated different systemic and non-systemic fungicides against fruit rot of chilli caused by *A. alternata in vitro*. They reported that systemic fungicide hexaconazole gave cent per cent growth inhibition followed by tridemorph (93.65 %) and propiconazole (91.42 %), whereas in non-systemic fungicides mancozeb and zineb gave cent per cent inhibition of *A. alternata.*

Vitner and Patel (2012) evaluated ten fungicides at different concentration *in vitro* against *A. alternata* causing leaf spot of marigold. They observed maximum growth inhibition (100 %) in hexaconazole at all five concentration tested. Next effective fungicides were mancozeb (3000 ppm), carbendazim + mancozeb (2500 ppm), metalaxyl + mancozeb (2500 ppm) and propineb (3000 ppm) which inhibited the fungal growth at 82.96, 79.26, 78.52 and 77.78 %.

Kumar *et al.* (2013) tested twelve different fungicides against *A. alternata* causing leaf spot of chilli. They reported that the fungicides *viz,* bavistin @ 0.1%, indofil M-45, chlorothalonil, vitavax and thiram (each @ 0.2%) completely inhibited mycelial growth of the pathogen. Rest of the fungicides in the order of merit were indofil Z-78 (97.00 %), captafol (94.10 %), ziram (90.30 %), captan (86.60 %), blitox-50 (81.40 %), kitazin (78.60 %) and topsin-M (72.60 %).

Maheshwari and Krishna (2013) evaluated *in vitro* efficacy of thirteen fungicides *viz.,* hexaconazole, mancozeb, captan, indofil Z-78, copper oxychloride, ziram, dodine, thiophanate methyl, propineb, carbendazim, benomyl, chlorothalonil and wettable sulphar and found hexaconazole was most effective fungicide with mycelial inhibition (100.00 %) followed by the mancozeb (89.60 %), indofil Z-78 (86.20 %), captan (83.90 %), copper oxychloride (80.90 %), ziram (76.80 %), dodine (73.90 %), thiophanate methyl (72.40 %), chlorothalonil (68.40 %), popineb (64.70 %), carbendazim (63.20 %), benomyl (60.10 %) and wettable sulphar (55.20 %).

Parveen *et al.* (2013) evaluated *in vitro* efficacy of systemic and non-systemic fungicides (each @ 200, 400, 600, 800, 1000 ppm) against *A. alternata* causing fruit rot and reported captan and zineb inhibited highest mycelial growth (each 69.33 %) followed by biteranol (65.33 %), myclobutanil (64 %), mancozeb (62 %) and hexaconazole (61.33 %).

Yadav *et al.* (2013) tested six systemic fungicides *viz.,* carbendazim, difenoconazole, hexaconazole, propiconazole, tebuconazole and thiophanate methyl (each @ 50, 100, 250, 500 ppm) against *A. alternata* causing purple blotch of onion under laboratory condition. They reported that hexaconazole found most effective with significantly highest mean mycelial growth inhibition (98.21 %) followed by propiconazole (97.32 %), difenoconazole (91.23 %) and tebuconazole (89.77 %) whereas, it was least with thiophanate methyl (18.41 %) and carbendazim (10.97 %).

Apet *et al.* (2014) screened five systemic and six non-systemic fungicides against *A. alternata* causing leaf spot of gerbera *in vitro* and found highest average mycelial growth inhibition of the systemic fungicide was with hexaconazole (94.44 %) followed by carbendazim (84.93 %), propiconazole (81.53 %), difenoconazole (75.97 %) and thiophanate methyl (50.21 %). Among non-systemic fungicides tested, mancozeb recorded highest average mycelial growth inhibition (92.21 %) followed by curzet (84.45 %), chlorothalonil (80.90 %), propineb (78.89 %) and copper oxychloride (74.03 %).

Kantwa *et al*. (2014) reported that mycelial growth and sporulation of *A. alternata* was completely inhibited by mancozeb at 1000 ppm which followed by copper oxychloride and captaf.

Ginoya and Gohel (2015) evaluated newer fungicides against *A. alternata* under *in vitro* condition by poisoned food technique. Results revealed that tebuconazole, hexaconazole and azoxystrobin 18.2 + difenoconazole 11.4 % at all three concentrations (500, 1000 and 1500 ppm) completely inhibited the mycelial growth of fungus. These were followed by fusilazole 12.5 + carbendazim 25 % (84.22, 87.47 and 90.71 %), tebuconazole 50 + trifloxysyrobin 25 % (82.83, 86.07 and 87.23 %), fenamidone 10 + mancozeb 50 % (64.04, 67.04 and 69.14 %) and isoprothiolane (47.09, 64.49 and 69.37 %), respectively at 500, 1000 and 1500 ppm, respectively.

Kalieswari *et al.* (2016) tested seven fungicides against leaf spot disease of ashwagandha caused by *A. alternata* in laboratory condition. *viz.,* carbendazim @0.05%, mancozeb @ 0.2%, copper oxychloride @ 1%, chlorothalonil @ 0.2%, fosetyl @ 0.1 %, ridomil MZ @ 0.05 % and dithane M-45 @ 0.05 % and maximum inhibition was recorded by mancozeb @ 0.2%.

Hans and Sharma (2017) assayed seven fungicides against *A. alternata* causing mouldy core, core rot of apple by poison food technique *in vitro.* The result revealed that maximum mycelial inhibition (cent per cent) was exhibited by difenoconazole followed by hexaconazole (92.80 %), trifloxystorbin (91.38 %) and tebuconazole (85.76 %) over control. The next effective fungicides were iprodione + carbendazim

(82.09 %) followed by dodine (78.31 %), propineb (73.50 %), metiram (67.35 %), mancozeb (64.08 %) and captan (47.03 %). Shield was found to be the least effective with only 4.17 per cent growth inhibition.

Jakatimath *et al.* (2017) studied the efficacy of eight fungicides with four different concentrations in laboratory against *A. alternata* causing fruit rot of brinjal revealed that difenoconazole and tebuconazole recorded 100 per cent inhibition at all the concentration tested followed by propiconazole (91.25 %) and carbendazim (85.41 %).

Kapadiya (2017) evaluated different fungicides *viz.,* mancozeb @ 0.2 %, copper oxychloride @ 0.2%, hexaconazole @ 0.0075%, tebuconazole @ 0.0375%, difenoconazole @ 0.025% and propiconazole @ 0.025% against Alternaria leaf blight on groundnut and found the mancozeb was most effective followed by difenoconazole and hexaconazole.

Sarkar *et al*. (2017) evaluated *in vitro* efficacy of six fungicides against *A. alternata* causing leaf spot of chill and reported that mancozeb inhibited maximum (78.99 %) radial mycelial growth of *A. alternata* followed by carbendazim (78.66 %), captan (74.78 %), propiconazole (74.42 %), hexaconazole (60.48 %) and chlorothalonil (52.12 %).

Rai *et al*. (2017) studied the *Alternaria spp.* causing Alternaria leaf spot of cabbage under *in vitro* condition with three fungicides like propiconazole, chlorothalonil and carbendazim and found propiconazole @ 0.1% was most effective in inhibition (93.36 %) followed by chlorothalonil @ 0.2 % (73.47 %).

Ajaybhai *et al.* (2018) tested nine fungicides at different concentrations (250, 500, 1000 ppm) by poison food technique *in vitro* condition for the management of Alternaria leaf blight of groundnut caused by *A. alternata*. They found two fungicides *viz.* propiconazole and hexaconazole showed cent per cent growth inhibition at all tested concentrations while, tebuconazole and difenoconazole + azoxystrobin gave 100 per cent growth inhibition at 500 and 1000 ppm concentrations.

Kadam *et al.* (2018) evaluated six systemic, five non systemic and two combine fungicides *in vitro* against *A. alternata* causing leaf and fruit spot in pomegranate. Among systemic fungicides, maximum average per cent inhibition of mycelial growth was recorded with propiconazole (100 %) followed by hexaconazole (99.38 %), penconazole (90.74 %) and difenoconazole (85.80 %). Among non-systemic and combi-fungicides, significantly highest average mycelial growth inhibition was with carbendazim 12 WP + mancozeb 63 WP (81.39 %), followed by mancozeb (78.05 %), copper oxychloride (70.13 %), copper hydroxide (66 %), propineb 70 WP (61.81 %) and chlorothalonil (50.14 %).

Kakraliya *et al.* (2018) studied the effect of fungicides against *Alternaria sp.* and maximum inhibition per cent of the mycelia growth was found in propiconazole (89.72 %) followed by hexaconazole (88.44 %) and vitavax (87.77 %).

Sharma (2019) revealed that hexaconazole (98.81 %) was the most effective in inhibiting the mycelium growth of *A. alternata* followed by tebuconazole + trifloxystrobin (94.36 %).

Suhas and Simon (2019) evaluated the efficacy of fungicide against *A. alternata* causing Alternaria blight of chickpea by poison food technique. They reported chlorothalonil was highly effective in inhibiting the growth of *A. alternata* as its produced (55.27 %) growth inhibition at 5% concentration followed by mancozeb (51.54 %), azoxystrobin (42.23 %) and thiabendazole (37.33 %).

Choudhary *et al*. (2020) reported that tebuconazole + trifloxystrobin (300 ppm) was found most effective in inhibiting cent per cent mycelial growth of Alternaria leaf spot of lehsua followed by hexaconazole (95.56 %).

Kaur *et al.* (2020) found that azoxystrobin and mancozeb were most effective and inhibiting the growth of *A. alternata in-vitro* with ED 50 value of 0.8 ppm and 74 ppm, respectively.

Meena *et al.* (2020) evaluated the efficacy of eight fungicide *in vitro* against *A. alternata* causing leaf blight of chandrasur. They observed tebuconazole and tebuconazole + trifloxystorbin (0.1%) were found most effective to inhibit the mycelium growth by 89.84 and 89.31 per cent, respectively over control followed by cuprous hydroxide (80.43 %), mancozeb (75.94 %), copper oxychloride (73.43 %) and azoxystrobin (25.17 %).

Shingne *et al.* (2020) assayed the efficacy of six fungicides against *A. alternata* causing leaf spot disease of niger *in vitro*. The result indicated that carboxin + thiram @ 0.3 per cent inhibits 100 per cent mycelial growth followed by carbendazim + mancozeb (93.15 %), copper oxychloride (92.22 %), propineb (90.00 %) and pyraclostrobin (80.37 %). Azoxystrobin were found least effective as recorded only (49.07 %) inhibition over the control.

Jewaliya *et al.* (2021) tested the efficacy of fungicides at different concentrations *viz.,* 100, 150, 200 and 500 ppm against *A. alternata* on PDA by poisoned food technique. They recorded maximum mycelial growth inhibition (97.96 %) with hexaconazole followed by tebuconazole + trifloxystrobin (94.17 %). Propineb recorded least per cent inhibition of mycelium growth at all concentrations with a mean per cent growth inhibition of (60.83 %). At 200 and 500 ppm, hexaconazole, propiconazole, azoxystrobin and tebuconazole + trifloxystrobin showed cent per cent inhibition of mycelium growth followed by carbendazim + mancozeb with (78.52 %) and (84.44 %) inhibition, respectively.

Madadi *et al.* (2021) evaluated the efficacy of five fungicide at different concentration *in vitro* condition against *A. alternata* causing potato brown spot at 200 ppm concentration results showed that growth inhibition by flutriafol 6.94 + tebuconazole 20.8 % was (63.25 %) followed by penconazole (61.54 %) followed by mancozeb causing growth inhibition by 17.95 per cent. On the other hand, carbendazim and copper oxychloride presented minimal inhibitory effects, expressing (2.56 %) and (0.00 %) growth inhibition, respectively. At 100, 300 and 500 ppm concentration flutriafol 6.94 + tebuconazole 20.8 % were highly effective and caused (100 %) inhibition of mycelial growth followed by penconazole which caused (100 %) inhibition of mycelial growth at both 300 and 500 ppm followed by mancozeb which caused (84.21 %) and (75.24 %) inhibition of mycelial growth at 500 and 300 ppm, respectively.

Khillare *et al.* (2022) tested the efficacy of seven fungicide *in vitro* against *A. alternata* causing Alternaria blight of pigeon pea by poison food technique and results revealed that the effective fungicide was carboxin 37.5 + thiram 37.5 % 75 WP @ 0.25 % (85.98 %) followed by tebuconazole 25% WG @ 0.2 % (83.57 %), captan 75 % WP @ 0.3 % (66.42 %), carbendazim 12 + mancozeb 63 % 75WP @ 0.25 % (63.33 %), pyroclostrobin 20 % WG @ 0.1 % (45.10 %), carbendazim 50 % WP @ 0.1 % (21.02 %) and thiophanate methyl 70 % WP @ 0.1 % (14.33 %) over control.

Nira *et al.* (2022) evaluated five fungicides against Alternaria leaf spot of broccoli caused by *A. alternata* at different concentration *viz.*,100 ppm, 150 ppm, 200 ppm, 250 ppm and 300 ppm *in vitro*. The result revealed that propiconazole and iprodione completely inhibited the growth of *A. alternata* at all the tested concentrations followed by mancozeb showed 36.61, 42.23, 48.55, 54.20 and 61.43 per cent inhibition of the mycelial growth at the 100 ppm, 150 ppm, 200 ppm, 250 ppm and 300 ppm concentrations, respectively. Next fungicide was metalaxyl +

mancozeb recorded 31.87, 35.20, 39.44, 46.23 and 52.29 per cent inhibition at 100 ppm, 150 ppm, 200 ppm, 250 ppm and 300 ppm concentrations, respectively. The least inhibition percentages of the mycelial growth were observed with carbendazim were 22.18, 29.12, 35.22, 43.32 and 49.80 per cent at the 100 ppm, 150 ppm, 200 ppm, 250 ppm and 300 ppm concentrations, respectively.

2.6. Screening of varieties/germplasms of pomegranate against leaf and fruit spot caused by *A. alternata* under field condition

Archana (2012) tested 17 different pomegranate genotypes by artificial inoculations of *A. alternata* revealed that none of the genotypes were resistant to leaf spot and fruit spot of pomegranate. Most of screened genotypes exhibited susceptible to highly susceptible reaction. The genotype Yercaud was to be moderately susceptible which recorded a PDI of 23.00 per cent. Among the susceptible genotypes *viz.*, A.K. Anar, Achook dana, Agah, Alah, Bedana sedana, Dorsata malas, Jalore seedless, Lupania and Siah shirin recorded a PDI range of 29.02 to 45.00 per cent. Genotypes Arakta, Jyothi, Kesar, Kabul, Gul-e-shah, Domain and Bedana suri were found highly susceptible with a PDI ranging between 56.00 to 70.02 per cent.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

3.1 Laboratory procedures

3.1.1 Cleaning of glassware

Standard quality glassware to be used for laboratory studies was washed with chromic acid solution (Potassium dichromate 80 g, sulphuric acid 400ml and water 300 ml). Thus, cleaned glassware was subsequently immerse in water for 24 hr. rinsed with tap water and finally with distilled water.

3.1.2 Sterilization of glassware

The clean glassware was sterilized at 180 ºC temperature for one hour in hot air oven. Culture media and water was sterilized in an autoclave at 121 ºC temperature at 1.1 kg/cm^2 pressure for 20 minutes.

3.2 Preparation of culture media

Potato dextrose agar (PDA) medium was used for the culturing of pathogen causing leaf and fruit spot of pomegranate.

Composition of Potato Dextrose Agar (PDA)

Peeled and sliced potatoes	:	200 g
Dextrose	:	20 g
Agar	:	20 g
Distilled water	:	1000 ml

The clean and peeled potatoes (200 g) was cut into small pieces. These pieces were boiled in 500 ml distilled water and the extract was collected by filtering through muslin cloth. Dextrose (20 g) were dissolved in another 500 ml of hot water. Both the solution was mixed in one container and the final volume of one liter was dispensed into conical flasks, plugged with non-absorbent cotton and finally wrapped with the help of Aluminum foil. Further, they were sterilizing in autoclave at 121 ºC temperature at 1.1 kg/cm^2 for 20 minutes.

3.3 Isolation of pathogen associated with leaf and fruit spot disease

Pathogen was isolated from infected leaves and fruits of pomegranate by tissue isolation technique on PDA. Diseased leaves or fruit were cut into small pieces along with growing margins of disease about 1.5 - 2 mm with the help of sterilized blade. Pieces was washed with sterilized distilled water and then pieces were surface sterilized with one per cent NaOCl solution for 45 second.Thus, obtain disinfect tissue were immediately wash thrice with sterilize distilled water and aseptically transfer on PDA plates. Inoculated Petri plates was incubated at 27 ± 2 ºC temperature. Mycelial growth was observed, later the bits of fungal growth were transferred to another PDA plates. These Petri plates were incubated at 27 ± 2 °C for one week to check the sporulation, resulted fungal culture were further purified by single spore isolation method/ hyphal tip culture method, pure culture was transferred periodically and maintained on PDA slant at 5 ± 2 ºC temperature. The periodical subculturing and multiplication were done on the same medium to keep the culture fresh and was used throughout studies.

3.4 Purification and maintenance of fungal culture

The fungal culture was purified by following hyphal tip method and culture obtained was maintained on potato dextrose agar (PDA) medium slants by adopting subsequent sub culturing at, regular intervals. Seven days old culture was used for further studies.

3.5 Identification of the pathogen associated with leaf and fruit spot disease

The cultural and morphological characters of the isolates were studied by microscopic examination. Morphological characters of *A. alternata* were carried out by observing slides stained with cotton blue under compound microscope. Measurements of fungal hyphae and conidial size were made with the help of stage micrometer, ocular micrometer and camera lucida. Observation on shape, colony, length, breath and also septation were recorded and based on these observation confirming the identify of the pathogen and necessary photomicrograph were taken.

3.6 Pathogenicity of pathogen associated with leaf and fruit spot disease

3.6.1 Detached leaf technique

Pathogenicity was proved by employing detached leaf technique. Healthy young leaves were selected and detached from the susceptible seedlings of pomegranate *Cv.* Bhagwa, washed thoroughly with tap water, swabbed with 0.1 % mercuric chloride and washed with distilled sterile water. Inoculated by dropping spore suspension of a 10^6 conidia/mL from purified seven days old culture were inoculated on approximately 2 weeks old leaves under aseptic conditions. Moist cotton swab was placed at the base of petiole. The inoculated leaves were kept in Petri plates, lined with moist blotting paper to maintain humidity. Further these Petri plates were placed in an incubator at 27 ± 2 °C. Regular observations were made for symptom development.

3.6.2 Pathogenicity on pomegranate fruit

To prove the Koch's postulates, semi-ripe pomegranate fruits was collected from of Agroforestry Research Station, S. D. Agricultural, Sardarkrushinagar the field and brought to laboratory. The fruits were washed thoroughly with tap water and then surface sterilize with 0.1 % mercuric chloride solution for about one minute followed by three washing with sterilized distilled water and was inoculated with the fungus established from diseased pomegranate fruits. The inoculations were carried out in following manner. The spore suspension (4×10^6 spores/ml) was prepared from the sporulating seven days old culture of *A. alternata* by homogenization of culture in sterilized distilled water. The sterilized ten pomegranate fruits were slightly injured with pin pricking and spore suspension was applied with the help of cotton swab on the surface of pomegranate fruits. The fruits were placed separately in sterilized, loosely tied polythene bags and the bagged fruits were kept at 27 ± 2 °C in an incubator Development of disease symptoms was observed regularly on inoculated fruit.

3.6.3 Pathogenicity on pomegranate plant

To prove the Koch's postulate, ten pots were taken and sterilized by dipping them in 2 per cent solution of formaldehyde for about one minute. The mixture of soil, sand and farm yard manure were sterilized by autoclaving it at 15 lb psi for one hour for three consecutive days. The pots were filled with mixture of sterilized soil, sand and FYM in the ratio of 50:25:25 and allowed to kept for 10 days to expel harmful gases. Six month old plants were used for inoculation purpose for leaf spot disease of pomegranate. The spore suspension (4×10^6 spore/ml) was prepared from the sporulating seven days old culture in distilled sterilized water sprays inoculation were carried out in early morning hours with newly fresh inoculum. The control pots without inoculation were sprayed with sterilized distilled water. The pots were watered up to soil saturation at the same time. All the inoculated and uninoculated plants were covered with polyethylene bags after spraying of spore suspension for 48 hours to maintain humidity. Development of disease symptoms was observed regularly on artificially inoculated plants.

After development of the symptoms on inoculates leaves, fruit and plants, the fungus was re-isolated from the diseased leaves and fruit. The morphological and cultural characters were compared with those of *A. alternata* which was previously isolated from diseased pomegranate fruit and leaves.

3.7 *In vitro* bio-efficacy of different bioagents against *A. alternata*

The effect of fungal and bacterial biological control agents was studied *in vitro* against *A. alternata*.

Experimental details

(a)	Experimental design	:	C.R.D (Completely Randomized Design)
(b)	Treatments	:	10 (Ten)
(c)	Repetitions	:	3 (Three)
(d)	Method	:	Dual culture technique (Dennis and Webster, 1971)

The *in vitro* efficacy of promising bio-agents against test pathogen was evaluated by dual culture methods as under:

Table 3.1: List of bio-agents tested against *A. alternata in vitro*

Sr. No.	Name of the bio-agent
1	*Trichoderma viride* (Anand)
2	*Trichoderma harzianum* (Anand)
3	*Trichoderma viride* (Sardarkrushinagar)
4	*Trichoderma harzianum* (Sardarkrushinagar)
5	*Pseudomonas fluorescence* (Sardarkrushinagar)
6	*Pseudomonas putida* (Sardarkrushinagar)
7	*Pseudomonas stutzeri* (Sardarkrushinagar)
8	*Bacillus amyloliquefaciens* (Sardarkrushinagar)
9	*Bacillus subtilis* (Sardarkrushinagar)
10	Control

3.7.1 *In vitro* efficacy of *Trichoderma* spp. against *A. alternata*

Various known bio-agents were evaluated *in vitro* against *A. alternata* by dual culture technique (Dennis and Webster, 1971). The test bio-agent and pathogen (*A. alternata*) were grown separately on PDA. Sterilized PDA (20 ml) was poured aseptically in 90 mm diameter sterilized Petri plate. Mycelial disc (5 mm diameter) from seven days old actively growing culture of bio-agents and the test pathogen was cut aseptically from the periphery of the colony with the help of sterilized cork borer and placed at equidistance, exactly opposite from each other on solidified PDA. Three replications of each treatment were kept and the Petri plates with only pathogen served as control. All inoculated Petri plates were incubated at 27 ± 2 °C temperature in an incubator. Observations on radial growth of the tested organism and the pathogen in each Petri plate were measured when control plate is fully covered with the growth of test pathogen. The per cent growth inhibition (PGI) was calculated by the following equation (Bliss,1934):

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

Where,

PGI = Per cent growth inhibition

C = Colony diameter (mm) in control

T = Colony diameter (mm) in treatment.

3.7.2 *In vitro* efficacy of *Pseudomonas* spp. and *Bacillus* spp. against *A. alternata*

To determine the antagonistic action of bacterial isolates *viz., Pseudomonas fluorescence* (Sardarkrushinagar), *Pseudomonas putida* (Sardarkrushinagar), *Pseudomonas stutzeri* (Sardarkrushinagar), *Bacillus amyloliquefaciens* (Sardarkrushinagar) and *Bacillus subtilis* (Sardarkrushinagar) against *A. alternata in vitro.* 20 ml of PDA medium was poured aseptically in each of the Petri plate and allowed to solidify. The bacterial antagonist was then streaked at one end of the Petri plate on PDA medium. The bacterial isolates were inoculated 24 hrs prior to pathogen inoculation. Just opposite to bacterial streak, 5 mm disc of *A. alternata* from seven days old culture was placed. Three replications were maintained. Petri plates with only pathogen served as control. The per cent mycelia growth inhibition of the fungus was measured as mention earlier (3.7.1).

3.8 *In vitro* bio-efficacy of different phytoextracts against *A. alternata*

The efficacy of ten plant products against the pathogen in laboratory were tested through poisoned food technique (Nene and Thapliyal,1993).

Experimental details

(a)	Experimental design	:	C.R.D (Completely Randomized Design)
(b)	Treatments	:	11 (Eleven)
(c)	Repetitions	:	3 (Three)
(d)	Method	:	Poisoned food technique (Nene and Thapliyal, 1993)

The details about the plant products tested against the pathogen with their three concentrations *i.e.,* 5, 10 and 20 per cent are given in the following table:

Table 3.2: List of tested phytoextracts against *A. alternata in vitro*

Treatment	Name	Scientific Name	Parts	Concentration (%)		
1	Neem	*Azadirachta indica* Juss.	Leaves	5	10	20
2	Tulsi	*Ocimum tenuiflorum* L.	Leaves	5	10	20
3	Onion	*Allium cepa* L.	Bulb	5	10	20
4	Garlic	*Allium sativum* L.	Bulb	5	10	20
5	Datura	*Datura stramonium* L.	Leaves	5	10	20
6	Nilgiri	*Eucalyptus globulus*	Leaves	5	10	20
7	Karanj	*Millettia pinnata* L.	Leaves	5	10	20
8	Lantana	*Lantana camera* L.	Leaves	5	10	20
9	Akdo	*Calotropis gigentia* L.	Leaves	5	10	20
10	Ardusa	*Justicia adhatoda* L.	Leaves	5	10	20
11	Control	-	-	-	-	-

Fresh and healthy 100 g plant parts of each plant species were washed thoroughly with tap water and then finally with sterilized distilled water. They were separately grind by adding 100 ml sterilized distilled water to obtain 1:1 extract in a sterilized pestle and mortar. The extract was first filtered through two layer of muslin cloth and subsequently filtered through Whatman No. 1 filter paper. This formed the standard plant extract solution (100 %). Phyto-extracts was tested for growth inhibition of the fungus by employing poisoned food technique. All the plant extracts mentioned above was used at 5, 10 and 20 per cent concentrations. Five ml of the plant extracts was added to 95 ml of the sterilized melted potato dextrose agar medium for 5 per cent concentration, 10 ml of the plant extracts was added to 90 ml of the sterilized melted potato dextrose agar medium for 10 per cent concentration and 20 ml of the plant extracts was added to 80 ml of the sterilized melted potato dextrose agar medium for 20 per cent concentration. Twenty ml medium was poured into the sterilized Petri plates under aseptic conditions. A five mm disc of seven days old culture of *A. alternata* was cut by means of a sterilized cork borer and placed at the center of the Petri plate. The plates were incubated at 27 ± 2 ºC. The medium without incorporating the plant extract was serve as control. Observation on radial mycelia growth of fungus was measured by averaging two diameters of colony at right to one another when the control treatment with pathogen reached full growth. Three plates were maintained for each treatment. The per cent mycelia growth inhibition of the fungus was calculated by the method described earlier (3.7.1).

3.9 *In vitro* bio-efficacy of different fungicides against *A. alteranata*

Bio-efficacy of fungicides at various concentrations was evaluated against *A. alternata* by using poisoned food technique (Nene and Thapliyal,1993). Six systemic, six non-systemic and six combined fungicides were tested against *A. alternata* at four different concentrations, on the basis of active ingredient (Table 3.3).

Table 3.3: List of fungicides evaluated against *A. alternata in vitro*

Sr.No.	Common name	Concentration (ppm)			
[A]	**Systemic fungicides**				
1	Carbendezim 50 WP	50	100	250	500
2	Tebuconazole 25 EC	50	100	250	500
3	Difenoconazole 25 EC	50	100	250	500
4	Hexaconazole 5 EC	50	100	250	500
5	Propiconazole 25 EC	50	100	250	500
6	Azoxystrobin 23 SC	50	100	250	500
[B]	**Non-systemic fungicides**				
1	Copper oxychloride 50 WP	500	1000	1500	2000
2	Mancozeb 75 WP	500	1000	1500	2000
3	Chlorothalonil 75 WP	500	1000	1500	2000
4	Propineb 70 WP	500	1000	1500	2000
5	Copper hydroxide 77 WP	500	1000	1500	2000
6	Captan 75 WP	500	1000	1500	2000
[C]	**Combine fungicides**				
1	Tebuconazole 50 + Trifloxystrobin 25 WG	500	1000	1500	2000
2	Metalaxyl 4 + Mancozeb 64 WP	500	1000	1500	2000
3	Azoxystrobin 11.5 + Mancozeb 30 WP	500	1000	1500	2000
4	Carbendazim 12 + Mancozeb 63 WP	500	1000	1500	2000
5	Carbendezim 25 + Iprodione 25 WP	500	1000	1500	2000
6	Azoxystrobin 18.2 + Difenoconazole 11.4 SC	500	1000	1500	2000

The measured quantities of different fungicides were incorporated separately in melted sterilized PDA medium in conical flasks aseptically to obtain desired concentrations of the fungicides at the time of pouring the medium. The medium was shaken well to give uniform dispersal of the fungicides and then poured into sterilized Petri plates under aseptic conditions. The Petri plates were inoculated in the centre by placing 5 mm seven days old mycelial disc and then incubated at 27 ± 2 ^{o} C temperature. Three replications of each treatment were kept. Simultaneously, a control was also maintained by growing the fungus on fungicide from PDA medium.

Radial mycelial growth of fungus was recorded on daily basis in control plates starting from the initiation of the fungal growth in correspondence to treatment plates till full mycelial growth of fungus was observed in control.

Per cent growth inhibition of the fungus was calculated by the following equation (Vincent, 1927):

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

Where,

PGI = Per cent growth inhibition

C = Growth of test fungus in untreated plates

T = Growth of test fungus in treated plates.

3.10 Screening varieties/germplasms of pomegranate against leaf and fruit spot caused by *A. alternata* under field condition

The experiment was conducted during *Mrig bahar*, 2020-21 at Agroforestry Research Station, S.D.A.U. Eighteen genetically diverse genotypes of pomegranate was selected. Eighteen varieties/germplasms were screened under field condition for resistance to leaf and fruit spot of pomegranate (Table 3.4). These varieties/germplasms were tested under natural conditions. Based on the field observation noted, varieties/germplasms were grouped in different categories, according to their reaction to *A. alternata*.

Table 3.4: List of varieties/ germplasms screened against *A. alternata in vivo*

Sr. No	Varieties/ germplasms	Sr. No	Varieties/ germplasms
1	Ak Anar	10	Ganesh
2	Achikdana (achirdana)	11	P-26
3	Afghan Kandhari	12	GP-4
4	Boshlinski	13	Jyoti
5	Bedana sadana	14	Mrudula
6	Bedana bosk	15	Bhagwa
7	Dholka	16	Super Bhagwa
8	Guleshah	17	Solapuri Red
9	Jodhpuri red	18	Kandhari-3

One tree per one varieties/ germplasm was selected for screening of varieties /germplasm of pomegranate against leaf and fruit spot disease caused by *A. alternata.* Four branches, one from each side of the tree was selected. Ten leaves or fruits from each branch was examined and rated on following 0-5 grade scale (Sharma and Sharma, 2013).

Table 3.5: Disease scoring 0-5 grade scale for Alternaria leaf and fruits graded into six categories

Grade	Infection
0	No spot on leaf and fruit (Healthy)
1	1-10 % leaf/fruit area covered
2	11-25 % leaf/fruit area covered
3	26-50 % leaf/fruit area covered
4	51-75 % leaf/fruit area covered
5	76-100 % leaf/fruit area covered

PDI was calculated as per formula given by Datar and Mayee (1981):

Per cent disease intensity =

$$\frac{\text{Sum of all individual disease rating}}{\text{Total numbers of leaves/fruit assessed} \times \text{Maximum rating}} \times 100$$

PDI was worked out as described above by using 0-5 grade scale and varieties /germplasms was classified into different grades as per their reaction to leaf /fruit spot disease (Sharma and Sharma, 2013).

Table 3.6: Catogarization of pomegranate varieties /germplasms for resistance against *A. alternata*

Per cent disease index (PDI)	Category
0	Immune (I)
1-5	Resistant (R)
6-20	Moderately Susceptible (MS)
21-50	Susceptible (S)
51-100	Highly Susceptible (HS)

RESULTS AND DISCUSSION

IV. RESULTS AND DISCUSSION

Present studies on leaf and fruit spot of pomegranate (*Punica granatum* L.) under North Gujarat condition were undertaken during 2020-22 on the aspects *viz.* isolation, identification, pathogenicity test, *in vitro* bio-efficacy of bioagents, phytoextracts, fungicides and *in vivo* screening of germplasms/varieties of pomegranate. The results obtained on all these aspects are being narrated and discussed herein this chapter.

4.1 Isolation, purification and identification of pathogen associated with leaf and fruit spot disease

4.1.1 Isolation of pathogen associated with leaf and fruit spot disease

Pomegranate leaves and fruits showing the typical leaf and fruit spot symptoms were collected from All India Co-ordinated Research Project on Arid Fruits, Agroforestry Research Station, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar as well as farmer's field. The infected diseased samples were brought to the laboratory for microscopic examination and tissue isolation. Isolation of pathogen from infected leaves and fruit of pomegranate showing typical symptoms were done by using tissue isolation method. After 24 hours of incubation the isolated fungus initially started to grow as white, gradually turned grey to black in colour on PDA. This culture was further purified to obtain pure culture.

4.1.2 Purification of pathogen associated with leaf and fruit spot disease

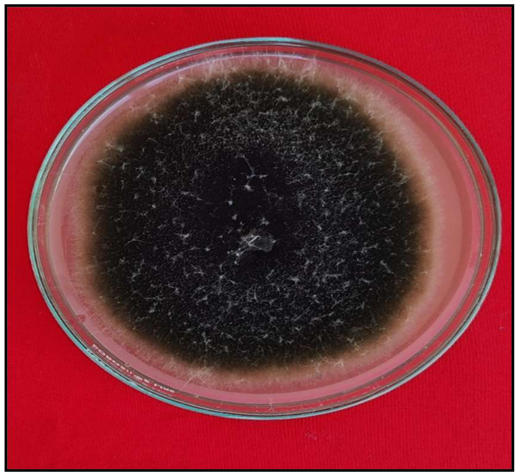
The culture was purified by single hyphal tip method and these cultures were further purified by single spore isolation method. This pure culture was maintained on PDA slants for further studies. The periodical sub-culturing and multiplication were made on PDA plates to keep the culture fresh and to use throughout the studies.

4.1.3 Identification of pathogen associated with leaf and fruit spot disease

After purification of the pathogen as described under materials and methods, cultural and morphological characters of the fungus grown on PDA, were studied for identification and compared with those described in the literature.

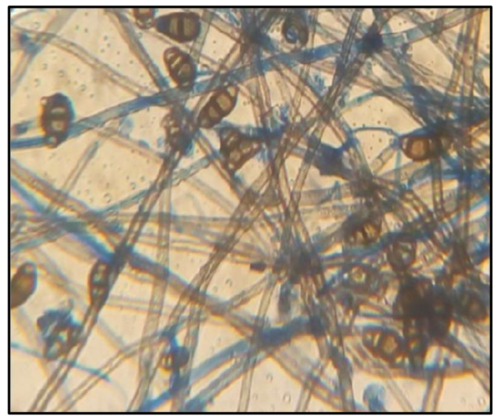
Cultural growth features

After eight days of incubation, the colony diameter of *A. alternata* was recorded as 90.00 mm. The cultural shows cottony growth with profuse aerial mycelium, colonies were surrounded by white young tip hyphae at the initial growth stage. The

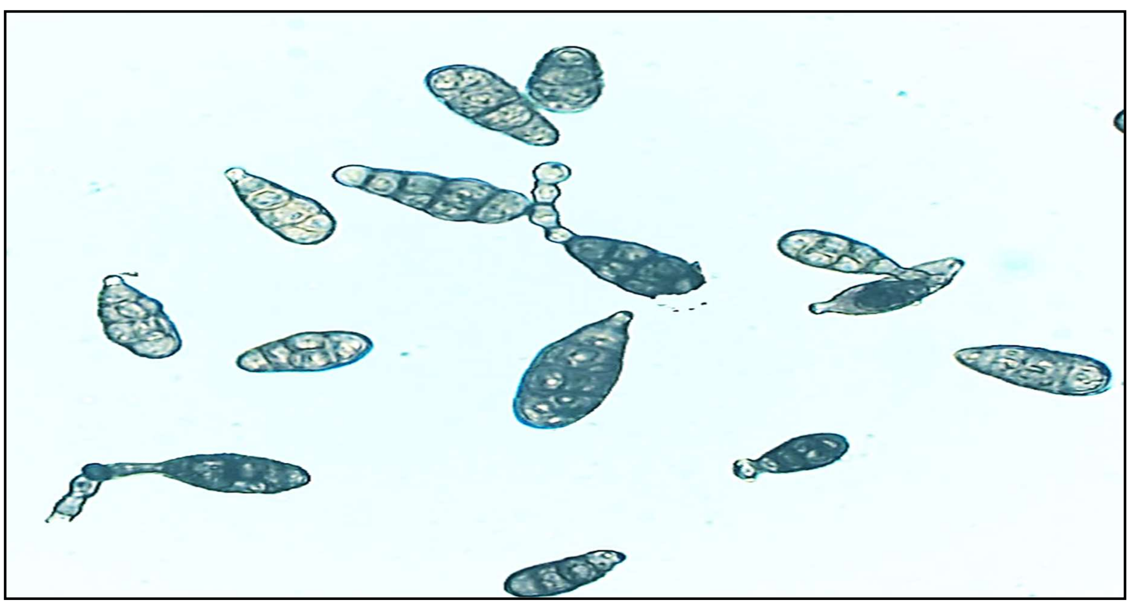


(A) Fully grown colony culture on PDA

(B) Spores of *A. alternata* in chain



(C) Septate mycelium



(D) Muriform Conidia at 100X

Plate I: Micrographs of *Alternaria alternata* isolate obtained from infected plant of pomegranate

(A) Typical symptoms of Alternaria Leaf spot

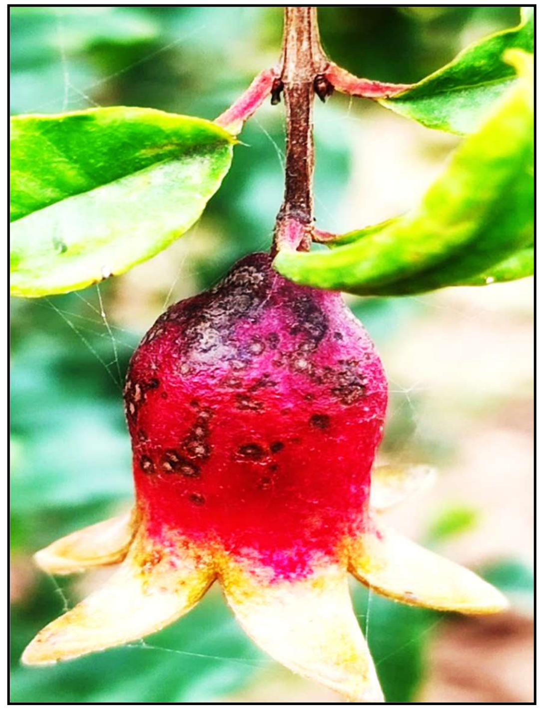
(B) Concentric ring formation on leaf

(C) Spotting at initial stage on leaf

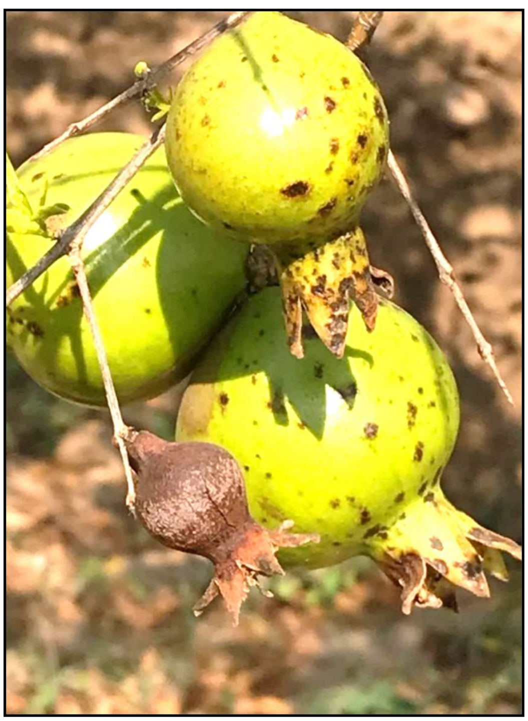
(D) Spotting at later stage on leaf

Plate II: Symptomatology of *A. alternata* on leaves of pomegranate

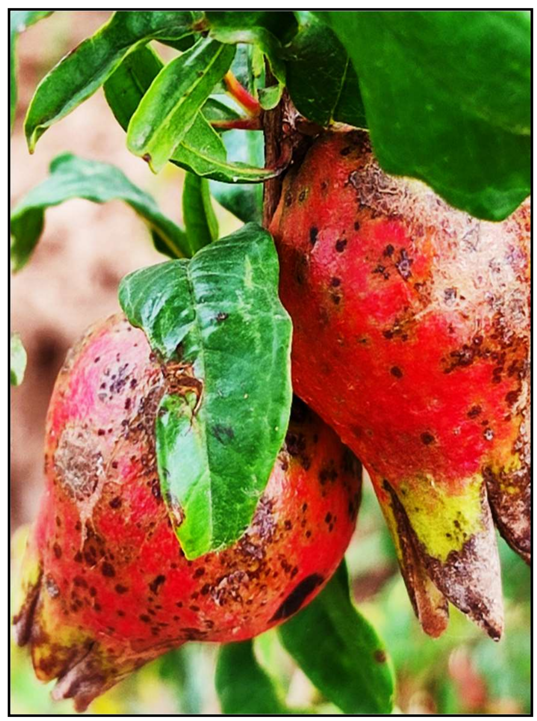
(A) Spot on flower and bud of pomegranate



(B) Initial spotting on bud



(C) Spotting on unripe fruit



(D) Spotting on ripe fruit

Plate III: Typical symptoms of *A. alternata* on pomegranate

Plate IV: Pathogenicity of *A. alternata* showing fruit spot symptoms pomegranate fruits by pin-pricking method

Plate V: Pathogenicity of *A. alternata* showing leaf spot symptoms on pomegranate leaf by detached leave technique

(A)Uninoculated plant of pomegranate (Control)

(B) Pathogenicity test of *A. alternata* showing leaf spot symptoms on pomegranate plant

Plate VI: Pathogenicity of *A. alternata* on pomegranate plant

fungal colony was initially white, which gradually turned grey to black colour with circular margins. Old culture appeared completely black with no aerial mycelium (Plate I-A).

Morphological observation

The microscopic study of *A. alternata* revealed that the mycelial was septate with colour ranged from raised ashy white to flat ashy green to raised blackish green with all having black reverse. Conidia are typically muriform (Plate I-D), dark brown, thick walled, in long branched chains (9 to 15) (Plate I-B). Hypha septated were brown in colour. Conidia was brown with length range from 31.5 to 62 µm and 9.4-14.6 µm wide. Some conidia were short rudimentary with dark brown beaks measuring 17.00 µm length and 8.09 µm in width. Conidia had 3 to 5 transverse septa and 0 to 3 longitudinal septa (Plate I-D). Based on the cultural and morphological characters observed under laboratory conditions were compared with those described in literature. Thus, it was confirmed as *A. alternata* (Fr.) Keissler which was responsible for leaf and fruit spot of pomegranate.

These characters agreed very closely with Keissler (1912), Mirkova and Konstantinova (2003), Ramjegathesh and Ebenezar (2012), Czajka *et al.* (2015) and Chethana *et al.* (2018). Keissler (1912) described the morphology of *A. alternata* found black or olivaceous black and sometimes grey the conidiophores produced singly or in small groups, simple or branched, straight or flexuous, sometimes geniculate, pale to mid olivaceous or golden brown, smooth measuring 50 µm long, 3-6 µm thick. Kirareia *et al*. (2019) found white compact aerial mycelium with black centre, other isolates had dense raised grayish white aerial mycelium with small dark concentric rings and circular margins. Conidial length ranged between 24.76 µm for the shortest to 75.14 µm for the longest and the width ranged from 6.82 µm to 14.78 µm. vaceous to dark brown. The conidia varied in shape from mostly muriform to ellipsoidal having smooth walls.

4.2 Pathogenicity test of pathogen associated with leaf and fruit spot disease

The pathogenicity of the isolate was proved by two different methods on leaves *viz.,* detached leaf method and artificial inoculation of the pomegranate plants whereas pathogenicity on fruit was proved by pin prick method on fruit with *A. alternata* which was carried out as explained in "Material and Methods".

4.2.1 Pathogenicity by detached leaf technique

On detached leaves, pathogenicity test was attempted by dropping of spore suspension of a 10^6 conidia/mL from purified seven days old culture were inoculated on approximately 2 weeks old leaves under aseptic conditions and placed in moist chamber at 27 ºC. Symptoms started appearing in after 48 hrs of incubation (Plate V). The diseased leaves (detached) of pomegranate were re-isolated and compared with original one on the basis of cultural and morphological characteristics which was similar in all respects. Hence pathogenicity was proved.

4.2.2 Pathogenicity on pomegranate fruit

Pathogenicity test of fungal pathogen causing fruit spot in pomegranate carried out by pin prick method as explained in "Material and Methods". By pin-prick the fruits to a 1 mm depth with a sterile needle, the seven days old culture was inoculated on the fruit in aseptic condition and placed in moist chamber at 27 ºC. Pathogenicity test (Plate IV) revealed that the test isolates *A. alternata* was able to infect the inoculated pomegranate fruit and developed the spots on fruits by pin pricking and pathogenic to pomegranate fruit (*Cv.* Bhagwa). Applying sterilized water by pin pricking method (Control) showed no symptoms on fruit. *A. alternata* induced typical symptoms like small oval to irregular brown to dark brown spots was observed on fruit after 7 to 10 days of inoculation. The symptoms induced by *A. alternata* on pomegranate fruits exhibited scars like lesions, slightly sunken which were occurs on upper surface of fruits, pericarp. The diseased fruit of pomegranate were re-isolated and compared with original one on the basis of their cultural and morphological characteristics which was similar in all respects. Hence pathogenicity was proved.

4.2.3 Pathogenicity on pomegranate plants

To confirm Koch's postulate, the pathogenic nature of the fungus (*A. alternata)* isolated from diseased plants was established by spraying spore suspension of a 10^6 conidia/mL on 6 months old plants. Plants were incubated in the mist house, where the relative humidity (80 %) and optimum temperature (27 ºC) were maintained for further development of symptoms. After 14 days of inoculation, the symptoms appeared as small light brown, regular to irregular spots (Plate VI). These spots enlarge, become oval in shape which was dark brown in colour. The brownish black concentric spot, enlarged and coalesced to cover large area of the leaf which resulted in defoliation of leaf. The pathogen was re-isolated from such inoculated leaves.

Cultural and morphological characters were compared with the original culture of the pathogen which was similar in all respects. Hence pathogenicity was proved.

Hence the causal agent of the disease was confirmed as *A. alternata* (Fr.) Keissler and the results presented in (Plate IV, V and VI) showed that the pathogenicity was proved positively in all the methods of inoculation.

These symptoms were found in agreement with the description made by Ezra *et al.* (2010) proved pathogenicity of *A. alternata* on both by leaves and fruit by detached leaf and fruit inoculation method. Symptoms appeared 48-72 hrs. later on detached leaf. On plant of pomegranate black spot was appeared on leaves one week after inoculation. Devappa and Thejakumar (2016) proved pathogenicity and symptoms of Alternaria leaf spot after, seven to nine days of inoculation on the leaves with a small, circular necrotic spot. Hosseinnia and Mohammadi (2018) observed symptoms after three to five days of spraying spore on the leaves and chlorosis was observed at the leaf surface. Aslam *et al.* (2019) tested pathogenicity and observed symptoms after 7 to 10 days of inoculation. Shafique *et al.* (2021) proved pathogenicity of *A. alternata*. They observed leaf spot symptoms after 10 days of inoculation which was similar to typical spot observed in field.

4.3 *In vitro* bio-efficacy of different bioagents against *A. alternata*

The hazardous effect of chemicals used in plant disease management has diverted plant pathologists to find out the alternative methods having little or no adverse effect on environment. Notable success of disease control through the use of antagonistic microorganism in the laboratory, glass house and field have been achieved during past several years and based on this information, there is a possibility of developing biological control of plant disease under field conditions. Nowadays the commercial formulations of some of the bio-agents are already made available in the market for farmers use. However, inadequate information on the performance of the antagonists under varying condition was a major constraint in the large-scale adoption of this technology.

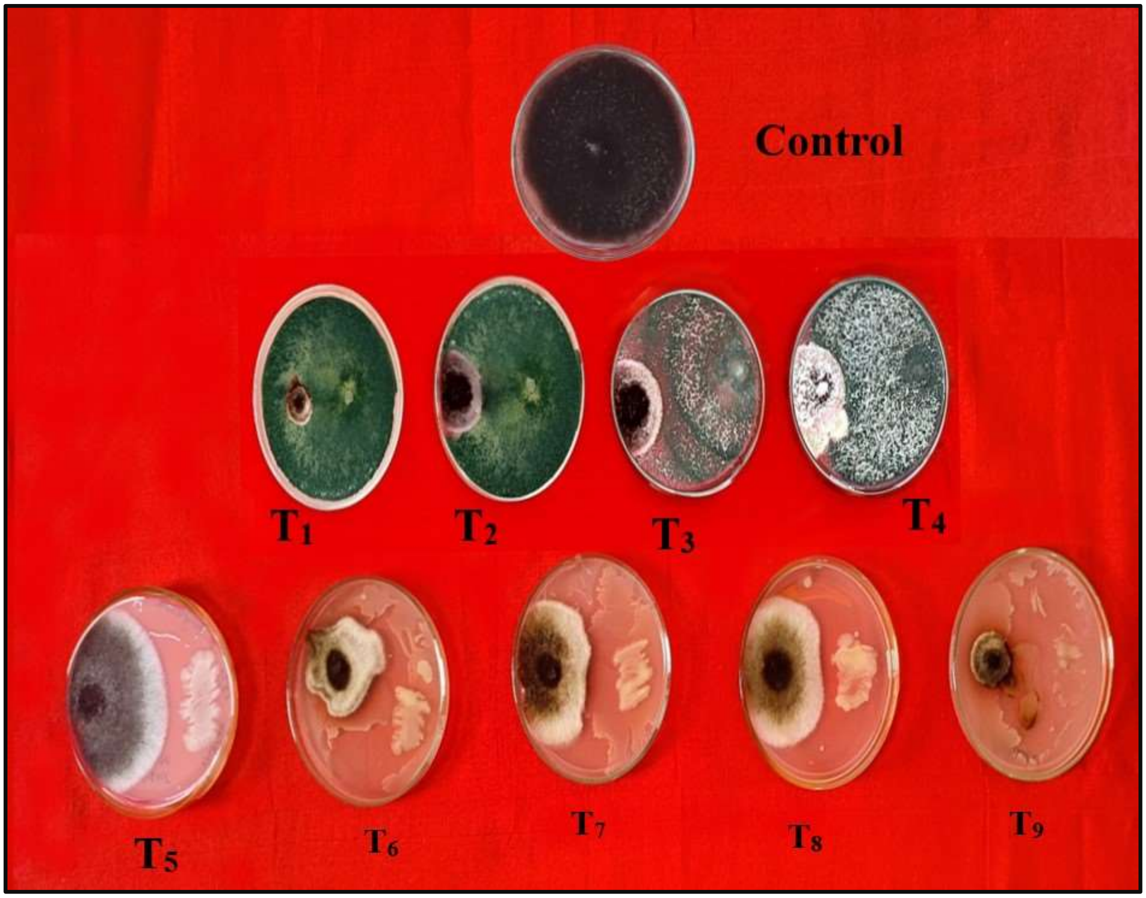
In this study, pure culture of *Trichoderma viride* (Anand), *Trichoderma harzianum* (Anand), *Trichoderma viride* (Sardarkrushinagar), *Trichoderma harzianum* (Sardarkrushinagar), *Pseudomonas fluorescence* (Sardarkrushinagar), *Pseudomonas putida* (Sardarkrushinagar), *Pseudomonas stutzeri* (Sardarkrushinagar), *Bacillus amyloliquefaciens* (Sardarkrushinagar) and *Bacillus subtilis* (Sardarkrushinagar) obtained from Department of Plant Pathology, Chimanbhai Patel College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar were tested *in vitro* by dual culture technique to find out the antagonistic effect.

Table 4.1: Effect of different bioagents on mycelial growth and per cent growth inhibition of *A. alternata in vitro*

Sr. No.	Test organism	Mycelial growth (mm)	Growth inhibition over control (%)
1	*Trichoderma viride* (Anand)	16.67	64.49[a] (81.48)[**]
2	*Trichoderma harzianum* (Anand)	29.98	54.73[c] (66.68)
3	*Trichoderma viride* (Sardarkrushinagar)	34.16	51.95[de] (62.04)
4	*Trichoderma harzianum* (Sardarkrushinagar)	40.91	47.59[f] (54.54)
5	*Pseudomonas fluorescence* (Sardarkrushinagar)	53.33	39.65[g] (40.74)
6	*Pseudomonas putida* (Sardarkrushinagar)	32.33	53.16[cd] (64.08)
7	*Pseudomonas stutzeri* (Sardarkrushinagar)	43.34	46.04[f] (51.85)
8	*Bacillus amyloliquefaciens* (Sardarkrushinagar)	36.34	50.53[e] (59.62)
9	*Bacillus subtilis* (Sardarkrushinagar)	20.89	61.17[b] (76.78)
10	Control	90.00	4.05[h] (0.00)
	S.Em.±		0.542
	C.D. at 5%		1.622
	C.V. %		1.799

[**]Figures in parentheses are original values and outside are arc-sine transformed values
Treatment means with the letter(s) common are not significant by Duncan's New Multiple Range Test at 5% level of significance

The results are presented in Table 4.1 and Plate VII revealed that, all the antagonists tested against *A. alternata* were significantly effective in checking the growth of *A. alternata*. Out of nine antagonists tested, least growth of the pathogen was recorded in *T. viride* (Anand) (16.67 mm) which was followed by *B. subtilis* (Sardarkrushinagar) (20.89 mm), *T. harzianum* (Anand) (29.98 mm), *P. putida* (Sardarkrushinagar) (32.33 mm), *T. viride* (Sardarkrushinagar) (34.16 mm),



Bio agents

T_1 - ***Trichoderma viride*** **(Anand)**

T_2 - ***Trichoderma harzianum*** **(Anand)**

T_3 - ***Trichoderma viride*** **(Sardarkrushinagar)**

T_4 - ***Trichoderma harzianum*** **(Sardarkrushinagar)**

T_5 - ***Pseudomonas fluorescence*** **(Sardarkrushinagar)**

T_6 - ***Pseudomonas putida*** **(Sardarkrushinagar)**

T_7 - ***Pseudomonas stutzeri*** **(Sardarkrushinagar)**

T_8 - ***Pseudomonas amyloliquefaciens*** **(Sardarkrushinagar)**

T_9 - ***Bacillus subtilis*** **(Sardarkrushinagar)**

T_{10} - **Control**

Plate VII: *In vitro* effect of different bio-agents on growth and inhibition of *A. alternata*

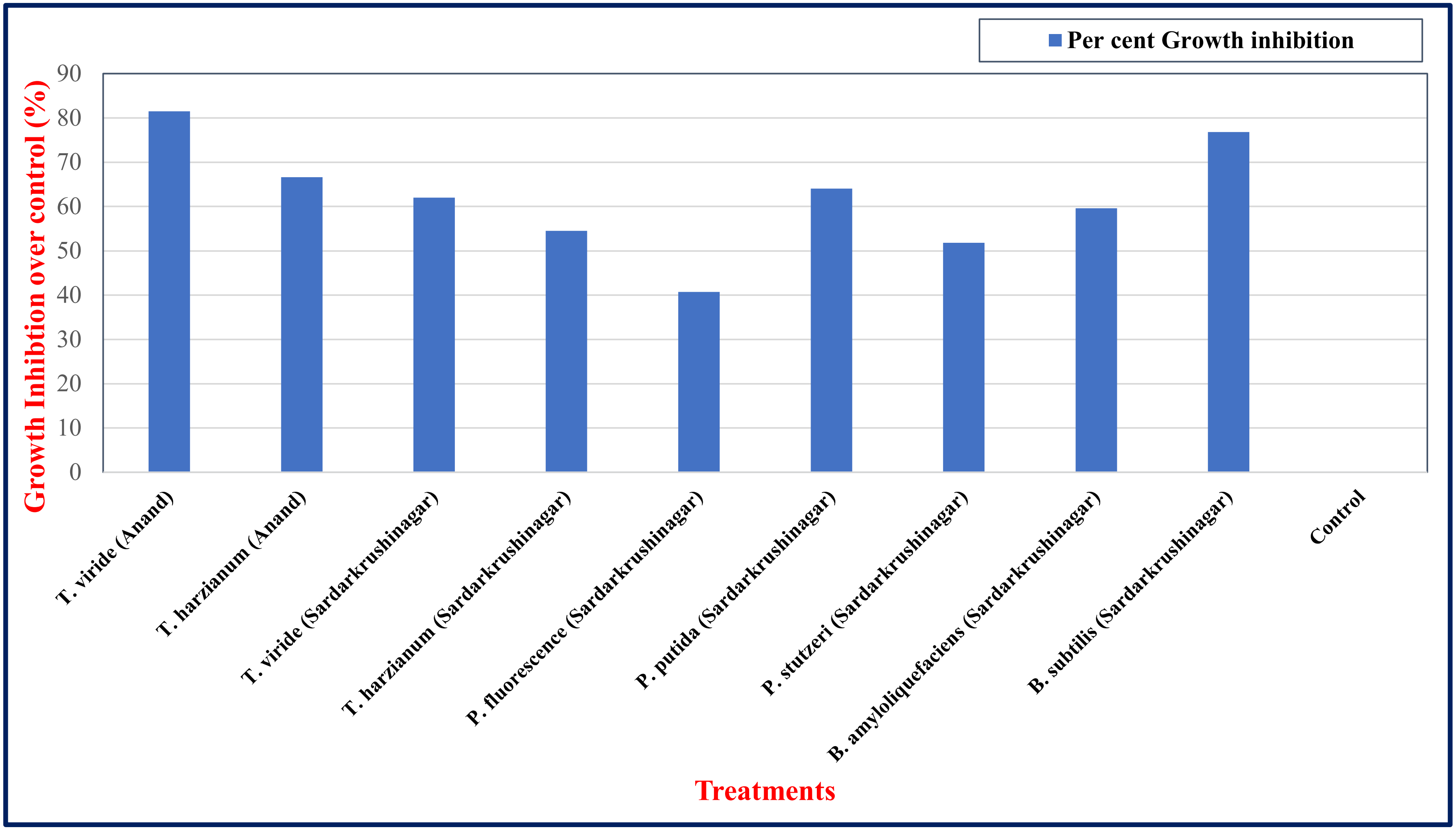


Fig. 4.1: Effect of different bioagents on per cent growth inhibition of *A. alternata in vitro*

B. amyloliquefaciens (Sardarkrushinagar) (36.34 mm), *T. harzianum* (Sardarkrushinagar) (40.91 mm), *P. stutzeri* (Sardarkrushinagar) (43.34 mm) and *P. fluorescence* (Sardarkrushinagar) (53.33 mm).

The per cent growth inhibition was computed in Table 4.1 and Fig. 4.1 revealed that maximum growth inhibition of the test fungus was achieved with *T. viride* (Anand) (81.48 %) followed by *B. subtilis* (Sardarkrushinagar) (76.78 %), *T. harzianum* (Anand) (66.68 %), *P. putida* (Sardarkrushinagar) (64.08 %), *T. viride* (Sardarkrushinagar) (62.04 %), *B. amyloliquefaciens* (Sardarkrushinagar) (59.62 %), *T. harzianum* (Sardarkrushinagar) (54.54 %) significantly inhibited the growth of the pathogen. Whereas *P. fluorescence* (Sardarkrushinagar) (40.74 %) was comparatively found least effective.It is evident from the study that among all the antagonists evaluated by dual culture method, *T. viride* (Anand), *B. subtilis* (Sardarkrushinagar) and *T. harzianum* (Anand) consistently showed strong antagonistic properties against *A. alternata* as compared to the other antagonists tested hence considered as potential antagonists.

These results are in harmony with earlier workers reported most effective. Rajput *et al.* (2013) reported maximum mycelial growth inhibition with *T. viride* IARI isolate followed by *T. viride* Navsari isolate and *T. harzianum* Junagadh isolate. Tagaram *et al*. (2015) found that the per cent growth inhibition of *A. alternata* by *T. viride* was superior over *T. harzianum*. Tapwal *et al.* (2015) observed that *T. harzianum* and *T. viride* found maximum mycelial growth inhibition of *A. alternata*. Ajayabhai *et al.* (2018) revealed that the maximum mycelial growth inhibition observed in *T. viride* followed by *T. harzianum* and *P. fluorescens* restricting growth of the *A. alternata*. Suhas and Simon (2019) who revealed that the maximum growth inhibition of *A. alternata* were observed in *T. viride* followed by *P. fluorescens* over control.

4.4 *In vitro* bio-efficacy of different phytoextracts against *A. alternata*

In recent years, there has been a major thrust on pesticide residue free organic pomegranate production. Taking the task into consideration, efficient botanicals need explored to fit into the management schedule. Use of phytoextracts for the management of various diseases of crop plants is eco-friendly and environmentally safe. Therefore, present investigation aimed to evaluate botanicals/ phytoextracts (*in vitro*) against *A. alternata* (Fr.) Keissler causing leaf spot of pomegranate. This information is certainly useful in exploiting inhibitory principle for developing botanical fungicide in plant disease management. In the present investigation, ten phytoextracts were tested at 5, 10 and 20 per cent concentration with suitable control by poisoned food technique *in vitro*. As plant extracts are cost effective means of management therefore an effort was made to know the efficacy of different phytoextracts against *A. alternata.*

4.4.1 Effect of phytoextracts on mycelial growth

Effect of phytoextracts

The results presented in Table 4.2 and Plate VIII revealed that all the phytoextracts at three different concentrations were found significantly inhibitory to mycelial growth. Among phyto-extracts tested, minimum mean mycelial growth was recorded in garlic clove extract (37.71 mm) followed by neem leaves extracts (44.99 mm) and karanj leaves extract (48.94 mm). The next effective phytoextract in order of inhibition was nilgiri leaves extract (50.90 mm), which showed lower mycelium growth and it was at par with ardusa leaves extract (51.44 mm), tulsi leaves extract (51.68 mm), onion bulb extract (52.11 mm), lantana leaves extract (52.33 mm), akdo leaves extract (53.66 mm) which showed significantly lower mycelial growth than rest of the phyto-extracts. Dhatura leaf extract (54.55 mm) was the least effective.

Effect of Concentration

The results revealed that various concentrations were significantly differ to each other and indicated that higher concentrations were significantly superior over lower doses of phytoextracts.

Interaction effect of phytoextracts and concentrations

The garlic clove extract at 20 per cent concentration was found most effective treatment (19.66 mm) followed by neem leaf extract 20 per cent (27.66 mm) and karanj leaf extract 20 per cent (30.33 mm). Tulsi and nilgiri extracts at 5 per cent were found least effective 75.40 mm and 68 mm, respectively.

Table 4.2: Effect of different phytoextracts on mycelial growth of *A. alternata in vitro*

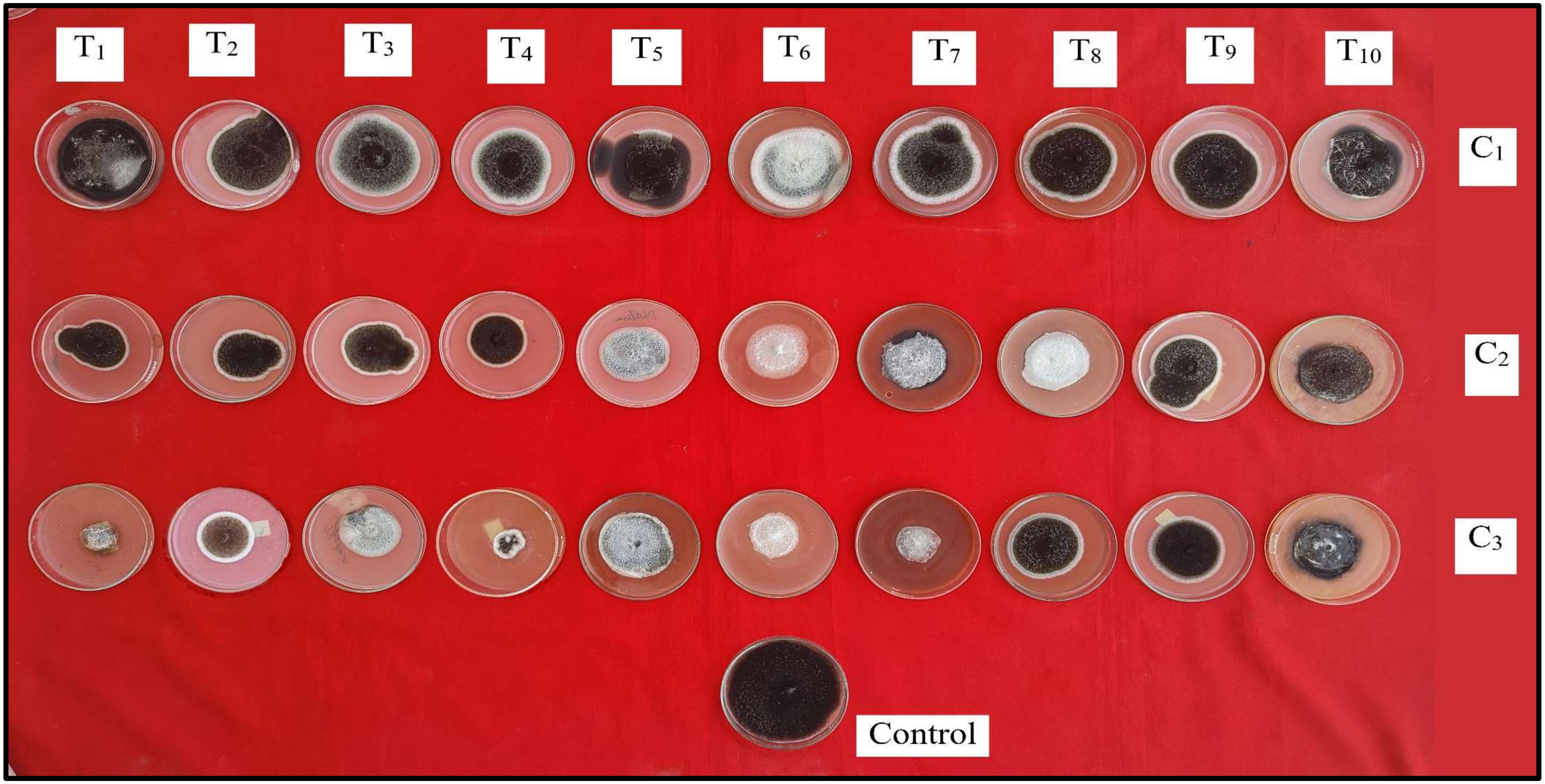
Sr. No.	Phytoextract	Part used	Mycelial growth (mm) Concentration (%) 5	10	20	Mean
1	Neem	Leaves	64.33 ^{e}	43.00 ^{l}	27.66 ^{p}	44.99 ^{f}
2	Tulsi	Leaves	75.40 ^{b}	44.66 ^{kl}	35.00 ^{n}	51.68 ^{d}
3	Onion	Bulb	66.33 ^{cde}	51.33 ^{hi}	38.66 ^{m}	52.11 ^{d}
4	Garlic	Clove	53.33 ^{h}	40.00 ^{m}	19.66 ^{q}	37.71 ^{g}
5	Dhatura	Leaves	64.33 ^{e}	55.66 ^{g}	44.66 ^{kl}	54.55 ^{b}
6	Nilgiri	Leaves	68.00 ^{c}	49.33 ^{ij}	35.33 ^{n}	50.89 ^{d}
7	Karanj	Leaves	67.33 ^{cd}	49.17 ^{ij}	30.33 ^{o}	48.94 ^{e}
8	Lantana	Leaves	65.00 ^{de}	46.33 ^{k}	45.66 ^{k}	52.33 ^{cd}
9	Akdo	Leaves	65.00 ^{de}	56.00 ^{g}	40.00 ^{m}	53.66 ^{bc}
10	Ardusa	Leaves	59.66 ^{f}	48.66 ^{j}	46.00 ^{k}	51.44 ^{d}
11	Control	-	90.00 ^{a}	90.00 ^{a}	90.00 ^{a}	90.00 ^{a}
Mean			66.45 ^{a}	51.74 ^{b}	40.72 ^{c}	-
			Treatment (T)	Concentration (C)		T × C
S.Em. ±			0.455	0.249		0.789
C.D. at 5 %			1.292	0.707		2.237
C.V. %			2.872			

Treatment means with the letter(s) common are not significant by Duncan's New Multiple Range Test at 5% level of significance

4.2.2 Effect of phytoextract on mycelial growth inhibition

Effect of phytoextracts

The result, present in Table 4.3, Fig. 4.2 and Plate VIII revealed that highest inhibition of mycelial growth was obtained by garlic clove (58.09 %) which was significantly higher over all the phytoextracts. Neem leaves extract (50.00 %) ranked second to inhibit the mycelial growth and recorded significantly higher over rest of the phytoextracts. It was followed by karanj leaves extracts (45.62 %), nilgiri leaves extract (43.44 %) and ardusa leaves extract (42.84 %). Rest of extracts *viz.,* tulsi leaves extract (42.57 %), onion bulb extract (42.09 %), lantana leaves extract (41.85 %), akdo leaves extract (40.74 %) and datura leaves extracts (39.01 %) were found less effective for inhibiting mycelial growth of the pathogen.



Phyto-extract

T_1 - Neem
T_2 - Tulsi
T_3 - Onion
T_4 - Garlic
T_5 - Datura
T_6 - Nilgiri
T_7 - Karanj
T_8 - Lantana
T_9 - Akdo
T_{10} - Ardusa

Concentrations

C_1 - 5%

C_2 - 10%

C_3 - 20%

Plate VIII: *In vitro* effect of different phytoextracts on growth and inhibition of *A. alternata* at different concentration

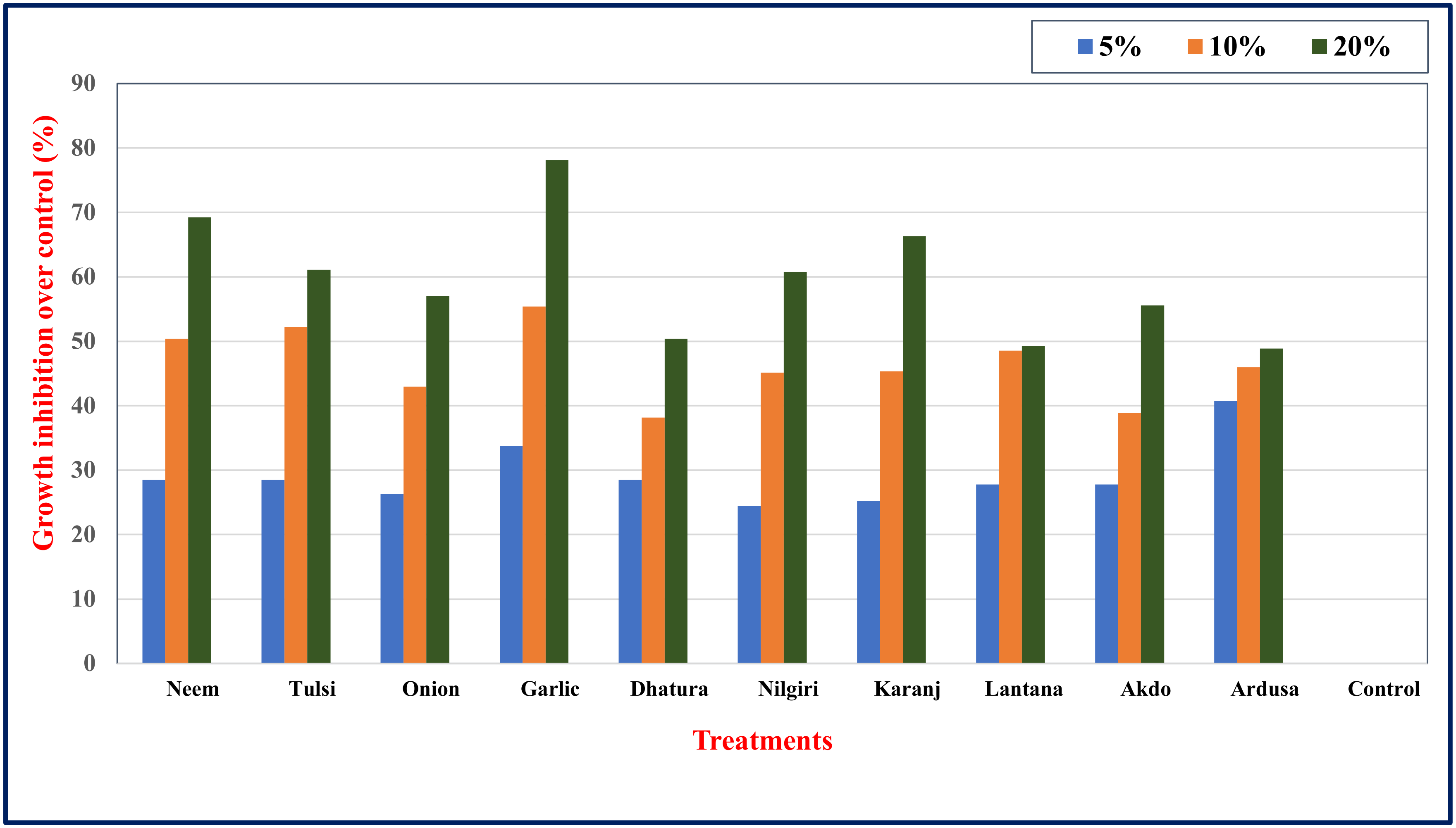


Fig. 4.2: Effect of different phytoextract on per cent growth inhibition of *A. alternata* at different concentration *in vitro*

Effect of concentrations

The result regarding effectiveness of various concentration found that the higher concentrations of the extracts were significantly superior over the lower doses of the same extract. The efficacy of the extract undeniably increased significantly with the increase of concentration.

Table 4.3: Effect of different phytoextract on per cent growth inhibition of *A. alternata* at different concentration *in vitro*

Sr. No.	Phytoextract	Name	Growth inhibition over control (%) Concentration (%) 5	10	20	Mean
1	Neem	Leaves	32.28 [m] (28.52)	46.27 [f] (52.22)	56.34 [b] (69.26)	44.97 [b] (50.00)**
2	Tulsi	Leaves	25.98 [q] (16.22)	45.21 [fg] (50.37)	51.42 [d] (61.11)	40.78 [cd] (42.57)
3	Onion	Bulb	30.84 [mnop] (26.29)	40.95 [ij] (42.96)	49.05 [e] (57.04)	40.28 [cd] (42.09)
4	Garlic	Clove	39.66 [jk] (40.74)	48.09 [e] (55.39)	62.14 [a] (78.15)	49.96 [a] (58.09)
5	Dhatura	Leaves	32.28 [m] (28.52)	38.15 [k] (38.15)	45.21 [fg] (50.37)	38.54 [f] (39.01)
6	Nilgiri	Leaves	29.61 [p] (24.44)	42.22 [i] (45.15)	51.20 [d] (60.74)	41.01 [cd] (43.44)
7	Karanj	Leaves	30.10 [np] (25.19)	42.34 [i] (45.37)	54.52 [c] (66.30)	42.32 [c] (45.62)
8	Lantana	Leaves	31.81 [mn] (27.78)	44.16 [gh] (48.53)	44.58 [fg] (49.26)	40.16 [de] (41.85)
9	Akdo	Leaves	31.81 [mn] (27.78)	38.58 [k] (38.89)	48.19 [e] (55.55)	39.53 [e] (40.74)
10	Ardusa	Leaves	35.49 [l] (33.71)	42.66 [hi] (45.93)	44.36 [g] (48.89)	40.82 [cd] (42.84)
11	Control		4.05 [r] (0.00)	4.05 [r] (0.00)	4.05 [r] (0.00)	4.05 [i] (0.00)
Mean			31.99 [c] (28.22)	42.86 [b] (46.29)	50.68 [a] (59.62)	-
			Treatment (T)	Concentration (C)		T × C
S.Em. ±			0.323	0.177		0.560
C.D. at 5 %			0.917	0.509		1.588
C.V. %			2.354			

**Figures in parentheses are original values and outside are arc-sine transformed values

Treatment means with the letter(s) common are not significant by Duncan's New Multiple Range Test at 5% level of significance

Interaction effect of phytoextracts and concentrations

Considering the overall results, garlic clove extract at 20 per cent (78.15 %) was found very effective in inhibiting mycelial growth of *A. alternata* and it was followed by neem leaves extract at same concentration (69.26 %), karanj leaves at 20 per cent (66.30 %), tulsi (61.11 %) and nilgiri leaves extract (60.74 %). Tulsi leaves extract at 5 per cent (16.22 %) was recorded least effective in mycelial growth of *A. alternata*.

Looking to the overall results, garlic clove extract at 20 per cent was reported highly effective against mycelial growth inhibition of pathogenic fungi, *A. alternata* followed by neem leaves extract. Dhatura leaves extract was found less effective than all the rest of plant extract tested. Different plant species substantially varied in their antifungal potential. These differences are expected since plant vary in their chemical composition, habitats and growth stages at which they collected.

In the present study, among all ten phytoextracts, garlic extract was found most effective which caused substantial inhibition (> 75 %) of *A. alternata*. Similar effect of the test botanicals/phytoextracts against *A. alternata* were reported to cause significant mycelial growth inhibition of *A. alternata*, earlier by several workers Jakatimath *et al.* (2017) revealed that the highest inhibition of *A. alternata* was recorded in garlic and onion bulb at 5 and 10 per cent concentration extract followed by neem seed kernel extract. Kadam *et al.* (2018) highest average mycelial growth inhibition was recorded with garlic followed by ginger, neem, nilgiri, turmeric, dhatura, neergudi and lantana whereas karanj, parthenium and *Bougainveilliea* spp. recorded comparatively least mycelial growth of the *A. alternata*. Rani *et al.* (2018) found that garlic clove extract was most effective inhibiting the growth of the fungus *A. alternata* followed by onion bulb extract and neem extract. Chaudhary and Singh (2021) who reported that the highest average mycelium growth inhibition of *A. alternata* was obtained by garlic followed by ginger and neem, while minimum inhibition was found by parthenium followed by dhatura. Sharma *et al*. (2021) found garlic extract showed maximum mycelium growth inhibition followed by neem extract against *A. alternata.*

4.5 *In vitro* bio-efficacy of different fungicides against *A. alternata*

Eighteen fungicides, *viz.,* six from systemic, six from non-systemic group and six combine formulations were evaluated at four different concentrations by poisoned food technique for their efficacy against *A. alternata.* The results are presented in Table 4.5, 4.7, 4.9, Fig 4.3, 4.4, 4.5 and Plates IX, X and XI indicated that all fungicides evaluated significantly reduced the growth of *A. alternata* as compared to control but all the fungicides and their concentrations significantly differ within themselves.

4.5.1 *In vitro* bio-efficacy of systemic fungicides against *A. alternata*

Six systemic fungicides tested at four different concentrations *viz.*, 50, 100, 250 and 500 ppm by poisoned food technique for their efficacy against *A. alternata*. Among all concentrations, the higher concentration of each fungicide produced maximum growth inhibition of the pathogen (Table 4.5, Fig.4.3 and Plate IX).

4.5.1.1 Effect of systemic fungicides on mycelial growth

Effect of fungicides

The results presented in Table 4.4 and Plate IX revealed that all the fungicides at four different concentrations were found significantly inhibitory to mycelial growth. Among systemic fungicide tested, minimum mean mycelial growth was recorded in propiconazole (5.66 mm) followed by difenoconazole (7.50 mm), hexaconazole (9.03 mm), tebuconazole (9.40 mm). Carbendazim recorded least effective which was (45.92 mm) followed by azoxystrobin (51.54 mm).

Table 4.4: Effect of different systemic fungicides on mycelial growth of *A. alternata in vitro*

Sr. No.	Fungicides	Mycelial growth (mm) Concentration (ppm)				Mean
		50	100	250	500	
1	Carbendazim 50 WP	64.33 ^{b}	57.66 ^{c}	50.50 ^{d}	33.66 ^{f}	51.54 ^{b}
2	Tebuconazole 25 EC	16.33 ^{gh}	12.16 ^{ijk}	6.23 ^{m}	2.90 ^{no}	9.40 ^{d}
3	Difenoconazole 25 EC	13.73 ^{g}	9.33 ^{hi}	5.00 ^{mn}	2.33 ^{no}	7.50 ^{e}
4	Hexaconazole 5 EC	17.89 ^{g}	14.46 ^{hi}	3.78 ^{mn}	0.00 ^{o}	9.03 ^{de}
5	Propiconazole 25 EC	11.37 ^{jkl}	9.16 ^{l}	2.10 ^{no}	0.00 ^{o}	5.66 ^{f}
6	Azoxystrobin 23 SC	56.50 ^{c}	45.33 ^{e}	49.00 ^{d}	32.83 ^{f}	45.92 ^{c}
7	Control	90.00 ^{a}	90.00 ^{a}	90.00 ^{a}	90.00 ^{a}	90.00 ^{a}
Mean		38.59 ^{a}	34.07 ^{b}	29.51 ^{c}	23.10 ^{d}	-
		Treatment (T)		Concentration (C)		T × C
S.Em. ±		0.469		0.383		0.937
C.D. at 5 %		1.336		1.091		2.673
C.V. %		4.946				

Treatment means with the letter(s) common are not significant by Duncan's New Multiple Range Test at 5% level of significance

Effect of concentrations

The results revealed that various concentrations were significantly differ to each other and indicated that higher concentrations were significantly superior over lower doses of fungicides. Successive reduction in mycelial growth was observed with subsequent increase in the concentration from 50 to 500 ppm concentration. The highest mycelial growth of 38.59 mm was recorded with 50 ppm concentration and least mycelial growth of 23.10 mm recorded in 500 ppm.

Interaction effect of fungicide and concentrations

Propiconazole and hexaconazole at 500 ppm concentration was found most effective treatment, which did not allow the pathogenic fungi to grow on treated culture plate (0.00 mm). The next best treatment was propiconazole 250 ppm (2.10 mm) and difenoconazole 500 ppm (2.33 mm). Carbendazim and azoxystrobin at 50 ppm were found least effective concentration which recored 64.33 mm and 56.50 mm mycelia growth, respectively.

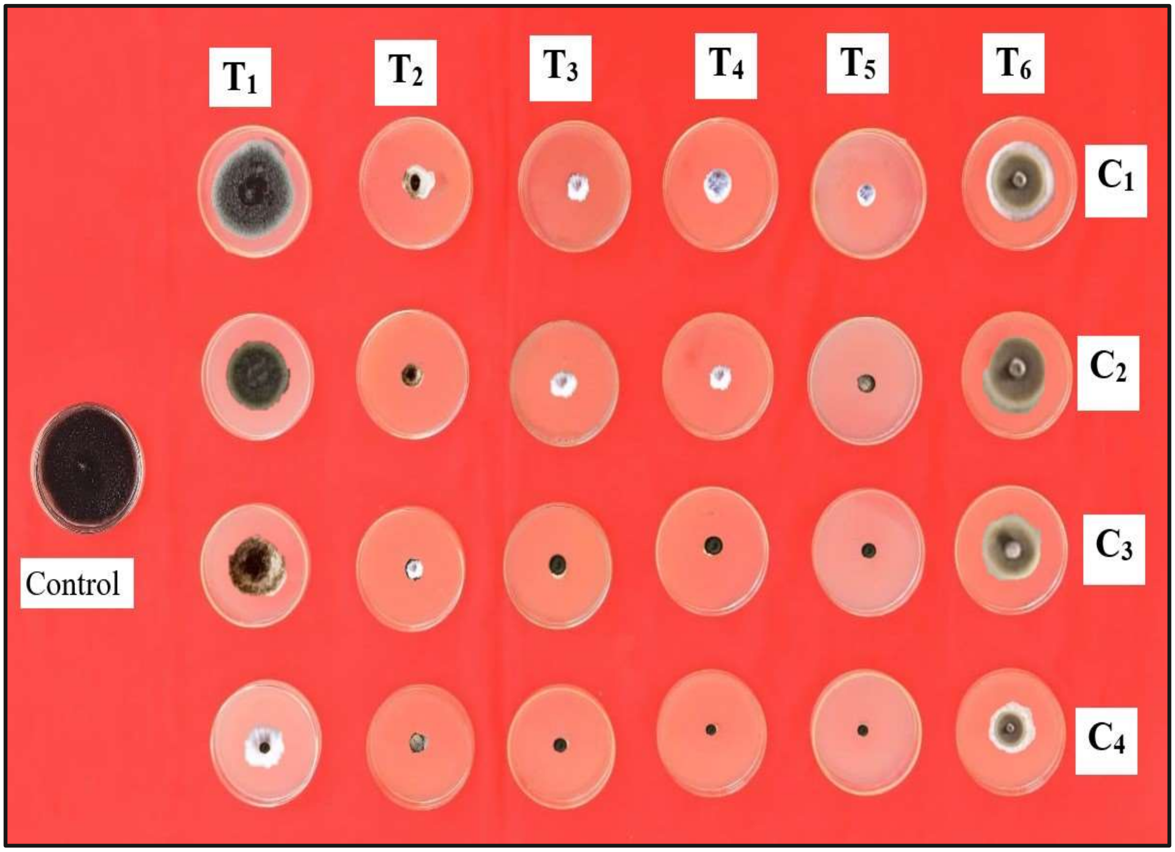
4.5.1.2 Effect of systemic fungicides on mycelial growth inhibition

Effect of fungicides

The results revealed that among the six systemic fungicides evaluated against *A. alternata,* average mycelial growth inhibition of the test pathogen was ranged from 42.82 per cent (carbendazim) to 93.81 per cent (propiconazole). The interaction effect of fungicide × concentration was also found significant. The highest mean growth inhibition of mycelial was obtained with propiconazole (93.81 %) followed by difenoconazole (91.68 %) which was at par with hexaconazole (89.71 %) followed by tebuconazole (89.55 %). Carbendazim recorded least effective (42.82 %) which was followed by azoxystrobin (48.96 %) (Table 4.5, Fig. 4.3).

Effect of concentrations

Among the systemic fungicides, all concentrations indicated significant effect on the growth inhibition of the fungus. Successive reduction in growth was observed with subsequent increase in the concentration from 50 to 500 ppm concentration. The highest inhibition of 86.70 per cent was recorded with 500 ppm concentration and least inhibition of 66.63 per cent recorded in 50 ppm.



Fungicides

T_1 : **Carbendazim 50% WP**

T_2 : **Tebuconazole 25% EC**

T_3 : **Difenoconazole 25% EC**

T_4 : **Hexaconazole 5% EC**

T_5 : **Propiconazole 25% EC**

T_6 : **Azoxystrobin 23% SC**

Concentrations

C_1: **50 ppm**

C_2: **100 ppm**

C_3: **250 ppm**

C_4: **500 ppm**

Plate IX: Growth inhibition of *A. alternata* by systemic fungicide at different concentration *in vitro*

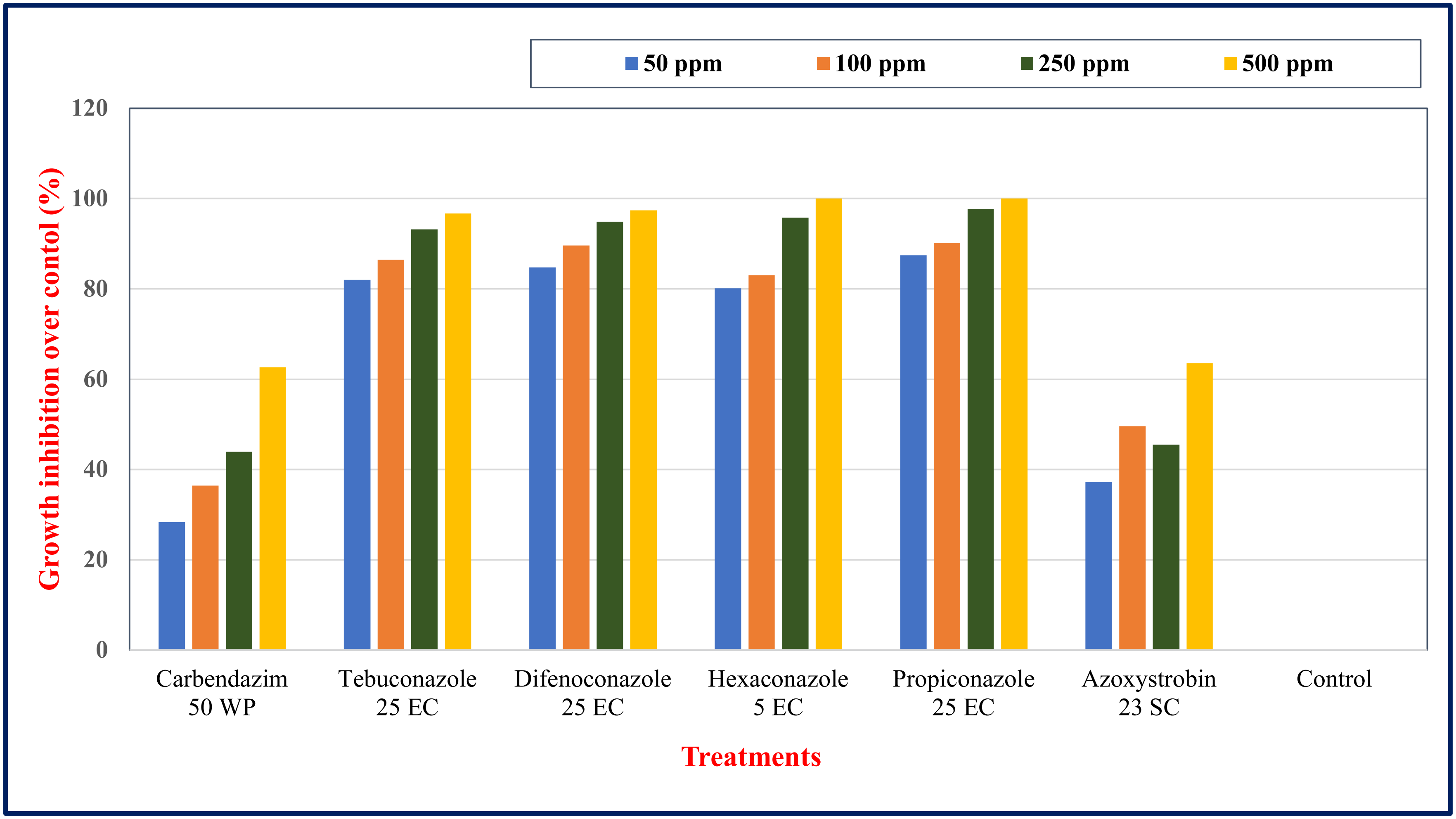


Fig. 4.3: Effect of different systemic fungicide on per cent growth inhibition of *A. alternata* at different concentration *in vitro*

Table 4.5: Effect of different systemic fungicide on per cent growth inhibition of *A. alternata* at different concentration *in vitro*

Sr. No.	Fungicides	Growth inhibition over control (%) Concentration (ppm) 50	100	250	500	Mean
1	Carbendazim 50 WP	32.16 [o] (28.34)	37.10 [n] (36.41)	41.51 [m] (43.93)	52.30 [k] (62.59)	40.77 [e] (42.82)**
2	Tebuconazole 25 EC	64.86 [ij] (81.96)	68.41 [h] (86.46)	74.70 [e] (93.22)	79.61 [bc] (96.74)	71.89 [c] (89.55)
3	Difenoconazole 25 EC	67.04 [hi] (84.78)	71.28 [fg] (89.63)	76.95 [d] (94.90)	80.78 [b] (97.41)	74.01 [b] (91.68)
4	Hexaconazole 5 EC	63.52 [j] (80.11)	65.76 [ij] (83.00)	78.12 [cd] (95.76)	89.43 [a] (100.00)	74.21 [b] (89.71)
5	Propiconazole 25 EC	69.22 [gh] (87.41)	71.77 [f] (90.18)	81.23 [b] (97.67)	89.43 [a] (100.00)	77.91 [a] (93.81)
6	Azoxystrobin 23 SC	37.60 [n] (37.22)	44.77 [l] (49.60)	42.40 [m] (45.48)	52.85 [k] (63.51)	44.40 [d] (48.96)
7	Control	4.05 [p] (0.00)	4.05 [p] (0.00)	4.05 [p] (0.00)	4.05 [p] (0.00)	4.05 [p] (0.00)
Mean		55.73 [d] (66.63)	59.85 [c] (72.54)	65.82 [b] (78.46)	74.06 [a] (86.70)	-
		Treatment (T)		Concentration (C)		T × C
S.Em. ±		0.382		0.312		0.764
C.D. at 5 %		1.091		0.891		2.182
C.V. %		2.079				

**Figures in parentheses are original values and outside are arc-sine transformed values

Treatment means with the letter(s) common are not significant by Duncan's New Multiple Range Test at 5% level of significance

Interaction effect of fungicide and concentrations

Efficacy of different fungicides for inhibtion of growth of *A. alternata* has been reported by several research workers. Gohel and Solanky (2011) found that propiconazole, difenoconazole, hexaconzole, copper oxychloride, mancozeb and propineb were highly fungitoxic to *A. alternata*. Rai *et al*. (2017) found that propiconazole was most effective in inhibition of *A. alternata* followed by chlorothalonil and carbendazim. Ajaybhai *et al.* (2018) who found two fungicides *viz.* propiconazole and hexaconazole showed maximum growth inhibition at all tested concentrations followed by tebuconazole. Kakraliya *et al.* (2018) who reported that maximum inhibition per cent of the mycelia growth was in propiconazole followed by hexaconazole and vitavax. Nira *et al.* (2022) observed that maximum growth inhibition of *A. alternata* was found with propiconazole and iprodione at all the tested concentrations *i.e.,* 100, 150, 200, 250 and 300 ppm *in vitro*. The present finding was in conformity with the finding of above research works.

4.5.2 *In vitro* bio-efficacy of non-systemic fungicides against *A. alternata*

Six non-systemic fungicides tested at four different concentrations *viz.*, 500, 1000, 1500 and 2000 ppm by poisoned food technique for their efficacy against *A. alternata*. (Table 4.7, Fig.4.4 and Plate X).

4.5.2.1 Effect of non-systemic fungicides on mycelial growth

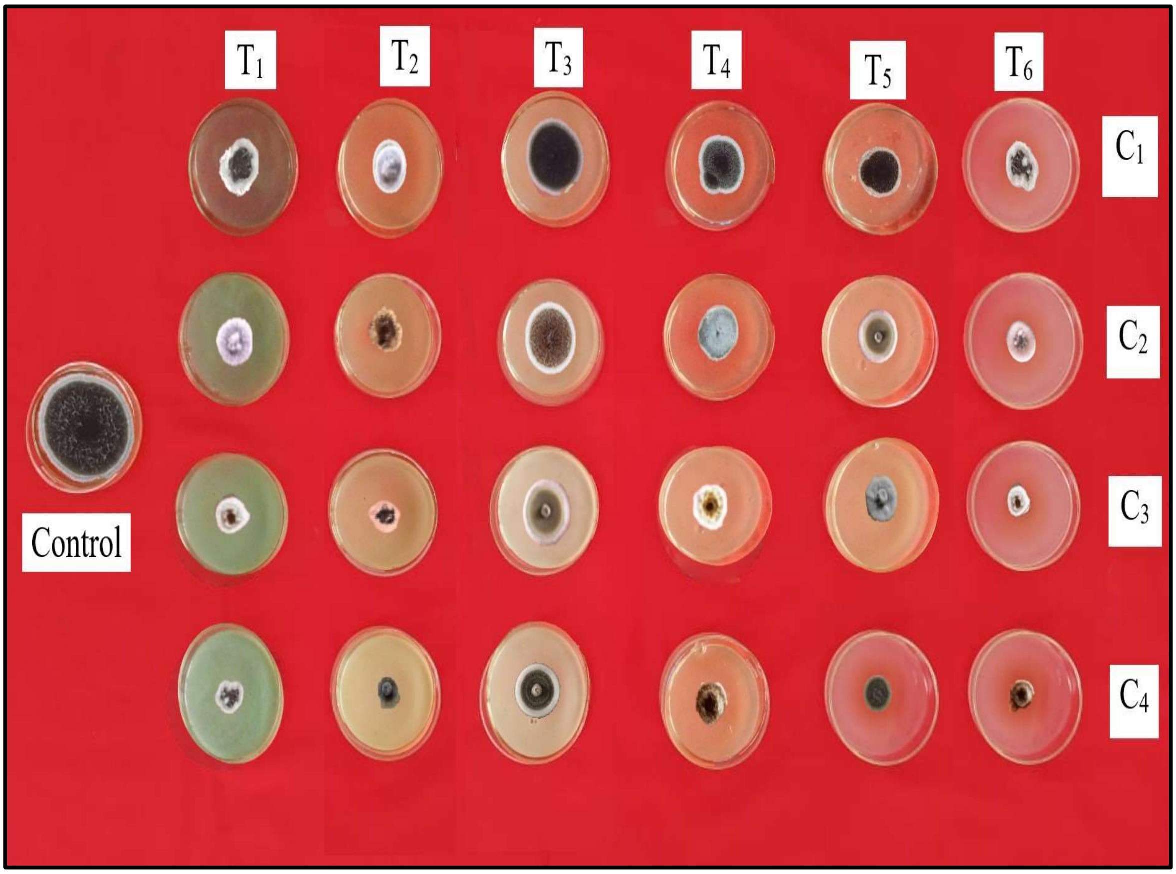
Effect of fungicides

The results presented in Table 4.6 reveaedl that all the fungicides at four different concentrations were found significantly inhibitory to mycelial growth. Among non-systemic fungicide tested, minimum mycelial growth was recorded in mancozeb (26.33 mm) followed by copper oxychloride (29.00 mm), captan (30.95 mm) and copper hydroxide (33.50 mm). Propineb and chlorothalonil recorded least effective which was 37.75 mm and 41.95 mm mycelial growth, respectively.

Table 4.6: Effect of different non-systemic fungicide on mycelial growth of *A. alternata in vitro*

Sr. No.	Fungicides	Mycelial growth (mm) Concentration (ppm)				Mean
		500	1000	1500	2000	
1	Copper oxychloride 50 WP	34.66 f	31.50 gh	27.00 kl	23.33 m	29.00 f
2	Mancozeb 75 WP	30.66 hi	28.50 jk	25.50 l	20.66 h	26.33 g
3	Chlorothalonil 75 WP	47.50 b	44.00 c	40.66 d	35.66 ef	41.95 b
4	Propineb 70 WP	45.00 c	41.00 d	34.33 f	30.66 hi	37.75 c
5	Copper hydroxide 77 WP	41.00 d	34.50 hi	31.00 d	27.33 f	33.50 d
6	Captan 75 WP	36.50 e	32.66 g	29.33 ij	25.33 l	30.95 e
7	Control	90.00 a	90.00 a	90.00 a	90.00 a	90.00 a
Mean		39.17 a	35.28 b	31.17 c	27.17 d	-
		Treatment (T)		Concentration (C)		T ×C
S.Em. ±		0.285		0.232		0.569
C.D. at 5 %		0.782		0.638		1.564
C.V. %		2.866				

Treatment means with the letter(s) common are not significant by Duncan's New Multiple Range Test at 5% level of significance



Fungicides

T_1: Copper oxychloride 50% WP

T_2 : Mancozeb 75% WP

T_3 : Chlorothalonil 75% WP

T_4 : Propineb 70% WP

T_5 : Copper hydroxide 77% WP

T_6 : Captan 75% WP

Concentrations

C_1: 500 ppm

C_2: 1000 ppm

C_3: 1500 ppm

C_4: 2000 ppm

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Plate X: Growth inhibition of *A. alternata* by non-systemic fungicide at different concentration *in vitro*

Effect of concentrations

The results revealed that various concentrations were significantly differ to each other and indicated that higher concentrations were significantly superior over lower doses of fungicides. Successive reduction in mycelial growth was observed with subsequent increase in the concentration from 500 to 2000 ppm concentration. The highest mycelial growth of 39.17mm was recorded with 500 ppm concentration and least mycelial growth of 27.17 mm recorded in 2000 ppm.

Interaction effect of fungicide and concentrations

Mancozeb at 2000 ppm concentration was found most effective treatment (20.66 mm) followed by copper oxychloride at 2000 ppm (23.33 mm) and captan at 2000 ppm (25.33 mm). Chlorothalonil and propineb at 500 ppm were found least effective at 500 ppm concentration.

4.5.2.2 Effect of non-systematic fungicides on mycelial growth inhibition

Effect of fungicides

The results revealed that among the six fungicides evaluated against *A. alternata,* average mycelial growth inhibition of the test pathogen was ranged from 53.38 per cent (chlorothalonil) to 70.53 per cent (mancozeb). The interaction effect of fungicide × concentration was also found significant. The highest mean growth inhibition of mycelial was obtained by mancozeb with 70.53 per cent followed by copper oxychloride with 67.63 per cent. Captan was found next effective to inhibit 65.60 per cent mycelial growth inhibition of the fungus and remained significantly superior over copper hydroxide (62.82 %) and propineb (57.61 %). Chlorothalonil recorded the least inhibition of only 53.38 per cent mycelial growth inhibition of the *A. alternata*. (Table 4.7, Fig. 4.3).

Effect of concentrations

Among all concentrations, the higher concentration of each fungicide produced maximum mycelial growth inhibition of the pathogen Among the non-systemic fungicides, all concentrations indicated significant effect on the mycelial growth inhibition of the fungus. Successive reduction in mycelial growth was observed with subsequent increase in the concentration from 500 to 2000 ppm concentration. The highest mycelial growth inhibition of 69.79 per cent was recorded with 2000 ppm concentration and least mycelial growth inhibition of 56.45 per cent recorded in 500 ppm.

Interaction effect of fungicide and concentrations

Mancozeb at 2000 ppm inhibited maximum mycelial growth of the fungus with 76.85 per cent. It was followed by copper oxychloride and captan at same concentration inhibited the mycelial growth with 73.87 and 71.85 per cent, respectively. Mancozeb at 1500 ppm found significantly superior to inhibited the mycelial growth with 71.66 per cent followed by copper oxychloride at same concentration recorded 69.98 per cent mycelial growth inhibition. Chlorothalonil and propineb at 500 ppm recorded the least inhibition of only 47.22 and 50.00 per cent mycelial growth, respectively. (Table 4.7, Fig. 4.4 and Plate X).

Table 4.7: Effect of different non-systemic fungicide on per cent growth inhibition of *A. alternata* at different concentration *in vitro*

Sr. No.	Fungicides	Growth inhibition over control (%)				Mean
		Concentration (ppm)				
		500	1000	1500	2000	
1	Copper oxychloride 50 WP	51.75^{hi} (61.67)	53.73^{gf} (65.00)	56.78^{c} (69.98)	59.27^{b} (73.87)	55.38^{b} (67.63)**
2	Mancozeb 75 WP	54.29^{efg} (65.93)	55.35^{de} (68.33)	57.84^{c} (71.66)	61.25^{a} (76.85)	57.18^{a} (70.53)
3	Chlorothalonil 75 WP	43.41^{l} (47.22)	45.64^{k} (51.11)	47.77^{j} (54.82)	50.98^{i} (60.37)	46.95^{f} (53.38)
4	Propineb 70 WP	45.00^{k} (50.00)	47.55^{j} (54.44)	50.66^{i} (59.81)	54.29^{efg} (65.93)	49.42^{e} (57.61)
5	Copper hydroxide 77 WP	47.55^{j} (54.44)	51.64^{hi} (61.67)	54.07^{efg} (65.56)	56.56^{cd} (69.63)	52.48^{d} (62.82)
6	Captan 75 WP	50.44^{i} (59.44)	52.95^{gh} (63.70)	55.19^{def} (67.41)	57.96^{bc} (71.85)	54.14^{c} (65.60)
7	Control	4.05^{m} (0.00)	4.05^{m} (0.00)	4.05^{m} (0.00)	4.05^{m} (0.00)	4.05^{g} (0.00)
Mean		48.74^{d} (56.45)	51.16^{c} (60.59)	53.72^{b} (64.87)	56.75^{a} (69.79)	-
		Treatment (T)		Concentration (C)		T×C
S.Em. ±		0.230		0.188		0.460
C.D. at 5 %		0.664		0.542		1.328
C.V. %		1.536				

**Figures in parentheses are original values and outside are arc-sine transformed values

Treatment means with the letter(s) common are not significant by Duncan's New Multiple Range Test at 5% level of significance

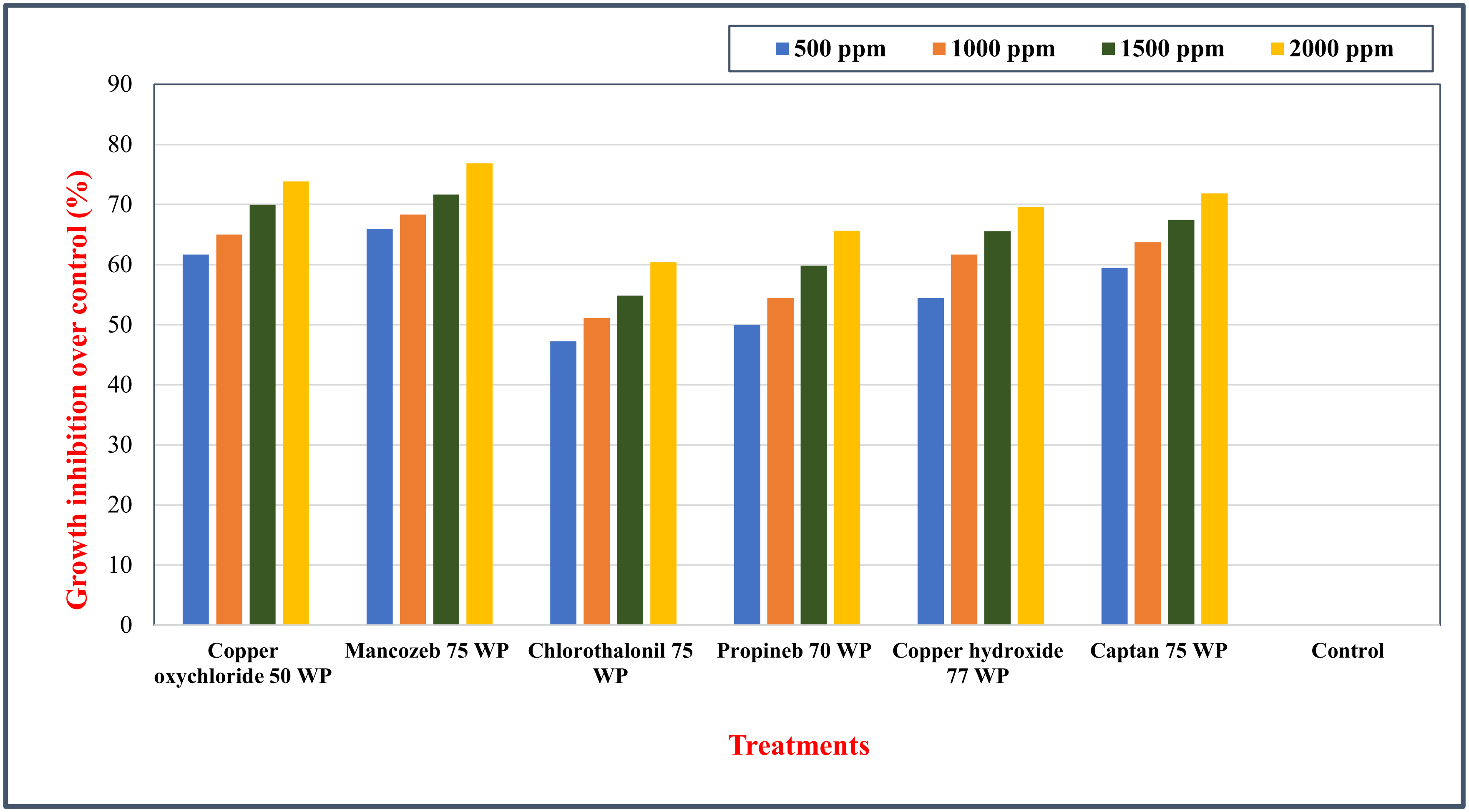


Fig. 4.4: Effect of different non-systemic fungicide on per cent growth inhibition of *A. alternata* at different concentration *in vitro*

The result of present data similar with Kantwa *et al*. (2014) reported that mycelial growth of *A. alternata* was completely inhibited by mancozeb which was followed by copper oxychloride and captaf. Kadam *et al.* (2018) who found highest average mycelial growth inhibition was with mancozeb followed by copper oxychloride, copper hydroxide, propineb and chlorothalonil.

4.5.3 *In vitro* bio-efficacy of combine fungicides against *A. alternata*

From combine fungicides (Table 4.9, Fig 4.6 and Plate XI), it is evident from the results that the growth inhibition of *A. alternata* increased as increase in the concentration of the chemicals. From the result, it was clear that all the six combine fungicide were inhibitory to the radial growth of *A. alternata.*

4.5.3.1 Effect of combine fungicides on mycelial growth

Effect of fungicides

The results presented in Table 4.8 revealed that all the fungicides at four different concentrations were found significantly inhibitory to mycelial growth. Among combine fungicide tested, minimum mycelial growth was recorded in azoxystrobin + difenoconazole was found most effective treatment which did not allow the *A .alternat*a to grow on treated culture plate followed by carbendezim+ iprodione (9.77 mm), azoxystrobin + mancozeb (13.47 mm), tebuconazole + trifloxystrobin (17.91 mm) and metalaxyl + mancozeb (33.87 mm). Carbendazim + mancozeb recorded least effective which was 38.87 mm mycelial growth.

Effect of concentrations

The results revealed that various concentrations were significantly differ to each other and indicated that higher concentrations were significantly superior over lower doses of fungicides. Successive reduction in mycelial growth was observed with subsequent increase in the concentration from 500 to 2000 ppm concentration. The highest mycelial growth of 39.28 mm was recorded with 500 ppm concentration and least mycelial growth of 28.88 mm recorded in 2000 ppm.

Interaction effect of fungicide and concentrations

Azoxystrobin + difenoconazole at 2000 ppm concentration was found most effective treatment which did not allow the *A. alternata* to grow on treated culture plate followed by carbendazim + iprodione at 2000 ppm (4.00 mm) and azoxystrobin + mancozeb at 2000 ppm (7.66 mm). Carbendazim + mancozeb and metalaxyl + mancozeb at 500 ppm were found least effective concentration.

Table 4.8: **Effect of different combine fungicide on mycelial growth of *A. alternata in vitro***

Sr. No.	Fungicides	Mycelial growth (mm) Concentration (ppm)				Mean
		500	1000	1500	2000	
1	Tebuconazole 50 + Trifloxystrobin 25 WG	27.36 [i]	19.30 [j]	14.00 [k]	11.00 [l]	17.91 [d]
2	Metalaxyl 4 + Mancozeb 64 WP	38.00 [d]	35.17 [e]	33.33 [f]	29.00 [h]	33.87 [c]
3	Azoxystrobin 11.5+ Mancozeb 30 WP	19.00 [j]	15.33 [k]	11.87 [l]	7.66 [m]	13.47 [e]
4	Carbendazim 12 + Mancozeb 63 WP	46.00 [b]	41.17 [c]	36.66 [de]	31.66 [g]	38.87 [f]
5	Carbendezim 25 + Iprodione 25 WP	15.33 [k]	11.00 [l]	8.73 [m]	4.00 [n]	9.77 [g]
6	Azoxystrobin 18.2 + Difenoconazole 11.4 SC	0.00 [o]	0.00 [o]	0.00 [o]	0.00 [o]	0.00 [a]
7	Control	90.00 [a]	90.00 [a]	90.00 [a]	90.00 [a]	90.00 [a]
Mean		39.28 [a]	35.32 [b]	32.43 [c]	28.88 [d]	-
		Treatment (T)		Concentration (C)		T×C
S.Em. ±		0.266		0.217		0.531
C.D. at 5 %		0.772		0.631		1.545
C.V. %		2.832				

Treatment means with the letter(s) common are not significant by Duncan's New Multiple Range Test at 5% level of significance

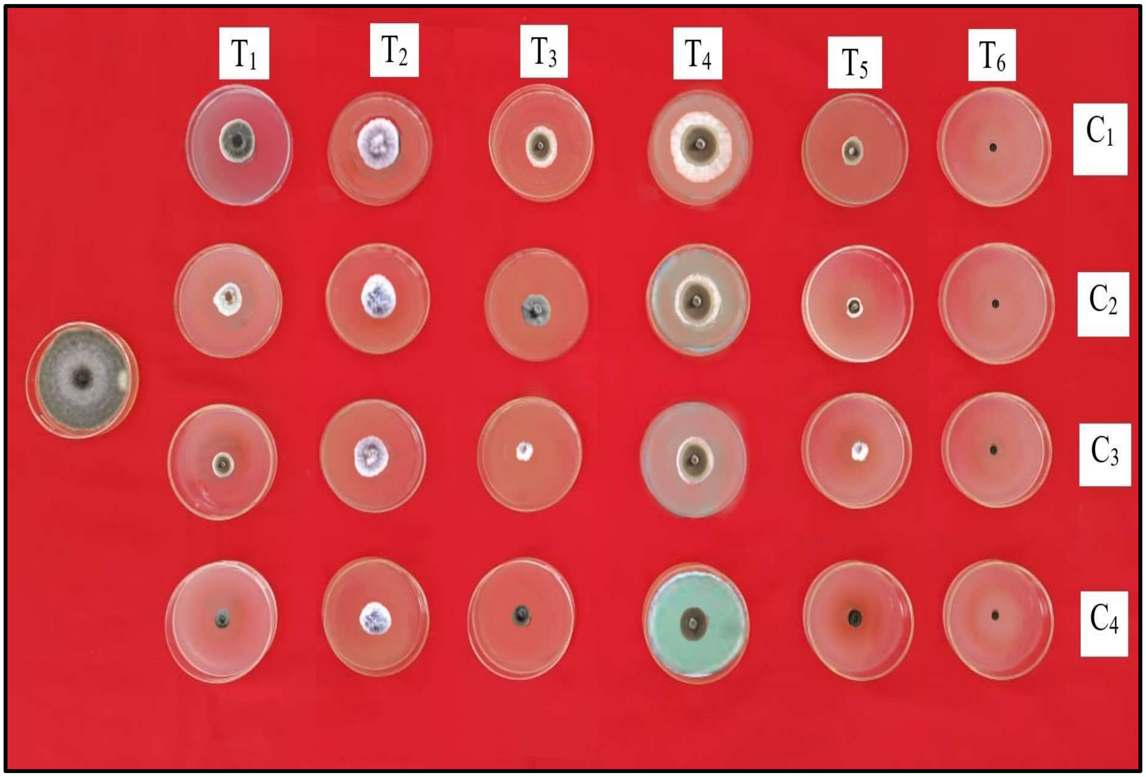
4.5.3.2 Effect of combine fungicides on mycelial growth inhibition

Effect of fungicides

The mean mycelial growth inhibition for these combined fungicides was as minimum as 56.81 to as high as 100 per cent. Among them, azoxystrobin + difenoconazole was found significantly higher than the rest of the combined fungicides with the cent per cent mycelial growth inhibition followed by carbendazim + iprodione, azoxystrobin + mancozeb, tebuconazole + trifloxystrobin and metalaxyl + mancozeb with percentages 89.10, 85.04, 80.09 and 62.36, respectively. Carbendazim + mancozeb recorded the least inhibition of only 56.81 per cent mycelial growth of test fungus (Table 4.9, Fig. 4.5 and Plate XI).

Effect of concentations

Among the combined fungicides, all concentrations indicated significant effect on the growth inhibition of the fungus. Successive reduction in growth was observed with subsequent increase in the concentration from 500 to 2000 ppm concentration.



Fungicides

T_1 : Tebuconazole 50% + Trifloxystrobin 25 WG

T_2 : Metalaxyl 4 %+ Mancozeb 64%WP

T_3 : Azoxystrobin 11.5% + Mancozeb 30% WP

T_4 : Carbendazim 12% +Mancozeb 63% WP

T_5 : Carbendezim 25 % +Iprodione 25 % WP

T_6 : Azoxystrobin 18.2% + Difenoconazole 11.4% SC

Concentation

C_1 : 500 ppm

C_2: 1000 ppm

C_3: 1500 ppm

C_4: 2000 ppm

Plate XI: Growth inhibition of *A. alternata* by combine fungicide at different concentration *in vitro*

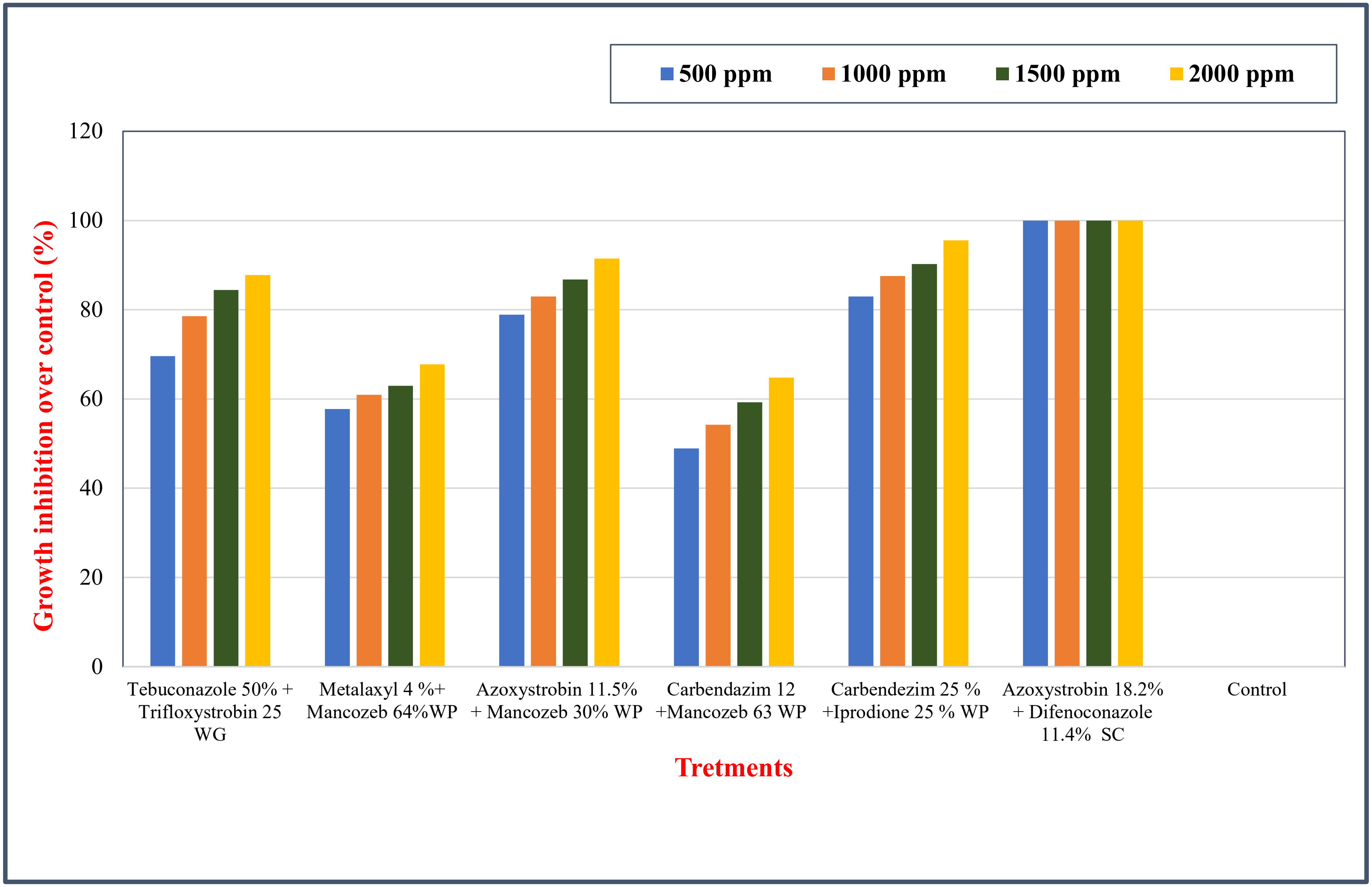


Fig. 4.5: Effect of different combine fungicide on per cent growth inhibition of *A. alternata* at different concentration *in vitro*

The highest inhibition of 84.56 per cent was recorded with 2000 ppm concentration and least inhibition of 73.02 per cent recorded in 500 ppm.

Table 4.9: Effect of different combine fungicide on per cent growth inhibition of *A. alternata* at different concentration *in vitro*

Sr. No.	Fungicides	Growth inhibition over control (%)				Mean
		Concentration (ppm)				
		500	1000	1500	2000	
1	Tebuconazole 50 + Trifloxystrobin 25 WG	56.54[i] (69.60)	62.40[h] (78.52)	66.78[f] (84.44)	69.55[e] (87.78)	63.82[d] (80.09)**
2	Metalaxyl 4 + Mancozeb 64WP	49.48[l] (57.78)	51.31[k] (60.92)	52.51[j] (62.96)	55.42[i] (67.78)	52.19[e] (62.36)
3	Azoxystrobin 11.5+ Mancozeb 30 WP	62.65[h] (78.89)	65.62[g] (82.96)	68.72[e] (86.82)	73.04[c] (91.48)	67.51[c] (85.04)
4	Carbendazim 12 + Mancozeb 63 WP	44.36[n] (48.89)	47.44[m] (54.26)	50.34[kl] (59.26)	53.62[j] (64.81)	48.94[f] (56.81)
5	Carbendezim 25 + Iprodione 25 WP	65.62[g] (82.96)	69.38[e] (87.59)	71.85[d] (90.28)	77.85[b] (95.56)	71.18[b] (89.10)
6	Azoxystrobin 18.2 + Difenoconazole 11.4 SC	89.43[a] (100)	89.43[a] (100)	89.43[a] (100)	89.43[a] (100)	89.71[a] (100)
7	Control	4.05[o] (0.00)	4.05[o] (0.00)	4.05[o] (0.00)	4.05[o] (0.00)	4.05[g] (0.00)
Mean		61.35[d] (73.02)	64.24[c] (80.30)	66.60[b] (80.63)	69.82[a] (84.56)	-
		Treatment (T)		Concentration (C)		T ×C
S.Em. ±		0.196		0.16		0.393
C.D. at 5 %		0.574		0.468		1.148
C.V. %		1.066				

**Figures in parentheses are original values and outside are arc-sine transformed values
Treatment means with the letter(s) common are not significant by Duncan's New Multiple Range Test at 5% level of significance

Interaction effect of fungicide and concentations

Azoxystrobin + difenoconazole at 500 ppm inhibited cent per cent mycelial growth and it was followed by carbendazim + iprodione at 2000 ppm (95.56 %) concentration and azoxystrobin + mancozeb at same concentration (91.48 %). Carbendazim + iprodione at 1500 ppm concentration (90.28 %) found very effective to inhibit the mycelial growth of fungus which was followed by tebuconazole + trifloxystrobin at 2000 ppm (87.78 %) concentration.

The present investigation is more or less similar to the work done by earlier workers *viz.,* Ginoya and Gohel (2015) revealed that azoxystrobin + difenoconazole at all three concentrations (500, 1000 and 1500 ppm) completely inhibited the mycelial growth of *A. alternata* under *in-vitro* condition. These were followed by fusilazole + carbendazim, tebuconazole + trifloxystrobin, fenamidone + mancozeb and isoprothiolane. Ajaybhai *et al.* (2018) found that among the combine fungicide, difenoconazole + azoxystrobin gave 100 per cent growth inhibition at 500 and 1000 ppm concentrations. Shingne *et al.* (2020) result indicated that highest mycelial growth inhibition of *A. alternata* was observed in carboxin + thiram followed by carbendazim + mancozeb, copper oxychloride, propineb and pyraclostrobin. Jewaliya *et al.* (2021) recorded maximum mycelial growth inhibition of *A. alternata* with hexaconazole followed by tebuconazole + trifloxystrobin.

4.6 Screening of varieties/germplasm of pomegranate against leaf and fruit spot caused by *A. alternata* under field condition

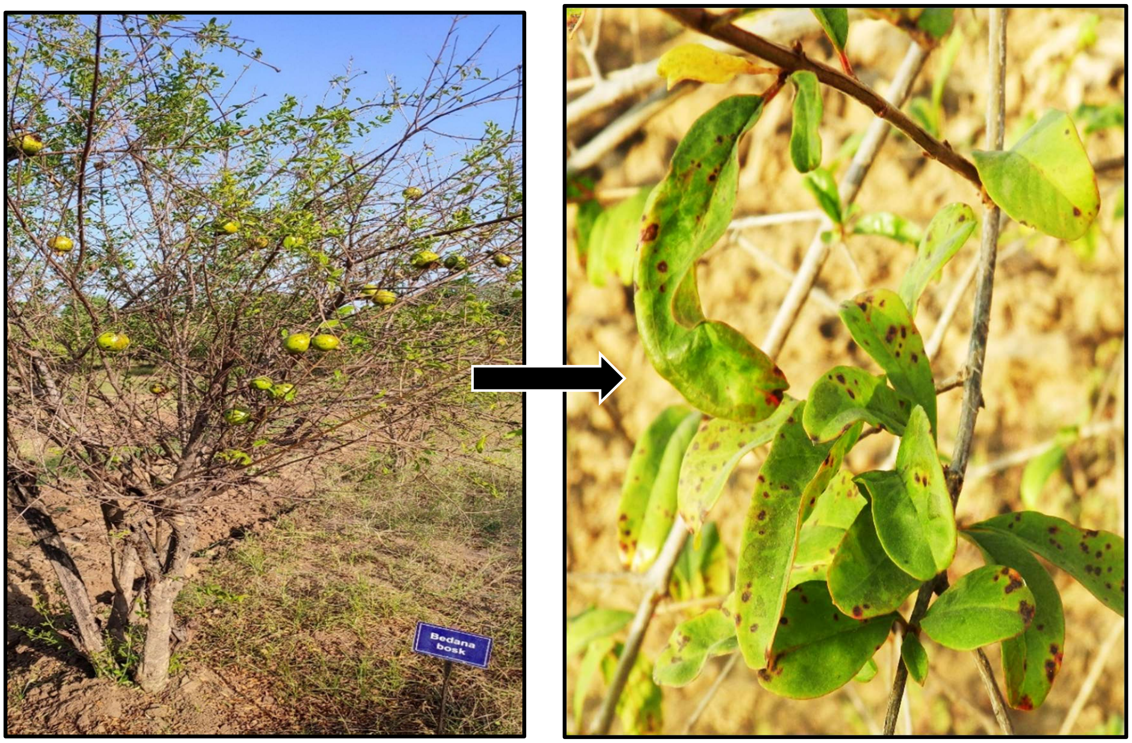
In the present investigation, eighteen varieties/genotypes were screened to identify the sources of resistance against *A. alternata*. The results with respect to leaf and fruit spot intensity presented in Table 4.10 and Table 4.11 revealed that fifteen varieties/germplasm (Ak Anar, Achikdana, Afghan Kandhari, Bedana sedana, Bedana bosk, Dholka, Guleshah, Jodhpuri red, Ganesh, P-26, GP-4, Jyoti, Mrudula, Solapuri Red and Kandhari-3) were susceptible to the leaf and fruit spot of pomegranate whereas, less than 20 per cent of disease intensity of leaf and fruit spot observed in Bhagwa and Super Bhagwa. These two varieties found moderately susceptible to the disease. Maximum leaf (55.5 %) and fruit spot (60.0 %) disease was observed in Boshlinski (Plate XII) which recorded as highly susceptible. Disease starts appearing after 3rd week of June and become more severe after 15th July during heavy monsoon rains.

The present investigation is more or less similar to the work done by earlier workers. Archana (2012) revealed that none of the genotypes were resistant to leaf spot and fruit spot of pomegranate. Most of screened genotypes exhibited susceptible to highly susceptible reaction. The genotype Yercaud was moderately susceptible which recorded a PDI of 23.00 per cent. Among the susceptible genotypes *viz.*, A.K. Anar, Achook dana, Agah, Alah, Bedana sedana, Dorsata malas, Jalore seedless, Lupania and Siah shirin recorded a PDI range of 29.02 to 45.00 per cent. Genotypes

(A) GP-4 variety of pomegranate

(B) Boshlinski variety of pomegranate

Plate XII: Screening of GP-4 and Boshlinski varieties of pomegranate under field conditions against *A. alternata*



(A) Bedana bosk variety of pomegranate

(B) Jyoti variety of pomegranate

Plate XIII: Screening of Bedana bosk and Jyoti varieties of pomegranate under field conditions against *A. alternata*

Arakta, Jyothi, Kesar, Kabul, Gul-e-shah, Domain and Bedana suri were found highly susceptible with a PDI ranging between 56.00 to 70.02 per cent.

Table 4.10: Screening of varieties/germplasms of pomegranate against leaf spot caused by *A. alternata* under field condition

Sr. No.	Varieties/germplasms	Per cent disease index Leaf spot	Reaction
1	Ak Anar	39.0	S
2	Achikdana (achirdana)	45.8	S
3	Afghan Kandhari	48.0	S
4	Boshlinski	55.5	HS
5	Bedana sadana	31.2	S
6	Bedana bosk	39.8	S
7	Dholka	44.2	S
8	Guleshah	35.5	S
9	Jodhpuri red	42.3	S
10	Ganesh	49.0	S
11	P-26	45.7	S
12	GP-4	48.3	S
13	Jyoti	49.2	S
14	Mrudula	47.5	S
15	Bhagwa	17.2	MS
16	Super Bhagwa	19.3	MS
17	Solapuri Red	32.2	S
18	Kandhari-3	31.7	S

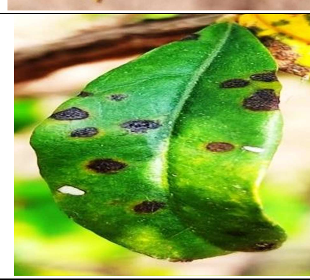
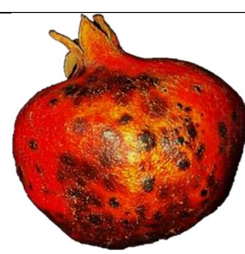
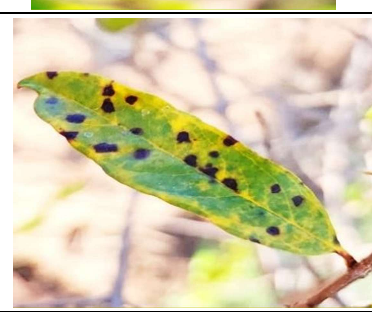
Grade 0		
Grade 1		
Grade 2		
Grade 3		
Grade 4		
Grade 5		

Plate XIV: Disease scoring 0-5 scale for Alternaria leaf and fruit spot

Table 4.11: Screening of varieties/germplasms of pomegranate against fruit spot caused by *A. alternata* under field condition

Sr. No.	Varieties/germplasms	Per cent disease index Fruit spot	Reaction
1	Ak Anar	37.3	S
2	Achikdana (achirdana)	47.3	S
3	Afghan Kandhari	37.3	S
4	Boshlinski	60.0	HS
5	Bedana sadana	29.3	S
6	Bedana bosk	40.7	S
7	Dholka	41.3	S
8	Guleshah	38.7	S
9	Jodhpuri red	42.0	S
10	Ganesh	55.3	S
11	P-26	49.3	S
12	GP-4	44.7	S
13	Jyoti	48.7	S
14	Mrudula	42.0	S
15	Bhagwa	16.7	MS
16	Super Bhagwa	20.0	MS
17	Solapuri Red	36.0	S
18	Kandhari-3	38.7	S

SUMMARY AND CONCLUSIONS

V. SUMMARY AND CONCLUSION

Pomegranate is an important fruit crop of semi-arid and arid regions of the world. It has both cultivated (*Punica granatum* L.) and wild types (*Punica protopunica*). The chromosome number varies among different cultivars of pomegranate; 2n=16 or 18. Pomegranate is fruit bearing shrubs or trees comes under small genus of *Punica* and family *Lythraceae*. Pomegranate is a native of Iran. Pomegranate cultivation is done in the countries like China, USA, India, Spain, Morocco, Egypt, Iran, Afghanistan, Baluchistan and Myanmar.

Isolation of pathogen from infected leaves and fruit of pomegranate showing typical symptoms were done by using tissue isolation method. After 24 hours of incubation the isolated fungus initially started to grow as white, gradually turned grey to black in colour on PDA. The culture was purified by single hyphal tip method and these cultures were further purified by single spore isolation method. These pure culture was maintained on PDA slants for further studies.

The microscopic study of *A. alternata* revealed that the mycelial colour ranged from raised ashy white to flat ashy green to raised blackish green with all having black reverse. Conidia are typically muriform, dark brown, thick walled, in long branched chains (9 to 15). Hypha septated were brown in colour. Conidia was brown with length range from 31.5 to 62 µm and 9.4-14.6 µm wide. Some conidia were short rudimentary with dark brown beaks measuring 17.00 µm and 8.09 µm in width. Conidia had 3 to 5 transverse septa and 0 to 3 longitudinal septa

Pathogenicity test revealed that the test isolates *A. alternata* was able to infect the inoculated pomegranate leaves, plant and fruit. On detached leaves, symptom started appearing after 48 hrs incubation period. Pathogenicity of fungus was confirmed through Koch's postulates by pin prinking method on fruits, *A. alternata* induced typical symptoms small oval to irregular brown to dark brown spots was observed on fruit after 7 to 10 days of inoculation. Pathogenicity of the pathogen was confirm through Koch's postulates by spraying spore suspension of 10^6 conidia/ml on 6 months pomegranate plants. After 14 days of inoculation, the symptoms appeared as small light brown, regular to irregular spot. These spots enlarge, become oval in shape which was dark brown in colour with brownish black concentric ring.

On re-isolation from diseased leaves, plant and fruits of pomegranate identical culture of *A. alternata* (Fr.) Keissler to that of original culture was obtained. Hence pathogenicity was proved.

All the bio-agent tested against *A. alternata* were effective in checking the growth of *A. alternata*. Out of nine bio-agent tested, least growth of the pathogen was recorded in *T. viride* (Anand) which was followed by *B. subtilis* (sardarkrushinagar), *T. harzianum* (Anand), *P. putida* (Sardarkrushinagar) and *T. viride* (Sardarkrushinagar). Whereas *P. fluorescence* (Sardarkrushinagar) was comparatively found least effective. It was evident from the study that among all the antagonists evaluated by dual culture method, *T. viride* (Anand), *B. subtilis* (Sardarkrushinagar) and *T. harzianum* (Anand) consistently showed strong antagonistic properties against *A. alternata* as compared to the other antagonists tested hence considered as potential antagonists.

Among phyto-extracts tested, minimum mycelial growth was recorded in garlic clove extract (37.71 mm) followed by neem leaves extracts (44.99 mm). The next effective phyto-extract in order of inhibition was karanj leaves extract (48.94 mm), nilgiri, ardusa leaves extract (51.44 mm), tulsi leaves extract (51.68 mm), onion bulb extract (52.11 mm), lantana leaves extract (52.33 mm), akdo leaves extract (53.66 mm) which showed significantly lower mycelial growth than rest of the phyto-extracts. Dhatura leaf extract (54.55 mm) was the least effective.

As plant extracts are cost effective means of management therefore an effort was made to know the efficacy of different phytoextracts against *A. alternata.* The results revealed that among the ten phytoextracts evaluated against *A. alternata* at different concentrations *viz.,* 5, 10 and 20 per cent The highest mean inhibition of mycelial was obtained by garlic clove (58.09 %) which was significantly higher over all the phytoextracts. Neem leaves extract (50.00%) ranked second to inhibit the mycelial growth followed by karanj leaves extracts (45.19 %), nilgiri leaves extract (43.44 %) and ardusa leaves extract (42.84 %). Rest of extracts viz., tulsi leaves extract (42.57 %), onion bulb extract (42.09 %), lantana leaves extract (41.85 %), akdo leaves extract (40.74 %) and datura leaves extracts (39.01 %) were found less effective for inhibiting mycelial growth of the pathogen.

Among systemic fungicide tested, minimum mycelial growth was recorded in propiconazole (5.66 mm) followed by difenoconazole (7.50 mm), hexaconazole (9.03 mm) followed by tebuconazole (9.40 mm). Carbendazim recorded least effective which was (51.54 mm) followed by azoxystrobin (45.92 mm).

In systemic fungicides cent per cent growth inhibition was recorded in propiconazole and hexaconazole at 500 ppm concentration. The next best treatment in order of merit was difenoconazole at 500 ppm with 97.41 per cent followed by tebuconazole at same concentration with 96.74 per cent growth inhibition followed by tebuconazole with 96.74 per cent growth inhibition. At 250 ppm concentration, propiconazole, hexaconazole, difenoconazole and tebuconazole recorded more than 90 per cent growth inhibition (97.67, 95.76, 94.90 and 93.22 %, respectively). Among these, carbendazim was the least effective as it recorded as high as 62.59 per cent growth inhibition even at the highest concentration.

Among non-systemic fungicide tested, minimum mycelial growth was recorded in mancozeb (26.33 mm) followed by copper oxychloride (29.00 mm), captan (30.95 mm), copper hydroxide (33.50 mm). Propineb and chlorothalonil recorded least effective which was 37.75 mm and 41.95 mm, respectively.

In non-systemic fungicides, maximum growth inhibition of the fungus was observed in mancozeb at 2000 ppm with76.85 per cent. It was followed by copper oxychloride and captan at same concentration inhibition mycelial growth with 73.87 and 71.85 per cent, respectively. Mancozeb at 1500 ppm found significantly superior to inhibited the mycelial growth with 71.66 per cent followed by copper oxychloride at same concentration recorded 69.98 per cent growth inhibition. Chlorothalonil and propineb at 500 ppm recorded the least inhibition of only 47.22 and 50.00 per cent mycelial growth, respectively.

Among combine systemic fungicide tested, minimum mycelial growth was recorded in azoxystrobin + difenoconazole was found to be most effective treatment, which did not allow the *A .alternat*a to grow on treated culture plate followed by carbendezim+ iprodione (9.77 mm), azoxystrobin + mancozeb (13.47 mm), tebuconazole + trifloxystrobin (17.91 mm) and metalaxyl + mancozeb (33.87 mm). Carbendazim + mancozeb recorded least effective which was 38.87 mm.

In combine fungicides, azoxystrobin + difenoconazole at 500 ppm inhibited cent per cent mycelial growth and it was followed by carbendazim + iprodione at 2000 ppm (95.56 %) concentration and azoxystrobin + mancozeb at same concentration (91.48 %). Carbendazim + iprodione at 1500 ppm (90.28 %) found very effective to inhibit the mycelial growth of fungus which was followed by tebuconazole + trifloxystrobin at 2000 ppm (87.78 %) concentration.

Eighteen varieties/genotypes were screened to identify the sources of resistance against *A. alternata*. The results indicated that fifteen genotypes (Ak Anar, Achikdana, Afghan Kandhari, Bedana sedana, Bedana bosk, Dholka, Guleshah, Jodhpuri red, Ganesh, P-26, GP-4, Jyoti, Mrudula, Solapuri Red and Kandhari-3) were susceptible to the pathogen whereas less than 20 per cent of disease intensity of leaf and fruit spot observed in Bhagwa and Super Bhagwa. These two varieties found moderately susceptible to the disease. Maximum leaf (55.5 %) and fruit spot (60.0 %) disease was observed in Boshlinski which recorded as highly susceptible. Disease starts appearing after 3rd week of June and become more severe after 15th July during heavy monsoon rains.

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