EVALUATION OF BOTANICALS, BIO-CONTROL AGENTS AND FUNGICIDES AGAINST TURCICUM LEAF BLIGHT OF MAIZE

by

Ms. Aghav Mukta Adinath
(Reg.No. 019/224)

A thesis submitted to the

MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722, DIST. AHMEDNAGAR,
MAHARASHTRA, INDIA

in partial fulfilment of the requirements for the degree

of

MASTER OF SCIENCE (AGRICULTURE)

in

PLANT PATHOLOGY

DEPARTMENT OF PLANT PATHOLOGY AND AGRICULTURAL MICROBIOLOGY

POST GRADUATE INSTITUTE
MAHATMA PHULE KRISHI VIDYAPEETH,
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APPROVED BY

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MAHARASHTRA, INDIA
2021

CANDIDATE'S DECLARATION

I hereby declare that this thesis or part
there of has not been submitted
by me or another person to any
other University or Institute
for a Degree or
Diploma

Place : MPKV, Rahuri. **(M. A. AGHAV)**

Date : / /2021

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Maharashtra(INDIA).

CERTIFICATE

This is to certify that the thesis entitled, **"EVALUATION OF BOTANICALS, BIO-CONTROL AGENTS AND FUNGICIDES AGAINST TURCICUM LEAF BLIGHT OF MAIZE"** submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar (Maharashtra) in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT PATHOLOGY**, embodies the results of a piece of bonafide research work carried out by **Ms. AGHAV MUKTA ADINATH,** under my guidance and supervision and that no part of the thesis has been submitted to any other University for Degree or Diploma.

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

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Date : / /2021 Research Guide

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Date : / /2021

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This is to certify that the thesis entitled, **"EVALUATION OF BOTANICALS, BIO-CONTROL AGENTS AND FUNGICIDES AGAINST TURCICUM LEAF BLIGHT OF MAIZE"** submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT PATHOLOGY**, embodies the results of a piece of bonafide research work carried out by **Ms. AGHAV MUKTA ADINATH,** under the guidance and supervision of **Dr. V. S. SHINDE**, Assistant Plant Pathologist, AICRP on Maize, Pulse Improvement Project, MPKV, Rahuri and that no part of the thesis has been submitted to any other University for Degree or Diploma.

Place : MPKV, Rahuri. **(P. N. Rasal)**

Date : / /2021

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I also feel immense pleasure in expressing my sincere thanks to members of my advisory committee ***Dr. T. K. Narute,*** *Head, Department of Plant Pathology and Agricultural Microbiology, MPKV, Rahuri;* ***Dr. S. V. Kolase,*** *Associate Professor, Department of Plant Pathology and Agricultural Microbiology, PGI, MPKV, Rahuri;* ***Dr. S. B. Latake****, Scientist (Plant Pathology), Pulse Improvement Project, MPKV, Rahuri for their useful comments and valuable guidance throughout the research work.*

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Place : MPKV, Rahuri.

Date : / / 2021 **(M. A. AGHAV)**

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LIST OF ABBREVIATIONS

@	:	At the rate of
°C	:	Degree Centigrade (S)
°E	:	Degree East
°N	:	Degree North
/	:	Per
%	:	Per cent
C. D.	:	Critical Difference
CRD	:	Completely Randomized Design
cm	:	Centimetre (s)
EC	:	Emulsifiable Concentrates
et al.	:	And other (et alli)
etc.	:	Et cetera
Ex.	:	Example
Fig.	:	Figure
g	:	Gram (s)
g/l	:	Gram per liter
h	:	Hours
i.e.	:	That is
kg/cm^2	:	Kilogram per centimeter square
kg/ha	:	Kilogram per hectare
lps	:	Low Pressure Steam
mg	:	Milligram
ml	:	Milliliter
mm	:	Millimeter
No.	:	Number

NSKE	:	Neem Seed Kernel Extract
psi	:	Pound per Square Inch
q/ha	:	Quintal per hectare
SC	:	Suspension Concentrates
SE	:	Suspoemulsion
S. E.	:	Standard Error of mean
spp.	:	Species
Sr.	:	Serial
t/ha	:	Tonn per hactare
Tr.	:	Treatment
µ	:	Micron
µl	:	Microliter
µm	:	Micrometer
viz.,	:	Namely
WP	:	Wettable Powder
w/v	:	Weight by Volume
w/w	:	Weight by weight

ABSTRACT

EVALUATION OF BOTANICALS, BIO CONTROL AGENTS AND FUNGICIDES AGAINST TURCICUM LEAF BLIGHT OF MAIZE

by

Ms. AGHAV MUKTA ADINATH

A candidate for the degree

of

MASTER OF SCIENCE (AGRICULTURE)

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PLANT PATHOLOGY

Mahatma Phule Krishi Vidyapeeth Rahuri – 413 722

2021

Research Guide	**:**	**Dr. V. S. Shinde**
Department	**:**	**Plant Pathology**

Maize **(***Zea mays* L.) is a cereal grain belonging to the family Gramineae/ Poaceae and is known as 'Queen of Cereals' because of its several uses. It is a widely distributed crop grown throughout the world in tropical region, sub-tropical and temperate region under irrigated to semi-arid condition. Being a versatile crop, it adapts to the wide range of production environments.

Maize cultivation reaches its boom in terms of acreage due to adoption of modern crop production practices like use of chemical pesticides, chemical fertilizers and cultivation of high yielding commercial hybrids. These factors in several ways led the maize crop to become vulnerable to pests and diseases at the farmers field. Among the different foliar diseases affecting maize, Turcicum leaf blight also called Northern corn leaf blight caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs. (syn. *Heliminthosporium turcicum* Pass.) is of worldwide importance.

The present investigation entitled **"**Evaluation of Botanicals, Bio-control Agents and Fungicides against Turcicum Leaf Blight of Maize", was carried out with a view to assess organism responsible for turcicum leaf blight of maize, its pathogenicity, cultural and morphological characteristics, efficacy of botanicals, bio-control agents and fungicides against the pathogen causing turcicum leaf blight.

The organism responsible for the disease was isolated from naturally infected turcicum leaf blight infected maize leaves.

Pathogenicity of the isolated fungus was proved on maize cultivar CM-202. Based on the disease symptoms under natural condition, pathogenicity test and morphological characters, the fungal pathogen responsible for turcicum leaf blight disease in maize was identified as *Exserohilum turcicum*.

There was variation in conidial growth of isolated pathogen (*Exserohilum turcicum*) on different cultural media. The colony colour varied from whitish grey to black. The isolated pathogen showed excellent growth on PDA and Oat meal agar media. Morphological characters

Abstract contd……

of isolated fungi indicated that the pathogen produced spindle shaped conidia having varied number of septa with protruding hilum.

Among all the treatments of botanicals, bio agents and fungicides, fungicidal treatments were found significantly superior in reducing mycelial growth of *Exserohilum turcicum*.

Among botanicals, aqueous extracts of *Parthenium hysterophorus* and *Allium cepa* were found effective against test pathogen (*Exserohilum turcicum*) at 5 and 10% concentration.

Out of six bio-control agents tested against *E. turcicum*, *Trichoderma harzianum* and *Trichoderma hamatum* were found superior and most effective in mycelial inhibition of pathogen with 77.07 and 73.51 per cent inhibition, respectively.

Among seven fungicides tested against *E. turcicum*, Carbendazim 12% + Mancozeb 63% was found most effective in reducing mycelial growth of test pathogen with 100 per cent inhibition of mycelial growth at 50% and 100% recommended concentration.

1. INTRODUCTION

Maize **(***Zea mays* L.) is a cereal grain belonging to the family Gramineae/ Poaceae. Due to its several uses it also called as 'Queen of Cereals'. The crop is native of Central America and Mexico by origin (Pursglove, 1972; Galaniant, 1976 and Dowswell *et al*., 1996). Maize is a widely cultivated crop and it is grown throughout the world in tropical, sub-tropical and temperate climate under irrigated to semi-arid condition. Maize is considered as a versatile crop as it adapts to the wide range of production environments. Maize grain contains about 70 per cent carbohydrates, 10 per cent protein , 4 per cent oil, 2.3 per cent crude fiber, 10.4 per cent albuminoides and 1.4 per cent ash. Maize also contains significant quantities of nicotinic acid, riboflavin and vitamin A with high content of carbohydrates, proteins, fats, vitamins and minerals. It is an important feed and food crop and it ranks third following wheat and rice not only in India but also in the world. It is perceived as a major cereal crop having great agro-economic value because of its expanded use in many agro-industries. It provides food, fodder, feed and serves as source of primary raw material for a number of industrial products *viz*., starch, oil, protein, alcoholic beverages, cosmetics, food sweeteners, bio-fuel, etc. Among all the cereal crops, maize has the highest average yield per ha and it ranks third after wheat and rice crop in total area under cultivation and production in the world. Maize is utilized as staple food by the lower strata of society and also used as a crop of par excellence for industrial use.

During the year 2019-2020, total world production of rice was 509.2 million tonnes, production of wheat was 761.5 million tonnes while that of maize was 1147 million tonnes (Anonymous, 2020). Since pre-historic times, maize has been basic food for majority of the people in Latin America, Mexico and Central America. In the world, important producer of the maize crop are USA (346 million tonnes), China (260.8 million tonnes), Brazil (102 million tonnes), European Union 27 (66.74 million tonnes), and India (26 million tonnes). USA ranks first in area (34 million ha), production (178 million tonnes) and productivity (5.4 t/ha) (Anonymous, 2020). Total cultivated area under maize in India is about 9.2 million hectares of total annual production of 27.8 million tones of grain and average yield of 3.02 tonnes per hectare which ranks third in production and contributes to about 2 per cent of the world production along almost 4 per cent share in world harvested area in the year 2018-19. In India, states *viz*., Karnataka, Andhra Pradesh, Maharashtra, Tamil Nadu, Rajasthan and Uttar Pradesh together contribute to 60 per cent of area and 70 per cent of maize production (Anonymous, 2020).

Maharashtra is emerging as a major maize growing state in the country, accounting for 10 per cent of total maize area with 0.92 million hectares equally contributing to the total maize production in the country. Maize cultivation reaches its boom in terms of acreage due adoption of modern crop production practices like, using chemical pesticides, chemical fertilizers and also

use of some commercial hybrids. These factors in various ways led the maize crop to become vulnerable to many pest and diseases at the farmers field. Among the different diseases infecting maize crop, foliar diseases are of significant importance and destroy the leaves of crop and result in great yield reduction.

Maize is prone to about 112 diseases in various parts of the world. These diseases caused by fungi, bacteria, viruses and nematodes, results in extensive damage and yield loss. In India, about 61 diseases have been reported which affects the maize crop (Payak and Sharma, 1985). On the basis of research studies conducted during the last few years under the All India Coordinated Maize Improvement Project, out of 61 diseases reported in country, about 16 diseases adversaly affecting maize crop have been recognized as major diseases. Among all the foliar diseases discovered in maize, the turcicum leaf blight (Northern corn leaf blight) caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs. (syn. *Heliminthosporium turcicum* Pass.) is of worldwide importance. Turcicum leaf blight is popularly known as Northern Corn Leaf Blight (NCLB) in the United States of America. Mild temperature and high humidity favoures the disease developement (Ullstrup, 1970). Environmental factors like heavy dews, frequent rains and cool temperature are favourable for disease development (Jordan *et al*., 1983). The disease and pathogen were first reported by Passerini in 1876 from Perma, Italy. In India, this disease was first recorded by Butler *et al*. (1920). The disease is more prevalent in the states *viz*., Andhra Pradesh, Himachal Pradesh, Karnataka and Maharashtra. Turcicum leaf blight is considered to be owe of the most destructive foliar diseases in Karnataka region and results in reduction of grain yield by 28 to 91 per cent (Harlapur, 2005). The pathogen causing turcicum leaf blight overwinters in the crop residues such as infected leaves, corn husks and other plant parts (Smith *et al*. 2004). The disease is known to affect crop from seedling stage up to harvest. Reduction in grain yield of crop is more if disease occurs at flowering, silking and grain filling stages of crop. The disease causes leaf necrosis and premature death of foliage which reduces the grain and fodder value of the crop. Symptoms of disease appear as a oval, water soaked, small spots on the leaves which gradually grow into long, elongate, elliptical, spindle shaped grayish green lesions ranging from 2.5 to 15 cm in length (Rai *et al*. 2010).

Use of fungicides for the control of plant diseases is a common practice. However, use of plant extracts in disease management is considered as eco-friendly, eliminating chances of environmental pollution and sustainable approach for disease management. Moreover, frequent use of fungicides in disease management has led to resistance development in various pathogens. No doubt in the past few decades fungicides have protected the plants from diseases, their continuous and over use resulted in some serious ecological problems, like residual effects, hazardous effects on beneficial organisms in soil and resistant strain development in pathogen. Now a days, some newer molecules are being available in market which are effective in disease

control and having less hazardous effect on environment. With this view present investigation was carried out to test newer fungicides, botanicals and bio-control agents against *Exserohilum turcicum* under laboratory conditions with the following objectives.

OBJECTIVES:

1. To isolate the pathogen causing Turcicum leaf blight in maize
2. To prove Koch's postulates
3. To study cultural and morphological characteristics of fungal pathogen
4. To test the efficacy of various botanicals, biocontrol agents and fungicides against the pathogen under *in vitro* conditions

2. REVIEW OF LITERATURE

2.1 Occurrence, Distribution and Losses

Northern leaf blight was first reported by Passerini on maize in Italy in 1876. Laxminarayana and Shankerlingam (1983) identified epidemic areas of Turcicum leaf blight in Karnataka, Maharashtra, Bihar, Andhra Pradesh and Uttarakhand.

Lal (1991) observed that disease is prevalent in the Himalayan region in *Kharif* season, in Peninsular India during *Kharif* and *Rabi* season and in plains of Eastern India particularly Bihar in *Rabi* season. Jha (1993) reported that due to epiphytotics in various parts of India severe losses in grain yield were observed and the losses ranges from 25 - 90 per cent depending upon severity of the disease.

Patil *et al*. (2000) observed that Turcicum leaf blight disease in maize can cause yield loss varying from 13.6 to 56.0 per cent depending on genotype. According to Harlapur (2005) reported tht among all the diseases infecting maize, Turciccum leaf blight can be recognized as one of the most destructive disease as its occurance and incidence results great significant reduction of grain yield by 28 to 91%.

Shivankar and Shivankar (2000) studied losses due to Turcicum leaf blight in grain yield of maize varieties under artificial inoculation and observed that losses in grain yield varied from 10.5 to 18.9 per cent.

Yeshitila (2003) reported yield losses up to 50 per cent especially when disease sets in early in the season. Babu *et al*. (2004) reported yield reduction of 83% during severe incidence of disease i.e. Turcicum leaf blight of maize in Uttaranchal, attaining epidemic proportion. Harlapue (2005) conducted survey in maize growing areas of Karnataka state and reported about 55.89% disease incidence.

2.2 Symptoms, isolation of pathogen and Koch's postulates

Ullstrup (1966) described the symptoms of the disease in United States . The disease is recognized by long elliptical grayish or tan lesions. When fully expanded, spots may be $1^{1/2} \times 6$ inches in size. These lesions appear first on the lower leaves and as the season progresses, the lesion number increases and all the leaves are covered. The plants look dead and gray. Richards and Kucharek (2006) observed the symptoms of *E. turcicum* as long elliptical lesions which firstly developes on the lower leaves and then progress upwards. The early symptoms of infection are noticed as a slightly oval, water-soaked small spots on the leaves. Later on, these spots grow into elongated, spindle shaped necrotic lesions. These lesions may appear first on lower leaves and increase in number as plant developes and this results in complete blighting of the foliage.

Harlapur *et al*. (2007) observed 16 isolates of *E. turcicum* for colony diameter, mycelial dry weight, sporulation and spore germination. Variation in the morphological and cultural

characters of 16 isolates of *E. turcicum* were observed. Harlapur *et al*. (2007) and Rashmi (2015) observed presence of different strains having virulence within *E. turcicum*. It was observed that highly virulent isolates exhibited higher infection types on host, whereas less virulent isolates were unable to cause more infection as compared to virulent isolates.

Ventura and Costa (2008) proved the Koch's postulates by transferring single hyphal tip of isolated fungal pathogen *Fusarium oxysporum* causing fusarium wilt disease of lettuce to carnation leaf agar. Geeta *et al*. (2019) investigated 32 isolate**s** of *Exserohilum turcicum* for morphological, cultural and pathogenic behaviour on diiferent host under identical conditions.

2.3 Cultural and Morphological Characters of Pathogen

Luttrell (1958) reported sexual stage of *Exserohilum turcicum* i.e. *Setosphaeria turcica* which is characterized by presence of ostiolate, dark brown coloured perithecia covered with short stiff spine like hairs on the upper third of the perithecial wall. The asci contain about one to six ascoaight having three to six septa and typically hyaline, whereas with aging they may become brown coloured and surrounded by a mucous like sheath.

Luttrell (1964) observed that the conidia of *Exserohilum turcicum* are 73-137 u long and 18-23 u wide with 4-9 septa and born singly at the tips of the conidiophores. Due to its protruding and truncate hilum *Exserohilum* can be separated from differential gramiicolous helminthosporoid genera. Mills (1970) noticed that *Cochliobolus sativus* consists of physiologic forms which differed in general appearance on cultural media.

Bolkvaldge (1977) reported that conidial formation in *Exserohilum turcicum* is associated with duration of dew, temperature and relative humidity. Increase in temperature accelerated the formation of conidiophores. Alcorn (1988) reported the conidia of *Helminthosporium* having strongly protruding hilum and are obclavate, fusoid, straight, or curved in shape. It was observed that conidia mainly germinate from one or both polar cells and rarely from intermediate cells. The conidiophores (7-11 × 165-283 u) are brown, irregularly cylindrical and septate having 3-7 septa. The conidiophores emerge in sets of two to six or more throught stomata or less frequently through the epidermis.

Aden (1991) studied the growth and sporulation of pathogen *Exserohilum turcicum* causing sorghum leaf blight disease on seven media (lactose casein hydrolysate agar, potato dextrose agar, sorghum leaf medium, sorghum leaf extract agar, maize leaf extract agar, maize grain extract agar and sorghum grain extract agar) at five different temperatures (15°C, 20°C, 25°C, 30°C, 35°C). The best temperature found for colony growth was 25°C (21mm), and for sporulation was 20°C (47,000 conidia/ml). Both colony growth and sporulation were found maximum between temperature of 20 and 30°C but was very poor at 35 and 15°C. At all temperatures studied, best medium for colony growth was lactose casein hydrolysate agar media (16.5 mm) and for sporulation best media found was sorghum leaf medium (53,000 conidia/ml).

The highest colony growth of pathogen after 12 days of incubation were noticed on lactose casein hydrolysate agar at 30°C (40 mm) followed by sorghum grain extract agar at 30°C (40mm), sorghum leaf extract agar at 25°C (38.5mm) and maize grain extract agar at 25°C (38.5mm).

Muiru *et al*. (2008) studied isolates of *E. turcicum* from different agro-ecological zones and observed that isolates showed differences in morphology, growth rate, sporulation rate and pigmentation in different media. The differential light regims had significant effect on both sporulation and growth rate of *E. turcicum* isolates. Type of media and incubation temperature also had a significant effect on growth rate of different isolates. The optimum temperature for growth of isolates was 25°C and among all isolates, only one isolate showed the minimal growth below 10°C and no mycelial growth was noticed in all the isolates at 40°C.

Harlapur *et al*. (2007) observed the growth of 16 isolates of *E. turcicum* and reported that among all isolates growth in five isolates, *viz.*, Et1, Et4, Et5, Et9 and Et11 was considered as profuse and fast growing. Excellent growth was obtained in two isolates that is Et2 and Et15. Growth of both Et6 and Et12 isolates was considered as good. Moderate growth was noticed in Et7, Et10, Et14 and Et16 isolates. Poor growth was observed in three isolates *viz*., Et3, Et8 and Et13. Maximum radial growth was showed by isolate Et1 with colony diameter of 87.33 mm followed by Et4, Et9 and Et15 (86.33 mm each). Minimum colony diameter of 52.00 mm was observed in Et13 isolate after 12 days of inoculation. Maximum dry mycelial weight of 315.34 mg was found in Et9 isolate followed by Et11 (314.67 mg) and Et4 (308.00 mg).

Harlapur and Kulkarni (2009) studied the conidial germination of *E. turcicum* [*Setophaeria turcica*], causing Northen leaf blight disease of maize at different incubation periods ranging from 4 to 36 h at an interval of 4 h. The maximum germination of conidia (94.20%) was seen after 36 h of incubation, while the minimum germination (7.67%) was observed after 4 h. More than 50% conidial germination was observed after 16 h of incubation. However, no significant increase in conidial germination was noticed from 28 to 36 h of incubation. Daniel and Narong (2006) investigated variations in morphological characters of different strains of *E. turcicum* and reported that conidia were elongated, blended and spindle in shape. The average conidial length and width were found to be 93.97 µm and 13.11 µm, respectively, while number of septations was found to range from 2-7.

Gowda *et al*. (2010) studied cultural and morphological variation of 13 isolates of *E. turcicum* causing turcicum leaf blight of maize. The cultural variability of isolates was carried out on five different solid media *viz*., Czapeck's dox medium, maize leaf extract medium, glucose peptone medium, potato dextrose agar medium and Richard's medium. Observations on the variation in mycelial weight, morphological characters and sporulation were recorded. The isolates collected from Almora, Bajaura and Nagenahalli were found to exhibit fastest growth

than other isolates. Isolates from Coimbatore and Udaipur were found to be slowest growing. The Nagenahalli, Almora, Hyderabad and Coimbtore isolates showed light brown to bright brown colored colony with compact growth, while the Jorhat, Jashipur and Udaipur isolates exhibited olive green colored colony. In general, most of the isolates showed better in respect of mycelia weight and sporulation

Bunker *et al*. (2011) studied cultural and morphological variability in five isolates of *Bipolaris maydis* from Rajasthan, Uttarakhand and Haryana and noticed variation in mean length and width of conidia in five isolates and it ranged from 55.02 to 81.80 µm and 12.45 to 16.70 µm, respectively.

2.4 Evaluation of the Botanicals, Bio-control Agents and Fungicides against Pathogen

2.4.1 Evaluation of Botanicals

Shekhavat and Prasad (1971) investigated antifungl properties of some plant extracts and reported about 100 per cent inhibition of mycelial growth and sporulation of *Helminthosporium* sp. in cold water extracts of *Allium cepa*, *Ocimum sanctum*, *Bougainvillea* sp., *Accia loculata*, and *Ficus religiosa*. Ahmed and Grainge (1982) studied 5280 plant species and reported that many of these plants possessed insecticidal, antiviral, fungicidal and antibacterial properties.

Girija Ganeshan and Jayachandra (1993) analyzed aqueous solution of parthenium (100-1000 ppm) isolated from *Parthenium hysterophorus* L. against *H. sativum* and observed that spore germination was reduced drastically. Meena and Mariappan (1993) tested some botanical extracts to know antifungal effect on seed borne mycoflora of sorghum *Curvularia lunata*. It was found that leaf extracts of neem, *Carthamus roseus* L. and *Lantana camara* L. were effective as compared to other plant extracts tested.

Ganguly (1994) reported that aqueous extracts of *Vinca rosea* L. leaves showed maximum inhibition of mycelial growth and spore germination of *H. oryzae* and *Pyricularia oryzae* followed by *Polyalthia longifolia* L. and *Azadirachta indica* Juss. when tested under laboratory condition.

Sharma and Jandaik (1994) and Sivakadadacham (1988) observed antifungal activity of extract of *Azadirachta indica* to have inhibitory effects on *Rhizoctonia solani*. Shivpuri *et al*. (1997) reported fungicidal property of extracts of some botanicals *viz*., *A. indica*, *Ocimum sanctum, Datura stramonium* and *Allis sativum*. Mistry and Vala (1998) conducted an experiment *in vitro* in which 19 plant extracts tested against *E. hawaiiensis.* It was reported that extracts of garlic, datura, eucalyptus, onion bulbs and soapnut inhibited the growth of the mycelium. Singh *et al*. (1998) evaluated leaf extracts of 10 plant species against *H. sativum*. They reported that *Azadirachata indica*, *Matricaria chamomilla* and *Mentha piperita* extracts inhibited growth under *in vitro* condition to the extent of more than 80 per cent. Meena *et al*. (2003) evaluated eight plant extracts *in vitro* and *in vivo* against banded leaf and sheath blight of

maize. The findings revealed that bulb extract of garlic (*Allium sativum*) at 5 per cent concentration completely inhibited mycelial growth of the fungus. The efficacy of all these plant extracts found to be increased when they were sprayed 24 hours before the inoculation.

Harlapur *et al*. (2007a) tested thirteen botanicals including *Allium sativum, Ocimum sanctum* and *Azadirachta indica* against *E. turcicum* causing turcicum leaf blight of maize under *in vitro* conditions. Significant reduction in the growth of *E. turcicum* was observed in respect of all the plant extracts tested. Neem seed kernel extract (NSKE) @ 5 per cent was highly effective showing significantly maximum inhibition of the test pathogen, onion bulb extract was also found effective in inhibiting the growth *E. turcicum*. The inhibitory action of NSKE may be due to azadirchitin which is present in seed kernel, it reduces the growth and activation of pathogen. The effectiveness of onion bulb extract may be due to presence of antifungal compounds such as carbohydrate propenyl sulphuric acid and cycloallin.

Ramegowda *et al*. (2007) evaluated seven plant extracts *in vitro* against *Alternaria macrospora* causing leaf spot of Bt-cotton. Higher mycelial growth inhibition (60%) was observed in garlic bulb extract followed by onion bulb extract (57.4%) at 7.5 per cent concentration. Satish *et al* (2007) investigated the antifungal activity in the leaf extract of *Datura stramonium* against various species of *Aspergillus*.

Hassanein *et al*. (2008) studied the efficacy of leaf extracts of chinaberry and neem against two tomato pathogenic fungi i.e. *Alternaria solani* and *Fusarium oxysporum* and he observed that all concentrations of liquid neem leaf extracts reduced the mycelia growth of the two test pathogens and effect was directly proportional to concentration and inhibition values were found higher for extracts of neem than those of chinaberry. Joseph *et al*. (2008) tested efficacy of different plant extracts *viz., A. indica, Eucalyptus globules, Ocimum sanctum, Artemessia annua* and *Rheum emodi in vitro* to control pathogen causing brinjal wilt and reported that all the plant extracts tested showed significant reduction in the growth of pathogen.

Ogbebor and Adekunle (2008) tested extracts of *Ageratum conyzoides, Azadirchta indica, Allium sativum, Ocimum basilicum* and *Jatropha curcas* for their botanical fungitixicants on *Drechslera heveae* and observed that extracts of *A. sativum, Ageratum conyzoides* and *O. basilicum* caused total conidial inhibition of the pathogen in liquid media. Prashanth *et al*. (2008) evaluated seven plant extracts *in vitro* against growth of pathogen (*Colletotrichum gloeosporoides*) causing pomegranate anthracnose They reported that mycelial growth inhibition was found more (93.3 and 88.1%) in eucalyptus leaf extract and garlic bulb extract respectively, at 10 per cent concentration. The next best treatments were Datura leaf extract (84.4%) and Tulasi leaf extract (84.4%). In Coat button leaf extract least inhibition of mycelial growth (52.2%) was observed.

Gawade and Suryawanshi (2009) evaluated five botanicals against *Colletotrichum*

truncatum causing soybean anthracnose. Among all the leaf extracts tested *in vitro*, neem was found highly effective with highest mean mycelial growth inhibition (72.56%) followed by parthenium (61.3%), mehendi (46.03%) and bogunveilia (28.98%). Least mycelial inhibition (9.99%) was observed in leaf extract of eucalyptus. Panchal and Patil (2009) tested nine phytoextracts *in vitro* against *Alternaria alternata* and observed garlic clove extract to be most effective followed by turmeric rhizome extract, neem leaf extract, ginger rhizome extract and dhatura leaf extract. Mesta *et al*. (2009) found that neem leaf extract and garlic bulb extract were effective against *Alternsris helianthi* causing sunflower blight.

Gachande *et al*. (2009) reported fungicidal properties of extracts of some botanicals *viz*., *A. sativum, A. vasica, A. indica, O.sanctum, Datura stramonium* and *Zingiber officinale* against *Alternaria solani*. Choubey and Patil (2009) evaluated nine plant extracts to know their efficacy for controlling Phomopsis fruit rot of aonla under *in vitro* conditions. Least mycelial growth was observed in garlic clove extract followed by ardusi and neem leaf extracts.

Dubey *et al*. (2010) evaluated five botanicals *viz*., neem leaves, onion, garlic, Ashok (*Polyalthia longifolia*) and tulsi leaves at 5% concentration for their efficacy against *Alternaria alternata* and reported that all the botanicals were significantly superior over the control in reducing the disease severity. Tandel *et al*. (2010) tested the plant extracts of eleven plant species *in vitro* against leaf blight of green gram and reported that maximum inhibition (98.14%) was observed in extracts of onion bulb followed by extracts of acacia, ginger, neem, garlic and karanj.

Dutta and Kalha (2011) evaluated nine phytoextracts under *in vitro* conditions against pathogen *Rhizoctonia solani* causing sheath blight of rice. *Melia azedarach* extract was found most effective with 46.5 per cent of mycelial inhibition, followed by *Cannabis sativa* (29.7%), *Allium cepa* (25.4%), *Ocimum tenuiflorum* (23.9%), *Aegle marmelos* (20.6%), *Lantana camara* (17.9%), *Murraya koenigii* (14.1%), *Parthenium hysterophorus* (13.4%) and eucalyptus (10.4%). Khedekar *et al*. (2012) studied the efficacy of some botanicals against *E. turcicum* by poisoned food technique under *in vitro* conditions and observed that Nimbicidin @ 5 per cent concentration was superior to all other treatments with the highest mycelial inhibition of 6.85 per cent followed by negunda (38.88%) and neem seed kernel extract (37.01%).

Kumar and Mauriya (2015) tested phytoextracts against *E. turcicum* and reported that all plant extracts inhibited mycelial growth of *E. turcicum*. Neem (*Azadirachta indica*) seed kernel extract @ 10% was found effective in reducing the growth of pathogen. Foliar spray of neem seed extract was also found most effective among all plant extract showing the minimum disease severity (25.4%) and maximum grain yield (24.1q/ha). While the *Eucalyptus citriodora* at 10% concentration was found less effective as compared to *Azadirachta indica* and *Ocimum sanctum* in inhibition of mycelial growth of *E. turcicum* and produced 72.8% lower grain yield as

comparision to *Azadirachta indica*. Manu *et al*. (2017) found that among the all plant extracts tested against *E. turcicum*, garlic bulb extract showed maximum inhibition of mycelial growth of pathogen.

2.4.2 Evaluation of Bio-control Agents

Biles and Hill (1988) reported that *Trichoderma harzianum* was antagonistic to *H. sativum* by reducing the sporulation capacity of pathogen. Ray *et al.* (1990) found that rice plants when treated with bacterial cell suspension of *Pseudomonas fluorescens* were found to be effective in reducing the growth of pathogen i.e. *D. oryzae*. Bopaiah *et al*. (1991) demonstrated that *A.fumigatus, Aspergillus niger* and *Penicillium islandicum* isolated from the phyllosphere of sorghum were antagonistic to *H. oryzae*. Growth of pathogen may have been reduced due to production of some antimicrobial compounds or due to competition for nutrients.

Mahmood *et al*. (1995a) reported that *Aspergillus* species and unidentified isolate No. 35 were most effective to the cultures of *Helminthosporium turcicum* causing leaf blight of maize. Mahmood *et al*. (1995b) found that *Trichoderma sp*., *Aspergillus sp*., *Arachniotus sp*., *Acremonium sp*., *Cladosporium sp*, and some unidentified fungi reduced the spore germination of *Helminthosporium turcicum.* Ramchandra (2000) evaluated antagonists against *E. hawaiiensis* causing leaf blight of Dicoccum wheat under *in vitro* and reported that *T. harzianum* and *T. viride* reduced the growth and sporulation significantly.

Biswas *et al*. (2000) reported that *Chaetomium globosum* was antagonistic to *Drechslera sorokiniana* through antibiosis. Harlapur *et al.* (2007a) tested eight bio-control agents under *in vitro* condition against *E. turcicum* to study their antagonism by using dual culture method. All the tested antagonistic fungi and bacteria were found to inhibit the growth of *E. turcicum* ranging from 19.30 to 65.17 per cent. Maximum mean per cent inhibition of mycelial growth was by *Trichoderma harzianum* (65.17%) followed by *T. viride* (56.95%) and *Bacillus subtilis* (49.57%), while *Psuedomonas fluorescens* was found to be least effective (19.30%).

Ramegowda *et al*. (2007) tested four bioagents, both indigenous and exogenous strains, against *Alternaria macrospora* causing leaf spot in cotton. Maximum inhibition was observed in *T. viride* (E) (62.3%) followed by *T. harzianum* (E) (60.3%). *T. harzianum* (I) and *T. viride* (I) inhibiting mycelial growth by 59.7 and 59.4 per cent respectively. The least inhibition of growth to the extent of 22.0 per cent was observed in *Bacillus subtilis* (PGPR). Prashanth *et al*. (2008) evaluated four bio-agents under *in vitro* conditions against *Colletotrichum gloeosporioides* causing antracnose of pomegranate. *T. viride* was found to be the best in inhibiting the mycelial growth of *C. gloeosporioides* with 79.1 per cent inhibition followed by *T. harzianum* (62.7%) and *P. flourescens* (54.7%). The least per cent inhibition of mycelial growth was noticed in *B. subtilis* (34.8%).

Gawade and Suryawanshi (2009) studied two bioagents against *Colletotrichum*

truncatum causing soybean antracnose. *T. viride* and *Verticellium lecanii* recorded mean mycelial growth inhibition of 41.79 and 23.75 per cent, respectively. Datta and Kalha (2011) tested three bio-agents *viz*., *T. viride*, *T. harzianum* and *P. flourescens* under *in vitro* conditions against *Rhizoctonia solani* causing sheath blight of rice and reported that isolates of *T. viride* were found most effective (55.5- 72.7% mycelial inhibition) than the isolates of *T. harzianum* (47-52.3% mycelial inhibition) and *P. flourescens* (19.1-31.8% mycelial inhibition).

Bhati and Singh (2012) in an experiment studied the antagonistic property of fluorescent *Pseudomonas* against *E. turcicum*, characterized by biochemical tests and 16S rDNA sequences. MBLK1, MBLK3, MBLK6, MBLK15, MBLK17, and MBLK22 bacterial strains out of 33 isolates were found to be antagonistic against *E. turcicum*. Khedekar *et al*. (2012) evaluated six bio-control agents under *in vitro* conditions against *E. turcicum* to test their antagonistic activity by using dual culture method. All the tested antagonistic fungi and bacteria inhibited the growth of pathogen i.e. *E. turcicum* ranging from 35 to 70 per cent. *T. harzianum* was found to be superior among all treatments showing 70.33 per cent mycelial inhibition followed by *T. viride* (62.93%), *T. konigii* (61.43%) and *T. virens* (59.23%). Least mycelial inhibition was observed in bacterial antagonistic organisms like *P. flourescens* with 35.13 per cent followed by *B. subtilis* with 39.20 per cent.

Azad *et al*. (2013) studied the effect of bio-control agents on pathogenicity of *C. gloeosporoides* and its potential to control fruit rot disease of banana. Addition of bio agents reduced radial growth, spore production, spore germination and appressoria production of *C. gloeosporoides*, *in vitro* by dual culture method. Among three bioagents *viz*., *T. harzianum, T. viride* and *T. virens*, *T. harzianum* was found to be the best to reduce the radial growth of *C. gloeosporoides* followed by *T. viridae*.

Singh and Singh (2014) evaluated antagonist potential of seventeen isolates of *Trichoderma harzianum* (Th 1, 2, 3, 4, 5, 6, 9, 12, 13, 14, 19, 22, 31, 32, 37, 39, 43) and ten isolates of *Pseudomonas fluorescens* (Psf 2, 3, 4, 12, 18, 25, 27, 82,101 and Psf Pant) against *Exserohilum turcicum in vitro* using dual culture technique. Th-39 and Psf-82 isolates gave maximum inhibition of mycelial growth of the pathogen by 77.11 and 56.00 per cent, respectively.

Vishwanath *et al*. (2017) studied the effect of bioagent *T. harzianum* against *Exserohilum turcicum* and it was found effective in inhibiting myclial growth of pathogen (64.1%) followed by *Trichoderma viride* with inhibition 58.7%. *T. harzianum* at the rate of 2% showed 32.3% disease reduction, where as *T. viride* at the same rate showed 29.2% disease reduction in field conditions.

Manu *et al*. (2017) tested antagonistic microorganisms *viz*. *Trichoderma harzianum* Rifai-1, *Trichoderma harzianum*-2, *Trichoderma viride* Pers. Ex. S.F. Gray, *Pseudomonas*

fluorescens Migula-1 and *Pseudomonas fluorescens* Migula -2 by dual culture technique for their antagonistic effect against *Exserohilun turcicum* under *in vitro* conditions. *Trichoderma harzianum*-2 showed maximum mycelia growth inhibition (98.65%) followed by *Trichoderma viride* (98.34%).

2.4.3 Evaluation of Fungicides

Cox (1956) reported that mancozeb formulations were most effective in minimizing the *H. turcicum* severity under field conditions followed by ziram, vacide and Z-65. These fungicides also increased the grain yield and seed quality. Sohi *et al*. (1965) evaluated six fungicides for the control of leaf blight on the maize hybrid Ganga-5 and observed that zineb was more effective in controlling disease with an increase in grain yield of 15.18 per cent followed by captan 10.19 per cent over check plots.

Kumar and Gupta (1977) reported that among eight fungicides tested, dithane M-45, unizeb and dithane Z-78 significantly reduced the maize leaf blight severity by 55, 47.4 and 44.43 per cent and increased grain yield by 8.54, 10.12 and 9.90 per cent respectively. Issa (1983) in Brazil reported that mancozeb @ 2 kg/ha as foliar spray was found to be effective in minimizing turcicum leaf blight severity in maize and also resulted in improved yield over untreated plots. Bowen and Pederson (1988) observed that three sprays of propiconazole at weekly interval were found to be effective in minimizing the rate of Turcicum leaf blight development in maize.

Kachapur and Hegde (1988) evaluated seven fungicides and revealed that mancozeb and captafol were the most effective fungicides in management of Turcicum leaf blight of maize caused by *E. turcicum*. Sharma and Mishra (1988) reported that infection of *E. turcicum* on maize was effectively controlled by 6 sprays of mancozeb at 0.2 per cent at 10 days interval starting from three days after inoculation at 30 days after sowing. Singh and Kaiser (1989) reported that fungicides bavistin and carboxin (vitavax) completely inhibited the mycelial growth and conidial germination of *E. turcicum*, which causes leaf blight of maize.

Sakhi *et al*. (1991) evaluated fungicides *in vitro* against *Helminthosporium turcicum* and revealed that, mycelial growth and conidial germination were completely inhibited by propineb even at low concentration of 10 ppm, whereas chlorothalonil and pyrifenox completely inhibited mycelial growth at 40 ppm, benomyl inhibited mycelial growth at 20 ppm. Raid (1991) studied efficacy of three fungicides i.e. mancozeb, propiconazole and chlorothalonil for the control of northen leaf blight and rust in maize crop and found that all fungicides could reduce disease severity.

Begum *et al*. (1993) tested five fungicides for control of artificial infections of *Exserohilum turcicum* on susceptible maize cultivars and reported that all the fungicides reduced

disease intensity and increased the grain yield with mancozeb being distinctly the most effective, followed by carbendazim, zineb, thiophanate methyl and lastly copper oxychloride.

Rahman *et al*. (1993) reported that propiconazole was effective against *E. turcicum* causing turcicum leaf blight in maize under *in vitro* conditions. Meli and Kulkarni (1994) evaluated ten fungicides against *E. hawaiinesis* causing leaf blight of wheat crop and reported that propiconazole gave complete inhibition followed by tridemorph.

Patil (2000) tested some systematic and non systematic fungicides *in vitro* against *E. hawaiiensis* causing leaf blight of wheat and reported that propiconazole, hexaconazole, tridemorph and difenconazole showed 100 per cent inhibition. Singh and Gupta (2000) studied the bioassay of fungicides against *Dreschlera sativus* causing leaf blight of wheat. Propiconazole was found to be most effective fungicide in inhibiting mycelial growth followed by dithane-M-45. Dharanendraswamy, (2003) reported carboxin and zineb were highly effective in inhibiting mycelia growth of *E. turcicum* causing leaf blight of maize.

Harlapur (2005) reported that corboxin (2 g/kg seed) as seed treatment and two sprays of mancozeb @ 0.25 % reduced leaf blight disease of maize caused by *E. turcicum*. Schwartz and David (2005) studied life cycle and seasonal history of Helminthosporium leaf blight and reported that fungicides for the management of the leaf blight were chlorothanil, propiconazole, EBDC and azoxystrobin.

Gonzalez *et al.* (2007) studied the efficacy of 75% chlorothalanil, 50% carbendazim, 25% azoxystrobin, 12.5% epoxiconazole, 9.45 flutriazole + 20% carbendazim, 0.5% flusilazole + 1% carbendazim, and 16% cyproconazole + 30% carbendazim against the turcicum leaf blight and found that 0.5% flutriazole + 1% carbendazim was most effective.

Harlapur *et al*. (2007a) tested twenty-three fungicides consisting of eleven systematic, ten non-systematic and two combination products for their efficacy against *E. turcicum* causing turcicum leaf blight of maize, under *in vitro* conditions. The systematic fungicides were tested at 0.1 per cent concentration and rest of the fungicides at 0.25 per cent concentration. It was reported that, maximum mean per cent inhibition of mycelial growth of *E. turcicum* was observed in mancozeb 0.25 per cent (100%) followed by carboxin power 0.1 per cent (99.16%) and propiconazole 25 EC @ 0.10 per cent (99.10%).

Ramegowda *et al*. (2007) tested five systematic and seven non systematic fungicides *in vitro* against *Alternaria macrospora*, causing leaf spot of Bt-cotton. Among systematic fungicides tridemorph and difeconazole at 0.075, 0.05 and 0.025 per cent concentrations and among non systematic fungicides iprodione and mancozeb at 0.15, 0.1 and 0.5 per cent concentration were found effective in inhibiting mycelia growth of *A. macrospora*.

Prashant *et al*. (2008) evaluated four systematic and four non systematic fungicides *in vitro* against *Colletotrichum gloesporioides*, the causal agent of anthracnose of pomegranate.

carbendazim, difeconazole and combination product, carbendazim + mancozeb were most effective in reducing the mycelial growth of pathogen at higher concentration (0.1%). Gawade and Suryawanshi (2009) tested five fungicides against *Colletotrichum tancatum* causing soybean antracnose at 100, 150 and 200 ppm each. They observed that carbendazim recorded highest mean inhibition (90.59%) of mycelia growth, followed by propiconazole (87.95%), hexaconazole (86.15%), difeconazole (84.71%) and chlorothalonil (70.23%).

Datta and Kalha (2011) studied five fungicides under *in vitro* conditions against *Rhizoctonia solani* causing sheath blight of rice crop @ 10, 25, 50, 100, 200 and 500 ppm each. Combination product mancozeb 63% + carbendzim 12% was the most effective fungicide which recorded maximum per cent inhibition even at 10 ppm followed by carbendazim (98.9%), vitavax (98.2%), propiconazole (74.8%) and hexaconazole (72.9%).

Reddy *et al*. (2013) evaluated seven fungicides *in vitro* against *E. turcicum* causing leaf blight of maize. Among the all treatments mancozeb (0.25%) and carbendazim + mancozeb (0.25%) were significantly superior over other treatments and can be recommended for the disease management under field conditions. The treatment mancozeb (0.25%) and combination treatments of carbendazim and mancozeb (0.25%) recorded the lowest percent disease index reducing the disease by 73.0% and 72.1% respectively. The treatment which had combination metriram + pyraclostrobin 0.3% was found to be the next best treatment in reducing the disease by 61.5% with PDI of 14.6 following propiconazole with PDI of 18.6.

Wathaneeyawech *et al*. (2014) evaluated three chemicals *viz*., mancozeb, chlorothanil and difeconazole at three concentrations i.e., half lower than recommended rate, recommended rate and half higher than recommended rate to study their efficacy to inhibit growth of isolates MHP5, TN3, MJ4, JT4 and JT5 of *E. turcicum* using poisoned food technique. It was revealed that the two contact fungicides, chlorothanil and mancozeb at three different concentrations gave complete (100%) growth inhibition to all five isolates. Whereas, difeconazole showed 100 per cent growth inhibition in isolates MHP5, TN3 and MJ4 at the all three concentrations, but gave 90 per cent inhibition in JT4 and about 94-96 per cent in JT5.

Kumar *et. al.* (2015) reported that among the six fungicides tested, zineb 75 WP @ 0.25% concentration was most effective in inhibiting the growth of *E. turcicum*, low in disease severity and ultimately produced higher grain yield of maize. Mancozeb 63% + carbendazim 12% @ 0.25% was found equally effective. Wani *et al*. (2017) studied integrated management of turcicum leaf blight of maize. It was reported that seed treatment with mancozeb 75 WP @ 0.25 per cent followed by two foliar sprays with propiconazole 25 EC @ 0.1 per cent at 40 and 50 days after sowing was most effective treatment with minimum disease intensity of 3.57 per cent and maximum grain and stover yield of 59.95 q/ha and 15.57 t/ha, respectively.

Manu *et al.* (2017) reported that among the systematic fungicides, tebuconazole completely inhibited the growth of pathogen at all the concentrations tested. In contact fungicides, Propineb was found highly effective as it inhibited the *E. turcicum* up to 83.89 per cent at 500 ppm and among combination products, only carbendazim 12% + mancozeb 63% at 500 ppm showed complete inhibition of mycelial growth of *E. turcicum* at higher concentration.

3. MATERIALS AND METHODS

The present research work entitled "**Evaluation of Botanicals, Bio-control Agents and Fungicides against Turcicum Leaf Blight of Maize."** was undertaken in the Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri. The different botanicals, bio control agents and fungicides were assessed for their antifungal activities against *Exserohilum turcicum*. Details of material required and methods followed during the period of investigation are described briefly in the succeeding paragraph.

3.1 Materials

3.1.1 Source of *Exserohilum turcicum* Culture

The disease samples of Turcicum leaf blight were collected from experimental fields of AICRP on maize, Mahatma Phule Krishi Vidyapeeth, Rahuri. The fungus was isolated from these samples on Potato Dextrose Agar (PDA) medium.

3.1.2 Glassware

Standard glasswares, *viz*. Petri plates, test tubes, conical flasks, spirit jar, measuring cylinder, volumetric flasks, beakers, glass rod, pipettes of 0.1 ml and 1 ml, microscopic glass slides, cover-slips etc. available at Department of Plant Pathology and Agricultural Microbiology, MPKV, Rahuri were used.

3.1.3 Seed

For pathogenicity test , seed of maize inbred line CM-202 was obtained from the AICRP on maize, Mahatma Phule Krishi Vidyapeeth, Rahuri.

3.1.4 Culture Media

Culture media used during the investigation was as below with their composition.

3.1.4.1 Composition of potato dextrose agar medium

Peeled Potato	200 g
Dextrose	20 g
Agar-Agar	20 g
Distilled water (Make up volume)	1000 ml

Used for isolation of *Exserohilum turcicum.*

3.1.4.2 Solid media for cultural study of pathogen

The growth characters of the fungus were studied on eight solid media as mentioned below,

1. Czapeck's Dox agar
2. Richard's agar
3. Malt extract agar
4. Potato dextrose agar
5. Subouraud's agar
6. Yeast extract agar

7. Oat meal agar
8. Corn meal agar

Composition of different media

1. Czapeck's dox agar

Dipotassium dihydratin phosphate (K_2HPO_4)	1.1009 g
Ferrous sulphate ($FeSO_47H_2O$)	0.01 g
Magnesium sulphate ($MgSO_47H_2O$)	0.05 g
Potassium chloride (KCL)	0.5g
Sodium nitrate ($NaNO_3$)	3 g
Sucrose ($C_{12}H_{12}O_{11}$)	30 g
Agar agar	20 g
Distilled water	1000 ml

2. Richard's agar

Ferric chloride ($FeCl_36H_2O$)	0.02 g
Magnesium sulphate ($MgSO_47H_2O$)	2.5 g
Potassium dihydrogen phosphate (KH_2PO_4)	5 g
Potassium nitrate (KNO_3)	10 g
Sucrose ($C_{12}H_{12}O_{11}$)	50 g
Agar agar	20 g
Distilled water	1000 ml

3. Malt extract agar

Malt extract	20 g
Agar agar	20 g
Distilled water	1000 ml

4. Potato dextrose agar

Potato	200 g
Dextrose	20 g
Agar agar	20 g
Distilled water	1000 ml

5. Sabouraud's agar

Dextrose	20 g
Peptone	10 g
Agar agar	20 g
Distilled water	1000 ml

6. Yeast extract agar

Soluble starch	10 g

Yeast ectract	1 g
Agar agar	20 g
Distilled water	1000 ml

7. Oat meal extract agar

Oat meal flakes	30 g
Agar agar	20 g
Distilled water	1000 ml

8. Corn meal agar

Corn meal extract	2 g
Agar agar	15 g
Distilled water	1000 ml

3.1.5 Experimental Site

The present experiments were carried out at Department of Plant Pathology and Agril. Microbiology, MPKV, Rahuri (19.3492^0N, 74.6461^0E).

3.1.6 Laboratory Equipments

The common laboratory equipments such as autoclave, Laminar air flow, microwave oven, BOD Incubator, refrigerator, microscope, centrifuge, camera, bacterial needle, weighing balance, spirit lamp, etc. were used.

3.1.7 Miscellaneous Materials

Polythene paper bags, Cotton wool, Blotter paper, Labels, Permanent marker, Sterile water, Micro pipettes of variable capacity (0.5-10 µl, 20-100 µl, 100-1000 µl), Tips of variable capacity (0.5-10 µl, 20-100 µl, 100-1000 µl), Test tube stand, Rubber band etc. were used as and when required during present investigation.

3.1.8 Bio-control Agents

Cultures of following bio-control agents were obtained from Department of Plant Pathology and Agricultural Microbiology. Following bio-control agents are used for present study.

1. *Trichoderma asperellum*
2. *Trichoderma harzianum*
3. *Trichoderma hamatum*
4. *Trichoderma konigii*
5. *Pseudomonas fluorescence*
6. *Bacillus subtilis*

3.1.9 Chemicals

The following chemicals are used for present study.

3.1.9.1 Fungicides

Sr.No	Fungicides	Trade Name
1	Mancozeb 75% WP	Dithane M-45
2	Kresoxim-methyl 44.3% SC	Ergon
3	Propiconazole 25% EC	Tilt
4	Azoxystrobin 18.2% w/w + Cyproconazole 7.3% w/w SC	Ampect^{TM}Xtra
5	Azoxystrobin 18.2% w/w + Difeconazole 11.4% w/w SC	Tag minister
6	Pyraclostrobin 133g/l + Epoxiconazole 50g/l SE	Opera
7	Carbendazim 12% + Mancozeb 63% WP	Saaf

3.1.9.2 Plant extracts

The aqueous extracts of eight plant species were analyzed for their antifungal activity under laboratory condition. The details of plant species used for extract preparation are as below

Sr. No.	Botanical name	Common name	Plant part used
1	*Azadiracta indica*	Neem	Leaves
2	*Lantana camara*	Ghaneri	Leaves
3	*Ocimum tenuiflorum*	Tulasi	Leaves
4	*Millettia pinnata*	Karanj	Leaves
5	*Vitex negundo*	Nirgudi	Leaves
6	*Allium cepa*	Onion	Bulb
7	*Parthenium hysterophorus*	Parthenium	Leaves
8	*Senna auriculata*	Tarvad	Leaves

3.2 Methods

3.2.1 Sterilization

Media, cotton wool etc. were sterilized in autoclave at 15 lbs pressure (121.6°C) for 15 min by following standard procedure. The glasswares were sterilized in hot air oven at 200°C for two hrs.

3.2.2 Isolation of *Exserohilum turcicum*

The diseased leaves showing typical symptoms were collected from experimental field of AICRP on maize, Mahatma Phule Krishi Vidyapeeth, Rahuri. The fungus was isolated by tissue

isolation technique. These leaves were first washed with tap water followed by sterile distilled water. The infected leaves were cut with the help of sterilized razor blade into several small sections of 1-2 mm square from the margin of the infected lesion to contain both diseased and healthy tissue. Then the bits were placed in surface sterilizing agent's solution (0.1% $HgCl_2$) for about 15-30 seconds. The sections were then rinsed thrice in sterilized distilled water to remove the traces of $HgCl_2$ solution, the sections were taken out aseptically with forceps and blotted dry on clean, sterile blotter paper and sections were transferred aseptically to petriplates containing potato-dextrose agar (PDA) medium and were incubated at 25±1°C in incubator.

3.2.3 Purification

The fungus isolated from the infected tissue was further subcultured by single spore isolation method. Spores were harvested from 10 days old culture of *E. turcicum* with the help of an inoculation needle and suspended in 1 ml of sterile distilled water in a culture tube. Then suspension was subjected to low speed centrifugation for making a uniform spore suspension. Concentration of the spores was adjusted by adding sterile distilled water. One ml of the spore suspension was taken and then it was uniformly spread on 2 per cent solidified water agar plates. The plates were incubated at 25±1°C for 12 hours. A single germinating spore was located by observiing the plate in an inverted position under compound microscope. Correspondingly a circle was drawn around the spore over the plate. A circular disc of the medium containing spore was picked up by using a sterile cork borer and transferred aseptically to petriplates containing PDA medium and incubated at 25±1°C for further growth.

3.2.4 Identification of the Fungure

In order to prove the identity of the fungus, conidia were observed under the high power (40X) microscope from both isolated culture and the infected leaves of maize plant.

3.2.5 Pathogenicity Test

3.2.5.1 Inoculation

Inoculation was undertaken to prove the pathogenicity of isolated pathogen. Seeds of maize from disease free plants of the previous season were surface sterilized with 0.1 per cent $HgCL_2$ for one minute and sown in the pots containing sterilized soil in order to raise healthy seedlings. The pathogen was multiplied by transferring a loopful of the stock culture to 250 ml of potato dextrose broth taken in 1000 ml flask. The inoculated flask was incubated at 25±1°C for seven days. The fungal culture growing on potato dextrose broth was passed through double layered muslin cloth. The spore suspension of pathogen was collected under aseptic condition in an automizer. Spore suspension was collected separately in an automizer and then sprayed on to the foliage at three to four leaf stage to susceptible maize genotype CM-202. Inoculated seedlings were covered with polythene bags to create required humidity for 24 hrs. The seedlings after spray inoculation were

kept in green house condition. Periodical observations were made to see the development of typical brown spots on the inoculated plants.

3.2.5.2 Re-isolation

The organism was re-isolated from artificially inoculated leaves of maize plants showing typical symptom of disease. The re-isolation was carried out on PDA medium and culture obtained from reisolation was purified and transferred on PDA slants.

3.2.5.3 Identification of culture

The reisolated culture was observed under microscope and compared with original culture.

3.2.6 Morpho-cultural Studies

The pathogen was identified on the basis of morpho-cultural characteristics. Sterilized petriplates containing equal quantity (20 ml) of PDA medium were inoculated with 5 mm culture disc of 10-15 days old culture of pathogen and incubated at 25±1°C. Various morphological traits both macroscopic (colony color, colony growth and colony type) and microscopic (shape and septation) were recorded.

3.2.6.1 Cultural characteristics of *E. turcicum* on different culture media

Pure culture of isolate was used to study the variation in growth of the pathogen on different nutrient media. Seven solid media *viz*., potato dextrose agar (PDA), malt extract agar (MEA), corn meal agar (CMA), Richard's agar (RA), Czapeck's dox agar (CDA), oat meal extract agar, yeast media and sabouraud agar (SA) were used to study the best nutrient medium for the radial growth of the test pathogen. Media were prepared and sterilized by autoclaving at 1.05 kg/cm^2 pressure (121.6°C) for 20 minutes. Each medium was poured in petridishes containing equal quantity and were inoculated with 5 mm culture discs taken from the 7 days old culture of the pathogen. Three replications were kept and the plates were incubated at 25±1°C. Radial mycelial growth of the colonies obtained after 7 days of inoculation on each culture medium were recorded and analyzed statistically to know the most suitable solid media for the growth of fungus. The colony characters of pathogen *viz*., shape and colour on each media were recorded.

3.2.7 *In vitro* Evaluation of Plant Extracts against *E. turcicum*

3.2.7.1 Preparation of aqueous plant extracts by crude extraction method

The crude extracts of eight plant spp. were prepared from their plant parts. Plant parts used for extract preparation (leaves, bulb) were collected and thoroughly washed with tap water and air dried. Ten gm of plant material was weighed and then crushed in mortar and pestle by adding 10 ml of sterile water (1:1 w/v). The macerated material was then filtered through muslin cloth and subsequently through Whatman No. 40 filter paper. The supernant was taken as

standard plant extract solution (100%). Further extract was diluted by adding sterilized distilled water to get 5, 10 per cent concentration.

3.2.7.2 *In vitro* evaluation of plant extracts against *Exserohilum turcicum*

The aqueous plant extracts were evaluated at 5 and 10 per cent concentration by poisoned food technique for their efficacy in inhibiting the mycelial growth of turcicum leaf blight pathogen i.e. *Exserohilum turcicum*. The plant extracts were tested at two concentrations i.e. 5 and 10% concentration and this was achieved by adding 5 ml and 10 ml supernatant of each plant extract into 95 ml and 90 ml of PDA media in conical flasks, respectively and such conical flasks containing PDA (food) and plant extract (poison) were sterilized by autoclaving at 15 psi for 20 minutes (Sood and Dohroo, 2003). Twenty ml PDA containing plant extract were poured into sterilized petriplate under aseptic conditions and allowed to solidify. PDA plates containing plant extracts were inoculated aseptically in the center with 5 mm disc of 10 days old younge sporulating culture of *Exserohilum turcicum.* Three replications were maintained for each treatment. Petri plates without any plant extract and inoculated with pathogen served as control. All the inoculated petri plates were incubated at 25±1°C in the laboratory. The radial growth of test fungus in the treated plates was measured when pathogen growth in control touched the edges of petri dishes. The per cent inhibition of fungal growth was evaluated by using the formula given by Vincent (1927).

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

Where,

I = Per cent inhibition

C = Colony diameter in control

T = Colony diameter in treatment

3.2.8 *In vitro* Evaluation of Bio-control Agents against *Exserohilum turcicum*

Bio-control agents were evaluated for their antagonistic potential against pathogen using dual culture technique (Morton and Stroube, 1955). Twenty ml of PDA per petri plate was poured and allowed to solidify. Five mm discs of *E. turcicum* taken from seven days old culture was placed at one end of petri plate and respective antagonistic organism (5mm) was inoculated at the opposite side of the petri plate. The test pathogen being relatively slow growing was incubated 48 hours prior to *Trichoderma* spp. to cope up with the fast growth of *Trichoderma* spp. In case of bacterial antagonist *E. turcicum* was placed at the one end of petri plate and the bacterial culture was streaked at the other half of the petri plate. Three replications were maintained for each treatment. Control having test pathogen only was kept for comparision. Petri plates were incubated for seven days at 25±1°C. The activity of antagonistic organisms was recorded after 96 hours by measuring colony diameter of test pathogen i.e. *E. turcicum* in each

treatment and compared with control. Per cent growth inhibition of the test pathogen over control was calculated according to the formula given by Vincent (1927).

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

Where,

I = Per cent inhibition

C = Colony diameter in control

T = Colony diameter in treatment

3.2.9 *In vitro* Evaluation of Fungicides against *Exserohilum turcicum*

Seven fungicides consisting of two systematic *viz*., kresoxim-methyl 44.3 SC, propiconazole 25 EC; one non systematic viz., mancozeb 75 WP and four combo- products *viz*., azoxystrobin 18.2 W/W + cyproconazole 7.3 W/W, azoxystrobin 18.2 W/W + difenoconazole 11.4 W/W, pyraclostrobin 133g/l + epoxiconazole 50 g/l and carbendzim 12 + mancozeb 63 WP were assayed for their efficacy against *E. turcicum* under *in vitro* condition. Poisoned food technique was adopted for *in vitro* testing of fungicides (Nene and Thapliyal, 1979). The calculated amount of fungicide was thoroughly mixed in the medium before pouring into the petri plates so as to get the desired concentration of active ingredient of each fungicide separately. Twenty ml of fungicide amended medium were poured in each of 90 mm sterilized petri plates and allowed to solidify. The plates were inoculated centrally with five mm disc of ten days old young sporulating culture of *E. turcicum*. Control plates without fungicides were also maintained. For each treatment three replications were maintained. The inoculated petriplates were incubated at 25±1°C in the laboratory. The colony diameter was measured when fungus touched the periphery in control plates. Per cent inhibition of growth were calculated by using formula given by Vincent (1927).

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

Where,

I = Per cent inhibition

C = Colony diameter in control

T = Colony diameter in treatment

3.3 Statistical Analysis

The results of all the experimental treatments were statistically analysed by using the appropriate statistical methods (Panse and Sukhatme, 1978).

4. RESULTS AND DISCUSSION

Maize (*Zea mays* L.) is an important cereal crop of India. Among the foliar diseases affecting maize crop, the Turcicum leaf blight (Northern corn leaf blight) is of worldwide importance. The diseases causing pathogen is identified as *Exserohilum turcicum*. Turcicum leaf blight is resulting in reduction of grains yield by 28 to 91 per cent. The pathogen developed resistant to commonly used fungicides. Therefore, attempt was carried out to find out eco-friendly management of the disease with different botanicals, biocontrol agents and newer fungicides. The results obtained during the course of research study on different aspects are elaborated as follow.

4.1 Symptoms, Isolation and Identification of Turcicum Leaf Blight Pathogen

The diseased leaves exhibiting typical symptoms of Turcicum leaf blight (Plate 1) were collected and brought to laboratory and isolation of fungal pathogen was carried out on PDA medium. Pure culture of the isolated fungus was obtained by single spore isolation method (Plate 2). Microscopic observations shown that mycelium was septate and branched. Conidiophores observed under microscope were simple, cylindrical and olivaceous brown. The conidia were elongated, spindle shaped with protruding hilum having brownish colour. The identified fungal pathogen was transferred to the PDA slants and maintained for further studies (Plate 3).

The morphological characters were identical to those observed earlier by Harlapur *et al.* (2007) and Geeta *et al*. (2019).

4.2 Pathogenicity Test by Koch's Postulates

The Pathogenicity test of the isalated fungus was carried out under glasshouse condition as described in materials and methods on susceptible maize variety CM-202 by following Koch's postulates. Typical symptoms of Turcicum leaf blight appeared on leaves on 14^{th} day after inoculation. Initially, small water-soaked lesions were observed on under surface of leaf. Later on, typical necrotic types of lesions were noticed on leaves. The disease symptoms observed on artificially inoculated plants in glasshouse were identical to those observed under natural field condition (Plate 4). The pathogen was re-isolated from the artificially inoculated leaves on PDA medium and compared with the original pathogen. The re-isolated pathogen was found identical to the original culture in growth and morphological characters.

Earlier, Geeta *et al*. (2019) had also proved the pathogenicity of *E. turcicum* by inoculating sorghum grains containing the pathogen (1×10^6 conidia/g) placed in leaf whorls of 25 days old maize seedling. CM-202 inbred line showed susceptible reaction to the isolated fungus.

Thus, based on typical disease symptoms of Turcicum leaf blight observed under field conditions and on artificially inoculated plants, pathogenicity test and morphological characters, fungus causing Turrcicum leaf blight of maize was identified as *Exserohilum turcicum*.

4.3 Cultural and Morphological Study of *Exserohilum turcicum*

4.3.1 Cultural Study of *Exserohilum turcicum*

The growth characters of isolated fungus on various nutrient media are presented in Table 4.1. The fungus was grown on eight nutrient media to analyze the efficacy of different media in growth promotion of *E. turcicum* (Plate 5). The cultural media assessed for growth characters showed varying degree of growth rates ranging from 66.03 to 90 mm in seven days. An excellent growth of mycelium (90 mm) was observed on PDA and Oat meal agar. Profused and good mycelium growth was observed on Malt extract agar (84.73 mm) and Czapeck's dox agar (82.23 mm) while Richard's agar, Yeast extract agar and Corn meal agar showed moderate mycelial growth. Minimum mycelial growth (66.03 mm) was recorded on Subouraud's agar.

Previous workers Nataraj (2014) and Rashmi (2015) reported that among different media tested PDA was found effective for growth and development of *E. turcicum*.

Table 4.1. Growth and cultural characteristics of *Exserohilum turcicum* on different solid media

Sr. No.	Culture media	Mean colony diameter (mm)	Characteristics of colony
1	Czapeck's dox agar	82.23	Profused growth withregular shaped, rough and slightly fluffy grayish mycelium
2	Richard's agar	78.83	Moderate growth, with regular shaped, rough and slightly fluffy whitish-grey mycelium
3	Malt extract agar	84.73	Profused growth withregular shaped, rough and slightly fluffy greyish- black mycelium
4	Potato dextrose agar	90.00	Profused growth withregular shaped, rough and slightly fluffy black mycelium
5	Subouraud's agar	66.03	Poor growth with irregular shaped, rough and slightly fluffy whitish-grey mycelium
6	Yeast extract agar	72.36	Moderate growth with irregular shaped, rough and slightly fluffy whitish-grey mycelium
7	Oat meal agar	90.00	Profused growth withregular shaped, rough and slightly fluffy grayish mycelium
8	Corn meal agar	75.86	Moderate growth with regular shaped, rough and slightly fluffy grayish black mycelium
	SE (m)±	0.52	
	CD P=0.05	2.59	

4.3.2 Morphological Study of *Exserohilum turcicum*

Morphological characters of *E. turcicum* have been described in Table 4.2. The mycelium was septate and branched (Plate 6). Conidiophores observed were simple, cylindrical and

olivaceous brown. The conidia were blended, elongated and spindle shaped with protruding hilum having brownish colour. The average conidial length was found 28.5 to 30.00 µm while the breadth ranged between 7.0 to 8.5µm. The number of septa ranged from 1 to 3 (Plate 7).

Earlier, Daniel and Narong (2006) had also observed similar morphological characters of *E. turcicum* and reported that conidia were elongated, blended and spindle in shape. The average conidial length and width were found 93.97 µm and 13.11 µm, respectively, while the number of septations was found to range from 2-7. These results are similar with findings of Harlapur *et al*. (2007) who noticed variation in morphological and cultural characters of 16 isolates of *E. turcicum*. He also studied colony character, colony diameter, mycelial dry weight,spore germination and sporulation.

Table 4.2 Morphological characteristics of *Exserohilum turcicum* causing Turcicum leaf blight of maize.

Sr. No.	Characteristic features	Characteristic features of *Exserohilum turcicum*
1	Growth type	Even
2	Mycelium	Initially white in colour then turning dark greyish to black.
3	Colony form	Circular
4	Margin type	Circular margin
5	Spore mass colour	Brownish
6	Conidia	Spindle shaped conidia with protruding hilum
7	Conidia length (µm)	28.5
8	Conidial breadth (µm)	7.5
9	Septa	1-3

4.4 *In vitro* Evaluation of Botanicals, Bio-control Agents and Fungicides

4.4.1 *In vitro* Evaluation of Botanicals against *Exserohilum turcicum*

The efficacy of eight plant extracts was tested at 5 and 10 % concentration to inhibit the radial mycelial growth of *E. turcicum*. The results obtained are presented as follow.

4.4.1.1 *In vitro* evaluation of different plant extracts @ 5% concentration against *Exserohilum turcicum*

From the data presented in Table 4.3, it is revealed that all the plant extracts were significantly superior over control in reducing the mycelial growth of *E. turcicum* at 5% concentration.

Table 4.3 ***In vitro*** **evaluation of different plant extracts @ 5% and 10% concentration against** ***Exserohilum turcicum*** **under** ***in vitro*** **condition**

Tr. No.	Plant spp.	Botanical name	Mean colony diameter (mm)		Per cent inhibition (%)	
			5%	10%	5%	10%
T_1	Neem	*Azadiracta indica*	43.33	31.60	51.85 (46.05)*	64.88 (53.65)*
T_2	Ghaneri	*Lantana camara*	56.17	34.17	37.59 (37.81)	62.03 (51.96)
T_3	Tulasi	*Ocimum tenuiflorum*	58.30	44.83	35.22 (36.40)	50.18 (45.10)
T_4	Karanj	*Millettia piñnata*	60.33	52.50	32.96 (35.03)	41.66 (40.20)
T_5	Nirgudi	*Vitex negundo*	63.63	62.50	29.29 (32.76)	30.55 (33.55)
T_6	Onion	*Allium cepa*	40.40	29.67	55.11 (47.93)	67.03 (54.95)
T_7	Parthenium	*Parthenium hysterophorus*	38.77	28.60	56.92 (48.97)	68.22 (55.68)
T_8	Tarvad	*Senna auriculate*	45.70	33.03	49.22 (44.55)	63.29 (52.70)
T_9	Control		90.00	90.00	0.00 (0.00)	0.00 (0.00)
	SE (m)±				0.52	0.42
	CDP=0.01				2.01	1.64

*figures in parenthesis are arc sine transformed values

Among all the treatments maximum inhibition of mycelial growth (56.92 %) was in *Parthenium hysterophorus* extract with least colony diameter of 38.77 mm and this treatment was followed by *Allium cepa*, *Azadiracta indica* and *Senna auriculata* extracts with 55.11, 51.85 and 49.22 per cent inhibition of mycelial growth respectively. Thus, plant extracts *viz*., *Parthenium hysterophorus*, *Allium cepa*, *Azadiracta indica* and *Senna auriculata* were at par with each other at 5% concentration in inhibiting the fungal growth. Among all plant extracts evaluated minimum inhibition (29.29 %) was recorded with *Vitex negundo* extract. Maximum colony diameter (90.00 mm) of fungus was recorded in control treatment (Plate 8).

4.4.1.2 ***In vitro*** **evaluation of different plant extracts @ 10% concentration against** ***Exserohilum turcicum***

The results given in Table 4.3 showes that all plant extracts at 10 % concentration significantly inhibited the mycelial growth over the untreated control.

Among all treatments the maximum inhibition of mycelial growth (68.22%) was found in *Parthenium hysterophorus* with minimum colony diameter of 28.60 mm. It was followed by *Allium cepa* (29.67 mm), *Azadiracta indica* (31.60 mm), *Senna auriculata* (33.03 mm) and *Lantana camara* (34.17 mm) extraxts showing 67.03, 64.88, 63.29 and 62.03 per cent inhibition ,

respectively. Thus, the plant extracts *viz*., *Parthenium hysterophorus, Allium cepa*, *Azadiracta indica*, *Senna auriculata* and *Lantana camara* were at par with each other at the concentration of 10 per cent in recording colony diameter of *E. turcicum*. Among the plant extracts evaluated, minimum inhibition (30.55 %) was found in *Vitex negundo* extraxt with 62.50 mm mean colony diameter. Maximum colony diameter (90.00 mm) was recorded in control treatment (Plate 9).

The present results are confirmed with the findings of Manu *et al*. (2017) who found that among the eleven botanicals tested against *E. turcicum*, garlic bulb extract showed maximum inhibition (79.63 %) of mycelial growth of *E. turcicum* followed by Parthenium leaf extract (64.14 %). Harlapur *et al*. (2007) noted that, neem seed kernel extract @ 10 % concentration showed maximum inhibition of mycelial growth (56.64 %) of test pathogen followed by *Aloe vera* @ 10 % (53.50 %). Similar results on antifungal activity of different aqueous plant extracts has been reported by Shivapuri *et al*. (1997) and Meena *et al*. (2003).

In present studies, all the botanicals significantly inhibited mycelial growth of test fungus *E. turcicum*. With increase in concentration of plant extracts, mycelial inhibition of fungus also increased and maximum inhibition was obtained at higher concentration i.e. 10 per cent.

4.4.2 *In vitro* Evaluation of Bio-control Agents against *E. turcicum*

The findings of *in vitro* experiment conducted to know the most effective bio-control agent against *E. turcicum* utilizing dual culture technique are presented in Table 4.4.

From the data presented it is revealed that all bio-control agents were significantly superior to inhibit the fungal growth over control. The per cent inhibition ranged from 47.96 to 77.07. The results revealed that *Trichoderma harzianum* was most effective antagonist exhibiting 77.07 per cent mycelial inhibition of fungus followed by *Trichoderma hamatum* (73.51 %). Moreover, *T. harzianum* and *T. hamatum* were at par with each other in inhibiting mycelial growth. Next effective antagonists reported were *T. konigii* and *T. asperellum* which gave mycelial inhibition of 68.33 and 65.14 per cent, respectively. Among all the treatments *Bacillus subtilis* exhibiting least mycelial inhibition (47.96 %) with 46.83 mm mean colony diameter of *E. turcicum*.

These results are in line with those reported by Manu *et al*. (2017) who reported maximum inhibitian of mycelial growth (98.65 %) by *T. harzianum*-2 followed by *T. viride* (98.34 %). Among the bacterial antagonists, *Pseudomonas fluorescence*-1 and *Pseudomonas fluorescence*-2 showed the mycelial inhibition of 95.49% and 94.24%, respectively. In present study bio-control agents were found effective in controlling the growth of test fungus under *in vitro* conditions. Earlier, Harlapur *et al*. (2007) and Khedekar *et al*. (2012) reported that *Trichoderma harzianum* was effective in inhibiting the mycelial growth.

Table 4.4 *In vitro* evaluation of bio-control agents against *Exserohilum turcicum*

Tr. No.	Antagonist	Mean colony diameter(mm)	Per cent inhibition (%)
T_1	*Trichoderma asperellum*	31.36	65.14 (53.81)*
T_2	*T. harzianum*	20.63	77.07 (61.38)
T_3	*T. hamatum*	23.83	73.51 (59.02)
T_4	*T. konigii*	28.5	68.33 (55.75)
T_5	*Psedomonas fluorescence*	37.16	58.7 (50.01)
T_6	*Bacillus subtilis*	46.83	47.96 (43.83)
T_7	Control	90.00	0.00 (0.00)
	SE (m)±		0.81
	CD P=0.01		3.42

*figures in parenthesis are arc sine transformed values

These results are further supported by the results of Singh and Singh (2014). Ramchandra (2000) studied anatagonist against *Exserohilum hawaiiensis in vitro* and reported that *T. viride* and *T. harzianum* decreased the growth and sporulation significantly.

4.4.3 *In vitro* Evaluation of Fungicides against *E. turcicum*

Seven fungicides including two systematic, one non systematic and four combi-product fungicides at concentration of 50% and 100% recommended were assessed to find out their efficacy in inhibition of mycelial growth of *E. turcicum* using poisoned food technique.

4.4.3.1 *In vitro* evaluation of fungicides against *E. turcicum* at 50 % concentration

Efficacy (*in vitro*) of seven fungicides at 50 % concentration evaluated using poisoned food technique is presented in Table 4.5.

The data presented showed that all the test fungicides significantly inhibited the mycelial growth of *E. turcicum*. Among all treatments carbendazim + mancozeb proved to be most effective fungicide exhibiting maximum mean mycelial growth inhibition of 100 per cent followed by azoxystrobin + cyproconazole which showed 96.66 per cent mycelial growth inhibition. Next efficacious treatments against *E. turcicum* were pyraclostrobin + epoxiconazole, mancozeb and propiconazole which gave 85.55, 84.96 and 83.32 per cent inhibition, respectively. Moreover, these three treatments were at par with each other in recording colony diameter. A mong the various fungicides evaluated, the least effective was kresoxim-methyl giving 27.15 per cent inhibition. Maximum colony diameter (90 mm) was recorded in control treatment (Plate 11).

Table 4.5 *In vitro* evaluation of fungicides against *Exserohilum turcicum* at 50% and 100% recommended concentration

Tr. No.	Fungicides	Conc.(%)		Mean colony diameter (mm)		Per cent inhibition (%)	
		50%	100%	50%	100%	50%	100%
T_1	Mancozeb 75% WP	0.125	0.25	13.53	10.9	84.96 (67.18)*	87.83 (69.58)*
T_2	Kresoxim-methyl 44.3% SC	0.05	0.1	65.56	63.83	27.15 (31.39)	29.07 (32.62)
T_3	Propiconazole 25% EC	0.05	0.1	15	12	83.32 (65.90)	86.66 (68.58)
T_4	Azoxystrobin 18.2% w/w + Cyproconazole 7.3% w/w SC	0.05	0.1	3	2.5	96.66 (79.47)	97.22 (80.39)
T_5	Azoxystrobin 18.2% w/w + Difenoconazole 11.4% w/w SC	0.05	0.1	18.5	15	79.44 (63.00)	83.33 (65.90)
T_6	Pyraclostrobin 133g/l + Epoxiconazole 50g/l SE	0.075	0.15	13	10.5	85.55 (67.65)	88.33 (70.02)
T_7	Carbendazim 12% + Mancozeb 63% WP	0.05	0.1	0.00	0.00	100.00 (90)	100.00 (90)
T_8	Control			90.00	90.00	0.00 (0.00)	0.00 (0.00)
	SE (m)±					0.53	0.41
	CD P=0.01					2.22	1.70

*figures in parenthesis are arc sine transformed values

4.4.3.2 *In vitro* evaluation of fungicides against *E. turcicum* at 100% recommended concentration

The data given in Table 4.5 revealed that all fungicides were significantly superior in reducing mycelial growth. Among different fungicides, carbendazim + mancozeb was found most eftective exhibiting maximum mean mycelial growth inhibiton (100 per cent). Next best treatmemt was azoxystrobin + cyproconazole with 97.22 per cent mycelial growth inhibition. It was followed by pyraclostrobin + epoxiconazole (88.33%), mancozeb (87.83%) and propiconazole (86.66%). Moreover, the fungicides pyraclostrobin + epoxiconazole, mancozeb and propiconazole were at par with each other at the recommended concentration in inhibiting mycelial growth of fungus. Least inhibition of mycelial growth was recorded in kresoxim-methyl (29.07%)

The results obtained during present study are in consonance with the findings of Reddy *et al*. (2013) who evaluated seven fungicides against *E. turcicum* under *in vitro* condition and reported that the lowest per cent disease index (PDI) was in treatment mancozeb @ 0.25 % and combination treatment of carbendazim and mancozeb, @ 0.25 % with per cent disease control of 73.0 and 72.1 %, respectively over control. Kumar *et al*. (2015) found that among the six

fungicides tested zineb 75 WP @ 0.25 % concentration was most effactive in inhibiting the growth of *E. turcicum*. Similarly, mancozeb 63 % + carbendazim 12 % @ 0.25 % was equally effective in reducing disease severity which can be used as an alternative to zineb. Many workers like Veerabhadraswamy *et al*. (2014) and Anand *et al*. (2013) reported that carbendazim 12% + mancozeb 63% and tebuconazole 50% + trifloxystrobin 25% were effective to inhibit growth of fungus at different concentrations. Similar results were reported by Manu *et al.* (2017) showing that amomg systematic fungicides, tebuconazole completely inhibited the pathogen at all the concentrations tested, while among contact fungicides, propineb was highly effective inhibiting 83.89 per cent mycelial growth of *E. turcicum* at 500 ppm. Among combi-products tested against *E. turcicum*, only carbendazim 12% + Mancozeb 63% at 500 ppm showed complete inhibition of mycelial growth of *E. turcicum*.

During present studies all the tested fungicides at various concentrations significantly inhibited the mycelial growth of *E. turcicum* over untreated control. As the concentration of fungicides was increased, mycelial growth inhibition also increased. Among all the tested fungicides maximum inhibition of pathogen was obtained with carbendazim + mancozeb at 50% and 100% recommended concentration. It was followed by azoxystrobin + cyproconazole and pyraclostrobin + epoxiconazole.

5. SUMMARY AND CONCLUSIONS

5.1 Summary

Maize (*Zea mays* L.) is one of the most important cereal crop which occupies an important place in agriculture worldwide due to its high yielding potential and great demand. Among the foliar diseases, Turcicum leaf blight caused by *Exserohilum turcicum* severely affect the growth and production of crop at pre-harvest stage. Turcicum leaf blight disease causes several losses about 25-90 % yield loss. Primarily, fungicides are utilized for the management of disease. Use of chemicals in agriculture causes several adverse, environmental hazards and residual toxicity. Therefore, the present research was carried out to evaluate some botanicals, bio control agents and fungicides against *E. turcicum*. The findings obtained during present investigation are summarized and concluded here under.

The disease was found to infect leaves, small water-soaked lesions observed on the under surface of leaf, later on, typical necrotic type of lesions appeared on the leaves.Turcicum leaf blight infected samples were collected for isolation of diseases causing fungus. Pathogen associated with diseased plant part was isolated on PDA media. The isolated pathogen was tested for pathogenicity on CM-202 maize variety in glasshouse and proved by using Koch's postulates. The isolated pathogen was identified as *Exserohilum turcicum* on the basis of morphological characteristics of pathogen and pathogenicity test.

There was variation in conidial growth of isolated pathogen (*Exserohilum turcicum*) on different cultural media. The isolated fungus on different nutrient media showed variation in colony growth characteristics as profused/ moderate/ poor/ rough/ fluffy/ regular or irregular growth. The colony colour varied from whitish grey to black. The isolated pathogen showed excellent growth on PDA and Oat meal agar. Morphological characters such as colony characteristics, spore size, septation, spore shape and pigmentation of isolated *E. turcicum* were studied.

In vitro evaluation of eight botanicals against *Exserohilum turcicum* was done by poison food technique. Plant extracts *viz*., parthenium, onion, neem and tarvad were found effective against *E. turcicum* at 5 and 10 % concentration. Among the various plant extracts, parthenium exhibited maximum inhibition of pathogen over untreated control.

In vitro evaluation of bio-control agents against *E. turcicum* showed that *T. harzianum* was most effective antagonist showing 77.07 per cent mycelial inhibition of *E. turcicum* followed by *T. hamatum* (73.51%) and *T. konigii* (68.33%), while, the least inhibition was recorded in *Bacillus subtilis* (47.96 %).

Seven fungicides were evaluated against *E. turcicum* under *in vitro* condition. It was recorded that among the various fungicides only combi fungicide Carbendazim + Mancozeb exhibited 100 per cent inhibition of mycelial growth of *E. Turcicum* at 50% and 100%

recommended concentration. It was followed by Azoxystrobin + Cyproconazole and Pyraclostrobin + Epoxiconazole. Least inhibition of mycelial growth was observed in Kresoxim-methyl.

5.2 Conclusions

1. Among the various nutrient media utilized for cultural studies, PDA and Oat meal agar media were found effective for profused mycelial growth of *E. turcicum*. Morphological characteristics indicated that the pathogen produced spindle shaped conidia having varied number of septa with protruding hilum.
2. Under *in vitro* conditions, botanicals were found effective and possessed fungistatic property against *E. turcicum*. Among the tested botanicals, *Parthenium hysterophorus* and *Allium cepa* were found effective against *E. turcicum* at 5 and 10% concentration.
3. Under *in vitro* condition, out of six bio-control agents tested against *E. turcicum*, *Trichoderma harzianum* and *Trichoderma hamatum* were found superior and most effective in mycelial inhibition of pathogen with 77.07 and 73.51per cent inhibition, respectively.
4. Among the seven fungicides tested against *E. turcicum*, Carbendazim 12% + Mancozeb 63% was found most effective in reducing mycelial growth of *E. turcicum* with 100 per cent inhibition of mycelial growth at 50% and 100% recommended concentration under *in vitro* condition.
5. The present studies clearly indicated that fungicides, botanicals and bio-control agents found effective in disease control. Botanicals and bio-control agents could be effective to control the Turcicum leaf blight infection in maize and thus can play a major role in eco-friendly management of the disease.

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7. VITAE

Ms. AGHAV MUKTA ADINATH

MASTER OF SCIENCE (AGRICULTURE)

IN

PLANT PATHOLOGY

(2021)

Title of thesis		:	"Evaluation of Botanicals, Bio-control Agents and Fungicides against Turcicum Leaf Blight of Maize"
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Plate 1: Symptoms showing Turcicum leaf blight on maize leaves

Plate 2: Pure culture of fungal isolate *Exserohilum turcicum*

Plate 3: Pure culture of fungal isolate (Slants) *Exserohilum turcicum*

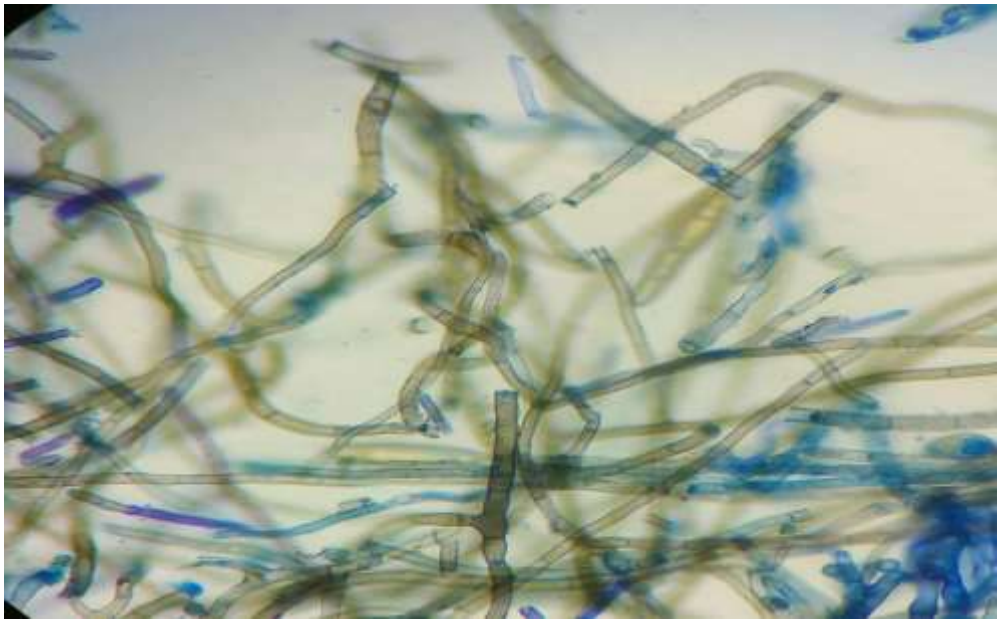

Plate 6: Mycelium of *Exserohilum turcicum*

Plate 7: Conidia of *Exserohilum turcicum*

Healthy plant **Infected plant**

Plate 4: Pathogenicity test of *Exserohilum turcicum*

MEA	Malt extract agar	SA	Sabouraud's agar
CMA	Corn meal agar	RA	Richard's agar
CDA	Czapeck's dox agar	OMA	Oat meal agar
PDA	Potato dextrose agar	YeEA	Yeast extract agar

Plate 5: *Exserohilum turcicum* colonies grown on different solid media

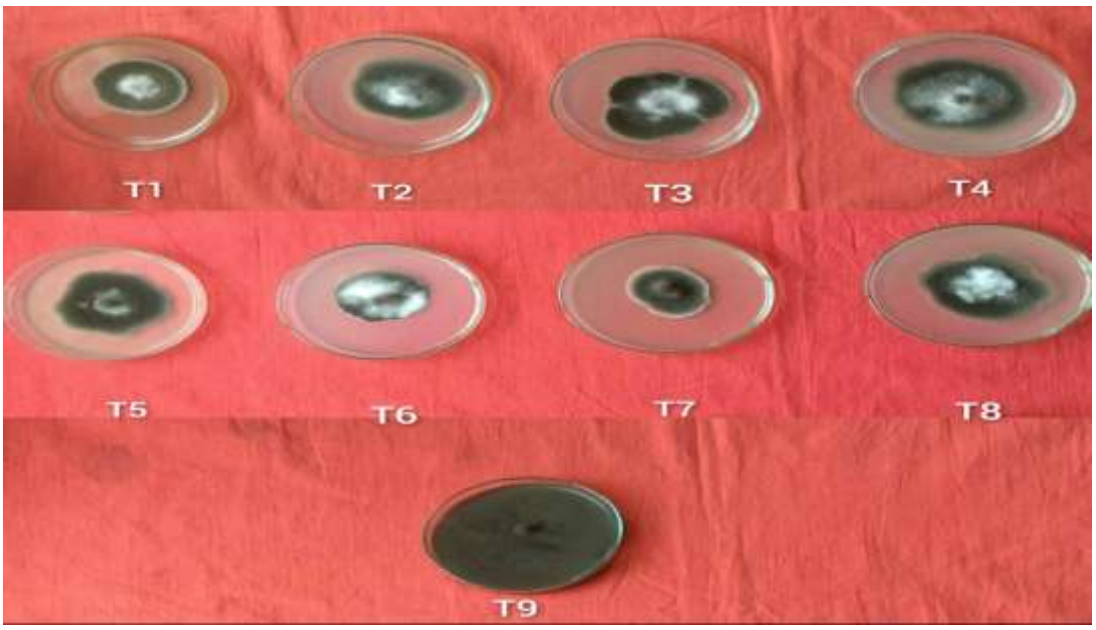

Plate 8:*In vitro* evaluation of different plant extracts @ 5% concentration against *Exserohilum turcicum*

Plate 9: *In vitro* evaluationof different plant extracts @ 10% concentration against *Exserohilum turcicum*

T_1 *Azadirachta indica*

T_2 *Lantana camara*

T_3 *Ocimum tenuiflorum*

T_4 *Millettia pinnata*

T_5 *Vitex negundo*

T_6 *Allium cepa*

T_7 *Parthenium hysterophorus*

T_8 *Senna auriculata*

T_9 Control

T_1 *Trichoderma asperellum*

T_2 *T. harzianum*

T_3 *T. hamatum*

T_4 *T. konigii*

T_5 *Pseudomonas fluorescence*

T_6 *Bacillus subtilis*

T_7 Control

Plate 10: *In vitro* evaluation of bio-control agents against *Exserohilum turcicum*

Plate 11: *In vitro* evaluation of fungicides against *Exserohilum turcicum* at 50% recommended concentration

Plate 12: *In vitro* evaluation of fungicides against *Exserohilum turcicum* at 100% recommended concentration

T_1 Mancozeb 75% WP

T_2 Kresoxim-methyl 44.3% SC

T_3 Propiconazole 25% EC

T_4 Azoxystrobin 18.2% w/w + Cyproconazole 7.3% w/w SC

T_5 Azoxystrobin 18.2% w/w + Difeconazole 11.4% w/w SC

T_6 Pyraclostrobin 133g/l + Epoxiconazole 50g/l SE

T_7 Carbendazim 12% + Mancozeb 63% WP

T_8 Control

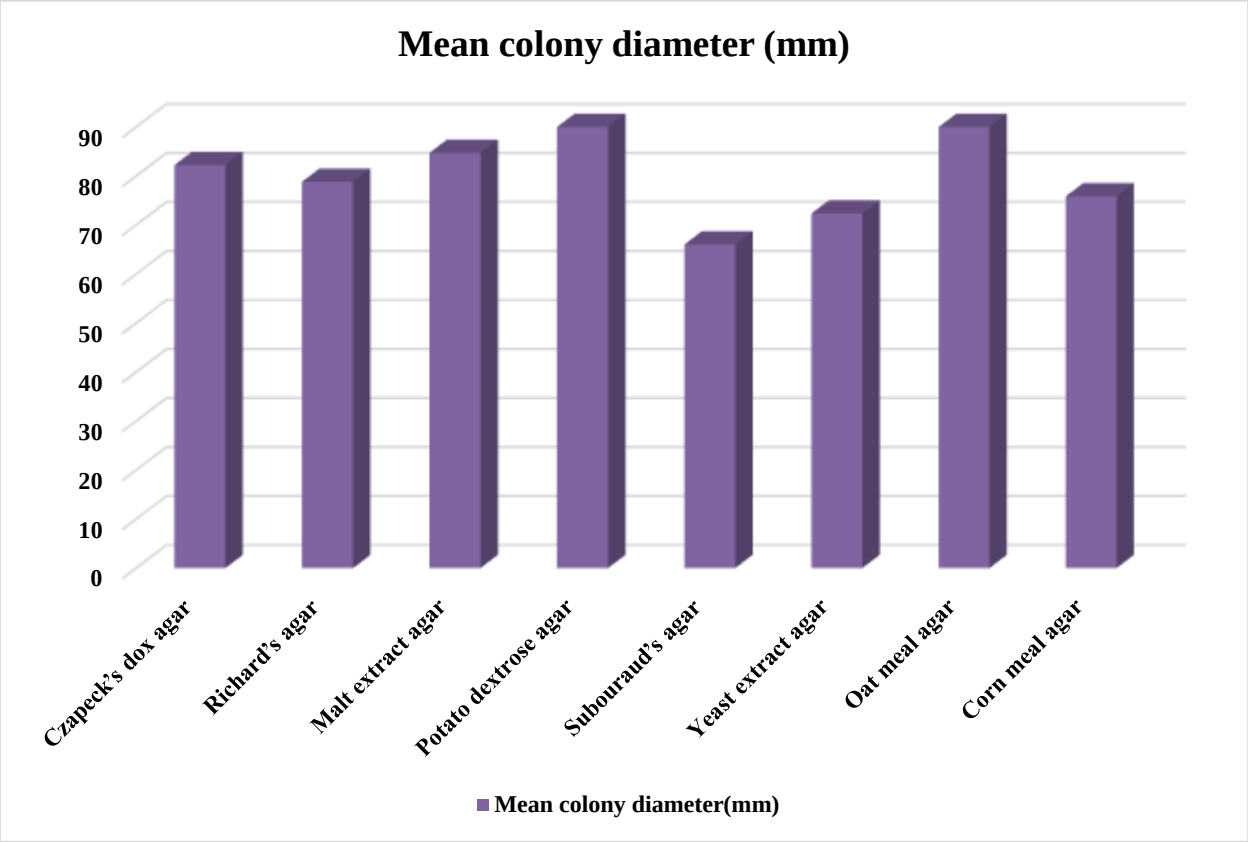

Fig. 1: *Exserohilum turcicum* colonies grown on different solid media

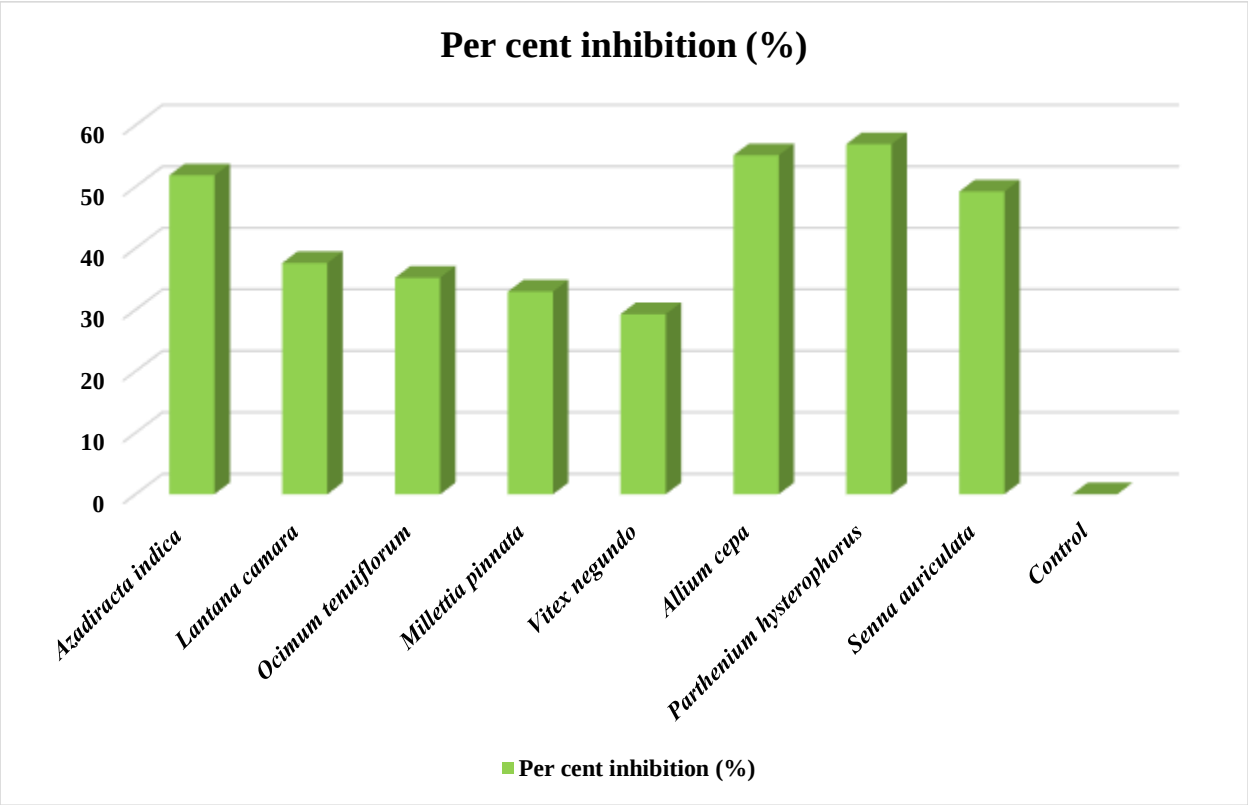

Fig. 2: *: In vitro* evaluation of different plant extracts @ 5% concentration against *Exserohilum turcicum*

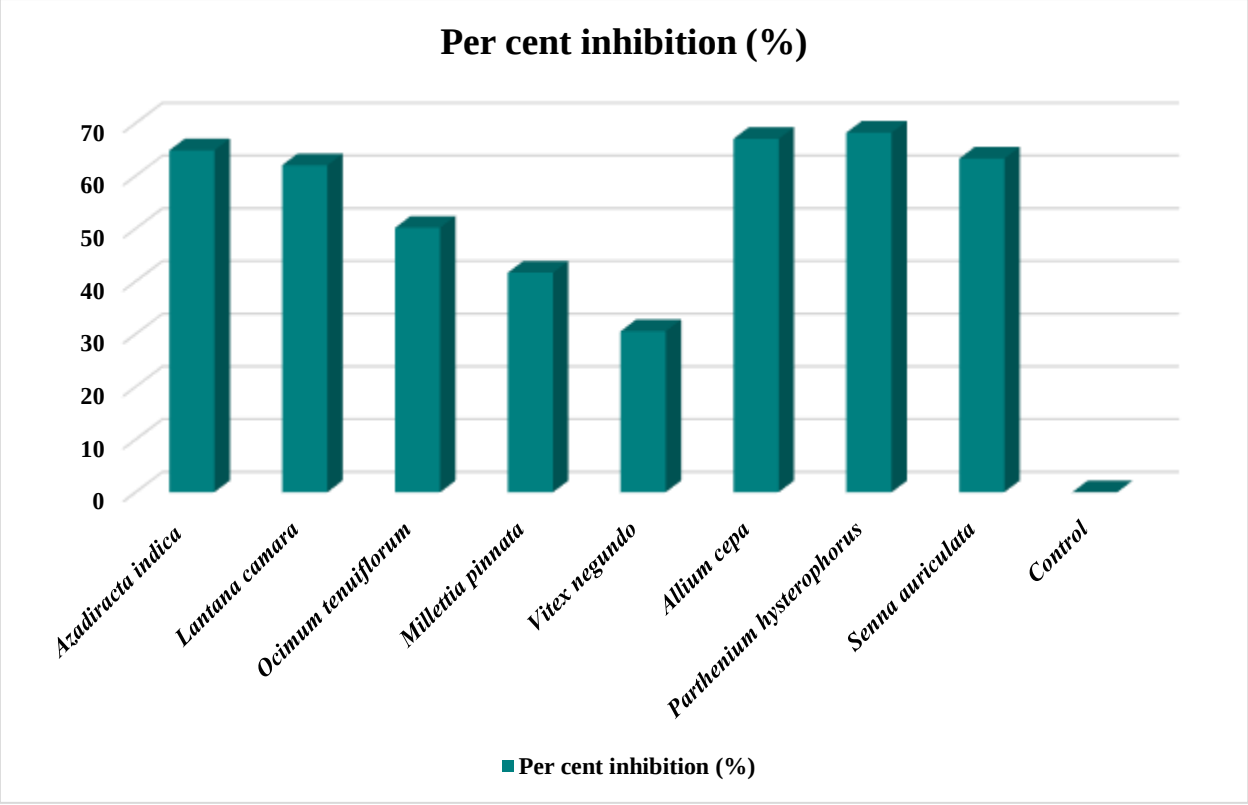

Fig. 3: *: In vitro* evaluation of different plant extracts @ 10% concentration against *Exserohilum turcicum*

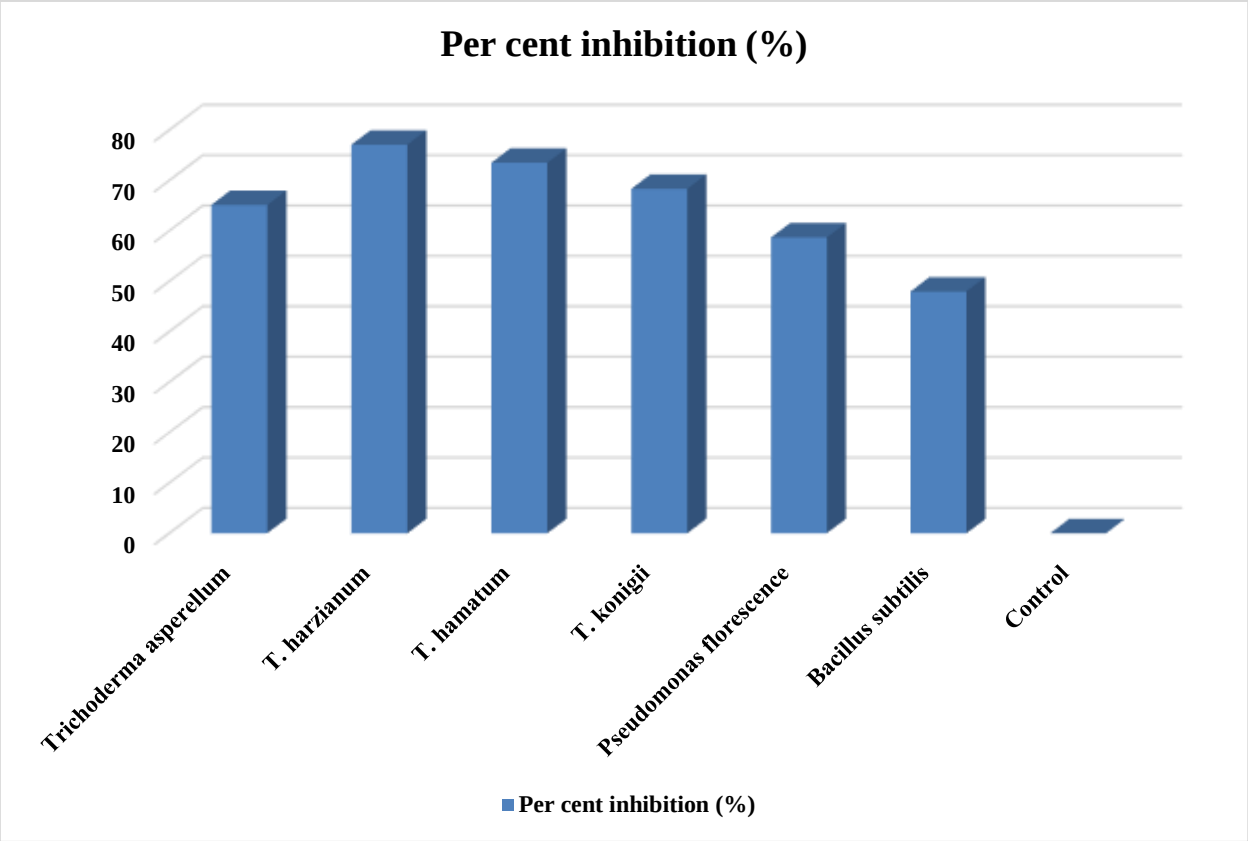

Fig. 4: *: In vitro* evaluation of bio-control agents against *E. turcicum*

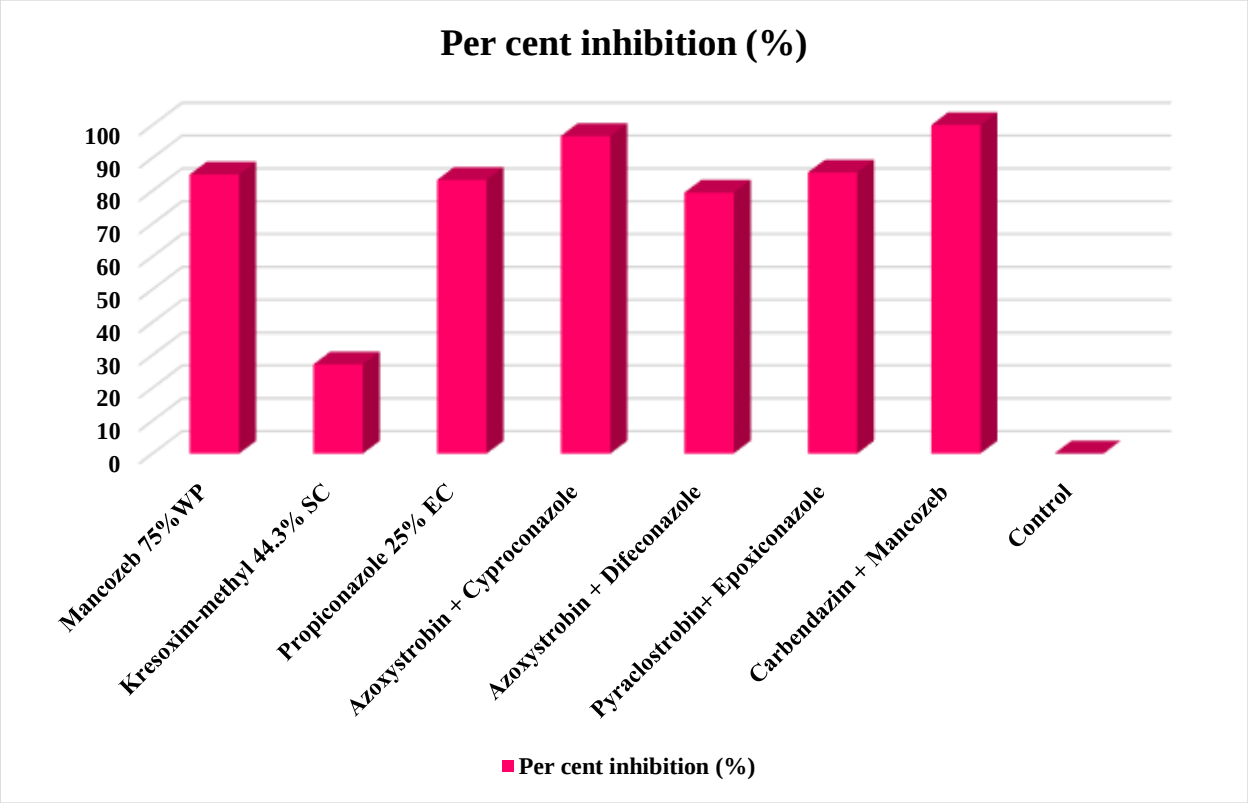

Fig. 5: *: In vitro* evaluation of fungicides against *Exserohilum turcicum* at 50% concentration

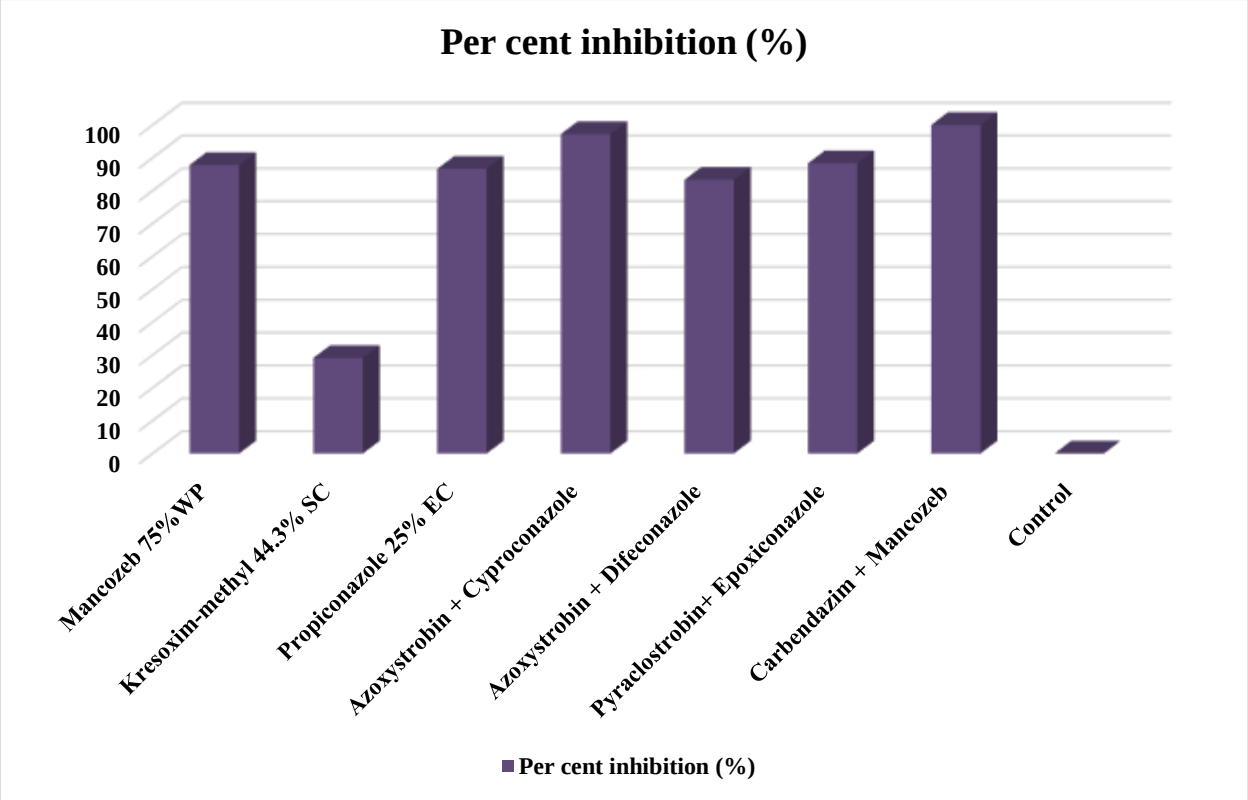

Fig. 6*: In vitro* evaluation of fungicides against *Exserohilum turcicum* at 100% recommended concentration