STUDY ON NORTHERN LEAF BLIGHT OF MAIZE CAUSED BY *Exserohilum turcicum* (Pass). Leonard and Suggs

PATIL LALIT PANDURANG
B.Sc. (Agriculture)

MASTER OF SCIENCE
IN
(Agriculture)

(PLANT PATHOLOGY)

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE, BADNAPUR
VASANTRAO NAIK MARATHAWADA KRISHI VIDYAPEETH.
PARBHANI-431402 (M.S.), INDIA.

2021

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BY
PATIL LALIT PANDURANG
B.Sc. (Agriculture)

A thesis submitted to
Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani
In partial fulfillment of the requirements
for the Degree of

MASTER OF SCIENCE
IN
(Agriculture)
(PLANT PATHOLOGY)

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I herby declare that the thesis entitled, **"STUDY ON NORTHERN LEAF BLIGHT OF MAIZE CAUSED BY *Exserohilum turcicum* (Pass). Leonard and Suggs."** submitted by me is based on the actual work carried out by me under the guidance and supervision of **G. P. JAGTAP** The extent of information derived from the existing literature have been duly cited and referenced. The existing research work or its any part is not submitted anywhere else for the award of any degree or diploma.

I also hereby declare that no sentence, equation, diagram, table, paragraph or section has been copied verbatim from previous work unless it is cited and duly referenced. There is no plagiarism; the work presented is original and own work of the researcher. No ideas, process, results or words of others have been presented as researcher's own work.

Place : Badnapur

Date : 4/08/2021

Signature of the Candidate

(L. P. PATIL)

Reg. No: 2019A18MB

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This is to certify that the thesis entitled **"STUDY ON NORTHERN LEAF BLIGHT OF MAIZE CAUSED BY *Exserohilum turcicum* (Pass). Leonard and Suggs"** submitted by **PATIL LALIT PANDURANG Reg. No. 2019A18MB** in partial fulfilment of the requirements for the award of the **MASTER OF SCIENCE (Agriculture)** in the subject of **PLANT PATHOLOGY** submitted to the Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani is a record of bonafide research work carried out by him under my guidance and supervision. The thesis or its any part has not previously formed the basis for the award of any degree, diploma or other similar title.

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URL: https://www.elsevier.es/es-revista-revista-argentina-microbiologia-372-articulo-selection-potential-biological-control-exserohilum-S0325754115000085
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URL: https://www.researchgate.net/publication/324834343_Effect_of_Different_Media_and_Tempe rature_on_the_Growth_of_the_Exserohilum_turcicum_Incitant_of_Turcicum_Leaf_Blight_in_Maize
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URL: https://www.researchgate.net/publication/349588589_TURCICUM_LEAF_BLIGHT_OF_MAIZE_ INCITED_BY_Exserohilum_turcicum_A_REVIEW
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URL: https://www.scitechnol.com/abstract/bioefficacy-of-botanicals-against-exserohilum-turcicum-causing-leaf-blight-of-sorghum-3755.html
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Integrated management of leaf blight of sweet sorghum caused by Exserohilum turcicum (Pass.) Leonard and Suggs
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URL: https://www.researchgate.net/publication/343826774_Morphological_and_Cultural_Variation_ of_Exserohilum_turcicum_isolates_in_Sorghum_Sorghum_bicolour_L 6Fetched: 7/27/2021 9:27:00
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Evaluation of different fungicides, bioagents and botanicals against Alternaria blight caused by Alternaria helianthi (Hansf) of sunflower
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CKNOWLEDGEMENT

I think it is a matter of pleasure to glance back and recall the way one traverse, the days of hard work and perseverance. It is still great at the juncture to recall the faces and spirits in the form of teachers, friends, near and dear once. In my opinion, this work is nothing more than incomplete, without attending to the task acknowledge ending, to overwhelming help I received during this endeavour of mine.

Everything has its beauty, but not everyone can see without critical observation and great vision. Inspiration is the best medicine that can make it possible to run for a crippled one and it is veritable gold of mine to get the talented and inspiring wilful guidance. Today I stand on the door of this vision due to my Guide ***Dr G. P. Jagtap*** *Associate Professor, National Agriculture Research Project, Aurangabad and Chairman of my advisory committee. Who in this unique way provided me with constant encouragement inspiring, scholastic guidance, love and affection offered to me during my study and research works would be a poor vehicle to communicate my sense of gratitude. For his constructive criticism, unflagging enthusiasm and precious guidance during the whole tenure of the investigation. It has his most co-operative and painstaking attitude, which made this thesis a reality.*

This memorable occasion provides me with a unique privilege to express my deep sense of respect and indebtedness to members of my advisory committee ***Dr D. G. Hingole*** *Associate Professor, Department of Plant Pathology, College of Agriculture, Badnapur,* ***B. V. Patil Associate*** *Professor, Department of Agricultural Entomology, College of Agriculture, Badnapur and* ***Dr C. B. Patil*** *Senior Scientist, Agronomy, Agricultural Research Station, Badnapur and may be regarded as the light hours for the ocean liners who have been kindly navigated my ship of academic pursuit and I would like to mention my gratitude to them. I owe my thanks to authorities of the V.N.M.K.V., Parbhani, honourable Vice-Chancellor* ***Dr A. S. Dhavan****, and* ***Dr. D. N. Gokhale*** *Dean Faculty of Agriculture, VNMKV, Parbhani and* ***Dr. D. P. Waskar,*** *Director of Research, VNMKV, Parbhani and* ***Dr R. D. Ahire****, Associate Dean and Principal, College of Agriculture, Badnapur for providing necessary facilities to conduct the research work.*

My sincere thanks to ***Dr D. K, Patil****, In-charge officer, ARS, Badnapur,* ***Dr. P. H. Ghante,*** *Senior Scientist ARS Badnapur,* ***Dr S. S. Mane****, Dept. of Soil Sci,* ***Dr K, T. Jadhav****, Dept of Agronomy,* ***Dr S. B. Sarode****, Dept. of Botany,* ***Prof.B. V. Patil****, Dept. of Entomology,* ***Shri. U. M. Naglot****, Dept. of Horticulture,* ***Shri P. L Sontakke*** *Senior research assistant, ARS, Badnapur,* ***Shri. S. J. Supekar,*** *Dept. of Engineering,* ***Shri. S. K, Raskar****, Dept. of Agronomy,* ***Dhage Sir, Zambare madam, Thorat madam, Seema madam*** *and all staff Member College of Agriculture,*

Badnapur and Agricultural Research Station, Badnapur for their positive backing and co-operation during the period of investigation.

I would like to thank my Pathology family circle ***Onkar, Madhav, Sagar, Sunil and my seniors Shubham, Pradnyashil, Chandramuni and Ganesh and junior's Sagar, Shivkanya, Dinesh, Mahesh, Aniket, Satish*** *for their encouragement and support throughout the M. Sc degree programme.*

I would also like to thank my M.Sc batchment ***Anirudha, vivek, Katare, Jeevan, Digambar, Atul, Swapnil, Jivraj, Nitin, Atul, Sudarshan, Abhishek, Shivaji, Lakhesingh and Gokul*** *for their encouragement and support throughout the M. Sc degree programme.*

I take this opportunity to thank my room owner ***Pawar*** *and* ***mess owner Dhakne*** *for their delicious and luscious food, tea, snacks and also love, affection, care and brotherly relationship towards me. My deepest and sincere gratitude goes out to* ***Shri. Jagannath Gaware.***

I would like to thank ***Pralay, Avon, Amol, Poonam, Bhavika, Aniket, Pallavi, Divya, Amruta, Pritam, Samadhan, Durgesh, Sagar and Bapu*** *who as a good friend, was always willing to help and give there best suggestions. Who encouraged and helped me at every stage of my personal and academic life and longed to see this achievement come true.*

I am also grateful to my late father ***Pandurang Amrut Patil*** *and my dearest mother* ***Saguna Pandurang Patil*** *who consistently provided me congenial environment for attiring total development to lead an empowered life. Words are insufficient to take account of encouraging and evergreen love. I wish to place on record the love and affection extended by my best elder Brother* ***Ram, Satish, Nitin, Sudhir and Pradip****. And my elder sister* ***Vandana and Jyoti****. And My best elder brother wife* ***Bhavini (wahini)*** *who always encourages, support me during my worst time.*

I special thanks to ***Google and Youtube*** *for providing me beneficial information in my thesis work and research work.*

Last but never least, I would like to place on record my sincere regard, deepest gratitude, soulful respect and a million thanks to the "God" the almighty, who helped me on every path of my life and made every step a great success and also my warm acknowledgements to farmers, soldiers, scientists and great social workers, who have devoted their whole life for the country. It is difficult to list all those who express my gratitude for their goodwill and moral support during my academic attainments, though nobody is mentioned none is forgotten too.

Place: Badnapur
Date:

(PATIL LALIT PANDURANG)

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ABBREVIATIONS

%	-	Per cent
@	-	At the rate of
a. i.	-	Active ingredient
µ	-	Microns
C.D.	-	Critical difference
CFU	-	Cell Forming Unit
Cm	-	centimeter (s)
Dia.	-	Diameter
e g.	-	Example Gratia (for Example)
et al.	-	*et alia* (and others)
etc.	-	Etcetera
Fig.	-	Figure (s)
Ha	-	Hectares (s)
Hrs	-	Hours
i.e.	-	That is
g	-	Gram
kg	-	Kilogram (s)
No.	-	number (s)
0C	-	degree Celsius
S.E.	-	Standard error
spp.	-	Species
T	-	Treatment
viz.,	-	videlicet (namely)
m	-	meter
mm	-	millimeter
N	-	Normal
pH	-	The potential of hydrogen ion
MT	-	Metric tonne
v/v	-	Volume by Volume

THESIS
ABSTRACT

"Study on Northern Leaf Blight of Maize Caused by (*Exserohilum turcicum* (Pass.) Leonard and Suggs."

Name	:	PATIL L. P.
Guide	:	JAGTAP G. P.
Department	:	Plant Pathology
College	:	College of Agriculture, Badnapur
Degree to be awarded	:	M. Sc (Agriculture)

ABSTRACT

Maize is a major cereal crop and India's third most significant crop after rice and wheat. The crop is afflicted by many fungal diseases, one of which is leaf blight, also known as northern maize leaf blight or turcicum leaf blight, which affects photosynthesis and reduces grain output by 28 to 91 %. Disease symptoms first emerge on the leaves at any stage of plant development, but most commonly at or after anthesis. The survey, variability, and management of turcicum leaf blight of maize caused by *Exserohilum turcicum* were studied. According to survey results, the district with the highest incidence and intensity of the disease had a mean maximum temperature of less than 30°C. The maximum incidence of 51.06 % was recorded in Phulambari teshil where the minimum incidence of 29.86 % was recorded in Aurangabad and The maximum intensity of 21.76 % was recorded in Phulambari teshil where the minimum intensity of 17.01 % was recorded in Kannad.

For the variability experiments, nine maize isolates of *E. turcicum* were used. All of the isolates differed in terms of cultural and physical characteristics. All nine isolates were classified into five groups based on colony colour. The pigmentation of the isolates SEt2, GEt3, KEt5, VEt6, SoEt9, and KhEt8 was noticeably different. Nine isolates had three different conidial shapes: curved, spindle, and elongated. Conidia has an average length of 73.79 µm and a width of 22.42 µm. The number of septa discovered ranged from 3 to 9. Conidia were found in all of the isolates. Conidia size was greatest in isolate PhEt7 (97.96 µm), with an average of 3-8 septation.

Temperature is a key component in regulating the fungus's development and reproduction. The effect of several temperatures on the mycelia growth of

Exserohilum turcicum, namely 20°C, 25°C, and 30°C, was investigated. Temperatures of 25-30° C promote larger mycelial growth of fungus, but temperatures of 20° C are less favourable.

The pathogenicity of all isolates was assessed using a spray inoculation approach on four maize varieties. There was significant variance among the isolates in terms of PDI, virulence index, lesion length, and latent duration. The isolate VEt6 from Vaijapur tehsil of Aurangabad district had the highest percent disease intensity (62.40), virulence index (6.16), lesion length (3.86 x 0.86), and shortest incubation period (4.36 days) among the 9 Aurangabad district isolates, followed by KEt5 from Kannad, District Aurangabad. The isolate SEt2 from the Aurangabad district's Sillod tehsil had the lowest PDI (36.06), the highest pathogenicity index (3.31), and the longest incubation period of 7.55 days.

In vitro testing of fungicides, bioagents, and botanicals revealed that systemic fungicides at 500, 1000, and 1500 ppm concentrations, Tebuconazole 25.9% EC, Carbendazim 50% WP, Propiconazole 25% EC, Azoxystrobin 23% SC, and Difenconazole 25% EC were effective in inhibiting the mycelial growth of the test fungus and contact fungicide Propineb 70 % WP (100 %) inhibited mycelia growth completely, followed by Merimain 50 per cent WP (78.92 %), Zineb 75 % WP (77.07), Mancozeb 75 % WP (74.51 %), Copper Hydroxide 77 % WP (70.10 %), and Copper Oxychloride 50 % WP (72.11). Dinocarp 48 % EC (60.88 %) demonstrated the least inhibition.

Among the all bioagent tested, maximum inhibition of mycelia growth (64.51 %) was observed in case of *T. asperellum* which was statistically at par with *T. harzhianum* (59.55 %), *Bacillus subtillis* (52.44 %) and *Pseudomonas fluorescens* (47.55 %) followed by *T. hamatum* (47.51%), *T. koningii* (43.77 %) and *Aspergillus niger* (46.03 %). Botanical study exposed that Garlic was found most operative at 5% % 10 %v concentration maximum inhibition of mycelial growth (73.70 %) was showed by Garlic followed by Aloevera (55.49 %) which was statistically at par with Neem (43.84 %) , onion (41.96 %) and Ghaneri (24.55 %).

Keywords : NLB (Northern leaf Blight), Cluster analysis, Isolate, bioagent, phytoextract and fungicide.

CHAPTER-I

INTRODUCTION

CHAPTER-I

INTRODUCTION

Maize (*Zea mays* L.) is among the most adaptable growing crops, with a wide range of adaptability under various agro-climatic conditions. Maize is regarded as the "Queen of Cereals" worldwide because it has the most significant genetic yield potential of any cereal. It is grown on about 150 million hectares in around 160 countries with diverse soil, environment, habitats, and management methods, accounting for 36 % (782 million tonnes) of the global grain supply. The United States of America (USA) is the world's largest producer of maize, accounting for approximately 35 % of overall demand, and maize is the engine that drives the US economy. The United States has the most significant productivity (> 9.6 t ha^{-1}) and is twice as productive as the rest of the world (4.92 t ha^{-1}). Maize, botanically known as Zea mays, is a member of the Gramineae grass family. It is a South American native (Mangelsdorf, 1974; Galinat, 1988) and has been the primary food for most people in Mexico, Central America, and Latin America since ancient times.

Maize is extensively grown in the world and has the highest yield of any cereal crop, with 972.40 million tonnes (Anonymous FAO, 2018 - 19). Maize was introduced to India from America in the early 17^{th} century, and it is now the third most valuable crop in India after rice and wheat. India's maize production is expected to be around 27.14 million tonnes (2018-19) (Anonymous FAO and Indiastat.com), with a productivity of around 25.6 q/ha (Anonymous USDA). It accounts for 9 % of the country's overall food grain intake. Maharashtra (10.5 million tonnes), Karnataka (3.3 million tonnes), Madhya Pradesh (2.6 million tonnes), Bihar (2.5 million tonnes), Telangana (1.8 million tonnes), and Uttar Pradesh (1.3 million tonnes) are the top maize growing states in India. Maize has a wider range of uses because of its worldwide distribution and relatively lower price. It is primarily used as human food, animal feed, and poultry feed, as well as a raw material in a variety of agricultural goods such as starch, food sweeteners, alcoholic drinks, cosmetics, gum, textiles, packaging and paper. The use of maize to produce ethanol, a replacement for petroleum-based fuels, is gaining popularity these days. Maize is also used to produce oil, which lowers blood cholesterol levels and is safe for human consumption.

Maize is a potential source of carotenoids like beta-carotene, lutein, zeaxanthin and cryptoxanthin which have highly varied health benefits such as

maintaining normal vision and lowering oxidative stress (Chaudhary *et al*. 2014). Maize demand in India is increasing as a result of evolving food preferences and diverse maize uses in industry. Maize is susceptible to 112 diseases in various parts of the world, which are caused by fungi, bacteria, viruses, and nematodes and cause significant damage. Approximately 61 crop diseases have been reported in India.These include seedling blights, stalk rots, foliar diseases, downy mildew and ear rots (Payak *et al*. 1973, Payak and Sharma, 1985). In contrast to America and Europe, India's maize production is very poor due to a variety of biotic and abiotic stresses.

Among the fungal diseases, *turcicum* leaf blight caused by *Exserohilum turcicum* (Pass.). Leonard and Suggs. (Synonyms: *Helminthosporium turcicum* (Pass.) Leonard and Suggs.) [Perfect stage: *Setosphaeria turcica* (Luttrell) Leonard and Suggs. (Synonym: *Trichometasphaeria turcica* (Luttrell)] is one of the important foliar disease causing a severe reduction in grain and fodder yield to the tune of 16 – 98 % (Kachapur and Hegde, 1988). Passerini (1876) from Italy and Butler (1907) from India were the first to describe the disease. This disease is prevalent in Maharashtra, Andhra Pradesh, Karnataka, Bihar, and Himachal Pradesh in India. In the United States of America, this fungus is known as “Northern Corn Leaf Blight” (NCLB), “White Blight,” and “leaf stripe.” Turcicum blight injures or destroys leaf tissues, reducing the region of green chlorophyll that produces food for the plant. When a large portion of the leaf area is destroyed, the vigour and yields are diminished. If much of the green area is killed, starch formation is restricted, and the kernels are chaffy. The blighted leaves are not suitable for fodder because of the lowered nutrition value. Pant *et al*. (2001) recorded a 91% reduction in photosynthesis rate when the intensity of turcicum leaf blight incidence in maize exceeded 50%. The disease is more prevalent in humid areas with moderate temperatures (Pataky *et al.* 2006). However, it is widely distributed; however, sporadic, and its development mainly depend on weather conditions, stage of plant growth, and resistance in maize cultivars (Perkins and Pedersen, 1987). The pathogen has a wide host range and high pathogenic variability (Muiru *et al.* 2010). The pathogen affects the whole plant, but the most visible symptoms/lesions are located on the foliage. Lesions defoliate the leaves, causing yield declines owing to a shortage of carbohydrate to fill the crops. Heavily infected fields present a scorched or burnt appearance resulting in the premature death of leaves (Harlapur et al. 2007). TLB causes extensive leaf damage and defoliation during the grain filling period, and yield losses due to necrosis or

chlorosis of leaves premature death of the leaves and loss of nutritive value even as fodder (Patil et al. 2000) has been reported. Yield losses of up to 28 to 91% due to TLB has been reported in Italy, mostly when heavy infection occurred before tasselling (FAO, 2010).

Several varieties with high yield potential that were recently released have been shown to be prone to leaf blight. With the availability of high yielding varieties and the need to achieve high grain yields and reasonable market prices, farmers began using unnecessary nitrogen and closer spacing to increase plant population, which predisposed the crop to turcicum leaf blight and resulted in low grain and fodder yields (Patil *et al.* 2000). Increased crop density and heavy use of a single nutrient can have an impact on the plant's photosynthetic behaviour by limiting light interception (Pant *et al.* 2001). Several management practices, such as resistant varieties (Lal, 1993), fungicides (Harlapur *et al.* 2007), and biocontrol agents (Harlapur *et al.* 2007), have been used alone or in tandem with high-input prices, lowering net returns.

Considering the disease's economic significance in the Marathawada region, especially in the Aurangabad district, the current research on "Study on Northern Leaf Blight of Maize Caused by (*Exserohilum turcicum* (Pass.) Leonard and Suggs." was undertaken with the following objectives.

1.1 OBJECTIVES

1. To survey the maize field to record the incidence and severity of Northern leaf blight in Aurangabad district.

2. To isolate, identify and prove the pathogenicity.

3. To study morpho-cultural, physiological and pathological variability among *E. turcicum* isolates.

4. To evaluate *in-vitro* efficacy of various fungicides, bioagents and botanicals against pathogen *E. turcicum*.

CHAPTER-II

REVIEW OF LITERATURE

CHAPTER-II

REVIEW OF LITERATURE

The following is a summary of research work conducted in India and abroad on different issues related to the current investigation, titled "Study on Northern Leaf Blight of Maize Caused by *(Exserohilum turcicum* (Pass.) Leonard and Suggs." has been reviewed and presented as under:

2. 1 Historical background and Nomenclature

Turcicum leaf blight of maize also known as Northern leaf blight of maize is caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs. The disease was first reported from Perma, Italy by Passerini in the year 1876. Earlier it was named *Helmin thosporium turcicum* Pass but later on, it was regarded to be the same as *Trichometas phaeria turcica* Luttrell by Pammel *et al*. (1910) and Drechsler (1923). It was further renamed as *Setosphaeria turcica* by Leonard and Suggs (1974) and described the conidial stage as *Exserohilum turcicum* (Pass.) Leonard and Suggs, having strongly protuberant conidial hilum. In India, the disease was first reported by Butler in 1907 from Bihar. Later it was reported from many parts of the country, *viz*., Himachal Pradesh (Chenulu and Hora, 1962), Lalmardi, Srinagar (Kaul, 1957), Kashmir valley (Payak and Renfro, 1968) and Punjab (Mitra, 1981). Lutrell, (1958) reported that *Trichometasphaeria turcica* Luttrel, the sexual stage of the fungus, rarely occurs in nature. The causal agent of turcicum leaf blight on maize is normally identified as *Exserohilum turcicum*, its imperfect stage. Leonard and Suggs (1974) have proposed the new nomenclature of the organism as *Exserohilum turcicum* (Pass.) Leonard and Suggs (imperfect stage) and *Setosphaeria turcica* (Lutrell) Leonard and Suggs (perfect stage). The systemic classification of *Setosphaeria turcica* (Lutrell) Leonard and Suggs are as follows:

Setosphaeria turcica (Sexual stage)

Kingdom	:	Fungi
Division	:	Ascomycota
Class	:	Dothidiomycetes
Order	:	Pleosporales
Family	:	Pleosporaceae
Genus	:	*Setospaeria*
Species	:	*S. turcica*

Exserohilum turcicum (Asexual stage)

Kingdom	:	Fungi
Division	:	Deuteromycota
Class	:	Hyphomycetes
Order	:	Moniliales
Family	:	Dematiaceae
Genus	:	*Exserohilum*
Species	:	*E.turcicum*

2. 2 Disease Survey and distribution

Jordhan *et al.* (1983) conducted a survey and collected turcicum leaf blight isolates from corn fields of Florida, Illinois, Indiana, Iowa, Minnesota, New York and Pennsylvania.

Laxminarayana and Shankerlingam (1983) surveyed in Arabhavi and Nagenahalli in Karnataka, Kolhapur in Maharashtra, Karimnagar in Andhra Pradesh, Dholi in Bihar, and Almora in Uttarakhand have all been listed as endemic areas (hot spots) for the disease.

Leonard *et al*. (1985) reported that the second week of August, an outbreak had spread through North Carolina, killing up to 75 % of the leaf region.

Gowda P. (1987) surveyed the major maize-growing areas of Southern Karnataka, disease incidence ranged from 10 % to 100 % on most hybrids and composites. The Arabhavi Centre conducted an AICRP maize survey, which showed that disease severity was mild to high and spread through all of Northern Karnataka's maize-growing regions.

Gowda *et al.* (1989) surveyed the occurrence of maize turcicum blight during 1987-1988 and found that disease severity was mild to extreme on commercially cultivated maize hybrids in Southern Karnataka.

Harlapur (2005) surveyed all Karnataka's maize-growing regions Belgaum district had the highest disease prevalence (55.89 per cent), followed by Mysore (54.76 percent), Davanagere (53.86 per cent), Mandya (53.85 percent), and Haveri (53.85 percent) (53.85 per cent).

Khedekar *et al*. (2010) reported that high percentage of disease severity (53.16 %) was noticed in Haveri taluk. In Belgaum district, the most prevalent disease was turcicum leaf blight in all the surveyed fields. Yargatti recorded the highest incidence (50.35 %) in Soudatti taluka. The lowest incidence was noticed at Kushtagi taluka (33.88 %) followed by Koppal (39.45 %) The survey, work indicated that in Bagalkot districts disease incidence observed was higher.

Geeta D. S. *et al*. (2018) reported that northern corn leaf blight (NCLB), also known as turcicum leaf blight, was found in almost every region surveyed. The highest disease prevalence was observed in fields in the Ballari district's Choornoor (40.00 %), Kottur (38.00 %), and Sovenahalli (34.00 %) villages. Due to continuous maize cultivation and strong rainfall in August and September, TLB was more prevalent in Ballari.

2.3 Symptomatology

Chenulu and Hora (1962) reported the first signs are small elliptical patches on the leaves that are greyish green in colour and have water-soaked lesions. Later spots become larger, eventually becoming a spindle pattern. Individual spots are usually 3/4 inch wide and 2 to 3 inches deep. On both sides of the spot, the fungus produces a large number of spores. The heavily infected field has a scorched look, with dead and grey trees.

Ullstrup (1966) described the disease's signs in the United States the disorder is characterised by long elliptical greyish or tan lesions. The spots may be 112” by 6”. These lesions begin on the lower leaves and grow in number as the season progresses, eventually covering all of the leaves.

Fredericksen, (1980) reported leaf blight lesions are elongated, elliptical lesions that measure 2.5 – 15 cm in length and 12 mm in width and appear on the leaf lamina.

Reddy and Bonman (1987) stated the severe blast epidemics caused by *P. oryzae* have recently occurred on rice in India and Egypt. The Indian Directorate of Rice Research survey teams registered blast on 120000 ha in the states of Andhra Pradesh, Karnataka, and Tamil Nadu during the wet and dry seasons of 1984 and 1985. Improved introduced IR 50, conventional introduced and improved locally produced NLR 9672, Tellahamsa, and TKM 9 were among the cultivars affected. According to the State Department of Agriculture, the explosion resulted in a gross loss of 140000 t of grain.

Vieira *et al*. (2014) Usually, small flecks appear at 3-4 days after favourable infection, while large distinctive lesions appear two weeks later. As the disease progresses, single lesions might join collectively to form vast blighted zones.

Li and Wilson, (2013) reported that symptoms vary from cigar-shaped lesions on lower leaves to vegetation death, reducing the amount of leaf surface area required for photosynthesis.

Sajeed and Chowdhury (2014) observed the first signs of TLB appear as small grey spots on the lower leaves that extend upwards. The spots gradually became larger and became spindle-shaped with pointed ends, a grey centre, and a dark brown border. the lesions ranged in length from 2.0 cm to 15 cm and width from 0.5 to 2.0 cm.

2.4 Pathogenicity

Chenulu and Hora (1962) reported that infection of *Exserohilum turcicum* on maize occurs from seedling to harvesting. However, maximum disease severity was noticed from the tasselling stage and six to eight weeks after a silking stage which resulted in heavy loss. The disease became well established before or at the silking stage.

Ullstrup (1966) stated that conidial germination and penetration of *E. turcicum* on the leaves took place in six to 18 hours when leaves are moist and temperature ranges between 65°F and 80°F.

Aden (1991) conducted pathogenicity tests of *E. turcicum* causing Sorghum leaf blight with three conidial concentrations (20,000, 10,000, and 5,000 conidia/ml) on sorghum showed that 20,000 conidia/ml caused the highest infection (>40 % leaf area damaged), while 5,000 conidia caused the lowest infection (10 % leaf area damaged).

2.5 Morpho- Cultural, Physiological and pathological studies

Nisikado (1927) observed that rice decoction agar was more favourable for mycelial growth compared to Hopkin's nutrient solution. The different light regimes had a significant effect on the sporulation and growth rate of *E. turcicum* isolates. The type of media and incubation temperatures had a significant effect on the growth rate of different isolates. The optimum temperature for growth was 25ºC and only one isolate showed minimal growth below 10º C and no mycelia growth was observed in all the isolates at 40º C.

Christensen (1929) studied the species of *H. sativum* and reported that the spores vary in length, width, shape and septations of conidia when grown on different media.

Champi (1939) reported good growth of the fungus on various standard media.

Pandey and Shukla, (1979) reported Best growth of the fungus was observed in Richard's and Czapek's media.

Luttrell, (1958) reported that the asci contain from one to six ascospores which are straight, three to six septate, and typically hyaline, although with ageing they may become brown and surrounded by a mucous-like sheath.

Bugnicourt (1955) studied *Helminthosporium* sp. and described morphological characters. He described cultural characters as, colonies were effuse, grey to blackish brown or grey, stromata sometimes formed in culture, erect, straight cylindrical and black. Hyphae to be pale to mild brown, smooth, septate and about 1-3 μm thick. Conidiophores were solitary flexuous, geniculate, septate pale to mid-brown measure 120 x 2-7 μm, whereas conidia were straight, ellipsoidal, oblong or cylindrical, round

at the ends, pale to mid-brown, 2-7 pseudo septate and measure 12-37 (24.5) x 5-11 (8.2) μm.

Robert (1960) and Rodriquez (1961) reported the physiologic specialization in maize and sorghum isolates was tested in their respective hosts and variations were observed in morphological and cultural characteristics of the isolates.

Robert and Sprague (1960) assessed the response of eight inbred lines of corn, to twenty-seven single-conidial strains of *E. turcicum* by rating disease on a scale from one to 11, the response of 27 strains to corn ranged from 1.1 cm^2 to 7.1 cm^2, along these lines showing an extensive variability of aggressiveness.

Misra and Singh (1963) reported Effect of temperature and humidity was studied on the development of a maize isolate of *Helminthosorium turcicum* and it was observed that the optimum temperatures for spore germination, growth of the fungus in the culture, and infection and development of disease were 20-30°C, 25-30°C, and 30°C, respectively.

Leonard and Suggs (1974) observed the conidia of *Exserohilum turcicum* were 18-23 u wide and 73-137 u long with 4-9 septa and born singly at the tips of the conidiophores. *Exserohilum* can be separated from different graminicolous *helminthosporoid* genera by a protruding and truncate hilum. The sexual morphology of *Exserohilum* has been set in *Setosphaeria.*

Levy and Cohen (1980) observed that maximum conidia were produced in a dew period of 30 hours at 20-25°C and 20°C was optimum for conidia formation whereas the optimum range varies between 11 and 35°C.

Alcorn (1988) observed that the conidia are fusoid, obclavate, linear, or angled, with a slightly protruding hilum. Conidia germinate mostly from one or both polar cells, and only occasionally from intermediate cells. The conidiophores are brown, irregularly cylindrical, and 3-7 septate (7-11 x 165-283 u). They appear in clusters of two to six or more from stomata or, less commonly, directly from the epidermis. *S. turcica*, the sexual stage of *Exserohilum turcicum*, is distinguished by the appearance of ostiolate, dark brown perithecia covered with short stiff spine-like hairs on the upper third of the perithecial wall.

Aden (1991) studied the optimal temperature for colony growth was 25°C (21mm), and the optimal temperature for sporulation was 20°C. After 12 days of incubation, the highest colony growth was observed on lactose casein hydrolysate agar at 30°C. Colony development and sporulation were at their peak between 20 and

30 °C, but were severely impaired at 35 and 15 °C. Lactose. casein Hydrolysate Agar was the perfect media for colony growth at all temperatures (16.5 mm)

Levy and Pataky (1992) demonstrated that strains from different geographical areas showed distinct parasitic behaviour, while strains from the same area showed less heterogeneity.

Daniel and Narong (2006) investigated there were variations in the morphological characteristics of *E. turcicum* strains, and the conidia were twisted, elongated, and spindly in shape. The average conidial length and width were found to be 93.97 m and 13.11 m, respectively, with a range of 2-7 septations.

Muiru *et al*. (2008) reported that different media, isolates from different agro-ecological zones differ in terms of growth, morphology, pigmentation, and sporulation rate.

Harlapur and Kulkarni (2009) The germination of conidia of *E. turcicum [Setosphaeria turcica*], which causes maize northern leaf blight, was measured at four-hour intervals from 4 to 36 hours. After 36 hours of incubation, the highest conidial germination (94.20 %) was observed, while the least germination (7.67 %) was observed after 4 hours. After 16 hours of incubation, more than half of the seeds germinated. However, no noticeable rise in conidia germination was observed from 28 to 36 hrs of incubation.

Gowda *et al.* (2010) studied the isolates of maize Northern Leaf Blight (NLB) caused by *E. turcicum* differed in their cultural and morphological characteristics. Czapek's medium, glucose peptone medium, maize leaf extract medium, potato dextrose agar medium, and Richard's medium were used to test cultural heterogeneity. Variations in mycelia weight, sporulation, and morphological characteristics were observed.

Muiru *et al*. (2010) stated that the virulence level of *E. turcicum* isolates varied. In isolates B3, B5, and G7, the lesions combined to form massive chlorotic and necrotic lesions, while in isolates Sorte 2, S62, and G5, the lesions persisted as small chlorotic specks confined in the regions protected by the drop of the spore suspension.

Bunker *et al.* (2011) pathogenic heterogeneity was discovered in five isolates of *Bipolaris maydis* from Rajasthan, Haryana, and Uttarakhand, and the isolates varied in virulence and aggressiveness on a collection of pot grown ten inbred maize

lines. Isolates from Udaipur, Rajasthan, and Pantnagar, Uttarakhand were the most virulent and violent, led by isolates from Undithal, Gorana, and Karnal, Haryana.

Reddy (2012) studied sporulation of eight *E. turcicum* isolates were divided into three classes. The pigmentation of *E. turcicum* isolates was divided into three categories: black, bluish-black, and greenish-black.

Kutawa *et al.* (2016) studied the development of five isolates at various pH levels ranging from 5, 7, and 9. However, pH 7 was found to be the best for growing *E. turcicum*, with 4.72 mm/day, 6.36 mm/day, and 6.96 mm/day on the third, fifth, and seventh days after incubation, respectively. This was followed by pH 9 with 4.84 mm/day, 6.14 mm/day, and 6.94 mm/day over the same incubation times. The least development was detected at pH 5 one week after incubation, with 3.58 mm/day, 5.0 mm/day, and 6.02 mm/day on the third, fifth, and seventh days, respectively.

Vinay M. R. and Sataraddi A. R. (2019) reported the district of Northern Karnataka yielded 20 isolates of *E. turcicum*. They were grown in Potato Dextrose Agar (PDA) and shown a range of cultural and morphological characteristics. All of the isolates were determined to be jowar pathogenic. The virulent isolate (E04) displayed major differences in colony colour, form, soil, and topography. Except for Et07, Et09, and Et17, conidia were found in all isolates. The PDA medium aided the isolate E04's maximum average development (7.35 cm)

Geeta D. S. *et al*. (2019) reported the northern leaf blight of maize is a significant economic foliar disease caused by *Exserohilum turcicum*. The Et 9 (Choornoor) isolate had the lowest mean radial growth of the 32 isolates tested for mycelia growth on PDA. The largest conidia were found in Et16 of Hadagali and Et28 of Kanakagiri. Inbred lines 9202B and 9208B demonstrated high resistance to mildly reactive reactions (1-3), while CI-4 and HS-2 demonstrated high resistance to a resistant reaction. The septa of 3-8 and 3-9 with protruding hilum is found in the majority of *E. turcicum* isolates.

2.6 In-vitro Management Studies

2.6.1 *In-vitro* evaluation of fungicides

Cox (1956) observed that the maneb formulations were most effective in minimising the *H.turcicum* severity under field conditions followed by ziram, vacide and Z-65. These fungicides increased grain yield and seed quality.

Miller (1970) observed that foliar application of mancozeb, zineb and propiconazole, was effective against southern leaf blight of maize caused by *H. maydis.* It was observed that seed germination also improved by seed dressing with maneb, captan, carboxin + thiram and benomyl + thiram.

Levy and Cohen (1980) observed that maximum conidia were produced in a dew period of 30 hours at 20-25°C and 20°C was optimum for conidia formation whereas the optimum range varies between 11 and 35°C.

Kachapur and Hegde (1988) observed that mancozeb and captafol were the most effective fungicides for controlling Northern leaf blight of maize among the seven fungicides tested.

Singh and Kaiser (1989) observed that the mycelial growth and conidial germination of *Exserohilum turcicum* causing leaf blight of maize was completely inhibited by bavistin (carbendazim) and vitavax (carboxin).

Rehman *et al.* (1993) observed that tilt (propiconazole) was effective against *Exserohilum turcicum* on maize in *in-vitro* conditions.

Begum *et al.* (1993) evaluated five fungicides for control of artificial infections *of E. turcicum* on susceptible maize cultivars. All the chemicals 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.

Meli and Kulkarni (1994) evaluated ten fungicides and confirmed that propiconazole gave complete inhibition followed by tridemorph against *E. hawaiinesis* causing leaf blight of wheat.

Dharanendraswamy (2003) Carboxin and zineb were highly effective in inhibiting mycelial growth of *E. turcicum* causing leaf blight of maize.

Harlapur *et al.* (2007) reported maximum mean per cent inhibition (100 %) of mycelial growth of *E.turcicum* was shown by mancozeb (0.25 %) followed by carboxin powder (0.1 %) which showed 99.16 % inhibition of mycelia growth.

Khedekar (2012) observed that among nine fungicides tested against *E. turcicum*, Cristol 56 SL and carboxin 200 FF were most effective at 0.025 %, 0.05 % and 0.1 % concentration with 100 % mycelial growth inhibition.

Wathaneeyawech *et al*. (2014) evaluated three fungicides chlorothalonil, difenoconazole and mancozeb were tested on *E. turcicum* using the poisoned medium method. The fungicides were found to inhibit the growth of isolates MHP5, TN3, MJ4, JT4 and JT5 of *E. Turcicum*.

Kumar and Mauriya (2015) evaluated Six fungicides were used (Metalaxyl 72 WP, Thiophanate methyl 70 WP, Zineb 75 WP, Propineb 70 WP, Copper oxychloride 50 WP, and Mancozeb 63 % + carbendazim 12 %). Zineb 75 WP at 0.25 % concentration was found to be the most effective in inhibiting the growth of *E.turcicum*, resulting in low disease severity and a higher grain yield of maize. Mancozeb (63 %) + Carbendazim (12 %) @ 0.25 %) is found to be similarly efficient and can be used as an alternative to Zineb. Zineb 75 WP inhibited mycelial growth 99.10 % more effectively and statistically than Mancozeb 63 % + carbendazim 12 %, which inhibited mycelial growth 98.40 % more effectively.

Manu *et al*. (2017) observed that tebuconazole, a systemic fungicide, totally inhibits pathogen development at all concentrations tested. Propineb was highly effective in contact fungicides, inhibiting *E. turcicum* up to 83.89% at 500 ppm, and the combi-product, Carbendazim 12% + Mancozeb 63 %, inhibited *E. turcicum* mycelial development completely at 500 ppm and higher concentrations.

Wani *et al.* (2017) observed that Propiconazole was found to be the most effective systemic fungicide in inhibiting the mycelial growth of *E. turcicum* (96.51 percent mean inhibition), whereas mancozeb was found to be the most effective non-systemic fungicide (95.23 percent mean inhibition), when tested in field conditions, two foliar sprays with a non-systemic fungicide, mancozeb 75 WP @ 0.25 % reduced the diseased intensity from

Bhatt and Kumar (2019) reported that eight fungicides were tested *in vitro* against the Northern Leaf Blight of Maize caused by *Exserohilum turcicum* (Pass) Leonard and Suggs. Propiconazole 25 % displayed the greatest inhibition of mycelial development (92.22 %) *in vitro* at 5 ppm concentration.

2.6.2 *In-vitro* evaluation of botanicals

Shivapuri *et al.* (1997) studied the plant extracts were tested for antifungal properties against five pathogenic fungi, and it was discovered that ethanol extracts of *Azadirachta indica, Allium cepa, Ocimum sanctum*, and *Polyalthia longifolia* inhibit pathogen development under laboratory conditions.

Meena *et al.* (2003) observed that the effectiveness of plant extracts improved when they were sprayed 24 hours before inoculation.

Mares *et al*. (2004) assayed *Tagetes patula* methanol extracts were tested against three phytopathogenic fungi: *Fusarium moniliforme, Botrytis cinerea*, and *Pythium ultimum*. The antifungal function was evaluated under both dark and light environments, using various lighting systems. The extracts had dose-dependent efficacy against all of the fungi, with a noticeable gap between treatments in the sun and treatments in the dark. Growing rose in the dark at concentrations of 5 and 10mg/ml.

Harlapur *et al.* (2007) assayed Neem seed kernel extract (NSKE) at 5 % was highly effective against *Exserohilum turcicum,* causing maximal growth inhibition. *In vitro* antifungal efficacy of *Polyalthia longifolia* aqueous extract (10-50 % concentration) against ten seed-borne fungi of paddy (*Oryza sativa.* L) was examined. At 50 % concentration in the extract, maximum inhibition was observed against A. alternata (92.88 percent), led by *F. solani* (87.10 percent) and *F.moniliforme* (88.10).

Lalitha *et al.*(2011) reported maximum inhibition in all the test fungi was recorded with solvent extract of petroleum ether extract at 1000 µl concentration.

Sharma and Sharma (2011) evaluated using a soxhlet assembly, the leaves of *Lawsonia inermis* Linn. and *Eucalyptus citriodora* Hook. were extracted with petroleum ether, benzene, chloroform, acetone, ethanol, and water. The poison food technique was used to monitor the antifungal activity. The inhibitory activity was important and superior to that of synthetic fungicides. The majority of the strains were resistant to fluconazole and amphotericin B.

Sharma *et al*. (2012) studied *Durenta erecta* L. has antifungal action against the following phytopathogenic fungi: *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus*, and *Penicillium sp*. The leaf extract was shown to have antifungal efficacy against all *Aspergillus spp.,* with the maximum inhibition reported against *A. fumigatus.*

Singh and Singh (2014) reported that garlic was found to be most effective in suppressing radial growth of *Exserohilum turcicum*. Jatropha, neem and garlic reduced disease severity by 41.01, 43.38 and 46.36 % respectively under glasshouse conditions. Increased number of sprays resulted in an improved reduction in disease severity.

Manu *et al.* (2017) reported the effect of plant extracts on the percent inhibition of *E. turcicum* mycelial growth at three concentrations varies considerably, and increasing the concentration of botanicals increases efficacy. Of the 11 plant extracts, garlic bulb extract had the highest mean percentage inhibition of mycelial growth (79.63 %). Ocimum leaf extract (7.59 %), Lucas leaf extract (12.78%), agarwood leaf extract (17.22 %), and noni leaf extract (30.19 %) displayed less percent inhibition against the pathogen. Garlic was found to be 100 % inhibitory of myelial development at a concentration of 10 %.

Singh and Dutta (2017) evaluated Lantana (*Lantana Camara*) and Datura (*Datura innoxia*) for their bio-efficacy against *E. turcicum* using a poisoned food technique. Among plant extracts, lantana (10 %) showed maximum inhibition of 43.87 %. While Datura 10 % and 20 % concentration was found less effective in comparison.

Vishwanath *et al.* (2017) observed that Neem seed kernel extract (NSKE) at the rate of 5% was effective in mycelia growth inhibition (64.1 %) of *Exserohilum turcicum* followed by aloe-vera at the rate of 10 % (17.7 %). Under field conditions NSKE@ 5 % showed disease reduction of 23.5 % followed by aloe-vera @10 % with 22.7 % and lantana leaf extract @10 % (11.8 %).

Bhatt and Kumar (2019) Among all the tested botanicals Heena (*Lawsonia inermis*) was found to be most effective in inhibiting mycelial growth (71.11 %) at 10 % concentration. Lemon tulsi oil was found best in inhibiting mycelial growth (71.30 %) of *E. turcicum*, among all the tested essential oils at 50 ppm concentration. Fungicides, plant extracts and essential oils showing good results under *in vitro* conditions were tested under glasshouse conditions.

2. 6. 3 *In-vitro* evaluation of Bioagents

Mahamood *et al.* (1995) reported the biological control agents such as *Trichoderma sp*., *Aspergillus sp*., and *Cladosporium sp*. were highly successful in inhibiting mycelial growth and sporulation of *E.turcicum*, which causes maize leaf blight.

Ramchandra (2000) evaluated antagonists against *E. hawaiiensis in vitro* and found that *T. viridae* and *T. harzianum* reduced the growth and sporulation of the fungus significantly.

Harlapur *et al.* (2007) recorded maximum mean per cent inhibition of mycelial growth by *Trichoderma harzianum* (65.17 %) followed by *T. viridae (*56.95 %) against *E.turcicum* among all the biocontrol agents tested *in vitro* conditions.

Singh and Singh (2014) studied 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 gave maximum inhibition of mycelial growth of the pathogen by 77.11 and 56.00 per cent respectively.

Kumar *et al.* (2016) evaluated under field and laboratory conditions, the effectiveness of four bioagents – *Pseudomonas fluorescence, Trichoderma viridae, Trichoderma harzianum*, and *Bacillus subtilis* – against brown spot of rice was investigated. Bioagents were used as seed treatments and foliar sprays in the area. Pseudomonas fluorescens @ 4g/kg seed+foliar spray *Trichoderma viridae* @10g/l water was found to be beneficial with a 34.67 percent reduction in disease incidence and a 42.96 q/ha increase in grain yield. Trichoderma viridae inhibited the development of *Helminthosporium oryzae* the most effectively (61.72 %) *in vitro.*

Vishwanath *et al*. (2017) studied the biocontrol agent *T. harzianum* was tested against *Exserohilum turcicum* and found to be successful in inhibiting mycelial development (64.1 percent), followed by *Trichoderma viridae* with a 58.7 percent inhibition. In field conditions, *T. harzianum* at a rate of 2% reduced disease by 32.3 percent, while *T.viridae* at the same rate reduced disease by 29.2 percent..

Manu *et al*. (2017) evaluated *Trichoderma harzianum* -2 inhibited mycelial development the most (98.65 %), followed by *Trichoderma viride* (98,34 %) of the five bioagents studied, *Trichoderm Rifai*-1 inhibited mycelianum development the

least (85.37 %). The authors argue that the analysis failed to classify the microorganisms responsible for the antagonistic effect against *Exserohilum turcicum in vitro.*

CHAPTER-III

MATERIALS
&
METHODS

CHAPTER-III

MATERIALS AND METHODS

The present study on "Study on Northern leaf Blight of Maize Caused by *Exserohilum turcicum* (Pass). Leonard and Suggs.' was conducted during 2020-2021. The experiments were conducted in the laboratory of the Department of Plant Pathology, College of Agriculture, Badnapur and National Agriculture Research Project, Aurangabad. The details of materials and methods adopted are described as under:

3.1 Laboratory experiments

3.1.1 Glassware, Chemicals and Equipment

Borosil made glassware was used throughout the present investigation. All the glassware were washed thoroughly with a detergent and rinsed in running tap water. Then they were soaked in a cleaning solution for 24 hrs and finally rinsed with distilled water 3-4 times and air-dried.

3.1.2 Composition of cleaning solution

Potassium dichromate (K2 Cr2O7) : 60 g

Concentrated Sulphuric acid (H2 SO4) : 60 ml

Distilled water : 1000 ml

Chemicals of guaranteed reagent (GR) and analytical reagent (AR) grades of standard made were used.

3.1.3 Types of equipment

A compound microscope was used for observing the fungi while for weighing chemicals, a single pan electronic balance with a sensitivity of 0.001g was used. A hot air oven was used for sterilisation of glassware and media was sterilised using Autoclave. BOD incubators were used for incubating cultures at desired temperatures and cultures were stored in a refrigerator at 4^{o} C.

3.1.4 Sterilisation of Glassware and Media

i. Petri plates were sterilised in a hot air oven at 160 º C for 90 minutes.

ii. Different media and water were sterilised at 15 psi (121.6ºC) for 20 minutes in an autoclave.

iii. Workbenches were sterilised using ethyl alcohol.

iv. Sodium hypochlorite (NaOCl) 2.0 % was used for surface sterilization of plant materials and rectified spirit for other equipment like inoculation needles, forceps, inoculation chamber and hands.

v. Cork borer, scalpel and inoculation loop were sterilised over a flame.

3.2 Laboratory techniques

The general laboratory techniques described by Nene and Thapliyal (1993), Dhingra and Sinclair (1995) were followed in the present research work for the preparation of media, sterilisation, isolation and maintenance of fungal cultures with slight modification wherever necessary. The poisoned food technique was used for testing the efficacy of fungicides and botanicals.

3.3 Survey

3.3.1 Disease status

Survey of maize growing areas of Aurangabad, Sillod, Gangapur, Paithan, Kannad, Vaijapur, Phulambri, Khultabad and Soegaon talukas of Aurangabad district was conducted in July-August during *Kharif* 2020 to collect the diseased samples for the isolation of *E. Turcicum* isolates and to assess the status of the disease. The Multistage Sampling Scheme technique was adopted for the survey. Three representative villages were taken from each of the talukas of the district and three random maize fields selected from each village. Plants from each field were selected randomly for recording on incidence and intensity of the disease. The details of the districts surveyed and the areas where diseased samples were collected for variability studies are given in Table 3.1. The total number of plants examined and the number of plants showing NLB symptoms was recorded from each plot and per cent disease incidence calculated by using the formula:

$$\text{Disease incidence (\%)} = \frac{\text{No. of a diseased plant in quadrate}}{\text{Total no. of plants assessed}} \times 100$$

$$\text{Disease severity(per cent)} = \frac{\Sigma \text{ of all numerical ratings}}{\text{The total number of plants observed} \times \text{maximum disease grade}} \times 100$$

Table 3.1: Details of the places surveyed for collection of turcicum leaf blight Isolate from Aurangabad district

Sr.No	Isolates code	Talukas
1	AEt1	Aurangabad
2	SEt2	Sillod
3	GEt3	Gangapur
4	PEt4	Paithan
5	KEt5	Kannad,
6	VEt6	Vaijapur
7	PhEt7	Phulambri
8	KhEt8	Khultabad
9	SoEt9	Soegaon

3.4 Isolation, purification, identification and pathogenicity of *Exserohilum turcicum pathogen*

3.4.1 Isolation of pathogen

Diseased maize leaf samples collected during a survey from different locations were attempted for the isolation of *E. turcicum*. The infected leaves were cut with help of a sterilised blade into pieces of 2-3 mm size having half healthy and half diseased tissues. The small pieces were sterilised with sodium hypochlorite solution 2% for 30 seconds and thoroughly washed in sterilized water three times. Then the pieces were placed between two layers of sterilized blotter paper to remove the excess water. These pieces were then transferred to slants and Petri plates containing PDA medium inside the laminar flow chamber under aseptic conditions, followed by incubation at 28±2º C. The growth of the fungus was conspicuous after 24 hrs of incubation. The pure colonies which developed from bits were transferred to PDA slants and incubated at 28±2 º C temperature for seven days. Seven days later when

abundant sporulation occurred; the pathogen was purified using the hyphal tip isolation technique.

3.4.2 Purification

The fungus isolates thus isolated from the infected tissue were further purified by the single spore isolation method as described by Ho and Ko (1997). Spores were harvested from a 10-day old culture with the help of an inoculation needle and suspended in one ml of sterile distilled water in a culture tube. The suspension was vortexed for making a uniform suspension. The concentration of the spores was adjusted by the addition of sterile distilled water. One ml of the spore suspension was transferred to Petri plates containing 15 ml of 2 % water agar medium. The plates were incubated at 25 ± 1 ºC for five hours. A well isolated single spore that initiated germination was located by observing the plate in an inverted position under a compound microscope. Correspondingly a circle was marked around the spore over the plate and a circular disc of the medium containing the spore was picked up with the help of a sterile needle and transferred aseptically to Petri plates containing PDA medium and incubated at 25 ± 1 º C for further studies.

3.4.3 Identification

The fungus was identified as *Exserohilum turcicum* based on morphological characteristics, cultural characters (colony colour, texture and appearance). To describe the hyphal morphology of the pathogen, photomicrographs were taken.

3.4.4 Pathogenicity Test

The spore suspension was prepared from a 10-day old culture of each isolated separately and the spore concentration was adjusted to 4×10^5 spores per ml with the help of a haemocytometer. The pathogenicity of isolates was tested by spray inoculating with an automizer on thirty days old plants of susceptible maize variety. The test plants were covered with a polythene bag for 18 hrs to maintain high humidity for initiating the fungal infection.

3.5 Morpho-cultural, physiological and pathological variability among *E. turcicum* isolates.

3.5.1 Morpho-cultural Studies

To study morphological characters of conidia of all the isolates of Exserohilum turcicum, slides were prepared from twelve days old culture. Temporary slides were prepared water mount using cotton blue. Data on length, width and septation of conidia was recorded by ocular micrometre by using a pre-calibrated compound microscope. One hundred spores were observed for each isolate to avoid the error while taking observations.

The potato dextrose agar medium was prepared and 20 ml of medium was poured into the Petri plates for solidification. Five mm discs of different isolates *of E. turcicum* were placed at the centre of each plate. These plates were incubated at 27 ± 1 ºC for 10 days. The variation in cultural characteristics of *E. turcicum* was investigated by selecting isolates of fungus. The cultural characteristics such as colony diameter, colony colour and pigmentation were recorded.

3.5.2 Physiological studies

The cultural characters of *Exserohilum turcicum* will be studied on the Potato Agar (PDA) media at different temperature *viz* 20º, 25 º and 30 ºC.

3.5.3 Pathological variability

Studies on pathogenic variability were carried out in pot at the College of Agriculture, Badnapur. Pathogenicity of the nine isolates was determined by spray inoculation of spore suspension of *E. turcicum*.

3.5.3.1 Preparation of Inoculum

The inoculum of each isolate was prepared by flooding 12-day old culture plates of *E. turcicum* grown on potato dextrose agar (PDA) medium with sterile distilled water and scrapping the spore mass with the scalpel. The spore suspension was filtered through a muslin cloth. The conidial concentration was adjusted to 4×10^5 spores/ml using a haemocytometer.

3.5.3.2 Inoculation

Thirty days old different plants of maize were inoculated with spore suspension (4×10^5 spores per ml) in the hand sprayer in the evening hours. The inoculated plant was be bagged with a polythene bag to create high humidity at 24 hrs interval for three days.

3.5.3.3 Observation

The pathological variability of each isolate was categorised based on five virulent scales, weak (DSI=1-10 %), mild (11-20 %), moderate (21-30 %), virulent (31-50 %) and highly virulent (> 50 %) (Shah et al., 2006).

Disease severity was estimated on inoculated leaves 14 days after inoculation by using the 1-5 disease rating scale of Payak and Sharma, 1983 as detailed below.

1.0 - Very slight to slight infection. One or two too few scattered lesions on the lower leaves.

2.0 - Light infection, a moderate number of lesions on lower leaves only.

3.0 - Moderate infection, abundant lesions on lower leaves, few on middle leaves.

4.0 - Heavy infection, lesions abundant on lower and middle leaves, extending to upper

Leaves.

5.0 - Very heavy infection, lesions abundant on almost all leaves, plants prematurely dry or killed by the disease.

3.5.6 Disease management strategies

3.6.1 *In-vitro* evaluation of Fungicides against *Exserohilum turcicum*

In-vitro, the efficacy of different fungicides against *Exserohilum turcicum* were studied by using the poison food technique. Seven systemic and seven contact fungicides were tested against *E. turcicum* on the potato dextrose agar media using poison food technique under in vitro conditions. The systemic fungicides were tested at 500, 1000 and 1500 ppm concentrations whereas contact fungicides were evaluated at 2000, 2500 and 3000 ppm concentrations. Information about fungicide formulations and the active ingredient is presented in a table.

Details of experiments :

Design : Completely Randomized Design (CRD)

Replication : Three

Treatment : Eight

Table 3.2 *In-vitro* evaluation of Fungicides against *Exserohilum turcicum*

Treatment No	Contact Fungicides	Trade Name
T1	Propineb 70 % WP	Antracol (Bayer)
T2	Merimain 50 %WP	Captan (Adama)
T3	Mancozeb 75 % WP	Dithane M-4 (Dow)
T4	Copper oxychloride 50 % WP	Blue copper (Syngenta)
T5	Dinocarp 48 % EC	Karathane (Dow)
T6	Copper Hydroxide 77 %WP	Kocide 101 (Dupont)
T7	Zineb 75 % WP	Indofil Z- 78
T8	Control	Untreated

Treatment No	Systemic Fungicides	Trade Name
T1	Azoxystrobin 23 % SC	Amistar (Syngenta)
T2	Carbendazim 50 %WP	Bavistin(BASF)
T3	Tebuconazole 25.9 % EC	Folicur (Bayer)
T4	Propiconazole 25 % EC	Tilt (Syngenta)
T5	Difenoconazole 25 %EC	Score (Syngenta)
T6	Hexaconazole 5 % SC	Contaf plus (Tata)
T7	Benomyl 50 % WP	Benofit (Coromandel)
T8	Control	Untreated

3.6.2 *In-vitro* evaluation of bio-agents

Fungal biocontrol agents known to antagonist were evaluated *in vitro* applying the Dual culture technique (Arora and Upadhay, 1978) on PDA medium in Petri plates. 20 ml PDA medium were poured into each of the sterilized Petri plates. On solidification 5 mm disc cut from 7 days old culture of both antagonistic and test pathogen *Exserohilum turcicum* were inoculated separately on half of Petri plate at the same time. All the Petri plates were kept for incubation at 27±10 º C.

The antagonistic action of various known species of fungal and bacterial biological control agents was determined by the dual culture technique suggested by Martyn and Stack (1990). Twenty millilitres of PDA was poured aseptically in each of

the Petri plates and allowed to solidify. Mycelial disc of 4 mm diameter of the i.e., each antagonist and *E. turcicum* were placed on media in the same Petri plates approximately 4 cm away from each other. In the case of bacterial antagonists, it was inoculated with an inoculation needle by the streaking method. Each treatment was replicated thrice. All the inoculated plates were incubated at 25± 10º C and were observed after seven days for the growth of antagonists and test pathogen.

Details of Experiment :

Design : Completely Randomized Design (CRD)

Replications : Three

Treatments : Eight

Table 3.3 *In-vitro* evaluation of bio-agents

Tr. No.	Name of bioagents
Fungal Antagonist	
T_1	*Trichoderma asperellum*
T_2	*Trichoderma harzhianum*
T_3	*Trichoderma hamatum*
T_4	*Trichoderma koningii*
T_5	*Trichoderma longibrachiatum*
T_6	*Aspergillus niger*
Bacterial Antagonist	
T_7	*Pseudomonas fluorescens*
T_8	*Bacillus subtillis*
T_9	Control

Observations were made on radial growth of test pathogen and antagonism was when fungus in control plate reached to the rim of the plate. The number of sclerotia formed and their formation type also was recorded 15 days after inoculation. The per cent growth inhibition of test pathogen was calculated by the following formula:

$$\text{Per cent growth inhibition (\%)} = \frac{C - T}{C} \times 100$$

Where,

C = Growth of test pathogen in controlled plate

T = Growth of test pathogen in treated plate

3.6.3 *In-vitro* evaluation of Phyto-extract / Botanicals

The botanicals reported earlier effective against many phytopathogens and which are locally available will be evaluated *in-vitro* against @ 5 % and 10 % V/V by applying poisoned food technique (Nene and Thaplial 1993) and using PDA as basal medium.

Details of an experiment:

Design	:	Completely Randomized Design (CRD)
Replications	:	Three
Treatments	:	Nine

Table-3.4 *In-vitro* evaluation of Phyto-extract / Botanicals

Tr.No.	Local name	Scientific name	Plant part used
T_1	Garlic	*Allium sativum*	Bulb extract
T_2	Neem	*Azadirachta indica*	Leaves extract
T_3	Tulsi	*Ocimum sanctum*	Leaves extract
T_4	Ghaneri	*Lantana Camara*	Leaves extract
T_5	Ashwagandha	*Withania somnifera*	Leaves extract
T_6	Onion	*Allium cepa*	Leaves extract
T_7	Aloe vera	*Aloe barbadensis*	leaves extract
T_8	Asoka	*Saraca asoca*	leaves extract
T_9	Control	Untreated	-

3. 7 Pot culture

Pot culture experiment was conducted during *Kharif* 2020-21 using a susceptible variety. For proving pathogenicity and pathological variability of different isolates collected from different talukas of Aurangabad district.

3.8 Statistical analysis

The data obtained in the laboratory and glasshouse were analysed statistically by Completely Randomised Design (CRD) using MS Excel statistical software). Comparison of data recorded was done using critical differences at a 1% level of significance. A dendrogram was produced by the unweighted pair group method for arithmetic average (UPGMA) in the SAHN program by cluster analysis (https://datatab.net/statistics-calculator/cluster).

CHAPTER-IV

RESULT
&
DISCUSSION

CHAPTER-IV

RESULTS AND DISCUSSION

Study on Northern leaf Blight of Maize Caused by *Exserohilum turcicum* (Pass). Leonard and Suggs. objectives was studied *viz*., survey, symptomatology, isolation, pathogenicity test, morphological, cultural, physiological, and pathological variability, in vitro evaluation of fungicides, bioagents and phyto-extract at Department of Plant Pathology, College of Agriculture Badnapur and National Agricultural Research Project (NARP) Aurangabad. During *Kharif* 2020-21. The results obtained are as follows.

4.1. Survey on incidence and severity of Northern Leaf Blight disease of maize under major growing areas of Aurangabad district.

A survey was conducted in 27 villages and 9 tehsils to determine the incidence and intensity of Northern Leaf Blight (NLB) of maize caused by *Exserohilum turcicum* (Pass). During *Kharif* 2020-21 in the Aurangabad district, Leonard and Suggs. obtained the results shown in Table 4.1, 4.2, PLATE 4.1, 4.2, Fig 4.1, 4.2, 4.3 & 4.4.

The incidence and intensity of Northern Leaf Blight of Maize were observed in all of the surveyed areas. The incidence and intensity of Northern Leaf Blight disease in Aurangabad district's major maize growing regions ranged from 26.5 to 57.2 % and 12.00 to 29.1 %. The highest incidence of NLB (57.2 %) was observed at Jategaon, Phulambari tehsil (District Aurangabad) and the highest intensity of NLB (29.1 %) was observed at Chinchkheda, Gangapur tehshil. In comparison, the lowest disease incidence (26.5 %) was observed at Waluj, Aurangabad Tehsil and the lowest disease intensity of NLB (12.0 %) was observed at Aurangabad tehsil.

4.1.1. Status of Northern Leaf Blight of maize under major growing areas of Aurangabad district.

The incidence and intensity of Northern leaf blight of maize in Aurangabad tehsil ranged from 26.5 to 34.1 % and 12.0 to 24.0 % respectively. Patoda had the highest incidence of disease (34.1 %), the highest intensity of disease (24.0 %) and Padhegaon had the lowest incidence of disease (26.5 %). The average incidence and intensity of Northern leaf blight disease were 29.86 % and 19.5 % respectively.

The incidence and intensity of Northern leaf blight of maize disease in Sillod tehsil, ranged from 28.1 to 32.2 % and 19.2 to 20.9% respectively. The highest incidence of disease (30.2 %) was observed at Balapur and the highest intensity of disease (20.9 %) was observed at Ajantha, while the lowest disease incidence (28.1%) was observed at Amantha and the lowest disease intensity (19.2 %) was observed at Amantha. The average Northern leaf blight disease incidence and intensity are 30.26 % and 20.26 % respectively.

The incidence and intensity of Northern leaf blight of maize disease in Gangapur tehsil ranged from 33.1 to 53.8 % and 19.4 to 29.1 %, respectively. Bolthan had the highest incidence (53.8 %) of disease and Chinchkheda had the highest intensity of disease (29.1 %). Dongaon had the lowest incidence of disease (33.1 %) as well as the lowest intensity of disease (19.4 %). The average incidence and intensity of Northern leaf blight of maize disease were 45.4 % and 25.8 %, respectively.

The incidence and intensity of Northern leaf blight of maize disease in Paithan Tehsil ranged from 40.2 to 54.3 % and 16.7 to 23.5 % respectively. Bidkin had the highest incidence of disease (54.3 %) and the highest severity of disease (23.5 %). Borgaon had the lowest incidence of disease (40.2 %) and Devgaon had the lowest intensity of disease (16.7 %). The average incidence and intensity of Northern leaf blight disease were 46.5 % and 20.36 % respectively.

The incidence and intensity of Northern leaf blight of maize disease in Kannad Tehsil is reported to be 38.9 to 51.2 % and 15.9 to 18.9 % respectively. Chincholi had the highest incidence of disease (51.2 %) and the highest intensity of disease (18.9 %). Borsar had the lowest incidence of disease (38.9 %) and the lowest severity of disease (15.9 %). The average incidence and intensity of Northern leaf blight disease were 46.53 % and 17.00 % respectively.

The incidence and intensity of Northern leaf blight of maize disease in Vaijapur tehsil ranged from 29.4 to 43.4 % and 15.4 to 25.3 % respectively. The highest incidence of disease (43.4%) was observed in Jategaon and the highest intensity of disease of maize (25.3 %) was observed in Jategaon. Khandala had the lowest disease incidence (29.4 %) and the lowest disease intensity (15.4 %). The

average incidence and intensity of Northern leaf blight disease were 37.00 % and 20.00 % respectively.

The incidence and intensity of Northern leaf blight of maize disease in Phulambri Tehsil ranged from 43.0 to 57.2 % and 17.6 to 26.2 % respectively. The highest incidence of disease (57.2 %) and the highest intensity of disease (26.2 %) were observed in Jategaon. Pathri had the lowest disease incidence (43.0 %) and Dhamangaon had the lowest disease intensity (17.6 %). The average incidence and intensity of Northern leaf blight disease were 51.06 % and 21.76 % respectively.

The incidence and intensity of Northern leaf blight of maize disease in Khultabad tehsil ranged from 38.6 to 52.3 % and 17.3 to 24.4 %, respectively. Salukheda had the highest incidence of disease (52.3 %) as well as the highest intensity of disease (24.4 %). Mesheshmal had the lowest disease incidence (38.6 %) and the lowest disease severity (17.3 %). The average incidence and intensity of Northern leaf blight disease were 45.73 % and 20.7 % respectively.

The incidence and intensity of Northern leaf blight of maize in Soegaon Tehsil, ranged from 25.00 to 35.2 % and 18.3 to 26.1 % respectively. Amkheda had the highest incidence of disease (35.2 %) and the highest severity of disease (26.1 %). Banoti had the lowest disease occurrence (25.00 %) and the lowest disease severity (18.3 %). The average incidence and intensity of Northern leaf blight disease were 30.13 % and 21.13 % respectively.

This result was similarly to the Geeta *et al*. (2018) surveyed the incidence of Northern leaf blight of maize in Ballari district.

Table No 4.1 : Record of Northern Leaf Blight (NLB) disease incidences and intensity at major maize growing area of Aurangabad district during Kharif 2020-21

Sr. No.	Name of Farmer	Village	Variety	Soil Type	Previous crop	Stage of Crop	Area (acres)	Disease Incidence (%)	Disease Intensity (%)
A. Aurangabad									
1	Raju Dongare	Patoda	P-3501	Black cotton soil	cotton	Vegetative	2	34.1	24
2	Shankar Navgire	Waluj	Pinnacol	Black cotton soil	ginger	Vegetative	2	26.5	22.5
3	Babasaheb Shinde	Padhegaon	Local	Medium soil	groundnut	Vegetative	1	29	12
Average Mean								**29.86**	**19.5**
B. Sillod									
4	Akshay Raosaheb Kolte	Balapur	Local	Black cotton soil	Cotton	Vegetative	1	30.5	20.7

5	Vinod Kale	Ajantha	Advanta	Black soil	Gram	Vegetative	2	32.2	20.9
6	Rahul Patil	Amthana	Pinnacol	Light soil	wheat	Vegetative	1	28.1	19.2
Average Mean								**30.26**	**20.26**
C. Gangapur									
7	Namdeo Shelke	Bolthan	Local	Light soil	Cotton	Vegetative	2	53.8	28.9
8	Anil Pawar	Chinhkheda	Local	Light soil	Cotton	Vegetative	2	49.5	29.1
9	Amol Gore	Dongaon	P-3501	Light soil	Groundnut	Vegetative	2	33.1	19.4
Average Mean								**45.4**	**25.8**
D. Paithan									
10	Kishor Rambhau Dharambe	Bidkin	Kargil 900M	Black cotton soil	Gram	Vegetative	1	54.3	23.5

11	Vilas Garad	Borgaon	P-3501	Light soil	Gram	Vegetative	2	40.2	20.9
12	Kamlakar Vansare	Devgaon	Pinnacol	Medium soil	cotton	Vegetative	2	45.5	16.7
Average Mean								**46.6**	**20.36**
E. Kannad									
13	Adinath Vishwanath Wagh	Chincholi	P-3501	Light soil	Gram	Vegetative	2	51.2	18.9
14	Ganesh Kathar	Borsar	Local	Medium soil	cotton	Vegetative	2	38.9	15.94
15	Tukaram Gore	Aurala	P-3501	Light soil	Gram	Vegetative	2	49.5	16.2
Average Mean								**46.53**	**17.01**
F. Vaijapur									
16	Sarjerao Rambhau Iname	Jategaon	Advanta	Black soil	Groundnut	Vegetative	1	43.4	25.3

17	Ramesh Shinde	Khandala	Advanta	Light soil	Cotton	Vegetative	1	38.2	15.4
18	Satiash Kumar	Dongaon	P-3501	Light soil	Cotton	Vegetative	2	29.4	19.5
Average Mean								**37**	**20.06**
G. Phulambari									
19	Sachin wagh	Jategaon	P-3501	Black cotton soil	Gram	Vegetative	2	57.2	26.2
20	Arun Jadhav	Dhamangaon	Local	Light soil	Gram	Vegetative	2	53	17.6
21	Raju thorat	Pathri	Kargil 900M	Light soil	Cotton	Vegetative	1	43	21.5
Average Mean								**51.06**	**21.76**
H. Khultabad									
22	Tukaram Kale	Salukheda	Pinnacol	Light soil	Gram	Vegetative	2	52.3	24.4

23	Kailas Bargal	Maheshmal	Pinnacol	Light soil	Cotton	Vegetative	1	46.3	20.4
24	Namdev Varpe	Nandrabad	Local	Medium soil	Gram	Vegetative	2	38.6	17.3
Average Mean								**45.73**	**20.7**
I.Soegaon									
25	Raosaheb Jadhav	Amkheda	Local	Black soil	Cotton	Vegetative	2	35.2	26.1
26	Raghunath Bargal	Banoti	Local	Light soil	Cotton	Vegetative	1	25	18.3
27	Ganesh Bhingare	Dabha	P-3501	Medium soil	cotton	Vegetative	2	30.2	19
Average Mean								**30.13**	**21.13**

PLATE-4.1

A. Location : Aurangabad tehsil

B. Location : Soegaon tehsil

Plate 4.1 Survey of NLB disease of maize on farmers' fields to record disease incidence and severity during *Kharif* 2020-21.

PLATE-4.2

C. Location : Khuldabad tehsil

D. Location : Kannad tehsil

Plate 4.2 Survey of NLB disease of maize on farmer's fields to record disease incidence and severity during *Kharif* 2020-21.

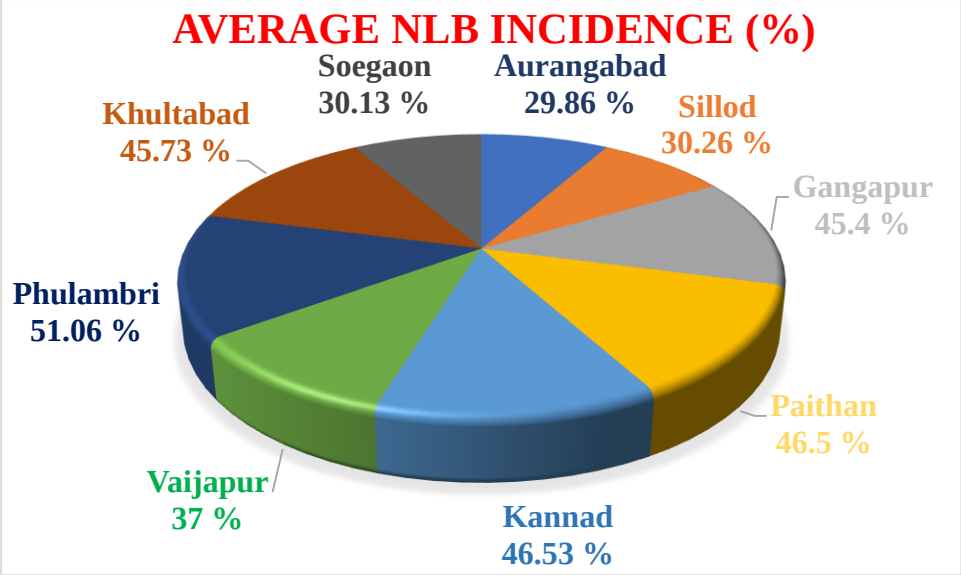


Figure 4.1. Percent NLB disease incidence in the Maize growing areas of Aurangabad district during *Kharif* 2020-2021.

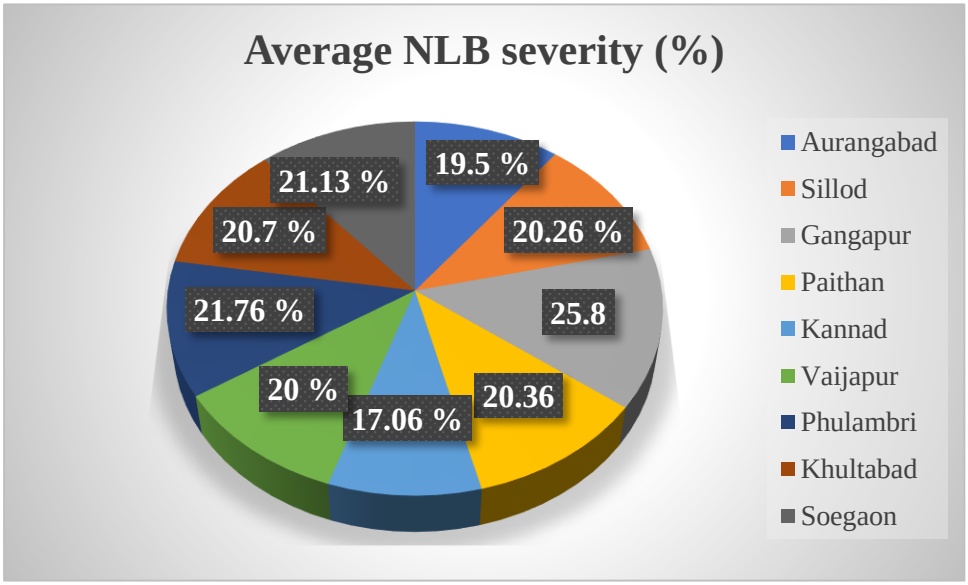


Figure 4.2. Percent NLB disease Severity in the Maize growing areas of Aurangabad district during *Kharif* 2020- 2021.

Figure 4.3. Disease map based on incidence of Northern leaf blight of maize in 09 tehsil of Aurangabad district during 2021.

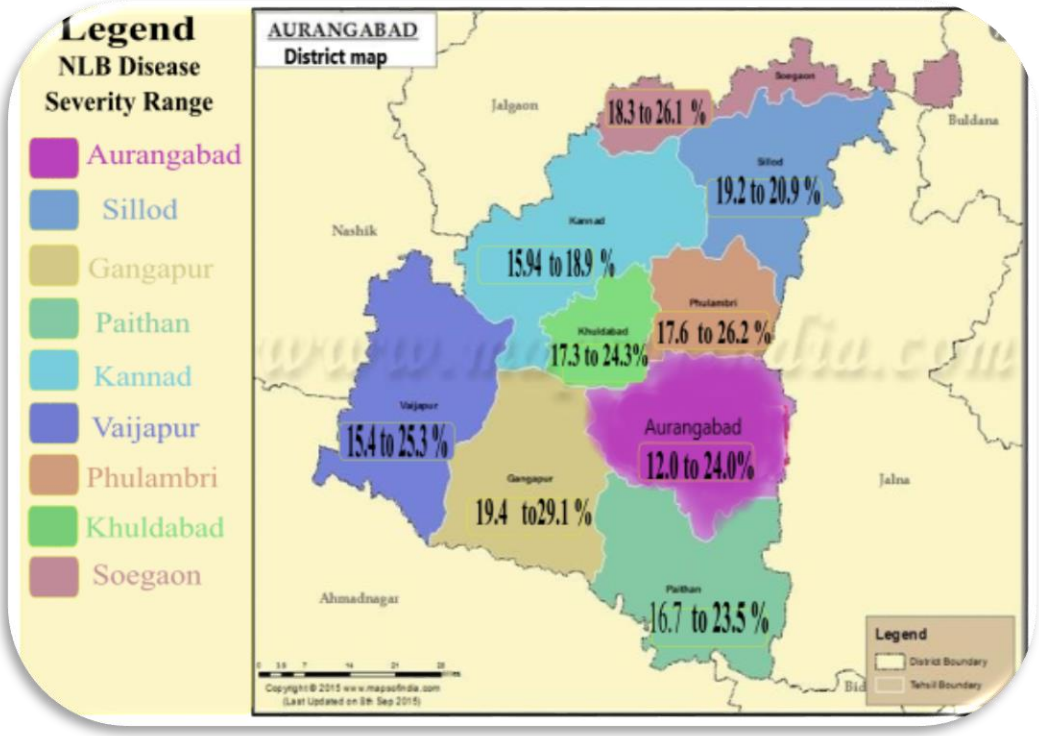


Figure 4.4. Disease map based on severity of Northern leaf blight of maize in 09 tehsil of Aurangabad district during 2020 -21.

Table 4.2 : Taluka-wise per cent disease incidence and severity of Northern leaf blight of maize in major growing areas of Aurangabad district during *Kharif 2020-21.*

Sr. No.	Name of Taluka	Average NLB incidence (%)	Incidence Range (%)	Average NLB severity (%)	Severity Range (%)
1	Aurangabad	29.86	26.5 to 34.1	19.5	12.0 to 24.0
2	Sillod	30.26	28.1 to 32.2	20.26	19.2 to 20.9
3	Gangapur	45.4	33.1 to 53.8	25.8	19.4 to 29.1
4	Paithan	46.5	40.2 to 54.3	20.36	16.7 to 23.5
5	Kannad	46.53	38.9 to 51.2	17.01	15.94 to 18.9
6	Vaijapur	37.00	29.4 to 43.4	20.00	15.4 to 25.3
7	Phulambri	51.06	43.0 to 57.2	21.76	17.6 to 26.2
8	Khultabad	45.73	38.6 to 52.3	20.7	17.3 to 24.4
9	Soegaon	30.13	25.0 to 30.2	21.13	18.3 to 26.1
9	**Overall Average**	**40.37**	**25.0 to 57.2**	**20.72**	**12.0 to 29.1**

4.2 Symptomology

The symptoms of disease mainly appear on the leaves. Small oval, water-soaked, slightly elliptical greyish colour spots appear on the leaves as initial symptoms of the disease. But in due course, these spots enlarge and become elongated spindle-shaped. These characteristic cigar-shaped lesions are straw-coloured in the centre with dark margins. Abundant spores develop on both sides of the spots. The straw-coloured centre of the lesion became darker during sporulation. Lesions enlarge, covering a more prominent area of leaves, giving a blighted appearance. These morphological characters of the fungus are similar to those described by (Fredericksen, 1980) observed tiny flecks at 3-4 days after favourable infection, while large distinctive lesions appear two weeks later. Chenulu and Hora (1962) observed the scorched appearance of leaves in the infected field. Ullstrup (1966) observed elliptical greyish or tan lesions as characteristic symptoms of the disease. With the increase in severity, the disease progressed on the upper side of the leaves, and the spots gradually increased in size, turned grey olivaceous to tan coloured and measured about 10-14 × 2-3 cm. Finally, the spots turned brown to straw coloured distinct spindle-shaped lesions and symptom produced by different isolates shown in, (Plate-4.3, 4.4) with greyish black mass, consisting of conidia and conidiophores of the fungus.

4.3 Isolation and identification of the pathogen

From the infected leaves, isolation was done on Potato Dextrose Agar (PDA) medium. The fungus isolated produces white coloured colony that was cottony and fluffy in an appearance on PDA medium. Similar results were reported by Reddy (2012) as greyish to black coloured and profuse growth of the fungus. The fungal growth later turns greyish to somewhat olivaceous black. Microscopic observations were made by preparing slides from fresh diseased samples and the fungus seven-day-old culture. Slides were prepared using lactophenol and were observed under a compound microscope. Mycelium and conidia of the fungus were observed under the microscope at 10X and 40X. The fungus was identified as *Exserohilum turcicum* based on septation of conidia and protruding hilum of conidia. (Plate 4.5).

PLATE- 4.3

A. Initial symptoms

B. Cigar shaped lesions

C. Symptoms at maturity

D. Blighting of leaves before silking

E. Blighting of leaves after silking

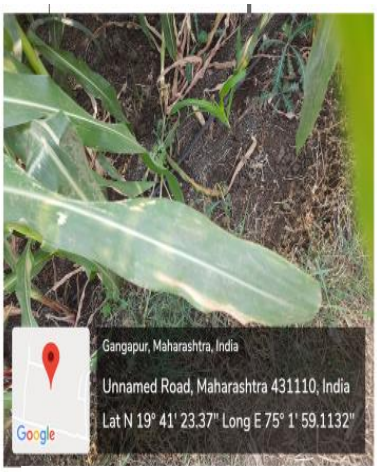
F. Mature NLB lesions associated with fungal spore

Plate- 4.3: Symptoms of Northern Leaf Blight (NLB) of maize caused by *Exserohilum turcicum.*

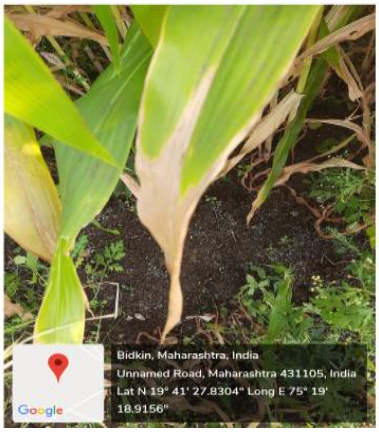
PLATE-4.4

AEt1

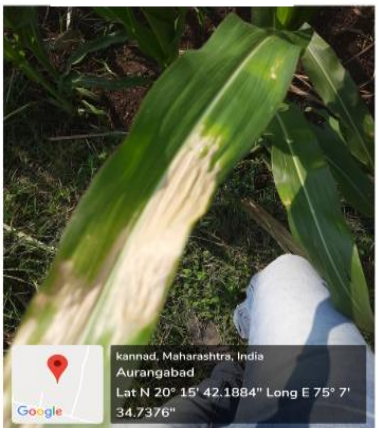
SEt2



GET3



PEt4



KEt5

VEt6

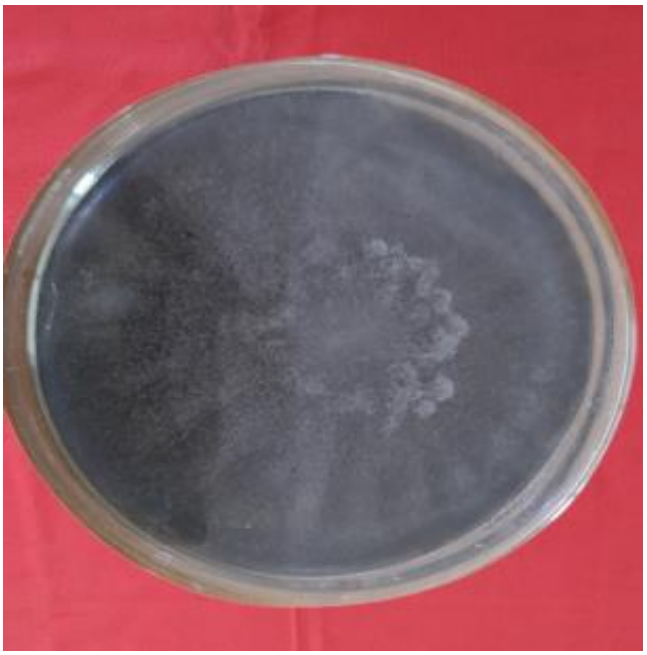
PhEt7

KhEt8

SoEt9

Plate- 4.4 : Photograph showing symptom of different isolate from Aurangabad district during survey.

PLATE- 4.5



A. Pure culture of *Exserohilum turcicum*

B. Slant of *Exserohilum turcicum*

Plate-4.5 : Identification of *Exserohilum turcicum* pathogen

4.3.1 Purification and maintenance of pathogen isolates

Twelve single spore cultures, isolated from NLB disease samples collected from 9 location were maintained on potato dextrose agar medium and slant prepared. The isolates were designated from AEt1 to SoEt9 (Table-4.3).

Table. 4.3 : Designation of *Exserohilum turcicum* isolates from nine tehsil from Aurangabad districts

Location	Isolates designation
Aurangabad	AEt1
Sillod	SEt2
Gangapur	GEt3
Paithan	PEt4
Kannad	KEt5
Vaijapur	VEt6
Phulambri	PhEt7
Khultabad	KhEt8
Soegaon	SoEt9

4.4 Morphological characteristics of the pathogen

The colour of the colony observed at 3-4 days after incubation was greyish white in colour but when observed on 7-8 days it appears olivacious brown, black and grey in colour. The colony was fluffy and cottony with regular margins. The hypha observed under the microscope was black or pale to light brown, smooth and septate. The conidia were 3 - 8 septate, olivaceous black or brown in colour. They are spindle-shaped, and have a protruding hilum. These results were in accordance with study conducted by Daniel and Narong (2006) as observed that conidia were elongated and spindle in shape with 2-7 septations.

The conidiophores were septate and measured about 140-350 x 6-14 μm in size. Brown to straw coloured conidia varied in shape from straight to slightly curved,

club-shaped or widest near the middle and tapering towards both ends with protruding hilum. The conidia were 2-7 septate and measured about 30-150 x 10-40 µm in size.

The chlamydospores were smooth produced terminally or intercalary and were borne singly or in chains. They were mostly spherical and measured about 6.5 15.5 µm in diameter. Conidia have a strongly protruding, truncate hilum.The conidial germination is bipolar (Table 4.4). On the basis of morphological and pathological characteristics as well as comparison with authentic descriptions, the fungus was identified as *Exserohilum turcicum* (Pass.) Leonard and Suggs (Syn. *Helminthosporium turcicum* Pass.), teleomorph; *Setosphaeria turcica* Luttrell (Syn. *Trichometasphaerium turcica* Lutterl). (Plate – 4.6)

Table 4.4 : Morphological characters of *Exserohilum turcicum*

Propagule	Colour	Shape	Size (µm)	Septation
Mycelium	Black or Light brown	Floccose, slendrical, branched	2.8-5.57 µm (wide)	Septate
Conidiophore	Light to dark Brown	Floccose, slendrical, branched	140-350 x 6-14 µm	Septate
Conidia	Black, Brown to straw coloured	Straight or slightly curved, cylindrical towards the ends with protruding hilum	30-150 x 10 -40 µm	2-7 septa
Chlamydospore	Brown	Smooth, mostly spherical, terminal borne singly or in chains	6.5-15.5 µm in diameter	-

4.5 Pathogenecity test

The pathogenicity test of *Exserohilum turcicum* was determined under glasshouse conditions. Twenty five days old plants were inoculated with spore suspension of *E. turcicum* culture. After inoculation plants were placed in moist chamber for 24 hrs and then transferred in glass house having a temperature of about 28±2°C. After inoculation, plants were regularly observed for the appearance of symptoms. (Plate 4.7)

PLATE -4.6

Uninoculated plant

Inoculated plant

Plate – 4.6 : Pathogenicity test of *Exserohilum turcicum*

4.6 Re-isolation

The fungus was again isolated from the leaves of artificially inoculated plants showing symptoms of the turcicum leaf blight of maize. The fungal growth obtained on PDA medium showed similar cultural and morphological characteristics as observed in the original culture of *Exserohilum turcicum.* The same was found identical to that of the original culture confirming the pathogenicity test.

4. 7 Morpho- Cultural, Physiological and pathological Variability

4. 7. 1 Morphological and Cultural variability

The pathogen was isolated and the fungal cultures on purification showed white colour and fluffy type of mycelium, which gradually turned into greyish, greenish or brownish as the culture started to produce conidia. On repeated isolation, it was found the association of *E. turcicum*. A total of nine isolates were obtained and designated with isolate code as Et according to their respective places of collection. The pathogen was identified by comparing with relevant literatures and by studying the morphological and cultural characters.

The cultural characteristics of isolates are presented in Table 4.6. The following observations were made on Incubation period (days) for maximum growth, Colony colour, Pigmentation, Sporulation, Colony texture, Surface texture and Edge of colony. (M. R. Vinay and A. R. Sataraddi, 2019) and (Geeta D. S., 2019).

4.7.1.1 Incubation period (days) for maximum growth

All the nine isolates produced good growth on PDA, but the period taken by different isolates to completely cover the 9 cm petridish were different based on aggressiveness of the isolate. Among isolates KEt5 and PhEt7 shown lowest Incubation period of 7 days and AEt1 shown highest Incubation period. In an average known to take 14 days of Incubation period to completely cover the 9 cm petridish.

4.7.1.2 Colony colour

The colony colour of fungus was recorded based on dominant spectral colour from Munsell's soil colour chart (1954), 14 days after incubation on PDA medium and the results are presented in Table 4.6. The colony colour varied from grey to black colour. Based on the colony colour all the nine isolates were grouped in 8

categories *i.e.*, dark Grey, light greenish, white to grey, yellow, blackish, creamy white, white to black. and black. The KEt5 (Kannad), KhEt8 (Khultabad) and SoEt9 (Soegaon) showed Black colony colour wheres isolate AEt1 shown Dark greyish to black colony colour.

The isolates SEt2 from sillod showed yellow colony colour and GEt3 from Gangapur showed light greenish colony colour which was distinctly different from all other isolates. The isolates PEt4 (Paithan) showed white to grey colony colour while creamy white colony colour was observed in the isolates VEt6 from Vaijapur. PhEt7 from phulambari showed white to black colony colour.

4.7.1. 3 Pigmentation

Based on the pigmentation *E. turcicum* isolates were grouped into 6 groups *i.e.*, Black, yellowish, light green, Brown, creamy, blackish, and Black red. The isolates SEt2, GEt3, KEt5, VEt6, SoEt9 and KhEt8 was in a distinctly different pigmentation *i.e* yellowish, light green, Brown, Creamy, yellowish and Black red. With regard to the isolate AEt1, PEt4 and PhEt7 showed Black to Blackish pigmentation.

Table. 4.5 : Morphological variability in different isolates of *Exserohilum turcicum*

Sr . no	Isolate code	Spore colour	No. of septa	Size of conidia µm (10X)	
				Length	Breadth
1	AEt1	Dark brownish	3-5	49.62	15.55
2	SEt2	Brownish	3-6	57.56	23.62
3	GEt3	Brownish	3-6	58.63	25.51
4	PEt4	Brownish	3-5	52.49	18.65
5	KEt5	Dark brownish	3-9	97.50	25.32
6	VEt6	Brownish	3-5	77.48	20.51
7	PhEt7	Brownish	3-8	97.96	26.52
8	KhEt8	Dark brownish	3-7	86.52	22.45
9	SoEt9	Dark brownish	3-7	86.43	23.68

4.7.1.4 Surface texture

On Potato Dextrose Media (PDA) majority of the isolates showed irregular shape. the isolates SEt2, VEt6, KhEt8 and SoEt9 produced rough texture and GEt3, PEt4 and PhEt7 produced fluffy texture whereas smooth surface texture produced by AEt1 and KEt6.

4.7.1.5 Colony texture

On PDA majority of the isolates produced distinct wavy and moderately wavy zonation are SoEt9, SEt2, VEt6 and KhEt8 and with regards to AEt1 which produced ad pressed colonies. GEt3, PEt4 and PhEt7 produced cottony colony growth and AKEt5 produced smooth colony growth.

4.7.1.6 Edge of colony

The isolates when grown on PDA shown sparsely branched to highly branched edges of the colony.The isolate having edge of colony sparsely branched are AEt1, PEt4 and PhEt7 and moderately branched edge of colony in isolate GEt3, VEt6 and KhEt8. Isolate SEt2 having branched edges of colony and isolate SoEt9 having heavily branched edges of colony. And one isolate that No branching edges of coloy *i.e* KEt5. The nine isolates did differ in different prospect such as Incubation period (days) for maximum growth, Colony colour, Pigmentation, Sporulation, Colony texture, Surface texture and Edge of colony. Such variations have been reported by Gowda *et al.,* (2010).

4.7.1.7 Microscopic studies

Nine isolates shown three types of conidial shapes *viz*., curved, spindle and elongated. The size of the conidia averaged 73.79 µm in length and 22.42 µm in width. The number of septa was found to range from 3 to 9. Conidia were observed in all the isolates. Morphological studies data of various location given in Table 4.5 and Plate – 4.9.

Among the isolates, conidia size was maximum in isolate PhEt7 (97.96× 26.52µm) with an average of 3-8 septation and minimum in isolate AEt14 (49.62×15.55 µm) with 3-5 septation.

Isolate AEt1 having Dark brownish spore colour, 3-5 septa and conidial size (49.62× 15.55).

Isolate SEt2 having brownish spore colour, 3-6 septa and conidial size (57.56× 23.62).

Isolate GEt3 having brownish spore colour, 3- 6 septa and conidial size (58.63 × 25.51).

Isolate PEt4 having brownish spore colour, 3-5 septa and conidial size (52.49 × 18.65).

Isolate KEt5 having dark brownish spore colour, 3- 9 septa and conidial size (97.50× 25.32).

Isolate VEt6 having brownish spore colour, 3- 5 septa and conidial size (77.48 × 20.51).

Isolate PhEt7 having brownish spore colour, 3- 8 septa and conidial size (97.96 × 26.52).

Isolate KhEt8 having dark brownish spore colour, 3- 7 septa and conidial size (86.52× 22.45).

Isolate SoEt9 having dark brownish spore colour, 3- 7 septa and conidial size (86.43× 23.68).

(The results show similarities with the conidial measurement reported by Bunker *et al.*, (2011).)

Table.4.6: Cultural characters of nine isolates *Exserohilum turcicum* on potato dextrose agar (PDA)

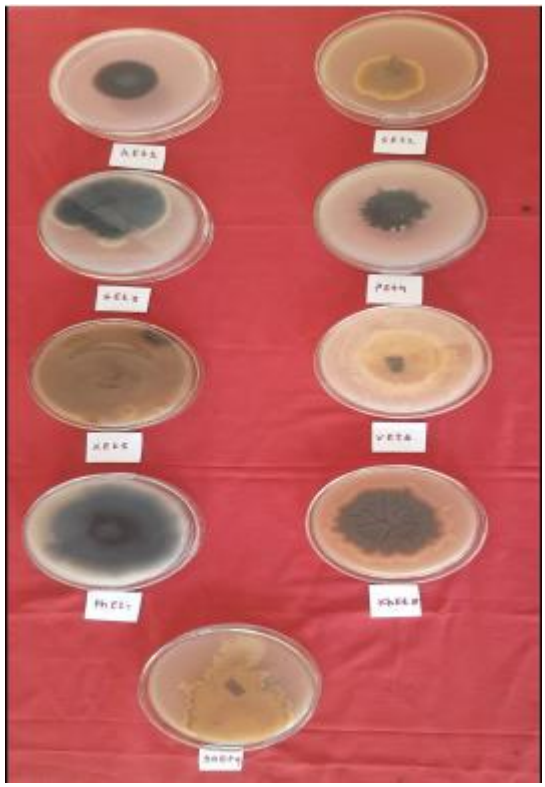
Location	Isolate	Incubation period (days) for max. growth	Mean Colony diameter (mm)	Colony colour	Pigmentation	Surface texture	Colony texture	Edge of colony
Aurangabad	AEt1	14	22	Dark grey	Black	Smooth	Pressed	Sparsely branched
Sillod	SEt2	12	25	Yellow	Yellowish	Rough	Moderately wavy	branched
Gangapur	GEt3	9	33	Light green	Light green	Fluffy	Cottony	Moderately branched
Paithan	PEt4	14	23	White to grey	Black	Fluffy	Cottony	Sparsely branched
Kannad	KEt5	7	90	Blackish	Brown	Smooth	Smooth	No branching
Vaijapur	VEt6	10	39	Creamy white	Creamy	Rough	Moderately wavy	Moderately branched
Phulambri	PhEt7	7	87	White to black	Blackish	Fluffy	Cottony	Sparsely branched
Khultabad	KhEt8	11	43	Black	Black to red	Rough	Moderately wavy	Moderately branched
Soegaon	SoEt9	10	63	Black	Yellowish	Rough	wavy	Heavily branched

PLATE-4.7 (A)

AEt1	Dark grey
SEt2	Yellow
GEt3	Light green
PEt4	White to grey
KEt5	Blackish
VEt6	Creamy white
PhEt7	White to black
KhEt8	Black
SoEt9	Black

Plate- 4.7 (A) : Growth of nine isolate on cultural media *i. e.*, PDA to observed the cultural variability of pathogen.

PLATE-4.7 (B)



AEt1	Black
SEt2	Yellowish
GEt3	Light green
PEt4	Black
KEt5	Brown
VEt6	Creamy
PhEt7	Blackish
KhEt8	Black to red
SoEt9	Yellowish

Plate- 4.7 (B) : Pigmentation of Nine isolate on culture media *i. e.*, PDA to observe cultural variability of pathogen.

4.8 Physiological variability

4.8.1 Effect of temperatures on growth of *Exserohilum turcicum* isolate

In order to culture pathogenic fungi in the laboratory it is necessary to furnish essential elements and compounds in the medium which are required for growth and other life processes and temperature is also one of the most important factor for regulating growth and reproduction of the fungus thus effect of different temperatures *i.e* 20°C, 25°C and 30°C on the mycelia growth of nine isolate of *Exserohilum turcicum* was studied. nine isolate were taken and each one of them was kept at three different temperatures and difference in colony diameter of the pathogen among them was observed. (Given in Table- 4.7 , Plate-4.8, Fig- 4.5).

Isolate AEt1 showed maximum colony diameter of 86.77 mm and Minimum mycelial growth in isolate KEt5 were 40.71 mm was observed at 30°C.

Isolate GEt3 showed maximum colony diameter of 85.22 mm and Minimum mycelial growth in isolate KEt5 were 34.45 mm was observed at 25°C.

Isolate GEt3 showed maximum colony diameter of 54.39 mm and Minimum mycelial growth in isolate KEt5 were 27.10 mm was observed at 20°C.

The cultural characteristics of isolates were also change according to temperature changes. *i.e* Incubation period (days) for maximum growth, Colony colour, Pigmentation, Sporulation, Colony texture, Surface texture and Edge of colony.

The study concludes that 25-30°C temperature supports greater mycelial growth of the fungus whereas 20° C is less favourable for the growth of fungus. These results were in accordance with the results obtained by Misra and Singh during 1963 observed that optimum temperature for growth of *E. turcicum* was 25-30°C.

Bergquist and Masias (1974) reported the optimum growth rate of sorghum and maize isolates of the fungus at 28°C while abundant sporulation was observed at 24°C.

Pandey and Shukla (1982) reported that optimum temperature for colony growth of maize isolate of *E. turcicum* was 20- 30°C, and no growth was observed at 40°C.

ogen

Table No :4.7 Effect of temperatures on growth of *Exserohilum turcicum* isolate

Sr No	Isolate	Temperature °C		
		20°C	25°C	30°C
		Colony diameter (mm)		
1	AEt1	31.34	70.36	86.77
2	SEt2	41.49	56.55	76.78
3	GEt3	54.39	85.22	85.45
4	PEt4	31.46	74.76	60.41
5	KEt5	27.10	34.45	40.71
6	VEt6	42.20	80.05	84.71
7	PhEt7	38.68	80.38	80.40
8	KhEt8	38.18	73.73	51.13
9	SoEt9	33.20	71.85	50.04
	SE ±	**0.64**	**0.40**	**0.45**
	CD @ 1%	**1.94**	**1.21**	**1.36**

4.9 Pathological variability

4.9.1 Reaction of maize variety against *E. turcicum* isolates

It is evident that the four variety of maize included in the present study were able to differentiate the virulence of the pathogen associated with geographical origin of the isolates. The results showed that irrespective of the isolates in susceptible local variety exhibited significantly more mean lesion size of, 1.31 cm^2 - 3.84 cm^2 respectively. Similarly, smaller mean lesion size 0.04- 0.97 cm^2 was recorded in resistant variety Pioneer 3501, Pinnacol and Advanta irrespective of the isolates. Across the maize variety *E. turcicum* isolates from Aurangabad (AEt1), Sillod (SEt2), Gangapur (GEt3), Paithan (Pet4) and Soegaon (SoEt9) produced higher mean lesion size of 3.84, 1.50, 1.31, 1.51 and 1.40 cm^2, respectively whereas small lesion size was noticed in Kannad (KEt5), Vaijapur (VEt6), Phulambari (PhEt7) and Khultabad (KhEt8) isolates are 0.52, 0.97, 0.26 and 0.76 cm^2 (Table- 4.8, Plate 4.9, Fig 4.6) On susceptible local variety, some of the

PLATE-4.8

20^0C

25^0C

30^0C

Plate-4.8 : Effect of temperature on radial growth of nine isolate of *Exserohilum turcicum* pathogen

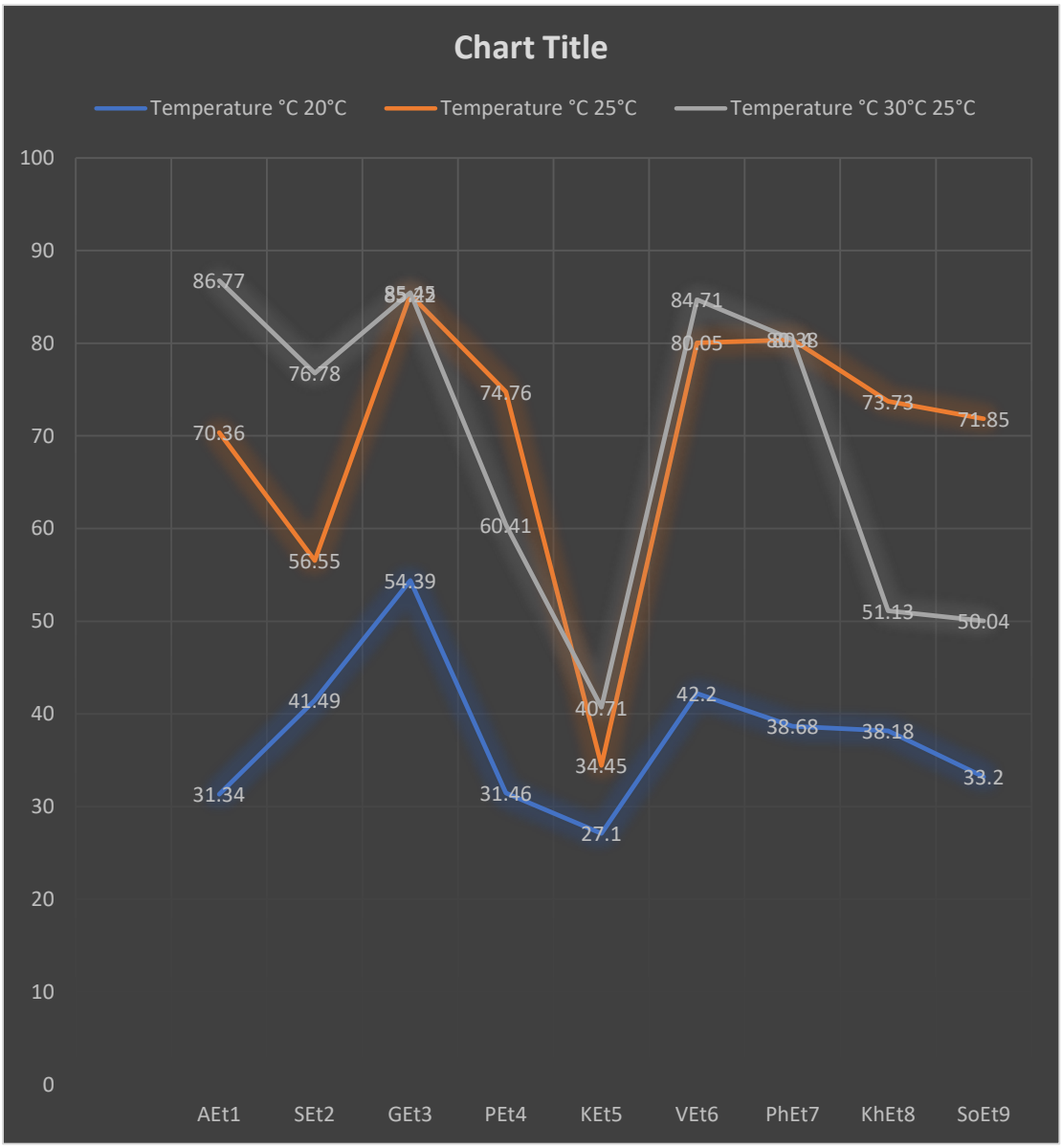


Fig- 4.5 : Effect of temperature on nine isolate of *E. turcicum* path

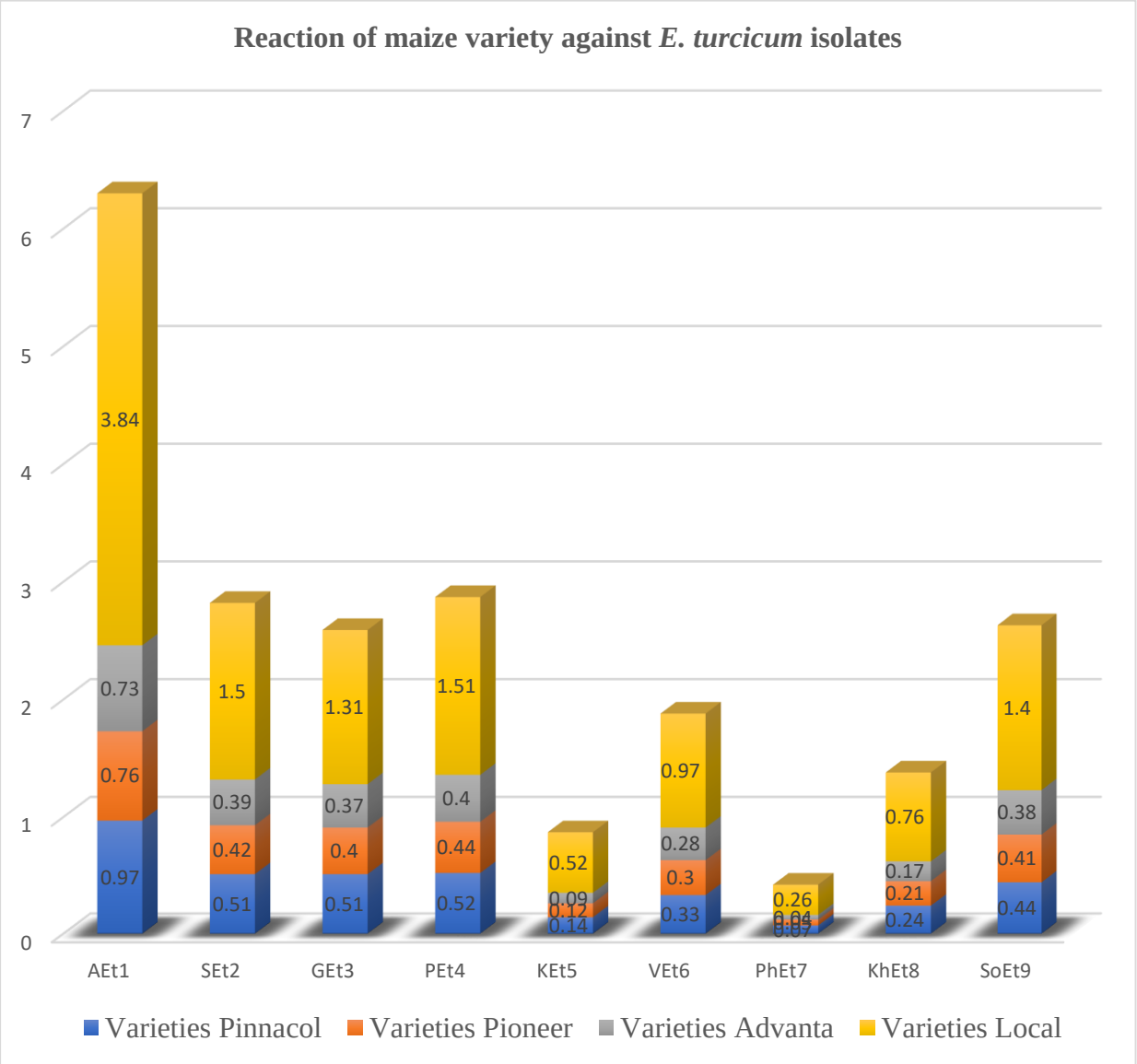


Fig- 4.6 : Reaction of maize variety against *E. turcicum* isolates

isolates such as Aurangabad, Sillod, Gangapur, Paithan and Soegaon produced maximum lesion size and could be considered as most virulent isolates. The results indicated that, there were three virulence patterns exhibited on four maize variety after inoculating with 9 isolates of *E. turcicum*. The isolates from Aurangabad, Sillod, Paithan and Soegaon were highly pathogenic on local variety.

The variety showing disease score between 0.04-0.14 were considered as resistant (R), 0.24-0.33 as moderately resistant (MR), 0.44-0.97 moderately susceptible (MS), 1.31-1.40 susceptible (S) and 1.50-3.84 as highly susceptible (HS).

Based on the disease reaction of these maize variety, the isolates could be grouped into three virulent types viz., the isolates has reaction 3.84 as most virulent, reaction between 1.31 -1.40 as moderately virulent and reaction upto 0.97 as less virulent type.

Therefore, the reason for lack of resistance in some of the commercial cultivars of maize may be attributed to the prevalence of virulent isolates of the pathogen *E. turcicum* similar observations were made by Gowda *et al.*,(1993).

The results of the present findings were supported by the Daniel and Narong (2006) Present findings are also in conformity with earlier findings (Nelson *et al.*, 1970 and Muiru *et al.*, 2008).

Table 4.8. Reaction of maize variety against *E. turcicum* isolates

Sr . no	Isolate code	Varieties				Average
		Pinnacol	Pioneer	Advanta	Local	mean
1	AEt1	0.97	0.76	0.73	3.84	**1.57**
2	SEt2	0.51	0.42	0.39	1.50	**0.70**
3	GEt3	0.51	0.40	0.37	1.31	**0.64**
4	PEt4	0.52	0.44	0.40	1.51	**0.71**
5	KEt5	0.14	0.12	0.09	0.52	**0.21**
6	VEt6	0.33	0.30	0.28	0.97	**0.47**
7	PhEt7	0.07	0.05	0.04	0.26	**0.10**
8	KhEt8	0.24	0.21	0.17	0.76	**0.34**
9	SoEt9	0.44	0.41	0.38	1.40	**0.65**
	Av. Mean	**0.41**	**0.34**	**0.31**	**1.34**	**0.60**
	SE±	**0.006**	**0.005**	**0.007**	**0.125**	**-**
	CD@ 1%	**0.019**	**0.016**	**0.021**	**0.374**	**-**
The variety showing disease score between 0.04-0.14 were considered as resistant (R), 0.24-0.33 as moderately resistant (MR), 0.44-0.97 moderately susceptible (MS), 1.31-1.40 susceptible (S) and 1.50-3.84 as highly susceptible (HS)						

4.9.2 Pathological variability

Reaction pattern of nine isolates of *E.turcicum* obtained from disease samples collected from different talukas of Aurangabed district was recorded on a four putative maize variety (Table- 4.9). The results revealed considerable pathogenic variability among the different isolates of *E. turcicum.* Cluster analysis (Fig.4.7) on the basis of similarity or dissimilarity in reaction types exhibited by these differential hosts, grouped the isolates into 5 pathogenic groups which were designated as Pathogenic Group I, II, III, IV and V (Table-4.10).

Table- 4.9 Pathogenic variability of *E. turcicum* isolates on four putative different maize variety

Isolate code	Variety			
	Pinnacol	**Pioneer**	**Advanta**	**Local**
AEt1	MS	MS	MS	HS
SEt2	MS	MR	MR	HS
GEt3	MS	MR	MR	S
PEt4	MS	MS	MR	HS
KEt5	R	R	R	MS
VEt6	MR	MR	MR	MS
PhEt7	R	R	R	MR
KhEt8	MR	MR	MR	MS
SoEt9	MS	MR	MR	S

Where,

Resistant (R), Moderately resistant (MR), Moderately Susceptible (MS), Susceptible (S) and Highly Susceptible (HS).

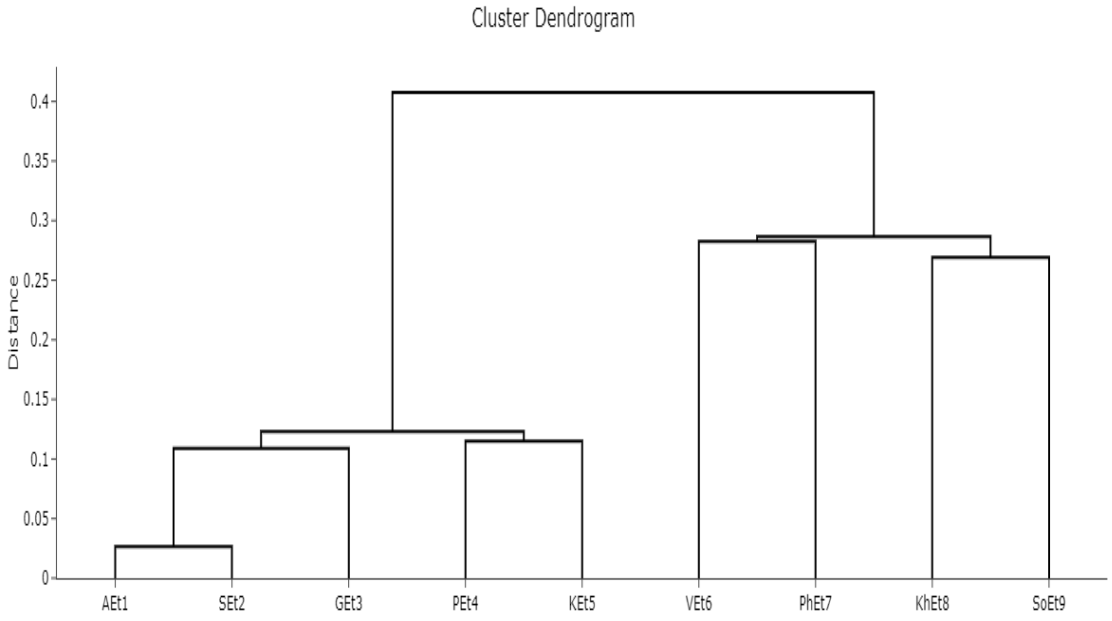


Fig- 4.7 : Cluster analysis : Dendrogram showing virulence similarity and successive clustering of nine isolates of E. turcicum on selected maize variety

Table-4.10 : Categorization of *E. turcicum* isolates on the basis of pathogenic variability

Pathogenic Groups	Isolate
Group- I	AEt1 and SEt2
Group- II	GET3
Group- III	PEt4 and KEt5
Group- IV	VET6 and PhEt7
Group- V	KhEt8 and SoEt9

The pathogenic Group-I comprised of isolates AEt1 and SEt2 by way of resistant response from differential hosts. AEt1 and SEt2 got moderate susceptible response with Pinnacol, Pionner and Advanta variety except local variety has highly susceptible respone.

The Group II comprised of only one isolate GEt3 acquired moderate susceptible and susceptible reaction from Pinnacol and local variety while isolate GEt3 acquired moderate resistance from Pioneer and Advanta.

Group III comprised of isolate PEt4 and KEt5. PEt4 having moderate sucetible response from two variety *viz.*, Pinnacol and Pioneer and highly susceptible reaction from local variety. KEt5 having Resistancr response from three variety *viz.*, Pinnacol, Pioneer and Advanta and moderate susceptible reaction from local variety.

The isolates VET6 and PhEt7 were clubbed under Group IV. VET6 comprised with moderate resistant response from three variety *viz.*, Pinnacol, Pioneer and Advanta and moderate sucetible response from local variety and The isolate PhEt7 exhibited resistance reaction with three variety *viz.*, Pinnacol, Pioneer and Advanta while as PhEt7 exhibited moderate resistance reaction with local variety.

Group V consisted of isolates KhEt8 with moderate resistant response from Pinnacol, Pioneer and Advanta variety and moderate susceptible response from local variety respectively while SoEt9 moderate resistant response from Pioneer and Advanta variety and moderate susceptible and sucetible response from Pinnacol and local variety.

During the present study a wide variation among the nine isolates of *E. turcicum* in terms of cultural characteristics, morphology and pathogenicity was observed when tested on a four different putative maize variety *viz,.* Pinnacol,

Pioneer, Advanta and Local variety. Cluster analysis on the basis of similarity or dissimilarity in reaction pattern exhibited by these differential hosts grouped the isolates into 5 pathogenic groups. The isolate PEt4 and KEt5 which belong to group III was most aggressive isolate which represented Paithan and Kannad tehsil followed by AEt1 and SEt2 from group I and GEt3 from group II. The isolate PhEt7 from group IV belonged to Phulambari Tehsil and isolate KhEt8 from group V belonged to Khultabad tehsil was least aggressive. (This observation are similar to the observation made by Malik R. A., 2016).

The pathological variability of each isolates will be categorised based on five virulent scales, weak (DSI=1-10%), mild (11-20%), moderate (21-30%), virulent (31-50 %) and highly virulent (> 50%) (Shah *et al.*, 2006).

Table-4.11 : Pathogenicity of 9 isolates of *E. turcicum* on 4 maize variety

Variety	Percent Disease Intensity									
	AEt1	**SEt2**	**GEt3**	**PEt4**	**KEt5**	**VEt6**	**PhEt7**	**KhEt8**	**SoEt9**	**Mean**
Pinnacol	30.6	10.4	26.8	12.5	32.3	28.5	6.8	18..8	11.0	**19.86**
Pioneer	27.4	17.2	25.2	20.4	30.0	12.9	8.6	19.5	11.7	**19.21**
Advanta	19.2	8.3	25.4	8.5	26.5	10.2	8.5	25.6	7.9	**15.56**
Local variety	54.0	36.0	52.1	50.5	60.6	62.4	43.9	51.9	50.6	**51.33**
Mean	**32.80**	**17.97**	**32.37**	**22.97**	**37.35**	**28.5**	**16.95**	**32.33**	**20.30**	**26.83**

On the basis of disease reaction of 4 variety with 9 different isolates of *E.turcicum* it was found that KEt5 showed highest average disease intensity (37.35 per cent) followed by KhEt8 (32.33), GEt3 (32.37), AEt1 (32.80) and VEt6 (28.5) (Table-4.11 Fig 4.8). The isolate PhEt7 was least aggressive with average disease intensity of 16.95 percent followed by PEt4 (22.97), SEt2 (17.97) and SoEt9 (20.30).

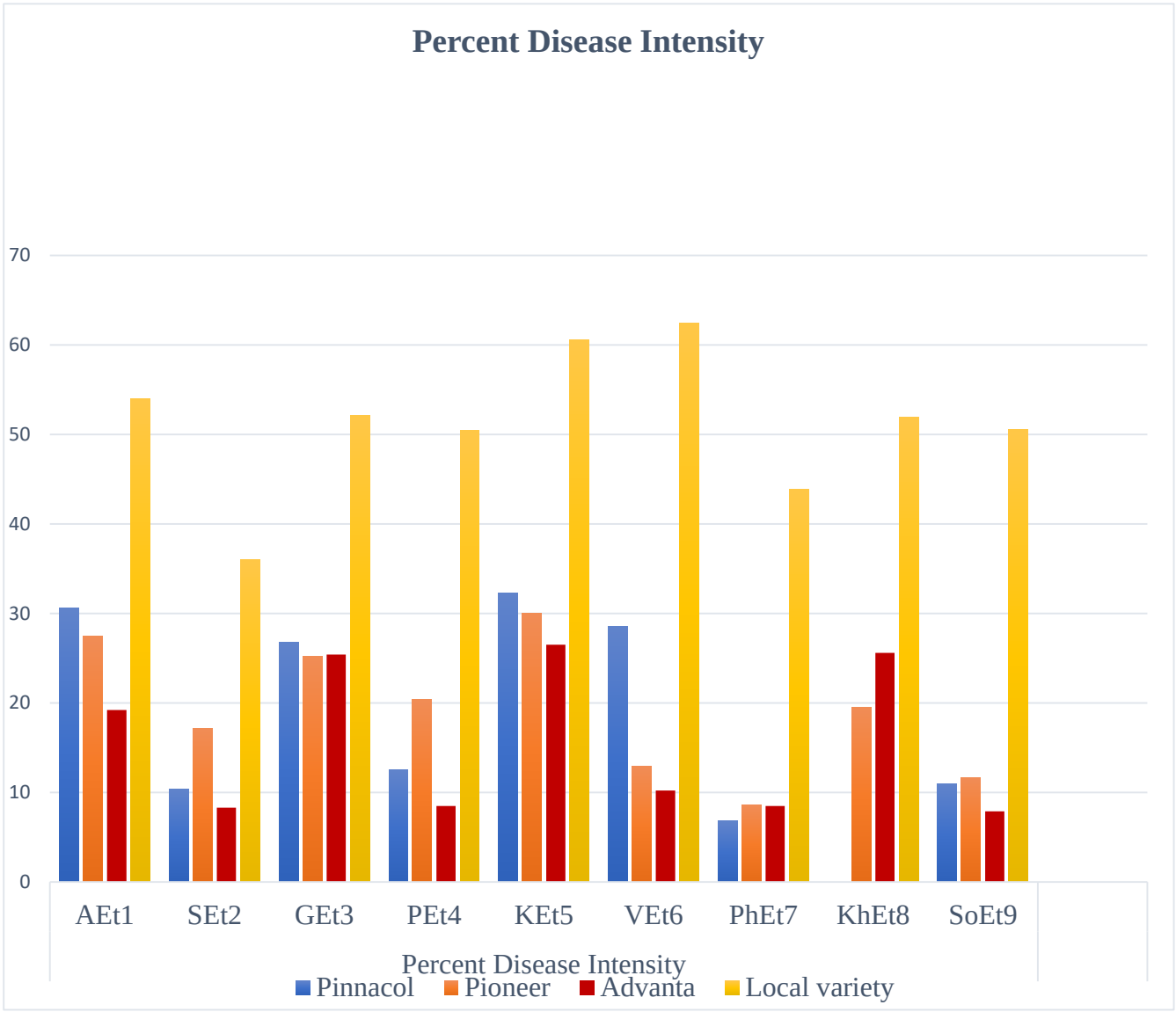


Fig- 4.8 : Pathogenicity of 9 isolates of *E. turcicum* on 4 maize variety showing percent disease intensity.

PLATE-4.9

Plate 4.9 : Variability studies of maize variety against Nine isolates *of E. turcicum* under controlled conditions

(Disease severity was estimated on inoculated leaves of four different variety with nine isolated collected from Aurangabad district, 14 days after inoculation by using 1-5 disease rating scale of Payak and Sharma, 1983.)

4.9.2 Virulence variability of Pathogen on suscetiple variety *i.e.*, Local variety

The isolates of *E. turcicum* tested in the present study exhibited considerable variation in the per cent disease intensity, virulence index, incubation period and lesion size of the local varietyof maize grown in pot culture. Among the 9 isolates of Aurangabad district the maximum percent disease intensity (62.40 %), virulence index (6.16), Lesion length (3.86 x 0.86) and shortest incubation period (4.36 days) was observed in isolate VEt6 from Vaijapur tehsil of Aurangabad district followed by KEt5 from Kannad, District Aurangabad. The isolate SEt2 from Sillod tehsil of Aurangabad district showed minimum PDI (36.06 %), virulence index (3.31), and longest incubation period of 7.55 days (Table-4.12).

The isolates showing shorter latent period were more virulent. Shorter latent period benefit the pathogen development (Agrios, 2005) while as longer latent period indicates the implication of dilatory resistance by the host as reported by Thakur *et al*. (2007).

Table-4.12 : Virulence of *Exserohilum turcicum* isolates of maize on Local variety

Isolate	per cent Disease intensity	Incubation period (Days)	Virulence Index	Lesion size (cm)	
				Length	breadth
AEt1	54.06	5.67	5.74	0.92	0.71
SEt2	36.06	7.55	3.31	0.67	0.80
GEt3	52.12	6.82	4.43	1.20	0.72
PEt4	50.53	6.94	3.20	1.25	0.67
KEt5	62.40	6.31	4.31	1.23	0.61
VEt6	60.68	4.36	6.16	3.86	0.86
PhEt7	43.90	6.87	2.52	0.42	0.30
KhEt8	51.96	5.76	4.37	0.67	0.80
SoEt9	50.63	7.11	3.74	1.20	0.68
SE±	**0.36**	**0.21**	**0.28**	**0.13**	**0.11**
CD@ 1%	**1.09**	**0.64**	**0.85**	**0.38**	**NS**

4.10 Disease management strategies

4.10.1 *In vitro* efficacy of different fungicides against *E. turcicum.*

The efficacy of certain fungicides against *E. turcicum* was tested in the laboratory using the poison food technique.

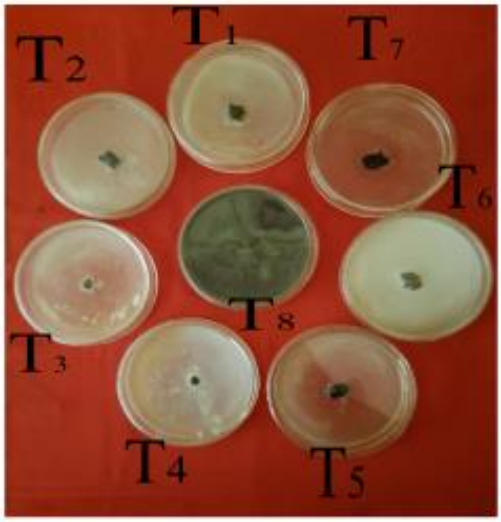
In vitro testing is an efficient tool for determining the effectiveness of fungicides against pathogens in a limited period of time. It is extremely useful in providing preliminary information and, as a result, acts as a reference for field evaluation. In the current study, systemic fungicides such as Azoxystrobin 23 % SC, Carbendazim 50 % WP, Tebenconazole 25.9 % EC, Propiconazole 25 % EC, Difenconazole 25 % EC, Hexaconazole 5 % SC, and Benomyl 50 % WP were tested against *E. turcicum* at three different concentrations (500, 1000 and 1500 ppm). Propineb 70 % WP, Merimain 50 % WP, Mancozeb 75 % WP, Copper oxychloride 50 % WP, Dinocarp 48 % EC, Copper Hydroxide 77 % WP, and Zineb 75 % WP were measured at three different concentrations (2000, 2500 and 3000 ppm).

Seven systemic fungicides and seven contact fungicide at three different concentrations were evaluated *in vitro* against *E. turcicum*. They exhibited a wide range of variation in mycelial growth and inhibition of the test pathogen. It was found that mycelial growth decreased drastically and inhibition increased with increased concentrations of fungicides tested. The result obtained are presented in Table 4.13, Table 4.14, Plate 4.10, Plate 4.11, Fig 4.9, 4.10, 4.11, 4.12, 4.13, 4.14, 4.15

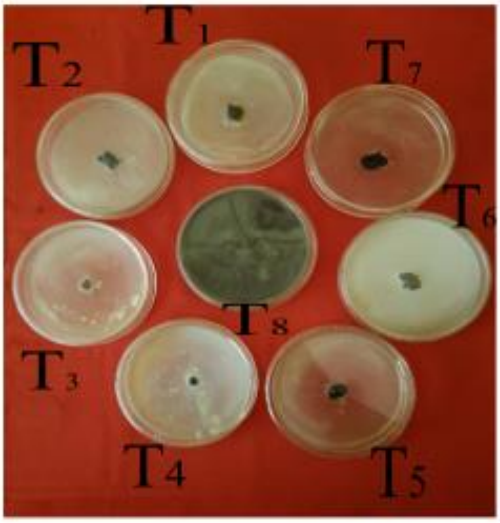
4.10.1.2 Effect of systemic fungicides on the growth of *Exserohilum turcicum*

At 500 ppm concentration, Tebuconazole 25.9 % EC, Carbendazim 50 % WP, Propiconazole 25 % EC, Azoxystrobin 23 % SC and Difenconazole 25 % EC were found effective in inhibiting the mycelial growth of the test fungus. Tebuconazole 25.9 % EC showed maximum inhibition (100 %), followed by Carbendazim 50 %WP (63.70 %) which is statistically at par with Propiconazole 25 % EC (63.10 %). Benomyl 50 % WP showed comparatively less mycelial inhibition (16.40) and Hexaconazole 5 % SC also showed comparatively less mycelial inhibition (18.88).

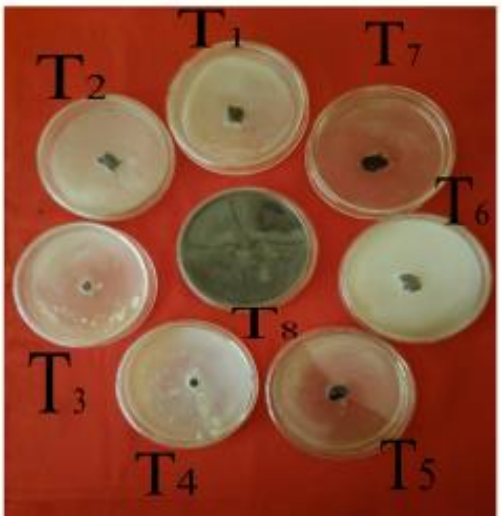
PLATE-4.10



500 ppm



1000 ppm



1500 ppm

Plate-4.10: ***In vitro*** **evaluation of Systemic fungicides on growth and inhibition of** ***Exserohilum turcicum.***

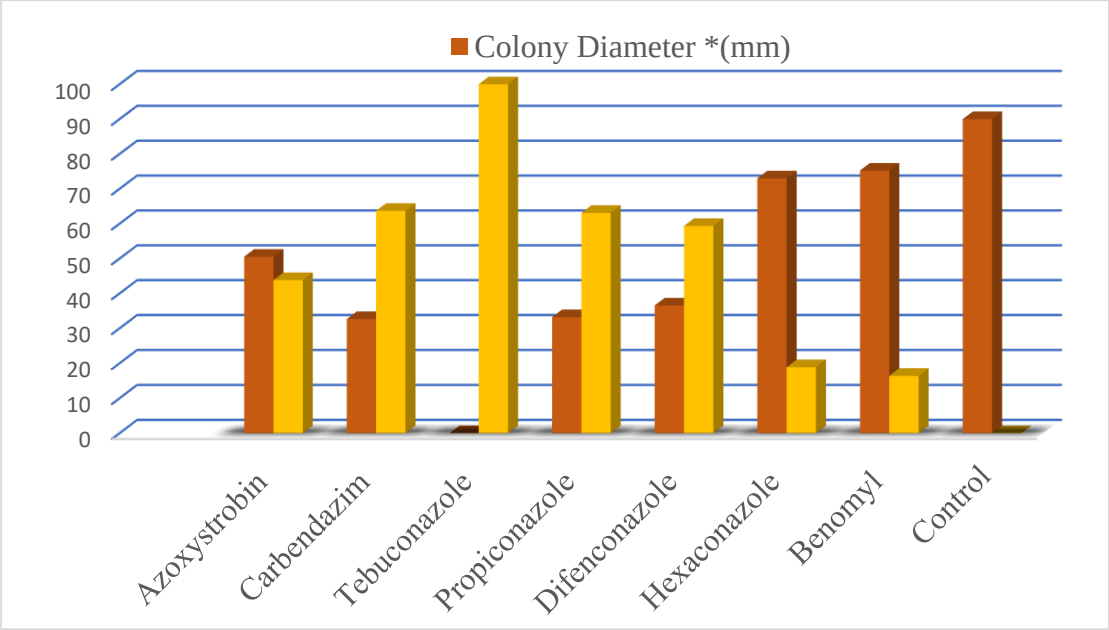


Fig-4.9. *In vitro* evaluation of different systemic fungicides (@ 500 ppm concentration) against *Exserohilum turcicum*.

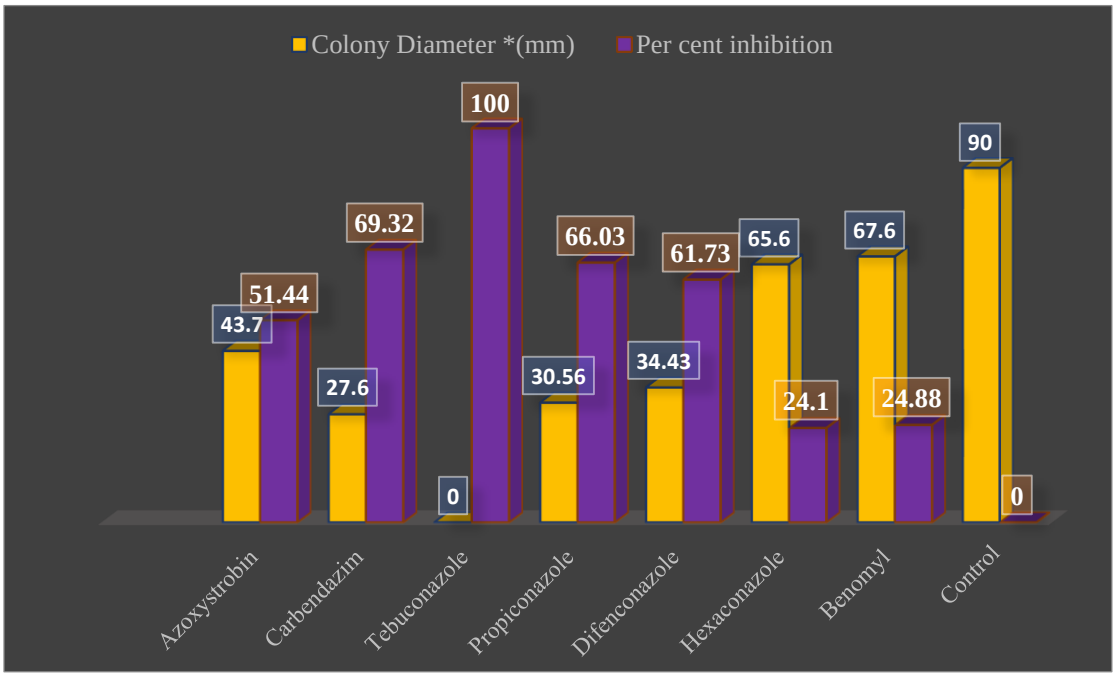


Fig- 4.10 : *In vitro* evaluation of different systemic fungicides (@ 1000 ppm concentration) against *Exserohilum turcicum*.

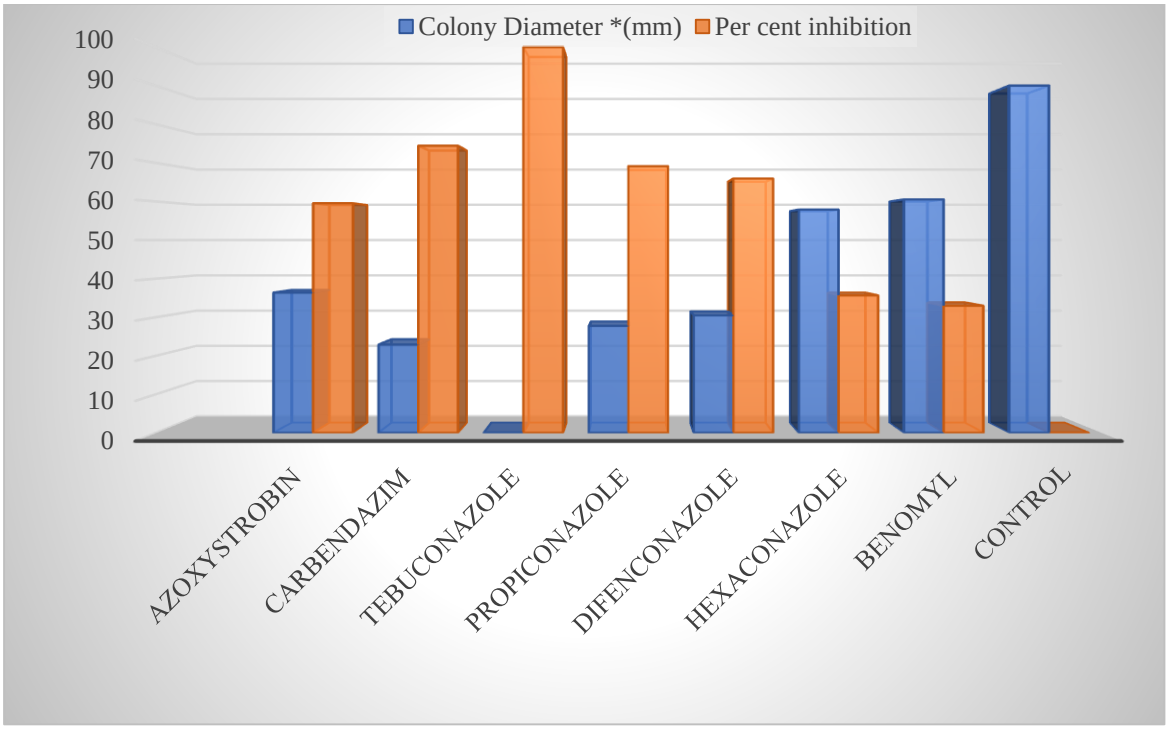


Fig- 4.11 : *In vitro* evaluation of different systemic fungicides (@ 1500 ppm concentration) against *Exserohilum turcicum.*

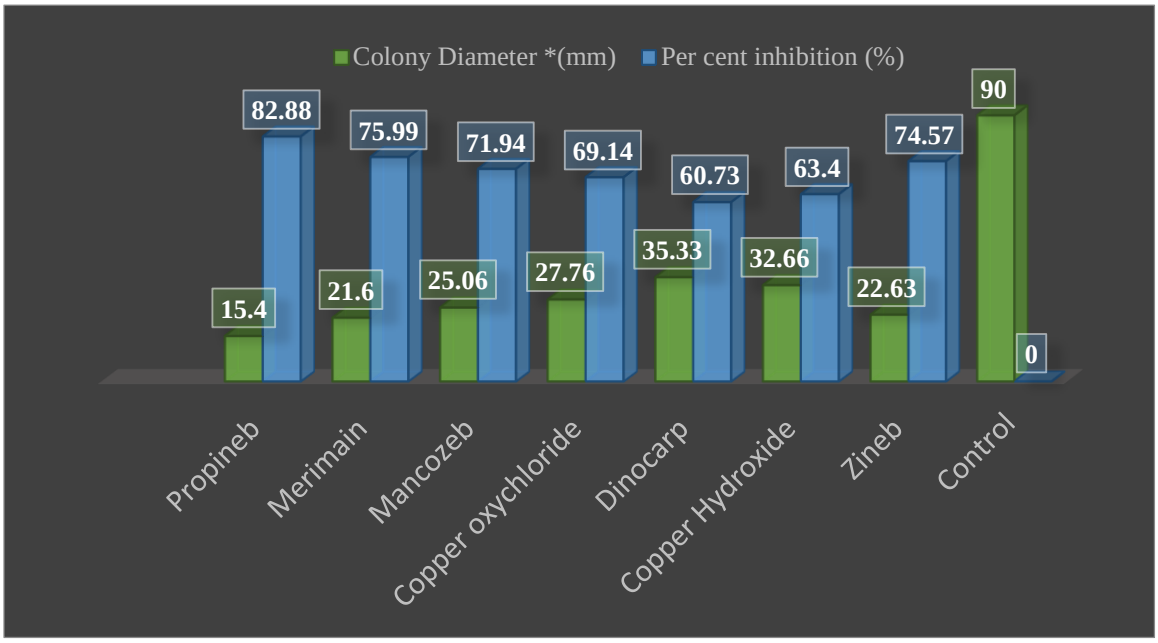


Fig- 4.12 : *In vitro* evaluation of different contact fungicides (@ 2000 ppm concentration) against *Exserohilum turcicum.*

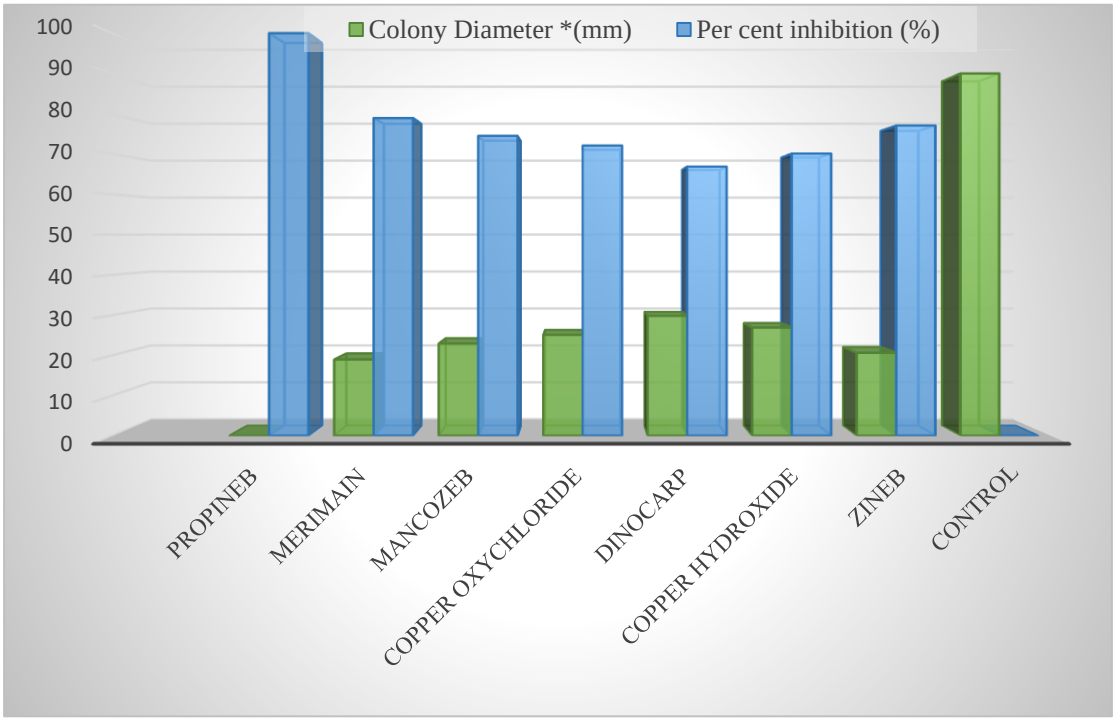


Fig- 4.13 : *In vitro* evaluation of different contact fungicides (@ 2500 ppm concentration) against *Exserohilum turcicum*.

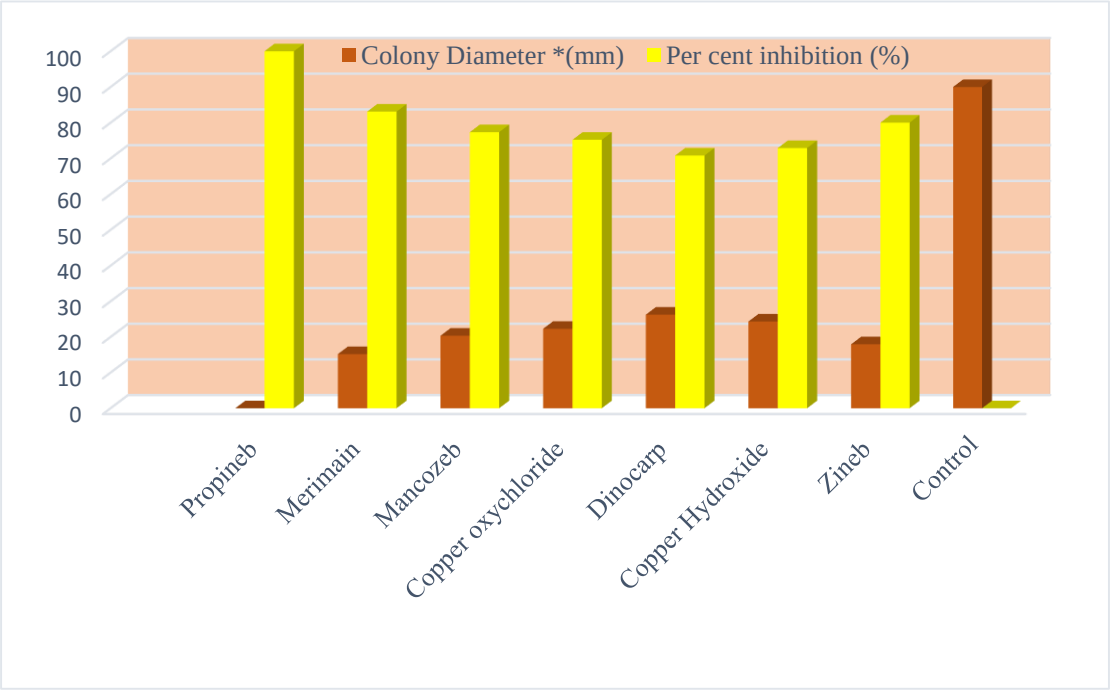


Fig-4.14 : *In vitro* evaluation of different contact fungicides (@ 3000 ppm concentration) against *Exserohilum turcicum*.

Table-4.13 : *In vitro* efficacy of systemic fungicides against *Exserohilum turcicum*

Tr No	Systemic Fungicides	Colony Diameter *(mm)				Per cent inhibition (%)			
		500	1000	1500	Mean	500	1000	1500	Average mean
T1	Azoxystrobin 23 % SC	50.53	43.70	36.40	65.5	43.84 (41.46)	51.44 (45.82)	59.55 (50.50)	51.61 (45.92)
T2	Carbendazim 50 %WP	32.66	27.60	22.90	27.72	63.70 (52.95)	69.32 (56.36)	74.44 (59.63)	69.15 (56.25)
T3	Tebuconazole 25.9 % EC	0.00	0.00	0.00	0.00	100 (90)	100 (90)	100 (90)	100 (90)
T4	Propiconazole 25 % EC	33.20	30.56	27.76	30.50	63.10 (52.59)	66.03 54.34()	69.14 (56.25)	66.09 (54.38)
T5	Difenconazole 25 %EC	36.56	34.43	30.50	33.83	59.36 (50.39)	61.73 (51.78)	65.94 (54.29)	62.34 (52.14)
T6	Hexaconazole 5 % SC	73.00	65.60	57.90	65.5	18.88 (25.75)	24.10 (29.40)	35.66 (36.66)	27.21 (34.44)
T7	Benomyl 50 % WP	75.23	67.60	60.53	67.78	16.40 (23.88)	24.88 (29.92)	32.99 (35.05)	24.75 (29.83)
T8	Control	90.00	90.00	90.00	90	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	SE±	**0.20**	**0.28**	**0.34**	**-**	**0.23**	**0.31**	**0.39**	**-**
	CD @ 1%	**0.63**	**0.86**	**1.03**	**-**	**0.70**	**0.96**	**1.19**	**-**

***Average of three replications and figures in parenthesis are arcsine transformation values.**

At 1000 ppm Tebuconazole 25.9 % EC showed per cent inhibition of mycelia growth followed by Carbendazim 50 %WP (69.32 %). Minimum inhibition was shown by Benomyl 50 % WP (24.88 %), followed by Hexaconazole 5 % SC (24.10 %).

At 1500 ppm concentration Tebuconazole 25.9 % EC (100 %) and Carbendazim 50 %WP (74.44 %) showed cent per cent inhibition of mycelial growth followed by Propiconazole 25 % EC (69.14 %), Azoxystrobin 23 % SC (59.55 %) and Difenconazole 25 % EC (65.94 %). Least mycelia growth inhibition per cent (32.99 %) and (35.66 %) was shown by Benomyl 50 % WP and Hexaconazole 5 % SC

The result can be concluded as Tebuconazole 25.9 % EC is most effective among all the other tested fungicides which is also in accordance with Manu *et al* (2017 who observed that Tebuconazole was effective against *E. turcicum* on maize *in vitro* conditions.

(Manu *et al.* (2017) observed that the systemic fungicide, tebuconazole completely inhibit the pathogen growth at all the concentrations tested.)

4.10.1.3 Effect of contact fungicides on the growth of *Exserohilum turcicum*

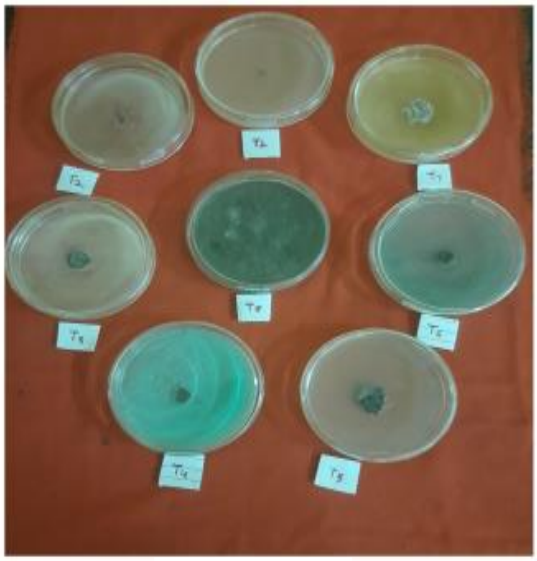
At 2000 ppm concentration, Propineb 70 % WP, Merimain 50 %WP, Zineb 75 % WP, Mancozeb 75 % WP, Copper Hydroxide 77 % WP and Copper oxychloride 50 % WP were found effective in inhibiting the mycelial growth of the test fungus. Propineb 70 % WP showed maximum inhibition (82.88 %), followed by Merimain 50 %WP (75.99 %) which is statistically at par with Merimain 50 %WP (71.94 %) and Zineb 75 % WP (74.57 %). Dinocarp 48 % EC showed comparatively less mycelial inhibition (60.73 %).

At 2500 ppm Propineb 70 % WP (100 %) showed per cent inhibition of mycelia growth followed by Merimain 50 %WP (78.92 %), Zineb 75 % WP (77.07 %), Mancozeb 75 % WP (74.51 %), Copper Hydroxide 77 %WP (70.10 %) and Copper oxychloride 50 % WP (72.11 %). Minimum inhibition was shown by Dinocarp 48 % EC (60.88 %).

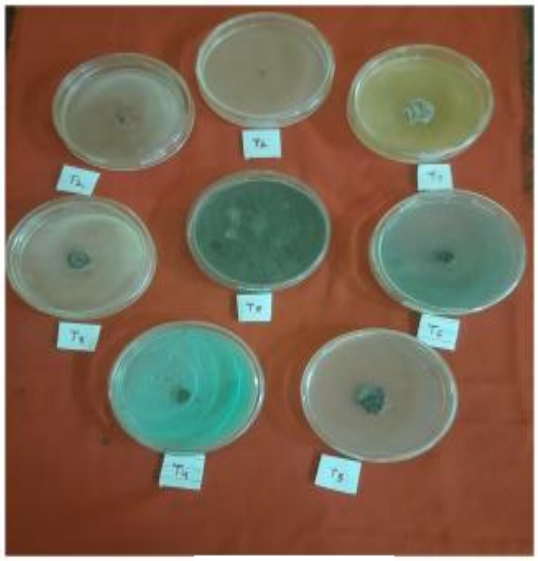
At 3000 ppm concentration Propineb 70 % WP (100 %) and Merimain 50 %WP (83.10 %) showed cent per cent inhibition of mycelial growth %), Zineb 75 % WP (80.03 %), Mancozeb 75 % WP (77.36 %), Copper Hydroxide 77 %WP (72.92 %) and Copper oxychloride 50 % WP (75.22 %). Least mycelia growth inhibition per cent (70.81 %) was shown by Dinocarp 48 % EC.

Among the Contact fungicides, propineb was highly effective at higher concentration as it inhibited the *E. turcicum* up to 82.88 %, 100 % and 100 % at 2000, 2500 and 3000 ppm respectively. Captan also found to be effective in inhibiting the mycelial growth up to 75.99, 78.92 and 83.10 % at 2000, 2500 and 3000 ppm concentration. Mancozeb was found to be less effective in inhibiting the growth of *Exserohilum turcicum* compared to other two contact fungicides as it inhibited mycelium growth up to 71.94, 74.5 and 77.36 % at 2000, 2500 and 3000 ppm concentration. Many workers like Harlapur *et al.* (2007) and Wathaneeyawech *et al.* (2015) reported that Mancozeb was effective in inhibiting the growth of the fungus but, the present investigation results were opposing to this which may be due to the variation in the isolates and due to the imprudent use of this mancozeb over a long

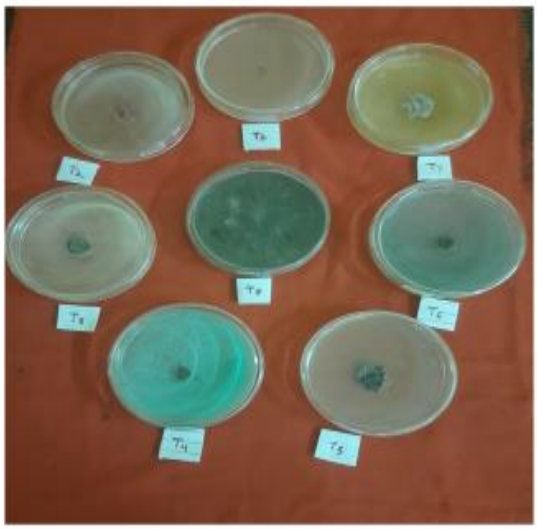
PLATE-4.11



2000 ppm



2500 ppm



3000 ppm

Plate-4.11 : *In vitro* evaluation of Contact fungicides on growth and inhibition of *Exserohilum turcicum.*

period of time which may result in development of resistant to the particular fungicides by the pathogen.

Propineb was highly effective at higher concentration as it inhibited the *E. turcicum* up to 82.88 %, 100 % and 100 % at 2000, 2500 and 3000 ppm respectively.. this result is similarly to the Conclusion given by Manu *et al.* (2017).

Table- 4.14 : *In vitro* efficacy of Contact fungicides against *Exserohilum turcicum*

Tr No	Contact Fungicides	Colony Diameter *(mm)				Per cent inhibition (%)			
		2000	2500	3000	mean	2000	2500	3000	Average mean
T1	Propineb 70 % WP	15.40	0.00	0.00	5.13	82.88 (65.55)	100 (92)	100 (90)	94.29 (76.17)
T2	Merimain 50 %WP	21.60	18.96	15.20	18.58	75.99 (60.65)	78.92 (62.66)	83.10 (65.72)	79.33 (62.95)
T3	Mancozeb 75 % WP	25.06	22.93	20.36	22.78	71.94 (58.01)	74.51 (59.67)	77.36 (61.58)	74.60 (59.73)
T4	Copper oxychloride 50 % WP	27.76	25.10	22.30	25.05	69.14 (56.25)	72.11 (58.12)	75.22 (60.14)	72.15 (58.14)
T5	Dinocarp 48 % EC	35.33	29.80	26.26	30.46	60.73 (51.19)	66.88 (54.86)	70.81 (57.29)	66.14 (54.41)
T6	Copper Hydroxide 77 %WP	32.66	26.90	24.36	27.97	63.40 (57.77)	70.10 (56.85)	72.92 (58.64)	68.80 (56.04)
T7	Zineb 75 % WP	22.63	20.63	17.96	20.40	74.57 (59.71)	77.07 (61.38)	80.03 (63.64)	77.22 (61.49)
T8	Control	90.00	90.00	90.00	90	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	SE±	**0.23**	**0.19**	**0.19**	**-**	**0.31**	**0.21**	**0.21**	**-**
	CD @ 1%	**0.72**	**0.59**	**0.57**	**-**	**0.95**	**0.65**	**0.64**	**-**

***Average of three replications and figures in parenthesis are arcsine transformation values.**

4.10.2 *In vitro* evaluation of bio-agents

4.10.2.1 Mycelial growth inhibition of *E. turcicum*

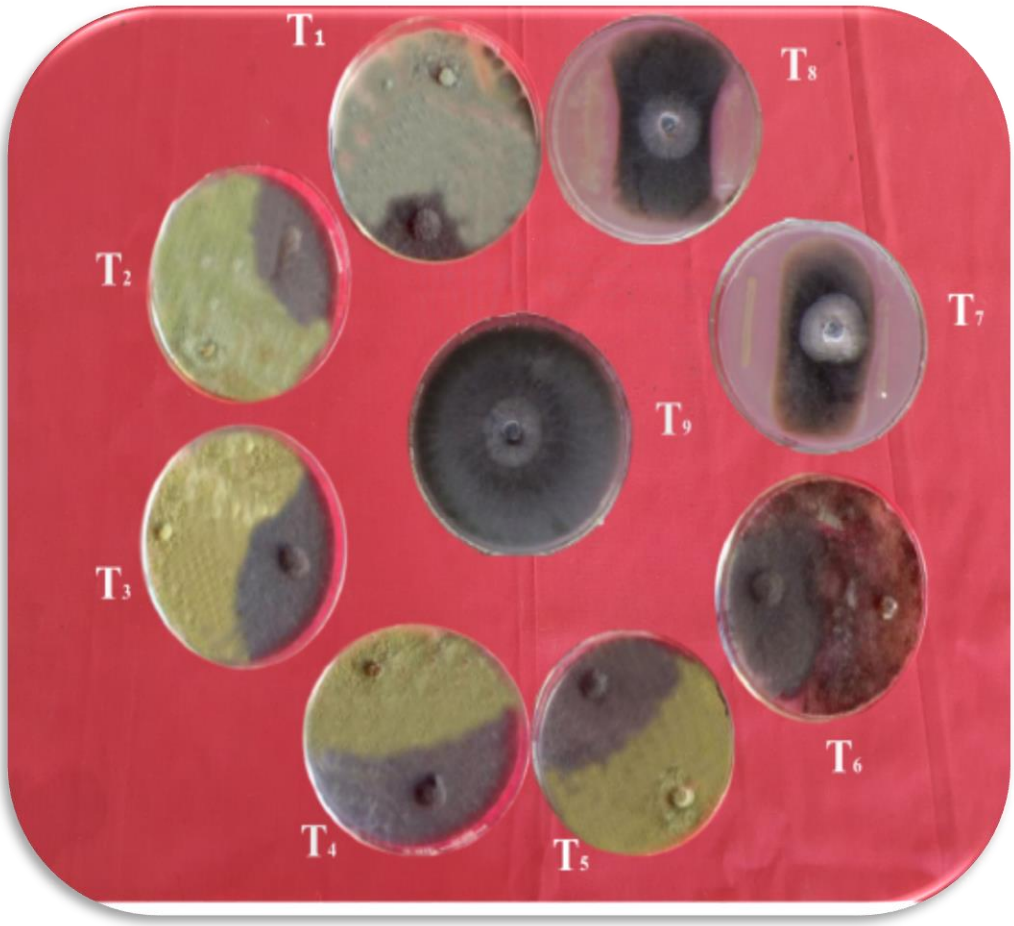
Use of bioagents for controlling plant diseases is an old practice in India. In the last two decades great emphasis has been given to antagonistic organisms to assess their potentiality for control of plant diseases using dual culture method. Antagonistic potential of 8 bioagent *viz., Trichoderma asperellum, Trichoderma harzhianum, Trichoderma hamatum, Trichoderma koningii, Trichoderma longibrachiatum, Aspergillus niger, Pseudomonas fluorescens* and *Bacillus subtillis* was evaluated against the pathogen *E.turcicum*. (shown Table 4.15, Plate 4.12 and Fig 4.15)

In dual culture all the isolates reduced the colony growth of the pathogen. Maximum inhibition of mycelia growth (64.51 %) was observed in case of *Trichoderma asperellum* which was statistically at par with *Trichoderma harzhianum* (59.55 %), *Bacillus subtillis* (52.44 %) and *Pseudomonas fluorescens* (47.55 %) followed by *Trichoderma hamatum* (47.51 %), *Trichoderma koningii* (43.77 %) and *Aspergillus niger* (46.03 %). Minimum inhibition of mycelia growth (41.96 %) was observed in case of Trichoderma longibrachiatum. The difference in inhibition of mycelial growth indicates the difference in their antagonistic potential for the test pathogen.

Results conclude that *Trichoderma asperellum* isolate is most effective in inhibiting the growth of the pathogen. This result was in accordance with Mahmood *et al.* (1995) reported that *Trichoderma sp* were highly effective in inhibiting mycelial growth and sporulation of *Helminthosporium turcicum* causing leaf blight of maize.

Trichoderma asperellum is most effective followed by Trichoderma harzhianum and *Bacillus subtillis*, this result is accordance with kumar (2010) .

PLATE-4.12



T1: *T. asperellum*
T2: *T. harzhianum*
T3: *T. hamatum*
T4: *T. koningii*
T5: *T. longibrachiatum*
T6: *Aspergillus niger*
T7: *Pseudomonas fluorescens*
T8: *Bacillus subtillis*
T9: Control

Plate-4.12 : *In vitro* evaluation of bio-agents against *Exserohilum turcicum*.

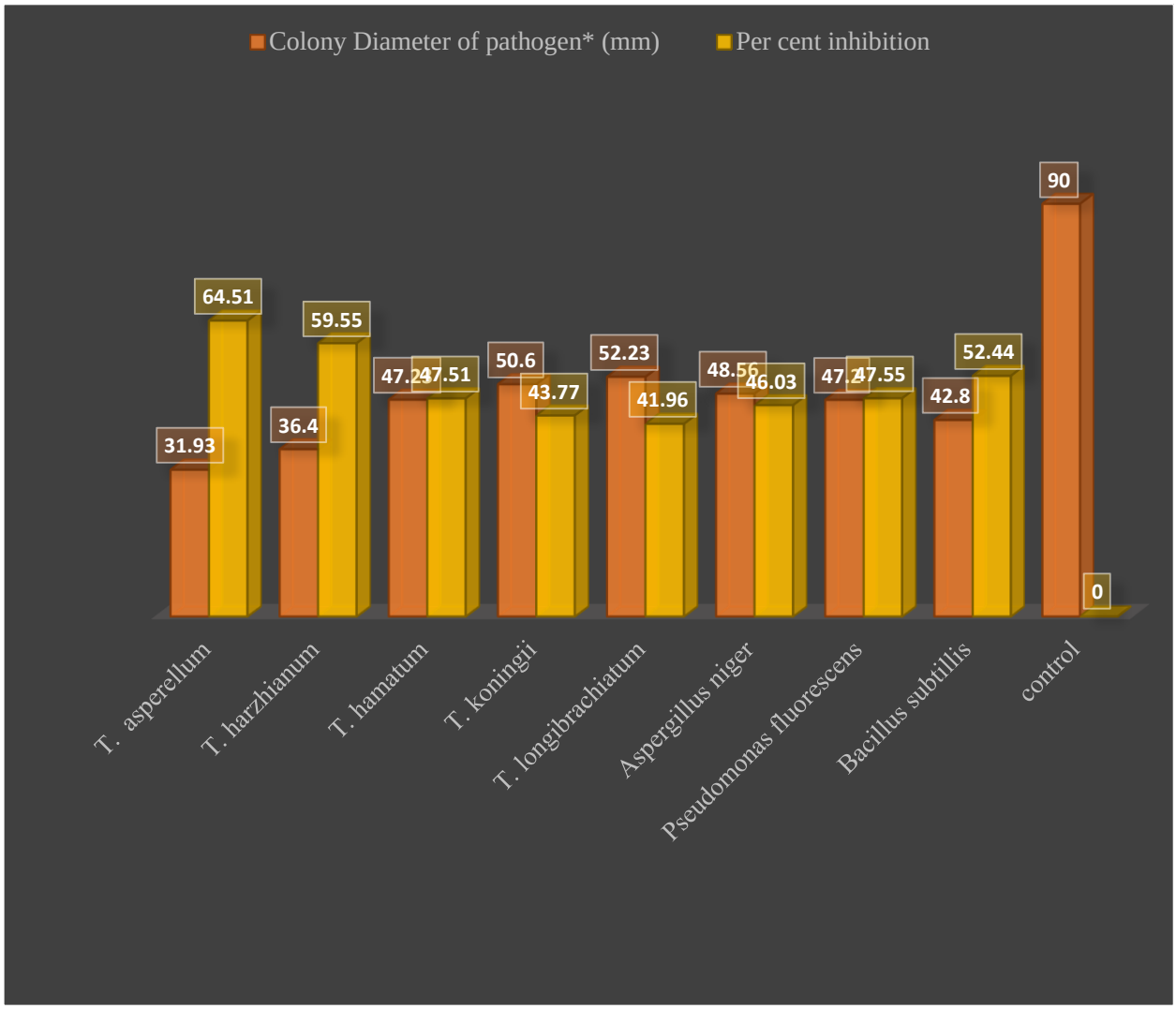


Fig- 4.15 : *In vitro* evaluation of different bio-agents against *Exserohilum turcicum*.

Table- 4.15: *In vitro* efficacy of different bio-agents against *Exserohilum turcicum* against *Exserohilum turcicum*

Tr. No.	Bioagents	Colony Diameter of pathogen* (mm)	Per cent inhibition (%)
T_1	*Trichoderma asperellum*	31.93	64.51 (53.43)
T_2	*Trichoderma harzhianum*	36.40	59.55 (50.50)
T_3	*Trichoderma hamatum*	47.23	47.51 (43.57)
T_4	*Trichoderma koningii*	50.60	43.77 (41.42)
T_5	*Trichoderma longibrachiatum*	52.23	41.96 (40.37)
T_6	*Aspergillus niger*	48.56	46.03 (42.72)
T_7	*Pseudomonas fluorescens*	47.20	47.55 (43.59)
T_8	*Bacillus subtillis*	42.80	52.44 (46.39)
T_9	Control	90.00	0.00 (0.00)
	SE±	**0.20**	**0.22**
	CD @ 1 %	**0.61**	**0.67**

***Average of three replications and figures in parenthesis are arcsine transformation values.**

4.10.3 *In vitro* efficacy of different Botanicals against *E.turcicum*

Plant extracts are cost effective and eco friendly means of management so an effort was made to assess antifungal activity of different plant extracts against *E. turcicum*. Nine plant extracts *viz*, Ginger, Neem, Tulsi, Ghaneri, Ashwagandha, Onion, Aloe vera, and Ashoka at two concentration 5 and 10 % (shown in Table 4.16 plate 4.13 and fig 4.16, 4.17).

At 5% concentration maximum inhibition of mycelial growth (73.70 %) was showed by Garlic followed by Aloevera (55.49 %) which was statistically at par with Neem (43.84 %) , onion (41.96 %) and Ghaneri (24.55 %). Minimum inhibition of mycelial growth was shown by Ashwagandha (4.59 %) followed by Ashoka (6.95 %) and Tulsi (7.03 %).

At 10% concentration garlic showed maximum mycelial growth inhibition (100 %) followed by Aloevera (81.07 %). Ashwagandha showed minimum inhibition of mycelial growth (5.92 %) followed by Ashoka (16.66 %) and Tulsi (9.29 %).

In this present investigation also garlic extract showed up to 73.70 % and 100 % inhibition at 5 % and 10 % concentration. In this study inhibition of mycelial growth of fungus by garlic is may be due to the presence of antibiotic allicin in garlic extract and parthenium leaf extract also found to inhibit the mycelial growth. This result is similarly to result of Manu *et al* (2017).

PLATE-4.13

5 %

10%

T1 : Garlic
T2 : Neem
T3 : Tulsi
T4 : Ghaneri
T5 : Ashwagandha
T6 : Onion
T7 : Aloevera
T8 : Asoka
T9 : Control

Plate-4.13 : *In vitro* evaluation of phyto-extracts against *Exserohilum turcicum.*

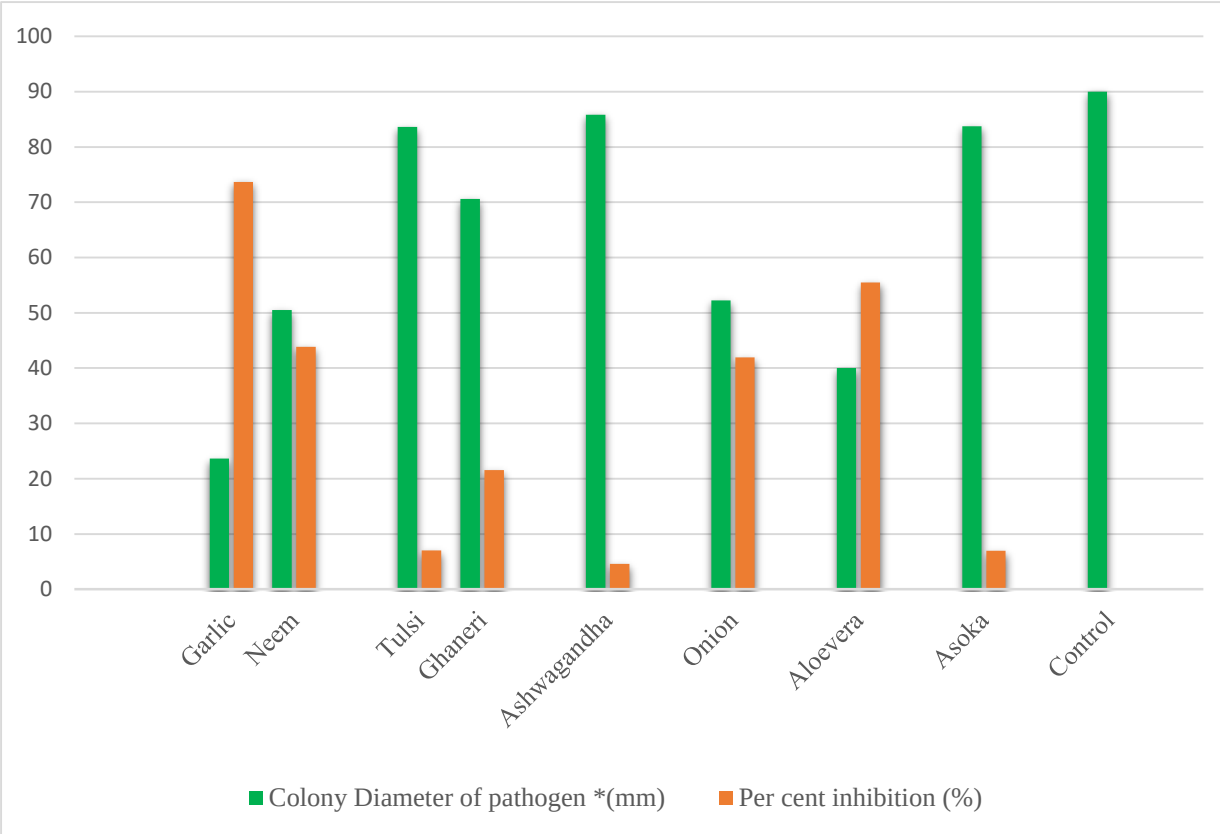


Fig-4.16: *In vitro* evaluation of different phyto-extracts (@ 5 % concentration) against *Exserohilum turcicum.*

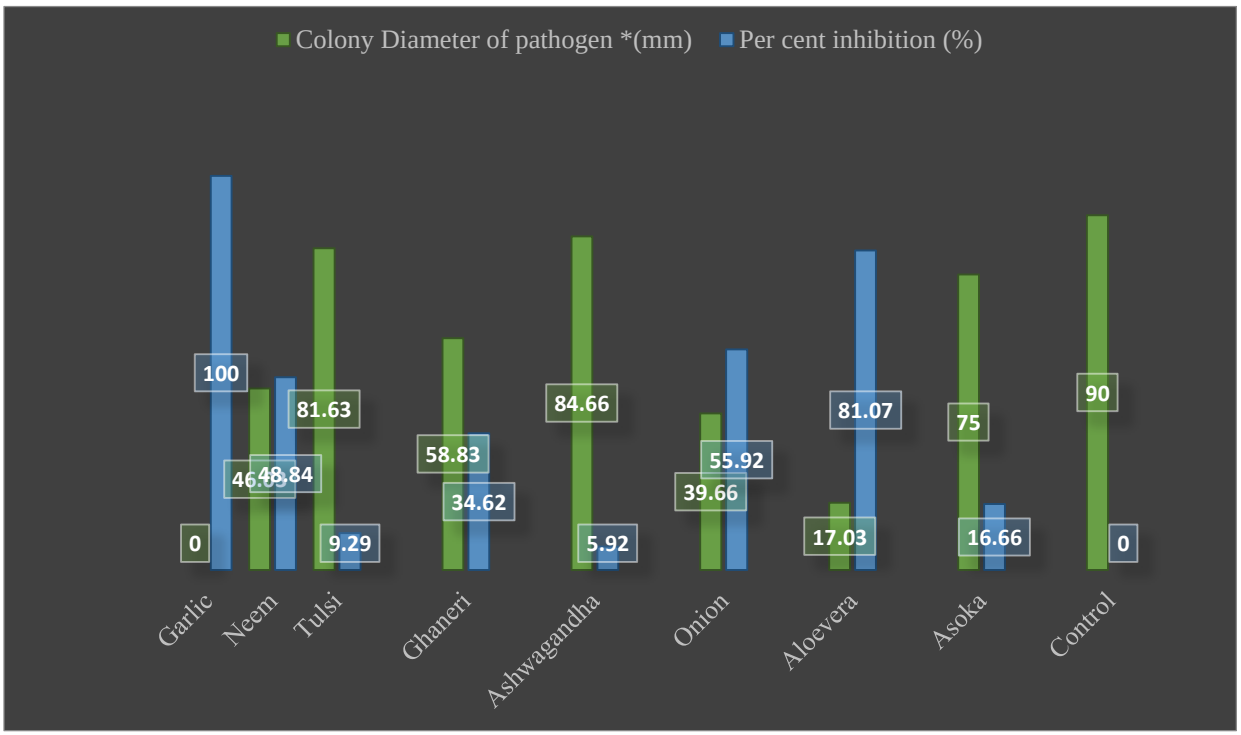


Fig-4.17 : *In vitro* evaluation of different phyto-extracts (@ 10 % concentration) against *Exserohilum turcicum.*

Table- 4.16 : *In vitro* efficacy of different plant extracts against mycelium growth and inhibition of *E. turcicum*

Tr.No.	Common name	Scientific name	Colony Diameter of pathogen *(mm) concentration		Per cent inhibition (%)		Average mean (%)
			5 %	10 %	5 %	10 %	
T_1	Garlic	*Allium sativum*	23.66	0.00	73.70 (59.14)	100.00 (90)	86.85 (68.73)
T_2	Neem	*Azadirachta indica*	50.53	46.03	43.84 (41.46)	48.84 (44.33)	46.34 (42.90)
T_3	Tulsi	*Ocimum sanctum*	83.66	81.63	7.03 (15.37)	9.29 (17.74)	7.16 (15.52)
T_4	Ghaneri	*Lantana camara*	70.60	58.83	21.55 (27.65)	34.62 (36.04)	28.08 (31.99)
T_5	Ashwagandha	*Withania somnifera*	85.86	84.66	4.59 (12.37)	5.92 (14.08)	5.25 (13.24)
T_6	Onion	*Allium cepa*	52.23	39.66	41.96 (40.37)	55.92 (48.39)	48.94 (44.39)
T_7	Aloevera	*Aloe barbadensis*	40.05	17.03	55.49 (48.15)	81.07 (64.20)	68.28 (55.72)
T_8	Asoka	*Saraca asoca*	83.74	75.00	6.95 (15.28)	16.66 (24.08)	11.80 (20.09)
T_9	Control	Untreated	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	SE±	-	**0.20**	**0.29**	**0.22**	**0.33**	-
	CD @ 1 %	-	**0.60**	**0.89**	**0.67**	**0.99**	-

***Average of three replications, Figures in parenthesis are arcsine transformed values.**

CHAPTER-V

SUMMARY
&
CONCLUSION

CHAPTER-V

SUMMARY AND CONCLUSIONS

Maize is one of the world's most valuable cereal crops, contributing to food security in the majority of developing countries. Maize is widely grown throughout the world and produces the most of any cereal crop, with 972.40 MT (Anonymous FAO, 2018-19). Many biotic and abiotic stresses reduce maize production and result in significant economic losses. Northern leaf blight of maize, caused by the Deuteromycetes fungus *Exserohilum turcicum* (Telomorph: *Setosphaeria turcica*), is one of the most serious fungal diseases affecting maize. The disease is found in almost all maize-growing areas and causes significant yield loss.

Given the disease's significance, the current research was conducted to classify the causal organism associated with northern leaf blight of maize. The research on "Study on Northern Leaf Blight of Maize Caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs." was conducted at the College of Agriculture, Badnapur, and the NARP, Aurangabad in 2020-21. Systematic research on morpho-cultural, physiological, and pathological variability was carried out. The *in vitro* efficacy of various fungicides, botanicals, and biogents against the test pathogen was evaluated at various concentrations, and the findings obtained are summarised.

Northern leaf blight of maize is one of the most significant limiting factors of maize production in the Marathwada region's Aurangabad district. During the years 2020-2021, research at the College of Agriculture in Badnapur and the NARP in Aurangabad studied the disease status and pathogenic variability of the causal agent. The survey, which was conducted in major maize growing areas of the nine tehsils of Aurangabad district, namely Aurangabad, Sillod, Gangapur, Paithan, Kannad, Vaijapur, Phulambari, Khultabad, and Soegaon during *Kharif* 2021, revealed the disease's prevalence in all surveyed locations, with varying levels of incidence and intensity ranging from 25.0 to 57.2 % and 12.0 to 29 %.

Symptoms begin as small oval, water-soaked, slightly elliptical greyish-colored spots, but later enlarge and become elongated spindle-shaped. The lesions

were cigar-shaped and straw-colored in the middle, with dark margins. These lesions grow in size, covering a greater region of the leaves and producing brighter symptoms. A microscopic examination of the test fungus was performed to determine the pathogen's morphological characteristics. Under the microscope, the hyphae were pale to light brown, smooth, and septate. The conidia were spindly in shape with a protruding hilum. Conidia were olivaceous brown in colour and had 3-8 septation.

The disease - causing fungus was isolated from diseased plant samples in pure culture and shown to be pathogenic. The pathogen isolated was identified as *Exserohilum turcicum* (Pass.) Leonard and Suggs (Syn. *Helminthosporium turcicum* Pass.), teleomorph; *Setosphaeria turcica* Luttrell based on the source of isolation, morpho-cultural, physiological, and pathological variability of different isolates in pure culture, as well as host, pathogenicity, symptomatology, and comparison with the authentic descriptions *(Syn. Trichometasphaerium turcica Lutterl*).

The pathogen was isolated, and after purification, the fungal cultures showed white colour and fluffy mycelium, which eventually turned greyish, greenish, or brownish as the culture began to produce conidia. The association of *E. turcicum* was discovered after repeated isolation. A total of nine isolates were collected and labelled with the isolate code Et based on their respective locations of collection. By comparing it to applicable literature and observing the morphological and cultural characteristics, the pathogen was identified. Nine isolates displayed three different conidial shapes: curved, spindle, and elongated. Conidia had an overall length of 73.79 μm and a width of 22.42 μm. The number of septa discovered ranged from 3 to 9. Isolate PhEt7 had the largest conidia (97.96 × 26.52μm) with an average of 3-8 septation, while isolate AEt1 had the smallest (49.62 × 15.55μm).

All the nine *E. turcicum* isolates produced good growth on Potato Dextrose Media (PDA) The period taken by different isolates to completely cover the 9 cm Petri dish were different based on the aggressiveness of the isolate. The colony colour varied from grey to black colour. The isolates when grown on PDA shown sparsely branched to highly branching edges of the colony. On PDA majority of the isolates that produced distinct wavy and moderately wavy zonation are SoEt9, SEt2, VEt6 and KhEt8 while adpressed colony produced by AEt1 isolate.

Temperature is also a significant factor in controlling the fungus's growth and reproduction. The effect of different temperatures, namely 20°C, 25°C, and 30°C, on the mycelia growth of nine *Exserohilum turcicum* isolates was investigated. At 30 °C, isolate AEt1 had a maximum colony diameter of 86.77 mm and isolate KEt5 had a minimum mycelial development of 40.71 mm.

E. turcicum isolates from Aurangabad, Sillod, Gangapur, Paithan and Soegaon were found to be highly pathogenic on local variety. On susceptible local variety, some of the isolates produced maximum lesion size and could be considered as most virulent isolates. The results indicated that there were three virulence patterns exhibited on four maize variety after inoculating with 9 isolates of *E. turcicum*. The isolates could be grouped into three virulent types viz., the isolates have reaction 3.84 as most virulent, the reaction between 1.31 -1.40 as moderately virulent and reaction up to 0.97 as less virulent type.

Results revealed that considerable pathogenic variability among the different isolates of *E. turcicum*. Cluster analysis based on similarity or dissimilarity in reaction types exhibited by these differential hosts grouped the isolates into 5 pathogenic groups. During the present study, a wide variation among the nine isolate in terms of cultural characteristics, morphology and pathogenicity was observed when tested on four different putative maize variety. The isolate PEt4 and KEt5 which belong to group III was the most aggressive isolate which represented Paithan and Kannad tehsil followed by AEt1 and SEt2 from group I and GEt3 from group II. The study found that *E. turcicum* it was found that KEt5 showed highest average disease intensity (37.35 per cent) followed by KhEt8 (32.33), GEt3 (32.37), AEt1 (32.80) and VEt6 (28.5) (Table-XI). The isolate PhEt7 was least aggressive with average disease intensity of 16.95 percent followed by PEt4 (22.97), SEt2 (17.97) and SoEt9 (20.30).

In the current study, the isolates of *E. turcicum* tested showed significant variation in the percent disease severity, virulence index, incubation time, and lesion size of a local variety of maize grown in pot culture. The isolate VEt6 from Vaijapur tehsil of Aurangabad district had the highest percent disease severity (62.40), virulence index (6.16), lesion duration (3.86 x 0.86), and shortest incubation period (4.36 days) among the 9 Aurangabad district isolates, followed by KEt5 from Kannad, District Aurangabad. The isolate SEt2 from the Aurangabad district's Sillod tehsil had the lowest PDI (36.06), the highest virulence index (3.31), and the longest incubation time of 7.55 days.

The poison food technique was used in the laboratory to measure the effectiveness of various fungicides against *E. turcicum*. It was discovered that as the concentrations of fungicides tested increased, mycelial growth decreased dramatically and inhibition increased. Seven systemic fungicides and seven contact fungicides) were tested at three different concentrations.

Tebuconazole 25.9 % EC was found to be effective in inhibiting the mycelial growth of the test fungus in a systemic fungicide at a concentration of 500 ppm. At 1000 ppm, it inhibited mycelia growth by 100%, followed by Carbendazim, which inhibited mycelia growth by 50 %. (69.32 %) Benomyl 50 % WP (24.88 %) inhibited the least, followed by Hexaconazole 5 % SC. Tebuconazole 25.9 % EC (100 %) and Carbendazim 50 % WP (74.44 %) inhibited mycelial development fully at 1500 ppm, followed by Propiconazole 25% EC (69.14%), Azoxystrobin 23 % SC (59.55 %), and Difenoconazole 25 % EC (69.14 %.)

Propineb was highly effective in contact fungicides at higher concentrations, inhibiting *E. turcicum* up to 82.88 %, 100 %, and 100 % at 2000, 2500, and 3000 ppm, respectively. Dinocarp 48 % EC inhibited mycelia development by the least amount (70.81 %). At 2000 ppm, Propineb 70 % WP inhibited the most (82.88 %), followed by Merimain 50 %WP (75.99 %).

Over the last two decades, much focus has been placed on antagonistic species in order to determine their capacity for plant disease control. The antagonistic potential of eight bioagents against the pathogen *E. turcicum* was investigated. Both of the isolates inhibited the colony growth of the test pathogen in dual culture. *T. asperellum* inhibited mycelia development the most (64.51 %), which was statistically comparable to other bioagents. *T. harzhianum* (59.55 %), *Bacillus subtillis* (52.44 %), and Pseudomonas fluorescens (47.55 %), followed by *T. hamatum* (47.51 %), *T. koningii* (43.77 %), and *Aspergillus niger* (47.55 %.)

Since plant extracts are a cost-effective and environmentally friendly method of management, an attempt was made to evaluate the antifungal efficacy of various plant extracts against *E. turcicum*. Garlic inhibited mycelial growth the most (73.70 %) at a 5 % concentration, followed by Aloe vera (55.49 %), which was statistically equal to Neem (43.84 %), onion (41.96 %), and Ghaneri (41.96 %). And more inhibition was observed at 10 % concentration.

CONCLUSIONS :

1. Northern leaf blight is present in all maize-growing areas of the Aurangabad district in varying degrees of severity.
2. The causal pathogen of the disease is *Exserohilum turcicum* Leonard and Suggs (Syn. *Helminthosporium turcicum* Pass.)
3. According to the survey results, Northern leaf blight disease is one of the most common and widespread diseases in all nine tehsils in the Aurangabad district during *Kharif* 2020-21. It was also discovered that the highest incidence of Northern leaf blight of maize (57.2 %) was found in Jategaon, Phulambari Tehsil, and the highest intensity of Northern leaf blight of maize (29.1 %) was found in Chinchkheda, Gangapur Tehsil, while the lowest disease incidence (26.5 %) was found in Waluj, Aurangabad Tehsil, and the lowest disease intensity of Northern leaf blight of maize (12.0 %) was found in Aurangabad tehsil.
4. The pathogen *Exserohilum turcicum* (Pass.) was isolated successfully on Potato Dextrose Agar from naturally infected Maize crop plants gathered during the survey. *Exserohilum turcicum* (Pass) isolated culture was collected, purified, and stored for future research.
5. *Exserohilum turcicum* isolate pathogenicity (Pass). Spore suspension inoculation in pot culture proved successful on vulnerable Maize varieties.
6. In terms of morpho-cultural, physiological, and pathological diversity, the different test isolates of *E. turcicum* varied greatly.
7. The significant interaction of four different maize varieties (pinacol, pioneer, advanta, and local) and nine isolates suggests some kind of specialisation in the fungus population because there are differences in both the resistance level of maize varieties and the aggressiveness of the pathogen isolates.
8. The presence and spread of diverse isolates of *E. turcicum* with wide pathogenic diversity in the field gives critical information for developing an appropriate disease control approach.
9. *In vitro* testing of various fungicides against *Exserohilum turcicum* revealed that systemic fungicides such as Tebuconazole 25.9 % EC, Carbendazim 50 % WP,

Propiconazole 25 % EC, Azoxystrobin 23 % SC, and Difenoconazole 25 % EC were effective in inhibiting mycelial growth of *Exserohilum turcicum* in Petri plates.

10. *In vitro* testing of various fungicides against *Exserohilum turcicum* revealed that contact fungicides such as Propineb 70 % WP, Merimain 50 % WP, Zineb 75 % WP, Mancozeb 75 % WP, Copper Hydroxide 77 % WP, and Copper Oxychloride 50 % WP were effective in inhibiting mycelial growth of *Exserohilum turcicum* in Petri plates.

11. Among the several bio-agents tested *in vitro*, the results show that *Trichoderma asperellum* isolate is the most effective at preventing pathogen growth. More promising bio-agents to block the mycelial growth of *Exserohilum turcicum* in Petri plates were *T. harzhianum*, followed by *Bacillus subtilis*.

12. In the case of Phyto-extracts, Garlic clove extract and Aloe vera extract were more effective at restricting *Exserohilum turcicum* mycelial growth in Petri plates at 5 % and 10 % concentration.

LITERATURE CITED

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Alcorn, J. L. (1988). The taxonomy of "*Helminthosporium*" species. *Annual Review of Phytopathology* 26, 37–56.

Aden, M. H. (1991*). Studies of sorghum leaf blight incited by Exserohilum Turcicum (pass.) Leonard & Suggs*. M. Sc. (Ag) Thesis. Acharya N G Ranga Agricultural University, Hyderabad, India.

Agrios, G. N. (2005). Plant diseases caused by fungi. In Plant Pathology Harcourt Asia PTE Ltd, 583 Orchard road, Singapore pp. 316

Arora, D. K., Upadhyay, R. K., (1978). Effect of fungal staling growth substances on colony interaction. *Pl. Soil*. 1978; 49:685- 690.

Anonomyous (2019) Indiastat.com. https://www.indiastat.com.

Anonymous USDA, (2015). United States Department of Agriculture. URL: http.//www.ers usda. gov/media/ 866543/cornusetable.html. (Retrieved : 03/7 /2015).

Anonomyous FAO, (2017) FAOSTAT 2015-2016 www. fao. org/faostat/en/#data.

Begum, H., Raj, R. B and Satyanarayana, E. (1993). Field evaluation of five fungicides to control turcicum leaf blight in maize. *Indian Journal of Plant Protection*. 21: 110-111.

Bolkvaldze, Z. A. (1977). Specialization of Helminthosporium turcicum Pass. Mikologiya Fitopatology 11(4): 345-346.

Bunker, R. N., Rathore, R. S and Kumawat, D. K. (2011). Pathogenic and morphological variability of Bipolaris maydis incitant of maydis leaf blight in maize. *Journal of Mycology and Plant pathology*. 41 (3): 418-421.

Bunker, R. N and Kusum Mathur. (2010). Pathogenic and morphological variability of *Exserohilum turcicum* isolates causing leaf blight in sorghum *(Sorghum bicolour) Indian Journal of Agricultural Sciences.* 80(10): 888-892.

Butler E.J. (1907). Some diseases of cereals caused by *Selenorpel graminicola*. Department of Agriculture, India, Botanical Survey. 2: 1-24.

Bugnicourt F. (1955). Deux especes nouvelles d' *Helminthosporium* isolates. De Riz. Genn. Bot. 62: 238-243.

Bhatt, B. and Kumar, P. (2019). Bioefficacy of fungicides, plant extracts and essential oils against *Exserohilum turcicum* (Pass.) Leonard and Suggs causing Northern leaf blight of maize, *Journal of Applied and Natural Science*, 11(2): 257262.

Champi, M. D. (1939). Helminthosporium turcicum Pass. *En la Republic Argentina.* Lilloa, 4: 532.

Chaudhary, D. P., Kumar, S. and Yadav, O. P. (2014). Nutritive Value of Maize improvements applications and constraints. Maize: Nutrition Dynamics and Novel Uses. pp 3-17.

Chenulu V. V. and Hora, T. S. (1962). Studies on losses due to Helminthosporium blight of maize. Indian Phytopathology, 15: 235-237.

Cox R., (1956). Control of Helminthosporium turcicum blight disease of sweet corn in south Florida. Phytopathology.5:68-70.

Christensen, J. J. (1929). The influence of temperature on the frequency of mutation in *Helminthosporum sativum*. *Phytopathology*. 19: 155-162.

Daniel, A. and Narong, S. (2006). Morphological, cultural and pathogenicity variation of E. turcicum (Pass) Leonard and Suggs isolates in maize (Zea mays L.). *Journal of Natural Science* 40, 341-352.

Dharanendraswamy, S. (2003). *Studies on turcicum leaf blight of maize caused by E. turcicum.* M.Sc. (Agri.) Thesis, University of Agricultural Sciences, Dharwad.

Dhingra, O. D. and Sinclair, J. (1999). Basic Plant Pathology Methods. Lewis, CRC Press, Florida.

Drechsler, C. (1923). Some graminicolous species of *Helminthosporium. Journal of Agricultural Research,* 24: 641-739.

Frederiksen, R. A. (1980) Sorghum leaf blight. in sorghum diseases. *A World review,* ICRISAT, Patancheru, India Pages 243- 248.

Galinat, W. C. (1988). The origin of corn. In Sprague, G. F. and Dudley, J. W. Eds. Corn and corn improvement. Agronomy Monographs No. 18; American Society of Agronomy: Madison, Wisconsin pp. 1-31.

Geeta, D. S., Aswathanarayana, M. K., Naik, M. K., and Kuchanur P.H., (2019). Cultural, Morphological and Pathogenic Variability of *Exserohilum turcicum* causing Turcicum Leaf Blight in Maize. *Int.J. Curr.Microbiol. App.Sci*. 8(11): 302-313.

Geeta, D. S Aswathanarayana, M. K Naik, Mallikarjun Kenganal and Prakash H Kuchanur (2018). Survey for the severity of turcicum leaf blight of maize in major maize growing regions of North Eastern Karnataka. International Journal of Chemical Studies 2018; 6(5): 2484-2486

Gowda, K. T. (1987). Status of breeding and management of turcicum leaf blight disease of maize. Progressive Report, 30th Annual Maize Workshop, April 1720, AICMIP, IARI, New Delhi. pp. 21-28.

Gowda, K. T. P., Gowda, B. J and Rajasekharaiah, S. (1989). Variability in the incidence of turcicum leaf blight disease of maize in southern Karnataka.Current Research. 18(8): 115-116.

Gowda, K. T. P., Mallikarjuna, N., Kumar, G. B. S., Manjunath, B., Kumar, B. R. (2010). Cultural and Morphological variation in the isolates of Exserohilum turcicum the incitant of turcicum leaf blight in maize. *Environment and Ecology*. 28: 3A. 1826-1830. 9 ref.

Harlapur, S. I., Kulkarni, S., Wali, M. C. and Kulkarni, S. (2007). Evaluation of plant extracts, bioagents and fungicides against Exserohilum turcicum (Pass) Leonard and Suggs. causing turcicum leaf blight of maize Karnataka Journal of Agricultural Sciences, 20 (3): 541-544.

Harlapur, S. I. (2005). *Epidemiology and management of turcicum leaf blight of maize caused by E. turcicum* (Pass) Leonard and Suggs. Ph. D. Thesis. University of Agricultural sciences. Dharwad, Karnataka, India.

Haralpur, S.I and Kulakarni, M.S. (2009). Conidial germination of *Exserohilumturcicum* (Pass.) Leonard and Suggs. Causing turcicum leaf blight of maize.Journal of Plant Disease Science. 4(2): 231-231.

Ho, W. C and Ko, W. H. (1997). A simple method for obtaining single spore isolation of fungi. Botanical Bulletin of Academia Sionica. 38: 41-44.

Jordhan, E.G., Perkins, J. M., Schalf, R. A and Pederson, W. L. (1983). Occurrence of race 2 of *E. turcicum* on corn in the Central and Eastern United States. *Plant Disease*. 67: 1163-1165.

Kuchanur, P. H., (2019). Cultural, Morphological and Pathogenic Variability of *Exserohilum turcicum* causing turcicum Leaf Blight in Maize. *Int. J. Curr. Microbiol.App. Sci.* 8(11): 302313.

Kachapur, M. R. and Hegde, R. K. (1988). Studies on turcicum blight of maize *(Zea mays L.*) caused by *Exserohilum turcicum* (Pass.) Leonard & Suggs with special reference to crop loss assessment. *Plant Pathology Newsletter*, 6(1-2): 35.

Kaul, T.N. (1957). Food and Agriculture Organization. Plant Protection Bulletin, 5: 93- 96.

Khedekar, S.A., Harlapur, S.I., Kulkarni, S. and Benagi, V.I. (2012). Evaluation of fungicides, botanicals and bioagents against Turcicum leaf blight of maize caused by *E. turcicum* (Pass.) Leonard and Suggs. *International Journal of Plant Protection*, 5 (1): 58-62.

Khedekar, S. A., Haralpur, S. I., Kulakarni, S., Benagi, V. I and Desphande, V. K. (2010). Survey of turcicum leaf blight of maize in Northern Karnataka. *Journal of plant disease sciences*. 5(1): 24250.

Kumar, H., Ahmad, S. and Zacharia, S. (2016). Efficacy of fungal, bacterial bioagent and Botanicals against brown spot (*Helminthosporium oryzae*) of rice (*Oryza sativa*) New Agriculturist, 27(2):343-348.

Kumar, S and Mauriya, A. K. (2015). Effect of fungicides and plant extracts For management of turcicum leaf blight of maize. *The Bioscan*, 10(4):1687-1690.

Kutawa, A. B., Sizam, K., Ahmad, K., Seman, Z. A, Razak, S. F. and Abdullah, N. (2017) Characterisation and pathological variability of *E. turcicum* responsible for causing northern corn leaf blight (NCLB) disease in *Malaysia Malaysian Journal of Microbiology*, 13 (1): 41-49.

Lalitha, V., Kiran, B. and Raveesha, K. A. (2011) Antifungal Activity of *Polyalthia longifolia* (Sonn.) The. against seed-borne fungi of Paddy (*Oryza sativa.* L). *Journal of Phytology*, 3(5):4-8.

Leonard, K. J., and Suggs, E. G. (1974). *Setosphaeria prolata*, the ascigerous state of *Exserohilum prolatum. Mycologia*, 66: 281-297.

Leonard, K. J., Levy, Y. and Smith, D. R. (1989). Proposed nomenclature for pathogenic races of *E. turcicum* on corn. *Plant Disease*, 73: 776-777.

Levy, Y. and Cohen, Y. (1980). Sporulation of *Helminthosporium turcicum* sweet corn effects of temperature and dew period. *Canadian Journal of Plant Pathology*, 2(2): 65-69.

Levy, Y. and Pataky, J. K. (1992). Epidemiology of northern leaf blight on sweet corn *Phytoparsitica* 20, 53-66.

Levy, Y. and Cohen, Y. (1983). Biotic and environmental factors affecting infection of sweet corn with *Exerohilum turcicum* *Phytopathology* 73: 722-725.

Levy, Y. (1984). The over wintering of *Exserohilum turcicum* in Israel. *Phytoparasitica*,12: 177-82.

Li B, and Wilson, W. A. (2013). Composition and methods for enhancing resistance to Northern leaf blight in maize.US Patent 20,130,061,355-free patents online.com.

Lutrell, E. S. (1958). The perfect stage of *Helminthosporium turcicum* Phytopathology, 48:281-28.

Laxminarayana, C and Shankarlingam, S. (1983). Turcicum leaf blight of maize, techniques of scoring for resistance to important disease of maize. Proceeding of All India Coordinated Maize Improvement Project. Indian Agriculture Research Institute.New Delhi. pp. 16-2.

Luttrell, E. S. (1964). Morphology of *Trichometasphaeria turcica. American Journal of Botany* 51:213- 219.

Mahamood, A., Javed, N., Ahmad, R. and Raheel, Z. I. (1995). Biological control of maize Leaf blight caused by *Helminthosporium turcicum* Pass *in vitro. Pakistan Journal of Phytopathology*, 7: 62-64.

Mangelsdorf, and Paul, (1974). Corn: Its origin, evolution and improvement. Havard University Press Cambridge, Massachusetts.

Manu, T. G., Naik, B. G., Sayipratap, B. R. and Balagar, M. S. (2017). Identification of Sources of Resistance against turcicum leaf blight of maize. *Chemical Science Review and Letters*, 6(24):2100-2107.

Malik, R. A. (2016). Pathogenic Variability of *Exserohilum turcicum* (Pass.) Leonard and Suggs, Causal Organism of Northern Leaf Blight of Maize, Dessertation submitted to Division of Plant Pathology, Faculty of Agriculture Sher-e-KashmirUniversity of Agricultural Sciences and Technology of Kashmir.

Martyn, R. D, Stack J. P (1990). Biological control of soil borne plant pathogens by antagonistic fungi. In: Laboratory Exercises in Plant Pathology (Ed. A.B.A.M. Baudoin), *Scientific Publisher*, Jodhpur. pp 64-74.

Mares, D., Tosi, B., Poli, F., Andreotti, E. and Romagnoli, C. (2004). Antifungal activity of *Tagetes patula* extracts on some phytopathogenic fungi ultrastructural evidence on *Pythium ultimum*. *Microbiological Research* 159(3):295-304.

Meena, R. L., Rathore, R. S. and Mathur, K. (2003). Evaluation of fungicides and plant extracts against banded leaf and sheath blight of maize. *Indian Journal of Plant Protection* 31: 94-97.

Meli, V. S. and Kulkarni, S. (1994). *In vitro* studies of fungicides *Exserohilum hawaiiensis* (Bugnicourt) Subram. and Jain. Ex. Ellis, M.B. *Karnataka Journal of Agricultural Sciences*, 7: 489-491.

Meneses, A. K., Marinez, R. I., Obregon, E. A., Vergara, N. V., Aguilar, G. A., Saiz, C. M., and Mishra, B., Sharma, R., Kabadwal, B. C., Negi, A., and Arya, A. (2018). *In vitro* evaluation of potential fungal and bacterial isolates against *Rhizoctonia solani* causing sheath blight of rice. *Crop Research*, 53:63-67.

Miller, P. R. (1970). Southern corn leaf blight. Plant Disease Reporter, 54: 1099-1136.

Muiru, W. M., Mutitu, E.W. and Ken, J. W. (2008). Distribution of turcicum blight of maize in Kenya and cultural variability of its causal agent, *Exserohilum turcicum.*

Muiru, W. M., Koopmann, B., Tiedemann, A.V., Mutitu, E.W.and Kimenju, J. W. (2010). Race typing and evaluation of Aggressiveness of *Exserohilum turcicum* isolates of Kenyan, German and Austrian origin. *World Journal of Agricultural Sciences* 6(3): 277-284.

Meli, V. S. and Kulkarni, S. (1994). *In vitro* studies of fungicides against *Exserohilum hawaiiensis* (Bugnicourt) Subram. and Jain. Ex. Ellis, M.B. *Karnataka Journal of Agricultural Sciences*, 7: 489-491.

Mitra, M. (1981). A comparative study of species and strains of *Helminthosporium* on Certain Indian cultivated crops.Trans. *British Mycological Society,* 15: 254- 293.

Nisikado, Y. (1927). Temperature relations to the growth of graminicolous species of *Helminthoporium* Effect of temperature on vegetative and reproductive growth of *H. turcicum* Pass and *H. maydis* Nisikado and Miyake, Ber, Ohara. Inst. 3:349-377.

Nene, Y. L. and Thapaliyal, P. N. (1973). Fungicides in Plant Disease Control; 3rd New Delhi, Oxford & IBH Publishing, 325 p.

Pandey, S. C. and Shukla, T. N. (1979). Comparative physiological studies on sporulation and colour of culture filtrates of six species of *Helminthosporium* from *Sorghum Vulgare*. *Indian Journal of Mycology and Plant Pathology*, 1980,9(1): 22-28.

Pandey, S. C and Shukla, T. N . (1982). Comparative physiological studies on growth and final pH of six species of *Helminthosporium* from *Sorghum Vulgare*. *Indian Botanical Society Journal*, 61: 156-160.

Patil, S. J., Wali, M. C., Harlapur, S. I and Prasanth, (2000). Maize Research in North Karnataka, University of Agricultural Science, Dharwad Pp 54.

Pant, S. K., Kumar, P. and Chauhan, V. S. (2001) Effect of turcicum leaf blight on photosynthesis in maize. *Indian Phytopathology*, 54: 251-252.

Pammel, N. B., King, D. N. and Bakke, A. K. (1910) Two barley blights. Bulletin Stav. Path. Veg. Deva, 21: 1-62.

Payak, M. M., and Renfro, B. L., (1968) Combating maize disease. Indian Farmer Disease, 1:3-58.

Payak, M. M., Sharma, R. C., and Lilaramani, J. (1973). How to control maize diseases.Indian Farming. 23: 20-23.

Passerini,. (1876). Lanebbia Delgranotur Co. Bol. Comiz, Agriculture. Parmense. 10:3.

Payak, M. M., and Sharma, R. C., (1980). An Inventory and Bibliography of Maize Diseases in Indian Agricultural Research Institute, New Delhi, pp.44.

Payak, M. M and Sharma, R. C. (1998). Maize diseases and approaches to their management. Tropical pest management. 31: 302-310.

Purschwitz, J., Muller, S., Kastner, C. and Fischer, R. (2006). Seeing the rainbow: light sensing in fungi. *Current Opinion in Microbiology*; 9, 566–571.

Kumar, (2010). studies on physiology and management of turcicum leaf blight (E*xserohilum turcicum* (pass.) leonard and suggs.) of maize, thesis submitted to the acharya n. g. ranga agricultural university.

Gowda K. T., Shekar Shetty, H., Jayaram Gowda, B., Prakash, H. S and Sangamlal.(1993). Efficacy of foliar fungicides against *Exserohilum turcicum* leaf blight of maize. *Mysore Journal of Agricultural Sciences* 27: 46-149.

Gowda, K. T. (1987). Status of breeding and management of turcicum leaf blight disease of maize. Progressive Report, 30th Annual Maize Workshop, April 1720, AICMIP, IARI, New Delhi. pp. 21-28.

Perkins, J. M. and Pedersen, W. L. (1987). Disease development and yield losses associated with northern leaf blight on corn. Plant Disease 71: 940-943.

Robert, A. L. (1960). Physiological specialization in *Helminthosporium turcicum Phytopathology* 50:217-220.

Rodriguez, A. F. (1961). Physiological specialization, morphological and cultural Variation in isolates of *Helminthosporium turcicum*.Dissertation Abstracts. 21:1701.

Robert, A. L and Sprague, G. F. (1960). Adaption of the corn leaf blight fungus to a resistant and susceptible corn host. *Phytopathology*. 50: 261-263.

Robert, S. L and Robert, P. S. (1983). Conversion of *Helminthosporium sacchari* Toxin to toxoids by β- galacto furanosidase from *Helminthosporium. Plant Physiology*. 72: 530-534.

Rahman, M. A., Begum, L. A., Alam, K. B. and Khan, A. L. (1993). Efficacy of fungicides to control *turcicum* leaf blight of maize. *Bangladesh Journal of Plant Pathology*, 9: 35-36.

Ramachandra, C. G. (2000). *Studies on leaf blight of Dicoccum wheat caused by Exserohilum hawaiiensis* (Bugnicourt). Subram. And Jain, Ex. Ellis, M.B. M.Sc. (Agri.) Thesis, University of Agricultural Sciences, Dharwad.

Reddy, V. (2012) *studies on variability and management of turcicum leaf blight (exserohilum turcicum (pass.) leonard and suggs) in maize* (doctoral dissertation, acharya ng ranga agricultural university rajendranagar, hyderabad).

Reddy, A. P. K and Bonman, J. M. (1987). Recent Epidemics of Rice Blast in India and Egypt. Accepted for publication 11 May 1987. Copyright 1987 *The American Phytopathological Society.* DOI: 10.1094/PD-71-0850E. Plant Dis. 71:850.

Sajeed, A. and Chowdhury, A. K. (2014). Histological studies on Turcicum leaf blight disease of maize in hill agro-ecological zones of the west. *Bengal Journal of Mycological Research* 113: 116.

Shah, S. S., Rahman, H., Khalil, I.H. and Rafi, A (2006). The reaction of two maize synthetics to maydis leaf blight following recurrent selection for grain yield. *Journal of Agriculture* 22, 263-269.

Singh, L. S. and Dutta, R. (2017). *In vitro* efficacy of native biocontrol agents from maize regime against *Exserohilium turcicum* in response to botanicals and fungicides. *The Bioscan*, 12(2): 775-779.

Singh, N. P. (2005). Maize in India: Production systems, constraints, and research priorities. *Indian Research Journal of Extension Education*, 42: 821-833.

Singh , R. D. N. and Kaiser, S. A. (1989). Seed treatment with bavistin and vita-vax on the incidence of *turcicum* blight of maize at the pre-tassel stage. *Indian Journal of Mycological Research*, 27: 31-35.

Singh, V. and Singh, Y. (2014). Evaluation of *Trichoderma harzianum* and *Pseudomonas Fluorescens* isolates for their antagonistic potential Against *Exserohilum turcicum* causing leaf blight of sorghum. *The Bioscan*, 9(3): 1171- 1175.

Singh, V. and Singh, Y. (2015). Bioefficacy of Botanicals against *Exserohilum turcicum* causing Leaf Blight of Sorghum. *Vegetos*, 28 (2): 148-152.

Sisterna, M. N. (1985). Study on the pathogenicity *of Exserohilum turcicum (Drechslera turcica*) in Argentina. *Revista de la Facultad de Agronomia, Universidad Nacional de La Plata,* 61-62: 169-174.

Shivapuri, A., Sharma, O. P. and Jhamaria, S. L. (1997). Fungitoxic properties plant extract against pathogenic fungi. *Journal of Mycology and Plant Pathology*, 27: 29- 31.

Sakhi, Muhammad., Dogra, M. A and Khan, M. A. (1991). Physiological studies and *in vitro* evaluation of fungicides against *Helminthosporium turcicum* Pass.*Journal of Agriculture*. 7(1):95-100.

Sanjeev, Kumar and Archana, Rani. (2009). Cultural and nutritional studies concerning growth and sporulation of *Helminthosporium turcicum. Annual of Plant Protection Sciences.* 17(1): 251-252.

Shankerlingam, S and Balasubramanian, K. A. (1984). The possible existence of variability in *Helminthosporium turcicum* incitant of leaf blight of maize in *India. Current Science*. 52(22): 1209-1210.

Sharma, J. P and Mishra, B. (1988). Effect of spray schedule of mancozeb (dithane M 45) on *Turcicum* leaf blight & impact on grain yield in maize. *Indian Journal of Plant Protection.* 16: 189-193.

Sharma, A . and Sharma, K. (2011). Assay of Antifungal Activity of Lawsonia inermis Linn. and Eucalyptus citriodora Hook. Journal of Pharmacy Research, 4(5):1313-1314.

Sharma, P., Khandelwal, S., Singh, T., and Vijayvergia, R. (2012). Phytochemical analysis and antifungal potential of Duranta erecta against some phytopatogenic fungi. International Journal of Pharmaceutical Sciences and Research, 3(8): 2686- 2689.

Thakur, R., Rao, V. P. W. B. M., Mathur, K., Tailor, H. C., Kushwaha. R. S., Dwivedi, R. R., Hiremat, R. V. and Indira, S. (2007). Genetic resistance to foliar anthracnose in sorghum and pathogenic variability in *Colletotrichum graminicola. Indian Phytopathology* 60: 13-23.

Ullstrup, A. J. (1966). Corn diseases in the United States and their control. A Handbook No.199, United States Department of Agriculture, p. 26.

Vieira, R. A., Mesquini R. M., Silva C. N. and Hata F.T. (2014). A new diagrammatic scale for the assessment of northern corn leaf blight. Crop Prot.56: 55-57.

Vinay, M. R. and Sataraddi, A. R. (2019). Studies on Cultural and Morphological Variability in Isolates of *Exserohilum turcicum*, Incitant of Turcicum Leaf Blight of Sorghum. *Int.J. Curr. Microbiol.App. Sci*. 8(12): 232 244. doi:https://doi.org/10. 20546/ijcmas.2019.812.033

Vincent, J. M. (1927). Distortion of fungal hyphae in the presence of certain inhibitors. Nature 59: 850 (cf: *Karnataka Journal of Agricultural Sciences* 20(3): 541 -544.

Vishwanath, V., Lal, A. A., Zacharia, S. and Simon, S. (2017). Efficacy of plant extracts and *Trichoderma sp*. against Turicum leaf blight of maize caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs. *Annals of Plant Protection Sciences*, 25(2):338 341.

Wani. T. A., Ahmad, M. and Anwar, A. (2017). Evaluation of fungicides, bioagent sand plant extracts against *Exserohilum turcicum* causing Turcicum Leaf Blight of *Maize. International Journal of Current Microbiology and Applied Sciences*. 6 (8): 2754-2762.

Warren, H. L. (1975). Temperature effects on lesion development and sporulation after infection by races O and T of B. maydis. *Phytopathology.* 65: 623-626.

Wathaneeyawech, S., Sirithunya, P. and Smitamana, P. (2015). Study of the host range of Northern Corn Leaf Blight disease and effect of *Exserohilum turcicum* toxin on sweet corn. *Journal of Agriculture Science and Technology*, 11 (4): 953-963.

APPENDIX

APPENDIX

1. PREPARATION OF CULTURE MEDIA

Sr No	Name of medium	Composition	Quantities
1	Potato Dextrose Agar	Peeled and sliced potato	200 g
		Dextrose	20 g
		Agar-agar	20 g
		Distilled water	1000ml

2. CONCENTRATION USED IN FORMULATION

1 percent	**10,000 ppm (parts per million)** **= 10 g/ lit** **= 10 g/kg**
0.1 percent	**= 1000 ppm = 1000 mg/liter**
0.01 percent	**= 100 ppm = 100 mg/liter**
0.001 percent	**= 10 ppm = 10 mg/liter**
0.0001 percent	**= 1 ppm = 1 mg/liter**

CURRICULAM
VITAE
(C.V.)

CURRICULUM VITAE

Name of Student : Patil Lalit Pandurang

Date of Birth : 03/06/1997

Nationality : Indian

Department : Plant Pathology

Permanent address : A/p. Mohadi, neri Bk, Tal. Jamner, Dist. Jalgaon-425114

Mobile Number : 9834404908

Email id : lp3436520@gmail.com

Title of thesis : **"Study on Northern Leaf Blight of Maize Caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs."**

Academic Qualification:

Course/ Degree	Name of the college/ institute	University/ Board	Year of passing	Percentage (%) /CGPA	Class/ Grade
SSC	L. F. E. M. S. Jamner	Nashik Board	2013	66.00 %	B
HSC	New English school junior college, Jamner	Pune Board	2015	57.45 %	B
B. Sc. Agri.	Aditya College of Agriculture, Beed	V. N. M. K. V. Parbhani	2019	7.3	1st