Studies on growth inhibition of *Exserohilum turcicum* (Pass) Leonard and Suggs, the causal organism of Northern leaf blight of maize

Thesis

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(Authoress)

CERTIFICATE

This is to certify that the thesis entitled **"Studies on growth inhibition of *Exserohilum turcicum* (Pass) Leonard and Suggs, the causal organism of Northern leaf blight of maize"** submitted in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE** with major in **PLANT PATHOLOGY,** of the College of Post-Graduate Studies, G.B. Pant University of Agriculture and Technology, Pantnagar, is a record of *bona-fide* research carried out by **Ms. Bhagyashree Bhatt, Id. No. 43693**, under my supervision and no part of the thesis has been submitted for any other degree or diploma.

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June, 2018

(Pradeep Kumar)
Chairman
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CERTIFICATE

We, the undersigned, members of the Advisory Committee of **Ms. Bhagyashree Bhatt, Id. No. 43693**, a candidate for the degree of **MASTER OF SCIENCE IN AGRICULTURE** with major in **PLANT PATHOLOGY**, agree that the thesis entitled "**Studies on growth inhibition of *Exserohilum turcicum* (Pass) Leonard and Suggs, the causal organism of Northern leaf blight of maize"** may be submitted in partial fulfilment of the requirements for the degree.

(Pradeep Kumar)
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Member

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Member

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ABBREVIATIONS

%	:	Per cent
±	:	Plus or minus.
@	:	At the rate of
Q	:	Quintal
ha	:	Hectare
Mt	:	Metric tonne
µm	:	Micrometer
nm	:	Nanometer
µl	:	Microlitre
ml	:	Millilitre
viz	:	namely
^{0}C	:	Degree Celsius
pH	:	Potenz of hydrogen ion
N	:	Normal
CD	:	Critical difference
CV	:	Coefficient of Variation
PDA	:	Potato Dextrose Agar
mm	:	millimetre
ppm	:	Parts per million
SEM	:	Standard Error Mean

Introduction

INTRODUCTION

Maize, also called corn is considered as 'Queen of Cereals' as it is one of the most important cereal crops of the world and contributes to food security in most of the developing countries. Maize, distinguished botanically as *Zea mays*, belongs to grass family Poaceae. It is native of South America **(Mangelsdorf, 1974 and Galinat, 1988)** and since ancient time, it has been the basic food for the majority of the people in Mexico, Central America and Latin America. Maize was introduced to India from America at the beginning of 17th century and now it is emerging as third most important crop after rice and wheat in India.

Maize is widely cultivated throughout the world and has maximum production among all other cereal crops in the world that is 972.40million MT **(FAO, 2015-2016).** USA is the largest producer of maize followed by China and Brazil. USA has the maximum production of about 344.3 m MT with productivity of 105.7 Q/ha **(USDA)**. India stands at 7th position with respect to production. Maize production in India is about 22.57million MT (2015-16) **(FAO and Indiastat.com)** with productivity of about 25.6 Q/ha **(USDA).** It accounts for ~9 per cent of total food grain production in the country. In India leading maize producing states are Karnataka (3.3 million MT), Madhya Pradesh (2.6 million MT), Bihar (2.5 million MT), Telangana (1.8 million MT) and Uttar Pradesh (1.3 million MT).

Maize has wider range of uses because of its worldwide distribution and relatively lower price. It is mainly used as human food, animal fodder, poultry feed and as raw material to large number of industrial products such as starch, food sweetners, alcoholic beverages, cosmetics, gum, textiles, package and paper industry. Maize for production of ethanol, a substitute for petroleum based fuels is gaining great attention now days. Maize is also used to produce oil which reduces the blood cholesterol concentration and is highly regarded for human consumption. Maize is a natural source of carotenoids such as beta-carotene, lutein, zeaxanthin and cryptoxanthin which have highly varied health benefits such as maintaining normal vision and lowering of oxidative stress **(Chaudhary *et al.,* 2014)**. Due to changing food habits and diversified uses of maize in industry there is increase in demands of maize in India.

Maize, being an important kharif fodder crop provides nutritionally rich and highly succulent fodder. It is also an important feed component of dairy cattle and poultry feed.

Maize produces good quality herbaceous fodder with high palatability. Maize grain contains about 10% protein, 70% carbohydrate, 4% oil, 2.3% crude fibre, 10.4% albuminoides and 1.4% ash. It contains adequate quantities of nicotinic acid, vitamin A, vitamin E and riboflavin. It is a major source of green fodder for the cattle. In Indian Agriculture, it occupies a prominent position and each part of the maize plant is put to one or the other use and nothing goes as waste.

The crop having high genetic variability, can easily adapt in tropical, subtropical, and temperate climates. The crop is primarily a rainfed crop and requires at least 50 to 90 cm of rainfall. It generally grows well under temperatures varying from 22^{o}C to 30^{o}C, although can tolerate temperatures as high as 35^{o}C. The crop requires well drained soils with pH 5.5 to 7.5. Main season for maize cultivation is rainy season and hence it is prone to many diseases and pests.

Maize production is greatly hampered by a number of abiotic and biotic stresses. Among the abiotic stresses moisture stress (low/excess soil moisture), temperature (high/low), salinity, sodicity, nutrient etc. whereas biotic stresses include insect pest and diseases which adversely affect crop yield. The crop is affected by a number of fungal, bacterial and viral diseases.

Maize crop suffers from various foliar and stalk rot diseases **(Payak and Sharma, 1980).** Among various foliar diseases affecting maize, Turcicum leaf blight of maize is one of the most devastating diseases as it results in reduction of grain yield by 28 to 91 per cent **(Kachapur, 1988** and **Harlapur *et al.*, 2000).** The Turcicum leaf blight also called as Northern leaf blight caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs (syn. *Helminthosporium turcicum* Pass.), is a disease of worldwide importance **(Carlos, 1997)**. There have been reports of this disease causing more than 50 per cent loss in grain yield in USA **(Robert, 1953; Raymundo and Hooker, 1981)**. In India, the disease is prevalent in majority of the maize growing areas such as Karnataka, Andhra Pradesh, Uttar Pradesh, Uttarakhand, Himanchal Pradesh, Orissa, and North Eastern Hill states. Epiphytotics causing severe yield loses have been reported in many parts of India and these losses vary from 25 to 90 per cent depending upon the severity of the disease **(Chenulu and Hora, 1962** and **Jha, 1993).**

The first report of Turcicum leaf blight disease of maize was made by **Passerini (1876)** from Perma, Italy. The genus *Exserohilum turcicum* for *Helminthosporium* species was established by **Leonard and Suggs (1974)** in which the conidial hilum was strongly protuberant. The perfect (ascigerous) state of *Exserohilum* was placed in the new genus *Setosphaeria.* The perfect state *Setosphaeria turcica* (Luttrell) Leonard and Suggs is rarely found in nature. The causal agent of Turcicum leaf blight of maize is normally the imperfect stage *Exserohilum turcicum.* In India, the disease was first reported from Bihar by Butler during 1907.

Turcicum leaf blight of maize is more severe in areas where the temperatures drop at night and there is high humidity. The disease is known to affect maize crop from seedling stage till harvest. If the disease occurs at flowering, silking and grain filling stage, grain yield loses will be more. On the leaves of susceptible plants, the lesions produced are normally large (4 -20 cm long and 1-5 cm wide), elliptical in shape and greyish green to tan in colour. Lesions may be covered with masses of dark conidia of the fungus under conditions with high relative humidity. The conidia are olive grey in colour, spindle shaped with 1- 9 septations. Turcicum blight injures or kills the leaf tissues and thus reduces the area of green chlorophyll which manufactures food for the plant, thereby adversely affecting the grain yield. About 91 per cent reduction in the rate of photosynthesis has been reported when severity of turcicum leaf blight incidence in maize exceeded 50 per cent **(Pant *et al*., 2001).** Because of the lowered nutrition value the blighted leaves are not suitable even for fodder purpose.

Since pathogen is directly linked with the disease thus studying the factors which hinder the growth of pathogen can be of great help in minimising the incidence of diseases. Various fungicides and botanicals can show effective growth inhibition of the fungus thus *in vitro* evaluation of different fungicides, botanicals and other organic compounds can help in identifying compounds which can show greater fungal growth inhibitions. As nowdays a lot of focus is on reducing the use environment contaminating fungicides there is a greater scope for use of more natural compounds which can be ecofriendly and could show satisfactory fungal growth inhibitions. Agricultural studies have focused on the biocontrol of plant disease for a long time.

Discovery of antifungal and germicidal compounds from plants is an efficient way to create new pollution-free pesticides. Many plants have antimicrobial activities due to the antimicrobial constituents, including alkaloids, terpenes, polysaccharide, esters, ketones, and quinines present in them. Effective components extracted from plants have promising potential for this purpose because of their high efficacy, low toxicity, and selective characteristics.

Since the disease is responsible for severe damage to crop and immense reduction in crop yield there is need for various effective management practices to overcome the yield loses. Integrated and eco friendly management of the disease need to be emphasized and thus identification of various chemicals, plant extracts, essential oils and biological control agents against the pathogen is required. Keeping in view of public health, environmental safety and the economic importance of the disease, the present investigation is aimed at following objectives:

1. Isolation and pathogenicity of *Exserohilum turcicum* causing Northern leaf blight of maize.
2. Identification and characterization of the test pathogen.
3. *In vitro* and *in vivo* evaluation of fungicides against the test pathogen.
4. *In vitro* and *in vivo* evaluation of botanicals, essential oils, biocontrol agents and cow urine against the test pathogen.

Review of Literature

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 *Helminthosporium turcicum* Pass but later on, it was regarded to be the same as *Trichometasphaeria 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 during 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 is 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 Symptomology

Initial symptoms are formation of small elliptical spots on the leaves, greyish green in colour and water soaked lesions. Later spots get bigger in size, finally attaining a spindle shape. Individual spots are usually 3/4" wide and 2" to 3" long. The fungus develops abundant spores on both sides of the spot. Heavily infected field gives a scorched appearance **(Chenulu and Hora, 1962).** The plants appear dead and grey. **Ullstrup (1966)** described the symptoms of the disease in United States. Long elliptical greyish or tan lesions are characteristic symptoms of disease. The spots may be 1½" by 6" in size. These lesions appear first on the lower leaves and as the season progresses, the lesion number increases and all the leaves are covered.

Leaf blight symptoms occur prominently on leaf lamina and lesions are elongated, elliptical, measure about 2.5 – 15 cm in length and 12mm in width **(Mishra, 1973).** Usually small flecks appear at 3-4 days after favourable infection, while large distinctive lesions appear two weeks later **(Fredericksen, 1980).** As the disease progresses, single lesions might join collectively to form vast blighted zones **(Vieira *et al*., 2014).**

2.3 Morphology

The conidia of *Exserohilum turcicum* are 18-23 u wide and 73-137 u long with 4-9 septa and born singly at the tips of the conidiophores **(Luttrell 1964).** *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* **(Leonard and Suggs, 1974).**

The conidia have strongly protruding hilum and are fusoid, obclavate, straight, or curved in shape. Conidia mainly germinate from one or both polar cells, rarely from intermediate cells **(Alcorn 1988).** The conidiophores (7-11 x 165-283 u) are brown, irregularly cylindrical and 3-7 septate. They emerge in groups of two to six or more through stomata, or less frequently directly through the epidermis.

Sexual stage of *Exserohilum turcicum* that is *Setosphaeria turcica* is characterised by presence of perithecia which are ostiolate, dark brown, and covered with short stiff spine like hairs on the upper third of the perithecial wall. The asci contain from one to six ascospores which are straight, three to six septate, and typically hyaline, although with aging they may become brown and surrounded by a mucous like sheath **(Luttrell, 1958)**.

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 by **Robert (1960) and Rodriquez (1961).**

Daniel and Narong (2006) investigated differences in morphological characters of *E. turcicum* strains and observed that the conidia were bended, elongated and spindle in shape. The average conidial length and width were found to be 93.97 µm and 13.11 µm, respectively, while the number of septations was found to range from 2-7.

Reddy (2012) studied eight isolates of *Exserohilum turcicum* and grouped them into 5 categories on the basis of colony colour - gray, dark grey, very dark gray, very darkish brown and black. *E. turcicum* isolates were grouped into 3 based on the pigmentation - black, bluish black, greenish black. All the eight *E. turcicum* isolates were classified into three groups based on the sporulation. These eight *E. turcicum* isolates were classified into 3 on the basis of mycelia growth *viz*., profuse growth, moderate growth and poor restricted growth. All *E. turcicum* isolates were classified in to 2 groups based on colony margin *viz*., regular margin and irregular margin.

2.4 Pathogenecity

The infection of *Exserohilum turcicum* on maize occurs from seedling to harvesting. However, maximum disease severity was noticed from tasselling stage and six to eight weeks after silking stage which resulted in heavy loss. The disease became well established before or at silking stage **(Chenulu and Hora, 1962; Ullstrup, 1966).**

It is a multiple cycle disease and for disease development new and repeated inoculations are required, thus it is highly dependent upon sporulation from other lesions **(Ullstrup, 1966).).**

Aden (1991) conducted pathogenecity 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).

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.

2.5 Host range

Specific isolates of *E. turcicum* (in maize) were noted in the Bajio region causing damage to sorghum. Field isolates (140) of the pathogen were inoculated to the seedlings of maize, sorghum and Johnson grass (*Sorghum halepense*) and on the basis of pathogenecity, their forms were distinguished as *E. turcicum* f.sp. *sorghi,* specific to sorghum and Johnson grass; *E. turcicum* f.sp. *zeae* specific to maize and *E. turcicum* f.sp. *complexa*, specific to sorghum and maize or sorghum and Johnson grass. None of the isolates were found pathogenic to all the three hosts **(Ayala Escobar, 1997). Sisterna (1985)** tested the pathogenicity *E. turcicum* isolates from maize and sorghum on a range of cereals in a green house, and found that only sorghum and maize were infected with similar symptoms.

Shankerlingam and Balasubramanian (1984) tested five isolates of *E. turcicum*, one from sorghum and four from maize on five cultivars of maize. Highest sporulation was observed in the isolate from sorghum which shows that the sorghum isolate of the fungus infects maize**. Bergquist and Masias (1974)** reported that isolates of the fungus which were pathogenic to only maize, sorghum or sudan grass were homokaryons. Isolates pathogenic to both sorghum and maize were heterokaryons.

Tsai *et al*. (2001) studied the pathogenicity of *Exserohilum rostratum* in Taiwan, causing leaf spot of maize and observed that the fungus was capable of causing leaf spot disease on weed hosts, *viz*., *Panicum maximum*, *Chenopodium album,* and cultivated crops namely sorghum, sugarcane, and wheat.

2.6 Physiological and Cultural studies

Conidial formation in *E. turcicum* is linked with duration of dew, relative humidity and temperature. Rapid formation of conidiophore with increase in temperature was observed by **Bolkvaldge (1977)**. It was observed that, maximum conidia were produced in a dew period of 30 hours at 20-25^{0}C and 20^0 C was optimum for conidia formation whereas the optimum range varies between 11 and 35^{0}C **(Levy and Cohen, 1980).**

Khedekar (2009) studied the effect of temperature level on the dry mycelia weight of various isolates of *Exserohilum turcicum*. It was observed that different temperature regimes show difference in the radial growth of *E. turcicum* isolates when compared to each other. The growth of these isolates was less at lower temperature i.e., 10^{o}C, 15^{o}C, 20^{o}C and 25^{o}C and was higher at 30^{0}C, thus it could be optimum range of temperature for the growth of *E. turcicum*.

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 culture, and for infection and development of disease were 20-30°C, 25-30°C, and 30°C, respectively **(Misra and Singh, 1963).**

Bergquist and Masias (1974) reported that 28^{o}C was optimum temperature for growth of sorghum and maize isolates of the fungus 24^{o}C temperature was optimum for abundant sporulation. **Pandey and Shukla (1982)** reported that the optimum temperature for colony growth of a sorghum isolate of the fungus was 20- 30^{0}C, and no growth was observed at 40^{o}C.

The physiology of *E. turcicum* was first studied by **Mitra (1923).** He observed that maize meal agar, barley agar, rice agar, Thaxter's hard potato dextrose agar and jowar meal agar respectively showed better growth of the fungus, than on nutrient plain agar, dextrose, glycerine agar and French agar.

Nisikado (1927) observed that rice decoction agar was more favourable for mycelial growth compared to Hopkin's nutrient solution. **Champi (1939)** reported good growth of the fungus on various standard media. Best growth of the fungus was observed in Richard's and Czapek's media **(Singh, 1958** and **Pandey and Shukla, 1979).**

Isolates from different agro-ecological zones showed differences in growth rate, morphology, pigmentation, and sporulation rate in different media **Muiru *et al*. (2008).** The different light regimes had 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 25^{o}C and only one isolate showed minimal growth below 10^{o}C and no mycelia growth was observed in all the isolates at 40^{o}C.

The germination of conidia of *E. turcicum* [*Setosphaeria turcica*], causing northern leaf blight of maize, was tested at various incubation periods starting from 4 to 36 h at an interval of 4 h. The maximum conidial germination (94.20 %) was observed after 36 h of incubation, while the least germination (7.67 %) was noticed after 4 h. More than 50 % germination was observed after 16 h of incubation. However, no significant increase in the germination of conidia was observed from 28 to 36 h of incubation **(Harlapur and Kulakarni, 2009**).

Aden (1991) studied the growth and sporulation of *Exserohilum turcicum* causing Sorghum leaf blight on seven media(lactose casein hydrolysate agar, potato dextrose agar, sorghum leaf extract agar, maize leaf extract agar, maize grain extract agar, sorghum grain extract agar, and sorghum leaf medium) at five temperatures (15^{o}C, 20^{o}C, 25^{o}C, 30^{o}C, 35^{o}C). The best temperature for colony growth was 25^{0}C (21 mm), and for sporulation was 20^{o}C (47,000 conidia/ml). Both colony growth and sporulation was maximum between 20 and 30°C, but were very poor at 35 and 15^{o}C. At all temperatures, the best media for colony growth was lactose casein hydrolysate agar (16.5 mm) and for sporulation was sorghum leaf medium (53,000 conidia /ml). The highest colony growth after 12 days of incubation were observed on lactose casein hydrolysate agar at 30^{o}C (40 mm), sorghum grain extract agar at 30^{o}C (40 mm), sorghum leaf extract agar at 25^{o}C (38.5 mm), and maize grain extract agar at 25^{o}C (38.5 mm).

In an experiment at 2-5 pH no germination occurred, at pH 3.0, 3-7% germination was observed, at pH 3.5 it was 46-56% and more than 95% at pH ranging between 4.7-7.0. Increased hydrogen ion concentration inhibited spore germination in inoculated leaves of maize of both susceptible and resistant maize varieties **(Mace and Veech, 1973).**

Kutawa *et al.*, (2016) studied growth of five isolates at different range of pH starting 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 at the 3rd, 5th and 7th day after incubation, respectively. And this was followed by pH 9 with 4.84 mm/day, 6.14 mm/day and 6.94 mm/day at the same incubation periods. Least growth was observed at pH 5 one week after incubation by having 3.58 mm/day, 5.0 mm/day and 6.02 mm/day at 3rd, 5th and 7th day, respectively.

Light is an important environmental factor for most living organisms, including fungi, which use light as a signal in many metabolic pathways. It regulates many metabolic activities conidiation, secondary metabolism, pigmentation and sexual development (**Purschwitz *et al*. 2006).** More than 100 fungal species have been found to be reactive to light **(Tisch and Schmoll 2010).** It regulates many metabolic activities, conidiation, pigmentation, secondary metabolism and sexual development **(Purschwitz *et al*. 2006).** Many researchers (**Idnurm and Heitman 2005; Purschwitz *et al*. 2006; Chen *et al*. 2009** and **Idnurm *et al.* 2010)** have studied the interaction between light and fungi but reports on the effect of light qualities on fungal pathogenicity or virulence are limited.

Yu *et al.* (2013) studied the effect of different light wavelengths on the mycelial growth and conidial germination of *Colletotrichum acutatum* using red, green, blue and white light sources. The mycelial growth was reduced under red and green light as compared with blue, white light and dark conditions. The least percentage of conidial germination was observed under blue light while the germination rate among white, red and green light, as well as in the dark, was insignificant.

Campbell *et al.* (2003) studied the effect of photoperiod and light quality on growth and sporulation of *Pyrenophora semeniperda* and observed that fungal colonies when exposed to clear or yellow light grew significantly more quickly (≈ 70 mm diameter after 14 days) than red and orange, which in turn significantly exceeded growth under blue and green light sources; colonies grown under purple light had the slowest growth of all (≈ 50 mm diameter) ($P < 0{\cdot}01$). Linear growth rates were observed irrespective of the photoperiod. Colony growth reaching 90 mm diameter after 14 days was significantly faster ($P < 0{\cdot}05$) when exposed to either total darkness or a 12 h photoperiod than under a 6 or 24 h photoperiod where colonies reached ≈ 50 mm diameter after 14 days.

2.7 *In vitro* Management Studies

2.7.1 *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 the 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.

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

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

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 (**Harlapur *et al.*, 2007)**

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)** tested three fungicides-chlorothalonil, difenconazole and mancozeb at 3 concentrations i.e., ½ lower than recommended rate, recommended rate and ½ higher than recommended rate for efficacy to inhibit growth of isolates MHP5, TN3, MJ4, JT4 and JT5 of *E. turcicum* using poisoned medium method. Two contact fungicides chlorothalonil and mancozeb at 3 concentrations gave cent per cent growth inhibition to all tested isolates. 100 per cent growth inhibition was showed against isolates MHP5, TN3 and MJ4 by difenconazole at all tested concentrations, where as it showed 90 per cent inhibition in JT4 and about 94-96 per cent in JT5.

Reddy *et al*. (2013) evaluated seven fungicides *in vitro* against *Exserohilum turcicum* causing leaf blight of maize. Mancozeb @ 0.25% and combination of carbendazim and mancozeb i.e. saaf @ 0.25% observed the lowest percent disease index (PDI) reducing the disease by 73.0% and 72.1% respectively. Combination of metiram + pyraclostrobin i.e. cabriotop 0.3% was found effective in reducing the disease by 61.5% with PDI of 14.6 followed by propiconazole with PDI of 18.6

Kumar and Mauriya (2015) evaluated six fungicides (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 @ 0.25% concentration was found most effective in inhibiting the growth of *E.turcicum,* low disease severity and ultimately produced higher grain yield of maize. Mancozeb 63%+Carbendazim 12% @ 0.25% were found equally effective which can be used as an alternative to Zineb. Zineb 75 WP showed 99.10% inhibition of mycelial growth was most effective and statistically at par with Mancozeb 63% + carbendazim 12% which inhibit the mycelial growth 98.40%

Manu *et al.* (2017) observed that the systemic fungicide, tebuconazole completely inhibit the pathogen growth at all the concentrations tested. In contact fungicides, propineb was highly effective as it showed inhibition of *E. turcicum* up to 83.89 per cent at 500 ppm and combi-product, Carbendazim 12% + Mancozeb 63% showed complete inhibition of the mycelial growth of *E. turcicum* at 500ppm and higher concentrations.

Wani *et al.* (2017) observed that among all the tested systemic fungicides, propiconazole was found best in inhibiting the mycelial growth of *E. turcicum* (96.51% mean inhibition), mancozeb among nonsystemic fungicides was found effective (95.23% mean inhibition), when tested in field conditions, two foliar sprays with non-systemic fungicide, mancozeb 75 WP @ 0.25 per cent reduced the diseased intensity from 20.45 per cent in control to 5.69 per cent and increased the grain yield from 45.20 q/ ha in control to 52.50 q/ha; two foliar sprays with systemic fungicide, propiconazole 25 EC @ 0.1 per cent reduced the diseased intensity to 6.11 per cent and increased the grain yield to 52.25 q/ha.

2.7.2 *In vitro* evaluation of botanicals

Shivapuri *et al.* (1997) studied the anti fungal properties of plant extracts against five pathogenic fungi and observed that ethanol extracts of *Azadirchata indica, Allium cepa, Ocimum sanctum* and *Polyalthia longifolia* reduces the growth of pathogens under laboratory conditions.

Leaf extract of *Mangifera indica* L. showed absolute inhibition of *H. sativum* causing leaf blight of wheat crop **(Singh *et al.,* 1999).** Aqueous extract of *Duranta repens* L. at five and ten per cent concentrations showed significant inhibition of mycelial growth of *E. hawaiiensis* in *in-vitro* and *in-vivo* **(Ramchandra, 2000).**

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

Mares *et al*. (2004) assayed methaonol extracts of *Tagetes patula* plant, against three phytopathogenic fungi: *Fusarium moniliforme*, *Botrytis cinerea* and *Pythium ultimum*. The antifungal activity was tested both in dark and in light conditions, using different lighting systems. Dose-dependent activity of the extracts was observed against all the fungi with a significant difference between treatments in the light than in the dark conditions. At the concentration of 5 and 10mg/ml in the dark, growth increased.

Harlapur *et al.* (2007) assayed antifungal activity of thirteen plant extracts (*Allium cepa, Allium sativum, Acacia concina , Azadirachta indica, Azadirachta indica* (Nimbicidin) Oil, Azadirachta indica (NSKE) *Bouganvillea spectabilis, Cassia serratia, Eucalyptus globus, Lantana camara, Ocimum sanctum, Aloe vera* and *Pongamia glabra*) reported that, neem seed kernel extract (NSKE) @ 5% was highly effective against *Exserohilum turcicum* and caused maximum inhibition of growth (56.64%) followed by *Aloe vera* @ 10 per cent (53.50%).

Polyalthia longifolia was tested for antifungal activity of aqueous extract (10-50% concentration) and solvent extract (500µl and 1000µl concentration) against ten seed borne fungi of paddy (*Oryza sativa*. L) *in vitro* condition. Maximum inhibition was observed against *A. alternata* (92.88%) followed by *F. solani* (87.10%), *F. moniliforme, D. halodes, F. oxysporum* and *C. lunata* at 50% concentration in aqueous extract compared to fungicide, Captan, Dithane M-45, Benlate, Thiram and Bavistin at 2% recommended dosage. Maximum inhibition in all the test fungi was recorded with solvent extract of petroleum ether extract at 1000 µl concentration **Lalitha *et al.* (2011)**.

Sharma and Sharma (2011) evaluated leaf extracts of *Lawsonia inermis* Linn. and *Eucalyptus citriodora* Hook. against some plant pathogenic fungal species *viz. Alterneria solani, Drechslera halodes (Helminthosporium halodes), Rhizoctonia solani* (ITCC no. 4574), *Fusarium solani, Curvularia lunata, Dreschlera graminae, Fusarium moniliformae* (ITCC no. 2927), etc. The dried and powdered leaves were successively extracted with petroleum ether, benzene, chloroform, acetone, ethanol and water using soxhlet assembly. The antifungal activity was tested by poison food technique. Petroleum ether extract of *E. citriodora* leaves and Acetone extract of *L. inermis* leaves showed highest inhibitory activity against all tested fungi. The inhibitory effect was significant and better than the synthetic fungicides used as most of the strains showed resistance against fluconazole and amphotericin B.

Sharma *et al*. (2012) studied the antifungal activity of *Durenta erecta* L. against some phytopathogenic fungi: *A. flavus, Aspergillus niger, A. fumigatus* and *Penicillium sp*. It was found that the leaf extract show antifungal activity against all *Aspergillus spp.* but highest inhibition was observed against *A. fumigatus*.

Singh and Singh (2014) studied antifungal effect of ten plant extracts obtained from different plant parts against *Exserohilum turcicum*, causing sorghum leaf blight. Garlic was found to be most effective in substantially suppressing the radial growth of the pathogen by 60.77%, followed by jatropha (59.66%), neem (52.22%), tulsi (50.00%) and bael (49.66%) under *in vitro* conditions. Three sprays of Jatropha, neem and garlic reduced disease severity by 41.01, 43.38 and 46.36% respectively under glasshouse conditions. Under field conditions garlic appeared best in reducing disease severity by 26.80% with three sprays. Increased number of sprays resulted in improved reduction in disease severity.

Manu *et al.* (2017) conducted an experiment to assess the antifungal activity of eleven plant extracts at three different concentrations. The effect of plant extracts on the per cent inhibition of mycelial growth of *E. turcicum* at three concentrations differs significantly and increasing the concentration of botanicals increases efficacy against the *E. turcicum*. Thus at 10 per cent concentration, cent percent inhibition of mycelial growth was observed in garlic bulb extract which was significantly superior over other plant extract followed by parthenium leaf extract (70.00%), ginger extract (63.15%), onion bulb extract (57.41%), pongamia leaf extract (51.11%), neem leaf extract (49.26%), nilgiri leaf extract (45.00%) and noni leaf extract (42.96%).

Least inhibition of mycelial growth was observed in ocimum leaf extract (9.44%) followed by leucas leaf extract (20.74%) and agarwood leaf extract (29.07%). Among the 11 plant extracts, mean maximum per cent inhibition of mycelial growth (79.63%) was recorded in garlic bulb extract which was significantly superior over all other botanicals tested, followed by parthenium leaf extract (64.14%), pongamia leaf extract (48.89%), ginger extract (46.79%), neem leaf extract (43.33%) and onion bulb extract (40.72%). Whereas, ocimum leaf extract (7.59%), leucas leaf extract (12.78%), agarwood leaf extract (17.22%) and noni leaf extract (30.19%) showed less per cent inhibition against the pathogen.

Singh and Dutta (2017) evaluated Lantana (*Lantana camara*) and Datura (*Datura innoxia*) for their bio-efficacy against *E. turcicum* using 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%).

Sharma and Sharma (2017) assessed the antifungal ability of *Polyalthia longifolia* by poison food method and agar well diffusion method against various fungal strains (*Fusarium spp).* The maximum extractive value of crude extract of *Polyalthia longifolia* plant leaves obtained in 50% alcohol of *Polyalthia longfolia*. 50% alcohol extract showed best inhibitory activity against the test pathogen. Result of study thus indicates that leaf of *Polyalthia longifolia* possess antifungal activity. *Polyalthia longiifolia* contains secondary metabolites i.e. phenols, flavonoids, alkaloid, steroid, volatile oils, tannins, carbohydrate etc which may be responsible for its antifungal activity.

2.7.3 *In vitro* evaluation of essential oils

Pattnaik *et al* (1996) tested essential oil of lemongrass, orange, palmarosa, agele, ageratum, citronella, eucalyptus, geranium, patchouli and peppermint for antibacterial activity against 22 bacteria and twelve fungi. All twelve fungi were inhibited by seven oils (agele, citronella, geranium, lemongrass, orange, palmarosa and patchouli). MIC values of palmarosa, eucalyptus, lemon grass and peppermint oil for twelve fungi ranged from 0.25 to 10ul/ml.

Kishore and Pandey (2007) tested clove oil, cinnamon oil, and five essential oil components (citral, eugenol, geraniol, limonene, and linalool) for growth inhibition of 14 phytopathogenic fungi. Complete inhibition of growth of *Alternaria alternata, Aspergillus flavus, Curvularia lunata, Fusarium moniliforme, F. Pallidoroseum* and *Phoma sorghina* was shown by Citral using paper disc agar diffusion assays. Spore germination of *Cercospora arachidicola, Phaeoisariopsis personata* and *Puccinia arachidis* was inhibited by >90% *in vitro* by lower concentration of about 0.01% (vol/vol) of Cinnamon oil, citral and clove oil.

Least antifungal activity was recorded in case of Limonene and linalool against the test fungi. Clove oil (1% vol/vol) when applied as a foliar spray 10 min before inoculation of *Phaeoisariopsis personata* reduced the severity of late leaf spot of peanut up to 58% when challenge inoculated with 10^4 conidia ml–1. This treatment was more effective (P = 0.01) than 0.5% (vol/vol) citral, cinnamon oil, or clove oil and 1% (vol/vol) eugenol or geraniol. In *Aspergillus niger*-infested soil, seed treatment with the test compounds had no effect on the incidence of crown rot in peanut. However, 0.25% (vol/wt) clove oil and cinnamon oil reduced the pre emergence rotting by 71 and 67%

and post emergence wilting by 58 and 55%, respectively, compared with the non-treated control, when applied as soil amendment. These treatments showed more effectiveness than geraniol eugenol and citral, on post emergence wilting and were more effective ($P < 0.01$) than geraniol on pre emergence rotting.

Katooli *et al.* (2011) evaluated antifungal activity of eucalyptus (*Eucalyptus camaldulensis* Dehnh.) essential oil and suppression in the mycelial growth of postharvest pathogenic fungi, *Aspergillus flavus, Colletotrichum gloeosporioides, Penicillium digitatum* and other soilborne pathogenic fungi, *Rhizoctonia solani, Pythium ultimum, Bipolaris sorokiniana* pathogenic fungi. The experiment was carried out with Whatman paper disc method at 25, 50, 75 and 100% concentration of essential oil on PDA culture at 25°C and mycelial growth was measured daily for 30 days. The antifungal activity was evaluated under a randomized completely factorial design with three replications. Complete inhibition of mycelial growth was observed only in *P. ultimum* and *R. Solani* at all concentrations of eucalyptus essential oil.

Sharma *et al.* (2016) studied the antifungal effects of essential oils *viz* clove, lemon grasss, mint and eucalyptus against wilt causing fungus, *Fusarium oxysporum* f.spp. *lycopersici* 1322. The inhibitory effect of oils showed dose dependent activity on the tested fungus. Complete inhibition of mycelial growth and spore germination at 125 ppm with IC50 value of 18.2 and 0.3ppm respectively was shown by clove oil. At higher concentrations lemon grass, mint and eucalyptus essential oils were inhibitory. Minimum inhibitory concentration of 31.25ppm was showed by clove oil in broth microdilution method. In pots, 5% aqueous emulsion of clove oil controlled *F.oxysporum f.sp. lycopersici* 1322 infection on tomato plants. Shrivelled hyphae of *F.oxysporum* f.sp. *lycopersici* 1322 as an effect of clove oil on its morphology was observed using Scan electron microscopy.

Singh *et al.* (2016) studied *in vitro* antifungal activities of thirty one essential oils against four different fungi (*Aspergillus niger, Fusarium solani, Rhizoctonia solani, Alternari brassicae*). The antifungal activity was performed by disc diffusion assay. Twenty seven oils showed antifungal activity to at least one out of four fungal species. Ginger oil showed maximum zone of inhibition of 57mm against *Aspergillus niger*. Different concentration of Peppermint oil showed inhibition of the hyphal growth and reduction in the number of spores. Citronella, thyme, cinnamon and palmarosa oils showed maximum zone of inhibition.

Gakuubi *et al.* (2016) confirmed fungicidal properties of *Eucalyptus camaldulensis* essential oils against five *Fusarium sp.* commonly associated with maize. The minimum inhibitory concentration and minimum fungicidal concentration of the essential oil on the test fungus were in the range of 7-8 µL/ml and 8-10 µL/ml, respectively.

Menses *et al.* (2017) carried out *in vitro* assay of two essential oils and three major components of essential oils on the spore germination and radial growth of *Fusarium verticillioides* and *Alternaria tenuissima.* For each treatment Minimum and half-maximal inhibitory concentrations (CMI and CI50) were calculated at 96 h. Lemongrass essential oil and citral recorded the highest inhibition for *A. tenuissima* (CMI of 1000 ul/l and CI50 of 10ul/l). Geraniol was found to be the most effective component for *F. verticillioides* (CMI and CI50 of 1000 and 250 ul/l, respectively). It was observed that essential oils delayed spore germination rate. The use of essential oils was effective to control these two fungal species in their different growth stages.

Mourão *et al.* (2017) evaluated antifungal effect of *Cymbopogon citrates* essential oil against *Curvularia lunata,* causative agent of the Curvularia Leaf Spot of Maize. It was found that 7.5 µL/ml to 50 µL/ml concentrations of essential oil were phytotoxic. Geranial (41.46%) and Neral (32.43%) were the main chemical components of the essential oil of *C. citrates*. 5 and 7.5 µl/mL concentration of oil inhibited 100% of conidia germination. None of the concentrations evaluated, effectively inhibited *C. lunata* mycelial growth in *in vitro* tests. The concentration of 7.5µL/ml was sufficient for the reduction in disease progress as preventive control.

2.7.4 *In vitro* evaluation of Bioagents

Biological control agents like *Trichoderma sp, Aspergillus sp* and *Cladosporium sp* were highly effective in inhibiting mycelial growth and sporulation of *E.turcicum* causing leaf blight of maize **Mahamood *et al.* (1995). 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,

Bhati and Singh (2012) studied antagonistic property of fluorescent Pseudomonas against *E.turcicum*, characterized by biochemical tests and 16S r DNA sequences. MBLK1, MBLK3, MBLK6, MBLK15, MBLK17, and MBLK22 bacterial strains out of total 33 isolates were found to be antagonistic against *E.turcicum*.

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 efficacy of four bioagents - *Pseudomonas fluorescence, Trichoderma viridae, Trichoderma harzianum* and *Bacillus subtilis* against brown spot of rice under field and lab conditions. In field conditions bioagents were applied as seed treatment and foliar spray. *Pseudomonas fluorescens* @ 4g/kg seed+foliar spray *Trichoderma viridae* @10g/l water was found effective with less disease severity of 34.67% and higher grain yield 42.96q/ha. *In vitro* conditions, most effective inhibition in the growth of *Helminthosporium oryzae* (61.72%) was shown by *Trichoderma viridae.*

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

Manu *et al*. (2017) evaluated antagonistic microorganism’s *viz*., *Trichoderma harzianum* Rifai-1, *Trichoderma harzianum*-2, *Trichoderma viride* Pers. Ex. S. F. Gray, *Pseudomonas fluorescens* Migula- 1 and *Pseudomonas fluorescens* Migula- 2 by dual culture technique for their antagonistic effect against *Exserohilum turcicum* under *in-vitro* conditions. *Trichoderma harzianum* -2 showed maximum inhibition of mycelial growth (98.65%), followed by *Trichoderma viride* (98.34%).

Among the bacterial antagonists, *Pseudomonas fluorescens*-1 and *Pseudomonas fluorescens*-2 showed the mycelial inhibition of 95.49% and 94.24% respectively. Among the five bio agents tested, least mycelial growth inhibition was observed in *Trichoderma harzianum* Rifai-1 (85.37%).

2.7.5 *In vitro* evaluation of cow urine

Basak *et al*. (2002) studied antifungal activity and comparative efficacy of fresh cow urine and cow dung *in vitro* conditions for controlling Sclerotinia rot caused by *Sclerotinia sclerotiorum* of cucumber. Mycelial growth inhibition was observed in treated and untreated sclerotia with these organic matters at different days of incubation. Results showed that mycelial growth was suppressed more effectively by cow urine even after 5 days of incubation in comparison to cow dung.

The highest inhibition 75.9% of mycelial growth was recorded in cow urine potato dextrose agar (CUPDA) after 3 days of incubation and least inhibition of 22.7% was observed in cow dung potato dextrose agar (CDPDA) after same days of incubation. Mycelial growth from sclerotia of *S. sclerotiorum* was also influenced by PDA medium mixed with cow urine and cow dung. Mycelial growth after 6 days of incubation in CUPDA was only 12.9 mm whereas in CDPDA and PDA the mycelial growth, 6 days after infection were 65.8 mm and 80.0 mm respectively. Sclerotia treated with cow urine had a very effective role on inhibition of mycelial growth compared to untreated one. No mycelial growth was observed in sclerotia treated with cow urine up to 4 days. After 5 days, only 0.9 mm mycelial growth was recorded in treated sclerotia, while in case of untreated sclerotia the growth was 42.6 mm. Application of cow dung and cow urine on growing plants inoculated with the pathogen at different concentrations also proved their inhibitive effects.

Paul (2011) evaluated distillates of cow urine against seven plant pathogens- *Rhizoctonia solani, Sclerotinia sclerotiorum, Sclerotium rolfsii, Phytophttora nicotianae, Fusarium oxysporum, Fusaarium solani* and *Colletotrichum capsici.* Hydro distillation method was used to prepare distillates and they were tested at 0.5, 2.0, 4.0, 6.0, 8.0 and 10% concentrations by using poisoned food technique. Cow urine distillate was less effective compared to botanical- cow urine distillates which showed more than 98% inhibition in mycelial growth of *P.nicotiane* and *S.sclerotiorum* and 93%

inhibition of *F. oxysporum, C. capsici, R. solani, F. solani and S. rolfsii* at 10% concentration and inhibition in rest of concentrations was 59.70-96.77%.

Rakesh *et al.* (2013) studied the antifungal effect of different concentrations of fresh and stored cow urine (3 months) *viz.,* 5, 10, 20 and 40% against two phytopathogenic fungi of ginger *viz.*, *Pythium aphanidermatum* and *Fusarium oxysporum* f.sp.*zingiberi* isolated from soft rot specimen of ginger. Poison food technique was used for evaluation and growth of test fungi was recorded and compared with the control.

Concentration dependent inhibition of test fungi was shown by both fresh and stored cow urine. Higher inhibition was recorded in case of stored cow urine than fresh cow urine. More than 50% inhibition of test fungi was recorded at 20% and higher concentrations of cow urine. *P. aphanidermatum* showed inhibition to higher extent than *F. oxysporum f.sp.zingiberi*.

Gotora *et al.* (2014) studied antifungal effect of cow urine at different concentrations (100u/ml, 200ul/ml, 300ul/ml and 500ul/ml) to inhibit the growth of *Fusarium lateritium,* causing Fusarium bark disease in coffee. The growth characteristics selected were conidial germination, germ tube length, mycelia growth rate. Distilled water was taken as negative control, undiluted cow urine was considered as positive control and Copper oxychloride 50% WP was taken as standard. The undiluted cow urine was found to be most effective in inhibiting fungal growth compared to rest of the cow urine concentrations showing dose dependent efficacy compared to the negative control ($P < 0.01$).

Jandaik *et al.* (2015) conducted an experiment to determine antifungal activity of three different concentrations (5, 10, and 15%) of cow urine against three fungal pathogens (*Fusarium oxysporum, Rhizoctonia solani, and Sclerotium rolfsii*) isolated from infected plants of Methi and Bhindi that showed symptoms of damping off and wilting disease, by using poison food technique. The extent of growth of test fungi was lesser in plates poisoned with cow urine when compared with the control plates. Among these concentrations cow urine at 15% concentration was most effective.

Maximum growth inhibition was observed in *Fusarium oxysporum* (78.57%) at 15% concentration of cow urine followed by *Rhizoctonia solani* (78.37%)

and *Sclerotium rolfsii* (73.84%). Finally it concluded that the cow urine has antifungal activities and the inhibitory activity thus can be used in the control of fungi.

Patel *et al.* (2015) evaluated comparative efficacy and *in vitro* activity of *Bos taurus* (cow) urine and dung for controlling red rot disease of sugarcane by determining percentage mycellial growth inhibition (MGI) of *Colletotrichum falcatum*. The percentage inhibition of mycelial growth suppressed differed greatly with respect to different isolates and days of incubation and kind of bio matters. In case of cow urine, highest percentage (48.76%) MGI was recorded for CHA8 isolate while GAN6 isolate showed minimum (26.08%) MGI.

Efficacy of cow dung was found to be varied greatly for all *C. falcatum* isolates. Cow dung showed 47.59% MGI for cfCHA8 while cfNAV showed minimum percent (21.14%) MGI. The percentage mycelial growth inhibition, varied with time of incubation. In majority of cases the maximum inhibition was observed after five days of incubation. The use of cow urine and cow dung was found cost-effective and eco-friendly approach for suppressing sugarcane red rot disease.

Materials and Methods

The present investigation was carried out in laboratory and glasshouse at Department of Plant Pathology, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar (Uttarakhand).

Pantnagar is located in the foot hills of Himalayan range and falls under humid subtropical climatic zone in the narrow belt called ‘Tarai’ and ‘Bhabhar’ region. Geographically it is situated at 29.5^o North latitude, 79.73^o East longitude at an altitude of 243.84m above the Mean Sea Level (MSL). The weather conditions such as mean temperature, relative humidity, rainfall etc are quite favourable for the normal growth and development of maize.

3.1 Laboratory experiments

3.1.1 Glassware, Chemicals and Equipments

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

3.1.2 Composition of cleaning solution

Potassium dichromate ($K_2Cr_2O_7$)	:	60g
Concentrated Sulphuric acid (H_2SO_4)	:	60ml
Distilled water	:	1000ml

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

3.1.3 Equipments

Compound microscope was used for observing the fungi while for weighing chemicals, single pan electronic balance with sensitivity of 0.001g was used. Hot air oven was used for sterilisation of glass wares 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 hot air oven at 160^{o}C for 90 minutes.
ii. Different media and water were sterilised in at 15 psi (121.6^{o}C) for 20 minutes in an autoclave.
iii. Work benches were sterilised using ethyl alcohol.
iv. Sodium hypochloride (NaOCl) 2.0% was used for surface sterilization of plant materials and rectified spirit for other equipments like inoculation needles, forceps, inoculation chamber and hands.
v. Cork borer, scalpel and inoculation loop were sterilised over 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 preparation of media, sterilisation, isolation and maintenance of fungal cultures with slight modification wherever necessary. Poisoned food technique was used for testing the efficacy of fungicides and botanicals.

3.3 Isolation, Identification and Pathogenicity of the Test Fungus

3.3.1 Symptomatology

The symptoms of northern leaf blight of maize were studied with respect to development of lesions (colour, shape, size) on leaves. The disease sign was studied for presence of mycelium, according to the detailed symptom.

3.3.2 Collection of infected leaves

Samples or specimen of turcicum blight infected leaves of maize were collected. The leaf samples were brought to the laboratory for its microscopic examination, isolation and for further studies.

3.3.3 Use of Media

Potato Dextrose Agar (PDA) was used for isolation, purification and maintenance of isolates.

Composition of Potato Dextrose Agar (PDA) medium

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

For preparing PDA medium, 200g potatoes were washed, peeled off and then sliced into small pieces. The sliced potatoes were boiled in 500ml water in a vessel for 20 minutes. Simultaneously 20 g of agar was mixed in 500 ml of water and boiled for 30 minutes. The potato extract was filtered through muslin cloth and was collected in a conical flask. 20 g of dextrose is added to potato extract, mixed thoroughly with potato agar mixture and volume is made into 1 litre with distilled water. The pH of medium was checked by using pH meter. Adjustment in pH was done by adding 1N HCl or 1N NaOH as per the requirement. The medium was sterilised in an autoclave at 15psi (121.6^o C) for 20 minutes.

3.3.4 Isolation of fungus

The infected leaves were cut with help of sterilised blade into pieces of 2-3mm size having half healthy and half diseased tissues. The small pieces were sterilised with Sodium hypochloride solution 2% for 30 seconds and thoroughly washed in sterilised water three times. Then the pieces were placed between two layers of sterilized blotter paper to remove excess of water. These pieces were then transferred to slants and Petri plates containing PDA medium inside laminar flow chamber under aseptic conditions, followed by incubation at 28 ± 2^o 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 hyphal tip isolation technique as described below.

3.3.5 Hyphal tip isolation

Spore suspension is prepared by adding 10ml of sterilised distilled water in petriplate containing 7 days old culture. The spore suspension obtained is diluted to obtain a suspension with 8-10 spores/ml. One ml of the suspension was spread uniformly on two per cent solidified agar plates and incubated at 28 ± 2 ^{o}C for 12 hrs the plates were marked with a marker on backside of the petriplate and spore was allowed to germinate. Such plates were periodically observed for spore germination under microscope. The hyphae coming from end cell of the single spore was traced and marked with marker. The tip of the hyphae was cut carefully and transferred to PDA plates in aseptic conditions in laminar chamber and incubated at 28 ± 2^{o}C for seven days.

3.3.6 Identification and maintenance of the test fungus

The fungus was identified as *Exserohilum turcicum* based on morphological characteristics, cultural characters (colony colour, texture and appearance). To describe hyphal morphology of pathogen, photomicrographs were taken. Pure cultures were maintained on PDA slants at 4^{o} C and subcultured on petriplates containing PDA medium for further experiments. Sufficient attention was given to maintain genetic purity of the isolates throughout the study.

3.3.7 Pathogenecity test

The test was conducted in glasshouse. The seeds of Surya variety of maize were sown in plastic pots containing sterilised soil with three replications. Twenty five days old leaves were rubbed with carborandum powder to make injury on the leaves and then the leaves were inoculated with spore suspension (5×10^{4}spores/ml) prepared in sterile distilled water from 10 days old culture of *Exserohilum turcicum* by using atomiser. Inoculated plants were covered with perforated plastic sheets for 24 hrs to maintain humidity for infection by pathogen. The organism was re-isolated from symptoms developed on artificially inoculated leaves. The culture obtained was compared with the original culture to confirm the identity of pathogen.

3.4 Cultural, physiological and nutritional studies

3.4.1 Effect of different media and different temperatures on radial growth

Fungi derive their food from the substrate upon which they grow. No universal medium is available on which all fungi could be grown satisfactorily. Therefore it is necessary to find out suitable medium which could support the growth and sporulation of the organism. Thus to support the fungal growth and sporulation different solid media were tested. The cultural characters of *Exserohilum turcicum* were studied on the following six different media.

1. Potato Dextrose Agar
2. Czapex Dox Agar
3. 2% Malt Extract Agar
4. Oat Meal Agar
5. Corn Meal Agar
6. Richard's Agar

The compositions of the above mentioned medias are given in Appendix-I. The ingredients of each medium were dissolved in 1000 ml of water and allowed to boil and make up the final volume to 1 litre by adding distilled water. The media prepared was transferred to 250ml conical flasks. The flasks were plugged in with non absorbent cotton. The media poured in flasks was then subjected to moist heat sterilisation in an autoclave at a temperature of 121.6 ^{o}C for 15 minutes.

Twenty ml of each above mentioned medium was poured in 90mm diameter petri plates. After solidification, 5mm discs of test fungus from actively growing culture were cut using a cork borer and a single disc was placed upside down at the centre of petriplate. Each set of experiment was replicated thrice and plates were incubated at different temperatures *viz*; 10, 15, 20, 25, 30, 35^{0}C. The measurements of the colony diameter were taken at 7 days after incubation.

3.4.2 Effect of different media and light quality on growth of *Exserohilum turcicum*

To assess the effect of different media and light quality on growth of *Exserohilum turcicum*, Petri plates were poured with six different types of media and allowed to solidify. After solidification, 5mm discs of *Exserohilum turicum* from actively growing culture were cut using a cork borer and a single disc was placed upside down at the centre of petriplate. These Petri plates were then incubated for seven days at 28^{0} C ± 2^{0}C enclosed in the chambers providing different coloured lights. Three replications were maintained for each treatment. These chambers were prepared covering them with coloured cellophane sheets of varying spectral characteristics. All types transmitted different spectra of visible light depending on cellophane colour: 400– 500nm (blue); 480–540nm (green); 500–700nm (yellow); 600–700nm (red). Observations of colony diameter in different treatment were taken from the third day of inoculation and final reading was taken on the seventh day of inoculation.

3.4.3 Effect of different pH on radial growth of *Exserohilum turcicum*

Five pH levels *viz.*, 5, 6, 7, 8, 9 were adjusted in Potato Dextrose Agar medium with the help of pH meter by adding 1 N Sodium hydroxide or 1 N Hydrochloric acid. The media having different pH values were sterilised and poured in petri plates. Three replication of each treatment were maintained and incubated at 28 ± 2^0 C. Colony diameter was measured from third till seventh day of incubation.

3.5 *In vitro* management studies

3.5.1 *In vitro* evaluation of Fungicides against *Exserohilum turcicum*

In vitro, efficacy of different fungicides against *Exserohilum turcicum* was studied by using poison food technique **(Sharvelle, 1961).** Four systemic and four non systemic 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 5, 10, 20, 30 and 50ppm concentrations where as non systemic were evaluated at 50, 100, 200, 500 and 1000ppm concentrations. Information about fungicide formulations and active ingredient is presented in table 3.1.

Table 3.1: List of systemic and non systemic fungicides evaluated *in vitro* against *E.turcicum*

Sl. No.	Trade name	Common name	Active ingredient (%)	Mode of action	Formulation	Manufacturer
1	Amistar	Azoxystrobin	23	Systemic	SC	Syngenta India Ltd.
2	Roko	Thiophenate methyl	75	Systemic	WP	Biostadt India Ltd.
3	Dhan	Propiconazole	25	Systemic	EC	Indofil Chemicals Ltd
4	Folicur	Tebuconazole	25.9	Systemic	EC	Bayer crop science
5	Captaf	Captan	50	Contact	WP	Rallis India Ltd.
6	Indofil Z-78	Zineb	75	Contact	WP	Indofil Chemicals Ltd
7	Curzate M8	Cymoxanil(8)+ Mancozeb(64%)	72	Contact+Systemic	WP	DuPont, India Pvt. Ltd.
8	Avtar	Zineb(68%)+ Hexaconazole (4%)	72	Contact+Systemic	WP	Indofil Chemicals Ltd

Poison food technique

Stock solution of 10,000 ppm concentration of each fungicide was prepared using distilled water in test tube. Required amount of solution was added into a beaker containing 60ml sterilised melted PDA, so as to get final required concentration of 5, 10, 20, 30 and 50ppm for systemic fungicides and 50, 100, 200, 500 and 1000ppm for non systemic fungicides. The medium was mixed thoroughly before plating. For each concentration three replications were maintained. Non toxicated media was poured in three petri plates and was kept as check. After solidification of media a 5mm disc of seven days old culture of the test pathogen was cut with a sterile cork borer and placed in centre of each petri plate. The petri plates were incubated at 28±2^{o} C. After seven days of incubation the radial growth was measured. The per cent inhibition in growth was determined with the help of mean colony diameter and calculated by using the following formula (**Vincent, 1947**).

Per cent inhibition=X-Y/X × 100

Where,

X= Colony diameter in control

Y=Colony diameter in treated medium

3.5.2 *In vitro* evaluation of botanicals against *Exserohilum turcicum*

Preparation of botanical extracts

The leaf extracts of Eucalyptus, Lantana, Ashok, Marigold, Duranta, Aloevera, Heena and rhizome extracts of Ginger and Turmeric were extracted using aqueous extract method. Fifty grams of thoroughly washed leaves or rhizome were macerated with 50ml of sterile distilled water in a blender for 10minutes. The macerate was first filtered through double layered muslin cloth. The filtered extract was then sterilized at 120°C for 30 minutes. The extract was preserved aseptically at 5°C for further use. For bioassay different concentration of extracts 5, 10, 15, 20 per cent were evaluated by adding them in culture media using poison food technique under *in vitro* conditions. Details about the botanicals and parts used are given in table 3.2.

Table 3.2: List of botanicals evaluated *in vitro* against *E.turcicum*

Sl. No.	Common name	Botanical name	Family	Parts used
1	Duranta	*Duranta goldiana*	Verbenaceae	Leaves
2	Marigold	*Tagetus erecta*	Asteraceae	Leaves
3	Eucalyptus	*Eucalyptus globules*	Myrtaceae	Leaves
4	Alovera	*Aloe vera*	Asphodelaceae	Leaves
5	Ginger	*Zingiber officinale*	Asteraceaea	Rhizome
6	Turmeric	*Curcuma longa*	Lythraceae	Rhizome
7	Heena	*Lawsonia inermis*	Annonaceae	Leaves
8	Rudraksh	*Elaeocarpus ganitrus*	Elaeocarpaceae	Leaves
9	Ashok	*Polyalthia longifolia*	Annonaceae	Leaves
10	Lantana	*Lantana camara*	Verbenaceae	Leaves

Bioassay procedure

Poisoned food technique (plant extract amended PDA medium) was used to screen different plant extracts *in vitro* **(Nene and Thapliyal, 1973)**, different concentrations (5, 10,15 and 20%) of plant extracts were incorporated in double strength concentration of PDA medium for inoculation of the test pathogen in sterilised petriplates. The 5mm disc of test pathogen grown on PDA medium was placed at centre of petriplates containing different concentration of the poisoned medium and incubated at 28±2^{o}C. Three replications were maintained and radial growth was taken when maximum growth occurred in the control plates. The inhibition per cent of radial growth over the control was calculated by using the following formula **(Vincent, 1947).**

Per cent inhibition=X-Y/X × 100

Where,

X=Colony diameter in check

Y=Colony diameter on amended medium

3.5.3 *In vitro* evaluation of essential oils against *Exserohilum turcicum*

Ten essential oils *viz* Ginger, Clove, Citronella, Lemon tulsi, Palmarosa, Geranium, Turmeric, Lemon grass, Patchouli and Eucalypus were evaluated *in vitro* against the test pathogen using poison food technique. All the oils were obtained from Central Institute of Medicinal and Aromatic Plants (CIMAP) Research Centre, Pantnagar, except Ginger, Eucalyptus and Turmeric oil which were extracted by using Clevenger apparatus. Details of essential oils and parts from which they are derived are given below:

Table 3.3: List of essential oils evaluated *in vitro* against *E.turcicum*

Sl. No.	Essential oil	Plant source	Family	Parts used
1	Clove	*Syzygium aromaticum*	Myrtaceae	Leaves
2	Ginger	*Zingiber officinale*	Zingiberaceae	Rhizome
3	Palmarosa	*Cymbopogon martini*	Poaceae	Leaves
4	Patchouli	*Pogostemon graveolens*	Lamiaceae	Leaves
5	Citronella	*Cymbopogon spp.*	Poaceae	Leaves
6	Peppermint	*Mentha piperata*	Lamiaceae	Leaves
7	Turmeric	*Curcuma longa*	Zingiberaceae	Rhizome
8	Lemon tulsi	*Ocimum africanum*	Lamiaceae	Leaves
9	Geranium	*Pelargonium graveolens*	Geraniaceae	Leaves
10	Eucalyptus	*Eucalyptus globules*	Myrtaceae	Leaves

Poison food technique

A suitable solvent was prepared for dissolving essential oils in PDA media. For preparing 1 litre of solvent 10ml of Tween 20 (1%) and 100ml of DMSO (20%) is needed to be mixed in 890ml of sterilised distilled water. Considering the ratios of components 250 ml of solvent was prepared. Then 10000ppm stock solution of essential oils was prepared by taking 100ul of desired oil in 10 ml of solvent. After that different concentration of oils *viz* 25ppm, 50ppm, 100ppm, 250ppm and 500ppm were prepared by adding desired quantity from stock solution to melted sterilised PDA media and poured in sterilised petriplates. Petriplates poured with sterisilised melted non toxicated PDA was taken as check. Three replications were maintained for each concentration. The 5mm disc of test pathogen grown on PDA medium was placed at centre of petriplates containing different concentration of the poisoned medium and incubated at 28±2^{o}C. Radial growth was taken when maximum growth occurred in the control plates. The inhibition per cent of radial growth over the control was calculated by using the following formula **(Vincent, 1947).**

Per cent inhibition=X-Y/X × 100

Where,

X=Colony diameter in check

Y=Colony diameter on amended medium

3.5.4 *In vitro* evaluation of *Trichoderma* isolates against *Exserohilum turcicum*

In vitro six isolates of *Trichoderma harzianum, viz.* Th, TCMS-36, PBAT-1, Th-17, Th-19 and TCMS-39 were used for experiments. Both bio-control agent and test fungus were cultured on PDA in order to get active growth of fungus. These bio-control agents were obtained from Bio-control Laboratory of Department of Plant Pathology, G.B. Pant University of Agriculture and Technology, Pantnagar.

Dual culture test

Bio-agents were evaluated against the test pathogen for their efficacy through dual culture technique. The bioagents and test fungus were inoculated side by side on a single petridish containing solidified PDA medium. Three replications were maintained for each treatment with one check by maintaining pathogen separately. Inoculated plates were incubated at 28± 2^{0}C. The diameter of colony of both bio-agent and the pathogen was measured in two directions and average was recorded. Per cent inhibition of growth of the test fungus was calculated by using the formula described by (**Vincent, 1947).**

Per cent inhibition=A-B/A×100

Where,

A=Diameter of fungal growth in check plate

B=Diameter of fungal growth in treatment

3.5.5 *In vitro* evaluation of cow urine against *Exserohilum turcicum*

Cow urine was collected from dairy cattle before milking in the morning from Instructional Dairy Farm, Nagla (Pantnagar). The efficacy of cow urine to inhibit test fungi was determined by poisoned food technique. PDA medium was amended with different concentrations of fresh cow urine *viz*., 1, 5, 10, 20, 30, 40 and 50%, sterilized by autoclaving and added to labelled Petri plates. After solidification of media, discs of 5 mm diameter of actively growing culture were cut from the periphery of seven days old culture and were inoculated aseptically on PDA plates poisoned with different concentrations of cow urine and incubated for 7 days at 28 ± 2^{o} C. The experiment was carried in triplicate and average colony diameter was recorded. The inhibition per cent of radial growth over the control was calculated by using the following formula **(Vincent, 1947).**

Per cent inhibition = X-Y/X × 100

Where,

X=colony diameter in check

Y=colony diameter on amended medium

3.6 Glasshouse experiment

Experiments were conducted in the glasshouse for evaluation of different methods of inoculation and to study the effect of different fungicides, botanicals, essential oil, bioagents and cow urine concentrations against *E.turcicum* causing northern leaf blight of maize on two varieties of maize Amar and Surya.

Two varieties Surya and Amar were sown under glasshouse conditions in separate pots and three replications were maintained for each treatment in case of both the varieties and were taken care of by regularly watering them. The experimental design used was CRD.

3.6.1 Evaluation of the Inoculation Methods

Three inoculation methods *viz.,* Spraying, Cotton swab method and Stem injection method were used to inoculate the maize plants under glass house conditions. Inoculation of pathogen was done to identify the most suitable method in order to study the host pathogen relationship. Plants were inoculated at twenty five days after sowing.

Spraying

The maize plants in pots under the glasshouse conditions were inoculated with spore suspension (5×10^4spores/ml) prepared in sterile distilled water from 10 days old culture of *Exserohilum turcicum* by using atomiser. Inoculated plants were covered with perforated plastic sheets for 24 hrs to maintain humidity for infection by pathogen

Stem injection method

Conidial suspension of the fungus was prepared by washing the conidia from full grown fungal culture plate in 10ml of sterilized distilled water and inoculated in the maize plants on pots. A puncture was made in stem of the plant with help of needle and one ml of the inoculum was injected into the hole using injection.

Cotton swab method

Conidial suspension of the fungus *Exserohilum turcicum* was prepared to inoculate the maize plants in the glasshouse. Absorbent cotton was soaked in the conidial suspension overnight for the proper absorption of the suspension. Soaked cotton swab was then wrapped around the leaf blade and sealed with parafilm to maintain the long duration exposure of leaf to the inoculum.

Observations were taken at three developmental stages after inoculation at knee high stage, tasseling stage and silking stage.

3.6.2 Evaluation of Fungicide, Botanicals, Essential oils, *Trichoderma i*solates and cow urine concentrations against the pathogen

Fungicides, Botanicals, Essential oils, Trichoderma isolates and cow urine concentrations which showed higher mycelial growth inhibition of *Exserohilum turcicum* under lab conditions were selected and evaluated under glasshouse conditions.

The causal organism *E.turcicum* was inoculated by foliar spray of spore suspension on 25 days old cultivars of maize. The inoculated plants were kept in humid conditions for 4-5 days by preparation of moist chambers to provide favourable conditions for growth of pathogen. The inoculated plants were observed regularly for development of symptoms.

Four fungicides (Propoiconazole, Tebuconazole, Avtar and Captan), four botanicals (Lawsonia, Duranta, Turmeric and Eucalyptus), four essential oils (Lemon tulsi, Clove, Citronella and Patchouli) and similar number of Trichoderma isolate (Th, PBAT-1, TCMS-36. TCMS-39) were selected for glasshouse experiment on the basis of *in vitro* superiority.

Fungicides were sprayed at the rate of 50ppm, Botanicals were sprayed at the rate of 5%, essential oils were sprayed at the rate of 50ppm, Trichoderma isolates were sprayed by preparing spore suspension of 1×10^6 conidia/ml.

Artificial inoculation was done five days prior to treatments by spraying spore suspension of *E.turcicum*. Two sprays were done for each treatment, first spray five days after inoculation of the pathogen and second spray ten days after the first spray. Observations were taken ten days after the first spray and ten day after the second spray.

Observations on the severity of disease were recorded on 1-5 scale **(Payak and Sharma, 1983)**

Table 3.4: Standard rating scale for Northern leaf blight of Maize

Scale	Infection Type	Disease Severity (%)
1.0	Very slight to slight infection, one or two to few scattered lesions on lower leaves, few on middle leaves.	1-10% Highly Resistant
2.0	Light infection, moderate number of lesions on lower leaves, few on middle leaves.	11-25% Resistant
3.0	Moderate infection, abundant lesions on lower leaves, few on middle leaves.	26-50% Moderately resistant
4.0	Heavy infection, lesions abundant on lower leaves, extending to upper leaves.	51-75% Susceptible
5.0	Very heavy infection, lesions abundant on almost all leaves, plant prematurely dried or killed by the disease.	>75% Highly susceptible

After disease appearance, disease rating was recorded. Twenty leaves were randomly selected from each pot and disease incidence and severity were recorded.

Following formula was used to calculate the per cent disease severity

$$\text{Per cent Disease Severity} \quad \frac{\text{Sum of all ratings X 100}}{\text{Maximum rating X No. of sample leaves}}$$

Disease incidence was calculated using the following formula

$$\text{Diseaese incidence} \quad \frac{\text{No. of plants infected}}{\text{Total no. of plants}} \text{X 100}$$

3.6.3 Statistical analysis

The data obtained in the laboratory and glasshouse were analysed statistically by Completely Randomised Design (CRD) using MS Excel and STPR programme (GBPUA &T statistical software). Comparison of data recorded was done by means of critical differences at 5% level of significance.

Results and Discussion

The detailed results of the investigation carried out in the laboratory and glasshouse conditions to determine the *in vitro* and *in vivo* evaluation of fungicides, botanicals, essential oils, bioagents and cow urine against *Exserohilum turcicum* causing northern leaf blight of maize are discussed. Experimental evidences are provided for the variations or agreement observed from the findings of various workers.

4.1 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 larger area of leaves giving blightened appearance. These morphological characters of the fungus are similar to those described by **(Fredericksen, 1980)** who observed small flecks at 3-4 days after favourable infection, while large distinctive lesions appear two weeks later**.** **Chenulu and Hora (1962)** observed scorched appearance of leaves in the infected field. **Ullstrup (1966)** observed elliptical greyish or tan lesions as characteristic symptoms of disease.

4.2 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 appearance on PDA medium. Similar results were reported by **Reddy (2012)** who observed greyish to black coloured and profuse growth of the fungus. The fungal growth later turns greyish to somewhat olivacious black in colour.

Microscopic observations were made by preparing slides from fresh diseased samples and from seven days old culture of the fungus. Slides were prepared using lactophenol and were observed under compound microscope. Mycelium and conidia of the fungus was observed under the microscope at 10x and 40x. The fungus was identified as *Exserohilum turcicum* on the basis of septation of conidia and protruding hilum of conidia.

4.3 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 black in colour. The colony was fluffy and cottony with regular margins. The hypha observed under the microscope was pale to light brown, smooth and septate. The conidia were 3 - 8 septate, olivaceous 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)** who observed that conidia were elongated and spindle in shape with 2-7 septations.

4.4 Pathogenecity test

The pathogenicity test of *Exserohilum turcicum* was determined under glass house conditions. Twenty five days old plants were inoculated with spore suspension of *Exserohilum 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^{o} C. After inoculation, plants were regularly observed for the appearance of symptoms. Reisolation was done from 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.*

4.5 Cultural and Physiological studies

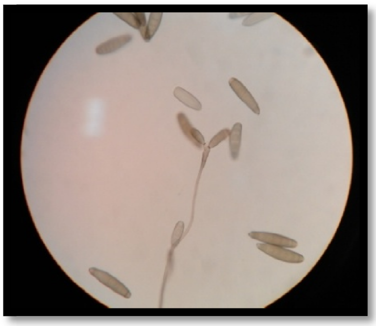
4.5.1 Effect of different media and temperatures on growth of *Exserohilum turcicum*

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 media at different temperatures on the mycelia growth of *Exserohilum turcicum* was studied. Six different media were taken and each one of them was kept at six different temperatures and difference in colony diameter of the pathogen among them was observed.

PLATE 1: Symptoms of Northern Leaf Blight of maize caused by *Exserohilum turcicum*

A. Initial symptoms B. Enlarged lesions C. Symptoms at maturity

PLATE 2: Morphological and cultural characters of *E.turcicum*



A. Mycelial growth B. Conidia with conidiophore C. Prominent hilum of conidia

PLATE 3: Experiments under glasshouse conditions

A. Maize plants grown in pots B. Inoculated plants

Potato Dextrose Agar showed maximum colony diameter of 87.33 mm at 30^{o}C followed by colony diameter of 70.00 mm at 25^{o}C. Minimum mycelial growth, 27.83 mm was observed at 10^{o}C.

Richard' Synthetic Agar showed maximum colony diameter of 77.17 mm at 30^{o}C followed by colony diameter of 73.33 mm at 35^{o}C. Minimum colony diameter of 20.50 mm was observed at 10^{o}C.

Oat Meal Agar showed maximum colony diameter of 86.67 mm at 30^{o}C followed by 85.33 mm at 25^{o}C. Minimum colony diameter of 46.17mm was recorded at 10 ^{o}C.

Corn Meal Agar showed maximum colony diameter of 75mm at 25^{o}C followed by 60mm at 30^{o}C. Minimum colony diameter of 23mm was observed at 10^{o}C.

2% Malt Extract Agar showed maximum mycelial growth of 41mm at 30^{o}C followed by 40.33mm at 15^{o}C. Minimum colony diameter of 27.17mm was observed at 10^{o}C.

Czapek's Dox Agar showed maximum colony diameter of 85mm at 30^{o}C followed by 63.60mm at 35 ^{o}C. Minimum colony diameter of 29.17mm was observed at 10 ^{o}C.

The results showed that Potato Dextrose Agar is most suitable medium compared to other media tested for the growth of *Exserohilum turcicum* at 30^{o}C. The study concludes that 25-30^{0}C temperature supports greater mycelial growth of the fungus whereas 10^{0} C is not favourable for the growth of fungus. These results were in accordance with the results obtained by **Misra and Singh (1963)** who observed that optimum temperature for growth of *Exserohilum turcicum* was 25-30^{0}C. **Champi (1939)** reported good growth of the fungus on various standard media.

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

Pandey and Shukla (1982) reported that optimum temperature for colony growth of sorghum isolate of *E.turcicum* was 20- 30^{0}C, and no growth was observed at 40^{o}C.

Table 4.1: Effect of different media and temperature on radial growth of *Exserohilum turcicum*

Sl. No.	Media	Temperature					
		10° C	15°C	20°C	25°C	30°C	35°C
		Colony diameter (mm)					
1	Potato Dextrose Agar	27.83	44.00	31.67	70.00	87.33	44.50
2	Richard's Synthetic Agar	20.50	53.83	42.67	57.50	77.17	73.33
3	Oat Meal Agar	46.17	63.67	54.83	85.33	86.67	64.67
4	Corn Meal Agar	23.00	53.17	31.83	75.00	60.00	54.00
5	2% Malt Extract Agar	27.17	40.33	28.17	35.00	41.00	38.00
6	Czapex Dox Agar	29.17	54.50	42.50	81.00	85.00	63.60
		Temperature (a)		Media (b)		Interaction (a×b)	
	SEM±	0.59		0.59		1.46	
	CD at 5%	1.68		1.68		4.13	
	CV	4.93					

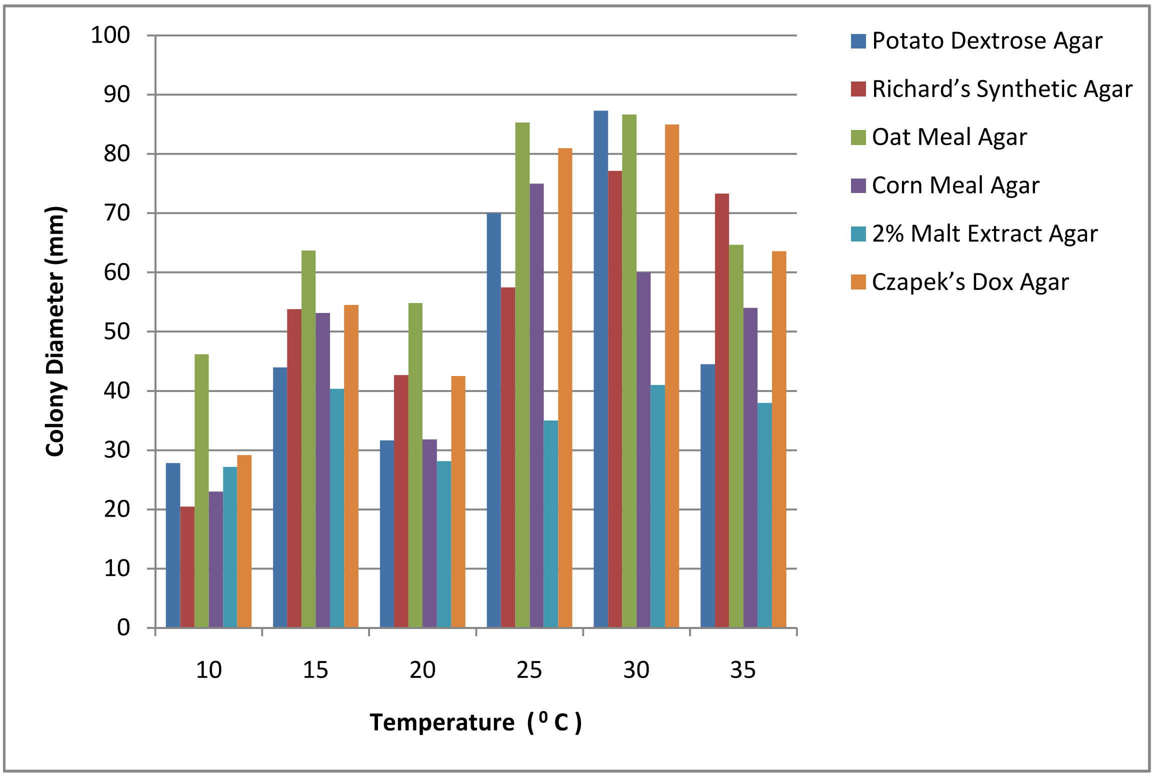
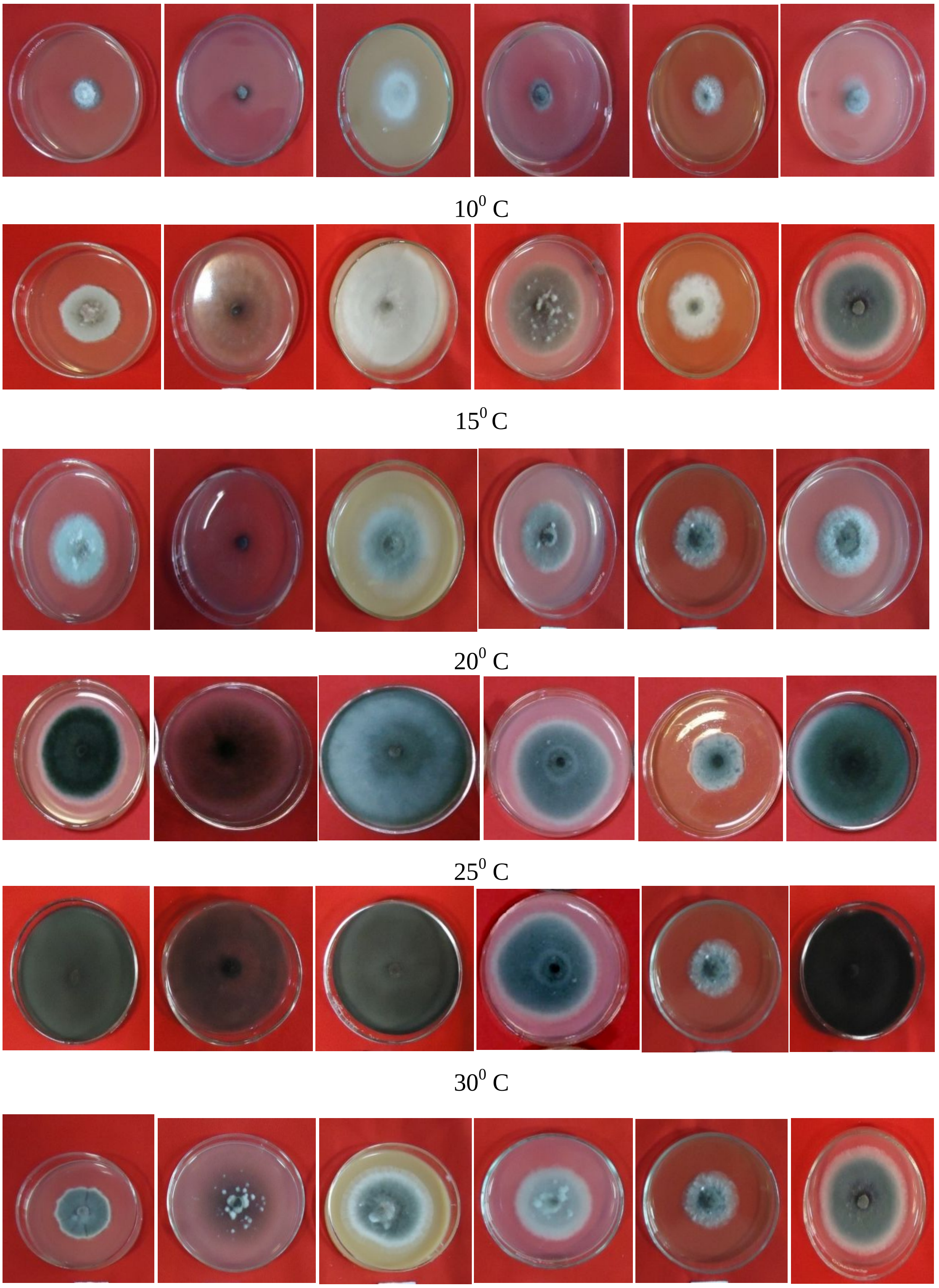


Fig 4.1: Effect of media and temperature on radial growth of *Exserohilum turcicum*

PLATE 4: Effect of media and temperature on radial growth of *Exserohilum turcicum*



PDA RSA OMA CMA 2% MALT CDA

PDA=Potato Dextrose Agar, RSA=Richard's Synthetic Agar, OMA=Oat Meal Agar, CMA=Corn Meal Agar, 2%MALT=2% Malt Extract Agar, CDA=Czapeck's Dox Agar

4.5.2 Effect of different media and different light quality on the growth of *E.turcicum*

Light plays an important role in growth and development of all organisms including fungi. Thus the influence of light wavelength on the mycelial growth of ***E.turcicum*** was investigated using red, green, blue and yellow light sources and difference in the colony diameter of the fungus obtained was recorded.

Potato Dextrose Agar showed maximum colony diameter of 51.83mm when kept under yellow light followed by blue light which showed colony diameter of 45.83mm. Minimum colony diameter of 44.01mm was observed under red light which is statistically at par with colony diameter of 44.5mm under green light.

Richard's Synthetic Agar showed maximum colony diameter of 84.80mm when kept under blue light followed by yellow light which showed colony diameter of 68.22mm. Minimum colony diameter (48.20mm) of the fungus was observed under green light followed by 55.53mm of colony diameter under red light.

Oat Meal Agar showed maximum colony diameter of 90mm under green light followed by 89.08mm colony diameter obtained under blue light. Minimum colony diameter of 88.67 was observed under red light which is statistically at par with colony diameter of 88.67 under yellow light.

Corn Meal Agar showed maximum colony diameter of 88.00mm under blue light followed by colony diameter of 82.17mm under yellow light which is statistically at par with colony diameter 81.33 under green light. Minimum colony diameter of 76.24mm was observed under red light.

2% Malt Extract agar showed maximum colony diameter of 55.67mm under red light which is statistically at par with colony diameter of 54.65 under yellow light. Minimum colony diameter of 51.50 mm was observed under blue light which is statistically at par with colony diameter of 51.93mm under green light.

Czapex Dox Agar showed maximum colony diameter of 89.67mm under yellow light followed by colony diameter of 88.03 under blue light which is statistically at par with colony diameter of 88.67mm under red and green light.

The results conclude that there is no fixed pattern of growth in different media with respect to different light wavelengths and Oat Meal Agar under green light is most favourable for the growth of the pathogen whereas Potato Dextrose Agar showed minimum growth of the test fungus when exposed to red light conditions.

Yu *et al.* (2013) studied the effect of different light wavelengths on the mycelial growth and conidial germination of *Colletotrichum acutatum* using red, green, blue and white light sources. The mycelial growth was reduced under green and red light as compared with blue light, white light and dark conditions.

Campbell *et al.* (2003) studied the effect of photoperiod and light quality on growth and sporulation of *Pyrenophora semeniperda* and observed that fungal colonies grew comparatively more quickly (≈ 70 mm diameter after 14 days) when exposed to clear or yellow light than that of red and orange, which in turn significantly showed more growth than under blue and green light sources; colonies grown under purple light had the slowest growth of all (≈ 50 mm diameter) (P < 001). Linear growth rates were observed irrespective of the photoperiod.

Table 4.2: Effect of different media and light wavelength on radial growth of *Exserohilum turcicum*

Sl. No.	Media	Light Quality			
		Red	**Yellow**	**Green**	**Blue**
		Colony diameter (mm)			
1	Potato Dextrose Agar	44.01	51.83	44.5	45.83
2	Richard's Synthetic Agar	55.53	68.22	48.20	84.80
3	Oat Meal Agar	88.67	88.93	90.00	89.08
4	Corn Meal Agar	76.24	82.17	81.33	88.00
5	2% Malt Extract Agar	55.67	54.65	51.93	51.50
6	Czapex Dox Agar	88.67	89.67	88.67	88.03
		Light Quality (a)	**Media (b)**	**Interaction (a×b)**	
	SEM±	0.52	0.43	1.06	
	CD at 5%	1.51	1.23	3.01	
	CV	2.59			

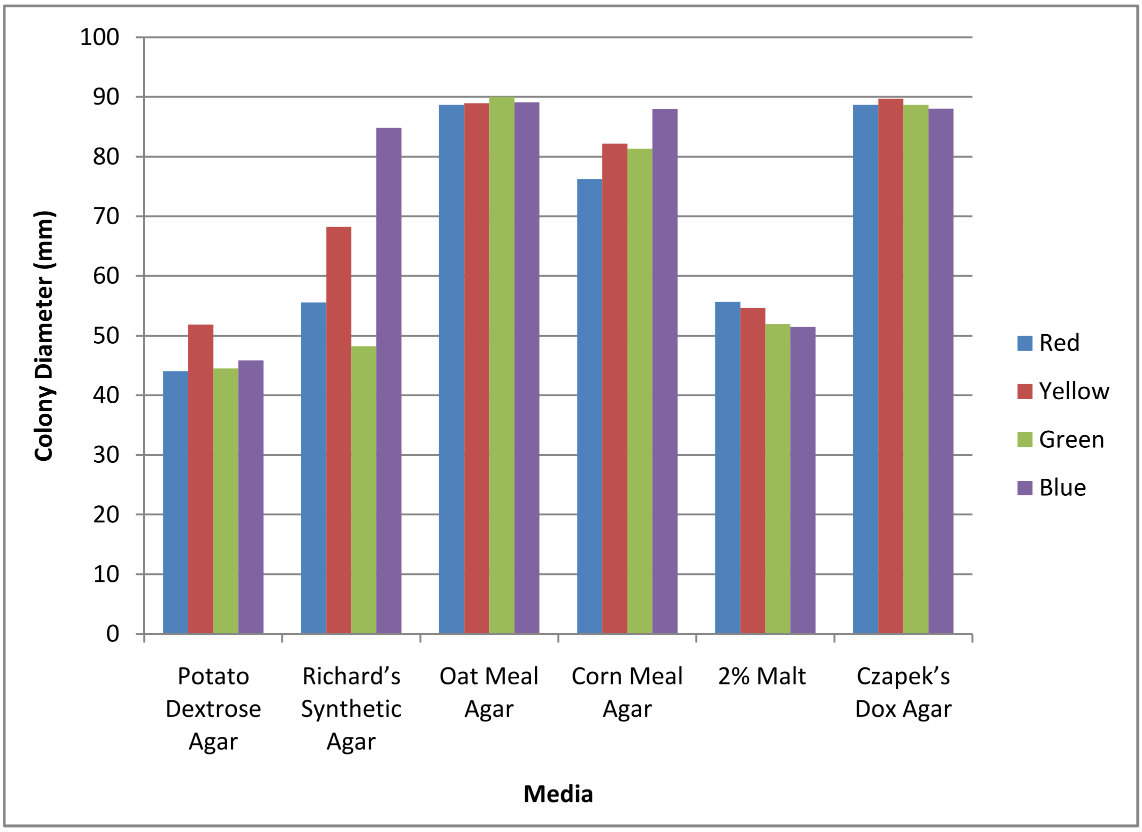


Fig 4.2: Effect of media and light wavelength on radial growth of *Exserohilum turcicum*.

4.5.3 Effect of pH on radial growth of *Exserohilum turcicum*

Fungi generally utilise substrates in the form of solution only if condition of solution is conducive to fungal growth and metabolism. Hydrogen ion concentration (pH) either in food or habitat is known to affect the growth and development of micro-organisms. In the present investigation acidic and alkaline pH were tested on the growth of *E.turcicum*. The data revealed that the maximum colony diameter (87.17mm) was at pH 7 followed by pH 9 (82.67mm) which was statistically at par with pH 8 (80mm). Minimum mycelial growth (63.67mm) was observed at pH 5. The growth gradually decreases with decrease in pH from 9 to 5, which is in conformity with the results obtained by **Kutawa *et al.*, (2016)** who observed that pH 7 is best for growing *E. Turcicum* and pH 5 showed the least growth at one week after incubation.

Table 4.3: Effect of pH on radial growth of *Exserohilum turcicum*

S.No.	pH	Colony diameter (mm)
1	5	63.67
2	6	72.33
3	7	87.17
4	8	80.00
5	9	82.67
	SEM±	2.16
	CD at 5%	6.81
	CV	4.85

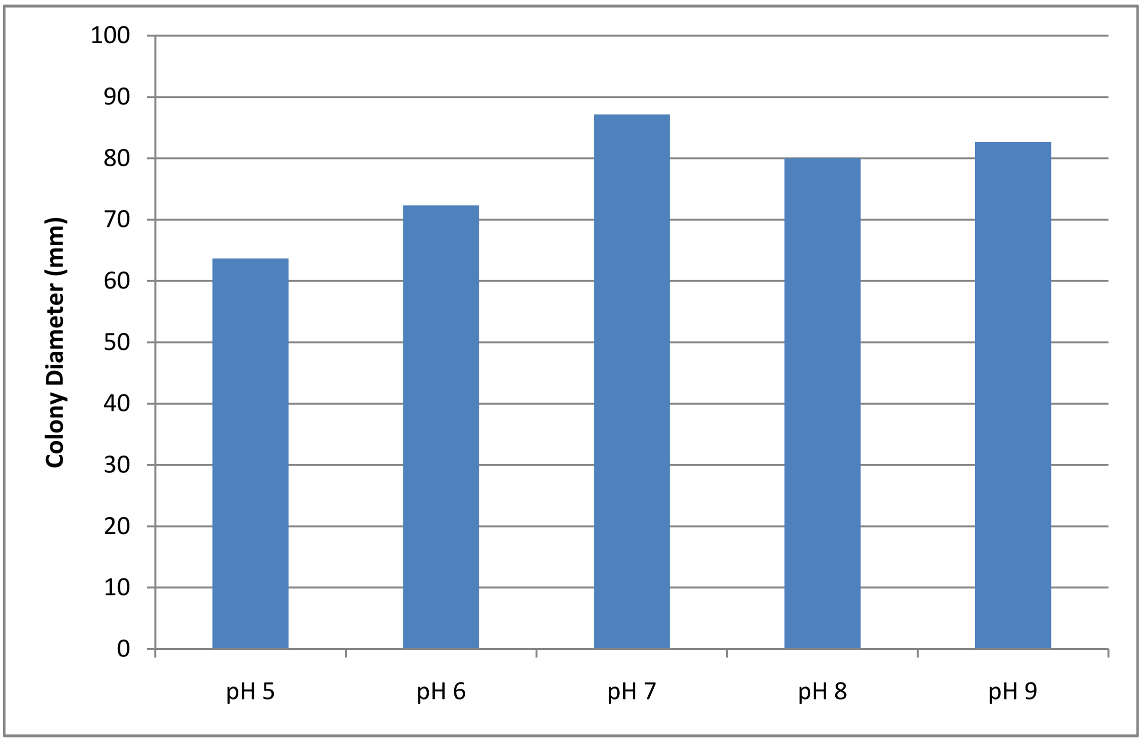


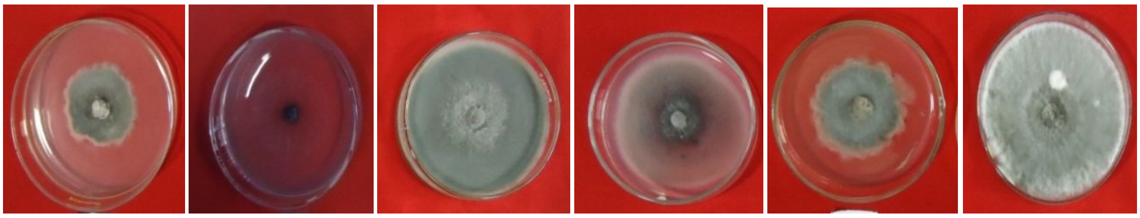
Fig 4.3: Effect of pH on radial growth of *Exserohilum turcicum*

4.6 *In vitro* management studies

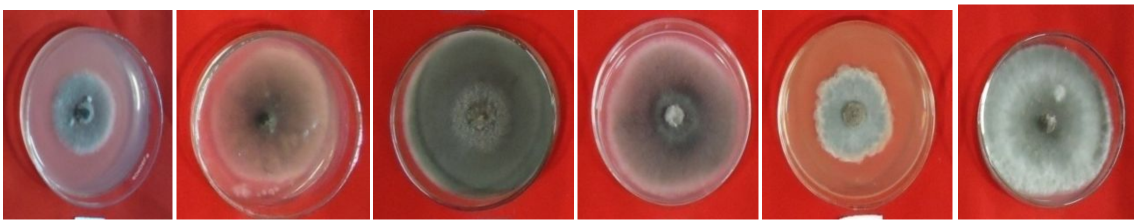
4.6.1 *In vitro* evaluation of fungicides against *Exserohilum turcicum*

The efficacy of some fungicides was evaluated in laboratory conditions against *E.turcicum* by using poison food technique.

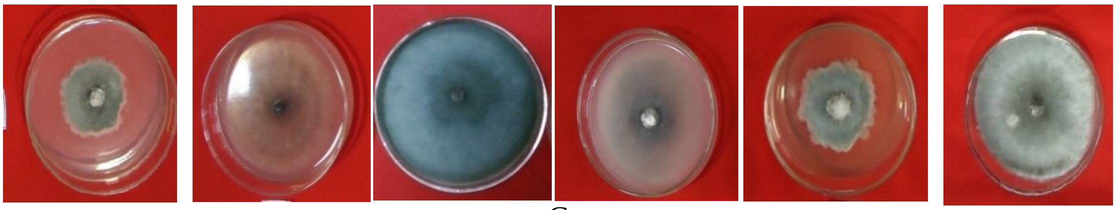
PLATE 5: Effect of media and light quality on radial growth of *Exserohilum turcicum*



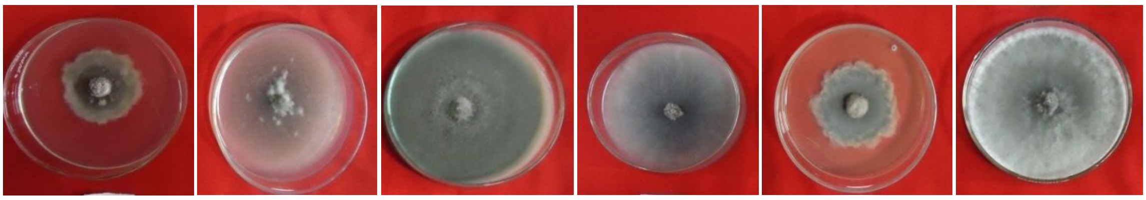
R



Y



G

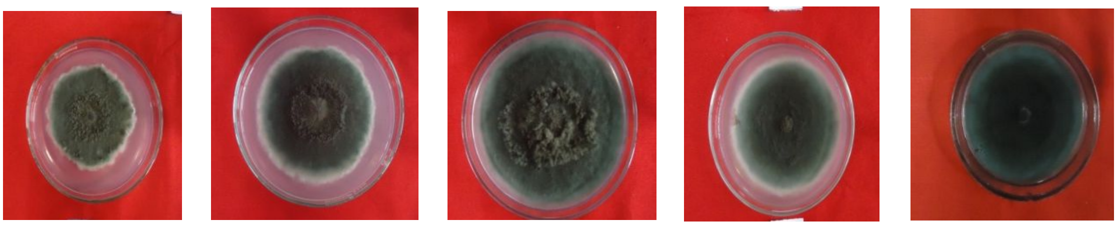


B

PDA RSA OMA CMA 2% MALT CDA

R=Red Light, Y=Yellow Light, G=Green Light, B=Blue Light

PLATE 6: Effect of pH on radial growth of *Exserohilum turcicum*



pH 5 pH 6 pH 7 pH 8 pH 9

In vitro evaluation is an effective method for testing efficacy of fungicides against pathogen in a shorter period of time. It is greatly helpful in providing preliminary information and thus serves as a guide for field evaluation. In the present investigation systemic fungicides *viz*., Amistar (Azoxystrobin 23%SC), Roko (Thiophenate methyl 75% WP), Folicur (Tebuconazole 25.9% EC) and Dhan (Propiconazole 25% EC) were tested against *E.turcicum* at five different concentrations (5, 10, 20, 30, 50ppm). Non systemic fungicides *viz*., Indofil Z-78(Zineb75%WP), Captaf (Captan 50%WP), Avtar72WP (Zineb 68%+ Hexaconazole 4%) and Curzate M8 72%WP (Cymoxanil 8%+ Mancozeb 64%), were tested at five different concentration (50, 100, 200, 500, 1000ppm).

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

At 5ppm

The results reveal that at 5ppm concentration, Propiconazole 25% EC, Azoxystrobin 23%SC and Tebuconazole 25.9% EC were found effective in inhibiting the mycelial growth of the test fungus. Propiconazole 25% EC showed maximum inhibition (92.22%), followed by Azoxystrobin 23%SC (76.48%) which is statistically at par with Tebuconazole 25.9% EC (74.44%). Thiophenate methyl 75% WP showed comparatively less mycelial inhibition (55.37mm).

At 10ppm

Propiconazole 25% EC showed cent per cent inhibition of mycelia growth followed by Tebuconazole 25.9% EC (87.78%). Minimum inhibition was shown by Thiophenate methyl 75 WP (64.81%) followed by Azoxystrobin 23% (79.26%).

At 20ppm

At 20ppm concentration Tebuconazole 25.9% EC and Propiconazole 25% EC showed cent per cent inhibition of mycelia growth followed by Azoxystrobin 23% (80.37%). Minimum per cent inhibition was showed by Thiophenate methyl 75 %WP (69.44%).

At 30ppm

At 30ppm concentration cent per cent inhibition was showed by Tebuconazole 25.9% EC and Propiconazole 25% EC followed by Azoxystrobin 23%SC (85%).

Minimum inhibition of mycelia growth (74.26%) was observed in Thiophenate methyl 75 %WP.

At 50ppm

At 50ppm concentration Propiconazole 25% EC and Tebuconazole 25.9% EC showed cent per cent inhibition of mycelial growth followed by Azoxystrobin 23%SC (87.41%). Least mycelia growth inhibition per cent (78.15%) was shown by Thiophenate methyl 75 %WP.

The result can be concluded as Propiconazole 25%EC is most effective among all the other tested fungicides which is also in accordance with **Rehman *et al.*** (1993) who observed that propiconazole was effective against *E. turcicum* on maize in vitro conditions.

Wani *et al.* (2017) observed that among systemic fungicides, propiconazole was found best in inhibiting the mycelial growth of *E. turcicum* (96.51% mean inhibition),

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

Table 4.4: Mycelial inhibition of *Exserohilum turcicum* by different Systemic fungicides

Systemic Fungicides	Per cent inhibition of radial growth *									
	Concentration									
	5ppm		10ppm		20ppm		30ppm		50ppm	
	G	I	G	I	G	I	G	I	G	I
Azoxystrobin 23% SC	21.17	76.48	18.67	79.26	17.67	80.37	13.50	85.00	11.33	87.41
Thiophenate methyl 75% WP	40.17	55.37	31.67	64.81	27.50	69.44	23.17	74.26	19.67	78.15
Tebuconazole 25.9%EC	23.00	74.44	11.00	87.78	0.00	100.00	0.00	100.00	0.00	100..00
Propiconazole 25% EC	7.00	92.22	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00
Check	90.00	-	90.00	-	90.00	-	90.00	-	90.00	-
	Fungicide(a)			Concentration (b)			Interaction (a×b)			
SEM±	0.72			0.72			1.61			
CD at 5%	2.04			2.04			4.58			
CV	9.76									

*Mean of three replications, G= Colony diameter in mm, I= Per cent inhibition

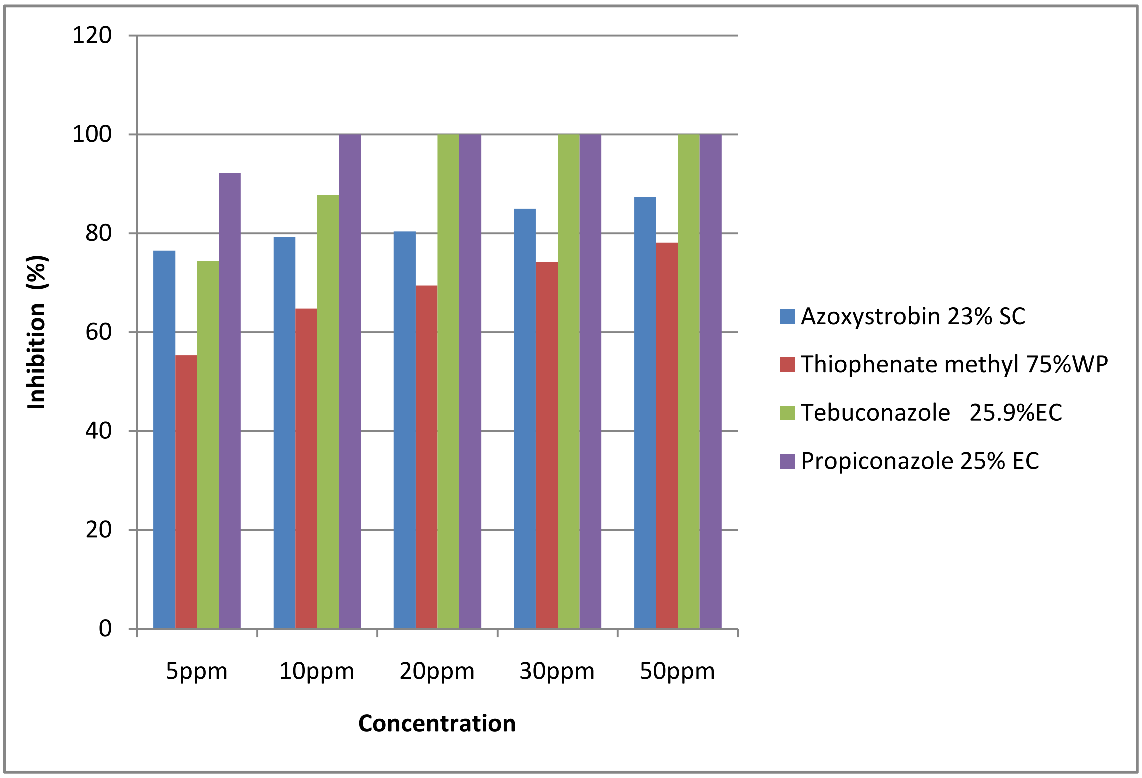


Fig 4.4: Mycelial inhibition of *Exserohilum turcicum* by different Systemic fungicides

4.6.3 *In vitro* evaluation of non systemic fungicides against *E.turcicum*

At 50ppm

At 50ppm concentration Avatar 72% WP showed maximum inhibition of mycelial growth (85.74%) followed by Captan 50% WP (74.44%) which is statistically at par with Curzate72%WP (72.59%). Minimum inhibition (65.37%) was showed by Zineb 75% WP.

At 100ppm

At 100ppm concentration maximum growth inhibition was showed by Avatar 72% WP (93.08%) followed by Captan 50% WP (78.7%). Least mycelial growth inhibition (65.74%) was observed in case of Zineb 75% WP followed by Curzate 72%WP (75.74%).

At 200ppm

At 200ppm concentration cent per cent inhibition was showed by Avatar 72% WP followed by Captan 50% WP (79.44%) which is statistically at par with Curzate 72%WP (77.78%). Zineb 75% WP showed minimum growth inhibition (72.03%).

At 500ppm

At 500 ppm concentration Avatar 72% WP showed cent per cent inhibition of mycelia growth followed by Captan 50% WP (86.11%) and minimum inhibition in mycelial growth (76.31%) was observed in case of Zineb75 %WP.

At 1000ppm

At 1000 ppm concentration Avatar 72% WP showed cent per cent inhibition of mycelia growth followed by Captan 50% WP (86.67%) and minimum inhibition in mycelial growth (76.37%) was observed in case of Zineb75 %WP.

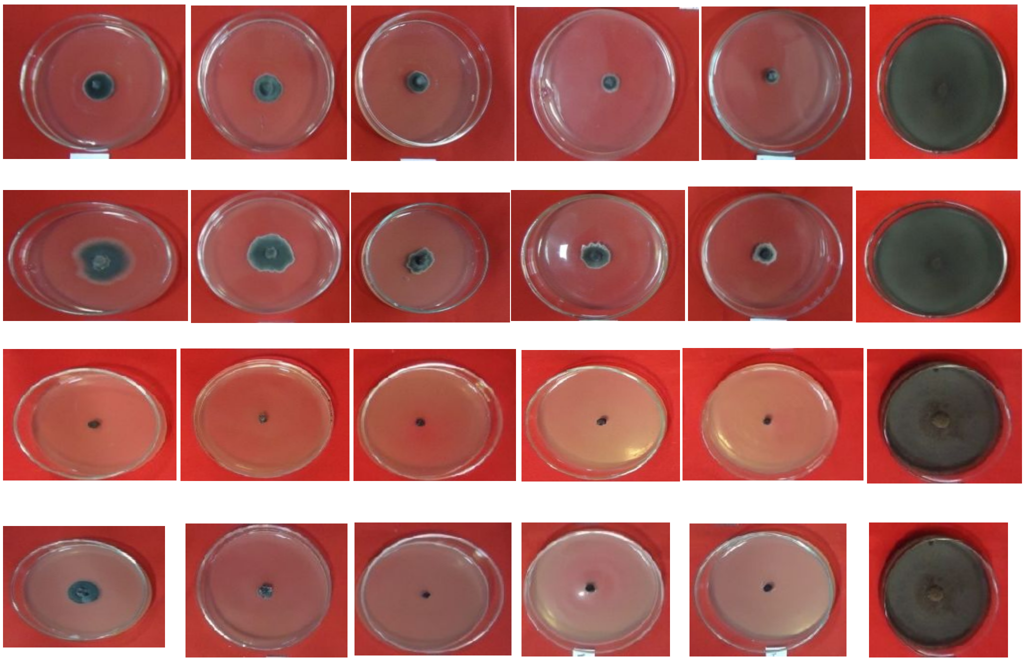
Kumar and Mauriya (2015) evaluated six fungicides (Metalaxyl 72WP, Thiophanate methyl 70 WP, Zineb 75 WP, Propineb 70 WP, Copper oxychloride 50 WP and Mancozeb 63% + carbendazim 12%). Zineb 75 WP @ 0.25 % concentration showed 99.10 % inhibition of mycelial growth of *E.turcicum*

Table 4.5: Mycelial inhibition of *Exserohilum turcicum* by different Non- Systemic fungicide

Non Systemic Fungicides	Per cent inhibition of radial growth *									
	Concentration									
	50ppm		100ppm		200ppm		500ppm		1000ppm	
	G	I	G	I	G	I	G	I	G	I
Zineb 75% WP	31.17	65.37	30.83	65.74	25.17	72.03	21.33	76.31	21.27	76.37
Captan 50% WP	23.00	74.44	19.17	78.70	18.50	79.44	12.50	86.11	12.00	86.67
Avatar (Zineb 68%+Hexaconazole 4%) 72% WP	12.83	85.74	6.23	93.08	0.00	100.00	0.00	100.00	0.00	100.00
Curzate (Cymoxanil 8%+ Mancozeb 64%) 72% WP	24.67	72.59	21.83	75.74	20.00	77.78	16.83	81.30	14.00	84.44
Check	90.00	-	90.00	-	90.00	-	90.00	-	90.00	
	Fungicide(a)		Concentration (b)			Interaction (a×b)				
SEM±	0.32		0.32			0.73				
CD at 5%	0.92		0.92			2.07				
CV	4.05									

*Mean of three replications, G= Colony diameter in mm, I= Per cent inhibition

PLATE 7: Mycelial inhibition of *Exserohilum turcicum* by different systemic fungicides at different concentrations



A

B

C

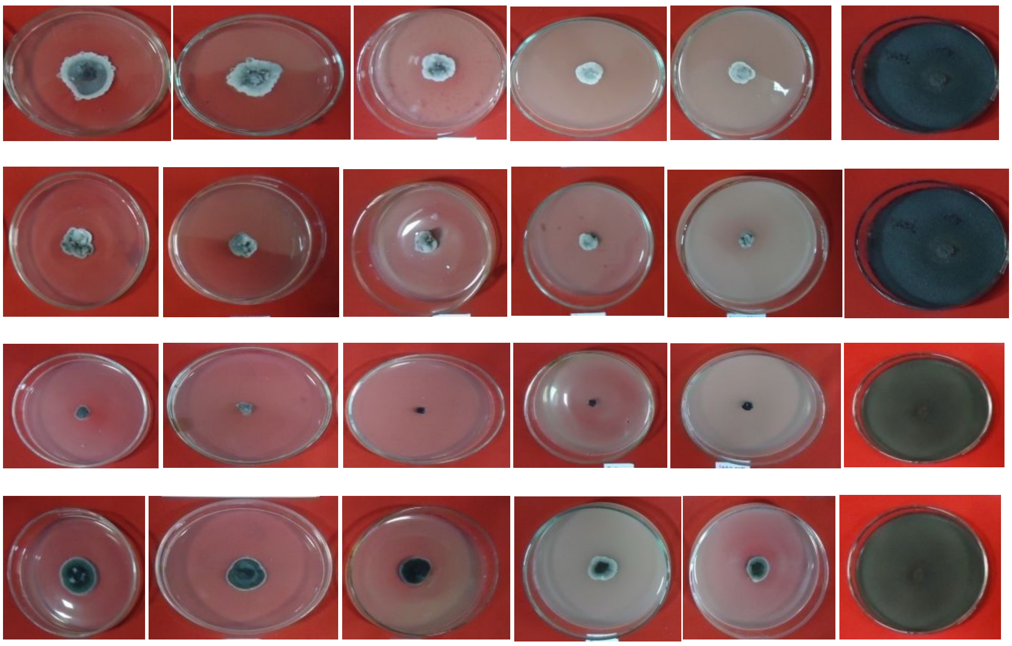
D

5ppm 10ppm 20ppm 30ppm 50ppm Check

A=Azoxystrobin 23%, B= Thiophenate methyl 75%, C= Propiconazole 25%

D= Tebuconazole 25.9%,

PLATE 8: Mycelial inhibition of *Exserohilum turcicum* by different non- systemic fungicides at different concentrations



A

B

C

D

50ppm 100ppm 200ppm 500ppm 1000ppm Check

A= Zineb 75%, B= Captan 50%, C= Zineb(68%)+ Hexaconazole (4%)
D= Cymoxanil(8)+ Mancozeb(64%)

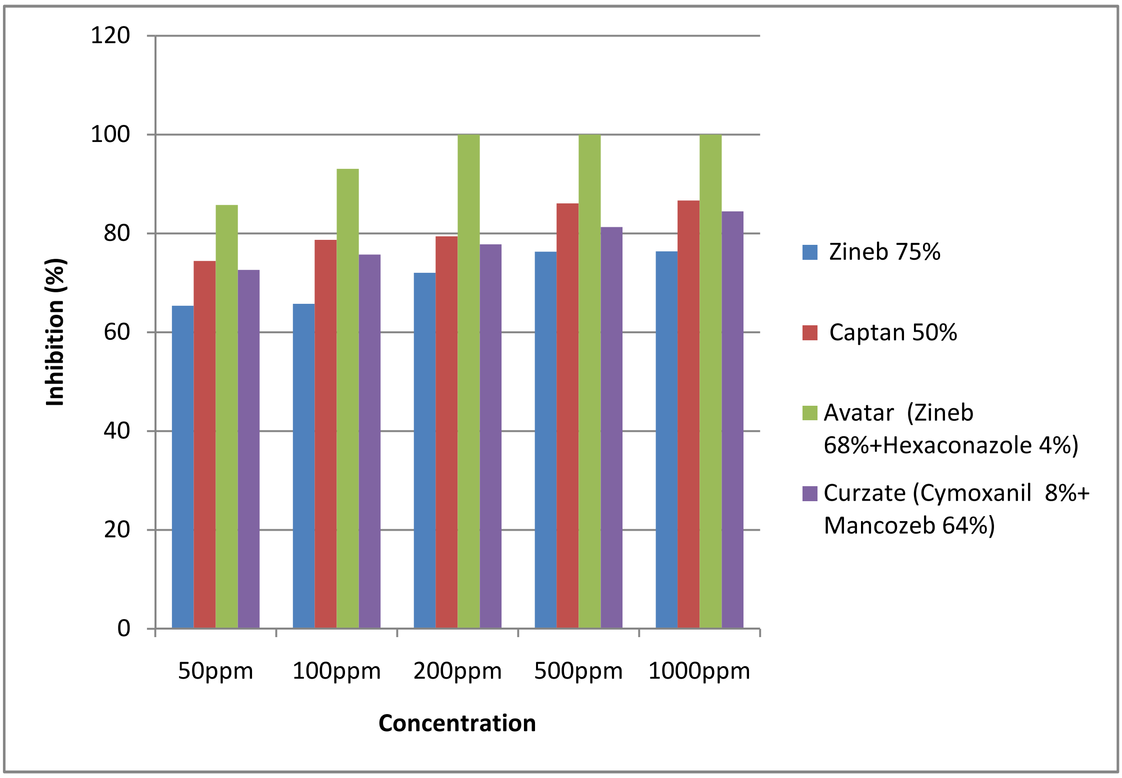


Fig 4.5: Mycelial inhibition of *Exserohilum turcicum* by different Non Systemic fungicides

4.6.4 *In vitro* evaluation of 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*. Ten plant extracts *viz*, Lantana, Eucalyptus, Ginger, Marigold, Turmeric, Heena, Rudraksh, Duranta, Ashok and Aloe vera.

At 5%

At 5% concentration maximum inhibition of mycelial growth (60.56%) was showed by Heena followed by Eucalyptus (54.81%) which was statistically at par with Duranta (53.7%). Minimum inhibition of mycelial growth was shown by Marigold (5%).

At 10%

At 10% concentration Heena showed maximum mycelial growth inhibition (67.59%) followed by Duranta (63.52%). Marigold showed minimum inhibition of mycelial growth (5.74%).

At 15%

At 15% concentration maximum inhibition of mycelial growth (68.94%) was recorded in Heena followed by Duranta (65.56%). Minimum inhibition of mycelia growth was recorded in Aloe vera (11.48%).

At 20%

At 20% concentration Heena showed maximum inhibition of mycelia growth (71.11%) followed by Duranta (67.04%). Aloe vera showed minimum inhibition of mycelial growth (13.7%).

From the data it can be summarised that, Heena was found to be most effective and significantly superior over all other treatments at all concentrations. Next to Heena, Duranta was found significantly effective at all the concentrations. Among the four concentrations 20% was found significantly superior over 15, 10 and 5% concentration in inhibiting the growth of the fungus.

The results obtained are in accordance with **Sharma and Sharma (2011)** who observed that leaf extract of ***Lawsonia inermis*** showed highest inhibitory activity against all tested fungi *viz. Alterneria solani, Drechslera halodes (Helminthosporium halodes), Rhizoctonia solani* (ITCC no. 4574), *Fusarium solani, Curvularia lunata, Dreschlera graminae, Fusarium moniliformae* (ITCC no. 2927), *Aspergillus flavus (Navjot 4 NSt), A. parasiticus var. globosus (MTCC No. 411), Trichophyton rubrum (MTCC 296), Aspergillus fumigatus (MTCC 2550) and Candida albicans (MTCC 227).*

Sharma *et al*. (2012) studied the antifungal activity of *Durenta erecta* L. against some phytopathogenic fungi: *A. flavus, Aspergillus niger, A. fumigatus* and *Penicillium sp*. It was found that the leaf extract of *Durenta erecta* show antifungal activity against all *Aspergillus spp.* but highest inhibition of the mycelial growth was observed against *A. Fumigatus.*

Manu *et al.* (2017) conducted an experiment to assess the antifungal activity of eleven plant extracts at three different concentrations and observed that increasing the concentration of botanicals increases efficacy of plant extract against *E. turcicum.*

Table 4.6: Mycelial inhibition of *Exserohilum turcicum* by different Botanicals

Name of Botanicals	Per cent inhibition of radial growth *							
	Concentration							
	5%		10%		15%		20%	
	G	I	G	I	G	I	G	I
Lantana	67.17	23.37	58.33	35.19	58	35.56	55.00	38.89
Marigold	85.50	5.00	84.83	5.74	75.67	15.93	74.83	16.85
Duranta	41.67	53.70	32.83	63.52	31	65.56	29.67	67.04
Eucalyptus	40.67	54.81	36.67	59.26	35.5	60.56	32.50	63.89
Lawsonia	35.50	60.56	29.17	67.59	27.95	68.94	26.00	71.11
Ginger	75.00	16.67	72.50	19.44	66.5	26.11	55.50	38.33
Ashok	83.33	7.41	74.67	17.04	66.33	26.30	58.50	35.00
Turmeric	50.83	43.52	43.83	51.30	36.67	59.26	35.67	60.37
Aloe vera	85.00	5.56	82.67	8.15	79.67	11.48	77.67	13.70
Rudraksh	66.17	26.48	55.17	38.70	46.17	47.96	37.17	58.70
Check	90.00		90.00		90.00		90.00	

	Botanicals (a)	Concentration (b)	Interaction (a×b)
SEM±	1.05	0.63	2.10
CD at 5%	2.95	1.78	5.91
CV	6.24		

*Mean of three replications, G= Colony diameter in mm, I= Per cent inhibition

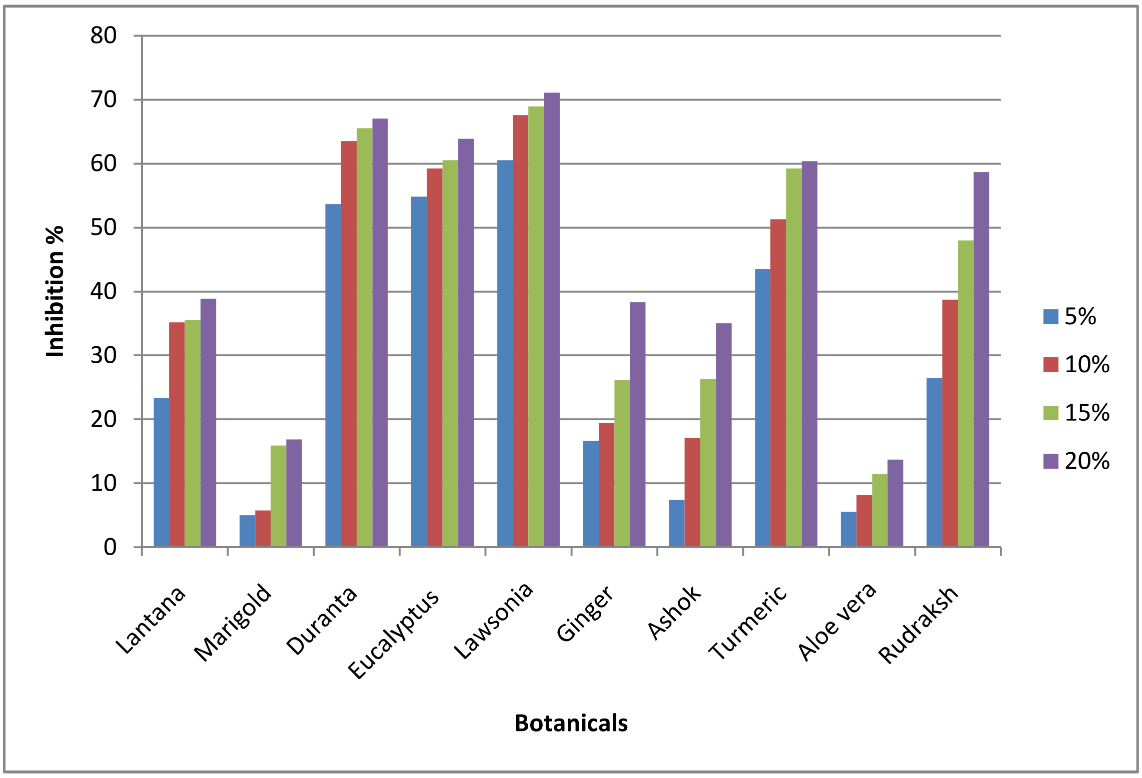


Fig 4.6: Mycelial inhibition of *Exserohilum turcicum* by different botanicals

4.6.5 *In vitro* evaluation of essential oils against *E.turcicum*

At 25pm

At 25ppm concentration maximum inhibition of mycelia growth (70.56%) was recorded in Lemon tulsi followed by Patchouli (67.04%) which was statistically at par with Ginger (66.85%). Minimum inhibition of mycelia growth was recorded in Peppermint (8.52%).

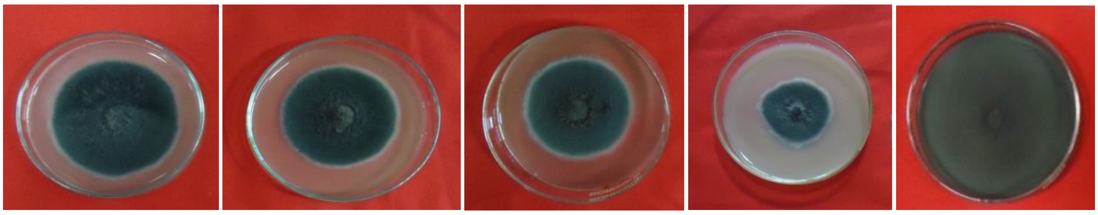
At 50ppm

At 50ppm concentration Turmeric showed maximum inhibition of mycelia growth (71.48%) which is statistically at par with Lemon tulsi (71.3%) followed by Ginger (68.33%) which was statistically at par with Patchouli (67.04%). Peppermint showed minimum inhibition of mycelial growth (8.15%).

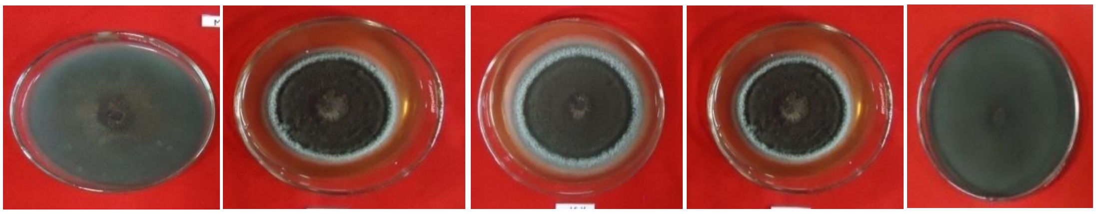
At 100ppm

At 100ppm concentration Lemon tulsi showed maximum inhibition of mycelia growth (74.26%) which is statistically at par with Turmeric (72.22%) followed by

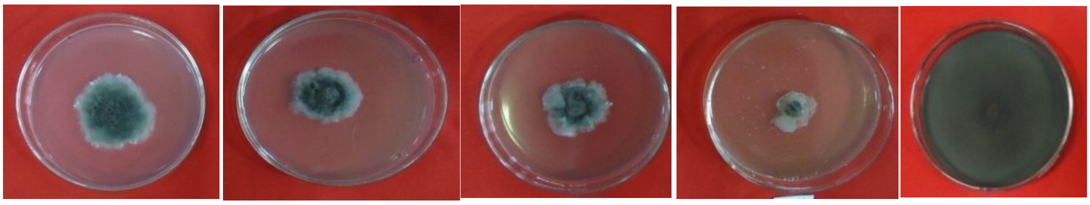
PLATE 9: Mycelial inhibition of *Exserohilum turcicum* by different botanicals at different concentrations



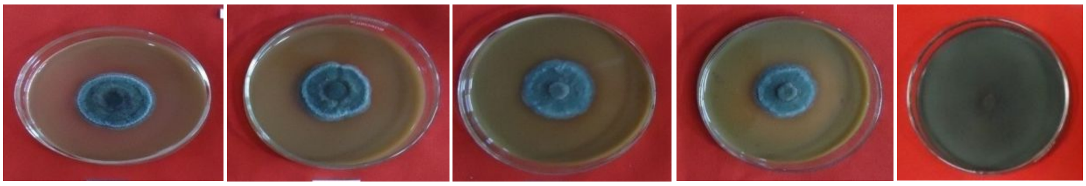
A



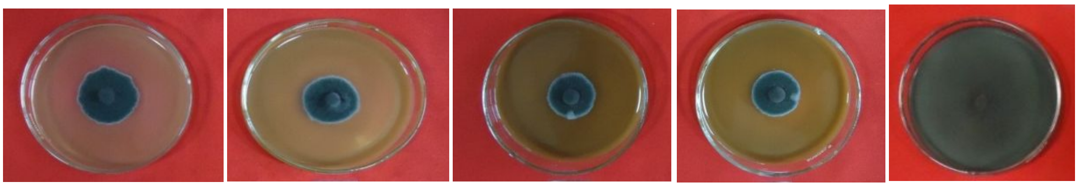
B



C



D

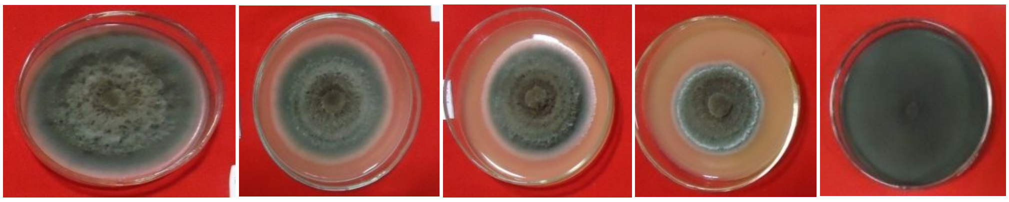


E

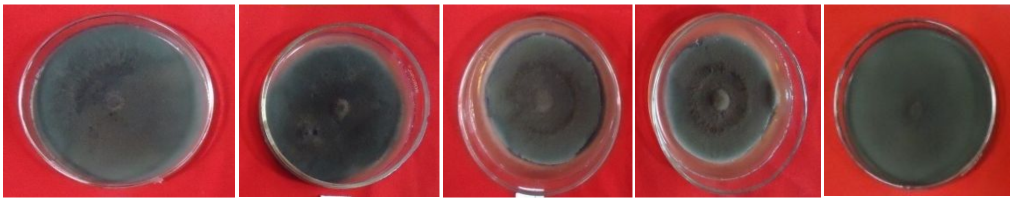
5% 10% 15% 20% Check

A= Lantana, B= Marigold, C= Duranta, D= Eucalyptus, E= Lawsonia

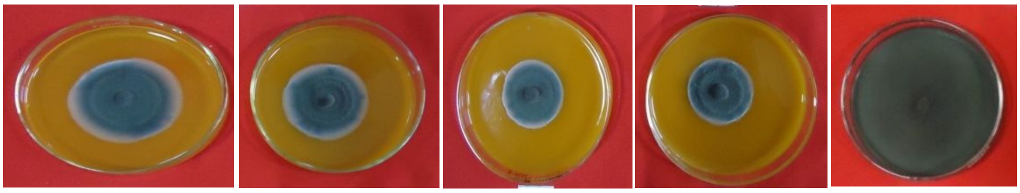
PLATE 10: Mycelial inhibition of *Exserohilum turcicum* by different botanicals at different concentrations



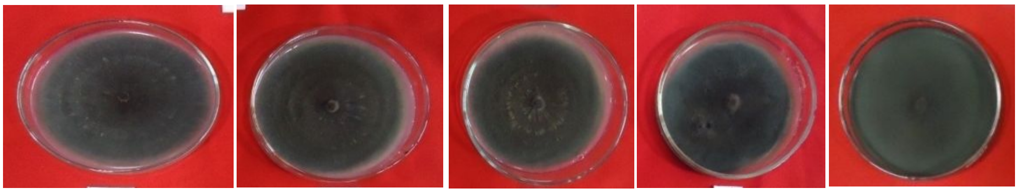
F



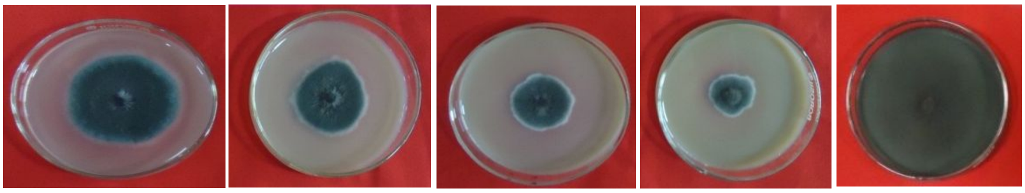
G



H



I



J

5% 0% 15% 20% Check

F= Ginger, G= Ashok, H= Turmeric, I= Aloe vera, J= Rudraksh

Clove (69.63) which was statistically at par with Ginger (68.52%) and Patchouli (67.22%). Minimum inhibition of mycelia growth was recorded in Peppermint (11.11%).

At 250ppm

At 250ppm maximum inhibition of mycelia growth was recorded in (87.96%) in Clove followed by Lemon tulsi (76.67%). Minimum inhibition of mycelial growth (18.15%) was recorded in Eucalyptus.

At 500ppm

At 500ppm concentration cent per cent inhibition was recorded in case of Lemon tulsi followed by Clove (88.89%) which was statistically at par with Citronella (87.78%). Minimum inhibition of mycelia growth was recorded in Peppermint (38.15%).

The result from the study conclude that Lemon tulsi oil is the most effective in inhibiting the mycelial growth followed Clove and Citronella, while Peppermint was least effective in inhibiting mycelial growth of the fungus. With increase in the concentration, efficacy of oils against fungus increases.

These results were in accordance with **Kishore and Pandey (2007**) who observed that clove oil shows antifungal properties and is very effective in inhibiting the growth of various fungal pathogens.

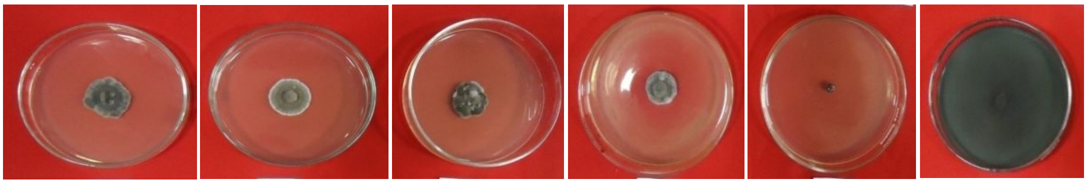
Sharma *et al.* (2016) studied the antifungal effects of essential oils *viz* clove, lemon grasss, mint and eucalyptus against wilt causing fungus, *Fusarium oxysporum* f.sp. *lycopersici* 1322. Complete inhibition of mycelial growth and spore germination was recorded at 125 ppm with IC50 value of 18.2 and 0.3ppm, respectively by clove oil. At higher concentrations lemon grass, mint and eucalyptus essential oils were inhibitory to growth and sporulation of the test fungus.

Table4.7: Mycelial inhibition of *Exserohilum turcicum* by different Essential oils

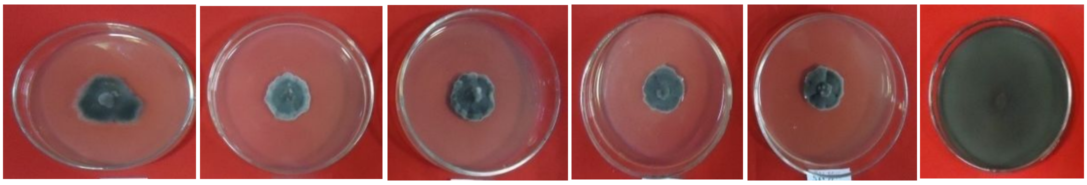
Essential oils	Per cent inhibition of radial growth *									
	Concentrations									
	25ppm		50ppm		100ppm		250ppm		500ppm	
	G	I	G	I	G	I	G	I	G	I
Lemon tulsi	26.50	70.56	25.83	71.30	23.17	74.26	21.00	76.67	0.00	100.00
Patchouli	29.67	67.04	29.67	67.04	29.50	67.22	23.83	73.51	21.50	76.11
Clove	72.00	20.00	62.33	30.74	27.33	69.63	10.83	87.96	10.00	88.89
Ginger	29.83	66.85	28.50	68.33	28.33	68.52	25.33	71.85	24.67	72.59
Citronella	67.50	25.00	67.00	25.56	61.83	31.30	44.67	50.37	11.00	87.78
Palmarosa	73.17	18.70	61.33	31.85	61.67	31.48	56.83	36.85	30.00	66.67
Geranium	75.17	16.48	70.00	22.22	59.33	34.07	59.17	34.26	55.67	38.15
Turmeric	31.83	64.63	25.67	71.48	25.00	72.22	23.67	73.70	22.50	75.00
Peppermint	82.33	8.52	82.67	8.15	80.00	11.11	66.83	25.74	56.67	37.04
Eucalyptus	81.17	9.81	80.00	11.11	79.00	12.22	73.67	18.15	52.23	41.85
Lemon grass	79.33	11.85	72.67	19.26	70.67	21.48	62.00	31.11	40.00	55.56
Check	90.00		90.00		90.00		90.00		90.00	
	Essential oils (a)			Concentrations (b)			Interaction (a×b)			
SEM±	0.98			0.63			2.20			
CD at 5%	2.75			1.77			6.16			
CV	7.51									

*Mean of three replications, G= Colony diameter in mm, I= Per cent inhibition

PLATE 11: Mycelial inhibition of *Exserohilum turcicum* by different essential oils at different concentrations



A



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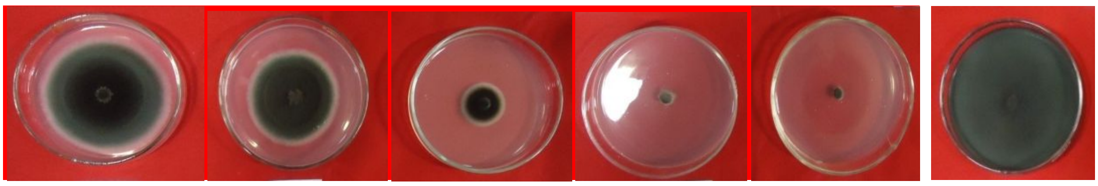
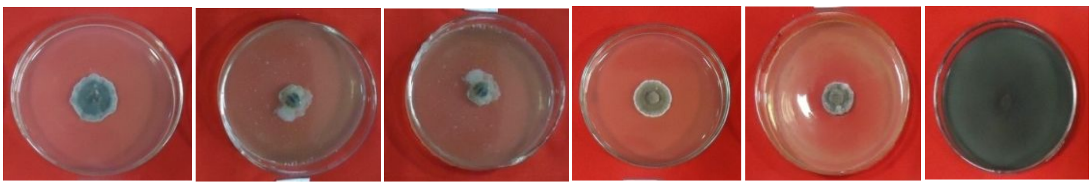
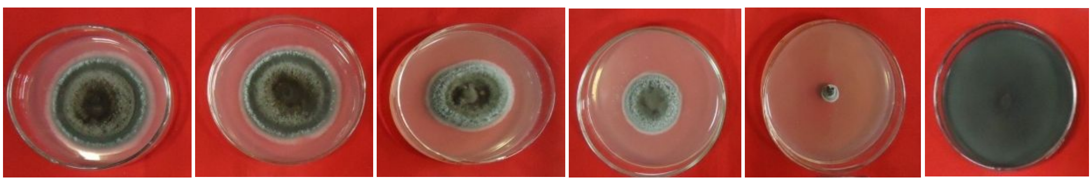


C



D

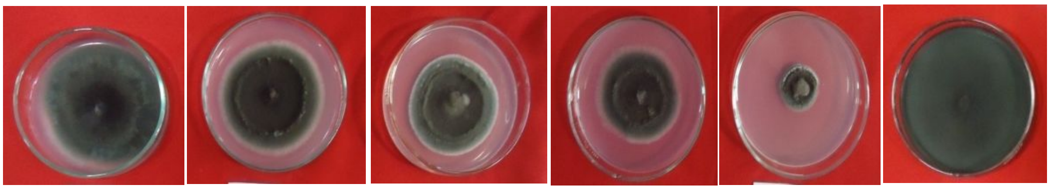


E

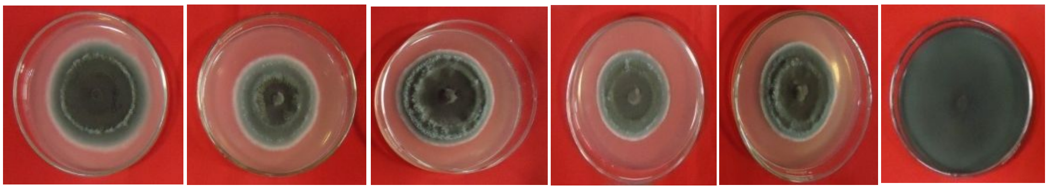
25ppm 50ppm 100ppm 250ppm 500ppm Check

A= Lemon tulsi, B= Patchouli, C= Clove, D= Ginger, E= Citronella

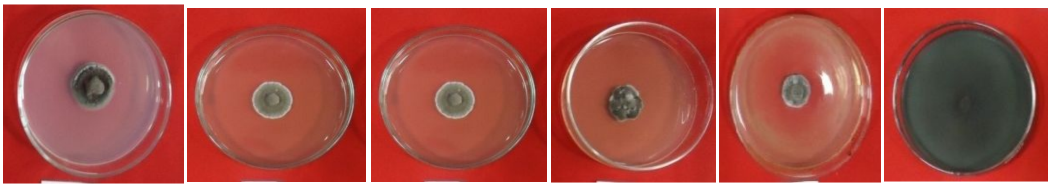
PLATE 12: Mycelial inhibition of *Exserohilum turcicum* by different essential oils at different concentrations



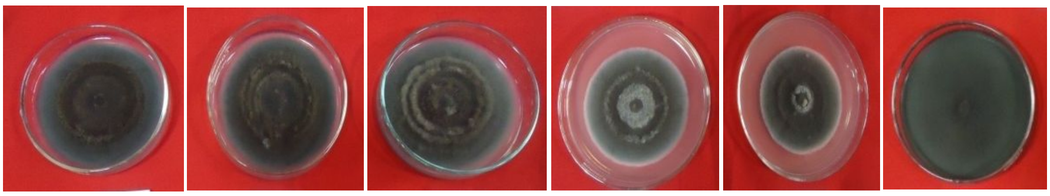
F



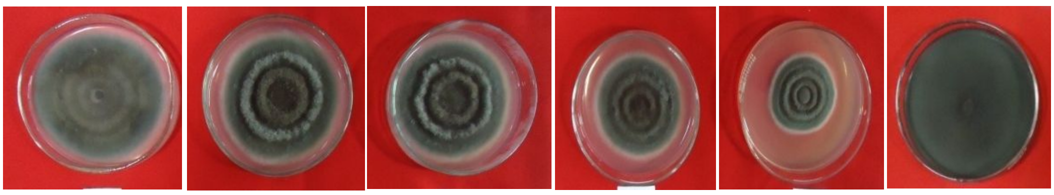
G



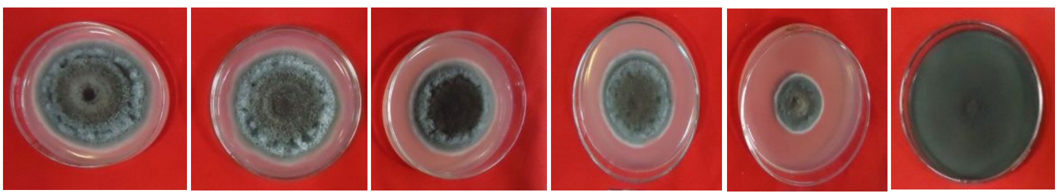
H



I



J



K

25ppm 50ppm 100ppm 250ppm 500ppm Check

F= Palmarosa, G= Geranium, H= Turmeric, I= Peppermint, J= Eucalyptus,

K= Lemon grass

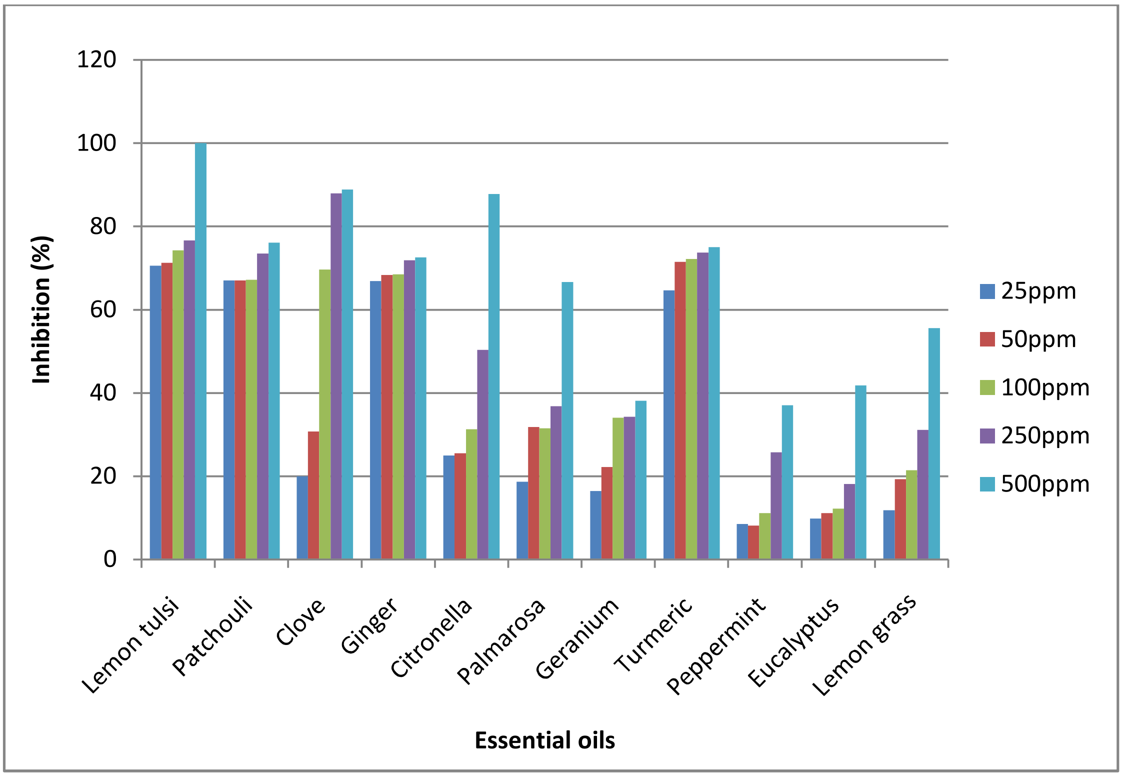


Fig 4.7: Mycelial inhibition of *Exserohilum turcicum* by different Essential oils

4.6.6 *In vitro* testing of antagonism between *T.harzianum* isolates and *E.turcicum* by dual culture method

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 6 isolates of *Trichoderma harzianum* was evaluated against the pathogen *E.turcicum*. In dual culture all the isolates reduced the colony growth of the pathogen. Maximum inhibition of mycelia growth (64.81%) was observed in case of Th which was statistically at par with TCMS-36 (64.44%) followed by PBAT -1 (57.04%). Minimum inhibition of mycelia growth (52.59%) was observed in case of Th-19. The difference in inhibition of mycelial growth indicates the difference in their antagonistic potential for the test pathogen.

Results conclude that Th isolate of Trichoderma is most effective in inhibiting the growth of the pathogen which is statistically at par with TCMS-36, while Th-19 was least effective in inhibiting the growth of the pathogen. These results were in accordance with **Mishra *et al*.** 2018 who observed maximum radial growth inhibition of *Rhizoctonia solani* by TCMS-36 isolate of *Trichoderma harzianum*.

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.

Harlapur *et al.* (2007) observed Maximum mean per cent inhibition of mycelial growth of *E.turcicum* was recorded in *Trichoderma harzianum* (65.17%) followed by *T. viridae (*56.95%).

Table 4.8: Mycelial inhibition of *Exserohilum turcicum* by different Trichoderma isolates

Sl. No.	Isolates of *Trichoderma harzianum*	Colony diameter (mm)	Inhibition (%)
1	Th	31.67	64.81
2	Th-17	41.67	53.70
3	Th-19	42.67	52.59
4	PBAT-1	38.67	57.04
5	TCMS -36	32.00	64.44
6	TCMS -39	40.69	54.81
7	Check	90.00	
	SEM±	1.37	
	CD at 5%	4.17	
	CV	5.26	

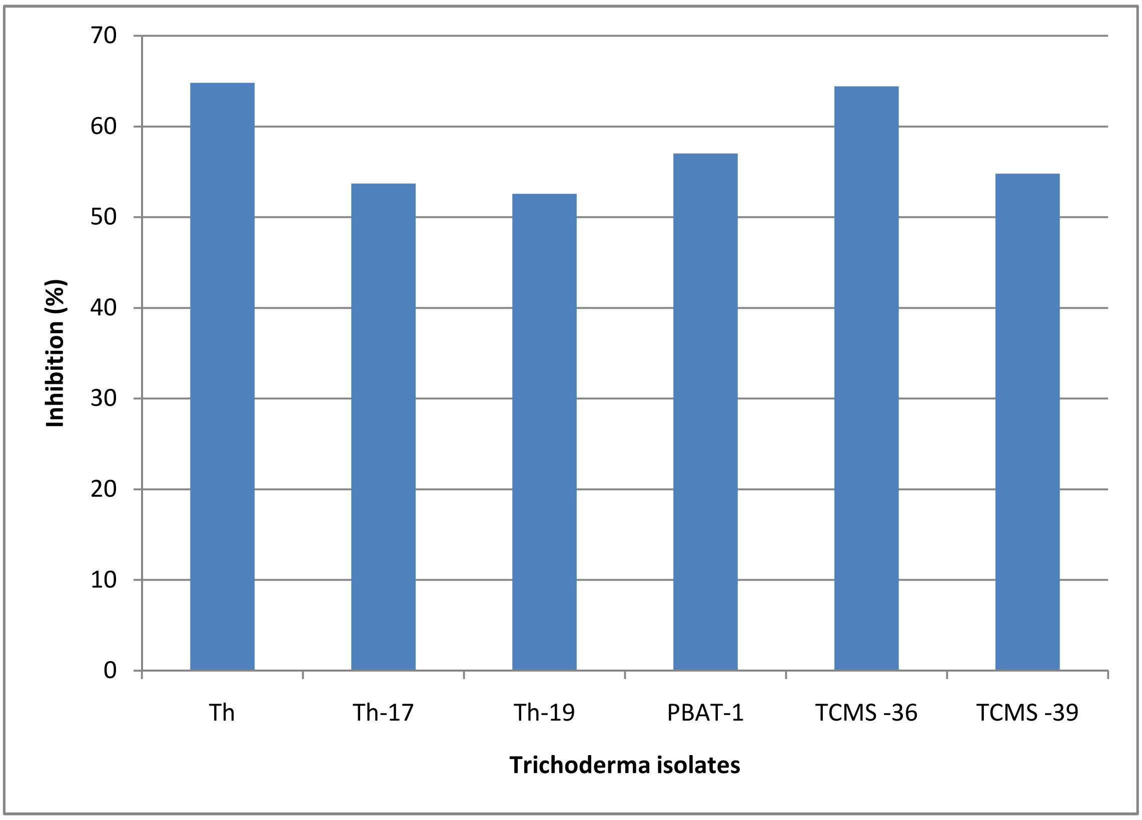


Fig 4.8: Mycelial inhibition of *Exserohilum turcicum* by different isolates of *Trichoderma harzianum*

4.6.7 *In vitro* evaluation of cow urine concentrations against *E.turcicum*

Five concentrations of cow urine 1, 5, 10, 20, 30, 40 and 50% were tested against *E.turcicum* and 10, 20, 30, 40 and 50% concentrations of cow urine showed cent per cent inhibition of mycelial growth. Minimum per cent inhibiton (35.56%) was observed at 1% concentration followed by 5% concentration of cow urine (55.56%).

Jandaik *et al.* (2015) conducted an experiment to determine antifungal activity of three different concentrations (5, 10, and 15%) of cow urine against three fungal pathogens (*Fusarium oxysporum, Rhizoctonia solani, and Sclerotium rolfsii*) and among all these concentrations cow urine at 15% concentration was most effective in inhibiting the fungal growth.

Gotora *et al.* (2014) studied antifungal effect of cow urine at different concentration (100u/ml, 200ul/m, 300ul/ml and 500ul/ml) to control the growth of *Fusarium lateritium*, causing Fusarium bark disease in coffee. The undiluted cow urine was most effective in inhibiting fungal growth.

Table 4.9: Mycelial inhibition of *Exserohilum turcicum* by different concentrations of cow urine

Sl No.	Concentration (%)	Colony diameter (mm)	Inhibition (%)
1	1	58	35.56
2	5	40	55.56
3	10	0	100
4	20	0	100
5	30	0	100
6	40	0	100
7	50	0	100
	Check	90	-
	SEM±	1.02	
	CD at 5%	3.06	
	CV	7.52	

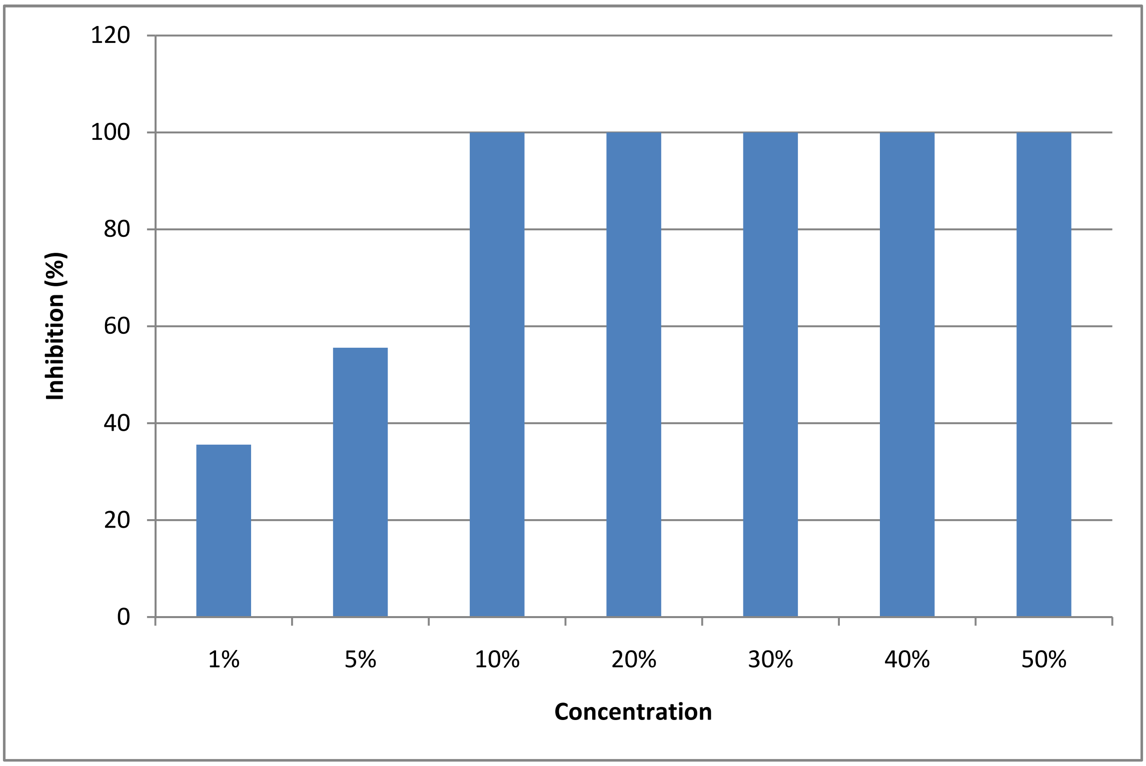
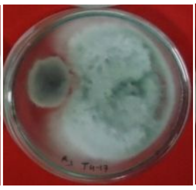
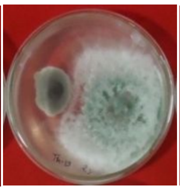


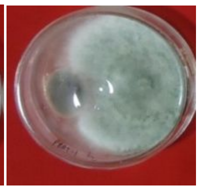
Fig 4.9: Mycelial inhibition of *Exserohilum turcicum* by different concentrations of cow urine

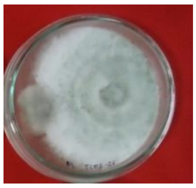
PLATE 13: Mycelial inhibition of *Exserohilum turcicum* by different *Trichoderma* isolates

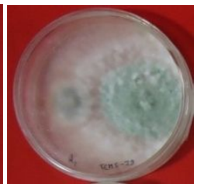

TH


TH-17


TH-19

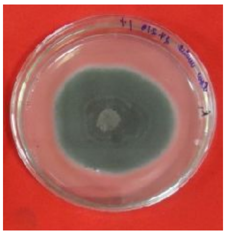

PBAT-1


TCMS-36


TCMS-39


Check

PLATE 14: Mycelial inhibition of *Exserohilum turcicum* by different concentrations of cow urine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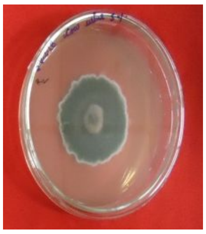
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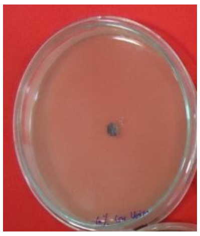
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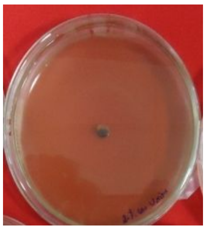
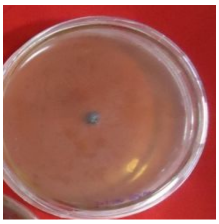

15%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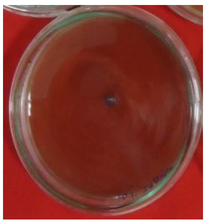
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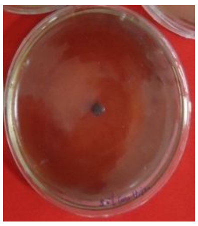
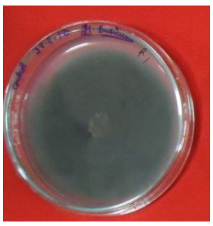

50%


Check

4.7 GLASS HOUSE EXPERIMENT

4.7.1 Evaluation of different methods of inoculation of *Exserohilum turcicum* on two varieties of maize

Maize plants in the pots under glasshouse conditions were inoculated by three different methods of inoculation: spraying, cotton swab and stem injection were used to inoculate the pathogen to identify the most suitable methods of inoculation. Observations were taken at different stages of development: Knee high stage (DVS 1), Tasseling stage (DVS 2) and Silking stage (DVS 3).

Disease incidence and disease severity was recorded for each treatment and following observations were made:

Variety: Amar

Spray

Maximum disease incidence (43.36%) was observed at silking stage followed by tasseling stage (34.36%). Minimum disease incidence (27.90%) was observed at knee high stage. Minimum disease severity (24.00%) was observed at knee high stage followed by tasseling stage (30.33%). Maximum disease severity (38.00%) was observed in case of silking stage.

Stem injection

Maximum disease incidence (28.30%) was observed at silking stage followed by tasseling stage (24.37%). Minimum disease incidence (18.67%) was observed at knee high stage. Minimum disease severity (17.67%) was observed at knee high stage followed by tasseling stage (21.67%). Maximum disease severity (27.00%) was observed in case of silking stage.

Cotton swab

Maximum disease incidence (34.23%) was observed at silking stage followed by tasseling stage (30.97%). Minimum disease incidence (25.72%) was observed at knee high stage. Minimum disease severity (22.00%) was observed at knee high stage followed by tasseling stage (26.33%). Maximum disease severity (29.00%) was observed in case of silking stage.

Table 4.10: Effect of different inoculation methods on Disease Incidence and Disease Severity on maize variety Amar

Amar						
Inoculation Methods	**Disease Incidence**			**Disease Severity**		
	Developmental Stages			**Developmental Stages**		
	DVS1	**DVS2**	**DVS3**	**DVS1**	**DVS2**	**DVS3**
Spray	27.90	34.36	43.36	24.00	30.33	38.00
Stem Injection	18.67	24.37	28.30	17.67	21.67	27.00
Cotton swab	25.72	30.97	34.23	22.00	26.33	29.00
Check	17.10	22.10	25.20	14.33	17.33	22.00
	Inoculation methods (a)	Developmental stages(b)	Interaction (a×b)	Inoculation methods (a)	Developmental stages(b)	Interaction (a×b)
SEM±	0.62	0.53	1.08	0.78	0.68	1.35
CD at 5%	1.81	1.57	3.14	2.28	1.98	3.95
CV	6.74			9.71		

DVS 1= Knee High stage, DVS 2 = Tasseling stage, DVS 3= Silking stage

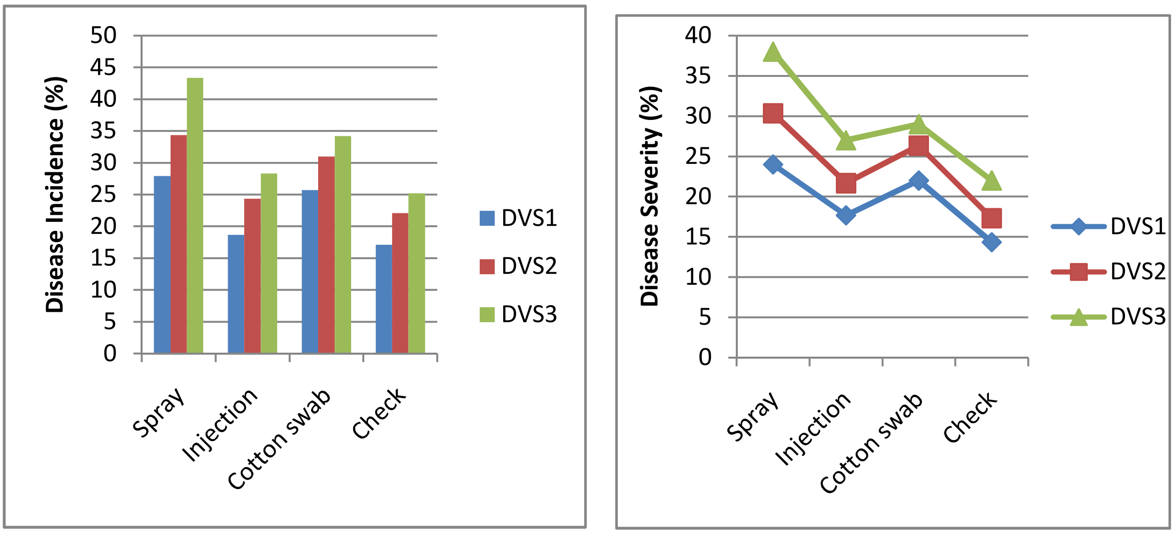


Fig 4.10: Effect of different inoculation methods on Disease Incidence and Disease Severity on maize variety Amar

Variety: Surya

Spray

Maximum disease incidence (54.30 %) was observed at silking stage followed by tasseling stage (45.66%). Minimum disease incidence (36.00%) was observed at knee high stage. Minimum disease severity (34.67%) was observed at knee high stage followed by tasseling stage (42.33%). Maximum disease severity (49.00%) was observed in case of silking stage.

Stem injection

Maximum disease incidence (44.63%) was observed at silking stage followed by tasseling stage (37.23%). Minimum disease incidence (34.33%) was observed at knee high stage. Minimum disease severity (32.67%) was observed at knee high stage followed by tasseling stage (37.33%). Maximum disease severity (42.67%) was observed in case of silking stage.

Cotton swab

Maximum disease incidence (40.22%) was observed at silking stage followed by tasseling stage (37.85%). Minimum disease incidence (32.99%) was observed at knee high stage. Minimum disease severity (31.33%) was observed at knee high stage followed by tasseling stage (36.33%). Maximum disease severity (38.00%) was observed in case of silking stage.

The results conclude that spraying of the spore suspension is most effective method of inoculation as compared to stem injection and cotton swab method. The results obtained were in accordance with **Frederiksen *et al.* 1975** who reported that spore suspensions of *E. turcicum* sprayed on leaves to be a successful method of inoculation.

Silking stage showed maximum disease severity as compared to tasseling and knee high stage. The results obtained were in accordance with the results concluded by **Chenulu and Hora, (1962); Ullstrup, (1966)** who observed that from tasseling and six to eight weeks after silking maximum disease severity occur leading to heavy yield losses.

Table 4.11: Effect of different inoculation methods on Disease incidence and Disease severity on maize variety Surya

Surya						
Inoculation Methods	**Disease Incidence (%)**			**Disease Severity (%)**		
	Developmental Stages			**Developmental Stages**		
	DVS1	DVS2	DVS3	DVS1	DVS2	DVS3
Spray	36.00	45.66	54.30	34.67	42.33	49.00
Injection	34.33	37.23	44.63	32.67	37.33	42.67
Cotton swab	32.99	37.85	40.22	31.33	36.33	38.00
Check	29.96	37.70	38.10	27.00	35.33	37.33
	Inoculation methods (a)	Developmental stages(b)	Interaction (a×b)	Inoculation methods (a)	Develop mental stages (b)	Interac tion (a×b)
SEM±	0.65	0.56	1.13	0.65	0.57	1.13
CD at 5%	1.90	1.64	3.29	1.90	1.64	3.29
CV	5.09			5.29		

DVS 1= Knee High stage, DVS 2 = Tasseling stage, DVS 3= Silking stage

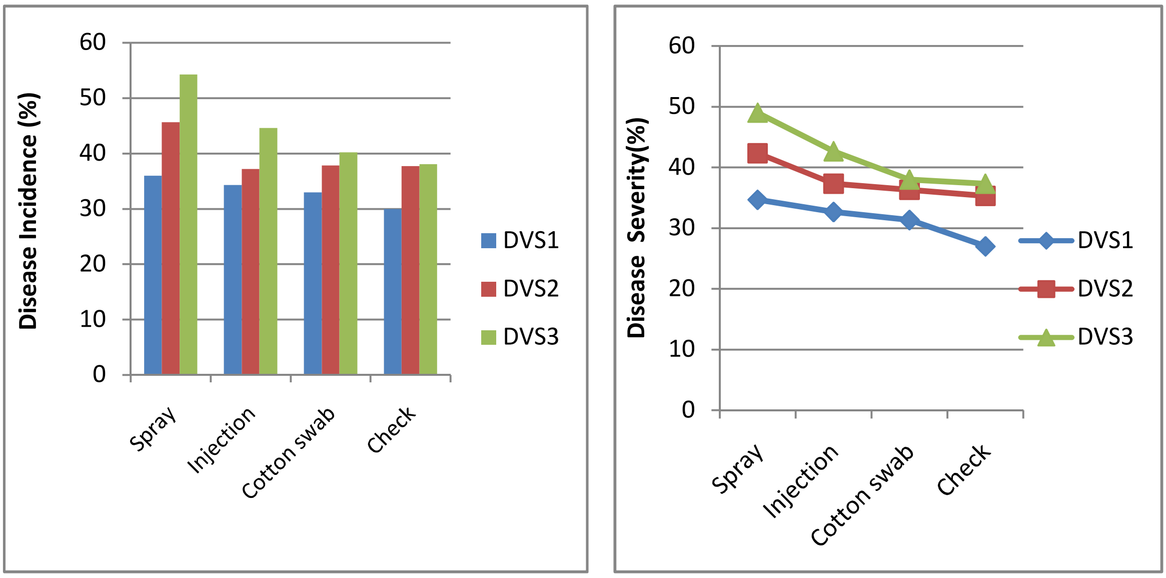


Fig 4.11: Effect of different inoculation methods on Disease incidence and Disease severity on maize variety Surya

4.7.2 Evaluation of fungicides against Northern leaf blight of maize on Amar and Surya variety of maize

Glasshouse experiments were conducted for screening of four fungicides *viz*., Propiconazole 25%EC, Tebuconazole 25.9%, Avtar 72%WP and Captan 50%WP against the pathogen. The data given in Table recorded that all treatments were significantly superior in reducing the disease over check.

Variety: Amar

Ten days after the first spray, minimum disease incidence (18.51%) and disease severity (17.67%) was observed in the plants treated with Propiconazole followed by disease incidence (23.33%) and disease severity (21.67%) in the pots sprayed with Tebuconazole. Maximum disease incidence (38.25%) and disease severity (30.00%) was recorded in check pots followed disease incidence (28.76%) and disease severity (23.67%) in the pots sprayed with Captan.

The results reveal that ten days after the second spray minimum disease incidence (23.33%) and disease severity (20.00%) was recorded from the pots sprayed with Propiconazole followed by disease incidence (28.50%) and disease severity (24.00%)in the pots sprayed with Tebuconazole. Maximum disease incidence (46.33%) and disease severity (37.00%) was recorded in check pots followed by the disease incidence (35.28%) and disease severity in pots sprayed with Captan.

Variety: Surya

The results revealed that ten days after the first spray minimum disease incidence (27.15%) and disease severity (25.33%) was recorded in pots sprayed with Propiconazole followed by disease incidence (27%) and disease severity (26.33%) in pots sprayed with Tebuconazole. Maximum disease incidence (45.33%) and disease severity (40.33) was recorded in check pots followed by disease incidence (37.48%) and disease severity (32.00%) in pots sprayed with Captan.

Ten days after the second spray minimum disease incidence (27.67%) and disease severity (26.67%) was recorded in pots sprayed with Propiconazole followed by disease incidence (30.48%) and disease severity (27.67%) in pots sprayed with

Tebuconazole. Maximum disease incidence (53.67%) and disease severity (47.67%) was recorded in check pots followed by disease incidence (38.37%) and disease severity (35.33%) in pots sprayed with Captan.

Wani *et al.* (2017) observed that two foliar sprays with systemic fungicide, Propiconazole 25 EC @ 0.1 per cent reduced the diseased intensity to 6.11 per cent and showed increase in the grain yield to 52.25 q/ha.

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 the most effective, followed by carbendazim, zineb, thiophanate methyl and lastly copper oxychloride.

Table 4.12: Effect of fungicides on Disease Incidence and Disease Severity of Northern leaf blight of Maize on Amar variety under glasshouse condition

Sl No.	Treatments	Disease incidence (%)		Disease severity (%)	
		10 days after first spray	10 days after second spray	10 days after first spray	10 days after second spray
1	Propiconazole 25%EC	18.51	23.77	17.67	20.00
2	Tebuconazole 25.9% EC	23.33	28.50	21.67	24.00
3	Zineb(68%)+ Hexaconazole (4%)	25.12	30.1	22.33	26.00
4	Captan 50% WP	28.76	35.28	23.67	27.00
	Check	38.25	46.33	30.00	37.00
	SEM±	1.36	0.99	0.86	0.73
	CD at 5%	4.31	3.14	2.69	2.30
	CV	8.84	2.27	4.23	4.71

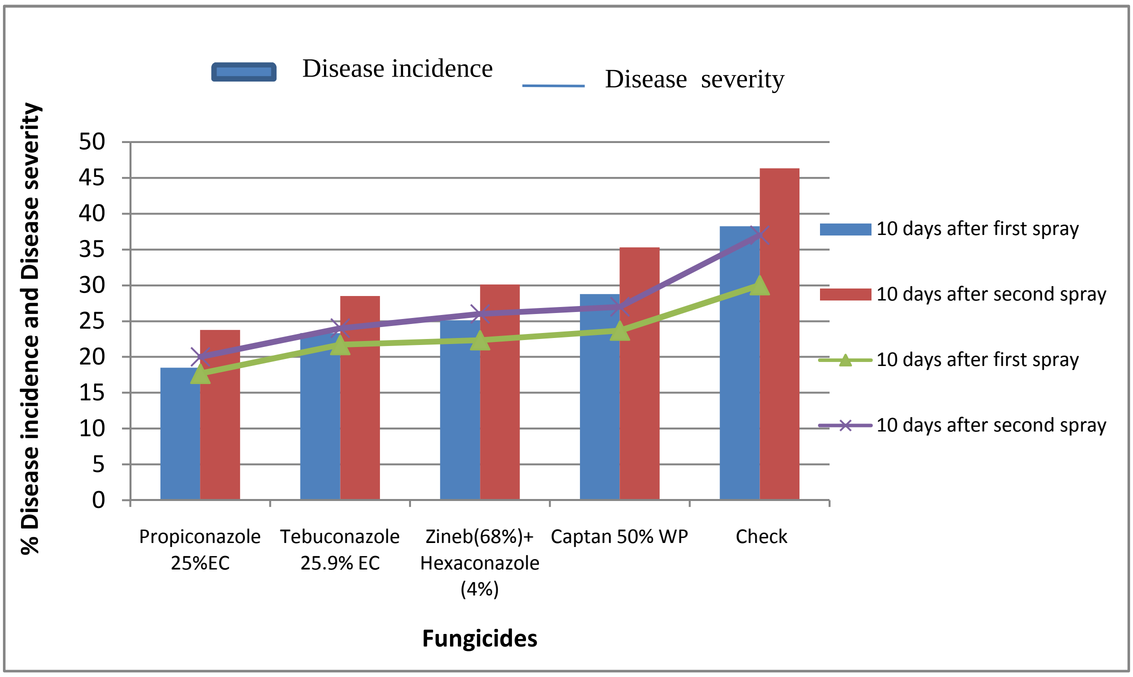


Fig 4.12: Effect of fungicides on Disease Incidence and Disease Severity of Northern leaf blight of Maize on Amar variety under glasshouse condition

Table 4.13: Effect of fungicides on Disease Incidence and Disease Severity of Northern leaf blight of Maize on Surya variety under glasshouse condition

Sl No.	Treatments	Disease incidence (%)		Disease severity (%)	
		10 days after first spray	10 days after second spray	10 days after first spray	10 days after second spray
1	Propiconazole 25%EC	27.15	27.67	25.33	26.67
2	Tebuconazole 25.9% EC	27.00	30.48	26.33	27.67
3	Zineb(68%)+ Hexaconazole (4%)	32.67	35.74	28.00	30.00
4	Captan 50% WP	37.48	38.37	32.00	35.33
	Check	45.33	53.67	40.33	47.67
	SEM±	1.43	1.39	1.06	1.11
	CD at 5%	4.53	4.36	3.35	3..48
	CV	7.34	6.45	6.06	5.72

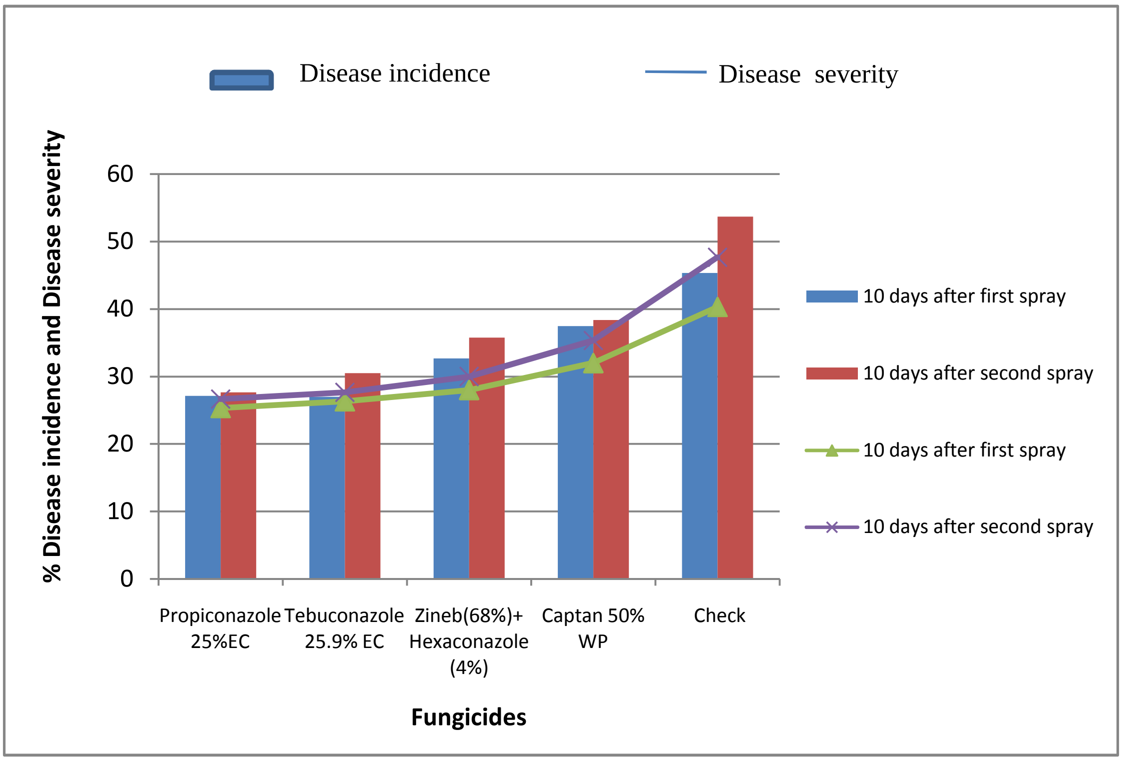


Fig 4.13: Effect of fungicides on Disease Incidence and Disease Severity of Northern leaf blight of Maize on Surya variety under glasshouse condition

4.7.3 Evaluation of Botanicals against Northern leaf blight of maize on Amar and Surya variety of maize

Variety: Amar

Ten days after first spray minimum disease incidence (24.12%) and disease severity (22.67%) was observed in pots sprayed with Heena followed by disease incidence (26.33%) and disease severity (24.33%) in the pots sprayed with Duranta. Maximum disease incidence (38.25 %) and disease severity (30.00%) was observed in check pots followed disease incidence (30.08%) and disease severity (27.33%) in pots sprayed with Turmeric.

The results reveal that ten days after the second spray minimum disease incidence (26.67%) and disease severity (25.00%) was observed in pots sprayed with Heena followed by disease incidence (29.50% and disease severity (28%) in the pots sprayed with Duranta. Maximum disease incidence (46.33%) and disease severity (37.00%) was observed in check pots followed by disease incidence (34.51%) and disease severity (32.33%) in pots sprayed with Turmeric.

Variety: Surya

Ten days after first spray, minimum disease incidence (32.48%) and disease severity (29.67%) was observed in pots sprayed with Heena followed by disease incidence (33.00%) and disease severity (30.33%) in pots sprayed with Duranta. Highest disease incidence (45.33%) and disease severity (40.33%) was recorded in check pots followed by disease incidence (37.54%) and disease severity (34.33%) in pots sprayed with Turmeric

After ten days of second spray, minimum disease incidence (34.13%) and disease severity (32.67%) was observed in pots sprayed with Heena followed by disease incidence (35.00%) and disease severity (34.33%) in pots sprayed with Duranta. Highest disease incidence (53.67%) and disease severity (47.67%) was recorded in check pots followed by disease incidence (44.11%) and disease severity (40.33%) in pots sprayed with Turmeric.

The results conclude that Heena (*Lawsonia inermis*) is effective in reducing disease incidence and disease severity of northern leaf blight of maize when sprayed to maize plants. These results are in accordance with **Natrajan and Lalithakumari, (1987)** who observed that *in vivo* spraying of leaf extract of *Lawsonia inermis* on rice leaves gives better control of *Dreschlera oryzae* than seed treatment.

Sharma and Sharma (2011) evaluated leaf extracts of *Lawsonia inermis* Linn. and observed that Acetone extract of *L. inermis* leaves showed highest inhibitory activity against all tested fungi. The inhibitory effect was significant and better than the synthetic fungicides used, as most of the strains showed resistance against fluconazole and amphotericin B.

Table 4.14: Effect of botanicals on Disease Incidence and Disease Severity of Northern leaf blight of Maize on Amar variety under glasshouse condition

Sl No.	Treatments	Disease incidence (%)		Disease severity (%)	
		10 days after first spray	10 days after second spray	10 days after first spray	10 days after second spray
1	Lawsonia	24.12	26.67	22.67	25.00
2	Duranta	26.33	29.50	24.33	28.00
3	Eucalyptus	27.48	31.16	25.00	30.00
4	Turmeric	30.08	34.51	27.33	32.33
	Check	38.25	46.33	30.00	37.00
	SEM±	1.12	1.17	0.86	0.69
	CD at 5%	3.53	3.69	2.69	2.20
	CV	6.64	6.05	5.73	3.97

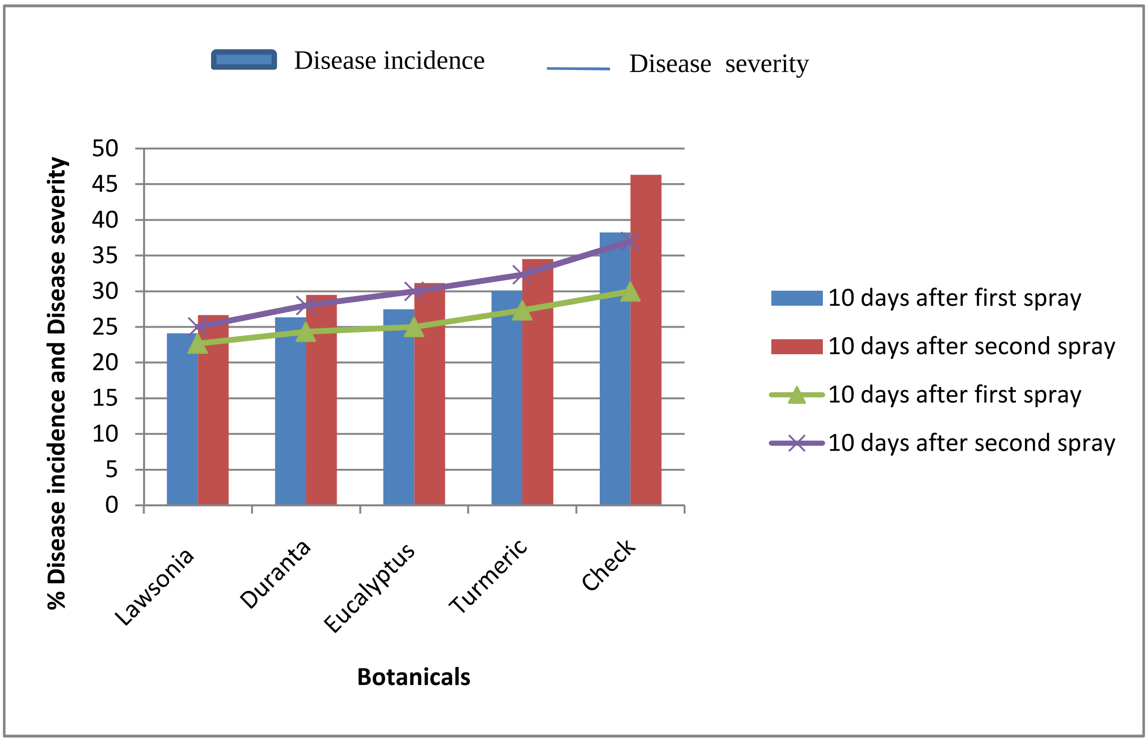


Fig 4.14: Effect of botanicals on Disease Incidence and Disease Severity of Northern leaf blight of Maize on Amar variety under glasshouse condition

Table 4.15: Effect of botanicals on Disease Incidence and Disease Severity of Northern leaf blight of Maize on Surya variety under glasshouse condition

Sl No.	Treatments	Disease incidence (%)		Disease severity (%)	
		10 days after first spray	10 days after second spray	10 days after first spray	10 days after second spray
1	Lawsonia	32.48	34.13	29.67	32.67
2	Duranta	33.00	35.00	30.33	34.33
3	Eucalyptus	35.15	38.72	33.33	37.00
4	Turmeric	37.54	44.11	34.33	40.33
	Check	45.33	53.67	40.33	47.67
	SEM±	1.44	1.38	1.12	0.83
	CD at 5%	4.53	4.34	3.51	2.61
	CV	6.79	5.80	5.71	3.74

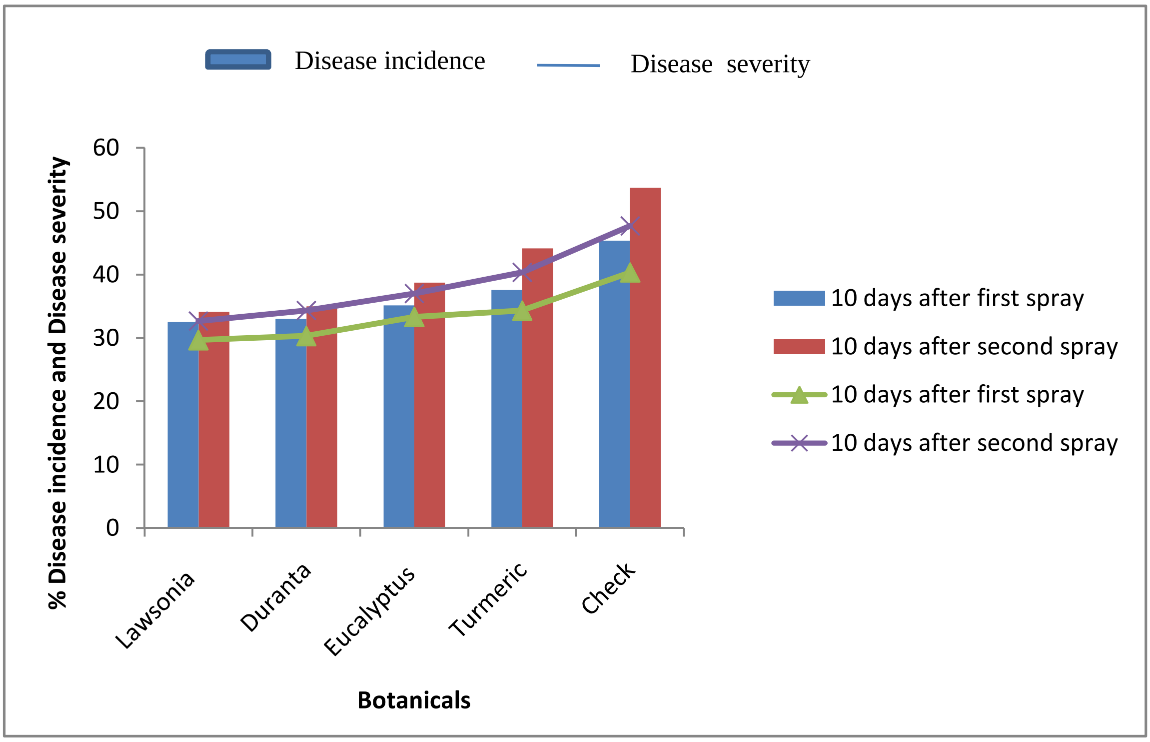


Fig 4.15: Effect of botanicals on Disease Incidence and Disease Severity of Northern leaf blight of Maize on Surya variety under glasshouse condition

4.7.4 Evaluation of Essential oils against Northern leaf blight of maize on Amar and Surya variety of maize

Essential oils which showed higher per cent inhibition of *E .turcicum* under lab conditions were tested under glasshouse conditions and following results were obtained:

Variety: Amar

Ten days after the first spray, minimum disease incidence (19.28%) and disease severity (18.33%) was recorded in pots sprayed with Lemon tulsi followed disease incidence (21.00%) and disease severity (20.33%) in pots sprayed with Clove oil. Maximum disease incidence (38.26%) and disease severity (30.00%) was observed in check pots followed by disease incidence (27.00%) and disease severity (25.00%) in pots sprayed with Patchouli.

The results revealed that ten days after the second spray, minimum disease incidence (24.17%) and disease severity (21.00%) was recorded in pots sprayed with Lemon tulsi followed disease incidence (27.07%) and disease severity (24.33%) in pots sprayed with Clove oil. Maximum disease incidence (46.33%) and disease severity (37.00%) was observed in check pots followed by disease incidence (38.42%) and disease severity (29.00%) in pots sprayed with Patchouli.

Variety: Surya

Ten days after the first spray, minimum disease incidence (29.67%) and disease severity (26.00%) was recorded in pots sprayed with Lemon tulsi followed disease incidence (34.27%) and disease severity (28.00%) in pots sprayed with Clove oil. Maximum disease incidence (45.33%) and disease severity (40.33%) was observed in check pots followed by disease incidence (38.33%) and disease severity (31.67%) in pots sprayed with Patchouli.

Ten days after the second spray, minimum disease incidence (36.05%) and disease severity (28.33%) was recorded in pots sprayed with Lemon tulsi followed disease incidence (38.42%) and disease severity (30.00%) in pots sprayed with Clove oil. Maximum disease incidence (53.67%) and disease severity (47.67%) %) was observed in check pots followed by disease incidence (42.88%) and disease severity (35.33%) in pots sprayed with Patchouli.

The results conclude that Lemon tulsi oil is effective in reducing the disease incidence and disease severity followed by clove oil. These results could be supported by studies made by **Kishore and Pandey (2007**) who tested clove oil, cinnamon oil, and five essential oil components (citral, eugenol, geraniol, limonene, and linalool) for growth inhibition of 14 phytopathogenic fungi. Clove oil (1% vol/vol) applied as a foliar spray 10 min before inoculation of *Phaeoisariopsis personata* reduced the severity of late leaf spot of peanut up to 58% when challenge inoculated with 10^4 conidia/ml.

Sharma *et al*. (2016) studied the antifungal effects of essential oils *viz* clove, lemon grasss, mint and eucalyptus were evaluated against wilt causing fungus, *Fusarium oxysporum* f.spp. *lycopersici* 1322. In pot 5% aqueous emulsion of clove oil controlled *Fusarium oxysporum* f.sp. *lycopersici* 1322 infection on tomato plants. This study demonstrated potential antifungal activity of clove oil that could be used a biofungicide for preventive and therapeutic effect.

Table 4.16: Effect of Essential oils on Disease Incidence and Disease Severity of Northern leaf blight of Maize on Amar variety under glasshouse condition

Sl No.	Treatments	Disease incidence (%)		Disease severity (%)	
		10 days after first spray	10 days after second spray	10 days after first spray	10 days after second spray
1	Lemon tulsi	19.28	24.17	18.33	21.00
2	Clove	21.00	27.07	20.33	24.33
3	Citronella	24.00	32.12	23.00	28.00
4	Patchouli	27.00	38.42	25.00	29.00
	Check	38.26	46.33	30.00	37.00
	SEM±	1.45	1.17	0.71	0.74
	CD at 5%	4.56	3.69	2.25	2.34
	CV	9.68	6.03	5.30	4.63

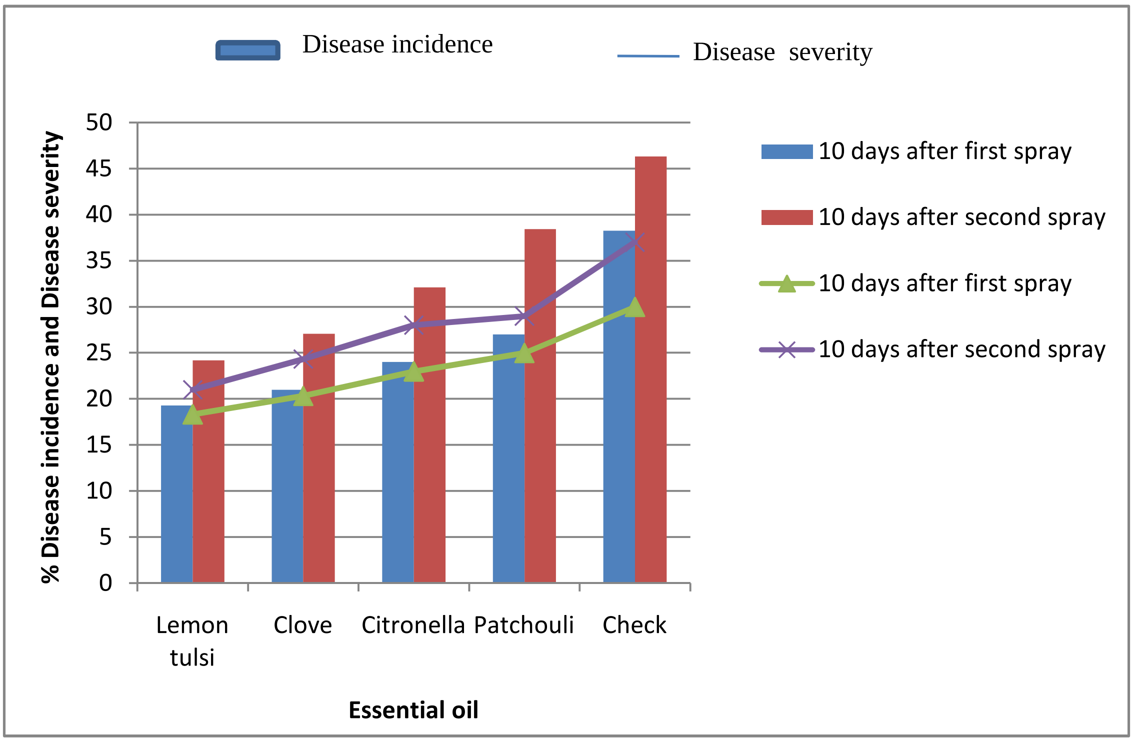


Fig 4.16: Effect of Essential oils on Disease Incidence and Disease Severity of Northern leaf blight of Maize on Amar variety under glasshouse condition

Table 4.17: Effect of Essential oils on Disease Incidence and Disease Severity of Northern leaf blight of Maize on Surya variety under glasshouse condition

Sl No.	Treatments	Disease incidence (%)		Disease severity (%)	
		10 days after first spray	10 days after second spray	10 days after first spray	10 days after second spray
1	Lemons tulsi	29.67	36.05	26.00	28.33
2	Clove	34.27	38.42	28.00	30.00
3	Citronella	36.75	40.67	31.00	34.00
4	Patchouli	38.33	42.88	31.67	35.33
	Check	45.33	53.67	40.33	47.67
	SEM±	1.22	1.29	0.88	0.86
	CD at 5%	3.85	4.07	2.78	2.69
	CV	5.74	5.28	4.86	4.22

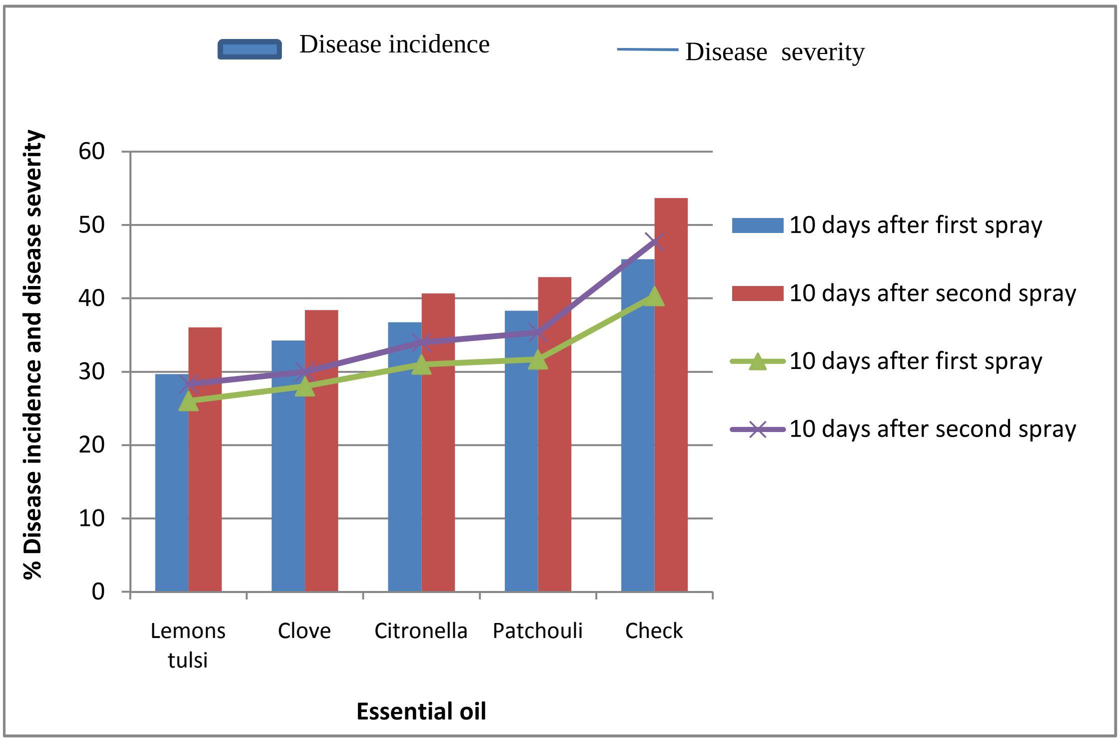


Fig 4.17: Effect of Essential oils on Disease Incidence and Disease Severity of Northern leaf blight of Maize on Surya variety under glasshouse condition

4.7.5 Evaluation of Bioagents against Northern leaf blight of maize on Amar and Surya variety of maize

Four Trichoderma isolates were tested for their efficacy against northern leaf blight of maize on two varieties of maize Amar and Surya and following results were recorded:

Variety: Amar

Ten days after the first spray minimum disease incidence (30.67%) and disease severity (27.09%) was recorded in pots sprayed with Th followed by disease incidence (31.94%) and disease severity (27.00%) in pots sprayed with TCM-36. Maximum disease incidence (38.26%) and disease severity (30.00%) was observed in check pots followed by disease incidence (34.67%) and disease severity (28.92%) pots sprayed with TCMS-39.

The results revealed that ten days after the second spray minimum disease incidence (33.80%) and disease severity (32.33%) was recorded in pots sprayed with Th followed by disease incidence (35.56%) and disease severity (33.14%) in pots sprayed with TCM-36. Maximum disease incidence (46.33%) and disease severity (37.00%) was observed in check pots followed by disease incidence (41.62%) and disease severity (35.02%) pots sprayed with TCMS-39.

Variety: Surya

Ten days after the first spray minimum disease incidence (35.13%) and disease severity (33.03%) was recorded in pots sprayed with Th followed by disease incidence (36.33%) and disease severity (35.48%) in pots sprayed with TCM-36. Maximum disease incidence (45.33%) and disease severity (40.33%) was observed in check pots followed by disease incidence (43.00%) and disease severity (39.67%) pots sprayed with TCMS-39.

The results revealed that ten days after the second spray minimum disease incidence (38.00%) and disease severity (37.67%) was recorded in pots sprayed with Th followed by disease incidence (35.48%). and disease severity (39.33%) in pots sprayed with TCM-36. Maximum disease incidence (53.67%) and disease severity (47.67%) was observed in check pots followed by disease incidence (47.67%).and disease severity (39.33%) pots sprayed with TCMS-39.

Vishwanath *et al*. (2017) studied the effect of biocontrol agent against *Exserohilum turcicum* and observed that *T. harzianum* at the rate of 2% showed disease reduction of 32.3%, where as *T.viridae* at the rate of 2% showed 29.2% disease reduction in field conditions.

Singh and Singh (2014) studied various isolates of *T. harzianum* against Leaf blight of sorghum caused by *Exserohilum turcicum* and five out of seventeen *T. harzianum* isolates found effective *in vitro* were further tested in glasshouse conditions. *T. harzianum* isolate Th-39 (38.74%) was found most effective in reducing the disease severity followed by Th-37 (36.92%), and Th-43 (34.77%). Th-39 isolate proved its worthiness in glasshouse as well as field conditions also reducing the maximum disease severity to 58.64% and 50.00% (glasshouse) and 35.59% and 30.30% (field) respectively using three sprays. Considering the biocontrol potential of these agents in disease suppression they can be recommended for management of disease.

Table 4.18: Effect of *Trichoderma* isolates on Disease Incidence and Disease Severity of Northern leaf blight of Maize on Amar variety under glasshouse condition

Sl No.	Treatments	Disease incidence (%)		Disease severity (%)	
		10 days after first spray	10 days after second spray	10 days after first spray	10 days after second spray
1	Th	30.67	33.80	27.09	32.33
2	TCMS- 36	31.94	35.56	27.00	33.14
3	PBAT-1	33.27	37.13	28.09	34.33
4	TCMS- 39	34.67	41.62	28.92	35.02
	CHECK	38.26	46.33	30.00	37.00
	SEM±	1.15	1.37	1.16	1.10
	CD at 5%	3.64	4.32	3.64	3.47
	CV	5.93	6.12	7.14	5.55

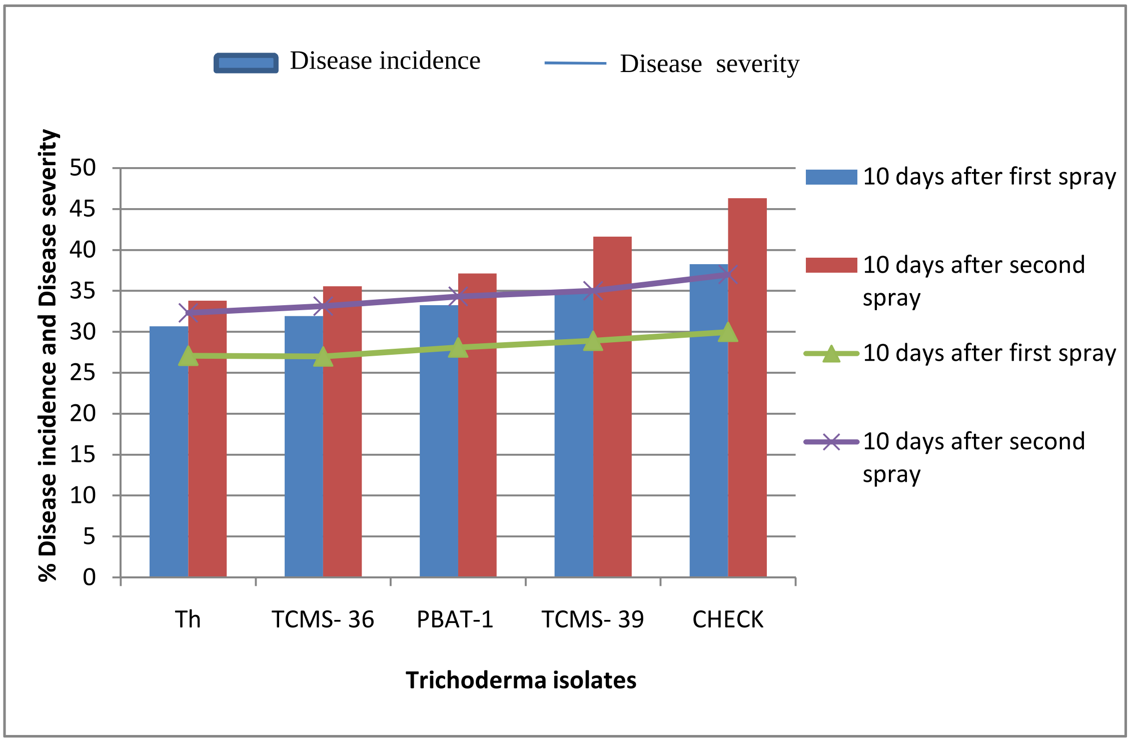


Fig 4.18: Effect of Trichoderma isolates on Disease Incidence and Disease Severity of Northern leaf blight of Maize on Amar variety under glasshouse condition

Table 4.19: Effect of *Trichoderma* isolates on Disease Incidence and Disease Severity of Northern leaf blight of Maize on Surya variety under glasshouse

Sl No.	Treatments	Disease incidence (%)		Disease severity (%)	
		10 days after first spray	10 days after second spray	10 days after first spray	10 days after second spray
1	TH	35.13	38.00	33.03	37.67
2	TCMS- 36	36.33	38.33	35.48	39.33
3	PBAT-1	39.31	43.95	37.00	43.33
4	TCMS- 39	43.00	47.67	39.67	45.33
	CHECK	45.33	53.67	40.33	47.67
	SEM±	1.41	1.23	1.24	1.23
	CD at 5%	4.45	3.87	3.93	3.87
	CV	6.15	4.32	5.82	4.99

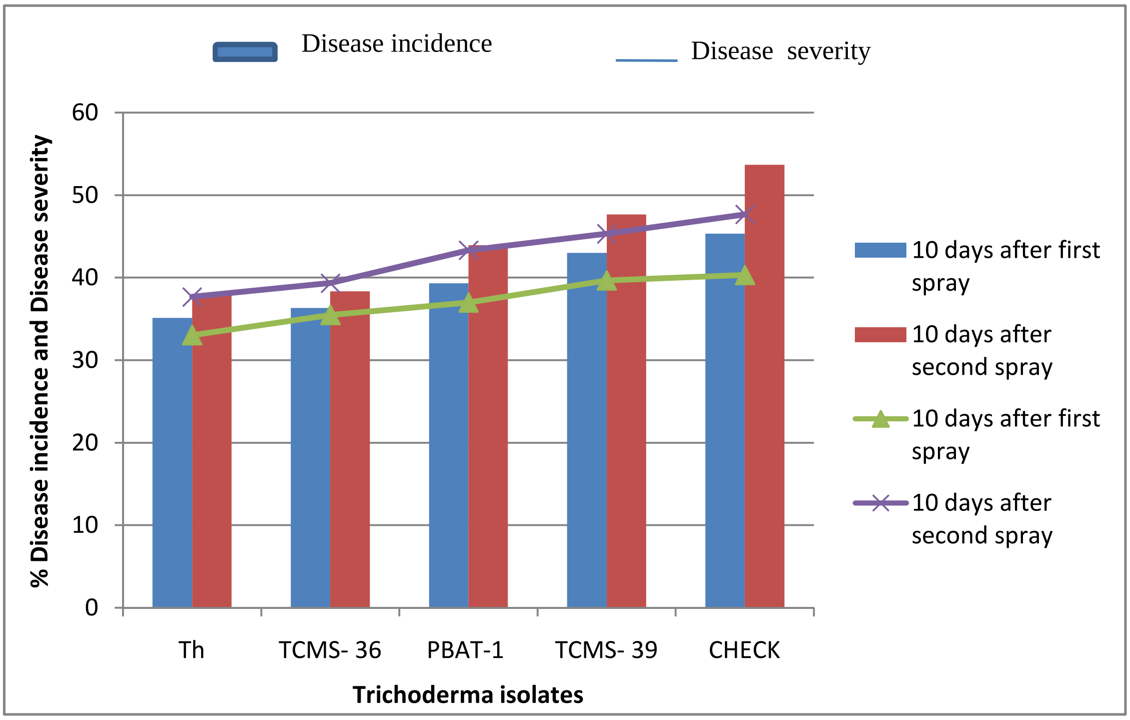


Fig 4.19: Effect of Trichoderma isolates on Disease Incidence and Disease Severity of Northern leaf blight of Maize on Surya variety under glasshouse

4.7.6 Effect of different cow urine concentrations on northern leaf blight of maize

Three concentrations (1%, 5% and 10%) of cow urine were evaluated in two varieties of maize Amar and Surya against northern leaf blight of maize and disease incidence and severity was recorded as followed:

Variety: Amar

Ten days after the first spray minimum disease incidence (22.33%) and disease severity (17.88%) was recorded in pots sprayed with 10% cow urine concentration. Maximum disease incidence (38.26%) and disease severity (30.00%) was observed in check pots followed by disease incidence and disease severity at 1% cow urine concentration.

Ten days after second spray, minimum disease incidence (27.08%) and disease severity (21.67%) was recorded in pots sprayed with 10% cow urine concentration. Maximum disease incidence (46.33%) and disease severity (37.00%) was observed in check pots followed by disease incidence (36.00%) and disease severity (27.67%) at 1% cow urine concentratio

Variety: Surya

Ten days after the first spray, minimum disease incidence (29.30%) and disease severity (26.48%) was recorded in pots sprayed with 10% cow urine concentration. Maximum disease incidence (45.33%) and disease severity (40.33%) was observed in check pots followed by disease incidence (36.03%) and disease severity (32.13%) at 1% cow urine concentration.

Ten days after second spray, minimum disease incidence (33.03%) and disease severity (28.00%) was recorded in pots sprayed with 10% cow urine concentration. Maximum disease incidence (53.67%) and disease severity (47.7%) was observed in check pots followed by disease incidence (39.00%) and disease severity (36.37%) at 1% cow urine concentration.

Basak *et al*. (2002) studied antifungal activity and comparative efficacy of fresh cow urine and cow dung for controlling Sclerotinia rot caused by *Sclerotinia sclerotiorum* of cucumber by performing mycelial growth inhibition test. Results showed that cow urine suppressed mycelial growth more effectively even after 5 days of incubation in comparison to cow dung.

Application of cow dung and cow urine at different concentrations on plants inoculated with the pathogen showed their inhibitory effects.

Table 4.20: Effect of different Cow urine concentrations on Disease Incidence and Disease severity of Northern leaf blight of Maize on Amar variety under glasshouse conditions

Sl No.	Treatments	Disease incidence (%)		Disease severity (%)	
		10 days after first spray	10 days after second spray	10 days after first spray	10 days after second spray
1	1%	28.33	36.00	25.33	27.67
2	5%	25.33	32.00	21.00	26.33
3	10%	22.33	27.08	17.88	21.67
4	CHECK	38.26	46.33	30.00	37.00
	SEM±	1.07	1.41	1.32	1.29
	CD at 5%	3.49	4.62	4.32	4.20
	CV	6.49	6.95	9.73	7.93

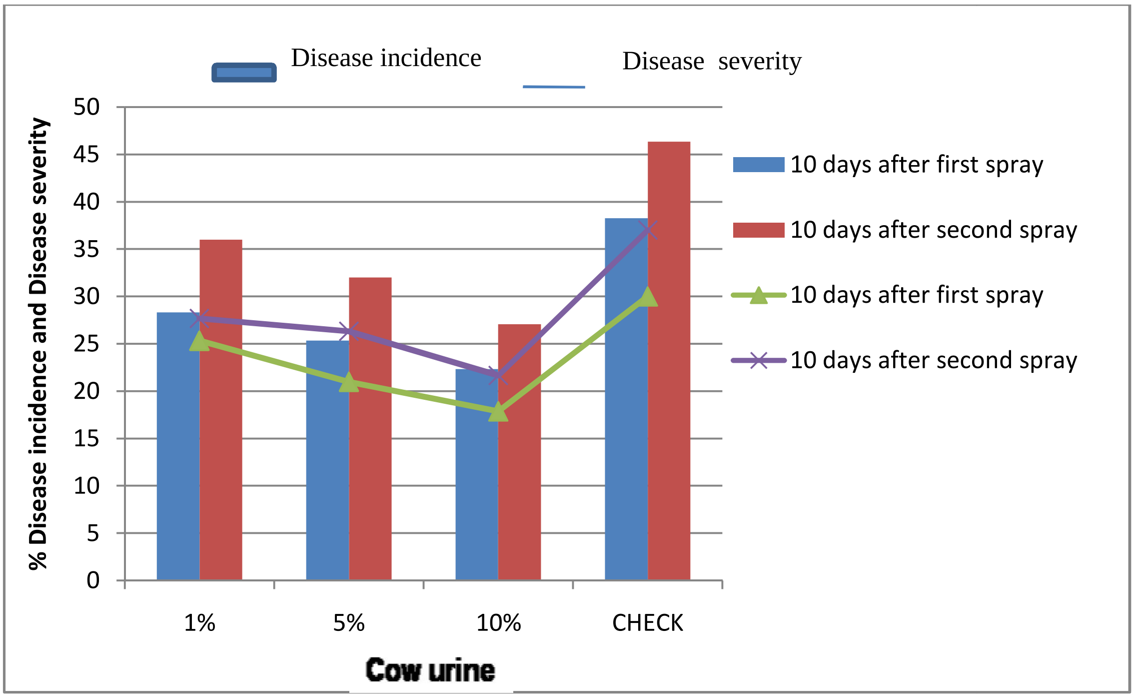


Fig 4.20: Effect of different Cow urine concentrations on Disease Incidence and Disease severity of Northern leaf blight of Maize on Amar variety under glasshouse conditions

Table 4.21: Effect of different Cow urine concentrations on Disease Incidence and Disease Severity of Northern leaf blight of Maize on Surya variety under glasshouse conditions

Sl No.	Treatments	Disease incidence (%)		Disease severity (%)	
		10 days after first spray	10 days after second spray	10 days after first spray	10 days after second spray
1	1%	36.03	39.00	32.13	36.37
2	5%	34.67	37.33	29.22	33.22
3	10%	29.30	33.03	26.48	28.00
4	CHECK	45.33	53.67	40.33	47.7
	SEM±	1.69	1.28	1.29	1.37
	CD at 5%	5.54	4.19	4.22	4.47
	CV	8.10	5.47	6.99	6.54

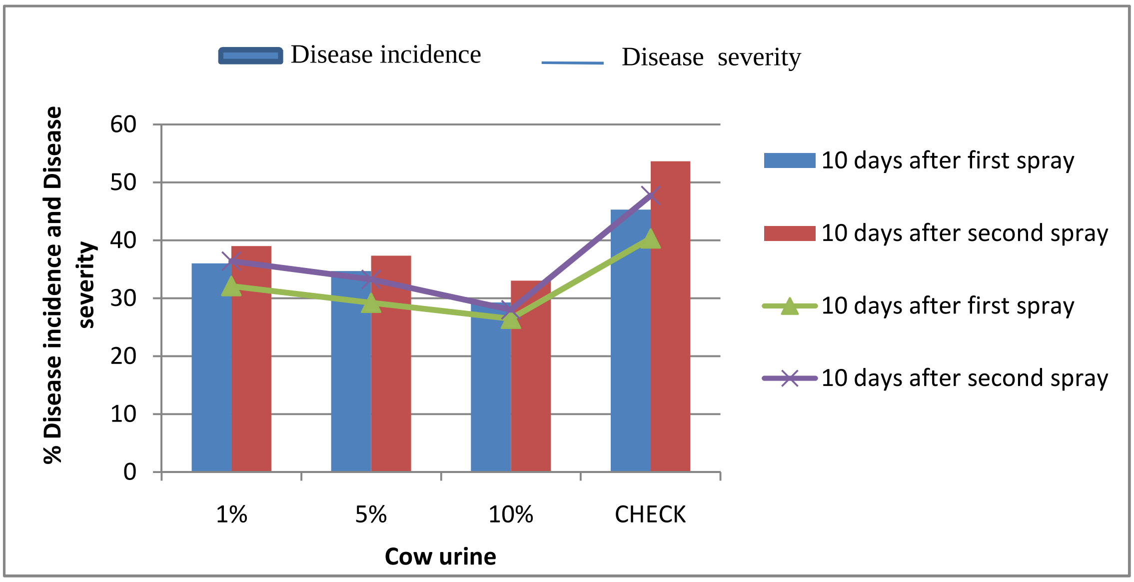


Fig 4.21: Effect of different Cow urine concentrations on Disease Incidence and Disease Severity of Northern leaf blight of Maize on Surya variety under glasshouse conditions

Summary and Conclusions

Chapter 5 SUMMARY AND CONCLUSION

Maize is one of the most important cereal crops of the world and contributes to food security in most of the developing countries. Maize is widely cultivated throughout the world and has maximum production among all other cereal crops in the world that is 972.40million MT **(FAO, 2015-2016).** Many biotic and abiotic stresses reduce maize production and cause huge economic losses. Northern leaf blight of maize caused by a Deuteromycetes fungus, *Exserohilum turcicum* (Telomorph: *Setosphaeria turcica*) is one of the most important fungal diseases of maize. The disease is prevalent in almost all the maize growing areas and cause severe yield loses.

Keeping in view the importance of the disease, the present investigations were carried out to identify the causal organism associated with northern leaf blight of maize. Systematic studies on morphological and physiological characters of pathogen were made. *In vitro* and *in vivo* efficacy of various fungicides, botanicals, essential oils, biogents and cow urine at different concentrations were evaluated against the test pathogen and the results obtained are summarised.

Initially symptoms are exhibited in the form of small oval, water soaked, slightly elliptical greyish colour spots but later spots enlarge and become elongated spindle shaped. The lesions were characteristically cigar shaped and straw coloured in the centre with dark margins. These lesions enlarge; covering larger area of leaves and produces blightened symptoms. The microscopic study of the test fungus was done for morphological characters of the pathogen. The hyphae observed under the microscope were pale to light brown, smooth and septate. The conidia were spindle-shaped, and had a protruding hilum. The conidia were olivaceous brown in colour with 3-8 septation. The colour of the colony was greyish white initially which later turned black coloured.

Cultural studies were conducted by testing six different media (Potato Dextrose Agar, Richard's Synthetic Agar, Oat Meal Agar, Corn Meal Agar, 2% Malt Extract Agar and Czapex Dox Agar) at six different temperatures (10, 15, 20, 25, 30, 35^{0}C). The results from the study concluded that among six media tested Potato Dextrose Agar showed maximum colony diameter 87.33mm at 30^{o}C. A temperature range of 25-30^{0}C was most suitable for growth of the test pathogen in all the media tested. Maximum mycelial growth of the test fungus in all the tested media was observed at 30^0C temperatures.

Effect of six different media (Potato Dextrose Agar, Richard's Synthetic Agar, Oat Meal Agar, Corn Meal Agar, 2% Malt Extract Agar and Czapex Dox Agar) and four different light wavelengths (Red, Yellow, Green and Blue) on growth of the test pathogen was studied and results conclude that Oat Meal Agar showed maximum colony diameter (90mm) under green light. Minimum colony diameter (44.0mm) was observed in case of Potato Dextrose Agar under red light.

The pathogen can grow at different pH levels. The study revealed that the radial growth was maximum (87.17mm) at pH 7 and minimum (63.67mm) was observed at pH 5.

In vitro studies were conducted to test the efficacy of different fungicides, botanicals, essential oils, *Trichoderma* isolates and cow urine concentrations against the test pathogen. Out of four systemic fungicides tested Propiconazole showed cent per cent inhibition of mycelia growth followed by Tebuconazole (87.78%) at 10 ppm concentration. Among four non systemic fungicides, Avtar (Zineb+Hexaconazole) showed cent per cent inhibition of mycelial growth followed by Captan (79.44%) at 200ppm concentration.

Among the ten botanicals tested Heena (*Lawsonia inermis*), showed maximum inhibition of mycelia growth (71.11%) followed by Duranta (67.04%) and minimum inhibition of mycelial growth (13.7%) was observed in case of Aloe vera.

Eleven essential oils were evaluated for antifungal activity against the test pathogen and the results revealed that at 500ppm concentration, cent per cent inhibition of mycelial growth was recorded in case of Lemon tulsi followed by Clove (88.89%) which was statistically at par with Citronella (87.78%). Minimum inhibition of mycelial growth was recorded in Peppermint (38.15%).

Among six *Trichoderma i*solates tested for their antagonistic potential against the test pathogen. Maximum inhibition of radial growth (64.81%) was observed in case of Th which was statistically at par with TCMS-36 (64.44%) followed by PBAT -1 (57.04%) while Th-19 showed minimum inhibition of mycelial growth (52.59%).

In vitro evaluation of cow urine against the test pathogen at different concentrations showed that 10% concentration of cow urine was highly effective in inhibiting the mycelial growth of the test fungus and showed cent per cent inhibition.

Glasshouse experiments were conducted to evaluate different methods of inoculation of *Exserohilum turcicum* on two varieties of maize Amar and Surya. Three different methods of inoculation- spraying, cotton swab and stem injection were used to inoculate the pathogen to identify the most suitable method of inoculation. Spray method of inoculation was found to be most effective method and showed maximum disease incidence at silking stage in both Amar (43.36%) and Surya (54.30%) variety of maize.

Different fungicides, botanicals, essential oils, *Trichoderma* isolates and cow urine concentrations which were effective *in vitro* were selected and tested against northern leaf blight of maize under glasshouse conditions on two varieties of maize Amar and Surya.

Among four fungicides tested Propiconazole 25 EC was the most effective and showed minimum disease incidence (23.33%) and disease severity (17.67%) in Amar variety. Reduced disease incidence (27.67%) and disease severity (26.67%) was observed in case of Surya variety. Out of four botanicals tested under glasshouse, Heena was found superior over all the treatments and minimum disease incidence (26.67%) and disease severity (25.00%) was observed in case of Amar variety and in Surya variety reduced disease incidence (34.13%) and disease severity (32.67%) was recorded.

Among four essential oils tested against *E.turcicum* under glasshouse conditions, Lemon tulsi oil was found to be most effective and showed minimum disease incidence (24.17%) and severity (37.00%) in case of Amar variety and reduced disease incidence (36.05%) and disease severity (28.33%) was observed in plants treated with Lemon tulsi oil in Surya.

Among four *Trichoderma* isolates tested, Th isolate was found most effective and showed minimum disease incidence (33.80%) and disease severity (32.33%) in Amar variety, while in Surya variety, Th isolate treated plants showed reduced disease incidence (38.00%) and disease severity (37.67%).

In vivo evaluation of different cow urine concentrations was done under glasshouse conditions. Among three concentrations tested 10% cow urine concentration was found to be most effective and showed minimum disease incidence (27.08%) and disease severity (21.67%) in Amar variety and reduced disease incidence (33.03%) and disease severity (28.00%) was observed in Surya variety.

The results from the studies conclude that in laboratory conditions, Potato Dextrose Agar media at 30^0C temperature was most suitable for the growth of *E. Turcicum.* pH 7 of Potato Dextrose Agar media was optimum for the growth of the fungus and pH 5 showed minimum mycelial growth of the pathogen among all the tested pH. Propiconazole 25 EC, Avatar 72% WP [Zineb (68%) + Hexaconazole (4%)] were most effective among all the tested fungicides in inhibiting the growth of the pathogen both *in vitro* and *in vivo*. Heena (*Lawsonia inermis*) showed maximum inhibition of mycelial growth among all the tested botanicals and was found to be effective under glasshouse conditions. Lemon tulsi (*Ocimum africanum*) was found most effective in inhibiting the mycelial growth of the pathogen, where as Peppermint was least effective in inhibiting the mycelia growth of the pathogen. *Trichoderma* isolate–Th showed maximum inhibition of the radial growth of the pathogen while Th-19 isolate was least effective in inhibiting the growth of the pathogen. Among all the tested concentrations 10% concentration of cow urine was most effective in inhibiting the growth of the fungus both *in vitro* and *in vivo* conditions where as 1% concentration of cow urine was least effective in inhibiting he growth of the fungus.

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Appendix

Medium used in studies: Composition and preparation

All the media used were sterilized by autoclaving at 121°C for 15 minutes. The pH of the medium was adjusted using NaOH or HCl before autoclaving.

A. Potato Dextrose Agar medium

Ingredients	**Quantity**
Potato (peeled and sliced)	200.0 g
Dextrose	20.0 g
Agar	20.0 g
Distilled water	1000.0 ml

B. Czapex Dox Agar medium

Ingredients	**Quantity**
Sucrose	30.0 g
Sodium nitrate	2.0 g
Dipotassium phosphate	1.0 g
Magnesium sulphate	0.50 g
Potassium chloride	0.50 g
Ferrous sulphate	0.01 g
Agar	15.0 g
Distilled water	1000.0 ml

C. 2% Malt Extract Agar medium

Ingredients	**Quantity**
Malt extract	20.0 g
Agar	15.0 g
Distilled water	1000.0 ml

D. Oat Meal Agar medium

Ingredients	Quantity
Oat Meal	60.0 g
Agar	20.0 g
Distilled water	1000.0 ml

E. Corn Meal Agar medium

Ingredients	Quantity
Corn meal infusion form	50.0 g
Agar	15.0 g
Distilled water	1000.0 ml

F. Richard's Synthetic Agar

Ingredients	Quantity
Potassium nitrate	10.0 g
Potassium dihydrogen phosphate	5.0 g
Magnesium sulphate	2.5 g
Ferric chloride	0.02 g
Saccharose (Sucrose)	50.0 g
Agar	15.0 g
Distilled water	1000.0 ml

VITA

The authoress Bhagyashree Bhatt of this manuscript was born on 27 November 1993 in Pithoragarh (Uttarakhand). She passed her high school examination from Beersheba Senior Secondary School, Pithoragarh (Uttrakhand) in 2009 and Intermediate from Beersheba Senior Secondary School, Pithoragarh (Uttrakhand)in 2011. She completed her degree of B.Sc. (Agriculture) from G B Pant University of Agriculture and Technology, Pantnagar in 2016. Then she joined GB Pant University of Agriculture and Technology, Pantnagar in July 2016 for pursuing M.Sc. (Ag) (Plant Pathology).

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ABSTRACT

Maize is one of the most important cereal crops of the world and contributes to food security in most of the developing countries. Northern leaf blight disease of maize caused by *Exserohilum turcicum* (Telomorph: *Setosphaeria turcica*) is one of the most important fungal diseases of maize. The disease is prevalent in almost all the maize growing areas and cause severe yield loses. Keeping in view the importance of the disease, *in vitro* and glasshouse studies were conducted to elucidate different aspects of pathogen biology and pathogenic growth inhibiting potential of various compounds. Pathogen produced white cottony mycelial growth which later turned black in colour. Conidia were spindle-shaped with 3-8 septation, and had a protruding hilum. Potato Dextrose Agar Media, 30^0 C temperature and pH 7 were found best for growth of *E.turcicum. In vitro* evaluation studies revealed that among fungicides tested Propiconazole 25 EC and Tebuconazole 25.9EC were most effective in inhibiting growth of the pathogen. Heena (*Lawsonia inermis*) among the botanicals, Lemon tulsi (*Ocimum africanum*) among essential oils and Th isolate of Trichoderma among the other tested isolates were found to be most effective in inhibiting mycelial growth of the pathogen *in vitro* conditions. Among different tested concentrations of cow urine, 10% concentration showed maximum growth inhibition of pathogen. Under glasshouse condition among three methods of inoculation spraying method gave best results both in Amar and Surya variety of maize. Four fungicides, botanicals, essential oils, Trichoderma isolates and three concentrations of cow urine which proved most effective under lab conditions were tested under glasshouse conditions. Propiconazole 25 EC showed maximum control over the disease and minimum disease incidence (23.33%) and disease severity (17.67%) was observed in case of Amar variety. Reduced disease incidence (27.67%) and disease severity (26.67%) was recorded in case of Surya variety.

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शोध शीर्षक : मक्का के नोर्दन पत्ती झुलसा रोग का कारण, *एक्सेरोहाइलम टर्सिकम* (पास) ल्योनार्ड और सग्स, के विकास अवरोध पर अध्ययन।

सलाहकार : डॉ0 प्रदीप कुमार

सारांश

मक्का दुनिया की सबसे महत्वपूर्ण अनाज फसलों में से एक है और अधिकांश विकासशील देशों की खाद्य सुरक्षा में योगदान देता है। *एक्सेरोहाइलम टर्सिकम* (टैलोमॉर्फः *सीटोस्फेरिया टर्सिकम*) के कारण मक्का की नोर्दन पत्ती झुलसा सबसे महत्वपूर्ण फफूँदीजनक रोगों में से एक है। यह रोग लगभग सभी प्रमुख मक्का उगाये जाने वाले क्षेत्रों को प्रभावित करता है तथा पैदावार घटाने का प्रमुख कारण है। नोर्दन पत्ती झुलसा बीमारी के महत्व को ध्यान में रखते हुये प्रयोगशाला एवं ग्लास हाउस में रोगजनक जीवविज्ञान के विभिन्न पहलुओं और विभिन्न यौगिकों की रोगजनक वृद्धि अवरोध क्षमता को स्पष्ट करने के लिए अध्ययन किया गया। *एक्सेरोहाइलम टर्सिकम* की कालोनी सफेद कवक जाल रूई के समान होती है जो बाद में काले रंग की हो जाती है। बीजाणु धुरी के आकार के होते हैं जिसमें 3–8 भाग होते हैं और उसमें प्रोमिनेन्ट हाइलम होता है। पोटैटो डैक्सट्रोस अगर माध्यम, 30^0से0 तापमान व पी0एच0 7, रोगकारक के कवकजाल की वृद्धि के लिए सबसे अच्छे पाये गये। प्रयोगशाला अध्ययन के दौरान प्रयोग किये गये कवकनाशक, प्रोपिकोनाजोल 25 ई0सी0 और टेब्यूकोनाजोल 25.9 ई0सी0 रोगजनक के विकास को रोकने में सबसे प्रभावी पाये गये। पादप रसों में से हिना, आवश्यक तेलों में से लेमन तुलसी और ट्राइकोडर्मा का टी0एच0 प्रथक प्रयोगशाला अध्ययन में रोगजनक के विकास को रोकने में सबसे प्रभावी पाये गये। गौमूत्र की विभिन्न परीक्षण सान्द्रता में 10 प्रतिशत सान्द्रता रोगजनक की वृद्धि में अधिकतम अवरोध देखा गया। ग्लास हाउस स्थिति में 3 तरीके के इनोक्यूलेशन में से छिड़काव के तरीके में मक्के की अमर और सूर्या विविधता दोनों में सर्वोत्तम परिणाम दिये। प्रयोगशाला में उत्तम परिणाम देने वाले 4 कवकनाशक, पादप रस, आवश्यक तेल, ट्राइकोडर्मा के प्रथम और गौमूत्र की 3 सांद्रता का परीक्षण ग्लास हाउस स्थितियों के तहत किया गया। प्रोपिकोनाजोल 25 ई0सी0 ने अमर प्रजाति में रोग पर अधिक नियंत्रण दिखाया और न्यूनतम रोग तीव्रता (23.33 प्रतिशत) और रोग गंभीरता (17.67 प्रतिशत) देखी गयी। सूर्या प्रजाति में भी कम रोग तीव्रता (27.67 प्रतिशत) और रोग गंभीरता (26.67 प्रतिशत) पाई गई।

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लेखिका