STUDIES OF SORGHUM LEAF BLIGHT INCITED BY *Exserohilum turcicum* (Pass.) Leo. & Suggs.

by

M.H. ADEN, B.Sc(Ag)

THESIS SUBMITTED TO THE
ANDHRA PRADESH AGRICULTURAL UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF

MASTER OF SCIENCE
IN THE FACULTY OF AGRICULTURE

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE
ANDHRA PRADESH AGRICULTURAL UNIVERSITY
RAJENDRANAGAR, HYDERABAD 500 030

CEREALS PATHOLOGY UNIT
CEREALS PROGRAM
ICRISAT
PATANCHERU
A.P. 502 324

AUGUST 1991

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C E R T I F I C A T E

Mr. Mohamed Hassan Aden has satisfactorily prosecuted the course of research and that the thesis entitled "Studies of sorghum leaf blight incited by Exserohilum turcicum (Pass.) Leo. and Suggs" submitted is the result of original research work, and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any university.

Date: 28 August 1991

L.K. Mughogho

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CERTIFICATE

This is to certify that the thesis entitled "Studies of sorghum leaf blight incited by Exserohilum turcicum (Pass.) Leo and Suggs." submitted in partial fulfilment of the requirment for the degree of Master of Science in Agriculture of Andhra Pradesh Agricultural University, Hyderabad, is a record of the bonafide research work carried out by Mr. Mohamed Hassan Aden under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

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

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DECLARATION

I, Mohamed Hassan Aden, hereby declare that the thesis entitled "Studies of sorghum leaf blight incited by *Exserohilum turcicum* (Pass.) Leo and Suggs" submitted to Andhra Pradesh Agricultural University for the degree of Master of Science in Agriculture is the result of original work done by me. I also declare that the material contained in this thesis has not been published earlier.

Date: 28.8.91

MOHAMED HASSAN ADEN

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A B S T R A C T

Experiments were conducted to study the following aspects of the sorghum leaf blight fungus Exserohilum turcicum (Pass.) Leo & Suggs: pathogenicity, mass production of conidial inoculum, inoculation methods, infection process, sorghum plant growth stages and their susceptibility to leaf blight, and the pathogenicity of sorghum and maize isolates on sorghum and maize genotypes.

Pathogenicity tests of three conidial concentrations (20,000, 10,000, and 5,000 conidia mL^{-1}) on sorghum showed that 20,000 conidia mL^{-1} caused the highest infection (>40%

leaf area damaged), while 5,000 conidia caused the lowest infection (<10% leaf area damaged).

The growth and sporulation of the fungus was tested 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°C, 20°C, 25°C, 30°C, 35°C). The best temperature for colony growth was 25°C(21 mm), and for sporulation was 20°C (47,000 conidia ml^{-1}). Both colony growth and sporulation were maximum between 20 and 30°C, but were very poor at 35 and 15°C. The best media for colony growth at all temperatures was lactose casein hydrolysate agar(16.5 mm), and for sporulation was sorghum leaf medium(53,000 conidia mL^{-1}).

The highest colony growth after 12 days of incubation were observed on lactose casein hydrolysate agar at 30°C (40 mm), sorghum grain extract agar at 30°C (40 mm), sorghum leaf extract agar at 25 °C (38.5 mm), and maize grain extract agar at 25°C (38.5 mm). It was poor on potato dextrose agar at 25°C (29 mm) and maize leaf extract agar at 25°C (34 mm). The highest sporulation after 12 days of incubation occured on sorghum leaf medium at 20°C (180,000 conidia mL^{-1}) followed by lactose casein hydrolysate agar at 20°C (113,300 conidia ml^{-}) and potato dextrose agar at 25°C

(73,000 conidia mL^{-1}). Sporulation was poor on sorghum grain extract agar at 25°C (13,300 conidia mL^{-1}), maize grain extract agar at 30°C (13,300 conidia mL^{-1}), and maize leaf extract agar at 25°C (20,000 conidia mL^{-1}).

Conidial suspension (20,000 conidia mL^{-1}) sprayed on the leaves of sorghum was the best inoculation method compared to the other methods tested: diseased sorghum leaves buried in soil, diseased sorghum leaves spread over soil, and diseased sorghum leaves placed into leaf whorls . Second to that was spore suspensions (20,000 conidia mL^{-1}) poured on the leaf whorls. The use of diseased leaves as inoculum did not cause disease.

The germination of conidia was polar and penetration was mostly through the cuticle and similar on the leaves of a leaf blight resistant sorghum cultivar(IS 8283) and a susceptible sorghum cultivar(Framida). Germinated conidia formed germ tubes, and appressoria were produced from these germ tubes before penetration. After penetration the fungal hyphae spread and branched in the leaf tissues of the susceptible variety Framida, but not in the resistant line IS 8283.

Sorghum was most susceptible to infection by E. turcicum at the 8-leaf stage. Other growth stages which were

susceptible were 5-leaf stage and flag leaf-visible stage, followed by 3-leaf stage and boot stage. Infection was lowest when plants were inoculated at the 50% flowering stage.

Four isolates of the fungus from sorghum and four from maize were cross inoculated into four varieties of maize and three of sorghum. It was found that sorghum isolates from Patancheru and Kurnool infected only sorghum. But isolates from Karimnagar and Momlapalli infected sorghum, and also maize varieties CM 500 and DH 103 respectively. Maize isolates from Patancheru and Amperbet infected maize, while isolates from Undavally and Biknoor infected maize, and also sorghum variety IS 2858.

INTRODUCTION

CHAPTER I

INTRODUCTION

Sorghum [Sorghum bicolor(L.) Moench] is one of the most important staple food crops of the world's poorest people, particularly those in the semi-arid tropics(SAT). The territories classified as semi-arid tropics include large areas of Africa, central India, and some regions of South America and South Asia. These areas are characterized by unpredictable rainfall distribution and variability of its frequency (Hulse et al. 1980). Over 55% of the world's sorghum production is in this ecological zone.

The total sorghum cultivated area of the world is 44.5 million hectares, with an annual production of 58 million tonnes (F.A.O. 1989). In India sorghum is the third important cereal after rice and wheat, and is currently grown on 16.4 million hectares with an annual production of 11.5 million tonnes (F.A.O. 1989). The main areas of sorghum cultivation in India are in the states of Maharashtra, Karnataka, Andhra Pradesh and Madhya Pradesh. Nearly 62% of this area is planted in the rainy season (kharif) and the rest in the postrainy season (rabi) (Ravindranath 1987).

Sorghum grain is used for both human food and animal feed. The stems and foliage are used for fodder, hay, silage

and pasture. In some areas the stem is used as building material and for fuel (House 1985).

Generally, sorghum grain yields on peasant farms are very low ranging from 500 to 800 kg ha^{-1} . The yield per hectare in the developing countries was 1028 kg ha^{-1} in 1989, which was below the world average of 1309 kg ha^{-1}, and well below the 3177 kg ha^{-1} of the developed countries (F.A.O. 1989).

Sorghum is a relatively undeveloped crop with great potential. Yields can be increased well beyond present levels, while the adaptation of sorghum to a wide range of ecological conditions is its greatest asset (Dogget 1988).

One of the major factors that reduce sorghum yield throughout the SAT is diseases. Sorghums are attacked by a wide range of stem, leaf, and panicle diseases (King 1972). Leaf diseases cause significant losses due to their reduction of the photosynthetic area of the affected leaves (Sharma and Jain 1975). Leaf blight caused by Exserohilum turcicum (Pass.) Leo & Suggs. is one of the important diseases affecting sorghum production. The disease is widespread in sorghum growing areas of the world, especially under humid conditions and it can do severe damage to foliage (Frederiksen et al. 1975). In India, leaf blight is

prevalent and widespread on sorghum, particularly in the states of Andhra Pradesh, Haryana, Maharashtra, Madhya Pradesh, Karnataka, and Tamil Nadu (Sundaram et al. 1972).

The present study had the following objectives :-

1) To produce mass conidial inoculum of the pathogen E. turcicum for inoculation of sorghum plants and to determine the most effective method of inoculation.

2) To study the infection process of E. turcicum on sorghum.

3) To determine the growth stages at which sorghum is more susceptible to leaf blight disease.

4) To investigate the pathogenicity of sorghum and maize isolates of the pathogen on sorghum and maize genotypes.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

2.1 THE FUNGUS

2.1.1 Taxonomy

Leaf blight of sorghum is incited by the fungus Exserohilum turcicum (Pass.) Leo. and Suggs [Syns. Helminthosporium turcicum (Pass.), Bipolaris turcica (Pass.) Shoemaker and Drechslera turcica (Pass.) Subram. & Jain.] (Commonwealth Mycological Institute 1971). Luttrell (1957) described the perfect stage of the fungus as Trichometasphearia turcica. Leonard and Suggs (1974) established the genus Exserohilum for Helminthosporium species in which the conidial hilum was strongly protuberant. They also placed the ascigerous (perfect) states of Exserohilum in the new genus Setosphaeria. The perfect state of Exserohilum turcicum, Setosphaeria turcica (Luttr.) Leo. & Suggs, is rarely found in nature.

2.1.2 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). The conidial shape is fusoid, obclavate, straight, or curved; the hilum is strongly protruding. Conidia germinate commonly 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 (Luttrell 1964).

2.2 THE DISEASE

2.2.1 Symptoms

Leaf blight lesions are elongated elliptical spots, measuring up to 12 mm wide and 2.5-15 cm long, and are present on both the lamina and leaf sheath; those on the lamina, however, are more prominent(Misra 1973). At first they have water soaked appearance, later turning into straw colour or brownish, the brownish colour being prominent, specially along the margins of the lesions. The centre of the lesions are ashy in colour in moist weather and straw-coloured in dry season due to sporulation (Misra and Mishra 1971b).

A distinctive feature of leaf blight is the timing of the appearance of symptoms. Small flecks appear, usually 3 to 4 days after a favourable infection period, but the large distinctive lesions do not appear until two weeks later (Frederiksen 1980).

2.2.2 Disease Cycle

Mycelia and conidia of this fungus from infected crop residue, in or on the soil, act as the primary inoculum for the next crop. Conidia can overseason by thickening of their walls to become chlamydospores. The secondary inoculum comes from lesions which produce conidia that are dispersed by wind and can be transported for long distances (Shenoi and Ramalingam 1983).

2.2.3 Distribution and Economic Importance

Leaf blight disease is widely distributed in all major sorghum growing areas of the world (Commonwealth Mycological Institute 1988).

In India the disease was first reported by Butler (1918) on sorghum and later by Mitra (1923) on both sorghum and maize from the Punjab.

Generally, the disease does not kill the plant except for seedlings exposed to prolonged attacks (Tarr 1962). However, by destroying the green photosynthetic tissue, and sometimes causing premature wilting and leaf death, it reduces or delays plant growth and development which results in reduced yield of both grain and fodder (Tarr 1962). A 45% yield loss of sorghum due to the disease was reported in India (Sharma 1980), and 22% in the Philippines (Elazegui

1971). Leaf blight is also an important disease of maize and up to 90% yield loss was reported in India (Chenulu and Hora 1962).

2.3 MASS PRODUCTION OF CONIDIAL INOCULUM

Different media and temperatures have been reported as suitable for the growth and sporulation of the fungus. These reports are reviewed below.

2.3.1 Effects of media

Rodriquez and Ullstrup (1962), Frederiksen et al. (1975), Tuleen and Frederiksen (1977), Leonard et al. (1988) reported good colony growth and sporulation of sorghum and maize isolates of Exserohilum turcicum on lactose casein hydrolysate agar.

Misra and Roy (1965) studied the effects of carbon and nitrogen sources on sorghum and maize isolates of the fungus. They recorded the highest mycelial growth in Richards and Czapeck's media, and maximum sporulation in glucose-peptone and Richards media. Furthermore, they reported the best colony growth with sucrose, glucose, and lactose as carbon sources and sporulation was highest with sucrose. Of the various nitrogenous sources that they tried, potassium

nitrate and asparagin proved the best for colony growth, and peptone was the best for sporulation.

Joshi et al. (1969) studied the growth of the fungus on sorghum seeds and observed mycelial growth within 72 hours, and abundant sporulation within one week. Pandey and Shukla (1979) reported poor sporulation of a sorghum isolate of the fungus on Richards medium, potato dextrose agar, and sorghum leaf extract. Pandey and Shukla (1982) also grew a sorghum isolate of the fungus on ten different media and observed that the best medium for growth was Richards medium, while Brown's starch medium was the poorest.

Shankerlingam and Balasubramanian (1983) suggested a simple method of inducing sporulation of the fungus by growing it on different media containing extracts of maize, sorghum, barley, and wheat grain, and leaves of highly susceptible cultivars. They found that the highest number of spores were observed on the medium containing leaf extracts of susceptible cultivars.

The literature reviewed above indicated that colony growth and sporulation of E. turcicum was influenced by the type of media.

2.3.2 Effects of temperature

Misra and Singh (1963) studied the effect of temperature and humidity on the development of a maize isolate of Helminthosporium turcicum and found 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.

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

2.4 INOCULATION METHODS

2.4.1 Diseased leaves as inoculum

Robert and William (1952) made field inoculations of maize leaf blight by placing dried infected leaves in the leaf whorls or scattering between the rows . They found that both inoculation methods caused infection . They highlighted that the use of diseased leaf material for inoculation to produce artificial epidemics not only saved laboratory work, but under favorable environmental conditions such as temperature and humidity, might also advance the development of

the disease by 7 to 10 days.

Drolsom and Dickson (1954) used different inoculation methods of the fungus on sudan grass and pointed out that the placement of diseased leaves over soil was a simple inoculation method that produced satisfactory results. Andrew et al. (1964) also used diseased leaves of maize as inoculum. Chenulu and Hora (1962) produced the initial inoculum for infection of maize by mixing a sufficient quantity of crushed pieces of diseased leaves containing spores and mycelium with seeds before sowing .

2.4.2 Spore suspension as inoculum

Spore suspensions of E. turcicum sprayed on leaves were reported to be a successful method of inoculation (Frederiksen et al. 1975, Tuleen and Frederiksen 1977, Tarumoto and Isawa 1972, Raymundo and Hooker 1981, Lipps and Hite 1982, Shree 1983, Leath and Pederson 1983 and 1986, and Abadi et al. 1989).

Gracen et al.(1971) suggested a rapid method to determine resistant and susceptible plants to leaf blight which consisted of spraying of spore suspensions on entire flats of plants in the greenhouse or rows in the field. Nelson et al. (1965) inoculated plants by spraying spore suspensions

on leaves or by pouring spore suspensions into leaf whorls. They also mentioned that pouring spore suspensions in leaf whorls eliminated inoculum drift. Robert and Sprague (1960) also inoculated maize plants by pouring about 10 mL of spore suspensions of the fungus into leaf whorls.

2.5 INFECTION PROCESS

Little work has been done on the infection process of E. turcicum on sorghum, but detailed studies have been done on the histopathology of maize leaf blight.

Jennings and Ullstrup (1957) examined hand sections of naturally infected sorghum leaves and found that the profuse fungal growth in the tissues was limited to the xylem. They suggested that histopathology of the fungus on maize and sorghum could be the same, since the pathogen caused similar symptoms on both hosts.

Shree (1987) studied the histopathology of the fungus on sorghum and his findings were similar to those of Jennings and Ullstrup (1957), and Hilu and Hooker (1964) on maize.

2.5.1 Spore deposition and germination

The germination of E. turcicum conidia was bipolar and occurred 8-12 hours after inoculation on leaves of sorghum

(Shree 1987) and 3-6 hours on maize (Hilu and Hooker 1964). Germination occurs in the presence of free moisture with the optimum temperature of 25°C. The percentage of conidial germination was not affected by the age of the plants and leaves (Levy and Cohen 1983b). After germination spores produced germ tubes which were 20 - 150u long and, in general, grew at an angle rather than parallel to the veins of the leaf. Germ tubes developed mostly from apical cells of the spores and produced simple or forked terminal appressoria (Hilu and Hooker 1964, Shree 1987). Appressoria played an auxiliary role in survival and increased the chances of successful infection(Emmet and Parbery 1975). However, Levy and Cohen (1983a) reported that the appressoria of E. turcicum were very sensitive to desiccation

Levy and Cohen (1983b) reported that appressoria were formed abundantly on leaf surfaces due to the stimulating effect of the leaves, but not on 2% water agar. From the appressoria infection pegs developed which grew into or between epidermal cells on either the dorsal or ventral sides of the leaf (Hilu and Hooker 1964, Shree 1987). Knox-Davies (1974) reported the formation of more than one infection hyphae from the appressorium of the fungus.

2.5.2 Penetration and colonization

The penetration was mostly direct and occurred 12-20 hours after inoculation, and was similar in resistant and susceptible leaves of young and mature sorghum and maize plants (Jennings and Ullstrup 1957, Hilu and Hooker 1964, Knox-Davies 1974, Shree 1987). After penetration the fungus produced a vesicle-like structure of 10-30 u diameter in or between the epidermal cells. Hyphae grew slowly in the mesophyl cells but rapidly in xylem cells. The entrance of the fungus into the xylem vessels and tracheids was observed 2-3 days after inoculation. Within 6 days a lesion occupied the area between 2-3 small veins, and later enlarged due to the growth of the hyphae from xylem to the surrounding healthy tissue (Hilu and Hooker 1964, Shree 1987). The hyphae of the fungus established early in the xylem of a susceptible variety with mycelial growth filling the vessels and tracheids, but in a resistant variety hyphae did not grow laterally or longitudinally and rarely branched (Jennings and Ullstrup 1957, Shree 1987).

Providing that inoculum was present the most important factor influencing infection was dew period (Levy and Cohen 1983b). Growth chamber studies showed that the minimal dew period, with the optimum temperature of 20^{o}C, required for infection of the fungus was 5 hours (Levy and Cohen 1983a).

2.6 PLANT GROWTH STAGES AND THEIR SUSCEPTIBILITY TO E. TURCICUM

Leaf blight can be serious on sorghum plants nearing maturity in humid conditions (Tarr 1962). Tuleen (1975) used five different sorghum genotypes at six growth stages and found that plants in the immature stages of plant growth were more susceptible to E.turcicuum than mature plants. Meenakashi and Ramalingam (1979) reported that sorghum leaf blight severity reached a peak when plants were in flowering stage. Shree (1983) reported that the fungus could reduce seed germination.

Shenoi and Ramalingam (1983) reported that leaf blight was more prevalent on vigorously growing sorghum plants in the post-flowering stage, and could develop into epidemic form. They also noticed that sorghum plants that had seedling resistance to the disease had adult susceptibility. Tuleen (1975) and Frederiksen (1980) reported that sorghum plants which were highly resistant to leaf blight developed some lesions when inoculated in the early seedling stages of growth.

2.7 HOST RANGE AND PATHOGENIC SPECIFICITY ON MAIZE AND SORGHUM

E.turcicum has a wide host range and under natural conditions it infects sorghum, teosinte, kodo millet , and maize, but in specific inoculations it attacks wheat, barley, oats, sugarcane, and rice (Shaw 1921, Mitra 1923, and Misra 1979). This is important in the epidemiology of the disease. It indicates the possibility that the initial inoculum could come from any of these hosts if the fungus lacks host specificity.

Isolates of E. turcicum that infected sorghum were different from those which infected maize (Shaw 1921), and differed culturally and pathogenically although they were similar in morphology (Mitra 1923). Lefebvre and Helen (1945) reported that isolates from sorghum failed to infect maize, while isolates from maize infected sorghum. Bhowmik and Prasada (1970) reported that isolates of the fungus from maize and sudan grass infected both of these hosts and Johnson grass but not sorghum. Isolates from sorghum were pathogenic to all of the four hosts tested. Arjunan et al. (1976) reported that sorghum isolates of the fungus infected Eleusine coracana, Pennisetum typhoides, Setaria italica, and Panicum maximum.

Masias and Bergquist (1974) reported that isolates of the fungus which are pathogenic to only maize, sorghum or sudan grass were homokaryons. Isolates which were pathogenic to both sorghum and maize were heterokaryons(when both mycelia and conidia contained different nuclei).

Misra (1979) reported that E. turcicum can infect maize, several millet species(such as Setaria italica, Eleusine coracana, and Paspalum scrobiculatum), sudan grass, Johnson grass, and teosinte. Shankerlingam and Balasubramanian (1984) reported that a sorghum isolate of the fungus infected maize. Sisterna (1985) tested the pathogenicity of isolates of E. turcicum from maize and sorghum on a range of cereals in a greenhouse, and found that only sorghum and maize were infected with similar symptoms. Hamid and Aragaki (1975) worked on 47 isolates of Setosphaeria turcica from sorghum and Johnson grass and observed that 18 isolates were virulent only to the host species from which each was isolated. The remaining 29 isolates were virulent to at least one other host.

Misra and Mishra (1971) made a comparative study of four sorghum isolates of the fungus from four widely separated localities in India. They observed that there is a difference among the isolates in their physiological characters, pathogenicity, viability and colony growth at different temperatures.

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

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1 ISOLATION OF THE FUNGUS

The fungus E. turcicum was isolated from typical blight lesions of naturally infected leaves of sorghum collected from the field at ICRISAT Center, Patancheru. Leaf bits of about 2 mm^2 which consisted of infected and healthy parts of the leaves were cut and surface sterilized with 0.15% mercuric chloride solution for 1-2 min., and washed in a series of sterile distilled water to remove the disinfectant. The pieces were transferred to sterilized filter paper in a Petri dish to remove the excess water; finally three to four bits were carefully plated on lactose casein hydrolysate agar (Rangaswamy 1984). Inoculated plates were incubated in a Percival incubator at 25°C with 12 hours of light followed by 12 hours of darkness.

After the fungus had grown on the media sporulation was observed. A spore suspension was prepared, a drop was put on a slide and observed under the microscope to verify the typical spores of the fungus. The dimensions (septation, length, width) of hundred spores was measured . Later pure cultures of the fungus were prepared by using the single

spore isolation technique(Jones and Clifford 1983), and maintained on lactose casein hydrolysate agar slants.

3.2 PATHOGENICITY TESTS

To test the pathogenicity of the isolate of E. turcicum from sorghum, three different conidial concentrations(5,000, 10,000, and 20,000 conidia mL^{-1}.) were Aelected. leaf blight susceptible variety of sorghum, Framida(IS 3167), was planted in the greenhouse in 18 cm diameter pots. The number of plants per pot was five.

3.2.1 Preparation of inoculum

The inoculum was prepared by washing the conidia from 14- days-old cultures of the fungus grown on lactose casein hydrolysate agar in Petri plates. About 5 mL of distilled water was added to each plate, and the conidia were loosened with a rubber policeman; the suspension was filtered through two layers of cheese cloth. To the suspension was added 0.1% of Tween 20 (polyoxyethylene sorbitan monooleate), a surfactant which spreads inoculum uniformity on leaves. Three conidial concentrations (5,000, 10,000, and 20,000 conidia mL^{-1}) were established by counting the spores on a haemocytometer. About 250 mL of each concentration was put in a separate plastic hand sprayer.

3.2.2 Inoculation of plants

Twenty-days-old plants were inoculated in the greenhouse at 18.00 hr by spraying conidial suspensions on both sides of the leaves with a hand sprayer. Each concentration was sprayed on three pots with five plants per pot, or a total of 15 plants for each treatment. About 15 mL of the suspension was sprayed on each pot. The control was sprayed only with distilled water.

After inoculation plants were kept in an inoculation chamber made up of polyethylene in the greenhouse for 48 hours in a condition where relative humidity was above 95% by using humidifiers. After that plants were taken out and kept inside the greenhouse and symptoms were observed. Disease scores were made on the upper leaves at 4 and 14 days after inoculation using a 1-5 rating scale (Table 1).

Table 1. The 1-5 leaf blight disease rating scale (AICSIP)

Rating value	% Leaf area infected	Description
1	0	Free from disease
2	<10	Slight symptoms
3	11-25	Moderate symptoms
4	26-40	Moderately severe symptoms
5	>40	Very severe symptoms

The data were analysed and comparisons made between treatments.

3.3 MASS PRODUCTION OF CONIDIAL INOCULUM

To produce abundant conidial inoculum for inoculation of host plants it was necessary to determine the media and temperature for sporulation of E. turcicum. The effect of seven media (Table 2) and five temperatures (Section 3.3.3) on colony growth and sporulation were investigated.

Table 2 Contents of the media

Name of the medium	Contents	
Lactose casein hydrolysate agar	Lactose	37.5 g
	Casein hydrolysate	3.0 g
	Potassium phosphate	1.0 g
	Magnesium sulphate	0.3 g
	Agar	15.0 mL
	Micro elements	2.0 mL
	(Ferric nitrate	723.5 mg
	Zinc sulphate	439.0 mg
	Manganese sulphate	203.0 mg)
	Distilled water	1.0 L
Potato dextrose agar(PDA)	PDA powder	39.0 g
	Distilled water	1.0 L
Sorghum and maize leaf extracts agar	Green leaf extract	20.0 mL
	Dextrose	20.0 g
	Agar	20.0 g
	Distilled water	1.0 L
Sorghum and maize grain extracts agar	Grain extract	20.0 mL
	Dextrose	20.0 g
	Agar	20.0 g
	Distilled water	1.0 L
Sorghum leaf medium	Chopped sorghum leaves	7.0 g

3.3.1 Preparation of the media

Contents of the media used are given in Table 2. Lactose casein hydrolysate agar and potato dextrose agar were prepared according to Tuite (1969). Sorghum leaf extract agar and maize leaf extract agar were prepared by putting green leaves of sorghum and maize (250 g each)in a 2000 mL beaker and 500 mL of water was added. After that it was boiled in a microwave oven for 10-15 minutes, then the solution was filtered in a cheese cloth to take out the extract. Later twenty mL of the extract were added to one litre of the medium. Sorghum grain extract agar and maize grain extract agar were prepared by the same method using grain of the respective hosts. One litre of each medium was prepared and put in 500 and 250 mL conical flasks, plugged with cotton wool and then sterilized in the autoclave for 15 minutes at 121.6^{o}C and 1.03 x 10^{4} Pa.

Leaf medium was prepared by collecting the green leaves of the leaf blight susceptible sorghum variety Framida. Leaves were chopped into 1-2 cm pieces. Seven grams of the chopped leaves were put in 100 mL conical flasks and 1 mL of sterilized distilled water was added to each flask. The flasks were plugged with cotton wool and then sterilized in the autoclave for 20 minutes at 121.6^{o}C and 1.03 x10^{4} Pa.

After sterilization about 10-15 mL of the liquid media were poured into sterilized plastic Petri plates in the Laminar flow. The plates were then allowed to solidify for 4-5 hours before inoculation.

3.3.2 Inoculation of the media

Plates were inoculated by placing at the center of each plate agar discs of 5 mm diameter. The discs were removed aseptically by using a sterilized cork borer from margins of a highly sporulating 14-days-old cultures of the fungus grown on lactose casein hydrolysate agar.

To measure the sporulation of leaf medium twelve flasks were inoculated to incubate at each temperature. Each flask was inoculated with an agar disc of 7mm size removed from cultures of the fungus by using a cork borer.

3.3.3 Incubation of inoculated media

The inoculated flasks and plates were incubated at five temperatures(15°C, 20°C, 25°C, 30°C, and 35°C) with 12 hours of light and dark alternately in Percival incubators.

3.3.4 Colony growth of *E. turcicum*

The radial colony growth of the fungus on different solid media under different temperatures was measured every

48 hours for a period of two weeks.

The data were taken by measuring the colony growth of three plates of each medium (each plate as a replication). The plates were taken out of the incubator and put upside down and observed under light. Radial mycelial growth was measured using a graduated ruler .

3.3.5 Sporulation of *E. turcicum*

The sporulation of the fungus was studied on the same six solid media and the sorghum leaf medium. Sporulation was determined once every four days for a period of sixteen days. Four plates of each medium was used for sporulation measurement.

To measure the spores on solid media, three disks of 7 mm size were removed from each plate by using a cork borer. Each disk was put in a test tube with 5 mL of distilled water and considered as one replication. The spore suspension was prepared by crushing the discs with a glass rod and agitating the suspension with a vortex mixer. The spore counts were made by putting a drop of the suspension on a haemocytometer and observing under the microscope. Then the number of spores per mL was estimated by using these spore counts made on the haemocytometer.

The quantity of spores in the sorghum leaf medium was measured by adding 50 mL of distilled water to each flask. The suspension was shaken on a vortex flask shaker. Then a drop of the suspension was put on a haemocytometer and observed under the microscope to count the spores and number of spores mL^{-1} estimated.

After all the measurements were completed, the data were analysed statistically by using Anova and the least significant difference (LSD) was calculated.

3.4 INOCULATION METHODS

To determine the most effective method of inoculation of sorghum with E. turcicum, five different inoculation methods were studied.

1) Diseased sorghum leaves buried in soil,

2) Diseased sorghum leaves spread over soil,

3) Diseased sorghum leaves placed in leaf whorls,

4) Conidial suspensions of the fungus sprayed onto leaves,

5) Conidial suspensions of the fungus placed in leaf whorls,

6)Control(no inoculation).

Two sorghum varieties susceptible to leaf blight (Framida and Local FSRP) were planted in 36 pots of 18 cm diameter(18 pots of each variety), with five plants per pot. The pots were watered every other day , 3 g of diammonium

phosphate was applied during planting. Carbofuran was applied at planting, and when plants were 10 days old for shootfly control. The experimental design was completely randomized. Treatments were five inoculation methods and two sorghum varieties replicated three times.

3.4.1 Diseased sorghum leaves buried in soil

Sorghum leaves naturally infected with leaf blight were collected from the field and air dried. The leaves were then stored in gunny bags in the laboratory, and crushed into small pieces in a grinding machine(Gley Creston micro-hammer mill) before use.

About 25 g of these leaves were buried at 5 cm. depth in soil of six pots of 18 cm diameter. After four days the pots were sown with two sorghum varieties, Framida and Local FSRP(3 pots for each variety, and 5 plants per pot) in a the greenhouse. After that the plants were observed daily and disease symptoms were recorded.

3.4.2 Diseased sorghum leaves spread over soil

About 25 g of diseased leaves collected as described above, were spread over the soil of six pots after the emergence of the plants and the appearance of disease symptoms on the leaves were recorded for two weeks.

3.4.3 Diseased sorghum leaves placed in leaf whorls

About 10 g of diseased leaves were placed in the leaf whorls of 20-days old plants of two varieties planted in pots in the greenhouse. After that the plants were put in the inoculation chamber of the greenhouse with above 95% relative humidity for 48 hours. Later pots were taken out, kept in the greenhouse and the disease incidence was observed and recorded. The disease was scored 4 and 14 days after inoculation.

3.4.4 Conidial suspensions of the fungus sprayed onto leaves

Conidial suspensions were prepared from cultures of the fungus grown on lactose casein hydrolysate agar (as described under section 3.2.1) and the conidial concentration was adjusted to 20,000 conidia mL^{-1}.

The conidial suspension was sprayed onto leaves of 20-days-old plants at the 5-leaf stage of the same two varieties planted in pots. After inoculation plants were kept in the inoculation chamber of the greenhouse for 48 hours. After that the plants were taken out and kept in the greenhouse, and daily observations were made for the appearance of symptoms. Disease scorings were made 4 and 14 days after inoculation.

3.4.5 Conidial suspensions of the fungus placed in leaf whorls

About 10 mL of conidial suspensions was poured in the leaf whorls of 20-days old plants of the same two varieties in six pots. After that plants were kept in the inoculation chamber of the greenhouse for 48 hours and taken out. Disease observations were taken in the same way as in the previous treatments.

3.4.6 Disease scoring

The first disease score of all the treatments was made 4 days after inoculation and two weeks later using a 1-5 rating scale (Table 1).

3.4.7 Data analysis

The data were statistically analyzed by using Anova and the least significant difference (LSD) was calculated. Then comparisons were made for different inoculation methods.

3.5 INFECTION PROCESS

To study the infection process of E. turcicum on sorghum, two cultivars of sorghum, Framida (leaf blight susceptible) and IS 8283 (leaf blight resistant) were planted in pots in the greenhouse. Three pots of each variety with five plants each were planted.

When plants were 25-30 days-old, pieces of about 12 cm^2 size were cut from the leaves of each variety. The leaf pieces were then put in sterilized Petri plates with moistened blotting paper to serve as moist chambers. About 2-3 pieces of each variety were kept in separate plates. After that each leaf piece was inoculated on both sides with a conidial suspension (40,000 conidia mL^{-1}) to which had been added a drop of a Tween 20 as a spreader. The suspension was prepared from 14-days-old cultures of the fungus grown on lactose casein hydrolysate agar. The inoculated leaf pieces were kept between two layers of moistened paper in the plates. The plates were incubated at room temperature.

The inoculated leaf pieces were sampled at hourly intervals for the first eight hours, and then at two-hour intervals for the next 24 hours, and finally one sample was taken every 12 hours for the next 120 hours. During sampling, three pieces of about 3 cm^2 each were cut from the center of the 12 cm^2 pieces of each variety using a scissor. Each piece was considered as one replication. The pieces were then put in glass vials of 15 mL size (three pieces in each bottle) which contained about 5 mL of Corny's solution(one part of glacial acetic acid and two parts of ethyl alcohol) and kept in an oven at 60°C for 8 hours for leaf clearing.

After clearing, Corny's solution was drained off and lactophenol (20 mL lactic acid, 20 mL phenol, 40 mL glycerol, and 20 mL of distilled water) was added and kept at 60°C for 5 hours . The leaf pieces were then taken out of lactophenol, transferred to Petri dishes, and stained with a solution of 0.2% trypan blue for 1.5 hours. After that the pieces were washed in distilled water 3 or 4 times to remove the excess colour of the stain, and the pieces were ready for observation. This procedures was used by Knox-Davies (1974), and Elazegui and Exconde (1973), but slight modifications were made in this investigation in reference to the staining procedures. In here samples were stained in 0.2% trypan blue.

Then each piece was put on one slide with the addition of a few drops of polyvinyl alcohol for mounting and covered with a cover slip and observed under a microscope (Olympus binocular phase). For observation of each sample from each variety, the following data were recorded: 1) number of spores germinated and type of germination, 2) germ tube and appressorial formation, 3) penetration, whether direct through the cuticle or through stomata, and 4) colonization. A photographic camera(Olympus C.35 AD) with black and white film was fixed on the microscope (Olympus binocular phase) to take photographs of different stages of the

infection process.

3.6 PLANT GROWTH STAGES AND SUSCEPTIBILITY TO E. TURCICUM

To determine the growth stage at which sorghum is more susceptible to leaf blight, two susceptible sorghum varieties (Framida and Local FSRP) were planted in pots of different sizes at ten days intervals in the greenhouse. Six growth stages of each variety from 10 to 60 days after emergence were as follows:

Stage 1: 3-leaf stage reached 10 days after emergence.

Stage 2: 5-leaf stage reached 20 days after emergence.

Stage 3: growing point differentiation approximately 8-leaf stage reached 30 days after emergence.

Stage 4: flag leaf visible reached 40 days after emergence.

Stage 5: boot stage reached 50 days after emergence

Stage 6: 50% flowering (half of the plants in this stage) reached 60 days after emergence.

The experimental design was a randomized complete block. Treatments were six growth stages of two sorghum varieties replicated three times. Each stage was planted in six pots, the number of plants per pot was five.

Plants were inoculated by spraying conidial suspensions of the fungus at a concentration of $2x10^4$ conidia mL^{-1} on both sides of the leaves. Plants were then kept in the

inoculation chamber of the greenhouse with above 95% relative humidity for 48 hours. After that plants were kept outside and the first appearance of symptoms, type of symptoms, and on which leaves symptoms appeared first were recorded. Disease scores using a 1-5 rating scale (Table 1) were made 4 and 14 days after inoculation. Data were analyzed and the mean disease incidence of different stages were compared.

3.7 *E. TURCICUM* ISOLATES FROM SORGHUM AND MAIZE AND THEIR PATHOGENICITY ON GENOTYPES OF BOTH HOSTS

3.7.1 Collection of the isolates

Isolates of the fungus from sorghum and maize were collected from different locations in Andhra Pradesh in October 1990. Maize isolates were collected from Undavelly, Amberpet, Biknoor, and Patancheru, and sorghum isolates were collected from Kurnool, Karimnagar, Momlapalli and Patancheru. The isolates were named after the place of collection .

The fungus was isolated from the typical lesions of leaf blight on the naturally infected leaves collected from both hosts. All the fungal isolates were grown on a lactose casein hydrolysate agar in Petri plates and pure cultures

were prepared.

The inoculum was prepared from washings of conidia from 14-days-old cultures in Petri plates, and the suspension was filtered through two layers of cheese cloth . The conidial concentration of the suspension for each isolate was adjusted to 20,000 conidia mL^{-1}. The inoculum of each isolate was used in a separate hand sprayer at the time of inoculation.

Four leaf blight susceptible varieties of maize (DH.103, CM.500, Ganga 5 and Aswani) and three leaf blight susceptible varieties of sorghum (Framida, Local FSRP, IS 2858) were sown in pots in the greenhouse. Each variety was sown in 24 pots.

3.7.2 Inoculation

Plants were inoculated 25 days later (at approx. 7-leaf stage) in the greenhouse by spraying conidial suspensions (20,000 conidia mL^{-1}) on both sides of the leaves. Each isolate was inoculated onto three pots of each variety with five plants per pot, or a total of 15 plants of each variety.

After inoculation the pots were kept in the inoculation chamber of the greenhouse at above 95% relative humidity for 48 hours. Thereafter plants were taken out, and daily observations were made for the appearance of disease symptoms for all varieties and data were taken if there was infection or not.

RESULTS

CHAPTER IV

RESULTS

4.1 PATHOGENICITY TESTS

The results obtained from pathogenicity tests of E. turcicum on sorghum with three different conidial concentrations are presented in Table 3 and Figure 9.

The highest disease score (above 40% leaf area damaged) was observed on plants sprayed with the highest conidial concentration (20,000 conidia mL^{-1}). But there was no significant difference between the infection caused by 20,000 and 10,000 conidia mL^{-1} . The lowest infection (less than 10% leaf area damaged) was seen on plants sprayed with the lowest conidial concentration (5,000 conidia mL^{-1}).

Table 3. Mean*disease score for pathogenicity tests of E. turcicum on sorghum variety Framida with three conidial concentrations.**

Number of conidia mL^{-1}	Mean disease score
20000	4.5
10000	4.0
5000	2.5
Control	1.0
SE	± 0.34
LSD 5%	0.58
LSD 1%	0.96

* 6 Replications
** 1-5 scale (Table 1)

4.2 MASS PRODUCTION OF CONIDIAL INOCULUM

4.2.1 Effects of temperature and media on colony growth of E. turcicum.

The results obtained from colony growth and sporulation of the fungus on lactose casein hydrolysate agar at different temperatures are presented in Table 4 and Figures 1 and 11A. Fastest colony growth was recorded at 30°C and the lowest colony growth at 35°C. Colony growth was good at 20 and 25°C, but poor at 15°C.

Table 4. Mean* colony growth of E. turcicum (mm) on lactose casein hydrolysate agar at different temperatures and days of incubation.

Temp.	Colony growth (mm) Days of incubation					
°C	2	4	6	8	10	12
15	1.0	3.0	5.5	7.0	8.0	10.0
20	4.0	8.5	16.0	22.0	33.0	38.0
25	5.0	11.0	17.0	25.0	31.0	33.5
30	10.0	24.5	38.5	40.0	40.0	40.0
35	2.5	3.0	3.0	4.0	5.0	6.0
SE						± 2.20
LSD 5%						6.14
LSD 1%						8.11

* 6 Replications

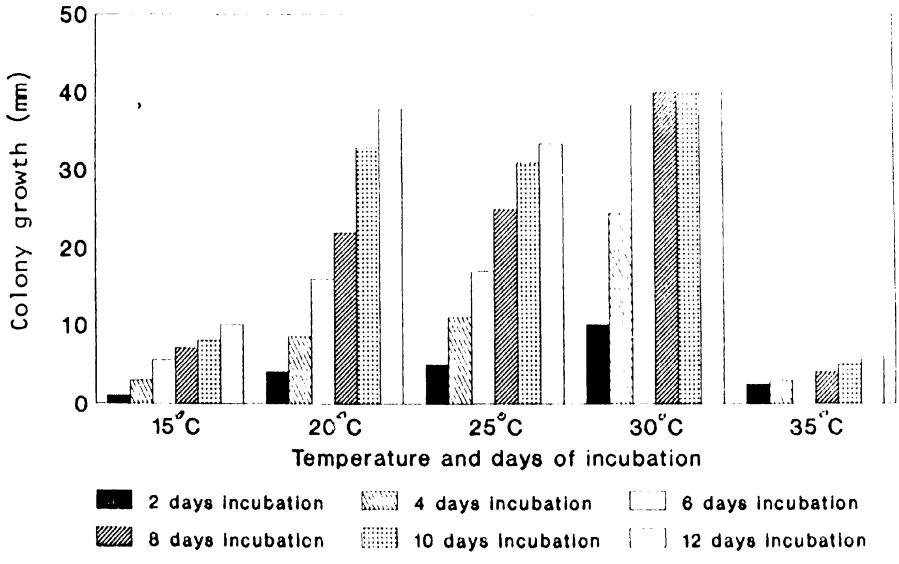


Fig.1. Colony growth of E.turcicum at different temperatures and days of incubation on lactose case in hydrolysate agar.

On potato dextrose agar the fastest colony growth of the fungus was observed at 30°C, but there was no significant difference between 20, 25 and 30°C. The lowest colony growth was recorded at 35°C (Table 5 and Figs. 2 and 10A).

Table 5. Mean* colony growth (mm) of E. turcicum on potato dextrose agar at different temperatures and days of incubation.

Temp. °C	Colony growth (mm) Days of incubation 2	4	6	8	10	12
15	1.0	2.0	3.5	5.0	8.5	10.0
20	3.0	6.0	12.5	15.0	17.5	24.0
25	3.5	9.0	19.0	23.0	27.0	28.0
30	3.5	6.0	11.0	15.0	20.0	28.5
35	1.5	2.0	2.0	2.0	2.0	2.0
SE						± 2.20
LSD 5%						6.14
LSD 1%						8.10

* 6 Replications

On sorghum leaf extract agar highest colony growth of the fungus was recorded at 25°C after 12 days of incubation. Colony growth was minimum at 35°C (Table 6 and Figs. 3 and 11C)..pa

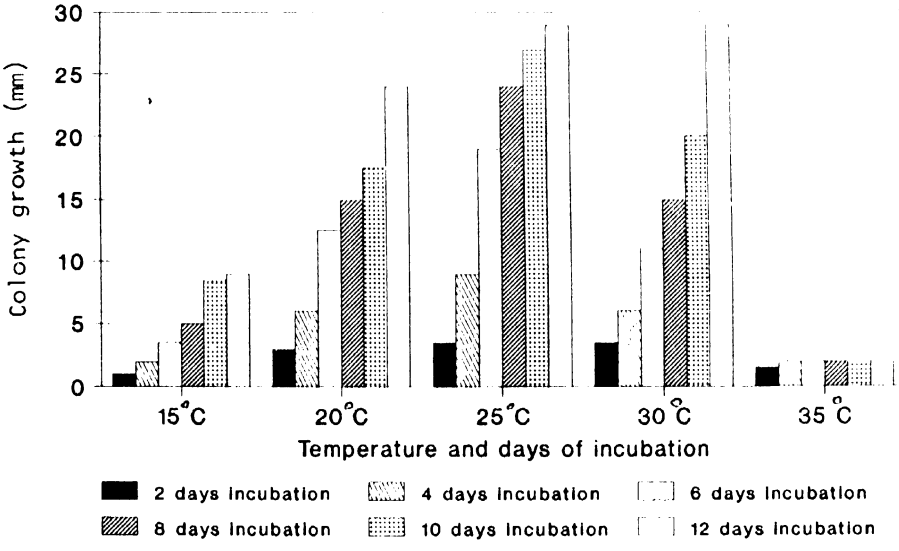


Fig.2. Colony growth of E.turcicum at different temperatures and days of incubatirn on potato dextrose agar.

Table 6. Mean* colony growth (mm) of E. turcicum on sorghum leaf extract agar at different temperatures and days of incubation.

Temp. °C	Colony growth (mm) Days of incubation 2	4	6	8	10	12
15	1.0	3.5	4.5	7.0	10.5	14.5
20	3.5	7.0	13.5	15.0	23.0	31.5
25	4.5	12.5	19.5	27.0	32.0	38.5
30	8.0	13.5	22.0	29.0	32.0	33.5
35	2.5	3.5	4.5	5.0	8.0	10.0
SE						± 2.20
LSD 5%						6.14
LSD 1%						8.10

* 6 Replications

The highest colony growth on maize leaf extract agar was recorded at 25°C and the lowest at 35°C Table 7 and Figures 4 and 11B.

On maize grain extract agar colony growth was maximum at 25°C and very poor at 35°C. (Table 8 and figures 5 and 11D).

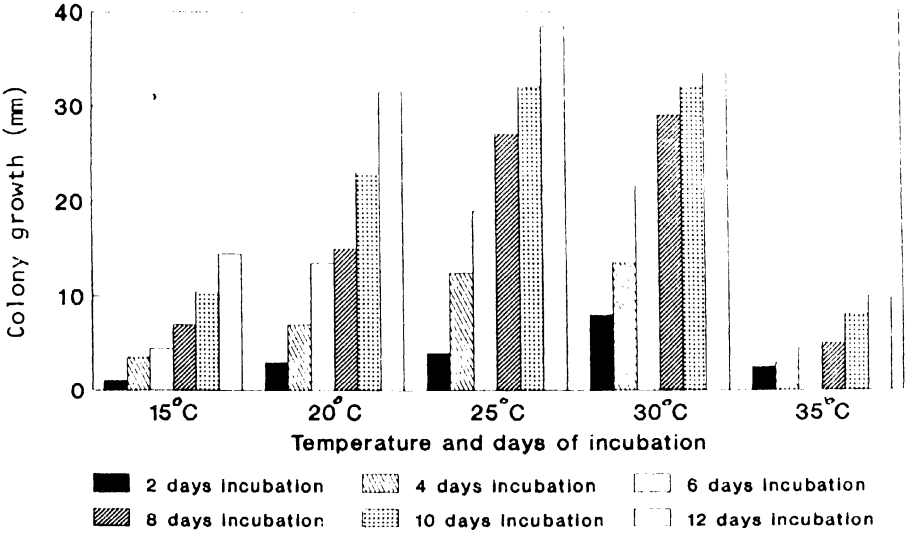


Fig.3. Colony growth of E.turcicum at different temperatures and days of incubation on sorghum leaf extract agar.

Table 7. Mean* colony growth (mm) of E. turcicum on maize leaf extract agar at different temperatures and days of incubation.

Temp. °C	Colony growth (mm) Days of incubation 2	4	6	8	10	12
15	1.0	4.0	6.5	9.0	10.5	13.5
20	2.5	5.5	10.0	14.5	19.0	24.0
25	3.5	8.0	17.0	21.0	26.5	34.0
30	4.0	6.5	12.5	16.0	20.0	27.0
35	2.5	3.0	3.0	3.5	4.5	5.5
SE						± 2.20
LSD 5%						6.14
LSD 1%						8.11

* 6 Replications

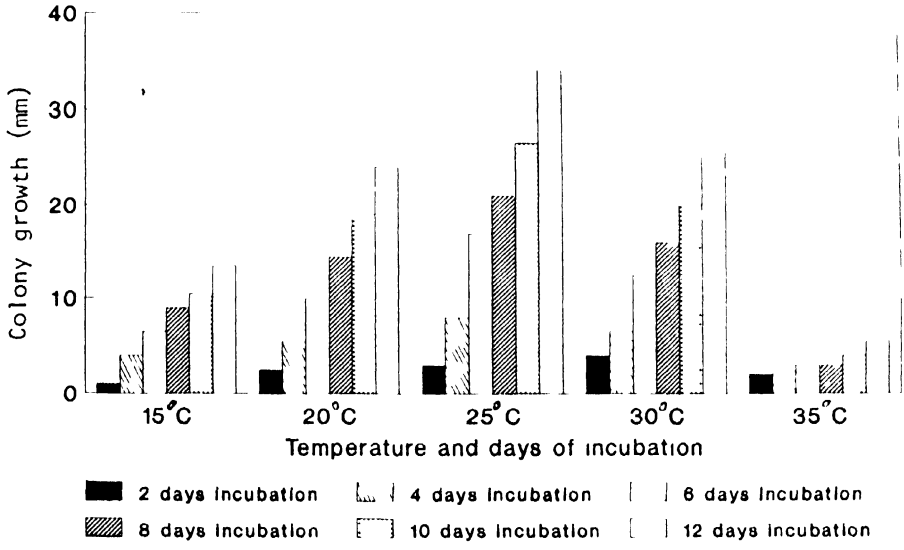


Fig.4. Colony growth of E.turcicum at different temperatures and days of incubation on maize leaf extract agar.

Table 8. Mean* colony growth (mm) of E. turcicum on maize grain extract agar at different temperatures and days of incubation.

Temp. °C	Colony growth (mm) Days of incubation 2	4	6	8	10	12
15	1.0	3.5	5.5	7.0	9.0	12.0
20	4.0	9.5	16.0	23.0	28.0	37.0
25	4.5	14.0	23.0	33.0	38.0	38.5
30	5.0	9.0	19.0	25.0	30.5	35.0
35	2.5	3.0	3.5	6.0	6.5	8.5
SE						± 2.20
LSD 5%						6.14
LSD 1%						8.10

* 6 Replications

The highest colony growth on sorghum grain extract agar was recorded at 30°C, but there was no significant difference between 25 and 30°C. The lowest colony growth was observed at 35°C (Table 9 and Figs. 6 and 9B).

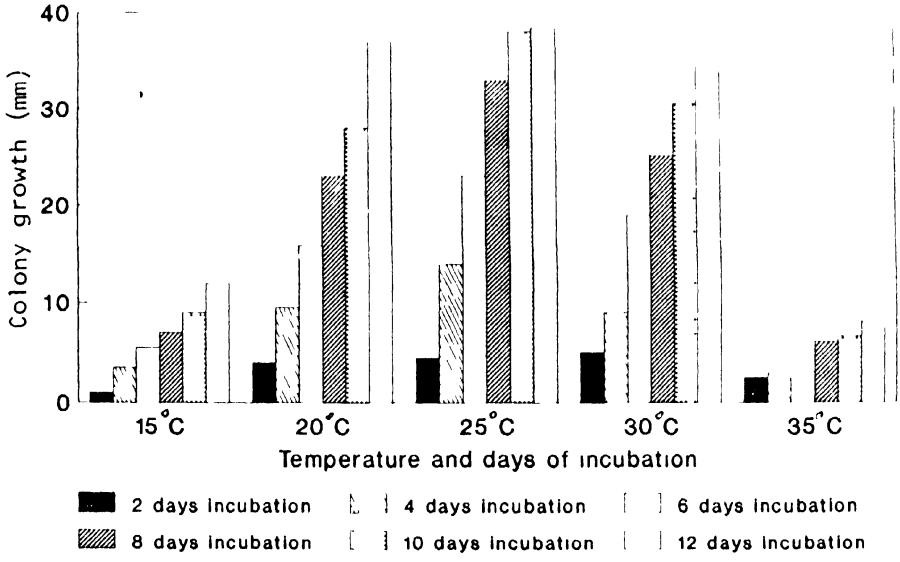


Fig.5. Colony growth of E.turcicum at different temperatures and days of incubation on maize grain extract agar.

Table 9. Mean* colony growth (mm) of E. turcicum on sorghum grain extract agar at different temperatures and days of incubation.

Temp.	Colony growth (mm) Days of incubation					
°C	2	4	6	8	10	12
15	1.0	3.5	6.0	8.0	12.5	15.0
20	3.5	8.0	13.5	20.0	26.5	31.0
25	3.0	10.0	19.0	24.5	35.0	39.0
30	3.5	7.5	21.5	33.0	39.0	40.0
35	2.0	3.0	3.0	5.5	6.0	9.0
SE						± 2.20
LSD 5%						6.15
LSD 1%						8.10

* 6 Replications

4.2.2 Effects of temperature and media on sporulation

The sporulation of the fungus on lactose casein hydrolysate agar was maximum at 20°C, very poor at 15°C, and there was no sporulation at 35°C (Table 10). The fungus started to sporulate after 4 days of incubation at 20 and 25°C.

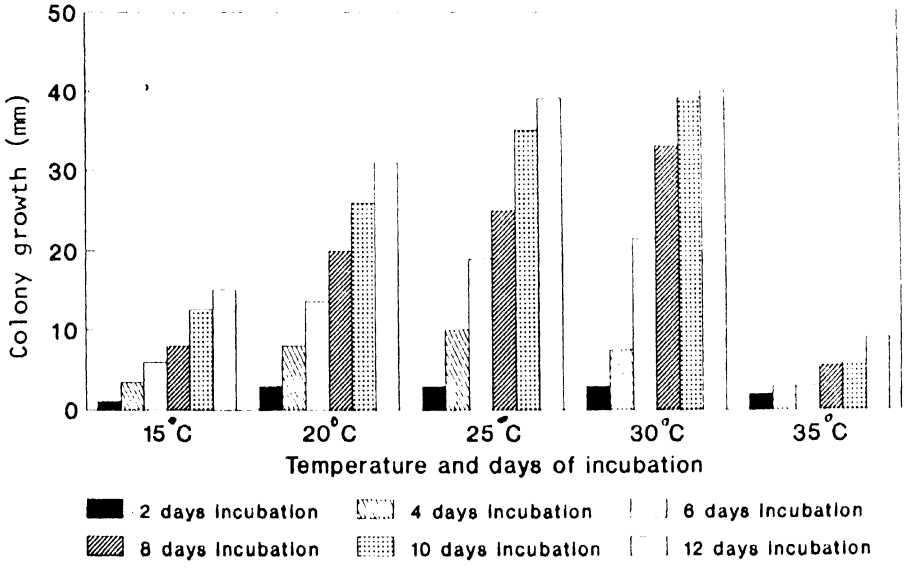


Fig.6. Colony growth of E.turcicum at different temperatures and days of incubation on sorghum grain extract agar.

Table 10. Mean* sporulation of E. turcicum as number of spores mL^{-1} on lactose casein hydrolysate agar at different temperatures and days of incubation.

Temp.	Number of spores mL^{-1} Days of incubation			
°C	4	8	12	16
15	0	10,000	13,300	16,700
20	10,000	80,000	113,000	213,300
25	20,000	36,700	50,000	80,000
30	0	13,300	16,700	53,300
35	0	0	0	0
SE				± 9,370
LSD 5%				26,230
LSD 1%				34,670

* 6 Replications

Maximum sporulation of the fungus on potato dextrose agar was observed at 25°C and there was no sporulation at 35°C (Table 11). Sporulation was good at 20 and 30°C and poor at 15°C.

Maximum sporulation of the fungus on sorghum leaf extract agar was recorded at 25°C. The sporulation was very poor at all the other temperatures (Table 12).

The sporulation of the fungus on maize leaf extract agar was very poor at all temperatures (Tables 13).

On maize grain extract agar sporulation was low at all temperatures (Table 14).

Table 11. Mean* sporulation of E. turcicum as number of spores mL^{-1} on potato dextrose agar at different temperatures days of incubation

Temp.	Number of spores mL^{-1} Days of incubation			
°C	4	8	12	16
15	0	10,000	10,000	23,300
20	6,700	46,700	60,000	190,000
25	6,700	40,000	73,300	230,000
30	0	3,300	26,700	53,300
35	0	0	0	0
SE				± 9,370
LSD 5%				26,230
LSD 1%				34,670

* 6 Replications

Table 12. Mean* sporulation of E. turcicum as number of spore mL^{-1} on sorghum leaf extract agar at different temperatures and days of incubation.

Temp.	Number of spores mL^{-1} Days of incubation			
°C	4	8	12	16
15	0	0	3,300	10,000
20	3,300	23,300	16,700	23,300
25	3,300	13,300	16,700	33,300
30	0	3,300	6,700	20,000
35	0	0	0	3,300
SE				± 9,370
LSD 5%				26,230

* 6 Replications

Table 13. Mean* sporulation of E. turcicum as number of spores mL^{-1} on maize leaf extract agar at different temperatures and days of incubation.

Temp.	Number of spores mL^{-1}			
	Days of incubation			
°C	4	8	12	16
15	0	3,300	6,700	10,000
20	0	13,300	13,300	16,700
25	6,700	20,000	20,000	23,300
30	0	0	3,300	3,300
35	0	0	0	3,300
SE				± 9,370
LSD 5%				26,230

* 6 replications

Table 14. Mean* sporulation of E. turcicum as number of spores mL^{-1} on maize grain extract agar at different temperatures and days of incubation.

Temp.	Number of spores mL^{-1}			
	Days of incubation			
°C	4	8	12	16
15	0	3,300	3,300	6,300
20	0	0	3,300	13,300
25	3,300	6,700	3,300	23,300
30	6,700	3,300	13,300	13,300
35	0	0	0	0
SE				± 9,370
LSD 5%				26,230

* 6 Replications

The sporulation of the fungus on sorghum grain extract agar was very poor at all temperatures(Table 15) .

Table 15. Mean* sporulation of *E. turcicum* as number of spores mL^{-1} on sorghum grain extract agar at different temperatures and days of incubation.

Temp.	Number of spores mL^{-1} Days of incubation			
°C	4	8	12	16
15	0	0	13,300	13,300
20	0	3,300	3,300	3,300
25	0	0	13,300	13,300
30	3,300	3,300	6,700	10,000
35	0	0	0	0
SE				± 9,370
LSD 5%				26,230

* 6 Replications

The results obtained from the sporulation of the fungus on sorghum leaf medium incubated at different temperatures and days are presented in Table 16.

The data show that the maximum sporulation of the fungus on this medium was recorded at 20°C followed by 25 and 30°C. The lowest sporulation was observed at 35°C. On this medium the fungus sporulated at all temperatures.

Table 16. Mean* sporulation of *E. turcicum* as number of spores mL^{-1} on sorghum leaf medium at different temperatures and days of incubation.

Temp. °C	Number of spores mL^{-1} — Days of incubation			
	4	8	12	16
15	16,700	20,000	23,300	23,300
20	30,000	46,700	180,000	200,000
25	43,300	53,300	83,300	150,000
30	13,300	20,000	36,700	26,700
35	26,700	16,700	30,000	20,000
SE				± 9,370
LSD 5%				26,230
LSD 1%				34,670

* 6 replications

4.3.3 Combined effects of temperature and media on colony growth and sporulation

The results showed that colony growth on all the six media tested were good at 20-30°C after 12 days of incubation. Highest colony growth on most of the media was recorded at 25°C and the lowest at 35°C (Table 17 and Fig. 7).

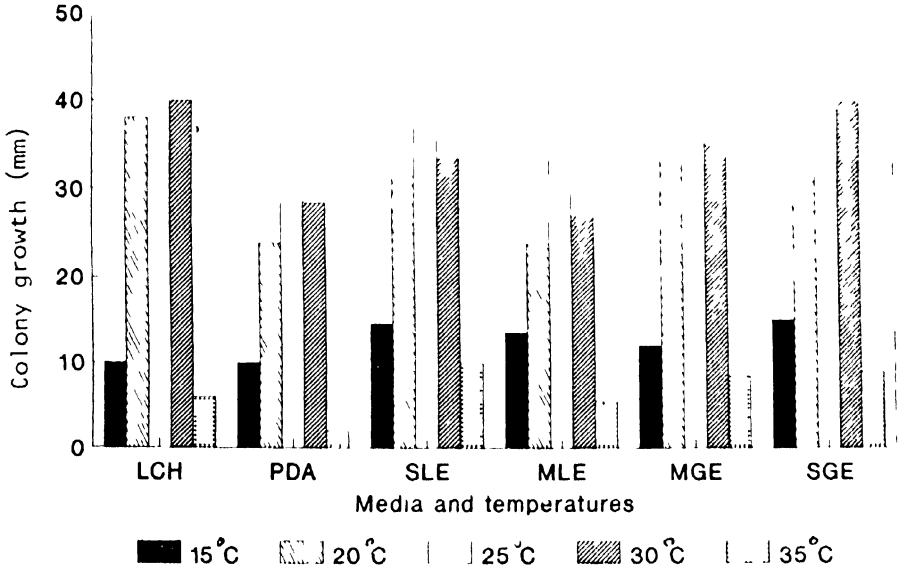
Table 17. Mean* colony growth of E. turcicum on six media and five temperatures after 12 days of incubation.

Temp.	Colony growth (mm) Media**					
°C	LCH	PDA	SLE	MLE	MGE	SGE
15	10.0	10.0	14.5	13.5	12.0	15.0
20	38.0	24.0	31.5	24.0	37.0	31.0
25	33.5	29.0	38.5	34.0	38.5	39.0
30	40.0	28.5	33.5	27.0	35.0	40.0
35	6.0	2.0	10.0	5.5	9.0	9.0
SE						± 2.2
LSD 5%						6.2
LSD 1%						8.1

* 6 Replications
** LCH = Lactose casein hydrolysate agar
PDA = Potato dextrose agar
SLE = Sorghum leaf extract agar
MLE = Maize leaf extract agar
SGE = Sorghum grain extract agar
MGE = Maize grain extract agar

Good colony growth was recorded on lactose casein hydrolysate medium followed by sorghum grain extract and maize grain extract media . Poorest colony growth for all media at all temperatures was observed on potato dextrose agar followed by maize leaf extract (Table 17 and Fig. 7) .

The sporulation of the fungus was good at 20-30°C on most of the media. Highest sporulation was recorded at 20°C followed by 25 and 30°C. There was no sporulation at 35°C



LCH: lactose casein hydrolysate, PDA: potato dextrose agar, SLE: sorghum leaf extract, MLE: maize leaf extract, MGE: maize grain extract, SGE: sorghum grain extract.

Fig.7. Colony growth of E.turcicum on six media at the incubation of five temperatures and 12 days.

for most of the media and it was very poor at 15°C (Table 18 and Fig. 8).

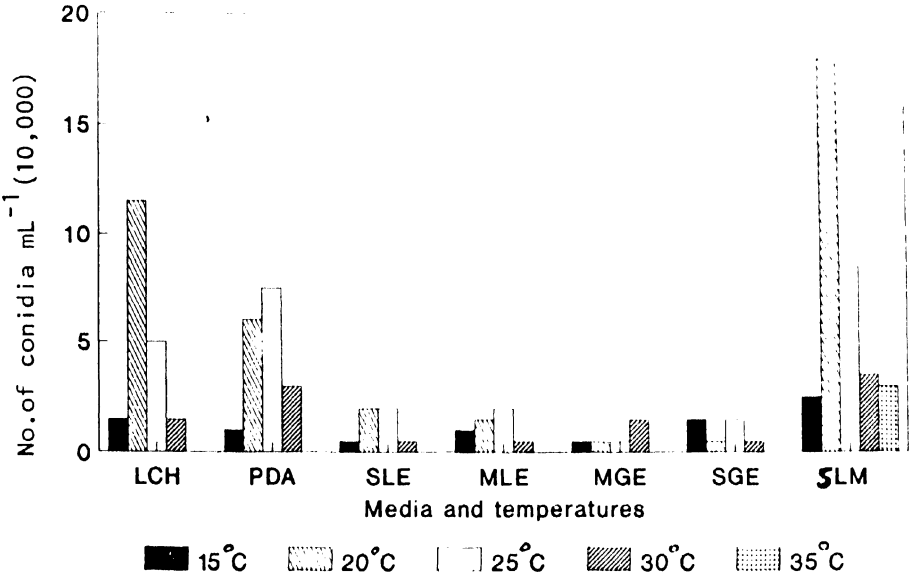
Sorghum leaf medium produced the highest sporulation at all temperatures followed by lactose casein hydrolysate and potato dextrose agar (Table 18).

The sporulation was very poor on sorghum grain extract, maize grain extract, maize leaf extract, and sorghum leaf extract media (Table 18 and Fig. 8).

Table 18. Mean* sporulation of E. turcicum as number of spores mL^{-1} on seven media and five temperatures after 12 days of incubation.

Temp.	Number of spores mL^{-1}						
	Media**						
°C	LCH	PDA	SLE	MLE	MGE	SGE	SLM
15	13,300	10,000	3,300	6,670	3,300	13,300	23,300
20	1,13,300	60,000	16,700	13,300	3,300	3,300	180,000
25	50,000	73,300	16,700	20,000	3,300	13,300	83,200
30	16,700	26,700	6,700	3,300	13,300	6,700	36,600
35	0	0	0	0	0	0	30,000
SE						±	9,370
LSD 5%							26,230
LSD 1%							34,670

* 6 Replications
** LCH = Lactose casein hydrolysate agar
PDA = Potato dextrose agar
SLE = Sorghum leaf extract agar
MLE = Maize leaf extract agar
SGE = Sorghum grain extract agar
MGE = Maize grain extract agar
SLM = Sorghum Leaf medium



LCH: lactose casein hydrolysate, PDA: potato dextrose agar, SLE: sorghum leaf extract, MLE: maize leaf extract, MGE: maize grain extract, SGE: sorghum grain extract.

Fig.8. Sporulation of E.turcicum on seven media at the incubation of five temperatures and 12 days.

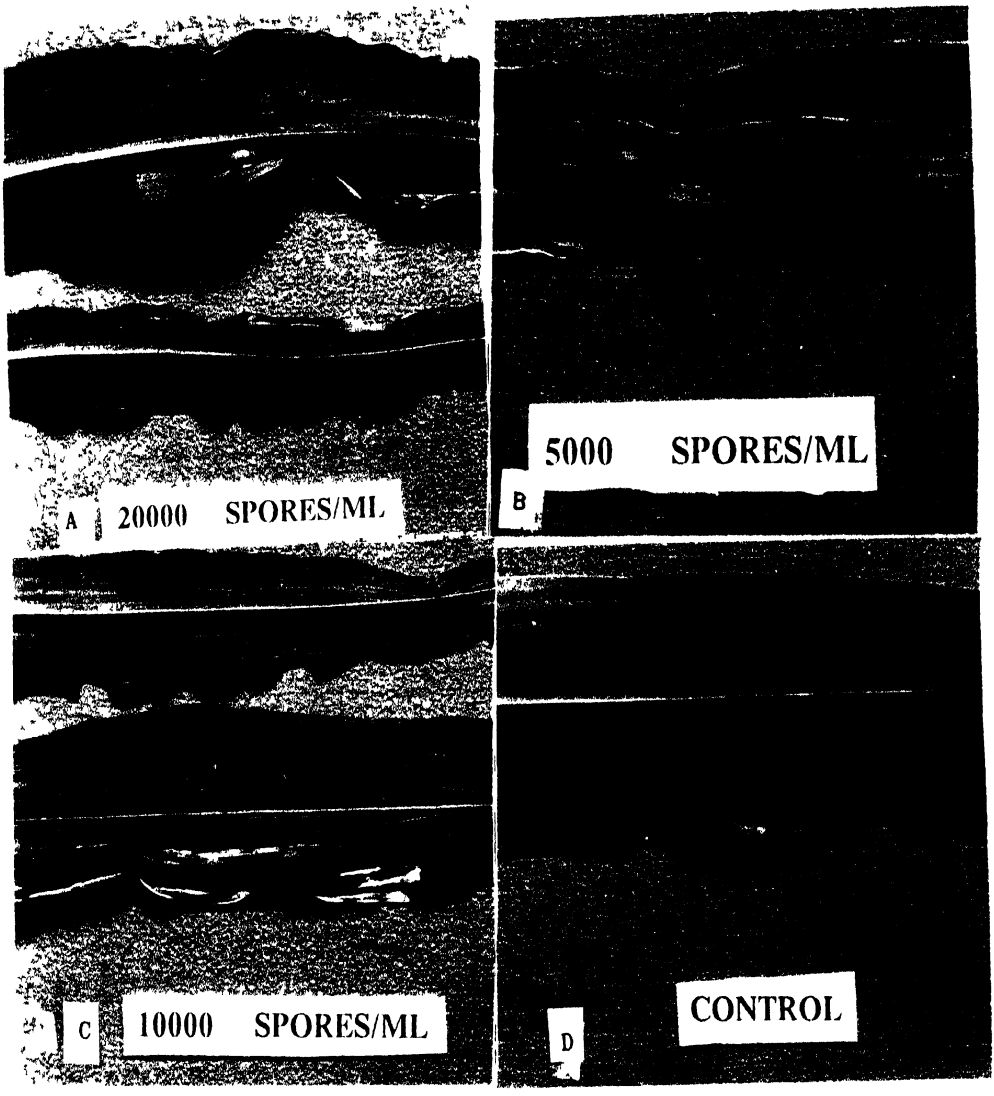


Figure 9: Blight lesions on sorghum (Framida) after 14 days sprayed with 20,000 conidia m^{-1} 1 (A), 5,000 (B), 10,000 (C), and control (D).

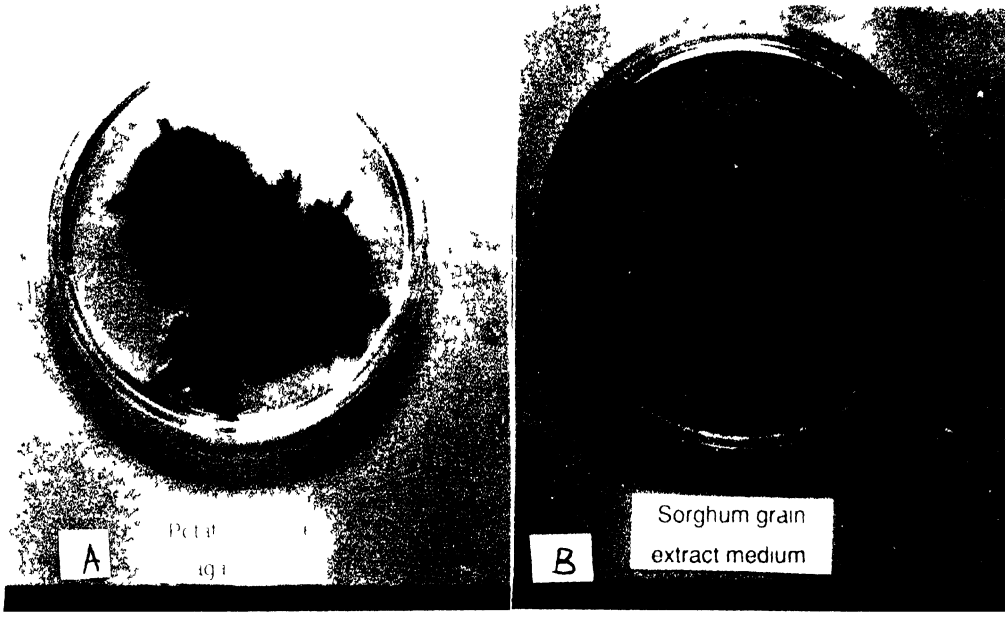


Figure 10. Colony growth of E. turcicum on potato dextrose agar(A) and sorghum grain extract agar(B).

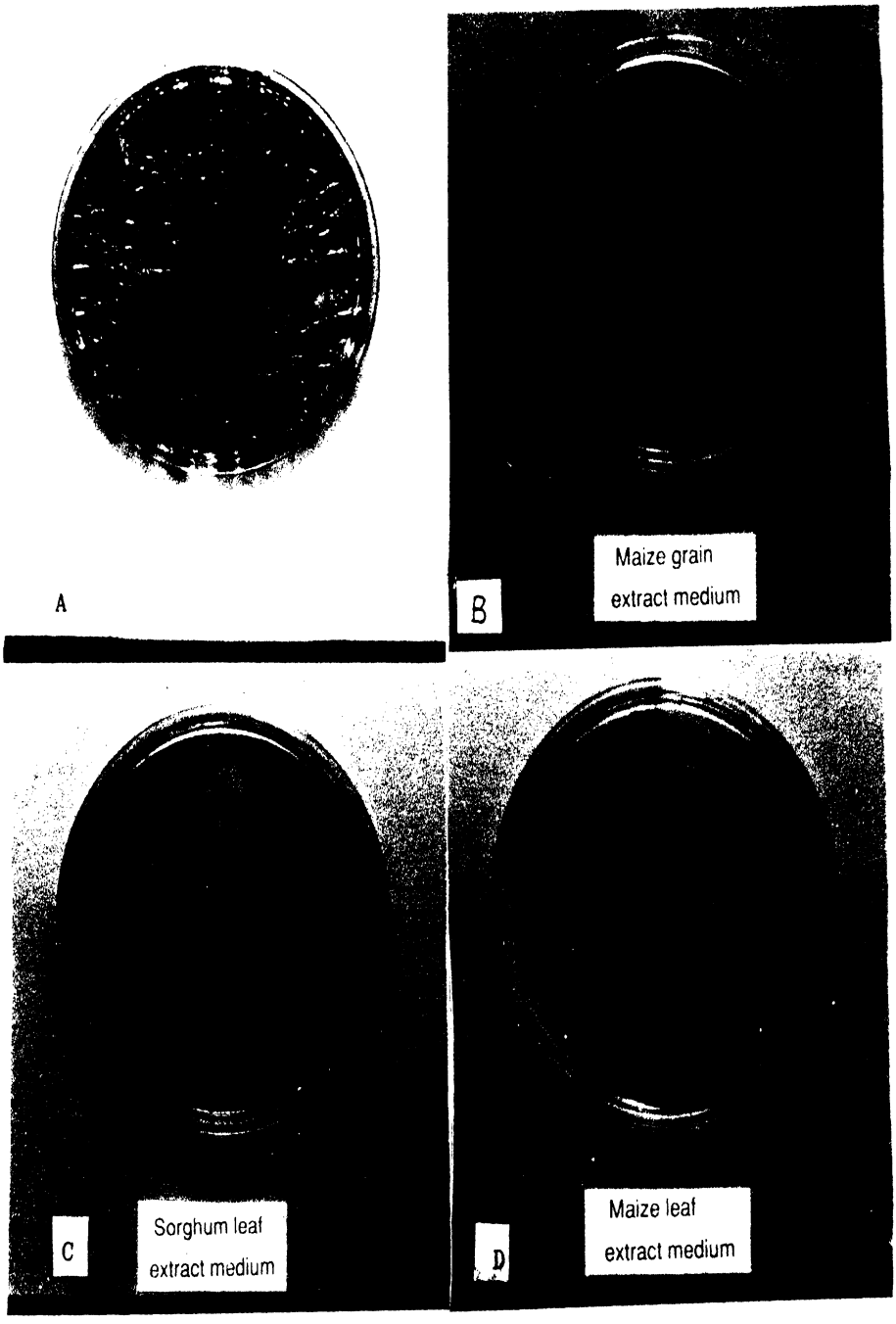


Figure 11: Colony growth of E. turcicum on lactose casein hydrolysate agar (A), Maize grain extract agar (B), sorghum leaf extract agar (C), and maize leaf extract agar (D).

4.3 INOCULATION METHODS

The results obtained from five different inoculation methods of E. turcicum on two sorghum varieties are presented in Table 19.

Table 19. Mean* disease score on two sorghum varieties (Framida and Local FSRP) and five different inoculation methods with E. turcicum.**

Inoculation method	Disease score		
	Framida	Local FSRP	Mean*
Diseased leaves buried in soil	1.5	1.5	1.5
Diseased leaves spread over soil	1.5	1.5	1.5
Spraying conidial suspension on leaves	4.2	3.8	4.0
Conidial suspensions poured in leaf whorls	3.2	2.8	3.0
Diseased leaves placed in leaf whorls	1.5	1.5	1.5
Control	1.0	1.0	1.0
SE	± 0.40	± 0.40	± 0.30
LSD 5%	0.85	0.85	0.60
LSD 1%			0.80

* 12 Replications
** 1-5 rating scale (Table 1).

The results showed that spraying of conidial suspensions on the leaves caused the highest infection (40% leaf area damaged) on the two varieties tested. The difference between this method and the others was highly significant. Spore suspensions poured in the leaf whorls caused the second highest infection.

The disease caused by the other inoculation methods was insignificant compared to the control.

4.4 INFECTION PROCESS

4.4.1 Spore deposition and germination

The number of spores observed and their types of germination on the leaves of the resistant and susceptible variety was similar (Table 21 and Fig. 12). The data showed that spores started to germinate on leaves of both varieties 5-8 hours after inoculation. Germination was bipolar and also unipolar and rarely from the sides (Table 20 and Figs. 12 and 13A).

Table 20. No. of conidia* of E. turcicum seen and their germination percentage on the leaves of susceptible (Framida) and resistant (IS 8283) sorghum varieties at different hours after inoculation.

Hours after inoculation	Framida (susceptible)		IS 8283 (resistant)	
	No. of conidia seen	Germination (%)	No. of conidia seen	Germination (%)
1	25	0	28	0
2	18	0	17	0
3	33	0	19	0
4	34	0	13	0
5	52	7	47	9
6	37	16	24	8
7	24	21	36	28
8	18	83	34	74
9	12	100	29	100

* 6 Replications

4.4.2 Germ tube growth and formation of appressoria

After germination the spores produced germ tubes which formed from the apical cells. Most of the germ tubes were slender shaped but sometimes branched ones were seen and lateral germ tubes were also observed but rarely (Figs. 13 A,C,D). From the germ tube spores started to form appressoria on the susceptible variety 12 hours after inoculation (Table 21) and on resistant variety after 14 hours (Table 22). After 16 hours 80% of the spores were forming appresso-

ria on both varieties. Appressoria were mostly round shaped or were oval and formed on the epidermal cells or on stomata (Fig. 13 B). Appressoria were not formed by all germ tubes. Penetration pegs developed from the appressoria.

4.4.3 Penetration and Colonization

Penetration started 14 hours after inoculation on the leaves of both varieties and was mostly direct through the epidermis (more than 90%) the remaining was through stomata (Tables 21 and 22 and Figs. 14 A & B). After penetration, colonization hyphae developed from the appressoria and formed vesicle like structures (Fig. 14 C&D).

Table 21. The number of condia* seen, percentage of conidia forming germ tubes, appressoria and penetration, and colonization by E. turcicum on the leaves of susceptible (Framida) sorghum variety at different hours after inoculation.

Hours after inocu-lation	No.of conidia seen	Conidia with germ tube %	Germ tubes with appres-soria %	Appressoria with penetra-tion pegs %	Coloni-zation
8	12	100	0	0	-
10	8	100	0	0	-
12	10	90	40	0	-
14	6	100	83	10	-
16	8	88	87	38	-
18	32	100	100	97	-
20	35	72	72	57	-
22	31	95	95	91	+
24	29	90	90	86	+

* 6 Replications
\- No colonization
\+ Colonization occurred

Table 22. The number of condia* seen, percentage of conidia forming germ tubes, appressoria and penetration, and colonization by E. turcicum on the leaves of resistant sorghum variety (IS 8283) at different hours after inoculation.

Hours after inoculation	No.of conidia seen	Conidia with germ tube %	Germ tubes with appressoria %	Appressoria with penetration pegs %	Colonization
8	24	100	0	0	
10	29	100	24	0	
12	9	77	44	0	
14	23	100	43	20	
16	16	100	95	75	
18	32	97	90	50	
20	29	93	90	89	
22	30	95	92	93	
24	18	100	98	94	

- No colonization
* 6 Replications

Colonization and spread of mycelium inside the leaf cells started 22 hours after inoculation on the susceptible variety (Tables 21 and 23), and after 48 hours on the resistant one (Table 24).

After penetration the mycelium branched and spread in the area around the point of penetration of the susceptible

variety. (Figs. 15A, 16 A&C, 17 A&C). A brownish red colour was also seen around the area after 48 hours (Fig. 18 A). On the resistant variety after penetration the mycelium did not branch or spread, in some cases it grew straight without branching (Figs. 15B, 16 B&D, 17 B&D) . The red brown colour near the point of penetration was seen after 96 hours.

Table 23. The number of conidia* seen, percentage of conidia that penetrated, colonized and spread of mycelium of E. turcicum inside the leaves of susceptible sorghum variety (Framida) at different hours after inoculation.

Hours after inoculation	No.of conidia seen	Appressoria with Penetration peg %	Colonization	Spread of mycelia
24	29	86	+**	+***
36	29	100	+	+
48	24	95	+	+
60	25	96	+	+
72	63	84	+	+
84	14	86	+	+
96	23	95	+	+
108	28	100	+	+
120	39	97	+	+

+** Colonization occurred
+*** Myclia spreading
* 6 Replications

Figure 12: Spore deposition on leaves (A), ungerminated spores (B), and germinated spores, bipolar (C) and unipolar (D), (x 1000).

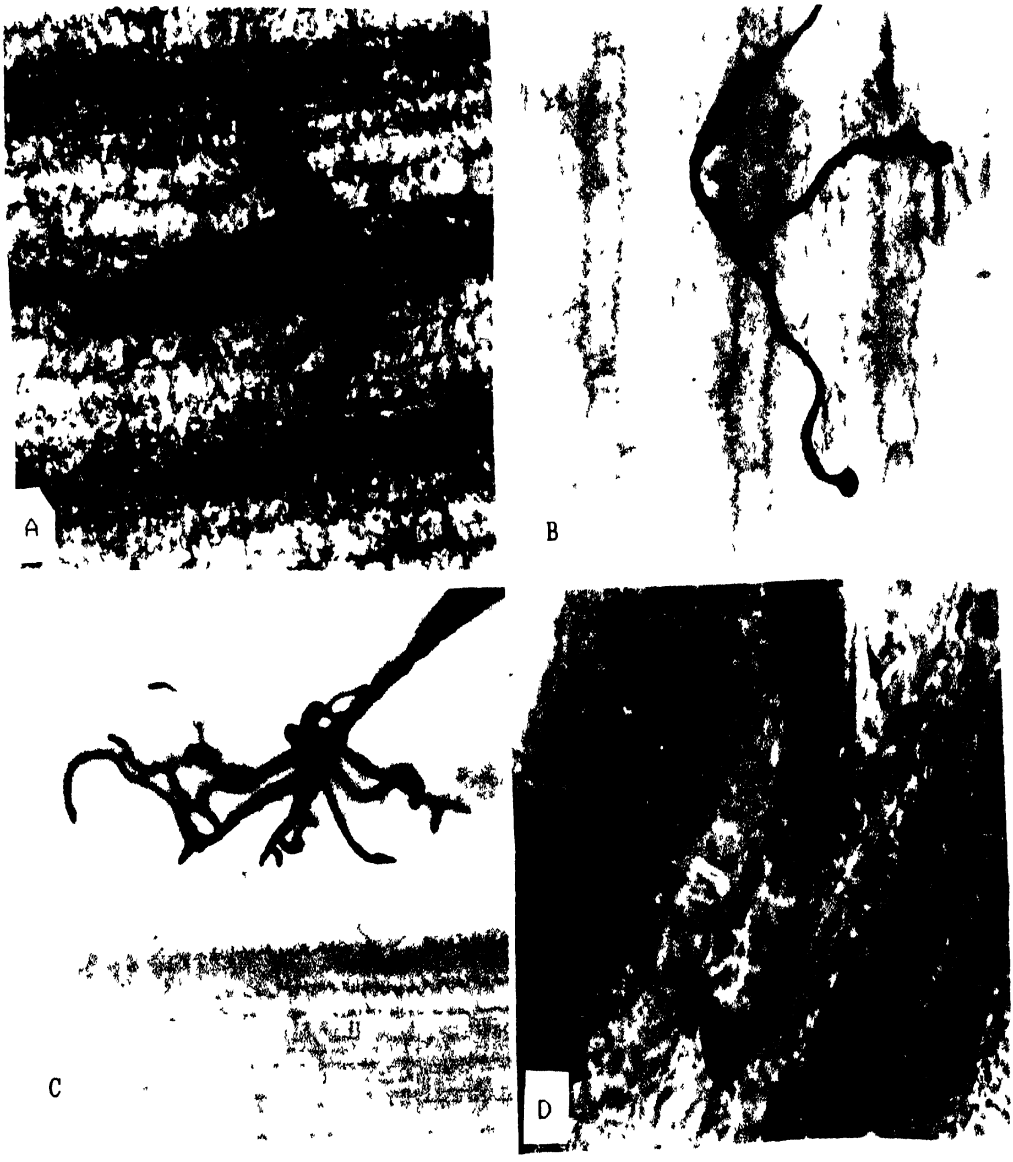


Figure 13: Side germination of spores (A), formation of appressoria (B), and germ tube branched (C), and unbranched (D). (X 500).

Figure 14: Penetration of the fungus on the leaves direct (A), through stomata (B) (X 1000), vesicle formation in susceptible variety (Framida) (C) and in resistant variety (IS 8283) (D) (X 2000).

Figure 15: Colonization hyphae after 36 hours in susceptible variety (Framida) (A) and resistant variety (IS 8283) (B), and after 60 hours in susceptible variety (Framida) (C), and resistant variety (IS 8283) (D) (X 1000).

Figure 16: Septate hyphae in susceptible variety (Framida) (A) and resistant variety (IS 8283) (B), colonization after 84 hours in susceptible variety (Framida) (C) and resistant variety (IS 8283) (D) (X 1000).

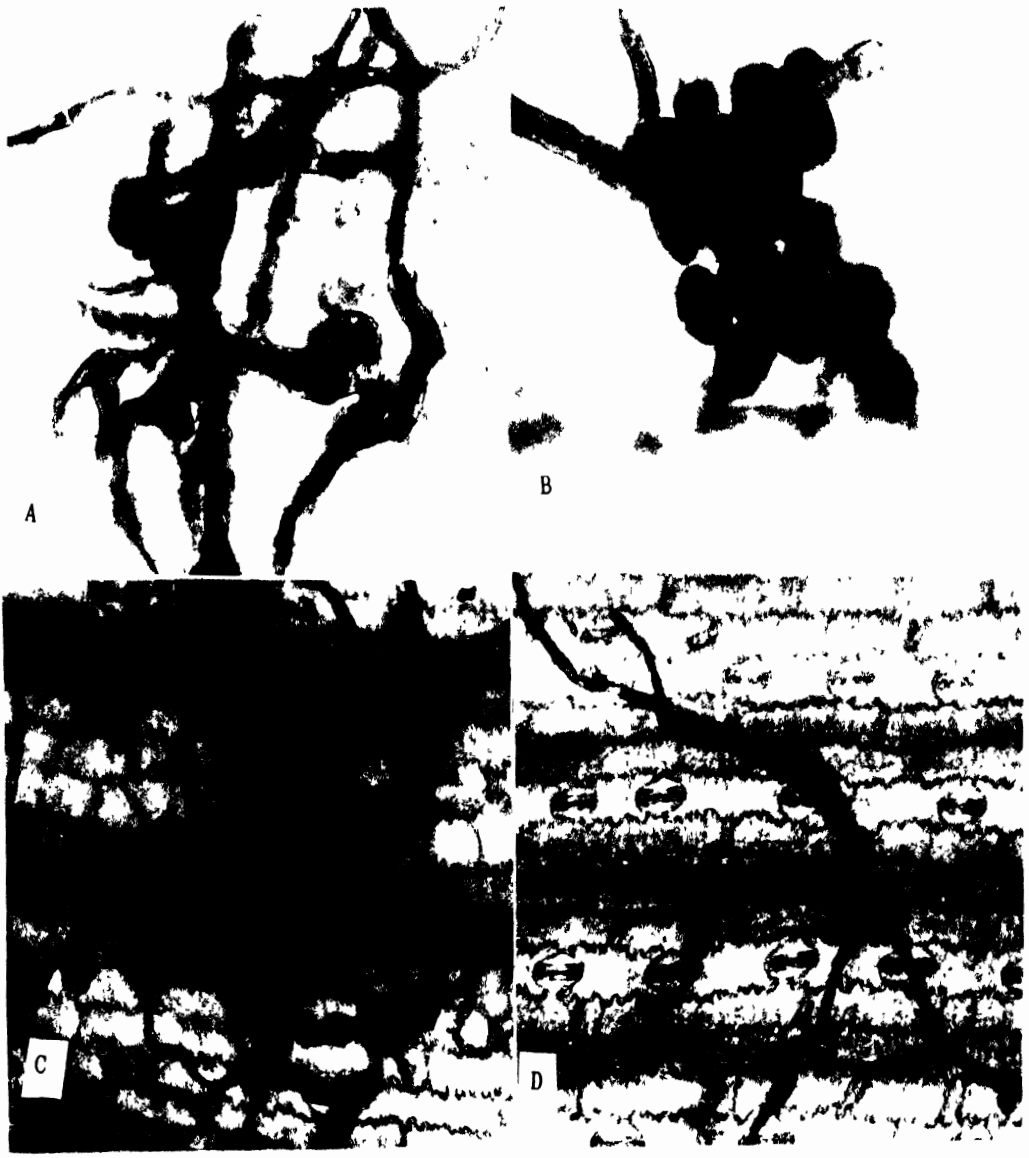


Figure 17: Colonization of the fungus after 108 hours in susceptible variety (Framida) (A) and resistant variety (IS 8283) (B) (X 1000), and after 120 hours susceptible variety (Framida) (C) and resistant variety (IS 8283) (D) (X 500)

Figure 18: Sympton appearance near the point of penetration (A) (X 500), mycelial growth in susceptible variety (Framida) (B) (X 1000).

Table 24. The number of conidia* seen, percentage of conidia that penetrated, colonized and spread of mycelium of E. turcicum inside the leaves of resistant sorghum variety (IS 8283) at different hours after inoculation.

Hours after inoculation	No.of conidia seen	Appressoria with Penetration peg %	Colonization	Spread of mycelia
24	18	94	-	-
36	28	86	-	-
48	42	95	+	-
60	12	92	+	-
72	9	66	+	+
84	25	92	+	-
96	17	94	+	+
108	18	100	+	+
120	14	93	+	-

\- Colonization or mycelia spread not seen
\+ Colonization or mycelia spread seen
* 6 Replications

4.5 PLANT GROWTH STAGES AND SUSCEPTIBILITY TO E. TURCICUM

The results obtained from the inoculation of sorghum at six growth stages with E. turcicum are presented in Tables 25 and 26.

The data indicated that highest disease incidence (>50% leaf area damaged) on the two varieties inoculated were observed when plants were inoculated at the 8th leaf stage. But 5th leaf stage and flag leaf visible stage were also susceptible, and there were no significant differences in disease incidence between these three stages. Other stages shown disease were 3 leaf stage and boot stage.

The lowest disease incidence was observed at 50 % flowering stage (Table 25).

Table 25. Mean* disease score for six sorghum growth stages of two sorghum varieties (Framida and Local FSRP) inoculated with E. turcicum.**

Crop growth stage	Mean disease score
3 leaf stage	3.0
5 leaf stage	4.5
8 leaf stage	5.0
Flag leaf visible	4.5
Boot stage	3.5
50% flowering, half of the plants at some stage of flowering	1.5
SE	± 0.29
LSD 5%	0.83
LSD 1%	1.10

** Disease score on a 1 to 5 scale (Table 1)
* 12 Replications

The data also shows that there were no significant difference between the two varieties tested at all stages (Table 26).

Table 26. Mean* disease score of leaf blight incidence of two sorghum varieties (Framida and local FSRP) at six growth stages.**

Crop stage	Mean disease score	
	Framida	Local FSRP
3 leaf stage	2.5	3.5
5 leaf stage	4.5	4.5
8 leaf stage	5.0	5.0
Flag leaf visible	4.5	4.5
Boot stage	4.0	3.0
50% flowering, half of the plants at some stage of flowering	1.5	1.5
SE		± 0.42
LSD 5%		1.17

* 6 Replications
** Disease score on a 1 to 5 scale (Table 1)

4.6 *E. TURCICUM* ISOLATES FROM SORGHUM AND MAIZE AND THEIR PATHOGENICITY ON BOTH HOSTS

The results obtained from cross pathogenicity of *E. turcicum* to sorghum and maize are presented in Tables 27 and 28 and Figure 19 .

4.6.1 Isolates from sorghum

Results showed that all the four sorghum isolates infected the sorghum varieties which were inoculated. In addition the sorghum isolate collected from Karimnagar infected the maize variety CM 500, while the isolate from Momlapalli infected the maize variety DH.103 (Table 27).

4.6.2 Isolates from maize

All the four maize isolates infected the maize varieties inoculated. Maize isolates collected from Undavally and Biknoor infected the sorghum variety IS 2858 (Table 28 and Fig. 19).

Table 27. Pathogenicity* of *E. turcicum* isolates from sorghum on maize and sorghum varieties.

Varieties	Sorghum isolate from			
	Patancheru	Karimnagar	Kurnool	Momlapalli
Sorghum				
Loc. FSRP	2.5**	3	2	4
IS2858	3.5	3.5	2.5	3.5
Framida	2	4	2	3
Maize				
Ganga 5	1	1	1	1
Aswani	1	1	1	1
CM 500	1	3	1	1
DH 103	1	1	1	3

* 6 Replications
** Disease score 1 to 5 scale (Table 1)

Table 28. Pathogenicity* of E. turcium isolates from maize on sorghum and maize varieties.

Varieties	Maize isolate from			
	'Undavally	Biknoor	Amperbet	Patancheru
Sorghum				
Loc. FSRP	1**	1	1	1
IS2858	3.5	3	1	1
Framida	1	1	1	1
Maize				
Ganga 5	3	4	3	2.5
Aswani	4	2	1	2
CM 500	2	2	3	3
DH 103	2.5	3	2	2

* 6 Replications
** Disease score 1 to 5 scale (Table 1)

Some maize isolates did not infected the maize variety Aswani this was might be the variety was tolerant to these isolates.

Figure 19: Sorghum variety (IS 2858) infected by maize isolates from Biknoor (A) and from Undavally (B).

DISCUSSION

CHAPTER V

DISCUSSION

The pathogenicity of three conidial concentrations tested on sorghum showed that 20,000 conidia mL^{-1} caused the highest infection followed by 10,000 and lowest by 5,000 conidia mL^{-1}. This indicated that the disease increased with increases in inoculum concentration, but further tests of lower or higher concentrations were not made here. The reason that the concentration with highest number of conidia mL^{-1} caused more disease might be because it created more inoculum pressure on the leaves. There was no significant difference to the disease caused by the two highest concentrations so both of them could be used for inoculations. The fact that the fungus caused disease even on low inoculum concentration shows that it was highly pathogenic to sorghum.

The fungus required different temperatures for optimum colony growth and for sporulation. Low (15°C) and high (35°C) temperatures both limited its growth. These results agree with the findings of Pandey and Shukla (1982), and Misra and Singh (1963) who reported maximum colony growth of the fungus at 20-30°C and no growth at 40°C. Bergquist and Masias (1974) on the other hand reported that the optimum

temperature for colony growth was 28°C and, sporulation was 24°C. These differences might be due to differences of the media and the fungal isolates used by different workers. Maximum colony growth on all media was recorded after 12 days of incubation and the lowest after 2 days of incubation, and on some media colony growth did not reach the end of the plates.

Lactose casein hydrolysate agar was the best for both colony growth and sporulation of all the solid media tested. It can be said that this medium contains the nutritional requirements of the fungus for both colony growth and sporulation. In contrast the media prepared from leaf and grain extracts of sorghum and maize supported maximum colony growth but poor sporulation. This shows that these media lacked the type of nutrients required by the fungus for sporulation but contained those which could support good colony growth. Shankerlingam and Balasubramanian (1983) reported that media containing leaf extracts of susceptible cultivars produced the highest number of spores. This is not supported by the results of this study perhaps due to the different isolates and cultivars used. Similarly Pandey and Shukla (1979) reported poor sporulation of the fungus on sorghum leaf extract agar.

Sorghum leaf medium produced maximum sporulation at all temperatures compared to other media, and it was the only medium in which the fungus sporulated at 35°C. The fungus started to sporulate on this medium after four days of incubation at all temperatures. This was so probably because the leaves of this medium were from a leaf blight highly susceptible variety of sorghum, which normally supports good sporulation in nature. Therefore, it can be said that sorghum leaf medium was the best medium for conidial inoculum production. In addition to that, the preparation of this medium was very simple and did not require the addition of other chemicals. This was the first time that this medium was compared to the other media for inoculum production. Lactose casein hydrolysate agar was the second best medium for sporulation of the fungus. Sorghum and maize leaf and grain extracts were the poorest.

Spraying of conidial suspensions on the leaves of sorghum was the best inoculation method followed by conidial suspensions poured on the leaf whorls. These results confirm the findings of Robert and Sprague (1960) and Nelson et al.(1965) on maize. They reported that spraying leaves and pouring of conidial suspensions on the leaf whorls were best inoculation methods of leaf blight fungus. Although they used a maize isolate, the fungus infected both maize and sorghum.

In this study, diseased leaves as inoculum did not cause disease. However, Robert and William (1952), Chenulu and Hora (1962) and Andrew et al. (1964) all used diseased leaves for inoculation and got enough infection. The use of diseased leaves as inoculum depends on the viability of the conidia and mycelium on those leaves. Levy (1984) reported conidia without chlamydiospores lost their viability . Hoppe (1962) also reported that conidia and mycelium of E. turcicum buried in soil lost their viability. It is this loss of viability in the diseased leaves that may explain the failure of diseased leaves to cause infection.

The quantity of inoculum (number of conidia) and the contact area of the inoculum to the host was different for the different inoculation methods. In respect to the area of contact, in the spraying method, spores were deposited over all parts of the leaves. In the other methods the contact was limited to small areas and the disease appeared only in those parts.

The infection process of E. turcicum on susceptible and resistant sorghum varieties was the same from spore germination to penetration. But difference started after penetration. In the susceptible variety, after penetration the

infection hyphae branched and spread laterally, while in the resistant one hyphae did not spread further and were not branched. Jennings and Ullstrup (1957) Hilu and Hooker (1964) made similar observations on maize in which hyphae of the fungus established early in the xylem of a susceptible variety with mycelium filling the vessels and tracheids. But in a resistant variety hyphae did not grow laterally and longititudinally and rarely branched. Although in this experiment cross sections of leaf tissues were not made, clear differences of stained infection hyphae inside the tissues of the two varieties were seen. Previous studies of the infection process of the fungus on maize suggested that the difference between resistant and susceptible varieties lies in the xylem tissues (Jennings and Ullstrup 1957, Shree 1987).

The 8-leaf stage was the most susceptible stage of the crop. The 5-leaf stage and flag leaf visible stages were equally susceptible. Similar results were obtained by Tuleen (1975) which reported immature sorghum growth stages were more susceptible to leaf blight than mature stages. However, Meenakashi and Ramalingam (1979) reported that the flowering stage was the most susceptible stage. This might be due to differences in the cultivars used, because different cultivars can have different susceptibility stages. In this

experiment it was found that younger stages were more susceptible than the older stages of the crop. These younger stages could be used for early screening to identify resistant lines in the greenhouse using large numbers of entries, particularly of breeding material.

E. turcicum isolates which attacked only hosts from which they were isolated were host specific, and those which attacked other hosts were not host specific and both cases were seen here. Levebvre and Helen (1945) reported isolates from maize infected sorghum. Shankerlingam and Balasubramanium (1984) reported that some sorghum isolates infected maize, as was found in this study. Robert (1960) and Rodriquez (1961) on the other hand reported the existence of host specific isolates of the fungus on sorghum and maize. In this study it was found that some sorghum isolates can infect maize and some maize isolates can infect sorghum. The lack of host specificity shown by some isolates of the fungus indicates that the primary inoculum for these isolates could come from both sorghum and maize. The epidemiological consequences of this need further study.

CONCLUSION

The most effective conidial inoculum concentration of the fungus that generated most disease was 20,000 conidia mL^{-1}.

The colony growth and sporulation of E. turcicum were excellent at 20-30°C. The optimum temperature for colony growth was 25°C and for sporulation 20°C. The poorest temperature for both colony growth and sporulation was 35°. Maximum colony growth and sporulation of the fungus on all media was reached after 12 days of incubation.

Lactose casein hydrolysate agar was the best medium for colony growth, followed by sorghum leaf extract agar, sorghum grain extract agar, and maize grain extract agar. The poorest colony growth was observed on potato dextrose agar, followed by maize leaf extract agar.

Sorghum leaf medium was the best medium for sporulation of the fungus, followed by lactose casein hydrolysate medium and potato dextrose agar. The sporulation was very poor on sorghum leaf extract agar, sorghum grain extract agar, maize leaf extract agar, and maize grain extract agar. Sorghum leaf medium was the most easily prepared medium for inoculum production of the fungus.

The most effective method of inoculation of E. turcicum on sorghum was the spraying of spore suspensions on the leaves, followed by spore suspensions poured on the leaf whorls. The other inoculation methods(diseased leaves buried in soil, spread over soil and placed on the leaf whorls) tested in this experiment were ineffective to produce disease.

The infection process of E. turcicum on sorghum was the same for the resistant and susceptible varieties studied(IS 8283 and Framida) at early stages of infection (such as conidial deposition, germination, germ tube and appressorial formation, and penetration). But was different after penetration. The colonizing hyphae of the fungus spread and branched inside the leaf tissues of the susceptible variety. While in resistant variety it did not spread and did not branch. The fungus also colonized early on the susceptible variety.

Sorghum was more susceptible to leaf blight at certain growth stages. The most susceptible stage was the 8-leaf stage followed by 5-leaf stage and the flag leaf visible stage. The other stages which were susceptible were 3-leaf stage and boot stage. Lowest disease incidence occured when

inoculation was done at the 50% flowering stage. Both the two varieties tested (Framida and Local FSRP) showed the same response to the disease.

The study of cross pathogenicity of isolates of the fungus to sorghum and maize showed that some sorghum isolates infected maize and some maize isolates infected sorghum. There were also some isolates which only infected either maize or sorghum.

SUMMARY

CHAPTER VI

SUMMARY

Laboratory and greenhouse experiments were carried out at ICRISAT Center to study the following: a) pathogenicity tests and inoculation methods of the fungus on sorghum, b) effects of temperature and media on growth and sporulation production of E. turcicum, c) infection process of the fungus on leaf blight resistant sorghum cultivar IS 8283 and leaf blight susceptible cultivar Framida, d) susceptibility to leaf blight of sorghum at different growth stages, e) pathogenicity of isolates of the fungus from maize and sorghum collected from different localities on maize and sorghum genotypes.

Results were as follows:

1) In pathogenicity tests the highest conidial suspension (20,000 conidia mL^{-1}) of E. turcicum caused the highest disease incidence when sprayed on sorghum leaves.

2) The optimum temperature for colony growth was 25°C, and for sporulation was 20°C. Both colony growth and sporulation were very poor at 15 and 35°C. Fastest colony growth occurred on lactose casein hydrolysate agar, sorghum leaf extract agar, sorghum grain extract agar, and maize grain

extract agar. Sorghum leaf medium produced maximum sporulation of the fungus compared to the other media. Lactose casein hydrolysate agar and potato dextrose agar were the second best for inoculum production. Sporulation was very poor on grain and leaf extract agar.

3) Conidial suspensions sprayed on the leaves of sorghum was the best inoculation method found in this study as it induced the most disease.

4) Observations of the infection process of E. turcicum on two sorghum varieties showed that spore deposition, germination, germ tube and appressorial formation, and penetration of the leaves were similar on resistant and susceptible varieties. But differences were noticed after penetration. The colonization hyphae grew abundantly in the leaf tissues of the susceptible cultivar, but not in resistant cultivar.

5) The most susceptible stage of sorghum to leaf blight was the 8-leaf stage. The susceptibility of the other growth stages in decreasing order were as follows: 5-leaf stage and flag leaf stage, 3-leaf stage, boot stage, and 50% flowering stage was the lowest. The two susceptible varieties tested (Framida and Local FSRP) showed the same response to the pathogen.

6) Four isolates of E. turcicum from maize and four from sorghum were cross inoculated to seven varieties of both hosts. Sorghum isolates infected sorghum, and maize isolates infected maize. In addition, sorghum isolates from Karimnagar and Momlapalli infected maize varieties CM 500 and DH 103, respectively. While maize isolates from Undavally and Biknoor infected the sorghum cultivar IS 2858.

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