STUDIES ON DIVERSITY OF SORGHUM ERGOT PATHOGEN ISOLATES OCCURRING IN INDIA

By

V.MUTHUSUBRAMANIAN

B.Sc. (Ag.)

THESIS SUBMITTED TO THE
ACHARYA N.G.RANGA 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
ACHARYA N.G.RANGA AGRICULTURAL UNIVERSITY
RAJENDRANAGAR, HYDERABAD-500030

DECEMBER, 2000

CERTIFICATE

Mr.V. MUTHUSUBRAMANIAN has satisfactorily prosecuted the course of research and that the thesis entitled **STUDIES ON DIVERSITY OF SORGHUM ERGOT PATHOGEN ISOLATES OCCURRING IN INDIA** 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 University

Date 11 12 2000

Place Hyderabad

(Dr. D. RAJA RAM REDDY)
Major Advisor

CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON DIVERSITY OF SORGHUM ERGOT PATHOGEN ISOLATES OCCURRING IN INDIA"** submitted in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE** of the Acharya N.G. Ranga Agricultural University, Hyderabad, is a record of the bonafide research work carried out by **Mr. V. MUTHUSUBRAMANIAN** 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 or has been published. The published part has been fully acknowledged. All the assistance and help received during the course of investigations have been duly acknowledged by the author of the thesis.

(Dr. D. RAJA RAM REDDY)
Chairman of the Advisory Committee

Thesis approved by the student's Advisory Committee

Chairman : **(Dr. D. RAJA RAM REDDY)**
Associate Professor
Department of Plant Pathology
College of Agriculture
Rajendranagar, Hyderabad-30.

Member : **(Dr. RANAJIT BANDYOPADHYAY)**
Senior Scientist (Pathology)
Genetic Resources and Enhancement Program
ICRISAT, Patancheru
Andhra Pradesh - 502 324.

Member : **(Dr. K. CHANDRASEKHARA RAO)**
Professor and Head
Department of Plant Pathology
College of Agriculture
Rajendranagar, Hyderabad-30.

Member : **(Dr.K.C. MOHAN KUMAR)**
Professor and Head
Department of Agricultural Microbiology & Bioenergy
College of Agriculture
Rajendranagar, Hyderabad-30.

LIST OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

Contd..

LIST OF PLATES

Contd

Contd..

Contd

LIST OF ABBREVIATIONS

@	-	at the rate of
A$	-	American dollar
ANOVA	-	Analysis of Variance
C	-	Celsius
cm	-	centimeter
°	-	degree
FAO	-	Food and Agriculture Organization
Fig	-	Figure
g	-	gram
h	-	hour(s)
ha	-	hectare
i e	-	that is
Kg	-	kilogram
l	-	litre
l s d	-	Least significant difference
mg	-	milligram
ml	-	millilitre
mm	-	millimeter
No	-	number
pH	-	Power of hydrogen ion concentration
PSI	-	Pounds per square inch
%	-	per cent
SEm	-	Standard error of means
µg	-	Microgram
µm	-	Micrometer

ACKNOWLEDGEMENTS

It is by the lavish and boundless blessing of the almighty that I have been able to complete my studies successfully hitherto and present this humble piece of work, for which I am eternally indebted.

Fervently and modestly, I extol the genuine cooperation, inspiration and affection offered to me by the Chairman of my advisory committee, ***Dr. D. Raja Ram Reddy****, Associate professor, Department of Plant Pathology, right from the initiation of the work to ship-shaping of the manuscript. The present work bears at every stage the impression of his wise counsel and concrete suggestions, careful, seasoned criticism and meticulous attention to details. It was indeed a rare privilege for me to work under his emending inspiration and indomitable spirit.*

I am greatly beholden beyond words to express my deep sense of gratitude and a great privilege to work under the able and highly exceptional guidance of ***Dr. Ranajit Bandyopadhyay****, Senior Scientist, Pathology, GREP, ICRISAT, for his brilliant counsel, constructive suggestions, indefatigable guidance, evincive criticism and inspiring encouragement to embellish the present study.*

I avail this opportunity to express my deep sense of reverence and profound gratitude to ***Dr. K. Chandrasekhara Rao****, Professor and Head of the Department, Department of Plant pathology and member of my advisory committee for his invaluable guidance, suggestions and support during the course of study.*

His keen interest, critical remarks and encouragements have enabled me to complete this work.

I feel immense pleasure in extending my deep sense of gratitude to ***Dr. K.C. Mohan Kumar****, Professor and Head, Department of Microbiology and Bio-energy, for his meticulous guidance, benign attitude, generous co-operation constructive criticism in the preparation and presentation of the thesis.*

I wish to express my sincere thanks to ***Dr. T. Vithal Reddy, Dr. R. Ravindra Babu, Dr. P. Narayan Reddy, Dr. Sikander Ali*** *and* ***Dr. B. Rajeshwari*** *for their generous help, valuable guidance during the course of study.*

I equally owe my deep sense of gratitude to ***Mr. S.S. Navi*** *(Scientific officer),* ***Mr. R. Kanaka Reddy*** *(Scientific officer), Cereal Pathology, GREP, ICRISAT and* ***Mr. Harikrishnan****, Research Associate, Statistic Division, ICRISAT for their precious suggestions, constant cooperation, and constructive criticism during the investigation.*

I place on record my profound and deep sense of gratitude to ***Dr. V. Bala Bhanumurthy****, Associate professor, EMP Scheme, College of Veterinary Science, Rajendranagar, Hyderabad, for providing me the stumps of grass species to carry out one of the objectives of my investigation.*

I proffer my sincere thanks to ***Mr. S.M. Ahmed****, Administrative Associate, GREP, ICRISAT for his help extended in innumerable ways during this investigation.*

I am highly thankful to ***Mr. D. Rangaswamy Reddy, Mr. B.B.Raju, Mrs. Andalu*** *and all staff members of Cereal Pathology, GREP, ICRISAT for their*

timely help and wholehearted cooperation in several occasions of my investigation at ICRISAT.

Diction is not enough to express my unboundful gratitude and affection to my grand mother ***Smt. Seetha****, beloved parents* ***Sri. Venkateshwaran*** *and* ***Smt. Alamelu****, brother-in- law* ***Sri. Ganesan*** *and Sister* ***Smt. Bharathi****, uncle Sundaram, cousins* ***Smt. Anuradha, Sri.Subramanian, Sri. Swaminathan*** *and Ravi who constantly inspired, guided and molded me to the present position. There is no match to the affection I show on Chotoos,* ***Sai Purushodh, Sai Siddharth, and Mani Bharathi,*** *who brought me a little lit of heaven on earth.*

Inexplicable is my sense of gratitude and affection to my ***Gopi*** *and my dear friend* ***Kanagaraj****, whose unparalled affection and persistent encouragement in shaping my career will go a long way throughout my life.*

With boundless affection and love, I would heartily offer my warm thanks to my colleagues, ***Venkat, Ashwani*** *and* ***Hared*** *for their delightful companionship and help throughout my course of study. The help and encouragement I received from them will remain fresh forever in my memory.*

I affectionately extend my heartfelt thanks to my dear friends ***Rajesh, Keshav, Prakash, Sheela, Sugila, Prabhakaran, Thiruchelvam, Selvakumari, Senthamizh, Nirmala, Sujatha, Nageshwari*** *for their splendid company, constant encouragement and inspiration.*

The words fail me in expressing my feelings to my batch-mates ***Sakthivel, Jayachndran, Senthil, Rajasekaran, Athinathan, Ebin, Vinay, Sachin,***

Saradh Chandra and my juniors Suresh, Dilip, Senthil, Gopi, Ashok, Muthu and Bakhi for their joyful company and needy help.

My special heartfelt thanks are also due to Mr. Vidhyasekaran, Photolab, ICRISAT for his kind cooperation and help rendered in taking photographs.

My study needs special acknowledgement to the Indian Council of Agricultural Research for providing finalcial assistance in the form of Junior Research fellowship during the course of my study, and to Acharya N.G. Ranga Agricultural University for giving me the opportunity to pursue my post graduation.

I convey my wholehearted thanks to many of my wellwishers and to other friends requesting their forgiveness for not mentioning them here by name.

(V.MUTHUSUBRAMANIAN)

DECLARATION

I, **Mr.V.MUTHUSUBRAMANIAN** hereby declare that the thesis entitled **STUDIES ON DIVERSITY OF SORGHUM ERGOT PATHOGEN ISOLATES OCCURRING IN INDIA** submitted to Acharya N.G. Ranga Agricultural University for the degree of **MASTER OF SCIENCE IN AGRICULTURE** is the result of the original work done by me. It is further declared that the thesis or any part thereof has not been published earlier in any manner.

Date. 11 12 2000

(V. MUTHUSUBRAMANIAN)

Place: Hyderabad

Author	V.MUTHUSUBRAMANIAN
Title of the thesis	STUDIES ON DIVERSITY OF SORGHUM ERGOT PATHOGEN ISOLATES OCCURRING IN INDIA
Degree to which it is submitted	MASTER OF SCIENCE IN AGRICULTURE
Faculty	AGRICULTURE
Major field	PLANT PATHOLOGY
Major advisor	Dr. D. RAJA RAM REDDY
University	ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY
Year of submission	2000

ABSTRACT

Ergot or sugary disease of sorghum, caused by *Sphacelia sorghi* McRae, is one of the destructive diseases of sorghum Two different species of *Claviceps, viz* , *C. sorghi* and *C. africana* have been reported to cause ergot in India However, the distribution of these two ergot pathogens from different parts of sorghum growing areas is not known Hence, the present investigation was taken up with 89 sorghum ergot isolates collected from major sorghum growing areas in Uttar Pradesh, Rajasthan, Gujarat, Maharashtra, Andhra Pradesh, Karnataka and Tamil Nadu to study the diversity among the isolates The isolates were maintained on sorghum male sterile line 296A by periodic spray inoculation with conidial suspension Each isolate was cultured on T_2 agar medium using young sphacelia obtained from fresh infected panicle of sorghum male sterile line 296A The isolates differed distinctly in their cultural characters on T_2 agar medium Of the 89 isolates studied, 84 isolates produced compact, fleshy, raised and non-sporulating colony on the medium (Group-I), whereas, only five isolates produced cottony, velvety, submerged and sporulating colony on T_2 agar medium (Group-II) Eight isolates *viz* , NI2, NI5, NI12, GUJ6, MH71, AP17, SK-20-24 and TN13 from Group-I and two isolates *viz* , NAP7 and MH74 from Group-II were selected representing different geographical locations of sorghum growing areas of the country for further studies, pertaining to *in vivo* variations in morphology of sphacelia, conidia and sclerotia, the effect of temperature and relative humidity on secondary conidia production, the extent of disease spread and host range

The isolates belonging to two distinct groups varied widely with respect to morphology of sphacelia. conidia and sclerotia Variations were also observed within the isolates of the same group The isolates belonging to Group-I produced smaller (2 92–3 6 × 1 9–2 26 mm), conical to spherical sphacelia, oblong to ellipsoidal, bigger sized (10 29-18 × 6 43-9μm) macroconidia, and spherical microconidia The isolates belonging to Group-II produced bigger sized (4 38–4 72 × 2.94–3 06 mm), oblong to cylindrical sphacelia, narrow, cylindrical to ellipsoidal, comparatively smaller (9–18 × 5 14–7 71μm) macroconidia and spherical microconidia

None of the isolates under study produced sclerotia even after incubation at 35°C and at 40 per cent relative humidity for 2 months in growth chambers Morphology of the sclerotia collected from field infected sorghum plants was studied for the seven ergot isolates *viz*, NAP4, NAP5, NAP7, NAP12, NAP13, AK1 and SK-20-24 The sclerotia of Group-I isolates were shorter than the Group-II isolates Sclerotia of Group-I isolates (AK1, NI12, and SK-20-24) were conical to spherical in shape and short measuring 4 1–4 42 × 1 4–1 82 mm in size whereas, the sclerotia of Group-II isolates (NAP4, NAP5, NAP7, and NAP13) were cylindrical to conical and long measuring 8 16–10 24 × 1 84–2 04 mm in size

Isolates differed distinctly in the extent of secondary conidia production Group-I isolates produced more number of secondary conidia, whereas, Group-II isolates produced least number of secondary conidia at all the temperatures (10,15,20,25,30 and 35°C) and relative humidity (RH) (80, 85, 90, 95 and 100%) levels tested Of the different temperature and RH levels tested, a temperature of 25°C and relative humidity of 100% were found to be the best for secondary conidia production Among the Group-I isolates NI2 produced more number of secondary conidia, and among Group-II isolates NAP7 produced least number of secondary conidia at 25C temperature and 100 per cent relative humidity

The pattern and spread of the disease of two distinct isolates (N12 and NAP7) have been tested in growth chambers The present investigation confirmed the aerial spread of the disease through secondary conidia produced by the pathogen The ergot incidence was more (71 21%) with N12 isolate than NAP7 isolate (15 15%) There was no definite pattern observed in the appearance of the disease as the test plants away or nearer from the central source of secondary conidia showed the same disease incidence The per cent of infected spikelets was high (5 36) with N12 isolate as against a very low per cent of infected spikelets with NAP7 isolate (0 42)

Of the 20 graminaceous plant species tested for their susceptibility to ergot pathogen under artifical inoculated conditions only *Sorghum arundinaceum, S. halepense, S. virgatum, S. versicolor* and *Pennisetum glaucum* were found to be infected by all the ten representative sorghum ergot isolates Microscopic examination of the honey dew collected from the infected hosts, revealed certain variations with respect to the shape of macroconidia The macroconidia on *P. glaucum* were showing dimorphism, with elliptical and elongated or spindle shaped macroconida, while the macroconidia on wild sorghum genotypes were thinner than those on sorghum There was significant reduction in the size of macroconidia on different host plants was observed, when compared to the macroconidia on sorghum male sterile line 296A, with all the representative isolates tested

Based on the studies conducted, it is evident that the isolates belonging to Group-I are putatively identified as *Claviceps africana* and isolates belonging to Group-II are putatively identified as *Claviceps sorghi*, indicating that in India the pathogen *C. africana* predominates in all the sorghum growing areas, whereas, the pathogen *C. sorghi* is restricted to certain regions of sorghum growing areas *viz* Adilabad region in Andhra Pradesh and Bori region in Karnataka Among the two distinct groups of isolates, variation persists within the groups, in all the characters studied, showing existence of greater diversity in ergot pathogen present in India

INTRODUCTION

CHAPTER I

INTRODUCTION

Sorghum, *Sorghum bicolor* (L) Moench, is an important cereal crop ranking fourth after rice, wheat and maize and is grown as a staple food in semi-arid tropics and sub-arids, throughout the world As a drought resistant crop, sorghum is adapted to a wide range of ecological conditions and produces reasonable grain yields under conditions unsuitable for most other cereals (Rosewich, 1996) The total area under sorghum cultivation is 44 82 million hectares with a production and productivity of 65 81 million tonnes and 1468 Kg ha^{-1} respectively (FAO, 1999) It is cultivated mainly for food, feed, beverage and fodder In India, area under cultivation of sorghum is 11 2 million hactares and is cultivated both in *kharif* and *rabi* seasons The major sorghum growing areas are distributed in Maharashtra, Karnataka, Andhra Pradesh, Uttar Pradesh, Gujarat, Tamilnadu and Rajasthan (Fig 1) The production of sorghum in India in 1999 was 11 million tonnes, as against 8 52 million tonnes, in 1998 The estimated productivity of sorghum in the year 1999 was 982 Kg ha^{-1}, as against 833 Kg ha^{-1} in the year 1998 (FAO, 1999) Increase in sorghum production and productivity over years, is mainly due to the cultivation of high yielding F_1 hybrids

Although the sorghum is hardy crop, it still suffers from various biotic and abiotic stresses Sorghum is susceptible to many fungal, bacterial and viral diseases at different stages of crop growth and development The major diseases which affect sorghum production are smuts (*Sphacelotheca sorghi, S. cruenta, S. reiliana* and *Tolyposporium ehrenbergii*), rust (*Puccinia purpurea*), downy mildew (*Peronosclerospora sorghi),* leaf blight *(Exserohilum turcicum),* anthracnose *(Colletotrichum graminicolum),* charcoal rot *(Macrophomina*

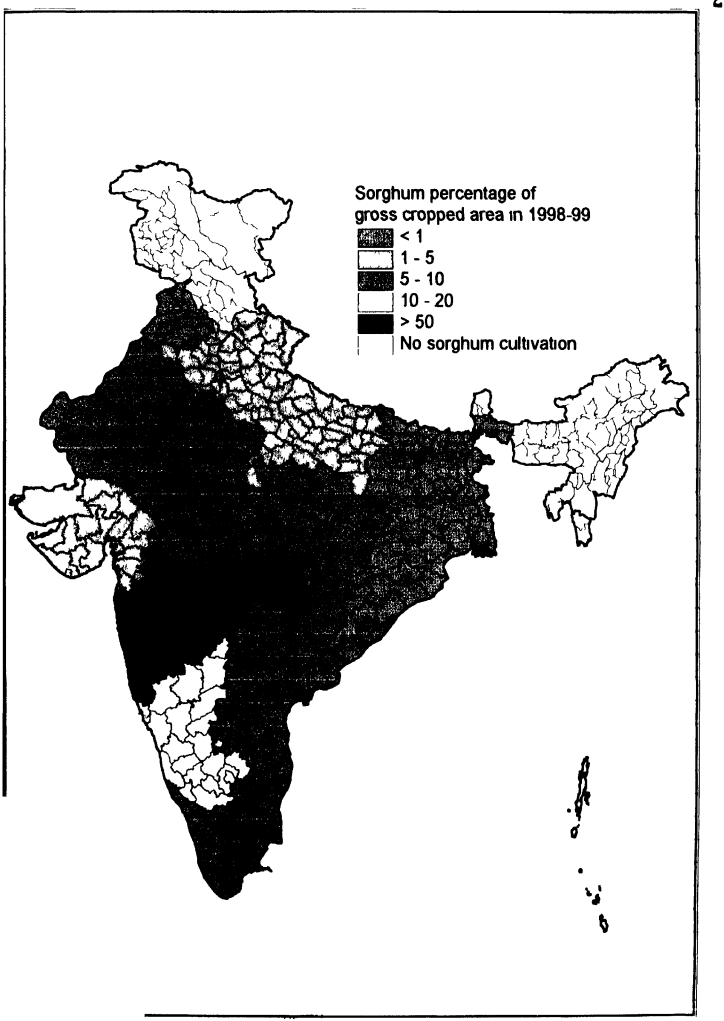

Figure 1 Sorghum growing areas in India

phaseolina), grain mold (complex etiology), ergot or sugary disease (*Sphacelia sorghi*) and stripe disease (a tenui virus transmitted by *Perigrinus maidis)*

Of these sorghum ergot is a serious threat for the production of F_1 hybrid seeds, particularly if 'nicking' is poor or seed set is delayed in male sterile lines In India, in Maharashtra state, where primarily hybrids are grown, and several seed companies are located, 10–80 per cent losses in seed yield have been reported due to ergot (Sangitrao *et al* 1997) In India, the disease is endemic in Maharashtra (Vidarbha and Ahmednagar regions), Karnataka, Andhra Pradesh and Tamilnadu In a recent survey conducted by ICRISAT in 1999-2000, the disease was found to occur in severe stage in major sorghum growing areas of Andhra Pradesh, Gujarat, Karnataka, Maharashtra, Rajasthan, Tamilnadu and Uttar Pradesh The epidemic outbreak of sorghum ergot has been observed consecutively for the last two years in few mandals of Mahabubnagar districts of Andhra Pradesh, in India In October 1999, towards the end of rainy season, the disease appeared in epidemic form in Machinenipally village, and in September 2000 it has spread to few more mandals showing greatest severity in Kalwakurthy mandal of Mahabubnagar district of Andhra Pradesh

Sorghum ergot is a disease of ovary and it reduces grain yield because infected flowers do not produce grains Instead of normal pollination, fertilization and production of seeds, ovaries are colonized by fungal hyphae, that develop into spore bearing fungal masses (sphacelia), which produce honeydew Later sphacelia are converted into sclerotia, when adverse environmental conditions prevail The disease poses threat not only to seed production, but also has health implications for livestock, due to potential toxicity of the alkaloids produced in the sclerotia

Sorghum ergot was reported first in India in 1917 (McRae, 1917) and subsequently in Kenya in 1926 (Mason, 1926). Three different *Claviceps* species have been reported to cause ergot disease in different parts of the world, *viz.*, *Claviceps sorghi* from India, *Claviceps africana* from Zimbabwe and *Claviceps sorghicola* from Japan. Recently Bogo and Mantle (1999) reported occurrence of *C. africana* in India. The imperfect stages of *C. sorghi* and *C. africana* are similar and are named as *Sphacelia sorghi* McRae. (Frederickson *et al.*, 1991).

In India, the occurrence of both *C. sorghi* and *C. africana* have been reported. However, the distribution of these two ergot pathogens from different parts of sorghum growing areas is not known. At the International crops Research Institute for the Semi-Arid Tropics (ICRISAT), a survey was being conducted to collect isolates of the sorghum ergot pathogen in different parts of India. Using these isolates, studies on diversity of sorghum ergot pathogen isolates, were carried out, with the following objectives:

Objectives of investigation:

1. To study the cultural characteristics and *in vivo* variations in sphacelia, conidia and sclerotia of different isolates.
2. To determine the extent of secondary conidia production in different isolates under a range of relative humidity and temperature regimes.
3. To explore the extent of disease spread in different isolates.
4. To explore the host range of different sorghum ergot isolates.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

The literature on different aspects of sorghum ergot disease is briefly reviewed under the following headings

2 1 Geographical distribution and Economic importance

2 2 Symptomatology

2 3 The pathogens

2 3 1 Cultural characters

2 3 2 Anamorph

(a) Sphacelia

(b) Macroconidia

(c) Microconidia

(d) Secondary conidia

2 3 3 Teleomorph

(a) Sclerotia

(b) Stroma, asci, ascospores

2 4 Pathogenicity

2 4 1 Inoculation methods

2 4 2 Isolation methods

2 5 Factors influencing secondary conidiation

2 6 Epidemiology

2 7 Disease spread

2.8 Host range

2.1 Geographical distribution and economic importance.

Ergot is an important floral disease of sorghum. It was first observed in India in 1915 in Madras state and was described by McRae (1917). In Africa, the disease was observed first in Kenya, in 1924 in Tanganyika by Mason (1926). In India the disease has been reported from the states such as Maharashtra (Ajrekar, 1926), Karnataka (Kulkarni, 1942), Tamil Nadu (Nath and Padwick, 1941, Thomas *et al*, 1945 and Ramakrishnan, 1947), Andhra Pradesh (Ramakrishnan, 1948, Sundaram, 1967,1969 and patil *et al.*, 1968) and Delhi and Haryana (AICSIP 1969–70). Kulkarni *et al.* (1976) observed the perfect stage of *Sphacelia sorghi* and described it as *Claviceps sorghi*.

In Africa the widespread occurrence of ergot disease was reported in Tanzania (Wallace and Wallace, 1949). In 1953 ergot was reported in South Africa (Doidge *et al.*, 1953), in Zambia in 1965 (Angus, 1965), in Botswana in 1974 (Molofe, 1975), and in Mozambique in 1984 (Plumb-Dhindsa and Mondjane, 1984). In Japan, sorghum ergot was first observed in Kyushu District in southern Japan in 1985 (Shimanuki, Kimigafukuro and Tsukiboshi, 1988). In 1986, ergot disease occurrence was confirmed in Zimbabwe, Swaziland and other African countries (de Milliano *et al.*, 1991). All reports and samples from Africa represent the distinctive pathogen *Claviceps africana* (Frederickson *et al.*, 1991).

In 1991, the ergot pathogen in Thailand was, contrary to expectations, identified as *Claviceps africana*, not *Claviceps sorghi* (Frederickson *et al.*, 1991), wheras Mantle and Hassan (1994) reported occurrence of *Claviceps sorghicola* parasitising sorghum in Japan. Reis *et al.* (1996) reported widespread occurrence of sorghum ergot disease in Brazil in 1995. Subsequently Ryley *et al.* (1996) reported the occurrence of *Claviceps africana* in Australia. Since then, the disease has been observed in rapid succession in South America, Central

America, the Caribbean, and North America The disease spread was rapid in Brazil, Mexico, the USA and Australia (Bandyopadhyay *et al*., 1996) Isakeit et al (1998) reported the occurrence of ergot disease on ratoon sorghum tillers in a field just North of the Riogrande near Progresso, Texas In late march1997, ergot was observed on ratooned tillers in a sorghum field just North of Rio Grande near Progresso, Texas By October 1997, the disease has spread throughout Texas and was recorded in Georgia, Kansas and Nebraska (Isakeit *et al*, 1998) Tsukiboshi *et al* (1999) reported the occurrence of distinctive ergot pathogen in Japan and described it as *Claviceps sorghicola* sp nov

The occurrence of *C. africana* in India, in addition to *Claviceps sorghi*, which is apparently confined to Indian sub-continent (Frederickson et al , 1991) was proved by Bogo and Mantle (1999), by their alkaloid analyses of sclerotia of Indian isolates through gas chromatography-mass spectrometry Pazoutova *et al.* (2000) confirmed the occurrence of *Claviceps africana* in Dharwad (Karnataka), Patancheru (Andhra Pradesh) and Akola (Maharashtra) of India, after confirming through random amplified polymorphic DNA (RAPD) banding pattern analyses, using the isolates collected from these regions Tooley *et al* (2000) reconfirmed the presence of *Claviceps africana* in India, by random amplified microsatellite (RAM) and amplified fragment length polymorphism (AFLP) analyses, using sorghum ergot isolates collected from Akola, Dharwad, and Patancheru, India

In India, losses of 10–80 % have been reported in hybrid seed production fields Similarly, ergot epiphytotics in Zimbabwe result in regular annual losses of 12–25 % and occasionally in total losses (Bandyopadhyay *et al*, 1998) Substantial losses in seed quality occur when honeydew oozing from infected florets contaminates surrounding grains, which are then

colonized by fungal saprophytes. Such seed may have decreased germination and seedling emergence and may be predisposed to other diseases (McLaren, 1993).

It was estimated that fungicidal control alone would cost approximately A$ 700 per hectare Cost related to seed processing would add another A$ 250 t^{-1} of seed Adequate and reliable supply of good quality seed will probably be jeopardized Ergot will cost the seed Industry, A$ 4 million annually, and an additional A$ 20 will be required to produce each 25 kg of seed (Bandyopadhyay *et al* , 1998)

2.2 Symptomatology

The characteristic symptoms of the disease have been adequately described by several workers (Sundaram, 1968, Molefe, 1975, Bandyopadhyay, 1992, Bandyopadhayay *et al* , 1996, Bandyopadhayay *et al.*, 1998) The pathogen only attacks unfertilized ovaries Few or all flowers in an inflorescence may be infected The most obvious external sign of the disease is the exudation from the infected flowers, of honeydew, a thin-to-viscous, sweet sticky fluid that gives the name 'sugary' or 'honeydew' disease to the malady

The ovary is infected much before the initiation of honeydew exudation Infact, the earliest symptoms of infection can be seen on the ovary if flowers are dissected 3-4 days after infection The infected ovary appears dull green and smaller (*Claviceps sorghi*) or larger (*Claviceps africana*) than the healthy, fertilized ovary which is dark green and round (Bandyopadhyay *et al* , 1996)

Superficial, mycelial growth initially appears at the basal end of the ovary and extends upwards as the pathogen colonizes ovary tissues both internally and externally Finally, the

complete ovary is converted into a white fungal mass or sphacelium that is visible between the glumes. Then, honeydew exudtion begins (Bandyopadhyay *et al.*, 1996).

Naturally infected sorghum plants produce honeydew secretion that is light brown, but when artificially inoculated, the secretion is pinkish (Molefe, 1975).

Newly formed honeydew droplets are colourless and transparent and become progressively opaque. With time honeydew may become uniformly yellow-brown to pink, or superficially white. Continued production of honeydew causes droplets to lengthen, smearing seeds and leaves, and falling to the ground below the panicle. When relative humidity is high honeydew droplets develop white covering as secondary conidia are produced. When infection is severe, affected panicles can be recognized from a distance. They may be white with fresh honeydew, or black if the honeydew is saprophytically colonized by *Cerebella sp.* Under warm dry conditions, sphacelia gradually harden to form solid dense sclerotia. But in moist conditions, the sphacelia shrivel and become fibrous, and fail to develop into sclerotia (Bandyopadhyay 1992; Bandyopadhyay *et al.*, 1996 and 1998).

2.3 The pathogens

Current evidence suggests that sorghum is uniquely host to three different ergot pathogens, viz, *Claviceps sorghi* B.G.P. Kulkarni, Seshadri & Hegde (Kulkarni *et al.*, 1976), *Claviceps africana* Frederickson, Mantle & de Milliano (Frederickson *et al.*, 1991) and *Claviceps sorghicola* Tsukib., Shiman. & T. Uematsu sp. nov. (Tsukiboshi *et al.*, 1999). The anamorphs of *Claviceps africana* and *Claviceps sorghi* are more similar and are named as *Sphacelia sorghi* McRae (McRae, 1917)

2.3.1 Cultural characters

The growth of the ergot fungus, *Sphacelia sorghi* on Kirchoff's medium is white in colour with plenty of aerial hyphae. But the rate of growth is very slow compared to that of ergot of rye. The growth found to be slower at 26–28°C than at 20–23°C (Ramakrishnan, 1948).

Chinnadurai (1972) confirmed the essentiality of micronutrient manganese for mycelial growth and iron for sporulation of *S. sorghi*, on Kirchoff's media. According to him the trace elements required for the growth and sporulation of the fungus are not the same and the effect of trace elements were more pronounced on the sporulation compared to the mycelial growth

The effect of molybdenum was confirmed to be highly inhibitory to the ergot fungus as its absence in the Kirchoff's medium gave maximum growth and sporulation (Brain and Hemming, 1950, Hawker, 1957, and Chinnadurai, 1972)

Nagarajan and Saraswathi (1975) reported the production of honeydew secretions by the culture in the Petriplates as well as in the test tube slants after 15 days of culturing and reported the change in the colour of the honeydew in the medium, from colourless at initial stage to palebrown or pinkish at later stages. According to them the honeydew secretions are less sticky contained numerous conidia which germinated in 4–6 h in water, producing germtubes from both ends The honeydew secretions obtained in the culture proved to be infective on sorghum male sterile line (CK 60 A)

Nagarajan and Saraswathi (1975) found sparse and delayed growth and sporulation of the sorghum ergot fungus when sterile sclerotial bits were inoculated on Kirchoff's medium. Attempts were made to maximize the growth and sporulation of the fungus in the medium, with certain modifications.

Addition of sucrose at 150 g l^{-1}, potassium nitrate at 1 5 g l^{-1} yeast extract at 10 g l^{-1} tocopherol acetate at 5 ml l^{-1} to Kirchoff's medium and adjusting the pH to 7 0, resulted in luxuriant growth and heavy sporulation after 12 days of culturing, as compared to sparse growth and low sporulation after 28 days in the original Kirchoff's medium (Nagarajan and Saraswathi, 1975)

Bogo and Mantle (1999) reported compact, white growth of *Clavıceps africana* which failed to produce spores on asparagines-sucrose-salts agar medium

Tsukiboshi *et al* (1999) descnbed the culture of *Clavıceps sorghıcola* as white to cream coloured on PDA medium, velvety colourless, with a hyphal growth of 1mm day^{-1} at 25°C According to them, the optimum temperature for hyphal growth ıs 25°C and some hyphal growth occurs even at 10°C and 35°C.

2.3.2 Anamorph

The anamorph of the three speciıes of *Clavıceps* that infect sorghum in different areas is *Sphacelia sorghı* McRae (Bandyopadhyay *et al*., 1998) The pathogen produces three types of single celled, hyaline spores, oblong to oval macroconidia (Tarr, 1962 and Kulkarnı *et al*, 1976), spherical microconidia (Mantle, 1968) and pear shaped secondary conidia (Frederickson *et al*, 1989)

(a) Sphacelia

Germinatıng spores produce hyphae that ınvade the ovary and replace it with a white fungal mass or sphacelium. Sphacelia are white rounded to egg shaped structure in the sorghum florets (Frederickson *et al* 1991)

The sphacelia of *Claviceps sorghi* are cylindrical, curved or strainght, bilaterally grooved, visible 1–2 days after honeydew exudation begins. At maturity, the protruding sphacelial portion of the parasitic biomass discoloured (Sangitrao and Bade, 1979b and Frederickson *et al.* 1991).

Frederickson *et al.* (1991) described sphacelia of *Claviceps africana* as bulky, soft, white, highly convoluted, oval to spherical fungal structure protruding between the glumes, measuring 5–8 mm long bearing macroconidia in discrete pockets

The slower rate of colonization of ovary by the fungi at lower temperature was reported by Bandyopadhyay *et al.* (1996). According to him the sphacelia appeared at the basal end of the ovary 5 days after inoculation (DAI) at 25°C, 6 DAI at 20°C and 8 DAI at 15°C. Development of sphacelia delayed at lower temperatures

(b) Macroconidia

Macroconidia of *Claviceps sorghi* are elliptic or ovate with smoothened ends, and with distinct vacuoles measuring 15 × 7μm in dimension (Sundaram, 1970).

The original discription of Kulkarni *et al.* (1976) describes macroconidia as 8–19 × 4–6μm. Bandyopadhyay *et al.* (1990) described macroconidia of *Claviceps sorghi* as hyaline, unicellular, elliptical to oblong with round ends and measured 5.1–7.7 × 7.7–23.0 μm (mean of 100 macroconidia: 7.3 × 13 2μm) in green house at a day and night temperature of 28/23°C with 90% RH for 16 h and a 5.1–7 7 × 6.4–17.9 μm (mean of 100 conidia: 6.9 × 10.9μm) at a day and night temperature of 38/28°C with 90% RH for 12 h, whereas, Frederickson *et al.* (1991) gave measurements of macroconidia of *Claviceps sorghi* as 8–19 × 4–6 μm.

Frederickson *et al.* (1991) described macroconidia of *Claviceps africana* as hyaline, mononucleate, oblong to oval measuring 9–17 × 5–8 μm, slightly constricted at the centre with two polar vacuoles.

The alteration in the macro conidial size and shpae of *Sphacelia sorghi*, when passed on to different hosts, was reported by several workers (Ramakrishnan, 1948, Chinnadurai and Govindaswamy, 1971 and Sangitrao and Moghe, 1995)

Sangitrao (1982) reported that the conidia of *Sphacelia sorghi* remained viable on the heads for one year when maintained at room temperature (max 29°C and min 17°C) When smeared on the seed surface 1–2% conidia germinated for upto 48 h, and lost viability completely in 72 h Conidia developed on modified Kirchoff's medium showed 80–100% germination

Manzarpour (1985) found that most conidia germinated to form microconidia at 24–30°C, whereas, at higher temperatures they germinated by forming hyphal germ tubes

Frederickson (1990) observed that macroconidia of *Claviceps sorghi* required 16 h to germinate *in vitro* at all temperatures, conidia germinated from 14°C to 37°C, optimally at 35°C Germination percentage did not vary significantly over 18–30°C range, but was always iterative, whereas in *Claviceps africana* macroconidia began to germinate after 12 h at 14–35°C The optimum temperature was 19°C and conidia failed to germinate at 37°C

Sangitrao and Moghe (1995) revealed that in the honeydew of *Dicanthium caricosum*, infected with *Sphacelia sorghi*, macroconidia were found to be triangular in shape

At 35°C, the germination of *Claviceps sorghi* was in the form of two, sometimes three germ tubes Macroconidia were able to germinate, even with 34% w/v sucrose in the media, whereas the macroconidia of *Claviceps africana* were unable to germinate once the sucrose content of the medium reached 10% w/v (Bandyopadhyay *et al* , 1996)

Germination of macroconidia of *Claviceps sorghi* was studied on water agar with or without amendment of suspension of stigmatic macerate at 15°C, 20°C, 25°C, 30°C and 35°C. Macroconidia germinated in two modes, iteratively from lateral sides by producing thin, slender, sporogenous germ tubes terminating in secondary conidia, and non-iteratively by producing thick germtubes, from both ends. Macroconidia almost always germinated non-iteratively in association with stigmatic macerate, and iteratively in the absence of stigmatic macerate (Bandyopadhyay *et al.*, 1996). Whereas, the germination of macroconidia of *Claviceps africana* was always iterative, i.e., producing a secondary conidium (Bandyopadhyay *et al.*, 1996).

Tsukiboshi *et al.* (1999) described macroconidia of *Claviceps sorghicola* as small, hyaline, ellipsoidal to oval measuring 5–11 3 × 2.5–3.8 µm.

(c) Microconidia

The honeydew of sorghum ergot infected by *Sphacelia sorghi* contains hyaline, round to obovate microconidia, and their number increases as the honeydew ages

Kulkarni *et al* (1976) in his original description of *Claviceps sorghi*, described the microconidia as spherical in shape measuring 2.5 µm in diameter. Frederickson *et al.* (1991) described microconidia of *Claviceps africana* as hyaline, spherical measuring 2–3 µm in diameter. Frederickson and Mantle (1988) noted microconidia of *Claviceps sorghi* germinating by one to several germ tube(s) within 16 h at 12–28°C. Bandyopadhyay *et al.* (1990) observed germination of microconidia after 24 h at 14–24°C, following inoculation onto the stigma.

Bandyopadhyay *et al.* (1990) described microconidia of *Sphacelia sorghi* as hyaline, spherical to obovate measuring 2 6–5.1 × 2.6–6.7µm (mean of 100 microconidia: 3 × 4 µm). A dense matrix of microconidia is found in a white, fragile crust around the developing sclerotia in

the sorghum genotype Tsukiboshi *et al* (1999) reported no production of microconidia by *Claviceps sorghicola.*

(d) Secondary conidia

Bandyopadhyay *et al* (1990) described secondary conidia of *Sphacelia sorghi* as hyaline, pyriform measuring 12–7 7 × 7 7–20 5 µm

The erect germtubes of conidiophores pierced through the hyphal mat and their proximate ends expanded into a bulb, which differentiated into secondary conidia The cytoplasm of the macroconidia passed through conidiophores into secondary conidia during their formation A mature secondary conidium was readily detached from the conidiophore by a constriction mechanism at its proximal end, which left a scar that distinguished it from a macroconidium (Bandyopadhyay *et al* , 1990)

Germ tube formation occurs as early as 6 h after the honeydew exudation from the stroma and the secondary conidia were formed within next 16 h (Bandyopadhyay *et al* , 1990)

Frederickson *et al* (1991) described secondary conidia of *Claviceps africana* as pear shaped borne on sterigma-like processes are 8–14 × 4–6 5 µm with a distinct, protruding hilum

Under natural conditions, secondary conidiation is a common feature of *Claviceps africana* and is occasionally observed in *Claviceps sorghi* but has never been seen naturally on honeydew of the Japanese species (Bandyopadhyay *et al* , 1998)

Tsukiboshi *et al.* (1999) reported the absence of secondary conidiation in the honeydew of *Claviceps sorghicola.*

2.3.3 Teleomorph

(a) Sclerotia

Production of sclerotia by *Sphacelia sorghi* was reported by Ajrekar (1926) which were overgrown with *Cerebella sp.* and yeast do not germinate

Ramakrishnan (1948) measured the sclerotia of *Sphacelia sorghi* to be 10–25 × 4–6 mm in dimension According to him obviously a cooler climate is necessary for sclerotial production, as he observed occurrence of the sclerotia at higher latitudes

Ramakrishnan (1948) observed the absence of ergotoxine in the sclerotia and reported the non toxicity of sorghum ergot sclerotia to cattle which are allowed to eat ergotized ears of sorghum

Four or five weeks period after inoculation are required for sclerotia to develop to maturity Sorghum florets that become infected at the end of the wet season and developed in the dry season produced sclerotia free from fungal parasites and the sclerotial development is very poor during the wet season owing to the heavy growth of fungal parasites in the honeydew (Futrell and Webster, 1966)

Sundaram (1968) mentioned in his report the occurrence of sclerotial bodies from many places in India, *viz* , Indore (Madhya Pradesh), Hyderabad (Andhra Pradesh), Coimbatore (then Madras state) and Jalna (Maharashtra state) According to him the sclerotia of *Sphacelia sorghi* are soft, tender, light grey in colour, long, up to 3 cm but are sparsely formed

Kulkarni *et al* (1976) and Alderman *et al* (1999) described the sclerotia of *Claviceps sorghi* as long, cylindrical, curved or straight in shape, soft or hard in texture, grey or light brown in colour and measuring 3–14 × 1–2 5 mm in size with two longitudinal grooves

According to Sangitrao and Bade (1979a) sclerotial formation takes place in about 1 to 2 months after the honeydew stage and the maximum sclerotial formation occurs in male sterile line Birds attacking grains do not disturb sclerotia, the sclerotial tapering end (part outside the glume) undergo weathering during rains and become papery and fall down (Sangitrao and Bade, 1979a)

The fungal parasites such as *Fusarium moliniforme*, *F. roseum fsp. cerealis*, *Cerebella sp* and *Cladosporium sp* were found on the honeydew and on the surface of the sclerotia Sangitrao and Bade (1979a) reported that sclerotial formation does not take place when honeydew is washed by rains Sclerotial production is also arrested by the fungus *Cerebella sp* which colonizes the sugary stage of the pathogen especially in *kharif* season

Sangitrao and Bade (1979b) and Frederickson *et al* (1991) observed the discoloration of protruding sphacelial portion of the parasitic biomass at its maturity stage whilst the proximal part, largely with in the glumes, contains the true sclerotium, composed of compact, white plectenchymatous tissue under a thin, red brown cortex

Bandyopadhyay *et al* (1990) showed that temperature and relative humidity affected honeydew formation and spore production, but not stromata development According to him temperature from 14–28°C combined with RH above 90% for 12–16 h/day are highly conducive to conidial production and hence pathogen spread, but not for differentiation of stormata into sclerotia In contrast, at 28–35°C and RH below 90% for 22 h/day stromata developed into sclerotia, but honeydew and spore production were suppressed

Frederickson *et al* (1991) described sclerotia of *Claviceps africana* as oval or spherical, hard, red brown, with few grooved fissures, measuring 4–6 × 2–3 mm Bandyopadhyay *et al* (1998) in his report differentiated the sclerotia of *Claviceps africana*, *Claviceps sorghi* and

Claviceps sorghicola The sclerotia of *Claviceps africana* are spherical, and largely confined with in the host glumes, whereas sclerotia of *Claviceps sorghi* are thin, elongate, and protruding and sclerotia of the *Claviceps sorghicola* are conical, elongate and purple black The alkaloid dihydroergosine is synthesized in the sclerotia of *Claviceps africana* and *Claviceps sorghicola* may contain small amounts of the alkaloid polyclavine, but the sclerotia of *Claviceps sorghi* do not synthesize alkaloids

According to Alderman (1999), sclerotia of *Claviceps africana* are not separate structure from sphacelia but are so closely physically associated that it is better to regard them only as different tissues Sclerotial tissue form, inside and to the base of sphacelial tissues, under dry condition Bogo and Mantle (1999) reported the production of roughly spherical sclerotia by Indian isolates which were typical of *Claviceps africana*

Tsukiboshi *et al.* (1999) described the sclerotia of *Claviceps sorghicola* as cylindrical to conical, straight or curved 2 5–20 mm long 1 9–3 5 mm wide, the true sclerotia purplish black, having longitudinal grooves on the surface, covered with white spherical tissues The hard texture, size and colour of scleorita different from sclerotia of *Claviceps sorghi* and *Claviceps africana*

(b) Stroma, asci and ascospore

Kulkarni *et al* (1976) described the teleomorphic stage of *Claviceps sorghi* They observed 2–3 stromata, stipe, and capitula of unspecified sizes Perithecia, 132 8–232 4 × 66 4–124 5 mm Asci (56–112 × 2 4 –3 2 µm), cylindrical with tapering ends and a hyaline apical cap, 8 ascospores, filiform and measuring 40–85 × 0 4–0 8 µm in size

The description of the teleomorphic stage provided by Sangitrao (1982) was almost similar to that of Kulkarni *et al* (1976) except for few minor details Frederickson *et al* (1991)

described the stipes of *C. sorghi* measuring 6–8 × 0.5 mm, burnished-bronze or deep terracotta in colour. The capitula 0 7 mm diameter, buff coloured but with darker, papillate perithecial ostioles, the stipe insertion point is surrounded by a white frill Perithecia measure 130–250 × 60–125 µm The white frills were not described by Kulkarni *et al* (1976) and Sangitrao (1982)

Frederickson (1990) and Frederickson *et al* (1991) described the stromatal origins of *Claviceps africana* as pale, globose stroma arising from sclerotia, fully extended stipes (8–15 × 0 3–0 6 mm) are pigmented purple, adjacent to the capitulum Capitula (0 5–1 3 mm) are subglobose, and intensely purle Perithecia are 86–135 × 123–226 µm, mature asci *in situ*, 140 × 3 2–4 2 µm, 8 ascospores measuring up to 45 × 0 8–1 2 µm

According to Tsukiboshi *et al* (1999) 1–4 stromata arise from one or two portions of the sclerotial surface of *Claviceps sorghicola* Stipes 3 5–17 mm long, brown to bronze, capitula globose to subglobose, 0 5–1 6 mm diameter, dark brown, distinctly papillate Perithecia ovate to pyriform, 215–300 µm long, 105–140 µm wide, embedded in the surface of capitula, ostioles evidently erumpent Asci cylindrical, hyaline, 122–215 µm long and 2 5–3 8 µm wide with thickened apical cap Ascospores filiform hyaline eight per ascus, measuring 92–205 µm long and 0 5–1 µm wide

2.4 Pathogenicity

2.4.1.Inoculation methods

The inoculation methods used by various workers for inducing the disease on sorghum heads are reviewed hereunder

Ramakrishnan (1948) used a suspension of the spores (macroconidia and microconidi) from the cultures for inoculation of young flower of sorghum and the suspension was sprayed, by an atomizer at the time of flower opening Subsequently, several workers have used the spore

suspension obtained by soaking the infected panicle in sterile water to release ergot conidia from the honeydew. The spore suspension of 10^6 conidia ml^{-1} was spray inoculated until run off, to the panicles when stigmas of the top 25% spikelets emerged (Puranik and mathre,1971, Frederickson *et al.*, 1989 and 1993, Thakur *et al.*, 1989, Bandyopadhyay *et al.*, 1990, Tegegne *et al* , 1994 and Tsukhiboshi *et al.*, 1999).

Futrell and Webster (1966), Musabyimana *et al* (1995) and Pazoutova *et al* (2000) followed dip inoculation method by immersing the unpollinated panicles in conidial suspension and bagging to induce infection on fresh stigmas

Atrificial inoculation on grass hosts and other graminaceous hosts was made by spray inoculation (Chinnadurai and Govindaswamy, 1971) as well as dip inoculation (Futrell and Webster, 1966).

Futrell and Webster (1966) made inoculation on maize with conidial suspension of *Sphacelia sorghi* by injecting a water suspension of conidia in to ear shoots with a hypodermic needle, seven days after the silk emerged and was maintained by bagging the inoculated inflorescence with a plastic bag.

Tegegne *et al* (1994) reported that ergot severity of non trimmed and trimmed panicles was similar, and one inoculation produced as much ergot as two inoculations. Bagged panicles had significantly more infected spikelets than non bagged panicles

According to Tegegne *et al.* (1994) a single inoculation when anthesis began at the tip of the panicle followed by bagging, was most appropriate inoculation technique with respect to convenience, biological significance and statistical inference.

Bandyopadhyay *et al* (1991) obtained a high degree of 52–95% infection of spikelets upon inoculation on 1–4 days before pollination and only 4–10% infection of spikelets when

inoculated 1–4 days after pollination. Bogo and Mantle (1999) used suspension of hyphal bits for inoculation of florets of a male-sterile sorghum to induce the disease

2.4.2. Isolation methods

The fungus causing ergot disease in sorghum was isolated and grown on various culture media such as Kirchoff's medium (Ramakrishnan, 1948; Chinnadurai, 1972; Nagarajan and Saraswathi, 1975) T_2 agar medium (Spalla, 1973 and Pazoutova *et al.*, 2000) and PDA medium (Tsukiboshi *et al*, 1999 and Pazoutova *et al*, 2000)

Spalla (1973), Nagarajan and Saraswathi (1975), Tsukiboshi *et al.* (1999) and Pazoutova *et al* (2000) used young sphacelia (five days old) for isolation of ergot pathogen which were surface sterilized for 15 min in 1 3% sodium hypochlorite, washed for 1 or 2 min in 95% Et OH, and rinsed for three times in distilled water and were placed on T_2 agar medium in plates, supplemented with 100 $\mu g\ ml^{-1}$ of ampicillin (Spalla, 1973)

Chinnadurai (1972) and Tsukiboshi *et al* (1999) obtained the isolates from honeydew by diluting to appropriate 10^5 spores ml^{-1} with sterile distilled water, and spread on the surface of water agar incubated in the dark at 25°C. The germinated spores were transferred to Kirchoff's medium (Chinnadurai, 1972) or PDA acidified to pH 4 5 with lactic acid (Tsukiboshi *et al*, 1999), using a sterile glass needle.

Pazoutova *et al.* (2000) confirmed that passage of the ergot pathogen through the plant ensured a higher success rate for obtaining pure culture than isolation from the field collected samples that were often heavily contaminated with saprophytes

2.5 Factors influencing secondary conidiation

Microcycle or secondary conidiation in a sorghum ergot pathogen was first recognized in the *in vitro* germination of auxenically produced conidia of an Indian isolate of *Claviceps sorghi*. At moderate temperature (24–30°C) most of the macroconidia germinated in this way whereas at 37°C spores germinated by forming a hyphal germ tube. (Manzarpour, 1985).

Rain appears to provide the high humidity which is conducive for secondry conidial production, and is evidenced by the appearance of classic symptom of whitened honeydew the day after rain (Frederickson *et al.*, 1989).

Frederickson (1990) reported the extension of sterigma like process bearing secondary conidium with increasing temperature to a maximum of five times the conidial length at 30°C.

A diurnal pattern of secondary conidia concentration was reported by several workers with greatest occurrence at nightfall coinciding with the sharp rise in RH and fall in temperature (Frederickson *et al.*, 1989, 1991, and 1993, Bandyopadhyay *et al.*, 1990).

Bandyopadhyay *et al.* (1990) reported the *in vivo* production of secondary conidia in Indian *Claviceps sorghi* in controlled environment cabinets and in glasshouse experiments. According to them, the production of honeydew and formation of secondary conidia were most profuse at a day and night temperature of 28/23°C, followed by 24/14°C and least at 35/28°C.

According to Bandyopadhyay *et al.* (1991) secondary conidia are produced on the soil surface if honeydew drips and falls on to wet soil. Such secondary conidia were able to infect plants in the field, soilborne secondary conidia may also have implication as a source of primary inoculum for disease initiation in the field.

Macroconidia inside the thick honeydew do not usually germinate because of the high osmotic potential caused by the high sugar concentration in the honeydew matrix. However,

being hygroscopic, the surface of the honeydew might absorb water from the atmosphere This would lower its osmotic potential and make the honeydew 'thin' As a result, under humid conditions, macroconidia on the honeydew surface germinate (Bandyopadhyay *et al* , 1996)

Bandyopadhyay *et al* (1996) reported a temperature of 20± 2°C as most favorable temperature for secondary conidia production and it does not occur when there were several hot, dry days in succession

Iterative germination of macroconidia does not occur on stigma and when in contact with stigmatic macerate agar, 96–100% macroconidia germinated non iteratively, at 15–30°C, but only 30% at 35°C after 48 h Germ tubes were 10 times smaller at 15°C than at 20°C On water agar, iterative germination was 47% at 15°C, 94% at 20°C, 6% at 25°C and 3% at 30°C and no germination was observed at 35°C (Bandyopadhyay *et al* , 1996)

2.6 Epidemiology

Although the disease is both soil borne and airborne, the airborne secondary conidia plays significant role in causing outbreaks Atmospheric temperature and relative humidity levels were reported to play a significant role in the occurrence of the disease

Kulkarni (1942) reported most severe ergot infections within the range of 21–31°C when humidity was high and most damage occurred in those fields which flowered during cooler periods According to Quinby (1958) cloudiness during anthesis aids in disease development probably due to delayed anther dehiscence, pollen deposition and activity under these condition

Futrell and Webster (1965) stated that when flowers of wheat , oats or barly open during a period of cold weather (35 to 40°F) the pollen becomes nonfunctional and sterile Ergot infection often occurs under these conditions and critical period for infection of sorghum by the ergot pathogen is from flower opening and on set of anthesis to fertilization

Futrell and Webster (1966) attributed incidence of honeydew to high RH as being favourable for infection of this disease and near 100% RH for 24 h during the anthesis of a male sterile line was optimal for infection. They also reported that no secondary spread of the ergot disease was observed during the dry season of very low humidity.

Futrell and Webster (1966) found that period of 12 and 36 h also favored infection, but no disease development on flowers held at higher humidity for 9 days (216 hrs).

Dogget (1970) emphasized hot, wet weather as favourable environment whereas Sundaram (1971) attributed cool (19±1°C), wet, cloudy weather during the period of crop development favours rapid spread of disease.

Several workers have shown that early sowing avoids ergot infection (Singh, 1964; Sangitrao *et al.*, 1979; Anahosur and Patil, 1982). Desai *et al.* (1979) and Sangitrao *et al.* (1979) proposed the most suitable seeding date for escape from sugary infection was around July 20. They related sowing dates of sorghum in India to ergot severity in male-sterile sorghum line CK 60A. Early sowing before 20 July, which resulted in flowering during the warm part of the season promoted disease escape. Maximum disease was recorded in those plots sown after the end of July.

Molefe (1975) attributed the cause of epiphytotic occurrence of sorghum ergot in Botswana was due to the heavy rain fall of over 750 mm during growing season and the dew which persisted for several days following the rain, though the RH was not very high at that period.

Molefe (1975) from Botswana reported occurrence of ergot in sorghum panicles that flowered when humidity was high and at minimum and maximum temperature of 14°C and

27°C, respectively. According to Sangitrao and Bade (1979) a minimum temperature of 13–18.7°C and relative humidity of 76–84 per cent are most favorable for disease development.

Anahosur and Patil (1982) reported that the sorghum male sterile lines *viz.*, 2199A, CK-60A, 2077A and 296A suffer seriously accounting to loss in grain yield. According to them a minimum temperature from 18.9°C–20.7°C and RH from 67–84 per cent during anthesis favoured the development of ergot in these lines. Whereas Sangitrao and Bade (1979a) felt that when humidity is high (>90 per cent), rainfall is not essential for ergot development

In India, weather conditions in 38th to 4th meteorological weeks were favourable for 80–100 per cent of disease incidence, and 35–70 per cent disease severity. Sclerotial formation was directly proportional to the severity of the sphacelial stage. Cool temperature, high humidities, rain splashes, and constantly changing wind direction are congenial for the spread of the disease (Sangitrao, 1982).

Frederickson *et al.* (1989) measured the secondary conidia concentration using Burkard spore trap and reported a concentration of less than 10/ m^3 at the initial period of secondary conidiation and a greater concentration of 200 /m^3 at conducive environmental condition of high RH following a rainy day, low temperature and at nightfall.

McLaren and Wehner (1990) determined that the optimum temperature for ergot disease development was 19 5°C and the upper maximum limit was at 28°C, above which disease severity was negligible. McLaren and Wehner (1992) also suggested that the severity of ergo infection, under a given set of climatic conditions during early anthesis, is dependent on the degree of predisposition of sorghum florets to infection, and identified pre-flowering cold stress (average minimum temperature <12°C) 23–27 days before flowering (coinciding with the

leptotene phase of male meiosis) as a critical period affecting pollen viability and hence predisposition to infection.

Frederickson *et al.* (1993) found that ergot severities were significantly higher at 20°C and 25°C than at 30°C. At 30°C ergot severities of 0.9 per cent were recorded, as opposed to 28 per cent at 20°C and 14 per cent at 25°C. Similarly, the latent period for infection was significantly shorter at lower temperature.

Frederickson *et al.* (1993) demonstrated how rapid epidemic development of *Claviceps africana* can result from secondary sporulation of the pathogen. They considered secondary conidia to be the primary epidemiological agent within their experimental area.

Frederickson *et al.* (1989 and 1993) reported a diurnal pattern of secondary conidia concentration, with the greatest occurrence at nightfall, coinciding with the sharp rise in RH and fall in temperature.

Frederickson *et al.* (1994) reported that chemically induced male sterility may aid and explore the gynoecial susceptibility in sorghum which naturally escaped ergot when in their native latitude or when induced to flower in more convenient experimental environments.

McLaren (1996) used the maximum daily temperature of 28°C (temperature above which disease incidence is minimum) and minimum temperature of 12°C (temperature below which total sterility/maximum predisposition occurs) to identify high and low-risk flowering periods, based on long and medium-term weather data for various production areas in South Africa.

2.7 Disease spread

Several workers have depicted and later confirmed different agents for disease spread of sorghum ergot pathogen. Wild grasses and wild sorghum genotypes were found to be a source

of inoculum for sorghum ergot and it was considered as an important factor in determining the spread of the disease (Tarr, 1962; Futrell and Webster, 1966; Boon-long, 1992).

Several workers have assumed wind driven rain splash to be an important agent in the spread of the disease, which dissolves the honeydew thus transmitting the conidia from diseased to fresh healthy panicle. (Tarr, 1962; Futrell and Webster, 1966; Mantle, 1968; Bandyopadhyay, 1991).

The role of insects on the dispersal of conidia was taken into consideration, as the prime factor in disease spread by several workers (Futrell and Webster, 1966; Sundaram, 1968; Mantle, 1968). According to Sundaram (1968) a number of insects, especially honeybees, flies and certain wasps are attracted by the honeydew which contains large number of fungal spores mixed with the sugary syrup. They act as the voluntary carrier of the disease to other healthy ears in the same field as well as in the neighbouring areas. The groups of insects that normally visit flowering panicles and panicles with developing grains are different (Bandyopadhyay *et al.*, 1991)

Bandyopadhyay *et al.* (1992) showed that insects such as thrips, *Orius sps.*, beetles, midges, head bugs and hymenopterous insects visit infected panicles, during when ergot conidia become attached to their bodies and are transmitted. But they failed to transmit the disease when released in cages containing susceptible panicles suggesting that insects do not play any role in the spread of the disease.

Spread of sphacelial conidia by head to head contact was assumed to be a probable means of disease spread by Tarr (1962) and Mantle (1968). Whereas, Sangitrao and Bade (1979a) reported the role of seclerotia on the perpetuation and spread of the disease and according to them, the removal of sclerotia from sorghum grain will help in checking the further spread.

Sundaram (1976) and Mantle (1968) depicted the role of airborne transmission of ascospore as a noval factor for the spread and development of ergot disease of sorghum.

Luttrell (1981) observed honeydew in the field with a sparse white layer of phialides projecting above the drops and bearing single conidia. Thus he assumed the possibility of the airborne secondary conidia, to be the causal agent of infection, but only as a subsidiary means of dissemination.

Frederickson *et al.* (1989 and 1993) confirmed the wind dissemination of secondary conidia as the most significant mode of dispersal for both the local and long distance spread of the sorghum ergot pathogen.

Bandyopadhyay *et al.* (1991) showed that secondary conidia are produced on the soil surface if honeydew drips and falls on to wet soil. Such secondary conidia were able to infect plants in the field. Soil borne secondary conidia may also have implication as a source of primary inoculum for disease initiation in the field

2.8 Host range

Several workers have examined the host range of *Sphacelia sorghi*. Tarr (1962) suggested that wild grasses may be a source of primary inoculum of ergot of sorghum. Futrell and Webster (1966) reported infection of *Zea mays* and guinea grass (*Panicum maximum*) following artificial inoculation with *Claviceps africana*. According to them *Pennisetum typhoides* is a non-host for *Sphacelia sorghi*. Reddy *et al.* (1968) reported the ability of sorghum ergot pathogen to infect *Pennisetum typhoides*. Chinnadurai and Govindaswamy (1971) reported infection of maize, *Chenchrus ciliaris* and *C. setigerus* and *Sorghum caffrorum* following artificial inoculation with *Sphacelia sorghi*. Sundaram (1970) observed the natural occurrence of *Sphacelia sorghi* on pearl millet and upon cross inoculation studies confirmed the infection of

pearl millet by *Sphacelia sorghi*. Loveless (1971) confirmed sudan grass to be the host for *Sphacelia sorghi*. Molefe (1975) confirmed the ability of *Sphacelia sorghi* to infect *Pennisetum typhoides* and *Panicum maximum*.

The comprehensive list of Bandyopadhyay (1992) indicates that collateral hosts of *Claviceps sorghi*, include *Chenchrus setigerous, Ischaemum pilosum, Pennisetum orientale, S. arundinaceum, S. caffrorum, S. halepense, S. membranaceum, S. nitens, S. verticilliflorum*, and *Zea mays* Boon-Long (1992) reported infection of sorghum in Thailand with conidia from *Panicum maximum, Dicanthium annulatum, Brachiaria mutica, Sorghum sudanensis, S. almum*, and *S. halepense*. Sangitrao and Moghe (1995) reported natural occurrence of *Sphacelia sorghi* McRae on *Dicanthium caricosum, D. annulatum, Ischaemum pilosum*, and *Sehima nervosum*. Alderman (1999) reported infection of *Sorghum halepense* and *Pennisetum glaucum* following artificial inoculation with *Claviceps africana* under green house condition, but reported the non-occurrence of the disease under field conditions

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The present investigation was carried out at the International Crops Research Institute for Semi Arid Tropics (ICRISAT), Patancheru, Hyderabad 502 324, Andhra Pradesh, India.

The general laboratory techniques followed for the present study were, those described by Rawlins (1933), Aneja (1993) and Dhingra and Sinclair (1993) for preparation of media, sterilization, isolation and maintenance of fungal cultures with slight modifications wherever necessary.

3.1 Sterilization

Liquid and semi-solid materials, such as media and distilled water were sterilized in an autoclave at 121 6°C and 15 PSI for 20 minutes. Glassware and other equipments were sterilized in hot air oven at 170° C for 1 h after wrapping in an aluminium foil. Sodium hypochlorite (NaOCl, 1%) was used for surface sterilization of sphacelia, whereas rectified spirit was used for sterilization of inoculation-needles, foreceps, inoculation chamber and hands etc.

3.2 Medium used

T_2 agar medium was used in the present investigation.

3.2.1 Composition of the medium

Sucrose	100 g
L-Asparagine	10 g
Yeast extract	0.1 g
KH_2PO_4	0.25 g
$MgSO_4\ 7H_2O$	0.25 g

$FeSO_4.7H_2O$	0 02 g
$ZnSO_4\ 7H_2O$	0 015 g
KCl	0 12 g
$Ca(NO_3)_2\ 4H_2O$	1 00 g
Agar	20 g
Distilled water	1000 ml
pH	5 2

3.2.2 Preparation of medium

T_2 agar medium was prepared by melting 20 g of agar in 500 ml of distilled water for about 3 to 4 minutes in a microwave oven (high power selection) The other components of the medium were added in another 500 ml of water and dissolved them by heating in a microwave oven (high power selection) for about 3 minutes Both the preparations were mixed thoroughly and sterilized in an autoclave at 15 PSI (121 6°C) for 20 minutes At the time of pouring into the Petri plates, medium was supplemented with 100 $\mu g\ ml^{-1}$ of ampicillin

3.3 Plant Material

An ergot susceptible sorghum male sterile line 296A was used throughout this investigation The plants were grown in 25 cm diameter plastic pots containing black soil, farmyard manure and sand in the ratio of 2 1 1

3.4 Source of Seed

The seeds of sorghum male sterile line 296A were obtained from Sorghum Breeding Unit, ICRISAT

3.5 Collection of ergot isolates

Isolates of sorghum ergot pathogen were collected during surveys conducted by ICRISAT, National Research Center for Sorghum (NRCS) and State Agricultural Universities from different major sorghum growing areas of Andhra Pradesh, Karnataka, Maharashtra, Uttar Pradesh, Gujarat, Tamil Nadu and Rajasthan during the year 1999-2000. The number of isolates collected and places of survey are given in Table 1 & Fig. 2. A total of 89 sorghum ergot isolates were collected from 59 locations in seven states in India. These isolates were used to study the cultural characteristics.

3.6 Identification of the pathogen

To identify the pathogen associated with the disease in collected sorghum earhead samples, the honeydew from the infected panicle was placed on a clean glass slide in a drop of sterile distilled water and observed under the stereobinocular microscope for the presence of macroconidia and microconidia Measurements of conidia of the fungus were taken with the help of ocular micrometer after calibration of the microscope. The fungus associated with the disease was identified based on descriptions as given by Frederickson *et al* (1991) Photomicrographs of the pathogen were taken using stereobinocular microscope and phase contrast microscope to describe spore morphology

3.7 Maintenance of ergot isolates

The collected earhead samples were dried in shade thoroughly along with the paper bags and stored in a cloth bag in dry condition. Sufficient attention was given to maintain the genetic purity of the isolates throughout the study

Table 1. Details of sorghum ergot isolates collected from different states of India.

ID No.	Location	District	State	Cultivar	Crop stage	Incidence (%)	Severity (%)
Ergot isolates collected from Andhra Pradesh (27)							
AP0	ICRISAT-Patancheru	Medak	AP	CSH 9	PM	2	50
AP12	Maachinenapally	Mahabubnagar	AP	Yellow Jowar	PM	100%	100
AP13	Maachinenapally	Mahabubnagar	AP	SSG 878	50%FL	100	100
AP14w	Maachinenapally	Mahabubnagar	AP	White Jowar	PM	100	100
AP14y	Maachinenapally	Mahabubnagar	AP	Yellow Jowar	PM	20-25	100
AP15	Maachinenapally	Mahabubnagar	AP	Yellow Jowar	PM	30-40	100
AP16	Maachinenapally	Mahabubnagar	AP	Yellow Jowar	PM	20-25	100
AP16rb	Chicholi	Nalgonda	AP	Yellow Jowar	NR	NR	NR
AP16vr	Lam Farm	Guntur	AP	Local	NR	NR	NR
AP17	Maachinenapally	Mahabubnagar	AP	SSG 898	PM	30-40	100
APAU1	Rajendranagar	Ranga Reddy	AP	27 B	PM	NR	NR
APAU2	Rajendranagar	Ranga Reddy	AP	296 B	PM	NR	NR
APAU3	Rajendranagar	Ranga Reddy	AP	DSV 3	PM	NR	NR
APAU4	Rajendranagar	Ranga Reddy	AP	CS 3541	PM	NR	NR
NAP1	Tammalonigudem	Ranga Reddy	AP	Local	PM	10	25
NAP2	Chakalisherupally	Nalagonda	AP	Yellow Jowar	PM	10	10
NAP3	Injamuru	Nalgonda	AP	Local	Post matrty	10	10
NAP4	Adigama	Adilabad	AP	CSH 9	PM	10	10
NAP5	Dongregaw	Adilabad	AP	CSH 9	PM	5	10
NAP6	Gudi hatnur	Adilabad	AP	CSH 9	Post maturity	10	10
NAP7	Sitagondi	Adilabad	AP	CSH 9	Post maturity	10	10
NAP8	Chintakunta	Medak	AP	Yellow Jowar	PM	2	5
NAP9	Kamalapuram	Adilabad	AP	CSH 9	PM	100	90
NAP10	Mannur	Adilabad	AP	CSH 9	PM	10	5
NAP11	Naradegonda	Adilabad	AP	CSH 9	PM	1	5
NAP12	Kupti	Adilabad	AP	CSH 9	PM	1	5
NAP13	Burkapalle	Adilabad	AP	JK 22	PM	10	15
Ergot isolates collected from Karnataka (24)							
KA41	Muttagi	Bijapur	Kar	White Jowar	HD	3	90
KA53a	Almel	Bijapur	Kar	Fodder sorghum	SD	60	60
KA57a	Navadagi	Gulbarga	Kar	Hybrid sorghum	PM	<1	1_2
KA57b	Bachnal/Rachnal	Gulbarga	Kar	Hybrid sorghum	SD-PM	10	20_40
KA58b	Jalasangi	Bidar	Kar	Yellow Jowar	SD-PM	10	2_100
KA58b-1	Jalasangi	Bidar	Kar	Hybrid sorghum	PM	40	100
KA61	ARS-Bidar	Bidar	Kar	DKR 9501	PM	1_2	10_15
KA61b	ARS-Bidar	Bidar	Kar	SVD 9601	PM	1_2	10_15
KA61c	ARS-Bidar	Bidar	Kar	DSV 2	PM	1_2	Oct-15
KA61d	ARS-Bidar	Bidar	Kar	SHD 9704	PM	1 to 2	10_15
KA61e	ARS-Bidar	Bidar	Kar	SPV 1333	PM	1_2	10_15
SK1	Kbettali	Mandya	Kar	Popcorn	FL-SD	<01	10
SK2	Chinakurali	Mandya	Kar	Yellow Jowar	SD	10	100
SK3	Thendekere	Mandya	Kar	Local	SD	2	100
SK5	Laxmipura	Mandya	Kar	Ogarujola	SD	5	100
SK-5-21	Neralur	Bangalore	Kar	Fodder sorghum	HD	80	100
SK7	Dansigatta	Hassan	Kar	Ogarujola	SD	10	100
SK12	Hirebennur	Chitradurga	Kar	CSH 5	MS-SD	<01	<1-10
SK13	Obavvanagathi halli	Chitradurga	Kar	Local	SD-HD	3	40-100
SK14-23	Hasalli	Shimoga	Kar	Ogarujola	HD-PM	<5-10	40
SK16	Talaku	Chitradurga	Kar	MSH 51	SD-HD	80-90	100
SK17	Bommadevanahalli	Chitradurga	Kar	MSH 51	HD	5_10	50-60
SK-20-24	Talaku	Chitradurga	Kar	MSH 51	Post-PM	80-90	100

SK-22-24	Amarapura	Bellary	Kar	GK 52	Post-PM	10	50-100
UASD1	Ag college, UASD	Dharwad	Kar	CSH 13	PM	<2	10
Ergot isolates collected form Maharashtra (12)							
AK1	Akola	Akola	MH				
MH67	Risode	Akola	MH	CSH 9	MS-HD	2	5
MH70	Mana	Akola	MH	Nilva local	SD-HD	80	50-100
MH71	Chembeli	Nagpur	MH	Motitura	SD-HD	2	5-100
MH72	Mahagaon	Nagpur	MH	Kaderu local	FL-MS	3	5-100
MH73	Salod	Wardha	MH	Pandharpuri	PM	<1	5-100
MH74	Bori	Yavatmal	MH	CSH 14	HD-PM	<1	1_10
MH75	Malegaon	Nanded	MH	Gulbhendi red	PM	<1	1_10
MH76	Limbgaon	Nanded	MH	Pandrapiwala	SD-PM	50	20-100
MH78	SRS-Parbhani	Parbhani	MH	CSH 9	PM	30	100
MH79	SRS-Parbhani	Parbhani	MH	SPV 1333	PM	20	80-90
MH80	SRS-Parbhani	Parbhani	MH	Nilwa local	FL-MS	80-90	100
Ergot isolates collected from Uttar Pradesh (10)							
NI1	Rampur	Rampur	UP	Local	SD	NR	5_50
NI2	Mouranipur(SRS)	Jhansi	UP	SBPR 94004	PM	<1	5_25
NI3	Mouranipur(SRS)	Jhansi	UP	ICSA 696 (Male sterile)	PM	<1	25
NI4	Pantnagar	Nainital	UP	ICSB 91002	HD	40	5_60
NI5	Pantnagar	Nainital	UP	2219 A	HD	55	5_75
NI6	Pantnagar	Nainital	UP	2219 A	HD	55	5_75
NI7	Droli	Moradabad	UP	Local	Harevst	100	80-95
NI8	Didoli/Deroli	Moradabad	UP	Local	Harvest	100	80-95
NI9	Kunapur	Rampur	UP	Local	Harvest	70	10-100
NI10	Sonali	Moradabad	UP	Local	Harvest	100	20-100
Ergot isolates collected from Rajasthan (3)							
NI11	RCA1	Udaipur	Raj	SU 1A	PM	NR	NR
NI12	RCA2	Udaipur	Raj	SU 14A	PM	NR	NR
NI13	RCA3	Udaipur	Raj	SU 27A	PM	NR	NR
Ergot isolates collected from Tamil Nadu (5)							
TN6	Naligankothapalli	Dharmapuri	TN	Yellow Jowar	PM	<0.1	10
TN10	Muniyappan Kovil	Namakal	TN	Solan local	MS	30-50	80-100
TN11	Totivalayam	Erode	TN	Manjal jolam local	SD	50	30
TN12	TNAU, Millets scheme	Coimbatore	TN	Doddamanja jola local	HD-PM	15-20	100
TN13	TNAU, Millets scheme	Coimbatore	TN	TNSH 39	PM	2	15-50
Ergot isolates collected from Gujarat (8)							
Guj1	Surat	Surat	Guj	NR	NR	NR	NR
Guj5	Surat	Surat	Guj	NR	NR	NR	NR
Guj6	Surat	Surat	Guj	NR	NR	NR	NR
Guj9	Surat	Surat	Guj	NR	NR	NR	NR
Guj15	Surat	Surat	Guj	NR	NR	NR	NR
Guj20	Surat	Surat	Guj	NR	NR	NR	NR
Guj34	Surat	Surat	Guj	NR	NR	NR	NR
Guj35	Surat	Surat	Guj	NR	NR	NR	NR

AP : Andhra Pradesh
UP : Uttar Pradesh
Guj : Gujarat
FL : Flowering stage

Kar : Karnataka
Raj : Rajasthan
PM : Physiological maturity
SD : Soft dough stage

MH : Maharashtra
TN : Tamil nadu
HD : Hard dough stage
NR : Not recorded

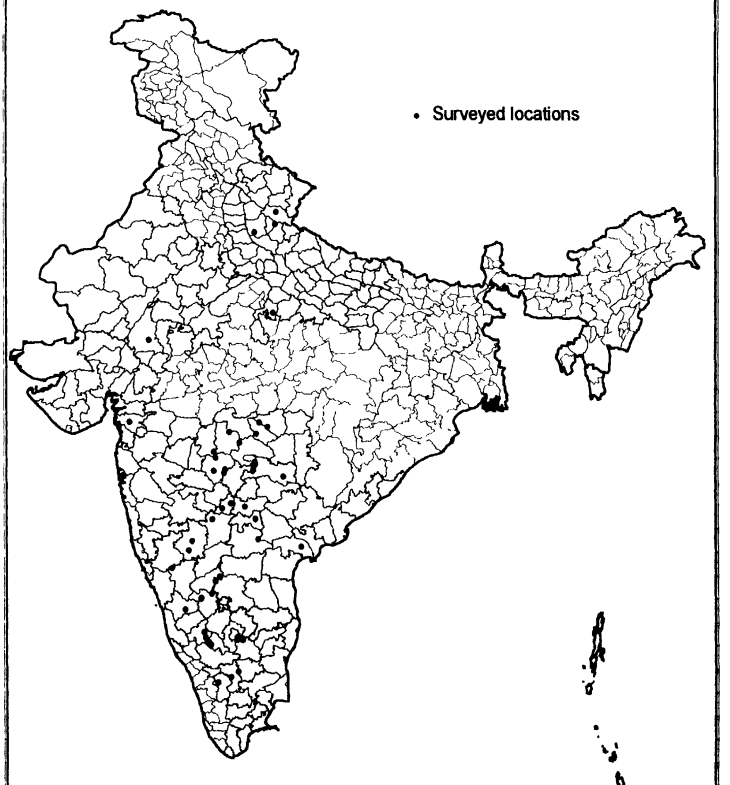

Figure 2. Areas surveyed for the collection of sorghum ergot isolates in 1999-2000

3.8 Pathogenicity test

Pathogenicity tests were carried out using susceptible sorghum male sterile line 296A. Panicles were covered with paper bags, as and when these emerged from boot leaves Bagging was carried out to avoid external inoculum from infecting the panicles Plants were spray inoculated with the desired isolate when the plants were in flowering stage with spikelets having fresh stigmas

(a) Preparation of inoculum

For each isolate, one rachis, branch containing infected flower showing typical symptom of ergot was taken from panicle collected from survey, and immersed in sterile distilled water for about one minute, to dissolve the honeydew completely The suspension was filtered through two layers of cheese cloth to get the conidial suspension without plant debris The resultant conidial suspension was diluted to get a spore load of 1×10^6 conidia ml^{-1}

(b) Inoculation of plants

The conidial suspension (1×10^6 conidia ml^{-1}) was spray inoculated (Plate 1) on sorghum male sterile line (296A) until runoff using a hand sprayer, when the stigmas of the top 50% spikelets emerged until runoff using a hand sprayer The inoculated panicles were covered with paper bags (Plate 2) to maintain high relative humidity (RH) inside and to avoid external contamination

(c) Incubation

Inoculated plants were kept in dew chambers (Plate 3) at 25°C and 100% RH for 24 h, to effect the infection process Later the plants were transferred to greenhouse and kept for incubation at 25°C and more than 80% RH, with bags enclosing the panicles, as they were Observations on honeydew formation were recorded as soon as the disease developed The

honeydew was further tested for the presence of macroconidia and microconidia and measurements of conidia were taken as described in Section 3.6 of Materials and Methods.

(d) Isolation of Pathogen

The infected spikelets from the panicles of inoculated sorghum male sterile line 296A plants were dissected to obtain young sphacelia five days after inoculation in a laminar air flow chamber. It was ensured that the sphacelia did not exude honeydew before dissection. The young sphacelia were surface sterilized using 1% sodium hypochlorite solution for 3 minutes, rinsed 5 times in distilled sterile water, and transferred to Petri plates containing T_2 agar medium The plates were sealed using a strip of parafilm sheet and incubated upside down for a period of 7 days and then incubated in its original position for further growth at 25 ± 1° C in an incubator The culture thus obtained on T_2 agar medium was used to study the cultural characteristics of each isolate. The ergot isolates were maintained on T_2 agar medium at 4°C and subcultured every 3 months

All the isolates were maintained on sorghum male sterile line 296A by periodic inoculation of conidial suspention (1×10^6 conidia ml^{-1}) prepared from honeydew collected from the infected sorghum panicles Inoculated plants were kept in growth chambers at 35±1°C and 40% RH for 30 days (Plate 4), to prevent secondary conidia formation and contamination among the isolates The plants were shifted to greenhouse when the consistency of the honeydew increased. To prevent the contamination of the inoculated plants by ants, antpans were kept at the bottom of the table. The inoculated plants were also sprayed with the insecticide monocrotophos (1 6 ml l^{-1}) at seven days interval to control other insect pests

3.9 Cultural characteristics of isolates

Observation on the diameter of the colony growth of different isolates was measured and recorded at 7-day interval on T_2 agar medium. For each isolate, the maximum colony growth, colour of the culture, colony type, puckering nature, pigmentation and sporulation on the medium were observed and recorded. Puckering nature of the colony of each isolate was recorded by using an arbitrary scale representing low, medium and high degree of puckering. Pigmentation was recorded using Munsell's colour chart for each isolate (APPENDIX I).

3.10 Grouping of isolates and selection of representative isolates

The ergot isolates were grouped based on the cultural characteristics, taking into consideration of colony type and sporulation on medium. After grouping the isolates, representative isolates were selected from each group from different geographical locations The representative isolates were used for further studies pertaining to *in vivo* variation in morphology of sphacelia, conidia and sclerotia, secondary conidia production, extent of disease spread, and host range.

3.11 *In vivo* variation of sphacelia, conidia, and sclerotia

To study *in vivo* variation in morphology of sphacelia, conidia and sclerotia, conidia of the representative isolates (obtained from infected plants) were spray inoculated on sorghum male sterile line 296A as given in Section 3 8 (a), (b) & (c) of Materials and Methods. The sphacelia and honeydew obtained from these inoculated plants were used to study the variation.

3.11.1 Sphacelial morphology

To study the sphacelial morphology, mature sphacelia were carefully dissected out from the infected spikelets, without damaging them, seven days after inoculation. Observations on colour, shape and size (length and breadth) of sphacelia were taken and recorded.

3.11.2 Conidial morphology

(a) Macroconidia

Seven days after inoculation, the bags covering the inoculated panicles were briefly opened and observed for honeydew secretion. Two-day old honeydew from different isolates was collected in vials and maintained individually to maintain genetic purity. Temporary aqueous mounts of conidial suspension were prepared after diluting the honeydew with sterile distilled water and observed under the stereobinocular compound microscope. Observations on shape, size, guttulation and constriction at the center were recorded.

(b) Microconidia

To study the morphology of microconidia 30 day-old honeydew (when it almost dry) was collected and aqueous mounts of conidial suspension were prepared by dissolving the honeydew in sterile distilled water and observed under stereobinocular microscope Observations on shape, size of microconidia were taken and recorded.

3.11.3 Sclerotial morphology

To study the morphology of ergot sclerotia, the sclerotia collected during the survey of sorghum growing areas were used. The study was made using the sclerotia with or without sphacelial cap. The sclerotia without sphacelial cap was obtained by immersing the sphacelial portion of the sclerotia into warm water and tearing out the sphacelial tissues from their tips using a foreceps, from their tips without causing damage to the sclerotial tissues. Observations on the size (length and breadth), shape and colour of the sclerotia were made and recorded.

3.12 Secondary conidia production

3.12.1 Effect of temperature

To determine the effect of temperature on secondary conidia production, ergot infected panicles from sorghum male sterile line 296A with 2-day old honeydew were taken. From each of the infected panicle, rachis with atleast six infected spikelets, were cut carefully without allowing the honeydew to drop. The base of the cut rachis was inserted into a 100 ml conical flask containing 2% sucrose solution covered with parafilm at the neck (Plate 5) For each isolates two such rachis were inserted per conical flask The flasks were incubated for 18 h in dew chambers at temperatures of 10, 15, 20, 25, 30 and 35°C keeping the RH constant at 100%, for secondary conidia production (Plate 5)

After incubation at different temperature regimes the rachis were taken out and four infected spikelets with honeydew were cut carefully and dispersed in 1 ml of sterile water to form a uniform suspension of germinated macroconidia From the suspension, samples were drawn and observed under stereobinocular compound microscope for germinated macroconidia Macroconidia that produced a germtube with a pointed tip was considered as those germinated, to produce secondary conidia The number of such germinated macroconidia was counted in 1000 macroconidia observed randomly The experiment was repeated thrice with three replications each The observations were recorded replication-wise for each temperature regime separately

3.12.2 Effect of relative humidity

To test the effect of relative humidity on secondary conidiation, ergot-infected panicles from sorghum male sterile line 296A with 2-day old honeydew were taken. From each of the infected panicle, rachis with atleast six infected spikelets were cut carefully without allowing the

honeydew to drop The base of the cut rachis was inserted into small vial containing 2% sucrose solution sealed with parafilm. For each isolate three such rachis were inserted in each vial These vials were incubated for 18 h at 80, 85, 90, 95, and 100% RH levels, keeping the temperature constant at 25°C for secondary conidia production (Plate 6–8)

After incubation at different RH regimes, the rachis were taken out and four infectd spikelets with honeydw were cut carefully and dispersed in 1 ml of sterile water to form a uniform suspension of germinated macroconidia From the suspension, samples were drawn and observed under stereobinocular compound microscope for germinated macroconidia The number of germinated macroconidia was counted in 1000 macroconidia observed randomly The experiment was repeated thrice with three replications each The observations were recorded replication-wise for each RH regime separately

3.11 Pattern and spread of the disease

To study the role of secondary conidia in the aerial spread of the disease an experiment was conducted using two distinct ergot isolates *viz*, NI2 from Group-I and NAP7 from Group-II in growth chamber with three replications The source plants (sorghum male sterile line 296A) raised in plastic pots were spray inoculated with conidial suspension (1×10^6 conidia ml^{-1}) of the two test ergot isolates separately at 50% flowering stage and incubated at 25°C and at 80 per cent relative humidity in greenhouse Inoculations were made one week before the commencement of the experiment The ergot inoculated plants were used as foci of infection (source of secondary conidia) in the experiment

The test plants (sorghum male sterile line 296A) were raised in 10" plastic pots in greenhouse and the panicles were covered with paper bags immediately after emergence to avoid

external contamination. The central foci of infection (source of secondary conidia) consisted of two artificially inoculated plants of highly susceptible sorghum male sterile line 296A showing the typical symptom (honeydew secretion) of the disease. Twenty two test plants were kept around the source plants after removing the paper bags covering the panicles (Plate 9). The plants were incubated at 25°C and at 97–100 per cent relative humidity to encourage secondary conidia production on the infected source plants and for infection of the test plants Both the test and source plants were exposed to 12 h light (6 a.m. to 6 p.m.) and 12 h dark (6 p.m. to 6 a.m.) for 10 days for complete appearance of the symptoms on the test plants. Care was taken to keep the growth chambers free of insects, to avoid spread of the disease by the insects. Observations on number of infected plants and number of infected spikelets were recorded for both the isolates separately as soon as the disease developed on test plants.

3.12 Host range

Investigations on host range of sorghum ergot pathogen was carried out with selected wild sorghum genotypes and other suspected host plants from graminaceous family. The list of host plants used for the study are given in the Table 2. The grass hosts were obtained from Dr. V. Bala Bhanu Murthy, Associate Professor (Agronomy), EMP Scheme, Rajendranagar, Hyderabad and were propagated through stumps in 25 cm diameter plastic pots containing black soil. The seeds of wild sorghum genotypes, pearl millet and maize cultivars (843A and Ashwini, respectively) were obtained from the Genetic Resources Unit, ICRISAT and were grown in 25 cm plastic pots filled with black soil, whereas, *Pennisetum glaucum* was grown in pots containing red soil. The panicles of grass hosts, sorghum wild genotypes and pearl millet cultivar and the cobs of maize were bagged upon emergence from boot leaves to avoid external contamination.

Table 2. List of graminaceous hosts used in the present investigation.

Sl. No.	Name of the host	Common Name
1	*Sorghum arundinaceum* (Desv.) Stapf	Wild sorghum
2	*S. halepense* (L.) Pers.	Wild sorghum
3	*S. versicolor* (Steud.) Stapf.	Wild sorghum
4	*S. virgatum* (Hack.) Stapf	Wild sorghum
5	*Pennisetum glaucum* (L.) R. Br.	Pearl Millet
6	*Pennisetum pedicellatum* Trin.	Deenanath grass
7	*Zea mays* L.	Maize
8	*Panicum maximum* Jacq.	Guinea grass (Riversdale)
9	*Panicum maximum* Jacq.	Giant guinea (variety)
10	*Panicum antidotale* Retz.	Blue panic
11	*Brachiaria mutica* (Forsk) Stapf.	Paragrass
12	*Brachiaria decumbens* (Forsk) Stapf.	Signal grass
13	*Cenchrus ciliaris* Linn.	Anjan grass/Buffel grass
14	*Cenchrus setigerus* Vahl.	Yellow anjan/Birdwood grass
15	*Andropogon gayanus* Kunth	Gamba grass
16	*Dichanthium annulatum* (Forsk.) Stapf.	Marvel grass
17	*Chrysopogon fulvus* (Spring.) Chiov	Dharaf grass
18	*Iseilema laxum* Hack.	Musal grass
19	*Bothriochloa pertusa* (L.) A. Camus	Bothriochloa grass
20	*Chloris gayana* Kunth	Rhodes grass

The grass hosts were dip inoculated whereas hosts like wild sorghum genotypes and pearl millet cultivar were spray inoculated For dip inoculation the panicles of different host plants at optimum stage (when the stigmas emerge from the spikelets and before anthers shed pollen) were immersed in the conidial suspension (1×10^6 conidia ml^{-1}) for 1 minute and bagged to maintain high humidity For spray inoculation the panicles of pearl millet cultivar and wild sorghum genotypes were sprayed with conidial suspension (1×10^6 conidia ml^{-1}) at 50% flowering stage and bagged immediately to maintain humidity In case of maize the cobs were dipped in ergot conidial suspension (1×10^6 conidia ml^{-1}) for 2 minutes and bagged immediately to maintain high humidity

The inoculated grass hosts and pearl millet plants were incubated at 25°C and 100% RH for 24 h in a dew chamber (Plate 10) for symptom expression, whereas, the tall growing sorghum wild genotypes and maize were kept in a greenhouse at 25°C and 85% RH for symptom expression

The inoculated panicles were observed for the disease symptoms and upon appearance of the symptoms, honeydew was collected from each of the infected host panicles and cross inoculated to sorghum male sterile line 296A at 50% flowering stage as mentioned in section 2 5(b) of Materials and Methods and observed for symptom expression Honeydew from infected panicles of sorghum male sterile line 296 A was collected and inoculated again to the host plants under study The experiment was repeated three times Honeydew collected from the infected host plants was observed for the presence of macroconidia and microconidia Observations on the shape, size, and colour of the conidia were recorded

3.13 Statistical analysis:

The data obtained in different laboratory experiments (*in vivo*) were statistically analysed by using completely randomized design (CRD) with one factor or two factors. The data pertaining to percentages were angular transformed and the values in numbers were square root transformed wherever necessary (Panse and Sukhatme, 1978). The analysis was done using computer package program GENSTAT (1986) version 5 (1994).

Plate 1 Spray inoculation of the panicle of male sterile line 296A with 1×10^6 conidial suspension of *Sphacelia sorghi*

Plate 2 Bagged inoculated panicles of sorghum male sterile line 296A

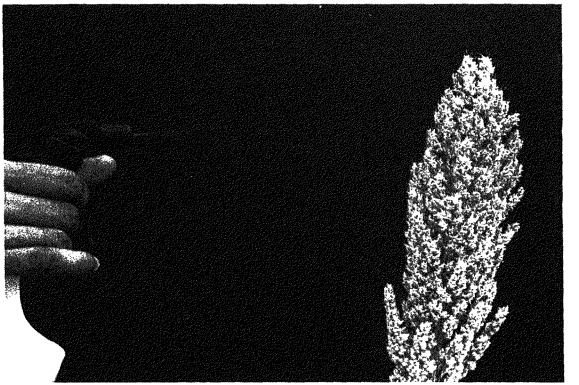

Plate 1

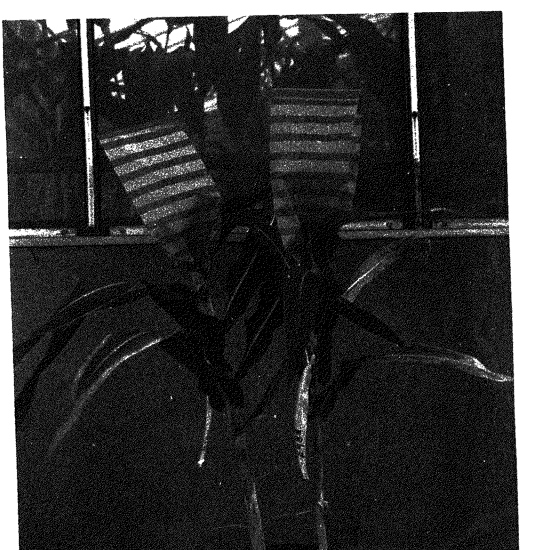

Plate 2

Plate 3 Inner view of the dew chamber with ergot inoculated sorghum plants

Plate 4 Ergot infected sorghum plants in a growth chamber

Plate 3

Plate 4

Plate 5 Infected sorghum (male sterile line 296A) rachis, with two-day old honeydew incubated in dew chamber for secondary conidia production at 20°C

Plate 6 Set of five single plant chambers, each containing ergot infected sorghum panicle rachis of ten representative isolates

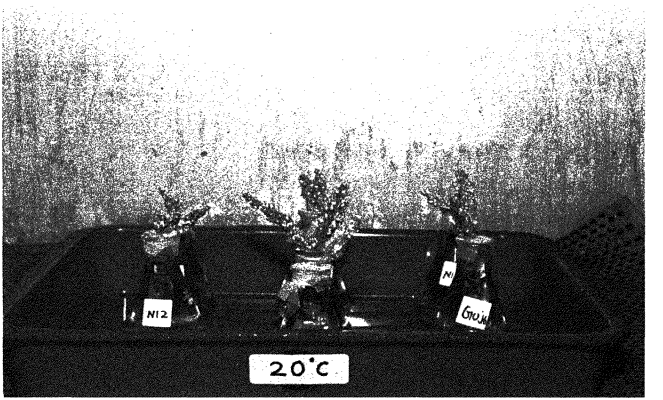

Plate 5

Plate 6

Plate 7: A closer view of the single plant chamber, showing infected sorghum panicle rachis incubated at 85% RH

Plate 8: A closer view of the single plant chamber, showing infected sorghum panicle rachis incubated at 100% RH

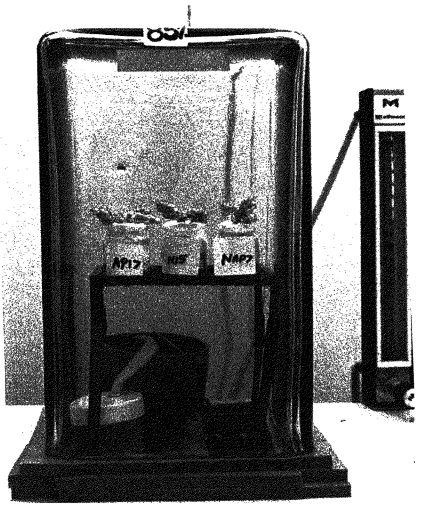

Plate 7

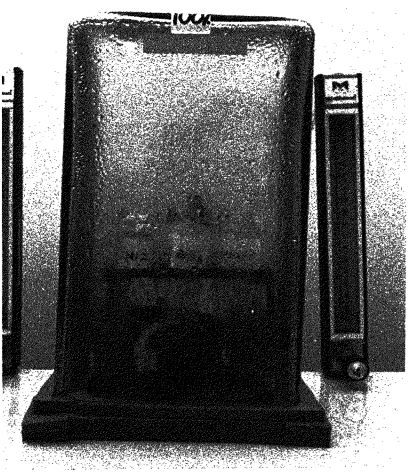

Plate 8

Plate 9: An inner view of growth chamber showing infected plants at the center and test plants at the periphery

Plate 10: An inner view of the dew chamber with inoculated grass plants

Plate 9

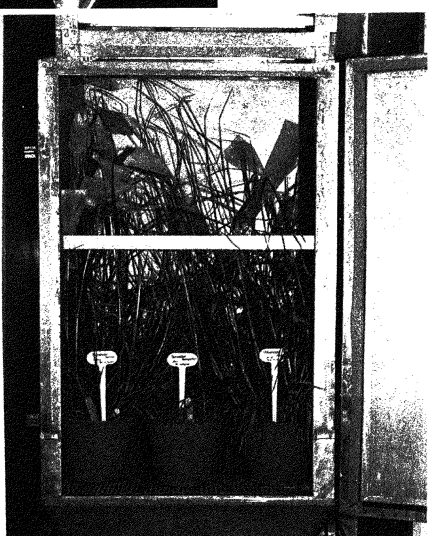

Plate 10

RESULTS

CHAPTER IV

RESULTS

The results of the experiments conducted in the present investigation on cultural characteristics, *in vivo* variation of sphacelia, conidia and sclerotia, effect of temperature and relative humidity on secondary conidia production, rate of disease spread, and host range, are presented hereunder.

4.1 Collection of ergot isolates

Eighty nine sorghum ergot isolates were collected from 59 locations distributed in seven states of India (Table 1). Of the 89 isolates, 27 isolates were collected from 18 locations in six districts of Andhra Pradesh *viz.*, Adilabad, Guntur, Mahabubnagar, Medak and Nalgonda and Rangareddy. The sorghum cultivars which were predominantly grown in Andhra Pradesh in 1999-2000 were CSH9 in Adilabad district, Yellow jowar in Mahabubnagar, Medak and Nalgonda districts. Whereas, local cultivars were grown predominantly in Guntur and Nalgonda districts. The incidence of sorghum ergot varied from 1–100 per cent and severity from 5 to 100 per cent in Andhra Pradesh. Cent per cent incidence and severity was observed in Maachinenapally mandal of Mahabubnagar district in Andhra Pradesh, where the disease occurred in an epidemic form during 1999–2000. From the surveys conducted, 24 ergot isolates were collected from 19 locations distributed in 10 districts of Karnataka viz., Bangalore, Bellary, Bidar, Bijapur, Chitradurga, Dharwad, Gulbarga, Hassan, Mandya and Shimoga. White Jowar, Yellow Jowar, Green Jowar, MH51 and hybrid sorghum were found to be the predominantly grown cultivars in Karnataka. The incidence of sorghum ergot ranged from 1 to 90 per cent and the severity ranged between 1 and 100 per cent. High incidence and severity (80–90% and 100%, respectively) of ergot occurred in Talaku mandal, Chitradurga district of Karnataka.

Twelve isolates were collected from Maharashtra from 9 locations distributed in six districts *viz.*, Akola, Nagpur, Nanded, Parbhani, Wardha and Yavatmal. The disease incidence ranged between <1 and 90%, and severity between 1 and 100%. In Parbhanbi the percentage of incidence and severity was found to be higher (90% and 100%, respectively) followed by Mana mandal of Akola district. Five sorghum ergot isolates were collected from 4 locations distributed in four districts of Tamilnadu *viz.*, Coimbatore, Dharmapuri, Erode and Namakkal. In most of the locations local cultivars were grown. The percentage of incidence and percentage of severity ranged from <0.1 to 50% and 10 to 100%, respectively. In Coimbatore and Namakkal higher percentage of disease incidence and severity were recorded (15–50% and 100%, respectively). In Uttar Pradesh, ten isolates were collected from 7 locations distributed in four districts *viz.*, Jhansi, Moradabad, Nainital and Rampur. In most of the surveyed locations in Uttar Pradesh, local sorghum cultivars were grown. Cent per cent disease incidence and severity was recorded in Moradabad district of Uttar Pradesh.

4.2 Identification of the pathogen

Microscopic examination of the honeydew collected from the infected sorghum earhead samples revealed the presence of macroconidia and microconidia. The macroconidia were oblong to elliptical, hyaline measuring 9–18 × 5.14–9 µm with mild constriction at the center. The microconidia were spherical, hyaline measuring 2.57–3.86 µm in diameter (Plate). On the basis of conidial morphology and their size, the fungus was identified as *Sphacelia sorghi* McRae and the morphological characters tallied with the descriptions given by Frederickson *et al.* (1991).

4.3 Pathogenicity test

The Koch's postulates were proved by spray inoculation with condial suspension (1×10^6 conidia ml^{-1}) of the pathogen on susceptible sorghum male sterile line 296Aat 50% flowering stage as mentioned in section 3.8(b) of materials and methods. Almost ninety to ninety-five per cent of the spikelets in an inflorescence were infected upon spray inoculation with conidial suspension (1×10^6 conidia ml^{-1}). The infected spikelets could be identified with flaccid stigma over the spikelets three days after inoculation. Six days after inoculation white, fungal mass or sphacelium emerged out of the spikelets between the glumes (Plate 11). Honeydew exudation was observed seven days after inoculation. Newly formed honeydew droplets were colourless and transparent with less consistency (Plate 12). When incubated at 90 to 100 per cent relative humidity secondary conidia production was observed on the surface of the honeydew as whitish layer (Plate 13). With time, the honeydew turned opaque and became uniformly yellowish brown to golden yellow in colour. Upon ageing the consistency of the honeydew increased (Plate 14).

4.4 Isolation of the pathogen

Isolation of the pathogen *Sphacelia sorghi* was made from the sphacelia taken from the infected spikelets of sorghum male sterile line 296A, in T_2 agar medium. Growth of the fungus when incubated at 25±1°C was observed 4–5 days after plating. The rate of growth of the culture was very slow in the medium. The maximum colony growth was obtained 25–30 days after plating. Thereafter the growth of the culture ceased. The culture was white, compact or cottony, raised or submerged depending on the isolate. Secondary growth of the culture was observed from the base of the compact colony 40–45 days after plating.

4.5 Maintenance of ergot samples and isolates

The collected shade dried ergot samples could be stored in dry condition in cloth bags at room temperature and the pathogen was viable in these samples throughout the study (more than 8 months). The culture of the pathogen could be maintained on T_2 agar medium by subculturing once in three months and storing at 4°C. The culture could be easily revived on T_2 agar medium whenever required

4.6 Cultural Characteristics

Studies on cultural characteristics of 89 ergot isolates grown on T_2 agar medium were made by recording the rate of colony growth, maximum radial growth, colony type, puckering nature and sporulation on the medium. The isolates differed distinctly in rate of colony growth, maximum radial growth, colony type, puckering nature and sporulation on the medium

4.6.1 Rate of colony growth

It is evident from the data presented (Table 3) that all the isolates under study differed significantly in the rate of colony growth which ranged between 0 03 and 0 12 mm day^{-1} Highest rate of colony growth (0 12 mm day^{-1}) was observed with sorghum ergot isolates NAP7, NAP13, and MH74 closely followed by isolates NAP4 and NAP5 The rate of colony growth was least (0 03 mm day^{-1}) in SK-20-24 and NI13 isolates All other isolates showed a slower growth rate ranging between 0 04–0 09 mm day^{-1}

4.6.2 Maximum radial growth

Significant difference was observed with respect to maximum colony growth of the different sorghum ergot isolates 30 days after incubation on T_2 agar medium (Table 3, Plate 15a-15j). Diameter of the colony on the medium ranged between 1 12 and 3 52 cm The isolate

Table 3. Cultural characteristics of various sorghum ergot isolates on T_2 agar medium.

Sl.No.	Isolate	Rate of growth (mm/day)	Maximum radial growth (cm)	Colony type	Colour of the culture	Puckering nature	Pigmentation*	Sporulation
1	NAP1	0.04	1.3	Compact	White	L	2.5YR 4/5	Absent
2	NAP2	0.04	1.21	Compact	White	M	2.5YR 5/4	Absent
3	NAP3	0.04	1.33	Compact	White	M/H	2.5YR 3/4	Absent
4	NAP4	0.11	3.27	Cottony	White	Nil	2.5YR 6/6	Present
5	NAP5	0.11	3.25	Cottony	White	Nil	2.5YR 6/6	Present
6	NAP6	0.07	2.1	Compact	White	L	2.5YR 5/6	Absent
7	NAP7	0.12	3.33	Cottony	White	Nil	2.5YR 5/4	Present
8	NAP8	0.04	1.4	Compact	White	M	2.5YR 5/6	Absent
9	NAP9	0.08	2.4	Compact	White	L	2.5YR 5/5	Absent
10	NAP10	0.05	1.64	Compact	White	M	2.5YR 4/6	Absent
11	NAP11	0.04	1.2	Compact	White	L	2.5YR 4/5	Absent
12	NAP12	0.04	1.32	Compact	White	M	2.5YR 5/6	Absent
13	NAP13	0.12	3.4	Cottony	White	Nil	2.5YR 5.5/4	Present
14	APAU1	0.05	1.52	Compact	White	L	2.5YR 5/6	Absent
15	APAU2	0.04	1.22	Compact	White	L	2.5YR 6/6	Absent
16	APAU3	0.06	1.95	Compact	White	L	2.5YR 6/6	Absent
17	APAU4	0.04	1.24	Compact	White	L	2.5YR 6/6	Absent
18	AP0	0.05	1.6	Compact	White	M/H	2.5YR 6/6	Absent
19	AP12	0.05	1.56	Compact	White	M	2.5YR 4/5	Absent
20	AP13	0.06	1.9	Compact	White	L	2.5YR 5/6	Absent
21	AP14w	0.06	1.73	Compact	White	L/M	2.5YR 5.5/6	Absent
22	AP14y	0.05	1.5	Compact	White	M	2.5YR 6/6	Absent
23	AP15	0.06	1.8	Compact	White	L	2.5YR 5/6	Absent
24	AP16	0.07	2.12	Compact	White	L	2.5YR 5/6	Absent
25	AP16RB	0.06	1.8	Compact	White	L	2.5YR 5/6	Absent
26	AP16Vr	0.04	1.25	Compact	White	L	2.5YR 4/6	Absent
27	AP17	0.05	1.6	Compact	White	L	2.5YR 5/6	Absent
28	KA41	0.04	1.2	Compact	White	L	2.5YR 6.5/6	Absent
29	KA53a	0.07	2.16	Compact	White	M	2.5YR 3/5	Absent
30	KA57b	0.06	1.75	Compact	White	L	2.5YR 5/6	Absent
31	KA58b	0.05	1.5	Compact	White	M	2.5YR 4/4	Absent
32	KA58b-1	0.06	1.75	Compact	White	M	2.5YR 5/6	Absent
33	KA61	0.06	1.77	Compact	White	M	2.5YR 5/6	Absent
34	KA61b	0.08	2.37	Compact	White	M	2.5YR 5/6	Absent
35	KA61c	0.04	1.35	Compact	White	L	2.5YR 3/5	Absent
36	KA61d	0.06	1.75	Compact	White	M	2.5YR 4/6	Absent
37	KA61e	0.08	2.53	Compact	White	L	2.5YR 4/6	Absent
38	SK1	0.06	1.9	Compact	White	L	2.5YR 5/6	Absent
39	SK2	0.05	1.52	Compact	White	M/H	2.5YR 3/2	Absent
40	SK3	0.04	1.25	Compact	White	L	2.5YR 5/6	Absent
41	SK5	0.05	1.4	Compact	White	L/M	2.5YR 4/4	Absent
42	SK7	0.08	2.43	Compact	White	L/M	2.5YR 4/6	Absent
43	SK12	0.05	1.52	Compact	White	M	2.5YR 3/4	Absent
44	SK13	0.05	1.6	Compact	White	M/H	2.5YR 3/3	Absent
45	SK16	0.06	1.9	Compact	White	M	2.5YR 4/5	Absent
46	SK17	0.05	1.16	Compact	White	L	2.5YR 6/6	Absent

47	SK-5-21	0.05	1.43	Compact	White	L	2.5YR 4/6	Absent
48	SK-14-23	0.05	1.65	Compact	White	L	2.5YR 5/6	Absent
49	SK-20-24	0.03	1.12	Compact	White	L	2.5YR 4/6	Absent
50	SK-22-24	0.04	1.42	Compact	White	M	2.5YR 4/6	Absent
51	UASD1	0.09	2.93	Compact	White	L	2.5YR 5/6	Absent
52	AK1	0.04	1.4	Compact	White	L	2.5YR 5/6	Absent
53	MH67	0.05	1.4	Compact	White	M	2.5YR 4/6	Absent
54	MH70	0.07	2.18	Compact	White	M	2.5YR 6/6	Absent
55	MH71	0.06	1.73	Compact	White	L	2.5YR 5/4	Absent
56	MH72	0.05	1.61	Compact	White	L	2.5YR 5/4	Absent
57	MH73	0.05	1.43	Compact	White	L	2.5YR 5/6	Absent
58	MH74	0.12	3.52	Cottony	White	Nil	2.5YR 4/4	Present
59	MH75	0.04	1.2	Compact	White	L	2.5YR 5/6	Absent
60	MH76	0.05	1.42	Compact	White	L	2.5YR 4/4	Absent
61	MH78	0.05	1.63	Compact	White	L	2.5YR 5/6	Absent
62	MH79	0.04	1.27	Compact	White	L	2.5YR 3/4	Absent
63	MH80	0.07	2.2	Compact	White	L	2.5YR 4/6	Absent
64	NI1	0.07	2.24	Compact	White	M	2.5YR 5/6	Absent
65	NI2	0.05	1.63	Compact	White	M/H	2.5YR 5/6	Absent
66	NI3	0.05	1.52	Compact	White	H	2.5YR 4/4	Absent
67	NI4	0.05	1.45	Compact	White	M	2.5YR 5/4	Absent
68	NI5	0.05	1.4	Compact	White	M	2.5YR 4/4	Absent
69	NI6	0.06	1.76	Compact	White	M	2.5YR 4/4	Absent
70	NI7	0.06	1.86	Compact	White	H	2.5YR 4.5/4	Absent
71	NI8	0.06	1.9	Compact	White	M	2.5YR 3/4	Absent
72	NI9	0.05	1.55	Compact	White	H	2.5YR 5/4	Absent
73	NI10	0.07	1.97	Compact	White	M	2.5YR 5/6	Absent
74	NI11	0.05	1.42	Compact	White	H	2.5YR 4.5/4	Absent
75	NI12	0.04	1.28	Compact	White	M/H	2.5YR 5/4	Absent
76	NI13	0.03	1.05	Compact	White	M	2.5YR 5/4	Absent
77	TN6	0.06	1.8	Compact	White	M	2.5YR 4/4	Absent
78	TN10	0.05	1.4	Compact	White	L	2.5YR 5/4	Absent
79	TN11	0.05	1.6	Compact	White	L	2.5YR 5/4	Absent
80	TN12	0.04	1.33	Compact	White	L	2.5YR 5/4	Absent
81	TN13	0.05	1.48	Compact	White	L	2.5YR 4/6	Absent
82	GUJ1	0.07	2.25	Compact	White	L	2.5YR 5/6	Absent
83	GUJ5	0.07	2.22	Compact	White	L	2.5YR 6/6	Absent
84	GUJ6	0.07	2.31	Compact	White	L	2.5YR 5/6	Absent
85	GUJ9	0.08	2.5	Compact	White	L	2.5YR 5/6	Absent
86	GUJ15	0.08	2.6	Compact	White	L	2.5YR 5/6	Absent
87	GUJ20	0.08	2.5	Compact	White	L	2.5YR 5/6	Absent
88	GUJ34	0.08	2.4	Compact	White	L	2.5YR 5/6	Absent
89	GUJ35	0.08	2.4	Compact	White	L	2.5YR 4/6	Absent
L: Low, M: Medium, H: High								
SEm+		0.009	0.089					
l.s.d. (5% level)		0.017	0.072					
CV%		18.1	11.3					
*As per Munsell's colour chart								

MH74 showed maximum growth (3 52 cm) followed by NAP7 (3 33 cm), NAP4 (3 27 cm) and NAP5 (3.25 cm) isolates. The isolate SK-20-24 showed the least colony growth (1.12 cm).

4.6.3 Colony type

Variation was observed with respect to colony type among the sorghum ergot isolates studied (Table 3) The fungus growth on the T_2 agar medium was cottony, velvetty and submerged with diffused margin (Plate 15a–15h) in ergot isolates NAP4, NAP5, NAP7, NAP13, and MH74 whereas it was compact, fleshy, raised with distinct margin in all other ergot isolates (Plate 15i & 15j)

4.6.4 Colour

There was no variation with respect to colour of the culture of different sorghum ergot isolates The colour of the colony of all the isolates was white on T_2 agar medium

4.6.5 Puckering nature

The isolates differed distinctly with respect to puckering nature of the colony Of the 89 isolates studied, only five ergot isolates *viz* , NAP4, NAP5, NAP7, NAP13, and MH74 showed no puckering (Plate 15i & 15j), whereas all other isolates showed low, medium and high degree of puckering (Table 3, Plate 15a–15h) Of these 47 isolates showed low degree of puckering, 27 isolates showed medium degree of puckering and 10 isolates showed high degree of puckering (Table 3)

4.6.6 Pigmentation on medium

Different sorghum ergot isolates produced pigmentation on medium ranging from dusky red to light red with a hue, value, chroma reading of 2 5YR 3/3 to 2 5YR 6/6, in Munsell's colour chart. There was no significant difference in pigmentation behaviour of the isolates (Table 3).

4.6.7 Sporulation

Sporulation was observed in cultures of NAP 4, NAP 5, NAP 7, NAP 13 and MH 74 isolates after 20–25 days of incubation on T_2 agar medium. All other isolates under study did not sporulate on the medium even after incubation at 25°C for 30 days. Sporulation was observed at the center of the colony (Plate 15i & 15j) as minute droplets of honeydew secretions containing both macroconidia and microconidia. The secretions were, pale brown in colour and were very thick in consistency

4.7 Grouping of isolates and selection of representative isolates

Grouping of ergot isolates was made on the basis of colony type and sporulation on T_2 agar medium Two distinct groups were made with one, having compact, raised colony with distinct margin, with no sporulation on the medium, and another having cottony or velvety, submerged colony with diffused margin and sporulation on the medium Of the 89 isolates, 84 isolates were placed in Group-I and 5 isolates were placed in Group-II based on cultural characteristics The isolates belonging to Group-I are distributed in 57 locations falling under seven states of India, *viz*, Uttar Pradesh, Rajasthan, Gujarat, Maharashtra, Andhra Pradesh, Karnataka and Tamilnadu, whereas, the isolates of Group-II are distributed in five different locations of two states *viz* Andhra Pradesh and Maharashtra (Tables 4 & 5) In Andhra Pradesh out of 27 ergot isolates collected, 23 isolates belong to Group-I, whereas, four isolates (NAP4, NAP5, NAP7 and NAP13) belong to Group-II, representing Adilabad district In Maharashtra state out of 12 ergot isolates collected, 11 isolates belong to Group-I and only one isolate (MH74) belongs to Group-II Of the 84 isolates eight isolates were selected from Group-I, representing different geographical locations in Uttar Pradesh, Rajasthan, Gujarat, Maharashtra, Karnataka, Tamil Nadu and Andhra Pradesh. Of the five isolates in Group-II, two isolates were

Table 4. Details of sorghum ergot isolates placed in Group-I

Sl.No.	ID No.	Location	District	State
1	AP0	ICRISAT-Patancheru	Medak	Andhra Pradesh
2	AP12	Maachinenapally	Mahabubnagar	Andhra Pradesh
3	AP13	Maachinenapally	Mahabubnagar	Andhra Pradesh
4	AP14w	Maachinenapally	Mahabubnagar	Andhra Pradesh
5	AP14y	Maachinenapally	Mahabubnagar	Andhra Pradesh
6	AP15	Maachinenapally	Mahabubnagar	Andhra Pradesh
7	AP16	Maachinenapally	Mahabubnagar	Andhra Pradesh
8	AP16rb	Chicholi	Nalgonda	Andhra Pradesh
9	AP16vr	Lam Farm	Guntur	Andhra Pradesh
10	AP17	Maachinenapally	Mahbubnagar	Andhra Pradesh
11	APAU1	Rajendranagar	Ranga Reddy	Andhra Pradesh
12	APAU2	Rajendranagar	Ranga Reddy	Andhra Pradesh
13	APAU3	Rajendranagar	Ranga Reddy	Andhra Pradesh
14	APAU4	Rajendranagar	Ranga Reddy	Andhra Pradesh
15	NAP1	Tammalonigudem	Ranga Reddy	Andhra Pradesh
16	NAP2	Chakalisherupally	Nalagonda	Andhra Pradesh
17	NAP3	Injamuru	Nalgonda	Andhra Pradesh
18	NAP6	Gudi hatnur	Adilabad	Andhra Pradesh
19	NAP8	Chintakunta	Medak	Andhra Pradesh
20	NAP9	Kamalapuram	Adilabad	Andhra Pradesh
21	NAP10	Mannur	Adilabad	Andhra Pradesh
22	NAP11	Naradegonda	Adilabad	Andhra Pradesh
23	NAP12	Kupti	Adilabad	Andhra Pradesh
24	KA41	Muttagi	Bijapur	Karnataka
25	KA53a	Almel	Bijapur	Karnataka
26	KA57a	Navadagi	Gulbarga	Karnataka
27	KA57b	Bachnal/Rachnal	Gulbarga	Karnataka
28	KA58b	Jalasangi	Bidar	Karnataka
29	KA58b-1	Jalasangi	Bidar	Karnataka
30	KA61	ARS-Bidar	Bidar	Karnataka
31	KA61b	ARS-Bidar	Bidar	Karnataka
32	KA61c	ARS-Bidar	Bidar	Karnataka
33	KA61d	ARS-Bidar	Bidar	Karnataka
34	KA61e	ARS-Bidar	Bidar	Karnataka
35	SK1	Kbettali	Mandya	Karnataka
36	SK2	Chinakurali	Mandya	Karnataka
37	SK3	Thendekere	Mandya	Karnataka
38	SK5	Laxmipura	Mandya	Karnataka
39	SK-5-21	Neralur	Bangalore	Karnataka
40	SK7	Darsigatta	Hassan	Karnataka
41	SK12	Hirebennur	Chitradurga	Karnataka
42	SK13	Obavvanagathi halli	Chitradurga	Karnataka
43	SK14-23	Hasalli	Shimoga	Karnataka
44	SK16	Talaku	Chitradurga	Karnataka

45	SK17	Bommadevanahalli	Chitradurga	Karnataka
46	SK-20-24	Talaku	Chitradurga	Karnataka
47	SK-22-24	Amarapura	Bellary	Karnataka
48	UASD1	Ag college, UASD	Dharwad	Karnataka
49	AK1	Akola	Akola	Maharashtra
50	MH67	Risode	Akola	Maharashtra
51	MH70	Mana	Akola	Maharashtra
52	MH71	Chembeli	Nagpur	Maharashtra
53	MH72	Mahagaon	Nagpur	Maharashtra
54	MH73	Salod	Wardha	Maharashtra
55	MH75	Malegaon	Nanded	Maharashtra
56	MH76	Limbgaon	Nanded	Maharashtra
57	MH79	SRS-Parbhani	Parbhani	Maharashtra
58	MH80	SRS-Parbhani	Parbhani	Maharashtra
59	NI1	Rampur	Rampur	Uttar Pradesh
60	NI2	Mouranipur(SRS)	Jhansi	Uttar Pradesh
61	NI3	Mouranipur(SRS)	Jhansi	Uttar Pradesh
62	NI4	Pantnagar	Nainital	Uttar Pradesh
63	NI5	Pantnagar	Nainital	Uttar Pradesh
64	NI6	Pantnagar	Nainital	Uttar Pradesh
65	NI7	Droli	Moradabad	Uttar Pradesh
66	NI8	Didoli/Deroli	Moradabad	Uttar Pradesh
67	NI9	Kunapur	Rampur	Uttar Pradesh
68	NI10	Sonali	Moradabad	Uttar Pradesh
69	NI11	RCA1	Udaipur	Rajasthan
70	NI12	RCA2	Udaipur	Rajasthan
71	NI13	RCA3	Udaipur	Rajasthan
72	TN6	Nallgankothapalli	Dharmapuri	Tamil nadu
73	TN10	Muniyappan Kovil	Namakal	Tamil nadu
74	TN11	Totivalayam	Erode	Tamil nadu
75	TN12	TNAU, Millets scheme	Coimbatore	Tamil nadu
76	TN13	TNAU, Millets scheme	Coimbatore	Tamil nadu
77	Guj1	Surat	Surat	Gujarat
78	Guj5	Surat	Surat	Gujarat
79	Guj6	Surat	Surat	Gujarat
80	Guj9	Surat	Surat	Gujarat
81	Guj15	Surat	Surat	Gujarat
82	Guj20	Surat	Surat	Gujarat
83	Guj34	Surat	Surat	Gujarat
84	Guj35	Surat	Surat	Gujarat

Table 5. Details of sorghum ergot isolates placed in Group-II

Sl.No.	ID.No.	Location	District	State
1	NAP4	Adigama	Adilabad	Andhra Pradesh
2	NAP5	Dongregaw	Adilabad	Andhra Pradesh
3	NAP7	Sitagondi	Adilabad	Andhra Pradesh
4	NAP13	Burkapalle	Adilabad	Andhra Pradesh
5	MH74	Bori	Yavatmal	Maharashtra

Table 6. Details of representative sorghum ergot isolates used in the investigation

Sl.No.	Isolates	location	District	State
	Group-I			
1	NI 2	Mouranipur	Jhansi	Uttar Pradesh
2	NI 5	Pantnagar	Nainital	Uttar Pradesh
3	NI 12	RCA2	Udaipur	Rajasthan
4	Guj 6	Surat	Surat	Gujarat
5	MH 71	Chembeli	Nagpur	Maharashtra
6	SK-20-24	Talaku	Chitradurga	Karnataka
7	AP 17	Maachinenapally	Mahbubnagar	Andhra Pradesh
8	TN 13	TNAU millet scheme	Coimbatore	Tamil Nadu
	Group-II			
9	NAP 7	Sitagondi	Adilabad	Andhra Pradesh
10	MH 74	Bori	Yavatmal	Maharashtra

selected representing different geographical locations in Andhra Pradesh and Maharashtra Details of the representative isolates and the locations are furnished in the Table 6

4.8 *In vivo* variation of sphacelia, conidia and sclerotia

4.8.1 Sphacelial morphology

Two days after inoculation, the stigmas of the infected spikelets present in the inoculated panicles became flaccid upon infection by the pathogen The fungus mycelium colonized the inner portion of the ovary and on third day, the ovary got sweller and became pale green (Plate 16b) Four days after inoculation, the appearance of the whitish mycelium at the basal end of the ovary noticed (Plate 16c) The mycelium grew on the outer surface of the ovary from the base towards the top On fifth day three fourth of the outer surface was covered by the mycelium (Plate 16d) Six days after inoculation the entire ovary was covered on its outer surface with the mycelial mat (Plate 16e) The sphacelium emerged out of the spikelets through glumes, seven days after inoculation (Plate 16f) in Group-I isolates, whereas it was light days in Group-II isolates

The perusal of the data presented in Table 7 indicates that there was significant difference in size of the sphacelia of ten representative isolates of *Sphacelia sorghi* (Plate 17 & 18) The length of sphacelia of the isolates varied from 2 92 mm to 4 72 mm and the width varied from1 9 mm to 3 06 mm The sphacelia of the isolates (NAP7 and MM 74) belonging to Group-II measured 4 72 × 3 06 mm and 4 38 × 2 94 mm respectively and differed significantly from all other isolates belonging to Group-I The size of the isolates belonging to Group-I ranged from 2 92 and 3 8 mm in length and 1 9 and 2 26 mm in breadth (Table 7) Among the Group-I isolates, length of sphacelia of the isolate N12 was the least (2 92 mm) while breadth of the sphacelia was the least in isolate SK-20-24 (1 9mm) The sphacelia of isolates belonging to

Table 7. Morphological characteristics of sphacelia of representative sorghum ergot isolates

Isolate	Length* (mm)	Breadth* (mm)	Shape	Colour
Group-I				
NI2	2.92^{g}	1.96^{e}	Elliptical to conical	White
NI5	3.02^{fg}	1.94^{e}	Elliptical	White
NI12	3.14^{ef}	2de	Elliptical	White
GUJ6	3.38^{d}	2.26^{c}	Spherical to elliptical	White
MH71	3.24^{de}	1.94^{e}	Conical	White
AP17	3.6^{c}	2.08^{d}	Elliptical to spherical	White
SK-20-24	3.16^{ef}	1.9^{e}	Elliptical	White
TN13	3.16^{ef}	1.92^{e}	Elliptical	White
Group-II				
NAP7	4.72^{a}	3.06^{a}	Oblong to elliptical	White
MH74	4.38^{b}	2.94^{b}	Oblong to elliptical	White
SEm+	0.06	0.042		
l.s.d. (5% level)	0.17	0.118		
CV%	8.7	9.7		

* Mean of 25 sphacelia

In a column means followed by a common letter(s) are not significantly different at 5% level.

Group-I (NI2, NI5 NI12 GUJ 6, MH 71, AP 17, SK-20-24, TN13) were elliptical or conical to spherical in shape, whereas, the shape of sphacelia of the isolates NAP 7 and MH 74 belonging to Group-II was oblong to elliptical (Plate 17 & 18). There was no distinct variation among the isolates with respect to colour of the sphacelia as the colour of the sphaceila of all the isolates was white.

4.8.2 Conidial morphology

4.8.2.1 Macroconidia

Significant variation was recorded with respect to shape and size of the macroconidia (Plate 19) of different representative isolates belonging to two different groups (Table 8). The length of the conidia of the different isolates varied from 9–18 µm and breadth from 5.14–9 µm The length of macroconidia of isolates NAP7 and MH74 of Group-II was ranged from 9–18 µm and breadth from 5.14–7.71 µm giving a typical cylindrical conidia (Plate 22) as against more oblong conidia in case of isolates belonging to Group-I (Plate 21), which measured 6.43–9 µm in breadth. The colour of the conidia of all the isolates was hyaline and showed a mild constriction at the center with two vacuoles (guttulations) one at each end.

4.8.2.2 Microconidia

There was no significant difference in the diameter of microconidia of sorghum ergot isolates belong to two distinct groups (Table 9). The diameter of the microconidia of different isolates ranged from 2.57–3.86 µm. The diameter of microconidia of the isolate NI2 was 2.78 µm while the diameter of microconidia of the isolates GUJ6, MH71, and AP17 showed a diameter of 2.57 µm. Microconidia were hyaline and spherical in shape irrespective of the isolates. The microconidia were observed on aged honeydew obtained from the infected panicles.

Table 8. Morphological characteristics of macroconidia of representative sorghum ergot isolates.

Isolate	Length (µm)*		Breadth (µm)*		Shape	Colour
	Range	Mean	Range	Mean		
Group-I						
NI2	12.85-18.00	15.14[a]	7.71-9.00	7.92[bc]	Oblong to ellipsoidal	Hyaline
NI5	12.85-18.00	15.39[a]	6.43-9.00	7.68[c]	Oblong to ellipsoidal	Hyaline
NI12	12.85-18.00	14.67[a]	6.43-9.00	8.3[a]	Oblong to ellipsoidal	Hyaline
GUJ6	10.28-18.00	12.9[b]	6.43-9.00	7.66[c]	Oblong to ellipsoidal	Hyaline
MH71	10.28-18.00	13.54[b]	6.43-9.00	7.76[c]	Oblong to ellipsoidal	Hyaline
AP17	10.28-18.00	13.11[b]	6.43-9.00	7.48[c]	Oblong to ellipsoidal	Hyaline
SK-20-24	12.85-18.00	15.21[a]	6.43-9.00	8.02[b]	Oblong to ellipsoidal	Hyaline
TN13	10.28-15.42	14.37[ab]	6.43-9.00	7.58[c]	Oblong to ellipsoidal	Hyaline
Group-II						
NAP7	9.00-18.00	13.43[b]	5.14-7.71	5.94[e]	Cylindrical to ellipsoidal	Hyaline
MH74	9.00-18.00	13.06[b]	5.14-7.71	6.25[d]	Cylindrical to ellipsoidal	Hyaline
SEm+		0.321		0.093		
l.s.d. (5% level)		0.892		0.261		
CV%		16.1		8.9		

* Mean of 50 macroconidia

In a column means followed by a common letter(s) are not significantly different at 5% level.

Table 9. Morphological characteristics of microconidia of representative sorghum ergot isolates

Isolate	Diameter (µm)		Shape	Colour
	Range	Mean*		
Group-I				
NI2	2.57-3.86	2.78[a]	Spherical	Hyaline
NI5	2.57-3.86	2.67[bcd]	Spherical	Hyaline
NI12	2.57-3.86	2.72[ab]	Spherical	Hyaline
GUJ6	2.57	2.57[d]	Spherical	Hyaline
MH71	2.57	2.57[d]	Spherical	Hyaline
AP17	2.57	2.57[d]	Spherical	Hyaline
SK-20-24	2.57-3.86	2.65[bcd]	Spherical	Hyaline
TN13	2.57-3.86	2.69[abc]	Spherical	Hyaline
Group-II				
NAP7	2.57-3.86	2.59[cd]	Spherical	Hyaline
MH74	2.57-3.86	2.62[bcd]	Spherical	Hyaline
SEm+		0.042		
l.s.d. (5% level)		0.116		
CV%		11.2		

* Mean of 50 microconidia

In a column means followed by a common letter(s) are not significantly different at 5% level.

4.8.3 Sclerotial morphology

None of the isolates under study produced sclerotia, even after incubating the inoculated plants at high temperature of 35°C and low RH of 40%, for two months, in the growth chambers. So, the sclerotia (Plate 23 & 24) of the isolates (NAP4, NAP5, NAP7, NAP12, NAP13, AK1 and SK-20-24) obtained through the survey conducted by ICRISAT during 1999-2000, were used to study morphological characters of sclerotia, both with sphacelial cap and without sphacelial cap.

4.8.3.1 Sclerotial morphology (with sphacelial cap)

The perusal of the data presented in Table 10 indicates that there was significant difference between the ergot isolates belong to two distinct groups with respect to the size of sclerotia with sphacelial cap. The length of sclerotia of different isolates varied from 4.1–9.6 mm. The isolates belonging to Group-II viz., NAP4, NAP5, NAP7, and NAP13 showed maximum sclerotial length (Plate 27) ranging from 8.16–9.6 mm, whereas the isolates belonging to Group-I such as NAP12, AK1 and SK-20-24 showed sclerotial length ranging from 4.1–4.42 mm, which was lesser than the length of Group-II isolates (Plate 27). The maximum length (9.6 mm) of the sclerotia was recorded with NAP13 isolate (Plate 26) collected from Sitagondi region in Adilabad district of Andhra Pradesh and minimum (4.1 mm) with SK-20-24 isolate (Plate 25) belonging to Group I collected from Talaku mandal in Chitradurga district of Karnataka. The breadth of the isolates, were ranging from 1–1.56 mm (Table 8). The shape of the sclerotia of the isolates belonging to Group-II was more cylindrical to conical whereas the shape of the sclerotia of the isolates belonging to Group-I was more conical to spherical. Colour of the sclerotia of different isolates was light yellowish brown with a hue, value, chroma, reading of 2.5Y 6/5 in Munsell's colour chart.

Table 10: Morphological characteristics of sclerotia (with sphacelial cap) of sorghum ergot isolates

Isolate	Length (mm)*	Breadth (mm)* Bottom	Middle	Top	Shape	Colour
Group-I						
NAP 12	4.42^{d}	1.82^{b}	1.00^{d}	1.06^{a}	conical to spherical	light yellowish brown
AK 1	4.40^{d}	1.40^{c}	1.00^{d}	1.06^{a}	conical to spherical	light yellowish brown
SK-20-24	4.10^{e}	1.94^{ab}	1.00^{d}	1.06^{a}	spherical to conical	light yellowish brown
Group-II						
NAP 4	9.40^{a}	1.84^{b}	1.56^{a}	1.10^{a}	cylindrical to conical	light yellowish brown
NAP 5	8.16^{c}	1.84^{b}	1.06^{d}	1.06^{a}	cylindrical to conical	light yellowish brown
NAP 7	9.12^{b}	2.04^{a}	1.38^{bc}	1.02^{a}	cylindrical to conical	light yellowish brown
NAP 13	9.60^{a}	1.82^{b}	1.34^{c}	1.06^{a}	cylindrical to conical	light yellowish brown
SEM	0.232	0.525	0.37	0.033		
l.s.d.	0.21	0.147	0.1	0.092		
CV%	15.2	14.3	14.8	15.4		

* Mean of 25 sclerotia

In a column means followed by same letter(s) are not significantly different at 5% level.

4.8.3.2 Sclerotial morphology (without sphcelial cap)

Significant differences were observed between the ergot isolates with regard to the size of sclerotia without sphacelial cap (Table 11 & Plate 28).The size of the sclerotia after dissecting out of sphacelial portion ranged from 2.2–3.9 mm in length and 1.25–2.3 mm in breadth. The length of the sclerotia ranged from 3.7–3.9 mm in respect of Group-II isolates which differed significantly with Group-I isolates as the length of the sclerotia of these isolates ranged from 2.2–2.3 mm. The isolates differed significantly with respect to the breadth of the sclerotia. The shape of the sclerotia of isolates belonging to Group-II was more oblong to cylindrical, whereas, it was conical to spherical in Group-I isolates. The colour of the sclerotia of the Group-II isolates, after the removal of the sphacelial cap, was reddish brown with a hue, value, chroma, reading of 5 YR 5/4 in Munsell's colour chart and the colour of the sclerotia of Group-I isolates NAP12, AK1 and SK-20-24 was reddish brown with a hue, value, chroma, reading of 5YR 4/4 which was still darker shade of the colour, when compared to the former.

4.9 Secondary conidia production

4.9.1 Effect of temperature

The perusal of the Table 12 indicates that the secondary conidia (Plate 20) production varied significantly at different temperature regimes among different sorghum ergot isolates. A temperature of 25°C was found to be optimum for secondary conidia production in all the isolates tested (Plate 29b). A maximum count of germinated conidia was recorded with NI2 isolate (128.78) and a minimum with NAP7 (4.44) isolate. The isolates belonging to Group-I, produced maximum number of secondary conidia, ranging from 19.22–128.78, as against a minimum count recorded with NAP7 and MH74 isolates belonging to Group-II (4.44 and 6.11 respectively). The difference in secondary conidia production was statistically significant

Table 11. Morphological characteristics of sclerotia (without sphacelial cap) of sorghum ergot isolates.

Isolate	Length* (mm)	Breadth* (mm)	Shape	Colour
Group-I				
NAP12	2.2[b]	1.3[c]	Conical to spherical	Reddish brown
AK1	2.2[b]	1.2[c]	Conical to spherical	Reddish brown
SK-20-24	2.3[b]	1.2[c]	Conical to spherical	Reddish brown
Group-II				
NAP4	3.8[a]	2[b]	Oblong to cylindrical	Reddish brown
NAP5	3.8[a]	2.1[a]	Oblong to cylindrical	Reddish brown
NAP7	3.7[a]	2.3[a]	Oblong to cylindrical	Reddish brown
NAP13	3.9[a]	2.2[a]	cylindrical	Reddish brown
SEm+	0.094	0.079		
l.s.d. (5% level	0.266	0.223		
CV%	9.1	13.5		

* Mean of 10 sclerotia (without sphacelial cap)

In a column means followed by a common letter are not significantly different at 5% level.

between the isolates tested (Table 12). The next best optimum temperature was found to be 20°C. At 20°C the secondary conidia production by all the isolates followed the same trend as that of at 25°C. At 15°C, a drastic reduction in secondary conidia production occurred in almost all the isolates, except, in AP17, which recorded a maximum number of secondary conidia (27.56) at this temperature. At 15°C, maximum number (27.56) of secondary conidia was produced by the isolate AP17, whereas, least number (0.22) of secondary conidia was produced by NAP7 isolate. At increased temperature of 30°C and 35°C, the secondary conidia production was drastically reduced. The secondary conidia production was meager at 35°C (Plate 29c) ranging from nil to 5.89 and at 10°C (Plate 29a) ranging from nil to 7.22 in all the isolates tested. The maximum secondary conidia production was recorded with the isolate TN13 (5.89), followed by SK-20-24 (4.78) and MH71 (3.44) isolates at 35°C. The maximum secondary conidia production, at 10°C was recorded with AP17 isolates (7.22), followed by NI2 isolates (3.00). The temperatures can be arranged in descending order based on its influence on secondary conidia production as 25°C, 20°C, 15°C, 30°C and 10°C. The effect of 30°C, 35°C and 10°C temperatures, on secondary conidia production are not statistically significant. Based on the secondary conidia production the isolates could be arranged in descending order as NI2, NI12, AP17, TN13, NI5, SK-20-24, MH71, GUJ6, NAP7 and MH74. The isolates NAP7 and MH74 from Group-II, differed significantly from rest of all other eight isolates in respect to secondary conidia production, being least at all temperature levels studied.

4.9.2 Effect of relative humidity on secondary conidia production

It is evident from the data presented in the Table 12 that maximum secondary conidia production occurred at 100% RH followed by 95%, 90%, 85%, and least at 80%, in all the isolates tested. The maximum count on secondary conidia production was recorded at 100% RH

Table 12. Effect of temperature on secondry conidia production of representative sorghum ergot isolates

Isolates	Number of germinated macroconidia*						
	10 C	15 C	20 C	25 C	30 C	35 C	Mean
Group-I							
NI2	3	12.78	85.89	128.78	5.11	0.89	39.41[a]
	[1.92]	[3.65]	[9.25]	[11.33]	[2.36]	[1.357]	[4.98]
NI5	0.56	2.78	20	50.67	1.67	1	12.78[cd]
	[1.219]	[1.908]	[4.549]	[7.169]	[1.559]	[1.35]	[2.96]
NI12	0.44	2.44	47.11	58	2.11	1.22	18.56[b]
	[1.173]	[1.78]	[6.81]	[7.61]	[1.73]	[1.46]	[3.43]
GUJ6	1.56	2.44	12.78	19.22	0.78	0.11	6.15[f]
	[1.56]	[1.81]	[3.68]	[4.47]	[1.31]	[1.05]	[2.31]
MH71	1.78	2.89	16.33	28.11	7.22	4.78	9.96[de]
	[1.62]	[1.94]	[4.13]	[5.36]	[2.79]	[2.03]	[2.98]
AP17	7.22	27.56	21.11	36.56	1.78	0.33	15.76[c]
	[2.85]	[5.21]	[4.68]	[6.05]	[1.64]	[1.13]	[3.59]
SK-20-24	1.22	3	23.44	31.33	7.22	4.78	11.83[cde]
	[1.42]	[1.92]	[4.87]	[5.64]	[2.79]	[2.33]	[3.16]
TN13	0.89	2.56	22.44	43.89	6.56	5.89	13.7[c]
	[1.35]	[1.86]	[4.82]	[6.68]	[2.69]	[2.49]	[3.32]
Group-II							
NAP7	0	0.22	1.22	4.44	0.44	0	1.06[g]
	[1]	[1.09]	[1.44]	[2.22]	[1.18]	[1]	[1.32]
MH74	0.33	0.67	1.78	6.11	1	0.22	1.69[g]
	[1.14]	[1.27]	[1.64]	[2.63]	[1.37]	[1.09]	[1.52]
Mean	1.7[d]	5.73[c]	25.21[b]	40.71[a]	3.39[d]	1.79[d]	
	[1.52]	[2.24]	[4.59]	[5.92]	[1.94]	[1.53]	

	Isolates	Temperature levels	Isolates x Temperature levels
Sem+	0.907 [0.076]	0.703 [0.059]	2.22 [0.188]
l.s.d. 5% level	2.521 [0.213]	1.953 [0.165]	6.175 [0.523]
CV%			50.9 [19.1]

*Figures in parenthesis are transformed values.

Mean of nine replications.

In a column or row means followed by the same letter are not significantly different at 5% level.

Table 13 Effect of relative humidity (RH) on secondary conidia production of representative sorghum ergotisolates.

Isolate	Number of germinated macroconidia*					
	80%	85%	90%	95%	100%	Mean
Group-I						
NI2	4.11	11.89	20	27.44	111.33	34.96[a]
	[2.2]	[3.57]	[4.57]	[5.31]	[10.58]	[5.25]
NI5	2.67	7.33	14.78	16.89	57.33	19.8[c]
	[1.89]	[2.86]	[3.95]	[4.19]	[7.62]	[4.11]
NI12	3.22	6.67	15.33	18.22	67.33	22.16[b]
	[2.02]	[2.74]	[4]	[4.35]	[8.26]	[4.27]
GUJ6	1.44	4.22	9.33	14.44	21.22	10.13[e]
	[1.52]	[2.25]	[3.18]	[3.91]	[4.66]	[3.1]
MH71	1.44	5	8.11	12.78	25.89	10.64[e]
	[1.55]	[2.41]	[2.97]	[3.66]	[5.15]	[3.15]
AP17	2.11	3.11	8.22	13.56	30.22	11.44[e]
	[1.73]	[1.99]	[2.99]	[3.78]	[5.55]	[3.21]
SK-20-24	2.67	7.11	11.67	13.44	37.67	14.51[d]
	[1.88]	[2.83]	[3.53]	[3.76]	[6.18]	[3.64]
TN13	1.22	2.33	4.89	11.89	33.33	10.73[e]
	[1.46]	[1.77]	[2.36]	[3.52]	[5.81]	[2.98]
NAP7	0.22	0.11	0.56	1.22	1.89	0.8[f]
	[1.08]	[1.05]	[1.23]	[1.45]	[1.67]	[1.29]
MH74	0.67	0.44	1.33	2.22	2.89	1.51[f]
	[1.27]	[1.18]	[1.49]	[1.75]	[1.94]	[1.52]
Mean	1.98[a]	4.82[b]	9.42[c]	13.21[d]	38.91[e]	
	[1.66]	[2.27]	[3.03]	[3.57]	[5.74]	
	Isolates		RH levels		Isolates x RH levels	
SEm+	0.631	[0.072]	0.446	[0.051]	1.411	[0.161]
l.s.d. (5% level)	1.755	[0.2]	1.241	[0.141]	3.924	[0.448]
CV%					31	[14.9]

*Figures in parenthesis are transformed values

Mean of nine replications

In a column or row means followed by same letter(s) are not significantly different at 5% level

with the isolate NI2 (111.33), followed by NI12 isolates (67.33) (Plate 29b). The ergot isolates NAP7 and MH74, produced least number of secondary conidia (1.89 and 2.89 respectively) even at 100% RH (Plate 29b). At cent per cent relative humidity maximum number of (111.33) of secondary conidia was produced by N12 isolate. Whereas least number (21.22) of secondary conidia was produced by GUJ6 isolate. The next best RH was found to be 95%, where even, a drastic reduction in secondary conidia production occurred and the maximum count was 27.44 in NI2 isolate. With decrease in RH value, the secondary conidia production showed a decreasing trend being least at 80% RH (Plate 30a). At 80% RH the secondary conidia production of different isolates varied from 0.22 (NAP7) to 4.11 (NI2). The effect of RH on secondary conidia production was significantly varying at all RH levels. Based on the secondary conidia production, the isolates can be arranged in descending order as NI2, NI12, NI5, SK-20-24, AP17, TN13, MH71, GUJ6, MH74 and NAP7. Among these isolates AP17, TN13, MH71 and GUJ6 did not differ significantly. The isolates NAP7 and MH74, belonging to Group-II, distinctly differed from rest of all other eight isolates with respect to least number of secondary conidia production at all RH levels tested.

Based on cultural characteristics, morphology of sphacelia, macroconidia, microconidia and sclerotia and secondary conidia production, the eight isolates (NI2, NI5, NI12, GUJ6, MH71, AP17, SK-20-24 and TN13) of Group-I are putatively identified as *Claviceps africana* and the two Group-II isolates (NAP7 and MH74) are putatively identified as *Claviceps sorghi.*

4.10 Pattern and spread of the disease

The pattern and spread of the disease of two distinct sorghum ergot isolates *viz.*, NI2, (Africana type) and NAP7 (sorghi type) has been tested in growth chambers. Data (Table 14) on aerial spread of the disease through secondary conidia revealed that the ergot disease

Table 14. Extent of disease spread in two distinct sorghum ergot isolates under artificial epiphytotic coniditions.

Isolate	Total plants*	Infected plants*	Total spikelets*	Infected spikelets*	% Incidence*	% Severity*
Group-I						
NI2	22	15.66[a]	860	46.12[a]	71.21[a] [57.8]	5.36[a] [12.83]
Group-II						
NAP7	22	3.33[b]	860	3.6[b]	15.15[b] [22.6]	0.42[b] [3.55]
SEm+		1.05		8.29	4.79 [3.39]	0.964 [1.111]
l.s.d (5% level)		4.139		25.16	18.81 [13.3]	2.925 [3.37]
CV%		19.2		94.3	19.2 [14.6]	94.5 [38.4]

* Mean of three replications

Figures in the parenthesis are transformed values.

In a column means followed by the same letter are not significantly different at 5% level.

incidence was more (71.21%) with NI2 isolate than NAP7 isolate (15.15%) (Plate 31 & 32). There was no definite pattern observed in appearance of the disease, as the test plants away or nearer from the central source of secondary conidia (foci of infection) showed the same disease incidence. The number of days taken for appearance of symptoms were seven days for NI2 isolate and eight days for NAP7 isolate, which were the same incubation periods, that of artificial inoculation of respective isolates. Typical symptom of the disease, such as exudation of honeydew was observed in all infected test plants. The number of plants infected was lower for NAP7 isolate in all the experiments conducted as 2–5 plants infected out of 22 plants tested. Whereas, 14–18 infected test plants out of 22 plants were observed with NI2 isolates.

Significant difference in percentage of infected spikelets was observed between the two isolates tested (Table 14). The per cent of infected spikelets was high (5.36) in case of NI2 isolate as against a very low per cent of infected spikelets in NAP7 (0.42) isolate.

4.11 Host range

The disease reaction of different graminaceous hosts upon artificial inoculation with ten representative sorghum ergot isolates are presented in Table 15. Of the twenty plant species tested, only *Sorghum arundinaceum, S. halepense, S. versicolor, S. virgatum* and *Pennisetum glaucum* were found to be infected by all ten representative sorghum ergot isolates (Plate 33–37). The infectivity in these test plants were cent per cent as all the inoculated plants showed the typical symptoms of the disease. Cross inoculation from infected host plant species to sorghum male sterile line 296A was found to be infective. On all the sorghum wild genotypes honeydew exudation was observed 7 days of inoculation and on *P. glaucum* honeydew exudation, was observed only after 10 days after inoculation. In *P. glaucum* there was no profuse exudation of honeydew, whereas, in wild sorghum genotypes, profuse honeydew exudation was observed.

Table 15. Reaction of various graminaceous hosts to ergot infection upon artificial inoculation with ten representative isolates.

Plant species	Disease reaction	Incubation period	Type of symptom observed
Sorghum arundinacium	+	7	Honeydew
S. halepense	+	7	Honeydew
S. versicolor	+	7	Honeydew
S. virgatum	+	7	Honeydew
Pennisetum glaucum	+	10	Honeydew
P. pedicellatum	-	-	-
Zea mays	-	-	-
Panicum antidotale	-	-	-
P. maximum	-	-	-
P. maximum	-	-	-
Brachiaria mutica	-	-	-
B. decumbense	-	-	-
Cenchrus ciliaris	-	-	-
C. setigerus	-	-	-
Andropogon gayanus	-	-	-
Dicanthium annulatum	-	-	-
Chrysopogon fulvus	-	-	-
Iseilema laxum	-	-	-
Bothriochloa pertusa	-	-	-
Chloris gayana	-	-	-

(+) Infected; (-) No infection

Table 16. Conidial measurements of sorghum ergot isolates (NI2) on different hosts.

Host	Macroconidia					
	Length (µm)		Breadth (µm)		Diameter of	
	Range	Mean*	Range	Mean*	microconidia*	Shape
Sorghum arundinaceum	10.28-15.42	13.6[a]	5.14-7.71	6.97[bc]	2.57[b]	Cylindrical
S halepense	12.85-18	14.73[a]	6.43-7.71	7.56[a]	2.59[b]	Cylindrical
S versicolor	12.85-16.71	14.66[a]	5.14-7.71	6.72[c]	2.57[b]	Cylindrical
S virgatum	12.85-16.71	17.04[a]	5.14-9	7.32[ab]	2.57[b]	Cylindrical
Pennisetum americanum	7.71-14.13	15.24[a]	5.14-8.99	7.48[a]	3[a]	Elliptical or spindle shaped
SEm+		1.811		0.132	0.045	
's d (5% level)		5.05		0.368	0.126	
CV %		14.5		12.7	11.9	

*Mean of 50 conidia

In a column mean followed by a common letter(s) are not significantly different at 5% level.

Table 17. Conidial measurements of sorghum ergot isolate (NAP7) on different hosts

Hosts	Macroconidia				Diameter of microconidia (µm)	Shape
	Length (µm)		Breadth (µm)			
	Range	Mean	Range	Mean		
Sorghum arundinaceum	7.71-15.42	9.53	5.14-6.42	5.78	2.57	Cylindrical
S. halepense	6.42-10.28	9.18	3.86-6.42	4.52	2.72	Cylindrical
S. versicolor	7.71-11.56	10.07	5.17-7.71	6.65	2.57	Cylindrical
S. virgatum	7.71-12.85	9.48	5.14-6.42	5.91	3	Cylindrical
Pennisetum glaucum	6.42-8.99	7.45	3.86-5.14	4.88	2.57	Elliptical to spindle
Sem+		0.992		0.528	0.037	
l.s.d. (5% level)		3.981		0.413	0.279	
CV%		16.3		14.7	12.4	

* Mean of 50 conidia

In a column mean followed by a common letter(s) are not significantly different at 5% level

The consistency of honeydew was very thick in case of all the infected plant species. Sclerotial formation was not, however, observed on any of the five infected hosts.

Microscopic examination of the honeydew collected from the infected hosts, revealed certain variations in size and shape of the macroconidia (Table 16 & 17). The length of the macroconidia on collateral hosts varied from 13.6–17.04 µm, and breadth from 6.97–7.56 µm. There was no variation with respect to the size and shape of the microconidia. There was a significant reduction in the size of macroconidia on different host plants, when compared to the macroconidia on sorghum male sterile line 296A, with all the representative isolates tested.

Variation in the shape of macroconidia on *Pennisetum glaucum* was observed. They showed dimorphism. Some were elliptical, while the others were elongated or spindle shaped. The shape of the macroconidia on wild sorghum genotypes did not show much variation, except the conidia were thinner than those on sorghum.

Plate 11: White sphacelia (fungal mass) emerging out of the infected spikelets of sorghum male sterile line 296A

Plate 12: Transparent honeydew exudation from the tip of the infected sorghum spikelets

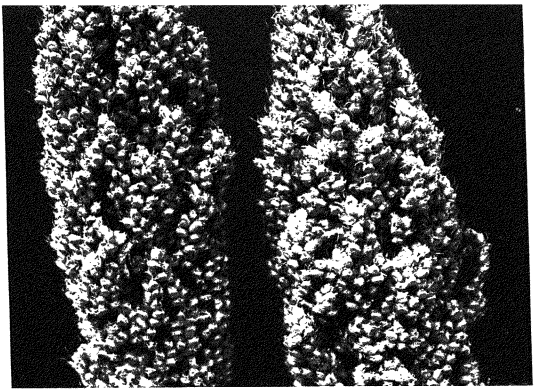

Plate 11

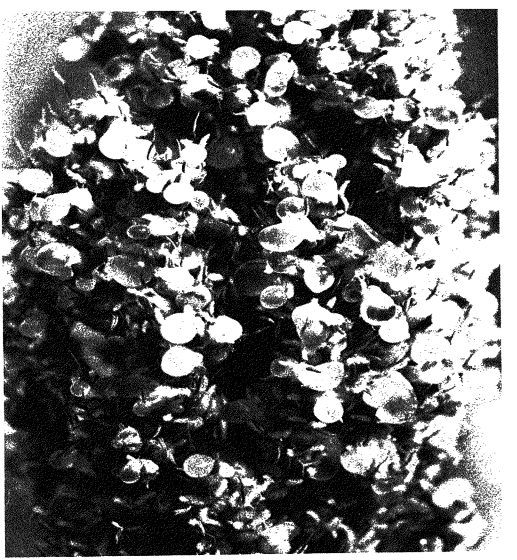

Plate 12

Plate 13: Whitish layer on the surface of honeydew showing secondary conidia production

Plate 14: Inoculated sorghum male sterile line 296A panicle showing golden yellow, thickened aged honeydew exudation

Plate 13

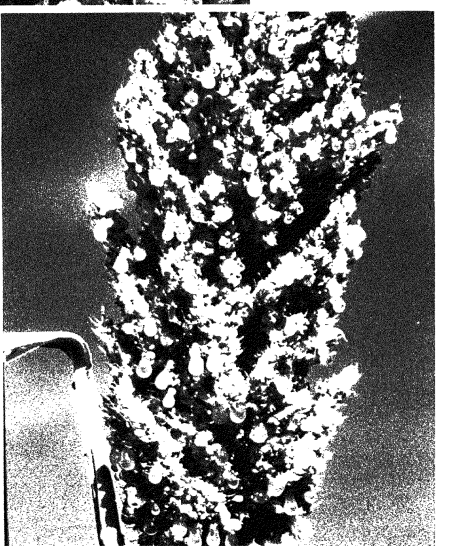

Plate 14

Plate 15: Growth of ten representative isolates of *Sphacelia sorghi* on T_2 agar medium
Plate 15a: Pure culture of NI2 isolate
Plate 15b: Pure culture of NI5 isolate
Plate 15c: Pure culture of NI12 isolate
Plate 15d: Pure culture of MH71 isolate
Plate 15e: Pure culture of AP17 isolate

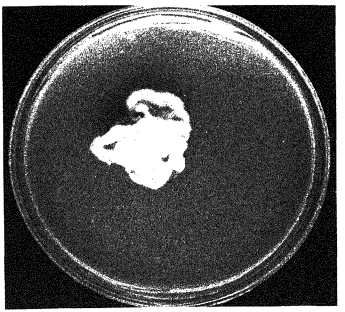

Plate 15a

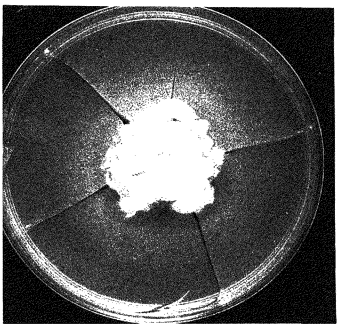

Plate 15b

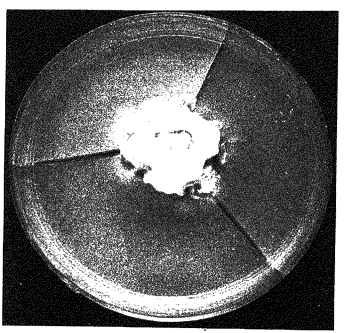

Plate 15c

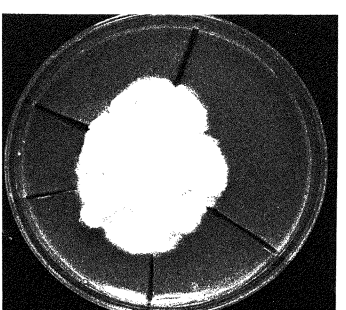

Plate 15d

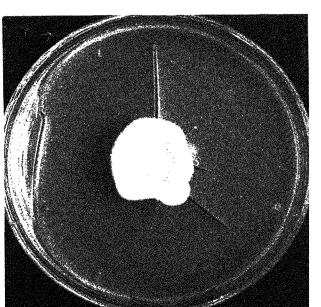

Plate 15e

Plate 15: Growth of ten representative isolates of *Sphacelia sorghi* on T_2 agar medium
Plate 15f: Pure culture of GUJ6 isolate
Plate 15g: Pure culture of SK-20-24 isolate
Plate 15h: Pure culture of TN13 isolate
Plate 15i: Pure culture of NAP7 isolate
Plate 15j: Pure culture of MH74 isolate

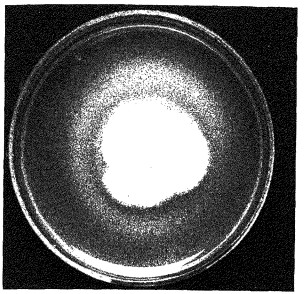

Plate 15f

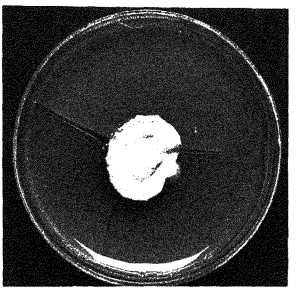

Plate 15g

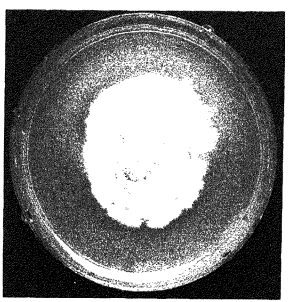

Plate 15h

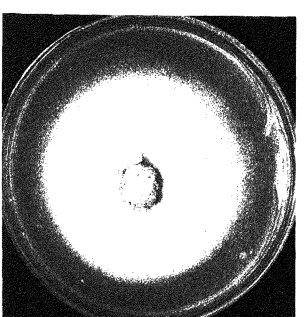

Plate 15i

Plate 15j

Plate 16: Development of sphacelia in *Sphacelia sorghi*
(a) uninfected ovary
(b) three days after inoculation
(c) four days after inoculation
(d) five days after inoculation
(e) six days after inoculation
(f) matured sphacelia

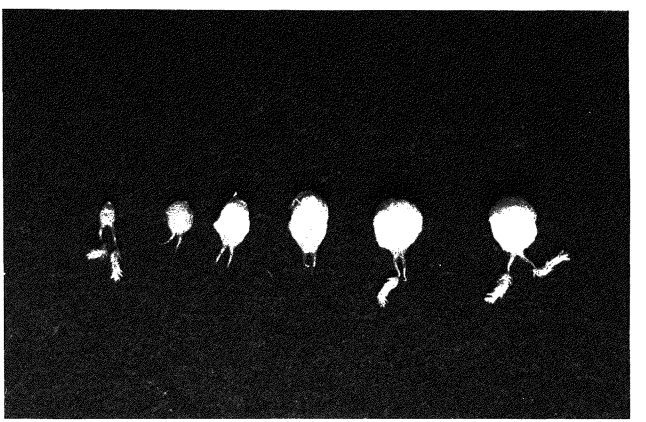

Plate 16

Plate 17: Mature sphacelia of ten representative isolates of *Sphacelia sorghi*

(a): Sphacelia of NI2 isolate
(b): Sphacelia of NI2 isolate
(c): Sphacelia of NI12 isolate
(d): Sphacelia of GUJ6 isolate
(e): Sphacelia of MH71 isolate
(f): Sphacelia of AP17 isolate
(g): Sphacelia of SK-20-24 isolate
(h): Sphacelia of TN13 isolate
(i): Sphacelia of NAP7 isolate
(j): Sphacelia of MH74 isolate

Plate 18: Mature sphacelia of two distinct groups

(a). Sphacelia of NAP7 isolate (Group-II)
(b): Sphacelia of NI2 isolate (Group-I)
(c): Uninfected ovary

Plate 17

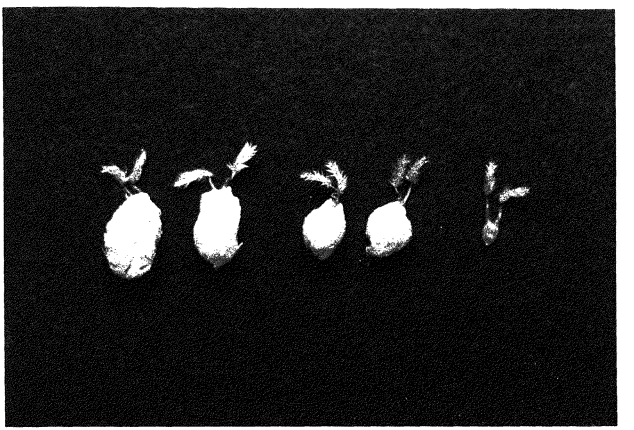

Plate 18

Plate 19: Photomicrograph showing macroconidia and microconidia of *Sphacelia sorghi*

Plate 20: Photomicrograph showing secondary conidia of *Sphacelia sorghi*

Plate 19

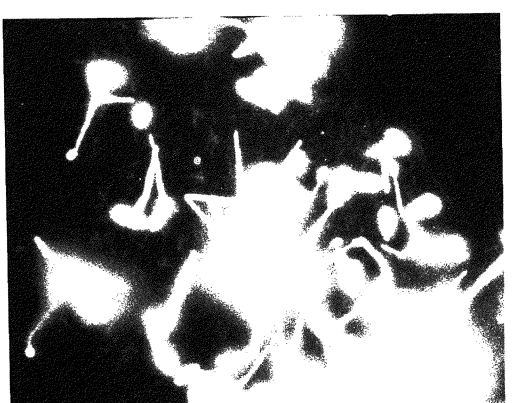

Plate 20

Plate 21: Photomicrograph showing macroconidia of NI2 isolate (Group-I)

Plate 22: Photomicrograph showing macroconidia of NAP7 isolate (Group-II)

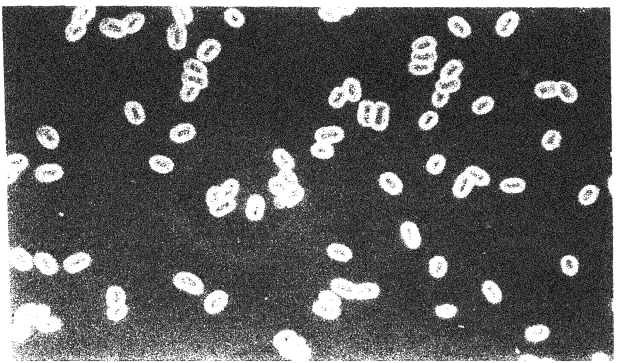

Plate 21

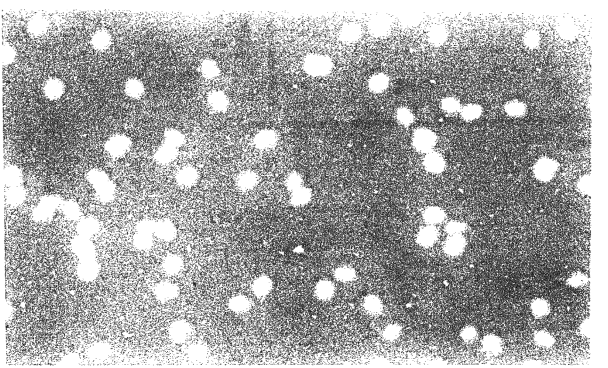

Plate 22

Plate 23: Naturally ergot infected sorghum panicle with sclerotia

Plate 24: Sclerotia of sorghum ergot pathogen
(a) Infected spikelet without sclerotium
(b) Infected spikelet with sclerotium
(c) Sclerotium without glumes
(d) Sclerotium without sphacelial tissues

Plate 23

Plate 24

Plate 25: Sclerotia of SK-20-24 ergot isolate (Group-I)
(a) with glumes
(b) without glumes

Plate 26: Sclerotia of NAP7 ergot isolate (Group-II)
(a) with glumes
(b) without glumes

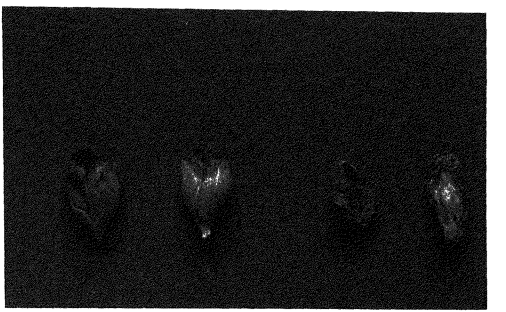

Plate 25

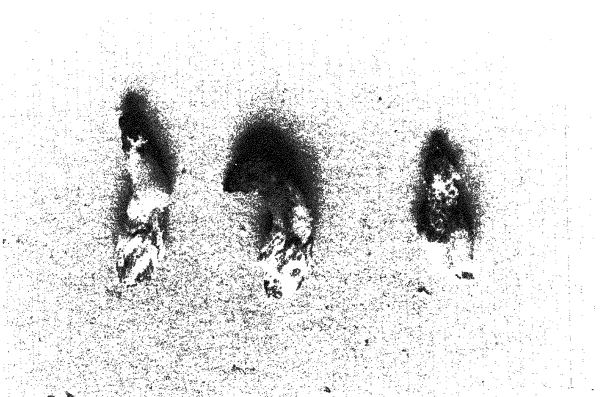

Plate 26

Plate 27: Ergot sclerotia with sphacelial cap
(a): Sclerotia of SK-20-24 isolate
(b): Sclerotia of NAP7 isolate

Plate 28: Ergot sclerotia without sphacelial cap
(a): Sclerotia of SK-20-24
(b): Sclerotia of NAP7

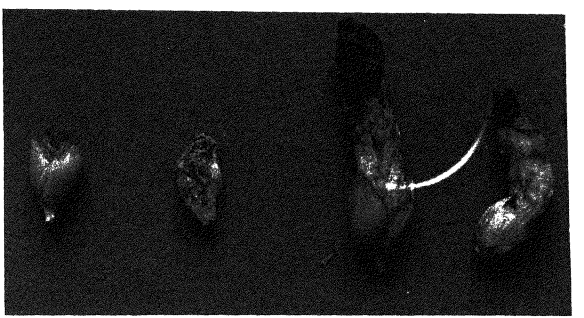

Plate 27

Plate 28

Plate 29: Effect of temperature on secondary conidia production
(a) No secondary conidia production on the surface of the honeydew incubated at 10°C
(b) Secondary conidia production on the surface of the honeydew incubated at 25°C
(c) No secondary conidia production on the surface of the honeydew incubated at 35°C

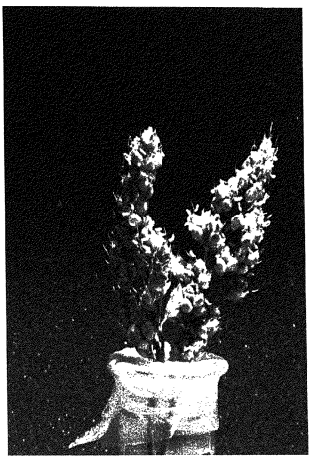

Plate 29a

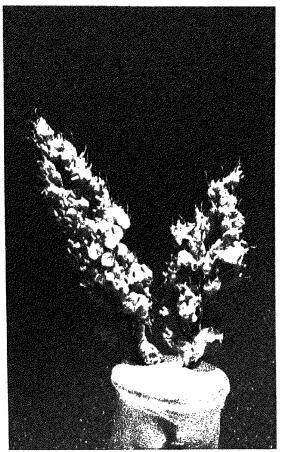

Plate 29b

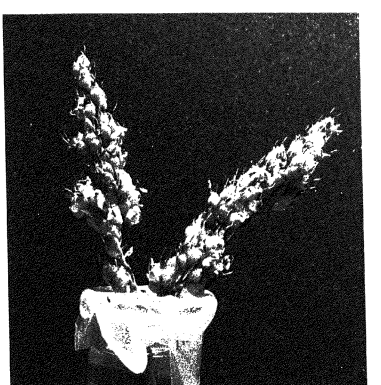

Plate 29c

Plate 30: Effect of relative humidity on secondary conidia production
(a) No secondary conidia production at 80% RH
(b) Secondary conidia production on the surface of the honeydew at 100% RH

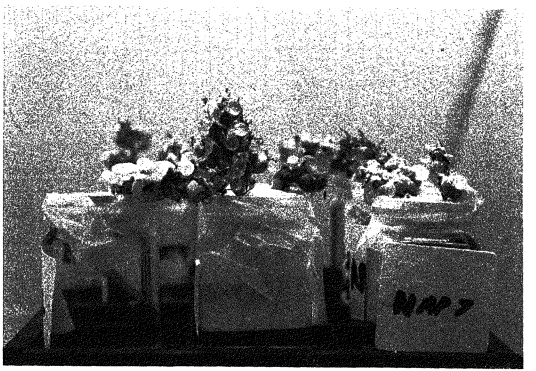

Plate 30b

Plate 30a

Plate 31: An inner view of the growth chamber, showing severe ergot infection of earhead wityh NI2 isolate

Plate 32: An inner view of the growth chamber, showing moderate ergot infection of earhead with NAP7 isolate

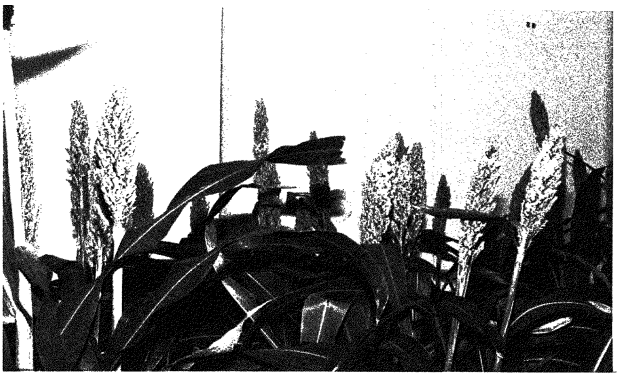

Plate 31

Plate 32

Plate 33: Ergot infected earhead of *Sorghum halepense*

Plate 34: Ergot infected earhead of *Sorghum arundinaceum*

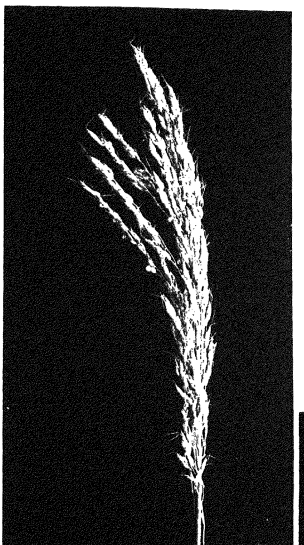

Plate 33

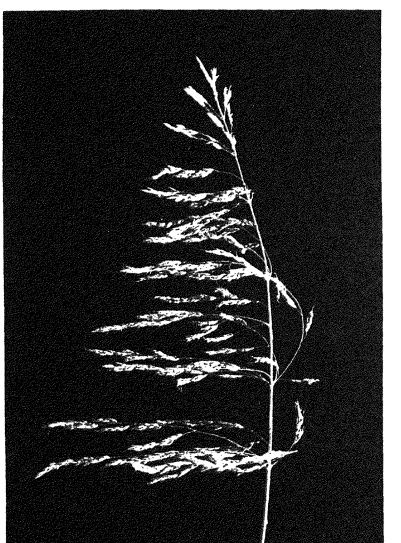

Plate 34

Plate 35: Ergot infected earhead of *Pennisetum glaucum*
(a) Control
(b) Infected earhead

Plate 36: Ergot infected earhead of *Sorghum versicolor*

Plate 37: Ergot infected earhead of *Sorghum virgatum*

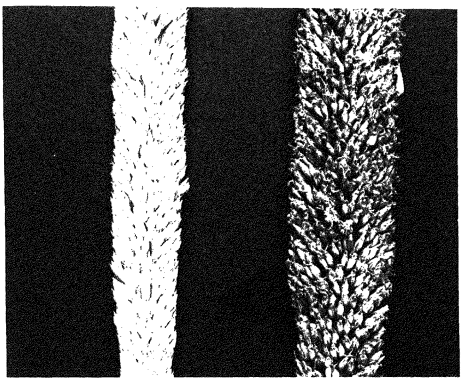

Plate 35

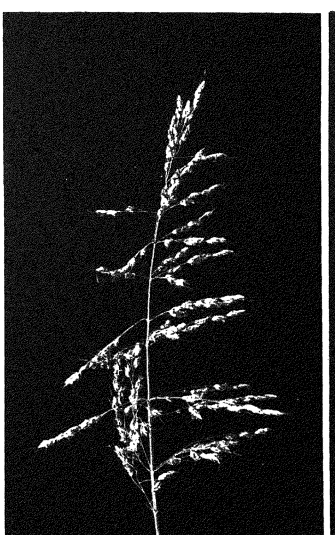

Plate 36

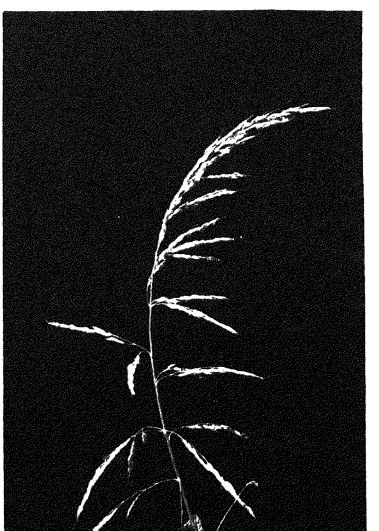

Plate 37

DISCUSSION

CHAPTER V

DISCUSSION

Ergot disease of sorghum is a serious constraint in the production of F_1 hybrid seeds, an essential input in productive sorghum cultivation systems causing 10–80% yield losses (Bandyopadhyay *et al*, 1991) The pathogen *Claviceps sorghi*, causing sorghum ergot is very much confined to India and was the only ergot pathogen reported by several earlier workers from India Recently, Bogo and Mantle (1999) reported the occurrence of *C. africana* in India, which was confirmed by Pazoutova *et al* (2000) and Tooley *et al* (2000) The earlier reports from India are known to describe the pathogen *C. sorghi* and the reports of 1980s and 1990s are describing more about *C. africana*, rather than *C. sorghi* This shows a gradual change in the species complex in India, over the period The species complex and distribution of the two ergot pathogens from different parts of sorghum growing areas remained unclear This prompted, taking up an investigation on studies pertaining to diversity of sorghum ergot pathogen occurring in India

An investigation was carried out with eighty nine isolates collected from the survey conducted in major sorghum growing areas of India Studies on cultural characters, *in vivo* variation in sphacelia, conidia and sclerotia, effect of temperature and relative humidity on secondary conidia production, extent of disease spread and host range of the pathogen were made The results of the present investigation were critically analyzed and discussed below with the light of past information available on this subject and conclusions are drawn under the following sections

5.1 Isolation of the pathogen

Like the earlier findings of Spalla (1973) and Pazoutova *et al.* (2000) the fungus associated with the ergot disease could be easily isolated on T_2 agar medium using sphacelia obtained from the infected sorghum spikelets. This suggests the best suitable medium and best suited isolation material to obtain pure culture of the sorghum ergot pathogen. Since, chances of contamination are more in isolation of ergot pathogen using sclerotia or honeydew, the choice of sphacelia proved to be the best way of isolation of *Sphacelia sorghi*.

5.2 Cultural characteristics

The growth of Sorghi type isolates was cottony, velvetty with aerial hyphae. Similar observations on the morphology of the fungus were reported by Ramakrishnan (1948) and Nagarajan and Saraswathi (1974), while describing the culture of *Claviceps sorghi*. Sorghi type isolates sporulated on the medium, which were infective on sorghum male sterile line 296 A. Similar results were obtained by Chinnadurai (1972) and Nagarajan and Saaswathi (1975), while working with *Claviceps sorghi*, the ergot pathogen of India. But in contrary to their reports on the profuse sporulation on Kirchoff's medium, the sporulation was sparse on the T_2 agar medium. This may be attributed to the differences in the medium used, as well as suggesting the possible genotypic variation in the pathogen isolates. There was no puckering, in the cultures of Africana type isolates. The cultural characters of isolates belonging to Africana type were quiet differing from Sorghi type isolates and are in agreement with the report of Bogo and Mantle (1999). The culture was compact, fleshy, white with low to high degree of puckering and failed to sporulate on the medium. This confirms the possible relatedness of these isolates to *Claviceps africana*. The growth rate of Africana type isolates were very less when compared to the isolates belonging to Sorghi type (Fig. 3 and the maximum diameter of the colony obtained was less in

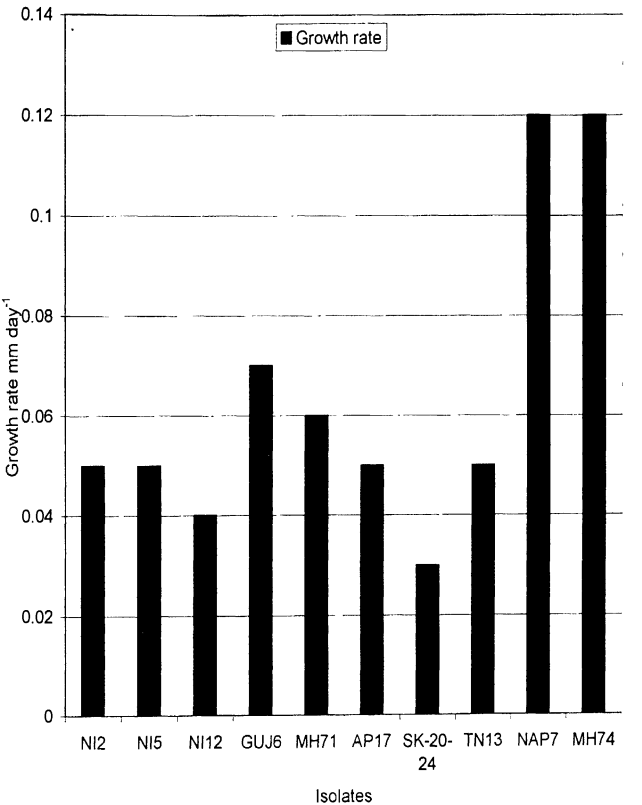

Figure 3. Growth rate of representative sorghum ergot isolates on T2 agar medium

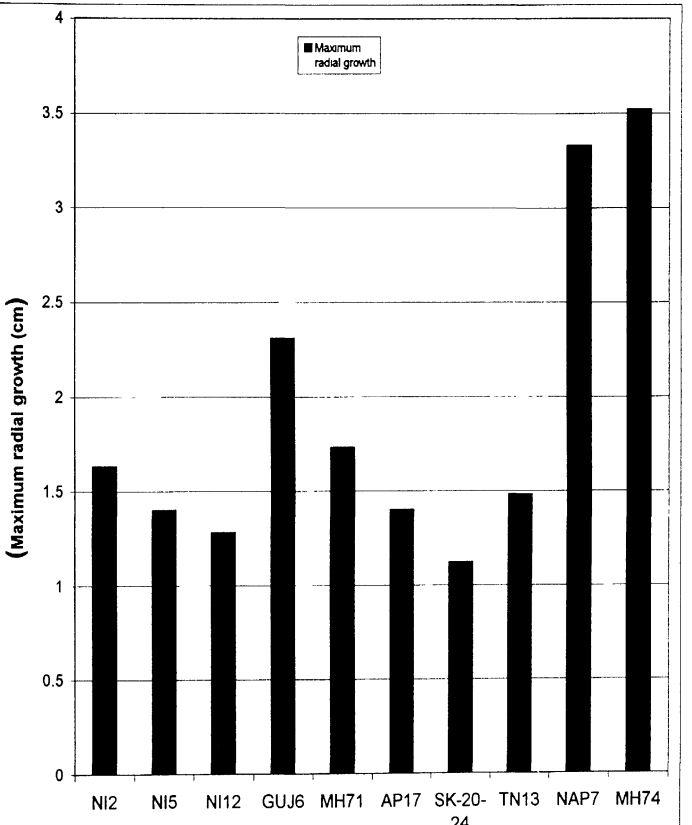

Figure 4. Maximum radial growth on T_2 agar medium in representative isolates of *Sphacelia sorghi*

Africana type due to its slow growth rate when compared to the isolates belonging to Sorghi type, which grows more faster than the former (Fig 4).

5.3 Sphacelial morphology

Sphacelia of Sorghi type isolates (NAP4, NAP5, NAP7, NAP13 and MH74) were bigger than the sphacelia of Africana type isolates with respect to length and breadth (Fig 5). This may be attributed to the fast as well as vigorous growth of Sorghi type isolates than Africana type isolates, thus covering the ovary more efficiently than the latter. The sphacelia of Sorghi type isolates were white, more cylindrical, bilaterally grooved. Similar observations were recorded by Sangitrao and Bade (1979b) and Frederickson *et al.* (1991) while working with *Claviceps sorghi*. Whereas, sphacelia of isolates belonging to Africana type (NI2, NI5, NI12, GUJ6, MH71, SK-20-24, AP17, TN13) were white, oval to spherical in shape, which are in agreement with the report made by Frederickson *et al.* (1991) while describing *Claviceps africana*. The length of the sphacelia of Africana isolates were 2.92–3.6 mm, which is in contrary with the observation (5–8mm) recorded by Frederickson *et al.* (1991). This may be due to the variation in the size of ovary of sorghum host genotype. The morphological studies of sphacelia of two types of isolates suggest the greater relatedness of isolates belonging to both *Claviceps sorghi* and *Claviceps africana.*

5.4 Conidial morphology

Macroconidia of isolates belonging to Africana type were oblong with more length and breadth (9–18 x 5.14–9µm) than macroconidia of Sorghi type isolates, which were more cylindrical with lesser breadth and length (9–18 x 5.14–7.71 µm) (Fig. 6). Similar observations on conidial morphology were made by Kulkarni *et al.* (1976) and Frederickson *et al.* (1991) while working with *Claviceps sorghi* and *Claviceps africana* respectively.

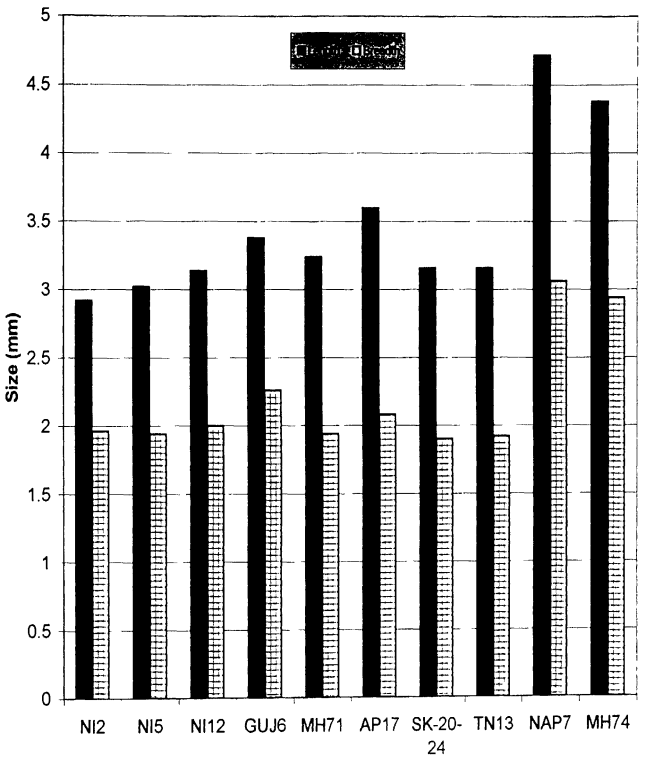

Figure 5. Size of sphacelia of representative isolates of *Sphacelia sorghi*

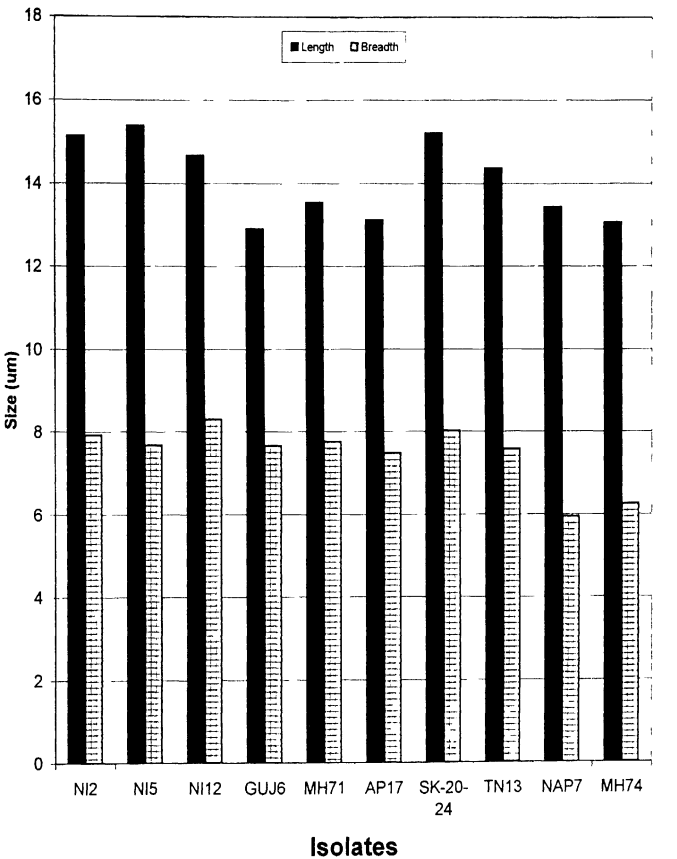

Figure 6. Size of macroconidia of representative isolates of *sphacelia sorghi*

The macroconidia are hyaline, mononucleate, slightly constricted at the centre with two polar vacuoles (guttulations). Similar observations were made by Sundaram (1970), Kulkarni *et al.*, (1976), Bandyopadhyay *et al.* (1990) and Frederickson *et al.* (1991).

5.4.2 Microconidia

In the present study, no variation in shape and size of microconidia was observed in different sorghum ergot isolates. Similar observations were made by Sangitrao (1982) and Frederickson *et al.* (1991) while describing microconidia obtained from difference sources from different regions. The microconidia were hyaline, spherical and measuring 2.5–3.8µm (Fig. 7). The shape and size of the microconidia recorded in the present investigation are tallying with the dimensions given by Kulkarni *et al.* (1976), Bandyopadhyay *et al.* (1990) and Frederickson *et al.* (1991).

5.5 Sclerotial morphology

None of the isolates under investigation has produced sclerotia in the infected sorghum male sterile line 296A on artificial inoculation, even after providing the optimum environmental conditions for sclerotial production in growth chambers. This may be attributed to unfavorable host genotype used in the study which failed to induce sclerotial production. However sclerotia are formed under natural condition in seven ergot isolates (NAP4, NAP5, NAP7, NAP12, NAP13, AK1, and SK-20-24) collected during the survey. The sclerotia obtained from the farmers' fields during the survey were formed on the sorghum cultivars viz., CSH9, MSH51 and JK22. This suggests the probable genotypes favouring the sclerotial production upon infection by the pathogen at optimum environmental condition. The sclerotia of isolates belong to Africana type were smaller in size (4.1–4.42 x 1–1.5 mm) when compared to the bigger sized sclerotia

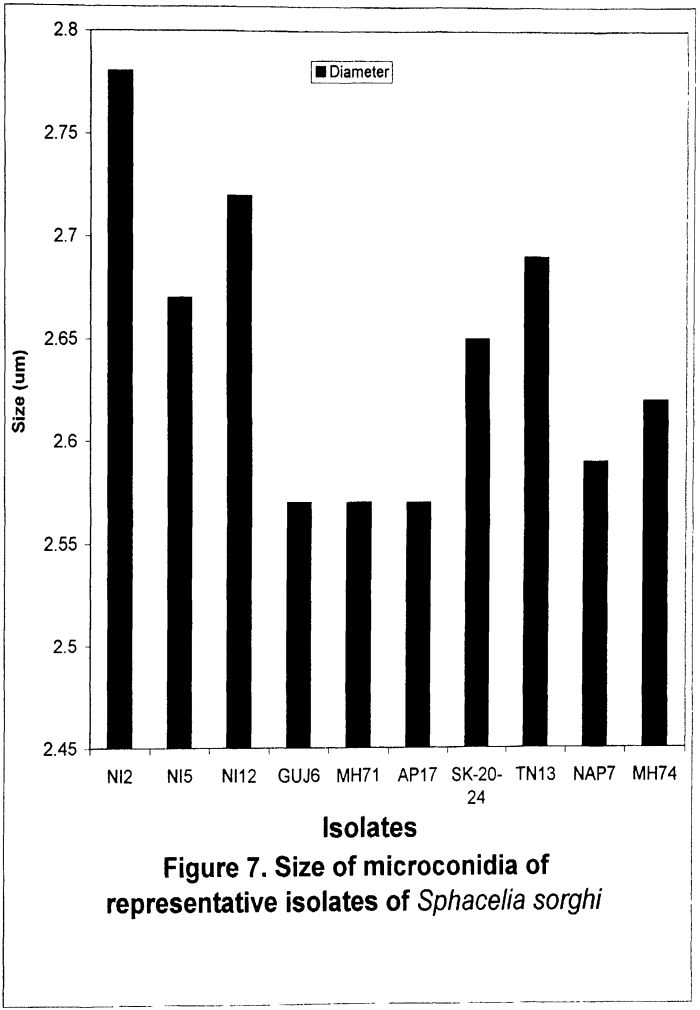

Figure 7. Size of microconidia of representative isolates of *Sphacelia sorghi*

(8.16–10.24 x 1–1.5 mm) of Sorghi type isolates (Fig.8), this may be due to the presence of lengthy sphacelial tissues on the surface of sclerotia of *Claviceps sorghi* than in *Claviceps africana* which has smaller sphacelia. Similar observations on variation in size of sclerotia were made by Kulkarni *et al.* (1976), Sangitrao (1982), Frederickson *et al.* (1991) and Bandyopadhyay *et al.* (1996). But, Sangitrao (1982) attributed the variation in the size of sclerotia to the variation in host genotypes which favor sclerotial production. But in our study sclerotia formed on the same sorghum cultivar CSH 9 infected by *C. sorghi* and *C. africana*, varied distinctly in their length, suggesting the differences in sclerotial size of *C. sorghi* and *C. africana*. The shape of the sclerotia were oblong to cylindrical in the Sorghi type isolates and were conical to elliptical in isolates belonging to Africana type. The size of the sclerotia without sphacelial cap were found to be bigger (3.7–3.9 x 2–2.3 mm) in Sorghi type isolates than Africana type isolates (2.2–2.3 x 1.2–1.3 mm) (Fig. 9). This disproves the influence of size of sphacelial tissue in increasing the size of sclerotial bodies of *Claviceps sorghi*, thus proving the sclerotia as itself is bigger in *Claviceps sorghi* than *Claviceps africana*. The sclerotia were further influenced by the amount and length of sphacelial tissue present on the surface of the sclerotia. The colour of the scleortia was reddish brown in both the type of isolates.

5.5 Secondary conidia production

5.5.1 Effect of temperature

In the present investigation, isolates belonging to both the distinct groups produced secondary conidia in controlled environmental condition. Production of secondary conidia is a noval phenomenon in *Claviceps africana* (Frederickson *et al.*, 1991) not observed in *Claviceps sorghi*. Bandyopadhyay *et al.* (1990) reported the production of secondary conidia in *C. sorghi*

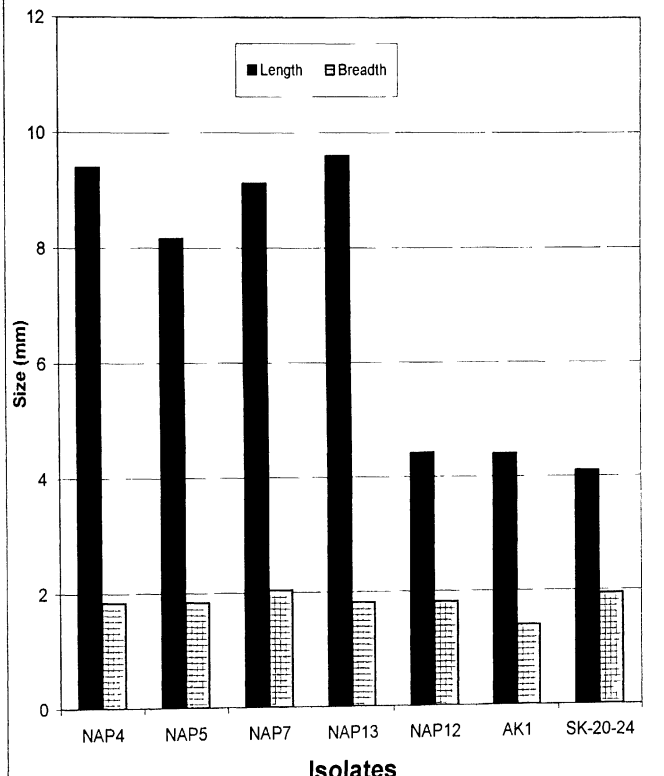

Figure 8. Size of sclerotia (with sphacelial cap) of sorghum ergot isolates

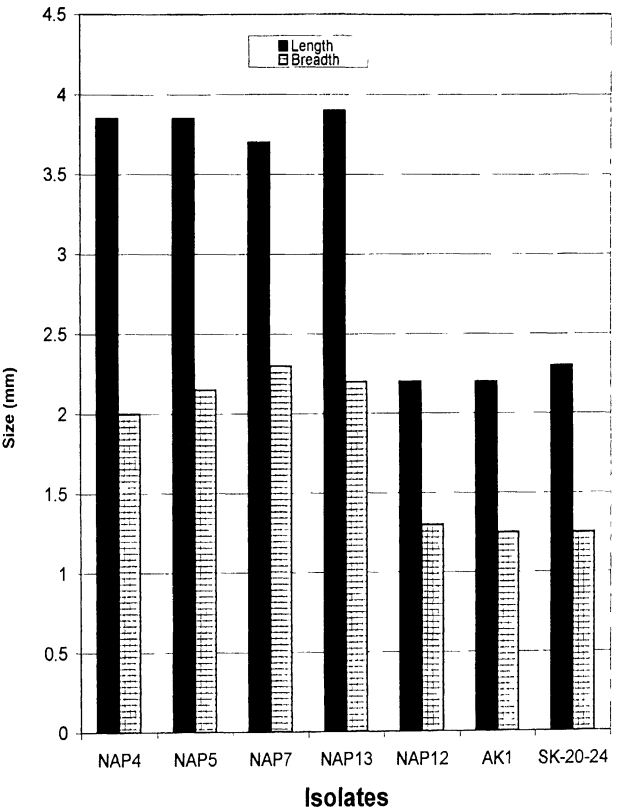

Figure 9. Size of sclerotia (without sphacelial cap) of sorghum ergot isolates

in controlled environment cabinets. In the present study the Africana type isolates produced more number of secondry conidia at all temperature levels (showing peak level at 25°C) when compared to the isolates of Sorghi type. Similar reports on the large number of secondary conidia production by *C. africana* were made by Frederickson *et al.* (1989) and Bandyopadhyay *et al.* (1996) while working with *C. africana*. The present study shows that the optimum temperature for secondary conidia production is 25°C (Fig. 10), which is in contrary with the temperature suggested by Manzarpour (1985) where he has proposed a temperature range of 25–30°C as favourable temperature. Although maximum number of secondary conidia was produced at 25°C, there was a strong interaction between temperature and production of secondary conidia by different ergot isolates. Bandyopadhyay *et al.* (1996) reported 20±2°C as most favourable temperature for secondary conidiation. In our study least secondary conidiation was observed in 35°C, which is in agreement with the report made by Bandyopadhyay *et al.* (1990 and 1996). Secondary conidiation starts declining with decrease in temperature below 25°C and increase in temperature above 25°C (Fig. 10). In our study the isolates belonging to North India (Uttar Pradesh and Rajasthan) produce more number of secondary conidia at 25°C, when compared to the isolates of South Indian origin. This may be attributed to the similar environmental conditions (near 20°C and 100% RH) which prevail in Northern India, to which the isolates would have got adapted. At a temperature of 35°C the isolates MH71, SK-20-24 and TN13 have produced more number of secondary conidia when compared to the other isolates. This may be due to the existence of higher temperature (>30°C) in the those regions of these isolates, to which they got adapted. The optimum temperature obtained in the present study was in contrary with the observations made by Bandyopadhyay *et al.* (1991) and other observations are in agreement with their results. This may be due to the *in vivo* condition provided for the secondary

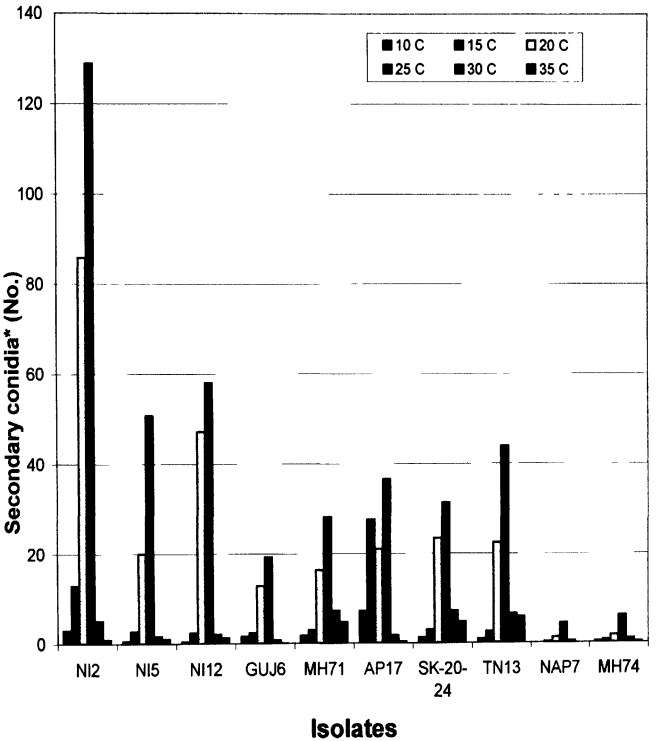

Figure 10. Effect of temperature on secondary conidia production of representative isolates of *Sphacelia sorghi*

*Out of thousand macroconidia counted

conidiation to occur, whereas in the study conducted by Bandyopadhyay *et al.* (1991), they provided *in vitro* condition by studying secondary conidiation on water agar surface

5.5.2 Effect of relative humidity

Secondary conidia production was found to increase with increase in relative humidity and was peak at saturation level of 100% (Fig 11) .Similar observations were recorded by Frederickson *et al.* (1989, 1991, and 1993) while working with *C. africana* and Bandyopadhyay *et al* (1990 and 1996) while working with *C. africana* and *C. sorghi* Supporting the report of Bandyopadhyay *et al* (1998) the rate of secondary conidia production was higher in all the isolates belonging to Africana type, whereas the secondary conidiation was least in isolates belonging to Sorghi type (Fig 11)

In all the experiments conducted, there were significant variations exist within the Africana type isolates as well as within Sorghi type isolates This may be attributed to their genetic nature of the isolates and to their adaptation to the different environmental condition, existing in different geographical locations

5.6 Pattern and spread of the disease

In present investigation the isolate belonging to Africana type (NI2) has shown higher percentage of disease incidence and percentage spikelets infected, when compared to the isolate belonging to Sorghi type (NAP7) Thus the rate of disease spread was more in case of former when compared to the latter (Fig 12)) This may be attributed to high degree of secondary conidiation in Africana type isolates when compared to the least secondary conidiation in the Sorghi type isolates The present study confirms the reports on role of secondary conidia in the

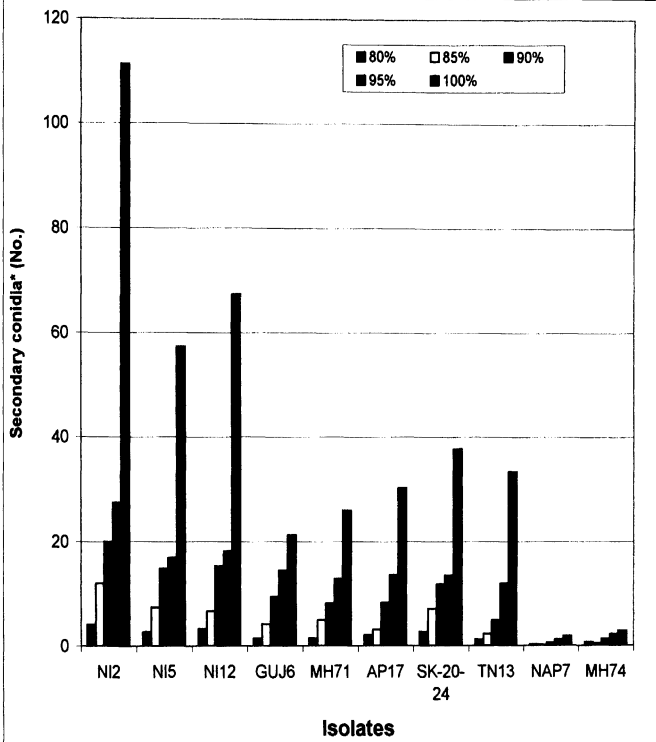

Figure 11. Effect of RH on secondary conidia prodction of representative isolates of *Sphacelia sorghi*

*Out of thousand macroconidia counted

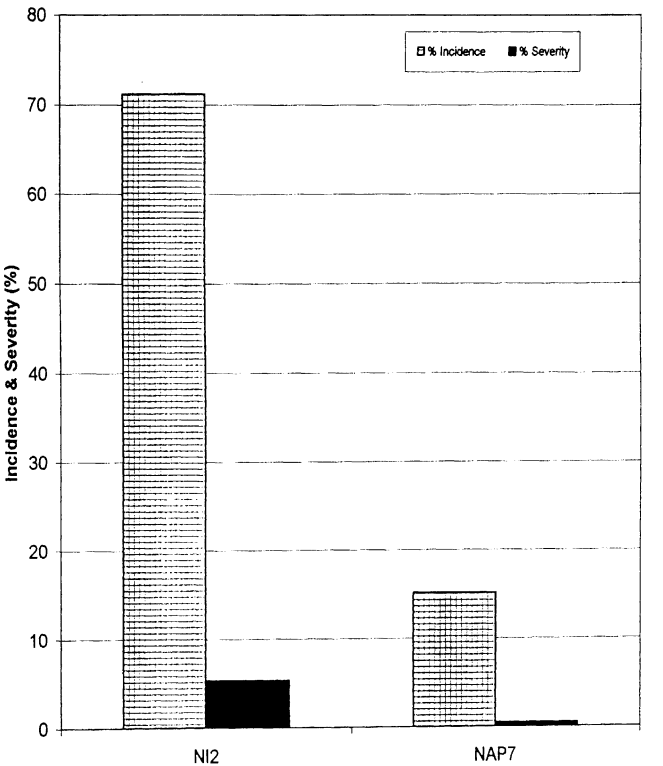

Figure 12. Extent of disease spread in two isolates of Sphcelia sorghi

aerial spread of the disease by Frederickson *et al.* (1989 and 1993) and Bandyopadhyay *et al.* (1991), under natural conditions.

5.7 Host range

Records of collateral hosts of *C. africana* and *C. sorghi* are numerous but inconsistent. In the present investigation an attempt has been made to study the host range of sorghum ergot isolates belonging to two distinct groups *viz.*, Sorghi type and Africana type. Of the twenty graminaceous host plants tested only five host plants *viz.*, *Pennisetum americanum*, *Sorghum arundinaceum*, *S. halepense*, *S. versicolor*, and *S. virgatum* were infected by all the ten representative isolates confirming their susceptibility to sorghum ergot pathogen. Ramakrishnan (1947), Sundaram (1970), Boon-long (1992) and Alderman (1999) also reported infection of these hosts by *Sphacelia sorghi*. In contrary to the reports made by Futrell and Webster (1966) and Chinnadurai and Govindaswamy (1971), the graminaceous hosts *Zea mays*, *Chenchrus ciliaris* and *C. setigerus* were found to be non-hosts to the sorghum ergot pathogen. Similar observations were made by Sundarm *et al.* (1970). In the present study, *Panicum maximum*, *P. antidotale*, *Dicanthium annulatum* were found to be non-hosts to the pathogen, which is in agreement with the report by Chinnadurai and Govindaswamy (1971) and Sundaram *et al.* (1970). But contary reports were made by Futrell and Webster (1966), Molefe (1975), Boon-long (1992) and Sangitrao and moghe (1995) as they reported, the plants to be hosts of the pathogen. The controversy over the host plants may be attributed to the differences in inoculation methods adapted and the type of genotype used by the workers. It is interesting to note that the conidial size and shape of *S. sorghi* were altered when passed on to different hosts. The shape of the macroconidia on wild sorghum genotypes did not show much variation, except the conidia were elongated and thinner than those on sorghum. The macroconidia, on *Pennisetum glaucum*

showed dimorphism. Some were elliptical, while the others were elongated or spindle shaped. Similar reports were given by Chinnadurai and Govindaswamy (1971), while describing the macroconida of *Sphacelia sorghi* on maize.

There was a reduction in the size of macroconidia of both the distinct groups of ergot isolates on different host plants tested. Similar reports on reduction in the size of macroconidia on other hosts are reported by Chinnadurai and Govindswamy (1971).

Based on cultural characters such as, colony diameter, colony texture, colony colour, nature of growth, pigmentation and sporulation on medium, sphacelial morphology, shape and size of macro and microconidia, secondary conidia production, sclerotial morphology and pathogenicity tests, the Group-I isolates (NI2, NI5, NI12, GUJ6, MH71, AP17, SK-20-24 and TN13), are putatively belonging to *C. africana* and the Group-II isolates (NAP7 and MH74) putatively belong to *C. Sorghi,* which were fast growers and had no puckered growth in culture compared to the isolates putatively belonging to *C. africana.* Majority of the isolates collected from different parts of the country had characters similar to that of *C. africana.* Of the 89 isolates collected and studied in the present investigation only five isolates *viz.*, NAP4, NAP5, NAP7, NAP13 and MH74 were of *C. sorghi* type and the rest of 84 isolates were of *C. africana* type, indicating predominance of *C. africana* occurrence in major sorghum growing areas of India.(Fig. 13) However there were a large number intermediate types, as considerable variations were noticed in their cultural characters. Thus the cultural characters appear to be a poor indicator of identity of sorghum ergot pathogen species. Further, confirmation on the identity of sorghum ergot species should be based on molecular characterization using DNA based methods *viz.*, random amplified polymorphic DNA analysis (RAPD), amplified fragment length polymorphism (AFLP), random amplified microsatellite (RAM) and radioactive amplified fragment analysis

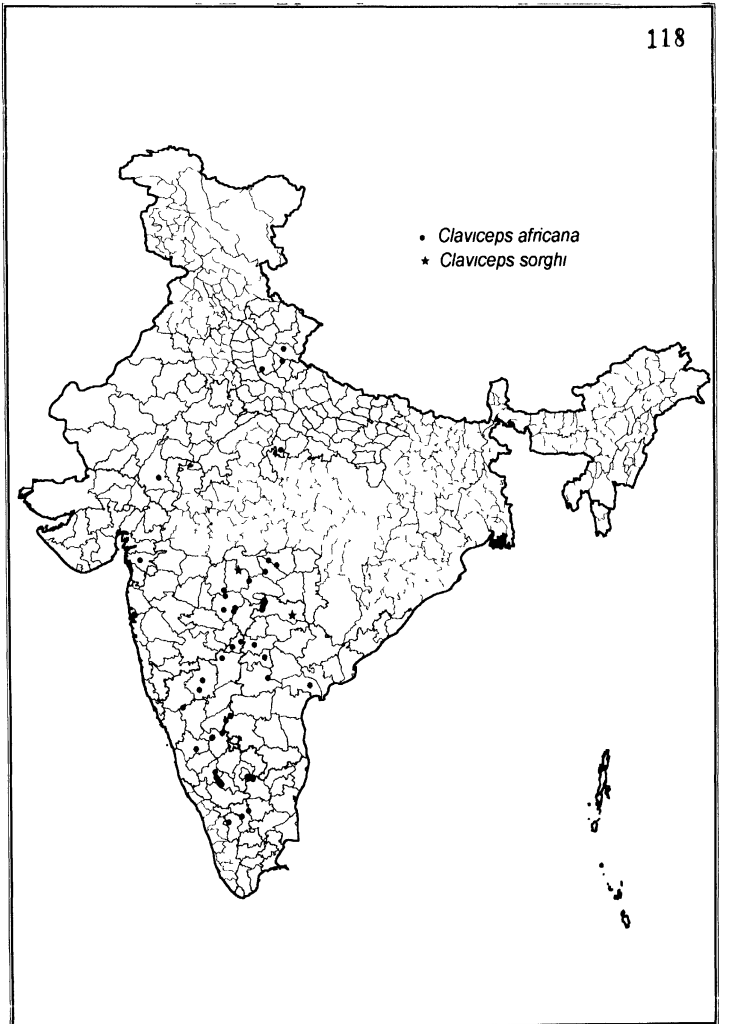

Figure 13. Distribution of sorghum ergot pathogens in India

(RAFS), which will throw more light on diversity of sorghum ergot pathogen isolates occurring in India.

Future lines of research

The results of the present study indicated the need for future research in the following areas.

- Estimation of dihydroergosine (DHE), a key factor which differentiates the pathogen at species level and standardization of procedure for the estimation of DHE.
- More emphasis on molecular characterization of the isolates through random amplified polymorphic DNA analysis (RAPD) or amplified fragment length polymorphism (AFLP) or random amplified microsatellite (RAM) or radioactive amplified fragments analysis (RAFs).
- Detailed studies on biology of sclerotia and stroma of *C. africana* of Indian isolates.
- Understanding the sources of primary inoculum and their relative relevance to initiate the disease at field level.
- Understanding the effect of environmental conditions, particularly low temperature, on infection and flowering biology and their interactions.
- Formulating disease forecasting model based on environment and host parameters to predict the occurrence of disease in seed production plots thus enabling judicious and need based use of fungicides.
- Intensifying search for resistance in male sterile lines of sorghum.

SUMMARY AND CONCLUSIONS

CHAPTER VI

SUMMARY

The present investigations on diversity of sorghum ergot pathogen isolates occurring in India were undertaken at the International Crop Research Institute for the Semi-arid Tropics (ICRISAT), Patancheru, Hyderabad 502 324, Andhra Pradesh.

Ergot or sugary disease of sorghum (*Sorghum bicolor* (L.) Moench) caused by *Sphacelia sorghi* McRae has attained international prominence since it affects sorghum cultivation as well as sorghum seed production throughout the world, wherever sorghum crop is grown. In India the disease occurs in severe form in all the major sorghum growing states. Three Clavicpes species causing the disease have been reported from different parts of the world: *Claviceps sorghi* in India, *Claviceps sorghicola* in Japan and *Claviceps africana* in all ergot-positive countries. In India occurrence of *Claviceps sorghi* and *Claviceps africana* has been reported and been confirmed by several workers. But the genetic diversity of the ergot pathogen and its distribution in India has not been investigated so far. Therefore, investigations were carried out with 89 ergot isolates collected from different major sorghum growing areas of Uttar Pradesh, Rajasthan, Gujarat, Maharashtra, Andhra Pradesh, Karnataka and Tamil Nadu. Of the 89 ergot isolates 27 isolates from Andhra Pradesh (18 locations), 24 isolates from Karnataka (19 locations), 12 isolates from Maharashtra (9 locations), five isolates from Tamil Nadu (4 locations), 10 isolates from Uttar Pradesh (7 locations), 3 isolates from Udaipur district in Rajasthan and 8 isolates from Surat district in Gujarat were collected. The pathogenicity were proved by spray inoculation of the conidial suspension (1×10^6 conidia ml^{-1}) to the fresh panicles of ergot susceptible sorghum male sterile line 296A and the re-isolated fungus from the diseased earheads resembled the original one. The isolates were maintained on sorghum male sterile line

296A by periodic spray inoculation with conidial suspension Each isolate was cultured on T_2 agar medium using young Sphacelia obtained from fresh infected panicle of sorghum male sterile line 296A The isolates were grouped based on cultural characters into two major distinct groups, one with compact, fleshy, raised, non-sporulating colony, with low colony growth rate and less colony diameter (Africana type) and another with cottony, velvety, submerged, sporulating colony with higher colony growth rate and more colony diameter (Sorghi type) on T_2 agar medium

Ten isolates representing all geographical locations (eight isolates from Africana type, *viz*, NI2, NI5, NI12, GUJ6, MH71, AP17, SK-20-24, TN13 and two isolates from Sorghi type *viz*, NAP7 and MH74) were selected for further studies pertaining to *in vivo* variations in morphology of sphacelia, conidia and sclerotia, effect of temperature and relative humidity on secondary conidia production, extent of disease spread and host range

Significant variation was observed with respect to size (length and breadth) and shape of the sphacelia of two distinct group isolates The sphacelia of Africana type isolates measured 2 92–3 6 × 1 9–2 26 mm, and the conical to spherical in shape, whereas, the sphacelia of Sorghi type isolates measured 4 38–4 72 × 2 94–3 06 mm, and were oblong to cylindrical in shape

Microscopic studies revealed that macroconidia belonging to two distinct groups of isolates varied widely in respect to their size and shape The macroconidia of isolates belonging to Africana type were oblong to elliptical measuring 10 28–18 × 6 43–9 µm whereas, the macroconidia of Sorghi type isolates were narrower, cylindrical, comparatively smaller in size measuring 9–18 × 5 14–7 71 µm The conidia of both the type of isolates were two guttulated and are slightly constricted at the center None of the isolates under study have produced sclerotia in controlled environmental conditions (at 35°C temperature and 40% RH) The

studies on sclerotial morphology using the sclerotia collected from the surveyed fields revealed the variations with respect to size and shape of the sclerotia of two distinct groups The sclerotia of Africana type (AK1, NI12 and SK- 20-24) isolates were always shorter than that of sorghi type (NAP4, NAP5, NAP7 and NAP13) isolates The sclerotia of Africana type isolates were conical to spherical, short measuring 4 1–4 42 × 1 4–1 82 mm, whereas, the sclerotia of Sorghi type isolates were cylindrical to conical measuring 8 16–10 24 × 1 84–2 04 mm Significant variations in morphological characters were observed within the ergot isolates of the same group

There was significant effect of temperature on secondary conidia production of Sphacelia sorghi Of the different temperatures tested, maximum sporulation recorded at 25°C and poor sporulation was observed at 10°C and at 35°C Statistically significant effect of humidity on secondary conidia production of the isolates was observed Maximum secondary conidia production occurred at 100 per cent humidity followed by 95, 90, 85 and 80 per cent humidity levels However, poor sporulation was recorded at 80 per cent relative humidity Humidity was found to be positively correlated with secondary conidia production in different ergot isolates

Secondary conidia production was found to be higher in the isolates belonging to Africana type than Sorghi type isolates at all temperature and relative humidity levels It is evident from the study that production of secondary conidia is affected to a greater extent by temperature and relative humidity factors Maximum secondary conidia production occurred at 25C and at 100 per cent relative humidity Highest secondary conidia production was recorded in NI2 isolate belonging to Africana type and least in NAP7 isolate belonging to Sorghi type

Studies on pattern and spread of the disease revealed the fact that isolates belonging to Africana type spreads more efficiently in an extensive manner than isolates of Sorghi type The present investigation also confirms the aerial spread of the disease through the air-borne

secondary conidia produced by the pathogen There was no definite pattern observed in appearance of the disease, as the test plants away or nearer from the central source of secondary conidia (foci of infection) showed the same disease incidence The percentage incidence and percentage severity was found to be higher (71 21 and 5 36, respectively) in NI2 isolate than in NAP7 isolate (15 15 and 0 42, respectively)

Host range of sorghum ergot pathogen was studied using twenty graminaceous plants including four wild sorghum genotypes Of the 20 hosts tested only five were found to be infected by all the representative isolates The wild sorghum genotypes *viz*, *Sorghum arundinaceum, S halepense, S versicolor, S virgatum* and *Pennisetum glaucum* showed the typical symptoms of the disease seven days after artificial inoculation All other grass plants, *Pennisetum pedicellatum* and *Zea mays* were found to be non-hosts of the pathogen The isolates belonging to two distinct groups were found to be equally infective to the collateral host species tested

Microscopic examination of the honeydew collected from the infected hosts, revealed certain variations in size and shape of the macroconidia

There was significant reduction in the size of macroconidia on different hosts plants, when compared to the macroconidia on sorghum male sterile line 296A, with all the representative isolates tested On P glaucum macroconidia showed dimorphism with elliptical and elongated or spindle shaped conidia However, the shape of the macroconidia on wild sorghum genotypes did not show much variation, except the conidia were thinner than those on sorghum

Based on cultural characters (colony diameter, colony texture, colony colour, nature of growth, pigmentation and sporulation on medium), sphacelial morphology, shape, and size of

macro and microconidia, secondary conidia production, sclerotial morphology and pathogenicity tests, the eighty four isolates putatively belong to *Claviceps africana* and five ergot isolates putatively belong to *Claviceps sorghi*, indicating the predominance of *C. africana* in all sorghum growing areas in India.

LITERATURE CITED

LITERATURE CITED

*Ajrekar S L 1926 Observations on a disease of Jowar (*Sorghum vulgare*) caused by *Sphacelia* (conidial stage *Claviceps*) Journal of the Indian Botanical Society 5 55-61

Alderman S C Frederickson D E Milbrath G Montes N Sanchez J N and Odvody G N 1999 A laboratory guide to the identification of *Claviceps purpurea* and *Claviceps africana* in Grass and Sorghum seed samples Publication of Oregon Department of Agriculture pp1-17

*All India Coordinated Sorghum Improvement Project (AICSIP) 1969-70 Progress Report of the All India Coordinated Sorghum Improvement Project 1969-70 Indian Council of Agricultural Research and Cooperative Agencies 138-146

Anahosur K H and Patil H S 1982 Effect of date of sowing on the incidence of ergot of sorghum Indian Phytopathology 35 507-509

Aneja K R 1993 Experiments in microbiology, Plant pathology and Tissue culture Wishwa Prakashan, New Delhi (A Division of Wiley Eastern Ltd) pp 471

*Angus A 1965 Annotated list of plant pests and diseases in Zambia (Northern Rhodesia) Part 7 (Cyclostyled)

Bandyopadhyay R 1992 Sorghum ergot Pages 235-244 in Sorghum and millets diseases a second world review W A J de Milliano, R A Frederickson, and G D Bengston (eds) Patancheru 502 324, Andhra Pradesh, India International Crops Research Institute for the Semi-Arid Tropics

Bandyopadhyay R Mughogho L K Manohar S K and Satyanarayana MV 1990 Stroma development, honeydew formation, and conidial production in *Claviceps sorghi* Phytopathology 80 812-818

Bandyopadhyay R Mughogho L K and Sharma H C 1991 Source of primary inoculum and spread of ergot Pages 24-25 in Cereals Program annual report 1990 Patancheru 502 324, Andhra Pradesh, India International Crops Research Institute for the Semi-Arid Tropics

Bandhyopadhyay R Mughogho L K and Tegegne G 1992 Role of pollination and inoculation on ergot development in sorghum. Phytopathology 82:241(Abstract).

Bandyopadhyay R Frederickson D E McLaren N W and Odvody G N 1996 Ergot—a global threat to sorghum. Int. Sorghum Millet Newsl. 37:1-32.

Bandyopadhyay R Frederickson D E McLaren N W Odvody GN and Ryley M J 1998 Ergot: a serious threat to sorghum in Americas and Australia. Plant Disease. 82:356-367.

Bogo A and Mantle P G 1999 *Claviceps africana* discovered in India. Plant Disease 83:79.

*Boon-Long T 1992 Sorghum diseases in Thailand. Pages 41–43 in sorghum and millets diseases: a second world review (de Milliano W A J Fredericksen R A and Bengston G D eds.) Patancheru 502 324, Andhra Pradesh India: International Crops Research Institute for Semi-Arid Tropics.

*Brian P W and Hemming H G 1950 Transactions of British Mycological Society 33:132-141.

Chinnadurai G 1972 Effect of certain trace elements on the growth and sporulation of *Sphacelia sorghi*. Indian Phytopathology 25:599-600.

Chinnadurai G and Govindaswamy C V 1971 Host range of sorghum sugary disease pathogens. Madras Agricultural Journal 58:600-603.

*de Milliano W A J Tavares Nogueira M F R Pomela L M Msiska F S Kunene S Matalaote B Mbwaga A M Kaula G M and Mtisi E 1991. New records of ergot of sorghum caused by *Sphacelia sorghi* in southern Africa. Plant Disease 75:215.

Desai K B Joshi H U Kikani B K and Rajkule P N 1979. Effect of seedling date on sugary disease appearance. Sorghum Newsletter 22:110-111.

Dhingra O D and Sinclair J B 1993 Basic plant pathology methods. CBS Publications and Distributors, New Delhi pp 335.

*Dogget H 1970 Sorghum. Longmans, Green & Co. Ltd., London and Harlow. 403 pp.

*Doidge E M Bottomley A M Van Der Plank J E and Panker G D 1953 A revised list of plant diseases in South Africa Science Bulletin Pretoria, South Africa, Department of Agriculture

FAO Quarterly Bulletin of Statistics 1999 12 33

*Frederickson D E 1990 Ergot disease of sorghum Ph D Thesis, University of London, London, UK

Frederickson D E and Mantle P G 1988 The path of infection of sorghum by *Claviceps sorghi* Physiological and Molecular Plant Pathology 33 221-234

Frederickson D E Mantle P G and de Milliano W A J 1989 Secondary conidiation of *Sphacelia sorghi* on sorghum, a novel factor in the epidemiology of ergot disease Mycological Research 93 497-502

Frederickson D E Mantle P G and de Milliano W A J 1991 *Claviceps africana sp* nov , the distinctive ergot pathogen of sorghum in Africa Mycological Research 95 1101-1107

Frederickson D E Mantle P G and de Milliano W A J 1993 Windborne spread of ergot disease (*Claviceps africana*) in sorghum A-lines in Zimbabwe Plant Pathology 42 368-377

Frederickson D E Mantle P G and de Milliano W A J 1994 Susceptibility to ergot in Zimbabwe of sorghums that remained uninfected in their native climates in Ethiopia and Rwanda Plant Pathology 43 27-32

Frederickson D E and Odvody G N and Isakeit T 1999 Sorghum Ergot Distinguishing sphacelia and sclerotia of *Claviceps africana* in seed Publication of Texas Agricultural Extension Service The Texas A & M University system pp1-5

Futrell M C and Webster O J 1965 Ergot infection and sterility in grain sorghum Plant disease Reporter 49 680-683

Futrell M C and Webster O J 1966 Host range and epidemilogy of the sorghum ergot ergot organism. Plant Disease Reporter 50 828-831

GENSTAT 1986 A general statistical program 1986 Rothamsted Experimental station, Harpenden, Herts Al 52 JQ, UK

Hawker L E 1957 The Physiology of Reproduction in Fungi Cambridge University Press

Isakeit T Odvody G N and Shelby R A 1998 First report of sorghum ergot caused by *Claviceps africana* in the United States Plant Disease 82 592

*Khadke V D 1975 Studies on sugary disease of sorghum M Sc Thesis Submitted to the MPKV,Rahuri (M S), India 71pp

Kulkarni G S 1942 Ergot in India Current Science 11 246

Kulkarni B G P Seshadri V S and Hegde R K 1976 The perfect stage of *Sphacelia sorghi* McRae Mysore Journal of Agricultural Science 10 286-289

Lutrell E S 1981 Tissue replacement diseases caused by fungi Annual review of Phytopathology 19 373-389

Loveless A R 1964 Use of the honeydew state in the identification of ergot species Transactions of the British Mycological Society 47 205-213

Mantle P G 1968 Studies on *Sphacelia sorghi* McRae, an ergot of *Sorghum vulgare* Pers Annals of Applied Biology 62 443-449

Mantle P G and Hassan H A G 1994 Widening geographical distribution of *Claviceps africana* an important ovary pathogen of grain sorghum International Sorghum and Millets Newsletter 35 97-98

*Manzarpour A 1985 Aspects of metabolism and physiology in *Claviceps* Diploma thesis, Imperial College, University of London, UK

*Mason E W 1926 On two species of *Tolyposporium* Worosin recorded on cultivated sorghum Transactions of the British Mycological Society 11 284-286

*McLaren N W 1993 Effect of sugary disease exudates on germination, seedling development and predisposition to seedling diseases of sorghum (*Sorghum bicolor*) S Afr J Plant Soil 10 12-16

*McLaren N W 1996 Components of resistance of sorghum to ergot Presented at the plant Breeding Symposium, ARC-Grain Crops Institute and Soputh African Plant Breeders Association 19-21 Mar 1996, South Africa

McLaren N W and Wehner F C 1990 Relationship between climatic variables during early flowering of sorghum and the incidence of sugary disease caused by *Sphacelia sorghi* Journal of Phytopathology 130 82-88

McLaren N W and Wehner F C 1992 Pre-flowering low temperature predisposition of sorghum to sugary disease (*Claviceps africana*) Journal of Phytopathology 135 328-334

*McRae W 1917 Notes on some south Indian fungi Pages 108-111 in Madras Agriculture Yearbook

Molefe T L 1975 Occurrence of ergot on sorghum in Botswana Plant Disease Reporter 59 751-753

Musabimana T Sehene C and Bandyopadhyay R 1995 Ergot resistance in sorghum in relation to flowering, inoculation technique and disease development Plant Pathology 44 109-115

Nagarajan K and Saraswathi V 1975 Production of honeydew line secretions in the culture of *Sphacelia sorghi* Indian Phytopathology 28 110

Nath P and Padwick G W 1941 Ergot in India Current Science 10 488-489

Panse V G and Sukhatme P V 1978 Statistical methods for Agricultural Workers Indian Council of Agricultural Research, New Delhi pp 136

Patil P Y Raut N K and Deshpande P M 1968 A note on the occurrence of ergot, sugary disease on the hybrid Jowar in Maharashtra (Parbhani) College of Agricultural Parbhani Magazine Vol IV 93-94

Pazoutova S Bandyopadhyay R Frederickson D E Mantle P G and Frederiksen R A 2000 Relations among sorghum ergot isolates from the Americas, Africa, India, and Australia Plant Disease 84 437-442

Plumb-Dhindsa P and Mondjane A M 1984 Index of plant disease and associated organisms of Mozambique Tropical Pest Management 30 407-429

Puranik S B and Mathre D E 1971 Biology and control of ergot on male sterile wheat and barley Phytopathology 61 1075-1080

*Quinby J R 1958 Grain sorghum production in Texas Texas Agricultural Experiment Station Bulletin no 912 College Station, Texas, USA Texas A & M University

Ramakrishnan T S 1947 The natural occurrence of ergot in south India Proceedings of the Indian Academy of Science B 26 136-141

Ramakrishnan T S 1948 Ergot sclerotia on *Sorghum vulgare* Pers Current Science 17 218

Ramakrishnan T S 1963 Diseases of millet Indian Council of Agricultural Research, New Delhi 152 pp

*Rama Sastri D V 1973 Studies of the sugary disease of sorghum caused by *Sphacelia sorghi* McRae Ph D Thesis Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi, India

Rawlins J E 1933 Phytopathological and Botanical Research Methods John Wiley and Sons Incorporation, New York pp 156

Reddy K Govindaswamy C V and Vidhyasekaran P 1969 Studies on ergot disease of Cumbu (*Pennisetum typhoides*) Madras Agriculture Journal 56 367-377

Reis E M Mantle P G and Hassan H A G 1996 First report in the Americas of sorghum ergot disease, caused by a pathogen diagnosed as *Claviceps africana* Plant Disease 80 436

Rosewich U L 1996 The population genetics of Colletotrichum graminicola from different ecosystems of sorghum Ph D Thesis Texas A & M University, USA, 150 pp

Ryley M J Alcorn J L Kochman J K Kong G A and Thompson S M 1996 Ergot on Sorghum spp in Australia Australian Plant Pathology 25 214

*Sangitrao C S 1980 Possibilities of spread and perpetuation of sorghum erogot in India A paper presented in 6th Annual Meeting of Indian Science

*Sangitrao C S 1982 Studies on ergot disease of sorghum in Vidarbha Ph D Thesis, Punjabrao Krishi Vidyapeeth, Akola, India 212 pp

Sangitrao and Bade (1979) Meteorological factors associated with honey dew development and sclerotial stage in sorghum ergot Sorghum Newsletter 22 107-108

Sangitrao C S and Bade G H 1979a Meteorological factors associated with honeydew development and sclerotial stage in sorghum ergot Sorghum Newsletter 22 107-108

Sangitrao C S and Bade G H 1979b Morphological studies of sclerotia of sorghum ergot Sorghum Newsletter 22 108

Sangitrao C S Taley Y M and Moghe P C 1979 Effect of planting date on the appearance of sugary disease Sorghum Newsletter 22 111

Sangitrao C S Indira S Bandyopadhyay R 1997 Sorghum ergot in India Pages 41-53 in Proceedings on global conference on ergot of sorghum, Sete Lagaos, Brazil

Sangitrao C S and Moghe P G 1995 Triangular conidia of *Sphacelia sorghi* on grass weed *Dicanthium caricosum* Indian Phytopathology 48 490-492

*Shimanuki T Kimigafukuro T and Tsukiboshi T 1988 Occurrence of ergot of sorghum caused by *Claviceps* species in Japan Abstracts of 5th International Congress of Plant Pathology 136 1403-1408

*Singh P 1964 Sugary disease of sorghum Pages 29-33 in Progress report of the Accelerated Hybrid Sorghum Project and the Millet Improvement Programme New Delhi, India Indian Council of Agricultural Research 81 pp

*Spalla C 1973 Genetic problems of production of ergot alkaloids in saprophytic and parasitic conditions, Pages 393-403 in Genetics of Industrial Microorganisms Z Vanek Z Hosalek and J Cudlin eds Elsevier, Amsterdam

Sundaram N V 1967 Sugary disease of sorghum Page 50 in Proceedings of the International Symposium on Plant Pathology, New Delhi, Dec 27, 1966 to Jan 1, 1967

Sundaram N V 1968 Sugary disease of Jowar-how to recognize and control it Indian Farming, April 1968 issue

Sundaram N V 1969 Current plant pathological problems in new agricultural strategy with special reference to sorghum and millets Indian Phytopathological Society Bulletin 5 62-72

Sundaram N V 1970 Sugary disease of sorghum Pages 435-439 in Plant Disease Problems Proceedings of the first International Symposium on Plant pathology (Raychoudhuri, S P *et al*, eds) New Delhi, India Indian Phytopathological Society

Sundaram N V 1971 Possible resistance to sugary disease in sorghum Indian Journal of Genetics and Plant Breeding 31 383-387

Sundaram N V 1974 Natural Occurrence of *Sphacelia sorghi* on *Pennisetum typhoids* Indian Phytopathology 27 124-125

Sundaram N V 1976 Sorghum diseases and their control Pesticides Information Oct-Dec 16-21

*Tarr S A J 1962 Diseases of Sorghum, Sudan Grass and Broom Corn Kew, Surrey, UK Commonwealth Mycological Institute

Tegegne G Bandyopadhyay R Mulau T and Kebede Y 1994 Screening for ergot resistance in sorghum Plant Disease 78 873-876

Thakur R P King S B and Rao V P 1989 Expression of ergot resistance in pearl millet under artificially induced epidemic conditions Phytopathology 79 1323-1326

Thomas K M Ramakrishnan T S and Shrinivasan K V 1945 Natural occurrence of ergot in South India Proceedings of the Indian Academy of Science (B) 21 93-100

Tooley P W Goley E D Carras M M Kuldau G Frederickson R D and Webber E 1999 Sequence analysis of the ITS and –tubulin intron 3 regions for differentiation of *Claviceps* species pathogenic on sorghum Phytopathology 89 S102 (Abstr)

*Tooley P W O'Neill N R Goley E D and Carras M M 2000 Assessment of Diversity in *Claviceps africana* and other *Claviceps species* by RAM and AFLP analyses Phytopathology 90 1126-1130

Tsukiboshi T Shimanuki T and Uematsu T 1999 *Claviceps sorghicola* sp nov , a destructive ergot pathogen of sorghum in Japan Mycological Research103 1403-1408

*Wallace G B and Wallace M M 1949 A revised list of plant diseases of economic importance in Tanganyika territory Mycological Paper 26 Kew, Surrey UK Commonwealth Mycological Institute

*Original not seen