STUDIES ON ERGOT OF JOWAR (*Sorghum vulgare* Pers.) CAUSED BY *Sphacelia sorghi* McRae IN MAHARASHTRA STATE

A Thesis submitted to the

MAHATMA PHULE KRISHI VIDYAPEETH

(AGRICULTURAL UNIVERSITY)

RAHURI, District : Ahmednagar,

(Maharashtra State)

In partial fulfilment of the requirements for the degree of

Master of Science (Agriculture)

In

Plant Pathology

By

Vijaykumar Digambar Khadke

B. Sc. (Agri.) First Class

Department of Plant Pathology

Post-Graduate School, Rahuri

October, 1974

" Affectionately Dedicated to

MY PARENTS

for their constant encouragement

and best wishes throughout my

educational career ".

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SHRI VIJAYKUMAR DIGAMBAR KHADKE

B.Sc.(Agri.) First Class.

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CERTIFICATE

This is to certify that the thesis entitled " Studies on Rogor of Lemon (Xanthomonas citri Hasse.) caused by Xanthomonas campestris pathovar citri (Hasse) Dye in Maharashtra State " submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth (Agricultural University), Rahuri, District : Ahmednagar (Maharashtra) in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE (AGRICULTURE) in PLANT PATHOLOGY, embodies the results of a piece of bonafide research work carried out by SHRI KHANDE VINAYKUMAR DIGAMBAR under my guidance and supervision and that no part of the thesis has been submitted for any other degree or publication.

Post Graduate School,
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Dated : 2074.

(D. K. KORI.)
Research Guide.

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INTRODUCTION

CHAPTER I

INTRODUCTION

Jowar (Sorghum vulgare Pers. synonymous to Andropogon sorghum Brot.) is one of the most important cereal crops in India which occupies over an area of about 18-19 million hectares. It contains a high protein and vitamin A-1 and provides a good quality fodder for the cattle. Besides India it is grown in many parts of the world thereby considered an important food grain crop. Considering an area, Maharashtra is the largest Jowar growing State where it occupies 35% of the total area. It is grown both in Kharif and rabi seasons.

In order to increase the production, the dwarf, short duration, fertiliser responsive hybrid varieties of Jowar have been evolved. Due to release of such varieties, many new and some severe diseases have appeared in India in general and Maharashtra in particular.

Although 50 diseases have been recorded on Jowar only 30 have been found in India. Amongst the more destructive ones grain smut (Sphacelotheca sorghi (Link) Clint.), loose smut (Sphacelotheca cruenta (Kuhn) Potter), long smut (Tolyposporium phyllogeni (Kuhn) Pat.), head smut (Sphacelotheca reiliana (Kuhn) Clint.), the downy mildew (Sclerospora sorghi (Kulk.) Weston and Uppal), rust (Puccinia purpurea Cke.), the various leaf spotting fungi like Helminthosporium, Gloeosporium, Colletotrichum and Curvularia are found to be attacking Jowar regularly. Very recently a new disease known as 'sugary' disease or 'target' has been found in Maharashtra in more severe form.

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The disease is well known as 'Chikatya' or 'Sakharya' meaning 'sticky' and 'sugary' respectively, incited by a fungus Sphacelia sp. The disease occurs in many parts of India including Maharashtra particularly in late sown kharif jowar. This is readily distinguished from the insect malady where it is restricted to earheads only.

Mehta (1917) was the first to report the disease from Madras where only the sphacelial stage or 'honey dew' or 'sugary' stage occurred while Ajrekar (1926) reported both sphacelial and sclerotial stages. Thereafter its regular occurrence has been reported from various States of India including Maharashtra where it is found in Sholapur, Satara, Poona, Sangli, Ahmednagar and Kolhapur districts.

Although, it attacks only ear heads about 30% incidence has been reported thereby reducing economic value of the crop. Instead of normal grain the sclerotia, containing various alkaloids are formed which cause hazards to human beings, cattle and birds. However, 'ergotamine' the most important medicinal drug derived from the ergot is particularly useful in the treatment of post natal haemorrhage (Kath and Podwick, 1941).

Farmers are inclined to use hybrids due to their high yielding quality and fertilizer response. It is also known that the higher doses of nitrogenous fertilizers increase severity of ergot of jowar (Chinnaduraî, 1971). Thus, due to adoption of susceptible hybrids on a large scale and increased use of nitrogenous fertilizers the disease occurs in an

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epiphytotic form in many parts of the State. It was therefore, thought necessary to investigate into the causal agent of the disease including host range, reaction to different varieties and selections and finding out a suitable and effective control measure. The results of these investigations are presented in the following pages.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Mehta (1917) observed for the first time a sugary disease of jowar (*Sorghum vulgare*) caused by *Sphacelia* sp. in Madras State and identified the causal fungus as *Sphacelia sorghi* McRae. Ajrekar (1926) reported from Poona a sugary disease of jowar caused by *Sphacelia* sp. and presumed to be the conidial stage of the genus *Claviceps*, in both sphacelial and sclerotial stages. He also recorded a similar disease on *Andropogon sorghum* var. *halepensis*, *Pennisetum glaucum*, and *Imperata cylindrica*. Belorme (1926) stated that for the control of 'ergot' of rye(*Claviceps purpurea*) it is advisable to avoid growing the rye too often on the same soil and sorting the diseased grains in every case.

Wilkinson (1927) reported from Kenya the occurrence of sugary disease on *Sorghum*. Rhind (1928) reported the incidence of this disease for the first time in a very severe form in Burma, where the sclerotia were also formed. He has also recorded a species of *Sphacelia* accompanied by *Sorosphella* on *Panicum punctatum* from Burma. Robertson (1928) also recorded similar results. Sundararaman (1930) reported that *Sphacelia sorghi* was capable of infecting the jowar heads both in the milk and flower stage but not after the grains are set.

Ramkrishnan (1937) reported the disease in sphacelial stage only from Coimbatore where the disease occurred almost

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annually. He further stated that the conidia were hyaline, oblong or oval with rounded ends and produced in a very large numbers and mentioned that the Sphacelia spores germinated readily in water in a period of 4 hours under laboratory conditions, the germtubes bearing at its end an oval secondary conidium. He reported some plants of Panicum sanguum infected with sugary disease, the spores of which were quite different in shape from those of Sorghum fungus.

Thirumalachar (1945) recorded sphacelial and sclerotial stages on Sphacelia sorghum, the former associated with abortive or immature sclerotia on Andropogon sorghum from Mysore. Thomas et al. (1945) reported that eighteen grasses were found to be infected with sugary disease of which fifteen were new records, the sphacelial and sclerotial stages being present on twelve, whereas only the sphacelial stage occurred on the remainder.

Ramkrishnan (1948) reported a heavy incidence of disease in Madras where there was profound sclerotial formation. He obtained the fungus in pure culture from sclerotia which produced spores on Kirchoff's medium within 15 - 20 days and a greater sporulation was resulted on sterilised young panicles of Sorghum. He proved the pathogenicity by inoculating the flowers at different stages of flower opening with the suspension of spores; the highest incidence occurred when inoculation was carried out at the time of flower opening. He described more or less similar symptoms as described by Ajrekar (1926) and reported the

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formation of cylindrical, elongated or slightly curved sclerotium having cream to grey colour, resembling in all appearances the sclerotia of 'ergot' of rye. Further, he attributed that the sclerotia were of various shapes and sizes. Some were like the conidia found in nature; others were smaller, oval or elongated and narrow. He had grown the fungus on Kirchoff's medium on which the growth was white in colour with plenty of aerial hyphae, but the rate of growth was very slow as compared to that of 'ergot' of rye. He also reported that the growth was faster at 20-25°C temperature than at laboratory temperature 26-28°C. He also reported that conidia of Sphacelia sorghi on artificial cultures varied a great deal when compared to natural conidia. He also mentioned thatt there was no sign of germination of sclerotia even after three months.

Wallace (1953) found the disease in Tanganyika territory. He also reported a destructive disease of Sorghum caused by Sphacelia sorghi from Eastern Province in 1952. Harrison (1955) also reported the same disease in Nigeria. Campbell and Preisen (1959) shown that it is possible to control 'ergot' in headland grasses by spraying with sulfa hydramide at the rate of 9 kg active ingredient per hectare. The suppression of heading prevents the development of 'ergot' sclerotia in the grasses of the headlands and eliminates the chief source of inoculum for the adjacent cereal crops.

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In the annual review of the Department of Agriculture of N. Nigeria (1959) Elizabeth Harrison reported that flotation in brine (sp.gr. 1.1) will prove successful in removing sclerotia of Sphacelia sp. from seeds of Pennisetum typhoides. Parr (1962) stated that 'ergot' of Sorghum caused by Sphacelia sorghi McRae was observed in India in 1915, Uganda and Kenya in 1924, Burma in 1928 and Tanganyika in 1949. Futrell and Webster (1965) found a high incidence of this disease in experimental plots at Samaru and reported that sugary disease of Sorghum can be controlled or markedly reduced in a cytoplasmic male sterile line by having an abundance of pollen from the pollen parent when sterile florets become receptive.

Pyne et al. (1965) noted from Northern Nigeria that all Sorghum varieties in the breeding plots were infected by Sphacelia sorghi. Futrell and Webster (1966) stated that at least 12 and preferrably 24 hours at 100% relative humidity after inoculation was necessary for infection, the incubation period being 7-9 days. They also reported successful infection of maize by Sphacelia sorghi from Nigeria. Emechebe et al. (1967) stated that the disease can develop on non pollinated flowers at any time of the year when conditions are suitable. Kulkarni (1967) mentioned that 'ergot' of bajra (Pennisetum typhoides) can be controlled by periodic and systematic ploughing of the infected fields.

Mantle (1968) found that the Nigerian strain of

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Sphacelia sorghi failed to develop the asexual state beyond production of stromatal initials. Reddy (1968) in his cross inoculation studies found that Sphacelia sorghi was able to infect Dactylactum fimbriatum.

Chinnaduraí and Govindaswami (1970) reported the disease from Madras in both sphacelial and sclerotial stages and mentioned that the disease can be effectively controlled by cyclohexamides. Chinnaduraí and Govindaswamy (1970) carried out inoculation experiments by spraying the unpollinated inflorescences with conidial suspension prepared from 15 day old culture as well as from honeydew of infected spikelets and found that conidia of Sphacelia sorghi germinate on the stigma, the fungus entering the ovary through the base of the style. They also reported that maximum infection occurred on 5th and 6th day of flowering and the incidence was highest at 28°C temperature.

Chinnaduraí et al. (1970 a) reported that conidia of Sphacelia sorghi from in-vitro cultures were less pathogenic to Sorghum florets than conidia from the honeydew of the infected spikelets and thus they postulated the presence of a substance in the honeydew stimulating conidial germination, the nature of which remained to be studied. They also (1970 b) reported that sugary disease of sorghum has recently became serious in India. Of the various species and varieties tested against the fungus in the glass house 'maKX 60-A' showed the maximum infection and 'K 3' the least.

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Chinnaduraí and Govindaswamy (1970) mainly recorded Sphacelia sorghum on Sorghum after inoculation of a male sterile variety highly susceptible to Sorghum 'ergot' fungus (Sphacelia sorghi) with no sclerotia developed even after 30 days. Sundaram and Shrivastava (1970) reported that Pennisetum typhoides x Pennisetum purpureum crosses showed infection in 12 days when inoculated with Sphacelia sorghi.

Chinnaduraí (1971) showed that the incidence of sugary disease of Sorghum caused by Sphacelia sorghi in India, significantly increased with increased nitrogen application and decreased with increased potash; phosphorus did not affect disease incidence but stimulated honeydew secretion.

Chinnaduraí and Govindaswamy (1971 a) noted that conidial size and shape of Sphacelia sorghi varied with host. Chinnaduraí and Govindaswamy (1971 b) in the course of cultural studies observed considerable variation in the spore size and shape as influenced by nitrogen nutrition. In all cases the conidial size was found to be less than that of naturally occurring conidia in Sorghum. They also reported differences in varietal reactions of Sorghum to 'ergot' fungus which were attributed to the nature of stigmatic secretions of different Sorghum varieties. Chinnaduraí and Govindaswamy (1971) recorded the sugary disease only on maize, Sorghum miliaria and Sorghum saccharum after inoculating 10 cereals and 14 wild grasses.

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MATERIAL AND METHODS

CHAPTER III

MATERIALS AND METHODS

1 ISOLATION, PATHOGENICITY AND IDENTIFICATION :

Isolation :

Several isolations were made from 'ergot' affected jowar flowers in the honey dew or sphacelial stage. These isolations were done by the usual laboratory methods. Ergot affected jowar samples were collected from Central Campus Farm of Mahatma Phule Krishi Vidyapeeth, Rahuri. Infected panicles showing sugary excretion were carefully removed from the earhead and disinfected in 1 : 1000 solution of mercury chloride for ½ to 2 minutes, followed by 3 washings in sterile water to remove traces of the poison. Two to 3 panicles were then transferred to a previously sterilized petridish containing a few drops of sterile water and teased with a flamed scalpel in order to release the conidia. After a few minutes such plates were poured with Kirchoff's agar, melted and cooled to 45°C. After solidification of agar, plates were inverted and incubated at room temperature (25° - 28°C). The growth of the fungus was distinctly noticed after 5 to 6 days, from which transfers were made on slants of Kirchoff's agar.

Pathogenicity :

The pathogenicity of the fungus was proved by inoculation experiments. For inoculation studies, the fungus

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medium. The media were prepared according to standard methods. The media were sterilised in an autoclave at 15 lb pressure for 15 minutes, cooled down to 45° C and poured into previously sterilised duplicate plates. The plates were inoculated aseptically with an uniform bit of a two week old culture of the fungus grown on Kirchoff's agar. The inoculated plates were incubated at 28±2° C for 15 days. The observations on nature of growth, sporulation and colony characters were recorded. The different media used were as follows.

1.	Czapek's agar	(6)	Richard's agar
2.	Cooper's agar	(7)	Lebeaumi's agar
3.	Kirchoff's agar	(8)	Synthetic nitrate agar
	a) (With 0.15 asparagine)	(9)	Ashby's agar
	b) (With 15 peptone)	(10)	Potato dextrose agar
	c) (With 0.15 asparagine and 15 peptone)	(11)	Oat meal agar
		(12)	Jowar grain extract agar
4.	Leonian's agar	(13)	Host (jowar carbonate) decoction agar
5.	M-2 agar	(14)	Plain agar

Following arbitrary grades were fixed for judging the sporulation.

Sr.No.	Grade	Symbol
1	Abundant	+ + +
2	Good	+ +
3	Poor	+
4	Nil	-

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ii. Growth on various liquid media

This experiment was undertaken to study the fungus growth on different liquid media, mostly those containing a high percentage of carbohydrates. The media were prepared according to standard methods, inoculated with an uniform bit of the fungus and incubated at 21-22°C for 15 days after which the observations were recorded on colony characters and sporulation. In order to get correct idea about the amount of growth the mycelial growth was filtered through the filter paper and dried at 60°C till constant weights are obtained. Following different liquid media were used, the results of which are presented in Table 2 .

1. Czapek's medium
2. Czapek's medium
3. Kirchoff's medium
   a. with 0.15 asparagine.
   b. with 1% proteose peptone
   c. with 0.1% asparagine & 1% proteose peptone
4. Leonian's medium
5. Richard's medium
6. Sabouraud's medium

4. MORPHOLOGICAL CHARACTERS:

i. Relation of temperature to the growth of the fungus

The relation of temperature to the growth of the fungus was studied on Kirchoff's agar in plates, inoculated at the centre with a small bit of the fungus and incubated at the temperature ranging from 0 to 40°C. The

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results were recorded 15 days after inoculation.(Table 3).

11.a Utilisation of carbon compounds.

This experiment was undertaken to determine the ability of the fungus to utilise different carbon compounds, mostly carbohydrates, as a source of energy. For this purpose basal Kirchoff's medium without sugar was prepared in bulk and distributed in 250 ml Erlenmeyer flasks in 100 ml quantities. To these 5% each of the various carbon compounds were separately added. The media were then sterilised and poured in duplicate plates. The basal medium served as a control. The plates were inoculated as usual with a young sporium of the fungus and incubated at 31-32°C. Observations on colony diameter, growth and sporulation were recorded 15 days after inoculation, which are given in Table 4.

The following different carbon compounds were used.

I. Monosaccharides :

A. Pentoses : i) Arabinose
ii) Rhamnose

B. Hexoses : iii) Fructose
iv) Galactose
v) Glucose
vi) Laevulose

II. Disaccharides : vii) Lactose
viii) Maltose
ix) Sucrose

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III. Trisaccharides : x) Raffinose

IV. Polysaccharides : xi) Dextrin

xii) Mannitol

V. xiii) Control (Kirchoff's medium without any sugar)

4.4.b Utilization of carbon compounds in liquid media

This experiment was made in order to compare and confirm the results of the experiment on solid medias and also to get correct idea about the exact amount of growth.

For this purpose a basal Kirchoff's liquid medium without sugar and agar was prepared in bulk and distributed in 250 ml Erlenmeyer flasks in 100 ml quantities. To these flasks 5% of the different carbon compounds were separately added. Kirchoff's liquid medium without any sugar served as control. The media were then sterilized at 15 lb pressure for 15 minutes, cooled, inoculated with an uniform bit of mycelium and incubated at 21-22° C for 15 days after which observations on colony characters and sporulation were recorded. The mycelial mat was filtered through the filter paper and dried at 60° C till constant weights were obtained. (Table 5).

Following different carbon compounds were used :

1) Dextrin	(5) Lactose	(8) Raffinose
2) Fructose	(6) Maltose	(9) Sucrose
3) Galactose	(7) Mannitol	(10) control (Kirchoff's liquid medium without any sugar).
4) Glucose		

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iii.a. Growth at different levels of sugar

This experiment was made to determine the optimum concentration of sugar in Kirchoff's medium. For this purpose, the fungus was grown on Kirchoff's liquid medium containing different levels of sugar viz. 2.5%, 5%, 6.25%, 7.5% and 10%, using asparagine (0.1%) and proteose peptone (1%) as nitrogen sources. The procedure was similar as described under liquid media. The observations were recorded after 12 days of incubation at 21-22°C and are presented in Table 6.

iii.a. Utilization of Nitrogenous compounds

In this experiment nitrogen metabolism of the fungus was studied. Kirchoff's medium without proteose peptone or asparagine was used as a basal medium. The medium was distributed in 100 ml quantities in 250 ml Erlenmeyer flasks to which 1% of the following inorganic and organic nitrogenous compounds were separately added . Kirchoff's medium without proteose peptone or asparagine served as a control. The following nitrogenous compounds were used for the study :

1) Inorganic nitrogenous compounds.

a) Ammonium nitrate
b) Ammonium sulphate
c) Ammonium tartarate
d) Ammonium oxlate
e) Ammonium acetate
f) Ammonium di-hydrogen phosphate
g) Asparagine
h) Proteose peptone
i) Potassium nitrate
j) Sodium nitrate
k) Sodium nitrite
l) Calcium nitrate

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ii) Organic nitrogenous compounds.

a) Gelatin (b) Urea

iii) a) Control (Kirchoff's medium without asparagine or protease peptone).

The medium was sterilized as usual poured in duplicate plates, inoculated at the centre with an uniform bit of a 15 day old fungus and incubated at 21-22°C temperature. Observations on colony diameter, growth characters and sporulation were recorded 15 days after inoculation. The results are given in Table 7.

iii.b. Utilization of different nitrogenous compounds in liquid media.

A parallel experiment with that of utilization of different carbon compounds in liquid media was carried out using 15 of the different nitrogenous compounds added one in each 250 ml Erlenmeyer flask containing 100 ml Kirchoff's liquid medium prepared without asparagine or protease peptone and agar. The procedure was the same as described under carbon compounds. The results are recorded in Table 8.

Following different nitrogenous compounds were used for this study.

1) Ammonium-di-hydrogen phosphate
2) Ammonium nitrate
3) Ammonium sulphate
4) Asparagine
5) Potassium nitrate
(6) Protease peptone
(7) Sodium nitrate
(8) Urea
(9) Calcium nitrate
(10) Control (Kirchoff's liquid medium without protease peptone or Asparagine).

iii.e. Growth of the fungus at different levels of asparagine and peptone :

After knowing that asparagine and proteose peptone are the most suitable nitrogenous compounds for the fungus, this experiment was laid out to know which is the better source of nitrogen and what should be its optimum concentration. However, peptone (bacterial) was used in absence of proteose peptone. The different levels of asparagine and peptone tried were 0.1%, 0.25%, 0.5%, 1% and 2%. The procedure was the same as recorded under carbon and nitrogen compound in liquid media. The results are given in Table 9.

iv) ENZYME PRODUCTION

The enzymatic activity of the fungus as evidenced by the production of extracellular enzymes in culture media was studied as per the method described by Grabill and Kest (1915). A basal medium of the following composition was prepared.

1.	Magnesium sulphate	0.5 g
2.	Potassium dihydrogen phosphate	1.0 g
3.	Potassium chloride	0.5 g
4.	Ferrous sulphate	Trace
5.	Agar	20.0 g
6.	Distilled water	1000 ml

The 100 ml stock solution was distributed in 250 ml Erlenmeyer flasks and the following synalytes were added as

weight basis to the flasks, separately.

1.	Gelatin	1 g
2.	Inulin	1 g
3.	Asparagine	1 g
4.	Potato starch	2 g
5.	Cellulose	2 g
6.	Casein	1 g
7.	Egg albumin	1 g

The medium was sterilised as usual and duplicate plates were poured with the medium containing each cymelyte separately. Petriplates were inoculated with an uniform bit of the fungus and incubated at 28-32°C temperature for 15 days. Uninoculated petriplates in each case served as a control. As the stock solution contains no carbon compounds it does not support the growth of the fungus. Production of enzyme was judged either by colour reaction or by an ability of the fungus to grow on a particular compound, which was added as a sole source of energy to the medium. The results obtained are given in Table 14.

v) STUDIES ON GERMINATION OF CONIDIA

An ability of conidia of the fungus to germinate under variet conditions of environment, was studied. The mode of germination and time required for germ tubes to come out was noted. Following experiments were undertaken.

a) Effect of temperature on germination of conidia

This experiment was conducted to study the

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germination response of conidia to a range of temperatures and to find out an optimum temperature for the good germination.

For this purpose, a set of clean glass slides, kept in petriplates seated with moist cotton, was prepared using sterile water. Conidia from fresh honey dew on diseased leaves were kept on the slides in a drop of sterile water and kept at different temperatures ranging from 0 to 40°C. Observations on the mode of the germination and counts of the germination percentage of conidia were recorded after 18 hours. The results are summarised in Table 13.

A) Effect of different substrata on germination of Conidia :

This experiment was made to determine the best substratum that would induce effective germination of conidia. A series of hanging drop cultures of 8 substrata viz. tap water, sterilised water, distilled water, Kirchoff's liquid medium, varying concentrations of sugar solutions and honey dew conidia. The conidia from culture were placed in the drop of each substratum and mounted over the cavity slides. The slides, were, then kept in the sterilised petridishes lined with moist cotton wool which prevented drying and provided sufficient moisture for the germination of conidia. The petridishes were then incubated at 25°C temperature. The counts of germination on percentage of conidia were recorded after 18 hours, under microscope. The results are presented in Table 14.

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(vi) HYDROGEN-ION CONCENTRATION IN RELATION TO FUNGUS GROWTH

The role played by hydrogen-ion concentration of the medium in enhancing or inhibiting the fungus growth was studied on Kirchoff's liquid medium adjusted to different pH values.

A stock solution of the liquid Kirchoff's medium was prepared and distributed in 100 ml quantities in 250 ml Erlenmeyer flasks. Hydrogen ion concentration of the liquid medium was adjusted colorimetrically to different pH values by adding to it approximate quantities of N/10 sulphuric acid and/or N/10 sodium hydroxide solutions. The accurate pH values before sterilization were determined with the help of Beckman's pH meter. Two test tubes of the medium for each pH level were sterilized along with the flasks as usual and after sterilization their final pH was similarly determined by testing the sample solution from the test tubes. The flasks were inoculated aseptically with a young mycelial growth of the fungus and incubated at 25-28° C for 21 days. The observations on nature of the growth and sporulation were recorded after 21 days incubation. The mycelial mat was filtered and dried at 60° C to constant weight to obtain dry weight of the mycelium. The results are given in Table XX

(vii) THERMAL DEATH POINT OF THE FUNGUS

The thermal death point of the fungus was determined by the following method. Test tubes of an uniform size and thickness were filled in with 10 ml of Kirchoff's liquid

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medium, sterilised and inoculated with a small mycelial bit of the fungus. The inoculated tubes were kept in the first test in the water bath at constant temperatures of 40, 45, 50, 55, 60 and 65°C for 10 minutes. The water in the water bath was constantly stirred in order to keep an uniform temperature of water and all possible care was taken to maintain the required temperature for 10 minutes in the water bath constantly. A test tube containing Kirchoff's liquid medium was used for keeping the thermometer for recording the temperature. After exposure for 10 minutes to the particular temperature, the tubes were immediately placed in cold water for a while and then incubated at 21-22°C temperature. No growth of the fungus was observed in tubes exposed to 60°C and above. The second test was repeated with temperature, 56, 57, 58 and 59°C to find out the exact thermal death point of the fungus. The results obtained are given in Table 14.

9. HOST RANGE

Host range studies were undertaken to determine the ability of the fungus to infect other hosts besides Jowar. The seed of commonly cultivated millets and some grasses were sown in 9 inch diameter earthen pots filled in with sterilized soil and flowering earheads of these test plants were inoculated with a spore suspension, daily in the evening for 4-5 days. Jowar served as control. The results are given in Table 15.

10. VARIETAL RESISTANCE

This experiment was undertaken to determine the

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reactions of different varieties and selections of jowar to the fungus and to determine the possibility of locating the source of resistance, if any. Seed of different varieties and selections of jowar received from Agricultural Research Stations in the State were sown in pots and at the time of flowering of the earheads were inoculated with the spore suspension, prepared either from a fresh honey dew or from the culture. Spraying was done with the help of an atomiser or hand spray pump and was repeated on alternate days for 4 to 5 times. Uninoculated seedlings served as a control. Observations were recorded after 15 days. The intensity of disease was recorded in the form of grades as given below.

Grade	A	:	No disease
Grade	B	:	1 to 5 spikelets infected on an earhead.
Grade	C	:	6 to 15 spikelets infected on an earhead.
Grade	D	:	16 to 30 spikelets infected on an earhead.
Grade	E	:	31 to 50 spikelets infected on an earhead
Grade	F	:	51 and above spikelets infected on an earhead.

The results are presented in Table 16.

IV. CHEMICAL CONTROL MEASURES

1) Effect of Different fungicides on the fungus in vitro :

The important object of Plant Pathological investigations is to find out suitable control measures for a

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ii) Effect of some fungicides on the control of disease in the field :

This experiment was undertaken to see the efficacy of some fungicides tested earlier in vitro test on the control of the disease in the field when used as a spray.

The field experiment was laid out in the completely randomised block design with four replications and 6 treatments viz. Bavistin, Diran, Captan, Dithane M-45, Agrosfungin, and control. However, this year the disease did not occur even after artificial inoculation. This might be due to unfavourable climatic conditions. Therefore no conclusive results were obtained.

Similarly, another field experiment was also carried out for studying the effect of different dates of sowing on the incidence of disease. This experiment was laid out in the completely randomised block design with four replications and five treatments viz. different dates of sowing with an interval of 10 days, starting from 20th June to 30th July, 1974. Since there was no natural incidence of the disease, the conclusive results could not be obtained.

8. BASIS SEPARATED FOR SEPARATING SUGAR 'UNGOT' SCLEROTIA

The 'ungot' sclerotia get mixed with the grain at the time of threshing. As they contain poisonous alkaloids they cause hazards to humanbeings, cattle and birds, when consumed. Also they are sown in the field with the seed and

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on return of favourable conditions they germinate and subsequently cause infection in the field. In order to avoid these it becomes very necessary to remove the sclerotia from the grain by 'sedimentation' process, which is based on the lower specific gravity of ergot sclerotia than the grain. For this purpose salt treatment experiment was carried out using last season's sclerotia, for finding out a suitable concentration of salt solution in which all the lumpy 'ergot' sclerotia will float without any exception.

For this purpose solutions of a common salt i.e. NaCl of different concentrations ranging from 5 - 30%, were prepared separately and 100 sclerotia were steeped into each solution and stirred for a while. After standing for about 5 minutes the number of sclerotia floated on the surface of the solution was counted in each case, the results of which are presented in Table 16.

EXPERIMENTAL RESULTS

CHAPTER IV

EXPERIMENTAL RESULTS

1. ISOLATION, PATHOGENICITY AND RE-ISOLATION :

The fungus was easily isolated from the sphacelial stage on jowar earhead and the growth was distinctly noticed after 5 to 6 days on Kirchoff's medium in petriplates at 25 - 28° C (room temperature).

Four to six days after artificial inoculation of freshly opened flowers of the jowar, the infected flowers started giving out the sugary secretion, the most prominent symptom of the disease, while control plants were healthy. Thus, the fungus proved pathogenic to jowar.

On re-isolation from infected sphacelia the fungus obtained was identical in all respects to the original culture used for inoculation.

1(a) SYMPTOMS OF THE DISEASE :

The first visible symptom of infection was coming out of a sticky, sugary liquid from ovaries of infected flowers on an earhead, which was the most prominent symptom of the disease. The sugary secretion appeared first as pearly drops which could be readily shaken off. The drops soon became turbid and whitish pink in colour. Later, they condensed and assumed the pinkish brown colour and consistency of honey and hence the name "honeydew" stage for this symptom. The honeydews were full of sphacelia of the fungus which were disseminated mechanically from diseased to healthy earheads

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PLATE I

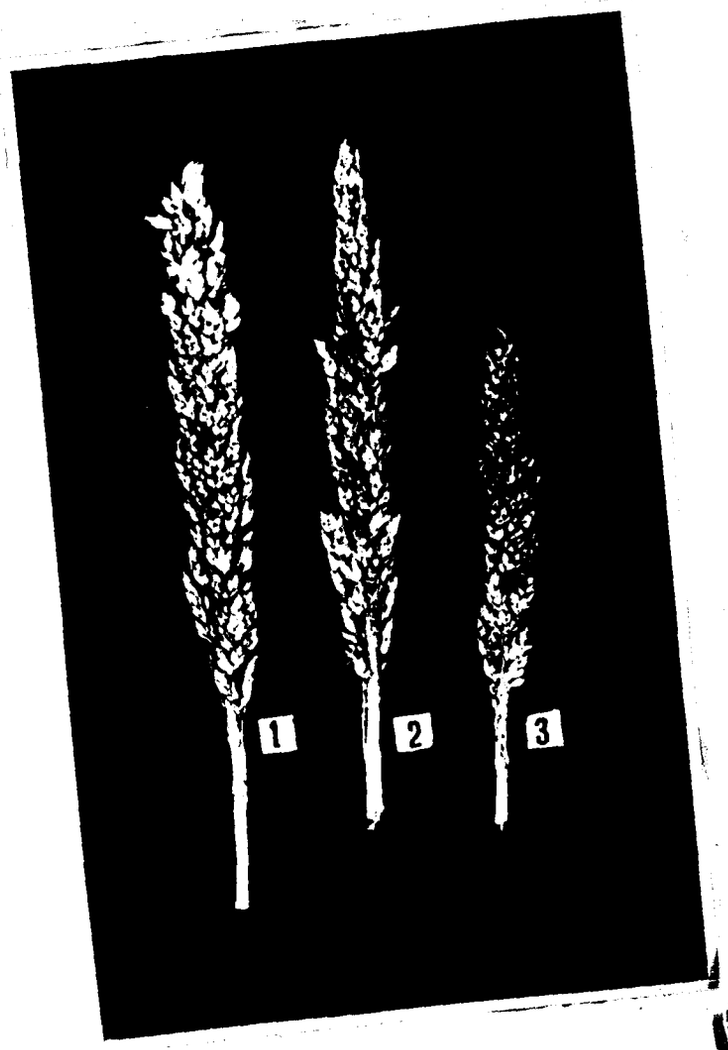
Pathogenicity of the fungus

H : Healthy plant

D : Diseased plant

D-1 : Diseased Earhead (Enlarged)

PLATE II



Earheads showing symptoms

1. Healthy earhead
2. Earhead showing sphacelial stage
3. Earhead showing immature sclerotia

by the ants, flies, wasps, beetles etc. being attracted by the sugary secretion. The duration of this stage depended upon weather conditions. In wet weather it continued for long time even up to 15 days and in dry weather the progress of the disease was often arrested after the drying up of the sugary secretion. The infection was localised and was very severe under most humid and cool weather conditions.

Following the honeydew usually sclerotia start developing in the infected flowers. In the present studies, however, as true, mature 'ergot' sclerotia were developed even after 20 days. This may be due to the slightly higher temperature (30 - 32°C) prevailing during the experimentation. Instead of greenish black to brown sclerotial bodies, greyish white elongated bodies were developed which on dissection found to consist of a mass of hyphae and the surface developed into number of ridges and furrows with closely packed conidiophores.

No other parts of the plant were found to be affected.

2. Morphological characters :

1. Mycelium : The mycelium was septate, irregularly branched, rich in food material, hyaline when young and turning to reddish when old. Mycelial growth was popular, always dense, matty and mostly raised in the centre. The mycelium penetrates the medium and colourless to pinkish droplets were seen on the mycelial colony, containing in most cases, conidia. The average width of the mycelium from the culture was 3.02 u (2.24 to 4.06 u).

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2. <u>Conidia</u> : The conidia were hyaline, single celled, oblong or oval with rounded ends with a vacuole like body at each end and were rich in food material when young. Conidia from culture showed slight variation in their size and shape. They were smaller, ovoid or rod shaped. The conidia from the host measured on an average 14.6 x 6.9 u (12.2 to 18.4 x 5.6 to 7.8 u) those from culture measured 7.5 x 3.2 u (3.8 to 10.0 x 2.1 to 5.5).

3. <u>Conidiophores</u> : Conidiophores were hyaline, single, septate, bearing conidia terminally. On the host, conidia were borne on mats of short conidiophores arranged in a compact palisade. Germ tubes of the conidia in most cases also bear an oval secondary conidia at their ends. Conidia were successively produced one after the other.

3. <u>Cultural character</u> :

<u>Table - 1</u> : <u>Cultural characters of jowar Sphacelia on various solid media</u>

Sr. No.	Name of the medium	Mean colony diameter after 15 days in mm	Sporulation	Growth characters
1	2	3	4	5
1	Coon's agar	44	+	Colony circular, flat with entire margin, dirty white in colour, poor, thin, partly submerged mycelial growth with loose aerial hyphae and whitish droplets scattered all over the colony.
2	Czapek's agar	49	+	Colony irregular, undulating with entire margin and dull white in colour, good, thick mycelial

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(Table 1 continued)

1	2	3	4	5
				growth with abundant aerial hyphae, bulged at the centre and tapering towards periphery with whitish droplets in the centre.
3	Kirchoff's agar A. (With 1% proteose peptone)	68	+ + +	Colony irregular, undulating with wavy margin and pinkish white in colour, very good, dense, matty mycelial growth, pink in the centre with loose, whitish aerial hyphae towards periphery and pinkish droplets scattered all over the colony.
	Kirchoff's agar B. (With 0.1% asparagine)	64	+ + +	Colony irregular, undulating with wavy margin and cottony white in colour, very good, dense, aerial mycelial growth.
	Kirchoff's agar C. (With 0.1% asparagine + 1% proteose peptone)	73	+ + +	Colony irregular, undulating with wavy margin and pinkish white in colour , abundant, dense, pinkish mycelial growth in the centre with cottony white aerial hyphae, thick in centre and tapering towards periphery with few yellowish droplets.
4	Leonian's agar	42	+	Colony irregular, flat with wavy margins and dirty white in colour, poor, thin, loose, partly submerged growth with few aerial hyphae.
5	M-2 agar	58	+ +	Colony circular, flat with entire margin and dull white in colour, good, dense, partly submerged mycelial growth.
6	Richard's agar	60	+	Colony circular, flat with entire margin and cottony white in colour, very good, dense matty mycelial growth with abundant aerial hyphae and yellowish droplets all over the colony.

(Table 1 continued)

1	2	3	4	5
7	Sabouraud's agar	52	♦ ♦	Colony irregular, undulating with wavy margin and whitish in colour, good, dense mycelial growth with loose aerial hyphae.
8	Synthetic Nitrate agar	40	♦	Colony irregular, undulating with wavy margin and dull white in colour, poor, slow, loose mycelial growth, slightly thick at the centre and thin towards periphery with whitish, loose aerial hyphae.
9	Ashby's agar	-	-	Scanty or No growth.
10	Potato dextrose agar	59	♦ ♦	Colony circular, flat with entire margin and dull white in colour, good, dense, aerial mycelial growth, with yellowish droplets all over the colony.
11	Oat meal agar	47	♦	Colony irregular, undulating, with wavy margin and whitish in colour, good, dense mycelia growth with loose aerial hyphae, thick in the centre and thin towards periphery.
12	Jowar grain extract agar	45	♦ ♦	Colony circular, undulating with entire margins and cottony white in colour, good, dense, aerial growth with yellowish droplets in the centre.
13	Root (jowar seedling) decoction agar	33	-	Colony irregular, undulating with entire margin and dull white in colour, very poor, submerged growth.
14	Plain agar	-	-	Scanty or no growth

Note : - : Nil ♦ ♦ : Good

♦ : Poor ♦ ♦ ♦ : Abundant

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From the results recorded in Table 1, it is observed that the fungus made good vegetative growth on Kirchoff's agar, Richards' agar, Potato dextrose agar, M-2 agar, Sabouraud's agar and Czapek's agar. The growth was fair on Oat meal agar, host seed extract agar and Coon's agar, and poor on Leonian's agar, host ear decoction agar and synthetic nitrate agar. The fungus could not grow on Ashby's agar and plain agar.

As regards sporulation, it was abundant on Kirchoff's agar and Potato dextrose agar whereas Sabouraud's agar, M-2 agar and host seed extract agar gave good, sporulation. It was poor in Richard's agar, Czapek's agar, Coon's agar, Leonian's agar, Synthetic nitrate agar and Oat meal agar.

The fungus produced flat colonies on potato dextrose agar, Richard's agar, Leonian's agar, M-2 agar and Coon's agar whereas in other cases the growth was undulating.

The fungus formed good aerial growth except in case of M-2 agar, Leonian's agar and host ear head decoction agar where it was partly submerged.

Thus, the fungus, though slow growing, derives its nutrition from a wide range of media especially those which contain a high percentage of Carbohydrates, with abundant production of spores in Kirchoff's agar and potato dextrose agar only.

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Table.no.2 : Cultural characters of Lunar Zabaglia on different liquid media

Sr. No.	Name of liquid medium	Dry weight of mycelial mat in mg	Sporulation	Growth characters
1	2	3	4	5
1	Coon's medium	835	+	Poor, thin, floating mycelial growth, cottony white in colour.
2	Czapek's medium	945	+	Medium to good growth, pinkish white in colour, partly submerged and partly floating.
3	Kirchoff's medium A. (with 0.1% asparagine)	1335	+ + +	Very good, matty, comparatively thin, submerged mycelial growth, pinkish white in colour.
	Kirchoff's medium B. (with 1% proteose peptone)	1255	+ + +	Very good, thick, matty, submerged growth cottony white in colour.
	Kirchoff's medium C. (with .1% asparagine and 1% proteose peptone)	1495	+ + +	Profuse, dense, matty, whitish mycelial growth.
4	Leonian's medium	660	+ +	Very poor, thin mycelial layer completely floating on the medium, white in the centre and pinkish towards periphery with pinkish droplets all over.
5	Richard's medium	1275	+	Good, dense, cottony white, matty mycelial growth.
6	Sabouraud's medium	850	+ + +	Poor, small, dirty white, scattered colonies.

Key.: - : Nil + + : Good
+ : Poor + + + : Abundant. ... Si

The results are more or less similar to those obtained on solid media. The fungus made abundant vegetative growth on Kirchoff's medium and Richard's medium indicating there by a high requirement of carbohydrate for vegetative growth. Sabouraud's medium , Czapeck's medium and Czapek medium gave good growth as they contain moderate percentage of carbohydrate. The growth was very poor on Leonian's medium as it does not contain any carbohydrate.

For abundant sporulation, a large amount of nitrogen, in the form of proteose peptone is essential as evidenced by the profuse sporulation obtained on Kirchoff's medium and Sabouraud's medium. It was poor in remaining media as they do not contain proteose peptone.

4. Physiological characters :

Table - 1 : Relation of temperature to the growth of the fungus.

Sr. No.	Temperature ° C	Mean colony diameter after 12 days in mm.	Sporulation	Nature of growth
1	0	-	-	No growth
2	5	-	-	No growth
3	10	-	-	No growth
4	15	22	+	Slow cottony white growth.
5	21 - 22	65	+ + +	Very good, mity growth, pinkish in the centre and cottony white on the periphery.

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(Table 3 continued)

1	2	3	4	5
6	25	30	+ + +	Good, cottony white growth.
7	28 - 30 (Room temperature)	32	+ +	Good, cottony white growth.
8	35	28	+	Slow, cottony white growth.
9	40	-	-	No growth.
10	45	-	-	No growth.

Key. : - : Nil + + : Good
: + : Poor + + + : Abundant

The results from the Table 3, indicate that the fungus had a different range of temperatures for growth. The maximum growth and sporulation occurred at 21 - 22 °C temperature. With the increase or decrease in temperature, there is a marked decrease in the growth and sporulation. The fungus could not grow below 10 °C and above 35 °C. Thus, the minimum, the optimum and the maximum temperature requirements of the fungus were 15 °C, 21 - 22 °C and 35 °C respectively.

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Table - 4 : Utilization of different carbon compounds by the fungus.

Sr. No.	Source of Carbon	Mean colony diameter in mm. after 15 days	Sporulation	Growth characters
1	2	3	4	5
I	Monosaccharides :			
	A. Pentoses.			
	1 Arabinose	35	-	Colony circular, flat with entire margin, dull white in colour, slow, dense aerial mycelial growth, thick at the centre and thin at periphery.
	2 Rhamnose	40	+	Colony irregular, undulating with wavy margin, loose, poor, partly submerged, whitish mycelial growth at the centre and spinkish at the periphery.
	B. Hexoses :			
	3 Fructose	40	-	Colony irregular, undulating, with wavy margin, pinkish white in colour, good, slightly thin growth in centre forming, concentric ring with plenty of aerial hyphae.
	4 Galactose	29	+	Colony irregular undulating with wavy margin, pinkish in colour, poor mycelial growth, raised in the centre.
	5 Glucose	38	+ +	Colony circular, flat with entire margin, pinkish white in

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(Table A continued)

1	2	3	4	5
				colour, abundant, dense, mycelial growth raised in the centre with plenty of aerial hyphae and yellowish droplets at several places on the colony.
6	6 Leevulose	39	-	Colony circular, flat with entire margin, cotton white in colour, good dense, aerial mycelial growth.
II	Disaccharides :			
	7 Lactose	48	+	Colony circular, flat, with entire margin, greyish in colour, good, thin partly submerged mycelial growth with loose aerial hyphae.
	8 Maltose	55	+ +	Colony circular, undulating with entire margin, pinkish white in colour, abundant, dense, cottony white aerial mycelial growth at the centre with thin towards periphery, pinkish in colour.
	9 Sucrose	63	+ + +	Colony irregular, flat with wavy margin, cottony white in colour, very good, thick, dense aerial mycelial growth.
III	Trisaccharides			
	10 Raffinose	55	+	Colony circular, undulating with wavy margin, dirty white in colour, good, dense, mycelial growth raised in the centre with plenty of aerial hyphae.

(Table A continued)

1	2	3	4	5
IV	**Polysaccharides**			
	11 Dextrin	24	++	Colony circular, flat with entire margin, whitish in colour, very poor, loose, whitish aerial mycelial growth.
	12 Mannitol	43	+	Colony irregular, flat with wavy margin, pinkish white in colour, good, little loose, mycelial growth with plenty of aerial hyphae.
	13 Control (Kirchoff's medium without any sugar)	-	-	Scanty or no growth

Key : - : Nil ++ : Good
+ : Poor +++ : Abundant

The results presented in the Table 4, indicate that the fungus could utilise carbon from almost all carbon compounds tried. Abundant growth was observed on sucrose, glucose, maltose and raffinose, indicating that these carbon compounds are good carbon sources for the fungus growth, while lactose, mannitol, rhamnose and fructose gave moderate growth. Laevulose, arabinose, galactose and dextrin were a poor source of carbon.

The fungus gave abundant sporulation on sucrose whereas it was good on glucose, maltose and dextrin. Rhamnose, galactose, lactose, raffinose and mannitol gave a poor sporulation, while arabinose, fructose, laevulose were unfavourable.

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Regarding production of aerial hyphae sucrose was found to be most favourable followed by glucose, fructose, raffinose and mannitol. Lactose and dextrin were the most unfavourable for the production of aerial hyphae.

Table.no.5 : Utilisation of different carbon compounds in liquid media by the fungus.

Sr. No.	Source of carbon	Dry weight of mycelial mat after 15 days in mg.	Sporulation	Growth characters
1	2	3	4	5
1	Dextrin	810	+ + +	Poor, whitish, partly floating and partly submerged growth.
2	Fructose	1520	+	Good, pinkish white mycelial growth, thin at the centre and thick at periphery.
3	Galactose	1480	+ +	Good, pinkish mycelial growth, thin at the centre and thick at the periphery.
4	Glucose	1575	+	Very good, submerged, dense, mity growth, white with pinkish tints in between.
5	Lactose	1675	+ +	Good, whitish, partly floating and partly submerged growth.
6	Maltose	1700	+ +	Good, dirty white, mity, submerged mycelial growth.
7	Mannitol	1535	+	Good, thin, submerged mycelial growth with pink colour.
8	Raffinose	2145	+	Very good, pinkish, thick, mity growth, partly floating and partly submerged.

... 37.

(Table 5 continued)

1	2	3	4	5
9	Sucrose	1870	+ + +	Very good, dense, matty, whitish with pink at periphery submerged growth.
10	Control (Richards's liquid medium without any sugar).	300	-	Very poor, cottony white, aerial mycelial growth.

The results given in Table 5, revealed that maximum growth was obtained on raffinose, glucose and sucrose ranking next in order of merit, indicating that these are best suitable carbon sources for the growth of the fungus. The growth was good on remaining carbon sources except dextrin on which it was poor.

Regarding sporulation, it was abundant on sucrose. Although dextrin was unfavourable for the growth it supported the sporulation. Maltose, lactose and galactose gave good sporulation while it was poor on others. Raffinose, though could give maximum growth proved unfavourable for the production of spores.

The order of merit for the growth was raffinose, glucose, sucrose, maltose, lactose, fructose, mannitol, galactose and dextrin.

The results obtained on different carbon compounds in liquid media were more or less similar to those on solid media.

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Table - 6 : Growth of Asper. niger on different levels of sucrose in Czapek's liquid medium with asparagine and proteose peptone as nitrogen sources.

% Cane sugar	Source of Nitrogen with percentage	Dry wt. of mycelial mat in mg. after 12 days	Sporulation	Growth characters
1	2	3	4	5
2.5	Asparagine 0.15	1195	+	Poor, thin, cottony white floating mycelial growth with aerial hyphae.
2.5	Proteose Peptone 15	1280	-	Poor, floating, pinkish white mycelial growth with abundant aerial hyphae.
5.0	Asparagine 0.15	1255	+	Good, whitish, dense, submerged growth
5.0	Proteose peptone 15	1350	+	Poor, dull white, thin, submerged growth.
6.25	Asparagine 0.15	1280	+	Good, submerged mycelial growth, white with slight pinkish tints at periphery.
6.25	Proteose peptone 15	14.10	+	Good pinkish white, dense, partly floating and partly submerged mycelial growth.
7.5	Asparagine 0.15	1525	+ +	Very good, profuse, cottony white, dense, thick matty submerged growth.
7.5	Proteose peptone 15	1405	+ +	Very good, dense, matty, dull white, submerged growth.
10.0	Asparagine 0.15	1755	+ +	Very good, white, submerged, matty growth.
10.0	Proteose peptone 15	1515	+ +	Very good, dull white, matty submerged growth.

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The results presented in the Table 6 indicate that the growth of the fungus goes on increasing at a increasing rate with the increase in concentration of sugar upto 7.5% and thereafter also the growth increases but at a decreasing rate. The sporulation was poor upto 6.25% concentration of sugar and was good thereafter. Therefore, the optimum concentration of sugar for maximum growth and good sporulation was 7.5% for both the nitrogen sources. Of the two nitrogen sources, asparagine was found to be superior.

Table.7 : Utilisation of Nitrogenous compounds by the fungus.

Sr. No.	Source of Nitrogen	Mean colony diameter after 15 days in mm.	Sporulation	Growth characters
1	2	3	4	5
	I. INORGANIC			
1	Ammonium nitrate	24	+	Colony irregular, undulating with wavy margin, cottony white in colour, poor, dense aerial mycelial growth.
2	Ammonium sulphate	30	-	Colony circular, undulating with entire margin, dirty white in colour, moderate, dense, partly submerge mycelial growth.
3	Ammonium tartarate	48	+	Colony irregular, undulating and wrinkled with wavy margin, dull white in colour, good, dense, thick matty, mycelial growth with few yellowish droplets scattered all over.

.... 40.

(Table 7 continued)

1	2	3	4	5
4	Ammonium oxlate	35	-	Colony circular, undulating and wrinkled with wavy margin, whitish pink in colour, good, dense, mutty mycelial growth, bulged in the centre with ridges and furrows and loose aerial mycelial growth at periphery with few whitish to yellowish small droplets on it.
5	Ammonium acetate	32	-	Colony irregular, undulating with entire margin, dull white in colony, slow, dense mycelial growth, white at the centre with loose pinkish aerial hyphae at periphery.
6	Ammonium-di-hydrogen phosphate	30	-	Colony irregular, flat with wavy margin, pinkish in colour, very slow, poor, few, submerged growth with no aerial hyphae.
7	Asparagine	60	◆◆◆	Colony circular, undulating raised and wrinkled with entire margin, whitish grey in colour, abundant, dense, cottony white mycelial growth, raised at the centre and tapering towards periphery with abundant aerial hyphae and plenty of yellowish droplets all over the colony.
8	Proteose peptone	65	◆◆◆	Colony circular, undulating, raised and wrinkled, with entire margin, cottony white with pinkish spots in between, abundant, thick, dense, mutty mycelial growth with plenty of aerial hyphae.
9	Potassium nitrate	44	◆	Colony circular, flat with entire margin, cottony white in colour, good, dense, mutty mycelial growth with plenty of aerial hyphae.

.... 41.

(Table 7 continued)

1	2	3	4	5
10	Sodium nitrate	45	++	Colony circular, undulating with entire margin, ashy white in colour, good, little loose mycelial growth, partly submerged with few aerial hyphae.
11	Sodium nitrite	52	+	Colony circular, flat with very margin, whitish in colour, slow, dense, partly submerged mycelial growth with ashy white aerial hyphae.
12	Calcium nitrate	-	-	No growth.
	XI. CREATIN			
13	Gelatin	53	++	Colony circular, flat with very margin, greyish in colour, good, dense, aerial mycelial growth.
14	Urea	37	+	Colony circular, undulating, raised and wrinkled, with very margin, dirty white in colour, poor, partly submerged, dense mycelial growth with no aerial hyphae.
15	Control (Kylsbef's medium without protease peptone or asparagine)	20	-	Colony irregular, undulating, with very margin, ashy white in colour, very poor partly submerged growth with few aerial hyphae.

Key :- - ; Nil ++ ; Good

\+ ; Poor +++ ; Abundant

From the results given in Table 7, it is revealed that fungus was able to utilize a limited range of nitrogenous compounds. The fungus could grow profusely on protease peptone and asparagine only, whereas growth was good on ammonium

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tartarate, gelatin, potassium nitrate and sodium nitrate. It was poor on other sources except calcium nitrate, on which the fungus failed to grow. Sporulation was abundant on proteose peptone and asparagine whereas good in gelatin and sodium nitrate. It was poor in ammonium nitrate, ammonium tartarate, potassium nitrate, sodium nitrate and urea while it was nil on remaining sources.

Among the nitrogenous compounds utilized, asparagine and proteose peptone produced maximum amount of aerial hyphae.

Table no 2 : Utilization of different nitrogenous compounds in liquid media by fungi Rhizoctonia.

Sr. No.	Source of Nitrogen	Dry wt. of mycelial mat after 15 days in mg	Sporulation	Growth characters
1	2	3	4	5
1	Ammonium di-hydrogen phosphate	835	+	Good, thick, submerged mycelial growth.
2	Ammonium nitrate	455	+ +	Poor, thin, dirty white, submerged mycelial growth.
3	Ammonium sulphate	1010	+	Good, dense, dirty white mycelial mat growth, partly floating and partly submerged.
4	Asparagine	1390	+ + +	Very good, profuse, dense, mity, thick, pinkish white, submerged growth.
5	Potassium nitrate	850	+	Good, mity, dense, dirty white, floating mycelial growth with abundant aerial hyphae

+++++ 43.

(Table 6 continued)

1	2	3	4	5
6	Proteose peptone	1955	+ + +	Very good, dense, murky, whitish with some pinkish patch, submerged growth.
7	Sodium nitrate	875	+ +	Good, dense, pinkish, floating mycelial growth with aerial hyphae.
8	Urea	915	+	Good, dense, cottony, white submerged mycelial growth.
9	Calcium nitrate	-	-	No growth at all.
10	Control (Ekhaloff's medium without proteose peptone or asparagine)	295	-	Very poor, white, scattered growth.

Key :- - : Nil + + : Good

\+ : Poor + + + : Abundant

The results given in Table 6 were similar to those on different nitrogen sources in solid media with few differences. It had become clear once again that the fungus could utilize a limited range of nitrogenous compounds, as exhibited by its abundant growth on proteose peptone and asparagine only indicating that these are the most suitable nitrogen sources for fungus growth. The growth was much lesser on other nitrogen sources as compared to that on asparagine and proteose peptone and it was medium to poor. The fungus preferred different nitrogen compounds in order of merit as proteose peptone, asparagine, ammonium sulphate, urea, sodium nitrate, ammonium di-hydrogen phosphate, potassium nitrate and ammonium nitrate.

... 44.

As regards the sporulation proteose peptone and asparagine gave abundant sporulation. It was good on ammonium nitrate, sodium nitrate and poor on others.

Table no 2 : Growth of Jowar ophiobolis at different levels of asparagine and proteose peptone.

Source of nitrogen	Concentration of Nitrogenous compound	Dry wt. of mycelial mat after 18 days in mg	Sporulation	Growth characters
1	2	3	4	5
1 Asparagine	0.1%	1600	++	Very good, cottony white, dense, matty, thick submerged mycelial growth.
Asparagine	0.25%	1540	++	Good, whitish, dense, matty, submerged growth.
Asparagine	0.5%	1400	+	Good, cottony white, thin, floating mycelial growth with aerial hyphae.
Asparagine	1.0%	1105	-	Poor, thin, floating, mycelial growth, cottony white in colour
Asparagine	2.0%	1225	-	Poor, cottony white, floating, mycelial growth.
2 Peptone	0.1%	1320	-	Poor, dull white, submerged mycelial growth.
Peptone	0.25%	1355	+	Poor, pinkish white, submerged growth.
Peptone	0.5%	1395	++	Poor, irregular, scattered, pinkish white mycelial colonies floating on the medium.

.... 45.

(Table 9 continued)

1	2	3	4	5
Peptone	1.0%	1500	++	Very good, whitish pink, dense, partly floating and partly submerged growth.
Peptone	2.0%	1505	++	Very good, dense, thick mats, submerged special growth with whitish pink colour.

Key :- - : Nil + : Poor ++ : Good

The results presented in the Table 9 indicate that there seems to be a negative correlation between the growth and sporulation on asparagine and peptone. Maximum growth was obtained at 0.1% asparagine and thereafter it steadily goes on decreasing with increase in concentration, whereas the growth on peptone goes on increasing with increase in concentration upto 2% and thereafter it increases but a decreasing rate. Similar is the case with sporulation. It was good upto 0.25% concentration of asparagine and above 0.5% concentration of peptone, poor at 0.5% asparagine and 0.25% peptone and nil above 1% asparagine and 0.1% peptone.

Of the two nitrogen sources, asparagine was found to be a better source of nitrogen for the growth of the fungus with an optimum concentration of 0.1%. The optimum concentration of peptone was found to be 1% .

b) Production of Enzyme

1 Amidase :- The fungus made very poor, submerged growth without any change in the colour of the medium, showing thereby its inability to produce amidase.

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2 Xylan :- The fungus made very poor, submerged, growth showing its inability to produce xylase.

3 Diastase :- The fungus made slow growth with distinct clear 'halo' around the colony when treated with iodine solution. This shows that the fungus is able to produce diastase.

4 Zymase :- The fungus made poor, submerged growth, without any zones below or around the colony, showing its inability to produce zymase.

5 Gelatinase :- The fungus made poor, submerged growth without 'halo' around the colony when treated with mercuric chloride solution. This shows that the fungus was unable to produce gelatinase.

6 Inulase :- The fungus made poor, submerged growth, showing thereby its inability to produce inulase.

7 Trypsin :- A poor, submerged growth of the fungus, without 'halo' around the colony showed its inability to produce trypsin.

The above observations are summarised in Table 10.

Table no.10 : Production of Enzymes by Asper. fumigatus

Sr. No.	Medium	Mean colony diameter in mm after 15 days	Presence of enzyme	Nature of growth
1	2	3	4	5
1	Asparagine pectic acid agar	14	-ve	Very poor, submerged growth
2	Cellulose agar	11	-ve	Very poor, submerged growth
3	Potato starch agar	25	+ve	Partly submerged, circular growth with clear 'halo'
4	Casein agar	19	-ve	Poor, submerged growth
5	Gelatin agar	23	-ve	Poor, submerged growth
6	Inulin agar	22	-ve	Poor, submerged growth
7	Egg-albumen agar	21	-ve	Poor, submerged growth

Key :- -ve = Negative +ve = Positive

Table 10 reveals that the fungus could utilise only a potato starch out of the different synthetics tried as shown by its ability to produce enzyme diastase and its inability to produce enzymes in other cases - indicating thereby the inability of the fungus to utilise them.

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Table . 11 : Growth of [illegible] on liquid Kirchoff's medium adjusted to different pH levels

Sr. No.	pH of the medium Before sterilization	pH of the medium After sterilization	Weight of dry mycelial mat after 21 days in mg	Sporulation	Nature of growth
1	2	3	4	5	6
1	2.0	2.35	-	-	No growth
2	3.0	3.55	1490	+	Poor, cottony white mycelial growth
3	4.0	4.50	1540	+ +	Good, dull white mycelial growth
4	5.0	5.20	1630	+ + +	Abundant, profusely growing pinkish white mycelial growth
5	5.5	5.60	1622	+ + +	Abundant, profusely growing pinkish white mycelial growth
6	6.0	6.05	1617	+ + +	Abundant, profusely growing pinkish white mycelial growth
7	6.5	6.50	1600	+ + +	Abundant, Profusely growing pinkish white mycelial growth
8	7.0	6.88	1575	+ +	Good, profusely growing pinkish white mycelial growth
9	8.0	7.55	1540	+ +	Good, cottony white mycelial growth
10	9.00	8.45	1520	+	Good, cottony white mycelial growth
11	10.0	9.20	1470	-	Poor, cottony white mycelial growth

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(Table 11 continued)

1	2	3	4	5	6
12	11.0	10.15	1443	-	Poor, cottony white mycelial growth
13	12.0	11.00	1405	-	Poor, cottony white mycelial growth

Note :- - = Nil; + = Poor; ++ = Good; +++ = Abundan

It is seen from Table 11, that the fungus could grow well within a wide range of pH, with a tendency to grow better in acidic condition. Maximum growth and abundant sporulation was obtained at pH 5.20. There was decrease in growth with increase in acidity (below 4.50) and alkalinity (above 8.85). However, the range of optimum pH was between pH 5.20 to 6.50.

Table - 12 : Thermal Death Point of the fungus

Sr.No.	Temperature °C	Fungus growth
1	40	Abundant growth
2	45	Good growth
3	50	Good growth
4	55	Poor growth
5	56	Poor growth
6	57	Poor growth
7	58	No growth
8	59	No growth
9	60	No growth
10	65	No growth

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It is revealed from the results given in Table 12, that the fungus mycelium was inactivated at 58°C and the thermal death point of the fungus, therefore, lied between 57° - 58°C.

v) Germination of conidia : Germination started after 4 to 5 hours and is almost completed in 16 to 22 hours. The conidia germinate readily by giving out germtubes from either the sides or ends with the formation of an oval or egg shaped secondary conidium at their ends, with no further growth.

Table no 13 : Effect of temperature on germination of conidia

Sr.No.	Incubation Temperature °C.	Germination after 18 hours
1	0	-
2	5	-
3	10	+
4	15	+ +
5	22	+ + +
6	25	+ + + +
7	28	+ + +
8	35	+
9	40	-

Note : - : Nil + : Poor
+ + : Medium + + + : Good
+ + + + : Very good

The results given in Table 13, indicate that the range of temperature for good germination of conidia was 22 - 28°C

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the optimum being 25°C. The germination goes on decreasing with increase or decrease in temperature and ceases below 10°C and above 40°C. The minimum and maximum temperature for germination of conidia was 10°C and 35°C respectively.

Table no 14 : Effect of different substrata on germination of conidia

Sr. No.	Substratum	Percent germination after 18 hours
1	Distilled water	67
2	Sterilised water	67
3	Tap water	33
4	Kirchoff's liquid medium	77
5	1% sugar solution	70
6	5% sugar solution	73
7	10% sugar solution	73
8	Honey dew conidia	90

The results presented in the Table 14 indicate that the best substratum which induced the maximum germination of spores was Kirchoff's liquid medium followed by sugar solution. As regards the concentration of sugar solution, germination was slightly increased with increase in concentration upto 5% and thereafter there was no difference in germination of conidia with increase in concentration of sugar solution. The germination was poorer in tap water than in distilled and sterilised water. The honey dew conidia gave highest germination, thereby indicating that honey dew

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is the most suitable substratum for germination of conidia.

Table - 15 : Host range of fungus

I. Cultivated crops

Sr. No.	Name of crop	Botanical name	Infection
1	2	3	4
1	Jowar	*Sorghum vulgare* Pers (control)	+ve
2	Bajra	*Pennisetum typhoides* (Stapf)	-ve
3	Maize	*Zea mays* L.	-ve
4	Paddy	*Oryza sativa* L.	-ve
5	Wheat	*Triticum vulgare* Vill.	-ve
6	Oats	*Avena sativa* L.	-ve
7	Barley	*Hordeum vulgare* L.	-ve
8	Rye	*Secale cereale* L.	-ve
9	Nachani	*Eleusine coracana* Gaerth	-ve
10	Shadli	*Panicum miliare* Dalz & Gibs.	-ve
11	Vari	*Panicum miliaceum* L.	-ve
12	Rala	*Setaria italica* Beauv.	-ve

II. Grasses

Sr.No.	Botanical name	Infection
1	2	3
1	*Cynodon dactylon* Pers.	-ve
2	*Chrysopogon montanus* Trin.	-ve
3	*Cenchrus ciliaris* L. Madras Anjan	-ve
4	*Dichanthium annulatum* Stapf.	-ve
5	*Dichanthium caricosum* A.Camus.	-ve

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(Table 15 continued)

1	2	3
6	*Chloris gayana* Kunth.	-ve
7	*Echinochloa crusgalli* Beauv.	-ve
8	*Iseilema laxum* Hack.	-ve
9	*Pennisetum polystachyon* Schult.	-ve
10	*Pennisetum orientale*	-ve
11	*Pennisetum purpureum* Schum & Thonn.	-ve
12	*Panicum antidotale* Retz.	-ve
13	*Panicum miliare* Lam.	-ve
14	*Sehima nervosum* Stapf.	-ve
15	*Sehima sulcatum* Camus	-ve
16	*Heteropogon contortus* Roem & Schult.	-ve

It is observed from the results presented in above Table 15 that the fungus under study could not infect any of the hosts tried, and thus it is very much specialised or restricted in its pathogenicity to *Sorghum vulgare* Pers. only.

Table No. [illegible] : Reactions of [illegible] varieties and selections to the fungus

Sr. No.	Variety/selection	No. of earheads inoculated	No. of earheads infected	Percentage of infection	Grade
1	2	3	4	5	6
1	CSH - 1	3	2	66	B
2	CSH - 2	5	3	60	B
3	CSH - 4 ([illegible])	5	5	100	D
4	PSH - 1	5	4	80	C
5	SPH - 1	3	1	33	C
6	SPH - 4	3	1	33	C
7	Swarna	5	4	80	D
8	J - 302	4	2	50	B
9	J - 604	5	-	-	A
10	R - 16 - 1	10	7	70	C
11	R - 16 - 2	9	6	66	D
12	R - 16 - 3	10	2	20	C
13	R - 16 - 4	5	1	20	B
14	R - 84	5	3	60	C
15	R - 30	5	3	60	C
16	R - 35 - 1	10	-	-	A
17	R - 47 - 3	5	-	-	A
18	P. J. 5 K	5	4	80	B
19	P. J. 8 K	5	4	80	C
20	P. J. 16 K	5	5	100	C
21	P. J. 24 K	5	4	80	C
22	P. J. 5 R	5	1	20	B

(Table 16 continued)

1	2	3	4	5	6
23	K. J. 156	5	3	60	C
24	K. J. 164	5	3	60	B
25	K. J. 1426	5	3	60	B
26	K. J. 1453/2	5	5	100	C
27	I. S. 84	5	2	40	B
28	I. S. 1088	5	1	20	B
29	I. S. 3924	5	3	60	C
30	I. S. 3945	5	1	20	B
31	I. S. 4046	5	1	20	B
32	I. S. 5490	5	3	60	B
33	I. S. 5554	5	1	20	B
34	I. S. 5651	5	2	40	B
35	[illegible] 60-A	5	5	100	B

Key :- A : No disease
B : 1 to 5 epidemic infected on an ear head
C : 6 to 15 epidemic infected on an ear head
D : 16 to 30 epidemic infected on an ear head

It can be seen from the Table 16 that jowar varieties viz. J. 604, K-35-1 and K-47-3 remained free from the disease while varieties K-36-3, K-36-4, P.J.S K, I.S.1088, I.S.4046 and I.S. 5554 showed less than 20% disease infection with a less intensity of the disease. This shows that these varieties were tolerant to the disease even under artificial inoculation. The remaining varieties were found to be highly susceptible.

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Table 17 : Effect of different fungicides and antibiotics on the growth and sporulation of the fungus Myrothecium roridum.

Sr. No.	Name of the fungicide/ antibiotic	Concentration used	Mean colony diameter in mm after 15 days	Sporulation
1	2	3	4	5
1	Bamlate	50 mg/50 cc (0.1%)	7	-
2	Blitox - 50	150 mg/50 cc (0.3%)	60	♦ ♦ ♦
3	Captan	200 mg/100 cc (0.2%)	9	♦
4	Difolatan	200 mg/100 cc (0.2%)	30	♦
5	Dithane Z-78	100 mg/50 cc (0.2%)	62	♦ ♦
6	Dithane M-45	100 mg/50 cc (0.2%)	38	♦
7	Dithane M-22	100 mg/50 cc (0.2%)	67	♦ ♦ ♦
8	Milstem	100 mg/50 cc (0.2%)	15	♦
9	Thiram	200 mg/100 cc (0.2%)	13	-
10	Vitavax	50 mg/50 cc (0.1%)	15	-
11	Zineb	200 mg/100 cc(0.2%)	12	-
12	Agrimycin	500 mg/100 cc(500 ppm.)	58	♦ ♦
13	Aureofungine	16.66 mg/100 cc(166 ppm.) 20 ppm.)Contd2 mg/100 cc	25	♦
14	Control	-	75	♦ ♦ ♦ ♦

Key :- - : Nil ♦ ♦ : Medium ♦ ♦ ♦ : Scanty

♦ : Scanty ♦ ♦ ♦ ♦ : Abundant

It would be seen from the Table 17 that Bamlate, Captan, Zineb, Thiram, Vitavax and Milstem inhibited the growth and sporulation of the fungus. Aureofungine, difolatan and Dithane M-45 also found effective in checking the growth

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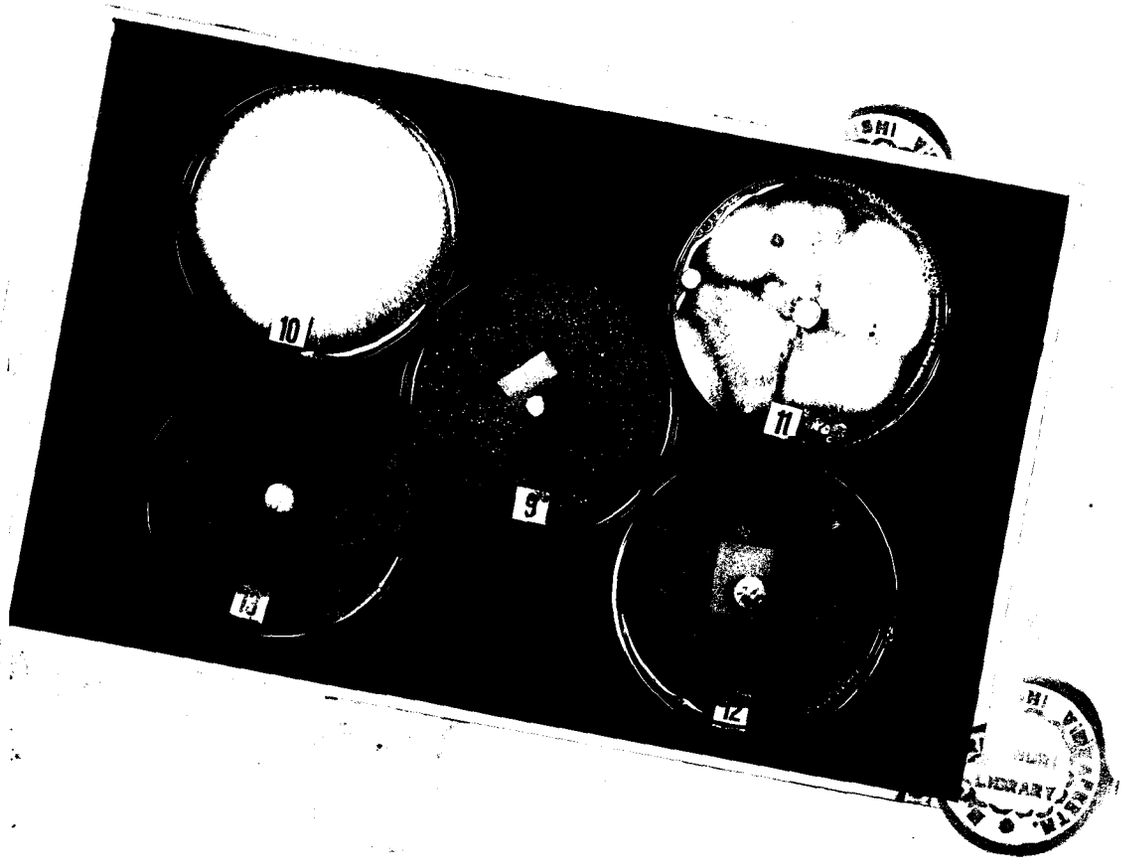
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and sporulation to some extent. Agrimycin, Mithane M-75, Mithane M-22 and Milton-60 were ineffective to check the growth and sporulation of the fungus.

Table 18 : Salt treatment for separation of *Sclerotium* from insect cadaver

Concentration	: No.of sclerotia floating (out of 100)
Water	69
1% salt solution	71
5% salt solution	77
10% salt solution	84
15% salt solution	93
20% salt solution	99
25% salt solution	100
30% salt solution	100

The results presented in Table 18, show that most of the sclerotia floated in 20% salt solution and all in 25% and above salt solutions. Therefore, 25% salt solution may be used for separation of *Sclerotium* sclerotia. However, as the sclerotia used in the present study were old they might have reduced in weight due to loss of moisture. Therefore, a salt solution of a slightly higher concentration i.e. 30% may be used a measure of safety for separation of *Sclerotium* sclerotia. Thereon sclerotization process was first worked out by Miller (1904) in Germany and according to him a 30 to 32% solution of common salt or 37% of potassium chloride is to be used for floating ergot bodies.

v.KAYONOMY*AND*NOMENCLATURE

CHAPTER V

TAXONOMY AND NOMENCLATURE

The production of the sexual stage by germination of sclerotia is a pre-requisite for the description of the Sorghum ergot fungus as a Claviceps species. If the sexual stage would have been formed by the fungus the nomenclature could have been as the species of Claviceps. However, as the disease was developed only in sphacelial stage with no formation of mature ergot sclerotia, in the present studies, the sclerotial germination studies could not be undertaken and hence its comparision with other ergot fungi on Sorghum species is based on measurements of conidia and other important morphological characters.

Literature review indicates that Kulkarni (1917) first observed the sphacelial stage on Sorghum halepense in India in 1915 and identified the fungus as Sphacelia sorghi Kulkarni in 1917. Later, Ajrekar (1926) recorded the disease in both sphacelial and sclerotial stages but all his attempts to find out the perfect stage of the fungus by germinating these dark green to a black sclerotium like bodies were failed. He therefore, considered this fungus as Sphacelia sp. and regarded it as an imperfect or conidial stage of the well known ergot genus Claviceps.

Ramakrishnan (1937) observed the disease in sphacelial stage only but in 1948 he reported both sphacelial and sclerotial stages. Although he tried the sclerotial

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germination he could not succeed. Therefore he named thefun fungus as Sphacelia sp.

A description of Sphacelia sp. reported by both the above workers is given in the following table for comparision.

Table - 12 : Comparative study of Sphacelia sp. described by Ajrekar and Ramkrishnan with the pathogen causing ergot of jowar (Sorghum vulgare Pers.) under study.

Characters	Sphacelia sp. described by Ajrekar S.L. (1926)	Sphacelia sp. described by Ramkrishnan T.S.(1937 & 1948)	Ergot of jowar under study.
1	2	3	4
Symptoms	The only earheads are infected. These are characterised by a sugary fluid being secreted on the apices of the flowers. With age there is formation of a dark green to a black sclerotium like body. Occassionally, instead of greenish black sclerotial bodies, there are developed elongated, greyish white bodies resembling grains affected by grain smut.	The disease is characterised by the formation of a big, pearly, turbid drop of stieky fluid, sweet to the taste, containing large number of conidia. On drying up of an infected panicle, diseased spikelet show a whitish oblong body. There was profuse sclerotial formation only at higher latitudes. The sclerotia were cylindrical, elongated or slightly curved, cream to grey in colour, resembling young sclerotia of ergot of rye.	The first visible symptom of infection is oozing ou of a stieky, sugary liquid from the ovaries of infected flowers in the form of pearly drops which soon become turbid and whitish pink in colour. Later, they are condensed and assumed the pinkish brown colour and consistency of honey. These honey dews are full of sphacelia of the fungus. With age these honey dews dry and elongated, greyish white bodies are developed. No true, mature ergot sclerotia are formed.
Conidia	Conidia are hyaline, oblong or oval with a vacuole like body	The conidia are hyaline, oblong or oval with rounded	Conidia are hyaline single celled, oblong or oval

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(Table 15 continued)

1	2	3	4
	at each end and measure 15 x 7.5 u on an average.	ends and produced in very large numbers.	with rounded ends with a vacuole like body at each end and rich in food material when young. Conidia from culture vary in size and shape. They are smaller, ovoid or rod shaped. Conidia from host measure 14.6 x 6.9 u and those from culture measure 7.5 x 5.2 u .
Germination of conidia	The lower Sphacelia spores germinate readily in distilled water by sending out the germtube from either the side or end, some-times from both ends of the spores, with no formation of secondary conidia.	Conidia germinate readily in water. The germtube is given off from the extremities or the sides and at its end an oval secondary conidium is produced but further growth did not take place.	The conidia germinate readily in most of the substrates especially in Kirchoff's liquid medium by sending out germtubes from either the sides or ends bearing at its end an oval secondary conidium.

Mantle (1968) obtained the defective germination which could not progress further beyond the production of stromatal initials.

Subsequently although, Wilkinson (1927), Khind (1928), Robertson (1928), Wallace (1955), Harrison (1955), Futrell and Webster (1965), Ryan et al. (1965) reported the occurrence of sugary disease on *Sorghum*, a detailed description of the fungus is not available.

Chinnadurai and Govindaswamy (1970) reported a sugary disease from Madras in Sphacelial stage with poorly developed

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sclerotia and they stated that formation of sclerotia is not a common phenomenon in this fungus.

The measurements of the conidia of *Sphacelia sorghi* reported from different places along with the fungus under study are presented in the Table 20, below.

Table - 20 : Comparision between *Sphacelia sorghi* parasitic on [illegible]

	Parasitic on	Measurement of spores	
		Microspores	Macrospores
	1	2	3
1	*Sorghum* [illegible] (1956) Uganda	3 - 5 u	6-8 x 13-18 u
2	*Sorghum vulgare* (1957) Burma	2.5 x 2.5-5.0 u	None
3	*Sorghum vulgare* (1955) Nigeria	3 - 5 u	6-7 x 12-20 u
4	*Sorghum halepense* (1960) Nigeria	None	6-8 x 18-27 u
5	*Sorghum vulgare* (1966) Nigeria	3 - 5 u	6-8 x 14-18.5 u
6	*Sorghum vulgare* (1967) England	3 - 5 u	5-7 x 10-18 u
7	*Sorghum vulgare* (1972) Madras (India)	None	Conidia from culture 5.6-9.3 x 2.2-5.0 u (7.5 x 3.0 u)
		None	Conidia from host 12.5-17.5 x 5-7.5 u (14.8 x 6.5 u)
8	*Sorghum vulgare* under study (1973-74) Maharashtra (India)	None	Conidia from culture 5.6-10.2 x 2.1-5.5 u (7.5 x 3.2 u)
		None	Conidia from host 12.5-18.4 x 5.6-7.8u (14.6 x 6.9 u)

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DISCUSSION

CHAPTER VI

DISCUSSION AND CONCLUSIONS

The present work deals with the investigations on the 'sugary' disease or 'ergot' of *Jowar*-caused by the fungus parasite of the genus *Sphacelia*.

The pathogen was readily isolated in pure culture from sphacelial stage of *Jowar* earheads on Kirchoff's agar medium and subsequently subcultured on slants and maintained in pure state for further study. The pathogenicity was proved by inoculating the earheads of *Jowar* at flowering stage by spraying conidial suspension of the fungus. While Ramakrishnan (1948) obtained the fungus in a pure form on Kirchoff's medium from surface sterilized sclerotial bits. However, Chinnaduraí and Govindaswamy (1970) were able to prove the pathogenicity of *Sphacelia sorghi* by inoculating unpollinated inflorescence of *Jowar*.

The disease appeared only on earheads, in the form of oozing out a sticky, sugary liquid which appeared initially as pearly drops, soon becoming turbid and whitish pink in colour, closely resembling honey and hence the name " honey dew " stage for this symptom. The disease manifested itself in the 'Sphacelial' or 'honey dew' stage with no true mature ergot sclerotia developed. Whereas Ajrekar (1926), and Ramakrishnan (1937 and 1948) described similar symptoms with formation of mature sclerotia.

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The morphological characters of the mycelium, conidiophores and conidia were studied and described. The conidia are single celled, oblong or oval with rounded ends or rod shaped and have variations in the measurements from the host and the culture medium. The conidia from the host measured on an average 14.6 x 6.9 u (12.2 to 18.4 x 5.6 to 7.8 u) and those from culture measured 7.9 x 3.2 u (3.8 to 10.0 x 2.1 to 5.5 u).

The cultural studies revealed that the fungus could derive its nutrition from a wide range of media, especially those which contain a high percentage of sugars. Although, the fungus preferred Kirchoff's agar, Potato dextrose agar, Richard's agar, M-2 agar, Sabouraud's agar and Czapek's agar to produce good growth but Kirchoff's agar only was found to be best for mycelial growth as well as abundant sporulation. Kirchoff's agar containing both 0.1% asparagine and 1% protease peptone gave maximum growth followed by 1% protease peptone and 0.1% asparagine. The fungus was found to be slow growing and sporulation was evident after 15 days. The growth on different liquid media was also more or less similar to that on solid media. Thomas Ambrishnam (1948) obtained the fungus in a pure form on Kirchoff's medium which produced spores within 15-20 days.

The physiological study revealed that the pathogen had an optimum temperature range from 21 to 25°C, though it produced good growth upto 28°C. The growth was faster at 20 to 23°C than at 26 - 28°C (Ambrishnam, 1948).

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The fungus could utilise carbon from most of the [illegible] carbon compounds tried. The fungus preferred sucrose, glucose, maltose and raffinose for abundant growth, whereas only sucrose gave abundant sporulation. Dextrin though found unfavourable for the growth, was found best for production of spores. The different carbon compounds tried in liquid media gave similar results except that the raffinose gave maximum growth followed by glucose, sucrose and maltose.

The optimum concentration of sucrose (cane sugar) for abundant growth and sporulation for both the nitrogen sources was found to be 7.5% .

The fungus was unable to utilise nitrogen from most of the nitrogenous compounds tried-except peptone peptone and asparagine. The fungus made profuse growth with abundant sporulation on proteose peptone and asparagine only. Though, ammonium tartrate favoured good growth, it was poor source for the production of spores. Among the organic nitrogenous compounds, gelatin gave good growth and sporulation. The results of the different nitrogenous compound tried in liquid media were similar to those on solid media. The fungus produced the maximum growth on asparagine and proteose peptone followed by ammonium sulphate and urea. Ammonium nitrate produced poor growth but profuse sporulation.

Of the two nitrogen sources viz. asparagine and peptone, asparagine with an optimum concentration of 0.1% was found to be the best for the profuse growth and abundant sporulation.

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The fungus produced only dikaryon and thus it was found very weak in enzymatic activity.

The present study revealed that the fungus was able to grow well over a wide range of pH, the optimum being 5.50 to 6.50, which means that it preferred acidic condition of the medium.

The thermal death point of the fungus lied between 57 - 58°C.

Spore germination studies revealed that the germination started after about 4 to 5 hours and was almost completed in 18 - 20 hours. The conidia germinated readily by the production of germtubes from either ends or sides bearing at their tips an oval, egg shaped secondary conidium. The range of temperature for good germination was found to be 22 to 28°C with an optimum of 25°C and the best substratum inducing maximum germination on Kirchoff's liquid medium. The hang dew conidia gave highest germination there by indicating the most suitable substratum for conidial germination. However, Ajrekar (1926) reported that the spores germinated readily in distilled water by sending out germtubes from either sides or ends with no formation of secondary conidia while Ramakrishnan (1937) reported that the spores germinate readily in water by sending out germtubes bearing at their ends secondary conidium. The present findings agree with those reported by Ajrekar (1926) and Ramakrishnan (1937).

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Host range studies have shown that the fungus was able to infect only jower while Ajrekar (1926) has recorded Sphacelia sp. on Andropogon sorghum var. saccharatus, Dimeria ciliata and Ischaemum pilosum. Besides, Mukherjee (1957) reported sugary disease on Panicum sumatrense the spores of which were different in shape from those of Sorghum fungus. Reddy (1960) found that Sphacelia sorghi was able to infect Dimeria hybrida. Chinnaduraj and Govindaswamy (1972) recorded that Sphacelia sorghi could infect maize, Sorghum ciliaris and Sorghum arundinaceum with the conidia varied in size and shape on different hosts. In the present study it was found that the fungus was unable to infect either maize, hairi (Dimeiatum hybridum) or Sorghum ciliaris. This may be due to the climatic variation.

In varietal reaction trial all the varieties and hybrids were found to be susceptible except J-604 and two local varieties viz. K-35-1 and K-47-3 which showed no infection as against 100% infection in CSH-4 and mSK 60-A. The varieties K-16-3, K-16-4, P.J.S 2, I.S. 1082, I.S. 4295 and I.S. 5534 were found to be tolerant to this disease. However, Chinnaduraj et al. (1970) reported mSK-60-A, CSH-1, CSH-2 as highly susceptible. The present work is in agreement with Chinnaduraj et al. (1970).

The literature review indicates that none of the workers has done any detailed work on the chemical control aspect of this disease. Campbell (1959) who used malic

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hydroxide to control 'ergot' in headland grasses reported that it inhibits the heading which may result in eliminating the chief sources of inoculum. Where as Chinnadurai and Govindaswamy (1969) reported that sugary disease of jowar can be controlled effectively by cycloheximides.

Under present investigation, *in vitro* tests, the growth and sporulation were inhibited by Bavistin, Ziram, Thiram and Vitavax by the recommended concentrations. Captan, Difolatan and Streptomycine (an antibiotic) were also proved effective in checking the growth as well as sporulation to some extent. On the basis of *in vitro* study, the disease may be controlled in the field by Bavistin (0.1%), Captan (0.2%) and Ziram (0.2%) too.

SUMMARY AND CONCLUSION

CHAPTER VII

SUMMARY

A regular occurrence of ergot of jowar (Sorghum vulgare Pers.) was reported from Satara, Sangli, Kolhapur, Poona, Ahmednagar and Sholapur districts of Maharashtra State causing heavy losses.

The diseased specimens of ergot of jowar were obtained from the Central Campus Farm, Rahuri. A species of Sphacelia was isolated and proved pathogenic to jowar. On inoculation, the fungus produces 'honey dew' stage on the ovaries within 3-4 days, followed by greyish white, elongated bodies.

Observations on morphological characters of mycelium, conidiophores and conidia are recorded in detail.

Cultural and physiological characters i.e. utilization of carbon and nitrogenous compounds were studied on both solid and liquid media. The fungus grew and sporulated best on both solid and liquid Kirchoff's medium. It required 7.5% cane sugar and 0.15% asparagine for the luxuriant growth. The fungus could not produce any enzyme except diastase.

The fungus could not grow below 10°C and above 35°C. The optimum temperature was found to be 21 - 25°C.

The fungus could grow better in pH 4.50 to 8.45 the optimum pH being 5.20 to 6.50. The thermal death point of

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the fungus lies between 37 and 38°C.

Conidia germinated best at 22 to 28°C with the production of germtubes bearing secondary conidia at their ends.

The fungus could not infect any other host except Zea. All hybrids and selections proved susceptible to this fungus.

In vitro test, Bavista, Ziram, Ceytam, Vitavax, Thiram and Milter effectively checked the growth and sporulation of the fungus. A 30% solution of common salt could separate all ergot sclerotia from Zea grains.

On the basis of measurements of conidia, their mode of germination and other morphological characters the fungus was identified as Sphacelia sorghi McRae.

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