OBSERVATIONS ON *Trichoconis padwickii* GANGULY WITH SPECIAL REFERENCE TO ITS PATHOGENICITY

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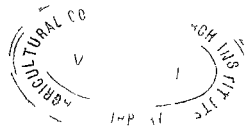
THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE IN AGRICULTURE
(PLANT PATHOLOGY)
OF THE UNIVERSITY OF KERALA

DIVISION OF PLANT PATHOLOGY
AGRICULTURAL COLLEGE AND RESEARCH INSTITUTE
VELLAYANI, TRIVANDRUM

1967



C E R T I F I C A T E

This is to certify that the thesis herewith submitted contains the results of bonafide research work carried out by Shri A.Vinayaga Murthi, under my supervision. No part of the work embodied in this thesis has been submitted earlier for the award of any degree.

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27th August 1967.

A C K N O W L E D G E M E N T S

I wish to place on record my deep sense of gratitude and indebtedness to Dr.J.Sam Raj, Professor of Plant Pathology, for suggesting the problem, able guidance and supervision during the course of this investigation and invaluable help in the preparation of the thesis.

I am extremely thankful to Dr.C.K.N.Nair, Principal and Additional Director of Agriculture(Research) for kindly providing the facilities for the conduct of this work and for other courtesies extended.

I am very much obliged to Dr.T.I.Wilson, Senior Lecturer cum Assistant Virologist for his constructive suggestions and helpful criticisms at every stage of the work.

I am indebted to Sri Abi Cheeran, Research Assistant for his help and valuable suggestions for the successful completion of this work.

The help rendered by all the members of the staff of the Plant Pathology Division during the period this investigation is gratefully acknowledged.

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C O N T E N T S

INTRODUCTION

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Stackburn disease of rice, caused by Trichoconis padwickii Ganguly, was first recorded in India by Padwick and Ganguly in 1945 as affecting earheads and grains. Padmanabhan(1949) found that seed infection in certain varieties can extend upto 76 per cent. In the U.S.A. from where the disease was first reported, Tisdale(1922) and Tullis(1936) found that the disease can cause considerable damage to grain before and during storage.

The disease is wide spread in Kerala and occurs in the first and second crops, but the greatest damage is done to the second crop. Eventhough the disease was previously considered only of minor importance, the damage done in recent years is considerable. The greatest damage is done to the grains, causing discolouration and chaffiness.

The importance of T.padwickii as a grain infecting organism was established by earlier workers like Tullis(1936), Martin(1939), Padwick and Ganguly(1946), Padmanabhan(1949), Dungicourt(1952), Heath(1956), Suryanarayana et al(1963) and Abi Cheeran(1963). Although

these workers observed that the seeds were attacked by T.padwickii, there is no report of the stage or stages of seed maturity during which the maximum infection could occur.

Since the severity of the disease and resultant effect on seed quality depend in part on the time when infection occurs, it was considered necessary to determine the most susceptible stage of grain maturity. This was done by inoculating earheads at different stages of maturity.

An attempt was also made to find out whether the percentage of embryo infection has any bearing on the stage of maturity of the grains at which infection take place. This was done by separating and examining the embryos of different lots of seeds by the method described by Abi Cheeran(1963) and also by seed germination testes.

The cultural characters and the sporulation of the organism in different media were also studied. During the course of this work a profusely sporulating saltant strain of T.padwickii was obtained and this is described separately.

REVIEW OF LITERATUR

REVIEW OF LITERATURE

In the early observations on Stackburn disease of rice, spores of the causal organism were not observed by Godfery(1910,1920) and by Tisdale(1922). The fungus was seem to produce a white mycelium and minute, black sclerotia on leaves, seedlings, and seed. Tisdale(1922) found that different strains of the fungus varied markedly in size and numbers of sclerotia formed and in the intensity of production of pink pigments. He thought that the fungus formed conidia which caused leaf infection, but it was not until Tulis pblished his account(1936) that a conidial stage was definitely established. He tentatively indentified this stage as Trichoconis caudata(App.&.str.) Clem.

Ganguly(1947) studied the disease in detail and found that the spores of the Indian strain were considerably different from those of T.caudata(App.& Str.)Clem. and described it as a new species of Trichoconis, Trichooonis padwickii Ganguly with the following description.

Mycelium well developed, profusely branched, hyaline at young stage, mature hyphae creamy-yellow,3.4 - 5.7 μ thick, septate at regular intervals of 20 - 25 μ, branches arising at rightangles to the main axis and constricted at the point of origin, the first septum being placed just near

the point of origin. Sclerotia black, almost spherical, partly embedded within the host tissue, with reticulated walls and connected by fibrils, measuring 124(52 -195)/u. Condiophores, not sharply distinguishable from mature hyphae, partly erect, 100 - 175/u long and 3.4 to 5.7/u broad, apex monosporous. Conidia elongately fusoid, with a long appendge at the tip, non-deciduous, 3 to 5 septate, creamy-yellow, constricted at septa, thick walled, straight, with second or third cell from the base larger than the rest, 103.2 - 122/u long including the appendage and 8.5 - 19.2/u broad; appendage at the tip of conidium is almost equally as long as the conidium proper, rigid, septate, 2 - 5/u thick, straight or slightly curved.

Ganguly(1947) carried out inoculation experiments on 180 plants each of seven varieties of three different age, by spraying with spore suspension and by placing mycelia on unwounded leaves and leaves wounded by pricking. The results showed low infection rates throughout. There was no evident correlation between the age of the seedlings and their susceptibility to the pathogen. The inoculation by wounding was most successful, leaf spots confined to small area appearing within three to five days, by the other methods after 9 - 12 days. By this experiment he proved that the fungus was a weak pathogen when infecting the leaves. Johnston(1958) also

in his inoculation tests with this fungus got only a low percentage of infection.

The seed-borne nature of the Stackburn disease of rice has been reported by many workers. Tullis(1936) reported Trichoconis caudata(App.& str.)Clem. as one of the organisms causing discolouration of rice grains in United States. Martin(1939) made isolations from 1200 affected kernels sterilized externally with mercuric chloride solution(1:1000), and found that fungi like Helminthosporium oryzae, Fusarium sp., T.caudata and Nigrospora sp. were obtained frequently in culture. Padwick and Ganguly(1946) reported that out of 40 rice seeds of normal or discoloured appearence sown, in Rouse tubes on cotton soaked in distilled water 21 failed to germinate, and of these six were found to be contaminated by H.oryzae four by Curuvularia lunata, seven by T.caudata and four by common moulds, Ganguly(1947) detected the sclerotia of the fungus in the endosperm and observed that the seedlings emerging from infected seeds under laboratory conditions, became rapidly infected, and the coleoptile, the first leaf and the roots were discoloured and bore, sclerotia in the tissues.

Padmanabhan(1949) reported that Trichoconis padwickii was the predominant fungus obtained

on oat-meal agar from the interior of externally healthy, surface sterilised rice grains. It occured in 51.3 to 76 percentage of the seeds. It was reported from the Central Rice Research Institute, Cuttack(1950-51) that T.padwickii was one of the main fungi causing rice grain spotting.

Bunglecourt(1952) found that T.padwickii was the dominant fungus on rice grains in Indo-China. Heath(1956) isolated T.padwickii from six samples of rice grains collected from different localities in Malaya. Johnston(1958) isolated T.padwickii from 7.9 per cent of the grains in the samples of rice seeds from five localities in Malaya.

Suryanarayana et al(1963) observed spores of T.padwickii in centrifuged rice seed-washings. They also isolated the pathogen both from unsterilised as well as surface sterilized seeds. Infection was also observed on the seeds germinated on sterilized moist filter paper and in sterilized sand and it was as high as 30 per cent.

Abi Cheeran(1963) isolated T.padwickii from all the tissues of the infected grains, including the embryos. He also recorded a low percentage germination of infected seeds.

Separation of embryo by chemical processing.

Only few reports are available, regarding the detection of fungus mycelium in the whole embryo by chemical processing. Skvortzoff(1937) separated the embryos of wheat grains and stained them with analine blue to detect the presence of mycelium of the loose smut fungus. Simmonds(1946) described a successful method, with whole embryo mounts, for loose smut determination in wheat and barley. Russel(1950) and Russel and Popp(1951) showed that these tests had a high correlation with green house and field indices. Later, Popp(1951,1958 and 1959) described an improved method for detecting loose smut mycelium in whole embryos of wheat and barley. The embryos were separated by boiling the kernels(barley 30 min., wheat 1 hr.) in 3 per cent NaOH + 12 per cent water glass and 0.04 per cent detergent, then floated off in more water glass. To clear they were boiled for 45 minutes in 12 per cent ethanol+ 15 per cent NaOH, washed, heated again for one minute in 3:1 ethanol: glacial acetic acid, and finally for 1 minute in 45 per cent lactic acid. They were then heated in 45 per cent acetic acid containing 0.1 per cent trypan blue, excess stain being removed by final heating and mounting in 45 per cent lactic acid. Morton(1960) described a quick method for preparing barley embryos for loose smut examination. About 600-700 barley

kernels were boiled to a gelatinous mass in 500 ml water with 25 gm NaOH + 70 ml commercial sodium silicate + a drop of detergent. The embryos were then separated by centrifuging in 50 per cent aqueous solution of sodium silicate. They were then washed in two changes of water and cleared by boiling in lactophenol for ten minutes. By using this method Malick and Batts(1960) studied the location of loose smut mycelium in the infected embryos of wheat and barley. Kavanagh and Mumford(1960) modified Popp's method of detection of loose smut mycelium in barley embryos for a routine observation. In a subsequent paper Morton(1961) described a technique with trypan blue and boiling lactophenol for detecting mycelium of Ustilago nuda(Jens.)Rostr. in barley embryos. Abi Cheeran(1963) was able to demonstrate the presence of the mycelium of Tricheconis padwickii in the embryo of rice seeds by using the technique of Popp(1958) and Morton(1960) with suitable modifications.

MATERIALS AND METHODS

MATERIALS AND METHODS

1. Isolation of the organism

Single spore isolate of *Trichoconis padwickii* were made from infected rice leaves and grains collected from the Agricultural College Farm, Vellayani.

Infected grains and leaf bits were washed in several changes of sterile water and then placed in sterile moist chamber for sporulation. After 48 hours spores were scraped out by means of a sterile scalpel and a spore suspension was prepared in sterile water. One drop of the spore suspension was transferred to melted agar(2 per cent) in test tubes, with the help of a sterile transfer needle. Two drops of 25 per cent lactic acid solution were then added to the melted agar to avoid bacterial contamination. The tubes were thoroughly agitated and the contents were plated in sterile Petri dishes. The Petri dishes were incubated at room temperature for two hours for the germination of the spores. The dishes were then inverted and viwed under the low power of the microscope. Single isolated spores were marked with ink, and agar bits containing single spore were transferred to potato dextrose agar slants by means of a sterile inoculation loop. The fungus was maintained on oat-meal agar with

yeast tablets since this medium was found to support good growth and sporulation.

2. Growth and sporulation on solid media.

The following media were used for this experiment.

Potato dextrose agar.

Peeled potato	200 gm
Dextrose	20 gm
Agar agar powder	15 gm
Distilled water	1000 ml

Oat-meal agar with yeast tablets.

Oat-meal	40 gm
Yeast tablets	15 gm
Agar agar powder	15 gm
Distilled water	1000 ml

Czapek's agar.

$NaNO_3$	2.00 gm
KH_2PO_4	1.00 gm
KCl	0.50 gm
$MgSO_4$	0.50 gm
$FeSO_4$	0.01 gm
Sucrose	30.00 gm
Agar agar powder	15.00 gm
Distilled water	1000.00 ml

Rice grain extract agar.

Rice grains	60 gm
Agar agar powder	15 gm
Distilled water	1000 ml

Preparation.

Sixty gm of the whole grains were powdered well and steamed in 700 ml of distilled water for one hour. The extract was then decanted and filtered through muslin cloth. Fifteen gm of agar agar powder was melted in 300 ml of distilled water. The two solutions were mixed together and made up the volume to 1000 ml.

Host-leaf extract agar.

Rice leaves	200 gm
Agar agar powder	15 gm
Distilled water	1000 ml

Preparation.

200 gm of leaves were boiled in 500 ml of distilled water for one hour. The clear solution was decanted and filtered. Agar(15 gm) was melted in 500 ml of distilled water and mixed with leaf extract. Made up the volume to 1000 ml.

Standard volume of 15 ml media were dispensed in test tubes and the tubes were plugged with cotton wool and

sterilized at 15 lb pressure for 20 minutes.

The pH of all the media were adjusted to six before autoclaving.

The comparative growth of the fungus on various media was studied in the following manner. The media were melted and poured in 10 cm Petri dishes(15 ml each)and allowed to set. A four m m culture disc of the fungus, cut with a sterile cork borer from actively growing region of a week-old culture grown on oat-meal agar with yeast tablets was placed in the centre of the medium in Petri dish. The dishes were incubated at room temperature and observations on the growth rate of the fungus, morphological as well as physiological characters like pigment production in the medium were made. The rate of growth of the fungus was obtained by measuring the diameter of the colony every day from the second day after inoculation upto nine days. By that time some of the colonies just reached the edge of the plate.

The intensity of sporulation was determined as follows:

A four m m agar disc from eight day old culture was put into two ml water and agitated. One drop of the spore suspension was placed on a slide and observed under the low power of the microscope.The average of four observations in

a colony was taken and the sporulation was graded as follows:

Number of spores in a field.	Grade.
50 and above	Good
26 to 49	Satisfactory
10 to 25	Sparse
Below 10	Poor

For measurements, conidiophores, conidia and mycelia were taken from eight day old cultures. Water mounts were used.

3. Germination of spores.

Spore germination in tap water, distilled water, rain water and rice leaf extract were studied. One drop of spore suspension was taken on each slide. Slides were carefully inverted and placed on two glass rods kept in Petri dish with a moistened filter paper at the bottom. These were incubated at room temperature and observations were taken at intervals of two hours for a period of eight hours.

4. Pathogenicity test.

The variety of rice used was Tainan-3. Seeds were obtained from Agricultural College Farm, Vellayani.

The pathogenicity of the fungus was tested by inoculating rice seedlings and earheads. The seedlings

were inoculated by the following methods.

1. Spraying the plants with a spore suspension from eight day old culture.

2. Spraying the plants with a spore suspension prepared from naturally infected grains.

3. Placing mycelial bits from eight day old culture, on the leaves with and without injury and covering with sterile moist cotton wool.

Inoculations were done on seedlings raised in earthern pots filled with a mixture of compost and soil. Four seedlings were raised in each pot. 20 day old seedlings, having four leaves each, were used for inoculation. The control plants were sprayed with sterile water. All the inoculations were conducted after six P.M. The inoculated seedlings were covered with polythene bags for 36 hours. The pots were kept on cement basins and two inches of water was maintained in it.

Earhead inoculation.

The earheads were inoculated at four stages of maturity.

1. Flowering stage
2. Milky stage
3. Dough stage
4. Mature stage

The earheads were inoculated by dipping them in spore suspension. The earheads of control plants were dipped in sterile water in a like manner. Inoculated and control earheads were covered with polythene bags for 36 hours. Observations were taken at the time of harvesting of earheads and the percentage of chaffy grains, infected full grains and healthy grains were recorded.

5. Seed germination

Germination studies were conducted using infected seeds collected from four sets of inoculated plants viz. those inoculated at flowering stage, milky stage, dough stage and mature stage. Seeds from control plants, collected at the respective stages were also used as the control for the germination study.

Twenty seeds were placed on moist filter paper on each Petri dish. The Petri dishes were kept at room temperature and germination counts were taken after five days.

Twenty seeds were sown in each pot. The pots were arranged in cement basins and two inches water was maintained in it. Germination counts were taken after seven days.

6. Detection of fungal mycelium in rice whole embryos.

Three lots of infected grains were used for this purpose. Each lot was collected separately from the earheads inoculated at different stages of maturity viz. flowering stage milky stage and dough stage. The embryos were separated by

chemical processing to detect the mycelium in the embryos.

The techniques described by Popp(1958) and Morton(1960) for the detection of mycelium in the wheat and barley embryos with modifications suggested by Abi Cheeran(1963) for separation of rice whole embryos were followed.

Kernels in lots of 250 were placed in 1000 ml beakers containing 600 ml of an extraction solution having the following formula.

Sodium hydroxide	60 gm
Water	600 ml
Sodium silicate (Commercial liquid glass)	84 gm
Teepol	Few drops

The kernels were vigorously boiled in the extraction solution with occassional stirring for one hour. The volume of the solution was maintained constant by periodical addition of hot distilled water. After an hour the boiling embryos got detached from the kernels.

Liquid glass was immediately added to the above solution in the beaker and it was slightly stirred. All embryos got floated. The floated embryos were skimmed off for further processing.

The embryos were washed twice in hot distilled

water, then placed on the surface of a 50 per cent solution of sodium silicate taken in centrifuge tubes and were centrifuged for two minutes at 4500 r.p.m. This removed all adhering particles from the embryos.

After washing, the embryos were transferred to a bleaching solution with the following formula which was adopted from Ainsworth and Sampson(1950).

Hydrochloric acid(Con.)	25 ml
Potassium chlorate	5 gm
Distilled water	75 ml

The embryos were kept in the above bleaching solution for two hours. Then the bleached embryos were removed, thoroughly washed in several changes of distilled water and the excess water was decanted.

The bleached embryos were then treated under five lb pressure for an hour in an aqueous solution containing 15 per cent sodium hydroxide and 12 per cent alcohol and then thoroughly washed in several changes of hot distilled water for about half an hour. They were further cleared by keeping them at five lb pressure for one to two minutes in a 3:1 mixture of rectified spirit and glacial acetic acid. Finally they were heated at five lb pressure for one minute in 45 per cent lactic acid.

The cleared embryos were placed in the staining solution and heated for 15 minutes at 10 lb pressure. The staining solution was the one that was used by Popp(1958) and with the following formula.

Glacial acetic acid	45 ml
Trypan blue	0.1 gm
Water	55 ml

The embryos were then placed in 45 per cent lactic acid and heated for one minute at five lb pressure to remove the excess stain.

The infected embryos could easily be detected when they were examined under stereomicroscope on account of the deep stain. The non-infected embryos took only a light stain.

The stained embryos were arranged on a 3" x 1" microscopic slides in rows with the help of a zero point camel hair brush and mounted in 45 per cent lactic acid.

The infected embryos were classified on the basis of the extent of the tissues invaded by the mycelium irrespective of the density of mycelial growth. An arbitrary scale was adopted in order to place the infection ratings on a numerical basis. This scale was the same as that suggested by Popp(1951) for determining loose smut infection

on wheat embryos and it is given below.

Grade 1: Traces of mycelium in the embryo.

Grade 2: About one fourth of the embryo invad d with mycellum.

Grade 3: About one half of the embryo invaded with mycelium.

Grade 4: About three fourth of the embryo invaded with mycelium.

Grade 5: The whole of the embryo invaded with mycellum.

RESULTS

EXPERIMENTAL RESULTS

1. Morphological characters of Trichoconis padwickii in culture.

i. Mycelium

The hyphae in young culture were hyaline, septate and highly branched. In old culture the colour of the mycelium turned to creamy yellow. The distance between septa varied from 8 to 45/u but it was generally 24 to 30/u. Diameter of the hyphn varied from 3.5 to 5.2/u.

ii. Conidiophore

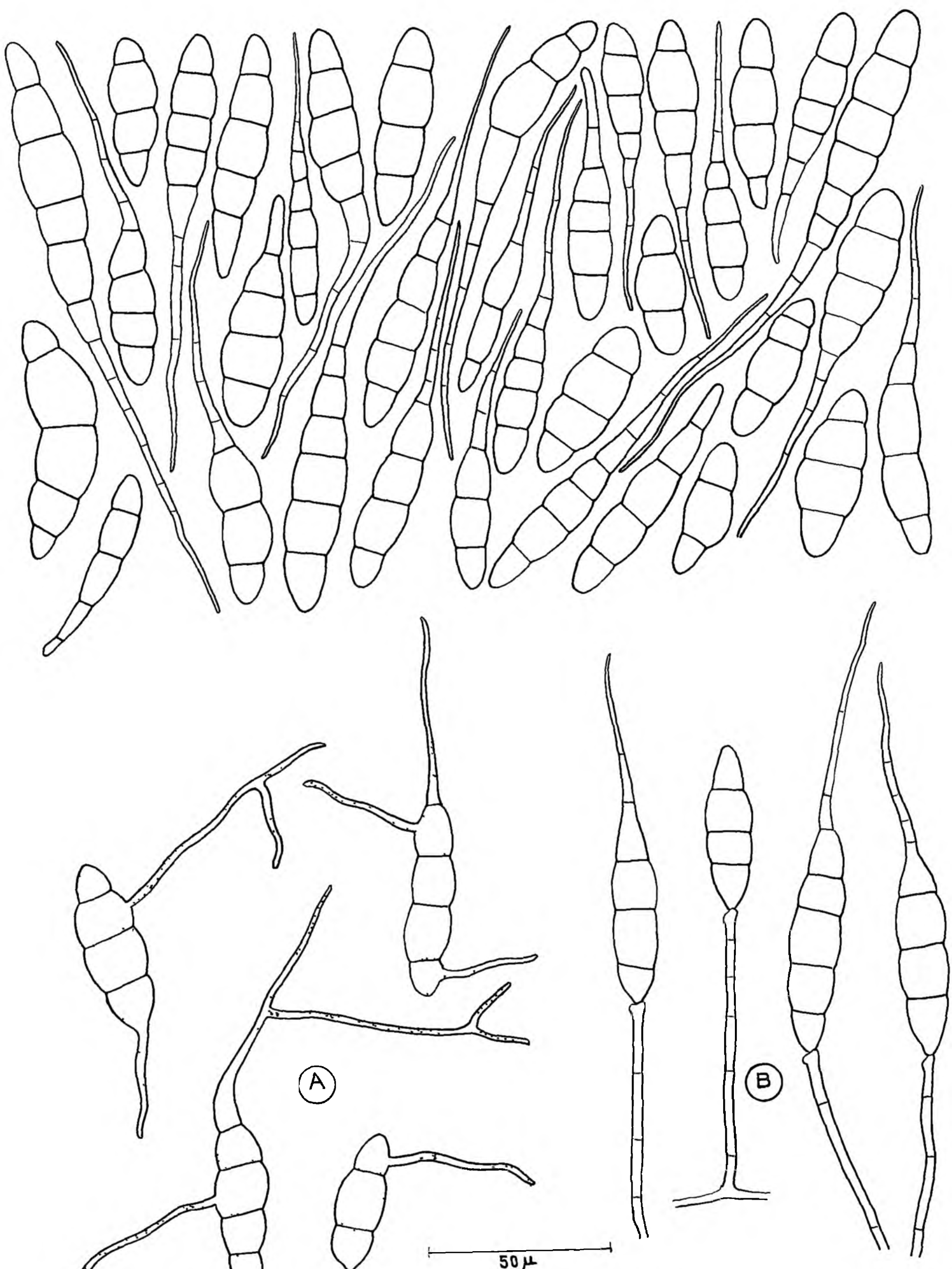
The conidiophores were not distinctly distinguisnable from the mature hyphae. They were straight or slightly curved, unbranched and septate. The number of septa varied from two to nine generally three to five(Fig:1). The distance between the septa varied from 12 to 27/u. Length of the conidiophore was found to be highly variable, ranging from 110 to 182/u. The diameter ranged from 3.5 to 5.2/u. Conidia were borne singly at the apex of the conidiophores.

iii. Conidia

Conidia were straight or slightly curved and hyaline to creamy yellow in colour. They were elongate to fusoid in shape with a long appendage at the tip. The appendage were absent in some of the conidia. Conidia were constricted at the septa and in most cases the second or third cell from the base was larger than the rest. In some conidia bulging was observed in other cells also.(Fig:1). Conidia varied considerably in size and number of septa. The septa were not clear in young conidia but became prominent in mature conidia. The length of the conidia was highly variable, ranging from 26 to 204 µ(including the appendage)the largest number being between 45 to 145 µ with an average of 101 µ(Table:1). The number of septa varied from two to seven, majority of spores having four to six septa and the average four septa(Table.3). Width of conidia varied from 8.8 to 17.6 µ and the average 13.8 µ(Table:2). The appendage at the tip of the conidium was straight or slightly curved, and septate. The length of the appendage varied from 10.56 µ to 133.7 µ with an average of 72 µ.

2. Growth and sporulation on different solid media

There was significant difference in the average daily radial growth of the fungus on different media.



CONIDIA OF *Trichoconis padwickii* FROM CULTURE

(A) GERMINATING CONIDIA

(B) CONIDIOPHORE WITH CONIDIA

Fig. 1

TABLE.1

Frequency distribution for length of conidia.

CLASS			FREQUENCY
18.25	-	36.25	8
36.25	-	54.25	16
54.25	-	72.25	11
72.25	-	90.25	18
90.25	-	108.25	6
108.25	-	126.25	19
126.25	-	144.25	10
144.25	-	162.25	7
162.25	-	180.25	5
180.25	-	198.25	3
198.25	-	216.25	3
Total			100

Maximum length - 204 /u
Minimum length - 26/u
Mean length - 101 /u
About 74% between 45 & 135/u

TABLE.2

Frequency distribution for width of conidia

CLASS			FREQUENCY
7.75	-	9.75	5
9.75	-	11.75	12
11.75	-	13.75	20
13.75	-	15.75	36
15.75	-	17.75	20
17.75	-	19.75	7
Total			100

Maximum width - 17.6 /u
Minimum width - 8.8 /u
Mean width - 13.6 /u
About 88% between 11&17 /u

Table.3

Frequency distribution for number of septa.

Number of septa	Frequency
1	0
2	3
3	9
4	36
5	25
6	25
7	2
Total	100

Maximum number of septa - 7
Minimum number of septa - 2
Mean number of septa - 4

Good growth was observed on potato-dextrose agar, followed in the descending order by grain extract agar, leaf extract agar, oat-meal agar with yeast tablets and Czapek's agar. Good sporulation was observed only on oat-meal agar with yeast tablets. Sporulation was very poor on all the other media(Table 4 and 5).

3. Germination of spores

Conidia germinated well in tap water, distilled water, rain water, and paddy leaf extract. Initial germination was slightly better in leaf extract. The growth of the germ-tube was also stimulated in leaf extract. But the percentage of germination at the end of eight hours was almost same in all the cases(Table 7).

Table.7

Percentage germination of spores in different substrates at different time intervals.

Time (hours)	Tap water	Distilled water	Rain water	Leaf extract
2	28	26	32	39
4	43	40	53	66
6	67	62	74	83
8	93	91	95	95

Conidia germinated by the production of germ-tubes from all the cells, and also from the apical

Table.4 Growth characters of T.padwickii on different solid media

Sl. No.	Media	Colony characters	Sporulation	Average daily radial growth
1.	Potato dextrose agar	White, profuse and compact, aerial mycelium, later turning to light grey, Concentric zonations were observed. Black, spherical sclerotia were observed on the reverse. Border was uniform.	Poor	9 m m
2.	Oat-meal agar with yeast tablets.	White, cottony, profuse and compact aerial mycelium, later turning to creamy yellow. Concentric zonations were observed. Numerous, black, spherical sclerotia were observed on the reverse. Border was uniform.	Good	7.1 m m
3.	Czapek's agar	Aerial mycelium thin, cottony, white and later turning to greenish grey. Concentric zonations were absent. Few, black, spherical sclerotia were reverse. Border was uneven.	Poor	5.2 m m
4.	Grain extract agar	Light grey, cottony and compact aerial mycelium. Concentric zonations were absent. Few black, spherical sclerotia were on the reverse. Border was uneven.	Poor	8.2 m m
5.	Host leaf extract agar	White, cottony, profuse and compact aerial mycelium. Concentric zonations were observed. Few, black spherical sclerotia were on the reverse. Border was uniform.	Poor	7.6 m m

Table.5 Radial growth of Trichoconis padwickii on different solid media (in millimeters)

Replication	A				B				C				D				E			
	Potato dextrose agar				Oat-meal agar				Czapek's agar				Host leaf extract agar				Grain extract agar			
	Days				Days				Days				Days				Days			
	2	3	4	5	2	3	4	5	2	3	4	5	2	3	4	5	2	3	4	5
1	14	21	30	41	12	19	25	34	9	13	19	25	11	18	25	34	12	20	28	37
2	13	22	29	41	12	20	24	35	10	14	19	24	11	19	26	34	12	19	28	36
3	14	23	31	42	13	20	25	34	9	13	18	25	12	18	25	35	13	20	26	37
4	14	22	32	41	12	20	24	34	9	13	19	25	11	18	24	34	12	19	27	38
Average	13.7	22	13.5	41.2	12.2	19.7	24.5	34.2	9.2	13.2	18.7	24.7	11.2	18.2	25	34.2	12.2	19.5	27.2	3[illegible]
Average daily radial growth	9				7.1				5.2				7.6				8.2			

TABLE.6

Analysis of variance table of the data in table 5

Source	S.S.	D.F.	Variance	F.	Inference
Total	761.20	19			
Between media	747.20	4	186.8	200.86	Significant
Within media	14.00	15	0.93		

C.D. 1.45

Ranks	Media	Mean	Difference	Inference
1	A	45		
			3	Significant
2	E	42		
			4	Significant
3	D	38		
			5	Significant
4	B	33		
5	C	28		

A E D B C

appendage. But majority of the conidia germinated by the production of germ-tubes from the end cells. In broken spores, the germination occurred by the production of germ-tubes from the broken end of the spore(Fig.1).

4. Pathogenicity tests.

i. Seedling inoculation:

Artificial inoculation of seedlings with spore suspension prepared from infected grains and also from culture gave only very low infection. After five to seven days, one or two small dark brown spots appeared on each leaf. These spots failed to develop further. Inoculation by placing mycelia on the leaf surface also gave the same type of infection. But inoculation by placing mycelia on wounded leaf surface produced spots within two to three days. These spots also did not develop further. Covering the plants with polythene bags before inoculation, before and after inoculation, and only after inoculation were also not effective to give more infection. In all cases the control plants remained healthy.

ii. Earhead inoculation:

Artificial inoculation of earheads at flowering stage, milky stage, dough stage and mature stage with spore suspension prepared from the culture were successful. However the intensity as well as the percentage

of infection were found to be different at different stages of earhead inoculation(Table 8 - Fig.2).

The percentage of infection was determined by counting the number of full grains with discoloured glumes and also the chaffy grains in each earhead.

The percentage of chaffy grains was very high when the earheads were inoculated during flowering and milky stages, being 39.6 and 38.7 respectively. The percentage of chaff in the corresponding controls were 8.2 and 7.9. This was reduced to 8.3 and 7.8 per cent in the plants inoculated during the dough and mature stages respectively. The percentage of chaff in the control plants were 8.1 in either case.

The highest percentage of infected full grains was noted in the plants inoculated during the flowering stage. While this was 44 per cent, the percentage of infected full grains in other stages of inoculation, namely, milky stage, dough stage, and mature stage were 42.9, 12.7 and 1.2 respectively. The control plants showed only very small percentage of infected full grains viz. 1.8, 1.3, 0.4 and 0 for the flowering, milky, dough and mature stages respectively.

Table 8. EFFECT OF EARHEAD MATURITY AT TIME OF INOCULATION ON THE PERCENTAGE OF INFECTION BY *Trichoconis padwickii*

Stages of inoculation		Chaffy grains	Infected full grains	Healthy grains.
		%	%	%
Flowering stage	Inoculated	39.6	44.0	16.40
	control	8.2	1.8	90.00
Milky stage	Inoculated	38.7	42.9	18.40
	Control	7.9	1.3	90.80
Dough stage	Inoculated	8.3	12.7	80.00
	Control	8.1	0.4	91.50
Mature stage	Inoculated	7.8	1.2	90.00
	Control	8.1	-	91.90

*15 earheads were inoculated for each stage and also for its control.

EFFECT OF EARHEAD MATURITY AT TIME OF INOCULATION
ON THE PERCENTAGE OF INFECTION BY T *paduvckii*

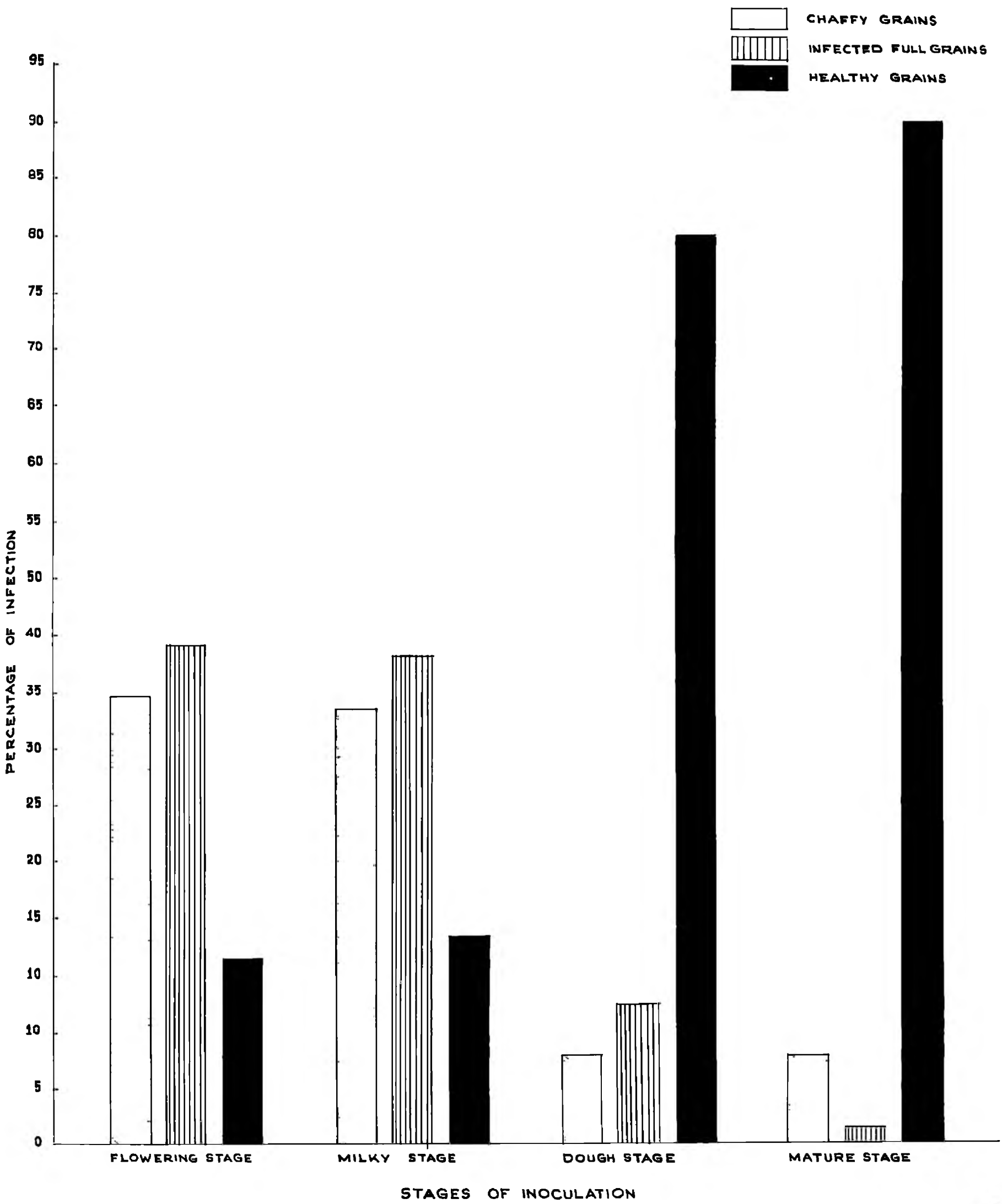


Fig 2

5. Seed germination

i. Moist chamber.

Infected seeds collected from the earheads inoculated at flowering, milky, dough and mature stages gave 72, 79, 92 and 97 per cent germination respectively. The seeds collected from the control plants of the corresponding stages gave 95, 97, 98 and 98 per cent germination(Table 9).

Growth of Trichoconis padwickii and other fungi like Curvularia, Aspergillus, Penicillium and Nigrospora species were observed on the surface of the infected seeds. Only spores of fungi other than Trichoconis padwickii were observed on the surface of the non-infected seeds. Decay of radicle and plumule was noted in some of the germinated seeds.

ii. Pots.

In pots, infected seeds collected from the earheads inoculated during flowering, milky, dough and mature stages gave 69, 75, 91 and 95 per cent germination respectively. The seeds collected from the control plants of the respective stages gave 93, 92,94 and 95 per cent germination(Table.9).

Table.9 PERCENTAGE GERMINATION OF THE INFECTED AND NON-INFECTED RICE SEEDS IN POTS AND PETRI DISHES

Stages of inoculation		In petri dishes		In pots	
		No.of seeds	Germination percentage	No.of seeds	Germination percentage
Flowering stage	Infect d seeds	200	72	200	69
	Healthy seeds	200	95	200	93
Milky stage	Infected seeds	200	79	200	75
	Healthy seeds	200	97	200	92
Dough stage	Infected seeds	200	92	200	91
	Healthy seeds	200	98	200	94
Mature stage	Infected seeds	200	97	200	94
	Healthy seeds	200	98	200	95

6. Detection of fungal mycelium in rice embryo.

The embryos of the infected grains collected from earheads inoculated during flowering and milky stages showed higher percentage of infection being 44.8 and 37.6 respectively. The percentage of embryo infection was low in the case of infected grains collected from earheads inoculated during dough stage, being 12.6. These figures do not take into account the embryos which were lost during processing. Details with grades of infection are given in the table 10.

Table.10. DETAILS OF THE PRESENCE OF MYCELIUM IN WHOLE RICE EMBRYO SEPARATED BY CHEMICAL PROCESSING

Stages of inoculation	No.of kernels used for processing.	Embryos obtained for final examination		Percentage of embryos invaded by mycelium. Grades*					
		No.	Percentage	1	2	3	4	5	1 - 5
Flowering stage	500	437	87.4	9.2	11.8	8.9	10.4	4.5	44.8
Milky stage	500	428	85.6	8.7	12.3	7.4	6.3	2.6	37.6
Dough stage	500	461	92.2	4.5	2.4	2.7	1.8	1.2	12.6

*
1. Traces of mycelium in Embryo.
2. About one fourth of the embryo invaded by mycelium.
3. About one half of the embryo invaded by mycelium.
4. About three fourth of the embryo invaded by mycelium.
5. Whole of the embryo invaded by mycelium.

TABLE.11

Analysis of variance table for full grains with infection.

Source	S.S.	D.F.	Variance	'F'	Inference
Total	32502.18	59			
Between stage	29773.38	3	9924.16	203.7	Significant
Error	2728.80	56	48.72		

C.D. = 5.09

Ranks	Stages	Mean	Difference	Inference
1	Flowering stage	57.87	4.74	Not significant
2	Milky stage	53.13	24.20	Significant
3	Dough stage	28.93	23.73	Significant
4	Mature stage	5.20		

Flowering stage — Milky stage — Dough stage — Mature stage

TABLE.12
Analysis of variance table for chaffy gains

Source	S.S.	D.F.	Variance	F.	Inference
Total	2157.65	59			
Between stages	18724.31	3	6241.44	143.64	Significant
Error	2433.34	56	43.45		

C.D. = 4.81

Ranks	Stages	Mean	Difference	Inference
1	Flowering stage	47.47	4.63	Not significant
2	Milky stage	45.84	28.67	Significant
3	Dough stage	11.06	00.93	Not significant
4	Mature stage	10.13		

Flowering stage Milky stage Dough stage Mature stage

DISCUSSION

Trichoconis padwickii usually does not sporulate well in some of the common culture media. A comparative study of the growth and sporulation of this fungus on five solid media was therefore made. Judging from the rate and density of growth, potato dextrose agar was found to be the best medium for vegetative growth followed by grain extract agar, leaf extract agar, oat-meal agar with yeast tablets and Czapek's agar media in the same order. But sporulation in potato dextrose agar was very poor.

Good sporulation was obtained only in oat-meal agar with yeast tablets. Abi Cheeran(1963) recorded good sporulation of T.padwickii in potato dextore agar and oat-meal agar, with thiamine and biotin. It is therefore possible that yeast tablets has provided these nutritional factors, thereby aiding better sporulation of the fungus.

The spores of the fungus germinated by producing germ-tubes from any of the cells and from the apical appenage, but more frequently from the end cells. Good germination was obtained in distilled water, tap water, rain water and in leaf extract. Though initially leaf extract gave a slightly higher percentage of germination the percentage germination was more or less similar in

all the substrates at the end of eight hours. This indicates that the host leaf extract does not exert any appreciable stimulatory effect on spore germination.

Artificial inoculation of rice seedlings with spore suspension from culture as well as from naturally infected grains and mycelial bits, produced only very few spots on the leaves. Even wounding the leaves before inoculation, did not have any effect in giving greater infection. It is possible that the fungus is only weakly pathogenic on the leaves of the variety of rice used. A similar observation has been made by Ganguly(1947) who also got only a very low percentage of infection on leaves when artificially inoculated.

Successful infection was obtained when earheads were inoculated. The percentage and intensity of infection were found to depend on the stage at which the earheads were inoculated. Highest percentage of infection was obtained when the inoculations were done at the flowering and milky stages. Inoculation at the dough stage gave a comparatively low percentage of infection, while that at the mature stage gave only negligible infection. The stage of maturity of the earhead, therefore seems to be an important factor in determining the extent of infection by the fungus. It is possible that soft and young seed

tissues offer little resistance to fungal penetration and that as these tissues mature, they become increasingly resistant. Early infection can bring about greater damage.

The above observations have been further corroborated by the results obtained in the experiment for the detection of mycelium in embryos and also by the percentage germination of infected seeds. A higher percentage of embryos of the infected seeds collected from earheads inoculated during flowering and milky stages showed the presence of mycelium. The percentage of embryo infection was low in the infected seeds collected from earheads inoculated during dough stage. A high percentage of seeds infected at flowering and milky stages failed to germinate both in pots as well as in Petri dishes. The germination percentage of seeds infected at dough stage was rather high and in the case of seeds infected during mature stage it was almost equal to the germination percentage of healthy seeds. The low germination percentage of seeds infected during flowering and milky stages may be due to the greater amount of mycelium present in the embryos of these seeds. Abi Cheeran(1963) observed failure of germination in seeds with half or more of their embryos invaded by mycelium. Early infection of earhead therefore

helps in the successful penetration of the fungus into the deeper tissues of the seeds resulting in the loss of viability in a high percentage of seeds.

SUMMARY

SUMMARY

Oat-meal agar with yeast tablets was found to be a good medium for the sporulation of Trichoconis padwickii, while mycelial growth of the fungus was better in potato dextrose agar.

Spores of Trichoconis padwickii germinated by the production of germ-tubes from any of the cells but more frequently from the end cells. Germination was equally good on tap water, distilled water, rain water and leaf extract.

The fungus was found to be only weakly pathogenic on the leaves of rice since only few spots were produced on artificial inoculation.

Earhead inoculation gave successful infection. The percentage and intensity of infection were found to depend on the stage at which the earheads were inoculated. Highest percentage of infection was obtained when the inoculations were done at the flowering and milky stages.

A high percentage of embryos of the infected

seeds collected from earheads inoculated during flowering and milky stages showed the presence of mycelium. It was low in the case of seeds infected during dough stage.

A high percentage of seeds infected at flowering and milky stages failed to germinate both in pots as well as in Petri dishes. The germination percentage of seeds infected at dough and mature stages were high. It is therefore concluded that early infection of earhead helps in the successful penetration of the fungus into the deeper tissues of the seeds resulting in the loss of viability in a high percentage of seeds.

A SALTANT STRAIN OF *Trichoconis padwickii*

During the course of this investigation, a sector of light grey colour was observed in a white colony of *Trichoconis padwickii* grown on oat-meal agar with yeast tablets. This area was immediately examined and transfers were made on potato dext ose agar slants since it appeared to be a saltant.

Single spore isolations were made and the saltant strain was maintained as a pure culture. The saltant strain maintained its characters and reversion to the parent strain did not occur on sub-culturing.

Typical symptoms of Stackburn disease were produced on rice leaves and earheads, when they were artificially inoculated with spores of the saltant strain. On reisolation the slatant strain maintained its characters. The saltant strain was compared with the parent strain for its morphological and cultural characters and pathogenicity.

Morphological characters.

i. Mycelium

The saltant produced a thin aerial mycelium

which consisted mostly of conidiophores and conidia, while the parent strain produced thick fluffy aerial mycelium. There was no difference between the two strains in other mycelial characters like septation, branching and width of the hyphae.

ii. Conidiophore

There was no difference in conidiophore characters between the two strains.

iii. Conidia

The conidial characters of both the strains were studied by taking conidia from eight day old culture. Hundred spores were measured in each case. The conidia of the saltant strain was distinctly smaller in size than those of the parent strain(Fig.3 and Table 13.15). They measured 19.4 to 169 by 7 to 12.3/u. The conidia of the parent strain measured 26 to 204 by 8.8 to 17.6/u. There was no significant difference in septation between both strains.

Growth and sporulation on different media.

The two strains were grown side by side on leaf extract,agar, grain extract agar, potato dextrose agar, oat-meal agar with yeast tablets and Czapek's agar

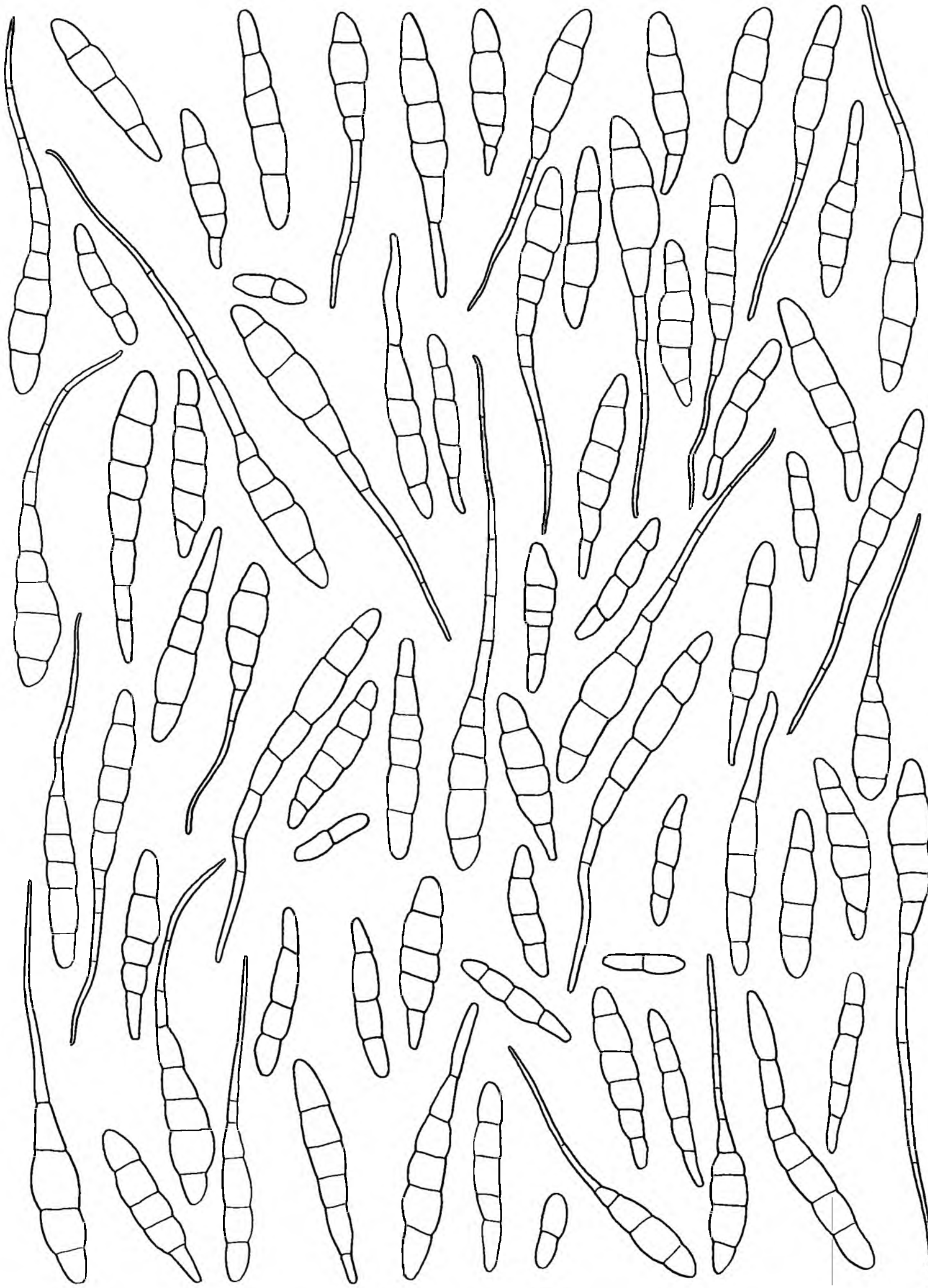


FIG 3

CONIDIA OF THE SALTANT STRAIN OF *Trichoconis padwickii* FROM CULTURE

TABLE.13

Frequency distribution for the length of Conidia

CLASS	FREQUENCY
18.25 - 36.25	10
36.25 - 54.25	38
54.25 - 72.25	35
72.25 - 90.25	4
90.25 -108.25	5
108.25 -126.25	3
126.25 -144.25	2
144.25 -162.25	2
162.25 -180.25	1
Total	100

Maximum length - 169 μ

Minimum length - 19.4 μ

Mean length - 60.4 μ

About 83% between 28 & 64 μ

TABLE.14

Frequency distribution for width of Conidia

CLASS	FREQUENCY
6.25 - 8.25	26
8.25 -10.25	26
10.25 -12.25	38
12.25 -14.25	10
Total	100

Maximum width -12.3 μ

Minimum width - 7 μ

Mean width - 9.5 μ

About 90% between 7 & 11 μ

TABLE.15

Frequency distribution for number of septa

Number of septa	Frequency
1	0
2	4
3	6
4	45
5	34
6	11
Total	100

Maximum number of septa - 6

Minimum number of septa - 2

mean number of septa - 4

media. On all the media the rate of growth of the saltant strain was faster than that of the parent strain(Tables 16-19; Fig.4 - 12).

The saltant strain sporulated profusely on all the media except Czapek's agar, wherein the sporulation was only moderate. Good sporulation of parent strain was observed only in oat-meal agar with yeast tablets and in all other media, sporulation was very poor.

Comparative pathogenicity tests.

The pathogenicity of the saltant strain was compared with that of the parent strain by artificial inoculation on rice seedlings. No differences in pathogenecity were noted.

Table.16. Growth characters of the mutant strain of T.padwickii on different solid media.

Sl. No.	Media	Colony characters	Sporulation	Average daily radia growth
1	Potato dextrose agar	Aerial mycelium thin, cottony, white and later turning to grey. Concentric zonations were observed. Border was uniform. Reverse was black.	Good	9.5 m m
2.	Oat-meal agar with yeast tablets.	Aerial mycelium thin, cottony, white and later turning to light grey. Concentric zonations were observed. Border was uniform. Reverse was light brown in colour.	Good	8.5 m m
3.	Czapek's agar	Aerial mycelium is very thin, cottony, white and later turning to olive green. Concentric zonations were not clear. Border was uneven. Reverse was black in colour.	Good	7.0 m m
4.	Grain extract agar	Aerial mycelium thin, cottony, white and later turning to light grey. Concentric zonations were not clear. Border was uneven. Reverse was dark brown in colour.	Good	7.6 m m
5.	Host leaf extract agar	Aerial mycelium thin, cottony, white in colour. Uniform border. Concentric zonations were not clear. Reverse was light orange in colour.	Good	9.3 m m

Table.17 Radial growth of saltant strain of T.padwickii on different media

(in millimeters)

Replications	A				B				C				D				E			
	Potato dextrose agar				Oat-meal agar				Czapek's agar				Host leaf extract agar				Grain extract agar.			
	Days				Days				Days				Days				Days			
	2	3	4	5	2	3	4	5	2	3	4	5	2	3	4	5	2	3	4	5
1	14	24	31	44	12	22	32	39	10	20	27	32	13	20	27	37	13	24	33	42
2	15	24	32	43	11	24	31	37	9	20	25	31	14	19	26	36	14	25	32	41
3	14	23	32	43	12	23	31	37	12	21	26	32	13	19	27	36	14	25	32	41
4	14	23	32	42	12	23	32	37	11	20	26	32	13	19	27	36	14	24	34	42
Average	14.2	23.5	31.7	43	11.7	23	31.5	37.5	10.5	20.2	26	31.7	13.2	19.2	26.7	36.2	13.7	24.5	32.7	41.5
Average daily radial growth	9.5				8.5				7				7.6				9.3			

TABLE.18

Analysis of variance table of the data in table 17

Source	S.S.	D.F.	Variance	F	Inference
Total	412.55	19			
Between media	399.80	4	99.95	117.8	Significant
Within media	12.75	5	0.85		

C.D. 1.39

Ranks	Media	Mean	Difference	Inference
1	A	53.25	5.75	Significant
2	E	47.50	5.25	Significant
3	B	45.25	3.25	Significant
4	D	42.00	1.00	Not significant
5	C	41.00		

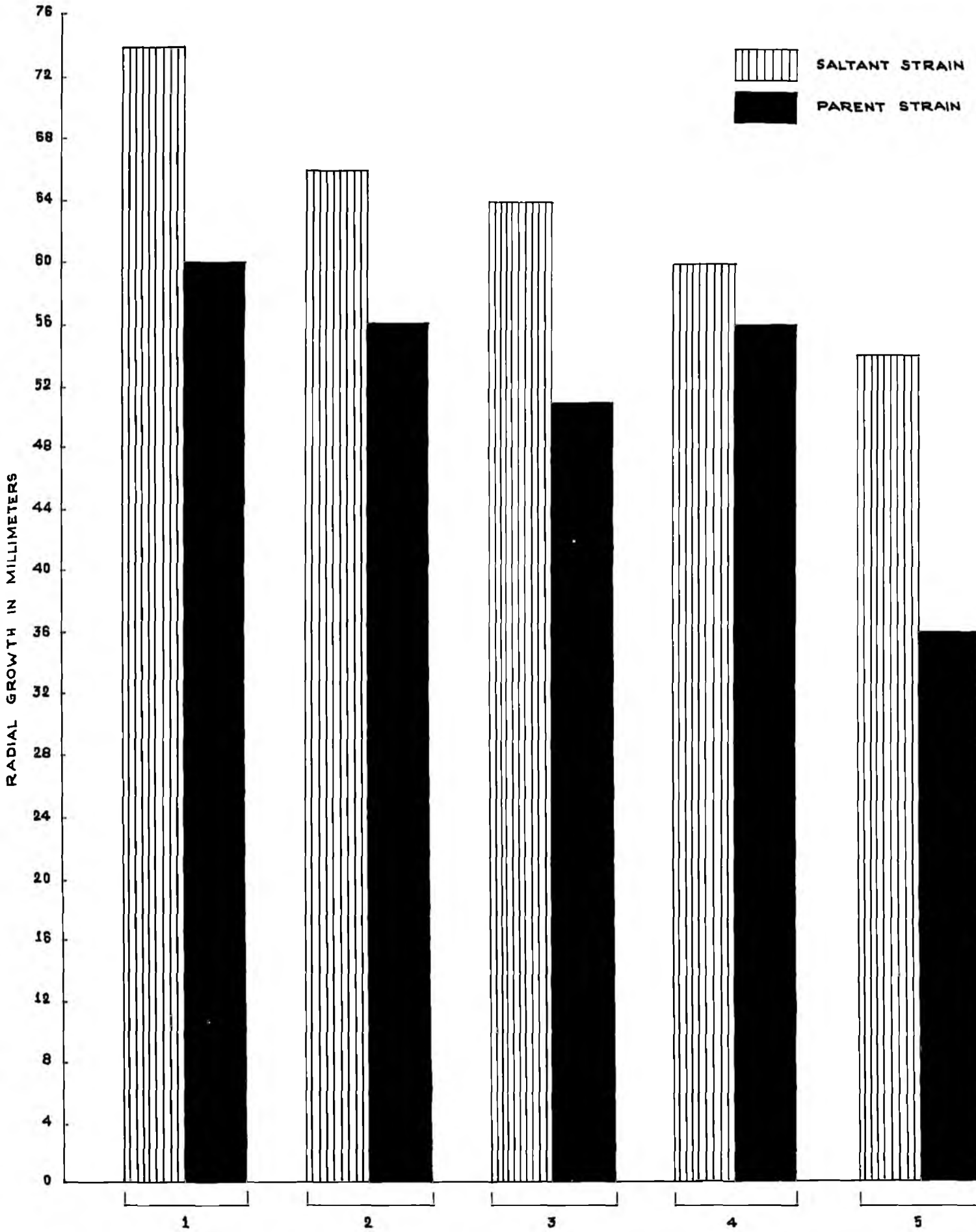
A E B $\overline{D\ C}$

Table.19. Comparative growth of parent and saltant strains of *Trichoconis padwickii* of different agar media (in m.m.)

	Leaf extract agar	Grain extract agar	Potato dextrose agar	Czapek's agar	Oat-meal agar
Parent strain	57	56	60	32	51
Saltant strain	60	74	73	54	65

* Average of four replications.

COMPARATIVE GROWTH OF PARENT AND SALTANT STRAIN
IN DIFFERENT SOLID MEDIA



1 P D A 2 GRAIN EXTRACT AGAR 3 OAT MEAL AGAR WITH YEAST TABLETS 4 LEAF EXTRACT AGAR
5 CZAPEK S AGAR

FIG 4

REFERENCES

REFERENCES

1. ABI CHERIAN.(1963). Observations on the Stackburn disease of rice with special reference to the detection of mycelium of Trichoconis padwickii Ganguly in the whole embryo. M.Sc. Thesis, University of Kerala, Trivandrum. 64 p.

2. AINSWORTH, G.C. & SAMPSON,K.(1950). The British Smut fungi(Ustilaginales). The Commonwealth Mycological Institute,Kew,Surrey. 137 pp.

* 3. BUNGICOURT,P.(1952). A contribution to the Systematic study of two Microfungi Parasitic on rice. Rev.Gen.Bot.59,702,413-420.

4. Combined Annual Report for 1949-50 and 1950-51 of the Central Rice Research Institute, Cuttack. Printed in India by the manager, Government of India Press, Nasik Road,33 pp. 1954.

5. GANGULY, D.(1946). Helminthosporium disease of paddy in Bengal. Sci. & Cult., 12,5, 220-223.

6. GANGULY, D.(1947). Studies on the Stackburn disease of rice and identity of casual organism. J.Indian bot.Soc.26,4,239.

7. GODFERY, G.H.(1920). A seed-borne sclerotium and its relation to a rice seedling disease. Abs.in Phytopathology,5,342-343.

8. GODFERY,G.H.(1916). Preliminary notes on a heretofore unreported leaf disease of rice. Abs. in Phytopathology,5,97.

9. JOHNSTON,A.(1958). Fungicidal treatment of paddy seed. Mal y agric.J., 41,4,282-289.

10. KAVANAGH, T. & HUMPHRD,D.E.(1960). Modification and adaptation of Popp's technique to routine detection of Ustilago nuda(Jens.) Rostr. in Barley embryos. Plant Dis.Reptr,44,7,591.

11. MALIK,N.M.S. & BATTS, G.C.V.(1960). The infection of barley loose smut (Ustilago nuda(Jens.) Rostr.The development of loose smut (Ustilago nuda) in the Barley plant, with observations on spore formation in nature and in culture. Trans.Brit.mycol.Soc.,43,1,117-131.

12. MARTIN, A.L.(1939). Possible Cause of black kernels in Rice. Plant Dis.Rept.,23,5,83-84.

13. MORGAN, D.J.(1960). A quick method of preparing barley embryos for loose smut examination. Phytopathology, 50,270-272.

14. MORGAN, D.J.(1961). Trypan blue and boiling lactophenol for staining and clearing barley tissues infected with Ustilago nuda. Phytopathology,52,27-29.

15. PADMANABHAN, S.Y.(1957), The relation between loss in viability and seed-borne microflora in rice. Proc. Indian Acad.Sci.sect.B., 46, 3,155-169.

16. PAMUK, G.N. & GANGULY,D.(1945). Stackburn disease of rice in Bengal. Curr.Sci.,14,12,328.

17. POPP,W.(1951). Infection of seeds and seedlings of wheat and barley in relation to development of loose smut. Phytopathology, 41,261-275.

18. POPP,W.(1958). A new procedure for embryo examination for Mycelium of Smut fungi. Phytopathology, 49,75-77.

19. POPP,W.(1959). A new approach for predicting loose smut of wheat in adult plants. Phytopathology, 49,75-77.

20. RUSSEL, R.C.(1950). The whole embryo Method of testing barley for loose smut routine test. Sci.Agri.,30,8,361-366.

21. RUSSEL, R.C. & POPP,W.(1951). The embryo test as a method for forecasting loose smut infection in barley. Sci.Agri.,31,12,559-565.

22. SIMMONDS, P.M.(1946). Detection of loose smut fungi in embryo of barley and wheat. Sci.Agri.,26,2,51-56.

23. SKOVORTZOFF, V.P.(1937). A simple method of detecting hyphae of loose smut in wheat grains. Pl.Prot., Leningr., 1937,15,90-91.

24. SURYANARAYANA,D. RAM NATH, & SHASHI PRABHA LAL(1963). Seed-borne infection of Stackburn disease of rice - its extent and control. Indian Phytopath. 16(2) 232-233.

25. TISDALE, W.T.(1922). Seedling blight and Stackburn of rice and the hot water treatment.U.S.Dept. of Agric.Bull.1116,11.

26. TULLIS, E.C.(1936). Fungi isolated from discoloured rice kernels. J.ch.Bull U.S.Dep.Agric.540,11.

27. TULLIS, E.C.(1940). Disease of rice. Frns'Bull.U.S.Dep.Agric.1854,17.

Fig.5. Growth of the parent strain of Trichoconis padwickii on potato dextrose agar medium.

Fig.6. Growth of the saltant strain of Trichoconis padwickii on potato dextrose agar.

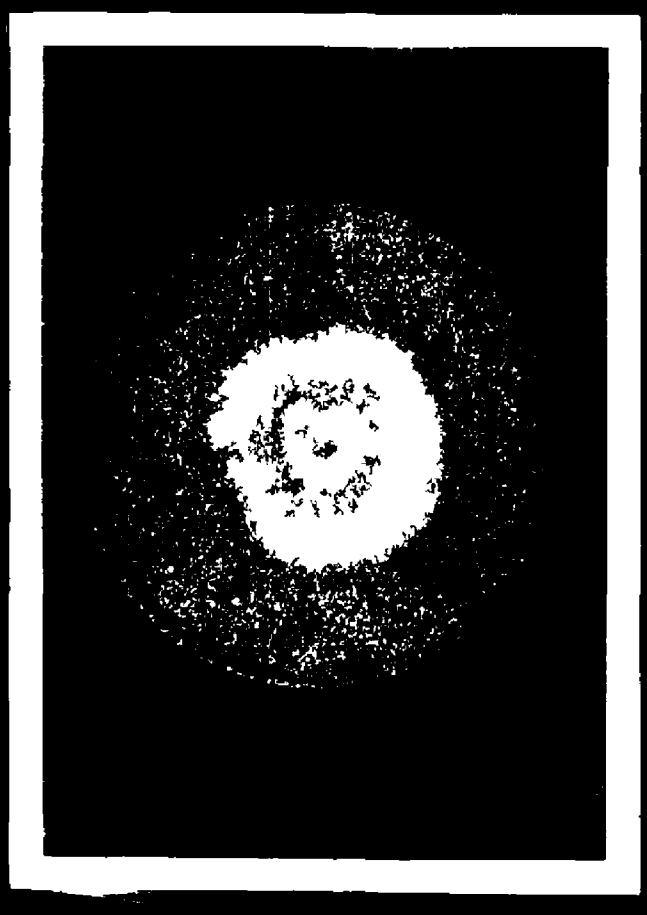


Fig.7. Growth of the parent strain of *Trichoconis padwickii* on leaf extract agar.

Fig.8. Growth of the saltant strain of *Trichoconis padwickii* on leaf extract agar.

Fig.9. Growth of the parent strain of Trichocconis padwickii on grain extract agar.

Fig.10. Growth of the saltant strain of Trichocconis padwickii on grain extract agar.

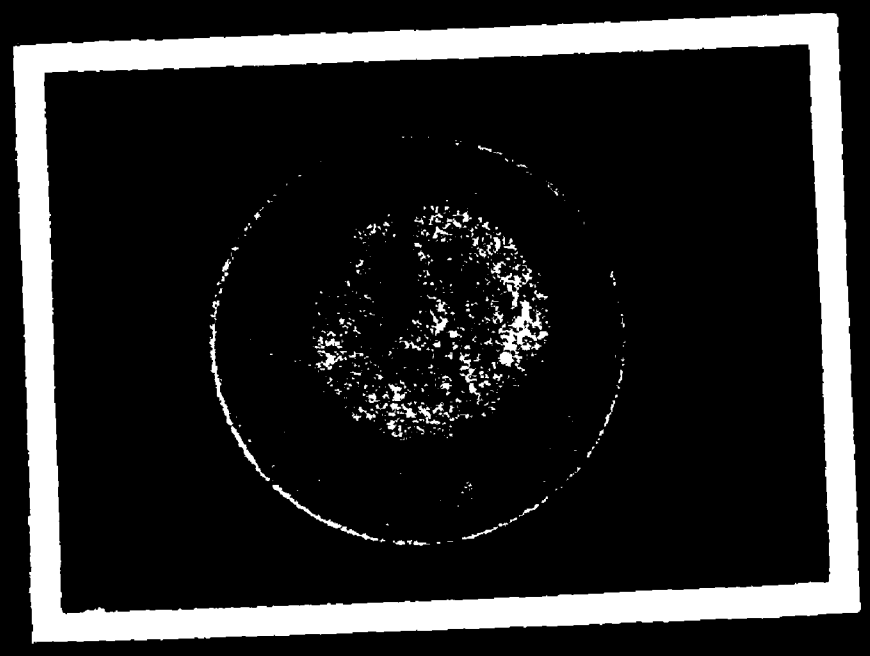


Fig.11. Growth of the parent strain of Trichocconis padwickii on oat-meal agar with yeast tablets.

Fig.12. Growth of the saltant strain of Trichocconis padwickii on oat-meal agar with yeast tablets.