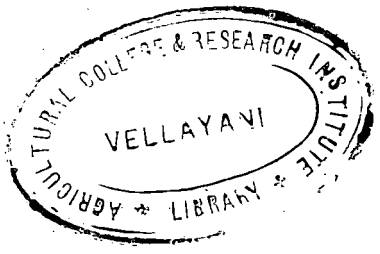
STUDIES ON THE HELMINTHOSPORIUM DISEASE OF RICE

1. DETECTION OF MYCELIUM OF THE FUNGUS IN DIFFERENT TISSUES OF THE SEED

2. OBSERVATIONS ON A SALTANT OF *Helminthosporium oryzae*.



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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 M.R. Krishnankutty Nair, 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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Fig. 1 Helminthosporium disease of rice. Infection on grains, leaf and sheath

INTRODUCTION.

The disease of rice caused by Cochliobolus miyabeanus Drechsler ex Dastur (Helminthosporium oryzae Breda de haan) is widespread in its occurrence and on account of its importance it has been studied extensively. This disease is known under the names"Helminthosporiose","Helminthosporium disease", "Brown-spot" and "Sesame leaf-spot". The crop is susceptible to attack by the organism at all stages of its growth. It can infect the germinating seedling, the growing and mature plants, the inflorescence and the grains.

In India, the disease causes serious loss in yield when it breaks out in an epiphytotic form. In 1922,there was a serious outbreak of this disease in the Godavari and Krishna delta resulting in heavy loss to the crop. The Bengal famine of 1942 was attributed to the incidence of this disease.

Earlier workers have established that the fungus is internally and externally seed-borne. But there is no clear indication as to the disposition of the mycelium in the different tissues of the seed especially whether it is present in the embryo. This is perhaps due to the unavailability to earlier workers of suitable techniques for the separation of the different tissues of the rice seeds for examination.

Abi Cheeran (unpublished 1963) has found that the techniques described by Popp (1958) and Morton (1960) in a modified form are applicable for the separation of the whole embryo of rice seed. Hence with the help of this technique and attempt was made to separate the embryos of infected seeds and examine them for the presence of fungus mycelium. Side by side with this, germinating embryos and other tissues of the infected grains were separated and cultured on nutrient medium to isolate the fungi present in the various tissues.

One of the measures recommended for the control of this disease is treatment of the seeds with organo-mercuric fungicides. This treatment is considered to improve germination and also reduce the incidence of the disease in the young seedlings. This recommendation is strictly insisted on by the Department of Agriculture of Kerala State. A study of this aspect, which is only a mere repetition of the earlier work, was also therefore undertaken since it was thought that this may not be out of place in a work which is mainly concerned with the study of the seed-borne nature of the disease.

During the course of the investigation a profusely sporulating saltant strain of H.oryzae was obtained and this is described separately.

In the present work the name Helminthosporium oryzae is used throughout in preference to the name of the perfect stage Cochliobolus miyabeanus for the sake of convenience.

REVIEW OF LITERATURE

Seed-borne nature.

It is now well recognised that Helminthosporium oryzae, the cause of the "Helminthosporium disease" on rice is seed-borne. Even as early as 1923 Tucker has pointed out that the fungus is so deep-seated in the grains that even polishing fails to remove the spots on the grains. Ocfemia (1924) reported that H.oryzae over-winters as dormant mycelium on the lemma and the palea and on the outer surface of the ovary wall, being attached in these situations by star shaped appressoria. Later Ito and Kuribayashi (1927) isolated H.oryzae from "rusted rice seeds" (in those having brownish or spotted surface) and matured perithecia developed from cultures from grains, pericarp and seeds. Suzuki (1930) observed that rice kernels internally infected by Ophiobolus miyabeanus (the name given to the perfect stage of the fungus at that time) showed a visible discolouration ranging from a small spot or streak to a large area covering the whole surface. He found that the pathogen could remain viable inside seeds for four years. Also he found that O.miyabeanus can infect young seedlings shortly after germination in spring and thus internally infected grains gave rise to infected seedlings. Ito (1932) observed that numerous

conidia (H.oryzae) and hyphae of Ophiobolus miyabeanus occur in and on the seeds collected in the diseased fields. Affected seeds may be entirely covered with a blackish growth but in relatively mild cases the hulls often show the rust coloured spots, a symptom readily overlooked, so that diseased seeds are frequently sown with healthy ones. Indoors the conidia and hyphae of O.miyabeanus persisted for about 2 and 3 years respectively. He noted that blighted seedlings developed from "rust paddy" seeds.

Tullis (1936) also attributed the light brownish tinge produced on rice kernels to infection by H.oryzae. When Martin (1939) made isolations from 1200 affected kernels sterilized externally with mercuric chloride solution (1:1000), the fungi frequently obtained were H.oryzae, Fusarium sp., Trichoconis caudata, and Nigrospora sp. It has been established by Thomas (1940) that seed and soil serve as a sources of infection of Ophiobolus miyabeanus, the incidence of disease contracted from either separately ranged from 14-20% and that derived from both simultaneously from 25 to 33%. Douglas and Ryker (1939-40) have observed that O.miyabeanus besides causing kernel peckiness, attacks glumes, which constituted a potential source of inoculum in plants used for seed. Later Nisikado and Nakayama (1943) observed that the infection was confined to the layers composed of seed coat and pericarp.

Padmanabhan et.al. (1948) found that the spots of the grains are frequently very deep as the mycelium penetrates the palea and lemmae. Subsequently Padmanabhan (1949) isolated O.miyabeanus from the interior of externally healthy surface sterilized seeds. Between 81.5 and 91% of all the grains harboured as internal pathogen. Later Hingorani and Prasad (1951) and also Merny (1957) isolated the fungus from infected kernels. Padmanabhan et.al. (1957) studied the viability of the rice seedling blight pathogen Cochliobolus (Ophiobolus) miyabeanus in India during the period between the end of growing season (December) and the beginning of the next season (June to July) and its pathogenicity under the conditions prevailing during the sowing season. It was found that from April to July the fungus retained its viability in five varieties of seeds stored at relative humidities below 90%.

Germination of infected seeds.

The view, that infection by H.oryzae reduces the germination percentage of rice seed has been expressed by many workers. Tucker (1923) reported that many of the seeds from diseased plants germinated poorly and were covered with mycelium. More than half the infected seedlings showed symptoms both on roots and coleoptile. Of the 207 infected seedlings 176 died before reaching a height of 6 inches. Secondary infections are believed to occur on leaves and

heads from the primary seedling infections which resulted from sowing diseased seeds. Disease free seeds planted in an isolated position in the field produced apparently healthy seedlings, which, however, developed the symptoms of the disease when they were 6 to 10 inches high. Ocfemia (1924) also believed that the disease is primarily a seedbed trouble arising from the planting of infected seeds. Nagai and Hara (1930) found that the seeds from diseased plant gave rise to exclusively diseased progeny. Cralley and Tullis (1937) studying the extent of seedling blight that may develop in a crop, found that it depends on weather conditions and the microflora of the rice seed and soil. His isolations from diseased seedlings gave most consistantly species of Fusarium (of which two most frequently isolated closely resembled Gibberella moniliformis and G.fujikuroi), a Rhizoctonia closely resembling R.(Corticium) solani, Curvularia lunata and H.oryzae (O.miyabeanus) all of which produced blighting of rice seedlings.

Thomas (1941) determined the influence of infected seed and soil, separately and combined, on the development of the disease. He found that infected seeds gave rise to 24.6 and 22.3 per cent disease in sterilized sand and soil, respectively while infected seeds in infected sand and soil gave rise to 31.9 and 26% infected plants respectively.

The incidence of the disease in the control plots (soil only) was 5.2%. He therefore concludes from these data that both soil and seed can serve as sources of infection. He also found that blight would assume a severe character in cold weather. Regarding the failure of germination of the infected seeds, Padwick and Ganguly (1945) found that out of 40 rice seeds of normal or discoloured appearance sown in Roux tubes on cotton soaked in distilled water 21 failed to germinate and of these 6 were found contaminated with H.oryzae (Ophiobolus miyabeanus). Orsinigo (1956, 1957) demonstrated that in culture H.oryzae liberates toxins which reduce the germinability of rice seeds and produce abnormalities in the seedlings. He was also successful in extracting and purifying the toxin which he named as Cochliobolin.

Seed treatment.

There are many reports about the usefulness of seed treatment with chemicals and hot water in controlling "Helminthosporium disease" of rice and increasing the germination of seeds. Of the chemicals suggested, mercuric compounds occupy the primary place. Nisikado and Miyake (1922) found mercuric chloride effective for controlling Helminthosporium disease. Subsequently they (1928) found that treatment with comparatively dilute solutions of

Upsulun (1 in 800 to 1200) for 48 hours at about 20°C. was also effective. Ito (1932) using mercuric chloride found that the conidia of H.oryzae died at 0.1% concentration. Later, Cralley and Tullis (1937) reported that experimental seed treatments with ethyl mercury phosphate and ethyl mercuric chloride gave consistent results. Mallamire (1949) suggested seed disinfection in 0.03% mercuric chloride for 24 hours and steeping 100 kgs. of paddy for 15 minutes in a solution of 50 gms. of ceresan in 10 litres of water for the control of the disease. Juillat and Tarquois (1950) found that the best effective fungicide on germination for rice grain was treatment by mercoran (a dust containing methoxy-ethyl mercuric silicate) which increased germination by 4 to 5% at 25° to 27°C. and over 25% at 14° to 18°C. Afterwards, Hashioka (1952) observed that slurry disinfection of rice seeds with ceresan (0.2% of seed weight) is almost as effective against the brown spot caused by C.miyabeanus as immersion in Upsulun, while arasan slurry is very inferior both in fungitoxicity and effect of seedling growth. Later it was reported from the Central Rice Research Institute, Cuttack (Anon. 1954) that the only effect of fungicidal treatment with agrosan GN. was in the improvement of germination of both healthy and spotted seeds. Padwick (1956) found that immersion of seed for 12 hours in 0.5%

Upsulun gave good control. In the same year Hashioka et.al. showed that a dust composed of 0.4% phenyl mercuric acetate plus slaked lime (ceresan lime) was the most effective for Helminthosporium disease control.

Regarding hot water treatment, Tisdale as early as 1922 has suggested that the best method for controlling seedling blight on rice, was the immersion of seed in hot water (54°C.) for 15 minutes, preceded by pre-soaking in tepid water for about 16 hours. Hot water prevents the development of fungi in the seed. Since then many workers have suggested hot water treatment as an effective control for the disease, but there are slight variations in the temperature and duration of the treatment recommended.

Ito (1932) recommended immersion in hot water at 54°C. for 5 minutes, while Saha (1946) recommended the immersion at this temperature for an long a period as 4 hours. Chowdhury (1948) at the same time recommended the immersion of the seeds at the above temperature for 10 to 12 minutes while Barat (1959) recommended a slightly longer period of 20 minutes. Bernal Correa (1940) suggested temperature ranging from 53°C. to 60°C. for varying periods and he found that treatment at 55°C. for 5 minutes reduced infection from 25 to 1%. Thomas (1941), on the other hand, recommended a temperature of 55°C. for 10 minutes.

Separation of embryo.

Only few reports are available regarding the detection of fungus mycelium in the whole embryos by chemical processing. Skvortzoff (1937) separated embryos of wheat and stained for detecting the presence of mycelium of the loose smut fungus. Subsequently, 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 indexes. Later, Popp (1951, 1958 and 1959) described an improved method for detecting loose smut mycelium in wheat and barley whole embryos. Morton (1960) suggested a quick method for preparing barley embryos for loose smut examination and by using this method Malik 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 embryo. Abi Cheeran (unpublished 1963) detected the presence of Trichoconis padwickii Ganguly in the embryo of rice by effecting suitable modifications of the techniques adopted by Popp (1958) and Morton (1960).

MATERIALS AND METHODS.

1. Seeds used.

PTB.23, PTB.31 and local Kochuvithu were the varieties of rice used for the present studies.

To determine the disposition of the mycelium of Helminthosporium oryzae in the grains, infected seeds of the above varieties were collected during 1964 from diseased fields at Kayamkulam in Alleppey District. Affected earheads were selected individually and the fully filled grains were dried in the shade and stored under dry conditions in the laboratory. For comparative purposes seeds of the above three varieties were collected from apparently disease-free fields also. Thus PTB.23 and PTB.31 seeds were collected from Pattambi and Kochuvithu from Alleppey.

The percentage infection of the rice seeds used for the study was determined by incubating samples of the seeds in moist chamber and also by culturing surface sterilized whole grains in potato dextrose agar. The seeds collected from diseased fields showed a very high percentage of infection. At the same time the seeds collected from apparently healthy fields also showed slight infection,

ranging from 3.25 to 3.75%. In the absence of completely disease-free samples, the above samples were used as control for purposes of comparison with the diseased sample. These samples are designated through out this work as control samples.

2. Separation of the tissues of the seed for examination.

a. Embryo.

The embryos of the seeds were separated by chemical processing. 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 (unpublished 1963) 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 gm.
Sodium silicate (Commercial liquid glass)	84 gm.
Teepol (detergent)	few drops.

The kernels were vigorously boiled in the extraction solution with occasional 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% solution of sodium silicate taken in centrifuge tubes and were centrifuged for 2 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 (concentrated)	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 5 lbs. pressure for an hour in an aqueous solution containing 15%

sodium hydroxide and 12% 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 5 lbs. pressure for one to two minutes in a 3:1 mixture of rectified spirit and glacial acetic acid. Finally they were heated at 5 lbs. 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 lbs. 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% lactic acid and heated for one minute at 5 lbs. pressure to remove the excess stain. The embryos were then arranged on 3" x 1" microscopic slides in rows with the help of a zero point camel hair brush.

b. Bran and Endosperm.

While the embryos were separated by the above technique the endosperms disintegrated and the bran layers got broken off into small bits. Hence modifications of the technique were attempted, but were not successful. It was, therefore, possible only to process the bits of these tissues

and examine them just for the presence of mycelium.

3. Isolation of fungi from different tissues of the seed.

In order to confirm the results obtained by chemical processing, an attempt was made to isolate and bring into culture, the organisms present in different tissues of the seed. Potato dextrose agar and Oat-meal agar media of the following composition were used for the purpose.

Potato dextrose agar.

Potato	200.00 gm.
Dextrose	20.00 gm.
Agar agar	20.00 gm.
Distilled water	1000.00 ml.

Oat-meal agar.

Oats	50.00 gm.
Agar agar	20.00 gm.
Distilled water	1000.00 ml.

a. Glumes.

The glumes were separated from the grains, washed and surface sterilized with 1:1000 mercuric chloride solution with one or two drops of a detergent for 45 seconds, and then washed in 4 changes of sterile water and finally transferred to the medium with a sterile forceps and incubated for 5 days at room temperature.

b. Separated plumule and radicle.

It was not possible to separate the embryo in the seed. The seeds were therefore allowed to germinate slowly and the radicle and plumule were separated. For the slow germination of the seed, the method described by Paul Neergaard and Adib Saad (1962) was followed.

Dehusked grains were soaked in water for about 40 minutes and washed with distilled water. The kernels were then surface sterilized using 1: 1000 mercuric chloride solution for 45 seconds and washed in 4 changes of sterile water, 15 minutes each. Finally the kernels were aseptically transferred to a sterile humid chamber as described below.

Filter papers of 9 cm. diameter were placed in Petri dishes of 10 cm. diameter and sterilized in hot air oven for 45 minutes at 160°C. The filter papers in the sterilized Petri dishes were moistened with 2,4-D solution of 0.1% strength. The kernels in the humid chamber were kept for 2 days. After that the germinated embryos (plumule and radicle) were dissected out carefully under the Steriomicroscope with fine pointed needle. Care was taken to remove most of the embryo tissues from the endosperm and also not to allow any part of the bran or endosperm to adhere on the embryo.

The separated embryos (plumule and radicle) were surface sterilised with 1:1000 mercuric chloride for 15 seconds, washed in 4 changes of sterile water for 15 minutes each. Embryos (plumule and radicle) were then transferred by a sterile forceps to the medium and incubated for 5 days at room temperature and then examined.

c. Bran and endosperm.

After separating the embryo one third of the kernels at the embryo end was cut and removed in order to make sure that no part of the embryo adheres. The remaining part of the endosperm with the bran was washed well in sterile water, surface sterilized and cultured as in the case of plumule and radicle.

d. Endosperm.

For the isolation of fungi from the endosperm, the grains were first dehusked carefully by hand and the bran layer was completely scraped off with a scalpel. One third of the kernel with the embryo was cut and removed. The remaining part of the endosperm was then surface sterilized and cultured.

4. Germination trials.

The percentage germination of infected and control seeds was tested in Petri dishes and also in pots of 6"diameter.

Seeds were sown at different depths of 0.5", 1", 1.5" and 2" in pots. Ten seeds were sown in each pot. When the seeds germinated, the non-germinated seeds were dug out, washed in sterile water and kept in moist chamber. After three days these seeds were examined for the presence of Helminthosporium oryzae. The seedlings were kept under observation upto the fourth leaf stage to note the number of seedlings infected.

5. Seed treatment.

Infected seeds were treated with different organo-mercuric compounds and also hot water to determine their comparative effectiveness in improving germination and reducing the incidence of the disease in the young seedlings.

Three hundred seeds each of PTB.23, PTB.31 and Kochuvithu, both infected and control, were used for every treatment. The mercuric fungicides used were agrosan GN. (1.5% mercury), Tafasan (6% mercury), ceresan (2.5% mercury). In the case of hot water treatment the seeds were pre-soaked in cold water for 16 hours and then dipped in hot water maintained at 55°C. for 5 minutes.

The treated seeds were divided into two lots. One lot was sown in 6" pots at the rate of 10 seeds per pot.

The other lot was placed in Petri dishes. Seeds were sown immediately after the treatment. Observations were taken upto fourth leaf stage in the pots. In all the cases, untreated seeds served as control.

RESULTS.

1. Percentage infection of the seeds used.

In the humid chamber 60.75 to 63.25% of the seeds collected from the diseased fields and 0.5 to 0.75% of the seeds collected from apparently disease-free fields yielded Helminthosporium oryzae.

When surface sterilized seeds were cultured on potato dextrose agar, a higher percentage of seeds were found infected. The percentage infection in the diseased and control samples were 66.5 to 71.5% and 3.25 to 3.75% respectively (Table I).

Other fungi like Curvularia sp., Rhizopus sp., Penicillium sp., Aspergillus sp., and Nigrospora sp. were also found on a number of grains.

2. Disposition of the mycelium in the different tissues of the seed.

a. Glumes.

On culturing, 66.75 to 70.25% of the glumes of infected grains yielded Helminthosporium oryzae either alone or in association with other fungi. In the sample selected as control 2.25 to 3.25% of the glumes yielded Helminthosporium oryzae (Table II).

Table I. Percentage infection by Helminthosporium oryzae noted on seeds in humid chamber and in culture

Variety.		No.of seeds used.	Seeds with Helminthosporium oryzae(%)	
			In humid chamber.	In culture.
PTB.23	Control	400	0.75	3.75
	Infected	400	60.75	71.50
PTB.31	Control	400	0.50	3.25
	Infected	400	63.25	68.75
Kochuvithu	Control	400	0.75	3.25
	Infected	400	62.25	66.50

Table II. Percentage infection of the glumes by Helminthosporium oryzae and other fungi in culture

Variety.		No.of glumes used.	Glumes with H.oryzae %	Glumes with other fungi %	Total of glumes with fungi %	Fungus-free glumes %
PTB.23	Control	400	3.25	35.50	38.75	61.25
	Infected	400	70.25	17.75	88.00	12.00
PTB.31	Control	400	2.25	30.50	32.75	67.25
	Infected	400	69.25	17.75	87.0	13.00
Kochuvithu	Control	400	2.25	31.50	33.75	68.25
	Infected	400	66.75	16.75	87.00	13.00

b. Embryo.

The mycelium of Helminthosporium oryzae was not present in the embryos of rice seed examined directly and by culturing.

Of the 4073 embryos of the infected seeds and 4070 embryos of the control seeds processed chemically and examined, only one showed the presence of mycelium. During chemical processing of the seeds, about 87.66 to 94% of the embryos were recovered. One embryo with mycelium was noted in the infected PTB.31 variety. It is possible that the mycelium belonged to some other fungi.

The results of culturing the embryos also confirm the above finding. Of the 2178 embryos cultured none showed the presence of Helminthosporium oryzae. It is therefore to be inferred that Helminthosporium oryzae is not carried through the embryo of the rice seeds.

c. Bran and endosperm.

During chemical processing the bran and endosperm could not be separated intact. But fungus mycelium was observed in the bits of bran and endosperm which remained after separation of embryos. (Fig. 2-7).

The kernels of the diseased seeds after removing the embryos, when cultured showed 62.25 to 63.75% infection

by Helminthosporium oryzae and 6.5 to 8.75% infection with other fungi. The kernels of the seeds used as control showed 1.75 to 2.75% infection by Helminthosporium oryzae and 16.25 to 20% infection by other fungi. The other fungi obtained in culture were Curvularia sp., Rhizopus sp., Aspergillus sp., and Penicillium sp.(Table III).

The infection noted could be due to the presence of the fungus in the endosperm or bran layer or both.

Endosperm without bran.

Twenty-one per cent of the endosperms of infected seeds yielded Helminthosporium oryzae alone or in association with other fungi and 2 to 3% yielded other fungi in culture. In the case of the sample taken as control, the endosperms did not yield Helminthosporium oryzae but 6.67 to 8.0% showed infection by other fungi. (Table IV). This indicates that the mild infection by Helminthosporium oryzae noted in the seed used as control was confined to the glumes and bran layers.

While removing the bran, the superficial layers of the endosperm might also have been removed. Similarly in some seeds at least, traces of bran layers might have been left. These would have affected the results.

Table III. Percentage infection of endosperms with bran by Helminthosporium oryzae and other fungi in culture.

Variety.		No.of endosperms with bran used.	Endosperms and bran with H.oryzae alone %	Endosperms and bran with H.oryzae and other fungi %	Endosperms and bran with other fungi alone %
PTB.23	Control	400	0.75	1.00	18.00
	Infected	400	55.50	6.75	8.75
PTB.31	Control	400	1.00	1.75	20.00
	Infected	400	59.75	3.50	6.50
Kochuvithu	Control	400	1.25	1.00	16.25
	Infected	400	58.25	5.50	8.00

Table IV. Percentage infection of the endosperms without bran by Helminthosporium oryzae and other fungi noted in culture.

Variety.		No.of endosperms used.	Endosperms with H.oryzae %	Endosperms with other fungi %	Total of endosperms with fungi %	Endosperms free from fungi %
PTB.23	Control	300	..	6.67	6.67	93.33
	Infected	300	21.00	2.00	23.00	77.00
PTB.31	Control	300	..	7.67	7.67	92.33
	Infected	300	21.00	3.00	24.00	76.00
Kochuvithu	Control	300	..	8.00	8.00	92.00
	Infected	300	21.00	3.00	24.00	76.00

The results show that the mycelium of Helminthosporium oryzae is present in the glumes, in the bran layers and also in the endosperm. The glumes showed the highest percentage infection, nearly 70%. The bran and the endosperm together showed upto 63.75% infection while the endosperm showed only 21% infection.

3. Germination trials.

The percentage germination of infected seeds was lower than that of the control seeds in moist chamber and also in pots. The percentage germination in the Petri dishes was, however, slightly higher than that in the pots.

a. Germination of seeds in moist chamber.

Infected seeds in the moist chamber gave only 82 to 84% germination. Helminthosporium oryzae was predominent in the non-germinated seeds. Control seeds gave 97 to 99% germination (Table V).

b. Germination in pots at different depths.

Seeds sown at 0.5" depth gave maximum number of plants emerging above the soil level and those at 2" depth showed minimum number of plants emerging above the soil level. In the infected seeds the rate of emergence decreased

Table V. Percentage germination of seeds in moist chamber*

Variety.		Germination. %	Germinated seeds with H.oryzae %	Non-germinated seeds with H.oryzae %
PTB.23	Control	98.33	..	0.33
	Infected	83.33	55.33	15.00
PTB.31	Control	99.00	0.33	0.66
	Infected	84.33	57.00	15.67
Kochuvithu	Control	97.67	0.66	0.66
	Infected	82.67	50.66	15.66

* Three hundred seeds of each variety were used for germination.

Table VI. Consolidated statement of the results

Variety.		Depth of sowing in inches.	Non germinated seeds			Total germinated seeds.
			Total non-germinated seeds.	H.oryzae with other fungi.	Other fungi alone	
			%	%	%	%
1		2	3	4	5	6
PTB.23	Control	0.5	3.00	..	3.00	97.00
		1.0	2.50	..	2.50	97.50
		1.5	3.00	..	3.00	97.00
		2.0	2.75	..	2.75	97.25
	Infected	0.5	12.50	11.25	1.25	87.50
		1.0	12.00	11.00	1.00	88.00
		1.5	13.00	11.50	1.50	87.00
		2.0	14.25	12.25	2.00	85.75
PTB.31	Control	0.5	2.25	..	2.25	97.75
		1.0	1.75	..	1.75	98.25
		1.5	1.75	..	1.75	98.25
		2.0	2.00	..	2.00	98.00
	Infected	0.5	11.75	10.00	1.75	88.25
		1.0	11.50	9.75	1.75	88.50
		1.5	13.00	11.75	1.25	87.00
		2.0	14.00	12.25	1.75	86.00
Kochuvithu		0.5	2.25	..	2.25	97.75
		1.0	2.00	..	2.00	98.00
	Control	1.5	1.75	..	1.75	98.25
		2.0	2.25	..	2.25	97.75
	Infected	0.5	10.50	9.50	1.00	89.50
		1.0	11.00	9.25	1.75	89.00
		1.5	13.00	12.25	0.75	87.00
		2.0	13.75	12.50	1.25	86.25

For sowing, 400 seeds from control and

from 78.5 to 64.75% when the depth increased from 0.5 to 2". The failure of emergence was partly due to pre-emergence blight. Pre-emergence blight was lower at 0.5" depth and greater at increasing depths. A high percentage of non-germinated seeds, when examined, showed the presence of H.oryzae. Similarly a high percentage of blighted plants, which were killed at the time of emergence or killed within four days after emergence, showed the presence of H.oryzae in the case of infected seeds. The seeds with pre-emergence blight showed the presence of H.oryzae predominently in association with other fungi like Fusarium sp., Rhizopus sp.,Penicillium sp., Aspergillus sp., and Curvularia sp. The percentage of seedlings which ultimately survived in the case of infected seeds sown at 0.5", 1", 1.5" and 2" depths were 65.25 to 70%, 66.5 to 68.75%, 64.5 to 66% and 63.5 to 64.25% respectively. The difference is thus not pronounced. At 0.5" depth, the post-emergence blight was greater whereas at increasing depths the pre-emergence blight was greater. (Table VI). In the case of control seeds, the depth of sowing did not practically exert any influence on the percentage germination.

The seedlings from the diseased and control seeds were kept under observation to note the leaf infection,

of germination studies at different depths.

Germinated seeds						Final standing seedlings
Pre-emergence blight			Post-emergence blight			
H.oryzae with other fungi	Other fungi alone	Total Pre-emergence blight	H.oryzae with other fungi	Other fungi alone	Total post-emergence blight	
%	%	%	%	%	%	%
7	8	9	10	11	12	13
..	1.25	1.25	..	0.25	0.25	95.50
..	1.75	1.75	..	0.75	0.75	95.00
..	1.75	1.75	..	..	..	95.25
..	2.75	2.75	..	..	..	94.50
8.25	1.25	9.50	10.25	0.25	10.50	67.50
10.75	1.50	12.25	7.00	..	7.00	68.75
16.25	1.00	17.25	3.50	0.25	3.75	66.00
19.50	0.75	20.25	1.25	..	1.25	64.25
..	0.75	0.75	..	0.25	0.25	96.75
..	1.50	1.50	..	0.50	0.50	96.25
..	3.25	3.25	..	0.50	0.50	94.50
..	3.50	3.50	..	..	..	94.50
8.75	1.75	10.50	12.00	0.50	12.50	65.25
10.75	1.25	12.00	10.00	..	10.00	66.50
18.50	1.25	19.75	2.25	0.50	2.75	64.50
20.25	1.00	21.25	1.25	..	1.25	63.50
..	2.25	2.25	..	..	..	95.50
..	2.50	2.50	..	1.25	1.25	94.25
..	3.25	3.25	..	0.25	0.25	94.75
..	3.75	3.75	..	..	..	94.00
9.75	1.25	11.00	8.25	0.25	8.50	70.00
10.00	2.00	12.00	8.00	0.25	8.25	68.75
16.00	1.50	17.50	3.50	..	3.50	66.00
18.25	2.00	20.25	2.50	0.25	2.75	63.50

infected lots were used in each depth.

if there is any. 10 to 13% of the plants from the infected seeds showed Helminthosporium spots on the first leaves. The spots appeared only after the leaves were fully unfolded. During 2nd, 3rd and 4th leaf stages a much larger number of plants showed leaf spots. In the control lot, infection was not noted on the first leaf. But infection was noted during 2nd, 3rd and 4th leaf stages (Table VII). The infection might have occurred from external sources. All the plants were kept at one place exposed and were, therefore, liable to natural infection. It is possible that the plants which showed spots on the first leaf might have contracted the infection from outside, quite likely from spores produced and liberated from infected seeds in the pots. Since there were no spots on the first leaves of the control plants, infection in the subsequent leaves in the seedling from the diseased seeds and also from the control seeds, might have spread from the initial infection in the first leaf. Hence the results can be taken to show only that the sowing of infected seeds can start infection.

14. Seed treatment.

Infected seeds when treated with mercuric fungicides and hot water gave a higher percentage of germination in pots and in Petri dishes. While untreated seeds gave a germination of only 82.33 to 85.33% the seeds treated with

Table VII. Percentage infection of seedlings at different stages of growth, by Helminthosporium oryzae.

Variety.		No.of seed-lings used.	Plants infected (%)			
			1st leaf stage	2nd leaf stage	3rd leaf stage	4th leaf stage
PTB.23	Control	375	..	1.06	4.26	6.93
		380	..	1.81	4.46	6.57
		375	..	1.86	4.26	5.86
		370	..	1.35	5.24	15.94
	Infected	282	13.83	22.70	30.14	36.62
		272	13.60	22.06	30.05	36.76
		261	13.41	21.46	31.04	38.70
		245	13.47	22.04	32.25	41.63
PTB.31	Control	376	..	0.53	3.48	5.32
		378	..	1.59	3.99	6.65
		372	..	1.85	3.71	6.20
		366	..	2.70	4.37	6.56
	Infected	281	13.45	20.28	28.50	34.88
		274	13.14	20.44	30.29	36.50
		262	13.36	23.66	30.42	40.07
		254	13.00	22.05	30.31	40.94
Kochuvithu	Control	371	..	2.15	4.85	7.00
		373	..	1.87	4.56	7.48
		375	..	1.87	4.83	8.27
		376	..	1.60	5.32	8.53
	Infected	280	14.29	19.64	27.86	35.36
		275	13.50	18.91	29.09	37.09
		264	14.01	23.10	29.13	36.36
		250	13.20	22.80	31.20	39.20

mercuric fungicides gave upto 90% germination and those treated with hot water gave upto 93% germination in Petri dishes (Table VIII).

In pots the germination was 82.0 to 84.64% and 88 to 89% for mercuric fungicides and for hot water respectively as against 75.33 to 77.66% for untreated seeds. (Table IX).

In the case of the sample of seeds which served as control, the improvement in the germination as a result of seed treatment was only very negligible.

Effect of seed treatment on disease control.

Treating infected seeds with organo-mercuric compounds and also with hot water helped to reduce the primary infection in the young seedlings. Seedlings from infected untreated seeds showed higher percentage of infection and those from treated seeds gave much lesser infection. While the percentage infection of seedlings from the infected untreated seeds was 12.9 to 14.4 at the first leaf stage, that in the seeds treated with organo-mercuric fungicides was only 3.8 to 4.8. The infection percentage, however, was as low as 0.6 to 1.3 in the case of hot water treatment.(Table X) The results show that the hot water treatment is better than organo-mercuric compounds in reducing the primary infection in the young seedling.

Table VIII. Percentage germination of seeds treated with various proprietory mercuric fungicides and hot water in Petri dish.

Variety.		No.of seeds used.	Germination of untreated seeds. %	* Germination of seeds treated with agrosan GN. %	** Germination of seeds treated with tafasan %	*** Germination of seeds treated with ceresan %	**** Germination of seeds treated with hot water %
PTB. 23	Control	300	97.67	98.33	98.00	96.67	98.67
	Infected	300	85.00	90.33	90.33	89.33	92.67
PTB.31	Control	300	98.00	98.33	98.33	97.33	98.33
	Infected	300	85.33	90.00	89.33	89.67	93.00
Koohuvithu	Control	300	96.33	96.33	96.67	96.33	97.00
	Infected	300	82.33	88.67	87.67	89.00	91.33

* Mixing thoroughly one part of agrosan GN. (1% mercury) with 400 parts of seed by weight.
** Dipping 100 gm. of seed in a solution of 0.1 gm. tafasan (6% mercury) in 100 ml.of water for 30 minutes.
*** Dipping 100 gm.of seed in 98 ml.of 0.1% ceresan wet (2.5% mercury) solution for 30 minutes.
**** Pre-soaking the seeds in cold water for 16 hours and then dipping the seeds in hot water maintained at 55°C. for 5 minutes.

Table IX. Percentage germination of seeds treated with various proprietory mercuric fungicides and hot water in pots.

Variety.		No.of seeds used.	Germination of seeds untreated. %	Germination* of seeds treated with agrosan GN. %	Germination** of seeds treated with tafasan %	Germination*** of seeds treated with ceresan %	Germination**** of seeds treated with hot water %
PTB.23	Control	300	95.67	95.00	95.67	96.33	96.33
	Infected	300	75.33	83.00	83.00	82.67	88.33
PTB.31	Control	300	95.33	96.00	95.67	96.00	95.33
	Infected	300	76.00	82.67	82.00	83.33	88.00
Kochuvithu	Control	300	96.00	96.33	96.67	96.00	96.33
	Infected	300	77.66	83.33	84.33	84.67	89.00

* Mixing thoroughly one part of agrosan GN. (1% mercury) with 400 parts of seed by weight.
** Dipping 100 gm. of seed in a solution of 0.1 gm. tafasan (6% mercury) in 100 ml.of water for 30 minutes.
*** Dipping 100 gm. of seed in 98 ml.of 0.1% ceresan wet (2.5% mercury) solution for 30 minutes.
**** Pre-soaking the seeds in cold water for 16 hours and then dipping the seeds in hot water maintained at 55°C. for 5 minutes.

Table X. Percentage infection noted in the seedlings raised from infected

compared with that of

Treatment	PTB.23							
			Infected plants					
	No.of seeds used.	Seeds germinated %	1st leaf stage %	2nd leaf stage %	3rd leaf stage %	4th leaf stage %	No.of seeds used.	Seeds germinated %
1	2	3	4	5	6	7	8	9
Untreated	300	75.33	14.40	20.40	27.00	35.00	300	76.00
Agrosan GN.	300	83.00	3.90	6.90	15.20	23.00	300	82.60
Tafasan	300	83.00	3.80	8.00	17.70	26.10	300	82.00
Ceresan	300	82.60	3.40	7.20	16.10	25.30	300	83.30
Hot water	300	88.33	1.00	4.10	12.40	26.40	300	88.00

rice seeds after treatment with mercuric compounds and hot water, the untreated seeds.

PTB.31						Kochuvithu.			
Infected plants.						Infected plants.			
1st leaf stage %	2nd leaf stage %	3rd leaf stage %	4th leaf stage %	No.of seeds used.	Seeds germinated %	1st leaf stage %	2nd leaf stage %	3rd leaf stage %	4th leaf stage %
10	11	12	13	14	15	16	17	18	19
12.90	19.30	29.80	36.00	300	77.60	13.30	17.60	27.40	34.30
3.00	6.45	16.80	25.00	300	83.00	4.00	7.20	16.80	24.40
4.80	7.70	15.90	27.20	300	84.30	3.90	8.30	15.02	30.04
4.20	8.00	17.20	28.40	300	84.60	3.80	7.80	17.50	23.60
1.33	4.70	14.40	25.90	300	89.00	0.60	6.00	14.98	27.00

DISCUSSION.

The seed-borne nature of Helminthosporium oryzae has been established by earlier workers like Tucker (1923) Ocfemia (1924), Ito and Kuribayashi (1927), Tullis (1936), Martin (1939), Padmanabhan et.al. (1948) and Merny (1957). Suzuki (1930) and Thomas (1940) found that infected seeds formed the source of primary infection. There is also specific mention of the presence of mycelium of the fungus in glumes (Douglas and Ryker 1940, Nakayama 1943), in bran (Ito and Kuribayashi 1927, Nakayama 1943) and in endosperm (Tucker 1923, Padmanabhan 1949). But in none of the reports there is any indication as to whether the fungus is present in the embryo of the infection seed. At the same time there are numerous reports of the low germination of the infected seeds (Tucker 1923, Padwick and Ganguly 1945), which could be improved by treating the seeds with chemicals or hot water. This claim, in the case of chemicals, naturally raises the question whether the fungus is after all deep-seated in the seed. On the other hand, the poor germination that is associated with the infected seeds points to the possibilities that the fungus could be present in the embryo also. Working on the Stackburn disease of rice, Abi Cheeran (1963) has established that the mycelium of the causal organism Trichoconis padwickii, is present in the embryo also and that this will result in

poor germination. To some extent this finding has provided the incentive for the present investigation.

As a result of the whole embryo examination and also culturing of the embryo in nutrient medium, it has been found that the mycelium of Helminthosporium oryzae is not present in the embryo. At the same time the infected seeds gave only a lower percentage of germination and Helminthosporium oryzae could be detected on the majority of the non-germinated seeds. The pre-emergence and post-emergence blights were also found to a large extent due to H.oryzae. The fungus, though absent in the embryos, is present in the glumes, bran and endosperms. It is, therefore, possible that the loss of germination may be due either to the invasion of the embryo by the fungus at the time the seeds begin to germinate or due to the inhibitory action of some metabolic products produced by the fungus. Orsinigo (1956, 1957) demonstrated that in culture Helminthosporium oryzae liberates toxins which reduce the germinability of rice seeds and produce abnormalities in the seedlings. He was also successful in extracting and purifying the toxin, which he named as Cochliobolin. However, further proof has to be adduced before concluding that some such substances are involved in inhibiting the germination of infected seeds.

About 62 to 63% seeds used in this work were found to be infected, of which 21% carried the mycelium in the endosperm. The loss of germination was 22 to 25% and this figure approximates the percentage of seeds with infected endosperms. But to what extent these two are correlated cannot be said with certainty with the existing evidence. It may be pointed out here that the method used for detecting the presence of infection of the endosperm has its draw backs. The technique followed for separating the whole embryos was found unsuitable for separating the endosperms and bran layers intact. Some of the improvements tried were also not satisfactory. Hence the determination of the percentage infection had to be made exclusively by culturing the endosperm. The method adopted for the preparation of the endosperms for culturing was also not very satisfactory and could have affected the results. Infected portions might have been lost in some cases while removing one third of the endosperm for eliminating the embryo. While scraping of the bran, portions of the outside layers of the endosperm carrying the fungus might have been removed. There is also possibility that minute traces of the infected bran layers might have remained on the endosperms, though maximum care was exercised to see that none of the above happened.

The glumes gave a higher percentage of infection than by the whole grains consisting of endosperms and bran

layers without the embryos. The endosperms gave the lowest infection. This points to the possibility that after infecting the glumes the fungus gradually penetrates to the deeper tissues though it was not able to establish itself in the embryo.

The depth of planting does not very much influence the ultimate number of seedlings that survive. In the control lot the difference is negligible. In the infected seeds, however, germination was slightly lower at 2" depth. The maximum difference in germination in the different seed samples at different depths was 6.5%. At higher depths the pre-emergence blight increased, but post-emergence blight decreased. This may be due to the fact that at higher depths the emerging seedlings take a longer time to come out, during which there was greater chance of infection.

Seedlings from control seeds showed no infection at the first leaf stage, but those from infected seeds showed 12.9 to 14.3% infection. Infection of the first leaf might have originated from the infected seeds sown. Infection in the subsequent leaves might have been continued from the infected first leaf.

The results of the treatment of diseased seeds with mercuric compounds and hot water agrees with the findings

of earlier workers like Hisikado and Miyake (1922, 1928) Tullis (1937), Bernal Correa (1940) and Padwick (1956). In either case the percentage germination was increased. All the mercuric compounds gave more or less similar results. Hot water treatment gave the highest germination. This may be because, the fungus present in the deeper tissues of the seed might have been successfully eliminated with the hot water treatment.

SUMMARY.

The presence of mycelium of Cochliobolus miyabeanus Drechsler ex Dastur (Helminthosporium oryzae Breda de haan) in the different tissues of the infected rice seed was determined by direct examination of the embryo and also by separating and culturing the different tissues. Whole embryos were separated by using the techniques initially described by Popp (1958) and Morton (1960) for the detection of mycelium in wheat and barley embryos as modified by Abi Cheeran (1963). This technique was not suitable for the separation of the other tissues of the seed.

Three varieties of rice seeds with infection ranging from 66.5 to 71.5% were used for the studies with suitable controls. The mycelium of H.oryzae was not noted in the embryos when examined directly and also when cultured. It was present only in the glumes, bran layers and endosperms. The presence of the mycelium of the fungus in these tissues was determined by culturing only. The glumes showed the highest infection namely 66.75 to 70.25%. The endosperms with the bran showed 62.25 to 63.25% infection. Endosperms alone showed only 21% infection.

The percentage of emergence of the seedlings from the infected seeds was lower than that of the control seeds.

The damage in the first instance was found to be due to the failure of the seeds to sprout. After sprouting, some of the seedlings were destroyed by pre-emergence and post-emergence blights. Planting the seeds at different depths of 0.5" to 2" showed that pre-emergence blight was lower at lower depths and greater at increasing depths.

Seedlings from infected seeds showed 13.45 to 14.29% infection at first leafstage, indicating thereby that primary infection can result from sowing infected seeds.

The percentage germination of the infected seeds was increased by treatments with organo-mercuric fungicides and with hot water. All the organo-mercuric fungicides gave more or less similar results. In pots the germination of treated seeds was 82.0 to 84.64% as against 75.33 to 77.66% for untreated infected seeds. Hot water treatment gave the highest percentage of germination namely 88 to 89%.

Treating infected seeds with organo-mercuric compounds and also with hot water helped to reduce the primary infection in the young seedlings.

OBSERVATIONS ON A SALTANT STRAIN OF *Helminthosporium oryzae*.

Introduction.

During the course of the isolation of *Helminthosporium oryzae* from infected rice kernels, on potato dextrose agar, a sector of a darker hue was observed in a colony grown out of the rice kernel. This area was immediately examined and transfers were made on potato dextrose agar slants since it appeared to be a saltant. The saltant strain was compared with the parent strain for its cultural characters, sporulation and pathogenicity.

Saltations in *Helminthosporium oryzae* have been frequently reported by earlier workers. Matsuura (1930) observed an island type of saltation in *H.oryzae* by which the black thick mycelium of the fungus was transformed to a white slender type. The change remained constant in various nutrient media. Hiroe (1937) found the development of dark sectors on pale colonies of the fungus and vice versa. He also observed the gradual or sudden reversion to the parent form. Chattopadhyay and Das Gupta (Miss) (1958) have induced saltation by subjecting the cultures of the fungus to different temperatures. They found 26°C. to be the optimum temperature at which maximum saltation occurred. They also found the reversion of the saltant to the parental strain.

Generally the sporulation in the saltants is considered to be fewer than that of the parent strains. Matsuura (1930) found no sporulation in the saltant. Chathopadhyay and Das Dupta (Miss) (1958) found that conidial production was practically absent in the saltants. The very few conidia produced were smaller than the normal.

Rate of growth, Colour and sporulation of the saltant.

Single spore isolates of the saltant were maintained at room temperature and it was compared to the parent strain. The saltant strain maintained its characters and reversion to the parent strain did not occur on sub-culturing. The two strains were grown side by side on leaf extract agar, Oat-meal agar, potato dextrose agar, Czapeck's agar and Richard's agar media. On all the media the rate of growth of the saltant was slow than that of the parent strain (Table XI Fig.10-19).

The mycelium of the saltant was much darker in appearance than the parent strain. The aerial mycelium of the saltant strain was only sparsely developed, while the parent strain produced thick fluffy aerial mycelium.

The saltant strain sporulated profusely where as the parent strain was only sparingly sporulating. On the leaf extract agar medium the sporulation was not profuse.

Table XI. Growth of the saltant strain of Helminthosporium oryzae on different media in five days old Petri plate cultures as compared to that of the parent strain (in cm.)

	Leaf extract agar.	Oat-meal agar.	Potato dextrose agar.	Czapek's agar.	Richard's agar.
Parent strain	6.42	6.35	4.20	3.90	3.80
Saltant strain	5.70	5.30	3.30	2.90	2.77

The conidial characters of both the strains were studied by taking conidia from 8 days old cultures. Two hundred spores were measured in each case. It was observed that the conidia of the saltant strain was distinctly longer than that of the parent strain. They measured 35.0 μ to 122.5 μ in length with an average of 83.5 μ while the conidia of the parent strain measured 21.0 μ to 98.0 μ in length with an average of 66.64 μ. There was no significant difference in breadth as well as septation between both the strains (Table XII Fig.8 and 9).

Comparative pathogenicity tests.

The pathogenicity of the saltant strain was compared with that of the saltant strain by artificial inoculation on rice seedlings.

Healthy seedlings of PTB.23 rice variety grown in pots were inoculated with both the strains of the fungus separately, when the plants were 20 days old having about 4 leaves each. Four pots each containing 5 plants were inoculated with both the strains and 4 pots were kept as control. The spore suspensions were prepared in sterile water from 10 days old cultures grown on potato dextrose agar in Petri dishes. The suspensions were standardised to contain between 55000 to 56000 spores per ml. Spore suspensions were sprayed on

Table XII. Comparison of the conidial size of saltant and parent strains of Helminthosporium oryzae from culture and leaf.

Age in days.	Saltant.						Parent.					
	Length in μ			Breadth in μ			Length in μ			Breadth in μ		
	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average	Maximum.	Minimum.	Average	Maximum.	Minimum.	Average
4	105.0	40.25	74.06	17.50	10.50	12.92	87.50	42.00	62.04	15.75	10.50	12.84
8	122.5	35.00	83.59	17.50	10.50	13.75	98.00	21.00	66.64	17.50	10.50	14.40
12	105.0	52.50	81.48	19.25	10.50	15.11	87.50	35.00	66.34	17.50	10.50	13.58
16	105.0	31.50	84.11	21.00	7.00	14.68	91.00	21.00	65.31	15.75	7.00	13.61
20	101.5	35.50	72.18	21.00	8.75	14.54	87.50	31.50	66.68	17.50	10.50	14.98
Leaf	105.0	63.00	82.51	17.50	10.50	13.93	87.50	27.00	72.33	17.50	7.00	13.75

the seedlings by means of an atomizer at the rate of 5 ml. per pot. The control plants were sprayed with sterile water. The inoculated as well as the control pots were kept in basins containing 3 inches of water and covered with bell jars. Symptoms of infection appeared 24 hours after inoculation. Observations were noted at 24 hours, 48 hours and 72 hours after inoculation and the spots were counted (Table XIII).

The results show that the saltant strain is much more virulent than the parent strain as evidenced by the number of spots which appeared on the rice seedlings. All the control plants remained healthy.

The saltant strain is interesting in that it is very profusely sporulating and more pathogenic than the parent strain. The spores are also longer than those of the parent strain.

Table XIII. Number of spots produced by the saltant and parent strains of Helminthosporium oryzae on rice seedlings.

Time.	No.of plants	Average No.of spots per plant.									
		Saltant					Parent				
		1st leaf	2nd leaf	3rd leaf	4th leaf	Total No. of spots per plant	1st leaf	2nd leaf	3rd leaf	4th leaf	Total No. of spots per plant
24 hours	20	14.20	27.60	73.20	123.20	238.20	9.60	22.00	43.60	84.50	159.70
48 hours	20	17.70	34.60	84.80	129.40	266.50	12.20	30.50	52.40	93.60	188.70
72 hours	20	20.30	37.40	88.80	131.90	278.40	15.10	32.16	57.06	89.60	193.92

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Fig. 2-7. Microphotographs of fungus mycelium in the bran.

Fig. 2

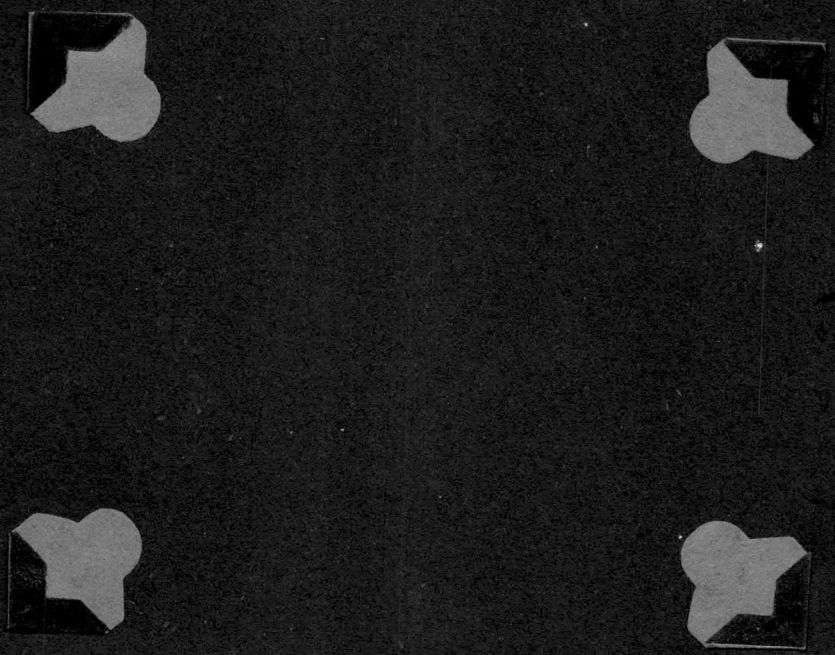

Fig. 3

Fig.4

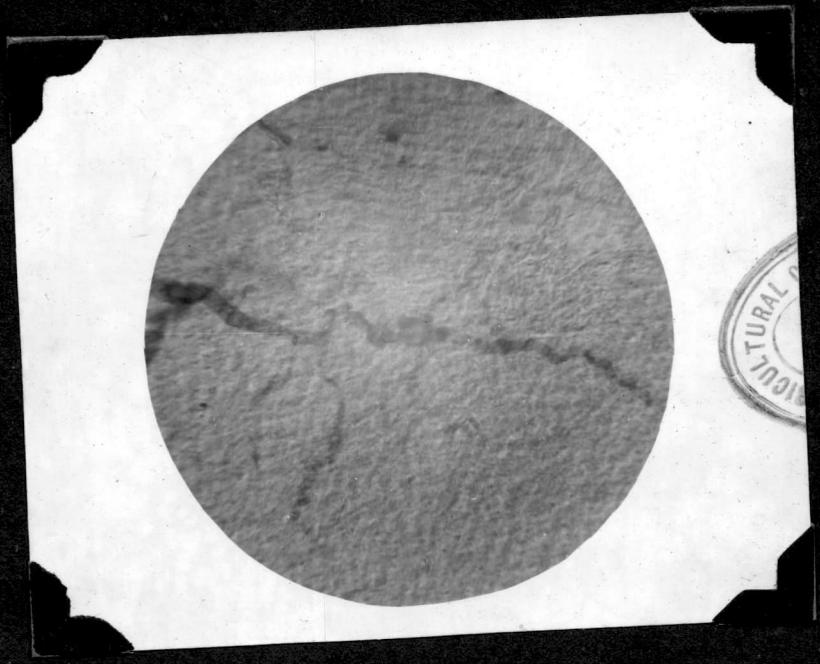

Fig.5

Fig.6

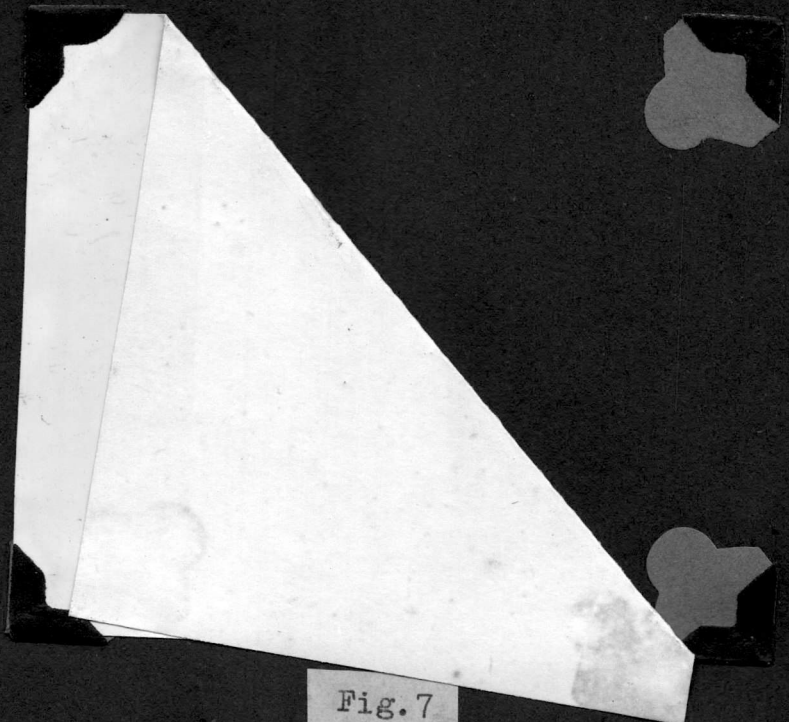

Fig.7

Fig. 8. Microphotograph of the conidia of the parent strain of Helminthosporium oryzae.

Fig. 9. Microphotograph of the conidia of the saltant strain of Helminthosporium oryzae.

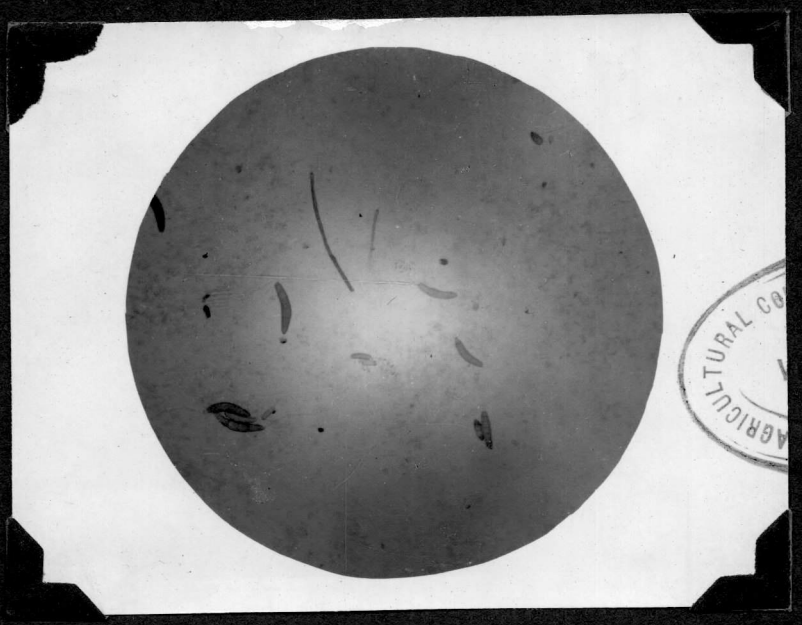

Fig.8

Fig.9

Feb. 10. Growth of the parent strain of Helminthosporium oryzae on rice leaf extract medium.

Fig. 11. Growth of the saltant strain of Helminthosporium oryzae on rice leaf extract medium.

Fig. 10

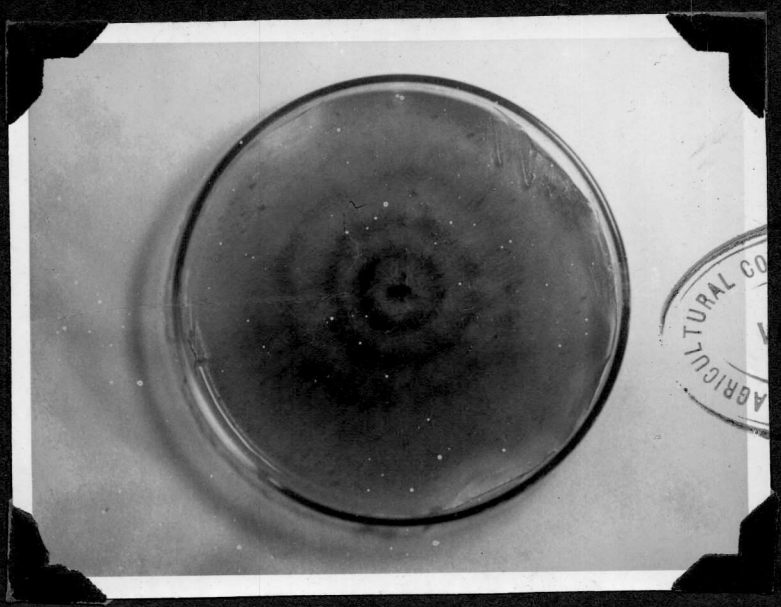

Fig. 11

Fig. 12. Growth of the parent strain of Helminthosporium oryzae on Oat-meal agar medium.

Fig. 13. Growth of the saltant strain of Helminthosporium oryzae on Oat-meal agar medium.

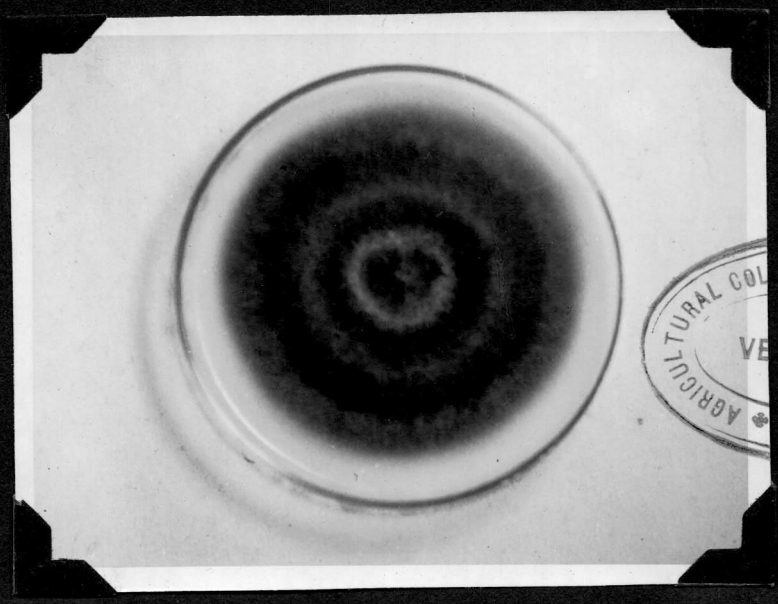

Fig.12

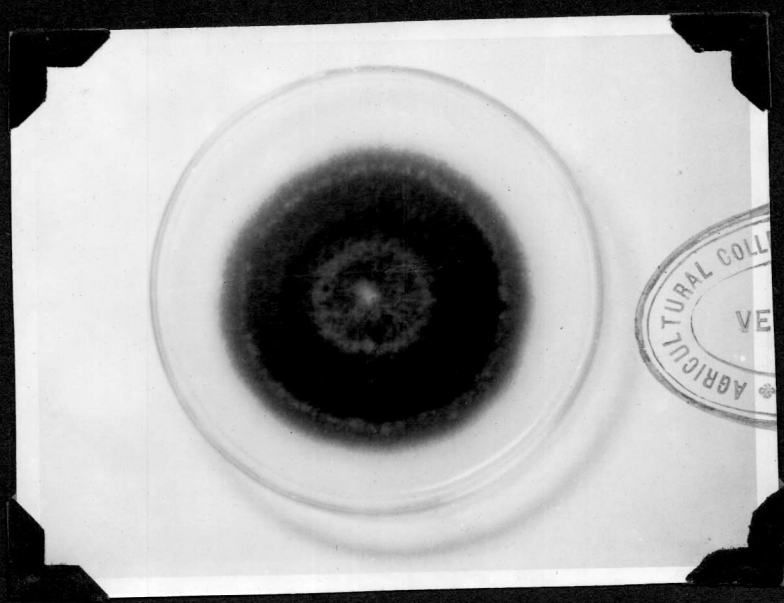

Fig.13

Fig. 14. Growth of the parent strain of Helminthosporium oryzae on potato dextrose agar medium.

Fig. 15. Growth of the saltant strain of Helminthosporium oryzae on potato dextrose agar.

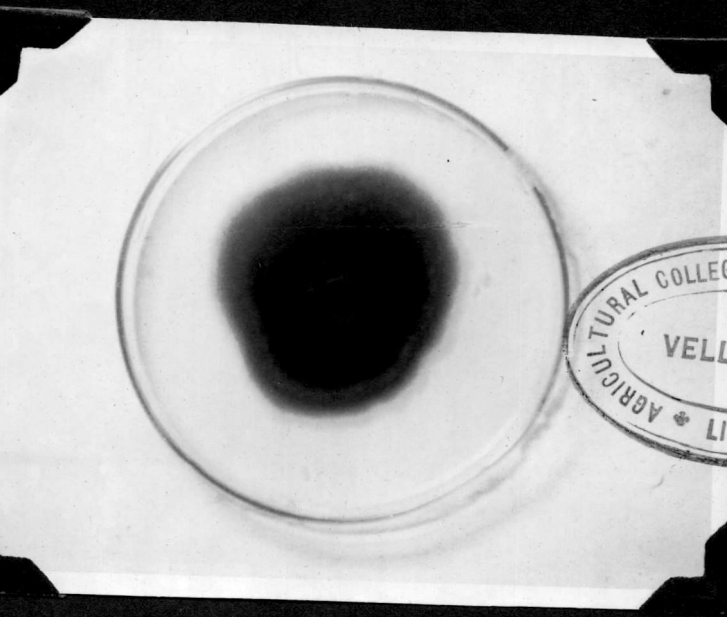

Fig.14

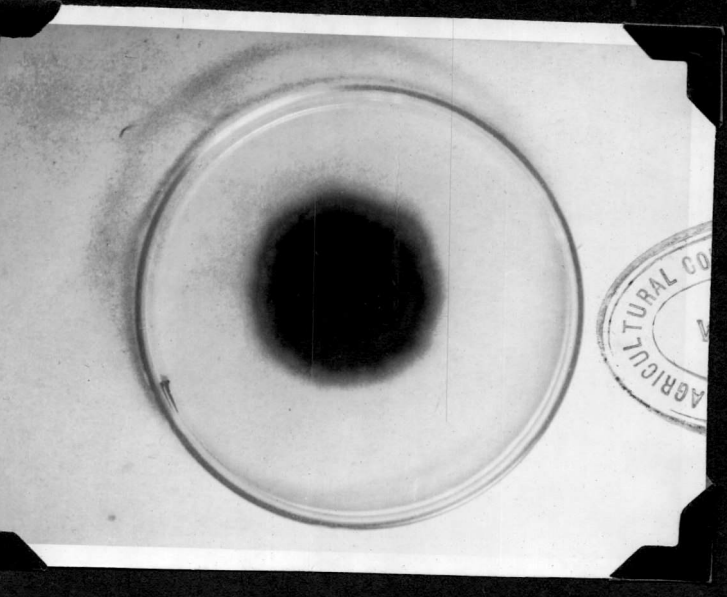

Fig.15

Fig. 16. Growth of the parent strain of Helminthosporium oryzae on Czapek's agar medium.

Fig. 17. Growth of the saltant strain of Helminthosporium oryzae on Czapek's agar medium.

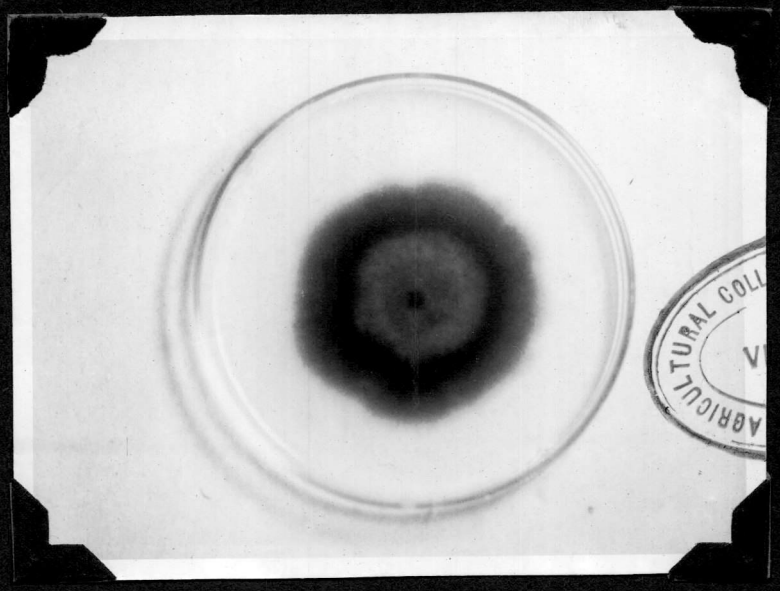

Fig. 16

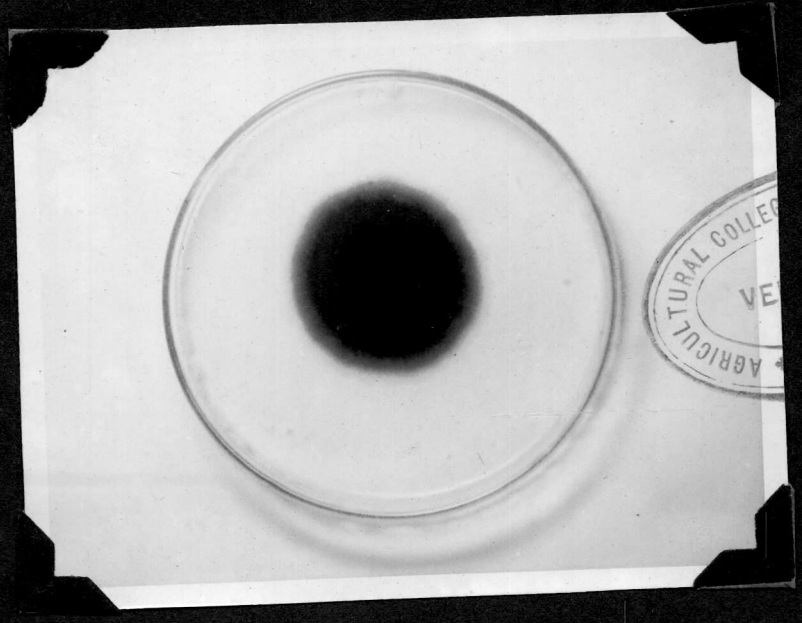

Fig. 17

Fig. 18. Growth of the parent strain of Helminthosporium oryzae on Richard's agar medium.

Fig. 19. Growth of the parent strain of Helminthosporium oryzae on Richard's agar medium.

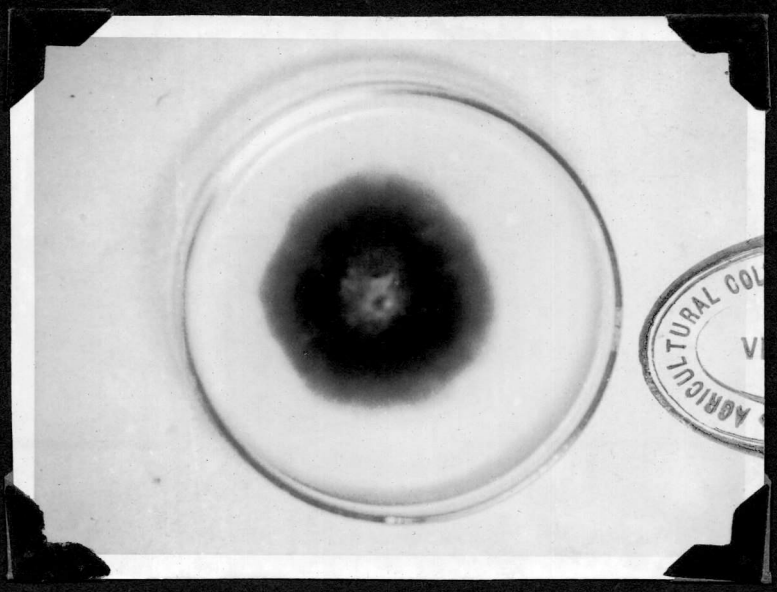

Fig. 18

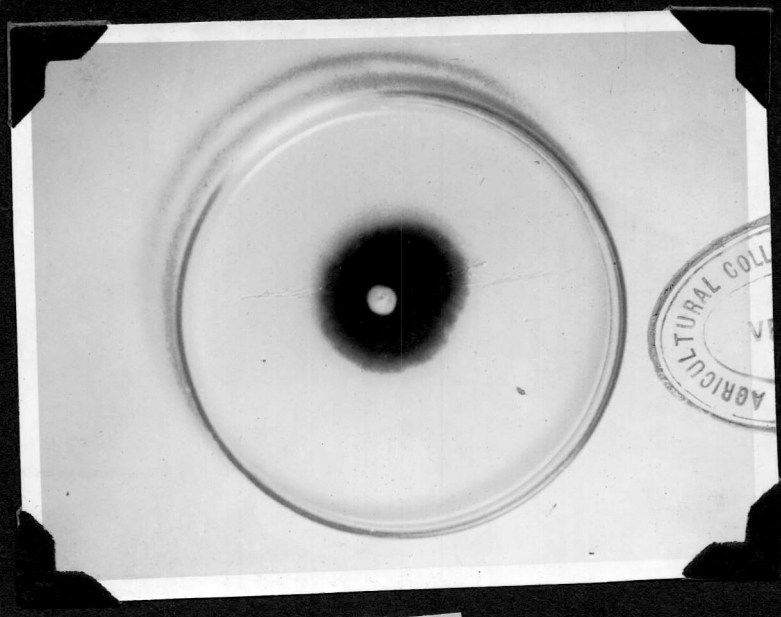

Fig. 19