MORPHOLOGICAL, CULTURAL AND PATHOGENIC VARIATION IN TOLYPOSPORIUM PENICILLARIAE BREF, THE CAUSAL AGENT OF SMUT OF PEARL MILLET IN NIGERIAN SAVANNA

BY

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CERTIFICATION

This thesis entitled: Morphological, Cultural and Pathogenic Variation in Tolyposporium penicillariae Bref., the Causal Agent of Smut of Pearl Millet in Nigerian Savanna by CHRISTIANA NCHETA ORJI meets the regulations governing the award of the degree of Master of Science, Crop Protection of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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D E D I C A T I O N

Affectionately dedicated

to

My parents, Francis and Anna, my brother and his wife, Mike and Celine and my sister, Lizzy for their love and all they have done for me.

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ABSTRACT

Twenty isolates of the pearl millet smut pathogen, Tolyposporium penicillariae, were collected from pearl millet growing ecological savanna zones of Nigeria and studied for morphological, cultural and pathogenic variations. All the isolates were used for morphological and cultural studies while 10 isolates were selected for pathogenicity tests.

The dimensions of the sporeballs of T. penicillarlae of the different isolates varied. The mean lengths and widths of 100 sporeballs from Jaji were the greatest (118.90 x 93.65 urn) while those of the isolate from Bakura were the smallest (87.20 x 70.00 u*m).* The sporeballs were dark brown and their shapes varied from circular to near polyhedral. The mean number of teliospores aggregated in a sporeball ranged from 549 for Jaji isolate to 758 for Bakura isolate. Teliospores were oval or spherical and yellowish brown. The mean lengths end widths of 100 teliospores varied from 8.36 x 7.46 um for Kano isolate to 10.9? x 9,57 u*m* for Jaji isolate. Germination of aggregated teliospores (sporeballs) in water occurred at 6 to 12 hours of incubation at 30°C.. The mode of germination was similar in all the isolates and the following patterns were observed within the isolates: sporidia borne laterally or/and terminally on promycelium; sporidia produced in clusters on promycelium and sporidia produced in branched chains directly by the teliospores without the

formation of promycelium, Sporidia were spindle shaped and hyaline and the mean lengths of 50 sporidia varied from 11.1+7 um to 19.15 um for Jalingo end Gombe isolates respectively. Mean incidence (percentage) of germination of aggregated teliospores (sporeballs) varied from 25.67 per cent for Karfi isolate to 64.33 per cent for Maigana isolate.

Some variations were observed in cultural characters of the pathogen grown on five different media. At 30 C maximum growth of T. penicillariae occurred on potato dextrose agar while minimum growth was observed on potato agar. Growth of the fungus remained sporidial and yeas-like even after several subculturing.

In the pathogenicity tests carried out with sporidial inoculum (2×10^{Q} sporidial/ml) from 1 - 1 0 day-old cultures and five pearl millet varieties, four pathogenic groups were identified, namely highly virulent, virulent, moderately virulent and weakly virulent. In general, no definite correlation could be established between the pathogenicity of the different isolates and their morphological or cultural characteristics.

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1. INTRODUCTION

Millets include a number of cereals of the grass family, Gramineae (Rachie, 1975). The principal millets consist of six genera, namely; Panicum, Paspalum, Pennisetum, Echinocloa, Setaria and Eleusine (Leonard and Martin, 1963). The principal species of these genera are Panicum miliaceum L. (common millet), Paspalum scrobiculatum L. (Ditch-millet), Pennisetum americanum (L.) Leeke (Pearl or Bulrush millet), Echinocloa frumentacea (Roxb) Link. (Barnyard millet), Setaria italica Beauv (Foxtail or Italican millet) and Eleusine coracana (Gaerth (Finger millet) (Rachie, 1975).

Among all these millets pearl millet (P. americanum (L.) Leeke) is the most important and widely cultivated species. It is an important common food grain and forage crop in the tropics (especially in the semi-arid tropical regions) and certain parts of the warm temperate regions of the world (Rachie, 1975; Vogel and Graham, 1978).

The most important pearl millet producing countries are China, India, Nigeria, Chad, Mali and Tanzania (Rachie, 1975). An average annual world production of pearl millet for the period, 1967 - 1971, has been estimated as 36 million tonnes (F.A.O., 1972). The average yields of traditional cultivars are estimated to

be 650 kg/ha in Africa and 468 kg/ha in India (Anonymous, 1976). In Nigeria about 5 million hectares are cultivated annually to millet in the Savanna Zones and an average yield of 564 kg/ha has been estimated (Thakare, 1980).

Pearl millet is second to sorghum in importance in the savanna zones of Nigeria but more important than sorghum in the Sahel Zone. It can adapt well to conditions of lighter soil and has low water requirement for growth and hence its predominance in the drier Sahel zone (Rachie, 1975; Kassam and Kowal, 1976). It is grown mostly in mixtures with sorghum and legume crops such as groundnuts and cowpea, except in the Sahel zone where it is grown as a sole crop (Kassam and Kowal, 1976).

Three types of pearl millet are grown in Nigeria, namely gero, maiwa and dauro pearl millets. Gero millets are the most popular and widely grown types due to their early maturing ability (Nwasike and Thakare, 1982). They are non-photoperiod sensitive, short seasoned types, sown early in May with beginning of the rainy season and mature between 80 to 90 days. Their cultivation stretches from Southern Guinea Savanna to Sahel Savanna areas. Gero millets account for over 80 per cent of all the millets grown in Nigeria (Ogunlela and Egharevba, 1981).

Maiwa millets account for a much smaller acreage, about 3 per cent of the total millet acreage (Ogunlela

and Egharevba, 1981). Their cultivation is mostly in the wetter areas between Southern Guinea Savanna and Sudan Savanna. Maiwa millet is a photoperiod sensitive, long seasoned type, and matures between 125 to 150 days. It is sown the same time as sorghum after the rains have established.

Dauro millet is grown in certain areas of Southern Guinea Savanna especially Southern Zaria area and part of Jos Plateau. It is a photoperiod sensitive type and matures between 100 and 150 days.

In Nigeria, pearl millet suffers from many diseases. In their decreasing order of importance, the most important diseases of pearl millet are downy mildew (Scherospora graminicola (Sacch) Schroet), smut (Tolyposporium penicillariae Bref. and ergot (Claviceps fusiformis Loveless (King, 1970; Selvaraj, 1978).

The prevalence and severity of these diseases vary in different pearl millet growing areas. Downy mildew is prevalent in all the millet growing zones while ergot is confined mainly to high rainfall areas. Smut, although common in drier areas, is found wherever millet is grown (Selvaraj, 1978).

Other diseases of pearl millet include foliage diseases such as blast (Pyricularia penniseti); rust (Puccinia penniseti); Dactuliophora leaf spot (Dactuliophora elongata); bacterial leaf blotch (Pseudomonas sp.); grain moulds (Fusarium spp.; Phoma sp., etc) (King, 1970; Selvaraj, 1978). Foliage diseases and grain moulds are more common in areas having an annual rainfall of about 600 mm or more (King, 1970; Selvaraj, 1978; Sundaram, 1981; Tyagi, 1983).

Among the diseases of pearl millet in Nigeria, attention seems to be geared mostly to the study of downy mildew, which is regarded as the most important disease of pearl millet. Smut has received comparatively less attention. Although, there is a dearth of published information on pearl millet smut in Nigeria, considerable work on smut resistance has been carried out and good sources of resistance have been identified (I.A.R., 1983).

The varieties of pearl millet grown in Nigeria show differential reaction to smut. Also, differences in reaction to smut have been observed on the same varieties grown at different locations (Tyagi, personal communication). Variability in the smut fungus might be one of the factors contributing to differences in susceptibility or resistance of these varieties planted at different locations.

Allard (1960) remarked, "knowledge of two closely interrelated phenomena, variations in the pathogenic capabilities of parasitic organisms and differences within host species in resistance to infectious disease, are basic to the intelligent planning of breeding programmes designed to develop disease resistant varieties".

For effective resistance breeding programme, it would be necessary to determine whether the differences in varietal response are due to pathogenic or environmental variation between the locations. Consequently, the present work was initiated with the following objectives:

1) To study the morphological and cultural variation of isolates of T. penicillariae from various locations where pearl millet is grown in Nigeria.

2) To test isolates of T. penicillariae from different savanna zones of Nigeria on a range of host varieties under the same environmental conditions.

It is hoped that this study would provide some information on variability (if any) in the pathogen which might be useful in pearl millet smut resistance breeding programme in Nigeria.

2. LITERATURE REVIEW

2.1 General

2.1.1 Historical

Smut of pearl millet is induced by Tolyposporium penicillariae Bref. The genus Tolyposporium was first described by Woronin in 1882(Fischer and Holton, 1957). The species, T. penicillariae, was described by Brefeld in 1895 on Penicillaria spicata Willd (Pennisetum typhoides Stapf and Hubb) Mundkur, 1940) which is now known as Pennisetum americanum (L.) Leeke.

2.1.2 Geographical distribution

The smut of pearl millet is prevalent in the semi-arid tropical regions of the world (Rachie and Majmudar, 1980) but it has also been reported from the United States of America (Wells et al., 1963). It is a common disease in various countries of Africa such as Senegal, Burkina Faso, Mali, Niger, Nigeria, Sudan, and Tanzania. (Anonymous, 1978). In Nigeria, smut is found in all the savanna zones where pearl millet is grown (King, 1970; Selvaraj, 1977).

2.1.3 Economic importance

Initially the smut disease of pearl millet was not considered economically important but the true magnitude of the losses has now been appreciated, at least in Africa and Asia (Ramakrishnan, 1963) and in North America (Wells et al., 1963).

In Senegal, some varieties of pearl millet went out of cultivation due to high damage caused by smut (Chevalier, 1931). Great yield losses of pearl millet crop due to smut have been reported in India by Ramakrishnan (1963), though no figures were provided. Also in northern India, smut has been observed and reported as a serious constraint in commercial plantings (Thakur et al., 1983a). In Nigeria, an annual yield loss of pearl millet by smut disease has been estimated at about 1 to 3 per cent (Selvaraj, 1978).

2.1.4 Symptoms

T. penicillariae is a floral infecting fungus which infects the florets and transforms them into large plump oval shaped sacs or sori which project beyond the glumes (Bhatt, 1946; Wells et al., 1963). The young sori from the infected grains are larger and greener than the non-infected grains. The sori are about 3mm broad which

is about twice the size of normal grain (1 to 2mm). As the sori mature, they become dark brown and their outer membranes are tough and made up of the host tissue.

The disease incidence in a pearl millet spike may vary between 1 - 2 sori per spike and up to 90 per cent infection in a spike composed of several hundreds of ovaries (Ramakrishnan, 1971). The infected grains occur individually or in groups and they are very often confined to one side of the ear or towards the base of the ear (Wells et al., 1963; Ramakrishnan, 1971).

2.2 Description of the Pathogen

The sori of T. penicillariae are dark or chocolate-brown, bluntly rounded or conical at the apex and are covered by a though membrane composed of the host tissue (Mundkur, 1940; Bhatt, 1946; Wells et al., 1963). The smut sori consist of masses of compact sporeballs of unequal size, ranging from 40 to 150 µm in diameter (Mundkur, 1940; Wells et al., 1963). Subba Rao and Thakur (1983) reported that sporeballs of T. penicillariae varied in size from 42 x 325 - 50 x 175 µm.

The spore balls contain several teliospores which are tightly pressed together and do not separate readily.

Teliospores are oval, ellipsoidal or spherical; light brown to antimony yellow ochre with a thin spore wall; 6.5 to 12.3 µm in diameter with a mean of 9 to 9.3 µm and the contents granular (Mundkur, 1940; Wells et al., 1963; Singh, 1978). Subba Rao and Thakur (1983) reported that the teliospores are mostly circular and measure 7 to 12 µm in diameter and that the numbers aggregated in spore balls vary from 200 to 1,400.

The teliospores germinate while still held in sporeballs to produce septate promycelia, maximum germination occurring at 30°C (Subba Rao and Thakur, 1983). Spindle-shaped sporidia which vary in length from 8 to 25 µm are borne on the promycelia, laterally and/or terminally and these reproduce by budding in chains.

2.3 Method of Infection

Bhatt's extensive study on the mode of infection by T. penicillariae revealed that its infection is not systemic. It was observed to occur through stigmas, in young florets before anthesis; infection decreases at or after anthesis (Bhatt, 1946).

Primary smut infection occurs through air-borne sporidia produced from germinating teliospores in the soil from the previous infected crop (Bhatt, 1946).

When these sporidia fall on young emerging florets of the crop they cause infection. Studies by Ajrekar and Likhite (1933) and Bhatt (1946) showed that no other part of the host plant is susceptible to smut infection except the florets. Thus, no other part of the host plant other than the grain develops the sori or show symptoms.

Vasudeva and Inyengar (1950) reported that secondary infection occurs mostly by the sporidia from the earlier infected ears which can be blown by wind to the florets of newly emerging ears. Thus, secondary infection is within a crop and is limited to only the nodal tillers.

Microscopic studies by Ajrekar and Likhite (1933) and Bhatt (1946) of the infected grains at various stages of the development have shown that the fungus occupies the space between the pericarp and the aleurone layer. It forms its sporeballs after gradually exhausting the starchy endosperm.

The mycelium is binucleate, inter and intra-cellular, exhibiting slight branching with two to four lobed haustoria. The downward advance is continued through the wall of the ovary to the thalamus. In this region it ramifies copiously, advancing towards the funicular end and finally invades the ovule. Before the whole tissue is invaded, the walls of the hyphae begin to gelatinize

to form the sporeballs. The entire process from the time of infection to the formation of the sporeballs or the appearance of the disease symptoms has been observed to take about two weeks, but this period may vary slightly according to the stage at which infection takes place (Bhatt, 1946).

2.4 Factors Affecting Disease Development

The extent of germination of the teliospores in the soil and the severity of smut infection generally depend on weather conditions during flowering in pearl millet.

Patel and Dessai (1959) have reported that high humidity up to 90 per cent is essential to secure high infection by artificial inoculation. In Nigeria, Selvaraj (1978), reported an increase in smut infection of pearl millet in 1974, which was attributed to favourable weather conditions especially cloudiness and high atmospheric humidity which prevailed that year. High infection is usually obtained by covering the ears with white parchment or polythene bags after inoculation (Patel and Dessai, 1959; Thakur et al., 1983a).

2.5 Artificial Culture

Ajrekar and Likhite (1933) reported that T. penicillariae can be easily cultured on corn meal, bajra meal and jowar meal agar as well as on boiled potato and carrot. The growth on all these media was mostly sporidial and very little mycelial growth was seen. It has also been observed by Subba Rao and Thakur (1983) that the pathogen grows well within three to five days on potato or carrot agar at 35°C and that its growth remains sporidial even after repeated subculturing.

2.6 Physiological, Morphological and Cultural Variation

Entities within morphologic species differ from each other in one or more physiologic characters and this may result in physiologic specialization (Christensen and Rodenhiser, 1940; Holton, 1953; Tarr, 1978). The main criterion for physiologic specialization in pathogenic fungi is variation in pathogenicity.

In cereal smuts, pathogenic specialization is common in many species and was first found in Ustilago hordei in Hordeum sativum (Faris, 1924, 1924b). It has also been found in Tilletia spp. in wheat (Faris, 1924a) and in the oat smuts (Reed, 1924) and U. maydis in corn

(Stakmen and Christesen, 1926). However, pathogenic specialization has been most extensively and intensively studied in Tilletia spp. which induce bunt or stinking smut of wheat and Ustilago spp. in cereals (especially U. maydis in maize) (Fischer and Holton, 1957).

Large variability is known to exist in some fungi causing diseases in pearl millet which include smut and ergot (Subba Rao and Thakur, 1983; Chahal et al., 1985). Many species of smut fungi are known to vary in their spore wall ornamentation and spore germination which has contributed greatly to the dynamic taxonomic history of smut fungi (Holton et al., 1968).

Subba Rao and Thakur (1983) observed large variability in cultural characteristics of multisporidial isolate of T. penicillariae grown on different media. This variation in cultural characteristics of the smut in this case, could be due to differences in the different media since only one isolate was used by these workers. Three different patterns of teliospore germination and formation of chlamydospores in the stored culture indicate the kind of variability that exists in T. penicillariae (Subba Rao and Thakur, 1983).

In view of this, it is probably likely that physiologic specialization exists in T. penicillariae.

However, this aspect needs to be carefully studied so as to understand the variability in this fungus.

2.7 Control

The control of smut of pearl millet has been attempted with the use of fungicides (Wells et al., 1963; King, 1970; Bhowmik and Sundaram, 1971; Phathak and Gaur, 1975; Selvaraj, 1978). Host plant resistance have also been used in control of pearl millet smut (Egharevba, 1978; I.A.R., 1983; Tyagi, 1983; ICRISAT, 1983). Frohlick and Rodewald (1970) have also attempted to control the disease through the use of cultural practices.

Most of the workers advocated the use of resistant varieties as the only satisfactory and economically feasible method to control smut. Tests conducted in Nigeria over 2 years showed that lines KARS 46, KARS 47, KARS, 69, KARS 75 and KARS 76 have good resistance to smut (I.A.R., 1983). In a multilocational testing by ICRISAT in 1982, lines ICMPS 101-1, 904-3, 1600-4 and 2001-2 were found to be highly resistant (ICRISAT, 1983).

Some workers have attempted to control smut by fungicidal sprays. In a test in India, Bhowmik and Sundaram (1971) reported that Plantvax and Vitavax at

2g/litre were very effective in reducing the number of sori in artificially inoculated ears of pearl millet. In Nigeria, King (1970) has reported the efficacy of Plantvax, Benlate, Vitavax, Ziram, Captan and Thiabendazole in controlling pearl millet smut.

3. MATERIALS AND METHODS

3.1 Collection of Smut Samples

Twenty samples of pearl millet smut were used in this study. The smutted heads were collected from different ecological savanna zones of Nigeria between July and September, 1984. At the time of collection, the smut sori were in various stages of maturity depending on millet variety and the dates of planting the crop. All the samples were collected from gero type of pearl millet except for Jalingo sample which was maiwa type. Dates and places of collection of the samples are given in Table 1.

Each sample was made up of a number of smutted heads. The samples were kept separately in labelled paper envelops in the laboratory during the period of this study.

3.2 Isolation of the Pathogen

The sori from the infected ears were first surface sterilized by soaking them for 12 - 72 hours in one per cent solution of cuprous sulphate to free them from surface contaminants (Fischer and Holton, 1957). Thereafter, they were teased in a Petri dish with the help of a sterilized needle to release the sporeballs.

Table 1. Sources of isolates of Tolyposporium penicillariae used in the study

Isolate no.	Date of Collection	Place of Collection
1.	28/7/84	Maiduguri(Borno State)
2.	9/7/84	Dambuo (Borno State)
3.	10/11/84	Bakura (Sokoto State)
4.	27/11/84	Gummi (Sokoto State)
5.	21/7/84	Kano (Kano State)
6.	28/8/84	Kadawa (Kano State)
7.	20/7/84	Samaru (Kaduna State)
8.	7/7/84	Zaria (Kaduna State)
9.	2/8/84	Jaji (Kaduna State)
10.	7/8/84	Yakasi (Kaduna State)
11.	7/8/84	Soba (Kaduna State)
12.	20/11/84	Mokwa (Niger State)
13.	2/11/84	Gombe (Bauchi State)
14.	11/8/84	Kari (Bauchi State)
15.	10/9/84	Jalingo (Gongola State)
16.	28/7/84	Bomo (Kaduna State)
17.	28/8/84	Kura (Kano State)
18.	2/8/84	Dumbi (Kaduna State)
19.	28/9/84	Maigana (Kaduna State)
20.	28/8/84	Karfi (Kano State)

The sporeballs were then squashed to release the teliospores which were subsequently surface sterilized by soaking in one per cent cuprous sulphate solution for 12 - 72 hours (Fischer and Holton, 1957).

With the aid of a compound microscope, single teliospores were picked up free hand and plated on potato dextrose agar in 9cm diameter Petri dishes. The plates were incubated at 30°C. For each of the 20 samples, a single teliospore culture was maintained on potato dextrose agar slants in MacCartney bottles.

3.3 Study of Morphological Variation

The morphological characteristics of the different isolates were studied microscopically.

3.3.1 Sporeballs

Measurements of length and width of the sporeballs in smut sori were taken. The sporeballs were mounted in a drop of distilled water on a microscope slide; their sizes were measured with the aid of an ocular micrometer under low power of a compound microscope (x10 objective lens). The measurements of 100 sporeballs were taken and the mean calculated for each sample.

The shape of the sporeballs of each sample was noted. With the aid of a tally counter, the number of teliospores in 100 sporeballs of each sample was counted under the low power of a compound microscope (x10 objective lens).

3.2.2 Teliospores

The teliospores were mounted in a drop of distilled water on a microscope slide and the measurements taken with the aid of an acular micrometer under high power of a compound microscope (x40 objective lens). One hundred teliospores were measured and the mean taken for each sample. The shape of the teliospores was also recorded.

3.3.3 Germination of aggregated teliospores (sporeballs)

Observations were made on the method of germination, time required for germination and the incidence (percentage) of germination of aggregated teliospores (sporeballs), of the different samples.

Glass slides containing drops of water, each with 100 sporeballs were maintained in moist chambers in an incubator at 30°C. The aggregated teliospores were microscopically examined for germination at hourly

intervals for 15 hours and germination percentage recorded. Camera lucida drawings of stages of the teliospores germination were made.

3.4 Study of Cultural Variation

To study cultural characteristics, the 20 isolates were grown on different media in 9cm diameter Petri dishes.

The following five media were used:

i) Potato dextrose agar (PDA): Peeled potato, 200g; dextrose. 20g; agar powder, 15g; distilled water, 1000ml (Tuite, 1969).

ii) Potato agar (PA): Peeled potato, 200g; agar powder, 15g; distilled water, 1000ml (Tuite, 1969).

iii) Carrot agar (CA): Peeled carrot, 200g; agar powder, 15g; distilled water, 1000ml (Tuite, 1969).

iv) Czapek-Dox agar (CDA): Magnesium glycerophosphate, 0.5g; sodium nitrate, 2.0g; potasium chloride, 0.5g; ferrous sulphate, 0.01g; potassium sulphate, 0.35; sucrose, 30g; agar powder, 15g; distilled water 1000ml (Tuite, 1969).

v) Sartoris 'Best' synthetic agar (SBSA): Calcium nitrate 0.4g; potassium nitrate, 0.2g; magnesium sulphate, 0.01g; potassium dihydrogen phosphate, 0.2g; malt extract, 3.0g; distilled water, 1000ml (Fischer and Holton, 1957).

All the media were autoclaved at 15 p.s.i. for 15 minutes. Thereafter, each medium was poured into steril Petri dishes and allowed to set in a microwave inoculating chamber.

Each Petri dish was inoculated centrally with 8mm sporidial disk of the pathogen growing on potato dextrose agar. Each treatment had three replications. The Petri dishes were incubated at 30°C for 10 days.

The type of growth, topography, margin, and colour of colony were visually observed and recorded at 10 days of incubation. The radial growth of the colony was measured daily for 10 days. The lengths of 50 sporidia on potato dextrose agar culture of each isolate were measured at 10 days of incubation and the mean calculated.

3.5 Study of Pathogenic Variation

Five following varieties of pearl millet were used in the pathogenicity tests for two consecutive years: MDN 131, MDN 137, MDN 143, MDN 145 and S6. These varieties are all gero type of pearl millet and are known to show differences in reaction to smut under field conditions, ranging from highly resistant to highly susceptible (Tyagi, personal communication).

Ten isolates of T. penicillariae were used in the study; they were selected to represent the different ecological zones of millet production in Nigeria.

3.5.1 Raising of test plants

Plants of each variety were raised in the glasshouse in clay pots (24cm diameter and 22cm deep) containing sandy loam soil. Three plants were raised per pot and there were three pots per variety, each pot serving as a replicate.

3.5.2 Preparation of inoculum

For each isolate, sporidial suspensions were prepared in sterile distilled water from 1 - 10 day old cultures of T. pencillariae grown on potato dextrose agar. The sporidial suspensions were adjusted to approximately 2×10^8 sporidia/ml.

3.5.3 Inoculation of test plants

Plants were inoculated using the method described by Thakur et al. (1983b). Five ml of an aqueous sporidial suspension (2×10^8 sporidial/ml) was injected into the space between the flag leaf sheath and the inflorescence at boot-leaf stage. The plants were immediately covered after inoculation with labelled white parchment bags.

Five heads were inoculated in each pot. Thus, a total of 15 heads (for three replicates) of each variety were inoculated with each isolate of the pathogen. Controls consisted of uninoculated plants covered with labelled white parchment bags at boot-leaf stage.

While still covered, the inoculated heads as well as the controls, were sprayed with water, two to three times everyday with CP-3 knapsack sprayer to maintain a high relative humidity. To avoid cross infection, the plants inoculated with respective isolates and the controls were kept separate from each other in different glasshouses.

3.5.4 Disease assessment

Twenty days after inoculation, the white parchment bags were removed and the smut serverity assessed by visual observation.

The heads were scored for percentage smut infection using the standard smut rating key, published by International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in 1982 (Appendix 8).

To indicate the degree of resistance or susceptibility of the five pearl millet varieties, the mean smut severities (percentage infection) were grouped as follows:

Smut incidence (%)	Reaction type
Up to 5	Resistant (R)
5.1 - 10	Moderately resistant (MR)
10.1 - 20	Moderately susceptible (MS)
> 20	Susceptible (S)

4. RESULTS

4.1 Morphological Variation in Isolates of Tolyposporium penicillariae

Variations in morphological characteristics, such as size, shape, colour, etc of sporeballs, teliospores and sporidia were studied for all the 20 isolates. The results are presented below.

4.1.1 Sporeballs

There were significant differences in the dimensions of sporeballs of the different isolates (Appendices 1a and 1b). The mean lengths and widths ranged from 87.20 x 70.00 µm for isolate 9 to 118.90 x 93.65 µm for isolate 3 (Table 2).

There was no difference in shape and colour of sporeballs of the different isolates. For all the isolates the sporeballs were circular to near polyhedral and dark brown.

The mean number of teliospores per sporeball ranged from 549 for isolate 9 to 758 for isolate 3 (Table 3).

Table 2. Dimensions of Sporeballs of 20 Isolates of Tolyposporium penicillariae

Isolate no.	Length (µm)		Width (µm)	
	Mean	Range	Mean	Range
1	$97.45^{cdefghi}$	40-190	$74.80^{efghijkl}$	40-130
2	94.75^{defghi}	40-175	83.10^{cdefg}	40.160
3	118.90^{a}	40.290	93.65^{a}	30-150
4	92.60^{defghi}	30-190	71.70^{hijkl}	30-140
5	101.55^{bcde}	50-150	$80.10^{defghij}$	30-130
6	$101.30^{bcdefgh}$	30-190	$77.00^{efghijk}$	30-140
7	$99.15^{bcdefghi}$	60-170	87.05^{abcd}	50-150
8	100.85^{bcdefg}	40-190	80.55^{defghi}	40-150
9	87.20^{j}	30-170	$70-00^{l}$	30-120
10	97.25^{defghi}	40-580	$79.90^{defghijk}$	40-180
11	101.50^{abc}	30-190	83.20^{cdef}	30-150
12	101.50^{abc}	50-250	$81.05^{cdefghi}$	30-150
13	93.85^{defghi}	40-190	$76.10^{efghijkl}$	30-140
14	95.45^{defghi}	35-190	$74.85^{efghijkl}$	30-125
15	91.05^{defghi}	40-206	72.20^{hijkl}	30-140
16	110.15^{abc}	50-170	93.45^{ab}	45-150
17	101.55^{bcde}	40-180	83.45^{cde}	40-140
18	111.30^{ab}	40-210	89.70^{abc}	40-150
19	96.80^{defghi}	40-170	$76.65^{efghijkl}$	35-130
20	93.80^{defghi}	30-170	$76.40^{efghijkl}$	30-140

Using Duncan's Multiple range test, means with the same superscript are not significantly different (P = 0.05).

Table 3. Mean number of teliospores per sporeball of 20 isolates of Tolyposporium penicillariae

Isolate no.	Number of teliospores per Sporeball	
	Mean	Range
1	734	203 - 1,532
2	664	317 - 1,052
3	758	254 - 1,214
4	694	284 - 991
5	751	267 - 1,291
6	729	296 - 1,695
7	754	712 - 1,666
8	698	336 - 1,562
9	549	301 - 1,056
10	708	296 - 1,005
11	694	431 - 983
12	620	325 - 967
13	743	282 - 1,123
14	664	256 - 976
15	582	267 - 998
16	695	425 - 1,102
17	693	318 - 1,223
18	678	215 - 972
19	695	240 - 1,018
20	690	315 - 992

However, differences in the number of teliospores per sporeball of the different isolates were not significant (Appendix 2).

4.1.2 Teliospores

The dimensions of the teliospores were significantly different between the isolates (Appendices 3a and 3b). The mean length and width of teliospores of isolate 9 were the greatest (10.92 x 9.58 µm) while those of isolate 5 were the smallest (8.36 x 7.46 µm) (Table 4).

The teliospores were oval, spherical or ellipsoidal and yellowish brown (vinaceous buff).

The time required for the germination of aggregated teliospores (sporeballs) ranged from 6 hours to 12 hours for the different isolates (Table 5). A sporeball was considered to have germinated when at least one promycelium or a sporidium had emerged (Fig. 1a). Camera lucida drawings of sporeballs of T. penicillariae showing different stages of germination of the sporeballs at different times are presented in Figs. 1 - 3.

In general, there was no germination up to 6 hours of incubation (Table 5). Sporidial formation usually occurred 1 to 2 hours after germination of the teliospores in all the isolates.

Table 4. Dimensions of teliospores of 20 isolates of *Tolyposporium penicillariae*

Isolate no.	Length (µm)		Width (µm)	
	Mean	Range	Mean	Range
1	9.36^{bcdefgh}	5.00-13.75	7.89^{efghijkl}	5.00-10.00
2	8.76^{fghijklmnopqr}	7.50-12.50	7.61^{hijkl}	5.00-10.00
3	9.08^{bcdefghijklmnop}	8.75-12.50	8.12^{bcdefgh}	5.00-10.00
4	9.36^{bcdefg}	6.25-15.15	8.29^{bcde}	6.25-12.50
5	8.36^{r}	5.00-12.50	7.45^{l}	5.00-10.00
6	8.99^{defghijklmnopq}	5.00-12.50	7.85^{efghijkl}	5.00-10.00
7	9.50^{bcde}	7.50-12.50	8.05^{bcdefghij}	5.00-12.50
8	9.35^{bcdefghi}	7.50-12.50	8.53^{b}	5.00-12.50
9	10.92^{a}	7.50-15.00	9.57^{a}	8.75-12.50
10	9.19^{bcdefghijklmno}	6.25-12.50	7.97^{bcdefghijkl}	5.00-10.00
11	9.21^{bcdefghijklm}	7.50-12.50	8.03^{bcdefghijk}	5.00-10.00
12	9.24^{bcdefghijk}	7.50-12.50	8.10^{bcdefghi}	6.25-11.25
13	9.69^{bc}	7.50-12.50	8.52^{bc}	5.00-10.00
14	9.71^{b}	7.50-12.50	8.50^{bcd}	5.00-10.00
15	9.60^{bcd}	5.00-15.00	8.21^{bcdef}	5.00-12.50
16	9.22^{bcdefghijkl}	7.50-12.50	8.13^{bcdefg}	5.00-12.50
17	8.93^{efghijklmnopqr}	7.50-12.50	7.51^{jkl}	5.00-10.00
18	9.41^{bcdef}	7.50-12.50	7.84^{efghijkl}	5.00-12.50
19	9.20^{bcdefghij}	7.50-12.50	7.90^{efghijkl}	5.00-10.00
20	9.32^{bcdefghij}	7.50-13.75	7.78^{efghijkl}	5.00-11.25

Using Duncan's multiple range test, means with the same superscript are not significantly different ($P = 0.05$).

Table 5. Time required for germination of sporeballs of 20 isolates of Tolyposporium penicillariae

Isolate no.	Time (hours)											
	1	2	3	4	5	6	7	8	9	10	11	12
1	-	-	-	-	-	-	-	-	-	+		
2	-	-	-	-	-	+						
3	-	-	-	-	-	-	-	-	-	+		
4	-	-	-	-	-	-	-	-	-			
5	-	-	-	-	-	-	-	-	+			
6	-	-	-	-	-	-	-	-	-	+		
7	-	-	-	-	-	-	-	-	+			
8	-	-	-	-	-	-	-	-	-	-	-	+
9	-	-	-	-	-	-	-	-	+			
10	-	-	-	-	-	-	-	-	-	-	+	
11	-	-	-	-	-	+	-					
12	-	-	-	-	-	-	-	+				
13	-	-	-	-	-	+						
14	-	-	-	-	-	-	-	-	+			
15	-	-	-	-	-	-	-	-	-	-	+	
16	-	-	-	-	-	-	-	+				
17	-	-	-	-	-	-	-	-	-	-	-	+
18	-	-	-	-	-	-	-	-	-	+		
19	-	-	-	-	-	+						
20	-	-	-	-	-	-	-	-	-	-	-	+

- = No germination

+ = Germination

There were no differences in the modes of germination of the sporeballs or aggregated teliospores of the isolates. However, there were three distinct modes of germination (resulting in sporidia production) of teliospores of each isolate. Firstly, the teliospores germinated to form promycelia from which sporidia arose laterally and/or terminally. Secondly, the teliospores germinated to produce promycelia from which sporidia were produced in clusters. Thirdly, teliospores produced sporidia directly in branched chains without producing promycelia (Figs. 1b, 2 and 3).

The germination percentages of aggregated teliospores (Table 6) were significantly different between the isolates (Appendix 4). The mean percentage germination of the teliospores ranged from 25.67 per cent for isolate 20 to 64.33 per cent for isolate 19.

4.1.3 Sporidia.

There were significant differences in the mean lengths of sporidia (Table 7) between the different isolates (Appendix 5). Isolate 13 had the greatest mean length (19.15 µm) while isolate 15 had the smallest (11.47 µm). For the isolates the sporidia were hyaline and single celled.

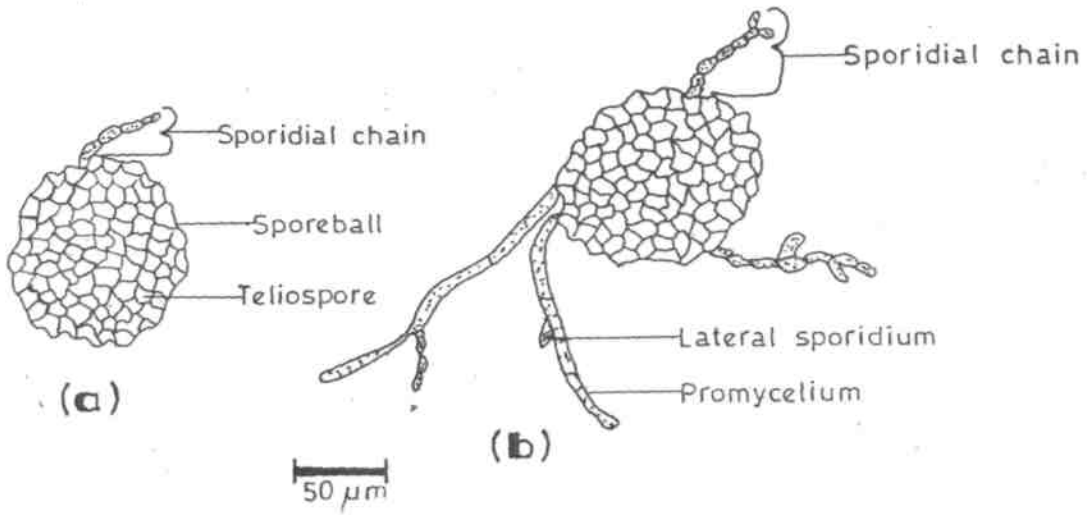


FIG. 1 Camera lucida drawings of sporeballs of Tolyposporium penicillariae showing :

(a) the early stage of teliospore germination within the first hour of the germination.

(b) sporidia production in branched chains without any promycelium and sporidia borne laterally on the promycelium between 1 and 2 hours after teliospore germination.

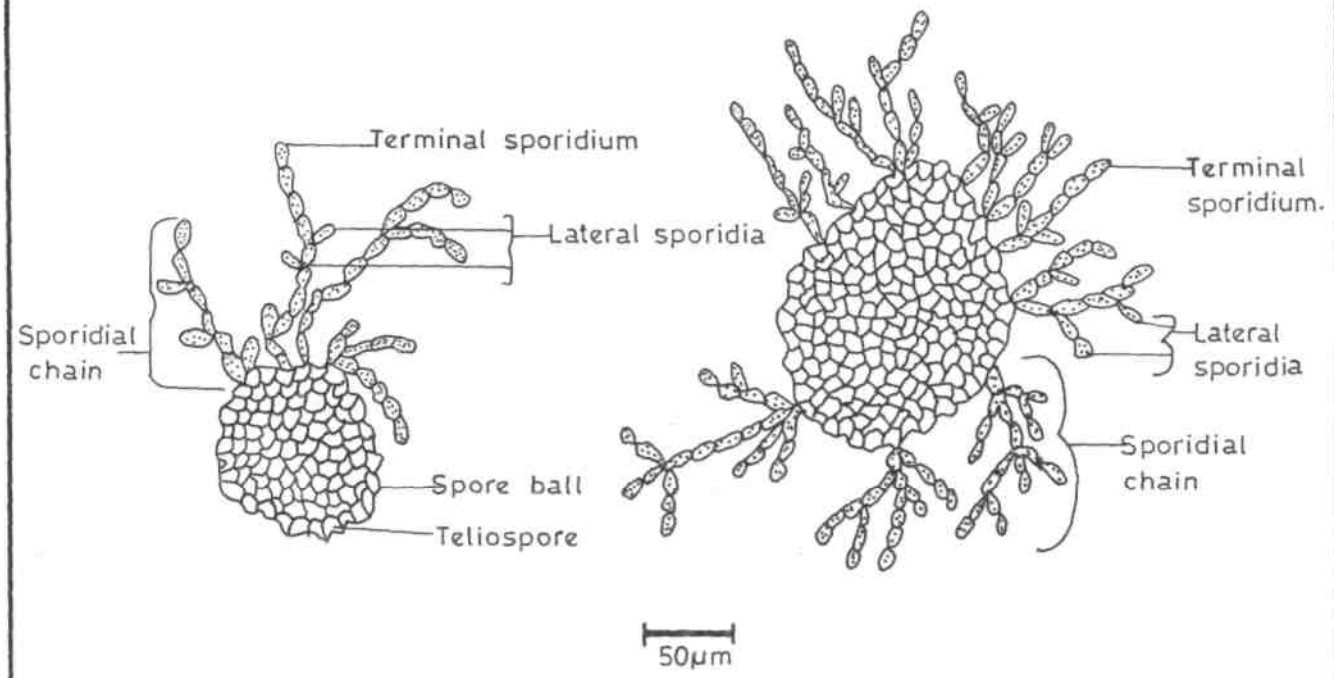


FIG. 2 Camera lucida drawings of sporeballs of Tolyposporium penicillariae showing sporidia production in branched chains without any promycelium, between 3 and 12 hours after teliospore germination.

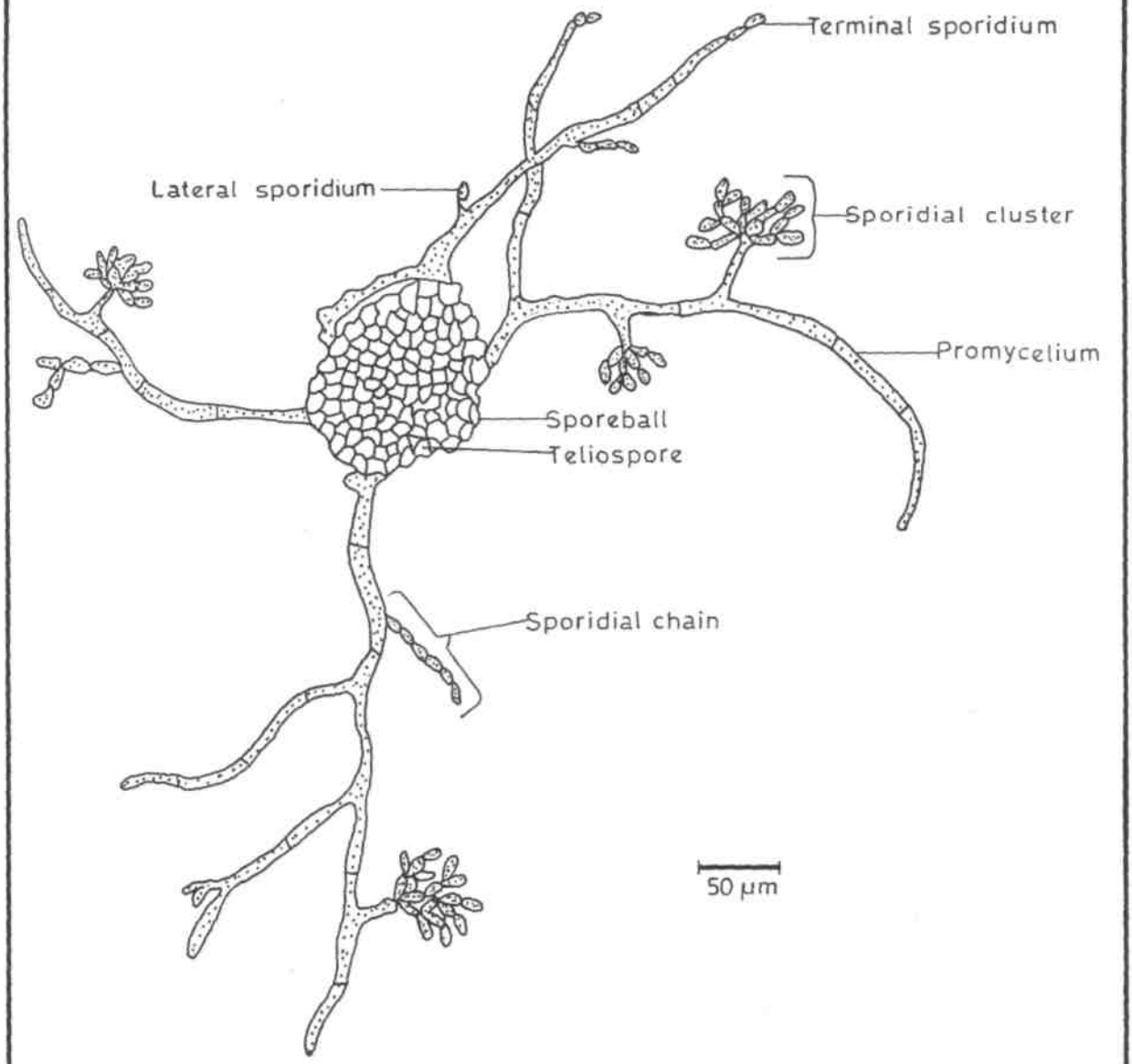


FIG. 3 Camera lucida drawing of sporeball of Tolyposporium penicillariae showing sporidia borne laterally and / or terminally and in clusters on promycelium between 3 and 12 hours after teliospore germination

Table 6. Mean germination percentage of aggregated teliospores (sporeballs) of 20 isolates of Tolyposporium penicillariae between 6 and 15 hours of incubation at 30°C.

Isolate no.	Germination percentage between 6 and 15 hours									
	6	7	8	9	10	11	12	13	14	15*
1	0	0	0	0	4.67	17.67	32.00	47.67	49.67	$50.00^{cdefghij}$
2	4	16.67	34.67	52.33	56.00	60.00	60.67	61.00	61.33	61.33^{abc}
3	0	0	0	0	3.67	20.00	29.00	36.67	37.67	$40.33^{ghijklmnop}$
4	0	0	0	3	12.67	24.33	41.00	42.33	43.33	$43.33^{efghijklmno}$
5	0	0	0	3.67	10.00	24.33	45.00	46.00	46.67	46.67^{eghijk}
6	0	0	0	0	7.33	9.67	17.33	25.33	39.33	$44.67^{eghijkl}$
7	0	0	0	6	12.00	26.00	40.00	49.00	50.67	$51.67^{bcdefgh}$
8	0	0	0	0	0	0	6.00	22.67	44.33	53.67^{abcdef}
9	0	0	0	6.67	12.33	20.33	28.67	44.00	50.67	$51.00^{cdefghi}$
10	0	0	0	0	0	7.00	12.67	25.67	35.33	$44.33^{efghijklmn}$
11	3.67	8.00	25.67	38.00	52.00	52.67	53.00	55.33	57.67	58.67^{abcd}
12	0	0	3.00	5.67	15.00	26.67	38.00	39.67	40.00	$40.00^{ghijklmnopq}$
13	6.67	14.33	35.33	51.00	58.00	59.67	61.33	63.00	63.00	63.33^{ab}
14	0	0	0	3.67	11.00	27.33	38.67	50.00	51.67	52.33^{bcdefg}
15	0	0	0	0	0	3.33	5.33	12.33	26.67	31.33^{pqr}
16	0	0	4.00	5.00	10.33	25.00	29.67	30.00	30.00	30.00^{pqrs}
17	0	0	0	0	0	11.67	30.67	45.00	51.00	54.33^{abcde}
18	0	0	0	0	6.67	14.67	27.00	41.33	43.33	$44.67^{efghijklm}$
19	6.67	22.67	42.33	57.67	62.00	63.00	64.00	64.00	64.33	64.33^{a}
20	0	0	0	0	0	0	3.33	3.67	10.00	25.67^{rs}
	LSD (0.05) = 10.20									

* Using Duncan's Multiple Range test, means in the last column with the same superscript (P=0.05) are not significantly different.

Table 7. Mean length of sporidia of 20 isolates of Tolyposporium penicillariae grown on potato dextrose agar

Isolate no.	Length of sporidia (um)	
	Mean	Range
1	$13.57^{defghijk}$	7.50-25.00
2	$14.05^{cdefghijk}$	7.50-27.50
3	$15.53^{bcdefgh}$	7.50-25.00
4	15.55^{bcdefg}	7.50-27.50
5	$12.95^{defghijk}$	7.50-25.00
6	$15.05^{bcdefghij}$	7.50-25.00
7	$15.15^{bcdefghi}$	7.50-27.50
8	17.55^{abc}	7.50-27.50
9	$13.89^{cdefghijk}$	8.75-27.50
10	$13.55^{defghijk}$	7.50-25.00
11	$14.30^{cdefghijk}$	7.50-25.00
12	16.15^{abcd}	7.50-27.50
13	19.15^{a}	7.50-25.00
14	$13.45^{defghijk}$	7.50-27.50
15	11.47^{k}	7.50-25.00
16	15.80^{abcde}	8.75-25.00
17	$14.35^{cdefghijk}$	7.50-25.00
18	15.65^{abcdef}	7.50-27.50
19	18.30^{ab}	8.75-27.50
20	$13.35^{defghijk}$	7.50-25.00

Using Duncan's Multiple Range test, means with the same superscript are not significantly different (P = 0.05).

4.2 Cultural Variation in Isolates of Tolyposporium penicillariae

The 20 isolates were grown on five culture media namely potato dextrose agar (PDA), potato agar (PA), carrot agar (CA), Czapek-Dox agar (CDA) and Sartoris 'Best' synthetic agar (SBSA). The cultural characteristics of these isolates on the different media are presented below.

4.2.1 Cultural characteristics on potato dextrose agar.

The results are presented in Table 8. The mean diameters of the different isolates were significant (P=0.05) from each other (Appendix 6a). Isolate 13 had the greatest radial growth (26.80mm) while isolate 20 had the smallest (14.35mm)

There was variation in the topography of the colonies of the different isolates. Thus, isolates 1, 3, 4, 5, 7 - 11, 13, 14, and 16 - 19 had finely ridged, raised colonies with thick centres, the colonies being thin at the periphery and appearing frilled. On the other hand, isolates 2, 6, 12, 15 and 20 had thick, raised, broadly ridged, frilled colonies (Plate 1).

The margin of the colonies ranged from wavy to lobate (Plate 1). Colonies of isolates 1, 2, 4, 6, 9 - 15, and 17 - 20 had wavy margin while the other isolates had colonies with both wavy and lobate margins.

The colour of colonies differred between the isolates. Isolates 1, 2, 4, 15, 17 and 20 had buff coloured colonies, while isolates 3, 6 - 9, 11, 13 and 14 had buff to cream colonies. Colonies with buff to pale luteous, buff to honey and buff to rosy-buff colouration were also observed in the other isolates.

Table 8. Cultural characteristics of 20 isolates of Tolyposporium penicillariae on potato dextrose agar

Isolate no.	Mean diameter of colony (mm)**	Topography**	Margin**	Colour of colony***
1	16.41^{ef}	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Buff
2	17.61^{def}	Broadly ridged, thick, raised and frilled	Wavy	Buff
3	21.53^{abcde}	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and lobate	Buff to cream
4	19.53^{cdef}	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Buff
5	23.31^{abcd}	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and lobate	Buff to Pale luteous
6	17.75^{def}	Broadly ridged, thick raised and frilled.	Wavy	Buff to cream
7	20.13^{bcde}	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and lobate	Buff to cream
8.	24.96^{abc}	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and lobate	Buff to cream
9.	21.31^{abcde}	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Buff to cream
10.	17.88^{def}	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Buff to cream

Table 8 (contd.)

11	16.07^{ef}	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Buff to cream
12	18.55^{def}	Broadly ridged, thick, raised and frilled.	Wavy	Buff to Rosy buff
13	26.80^{a}	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Buff to cream
14.	17.81^{def}	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Buff to cream
15	18.76^{cdef}	Broadly ridged, thick, raised and frilled.	Wavy	Buff
16	21.47^{abcde}	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and lobate	Buff to Pale luteous
17	19.72^{cdef}	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Buff
18	18.22^{def}	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Buff to Pale luteous
19	26.78^{ab}	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	White to Buff
20	14.35^{f}	Broadly ridged, thick, raised and frilled.	Wavy	Buff

* Using Duncan's Multiple Range test, means with the same superscript (P=0.05) are not significantly different.

** Topography and margin descriptions are based on descriptions by Subba Rao and Thakur (1983).

*** Colour description is based on Commonwealth Mycological Institute (CMI) Mycological colour charts (Rayner, 1970).

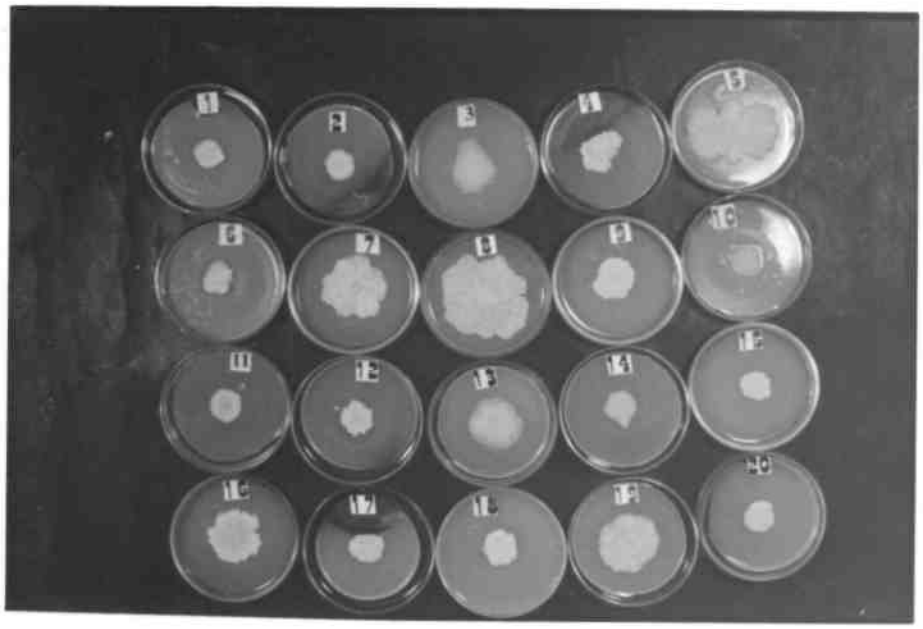


Plate 1: Cultural characters of 20 isolates of Tolyposporium penicillariae on potato dextrose agar.

4.2.2 Cultural characteristics on potato agar

The results are presented in Table 9. The mean colony diameters were not significantly different (P=0.05) between the isolates (Appendix 6b).

All the isolates had finely ridged colonies which were raised, thick in the centre, thin at the periphery and frilled except isolate 17 which had thick, raised broadly ridged frilled colony.

The margins of the colonies in isolates 1, 5 - 7, 10, 11, 13 - 16, 18 and 20 were wavy and lobate while those of the other isolates were wavy. The colonies were dull white. buff or honey.

Table 9. Cultural characteristics of 20 isolates of Tolyposporium penicillariae on potato agar.

Isolate no.	Mean diameter of colony (mm)	Topography**	Margin**	Colour of colony***
1	17.01	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and	Dull white
2	15.65	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Honey to Dull white
3	16.71	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Dull white
4	16.65	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Dull white
5	16.03	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and lobate	Dull white
6	16.44	Finely ridged, raised, thick in the centre, thin at the periphery and frulled.	Wavy and lobate	Honey to Dull white
7	16.01	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and lobate	Dull white
8	17.46	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Honey
9	15.98	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Honey
10	17.46	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and lobate	Dull white

Table 9 (contd.)

11	16.70	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and lobate	Dull white
12	16.07	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Buff
13	16.50	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and lobate	Dull white
14	15.45	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and lobate	Dull white
15	15.98	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and lobate	Dull white
16	16.10	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and lobate	Dull white
17	16.15	Broadly ridged, thick, raised and frilled.	Wavy	Buff
18	15.23	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and lobate	Buff
19	13.65	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and lobate	Buff
20	13.60	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and lobate	Dull white

** Topography and margin descriptions are based on descriptions by Subba Rao and Thakur (1983).

*** Colour description is based on Commonwealth Mycological Institute (CMI) Mycological colour charts (Rayner, 1970).

4.2.3 Cultural characteristics on carrot agar

The results are presented in Table 10. The mean colony diameters of the different isolates were not significantly ($P=0.05$) different from each other (Appendix 6c).

Isolate 13 had broadly ridged, thick, raised and smooth colony while all the other isolates had finely ridged, raised, smooth colonies with thick centres and thin peripheries.

The margins of the colonies of all the isolates were wavy. The colonies were pale-luteous, pale-luteous and white, buff and cream or buff and white.

Table 10. Cultural characteristics of 20 Isolates of Tolyposporium penicillariae on carrot agar

Isolate no.	Mean diameter of colony (mm)	Topography**	Margin**	Colour of colony***
1	14.70	Finely ridged, raised, thick in the centre, thin at the periphery and smooth.	Wavy	Pale luteous
2	15.98	Finely ridged, raised, thick in the centre, thin at the periphery and smooth.	Wavy	Pale luteous
3	15.21	Finely ridged, raised, thick in the centre, thin at the periphery and smooth.	Wavy	Pale luteous
4	16.97	Finely ridged, raised, thick in the centre, thin at the periphery and smooth.	Wavy	Buff to white
5	16.01	Finely ridged, raised, thick in the centre, thin at the periphery and smooth.	Wavy	Buff to cream
6	14.98	Finely ridged, raised, thick in the centre, thin at the periphery and smooth.	Wavy	Pale luteous
7	16.52	Finely ridged, raised, thick in the centre, thin at the periphery and smooth.	Wavy	Pale luteous
8	18.40	Finely ridged, raised, thick in the centre, thin at the periphery and smooth.	Wavy	Buff
9	17.70	Finely ridged, raised, thick in the centre, thin at the periphery and smooth.	Wavy	Buff
10	15.81	Finely ridged, raised, thick in the centre, thin at the periphery and smooth.	Wavy	Buff to white

Table 10 (contd.)

11	15.61	Finely ridged, raised, thick in the centre, thin at the periphery and smooth.	Wavy	Pale luteous
12	16.41	Finely ridged, raised, thick in the centre, thin at the periphery and smooth.	Wavy	Buff to cream
13	19.26	Broadly ridged, thick, raised and smooth	Wavy	Buff to Rosy Buff
1414	15.48	Rinely ridged, raised, thick in the centre, thin at the periphery and smooth.	Wavy	Pale luteous
15	17.40	Finely ridged, raised, thick in the centre, thin at the periphery and smooth.	Wavy	Pale lut- to white
16	15.08	Finely ridged, raised, thick in the centre, thin at the periphery and smooth.	Wavy	Pale luteous
17	16.60	Finely ridged, raised, thick in the centre, thin at the periphery and smooth.	Wavy	Pale lut- to whi.
18	15.89	Finely ridged, raised, thick in the centre, thin at the periphery and smooth.	Wavy	Pale luteous
19	15.80	Finely ridged, raised, thick in the centre, thin at the periphery and smooth.	Wavy	Pale luteous
20.	14.55	Finely ridged, raised, thick in the centre, thin at the periphery and smooth.	Wavy	Pale luteous

** Topography and margin descriptions are based on descriptions by Subba Rao and Thakur (1983).

*** Colour description is based on Commonwealth Mycological Institute (CMI) Mycological colour charts (Rayner, 1970).

4.2.4 Cultural characteristics on Czapek-Dox agar

The results are presented in Table 11. The differences between mean colony diameters of the isolates were significant (P=0.05) (Appendix 6d). Isolate 3 had the greatest radial growth (20.36mm) while isolate 20 had the smallest (12.61mm).

All the isolates had finely ridged, raised, frilled colonies with thick centres and thin peripheries (Plate 2). The margins of colonies in isolates 9, 11, 12 and 16 were wavy and lobate while those of the other isolates were only wavy.

Colony colour was buff for isolates 1, 16 and 20; rosy buff for isolates 2, 3, 6, 15 and 18; and rosy buff to buff for isolates 4, 8, 9, 11, 12 and 14. In the other isolates the colonies were honey, rosy buff to honey, rosy buff to salmon or honey to dull white.

Table 11. Cultural characteristics of 20 Isolates of *Tolyposporium penicillariae* on Czapek-Dox agar

Isolate no.	Mean diameter of colony (mm)*	Topography**	Margin	Colour of colony ***
1	12.98[b]	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Buff
2	13.21[b]	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Rosy Buff
3	20.36[a]	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Rosy Buff
4	18.23[ab]	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Rosy Buff to Buff
5	13.60[b]	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Salmon to Buff
6	14.66[b]	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Rosy Buff
7	13.91[b]	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Rosy Buff to Salmon
8	16.23[ab]	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and lobate	Rosy Buff to Buff
9	14.20[b]	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and lobate	Rosy Buff to Buff
10	12.91[b]	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Honey

Table 11. (contd.)

11	14.95[b]	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and lobate	Buff to Rosy Buff
12	14.73[b]	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and lobate	Buff to Rosy Buff
13	13.55[b]	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Honey to Dull white
14	13.78[b]	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Buff to Rosy Buff
15	16.52[ab]	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Rosy Buff
16	13.74[b]	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and lobate	Buff
17	14.51[b]	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Honey
18	14.18[b]	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Rosy Buff
19	13.61[b]	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Rosy Buff to Salmon
20	12.60[b]	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Buff

* Using Duncan's Multiple Range test, means with the same superscript (P=0.05) are not significantly different.

** Topography and margin descriptions are based on descriptions by Subba Rao and Thakur (1983).

*** Colour description is based on Commonwealth Mycological Institute (CMI) Mycological colour charts (Kayner, 1970).

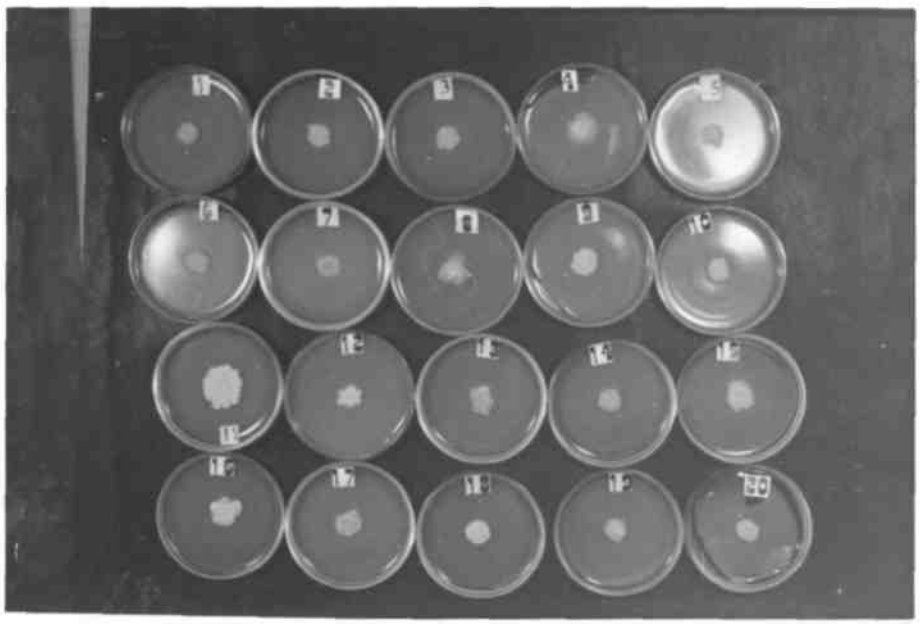


Plate 2 Cultural characters of 20 isolates of Tolyposporium penicillariae on Czapek-Dox agar.

4.2.5 Cultural characteristics on Sartoris 'Best' synthetic agar.

The results are presented in Table 12. There was no significant difference (P=0.05) in the mean colony diameters of the different isolates (Appendix 6e).

Isolates 3, and 16 - 20 had broadly ridged, thick, raised and frilled colonies, while the other isolates had finely ridged, raised, frilled colonies with thick centres and thin peripheries.

The margins of the colonies were wavy for isolates 3 - 12, 17, 18 and 20. Isolates 1, 11, 14, 16 and 19 had wavy and lobate margin while isolate 2 had an entire margin.

The colony colour was rosy buff for isolates 1 - 3, 7, 8, 10 - 12, 14 - 16 and 19; rosy buff to white for isolates 4, 6, 9, 13, 17, 18; and white for isolate 5.

Table 12. Cultural characteristics of 20 isolates of *Tolyposporium penicillariae* on Sartoris 'Best' synthetic agar.

Isolate no.	Mean diameter of colony (mm)	Topography**	Margin**	Colour of colony***
1	15.10	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and lobate	Rosy Buff
2	16.40	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Entire	Rosy Buff
3	17.05	Broadly ridged, thick raised and frilled.	Wavy	Rosy Buff
4	18.30	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Rosy Buff to white
5	14.10	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	White
6	15.94	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Rosy Buff to white
7	16.20	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Rosy Buff
8	16.05	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Rosy Buff
9	15.31	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Rosy Buff to white
10	17.36	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Rosy Buff
11	17.71	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and lobate	Rosy Buff

Table 12 (contd.)

12	16.47	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Rosy Buff
13	14.97	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy	Rosy Buff to white
14	16.05	Finely ridged, raised, thick in the centre, thin at the periphery and frilled.	Wavy and lobate	Rosy Buff
15	17.01	Finely ridged, raised, thick in the centre, thick at the periphery and frilled.	Wavy	Rosy Buff
16	18.12	Broadly ridged, thick, raised and frilled.	Wavy and lobate	Rosy Buff
17	17.78	Broadly ridged, thick, raised and frilled.	Wavy	Rosy Buff to white
18	15.70	Broadly ridged, thick, raised and frilled.	Wavy	Rosy Buff to white
19	16.67	Broadly ridged, thick, raised and frilled.	Wavy and lobate	Rosy Buff
20	15.50	Broadly ridged, thick, raised and frilled.	Wavy	Rosy Buff to buff

** Topography and margin descriptions are based on descriptions by Subba Rao and Thakur (1983).

*** Colour description is based on Commonwealth Mycological Institute (CMI) Mycological colour charts (Rayner, 1970).

4.2.6 General comparison of the growth of 20 isolates of Tolyposporium penicillariae on five culture media.

The growth of the 20 isolates of the pathogen on the above five media was purely sporidial and yeast-like. The radial growth of all the isolates was greatest and fastest on potato dextrose agar while it was least and lowest on potato agar (Fig. 4). The cultural characteristics of Zaria, Dambuo, Bakura, Mokwa and Jalingo isolates on the five media are shown in Plates3 - 7.

4.2.7 Comparison of the growth of 3 isolates of Tolyposporium penicillariae on potato dextrose agar and potato agar media.

The growths of Gombe, Maigana and Bakura isolates on potato dextrose agar and potato agar were evaluated. All the three isolates had more growth on potato dextrose agar than on potato agar (Plate 8).

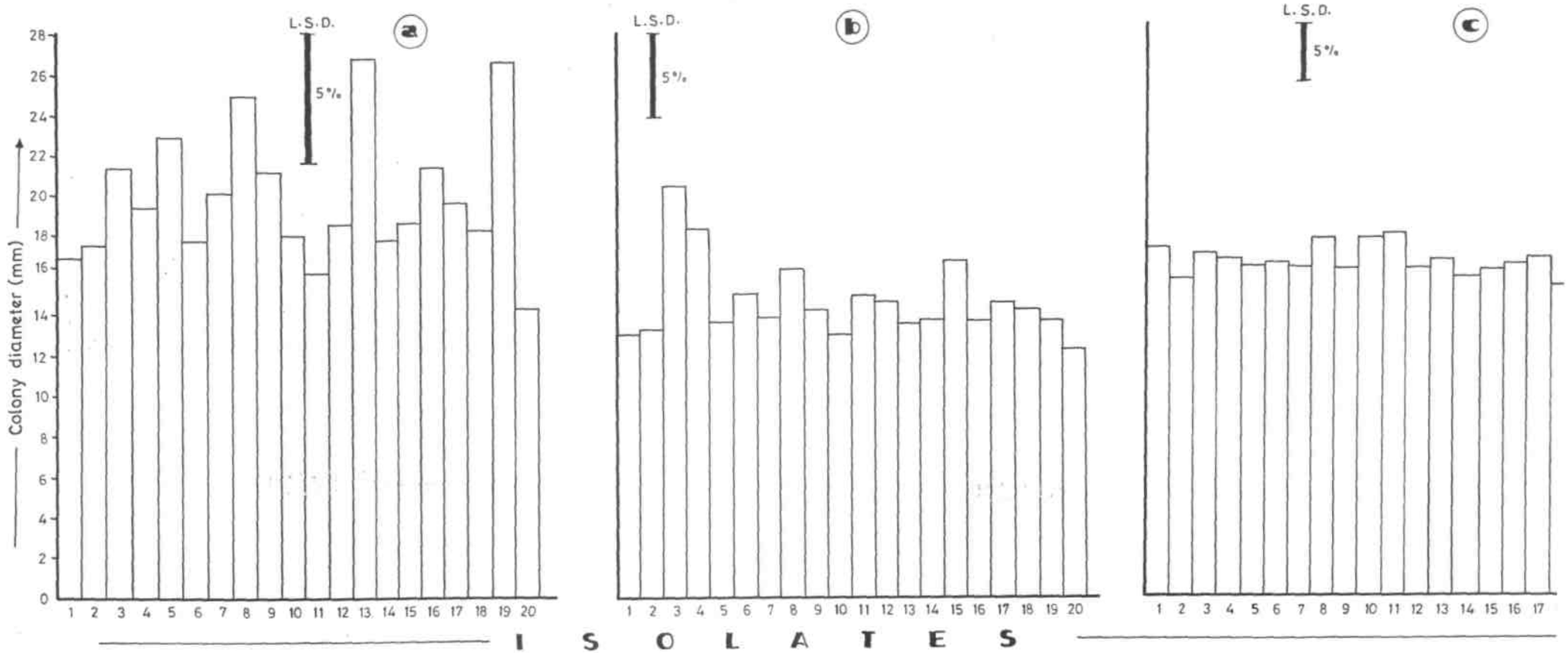


FIG. 4 Diameter of 10 - day old cultures of 20 isolates of Tolyposporium penicillariae at 30°C on (a) Potato dextrose agar, (b) Czapek-Dox agar and (c) Potato agar.



Plate 3. Cultural characters of Zaria isolate on five culture media.

Key to culture media used

1 = Potato dextrose agar.

2 = Potato agar.

3 = Carrot agar.

4 = Czapek-Dox agar.

5 = Sartoris 'Best' synthetic agar.



Plate 4. Cultural characters of Dambuo isolate on five culture media.

Key to culture media used

1 = Potato dextrose agar.

2 = Potato agar.

3 = Carrot agar.

4 = Czapek-Dox agar.

5 = Sartoris 'Best' synthetic agar.



Plate 5. Cultural characters of Bakura isolate on five culture media.

Key to culture media used

1 = Potato dextrose agar.

2 = Potato agar.

3 = Carrot agar.

4 = Czapek-Dox agar.

5 = Sartoris 'Best' synthetic agar.

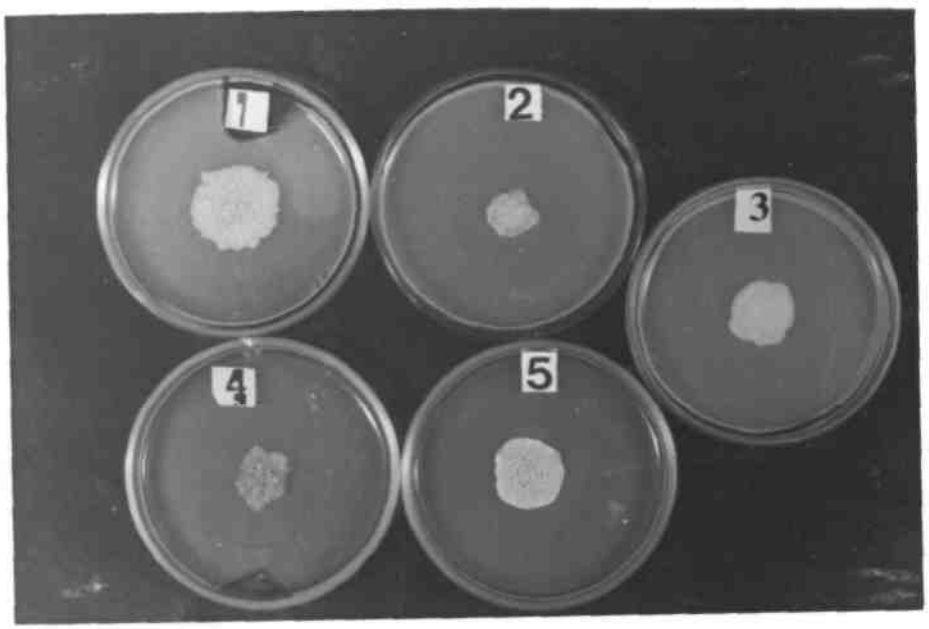


Plate 6. Cultural characters of Mokwa isolate on five culture media.

Key to culture media used

1 = Potato dextrose agar.

2 = Potato agar.

3 = Carrot agar.

4 = Czapek-Dox agar.

5 = Sartoris 'Best' synthetic agar.

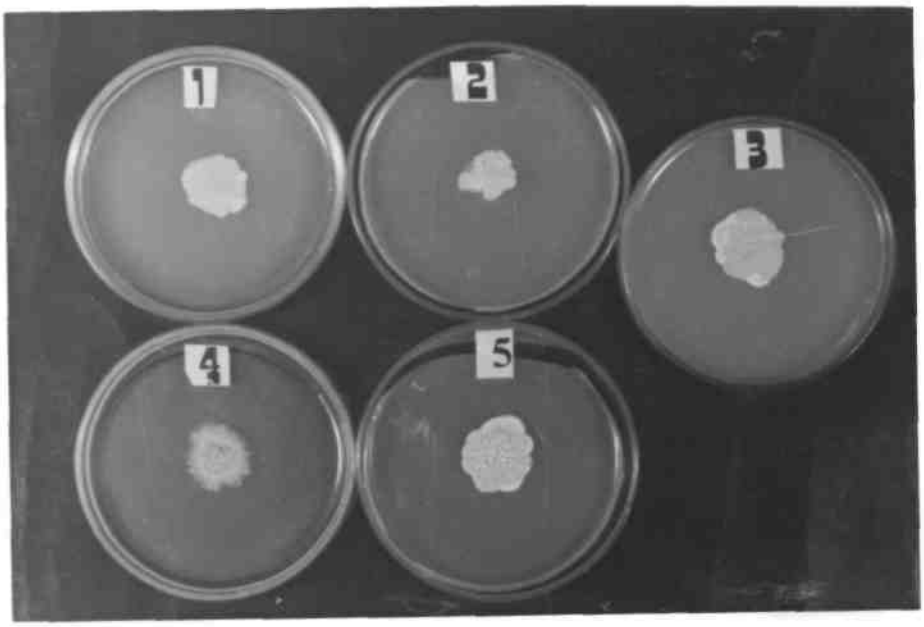


Plate 7. Cultural characters of Jalingo isolate on five culture media.

Key to culture media used

1 = Potato dextrose agar.

2 = Potato agar.

3 = Carrot agar.

4 = Czapek-Dox agar.

5 = Sartoris 'Best' synthetic agar.

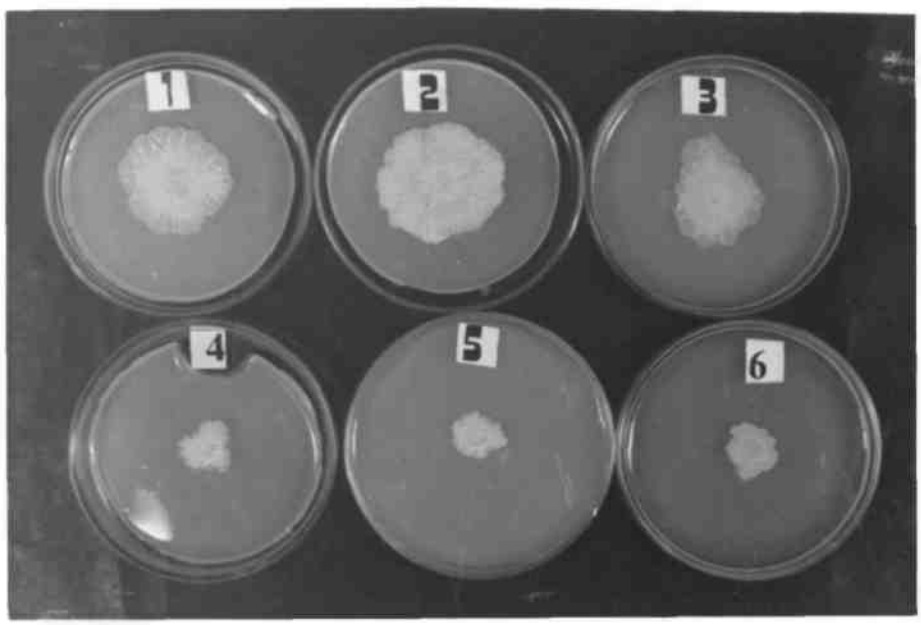


Plate 8. Comparison of growth rate of three isolates of T. penicillariae on potato dextrose agar and potato agar.

1 = Gombe isolate on potato dextrose agar.

2 = Maigana isolate on potato dextrose agar.

3 = Bakura isolate on potato dextrose agar.

4 = Gombe isolate on potato agar.

5 = Maigana isolate on potato agar.

6== Bakura isolate on potato agar.

4.3 Pathogenic Variation in Isolates of Tolyposporium penicillariae

Five varieties of pearl millet were tested for their reaction to inoculation with some of the above isolates in 1984 and 1985.

4.3.1 Pathogenicity of six isolates of Tolyposporium penicillariae in five varieties of pearl millet in 1984

Six isolates were used in a preliminary study in 1984. The mean smut severities of the six isolates in five varieties of pearl millet are presented in Table 13. The results indicate that there were significant differences ($P=0.01$) between the isolates (Appendices 7.1a - 7.1e).

The reaction types of the five varieties of pearl millet to six isolates of T. penicillariae are given in Table 14.

Varieties MDN 145, MDN 143, and S6 were susceptible to all the isolates. MDN 131 was susceptible to Zaria, Samaru, Dambuo and Maiduguri isolates, and moderately susceptible to Kano and Jalingo isolates. MDN 137 was moderately susceptible to Samaru, Dambuo and Maiduguri isolates; it was moderately resistant to Zaria and Kano isolates and resistant to Jalingo isolate.

Table 13. Mean smut incidence (%) of six isolates of Tolyposporium penicillariae on five varieties of pearl millet artificially inoculated in 1984

Isolate	Mean incidence of smutted grains (%) in varieties				
	MDN 131	MDN 137	MDN 145	MDN 143	S6
Zaria	20.9 (23.0)^{abc} *	5.6 (9.6)^{abcd} *	65.0 (57.4)^{bcdef} *	51.7 (48.7)^{abcde} *	79.7 (69.5)^{ab} *
Samaru	16.1 (20.1)^{abcd}	6.8 (11.7)^{abc}	73.3 (60.8)^{ab}	63.3 (56.8)^{ab}	82.8 (69.5)^{abc}
Kano	14.5 (16.9)^{abcde}	3.7 (8.6)^{abcd}	70.3 (60.1)^{abcde}	62.0 (52.9)^{abc}	51.0 (47.5)^{de}
Dambuo	22.0 (25.6)^{ab}	11.5 (16.0)^{a}	84.2 (69.9)^{a}	70.7 (59.3)^{a}	90.3 (76.5)^{a}
Maiduguri	22.8 (27.3)^{a}	7.6 (13.3)^{ab}	71.3 (60.2)^{abcd}	53.0 (46.6)^{bcdef}	56.7 (51.0)^{d}
Jalingo	5.0 (10.1)^{de}	1.2 (4.9)^{bcd}	38.3 (37.3)^{g}	30.7 (32.9)^{g}	21.0 (26.8)^{f}

Using Duncan's Multiple Range test, means in each column with the same superscript are not significantly different (P=0.01)

* Mean of three replicates.

Values in bracket = Angular transformed values.

Table 14. Reaction types of five varieties of pearl millet to six isolates of Tolyposporium penicillariae.

Isolate	Reaction type				
	MDN 131	MDN 137	MDN 145	MDN 143	S6
Zaria	S	MR	S	S	S
Samaru	S	MS	S	S	S
Kano	MS	MR	S	S	S
Dambuo	S	MS	S	S	S
Maiduguri	S	MS	S	S	S
Jalingo	MS	R	S	S	S

4.3.2 Pathogenicity of 10 isolates of *Tolyposporium penicillariae* in five varieties of pearl millet in 1985.

Ten isolates were used in a more detailed study in 1985. The results presented in Table 15 indicate that there were significant differences (P=0.01) between the pathogenicities of the isolates (Appendices 7.2a - 7.2e).

The reaction types of the five varieties of pearl millet to the ten isolates of *T. penicillariae* are given in Table 16.

Varieties MDN 145, MDN 143 and S6 were susceptible to all the isolates. MDN 131 was susceptible to Zaria, Gombe, Dambuo and Maiduguri isolates; it was moderately susceptible to Samaru, Bakura, Gummi and Kano isolates; resistant and moderately resistant to Jalingo and Mokwa isolates respectively. MDN 137 was susceptible to Gombe and Dambuo isolate and moderately resistant to Bakura, Gummi and Kano isolates. It was resistant to Jalingo and Mokwa isolates.

The Jalingo isolate could be easily differentiated from the others because it was least pathogenic to MDN 131 and MDN 137. The interactions between the isolates and the five varieties are shown in Fig. 5. On the basis of the apparent differential interactions between the isolates of the pathogen and two of the five varieties, namely MDN 131 and MDN 137, the isolates can be placed in the following

four pathogenic groups:

Group I	:	Gombe and Dambuo.
Group II	:	Maiduguri, Zaria and Samaru.
Group III	:	Bakura, Gummi and Kano.
Group IV	:	Jalingo and Mokwa.

Table 4. Mean smut incidence (%) of 10 isolates of Tolyposporium penicillariae on five varieties of pearl millet artificially inoculated in 1985.

Isolate	Mean incidence of smutted grains (%) in varieties				
	MDN 131	MDN 137	MDN 145	MDN 143	S6
Zaria	44.0(41.3)* ab	5.3(10.9)* abcd	77.0(64.7)* abcd	58.9(53.1)* abcdefg	58.7(53.9)* bcde
Samaru	12.8(17.0)* def	9.3(12.9)* abcd	67.3(55.6)* bcdefg	55.0(48.0)* abcdefghi	64.7(54.0)* bcd
Jalingo	1.3(4.7)* f	1.0(4.8)* cd	27.3(30.6)* i	29.3(31.7)* j	15.3(19.7)* j
Gombe	53.3(47.0)* a	19.7(23.8)* a	73.0(60.1)* abcdef	72.3(59.7)* abcd	76.7(63.7)* abc
Dambuo	18.4(21.8)* cd	17.7(21.8)* ab	87.1(71.7)* a	70.3(60.4)* a	88.0(75.3)* a
Maiduguri	32.0(33.4)* bc	10.9(17.2)* abc	77.7(66.7)* ab	59.0(50.3)* abciefgh	60.7(53.6)* bcdef
Bakura	9.5(13.9)* def	3.3(6.5)* cd	51.7(46.0)* gh	47.7(43.4)* ghji	37.7(37.3)* ghi
Gummi	15.4(19.2)* de	5.1(9.3)* bcd	77.3(66.4)* abc	67.0(57.6)* abcdef	70.9(66.1)* ab
Mokwa	7.5(9.4)* def	0.2(1.2)* d	45.7(42.3)* ghi	70.3(59.9)* ab	40 3(39.7)* gh
Kano	14.9(16.9)* def	4.3(9.1)* bcd	71.2(60.9)* abcde	70.1(58.8)* abcde	45.3(42.6)* defg

Using Duncan's Multiple Range test, means in each column with the same superscript are not significantly different (P=0.01).

* Mean of three replicates.

Table 16. Reaction types of five varieties of pearl millet to 10 isolates of Tolyposporium penicillariae.

Isolate	Reaction type				
	MDN 131	MDN 137	MDN 145	MDN 143	S6
Zaria	S	MS	S	S	S
Samaru	MS	MS	S	S	S
Jalingo	R	R	S	S	MS
Gombe	S	S	S	S	S
Dambuo	S	S	S	S	S
Maiduguri	S	MS	S	S	S
Bakura	MS	MR	S	S	S
Gummi	MS	MR	S	S	S
Mokwa	MR	R	S	S	S
Kano	MS	MR	S	S	S

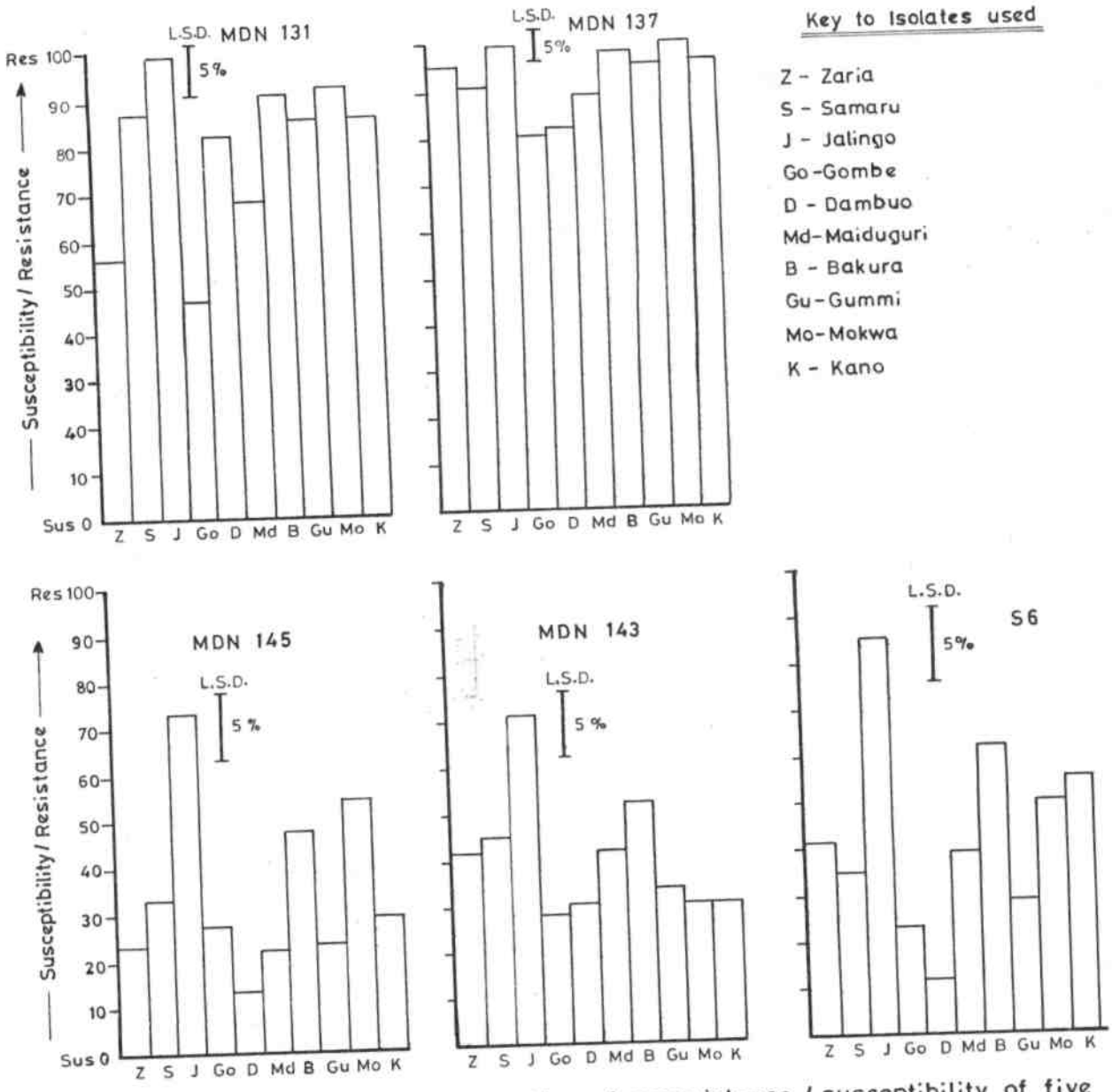


FIG. 5 A diagramatic representation of resesistance / susceptibility of five pearl millet varieties to 10 isolates of Tolyposporium penicillariae.

5. DISCUSSION

The results of this study have shown that some variations exist in the pearl millet smut pathogen, T. penicillariae, with respect to morphological and cultural characteristics as well as with regards to their pathogenicities in five millet varieties.

Morphological characteristics such as spore wall ornamentation, spore colour and size, and spore germination are known to contribute greatly to the dynamic taxonomic history of smut fungi (Holton et al., 1968). In Tilletia tritici in wheat, the spores of some races are more prominently reticulate than those of other races (Holton, 1935). Races of Sphacelotheca sorghi have been reported to exhibit some differences in length, colour and manner of rupturing of the sori (Melchers et al., 1930).

In this study, morphological variation in T. penicillariae was reflected in sizes of sporeballs, teliospores and sporidia. No variation was found in the number of teliospores per sporeball.

Subba Rao and Thakur (1983) reported variation in the pattern of germination of teliospores aggregated in sporeballs of T. penicillariae. In this study, three different patterns of teliospore germination were observed

in all the isolates, namely sporidia borne laterally or/ and terminally on the promycelium, sporidia produced in clusters on promycelium and sporidia produced in branched chains directly from the teliospores without any promycelium.

It has been reported by Subba Rao and Thakur (1983) that no germination of aggregated teliospores occurred up to 9 hours of incubation at 15°C to 40°C in water. They also reported that promycelia became visible by 10 hours, and that by 16 hours most sporidia were liberated from the promycelia. By contrast, results of the present study showed that germination of aggregated teliospores of some isolates occurred at 6 hours of incubation at 30°C in water. However, the time required for germination of the aggregated teliospores in water at 30°C varied between the different isolates. For instance, the teliospores of some isolates germinated within six hours while others germinated six hours later. There was also some variation in the viability of the teliospores. In general, after the onset of germination time of teliospores, there was a gradual increase in germination percentage between one and three hours. This was followed by a rapid increase in germination between four and six hours of onset of germination.

Variation in smut fungi as expressed by differences in cultural characters is common in many species (Fischer and Holton, 1957). This is usually exhibited in the rate of growth, type of growth, colour of colony and consistency (that is, whether predominantly sporidial or mycelial). Rodenhiser (1928) has reported that some races of loose smut of wheat, Ustilago nuda differ widely in growth rate in culture at the same temperature. Specialization based on cultural characteristics has also been reported in long smut of sorghum, Tolyposporium ehrenbergii (Kamat, 1933).

In the present study, variations in growth rate, colour, margin and topography of colonies of the isolates were observed. These cultural variations appeared to depend on the media on which the isolates were grown. Thus, these differences were best manifested on potato dextrose agar and Czapek-Dox agar, that is, a wide range of cultural types occurred in these two media.

In discussing variations in cultural characteristics among isolates of smut fungi, Fischer and Holton (1957) concluded that they are a function of the genetics of the organism as well as the type and constituents of the culture media used in different studies. Fischer and Holton (1957) demonstrated the phenomenon in grass smuts including Ustilago bullata, which grew better on potato

dextrose agar and Blakeslee's agar than they did on the other media studied by them.

Subba Rao and Thakur (1983) reported that T. penicillariae grew better on potato and carrot agar media than on potato dextrose agar. This is contrary to the observations made in the present study. All the isolates showed more growth on potato dextrose agar than on potato agar or carrot agar. The discrepancy between results obtained in this study and those of Subba Rao and Thakur (1983) may possibly be due to genetic differences between India and Nigeria isolates of the pathogen. On the other hand, the observations in the present study that growths of all the isolates on all the media were purely sporidial and yeast-like (even after repeated subculturing) are in conformity with those of Subba Rao and Thakur (1983).

Pathogenic variation has been observed to be common in many species of cereal smuts and have been reported by previous workers in Tilletia spp. (Faris, 1924), Ustilago zeae (Stakman and Christensen, 1926), Sphacelotheca sorghi (Melchers et al., 1930) and in Ustilago bullata (Meiners and Fischer, 1952).

The pathogenicity tests carried out in this study with isolates from different Savanna zones of Nigeria provided evidence that T. penicillariae is specialized with respect to the varieties and types of pearl millet it attacks. Two varieties, viz MDN 131 and MDN 137 proved to be better than the other three varieties in manifesting the pathogenic differences between isolates of the fungus. In this respect, the two varieties were classified as susceptible or moderately susceptible to isolates from Gombe, Maiduguri, Dambuo, Zaria and Samaru while they were resistant to Jalingo isolate.

On the basis of their interactions with the two varieties, the 10 isolates may be divided into four pathogenic groups, namely highly virulent (Gombe and Dambuo isolates); virulent (Maiduguri, Zaria and Samaru isolates); moderately virulent (Bakura, Gummi and Kano isolates) and weakly virulent (Jalingo and Mokwa isolates). All the isolates were however, virulent in the three other host varieties. It is interesting to note that the two least virulent isolates were from the most humid areas. In addition, the Jalingo isolate was obtained from maiwa (a photoperiod sensitive) type of pearl millet while the rest of the isolates were from gero (a non-photoperiod sensitive) type. This could possibly be one of the reasons for its low virulence.

The diagramatic representations of the interactions between the isolates and the five varieties appear to suggest that there were differential interactions between some isolates and the varieties. For example MDN 145 appears to possess vertical resistance (Sensu Van der Plank, 1963; 1982) to Jalingo, Mokwa and Bakura isolates. The resistance of the same variety to Zaria, Gombe, Dambuo, Maiduguri and Gummi isolates and to some extent Samaru, and Kano isolates appears to be about the same; that is, it is approximately uniformly resistant to these isolates thereby satisfying Van der Plank (1963; 1982) concept of horizontal resistance. A similar conclusion can be made about the interaction between MDN 131, MDN 137, MDN 143 and S6 and the isolates although the isolates to which the respective varieties are vertically or horizontally resistant are not necessarily the same. It appears therefore, that these varieties possess both vertical and horizontal resistance to the isolates of T. penicillariae.

The degree of virulence of Gombe and Jalingo isolates could probably be correlated with their rate of growth on potato dextrose agar and on the size of sporidia produced on the same medium. For example, the Gombe isolate was the most pathogenic and it had

relatively long sporidia and the highest growth rate. The Jalingo isolate, on the other hand, was the least pathogenic and had relatively short sporidia and the lowest growth rate. However, all this could possibly be incidental observations, since corresponding characteristics are not shared by other isolates in the same pathogenic groups as the Gombe and Jalingo isolates. For instance, Dambuo isolate was as virulent as the Gombe isolate but did not possess the above cultural and morphological characteristics as the latter. Also, Mokwa isolate was as weakly virulent on MDN 131 and MDN 137 as the Jalingo isolate, although it was not culturally or morphologically similar to the Jalingo isolate.

Thus, no definite correlation could be established between either morphological or cultural characteristics of the different isolates and their pathogenicity. It could be that morphological and cultural characters have no definite relationship with virulence in T. penicillariae.

This study has provided basic information on T. penicillariae in Nigeria, and also raised some questions for further investigations. The information gathered should be used by Nigerian millet researchers in careful planning of the breeding programme for incorporating resistance against pearl millet smut. In particular, the already

existing pathogenic variation within the pathogen should be taken into consideration in preparing inoculum for artificial inoculation of pearl millet lines during screening for resistance. For instance, it is important that such inoculum includes one of the most virulent isolates.

Whether or not it is necessary to use a mixture consisting of representatives of the four pathogenic groups is a matter for future research, especially in respect of the compatibility or otherwise of the isolates. Also, the method of inoculation, that is, whether isolates should be mixed and applied simultaneously or whether the components of the mixture should be applied sequentially should be determined by future research.

6. SUMMARY AND CONCLUSIONS

Morphological, cultural and pathogenic variations in T. penicillariae, the inducing organism of smut of pearl millet were investigated. Smut specimens were collected from various millet growing ecological zones in northern Nigeria. Twenty isolates were used to study morphological and cultural variations while 10 of them were selected for pathogenicity tests.

Morphological variation was reflected in the sizes of sporeballs, teliospores and sporidia. The time required for germination of teliospores and the viability (as reflected by percentage germination) of the aggregated teliospores of the different isolates also varied.

In culture, the fungus showed variation in topography, margin and colour of colonies in the following five artificial media: potato dextrose agar, potato agar, carrot agar, Czapek-Dox agar and Sartoris 'Best' synthetic agar. All isolates of the fungus grew well on potato dextrose agar, followed by Czapek Dox agar, while their growths on the other three media were not significantly different. The growth of all the isolates of the pathogen was sporidial and yeast-like on all the media.

Pathogenic variation was reflected by the reaction of five varieties of pearl millet (MDN 131, MDN 137, MDN 143, MDN 145 and S6).to ten isolates of the pathogen. On the basis of their interaction with MDN 131 and MDN 137, the ten isolates could be placed in four pathogenic groups, namely highly virulent (Gombe and Dambuo isolates), virulent (Maiduguri, Zaria and Samaru isolates), moderately virulent (Bakura, Gummi and Kano isolates) and weakly virulent (Jalingo and Mokwa isolates). By contrast, all the isolates were pathogenic in MDN 143, MDN 145 and S6.

In general, however, no definite relationship could be established between either morphological or cultural characteristics and the pathogenicity of the different isolates.

The relevance of this study in breeding pearl millet for resistance to smut has been discussed. However, some more work is needed before a reliable methodology can be evolved for screening millet cultivars for effective resistance to smut disease.

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APPENDICES

Appendix I. Analysis of variance of dimensions of sporeballs of the 20 isolates of *T. penicillariae*

(a) Length of spore ball

Source	DF	SS	MS	F
Treatments	19	10486	55.89	3.66*
Error	180	27114.50	150.64	
Total	199	37600.50		

(b) Width of spore ball

Source	DF	SS	MS	F
Treatments	19	8746.75	460.36	5.49*
Error	180	15098.80	83.88	
Total	199	23845.50		

* - Significant at 5% level.

Appendix 2. Analysis of variance of mean number of teliospores per sporeball of the 20 isolates of T. penicillariae

Source	DF	SS	MS	F
Treatments	19	285264	15013.90	0.91^{NS}
Error	80	131953	16494.20	
Total	99	16048		

NS = Not significant at 5% level.

Appendix 3. Analysis of variance of dimensions of the Teliospores of the 20 isolates of T. penicillariae

(a) Length of Teliospores:

Source	DF	SS	MS	F
Treatments	19	46.04	2.42	6.17*
Error	180	70.75	0.39	
Total	190	116.79		

(b) Width of Teliospores:

Source	DF	SS	MS	F
Treatments	19	40.88	2.15	7.24*
Error	180	53.56	0.29	
Total	190	94.44		

* = Significant at 5% level.

Appendix 4. Analysis of variance of mean germination percentage of aggregated teliospores of 20 isolates of T. penicillariae between 6 and 15 hours of incubation at 30°C.

Source	DF	SS	MS	F
Hours	9	167755	18639.40	458.63**
Isolates	19	98183.30	5167.54	127.15**
Hours x Isolates	171	37018.20	216.48	5.33**
Error	400	16256.70	40.64	
Total	599	319213		

** = Highly significant at 1% level.

Appendix 5. Analysis of variance of the mean length of sporidia of the 20 isolate of T. penicillariae grown on potato dextrose agar medium

Source	DF	SS	MS	F
Treatments	19	332.40	17.49	2.84*
Error	80	492.44	6.16	
Total	90	824.84		

* = Significant at 5% level.

Appendix 6. Analysis of variance of diameter of colonies of the 20 isolates of **T. penicillariae** on five different media

(a) Diameter of colonies on potato dextrose agar medium

Source	DF	SS	MS	F
Treatments	19	664.37	34.97	2.23*
Error	40	626.84	15.67	
Total	59	1291.21		

(b) Diameter of colonies on potato agar medium

Source	DF	SS	MS	F
Treatments	19	59.33	3.12	1.06^{NS}
Error	40	118.29	2.96	
Total	59	177.63		

(c) Diameter of colonies on carrot agar medium

Source	DF	SS	MS	F
Treatments	19	86.65	4.56	0.32^{NS}
Error	40	573.65	14.34	
Total	59	660.30		

(d) Diameter of colonies on (zapek-Dox agar medium

Source	DF	SS	MS	F
Treatments	19	208.681	10.983	1.529*
Error	40	287.265	7.181	
Total	59	495.947		

Appendix 6. Contd.

(e) Diameter of colonies on sartoris 'Best' synthetic agar medium

Source	DF	SS	MS	F
Treatment	19	71.99	3.788	1.067^{NS}
Error	40	141.96	3.549	
Total	59	213.951		

* = Significant at 5% level.

NS = Not significant at 5% level.

Appendix 7. Analysis of variance of Pathogenicity of isolates T. penicillariae of five varieties of pearl millet

Appendix 7.1 For 1984

(a) Variety: MDN 131

Source	DF	SS	MS	F
Isolates	6	8401.90	1400.30	8.14**
Error	98	16862.20	172.10	
Total	104	25264.10	242.90	

(b) Variety: MDN 137

Source	DF	SS	MS	F
Isolates	6	2599.23	433.20	5.31**
Error	98	7991.66	81.55	
Total	104	10590.89	101.84	

Appendix 7.1 Contd.

(c) Variety: MDN 145

Source	DF	SS	MS	F
Isolates	6	45759.10	7626.50	29.59**
Error	98	25255.60	257. 70	
Total	104	71014.60	682.80	

(d) Variety: MDN 143

Source	DF	SS	MS	F
Isolates	6	34975.00	5829.20	20.18**
Error	98	28307.90	288.90	
Total	104	63282.90	608.50	

(e) Variety: S6

Source	DF	SS	MS	F
Isolates	6	63603.50	10600.60	41.06**
Error	98	25300.60	258.60	
Total	104	88904.10	854.80	

** = Highly significant at 1% level.

Appendix 7.2 [illegible]

(a) Variety: MDN 131

Source	DF	SS	MS	F
Isolates	10	31978.40	3197.80	20.31**
Error	154	24242.70	157.40	
Total	164	56221.20	342.80	

Appendix 7.2 Contd.

(b) Variety: MDN 137

Source	DF	SS	MS	F
Isolates	10	9049.47	904.95	10.23**
Error	154	13620.88	88.45	
Total	164	22670.35	138.23	

(c) Variety: MDN 145

Source	DF	SS	MS	F
Isolates	10	53097.80	6309.80	27.27**
Error	154	35632.30	231.40	
Total	164	98730.10	602.00	

(d) Variety: MDN 143

Source	DF	SS	MS	F
Isolates	10	48118.90	4811.90	22.65**
Error	154	32711.20	212.40	
Total	164	80830.10	492.90	

(e) Variety: 56

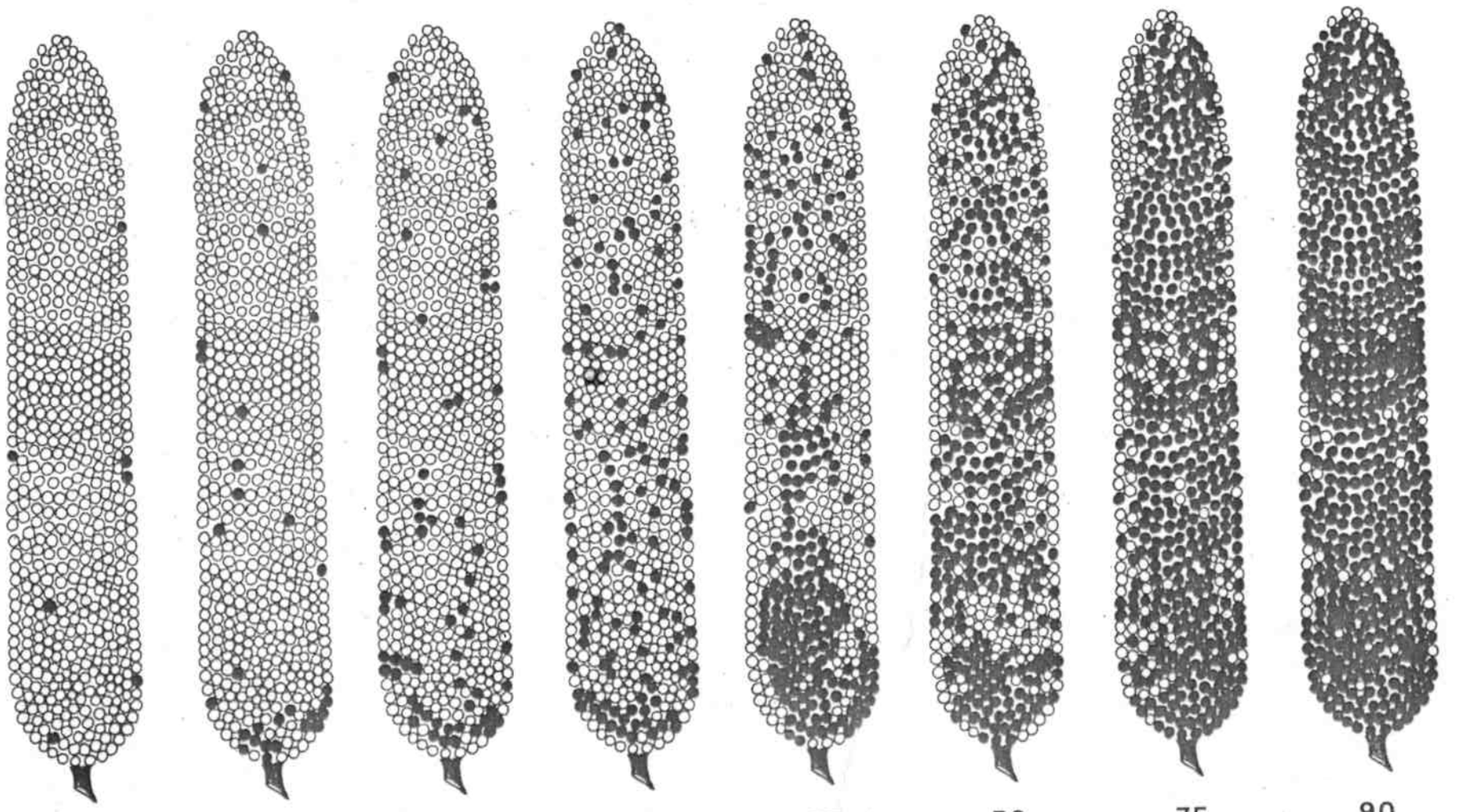
Source	DF	SS	MS	F
Isolates	10	67359.40	6735.90	24.33**
Error	154	42630.00	276.80	
Total	164	109989.40	670.70	

** = Highly significant at 1% level.

Appendix 8

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PEARL MILLET SMUT SEVERITY ASSESSMENT KEY



ICR 81-0073