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Microorganisms asssociated with postharvest spoilage of yams

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ABSTRACT

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This study isolated the microorganisms causing major spoilage of yams collected during a two-year period from various markets in Orissa, India. Seven fungal and two bacterial species (*Erwinia* sp. and *Serratia* sp.) were isolated from rotted yam tubers. *Aspergillus awamori*, *A. versicolor*, *Botryodiplodia theobromae*, *Fusarium solani*, *Penicillium decumbens* and *P. purpurogenum* caused primary dry rot while *Rhizopus oryzae* and *Serratia* sp. caused primary soft rot. *Erwinia* sp. was responsible for the secondary soft rot. The nature of the fungal rot was dependent on secondary infection by *Erwinia*: soft or wet rot when there was secondary infection by *Erwinia* sp. and dry rot when there was none.

Keywords: microorganisms. spoilage. tropics. yams.

INTRODUCTION

Yams, the starchy tubers of the genus *Dioscorea,* are among the important tuber crops in the tropics. There is hardly any tropical country, apart from those in the most arid, where yams are not grown. The genus *Dioscorea* has several species, of which *D. alata* (greater yam) and *D. esculenta* (lesser yam) are very common in India. The crop is harvested once a year (June to December), and usually stored using traditional methods (Ghosh *et al.*, 1988).

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Postharvest losses are manifested by a loss of quantity or quality or both due to pathological, physiological and mechanical damages or various combinations of these factors (Coursey and Booth, 1972). In yams, these factors are interrelated since mechanical injury and physiological stress may greatly influence the susceptibility of the tubers to diseases.

The largest postharvest losses in yams result from microbial attacks but the pathogenicity of the fungi and bacteria reported as "associated" with yam rots in the tropics has not been established (Ghosh *et al.*, 1988). Moreover, some species may be regarded as important pathogens in one country but as minor or non-pathogens in another. The discrepancies can be due to the differences in climate, experimental material, environmental conditions and investigation methods.

Reports from Africa revealed that the most common yam rots there include *Aspergillus* rot caused by *Aspergillus niger* V. Tieghem (Ogundana *et al.*, 1970; Ikotun, 1983), blue green mould rots caused by *Penicillium* sp. (Adeniji, 1970) and *Botryodiplodia* rot caused by *Botryodiplodia theobromae* Pat (Adeniji, 1970); Ogundana, 1983; Aderiye and Ogundana, 1984). There have also been reports on spoilage of yams exported to the UK from Ghana, Nigeria and West Indies (Noon and Colhoun, 1979; Plumbley *et al.*, 1985). In India, only few studies have been conducted on yam spoilage, although Maheswari *et al.* (1983) reported *Aspergillus* rot as a common postharvest rot of yams during transport and marketing.

This study presents the isolation and identification of the major spoilage microorganism of yams collected from various markets in Orissa, a major yam growing state in India.

MATERIALS AND METHODS

Dioscorea tubers were sampled during a two-year period (January 1996 - January 1998) from various markets in Orissa, India. Samples of diseased materials (8-10 per lot showing identical symptoms) were brought to the laboratory, where isolations were made and isolates were tested for pathogenicity (Ray and Misra, 1995).

Isolations were done aseptically inside a laminar flow inoculation chamber using the advancing edges and from the center of lesions. Pieces of diseased tissues exposed by cutting were transferred to potato dextrose agar (PDA) and malt dextrose agar (MDA) plates and kept in a biological oxygen demand

(BOD) incubator at 28 °C. The fungi (on PDA) or bacteria (on MDA) which developed were repetitively sub-cultured until pure and then grown on agar slants. Cultures from single spores or single hyphal tips were made before testing for pathogenicity.

Yam tubers which were free from cuts and bruises were washed in running tap water for 10 min, surface disinfected with a 10% (v/v) sodium hypochlorite solution for 1 min, rinsed in water and air dried at ambient temperature (28 ± 2°C). Each tuber was then sliced into 5 cm circular pieces. Two inoculations were made on each tuber slice under laminar flow. This was accomplished by placing 5 mm diameter agar discs taken from the margin of 5-day old cultures on PDA or MDA, into cavities produced by inserting a sterile 5 mm diameter cork borer at about 3 cm deep into the tubers to remove the cores of tissue. Cores were then replaced and the wounds were sealed with liquid parafin wax at the lowest possible temperature. Controls consisting of sterile agar discs were likewise placed into the cavities. There were 4 successive experiments involving 10 tubers each for a total of 80 infection sites scored per isolate. Inoculated and control tubers were placed in a previously disinfected BOD incubator at 28°C arranged for air circulation around the tubers. At the end of the incubation period (varying between 7 & 21 days), the tubers were cut diametrically across the point of inoculation and the symptoms of any rot which had developed were recorded. Rot incidence (I) was calculated by the following formula:

$$\text{Rot incidence (I) Frequency} = \frac{\text{No. of infected tuber units} \times 100}{\text{Total no. (healthy \& infected) of units assessed}}$$

An isolated microorganism was considered pathogenic when after inoculation, it was recovered from the decayed tissue of at least one site/tuber. Each isolate was re-isolated from about 80% of the tubers inoculated. The fungal isolates were sent to the Institute of Microbial Technology, Chandigarh, India for identification. The bacteria were identified as *Erwinia* sp. and *Serratia* sp. following the method described in Bergey's Manual of Systematic Bacteriology, Vol. I (Krieg, 1984).

In follow up experiments, inoculations were made by placing agar discs taken from individual fungal cultures on PDA and a bacterial (*Erwinia* or *Serratia* sp.) culture on MDA simultaneously, and icubating them at 28°C as described above to distinguish between primary and secondary infections. Controls consisting of sterile agar discs were maintained as above.

RESULTS AND DISCUSSION

Eight fungal isolates belonging to 7 different species and 2 bacteria were isolated from diseased yam tubers (Table 1). All the fungi isolated were pathogenic and caused primary dry rots, except *R. oryzae* which caused soft rot, whereas *Serratia* sp. which produced an orange-red pigment over the affected surface, caused extensive primary soft rot. Furthermore, the disease symptoms on tuber slices inoculated with putative pathogens were similar to those observed on the original diseased tubers.

Table 1. Fungi and bacteria isolated from spoiled yam tubers

Microorganisms isolated	IMT code number
Aspergillus awamori Nakazawa	2879
A. versicolor (Vuill) Tiraboschi	2936
Botryodiplodia theobromae Pat.	2892
Fusarium solani (Martius) Sacc.	2935
Penicillium decumbens Thom.	2881
P. purpurogenum Stoll (isolate 1)	2877
P. purpurogenum Stoll (isolate 2)	2878
Rhizopus oryzae Went & Prins. Geerl.	2880
Erwinia sp.	-
Serratia sp.	-

IMT - Institute of Microbial Technology, Chandigarh, India

All the fungi, except *R. oryzae* and *F. solani,* were present in more than 50% of the samples (Table 2). *Rhizopus* rot caused by *Rhizopus oryzae* or *R. stolonifer* had been recorded in Nigeria, Ivory Coast (Snowdon, 1991), Brazil (De Moura, 1980) and also on yams exported to the USA from Puerto Rico (Snowdon, 1991). The symptoms of *Rhizopus* rot (Fig. 1) were similar to those reported in sweetpotato (Ray *et al.*, 1994). It is sometimes known as watery rot as infected tissue is mottled down and soft, and in a humid atmosphere the cut surface is covered by a copious growth of coarse white mould strands.

Botryodiploida rot is common in tuber crops (Ray *et al.*, 1994). However, it had not been reported earlier in yams from India. The affected tissues were found to be dark brown or black and the margin between diseased and healthy tissues was a distinct brown line (Fig. 2). Minute black bodies

Table 2. Incidence of storage rots caused by pathogens in yams collected from different markets

Pathogens	% incidence of rots
Aspergillus awamori	56.8 ± 8.2
A. versicolor	54.3 ± 3.6
Botryodiplodia theobromae	66.9 ± 10.1
Fusarium solani	42.3 ± 3.8
Penicillium decumbens	69.8 ± 8.0
P. purpurogenum	66.4 ± 6.8
Rhizopus oryzae	32.0 ± 2.6
Erwinia sp.	66.8 ± 4.6
Serratia sp.	13.6 ± 2.8

± standard error

(Pycnidia) eventually developed on the surface, as reported in sweetpotato (Ray & Punithalingam, 1996). *B. theobromae* produced a dry rot, however, secondary infection by *Erwinia* sp. led to soft rot.

Aspergillus niger V. Thieghem (Ikotun, 1983; Snowdon, 1991) has been reported as a pathogen of yam tubers. Two other species, *A. awamori* and *A. versicolor*, more or less cause similar symptoms caused by *A. niger*. The affected flesh was either fawn or brown (Fig. 3). Lesions were generally firm, unless secondary infection by *Erwinia* sp. made the tubers soft.

Penicillium rot (blue and green mould rots) is very common in yams exported to the UK from Nigeria. The casual agent is *Penicillium crustosum* Thom., while *P. gladioli* Mc Culloch & Thom. has been found on tubers exported to the USA from Cuba and Puerto Rico (Ricci *et al*., 1978). *P. sclerotigenum* Yamam was described in Japan (Plumbly *et al*., 1985). However, the incidence of blue and green mould rot of yams caused by *P. purpurogenum* Stoll and *P. decumbens* Thom. is unknown. The symptoms were more or less similar. Rotted tissue was pale to dark brown inside and the other affected surface showed blue or pale green color (Fig 4). Secondary *Erwinia* sp. infection could result in wet rot. Among the strains of *Penicillium purpurogenum* isolated, the culture MTCC 2878 produced more mycelial mass than the culture MTCC 2877 (Anantapadmanabhan, pers. comm.).

F. moniliforme has been recorded as a pathogen in dry rots of yams in Nigeria (Nwakiti and Arene, 1978; Ogundana and Dennis, 1981). *F. oxysporum* in Puerto Rico and *F. solani* in India (Sharma and Chatterjee,

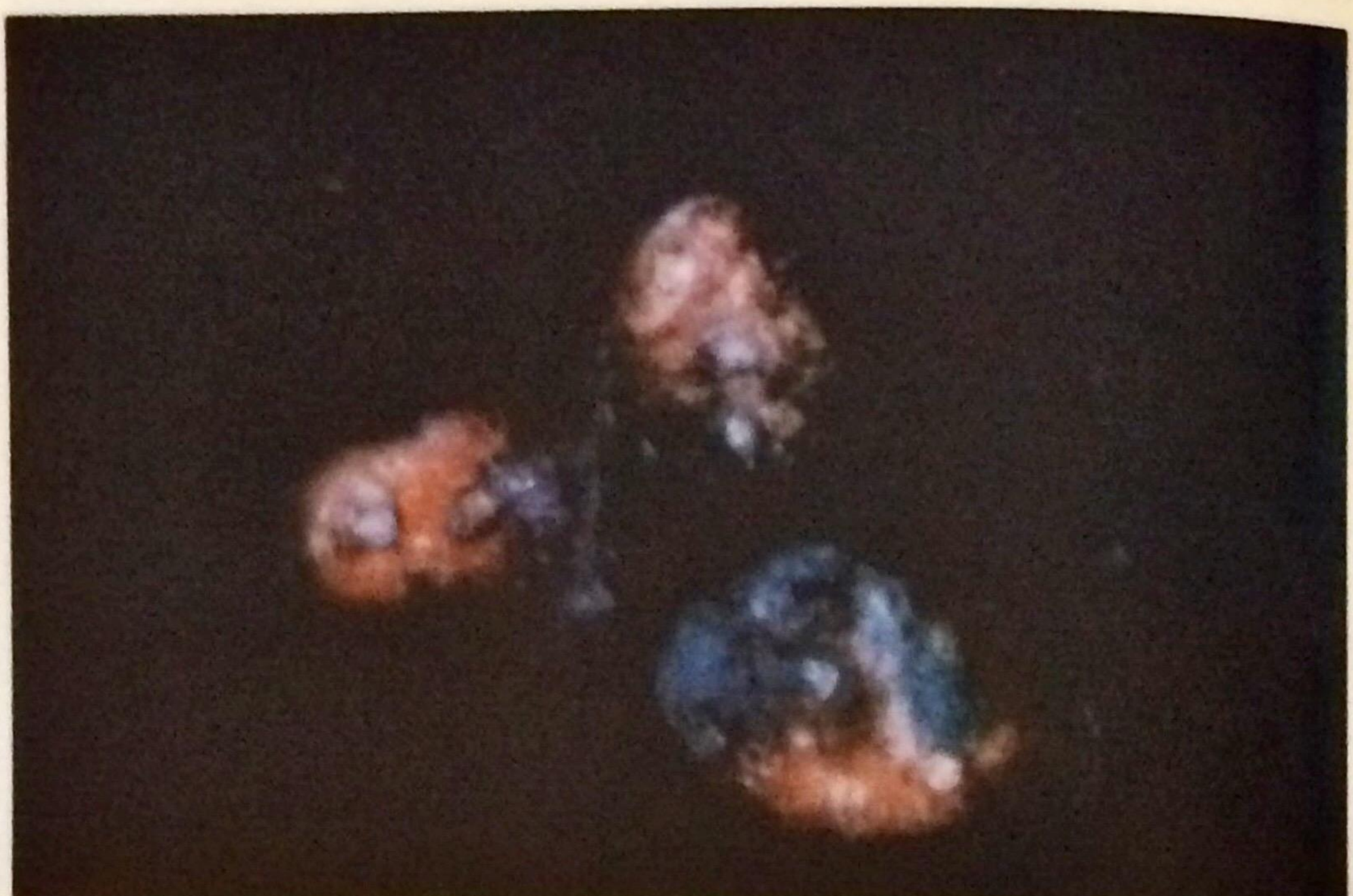

Figure 1. Yam tubers showing infection by *Rhizopus oryzae*

Figure 2. Yam tubers showing infection by *Botryodiplodia theobromae*

Figure 3. Yam tubers showing infection by *Aspergillus awamori*

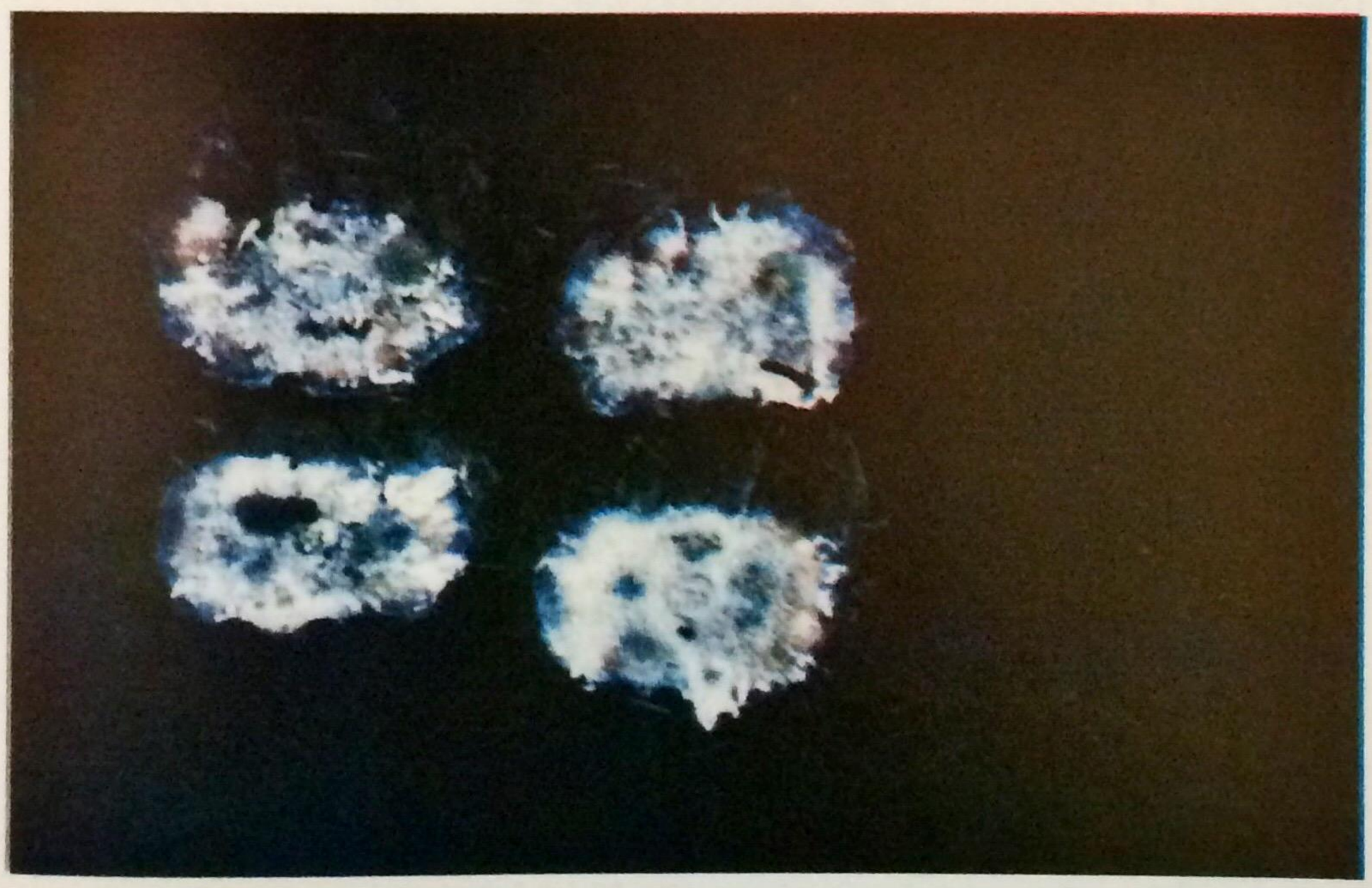

Figure 4. Yam tubers showing infection by *Penicillium purpurogenum*

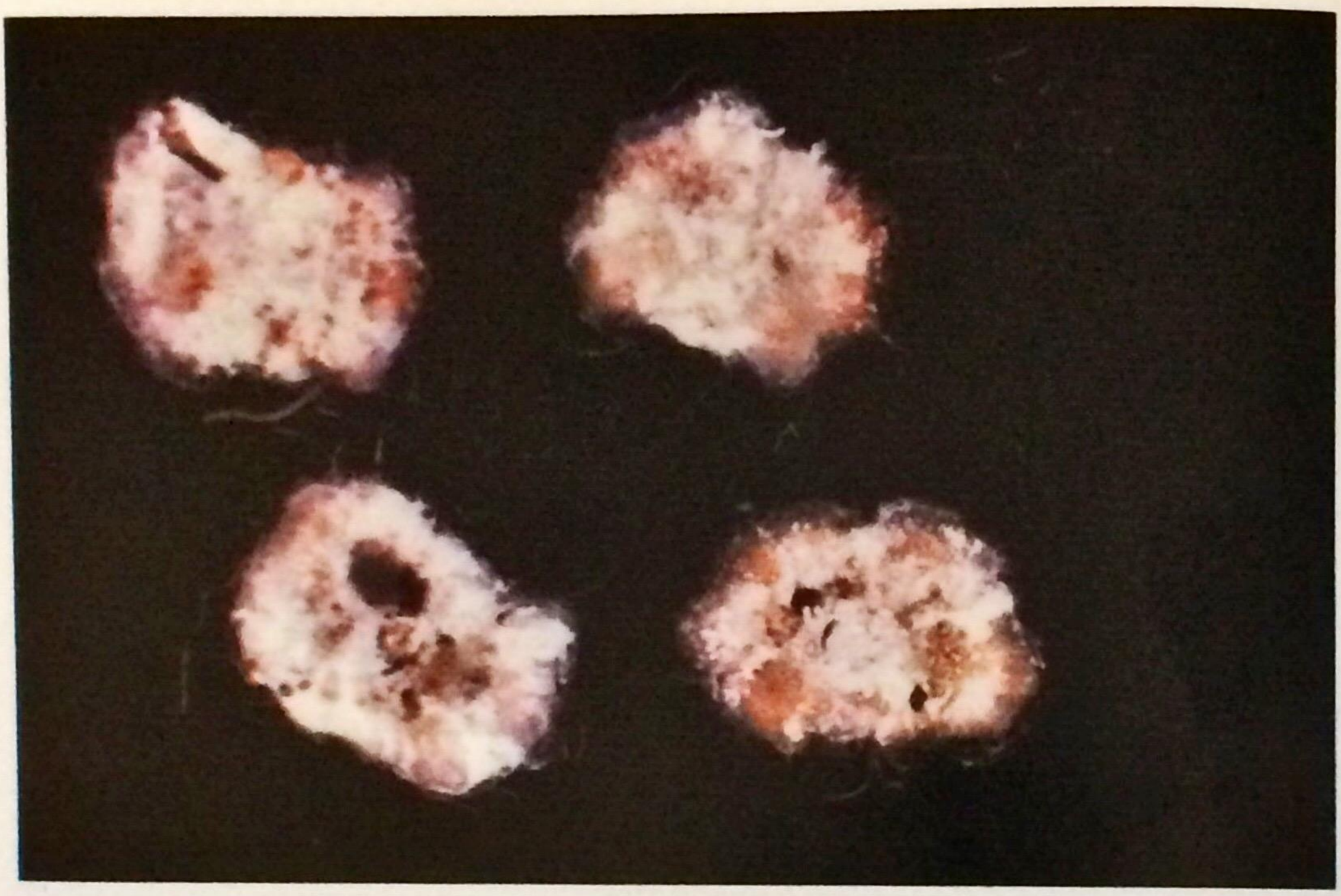

Figure 5. Yam tubers showing inffection by *Fusarium solani*

1982). Our observations confirm the previous findings of Sharma and Chatterjee (1982) identifying *F. solani* as a dry rot pathogen of yams in India. As in sweetpotato, the affected tissue was dry, pale in color and bordered by a brown margin (Ray amd Misra, 1995). In humid conditions, the surface of the tuber was covered with tufts of dense white moulds (Fig. 5). However, secondary infection by *Erwinia* sp. resulted in wet rot.

The results of the above work showed the different microorganisms associated with the postharvest spoilage of yams in India. To the best of the authors' knowledge, fungi like *Aspergillus awamori*, *A. versicolor*, *Penicillium decumbens* and *P. purpurrogenum* have been reported here for the first time as the causal fungi of postharvest yam rots.

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