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STUDIES ON FRUIT ROT OF PAPAYA CAUSED BY RHIZOPUS ORYZAE (WENT. AND JEERL.)

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As a result of surveys undertaken in various papaya growing areas in south-west Pakistan, fruit rot was found to be widely prevalent, mainly as a market disease. The causal organism was determined as *Rhizopus oryzae* (Went. and Jeerl.). Comparative pathogenicity tests showed that other species locally available viz. *R. nigricans* (Ehrenbag) and *R. arrhizus* (Fisher) could also cause the disease.

Studies made on the protopectinase activity of the pathogen showed that it secreted active protopectinase enzyme in the presence of pectin causing maceration of tissues in 3 hr.

Papaya (*Cairca papaya* L.), occupies an important place among the fruit plants of the world. Soils and climatic conditions of south-west Pakistan are well-suited for its production. According to recent estimates, it is being grown over an area of 4000 acres which is likely to increase as more development takes place.

Papaya suffers from a number of diseases viz. damping off and stem rot: *Pythium aphanidermatum* (Edson) Fitzp, charcoal rot: *Macrophomina phaseoli* (Maubl) Ashby, Anthracnose: *Colletotrichum dematium* (Pers. ex. Fr.) Grove, Fruit rot: *Rhizopus oryzae* (Went. and Jeerl.); and virus diseases such as leaf curl and bunchy top (Kamal and Moghal).[2] Among these fruit rot was first observed by the authors during 1964; and species of *Rhizopus*, later identified as *Rhizopus oryzae* (Went. and Jeerl.), was found to be invariably associated with the rotted fruits. It was considered worth while to undertake further investigations and as far as known to the authors this forms the first report of studies on the disease in Pakistan.

Fruit rot of papaya was first reported from Queensland caused by *R. nigricans* Ehrb. as a market disease.[6] Fruit rot caused by *R. oryzae* does not appear to have been reported previously although there are several reports of rotting in case of sweet potato[1] and banana.[6] The studies reported in this paper relate to the occurrence of the disease, pathogenicity tests, growth behaviour of the pathogen and production of protopectinase.

Material and Methods

Surveys.—Surveys of markets and papaya plantations were made at various places of Tandojam, Tando Allahyar, Mirpur Khas, Hyderabad and Karachi during 1964–65. Prevalence of the disease was recorded by counting healthy and diseased fruits. Large-scale isolations were made from diseased fruits on PDA medium using standard methods.

Pathogenicity Tests.—Pathogenicity tests were carried out both under laboratory and field conditions using pure culture of the pathogen. Selected healthy ripe fruits were surface sterilised with methlyated spirit followed by 3 washings with sterilized water. Fruits injured by needle pricks or uninjured fruits were inoculated by spraying with spore suspension. Inoculum was also introduced into the fruits by syringe. Control tests in each case were also run. The tests were made under sterilised bell jars at room tertemperature of 28–33°C. Regular observations were made and fruits showing distinct lesions were taken as diseased fruits. Reisolations of the pathogen were also made.

Studies on Protopectinase Activity.—Studies made on the physiological behaviour of the pathogen had shown that best growth of the fungus was obtained on nutrient dextrose medium prepared according to the formula given by Riker and Riker.[4] This medium was used for determining protopectinase activity of the fungus in the presence of pectin. Forty ml of the medium was poured in 250-ml-capacity medicinal bottles. The bottles were inoculated with 2 mm discs each taken from the margin of a 2-day old culture of *R. oryzae*. After incubation at 30°C for ten days dry mycelial weight was recorded after removing the mycelial mat over muslin cloth and drying for 24 hr at 70°C. Three replications were used for each treatment. Protopectinase activity of the culture filtrate was determined according to the methods used by Kamal and Wood.[3]

Experimental Results

(1) *Survey and Isolations.*—The disease usually appeared on the surface of ripe fruits as water soaked lesions which enlarge to cover the entire fruit. White mycelial growth can be observed both on the upper surface and interior of the fruits. The fungal growth turns brown and in the final stage the entire fruit is infested with the fungus and shows rotting of the tissues.

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TABLE 1.—INCIDENCE OF DISEASE IN MARKET LOTS.

Location	No. of fruits counted		Total	% rot
	Healthy	Diseased		
Tandojam	105	45	150	30.0
Tando Allahyar	82	38	120	31.7
Mirpur Khas	77	31	108	28.7
Kunri	50	35	85	41.0
Hyderabad	110	70	180	38.8
Karachi	90	85	175	48.6

TABLE 2.—ISOLATIONS FROM ROTTED PAPAYA FRUITS.

Fungi isolated	% frequency
Rhizopus oryzae	70.2
Penicillium sp.	10.0
Aspergillus sp.	7.5
Alternaria sp.	3.2
Bacteria	2.5
Unidentified	6.6

The greatest extent of damage was found in markets, but was negligible in the orchards. This is because the fruits are plucked just before ripening which is not the optimum stage for disease development. The extent of damage in markets survey is presented in Table 1.

It can be seen from the above table that the incidence of fruit rot in market lots at different places varied from 28.7–48.6% with an average of 36.5%.

Isolations were made from 200 diseased fruits taking 4 pieces from each fruit and the results are given in Table 2.

It was observed that *R. oryzae* came out with maximum frequency. Pure cultures of the isolate were made for further studies. The culture was also sent to Commonwealth Mycological Institute, Kew Garden, England where the identity was confirmed as *Rhizopus oryzae* (Went. and Jeerl.)

(ii) *Pathogenicity Tests.*—Pathogenicity tests were carried out using different inoculation techniques. The results obtained are given in Table 3.

Initial symptoms of the disease were observed within 48 hr and rotting became distinct after 72 hr in wound inoculations. Inoculations in uninjured fruits also produced the disease but its development was delayed. In some cases 100% rotting developed in all the inoculated fruits. Check fruits under similar conditions in the absence of inoculum remained completely healthy (Fig. 1). Injuries favoured the process of the infection but the pathogen could also cause rotting in uninjured fruits provided optimum conditions were available.

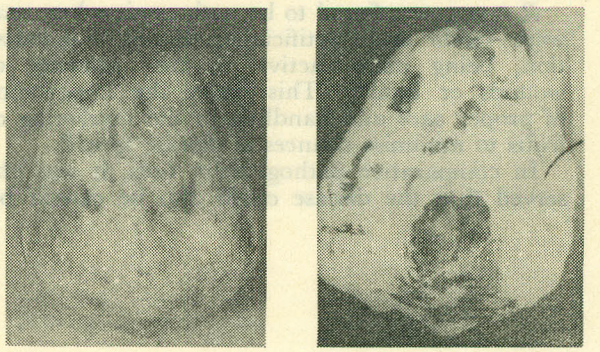
Other *Rhizopus* sp. were also tested for the production of disease in healthy fruits. *R. nigricans*, *R. arrhizus* and *R. oryzae*, isolated from cotton-bolls rotted apples and rice seeds, respectively, were used for inoculating the fruits and the results obtained are shown in Table 4.

TABLE 3.—PATHOGENICITY TESTS WITH PAPAYA FRUITS.

Treatments	No. of fruits inoculated	No. of fruits rotted
Uninjured fruits with spore spraying	15	15
Injured fruits with spore spraying	15	15
Introducing inoculum in injured fruits	15	15
Control (in each case)	15	0

TABLE 4.—ROTTING OF PAPAYA FRUITS BY DIFFERENT *Rhizopus* spp.

Treatment	No. of fruits inoculated	No. of fruits showing rotting
R. oryzae	15	15
R. nigricans	15	11
R. arrhizus	15	9
Control	15	0



(a) (b)

Fig.1.— (a) Uninoculated healthy fruit (b) Inoculated fruit showing rotting and over growth by the pathogen.

All the species tested were able to cause rotting of the fruits in varying degrees of intensity. *R. oryzae* was found to be the most virulent followed by *R. nigricans* and *R. arrhizus*.

(iii) *Studies on Protopectinase Activity.*—Since protopectinase is known to be the enzyme involved in the rotting of tissue,[1] protopectinase activity under various concentrations of pectin was esti-

TABLE 5.—EFFECT OF DIFFERENT CONCENTRATIONS OF PECTIN ON GROWTH AND PROTOPECTINASE ACTIVITY OF *R. oryzae*.

Pectin conc. %	Av. dry mycelial wt (mg)	Protopectinase activity (hr)
Basal medium alone	155	10
0.5	170	6
1.0	192	4.5
1.5	205	3.5
2.0	228	3.0

mated using the technique adopted by Kamal and Wood.[3] The results are given in Table 5.

Maximum protopectinase activity was obtained when a concentration of 2% pectin was incorporated in the medium, causing maceration of tissues in a reaction time of 3 hr. Mycelial growth also increased with the addition of pectin.

Discussion

Fruit rot disease of papaya was found to be widely prevalent in markets. The casual organism was found to be *Rhizopus oryzae* (Went. and Jeerl.), which has not been reported previously in association with papaya.

R. oryzae was found to be pathogenic when tests were made under artificial conditions of inoculation, being more active in the presence of wounds or injury. This shows the importance of proper care and handling in the marketing of fruits to minimise chances of disease spread.

In comparative pathogenicity tests, it was observed that the disease could also be caused by other species of *Rhizopus* which are locally available viz. *R. nigricans* and *R. arrhizus*, although the intensity of disease in these cases was less. This indicates the possiblity of the fruits being attacked by any of the other species provided inoculum and optimum conditions for disease development are present. *R. nigricans* was reported to be the main organism causing fruit rot of papaya in Queensland.[5]

Protopectinase enzymes are known to be important in the rotting mechanism by various pathogens.[1,3] Preliminary studies made with *R. oryzae* have shown that the pathogen is capable of secreting active protopectinase enzyme in the presence of pectin which brings about maceration of tissues. Further studies on the properties of this enzyme are likely to provide more information on the disease mechanism.

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