TABLE I

Cellulolytic activity of different streptomycete isolates

Culture No.	Sporophore type	Mycelial dry wt (mg/100 ml)	(mg glucose/100 ml) Sodium acetate buffer (pH 5·2)		Sodium phosphate buffer (pH 8·0)	
			CMC	Cellulose	CMC	Cellulose
S. 1	Open-loop spiral ..	159	4·33	0·62	2·21	0·27
S. 2	Open-loop ..	203	1·67	0·58	1·18	0·13
S. 3	Open-spiral ..	72	2·15	0·36	0·48	0·18
S. 4	Flexuous ..	82	0·27	0·04	0·35	..
S. 6	Open-spiral ..	141	1·15	0·38	0·34	0·18
S. 7	Flexuous to fascicled ..	53	0·35	0·60	0·13	0·12
S. 13	Flexuous ..	195	1·02	0·34	0·50	..
S. 16	Straight ..	95	1·45	0·62	0·68	0·08
S. 21	Straight ..	201	0·12	0·26	..	..
S. 27	Closed-spiral ..	101	1·06	0·60	0·73	..
S. 28	Closed-spiral ..	161	1·18	0·55	0·78	..
S. 32	Open-spiral ..	152	4·08	0·93	1·28	0·55
S. 35	Closed-spiral ..	107	1·04	0·36	0·69	..
S. 36	Flexuous ..	123	0·60	0·54	..	..
S. 38	Open-spiral ..	53	0·57	0·33	0·49	..

cellulase production (Table I). Hence cellulase production may be ascribed to the inherent capacity of the isolates rather than the growth. When compared to terrestrial Streptomycetes[10], many of the marine isolates are found to be active cellulose decomposers. From the present investigation, it is clear that marine actinomycetes may also take an active role in the deterioration of cellulosic substances in the marine environment.

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EXPERIMENTAL STUDIES ON LEAF ANTHRACNOSE OF *FICUS ELASTICA* ROXB.

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ABSTRACT

The seasonal incidence, development and disease symptoms caused by *Gloeosporium elasticae* on leaves of *Ficus elastica* Roxb. have been described. The pathogenicity of the fungus was demonstrated on the host leaves in field as well as under laboratory conditions. It was concluded that the entry of the fungus is through injury. The production of cellulase enzyme by the fungus on different cellulosic substrates was studied, suggesting thereby that pathogen by its enzyme producing capacity disintegrate the structural components of the host cells.

INTRODUCTION

ANTHRACNOSE of *Ficus elastica* Roxb. is present in most of the rubber producing areas of the south-east India. In many of these regions severe epidemics of the disease have at one time or other caused substantial losses. A careful diagnosis revealed sporulating acervuli of *Gloeosporium elasticae* Cooke and

Massee parasitizing several diseased leaves. From India the disease was earlier reported by Uppal *et al.*[6] from the Rubber plantation at Poona. Grove[2] gives it under *Colletotrichum ficus* Koord. Some other species of *Gloeosporium* are also known in India to parasitise *Areca catechu* Thirumalachar[5] and *Anthocephalus cadamba* Lal and Tandon[3]. Besides these reports, little further information about anthracnose of *F. elastica* has appeared in the literature since 1937. The present paper describes the occurrence and symptoms of anthracnose of *F. elastica* in the central part of India, and furnishes the first detailed account of the conidial stage of *G. elasticae*. It also includes the role of cellulolytic enzymes in the disease cycle. Observations reported here were made in 1969–70 on rubber plants growing in the Botanical garden of Saugar University, M.P., India.

Isolation Method

Leaves of *F. elastica* with leaf spots were collected from field trees. Infected tissues were cut into pieces about 3–6 mm. square, the surface of the pieces were sterilized in 0·1% mercuric chloride solution for 3 minutes and rinsed by several changes in sterile water. The pieces were then plated on Potato Dextrose Agar (PDA) ; three pieces per Petri dish. Twelve isolations were made from each leaf. The plates were incubated at 25° C for 6–8 days ; by that time the colonies growing out of the leaf pieces had begun to sporulate. The fungal colonies were examined and subcultured. In most of the plates pieces cut from a leaf yielded the same fungus. The fungus was identified as *Gloeosporium elasticae* Cooke and Massee.

Symptoms and Seasonal Occurrence of the Conidial Stage :

In the year 1969, several leaves infected with anthracnose were first observed during the month of September but during the latter part of October an average of thirty per cent of the leaves on all the trees examined were infected. The initial symptoms appeared as minute yellowish-brown coloured areas which continued to enlarge (Plate 1, A) until the whole leaf was of a blackish-brown colour. The leaves were dotted with small flesh coloured areas which were conidial masses or acervuli rupturing the host epidermis. Finally these acervuli turned black, due to change in colour of the mycelium. The acervuli often appeared on the sides of the midrib (Plate 1, B) and folded surfaces of leaves not exposed to the open air. These observations showed that the disease incidence was not very common during the period of May and June 1970 but appeared on the leaves in the autumn. The fungus overwintered in leaves which had been buried under the leaf-litter and sporulated during the following spring producing viable conidia ; the ascogenous stage of the pathogen was not observed during these observations. The first symptoms in most of the leaves appeared at the apical portions.

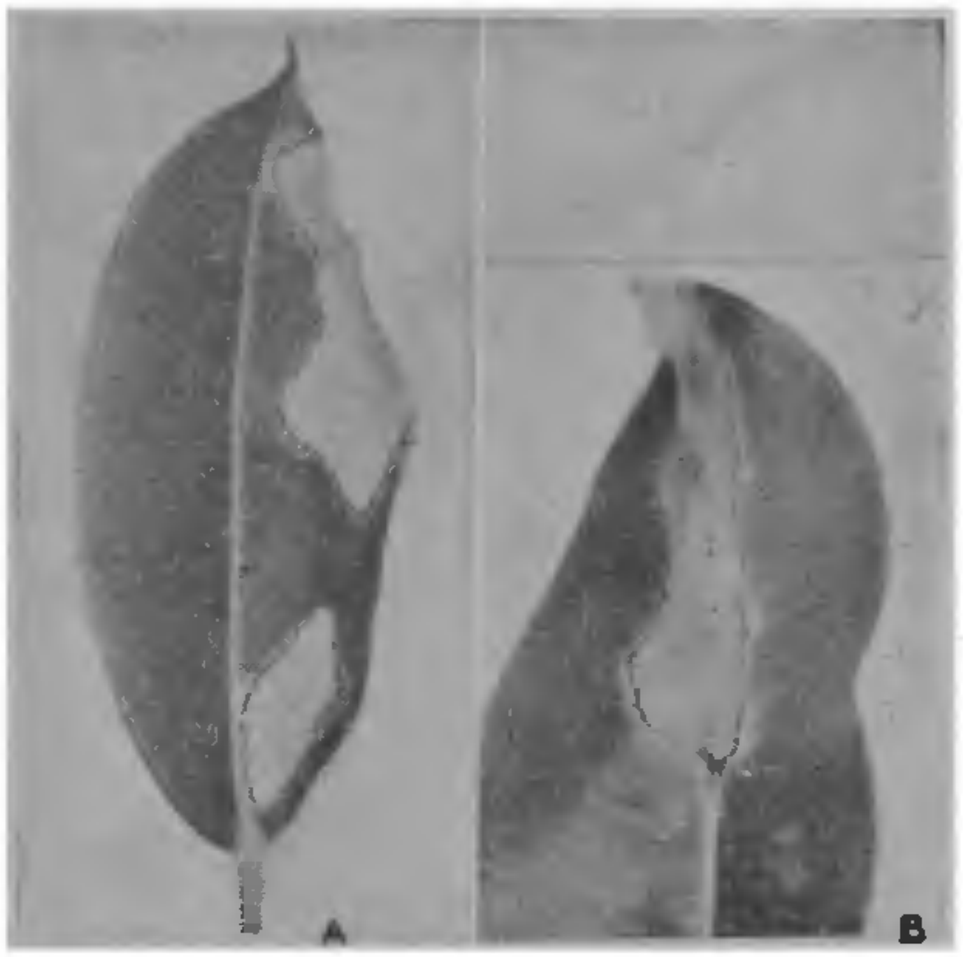


Plate 1. A–B. A. Leaf of *Ficus elastica* Roxb. showing initial symptoms of anthracnose caused by *G. elasticae*. B. *F. elastica* leaf showing black acervuli of *G. elasticae on* the sides of the midrib.

Pathogenicity Tests

Tests for pathogenicity were carried out on the leaves in the laboratory as well as on the field trees. Three methods were used :

Method 1

Surface sterile healthy leaves were taken in the 6" diam. Petri dishes. Inoculum was prepared from 8-day old culture of *G. elasticae* on Potato Dextrose Agar (PDA). After pricking the upper epidermis of the leaves with the help of sterile needle, an inoculum disc of 6 mm. diam. was placed on the leaf surface. To provide suitable moisture for initial penetration of the germ tube, some sterile cotton dipped in sterile water was kept on inoculum disc and also at a side of the Petri dish. The dishes were covered with bell jar and left in this condition for seven days at room temperature. In all, five leaf samples of different ages were inoculated by this method.

Method 2

Instead of inoculum disc the suspension of conidia and hyphal fragments in sterile water was sprayed on the upper surface of the leaves. Five leaf samples of different ages were sprayed and kept in Petri dishes provided with suitable temperature.

Method 3

Inoculation experiment was done on field trees. The inoculum disc was kept after pricking the upper epidermis of the leaves as described in method 1. Leaves were covered with polythene bags to avoid air contamination. In all five healthy leaves of different ages were inoculated.

Results

After seven days, anthracnose symptoms appeared on those leaf samples in which inoculum disc was kept after pricking the leaf epidermis. The number of acervuli was greater on the upper surface. Lesions were elliptical to elongate with a dark brown to black margin which was not clearly defined. The midrib of the leaf appeared to restrict the extension of the lesion on the other half of the leaf. The lesions were extended more towards the margin of the leaf in most of the leaf samples. Infection occurred in all the five leaves. Isolations from the lesions yielded *G. elasticae* in all cases. Acervuli appeared around the inoculated areas, but not elsewhere on the leaves. The acervuli were identical to early stages of leaf anthracnose of ficus leaves on naturally infected plants in the field.

Leaf samples on which suspension of conidia and mycelial fragments was sprayed were found unaffected. Out of five not even a single leaf showed the disease symptoms.

Disease symptoms appeared after four days on the inoculated young leaves of field trees. The number of acervuli was greater on the young soft leaves. Much less area was occupied by the pathogen on the older leaves.

Cellulase Enzyme Produced by G. elasticae

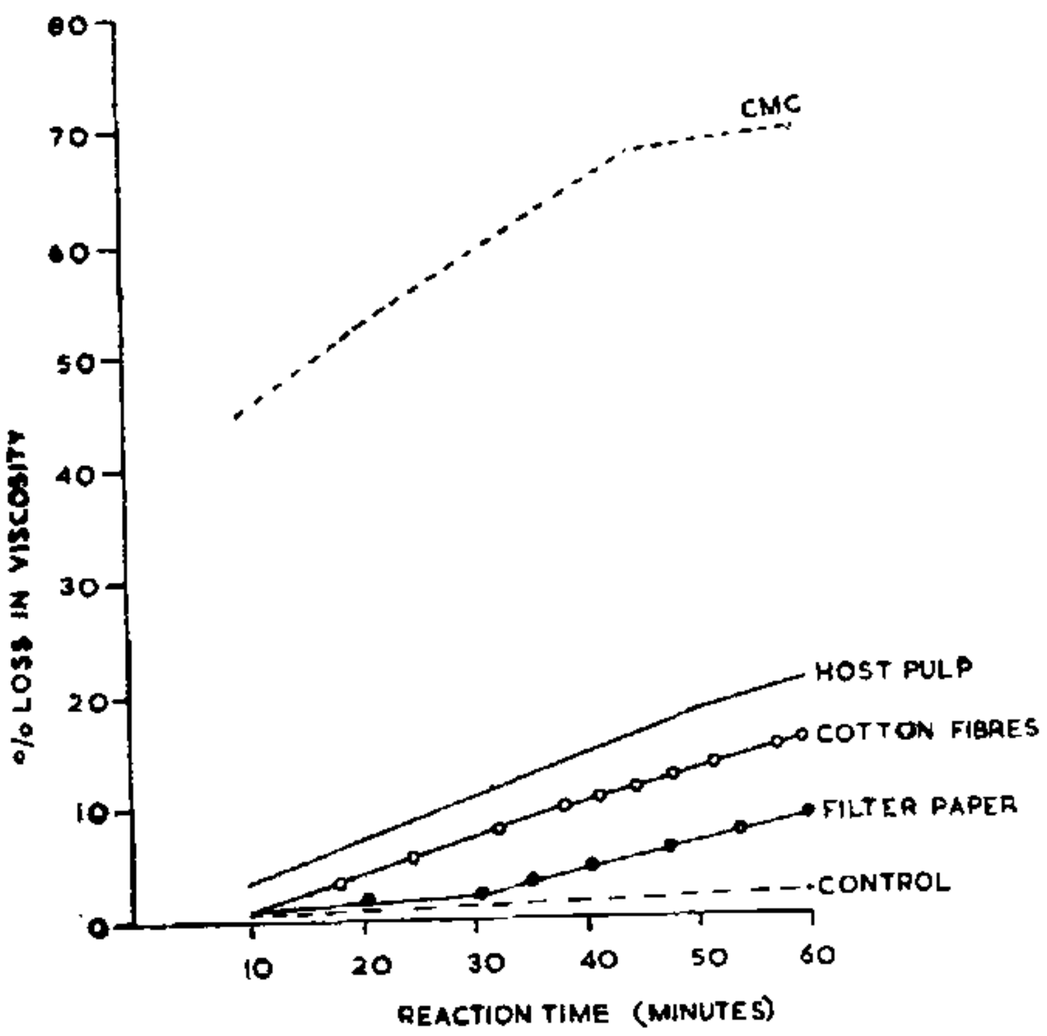
Number of species of *Gloeosporium*, including some plant pathogens have been known to be cellulolytic. For this reason, the cellulolytic activity of *G. elasticae* and the role of cellulase enzyme in the causation of anthracnose on leaf have been studied. Enzyme assay was done in culture filtrate of *G. elasticae* to test their ability to decompose native cellulosic material.

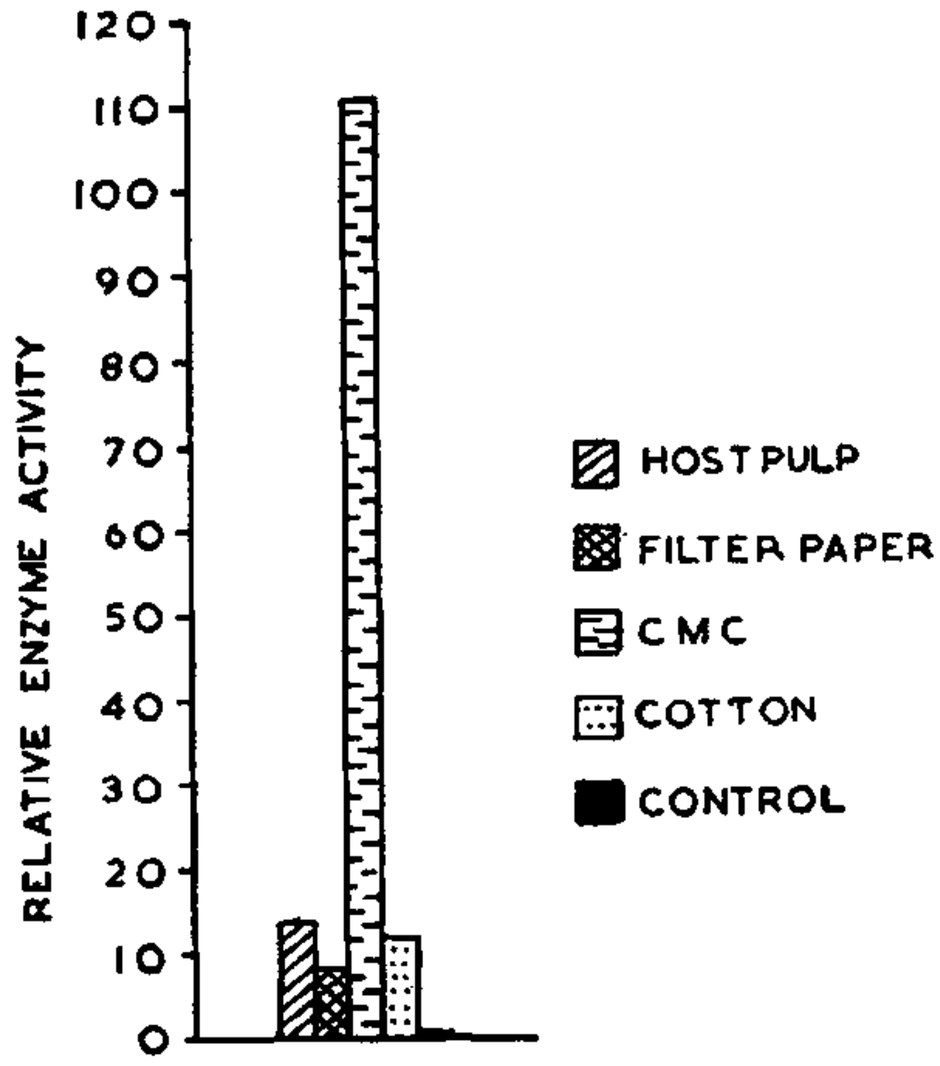
Materials and Methods

The fungus was cultured on a basal modified Czapek's Dox Medium in which the carbon source was partly substituted with different cellulose sources. Cellulose sources used singly in the culture media were host leaf pulp, filter-paper strips, carboxymethyl cellulose (CMC) and cotton fibres. In one flask only basal medium was taken as control. 35 ml. of sterile medium in 150 ml. conical flask was inoculated with test fungus by adding a disc of 6 mm. diam. and grown in a incubator for 15 days at 26°C. The filtrate was centrifuged and the clear supernatant liquid was taken as the enzyme sample for studies of cellulase production. The enzyme assay was done viscometrically with a size 300 Fenske-Ostwald Viscometer, as earlier described by Agrawal[1]. Activity of cellulase was determined by the loss in viscosity of solution of carboxymethyl cellulose. The viscosity measurements of reaction mixture were recorded in a water-bath at 30° C, at intervals of 10 minutes over a total period of 60 minutes. The reaction mixture was having enzyme preparation, solution of carboxymethyl cellulose, citrate buffer (pH 5·5) and water.

Results

The fungus produced a large amount of enzyme on media containing cellulose. The culture filtrate obtained from a medium containing CMC showed a rapid loss in viscosity of the substrate and a total of 70% loss in viscosity was recorded within 60 minutes (Fig. 1). In this case maximum Relative Enzyme Activity (REA) was 111 (Fig. 2). Cellulase activity was also quite high, *i.e.*, 22% loss in viscosity, when the fungus was grown on host leaf pulp as a cellulose source, a REA value of 14 was obtained, the culture filtrate filtered from media containing cotton fibres and filter-paper strips showed still less cellulase activity with 16% and 9% loss in viscosity respectively (Fig. 1) and REA value of 12 and 8 were obtained in them (Fig. 2). Very little (2% loss in viscosity) enzyme activity was found to be present in cellulose free media, *i.e.*, in control flask. Fungal mycelium was produced in all the flasks containing native cellulosic sources but greatest enzyme production was recorded on CMC.





FIGS. 1-2. Fig. 1. Activity of the cellulase of *G. elasticae* as per cent loss in viscosity of reaction mixture in different cellulose sources. Fig. 2. Activity of the cellulase of *G. elasticae* as relative enzyme units in different cellulose sources.

Discussion

In the pathogenicity experiments it was revealed that the symptoms produced after inoculation of leaves in the Petri dishes in laboratory were the same as those seen in nature as well as when the tree leaves were inoculated in the fields. However, the inoculation when done by spraying in the form of conidial and mycelial suspension on healthy leaves was not successful in any case. This may be due to the presence of waxy leaf surface and thick epidermis which resist the direct penetration of the pathogen. It appears that the infections in nature occur only through injured portions of the leaves. When leaves of different ages were taken it was found that ready infection appeared only in case of younger leaves. When infection spreads it covers usually the area on one side of the midrib the latter appears to restrict it by some resistance. It is only at a late stage that the midrib is also affected probably due to the production of some cellulolytic enzymes or some other metabolites which weaken this resistance.

From the study of the enzyme produced by the pathogen, it could be demonstrated that *G. elasticae* is a cellulolytic fungus and is capable of degrading native cellulose (host leaf pulp, cotton fibres and filter-paper strips) as well as derived soluble cellulose (CMC). It is further seen that *G. elasticae* produces both the C_1 and C_x enzymes[4], suggesting thereby that it produced cellulase on infected leaves of the host plant, thus helping in the disintegration of structural components of the host cells where cellulose is present as the skeletal substance of the cell walls. This action of cellulase enzyme facilitates the penetration and spread of the fungal mycelium in the host tissues, and cause the collapse of parenchymatous tissues with consequent disintegration of the cellular structure, thus aiding the fungus in the production of disease.

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