with dead rotten areas presenting a ragged appearance (Fig. 1–B). Such diseased material was macerated, diluted with sterile distilled water and used as inoculum in the preliminary inoculations. The causal organism was isolated under asceptic conditions in nutrient agar and purified subsequently by dilution plate method. Artificial inoculation with the organism produced water-soaked areas along the midrib and veins (Fig. 1–A) as observed under field infections (if heavy cell

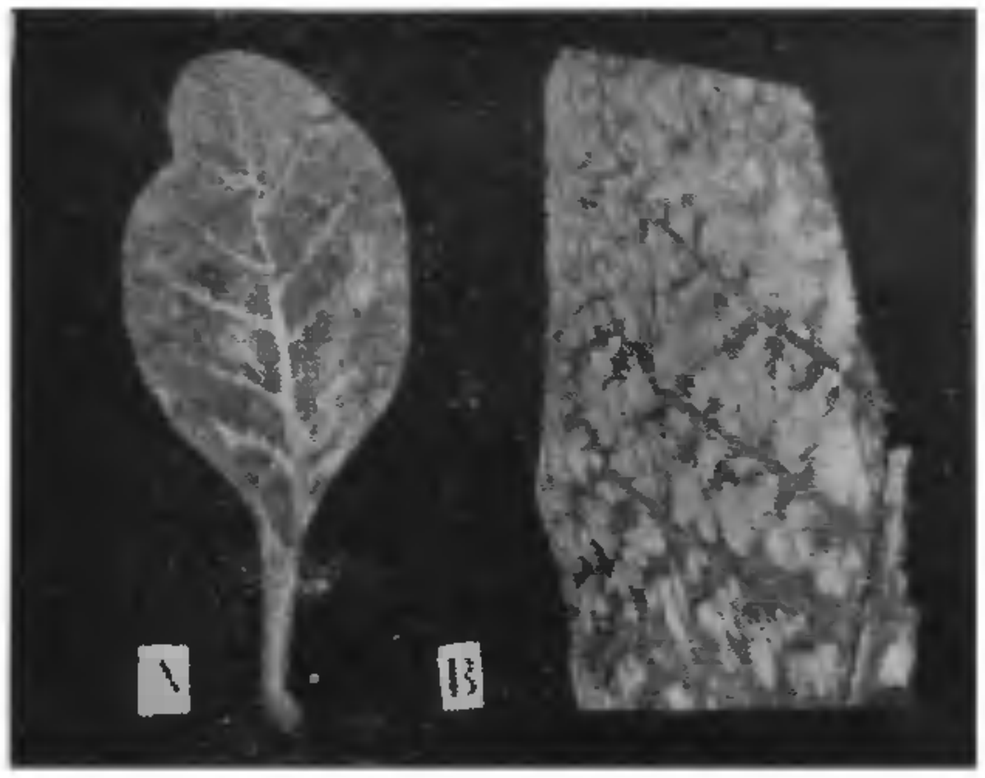

FIG. 1 A–B

concentration was used in inoculum) and also lesions consisting of a small central dead area surrounded by a broad yellow halo[3]. Under artificial conditions, the lesions required about a week to develop fully. In repeated pathogenicity tests the organism produced similar symptoms on tobacco. Inoculation of the pathogen upon *Capsicum annuum*, *Arachis hypogaea*, *Solanum melongena* and *Lycopersicum esculentum* gave negative results.

The pathogen is a rod-shaped bacterium with size of the cells varying from 2·0 to ·4·5 × 0·9 to 1·6 μ and average size being 3·2 × 1·1 μ. The organism is motile by a single polar flagellum. It is gram negative and, however, is not acid fast. It produces acid but not gas in sugars like glucose, galactose, fructose, xylose, sucrose, glycerol and mannitol; does not produce indole; produces a diffusable green, fluorescent pigment in King's medium B[6]. It is oxidase negative but liquefies gelatin; the organism does not hydrolyse starch but reduces nitrates to nitrites. The process of denitrification is rather difficult to demonstrate in yeast extract-peptone broth but can be readily detected in a synthetic medium[4]. From the above description of this phytopathogenic bacterium and from its capability to initiate disease symptoms on tobacco alone, the organism is identified as *Pseudomonas tabaci* (Wolf and Foster) Stevens[1,2,5], the incitant of tobacco wildfire disease. The authentic culture has been deposited at Type culture collection at Indian Agricultural Research Institute, NewDelhi and in the type culture collection at the Tamil Nadu Agricultural University (item No. 56). This appears to be the first time this pathogen has been found in India.

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A COMPARATIVE STUDY OF EXTRACELLULAR ENZYMES PRODUCED BY PATHOGENIC FUNGI

SINCE not much is known about the enzyme make up of pathogenic fungi, particularly, the enzymes produced extracellularly, the present investigation was undertaken to study the different kinds of enzymes produced by some of these fungi in relation to their possible role in pathogenicity with the respective host. Extracellular amylase production has been reported from phytopathogenic fungi[1,2]. Cellulase enzymes necessary for the breakdown of cellulose of higher plant cell walls were also known to have been produced extracellularly by a number of phytopathogenic fungi[3-5]. Ribonuclease and deoxyribonuclease, though not related directly to the penetration of the pathogen into the host play an important secondary role of disrupting the protein metabolism of the host and host DNA degradation[6-8]. Little work however has been done so far, to correlate extracellular enzyme production by non-obligate fungi with pathogenicity.

The fungal strains (Table I) obtained from the Indian Agricultural Research Institute, New Delhi, India, were maintained on Czapek–Dox[9] medium and tested for their pathogenic activity prior to enzyme assay. Chemicals used were Na-salt of carboxymethyl cellulose (I.C.I. Ltd.), DNA of calf thymus and Yeast RNA (Nutritional Biochemicals Corporation), Soluble starch (E. Merck), Uranyl acetate (B.D.H.).

The cultures were grown in 100 ml capacity Erlenmeyer flasks containing 25 ml of Peptone-Glucose medium[10] for 10 days at 22° C and 30° C. The supernatant clear culture filtrates obtained after the removal of mycelial debris by

TABLE I

Enzyme activity (zone in mm ± S.E.) of different pathogenic fungi

Organisms	Enzyme activity*							
	Amylase		Cellulase		DNase		RNase	
	22° C	30° C	22° C	30° C	22° C	30° C	22° C	30° C
Alternaria brassicola	—	21·00±1·20	15·50±0·60	13·25±0·39	+	—	14·25±0·39	13·75±0·45
Alternaria crassa	—	—	12·00±0·12	21·00±0·99	—	—	—	—
Alternaria solani	17·90±0·25	13·66±0·53	—	—	—	—	—	14·50±0·81
Aspergillus fumigatus	16·37±0·35	23·25±0·21	16·50±0·30	15·50±0·91	23·25±0·80	—	26·50±0·90	16·75±0·43
Curvularia lunata	18·00±0·12	19·00±0·81	20·00±0·75	31·25±0·70	—	—	—	—
Fusarium oxysporum	—	—	—	14·50±0·72	—	—	—	—
Fusarium udum	—	—	—	—	—	—	17·77±0·20	19·25±0·25
Helminthosporium sativum	15·37±0·43	17·50±0·32	20·25±1·02	14·00±0·05	13·25±0·23	—	15·75±0·62	13·75±0·53
Helminthosporium oryzae	16·87±0·32	18·65±0·30	21·40±1·02	—	15·13±0·54	—	16·00±0·50	13·63±0·23
Rhizoctonia bataticola	—	—	—	18·50±0·09	—	—	18·80±0·53	15·50±0·32
Rhizoctonia solani	14·50±0·08	—	16·50±1·12	17·50±0·07	—	—	16·75±1·02	14·50±0·83
Ustilaginoidea virens	20·25±1·12	22·60±0·04	22·25±0·23	16·00±0·33	—	—	25·25±0·32	15·00±0·12

*Zone of clearing in mm; + presence of activity; — absence of activity.

centrifugation were used to assay for amylase, cellulase, deoxyribonuclease, and ribonuclease enzyme by cup-plate assay method[11]. Ten replicates were taken for each enzyme of a series. The uninoculated broth tested similarly served as control. Enzymes were assayed using 0·1 ml quantity of the supernatant culture broth pipetted to 7 mm diameter cup in each test plate containing 20 ml of the medium.

For the assay of amylase nutrient, agar with 1% starch was used[12]. The plates were incubated for 2 h at 37° C and then developed with Gram's Iodine solution[12]. Cellulase was assayed using carboxymethyl cellulose in acetate buffer of pH 5·6[12]. Plates were incubated for 18 h at 37° C and then developed with chloroiodide of zinc solution[13]. For RNase and DNase assay, the medium was plated after adding yeast RNA and calf thymus DNA respectively, incubated for 2 h at 37° C and then developed with uranyl reagent[10]. In each case the diameter of the zones of clearing were measured in mm.

Of the 12 pathogenic strains studied, 8 gave positive results for amylase, 10 for cellulase, 3 for DNase and 9 for RNase (Table I).

It was observed that the effect of temperature on enzyme production and the quantity produced varied from organism to organism. In some cases a lower temperature of 22° C was proved conducive to enzyme production while in others a higher temperature of 30° C was found more effective.

In *Alternaria* sp. at 28–30° C encouraged amylase and cellulase production. In the case of *Fusarium* sp. 17–29° C was reported to be favourable for disease development[14]. Optimum production of cellulase and ribonuclease was obtained at a temperature of 22° C in these species. Also, it has been found that seedlings emerge slowly between 16–24° C and the maximum seedling infection by

Helminthosporium sp. is induced between 16–24° C[15]. In the present study it was found that optimum cellulase, DNase and RNase production occurred at 22° C (Table I) which falls within the temperature range optimum for disease development. These studies reveal that the significant activities of various enzyme systems indicate encouraging possibilities for their quantitative estimation in relation to pathogenecity.

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TOXICITY OF A NEW RODENTICIDE, VACOR TO LESSER BANDICOOT RATS, *BANDICOTA BENGALENSIS*

DAMAGE, losses and disease attributable to rodents in India are well documented[1,2]. *B. bengalensis* is one of the most common agricultural and commercial pests, ranging over almost the whole of India except for the drier regions of Rajasthan and Gujarat[3]. Despite recognition of the serious losses to this species, little information is available on its susceptibility to rodenticides.

Of acute poisons used against *B. bengalensis*, only zinc phosphide and aluminium phosphide are effective and readily available; and of these, only zinc phosphide can be given with bait[3]. Various chronic anticoagulants have also been tested[4,5], but *B. bengalensis* shows considerable variation in susceptibility to warfarin and difenacoum[6,7]. If only anticoagulants are used to control *B. bengalensis*, problems of resistance may become important[6,8].

There is thus urgent need for the development of new effective acute poisons as an alternative to zinc phosphide. Vacor (N-3-pyridymethyl N[1]-*p*-nitrophenyl urea) is such a rodenticide recently developed by Rohm and Haas, USA; it is thought to be a nicotinamide antagonist, and is highly effective against a wide variety of rodents[9]. Laboratory tests were therefore made of its effectiveness against *B. bengalensis*; field trials in rice and sugarcane are also being conducted.

B. bengalensis were caged singly in metal cages, 60 × 30 × 30 cm, with water always available. For four days, rats were fed weighed amounts of rice flour + groundnut oil (95 : 5 by weight); on the fifth day, Vacor was added to the food in concentrations of 1% or 0·25% for different groups. Consumption corrected for spillage was measured each day to 0·1 g and converted to 100 g body weight for analysis.

Results on the toxicity of Vacor are summarised in Table I. All rats died in less than 18 hours; symptoms were first noticed after 2–3 hours and involved a noticeable reduction in general activity, with later partial paralysis of the hind legs, pulmonary distress and coughing. Dead rats were often found with fore and hind limbs held rigid out from the body so that the corpse was supported off the cage floor.

In a second experiment, three male and two female *B. bengalensis* (mean weight 170 g) were caged singly as before and fed rice flour + 5% groundnut oil. After consumption had stabilized, rats were weighed, starved for 30 hours, and then