

mented at 9,000 g for 20 min at 4°C and the washed pellets were resuspended in ST buffer having about 5 mg/ml protein.

Protein, RNA and phospholipid estimations were done according to standard methods⁴.

Experiments conducted under *in vitro* conditions show (Table I) that NADPH and DMSO do not degranulate the reticular membranes. However, isatin caused degranulation around 7% in the absence of NADPH and around 40% in the presence of NADPH. Thus the function of isatin to act as a carcinogen seems to be dependent on NADPH, required for the conversion of a number of carcinogens to their electrophiles by the microsomal hydroxylase system^{2,3}. This conversion has also been reported to be favoured by the presence of smooth membrane vesicles⁵ which are present in our preparations. It may be possible that the -NH group present in isatin is converted to -NOH by the action of microsomal hydroxylase system, thereby producing an active carcinogen. Ascorbic acid has been reported to be antagonistic to the carcinogenic activity of nitrosamine⁶. Our results also demonstrate the anticarcinogenic activity of ascorbic acid under *in vitro* conditions, which corroborates the results of our earlier communication⁴.

Results presented in Table I show that about 42% ribosomal loss is observed in microsomes prepared from livers of animals treated with isatin both on the bases of RNA/protein and RNA/phospholipid ratios. But in the third group of rats ascorbic acid treatment antagonized the effect of isatin and the microsomal degranulation in this group was reduced to negligible levels.

Data reported in this paper show that isatin can act as a carcinogen and ascorbic acid antagonizes that activity.

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LIGNOCLASTIC ACTIVITY OF *ASPERGILLUS CLAVATUS*, *PENICILLIUM MARTENSII* AND *PYTHIUM PROLIFERUM*

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LIGNIN decomposing fungi, *Aspergillus clavatus*, *Penicillium martensii* and *Pythium proliferum* deBary were isolated from soil. The cultures were tested for their efficacy in decomposing wheat straw lignin in liquid medium as well as in soil. The major loss of lignin occurred within 4 weeks of inoculation and later the rate of decomposition was sluggish. *Aspergillus clavatus* was found to have comparatively higher lignoclastic activity.

Introduction

The decomposability of plant material in soil is largely determined by its nitrogen and lignin contents. Lignin, besides being resistant to decay, imparts a protective covering over easily decomposable plant constituents like celluloses and hemicelluloses. Among lignin decomposing organisms Basidiomycetes such as *Polystichus*, *Polyporus*¹ have been well known. Some fungi imperfectii like *Aspergillus*, *Penicillium* and ascomycetes also metabolize lignin^{2,4,7}. These heterotrophic microorganisms have great practical significance. The efficient strains of these microorganisms may be utilized to hasten the decomposition of crop residues in compost and manure pits.

Experimental

Lignin decomposing fungi were isolated from soil, compost and decaying wood on Czapeck's agar medium containing tannic acid. The colonies showing brown halo round them were considered as lignin decomposers (Fig. 1). The selected isolates *Aspergillus clavatus*, *Penicillium martensii* and *Pythium proliferum* deBary were tested for their efficiency in decomposing lignin in liquid medium containing (NH₄)₂ HPO₄ (0.1%), MgSO₄ (0.02%), KCl (0.002%) and also in soil. Wheat straw was uniformly cut to size (1 cm) and 4 g. samples were taken in 50 ml liquid medium in 250 ml conical flasks. In another series, 5 g wheat straw

mixed with 200 g soil and moistened to 2/3 water holding capacity, was taken. The contents of the flasks were sterilized at 15 lb pressure for half an

hour, inoculated with uniform suspension of specific cultures and incubated at 30° C for 12 weeks. The loss of moisture was maintained regularly with sterile water and ash-free sulphuric acid lignin of residual wheat straw was determined² at 4, 8 and 12 weeks gravimetrically. The soil used for this study was an acid red loam, pH 5.8, organic carbon —0.31% and nitrogen —0.034%. The wheat straw contained total carbon —40.69%, cellulose 40.94%, lignin —19.32% and nitrogen —0.81%.

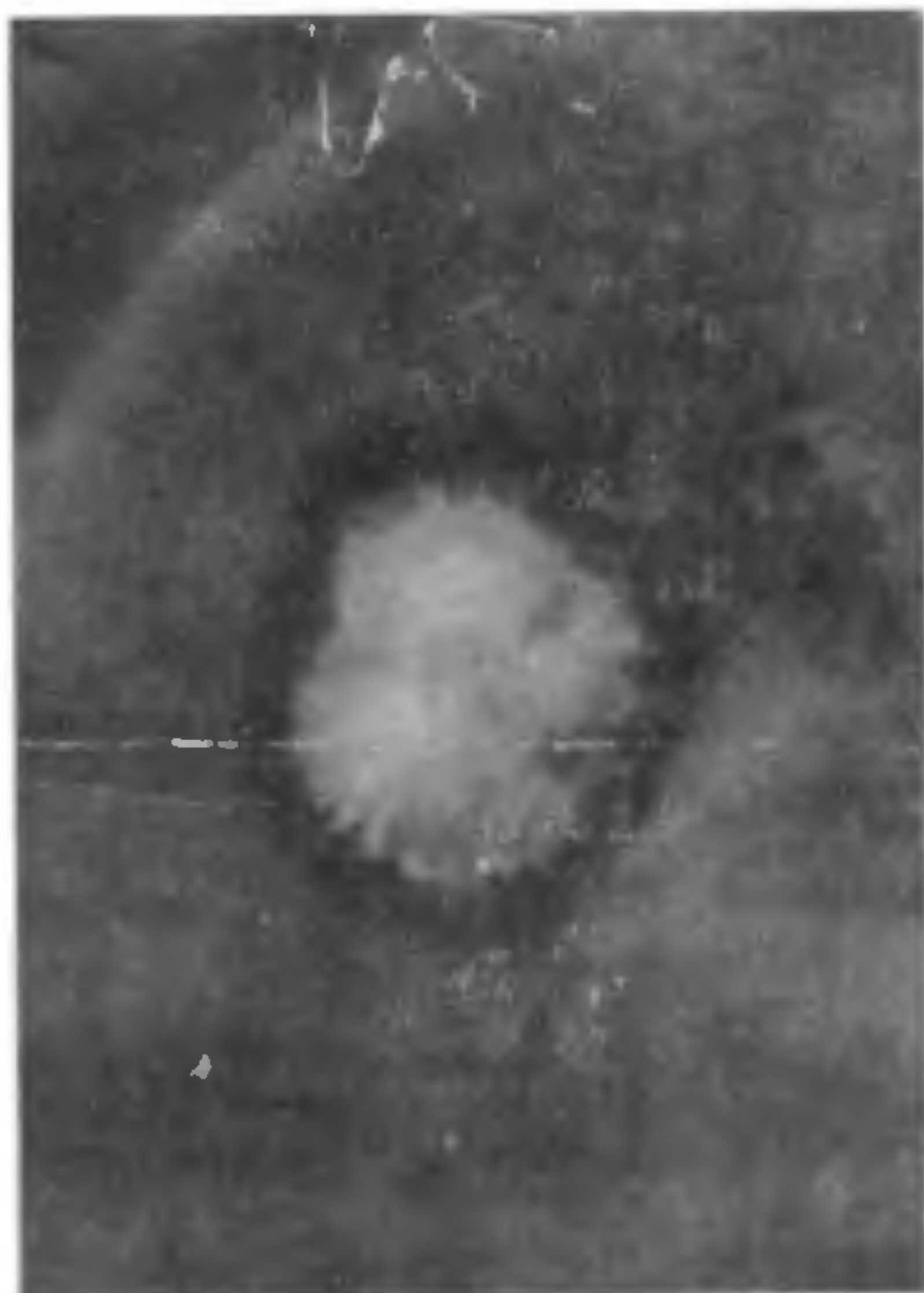
Results and Discussion

The browning of tannic acid caused due to its oxidation by extracellular enzymes excreted by microorganisms has been taken as a criterion for isolating lignin decomposers. This, being an indirect evidence, has been criticized by Hendersen⁵. However, Higuchi⁶ has shown that microorganisms which split tannic acid or other phenolic compounds possessed lactase and phenolase which also oxidized polyphenols in lignins. The decomposition of wheat straw in liquid medium and soil as determined by its lignin content at various stages of decomposition has been given in Table I.

The major loss of lignin due to inoculation was within 4 weeks and later the rate of decomposition was slower. Schobinger⁸ reported that 65% of the lignin in wheat straw was lost in about 180 days after which no further loss was observed upto 410 days. The rapid loss of lignin in wheat straw may be due to



(a)



(b)



(c)

FIG. 1. Lignin decomposition by (a) *A. clavatus*, (b) *P. nartensii* and (c) *Pythium proliferum* deBary.

TABLE I
Lignin content (%) of wheat straw at different stages of decomposition

| Treatments | Initial | Period in weeks | | | | | |
|--|---------|-----------------|------|---------------|------|---------------|------|
| | | 4 | | 8 | | 12 | |
| | | Liquid medium | Soil | Liquid medium | Soil | Liquid medium | Soil |
| Control | 19.3 | 17.8 | 17.8 | 17.5 | 17.7 | 17.4 | 17.6 |
| Inoculated with <i>Aspergillus clavatus</i> | 19.3 | 10.9 | 11.8 | 10.5 | 11.5 | 10.0 | 10.7 |
| Inoculated with <i>Penicillium martensii</i> | 19.3 | 12.5 | 13.0 | 12.1 | 12.7 | 11.8 | 12.0 |
| Inoculated with <i>Pythium proliferum</i> deBary | 19.3 | 14.3 | 15.2 | 14.0 | 14.9 | 13.6 | 14.2 |

higher content of cellulose (40.9%) than lignin (19.3%). The cellulosic material might have served as initial source of energy for rapid proliferation of lignin decomposing fungi which later accelerated the decomposition of lignin. *Aspergillus clavatus* was most efficient in lignin decomposition followed by *Penicillium martensii* and *Pythium proliferum* deBary. Several soil fungi imperfecti commonly occurring in soil decompose lignin³ but there appears to be no report on *Pythium* sp. The relatively lower efficiency of this organism may be attributed to the lack of proper enzyme system needed for the decomposition of lignin.

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A NEW LEAF BLIGHT DISEASE OF CITRONELLA GRASS

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CITRONELLA (*Cymbopogon winterianus* Jowitt.) is an aromatic, perennial grass largely grown for its essential oil, "Citronella oil". It is cultivated over large areas in South India. A new leaf blight disease was observed during June, 1978 at the Forest Research Station, Gottipura near Bangalore. The disease manifests in the form of irregular, minute, brownish spots, scattered all over the leaf lamina which later turn reddish brown. When the disease incidence is severe, the leaf spots coalesce causing extensive blighting of the leaf. Death of the tissue is striking at the centre of the leaf which eventually spreads to the entire leaf surface.

When the affected tissues were kept in the humid chamber, numerous conidia emerged from the dead portion of the leaf. A large number of tissue isolations from leaves invariably yielded a culture of the fungus akin to *Drechslera* sp.

One week old sporulating culture of the fungus was used successfully to produce infection on a 10 week-old, healthy potted citronella plant. Characteristic symptoms were observed on the leaves on the 7th day after inoculation. Profuse sporulation was observed in a week's time on PDA at 25°C.