

TABLE II
Nitrogen fixing capacity of *Azotobacter* (mg/g mannitol)
associated from different plant parts

Plant parts	Rhizo- sphere	Phyllo- sphere	Geocarpo- sphere
Healthy	12.1	17.3	20.0
Virus-infected	8.6	8.6	8.9

unhealthy or diseased plants. The data in Table II also indicate that *Azotobacter* associated with geocarposphere has higher nitrogen fixing potentiality than that found in rhizosphere. This, therefore, seems to suggest clearly that different parts of the plant harbour different strains of *Azotobacter* and that geocarposphere is a potential source of desirable strains of *Azotobacter* for use as biofertilizers.

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FUNGI ASSOCIATED WITH DETERIORATING SEEDS OF *CANNABIS SATIVA* L.

THE fungi associated with seed surfaces of *Cannabis sativa* L. and those invading their deeper tissues may be of some concern to the producers and manufacturers of narcotics for medicinal purposes, as they may influence not only the quality but even the quantity of the seeds. Keeping this in mind, the present investigations were taken up.

For isolation of mycoflora, nearly 400 seeds were randomly selected from 6 months old seed lot. The techniques of seed washing and agar platings were followed as suggested by ISTA (Anonymous¹). For agar plate methods the seed surfaces were sterilized with 0.1% HgCl₂. The inoculated plates were incubated for a week at 20°C. The isolates were purified following Ricker and Ricker⁴ and identified with the help of stock cultures of the Department and books.^{2,3,5} For determining the pathogenic nature of internally borne fungi, the seeds were soaked for few hours in spore suspension and then incubated on sterilized blotting paper at 28°C. The respective fungal spores were also sprayed on leaf surfaces of 10–15 days old plants in glass house in order to determine their pathogenicity on adult crops. In all cases, care was taken to fully satisfy the Koch's postulate; corresponding controls were also maintained for which the seeds were freed from fungi by heat therapy.

From seed washing 16 fungal species were isolated, viz., *Aspergillus niger* Van Tieghem, *A. flavus* Link ex Fries, *A. tamarii* Kita, *A. Sulphureus* (Fres) Wehmer, *A. repens* (Corda) de Bary, *Penicillium chrysogenum* Thom., *Alternaria tenuis* Nees, *Alternaria geophila* Daszewska, *Fusarium javanicum* Koorders, *Curvularia lunata* (Walker) Boedijn, *Cladosporium herbarum* Persoon Link, *Monilia sitophila* Sacc, *Mycelia sterilla* Cook, *Trichoderma album* Preuss, *Cephalosporium curtipes* Sacc., *Streptomyces* sp.

Ten fungal species were isolated from surface sterilized seeds, viz., *Aspergillus niger*, *A. sulphureus*, *Cladosporium herbarum*, *Penicillium chrysogenum*, *P. chermesinum* Biourge, *P. frequentans* Westling, *P. lavitum* Raper and Fennell, *P. fellutanum* Biourge, *P. chrlichii* Klebahn, and *Cephalosporium curtipes*.

Five fungal species, viz., *Aspergillus niger*, *A. sulphureus*, *Penicillium chrysogenum*, *Cladosporium herbarum* and *Cephalosporium curtipes* were present both externally and internally.

Out of 10 internally borne fungi only *Penicillium* were found to be pathogenic. The highest degree of seed spoilage was, however, exhibited by *Penicillium chrysogenum* and *P. frequentans* and the rest was moderately pathogenic. The fungi other than *Penicillium* species appear to saprophytic and probably came as a result of secondary infections. *Penicillium*

chrysogenum also produced burning type of symptoms on leaves of adult plants and thus exhibited localised aerial infections and later caused wilting of the twig itself but never of the old plant.

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A TECHNIQUE FOR THE REVIVAL OF HERBARIUM SPECIMENS FOR FLORAL DISSECTIONS AND ANATOMICAL STUDIES

HERBARIUM specimens for floral dissections or anatomical studies are usually softened by boiling them in water or soaking in a detergent solution¹ or treating with 2.5–5.0% sodium hydroxide or sodium hypochlorite². Aerosol OT solution, was sponsored as a softener for herbarium specimens^{3,4}. Being a wetting agent, Aerosol functions like any similar chemical. No single technique is equally suitable to revive all plant parts and/or species. In an attempt to devise an alternative softening agent, the present author tried for over five years, a solution of the following composition and found it to be very satisfactory in reviving herbarium specimens of various plant groups.

The mixture is made of glycerine 20 ml, glacial acetic acid 10 ml, EDTA (0.292% aqueous solution) 10 ml, sodium lauryl sulphate (5% aqueous solution) 10 ml and distilled water 50 ml. The duration of soaking the material in this solution depends on its hardness. No heating is required at any stage. The solution does not deteriorate on storage or repeated use. After the desired degree of softening the flowers can be dissected and retained in the same solution without the risk of their drying or rotting. The softened material can be returned to the herbarium sheet, after a brief washing and drying. The softened material can be sectioned free hand or on a microtome, after thoroughly washing it with distilled water. Conventional methods for microtoming and staining may be adopted. Refractory material already embedded in paraffin can be softened in this solution by slicing the wax away

exposing the material at one end to imbibe the solution.

Mixtures without acetic acid and/or EDTA were very unsatisfactory. EDTA chelates with divalent metal ions from the middle lamella and the cell walls thus softening the wall material. Glycerine also acts as a softening agent while acetic acid functions as a preservative without making the material brittle like formaldehyde. Sodium lauryl sulphate serves as a wetting agent and brings down the surface rigidity of the material. The chelation of the divalent metal ions may reduce the intensity of staining to a little extent. The addition of a suitable mordant after sectioning, will restore the intensity of staining.

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SOME ADDITIONS TO THE LICHEN FLORA OF INDIA

V. Genera *Phaeographis* and *Phaeographina* (Family: Graphidaceae)

TAXONOMIC investigations on the lichen flora of Western Ghats, south-western India, carried out during 1973–77 have resulted in the additions of several taxa as new reports for the country and many new species. Some of these have already been reported earlier (Patwardhan and Kulkarni², Patwardhan and Prabhu⁴) and seven species of the genus *Phaeographis* and two species of the genus *Phaeographina* are being reported here in this note.

Chemical studies were carried out by thin layer chromatography (Culberson¹). Specimens referred to in the text are deposited in the Lichen Unit of the Ajrekar Mycological Herbarium (AMH).

1. *Phaeographis angulosa* Muell. *Arg. Rev. Mycol.* 9: 81, 1887. Thallus thick, epiphloeodal; apothecia lirelline, immersed, 0.5–3.0 mm long, ends subacute, angulose; disc wide open, slaty; exciple non-carbonized; ascospores 8/ascus, 5–8 loculate, brown, 6–10 × 30–40 μm in size. Chemistry: K + yellow to red P + orange, norstictic acid is present.

Specimens examined: Tamil Nadu, Nilgiris—73-303, 496, 871, 1253, 3116, Palni hills, Kodaikanal—73-1968.

Distribution: New Caledonia and now India.