Endophytic fungal infection in a moss from Nepal

Nupur Bhowmik and Vineeta Rai

Botany Department, Allahabad University, Allahabad-211002

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Cushions of *Fissidens anomalus* Mont. growing on soil, constantly wetted by dripping water along road cuttings on hill slopes on way to Phulchowki, in Kathmandu (Nepal), frequently show signs of fungal infection in rhizomes, aerial stems and leaves. The cushions grow in close association with population of *Pellia endiviaefolia* (Dicks.) Dum. The infected plants of *Fissidens* are evident by their brownish black coloured swollen stems and spots on leaves. In case of intensive infection, destruction of vaginant lamina and apical lamina may also take place. Fungus enters the plant through rhizoids infecting the base of shoot and often moving up towards shoot tip. The endophytic fungus is abundantly present usually in cortial tissue in the form of nonseptate hyphae, arbuscules and vesicles. Besides these features, the paper enumerates several other changes caused in the plant due to infection of the endophyte. Plants of *Pellia* growing in close association have also been studied to observe morphological changes, if any, due to the fungal infection.

Key-words*—Fissidens*, Vaginant lamina, Cortex, Endophytic fungus, Arbuscules.

INTRODUCTION

DIVERSE fungal species known as endophytes are reported to be present in and on leaves and stems of plants which often show no symptoms of harbouring the fungi and may actually benefit the host from their presence (see Carrol 1988, 1992; Clay 1988; Cooke & Whipps 1993). Similarly, a variety of fungi also known to form mutual relationship with plants are the mycorrhizae, that develop between the fungi and roots of higher plants usually in phosphate deficient habitats. In bryophytes too, sporadic reports of moss parasitising on either aerial gametophores or sporophyte *Funaria hygrometrica, Brachythecium* sp., *Climacium* sp., *Hypnum* sp., *Plagiothecium* sp., etc. by Mastigomycotina, Ascomycotina and Basidiomycotina are known (Britton 1911; Dunham 1916; Prior 1966; Wolf 1954; Richardson 1981). Likewise, rhizoids and cells of several thalloid liverworts harbouring endophytes similar to those of functional vesicular - arbuscular (VA) endomycorrhiza have also been reported by many including Stahl (1949) and Harley and Smith (1983). Johnson (1977) showed the vesicular-arbuscular endophyte *Glomus tenuis* harbouring several liverwort species of New Zealand, where the infection was in the axis in *Haplomitrium gibbsiae, Lophocolea leucophylla* and *Trichocolea mollissima* and in the lower part of thallus of *Riccardia* sp., *Pallavicina xiphoides, Symphyogyna hymenophyllum,* etc. Rhizoids were also shown to be infected in *Marchantia* sp., *Schistochila* sp. and *Hymenophyton.* sp.

However, little is known about the mycorrhizal status in mosses, except for stray reports of occurrence of VAM fungus *Glomus tenuis* along leaves and stems of a *Pogonatum* -like moss by Rabatin (1980). She observed rhizoids free of fungal infection and the fungus "seemed most common on senescent mosses".

Another instance of moss-fungus association (not vesicular-arbuscular mycorrhizae) in hydroids of underground stems of *Polytrichum commune* Hedw. was reported by Grasso and Scheirer (1981) but whether the moss-fungus association was pathogenic or mycorrhizae-like was not determined. Concerning fungi in axes of *Fissidens anomalus* Mont. there are no reports to our knowledge, and so the present study was undertaken to report presence of VA mycorrhiza in gametophores of the plant and record all morphological changes brought about in the gametophores as a result of the endophytic infection. Preliminary observations revealed the association of endophyte to be antagonistic rather than beneficiary. Thalli of *Pellia endiviaefolia* (Dicks.) Dum. growing in close proximity of the moss were also studied to observe morphological changes as a result of VA mycorrhiza infection. Surprisingly, the association did not seem to adversely affect the gametophytes of *Pellia* sp. as it did in the case of gametophores of *Fissidens* sp.

Fig. 1. *Fissidens anomalus* Mont. showing normal and infected plants. 1-2. Normal plant (1. x3; 2. x12) 3-4. Showing infected plants (x3). 5-6. Part of infected shoots magnified to show damage caused to leaves (x12).

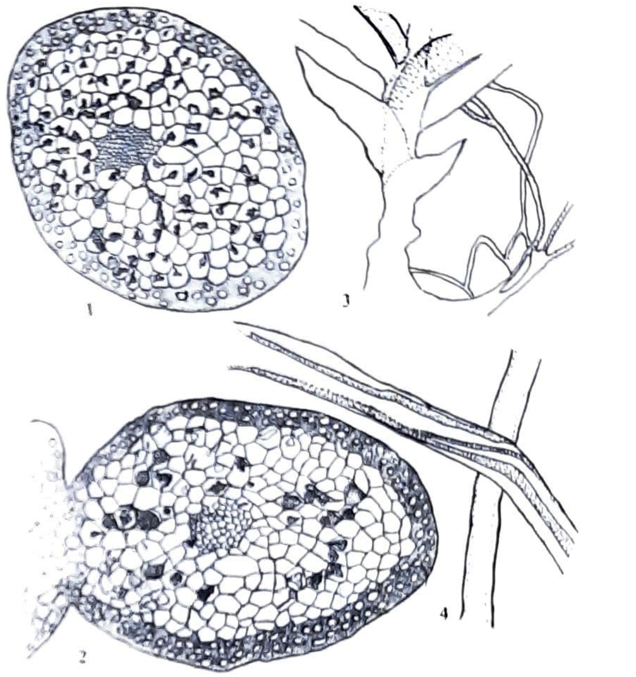

Fig. 2. *Fissidens anomalus* Mont. 1-2. Transverse sections of basal and upper parts respectively, of shoots showing intensity of infection by the abundant presence of aseptate hyphae, arbuscules and vesicles (x125); 3. Basal part of a plant showing rhizoids harbouring the fungus (x15); 4. Rhizoids of 3, further magnified to show aseptate hyphae inside (x600).

MATERIAL AND METHOD

The present investigation on infection in gametophores of *Fissidens anomalus* Mont. by VA mycorrhiza is based chiefly on collection of material at various points on way to Phulchowki, Kathmandu, Nepal. The infected plants growing intermixed with normal ones could be identified by their dark coloured axes and damaged leaf lamina. Plants were fixed in F.A.A. solution. Hand sections of stems and leaves of both normal and infected plants were cut and sections were stained in Cotton blue-lactophenol solution and mounted in glycerine for observation. Sections of *Pellia* thalli were also prepared for observation. Photomicrographs were made with a Leitz Wetzlar Periplan microscope.

OBSERVATION AND DISCUSSION

Plants of *Fissidens anomalus* Mont. growing on wet mud along road cuttings on way to Phulchowki looked seemingly normal but on closer examination quite a number of them showed brownish black coloured axes and torn up or eaten up leaf lamina where in extreme cases only the costa persisted (figs. 1, 3-6).

The infection showed a vertical zonation, being found abundantly at the base of the shoot, frequently moving upwards attacking more than half of the stem. Quite a number of leaves on these shoots also exhibited infection and close examination of laminar surfaces revealed circular to subcircular lacunae formed on account of laminar cell disintegration.

The fungus did not significantly alter the external morphology of the host by producing fruiting bodies or a "mantle" or Hartig network, conversely the fungal hyphae grew between and into cortical cells by penetrating the cell wall.

Internal anatomy of the infected plants was stud-

PLATE I

Fissidens anomalus Mont. 1. Basal part of axis showing normal and infected rhizoids (x45); 2. Part of a rhizoid in 1, magnified to show endophytic nonseptate hyphae inside (x600); 3, 5. Transverse sections of axes showing arbuscules and hyphae (x100); 4. Part of 3, further magnified to show disintegrated central strand cells and nonseptate hyphae traversing lacuna caused after cell disintegration (x630).

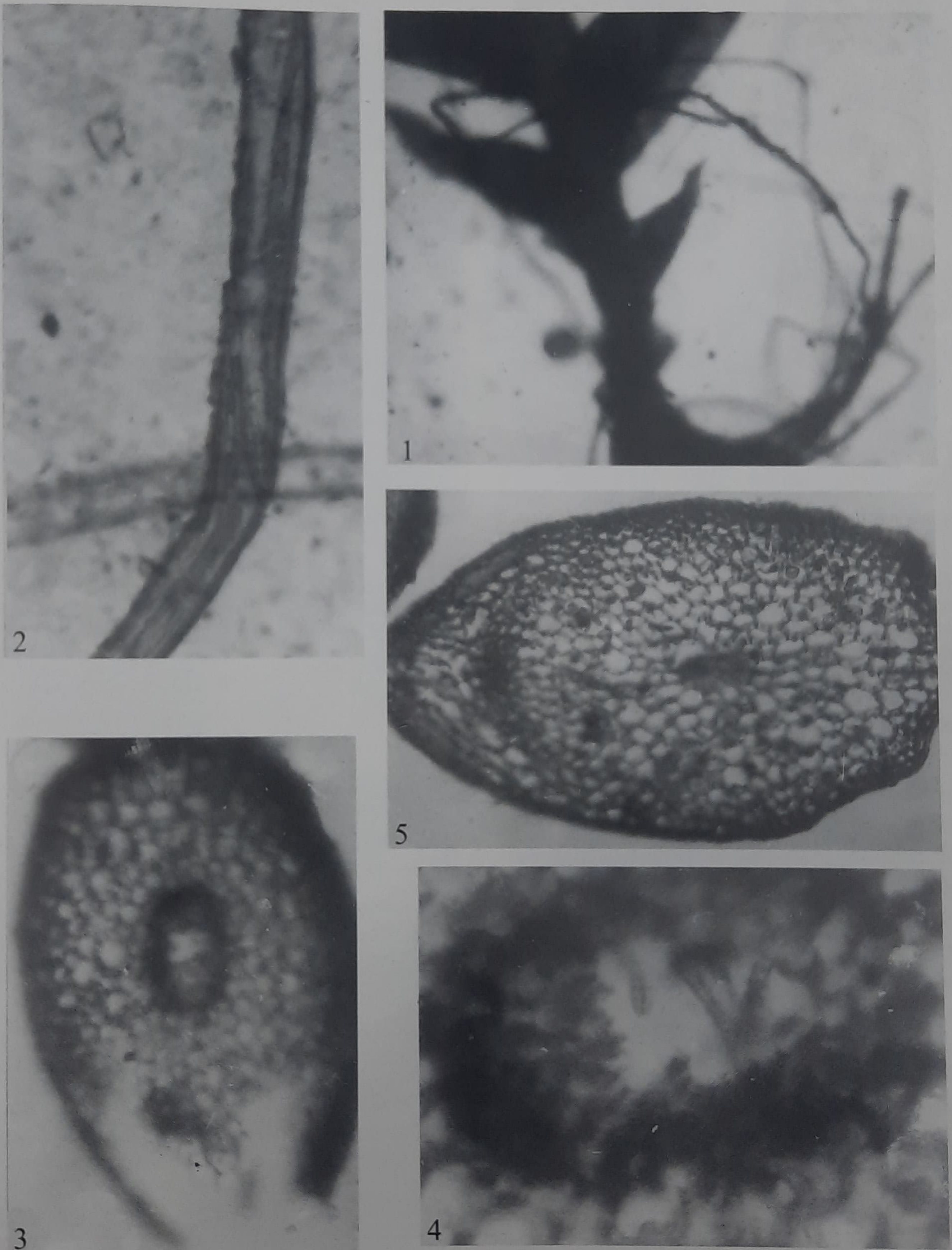

PLATE 1

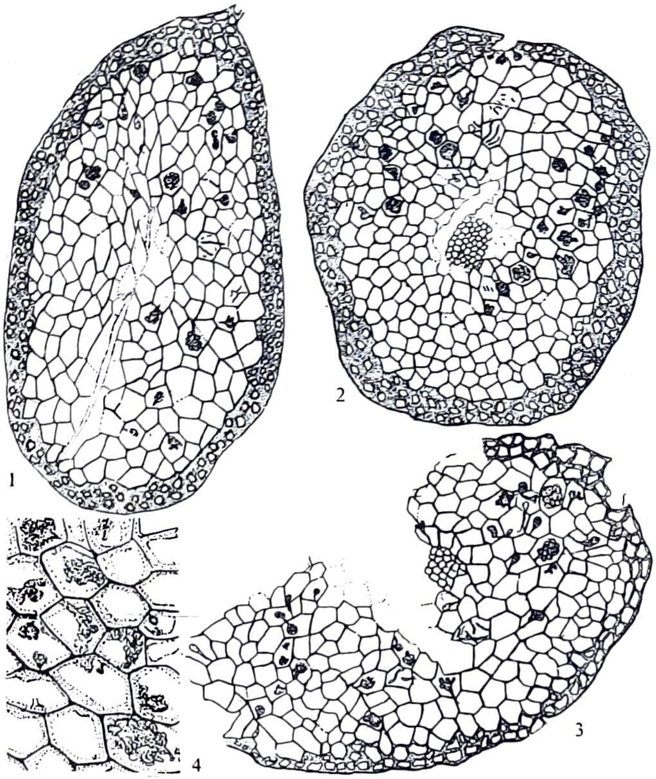

Fig. 3. *Fissidens anomalus* Mont. 1-3. Transverse sections showing disintegration of cells of central strand and cortex. Cells also show aseptate hyphae, young and old arbuscules and vesicles (x125); 4. Showing magnified view of cortical cells containing young and old arbuscules (x650).

ied after cutting sections of stems and leaves and staining them in Cotton blue-lactophenol solutions.

Microscopic examination of stained sections of axes and leaves of infected plants showed the presence of endophyte in the form of intracellular nonseptate hyphae, vesicles and arbuscules. The fungal hyphae, 6.5-7.5 μm in diameter, appeared to enter the gametophores through rhizoids. A single rhizoid often showed two to three hyphae inside its cavity. The hyphae penetrated the host mechanically by their pointed tips and in the same manner passed from one cell to another apparently by penetrating lateral walls and establishing themselves intracellularly. They produced coils of highly branched haustorium- like structure called arbuscules and in some cases large terminal swellings called vesicles. The endophyte was abundantly present in the cortical region sometimes even inhabiting the thick walled cells of outer cortex. In some cases the hyphae were also present in the small cells of central strand. The hyphae clumped together in host cells to form young arbuscules that fused to form larger ones. However, arbuscules remained alive for a short period only as they disintegrated and appeared to be digested. The mycorrhizal association showed a continuous sequence of development and disintegration of arbuscules. The hyphae also produced spherical to ovoid swellings of various sizes terminally, called vesicles. Younger vesicles were dark coloured having dense cytoplasm and older ones lighter coloured. Vesicles were thought to function as energy stores for use by fungus when supply of host metabolite was low (Alexopolous *et al.* 1996). No fungal reproductive structures were observed in the cells of the gametophores.

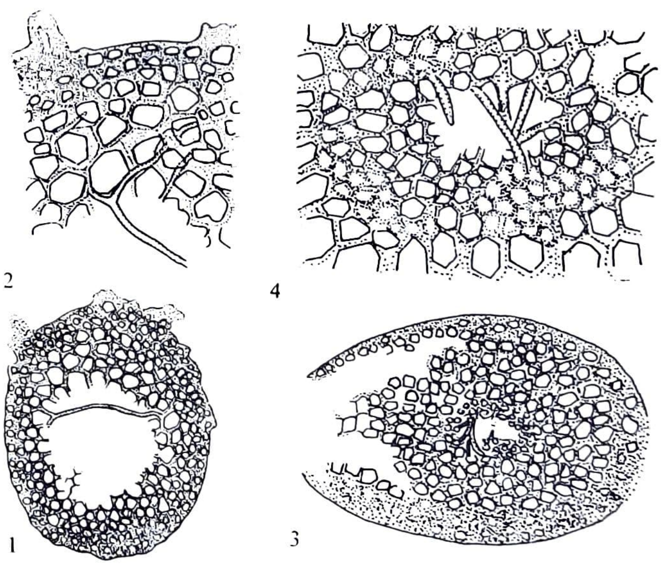

Fig. 4 : *F. anomalus* Mont. 1-4, transverse sections of axes. 1, section of basal part of axis showing non-septate hypha and central lacuna after disintegration of central strand and cortical cells. (1, x 125). 2, same section further magnified (2, x 400). 3, transverse section of axis, a little above base showing degeneration of central strand cells and non-septate hyphae (3, x 100). 4, same section further magnified (4, x 630).

PLATE-II

Fissidens anomalus Mont. 1-5. Transverse sections of axes and leaves showing infection; 1, 3. Showing various stages of cell disintegration caused by the endophytic fungus. Almost all cells contain arbuscules and vesicles (x100); 2. Transverse section of axis showing mature vesicles and arbuscules in cells of cortex and central strand (x100); 4. Part of transverse section of cortex of an axis magnified to show cells containing hyphae, young and old arbuscules (x1000). 5. Transverse section of a part of leaf showing infected cells (x250).

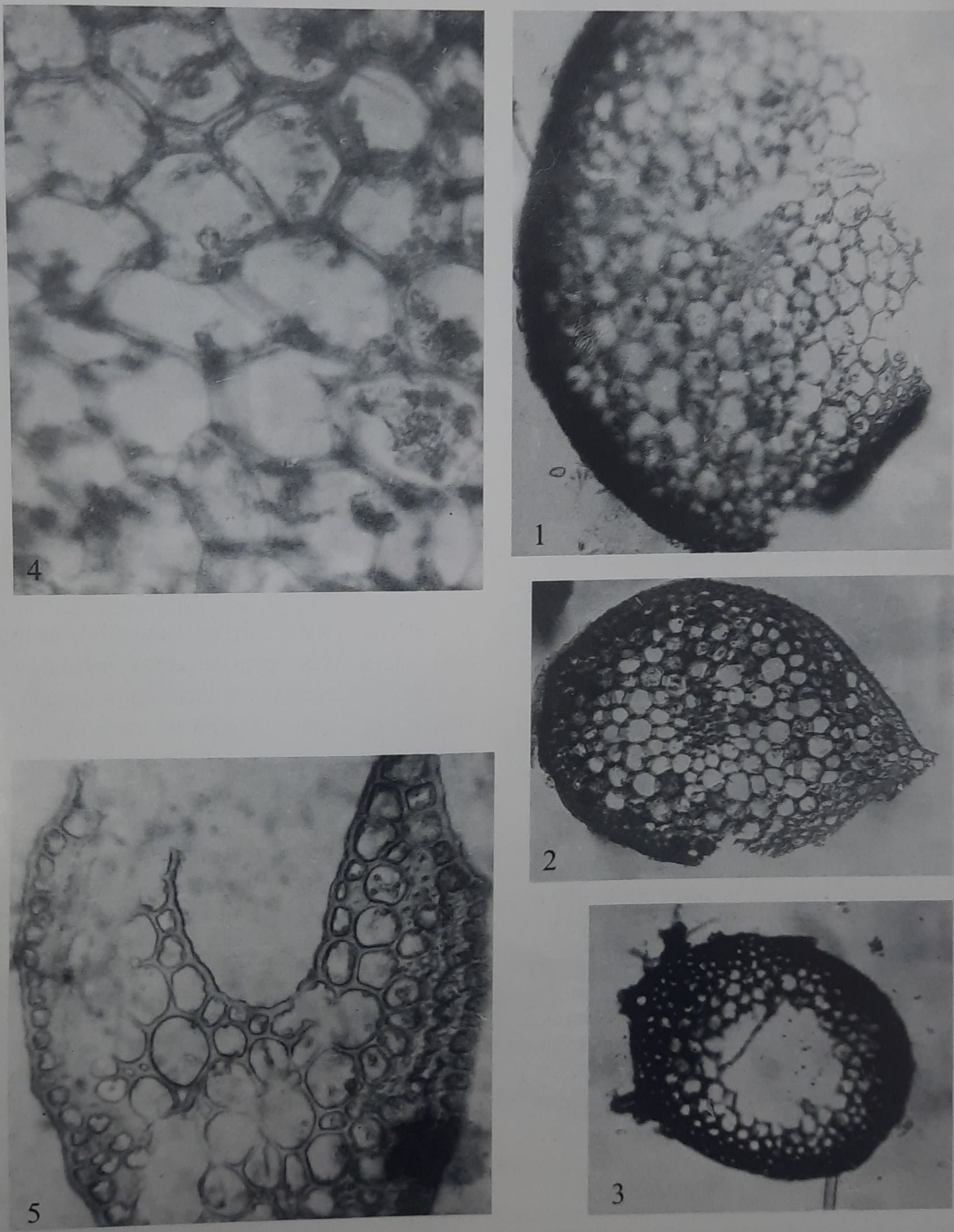

PLATE-II

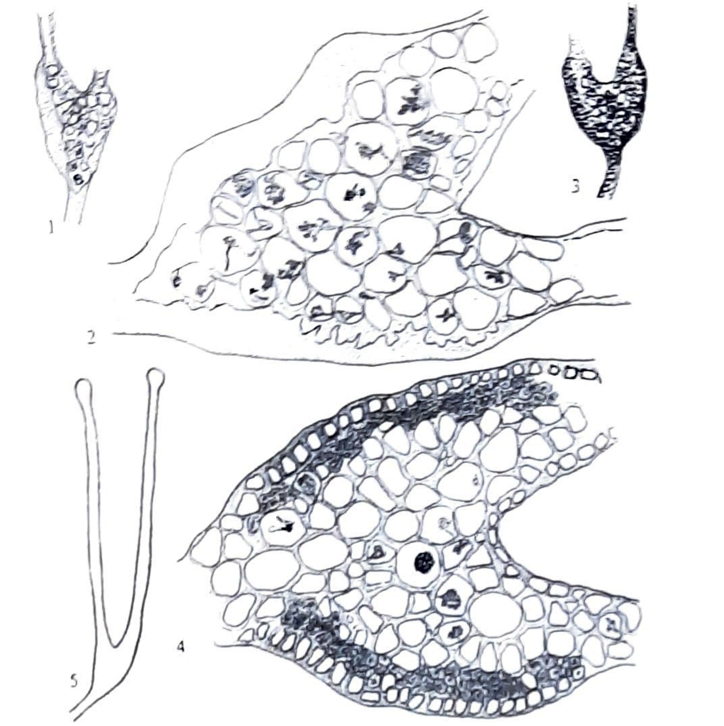

Fig. 5. *Fissidenis anomalus* Mont. 1, 5, showing transverse sections of leaves. 1, 2 showing almost all leaf cells harbouring endophytic fungus in the form of aseptate hyphae, arbuscules and vesicles. (1, x 75) 2, same section further magnified (2, x 350). 3, 4 sections of another leaf showing thick walled cortical cells and thin walled inner cells containing arbuscules, (3, x 75); 4, part of 3 further magnified (4, 350). 5, transverse section of normal leaf. (5, x 50).

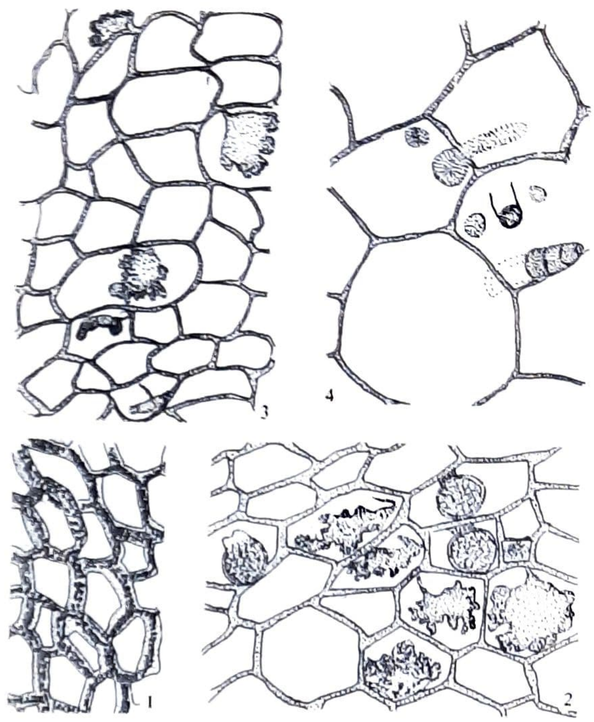

Fig. 7. *Pellia endiviaefolia* (Dicks.) Dum. 1-5. Showing V.T.S. of thalli. 1; showing epidermal cells (x 630) 2, cells showing stalked vesicles and large arbuscules (x 630). 3, 4, cells of some thallus showing arbuscules and conidia-like structures (3, x 630; 4 x 1000).

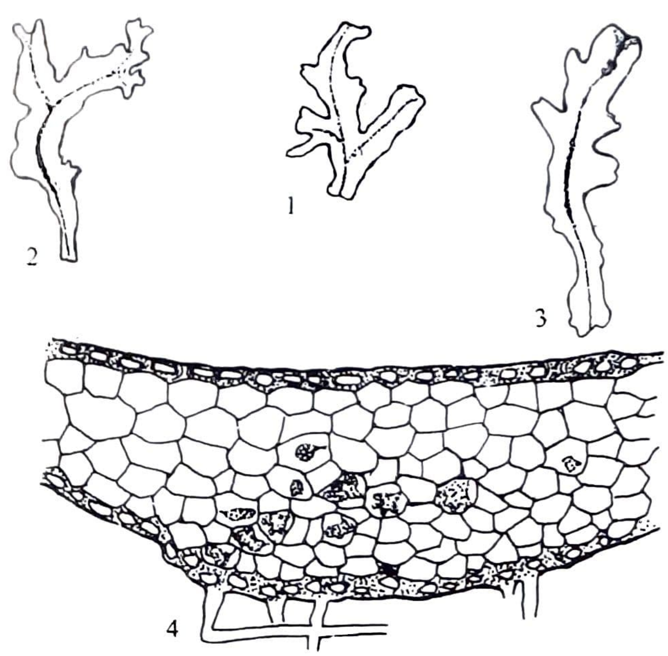

Fig. 6: *Pellia endiviaefolia* (Dicks.) Dum., 1. Normal thallus (x2); 2,3. Thalli showing presence of fungus in darker regions (x 2). 4, V.T.S. of thallus showing arbuscules and vesicles inside thallus cells (x 100).

Although the occurrence of non-septate hyphae, arbuscules and vesicles in the present material indicated affinities with members of Glomales of Zygomycotina, which are often referred to as VAM fungi or endomycorrhizae and which are known to form mycorrhizic relationships with agronomically important angiosperms, some gymnosperms, as well as certain bryophytes, pteridophytes and even a few algae, they are also reported not to significantly alter external morphology of their higher plant partners (Alexopolous *et al.* 1996). However, the present study revealed features which are a departure from what is known concerning mycorrhizae of higher plants. The VA mycorrhizae in gametophores of *Fissidens* not only digested the cells of the central strand and in many instances also the surrounding inner cortical cells causing large central cavities in the stem but in some sections nonseptate hyphae were even visible dangling in the centrally formed cavities. Besides destroying the stem, the VA mycorrhiza also seemed to cause extensive damage to the leaves by seemingly devouring the cells of the lamina where in extreme situations only the mid rib seemed to survive the attack.

The moss-fungus association described in the present paper appears to be pathogenic rather than mycorrhizal-like, since in higher plants it never invades the xylem or central strands Grasso and Scheirer 1981). In case the association is one of host-parasite, breakdown of moss tissue is not rapid and the parasitized plants are not killed and may eventually recover and regenerate. This pathogenic activity of the VA mycorrhiza in plants of *Fissidens* is perhaps the first report of its kind and in order to study whether the VA mycorrhiza present in the thallus cells of *Pellia endiviaefolia* (Dicks) Dum. growing in close proximity of *Fissidens anomalus* plants had a similar parasitic effect on the liverworts, morphology of thalli of *Pellia endiviaefolia* were also studied. Surprisingly the liverwort-fungus association in *Pellia endiviaefolia* exhibited no symptoms of parasitism even when the thalli were intensely infected with the VA mycorrhiza. The plants looked normal and vibrant. Hand sections of the thalli showed various features of the VA mycorrhiza including nonseptate intracellular hyphae, large and small arbuscules and very large vesicles. In addition to these the hyphae in some of the cells also produced conidia-like structures resembling the arthric conidia (Deuteromycetes).

In nature phosphate is likely to be an element regulating growth of many bryophytes, but there have been very few studies on this problem and there is no evidence to suggest that mycorrhizal infection occurring in bryophytes improved their phosphate status (Brown 1982). There have been instances where endotrophic mycorrhiza in liverworts (Harley 1959; 1969) particularly those similar to functional vesicular arbuscular endomycorrhiza derived from a range of liverworts have been used to induce typical VA infection in higher plants (Stahl 1949; Johnson 1977; Rabatin 1980) and in rare instances plants of *Philonotis glaucencens* showed more prolific growth when a mixture of VA mycorrhizal fungi was added to their plant mixture (Brown 1982) yet there are no reports, as far as we know, of VA mycorrhiza causing harm to the host as seen in the plants of *Fissidens.* Destruction of the host cells and tissues due to the production of certain chemicals by the endophyte whose nature may be quite similar to fungal phytotoxins produced by some plant pathogenic fungi cannot be overruled but ambiguous behaviour of the VA fungus in different bryophytic hosts growing under similar environmental conditions is thought provoking and calls for more detailed study.

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