

7. Heston, W. M., (Jr.), Franklin, A. D., Hennely, E. J. and Smyth, C. P., *J. Am. Chem. Soc.*, 1950, **72**, 3443.
8. Glasstone, S., Laidler, K. J. and Eyring, H., *The Theory of Rate Processes*, McGraw-Hill, New York, 1941, p. 548.
9. Davidson, D. W., *Can. J. Chem.*, 1961, **39**, 571.
10. Deogaonkar, V. S., Adgaonkar, C. S. and Jajoo, S. N., *Indian J. Pure Appl. Phys.*, 1979, **17**, 745.
11. Deogaonkar, V. S., Adgaonkar, C. S. and Jajoo, S. N., *Indian J. Pure Appl. Phys.*, 1982, **20**, 617.
12. Mathur, A., Sharma, S. N., Saxena, M. C., *Indian J. Pure Appl. Phys.*, 1974, **12**, 370.
13. Dhar, R. L. and Shukla, J. P., *Z. Phys. Chem. Neue, Folge*, 1973, **84**, 25.
14. Gandhi, J. M. and Sharma, G. L., *J. Mol. Liquids (Netherlands)*, 1988, **38**, 23.
15. Madan, M. P., *J. Mol. Liquids (Netherlands)*, 1987, **33**, 203.

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Mycorrhiza: A possible deterrent in artificial cultivation of morels

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We report, for the first time, four species of morels that are in mycorrhizal association with the roots of some herbaceous plants in the northwestern Himalayas. It appears that the perhaps obligate mycorrhizal association of these morels may be responsible for the failure of earlier attempts to cultivate them artificially.

MORELS are a cherished delicacy and an important minor forest produce, with enormous economic potential, presently being sold at Rs 2200–2500 per kg in the Indian market¹. Various attempts have been made to domesticate this natural commodity since very early times but with little success². The indoor artificial cultivation of mushrooms has been successfully achieved in about fifteen species the world over³. This has, however, been possible only in the case of non-mycorrhizal mushrooms, where a successful imitation of natural processes has been possible.

The morels were not known to enter into any mycorrhizal relationship till we briefly reported this association in *Morchella esculenta*⁴. Later a similar but more detailed and definitive relationship between *M. rotunda* (Pers.) Boudier and some living trees and herbaceous plants was described and discussed by Buscot and Roux⁵. In the meantime we observed other morel species to enter into mycorrhizal relationship. The observations on the same relationship are being presented here.

Mycorrhizal connections were established by digging the soil and tracing the roots of the plant species to the fructifications following Young^{6,7}, and Zak^{8,9}. The

roots of plants associated were thoroughly washed with tap-water, and after fixing them in FAA for 24 h they were preserved in 70% alcohol. In order to confirm the formation of mycorrhizae, both stained (cotton blue in Lactophenol) and unstained sections were prepared and examined microscopically.

Observations for mycorrhizal association between the sporocarps of *Morchella deliciosa*, *M. esculenta* and *M. conica* and the roots of strawberry, grasses and fern rhizomes were made visually. The sporocarps were taken out carefully along with some mass of soil. These were allowed to dry and then freed from soil particles or they were washed slowly in running water to remove the dirt. The subterranean portion of the carpophore was seen to form a loosely woven cord near its base, which was gradually transformed into a more compact and shapely cord, producing some short roots on way, and tapering at the distal end and there getting connected to the farther end of the roots of these plants, forming a 'mycorrhizal bridge' (Figure 1, a–f; Figure 2, a–c).

Anatomical observations made on sections stained with cotton blue, show that morel hyphae penetrate into all the tissues of roots except xylem (Figure 2, d). The hyphae penetrate into the cells where they mostly grow near the cell wall but they also sometimes grow more deeply within the lumen. The root hairs are mostly absent. The typical fungus mantle was also absent. The hyphae have not been seen to grow intercellularly. In longisections of the root, the hyphae were seen to form a loose web on the root periphery; some of them penetrating directly into the cell lumens (Figure 2, e). The short roots also show a similar structure and they are almost evenly distributed all along the long root and its branches.

As has been observed in the present investigations, Buscot and Roux⁵, also had noted that ascocarps of *Morchella rotunda* are joined by subterranean hyphal systems, the 'mycelial muffs', surrounding living roots of various plants. Anatomically, the fungus has been found to penetrate almost all cells lying outside the xylem. They noted that from the 'mycelial muffs' the fungus penetrates into the root tissues as far as the vascular cambium, and grows predominantly intracellularly, especially within young secondary phloem. The formation of this close association with plant roots may represent a critical stage, concerned with elaboration of the ascocarps in the life cycle of this, otherwise 'putatively saprophytic fungus'. They mention that the localization of 'muffs' on parts of roots that are non-absorptive, suggests that the association is not truly mycorrhizal. However, the association that we have observed between species of morels and the herbaceous plants during the present studies, seem to be doubtlessly mycorrhizal as the fungus has been found to be associated with young absorbing roots.

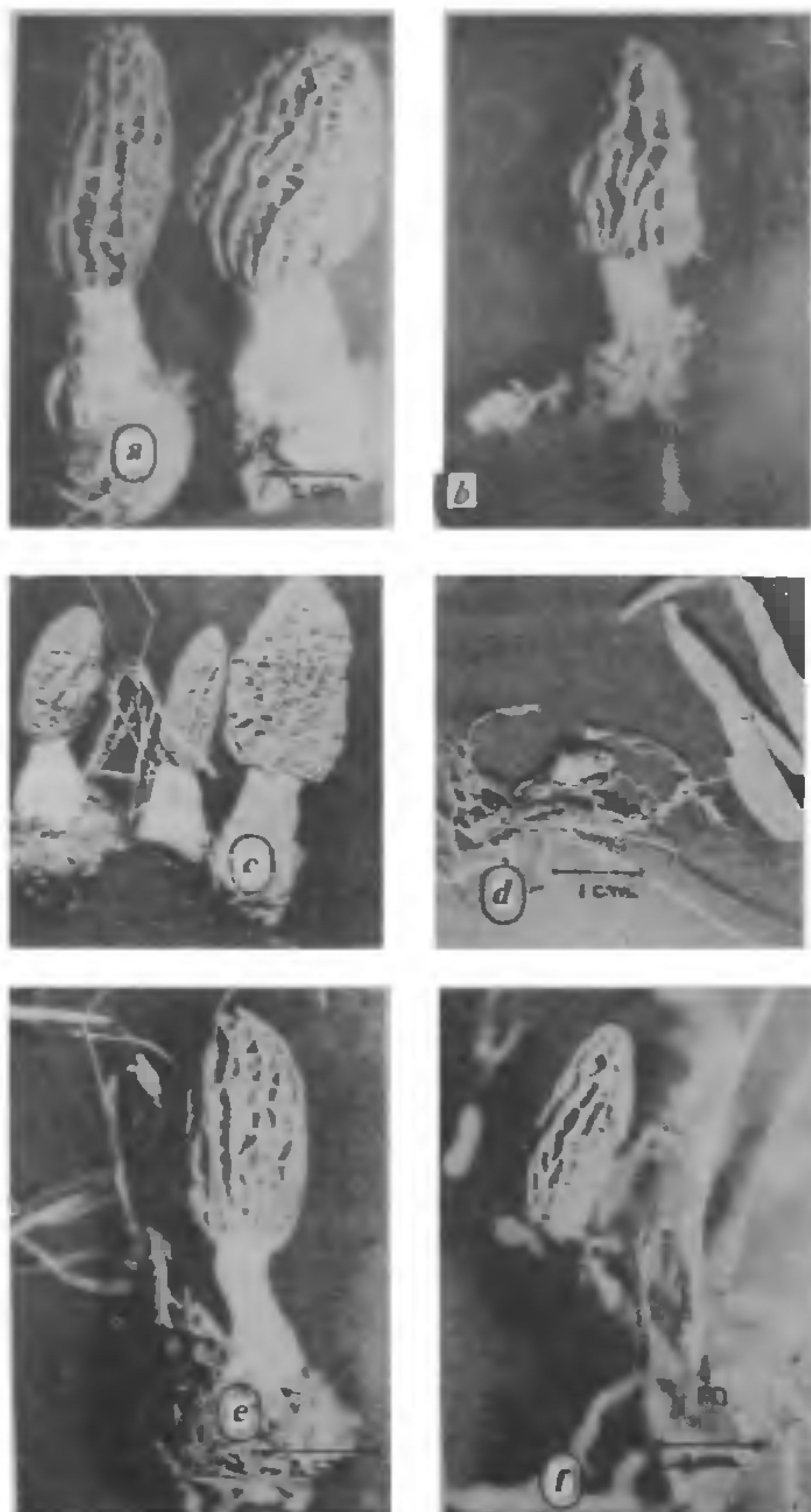


Figure 1. **a**, Uprighted *Morchella crassipes* showing mycelial cord formed with grass roots. **b**, *Morchella conica* showing mycelial cords with grass. **c**, *Morchella crassipes* in mycorrhizal association with grass roots. **d**, *Morchella deliciosa* in mycorrhizal association with fern. **e**, *Morchella esculenta* in mycorrhizal association with grass. **f**, *Morchella esculenta* in mycorrhizal association (m) with fern.

Buscof and Roux⁵, also suggest that this morel may possess a parasitic or symbiotic phase in its life cycle and that consistent with this possibility is the direct relationship between the ascocarp and the 'mycelial muffs' exclusively located on subterranean organ that conduct elaborated metabolites and the preferential attack of tissues within that are concerned with their transport. They further pointed out that on the other hand, the limited area colonized by the fungus and the saprophytic potential of its mycelium suggests that

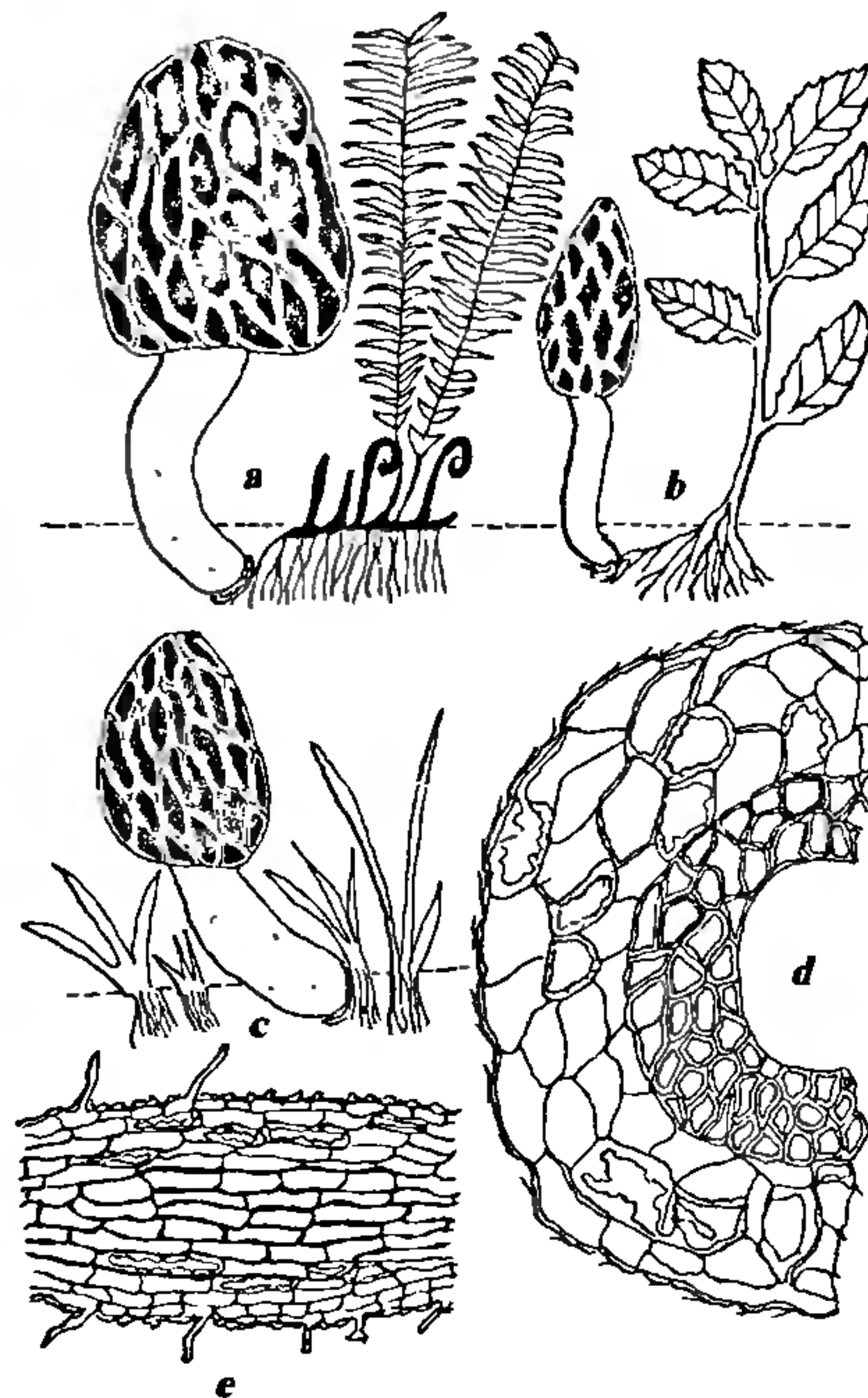


Figure 2. **a**, *Morchella esculenta* in mycorrhizal association with fern. **b**, *Morchella conica* in mycorrhizal association with raspberry. **c**, *Morchella deliciosa* in mycorrhizal association with grass. **d**, TS mycorrhizal root of fern. **e**, LS mycorrhizal root of fern.

either this parasitism is temporary or that the association was more to do with stimulation of ascocarp formation than nutrition *per se*.

Though all these morel species were collected from forested areas with many species of conifers predominating, their association with trees could not be established. Circumstantial evidence does suggest that some species may form mycorrhizae with apple trees. The fruiting bodies of morels have been frequently observed to crop up in the apple orchards, especially around the apple tree basins. In the forests where the fruiting bodies come up, they apparently do not seem to form any mycorrhiza with them. However, considering reports of Schramm^{9,10}, and Maroneck *et al.*¹¹ that the mycorrhizal strands of *Pisolithus tinctorius* may extend 5–10 m from the seedlings to the basidiocarps, the association of morels also need to be examined more closely and carefully to negate any such link. If it was purely an association with herbaceous flora, then these should be expected to come up in areas which do not have trees but abundant herbaceous flora. We have observed them

to come up in pastures, but not in that great abundance.

The mushrooms that enter into symbiotic mycorrhizal relationship with different plants are usually host-specific and hence cannot be grown indoors. In such cases field cultivation has been suggested and practised in some instances. Attempts to obtain fruiting bodies of mycorrhizal mushrooms without host plants have never been successful because such mushrooms accomplish their biological cycle jointly with the host plant, giving rise to a complex nutritional relationship.

The observations that morels also enter into mycorrhizal association are of utmost significance. These are a pointer to the fact that may be it is the mycorrhizal specificity which has so far foiled all attempts at artificial cultivation of morels. It seems now imperative to direct attention on this aspect and if it is so, to alternatively try their cultivation outdoors with specific host plants.

1. Lakhanpal, T. N. and Shad, O. S., *Indian J. Mush.*, 1986-87, 12-13, 5.

2. Ower, Ra, *Mycologia*, 1982, 74, 142.
 3. Chang, S. T. and Miles, P. G., in (eds. Chang, S. T. and Quimo, T. H.), *The Tropical Mushrooms, Biological Nature and Cultivation Methods*, The Chinese Univ. Press, Hong Kong.
 4. Lakhanpal, T. N., in *The Souvenir*, NCMRT Solan, India, 1986, pp. 23-25.
 5. Buscot, F. and Roux, J., *Trans. Br. Mycol. Soc.*, 1987, 89, 249.
 6. Young, H. E., *J. Aust. Inst. Agric. Sci.*, 1936, 2, 32.
 7. Young, H. E., *J. Aust. Inst. Agric. Sci.*, 1940, 6, 21.
 8. Zak, B., in *Mycorrhizae*, (ed. Hacskeylo, E.), U.S. Govt. Printing Office, Washington, 1971, pp. 38-53.
 9. Zak, B., in *Ectomycorrhizae, Ecology and Physiology*, (eds. Marks, G. C. and Kozlowski, T. T.), Academic Press, New York, 1973, pp. 43-78.
 10. Schramm, J. E., *Am. Philos. Soc.*, 1966, 56, 1.
 11. Maroneck, D. M., Hendrix, J. W. and Kiernan, J., *New Phytol.*, 1981, 102, 85.

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