VOLATILE METABOLITES OF SOIL FUNGI IN RELATION TO SPORE GERMINATION AND MYCELIAL GROWTH

VOLATILE metabolites of fungi have received considerable attention in the recent years and they have been implicated in the growth and development[2,3,6,8,9] and ecology of soil microbes[1,4,5,6,7,10] One such sphere of microbial ecology is that of soil fungistasis in which our laboratory has been keenly interested[7,10].

Volatiles emitted by *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* van Tieghem, *A. terreus* Thom, *Penicillium chrysogenum* Thom, *P. jenseni* Zaleski, *P. nigricans* Bainier, and *P. notatum* Westling were studied in liquid and agar Czapek's medium employing the flask technique or chambers made of paired Petri dishes. In the former Erlenmeyer flasks of 150 ml capacity were used and 25 ml of liquid medium was dispensed in each. After autoclaving, each flask was inoculated by the spore suspension prepared from 6–8 day-old culture of soil fungi. The mouth of the flask was closed by cork plug through which a soft copper wire was pierced whose inside end was made into a loop ; the loop was big enough to hold a single water agar disc (2 mm thick and 8–10 mm diam) which was exposed to the volatiles of soil fungus growing in the medium for 10 days. For germination study, the exposed agar disc was taken out for a brief period and after placement of spore suspension, was returned to its oiginal place ; germination counts for at least 200 spores were made after a further incubation of 24 hr. Effect of volatiles on spore germination and mycelial growth was also evaluated in paired Petri dish chambers[2]. In this technique, soil fungi were grown in Czapek's agar medium for a period of 10 days. The upper lid was then replaced by another lid of the same size which also contained agar medium and a centrally-placed inoculum disc of the test fungus or spores. The radial mycelial growth was recorded after a further incubation of 6 days. In the control set, the lower lid contained uninoculated medium. The two lids of Petri dish were sealed off, using cellotape to avoid any outward diffusion of the volatiles. Test fungi included, *Alternaria tenuis* Nees, *Curvularia geniculata* (Tracy and Earle) Boedijn, *Helminthosporium rostratum* Drechsler, and *Pestalotia* sp. ; all the four are common soil inhabitants. All manipulations were made under aseptic conditions and experiments were run in triplicate at least.

Inhibition of spore germination was quite marked in the case of *A. fumigatus* and *A. terreus* and the values ranged between 50–90% (Fig. 1) ; spores of *Pestalotia* were, however, inhibited to a smaller degree against these two soil fungi. *Aspergillus niger* and *A. flavus* were not as effective as the other two species. These observations are in agreement with those of Johri and Singh[7]. All the four species of *Penicillium*, on the other hand, could inhibit spore germination of *Alternaria, Curvularia,* and *Helminthosporium* to a marked degree (60–90%). *Penicillium jenseni* was the most active producer of volatiles, since this organism inhibited 80–90% of the test spores ; our experience with this fungus has shown that it is an equally potent producer of non-volatile inhibitors of spore germination. As noted against species of *Aspergillus,* spore germination of *Pestalotia* was also not strongly inhibited by

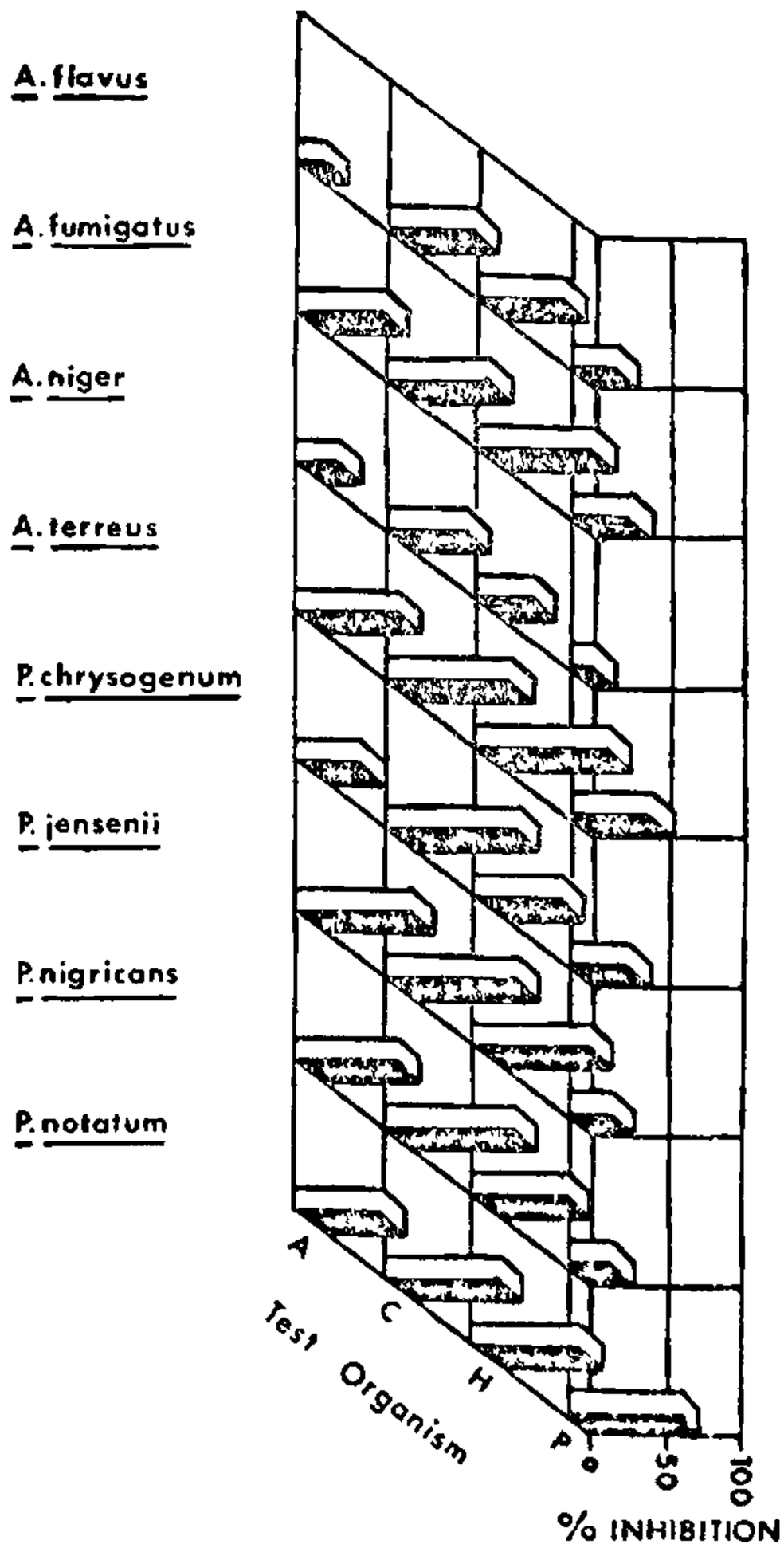

FIG. 1. *In vitro* production of volatile inhibitors by soil fungi in liquid Czapek's medium. Erlenmeyer flask-method was used for assessing the spore germination of test fungi. In this and the figures which follow, A, C, H, P, denote *Alternaria, Curvularia, Helminthosporium,* and *Pestalotia.*

these four Penicillia; *P. notatum* alone suppressed spore germination to a marked degree (80%).

Soil colonization studies have shown that *A. fumigatus* is the most dominant fungus in local soils and, therefore, an experiment was run in which sodium nitrate in Czapek's medium was replaced by an equivalent amount of nitrogen in the form of asparagine, ammonium chloride, ammonium nitrate, potassium nitrate, sodium nitrite, and sodium nitrate. In general it was noted that volatile production was directly proportional to the quantity of nitrogen utilized. Thus, asparagine and sodium nitrite supported good mycelial growth and volatile production of *A. fumigatus* (Fig. 2); growth was poor on ammonium chloride, ammonium nitrate, and potassium nitrate and consequently the levels of volatiles were low resulting in poor inhibition of spore germination.

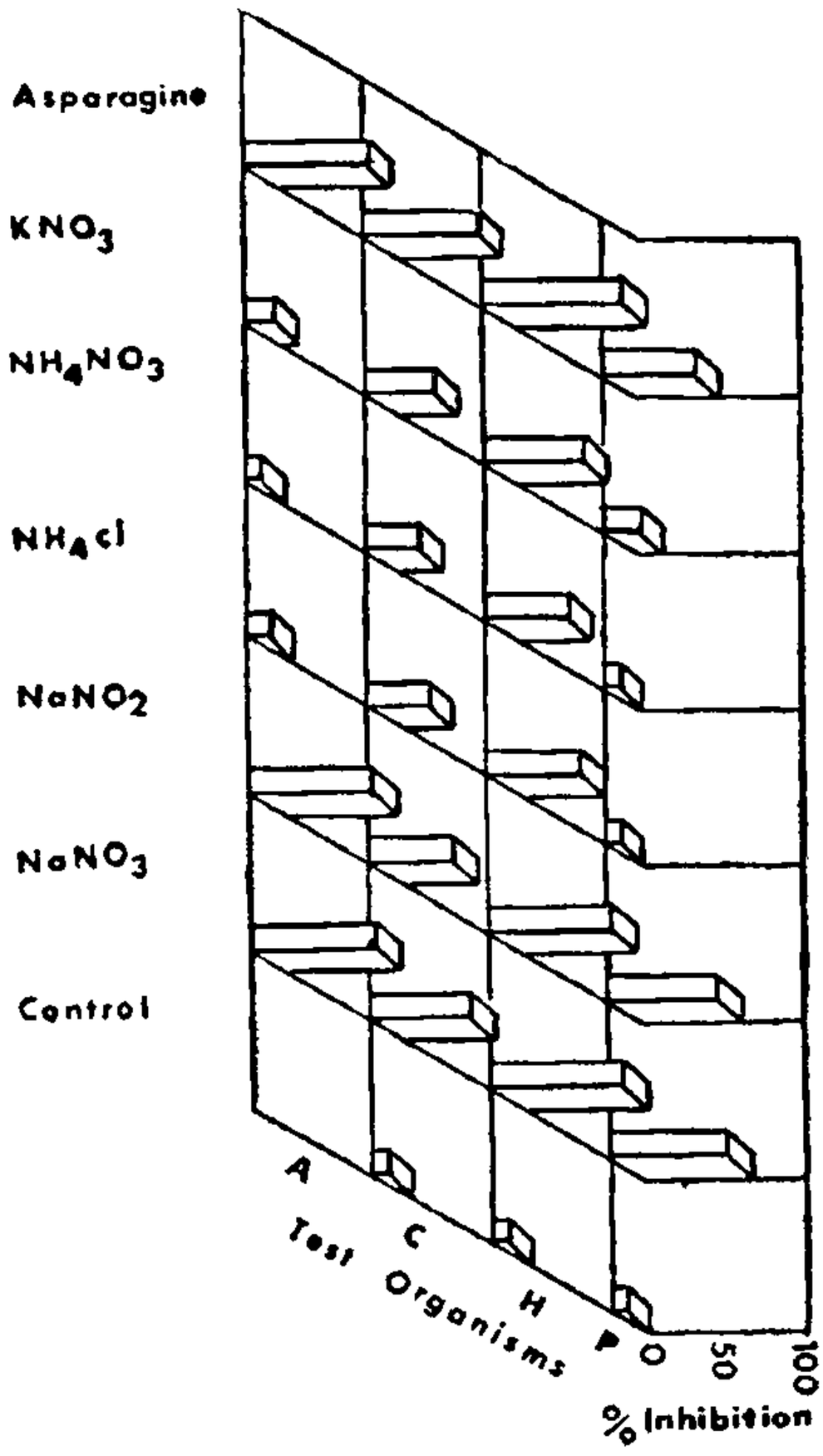

FIG. 2. *In vitro* evaluation of the effect of various nitrogenous sources on the production of volatile inhibitors by *Aspergillus fumigatus*. Nitrogen was added at a concentration equivalent to that of 2 g sodium nitrate. Control flask did not receive any nitrogenous substrate.

The release of volatiles in agar medium was comparable to that noted in liquid medium (Fig. 3). *Aspergillus fumigatus* and *Penicillium jenseni* dominated over other members as inhibitors of spore germination of *Alternaria, Curvulara,* and *Helminthosporium.* In contrast to its behaviour under liquid culture conditions, *P. nigricans* was quite effective in emitting volatiles in agar medium; spore germination of *Pestalotia* was once again least affected. The chief difference between the release of volatiles in liquid and agar medium was the comparatively low inhibition recorded in the latter.

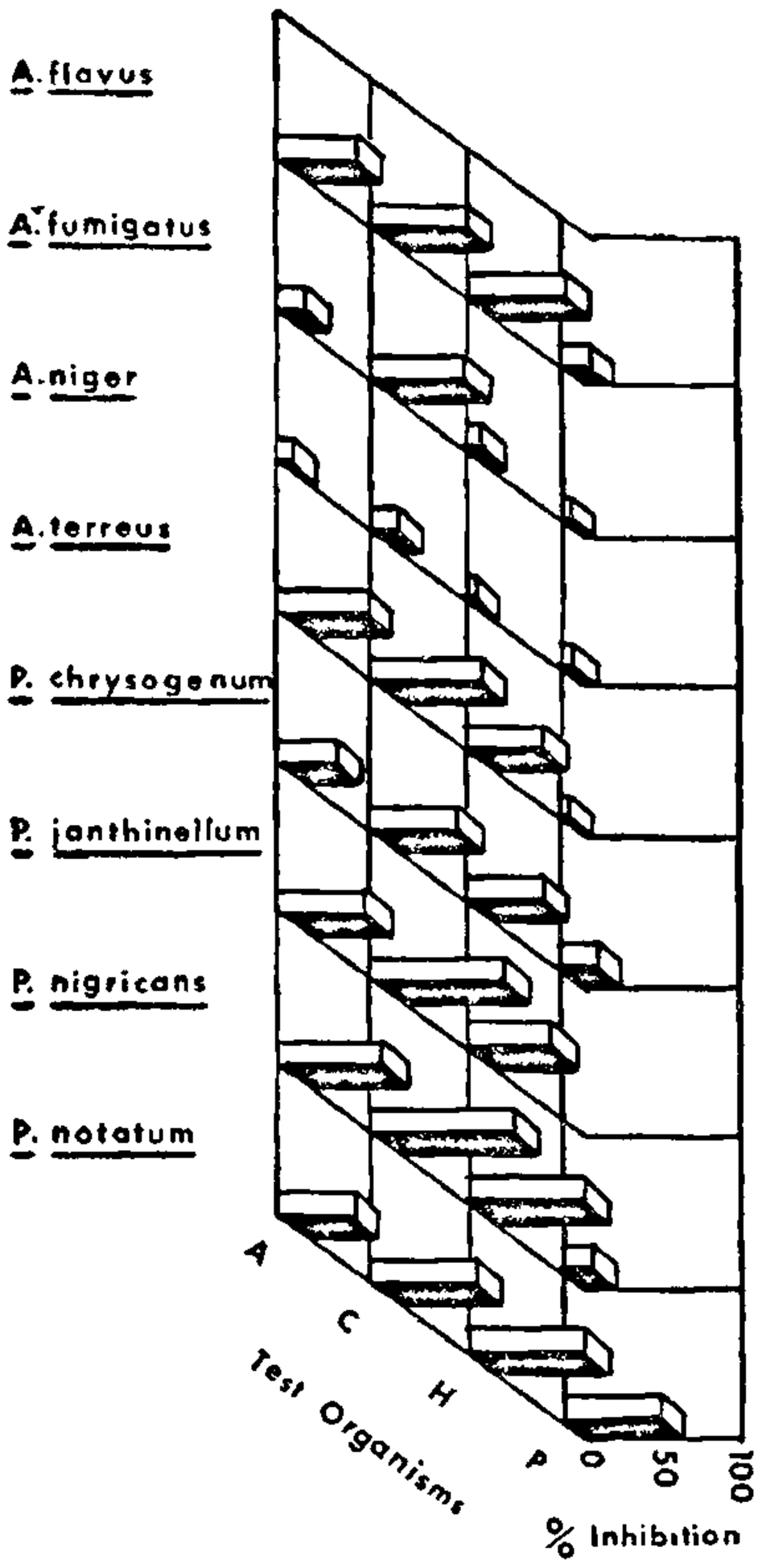

FIG. 3. *In vitro* production of volatile inhibitors of spore germination by soil fungi on agar medium. Paired Petri dish chambers were used for this experiment. Details in the text.

The effect of volatiles on mycelial growth of test fungi was less feeble than the inhibition of spore germination. Radial growth of *Curvularia* alone was

reduced to an appreciable extent (Table I); even for this test organism, only *A. fumigatus* and *P. jenseni* could inhibit mycelial growth to an extent of 30–40%. Some inhibition of mycelial growth of *Alternaria*, *Helminthosporium*, and *Pestalotia* was also noted but the values were considerably low (5–10%).

TABLE I

Effect of volatile inhibitors on the mycelial growth of test fungi

Soil fungi	Radial mycelial growth (mm)			
	Alternaria	*Curvularia*	*Helminthosporium*	*Pestalotia*
Control (Uninoculated agar)	80	70	70	85
Aspergillus flavus	75	65	55	80
A. fumigatus	70	60	45	75
A. niger	75	70	60	85
A. terreus	75	70	55	85
Penicillium nigricans	75	65	50	80
P. notatum	70	65	50	80
P. chrysogenum	75	70	55	85
P. jenseni	65	60	45	75

Soil fungi were grown in Czapek's agar medium for 10 days in chambers made of paired Petri dishes; the growth of test fungus was measured 6 days after placement of the inoculum disc.

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SHORT SCIENTIFIC NOTES

A New Species of *Acrosporium* Nees ex Gray with a Note on *Oidium pedilanthi* Mathur *et al.*

During survey of plant pathogenic fungi in and around Jabalpur, M.P., in 1973 and 1974 the authors came across a powdery mildew on the leaves of *Scoparia dulcis* L. Mildew appears on both the sides of leaves predominantly on upper side. Gradually necrosis develops in affected parts and leaves defoliate. The pathogen was identified as *Acrosporium* sp. We feel considerable difficulty while disposing-off this collection of *Acrosporium* under the known species, because cleistothecia were not observed in the collection, whereas the most useful classifications are based on their cleistothecial states[1,5]. Moreover in *Acrosporium* the delimitation of species is based largely and primarily on the host plant attacked[8]. So far there is no record of any species of it on *Scoparia* or any other member of the family Scrophulariaceae[2,4,9]. It is, therefore, proposed to report the present fungus as a new species.

The specimen has been deposited in the herbarium of Department of Plant Pathology, J.N. Agricultural University, Jabalpur.

Acrosporium scopariae sp. nov.

Colonies sparse; mycelium superficial, branched, hyaline, unequal in thickness, haustoria globose; upto 5 μ wide; conidiophores simple, erect, clavate, upto 6-septate, 60–120 × 8–11 μ, conidia hyaline, granulated internally, oval to elliptical, 1-celled, usually in chains of 3–4, 25–37 × 12–19 μ.

On leaves of *Scoparia dulcis* L. (Scrophulariaceae) Experimental Fields, Agric. Univ. Adhartal, Jabalpur, December, 1973, Leg. N. D. Sharma, H. P. P. JNKVV No. 15.