Figure 1. Showing the infection of *Alternaria alternata* on radish seedlings.

sules are also shrivelled, light in weight and discoloured. The infected seeds on germination develop greyish to brownish necrotic spots near the collar region which in advanced stages girdle the stem (figure 1) and results in the death of the seedling or the spots later spread towards the stem, branches and leaves.

Such seeds after microscopic examination were found infected with *Alternaria* sp. Such seeds were further asseyed by agar and blotter plate methods.[1] The seeds were surface-sterilized with 0.1% mercuric chloride for studying the extent of internal infection. The percentage of external and internal infection was 17 and 21 in potato dextrose agar (PDA) and 8 and 13 in blotter paper method respectively. The *Altenaria* sp. isolated was purified by single spore isolation technique and maintained on PDA medium.

The characteristics of the pathogen which was isolated from the seeds show greyish colonies turning olive green to black, effuse, conidiophores simple, straight, pale to dark brown, smooth, septate, geniculate with one or more conidial scars 25-55 μm ×. 3-5 μm. Conidia catenulate simple or branched ovoid, ellipsoidal obclavate obpyriform, pale to dark brown rostrate, smooth or verriculose with upto 6 or 7 transverse and or also longitudinal and oblique septa 20-40 μ m × 10-12 μ m beak pale measuring 3-10 μ m ×3 μm. On the basis of these characters the fungus was identified as *Alternaria alternata* (Fr) Keissler.

The pathogenicity of the fungus was proved by inoculating the seeds and leaves with actively sporulating culture from PDA medium. Typical symptoms of the disease develped after 8-11 days on leaves and on collar region of the seedlings. The pathogen was reisolated from these infected tissues which resembled the original fungus used for inoculation. *A. brassicae, A. brassicicola, A. raphani* and *A. cheiranthi* have been reported on seeds of radish[2,3] and mustard[4,5]. The present report describes internal seed borne nature of *A. alternata* in radish for the first time. *Alternaria* sp. nearing to *A. tenuis* group has also been reported on radish[6].

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ULTRASTRUCTURE OF GERMINATING SPORANGIOSPORES OF *RHIZOPUS RHIZOPODIFORMIS* (GOHN) ZOPF, A THERMOPHILE

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BROCK[1] had suggested that the ability of eukaryotic thermophiles to grow only upto 62° C is due to the membrane disintegration at elevated temperatures. However, a few studies of ultrastructural organization among thermophilic moulds have been undertaken[2]. We have earlier reported[2,3] that the thermophile *Rhizopus rhizopodiformis* (Gohn) Zopf can serve as a good experimental material due to synchrony in spore swelling and germination. While mesophilic species of *Rhizopus* have been examined at ultrastructural

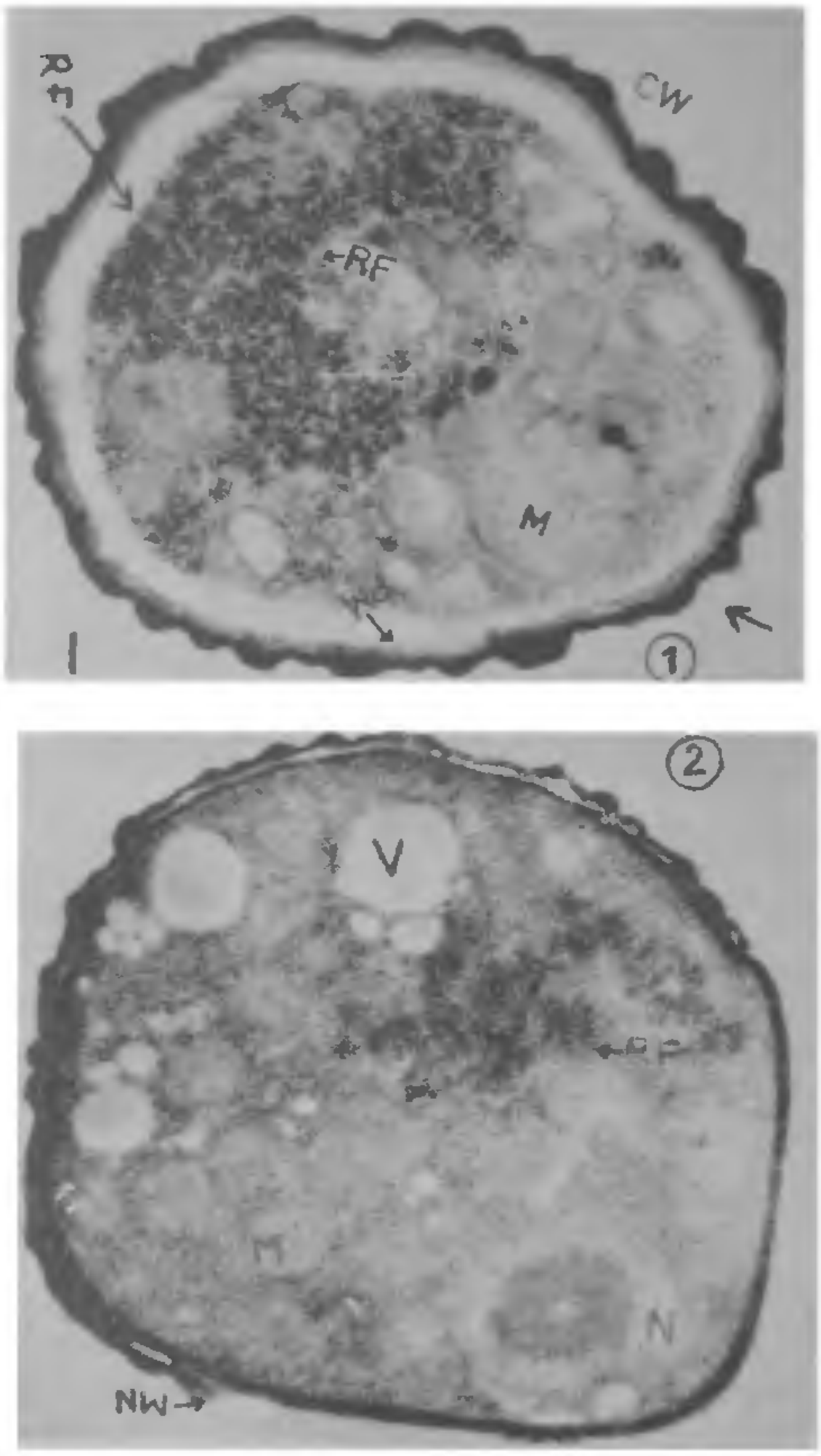
level[4–7], no thermophilic counterpart has been examined.

The coal mine isolate of *R. rhizopodiformis* used in this study was maintained on glucose-yeast extract-agar[8]. Spores from 4-day old slants were germinated in glucose-asparagine broth: glucose 20 g, asparagine 5 g, KH_2PO_4 1 g, $MgSO_4.7H_2O$ 0.5 g, microelement solution 2 ml ($Fe(NO_3)_2.9H_2O$ 724 mg, $ZnSO_4.7H_2O$ 440 g, $MnSO_4.4H_2O$ 200 mg per 1000 ml distilled water), vitamin 1 ml (thiamine 10 mg, biotin 5 mg per 100 ml stock solution). Media were sterilized at 15 psi for 15 min; pH of the final medium was 6.5. Fifteen ml aliquots were dispensed in 150 ml Erlenmeyer flasks and after sterilization each was inoculated to provide a density of 2×10^5 spores per ml. Flasks were incubated at 45° C in a shaking reaction incubator. Maximum swelling in broth was reached in 4.5 hr and germination ensued at 6 hr[9]. Germination process was checked by examining the wet mounts under phase contrast on a Leitz microscope.

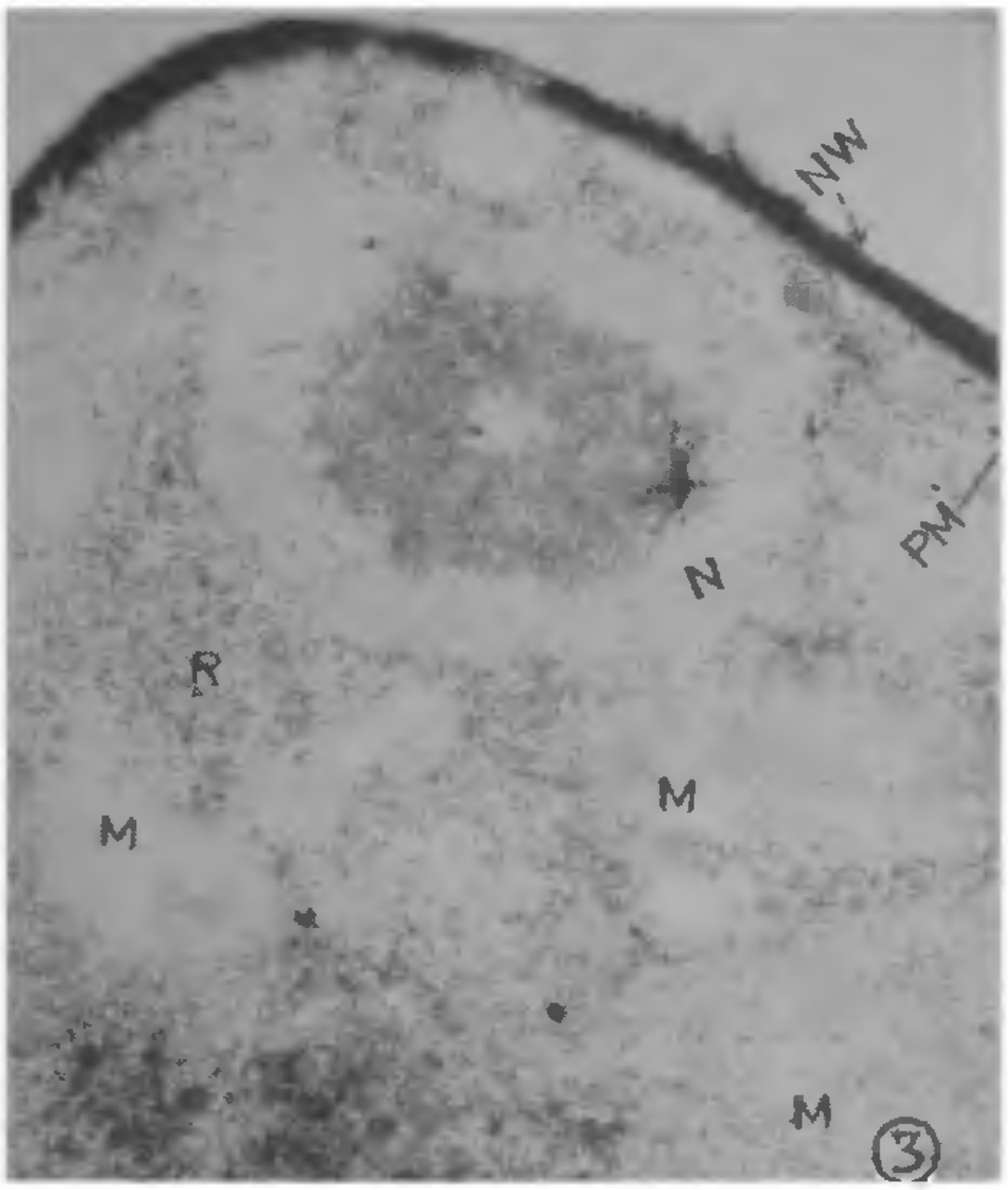
For thin sectioning, swollen/germinated spores were collected by centrifugation and suspended in 1% bacto agar at 45° C. Agar blocks (1 mm^3) were cut and fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0). The fixative was discarded and the preparation washed with fresh water. It was post fixed in 2% OsO_4 and dehydrated in graded series of ethyl alcohol. Spores were embedded in araldite and sections were cut on an KLB Ultramicrotome. Staining was completed in 2% uranyl acetate followed by 0.2% lead citrate. The preparations were examined and photographed with a Phillips 201 EM.

Thin sections of ungerminated spore showed wavy and thick outer wall of the spore. It possessed normal complement of mitochondria, vacuoles, lipoidal bodies and unidentified organelles besides heavy accumulation of endogenous reserve material (figure 1), which often masked the single nucleus in dormant spores. Germ tube initiation (4.30 hr) was followed by the disappearance of food material, movement of the single nucleus towards the tip, appearance of prominent vacuoles, aggregation of mitochondria and the formation of a new wall layer (figures 2, 3). Germ tube reached a length closely approaching spore diam in 6 hr. The first nuclear division was completed at this stage; vacuoles became more prominent but they were not markedly visible in the young germling (figures 4 and 5). Mitochondria and reserve food materials were aggregated around the nucleus in the tip of germ tube and also in the spore body itself (figures 5 and 6).

The wall pattern in *R. rhizopodiformis* was closely similar to that in mesophilic *R. arrhizus*[6] but some variation was clearly evident. Thus, as opposed to the mesophilic species, ridges did not completely disappear at the time of spore germination. This could be due to disproportionate stretching of the wall material within ridges and furrows.



Figures 1, 2. 1. Thin section of an ungerminated sporangiospore of *R. rhizopodiformis* to show deep ridges and furrows of the cell wall (CW), accumulation of endogenous reserve food (RF) and mitochondria (M) and a distinct plasma membrane. Nucleus can not be seen due to food material. × 22,000. **2.** Thin section of a swollen spore after placement in glucose-asparagine broth for 4.30 h. Note rupturing of outer wall and initiation of germ tube by formation of a new wall layer. A distinct nucleus has occupied the apical position. RF is less dense and vacuoles (V) have appeared in the basal portion of the spore. Abundant mitochondria can also be seen suggesting strong metabolic activity. × 22,000



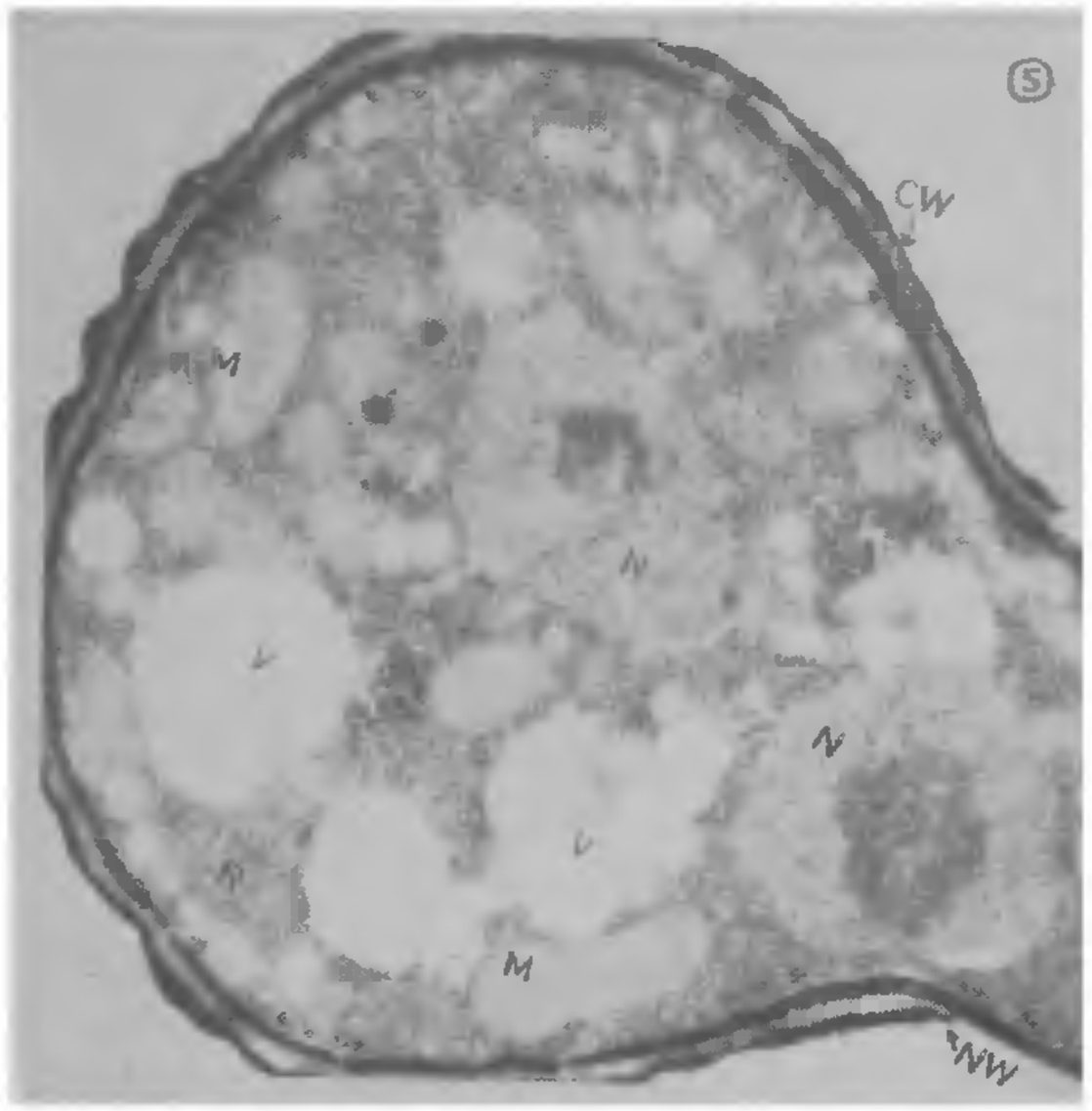
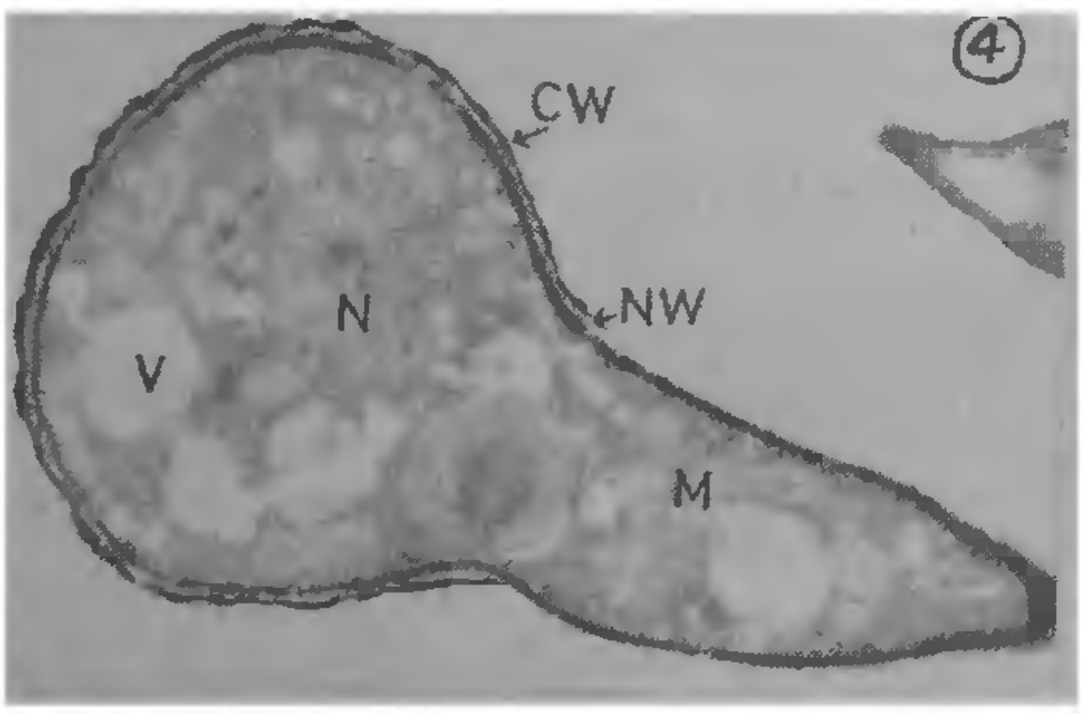
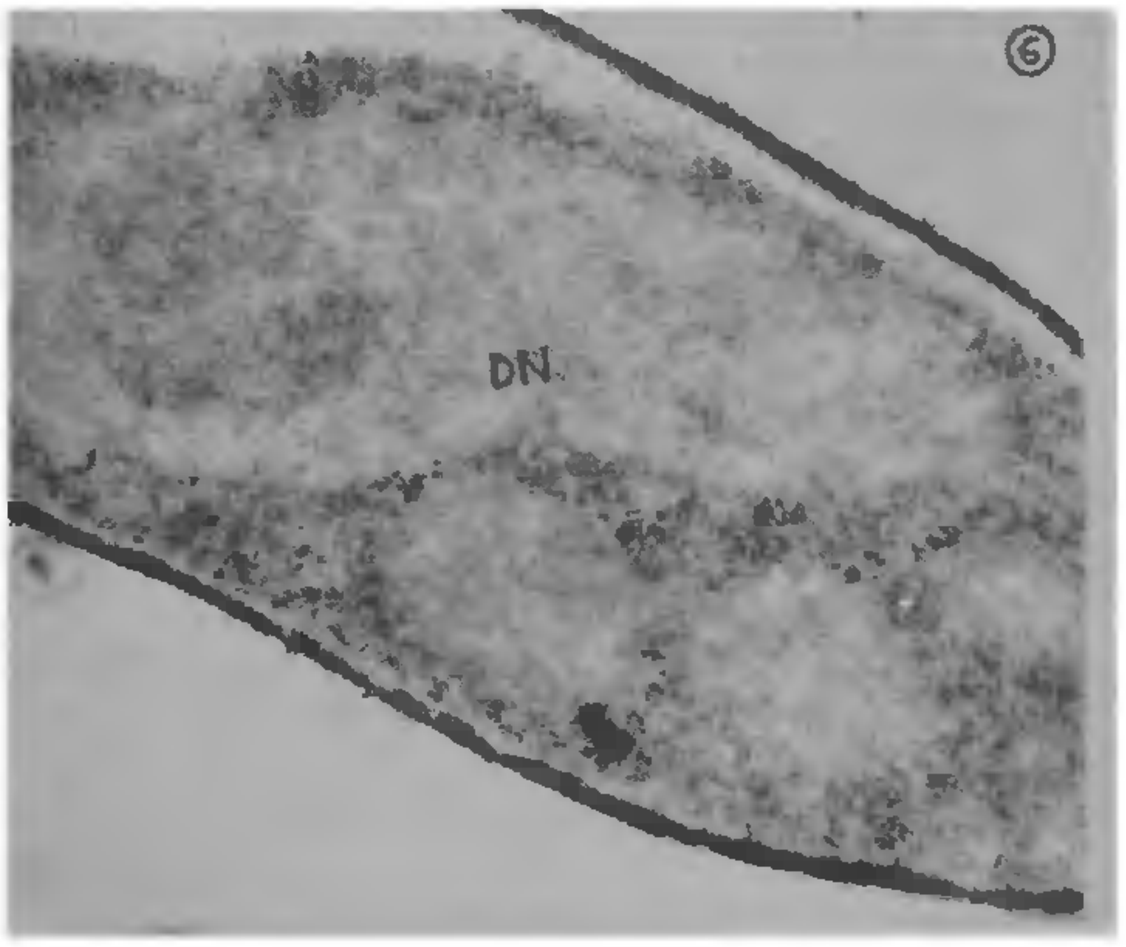


Figure 5. Enlarged view of the basal area of the spore in figure 4 to show small and large vacuoles (V), mitochondria and ribosomes. Note that the position of upper nucleus has remained nearly unchanged while germ tube tip has grown (cf. Figure 2). × 36,000





Figures 3, 4.3. An enlarged view of the germ tube shown in Figure 2. Note heavy distribution of ribosomes (R), few vesicles (V), distinct plasma membrane (PM) and variously shaped small and large mitochondria (M). Darker area of RF is gradually disappearing. × 44,800. **4.** Thin section of a spore after 6.0 h to show vacuolated area and divided nucleus after completion of the first nuclear division. Although some stretching of the outer wall has occurred, the wavy nature is still evident. × 12,000

Figure 6. Thin section of a germ tube after placement of the spore in glucose-asparagine broth for 7.30 h. Note the nucleus completing second division. Most of the vesicles (cf. Figures 5, 6) have now disappeared and mitochondria are fewer too (cf. Figure 2). × 48,600

The formation of an additional wall layer was reported by Ekundayo[4] in *R. arrhizus* but this appears to be a common feature in the majority of fungi[9, 10]. As opposed to multinucleate condition in mesophilic species of *Rhizopus* only single nucleus was seen in *R. rhizopodiformis*; the distribution of other organelles was, however, closely similar. There was copious amount of endogenous reserves which could be lipoidal in nature; vegetative hyphae of thermophilic *Humicola* have been shown to possess similar lipoidal bodies[11]. Hyphae tips of fungi exhibit a wide array of vesicle arrangement. While studying *Gilbertella persicaria*, Bracker[9] showed that germ tubes contained only few apical vesicles at the growing tip; they increased in number with ensuing growth. Though the present study was limited to 6 hr germ tube growth, none or very few vesicles were present at the tip. This limited study suggests that the ability to grow at elevated temperatures is coupled with more endogenous reserves, less vacuolation and disproportionate stretching of the cell wall. Whether this generalization applies to the thermophiles as a whole could be ascertained by a study on other thermophilic fungi.

5 January 1982

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ACHAETOMIUM THERMOPHILUM SP. NOV., A NEW THERMOPHILLIC SPECIES OF GENUS *ACHAETOMIUM* FROM INDIA

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DURING studies on thermophilic fungi, an interesting ascomycetous fungus belonging to genus *Achaetomium* was isolated from a sample of leaf litter in December 1977. This genus was established by Rai *et al*[1] and at present it includes 10 species, all reported from Indian soil[2-6]. This paper describes a new thermophilic species.

Achaetomium thermophilum sp. nov.

Coloniae in agar farinae avenae crescentes celeriter in 45° C, floccosae, primo albae, flavescentes ad maturitatem quando primum excultae, sed remanent quasi albae in culturis subsequentibus; pars aversa incolor; ascocarpi pulle nigri, subglobosi vel elongati, circumdati hyphis longis, brunneis, septatis, asperis, flexuosis, cum pariete clare definito, usque 350 μm diametro, ostiolati, superficiales; asci in fasciculis, clavati, evanescentes, habentes 8 ascosporas, 40–68 μm × 13–20 μm, aporati; ascosporae ellipsiformes, magnae, primum hyalinae, vertentes rave brunneae ad maturitatem, pleraeque cum una guttula magna olei, interdum cum pluribus guttulis, 19–20 μm × 9–11 μm, cum duobus foraminibus germinalibus distinctis. Forma conidialis ignota.

Lectus e stramento foliarum mense Decembri 1977 a Bhatni, U.P., India. Cultura posita in Department of Botany, Allahabad University.

Fungus thermophilus cuius temperatura optima est 45° C, maxima 50–55° C et minima 20° C.

Colonies on oat meal agar growing rapidly at 45° C, floccose, at first white, turning to yellowish at maturity when freshly isolated but after subsequent culturing mature colonies remain white; reverse uncoloured; ascocarps dispersed, sometimes in small groups, grayish black, subglobose to elongated, surrounded by long, brown, septate, rough, flexuous hyphae, with well defined wall, upto 350 μm in diameter, ostiolate, superficial; asci in fascicles, clavate, evanescent, having 8 ascospores, 40–68 μm by 13–20 μm, aporate; ascospores elliptical, greyish-brown, apicultate at both the ends, mostly with one large oil drop, sometimes more, 19–20 μm by 9–11 μm, with two distinct polar germ pores. No conidial stage was observed.

Isolated from leaf litter in December 1977 from Bhatni, U.P., India. Culture deposited in the Department of Botany, Allahabad University.

A thermophilic fungus having optimum temperature at 45° C, maximum 50–55° C and minimum 20° C.

The present species differs from other known species of the genus *Achaetomium*. However, it comes somewhat closer to *A. macrosporum* Rai *et al*[2], especially in size range of ascocarps and shape and size of ascospores but mainly differs in shape of ascocarps which is subglobose to elongated instead of flask shaped; much smaller size of asci, ranging from 40–68 μm × 13–20 μm instead of 53–96 μm × 16–17 m; slight difference in length of the ascospore size which ranges from 19–20 μm instead of 16–19 μm and with two distinct polar germ pores in each asco-