OCCURRENCE AND ISOLATION OF THERMOPHILIC FUNGI

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THE fungi which thrive at temperatures of 50° C. and higher but are unable to grow at 20° C. have been called thermophilic. Thirteen species of such fungi belonging to 9 genera were monographed for the first time by Cooney and Emerson in 1964. Since then new species and additional sources of occurrence of thermophilic fungi have been recorded (Apinis, 1963; Fergus, 1964; Apinis and Eggins, 1966; Semeniuk and Carmichael, 1966; Okafur, 1966; Apinis and Pugh, 1967). There appears to be no report of such fungi from India. This communication reports on the thermophilic fungi isolated from samples collected locally.

The fungi were isolated from dung of herbivores, compost and sewage manure. Samples of these substrates were placed in moist containers and incubated at 40-60° C. for periods up to two weeks to provide enrichment environment for thermophilic microflora. Fungi were then isolated on nutrient media to which streptomycin or crystal violet was added to prevent bacterial growth at 45-50° C. Yeast-Starch agar (YpSs) (Cooney and Emerson, 1964) proved the best medium for growth and reproduction of thermophilic fungi. That fungi isolated were thermophilic and not thermotolerant was proved by their inability to grow at 20° C. on YpSs, oat meal and Czapek's agar. The fungi isolated are listed in Table I.

In general morphology, cultural characteristics and temperature requirements for growth of all isolates agreed closely with those described by Cooney and Emerson (1964). Although the present isolate of *Talaromyces thermophilus* Stolk (Fig. 1) did not produce the perfect stage, however, the close resemblance of its imperfect stage with the diagnosis left no doubt that this is the same fungus which has synonymy of *Penicillium duponti* (Griffon et Maublanc and *Talaromyces duponti* (Griffon et Maublanc) Emerson (*nomen invalidum*) Cooney and Emerson (1964) had reported that this fungus produces cleistothecia on nutrient media under conditions of reduced oxygen tension whereas they are profusely formed on moist, chopped guayule shrub. According to Stolk (1965) cleistothecia are not produced on agar media but are regularly formed on sterilized oat grains at 45° C. However, present attempts to induce the development of cleistothecia on sterilized oats or wheat straw and other plant materials did not succeed. This fungus often showed the presence of swollen cells, with smooth walls borne singly on sympodially branched or unbranched hypha in the substrate mycelium (Fig. 2). Furthermore, certain hyphal cells were conspicuously swollen towards one end of the septum (Fig. 3). These features have not been mentioned by Cooney and Emerson (1964).

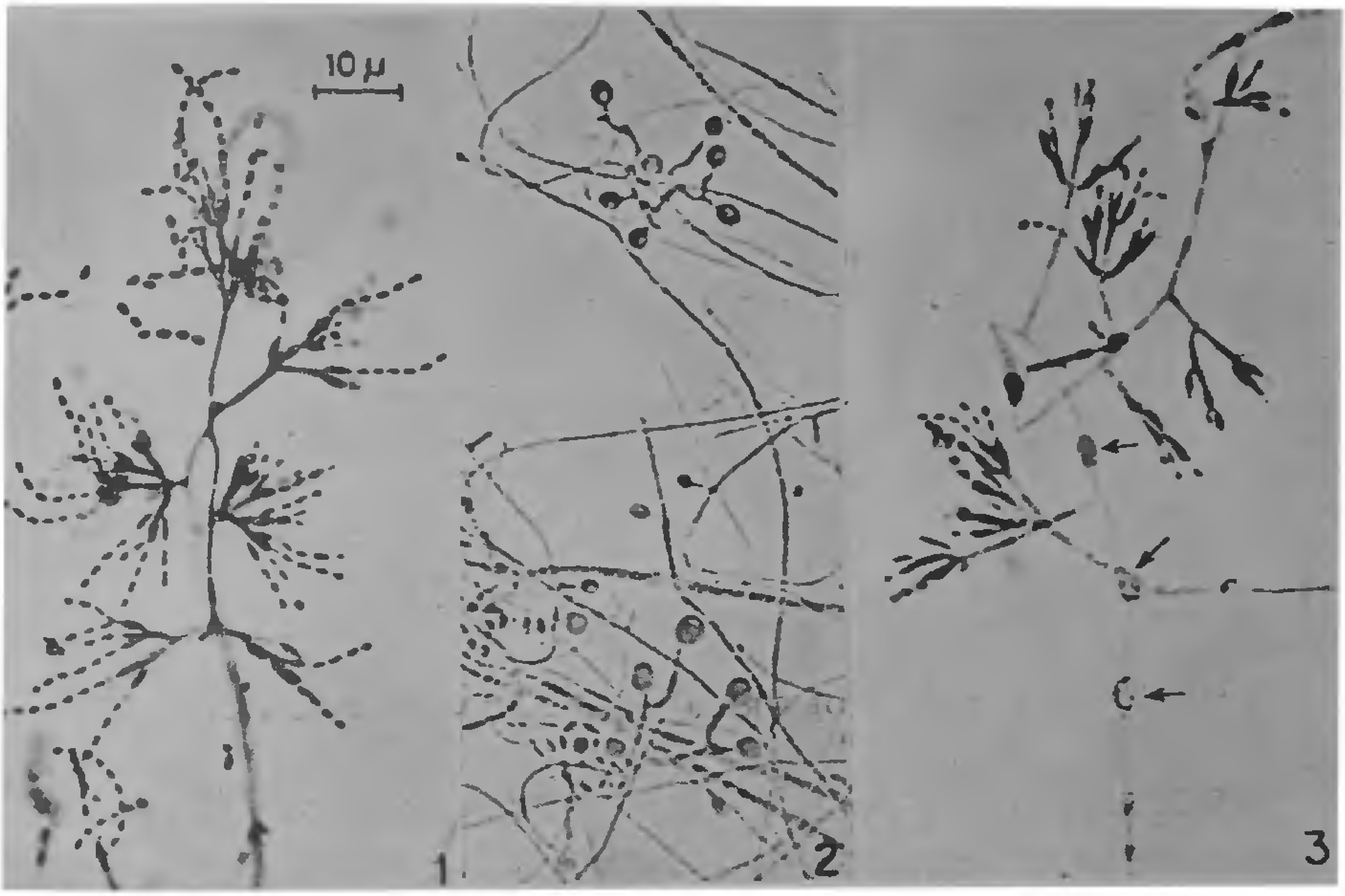
TABLE I
Occurrence and temperature relation of thermophilic fungi

Fungi	Growth temperature (°C.) Minimum	Growth temperature (°C.) Maximum	Substrate
Chaetomium thermophile La Touche			
var. *coprophile* Cooney & Emerson	27	58	Dung
var. *dissitum* Cooney & Emerson	27	58	Dung, manure
var. *thermophile* La Touche	27	58	Dung, compost
Humicola insolens Cooney & Emerson	23	55	Compost, manure
H. lanuginosa (Griffon et Maublanc) Bunce	30	60	Dung, compost
H. stellata Bunce*	..	..	Compost
Malbranchea pulchella Sacc. & Penzig var. *sulfurea* (Miehe) Cooney & Emerson	27	55	,,
Talaromyces thermophilus Stolk	30	60	Dung, compost

* Not isolated in culture.

In addition to fungi listed in Table I, three other fungi, *Absidia ramosa*, *Aspergillus fumigatus*, and *Chrysosporium* sp., capable of growing at 40-45° C., were isolated from compost. These were capable of growing below 20° C. although optimum growth occurred at about 40° C. They may be considered psychrotolerant.

The incubation of samples from compost, dung and manure in moist containers at 40-60° C. often resulted in mycelial growth and



FIGS. 1–3. *Talaromyces thermophilus* Stolk. Fig. 1. Conidiophores. Fig. 2. Swollen cells resembling aleuriospores in substrate mycelium. Fig. 3. Hyphal swelling (indicated by arrows).

sporulation of some thermophilic fungi. Particularly, the partially decomposed materials from compost (chiefly of garden and kitchen wastes) supported profuse mycelial growth of *Humicola insolens, H. lanuginosa* and *Talaromyces thermophilus.* Microscopic examination of decomposing material also revealed *H. stellata* which, however, could not be obtained in culture.

Measurements of temperature in the compost confirmed that higher temperatures required for growth of thermophiles exist in nature. Temperatures 5 to 20° C. higher than the ambient temperature were recorded inside a compost during monsoon season when the microbial decomposition of organic matter appeared to be at maximum due to the high level of moisture present therein. Temperature was highest (45–50° C.) in those sections of the compost where the decomposable material was relatively fresh. *H. insolens, H. lanuginosa, T. thermophilus* and *Malbranchea pulchella* var. *sulfurea* were conspicuous in the compost. These observations support the suggestion of Cooney and Emerson (1964) that thermophilic fungi are importantly involved in composting action.

The possibility was considered that complex nutrients in natural substrata may support growth of these fungi at lower temperatures than the media commonly used in the laboratory. *H. lanuginosa* and *T. thermophilus* did not grow at 20° C. on nutrient media containing vitamins, growth hormones, complex nutrients such as coconut milk and yeast extract even after prolonged incubation. Addition of extracts of composting plant material was also ineffective in inducing growth at 20° C. of all thermophiles isolated except *H. insolens.* Present isolate of this fungus grew well at 20° C. after a lag of 10 days and sporulated on an agar medium containing an extract of decomposing plant tissue, yeast extract, peptone, KH_2PO_4, $MgSO_4 . 7H_2O$ and sucrose. Recently, it has been reported that fungi which grow luxuriantly on a sucrose-salt medium at 50° C. but fail to grow on it below 30° C. will grow well at 22° C. on complex natural media (Tendler, Korman and Nishimoto, 1967). A requirement for high temperature may then be eliminated by nutritional environment.

This study is in agreement that thermophilic fungi have an ubiquitous distribution. However, in the present study only such sources were explored where microbial thermogenesis itself produces temperature favouring thermophiles. Future studies in the tropics should explore surface soils and pond waters to determine the frequency of occurrence of thermophiles in such places.

Thanks are due to University Grants Commission for the award of a Senior Research Fellowship, and to Professor D. G. Cooney of University of Nevada for sending a culture of *Chœtomium thermophile* var. *dissitum* for comparison.

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RESONANCE ENERGIES OF TRICARBONYLARENECHROMIUMS

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THE order of stability of the series of π-complexes, the chromium tricarbonyl derivatives of benzene, biphenyl and phenanthrene seems on primary considerations to depend on the π-bonds in the series. Delocalisation of π-electrons takes place extensively in these compounds as manifested by their dipole moments,[1] infrared spectra[2] and other properties.[3] In bipehnyl and phenanthrene there are neighbouring ring π-electrons and the extent of participation of such electrons in the bonding becomes important. The Effective Atomic Number (E.A.N.) rule does not permit any such participation but one of us has suggested that in molecular orbital theory the interaction of all the π-orbitals must be taken into account.[4] Thus it is possible to explain the greater stability of phenyl substituted complexes such as tricarbonyl-π-tetraphenylcyclobutadieneiron.[5] The fact that in both tricarbonyl-π-biphenylchromium and tricarbonyl-π-phenanthrenechromium planar structures are maintained[6] and that considerable delocalisation takes place in free biphenyl and phenanthrene indicates the necessity of including the interaction of all π-electrons.

In view of this, we have used semiempirical molecular orbital theory for obtaining the resonance energies of the arene-metal fragments in these complexes. The interaction of all the π-electrons of the respective arene have been included in such calculations. X-ray work has shown that the aromatic moieties in these complexes are planar[6] but there are considerable and random variations of the C–C distances. Hence planar structures are assumed for the aromatic moieties with all the C–C distances taken as 1·39 Å. The inter-ring bond in biphenyl is 1·48 Å.[7] The C–Cr distance is 2·2 Å, the carbon atoms being those of the ring to which the chromium is bound. Further, in the phenanthrene complex the bonding is to one of the end rings.[8]

The highest filled levels amongst the M O's of the arenes are identified with the corresponding ionisation potentials :[9]

biphenyl $H(4a_1, 4a_1) = -8 \cdot 27$ e.v.
phenanthrene $H(4a_1, 4a_1) = -8 \cdot 03$ e.v.

Other levels are calculated with respect to these energies. The coulomb terms are taken from Berry's spectroscopic data :[10]

$$H(4p, 4p) = -3 \cdot 86 \text{ e.v.}$$
$$H(4s, 4s) = -5 \cdot 76 \text{ e.v.}$$
$$H(3d, 3d) = -6 \cdot 76 \text{ e.v.}$$

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