

in sterile distilled water to a final count of 2×10^8 spores/ml. This suspension was used for studies on growth and amylase production.

The spore suspension (2 ml) was inoculated in Czapeck-Dox medium with individual carbon sources and incubated at $30 \pm 2^\circ \text{C}$ for a number of days. It was found that the growth was poor on this medium and hence for subsequent experiments yeast extract (0.5%) was added. The individual carbon sources used were starch, sucrose, glucose, fructose and carboxy-methyl cellulose. The growth in submerged culture continued till 8 days and 5 days with starch (3%) and sucrose (3%) as sole carbon sources respectively. The pH of the medium also dropped during the growth and became normal (pH 5.2) in the later phase of the growth, indicating the production of organic acids during growth and their subsequent re-utilization. The identification of the organic acids is in progress.

Amylase production by *Syncephalastrum* sp. was studied in submerged culture with starch and sucrose as carbon sources. The culture filtrate was used as a source of crude enzyme. The dinitro salicylic acid method (DNSA) was used for the determination of amylase activity⁸. From the results obtained it was found that the amylase production by *Syncephalastrum* sp. continued till eight days with starch as sole carbon source. However, it was found that when sucrose was used as sole carbon source the enzyme production continued only for two days, with a maximum of 38.3 Units/flask. The maximum production of amylase with starch as sole carbon source was achieved on 6th day of growth (520 Units/flask). The enzyme activity steadily increased from the first day till the 7th day and then decreased significantly on the 8th day. These results suggest the inducible production of amylase, though some constitutive production cannot be overruled.

Although the appearance of amylase in the young cultures filtrates of this fungus is only *prima facie* evidence regarding extracellularity, it suggests that this amylase may be true extracellular enzyme. Similar results were obtained by Somkutti and Babel⁹ with *Mucor fusillum* lipase. Further work regarding the conditions of induction of this enzyme is in progress. During the production of amylase the pH of the medium also decreased from pH 5.8 to 4.2, suggesting the production of organic acids.

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1. Corman, J. and Tsuchiya, H. M., *Cereal Chem.*, 1951, 28, 280.
2. Pool, E. L. and Underkofler, L. A., *J. Agri. Food Chem.*, 1953, 1, 87.
3. Windish, W. W. and Mhatre, N. S., *Advances in Appl. Microbiol.*, Ed. Umbreit, W. W., Academic Press, New York, 1965, 7, 273.
4. Mangalam, S., Subramanyan, A. and Gopal-krishnan, K. S., *Curr. Sci.*, 1977, 46, 16.
5. Prescott, S. C. and Dunn, C. G., *Industrial Microbiology*, McGraw-Hill, New York, 1958, p. 674.
6. Cochrane, V. W., *Physiology of Fungi*, John Wiley and Sons, New York, 1958, p. 110.
7. Ainsworth, C. G. and Sussman, A. S., *The Fungi*, Academic Press, New York, 1965, 1, 487.
8. *Manual on Amylase*, Sarabhai Research Centre, Baroda.
9. Somkutti, G. A. and Babel, F. J., *Applied Microbiology*, 1968, 16, 617.

FUNGISTASIS DUE TO VOLATILE INHIBITORS PRODUCED BY SOME *PENICILLIUM* SPECIES

It is well known that all living things elaborate volatiles at the normal temperatures of their environments. Through several compilations of the extractions of volatiles from various sources have appeared^{4,6,7,13}. Only limited studies have been made with regard to their possible significance in the realm of microbial ecology. One such sphere of microbial ecology is that of soil fungistasis in which, the term 'fungistasis' was coined³ to explain the phenomenon of the viability of fungal propagules in the soil which was affected even in the realm of conditions which were otherwise favourable for the germination of spores. Recent years have seen the reports of workers^{1,5,8} who explained the widespread presence of volatile inhibitors amongst various groups of microorganisms and their significant role in maintaining soil fungistasis. Workers from our laboratory^{9,11,12} have reported that many fungi, especially the species of *Aspergillus* and *Penicillium* can produce substantial amounts of volatile inhibitors which can cause fungistasis in soil.

The present study deals with the effect of volatiles produced by 5 selected species of *Penicillium*, viz., *P. janthinellum*, *P. canescens*, *P. expansum*, *P. granulatum* and *P. duclauxi*, against a set of pathogenic and saprophytic fungi in relation to (a) spore germination, (b) culture growth and (c) mycelial weight.

The study of the effect of volatiles produced by penicillia on spore germination of test fungi was followed

by cellophane agar disc technique¹⁰. The spore suspensions of the test fungi were placed on 2% glucose agar discs and exposed to the volatiles emitted by 5 days old cultures of *Penicillium* species grown on Czapek's agar medium. The per cent inhibition of spore germination of test fungi caused by volatiles produced by penicillia was calculated in comparison to the spore germination rate of test fungi in control. For the study of the effect of volatiles produced by penicillia on the culture growth of test fungi, paired petri plate method⁹ was employed. The radial growth of the test fungi exposed to the volatiles produced by 5 days old cultures of penicillia on Czapek's agar

medium for 5 days, was compared with that of control in which the test fungi were grown alone and the per cent inhibition of culture growth caused by volatiles produced by penicillia was calculated. The effect of these volatiles on mycelial weight of test fungi was also studied by the use of 150 ml Erlenmeyer flasks consisting of side arms. The flasks containing 25 ml of Czapek's broth medium were inoculated with the test fungi and were connected to the other set of flasks consisting of 5 days old cultures of the antagonist through sterilized rubber tubes and incubated for another 5 days. The per cent inhibition of mycelial weight of test fungi caused by volatiles produced by

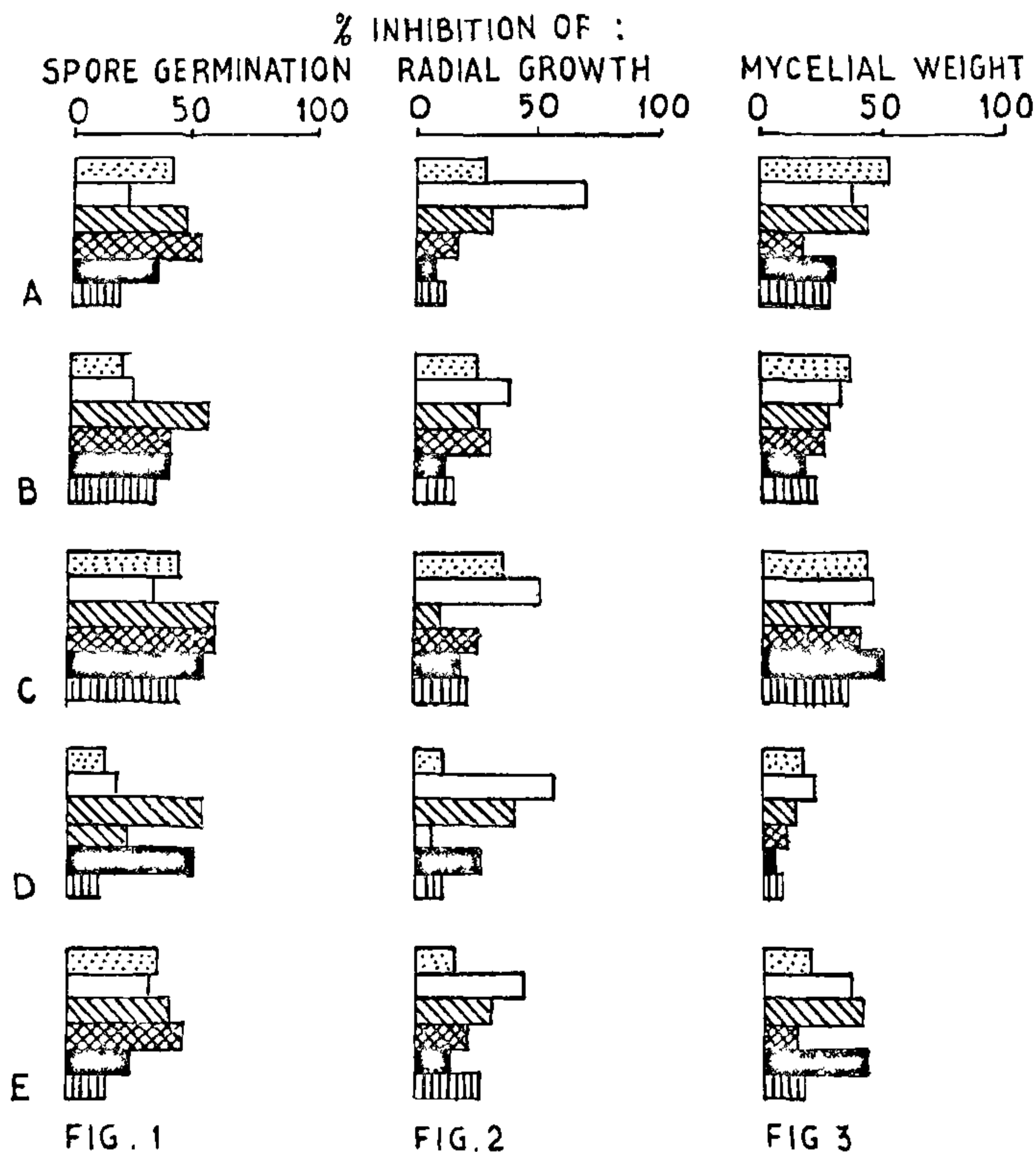


FIG. 1 FIG. 2 FIG. 3

A - *P. JANTHINELLUM* B - *P. CANESCENS* C - *P. EXPANSUM*
D - *P. GRANULATUM* E - *P. DUCLAXI*

F. MONILIFORME	T. REESEI	COLLETOTRICHUM SP.
CERCOSPORA SP.	A. PALANDUI	R. NIGRICANS

penicillia was calculated as above in comparison to control.

In all these cases, it was observed that the nature of volatiles produced by penicillia were fungistatic to all the test fungi varying in the degree of their activity. Figs. 1, 2 and 3 show the values of the per cent inhibition of test fungi in terms of spore germination, culture growth and mycelial weight caused by the volatiles produced by penicillia.

In the case of the fungistatic spectra on spore germination, *P. expansum* caused an average 50% inhibition of test fungi. The other fungi, i.e., *P. janthinellum*, *P. canescens*, *P. duclauxi* and *P. granulatum* caused an average 35%, 32.5%, 32% and 29% inhibition of test fungi respectively. In the case of fungistatic spectra on culture growth, *P. janthinellum* caused an average 27.1% inhibition of test fungi. The other fungi, i.e., *P. expansum*, *P. duclauxi*, *P. granulatum* and *P. canescens* caused an average 26.6%, 25.3%, 25.1% and 23.6% inhibition of test fungi respectively. The fungistatic effect on mycelial weight reveals an average 39% inhibition caused by *P. expansum*. The other fungi, i.e., *P. janthinellum*, *P. duclauxi*, *P. canescens* and *P. granulatum* caused an average 35.1%, 27.3%, 27.2% and 11.6% inhibition of test fungi respectively.

The species of *Penicillium* which occupy a ubiquitous and abundant occurrence in the natural environments and which are well known for the effective production of non-volatile antibiotics are also reported through these studies to be producing substantial amounts of volatile inhibitors which can play a significant role in the phenomenon of fungistasis. Many of the non-volatile antibiotics of penicillia including that of the well-known 'wonder drug' penicillin, which are isolated in quantities are now occupying a significant place in medical sciences in combating the ever-increasing diseases caused by microbes. Hence, in the same way, there is a great possibility of exploiting the volatile inhibitors of these organisms for the good of man in many possible ways. The field is now open to isolate and identify the volatile compounds produced by penicillia.

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1. Balis, C. and Kouyeas, V., *Ann. Inst. Phytopath.*, Benaki, N.S., 1968, 8, 145.
2. Dennis, C. and Webster, J., *Trans. Brit. mycol. Soc.*, 1971, 57, 41.

3. Dobbs, C. G. and Hinson, W., *Nature*, 1953, 172, 197.
4. Foster, J. W., *Chemical Activities of the Fungi*, Academic Press, New York, 1949.
5. Hutchinson, S. A., *Trans. Brit. mycol. Soc.*, 1971, 57, 185.
6. Lebeau, J. B. and Dickson, J. C., *Phytopath.* 1953, 43, 581.
7. Miller, M. W., *The Pfizer Hand-Book of Microbial Metabolites*, McGraw-Hill Book Co. Inc., 1961.
8. Robinson, P. M., Park, D. and Garrett, M. K., *Trans. Brit. mycol. Soc.*, 1968, 51, 113.
9. Satyanarayana, T. and Johri, B. N., *Hind. Antibiot. Bull.*, 1974, 16, 215.
10. Scheupp, H. and Green, J. R., *Phytopath.*, 1964, 54, 906.
11. Singhai, K., *Ph.D. Thesis*, University of Saugar, 1973, p. 146.
12. Sundara Singh, B., *Ph.D. Thesis*, University of Saugar, 1979, p. 79.
13. Ward, E. W. B., *Can. Jour. Bot.*, 1964, 40, 85.

A NEW SPECIES OF *PHOLIOTA* FROM INDIA

DURING the survey of Mushrooms from South West India, a species of *Pholiota* was collected from Mahabaleshwar plateau—a place 120 km from Poona, which on detailed characterization was found to belong to a new specific taxon on account of the reasons discussed below. It is, therefore, proposed to accommodate the present specimen under a new species of *Pholiota*, viz., *mahabaleshwarensis* and is described below along with its Latin diagnosis.

Pholiota MAHABALESHWARENSIS sp. nov. Sathe and Deshpande (Figs. 1 and 2)

HABIT : Pholiotoid.

PILEUS : 2.4–3.7 cm diam. in fresh; pale luteus to orange saffron; darker at centre becoming olive-grey at maturity; convex becoming plane with age; margin entire, inflexed when young; fleshy; non-hygrophanous; pileal veil absent; pileal surface non-viscid, glabrous; an epicutis of thin parallel repent hyphae with clamp connections, mixed with incrustated hyphae, incrustations weakly dextrinoid in Melzer; hyphae 5.72–8.58 μ m broad; pileal hairs absent; pileocystidia absent; context 1–2 mm thick, pale luteous, confluent with stipe.

LAMELLAE : adnexed; unequal with 3 sets of lamellulae; pale luteus to amber; becoming dark amber with maturity; fleshy; 2–3 per mm; 3–4 mm broad; more or less ventricose; thickness 133.2 μ m at base, 55.5 μ m at apex; margin entire; non-separable from pileus; hymenophoral trama homiomeric; regular, monomitic with thin walled generative