EFFECT OF ENVIRONMENTAL CONDITIONS ON SCLEROTIA AND CLEISTOTHECIA PRODUCTION IN ASPERGILLUS

by

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(with 4 plates)

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Introduction

Ludwig (1892) proposed "to apply a separate generic designation (*Euaspergillus* Ludwig) to all Aspergilli producing sclerotia". His attempt is only of historical importance, since sclerotia probably do not constitute a very significant taxonomically diagnostic feature. Thom & Raper (1945) recorded sclerotia in *Aspergillus candidus*, *A. niger*, *A. wentii*, *A. tamarii*, *A. flavus-oryzae* and *A. ochraceus* groups. In *A. flavipes* (Thom & Raper, 1945), *A. paradoxus* Fennell & Raper and *A. terreus* var. *africanus* Fennell & Raper (Fennell & Raper, 1955), sclerotium-like bodies of insufficient compactness have been described. Sappa (1955) described sclerotia in an *Aspergillus* of uncertain group affinities. Rai & Tewari (1961) described more sclerotia-like bodies in an Indian isolate of *A. terreus* var. *africanus*. Sclerotial development (Rudolph, 1962) in *A. alliaceus* Thom & Church conforms to the strand-type of Townsend & Willets (1954).

Dangeard (1907) "claimed to have found ascospores within the sclerotia of *A. niger* but failed to describe them". Raper, Fennell & Tresner (1953) described *A. citrisporus* von Höhnel and *A. ornatus* Raper, Fennell & Tresner, in which individual immature cleistothecia were suggestive of developing sclerotia. Fennell & Warcup (1959) described in *A. alliaceus* multiple discrete thin-walled cleistothecia, developing within a sclerotioid stromatic body. Rudolph (1962) on the basis of above and other experimental evidence suggested that sclerotia in *Aspergillus* are "sterile stromata".

The present studies include the effect of certain environmental and cultural conditions on sclerotia and cleistothecia production

in certain representative Aspergilli. The environmental conditions, effects of which have been described in the present paper, were pH of the medium, temperature of incubation, light, oxygen-deficient conditions and various relative humidity values.

Materials and Methods

Sclerotial and cleistothecial Aspergilli, viz. *A. niger* van Tieghem (two strains), *A. flavus* Link (two strains), *A. sclerotiorum* Hüber (one strain), *A. paradoxus*, Fennell & Raper (one strain) and *A. nidulans* (Eidam) Wint. (one strain)used in the present studies were all isolated from Indian soils. Cultures were grown on Czapek-Dox agar (pH 7.5 prior to autoclaving), unless otherwise stated, in 250 ml Erlenmeyer flasks and petri dishes. The inoculum consisted of a loopful of freshly germinated conidia. The cultures, throughout the experiment, were incubated at $28 \pm 1°$ C in dark for a week after which the observations were recorded. For studying the effect of pH, Czapek-Dox agar of different pH values (5.0, 7.5, 9.0 & 11.0) was used. The pH was adjusted with citric acid or sodium hydroxide before steam-autoclaving.

Effect of temperature was studied by keeping the cultures in incubators adjusted at different temperatures ($\pm$ 1° C) as mentioned in the text.

For studying the effect of light, cultures were kept in diffused light near the laboratory window and subjected to diurnal illumination.

Two simple methods were devised for studying the effect of oxygen-deficient conditions. The first method consisted of keeping the petri-dish cultures in a vacuum dessicator, the bottom of which contained 200 ml of 4.0 % pyrogallic acid. The vacuum dessicator was then connected to a suction pump and evacuated for 15 minutes. It was then connected to the side tube of a flask containing 250 ml of pyrogallic acid which had a capillary tube dipping into the solution and opening to the atmosphere. This proved to be a suitable arrangement for slowly bubbling the partially oxygen-free air back into the vacuum dessicator. After the bubbling stopped, the stopcock of the dessicator was closed. Most of the remaining oxygen in the dessicator should have been absorbed by the pyrogallic acid solution filled in its bottom. This was termed as partially oxygen-deficient condition. Pyrogallic acid solution was prepared in rigidly cleaned "Pyrex" glass-ware using double-glass distilled water. Controls were run in an ordinary dessicator with its bottom filled with glass distilled water. Lid of this dessicator was opened daily for half-an hour to allow proper aeration of the control cultures.

In the second method, the petridishes after inoculation were covered with sterile liquid paraffin layer. Controls were run uncovered.

A modification of methods used by Teitell (1958) and Ezzeldin & Sharabash (1959) was adopted for studying the effect of

TABLE I.

Solution used (Aqueous)	Concentration	Relative humidity (%)
Magnesium chloride	Saturated	32.0
Potassium bicarbonate	Saturated	42.0
Sodium chloride	32%	80.0
Sodium chloride	24%	85.1
Sodium chloride	16%	90.0
Sodium chloride	8%	95.1
Distilled Water	—	100.0

relative humidity on sclerotia and cleistothecia production. After inoculation, lower piece of the petridish was inverted on to another lower piece from a different pair which contained the salt solution. Rims of the two pieces were then sealed by means of a sealing tape. Salt solutions used have been indicated in Table I.

All cultures were run in triplicate. The results are presented as a mean of these three treatments.

OBSERVATIONS

Effect of pH

Colony diameter, extent of sporulation and number of sclerotia or cleistothecia in 7–day old cultures on Czapek-Dox agar of different pH values have been recorded in Table II (Plate I, Figs. 1 and 2).

It is evident from the table that maximum number of sclerotia or cleistothecia were produced at pH 7.5. An increase or decrease in this pH value brought about a marked reduction in the number of sclerotia or cleistothecia. The colony diameter behaved similarly. The extent of sporulation had no relation with the number of sclerotia or cleistothecia, although it increased in all the species with increase in the pH value.

Effect of temperature of incubation

Effect of temperature was studied in two strains of *A. flavus* on Czapek-Dox agar + 0.5 % yeast and the results have been recorded in Table III.

A. flavus strain 1 showed maximal sclerotia production at 27° C (Table III, Plate II, Fig. 3) while strain 2 showed it at 38° C (Plate II, Fig. 4). Both the strains showed maximal colony diameter at these temperature optima.

Effect of light

Cultures of *A. paradoxus* incubated at 22–25° C in dark showed no conidial heads and the colony was full of sclerotia. Presence of

TABLE II.

Showing the effect of pH of the medium on colony diameter, extent of sporulation and number of sclerotia or cleistothecia produced in 7-day old cultures on Czapek-Dox agar.

Strains	pH of the medium											
	5.0			7.5			9.0			11.0		
	Colony diameter (cm)	Extent of sporulation	Number of sclerotia or cleistothecia	Colony diameter (cm)	Extent of sporulation	Number of sclerotia or cleistothecia	Colony diameter (cm)	Extent of sporulation	Number of sclerotia or cleistothecia	Colony diameter (cm)	Extent of sporulation	Number of sclerotia or cleistothecia
A. niger strain 1	3.1	++	320	7.1	+++	1240	5.2	++++	800	4.3	++++	660
A. niger strain 2	2.3	+	200	8.4	++	2592	6.5	+++	2240	5.4	+++	960
A. nidulans	4.6	+++	550	8.5	++++	8320	6.8	++++	4720	5.9	+++	250

\- No heads
\+ Poor
++ Moderate
+++ Good
++++ Excellent

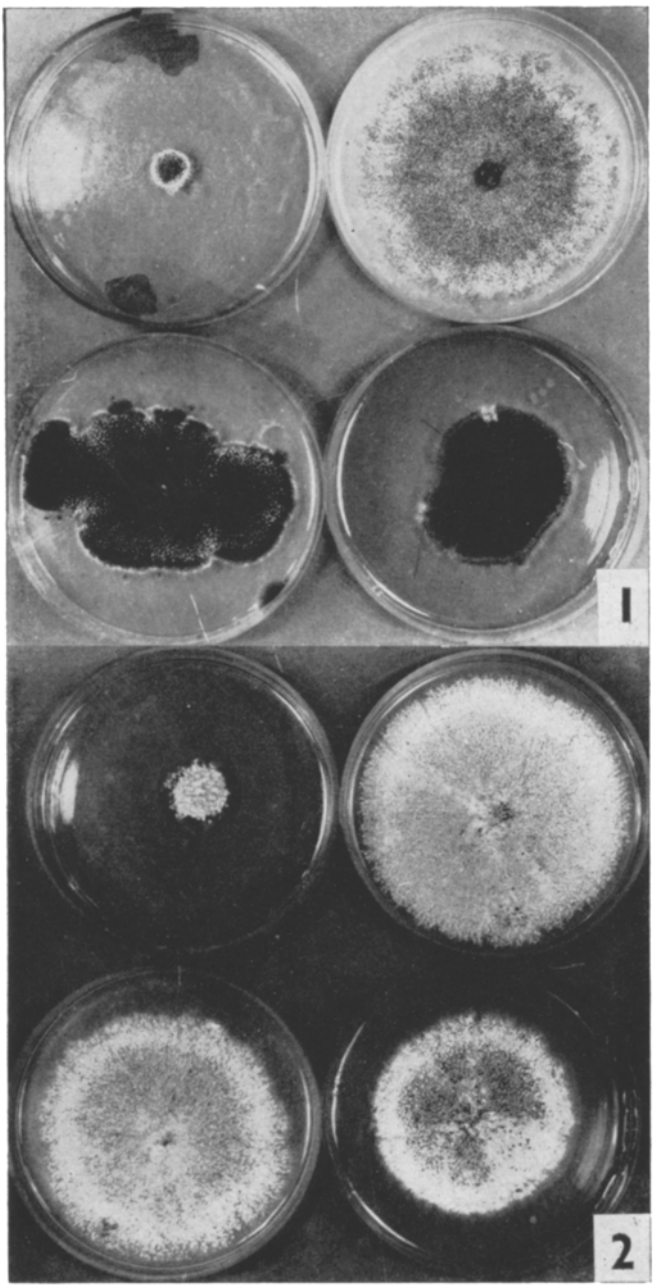
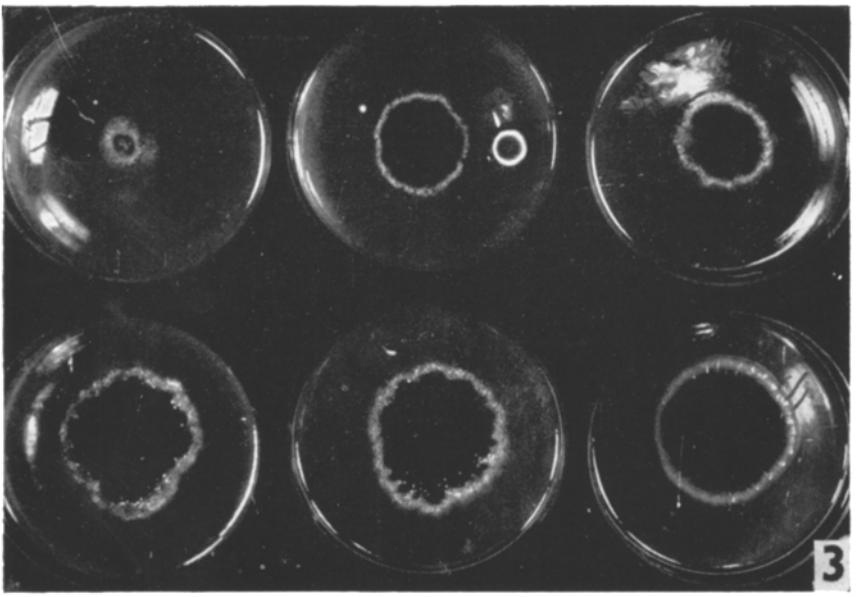


PLATE I.

Figs. 1–2. *Aspergillus niger*. Cultures grown on Czapek-Dox agar of various pH values and incubated at 28 ± 1° C for seven days. Top rows (left to right). 5.0, 7.5. Bottom rows (left to right). 9.0, 11.0. 1. Strain 1. 2. Strain 2.



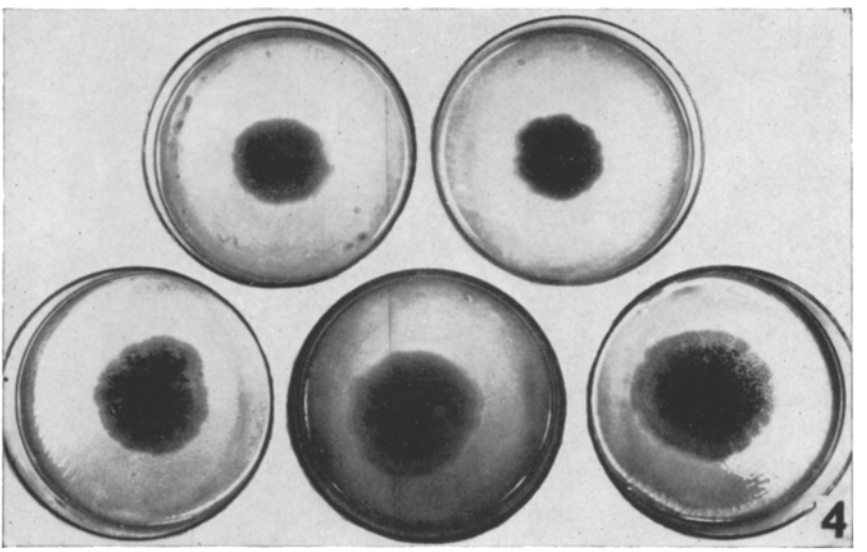


PLATE II.

Figs. 3–4. *A. flavus*. Cultures grown on Czapek-Dox agar + 0.5% yeast (pH 7.5) and incubated at different temperatures (± 1° C) for seven days.

3. Strain 1. Top row (left to right). 15, 20, 25.
   Bottom row (left to right). 27, 30, 38.
4. Strain 2. Top row (left to right). 22, 25.
   Bottom row (left to right). 27, 30 38.

TABLE III.

Showing the effect of temperature of incubation on colony diameter, extent of sporulation and number of sclerotia produced in 7-day old cultures of Aspergillus flavus Strain 1 on Czapek-Dox agar + 0.5% yeast.

Temperature of incubation (± 1°C)	Colony diameter (cm)	Extent of sporulation	Number of sclerotia
15	1.5	++	0
20	3.6	++++	0
25	3.6	++++	0
27	5.0	++++	130
30	4.9	++++	50
38	5.0	++++	0

Signs same as in Table II.

light at these temperatures stimulated conidia production and retarded the production of sclerotia (Plate III, Fig. 5). There was no growth beyond 30° C. However, at lower temperatures even in dark a good number of the conidial heads were produced along with the sclerotia. Sclerotia produced at higher temperatures were harder, bigger and more definite in shape than those produced at lower temperatures. On potato-dextrose agar the sclerotia were brownish, harder and bigger. Production of yellow pigment in the medium also behaved more or less the same as the conidial heads. Some of these facts have already been noticed by FENNELL & RAPER (1955).

A. flavus strain 1 in contrast to *A. niger* strain 1 did not produce sclerotia (Table IV) on Czapek-Dox agar + 0.5 % yeast placed in light at room-temperature (28—34° C) for eight days.

TABLE IV.

Showing the effect of light on the extent of sporulation and number of sclerotia produced in 7-day old cultures on Czapek-Dox agar + 0.5% yeast.

Strain	Placed in	Number of sclerotia	Extent of sporulation
A. niger strain 1.	Darkness	13248	++++
	Diurnal light	13152	++++
A. flavus strain 1.	Darkness	3808	++++
	Diurnal light	0	++++ (more conidial heads)

Signs same as in Table II.

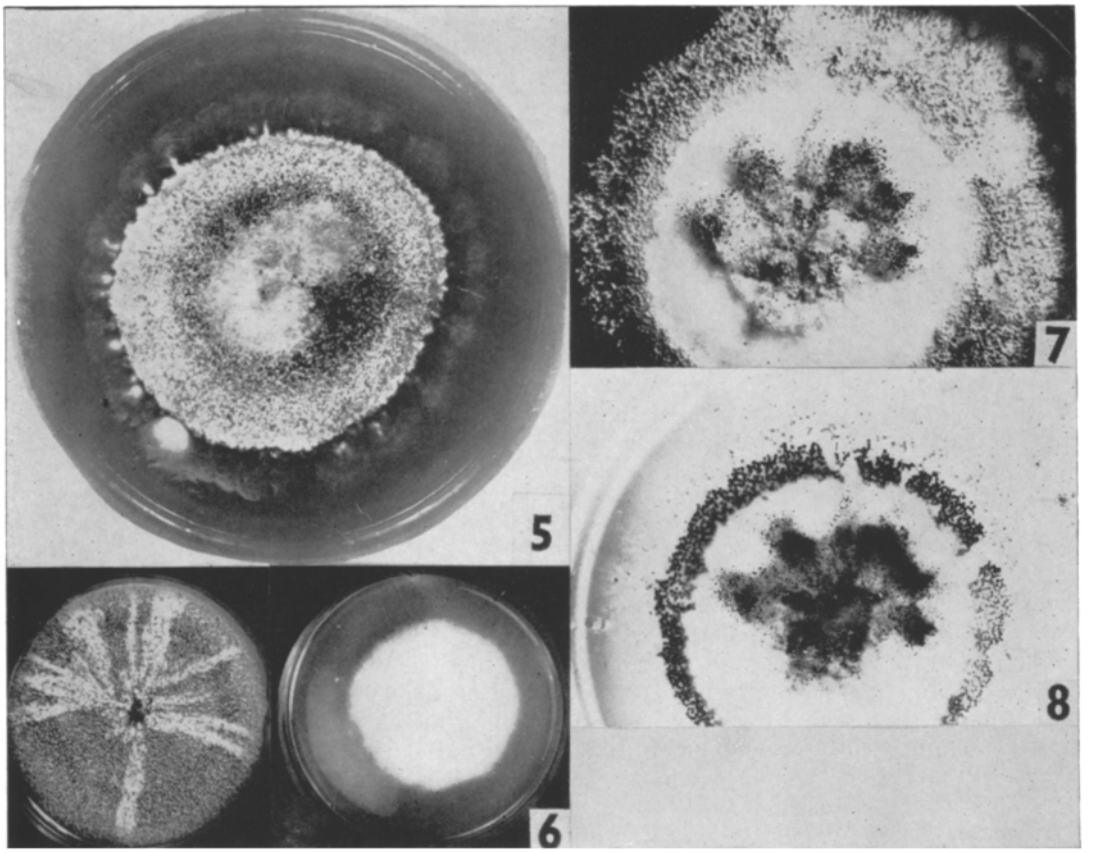


PLATE III.

Fig. 5. *A. paradoxus*. Culture on Czapek-Dox agar + 0.5% yeast (pH 7.5) first incubated in dark at 28 ± 1° C and then kept in diurnal light at room temperature.
Fig. 6. *A. niger* Strain 2. Cultures grown on Czapek-Dox agar (pH 7.5) for 7 days showing the effect of partially oxygen-deficient conditions using pyrogallic-acid method. left: Control, right: Test.
Figs. 7–8. *A. niger* Strain 2. Cultures on Czapek-Dox agar (pH 7.5), first kept under oxygen-deficient condition for 7 days and then kept in open for a few days. Fig. 7. Culture in reflected light. Fig. 8. Same in transmitted light showing silhouettes of conidial heads and sclerotia.

Effect of oxygen-deficient conditions

Colony diameter, extent of sporulation, and number of sclerotia or cleistothecia produced in 7–day old cultures as affected by oxygen-deficient conditions using the pyrogallic acid method have been recorded in Table V.

It is clear from the table that oxygen-deficient conditions markedly reduce or even inhibit sclerotia or cleistothecia formation. In

A. niger strain 2 (Fig. 6) and *A. flavus* strain 2, mycelial knots and immature sclerotia produced in oxygen-deficient environment, when kept under well-aerated conditions, matured into normal sclerotia while normal sclerotia were produced in the freshly grown regions of the colony.

In *A. niger* strain 2 when the cultures were kept under aerated conditions in addition to the maturation of the sclerotial rudiments, development of some fresh sclerotia, out of apparently undifferentiated sclerotial initials, was also observed (Plate III, Figs. 7 & 8). While this matter will be discussed at length in the latter part of this paper, it may be pointed out that *A. flavus* strain 1 and *A. sclerotiorum* were different from the rest of the Aspergilli tested here, with reference to their critical minimum oxygen concentration for

TABLE V.

Showing the effect of oxygen- deficient conditions on colony diameter, extent of sporulation and number of sclerotia or cleistothecia produced in 7-day old cultures on Czapek-Dox agar by pyrogallic acid method.

Strain	Condition	Colony diameter (cm)	Extent of sporulation	Number of sclerotia or cleistothecia
A. niger strain 2	Control	9.0	++++	2016
	Oxygen-deficient	5.0	+	20*
A. flavus strain 1	Control	7.2	++++	400
	Oxygen-deficient	5.0	++++	0**
A. flavus strain 2	Control	6.9	+	2400
	Oxygen-deficient	3.0	—	22*
A. sclerotiorum	Control	3.6	+++	4200
	Oxygen-deficient	2.3	—	0**
A. nidulans	Control	8.9	++++	8300
	Oxygen-deficient	4.7	—	0**

*) Light yellowish or whitish sclerotial rudiments, resembling mycelial knots and devoid of outer dark brown rind, developed into normal sclerotia on keeping for 2—3 days under aerated conditions. In addition to these, a number of sclerotia, apparently arising from already existing sclerotial initials, developed after this treatment in the already grown region of the colony.

**) After keeping the cultures under aerated conditions for 2—3 days (in darkness), sclerotia or cleistothecia developed in the freshly grown region of the colony and not in the already grown region.

Signs same as in Table II.

TABLE VI.

Showing colony diameter, extent of sporulation and number of sclerotia or cleistothecia produced in 7-day old cultures of Aspergilli grown on Czapek-Dox agar at different relative humidity values.

Relative humidity values (%)	*A. niger* strain 2			*A. flavus* strain 2			*A. sclerotiorum*			*A. nidulans*		
	Colony diameter (cm)	Extent of sporulation	Number of sclerotia	Colony diameter (cm)	Extent of sporulation	Number of sclerotia	Colony diameter (cm)	Extent of sporulation	Number of sclerotia	Colony diameter (cm)	Extent of sporulation	Number of cleistothecia
32	0.4	—	0	0.5	—	0	0.2	—	0	0.6	+	0
42	4.2	++	0**	4.2	+	0	1.6	+++	200	2.0	+	0
80	4.5	+	28	4.6	+++	5	2.0	+++	460	3.2	+++	268
85.1	5.1	+	140	4.8	+++	16	2.5	+++	1800	4.6	++++	460
90	5.5	++	168	5.5	+++	120	2.8	+++	2432	6.5	++++	500
95.1	6.4	++	1248	5.8	+++	1172	3.0	+++	2896	7.5	++++	1256
100	8.8	+	1700	6.1	+++	2388	3.2	++++	3500	8.5	++++	6800

Signs same as in Table II

**) Mycelial knots or immature sclerotia were produced in plenty.

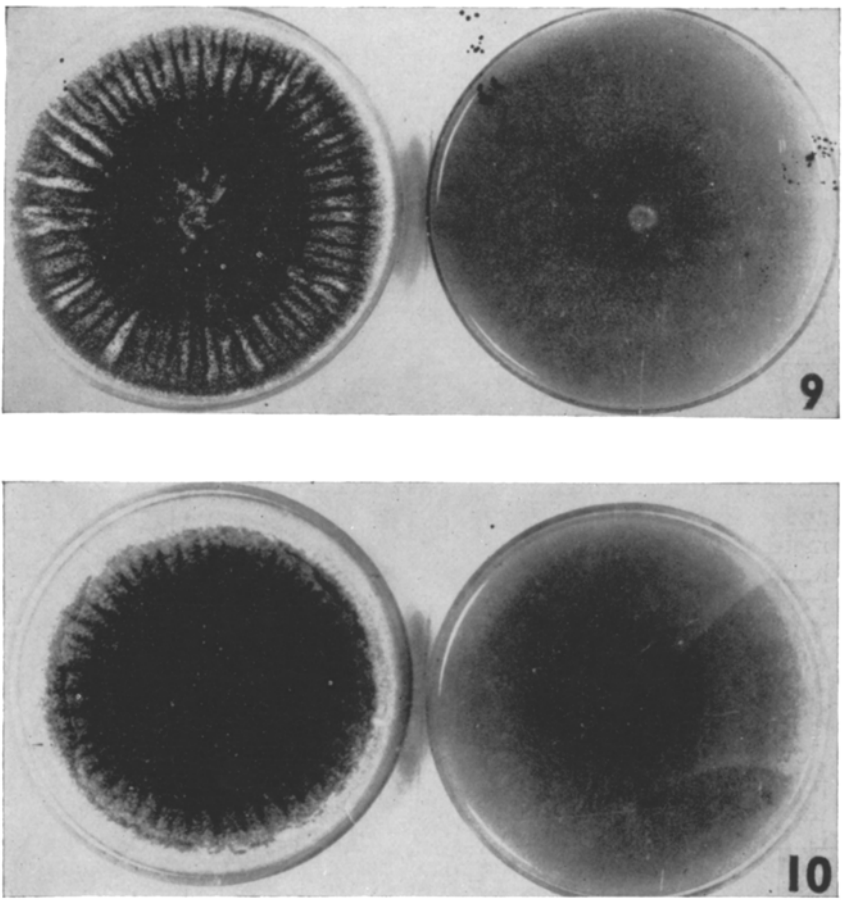


PLATE IV.

Figs. 9–10. Cultures grown on Czapek-Dox agar + 0.5% yeast for 7 days at room temperature (26–32°C) showing the effect of oxygen-deficient conditions using the liquid-paraffin method.
Fig. 9. *A. niger* Strain 2. left: Control, right: Test
Fig. 10. *A. flavus* Strain 2. left: Control, right: Test.

inhibition of sclerotia formation. Cleistothecia production in *A. nidulans* responded similarly to the sclerotia formation in *A. flavus* strain 1 and complete inhibition of cleistothecia production took place in already grown regions of the colony.

Cultures covered with sterile liquid paraffin layers even after a fortnight showed smaller frequency of sclerotial rudiments (Plate IV, Figs. 9 & 10). However, on ageing the sclerotia in liquid paraffin covered cultures matured and showed a tendency of producing submerged sclerotia.

Effect of relative humidity

Colony diameter, extent of sporulation and number of sclerotia or cleistothecia produced in 7–day old cultures grown at different relative humidity values have been recorded in Table VI.

It will be clear from the data presented in Table VI that number of sclerotia in the three species viz. *A. niger*, *A. flavus* and *A. sclerotiorum* increased with an increase in the relative humidity values. In all cases, sclerotia or cleistothecia production was completely suppressed at the lowest percentage relative humidity used i.e. 32 %. At this condition very little vegetative growth was observed. In *A. niger* strain 2 light yellow knob-like structures (mycelial knots) devoid of dark brown outer rind were produced in abundance when the relative humidity was maintained at 42 %. At 42 % in *A. flavus* strain 2 and *A. nidulans*, in contrast to *A. sclerotiorum*, no sclerotia or cleistothecia respectively were formed. Maximum number of sclerotia and cleistothecia were produced at 100 % relative humidity, other values showing intermediate numbers of sclerotia and cleistothecia. Vegetative growth as measured by colony diameter also increased with the increase in the percentage relative humidity. Production of sclerotia and cleistothecia was observed to be related to the extent of sporulation except in *A. niger* strain 2.

Discussion

Rudolph (1962), quoting the works of Klebs (1896, 1900), Townsend (1957), Hawker (1957) and Fennell & Warcup (1959) presented an excellent discussion in favour of his hypothesis that the sclerotium of *Aspergillus* is a "sterile stromata". This hypothesis is, however, not a new one. Thom & Raper (1945) after failing to confirm the results of Dangeard (1907) remarked that "it may be assumed that such sclerotia may be the homologue of structures which under some conditions might become perithecia". Some similar views were also expressed by Raper, Fennell & Tresner (1953) and Fennell & Warcup (1959). In favour of his hypothesis Rudolph (1962) has drawn attention to his UV-studies as is also apparent from the works of Sakaguchi, Suzuki & Iizuka (1955), Fennell & Warcup (1959) and Coats (1959). The present study reveals as Rudolph (1962) has also observed, that the vegetative growth in *Aspergillus* is not always accompanied by the formation of sclerotia, indicating that "the sclerotia in *Aspergillus* may be fundamentally different from those found in many other fungi, where they often are the important vegetative structures." In addition, evidence has been gathered that in many respects the physiological conditions (especially the environmental conditions) similarly affect the sclerotia and cleistothecia production by Aspergilli. Results of the present studies have been discussed below under separate headings:

Effect of pH

Optimum pH for the production of sclerotia and cleistothecia was 7.5. Within the range tried, pH values higher or lower than this brought about a reduction in sclerotial or cleistothecial numbers. Sporulation increased with increase in the pH.

Effect of temperature of incubation

Two strains of *A. flavus* behaved differently as to their temperature optima for sclerotia production. *A. flavus* strain 1 showed sclerotia formation only at 27° and 30° C, the former being the optimum. In *A. flavus* strain 2 the sclerotia formation increased gradually from 22—38° C. This temperature at which maximum number of sclerotia were produced always coincided with the growth optima, as judged by the colony diameter. RUDOLPH (1962), taking colony diameter as a measure of vegetative growth, also found that "in all cases, except for strain 4327, the production of sclerotia was maximal at or slightly below the optimum temperature for mycelial growth". In *A. flavus* strain 4327, RUDOLPH (1962) observed that "sclerotia were formed only at 38° C, an unusual response".

PETERS & RIPPEL-BALDES (1949) isolated from German soils "purely sclerotial strains" of *A. niger* "hitherto known only in the tropics" and observed that they either loose the capacity for sclerotia formation after sub-culturing a few times or produce them at lower minimum temperature than the tropical strains.

In *A. paradoxus*, in general, higher temperature and darkness favoured sclerotia formation.

Effect of light

Light had no effect on sclerotia formation of *A. niger* strain 1. On the contrary in *A. flavus* strain 1, sclerotia formation was inhibited by the presence of light. Both these strains showed a little more sporulation in presence of light. TATARENKO (1954) noted retardation of sclerotia formation by intense light in a few Aspergilli including *A. flavus*. RUDOLPH (1962) in six species of Aspergilli (including some previously investigated by TATARENKO, 1954) found no significant difference in production of sclerotia and conidia between cultures grown in continuous darkness and in continuous white light. It thus appears that species of *Aspergillus* and even strains of the same species have different responses to light.

In *A. paradoxus* conditions leading to maximal sclerotia production were generally those that retarded (or even inhibited) the formation of conidial heads and the yellow pigment in the medium. Light inhibited or retarded sclerotia formation in *A. paradoxus*.

Effect of oxygen-deficient conditions

BRANCATO & GOLDING (1953), BARINOVA (1953), STOVER (1955) and others have studied gas requirements of Aspergilli, but so

far as the authors are aware, no study has been made with reference to sclerotia formation except for that of COATS (1959) with respect to *A. niger*.

A. niger strain 2 and *A. flavus* strain 2 showed marked reduction in the number of sclerotia produced (those produced were underdeveloped) under partially oxygen-deficient conditions. Under similar conditions *A. flavus* strain 1 and *A. sclerotiorum* showed no sclerotia formation. On keeping for sometime, the culture plates previously grown in partially oxygen-deficient conditions, in open, two types of responses were observed: (a) in *A. niger* strain 2 and *A. flavus* strain 2, the underdeveloped sclerotia matured and fresh ones also developed all over the colony, (b) in *A. flavus* strain 1 and *A. sclerotiorum* sclerotia were produced only in the freshly grown and not in the previously grown regions of the colony. *A. nidulans* behaved similarly with respect to cleistothecia formation.

Taking into account limitations of the methods used, it is appreciated that a little gaseous oxygen and a good amount of oxygen dissolved in the medium may have been available to the fungus culture. The results are, thus, only indicative of differential oxygen requirement of various Aspergilli for sclerotia formation. It appears that formation of sclerotia all over the colony (and maturation of the underdeveloped ones) and only in the freshly grown regions of the colony are significant in this connection, strains showing the former response having a lower and the latter comparatively higher critical minimum oxygen concentration for the inhibition of sclerotia formation. Based on this interpretation, it is concluded that in *A. flavus* strain 1, *A. sclerotiorum* and *A. nidulans* under concentration of oxygen used in this experiment, even the formation of sclerotial or cleistothecial initials was inhibited. In others (which probably have a lower critical minimum oxygen concentration for the inhibition of sclerotia formation) however, the initiation of sclerotial initials or even their development upto a certain stage (e.g. in *A. niger* strain 2 and *A. flavus* strain 2 which had a lesser frequency of underdeveloped sclerotia) took place.

COATS (1959) working with *A. niger* found that at 2.0 % oxygen level no sclerotia formation occurred and that oxygen uptake of a sclerotial strain prior to sclerotization and that of a non-sclerotial strain in the similar stage was not significantly different. He thus concluded that oxygen level does not control sclerotia initiation but controls pathways leading to sclerotization.

Effect of relative humidity

BONNER (1948) reported that in *A. niger* optimum conditions for growth were at a relative humidity near 93 % and at a temperature near 40° C while at 100 % relative humidity the optimum temperature was near 30° C. Results of the present investigations also showed that optimum relative humidity value, within the range tried, for growth and sclerotia and cleistothecia production was

100 % at 28° C. Thus, observations reported here for sclerotial and cleistothecial Aspergilli are more or less parallel to those of BONNER (1948). The observations reported here also show that both sclerotia and cleistothecia formation were inhibited at low relative humidity, thus confirming the observations of COATS (1959) for *A. niger*, NRRL 346. This however, should be expected in view of the fact that low relative humidity value would not permit normal development of sclerotial and cleistothecial initials and even the vegetative growth should be greatly affected through dehydration of culture medium.

In all the experiments except that of relative humidity, no definite correlation could be obtained between the number of sclerotia formed and the extent of sporulation. In case of relative humidity experiment, production of sclerotia and cleistothecia was observed to be related to the extent of sporulation except in *A. niger* strain 2.

Parallel response of some of the sclerotial and cleistothecial strains of *Aspergillus* to certain environmental conditions lends further support to RUDOLPH's (1962) hypothesis that sclerotia of *Aspergillus* are "sterile stromata".

Summary

Literature pertaining to sclerotial Aspergilli has been reviewed in brief. Observations on the effect of certain environmental conditions viz. pH, light, temperature of incubation, oxygen-deficient conditions and various relative humidity values on sclerotia production by *Aspergillus niger* VAN TIEGHEM, (two strains), *A.flavus* LINK (two strains), *A. sclerotiorum* HÜBER (one strain) and *A. paradoxus* FENNELL & RAPER (one strain) and on cleistothecia production by *A. nidulans* (EIDAM) WINT. (one strain) have been presented. Optimum pH for sclerotia or cleistothecia production was 7.5. In other respects sclerotia and cleistothecia behaved similarly. In general, condition showing maximum sclerotia or cleistothecia production was the one that showed maximum vegetative growth. Certain strains of the same species reponded differently to the same condition. Light completely inhibited sclerotia formation in one strain of *A. flavus*. In *A. paradoxus*, in general, conditions favouring sclerotia production were those that inhibited (or retarded) the formation of conidial heads and the yellow pigment in the medium. Oxygen-deficient conditions inhibited or retarded sclerotia or cleistothecia formation. Production of sclerotia and cleistothecia increased with an increase in relative humidity values. No definite correlation could be observed between extent of sporulation and sclerotia or cleistothecia production except in case of relative humidity. Parallelism in the behaviour of sclerotia and cleistothecia production in *Aspergillus* lends further support in favour of the hypothesis that in this genus sclerotia are "sterile stromata".

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Addendum

Aspergillus niger van Tieghem strain 2 used in the present investigation was sent to Prof. K. B. Raper in 1962 who identified it as an extremely sclerotial strain of *A. niger* and accessed it as Wis 4700 (in lit. May 11, 1962). The same strain has since then been considered to be *A. tubingensis* (Schöber) Mosseray (Raper, K. B. & Fennell, D. I. 1965. The Genus Aspergillus. The Williams & Wilkins Co., Baltimore). Hence, in this paper *A. niger* strain 2 should be read as *A. tubingensis*.

* Originals not seen.