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STUDIES ON THE PHYSIOLOGY OF *ACHLYA MEGASPERMA* HUMPHREY OCCURRING IN PONDS ON THE ACCRA PLAINS WITH SPECIAL REFERENCE TO ITS TOLERANCE TO ORGANIC AND INORGANIC POLLUTION

A thesis presented by

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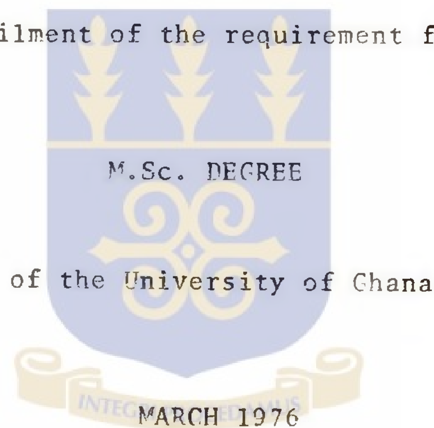
in fulfilment of the requirement for the



M.Sc. DEGREE

of the University of Ghana

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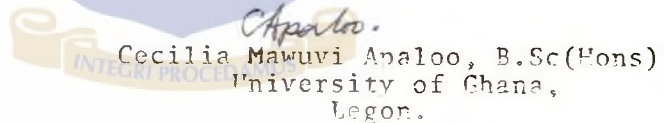
This is to certify that the work presented in this thesis:

"Studies on the physiology of *Achlya megasperma* Humphrey occurring in ponds on the Accra Plains with special reference to its tolerance to organic and inorganic pollution" was done entirely by me in the Botany Department, University of Ghana from October 1974 to March 1976.

This work has never been presented, either in part or completely, for any degree of this University or elsewhere.



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ABSTRACT

Achlya megasperma sporulated best at 30°C and at pH 7.0 to 9.0. Light had no effect on formation of the sporangia.

Certain concentrations of $CaCl_2$, Dulcitol, Mg $SO_4.7H_2O$, Malt extract, Peptone and Yeast extract enhanced sporangial formation. The mineral salts, $CuSO_4.5H_2O$, $FeCl_3$, $MnSO_4.4H_2O$ and Zn Cl_2 depressed sporangial formation. Gemmae were produced in great quantities in solutions of 0.1, 0.2 and 0.4 per cent haemoglobin and 1x0^{-7} and 1x10^{-5}M Zn Cl_2. Sporangial formation was also inhibited by the carbohydrates, Fructose, Galactose, Glucose, Lactose and Mannose, the polyhydrol alcohols Glycerol and Mannitol and, by Potato dextrose broth. Vegetative growth was, however, greatly improved by Potato dextrose broth, Fructose, Glucose, Mannose, Malt extract, Peptone and Yeast extract, and there was considerable hyphal branching in solutions of Fructose Glucose, Malt extract, Peptone and Yeast extract and in Potato dextrose broth. In the mineral salt solutions the range of concentration permitting sporangial formation was narrower than that supporting vegetative growth.

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Sporangia which had been delimited from the supporting hyphae were prevented from further development by pH4, provided by distilled water adjusted with 0.5N HCl, and by cow-dung solution of 10 and 20g dung per litre of distilled water. There was total inhibition of sporangial formation at dung concentration of 30g per litre of suspending medium. Sporangia formed under conditions of poor sporulation were markedly smaller in size. A particularly pronounced effect was produced by $20^{o}C$, pH 5, $1x10^{-3}$ to 0.1M $CaCl_2$, $1x10^{-7}$ and $1x10^{-5}$M $CuSO_4.5H_2O$ and $MnSO_4.4H_2O$, $1x10^{-5}$M $FeCl_3$ and $1x10^{-7}$M $ZnCl_2$ and by the alcohols, Glycerol and Mannitol.

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INTRODUCTION

Fitzpatrick (1930) separated the members of the class Phycomycetes into eight orders, Chytridiales, Blastocladiales, Monoblepharidales, Saprolegniales, Pe nosporales, Mucorales and Entomophthorales.

Bessey (1950), on the other hand, recognized 12 orders. The additional orders were Hyphochytriales, Lagenidiales, Protomycetales, Eccrinales and Zoopagales.

The orders Chytridiales, Blastocladiales, Hyphochytriales, Monoblepharidales, Lagenidiales and Saprolegniales are largely aquatic and semi-aquatic. Man species are saprophytic while some are parasitic on flowering plants, algae, animals and other fungi.

The order Saprolegniales consists of fungi with well-marked hyphal development. In fact, the largest fungu hyphae known are to be found here. Thus Monsona (1937) found on hemp seed in water, hyphae of _Achlya oblongata_ de Bary var _globosa_ Humphrey that attained a diameter of 270 µm near the base and were so stiff that, on removing the seed from the water, the hyphae stood out straight to a length of 15 mm. Members of this order are saprophytic on dead plant or animal matter in the soil or in fresh water, or parasitic in algae or small animals or even fish, and in some cases in the roots of plants in the soil.

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Although spoken of usually as water moulds perhaps the majority are inhabitants of moist soil.

Of the three families of Saprolegniales: Saprolegniaceae, Leptomitaceae and Rhipidiaceae, the family Saprolegniaceae is by far the largest, and the occurrence and distribution of the members in fresh water bodies have been extensively studied.

Perrot (1960) noted that the occurrence of aquatic Phycomycetes depends not only on the nature and pH of the substratum, but also on the temperature and time needed for maturation of the resting spores. As a result of these two last factors there is a distinct seasonal periodicity of growth.

That certain species in the Saprolegniaceae exhibit a seasonal periodicity of occurrence and variation in abundance was first pointed out by Coker (1923). In an attempt to elucidate this seasonal occurrence he presented a periodicity table, based on species of Saprolegniaceae isolated from 593 water samples collected in the Chapel Hill, North Carolina region over 2 years, 1912-13. Data included in this table show that some species, *Saprolegnia ferax* and *Achlya racemosa*, for example appeared more frequently in samples taken at one time of the year - spring

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than in those collected at other times. Coker pointed out that in North Carolina there is no 'closed season' for water moulds, but he indicated that for most species spring was the most favourable season for growth. Coker concluded his discussion of seasonal periodicity by stating, 'if we compare the six cold and the six warm months we find little or no difference, ten species appearing more frequently in the warm and nine in the cold seasons'. He did not attempt to determine any relationships or distinctions that might exist between the species groups exhibiting variation in occurrence or abundance at different periods of the year, nor did he offer any speculation as to the factors which might be responsible for seasonal periodicity.

Lund (1934), in his extensive study of Danish freshwater Phycomycetes, discussed seasonal periodicity briefly, noting that *Achlya* spp. thrived best in rather cold waters while *Saprolegnia* spp. did not seem so dependent on temperature.

Accounts that followed these earlier reports provided greater details on the effect of temperature on the occurrence of members of the Saprolegniaceae.

A state-wide survey of the Saprolegniaceae of Florida,

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U.S.A., in 1958 by Ziegler (1958) showed that the species fell into four categories, species that grew best in cool weather, cold weather, hot weather or occurred at the same frequency in all seasons. Species isolated in cool weather were Achlya apiculata, Achlya colorata, Achlya megasperma, Achlya mucronata, Achlya treleaseana, Isoachlya intermedia, Isoachlya toruloides, and Isoachlya unispora. The cold weather species were Achlya conspicua, Achlya hypogyna, Leptolegnia caudata, and the following five species of Saprolegnia, S. delica, S. declina, S. ferax, S. litoralis and S. megasperma. Ten species were abundant in hot weather, the Achlya species, A. americana, A. dubia A. klebsiana, A. oblongata, A. recurva and A. rodrigueziana the species of Brevilegnia, B. declina, B. linearis and B. unisperma and Thraustotheca clavata.

Hughes (1962), in a similar investigation in the South-eastern United States, examined Saprolegniaceae in pond samples of different temperatures and produced results closely identical to those of Ziegler. The ponds contained A. dubia and T. clavata when their temperatures were 17° to 37°C in summer, species found during the hot weather in Florida. In winter, with temperatures ranging from 7° to 22°C, the ponds contained again only cold or cool weather species

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of Florida, A. colorata, A. treleaseana, I. intermedia, I. unispora, S. delina, S. declina, S. ferax, S. litoralis and S. megasperma.

An analysis of the result obtained by Roberts (1963) from five habitats in England sampled regularly also gave evidence that Saprolegniales show seasonal fluctuations in their occurrence. Three of the habitats Malham Tarn, Molinetum pool and Tarn Moss were sampled every 2 months from November 1956 to November 1957. In the other two habitats, South Pond F and Chobham common bog, sampling was continued until summer 1958.

He divided the Saprolegniales studied into Winter "Species", Summer "Species" and Constant "Species" depending on the season in which they were found. Of the 27 species isolated, 13 species were classified as Winter "Species", which commonly occurred in large quantities in winter. Other periods of the year when they were absent, however differed with species. Thus, the winter "Species" Achlya polyandra, Isoachlya toruloides and Aphanomyces laevis were absent from May to September, whilst Saprolegnia monoica was absent in a shorter period from June to September. Saprolegnia asterophora S.declina and Saprolegnia mixta were absent over a longer period from June to November.

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The six summer "Species" which were absent from January to March consisted of four Achlya species, A. flagellata, A. hypogyna, A. oligocantha and A. treleaseana and two species of Saprolegnia, S. ferax and S. litoralis.

The constant "species" occurred throughout the year. This group was made up of eight species and, species mentioned in the report were A. americana, A. apiculata, A. colorata, S. delica and Dictyuchus sterile. The first four, in contrast, belonged to either the cool or cold weather in Ziegler's (1958) list. Species found in all seasons by Ziegler were the Achlya species, A. flagellata, A. lobata, A. orion, A. prolifera, A. proliferoides and A. racemosa, the Aphanomyces species, A. laevis, A. scaber and A. stellatus, the Dictyuchus species, D. missouriensis, D monosporous and D pseudodictyon and, Thraustotheca clavata.

Alabi (1971) provided the most comprehensive pertinent work done in West Africa. He placed the aquatic Saprolegniaceae found in ponds around the city of Ibadan in Nigeria into three categories according to the season. There were two contrasting seasons, with characteristic species, the rainy season with comparatively lower temperatures and, dry season with attendant higher temperatures. Some species constituting a third group were found throughout the year.

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This group was made up of the following Achlya sp

A. abortiva, A. dubia, A. prolifera and A. debary

The dry season extended from October to April

was characterised by the greatest number of specie

presumably because of the higher water temperature

this period. Measurements at some of the collectin

showed that the temperature ranged from 27° to 32°C

January to April at one locality and from 28° to 31°

at another site during the dry season (October-April)

In contrast, the temperature fell to 26° - 28°C durin

the rainy season, from April to October. Species foun

the dry season were A. treleaseana, Achlya caroliniana

Brevilegnia bispora, Brevilegnia linearis, Brevilegnia

unisperma, D. monosporus, Dictyuchus achlyoides, A. laev

Pythiopsis cymosa and P. clavata four species of Achlya,

A. klebsiana, A. megasperma, A. proliferoides and A racem

were characteristic of rainy season from April to Octobe

Fluctuations in the pH of the environment could also

influence the abundance of members of the Saprolegniales.

The pH of the pond sampled by Alabi clearly fluctuated with

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dry season, October to April, in one particular pond; approximately double the number found in the rainy season. The respective pH's of the ponds during these seasons were pH 8.0 and pH 7.0.

Alabi, further showed the role of another important factor, organic matter, in the frequency of occurrence of Saprolegniales in pond. He found out that, the organic matter of the pond decreased from 7.2 g/l in the dry season, October to April, as a result of floods during rainy season May to September to 3.13 g/l. The average number of species found in the four ponds at flooding was two. The peak of flooding varied with the station: it was September in station A, June to September in B, June to July in C and May to July in station D. In each case, the minimum number of species coincided with the peak of flooding. As soon as the rains stopped, the level of soluble organic matter rose accompanied an increase in the number of species from an average of two to an average of five species.

Out of a total of 44 species, isolated in a similar investigation elsewhere, Perrot ([illegible]) noted that 37 species mostly belonging to the genus _Monoblepharida_ grew in neutrally acid water of pH 6.5 - 7.7. The most commonly occurring species among these, _Apoda chlya purifera_, was found in water with pH beyond 7.5.

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Roberts (1963) recognised three groups according to the pH preference of the species. Acid group "species" found in pH 3.6 to 5.2 were A. americana and the following Saprolegnia species. S. latviaca, S. asterophora S. treleaseana and S. litoralis. The alkaline group "species" occurred in waters with a pH above 7.8 and included A. oligocantha A. polyandra and S. ferax. Species placed in neutral group flourished at pH 5.2 - 7.4 with the greatest number of the different species occurring in the neutral range. The neutral group species were P. cymosa, A. flagellata, Achlya radiosa and A. laevis.

It is well known that certain fungal species are closely associated with the specific environmental conditions. For example, Harvey (1952) comparing fungi from polluted and non-polluted waters found that the largest numbers of true fungi were isolated from non-polluted waters. Achlya and Saprolegnia were never found in grossly polluted waters and rarely in partially polluted waters. Pythium and Geolegnia were like wise absent.

Cooke (1954) found that of the 105 species isolated, six including Aspergillus fumigatus, Geotrichum candidum Penicillium and Trichoderma viride were present at all stations on all monthly occassions in the organically

polluted Lythe Creek. Pollution was caused by domestic sewage and by organic matter from artificial silk and sugar factories, breweries and paper mills.

There are very detailed records of the influence of pH on the distribution of Saprolegniaceae in soil. Dick (1963) was able to show in his studies on the occurrence and distribution of Saprolegniaceae in South East England that, there existed characteristic groups of species in the various habitats as defined by the pH of the soil. The moderately acid soils had a higher incidence of species than was found in the highly acid or slightly alkaline soil. *S. asterophora*, *Saprolegnia turfosa* and *S. litoralis* occurred only in soils with pH below 4.2. *T. clavata*, *A. caroliniana*, *Saprolegnia anisospora*, *Calyptralegnia inflata* were specifically characteristic of soils with hydrogen ion concentration beyond pH 6.0. Only two species *Aplanopsis spinosa* and *Aplanopsis terrestris* among the 27 isolated, were found in soils with pH's ranging from below pH 4.2 to over 6.0. The rest occurred at pH 4.3 to 6.0.

It is clear that members of the Saprolegniales and, most likely other aquatic fungi, react to changes in both physical and chemical conditions of water bodies in which they occur.

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It should be possible then, to supplement existing methods, to employ water moulds deliberately introduced into a water body as a test organism for defining the characteristics of the environment and particularly establishing level of pollution.

This, however, is impossible without knowledge on the biology and physiology of the test fungus. The species must be of wide distribution and must be easy to obtain, should be fast growing to provide quick answers, must have clearly diagnostic features and the test must not involve elaborate methods.

This suggestion is based on the frequent difficulty of establishing degree of pollution by characteristic flora and fauna. Hawkes (1962), for example, pointed out that the discharge of a sewage effluent, which brings about de-oxygenation of a stony-stretch of river may produce luxuriant growths of sewage fungus colonized by blood worms. The discharge of a similar load of another organic waste, which whilst producing a similar growth of sewage fungus, does not result in a serious de-oxygenation of the rapid reach; here one would find Gammarus and even species of may-fly, as well as Asellus, amongst the sewage fungus.

Conversely, the same degree of de-oxygenation may

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exist in the recovery zone of a seriously polluted river as is found in the zone immediately below the effluent in the case of mild pollution. In the recovery zone, however, the more tolerant invertebrates such as _Asellus_ and _Hydropsyche_ are usually associated with profuse algal growth that are encouraged by the increased mineral salts. In the mild pollution, although similar oxygen condition enables a similar macro fauna to be present, the organic matter supports saprobic growths instead of algae. Thus, because of quantitative and qualitative differences in organic discharges which affect the several factors involved differentially, it is not possible to define precisely stream communities typical of different degrees of organic pollution. Conversely two similar communities may not be supported by the same degree of organic pollution. The identification of all the species is besides a very lengthy procedure.

There is another reason. A particular condition may not necessarily have clearly identifiable associated fauna and flora, particularly, where conditions are not retained at specific levels for very long periods.

The use of organisms to test level of pollution, as employed in coliform test, is encouraged generally.

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Hawkes (1962) wrote on Biological Aspects of River Pollution in the book "River pollution II Causes and Effects" (Klein 1962)......"Although river pollution may be said to be caused by chemical or physical agencies, it is essentially a biological phenomenon. In the past attempts have been made to measure pollution in terms of purely biochemical, chemical and physical standards. After years of chemical and physical testing of river waters the boards are today experiencing difficulties in setting of standards for effluents, and it will only be in the light of biological surveys and tests that these standards will eventually be successfully determined. A knowledge of the biological aspects of pollution is then not only of interest but essential to the problem of river pollution".

The response of a member of the Saprolegniaceae that commonly occurs in ponds in the Accra Plains, Achlya megasperma Humphrey, has been studied for this purpose. Because of the quick production of the sporangia, 4 days after inoculation under optimal conditions, asexual reproduction in this species was adopted as a criterion of response. The report will show how various aspects of the sporangium can be employed, viz the number of sporangia formed and the size of the sporangia. Sexual reproduction

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was not employed as this requires the presence of male and female hyphae.

This investigation has revealed some significant advantages in employing such a fungus. It can be used

a) to examine different types of pollution, and (b) to establish approximately, the concentration of chemicals and other pollutants in the medium.

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II. LITERATURE REVIEW

Achlya megasperma has an extensive geographical distribution. It has been isolated several times in the United States of America (Coker, 1927; Slifkin, 1961; Ward, 1939; Ziegler, 1958) since its first description from specimens collected in 1893 in the U.S.A. by Humphrey (1893). It has been recorded in collections in England (Forbes, 1935; Ivimey-Cook and Forbes, 1933), Germany (Höhnk, 1935), Japan (Nagai, 1931) and Nigeria (Alabi, 1971) and, it occurs abundantly in ponds in the Accra Plains in Ghana.

Members of the genus *Achlya* generally reproduce asexually by two methods, Sporangia and gemmae. Morphological and cytological details of sporangium formation have been described for several Saprolegniaceae species (Bessey, 1950; Johnson, 1956; Sparrow, 1960). Although some of the species are serious parasites, *A. flagellata* (Tiffney and Wolf, 1937; Wolf 1939) and *Saprolegnia parasitica* (Powell, Scott and Krieg, 1972) are parasites of fish and *A. americana*, *A. flagellata* *Achlya oryzae* and *A. prolifera* cause rot disease of the seeds and seedlings of rice (Abe, 1927; Hemmi and Abe, 1928; Ito and Nagai, 1931), Literature on the physiology and biochemistry of the

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sporangium that bears the principal dispersal agents, zoospores, is scanty.

Lee (1962) observed that S. parasitica produced maximum number of sporangia at 15°C and beaded hyphal tips were observed in place of normal zoosporangia near the extremes of the temperature range, zero to 36°C. Cotner (1930) noted that the optimum temperature for zoospore formation and liberation in Isoachlya paradoxa and Achlya conspicua lay from 23°C to 25°C and from 23° to 27°C, respectively. Zoospores were not formed beyond 34°C in I. paradoxa. The influence of temperature on the formation of the sporangium was not indicated in the report.

A remarkable effect of temperature on another form of reproduction in A. colorata, oogonium formation was found by Reischer (1949a). Oogonia were never formed in abundance at 10°C and those formed were somewhat atypical and aborted. At 15°C papillate Oogonia were produced. The papillae, 6-10 um long, were set regularly about the entire surface of the oogonium. The Oogonia, irregularly set with short papillae or merely roughened, were typical of about 90 percent of those appearing at 20°C. Oogonia typical of 25°C were rough or with one or two short papillae. None was as papillate as any of the Oogonia produced in the

15°C cultures but a completely smooth Oogonium was rarely seen.

Another report of Reischer (1949b) also showed a relationship between the size of the Oogonium of Achlya sparrowii and temperature. More than 70 percent of the sporangia formed at 15°C were 28.6 - 30.2 µm in diameter whilst the same proportion of oogonia that developed at 25°C were 30.8 - 35.2 µm in diameter.

The effect of hydrogen ion concentration on sporangium formation has been studied in S. parasitica. Lee (1962) obtained no sporangia in cultures at pH 3.5 and 9.0. The greatest number of sporangia were formed at pH 7.0, 620 sporangia per microscope field whilst cultures at pH 4.0 and 8.0 produced 210 and 400 sporangia respectively, in a microscope field. Lee observed beaded hyphal tips in place of normal sporangia at pH's close to pH 4.0 and 8.3.

Cultures of S. parasitica grown in continous light produced twice the number of zoosporangia compared with those grown in complete darkness (Lee, 1962).

Griffin (1966) used an unspecified Achlya species in his investigations on the effect of electrolytes on both sporangium and gemmae formation. Concentration of 0.001 to

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to 1.0 mM of ethylenediaminetetra-acetic acid (EDTA) and low concentrations, 0.001 and 0.01 mM of $CaCl_2$ did not support sporangium formation. The sporangia were formed at higher $CaCl_2$ concentrations of 0.1 to 10.0 mM, with best sporangium development at 0.1 and 1.0 mM.

Barksdale (1962) examined the effect of various carbon compounds on oogonial development in Achlya ambisexualis, but not on sporangium formation. Glucose, D- levulose, maltose and sucrose were the best carbon sources supporting 1625 to 2250 oogonia per microscope field, using a dissecting Microscope. D-arabinose, inositol, lactose, mannitol and raffinose were poor carbon sources. Cultures supplied with these compounds supported less than 156 oogonia per microscope field whilst D-galactose, D-mannose, D-xylose and sodium acetate did not support any oogonium formation. When different nitrogen concentrations' were tested, oogonial density increased with increase in nitrogen concentration from zero to 8 mg per 100 ml of medium, but at higher concentrations of nitrogen, the number of oogonia fell sharply.

Other aspects besides relationship between external environmental factors and sporangium development have been studied by Griffin and Breuker (1969). By treating

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cultures of unspecified Achlya species with Actinomycin D, they were able to elucidate the role of ribonuceleic acid (RNA) in sporangium development. Actinomycin D attaches itself to the side of the DNA molecule where mRNA synthesis takes place and thus prevents this synthesis (Brock, 1970). Actinomycin D inhibited all stages of the sporulation process, differentiation of the hyphal tips into sporangia, cleavage and discharge of zoospores and the emergence of zoospores from the cystospores at the mouths of the sporangia. They interpreted this inhibition as indicating a requirement for DNA - dependent RNA synthesis for these processes. The degree of inhibition of later events in the differentiation process was greater for any specified time of addition of the drug than for earlier events in differentiation. Thus there was a continuing need for RNA synthesis for each stage in the process. This was quite different from the situation observed by Murphy and Lovett (1966) in Blastocladiella emersonii. They found that RNA synthesis ceased during the differentiation of sporangia before the formation of the discharge papillae, so that the processes of papilla formation, spore cleavage and discharge occurred in complete absence of RNA synthesis.

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There are no reports in the relevant literature on the physiology of sporangium formation in _A_. _megasperma_.

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III. MATERIALS AND GENERAL METHODS

Material

The isolate of *A. megasperma* was obtained from a pond at Sohenko farm near the Accra-Aburi Road, about 4 kilometers from the University of Ghana, Legon.

The pure culture obtained after isolation was maintained on hemp seeds throughout the period of this investigation. Fresh cultures were prepared every fortnight and incubated at 22°C.

The fungus was isolated by placing pieces of sterile hemp seeds in pond samples in glass bowls (15 cm diameter 5cm deep) kept on a bench in the laboratory until sporulating mycelia were observed. Approximately, seven days after introduction of the hemp seeds, the fungi were transferred into sterile distilled water in petridishes and examined microscopically. *A. megasperma* was originally selected from among a number of aquatic fungi because of its high frequency. A piece of hemp seed with *A. megasperma* was separated for the preparation of a pure culture. The mycelium was washed in 12 changes of distilled water to remove the masses of protozoa. It was next rinsed in 12 changes of sterile distilled water, to remove as much as possible bacteria growing on the hyphae. For thorough washing, the mycelium was held with a pair of flamed sterile forceps and shaken vigorously in each dish.

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The mycelium was transferred after rinsing in the last change of sterile distilled water into sterile distilled water containing streptomycin and incubated in this medium for 24 hours at room temperature (26°C) after which washing in the sterile distilled water was repeated.

GENERAL METHODS

a) Preparation of stock cultures and Experimental Inoculum.

Both the stock culture and experimental inocula consisted of *A. megasperma* growing on bits of cotyledon of hemp seed. Fairly large pieces of the hemp seed cotyledon, usually one half of a half pair of the cotyledon, were used for the stock culture (Plate 1), but considerably smaller bits, exactly one sixth of a half pair of the cotyledon, were used for raising the experimental inocula in order to reduce to the minimum introduction of other factors beside a particular treatment under investigation. The hemp seed pieces were inoculated by placing 10 pieces in a circle around an established mycelium on hemp seed lying in the centre of a petridish with sterile distilled water (Plate 2). Zoospores readily invaded the baits and hyphae became visible within 24 hours. Each bait was then transferred with a pair of flamed sterile fine forceps into petridishes, 7 cm diameter, containing 20 ml of the test medium.

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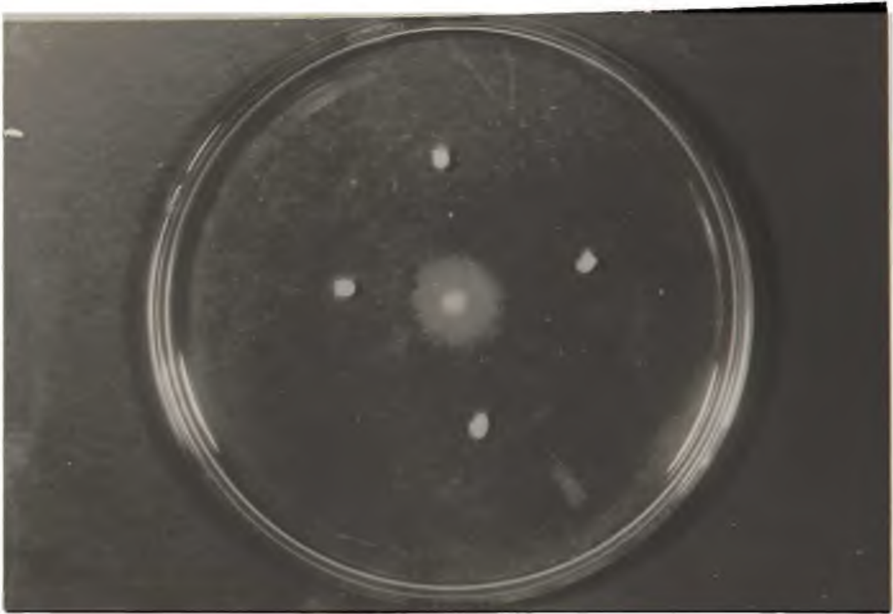

Plate 1: Preparation of Stock Culture.

Photograph showing the arrangement of substratum, fairly large pieces (half pair) of the hemp seed cotyledon, around an established mycelium of A. megasperma in sterile distilled water.

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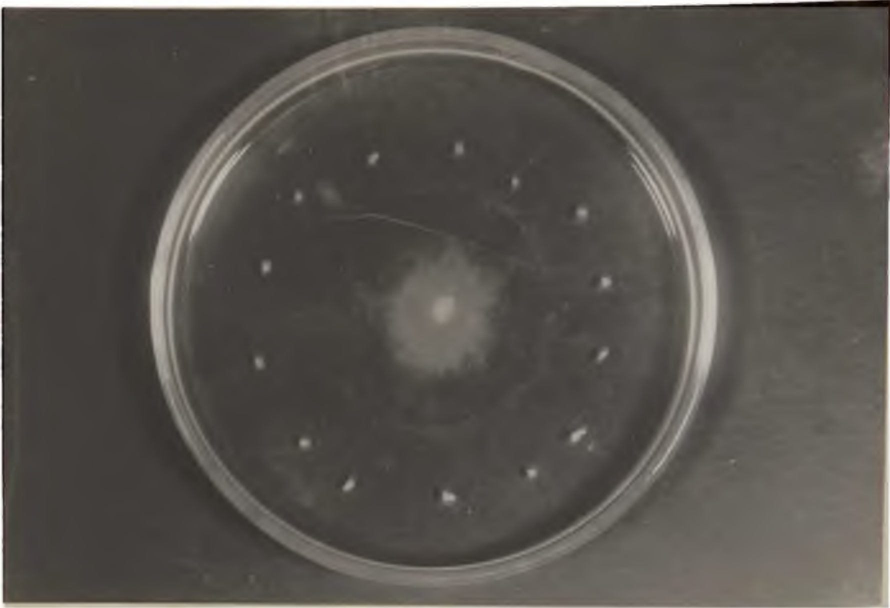

Plate 2. Preparation of Experimental Inoculum.

Photograph showing the arrangement of smaller baits of hemp seed cotyledon (exactly one sixth of a half pair of the cotyledon) around an established mycelium of *A. megasperma* in sterile distilled water.

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Inoculated hemp seeds acting as stock cultures were kept in sterile distilled water.

b) Sporulation tests

All tests were carried out with 7 cm diameter petri dishes, each dish held 20 ml of the test medium. Sporulation was studied throughout in a liquid medium. There were five replicates for each treatment and both the period and temperature of incubation are indicated at appropriate places in the text. At the end of the incubation period, a drop of dilute formalin was added to each petridish as some experiments were extensive and required more than a day to record the results. The cultures were never lifted from the media they were examined and sporulation assessed on cultures retained in the petri dish under low power of the microscope.

c) Incubation

Petri dishes containing experimental cultures were incubated mainly in electrically - controlled incubators and on occasions, as indicated at the relevant places in the thesis, on the laboratory benches.

Petri dishes with cultures were placed in card boxes and the boxes covered with black paper whenever dark conditions were required. Light was provided, where continuous illumination was desired, by day-light fluorescent tubes placed 6 feet above the incubated cultures.

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The light intensity for this particular experiment has been indicated during the description of the experiment in the results.

d.) Assessment of sporangium formation.

By gently removing some of the liquid medium at the end of the incubation with a pipette and during observation of the cultures, it was easy to bring the culture into contact with the bottom of the petri-dish and thereby kept stationary for examination. Common to similar studies on aquatic fungi (Reischer 1949b Zielgler and Gilpin 1954) the number of sporangia in several microscope fields under low power objective was counted and the mean number of sporangia per microscope field calculated. The size of the sporangium was assessed by measuring, with the aid of an eye-piece micrometer, the length and width of 100 sporangia for each treatment.

e.) Determination of pH of culture Media.

The pH of all liquid media was tested with PYE - UNICAM Model 290 pH Meter, after autoclaving. After incubation, the amount of fluid was too small for individual measurements. The five replicates of each treatment was therefore pooled to provide enough fluid for a single measurement.

f.) Culture media.

All chemicals used in the preparation of media were either of the Analar,British Drug House (BDH) or Oxcid grade.

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Composition of the media varied with the experiment and this is described in the appropriate places in the text.

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g.) Buffer Solutions

Mcllvaines buffer solution was used.

(Hale 1966) See TABLE I

Table I

Mcllvaine's Standard Buffer Solutions

Stock solution A : 0.1 M Citric acid ($C_6H_8O_7$).
Stock Solution B : 0.2 M disodium hydrogen Orthophosphate (Na_2HPO_4).

pH	Solution A (Ml)	Solution B (Ml)
2.2	19.60	0.40
3.0	15.89	4.11
4.0	12.29	7.71
5.0	9.70	10.30
6.0	7.37	12.63
7.0	3.53	16.47
8.0	0.55	19.45

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h). Methods of Sterilization

All media in conical flasks, test-tubes and medicinal flats were plugged with non-absorbent cotton wool and heated in an autoclave for normally 15 minutes at 15 pounds per square inch in steam pressure unless otherwise stated.

Dry petri-dishes and pipettes were sterilized by heating at 160°C for 6 hours in an electrically heated oven.

The inoculation room was sterilized by spraying with 5% dettol solution immediately before use.

i). Coliform test.

An estimation of the number of coliform bacilli in the pond water and in experimental media containing fresh cow dung was made using the Multiple Tube Fermentation Technique. They were the Presumptive, Confirmed and Completed Test.

i. Presumptive test.

The test was carried out in a Finhorn fermentation tube comprising of test-tube holding a Durham's tube, and the medium used was lactose broth.

Aliquot of 10 ml lactose broth was dispensed into each of 20 test-tubes containing Durham tubes and then autoclaved. After sterilization, five tubes were each inoculated with 10.0 ml pond water another set of five tubes with 1.0 ml and a third of five tubes with 0.1 ml pond water. Five tubes uninoculated with pond water, served as control.

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They were incubated in the dark at 35°C for 24 and 48 hours. The level of faecal pollution was then assessed. The data on the Most Probable Number are presented in the appropriate chapter in the results.

ii. Confirmed test.

The contents of tubes containing gas in the Durham tubes were streaked on Eosin Methylene Blue agar and incubated at 35°C for 24 hours.

iii. Completed test

Inoculum from colonies of coliform bacilli on the plates of the confirmed test was transferred into lactose broth for 24 hours and the resultant culture streaked onto nutrient agar slants which were incubated at 35°C for 24 hours.

iv. Gram Stain (Hucker's Modification)

The completed test for coliform group organisms included the determination of Gram Stain Characteristics of the organism isolated.

The following procedure was followed in the Gram Stain test using the culture of the nutrient agar. The slide was flooded with Oxalate-crystal violet dye for one minute and washed in water. It was again flooded with Lugol's iodine for one minute. It was then decolorized with 95 per cent ethyl alcohol for 30 seconds and counter-stained with one per cent safranin for 10 seconds. The stain was washed off and the slide air dried.

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Examination was made under oil immersion objective of the microscope.

v. Media for Coliform test

1. Lactose broth (Oxoid)

Thirteen grammes of Oxoid Lactose Broth were dissolved in 1,000 ml of distilled water and 0.5 ml of 0.1 per cent bromothymol blue was added.

2. Eosin Methylene Blue Agar. (E.M.B.A.)

The procedure describedby Cruickshank (1968) was adopted here. The medium summarized below was adjusted to pH 6.8 before autoclaving.

Peptone, 3.0 g; Dipotassium hydrogen phosphate, 0.6 g; Eosin Yellow, 0.4; Methylene Blue, 0.065g; Lactose, 20g; Agar, 13g; Distilled water, 1,000 ml.

3. Nutrient Agar

This consisted of Oxoid Nutrient Agar dissolved in water as shown:

Oxoid Nutrient Agar	28g
Distilled water	1,000 ml

J. Differentiation of the members of the Coliform.

In order to identify the coliform group, vis: E. Coli, Aerobacter aerogenes and Escherichia freundii, the following tests were made: Indole, Methyl Red, Voges-Proskauer and Sodium Citrate Tests.

i. Indole test:

Cultures from E.M.B. Agar were subcultured onto the following Peptone water medium:

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Peptone, 10g; Sodium Chloride, 5g; Distilled water, 1,000 ml; and incubated at 35°C for 48, 72 and 96 hours. After incubation, five to six drops of Kovac's Reagent in one test and Erlich's Reagent in another test were added to each culture. A rose pink colour developed in the presence of Indole. The composition of the Kovac's Reagent was: P - dimethyl - aminobenzaldehyde, 5.0g; Amyl alcohol, 75.0ml; Concentrated Hydrochloric acid, 25ml (Cowan and Steel,1965). The Aldehyde was dissolved in the Alcohol by gently warming in a water bath at 50-55°C. This was allowed to cool before adding the HCl. The reagent was wrapped in black paper and stored in a refrigerator to avoid decomposition.

The Erlich's reagent was prepared as follows: One gramme of P- dimethylaminobenzaldehyde was dissolved in 95ml absolute ethanol. Twenty millilitres of concentraded Hydrochloric acid was then added to the mixture and kept in refrigerator. The container was in this case also wrapped in black paper.

ii. Methyl Red test.

Test tubes, each containing 5ml Glucose phosphate Peptone water medium (Peptone, 5.0g; Glucose, 5.0g; Dipotassium hydrogen phosphate, 5.0; Distilled water 1,000 ml) and the pH adjusted to 7.6 (Cruickshank,1968) were inoculated with cultures from Positive E.M.B. agar plates and incubated at 35°C for 2,3,4 and 5 days respectively.

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Five drops of Methyl Red solution (Methyl red 0.1g Ethanol, 300 ml; Distilled water, 200ml) were added to the contents of each test tube after incubation. A bright red coloration indicated a positive reaction. Indicator colour for negative reaction was yellow.

iii. Voges - Proskauer test:

Aliquot of 5ml Glucose phosphate peptone water (see Methyl Red Test) in test tubes were inoculated with Coliform cultures from E.M.B. agar plates and incubated for 72, 96 and 120 hours, respectively.

Two tests were carried out thereafter using Barritt's reagent in one test and O'Meara's reagent in another. In each case, 5 drops of the reagent were added to the contents of each test-tube. A pink coloration was the expected positive reaction.

Barritt's Reagent was a mixture of 1 ml Potassium hydroxide (40 per cent), and 3 ml of 5 per cent solution of X-naphthol in absolute Ethyl Alcohol. O'Meara's Reagent consisted of Potassium hydroxide (KOH), 40.0g; Creatine, 0.3; and 100 ml distilled water.

iv. Citrate test

Koser's and Simmons' citrate media were inoculated with cultures from positive E.M.B. agar plates and incubated for 48, 72 and 96 hours, respectively.

The Simmons' Citrate agar medium turned blue for positive reaction and green for a negative reaction.

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The Koser's medium remained clear in the event of inability to use citrate and became turbid when the cells used citrate and therefore grew.Koser's Medium was prepared by dissolving 2.5g NaCl, 0.1g Mg $SO_4.7H_2O$, 0.5g ammonium dihydrogen phosphate and 2.5 g. sodium citrate in 500ml distilled water. The pH of the solution was adjusted to 6.8

The Simmons' Medium was prepared by adding 10g of agar and 20 ml of a 1 in 500 aqueous solution of bromothymol blue to 500 ml of Koser's Medium.

k) Physical and Chemical Parameters of Sohenko pond.

i. Sample Collection.

Water sample was collected from the pond in a two litre sterilized bottle with a stopper. The bottle was plunged mouth-downwards into the pond with the stopper still in position and then inverted and held in oblique position when it was fully submerged and allowed to fill by removing the stopper. The filled bottle was pulled out of the pond and stoppered immediately. The sample was taken directly to the laboratory for analysis.

ii. Temperature

The temperature of the pond was measured at each sampling time with an ordinary laboratory thermometer.

iii. pH

The pH of the pond water was measured with PYE-UNICAM Model 290 pH Meter.

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iv. Acidity

The various tests for Acidity, Alkalinity and level of Calcium, Chloride, Copper, Iron, Magnesium, Manganese, Nitrate, Phosphate and Sulphate used the procedures described in 'Standard Methods for the examination of water and Waste Water' (APHA, AWWA and WPCF 1965), and modified by the Institute of Aquatic Biology, C.S.I.R., Accra. For test of acidity, ten millilitres of water sample was titrated against standard 0.IM Na_2CO_3 using phenolphthalein indicator.

v. Alkalinity

One or two drops of methyl orange were added to 10 ml sample and titrated with 0.02N H Cl.

vi. Hardness

The following procedure was adopted to determine Total Hardness.

A few crystals of Eriochrome Black T was added to a mixture of 10ml of pond sample and 0.5 ml buffer solution (Ammonium Chloride, 16.9g; Magnesium salt of E.D.T.A., 1.25 g; Concentrated Ammonium hydroxide, 143 ml; Distilled water, 57 ml). This was titrated with standard 0.IM EDTA until the last trace of purple disappeared and the colour turned a bright blue. The 0.IM E.D.T.A. titrant was standardized with Standard Calcium carbonate solution and the hardness expressed in terms of Calcium carbonate.

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vii. Calcium

Using the EDTA complexometric Method, 10ml of the sample was pipetted into a test tube and 0.5ml of 1N NaOH solution added. A few grains of Murexide indicator (ammonium purpurate 0.2g; Sodium Chloride, 100g;) was added. The mixture was titrated with 0.02N EDTA solution until the colour changed from Salmon to orchid purple.

viii. Chloride

The Argentometric Method was used. One or two drops of Potassium chromate indicator solution was added to a 10ml pond sample. This was then titrated with 0.0141 N standard silver nitrate solution to a pinkish yellow end-point. The silver nitrate was standardized with distilled water (blank) and blank value determined.

ix. Copper

Copper was pre-concentrated by passing 2 litres of water sample down a 10 inch Chelex - 100 resin in a 25ml burette, and then eluted with 25ml of 2N HNO_3. The eluate was then analysed in an Atomic Absorption Spectrophotometer Perkia Elymer Model 303 using wave length 325 mu

x. Iron

Iron content was determined according to the Potassium thiocyanate Method.

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A series of concentrations in p.p.m. of standard iron was prepared in test-tubes from pure metal for the construction of the Photometric Calibration curve, after they have been appropriately treated.

Each solution was acidified by adding 0.5ml 50:50HCl solution and allowed to stand for 5 minutes. One or two drops of Potassium cyanate and 15ml of H_2O_2 were added. The concentration of iron in each was determined photometrically with Unicam Model S P 500 series 2 Ultra Violet and Visible Spectrophotometer using wavelength 510 mu.

Pond water and distilled water (blank) were similarly treated and their absorbance read. The level of iron was then estimated from the Photometric Calibration Curve provided by the standard.

This Spectrophotometer was used in all subsequent tests for estimation of absorbance.

xi. Magnesium

The level of Magnesium was calculated using the formula below.

$$Mg\ mg/l = 0.243\ (A - B)$$

where A = EDTA hardness expressed in terms of $CaCO_3$

B = Calcium hardness by EDTA Titrametric Method expressed in terms of mg/litre of $CaCO_3$.

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xii. Manganese

Using the Persulphate Method for the determination of Manganese, one gramme of ammonium persulphate and five millilitres of the special reagent (Mercuric Sulphate, 75g; was dissolved in 400 ml of Concentrated HNO_3 and 200ml distilled water; 200ml 85 per cent phosphoric acid and 0.035 grammes silver nitrate were added. The mixture was cooled and diluted to 1,000ml) were added to 90ml of the pond sample, boiled for one minute and then cooled for another one minute under the tap. This was diluted to 100ml with distilled water and the absorbance read at 525 mμ. The absorbance standards containing 0.005, 0.01 to 1.5 mg Manganese prepared by treating various amounts of standard Manganese solution in the same way was also read and the photometric calibration curve constructed.

The interfering colour or turbidity in the sample was corrected by mixing 0.5ml of 30 per cent Hydrogen Peroxide solution directly with the sample in the optical cell and repeating the absorbance measurements on the decolorized solution. The interference value was substracted from the actual Manganese value.

xiii. Nitrate

In the Phenoldisulphide Acid Method used, 10ml of the pond sample, distilled water and standard nitrate solutions of different concentrations were pipetted into separate

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beakers. The Standards, pond sample and distilled water (blank) were then evaporated to dryness over water bath. Half a millilitre of Phenoldisulphonic acid reagent (Phenol, 25g; concentrated H_2SO_4, 150 ml; fuming H_2SO_4 (15% free SO_3), 75ml) was added to each residue to dissolve it. To each beaker was finally added 10ml distilled water and 2ml Concentrated Ammonium hydroxide. The absorbance of each Solution was measured at 480 mu.

xiv. Phosphate

The phosphate concentration was determined by the stannous chloride method. The pond sample was first centrifuged to obtain a clear sample for the test. Each test tube during this test contained 10ml of either clarified pond sample, distilled water (blank) or solutions of Phosphate Standard (prepared with KH_2PO_4): One or two drops of stannous chloride reagent (fresh $SnCl_2$ $2H_2O$, 2.5g; glycerol, 100ml) and 0.5 ml Ammonium Molybdate reagent (a mixture of 25g $(NH_4)_6Mo_7O_{24}4H_2O$, and 280ml Concentrated H_2SO_4, was cautiously added to 400ml distilled water, cooled and finally made up to 1,000 ml with distilled water) were added to the content of each test tube. The absorbance of each was determined at 560 mμ within ten minutes.

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xv. Sulphate

Test tubes held 10ml of either pond water, distilled water (blank) or solutions of standard of Na_2SO_4 and to each was added 2ml of acid salt reagent (sodium chloride, 240g; distilled water containing 20ml concentrated Hydrochloric acid, made up to 1 litre). A few drops of 50 per cent Glycerol and a spoonful of $BaCl_2 . 2H_2O$ were added and the contents of the test tube thoroughly stirred for exactly one minute. This was allowed to stand for 5 minutes before finally adding Barium chloride crystals. The absorbance was read at 420 mμ.

l) Statistical Analysis.

Experimental results were statistically analysed where necessary.

m) Experimental Precaution

i. Glassware was kept scrupulously clean. They were rinsed twice in tap water after washing with detergents and a third time with distilled water and allowed to dry before use.

ii. After the establishment of the optimum temperature for sporangium formation one incubator was kept exclusively for this investigation. There was, therefore, absolutely no disturbance of the cultures and the hyphae always grew out radially from the hemp seed and free from entanglement which could result on violent

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shaking of the culture.

iii. The concentration of the formalin was arrived at after careful preliminary trial, as higher concentrations caused shrinkage of the mycelium and sporangium which made measurements of the sporangia impossible or inaccurate.

iv. The sporangia are normally formed at the apices of the hyphae. Branching was never profuse and the apices of the sparsely branching hyphae growing out from the hemp seed tissue formed an easily recognisable perimeter of sporulating zone. Counting of the sporangia formed in each treatment was always restricted to this zone of densest sporulation to provide a true response of the fungus to the particular treatment.

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IV. RESULTS

A. EFFECT OF TEMPERATURE ON SPORANGIAL FORMATION.

Temperature is one of the most important environmental factors which influence the abundance, speed and sometimes the morphology of the reproductive apparatus. In general, the rate of sporulation is greatest at the optimum temperature and decreases as the temperature is moved away from the optimum.

In the present investigation, it was considered essential to find the most favourable temperature for growth and sporangial formation of. *A. megasperma* to be used in the subsequent physiological investigations.

Very young cultures on pieces of hemp seeds exactly 48 hours old were transferred into sterile distilled water in petri dishes and incubated at 20°, 25°, 30°, 35° and 40°C, respectively, for 10 days.

This range of temperature was chosen to cover the possible range of temperatures the organism might be subjected to in the tropics. Readings were made according to the procedure outlined under Materials and General Methods, to assess the extent of sporangium development at each temperature level.

The length and width of the sporangia were also

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measured and the extent of vegetative growth was noted, qualitatively. The results are presented in Tables 2 to 5 and in Fig.1 to 3. The class distribution of length of the sporangia is illustrated in Fig.3. Variations in size of the sporangia observed are illustrated by means of camera lucida drawings in Fig.4.

The results show that the best vegetative growth and the greatest production of sporangia occurred at 30°C where the mean number of sporangia formed per microscope field after 10 days was 18 as compared to 12 sporangia at 25° and 35°C. The largest sporangia, were also formed at 30°C. The mean length of the sporangia was 382.8 um whilst very short sporangia, 232.3 and 274.6 µm, respectively, were produced at 20° and 35°C. <u>A</u>. <u>megasperma</u> did not grow at 40°C.

The optimum temperature from this study, 30°C, was therefore adopted for the subsequent experiments.

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TABLE 2

Effect of temperature on growth and Sporangial production in *A. megasperma* incubated at different temperatures for 10 days

Temperature (°C)	Vegetative growth	Sporangial Production
20	Slight	Scanty
25	Moderately Abundant	Moderate
30	Abundant	Abundant
35	Moderate	Moderate
40	None	None

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TABLE 3

Formation of Sporangia of A. megasperma incubated at different temperatures for 10 days.

Temperature (°C)	Number of Sporangia observed in 100 microscope fields after indicated days of incubation.			
	5		10	
	Total	Mean per field	Total	Mean per field
20	174	2	531	5
25	673	7	1237	12
30	1132	11	1820	18
35	860	9	1193	12
40	-	-	-	-

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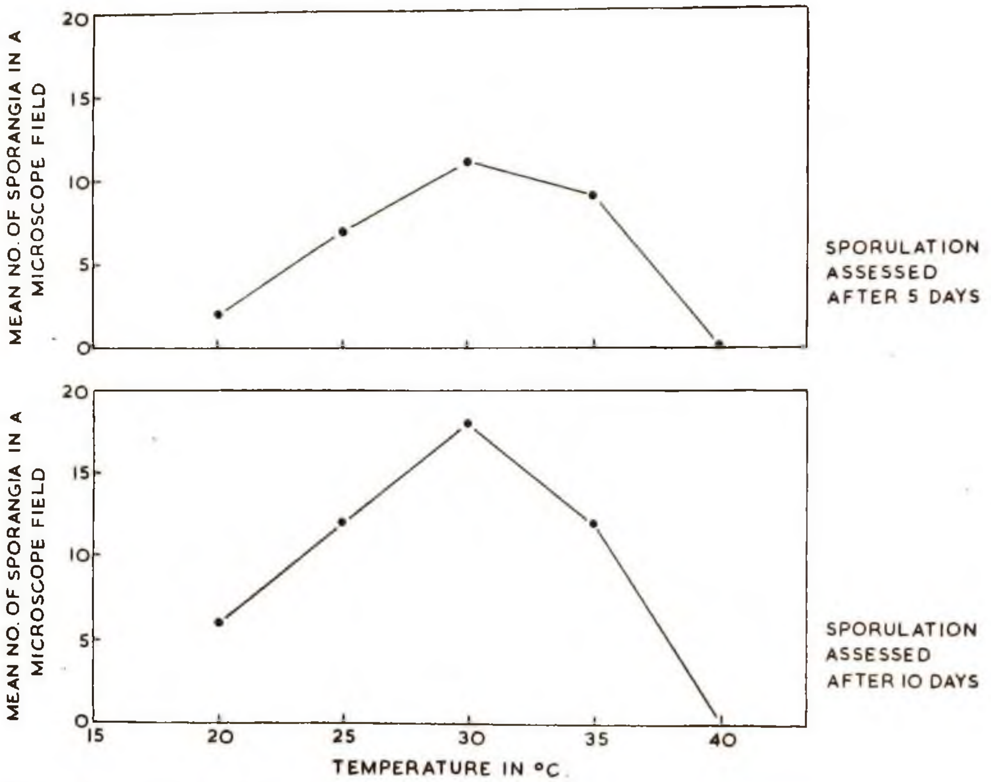

FIG. I DEVELOPMENT OF SPORANGIA OF *ACHYLA MEGASPERMA* INCUBATED AT DIFFERENT TEMPERATURES FOR TEN DAYS.

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TABLE 4

Dimensions of sporangia of *A. megasperma* formed at different temperatures for 10 days.

Temperature (°C)	Mean length of 100 Sporangia (μm)	Mean width of 100 Sporangia (μm)
20	232.3 ± 10.6	20.3 ± 0.03
25	306.2 ± 13.5	21.6 ± 0.05
30	382.8 ± 9.3	23.2 ± 0.05
35	274.6 ± 10.6	21.9 ± 0.06
40	-	-

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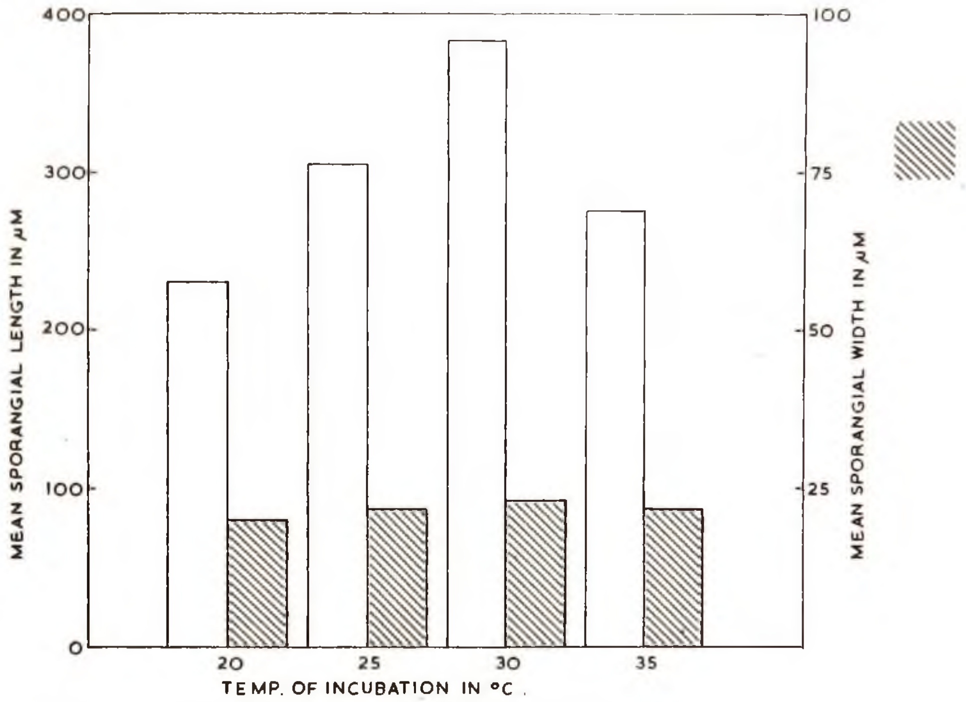

FIG. 2 DIMENSION OF SPORANGIA OF *ACHYLA MEGASPERMA* FORMED AT DIFFERENT TEMPERATURES FOR 10 DAYS

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TABLE 5

Percentage frequency of class length of sporangia of *A. megasperma* incubated at different temperatures for 10 days.

Class Length	Percentage frequency at different temperatures (°C)				
	20	25	30	35	40
125-205	48	16	4	26	-
205-285	24	22	16	20	-
285-365	26	34	36	44	-
365-445	2	22	32	10	-
445-525	-	6	4	-	-
525-605	-	-	8	-	-

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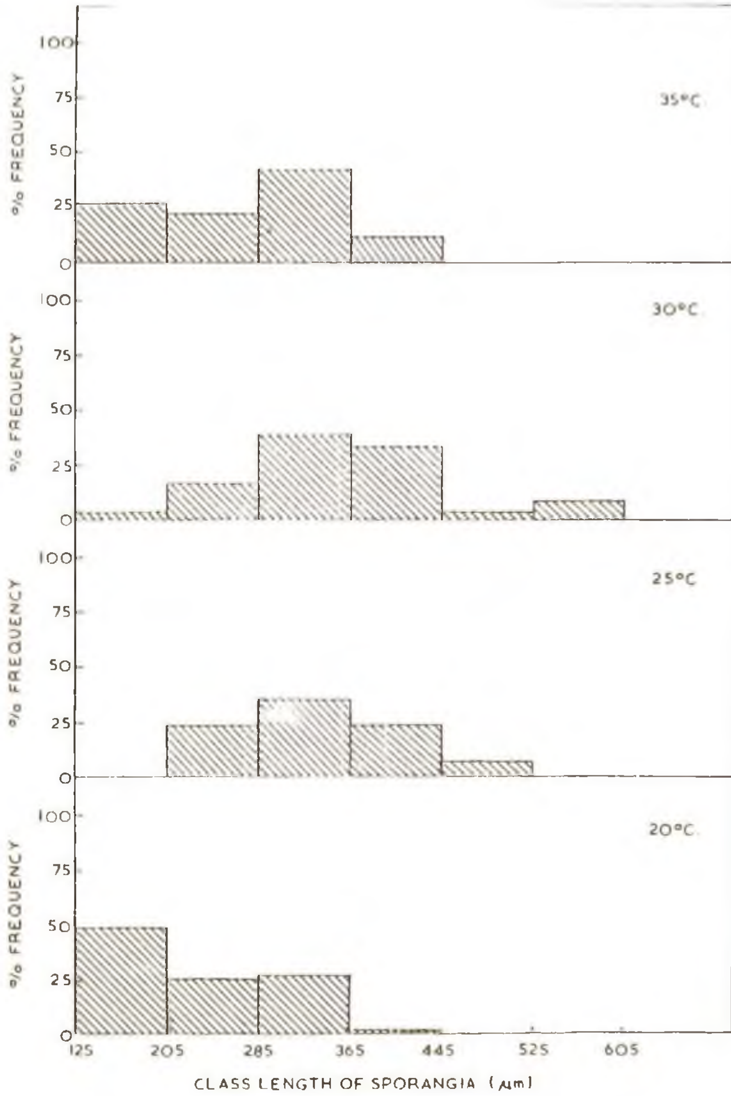

FIG. 3 CLASS DISTRIBUTION OF LENGTH OF SPORANGIA OF *ACHLYA MEGASPERMA* FORMED AT DIFFERENT TEMPERATURES FOR 10 DAYS

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FIG. 4 CAMERA LUCIDA DRAWINGS OF SPORANGIA OF A. MEGASPERMA INCUBATED AT DIFFERENT TEMPERATURES FOR 10 DAYS
NOTE VARIATION IN SIZE OF SPORANGIA AT DIFFERENT TEMPERATURES

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B. EFFECT OF LIGHT ON SPORANGIAL FORMATION

During the previous investigation, the cultures were incubated under different light conditions. Some were incubated in air-conditioned rooms (20°C) and therefore exposed to light during the day whilst the rest were kept in incubators and practically in darkness throughout the incubation period.

Light is a potent factor in the reproduction of fungi, and there are instances of its effect on both rate of development of the reproductive structures and their morphology. The pertinent literature has been excellently reviewed by Carlile (1965).

An examination of the effect of light on sporangium formation in _A. mesgasperma_ was judged necessary to find out how far the different light conditions in the preceding experiment might have affected the results obtained.

Twenty petri-dishes containing sterile distilled water were inoculated and incubated at room temperature (26°C) for 5 and 10 days, respectively. Ten plates were wrapped in black paper and thus kept in continuous darkness and the rest placed on the tables in the research laboratory and exposed to flourescent light of 250 ft.c. The number of sporangia formed were counted after 5 and 10 days incubation.

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The data in Table 6 show that cultures grown in constant light and darkness sporulated to the same extent. Cultures kept in darkness produced an average of 9 and 16 sporangia after 5 and 10 days incubation, respectively, and those in constant light produced 9 and 15 sporangia, respectively, within the same periods.

Apparently sporangium formation is independent of light.

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TABLE 6

Formation of sporangia by *A. megasperma* in light and in darkness at 26°C.

Time of Incubation (Days)	Treatment	No of sporangia observed in 100 microscope field		Mean length of 100 Sporangia (μm)
		Total	Mean	
5	Light (250 ft.c.)	889	9	325.1 ± 10.5
	Dark	913	9	348.3 ± 12.6
10	Light (250 ft.c.)	1528	15	357.5 ± 12.9
	Dark	1594	16	361.2 ± 14.2

C. EFFECT OF pH ON GROWTH AND SPORANGIAL FORMATION

The hydrogen ion concentration of the environment is one of the most important external factors influencing growth and reproduction of fungi. It is in fact very critical in the ecological distribution of fungi (Wilkins, Harley and Kent, 1938; Dick, 1963). If A. megasperma possesses a restricted range of pH for growth and particularly for sporangium formation, it would be a very useful test organism.

Whilst investigating the relationship between sporangium formation in A. megasperma and pH, it was borne in mind that the results could be altered by other factors such as temperature (Person, Olson and Martin 1948) growth factor supply (Lilly and Barnett, 19[illegible]7) Calcium and Magnesium levels (Venning and Crandall 1954) and nitrogen source (How, 1940).

a. Experiment using McIlvaine's Buffer Solutions.

McIlvaine's buffer solutions (see Table 1) were used as the growth medium into which were placed 48 hour-old cultures of A. megasperma on pieces of hemp seeds. The cultures were incubated at 30°C for 10 days. The hyphae surprisingly disintegrated in all the buffer solutions.

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b. Experiment using distilled water adjusted with Hydrochloric acid and Sodium hydroxide.

A new experiment on pH- sporulation relationship, using distilled water adjusted to different pH levels with 0.5 N HCl and 0.5N NaOH was set up, following the destruction of the hyphae by McIlvaine's buffer solutions. It was presumed that destruction of the hyphae was due to the effect of the components of the buffer *per se* rather than to the pH of the medium since growth and sporulation occurred in distilled water at a pH (pH 6.4) included in the range provided by the buffer solutions.

Distilled water was adjusted with 0.5 N HCl and 0.5N NaOH to give the following pH levels pH 3, 4, 5, 6, 7, 8, 9 and 10. The pH was determined accurately with a pH meter before autoclaving, and at the end of the growth period.

The results are presented in Tables 7 to 9 and in Fig.5.

The results show that there was no growth or sporulation at pH 3 and 10.

There was abundant vegetative growth at pH 5 to 8 and moderate growth at pH 4 and 9. The optimum pH for sporangium formation was clearly pH 9. There was a mean of 21 sporangia in a microscope field compared to the lowest record of 8 at pH 5. Although the fungus grew at

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TABLE 7

Effect of pH of medium adjusted with 0.5N HCl and 0.5N NaOH on growth and sporangial formation in *A. megasperma* incubated in dark at 30°C for 10 days.

pH		Vegetative growth	Sporangial production
Initial	Final		
3.0	3.0	-	-
4.0	3.7	Moderate	-
5.0	6.8	Moderate	Moderate
6.0	6.5	Moderate	Moderate
7.0	6.7	Abundant	Abundant
8.0	7.2	Very Abundant	Abundant
9.0	7.5	Moderately abundant	Very abundant
10.0	9.6	-	-

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pH 4 the sporangia produced were non-viable. The sporangium did not develop any further following the laying down of the separating septum.

Initial pH's of 6, 7, 8 and 9 produced sporangia more than 300 um long: 310.2, 319.4, 355.1 and 322.1 µm, respectively. The sporangium was considerably shorter, 255.7 µm, at pH 5.0.

The drift of the pH of the growth medium was not in the same direction for all cultures. Whilst the initial pH 5 and 6 moved slightly to alkaline side, pH 6.8 and 6.5, respectively, the pH's 7, 8, 9 and 10 drifted to the acidic side, with the greatest drift at pH 9 to a final pH 7.5.

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TABLE 8

Effect of pH of medium adjusted with 0.5N HCl and 0.5N NaOH on the formation of sporangial by A. megasperma incubated in dark at 30°C for 10 days.

pH		Number of Sporangia observed in 100 microscope fields	
Initial	Final	Total	Mean
3.0	3.0	-	-
4.0	3.7	-	
5.0	6.8	831	8
6.0	6.5	1116	11
7.0	6.7	1689	17
8.0	7.2	1644	16
9.0	7.5	2119	21
10.0	9.6	-	-

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TABLE 9

Dimensions of sporangia of *A. megasperma* formed in dark at different pH's for 10 days at 30°C

pH		Mean length of 100 sporangia (µm)
Initial	Final	
3.0	3.0	-
4.0	3.7	268.0 ± 6.9*
5.0	6.8	255.7 ± 13.6
6.0	6.5	310.2 ± 9.2
7.0	6.7	319.4 ± 11.9
8.0	7.2	355.1 ± 12.0
9.0	7.1	322.1 ± 13.9
10.0	9.4	-

* Inviable sporangium

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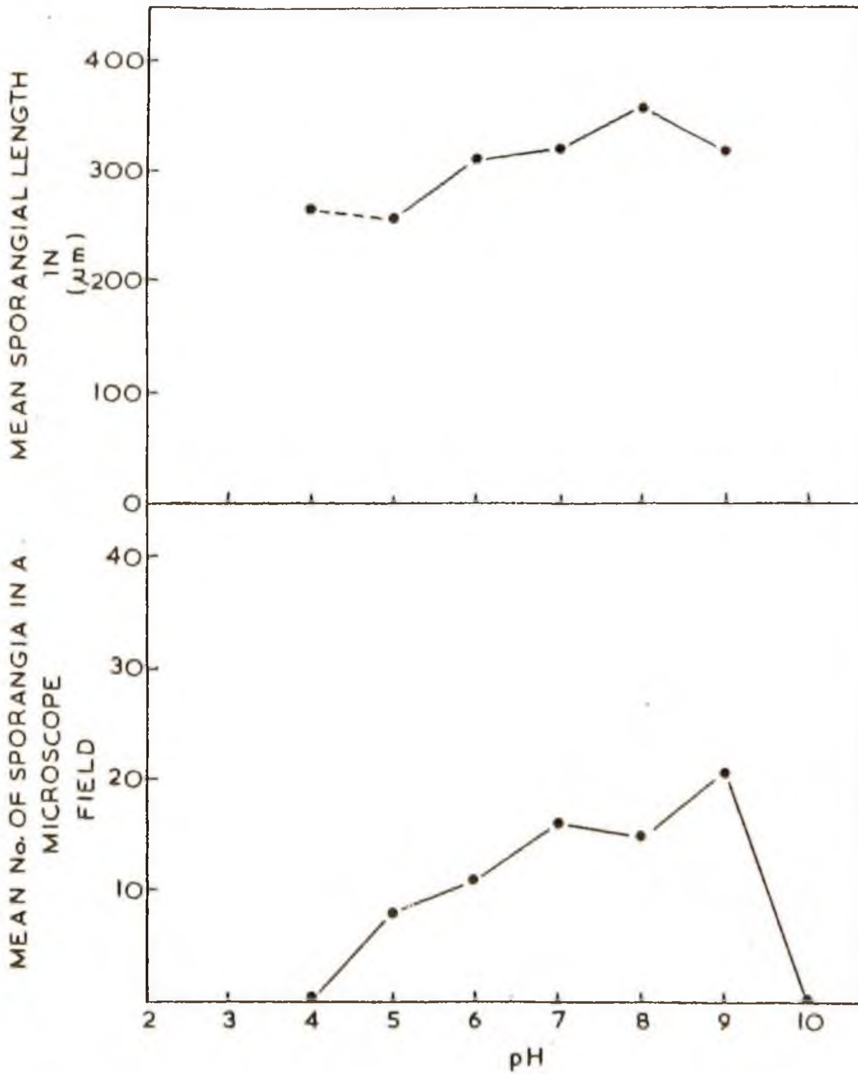

FIG. 5 . EFFECT OF pH ON THE DEVELOPING OF SPORANGIA OF *ACHLYA MEGASPERMA* INCUBATED AT 30°C FOR 10 DAYS

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D. PHYSICAL AND CHEMICAL PARAMETERS OF SOHENKO POND

The physical and chemical characteristics of fresh water bodies are major features that influence life in these environments and their levels also determine the healthiness of the water. The physical and chemical parameters are always measured in any study related to the healthiness of fresh water bodies, generally by very laborious methods.

Major ions in natural waters may vary in concentrations even when uncontaminated by products of human activities from a fraction of a milligram to several thousand milligrams per litre whilst the range of minor elements is for most parts a tenth of a microgram per litre.

A megasperma naturally tolerates the levels of the different ions in the Sohenko pond. After these levels had been determined the ability of the fungus to withstand higher concentrations would be investigated. This chapter contains report on the investigations carried out to provide the physical and chemical parameters of the Sohenko pond.

The various methods used have been fully described at pages 35 to 40.

The following parameters were determined and the results

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tabulated in Tables 10 and 11.

a. Temperature

b. Hydrogen ion concentration

c. Acidity, as mg/l of $CaCO_3$ $= \frac{A \times N \times 50{,}000}{\text{Milli litres of sample}}$

where A=Millilitres of Na_2CO_3

N=Normality of Na_2CO_3.

d. Alkalinity, as mg/l of $CaCO_3$ $= \frac{A \times N \times 50{,}000}{\text{Millilitres of sample}}$

where A=Millilitres of titrant HCl

N=Normality of HCl titrant

e. Calcium = Volume of 0.01M EDTA titrant x 40

f. Chloride: mg/l Cl $= (A-B)\frac{N \times 35{,}450}{\text{Millilitres of sample}}$

where A=Millilitre titration for sample

B=Millilitre titration for blank

N=Normality of $AgNO_3$.

g. Copper: mg/l Cu $= \frac{Cu^{++}\ \text{ppm} \times V \times \text{Dilfactor}}{10^3}$

h. Ferric: mg/l Fe $= \frac{\text{mg } Fe^{+++} \times 1{,}000}{\text{Millilitre of sample}}$

i. Magnesium: mg/l $= 0.243(A-B) \times 100$

where A= EDTA hardness expressed in terms of $CaCO_3$.

B= Calcium hardness by EDTA titrametric method expressed in terms of Mg/l $CaCO_3$.

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j. Manganese: mg/l Mn $= \frac{\text{mg Mn} \times 1{,}000}{\text{Millilitre of sample}}$

k. Nitrate: mg/l N $= \frac{\text{mg nitrate N} \times 1{,}000}{\text{Millilitre of sample}}$

l. Phosphate: mg/l PO_4 $= \frac{\text{mg } PO_4 \times 1{,}000}{\text{Millilitre of sample}}$

m. Sulphate: mg/l SO_4 $= \frac{\text{mg } SO_4 \times 1{,}000}{\text{Millilitre of sample}}$

The temperature was taken in the field any time a sample was collected. The series of temperature and pH measurements of the pond are shown in Table 10.

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TABLE 10

Temperature and pH of Sohenko pond during collection of sample for chemical analysis.

Date (Sample Collected)	Temperature (°C)	pH
3rd Sept. 1974	27.0	7.87
24th Sept. 1974	26.5	7.35
9th Oct. 1974	27.0	7.50

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TABLE II

Concentration of some ions in Sohenko pond.

Ions	Concentration mg/1
Acidity	35.0
Alkalinity	25.0
Ca^{++}	14.0
Cl^{-}	60.0
Cu^{++}	.022
Fe^{+++}	0.0
K^{+}	24.0
Mg^{++}	1.3
Mn^{++}	1.5
Na^{+}	47.0
NO_3^{-}	99.2
N_2 (Ammonia)	41.0
PO_4^{-}	46.5

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The ions occurred in very varying concentrations whilst no iron was detected, other ions, for example, Chloride (60 . mg/l) and Nitrate (99.2mg/l) were present in very high concentrations. Magnesium and Manganese on the other hand, occurred in very low concentrations, 1.3 and 1.5 mg/l litre, respectively.

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E. EFFECT OF MINERAL SALTS ON GROWTH AND SPORULATION

Mineral salt requirements vary from species to species but generally some ions are commonly toxic to most water moulds and fungi in general and they affect various aspects of fungal life. For example, Fe^{+++}, Cu^{++} and Zn^{++} at concentrations just above II.0 p.p. m were found to be toxic to Allomyces (Ingraham and Emerson 1954). Copper sulphate and Ferric chloride at the extremely low concentrations of 0.00IM completely inhibited germination of conidia of Cercospora canescens (Teyegaga, 1970) and the zoospores of Phytophthora palmivora disintegrated instantly in 1.0 mM solution of Copper Sulphate (Bimpong and Clerk, 1970).

The observation at pages 55 to 56 that the levels of Citric acid and Na_2HPO_4 in McIlvaines Buffer solution, inhibited the growth and sporulation of A. Megasperma suggested that the fungus could be particularly sensitive to ions. This belief was found to be accurate during studies on the effect of various mineral salts on growth and sporulation. Citric acid, an organic compound, which was a component of McIlvaine's Buffer solution, was added to the list.

Forty-eight-hour cultures of A. megasperma on hemp seeds were transferred into aqueous solutions of Calcium

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chloride ($CaCl_2$), Citric acid, Copper Sulphate ($CuSO_4.5H_2O$), Ferric Chloride ($FeCl_3$), Magnesium Sulphate ($MgSO_4.7H_2O$), Manganese Sulphate ($MnSO_4.4H_2O$), di-potassium hydrogen phosphate (K_2HPO_4), Sodium Chloride ($NaCl$), di-ammonium hydrogen phosphate ($(NH_4)_2HPO_4$) and Zinc Chloride ($Zn\ Cl_2$) each at concentrations of 1×10^{-7}, 1×10^{-5}, 1×10^{-3}, 0.1 and 0.5 M and incubated at 30°C for 10 days, after which growth and extent of sporulation were assessed. The pH of the media was measured immediately after autoclaving and at the end of the incubation period. The results are indicated in Tables 12 to 21 and in Figs .6, 7 and 8.

The data in Tables 12 to 21 and in Fig.6 show that A. megasperma was very sensitive to the mineral salts used. Concentrations of all salts at 0.1 and 0.5 M inhibited growth and sporangial production with the exception of $CaCl_2$ (Table 12) and $MgSO_4.7H_2O$ (Table 17) which allowed the low production of only one sporangium per microscope field at 0.1M concentration. The hyphae of the inocula disintegrated in all the Citric acid solutions.

The effect of the lower concentrations of the salts was variable. The average number of 13 - 16 sporangia per microscope field in distilled water was improved only

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by 1 x 10^{-7} and 1 x 10^{-5}M $CaCl_2$(Table 12), 1 x 10^{-5}M K_2 HPO_4 (Table 16) and 1 x 10^{-7}, 1 x 10^{-5} and 1 x10^{-3} M $MgSO_4.7H_2O$ (Table 17). Sporulation in all remaining cultures that produced sporangia was inferior to that in water.

Sporulation occurred at all three concentrations (1 x10^{-7}, 1 x 10^{-5} and 1 x 10^{-3}M) in solutions of $CaCl_2$ (Table 12), K_2HPO_4 (Table 16), Mg $SO_4.7H_2O$ (Table 17), NaCl (Table 19) and $(NH_4)_2HPO_4$ (Table 20). whilst sporangia were produced at the two lowest concentrations (1 x10^{-7} and 1 x10^{-5}M) in solutions of $FeCl_3$ (Table 15) and $MnSO_4.4H_2O$ (Table 18), and at the lowest concentration only in $CuSO_4$ (Table 14) and $ZnCl_2$ (Table 21).

The size of the sporangia was also affected by the salts. No treatment induced a significantly larger sporangium than distilled water. The size of the sporangia was, however, extensively reduced at certain concentrations of some of the salts. The mean sporangium length of cultures in distilled water ranged from 302.1 to 377.9 um. Conspicuosly smaller sporangia were produced at 1 x 10^{-3}M $CaCl_2$ and 0.1M $CaCl_2$ (Table 12), 1 x 10^{-5}M $FeCl_3$(Table 15), 1 x10^{-5}M $MnSO_4.4H_2O$ (Table 18) and 1 x 10^{-7}M $ZnCl_2$ (Table 21) where the mean lengths of

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TABLE 12

Effect of $CaCl_2$ on growth and sporangia production in *A. megasperma* incubated at 30°C for 10 days.

Concentration (M)	pH		No of sporangial observed in 100 microscope fields		Mean length of 100 sporangia (μm)
	Initial	Final	Total	Mean	
0.00	6.4	8.1	1268	13	302.1 ± 7.9
$1x10^{-7}$	7.1	7.8	1850	19	294.1 ± 10.2
$1x10^{-5}$	6.8	7.3	2083	21	312.5 ± 12.0
$1x10^{-3}$	6.6	7.5	715	7	221.3 ± 10.5
0.1	6.3	7.8	105	1	175.8 ± 6.4
0.5	6.2	6.0	-	-	-

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TABLE 13

Effect of Citric acid on growth and sporangial formation in *A. megasperma* incubated at 30°C for 10 days.

Concentration (M)	pH		No.of sporangial observed in 100 microscope fields		Mean length of 100sporangia (µm)
	Initial	Final	Total	Mean	
0.00	6.4	8.1	1429	14	315.3± 9.3
$1x10^{-7}$	6.5	5.2	-	-	-
$1x10^{-5}$	5.2	5.1	-	-	-
$1x10^{-3}$	3.5	3.3	-	-	-
0.1	2.4	2.2	-	-	-
0.5	2.0	1.8	-	-	-

0.00 : Sterile distilled water.

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TABLE 14

Effect of $CuSO_4 \cdot 5H_2O$ on growth and sporangial formation in *A. megasperma* incubated at 30°C for 10 days.

Concentration (M)	pH		No.of sporangia observed in 100 microscope fields		Mean length of 100 sporangia (μm)
	Initial	Final	Total	Mean	
0.00	6.4	8.1	1394	14	321.3±10.5
$1x10^{-7}$	6.7	6.8	256	2	279.4± 9.8
$1x10^{-5}$	6.4	6.9	-	-	-
$1x10^{-3}$	5.5	5.6	-	-	-
0.1	3.7	3.9	-	-	-
0.5	3.1	3.3	-	-	-

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TABLE 15

Effect of $FeCl_3$ on growth and sporangial formation in A. megasperma incubated at 30°C for 10 days.

Concentration (M)	pH		No.of sporangia observed in 100 microscope fields		Mean length of 100 sporangia (µm)
	Initial	Final	Total	Mean	
0.00	6.4	8.1	1254	13	319.9±10.6
$1x10^{-7}$	6.6	6.8	1164	12	320.5±11.8
$1x10^{-5}$	6.3	6.5	795	8	252.9± 7.2
$1x10^{-3}$	6.3	6.2	-	-	-
0.1	1.5	1.6	-	-	-
0.5	1.2	1.2	-	-	-

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TABLE 16

Effect of K_2HPO_4 on growth and sporangial formation in *A. megasperma* incubated at 30°C for 10 days

Concentration (M)	pH		No.of sporangia observed in 100 microscope fields		Mean length of 100 sporangia (μm)
	Initial	Final	Total	Mean	
0.00	6.4	8.1	1262	13	333.2±7.3
$1x10^{-7}$	6.3	6.9	1199	12	298.8±7.8
$1x10^{-5}$	6.9	7.2	1733	17	305.9±13.5
$1x10^{-3}$	7.8	7.6	1101	11	378.3±10.5
0.1	8.9	8.3	-	-	-
0.5	9.2	8.5	-	-	-

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TABLE 17

Effect of $MgSO_4.7H_2O$ on growth and sporangial formation in *A. megasperma* incubated at 30°C for 10 days.

Concentration (M)	pH		No. of sporangia observed in 100 microscope fields		Mean length of 100 sporangia (μm)
	Initial	Final	Total	Mean	
0.00	6.4	8.1	1600	16	377.9± 9.8
$1x10^{-7}$	6.3	7.7	1801	18	382.6±11.5
$1x10^{-5}$	6.4	7.5	1921	19	360.4±12.5
$1x10^{-3}$	6.6	8.2	2445	24	383.6±10.2
0.1	4.7	6.3	87	1	287.6± 9.1
0.5	4.2	7.4	-	-	-

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TABLE 18

Effect of $MnSO_4.4H_2O$ on growth and sporangial formation in *A. megasperma* incubated at 30°C for 10 days.

Concentration (M)	pH		No.of Sporangia observed in 100 microscope field		Mean length of 100 sporangia (µm)
	Initial	Final	Total	Mean	
0.00	6.4	8.1	1633	16	361.7 ± 12.5
$1x10^{-7}$	6.7	6.8	889	9	274.0 ± 7.9
$1x10^{-5}$	6.6	7.5	803	8	208.6 ± 8.3
$1x10^{-3}$	6.4	6.2	-	-	-
0.1	5.9	6.2	-	-	-
0.5	5.2	5.7	-	-	-

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TABLE 19

Effect of NaCl on growth and sporangial formation in A. megasperma incubated at 30°C for 10 days.

Concentration (M)	pH		No. of Sporangia observed in 100 microscope fields		Mean length of 100 sporangia (µm)
	Initial	Final	Total	Mean	
0.00	6.4	8.1	1569	16	353.8 ± 12.8
$1x10^{-7}$	6.4	6.6	1267	13	354.0 ± 13.5
$1x10^{-5}$	6.4	7.9	1457	15	356.1 ± 11.2
$1x10^{-3}$	6.3	6.7	1431	14	330.7 ± 12.4
0.1	6.1	6.4	-	-	-
0.5	5.8	6.1	-	-	-

TABLE 20

Effect of $(NH_4)_2\ HPO_4$ on growth and sporangial formation in _A. megasperma_ incubated at 36°C for 10 days.

Concentration (M)	pH		No of sporangia observed in 100 microscope fields		Mean length of 100 Sporangia
	Initial	Final	Total	Mean	
0.00	6.4	8.1	1321	13	374.5 ± 10.8
$1x10^{-7}$	8.6	7.9	1128	11	338.1 ± 12.2
$1x10^{-5}$	8.5	7.8	1169	12	378.8 ± 10.5
$1x10^{-3}$	8.0	7.4	102	1	308.9 ± 11.7
0.1	7.4	7.3	-	-	-
0.5	7.0	7.1	-	-	-

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TABLE 21

Effect of Zn Cl_2 on growth and sporangial formation in *A. mesgasperma* incubated at 30°C for 10 days.

Concentration (M)	pH		No. of sporangia observed in 100 microscope fields		Mean length of 100 sporangia (µm)
	Initial	Final	Total	Mean	
0.00	6.4	8.1	1477	15	349.1 ± 10.5
$1x10^{-7}$	6.5	6.9	348	3	245.0 ± 11.8
$1x10^{-5}$	6.5	7.2	-	-	-
$1x10^{-3}$	6.1	7.6	-	-	-
0.1	6.1	8.3	-	-	-
0.5	5.2	8.5	-	-	-

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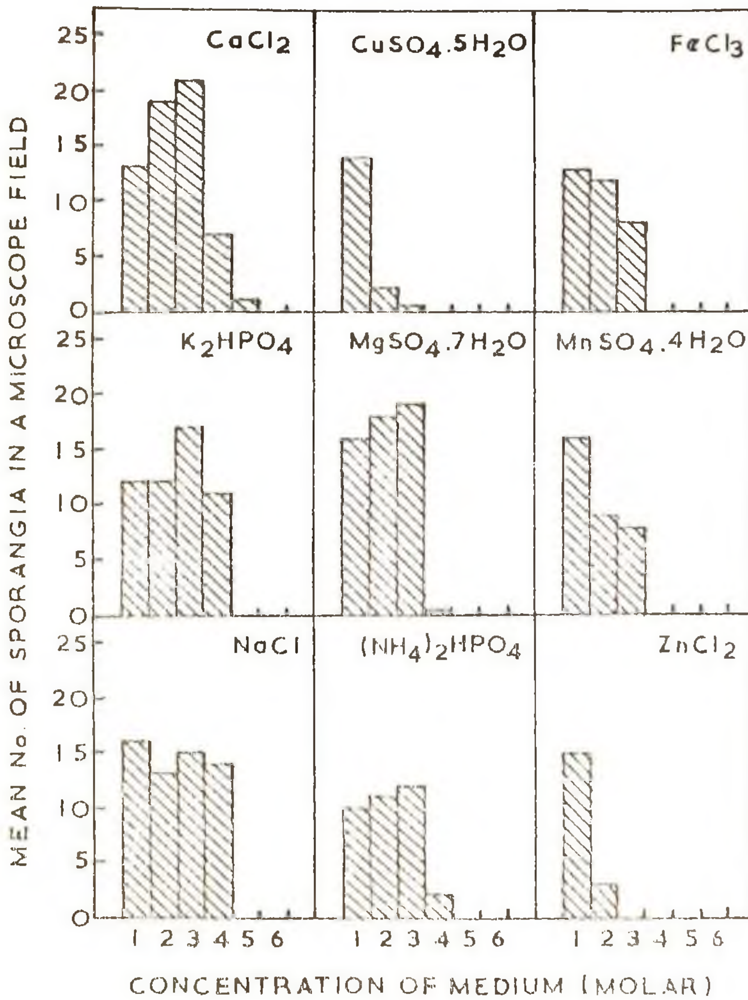

FIG. 6 EFFECT OF MINERAL SALTS ON SPORANGIAL FORMATION IN ACHLYA MEGASPERMA INCUBATED IN DARK AT 30°C. FOR 10 DAYS

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Plate 3: Photomicrograph of small and medium sized sporangia of *A. megasperma* produced by culture in 1×10^{-3}M. $CaCl_2$ solution and incubated at 30°C.

x 300

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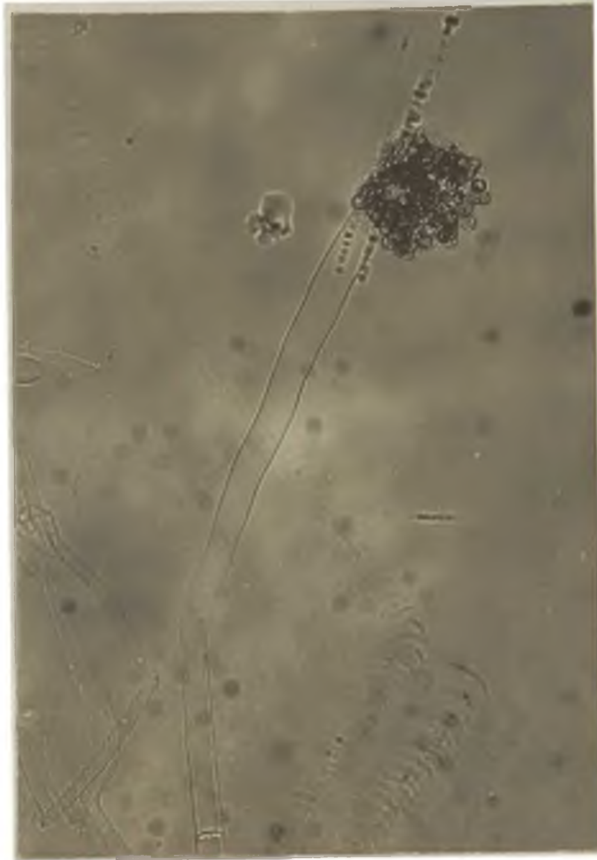

Plate 4: Photomicrograph of large-sized sporangia of A. megasperma produced by cultures in 1×10^{-5} $MgSO_4 \cdot 7H_2O$ solution and incubated at $30^{\circ}C$.

x 300

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sporangium recorded were 221.3, 175.8, 252.9, 208.6 and 245.0 µm, respectively.

There were some noticeable effects of some of the salts on gemmae structure. Those formed in distilled water were Obovate clavate or fusiform and generally occurred in chains at the apices of the hyphae. Gemmae formed in solutions of 1×10^{-7} and 1×10^{-5}M $FeCl_3$, 1×10^{-7} and 1×10^{-5}M $MnSO_4$ and 1×10^{-7}M $ZnCl_2$, which incidentally were the only concentration levels that permitted growth in these salts, were markedly different.

Gemmae in the $ZnCl_2$ solutions were very irregularly shaped and contained extremely dense protoplasm (see plate 5 & 6).They occurred mostly singly at the ends of the hyphae.

Those in the other solutions were spherical in shape and were borne in chains. Apparently the contents of a gemma is transferred into the suceeding developing gemma resulting in a chain of empty gemmae supporting a terminal protoplasm-filled gemma (see plate 7).

The pH of the media changed with growth of the cultures. Four clear types of drift of pH could be distinguished.

a. The pH drifted to the alkaline side in solutions of $CaCl_2$ (Table 12), $MgSO_4.7H_2O$ (Table 17) NaCl (Table 19),

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Plate 5: Photomicrograph of irregular-shaped gemmule of <u>A. megasperma</u> in 1 x 10^{-5}M $ZnCl_2$ solution at 30°C.

x 330

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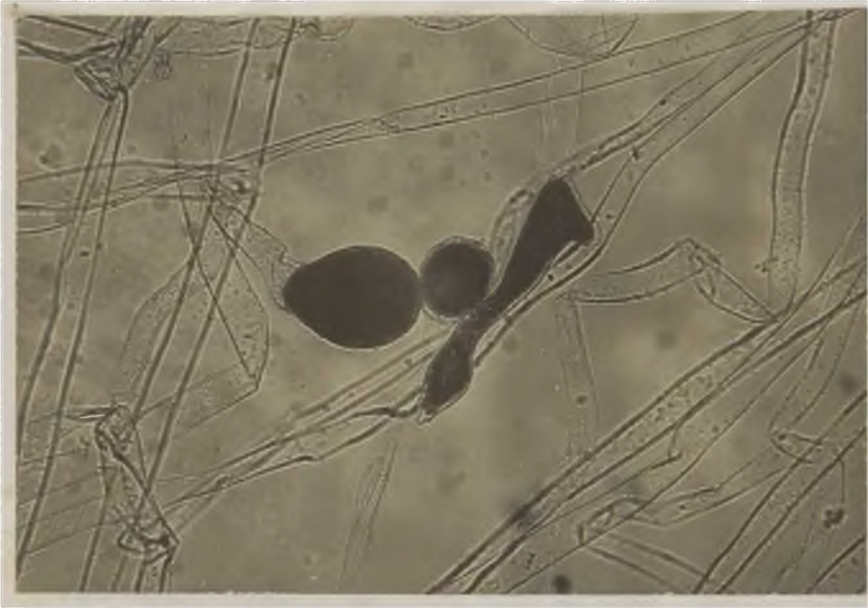

Plate 6: Photomicropgraph showing different forms of gemmae produced by A. megasperma in 1×10^{-7}M. $ZnCl_2$ solution at 30°C. Photograph taken after 10 days incubation.

x 350

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Plate 7: Photomicrograph of spherical gemmae formed by *A. megasperma* in 1×10^{-5} $FeCl_3$ solution at $30^{o}C$. Photograph taken after 10 days incubation.

Note the loss of content on the development of the succeeding gemma.

x 330

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$ZnCl_2$ (Table 21) and very slightly in $CuSO_4 . 5H_2O$ (Table 14).

b. The pH drifted to the acidic side in solutions of $(NH_4)_2HPO_4$ (Table 20) and very slightly in Citric acid (Table 13).

c. The pH drift was not uniform for all the solutions of K_2HPO_4 (Table 16). Whilst the pH drifted to the alkaline side at 1×10^{-7} and 1×10^{-5} it moved to the acidic side at 1×10^{-3}, 0.1, and 0.5M.

d. Culture solutions of $FeCl_3$ (Table 15) and $MnSO_4 . 4H_2O$ (Table 18) did not show any clear drift.

The graph in Fig.7 is a summary of the effects of the salts in sporangial formation $CuSO_4 . 5H_2O$, $FeCl_3$, $MnSO_4 .$ $4H_2O$ and $ZnCl_2$ could be placed in one group which could be considered highly inhibitory whilst the remaining salts form another group that permitted sporulation at higher concentrations.

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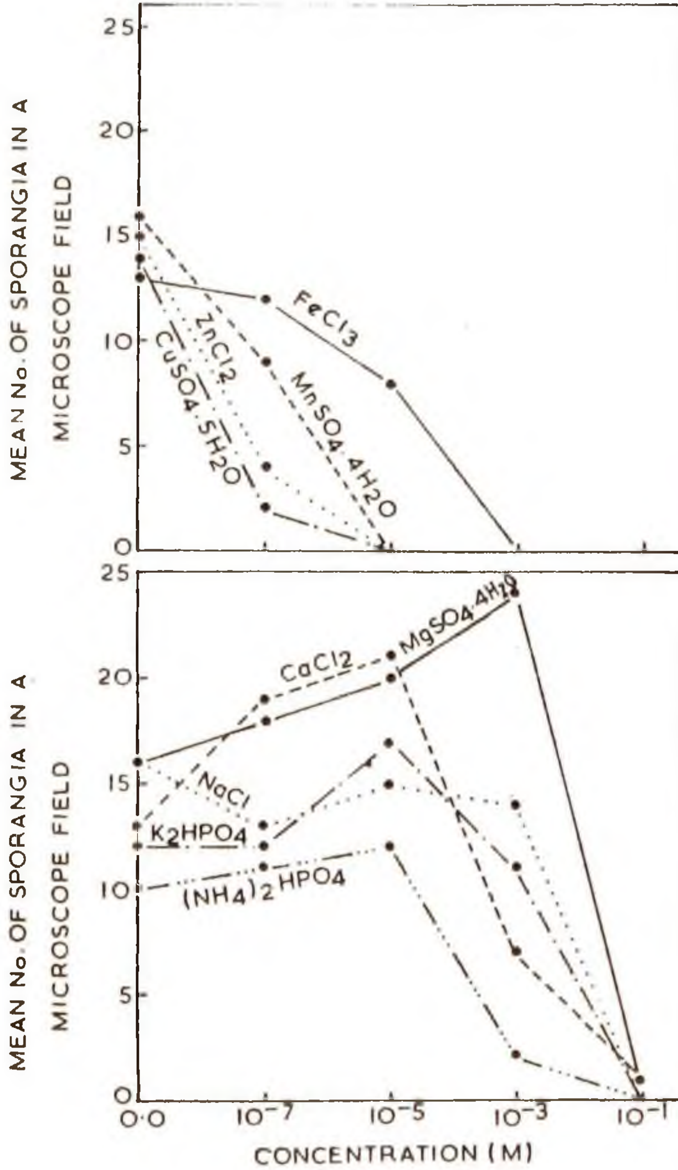

FIG. 7 EFFECT OF MINERAL SALTS ON SPORANGIAL FORMATION IN A. MEGASPERMA INCUBATED AT 30°C. FOR 10 DAYS

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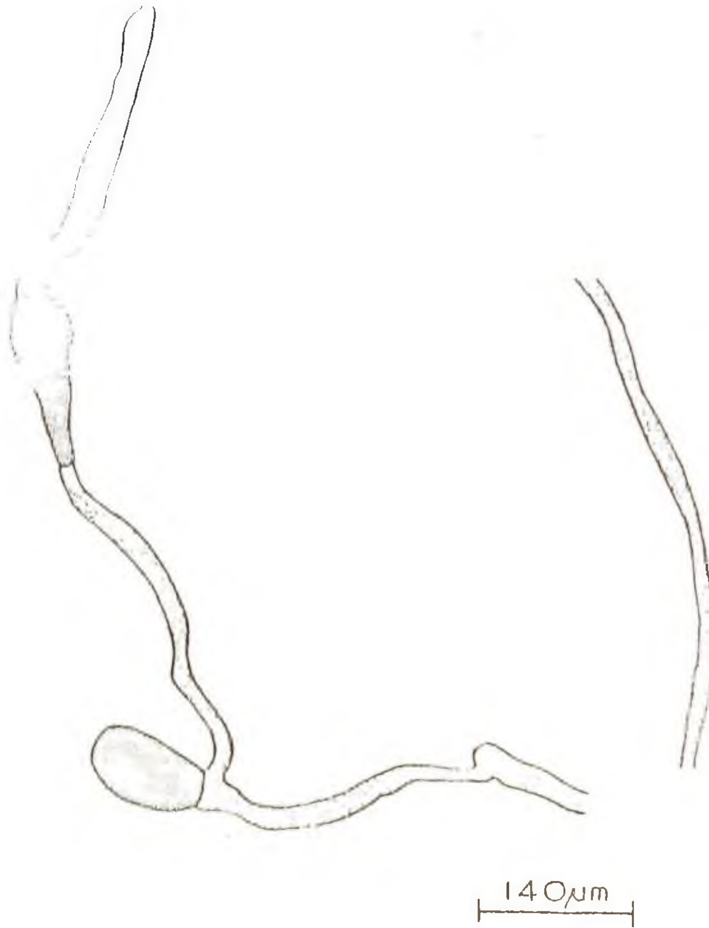

FIG. 8 CAMERA LUCIDA DRAWIGS OF GEMMAE OF A. MEGASPERMA IN 1×10^{-5} M ZINC CHLORIDE SOLUTION INCUBATED AT 30°C. FOR 10 DAYS

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F. VEGETATIVE GROWTH AND SPORANGIAL DEVELOPMENT IN SOLUTIONS OF SELECTED MINERAL SALTS AMENDED WITH POTATO DEXTROSE BROTH

Fungi never show just a simple relationship with mineral elements supplied, and like other nutritional requirements, could be influenced by several factors. Thus, Steinberg and Bowling (1939) found that the optimum concentration of magnesium for growth of *Aspergillus niger* was dependent on the concentration of the carbon source and, *Aspergillus terreus* in surface culture required a higher optimum concentration of magnesium than it did in shaken culture (Lockwood and Nelson, 1946; Lockwood and Reeves, 1945).

From the results of experiments of the previous chapter, copper sulphate, Manganese sulphate and Zinc chloride at $1x10^{-5}$ M were clearly inhibitory and prevented both growth and sporangial formation. Ferric chloride at this concentration supported only scanty growth and low sporulation. This experiment examined the effect of nutrients in the presence of these inhibitory salts on both growth and sporangial formation in *A. megasperma*, using Potato Dextrose Broth of standard concentration and at two dilutions, 1:10 and 1:100.

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Double concentrations of both the nutrient broth and salts were prepared in order to achieve the desired concentrations on mixture of equal volumes of the two. Pieces of hemp seeds with 48 hr-culture of mycelium were then placed in the sterilized media and incubated at $30^{o}C$ for 10 days. Growth of the mycelium, mean numbers and lengths of sporangia produced in the various treatments are presented in Tables 22 to 25.

The results indicate marked effects of the nutrient. The mycelium grew in spite of the presence of the inhibitory levels of the salts, but to varying degrees depending on both the salt and concentration of the Potato Dextrose Broth. The standard concentration of the broth was mostly less suitable than the lower concentrations (Tables 22, 23 and 25). The best broth concentrations for growth were 1:100 dilution in $FeCl_3$ and $Zn\ Cl_2$ solutions (Tables 23 and 25) and 1:10 dilution in $CuSO_4.5H_2O$ solution (Table 22). The Potato dextrose broth induced branching of the hyphae and Figs 9 and 10 illustrate some forms of branching observed.

A greater number of sporangia was formed in distilled water than in salt solutions supplemented with Potato Dextrose Broth. No sporangia were formed at the standard broth concentration in any treatment nor at a broth dilution

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of 1:10 in $FeCl_3$ (Table 23) and $ZnCl_2$ (Table 25) solutions. The mean number of sporangia per microscope field in the remaining treatments ranged from one to seven compared to 15 and 16 in distilled water. The sporangia were further-more correspondingly smaller, 118.3 to 260.3 µm long, than those in distilled water, 315.5 to 318.6 µm in length. Potato Dextrose Broth without any of the salts remarkably did not support any sporulation.

A change in viscosity of the different solutions except distilled water was observed. Solutions containing the Potato Dextrose Broth became noticeably viscid. No quantitative measurements in change of density of the solutions were, however, made.

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TABLE 22

Vegetative growth and sporangial formation in *A. megasperma* incubated in $1x10^{-5}$ M $CuSO_4 . 5H_2O$ solution supplemented with Potato Dextrose Broth at 30^oC for 10 days.

Potato Dextrose Broth Concentration	pH		Vegetative growth	Sporulation	
	Initial	Final		Mean No. of sporangia per microscope field	Mean sporangial length (µm)
Standard					
Amended	5.8	5.0	Scanty Branched hyphae	-	-
Unamended	6.2	5.5	Moderate Branched hyphae	-	-
Dilution 1:10			Fairly abundant.		
Amended	6.2	6.2	Highly branched hyphae	6	169.5± 6.5
Unamended	6.5	6.9	Abundant Branched hyphae	-	-
Dilution 1:100					
Amended	6.5	7.0	Moderate Branched hyphae	4	182.2±8.8
Unamended	6.5	7.0	Abundant Branched hyphae	-	-
Distilled water	6.4	8.1	Moderate No branching	15	318.6±13.[illegible]

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TABLE 23

Vegetative growth and sporangial formation in *A.megasperma* incubated in $1x10^{-5}$M $FeCl_3$ Solution supplemented with Potato dextrose Broth at 30°C for 10 days.

Potato Dextrose Broth Concentration	pH		Vegetative growth	Sporulation	
	Initial	Final		Mean No of sporangia Per micro scope field	Mean sporangial length (µm)
Standard					
Amended	6.5	7.1	Moderate Branched Hyphae	-	-
Unamended	6.2	5.5	Moderate Branched Hyphae	-	-
Dilution 1:10					
Amended	6.8	7.2	Fairly abundant Branched Hyphae	-	-
Unamended	6.5	6.9	Abundant Branched Hyphae	-	-
Dilution 1:10					
Amended	6.4	8.1	Moderate No branching	7	218.6±9.2
Unamended	6.5	7.0	Abundant Branched Hyphae	-	-
Distilled water	6.4	8.1	Moderate No branching	15	315.6±13.2

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TABLE 24

Vegetative growth and sporangial formation in *A. megasperma* incubated in $1x10^{-5}$ M $MnSO_4 . 4H_2O$ solution supplemented with Potato Dextrose Broth at 30°C for 10 days.

Potato Dextrose Broth Concentration	pH		Vegetative growth	Sporulation	
	Initial	Final		Mean No. of sporangia per microscope field	Mean sporangial length (µm)
Standard					
Amended	6.2	6.3	Moderate Branched hyphae	-	-
Unamended	6.2	5.5	Moderate Branched hyphae	-	-
Dilution 1:10					
Amended	6.5	6.7	Moderate Branched hyphae	3	208.6±9.7
Unamended	6.5	6.9	Abundant Highly branched hyphae	-	-
Dilution 1:100					
Amended	6.3	6.7	Moderate. Branched hyphae	4	260.3±10.5
Unamended	6.5	7.0	Abundant Branched hyphae	-	-
Distilled water	6.4	8.1	Moderate. No branching	15	315.5±13.2

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TABLE 25

Vegetative growth and sporangial formation in *A. megasperma* incubated in $1x10^{-5}$ M $ZnCl_2$ solution supplemented with Potato Dextrose Broth at 30°C for 10 days.

Potato Dextrose Broth	pH		Vegetative growth	Sporulation	
	Initial	Final		Mean No of sporangia per microscope field	Mean Sporangial length (µm)
Standard					
Amended	6.5	6.2	Moderate Branched hyphae	-	-
Unamended	6.2	5.5	Moderate Branched hyphae	-	-
Dilution 1:10					
Amended	6.3	6.5	Moderate Branched hyphae	-	-
Unamended	6.5	6.9	Abundant Branched hyphae	-	-
Dilution 1:100					
Amended	6.3	7.0	Abundant Branched hyphae	1	110.2±7.5
Distilled Water	6.4	8.1	Moderate No branching	15	216.0±11.2

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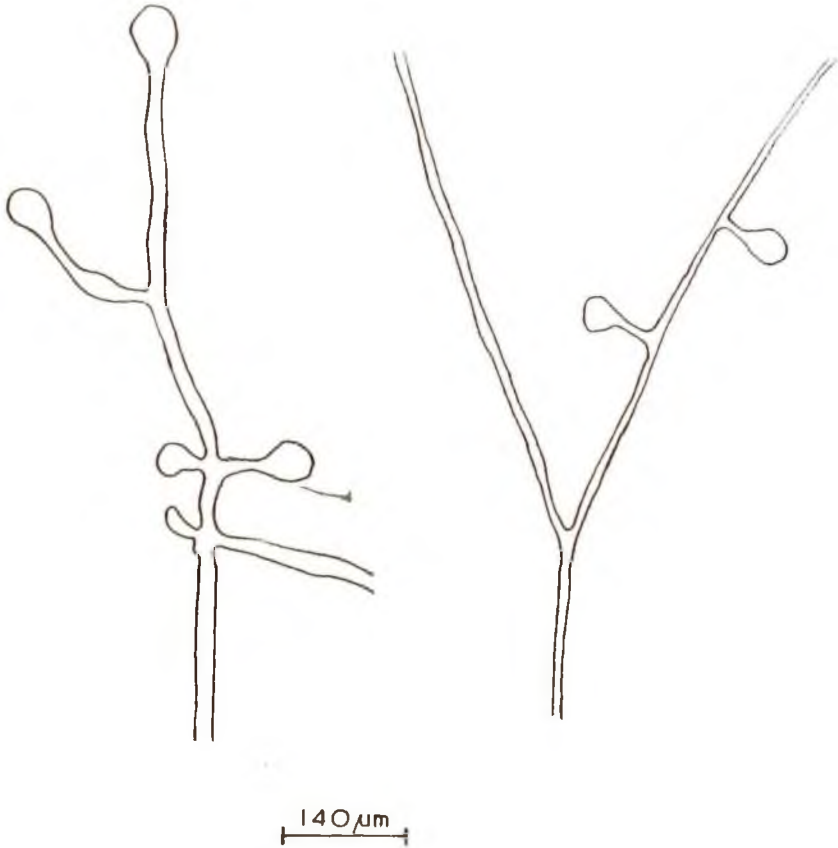

FIG. 9 CAMERA LUCIDA DRAWINGS OF *A. MEGASPERMA* INCUBATED IN 1:100 BROTH DILUTION PLUS 1×10^{-5} M $MnSO_4 \cdot 4H_2O$ AT 30°C. FOR 10 DAYS

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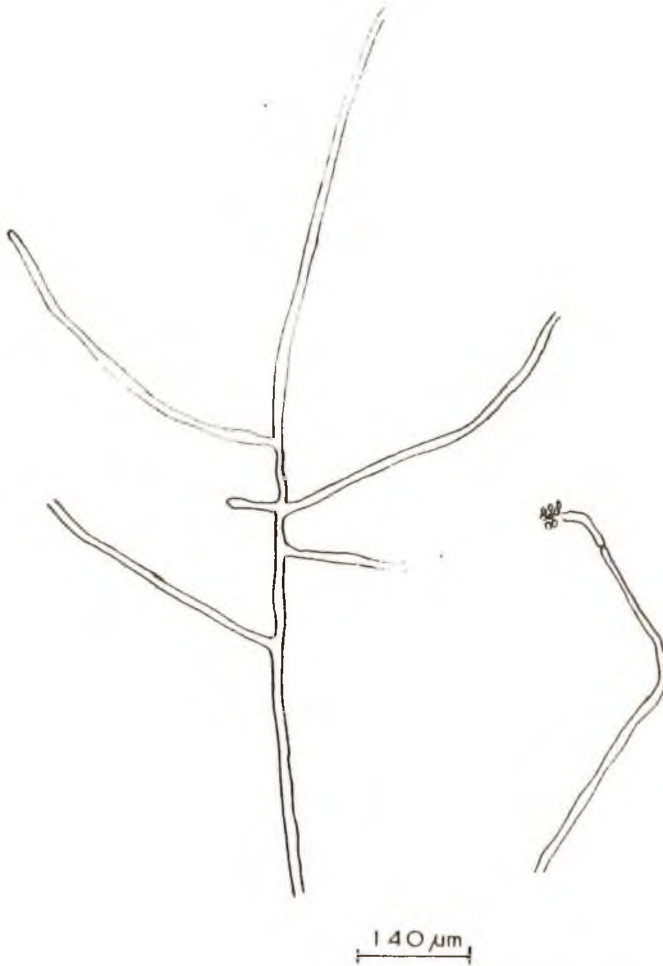

FIG. 10 CAMERA LUCIDA DRAWINGS OF *A MEGASPERMA* INCUBATED IN 1×10^{-5} M. $CuSO_4 \cdot 5H_2O$ AND 1:100 BROTH DILUTION AT 30°C FOR 10 DAYS.

NOTE THE HIGHLY BRANCHED HYPHA AND VERY SMALL SPORANGIA.

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G. INFLUENCE OF CARBOHYDRATES ON GROWTH AND SPORANGIAL FORMATION

Sporangial formation in *A. megasperma* was exceptionally sensitive to high nutrient concentration as the fungus failed to form sporangia in Potato Dextrose Broth, a medium that generally supports sporulation in numerous fungi. The active principle responsible for the suppression of sporulation could not be inferred because of the complex composition of the broth. In addition to the high levels of carbohydrates, supplied as glucose and also derived from the potato, proteins and aminoacids although at lower concentrations were naturally present in the medium.

Experiments described here and in subsequent chapters provide information, first, on the response of *A. megasperma* to various carbohydrates and proteins and secondly, on the contribution of some of these, for example glucose to the observed influence on sporangial formation by the Potato Dextrose Broth. These organic compounds are besides major constituents of organic pollution.

The carbohydrates, Fructose, Galactose, Glucose, Lactose, Mannose and Sucrose were used at the concentrations 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 per cent (W/V).

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The media, inoculated with 48-hour inoculated hemp seeds, were as usual kept at 30°C for 10 days.

The response of *A. megasperma* to these compounds are recorded in Tables 26 to 31 and in Fig.11

The fungus grew at all concentrations of every carbohydrate and there was very abundant vegetative growth in all solutions. It, however, sporulated to varying degrees in the presence of the various carbohydrate compounds. Sporulation at all the concentrations used was less than that in distilled water. The greatest number of sporangia was obtained at the lowest concentration in each case and the number decreased with increase in concentration.

The following consideration could be employed as the principal basis for assessing the effect of the Carbohydrates: the number of sporangia produced at the best concentration (0.1 per cent) and, the range of concentration permitting sporulation.

Galactose was, therefore, the least inhibitory, for it permitted sporangial formation over the widest range, 0.1 to 4.0 per cent and supported the largest number of sporangia, 10 per microscope field, at 0.1 per cent concentration (Table 27).

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Lactose was closely similar in effect. A mean of eight sporangia was obtained for a microscope field at 0.1 per cent concentration, and the remaining concentrations, 0.5, 1.0 and 2.0 per cent provided mean number of sporangia of 6, 6 and 5, respectively (Table 29).

Glucose, on the other hand, was the most inhibitory. Sporangia were formed at the concentrations of 0.1, 0.5 and 1.0 per cent only and in each case, the mean per microscope field was less than one (Table 28). The effects of the remaining compounds, Fructose, Mannose and Sucrose (Tables 26, 30 and 31) were median, between those of Lactose and Glucose.

The sporangia were smaller in all the solutions. The extent of reduction , however, varied with the compound. There was very marked reduction in length in solutions of Galactose, at the higher concentrations of 2.0 to 4.0 per cent which gave mean sporangial length of 174.2, 166.5 and 132.0 µm and Mannose at all concentrations which permitted Sporulation (Table 30). The mean sporangial length ranged from 149.9 to 166.3 µm in the Mannose solutions in contrast to a mean value of 384.1 µm in distilled water. The influence of the other treatments was less severe and the sporangial length ranged from 231.6 µm (Table 31) to 336.6 µm (Table 29).

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TABLE 26

Influence of Fructose on sporangial production in *A. megasperma* incubated in dark at 30°C for 10 days.

Concentration %	pH		No. of sporangia observed in 100 microscope fields		Mean length of 100 sporangia (μm).
	Initial	Final	Total	Mean	
0.0	6.4	8.1	1463	15	394.0 ± 9.8
0.1	5.9	6.9	481	5	274.0±18.6
0.5	5.6	6.3	470	5	252.0±13.4
1.0	6.0	6.3	20	1	237.6±11.0
2.0	5.4	5.6	-	-	-
3.0	5.2	5.0	-	-	-
4.0	4.6	4.6	-	-	-
5.0	4.5	4.6	-	-	-

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TABLE 27

Influence of Galactose on sporangial production in *A. megasperma* incubated in dark at 30°C for 10 days

Concentration %	pH		No.of sporangia observed in 100 microscope fields		Mean length of 100 sporangia (µm)
	Initial	Final	Total	Mean	
0.0	6.4	8.1	1638	16	390.2±10.2
0.1	5.8	6.8	981	10	298.3±13.5
0.5	5.7	6.9	793	8	285.1±13.1
1.0	5.4	6.6	268	3	248.2±19.0
2.0	4.9	6.9	171	2	174.2±15.2
3.0	4.9	6.4	62	1	166.5±6.6
4.0	4.7	5.9	15	1	132.0±7.2
5.0	4.5	5.4	-	-	-

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TABLE 28

Influence of Glucose on sporangial production in *A. megasperma* incubated in the dark at 30°C for 10 days.

Concentration %	pH		No. of sporangia observed in 100 microscope fields		Mean length of 100 sporangia (μm)
	Initial	Final	Total	Mean	
0.0	6.4	8.1	1511	15	380.9±12.5
0.1	6.5	6.6	61	1	265.0± 5.9
0.5	6.1	6.4	10	1	261.0± 6.2
1.0	5.4	6.5	26	1	264.2± 5.4
2.0	5.2	6.2	-	-	-
3.0	4.9	6.8	-	-	-
4.0	5.1	6.6	-	-	-
5.0	5.1	6.8	-	-	-

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TABLE 29

Influence of Lactose on sporangial production in *A. megasperma* incubated in the dark at 30°C for 10 days.

Concentration %	pH		No. of sporangia observed in 100 microscope fields		Mean length of 100 sporangia (µm)
	Initial	Final	Total	Mean	
0.0	6.4	8.1	1466	15	386.0±11.8
0.1	6.2	6.6	834	8	336.6±16.1
0.5	5.6	6.1	643	6	322.1±14.6
1.0	5.7	6.5	575	6	270.6±15.2
2.0	5.3	7.1	534	5	287.8±16.9
3.0	5.0	6.4	-	-	-
4.0	4.8	6.2	-	-	-
5.0	4.9	5.7	-	-	-

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TABLE 30

Influence of Mannose on sporangial production in <u>A. megasperma</u> incubated in the dark at 30°C for 10 days.

Concentration %	pH		No. of sporangia observed in 100 microscope fields		Mean length of 100 sporangia (µm)
	Initial	Final	Total	Mean	
0.0	6.4	8.1	1621	16	384.1±13.3
0.1	5.9	6.2	383	4	149.9±15.2
0.5	5.6	6.0	299	3	166.3±14.7
1.0	5.2	5.6	75	1	158.0±16.2
2.0	4.8	5.0	-	-	-
3.0	4.6	4.5	-	-	-
4.0	4.7	4.7	-	-	-
5.0	4.3	4.4	-	-	-

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TABLE 31

Influence of Sucrose on Sporangial production in *A. megasperma* incubated in the dark at 30°C for 10 days.

Concentration %	pH		No.of sporangia observed in 100 microscope fields		Mean length of 100 sporangia (µm)
	Initial	Final	Total	Mean	
0.0	6.4	8.1	1498	15	386.4±10.9
0.1	7.2	6.1	316	3	278.0±15.0
0.5	7.3	5.9	231	2	251.4±17.2
1.0	7.2	5.7	268	3	250.0±20.3
2.0	7.0	5.6	14	1	231.6±18.5
3.0	6.9	5.7	-	-	-
4.0	6.3	5.6	-	-	-
5.0	5.4	5.2	-	-	-

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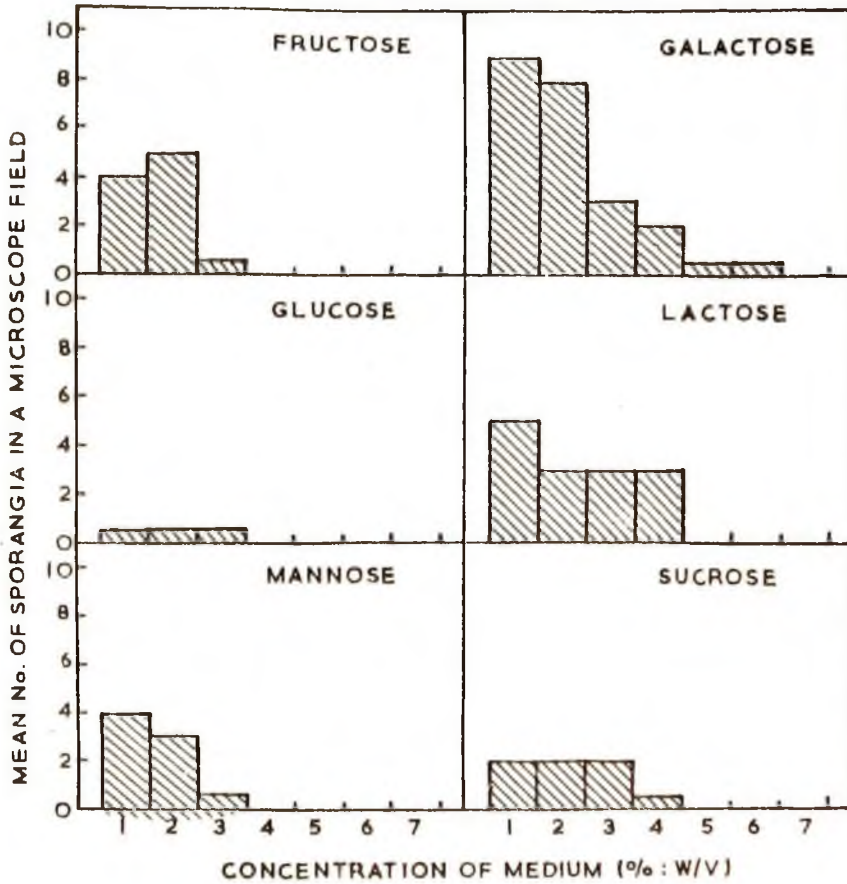

FIG. 11 EFFECT OF SUGARS ON SPORANGIAL FORMATION IN A. MEGASPERMA INCUBATED IN DARK AT 30°C. FOR 10 DAYS MEAN No. OF SPORANGIA IN WATER : 18

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There was a uniform trend in carbohydrate effect. The length of the sporangium decreased with increase in concentration of all Carbohydrates.

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H. INFLUENCE OF NITROGEN-COMPOUNDS ON GROWTH AND SPORULATION

Fungi generally require nitrogenous compounds at lower concentrations than the levels of carbohydrates at which they both grow and sporulate. The present investigation which examined the response of A. megasperma to nitrogenous compounds therefore employed low concentrations of the compounds selected. The maximum concentration was 1.0 per cent. The compounds tested consisted of both inorganic and organic nitrogenous compounds for, Whiffen (1945) had recorded no growth of Saprolegniaceous fungi on $(NH_4)_2 SO_4$ or KNO_3 but obtained adequate growth on the organic nitrogen sources. Reischer (1951) also reported that inability to utilize nitrate is common in the Saprolegniaceae. Eleven species of Saprolegniaceae made excellent growth on media containing ammonia salts but grew poorly on nitrate media unless fumarate, malate and succinate were included in the medium. A. megasperma was grown in the present study in solutions of Ammonium nitrate, Haemoglobin, Malt extract, Peptone and Yeast extract at concentrations of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 per cent (W./V.). The inoculated media were incubated at 30°C for 10 days.

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No sporangia were produced in any of the media, whilst the culture in the distilled water fructified, producing normal sized sporangia, averagely 14 sporangia per microscope field.

The mycelia in the different treatments and in distilled water grew to the same extent, the nitrogenous compounds, however inducing considerable branching of the hyphae.

The media of nitrogenous compounds were found to be more viscid in consistency at the end of the incubation period. The drift of the pH of the media during this period is summarised in Table 32.

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TABLE 32

Changes in pH of media of nitrogenous compounds during growth of *A. megasperma* incubated at 30°C for 10 days.

Medium	Concentration (per cent)	pH Initial	pH Final
Ammonium nitrate	0.1	6.3	6.6
	0.2	6.7	6.4
	0.4	5.9	6.1
	0.6	5.3	6.0
	0.8	5.4	5.9
	1.0	4.8	5.7
Haemoglobin	0.1	6.4	7.0
	0.2	6.0	7.0
	0.4	6.6	7.7
	0.6	6.5	7.9
	0.8	6.8	8.0
	1.0	6.9	8.1
Malt extract	0.1	5.9	7.0
	0.2	5.7	7.2
	0.4	5.6	6.9
	0.6	5.8	7.1
	0.8	5.4	6.7
	1.0	5.4	6.5
Peptone	0.1	6.4	7.1
	0.2	6.5	7.4
	0.4	6.1	7.3
	0.6	6.0	7.6
	0.8	5.9	7.9
	1.0	5.8	8.3

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TABLE 32 Cont'd.

Changes in pH of media of nitrogenous compounds during growth of *A. megasperma* incubated at 30°C for 10 days.

Medium	Concentration (per cent)	pH	
		Initial	Final
Yeast extract	0.1	6.2	7.3
	0.2	5.6	7.2
	0.4	5.3	7.5
	0.6	5.4	7.8
	0.8	5.1	8.5
	1.0	4.8	8.2
Distilled water	0.0	6.4	8.1

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I. INFLUENCE OF LOW CONCENTRATIONS OF NITROGENOUS COMPOUNDS ON GROWTH AND SPORULATION

In order to obtain somewhat more precise information on the response of A. megasperma to nitrogen sources, the experiment was repeated with very low concentrations of three of the compounds tested in the previous experiment, Malt extract, Peptone and Yeast extract. The fungus was grown in media of these compounds at the concentrations 0.01, 0.05, and 0.1 per cent (w/v) for 10 days at 30°C. The results are presented in Table 33.

Peptone remained inhibitory at the very low concentration of 0.01 per cent, although the mycelium grew well in this and in all the other media.

The remaining two compounds at 0.01 and 0.05 per cent produced contrasting effects. Whilst malt extract depressed sporangial formation, supporting respective means of 11 and 6 sporangia per microscope field as compared to 17 in distilled water, yeast extract stimulated sporangium formation, more than doubling the rate of sporulation, with a mean of 43 per microscope field, at 0.01 per cent. The sporangia in the yeast-extract medium were also found to be larger than those in distilled water.

There was a uniform drift of the pH to the alkaline side.

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TABLE 33

Influence of low concentrations of nitrogenous compounds on sporangial formation in _A. megasperma_. Incubated in dark at 30°C for 10 days.

Medium	Concentration (per cent)	pH		No. of Sporangia observed in 100 microscope fields		Mean length of 100 sporangia (µm)
		Initial	Final	Total	Mean	
Malt extract	0.01	5.6	6.7	1135	11	275.3±10.4
	0.05	5.9	6.9	568	6	1[illegible].6±[illegible].5
	0.1	6.1	6.8	-	-	-
Peptone	0.01	6.1	7.1	-	-	-
	0.05	6.5	7.9	-	-	-
	0.1	5.[illegible]	8.6	-	-	-
Yeast extract	0.01	5.7	7.9	4257	43	332.3±13.7
	0.05	5.9	7.8	2103	20	317.5±13.5
	0.1	6.2	7.2	-	-	-
Distilled water	-	6.4	8.1	1735	17	302.0±12.5

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J. FURTHER EXPERIMENTS ON EFFECT OF LOW CONCENTRATIONS OF PEPTONE ON GROWTH AND SPORULATION

The results of the last experiment showed that whilst Malt extract and Yeast extract permitted sporulation at a concentration of 0.01 per cent, Peptone at that concentration was still totally inhibitory . It was found necessary to examine further the effect of very low concentration of peptone on sporulation, by using concentrations below 0.01 per cent viz, 0.001 and 0.005 per cent.

Table 34 shows the effect of peptone at these concentrations on both the rate of sporangial formation and size of the sporangia at the end of the incubation period of 10 days at the optimum temperature of 30°C.

Concentrations of 0.001 and 0.005 per cent were definitely below the inhibitory level of peptone. _A. megasperma_ formed sporangia in both media and the amount produced was even greater at 0.001 per cent than in distilled water. Again the sporangia formed at the higher concentrations 0.005 per cent were smaller (295.5 µm long) and in distilled water (382.5 µm long).

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TABLE 34

Influence of very low concentrations of Peptone on Sporangial formation in *A. megasperma* incubated in dark at 30°C for 10 days.

Medium	Concentration per cent	pH		No. of sporangia in 100 microscope fields		Mean length of 100 sporangia (µm)
		Initial	Final	Total	Mean	
Peptone	0.001	6.4	7.6	2604	26	302.3±14.5
	0.005	6.4	7.6	1226	12	295.5±12.1
Distilled Water	-	6.4	8.1	1831	18	382.5±15.2

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K. INFLUENCE OF POLYHYDRIC ALCOHOLS ON GROWTH AND SPORULATION

Because of the teeming population of bacteria in any freshwater body, it is unlikely that sugars deposited into these bodies would remain unattacked for long since the sugars constitute major substrates for microorganisms. Any tests of sensitivity to sugars under these conditions would, therefore, invariably involve sugar alcohols that have accumulated through reduction of sugars.

The notable polyhydric alcohols produced in such manner are D-sorbitol which arises by reduction of D-glucose, and D-mannitol by reduction of D-fructose or D-mannose. Glycerol may be considered as a reduction product of a 3-carbon sugar.

The polyhydric alcohols could be important in metabolism. Glycerol and mannitol may be expected on oxidation to yield respectively, fructose and glyceraldehyde or dihydroxyacetone, all of which can after phosphorylation enter known respiratory path ways.

Data on the utilization of polyhydric alcohols by fungi are insufficient for generalisation. For example, although in the lower phycomycetes a few species including _Saprolegnia delica_ and _Pythiogeton_ spp. grow poorly on

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mannitol (Bhargava, 1945; Cantino, 1949), this alcohol is equivalent to glucose as carbon source for a number of species, such as Memnoniella echinata (Perlman, 1948) and Stachybotrys atra (Jermyn, 1953).

The growth and formation of sporangia in solutions of the polyhydric alcohols, Dulcitol, Glycerol, Mannitol and Sorbitol in A. megasperma were studied using the following concentration levels for each alcohol, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 per cent. The cultures were grown at 30°C for 10 days. The results are presented in Tables 35 to 38 and graphically in Fig. 12.

Observations made could be summarised as follows:-

(a). There were 15-16 sporangia per field in distilled water and the mean lengths of the sporangia ranged from 369.6 - 374.6 µm.

(b). Sporangia were formed at all concentrations of Mannitol and Sorbitol (Tables 37 and 38), but were not formed at 1.0 per cent Dulcitol (Table 35) and at 0.8 and 1.0 per cent of Glycerol (Table 36).

(c) Glycerol was very inhibitory. Not more than two sporangia per microscope field were formed at any concentration and the sporangia were less than

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TABLE 35

Influence of Dulcitol on Sporangial Formation in *A. megasperma* incubated at 30°C for 10 days.

Concentration per cent	pH		No. of sporangia observed in 100 microscope fields		Mean length of 100 sporangia (µm)
	Initial	Final	Total	Mean	
0.1	7.0	6.4	3261	33	412.6±13.2
0.2	7.2	6.3	2805	28	399.4±14.6
0.4	7.4	6.3	2301	23	379.5±16.5
0.6	8.0	6.4	1725	17	312.5±13.5
0.8	8.3	6.6	1529	15	351.3±15.2
1.0	8.5	6.8	-	-	-
Distilled Water	6.4	8.1	1638	16	372.0±11.8

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TABLE 36

Influence of Glycerol on sporangial formation in *A. megasperma* incubated at 30°C for 10 days.

Concentration per cent	pH		No. of sporangia observed in 100 microscope fields		Mean length of 100 sporangia (μm)
	Initial	Final	Total	Mean	
0.1	7.3	6.6	170	2	162.6±9.8
0.2	7.5	6.4	213	2	161.9±11.5
0.4	6.2	6.3	117	1	163.1±10.3
0.6	6.0	6.4	157	2	162.8±10.5
0.8	5.6	6.4	-	-	-
1.0	5.4	6.1	-	-	-
Distilled water	6.4	8.1	1599	16	370.5±12.7

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TABLE 37

Influence of Mannitol on sporangial formation in *A. megasperma* incubated at 30°C for 10 days.

Concentration per cent	pH		No. of Sporangia observed in 100 microscope fields		Mean length of 100 sporangia (µm)
	Initial	Final	Total	Mean	
0.1	7.1	6.8	1229	12	232.3±15.8
0.2	7.1	6.6	1409	14	255.3±19.3
0.4	6.9	6.6	899	10	277.9±14.7
0.6	7.8	6.7	619	6	213.9±12.1
0.8	7.5	6.9	484	5	198.0±13.8
1.0	7.9	6.6	351	5	175.0±13.5
Distilled Water	6.1	8.4	1538	15	364.6±12.5

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TABLE 38

Influence of Sorbitol on sporangial formation in *A. megasperma* incubated at 30°C for 10 days.

Concentration per cent	pH		No. of sporangia observed in 100 microscope fields		Mean length of 100 sporangia (μm)
	Initial	Final	Total	Mean	
0.1	7.6	6.6	1488	15	389.6±14.3
0.2	7.3	6.8	1493	15	371.8±13.1
0.4	7.2	6.5	1475	15	325.1±17.5
0.6	7.4	6.3	1571	16	355.1±13.4
0.8	7.6	6.5	672	7	325.1±14.7
1.0	7.3	6.6	610	6	296.3±15.2
Distilled Water	6.4	8.1	1472	15	369.6±16.1

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half (161.9 - 163.1 μm) the length of sporangia formed in distilled water (Table 36).

(d) Production of sporangia was slightly suppressed in Mannitol solutions (Table 37) and at the higher concentrations of Sorbitol (Table 38). There were 12 sporangia per microscope field at the best concentration of 0.1 per cent of Mannitol and the sporangia were 232.3 μm long. Only five sporangia were recorded in a microscope field at the highest concentration (1.0 per cent) and the sporangia were short, 175.3 μm long.

Rate of sporangial formation and size of the sporangia at 0.1 to 0.6 per cent sorbitol were similar to those of distilled water. Sorbitol reduced sporulation at 0.8 to 1.0 per cent (Table 38).

e. Dulcitol was stimulatory at 0.1 to 0.4 per cent and supported greater level of sporulation (23-33 sporangia per microscope field) and larger sporangia (379.5 - 412.6 μm) than distilled water (Table 35).

f. The pH of the media generally drifted towards the acid side during growth of the fungus.

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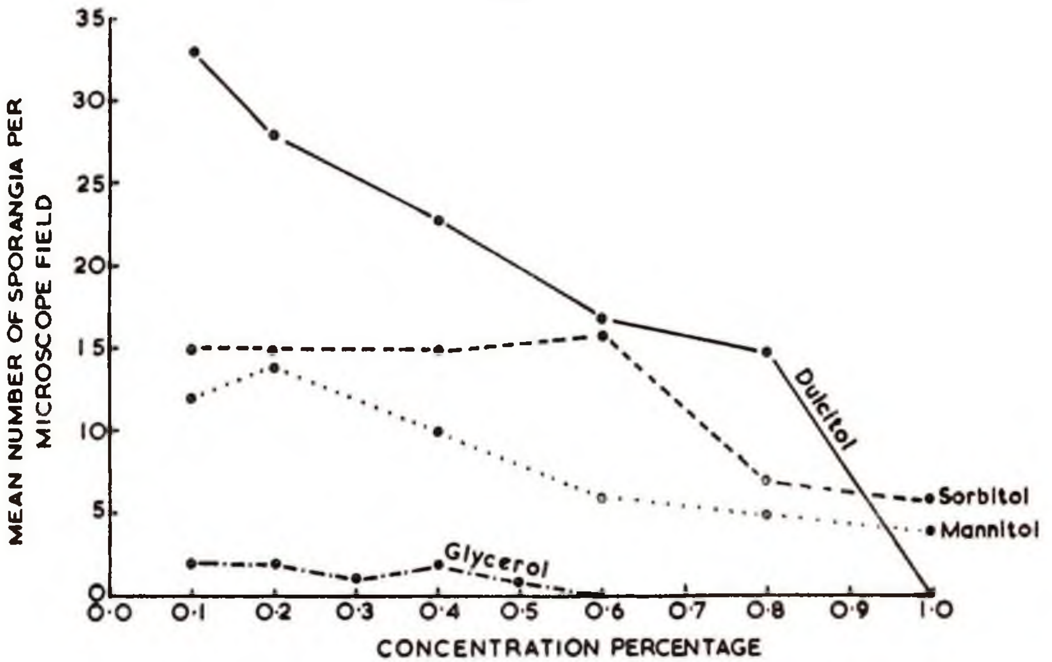

FIG.12 EFFECT OF POLYHYDRIC ALCOHOLS ON SPORANGIAL FORMATION IN _A. MEGASPERMA_ INCUBATED AT 30°C. FOR 10 DAYS

L. GROWTH AND SPORANGIAL FORMATION IN MEDIA OF APPROXIMATELY UNALTERED CONSISTENCY

It has been shown in preceding chapters (F and H), that a change in the consistency of the growth medium was noticeable during incubation of the fungus in some of the media. The media became more viscid. Under such circumstances, the fungus was influenced by two major factors, the nutrient being tested and, increased medium density. The contribution of the higher density of the medium could not be assessed from those results.

A preliminary investigation, that used a series of distilled water containing varying amounts of pyridine-washed (nutrient-free) agar of sufficiently small quantities to avoid setting but to provide media of greater density than distilled water, indicated by the data in Table 39 that distilled water containing agar as much as one gram per litre of medium did not depress sporangial formation.

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TABLE 39

Formation of sporangia by *A. megasperma* incubated in distilled water containing varying quantities of pyridine-washed agar to raise the density of the medium at 30°C for 10 days.

Amount of Agar (g./1.)	pH		No.of sporangia observed in 100 microscope fields		Mean length of 100 Sporangia (μm)
	Initial	Final	Total	Mean	
0.0	6.4	8.1	1520	15	335.6 $\pm$ 15.0
0.1	6.3	7.0	1612	16	312.6 $\pm$ 18.7
0.2	6.3	6.9	1577	16	328.5 $\pm$ 23.91
0.4	6.3	6.9	1579	16	352.6 $\pm$ 17.6
0.6	6.4	7.1	1536	15	318.1 $\pm$ 16.0
0.8	6.5	7.4	1457	15	316.8 $\pm$ 13.7
1.0	6.8	7.5	1523	15	326.6 $\pm$ 23.0

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The agar was washed prior to the test to remove any nutrients present according to the procedure of Robbins (1939). Hundred millilitres of 5.0 per cent aqeous pyridine was added to 10 g of agar at room temperature for 48 hours. The mixture was strained through muslin and agar trapped on the muslin was washed twice with 95 per cent ethyl alcohol and air dried.

Petri dishes with distilled water containing 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 g agar/litre, respectively, were inoculated with 48-hour cultures and incubated at 30°C for 10 days.

The experiment could not be extended as media with agar exceeding 1.0 g/l began to set.

An experiment was therefore designed, using two carbohydrates, one nitrogenous compound and one polyhydric alcohol that maintained as far as possible the original density of the medium throughout the period of growth. Higher viscosity, as a factor, was therefore eliminated.

The compounds used were Glucose, Lactose Yeast extract and Dulcitol at the concentrations of 1.0, 2.0, and 4.0 per cent for carbohydrates 0.1, 0.2 and 0.4 per cent for Dulcitol and 0.01, 0.05 and 0.1 per cent for Yeast extract. The solutions of these compounds became strongly viscid in

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the previous experiments. Ten replicates were prepared for each treatment. Five of these were kept undisturbed throughout the period of incubation of 10 days. The medium in the other five dishes was changed aseptically every 48 hours. The fluid was gently removed at each 'changing' time with a sterile pipette without distorting the hyphae of the culture, and replaced very gently with a fresh medium again using a sterile pipette. Changing was always done in the inoculation chamber. Changing was carried out after 2, 4, 6 and 8 days following inoculation and the response of the fungus was assessed after 10 days. Because of the changing of the media at these short intervals, pH recording of the growth medium was dispensed with.

The stock medium was kept in the incubator at 30°C the temperature at which the cultures were grown to obviate a change in temperature when it was added to the mycelium at changing time.

The results are presented in Table 40. Cultures in the unchanged media provided details remarkably close to earlier studies of Dulcitol (Table 35), Glucose (Table 28), Lactose (Table 29) and Yeast extract (Table 33). Glucose was again strongly inhibitory whilst Lactose was moderately so.

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Dulcitol was stimulatory at all concentrations and Yeast extract at the two lowest concentrations, 0.01 and 0.05 per cent.

Sporangial formation in the Dulcitol, Glucose and Yeast extract solutions did not show any difference between the two treatments, and change in viscosity during growth might not have played any significant role. In the Lactose media, however, the differences observed were large enough to be be attributed at least in part to the change in viscosity. The unchanged media of 1.0 and 2.0 per cent concentration supported, respectively, 665 and 455 sporangia counted in 100 microscope fields, which were far less than 889 and 810 sporangia, respectively, in dishes in which the solution was changed every 48 hours.

Sporangial size and mycelial growth were the same for both types of cultures in all cases.

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TABLE 40

Formation of sporangia by *A. megasperma* in Media of different levels of viscosity incubated at 30°C for 10 days.

Medium	Treatment (Medium)	Concentration (per cent)	No. of sporangia observed in 100 microscope fields		Mean length of 100 sporangia (µm)
			Total	Mean	
Dulcitol	changed every 48 hours	0.1	3899	40	365.5± 17.6
		0.2	2722	27	342.3±14.8
		0.4	2518	25	336.5±18.2
	Unchanged	0.1	3575	36	387.8±21.5
		0.2	2483	25	355.8±15.7
		0.4	2015	20	325.1±12.5
Glucose	changed every 48 hours	1.0	-	-	-
		2.0	-	-	-
		4.0	-	-	-
	Unchanged	1.0	5	1	105.6± 10.2
		2.0	-	-	-
		4.0	-	-	-
Lactose	changed every 48 hours	1.0	889	9	271.6±10.5
		2.0	810	8	242.4±11.0
		4.0	-	-	-
	Unchanged	1.0	665	7	268.5± 10.6
		2.0	455	5	253.2± 12.8
		4.0	-	-	-
Yeast extract	changed every 48 hours	0.01	4501	45	351.2± 12.6
		0.05	2435	24	333.5± 11.2
		0.1	-	-	-
	Unchanged	0.01	4366	44	373.5± 16.5
		0.05	2263	23	247.4± 9.5
		0.1	-	-	-
Water	Changed every 48 hours	0.0	1738	17	322.8± 12.2
	Unchanged	0.0	1668	17	315.5± 10.3

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M. EFFECT OF COW DUNG ON GROWTH AND SPORULATION

The most important fresh water pollution problem in rural West Africa is without doubt sewage dumping. Faecal pollution is a major health hazard as organisms causing intestinal infections are often excreted in large numbers in the faecal material. Because of the virtually universal presence of Escherichia coli in human and animal intestinal tracts, and because of the ease with which it can be identified and counted in a water sample, the presence of this organism in a water sample is usually used as an index of faecal pollution of the water. The presence of E. coli can be determined by standard method based on the ability to ferment lactose with the production of acid and gas. Negative results procedures of this sort, although highly standardised, are not always perfect indication of the absence of faecal matter, and therefore, the absence also of non-bacterial intestinal disease agents such as poliovirus. For bacterial cells may not be detected because of sedimentation of the cells onto the bottom of the pond or stream, destruction of the cells by chemical factors of the environment and by ultraviolet radiation and natural mortality. The series of experiments presented here examined the response of A. megasperma to faecal matter in the culture medium and affords a possible supplementary method

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of assessment of level of faecal matter in a fresh water body.

The Sohenko Pond in which the isolate of *A. megasperma* studied in this investigation flourishes was first examined to determine the level of faecal matter the fungus normally exists with.

i. FAECAL POLLUTION OF SOHENKO FARM

(a) PRESUMPTIVE TEST

Samples of the pond water were collected immediately after a visit by the herd of cattle which use the pond for watering, when the pond was most likely to contain freshly excreted dung.

The Presumptive Test was carried out as outlined at page 29 under Materials and General Methods. The results obtained after incubation of lactose broth in test tubes with Durham tubes and inoculated with varying quantities of pond water at 35°C for 48 hours are presented in Table 41.

Gas was produced in all the tubes. Sohenko Pond was polluted with faecal matter. The estimated number of coliform bacilli, expressed in terms of the Most Probable Number was over 1,800 bacteria cells per 100 ml of water sample.

(b) CONFIRMED TEST.

Ten plates of Eosin Methylene Blue Agar (E.M.B.) were inoculated with contents of test tubes which were positive for the Presumptive Test. All the plates had pink colonies

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TABLE 41

Presumptive Coliform Test using water samples from Sohenko Pond. (Inoculated Lactose broth incubated in darkness at 35°C for 48 hours).

Volume of Pond Water added (ml)	No. of tubes showing gas/No.of tubes inoculated	M.P.N Index per 100 ml of sample
0.1	5/5	
1.0	5/5	1,800
10.0	5/5	
Sterile distilled Water		
1.0	0/5	0

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characteristic of coliform bacilli after 24 hours of incubation at 35°C. Three plates showed opaque black colonies with metallic sheen typical of *E. coli*.

(c) COMPLETED TEST

Growth on Nutrient Agar

Colonies on nutrient agar were greyish white in colour. They were circular dome shaped discs expected of colonies of *E. coli*.

Gram Stain

The cells were found to be Gram-negative rods 1.5 - 4.0. um long and 0.5 um broad.

(d) DISTINGUISHING THE TYPES OF COLIFORM BACTERIA

It was found desirable after establishing the presence of Coliform bacteria in the pond water to distinguish the types present. The identification of the Coliform bacteria, for example, *E. coli*, *Aerobacter aerogenes* and *Escherichia freundii*, was based on ability to break down proteins and sugar and to utilize citrate. The tests employed were Indole, Methyl Red, Voges-Proskauer (V.P.) and citrate utilization - the mnemonic I.M. Vi C.

Indole.

Some Coliform organisms decompose the amino acid Tryptophan to indole which accumulates in the medium. The presence of

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indole can be detected by either testing with Kovac's or Ehrlich's reagent which changes the medium from creamish yellow to pink. The data presented in Table 42 provide the results for all tubes inoculated with 24 hour culture from the E.M.B Agar and tested after 48, 72 and 96 hours incubation at 35°C. Tubes which did not produce any colour change with the test reagent when cold were immersed in a water bath and boiled for 15 minutes and observed for any colour change.

There was no colour change after 48 hours when the medium was cold. On heating however one tube became slightly pink.

The positive reaction observed indicated the presence of E. coli and Proteus vulgaris.

Methyl Red Test.

Methyl Red test is employed to detect the production of sufficient acid commonly acetic acid, formic acid, lactic acid and succinic acid during fermentation of glucose. The acids give the medium a final pH of less than 4.5 and on addition of methyl red, a red colour is produced.

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TABLE 42

Reaction of Peptone - Water medium inoculated with culture from F.M.R. Agar to Kovac's and Ehrlich's Reagents. (Inoculated media incubated at 35°C for 48, 72 and 96 hours).

Treatment after Incubation	Period of Incubation (Hours)	No of tubes giving positive reaction/No. of tubes tested			
		Test with Kovac's Reagent		Test with Ehrlich's Reagent	
		Inoculated Medium	Control	Inoculated Medium	Control
No heating	48	0/2	0/2	0/2	0/2
	72	2/2	0/2	1/2	0/2
	96	2/3	0/2	1/2	0/2
Heating for 15 minutes	48	1/2	0/2	0/2	0/2
	72	2/2	0/2	1/2	0/2
	96	2/2	0/2	2/2	0/2

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TABLE 43

Reaction of Glucose Phosphate Medium inoculated with culture from E.M.B. Agar to Methyl Red. (Inoculated media incubated at 35°C for 48, 72 and 96 hours).

Period of Incubation (Hours)	Culture Replicate	Colour on addition of Methyl Red
48	1	Yellow
	2	Orange
	3	Yellow
	4	Yellow
	Control (no culture)	Yellow
72	1	Yellow
	2	Red
	3	Yellow
	4	Orange
	Control (no culture)	Yellow
96	1	Red
	2	Orange
	3	Orange
	4	Orange
	Control (no culture)	Yellow

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Table 43 summarises observations made on Glucose Phosphate Medium inoculated with culture of E.M.B. agar and incubated at 35°C for 48, 72 and 96 hours.

One tube gave a positive reaction - red colour - after 72 hours and after 96 hours showing the presence of E. coli but at very low density, since the majority of the tubes were either negative or doubtful.

Voges - Proskauer Test.

Voges - Proskauer test was employed to detect the formation of acetyl methyl carbinol from dextrose by few members of the family Enterobacteriaceae. Most Coliform bacilli like the Escherichia and Proteus groups are not able to produce acetyl methyl carbinol from dextrose. Acetyl methyl carbinol produced in the medium is oxidised by the test reagent, Barritt's reagent to diacetyl which produces a pink colour.

Glucose-Phosphate Peptone Medium inoculated in similar manner as the Methyl Red test was incubated for 72 and 120 hours at 35°C. None of the cultures showed the presence of Acetyl methyl carbinol and, therefore, of Klebsiella species on adding the Barritt's Reagent.

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Citrate Test.

The media used for this test was prepared as described at page 33 . Koser's and Simmons' media were inoculated with colonies from E.M.B. agar plates and incubated at 35°C for 48, 72 and 96 hours. Observations at the end of the respective incubation periods are presented in Table 44.

Simmons' and Koser's media retained their normal coloration of green and clear respectively after 24 hours incubation. Simmons medium began to turn blue after 48 hours whilst Koser's medium became turbid. A complete change of Simmons' medium to blue was noticed after 72 hours. This again indicated the presence of *Citrobacter freundii* and *Klebsiella aerogenes*.

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TABLE 44

Observed changes in Koser's and Simmons' media inoculated with culture from E.M.P. Agar during Citrate Test. (Inoculated media incubated at 35°C for 48, 72 and 96 hours).

Period of Incubation (Hours)	No. of tubes giving positive reaction/No. of tubes tested			
	Test with Koser's Medium		Test with Simmons' Medium	
	Inoculated Medium	Control	Inoculated Medium	Control
48	1/2	0/2	2/2	0/2
72	2/2	0/2	2/2	0/2
96	2/2	0/2	2/2	0/2

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II. COLIFORM BACILLI POPULATION IN MEDIA OF KNOWN COWDUNG CONTENT

The sensitivity of *A. megasperma* to faecal matter can only be quantitatively assessed if the precise level of cowdung in the medium is known. The fungus flourishes in pond water with a certain level of dung pollution. The amount of dung that may be critical should therefore lie above that of the pond.

It is not easy to determine directly from the M.P.N. estimation the actual level of dung content of the pond, since there are other sources of the coliform bacteria, principally the soil. The fungus is therefore not subjected to as much dung matter as depicted by the M.P.N. estimation. This proposition was verified in two subsequent experiments. First to find out minimum quantity of dung in distilled water that will give an M.P.N. index of 1800 and secondly, to examine the response of *A. megasperma* to this level of dung concentration.

The Presumptive Test was carried out using the following dung-distilled water mixtures: 0.001, 0.01, 1.0, 10.0 and 20.0 g/l. The results in Table 45 show the production of gas at all the dung concentrations. Concentrations of 10g and 20g per litre produced gas in lactose broth equivalent to that produced with pond water from Sohenko.

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TABLE 45

Coliform Bacilli population of cowdung-distilled water mixtures obtained by Presumptive test.

(Inoculated Lactose broth incubated at 35°C for 48 hours).

Concentration in (g/l)	No.of tubes	Volume of water inoculated (ml)	No.of Positive tubes	M.P.N index PER 100 mls Sample
0.0	5	0.1	0	0
	5	1.0	0	
	5	10.0	0	
0.001	5	0.1	0	
	5	1.0	5	240
	5	10.0	5	
	5	0.1	2	
0.01	5	1.0	5	542
	5	10.0	5	
	5	0.1	2	
0.1	5	1.0	5	542
	5	10.0	5	
	5	0.1	2	
1.0	5	1.0	5	542
	5	10.0	5	
	5	0.1	5	
10.0	5	1.0	5	1800
	5	10.0	5	
	5	0.1	5	
20.0	5	1.0	5	1800
	5	10.0	5	

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Dung Concentrations between 0.001g/l and 1.0g/l were clearly below the level of pollution observed in the pond immediately after a visit by the cattle.

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EFFECT OF KNOWN CONCENTRATIONS OF COWDUNG ON GROWTH AND SPORULATION

The response of *A. megasperma* to the highest level of dung pollution in the preceeding experiment to give an M.P.N. under 1800 and further higher concentrations of dung was next examined, by culturing the fungus in solution of sterile distilled water and known quantities of non sterile cowdung of the following concentrations: 0.1, 1.0, 10.0, 20.0, 30.0, 40.0, 50.0 and 60.0 g/litre. The inoculated petridishes were incubated at 30°C for 10 days. The results are presented in Table 46.

The fungus was very sensitive. Although there was vegetative growth in all the media, sporangial formation was inhibited at the dung concentration of 30g/litre. Sporulation formed at 1.0 and 2.0 per cent were also mainly non-viable. Sporulation was, however, high at the lowest dung level used; there were 22 and 26 sporangia per microscope field at 0.1 and 1.0g/l, respectively.

The size of the sporangium decreased with increase in concentration of the dung.

Finally, there was a clear drift of the pH towards the alkaline side in all cultures.

Table 47 contains data obtained when the experiment was repeated with sterile dung solution, which excluded the possible influence of the constituent bacteria and microfauna of the dung.

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TABLE 46

Effect of cowdung on sporangial formation in *A. megasperma* incubated at 30°C for 10 days.

Quantity of cowdung (g/1)	pH		No. of Sporangia observed in 100 microscope fields		Mean length of 100 Sporangia (um)
	Initial	Final	Total	Mean	
0.1	6.4	8.0	2315	23	382.8± 10.5
1.0	6.2	8.1	2593	26	290.4± 13.8
10.0	6.8	6.1	600	6	278.5± 10.2
20.0	6.6	8.0	-	-	-
30.0	7.1	8.6	-	-	-
40.0	7.5	8.5	-	-	-
50.0	7.5	8.5	-	-	-
60.0	7.6	8.9	-	-	-
Distilled Water	6.4	8.1	-	-	345.6± 9.8

* There were few developed sporangia but very numerous sporangia with arrested development. Average number of such sporangia per microscope field was 18.

** Undeveloped Sporangia
Average number per microscope field was 17.

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Viable sporangia although in very few numbers, were formed at dung concentrations of 30-60 g/l which were completely inhibitory in the non-sterile mixtures.

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TABLE 47

Sporangial formation in sterile aqueous cowdung solution by *A. megasperma* at 30°C for 10 days

Quantity of Cowdung g/l	pH		No. of sporangia observed in 100 microscope fields		Mean length of 100 Sporangia (μm)
	Initial	Final	Total	Mean	
0.1	5.5	6.3	1769	18	361.5±14.5
1.0	5.9	6.4	3319	33	358.2±12.8
10.0	6.0	7.5	978	10	274.5±14.3
20.0	6.3	7.8	324	3	268.6±13.5
30.0	6.5	8.2	179	2	195.3± 9.2
40.0	6.7	8.3	235	2	219.8±11.9
50.0	6.8	8.6	218	2	251.4±13.2
60.0	6.9	8.3	158	2	215.5± 8.5
Distilled Water	6.4	8.1	1296	13	341.7±15.1

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IV. FURTHER EXPERIMENTS ON THE COLIFORM BACILLI POPULATION AND SPORANGIAL FORMATION BY *A. MEGASPERMA* IN MEDIA OF KNOWN COWDUNG CONTENT

Sporangial formation was suppressed to some extent in non sterile solutions by a dung concentration of 10g./1 as shown in Table 46, the lowest concentration to give an M.P.N. of 1800 . It is not likely that this is the equivalence of dung content of the pond which also gave the same M.P.N. value (See Table 44) since the fungus thrives well in the pond. There is a strong likelihood that some concentrations between the levels 1.0 and 10.0g/1 might give M.P.N. Value of 1800 and yet be able to support extensive sporangial formation as occurs in the Sohenko Pond. This supposition was supported by the data obtained and presented in Table 48. A concentration of 8.0g/1 supported good sporangium formation. There were 19 sporangia per microscope field.

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TABLE 48

Sporangial formation in non sterile aqueous cowdung solution by *A. megasperma* at 30°C for 10 days.

Quantity of Cowdung (g/l)	M.P.N. Index per 100 ml of sample	pH of growth Solution		No. of sporangia observed in 100 microscope fields		Mean length of 100 Sporangia (µm)
		Initial	Final	Total	Mean	
1.0	240 * (5:5:0)	6.3	7.9	1680	17	329.0±23.0
2.0	918 (5:5:3)	6.4	7.5	1614	16	299.2±18.4
4.0	1800 (5:5:5)	6.4	8.1	1638	16	312.0±18.7
6.0	1800 (5:5:5)	6.5	7.8	1705	17	333.2±19.4
8.0	1800 (5:5:5)	6.6	7.6	1885	19	351.1±17.6
10.0	1800 (5:5:5)	6.5	7.5	781	8	263.2±23,9
Distilled Water		6.4	8.1	1681	17	328.5±16.8

*Number of Tubes giving Positive Reaction out of 5 tubes of 10ml, 1ml, and 0.1ml, respectively.

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V. GENERAL DISCUSSION

Pollution is now a well known subject and has received considerable attention in numerous studies. Various types of water pollution have been identified. Identification of the level of most types is by chemical analysis which require expertise which is not always available; secondly, it could be an expensive operation under circumstances that require regular routine tests. The use of the response of organisms to pollution, bioreagents, is being advocated for by several workers as it is considered to be a very easy method and besides an inexpensive way of assessing levels of specifically known causes of pollution.

Pertinent studies so far have been mostly concerned with reaction of animals as test organisms, as illustrated by the following reports on some common inorganic salts. Blue gills, Lepomis pallidus, was shown to be killed in 3½ hours by concentrations of 66 p.p.m. of $(NH_4)_2SO_4$ in distilled water (Wells 1915). A concentration of 2,275 p.p.m. of $CaCl_2$ in distilled water and 476 p.p.m $MgSO_4.7H_2O$ killed straw coloured minnows, Notropis blennius, in 2 to 4 days and 4 to 6 days, respectively (Garrey 1916). In an investigation on the lethal action of soluble metallic salts on fishes, Capenter (1927) observed that concentrations

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of 399 p.p.m. of $CuSO_4.5H_2O$ in distilled water, 270 p.p.m of $FeCl_3$ and 404 p.p.m. of $ZnSO_4$ killed minnow, Leuciscus phoxinus, in 62, 90 and 20 minutes, respectively. Ellis (1967) also observed that 1 p.p.m. of $CuSO_4.5H_2O$ killed Daphnia magna in 1½ to 2 hours. His other observations included death of D. magna in 1 p.p.m $NaCl_2$ solution in 3 hours, death of gold fish, Carassius auratus, in 100 p.p.m. of $FeCl_3$ solution in one hour and in 100 p.p.m. of $ZnCl_2$ solution in 5 days. He was able to show the survival time of C. auratus in different concentrations of NaCl solution. The respective periods needed to kill it in solutions of concentrations of 10,000, 12,500, 14,925, 20,000 and 50,000 p.p.m. were 4 - 10 days, 24 - 36 hours, 1 - 2 hours and 30 - 40 minutes.

The present investigation was carried out to examine the possibility of using A. megasperma, an aquatic fungus of wide distribution, as a bioreagent for assessing level of water pollution. Information has in addition been obtained on some aspects of the biology of the fungus.

A. megasperma was cultured over a temperature range of 20° to 40°C. Growth occurred at all the temperatures, except 40°C, with 30°C as the optimum (see Table 3). The usefulness of A. megasperma as a bioreagent depends on its

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ability to grow in temperatures of local fresh water bodies. There are records of temperature of certain lakes in Ghana. In studies of the Physico-Chemical Condition in Lake Bosumtwi, a meteoritic crater lake 250 Km North of Legon, carried out from April, 1966 to April, 1967, Whyte (1976) obtained the highest temperatures of 31 to 32°C of the surface water in the months of April and May, 1966, October and November, 1966, and in March and April, 1967. The lowest temperatures obtained ranged from 28°C to 29°C in August and in the first two weeks of September, 1966 and from December, 1966 to February, 1967. The same pattern was shown by water collected at a depth of 5 meters. The highest temperature in this case, however, ranged between 30° to 31°C. The range of temperature of Lake Bosumtwi thus included the optimum temperature for growth and sporulation of _A. megasperma_, 30°C, and the extremes of the range were close to this optimum.

Observations were also made by Biswas (1966) on the Volta Lake at Ajena from June to December, 1965 and from January to June, 1966. The monthly average temperatures recorded commencing from June, 1965 were 29.45°, 28.60°, 27.95°, 28.30°, 29.28°, 30.15°, 29.56°, 28.90°, 28.95°, 30.30°, 30.44°, 30.37° and 29.82°C.

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Temperature of fresh water bodies at least in Ghana would therefore permit the use of A. megasperma as a pollution detector in situ.

Temperature has been found to affect the morphology of reproductive organs of fungi. Examples are the finding that the conidia of Cercospora sesami were larger at an intermediate temperature than at higher or lower temperatures (Chowdhury , 1944) and the effect of temperature on the morphology of the asexual reproductive structures of Aspergillus janus (Raper and Thom, 1944). The influence of temperature on papilla development on the oogonial wall of A. colorata has been mentioned earlier in this text (see page 16). The oogonia were papillate when formed at 15°C and smooth walled when formed at 20°C (Reischer,1949a). Thus, temperature may alter characters usually regarded as diagnostic. The sporangia of A. megasperma formed at 30°C (see Table 4) were almost twice as long, 382.8 um averagely, as those produced at 20°C, 232.3 um averagely (see Fig.4). The difference in width of the two types of sporangia was rather small. Naturally more zoospores will be formed by the larger sporangia. The clumping of the encysted zoospores at exit pore did not, however, permit actual counting of the zoospores of each type of sporangia.

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Perhaps that information could be obtained by counting the nuclei of giemsa - stained sporangia. Whether these changes in morphology were attended by any physiological modifications in either the sporangia or the zoospores should be established in future investigations. Other environmental factors altered the morphology of the sporangia and these have been commented on elsewhere in this chapter.

Ziegler (1958) classified isolates of A. megasperma obtained in Florida in the U.S.A. as a 'cool' species. The optimum temperature of 30°C of the Ghana isolates is certainly high and the local isolate may be showing an adaptation to the tropical environment.

A. megasperma derives an advantage from this ability to produce larger sporangia and hence greater number of zoospores at the optimum temperature. It would prevent excessive wastage of zoospores at temperatures that would not sustain subsequent good vegetative growth after the germination of large number of zoospores.

A. megasperma has been found to be rather sensitive to inorganic ions. Experiments carried out to find the effects of some inorganic salts on growth and sporangial formation of the fungus showed that a concentration of 0.5M of all the salts tested inhibited growth. A. megasperma thus responded to high salt concentrations in common with several other reported instances.

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For example, Chesters and Rolinson (1951) found the optimum with regards to Zinc supply to fungi to be 0.001 to 0.5 p.p.m whilst Jarvis and Johnson (1950), Pisano, Olson and San Clemente (1954) and Steinberg (1946) regarded potassium concentrations of 0.001 to 0.004M adequate.

Considering sporangial formation over the concentration range of $1x10^{-3}$ to 0.1M, the salts tested could be separated into three categories. $CaCl_2$ and $MgSO_4.7H_2O$ fell into one category of salts well tolerated by the fungus. A. megasperma not only sporulated over the entire concentration range but also at certain concentrations, that is $1x10^{-5}$ and $1x10^{-3}M$ for $CaCl_2$ and $MgSO_4.7H_2O$, respectively, sporangial formation was actually stimulated.

The number of sporangia produced in each instance, a mean of 21 and 24 per microscope field, respectively (see Tables 12 and 17), was greater than the number found in distilled water, a mean of 16 per microscope field.

$CuSO_4.5H_2O$ and $ZnCl_2$ constituted another category of extremely inhibitory salts. Very few sporangia, a mean of 2 to 3 per microscope field, were produced in solutions of $CuSO_4.5H_2O$ and $ZnCl_2$ at a concentration of $1x10^{-7}M$. The remaining concentrations were completely inhibitory (see Tables 14 and 21). The remaining five salts $FeCl_3$, K_2HPO_4, $MnSO_4.4H_2O$, NaCl and $(NH_4)_2PO_4$ formed a median group of

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slightly inhibitory salts. The degree of suppression, varied among the group. Whilst K_2HPO_4, NaCl and $(NH_4)_2PO_4$ permitted sporangial formation at $1x10^{-3}M$ (see Tables 16, 19 and 20) $FeCl_3$, $MnSO_4.4H_2O$ were less favourable and the highest concentration permitting sporangial formation was $1x10^{-5}M$.

It is generally recognized that there is no qualitative difference in the inorganic requirement for fungal sporulation and growth. Quantitatively, however, there are differences, most of which are subsumed in the generalization that sporulation is possible over narrower range than growth.

A. megasperma also mostly grew over a much wider range of salt concentration than it did sporulate. The only exceptions were $CaCl_2$ K_2HPO_4 and $MgSO_4.7H_2O$. A summary of the observations on vegetative growth and sporangial formation in the various salt solutions is shown in Fig..13....

Two factors were likely to have operated in the response of the fungus to the various salts. Experiments on pH response showed that growth was possible from pH 5.0 to 9.0. Growth was best at pH 8.0 (see Table 7). The greatest number of sporangia was, however, formed at pH 9.0, in experiments in which the medium, distilled water, was adjusted with either 0.5N HCl or 0.5N NaOH (See Table 8). Large sporangia,

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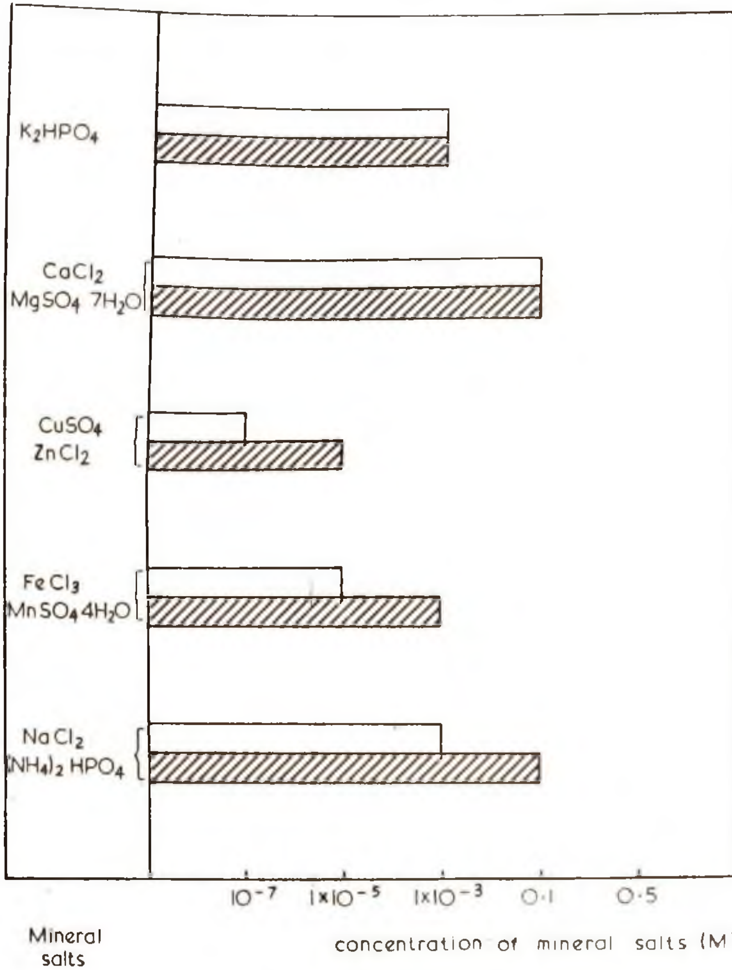

FIG 13 Diagrammatic illustration summarising vegetative growth and sporangial formation by A. megasperma in solutions of various salts

[ ] Sporangial formation [hatched] Vegetative growth

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310.2 to 355.5 um long averagely, were formed at pH 6.0 and 8.0 whilst the sporangia at pH 4.0 and 5.0 were significantly smaller, a mean of 263.0 and 255.7 um, respectively (See Table 9). Furthermore, the sporangia produced at pH 4.0 were inviable.

The sensitivity of A. megasperma to acidic pH was likely to have influenced the response of the fungus to some of the inorganic salts. Particularly $CuSO_4.5H_2O$ 0.1 to 0.5M (pH 3.1 to 3.7) and $FeCl_3$, 0.1 to 0.5M (pH 1.2 to 1.6) (See Tables 14 and 15). The inhibitory nature of $CuSO_4.5H_2O$ and $FeCl_3$ was most probably more critical than pH at those concentrations. For, in each instance, lower salt concentrations with more favourable pH, $1x10^{-5}M$, $CuSO_4.5H_2O$ (Initial pH6.4) and $1x10^{-3}M$ $FeCl_3$ (Initial pH 6.3) were still completely inhibitory.

Zinc at the higher concentrations of $1x10^{-3}$ to 0.5M was found to be toxic. Nickerson and Chadwick (1946) found that Zinc was toxic to fungi at higher concentration especially at high pH. The recorded pH of 7.0 to 8.0 could be high enough to make Zinc chloride at these concentrations exceptionally toxic.

The utilization of many salts is influenced by other compounds in the medium. The rate of absorption of Phosphorus

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is retarded if available Carbon or Nitrogen is made limiting (Cochrane, 1958). The optimum concentration of Magnesium is dependent on the concentration of the Carbon source (Steinberg and Bowling; 1939). During these studies the mycelium was completely submerged in solutions of the salts. It is impossible at present to relate the response of the salts to the level of Carbohydrates present in the hemp seeds as this has not been estimated.

The method used here, however, provided more satisfactory conditions nutritionally than studies of Rao, Desai and Kulkarni (1967) where the effects of the salts were studied in the absence of any nutrients and the present results should be a true response of the fungus to the different salts. Rao *et al* (*op. cit.*) immersed bits of mycelium of one week old cultures of *Phytophthora palmivora* and *Phytophthora parasitica* var *macrospora* in the salt solutions and examined them under the microscope after three days for sporangial formation. They could under those condition clearly distinguish the effects of the different salts. There was abundant sporangial formation by both fungi in N/10 solutions of Potassium nitrate, Potassium permanganate, Sodium chloride and Sodium thiosulphate. No sporulation occurred in each fungus in the solutions of Ammonium oxalate, Copper sulphate, Ferrous sulphate, Potassium chromate, Potassium dichromate,

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Strontium sulphate and Zinc sulphate.

Results of the physical and chemical examination of Sohenko Pond and the data obtained in similar studies of other lakes in Ghana (See Table 49) show the approximate content of ions in local fresh waters, and usefulness of the concentrations of the salts employed in these studies. _A_ _megasperma_ is most likely to grow and sporulate in the two examples judging by their composition and hence its suitability to test for pollution in them.

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TABLE 49

Concentration of ions (mg/1) in Sohenko pond, Lake Bosumtwi and the Volta Lake.

Ions	Concentration in mg/1		
	Sohenko Present Studies	Bosumtwi (Whyte,1976)	Volta (Entz,1966)
Acidity	35.0	-	-
Alkalinity	25.0	-	-
Ca^{++}	14.0	16.0	7.91
Cl^{-}	60.0	71.0	1.61
Cu^{++}	0.022	-	-
Fe^{+++}	0.0	0.0	0.0
K^{+}	24.0	26.0	4.73
Mg^{++}	1.3	16.0	1.87
Mn^{++}	1.5	-	-
Na^{+}	47.0	480.0	3.49
NO_3^{-}	99.2	0.013	-
N_2 (Ammonia)	41.0	0.00	0.02
PO_4	46.5	0.10	-

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No chemical standards of quality for drinking water have been put forward officially in this country. In the U.S.A., however, where the quality of natural waters is much more variable, various physical and chemical standards in addition to bacteriological standards, have been published from time to time. Examples of the maximum permitted concentration published by the United States Public Health in 1946 are 1.00, 0.3, 125.0, 0.1, 250.0 and 15.0 p.p.m. for Copper, iron, Magnesium, Manganese, Sulphate and Zinc, respectively. Any standards that will be adopted in future in this country would be close to these figures. The response of *A. megasperma* to these salts indicate that it could be used to detect excesses of these salts beyond these limits. For, the highest concentrations of $CuSO_4.5H_2O$, $FeCl_3$, $MgSO_4.7H_2O$, $MnSO_4.4H_2O$ and $ZnCl_2$ permitting sporangial formation were 0.025 p.p.m. ($1x10^{-7}M$ $CuSO_4.5H_2O$), 0.16 p.p.m. ($1x10^{-5}M$ $FeCl_3$), 246.4 p.p.m. ($1x10^{-3}M$ $MgSO_4.7H_2O$), 0.22 p.p.m ($1x10^{-5}M$ $MnSO_4.4H_2O$) and 0.014 p.p.m. ($1x10^{-7}M$ $ZnCl_2$).

In addition to the possibility of using *A. megasperma* to detect specific levels of mineral salts, it could clearly be employed to assess concentrations of organic compounds. Concentrations of sugars in fresh water bodies rise to high levels due to waste waters from local industries such as dairies, breweries, sugar factories and from wood processing.

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Starch comes from wood processing and textile industries and other organic materials are produced by pharmaceutical plants and glue and gelatine plants.

A. megasperma was particularly sensitive to Fructose, Glucose, Lactose, Mannose and Sucrose, where in each case 2.0 per cent concentration was approximately the inhibitory level. The average number of sporangia produced per microscope field for the highest sugar concentration that allowed sporulation was mean of one sporangium per microscope field for 1.0 per cent Fructose, Glucose and Mannose (See Tables 26, 28 and 30). A mean number of one sporangium per field was produced in 4.0 per cent Galactose and 2.0 Lactose solutions. The pH in each case, Fructose (Initial pH 6.3), Glucose (pH 6.5) and Mannose (pH 5.6) was not likely to have contributed to the low sporulation in the sucrose and fructose solutions as they fell within the favourable pH range. It is not unlikely that pH's close to pH 5.0 in Glucose (See Table 28), Lactose (See Table 29) and Mannose solutions (See Table 30) might have suppressed fructification.

The variation in response to the different Carbohydrates is another typical example of the inability of fungi to use different carbon compounds to the same extent. One per cent solutions of Dextrose, Fructose, Galactose, Glucose and Laevulose, for example, supported abundant sporangial formation

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in P. palmivora and P. parasitica var macrospora whilst maltose, sucrose and xylose supported just moderate sporulation in P. palmivora and very scanty sporangial formation in P. parasitica var macrospora (Rao et. al 1967).

The general suppression of sporangial formation by high concentrations of carbohydrates could have, besides other factors been due to high osmotic concentration of the solutions. All the concentrations of the sugars used, contrary to their effects on sporulation, supported abundant vegetative growth.

Possible polyhydric alcohols that could be produced from these sugars in the natural habitat through the action of microorganisms also showed varying effects. Thus, Mannitol and Sorbitol were inhibitory only at higher concentrations of 0.6 and 0.8 per cent, respectively (See Tables 37 and 38), whilst Glycerol greatly reduced sporangial formation even at 0.1 per cent supporting a mean of two sporangia per microscope field (See Table 36) and Dulcitol, on the other hand supported greater sporangial formation at concentrations of 0.1 and 0.4 per cent, 33 and 23 mean sporangia per microscope field, respectively, (See Table 35) than the mean of 15 sporangia in distilled water.

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If the conversion of sugars into these alcohols is a common phenomenon in fresh water bodies, tests using *A. megasperma* as a detector of sugar levels are likely to be upset not only by reduction in original levels of the sugars but also by the presence of the accumulating polyhydric alcohols.

The influence of nitrogenous compounds and complex nutrients provided by Potato dextrose broth was considerable and very varied. Potato dextrose broth would not support sporulation (See Tables 22 to 25) neither would peptone at the low concentrations of 0.01 to 0.1 per cent (See Table 33) and Ammonium nitrate, Haemoglobin and Malt extract at 0.1 per cent concentration (See Table 32). The formation of sporangia at lower concentrations of 0.001 per cent peptone, that supported a mean of 26 sporangia and at 0.05 per cent malt extract (See Table 34) and 0.05 per cent yeast extract (See Table 33) which gave the respective mean number of sporangia of six and 20 per microscope field, indicated that concentrations of 0.01 per cent and above fell outside the suitable range for these compounds. This view is supported by the greater number of sporangia, a mean of 43 sporangia per microscope field, at 0.01 per cent concentration of Yeast extract (See Table 33) than in water (a mean of 17 sporangia), presumably the optimum or close to the optimum for Yeast extract.

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The effect was directly on the mechanism of sporulation since the mycelium grew well and even induced branching of the hyphae in potato dextrose broth (See fig 10). It is to be expected, however, that in common with other fungi sporulation would be low in the event of profuse vegetative growth.

The bacteriological examination of the pond water revealed the presence of E. coli, which is an indicator of faecal pollution and other coliform bacilli namely P. vulgaris, C. freundii and K. aerogenes. Differentiation of these members was done by the I.M.Vi.C. test. Positive Indole result (See Table 42) showed the presence of E. coli and P. vulgaris. The presence of E. coli was also confirmed by a Positive Methyl Red test (See Table 43). C. freundii and K. aerogenes were also shown to be present by a positive result with citrate medium. The inability of E. coli to produce acetyl methyl carbinol from dextrose was shown by the negative results in the Voges-Proskauer Test.

When Coliform Test was carried out, the pond was found to contain sufficient dung matter to give an M.P.N value of 1800. Results of the various tests provided in Tables 47 and 48 indicated that the dung level might be approximately equivalent to concentration of 8.0 g/1. A concentration that gave an M.P.N value of 1800 and also supported sporangia formation a feature that allows the fungus to thrive in the

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pond. Dung level of 20 g/l was definitely inhibitory (See Table 47).

Dung and sewage contain both mineral and organic matter, both in colloidal dispersion and in true solution. Unfortunately these substances may show profound differences in concentration from one locality to another and also at different times in the same area. Thus the organic and mineral matter of dung may vary considerably in composition and strength due, for example, to difference in diet. _A. megasperma_ as a bioreagent for testing level of dung or sewage may therefore be quite unreliable and cannot be recommended as a test organism for dung pollution. In most cases its response would be more directly related to these compounds to which it is relatively sensitive. For instance, sporulation would be inhibited in the presence of a small quantity of dung with high mineral content, but sporangia would be formed in solutions with comparatively larger quantity of dung containing low mineral salt component.

There were two significant effects of some of the treatments on some aspects of sporangial formation other than the quantity of sporangia produced.

At pH 4.0 (See Table 8) and at certain dung concentrations, for example 20 g/l (See Table 47), the sporangia never matured.

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Information is not available now to enable a suggestion of the possible roles of these factors in the sporangial process. A parallel observation of the sporangium in which environmental factors influenced the physiology was made in P. palmivora and Phytophthora capsici under very different culture conditions by Hendrix (1967). P. palmivora produced large numbers of sporangia in response to light and cholesterol. In the dark this fungus produced a few sporangia in response to cholesterol. On sterol - free media exposed to light, P. palmivora sporangia initially appeared normal, although they were generally smaller and fewer in number than those produced on sterol - containing media. After a few days, however, these sporangia degenerated. The P. capsici isolate responded similarly. The abortive sporangia produced on sterol - free media exposed to light were extremely numerous.

In A. megasperma, pH 4.0 might have created an unfavourable environment for the processes of zoospore formation to proceed. It is likely that a component or some components of dung might have at high concentration become uncongenial. It can be inferred from these observations that delimitation of the sporangia was a less sensitive mechanism to these factors than the later processes of zoospore differentiation.

Particularly remarkable was the strong morphogenic effect of $CuSO_4.5H_2O$ and P.D.B. mixture, Galactose, Glycerol,

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Malt extract, Mannitol, Mannose and $ZnCl_2$ and P.D.B. mixture. The sporangia were markedly shorter especially at the higher concentrations of these substances. The average length of sporangia in $CuSO_4$ $1x10^{-5}M$ and 1:10 dilution P.D.B. mixture, 4.0 per cent galactose, 0.1 per cent glycerol, 1.0 per cent mannitol, 0.1 per cent Mannose and $1x10^{-5}M$ $ZnCl_2$ and 1:100 dilution of P.D.B. mixture were 169.5, 132.0, 162.6, 175.0, 149.3 and 118.3 um, respectively, in comparison to a length of 335 um in distilled water (See Tables 22, 27, 36, 37, 30 and 25).

In a few instances the effect was opposite. The length of sporangia in 0.1 and 0.2 per cent Dulcitol solutions were longer, 417.6 and 399.4 um averagely, than those in distilled water (372.0 um)(See Table 35). Any reports on investigations concerned with sporulation of A. megasperma must, therefore, clearly state the conditions of experimentation.

Sporangia of Phycomycetes seem to be rather plastic and their size and morphology are modified materially by environmental factors. Klotz and Fawcett (1934) found that sporangia of Phytophthora citrophthora from mycelium grown in glucose potato broth were smaller than those from prune juice.

The observations on P.capsici, Phytophtora phaseoli, Phytophthora mexicana and Phytopthora omnivora by Leonian (1971) were remarkable. The sporangia of P.capsici produced

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on potassium nitrate medium were largest, followed by those formed on egg plant (Solanum melongena L.). Next were those produced on bell pepper (Capsicum sp.) with the smallest sporangia formed on tomato (Lycopersicum esculentum Mill). P. phaseoli formed larger sporangia on bell pepper than on lima bean (Phaseolus lunatus L.). The sporangia of P. mexicana formed on egg plant were ellipsoidal and large; they were rounded and large when formed on potassium nitrate medium and extremely small and rounded on tomato. P. omnivora showed very different response to the same host plants. The sporangia on tomato were ellipsoidal while they were comparatively more rounded on potassium nitrate medium.

There are sufficient instances on record demonstrating that many other fungi can be made to alter their morphological features. Perhaps one of the most notable work along this line was that of Welles (1925) on the genus Cercospora. Through many extensive experiments he found that the size and septations of conidiophores and conidia were modified by the environmental changes and by the effect of different hosts to such an extent that one species would readily pass for an entirely different species. Welles concluded, although too strongly, that...... "so far as the genus Cercospora was concerned, morphological characteristics have no value." Taubenhaus (1915) showed that, when sweet potato (Ipomoea batatas Lam.)

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was inoculated with Diplodia gossypii, the fungus assumed the characteristics of the supposed genera of Lasiodiplodia, Chaetodiplodia Botryodiplodia and Diplodiella. The same was found to be true when sweet potato was inoculated with Lasiodiplodia. A mere change of host plants, therefore, produced or abolished generic differences. There are examples in rust fungi where morphological characteristics are by no means constant or reliable. Long (1914) observed that the influence of host plants upon the morphological characters of the rusts, Puccinia ellisiana and Puccinia andropogonis was very startling. P. ellisiana infects both Viola and Pentstemon, but when it passed through the latter host its characteristics entirely changed and it became like P. andropogonis. When species of Viola were inoculated with P. andropogonis; the ensuing fungus became, so far as could be determined, P. ellisiana . Stakman and Levine (1918) and Levine (1923) stated that uncongenial host as well as adverse environmental conditions tended to decrease the size of the uredium and uredospores of Puccinia graminis.

Another evidence has been provided here to support the belief that morphological characteristics at least in some fungi, should not form the exclusive basis for the separation of species.

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These studies have shown that it would be possible to use *A. megasperma* under certain circumstances to test for both the presence and the level of mineral salts, carbohydrates and nitrogenous compounds in fresh water bodies by assessing its degree of sporulation. This could be expressed, in the presence of these compounds, by a suppression of sporangial formation or by enhanced sporulation when some compounds are present.

In certain treatments, it was observed that the quantity of gemmae formed was influenced by the compounds. There were numerous gemmae in 0.4, 0.2 and 0.1 per cent Haemoglobin solutions and 1×10^{-5}M Zinc chloride solution (See plates 5 & 6). Since gemmae formation was not the primary objective of this study, detailed information has not been provided. Future investigations should critically assess formation of the gemmae as a possible additional habit to be employed in detecting pollution.

A. megasperma sporulated to the same degree in both light of 250 ft. C. and dark (See Table 6). Tests conducted in the field with this fungus would, therefore, not be affected by light conditions. Tests could therefore be located in water bodies at both shaded or exposed sites.

The procedure of the use of standard test animals is recognized in other fields of biological investigation as well as in detection of chemicals in water bodies.

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No single test organism combines all of the desirable qualities, and in making a choice both availability and physiological suitability for the problem in hand must be considered.

An arbitrary application of lethality or inhibition data to specific pollution problem is, however, absolutely impossible owing to many limiting factors. In every test the response of the organism to all other factors in the habitat apart from the specific feature under investigation must be clearly known. The differences observed between sporangial formation in *A. megasperma* in sterile and non-sterile dung solutions (See Tables 46 and 47) illustrated the interference of other factors - microorganisms to the response of the fungus to dung. Non-sterile dung solution of the concentration 20g per litre inhibited formation of normal sporangia and supported production of a mean of 26 sporangia per microscope field at a concentration of 1g. per litre. The corresponding values for the sterile dung solution were two and 33, respectively.

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VI. SUMMARY

1. *A. megasperma* grew best and produced the largest number of sporangia at 30°C.

2. Sporangia were formed over the range 20°C to 35°C but not at 40°C

3. Temperature influenced sporangial length. The sporangia were averagely 382.8 µm long at 30°C and 232.3 µm long at 20°C

4. The same number of sporangia was formed in light and dark.

5. McIlvaines Buffer solutions of pH 2.2 to 8.0 inhibited growth but the fungus sporulated from pH 5.0 to 9.0 with an optimum at pH 9.0 in distilled water adjusted with 0.5N HCl and 0.5N NaOH.

6. Sporangia were delimited at pH 4.0 but never completed development and never produced zoospores.

8\. The effects of mineral salts $CaCl_2$, $CuSO_4.5H_2O$, $FeCl_3$, K_2HPO_4, $MgSO_4.7H_2O$, $MnSO_4.4H_2O$ NaCl, $(NH_4)_2HPO_4$ and $ZnCl_2$ were very variable.

9\. There was no growth at a concentration of 0.5M of all the salts.

10\. The highest salt concentrations which supported vegetative growth were:

(a) 0.1M: $CaCl_2$, $MgSO_4.7H_2O$ NaCl and $(NH_4)_2HPO_4$.

(b) $1x10^{-3}$M: $FeCl_3$, K_2HPO_4 and $MnSO_4.4H_2O$.

(c) $1x10^{-5}$M: $CuSO_4.5H_2O$ and $ZnCl_2$.

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11. There was sporulation at the following salt concentrations:
    (a) $1x10^{-7}M$: $CuSO_4.5H_2O$ and $ZnCl_2$.
    (b) $1x10^{-7}$ to $1x10^{-5}M$: $FeCl_3$ and $MnSO_4.4H_2O$
    (c) $1x10^{-7}$ to $1x10^{-3}M$: K_2HPO_4, NaCl and $(NH_4)_2HPO_4$.
    (d) $1x10^{-7}$ to 0.1M: $CaCl_2$ and $MgSO_4.7H_2O$.
12. Fewer sporangia were produced at all concentrations of $CuSO_4.5H_2O$, $FeCl_3$, $MnSO_4.4H_2O$ and $ZnCl_2$ which supported sporulation than in distilled water.
13. A greater number of sporangia was produced in solutions of certain concentrations of $CaCl_2$ and $MgSO_4.7H_2O$ than in distilled water.
14. At their most favourable concentrations, sporulation in solutions of K_2HPO_4, $NaCl_2$ and $(NH_4)_2HPO_4$ was similar to that in distilled water.
15. The sporangia formed in many of the salt solutions especially at the higher concentrations were markedly shorter than those formed in distilled water. $ZnCl_2$ also altered the morphology of the gemmae.
16. Sporangia were not formed on Potato dextrose broth, but a few were produced when Potato dextrose broth was mixed separately with $CuSO_4.5H_2O$, $FeCl_3$ or $MnSO_4.4H_2O$. The fungus, however, grew very well in all the media.
17. Sporulation in solutions of the carbohydrates fructose, galactose, glucose, lactose, mannose and sucrose was poorer than in water and 3.0 per cent concentration of this sugar except galactose inhibited sporangial formation.

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18. A concentration of 0.1 per cent of the nitrogenous compound, Ammonium nitrate, Haemoglobin, Malt extract, Peptone and Yeast extract inhibited sporangial formation.
19. Low concentrations of yeast extract, 0.01 and 0.05 per cent supported abundant sporangia: Mean number per microscope field 43 and 20 respectively.
20. The suitable concentration of Peptone was extremely low. The highest concentration of peptone permitting sporangium formation was 0.001 per cent.
21. Vegetative growth was profuse in P.D.B, and in solutions of various carbohydrates and Nitrogenous compounds used.
22. The fungus sporulated to a varying degree in various polyhydric alcohols.
23. Dulcitol was the most favourable alcohol tested. The mean number of sporangia per microscope field at Dulcitol concentrations of 0.1, 0.2, 0.4 and 0.6 per cent was 33, 28, 23 and 17, respectively.
24. Sporulation was moderate in Mannitol and Sorbitol solutions.
25. Poorest sporulation occurred in Glycerol. The most favourable concentration was 0.2 per cent which supported a mean of two sporangia only per microscope field.
26. Changes in density by addition of 0.1 to 1.0 g/l pyridine-washed agar neither suppressed nor improved sporangial formation in comparison with sporulation in distilled water.
27. Solutions of Carbohydrate and Nitrogenous compounds became viscid during growth of the fungus but this change in medium density was not found to influence sporulation.

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28. Coliform test showed that the Sohenko pond was highly polluted with faecal matter, particularly immediate after the visit of the cattle.
The M.P.N. index per 100 ml of the water sample was 1800.
29. Differentiation of members of the coliform by I.M.Vi.C test showed the presence of E. coli, P. vulgaris, C. freundii and K. aerogenes.
30. Concentration of dung solution which gave an M.P.N. equal to 1800 and supported sporulation was 8g/l.
31. The results showed that A. megasperma could be used to assess concentration of mineral salts, carbohydrates and nitrogenous compounds in fresh water bodies but would not be a desirable bioreagent for assessing faecal pollution.
32. Because of the considerable effect of environmental factors on morphology of the sporangium, conditions of study of this fungus in any report must always be very clearly indicated.

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