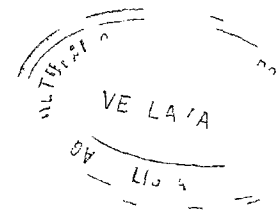
MORPHOLOGICAL & PHYSIOLOGICAL STUDIES ON *Helminthosporium halodes* DRECHS. THE 'LEAF ROT' FUNGUS OF COCONUT



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THESIS

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C E R T I F I C A T E.

This is to certify that the thesis herewith submitted contains the results of bonafide research work carried out by Shri S.N.Shanmughom, under my supervision. No part of the work embodied in this thesis has been submitted earlier for the award of any degree.

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S.N. S H A N M U G H O M.

CONTENTS

INTRODUCTION.

The genus Helminthosporium includes a large number of species distributed widely in nature. A good many species are parasitic on higher plants. Almost all the cereals and a number of other cultivated plants are attacked by one or more species of Helminthosporium.

Only three species of this genus namely, H.halodes on coconut, H.oryzae (Cochliobolus miyabeanus) on rice and H.heveae on rubber are economically important in Kerala. H.oryzae because of its occurance in almost all the rice growing countries of the world has been studied extensively. But our knowledge about H.halodes is far from complete.

This fungus causes the leaf rot of the coconut palm, which occurs in a severe form in the southern Districts of Kerala especially along the coastal belt.

H.halodes was first described by Drechsler(1923) on Distichlis spicata. A comparison of the coconut isolate with Drechsler's original description indicated that the former differed from the latter in certain important respects. The spores of the coconut fungus were markedly larger and it produced secondary conidia in abundance, on short conidiophores, which were formed at the apex of the primary conidia, even while they were attached to the primary conidiophores. It was therefore thought that the fungus may not be the same as that originally described by Drechsler as H.halodes. An attempt was therefore made to compare the coconut fungus with the other isolates of H.halodes

already described.

Cultures or herbarium materials of Drechsler's fungus were not available for comparison. However, a culture of H.halodes isolated by Subramaniam(1936) from sugarcane seedlings was available at the Centraal Bureau Voor Schimmelcultures, Netherland, and this culture was used for comparison.

An attempt was also made to study the growth and sporulation of the fungus in different common media and also on the media at different pH and containing different carbon and nitrogen sources.

REVIEW OF LITERATURE.

The validity of the genus Helminthosporium.

Link(1809) published the genus Helmisporium based on H.valutinum Link ex S.F.Gray, which was validated in 1821 by S.F.Gray's publication. In 1822 Persoon altered the spelling of the name to Helminthosporium. The name Helminthosporium has been recognised by Saccardo(Sylloge Fungorum) and Lindau(Die Pilze Deutschlands, Oesterreichs Und Der Schweize) and has been adapted by Drechsler(1923) in his monographic treatment on the graminocolous species of Helminthosporium. Since then the mycologists throughout the world have generally adapted this name.

Recently, Shoemaker(1959)challenged the validity of the generic name Helminthosporium and proposed certain important changes in the nomenclature of the graminicolous species of the genus. He regards Helminthosporium only as an orthographic variant of Helmisporium. So he suggests that the generic name Helmisporium Link ex S.F.Gray is a more legitimate one. But he restricts this name to the generic type and its chiefly lignicolous allies, all of which form conidia both apically and laterally on the conidiophore. He recognises another generic name Drechslera which was originally proposed by Ito(1930) and includes sixteen species with cylindrical conidia that germinate from all cells. Shoemaker has also proposed another generic name Bipolaris

to include the major groups of graminicolous species of Helminthosporium which form fusoid phragmospores and exhibit bipolar germination. This change in the nomenclature of Helminthosporium is yet to be approved and adapted by mycologists in general.

Helminthosporium halodes Drechsler

Helminthosporium halodes was first described by Drechsler in 1923. During the later part of growing season of 1920 he kept under observation a stand of Distichlis spicata(L). Greene, growing on a salt marsh near Douglaston, N.Y., on the northern coast of Long Island. Microscopical examination of dead affected foliar parts revealed a species of Helminthosporium. The investigator also succeeded in isolating pure culture of this fungus from the diseased material. He has compared this fungus with H.sativum to which it showed certain similarities. The symptoms and the types of conidia were found to be more or less similar. But the conidia of the new fungus were narrower and not as regularly crescentric, being more frequently straight or irregularly curved. In colour they were usually brownish yellow instead of olivacious. In a certain proportion of the spores, moreover, the end cells were less deeply coloured and the basal and distal septa appeared conspicuously darker or heavier than the intermediate cross walls. When developed in a damp chamber on

diseased host materials, the conidia approached those of H.sativum in depth of colouration; but the distinction between the dark intermediate segments and subhyaline or fuliginous end segments set off by the conspicuously accentuated septa became constant characteristics.

The peripheral wall of the conidium of this new Helminthosporium varied more or less in thickness. The darker and more mature conidia usually had the wall of at least moderate thickness. As in H.monoceras, the wall was very thin at the apical end and over a narrow zone at the proximal end immediately adjacent to the hilum. Germination regularly occured by the production of two polar germ tubes, one from each of these thin walled regions, however, the immature spores which were brownish yellow in colour and the peripheral wall was thinner, germinated by the production of lateral germ tubes from the intermediate segments as well as by polar germ tubes from the end cells. But the polar germination was regarded as typical by Drechsler for this species.

The fungus was cultivated readily in artificial media and produced a luxuriant dark olivaceous aerial growth, consisting of a variable quantity of mycelium bearing on abundance of sporophores. The spores were attached to the sporophores at short intervalsa at wide angles and in

moderately compact reoemose arrangement. The conidia usually exhibit irregularities in shape, including flattening or bifurcations of the apical portion.

The author has further compared this fungus with H.Leersii and more especially with H.Oryzae and H.rostratum. From H.leersii it may be distinguished by the sub-hyaline end-cells, and accentuated and septa and the protruding hila. H.Oryzae the rice parasite produced spores which are of greater dimensions and the position of the hilum was within the contour of the peripheral wall of the spores, although the end cells of H.oryzae also were sub-hyaline. Moreover, while the spores of H.oryzae tend to taper strognly towards the tip and to a smaller extent towards the base, those of the new fungus showed relatively slight tapering towards the abruptly rounded apex, while the proximal portion usually tapers markedly towards the base. The resumblance of H.rostratum to the new Helminthosporium is attributable to the similarity in colour of the conidia, protruding hila and accentuated proximal and distal septa. The new fungus, however, had inferior dimensions, both for spores and sprophores. The rostrate modification of the apical portion of the spores found in H.rostratum was also not present in the new fungus. Drechsler gave the specific name halodes to this, hitherto undiscribed species of Helminthosporium taking into consideration of the frequently associated nature of its host to sea water.

Drechsler gives the diagnosis of the fungus as follows:-

Conidiophores arising singly or in groups of two; measuring generally 4 to 7 u in diameter by 60 to 150 u in length producing first spores usually 60 to 100 u from the base successive spores at intervals of 5 to 15 u at the apices of geniculate irregularities; 1 to 5 septate, the length of segments highly variable typically 15 to 30 u. Conidia as produced under natural conditions, straight or curved, measuring 10 to 14 u in width by 20 to 105 u in length, 1 to 12 septate, brownish yellow, the end segments some times lighter in colour or subhyaline and delimited by accentuated septa; tapering slightly towards the broadly rounded apex and more markedly towards more narrowly rounded or somewhat acuminate basal end, which is distinguished by protruding hilum. Mature spores germinating by the production of two polar germ tubes at either ends, immature spores sometimes atypically by lateral germ tubes also.

On artificial media not rich in organic food material conidiophores arising as lateral branches from prostrate hyphae bearing conidia in moderately recemose arrangement at geniculate irregularities occurring at short intervals conidia more or less like that on natural substratum.

As per Shoemaker(1959) H.Halodes Drechs comes under the genus Bipolaris and the new combination is B.halodes.

Subramaniam(1936) isolated two species of

Helminthosporium from dying sugarcane, one of which was identified as H.halodes by its spore dimensions(20 to 104.5 u by 9.5 u and 3 to 10 septa). The author states that H.Halodes caused a very high percentage of infection not only on different cane varities but also on maize, sorghum, wheat and barley. This is believed to be the first record of H.Halodes on sugarcane. He deposited a culture of the fungus in the CENTRAAL BUREAU VOOR SCHIMMEL-CULTURES, Netherland. (This culture was obtained for comparision in the present study). H.halodes has also been isolated by Putterill(1954) from the foot rot affected wheat plants from South Africa.

Menon and Nair(1948) who have studied the 'leaf rot' disease of the coconut palm in some details, reported that H.halodes, Colletotrichum paucisetum and Glococladium roseum sp. are commonly found on rotted coconut leaves. Among the three, H.Halodes has been reported to be the most virulent one on the basis of inoculation experiments conducted by these authors. The measurement of conidia of this fungus isolated from Travancore area was 36 to 98 u x 10 to 18 u.

Varieties of H.halodes.

Since Drechsler's original publication of this species, two new varieties of this fungus have been described. Mitra(1931) isolated and described H.halodes var.tritici n.var. from foot rotted wheat obtained from the Central Provinces. Its conidiophores were similar to those of the type species; but

the conidia differed in size and septation. The conidia were cylindrical or elliptical with the distal end abruptly rounded and the proximal end tending to taper to an acute base; the basal cell was thus somewhat triangular with a prominant hilum at the end. The spores were widest near the middle, straight or slightly curved, two to nine septate (average,six) light greenish brown to brown or smoky brown, the two end cells being slightly lighter, their extreem measurements were 23 to 73 u by 13 to 20 u(mean- 52 u by 16.5 u). The same organism was later noted by Hynes(1937)on wheat affected with root rot in Australia.

Kovachich(1954) reported another variety of this fungus, namely H.halodes var.elaeicola on oil palm (Elacis guineesis) in Belgium Congo. He described the diagnostic features of the fungus as follows:-

Hyphae-hyaline to pale brown, three to five u wide. Conidiophore single or in small groups emerging through the epidermal cells, straight or flexuous, sometimes geneculate, brown, pale at the apex with one or more well defined scars, 25 to 150 u long, five to eight u wide with a swollen basal cell 12 to 17 u wide. Conidia - straight or slightly curved elliptical to obclavate olive brown, 33 to 125 u by 10 to 18 u with a protruding hilum 2 to 3 u wide, 4 to 12 septate, the end septam accentuated. In culture conidia were cylindrical to elliptical; but never becoming obolavate. They were 32 to 96 u x 10 to 16 u, 5 to 10 septate. The mature conidia germinted typically by production of two polar germ tubes. The

author pointed out that this fungus although indistinguishable from H.halodes in culture. The production of obclavate conidia on the host serves at once to distinguish the variety. He has also referred to H.halodes var.tritici of Mitra(cf.Mitra,1931) and pointed out the differences in spore dimensions.

Leaf rot disease of the coconut palm.

According to Menon and Nair(1948) this serious disease was known to Cochin as early as 1914, and Dr.McRae in 1916 and later Sundararaman in 1924 have made some investigations on this disease. Dr.E.J.Butler has reported the occurrence of this disease in Travancore area as early as 1908. But a detailed investigation on this disease has been made only after the lapse of some years, by Menon and Nair (1948). They have studied this disease for its distribution, economic importance, symptoms, causes and pathogenecity. After isolation and inculation experiments both in the laboratory and in the field conditions, they have proved that three fungi, namely, H.Halodes, Colletotrichum paucisetum and Gloeocladium roseum are responsible for this disease, the first one being the most virulent.

Later on, the same workers(1951) made aeroscope studies and investigations on the physiology of the three causal organisms, viz.H.halodes, G.roseum and Gloeosporium sp. which were reported to be the causal agents. Prasannakumari et.

FIG.1. LEAF ROTTED PALMS

al(1960) have studied the control aspect of the disease and Radha et al (1961) have studied this in relation to the environmental conditions.

Symptoms(Fig.1)

More detailed investigations on this disease was made by Menon and Pandalai(1958). They described the symptoms and also factors favouring infections.

The first visible symptom of this disease is a blackening and shrivelling up of the distal ends of the leaflets in some of the inner whorls of leaves. On drying up, they are broken off into bits by the winds and the infected leaves assume a fan like appearence which is charateristic of the disease. The central spindle also gets infected. Reddish brown spots and patches appear on the tender leaves and these penetrate to some distance into the interior of the shoots. The spots enlarge in size and a soft rot of the central shoot is developed. High humidity favours infection. When weather gets hot and dry, the rotten portion dry up and turn black and fall off in the wind. In severe cases the rotten portions dry up,will be so completely cemented together, that the central shoot is prevented from opening out. The leaf rot does not kill the palm out right; but it progresses slowly and steadily until finally the tree succumbs to the disease. Reduction in leaf area and destruction of the leaves and consequent reduction in the photosynthesis causes a considerable reduction in the yield.

Physiology

Synthetic media are usually employed for the study of the growth requirements of fungi. Raulin(1869) was the first to devise a synthetic medium (Lilly and Barnett, 1951). Various authors followed this pioneer and suggested different types of artificial media which are now commonly employed for the in vitro studies of fungi.

They are selective as regard to their carbon nutrition. Response of fungi to various carbon sources varies considerably. In case of Sclerotinia Sclerotiorum on the rate of radial growth. But carbohydrates were essential for the formation of sclerotium. Growth were equally good when dextrose, sucrose, raffinose and potato starch were used (Tanrikut and Vaughan 1951). Wardlaw(1932) reported the marked capacity of Botryodiplodia theobromae Pat.for its growth on high concentration of starch, sucrose and other carbohydrates. According to Steinberg(1959 b) sucrose,d-glucose, d-fructose, d-mannose and l-sorbose were excellent sources of carbon for Aspergillus niger, whereas glycerol, d-mannitol, d-lactose, d-galactose proved quite ineffective. Schade(1940) found that sugars are unavailable to Liptomitus while Apodachlya grows well on dextrose, levulose and sucrose, but not at all on galactose and maltose. The best carbon source for Ophiobolus graminis was found to be maltose, 2% dextrose was optimum

(Gilpatrik and Henry 1950). Beckmen et al (1953) have reported the comparative yield of Chalara quercina(the Oak wilt fungus) as follows:- The yield on dextrose and xylose were best, on maltose, fructose, cellobiose, galactose, glucose, raffinose, mannose, sucrose and xylan were good; on pectin, arabinose and melibiose were fair; on rhamnose, starch, lactose and cellulose were poor. Tanak (1956) studied the growth of Cochlioblus miyabeanus and found that the growth was best with maltose followed in descending order by fructose, sucrose, galactose, glucose xylose and lactose. Thind and Sandhu (1956) have reported that arabinose, dextrose, fructose, sorbose galactose, sucrose and starch are good carbon sources for the growth of Gloeosporium psidii. Grewal (1957) has shown that out of 19 carbon compounds studied arabinose, rhamanse, lactose, sucrose, glucose, and erytheritol supported good growth of Gloeosporium musarum, G.papayae and Colletotrichum papayae.

Like carbon sources fungi may be specific about the nitrogen source also. One serious problem in the study of nitrogen nutrition of fungi is that there is no optimum amount of nitrogen for a culture; the demand depend in the first instance upon the carbon supply, but in principle atleast any factor may change the apparent optimum concentration of the nitrogen source (Cochrane, 1958).

Fungi can utilise organic and inorganic nitrogen. But specificity in the ability of utilizing the various nitrogen sources by different fungi is conspicuous.

Steinberg (1939a) in his work on Aspergillus niger concluded that nitrates, ammonium salts and nitrohydroxylaminic acid salt were found to be the best sources of inorganic nitrogen for its growth. Nitrite, hyponitrite, hydrozine, azide and nitrous oxide were useless for growth. Hydroxylaminic acid was a poor source of nitrogen. Ammonia was concluded to be the primary source of nitrogen for conversion to organic nitrogen. In his nutritional investigations of Leptomitus lacteus and Apodaclya brachynema Schade (1940) has reported that neither nitrate nor ammonia is a suitable nitrogen source for either species. But the single amino acids d,l-alanine and l-leucine supported the growth of both species while glycine did not. Glycine and asparagine were untiliged by both fungi if acetate is present. As per the report of How (1940), while nitrate, asparagine, peptone and gelatin can serve as nitrogen sources for Boletus elegans, optimum growth is only obtained on inorganic ammonium salts. In the case of Ophiobolus graminis Sacc. peptone and asparagine proved to be the best sources of nitrogen(Gilpatrik and Henry,1950) The findings of Haskins and Weston Jr (1950) is that Karlingia(Rhizophlyctis) rosea prefers ammonia nitrogen to nitrate nitrogen when both are present, as with NH_4NO_3, though it can grow equally well on either when the other is absent. Of the nitrogen sources tested by them urea supported the best growth. It is found by Tanrikut and Vaughan (1951) that three organic forms of nitrogen had quite different effects on the growth of Sclerotinia sclerotiorum. When cystine is used it was equal

to the growth on no nitrogen. In asparagine growth was luxuriant. Urea was toxic to the fungus at concentrations greater than 0.5gm/lit. At 1gm/lit growth retarded at 3.5gm no growth at all. The best nitrogen sources for the Oak wilt fungus were arginine, asparagine and aspartic acid. Alanine, urea, meteonin and ammonium salt promoted growth. Phenylalanine glycine and histidine allowed sparse growth while nitrate salts, isoleucine, threonine, hydroxypyroline, tryptophane, tryosine, cystine, lysine and leucine promoted very little or no growth. Saksena et al (1956) established the capacity of various species of Pythium to assimilate nitrogen from sodium nitrate, ammonium chloride or from a single aminoacid but not from a nitrite. After a very detailed study on the assimilation of inorganic nitrogen by Scopulariopsis brevicaulis and some physiologically similar species, Morton and MacMillan (1954) found that several external factors influence the nitrogen assimilation capacity of these fungi. Their failure to assimilate nitrogen from ammonium sulphate has been shown to be due to the fall of pH of the medium induced by the initial uptake of ammonia. They have also found that in corresponding conditions S. brevicaulis assimilates ammonia more rapidly than nitrate over a wide range of conditions. Ammonia even in a very low concentration completely supresses nitrate assimilation when both sources of nitrogen are present together. Nitrate, however, is assimilated simultaneously with ammonia, It is therefore concluded that ammonia completely blocks the reduction of nitrate by the fungus. The supression of nitrate

assimilation in the presence of ammonia is common to many mould fungi besides S.brevicaulis and is believed to have adaptive significance in natural habits. Thind and Sandhu (1956) have reported that of the 36 nitrogen compounds, both organic and inorganic nitrate proved to be better sources of nitrogen than ammonium nitrogen for the growth of Gloeosporium psidii. Quite unexpectedly, the fungus gave good growth on nitrates of sodium and potassium which usually inhabited the growth of some fungi.

Under a given set of conditions a fungus will grow maximally over a certain range of pH values of the medium, and will fail to grow at high or low extremes. Furthermore, pH is affected during growth by metabolic activities - raised by absorption of anions or production of ammonia from nitrogenous compounds, lowered by formation of acids or absorption of cations (Cochrane, 1958). A double optimum is also sometimes been reported for certain fungi.

Dosdall(1923) reports that spores of Helminthosporium sativum germinate through a wide range of pH in phosphoric acid potassium hydroxide solution. He has observed a double optimum both on alkaline side viz, pH 8.2 and 9.2. In his trial, the maximum germination in Czapeks solution took place at pH 6 and 8. He has stated that in general, spores are more tolerant to alkalinity than acidity. Studies of Mitra and Mehta (1934) shows that in bactopeptone solutions the spores of H.nodulosum tolerated a pH range from 3.8 to 10, optimum for germination

and elongation of germ tube lying between pH6.5 to 6.9. The authors have also shown that the optimum pH for the growth (by dry weight) of H.nodulosm was pH 7.1 and for H.leucostylum 6.7Aoki(1937) states that Ophiobolus miyabeanus germinated through a wider range of pH than Piricularia oryzae which developed better at pH 5 to 6 and 8 to 9, than in medium adjusted to neutrality. Mycelial growth in the latter fungus is almost equally abundant at pH 4.6 and 9.6, so that the vegetative development and conidial germinability are evidently distinct phenomena. He also states that pH concentration chiefly effects the germ tube length rather than the percentage of germination in both fungi.

Wolf, Bryden and MacLaren(1950) have reported that, Monosporium apiospermum can grow within the pH range 3.6 to 10.8, with an optimum of 7.00 to 7.8. A lower chytridiales Karlingia (Rhizophlyctis)rosea has been reported by Haskins and Weston Jr.(1950) is able to grow within the rage of pH 3.4 to 8.0. Maximum growth occurred around pH 6.8 to 7.0. According to Tanrikut and Vaughan(1951) the limits in which Solerotinia sclerotiorum can grow appear to be pH 2.4 and 9.6, a tremendous range from extremely acid to very alkaline condition.

It is found by Saksena et al (1953) that various species of genus Pythium is able to raise the pH of the medium after incubation, and it is shown by them is due to the accu-

mulation of ammonia.

Many workers have made investigations on the influence of temperature on the growth and sporulation of fungi. The temperature growth curves have generally a characteristic linear portion in which the growth increases directly with temperature, there will be an optimum range though a narrow one and then a steeply descending limb (Cochrene,1958).

The cardinal temperature growth andreproduction of fungi show marked variation, Halma and Fawcett (1925) have reported that Helminthosporium sacchari could grow at all temperatures between 13.5 to 36° C and the optimum temperature for development was between 20 and 29°C. Nisikado (1927) has reported that growth of H.turcicum started between 5° and 8° C and ceased between 30° and 35° C with an optimum at 27° to 30° C. He has reported the minimum temperature for the mycelial growth of H.mayadis as 10° C, maximum 35° and optimum 30°C. He observed profuse sporulation in both the species at about 23° C. Shands (1934) has reported maximum, minimum and optimum temperatures for H.grameneum on potato dextrose agar as 32°C, below 8° C and 25°C respectively, but he observed that the highest incidence of disease occured in the field at a low temperature (12 to 16° C) than this optimum. Dosdall (1923) has observed variations in spore length for H.sativum at different temperature, shortest spores with mean length 55.98 u at 28° C and largest

with a mean length of 67.32 u at 14°C in potato agar medium.

Vitamin requirements differ from one kind of living organism to another and fungi are no exceptions to this. Mathur, Burnett and Lilly (1950) have shown that Colletotrichum lindemuthianum is partially deficient in inositol and biotin. Pyridoxine alone has no effect either on the rate, or amount of growth. Beekman et al (1953) found that a mixture of biotin, thiamine and inositol promoted growth of oak wilt fungus Chalara quercina. Gilpatrik and Henry (1950) studied on the nutrition of Ophiobolus graminis and found that biotin is necessary for its early growth and thiamine for further mycelial development. Timnick et al (1951) studied the effect of thiamine, biotin, inositol andpyridoxine on Diaporthe phaseolorum. The fungus showed partial deficiency for inositol but thiamine alone depressed the rate of growth. Leahart (1956) has demonstrated that some species of Leptographium associated with pole blight of western white pines are completely autotrophic in respect of vitamins, or do not require them for growth.

MATERIAL AND METHODS.

Cultures received from three sources were used in the present study:

(i) From the Central Coconut Research Station, Kayamkulam.(This will be referred to as 'C' hereafter).

(2) Isolate of Subramoniam(1936) from Sugarcane seedlings received from the Centraal Bureau Voor Schimmelcultures, Netherland.(This will be referred to as 'S').

(3) Isolate made directly from the diseased coconut leaf collected from Kayamkulam area.

Single spore isolates were made in all the cases. Morphological studies were also made for fungus on natural substrate. Attempts to obtain the original material reported by Drechsler(1923) from the American type Culture collections, Washington 7, were not succesful. Above mentioned isolate of Subramoniam(culture 'S') was therefore used for comparative purposes.

The morphological characters of the fungus were studied on the host andon potato dextrose agar medium. For comparison of the culture 'C' with 'S', both were inoculated on PDA plate cultures on the same day and incubated at room temperature on the laboratory table. Cultural characters were studied by taking radial growth measurements everyday from the second day after inoculation till the colonies just reached the edge of the plate and

observing the extent of sporulation and other characters under a stereomicroscope. Colour comparisons were made with 'A dictionary of colour' by Maerz and Rea' paul(1950). Conidiophores, conidia and mycelium for measurement were taken from 20 days old cultures. Water mounts were used for taking measurements. For obtaining spores from the host material, the following procedure was adapted:-

Bits of decaying leaves bearing conidiophores and conidia were collected from the field, brought to the laboratory and kept in humid chamber overnight. Scrapings taken from this were mounted in water and measurements made.

Germination tests were conducted at room temperature in hanging drops. For this, filter paper with a circular hole of 5 mm. diameter was placed in the bottom of the Petri dishes. Moistened with distilled water. A drop of spore suspension was centrally placed on a perfectly clean glass slide. This was then inverted and rested on two bits of glass rodes placed in the Petri dish in such a manner that the hanging drop remained over the hole. Germination in different substrates, namely, distilled water, tap water, sucrose and glucose solutions and coconut leaf extract were studied. The glucose solutions were used at 0.1 M concentration. The spore suspensions were so prepared as to give about 30 spores per field when observed under low power(16 mm.) objective with 10X ocular.

Physiological

The single spore isolate of the organism, made out of the culture received from C.C.R.S., Kayamkulam(culture C) was used for the study of the nutritional requirement and the influence of environmental factors on the growth&sporulation of the fungus.

Generally, Phytopathological and Botanical Research Methods by Rawlins(1933) and Introduction to Research on Plant diseases by Ricker and Ricker(1936) were referred to for the fomulae,. preparation of media and other laboratory techniques. Pyrex glasswares and reagent quality chemicals were used throughout the experiment. For solid media, nine-centimetre petri dishes and for liquid media 100 ml. Elenmayer flasks were used. In dishes 15 ml. and in flasks 20 ml. media were used as a standard. In the case of solid media, the standard volumes of 15 ml. media were first transferred into test tubes with pipette and were plugged with cotton wool and autoclaved. These media in tubes were then melted in waterbath and poured into the petri dishes aseptically. In the case of liquid media, the standard volumes of 20 ml.media were transferred into the culture flasks with pipette, plugged with cotton wool and then autoclaved. Generally, autoclaving was done for 20 minutes at 15 lb.pressure; but those media in which hydrolysis of sugars was expected, were sterlized in Arnold Steam Sterilizer, 2 hours each for 3 consequitive days.

Inoculation of solid culture was done by centrally placing circular discs of 5 mm. diameter cut out from actively growing zone of a plate culture. Liquid culture were also inoculated in the same manner with mycelial discs. In all cases except for the temperature study, the cultures were incubated for a period, till the growing edge of the eolony just reached the edge of the plate. Liquid culture were incubated for 10 days unless otherwise mentioned.

Measurements of the colony of plate culture were made by placing the plate on a linear scale. The radial length from the edge of the inoculam disc to the edge of the growing colony was taken as the measurement of growth. Average of four such readings was taken for each colony. The measurements were taken everyday from the second day of inoculation till the growing colony just covered the plate. In ease of liquid cultures the dry weights of the mycelium was taken as the criterion for measuring the growth. After the said period of incubation, the culture of each flask were vacuum filtered through previously weighed filter papers(Whatman No.1). Mycelial mat in the filter papers washed with distilled water, dried in hot air oven for 24 hours at 80°C along with the filter papers. After they have cooled down to room temperature, they were weighed to find out the dry weights of the mycelium, by deducting the weights of filter papers from the final weights. With

all batches 4 weighed filter papers without mycelium were also dried to find out whether there was any loss in weight of filter papers during drying. It was found that in no case the loss in weight of each filter paper exceeded one mg.

A rough apprisal of the sporulation was done by examining the plate cultures under stereo microscope. The sporulation was expressed only in grades such as profuse, good, fair and poor, according to the density of population of spores in a field of the stereo microscope.

The distilled water used for the preparation of media was obtained from a Manesty Water Still made of cast iron.

For adjusting the pH of the media, 6N NaOH or or 6N HCl solution was added to the medium. Determination of pH was done by using Universal Indicator(BDH) except in the case of pH study in which case pH-meter was employed.

Different Media.

The following media were tested for the growth and sporulation of the fungus under study. pH of all the media were adjusted at 6.0.

Liquid media

1.Potato Dextrose Solution

Sliced potato	200	gm.
Dextorse	20	..
Distilled water	1000	ml.

2.Richard's

KNO_3	10 gm.
KH_2PO_4	5 gm.
$MgSO_4$	2.5 gm.
$FeCl_3$	0.02 gm.
cane sugar	50.0 gm.
Distilled water	1000 ml.

3.Barne's

K_3PO_4	10 gm.
NH_4NO_3	1.0 ,,
KNO_3	1.0 ..
Glucose	1.0 ..
Distilled water	1000 ml.

4.Coon's

Saccharose	7.2 gm.
Dextrose	3.6 ..
$MgSO_4$	1.23 ..
Pot.acid phosphate.	2.72 ..
KNO_3	2.02 ..
Distilled water	1000 ml.

5.Czapeks

$MgSO_4$	0.5 gm.
KH_2PO_4	1.0 ..
Kcl	0.5 ..
$FeSO_4$	0.01 ..
$NaNO_3$	2.0 ..
Sucrose	30.0 ..
Distilled water.	1000 ml.

6.Czapek's Dox.

Sucrose	30.0 gm.
$NaNO_3$	2.0 ..
KH_2PO_4	1.0 ..
$MgSO_4$	0.5 ..
$FeCl_3$	0.5 ..
$FeSO_4$	0.01 ..
Distilled water	1000 ml.

Solid media

All the above media except Czapek's-Dox medium were used as solid media also. For this 20 gm. of agar per litre of media was also incorporated in addition to the other constituents of the media.

Deficient media.

To determine the importance of the different constituents in a medium, a study was made in Richard's solution. The treatment were so arranged that each one was deficient in one or the other constituents of the medium. For this basal solutions were first prepared for each constituents. Then these solutions were mixed in such a way that each was deficient in one of the constituents. A treatment with complete media was kept as control. The treatments and eliminations were as follows:

Treatments	Constituents eliminated
C	Control(no elimination)
1	KNO_3
2	KH_2PO4
3	MgSO4
4	$FeCl_3$
5	Sucrose

Different Carbon and Nitrogen sources:

This experiment was aimed at finding out the most suitable carbon and nitrogen sources that the

fungus under study can utilise. Czapek's medium was used as the basal medium changing the carbon and nitrogen sources in each treatment. Different sources of nitrogen were so selected as to supply ammonium nitrogen, nitrite nitrogen, nitrate nitrogen and organic nitrogen. One treatment was kept as a control without any nitrogen source. The quantities of nitrogen sources were so adjusted in all cases as to contain nitrogen equivalent to that in 2.0 gms. of sodium nitrate per litre, the nitrogen source of the standard Czapek's medium. In the case of carbon sources mono, di and poly saccharides were selected. Here also the quantity of carbon sources were so adjusted in all the cases as to contain carbon equivalent to that contained in 30 gms. of sucrose per litre, the carbon source of the standard Czapek's medium. In the study of carbon sources the media were steam sterilised for three consecutive days for a period of 2 hours per day. Studies were made on solid and liquid media and in all the cases The pH of the media were adjusted at 6.0. The following were the carbon and nitrogen sources used:-

Carbon sources

Treatments.	Carbon sources.	Wt.in gm/lit of medium
0	No carbon	00.000
1	Sucrose	30.000
2	Fructose	31.575
3	Glucose	31.575

Treatments.	Carbon sources	Wt.in gms/lit of medium
4	Maltose	31.575
5	Lactose	30.000
6	Starch	14.360
7	Collulose	14.360

Nitrogen sources

Treatment	Nitrogen source	Wt.in gms/lit of medium.
0	No nitrogen	0
1	Amm.sulphate	1.564
2	Amm:carbonate	1.130
3	Amm:nitrate	0.943
4	Sodium nitrite	1.150
5	Sod.nitrate	2.000
6	Urea	0.710
7	Asparagine	1.775
8	Gelatin	2.000
9	Peptone	2.000

Hydrogenion concentration

Czapek's solution was used in this study also. The pH of the media were first roughly adjusted with Universal indicator(BDH). 6N NaOH or 6N HCl was used to alter the pH. Each batch of the medium was then transferred to six culture flasks at the rate of 20 ml per flask, plugged with cotton wool and autoclaved. After

autoclaving one flask from each batch was drawn and the pH estimated with pH meter, correct to the first decimal place. Other five flasks of each batch were used for inoculation. After incubating the culture for thirteen days vaccum filtered and the pH of the filterate of each treatment determined with the pH meter.

Temperature

Czapek's agar media adjusted at pH 6.0 was poured in Petri dishes, inoculated with mycelial discs. These dishes were incubated at the temperatures, 0°,5°, 10°,15°,20°,25°,30° and 35°C in a cooling incubator. The above treatment were done one after another, because only one incubator was available. No light was provided in the incubator. The cultures got light every day for few minutes while the measurements were taken. After this it was found that the optimum range was between 25° and 30°C. Further study were therefore made at the temperatures 25°,26°,27°, 28°,29°, and 30°C.

Vitamins

Biotin and thiamine were tested at different concentrations for their effects on growth and sporulation. Czapek's solution adjusted at pH 6.0 was used as basal medium. After autoclaving the media, vitamins were added aseptically with sterile pipettes. For both the vitamins the concentrations of 5 ppm.,50 ppm.and 100 ppm. were tested. Control was also kept without vitamin.

RESULTS.

Morphological Characters of Culture.C.

The fungus is readily culturable in standard artificial media like potato dextrose agar, Czapek's and Richard's media. Colour of the colony yew green when young generally with hyaline growing region, colour becoming more or less black with age, reverse appear black. Grows very rapidly (7 to 9 mm.radial growth per day). Very little aerial growth, even that little growth consists of sporophore and spores. Growth is thick at the upper portion of the media and becoming sparse with depth. In liquid cultures it produce surface growth of black and thick mycelial mat with submerged hyaline mycelium.

Mycelium

In potato dextrose agar culture aerial mycelium sparsely developed, Mycelium mainly submerged with thickly growing mycelial mat on the surface of the medium, hyaline when young, changing to yellowish brown and then to dark brown with ageing. Hypha profusely branched and septate. The distance between the septa varies considerably from 8 to 50 U or more, generally 20 to 25 u. Diameter of the hypha from 3 to 6 u. The prostrate hyphae from which the conidiophores usually arise generally broader than other hyphae, and in these the cells sometimes are swollen to form barrel shaped or more or less rounded cells, near the region from where the conidiophores arise and sometimes in other

places also(Fig.2 and 3). Medium not coloured,but appear dark on account of the colour of the colony.

Conidiophore

The conidiophores arise mainly from the thicker prostrate hyphae, single, straight or curved and flexuous, often prominently geniculate, with dark scars on the geniculations where the conidia are borne on conidiophore, brown with lighter, subhyaline distal region, 2 to 16 septate generally 5 to 12 septate. Distance between the septa highly variable generally from 17 to 30 u sometimes even 60 u or more. Length of conidiophores also highly variable from 60 to 350 u generally between 100 to 200 u(Fig.2,3 and 4). Conidia are borne apically and laterally, younger conidia usually being on the apex. They are borne in clusters of 4 to 10 or sometimes even more, in recemose arrangement(Fig.2 and 3).

Conidia

Conidia vary in size and shape considerably. They are straight or curved cylindrical to elliptical, more often elliptical than cylindrical. Some spores are abnormally curved to make even a right angle at the curve. (Fig.3.). Brownish yellow to cocoa brown or deeper and generally the end cells are lightly coloured or subhyaline in mature spores. Colour of the spore is more deep when the concentration of sugar in the medium is high. The immature spores are lighter in colour, brussels brown without colour difference between

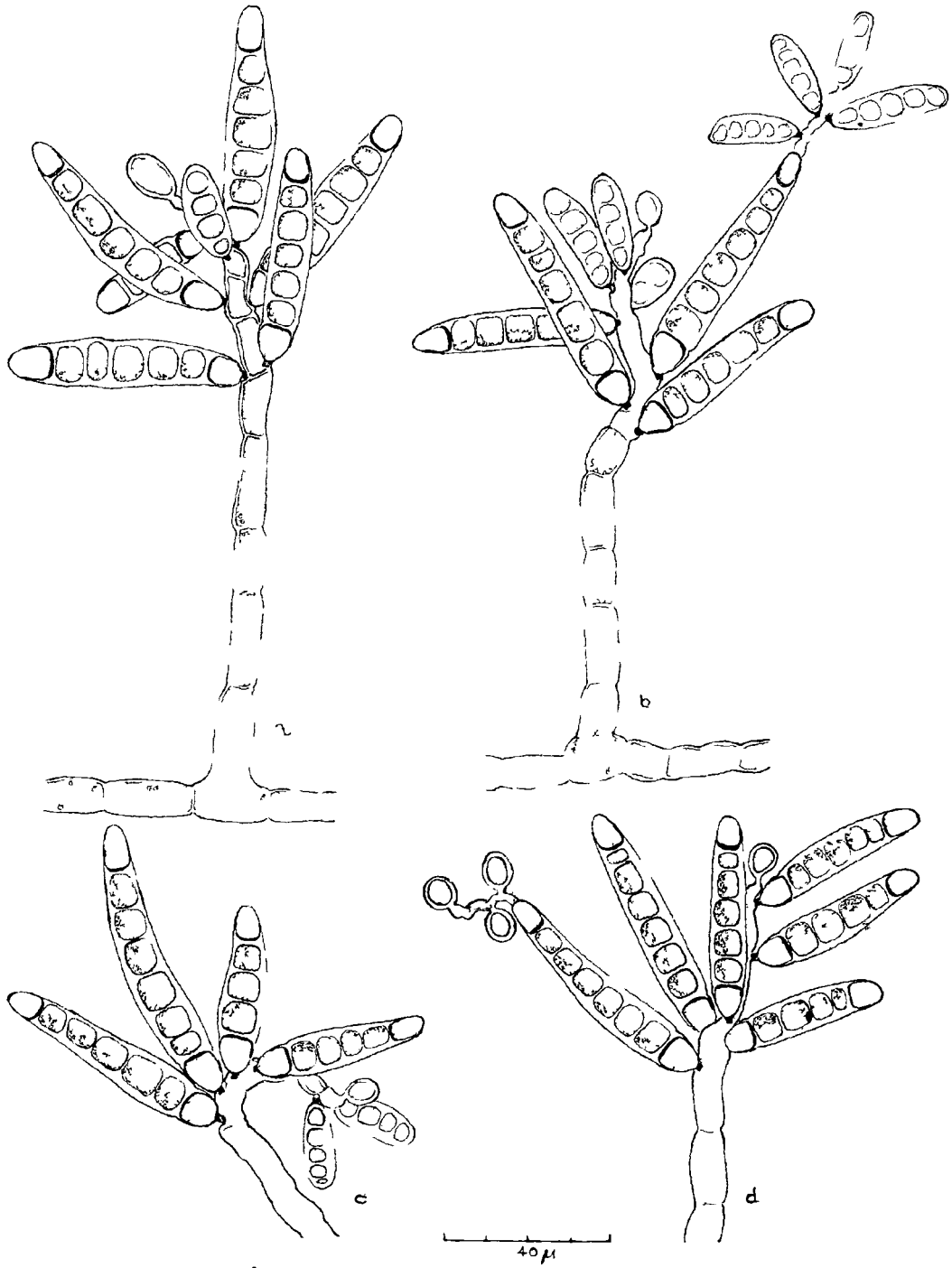


FIG 2 CULTURL 'C' CONIDIOPHORE & CONIDIA, b c d WITH SECONDARY SPORES

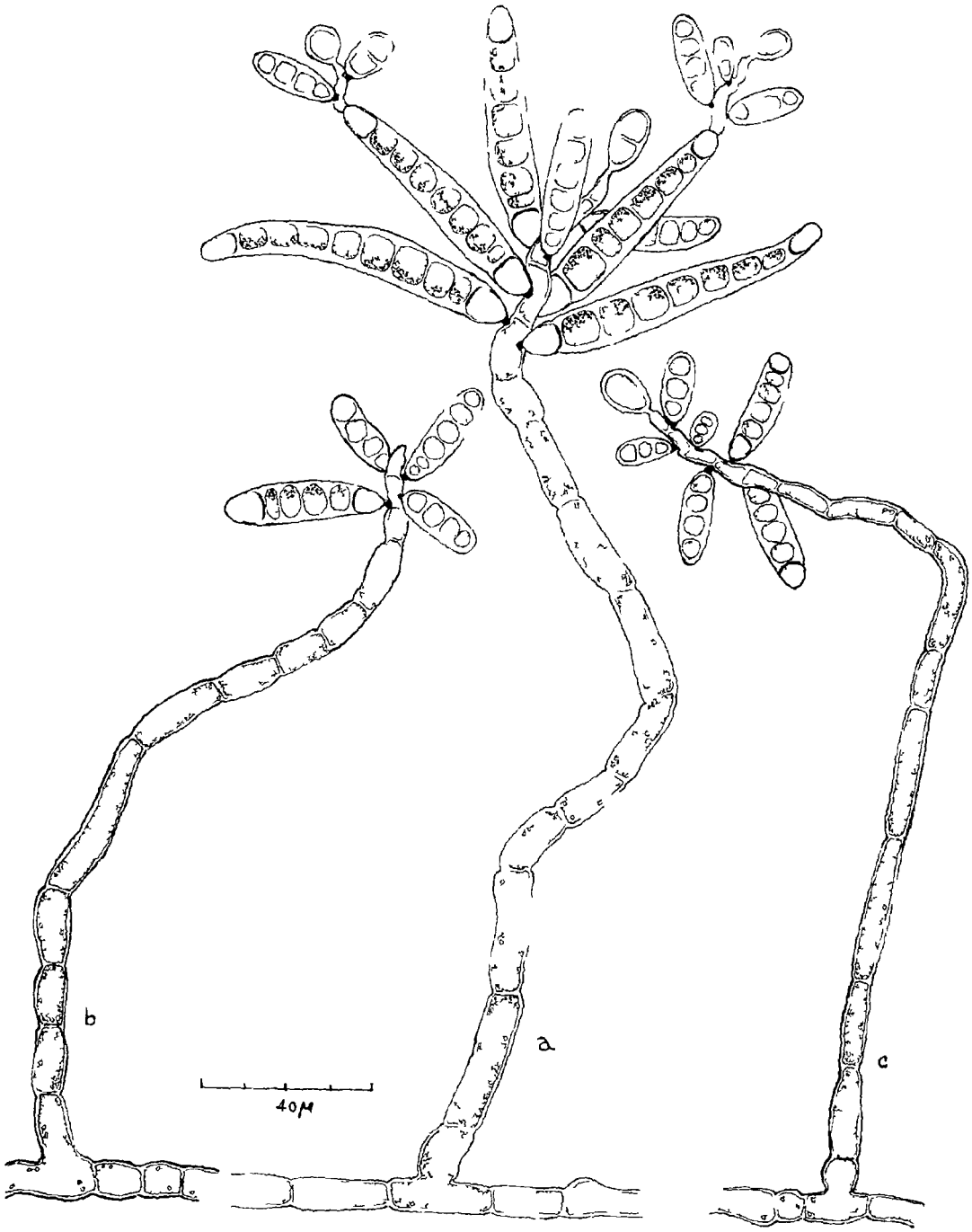


FIG 3 CULTURE C CONIDIOPHORES AND CONIDIA
a MATURE b c YOUNG

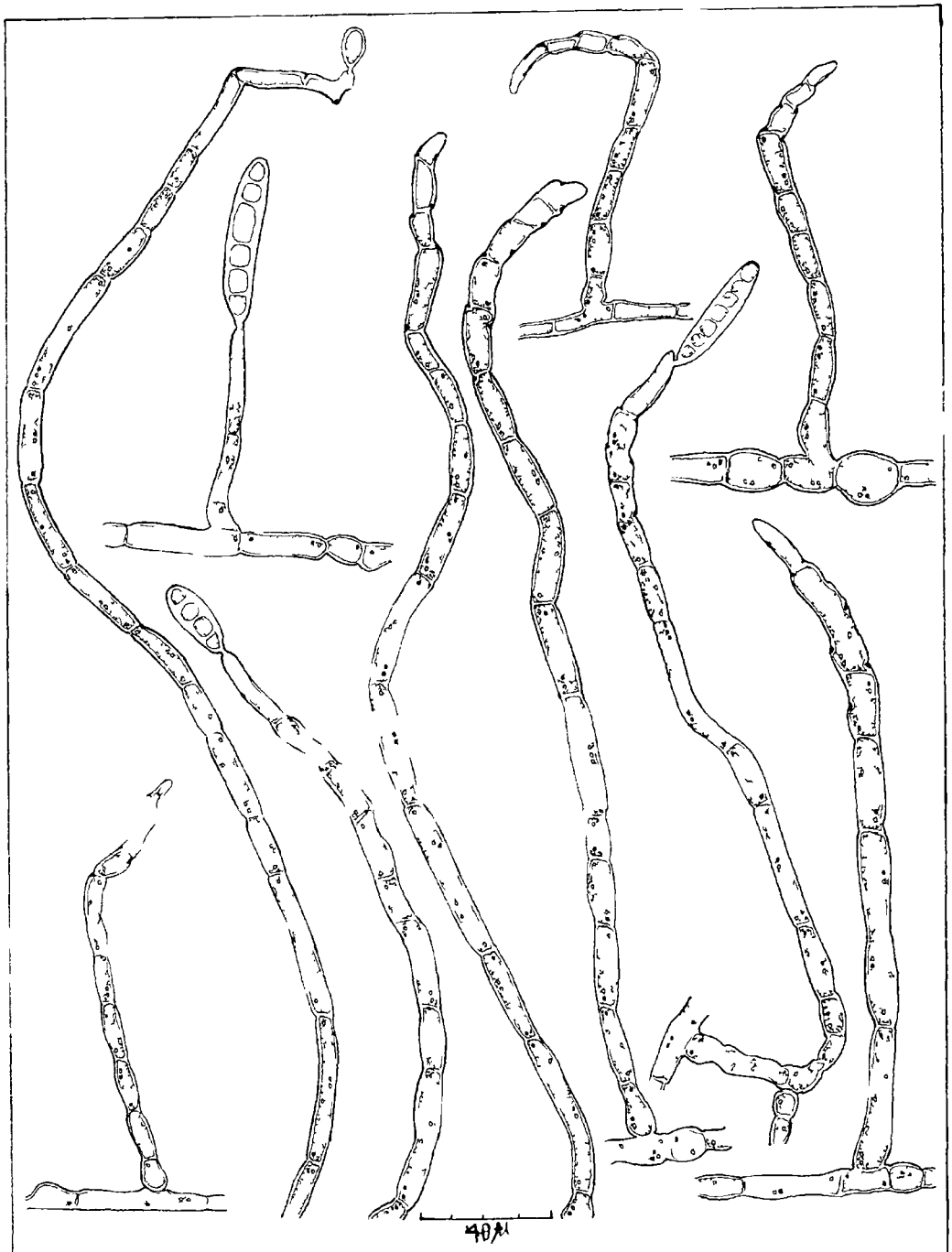


FIG 4 CULTURE C CONIDIOPHORE

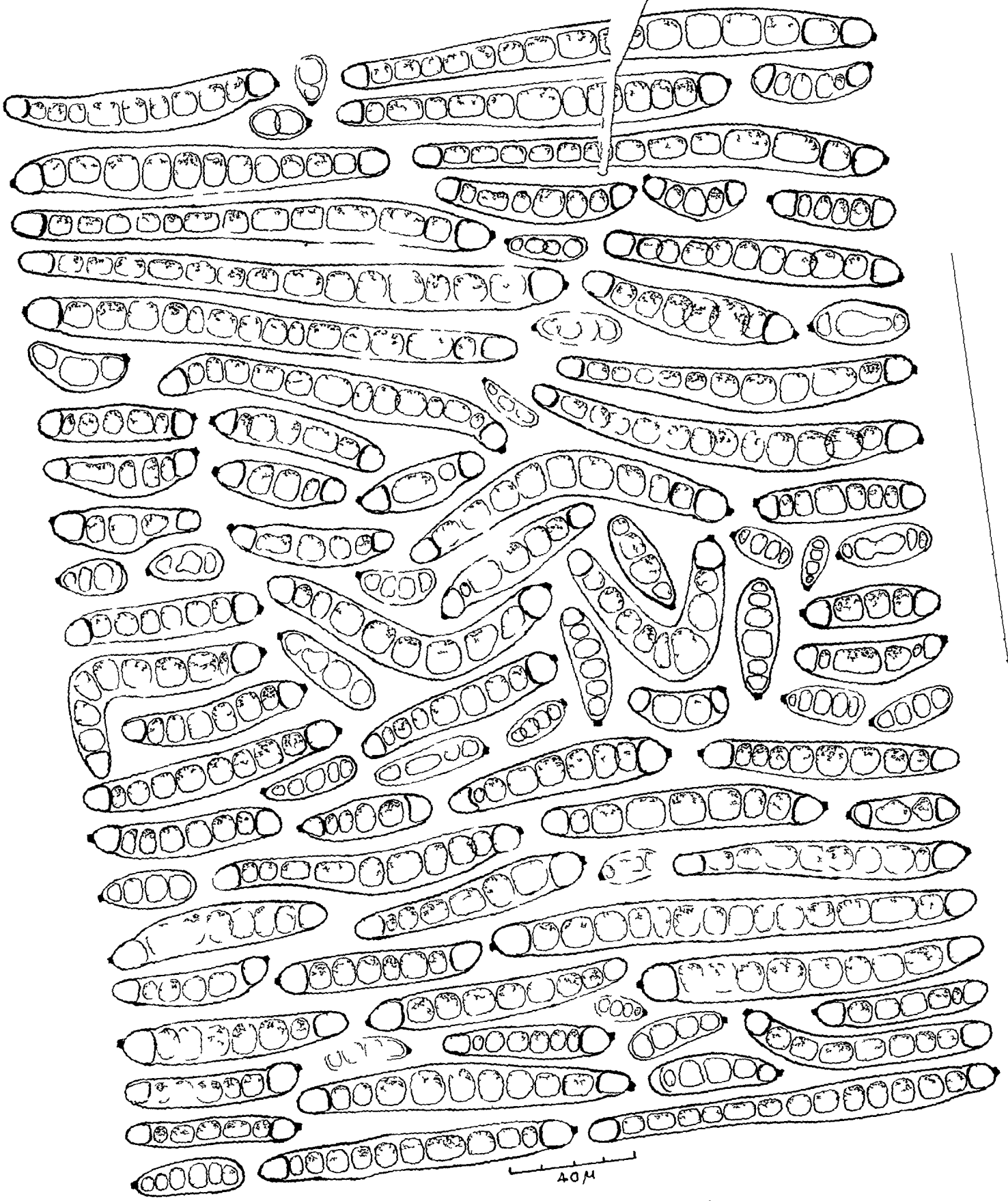


FIG 5 CULTURE C CONIDIA

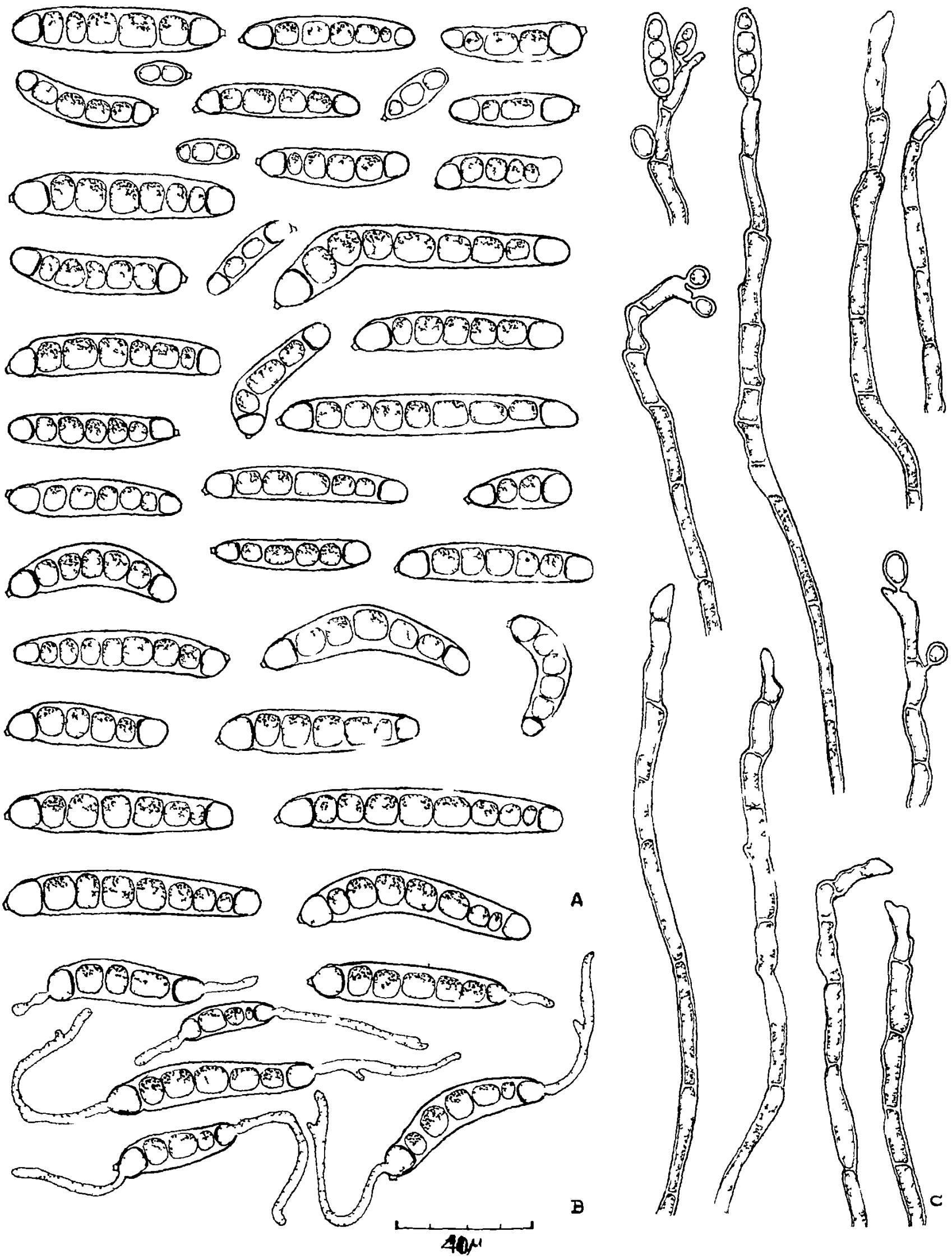


FIG 6 CULTURE S A CONIDIA B GERMINATING CONIDIA C CONIDIOPHORE

the two end cells and the other middle cells. The septa are not clear in young spores but prominent in mature spores, the two end septa which delimit the subhyaline end cells are generally accentuated rarely two or three basal septa are accentuated. The number of septa vary from 1 to 17 majority being 6 to 10 septate and average 8 septa(Table 1 & 4). Often there is a slight constriction of the peripheral wall at the first accentuated basal septum. Elliptical spores slowly tapering towards the broadly rounded apex and more abruptly towards the base. The maximum width of the spore being usually at a region between 1/3 to 1/2 from the basal end. Width of conidia varying from 6.5 to 22.7 u, usually between 12 to 18 u and average 15.8 u (Table 1 & 3). Length highly variable from 16.2 to 198.8 u the largest number being between 50 and 115 u and the average 93.6 u (Table 1 & 2). Somewhat acuminate basal end regularly show a prominent hilum 1.5 to 2 u long and 2 to 3 u wide. The conidia from the diseased host material are smaller, 27.2 to 126 u long(average 63.2 u) 11.9 to 20.4 u wide(average 15.7 u) and 2 to 11 septate.

Secondary conidia

An interesting phenomenon observed in this organism is the formation of secondary conidia. In this process a short sporophore proliferates from the apex of the mature conidium, which then produces variable number of secondary spores on the geniculations. They are smaller in dimensions and the

TABLE.1

Measurement of Conidia from 20 days old culture of Helminthosporium halodes in P.D.A.(Culture C)

(in microns)

Length	Width	No.of septa.	Length	Width	No.of septa.	Length	Width	No.of septa.
158.5	16.2	13	40.5	13.0	3	97.2	16.2	9
97.2	16.2	9	139.3	13.0	11	42.1	16.2	7
61.6	16.2	7	42.1	13.0	3	61.6	16.2	7
71.3	13.0	7	198.3	13.0	12	64.8	16.2	7
170.4	16.2	13	126.4	19.4	10	170.4	13.0	17
74.5	17.5	5	71.3	16.2	7	139.3	9.7	13
55.1	16.2	6	90.7	13.0	8	113.4	13.0	10
64.8	13.0	7	42.1	13.0	5	177.5	13.0	13
71.3	13.0	7	16.2	9.7	1	170.4	13.0	13
74.5	19.4	6	64.8	13.0	7	113.4	14.6	10
68.0	19.4	6	149.0	13.0	15	113.4	16.2	10
158.8	16.2	15	87.5	16.2	8	87.5	13.0	7
48.6	21.1	7	63.2	16.2	7	77.8	19.4	8
28.2	8.1	4	132.8	16.2	10	158.8	16.2	13
97.2	16.2	8	139.3	13.0	12	132.8	16.2	12
198.8	13.0	15	152.3	13.0	10	155.5	14.6	15
97.2	16.2	8	81.0	13.0	9	71.3	19.4	6
87.5	13.0	9	81.0	19.4	7	58.3	19.4	5
68.0	13.0	8	170.4	13.0	13	184.6	16.2	14
170.4	16.2	13	87.5	16.2	8	61.6	16.2	7
64.8	13.0	8	103.7	13.0	8	68.0	19.4	2
81.0	14.6	7	97.2	14.6	8	61.6	16.2	4
77.8	16.2	8	129.6	13.0	10	42.1	13.0	4
74.5	19.4	7	84.2	11.2	9	29.2	22.7	4
100.4	14.6	11	74.5	19.4	7	90.7	22.7	7

TABLE.1(contd)

Length	Width	No.of septa.	Length	Width	No.of septa.	Length	Width	No.of septa
68.0	22.7	5	71.3	16.2	7	61.6	16.2	7
155.5	19.6	11	165.2	16.2	12	74.5	19.4	6
87.5	16.2	9	110.2	16.2	10	40.1	19.4	8
74.5	13.0	7	74.5	16.2	6	58.3	22.7	6
87.5	13.0	9	55.1	16.2	6	119.9	16.2	10
168.5	13.0	13	68.0	19.4	6	136.1	16.2	12
74.5	13.0	8	55.1	19.4	6	55.1	9.7	7
77.8	16.2	8	68.0	22.7	6	42.1	16.2	4
106.9	16.2	9	61.6	19.4	5	116.6	22.7	9
25.9	9.7	3	38.9	9.7	5	162.0	13.0	13
38.9	13.0	4	58.3	19.4	6	149.0	13.0	12
94.0	16.2	8	29.2	9.7	3	113.4	16.2	4
198.8	13.0	15	171.7	13.0	14	94.0	16.2	8
142.6	16.2	11	77.8	16.2	7	142.6	13.0	11
77.8	13.0	7	61.6	16.2	7	94.0	16.2	8
55.1	13.0	7	100.4	13.0	9	100.4	13.0	10
35.6	9.7	4	74.5	13.0	9	84.2	13.0	8
97.2	13.0	11	116.6	16.2	10	184.6	13.0	15
64.8	13.0	8	32.4	9.7	4	71.3	16.2	8
64.8	13.0	7	38.9	9.7	4	68.0	16.2	7
64.8	13.0	6	106.9	13.0	11	55.1	13.0	5
152.3	16.2	10	87.8	9.7	6	100.4	16.2	9
171.7	16.2	13	165.2	16.2	13	74.5	16.2	7
77.8	13.0	7	64.8	16.2	7	152.3	13.0	11
55.1	16.2	6	64.8	16.2	7	142.6	13.0	10

TABLE.1 (contd.)

Length	Width	No.of septa.	Length	Width	No. of septa
116.6	16.2	11	51.8	16.2	6
61.6	13.0	7	113.4	16.2	10
38.9	9.7	5	168.5	16.2	15
58.3	19.4	6	74.5	13.0	7
61.6	16.2	7	68.0	13.0	6
90.7	13.0	9	90.7	16.2	7
71.3	16.2	7	106.9	9.7	10
55.1	13.0	7	64.8	16.2	7
145.1	13.0	13	184.6	16.2	15
81.0	16.2	9	90.7	13.0	9
81.3	16.2	7	71.3	22.7	6
22.7	9.7	4	22.7	6.5	2
77.8	13.0	7	100.4	13.0	8
84.2	19.4	8	129.6	13.0	10
48.6	13.0	7	71.3	13.0	7
84.2	13.0	9	145.8	13.0	4
51.8	9.7	7	129.6	13.0	9
110.2	13.0	9	64.8	11.3	7
81.0	16.2	8	84.2	13.0	9
97.2	16.2	9	149.0	13.0	12
136.1	16.2	11	165.2	16.2	13
84.1	16.2	8	103.7	16.2	9
77.8	13.0	7	113.4	16.2	9
71.3	16.2	7	97.2	13.0	8
145.8	13.0	12	64.8	9.7	6

TABLE.2

Frequency distribution for the length of Conidia.

(Culture C)

CLASS	FREQUENCY
15.5 - 25.5	4
25.5 - 35.5	5
35.5 - 45.5	11
45.5 - 55.5	5
55.5 - 65.5	33
65.5 - 75.5	30
75.5 - 85.5	19
85.5 - 95.5	15
95.5 - 105.5	15
105.5-115.5	11
115.5-125.5	4
125.5-135.5	6
135.5-145.5	8
145.5-155.5	10
155.5-165.5	5
165.5-175.5	12
175.5-185.5	4
185.5-195.5	0
195.5-205.5	3
Total	200

Maximum length - 198.8 u
Minimum length - 16.2 u
Mean length - 93.6 u
About 64% between 50 & 115 u

TABLE.3

Frequency distribution for width of Conidia

(Culture C)

CLASS	FREQUENCY
6-8 u	1
8-10	16
10-12	2
12-14	74
14-16	6
16-18	75
18-20	18
20-22	1
22-24	7

Maximum width - 22.7 u
Minimum width - 6.5 u
Mean width - 15.8 u
Highest frequency for width between 12-18 u
about 77%

TABLE.4

Frequency distribution for Number of septa

(Culture C)

No.of septa	Frequency
1	1
2	2
3	4
4	10
5	8
6	20
7	47
8	25
9	22
10	16
11	12
12	8
13	14
14	2
15	8
16	0
17	1
Total	200

Maximum number of Septa - 17

Minimum ,, ,, - 1

Mean number of Septa - 8.4

Maximum frequency for septa number - 7

65% between 6 and 10 septa.

TABLE.5

Measurement of Conidia of 20 days old culture S

(in microns)

Length	Width	No.of septa.	Length	Width	No.of septa.	Length	Width	No.of septa
56.1	13.6	7	86.9	15.3	9	59.5	15.3	5
61.2	11.9	9	40.8	13.6	7	57.8	10.2	8
8.5	8.5	0	64.6	11.9	7	71.4	10.2	7
39.1	15.3	4	66.3	15.3	9	51.0	11.9	7
85.0	13.6	8	66.3	15.3	9	41.8	15.3	9
13.6	8.5	1	54.4	13.6	7	74.8	15.3	8
42.5	11.9	6	76.5	15.3	8	47.6	11.9	7
68.0	13.6	8	69.7	15.3	8	93.5	15.3	9
79.9	13.6	9	34.0	10.2	4	85.0	13.6	9
10.2	8.5	0	57.0	11.9	7	61.2	15.3	7
51.0	13.5	7	59.5	13.6	7	37.4	10.2	4
30.6	10.2	5	59.5	13.6	7	61.2	11.9	8
64.6	13.6	7	69.7	13.6	7	85.0	13.6	9
45.9	11.9	8	68.0	13.6	8	54.4	13.6	7
49.3	11.9	7	18.7	10.2	2	34.0	13.6	5
45.9	11.9	7	66.3	13.6	7	56.1	13.6	7
51.0	11.9	7	66.3	10.2	7	49.3	10.2	6
76.5	15.3	7	68.0	11.9	7	57.8	15.3	7
66.3	11.9	7	73.1	15.3	8	13.6	8.5	0
74.8	11.9	7	47.6	10.2	7	45.9	13.6	5
25.5	10.2	4	62.9	11.9	7	76.5	17.0	7
66.3	10.2	7	74.8	13.6	7	64.6	11.9	
76.5	11.9	7	54.4	10.2		54.4	11.9	7
32.3	10.2	4	83.3	15.3	9	76.5	17.0	9
45.9	11.9	6	69.7	13.6		32.3	13.6	3

TABLE.5(contd.)

Length	Width	No.of septa.	Length	Width	No.of septa	Length	Width	No.of septa.
52.7	15.3	7	68.0	13.6		78.2	15.3	9
85.0	13.6	9	51.0	13.6	5	15.3	8.5	1
89.5	13.6	7	30.6	11.9	3	47.6	10.2	7
37.4	10.2	4	76.5	11.9	7	73.1	11.9	9
59.5	15.3	6	76.5	10.2	7	51.0	11.9	7
86.7	11.9	9	64.6	13.6	7	15.3	8.5	1
74.8	15.3	9	91.8	13.6	8	51.0	13.6	6
47.6	11.9	7	28.9	10.2	2	52.7	13.6	7
54.4	13.6	7	62.9	13.6	8	32.7	13.6	4
76.5	11.9	6	68.0	13.6	7	40.8	10.2	6
61.2	15.3	5	68.0	13.6	8	51.0	10.2	7
83.3	17.0	9	68.0	15.3	8	42.5	11.9	5
73.1	15.3	9	34.0	11.9	3	23.8	10.2	3
28.9	8.5	3	76.5	11.9	7	62.9	13.6	7
62.9	10.2	7	54.4	13.6	5.	49.3	10.2	5
76.5	11.9	7	76.5	15.3	9	68.0	11.9	7
62.9	11.9	7	49.3	13.6	8	71.4	13.6	9
45.9	10.2	7	13.6	8.5	1	66.3	15.3	7
76.5	17.0	8	13.6	8.5	1	59.5	15.3	7
47.6	11.9	7	51.0	10.2	6	34.0	10.2	3
68.0	11.9	7	23.8	8.5	2	73.1	15.3	8
54.5	13.6	7	35.7	10.2	4	25.5	8.5	2
78.2	11.9	9	74.8	15.3	10	54.4	11.9	7
61.2	13.6	6	42.5	13.6	5	51.0	10.2	7
27.2	10.2	3	90.1	13.6	10	42.5	10.2	5

TABLE.5.(Contd.)

Length	Width	No.of septa.	Length	Width	No.of septa.
37.4	10.2	5	59.5	11.9	7
52.7	11.9	7	28.9	11.9	7
59.5	13.6	7	66.3	11.9	7
83.3	17.0	9	37.4	10.2	6
27.2	10.2	4	57.8	15.3	6
17.0	8.5	1	27.2	10.2	3
59.5	11.9	7	57.8	13.6	8
62.9	13.6	7	76.5	13.6	9
47.6	11.9	5	68.0	13.6	7
42.5	10.2	5	45.9	11.9	7
76.5	13.6	9	34.0	10.2	5
69.7	15.3	8	51.0	11.9	7
32.3	10.2	5	56.1	13.6	7
40.8	10.2	6	59.5	11.9	6
68.0	11.9	7	52.7	10.2	7
68.0	11.9	8	42.5	11.9	5
13.6	8.5	1	25.5	10.2	3
11.9	8.5	1	42.5	11.9	4
25.5	10.2	4	93.5	17.0	9
42.5	11.9	7	42.5	11.9	6
59.5	15.3	7	68.0	15.3	9
10.2	8.5	0	30.6	10.2	3
71.4	15.3	8	8.5	8.5	0
25.5	10.2	3	51.0	10.2	6
27.2	10.2	4	49.3	11.9	7

TABLE.6

Frequency distribution for the length of Conidia(Culture S)

Class	Frequency
5.5-15.5	12
15.5-25.5	7
25.5-35.5	20
35.5-45.5	19
45.5-55.5	41
55.5-65.5	33
65.5-75.5	37
75.5-85.5	24
85.5-95.5	7
Total	200

Maximum length - 93.5 u

Minimum length - 8.5 u

Mean length - 53.4 u

65% of the spore is between 35 & 75 u length.

TABLE.7

Frequency distribution for width of Conidia (Culture S)

Class	Frequency
8-10	16
10-12	96
12-14	50
14-16	32
16-18	6
Total	200

Maximum width = 17.0 u

Minimum width = 8.5 u

Mean width = 12.4 u

73% of the sporesare between 10 and 14 u width

TABLE.8

Frequency distribution for Number of septa (Culture S)

No.of septa.	Frequency
0	5
1	8
2	4
3	11
4	12
5	17
6	15
7	75
8	21
9	26
10	2
Total	196

(Septalin not clear in 4 spores).

Maximum Number of septa.=10

Minimum number of septa = 0

Mean septa =6.19

65% of the spores are of 5 to 8 septate.

accentuated end septa and subhyaline end cells common to the normal spores are not present in them. These spores are usually lighter in colour. The secondary sporophores are thinner 3 to 6 u in width and variable in length from 10 to 60 u or even more, septate and with characteristic geneculations and scars at regular intervals. They are yellowish brown with subhyaline distal end. These secondary sporophores bear 1 to 10 smaller secondary conidia.(Fig.7)

Germination of conidia(Fig.8)

Conidia germinate well in distilled water, tap water, glucose and sucrose solutions and coconut leaf extract. Germination is rapid in solutions containing sugars and leaf extract than in water. The growth of germ tube is also stimulated in these solutions, however the percentage of germinations at the end of 24 hours is almost the same in all cases. (Table 9).

TABLE 9.

Percentage germination of spores in different substrates at different time intervals.

Time (hours)	Distilled water.	Tap water	Sucrose solution	Glucose solution	Leaf extract
2	0	0	2	3	2
4	12	12	25	38	33
6	22	25	43	54	45
8	34	36	56	66	62
24	93	94	95	94	94

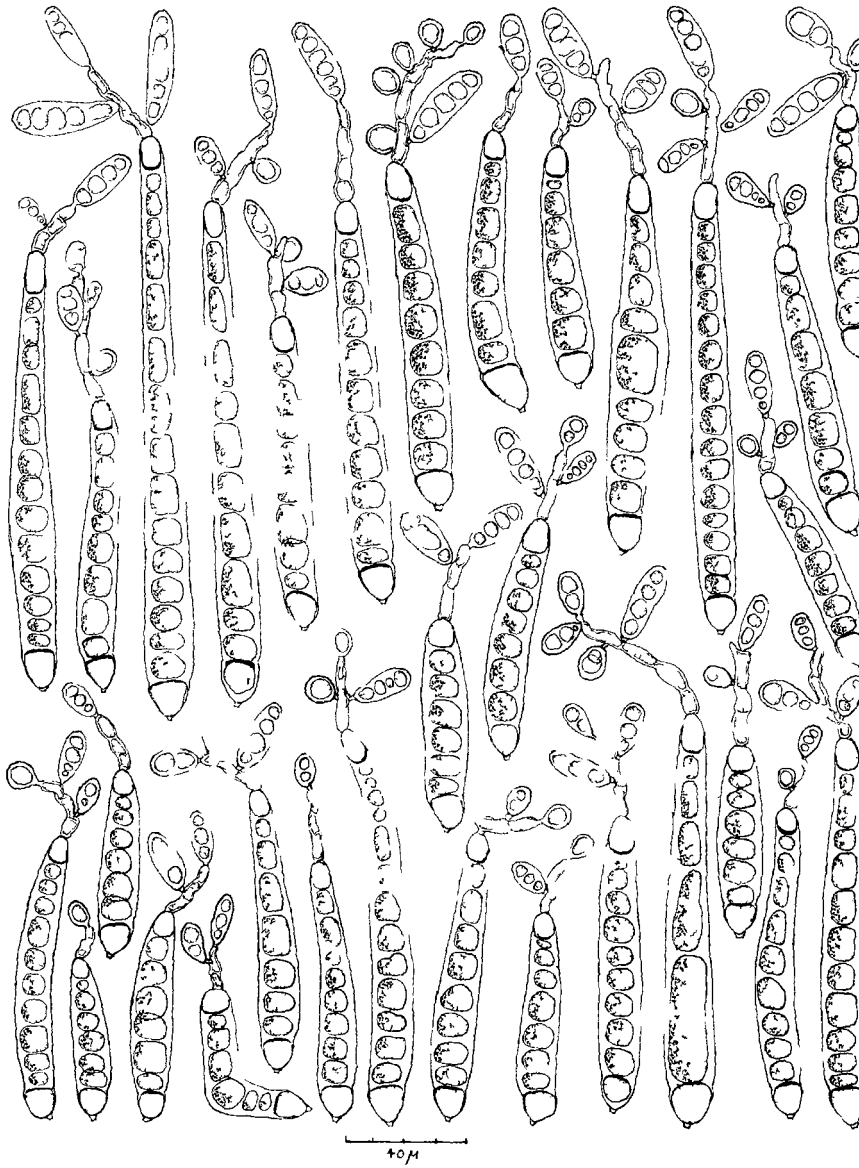


Fig 7 CULTURE C PRIMARY AND SECONDARY SPORES

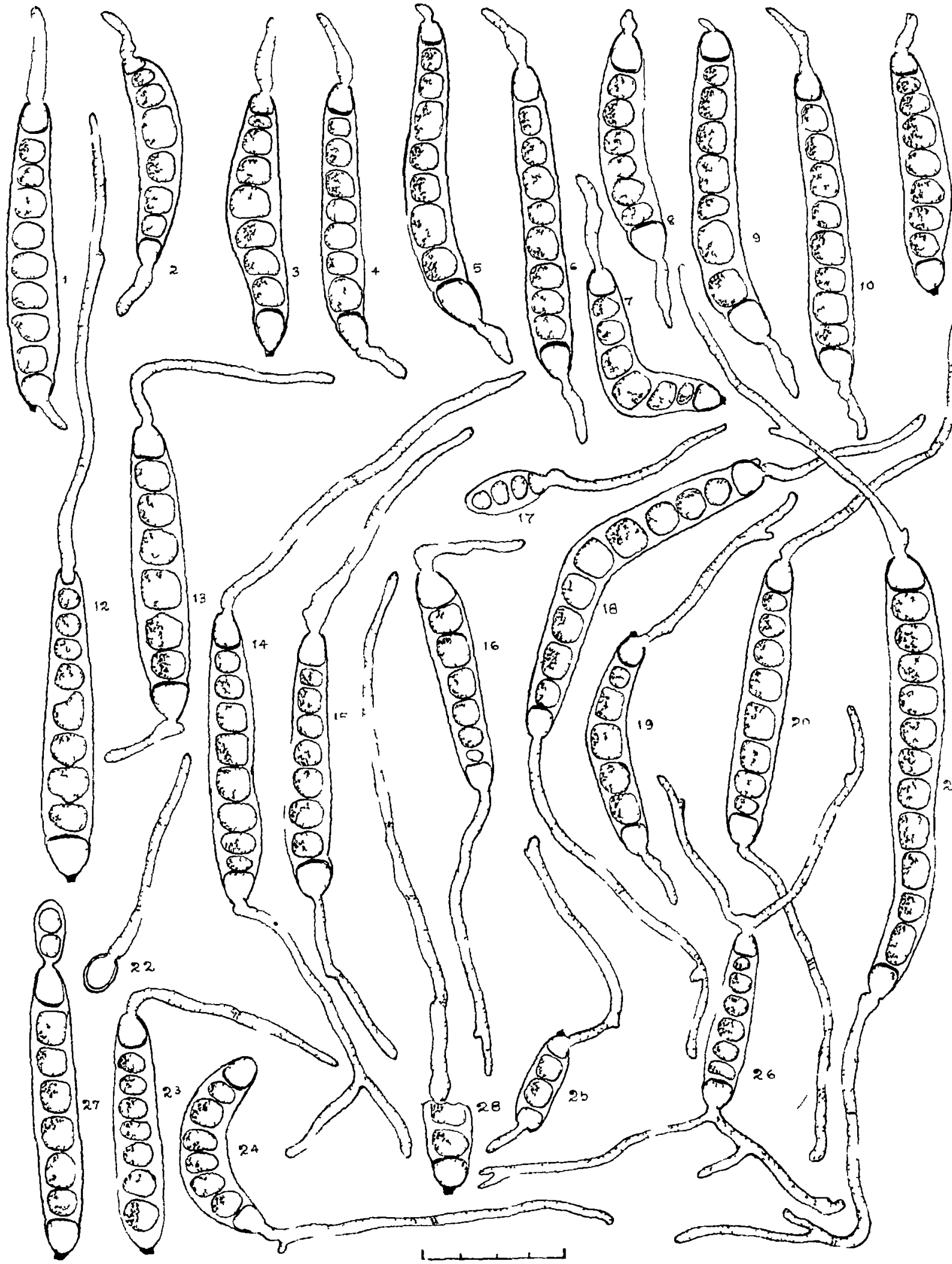


FIG 8 CULTURE OF GERMINATING CONIDIA, 1-11 after 4 hour
12-26 after 6 hour 27 and 28 Germ tube, broken conidia

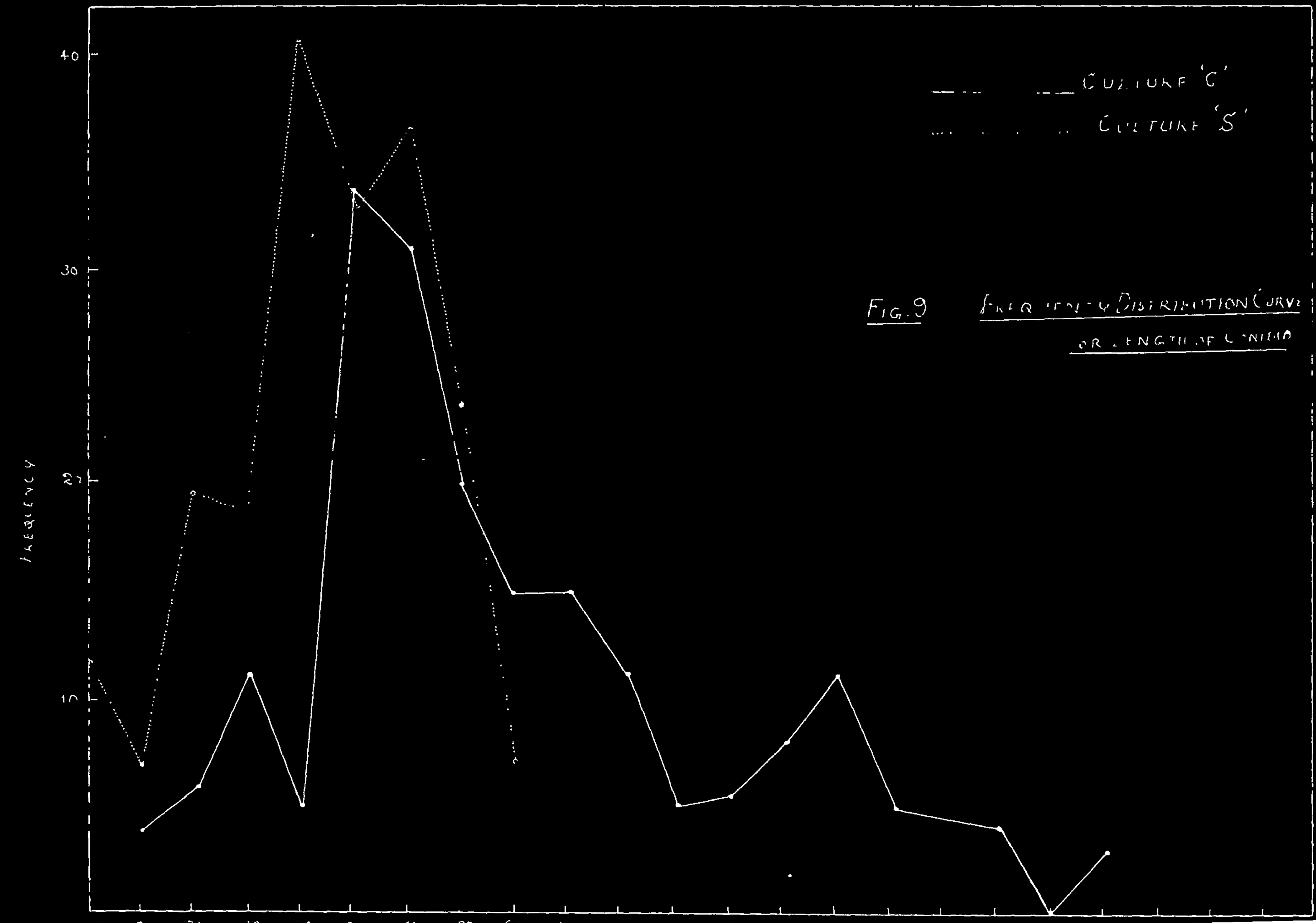


Fig. 9 Frequency Distribution Curve for Length of Conidia

FIG 10. FREQUENCY DISTRIBUTION FOR SEPTH NUMBER

Germination is by the production of two polar germ tubes one each from the subhyaline end cells delimited by accentuated end septa. The germ tubes emerged out from the thin walled regions of these cells. In the proximal cells it is usually from the apex and in the basal cell it is from a region near the hilum, or from the hilum region itself where the spore wall is perceiptatably thin. The germination from other cells than from these end cells as reported by Drechsler(1923) for immature spores has not been observed in the present study.

In sugar solution budding of spores was observed though only very rarely(Fig.8) In sugar,glucose and leaf extract solutions the growths of germ tubes were rapid and sporulated in 12 to 24 hours, maximum being observed in case of glucose solution. These spores were small and pale and borne on short conidiophores in small numbers, one or two usually.

In broken spores, the germination occured by production of germ tube from the broken end of the spore(Fig.8)

Comparison with culture S.

Culture C is compared with culture S. Considerable differences have been observed in spore measurements and in some other morphological characters. The details of spore measurements of culture S are given in tables 5,6 and 7. The spore measurements of various isolates of H.halodes and its varieties are presented in Table 10. In general, it is seen that the

TABLE.10.

Spore measurements of different isolates of H.halodes and its varieties.

(in microns)

Measurement	Original report of Drechsler	Culture C. on host.	Culture C. on PDA	Culture S on PDA	Report of Subramoniam	Report of Mitra.	Report by Kovachich on host.	Report by Kovachich on Culture
Maximum length	105	126.0	198.8	93.5	104.5	73.0	125	96
Minimum length	20	27.2	16.2	8.5	20.0	23.0	33	32
Mean length	--	63.2	93.6	53.4	--	52.0	--	--
Maximum width	14	20.4	27.7	17.0	--	20.0	18	16
Minimum width	10	11.9	6.5	8.5	--	13.0	10	11
Mean width	--	15.7	15.3	12.4	9.5	16.5	--	--
Maximum septa No.	12	11	17	10	10	9	12	10
Minimum septa No.	1	2	1	0	3	2	4	5
Mean septa No.	--	7.2	8.4	6.2	--	6	--	--

organism under study is significantly superior in spore dimensions to the culture S and other H.halodes reported.

In general morphology, the difference was not conspicuous. The seoondary spore formation was not observed in the culture S or in any other reports on H.holodes or its varieties.

In cultural characters also there were some differences between culture C and S. The colour of the colony of culture C in P.D.A. was dark yew green but that of culture S was brownish yew green. On aging the colony of culture C was changed to black, but that of culture S remained brownish. The aerial growth of culture S was more than that of culture C.

Physiology

Growth on different media

Five solid media and six liquid media were tested for the growth and sporulation of the organism. There was no appreciable difference in the average daily radial growth in the solid media. It ranged from 8.6 mm. in PDA and Czapek's media to 7.5 mm. in the Richard's agar(Table 11 and Fig.12). However, marked difference in the type of growth andcolour of the colony was noted.(Fig.11). Growthwas dense and dark in PDA and very sparse and light in Barne's. Intensity of sporulation also differed much. Maximum was observed in PDA followed by Czapek's and Richard's. In Coon's the

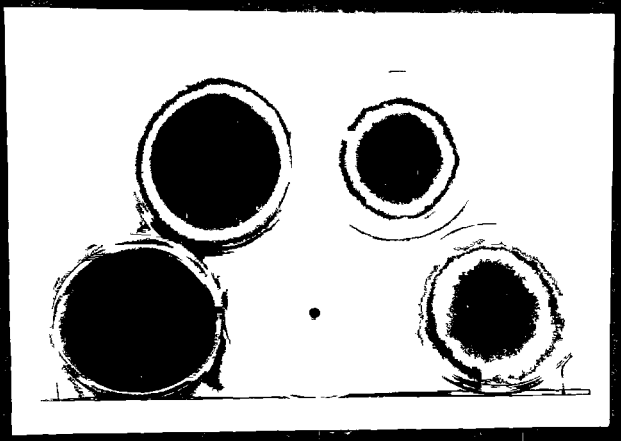


FIG.11. Different Media 1. P.D.A., 2. Barne's 3.Coon's, 4. Czapeks, 5.Richard's.

TABLE.11

Radial growth of the colonies in different media
(in millimetres)

Replications.	1 P.D.A.				2 Barne's				3 Coon's				4 Czapek's				5 Richard's			
	Days				Days				Days				Days				Days			
	2	3	4	5	2	3	4	5	2	3	4	5	2	3	4	5	2	3	4	5
1	17	25	33	43	13	21	30	39	16	25	34	42	18	27	37	43	14	23	32	37
2	18	26	35	43	11	20	38	36	16	26	35	43	18	26	36	43	15	24	34	38
3	18	27	37	43	13	21	29	39	16	25	35	42	17	26	37	43	17	25	34	38
4	17	26	36	42	12	21	29	39	16	25	35	43	18	27	37	43	16	24	33	37
5	18	26	34	43	13	21	29	38	17	26	36	43	17	26	37	44	16	24	33	38
Average:	17.6	26.0	35.0	42.8	12.4	20.8	29.2	38.2	16.2	25.6	35.0	42.6	17.6	26.4	36.8	43.2	15.6	24.0	33.2	37.6
Average daily radial growth.	8.6				7.6				8.5				8.6				7.5			

FIG. 12

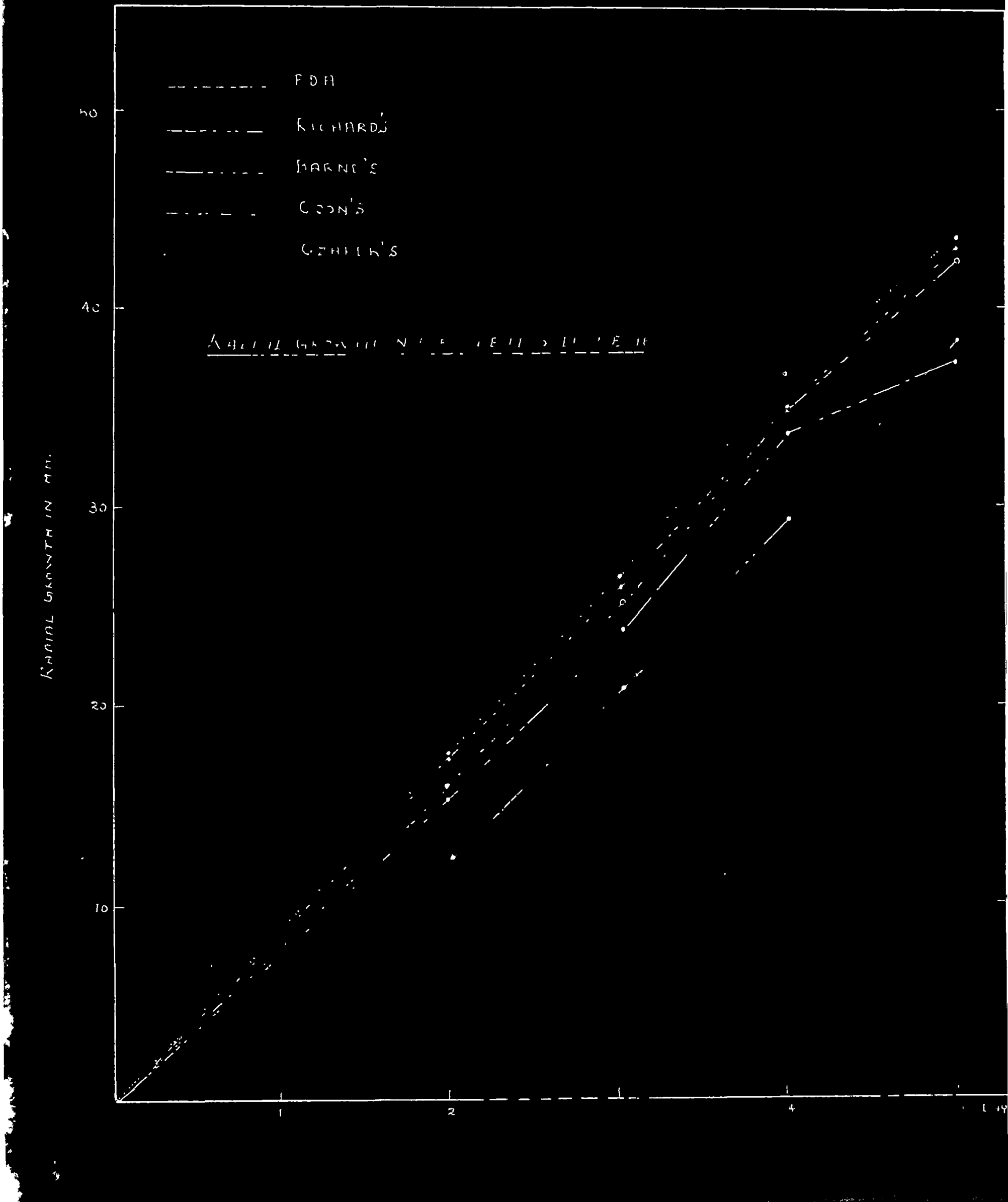

FDH
RICHARDS
BARNES
COON'S
GEHRIG'S
RADIAL GROWTH IN
50
40
30
20
10
1
2
4

TABLE.12

Dry weight of the mycelium in different liquid media after 10 days incubation.(in milligrammes)

Replications.	Media					
	Potato Dextrose	Richard's	Barne's	Coon's	Czappek's	Czapek's-Dox
I	148	137	12	90	130	20
II	156	130	14	94	141	21
III	160	125	18	86	140	25
IV	158	128	13	84	128	18
V	164	146	11	79	120	19
Total	786	666	68	433	659	103
Average	157.2	133.2	13.6	86.6	131.8	20.6

FIG. 13. DRY WEIGHT IN DIFFERENT LIQUID MEDIA

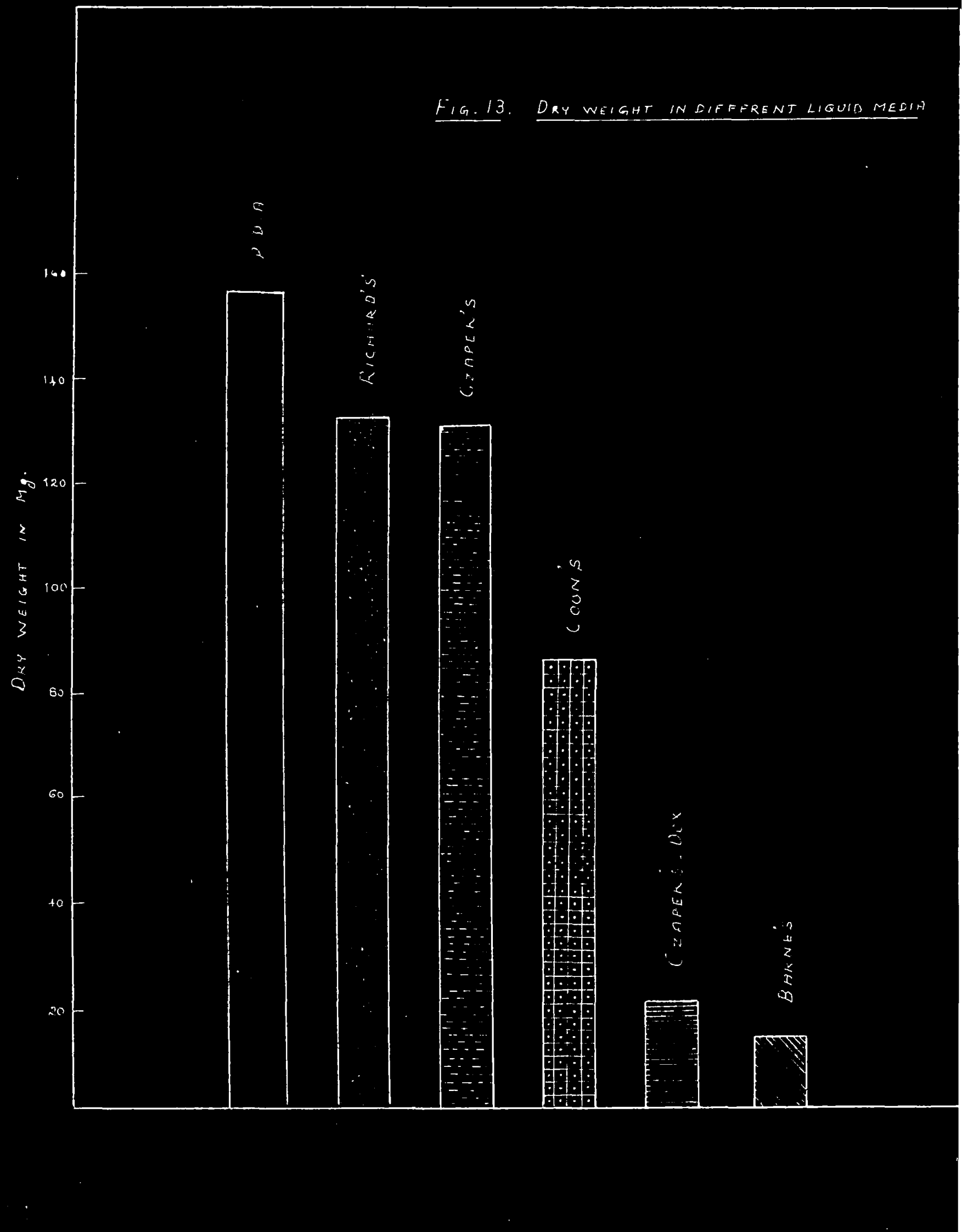


TABLE.13

Analysis of variance - Different media.

Analysis of variance table.

Source	SS	DF	Variance	F	Inference
Total	94854.5	29			
Treatment	93963.5	5	18792.7	506.5	Highly significant
Error	891.0	24	37.1		

Critical difference 7.95

Rank	Treatment	Total dry wt.of the mycelium	Mean	Difference	Inference
1	P.D.Solution	786	157.2		
				24.0	Significant
2	Richard's	666	133.2		
				1.4	Not significant.
3	Czapek's	659	131.8		
				45.2	Significant
4	Coon's	433	86.6		
				60.0	Significant
5	Czapck's-Dox	103	20.6		
				7.0	Not significant.
6	Barne's	68	13.6		

Result:-

PDS Richard's Czapck's Coon's Czapck's-Dox Barnes

sporulation was moderate and in Barne's very poor.

There was significant difference in the dry weight of mycelium in different liquid media, maximum in potato dextrose solution(157.2 mg.) and minimum in Barne's(13.6 mg.). Good growth was obtained in Czapek's and Richard's solutions also, though significantly less than that in potato dextrose solution. Coon's solution also promoted some growth. But Barne's and Czapek's-Dox solution were found to be unsuitable for the growth of the fungus(Table 12 and Fig.13).

When the gain in dry weight of mycelium was statistically analysed(Table 13) it was found that the growth in Richard's and Czapek's solution were not significantly different, but differed significantly from that on potato dextrose solution.

Characters of the colonies in different solid media.

(1)P.D.A. Colony thick, dark yew green (darker than colonies in other media) becomes black in ten days, reverse appear blackish, aerial growth very sparse, consisting mainly of conidiophores and conidia. Mycelial mat is thicker at the surface to a depth of about 600 u becoming sparse below, reaching upto the surface of the plate. Mycelium of the growing zone of the colony is hyaline, about 6 to 8 mm, becoming light brown with age. Conidiophore light brown, usually darker than the mycelium, unbranched, erect, curved or flexuous, arising from slightly submerged and prostrate surface mycelium. Sporulation very profuse. Conidia numerous per conidiophore,

light brown when young becoming darker when mature. Secondary spore formation also is profuse. Growth of the colony very rapid attaining a radial growth of 42 to 43 mm. in 5 days. Daily growth is marked with zonations. No odour, no exudate, colour of the media not altered.

(2)Barne's. Growth very sparse, colony hyaline, sporulation very poor. Mycelium hyaline when young becoming light yellow on maturity. No aerial growth, submerged growth is also very sparse. Conidia yellowish in colour without prominent accentuated end septa or hyaline end cells. Inferior in dimensions, no secondary spore formation. Only few conidia per conidiophore. Reverse colourless. Radial growth fair, attaining about 38 mm in five days. No odour or exudate.

(3)Coon's. Colony light yew green, not as dark as on P.D.A. or on Richard's colour not even, darker at the centre and radiating to the periphery, with dark specks here and there. No marked white growing zone unlike that in PDA or Richard's. Growth fair, not dense, but radial growth fairly well attaining 42 to 43 mm.in five days. Aerial growth slightly more than that in other media. Thick mycelial mat at the surface to a thickness of about 400 u. Conidiophore and conidia more or less like that on PDA, but secondary conidia formation not so frequent. Conidia are generally not as long as that on PDA,conidia light brown. Accentuated end septa and sub-hyaline end cells are prominent in majority of the spores. Colour of medium not altered, no odour or exudate.

(4) Czapek's Next to PDA, luxuriant growth and sporulation is obtained in Czapek's agar. Colony dark yew green with marked growing zone of hyaline mycelium about 8 to 11 mm. Thick growth at the surface about 500 u, becoming sparse down wards touching the plate, Young mycelium hyaline, becoming light brownish with age, conidiophores and conidia same as on PDA, conidia numerous per conidiophore, secondary conidia formation fairly good. Conidia light brown when younger and becoming darker at maturity with clear accentuated end septa and subhyaline end cells.Colony becoming black with age, reverse appear black but medium not coloured. Growth rapid like that on PDA reaching a radious of 42-43 mm. in five days. Daily growth is not marked by zonations unlike that on PDA. No odour or exudate.

(5) Richard's Colony dense as on PDA. No aerial growth. Dense growth to a depth of 550 u. Colony dark yew green but not as dark as that on PDA, marked growing zone of hyaline mycelium about 10 to 12 mm, but not as well marked as that on PDA, colony becoming dark with age, reverse appear black though the medium is not coloured. Mycelium hyaline when young, becoming yellowish brown with age, conidiophores and conidia like those on PDA, secondary spore formation fairly well. Spores are slightly shorter. Radial growth not as fast as that on other media, attaining 37-38 mm. in five days. No odour or exudate.

Colony characters in different liquid media

(1). Potato dextrose solution. Surface growth dark, submerged mycelium nearly hyaline, aerial growth not conspicuous, mycelial mat thick and continuous surface uneven, sporulation very good.

(2). Richard's solution. Colony greenish black, greyish brown at the growing edge. Forms a thick mat on the surface of the media. Aerial growth not prominent. Sporulation very good.

(3). Barne's solution. Growth very poor, colour brownish green, growing part brownish, mycelium forming a very thin mat on the surface of the solution. No aerial growth. Sporulation very slight.

(4). Coon's solution. Growth some what fair, not luxuriant, Colour yew green with blackish tinge submerged growth limited. No aerial growth, mycelial mat not dense. Sporulation fairly good.

(5). Czapek's solution. Growth very good, more or less similar to that of Richard's solution. Colour not as dark as that on Richard's. Sporulation very good. Dense mycelial mat on the surface. No aerial growth, fairly good submerged growth, reaching nearly upto the bottom of the flask.

(6). Czapek's-Dox solution. Growth very poor, only a very thin mycelial mat on the surface of the solution. Colour yellowish green. No aerial growth. Sporulation very shy.

Deficient media

The total dry weight of mycelium in different deficient media, except in that in which $FeCl_3$ was deficient was very low when compared with the complete medium. While the mean dry weight in the complete medium was 44.0 mg, that of sucrose deficient medium was as low as 3.8 mg. In the medium in which KNO_3 was omitted the dry weight was equally low being 4.8 mg. The dry weight in Potassium phosphate and $MgSO_4$ deficient media were 19.0 mg and 22.8 mg. respectively. However, in the medium in which $FeCl_3$ was omitted the dry weight was 54.4 mg. which is 10.8 mg. greater than that of control (Table 14 and 15, Fig 14). The cultural characters also exhibited some differences especially in colour and in intensity of sporulation.

Nature of growth in deficient media (Richards solution)

(c) Control Growth good, getting 44.0 mg. of dry mycelium in 7 days. Colour dark brown with greenish tinge, with a zone of light brown colour around the growing colony. Fairly good submerged growth. Mycelium yellowish brown. Sporulation good.

(1) -KNO_3 Growth very slight andsparse only 5.6 mg. of dry mycelium in 7 days. Colour light yellowish brown. No submerged growth. Sporulation very slight.

(2) -Potassium phosphate. Fairly good growth, but conspicuously less than that on the control, 19.0 mg dry mycelium in 7 days. Colour lighter when compared to the control, but

TABLE.14

Dry weight of mycelium in deficient media (liquid) after 7 days incubation at room temperature

Replication.	Treatments					
	C Complete	1 -KNO_3	2 -KH_2PO_4	3 -$MgSO_4$	4 -$Fecl_3$	5 Sucrose
1	49	4	16	22	50	5
2	38	5	20	23	67	3
3	46	6	18	24	53	3
4	45	4	21	24	48	5
5	42	5	20	21	56	3
Total	220	24	95	114	274	19
Mean	44.0	4.8	19.0	22.8	54.8	3.8

FIG 14 DRY WEIGHT IN DEFICIENT MEDIA

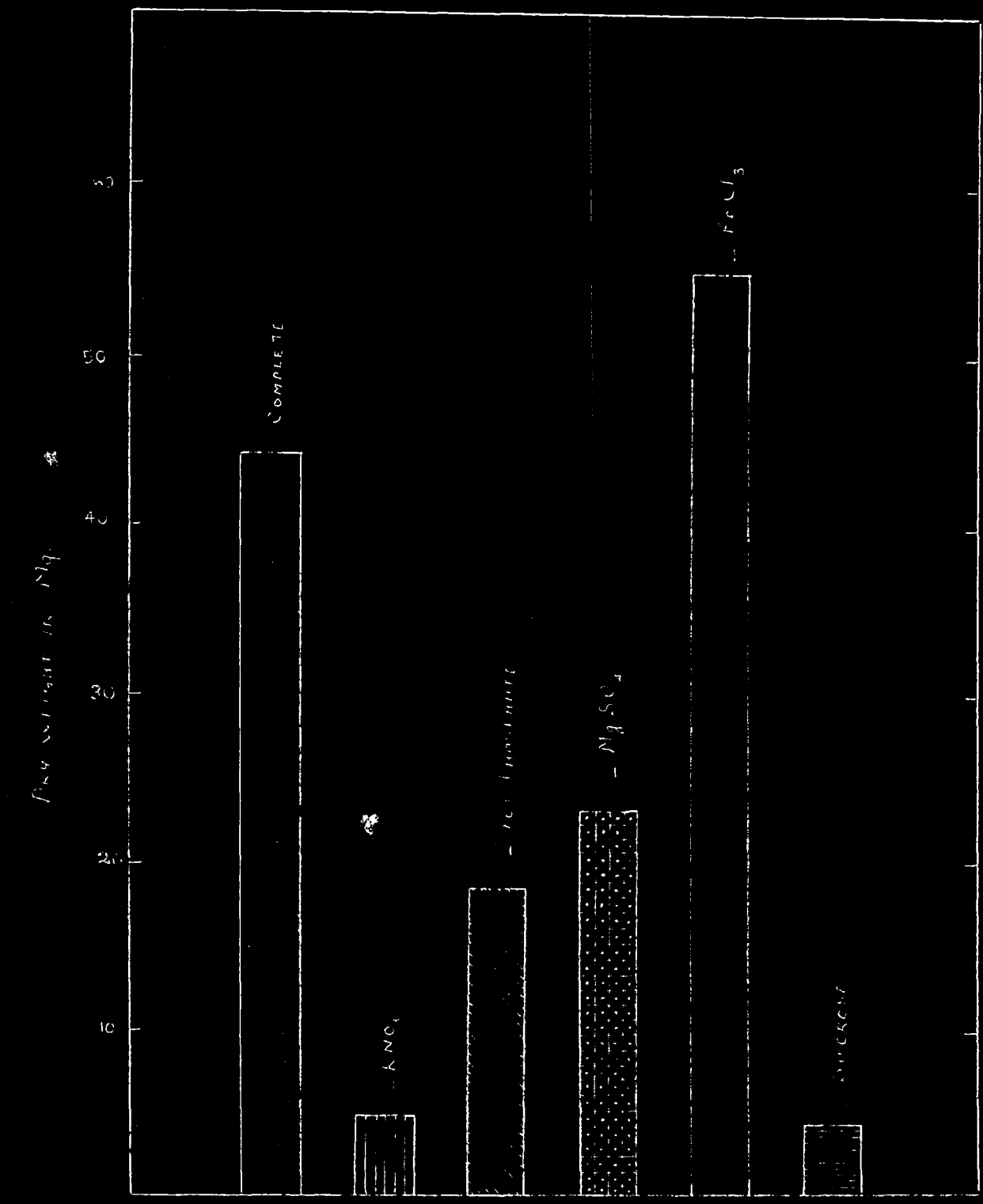


TABLE.15

Analysis of Variance

for the treatments m deficient media

Source	SS	DF	Variance	F	Inference
Total	11039.47	29			
Treatment	10736.27	5	2147.25	170.0	Significant
Error	303.20	24	12.63		

Critical difference: 7.95

Ranks	Treatments	Total dry wt. mycelium	Mean	Difference	Inference
1	4	274	54.8		
				10.8	Significant
2	C	220	44.0		
				21.2	Significant
3	3	114	22.8		
				3.8	Not significant
4	2	95	19.0		
				14.2	Significant
5	1	24	4.8		
				1.0	Significant
6.	5	19	3.8		

Result: 4 C $\overline{3\ 2}$ $\overline{1\ 5}$

darker than the treatment No.1. Submerged growth fairly good. No light coloured halo around the colony. Sporulation fairly good.

(3)-$MgSO_4$. Growth better than treatment No.2. 22.8 mg. dry mycelium in 7 days. Colour of the colony blackish, fairly good submerged sporulation good.

(4)-$FeCl_3$. Growth very good. Better than that of control, 55.4 mg. of dry weight in 7 days. Colour darker than control, tending to dark green. Good submerged growth. Sporulation profuse.

(5)-Sucrose. Growth very slight, confined to a small zone around the inoculam disc only. Colour light brown. No submerged growth. Dry weight of the mycelium only 3.8 mg in 7 days. Sporulation practically nil.

In all the treatments no aerial growth was observed.

Different Nitrogen Sources.

This experiment clearly demonstrated that the fungus has varying ability to utilise different nitrogen sources - organic and inorganic. The difference in radial growth and dry weight were conspicuous and it was more pronounced in the latter case. The average daily radial growth of the colonies in culture with no nitrogen, sodium nitrate, urea, asparagine, gelatin and peptone were more or less similar, ranging between 8.25 and 8.81 mm. The

cultural characters were also more or less same in these nitrogen sources except in the no nitrogen treatment in which growth was very sparse and sporulation very poor. The radial growth in the ammonium sulphate and ammonium nitrate were very low(Table 16), being 2.50 mm. and 2.75 mm. per day respectively. Growth habit of the colony was also altered in these two treatments(Fig.15,Nos.1 and 3). Colonies in these were brownish with fair aerial growth, margin undulated. Sporulation poor and spores very small.

Growth by dry weight of mycelium was lowest in the control being 21.0 mg. and maximum dry weight 305.6 mg. was obtained with asparagine followed by peptone and urea, with 282.0 mg and 262.mg. respectively. Then came sodium nitrate, sodium nitrate and ammonium carbonate, ammonium nitrate, gelatin and ammonium sulphate in descending order(Table 17). It was also found that the differences among these treatments were significant on statistical analysis(Table 18). Differences in the characters of the colonies were also noticed in case of control, ammonium sulphate and ammonium nitrate. In the control the colony was sparsely developed with no submerged or aerial growth. In ammonium sulphate and ammonium nitrate the colonies were developed in clumps, fairly good aerial growth and no submerged growth, colour brownish.

Different carbon sources

No appreciable difference in radial growth was observed for different carbon sources on solid media. In all

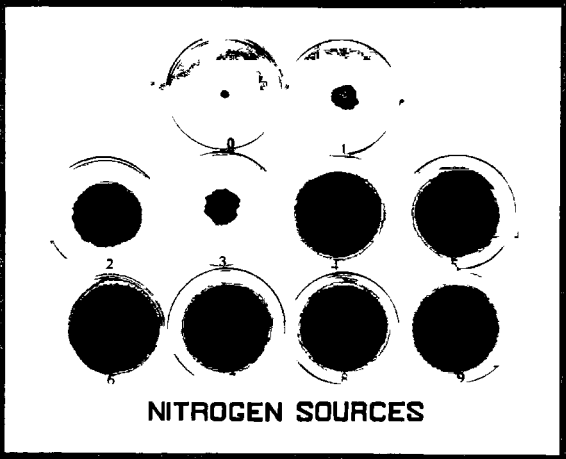


FIG.15. 0. No nitrogen, 1.Amm. sulphate,
2. Amm.carbonate, 3.Amm.nitrate,
4. Sodium nitrite, 5.Sodium nitrate,
6. Urea, 7.Asparagine, 8.Gelatin
9. Peptone.

TABLE.16

Measurement of daily radial growth of colonies in solid media with different Nitrogen sources.

(in millimeters)

Treatment	Replications	Days			Average daily growth
		2	3	4	
0-No Nitrogen	I	15	23	33	
	II	14	23	33	
	III	16	25	34	
	IV	15	25	34	
Average		15.0	24.0	33.5	8.38
1-Ammonium sulphate	I	5	7	9	
	II	7	12	13	
	III	5	8	9	
	IV	4	8	9	
Average		5.3	8.8	10.0	2.50
2-Ammonium carbonate	I	10	20	28	
	II	11	19	27	
	III	11	21	27	
	IV	10	21	28	
Average		10.5	20.3	27.5	6.88
3-Ammonium Nitrate	I	9	10	12	
	II	8	9	11	
	III	5	6	8	
	IV	9	11	13	
Average		7.8	9.0	11.0	2.75
4-Sodium Nitrite.	I	14.	25	37	
	II	14	27	38	
	III	15	25	36	
	IV	14	25	35	
Average		14.3	25.5	36.5	9.13

TABLE 16(contd.)

Treatment	Replications.	Days 2	3	4	Average daily growth
5-Sodium nitrate	I	15	26	35	
	II	15	26	35	
	III	15	25	36	
	IV	15	25	35	
Average		15.0	25.5	35.3	8.81
6-Urea	I	13	24	35	
	II	13	24	35	
	III	13	24	35	
	IV	13	24	36	
Average		13.00	24.0	35.3	8.81
7-Asparagine	I	13	23	33	
	II	13	23	34	
	III	14	24	35	
	IV	13	24	35	
Average		14.3	23.5	34.3	8.56

TABLE 16 (Contd.)

Treatment	Replications.	Days			Average daily growth
		2	3	4	
8-Gelatin	I	14	23	33	
	II	14	22	32	
	III	15	24	33	
	IV	16	25	34	
Average		14.8	23.5	33.0	8.25
9-Peptone	I	14	23	33	
	II	14	23	34	
	III	14	23	33	
	IV	14	24	34	
Average		14.0	23.3	33.5	8.38

TABLE.17

Dry weight of the mycelium in Czapcks solution with different nitrogen sources after 12 day incubation at room temperature.(in mg.)

	Treatments *									
Replication	0	1	2	3	4	5	6	7	8	9
I	20	60	177	134	212	242	280	300	105	285
II	22	62	175	135	210	249	260	304	95	295
III	21	66	176	121	236	250	266	311	119	260
IV	23	65	181	132	227	240	268	315	112	278
V	19	70	178	128	218	248	270	298	110	292
Total	105	323	887	650	1103	1229	1344	1528	541	1410
Average	21.0	64.6	177.4	130.0	220.6	245.8	268.8	305.6	108.2	282.0

* Treatments

0 - No nitrogen

1 - Ammonium sulphate

2 - Ammonium carbonate

3 - Ammonium nitrate.

4 - Sodium nitrite

5 - Sodium nitrate.

6 - Urea

7 - Asparagine

8 - Gelatin

9 - Peptone.

FIG. 16 DRY WEIGHT IN DIFFERENT N SOURCES

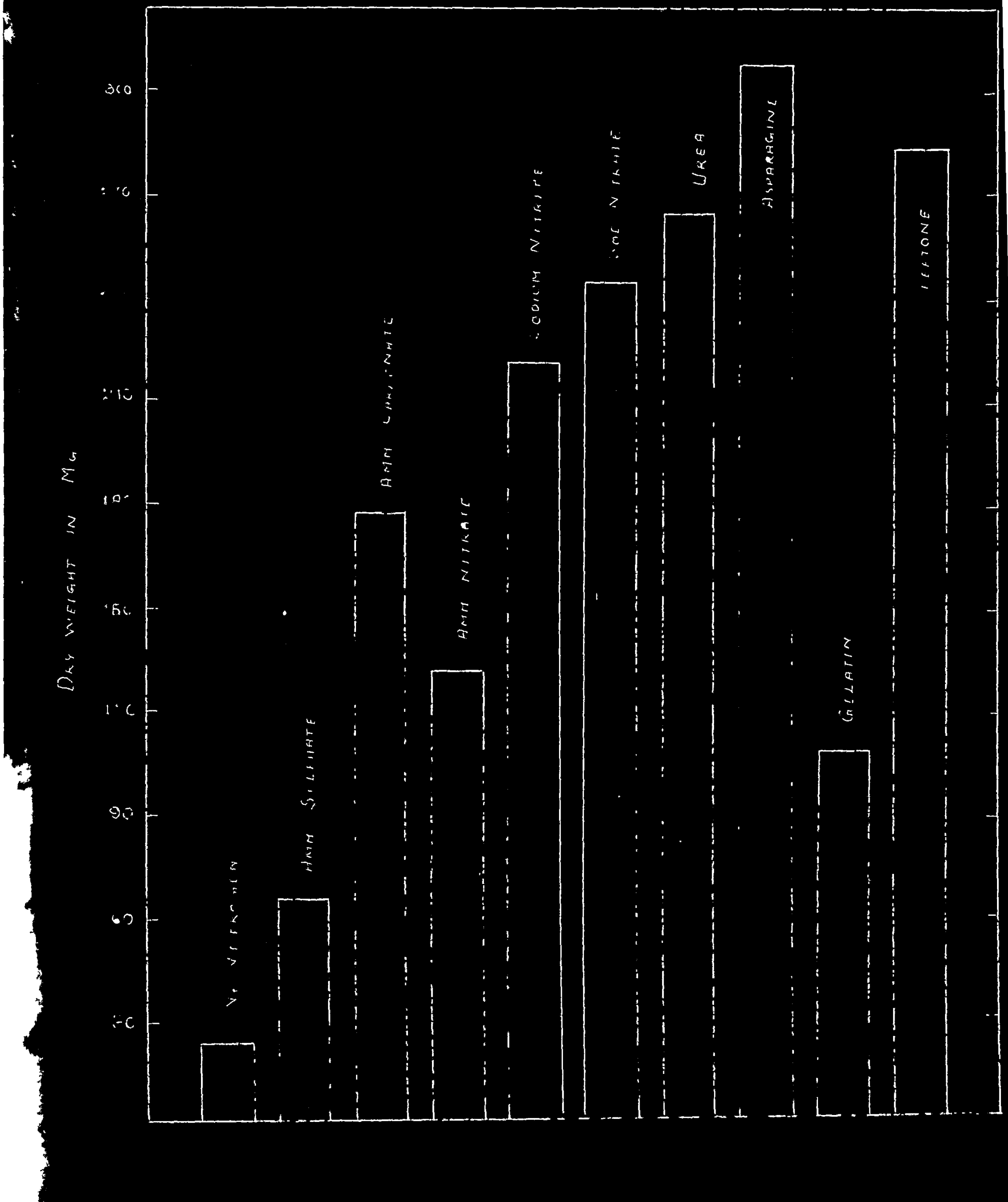


TABLE.18

Analysis of variance for the treatments with different Nitrogen sources

Source	SS	DF	Variance	F	Inference
Total	435918.0	49			
Treatment	431232.8	9	47914.76	409.0	Significant
Error	4685.2	40	117.13		

Critical difference=13.82

Ranks	Treatments	Total dry wt.of mycelium	Mean	Difference.	Inference
1	7-Asparagine	1528	305.6		
				23.6	Significant
2	9-Peptone	1410	282.0		
				13.2	Not significant
3	6-Urea	1344	268.8		
				23.0	Significant
4	5-Sodium nitrate	1229	245.8		
				25.2	,,
5	4-Sodium nitrite	1103	220.6		
				43.2	,,
6	2-Amm:carbonate	887	177.4		
				47.4	,,
7	3-Amm:nitrate	650	130.0		
				21.8	,,
8	8-Gelatin	541	108.2		
				43.6	,,
9	1-Amm:sulphate	323	64.6		
				43.6	,,
10	0-No nitrogen	105	21.0		

Result 7 $\overline{9\ 6}$ 5 4 2 3 8 1 0

cases the radial growths were slightly more than that in the control with no carbon source except in cellulose in which the radial growth was lower than that of the control. While the radial growth of the control was 7.6 mm. per day, in sucrose and glucose it was 9.6 mm. and in starch 9.mm., Growth in maltose was slightly more being 10.1 mm. The lowest radial growth was 4.9 mm. per day in the case of cellulose(Table 19). Colonies in the control and in cellulose were sparse and sporulation very meager. In lactose also the colony was slightly sparse and sporulation less.

The difference in growth by dry weight was more conspicuous. Maltose gave maximum dry weight, 197.8 mg. This was followed by sucrose, with 155.6 mg. which is significantly less than the growth on maltose. In gluoose, fructose, starch and lactose the dry weights were more or less the same without any significant difference among themselves(Table 20+21).

Different pH

Dry weight of the mycelium was maximum at pH 5.5. Next highest weight in pH 4.5, followed by that at pH 6.5. They were 550.2 mg, 422.2 mg and 297.2 mg. respectively. Below pH 4.5 and above 6.5 growth were low. An appreciable increase in growth was again observed at pH 9.2(310.0 mg.). But at this pH sporulation was very poor. The dry weight was minimum at pH 1.4, which was 20.5 mg. At pH 10.6 also the dry weight was very low. When the pH of

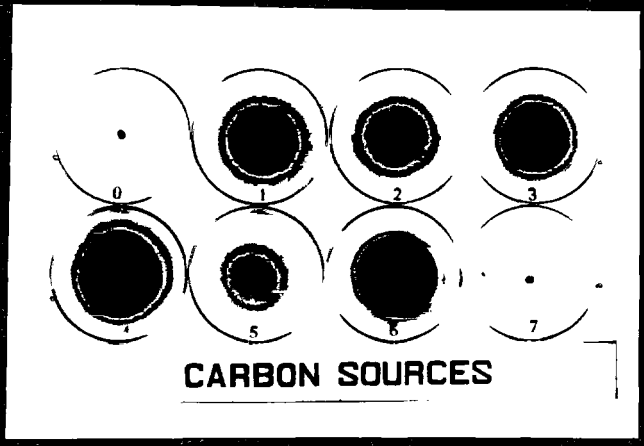


FIG.17. 0. No carbon, 1.Sucrose, 2.Fructose,
3. Glucose, 4.Maltose, 5.Lactose,
6. Starch, 7.Cellulose.

DIFFERENT CARBON SOURCES

TABLE 19

Measurement of daily radial growth of colony in solid media (Czapek's agar) with different carbon sources (in millimeters).

Treatment	Replications.	Days 2	3	4	Average daily growth
0-No carbon	I	14	22	30	
	II	14	22	30	
	III	13	23	31	
	IV	13	23	30	
Average		13.5	22.5	30.3	7.6
1-Sucrose	I	15	27	38	
	II	16	28	39	
	III	15	27	39	
	IV	16	28	38	
Average		15.5	27.5	38.5	9.6
2-Fructose	I	12	24	35	
	II	13	23	34	
	III	14	24	34	
	IV	14	25	36	
Average		13.3	24.0	34.8	8.7
3-Glucose	I	15	27	38	
	II	16	28	39	
	III	16	27	38	
	IV	15	27	38	
Average		15.5	27.3	38.3	9.6

TABLE.19 (contd)

Treatment	Replications.	Days 2	3	4	Average daily growth
4-Multose	I	17	28	41	
	II	17	28	41	
	III	18	27	40	
	IV	19	28	40	
Average		17.0	27.8	40.5	10.1
5-Lactose	I	15	26	36	
	II	16	26	35	
	III	16	26	35	
	IV	17	26	36	
Average		16.0	26.0	35.5	8.9
6-Starch	I	17	27	38	
	II	16	27	38	
	III	16	27	38	
	IV	16	26	37	
Average		16.3	26.8	37.8	9.5
7-Cellulose	I	10	14	18	
	II	10	14	18	
	III	10	15	17	
	IV	9	13	18	
Average		9.8	14.0	17.8	4.9

TABLE.20

Dry weight of the mycelium in liquid media (Czapcks) with different carbon sources after 10 days incubation at room temperature

(in milligramms)

Replication	Treatments							
	0	1	2	3	4	5	6	7
I	33	158	125	132	193	130	125	70
II	25	168	130	128	185	121	120	50
III	35	155	128	133	214	120	123	65
IV	40	145	120	120	209	132	136	58
V	31	152	135	131	188	115	132	56
Total	164	778	638	644	989	618	636	299
Average	32.8	155.6	127.6	128.8	197.8	123.6	127.2	59.8

Treatments

0 - No sugar

1 - Sucrose

2 - Fructose

3 - Glucose

4 - Maltose

5 - Lactose

6 - Starch

7 - Cellulose

FIG 18 DRY WEIGHT IN DIFFERENT CARBON SOURCES

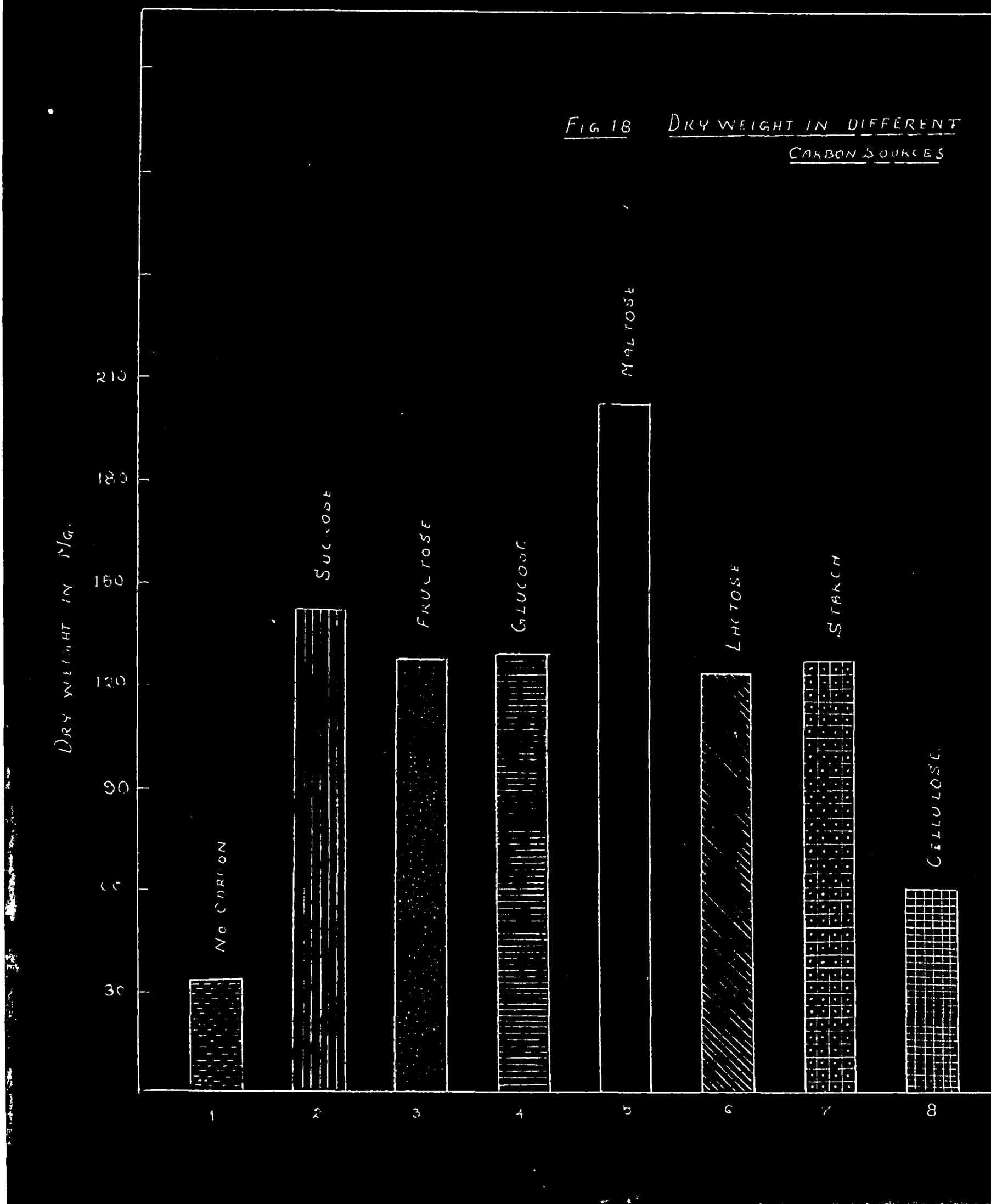


TABLE.21

ANALYSIS OF VARIANCE FOR DIFFERENT CARBON SOURCES - LIQUID MEDIA

Source	SS	DF	Variance	F	Inference
Total	95654.1	39			
Treatment	93716.5	7	13388.09	221.1	Significant
Error	1937.6	32	60.55		

Critical difference=10.03

Rank	Treatment	Total dry wt of mycelium	Mean	Difference	Inference
1	4-Maltose	989	197.8	42.2	Significant
2	1-Sucrose	778	155.6	26.4	Significant
3	3-Glucose	644	128.8	1.2	Not significant
4	2-Fructose	638	127.6	0.4	,,
5	6-Starch	636	127.2	3.6	,,
6	5-Lactose	618	123.6	63.8	Significant
7	7-Cellulose	299	59.8	27.0	,,
8	0-No sugar	164	32.8		

Result 4 1 $\overline{3\ 2\ 6\ 5}$ 7 0.

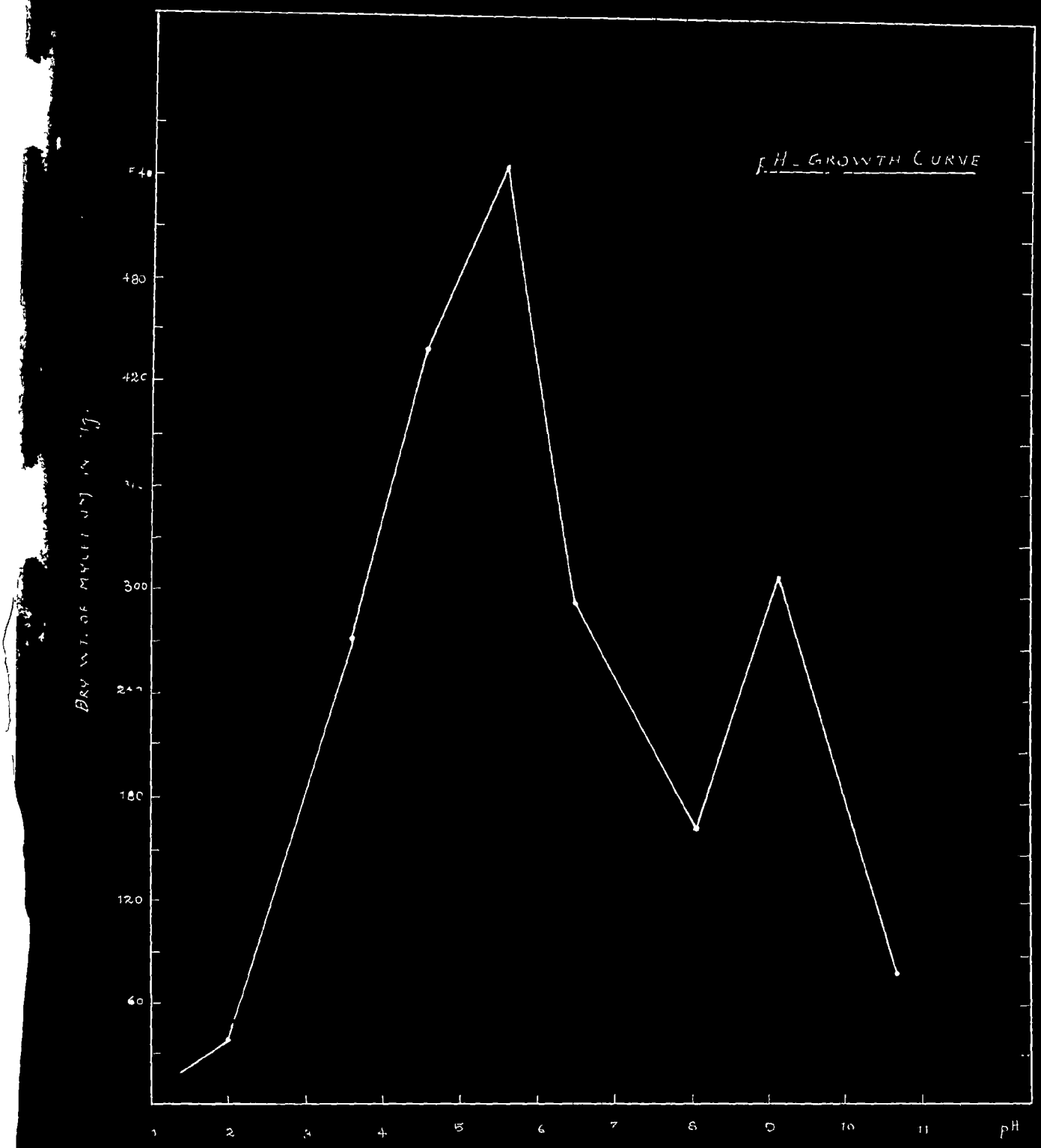
TABLE.22

Dry weight of the mycelium in liquid media (Czapcks) of different PH, after 13 days incubation at room temperature.

(in milligrammes)

Initial PH	Replications					Total dry wt. of the mycelium	Mean	Final PH
	I	II	II	IV	V			
1.4	22	18	23	19	20	102	20.5	1.3
2.0	39	40	37	47	41	194	38.8	1.5
3.6	299	285	273	265	280	1402	280.4	7.0
4.5	414	424	412	435	426	2111	422.2	7.2
5.5	567	540	532	560	552	2751	550.2	7.0
6.5	322	300	287	265	312	1486	297.2	7.0
7.0	221	274	237	298	266	1296	259.2	8.0
8.1	170	166	162	160	148	806	161.2	8.1
9.2	339	286	295	294	340	1554	310.8	8.1
10.0	187	174	172	181	176	890	178.0	8.6
10.6	84	69	76	80	82	391	78.2	9.4

FIG 19


pH - GROWTH CURVE
DRY WT. OF MYCELIUM IN mg.
480
420
300
180
120
60
1
2
3
4
5
6
7
8
9
10
11
pH

the filterate was determined it was found that generally the pH of the acidic media were increased to neutrality when there were good growth of thefungus. But wherever the growth was poor the pH alterations were also neglegible. But in case of alkaline media especially in pH 9.2 and above the pH were lowered. pH 9.2, 10.0 and 10.6 were lowered to 8.1,8.6 and 9.4 respectively. The initial pH 8.1 remained unchanged. In this treatment the growth was very low(Table 22 Fig.19) Sporulation was higher at the reactions from 3.6 to 7, and it was considerably reduced, above these points. Even at pH 9.2 were the growth was fair,sporulation was very shy.

Different Temperatures

The fungus grow from 10° to 35°C, but at 10°C the radial growth rate was very low, only 0.6 mm. per day. There was no growth at 0C, though the fungus remained viable at this temperature. The growth was considerably reduced at 35°C, a radial growth of 2.4 mm. per day only was observed at this temperature. The maximum growth occured between 25° and 30°C. There were no appreciable difference in radial growth at temperatures between 25 and 30°C, but a maximum was noted at 28°C, suggesting that the optimum temperature for this fungus probably lies very near to 28°C(Table 23 and 24,Fig.20). Difference in cultural characters were also observed at different temperatures. At low temperatures the growth was sparse and sporulation shy. At the optimum range dense growth and profuse sporulation took place. But at the high temperatures the

TABLE.23

Radial growth of colony at different temperature (Solid media Czapeks) in millimeters.

Replications.	Temperatures									
	0°C	5°C	10°C				15°C			
	Days	Days	Days				Days			
	Upto 7 days	upto 7 days.	4	6	8	10	2	3	4	5
I	No growth	No growth	1	3	5	7	4	8	14	19
II	,,	,,	-	2	4	6	5	9	15	20
III	,,	,,	-	1	3	5	7	11	16	21
IV	,,	,,	1	3	6	7	6	10	15	20
V	,,	,,	1	2	4	6	7	10	15	20
Average	0	0	0.6				4.0			

TABLE.23 (contd)

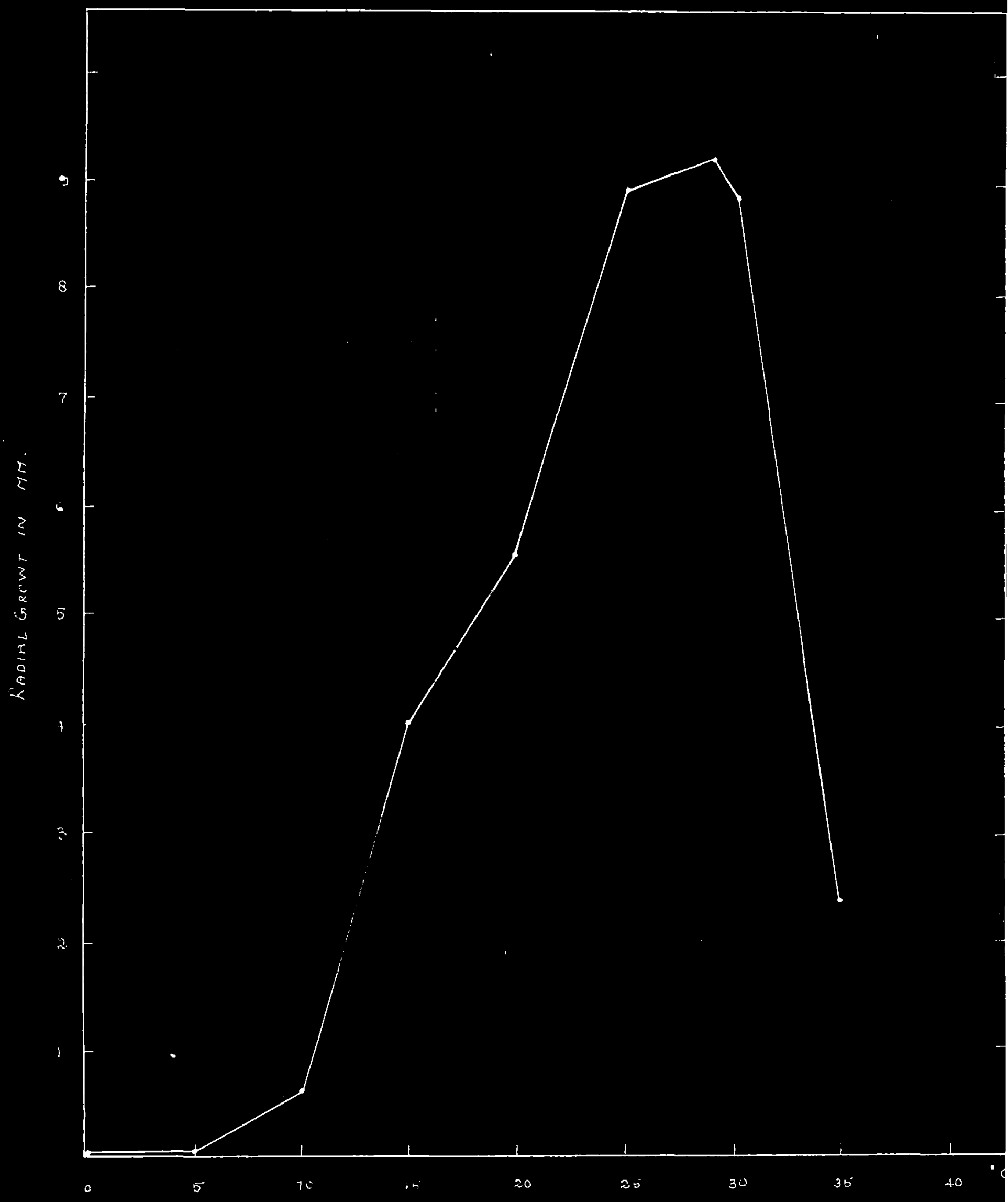
Replications	Temperatures															
	20°C				25°C				30°C				35°C			
	Days				Days				Days				Days			
	2	3	4	5	2	3	4	-	2	3	4	-	2	3	4	5
I	9	15	22	28	14	25	36	-	15	26	35	-	7	9	10	11
II	9	15	22	28	14	24	35	-	15	26	35	-	7	9	10	11
III	8	14	21	27	14	24	35	-	16	27	36	-	9	10	11	13
IV	9	15	22	28	14	24	35	-	14	25	35	-	7	9	12	13
V	8	15	22	29	14	24	36	-	15	25	36	-	8	10	12	13
Average			5.6				8.9				3.9				2.4	

TABLE.24

Radial growth of colony at different temperatures from 25°C to 30°C (in mm.)

Replications.	Temperature																	
	25°C			26°C			27°C			28°C			29°C			30° C		
	Days			Days			Days			Days			Days			Days		
	2	3	4	2	3	4	2	3	4	2	3	4	2	3	4	2	3	4
I	15	26	35	16	26	35	16	26	36	16	26	37	15	26	35	14	26	34
II	15	27	36	15	26	35	16	25	35	15	26	36	16	26	35	15	26	36
III	15	26	35	15	27	36	15	26	35	16	27	38	15	25	36	15	26	35
IV	14	26	35	15	26	36	16	26	37	16	26	36	15	25	37	15	25	35
Average	8.8			8.9			8.9			9.2			8.9			8.8		

FIG. 20 TEMPERATURE. GROWTH CURVE



growth and sporulation were reduced and the colour of the colony became brownish and the conidia smaller.

Different vitamins

When different concentrations viz. 5 ppm, 50 ppm, and 100 ppm each of biotin and thiamine were used with the culture solution the growth obtained were more or less similar (Table 25 and 26) to that of the control with no vitamins. The sporulation and the culture characters were also similar.

Studies on five common species of

Pestalotia with special reference

to their identity

VITAMINS

Table.25

Dry weight of the mycelium in media with different concentrations of Biotin (in milligrams.)

Replications.	Concentration of Biotin			
	0	5 ppm	50 ppm	100 ppm
I	237	228	234	244
II	230	244	246	229
III	228	238	241	236
IV	240	230	232	220
Average	233.8	235.0	238.3	232.2

Table 26

Dry weight of the mycelium in media with different concentrations of Thiamine (in milligrams)

Replications.	Concentration of Thiamine			
	0	5 ppm	50 ppm	100 ppm
I	224	247	252	242
II	228	216	242	230
III	242	237	246	237
IV	229	240	230	228
Average	237.5	235.0	242.5	234.3

DISCUSSION

Helminthosporium halodes is considered to be the fungus which was mainly involved in the leaf rot disease of the coconut palm(Menon and Nair,1948). A comparative study of the available cultures of the fungus and also the published reports indicate that the isolate from the coconut palm shows certain characteristics which differentiate it from others. The most prominent difference is the occurance of the secondary conidia in the coconut isolate. At the same time there are certain similarities in the cultural characters which indicate the relationship of the coconut isolate with the type species described by Drechsler.

The colour of the mycelium and spores is more or less similar. The colour of the colony in PDA is also more or less similar. There was slight difference in the aerial growth, which was pronounced in culture S, giving a floculant apperance. The important distinguishing characters presented by Drechsler(1923), mainly the dark intermediate segments and sub-hyaline or fuliginous end segments set off by conspecuously accentuated septa, the characters mentioned in all subsequent reports, had been found to be constant characteristics of the culture C also. Germination which is typically by the production of two polar germ tubes had also been found to be similar in all the cases. The general contour and shape of the spores and sporophores were similar.

In some important characters the isolate C differed significantly from the original report of Drechsler and from all the other reports, and from the isolate of Subramoniam, used in the present study for comparison. Differences in measurements were found to be appreciable. The conidial measurements of isolate C were far superior to that of isolate S and reported measurements of other isolates (Table 10 gives the comparison of the measurements of conidia of various isolates). While the maximum length of conidia on natural substrate given in Dreschler's report was 105 u, that of isolate C was 126 u. The difference may not be appreciable. But the difference in spore size in the culture is, however, very prominent. Dreschler had reported a shortening of the spore in artificial media. The maximum length of spore of culture S on PDA was only 93.5 u (Table 10), where as, the culture C had a maximum length of 199 u. Ther is, therefore marked difference in the size of spores of isolates C and S in culture. The length of conidia in natural substrate is greater for isolate C. While the artificial culturing reduced the spore length of other isolates it is considerably increased in isolate C. Differences in width of spores and septa number were also found to be conspecuous (Table 10). Frequency distribution curves (Figre 9 and 10) also shows that the two groups of spores cannot be from the same population.

When isolate C was compared with the Mitra's (1931)

fungus H.halodes var.tritici difference in spore measurement was found to be more prominent. While the maximum length of conidia of isolate C is 126 u that of Mitra's is only 73 u, and the maximum width is 27.7 u and 20.0 u respectively, the number of septa of culture C and the variety of Mitra are 17 and 9 respectively.

The maximum length of spores reported by Kovachich (1954) for H.halodes var.elaeicola is 125 u which is not significantly different from the maximum length of spores of culture C (ie.126 u), but in other measurements this organism differs from that of Kovachich's. The maximum length of conidia in artificial culture is reduced to 96 u for his organism, but it is increased considerably (to 199 u) for culture C.(Table 10).

The secondary spore formation observed in culture C has not been described for the type species or for any of its varieties. It is absent in colture S also. Such an important character cannot be over looked in fixing the taxonomic position of an organism. The secondary spore formation in this organism is more or less similar to that reported for H.gramineum (Drechsler,1923). But for this character, there are no other similarities between H.gramineum and the organism under study. There is considerable difference in the spore measurements also. The sub-hyaline end cells and accentuated end septa are not presentin H.gramineum.

In the light of these observations the coconut fungus is considered as a new variety of H.halodes. It is designated as H.halodes var.nuciferae. The diagnosis of the fungus is given below:-

Helminthosporium halodes var.nuciferae var.nov.

Mycelium well developed, septate and branched, hyaline when young changing to yellowish brown to dark brown with aging, 3 to 6 u in width. Conidiophore straight or curved and flexuous, often geniculate, brownish in colour, with sub-hyaline distal region; length highly variable, 60 to 350 u, generally 100 to 200 u. Conidia are borne apically and laterally in clusters of 4 to 10, in racemose arrangement. Conidia straight or curved, cylindrical or elliptical, more often elliptical than cylindrical; brownish yellow to cocoa brown, with sub-hyaline end cells and accentuated end septa, 27 to 126 u by 12 to 20 u, 2 to 11 septate when produced in natural substrate, and becoming longer when cultivated on PDA. On PDA the conidia are 16 to 119 u long 6.5 to 22.7 u wide and 1 to 17.septate. Secondary conidia are borne on a short secondary conidiophore proliferated from the apex of mature conidia. Secondary conidiophores are thinner 3 to 6 u in width and 10 to 60 u in length, with characteristic geniculations. Secondary conidia are smaller in dimensions and lighter in colour. Conidia germinate by production of two polar germ tubes. Readily cultivable in standard artificial media giving a surface colony, yew green when young and becoming dark with age.

The present investigation on the nutritional requirements of H.halodes are only of a preliminary nature. It is therefore not possible to arrive at any definite conclusions. The results can be interpreted only in such a way as to provide certain broad indications. The mycelial discs used as inoculam might have influenced the results to some extent, especially in such cases where the complete elimination of certain nutrients was intented.

When different solid media were used for determining the radial growth it was found that there was no appreciable difference in the rate of growth in different media. However, considerable difference in dry weights were observed when the above media were used in the liquid form. The average dry weights in liquid media ranged from 13.6 mg. in Barne's to 157.2 mg. in potato dextrose solution, while the radial growths were almost identical. Though there may be correlations between the rate of growth and dry weight in the cases of certain fungi it is not true of H.halodes as also of many other fungi. Radial growth alone cannot therefore be taken as a criterion for determining the suitability of a medium for the growth of H.halodes. Though the radial growth does not give any clue as to the suitability of any media for the growth of this fungus by observing the pigmentation and type of growth and darker colouration were associated with better growth and this was confirmed by the dry weights. Fig.11 gives an idea of the gradual transition from dark to light pigmentation in the

different media and this fall in the intensity of pigmentation can well be correlated with the fall in the dry weight given in Table 12.

Poorest growth in Barne's medium may be attributed to the very low concentration of the nutrients it contains. In fact this medium was selected for its low nutrient contents. Though the Coon's medium contains all the nutrients in fairly high concentrations its carbohydrate content is very low in comparison to PDA, Czapek's and Richard's media in which the fungus grow well. This may be the reason why the fungus grow poorly in Coon's medium. In Czapek's-Dox medium the growth was very poor. The constituents of this medium are similar in every respect to those of the Czapek's medium, except that the former additionally contains 0.5 gms of $FeCl_3$. While the dry weight in the Czapek's was 131.8 mg, that in Czapek's-Dox medium was as low as 20.6 mg. It it possible that the higher concentration of iron in the latter medium in the form of $FeCl_3$ may have adversely affected the growth of this fungus. A slight excess of iron may be toxic to this fungus. This finding is again supported by the study in which Richard's medium deficient in $FeCl_3$ was used, the dry weight was higher than that in the complete medium.

These findings do not necessarily go to prove that by omitting the iron source, iron was completely eliminated from the medium, or that the fungus does not require iron. Iron might have been present as contaminant in the distilled

water or the other chemicals used in the experiment or provided by the inoculam disc. It may be recalled in this connection that Peterson and Katznelson (1954, 1956) have definitely proved that this fungus, - though not this particular strain, requires iron for its proper growth. The results of the present studies perhaps go to show only that the fungus cannot tolerate higher concentrations of iron.

The elimination of one or the other constituent except the iron source of the Richard's medium considerably reduced the growth of H.halodes. This was especially very prominent for the deficiency of carbon and nitrogen. Definite conclusion regarding the extend to which the deficiency of individual constituent influenced the growth and sporulation can be reached only after further more eloberate experimentations.

The fungus can utilise the organic and inorganic forms of nitrogen. Among the organic forms asparagine was the most favoured one followed by peptone and urea. Among the inorganic forms the fungus favoured sodium nitrate followed by Sodium nitrite, to ammonium carbonate, ammonium nitrate and ammonium sulphate. Though certain amount of growth was observed in ammonium nitrogen it was significantly lower than that in other organic and inorganic nitrogen sources. This definitely shows that the organism can utilise ammonium nitrogen, but some facters block the way of ammonium utilisation. Hacskyle et al (1954) who have studied the nitrogen utilisation of 25 species of fungi, from ammonium sulphate found sharp pH drops

in all cases, which accounted for a retardation in growth resulting in a considerable reduction in dry weight. Cochrane (1958) after making an eloborate review of more than 20 authors came to a conclusion that the ammonium utilisation from salts of ammonia, caused a very rapid and large drop of pH, due to preferential utilisation of the cations from the ammonium salts. In the light of these observations it can be concluded that a similar situation possibly exists in the case of H.halodes also.

The ability of a fungus to utilise different types of carbohydrate depends upon both the configuration of the carbohydrate and the ability of the specific organism to form adaptive enzymes. Generally monosaccharides are utilised by many fungi than di and polysaccharides. Glucose has been reported to be a good carbon source for many fungi. In the present study it has been found that H.halodes has marked ability to assimilate various types of carbon sources like mono, di, and polysaccharides. However, the soluable forms of these carbohydrates were preferred by this organism. Maltose, a disaccharide was found to be the best carbon source for the maximum rate and amount of growth. This was followed by sucrose, also a disaccharide. The two monosaccharides tested namely, glucose and fructose were also utilised fairly well. Among polysaccharides the starch was available to the fungus as good as glucose or fructose. Cellulose was not a favourable carbon source though the fungus could utilise it to a certain extant. This is evident from the fact that the dry weight when is grown

in medium containing cellulose was significantly greater than that in which carbon source was omitted.

The pH of the medium usually have direct influence on the growth and reproduction of the fungi; simultaneously the metabolites produced by the growing organism may also exert some influence which may account for an alteration of the pH. The constituents of the medium also may have some interrelation with the pH of the medium and the growth of the organism. The substance with buffering action are of special importance in this respect. In addition certain external factors like temperature etc. may also influence the pH. Cochrane(1958) belives that almost any factor in the environment may change the pH growth curve.

In the present study it was found that the optimum range of pH for H.halodes was between 4.5 and 6.5, maximum growth was observed at 5.5. The optimum pH for this fungus may therefore lie around 5.5. Above the optimum point of pH the growth fell sharply, but a second increase was again noted at pH 9.2, though it was significantly less than that at 5.5. This phenomenon though not very common has been reported for certain other species of fungi as well. Scott(1924) has reported a double pH optimum for Fusarium lycopersici. Desdall(1923) has reported double pH optimum for the germination of spores of Helminthosporium sativum but both on alkaline side at pH 8.2 and 9.2.

Above pH 5.5 the sporulation was considerably reduced. Even at 9.2 where another growth increase was noticed, the sporulation was almost absent. The second optimum was therefore not a favourable pH for sporulation.

It was noted that the initial pH of the media was altered by the growth of this organism. When the initial pH was very low growth was very poor and the alteration of pH also was not significant. But when the initial pH was between 3.5 and 6.5 where the growth was good or fair, the final pH was raised to neutrality. This possibly indicates that metabolic products of this organism might have been basic when grown on acidic medium. Similar situation has been reported by Saksena et al(1953) for various species of Pythium which raised the pH of the medium after incubation. It was attributed to the production of ammonia. Henry and Anderson (1948) have also observed a similar relationship in the case of Piricularia oryzae. Another possible explanation given by Cochrane(1958) is that the metobolic activities of the fungi raised the pH of the medium by absorption of anion. In the present study pH 7.0 was increased to 8.0. Here also the same explanations hold good. At pH 8.1 the growth was poor and there was no change of pH. Above this point a lowering of pH was generally noted. pH 9.2 was lowered to 8.1, a fair growth occurred at this pH. The lowering of pH here may be due to the formation of organic acids or due to the absorption of cations, (Cochrane,1958). The absorption of carbon dioxide

by sodium hyroxide added to the medium might also have helped to lower the pH.

H.halodes can grow from 10°C to slightly more than 35°C and the optimum ranged from 25 to 30°C. The optimum temperature for growth lies around 28°C, the maximum slightly above 35°C and the minimum between 5 and 10°C. The fungus can withstand exposure to 0°C for seven days or more.

The growth of this fungus was not influenced by biotin and thiamine at the concentrations tried, viz. 5 ppm, 50 ppm, and 100 ppm. This suggests that either the fungus is autotrophic with respect to these two vitamins or it does not require them for its growth.

SUMMARY AND CONCLUSION.

The strain of Helminthosporium halodes occuring in Kerala and causing leaf rot disease of the coconut palm was found to differ in certain respects from the type species and also from its known varieties. The dimensions of the spores of this organism were far superior to that of others which were reported as H.halodes or as its varieties. The differences were very prominent in culture. Formation of secondary spores on definite secondary sporophores, proliferated from the primary spores occurs only in the coconut strain not in the type species or its varieties. The fungus is therefore considered as a new variety of H.halodes and designated as H.halodes var.nuciferae var. nov.

The fungus was readily cultivable in standard liquid and solid media and grow well in PDA, Czapek's and Richard's medium. In most of the cases it was found that the radial growth was not correlated with the dry weight, suggesting that the radial growth is not a dependable criterion as a measurement of growth of this organism.

Deficiency of nitrogen or carbon reduced the growth and sporulation. The fungus seems to prefer only a very low concentration of iron and an increase in concentration was found to affect the growth adversely.

Of the nitrogen sources tested the fungus utilised asparagine best followed by peptone, urea, sodium nitrate and sodium nitrite. Ammonium nitrogen sources, ammonium sulphate

and ammonium nitrate, though utilised to a certain extent were found to be a poor nitrogen sources.

Maltose was found to be the best carbon source followed by sucrose, glucose, fructose, starch and lactose, were also utilised fairly well. Cellulose was an unsuitable carbon source, it being only slightly utilised.

This fungus can grow well under a wide range of pH. The optimum pH lies at about 5.5. But a second optimum was also noticed near pH 9.2. It was found that the fungus can alter the pH to neutrality when grown on acidic medium. When the initial reaction was alkaline there was only very slight lowering of pH.

The optimum temperature for growth was 28°C.

This fungus showed no response to biotin and thiamine at the concentration tried. Either the fungus is autotrophic with respect to these two vitamins or it does not require them for its growth.

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