CHAPTER - IV

CYTOTAXONOMICAL, SEXUALITY, CULTURAL AND DEVELOPMENTAL STUDIES OF -

a) *Leptosphaerulina alysicarpii* Dhage and Barge.

b) *Salmonomyces kamatii* Chiddarwar

CYTOTAXONOMICAL, DEVELOPMENTAL AND CULTURAL STUDIES IN *LEPTOSPHAERULINA ALYSICARPII* DHAGE AND BARGE.

During the course of field work, I collected infected leaves of Alysicarpous rogosus Baker. [Photo No 1] The fungus was studied in laboratory and it was found to be Leptosphaerulina. This was determined on the basis of the structure of the apical wall of the ascus. It was dome shaped and resembelled a hat like structure.

Barge (1975) had reported one unidetified species of Leptosphaerulina on seme host, so I studied my collected material in detail.

MORPHOLOGY :

Perithecia on dead necrotic areas, epiphyllous, immersed pseudoparenchymatous, stoma surrounded by dark brown cells. Perithecia - 103 - 150 µm X 110 - 150 µm. [Photo. No. 2.].

Asci 3 - 6, club shaped to saccate, bitunicate and apex dome shaped, 45 - 90 µm X 25 - 45 µm. Ascospores 8, ellipsoidal, hyaline, with generally five transverse septa and 2 to 4 longitudinal septa restricted to middle cells giving dictyosporous appearence, ascospore 25 - 40 µm X 6 - 15 µm, on living leaves of host Alysicarpous rogosus Baker, Satara, on August 15, 1988 (Photograph No. 3).

Barge (1974) has not reported giving Latin diagnosis to this species Leeptosphaerulina hence I accomodated it in a new species as Leptosphaerulina alysicarpii, Dhage and Barge.

infectio epiphyllae, periethecium in necrasis areae de folium, emergoe re, stromatis pseudoparenchymatis, circumvallatus juxtaatra brunneo cellulis, perithecie 103 - 150 X 110 - 150 µm, Ascis 3-6, Clavatus, Saccatus, bitunicatus apicis forma domus, 45 - 90 X 45 - 45 µm, actosporae, sporae ellipticus, hyalinus, generalis 5 - traniversalis septatus, gue 2-4 longitudinalis septatus atricum medins cellulis, dictyosporosis aspectum, 25 - 40 X 6 - 15 µm.

Typus lectus infoliis
Alysicarpus rogasus Bak., Satara
(M.S.) 15-8-88. Leg.
H.C.I.O.
The type material is being deposited at H.C.I.O., New Delhi.

CULTURAL STUDIES :

Barge (1975) studied the culture of this species on P.D.A. and found :-

1) The development of mycelium was in circular rings.
2) The reproductive hyphae was broader than vegetative hyphae.
3) Perithecia were developed in culture.
4) The breadth of the ascospores and the number of transverse septa showed constancy both in natural and cultural structures.

The pathogenic fungi are selective in their choise of food. We studied influence of nitrogen source and influence of vitamins and growth hormones on the growth of this fungus Leptosphaerulina alysicarpii.

Nitrogen Sources :

All the nitrogen sources are equally suitable for different fungi. Steinberg (1950) has classified the fungi according to their ability to utilize the different sources of nitrogen. We studied organic sources of nitrogen compounds such as (1) Monoamino monocarboxylic acids as Phenylalanine and Glysine and (2) Monoamino dicarboxylic acids such as Aspartic acid and Glutamic acid.

Among the aminoacids the growth was quite satisfactory in Phenylalanine and Glysine appeared to be a poor source of nitrogen. Tondon (1961) observed good growth for Gloeasporium, Pestalotia in Phenylalanine while Converse (1953) observed poor growth for Helminthosporium with Glysine. With aromatic amino acid Tyrosine growth was found to be satisfactory in Mycena, Nicholas (1965), (Photograph No. 6,5). In the culture of Leptosphaerulina the response to Tyrosine was poor. The importance of vitamins in nutrition of fungi has been shown by various workers like Sadasivan and Subramanian (1954), Tondon and Bilgrami (1957). Ascorbic acid or Vitamin C when added in the concentration of 10 ppm - 20 ppm an appreciable

decrease in growth was observed. Similar concentration in case of Phyllosticta (Tandon,1961). The growth was found to be satisfactory in Gibberlic Acid and Indole Acetic Acid (Photograph No.7).

SEXUALITY AND DEVELOPMENT :

In the culture of Leptosphaerulina, after innoculation thin, elongated, branched, mycelia were developed after 5 - 6 hours. The cells of these mycelia were narrow (2 - 6 µm) and elongated (8 - 20 µm), mostly uninucleate, rarely bi or multinucleate. Lateral branches were usually bifurcated.

Any sort of reproductive structures or their initiation was not observed on these mycelia. So here, it was referred as vegetative mycelium.

20-24 hours after innoculation, wider branches 7-13 µm broad and 7-14 µm long, having fleshy appearence made their appearence within network of vegetative mycelia. The fleshy appearence was due to their shorter cells and swollen nature. These branches were brancehed, branches were fleshy and 1-5 cells in length (6-30 µm length). Very rarely secondary branching was observed. The cells of wider branches were mostly uninucleate but binucleate cells were also frequent. Multinucleate cells occurred very rarely. Within 2-3 hours after appearence of wider branches, small knots (12-18 µm diam.) appeared at the apices of shorter branches of wider mycelia. These were singly

developed. Knots were of two types (Photograph No.9(b). In formation of some knots two lateral branches of different mycelia were involved, while in others, only single lateral branch was involved. Here I referred them as bihyphal and unihyphal knots respectively. Reproductive cycle began here on wider mycelia hence these were referred as reproductive mycelia.

It is observed that two apical cells of two lateral branches of different mycelia came in close association and divided and redivided together to form bihyphal knots. But the fusion of these two cells or migration and fusion of their nuclei were not observed during formation of knots. Unihyphal knots were developed by internal periclinal divisions of apical cells of lateral branches.

Whemeyer (1955-a) reported development of such structures formed by irregular coilling of branches and called them as stromatal or ascocarp primordia in Pseudoplea gaeumannii. He observed that the protoplast of active cells divide by cleavage into number of endogenous protoplast accompanied by nuclear division and cell division to form cells with one or two nuclei.

Miyabe (1889-90) reported a row of short cells cutting into slender branches which anastomosed to form a parenchymatic plexus of cells in Macrosporium parasiticum.

Here in our material cell divisions continued in both types of knots, but unihyphal knots enlarged up to certain

extent (48-58 um diam.) and stopped its development. Then outer wall became thick and brown coloured. At this stage this knot appeared fleshy due to its large fleshy cells and showed less affinity for nuclear stains (Photograph No. 9). Bihyphal knots continued their development and underwent differentiation of wall cells and internal cells. Cells of these knots were small with dense granular protoplasm and showed more affinity for nuclear stains (Photograph No. 10). By continued cell divisions in bihyphal knot a pseudoparenchymatous stroma was developed.

Wehmeyer (1955 a) observed progressive tendancy in stromatal primordia having which showed deeply staining affinity of their protoplasm and development of them into ascocarp and retrogressive tendency in some primordia which showed less staining affinity and which failed to develop further in Pseudoplea gaeumannii.

Cavara and Mollica (1907) reported fusion of two gametic cells or hyphae before stroma formation and two nuclei of these hyphae also fused in Pleospora herbarum. The ascostromata were then formed by enveloping hyphae, the original 'oogonial' cells supposedly dividing to form chains of binucleate cells. But he observed that binucleated cells of paraphyses became large and developed into young asci within which two nuclei fused.

Here in our material binucleated cells of paraphyses and fusion of their two nuclei after enlargement was not

observed but in mature, bihyphal knots (stroma) well differentiated uninucleated sex organs were developed at the centre (Photograph Nos.11,12,13). These sex organs showed dense granular protoplasm, enlarged nucleus and presence of vacuole below nucleus in both sex organs. These sex organs were stalked. The size of stroma at this stage was 45-91 µm in diam. and 8-11 cells in width. The size of antheridium was 12-21 µm X 8-10 µm and that of ascogonium was 10-17 µm X 8-13 µm.

Formation of ferlization tube between the sex organs was also observed. In some sections binucleated ascogonium in association with empty antheridium in the centre of stroma were located. Though actual migration of antheridial nucleus along with protoplasm was not observed, these structures indicated the existance of act of fertilization (dikaryotization) and functional nature of antheridium.

At this stage outer one or two cell layers of stroma (pseudoperithecium) underwent differentiation and became thick walled and brown. These cells and also inner cells around sex organs, started showing less affinity to nuclear stains.

The binucleated ascogonia then underwent enlargement and produced short branched filament of cells, probably this was the initiation of ascogenous filament. Further development of this ascogenous filament was not practically tracable but what we observed in later development was presence of 2-7 bi-bucleated cells at the centre of speudothecium. These cells

showed more affinity for cytoplasmic and nuclear stains than the surrounding nutritive pseudoparenchymatous cells and ascogenous filaments.

Wehmeyer (1954) admitted that it is almost imposible to follow exact nuclear behaviour in side these protoperithecia in Pleospora trichostoma.

These binucleated cells then started enlargement and grew vertically to develop in to ascus mother cells (Photograph No. 16,17). At this stage size of pseudothecium was 83-102 um in diam. and 13-18 cells in width. The two nuclei in them started enlargement and migration towards apical region of ascus mother cells. These two nuclei then came in close association and got enveloped by common halo around them. These two nuclei then fused to form fusion nucleus (Photograph No. 18). Thus, karyogamy was observed here in ascus mother cell. At this stage in most of the asci two large vacuoles were observed below the fusion nucleus. Thus, 2-7 asci started their development in the pseudothecium.

DEVELOPMENT OF ASCUS AND ASCOSPORE FORMATION :

Fusion nucleus in the developing ascus was homogenous and undifferentiated. It underwent resting period and after resting period it was differentiated into chromatin material and large spherical to elliptical nucleolus (Micro-Photograph No. 19).

This fusion nucleus then underwent meiotic division followed by mitotic division. Here these divisions were referred as Division I, II and III. Division I was comparatively slow and it ended by forming two haploid daughter nuclei in the ascus.

The II and III Divisions were rapid. Division II was ended by forming 4 haploid nuclei while division III by forming 8 haploid nuclei in the ascus. The detailed account of nuclear division is given separately.

The size of ascus and pseudothecium increased rapidly simultaneously with nuclear changes in the ascus. After formation of 8 haploid nuclei in ascus, protoplasm of ascus started accumulating around 5-8 nuclei which was accompanied by vacuolization in the ascus. Thus, ascosporogenesis was by free cell formation and by vacuolization.

Thus, 5-8 ascospores were produced in the ascus (Photograph No.22,23). These ascospores were uninucleate, one celled and almost spherical to elliptical.

Each one celled ascospores then started septation, the nucleus in it continued to divide further mitotically. It was observed that septa were laid down in two fashions. In one type, first 2-3 septa were laid transversely to form a raw of four or five cells, in side the ascospore wall. The apical and basal cells were cap like. This development type referred as phragmosporous. In other type, first septum was laid down transversely but second septum was laid down perpendicular to previous septum to form quadrately arranged spores in cruciform fashion, in side the spore wall. Further septa were laid down only transversely to form two vertical rows of cells inside the wall of ascospore. Each row contained 3-4 cells in matured ascospore. The basal and apical cells were almost hemispherical in shape. This development type I refered as muriform. Early in development, asci had broad gelatinous membrane but later it became thicker and thicker at apex.

Formation of two celled ascospore :

In most of the asci one celled ascospore then started elongation in vertical direction, after elongation a constricted ring appeared on its lateral wall at its centre. The nucleus in side it continued to divide mitotically to form 2, 4, 6 or 8 daughter nuclei. The planes of divisions were either parallel or oblique or right angle to the long axis of spore. Then,

transverse septum was laid down at the region of constricted ring. Thus, ascospore became two celled. Each cell received 1-4 daughter nuclei. This type of behaviour was seen in pharagmosporous type of ascospore development (Photograph No.23).

But in few asci one celled ascospores started enlargement instead of elongation and remain elliptical or spherical. Nucleus in it also continued to divide mitotically as in phragmosporous type. Septum was laid down and ascospore became two celled. This type of behaviour was seen in muriform type of ascospore development (Photograph No. 26).

Formation of four celled ascospore :

In phragmosporous type of development the two cells in diad continued enlargement and elongation the nucleus or nuclei in them continued mitotic division and septum was laid down - transversely (parallel to previous septum) in each cell to form a vertical row of four cells (1+1+1+1). Each cell received 1-4 nuclei (Photograph No. 24).

In muriform type of development the two cells in diad continued enlargement along with mitotic division of their nuclei and septum was laid perpendicular to previous septum to form quadrately arranged 4 cells (2+2) in cruciform fashion (Photograph No. 27).

Formation of six celled ascospore :

The phragmosporously developed 4 cells continued elongation and enlargement along with nuclear divisions

of their nuclei and vertical septa were laid down in the middle two cells along the same line or plane. The basal and apical cell of row remain undivided. Thus ascospore became 6 celled (1+2+2+1). One cap like cell at basal and apical ends and two pairs of cells in the centre (Photograph No. 24).

Muriformly developed 4 cells also continued their enlargement along with nuclear divisions of their nuclei. A transverse septum was laid down only in one pair of cells either basal or apical to form 6-celled ascospore with 3 pairs of cells placed one above the other in vertical direction (2+2+2) (Photograph No. 27).

Formation of eight celled or more celled ascospore :

Phragmosporously developed spores continued elongation and enlargement along with nuclear divisions of their nuclei and sometimes either one or both the middle cells underwent one more transverse division each to form a strip of (1+2+2+2+1) 8 or (1+2+2+2+2+1) 10 cells in the ascospore. In further development vertical septa were laid down in one or more middle cells except basal and apical one to form a strip of (1=2+2+3+1) 9 or (1+2+3+3+1) 10 or (1+3+3+3+1) 11 or (1+2+2+2+3+1) 11 or (1+2+2+3+3+1) 12 or (1+2+3+3+3+1) 13 or (1+3+3+3+3+1) 14 or ... (1+4+4+4+4+1) 18 cells in the ascospore.

By transverse division of both pairs of cells octant stage was reached in muriform development type (2+2)→(2+2+2+2)

-). But in this type of development further increase in number of cells was not observed.

Thus the stage of mature ascospore with upto 5 transverse septa and upto 3 vertical septa was reached and each cell received 1-3 nuclei.

During all these changes the size of ascus and pseudothecium increased rapidly and 3-4 wall layers of pseudothecium underwent thickning of their cell walls, inner layers remained thin walled.

Though 2-7 ascus mother cells started growing at the centre of ascocarp 1-4 of them attained maturity while others were degenerated in the course of development. In some ascocarps two types of asci, one producing muriformly formed ascospores and other producing phragmosphorously formed ascospores were observed. But very less number of asci were seen which produce muriform ascospores. Pseudo parenchymatous septa were also observed in between the developing asci.

During the course of development a meristematic tissue was developed below the wall layers at the apical region of pseudopthecium. Due to pressure of these cells necklike structure was differentiated at the apical region of pseudothecium (Photograph No.28/29). Due to continuous pressure from inside the overlying wall cells around the neck were destroyed.

These cells inside the neck were then destroyed by lysis of cells due to continuous pressure from below exerted by fastly growing asci.

CYTOLOGICAL CHANGES :

The cell of vegetative mycelium were mostly uninucleate, rarely binucleate or multinucleate. These cells of mycelia were 2-5 µm broad and 8-20 µm long. The cells of the reproductive mycelia were mostly uninucleate or rarely binucleate or multinucleate. These cells were 7-13 µm broad and 7-16 µm long.

The unihyphal knots had fleshy cells and showed less affinity for the nuclear stains. The bihyphal knots had small cells with dense cytoplasm and these cells showed more affinity for nuclear stains. Rarely binucleate cells were observed in the peripheral region of unihyphal and bihyphal knots, but their positions were not constant.

The ascoganium and antheridium were uninucleate. The ascogonial nucleus (1-2 µm) was larger than antheridial nucleus (0.5-1.5 µm). In both the sex organs nuclei were located in the apical region where cytoplasm was highly granular. The characteristic feature of both sex organs was that both the sex organs enclosed a large elongated vacuole below the nucleus (Micro-photograph No. 12,13).

Binucleate ascogonium was seen along with empty antheridium on its one side (Micro-photgraph No. 15). In most of the cases two elongated vacuoles were present below the two nuclei in the ascogonium. The surrounding cells started showing less affinity for nuclear stains. In the later stage of development

binucleated cells with dense granular cytoplasm appeared at the centre of pseudothecium. Intermediate stages were not tracable. Without forming crozier the binucleate ascus mother cell enlarged and became vertically elongated. Along with elongation of ascus mother cells the two nuclei in them underwent enlargement. Out of the two nuclei one nucleus (2.5 µm diam.) was larger than the other nucleus (1.5 µm diam.). In later stage the two nuclei came near to one another, in the apical region of ascus mother cell and a common halo appeared around them. Then these two nuclei became fused, the size of fusion nucleus was 4 um in diam. Fusion nucleus was homogenous and showed more affinity for nuclear stains. Later on it was differentiated into darkly stained nucleolus (2.5 µm diam.) and chromatin material in the form of network. A large vacuole was observed in the ascus below the fusion nucleus. Superficial one or two layers of cells of pseudothecium underwent differentiation to form wall layers.

Fusion nucleus underwent three divisions, out of which first division was reductional and second and third divisions were equational (mitotic).

During first division, the plane of division of the nucleus was oblique or parallel to long axis of the ascus

This division was slow and at the diplotene stage - three short, thick, bivalents were observed (so the

chromosome number is n = 3) and longest bivalent was in association with nucleolus.

During the second division, the divisional planes of nuclei were either parallel or perpendicular or oblique to the long axis of the ascus. But in most of the asci the planes of divisions of two nuclei were parallel to each other).

During third division the plane of the division of nuclei were oblique to the long axis of the ascus. Divisional stages and chromosomes were difficult to identify due to compact network of dense, granular cytoplasm and fast rate of division. Third division ended with formation of 8 haploid nuclei (0.5 to 1.5 µm in diam.). It was observed that the size of the daughter nuclei decreased during first, second and third nuclear divisions. It was also observed that during first, second and third nuclear divisions, vacuole or vacuoles of different size were present inside the ascus.

Ascosporogenesis occurred by free cell formation and by vacuolization. During ascosporogenesis 5 to 8 nuclei were surrounded by dense, granular cytoplasm and small vacuoles appeared around them.

The young ascospores were one celled, thin walled and uninucleate. The nucleus in the ascospore showed strong affinity for nuclear stains. This nucleus then underwent

nuclear divisions by mitosis. The planes of division were either parallel or oblique or perpendicular to long axis of the spore. In some ascospores septum was laid down when the ascospore became binucleate, while in some spores septum was laid down after quadri or hexa or octanucleate stage.

Later on the ascospore became multicelled in phragmosporous manner or muriform manner by formation of vertical and transverse septa. During all these changes each cell received 1-3 daughter nuclei. During septation of ascospore the outer wall of it gradually became thicker and thicker. The details of nuclear divisions in the multicelled ascospore could not be well traced. Wehmeyer (1955-b) reported in Pleospora armeriae, spore wall became so thick and deeply coloured that no nuclear detail could be seen within.

DISCUSSION :

The development of L.trifolii was worked out by Muller (1951) as L.australis; by Wehmeyer (1955-a) as Pseudoplea gaeumannii and by Denison and Carlstrom (1968) as L.argentinensis.

Barge (1974) while doing cultures of various species of Leptosphaerulina, he found that ascogonia were formed at apical or lateral or intercalory positions of broad reproductive mycelia while antheridia were formed only on lateral or terminal positions. But he did not provide any figures or photographs. In our material such structures i.e. antheridia and ascogonia were not observed at their early stage of development of mycelium.

Wehmeyer (1955-a) reported presence of stromatal or ascocarp primordia formed by irregular coiling of short side branches of reproductive mycelia in Pseudoplea gaeumannii. But he said that there is no indication of any differentiation of gametic branches or transfer of nuclei at this stage. He also reported (1955-b) the presence of complex knots formed by coiling of short contarted branches in Pleospora armeriae. But he remarked that no sexual function was observed at this stage. He reported that the knots which he observed in his material were similar to the figures given by Miyabe (1989). Von Arx (1974) drew the knot like structures for his cultured Leptosphaerulina and Pleospora but did not show any indication of differentiation of any gametic branches.

In this material we observed two types of knots on the bases of their behaviour and named them as unihyphal and bihyphal knots. Unihyphal knots showed larger fleshy cells and showed less affinity for nuclear stains. In the formation of bihyphal knots two lateral branches of reproductive mycelia were involved. The cells of these knots were small and showed granular cytoplasm and affinity for nuclear stains. The unihyphal knots were abortive because these knots developed upto certain extent and did not show formation of any reproductive cells.

This type of behaviour was also observed by Wehmeyer (1955-a) in Pseudoplea gaeumannii in stromatal or ascocarp primordia. He referred it as progressive and retrogressive tendency.

Here in our material, the unihyphal knots showed retrogressive tendency and I am of opinion that these unihyphal sterile knots were formed by ascogonial branch tips, when they did not meet their opposite mate and failed to get fertilized.

Wehmeyer (1955 a & b) did not observed antheridia and ascogonia in Pseudoplea gaeumannii and Pleospora armeriae and commented on Muller (1951) that figures as those of Muller indicating antheridia, ascogonia and trichogyne, appeal to the writer as wishful thinking stemming from the hypothetical origin

of the ascomycetes from the red algae.

But here author did not support his comments because :-

(1) Here we observed well differentiated sex organs at the centre of ascocarp.

(2) These sex organs were uninucleate in the begining.

(3) Presence of fertilization tube or connection in between ascogonium and antheridium.

(4) Occurence of binucleate ascogonia with empty antheridium on its lateral side supports fertilization of ascogonium by antheridium.

(5) Nuclear fusion took place in the young ascus.

SUMMARY AND CONCLUSIONS :

This fungus was cultured in artificial medium, P.D.A. and effect of different concentrations of five aminoacids and different concentrations of vitamins and growth substances on the growth of Leptosphaerulina alysicarpii was studied and following conclusions were drawn.

1. Fungus developes rapidly in P.D.A. with 50 ppm Aspartic acid, P.D.A. with 50 ppm Phenyl alanine.
2. It also grows rapidly in P.D.A. with 10 ppm Indole Acetic Acid and 20 ppm Gibberlic Acid.
3. Glycine and Ascorbic Acid supress the growth of this fungus.

The development of this fungus was studied and following points were noted.

1. Presence of two types of knots (i) unihyphal sterile knots and (ii) bihyphal fertile knots.
2. Presence of well developed uninucleate sex organs i.e. antheridia and ascogonia.
3. Large vacuole was observed below the nucleus of ascogonium and antheridium.
4. Presence of fertilization tube and occurence of binucleate ascogonium with empty antheridium in association.
5. Dikaryotization occurred in ascogonia.
6. Stroma developed first and sex organs differentiated later.

7. Asci were formed in the locule formed by disintegration of centrum.
8. Karyogamy took place inside the young ascus.
9. Asci were bitunicate.
10. Out of the two nuclei in the ascus mother cell one nucleus was larger than the other.
11. Crozier formation was not observed.
12. Fusion nucleus underwent three divisions, first division was reductional, second and third divisions were equational.
13. Presence of single nucleolus in the nucleus. Longest chromosome was associated with this nucleolus.
14. Three bivalents were observed in diplotene stage.
15. During ascosporogenesis many small vacuoles observed in the protoplasm of ascus.
16. Ascospores became multicelled by two types developments, Phragmosporous type and muriform type.
17. The number of muriformly formed ascospores was very less than the number of phragmosporously formed ascospores.

From these observation following conclusions are drawn.

(1) Presence of two types knots and two types of spore development suggests presence of two strains and heterothallism in Leptosphaerulina alysicarpii.
(2) Sexual reproduction is by gametangial contact.
(3) Antheridium is functional.
(4) Development of centrum is of Dothidea type.

(5) Chromosome number is n=3. Longest chromosome is a nucleolar chromosome.

(6) Ascosporogenesis is by free cell formation and by vacuolization.

Photograph No.1 - Infected leaves of Alysicarpous rogosus

Photograph No.2 - Perithecium showing well developed neck

Photograph No.3 - Bitunicate ascus with phragmosporous ascospores

Photograph No.4 - Four day culture cycle seen in P.D.A.

Photograph No.5 - Effect of five different amino acids of 50 ppm concentration on the growth of Leptosphaerulina alycicarpii cultured in P.D.A. (Three days old cultures).

Photograph No.6 - Effect of five different amino acids of 25 ppm concentration on the growth of Leptosphaerulina alycicarpii cultured in P.D.A. (three days old cultures).

Photograph No.7 - Effect of three different growth substances of 10 ppm concentration on the growth of Leptosphaerulina alycicarpii cultured in P.D.A. (two days old cultures).

Photograph No.8 - Reproductive mycelium showing short fleshy swollen cells and short lateral branches

Photograph No.9 - Unihyphal knots - Cells of knot showing less affinity for nuclear stain

Photograph No.10 - Bihyphal knot - Cells of knot showing granular cytoplasm and more affinity for nuclear stain

Photograph No.11 - Bihyphal knot showing unicucleate ascogonium and uninucleate antheridium at its centre

Photograph No.12,13 - Unicucleate stalked sex organs at the centre of byhyphal knot. Presence of large vacuole below the nucleus in both the sex organs. Differentiation of wall layers in the knot

Photograph No.14 - Knot showing formation of fertilization tube between antheridium and ascogonium

Photograph No.15 - Knot showing binucleate ascogonium and empty antheridium

Photograph No.16 - Differentiation of binucleate ascus mother cells at the centre of pseudothecium

Photograph No.17 - Pseudothecium with vertically elongated ascus mother cell with two nuclei approached near each other at centre

Photograph No.18 - Pseudothecium showing ascus mother cell with fusion nucleus. Differentiation of 2-3 layered wall in pseudothecium

Photograph No.19 - Ascus showing large darkly stained nucleolus and three bivalent chromosomes

Photograph No.20 - Eight nucleate ascus - only five nuclei are covered in photograph

Photograph No.21 - Left ascus showing large nucleolus and three bivalent chromosomes. A large vacuole is seen below the nucleolus - in the same ascus. Right ascus showing one celled unicucleate and multinucleate phragmosporous ascospores

Photograph No.22 - Asci containing one celled uninucleate and multinucleate ascospores

Photograph No.23 - Left ascus containing bicelled phragosporous ascospores. Right ascus containing multinucleate one celled phragmosporous ascospores. Differentiation of meristematic cells is seen at the apical region of pseudothecium

Photograph No.24 - Ascus containing multicelled phragmosporous ascospores

Photograph No.25 - Asci containing one celled unicuc leate and multinucleate muriform ascospores

Photograph No.26 - Asci containing one celled, two celled and four celled muriform ascospores

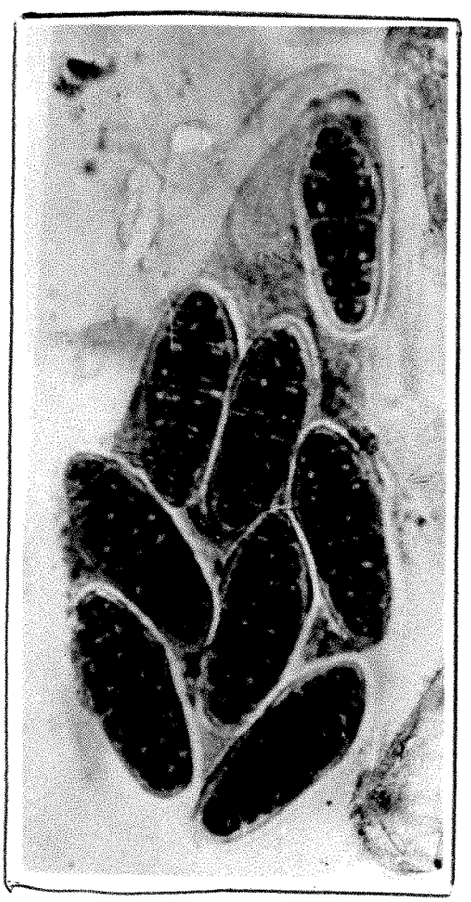
Photograph No.27 - Pseudothecium containing two asci, one containing multicelled muriform ascospores

Photograph No.28 - Pseudothecium showing differentiation of meristematic tissue below the wall layers in its apical region

Photograph No.29 - Pseudothecium with well developed neck

1

2



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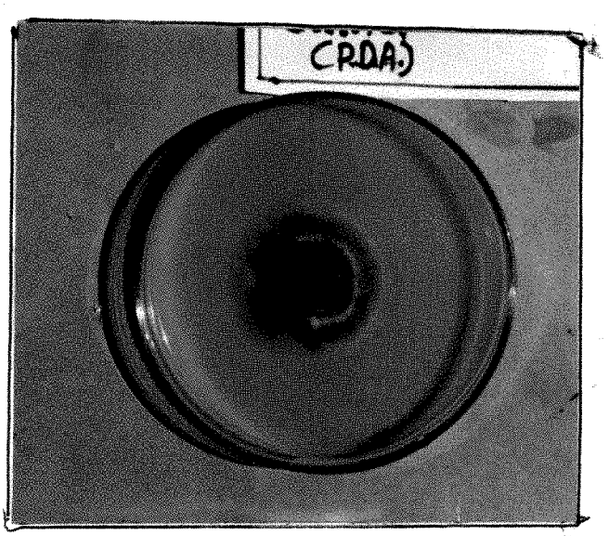

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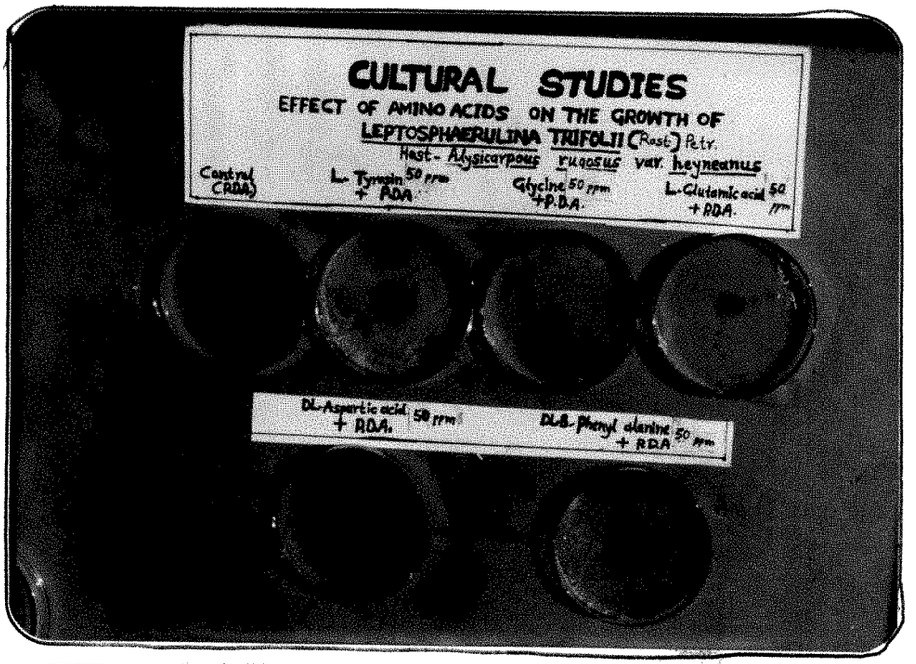
CULTURAL STUDIES
EFFECT OF AMINO ACIDS ON THE GROWTH OF
LEPTOSPHAERULINA TRIFOLII (Rost.) Petr.
Host - Alysicarpous rugosus var. heyneanus
Control (PDA)
L. Tyrosin 50 ppm + PDA.
Glycine 50 ppm + P.D.A.
L. Glutamic acid 50 ppm + PDA.
DL-Aspartic acid 50 ppm + PDA.
DL-β-Phenyl alanine 50 ppm + PDA.

5 5


CULTURAL STUDIES
EFFECT OF AMINO ACIDS ON THE GROWTH OF
LEPTOSPHAERULINA TRIFOLII (Rost.) Petr.
Host - Alysicarpous rugosus var. heyneanus
Control (PDA)
L. Tyrosin 25 ppm + PDA
Glycine 25 ppm + P.D.A.
L. Glutamic acid 25 ppm + PDA.
DL-Aspartic acid 25 ppm + PDA.
DL-β-Phenyl alanine 25 ppm + PDA.

6 6


CULTURAL STUDIES
EFFECT OF GROWTH SUBSTANCES ON THE GROWTH OF
LEPTOSPHAERULINA TRIFOLII (Rost.) Petr.
Host - Vigna unguiculata (Linn.) Walp
Control (PDA)
PDA + GA 10 ppm.
PDA + IAA 10 ppm
PDA + AA 10 ppm.

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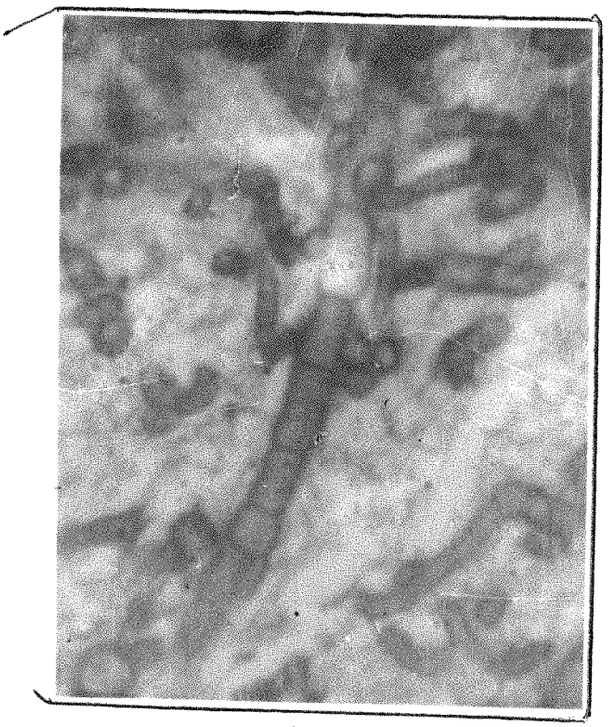

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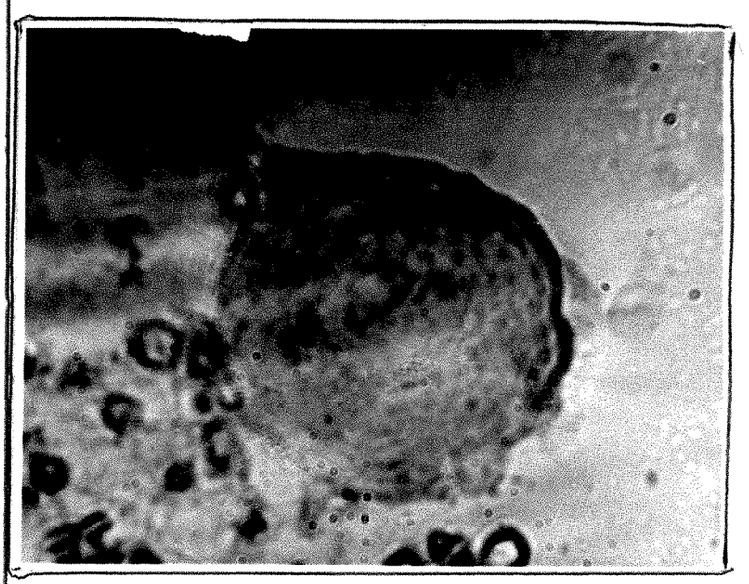

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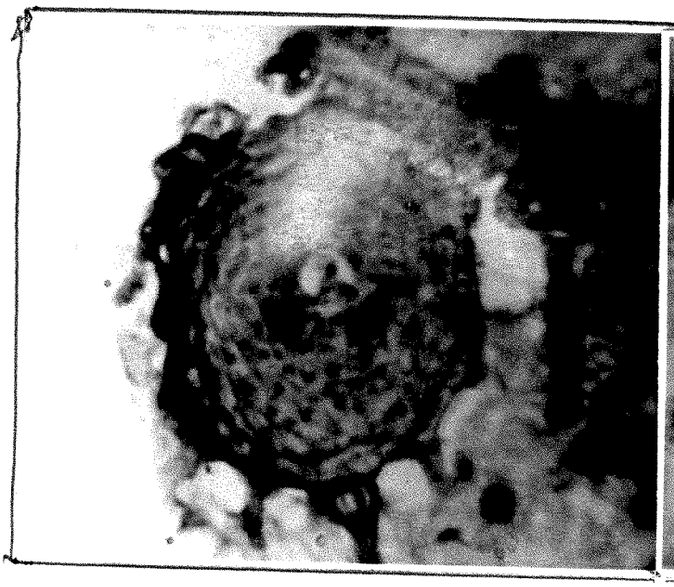

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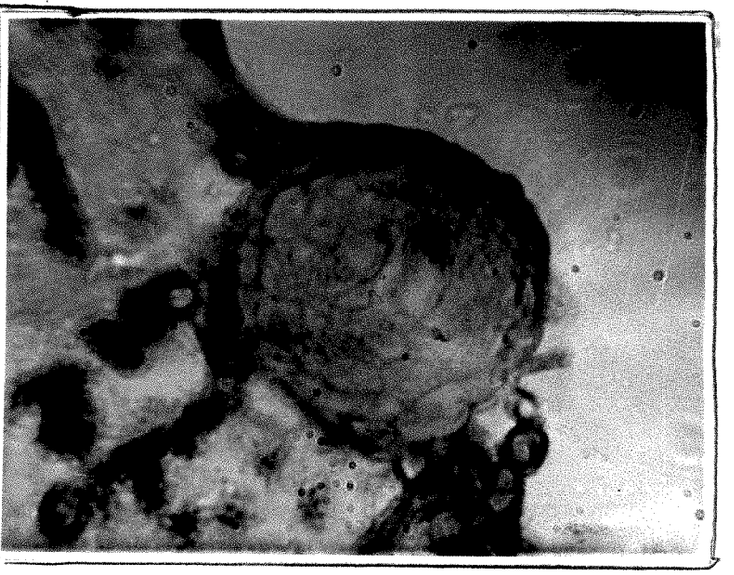

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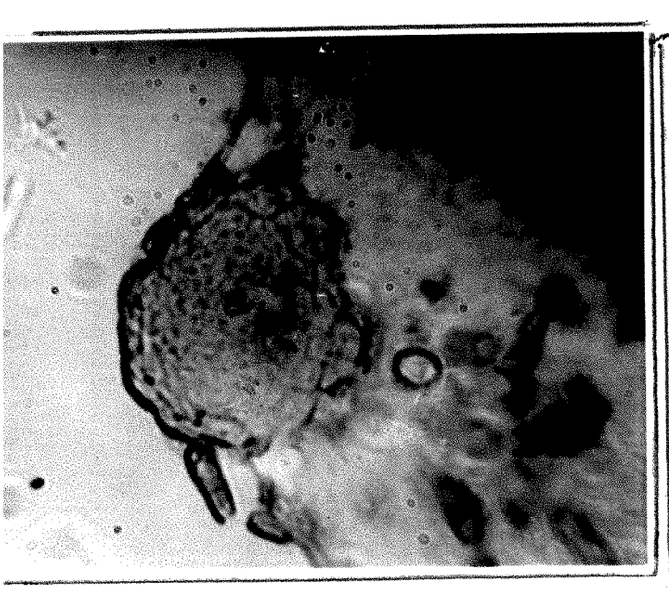

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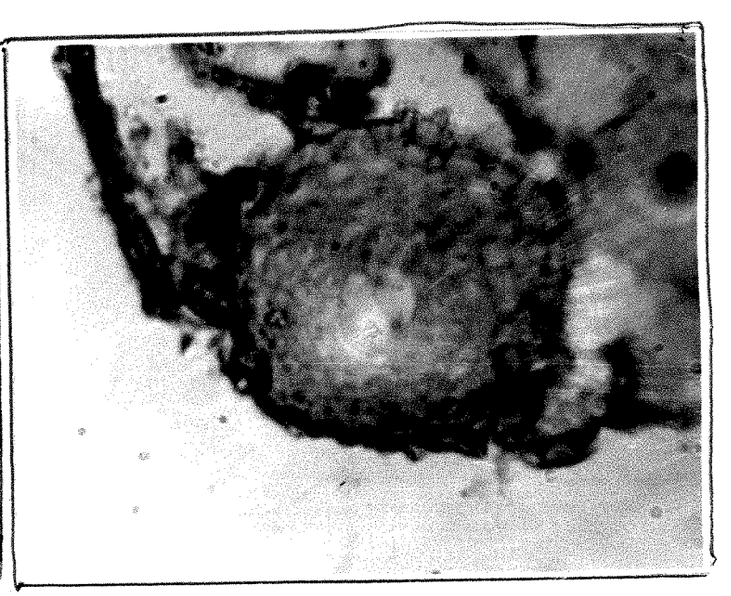

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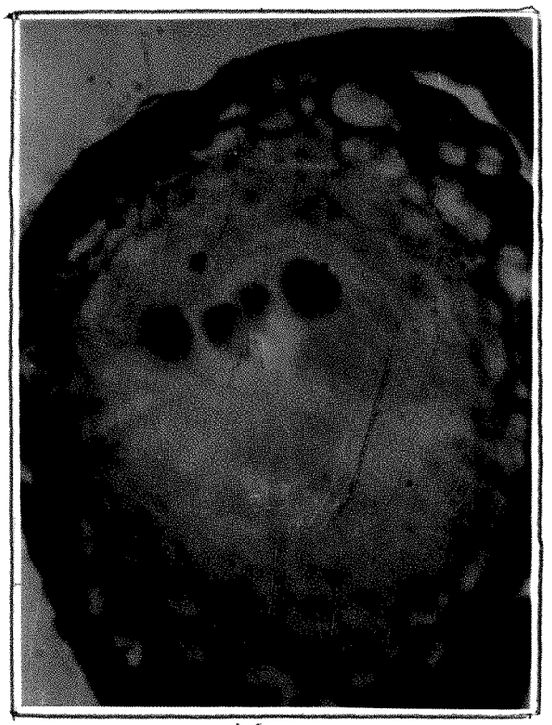

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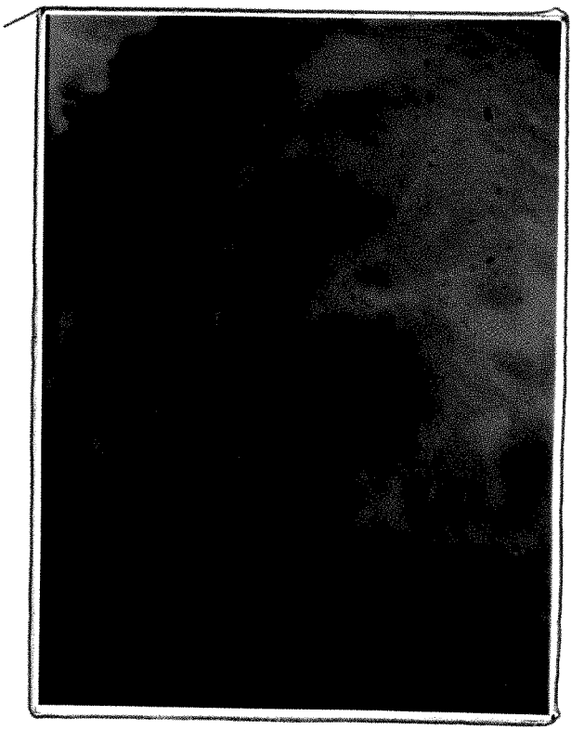

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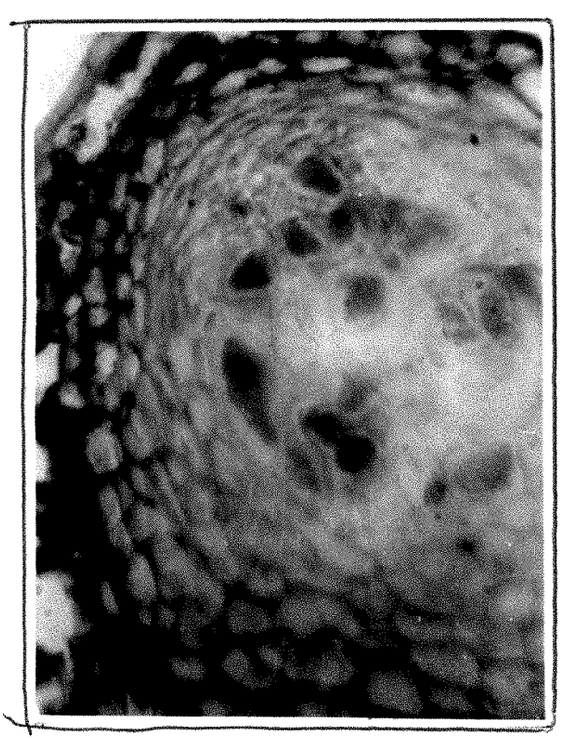

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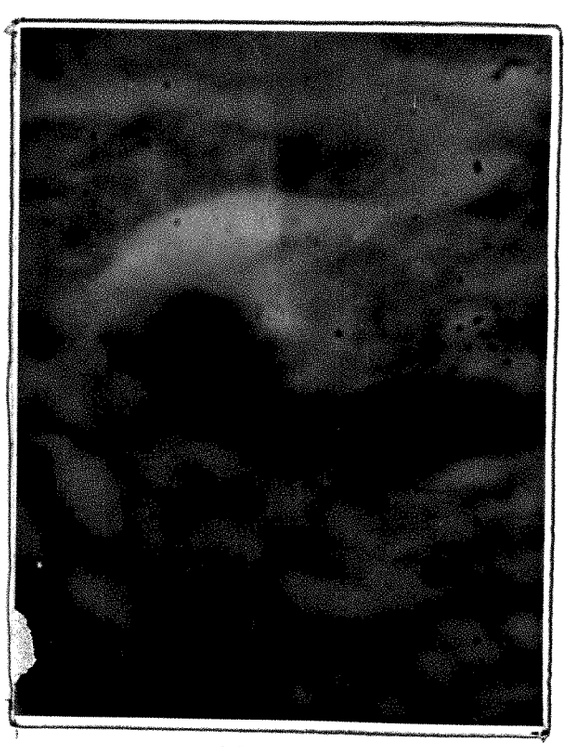

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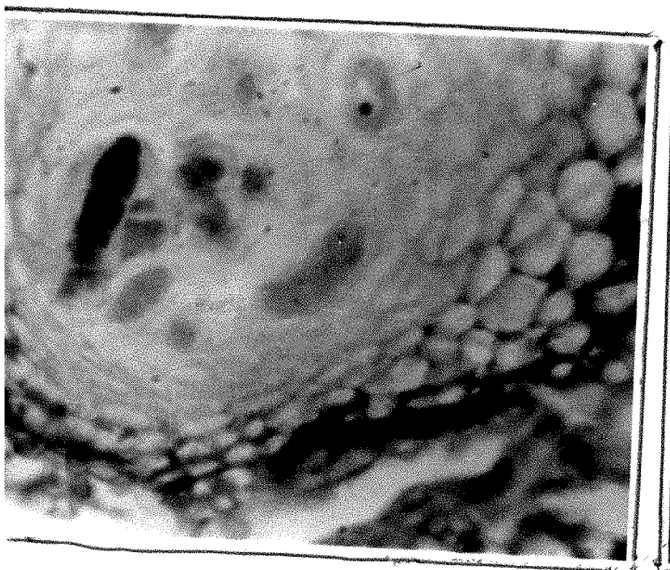

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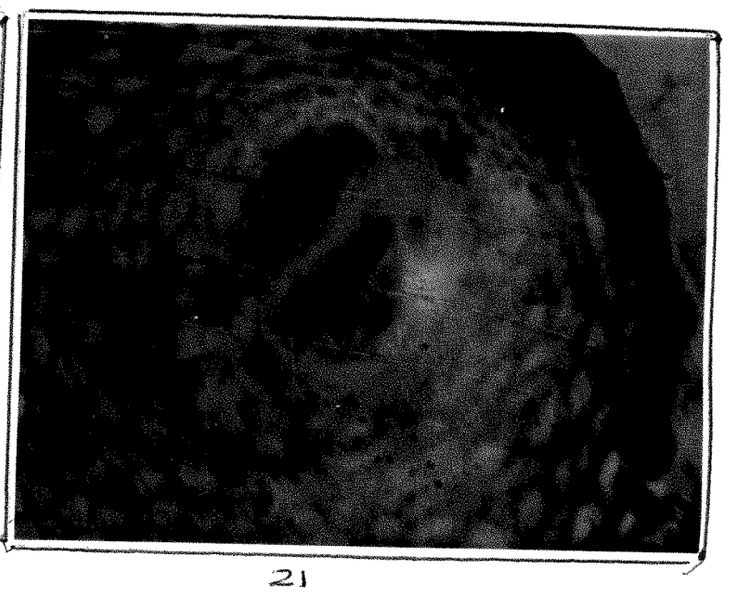

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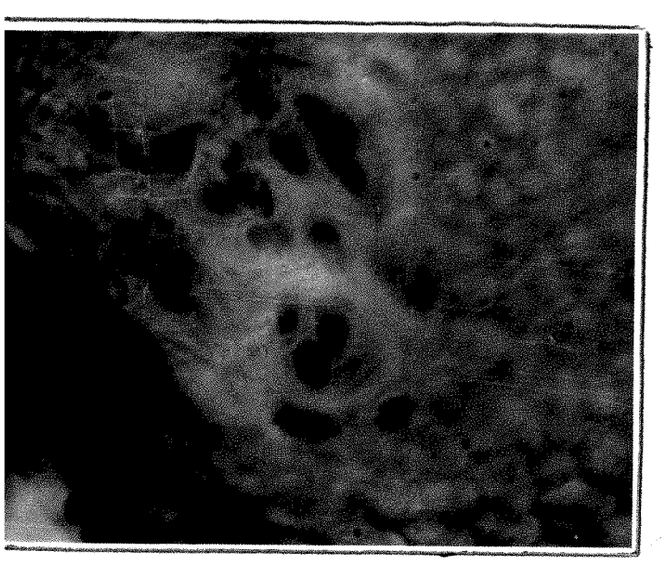

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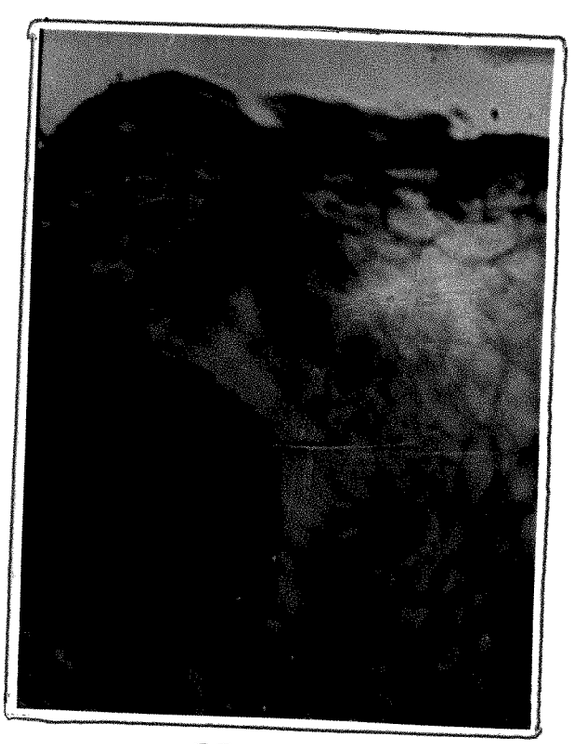

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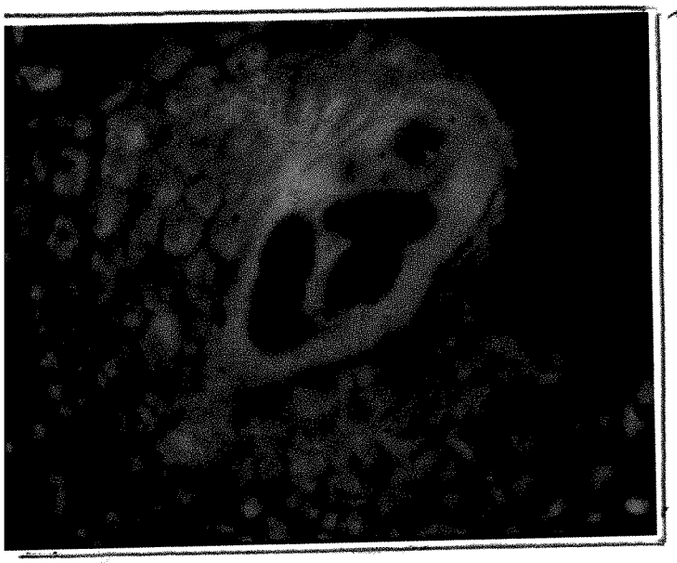

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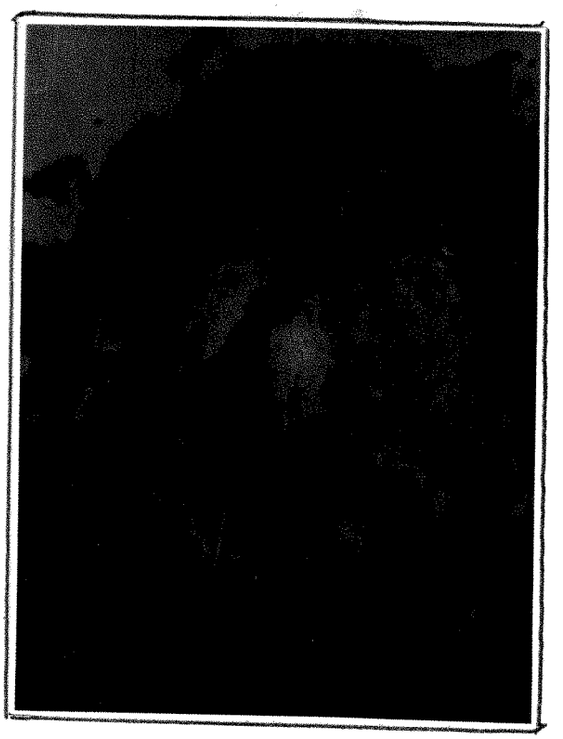

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OoO

CYTOTAXONOMICAL, SEXUALITY AND DEVELOPMENTAL STUDIES OF THE FUNGUS *SALMONOMYCES KAMATII* CHIDDARWAR.

During the course of my collection I came across a powdery mildew, on the living leaves of Acalypha ciliata Forsk. (Euphorbiaceae). The description of it is as follows.

DESCRIPTION :

Infection - spreaded, covering part or whole of the leaf surface amphiphyllously. [Photo. No. 1]

Mycelium - broad, superficial, ectophytic, creeping, persistent, white, later appearing scanty colourless. Diameter of hyphae 1-3 µm, an average 2 µm, septate, cells 33 - 36 µm X 3-6 µm. Haustoria - globular, stalked penetrating into epidermal cells. Conidiophore spring from superficial mycelium, many straight, septate, basal cell - swollen or 6-7 µm wide slender, 36-54 µm long; Conidia - produced in chains, 18 X 12-15 µm, globular to elliptical shape more or less constant, though the size is variable but L/B ratio of conidia is almost constant.

Reproductive structures cleistothecia, 72-153 µm diameter, average diameter 99 µm. Perithecia scattered, dark, brown, globular in shape having 8-28 appendages, an average 21. Appendage tips swollen or flat or pointed, shrivelled or curved tips less than 1%. Appendages rigid, hard, brown, 66-165 µm long, average 104 µm, 1-4 septate, Basal cell - swollen 6-9 µm but not globose or bulbose, epibasal cell 3 - 6 µm wide. Cells of perithecial wall polygonal, thick walled, hard, dark brown. [Photo. No. 2, 3, 4, 5].

Asci - many, 5-7. Ascus - broadly ovate to sub-ovate, 33-39 X 15-21 µm, average 34 X 17 µm, basal - narrow tubular region 6 X 3 µm. Ascospore - 1, one-celled, globose to sub-globose, 6-9 µm, average 7 µm diam. [Photo. No 11, 12, 14, 15].

Collected on the living leaves of Acalypha ciliata Forsk. (Euphorbiaceae) by Dhage R.B., at Satara, on Sept.15, 1988. The material is deposited in Herbarium of Botany Department of Y.C.Institute of Science, Satara, Maharashtra, India.

SEXUALITY AND DEVELOPMENT :

The cells of vegetative and reproductive mycelium were uninucleate. Binucleate cells were seen only occassionally. Sex organs could be recognised by their sizes, shapes and granular cytoplasm. Antheridia and Ascogonia were produced at the ends of specialised hyphae or on lateral sides of them. Both were uninucleate. The two initials were morphologically similar but after formation of Septa ascogonial branch and antheridial branch could be differentiated. Ascogonial branch appeared broader and shorter than antheridial one. Both branches had now developed staining affinity than the vegetative mycelium. The distal part of antheridial branch bent over the ascogonial branch and became closely appressed over the distal end of it (Photograph No.7,8). By dissolution of intervenning walls at the point of contact between them a narrow fertilization tube was formed. There was no twisting between the sexual branches. There was also almost no difference in the size of antheridical and ascogonial nucleus. Ascogonial cell was larger than its stalk cell. The apical cells of the antheridial branch i.e. antheridium was comparatively smaller in breadth and longer than ascogonial length. there trichogyne was not formed. Uninucleate sex organs at the beginning, presence of open fertilization tube between antheridium and ascogonium and occurence of binucleate ascogonium and empty antheridium in association, indicated that antheridium was functional (Photograph No. 8). The migrated

antheridial nucleus in the fertilization tube was not seen but the shifting of antheridial nucleus towards apical region was observed. The Karyogamy could not be located in ascogonium.

PERITHECIAL DEVELOPMENT :

Protective covering was developed by hyphae growing over the sex organs from their stalk cells. The initiation of protective sheath was started before or after dikaryotization. There was no nuclear fussion inside the ascogonium. The size of knot at this stage was 12-15 µm in diameter.

The ascogonium continued to elongate and became curved. The two nuclei of similar sizes are recognised in dense, granular cytoplasm. These nuclei divided by conjugate nuclear division and 4 nuclei were formed, cell walls were laid down and ascogonium became 3 celled. Out of these three cells, the central one was binucleate and upper and basal ones were uninucleated. At this stage the protective cover had became 2-3 layered.

From this binucleated cell of ascogonial strip lateral branches were formed. These were the ascogenous hyphae (Photograph No. 9). Ascogenous hyphae branched, bent inwards and grew in centripetal fashion and finally radial rows of ascogenous hyphal branches occupied central portion of the perithecium (Photograph No. 9). Most of the cells in the ascogenous hyphae were uninucleate but binucleate cells were also seen in terminal and subterminal positions. Regular

conjugate nuclear divisions were not observed. The size of the perithecium at this stage was 21-24 µm in diameter. Terminal or subterminal binucleate cells of ascogenous filament towards central region of the perithecium began to grow into young ascus mother cells.

Binucleate ascus mother cells then grew vertically or obliquely into young ovate or sulovate structures with narrow bases. These were surrounded by 2-3 layers of pseudoparenchymatous hyphal cells with dense cytoplasm and clearly marked nuclei. These cells were mostly uninucleate, rarely binucleate or even trinucleate. Probably these cells provide nourishment to the developing asci (Photograph No.13,14).

At this stage perithecial wall started differentiation, some superficial equatorial or subequatorial cells started developing uniseriate, multicellular appendages that later matured and become rigid, hard and brown. Basal cell was larger in width but not bulbose.

Later binucleate ascus mother cell get enlarged. At this stage there was no difference in the sizes of nuclei. Then these two nuclei approached each other almost at the middle region of ascus mother cell and lied in common large halo.

It was followed by Karyogamy. Thus, diploidization occurred in the ascus mother cell. This fusion nucleus showed

its size equal to the sum total of sizes of two unfused nuclei. Wall of the perithecium became thicker at this stage; thickening initiated from peripheral layers and gradually proceeded inward in centripetal manner. Perithecial wall at this stage was 1-2 layered.

Enlargement of ascus continued till 8 nucleate state. Correlation was observed between the size of ascus and divisional stage of diploid nucleus inside it. All the asci in the perithecium did not show simultaneous nuclear divisions.

Measurement Table

Nuclear No. (Stage)	Perithecium size in µm	Ascus size in µm
Fussion nucleus	90 - 99	30 - 33 X 15
Binucleate stage	99 - 100	33 - 36 X 15-18
Four nucleate stage	100 - 105	36 - 39 X 18
Eight nucleate stage	105 - 111	39 - 42 X 21

The diploid nucleus underwent three divisions and formed 8 nucleate ascus. First division was reductional, second was equational and third was mitotic. During divisional stages the chromosome number was found to be $n = 5$.

ASCOSPOROGENESIS :

At the end of third division 8 haploid dauthter nuclei were formed, out of these nuclei 6-7 nuclei degenerated before ascosporogenesis. After degeneration of 6-7 nuclei ascosporogenesis started by vacuolization in the ascus and protoplast started condensation arouńd only one nucleus. It was then followed by secretion of wall around itself to form one ascospore in the ascus (Photograph No.).

Thus, ascospore formation is by free cell formation.

Special mechanism for liberation of ascospore or dehiscence of perithecium was not noticed.

The observation of perithecium development showed that development of centrum is of Phyllactinia type (Luttrell,1951).

CYTOLOGICAL CHANGES :

The superficial, ectophytic mycelium was 1-3 µm broad and showed generally one nucleus in the cells, occasionally two nucleated cells were noted, nuclei were prominent, globular or elliptical.

Ascogonium, antheridium showed dense granular cytoplasm (Photograph No. 6,7). The nuclei of antheridia and ascogonia were of similar sizes, dikaryo tisation occurred in ascogonium, initially sex organs were uninucleate but occurence of binucleate ascogonium with empty antheridium in association and presence of open fertilization tube indirectly supported migration of antheridial nucleus in to ascogonium. Even in dikaryotic condition of ascogonium or binucleate cells of ascogenous hyphae or even in ascus mother cell, nuclei were equal in size. Before, diploidization when two nuclei approached each other a common halo appeared around the two nuclei and the fusion nucleus showed a size equal to the sum total of the sizes of the two equal haploid nuclei placed together (Photograph No. 11).

The diploid nucleus underwent three divisions, first of them was reductional. The nucleolus was distinct and fully expanded at pachytene and gradually disappear at the end of 1st prophase. The chromosome complement showed n = 5 and largest bivalent was associated with nucleolus (Photograph No. 16).

DIVISION - I :

Resting diploid nucleus was homogenously stained. This fusion nucleus after resting period showed a definite reticulum or chromatin network and a large spherical to elliptical heavily stained nucleolus. The nucleoli were located near the wall of nucleus but were not of protruding type.

The thin elongated chromatin threads at leptotene gradually started pairing at terminal or at middle parts, where they were associated. Contraction and condensation was followed by deeper staining affinity of bivalents as well as nucleolus (Photograph No. 13.16). The condensation continued in diplotene and 4 bivalents of different lengths were noted. The longest bivalent was nucleolar chromosome pair, at the end of prophase nucleolus and nuclear membrane disappeared.

At metaphase 1st, five bivalents formed an equatorial plate. At anaphase-I and telophase - I the axis of spindle was mostly parallel to the long axis of the ascus. The nucleolus and nuclear membrane reappeared at ehe end of the telophase.

DIVISION II AND III :

These divisions were rappid and showed same synchronization in their behaviour. The planes of the divisions of nuclei were varied - parallel, oblique, perpendicular to long axis.

The spindle fibers being well stained in smears with aceto-orcein than in FAA fixed material, stained with Haematoxylene.

During successive nuclear divisions II and III, progressive reduction in size of nuclei was observed, some nuclei had been observed to have failed to take part in the division and 5-7 nucleated conditions were observed at the end of division III. Only one nucleus was used to develop ascospore. The remaining nuclei disappeared before ascosporogenesis. Ascosporogenesis occurred after vacuolization in ascus. And ascospore is formed by free cell formation.

CYTOKINESIS :

The interphases between I and II Division and between II and III Division were verily brief, so there cytokinesis between them was absent. Cytokinesis by free cell formation occurred only at the end of division III. The cytoplasm underwent condensation around the single undergenerated nucleus to form globose to subglobose cytoplasmic mass. This uninucleated protoplast secreted a wall around itself.

1. CYTOLOGICAL CONCLUSIONS :

1) Mode of sexual reproduction is by gametangial contact of ascogonia and antheridia. Antheridia are functional and trichogyne is absent.

2) The protective cover is formed from stalk cells of both antheridia and ascogonia, before or after fertilization.

3) Centrum consists of ascogenous hyphae and asci, and is developed inside the pseudoparenchymatous nutritive tissue, the development of centrum is of Phyllactinia type.

4) Asci unitunicate in a group of 5-7 forming basal hymenium, asci develop from terminal or subterminal binucleate cells of ascogenous hyphae.

5) Ascosporogenesis by free cell formation, only one ascospore is formed per ascus. The remaining nuclei degenerate before ascosporogenesis.

6) The number of chromosome is n = 5 and longest chromosome is nucleolar chromosome.

2. TAXONOMIC DISCUSSION AND CONCLUSION :

This fungus was referred to Salmonomyces by Chiddarwar (1959), Pirozynski (1965) referred it to Uncinula; Ainsworth (1971) also included in Uncinula. But Kamat and Patwardhan (1967)

recognised the validity of the genus Salmonomyces. Sathe (1969) referred it to Erysiphopsis, Braun (1981) felt to follow Pirozynski in the absence of the study of type collections.

The position of the fungus on the host Acalypha ciliate Forsk (Euphorbiaceae) could not be therefore decided on the morphological ground, because the appendages have been variously interpreted, as very rigid, to slightly curved and though slightly broader at the base they were not bulbose and though they were very gradually tapering at apex they were strictly not acicular. Hence, these could not be accomodated strictly into Erysiphe, Uncinula or Phyllactinia and even though Pirozynski (1965) and Braun (1981) are inclined to include it in Uncinula, the treatment of Kamat and Patwardhan (1967) in keeping under separate genus Salmonomyces created by Chiddarwar (1959) is fairly correct on the basis of nature of the appendages (Homma,1937), the cytological evidence supports this separate identity, because Uncinula shows the basic chromosome number n=4, Erysiphe n=4 while in Phyllactinia n=4 or 5. Even development of centrum here is of Phylloctinia type. It is likely that Erysiphe like genera, have in the evolution lead to Phyllactinia like genera through two routes - (a) Erysiphe with n=4 chromosomes to Phyllactinia with n=4 chromosomes and (b) Erysiphe with n=4 chromosomes to Phyllactinia with n=5 chromosomes, through intermediate forms like Salmonomyces with n=5 chromosomes.

The modification in the evolution is always change in structure due to change in function and change in function is determined by genes or chromosomes. Salmonomyces is thus probably representing such vestigial intermediate stage which has n = 5 chromosomes as a cytological change and swollen bases and tapering tips of appendages as morphological changes. Salmonomyces shows one ascospore per ascus as in Phyllactinia. Similar transitions have been observed by Hirata (1976) between Erysiphe - Microsphaera Uncinula (Braun 1981). This conclusion is also supported by the fact that only Phyllactinia is represented on family Euphorbiaceae, but no Uncinula is reported on its members anywhere in world and on the ground of host specificity and obligate parasitism, Salmonomyces is more related to Erysiphe - Phyllactinia Complex than that of Erysiphe - Uncinula complex.

Photograph No.1 - Infected leaves of Acalypha ciliata Forsk showing white powdery patches

Photograph No.2 - Cleistothecium with exposed asci

Photograph No.3 & 4 - Appendages with swollen bases and showing broad or pointed tips

Photograph No.5 - Cleistothecium with several appendages showing straight tips

Photograph No.6 & 7 - A knot showing uninucleate sex organs at its centre

Photograph No.8 - A knot showing binucleate ascogonium and empty antheridium at its centre

Photograph No.9 - A knot showing ascogenous hyphae. Few cells of ascogenous hyphae lying at the centre of the knot are seen binucleate

Photograph No.10 - Perithecium showing binucleate ascus mother cells at its centre

Photograph No.11 - Perithecium showing asci with fusion nucleus. Perithecium with 3 asci, left and right ascus showing fusion nucleus

Photograph No.12 - Ascus showing large nucleolus and 5 bivalent chromosomes

Photograph No.13 - Perithecium with right ascus containing nucleolus and chromatin network

Photograph No.14 - Perithecium with binucleate asci

Photograph No.15 - Perithecium containing two asci, one with fusion nucleus, other with 4 nuclei, few binucleate nutritive cells are seen in nutritive tissue

Photograph No.16 - Perithecium with 3 asci, left ascus with nucleolus and 5 bivalent chromosomes

Photograph No.17 - Perithecium showing 7 asci in T.S.

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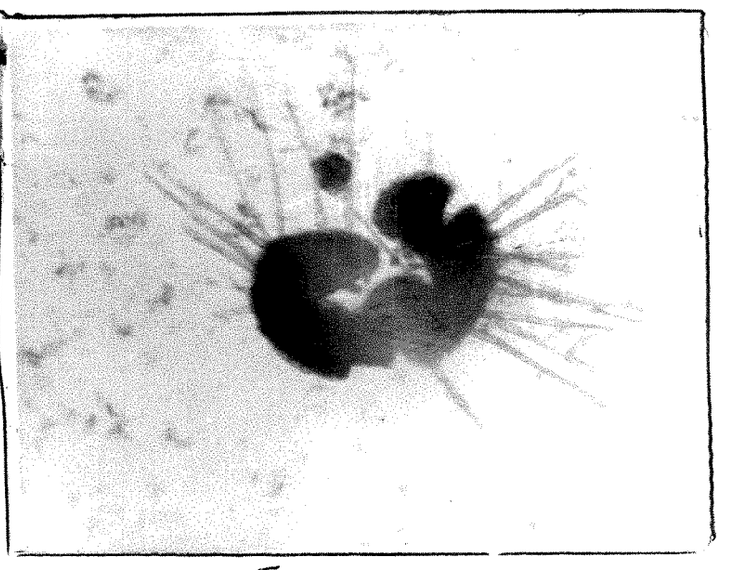

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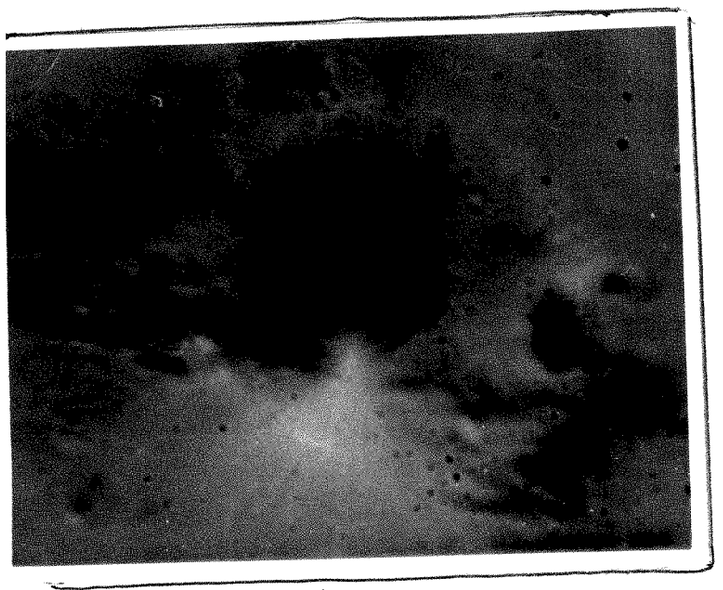

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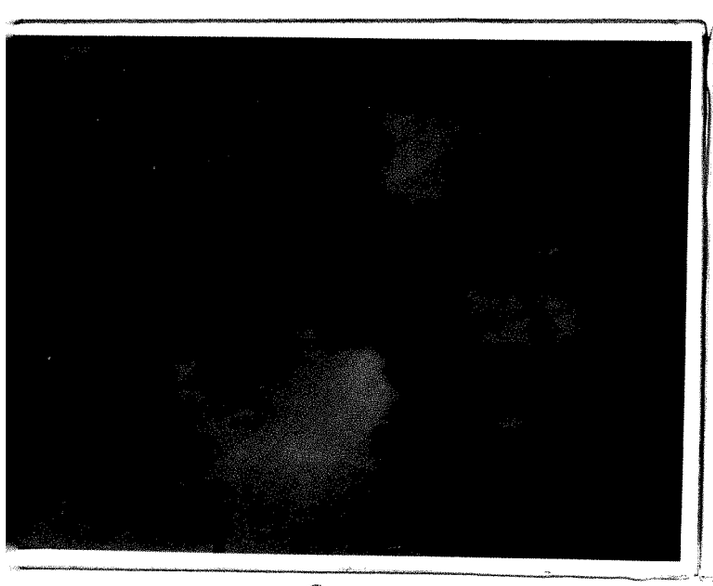

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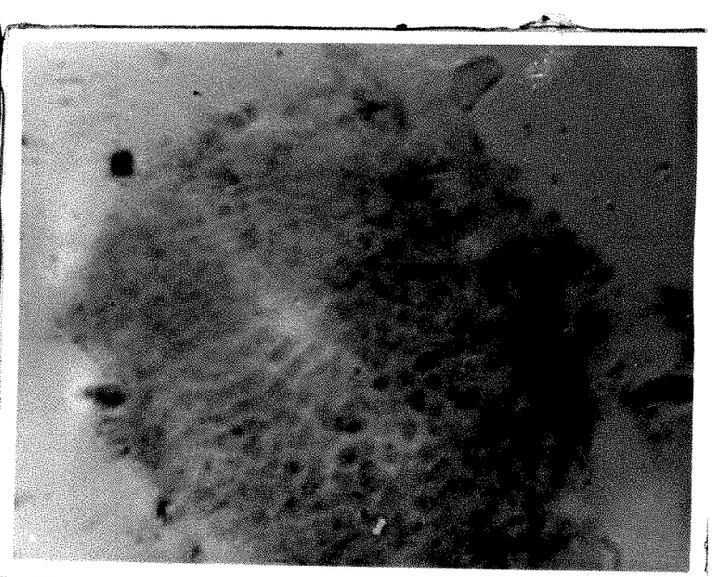

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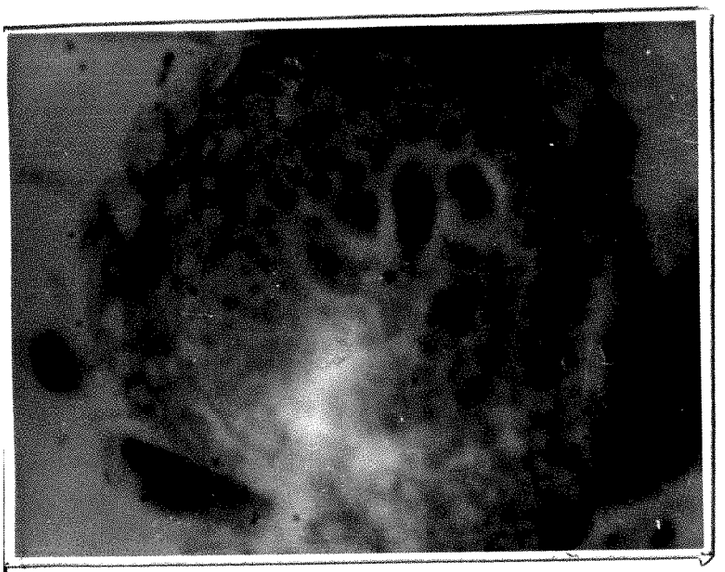

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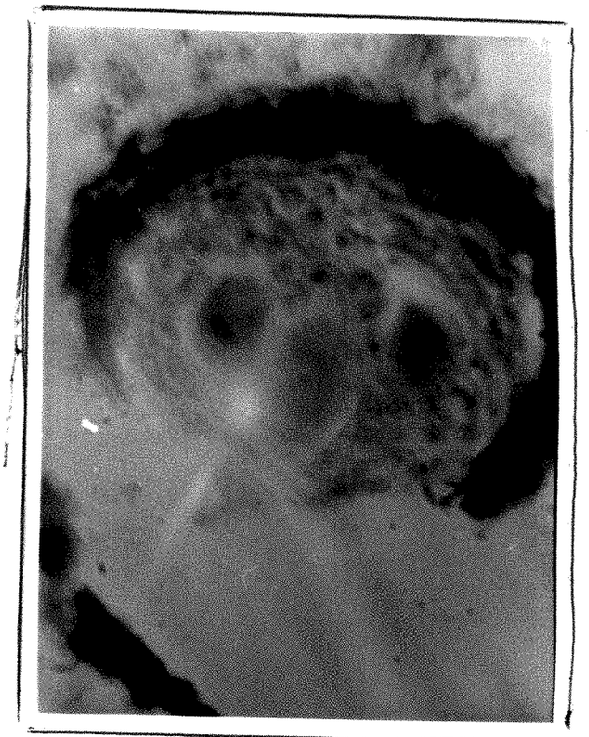

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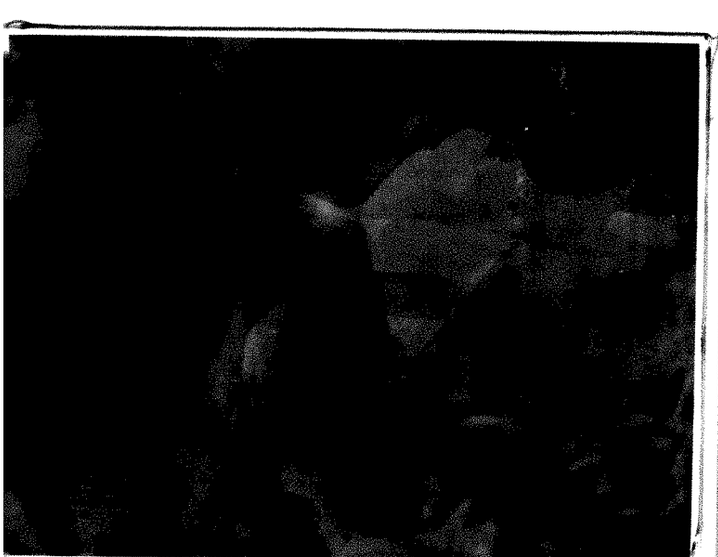

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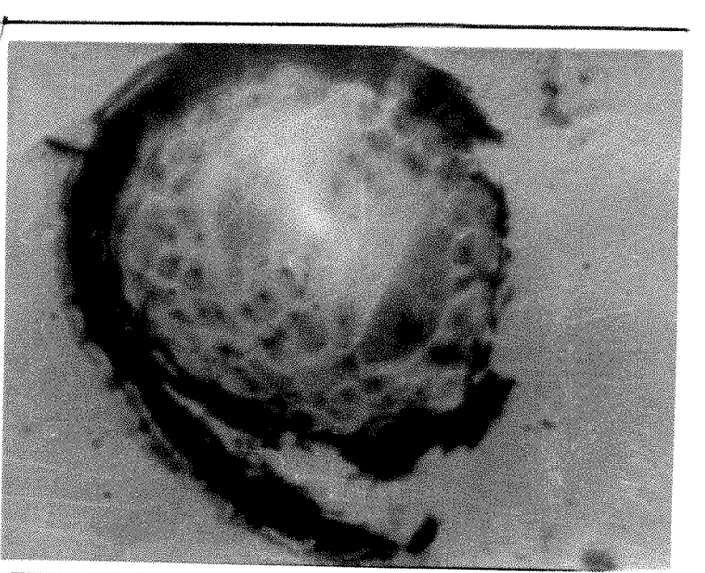

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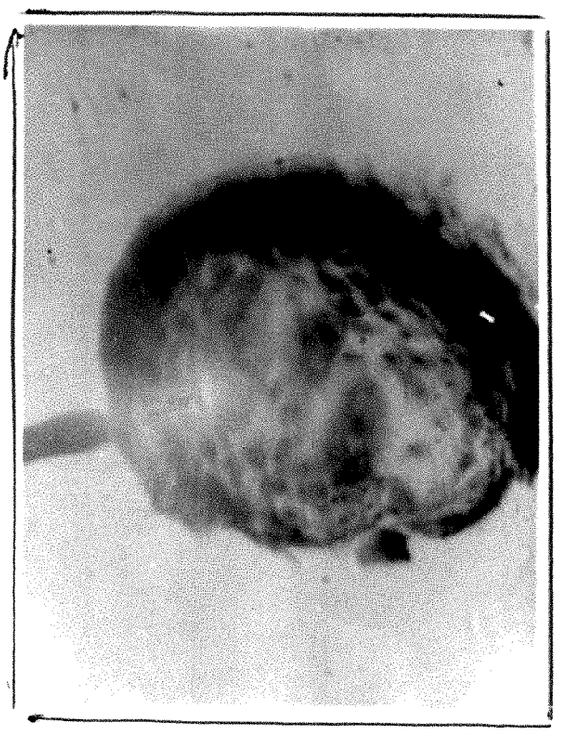
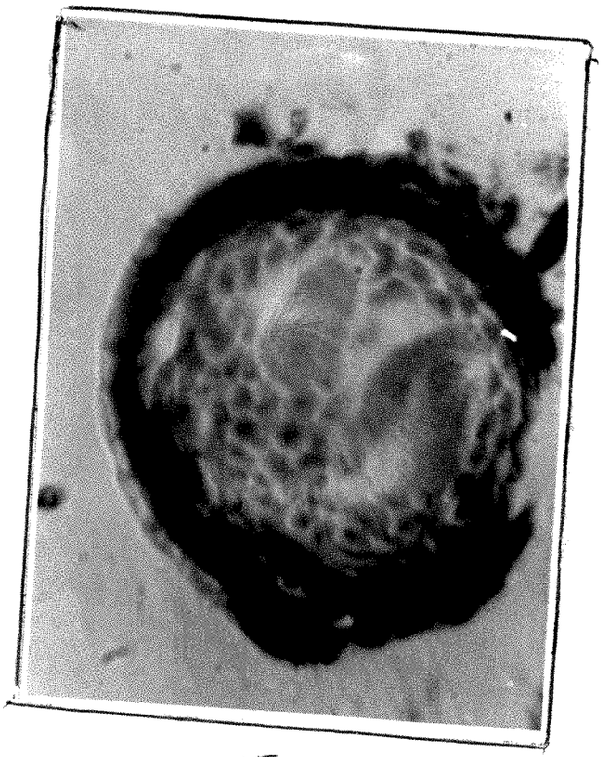


14



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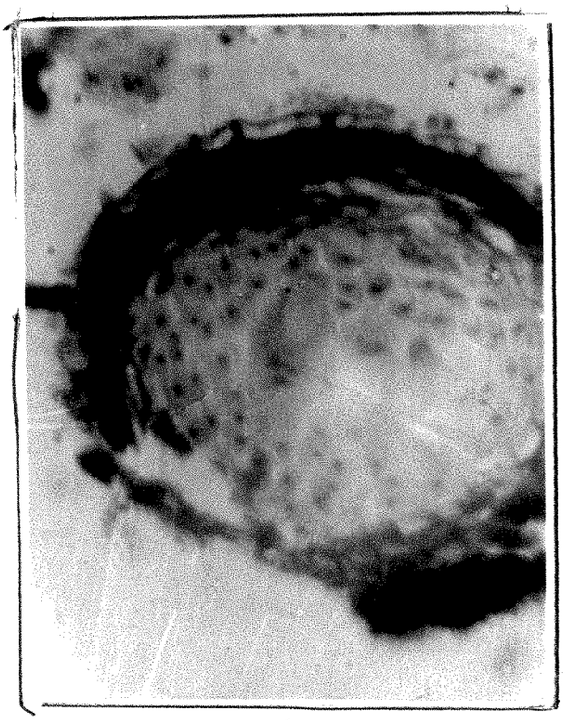

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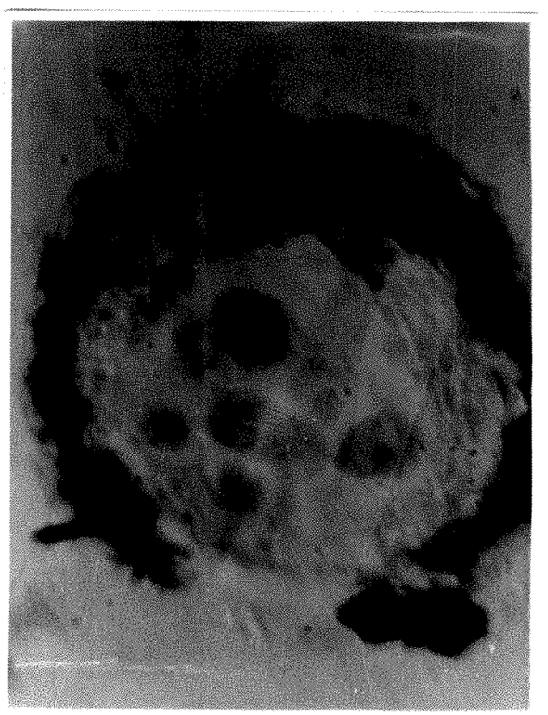

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