INDUCTION OF SPOROGENESIS IN GRACILARIA SPP (GIGARTINALES, RHODOPHYTA) BY ENVIRONMENTAL MANIPULATION

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Certified that the thesis entitled "INDUCTION OF SPOROGENESIS IN *GRACILARIA* SPP (GIGARTINALES, RHODOPHYTA) BY ENVIRONMENTAL MANIPULATION" is a record of independent bonafide research work carried out by **Mr. P. Saravanane** during the period of study from September 1999 to August 2001 under our supervision and guidance for the degree of **Master of Fisheries Science (Mariculture)** and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title.

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DECLARATION

I hereby declare that the thesis entitled "**INDUCTION OF SPOROGENESIS IN *GRACILARIA* SPP (GIGARTINALES, RHODOPHYTA) BY ENVIRONMENTAL MANIPULATION**" is an authentic record of the work done by me and that no part thereof has been presented for the award of any degree, diploma, associateship, fellowship or any other similar title.

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सारांश

अत्यधिक पुनरुत्पादक क्षमता के कारण ग्रेसिलेरिया जाति सबसे महत्वपूर्ण एगारोद्भिद मानी जाती है. समुद्री शैवालों के बीजाणुओं की कृषि को प्राकृतिक सम्पदा की रक्षा व एगार उद्योगों के लिये कच्चे माल की बढती मांग को पूरा करने के लिये विकल्प के तौर पर प्रयोग किया जा सकता है. सही समय पर परिपक्व फलबीजाणु सहित मादा जननीय पौधों की अनुपलब्धता जननीय प्रवर्धन में साधारण व्यवरोध पैदा करती है. इस शोध कार्य में फलबीजाणुजनन की विकासी अवस्थाऐं , बीजाणु जनन प्रेरण, बीजाणु उत्पादन, आरम्भिक वृद्धि और बीजाणुओं की उत्तरजीविता का अध्ययन शामिल है. ग्रेसिलेरिया इड्युलिस और ग्रे. कोलिफेरा के पौधों को बीजाणुजनन प्रेरण के लिये विभिन्न वातावरणीय प्राचलों जैसे प्रकाशावधि, प्रकाश स्पेक्ट्रम, प्रकाश तीव्रता और तापमान के तहत रखा गया. ग्रे. कोलिफेरा में बीजाणुजनन की शुरुआत लम्बी अवधि के दिन, 2000 लक्स प्रकाश तीव्रता व 25°C तापमान पर देखी गई जबकि ग्रे. इड्युलिस में बीजाणुजनन की शुरुआत लाल स्पेक्ट्रम (630-670 nm) पर हुई. 630-670 नैनीमीटर पर क्लोरोफिल, फाइकोएरिथिन व फाइकोसायनिन की मात्रा में बढती देखी गई. ऊतकीय प्रेक्षण में जनिफलिका की विकासीय अवस्थायें साफ तौर पर दर्शित हुई. अधिकतम बीजाणु उत्पादन 25 डिग्री सेल्सियस पर छोटी प्रकाशावधि के दिनों में पहले दो दिन तक देखा गया तत्पश्चात पतन दर्शित हुआ. दोनों जातियों के बीजाणु 25-26 डिग्री सेल्सियस तापमान पर संधारित पी.ई.एस. माध्यम में आठ दिनों तक अच्छी वृद्धि दरों पर पाले जा सके.

ABSTRACT

Gracilaria spp have been regarded as the most important agarophytes for cultivation due to their high regenerative capacity. Seaweed farming from spores of *Gracilaria* is considered as an alternative tool to protect the natural stock and to meet the demand of raw materials for the agar industries. Non-availability of female reproductive plants with mature carpospores during favourable period of cultivation is a general constraint of reproductive propagation. The present work includes study of developmental stages of carposporogenesis, induction of sporogenesis, spore output, early growth and survival of spores. *Gracilaria edulis* and *G. foliifera* were subjected to various environmental parameters such as photoperiod, light spectra, light intensity and temperature for induction of sporogenesis. *G. foliifera* showed initiation of sporogenesis under long day at 2000 lux light intensity and temperature of 25^0 C, while in *G. edulis* sporogenesis was initiated under red spectra (630-670nm). The chlorophyll a, phycoerythrin and allophycocyanin contents showed increase under 630-670nm. The histological observation clearly showed the developmental stages of cystocarp in *G. edulis* and *G. foliifera.* Maximum spore output was observed at 25^0 C and short day photoperiod for both the species ($P>0.01$) for first two days and declined subsequently. The spores of *G. edulis* and *G. foliifera* have grown well up to 8 days in PES medium maintained under temperature range of 25–26^0 C ($r=0.99$).

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INTRODUCTION

1. INTRODUCTION

During the past few years, phycoculture has gained importance in view of increasing demand for manufacture of variety of goods from seaweeds. Seaweeds are marine algae, saltwater dwelling, and simple organisms that fall into the rather outdated general category of plants. Most of them are the red (6000 species), brown (2000 species), and green (1200 species) and most of them are attached by the holdfasts, which only have an anchorage function. This plant-like organisms are found throughout the world's oceans and seas and none is known to be poisonous. Many are in fact eaten and considered to be delicacy.

It is estimated that the total standing crop of all seaweeds in Indian waters is more than 100,000 tones wet weight consisting of 6,000 tones of agar yielding seaweeds, 16,000 tones of algin yielding seaweeds, 8,000 tones of carrageenan yielding seaweeds and the remaining 70,000 tones of edible and green seaweeds (Devaraj *et al.*, Unpub.).

The total production of cultivated seaweed in the world is about 3,250 million tonnes wet weight and the total cultivation area is estimated as 200 x 10^3 hectare. China leads in harvests from aquaculture with 4.8 million tons followed by South Korea (650,000 tons), Japan (570,000 tons) the Philippines (465,000 tons) and North Korea and Indonesia with 110,000 tons in each during 1995 (Buendla, 1998). The annual total value of cultivated seaweeds has been reported to be about 3 billion US dollars (Geng *et al.*, 1999). Much of world's agar is processed in Japan but the resources are obtained from elsewhere.

Of the major classes of macro algae red algae, assume greater importance due to their wide utilization. Rhodophyta appears to have 675 genera. Family *Gracilariaceae* of order Gigartinales and group Rhodophyta has six genera and 130 species, of which more than 120 species belong to worldwide agarophytes. The cell wall polysaccharides of Rhodophyceae known as agar-agar and carragenan are used in pharmaceutical, food and cosmetic

industries as a gelling or thickening agent. Because of the polysaccharide composition of these algae, *Gracilaria* is one of the important agarophytes of India presently exploited only from the natural resource to meet the demand of agar industry. Besides its use as an agarophyte, species of *Gracilaria* are also used as medicine (Chapman, 1950), fertilizer (Zaneveld, 1959), human food (Leving *et al.*, 1969), tertiary treatment of sewage (Ryther *et al.*, 1979), food for invertebrate cultivation (Chiang, 1981) and for biogas production (Hanisak, 1981).

Asia is the leading in the supply of *Gracilaria* spp for the manufacture of agar (Doshi *et al.*, 1992). The current international market price for the raw material of *Gracilaria* species range from US $ 1200-1300/ton.

There is luxuriant growth of seaweeds along the southeast coast of India, Gujarat coast, Lakshadweep Island and the Andaman and Nicobar group of islands. Fairly rich seaweed beds are present in the vicinity of Bombay, Ratnagiri, Goa, Karwar, Visakhapatnam and few other places such as Chilka and Pulicat lakes (Chennubhotla *et al.*, 1987). In India, the natural agarophyte resources are found in Mandapam and Tuticorin area of Gulf of Mannar, Pulicat lake, Andhra Pradesh coast, Chilka lake, Arabian Sea near Kanyakumari of Kerala coast, Lakshadweep island and Andaman Nicobar Islands.

The area between Keelakari and Rameswaram in southeast coast of Tamilnadu of India forms the nodal region for commercial exploitation of *Gracilaria spp* (Umamaheswarrao, 1969). Demand of *Gracilaria* has increased significantly over the last ten years and this led to over exploitation of the natural stocks in several geographical areas (Paramasivam and Devadoss, 1985). The decline of the natural beds of *Gracilaria* in recent years prompted the development of several restoration techniques.

Farming of *Gracilaria* is a reliable method of increasing volume and quality. *Gracilaria* has very high regenerative capacity and hence vegetative propagation by cutting is presently done on nets and ropes placed in ponds and inshore waters. However, nursery produced seedlings from spores is better for cultivation because of uniform growth and survival rate (Trono, 1987).

Looking into the life cycle of *Gracilaria*, the asexual diploid individuals bear tetrasopores during the formation of which reduction division occurs, and these spores give rise to a perfectly similar haploid individual bearing the sex organs. The gametes of these individual plants unite together and produce gonimoblast representing diploid phase, from which carpospores are formed. These carpospores grow into diploid individuals bearing tetraspores (Diplobiontic forms) (Fritsch, 1945).

The life history and reproduction of *Gracilaria spp* were studied by Jaffe (1958), Jones (1959), Umamaheswaraao (1974), Bird and McLachlan (1976), Evans *et al.*, (1982), Chennubhotla *et al.* (1986), Breeman and Guiry (1989), Figuero *et al.* (1995) and West *et al.* (1996). Nutrients, temperature, salinity, wavelength and intensity of light play importance roles on reproduction and life history and life history of seaweeds.

Among the total population of *G. corticata* in natural environment, about 77% were in fruiting, of which 17% were of cystocarpic, 16% of antheridial and 45% of tetrasporic plants (Subbarangaiah, 1983). Considering that the fronds of *Gracilaria* sp bearing reproductive structures are rare in nature, the induction of sporogenisis by environmental manipulation has been identified and analyzed for suitability and viability. This technology could be applied to promote the establishment of *Gracilaria* cultivation from spores as an alternative to vegetative propagation. Eventually it will help to conserve the existing natural resource of *Gracilaria.* The cultivation of *Gracilaria* will also increase the production of agar, agar products and other seaweed products and can give job opportunity to the rural fisher folk.

Objectives of the study mainly include

1. Induction of the sporogenesis
2. Studies on developmental stages during carposporogenesis
3. Identification of ideal environmental conditions to induce sporogenesis and spore output
4. Increase the survival percentage of the spores.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Gametogenesis and sporogenesis have been known to play major roles in the reproduction of seaweeds like any other reproductive plants, in addition to vegetative reproduction method. Correlation between the environmental factors and sporogenesis in seaweeds has been well studied and established by many researchers. These studies are expected to support the seaweed farming through continuous availability of sporelings.

2.1 Gametogenesis

Antheridial mother cells are easily distinguished by their dense cytoplasm containing few vacuoles and a large central nucleus with active perinucleus golgibodies (McCully, 1968; Berkaloff and Rousseau, 1979). The carpogonium consists of an enlarged basal portion harboring the ovum and a long narrow process known as trichogyne (Venkataraman *et al*, 1974).

In *Fucus*, the cytoplasm cleavage proceeds by fusion of vesicles resulting eventually in 64 almost isodiametric cells. And the oogonial mother cell divides further and at the 8 nuclei stage, the nuclei are dispersed throughout the cytoplasm, which cleaves into eight individual eggs. Eggs are separated within the oogonium by a sulphated polysaccharide mucialiage (Evans *et al*, 1982).

In *Scinaia forcellata*, BelaBhatia and Vijayaraghavan (1995) studied that the Spermatangia are borne at the tip of 3 or 4 celled spermatangial filaments that project beyond the epidermal cells. They noted that the one of the hypodermal cells cuts off one or two cells; each of which produces one or two spermatangial mother cells and two or three spermatangia are produced from each spermatangial mother cell.

In *Cystoseria indica*, the presumptive oogonium initially possesses dark cytoplasm, large nucleus and a few chromatophores and later divides by a transverse wall into two cells and the upper cell acts as the oogonial mother cell and lower cell as a stalk cell (Sahoo and Vijayaraghavan, 1995).

Subbarangaiah and Vanillakumari (1999) found the carposprophytic antheridial and tetrasporophytic plants in all months of the year

and maximum development of these saprophytic and gametophytic phases were also found in two seasons in the periods from July to August and November to December.

2.2 Fertilization

Cook *et al.*, (1951) showed that a variety of simple hydrocarbons including n-hexane, ethers and esters would mimic the natural chemo-attractant for the sperms towards the egg. In *Fucus*, the secretion of sperm chemo attractant mediates the initial interaction between the gametes by the eggs, and the development of a strong chemotactic response has been shown to parallel the evolution of anisogamy and oogamy (Muller and Saferiadis, 1977).

Sperm appears to "probe" the egg surface and attach itself with the egg by its anterior flagella. Then the sperm moves further for fusion and fertilization occurs. Within the seconds of gamete fusion the egg cell releases the β-linked polyuronide, alginic acid, help within cytoplasmic vesicles, to form an incipient cell wall to the developing zygote (Evans *et al.*, 1982).

2.3 Sporogenesis

Sjoestedt (1926) observed that the carpogonium in *G. confervoiders* (=*G. verucosa*) and *G. robusta* is two celled and is developed from a bearing cell arising from a primary cortical cell. He found that the fertilized carpogonium fused progressively with neighbouring cells and formed a fusion cell, which later produced the gonimoblast in *Gracilaria* (Oza, 1976; Edelstein *et al.*, 1978).

The fertilized carpogonium of *Gracilaria* fuses progressively with the adjacent multinucleate nutritive cells. Meanwhile the cortical cells above divide copiously to produce the cystocarp-wall; the inner cells of the rows thus formed, however, constitute a nutritive tissue around the fusion cell. The several goimoblast-initials develop into a dense aggregate, the inner part of which forms a compact pseudo-parenchyma bearing the rows of successively ripening carposporangia (Fritsch, 1945). Smith (1954) reported that the production of an auxiliary cell produced by the supporting cell in *G. foliifera* with which fertilized carpogonium was connected by a short connecting filament.

Oza (1976) observed that in *Gracilaria corticata* after fertilization, the trichogyne shrivels up and the carpogonium fuses with lower cells of the carpogonial branch, the supporting cell and sterile cells resulting in a large fusion cell. He noted that the gonimoblast are initiated from the fusion cell as a number of lobes and these become delimited as cells, which give rise to dichotomous branching filaments. From the end cells of these filaments, carpospores are formed in chain, borne on slightly enlarged stalk cells. The carpospores are more or less spherical 18 - 20μ in diameter and have granular contents and a single nucleus. A mature cystocarp shows the presence of irregular filaments extending from gonimoblast to the base of the pericarp; these are the so called nutritive filament.

Joseph and Krishnamurthy (1977) found that the young cystocarps are solitary but sometimes during the development, two or three unite together to form a compound cystocarp in *G. corticata*. They observed that carpospores are reddish brown in colour and 20-30 μ in diameter and the maximum number of spores were liberated on the first and second day of the experiment. Yamamoto (1978) found the tip of the gonimonema is to have pit connection with cells of the pericarp. However in any of the Indian works, such pit connection could not be observed in the spores *of Gracilaria*.

At least the majority of carpospores within a single cystocarp mature simultaneously resulting in the release of almost the total potential spore production from the cystocarp within a single short period in *Polycsiphonia* and other seaweeds (Ngan and Price, 1983). It has been observed that 51.33-57.33% of cystocarpic plants, 34.17% of tetrasporophytes and 12.67-14.50% of male plants of *Gracilaria textorii* collection occur in natural bed. *G. textorii* have a short life span of 3.5 months and produce more cystocarps plants than tetrasporophtes (Subbarangaiah, 1984). In *Centroceras clavulatum*, Sahoo (1989) observed that the bisporangia and tetrasporangia (both tetrahedral and cruciate type) are found in the same plant and all the cells are connected with pit connections.

In *Caulacanthus ustulatus* (red algae), following presumed fertilization, the diploid nucleus is transferred to the auxiliary cell, which expands

and cuts off several multinucleate gonimoblast initials. The gonimoblast initials are distributed around the periphery of the cystocarpic cavity and divide further to produce generative gonimoblast cells and the later cleave repeatedly to form clusters of carpospore. The first formed gonimoblast cell is transformed into a strong gonimoblast cell containing large quantities of starch granules and presumably functioning to supply nutrients (Delivopoulos and Diannelidis, 1990). Kain and Destombe (1995) found that the plants could bear both gametangia and tetrasporangia, either on separate parts of the thallus or on the same in *Gracilaria*.

2.4 Influence of environmental factors on sporogenesis

Jaffe (1958) observed that the strong unilateral illumination induces the formation of the rhyzoidal protrusion from the shedded side, i.e., the region of least absorption, whilst unilateral illumination of moderate intensity (10^{-15} to 10^{-16} Quanta cm^{-2}) causes the protrusion from $90^0 - 135^0$ away from the source.

Jones (1959) suggested that in *Gracilaria verucosa* the growth rates of cystocarpic and tetrasporic plants were not always the same in the wild population. Tetrasprophytes occur at Rameswaram area for 7-9 months with peak number of fertile plants between March/April and June and again between August and October in *Gelidiella acerosa* (Umamaheswararao, 1974).

Photoperiodic control of receptacle initiation has been demonstrated in *F. distichus* subsp. *distichus* (Bird and McLachlan, 1976) and *Ascophyllum nodosum* (Terry and Moss, 1980). Both species form receptacles under 8 and 12 h photoperiods but a 16 h photoperiod was inhibitory (Evans *et al.*, 1982). In *Gracilaria*, the fertile thalli predominated over the sterile thalli during months of high salinity (30 – 35 ppt) regimes seemed to favour reproduction. The population is mostly vegetative when the salinity is 20 – 25 ppt (Trono and Corrales, 1981).

Evans *et al.* (1982) reported that light, chemicals, gradients and electrical fields are the main vectors for polarity. Other factors, which can induce the polarity include direction of seawater flow, the presence of other zygotes, H^+

and centrifugation. Friedlander and Dawes (1984a) reported that the release of carpospores was negatively correlated with temperature under dark or low light conditions ($r = -0.883$, $P<0.05$) in *Gracilaria foliifera*.

Chennubhotla *et al.*, (1986) showed that the tetrasporophytes were more abundant than carposporophytes in *Gracilaria foliifera*. The total population was tetrasporophytic plants during the month of February and maximum output of tetraspores and carpospores were recorded on the first day. In *Gracilaria* spp tetraspores cultured without aeration developed into plants bearing spermatangia only; tetraspores cultured with aeration developed into 1:1 female and male gametophytes (Estela and Oliveira, 1988).

Breeman and Guiry (1989) recorded that high water of spring tides falling at the beginning and end of the day reduced effective day length for the plants and caused an early onset of reproduction in *Bonnemaisonia hamifera*. Photoperiodic induction was affected by tides, principally by the time of day at which high and low water occurred through the spring/neap cycle. High water of spring tides falling at the beginning and end of the day reduced the effective day length for plants and caused an early onset of reproduction in *Bonnemaisonia hamifera*.

In red algae, light has been reported to induce developmental and morphological changes in the phenomenon of photoperiodism (dependence on day length), of photomorphogenesis (dependence on spectral range) and in other phenomena like phototropism (Luning, 1990). Guiry and Dawes (1992) reported in *Asparagopsis armata* that at a day length of 8 h, Irish plants formed tetrasporangia only at 17^0 C, Italian plants at 17 - 21^0 C and Australian plants at 13 - 17^0 C. However, after an incubation period of 5 weeks, only the Italian strain consistently showed 100% reproduction under these conditions. At 17^0 C, the critical day length for tetrasprogenesis is 8-9h for the Irish and Australian strain and 9-10h for the Italian strain. Arsenic and Iodine supplementation to N and P enriched seawater appeared to be necessary for reproduction in the Irish strain.

Figuero *et al.* (1995) recorded that the light absorption around the maximal transmission of the blue light used (440- 480nm) was 1.2 -1.5 times smaller than that around the maximal transmission of the red light used

(630 – 670nm) in *Porphyra umbilicalis*. The better growth rate in red than in blue light was probably due to higher photosynthetic efficiency and quantum yield in the red light. Reed *et al.* (1996) recorded that the reproduction is closely tied to environmental conditions and the availability of resources and thus typically varies with season in seaweeds. They explained reproductive allocation and spore standing stock in *Macrocystis* were negatively correlated with seawater temperature and positively correlated with the nitrogen content of adult plants.

West *et al.* (1996) showed that in *Stictosiphonia hookeri,* the temperature required to induce sporogenesis correlates with the range of water and air temperatures in the natural habits. Buchhloz and Luning (1999) observed that the pieces of young tissue developed sorus under various light regimes and a range of temperatures in seaweeds. They noted that in contrast to *Laminaria digitata , L. saccharina* tissue required short day conditions for sorus formation and nutrient seawater accelerated sporogenesis compared to the pure seawater. Edding *et al.*(1987) reported that the extremes in temperature (10 and 30° C) inhibited development of *Gracilaria* spp.

2.5 Pigmentation

In *Gracilaria verucosa*, the parts of the plant under the brightest area of the field becoming lighter in colour while the remainder in the less intense light lost less of their pigments (Jones, 1959). The concentration of both chlrophyll a and phycoerythrin, which were found to be the main pigments in *Gracilaria* spp., decreased with increasing photon fluence rate, while phycocyanin was more stable (Beer and Levy, 1983).

In *Gracilaria foliifera,* Friedlander and Dawes (1984b) found that the chlorophyll a and phycoerythrin levels were higher sporelings preincubated at 30 ppt as compared to lower salinities. The strains, which had higher chlorophyll, content showed higher growth rate performances in *Gracilaria* spp. (Levy and Friedlander, 1990).

In red algae, under condition of low light higher pigment content is required to enhance the antenna molecules of the photosynthesis. In strong light the pigment content should be lower in order to avoid photo damage (irreversible

inhibition of the photosynthesis apparatus) and photo inhibition (Reversible inhibition) (Luning, 1990). Rodrigo and Robaina (1997) found that the pigment composition of sporelings promotes a better photosynthetic performance under chlorophyll a excitation.

2.6 Shedding of spores

Oza and Krishnamurthy (1968) indicated that apart from tetraspore, the maturity of the specimen has a great influence on the spore discharge rates in *Gracilaria verrucosa*. Boney (1975) recorded that the mucilage sheaths occupied 52.8 – 87.7% of the combined spore plus mucilage volumes depending on the species. The peak shedding of tetraspores in *Gracilaria corticata* were observed at the early morning hours from 02.00 to 06.00 h (Umamaheswararao, 1976; Subbarangaiah and Umamaheswararao, 1983).

The mean diameter of carpospores and tetraspores of *Hypnea musiformis* were 26.9 ± 0.6 and 24.2 ± 0.4 µm respectively (Mshigeni and Lorri, 1977. In most of the red algae, the size of the spores produced by a specific plant apparently depends upon the type of sporangia it produces (Okuda and Neushul, 1981). The smallest spore diameter of 3.0µm has been reported for tetraspores of *pseudogloiophlora confusa* with the largest size of 182.5µm for a tetraspore of *Leveillea jungermannioides* (Ngan and Price, 1979).

Subbarangaiah and Umamaheswararao (1983) observed that carpospores shedding occurred for 5 to 10 days whereas tetraspore shedding was seen only for 3 days and the maximum number of carpospores was seen at midnight 22.00 to 02.00hrs and early morning hours 02.00 to 06.00 hours respectively in *Hypnea valentia*. Sylvester and Waaland (1984) showed that the freshly released tetraspores average 15-18µm in diameter in the *Gigartina exasperata*. In *Gelidium pusillum,* shedding of tetraspores was seen for 4 days and carpospores continuously for 20 days under laboratory condition (Kaliaperumal and Umamaheswararao, 1986).

In *G. edulis*, Mal and Subbaramaiah (1990) suggested that time between 19.00 and 01.00 h as ideal for bulk collection of carpospores for mass cultivation. In the laboratory experiments 30 to 40% salinity submergence of

fronds and 27 to 44 μE m^{-2} s^{-1} light intensity were found to be favorable condition for the maximum liberation of spores (Narasimharao and Subbarangaiah, 1991).

Sundar *et al.* (1991) observed that the spore shedding took place for 5 to 12 days only and maximum shedding of carpospore in *Gracilaria crassa* was seen on the first day. Carpospores apperared on slides as dark red, spherical cells, approximately 20μm in diameter with some undergoing cell division after 72 hrs (Glenn *et al.*, 1996). Subbarangaiah and Vanillakumari (1999) reported that the tetraspore shedding varied from 4,240 to 5,28,461 spores/g fr. wt/day on day one in *Amphiroa fragilissima*.

Banumathi and Subbaramaiah (1990) reported that type wise maximum peak spore shedding occurred generally in March as monospores from 12.30 to 1.30pm; bispores and polyspores from 1.30 to 2.30pm; tetraspores from 11.30 to 12.30pm under laboratory conditions in *Gelidiella acerosa*.

2.7 Influence of environmental factors on shedding of spores

Ramarao and Thomas (1974) reported that the daily periodicity was always maximum in the first day and decreased gradually but showed a second maximum after fourth or fifth day in *G. edulis*. No shedding is seen when the seawater reaches higher temperature i.e., 29-33^0 C in April – May. In red algae, hydrostatic pressure on the pericarp walls will direct the expanding spore mass towards the terminal canal leading to the ostiole (Boney, 1975).

Umamaheswararao (1976) pointed that the submerged condition is more favourable for spore liberation and any increase in the duration of exposure caused by tides would greatly affect the spore shedding in *Gracilaria corticata* especially in cystocarpic plants. It may be noted that in *Gracilaria corticata* maximum liberation of spores is seen while keeping the plants in submerged condition and in a dark chamber.

Nagan and Price (1983) noted that the peak liberation of tetraspores was delayed in *G. pusillum* and *G. Variabilis* when seawater temperature was below 30C. The majority of taxa investicated maximum spore output took place between 05.00 and 09.00 h on the day following collection, during the prediched flood tide period.

Submerged condition of plants, long day condition at low illuminance (500 lux), seawater of 30 to 40 ppt salinity and 25^0 to 30^0 C temperature were found to be favorable for maximum shedding of spores in *Gellidium pusillum, Pterocladia heteroplatos* and *Gelidiopsis variabilis* at Visakhapatnam (Umamaheswararao and Kaliaperumal, 1983). Ruiz *et al.*, (1989) found no correlation between spore release and salinity or the number of reproductive pinnules present in *Gigartina canaliculata.* The release of carpospores from the cystocarp via the ostiole occurred precipitously during the first two minutes after the plants were put in water.

Ganesan *et al.* (1999) identified that the UV radiation enhances spore liberation initially during short time and inhibits spore release during long time exposure. Low illumination 26°C and short day (8:16h LD) with submerged condition of plants and seawater of 35 ppt salinity for maximum liberation of tertraspores. A combination of 26^0 C and short days (8 L : 16 D) gave the highest carpospore discharge per cystocarp in *Gracilaria cornea* (Vriosteegui *et al.*,1999). At 31^0 C spore release and sporeling growth were significantly depressed regardless of irradiance in *Gracilaria cornea* (Rojas and Robledo, 1999).

In *Polysiphonia platycarpa,* Subbarangaiah and Sudhakar (1999) reported that the effects of different photon flux densities from 0 to 52 $\mu E\ m^{-2}\ s^{-1}$ and different periods of exposure to air from 15 to 60 minutes and different salinities from 10 to 40 ppt did not change the normal peak period of shedding of spores. However, the effect of cold treatment at 10^0 C showed significant variation in the peak shedding of spores. The maximum spore shedding was observed between 1400 and 1800 hrs.

2.8 Spore settlement

In red algae, Okuda and Neushul (1981) pointed out that the sinking rate might change as mucilage released from the cell. Hoffmann and Camus (1989) observed that the sinking rate of spores measuring <15μm in diameter was significantly lower than that of spores >15μm ($P<0.01$) in benthic algae. They showed that spores were viable from 4 to 11 days, depending upon

the species. Spores remained suspended near the water surface for >12h but their concentration decreased to >50% of the initial value after 24 hours.

There may be developmental timing mechanisms in the cells for settlement activity in *Pterygophora* (Amsler, 1990). The spores released from 4 and 6 cm above the substrate show a normal disc development than 2 cm in *Gracilaria chilensis* (Herrera *et al.*, 1997).

Santelices and Dedo (1999) found that the artificial substrate coated with polylysine retained 4 –10 times more spores than uncoated controls, both in field exposed and in nursery experiments in *Mazzaella laminarioides, Lessonia nigrescens* and *Ulva rigidia.*

2.9 Spore growth.

Mshigeni and Lorri (1977) showed that the spores were segmented into 2 cells by formation median wall, then a second wall was formed, usually perpendicular to the first one and further walls were added, until a morula like mass of cells was produced in *Hypnea musiformis.*

Gallagher and Humm (1983) noted that the algal spores settle and grow well on dacron twine, meshnet, fibreglass screen and mollusk shells. Out planting of sporophytic stages at various times during the year indicated only sporophytes prepared from autumn and winter could survive summer conditions in *Laminaria saccharina* (Lee and Brinkhuis, 1988).

In *G. edulis,* Reeta (1992) reported that an erect frond developed from the Paranchymatous disc of the dividing, spores within 15-17 days of their output when the size of the circular Parenchymatous disc grew to 557µm in diameter. Tassende and Fraga (1997) recorded that the growth of young shoots were attained in swn-T medium containing 1 mg l^{-1} of bezylaminopurine under an irradiance of 20µ mol Photons $m^{-2}s^{-1}$ in the development of *Chondrus crispus* stack house.

The spores are microscopic in nature with a size of 0.019 mm during liberation, got attached to the substratum and started dividing to form an uniform Parenchymatous disc or the hold fast in *G. edulis* (Reeta and Ramamoorthy, 1997). It was found that pre – rinsing the blades with tap water

produced marked sporulation without affecting germination and survival in *Lessonia* (Fonck *et al.*, 1998). In *Kappaphycus alvarezii,* Azane and Aliaza (1999) recorded that the high levels of nutrients (F/2, F/20) enhanced growth of contaminants and reduced carpospore viability. He also noted that the germ ling growth was highest in the more enriched medium (F/2).

2.10 Life history

A triphasic cycle is one in which a haploid gametophtye produces a diploid carposporophyte which in turn gives rise to diploid free living tetraporophyte (Drew, 1955; Kylin, 1956). Members of the *Gracilariaceae* are generally thought to have the standard *Polysiphonia* type life history (Lobban and Wynne, 1981).

Diploid carpospores and haploid tetraspores are to be produced during the process of life cycle (Santelices and Doty, 1989). The triphasic life with isomorphic gametophytic and tetrasporophytic generations in which meiosis takes place in the tetrasporangium (Yamanouchi, 1906). In *Gracilariceae*, the period to complete the life history *in Vitro* varies not only with the species, but also with the culture conditions (Oliveira and Estela, 1994).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Sample collection

3.1.1. Collection area

Species of *Gracilaria* were collected from the Southeast coast of Tamilnadu near Mandapam. *G. foliifera* and *G. edulis* were collected from Rameswaram and Thonithurai respectively ($70^0$11'E and $9^0$17'E). The *G. edulis* was also collected from the landing centers at Vedalai village, which were collected from Hare Island by the fishermen. Samples collected from the respective collection centers during low tide in the morning and transported to the laboratory of Mandapam Regional Center of CMFRI. Immediately after the collection, the plants were cleaned well in running seawater for 3-4 times to remove the epiphytes and kept under running seawater in fiberglass tank in the greenhouse.

3.1.2. Transportation

Fresh seaweeds were sorted out and packed in the perforated polythene bags. The bags were kept in a bucket filled with a required level of enriched seawater (Walnes, 1974) and transported to Kochi headquarters of CMFRI.

3.1.3 Treatment of plant

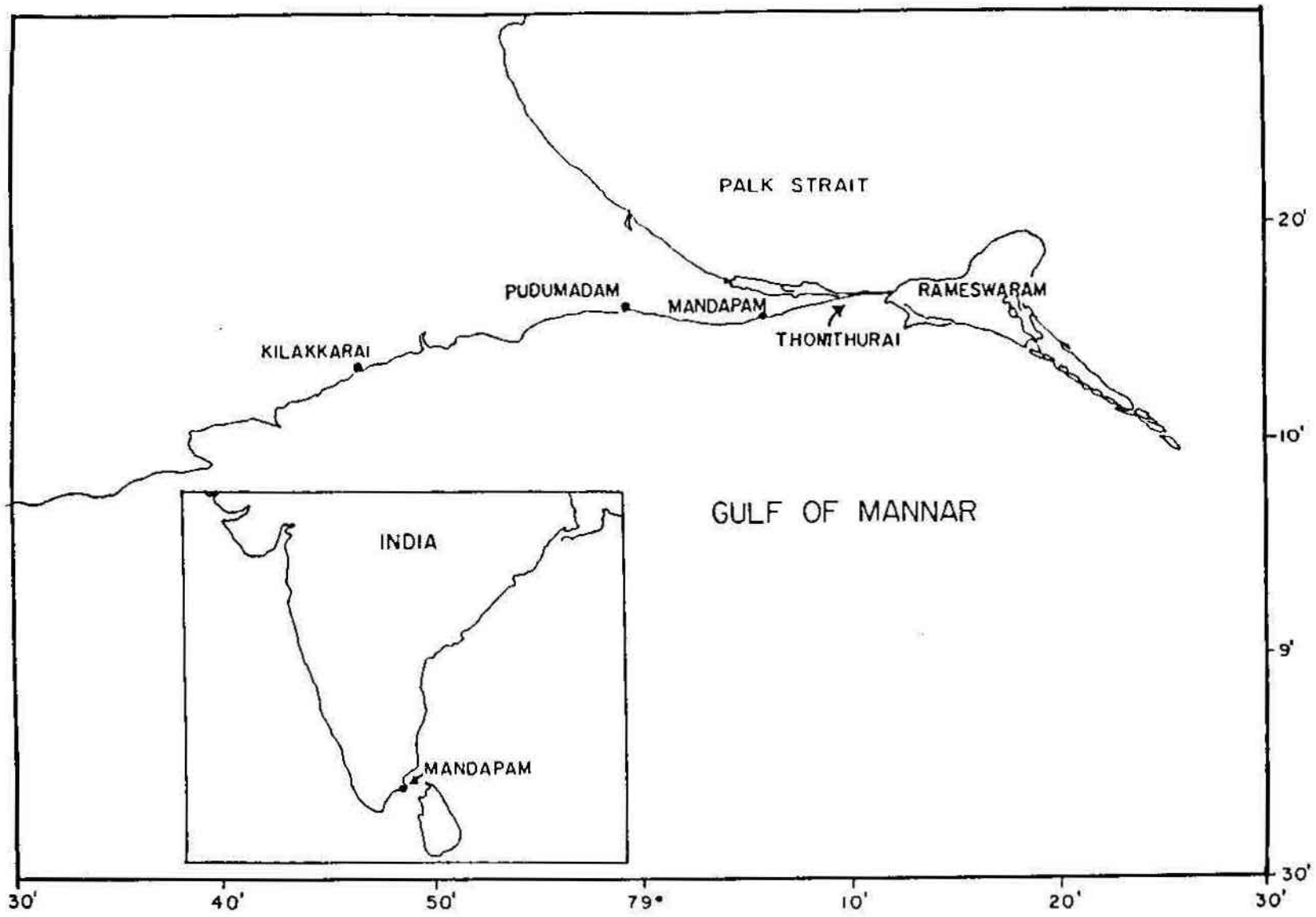
The plants were kept for few hours under vigorous aeration to overcome the transportation stress. The plants were washed thoroughly in sterilized seawater in order to remove the debris, and brushed to clean the visible epiphytes. Surface sterilization of the plants was carried out by sodium hypochlorite solution (1 ppm) and antibiotics mixture of ampicilin, chloramphenical, gentamycin and streptomycin (0.2 mg/ml)).

3.2 Influence of environmental factors on sporogenesis

3.2.1 Photoperiod

This experiment was set up to know the development of sporogenesis in the vegetative plants by exposing them to the short day (SD)

Fig.1 Location of sample collection area



photoperiod in the growth chamber (8L:16D). Three, 2 L capacity semi-transparent plastic box were filled with Provasoli Enriched Seawater (PES) medium (35ppt). The vegetative plants of *G. edulis* and *G. foliifera* were thoroughly washed with sterilized seawater and kept in the box under different light intensities. The temperature of the growth chamber was maintained at 25^0C.

Another long day (LD) experiment was also done by exposing the vegetative plant to the long day photoperiod in the growth chamber (16L:8D). All other conditions were maintained as above.

3.2.2 Light spectra

The vegetative plants of *G. edulis* and *G. foliifera* were thoroughly washed with sterilized seawater and kept in the semitransparent box filled with PES medium. Plastic boxes covered with different colour paper. They were subjected to different light spectra such as green (450 – 610 nm) red (630 – 670nm) and plants exposed to white light were treated as control.

The whole set up was kept inside the growth chamber for a period of 20 days with an automatic control of 8 h light and 16 h dark provided at 25^0C. Once in a 5 day, the medium exchanged and at the end of the experiment the section cutting and pigment estimation were performed. The estimation of pigments such as chlorophyll a, Phycoerythrin (PE) Phycocyanin (PC) and Allophycocyanin (APC) was carried out by the procedures of Jefferry and Humphery (1975).

3.3. Developmental stages of cystocarp

Histological observations were also made from the sections of cystocarp of wild collected plants of *G. edulis* and *G. foliifera.* The very fine sections were selected and stained with cotton blue or eosin for 1-2 minutes. The fine sections were selected and mounted on mixture of glycerine and seawater (1:1). Then it was observed under the light microscope and scanned using a digital camera and a computer connected with the microscope (Zeiss, 400x and 100x magnification).

Plate 1. Experimental setup

Plate 1.1 Spore shedding in outdoor environment

Plate 1.2 Spore shedding in controlled condition

3.4. Spore shedding

Two 50 L capacity plastic troughs were filled with Grund medium enriched filtered and sterilized seawater. Cleaned slides were kept at the bottom of troughs as substrate for adhesion of spores to the slides. The pre-treated cystocarpic plants of *G. edulis* and G. *foliifera* were kept on a net cloth, suspended on the surface water, keeping a gap of 15 cm from the substrate. Aeration was provided for the uniform distribution of spores. After 24 hours of incubation the slides were taken and observed under microscope for the spore output and attachment.

3.4.1. Spore output in growth chamber

3.4.1.1 Effect of short day on spore output (8 L:16 D h)

The two 1000ml beakers were taken and filled with sterilized seawater and two slides were placed at the bottom of beaker. Cystocarpic plants were suspended on a nylon net just immersed on the water. They were maintained in a growth chamber at 20^{0}C with a photoperiod of 8L:16D. A control under room temperature was maintained and the spore shedding was observed once in 24 h by keeping new slides in the beaker after each observation and continued for 5 days. After 5 days, the set up was exchanged with new cystocarpic plants of all the above five species and kept at 25^{0}C. The observation was taken continuously for five days with an interval of 24h for spore shedding.

3.4.1.2 *Effect of Long day on spore output* (16 L:8 D h)

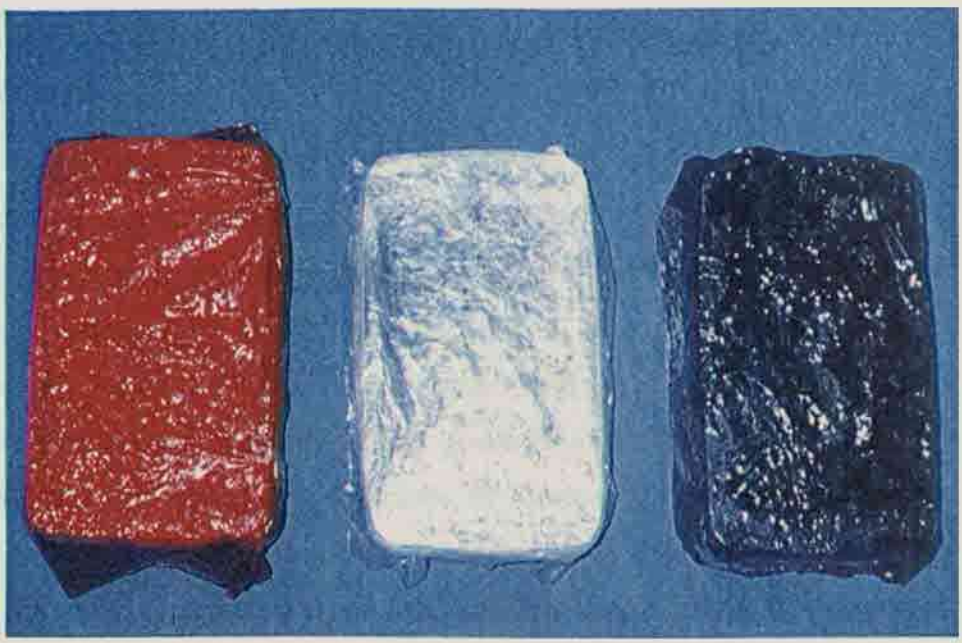
The long day experiment was conducted as like short day but a 16 h light and 8 hrs dark was given to attain the long day. The observation was taken once in 24 h for 5 days at 20^{0}C. After 5 days the fronds were exchanged with new fronds and kept at 25^{0}C for spore shedding. The observation was continuously recorded once in a 24 hrs for a period of 5 days.

3.5. Growth of spores

This experiment was conducted to analyze the efficacy of the different growth medium. Different culture media were prepared such as

Plate 1.3 Sporogenesis in controlled condition

Plate 1.4 Spore growth in enriched media



Provosoli (1968), Walne (1974), and Grund (von Stosch, 1963) with sterilized seawater (30ppt).

Different enriched medium was poured into culture tube (100ml capacity) approximately a half level to the tube depth. One slide with good spore attachment was immersed in each tube. The open end of the culture tube was covered with aluminium foil paper and kept under controlled temperature (24 – 26^{0}C). The enriched medium was exchanged once in a 5-day interval. The slides were observed daily under Olympus compound microscope for the growth of the spore. The number of the spores was recorded under a particular field either in100x or 400x magnification. The size of the spores was measured by using ocular micrometer and the diameter was expressed in millimeter.

3.6. Image documentation

All the anatomical sections of the developing cystocarp were documented using a Olympus zoom camera with Kodak film. The stills of spore shedding and growth of spores were taken using a compound microscope (Zeiss) provided with a digital camera and attached with a computer for imaging. The image was clearly adjusted and further edited with the aid of computer to get a clear picture.

3.7 Statistical analysis

The data on the spore shedding were analysed statistically using ANOVA test (t – test) and the growth studies data were subjected to regression analysis. The significance was checked with table values.

RESULTS

4. RESULTS

4.1 Influence of environmental factors on sporogenesis

4.1.1 Photoperiod

In this particular experiment *G. edulis* and *G. foliifera* were subjected to different light intensities under 8L:16D (short day) and 16L:8D dark (long day) photoperiod. The plants did not show any change in the development of carposporangia on the thallus when exposed to short day. On the other hand plants exposed to long day condition showed initiation of cystocarps in *G.foliifera* under low light intensity of 2000 lux (Plate 2.1).

4.1.2 Light spectra

Both the species of *Gracilaria* were also subjected to different light spectra showed prominent cystocarp in *G. edulis* when exposed to red light. (Plate 2.2) *G. foliifera* did not show any encouraging results. The two species of *Gracilaria*, when exposed to different light spectra (green 450 - 610nm and red 630 - 670 nm) showed remarkable variation in their pigment levels. In *G. edulis*, the chlorophyll a content was higher under red spectrum than that of green spectrum. Similarly the PC and APC content also showed an increasing trend, which were almost 20 and 15 times greater than that of green spectrum respectively. The PE content under green spectrum was higher than that of red spectrum (Fig. 1).

In *G. foliifera*, the plant showed very high difference of PE content under green from that under red light (about 200 µg/g plant). PC was also very high under red light (about 9 times greater than that of green). In contrast, the difference in pigment contents, especially those of PE, PC and APC showed high difference from one another (Fig.2).

Fig. 2. Pigment composition of *G. edulis* under different wavelengths of light

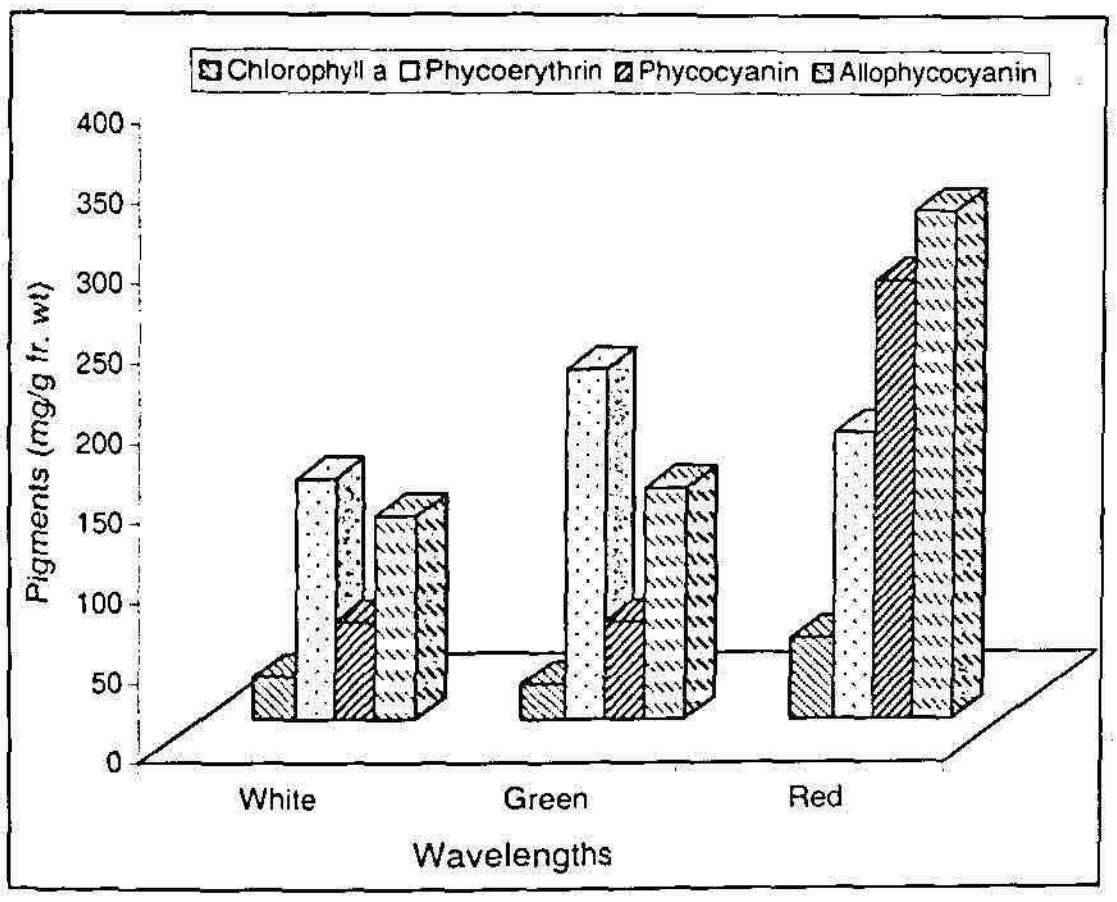


Fig. 3. Pigment composition of *G. foliifera* under different wavelengths of light

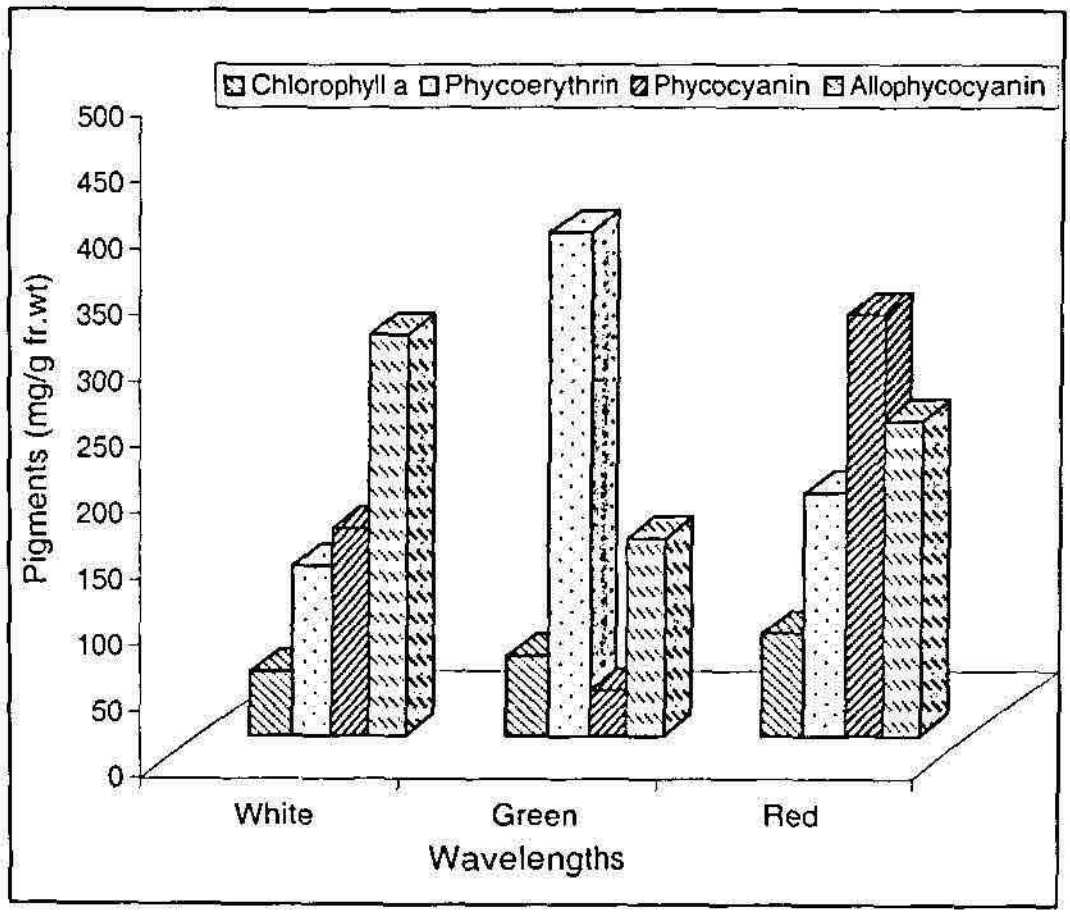
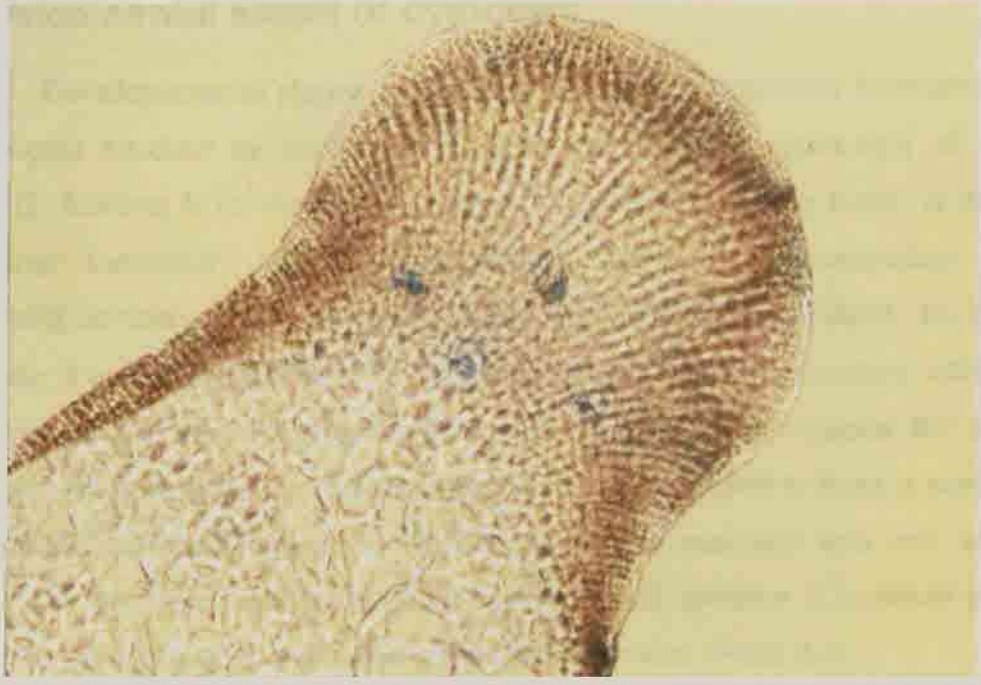
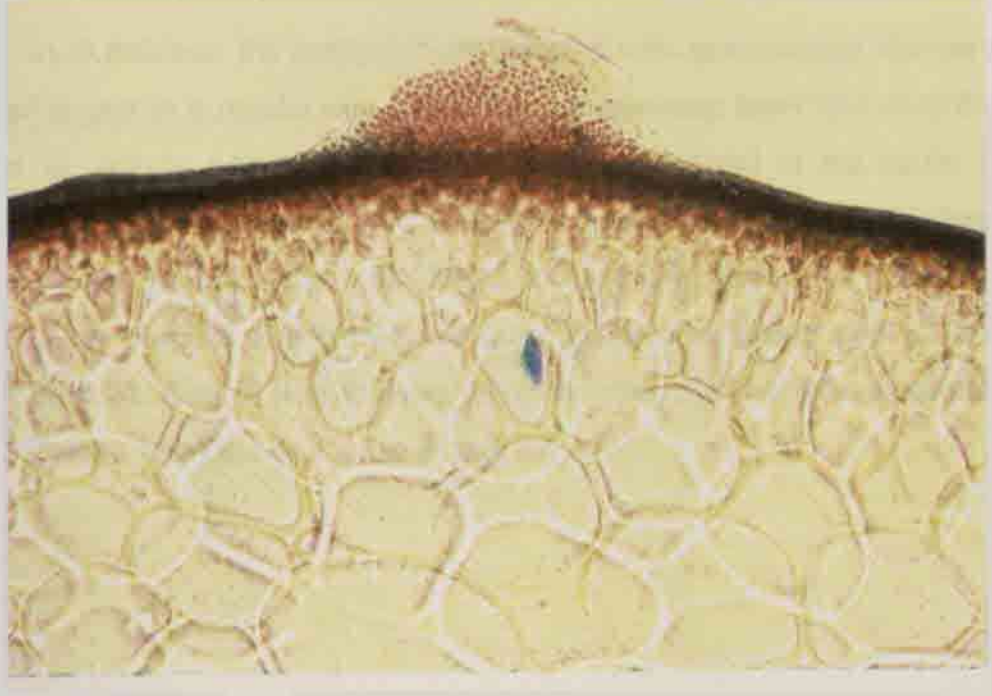


Plate 2. Cross section of initiation of sporogenesis

Plate 2.1 Early growth of development of cystocarp in *G. edulis*

Plate 2.2 Early growth of development of cystocarp in *G. foliifera*





4.2 Developmental stages of cystocarp

Developmental stages of cystocarps and carposopore formations were thoroughly studied by making histological sections of cystocarps of *G. edulis* and *G. foliifera* from the apical portion of the thallus to the base. It was observed that formation of carposoporangium starts by accumulation of chromatophore in the apical cell, which form the carpogonium initial. In the present study, it was not possible to find out the stage prior to fertilization, where the trichogyne of the female reproductive organ acts as the receptor for the spermatangia. After fertilization, the carpogonium initial divided to form a series of gonimoblast filament (Plate 2.1). At this stage the pericarp was not well developed. The pericarp was formed by the repeated division of cortical cell layer, which formed the covering to the carpogonium initial (Plate 2.2).

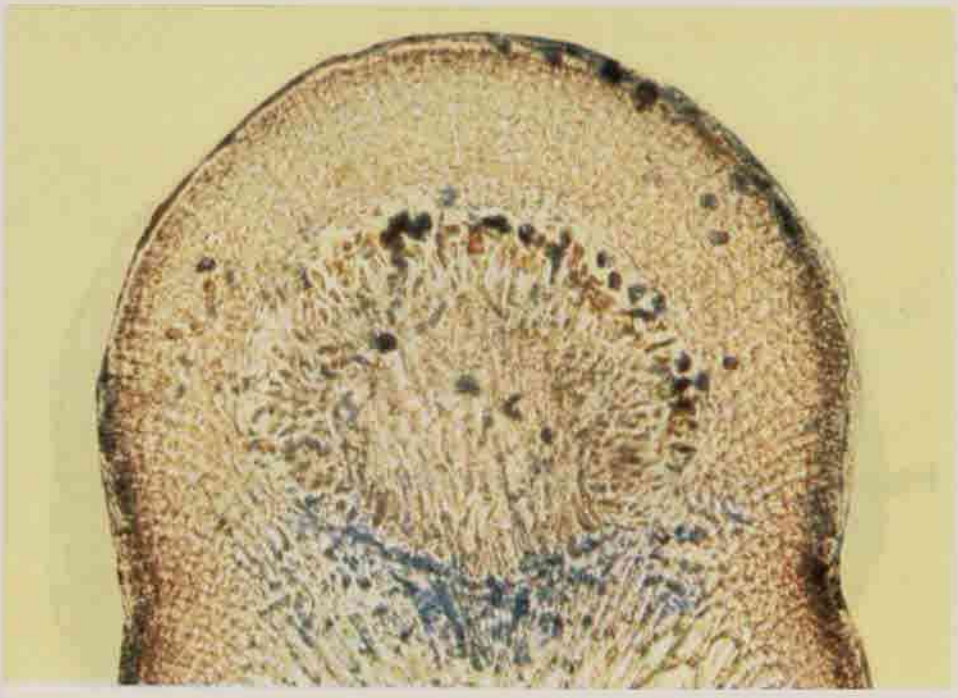
In *G. edulis,* the pericarp was very uniform throughout the cystocarp and it formed a narrow constriction at the base (Plate 3.1). The gonimoblast filament was intermingled with nutritive tissue, but no definite gonimonema was observed which connects the sterile cells with the pericarp. The apical gonimoblast filament formed the carpospore initial and a series of carpospores developed by repeated divisions (Plate 3.2). The ostiole opening was formed when the carpospores were matured and ready to get released from the cystocarp (Plate 3.3). The ostiole was more prominent, which explains the spent stage of cystocarp of *G. edulis* (Plate 3.4).

In *G.foliifera*, the carpogonium initiated with gonimoblast filament, which were arranged in a regular way (Plate 4.1). Its pericarp layer was very thin compared to *G. edulis* and a sharp constriction was found at the base of cystocarp (Plate 4.2). No prominent gonimonema was observed in *G. foliifera.* The nutritive filment of *G. edulis* was found to be more elongated unlike *G. foliifera*, where the cortical cells were protruding inside the sterile layer of the cystocarp (Plate 4.3). Ostiole was formed when the carpospores were matured and ready to liberate from the cystocarp (Plate 4.4).

Plate 3. Developmental stages of carposporogenesis in *G. edulis*

Plate 3.1 The gonimoblast filament with definite pericarp

Plate 3.2 Carpospores and nutritive filament in the cystocarp



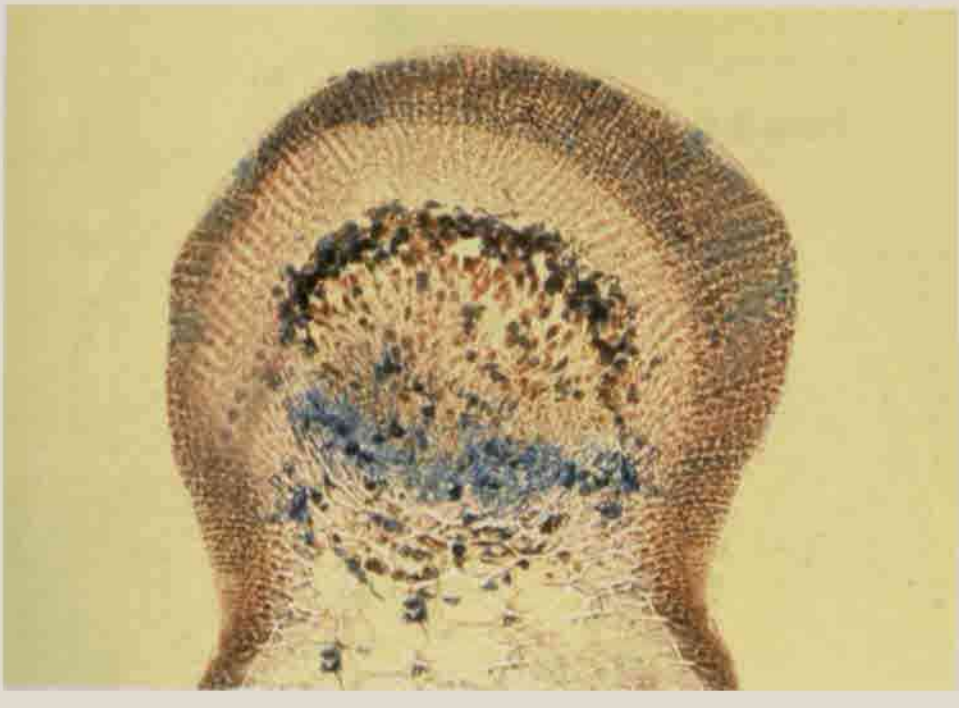


Plate 3.3 Mature cystocarp with prominent ostiole

Plate 3.4 Spent stage of cystocarp

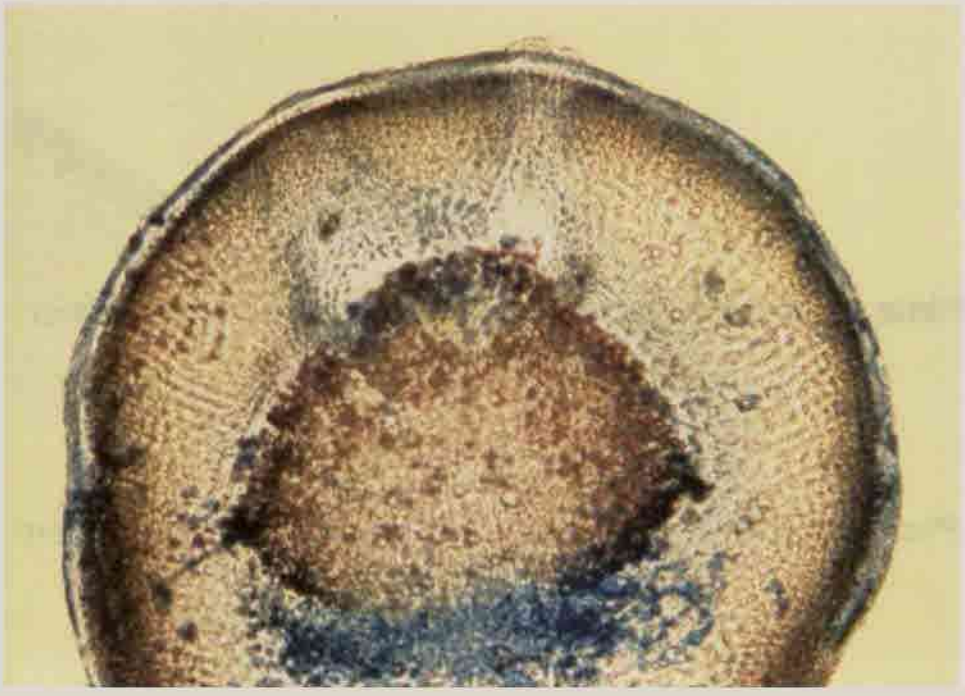
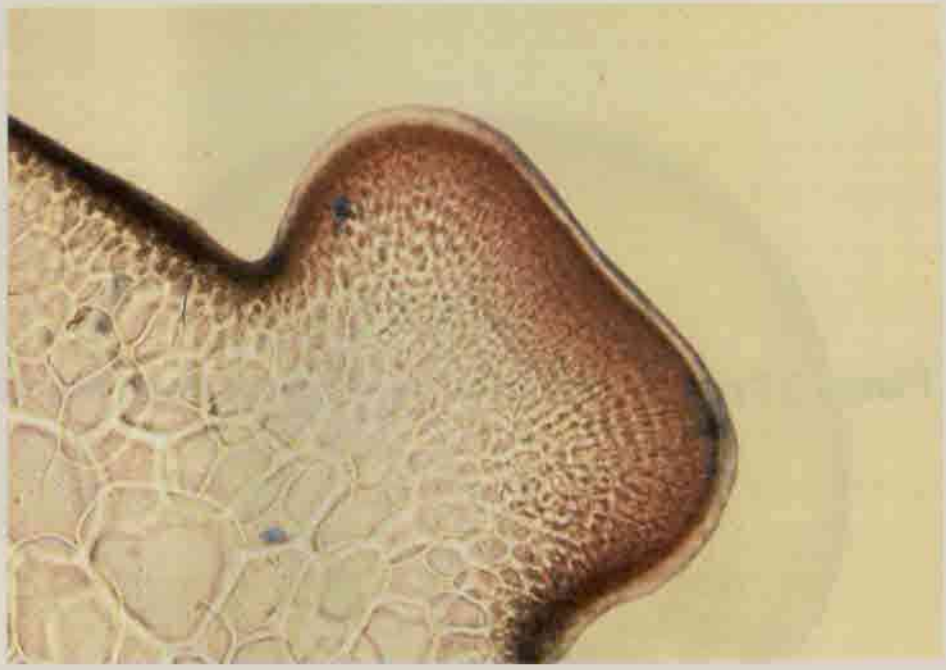


Plate 4. Developmental stages of carposporogenesis in *G. foliifera*

Plate 4.1 The gonimoblast filament with definite pericarp

Plate 4.2 Cystocarp with sharp constriction in the basal end



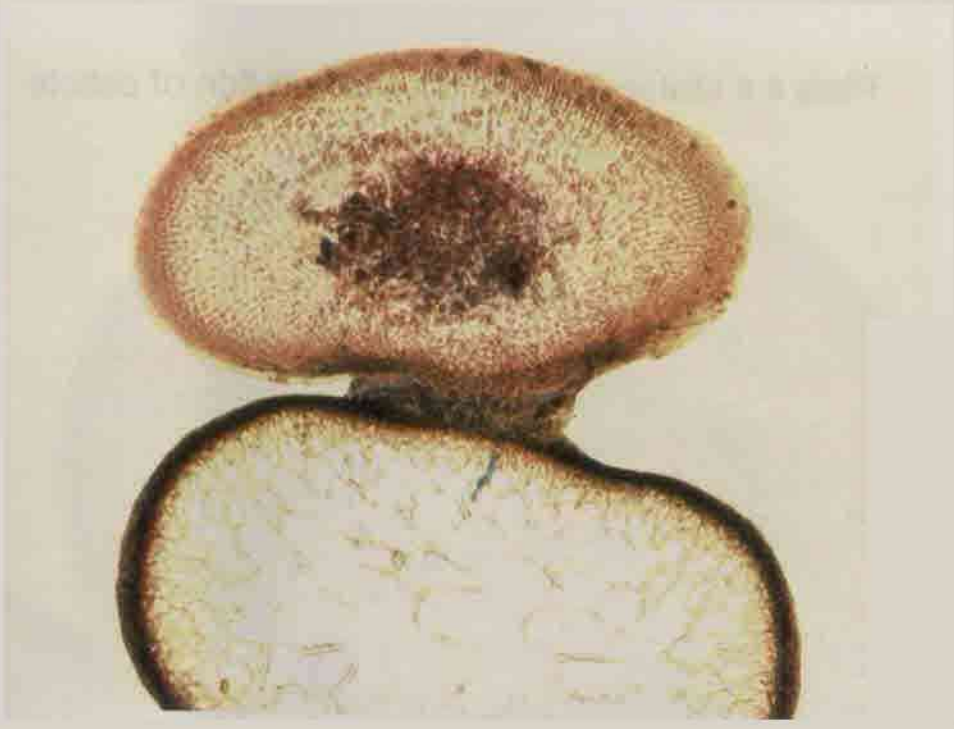


Plate 4.3 Cystocarp formation by the gonimoblast filament

Plate 4.4 Matured cystocarp with initiation of ostiole

4.3. Spore shedding

Gracilaria edulis when placed under different photoperiods and temperature showed variations in spore shedding. The spore shedding was higher than the control under short day (8L:16D) photoperiod with 25^0 C. *G. edulis* showed an increase in spore shedding during the first three days, which then declined drastically. In all the other three treatments (SD 20^0 C, LD 20^0 C and LD 25^0 C), G. *edulis* showed less spore shedding than that of control. However, a slight increase in the spore shedding could be witnessed in SD 20^0 C and LD 25^0 C at the fag end of the experiment period (Fig.3).

In *G. foliifera*, treated plant exhibited higher spore shedding than that of control. However, the highest spore shedding was observed in the SD 25^0 C except on the last day of the experiment followed by LD 25^0 C with more or less same trend as like as SD 25^0 C (Fig. 4). This difference was statistically significant ($P>0.01$). Considering the overall trend, SD 25^0 C and LD 25^0 C showed an increase during the first two days with a decrease for the remaining days. The percentage deviation on spore shedding from the control has been given for both the species of *Gracilaria* in Table 1.

4.4. Early growth and survival of spores

The spores of *G. edulis* and *G.foliifera* were grown in three different nutrient media (PES, Walne's and Grund) immediately after spore output. In *G.edulis* the survival rate of the spores during early stage was more in Walne's medium followed by PES and Grund medium. On the other hand, the growth was more encouraging in PES medium (Fig. 5). The relative growth rate of *G. edulis* did not show much variation in the growth in PES and Walne's medium but PES medium favoured the growth up to 8 days (Fig.6)(Plate 5.1 &5.2).

The number of spores and survival rate of *G. foliifera*, were more in PES medium followed by Walne's and Grund medium. There was a sudden decline in the number of spores within third day of spore output in PES and Grund medium but the decline was gradual in Walne's medium (Fig.7). The relative growth rate showed high value in PES medium throughout the culture

Table1. The percentage deviation of spore shedding from the control

Gracilaria edulis					
No. of days	Short day		Long day		Control (no. of spores)
	25^0 C (%)	20^0 C (%)	25^0 C (%)	20^0 C (%)	
1	28.84	0	0	-41.57	2.67
2	42.21	-34.6	-11.42	-50.17	2.89
3	53.93	-16.85	-37.45	-41.57	2.67
4	27.87	45.9	18.03	-27.05	1.22
5	49.25	98.51	82.09	-16.42	0.67
Gracilaria foliifera					
No. of days	Short day		Long day		Control (no. of spores)
	25^0 C (%)	20^0 C (%)	25^0 C (%)	20^0 C (%)	
1	17.46	-11.64	11.64	-23.81	1.89
2	69.44	38.89	54.17	23.61	1.44
3	54.17	-22.92	38.87	0	1.44
4	165.67	65.67	132.84	65.67	0.67
5	32.84	65.67	82.09	49.25	0.67

Fig. 4. Spore shedding in relation to different photoperiods and temperatures in *G. edulis*

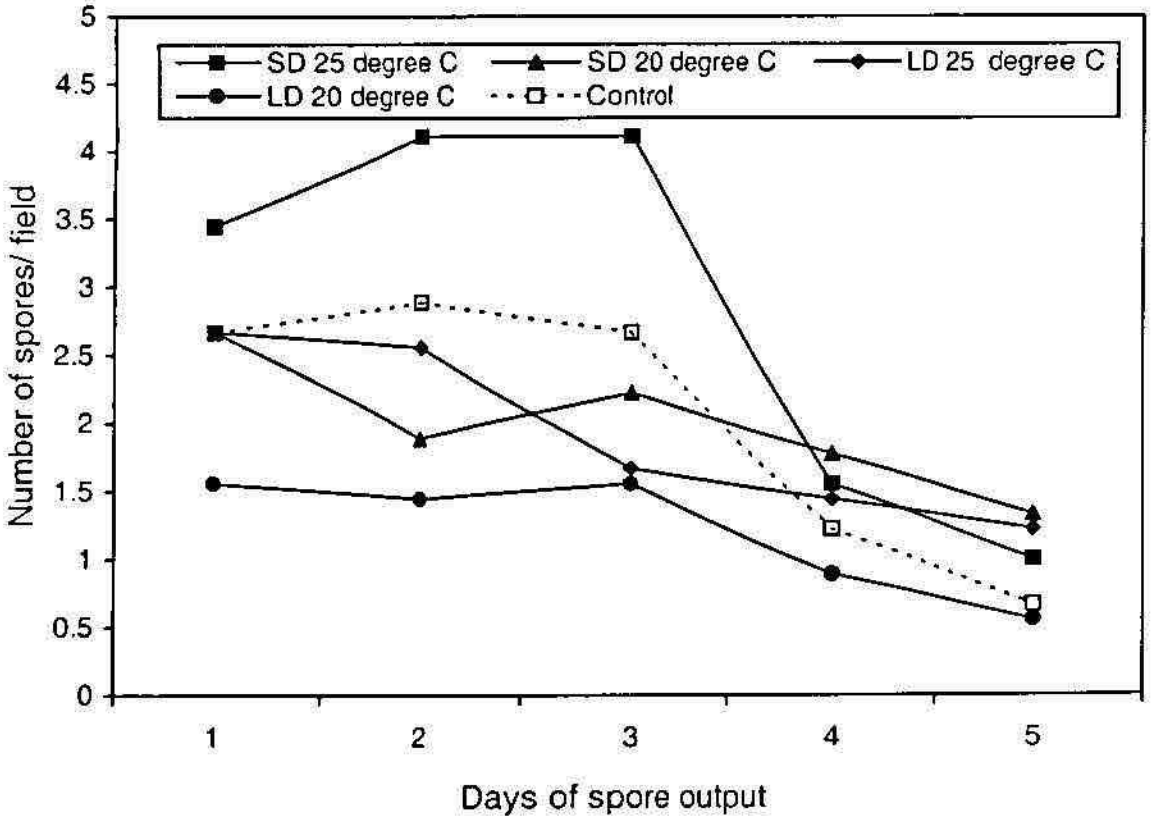


Fig. 5. Spore shedding in relation to different photoperiods and Temperatures in *G. foliifera*

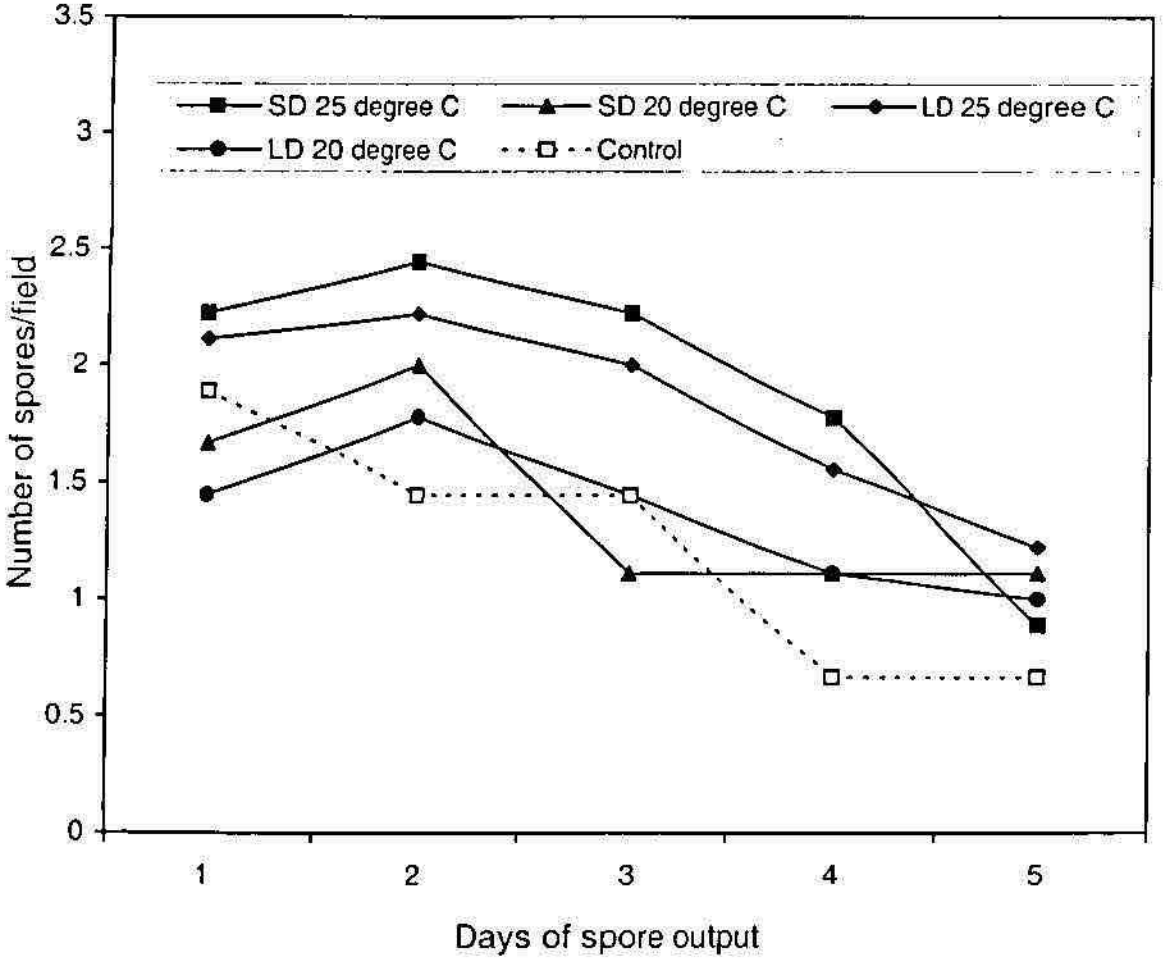


Table 2. Survival rate of spores in different growth media

Gracilaria edulis			
No. of days	Average survival (%) of spores in different growth media		
	PES	Walne's	Grund
1	98.7	84.05	79.31
2	50.4	68.23	39.59
3	48.96	48.28	27.92
4	20.16	27.59	20.3
5	12.96	11.03	12.69
6	9	9.2	12.69
7	3.96	0	0
Gracilaria foliifera			
No. of days	Average survival (%) of spores in different growth media		
	PES	Walne's	Grund
1	86.04	69.78	98.64
2	66.06	42.83	96.14
3	45.83	29.08	37.29
4	34.85	24.73	15.68
5	16.52	9.89	9.04
6	15.69	4.35	4.19
7	9.08	0	0

Fig. 6. Early growth and survival rate of *G. edulis*

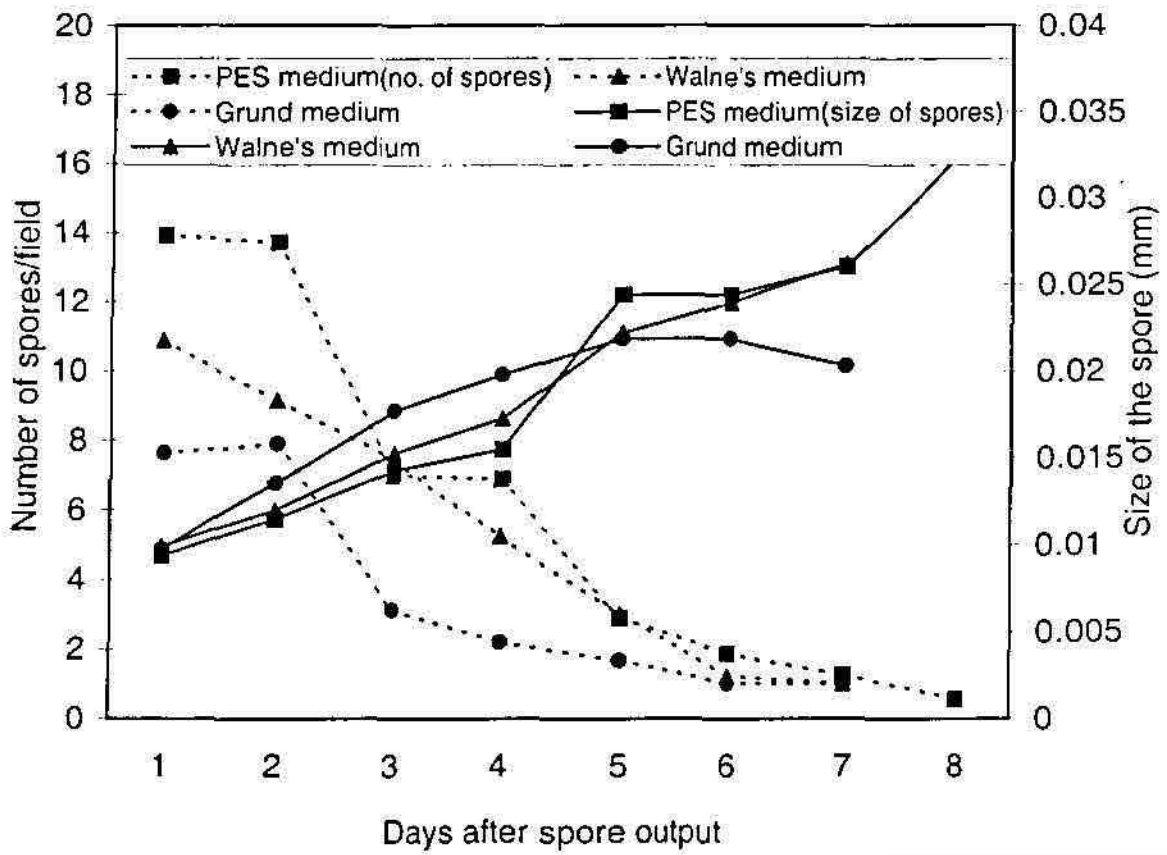


Fig. 7. Early growth and survival rate of *G. foliifera*

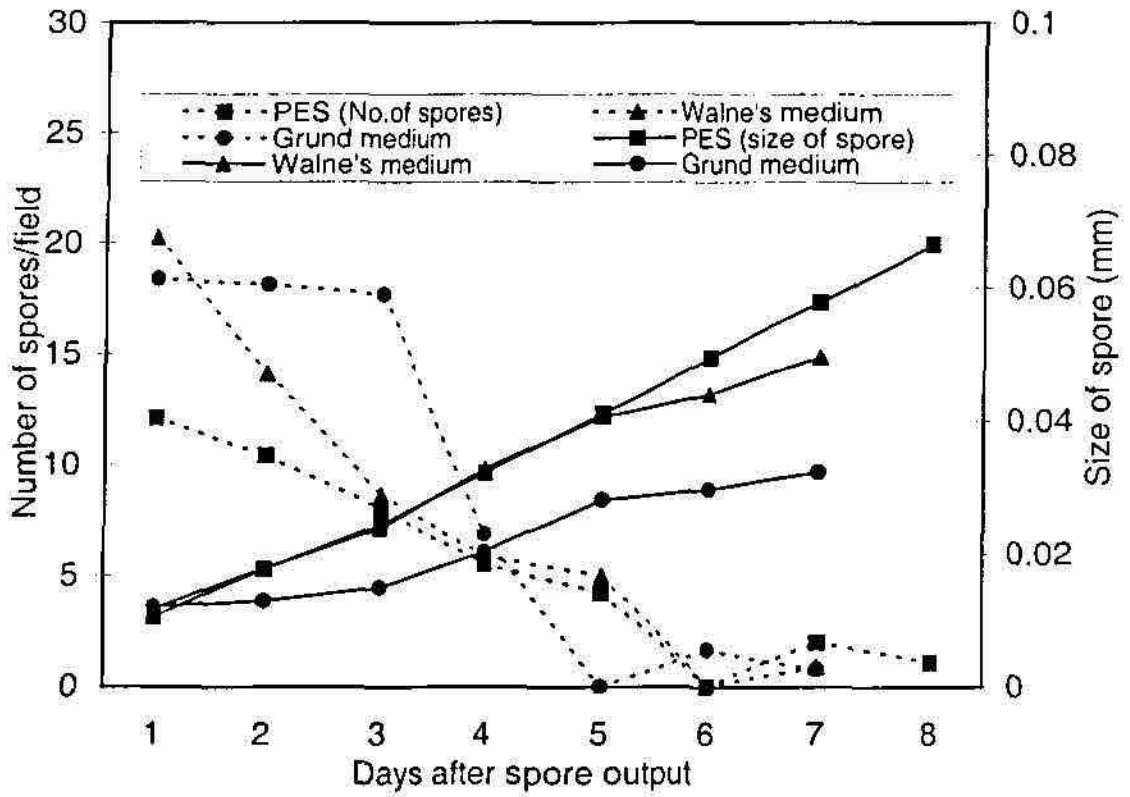


Fig. 8. Relative growth rate of *G. edulis* in different culture media

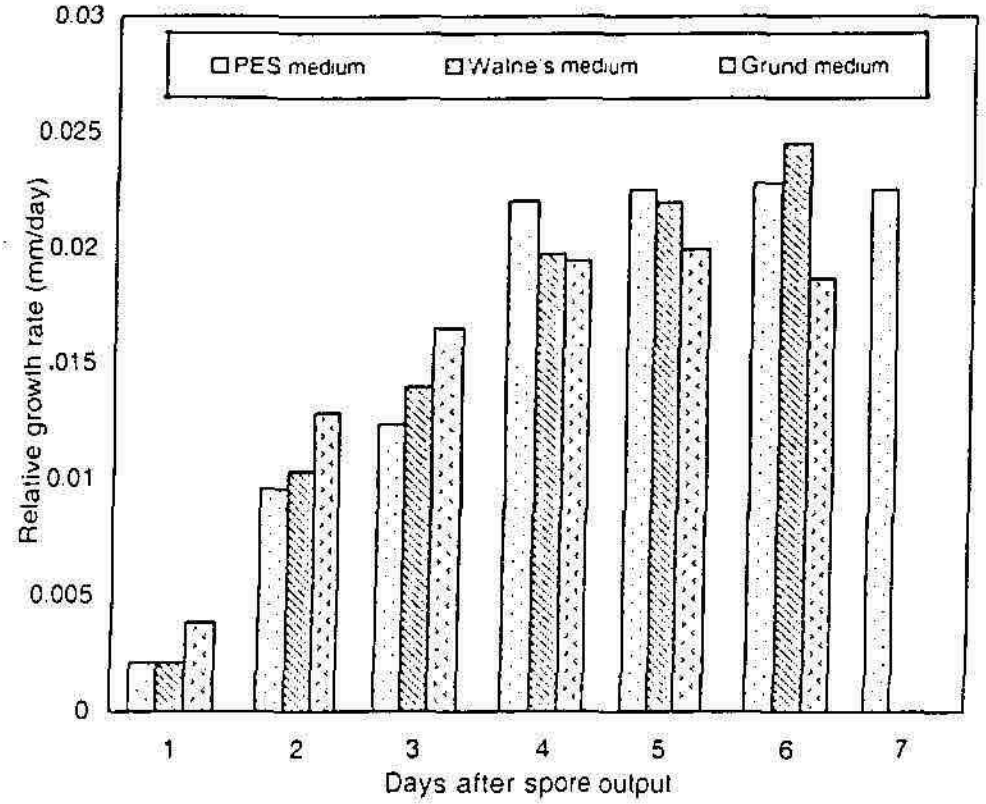


Fig. 9 . Relative growth rate of *G. foliifera* in different culture media

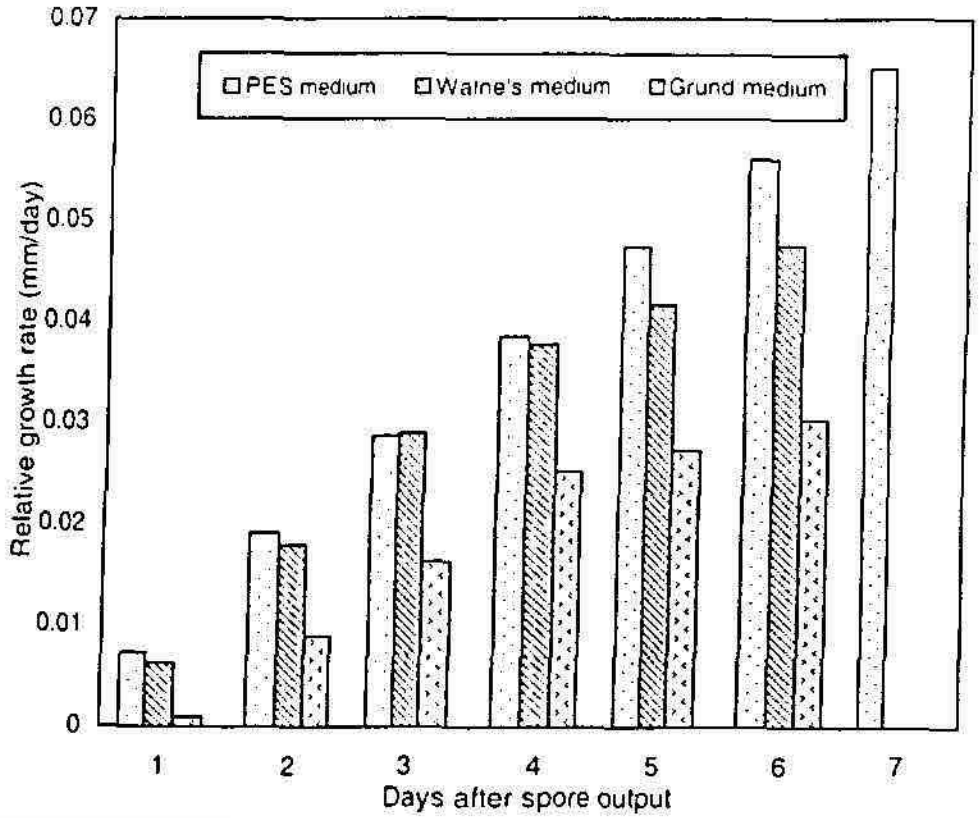
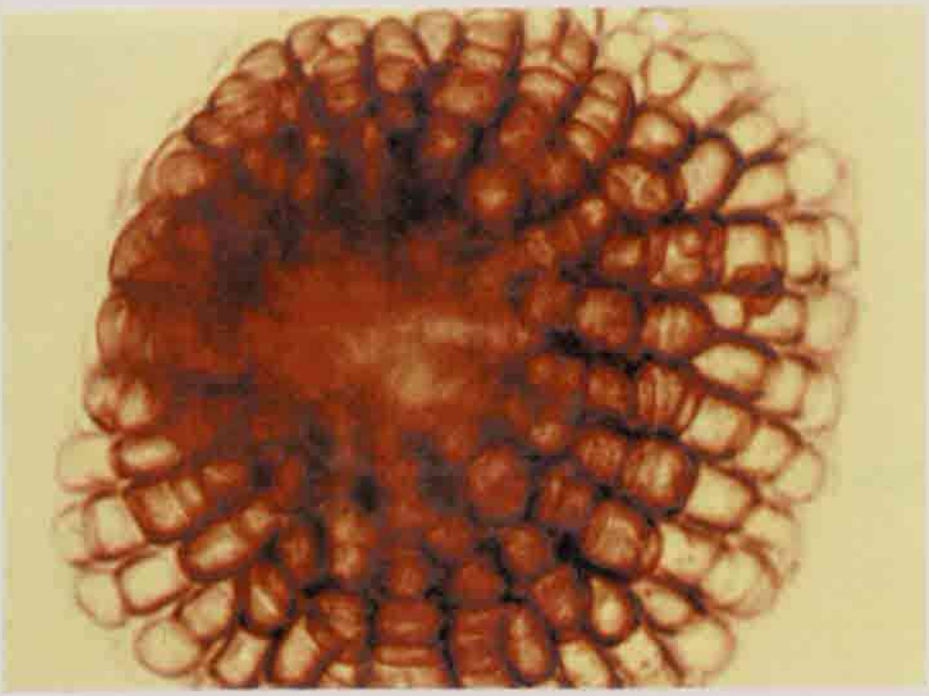


Plate 5. Early growth of *G. edulis*

Plate 5.1 Fifth day after spore output (400x)

Plate 5.2 Seventh day after spore output (100x)



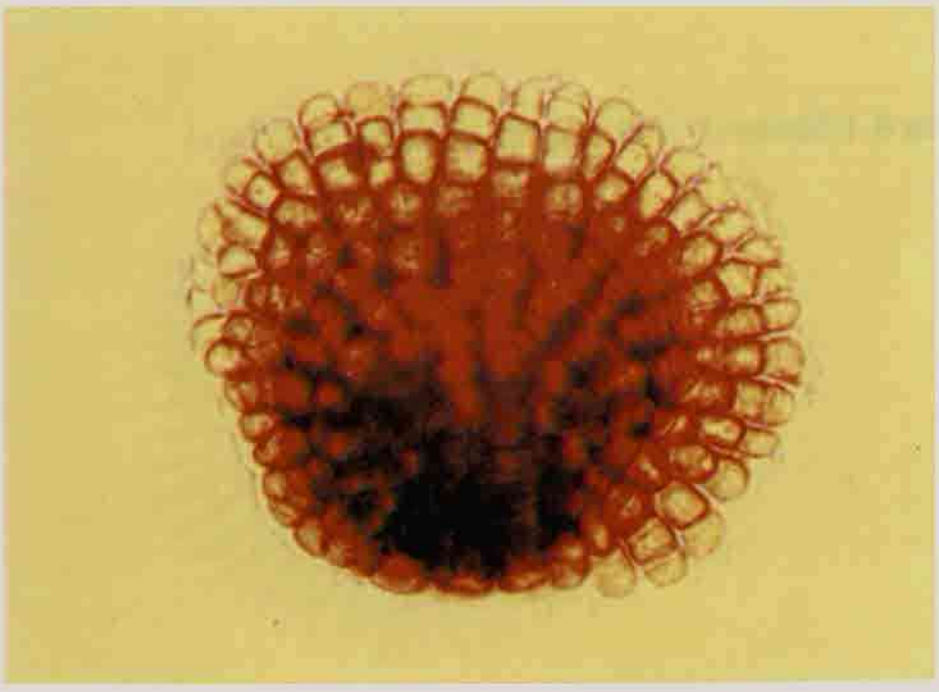
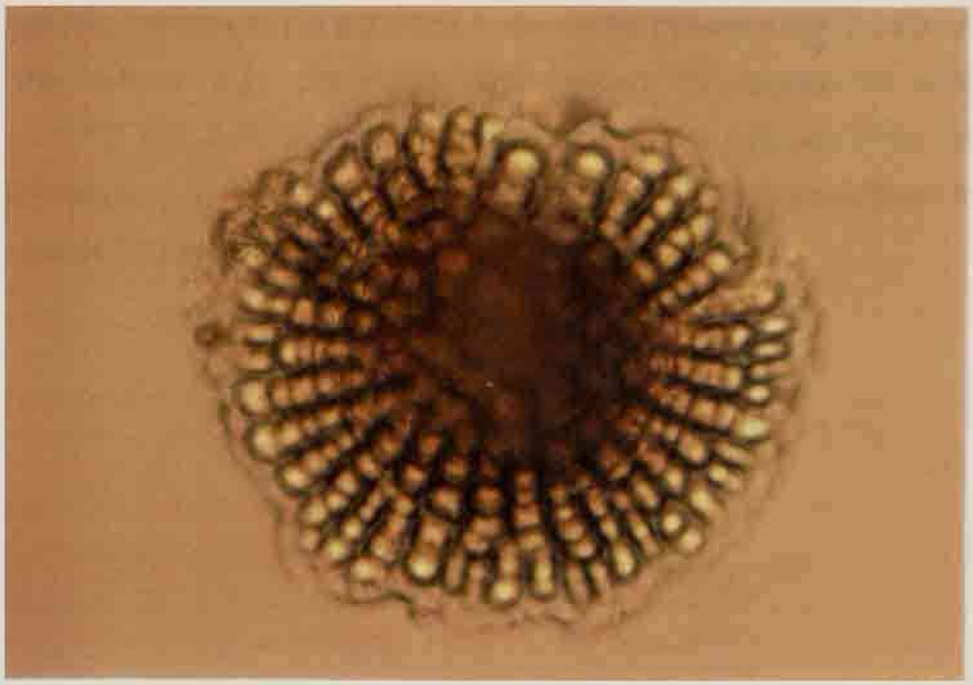
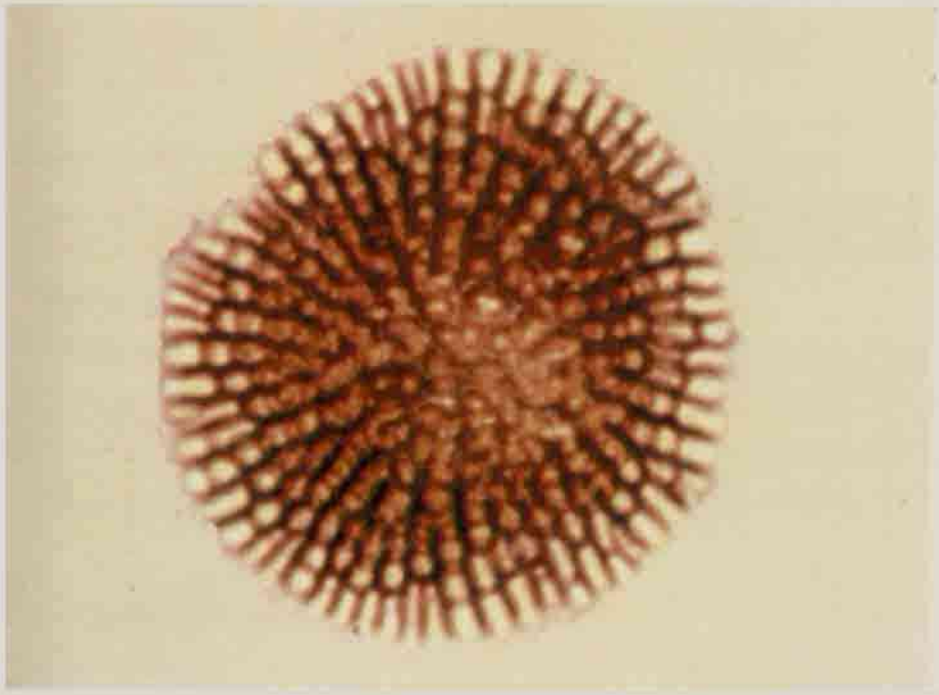


Plate 6. Early growth of *G. foliifera*

Plate 6.1 Fifth day after spore output (400x)

Plate 6.1 Seventh day after spore output (100x)





period (Fig.8)(Plate 6.1 & 6.2). The relationship between the growth and duration of the culture was statistically established by regression analysis. The relationship was very highly significant for both the species when grown in the PES medium (r=0.99). The percentage of and survival rate is given for both the species of *Gracilaria* in Table 2.

DISCUSSION

5. DISCUSSION

Seaweed farming has great potential to develop into an industry considering the importance of certain specific products derived form the seaweeds. Seaweed culture has many advantages over other types of mariculture practises. Apart from less labour intensive and eco-friendly nature, seaweed culture is known to yield products, which are very essential for food and pharmaceutical industries world wide as on date. Oliveira and Estela (1994) has stressed the economic importance of seaweeds and recommended for the farming of macro algae. During 1980's, the demand for seaweeds particularly *Gracilaria* spp was widely met by the harvesting from the wild, which has caused a decline in natural production (Rajadurai, 1990; Guanzon and Castro, 1992). In order to maintain and stabilize the supply of *Gracilaria* for many industrial purposes mass cultivation is required.

Doty (1973) opined that tropical areas are more suitable for seaweed farming as productivity is higher in warmer than in colder latitudes. In this context, seaweed farming has considerable relevance with reference to India, a tropical nation blessed with several species of this macro algae.

Gracilaria spp are known to multiply through vegetative propagation as well as by spores. Vegetative propagation has been widely practised for *Gracilaria* spp. in pond culture due to their very high regenerative capacity for the plant and simplicity of the method (Trono, 1981). However this has declined due to obvious reasons. The planting rate of *Gracilaria* has been 4 ton/ha under polyculture and 5 ton/ha for monoculture (Shang, 1976). Under these circumstances, the availability of the huge quantity of plants for the vegetative propagation to undertake mariculture of seaweeds is very limited. The constant growth of epiphytes and continuous grazing by other aquatic organisms were also found to reduce the intensity of vegetative propagation. In this context reproduction through spores under hatchery conditions is promising for the continuous supply of stocking seedlings for the pond culture of *Gracilaria* spp (Mathieson and Norall, 1975).

Environmental manipulation for better spore production has been found possible in *Gracilaria* spp. by many authors worldwide (Doty, 1973; Ramarao and Thomas, 1974; Ngan and Price, 1983; Ruiz *et al.*, 1989; Vriosteegui *et al.*, 1999; Ganesan *et al.*, 1999). In the present study also, environmental factors such as photoperiod, light intensity, temperature and different light spectra were found to influence the sporogenesis in *G. edulis* and *G. foliifera*.

There was no initiation of sporogenesis as well. Exposures of *Gracilaria* spp to high and medium light intensities for longer day duration was found to cause discolouration, while at low intensity the plants were not affected. The above finding can be well compared with earlier studies as the influence of photoperiod on sporogenesis or plant health. The plants grown in shade has shown higher photosynthetic performance and pigment accumulation at low light intensities (Boardman, 1977). The findings in the present study correlate with the findings of Luning (1990) also, who stated that for photon absorption, the plants tend to have higher pigments at lower light intensities, which would be reversed at higher intensities.

Luning (1990) also stated that the plant would show developmental and morphological changes when exposed to different light intensities. This has been found true under low intensity of light exposed for longer duration (LD). The contention of the Charnofsky *et al.* (1982) with regard to the above was also found true in the present study, as *G. foliifera* has shown initiation of sporogenesis at low light intensity.

Ramus *et al.* (1976) have reported that both light intensity and spectral distribution affected the red algae. Although the red algae have been reported to utilize low light intensities and different light spectra, the level of utilization and influence on the plants were found to vary from species to species, within the species at various intensities, in their growth habits and even in same thallus (Brinkhuis and Jones, 1974; Mathieson and Norall, 1975; Dawes *et al.*, 1976, 1978; Durako and Dawes, 1980; Gleen and Doty, 1981).

Various authors while working on the influence of light spectral distribution on the red algae were of the opinion that the red algae were well

adapted to a large variety of light spectra. The absorption spectra of the thalli have been reported to be a response of light quantity rather than to spectral distribution (Dring, 1981; Beer and Levy, 1983). Therefore, the observation in the present study may be specific to *G.edulis* under the present laboratory conditions tried.

Liu *et al.* (1981) already opined that in *Gracilaria Spp* chlorophyll b, c and d were either absent or in low quantity. *Gracilaria Spp* were reported to have absorption spectra in the range of 400-650nm, due to the presence of chlorophyll a. In the present study, both the species studied showed the dominance of the chlorophyll a under red light spectrum than the green light spectrum. This finding coincides with the statement of Luning (1990) and Reeta and Kulandaivelu (2000). The inactivity of chlorophyll a in red algae under unfavourable light spectra was earlier reported by Haxo and Blinks (1950) as the "red drop" and "blue drop". Dring (1981), after studying the red algae in coastal water, has stated that the photosynthetic effectiveness decreased with depth in green algae increased in red algae due to high content of phycoerythrin.

In the present investigation it was found that both the species of *Gracilaria* showed an increased PC and APC content of under red light is in accordance with the findings of Figuero *et al.*, (1995). Beer and Levy, (1983) while studying the effect of photon fluence rate and light spectrum composition on photosynthesis and pigments relation in *Gracilaria* species found that there was a ratio between chlorophyll a and phycoerythrin, which decreased with increasing wavelength. They also reported a negative relationship between chlorophyll a and phycoerythrin when the wavelength increased. This condition was found true in the present study, where plants at lower wavelength appeared to be red while the plants under higher light were of greenish colour, which could be attributed to the dominance of PE at lower wavelengths and chlorophyll a at higher wavelengths.

G.edulis and *G.foliifera* are morphologically different, one being cylindrical and the other flat and compressed. They also show different anatomical variations in the development of cystocarps. Developmental stages during carpospore differerentiation are of significance because they have

phylogenetic implications. Occurrence of nutritive tubular filament a characteristic feature reported in *G. conferoides* and *G. compressa* (Sjoestedt, 1926) is not prominent in *G. edulis* and *G. foliifera.* Yamamoto (1978) classified gonimonemata in two major types. The common gonimonema like structure, which is tubular cell with swollen at the top was more evident in *G. edulis,* but the other gonimonema which joins the pericarp layer were not evident in both the species of *Gracilaria* in the present study.

The reproductive potential of the red algae can be measured by the quantity of spores that are released during spore shedding. The environmental conditions are known to influence the reproductive activity of the algae (Guzman *et al.,* 1972; Umamaheswararao, 1974; 1976). The relationship between spore shedding and seawater temperature studied by Suto (1950) is similar to that in the present study. He found that when the seawater was 24^{0}C, the shedding of carpospores started in *Gelidium* species and abnormal temperature in the seawater delayed or hasten the spore shedding.

The difference in photoperiod (L:D) was found to influence the carpospore production in *G.corticata* (Umamaheswarar*ao,* 1976). Present results in this study are also conformity with this observation. *G.edulis* when placed under short day (SD) at 25^0 C, shedded the carpospores at a higher rate than the control. Longer day and lower temperature were found to lesser spore shedding, which can be compared to the findings of Joseph and Krishnamurthy (1977). They found that cystocarpic plants of *G.verucosa* had peak season of sporulation during December at Mandapam, which coincides low daytime water temperature of almost 22 - 25^{0}C.

On the contrary, *G. foliifera* in the present study showed positive response of spore shedding only at higher temperatures irrespective of the photoperiod. Chennubhtola *et al.,* (1986) while studying the carpospore output in *G.foliifera* found that maximum spore output occurred on the first day and maximum quantity of spore liberated in the month of March. This was similar to the present study, where the maximum output of spores was in the first day, which declined gradually. Long day exposure has greatly favoured spore shedding, there was a positive correlation between the temperature and quantity

of spores released. Generally at both photoperiods the number of spores liberated was lesser at 20^{0}C. This finding is contrary to the finding of Friedlander and Dawes (1984a) who found that release of carpospore was negatively correlated with temperature but at low light condition. It could be assumed that by giving proper photoperiod, *G.foliifera* can be made to liberate the spores even at higher temperature.

Maximum shedding of carpospores by *G.corticata* was reported to be on the first day by Subarangaiah (1983). He has observed that the quantity of spores declined drastically during the course of his observation. In the present study, the control plant behaved as above while the alteration in photoperiods and temperature caused greater variation. Though there had been very poor release of spores on the first day at all the experimental condition, the number of spores released was found positively related to the temperature rise exhibiting a peak on the 3rd and 4th day.

Similar trend was also reported by Joseph and Krishnamurthy (1977), who noticed more than one peak in the spore liberation in *G. corticata.* Therefore it could be understood that the plant is influenced by photoperiod and temperature, which can be suitably manipulated to get maximum spore output. The above authors also reported that 25^0 C could be considered as ideal temperature for *G. corticata* to release spores as most of the spores could be seen in the plant collected during October and November.

All the experimental photoperiod and temperature tried for *G. crassa* have resulted in poor spore shedding than that of control indicating the plants poor response to the optimum conditions existing in the control (27^0 C $\pm$ 2^0 C with 14:10 h). There may be favourable temperature and photoperiods other than the one tried in the present study, which may be examined in future.

Studies on the growth and survival of spores of seaweeds under different culture media are scarce. Influence of growth regulators on the growth of *Gracilaria* species has also been studied. McLachlan and Bird (1986) highlighted the importance of nutrient concentration for growth. Oliveira and

Estela (1994) have reported that except for a few cold-water species, the optimum temperature for *Gracilaria* species is around 25^0 C.

In the present study with the spores of *G. edulis*, the three culture media used have promoted the spore growth (increase in size of the spore) uniformly. However, early stage of spore growth showed a high stage of mortality in the initial stage of development. The PES medium showed better performance than other two media tried in terms of longer duration support for spore growth and survival. The Grund medium could not support the survival of the spores as that of other two indicating poor availability of required nutrients for the spores. The percentage of survival as well as the growth rate was more in Prosoli's medium followed by Walne's medium. Grund medium did not show encouraging result due to the lack of essential vitamins such as biotin, thiamine HCl and trace elements. PES medium has most of the essential vitamins, trace elements and Tris in optimal quantities. Grund is having ten times more enrichment than PES in concentration and did not result in high growth rate. PES may be a better enrichment medium for the nursery rearing of spores in the early stage of growth and found to be more economical with high growth rate. From the study, it is evident that with right quantity and quality of nutrients, the spore growth can be enhanced with increased survival. From the experiments with both species, PES medium can be adjudged as the most suitable medium for the spore growth.

SUMMARY

SUMMARY

Seaweeds, particularly red algae are considered economically important due to their products, which find application in human food, food processing and industrial uses. Seaweed farming has been advocated considering the fast rate of depletion of natural stock due to indiscriminate wild harvesting. Research on sporogenesis is thought to be a tool to find out various solutions for encouraging seaweed culture from the spores and to fulfill the demand of seaweeds for the variety of applications.

Gracilaria spp reproduce both vegetatively and sexually. While vegetative reproduction requires a large quantity of plants, reproduction through spores can be done with limited quantity of plants, provided all conditions are favourable. Under the above circumstances, the present study was taken up to find out the influence of environmental parameters such as photoperiod, light intensity, light spectrum and temperature on the sporogenesis and spore output and on the early growth of spores in laboratory conditions.

The studies on photoperiod assume importance in the context of seasonal influence on the sporogenesis. The complementary factors such as light intensity, light spectra and temperature will be useful to find out at what depth in the sea the sporogenesis can be promoted under natural conditions. It is confirmed that only at low light intensity in long day exposure, *G.foliifera* showed initiation of sporogenesis. *G. edulis* showed positive initiation of sporogenesis under red light spectrum.

The histology of carposporogenesis showed the remarkable variation during the course of development of the carpospore. The elongation of the gonimoblast and the expansion of the cystocarp, presence and absence of nutritive filaments was also clearly represented the developmental stages of cystocarp. Histological development pattern of cystocarp can also be a tool for taxonomical ratification of *Gracilaria* spp. Various reports have clearly shown that the pigment

content of *Gracilaria* spp is affected by different light spectra. In the present study also, phycoerythrin (PE) showed an increased level under green spectrum, which is because of the absorption efficiencies of PE under low photon influence rate. All the other pigments were found at increased levels under red spectrum.

Spore shedding is an important activity following the sporogenesis in *Gracilaria* spp. In the present study, 25°C under short day was found optimum under all conditions tried for spore shedding in both the species and it was statistically significant (P>0.01). Though both the plants have shown spore shedding, there had been variation in the amount of spore shedding on different days of the experiment. This clearly indicates the interaction and influences of environmental parameters such as photoperiod and temperature on spore shedding in *Gracilaria* spp. Favourable results obtained at 25°C indicates that both species studied have more spore shedding activity in the months of higher temperature than at lower temperature. This hypothesis has been well supported by many researchers.

Nutrient supply and availability have been regarded as major factors determining the growth of the plants under natural environment. In an attempt to know the growth of the spores based on the nutrient supply, it was found that the PES medium has supported the growth of the spores better than Grund and Walne's media. The calculated r-values for both the species were highly significant (r= 0.99). This was found applicable to the survival of plants also. Optimal nutrient supply and enhancement of growth of spores would help in a long way for hatchery production of viable spores in order to support the seaweed farming.

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