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# **Original Research Article**

# Factors effecting on germination of oospores in sargassum ilicifolium along the Visakhapatnam coast

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**Abstract:** The species of Sargassum are abundant among the brown algae occurring along the Indian shores and these are the chief sources for the extraction of alginic acids. In view of the importance of brown algae as a source of algin and as food, fodder and fertilizer, special efforts were made in India since 1950 to study the chemical composition and algin content of brown seaweeds. At present detailed ecological studies on the Sargassum ilicifolium in two different localities were made for a period of two and half years from December, 1995 to May, 1998. During this period data on germination of oospores. Laboratory experiments on the environmental factors have also been carried out to understand the germination. Some preliminary observations were also made on the growth and survival of Sargassum germ lings in different photon flux densities and salinities. Results obtained on the above aspects are presented and discussed in this paper. The percentage of germination varied from 70 to 80%. Maximum growth was observed in the germ lings of S.ilicifolium at photon flux densities of 18 and  $36\mu \text{ E m}^{-2}\text{s}^{-1}$ . The rate of survival of germ lings was 88-99% between  $9\mu \text{ E} \text{ m}^{-2}\text{s}^{-1}$  and  $36\mu \text{ E m}^{-2}\text{s}^{-1}$ . Growth and survival of germ ling's tolerance was optimal at 30% salinity. **Keywords:** Sargassum ilicifolium (Turner) C.Agardh, Oospores germination, Germ ling's growth, Photon flux density,

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## **INTRODUCTION**

The species of Sargassum are abundant among the brown algae occurring along the Indian shores and these are the chief sources for the extraction of alginic acids in the country [1]. From Visakhapatnam 4 species of Sargassum viz., S.ilicifolium, S.polycystum, S.tenerrimum and S.vulgare were reported [2]. The above four species have also been found in other localities along the coast of Visakhapatnam (Fig.1). Sargassum species and other brown algae of the tropical shores are less investigated when compared with ecological, biological and biochemical aspects studied on the members of laminar ales and Fucales of temperate shores. Sargassum muticum species introduced from Japan, has received much attention in recent years and many aspects relating to its distribution, growth and development, fruiting behaviour, dispersal and colonization have been studied in details by [3, 4, 5] and [6-9]. In other geographical areas also ecological and other investigations on the species of Sargassum were made in recent years. In view of the importance of brown algae as a source of algin and as food, fodder and fertilizer, special efforts were made in India since 1950 to study the chemical composition and algin content of brown seaweeds by [10-19]. At Visakhapatnam some preliminary

observation were made on the seasonal changes in the abundance of brown algae in a general ecological study of the intertidal algae [20, 21]. During this period data on germination of oospores, laboratory experiments on the environmental factors have also been carried out to understand the germination. Some preliminary observations were also made on the growth and survival of germ lings in different photon flux densities and salinities. Results obtained on the above aspects are presented and discussed in this paper.

## MATERIALS AND METHODS

Visakhapatnam is situated on the east coast of India between  $1\overline{7}^{\circ}$  40' 30'' and 17° 45' N latitude and 83° 16' 25" and 83° 21' 30"E longitudes. The coastal line in the vicinity of Visakhapatnam i.e. from Bhimunipatnam to Pudimadaka is sandy without crops of rocky boulders in different shapes and sizes in the intertidal and sub tidal regions. Species of Sargassum occur in many coastal localities between Bhimunipatnam to Pudimadaka. Two coastal localities were selected for the present study namely from Gangavaram and Pudimadaka. They are located 10 and 30 Kms, South of Visakhapatnam respectively. These two stations are directly exposed to the open sea.

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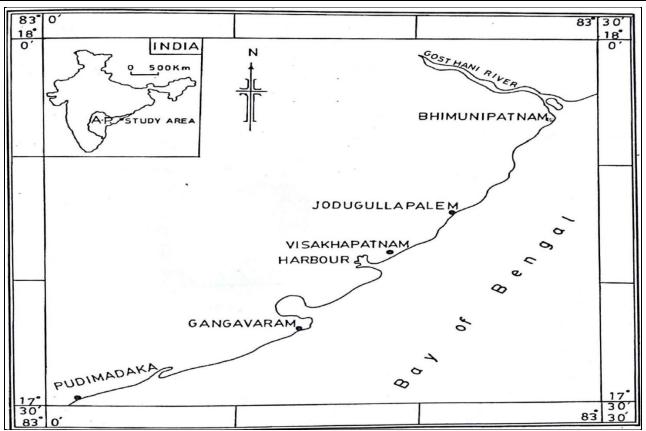


Fig.1. Coast line showing different study sites viz., Gangavaram and Pudimadaka

## PLATE-I



Sargassum ilicifolium (Turn) C.Agardh

The present investigations were carried out on Sargassum ilicifolium (Turner) C.Agardh. The

photograph of the herbarium specimen of the species of Sargassum are shown in plate – I. Depending upon the

availability and density of Sargassum ilicifolium (Turner) C.Agardh occurring in any two localities (field stations) has been collected from Gangavaram and Pudimadaka. Collection of samples was made at fortnight intervals from the same rocky area throughout the period of the investigation. The samples collected from each field station were immediately transported to the laboratory in separate polythene bags filled with seawater. The samples thus collected were used for growth analysis.

#### **Germination of oospores**

While counting the oospores in various laboratory experiments conducted in the present study, data on the percentage frequency of the dividing oospores was collected separately to get a general idea of the germination of oospores (with 24 hours duration) during the fruiting periods of the brown algae investigated and also in the experiments on the environmental factors associated with intertidal region.

## Effect of different photon flux density and salinity on the growth and survival of germ lings

Data on the growth and survival of the germ lings of Sargassum ilicifolium collected under different photon flux densities and salinities. Oospores of S. ilicifolium, liberated and settled on glass cover-slips, were used to follow the growth and survival rate of germ lings. The cover-slips along with germ lings were placed in Petri-dishes containing sterile seawater were exposed to 0, 9, 18, 36, 54, 72 and 90  $\mu$  E m-<sup>2</sup>s-<sup>1</sup> photon flux densities for a period of 8 h in a day (i.e., 8 : 16 L: D cycle) in light chambers at room temperature.

The initial number of germ lings present on each cover-slip and their length were recorded before

conducting these experiments in different photon flux densities. Data on the number of germ lings survived on the cover-slips and their length were collected at 5 day intervals and seawater in the Petri-dishes was replenished while measuring the germ lings.

The effect of different salinities ranging from 10 to 80 ‰ on the germ lings growth and survival was also recorded by exposing the Petri- dishes at a photon flux density of 18  $\mu$  E m-<sup>2</sup>s-<sup>1</sup> at ambient temperature and at a day length of 8 h (8 : 16 L: D cycle) following the method given above.

#### RESULTS

Results obtained on oospores germination and environmental factors influencing on germination of oospores and Germlings growth and survival of Sargassum ilicifolium are presented in this paper.

## Germination of oospores

#### Effect of exposure to air (Desiccation)

Percentage of oospores germinated in the desiccation experiments conducted under laboratory conditions are shown in Fig.2. Though oospores output decreased from 0 to 30 minutes of exposure to air at room temperature, germination rate was high in receptacles of Sargassum ilicifolium, exposed from 0 to 30 minutes. From 30 minutes onwards germination rate decreased rapidly with increasing exposure onwards germination rate decreased rapidly with increasing exposure. Nearly 50% of germination of oospores was observed in the receptacles exposed for 120 minutes and from there onwards the percentage of germination was decreased and complete inhibition and viability of oospores was observed after 180 minutes of exposure.

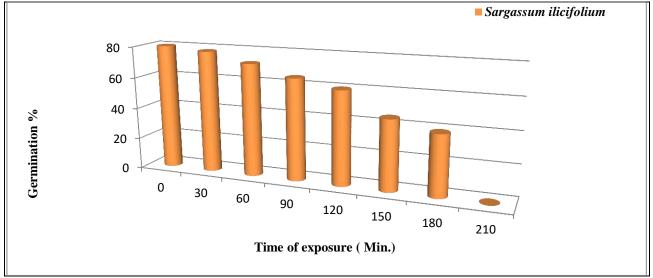


Fig.2. Effect of exposure to air on the percentage germination of oospores of Sargassum ilicifolium (Turner) C. Agardh.

## Effect of salinity

Variations in the percentage germination of oospores of S.ilicifolium at different salinities are shown in Fig.3. Within the salinity tolerance range of the two alginophytes from 10 to 60‰, the germination rate also varied markedly. Though maximum percentage of germination (about 55% in S.ilicifolium) was observed at 30‰ salinity in S.ilicifolium, the rate of germination was also considerably high at 20‰ and 40‰ salinities.

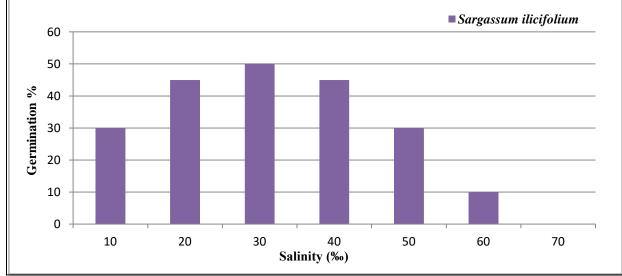


Fig.3: Effect of salinity on the percentage germination of oospores of Sargassum ilicifolium (Turner) C. Agardh.

## Effect of light intensity (Photon flux density)

Germination rate varied in S.ilicifolium were investigated (Fig.4) with higher rates of germination of oospores (about 85% in S.ilicifolium) up to 36  $\mu$  E m<sup>-2</sup> s<sup>-1</sup> photon flux density. The rate of germination

increased from dark (0 photon flux density) to 18  $\mu$  E m<sup>-2</sup> s<sup>-1</sup> in S.ilicifolium and from there onwards the percentage of germination was decreased upto 72  $\mu$  E m<sup>-2</sup> s<sup>-1</sup> and at 90  $\mu$  E m<sup>-2</sup> s<sup>-1</sup>, completes inhibition of oospores liberation and germination was observed.

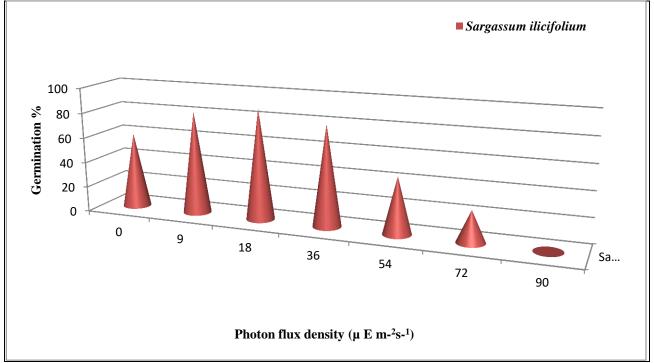


Fig.4. Effect of different photon flux densities on the percentage germination of oospores of Sargassum ilicifolium (Turner) C. Agardh.

## Effect of temperature

In experiments conducted at different temperatures, maximum percentage of germination S.ilicifolium (Fig.5). The values obtained at 20 and  $30^{0}$ C are also considerably high. As observed in the oospores liberation, the oospores germination was also seen at  $10^{0}$ C and  $35^{0}$ C (Fig.4).

Data collected regarding the percentage of germination of oospores in S.ilicifolium is shown in Fig.5. The percentage of germination in the controls (i.e., untreated receptacles), was maximum (about 65%

to 95%, Fig.5 A, B and C). The rate of germination gradually decreased in receptacles exposed to different periods of thermal shock, both at low and high temperatures. About 50 to 60% of germination of oospores was seen up to the receptacles exposed for 2 hours at 0, 5 and 40<sup>o</sup>C (Fig.5 A, B and C) and after 2 hours of exposure the percentage of germination was decreased slowly. No germination of oospores was seen after 5 hours of exposure of receptacles at 0<sup>o</sup>C and 40<sup>o</sup>C, but whereas at 5<sup>o</sup>C, 20 to 30% of the germination of oospores was seen in the receptacles exposed upto 8 to 10 hours in S.ilicifolium and S.vulgare respectively.

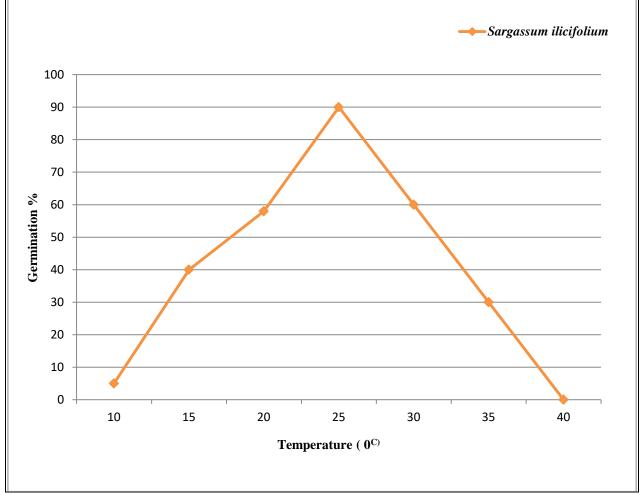


Fig.5. Effect of temperature on the percentage germination of oospores of Sargassum ilicifolium (Turner) C. Agardh.



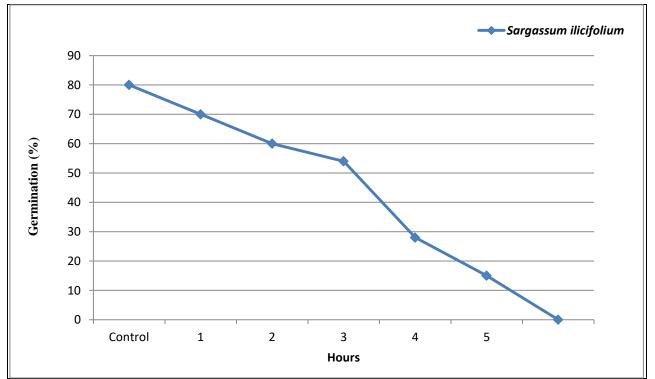
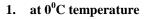


Fig.5. Effect of short treatment in the percentage germination of oospores of and Sargassum ilicifolium (Turner) C. Agardh.



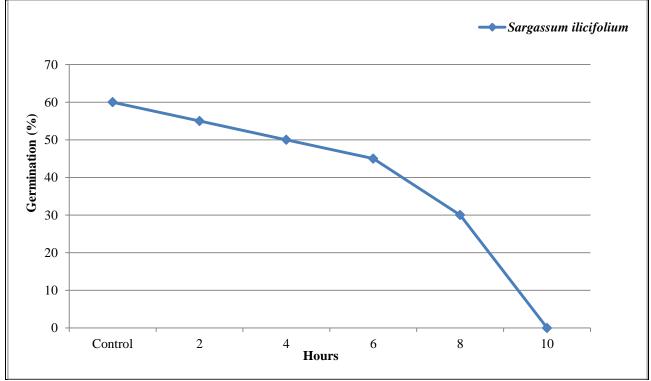


Fig.5. Effect of short treatment in the percentage germination of oospores of and Sargassum ilicifolium (Turner) C. Agardh.

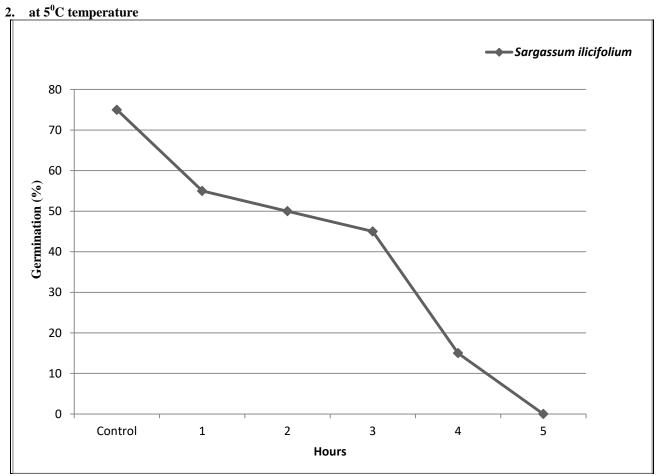


Fig.5. Effect of short treatment in the percentage germination of oospores of and Sargassum ilicifolium (Turner) C. Agardh.

# 3. at 40<sup>°</sup>C temperature

## Germlings growth and survival

Growth data obtained from germ lings of S.ilicifolium under varying conditions of salinity and photon flux density are shown in Figs.6-9.

In the salinity experiments (Fig.6 and 7) maximum growth was observed in the germ lings of S.ilicifolium at 30‰ salinity, coinciding with the optimal salinity noticed in shedding and germination of oospores. The growth rate at 20 and 40 ‰ salinity was slightly less than at 30 ‰, indicating that this alginophyte can thrive well in salinities around 20 and 40 ‰. Germlings growth was minimum at 10 and 50 to 60‰ salinities (Fig.6). High percentage of survival and germination was found at 30‰ in Sargassum ilicifolium (Fig.7). But at 40, 50 and 60‰ salinities, the germ lings were found in degenerating condition after 25 days under laboratory conditions.

In experiments conducted at different photon flux densities, maximum survival and growth was observed in the germ lings of S.vulgare and S.ilicifolium at 9 to 36  $\mu$  E m<sup>-2</sup> s<sup>-1</sup> (Fig.30A and B; 31 A and B). Minimum growth was obtained at 0 and 72  $\mu$  E m<sup>-2</sup> s<sup>-1</sup> (Fig.31 A and B). At low photon flux densities i.e., at 9 and 18  $\mu$  E m<sup>-2</sup> s<sup>-1</sup>, 98% of germ lings were survived up to 30 days in the laboratory conditions. About 40 to 50% of germ lings were survived up to 30 days at dark in laboratory conditions (Fig.30A and B).

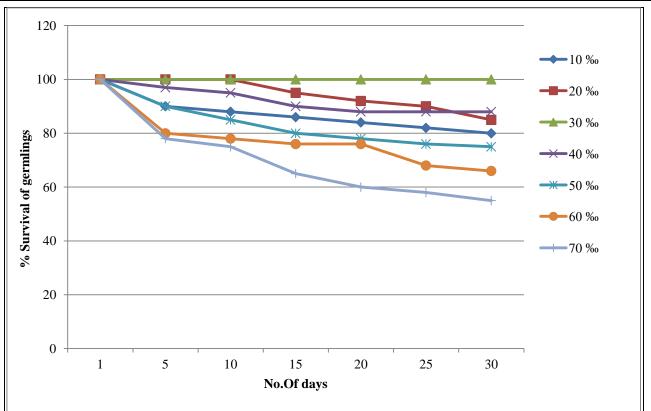


Fig. 6: Effect of different salinities on the percentage survival of germ lings of Sargassum ilicifolium (Turner) C.Agardh

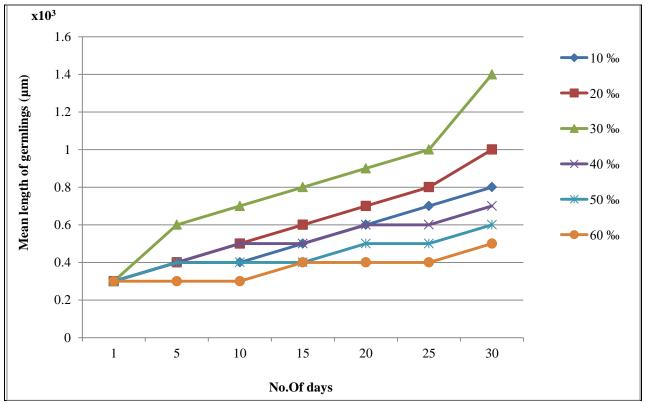


Fig.7: Effect of different salinities on mean length of germ lings of Sargassum ilicifolium (Turner) C.Agardh.

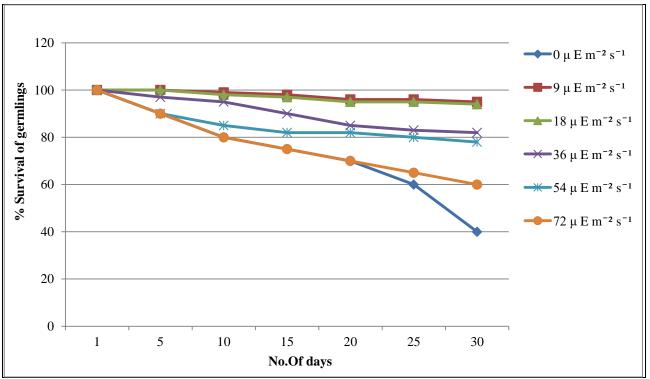


Fig.8: Effect of different photon flux densities on the percentage survival of germ lings of Sargassum ilicifolium (Turner) C.Agardh.

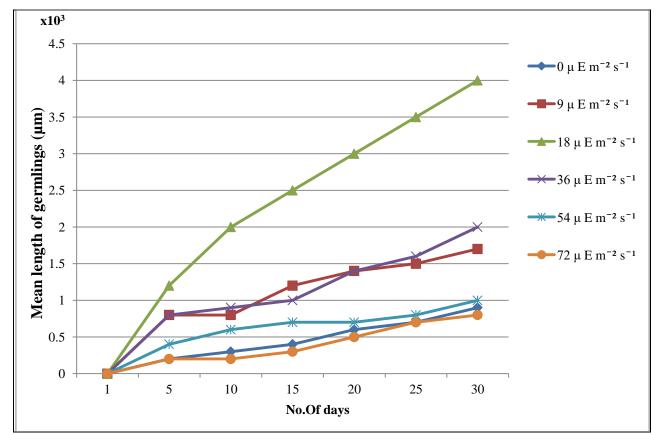


Fig.9: Effect of different photon flux densities on the mean length of germ lings of Sargassum ilicifolium (Turner) C.Agardh.

## DISCUSSION

In the present study information was gathered on germination of oospores of Sargassum ilicifolium. Effect of salinity and Photon flux density on the growth and survival of germ lings were also studied.

## Germination of oospores

Studies regarding the effect of environmental factors on germination and further growth of germ lings of brown algae are very scanty [22, 4] observed optimum growth in the germ lings of Sargassum species between 30-35 ‰ salinity. Growth of zygotes and tetra spores of Padina Japonica was maximum at 20-50‰ salinities [23]. The optimum salinity observed for the growth of germ lings of Dictyota australis [24] was 30-32 ‰. In the present study the growth and survival of germ lings of Sargassum species were optimal at 30% salinity (Fig. 7). These results agree with the salinity optimal obtained for Sargassum species [22, 4] and Dictyota australis [24] and the members of Gigartinales [25]; [26], Ceramiales [27] and Crytponemiales [28], [29] Found maximum development of Fucus germ lings between 5 and 15k lux on 10: 14 L D cycle. [30] Reported that 2000 lux light intensity was optimum for their growth of germ lings of Padina gymnospora. [31] Observed 94% survival of germ lings of Cystoseira indica at low light intensities of 480-580 lux. [32] did not observed development of carpospores of Gracilaria verrucosa, when exposed to high light intensity for 4 to 5 h daily. [33] Did not find germination in Acrochaetium endophyticum in dark as well as in high photon flux densities of 480-500 lumens/Sq. ft. (5167-5382 lux). In Calithamnion hookerii, [34] found that the percentage germination was not influenced by photon flux density. In Gelidium pusillum and Pterocladia heteroplatos, [26] reported best germination at light intensity of 1500 to 3000. [35], [36] reported a light intensity of 1500 lux was favourable for the optimum germination of Gracilaria corticata and Hypnea valentiae. In Wrangelia argus and Centroceros clavulatum, the maximum percentage of tetra spores and carpospores germination was observed at 34 and 23  $\mu \text{Em}^{-2}\text{s}^{-1}$  respectively [27]. The maximum percentage of germinating tetra spores and carpospores of Amphiroa fragilissima and Grateloupia lithophila was observed at  $54\mu \text{Em}^{-2}\text{s}^{-1}$  [28].

In the present study, the maximum percentage of oospores of S.ilicifolium germinated at 9 to  $54\mu\text{Em}^{-2}\text{s}^{-1}$ , which agrees with the conditions observed in the members of Gelidiales [26], Gigartinales, [35, 36] and Ceramiales [27] of the same coast, but required slightly lower photon flux densities, when compared to the members of Cryptonemiales [28] of the same coast.

Contrary to be above findings, there was no much difference in the percentage germination of oospores in the present study between 9 and  $54\mu E m^{-2} s^{-1}$  (Fig. 8). However, the minor variations in the light intensity of the above orders may be attributed to the variations in

the temperature and other conditions of the environment in which the experiments were conducted. The percentage of germination of oospores of S.ilicifolium of the present study at different exposure period (Desiccation), salinity, photoperiod, temperature, closely agrees with the findings of [35], [36] on Gigartinales, [26] on Gelidiales, [27] on Ceramiales and [28] on Cryptonemiales of the same coast.

## Germ ling's growth and survival

Even though much emphasis was not given on the germination and survival of oospores due to some difficulties encountered in the experimental work, the data obtained show that the germination rate in oospores of S.ilicifolium did not very much in different months of the year. The percentage of germinating oospores varies from 64% to 90% in all the months of their occurrence in the field. Though the literature related is scanty, it indicates that the oospores germination varies in different months of the year. In Gracilaria corticata, Hypnea valentiae [35, 36] Wrangelia Argus and Centroceros clavulatum [27], Amphiroa fragilissima and Grateloupia lithophila [28] also, the highest percentage of dividing tetra spores and carpospores, was observed during the peak period of shedding. In Gelidium robustum, though fruiting plants were found throughout the year, spore germination was observed during the spring and early summer [37] Similarly [38] reported seasonal variation in the growth of germ lings of Chondrus crispus. In Gelidium pusillum and Pterocladia heteroplatos also the highest number of dividing spores was observed during and after the peak period and the rate of germination was low in Pterocladia heteroplatos than in Gelidium pusillum as germination was not observed in Gelidiopsis variables within 24h [26]. Wrangelia argus, Centroceras clavulatum, [27]. Amphiroa fragilissima and Grateloupia lithophila [28], exhibited a high rate of germination of 90% compared to Gigartinales (60%) [35, 36] and Gelidiales (40%) [26]. In the present study shows nearly about 90% of germ lings in the first week. Though the observations are preliminary, but it suggests that all the spores liberated throughout the year may not germinate within 24h and their viability is high in certain periods or during or immediately after the peak But the viable and germinating growth periods. oospores are evident in all the months (4 to 5 months) of their occurrence in present study, as also observed by [39] in Hypnea valentiae, and Gracilaria corticata and [35, 36], Wrangelia argus, and Hypnea valentiae Centroceras clavulatum [27], Amphiroa fragilissima and Grateloupia lithophila [28]. The above workers had calculated the percentage of germinating spores on day one or day two. But in the present study, the percentage of germination of oospores on Sargassum ilicifolium in different sites have been calculated from oospores liberated for nearly two weeks. However, more detailed studies are needed and the experiments should be continued for more number of days to see the

percentage germination in different months of the year up to a considerable growth of the germ lings.

## CONCLUSION

Ecological studies were made from December 1995 to May 1998 on Sargassum ilicifolium growing along the Visakhapatnam and nearby localities. Laboratory experiments were carried out during the period of this study with Sargassum ilicifolium (Turner) C. Agardh to know in detail preliminary data collected on the germination and growths of oospores were presented in this paper.

The percentage of germination varied from 70 to 80%. Maximum growth was observed in the germ lings of S.ilicifolium at photon flux densities of 18 and  $36\mu \text{ E m}^{-2}\text{s}^{-1}$ . The rate of survival of germ lings was 88-99% between  $9\mu \text{ E m}^{-2}\text{s}^{-1}$  and  $36\mu \text{ E m}^{-2}\text{s}^{-1}$ . Growth and survival of germ ling's tolerance was optimal at 30% salinity.

From this study on different factors, it can be concluded that the submerged condition of fronds, photon flux density of 9  $\mu$  E m<sup>-2</sup>s<sup>-1</sup> (long days at low photon flux densities and short days at higher photon flux densities), salinities around normal sea water (30 ‰) and temperatures around 25°C are favourable for germination and growth of S.ilicifolium. These experimental findings closely agree with the environmental conditions existing in the intertidal habitats of the Visakhapatnam coast.

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