INFLUENCE OF AQUEOUS EXTRACT OF MACROALGA *HYPNEA MUSCIFORMIS* LAMOUR AND *CODIUM TOMENTOSUM* (HUDS.) STACKHOUSE AT DIFFERENT GROWTH PHASES OF MICROALGAE *CHLORELLA MARINA* (CHLOROPHYTA)

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Abstract

The microalgae *Chlorella marina* is used as live feed for feeding larvae and post-larvae of fish and shell-fish in hatcheries or aquaculture. Considering the different bioactivities of the extracts of *Hypnea musciformis* and *Codium tomentosum*, experiments were conducted to evaluate their influence on growth promotion in *C. marina*. The growth response of microalgae *Chlorella marina* was evaluated using Walne's medium supplemented with different dilutions of the aqueous extract of *H. musciformis* and *C. tomentosum*. Results indicated that 0.1% w/v of the extract of both macroalgae reduced the growth of *C. marina* to an extent of 25% to 45% initially at its lag phase. A marked increase in growth was observed with the increasing concentrations of the extract compared to the control sets. At its exponential phase, *C. marina* registered 57.5% increase and 5% decrease in growth in 1% w/v of the extract of *H. musciformis* and *C. tomentosum* respectively. The higher concentration of 10%w/v and the aqueous extract as such (100% w/v) of both the macroalgae increased the growth rate to 43.8 % and 56.3% respectively at the log phase as against the control group. Further experiments at the higher concentrations of extract indicated a decreasing trend of growth of *C. marina* markedly in its declining phase. The increased growth rate in the exponential phase in the presence of the extracts could be attributed to the high metabolic activity of microalgae concomitant with the maximum absorption of nutrients. The results of this study bring to light the possibilities of effective utilization of macroalgal aqueous extract to increase the growth of microalgae used as live feed in aquaculture.

Key words : Aqueous extract, Hypnea musciformis, Codium tomentosum Chlorella marina

Introduction

Large scale production of microalgae is an integral part of aquaculture as they form the basis of the food chain in aquaculture ventures. (Laing, 1987, De Pauw et al., 1988, Metting, 1996, Sorgeloos, 1996). Considering their nutritional quality, microalgal feeds are valuable for the early stages of finfish and shell fish species. Marine microalgae have also been suggested as a new source of single cell protein. (Fabreoas and Herrero, 1985).

Research results indicated that microalgae at the base of food chain are the primary source of long chain fatty acids; EPA (Eicosapentaenoic acid) and DHA (Docosa hexaenoic acid) for the growth and maturation of marine fish and shell fish (Langdon and waldock, 1981, Devresse et al., 1990).

Species of microalgae belonging to the genera *Chlorella* and *Nanochloropsis* have been widely utilized in marine fish hatcheries in view of transferring the essential fatty acids and other dietary component from the algae via the rotifers to fish larvae (James et al., 1987, James and Aburezag, 1988).

The present study was aimed to investigate the growth pattern of microalgae on addition of two different macroalgae *Hypnea musciformis* and *Codium tomentosum* as supplement at different dilutions using cost effective conventional method.

Materials and Methods

Stock culture (Microalgae) maintenance

The microalgae *Chlorella marina* maintained at their log phase was obtained from Central Marine Fisheries Research Institute (CMFRI), Vizhinjam, Trivandrum. Healthy culture of *C. marina* retrieved at their log phase (12 days old) was used as inoculum for stock culture maintenance. Stock culture of *C. marina* was prepared using 200 ml sterile sea water (28 to 32 ppm) to which 40 ml of inoculum was added and further enriched with standard Walne's medium. The setup was left undisturbed at controlled room temperature of 26 ± 2° C and pH 7.5 - 8.0. Periodic monitoring of the growth phases of microalgae was noted using haemocytometer.

Preparation of Aqueous Extracts of Macroalgae

The macroalga *Hypnea musciformis* (red algae) and *Codium tomentosum* (green algae) were handpicked off the coast of Kanyakumari and immediately transferred to the laboratory. Upon collection, the macroalgae was washed thoroughly with sterile seawater to remove the transient bacteria and entangled fauna. Fifty gram of each macroalgae were ground with 15ml of phosphate buffer (pH 7) in a mixer grinder. The homogenate (100%w/v) was filtered using muslin cloth and different dilutions of 10%, 1% and 0.1% were prepared for further studies.

Culturing of Microalgae

Exponential phase of *C. marina* from stock was used to inoculate two sets of triplicates maintained for growth characteristic study. The stock inoculum was added along with Walne's medium and different dilutions of aqueous extracts of two selected macroalga *H. musciformis* and *C. tomentosum.* The triplicates were placed on racks illuminated with fluorescent tubes (1000 lux) with photoperiod of 16h light and 8h dark simultaneously. Aeration was not provided but the flasks were swirled manually twice a day. Inoculum density and cell count were measured every 3days for each flask including stock using haemocytometer and the results recorded.

Results

The growth pattern of the microalgae *Chlorella marina* in Walne's medium supplemented with different dilutions of the aqueous extract of *Hypnea musciformis* and *Codium tomentosum* are presented in Fig. 1 to 4.

Fig. 1

Growth of *C. marina* culture in Walne's medium supplemented with different dilutions of aqueous extract of *Hypnea musciformis*

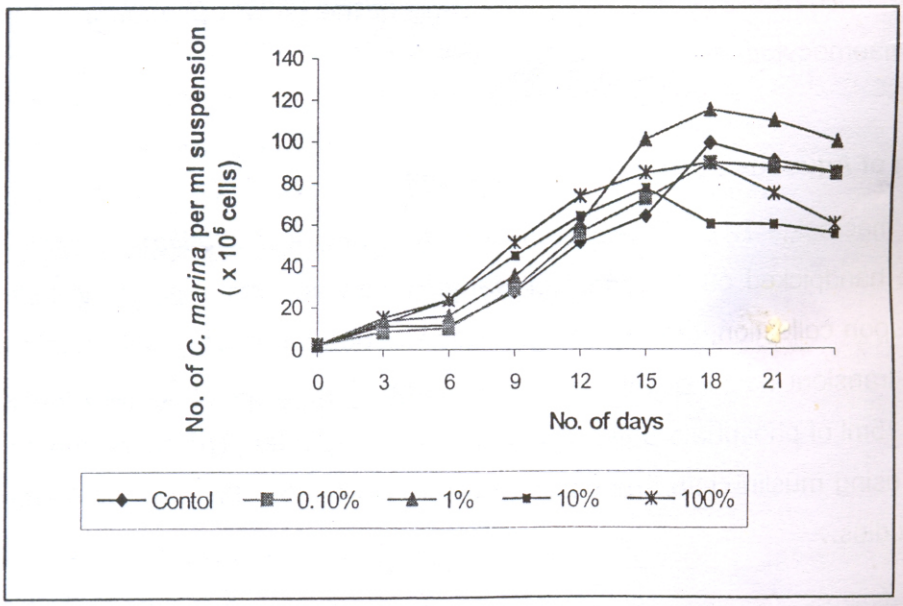


Fig. 2

Comparative increase / decrease in growth of *C. marina* to different dilutions of aqueous extracts of *Hypnea musciformis* over control

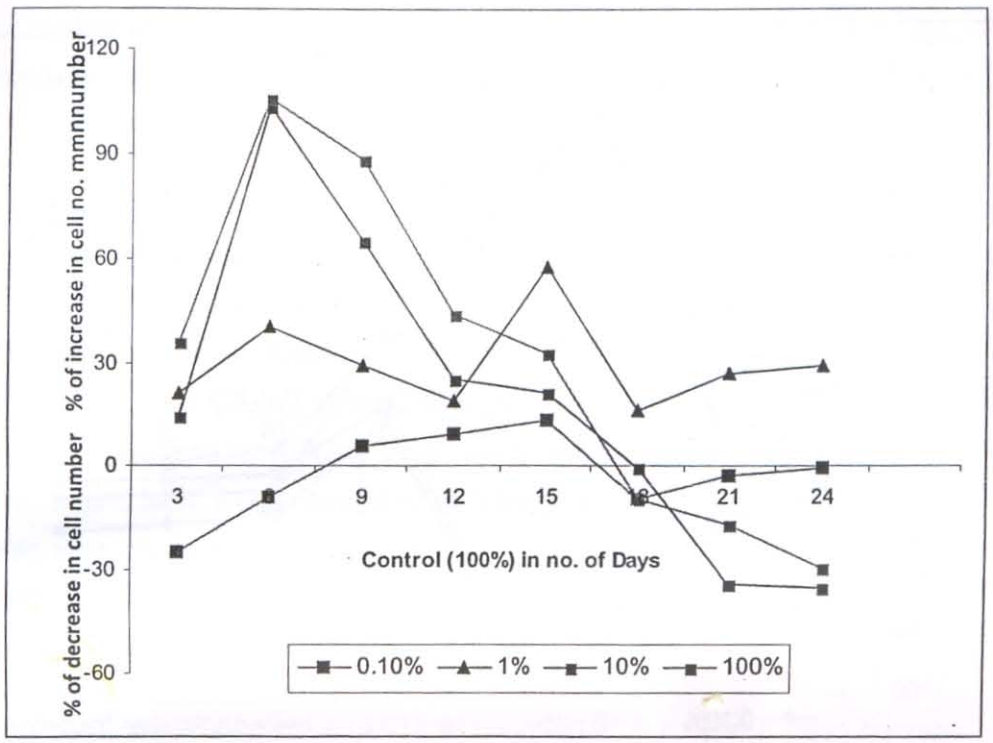


Fig. 3

Growth of *C. marina* culture in Walne's medium supplemented with different dilutions of aqueous extract of *Codium tomentosum*

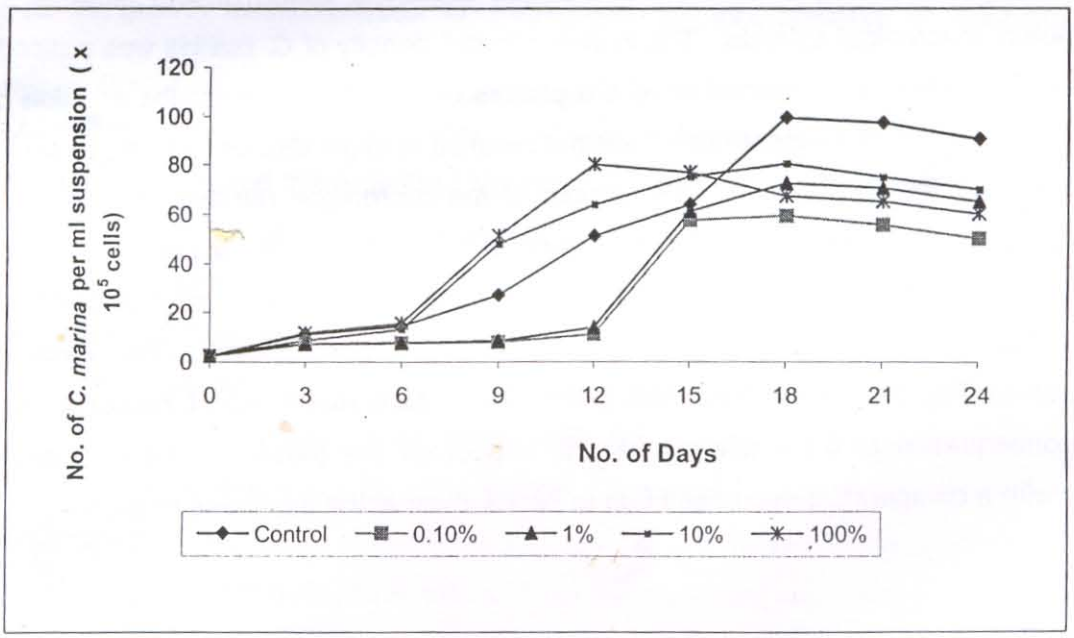
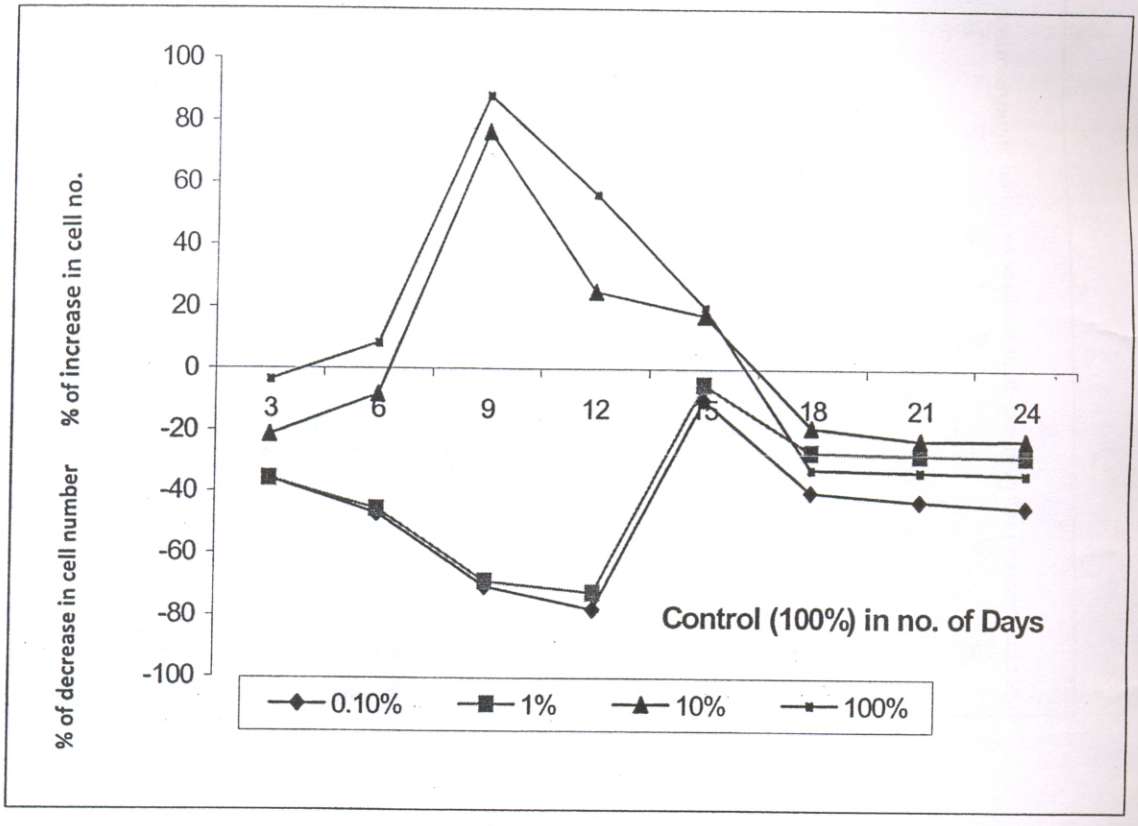


Fig. 4

Comparative increase / decrease in growth of *C. marina* to different dilutions of aqueous extracts of *Codium tomentosum* over control



As seen from fig. 1 to 4 the microalgae displayed varied growth patterns in the presence of macroalgal extracts. The increase in cell density of *C. marina* was noticed with 1% w/v of *H. musciformis* extract at all the phases of growth. However the addition of the same concentration of *C. tomentosum* extract resulted in slight decrease in its growth when compared with the control. The cell number of the microalgae remained in a decreased state as against the control at 0.1 % w/v concentration of *H. musciformis* at initial log phase. The log phase had seen an increased cell concentration with the least dilution of 0.1% w/v, and further decreased when the cells reached its decline phase. The case of *C. tomentosum* (Fig. 3 and 4) is in contrast to the observations made with *H. musciformis*. The least concentration of 0.1% w/v showed no impact on the growth of the microalgae *C. marina* with a comparative decrease of up to 78.1% even at the log phase of growth.

C. marina reached a maximum multiplication phase with the increased concentrations of *H. musciformis*. The 10% w/v and 100% w/v of the extract of the same produced 64.7% w/v and 88.2% increase in growth respectively. In case of the extract of *C. tomentosum* the increased concentration (10%, w/v, 100% w/v) of the extract brought about a market increase in growth (76.5% w/v and 88.2%, w/v respectively). Further, a decrease in growth was noticed with the start of the decline phase when compared to the control.

Discussion

Macroalgae, being a rich source of organic and inorganic nutrients were analyzed for their influence on growth promotion of the microalgae *C. marina*. They are the potential renewable source of food, fertilizer and renewable energy (Kirkman and Kendrick, 1997). Vinoj and Kaladharan (2007) proved that the macroalgae are rich sources of minerals, vitamins and polyunsaturated fatty acid which has been used as an alternate source of protein for animal feed. Kannathasan et al., (2008) also proposed the presence of increased Gibberellic acid content in *H. musciformis and* Reeta et al., (1999) reported the nutrient content of *Codium tomentosum* which further credited to the usage of those macroalgae as an enricher for the growth of microalgae.

In recent years, applied aspects of microalgae & research have become more and more important. Experiments on microalgae cultures are conducted to obtain basic background on maximal microalgae growth under optimal environmental conditions (Goldman et a1, 1975). The wide distribution and diversity too attributed to the fact of usage of macroalgae as supplement. The study was initially mooted to gain insights into the difference in growth characteristics of microalgae on incorporation of supplemental macroalgae.

Addition of different dilutions of the extracts of *H. musciformis* and *C. tomentosum* to Walne's medium promoted the growth of the microalgae to a certain extent. Both the lower and higher concentration of *H. musciformis* extract could bring about significant impact on the growth of the microalgae. However, when compared with the higher dilution, the effect of the lower dilutions was seen hardly in all the phases of growth. The growth maxima observed at the exponential phase of growth in both the cases could be attributed to the high metabolic activity of microalgae concomitant with the maximum absorption of nutrients. However it is reverse in case of *C. tomentosum*. The study made it clear that the lower concentrations of the extract of *C.tomentosum* upto 1% w/v did not favour the growth and multiplication of microalgal cells, as is evident from the increased cell number in the case of control than the experimental groups. The superiority of the control as against the lower

concentrations of *C. tomentosum* extract clearly indicated the inhibition of the microalgal growth at this particular concentration of the aqueous extract. It is possible that *C. tomentosum* at the low concentration could have organic compounds which could inhibit the growth of microalgae. Earlier observation by Provasoli (1963) also indicated the growth of microalgae being affected by the presence of organic compounds. In contrast to these observations, the higher concentrations of the same brought about marked increase in the cell number of *C. marina*, which could be attributed to the possible availability of more amounts of organic compounds acting as chelators, thus enhancing the growth. Similar observations were reported by Mary et al., (1995) on using the organic extracts of *Gracilaria eucheumoids* against *Nitzschia sp. and S. costatum*. Neilands (1967) also reported the influence of organic compounds in the growth regulation of micro organisms.

The macroalgae *Hypnea musciformis* and *Codium tomentosum* are proved to be rich sources of minerals like Calcium, Magnesium, Sodium, Potassium and plant growth regulators like auxin, cytokinin and gibberellins which are found to be highly essential for the metabolic activities of *Chlorella marina* (Johri and Mitra 2001). The microalgal growth could be attributed to the nutrients contained in the macroalgalextract (Mumford and Miura 1998).

The use of this macroalgae with high protein and mineral content could be a promising way for the utilization of this marine resource that remains under exploited in our coasts. This would pave way for developing a low cost culture medium for raising microalgae using such cheap and commonly available resources which can further reduce the production cost.

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