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STUDIES ON THE AGAROPHYTE, *GRACILARIA EDULIS* – EXPERIMENTAL FIELD CULTIVATION AND METHODS OF IMPROVING YIELD AND QUALITY OF AGAR.

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Abstract : Preliminary investigations on the extraction of phycocolloids from five common red algae of Sri Lanka revealed that *Gracilaria edulis* forms a suitable source for the production of agar. This coupled with the relative abundance of this species prompted the investigations on the cultivation of this alga and on the methods of extraction of agar from this species. Vegetative fragments from the apex of the plant were used as "seed" material for planting. Planting of alga was carried out from October 1982 to June 1983 and each month an experiment was set up using algae collected in that month. The algae grew to its maximum size in about 2½ to 3 months attaining a length of 30 – 35 cm and fresh and dry weights of 20 – 30 g and 1.5 to 2.5 g respectively. The agar content and gel strength of agar obtained from cultured *G. edulis* were determined. The amount of agar increased with increase in growth of alga but there were no significant differences in the gel strength of agar. These data compare well with those obtained for naturally occurring *G. edulis*. The effects of different pre-treatments of naturally occurring *G. edulis* on the yield and gel strength, have been studied. While prior wet grinding increased both yield and gel strength of agar, prior soaking did not have any improvement on the agar. Extraction under pressure resulted in a product with increased yield and gel strength. Prior alkali treatment of the sea weed was found to increase the gel strength and pretreatment with KCl upto a concentration of 4% increased the gel strength markedly.

1. Introduction

Marine macroscopic algae which are popularly known as seaweeds are gaining importance as food and also as source of commercially important polysaccharides such as agar, carrageenan and alginic acid. Seaweeds have also been used as a source of biomass and in a number of pharmaceutical products[6,7]. A large number of seaweeds that could be utilised for these purposes are found along the coasts of Sri Lanka[3,4]. Of these seaweeds, species of *Sargassum* and *Gracilaria* have been reported to be present in appreciable quantities. The former could be utilised for the extraction of alginic acid and the latter for agar. Thus there is considerable scope for the utilization of these seaweeds in Sri Lanka.

Several species of red algae occurring in Sri Lanka have been found to be potential sources of agar[1,2] and two species of *Gracilaria* namely *Gracilaria edulis* and *Gracilaria verrucosa* are found in appreciable quantities. However, the estimated quantity of available *Gracilaria*[3] is far below the requirements of an industry for the production of agar. Therefore exploitation of these algae at industrial level cannot be based on the raw materials that could be collected from natural beds alone. In addition indiscriminate harvesting of *Gracilaria* for export as raw sun dried seaweeds by private small scale exporters makes this small resource even smaller. These factors therefore limit the proper utilization of these red seaweeds for commercial purposes in Sri Lanka.

Under these circumstances the only alternative is to increase the amount of raw material by artificial cultivation. Cultivation of seaweeds have been successfully practised for a long time in countries like Japan, Philippines, Indonesia and India. A preliminary investigation on the cultivation of *Gracilaria lichenoids (= G. edulis)* has been carried out at Puttalam lagoon in Sri Lanka too.[10] Further, Sivapalan and Theivendirarajah[11] have shown that this species could also be cultivated in other parts of the country. The main reason for undertaking the cultivation of seaweeds such as *Gracilaria* is the increase in demand for this valuable raw material for economic exploitation. The present work on the cultivation of *G. edulis* was carried out at Mandaitivu, Jaffna.

Extraction of agar from Sri Lankan seaweeds have been investigated previously.[1,2] In many instances it has been observed that many red algae yield little agar and the gel strength of the agar is also low. It is possible to increase the yield and gel strength of agar by various pretreatments. A systematic study on improving the yield and gel strength of agar obtained from *G. edulis* has also been undertaken during the present investigation.

2. Materials and methods

G. edulis was cultivated at Mandaitivu which is an island situated on the west of Jaffna peninsula. This area was selected for cultivation as it supported good natural growth of *G. edulis* and the seawater environment is also ideal for the growth of this species. Further, this alga grows well in shallow water and the area selected formed the required habitat.

Seaweeds are cultivated by means of spore production and by vegetative fragmentation and the latter has been found to be much easier and quicker for *G. edulis*.[9] Several methods for propagating *Gracilaria*, including vegetative fragmentation on coir ropes[9] coir net frames[15] and on coral stones[13] have been carried out. In this investigation all these methods were tried out, where vegetative fragments of about 2 cm long, usually taken from

the apical portions of the *Gracilaria* plant were employed. This "seed" material was inserted into the twists of coir ropes or coir net frames at regular intervals. The long line coir ropes or coir net frames with the algae planted were attached to wooden poles and suspended under water. In the case of coral stones, weeded coral stones were tied with coir ropes containing the planting material by nailing the ropes. These were then submerged under water. The level of water above the planting material was maintained throughout the investigation. However, during low tides the planting materials were exposed, but this was only for a short period.

The project was carried out from October 1982 to June 1983 and each month a fresh set of planting was made using fragments from a number of plants collected that month in order to ascertain the best period for the cultivation and harvesting of *G. edulis*. Observations were made every week after planting on the increase in linear growth, wet weight and dry weight of the algae. The mean length was determined by measuring the length of 20 plants selected at random. The wet and dry weights were determined by removing all the algae from one meter length of coir ropes or one square meter area of coir net frames and the results were related to the weights per plant or per metre length area or square metre area of coir ropes. The plants that attained maturity were clipped at the bottom leaving a fragment on coir rope. Regeneration studies on these fragments were also made using the same parameters. During the period of investigation physical parameters of the sea water such as salinity, oxygen concentration and temperature were also recorded.

The cultured *G. edulis* was analysed for its agar content and gel strength of agar. The gel strength was determined by a penetrameter and expressed as g cm^{-2}. These values were compared with the values obtained for *G. edulis* grown naturally.

Detailed studies on improving the yield and quality of agar were made on naturally grown *G. edulis*. For the purpose of extracting the phycocolloid unless stated otherwise, the following method was employed. In each experiment 10g of powdered *G. edulis* was soaked in distilled water overnight and the excess water was drained off. Fresh distilled water was added to the seaweed in the ratio of 1:20 (w/v) and the pH of the mixture was adjusted to 5. The mixture was boiled for 30 min and after boiling the seaweed was filtered through double layers of muslin cloth. The filtrate was frozen overnight at −10°C in the deep freeze. The frozen sample was subsequently thawed at room temperature and the excess water was drained off. The agar extracted was spread into thin films over a polythene sheet and dried at 50–55°C in a drier. The dried agar was ground in a micromill and used to determine the yield, and its gel strength and the results are expressed as % agar and g cm^{-2} respectively.

3. Experiments and Results

Analysis were carried out initially on the agar obtained from five red algae namely *G. edulis, G. crassa, Hypnea musciformus, Gelidiella acerosa* and *Laurencia obtusa.* It was found that *G. edulis* produced the highest yield of agar. Agar solution of different concentrations (1%, 1.5% & 2%) were prepared using agar obtained from the five algae under investigation and the gel strength, melting temperature and setting temperature were determined. These values were compared with those obtained for a sample of Difco agar (Table 1).

It is apparent from the result that *G. edulis* shows superior quality with respect to qualities of agar and because of this fact and the relative abundance of this species it was decided to carry out an extensive investigation on field cultivation of *G. edulis* and to improve the qualities of agar obtained from it.

The cultivation programme was initiated on 09.10.1982. Fragments of the alga obtained from a few plants were planted and weekly observations were made on the growth of the alga from the 2 cm fragments in terms of increase in linear growth, increase in wet and dry weights (Table 2).

The results show that growth of *G. edulis* from 2 cm fragments is remarkable as the plant attained about 12 cm in eight weeks with a growth rate of about 0.6 cm per day. Similar observations were made on fresh planting that were carried during subsequent months. It is revealed (Table 3) that cultivation programme can be started during every month of the year. This is in accordance with the observations that *G. edulis* can be collected from its natural habitat at Mandaitivu throughout the year. However, algae planted during October 1982 and January 1983 grew faster and luxuriantly. The conditions during the months of October and January seem to favour faster growth and these months could be selected for any extensive cultivation of the algae.

Further observations indicate that *G. edulis* attains its maturity in about 2½ — 3 months reaching a length of 20 — 30 cm and fresh and dry weights of 20 — 30g and 1.5 — 2.5g respectively (Table 4).

Analysis of agar obtained from cultivated *G. edulis* during its different stages of growth indicates that with increase in age of the plant the agar content also increased but the gel strength remained unchanged (Table 5). Comparison of the qualities of agar obtained from cultured and naturally obtained *G. edulis* revealed no difference in the agar content or gel strength of agar. However, processing of alga for agar extraction was much easier with cultivated alga as it was relatively free from calcium deposits and other extraneous materials.

Table 1 : Properties of phycocolloid obtained from some red algae

Alga	% moisture	% agar	strength of agar solution (% w/v)	Setting temp./°C	Melting temp./°C	Gel strength g/cm^{-2}
Gracilaria edulis	86.2	40.0	1.0*	38*	56*	80.2*
			1.5	39	60	140.8
			2.0	40	63	199.3
Gracilaria crassa	84.8	38.4	1.0	41	68	66.5
			1.5	43	70	138.0
			2.0	42	72	168.5
Hypnea musciformis	89.2	34.5	1.0	39	58	95.8
			1.5	42	61	130.3
			2.0	43	60	149.0
Gracilaria acerosa	68.0	20.0	1.0	38	53	65.3
			1.5	37	56	80.3
			2.0	35	58	118.5
Laurencia obtusa	85.8	29.5	1.0	36	56	72.8
			1.5	38	53	88.6
			2.0	34	58	102.3
Difco agar	—	—	1.0	35	58	280.0
			1.5	40	65	392.0
			2.0	43	72	495.0

* The three values in columns 4, 5, 6 and 7 under each algal species refer to the setting temperature, melting temperature and gel strength of 1.0%, 1.5% and 2.0% of agar solutions prepared from the extracted agar of the alga).

Table 2. Growth of *Gracilaria edulis* after different periods

Age of Plant (weeks)	Length (cm)	Fresh weight (g)	Dry weight (g)
1	3.8	1.01	0.11
2	4.2	1.83	0.21
3	5.4	2.42	0.26
4	6.9	2.88	0.31
5	7.4	3.10	0.35
6	9.3	9.80	0.48
7	11.5	13.50	0.99
8	12.3	14.20	1.30

Table 3. Growth of alga (increase in dry weight) planted at different periods of the year

Algae planted on	Dry weight (g) at the end of week							
	1	2	3	4	5	6	7	8
1st Oct. 1982	0.41	0.54	0.75	1.18	1.38	1.67	1.48	1.82
29th Oct. 1982	0.11	0.21	0.26	0.31	0.35	0.48	0.99	1.30
3rd Dec. 1982	0.20	0.32	0.48	0.71	0.95	1.02	1.18	1.26
12th Jan. 1983	0.24	0.31	0.33	0.46	0.54	1.31	1.81	2.27
18th Feb. 1983	0.11	0.20	0.41	0.54	0.72	0.92	1.01	1.21
13th Mar. 1983	0.10	0.27	0.22	0.51	0.63	0.83	1.02	1.31
12th Apr. 1983	0.12	0.34	0.44	0.56	0.62	0.86	1.01	1.02
8th May 1983	0.30	0.39	0.49	0.67	0.97	1.09	1.21	1.48

Table 4. Growth of *Glacilaria edulis* at the time of harvest

Date of planting	Date of harvest	Length (cm)	Fresh weight (g)	Dry weight (g)
1st Oct. 1982	12th Jan. 1983	34.5	20.6	1.40
3rd Dec. 1982	13th Mar. 1983	31.7	24.2	2.30
18th Feb. 1983	8th May 1983	29.3	29.7	1.82

Table 5. Yield and gel strength of agar obtained from *Gracilaria edulis* after different periods of growth.

Age of plant (weeks)	% yield	Gel strength (1.5% solution)
4	20.0	125.8
5	20.5	131.2
6	24.8	120.4
7	29.0	134.3
8	31.0	118.8
9	37.8	102.6
10	41.1	114.2
11	40.2	122.6
12	43.2	112.8

Observations so far have indicated that *G. edulis* could be grown successfully on artificial substrata by vegetative propagation and that the quality of agar obtained from the cultured agar is comparable to that of the agar found in natural beds. Thus artificial cultivation together with the natural raw material cast ashore due to wave action will provide adequate seaweeds or commercial extraction of agar.

With the information available it was decided to study the properties of agar on the different methods of extraction. In the initial studies analysis of agar obtained from *G. edulis* was carried out on air dried algal thallus without powdering before extraction. It was noticed that there was considerable increase in the yield of agar by powdering the seaweeds prior to extraction, however the gel strength did not improve by this pretreatment (Table 6).

When the effect of soaking the seaweeds prior to extraction was investigated it was found that prior soaking did not enhance either the yield of agar or the gel strength of powdered seaweed (Table 7).

An experiment was undertaken to determine the time of extraction that gives the best yield. It was observed that with increase in the time of extraction both the yield and gel strength of agar increased (Figure 1).

Extraction of agar using different methods were tried out. Seaweeds were either boiled for 25 mins or autoclaved at 15 lbs pressure for 25 mins. This experiment involved powdered/unpowdered and soaked/unsoaked treatments.

The results (Table 8) indicate that extraction of agar by autoclaving increased both the yield and gel strength irrespective of whether the seaweed was powdered or soaked.

The requirements of a suitable pH for extraction of agar was subsequently examined and it was found that pH near 5 – 6 gave higher gel strength but the amount of agar produced was greater in the more acidic conditions (Figure 2).

Finally the effect of concentration of initial seaweed water mixture prior to extraction was investigated. It was observed that with increase in the quantity of water the yield of agar also increased but the gel strength was better with a relatively concentrated solution (Table 9).

Several reports have indicated that pre-chemical treatments of either the seaweed or agar improved the quality of agar. These aspects were explored in the following experiment.

Table 6. Effect of powdering *Gracilaria edulis* on the yield and quality of agar.

Treatment	% agar	Gel strength (1% solution)
Powdered	32.8	81.2
Unpowdered	20.3	85.6

Table 7. Effect of prior soaking of powdered seaweed on the yield and quality of agar.

Treatment	% agar	Gel strength (1% solution)
Soaked	25.7	79.3
Unsoaked	23.5	82.5

Table 8. Effects of different methods of extraction on the yield and quality of agar.

Treatment	Extraction by	% agar	Gel strength (1.5% solution)
Soaked powdered	boiling	14.4	146.0
	autoclaving	27.2	168.5
Soaked unpowdered	boiling	19.0	150.4
	autoclaving	32.0	178.3
Unsoaked powdered	boiling	12.2	166.0
	autoclaving	20.4	180.0
Unsoaked unpowdered	boiling	14.6	160.0
	autoclaving	24.2	174.0

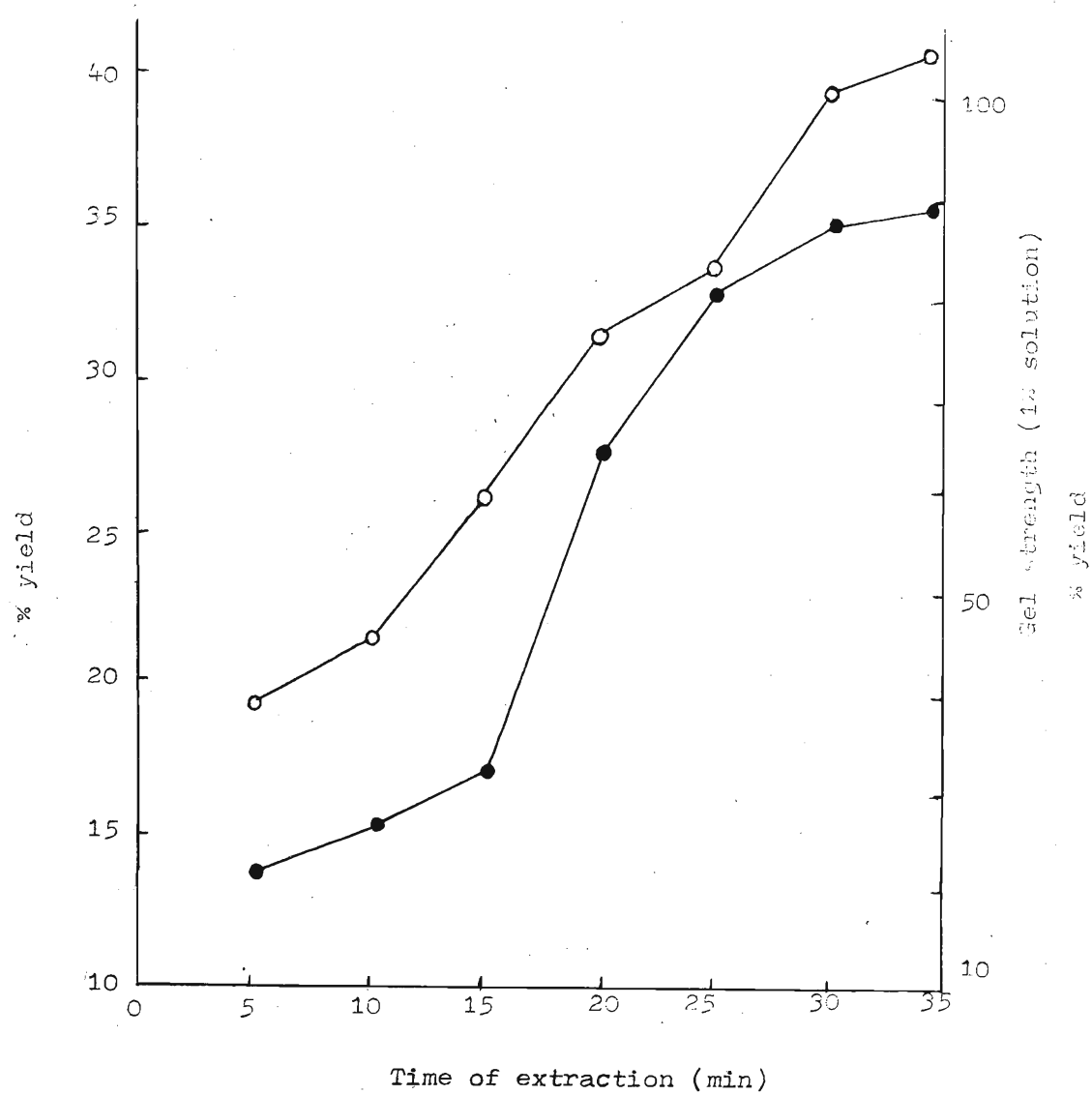

Figure 1. Effect of different periods of extraction on the yield and quality of agar.

o—o — % yield

●—● — Gel strength g cm^{-2}

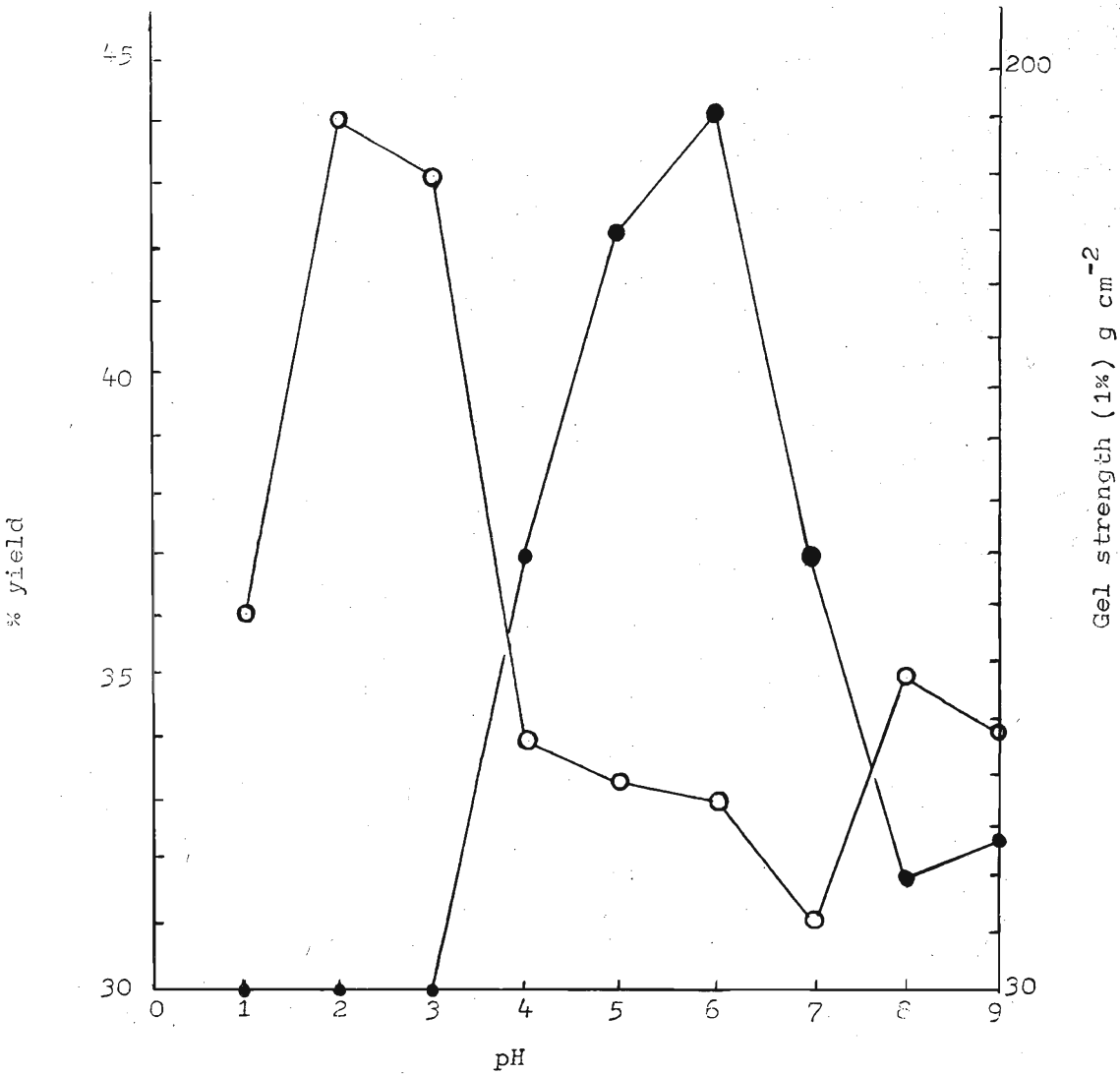

Figure 2. Effect of pH on the yield and quality of agar

— % yield

— Gel strength g cm^{-2}

Table 9. Effect of seaweed: water ratio on the yield and quality of agar.

Ratio of alga to water (w/v)	% agar	Gel strength (1% strength)
1 : 10	5.3	31.5
1 : 20	14.5	58.2
1 : 30	19.3	152.8
1 : 40	23.4	110.8
1 : 50	31.2	113.2

Table 10. Effect of pretreating the seaweed with alkali on the gel strength of agar.

Contration of solution (%)	Gel strength of agar (1% solution) KCl treated	$BaCl_2$ treated
0 (control	70.4	70.2
1	73.5	82.5
2	84.5	89.0
3	99.0	103.5
4	138.5	123.8
5	95.5	98.5

Table 11. Effect of pretreatment of agar with alkali on the gel strength.

Concentration of the solution (%)	Gel strength of agar (1% solution) KCl treated	$BaCl_2$ treated
0 (control)	96.2	96.2
1	136.9	128.0
2	121.4	110.2
3	102.4	102.3
4	98.4	93.8
5	90.5	88.8

Powdered samples of seaweed were soaked for 1h separately in 50 ml KCl and $BaCl_2$, solutions of concentration 1%, 2%, 3%, 4% and 5%. After soaking the seaweeds were washed in distilled water, the agar extracted and the gel strength of agar determined (Table 10).

It is apparent that pretreatment with alkali improved the quality of agar but higher concentration of alkali seemed to lower the gel strength. Pretreatment with $BaCl_2$ appears to be more effective than with KCl solution.

Subsequently agar was extracted from *G. edulis* according to the usual procedure and about 2g of agar was soaked separately in KCl and $BaCl_2$ solution for 1h and washed in water thoroughly after soaking. The gel strength of the agar was then determined (Table 11).

Pretreatment of agar with alkali appears to produce high gel strength agar compared to that of the control but here too higher concentrations of alkali lower gel strength.

5. Discussion

During a preliminary laboratory study on the phenology and life history of *G. edulis* to investigate the selection of seed material, time and method of growth, nature of substrata and rate of growth of alga it was found that the alga can be propagated vegetatively much easily. Observations indicated that vegetative fragments from the basal and middle portions also can grow although not vigorously as the fragments from the apex. During this investigation and observations over many years no carposporic plants of *G. edulis* are observed and this alga appears to be "sterile".

The present investigation reveals that *G. edulis* could be cultivated by the three methods employed but the coral stones supported good growth of the alga. This may be because of the firm support the alga received compared to the coir ropes and nets where the alga is subjected to wave action, as a result portions of algal thalli become detached from the substratum and lost.

Although *G. edulis* could be grown during any month of the year frequent growth of other algae occurred on the coir ropes which prevented the growth of this alga. This was noted with the over growth of *Chaetomorpha sp.* during November 1982, *Struvea sp.* during January 1983 and *Jania sp.* during April 1983. During these periods frequent weeding out of these seaweeds was essential for the better growth of *G. edulis*. During weeding portions of thalli of *G. edulis* were also sometimes removed from coir ropes and lost. The major algae that grew among *G. edulis* on coral stones were species of *Acanthophora* and *Caulerpa.*

One other problem facing cultivation of *G. edulis* is that of grazing by fish particularly species of Siganids. This was evident from the observation that damaged or browsed seaweeds had truncated apical tips as opposed to the tapered apical tips of intact thalli. Stephen *et al* (1981) showed similar browsing of *G. edulis* by herbivore fishes. The present investigation reveals that *G. edulis* could be grown on artificial substrata from vegetative fragments to harvestable size in about three months. Regeneration studies from the harvested algae indicate that plants are able to grow at the same rate as the fresh planting material thus indicating its remarkable regeneration capacity. This makes three harvests possible in an year. Further it is possible to harvest about 1 kg wet *G. edulis* from one metre length of coir ropes.

Of the five species of red algae studied *G. edulis* was found to be superior in terms of yield of agar and the ease with which the agar could be extracted. This alga has been found to be the most abundant of the algae studied. Thus with the indigenous supply of *G. edulis* together with cultivation on artificial substrate it appears that this alga could be made useful for the commercial production of agar in this country. Similar claim has been made elsewhere.[7] *Gelidiella acerosa,* the principal agarophyte in several countries including India has been claimed to yield good quality agar. Similar observation has not been observed with the *G. acerosa* studied and this observation substantiates the observations recorded by Dantanarayana *et al.*[2]

Several reports claim that there is seasonal variation in agar content and gel strength of agar obtained for different months.[8] Similar variations have also been observed in this investigation and the agar obtained during April were of better quality. Raju and Thomas[9] reported that the quality of agar was better in algae obtained in the second and third harvest compared to the first. However, such an improvement was not observed during the present investigation.

Effects of pretreatment of *G. edulis* on the yield and quality of agar have been investigated.[12] During the extraction of agar grinding seaweeds prior to extraction has certainly increased the yield and quality of agar from *G. edulis.* However prior soaking did not improve the quality of agar significantly as indicated by Kappanna and Visweswara Rao.[5] It can be concluded that wet extraction of the ground seaweed at a pH of 5.6 for relatively longer period give better yields of agar with good gel strength. Experiments have also shown that the gel strength of agar could be improved by prior alkali treatments.

While *G. edulis* appears to be the most promising of the red algae studied, other algal species too have been found to yield agar suitable for commercial use in the food industry.

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