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Effect of environmental factors on algal farming of *Spirulina platensis* and *Chlorella vulgaris* in two selected sites of Chennai: Ennore and Manali.

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Abstract:
The present study was designed for pilot scale productions of *Spirulina platensis* and *Chlorella vulgaris* were accomplished at two selected sites of Chennai: Manali and Ennore owing to their location on the coastal belts, among the highly polluted sites, being the native occurrence of the mother culture. The results show that both *Spirulina platensis* and *Chlorella vulgaris* showed maximum production of biomass and metabolites in Ennore site compared to the Manali site. *Spirulina platensis* produced about 1.03g/l and 1.30g/l of biomass in Manali and Ennore respectively, whereas *Chlorella vulgaris* produced about 0.91g/l and 1.00g/l of biomass in Manali and Ennore sites. This study concluded that effect of various environmental study factors in Ennore is very suitable for both *Spirulina platensis* and *Chlorella vulgaris* pilot scale biomass production when compare to Manali.

Key words: Ennore, Manali, *Spirulina platensis, Chlorella vulgaris*, Biomass, Algae, Farming

INTRODUCTION:

Various forms of energy may produce from the natural fossils. Energy produced from natural resources is not enough to meet the demands of the high density populations. Many researches studies are under process to produce energy from alternative feedstock's. Hence algal farming is one of the alternative feedstock's to produce energy (Alpesh and Nirvesh, 2015). To identify the potential sites for algae biomass production, considering atmospheric carbon level, area of the land and level of carbon were measured with respect to biomass production (Jason *et al.,* 2012). The choice for finding algae farming in a chosen location, as with numerous different advances, begins with assessing the resources potential. Positive atmosphere conditions and accessibility of water, CO_2, and different supplements (primarily nitrogen and phosphorous) must be adjusted to appropriate land qualities geography, soil, and use to decide the most optimal area for cultivation. Since these resources change considerably with one geographic area then onto the next, ideal location of algae cultivating frameworks requires learning of the particular resources accessibility, magnitude, and changeability at any selected location. The effect of atmosphere on algae growth is practically identical to the effect of atmosphere on aquatic plants. Like higher plants, algae require unlimited daylight and have contrasting acceptance to temperature. Closed light bioreactors show less significance to atmosphere changeability than open ponds because of their environment controlled up to some extent. Sunlight intensity and the quantity of daylight hours specifically influence algal growth efficiency; precipitation and vanishing influence water supply, and extreme climate impacts water quality. Another imperative atmosphere component influencing algal growth development is the quantity of daylight hours per day. In many parts of India, clear sunny climate is seen about 250 to 300 days for every year with the daylight hours going somewhere around 2,300 and 3,200 every year depends on the locations (Muneer *et al.,* 2004).Water loss by evaporation is an essential variable to consider, while picking areas for open lake cultivating in light of the fact that it can altogether increase the working expenses. Rate of evaporation take after firmly climatic seasons, they are low amid the winter (2–6 mm/day), monsoon (4–10 mm/day), and post-monsoon (4–8 mm/day) months and achieve their peak in the mid year months of April and May (5–16 mm/day) (NIH, 2009). Saline water surfaces vanish not as much as freshwater surfaces. In this way, algal growth ponds utilizing harsh or saline water should to be considered in area where the evaporation rates are very high. The renewable water resources in India are evaluated at 1,869 km3 every year (MWR, 2009). India has more than 20 river streams giving water system, drinking water, transportation, power, and the live hoods for many populations throughout the nation. One of the advantages of developing algae farming is that it can use low-quality water with few contending uses, for example, bitter/saline groundwater, "co-delivered water" from oil and gas wells, and wastewater

released from household, modern, and horticultural exercises. Accordingly, algal cultivation does not require extra request on freshwater supplies. Utilizing low quality water for algal cultivations can fill two needs: one is to discard water that would some way or another be exorbitant (financially and ecologically) to arrange, and the other is to use low quality water to make items that have high economic values—algal biofuels and biomass for animal nourishment or compost. High salt concentration in ground water were normally seen in a few sections of India, for example, the coastal regions, however as a rule this has been increased by human exercises quicken the activation and amassing of salt. Numerous types of algal growth have a high resistance to saltiness; in this manner, addition to coastal regions, the algae farming can target parts of the nation where the nearness of saline groundwater resources keeps their immediate use in different applications. On the off chance that harsh/saline water is utilized for algal cultivation, an essential thought is the place and how algal culture media is discarded after cultivation. Wastewater released from residential, modern, and rural exercises is another source of low-quality water that could be utilized for algal cultivation. Utilizing wastewater as culture medium in co-found algal farms with offices treating sewage, modern wastewater, and agrarian seepage waters gives a savvy arrangement to water as well as to land and supplements contemplations in light of the fact that the wastewater treatment capacity would take care of almost all expenses. Ideal algal cultivation happens in a high CO_2 environment. Thusly, algal cultivation gives an incredible chance to the use of carbon emanations and serves as a supplement to subsurface sequestration. India was the fourth-biggest emitter of CO_2 in 2006, discharging 1,510 Mt into the air. The biggest anthropogenic wellspring of CO_2 emanations in India is the burning of fossil energizes utilized as a part of power generations, modern procedures, transportation, agribusiness, business administrations, and the private division. All atmospheric CO_2 are not appropriate for algae. This is relevant to expansive stationary sources with high centralizations of CO_2. In India, these incorporate warm power plants, steel plants, concrete plants, compost plants, refineries, and petrochemical plants. The idea of co-finding high CO_2 location with algal cultivating gives a compelling way to deal with reuse the CO_2 into a useable product. The accessibility of land for algal cultivation in India will rely on upon numerous physical, monetary, legitimate, social, and political elements. Physical qualities, for example, geography and soil, could restrict the land accessible for algal cultivating in a few areas. Geology would be a constraining variable for these frameworks since the establishment of substantial shallow ponds requires moderately level territory. Larger part of the nation is appropriate for algal cultivation from geography's viewpoint with the exception of the mountain zones in the north, east, and some inner parts of the nation. Different contemplations that may confine the land accessible for algal cultivations are land possession and land cost. Land ownership data gives significant bits of knowledge on which strategies and gatherings could influence project development. Information ashore costs in India are hard to get because of the high fluctuation between and within the states. Land costs are difficult to sum up in light of the fact that they rely on upon various elements including area, accessibility of water, and closeness to transportation infrastructure (Anelia and Eric, 2010).

MATERIALS AND METHODS:

Isolation and Identification:

Algae were isolated from the Ennore Estuary water samples by serial dilution and the samples were streaked on Petri plates containing agar with algae growth medium. *S. platensis* was cultured in Zarrouk medium (1966) and *C. vulgaris* was cultured in Bold basal medium (Ilavarasi *et al.,* 2011). Algal colonies grown were isolated and sub cultured in fresh agar plates. Later colonies were inoculated in freshly prepared liquid culture medium. All microalga genera in the water samples were identified based on their morphological characters such as colour of scum, fibrous nature, floating (or) deposited filaments, heterocyst and shape using low and high power objectives of the compound microscope. Due to the high dominance of *S.platensis* and *C.vulgaris* in the collected water samples, further investigations were carried out with the same.

Mass cultivation of test algae on biomass production during different months:

Due to the various environmental factors and the other foreign organism's contaminations, the cultivation of algae is a complicated task. High biomass productivity may be affected by various environmental factors, so it's very important to find out the suitable temperature, pH and light intensity. Temperature along with pH and light intensity are the important restrictions for high biomass productivity. Ennore and Manali are the two sites chosen for biomass production in outdoor conditions.

Culture Conditions:

Algal farming in selected Chennai sites was performed in 50 liters capacity plastic barrels. Clean environment is playing an important factor for avoiding cross contaminations. Places near culturing barrel were cleaned from dust, leaves and other particles. Algae cultures may get harm by direct sunlight, so all the cultured barrels were kept in shadow. 1 liters of culture medium was prepared and 100ml of mother cultures were inoculated in the respective barrels. Both *S. platensis* and *C. vulgaris* culturing barrels were kept away from each other and also all other aids like mixing rods, collection jugs, and conical flasks were kept separate for both cultures to avoid cross contamination from each other. The volume of cultures was increased up to 40 liters with respective mediums after reaching appropriate growth. Loss of water level by evaporations was compensated by adding freshly prepared culture medium.

Effect of environmental factors:

The current experiments are to evaluate the effect of various environmental elements on the biomass productivity of test algae in selected sites. Temperature and light intensity were two important environmental elements that affect biomass productivity because these two elements may highly vary in the natural conditions in different months of the year. Effect of these two parameters were analysed for three months in summer (March, April and May) and also three months in monsoon (September, October and November).

Temperature:

One of the very essential climatic changes that affect the algal growth is temperature. Temperature lesser than 16°C and greater than 36°C may slow down the growth of culture. Temperature in Chennai ranges from 20°C - 25°C in March and gradually increased up to 40°C in the month of May. In monsoon period the temperature decreased to 30°C and that prolongs till November. Temperature was measured using centigrade thermometer for all cultures in various months of summer and monsoon in the two selected sites of Chennai.

Light intensity:

Another important growth limiting factors is light. Algae required light for its normal growth, but direct sunlight is not the best choice of illumination. Light intensity was increased during the summer season that will affect the normal growth. Measures like mixing the cultures barrels multiple times during the day, keeping culturing barrels under shadow were done to overcome the above problem. During the monsoon the cultured barrels were closed with plastic covers during raining to maintain the water levels. Digital lux meters were used to record the light intensity on constant interval during the growth period in summer and winter months.

pH:

pH is also a vital factor that affects the biomass productivity of a test alga. The pH of the culture should be between 9.0 to 11.0 for the normal growth of the algae. Various contaminations may appear if the pH of the culture is changed during biomass productions. pH was determined using digital pH meter at regular intervals during the culture period in various months of summer and monsoon seasons at the selected Chennai sites.

Harvesting:

Morning is the best time for harvesting algae, because of having enough time to dry the wet algae biomass on the same day. On the 20^{th} day following inoculation, the biomass was harvested using gravity filtration method. Algae cultures were first filtered by normal cloth to remove dust and leaves etc. Secondly the cultures were filtered by using 380-500 mesh cloth made by nylon. Biomass slurry was washed with tap water to remove excess salts.

Drying:

The filtered biomass was dried in the room temperature for 24 hours or more depending on the month of cultivation. Dried algal flaks were grinded in a mixer to get fine algal powder, which will be stored in plastic containers for further

analysis. The biomasses obtained from various months were examined for growth studies and other biochemical metabolites were analysed.

Assay on algal metabolites:

Growth of cultures was estimated by OD method (Vonshak, 1997). Biomasses were estimated by filtration method (Sivakumar and Rajendran, 2013). Total Protein content was estimated by Lowry's method (Lowry *et al.,* 1951). Total carbohydrate content was determined by anthrone method (Roe. 1955). Total lipid content was determined by solvent extraction method (Bligh and Dyer, 1959). Total chlorophyll content was calculated by Tandeu and Houmard. 1988. Total carotenoids content was calculated by Saleh *et al.,* 2011. Total phycocyanin content in *S.platensis* was estimated by Bennet and Bogorad, 1973.

RESULTS:

Isolation and Identification:

A total of 62 isolates of *S.platensis* and 54 isolates of *C.vulgaris* were obtained from their selective medium respectively. Based on the morphological characters, 35 isolates of *S.platensis* and 28 isolates of *C.vulgaris* were identified contributing to their high dominance of 56% and 51% respectively. Hence, further investigations were carried with the above respective strains. The isolation and identification of *S.platensis* and *C.vulgaris* by agar plating method are presented in plate 1.

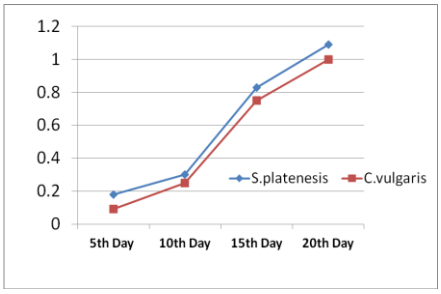
Preliminary growth studies of unialgal cultures:

Biomass production of unialgal mother cultures of *S.platensis* and *C.vulgaris* were presented in table 1 and fig1 shows that there was a rapid increase in biomass was observed, that maximal slope indicating maximum growth was reached on 20th day during log phase for both algal strains. The stationary phase is reached where there is no change in the biomass leading to decline phase.

Table: 1 Growth studies of unialgal *S.platenesis* and *C.vulgaris*. Each value is a mean of 4 individual analysis with standard error.

Days	*S. platensis* Biomass (g/l) (Mean ± SEM)	*C. vulgaris* Biomass (g/l) (Mean ± SEM)
5th	0.18 ± 0.009	0.09 ± 0.005
10th	0.30 ± 0.040	0.25 ± 0.018
15th	0.83 ± 0.012	0.75 ± 0.040
20th	1.09 ± 0.081	1.00 ± 0.021

Fig1. Biomass production of uni algal *S.platensis* and *C.vulgaris*.the culture period at Ennore and Manali.



Biomass cultivation of *S. Platensis* and *C. Vulgaris*:

To identify the optimal site for algal farming two sites were selected in Chennai for algal biomass production in various seasons of both sites. The biomass production of *S. platensis* and *C.vulgaris* at Ennore are presented in plate 2.

Environmental Factors and Algae Metabolites:

Environmental factors such as temperature, light intensity, pH and the biomass production of *S.platenesis* and *C.vulgaris* grown under outdoor conditions at Ennore and Manali are presented in table 2 & 3. The mean values of *S.platenesis and C.vulgaris* metabolites at the end of the culture period in Ennore and Manali are presented in table 4 & 5.

Table: 2 Environmental factors such as temperature, light intensity, pH and the biomass production of *S.platenesis* and *C.vulgaris* grown under outdoor conditions at Ennore.

	Environmental factors		*S. platensis*		*C. vulgaris*	
Month	Temperature (° C)	Light (Lux)	Final pH (Mean ± SEM)	Biomass (g/l) (Mean ± SEM)	Final pH (Mean ± SEM)	Biomass (g/l) (Mean ± SEM)
March	25.4– 28.0	67500- 69000	9.00± 0.10	0.88 ± 00.18	8.00 ± 0.05	0.61 ± 00.24
April	31.7 - 36.4	72600- 75100	9.57 ±0.05	1.19 ± 00.47	8.84 ± 0.22	0.84 ±00.17
May	33.8 – 39.1	74800- 77200	11.67 ±0.11	1.30 ± 00.21	9.10 ± 0.19	1.00 ±00.06
October	26.0 – 30.8	59000- 62400	11.21 ±0.08	1.15 ±00.08	8.29 ± 0.07	0.92 ± 00.31
November	25.1 – 28.3	55600 -58100	10.51 ±0.19	0.80 ± 00.15	8.21 ± 0.24	0.99 ± 00.26
December	23.4 – 27.9	56800 – 57000	10.00± 0.14	0.72 ±00.29	8.74 ± 0.31	0.80 ±00.10

From the Table: 2, it is inferred that standard error mean of *S. Platensis* and *C. Vulgaris* shows the significance in their biomass production in Ennore. Since the P value is less than 0.05 the biomass production of *S. Platensis* and *C. Vulgaris* shows significance with environmental factors during the various month of cultivation in Ennore.

Table: 3 Environmental factors such as temperature, light intensity, pH and the biomass production *of S.platenesis* and *C.vulgaris* grown under outdoor conditions at Manali.

	Environmental factors		*S. platensis*		*C. vulgaris*	
Month	Temperature (° C)	Light (Lux)	Final pH (Mean ± SEM)	Biomass (g/l) (Mean ± SEM)	Final pH (Mean ± SEM)	Biomass (g/l) (Mean ± SEM)
March	26.2– 30.5	66000- 68500	8.09± 0.24	0.80 ± 00.22	8.28 ± 0.11	0.73 ± 00.10
April	30.7 - 34.0	70400- 73000	9.12 ±0.41	0.93 ± 00.17	8.94 ± 0.30	0.80 ±00.27
May	31.9 – 37.2	72700- 76000	10.82 ±0.09	1.03 ± 00.05	9.58 ± 0.07	0.91 ±00.20
October	25.5 – 27.0	56200- 60100	10.71 ±0.18	0.84 ±00.11	8.20 ± 0.05	0.72 ± 00.09
November	23.7 – 27.4	52400 -55000	10.60 ±0.30	0.77 ± 00.26	8.19 ± 0.18	0.65 ± 00.20
December	20.3 – 26.1	53000 – 55600	10.39± 0.25	0.69 ±00.09	8.55 ± 0.22	0.60 ±00.04

From the Table: 3, it is inferred that standard error mean of *S. Platensis* and *C. Vulgaris* shows the significance in their biomass production in Manali. Since the P value is less than 0.05 the biomass production of *S. Platensis* and *C. Vulgaris* shows significance with environmental factors during the various month of cultivation in Manali

Table: 4 The mean values of *S.platenesis* metabolites end of the culture period at Ennore and Manali.

	Ennore						Manali					
Month	Carbo (mg/g) Mean	Protein (mg/g) Mean	Fat (mg/g) Mean	Chlo (mg/g) Mean	Caro (mg/g) Mean	Phyco (mg/g) Mean	Carbo (mg/g) Mean	Protein (mg/g) Mean	Fat (mg/g) Mean	Chlo (mg/g) Mean	Caro (mg/g) Mean	Phyco (mg/g) Mean
March	198.2	540.4	35.4	8.4	1.0	108.0	182.0	503.	29.0	6.9	0.9	89.0
April	200.9	570.0	38.0	8.9	1.8	114.8	191.8	533.5	35.4	7.8	1.3	97.8
May	210.4	600.5	40.7	10.0	2.4	120.7	200.2	590.0	38.1	8.4	2.0	113.9
October	201.2	592.8	38.5	9.5	2.2	118.4	195.1	564.1	33.7	8.0	1.8	100.0
November	197.4	561.3	37.0	9.0	1.5	101.3	192.4	529.8	30.4	7.5	1.2	94.2
December	190.2	535.7	34.8	8.7	1.4	99.7	189.0	517.0	25.9	7.2	0.8	89.4

From the Table: 4, it is inferred that standard error mean of *S. Platensis* metabolites shows the significance in their production, Since the P value is less than 0.05 *S. platensis* metabolites production shows significance with environmental factors during the various month of cultivation in Ennore and Manali.

Table: 5 The mean values of *C.vulgaris* metabolites end of the culture period at Ennore and Manali.

	Ennore					**Manali**				
Month	Carbo (mg/g) Mean	Protein (mg/g) Mean	Fat (mg/g) Mean	Chlo (mg/g) Mean	Caro (mg/g) Mean	Carbo (mg/g) Mean	Protein (mg/g) Mean	Fat (mg/g) Mean	Chlo (mg/g) Mean	Caro (mg/g) Mean
March	264.0	500.8	66.2	9.4	3.7	260.0	479.0	54.7	8.0	1.9
April	279.5	546.7	69.1	11.0	4.0	269.5	500.4	58.0	8.5	2.5
May	300.7	590.0	71.7	12.0	4.9	280.3	520.2	60.9	9.2	3.1
October	288.0	582.3	60.9	10.4	4.2	259.1	509.4	52.1	8.4	2.0
November	265.8	587.4	57.2	10.0	3.5	241.7	471.7	44.8	7.2	1.4
December	200.0	520.5	51.0	8.2	2.9	199.2	420.0	41.2	6.5	1.0

From the Table: 5, it is inferred that standard error mean of C. vulgaris metabolites shows the significance in their production, Since the P value is less than 0.05 C. vulgaris metabolites production shows significance with environmental factors during the various month of cultivation in Ennore and Manali.

Temperature:

Temperature recorded during different months at Ennore and Manali are presented in fig. 2. The data obtained show that the highest temperature (39.1°C) was recorded in the month of May and lowest temperature (27.9°C) was recorded in the month of December at Ennore. In Manali that the highest temperature (37.2°C) was recorded in the month of May and lowest temperature (26.1°C) was recorded in the month of December.

Light intensity:

Light intensity measured during day in different months in Ennore and Manali are graphically shown in fig. 3. The measured data show that in Ennore the highest light intensity (7.72k lux) was recorded in the month of May and

lowest light intensity (5.70k lux) in the month of December. In Manali the highest light intensity (7.60k lux) was recorded in the month of May and lowest light intensity (5.50k lux) was recorded in the month of November.

pH:

The final pH recorded on the harvesting day during different months in Ennore and Manali were represented in fig. 4 and 5. The obtained data show that in Ennore the highest pH of *S.platensis* culture (11.67) was recorded maximum in the month of May and lowest pH (9.00) was recorded in the month of March. In Manali the highest pH of *S. platensis* (10.82) was 91 recorded in the month of May and lowest pH (8.09) was recorded in the month of March. Similarly, in Ennore the highest pH (9.10) of *C. vulgaris* culture was recorded in the month of May and lowest pH (8.00) was recorded in the month of March. In Manali highest pH (9.58) of *C. vulgaris* culture was recorded in the month of May and lowest pH (8.19) was recorded in the month of November.

Fig 2 Changes in temperature during the culture period at Ennore and Manali.

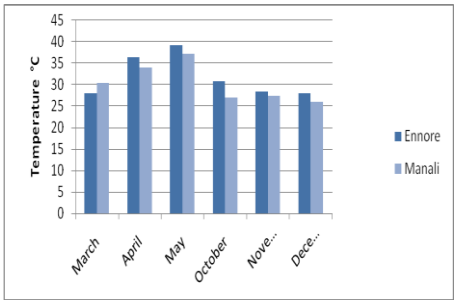
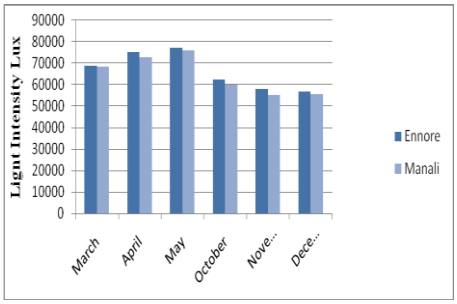


Fig 3. Changes in light intensity during the culture period at Ennore and Manali.



4. Changes in culture pH of *S. platenesis* during the culture period at Ennore and Manali.

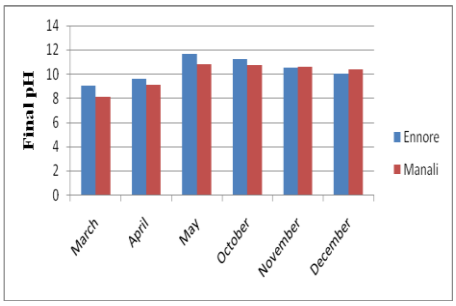
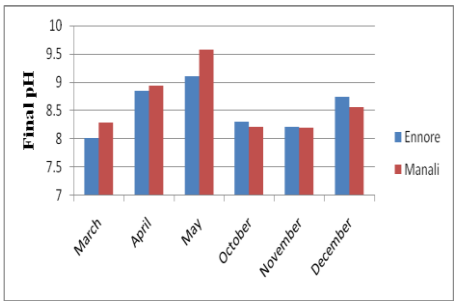


Fig 5. Changes in culture pH of *C.vulgaris* during the culture period at Ennore and Manali.



Biomass production:

Biomass production of *S.platensis* and *C.vulgaris* at selected Chennai sites on different seasons was graphically shown in fig. 6 & 7. Data collected on the *S.platensis* biomass estimation showed that the maximum biomass production (1.30g/l) was obtained in the month of May and minimum biomass production (0.72g/l) was obtained in the Month of December at Ennore. The data clearly show that the highest biomass production (1.03g/l) of *S. platensis* was recorded in the month of May and lowest biomass production (0.69g/l) was observed in the month of December at Manali. Data show that the maximum biomass production (1.00g/l) of C.vulgaris was obtained in the month of May and minimum biomass production (0.61g/l) was obtained in the month of March at Ennore. Result shows that the maximum biomass production (0.91g/l) of *C.vulgaris* was obtained in the month of May and minimum biomass production (0.60g/l) was obtained in the month of December at Manali.

Fig 6. Biomass production of *S.platenesis* end of culture period at Ennore and Manali

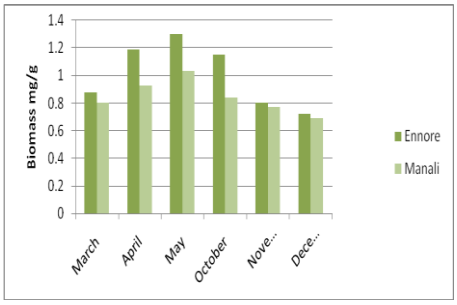
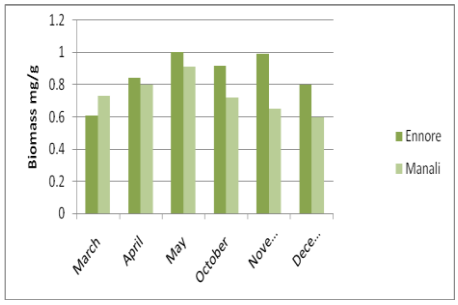


Fig 7. Biomass production of *C.vulgaris* end of culture period at Ennore and Manali.



Carbohydrate content:

The data on the carbohydrate content of *S.platensis* and *C.vulgaris* at selected Chennai sites on different seasons were graphically shown in fig. 8 & 9. Data show that the maximum carbohydrate (210.4mg/g) content of *S.platensis* was observed in the month of May and minimum carbohydrate content (190.2mg/g) was observed in the month of December at Ennore. The highest carbohydrate content (200.2mg/g) of *S. platensis* was obtained in the month of May and the lowest carbohydrate content (182.0mg/g) was observed in March at Manali. It is clear that the maximum carbohydrate content (300.7mg/g) of *C.vulgaris* was observed in the month of May and minimum carbohydrate content (200.0mg/g) was observed in the month of December at Ennore. Evidence show that the highest carbohydrate content (280.3mg/g) of *C.vulgaris* was obtained in the month of May and lowest carbohydrate content (199.2mg/g) was observed in month of March at Manali.

Fig 8. Carbohydrate content of *S.platenesis* end of culture period at Ennore and Manali.

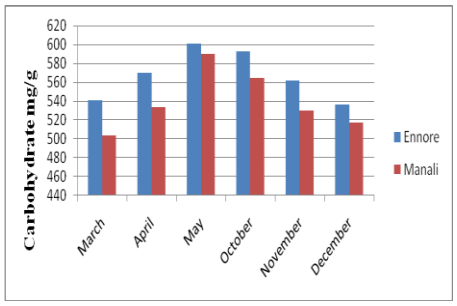
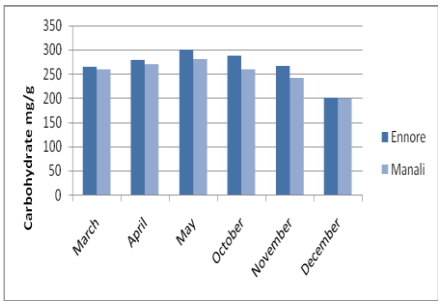


Fig 9. Carbohydrate content of *C.vulgaris* end of culture period at Ennore and Manali.



Protein content:

Data on protein content of *S.platensis* and *C.vulgaris* at selected Chennai sites on different seasons were shown in fig. 10 & 11 respectively. Data show that the highest protein content (600.5mg/g) of *S.platensis* was observed in the month of May and the lowest protein content (535.7mg/g) was observed in the month of December at Ennore. The highest protein content (590.0mg/g) of *S.platensis* was obtained in the month of May and the lowest protein content (517.0mg/g) was observed in the month of March at Manali. It is evident that the maximum protein content (590.0mg/g) of *C.vulgaris* was observed in the month of May and minimum protein content (500.8mg/g) was observed in the month of March at Ennore. It is evident from Fig. 11 that the highest protein content (520.2mg/g) of *C.vulgaris* was obtained in the month of May and the lowest protein content (420.0mg/g) was observed in month of March at Manali.

Fig 10. Protein content of *S.platenesis* end of culture period at Ennore and Manali.

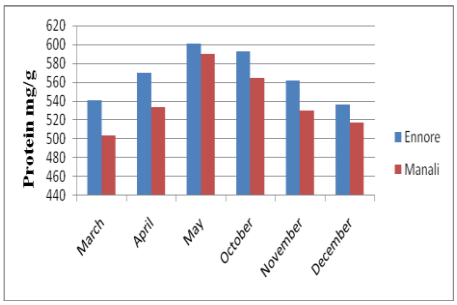
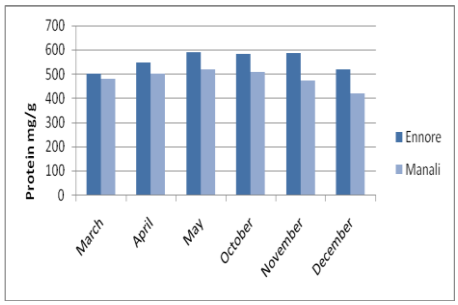


Fig 11. Protein content of *C.vulgaris* end of culture period at Ennore and Manali.



Fat content:

Fat content of *S.platensis* and *C.vulgaris* at selected Chennai sites on different seasons were studied and data are graphically shown in fig. 12 & 13. From the table 4, it shows that the highest fat content (40.7mg/g) of *S.platensis* was observed in the month of May and the lowest fat content (34.8mg/g) was observed in the month of December at Ennore. Fig.12 clearly shows that the highest fat content (38.1mg/g) of *S.platensis* was obtained in the month of May and the lowest fat content (517.0mg/g) was observed in the month of March at Manali. Table 5, it clearly shows that the maximum fat content (71.7mg/g) of *C.vulgaris* was observed in the month of May and minimum fat content (51.0mg/g) was observed in the month of December at Ennore. Evidence from Fig. 13, show that the highest fat content (60.9mg/g) of *C.vulgaris* was obtained in the month of May and the lowest fat content (41.2mg/g) was observed in month of December at Manali.

Fig 12. Fat content of *S.platenesis* end of culture period at Ennore and Manali.

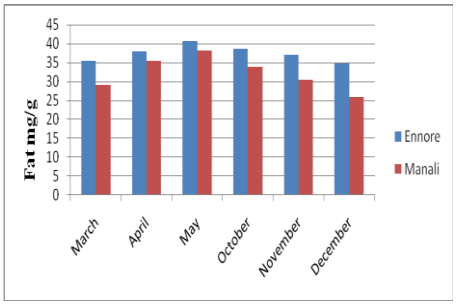
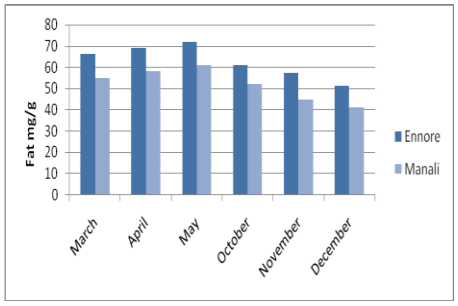


Fig 13. Fat content of *C.vulgaris* end of culture period at Ennore and Manali.



Chlorophyll content:

The data on chlorophyll content of *S.platensis* and *C.vulgaris* at selected Chennai sites on different seasons were represented in fig. 14 &15. From the table 4, it is observed that the maximum chlorophyll content (10.0mg/g) of *S.platensis* was observed in the month of May and minimum chlorophyll content (8.4mg/g) was observed in the month of March at Ennore. Fig. 14 clearly shows that the maximum chlorophyll content (8.4mg/g) of *S.platensis* was obtained in the month of May and minimum chlorophyll content (6.9mg/g) was observed in the month of March at Manali. Table 5, it is clearly shows that the maximum chlorophyll content (12.0mg/g) of *C.vulgaris* was observed in the month of May and minimum chlorophyll content (8.2mg/g) was observed in the month of December at Ennore. It is clear from fig. 15 that the maximum chlorophyll content (9.2mg/g) of C.vulgaris was obtained in the month of May and minimum chlorophyll content (6.5mg/g) was obtained in month of December at Manali.

Fig 14. Chlorophyll content of *S.platenesis* end of culture period at Ennore and Manali.

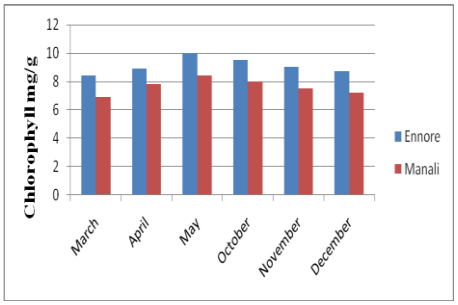
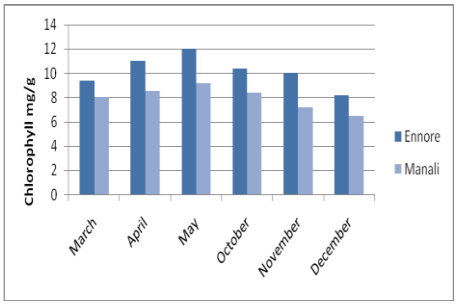


Fig 15. Chlorophyll content of *C.vulgaris* end of culture period at Ennore and Manali.



Carotenoids content:

The data on carotenoids content of *S.platensis* and *C.vulgaris* at selected Chennai sites on different seasons were given in fig. 16 & 17. Table 4, shows that the maximum carotenoids content (2.4mg/g) of *S.platensis* was observed in the month of May and minimum carotenoids content (1.0mg/g) was observed in the month of March at Ennore. Fig. 16 clearly shows that the maximum carotenoids content (2.0mg/g) of *S.platensis* was obtained in the month of May and minimum carotenoids content (0.8mg/g) was observed in the month of December at Manali. The table 5 shows that the maximum carotenoids content (4.9mg/g) of *C.vulgaris* was observed in the month of May and minimum carotenoids content (2.9mg/g) was observed in the month of December at Ennore. Fig. 17 shows that the maximum carotenoids content (3.1mg/g) of *C.vulgaris* was obtained in the month of May and minimum carotenoids content (1.0mg/g) was observed in month of December at Manali.

Fig 16. Carotenoids content of *S.platenesis* end of culture period at Ennore and Manali.

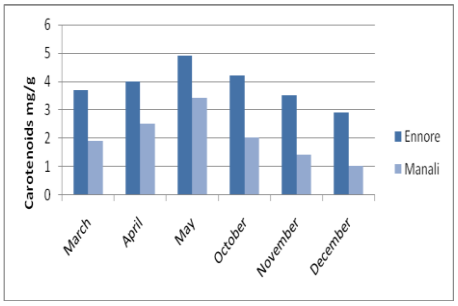
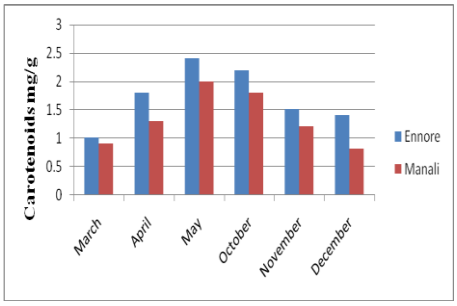


Fig 17. Carotenoids content of *C.vulgaris* end of culture period at Ennore and Manali.



Phycocyanin content:

The data on phycocyanin content of *S.platensis* at selected Chennai sites on different seasons was graphically shown in fig. 18. From the table 4, it is clear that the maximum phycocyanin content (120.7mg/g) of *S.platensis* was observed in the month of May and minimum phycocyanin content (99.7mg/g) was observed in the month of December at Ennore. Fig. 18 clearly shows that the maximum phycocyanin content (113.9mg/g) of *S.platensis* was obtained in the month of May and minimum phycocyanin content (89.0mg/g) was observed in the month of March at Manali.

Fig 18. Phycocyanin content of *C.vulgaris* end of culture period at Ennore and Manali.

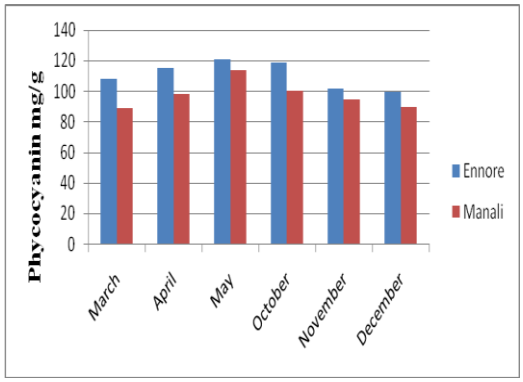


Plate 1. Isolation and Identifcation of *S. platensis* and *C. vulgaris*

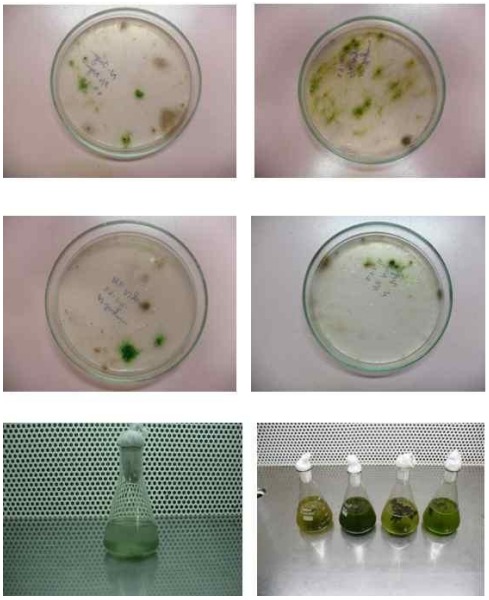


Plate 2. Out door cultivation of *S. platensis* and *C. vulgaris*

Discussion:

Following the metabolic assays of chosen algal strains under laboratory scale, a pilot scale study for commercial production of *S. platenesis* and *C.vulgaris* was initiated in 50 liters barrel in outdoor conditions at Ennore and Manali under various seasons. Various factors influence the algal growth like clarity of the water, time of day, effective range of sunlight, shape, color or transparency, wall thickness and size of the container (Lee and Palsson, 1994) among which the following environmental factors temperature, light intensity and pH of the cultures are highly significant. The highest biomass was recovered during the month of May at Ennore compared to Manali signifying the influence of above said optimal environmental factors (Table 2 & 3). Studies infer that the optimal growth temperatures are ranging from 24 to 42°C (Tomaselli and Vonshak, 2000).The highest biomass was recovered during May when the maximal temperature reached about 39°C and lowest biomass during December acclaiming a minimal temperature of 26°C. Supportively Barsanti and Gualtieri (2014) reported that temperatures lower than 16°C will slow down growth

whereas those higher than 35°C are lethal for a number of species. Richmond (1986) had also concluded that minimal temperatures for production of *S. platenesis* and *C.vulgaris* at outdoor was 18°C. Light intensity is affected by culture depth and density of the algae affecting the overall biomass productivity as well as pigment biosynthesis (Barsanti and Gualtieri, 2014; Carvalho and Malcata, 2003; Danesi et al; (2004). Light intensity is also closely associated with infrared component of sunlight, being responsible for the high temperature (Vonshak *et al*., 1982). Maximum light intensity in May in dense cultures are responsible for higher growth rates while lower daytime and temperature in winter results lower growth rate of the chosen algae. Past researches have confirmed high tolerance to light and temperature can increase microalgae productivity than in suboptimal condition (Vonshak *et al*., 1982; Renaud *et al*., 2002; Danesi *et al*., 2004). Considering pH, no system for pH control was established during this pilot study. Yet, the final pH does not exceed 11.6 and 9.58 for *S.platenesis* at Ennore and *C.vulgaris* at Manali respectively (Fig, 4 & 5). Maintaining the pH above 9.5 is mandatory in large open ponds in order to avoid contamination with other microalgae (e.g., diatoms) and exceeding above 12.5 leads to their decline phase (Fox. 1996; Belay; 1997). Accordingly the pilot scale production observed a stationary pH below 10.0, during monsoon for both the algal strains. *S.platenesis* is an alkaliphile, and its optimal pH is reported to be 9 – 11 (Clement *et al*., 1980); Gajraj (1994) and Venkataraman *et al*., 1995)). It is also reported that the algae can survive up to pH 13.5 (Zarrouk, 1966). Pandey and Amit (2010) had examined the influence of pH on *S. platensis* yield dry weight of 0.91g/500ml whose protein and Chlorophyll- A content were 64.3% and 13.2mg/gm respectively at pH 9. Feng *et al*., (2011) reported that the cultures of *C. zofingiensis* reached higher biomass productivity during spring compared to autumn that could be influenced by the differences in temperature and light intensity during the two seasons (Carlsson *et al*., 2007). Additionally, the proper agitation of cultures was also found helpful in controlling the photo inhibition (Hu and Richmond 1996) and agitating twice a day for 2 minutes stimulated the production rate (Soni *et al*., 2006). Agitation helps in the uniform exposure of the algae to sun light and uniform distribution of nutrients. The biomass production of *S. platenesis* and *C.vulgaris* was reduced by 13% and 18 % when compared to biomass obtained from lab cultures respectively (Table: 2 & 3). The reduction in biomass production obtained in this study was however not as high as compared to the studies conducted by Liang *et al*., (2009) and Widjaja *et al*., (2009), who had reported 38% and 50% of reduction respectively. But Lourduraj, 2016 showed that the outdoor biomass production of *Chlorella* was 1.69g/l which was higher than the biomass obtained in Ennore and Manali. The impact of seasonal factors are further supported by Sigita *et al*., (2014) showing a similar result of highest outdoor biomass production of *S.platenesis* in the month of May, when compared to other months in Agra, also showed high biomass production in the cultures growing in high temperature. The biochemical composition of *S.platenesis* and *C.vulgaris* grown in Ennore and Manali sites was analysed and found that the Ennore site yielded higher product rates of biochemical components. Higher protein content of *S. platenesis* and *C.vulgaris* was obtained during May at Ennore, the values being 60.5% and 59% respectively, compared to cultures at Manali, the values are being 50.3% and 52%. Further, the cultures harvested during March and December yielded a lower protein content of 50.3% for *S. platenesis* and 42% for *C.vulgaris* at Manali respectively. Similar results were obtained by Tolga goksan *et al*., 2007 and Affan *et al*., (2016) in *S. platenesis* (56.5% protein) in outdoor biomass cultivation. Few other supportive evidences were also found from studies of Heberto et al., (2016) yielding 49.8% protein and Abreu *et al*., (2012) yielding 47.4% protein in *C.vulgaris*. However the highest protein content obtained in our study was significantly higher than that (26%) found in *C. vulgaris* cultivated in optimized mixotrophic medium with pure glucose as carbon source (Kong *et al*., 2011). Likewise the carbohydrate content of *S. platenesis* and *C.vulgaris* obtained during May at Ennore were higher as 21% and 30% respectively compared to cultures at Manali with 20% and 28% of carbohydrate content respectively. Further, the cultures harvested during December yielded a lower carbohydrate content of 18.9% for *S. platenesis* and 19.9% for *C.vulgaris* at Manali respectively (Table 4 & 5). Similar studies in *S. platenesis* by Kamil *et al*., (2012), Piorreck *et al*., (1984), Soni *et al*., (2012) reported that 24.5%, 26% and 14.5% of carbohydrate respectively. In *C.vulgaris* similar studies made by Spolaore *et al*., 2006 and Yasmin et al., 2011reported that 17%, and 29.8% of carbohydrate respectively. Hitchcock (1980) highlighted that, the carbohydrate content of microalgae is related to light intensity, temperature and stationary phase of the culture medium. Higher fat content of *S. platenesis* and *C.vulgaris* were obtained during May at Ennore of 4% and 7.1% respectively compared to cultures at Manali the values being 3.8% and 6%. Further, the cultures harvested during March and December yielded a lower fat content of about 2.5% for *S. platenesis* and 4.1% for *C.vulgaris* at Manali respectively. Wrede *et al*., (2014) and Fedekar *et al*., (2012) reported 5.25% and 6.5% of fat in outdoor cultures of *S. platenesis* respectively. Koru and Cirik (2003) reported that *S. platenesis* culture grown at the temperatures of 28°C to 45°C had the lipid content of 7.4% and 11.3% respectively. Supportive results by Attilio *et al*., in (2009) and Liliana Rodolfi *et al*., (2008) showed 14.7% of lipid content in outdoor

C.vulgaris cultures. The production of pigments like chlorophyll, phycocyanin and carotenoids are highly influenced by environmental factors as differing conditions of light intensity affects the algal photosynthetic apparatus altering the rate of biosynthesis of these supra molecular complexes of thylakoids. The cyanobacterium *S.platensis* was found to be an attractive alternative source of the pigment chlorophyll, which should be used as a natural color in food, cosmetic, and pharmaceutical products. Obtained data showed that the maximum chlorophyll content (0.1% and 0.08%) of *S.platensis* was seen in the month of May and the lowest chlorophyll content (0.08% and 0.06%) was seen in the month of March at Ennore and Manali. The highest chlorophyll content (1.2% and 0.9%) of *C.vulgaris* was observed in the month of May and the lowest chlorophyll content (0.8% and 0.6%) was 169 observed in the month of December at Ennore and Manali. These results agree with those of Devanathan *et al*., (2013), who found that the best cellular growth and highest chlorophyll content (0.05%) were observed in *S.platensis* in lab conditions. Similar results were obtained by Kim *et al*., (2007) reporting 0.07% of chlorophyll production using underground water in open ponds. High irradiance in outdoor cultures of *Dunaliella salina* resulted in 70% high biomass with massive accumulation of β-carotene under a real densities of 35–45 g m^2 (Grobbelaar, 1995). However, it is reported that higher light intensities causes photolysis and optimal conditions prevents or reduces the photo inhibitory damage. Pisal and Lee, (2003) reported that the beta carotene is a secondary metabolite and these molecules are produced by the algae in various stress condition as cell protecting mechanism. Among the tested biomass the highest Carotenoid content (0.24% and 0.20%) of *S. platenesis* was observed in May and the lowest Carotenoid content (0.1% and 0.08%) was seen in March at Ennore and in December at Manali (Fig. 16 & 17). Similarly the highest Carotenoid content (0.49% and 0.31%) of *C.vulgaris* was observed in May and the lowest carotenoid content (0.29% and 0.10%) was seen in December at Ennore and Manali. El-Baz *et al*., (2002) stated that the mechanism suggested for the accumulation of carotenoids in *C.vulgaris* to grow under various stress conditions also applies to *S. platenesis*. Additional carotenoids is produced in order to protect the algae cells and to continue their growth (Costantini *et al*., 2008). Manish *et al*., (2017) found the similar concentration of Carotenoid in *S. platenesis* and the result indicates that biomass, chlorophyll and Carotenoid produced by the organism were higher during the summer, when compare to monsoon season as observed in the present study. Ehsan *et al*., (2015) also obtained similar results, where they produce 0.3% of carotenoids from *S. platenesis* in control culture conditions. The experiments conducted by Cordero *et al*., (2011) states that 0.24% of carotenoids was produced in *C.vulgaris* while studying the effect of environmental factor on cell growth and Carotenoid production. Dong and Zhao, (2004) reported that *C.vulgaris* was produced high concentration of Carotenoid in the month of March. According to Imamoglu et al., (2009), light is essential for Carotenoid formation and growth of the alga. However, continuous illumination rather than light/dark illumination cycles has been shown to be more favorable for Carotenoid production by *C.vulgaris*, indicating that light quantity is more important than light intensity for production of this compound (Kobayashi *et al*., 1992). Above studies are highly supportive, where the highest Carotenoid production was observed in the month of May. The use of continuous illumination instead of light/dark cycles might represent an additional source of stress that could accelerate the process of astaxanthin accumulation (Fabregas *et al*., 2001). Sandeep *et al*., (2015) conducted outdoor cultivation of *S. platenesis* using Inline saline water and hatchery waste water as culture medium. The culture was mixed using air injection tube and tank was covered with polythene sheets. The highest phycocyanin content (5%) was observed at end of experiments in both medium when compared to zarrouk medium. But the results obtained from our study shows that the highest phycocyanin content (12% and 11%) in the month of May at Ennore and Manali. Murugan *et al*., (2014) also reported 34% of phycocyanin in *S. platenesis* in sea water medium. The secret of multi facet benefits of *S. platenesis* and *C.vulgaris* lies in the fact that it is rich in nutrient contents in its cellular composition. *S. platenesis* and *C.vulgaris* are photoautotrophic alga so light affects their growth and there is also a huge chance of change in metabolite production. Temperature is the most important climatic factor influencing the growth rate of *S.platenesis* and *C.vulgaris*. Below 20°C, growth is practically nil but *S.platenesis* and *C.vulgaris* do not die. The present work deals with monthly variation of metabolites of *S.platenesis* and *C.vulgaris*.

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