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***Full Length Article***

# Replacement of Expensive Pure Nutritive Media with Low Cost Commercial Fertilizers for Mass Culture of Freshwater Algae, *Chlorella vulgaris*

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## ABSTRACT

*Chlorella* sp., was isolated from wild water and purified on agar plates. *Chlorella* culture was further extended in Erlenmeyer's flasks on pure nutrient media (reagent grade chemicals) and then maximized in aspirators. *Chlorella* was then cultured on mass scale in polyethylene bags and 1000 L fiber glass tanks on commercial fertilizers (urea, nutri-calcium, ammonium sulfate, phosphorus plus ($P^+$), potash-plus ($K^+$), nitro-20 and di-ammonium phosphate (DAP) instead of pure nutrient media as practiced in flasks and aspirators. Various combinations of N, P and K were prepared empirically from aforementioned fertilizers and added to polythene bags and fiber glass tanks in fixed ratios for propagation of *Chlorella vulgaris*. Combined applications of urea, $P^+$ and $K^+$ (N:P:K; 16:4:6) produced the highest cell number ($34.05\times10^6$ cells $mL^{-1}$) and were far higher ($p<0.05$) than control group ($8.5\times10^6$ cells $mL^{-1}$). Duration of the log phase of *Chlorella* varied among containers. The polythene bags showed the best average (200%) and median (178%) growth rates of natural increase at exponential phase. Growth rate per day (244%), density ($34.05\times10^6$ cell ) and division's $day^{-1}$ (2.9) were also highest in polythene bags. Fiberglass tanks were second in production and average algal growth, median growth, maximum growth rate $day^{-1}$, maximum cell density, divisions $day^{-1}$, generation time $day^{-1}$ $h^{-1}$ were 1.13 (113%), 1.19 (119%), 1.19 (119%), $23.15\times10^6$ cells $mL^{-1}$, 1.63, 0.57 and 14.7, respectively. *Chlorella* in aspirators exhibited the poorest growth. The average and median growth rates were 34.95% and 39.93%, respectively. Growth rate $day^{-1}$ was 57.64%, and maximum cell density did not exceed $8.5\times10^6$ cells $mL^{-1}$ and divisions $day^{-1}$ were only 0.504. Strong positive correlation was observed between number of cells and number of days. It was the highest in polythene bags ($R^2$=0.9724) and the lowest in aspirators ($R^2$=0.7539), while correlation values for fiberglass tanks fell in between these two extremes ($R^2$=0.8355). Ciliates, major algae consumers were successfully controlled by the application of quinine sulfate @ 80 mg $L^{-1}$ of water after 3 h of administration with no effect on algal cells. 

**Key Words:** *Chlorella;* Growth rate; Starter culture; Exponential phase; Commercial; Fertilizers; Ciliates; Tetracycline; Quinine sulfate

## INTRODUCTION

Algae are primary producers of the oceans, rivers, streams and lakes (Stottrup & McEvoy, 2003). They are mainly composed of microscopic free floating or suspended algae such as diatoms, blue green algae and flagellates (Apt & Behrens, 1999). Micro-algae manufacture more complex nutritive molecules including proteins, starches, fatty acids and oils in reasonable quantities hence is an important part of crustacean and fish larval nutrition (Brown *et al*., 1997; Stahl, 2009). It provides vital bio-pigments (Sukenik *et al*., 1987; Sukenik *et al*., 2002), which are required for the development of proper coloration in rainbow trout fry. An adequate supply of nutrients and carbon dioxide enables algae to transform successfully the light energy of the sun into energy rich chemical compounds through photosynthesis (Turner, 1979).

Micro-algae directly or indirectly are an excellent first food for early stages of filtering mollusks (Muller-Feuga, 2004), farmed shrimp (Rosenberry, 1998) and marine fish. It is the most important dietary source during larval stages in the wild and contributes to the nutrient supply for post larval

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and juveniles in estuaries. Unlike bivalve and crustacean larva, which are regular or transient micro algal feeders, most marine fish larva do not feed directly on micro alga and can not survive in pure micro algal cultures or on exclusive phytoplankton diets. However, when phytoplankton was included in the larval rearing tanks, the survival, growth and food conversion index of more than 40 species were better than in clear water conditions (Tamaru *et al*., 1994; Dhert *et al*., 2001; Papandroulakis *et al*., 2002).

More than 40 different species of micro-algae are cultured intensively for direct or indirect feeding through production of zooplanktons and *Artemia* nauplii. The most common algal species cultured are diatoms, *Skeletonema costatum*, *Thalassiosira psuedonanna, Chaetoceros gracilis, Chaetoceros calcitrans*, the flagellates *Isochrysis galbana*, *Tetraselmis suecica*, M*onochrysis lutheri* and Chlorococcalean, *Chlorella*. All of them *Chlorella* has become an important source of food for rotifers due to its nutritional value and physical compatibility (Sen *et al*., 2005). *Chlorella* was first studied as a possible food source in Japan, United States and Germany after World War II (Miyachi, 1995). *Chlorella* is mono cellular (2-5 µm in size) and is non-motile. *In vitro* or in situ *Chlorella* is produced on pure chemical media, which is really expensive if algae are to be produced on mass scale (Okauchi, 1991). Therefore, there is always search for cheaper nutrient sources.

Commercial fertilizers (both organic & inorganic) are heavily used in current fish production practices (Yusof & McNabb, 1989). Highly significant production increments and returns have been obtained with accurate applications of fertilizers in conjunction with supplementary feeds in carp poly culture system. Fertilizers in ponds augment the production of plankton by stimulating both auto and hetero-trophic food levels (Grag & Bhatnagar, 2000). Like pure nutrient media, the nutrients in fertilizers are one of the major factors that influence the growth and production of phytoplankton in the ponds. Taiganides (1978) further verified that animal manure contains all of the major inorganic nutrients (N, P, K) and required trace elements for algae such as Ca, Cu, Zn, Fe and Mg. As much as 72-79% N, 61-78% P and 82-92% K can be conveniently recovered from animal excreta.

*Chlorella* and *Nannochloropsis* are extensively cultured all over the world under controlled conditions implying different protocols. Refined and expensive chemicals have been mainly used for its mass propagation, which is not cost effective when algae are required in abundance. Therefore, efforts have been made to replace pure and expensive nutrient media with cheaper commercial fertilizers following the similar protocol as exercised in pure nutrient media. Therefore, purpose of the present studies was to culture *Chlorella* with the manipulation of various commercial fertilizers for its cost effective mass scale production at fish and crustacean hatcheries. Control of ciliates, a major factor limiting algal growth, was also attempted.

## MATERIALS AND METHODS

**Experimental details:** Studies were conducted at Fish Seed Hatchery Satyana Road Faisalabad, Pakistan. Studies were planned following Completely Randomized Statistical Design and consisted of two trials. First trial was executed in 70 L polythene bags and 1000 L fiberglass tanks, while 2nd trial was only run in polythene bags. There were six treatments in the former trial, while only three in later. The pure nutrient media (N-8) (Vonshak, 1986) served as control, while treatment groups received only commercial fertilizers for algal growth. All the treatments including control had three replicates and studies were continued for three weeks. The various NPK combinations were prepared by drawing different quantities from liquid and solid inorganic fertilizers in fixed proportions (Table I & II) following chemical composition labeled on the bag.

**Collection and purification of *Chlorella*:** A sample of green water composed of varieties of microscopic organisms, was collected from fish culture ponds. Isolation of *Chlorella* sp., from sample was achieved by series of washings in a sterile solution following plating methods (Pringsheim, 1946). An agar media was prepared in a conical flask containing 7.5 g bacteriological grade agar in 500 mL distilled water. After boiling/sterilization, the media was left to cool. Media then was transferred to petri dishes (15 mL petri dish$^{-1}$). After half an hour, water sample from pond was streaked on agar. The petri dishes were placed up side down in glass racks placed under 3000 lux cool white fluorescent light at an intensity of 40 µmole photon m$^{-2}$ s$^{-1}$. Except any accidental breakdown petri dishes were continuously exposed to light and maintained at an ideal temperature (25±2.0$^{o}$C). Algal colonies appeared after 5-20 days. *Chlorella* colonies were picked up selectively and inoculated into 20 mL test tubes containing same chemical media as used in petri plates. Presence of *Chlorella* was adjudged by appearance of green color in test tubes after 10-15 days of incubation.

**Starter culture (in flasks):** Starter culture of *Chlorella* was accomplished in 1000 mL Erlenmeyer flasks. Flasks were cotton plugged and aluminum foil was wrapped around it. Flasks were autoclaved and 50 mL aliquot of green water containing pure *Chlorella* ($4.2\times10^6$ cells mL$^{-1}$) was transferred into each flask containing the same chemical media with similar solution ratios as used above for purification of *Chlorella*. Flasks were placed in front of 2-4 fluorescent tubes (40 watt each), which emitted and provided 1000-1500 lux light during culture period. Flasks were shaken gently manually daily to meet their oxygen demands and keep the cells in suspension for un-interrupted uniform multiplication of algal cells.

**Intermediate Culture (aspirators/jars):** After addition of 8 L filtered water and pure nutrient media (Okauchi, 1991), ozone ($O_3$) gas was passed in each aspirator for 30 minutes to avoid bacterial and ciliate contamination. Then 550 mL inocula from 1000 mL Erlenmeyer flasks were transferred

**Table I: Composition of N-8 nutritive (chemical) media (Vonshak, 1986)**

| S. No. | | Name of chemical used | Quantity/mL distilled $H_2O$ | Required per L (final Conc.) |
|---|---|---|---|---|
| 1. | | Sodium Nitrate | 15 g/500 mL | 10 mL |
| 2. | | Potassium phosphate | 0.4 g/200 mL | 5 mL |
| 3. | i | Iron Sulfate | 0.3 g/100 mL | |
| | ii | Citric Acid | 0.3 g/100 mL | 1 mL |
| | iii | Boric Acid | 0.15 g/100 mL | |
| | iv | Manganese Chloride | 0.15 g/100 mL | |
| 4. | i | Zinc Sulfate | 0.022 g/100 mL | |
| | ii | Copper Sulfate | 0.079 g-do | |
| | iii | Ammonium Molybdate | 0.015 g -do- | 0.1 mL |
| | iv | Ammonium Vanadate | 0.023 g -do- | |
| | v | EDTA | 0.25 g -do- | |
| | vi | Cobalt chloride | 0.012 g -do- | |
| 5. | | Vitamin $B_{12}$ | 0.007 g/100 mL | 20 mL |
| 6. | | $Na_2$ EDTA | 3 g/100 mL | 20 mL |

in each aspirator. Aspirators were placed in a temperature controlled conditions, exposed continuously to 2000-2500 lux cool white fluorescent light with gentle continuous aeration until *Chlorella* attained exponential growth phase ($3.5*10^6$ cells $mL^{-1}$), which took about 5-6 days.

**Maximizing culture:** From this step onward use of pure chemicals was totally abandoned and commercial fertilizers were applied in polythene bags and fiber glass tanks instead.

**Culture in polythene bags:** There was no difference in culture conditions. Only volume of water was increased up to 70 L including 10 L of inoculum from the aspirators. Commercial fertilizers were added as nutrient media instead of pure chemicals. The types of fertilizers used and their various combinations have been given in detail in Table I and II. Nitrophos and urea were purchased locally, while liquid fertilizers (nutri-calcium, $P^+$ & $K^+$) were procured from Sitara Chemicals Faisalabad, Pakistan.

**Culture in 1000 L fiberglass tanks:** Culture containers were well cleaned with bleach and were rinsed until bleach smell had totally gone off (A suitable de-chlorinator can work to neutralize the bleach although not used in this setup). Then fiberglass tanks were filled with tap water up to 550 L and inoculated with 20 L green water from the aspirators. Same commercial fertilizers with variable combinations, as administered in polythene bags, were used as nutrient media. The tanks were vigorously aerated to provide required quantity of oxygen and to keep cells and media in suspension. Required concentration of algae developed after 5-6 days of inoculation. The tanks were kept in open under 100% outdoor light exposure. Temperature remained at $30\text{-}35^0$C during growth period.

**Counting of algal cells:** Sampling was done daily basis using 10 mL capacity vials. *Chlorella* cells in each vial were preserved by adding 2-3 drops of formalin. One mL of sample was carefully filled in Neubauer Hemocytometer groove (Bauer, 1990; APHA, 1992) and covered with glass slide. The cells were enumerated under compound microscope. Hand tally counter was used for reliable counting. Algal cells were calculated by the following mathematical expression.

$$\text{Cells } (mL^{-1}) = \text{Total number of cells counted}/10*4*10^{-6}$$

**Determination of algal growth rate:** The growth rate, divisions per day, and generation time or doubling time was calculated following Fogg and Thake (1987).

$$\text{Growth rate; } K' = \text{Ln } (N_t/N_o)/(t_2 - t_1).$$
$$\text{Divisions per day; } Div.day^{-1} = K'/Ln2.$$
$$\text{Generation time (days); } Gen't = 1/Div.day^{-1}.$$
$$\text{Generation time (hours); } Gen't = 24(1/Div.day^{-1}).$$

Where,

$N_o$ and $N_t$ = final and initial populations at time $t_1$ and time $t_2$, respectively.

Since sample was collected daily, therefore, $t_2 - t_1 = 1$.

**Control of ciliates:** Ciliates have been a major problem during algae throughout the culture period. They have very high ingestion, grazing and growth rate and result in total destruction of dense cultures of algae within 48-72 h. Numerous algae crashes were observed due to their contamination. Several chemicals were applied for their control other than ozone sterilization. Types of chemicals/medicines, dosages used with their outcomes (VI, a–d).

**Chemical assessment tests:** For toxicity tests sterile 500 mL glass beakers were used. All the tests were performed in triplicate. Measured concentrations of chemicals/medicines were added to the beaker. Survival of ciliates was checked at different time intervals for assessment of status of ciliates and algae. Turning of chlorophyll color to brown and immobility of ciliates, observed under microscope, was used as criterion for death of these organisms.

## RESULTS AND DISCUSSION

Numerous nutrient media are in vogue for culture of pure *Chorella* sp. (Corsini & Karydis, 1990) Most of those are for laboratory use and/or for low grade production of algae. Majority of these media is composed of pure nutrients (N-8). Commercial fertilizers are least considered for *Chlorella* culture because of the conception that they do not provide requisite nutrients for algal growth and are mostly suitable for crop (land) agriculture. Unlike the previous misconceptions, we used commercial fertilizers for *Chorella* production and tried to replace expensive nutrient media (N-8) with cheaper commercial fertilizers for culture of desired algal species at crustacean and fish hatcheries.

We used various combinations of locally available and cheaper commercial fertilizers maintaining various NPK ratios (Table I). *Chlorella* study comprised of two trials. In the first trial, algae were cultured simultaneously in polythene bags and fiberglass tanks. Due to comparatively less density in fiberglass tanks, algae were dropped in the second trial and studies were continued solely on polythene bags. Algal culture in pure nutritive media always served as control. When *Chorella* production was compared among various NPK ratios, the output was always higher in

**Table II: Type and composition of commercial fertilizers used for *Chlorella* culture in fiberglass tanks**

| Name of fertilizer | Chemical composition (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | N | P | K | Iron | Ca | Zn | Mg | S |
| Urea | 46 | - | - | - | - | - | - | - |
| Nutri-Calcium | 5 | - | - | - | 8 | - | - | - |
| Ammonium Sulphate | 21 | - | - | - | - | - | - | 24 |
| Phosphorous-plus | - | 10 $P_2O_5$ | - | - | Minor | - | Minor | - |
| Potash-plus | 20 | - | 24 | - | - | - | - | - |
| Nitro-20 | Ammonical N 20 | - | - | 2.5 | - | 2.5 | - | - |
| D.A.P | 18 | 46 $P_2O_5$ | - | - | - | - | - | - |

**Table III: Effect of various fertilizer combinations on the production of *Chlorella***

| **Trial 1 (a) fiberglass tanks** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Group No.** | **N.P.K. ratios** | | | **Type and quantity of fertilizers used** | | | **Algae cells $mL^{-1}$** |
| | **N** | **P** | **K** | **Urea** | **$P^+$** | **Potash$^+$** | |
| 1 | 8 | 4 | 2 | 8.2 g | 24 mL | 5 g | $23.15\times10^6\pm3.5\times10^{6a}$ |
| 2 | 18 | 8 | 4 | 19.1 g | 48 mL | 10 g | $05.15\times10^6\pm1.2\times10^{6b}$ |
| 3 | 6 | 8 | 4 | 5.8 g | 48 mL | 10 g | $12.25\times10^6\pm2.3\times10^{6c}$ |
| | | | | Water Quality parameters | | | |
| **pH** | **DO (ppm)** | | | **Light Intensity (Lux)** | | | **Temp. (°C )** |
| 8.5 | 5.3 | | | 184 × 10 | | | 18.2 |
| **(b) Polythene bags** | | | | | | | |
| **Group No.** | **NPK ratios** | | | **Type and quantity of fertilizers used** | | | **Algae cells $mL^{-1}$** |
| | **N** | **P** | **K** | **Urea** | **$P^+$** | **Potash$^+$** | |
| 1 | 8 | 4 | 4 | 8.43 g | 24 mL | 10 g | $25.46\times10^6\pm6.1\times10^{6a}$ |
| 2 | 12 | 6 | 4 | 13.65 g | 36 mL | 10 g | $31.0\times10^6\pm 5.7\times10^{6b}$ |
| 3 | 8 | 8 | 4 | 8.43 g | 48 mL | 10 g | $6.77\times10^6\pm 1.8\times10^{6c}$ |
| | | | | Water Quality parameters | | | |
| **pH** | **DO (ppm)** | | | **Light Intensity$10^6$ (Lux)** | | | **Temp. (°C)** |
| 8.3 | 4.5 | | | 255 × 10 | | | 25 |
| **Trial 2 Polythene bags** | | | | | | | |
| **Group No.** | **NPK ratios** | | | **Type and quantity of fertilizers used** | | | **Algae cells/mL** |
| | **N** | **P** | **K** | **Urea** | **$P^+$** | **Potash$^+$** | |
| 1 | 16 | 8 | 6 | 17.86 g | 24 mL | 15 g | $34.05\times10^6\pm7.9\times10^{6a}$ |
| 2 | 12 | 6 | 4 | 13.65 g | 36 mL | 10 g | $27.07\times10^6\pm8.1\times10^{6a}$ |
| 3 | 12 | 8 | 4 | 13.65 g | 48 mL | 10 g | $17.85\times10^6\pm4.7\times10^{6b}$ |
| **Water Quality parameters** | | | | | | | |
| **pH** | **DO (ppm)** | | | **Light Intensity (lux)** | | | **Temperature (°C )** |
| 8.7 | 4.6 | | | 315 × 10 | | | 28 |

**Table IV: Pattern of *Chlorella* growth**

| **(a) Aspirators** | | | | |
|---|---|---|---|---|
| **No. of days in log phase in aspirators = 09days** | | | | |
| **$N^{th}$ Days of log phase** | **Growth rate** | **Divisions per day** | **Generation Time** | |
| | | | **Days** | **Hours** |
| 3rd | 0.576 | 0.832 | 1.203 | 28.86 |
| 4th | 0.418 | 0.604 | 1.656 | 39.75 |
| 5th | 0.428 | 0.618 | 1.618 | 38.83 |
| 6th | 0.380 | 0.548 | 1.824 | 43.77 |
| 7th | 0.360 | 0.520 | 1.923 | 46.15 |
| 8th | 0.076 | 0.109 | 9.178 | 220.28 |
| 9th | 0.492 | 0.710 | 1.409 | 33.81 |
| 10th | 0.064 | 0.093 | 10.762 | 258.29 |
| **(b) Polythene Bags** | | | | |
| **No. of days in log phase in polyethylene bags = 07days** | | | | |
| 2nd | 2.38 | 3.4 | 0.28 | 6.59 |
| 3rd | 2.44 | 3.5 | 0.28 | 6.8 |
| 4th | 1.78 | 2.57 | 0.39 | 9.35 |
| 5th | 1.8 | 2.6 | 0.38 | 9.1 |
| 6th | 1.6 | 2.3 | 0.43 | 10.29 |
| 7th | 0.4 | 0.57 | 1.75 | 41.98 |
| **(c) Fiber glass tanks** | | | | |
| **No. of days in log phase in fiber glass tanks = 04 days** | | | | |
| 4th | 1.16 | 1.67 | 0.43 | 14.35 |
| 5th | 1.19 | 1.71 | 0.58 | 14.02 |
| 6th | 1.05 | 1.51 | 0.66 | 15.8 |

polythene bags ($34.05\times10^{6}$ cells $mL^{-1}$) (Table IIIa, trial I) than fiberglass tanks (Table II) and control ($8.5\times10^{6}$ cells $mL^{-1}$) group (pure media). Significantly higher ($p<0.05$) algal production in polythene bags than its counterparts might be due to available conducive environment, which accelerated rate and intensity of algae production. Other parameters like growth rate, divisions $day^{-1}$ and generation time followed the same trend and all these values were higher in polythene bags than fiberglass tanks and control. Further those combinations, which contained 50% P to N or if there was slight increase in K ratio, always produced higher algal densities than their counterparts. Singh *et al*. (2000) concluded that phosphorus is one of the limiting nutrients for growth of *Dunaliella salina* because phosphate-rich cultures were 2-3 times denser than control ones. Tawfiq *et al*. (2010) recently observed in his studies that high cell density of *D. salina* in high phosphate media indicates that this species prefers high phosphate concentrations in culture media. Our findings are quite in line with earlier investigations and further confirm that phosphorus is a limiting factor not only in *Dunaliella* culture but in *Chlorella* too. Nevertheless, high phosphates do support algal growth to certain level (50% of N) but when concentration increased beyond this level or became equivalent to N it showed growth suppression (Table II). The reasons of this decline in cell density at higher ratios need further explorations.

Apparently current method seems quite non-conventional and retracted from the norms of algae culture system but it really worked very well. Grigorios *et al.* (2002) used Chemix * Standard-Kanlis formula [$NaNO_3$, EDTA, $H_3BO_3$, $NH_2PO_4$, $NH_4Cl$, $FeCl_3$, $MnCl_2$, $CoCl_2$, $CuSO_4$, $(NH_4)$, $Mo_2O_4$] in various combinations. They successfully produced *Nannochlorosis* sp., *Chorella* sp., and *Isochysis* sp., in the concentrations of $40\times10^{6}$, $40\times10^{6}$, $2\times10^{6}$ and $17\times10^{6}$ cells $mL^{-1}$, respectively. We were very close to this pure media. Aftab-Uddin and Zafar (2006) cultured *Skeletonema* in f/4 nutrient media and commercial fertilizers. They could not touch even the lowest density we achieved. Their values were only $1.2\times10^{6}$ and $0.75\times10^{6}$ cells $mL^{-1}$ in pure and commercial fertilizer media respectively against ours ($5.15\times10^{6}$; the lowest value). Wang *et al*. (2009) grew *Chlorella* on various categories of waste water and noted maximum average specific growth rate of 0.948 $day^{-1}$ against 3.5 in our studies (Table III). Lower specific growth rates in their studies may be due to several reasons, heavy metal toxicity and poor water quality can be the major ones. Further nutrient combination was not cared for, higher nutrient level displayed higher algae growth. It is likely that imbalanced ratios of nutrients suppressed the growth and showed negative trend in *Chorella* propagation. Contradictory to these studies water quality parameters were totally under control in our studies and water used was totally pollution free. This set up provided an ideal environment for algae culture and mass scale production. Sukumaran *et al*. (2005) tried algae growth on cow dung, poultry manure, swine manure, biogas spent slurry, vermi-compost and bio-dynamic compost for 16 days and compared it with inorganic culture medium (Bristol's medium). Poultry manure showed the best algae growth ($3.6\times10^{6}$ cells $mL^{-1}$). It was quite equal to their control (Bristol's medium) but significantly lower ($p<0.05$) than we observed (Table II). Organic matter has its own limitations and cannot compete with pure nutrients. In one of our trial its performance was very poor too. Therefore, it can be concluded that media prepared for algae culture and the system developed is more productive, effective, dependable and free from pollutants. It is very cost effective and can be used reliably and conveniently for mass production of algae at crustacean and fish hatcheries.

**Control of ciliates:** We applied several chemicals to control ciliate contamination (Table V, a−d). Sodium chloride was effective at very high concentrations (120 g $L^{-1}$) though it killed all the ciliates within two hours of application but it had undesirable effect on algae too. Though algae survived to the stage, where ciliates were dead but soon after addition of salt the dark green color started turning to light green. It continued and ultimately turned to brown and all the algae were dead after 24 h. Menezes-Benavente *et al*. (2004) tested different salinity levels (0, 50, 100, 150, 200 & 250 mM) of sodium chloride concentration. The growth remained steady in C. *vulgaris* in these concentrations but decreased in *C. humicola*. This indicates that different species of algae exhibit variable response to high salinity. Contradictory to previous studies (Menezes-Benavente *et al*., 2004) effect of high salinity was very prominent on *C. vulgaris* in our studies (Table VI, a).

Falcon killer powder (pyrethyroid 0.5% w/w) is a mild poison and is used to control various insects present in and on indoor installations. It was applied to eradicate ciliates @ 0.5, 1, 2 and 4 g/L. Its effectiveness was continuously monitored up to 24 h but it failed to kill ciliates neither it had any negative effect on algae (Table VI, b). Tetracycline was another option and was thought a solution to this problem (Li-hong *et al*., 2002). But unfortunately, our findings were not different from those observed in earlier studies (Agrawal & Manisha, 2007), where it suppressed auto-spore mother cells to dehisce (the ratio of auto-spore mother cells to vegetative cells increased). Discoloration of chloroplast and pyrinid clearing were evident prior to cell death in the presence of streptomycin in current studies. Like previous studies, all the algae died after 3 h of antibiotic application @ 3 mL $L^{-1}$) with no effect on ciliates. Earlier Kovacevic *et al*. (2005) reported that cinoxacin (antibiotic) had remarkable effect on the cellular and sub-cellular structure of *Chlorella* sp., including pronounced anti-chloroplastal and microbial effect even at far lower concentration then used in the current studies. Our observations favorably corroborate with previous studies and further confirm that antibiotic is not a pragmatic for ciliate eradication.

Quinine sulfate was fourth option implied to get rid of

**Table V: Descriptive statistics of the recorded data**

| | Aspirators | | Polythene bags | | F.G. Tank | |
|---|---|---|---|---|---|---|
| Source of Light | 1750 (lux) | | 2000 (lux) | | Sunlight | |
| Capacity of containers (litres) | 8 | | 70 | | 550 | |
| Chemicals | Reagent Grade | | Commercial fertilizers | | Commercial fertilizers | |
| Average Growth Rate | 0.3495 | 34.95% | 2 | 200.00% | 1.13 | 113.00% |
| Median Growth Rate | 0.3993 | 39.93% | 1.78 | 178.00% | 1.19 | 119.00% |
| Maximum Growth Rate per Day | 0.5764 | 57.64% | 2.44 | 244.00% | 1.19 | 119.00% |
| Maximum Density (ind/ml) | $8.5x10^{6}$ | | $34.05x\ 10^{6}$ | | $23.15x\ 10^{6}$ | |
| Divisions per day | 0.504 | | 2.9 | | 1.63 | |
| Generation time(days) | 1.98 | | 0.35 | | 0.57 | |
| Generation time (hours) | 47.60 | | 8.4 | | 14.7 | |
| R2 | 0.75 | | 0.97 | | 0.84 | |

**Table VI: Effect of various chemicals/medicines on control of ciliates**

**(a) Sodium Chloride**

| Time | Dose/L | | | |
|---|---|---|---|---|
| | 30 g | 60 g | 90 g | 120 g |
| After 30 min. | No effect on Ciliates | No effect on Ciliates | No effect on Ciliates | No effect on Ciliates |
| After 1 h | No effect on Ciliates | No effect on Ciliates | No effect on Ciliates | No effect on Ciliates |
| After 2 h | No effect on Ciliates | No effect on Ciliates | No effect on Ciliates | Ciliates dead |
| After 3 h | No effect on Ciliates | No effect on Ciliates | No effect on Ciliates | Ciliates dead |
| After 4 h | No effect on Ciliates | No effect on Ciliates | No effect on Ciliates | Ciliates dead + algae color became faint |
| After 5 h | No effect on Ciliates | No effect on Ciliates | Ciliates dead, alga color became faint | Ciliates dead + algae dead |
| After 24 h | No effect on Ciliates | Ciliates dead | Ciliates dead +algae dead | Ciliates dead +algae dead |

**(b) Falcon Killer Powder (Pyrethroid 0.5 % w/w)**

| Time | Dose/L | | | |
|---|---|---|---|---|
| | 0.5 g | 1 g | 2 g | 4 g |
| After 30 min. | No effect on Ciliates | No effect on Ciliates | No effect on Ciliates | No effect on Ciliates |
| After 1 h | No effect on Ciliates | No effect on Ciliates | No effect on Ciliates | No effect on Ciliates |
| After 2 h | No effect on Ciliates | No effect on Ciliates | No effect on Ciliates | No effect on Ciliates |
| After 3 h | No effect on Ciliates | No effect on Ciliates | No effect on Ciliates | No effect on Ciliates |
| After 4 h | No effect on Ciliates | No effect on Ciliates | No effect on Ciliates | No effect on Ciliates |
| After 5 h | No effect on Ciliates | No effect on Ciliates | No effect on Ciliates | No effect on Ciliates |
| After 24 h | No effect on Ciliates | No effect on Ciliates | No effect on Ciliates | No effect on Ciliates + algae |

**(c) Oxytetracycline (Liquid) concentration**

| Time | Dose/L | | |
|---|---|---|---|
| | 1 mL | 2 mL | 3 mL |
| After 30 min. | Ciliates alive | Ciliates alive | Ciliates alive |
| After 1 h | Ciliates alive | Ciliates alive | Ciliates alive |
| After 2 h | Ciliates alive | Ciliates alive | Ciliates alive |
| After 3 h | Ciliates alive | Ciliates alive | Ciliates alive+algae dead |
| After 4 h | Ciliates alive | Ciliates alive | Ciliates alive+algae dead |
| After 5 h | Ciliates alive | Ciliates alive | Ciliates alive+algae dead |
| After 24 h | Ciliates alive | Ciliates alive | Ciliates +algae dead |

**(d) Quinine Sulfate**

| Time | Dose/L(ppm) | | |
|---|---|---|---|
| | 40 mg | 80 mg | 120 mg |
| After 30 min. | Ciliates alive | Ciliates alive | Ciliates alive |
| After 1 h | Ciliates alive | Ciliates alive | Ciliates dead+algae alive |
| After 2 h | Ciliates alive | Ciliates alive | -do- |
| After 3 h | Ciliates alive | Ciliates dead+ algae alive | -do- |
| After 4 h | Ciliates alive | -do- | -do- |
| After 5 h | Ciliates alive | -do- | Ciliates dead+ algae dead |
| After 24 h | Ciliates alive | Ciliates dead + algae alive | -do- |

ciliate contamination. It worked really very well and killed all the ciliates after 3 h of application. Its effect was really quick at 120 $L^{-1}$, where it killed all ciliates population within 1 h of its administration but due to possible risk of chlorophyll destruction, higher dosages should be avoided. Earlier Marino-Garrido and Canavate (2001) tested quinine sulfate (10 ppm) successfully to control the ciliates without harming *D. salina*. Like previous findings *Chorella* sp., was quite safe even at much higher concentrations in the current studies. This resistance may be attributed to the presence of high contents of organic osmolytes present in marine (Kirst, 1989) and fresh water alga (Cruz *et al*., 2001). Quinine sulfate block potassium channels (Marino-Garrido & Canavate, 2001) and there is possibility that this effect induces malfunctions in the osmotic regulation which resulted in mass mortality of ciliates. Previously, Heussler *et al*. (1978) successfully used metronidazole for disinfection of *Scenedesmus* sp. and eradication of zooflagellates

(*Amphelidium* sp.) but could not get wide applicability. Nevertheless, our and previous studies agreed upon a common conclusion that quinine sulfate is most appropriate and pragmatic option to obviate ciliate contamination in algae culture.

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