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# Growth, Survival and Reproduction in *Chlorella vulgaris* and *C. variegata* with Respect to Culture Age and under Different Chemical Factors

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**ABSTRACT.** Batch cultures of *Chlorella vulgaris* and *C. variegata* reproducing about twice every 5 d within 0–15 d had vegetative cells and autospore mother cells in the ratio of about 19 : 1. Continuous slow or negligible and/or no growth in >15-d-old control cultures or in young cultures supplied with the antibiotics streptomycin, penicillin, amoxycillin (10–1000 ppm) or tetracycline (10, 100 ppm), and pesticides carbofuran, gammaxine, moticop or iralon (1–100 ppm) was due to slow autospore mother cells dehiscence (leading to an increase in their percentage); while negligible and/or no growth of both algal species in sewage water (100, 25 %), detergent (0.1–1 %), petrol or kerosene (5–20 %), benzene, toluene or phenol (5, 10 %) and pesticides rogor or endosulfan (1, 10 ppm) was due to vegetative cells failure to differentiate into autospore mother cells (leading to decreased/zero autospore mother cells percentage) and/or rapid death of all cells. *C. variegata* was equally or slightly more sensitive to different chemical stress than *C. vulgaris.*

*Abbreviations*

| | | | | | |
|---|---|---|---|---|---|
| *C.var.* | *Chlorella variegata* | *C.vul.* | *Chlorella vulgaris* | | |
| Cbf | carbofuran | Esf | endosulfan | Gmx | gammaxine |
| Ira | iralon | Mtc | moticop | Rgr | rogor |

*C.vul*. was found to grow on sludge and in retention basins of sewage treatment plants (Palmer 1969) and has been employed successfully for the treatment of nitrogen industry effluents and dairy animal waste (Przytocka *et al*. 1984; Hosetti *et al*. 1985); *C. pyrenoidosa* was used for the treatment of soybean waste (Wong and Lay 1980). *C.vul*. was found to remove Cu from the medium through intrinsic adsorption kinetics or by film diffusion (Chu and Hashim 2004), the uptake being influenced by Cu concentration and the capacity of the cells to accumulate it (Fathi *et al*. 2005). Since very little or nothing is known about the growth, survival and reproduction of *Chlorella* with respect to culture age and under different chemical stress conditions such as sewage water, detergents, hydrocarbons and petrochemicals (petrol, kerosene, benzene, toluene, phenol), antibiotics (streptomycin, penicillin, amoxycillin, tetracycline) and pesticides (Cbf, Gmx, Mtc, Ira, Rgr, Esf), we report these data for *C.vul*. and *C.var.*

## MATERIAL AND METHODS

*Chlorella vulgaris* BEYERINCK was isolated from a free floating clump in a small concrete pond in a local park. Its clonal cultures were raised through autospores. *C. variegata* BEYERINCK clonal culture was obtained from *Indian Agriculture Research Institute*, New Delhi. They were maintained in BG11 medium (Staniet *et al*. 1971), adjusted to pH 7.5, at 22 ± 1 °C and light intensity of ≈40 μmol $m^{-2}$ $s^{-1}$ for 16 h per day in a culture chamber. *C.vul*. vegetative cells were spherical, dark-green, 5.0–7.5 μm in diameter while *C.var.* cells were spherical, light-green, ≈3.3 μm in diameter. They reproduced through the formation of autospore mother cells, measuring ≈10–20 μm in diameter in *C.vul*. and ≈8–10 μm in *C.var.* In 15-d-old cultures of both algal species, ≈5–6 % of all cells were autospore mother cells. Autospore mother cells divided into 4 or 8 daughter protoplasts. Each protoplast secreted a wall and was released into the medium as a spherical autospore following rupture of a mother cell wall. Autospores grew to become new vegetative cells.

A loopful of 7-d-old actively growing algal material, obtained by centrifugation of control culture, was inoculated into 10 mL of either fresh liquid media, sewage water, and/or liquid media containing different chemical effectors.

**Table I.** $A_{660}$ increase ($\times 10^3$) in batch cultures of *C. vulgaris* (*1st lines*) and in *C. variegata* (*2nd lines*) in control medium (BG 11), and in the presence of detergent, hydrocarbons and petrochemicals (petrol, kerosene), antibiotics (streptomycin, penicillin, amoxycillin, tetracycline) and pesticide (carbofuran)[a]

| Treatment or effector and its concentration | | Time, d | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 5 | 10 | 15 | 30 | 45 |
| **Control**[b] | | | | | | | |
| BG 11 medium | | 20 | 40 | 83 | 140 | 202 | 220 |
| | | 10 | 23 | 41 | 76 | 101 | 110 |
| **Detergent** | | | | | | | |
| Surf Excel | 0.1 % | 23 | 38 | 60 | 79 | 122 | 163 |
| | | 10 | 17 | 25 | 37 | 54 | 76 |
| **Hydrocarbons and petrochemicals** | | | | | | | |
| Petrol | 5 % | 24 | 29 | 35 | 41 | 40 | 36 |
| | | 11 | 13 | 17 | 21 | 21 | 20 |
| Kerosene | 5 % | 18 | 21 | 23 | 25 | 27 | 26 |
| | | 9 | 11 | 14 | 14 | 18 | 16 |
| **Antibiotics** | | | | | | | |
| Streptomycin | 10 ppm | 20 | 38 | 41 | 50 | 60 | 60 |
| | | 11 | 19 | 20 | 30 | 40 | 52 |
| | 100 | 22 | 30 | 30 | 39 | 46 | 48 |
| | | 8 | 10 | 13 | 17 | 28 | 30 |
| | 1000 | 21 | 29 | 30 | 38 | 10 | – |
| | | 10 | 12 | 12 | 11 | 10 | – |
| Penicillin | 10 ppm | 23 | 37 | 40 | 49 | 60 | 54 |
| | | 10 | 18 | 30 | 30 | 28 | 20 |
| | 100 | 26 | 28 | 32 | 38 | 40 | 30 |
| | | 10 | 14 | 20 | 26 | 22 | 16 |
| | 1000 | 21 | 23 | 21 | 10 | 8 | – |
| | | 9 | 14 | 10 | 7 | – | – |
| Amoxycillin | 10 ppm | 20 | 30 | 43 | 51 | 52 | 49 |
| | | 11 | 18 | 21 | 20 | 16 | 16 |
| | 100 | 20 | 37 | 50 | 36 | 29 | 18 |
| | | 10 | 18 | 25 | 16 | 11 | 8 |
| Tetracycline | 10 | 22 | 38 | 40 | 41 | 23 | 21 |
| | | 11 | 17 | 30 | 28 | 22 | 12 |
| **Pesticide** | | | | | | | |
| Carbofuran | 1 ppm | 20 | 39 | 50 | 58 | 40 | 38 |
| | | 8 | 17 | 31 | 40 | 32 | 21 |
| | 10 | 18 | 35 | 46 | 49 | 38 | – |
| | | 10 | 17 | 29 | 26 | 20 | – |
| | 100 | 19 | 31 | 21 | 20 | – | – |
| | | 10 | 15 | 26 | 22 | – | – |

[a]Rounded mean of triplicates; there was no $A_{660}$ increase (growth) in either algal species in 100, 25 % sewage water; ≥0.5 % detergent; ≥10 % of petrol or kerosene; ≥5 % benzene, toluene or phenol; 1000 ppm amoxycillin, 100 ppm tetracycline; and ≥1 ppm of Gmx, Mtc, Ira, Rgr or Esf.

[b]Values after 60, 90, and 120 d for *C.vul*. and for *C.var.* are 242, 260, 281, and 124, 131, 140, respectively.

*Sewage water* was collected from a open sewage tunnel at Allahabad City. Its physico-chemical properties were assessed in *Water Analysis Laboratory, Indian Farmers Fertilizer Cooperative* (Allahabad) and were reported to be: pH 7.65, total hardness 474 ppm, turbidity 155 nephelometric turbidity unit, conductivity 1550 μΩ/cm, nitrate 3.8 ppm, total inorganic phosphate 11.02 ppm, total ammoniacal nitrogen

21.82 ppm, dissolved oxygen 0.74 ppm, iron 7 ppm, zinc 4 ppm. Since sewage water was found to be very toxic to both algal species, it was diluted 4× with BG11 medium.

*Detergent*. Surf Excel powder (*Hindustan Lever*, India) was added into sterilized culture medium so as to prepare 0.1–1 % solutions (pH adjusted to 7.5).

*Hydrocarbons and petrochemicals*. Petrol or kerosene (100 %; Governmental supply, India), benzene (99.7 %; *Merck*, India), toluene (99 %; *Ranbaxy*, India) or phenol (99.5 %; *Sisco Research Lab*, India) were added separately to sterilized culture medium to prepare a 5–20 % suspension or solution (pH adjusted to 7.5).

*Antibiotics*. Streptomycin sulfate I.P. (0.75 g; *Sarabhai Piramal*, India), benzyl penicillin ($1 \times 10^5$ U per vial; *Alembic*, India), tetracycline hydrochloride I.P. (500 mg; *Synbiotics*, India) or amoxycillin trihydrate I.P. (250 mg; *Seagull Pharmaceuticals*, India) were added separately to autoclaved cooled culture medium to prepare 10–1000 ppm solutions (pH adjusted to 7.5).

*Pesticides*. Cbf (3 %; *Rallis*, India), gammaxine (5 % malathion; *Pentapharma*, India), Mtc (50 % copper oxychloride; *Motilal Pesticides*, India), Ira (75 % isoproturon; *Irachem*, India), Rgr (35.5 % dimethoate; *Rallis*, India) or Esf (39 %; *Swadeshi Pesticides*, India) were added to culture medium at 1–100 ppm (pH adjusted to 7.5).

Inoculated culture tubes were placed in the culture chamber and examined periodically to determine (*i*) the growth of both algal species by measuring $A_{660}$ in a *Systronic* spectrophotometer with respect to uninoculated culture media or culture media with an effector blank and (*ii*) the survival of vegetative cells and differentiation of vegetative cells into autospore mother cells by counting their numbers as well as dead (empty, hyaline) cells if any, from a total of ≈5000–6000 cells counted in triplicate.

## RESULTS AND DISCUSSION

*Culture age. C.vul*. and *C.var.* batch cultures grew to ≈2× $A_{660}$ every 5 d within 0–15 d (Table I) and possessed vegetative cells and autospore mother cells in a initial ratio of ≈19 : 1, which gradually dropped to 2 : 1 (Table II). The percentage of autospore mother cells increased from about 5.5 to 7.5, 10, 15, 22 and 28 in *C.vul*. and from about 5 to 8, 10, 14, 21 and 26 in *C.var.* as the cultures aged from 15 to 30, 45, 60, 90 and 120 d, respectively (Fig. 1A,B). This was due to their continuous differentiation but slow dehiscence, a reason for slow growth of old age cultures. In both species, the percentage of dead cells increased from 0 to ≈16–20 as the cultures aged from 15 to 120 d. Autoinhibition of growth in old-age cultures of *C.vul*. was reported to be due to the release and accumulation of specific metabolic products, chlorellin, peroxides of unsaturated fatty acids, released and accumulated (Pratt and Fong 1940; Scutt 1964). Insufficient Ca ion level in the culture medium (Soeder and Thiele 1967) or increased salinity of the culture medium (Agrawal and Singh 2001) retard the release of daughter cells from mother cells in *C. fusca* and *C.vul*., respectively. On the other hand, a higher content of inorganic salts did interfere with the growth and survival of green alga *Rhizoclonium crassipellitum* (Gupta and Agrawal 2006).

*Sewage water.* Vegetative *C.vul*. and *C.var.* cells did not grow and died rapidly without differentiation into autospore mother cells in both 100 and 25 % sewage water (Fig. 1C). Thus both algal species were very sensitive to low dissolved oxygen content and high ammonical nitrogen of sewage water.

*Detergent.* Cells grew slowly in 0.1 % detergent and not at all in ≥0.5 of detergent; this was because of the failure of vegetative cells to differentiate into autospore mother cells (whose percentage dropped with increasing detergent concentration and time period) and ultimately cell death (Fig. 1D). At 0.5 and 1 % detergent, all cells of *C.vul*. died within 15 and 12 d, and those of *C.var.* within 15 and 10 d. On the other hand, Maloney (1966) observed an increased growth of *Chlorella* sp. by sodium triphosphate, an ingredient of synthetic detergent. Detergent stopped diatom movement (Drum and Hopkins 1966) and prevent *Pinnularia viridis* vegetative cells from separating during cell division (Hopkins 1969). Sodium dodecylsulfate produced no significant effect to growth of *Dunaliella salina* at 1 ppm level but a suppression effect was observed at 10 ppm (Markina and Aizdaicher 2005).

*Hydrocarbons and petrochemicals.* Very slow growth of both species at 5 % petrol or kerosene and no growth at 10 % was due to the failure of vegetative cells to differentiate into autospore mother cells and finally the death of all cells. Both species died within 15 and 10 d at 10 and 20 % petrol, respectively; with 10 and 20 % kerosene *C.vul*. died within 15 and 10 d and *C.var.* within 10 d (Fig. 1E,F). Petroleum products produced a significant reduction of algal growth (Vandermeulen and Ahorn 1976) as well as zooplankton population (Saha and Konar 1986) and macroinvertebrates (Pettigrove and Hoffmann 2005). Studies on the growth of algae on hydrocarbons might be of ecological importance, particularly because of widespread oil spills.

**Table II.** Survival (%) of vegetative *C. vulgaris* and *C. variegata* cells (VS) and autospore mother cells (AM) (the rest is dead cells, if any) as dependent on culture age in control, sewage water, detergent, hydrocarbons and petrochemicals, antibiotics and pesticides[a]

| Concentration and/or treatment | | Time, d | *C. vulgaris* | | *C. variegata* | |
|---|---|---|---|---|---|---|
| | | | VS | AM | VS | AM |
| **Control** | | | | | | |
| BG 11 medium | | 5 | 95 | 5.0 | 95 | 5.0 |
| | | 10 | 95 | 5.0 | 95 | 4.5 |
| | | 15 | 94 | 5.5 | 95 | 5.0 |
| | | 30 | 90 | 7.5 | 88 | 8 |
| | | 45 | 86 | 10 | 85 | 10 |
| | | 60 | 76 | 15 | 78 | 14 |
| | | 90 | 67 | 22 | 66 | 21 |
| | | 120 | 56 | 28 | 54 | 26 |
| **Sewage water** | | | | | | |
| % | 100 | 5 | 53 | 2.5 | 48 | 2 |
| | | 10 | 41 | 2 | 38 | 1 |
| | | 15 | 36 | 1 | 23 | 0 |
| | | 30 | 0 | 0 | 0 | – |
| | 25 | 5 | 73 | 4 | 63 | 3 |
| | | 10 | 68 | 3 | 58 | 3 |
| | | 15 | 56 | 2 | 47 | 2 |
| | | 30 | 0 | 0 | 0 | 0 |
| **Detergent** | | | | | | |
| % | 0.1 | 15 | 82 | 3 | 84 | 3 |
| | | 30 | 80 | 2 | 82 | 3 |
| | | 45 | 79 | 1 | 72 | 1 |
| | 0.5 | 5 | 86 | 3 | 80 | 3 |
| | | 10 | 65 | 1 | 64 | 2 |
| | | 15 | 0 | 0 | 0 | 0 |
| | 1 | 5 | 76 | 2.5 | 70 | 2 |
| | | 10 | 26 | 1 | 0 | 0 |
| | | 15 | 0 | 0 | – | – |
| **Hydrocarbons and petrochemicals, %** | | | | | | |
| Petrol | 5 | 15 | 85 | 4 | 80 | 4 |
| | | 30 | 80 | 2 | 76 | 2 |
| | | 45 | 73 | 1.5 | 71 | 2 |
| | 10 | 5 | 80 | 4 | 80 | 4 |
| | | 10 | 49 | 2 | 47 | 2 |
| | | 15 | 0 | 0 | 0 | 0 |
| | 20 | 5 | 64 | 4 | 50 | 4 |
| | | 10 | 0 | 0 | 0 | 0 |
| Kerosene | 5 | 15 | 82 | 3 | 77.5 | 2 |
| | | 30 | 77 | 2 | 68 | 2 |
| | | 45 | 64 | 1 | 59 | 1 |
| | 10 | 5 | 76 | 3.5 | 75 | 3 |
| | | 10 | 49 | 2 | 0 | 0 |
| | | 15 | 0 | 0 | – | – |
| | 20 | 5 | 45.5 | 2.5 | 38 | 2.5 |
| | | 10 | 0 | 0 | 0 | 0 |
| Benzene | 5 | 5 | 68 | 2 | 59 | 2 |
| | | 10 | 47 | 2 | 32 | 2 |
| | | 15 | 20 | 1 | 0 | 0 |
| | 10 | 5 | 60 | 2 | 47 | 2 |
| | | 10 | 25 | 1 | 0 | 0 |
| | | 15 | 0 | 0 | – | – |
| Toluene | 5 | 5 | 70 | 3 | 64 | 2.5 |
| | | 10 | 58 | 2 | 57 | 2 |
| | | 15 | 38 | 0.5 | 35 | 1 |
| | | 30 | 0 | 0 | 0 | 0 |
| | 10 | 5 | 56 | 4 | 53 | 3 |
| | | 10 | 47 | 2 | 40 | 2 |
| | | 15 | 31 | 2 | 30 | 2 |
| | | 30 | 0 | 0 | 0 | 0 |
| Phenol | 5 | 5 | 62 | 2 | 56 | 2 |
| | | 10 | 40 | 1 | 29 | 1.5 |
| | | 15 | 0 | 0 | 0 | 0 |
| | 10 | 5 | 14 | 2 | 11 | 1 |
| | | 10 | 7 | 1 | 0 | 0 |
| | | 15 | 0 | 0 | – | – |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Antibiotics, ppm** | | | | | | |
| Streptomycin | 100 | 15 | 85 | 7 | 78 | 8 |
| | | 30 | 79 | 9 | 77 | 10 |
| | | 45 | 65 | 13 | 60 | 14 |
| | 1000 | 10 | 82 | 7 | 81 | 7 |
| | | 15 | 81 | 8 | 79 | 8 |
| | | 30 | 57 | 11 | 61 | 11 |
| | | 45 | 0 | 0 | 0 | 0 |
| Penicillin | 100 | 15 | 77 | 11 | 76 | 10 |
| | | 30 | 71 | 15 | 70 | 14 |
| | | 45 | 69 | 16 | 68 | 15 |
| | 1000 | 10 | 50 | 8 | 41 | 10 |
| | | 15 | 32 | 8 | 35 | 11 |
| | | 30 | 18 | 15 | 0 | 0 |
| | | 45 | 0 | 0 | – | – |
| Amoxycillin | 100 | 15 | 70 | 10 | 73 | 10 |
| | | 30 | 57 | 10 | 62 | 12 |
| | | 45 | 34 | 13 | 39 | 13 |
| | 1000 | 10 | 31 | 8 | 12 | 7 |
| | | 15 | 0 | 0 | 0 | 0 |
| Tetracycline[b] | 10 | 15 | 74 | 10 | 75 | 8 |
| | | 30 | 70 | 11 | 62 | 10 |
| | | 45 | 62 | 13 | 51 | 13 |
| | 100 | 5 | 65 | 8 | 54 | 7 |
| | | 10 | 49 | 8 | 0 | 0 |
| | | 15 | 0 | 0 | – | – |
| **Pesticides, ppm** | | | | | | |
| Carbofuran | 1 | 15 | 76 | 13 | 81 | 7 |
| | | 30 | 72 | 14.5 | 78 | 10 |
| | | 45 | 61 | 15 | 63 | 12 |
| | 10 | 15 | 70 | 16 | 77 | 9 |
| | | 30 | 62 | 17 | 59 | 10 |
| | | 45 | 0 | 0 | 0 | 0 |
| | 100 | 10 | 57 | 20 | 52 | 10 |
| | | 15 | 50 | 23 | 44 | 15 |
| | | 30 | 0 | 0 | 0 | 0 |
| Gammaxine | 1 | 15 | 76 | 12 | 70 | 10 |
| | | 30 | 72 | 14 | 57 | 11 |
| | | 45 | 62 | 17 | 41 | 13 |
| | 10 | 15 | 63 | 12 | 58 | 11 |
| | | 30 | 54 | 14 | 0 | 0 |
| | 100 | 10 | 62 | 12 | 58 | 10 |
| | | 15 | 46 | 14 | 43 | 11 |
| | | 30 | 34 | 14 | 0 | 0 |
| | | 45 | 0 | 0 | – | – |
| Moticop | 1 | 15 | 53 | 12 | 37 | 12 |
| | | 30 | 40 | 12 | 24 | 12 |
| | | 45 | 27 | 14 | 13 | 10 |
| | 10 | 10 | 62 | 10 | 47 | 10 |
| | | 15 | 42 | 12 | 27 | 13 |
| | | 30 | 25 | 13 | 0 | 0 |
| | | 45 | 0 | 0 | – | – |
| | 100 | 10 | 52 | 11 | 35 | 12 |
| | | 15 | 34 | 11 | 0 | 0 |
| | | 30 | 0 | 0 | – | – |
| Iralon | 1 | 15 | 65 | 12 | 53 | 10 |
| | | 30 | 47 | 13 | 25 | 12 |
| | | 45 | 25 | 14 | 0 | 0 |
| | 10 | 10 | 49 | 12 | 33 | 11 |
| | | 15 | 29 | 14 | 0 | 0 |
| | | 30 | 29 | 15 | – | – |
| | | 45 | 0 | 0 | – | – |
| | 100 | 5 | 63 | 10 | 47 | 10 |
| | | 10 | 42 | 12 | 0 | 0 |
| | | 15 | 0 | 0 | – | – |
| Rogor[c] | 1 | 5 | 31 | 2 | 29 | 1.5 |
| | | 10 | 4 | 0 | 5 | 0 |
| | | 15 | 0 | – | 0 | – |
| Endosulfan[c] | 1 | 5 | 20 | 1 | 8 | 0 |
| | | 10 | 0 | 0 | 0 | – |

[a]About 5000–6000 cells were assessed for each triplicate; data represent rounded means; for details *see Material and Methods*.

[b,c]At 1000 ppm tetracycline, and at 10 ppm Rgr or Esf, respectively, all cells of both species died within 5 d.

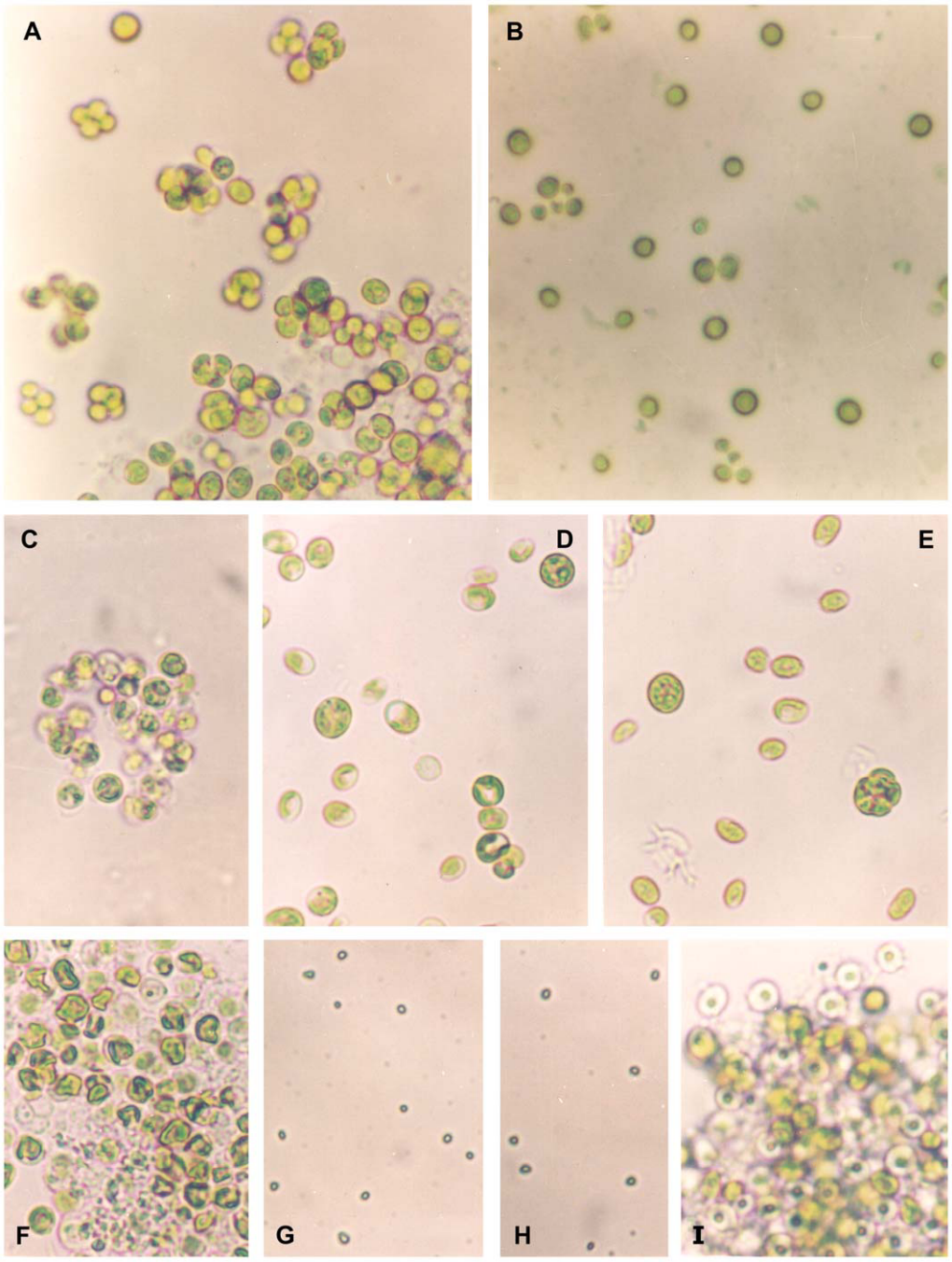


**Fig. 1.** **A**: *C. vulgaris* vegetative cells and autospore mother cells in 90-d-old control culture, magnification ×600;
**B**: *C. variegata* vegetative cells and ruptured autospore mother cells in 45-d-old control culture, ×840;
**C**: *C.vul*. in 100 % sewage water on 15 d, cells show shrinkage of chloroplast and discoloration, ×700;
**D**: *C.vul*. in 0.1 % detergent on 50 d, cells are discolored and hyaline, ×660;
**E**: *C.vul*. in 10 % petrol on 10 d, cells are discolored, also an autospore mother cell with 8 autospores, ×660;
**F**: *C.vul*. in 20 % kerosene on 7 d, cells show shrinkage and discoloration, ×700;
**G**, **H**: *C.var*. all cells dead and highly shrunk in 10 % toluene on day 30 and in 10 % phenol on day 10, respectively, ×660;
**I**: *C.vul*. in 100 ppm streptomycin on 60 d, cells show discoloration and pyrenoid clearing, ×700.

At ≥5 % of benzene, toluene and phenol neither culture grew, vegetative cells dying rapidly without differentiation into autospore mother cells (Fig. 1G,H). Phenol was found to be algicidal (Palmer 1956), according to us more than benzene and toluene. Hexachlorobenzene depressed the growth of *Chlorella pyrenoidosa* (Geike and Parasher 1976; Parasher *et al*. 1978). Toluene acts as a barrier between the air and the surface of algal specimen and has been found to act as accelerator of toxin release from *Microcystis* cells to their environment (Chantara *et al*. 2004).

*Antibiotics*. Growth inhibition at 10 and 100 ppm streptomycin, penicillin or amoxycillin and at 10 ppm tetracycline, and more particularly at 1000 ppm streptomycin or penicillin and no growth at 1000 ppm amoxycillin and 100 ppm tetracycline was due to suppression or failure of autospore mother cells to dehisce (the ratio of autospore mother cells to vegetative cells increased) and cell death. Discoloration of chloroplast and pyrenoid clearing were evident prior to cell death in the presence of streptomycin (Fig. 1I). Penicillin up to certain level favored the growth of *Pediastrum tetras* (Srivastava and Nizam 1969) and akinete germination in *Stigeoclonium pascheri* (Agrawal and Sarma 1980). Antibiotic cinoxacin affected remarkably the structure and ultrastructure of *Chlorella* sp. including, *e.g*., pronounced antichloroplastal and antimitochondrial effect (Kovačević *et al.* 2005).

*Pesticides. C.vul*. and *C.var.* grew to a limited extent at 1–100 ppm Cbf, but not at all at 1 ppm Gmx, Mtc, Ira, Rgr or Esf. An increased ratio of autospore mother cells to vegetative cells prior of the death of all cells in the presence of 1–100 ppm Cbf, Gmx, Mtc or Ira indicates that autospore mother cells dehisced slowly in their presence (or they were slightly more tolerant than vegetative cells). However, vegetative cells of both species rapidly died and no autospore mother cells differentiated in the presence of 1 and 10 ppm of Esf and Rgr. Gmx inhibited the growth of *Chlorogloea fritschii* (Da Silva *et al*. 1975), *Nostoc muscorum* and *Wollea bharadwajae* (Kar and Singh 1979). Esf at >1 ppm was algicidal to *Anabaena flos-aquae* (Chinnaswamy and Patel 1983) and Rgr at ≥20 ppm to *Westiellopsis prolifica* (Adhikary 1989). Cbf at 1–50 ppm inhibited the survivability and formation of akinetes in *Pithophora oedogonia* and zoosporangia in *Cladophora glomerata* and *Rhizoclonium hieroglyphicum* (Agrawal and Misra 2002), 25–1000 ppm captan and 2,4-D induced morphological changes leading to a formation of round motile and round nonmotile forms of *Euglena ignobilis* (Gupta and Agrawal 2005).

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