SUMMARY

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SUMMARY

Fresh water cyanobacterial blooms flourishing in and around Calicut University Campus and in the 'Manachira pond, of Calicut city during summer season were considered in the present investigation. The first part of the present study is on the characterization of fresh water cyanobacterial bloom forming members, isolation and in vitro culture. The second part of the present work mainly emphasizes, how different dosages of crude extracts of eight potentially toxic species of cyanobacteria namely Anabaena circinalis var. crassa Ghose, Microcystis aeruginosa Kütz., Nostoc carneum Agardh ex Born. et Flah., N. commune Vaucher ex Born. et Flah., N. spongiaeforme Agardh ex Born. et Flah., Oscillatoria obscura Brühl et Biswas, O. princeps Vaucher ex Gomont and Phormidium tenue (Menegh.) Gomont affected the different stages of cell cycle in A. cepa root tip meristem. Cytotoxic properties of different dosages of crude extracts were evaluated at different time intervals by using Allium cepa assay.

The cyanobacterial taxa were identified up to their species level and one strain up to variety, with the help of keys and classical monographs of Geitler, Desikachary and Anand on cyanobacteria.

The isolation was carried out by the streak and spread plate methods. Subculturimg was done repeatedly, whereby pure cultures of the cyanobacterial species (unicyanobacterial isolates) were obtained on specific BG-11 medium [with a nitrogen source for non-heterocystous cyanobacteria and without a nitrogen source (nitrogen free medium) for heterocystous types]. Unicyanobacterial cultures were studied based on their morphological features and were characterized under laboratory conditions also.

30-40 days old cultures were used for the cytotoxic experiments. The unicyanobacterial cultures were harvested by filtration. Cyanobacterial cell
extracts of the eight potentially toxic species were prepared following standard protocols. Different concentrations of the cyanobacterial crude extract such as 0.01%, 0.02%, 0.05% 0.075% and 0.1% (v/v) were prepared in distilled water. Roots of *A. cepa* growing for ½ h, 1 h, 1 ½ h and 2 hours in the presence of 0.01%, 0.02%, 0.05%, 0.075% and 0.1% of cyanobacterial extracts of all the eight species showed mitotic abnormalities. Meristematic cells treated with the eight cyanobacterial extracts revealed a dose- and time-dependent decrease of mitotic indices. The *Allium* test revealed that all concentrations (0.01% to 0.1%) of the tested substance lowered the frequency of dividing cells and induced an array of clastogenic and non-clastogenic abnormalities. The frequently observed clastogenic abnormalities are, aberrant and bizarre nucleus, bizarre metaphase, anaphase and telophase, nuclear appendage formation / nuclear budding, nuclear lesions and microlesions, pulverization, dissolution of chromatin, abnormal condensation of chromatin, abnormal condensation of chromosomes, giant cells, stickiness of chromosomes, chromosome fragments and chromosome bridges. Rarely observed clastogenic abnormalities include nuclear diminution, nuclear disintegration, nuclear extrusion, cytornixis, nuclear fragmentation, hyperchromasia, nuclear erosion, contorted chromatin, ring chromosomes, coagulation / adhesion of chromosomes, chromosome gaps, telosome formation, convoluted chromosomes and deproteinized chromosomes. The frequently observed non-clastogenic abnormalities are micronuclei, bi-, tri-, tetra- and multinucleate cells, clumping of chromosomes, ball shaped arrangement of chromosomes, C- metaphase, stellate arrangement of chromosomes, misorientation of chromosomes, polyploidy, diagonal arrangement of chromosomes, disturbed metaphase, anaphase and telophase, chromosome laggards, abnormal movement of chromosomes and stathmo - anaphase. Rarely observed non-clastogenic abnormalities include blebbing, stratified metaphase, scattering of
chromosomes, bouquet formation of chromosomes, "lollypop" metaphase, abnormal grouping of chromosomes, equatorial separation of chromosomes, shift in microtubule organizing centres and unequal division and formation of macro and micro cells.

Out of the eight cyanobacterial extracts, the extracts of Oscillatoria obscura, Microcystis aeruginosa, and Anabaena circinalis var. crassa were able to induce maximum clastogenic and non-clastogenic abnormalities and various morphological cell death features in Allium cepa root cells. Moderate cytotoxicity is noticed with the extracts of Nostoc carneum, N. commune, Nostoc spongioforme and O. princeps and comparatively low cytotoxicity is observed with the extracts of N. carneum and Phormidium tenue. These data indicate that crude extracts of cyanobacteria have cytotoxic, mitodepressive, turbogenic and apoptotic effect on cell cycle of A. cepa root meristem. The various clastogenic aberrations might have been caused by the damage induced by the toxic cyanobacterial extracts on the shape, size and structure of nuclei and chromosomes during different stages of the cell cycle, through various endogenous mechanisms of action in the G1, S, G2 and M stages. The different non - clastogenic or physiological aberrations might have occurred due to the spindle poisoning effects of the cyanobacterial extracts, affecting the cytoskeleton and non - chromosomal components of the cell during different stages of cell cycle in A. cepa. Thus it can be concluded that the cyanobacteria which flourishes during the summer season in the surface waters in the aquatic ecosystems in and around Calicut University campus and in 'Mananchira pond ' at Calicut city are toxic. Allium cepa assay is a reliable assay for testing cytotoxicity of cyanobacteria. The results indicate that cyanobacteria contain potentially harmful bioactive compounds which possess cytotoxic, antimitotic and apoptotic effects on plants, capable of causing lethal effects in higher organisms including man.