CHARACTERIZATION, *IN VITRO* CULTURE AND CYTOTOXIC ACTIVITIES OF SOME CYANOBACTERIA

Thesis
submitted to the University of Calicut for the degree of
DOCTOR OF PHILOSOPHY IN BOTANY

By

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2007

Dedicated

to

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CERTIFICATE

This is to certify that the thesis entitled "**Characterization, *in vitro* culture and cytotoxic activities of some cyanobacteria**" is an authentic record of work carried out by Sandhya Rani R. during 2001-2007 under my supervision and guidance and that no part thereof has been presented earlier for any other degree or diploma.

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DECLARATION

I hereby declare that the thesis entitled "**Characterization, *in vitro* culture and cytotoxic activities of some cyanobacteria**" submitted for the degree of Doctor of Philosophy in Botany of Calicut University is a research work done by me under the guidance of **Dr. John E. Thoppil**, Reader, Genetics and Plant Breeding Division, Department of Botany, University of Calicut. This has not been submitted earlier for any other degree or diploma.

Calicut University,
27.09.2007.

Sandhya Rani R.

Acknowledgement

With proud respect and admiration, I express my gratitude to my guide Dr. John E. Thoppil, Reader, Genetics and Plant Breeding Division, Department of Botany, University of Calicut for having consented to supervise my research work. He has been of immense help and a source of inspiration at every step of my work. But, for his guidance and assistance, the work would never have seen the light of the day. I thank him for his keen interest, generous availability, infinite patience, valuable suggestions, unfailing guidance and understanding throughout the investigation.

I place on record my gratitude to Dr. M. Sivadasan, Head of the Department of Botany, University of Calicut for providing necessary facilities.

I wish to thank, Dr. P. V. Madhusoodanan and Dr. S. Nandakumar, former Heads of the Department of Botany, University of Calicut, for their sincere comments and valuable suggestions.

I owe a special debt of gratitude to Dr. Sabu M., Reader, Department of Botany, University of Calicut for his valuable help during the earlier part of my research.

I am happy to record my sincere thanks to Prof. A. *Kuttialikutty, Principal, Farook College, Calicut and former Principal Dr. P. M. Mubarak Pasha, for permitting me to do my Ph. D. work.*

I also extend a special thanks to UGC for financial assistance through the Faculty Improvement Programme.

I wish to thank Dr. Uma Maheswari, N. A., Head of the Department of Botany, Carmel College, Trichur for her sincere comments and valuable suggestions.

I would like to thank Mrs. Geetha Nair, Librarian, for her valuable help during this study.

I would like to express my heartfelt thanks to lab mates, Mr. Jojo Joseph, Mr. Jose Mary Das, Sr. Betty and Asha Ramachandran for their valuable help.

I also acknowledge the encouragement of Mr. R. V. Ibrahim, Head, Department of Botany, Farook College and all my colleagues, during the course of my work.

Finally I owe my special thanks to my children for sacrificing their comfort and giving me full support and encouragement during the entire period of study.

Sandhya Rani R.

CONTENTS

INTRODUCTION

Cyanobacteria are one of the most primitive (Brock, 1973) and ubiquitous families of photosynthetic organisms. They are considered to be pioneers of early earth (Brock, 1973; Schopf, 1996). Cyanobacteria, strikingly similar to the present day counterparts were found fossilized in sedimentary rocks formed 3,500 million years ago (Bryant, 1994). They are members of the kingdom Prokaryote, division Gracilicutes (bacteria with gram negative cell wall), class Photobacteria, subclass Oxyphotobacteria and order Cyanobacteriales (Gibbons and Murray, 1978). The cyanobacteria are a remarkably widespread and successful group, colonizing fresh water, marine and terrestrial ecosystems, including extreme habitats such as Antarctic lakes, salt fields and hot springs (Fogg *et al.*, 1973). Morphologically, physiologically and metabolically this group is one of the most diverse groups of prokaryotes (Codd, 1994). The rapid evolution of cyanobacteria in different water and land environments is related to their capacity for both aerobic and anaerobic photosynthesis. They have been known to occur in the sediments from the early Precambrian period as components of stromatolitic microbial mats (Schopf, 1983, 1992). This fact serves as a clue that these microorganisms might have played a major role in the evolution of an oxygenic environment (Fay, 1983). Their long evolutionary history is considered as a reason for the success of cyanobacteria in many habitats and wide ecological tolerance (Whitton and Potts, 2000).

Cyanobacteria are related to eubacteria in their prokaryotic cellular organization, possession of a gram negative cell wall made up of peptidoglycan and a gelatinous sheath liable to lysosome treatment, absence of sexual reproduction, tolerance to high temperature and susceptibility to antibiotics, high degree of adaptability and non-sexual genetic recombination.

They contain chlorophyll-a, carotene, xanthophylls, c-phycocyanin, allophycocyanin and c-phycoerythrin. The last two pigments can only be found in cyanobacteria (Benson, 1969). Their photosynthetic organ and mechanism of photosynthesis are similar to algae but, unlike eukaryotic microalgae, cyanobacteria do not possess membrane bound sub-cellular organelles like chloroplasts. The pigments are embodied in phycobilisomes, which are found in rows on the surface of the thylakoides (Douglas, 1994).

Cyanobacteria have several characteristics that allow them to out-compete more complex organisms. Due to minimal need for nutrients, cyanobacteria can inhabit a wide range of environments. Cyanobacteria are able to maintain photoautotrophic growth in relatively lower light intensities than their potential competitors (Reynolds and Walsby, 1975; Ganf *et al.*, 1991). In addition, cyanobacteria have been shown to be both heterotrophic and photoheterotrophic and therefore able to survive in conditions of very low light intensity, such as at the bottom of euphotic zone of lakes and in lake sediments (Ganf and Oliver, 1982) and in caves (Prescott, 1968), or in places where there is no light at all. Some cyanobacteria not only survive high levels of visible light but also survive damage by near ultra violet light (UV), even being capable of utilizing these wave lengths for photosynthesis (Pearl *et al.*, 1985; Tilzer, 1987). The amazing combination of properties found in algae and bacteria which these organisms exhibit, have been a source of fascination and attraction for many scientists.

A noteworthy feature of the cyanobacteria is the ability of some species to fix elementary nitrogen dissolved in water, and many species are capable of living in water with low levels of combined N_2. Using the enzyme nitrogenase, they convert N_2 directly into ammonium in aerobic conditions. Nitrogen fixing cyanobacteria are wide spread among filamentous, heterocyst forming genera such as *Anabaena, Nostoc* and *Aphanizomenon* (Ressom *et*

al., 1994; Adams, 2000). When cyanobacteria are present even in low numbers, their nitrogen-fixing capabilities may enhance the fertility of marine and freshwater environments; in fact, cyanobacteria are sometimes used to fertilize paddy (Singh, 1976; Bold and Wynne, 1985; Singh and Bisoyi, 1989). Cyanobacteria are most commonly found in neutral to alkaline surface waters (Moss, 1973) and are also able to utilize both free CO_2 and bicarbonate ions as a source of inorganic carbon for photosynthesis (Talling, 1976).

In contrast to true algae, many species of planktonic cyanobacteria possess specialized intracellular gas vesicles. Stacks of minute (<300nm) proteinaceous hollow cylinders maintain a gas-filled space in the cell, which enable regulation of buoyancy of cells and colonies, and optimize their vertical position in the water bodies. This in turn enables them to find suitable niche for survival and growth. The buoyancy of some cyanobacteria is responsible for intensive formation of blooms at the surface of water. The process of nitrogen fixation and occurrence of gas vesicles are especially important for the success of noxious species of cyanobacteria. The slow growth rate of cyanobacteria in comparison to eukaryotic microalgae is compensated by a higher affinity for phosphorus and nitrogen, substantial phosphorus storage capacity, and low losses to grazing by zooplanktons as a result of the formation of large colonies (Reynolds, 1987).

The cyanobacteria also provide an extraordinarily wide ranging contribution to human affairs in every day life (Tiffany, 1958) and are of economic importance (Mann and Carr, 1992). Both the beneficial and detrimental features of the cyanobacteria are of considerable significance. They are important producers and their general nutritive value is high. They are a rich source of several phytopharmaceuticals (Schwartz *et al.*, 1990). The nitrogen fixing species contribute globally to soil and water fertility (Rai,

1990). However, cyanobacteria have the potential to produce mass population in natural and controlled water bodies. Such development leading to cyanobacterial blooms, scums and mats, is a common consequence of eutrophication. Abundant growth of cyanobacteria in water reservoirs creates severe practical problems for water supplies. Furthermore, cyanobacteria are well documented in being able to potentially synthesize a large number of low molecular weight compounds called cyanobacterial toxins or cyanotoxins.

Cyanobacterial blooms are usually observed during spring time or during late summer time. The following factors are responsible for the predominance of bloom forming cyanobacteria during the summer period: water temperature above 25°C, low light intensity in water, low N:P ratio and stability of the water column. A cyanobacterial bloom, occurring mainly under conditions of high water temperatures and reduced turbulence, show a buoyant migration to the water surface (Hutchinson, 1967; Reynolds and Walsby, 1975; Robarts and Zohary, 1987; Pearl, 1996). An explanation for cyanobacterial dominance may be their ability to control their buoyancy and to photosynthesize under low light conditions (Reynolds and Walsby, 1975). Many cyanobacteria are able to make controlled migrations to specific depths and thereby possess an advantage to obtain the otherwise unavailable nutrients retained there (Fogg, 1969; Reynolds and Walsby, 1975).

Most cyanobacterial blooms tend to occur in summer months, in temperate climates and it is reasonable to mention that elevated temperature would be a major factor in population growth and possible subsequent bloom formation (Tilman and Kiesling, 1984). Bloom forming cyanobacteria mainly belong to the genera *Anabaena, Aphanizomenon, Anabaenopsis, Arthrospira, Cylindrospermopsis, Oscillatoria, Nodularia* and *Microcystis* (Reynolds and Walsby 1975; Oliver and Ganf, 2000).

TOXIC CYANOBACTERIA

Cyanobacteria are the integral parts of many ecosystems and as such cause no problem. A small group of genera however, produce toxins which cause sporadic but repeated cases of animal poisoning (Schwimmer and Schwimmer, 1964, 1968; Carmichael, 1992 a, b). Because of wide spread eutrophication of lakes, ponds and some parts of oceans, cyanobacteria often form blooms, which lead to water hygienic problems (Henning and Kohl, 1981; Skulberg *et al.*, 1984; Bell and Codd, 1994; Chorus and Bartram, 1999; Day *et al.*, 2000). Several genera of cyanobacteria form toxic water blooms and different cyanobacterial toxins have been characterized (Carmichael, 1992c, 1994). Possibly the synthesis of highly active toxins is a defense option of cyanobacteria against attack by other organisms like bacteria, fungi, zooplankton and eukaryotic microalgae. Carmichael (1994) found that cyanobacterial toxins can be extremely harmful to zooplanktons that feed on cyanobacteria. They may be directly lethal or they may reduce the number of their offspring.

The incredible variety of niches inhabited by toxic cyanobacteria indicates a high degree of biological adaption which has enabled these organisms to thrive and compete effectively in nature. One reason toxic cyanobacteria have been so successful in wide spectrum of niches is their ability to produce a unique range of defensive secondary metabolites, the cyanobacterial toxins (cyanotoxins). Toxic cyanobacteria are well known for their ability to produce cyanotoxins, which have been responsible for numerous animal deaths (Schwimmer and Schwimmer, 1968; Carmichael *et al.*, 1985; Beasley *et al.*, 1989; Kuiper- Goodman *et al.*, 1999). Of more than 50 genera of cyanobacteria, at least 19 of them, comprising 41 species, have been shown, to possess toxic properties (Scott, 1991). The best characterized genera are *Microcystis, Anabaena, Aphanizomenon, Cylindrospermopsis,*

Gloeotrichia, Lyngbya, Nodularia and *Oscillatoria* (Scott, 1991; Carmichael, 1992 a) as it is these genera which have been most frequently implicated in poisoning animals and causing illness in humans (Carmichael, 1992 a, b).

Of the cyanobacterial blooms tested to date, 50-75% have been toxic (Codd, 1995). In fact, toxicities of blooms of the same species can vary markedly both geographically and with time (Carmichael and Gorham, 1981). At least 46 species have been shown to cause toxic effects in vertebrates (Sivonen and Jones, 1999). In cyanobacterial blooms often only one species comes up to more than 95% of the population. Though this has been interpreted as a result of competition between species, the dominance of one species could be a hint for the formation of metabolites with cyanobacterial activity. The common toxic cyanobacteria in fresh water are *Microcystis* spp., *Cylindrospermopsis raciborskii*, *Planktothrix* (syn. *Oscillatoria*) *rubescens*, *Synechococcus* spp., *Planktothrix* (syn. *Oscillatoria*) *agardhii*, *Gloeotrichia* spp., *Anabaena* spp., *Lyngbya* spp., *Aphanizomenon* spp., *Schizothrix* spp. and *Synechocystis* spp. Among the toxic cyanobacteria, *Microcystis* is the most common genera producing microcystins, a group of toxins with strong hepatotoxicity (Carmichael, 1994). Acute hepatotoxic and chronic hepatocarcinogenic effects of microcystins have been studied intensively in mammals (Dawson, 1998; Dietrich and Hoeger, 2005) and human health risks resulting from the presence of microcystin in drinking and recreational water have been recognized (Codd *et al.*, 2005). While initially toxicity appeared to be restricted to planktonic cyanobacteria, benthic forms which form mats in water bodies have also been shown to be toxic (Edwards *et al.*, 1992; Carmichael *et al.*, 1997; Metz *et al.*, 1997).

Growth of cyanobacteria occurs all year round in some tropical lakes, however in temperate regions growth and blooms of cyanobacteria exhibit a characteristic seasonality (Reynolds and Walsby, 1975). Filamentous forms

such as *Aphanizomenon, Gloeotrichia* and *Nodularia* are first to appear, while colonial forms such as *Microcystis* generally appear later (Ganf and Oliver, 1982). All these genera overwinter in the sediment, either as akinetes or spores in the case of *Anabaena* and *Aphanizomenon* or as vegetative colonies in the case of *Microcystis* (Reynolds and Walsby, 1975). The ability to switch from photoautotrophy to heterotrophy, based on stored carbohydrate, combined with a reduction in respiration rate, is the likely reason that these cyanobacteria can survive in the bottom sediments for several months over winter (Reynolds and Walsby, 1975).

Contamination of water by toxic blooms of cyanobacteria has occurred widely in many regions of the world. Anthropogenic eutrophication can exacerbate the risks, allowing toxic cyanobacteria to grow unchecked and resulting in harmful algal blooms with potentially serious economic and health related impacts. The blooms are frequently found in bodies of warm, stagnant water such as drinking water reservoirs and ponds that serve as drinking holes for domestic or wild animals. Contact with or ingestion of water containing cyanobacterial cells or toxins can cause skin irritations, allergic responses, blistering of mucosa, hay fever symptoms, diarrhoea, acute gastroenteritis, and liver and kidney damage (Ressom *et al.*, 1994; Falconer, 1994; Bell and Codd, 1996; Pilotto *et al.*, 1997; Codd, 2000).

Blooms of blue-green algae are potential hazard to human beings due to the fact that extensive growth of cyanobacteria in water supply reservoirs lasts for several months per year (Carmichael and Falconer, 1993). It is also important to note that mass occurrences of toxic cyanobacteria are not always associated with human activities causing pollution or "cultural eutrophication".

IMPORTANCE OF PRESENT STUDY

The toxic blooms of cyanobacteria develop and flourish worldwide, both in freshwater and in marine environments (Carmichael and Falconer, 1993). There is evidence that these toxic organisms are on the increase perhaps as a result of global pollution. The frequency, intensity and geographic distribution of toxic species in aquatic environment seem to be increasing in recent decades due to the proliferation of harmful cyanobacteria (Gago-Martinez *et al.*, 2003).

One of the major problems arising from harmful cyanobacterial blooms is the production of cyanotoxins. The hepatotoxic microcystin are amongst the most frequently reported cyanotoxins (Sivonen and Jones, 1999), as they are not only associated with *Microcystis* blooms, but also with blooms of *Anabaena, Nostoc* and *Oscillatoria* (Bartram *et al.*, 1999). These compounds represent, by different exposure routes, a significant health hazard to humans and livestock (Kuiper-Goodman *et al.*, 1999), being involved in several intoxication episodes throughout the world, and even in death of humans exposed through haemodialysis (Jochimsen *et al.*, 1998). The observed hepatotoxicity of microcystins is leading to acute liver failure via disruption of hepatocyte cytoskeletal components (Fastner *et al.*, 1999). Furthermore, microcystins have been shown to act as tumor promoters (Nishiwaki-Matsushima *et al.*, 1992) and have been considered as a major risk factor contributing to the high rate of hepatocellular carcinoma in south-east China (Ueno *et al.*, 1996).

Health hazards of cyanotoxins have alerted water authorities and even the public, the need for detailed research on potentially harmful cyanobacteria. Indeed, an estimated 50% of cyanobacterial blooms contain toxins, and some of these toxins are not destroyed by the chlorine treatment or filtration methods often used by water treatment plants and can therefore be a

threat to humans (Dale and Yentsch, 1978). The ability of cyanobacterial population to produce potent toxins and examples of associated human and animal health problems have raised the position of cyanobacteria in the priorities for the management and production of water quality. Consequently the early detection in waters used for drinking is highly desirable so that measures to minimize or prevent exposures can be implemented. Reports of toxicity and associated health risks of cyanobacteria in waters used for domestic supply, agriculture and recreation have resulted in increased level of awareness and monitoring to detect potentially toxic species. In addition, blue green algae are used in many parts of the world as fertilizers, and several types are even used as side dishes ([5]http) and as a dietary supplement in countries like the U.S.A. Furthermore, blue green algae are used to treat children with Attention Deficit Disorder ([4]http) and also used for pets ([6]http). About a million people in the U.S.A. and Canada consume dietary supplements containing blue-green algae ([2]http).

Although in recent years, toxic cyanobacterial blooms have been reported with an increasing frequency in different countries worldwide, often associated with the production of microcystins, such toxic occurrences are poorly documented in India. Most work has been done in the field of water bloom ecology and taxonomy. Few studies have addressed on the cytotoxic effects of cyanobacteria on plant growth.

OBJECTIVES OF THE STUDY

The present study is aimed to assess the cytotoxicity of some selected cyanobacterial strains of algal blooms of water samples collected from a water reservoir of Calicut city, 'Mananchira pond', water bodies and garden pond of the Calicut University campus, rice fields and adjoining areas of Calicut and Malappuram Districts in a random way. The study also includes:

1. Characterization of potentially toxic cyanobacteria at species level based on field collection.

2. Isolation and identification of selected cyanobacterial strains in uni cyanobacterial cultures under laboratory conditions.

3. Cytotoxic activities of different dosages of crude extracts of toxic cyanobacteria are evaluated at different time intervals by using *Allium cepa* assay.

4. Cytotoxic potentiality of all the isolated toxic cyanobacteria will be analysed based on the clastogenic and non-clastogenic aberrations induced by them on *Allium cepa* root tip meristem.

5. Detection of the probable causes for all the clastogenic and non-clastogenic abnormalities.

6. Detection of the level of the toxicity of the studied samples of Cyanobacteria based on the results of the cytotoxic assays.

REVIEW OF LITERATURE

Characterization of cyanobacteria

Cyanobacteria are prokaryotic organisms with unique quality of nitrogen fixation and oxygenic photosynthesis. They comprise a large group of structurally complex and ecologically significant gram-negative prokaryotes. The cyanobacteria are a highly successful group, which establish themselves easily on a new habitat (Fritsch, 1945). In their habitat and distribution, they are equaled to bacteria (Fogg *et al.,* 1973). Cyanobacteria are included under two broad categories of morphological organizations namely, unicellular or coccoid forms and filamentous forms. The filamentous forms are separated on the basis of the presence or absence of specially differentiated cells called heterocysts, into heterocystous or non-heterocystous filamentous forms. The heterocystous forms may be unbranched or branched. If branched, they exhibit various types of true or false branching.

The numerous cyanobacterial taxa scattered in the literature are critically compiled by Geitler (1932), Elenkin (1936) and Bourrelly (1970) on the basis of their morphological diversity. The taxonomy of cyanobacteria has long been in a highly confused state (Gibbons and Murray, 1978). Rippka *et al.* (1979) separated the cyanobacteria from algae and included them under a special group of bacteria called cyanobacteria. Different phycologists have attempted and erected a large number of new species and genera on trivial grounds that made the taxonomy of cyanobacteria extremely cumbersome to define especially at generic and species level (Castenholz, 1992).

Morphological characteristics of cyanobacteria formed the basis of the classification in both classical and modern systems. The grouping of these organisms under various orders according to Geitler (1932), Fritsch (1945)

and Desikachary (1959) or under separate families without orders as done by Drouet (1981) were based on mere morphology. The various revisions made in various groups of the algae, namely coccoid forms (Drouet and Daily, 1956), Oscillatoriaceae (Drouet, 1968), Nostocaceae with cylindrical trichome (Drouet, 1973) and Nostocaceae with constricted trichome (Drouet, 1978) were based on nomenclatural synonymy reducing the number of taxa to very few. The ranking of blue green algae to a special group, namely cyanobacteria (Rippka *et al.,* 1979) was mainly based on their prokaryotic organization of the cell structure as revealed by electron microscopic investigation. However, the morphological characteristics is highly variable in culture. In many case, its expression is determined by culture conditions (Stanier and Cohen-Bazire, 1977). Stanier *et al.* (1971) tried another new and entirely different method in cyanobacterial taxonomy. They studied these prokaryotic organisms using bacteriological methods and they proposed a cyanobacterial taxonomy on the basis of characters evident in pure cultures.

Anagnostidis and Komarek (1985, 1990) pointed out that cultures of cyanophytes are very useful in taxonomy since they furnish additional information about the taxa. Anand (1988) emphasized on the precise study of the variability of culture material combined with perfect knowledge of the natural populations, since these are important for the correct taxonomic classification of the cyanophytes.

Rippka *et al.* (1979) tried to modify Geitlerian taxonomy of Cyanobacteria based on morphological, physiological, developmental and DNA base composition studies, and the organisms were divided into five sections. Herdman *et al.* (1979), however found that there is no correlation between DNA composition and structural and developmental diversity of the organisms that distinguish the five sections of Rippka *et al.* (1979). Nevertheless, it is undoubtedly sure that the taxonomic information resides in

the DNA molecule (Krogmann, 1981). In short, a satisfactory taxonomy of cyanobacteria is lacking even now, which in turn poses problems to identify various strains (Roger *et al.*, 1991).

The taxonomy of the Cyanophyceae (cyanobacteria) has been formulated under the International Code of Botanical Nomenclature and based primarily on phenotypic characters (Bourrelly, 1985; Desikachary, 1959; Geitler, 1932). Individual cyanobacterial cells are identified in environmental samples on the basis of their pigmentation and morphology.

Desikachary (1959) distinguished 18 species of *Microcystis*, 25 species of *Anabaena,* 23 species of *Nostoc*, 76 species of *Oscillatoria* and 45 species of *Phormidium*. According to Desikachary (1959), the young colonies of *Microcystis aeruginosa* seem to be rounded, slightly longer than broad and solid. But old colonies were clathrate, with distinct colonial mucilage. The cells were 3 - 7 µm in diameter, spherical, generally with gas vacuoles.

The thallus of *Anabaena circinalis* was frothy, floating and trichomes were circinate, without a sheath and 8 - 14 µm broad. Cells were barrel shaped or spherical, shorter than broad with gas vacuoles; heterocysts were subspherical, 8 - 10 µm broad; spores cylindrical, ends rounded, 16 - 18 µm broad upto 43 µm long, ordinarily away from the heterocyst and epispore smooth and colourless.

The thallus of *Anabaena circinalis* var. *crassa* has free swimming, single, semicircular, loosely coiled trichomes, cells nearly spherical, generally shorter than broad, 5 - 7 µm in diameter, with pseudovacuoles; heterocysts globose, up to 8 µm broad and spores were absent.

In *Nostoc carneum,* the thallus was leathery, irregularly expanded, gelatinous, flesh coloured, reddish brown, violet, rose or blue to olive green; filaments were loosely contorted and flexuous; sheath indistinct, colourless;

trichome 3.5 - 4 μm broad; cells oblong cylindrical, upto twice as long as broad; spores oval to ellipsoidal, 6 μm broad, 5 - 10 μm long and epispore smooth and hyaline.

Nostoc commune has a firm thallus, gelatinous, blue-green, olivaceous or brown; filaments flexuous, entangled; sheath distinct at the periphery, thick and yellowish brown. Trichomes were 4.5 - 6 μm broad, cells short barrel-shaped or nearly spherical, shorter or little longer than broad, 5 μm long, heterocysts nearly spherical, about 7 μm broad; spore as big as vegetative cell and epispore smooth and colourless. The species was observed on moist soils, rocks and in stagnant waters.

In *Nostoc spongiaeforme*, the thallus was gelatinous, light blue- green, violet or brownish, filaments flexuous, loosely entangled, sheath diffluent in the inside, more or less distinct along the periphery, yellowish - brown, trichome about 4 μm broad, blue green to violet; cells partly cylindrical and up to 7 μm long, partly short barrel shaped; heterocysts subspherical or oblong, 7 - 8 μm broad; spores oblong, 6 - 7 μm broad and 10 - 12 μm long, epispore smooth, at first colourless later became yellowish.

In *Oscillatoria obscura*, trichomes were broad, attenuated at the apex, rounded, slightly bent or nearly straight, blue-green, not constricted at the cross walls; cells about 1/5 as long as broad or shorter and cross - walls were granulated. It is normally observed in ponds and puddles.

In *Oscillatoria princeps,* trichomes were blue - green, more or less brownish, violet or reddish, mostly straight, not constricted at the cross walls, 16 - 60 μm broad, commonly 25 - 50 μm, blue-green to dirty green, slightly attenuated at the apices and bent; cells slightly capitate with or without thickened membrane. This species was observed in fresh water, sea water and

in moist soil. Geitler (1932) considers this species as a mixture with variations in colour, cell dimensions, *etc.*

In *Phormidium tenue*, the thallus was pale blue-green, thin membraneous, expanded; trichome straight or slightly bent, densely entangled, slightly constricted at the cross- walls, attenuated at the ends, 1 - 2 μm broad, pale blue-green, sheath thin, diffluent, cells up to 3 times longer than broad, 2.5 - 5 μm long, septa not granulated, cross-walls not commonly visible, end cell acute - conical, and calyptra absent. This species was reported on moist surfaces and along other algae, fresh water and salt water bodies.

In a botanical approach Koma´rek and Anagnostidis (1999) distinguished 21 morphospecies in the genus *Microcystis* based on characteristics such as cell diameter, border and consistency of the mucilage and number of cell division planes. Anand (1989) distinguished 3 species in the genus *Microcystis* based on characteristics such as cell diameter, shape of the colony and nature of the colonial mucilage. He has identified *Microcystis aeruginosa*, *M. flos-aquae* and *M. pulverea*. He has described the colonies of *Microcystis aeruginosa* as clathrate and the size of cells as 6.5 μm broad.

Toxicity of cyanobacteria

Occurrence of cyanobacterial blooms in freshwater, brackish and coastal marine ecosystems has become a worldwide problem. The production of potent toxins by bloom -, scum - and mat - forming cyanobacteria, in fresh, brackish and marine waters, appears to be a global phenomenon. Cyanobacterial toxins can also be produced by cyanobacteria from terrestrial sources. Experts recommend that all cyanobacterial blooms be considered potentially toxic until tested ([3]http).

Many of the cyanobacterial species, however, which are classified as freshwater cyanobacteria, have also been isolated from marine sources. This is either as a direct result of run-off from river ways and estuaries, or due to the fact that these species are able to survive and indeed flourish within the marine environment. It is impossible to completely separate both freshwater and marine cyanobacteria, due to the fact that the same species may be able to grow within both environments and produce similar or different natural products (Moore and Entzeroth, 1988).

There are three Cyanophyte orders that produce the vast majority of toxic compounds, these being Chroococcales, Oscillatoriales and Nostocales. Over 40 different Nostocales species, majority of which are *Anabaena* and *Nostoc* sp. produce over 120 natural products. These almost entirely being secondary metabolites, can be classified as biotoxins. The associated activities, such as anticancer, antifungal, antimalarial, anti-HIV and antimicrobial, are a direct result of their cytotoxic activity. In a related study, several cytotoxic antibiotics were isolated by Patterson *et al.* (1991). The main orders of cyanobacteria found to produce these compounds were Nostocales and Stigonematales.

Chauhan *et al.* (1992) studied the effect of growth of some cyanobacteria and a green alga when cultured along with an axenic planktonic *Oscillatoria* sp. The growth of other aquatic organisms was suppressed owing to release of an algicidal metabolite (antibiotic). Growth inhibition was also detected on exposure to a cell-free extract of *Oscillatoria* or to an ether extract. The cyanobacterial product also suppressed growth of some natural isolates of cyanobacteria and green algae in controlled conditions and decreased the population density of a green alga (phytoplankton) in nature. Growth of higher plants was also affected, though only when the *Oscillatoria* extract was applied to leaves.

Fastner *et al.* (1995) examined the cytotoxicity induced by cyanobacteria harboring 12 eutrophic waterbodies in Germany and four laboratory strains of cyanobacteria. They had estimated the content of microcystins in these species and the level of toxicity was assessed with various bioassays. Altogether nine of twelve waterbodies and two laboratory strains contained up to seven different microcystins and were found to be severely cytotoxic. Probably other substances from the algae or their environment cause reactions which are synergistic with or additive to microcystins.

An extensive study was conducted in Lake Sammamish in the summer and fall of 1999 ([1]http). This study revealed a toxin-producing bloom despite the absence of visible cyanobacterial biomass. The toxic cyanobacteria was later on characterized as *Microcystis aeruginosa.*

Fifty cyanobacterial strains from different habitats (symbioses, soil, fresh and marine waters) belonging to the genus *Nostoc* were cultured and tested for bioactivity by Piccardi *et al.* (2000). Thirty-seven strains were isolated in the laboratory, the remaining were obtained from official culture collections. All the organisms were grown under controlled laboratory conditions. Crude extracts were found to exhibit antifungal (against *Penicillium expansum* and *Rhizoctonia solani*) and antibacterial activity (against *Agrobacterium vitis*, *Escherichia coli* and *Staphylococcus epidermidis*) and were also found to be cytotoxic. Twenty-four strains showed activity against at least one of the target organisms. The presence of bioactivity was independent of the strain origin.

Romanowska-Duda *et al.* (2002) evaluated the influence of the toxic cyanobacterial extract containing microcystins from the "algal bloom" dominated by the main species, *Microcystis aeruginosa* on the growth and morphology of a water plant *(Spirodela oligorrhiza)* and it's cytotoxicity on

animal cells. The cyanobacterial extract at higher concentrations reduced the number of fronds by about 50% in comparison with the control. The results of the study confirm the high toxic effect of blue-green algal bloom from Sulejow reservoir on both plants and animals. The eutrophication of the Sulejow reservoir dam in Poland is connected with the problem of toxicity of cyanobacterial blooming (blue-green algal blooming).

According to Surakka *et al.* (2005), benthic cyanobacteria from aquatic environments produce biologically active metabolites. The toxicity and other biological activities were studied on 21 strains of cyanobacteria belonging to *Anabaena, Calothrix, Nodularia, Nostoc* and *Phormidium* that were isolated from the benthic littoral habitats of the Baltic Sea. Studies were made to find out whether benthic cyanobacterial extracts caused cytotoxicity. Results indicate that benthic Baltic cyanobacteria contain potentially harmful cytotoxic compounds even though they do not produce microcystins or nodularins.

Based on a broad phytoplankton sampling program conducted by Marshall *et al.* (2005), 30 potential toxin producing species have been identified in the Chesapeake Bay estuarine system or the tidal regions of its tributaries in Virginia, U. S. A. Significant number of toxic cyanobacteria have been identified within the tidal freshwater and oligohaline regions of these rivers. Over the past decade there is an increase in the distribution and magnitude of blooms mainly from *Microcystis aeruginosa*. Other taxa, although rarely noted, still remain to be a potential threat as toxin producers.

Research into the ecological state of the Vistula Lagoon was carried out by Rybicka (2005). Results reveal the occurrence of blooms of several potentially toxic species of blue-green algae, *viz.*, *Anabaena flos-aquae, A. spiroides, A. lemmermannii, A. mendotae, A. circinalis, A. crassa, Aphanizomenon* sp., *Microcystis botrys, M. wesenbergii* and *Nodularia*

spumigena. Anabaena spp. was the significant component of mixed blue-green algae blooms in summer seasons.

Studies were carried out by Kobos *et al.* (2005) in the Kociewskie District on Lakes Słone, Czarne Południowe, and Kałębie of Northern Poland, which are used for recreational purposes. Based on microscopic analyses the most frequently occurring toxic cyanobacterial species belonged to the genera: *Pseudanabaena*, *Phormidium*, *Woronichinia*, *Oscillatoria* and *Anabaena*.

Bucka and Wilk-Woźniak (2005), reviewed certain selected field and laboratory works comprising both, the foreign and Polish literature data concerning the toxin production by some cyanobacteria being recognized as toxic species, *i.e.*, *Microcystis aeruginosa*, *Anabaena* spp. - *Anabaena spiroides*, *A. flos-aquae*, *Aphanizomenon flos-aquae* and *Oscillatoria agardhii*. They reported the extreme toxicity of these cyanobacterial species.

During the summer-autumn of 2004, phytoplankton as well as potential toxic blue green algae and their microcystin dynamics were studied by Kasperoviciene *et al.* (2005) in the shallow hypertrophic Lake Gineitiskes of South-eastern Lithuania. Totally 136 algae species and varieties were found, fourteen of them were found to be potentially toxic. Water bloom was caused by cyanoprokaryotes, particularly from *Anabaena* and *Microcystis* genera. Even though the bloom in the studied lake was non-toxic, the surface scum formation in midsummer might increase the risks to animals and human, especially taking into account the cumulative microcystin toxicity.

The most common cyanotoxins are described chemically as cyclic peptides, alkaloids, and lipopolysaccharides (Mur *et al.*, 1999). In the aquatic environment these toxins usually are contained mainly within the cyanobacterial cells and are released in substantial amounts during cell lysis

(Sivonen and Jones, 1999). Most cyanobacterial poisonings are caused by a group of small molecular-weight cyclic peptides known as microcystins (MCs) and nodularins (Carmichael, 1997). These are produced mainly by the genera *Microcystis, Oscillatoria, Anabaena, Nodularia, Nostoc, Umezakia* (Carmichael, 1994, 1997; Harada, 1994; Codd, 1995; Sivonen, 1996; Falconer, 1999; Singh *et al.*, 1999; Chorus *et al.*, 2000; Codd, 2000), *Arthrospira and Phormidium* (Ballot *et al.*, 2004). *Microcystis aeruginosa* is the species most often identified with fresh water cyanobacterial poisonings. They are distributed in most parts of the world in substantial quantities (Ueno *et al.*, 1998). These substances are natural endotoxins and their high concentration in water can result from cell lysis.

The effects of microcystic cyanobacterial extracts (MCE) collected from a contaminated water source on the organization of cellular microtubules (MTs) and microfilaments (MFs) in hepatocytes were made by Ding *et al.* (2000). The results suggest that MCEs induced intense intracellular cytotoxicity and were found to be responsible for an array of cytoskeletal changes. The results also reveal the spindle inhibition capacity / spindle poisoning effects of MCEs.

Studies conducted by Ding *et al.* (1999) also showed that microcystic cyanobacterial extract of water source in China had potent genotoxicity. This shows that the microcystin containing cyanobacteria are capable of inducing non-clastogenic (physiological) aberrations in test organisms.

Microcystin-disrupted microfilaments have also been reported in animal cells by Eriksson *et al.* (1989). Cellular microtubules, intermediate filaments and microfilaments were found to be disrupted by microcystin present in microcystic cyanobacteria (Wickstrom *et al.*, 1995).

MCLR is the most widely distributed variant of all MCs (Carmichael, 1992a), and exposure to MCLR has resulted in toxicities in the animal species (Fawell *et al.,* 1993). MCLR is produced as a secondary metabolite mostly by *M. aeruginosa* and by other blue green algae (Bishop *et al.,* 1959; Dittman *et al.*, 1997; Nishizawa *et al.,* 1999). Exposure to MCs was reported worldwide in animals and in humans for over a century (Francis, 1878). Kuiper-Goodman *et al.* (1999) and Carmichael (1992a) described several instances of cyanotoxin-related illnesses in humans beings.

MCLR is hepatotoxic and is a potent tumour promoter. The primary target organ of MCLR is the liver (Nishiwaki *et al.,* 1994). Exposure to MCLR was also found to result in cytoskeletal damage, resulting in apoptosis and in tumour promotion. Clearly MCLR is distributed widely and is a highly toxic biotoxin. Recognizing its potential health effects, the World Health Organization (WHO) has set a provisional guideline of 1 μg/L for MCLR in fresh water (WHO, 1998).

Lankoff *et al.* (2003) examined the influence of microcystin-LR on cell cycle progression, onset of anaphase, segregation of chromosomes by mitotic spindle and apoptosis in Chinese hamster ovary cells. Cells treated with these toxins revealed a dose and time - dependent increase of mitotic indices, accumulation of abnormal G(2) / M figures with hypercondensed chromosomes, abnormal anaphases with defective chromosome separation and polyploid cells. Since spindle check point is a fundamental regulatory mechanism that assures the onset of anaphase and subsequent exit from mitosis, the spindle organization in microcystin-treated cells were examined. The mitotic cells showed monopolar and multipolar spindles (multiple asters). Microtubule bundles were present in interphase cells. The results indicate that microcystin-LR induces apoptosis and necrosis in a dose and time-dependent manner.

Recently, research has also been aimed at the effects of crude extract of cyanobacteria to the early life stages of organisms. The effect of crude extract of cyanobacteria on the development of fish and amphibians were studied (Oberemm *et al.*, 1997, 1999; Wiegand *et al.*, 1999). These authors described deformation of nucleus, moving of nuclei to the side of nuclear membrane and convolution of the nuclear membrane.

Microcystic cyanobacterial extract (MCE) collected from a contaminated water source affected the organization of cellular microtubules and microfilaments in primary cultured rat hepatocytes in a time-dependent manner. MCE caused aggregation of MTs and MFs and a severe loss of MTs in some cells. Moreover, MCE-induced cytoskeletal alterations. These two changes could be the main cytotoxic effect of MCE. MCLR exposure results in reorganization of actin MFs, resulting in aggregation of MFs, and consequently in plasma membrane bleb formation and invagination (Hooser *et al.*, 1990, 1991).

Toxic cyanobacteria not only affect sensitive organisms and populations, but also damage fundamental ecological processes, e.g., primary production and microbial activity (Lindholm *et al.*, 2006).

Sanevas *et al.* (2006) investigated the phytotoxic activities of the crude extract from the cyanobacterium, *Hapalosiphon* sp., on the initial growth and root cell division of several plant species. Although the germination percentages of the plants were not affected by the extract, their root and shoot growth were remarkably suppressed, depending on the concentrations of the extract. Roots were more sensitive to the extract than shoots. The root tips of susceptible species became swollen and turned to a brownish color when exposed to higher concentrations. The effect of the algal extract on mitosis was investigated in wheat and onion. The extract caused a decrease of the MI in a concentration-dependent manner in onion, but not in wheat. The strong

growth reduction of the onion's primary root might be related to a reduction of cells in mitosis. However, the extract is considered to cause inhibition of cell elongation of the roots because the root length of wheat was suppressed with a higher concentration. The mitotic profiles of the treated onion and wheat cells were compared with the untreated control. The profiles of the wheat roots were not affected by the extract, but those of onion became abnormal. For onion, the number of cells in prophase, metaphase, anaphase, or telophase decreased remarkably when exposed to the extract. Generally, the compounds inhibiting mitosis (M phase) affect microtubule configuration, which resulted in an increased proportion of cells arrested in the metaphase (Armbruster *et al.*, 1991; Lazareva *et al.*, 2003). In the extract-treated cells, there was no increase in the number of cells in all mitotic phases. This suggests that the extract might not directly affect the process in mitosis but inhibit particular phases of the cell cycle, resulting in the prevention of the cell's entry into mitosis. The possible factors related to this action are the inhibition of DNA synthesis in the S phase, blocking the cell cycle progress in the G1/S or G2/M interphase, fragmentation of DNA and proteolysis of cell cycle regulators (Planchais *et al.*, 2000). Chen *et al.* (2003) reported that calothrixin A, a cyanobacterial metabolite, caused the induction of DNA fragmentation, blocking G2/M stage of cell cycle and cleavage of plasmid DNA. Thus the *Hapalosiphon* sp. crude extract induced the suppression of seedling growth by causing cessation of cell division in plant roots.

Microcystins (MCs) are cyclic heptapeptides produced by many species of cyanobacteria. MCs have been shown to cause adverse effects on animals as well as plants and therefore methods are needed for analysing MCs in different matrices. Järvenpää *et al.* (2007) assessed the effects of MC exposure on broccoli (*Brassica oleracea* var. *italica*) and mustard (*Sinapis alba*) by watering the seedlings of the plants with water containing MCs (at concentrations typically found in natural waters). Morphological,

physiological and cytological aberrations were detected in broccoli and mustard.

Jang *et al.* (2007) examined cyanobacterial toxin production in response to direct exposure to an axenically cultured aquatic plant (*Lemna japonica*) using two toxic monoclonal strains of *Microcystis aeruginosa* (NIES strains 103 and 107). The exposure to toxic *M. aeruginosa* inhibited growth in *L. japonica* and exposure to the aquatic plant caused an increase in microcystin (MC) production and inhibited growth in *M. aeruginosa*. Increased MC production in response to direct exposure to this aquatic plant could be an induced defense, mediated by the release of allelochemicals from the plant. This study revealed reciprocal allelopathic responses between *Microcystis* and *Lemna*, which can be applied to the management of eutrophic waters as well as an important information concerning strategies for recovering eutrophic waters.

MATERIALS AND METHODS

The cyanobacterial species collected from natural habitats around Calicut University and a water reservoir 'Mananchira pond' of Calicut city were isolated and considered in the present investigation. Cyanobacteria bloom abundantly in surface waters of ponds and water reservoirs (Fig. 1-3). They grow together with the rice plants and form yellowish green or brownish green mass on the surface of water (Fig. 4). Samples of such visible cyanobacteria were collected from water blooms, paddy fields and moist soils in plastic vials. The nature of habitat and habit of the thallus were noted for each sample. Standard microbial techniques were employed for isolation and subsequent culturing of cyanobacteria. A portion of the samples was stored in 4% formaldehyde and the remaining was used for culturing of cyanobacterial species using the standard isolation techniques. The taxa were identified up to their species level with the help of keys and classical monographs of Geitler (1932), Desikachary (1959) and Anand (1989) on cyanobacteria. Microphotographs of the species were taken under an Olympus CX 21 microscope attached with Olympus Camedia C- 4000 digital camera.

Culture studies

The fresh field samples were subjected to enrichment cultures in culture tubes and agar petriplates in sterile medium. The visible cyanobacterial masses were collected and washed in sterile water. A small quantity of the cyanobacterial aggregate was put in 10 ml of sterile culture medium and used as initial inoculum. The isolation was carried out by the streak and spread plate methods (Stein, 1973; Kaushik, 1987). Subculturing was done repeatedly, whereby pure cultures of some species (unicyanobacterial isolates) were obtained. The cultures were periodically examined under research microscope for assessing their pure nature. Liquid

cultures were maintained in conical flasks and solid cultures in agar plates/ slants.

Culture methods

The glass tubes and conical flasks used for isolation and purification of cyanobacteria were cleaned in liquid detergent and dried in a hot air oven before use. After cooling, they were immersed in chromic acid (Potassium dichromate in conc. H_2SO_4 1% w/v) overnight followed by repeated washing in tap water and finally with distilled water.

The culture medium selected for the study was BG-11 medium with a nitrogen source for non- heterocystous cyanobacteria and without a nitrogen source (nitrogen free medium) for heterocystous types. The composition of the medium is given below.

BG-11 Nitrogen free medium-Composition (Rippka *et al.*, 1979).

Chemicals	**Concentration (g/l)**
$K_2HPO_4.\ 3H_2O$	0.040
$MgSO_4.\ 7H_2O$	0.075
$CaCl_2.\ 2H_2O$	0.036
Citric acid	0.006
Ferric Ammonium Citrate	0.006
EDTA	0.001
Na_2CO_3	0.02
A6 Micronutrient solution	1 mg/l

For non-heterocystous cyanobacteria, 1.5g $NaNO_3$ is added to BG-11 medium as nitrogen source (pH of the medium was adjusted to 7.4).

Composition of trace metal solution (Rippka *et al.*, 1979).

Chemicals	Concentration (g/l)
H_3BO_3	2.86
$MnCl_2.4H_2O$	1.81
$ZnSO_4.7H_2O$	0.222
$Na_2MoO_4.2H_20$	0.390
$CuSO_4.5H_2O$	0.079
$Co(NO_3)_2.6H_2O$	0.0494

Sterilization

The glassware and culture medium used for the experiment were sterilized properly for 20 minutes in a pressure cooker.

Culture conditions:

The cultures were incubated at 26± 2 °C under illumination of cool day light fluorescent tubes (intensity 14.4 W m-2) on surface of the culture vessels with a 14/10 h light/ dark cycle. The cultures in the liquid medium were hand shaken daily to prevent sticking of the cyanobacteria to the wall of the container. The volume of the medium has been maintained less than half of the culture vessel volume in order to provide adequate air supply and eliminate contamination due to slashing during shaking. Growth was visible in tubes after 10 days of incubation. Each of such clonal culture was transferred to fresh BG-11 medium to obtain desirable population size.

Maintenance of culture

The stock cultures of cyanobacteria were maintained in agar slants and petriplates. The pure cultures of each organism was transferred into a conical flask (250 ml) containing 100 ml fresh sterile medium from the agar slants/

petriplates and these cultures were incubated and each of the cultures were used during the phase of maximum growth (30-40 days).

Characterization under laboratory conditions

Unicyanobacterial cultures belonging to the genera *Microcystis, Anabaena, Nostoc, Oscillatoria* and *Phormidium* were studied based on their morphological features. The various morphological parameters, such as, nature and colour of the thallus, pattern of growth and other features were considered (Desikachary, 1959).

Preparation of crude extract of cyanobacterial samples

30-40 days old cultures were used for the experiments. Homogenized suspensions of each of the cyanobacterial species were prepared using a sterile glass tube homogenizer. The cyanobacteria were harvested by filtration. Cyanobacterial cell extracts were prepared following the methods of Shibib *et al.* (1993). For this purpose 5 g fresh weight of cyanobacterial mass of each species was blended in 10 ml of 95% methanol and left at room temperature with occasional shaking for 48 h. Methanol has been advocated as the most suitable solvent because it gives good extraction efficiency and has the added advantage of allowing rapid sample concentration through evaporation. The suspension was centrifuged at 6000 rpm for 15 minutes at room temperature. The supernatant obtained was filtered through cheesecloth and the filtrate was evaporated at 40-50°C to a residue. On the day of experimentation, 0.25 g of the residue of each one was dissolved in 25 ml of distilled water and thus 1% stock solution was prepared. From the stock solution of each of the cyanobacterial species 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.

The test material used for cytotoxic assay was *Allium cepa* L. Healthy and uniform sized certified bulbs of *Allium cepa* L. were selected and washed in distilled water. These bulbs were planted in sandy soil and watered adequately. Germinated bulbs of *Allium cepa* L. with healthy roots were collected at the time of peak mitotic activity (9.00 am -10.00 am). The collected bulbs were washed thoroughly in distilled water and were treated with different concentrations of cyanobacterial extracts taken in bottles, in such a manner that only the roots were immersed in the solution. Root tips were cut off from few samples of each concentration at different time intervals such as ½ h, 1 h, 1½ h and 2 h, washed thoroughly with distilled water and immediately fixed in modified Carnoy's fluid (1 acetic acid : 2 ethyl alcohol) for 1 h. After fixing, all the root tips were thoroughly washed in distilled water and were stored in 70% ethyl alcohol under refrigeration.

Preparation of stain

This was done according to the procedure of Sharma and Sharma (1990). 45% acetic acid was poured into a reflex condenser and allowed to boil for 30 minutes. When the mixture started boiling, 2 grams of orcein powder was added little by little. When the powder was well dissolved in the solution, it was cooled and filtered into an amber coloured bottle and stored in a refrigerator.

Squash preparation

Mitotic squash experiments were conducted with the help of improved techniques (Sharma and Sharma, 1990). The stored root tips were washed with distilled water and hydrolysed in 1N HCl for 2-3 minutes to separate the cells during squashing. The root tips were washed thoroughly with distilled water and stained with 2% aceto-orcein for 3 h. After staining, the root tips

were destained with 45% acetic acid, squashed and mounted on clean microslides.

Mitotic index (%) and abnormality percentage were calculated using the following formulae:

$$\text{Mitotic index (\%)} = \frac{\text{Total number of dividing cells}}{\text{Total number of cells observed}} \times 100$$

$$\text{Percentage of abnormalities} = \frac{\text{Total number of abnormal cells}}{\text{Total number of cells observed}} \times 100$$

All the slides were scanned under Olympus CX 21 microscope, tabulated and photomicrographs were taken with Olympus Camedia C-4000 digital camera. During tabulation, normal and abnormal cells of divisional and non-divisional stages were scored except normal interphase. However, normal interphase cells were also counted under the total number of cells observed in each treatment. Bar represents 10μm in all figures from Plates 2 - 21.

PLATE – 1

Figs. 1-4. Cyanobacterial blooms formed in various water reservoirs, rice fields and associated areas adjacent to Calicut University.

Fig. 1. Cyanobacterial bloom formation in 'Mananchira pond' at Calicut.

Fig. 2. Cyanobacterial bloom in a private pond at Malappuram District.

Fig. 3. Bloom formation by cyanobacteria in a water logged area in Malappuram.

Fig. 4. Blooming cyanobacterial mass in a rice field near Calicut University.

PLATE - 1


1


2


3


4

RESULTS

Eight cyanobacterial species coming under 5 genera of 3 families with an elevated occurrence of *Phormidium*, *Oscillatoria* and *Anabaena* genera were isolated from the sampling sites (Plate 1). Individual cyanobacterial species (Plates 2 & 3) were identified both in environmental samples and culture samples. Out of the eight cyanobacterial species identified, four of them are capable of motility. Separate taxonomic key is provided only in those areas, where more than one taxon is characterized in a group.

TAXONOMIC TREATMENT

Key to the various orders of Cyanobacteria characterized

1. Forms unicellular, not attached ..... **Chroococcales**
1. Forms filamentous, psuedoparenchymatous growth absent, unbranched or showing false branching ..... **Nostocales**

Order : **CHROOCOCCALES** F. Wettst.

Forms unicellular or colonial, sometimes forming a psuedofilamentous colony, never with a trichome organization, no differentiation into base and apex.

Family : **CHROOCOCCACEAE** Nägeli

Cells mostly spherical, ellipsoidal, cylindrical, seldom spindle shaped, single or forming colonies, not forming filament like growth; membrane thick, mucilaginous, often lamellated, with an overall formation of amorphous mucilaginous masses; colony shapeless, spherical, ellipsoidal or hemispherical; cell-division in two or three directions, in elongate cells often

only in one direction - transverse, cells of many generations in a single parent sheath; multiplication by division, sometimes through nannocysts.

MICROCYSTIS Kütz.

Colony spherical, ellipsoidal or irregularly overlapping net-like; cells irregularly arranged, many in a colony, spherical or elongated in homogeneous colourless, diffluent mucilage, densely packed, gas vacuoles commonly seen.

Microcystis aeruginosa Kütz.

Colonies are blue green, when young round or slightly longer than broad, when old becoming clathrate with distinct hyaline colonial mucilage; cells 3 - 7 μm in diameter, spherical, generally with gas vacuoles (Fig. 6).

Observed as water bloom in a paddy field near Calicut University and in a private pond at Malappuram District.

Order : **NOSTOCALES** Geitler

Plants filamentous, hormogones present, heterocysts and akinetes present, true branching absent, false branching present.

Key to the various families of Nostocales characterized

1. Filaments heterocystous with spores, showing uniform width, unbranched ..... **Nostocaceae**
1. Filaments non heterocystous without spores ..... **Oscillatoriaceae**

Family : **OSCILLATORIACEAE** Kirchn.

Trichome with a single row of similar and uniformly broadened cells, sometimes tapering at the extreme ends; not branched, with or without diffluent mucilage or a homogenous or more or less lamellated firm sheath; occasionally branched in genera with a firm sheath; trichome straight or bent at the apex, or regularly or irregularly spirally coiled, single or many within a sheath; heterocysts and spores absent; hormogones present; majority show a spiral movement by rotation along the longitudinal axis.

Key to the various genera of Oscillatoriaceae characterized

1. Trichome with a sheath, single within a sheath, sheath mucilaginous ..... ***Phormidium***
1. Trichome without a sheath, straight or bent slightly at the apex, cells mostly shorter than broad ***Oscillatoria***

PHORMIDIUM Kütz.

Filaments blue-green, coiled or entangled or parallel; many forming a leathery stratum; sheath thin, colourless, often diffluent; trichome cylindrical; trichome apices attenuated, straight or apex slightly bent, with or without calyptra.

Phormidium tenue (Menegh.) Gomont

Thallus pale blue-green, thin, expanded, membraneous; filaments flexuous, densely entangled; sheath thin, diffluent; trichome straight, not constricted and not granulated at the cross walls, cross walls not visible; cells 1.5 - 2.5 μm broad, 3.5 – 7 μm long; ends straight, end cell acute conical, more or less pointed, calyptra absent (Fig. 12).

Observed as planktonic form in stagnant water of a pond near the Calicut University Campus.

OSCILLATORIA Vaucher

Trichome solitary and scattered or in groups forming an expanded mass; sheath absent or thin, motile either by oscillation or creeping; end cell of the trichome distinctly marked, pointed or blunt, with or without calyptra; hormogones present.

Key to the various species of *Oscillatoria* characterized

1. Trichome not constricted at the septa, end cell rounded at the apex, about 4 μm broad ..... ***O. obscura***
1. Trichome not constricted at the septa, more than 20 μm broad .... ***O. princeps***

Oscillatoria obscura Brühl *et* Biswas

Trichome deep blue green in colour, about 4-5 μm broad, attenuated at the apex, rounded, slightly bent or nearly straight, not constricted at the cross-walls; cross-walls granulated (Fig. 10).

Observed as planktonic form in the water reservoir, Mananchira pond at Kozhikode.

Oscillatoria princeps Vaucher ex Gomont

Trichomes blue-green, straight, not constricted at the cross-walls, 16 – 50 μm broad, 3.5 - 6.5 μm long; end cells flatly rounded, slightly capitate (Fig. 11). Observed as planktonic form in rice fields of Kozhikode.

Family : **NOSTOCACEAE** Kütz.

Trichome free or in a common mucilage to form colonies of various shapes; filamentous, cells generally similar throughout, end cells attenuated; sheath thick or thin, mucilaginous or firm; hormogones present, heterocyst present or absent, if present intercalary or terminal, single or in chains of 2 or 3; spores present or absent, single or in series beginning from near the heterocyst or in between two of them.

Key to the various genera of Nostocaceae characterized

1. Trichome without firm sheath, heterocysts intercalary, rarely terminal, spore adjoining or not, filaments free or in a formless gelatinous mass . ... ***Anabaena***
1. Trichome without firm sheath, heterocysts intercalary, rarely terminal, spore adjoining or not, filaments entangled in a definite colony.....***Nostoc***

NOSTOC Vaucher

Thallus mucilaginous, globose, foliose or irregular lobed colony, trichome uniseriate, unbranched, torulose; sheath sometimes distinct, generally diffluent; cells barrel shaped, spherical or cylindrical; heterocysts intercalary or terminal; spores spherical or oblong, formed singly or centrifugally in series in between the heterocysts.

Key to the various species of Nostoc characterized

1. Thallus with a firm outer layer, heterocysts terminal.....***N. commune***
1. Thallus without firm layer, soft and formless, trichome not densely coiled, cells clearly visible 2
2. Thallus macroscopic, cells cylindrical, trichome 4 - 4.5 μm broad ....***N. spongiaeforme***

2. Thallus macroscopic, cells cylindrical, trichome 3- 4 μm broad, heterocyst 6 μm broad ..... ***N. carneum***

Nostoc commune Vaucher ex. Born. *et* Flah.

Thallus firm, gelatinous, at first globose, later irregularly expanding, membranous, blue-green to brown; trichome 5 - 6 μm broad, cells short, barrel shaped, 5 - 5.5 μm long; heterocyst nearly spherical, 5.5 - 7 μm broad, terminal; spores not observed (Fig. 8).

Observed in paddy fields of Malappuram district of Kerala.

Nostoc spongiaeforme C. Agardh ex Born. *et* Flah.

Thallus first globose, later expanding, light blue green or brownish; filaments flexuous, loosely entangled; sheath diffluent and distinct towards the periphery; trichomes 4 - 4.5 μm broad, blue-green; cells elongated, cylindrical, 6 - 9 μm long or short, barrel shaped; heterocysts sub spherical or oblong, 4.5 - 5.5 μm broad, 7 – 8 μm long; spores oblong, in chains, 5 – 6 μm broad, 7.5 – 11 μm long, epispore smooth, yellowish (Fig. 9). Observed in algal blooms present in a pond and in rice fields of Malappuram.

Nostoc carneum C. Agardh ex Born. *et* Flah.

Thallus globose, blue / brownish blue coloured; filaments loosely entangled; trichome 3 - 4 μm broad, cells oblongo-cylindrical, up to twice as long as broad; with rounded ends, cells longer than broad, heterocysts oblong, 6 μm broad, spore oval to ellipsoidal, 6 μm broad (Fig. 7).

Observed as epiphytic in paddy fields of Calicut.

ANABAENA Bory

Trichomes uniformly broad throughout or apices alone somewhat attenuated, sheath absent or more or less diffluent, forming a free, torn or floccose or soft mucilagenous thallus; heterocysts generally intercalary; spores single or in long series, formed from near the heterocysts or in between the heterocysts.

Anabaena circinalis Rabenhorst ex Born. *et* Flah. var. ***crassa*** Ghose

Trichome blue green in colour, free-swimming, single, mostly circinate or semi-circular, loosely coiled up to 4 times; cells nearly spherical, but generally shorter than broad, 5-8 μm in diameter, with pseudovacuoles; heterocysts globose, up to 8 μm broad; spores not seen (Fig. 5).

Observed as planktonic form in water blooms of Calicut .

Characterization under laboratory conditions

The eight cyanobacterial species viz., *Microcystis aeruginosa, Anabaena circinalis* var. *crassa, Nostoc carneum, N. commune, N. spongiaeforme, Oscillatoria obscura, O. princeps* and *Phormidium tenue* flourished well in the BG-11(liquid and solid) medium [with a nitrogen source for non-heterocystous species of cyanobacteria and without a nitrogen source (nitrogen free medium) for heterocystous types] under laboratory conditions.

Growth and thallus characteristics of cyanobacterial species in culture medium (both liquid and solid)

The study showed all the species grew well in BG - 11 media. *Anabaena circinalis* var. *crassa, Nostoc carneum, N. commune and N. spongiaeforme* exhibited better growth in BG - 11 nitrogen free media.

Colony initiation was noticed 7 - 8 days after inoculation in *Nostoc carneum, N. commune, N. spongiaeforme* and *Anabaena circinalis* var. *crassa* and they showed complete growth within 30 days after colony initiation. But in *Microcystis aeruginosa, Oscillatoria obscura, O. princeps* and *Phormidium tenue* colony initiation was noticed 13-15 days after inoculation. After the formation of colonies the growth was slow compared to the heterocystous species. 40 days were needed for their complete growth.

In liquid medium thallus of most of the species especially *Nostoc commune,* showed a greater tendency to stick on to the wall of the containers and such a tendency is least exhibited by *Microcystis aeruginosa.* The colour of the thallus vary from blue green to brownish green. The thalli were blue - green except for *Nostoc carneum, N. commune, N. spongiaeforme,* which were brownish green in colour.

CYTOTOXIC STUDIES

Cytotoxicity of crude cyanobacterial extracts in *Allium cepa* L.

The cytotoxic effects of different treatments with the extracts of eight species of cyanobacteria on mitotic divisions in the root tip meristems of *Allium cepa* in four different time durations (½, 1, 1 ½ and 2 hrs) are given in plates 5-21, tables 1-32 and graphs 1-8. The species studied were *Microcystis aeruginosa, Anabaena circinalis* var. *crassa, Nostoc carneum, N. commune, N. spongiaeforme, Oscillatoria obscura, O. princeps* and *Phormidium tenue.*

In the present investigation, *Allium cepa* L. root tip cells showed normal mitotic divisions when treated with distilled water as control (Plate 4). Roots tips treated for ½ h, 1 h, 1½ and 2 hours in the presence of 0.01%, 0.02%. 0.05%, 0.075% and 0.1% extracts of eight cyanobacterial species showed rigorous cytotoxicity, mitotic inhibition and malformations in the nuclear structure, including changes in shape, budding, progressive

disappearance of the nucleus and chromatin, damage of the nuclear envelope and many other mitotic abnormalities.

The *Allium* test revealed that all concentrations (0.01% to 0.1%) of the cyanobacterial extracts lowered the frequency of dividing cells and induced an array of mitotic abnormalities. The extracts caused a decrease of the mitotic index in a concentration and time - dependent manner in *Allium cepa* L. root meristem. The percentage of abnormality showed a general increase as the concentration of the extract increased. However an increase in the concentration of the extract caused a decrease in mitotic index when compared with the control (Tables 1-32).

A wide spectrum of cytological aberrations were found to be induced after treatments with the aqueous extracts of eight cyanobacteria. Many clastogenic and non-clastogenic abnormalities were detected during the different treatments (Plates 5 – 21). The major clastogenic abnormalities observed were cytomixis (Fig. 19; Tables 29 - 32), aberrant and bizarre nucleus (Figs. 27, 30, 38, 50-53, 55-58, 70, 84, 85; Tables 9 – 32), bizarre metaphase, -anaphase and -telophase (Figs. 130-133, 197, 198, 211-213; Tables 1-8, 21-28). The other classes of clastogenic aberrations include nuclear appendage formation / nuclear budding (Figs. 33-35; Tables 1-8, 29-32), nuclear diminution (Fig. 36; Tables 21-24), nuclear disintegration (Figs. 37, 81; Tables 1-8, 21-24), nuclear extrusion (Figs. 40, 41, 77; Tables 1-4), nuclear fragmentation (Figs. 42-44, 215; Tables 17-24, 29-32), nuclear lesions and microlesions (Figs. 45, 61-63, 65, 66, 68; Tables 9-12, 25-32), pulverization (Figs. 46, 47, 69, 79, 80, 107-109, 181, 184-187, 209; Tables 1-4, 21-24, 29-32), hyperchromasia (Fig. 48; Tables 21-24), dissolution of chromatin (Figs. 49, 54, 76, 78; Tables 5-8, 21-24, 29-32), nuclear erosion (Fig. 64; Tables 5-8), abnormal condensation of chromatin (Figs. 33, 66, 71, 73-75; Tables 1-32), contorted chromatin (Fig. 72; Tables 9-12), abnormal

condensation of chromosomes (Figs. 86, 88, 108, 122, 172, 201, 202, 206, 218; Tables 5-16, 25-32), giant cells (Figs. 67-70, 119-126, 159-166, 199-202; Tables 13-20, 29-32), stickiness of chromosomes (Figs. 87, 100, 103, 104, 122, 134, 137, 140, 143, 150, 152, 183, 188-192, 199, 207, 208, 214; Tables 1-32), ring chromosomes (Figs. 97, 98, 168; Tables 29-32), coagulation / adhesion of chromosomes (Figs. 105, 193; Tables 29-32), chromosome gaps (Figs. 112, 124; Tables 5-8, 21-24), telosome formation (Fig. 115; Tables 1-4), convoluted chromosomes (Fig. 116; Tables 29-32), deproteinized chromosomes (Figs. 118, 194-196; Tables 5-8, 29-32), chromosome fragments (Figs. 110-112, 115, 124, 156, 161, 165, 167, 168, 177, 180; Tables 1-8, 21-24) and chromosome bridges (Figs. 21, 137-146, 153, 160, 176, 187; Tables 1-4, 9-28).

The chief non-clastogenic abnormalities observed were micronuclei (Figs. 20-24, 28, 29, 44, 58; Tables 1-8, 17-24) bi-, tri-, tetra- and multinucleate cells (Figs. 25-32, 41, 59, 60, 117; Tables 1-4, 9-20, 25-28) clumping of chromosomes (Figs. 89, 106, 126, 191; Tables 5-12, 17-28), stellate arrangement of chromosomes (Figs. 96, 145-150, 162, 163, 200, 205; Tables 1-4, 9-16, 21-28), ball shaped arrangement of chromosomes (Figs. 99, 100, 122, 135, 136; Tables 5-8, 17-24), C-metaphase (Figs. 101, 102, 104, 109, 113, 123, 125, 126; Tables 1-4, 13-16, 29-32), blebbing (Fig. 39; Tables 29-32), abnormal grouping of chromosomes (Figs. 83, 129, 195, 210; Tables 13-24), equatorial separation of chromosomes (Figs. 151-153, 204; Tables 17-20, 29-32), stratified metaphase (Figs. 90, 91, 120; Tables 21 - 24), scattering of chromosomes (Figs. 92, 93, 169; Tables 13-20), lollypop metaphase (Fig. 95; Tables 13-16), bouquet formation of chromosomes / chromatin (Figs. 82, 94; Tables 25-28), misorientation of chromosomes (Figs. 113, 119, 127, 128, 157, 164, 170, 208; Tables 13 - 20, 29 - 32), polyploidy (Figs. 114, 115, 121, 124, 177; Tables 1-4, 21-24), diagonal arrangement of chromosomes (Figs. 118, 123, 143, 178-181, 192, 193, 199, 203, 206; Tables 9-32), disturbed

metaphase, -anaphase and -telophase (Figs. 121, 144, 173-177; Tables 1-4, 13-24), chromosome laggards (Figs. 136, 149, 154, 174, 186, 194; Tables 1-4, 9-12, 21-28), abnormal movement of chromosomes (Figs. 155, 156, 158, 159, 190, 196; Tables 9 – 28), stathmo - anaphase (Figs. 166, 182-184; Tables 21-24, 29-32), shift in microtubule organizing centers (Figs. 165, 171; Tables 13-16) and unequal division and formation of macro and micro cells (Figs. 216, 217; Tables 25-28).

Mitotic indices in the various treatments with the cyanobacterial extracts (Tables 1-32) were found to be less than that of control. Mitotic index showed an inverse relationship with the increase in the concentration of the extract, except in a few species. There is a positive correlation between concentration of extract and the frequency of aberrations. The frequency of abnormalities were found to increase with the increasing concentration of the extract and time duration (Graphs 1 – 8).

The percentage of cytotoxicity and mitotic inhibition was found to be different in various cyanobacterial extracts in the present investigation. Severe cytotoxicity, mitotic inhibiton and nuclear malformations were noticed with the cyanobacterial extracts of *Microcystis aeruginosa, Anabaena circinalis* var. *crassa,* and *Oscillatoria obscura*, moderate cytotoxicity and mitotic inhibition were noticed with the extracts of *Nostoc commune, N. spongiaeforme,* as well as *O. princeps* and comparatively low cytotoxicity and mitotic inhibition were noticed with *Phormidium tenue* and *N. carneum.*

***Anabaena circinalis* var. *crassa* Ghose**

Abundance of cytological aberrations was observed with 0.01%, 0.02%, 0.05%, 0.075% and 0.1% extract of *Anabaena circinalis* var. *crassa* in the root tip meristems of *Allium cepa* during four different time durations (½, 1, 1 ½ and 2 hrs).

The major abnormalities observed were abnormal disintegration of nuclear membrane, differential condensation, nuclear extrusion, sticky metaphase, C- metaphase, double metaphase in a binucleate cell, chromosome fragments, polypoidy, chromosome bridges, disturbed early anaphase with laggards, chromosome fragments and telosomes in polyploid and diploid cells, bizarre telophase, stellate telophase, pulverization of chromosomes, sticky cytokinesis, binucleate cell, nuclear budding, macro and micronucleus with a bridge like connection, micronuclei and crescent shaped nucleus. High frequency of micronuclei and crescent shaped nuclei were observed especially at 2 h duration with 0.1% concentration of the extract.

In the various treatments with extracts of *A. circinalis* var. *crassa,* the frequency of aberrations were found to be high. Mitotic index ranges from 21.37% to 6.31% and frequency of aberrations from 17.56% to 35.46% (Graph 1; Tables 1-4).

Microcystis aeruginosa Kütz.

A wide spectrum of cytological aberrations were scored with 0.01%, 0.02%, 0.05%, 0.075% and 0.1% extract of *Microcystis aeruginosa* in the root tip meristems of *Allium cepa* during four different time durations (½, 1, 1½ and 2 hrs). Condensed chromatin, abnormal disintegration of nuclear membrane, dissolution of chromatin, sticky metaphase, chromosome gaps and fragments, bizarre metaphase, ball metaphase, hyper condensed chromosomes, clumped sticky anaphase, bizarre anaphase, deproteinized chromosomes, extremely sticky telophase, abnormal cytokinesis, condensed chromatin, nuclear erosion, nuclear budding and micronuclei were the major abnormalities observed. The frequency of micronuclei were found to be highest at 2 hour time duration with the extract.

In the various treatments with the extracts of *M. aeruginosa,* the frequency of aberrations were maximum. Mitotic index ranges from 20.37% to 8.4% and frequency of aberrations from 10.09% to 35.63% (Graph 2; Tables 5-8).

Nostoc carneum C. Agardh ex Born. *et* Flah.

Treatments with extracts of *Nostoc carneum* induced large number of cytological aberrations. The major abnormalities observed were differential condensation, contorted chromatin, clumping, abnormal condensation, contorted metaphase, anaphase with a single bridge, chromosome laggard, early movement of chromosomes, delayed movement of chromosomes, stellate anaphase, diagonal anaphase, giant cells, bizarre telophase, diagonal telophase, stickiness, nuclear lesions, binucleate cell and abnormal interphase. Nuclear lesions were noticed in high frequency in long time treatments.

The frequency of aberrations were comparatively low in various treatments. However, there was a decline in the mitotic index in various treatments. Mitotic index ranges from 19.57% to 7.07% and frequency of aberrations from 5.26% to 16.3% (Graph 3; Tables 9-12).

Nostoc commune Vaucher ex Born. *et* Flah.

An array of cytological aberrations were scored with 0.01%, 0.02%, 0.05%, 0.075% and 0.1% extract of *Nostoc commune* at various time durations.

The main abnormalities observed were bizarre nuclei, differential condensation, unequal grouping of chromatin, lollypop metaphase, scattering and grouping of chromosomes, giant cell with C-metaphase, double metaphase in a binucleate cell, early movement of chromosomes, giant cell with a bridge, disturbed anaphase, shift in MTOC and unequal separation,

misorientation of chromosomes, stellate anaphase in a giant cell, stellate telophase in a giant cell, diagonal sticky telophase, condensed diagonal telophase, hyper condensation, bizarre form of nucleus, club shaped nucleus and giant cell with nucleus at the periphery. The most frequently observed aberrations were club shaped nuclei and bizarre forms of nuclei during interphase.

The frequency of aberrations were moderate in various treatments. Mitotic index ranges from 19.40% to 5.04% and frequency of aberrations from 10.45% to 19.31% (Graph 4; Tables 13-16).

Nostoc spongiaeforme C. Agardh ex Born. *et* Flah.

The chief abnormalities observed with various concentrations of the extract of *Nostoc spongiaeforme* were condensed chromatin, scattering of chromosomes, bizarre form of nucleus, ball metaphase, giant cell with misoriented chromosomes, displaced chromosomes, sticky diagonal anaphase, disturbed spindle organization at one pole, scattered movement of chromosomes, diagonal sticky telophase, abnormal grouping of chromosomes, equatorial separation of chromosomes, fragmentation of one daughter nucleus, binucleate cell, elongated nucleus with micro nucleus and giant cell with nucleus at the periphery.

The frequency of aberrations were high in various treatments. Mitotic index ranges from 18.59% to 9.33% and frequency of aberrations from 12.87% to 21.2% (Graph 5; Tables 17-20).

Oscillatoria obscura Brühl *et* Biswas

Various drastic chromosomal aberrations were noticed in the treatment with extracts of *O. obscura*. The chief abnormalities observed were condensed chromatin, nuclear disintegration, pulverized prophase, bizarre

prophase, sticky metaphase, pulverized metaphase, chromosome gaps and fragments, stratified metaphase, bizarre metaphase, polyploid cell, sticky anaphase with bridges, ball anaphase, disturbed anaphase with laggards, stathmo-anaphase, diagonal pulverized anaphase, unequal movement of chromosomes, bizarre telophase, stellate telophase, abnormal grouping of chromosomes, stickiness during cytokinesis, dissolution of chromatin, nuclei showing hyperchromasia, nuclear pulverization, nuclear diminution, nuclear fragmentation and micronuclei. Highest frequency of micronuclei were noticed in 2 hour duration with 0.1% concentration of the extract.

The frequency of aberrations were severe in various treatments. Mitotic index ranges from 19.38% to 13.51% and frequency of aberrations from 18.10% to 37.84% (Graph 6; Tables 21-24).

Oscillatoria princeps Vaucher ex Gomont

Treatments with extracts of *Oscillatoria princeps* induced severe abnormalities. Differential condensation, non synchronized condensation, clumping, abnormal condensation, bouquet formation of chromosomes, anaphase with a single bridge, chromosome laggard, early movement of chromosomes, delayed movement of chromosomes, stellate anaphase, diagonal anaphase, bizarre telophase, diagonal telophase, unequal division, nuclear lesions, binucleate cell and abnormal interphase were visualized.

The frequency of aberrations were comparatively low in various treatments. Mitotic index ranges from 8.57% to 5.21% and frequency of aberrations from 9.72% to 18.48% (Graph 7; Tables 25-28).

Phormidium tenue (Menegh.) Gomont

The major abnormalities observed were condensed chromatin and appendage formation, bizarre prophase, dissolution of chromatin, ring

chromosomes, hyper-stickiness, convoluted chromosomes, misoriented C-metaphase, adhesion of chromosomes, deproteinized diagonal metaphase, equatorial separation of chromosomes, stathmo-anaphase, pulverized anaphase, sticky anaphase, fragmentation of daughter nuclei, pulverized chromosomes, equatorial separation, hyper condensation at cytokinesis, aberrant nucleus, cytomixis, blebbing and giant cell with several lesions.

The frequency of aberrations were comparatively low in various treatments. Mitotic index ranges from 10.01% to 6.69% and frequency of aberrations from 6.24% to 15.48% (Graph 8; Tables 29-32).

PLATE – 2

Figs. 5-8. Habit of various cyanobacteria characterized in the present investigation

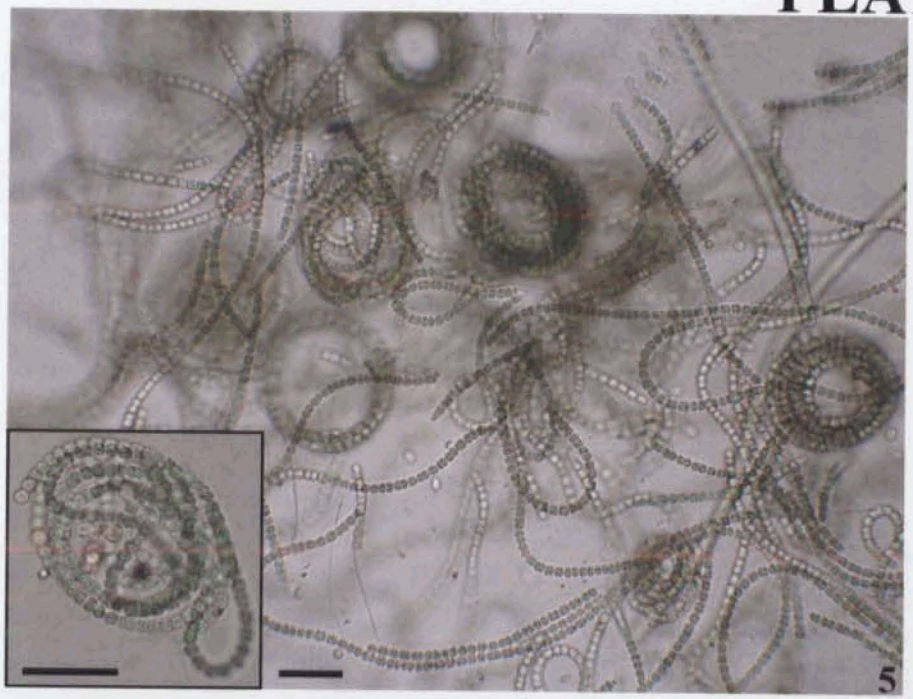
Fig. 5. *Anabaena circinalis* var. *crassa.*

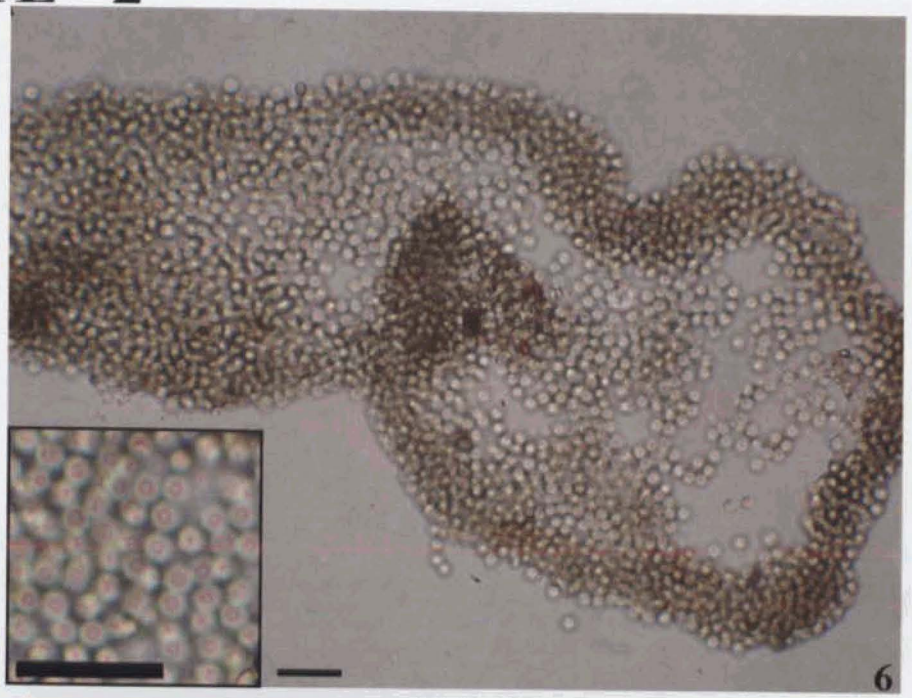
Fig. 6. *Microcystis aeruginosa.*

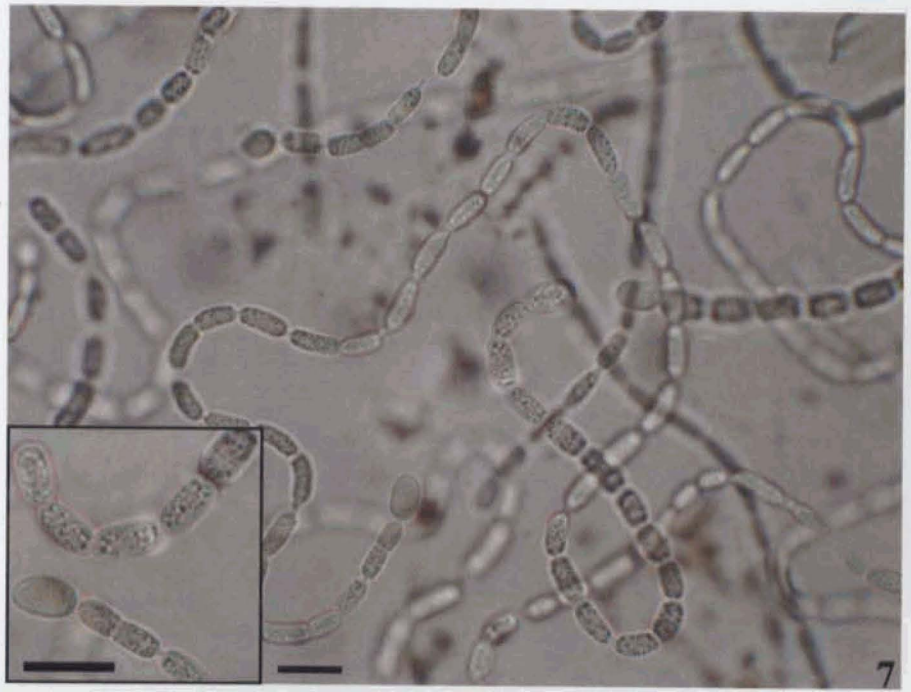
Fig. 7. *Nostoc carneum.*

Fig. 8. *Nostoc commune.*

PLATE - 2







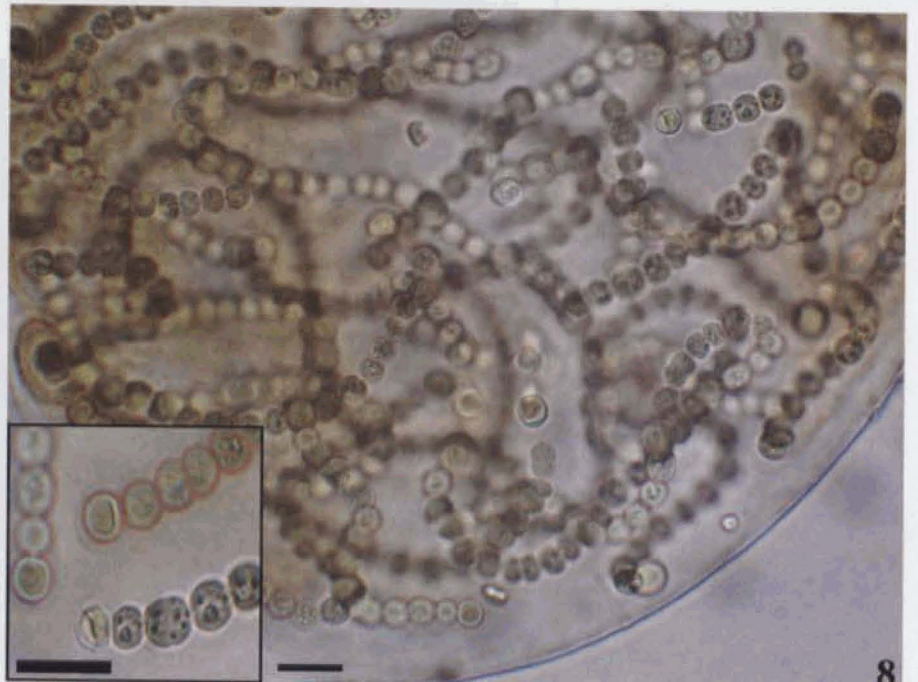


PLATE – 3

Figs. 9-12. Habit of various Cyanobacteria characterized in the present investigation.

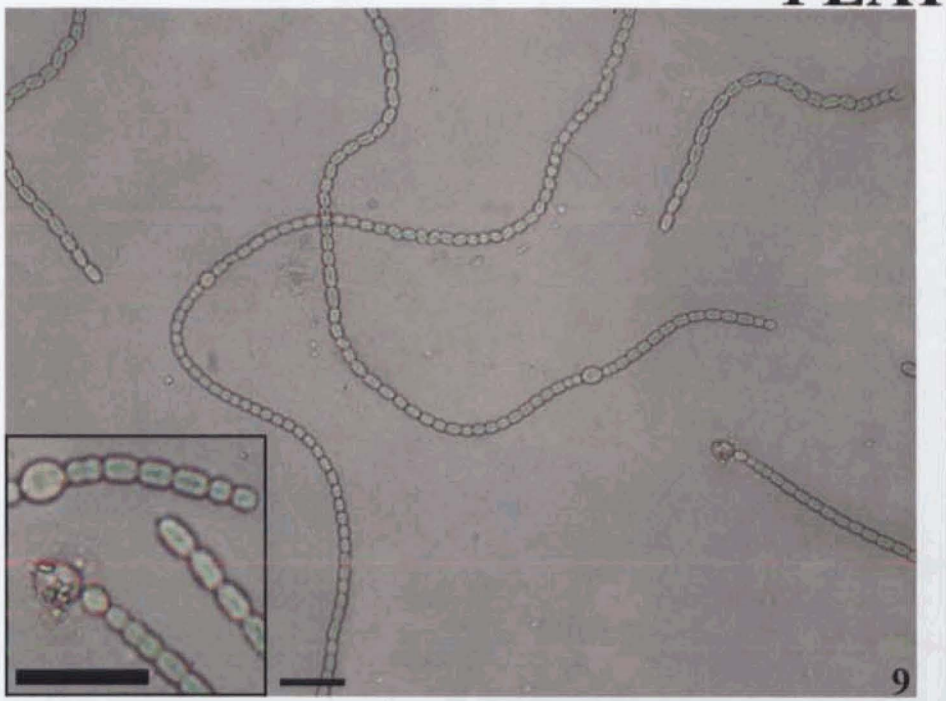
Fig. 9. Nostoc *spongiaeforme*

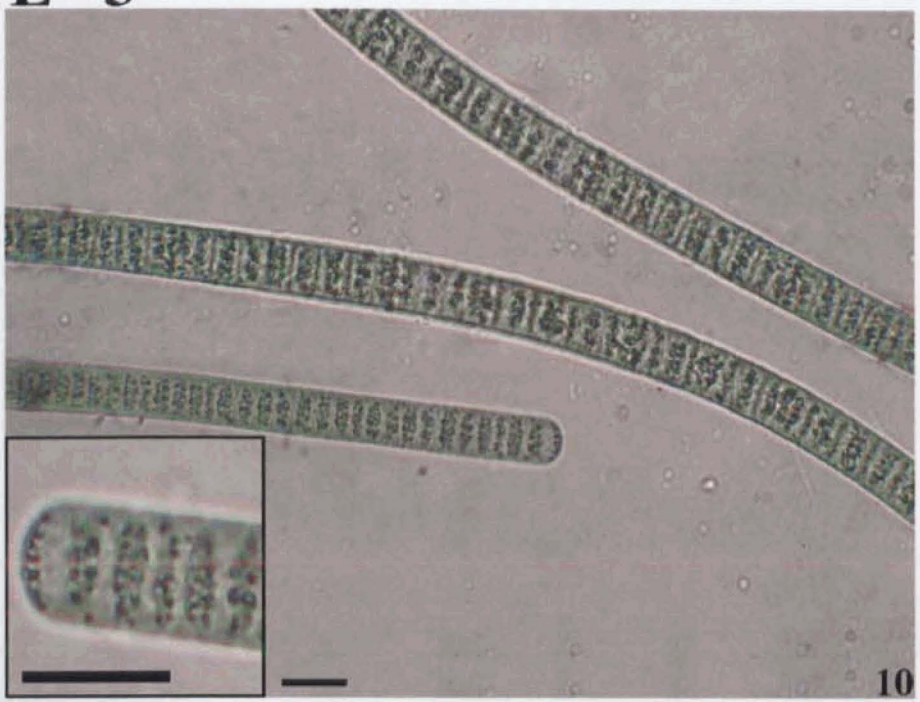
Fig. 10. *Oscillatoria obscura*

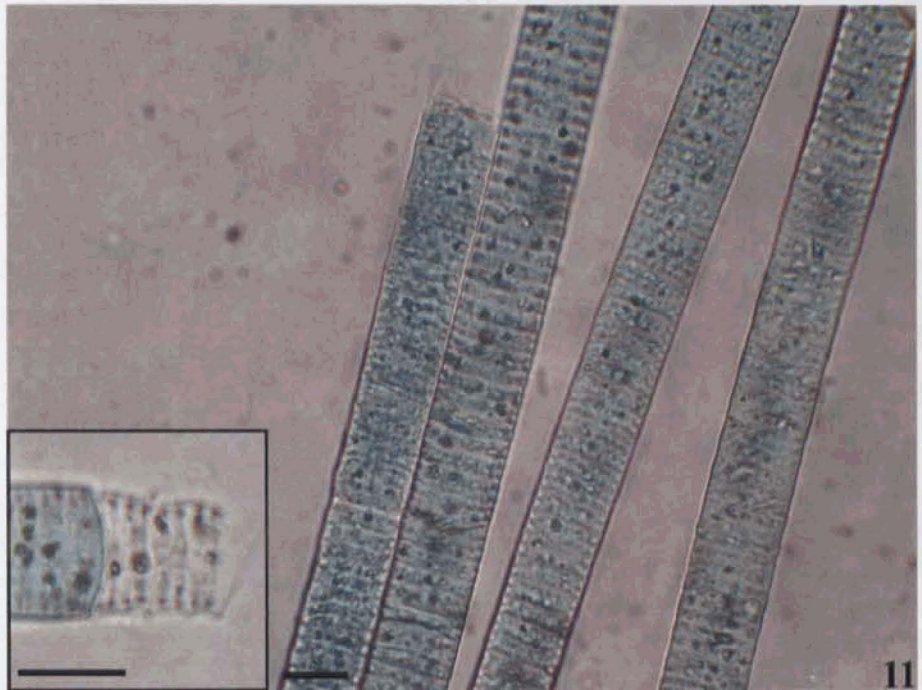
Fig. 11. *Oscillatoria princeps*

Fig. 12. *Phormidium tenue*

PLATE - 3







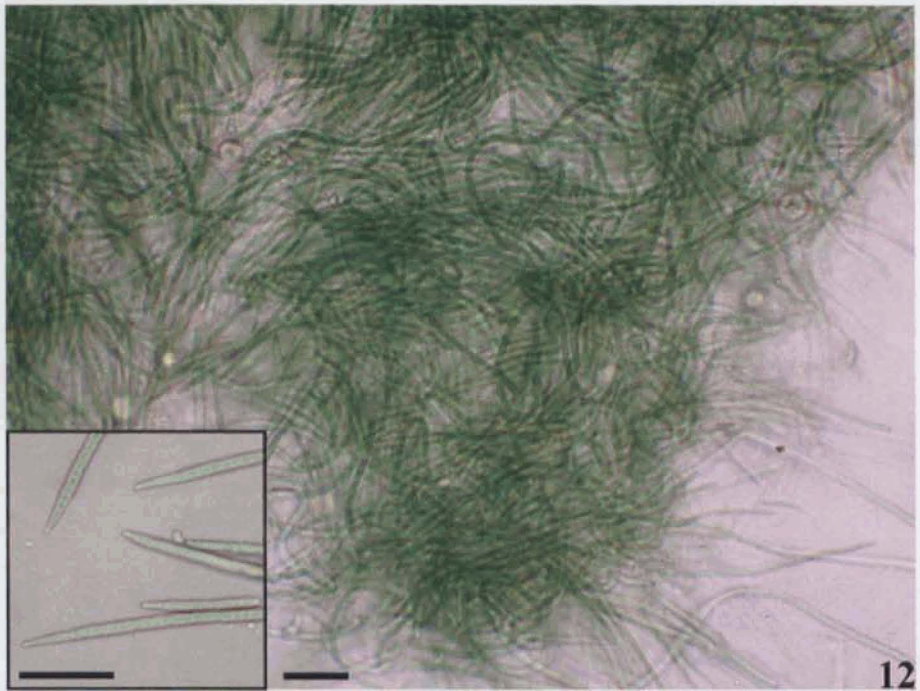


TABLE 1: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Anabaena circinalis* var.*crassa* Ghose in *Allium cepa* L. root tips after ½ hour treatment

Treatment	Total No. of Cells	Prophase				Metaphase						Anaphase				Telophase & Cytokinesis						Interphase					Mitotic Index (%)	Abnormality (%)
		Normal	Abnormal disintegration of nuclear membrane	Differential condensation	Nuclear extrusion	Normal	Sticky metaphase	C- metaphase	Double metaphase in a binucleate cell	Chromosome fragments	Polyploidy	Normal	Chromosome bridges	Disturbed early anaphase with laggards	Chromosome fragments and telosomes in a polyploid cell	Normal telophase	Bizarre telophase	Stellate telophase	Pulverization of chromosomes	Normal cytokinesis	Sticky cytokinesis	Binucleate cell	Nuclear budding	Macro and micro nucleus	Micronuclei	Crescent shaped nucleus		
Control	621	65	--	--	--	34	--	--	--	--	--	23	--	--	--	20		--	--	13	--	--	--	--	--	--	24.9	--
0.01	1310	101	--	34	--	39	56	--	--	--	--	2	18	12	--	8	8	--	--	--	2	42	--	--	--	58	21.37	17.56
0.02	1110	50		59	5	--	--	15	--	10	5	--	11	--	--	--	25	5	4	--	1	21	10	--	19	20	17.12	18.92
0.05	1170	42	4	--	--	--	30	9	11	10	10	8	10	--	--	--	26	--	--	--	--	39	51	22	48	--	13.68	23.08
0.075	1040	20	--	40	2	--	15	--	--	25	--	--	6	--	3	--	28	--	1	--	--	--	40	7	73	--	13.46	23.08
0.1	1260	90	--	--	--	--	--	--	--	30	--	--	10	--	--	--	30	--	5	--	5	44	67	20	62	34	13.49	24.36
Average of treatments																											15.82	21.4

TABLE 2: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Anabaena circinalis* var.*crassa* Ghose in *Allium cepa* L. root tips after 1 hour treatment

Treatment	Total No. of Cells	Prophase				Metaphase						Anaphase				Telophase & Cytokinesis						Interphase					Mitotic Index (%)	Abnormality (%)
		Normal	Abnormal disintegration of nuclear membrane	Differential condensation	Nuclear extrusion	Normal	Sticky metaphase	C- metaphase	Double metaphase in a binucleate cell	Chromosome fragments	Polyploidy	Normal	Chromosome briges	Disturbed early anaphase with laggards	Chromosome fragments and telosomes in a polyploid cell	Normal telophase	Bizarre telophase	Stellate telophase	Pulverization of chromosomes	Normal cytokinesis	Sticky cytokinesis	Binucleate cell	Nuclear budding	Macro and micro nucleus	Micronuclei	Crescent shaped nucleus		
Control	759	76	--	--	--	49	--	--	--	--	--	22	--	--	--	20	--	--	--	13	--	--	--	--	--	--	23.72	--
0.01	1350	130	4	6	--	--	30	49	--	11	10	--	15	--	5	--	--	--	10	--	--	79	--	--	--	21	20	17.78
0.02	1320	120	--	12	--	--	38	--	--	10	--	--	9	--	1	10	20	9	--	--	1	36	34	4	20	46	17.4	18.18
0.05	1570	70	1	19	--	--	30	25	--	5	--	--	10	2	--	10	2	2	2	--	2	30	52	8	31	49	13.14	19.71
0.075	1650	41	--	28	--	--	32	--	10	18	12	9	10	--	--	--	--	7	1	--	2	--	79	1	132	48	10.30	23.03
0.1	1050	30	--	--	6	--	40	15	--	21	4	--	--	--	1	5	15	14	1	4	5	50	71	--	119	--	10.39	23.35
																										Average of treatments	14.25	20. 41

TABLE 3: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Anabaena circinalis* var.*crassa* Ghose in *Allium cepa* L. root tips after 1½ hour treatment

Treatment	Total No. of Cells	Prophase				Metaphase						Anaphase				Telophase & Cytokinesis						Interphase					Mitotic Index (%)	Abnormality (%)
		Normal	Abnormal disintegration of nuclear membrane	Differential condensation	Nuclear extrusion	Normal	Sticky metaphase	C- metaphase	Double metaphase in a binucleate cell	Chromosome fragments	Polyploidy	Normal	Chromosome bridges	Disturbed early anaphase with laggards	Chromosome fragments and telosomes in a diploid cell	Normal telophase	Bizarre telophase	Stellate telophase	Pulverization of chromosomes	Normal cytokinesis	Sticky cytokinesis	Binucleate cell	Nuclear budding	Macro and micro nucleus	Micronuclei	Crescent shaped nucleus		
Control	680	60	--	--	--	40	--	--	--	--	--	20	--	--	--	15	--	--	--	5	--	--	--	--	--	--	20.59	--
0.01	1060	49	7	53	--	11	1	9	--	--	--	--	--	--	--	--	10	--	--	--	--	76	--	--	--	64	13.21	20.75
0.02	2050	60	9	31	--	18	10	15	9	12	30	2	10	--	--	--	10	2	11	--	1	20	75	5	175	35	11.22	22.44
0.05	910	30	--	11	9	5	8	5	--	1	4	5	5	4	1	--	22	--	--	--	--	26	40	14	120	--	12.09	29.67
0.075	820	30	--	6	4	--	14	14	--	--	6	--	5	3	2	--	26	--	--	--	--	--	50	--	130	--	13.41	31.71
0.1	1160	40	--	9	11	--	26	9	--	--	11	--	--	--	--	10	44	--	8	--	2	41	72	12	98	47	14.66	33.62
Average of treatments																											12.92	27.64

TABLE 4: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Anabaena circinalis* var.*crassa* Ghose in *Allium cepa* L. root tips after 2 hour treatment

Treatment	Total No. of Cells	Prophase				Metaphase						Anaphase				Telophase & Cytokinesis						Interphase					Mitotic Index (%)	Abnormality (%)
		Normal	Abnormal disintegration of nuclear membrane	Differential condensation	Nuclear extrusion	Normal	Sticky metaphase	C-metaphase	Double metaphase in a binucleate cell	Chromosome fragments	Polyploidy	Normal	Chromosome bridges	Disturbed early anaphase with laggards	Chromosome fragments and telosomes in a diploid cell	Normal telophase	Bizarre telophase	Stellate telophase	Pulverization of chromosomes	Normal cytokinesis	Sticky cytokinesis	Binucleate cell	Nuclear budding	Macro and micro nucleus	*Micronuclei*	Crescent shaped nucleus		
Control	808	62	--	--	--	42	--	--	--	--	--	30	--	--	--	28	--	--	--	18	--	--	--	--	--	--	22.28	--
0.01	1410	42	--	20	--	38	40	18	--	--	9	10	16	4	--	--	--	--	--	--	--	64	36	--	110	--	13.97	22.48
0.02	1840	40	5	45	--	5	--	22	24	12	12	5	17	3	10	--	10	16	12	1	1	1	62	6	154	38	13.04	24.46
0.05	1340	35	1	11	--	24	--	8	--	10	2	21	10	--	--	10	8	--	10	11	--	98	39	8	120	95	12.01	31.34
0.075	1440	16	4	--	6	7	20	15	--	13	12	4	16	--	12	2	11	18	12	1	--	--	84	10	167	109	11.74	35.35
0.1	1410	6	2	--	--	1	--	22	--	12	10	1	6	2	1	1	18	4	2	--	1	--	50	--	201	169	6.31	35.46
Average of treatments																											11.41	29.82

TABLE 5: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Microcystis aeruginosa* Kütz. in *Allium cepa* L. root tips after ½ hour treatment

Treatment	Total No. of Cells	Prophase				Metaphase					Anaphase					Telophase & Cytokinesis					Interphase				Mitotic Index (%)	Abnormality (%)
		Normal	Condensed chromatin	Abnormal disintegration of nuclear membrane	Dissolution of chromatin	Normal	Sticky metaphase	Chromosome gaps and fragments	Bizarre metaphase	Ball metaphase	Normal	Hyper condensed chromosomes	Clumped sticky anaphase	Bizarre anaphase	Deproteinized chromosomes	Normal telophase	Hyper condensed chromosomes	Extremely sticky telophase	Normal cytokinesis	Hyper condensed cytokinesis	Condensed chromatin	Nuclear erosion	Nuclear budding	Micro nuclei		
Control	680	56	--	--	--	40	--	--	--	--	19	--	--	--	--	20	--	--	4	--	--	--	--	--	20.44	--
0.01	1090	150	50	--	--	--	12	--	--	4	2	1	--	1	--	--	1	--	--	1	34	--	--	6	20. 37	10.09
0.02	1160	101	24	14	12	--	34	2	8	6	--	2	4	--	1	--	1	1	9	1	30	--	--	10	18.97	12.93
0.05	910	40	16	4	--	--	63	2	2	13	--	2	4	1	--	10	1	1	--	1	38	2	2	18	17.58	18.68
0.075	1680	132	47	3	10	10	58	3	2	7	10	--	--	--	--	--	--	--	8	--	67	4	4	105	17.26	18.45
0.1	1090	90	38	2	--	--	29	1	--	--	--	1	7	1	1	--	4	3	--	3	48	1	6	65	16.51	19.27
																									18.14	15.88

Average of treatments

TABLE 6: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Microcystis aeruginosa* Kütz. in *Allium cepa* L. root tips after 1 hour treatment

Treatment	Total No. of Cells	Prophase				Metaphase					Anaphase					Telophase & Cytokinesis					Interphase				Mitotic Index (%)	Abnormality (%)
		Normal	Condensed chromatin	Abnormal disintegration of nuclear membrane	Dissolution of chromatin	Normal	Sticky metaphase	Chromosomes with gaps and fragments	Bizarre metaphase	Ball metaphase	Normal	Hyper condensed chromosomes	Clumped sticky anaphase	Bizarre anaphase	Deproteinized chromosomes	Normal telophase	Hyper condensed chromosomes	Extremely sticky telophase	Normal cytokinesis	Unequal division at cytokinesis	Condensed chromatin	Nuclear erosion	Nuclear budding	Micro nuclei		
Control	740	60	--	--	--	34	--	--	--	--	30	--	--	--	--	20	--	--	6	--	--	--	--	--	20.27	--
0.01	980	50	48	10	--	--	29	--	2	--	--	1	3	6	1	--	--	--	--	--	70	--	60	--	15.31	23.47
0.02	1070	24	34	--	16	--	20	1	15	4	10	2	3	5	--	--	6	3	6	1	62	4	36	48	14.02	24.3
0.05	1240	31	62	6	2	10	39	2	18	1	--	--	--	--	--	--	--	--	--	--	60	5	60	55	13.8	25
0.075	1100	32	29	1	--	10	--	4	27	9	--	--	--	--	--	--	4	3	8	3	40	8	54	98	11.82	25.45
0.1	1090	12	35	5	--	--	45	5	16	4	--	--	--	---	--	--	--	--	--	--	20	10	61	101	11.19	27.71
																									13.23	25.19

Average of treatments

TABLE 7: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Microcystis aeruginosa* Kütz. in *Allium cepa* L. root tips after 1½ hour treatment

Treatment	Total No. of Cells	Prophase				Metaphase					Anaphase					Telophase & Cytokinesis					Interphase				Mitotic Index (%)	Abnormality (%)
		Normal	Condensed chromatin	Abnormal disintegration of nuclear membrane	Dissolution of chromatin	Normal	Sticky metaphase	Chromosome gaps and fragments	Bizarre metaphase	Ball metaphase	Normal	Hyper condensed chromosomes	Clumped sticky anaphase	Bizarre anaphase	Deproteinized chromosomes	Normal telophase	Hyper condensed chromosomes	Extremely sticky telophase	Normal cytokinesis	Unequal division at cytokinesis	Condensed chromatin	Nuclear erosion	Nuclear budding	Micro nuclei		
Control	720	50	--	--	--	28	--	--	--	--	25	--	--	--	--	25	--	--	12	--	--	--	--	--	19.44	--
0.01	1470	36	30	11	19	4	12	2	7	9	--	16	4	--	--	--	--	--	--	--	137	1	16	106	10.2	25.17
0.02	1210	24	32	8	--	3	--	2	26	22	3	--	--	--	--	--	--	--	--	--	47	1	18	154	9.92	25.62
0.05	1310	10	36	--	4	--	19	1	12	4		3	--	1	--	6	2	5	4	3	72	2	16	200	8.4	29.01
0.075	1390	24	64	6	10	6	9	1	--	--	--	--	--	--	--	--	--	--	--	--	168	1	19	132	8.63	29.5
0.1	1340	21	43	--	7	--	16	6	18	10	--	3	3	2	2	--	--	--	--	--	129	--	40	121	9.78	29.85
																									9.39	27.83

Average of treatments

TABLE .8: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Microcystis aeruginosa* Kütz. in *Allium cepa* L. root tips after 2 hour treatment

Treatment	Total No. of Cells	Prophase				Metaphase					Anaphase					Telophase & Cytokinesis					Interphase				Mitotic Index (%)	Abnormality (%)
		Normal	Condensed chromatin	Disintegration of nuclear membrane	Dissolution of chromatin	Normal	Sticky metaphase	Chromosomes with gaps and fragments	Bizarre metaphase	Ball metaphase	Normal	Hyper condensed chromosomes	Clumped sticky anaphase	Bizarre anaphase	Deproteinized chromosomes	Normal telophase	Hyper condensed chromosomes	Extremely sticky telophase	Normal cytokinesis	Unequal division	Condensed chromatin	Nuclear erosion	Nuclear budding	Micro nuclei		
Control	640	50	--	--	--	40	--	--	--	--	30	--	--	--	--	28	--	--	2	--	--	--	--	--	23.4	--
0.01	1900	46	68	1	1	21	19	1	--	20	8	13	7	--	--	--	2	7	--	1	154	--	79	167	11.32	28.42
0.02	1530	80	18	2	--	--	9	2	28	31	--	--	--	--	--	--	--	--	--	--	198	2	--	210	11.11	32.68
0.05	1050	36	47	--	3	6	--	--	1	8	4	--	--	1	--	--	--	--	4	--	--	--	98	192	10.48	33.33
0.075	850	30	--	--	--	--	38	--	10	--	--	--	--	--	2	--	--	--	--	--	99	--	51	100	9.41	35.29
0.1	1580	20	33	9	1	8	51	--	17	--	2	--	--	--	--	--	1	1	--	--	102	3	65	280	9.05	35.63
																								Average of treatments	10.27	33.07

TABLE 9: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Nostoc carneum* C. Agardh ex Born. *et* Flah. in *Allium cepa* L. root tips after ½ hour treatment

Treatment	Total No. of Cells	Prophase			Metaphase				Anaphase								Telophase & Cytokinesis					Interphase			Mitotic Index (%)	Abnormality (%)
		Normal	Differential condensation	Contorted chromatin	Normal	Clumping	Abnormal condensation	Contorted metaphase	Normal	Anaphase with a single bridge	Chromosome laggard	Early movement of chromosomes	Delayed movement of chromosomes	Stellate anaphase	Diagonal anaphase	Giant cell	Normal telophase	Bizarre telophase	Diagonal telophase	Normal cytokinesis	Sticky cytokinesis	Nuclear lesion	Binucleate cell	Abnormal interphase		
Control	900	76	--	--	40	--	--	--	30	--	--	--	--	--	--	--	30	--	--	14	--	--	--	--	21.11	--
0.01	760	34	--	--	26	--	--	--	17	--	4	2	3	--	--	1	19	--	--	11	--	15	7	8	15.39	5.26
0.02	820	29	--	--	21	9	--	11	13	--	--	--	--	--	--	--	18	--	--	12	--	21	9	10	13.78	7.32
0.05	880	31	--	--	9	--	19	11	10	--	--	--	--	--	--	--	18	2	7	2	1	31	7	2	12.50	9.09
0.075	680	21	--	--	9	--	9	21	--	--	--	--	--	--	--	--	--	5	3	--	2	28	1	1	10.29	10.29
0.1	1000	18	6	4	13	10	--	--	12	14	1	1	1	2	1	--	7	--	--	4	--	41	11	19	9.4	11.1
Average of treatments																									12.27	8.61

TABLE 10: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Nostoc carneum* C. Agardh ex Born. *et* Flah. in *Allium cepa* L. root tips after 1 hour treatment

Treatment	Total No. of Cells	Prophase			Metaphase				Anaphase								Telophase & Cytokinesis					Interphase			Mitotic Index (%)	Abnormality (%)
		Normal	Differential condensation	Contorted chromatin	Normal	Clumping	Abnormal condensation	Contorted metaphase	Normal	Anaphase with a single bridge	Chromosome laggard	Early movement of chromosomes	Delayed movement of chromosomes	Stellate anaphase	Diagonal anaphase	Giant cell	Normal telophase	Bizarre telophase	Diagonal telophase	Normal cytokinesis	Sticky cytokinesis	Nuclear lesion	Binucleate cell	Abnormal interphase		
Control	930	73	--	--	33	--	--	--	25	--	--	--	--	--	--	--	30	--	--	22	--	--	--	--	19.67	--
0.01	920	71	4	4	49	9	--	1	10		5	6	3	--	2	1	8	1	2	2	2	22	28	--	19.57	9.78
0.02	900	69	5	3	33	11	--	9	21	9	5	4	--	--	--	2	--	--	--	--	2	51	9	--	19.22	12.22
0.05	960	77	6	4	48	5	3	2	26	--	6	--	--	2	2	--	--	--	--	--	--	60	18	12	18.85	12.5
0.075	880	90	--	6	10	17	3	10	--	--	7	--	2	--	--	--	7	--	1	3	4	41	15	8	18.18	12.95
0.1	960	64	--	--	21	18	--	2	12	9	8	--	--	3	4	--	6	5	7	4	10	31	19	17	18.02	13.85
Average of treatments																									18.77	12.26

TABLE 11: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Nostoc carneum* C. Agardh ex Born. *et* Flah. in *Allium cepa* L. root tips after 1 ½ hour treatment

Treatment	Total No. of Cells	Prophase			Metaphase				Anaphase								Telophase & Cytokinesis					Interphase			Mitotic Index (%)	Abnormality (%)
		Normal	Differential condensation	Non synchronized condensation	Normal	Clumping	Abnormal condensation	Contorted metaphase	Normal	Anaphase with a single bridge	Chromosome laggard	Early movement of chromosomes	Delayed movement of chromosomes	Stellate anaphase	Diagonal anaphase	Giant cell	Normal telophase	Bizarre telophase	Diagonal telophase	Normal cytokinesis	Sticky cytokinesis	Nuclear lesion	Binucleate cell	Abnormal interphase		
Control	740	49	--	--	30	--	--	--	35	--	--	--	--	--	--	--	25	--	--	11	--	--	--	--	20.27	--
0.01	800	26	--	--	14	12	--	8	11	6	4	--	--	3	--	--	8	--	--	2	--	42	4	--	11.75	9.88
0.02	960	21	--	--	16	--	--	10	8	10	6	1	--	--	--	1	--	1	1	--	--	52	18	--	7.81	10.42
0.05	820	14	11	9	10	14	6	--	--	--	--	--	--	--	--	--	--	--		--	--	55	3	--	7.8	11.95
0.075	840	20	--	--	5	8	2	--	--	4	--	5	4	3	--	1	10	1	1	--	1	63	--	7	7.74	12.02
0.1	780	20	--	--	--	19	1	--	--	10	6	--	--	--	4	--	--	--	--	--	--	57	--	3	7.69	12.82
Average of treatments																									8.56	11.42

TABLE 12: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Nostoc carneum* C. Agardh ex Born. *et* Flah. in *Allium cepa* L. root tips after 2 hour treatment

Treatment	Total No. of Cells	Prophase			Metaphase				Anaphase								Telophase & Cytokinesis					Interphase			Mitotic Index (%)	Abnormality (%)
		Normal	Differential condensation	Contorted chromatin	Normal	Clumping	Abnormal condensation	Contorted metaphase	Normal	Anaphase with a single bridge	Chromosome laggard	Early movement of chromosomes	Delayed movement of chromosomes	Stellate anaphase	Diagonal anaphase	Giant cell	Normal telophase	Bizarre telophase	Diagonal telophase	Normal cytokinesis	Abnormal cytokinesis	Nuclear lesion	Binucleate cell	Abnormal interphase		
Control	860	65	--	--	45	--	--	--	34	--	--	--	--	--	--	--	22	--	--	12	--	--	--	--	20.7	--
0.01	980	19	--	--	14	10	--	--	8	9	1	5	1	--	--	2	9	1	1	8	--	71	29	--	8.98	13.26
0.02	840	18	2	8	11	7	--	3	10	--	4	6	--	2	1	--	--	--	--	--	--	73	--	7	8.57	13.45
0.05	820	22	--	--	8	5	8	7	4	--	--	--	--	--	--	--	7	---	--	5	--	82	4	14	8.05	14.63
0.075	909	10	6	4	10	9	6	15	--	--	--	2	4	1	1		--	--	1	--	1	38	12	40	7.7	15.4
0.1	920	20	8	7	--	20	--	--	--	2	2	--	--	4	1	1	--	--	--	--	--	51	15	39	7.07	16.3
Average of treatments																									8.07	14.61

Table 13: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Nostoc commune* Vaucher ex Born. *et* Flah. in *Allium cepa* L. root tips after ½ hour treatment

Treatment	Total No. of Cells	Prophase				Metaphase					Anaphase							Telophase & Cytokinesis						Interphase			Mitotic Index (%)	Abnormality (%)
		Normal	Bizarre form of nucleus	Differential condensation	Unequal grouping of chromatin	Normal	Lollypop metaphase	Scattering and grouping of chromosomes	Giant cell with C-metaphase	Double metaphase in a binucleate cell	Normal	Early movement of chromosomes	Giant cell with a bridge	Disturbed anaphase	Shift in MTOC & unequal separation	Misorientation of chromosomes	Stellate anaphase in a giant cell	Normal telophase	Stellate telophase in a giant cell	Diagonal sticky telophase	Condensed diagonal telophase	Normal cytokinesis	Hyper condensation during cytokinesis	Bizarre form of nucleus	Club shaped nucleus	Giant cell with nucleus at the periphery		
Control	620	35	--	--	--	30	--	--	--	--	28	--	--	--	--	--	--	25	--	--	--	4	--	--	--	--	19.68	--
0.01	2010	310	24	16	--	--	2	8	10	--	--	11	3	--	1	5	--	--	--	--	--	--	--	46	84	--	19.4	10.45
0.02	1350	110	19	--	1	20	1	9	15	5	--	--	5	16	--	--	4	8	6	9	10	2	--	13	57	--	17.78	12.59
0.05	1970	130	--	2	8	9	--	2	27	13	2	12	8	18	4	16	8	20	--	12	22	--	18	48	32	--	16.8	12.69
0.075	1730	50	27	--	3	--	--	6	24	--	--	--	16	10	--	7	13	30	14	18	12	--	54	--	30	6	16.42	13.87
0.1	1050	30	1	--	9	--	--	--	10	--	--	12	--	4	10	--	8	--	6	10	10	--	61	11	8	--	16.29	15.24
Average of treatments																											17.34	12.97

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TABLE 14: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Nostoc commune* Vaucher ex Born. *et* Flah. in *Allium cepa* L. root tips after 1 hour treatment

Treatment	Total No. of Cells	Prophase				Metaphase					Anaphase							Telophase & Cytokinesis						Interphase			Mitotic Index (%)	Abnormality (%)
		Normal	Bizarre form of nucleus	Differential condensation	Unequal grouping of chromatin	Normal	Lollypop metaphase	Scattering and grouping of chromosomes	Giant cell with C-metaphase	Double metaphase in a binucleate cell	Normal	Early movement of chromosomes	Giant cell with a bridge	Disturbed anaphase	Shift in MTOC & unequal separation	Misorientation of chromosomes	Stellate anaphase in a giant cell	Normal telophase	Stellate telophase in a giant cell	Diagonal sticky telophase	Condensed diagonal telophase	Normal cytokinesis	Hyper condensation during cytokinesis	Bizarre form of nucleus	Club shaped nucleus	Giant cell with nucleus at the periphery		
Control	1400	115	--	--	--	61	--	--	--	--	39	--	--	--	--	--	--	40	--	--	--	15	--	--	--	--	19.29	--
0.01	1170	130	--	18	2	--	1	--	10	--	--	--	--	5	7	1	3	20	--	4	--	1	9	31	46	3	18.03	11.97
0.02	950	70	8	21	--	--	--	3	8	--	--	5	5	--	--	--	--	10	--	--	--	--	10	22	37	1	14.74	12.63
0.05	1220	39	16	14	3	--	1	6	27	3	--	6	--	--	--	1	4	--	2	4	2	1	1	25	40	5	10.66	13.11
0.075	1050	20	21	--	9	--	--	--	40	1	--	--	8	2	--	--	--	--	--	--	--	--	9	60	9	1	10.48	15.24
0.1	1390	10	--	8	2	--	--	--	17	3	--	10	--	--	--	--	--	--	5	7	8	--	--	91	89	10	5.04	17.99
Average of treatments																											11.79	14.19

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TABLE 15: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Nostoc commune* Vaucher ex Born. *et* Flah. in *Allium cepa* L. root tips after 1½ hour treatment

Treatment	Total No. of Cells	Prophase				Metaphase					Anaphase							Telophase & Cytokinesis						Interphase			Mitotic Index (%)	Abnormality (%)
		Normal	Bizarre form of nucleus	Differential condensation	Unequal grouping of chromatin	Normal	Lollypop metaphase	Scattering and grouping of chromosomes	Giant cell with C-metaphase	Double metaphase in a binucleate cell	Normal	Early movement of chromosomes	Giant cell with a bridge	Disturbed anaphase	Shift in MTOC & unequal separation	Misorientation of chromosomes	Stellate anaphase in a giant cell	Normal telophase	Stellate telophase in a giant cell	Diagonal sticky telophase	Condensed diagonal telophase	Normal cytokinesis	Hyper condensation during cytokinesis	Bizarre form of nucleus	Club shaped nucleus	Giant cell with nucleus at the periphery		
Control	970	63	--	--	--	47	--	--	--	--	34	--	--	--	--	--	--	36	--	--	--	19	--	--	--	--	20.52	--
0.01	890	84	--	9	1	26	--	--	--	1	--	--	--	--	--	--	--	--	--	--	--	--	9	48	42	--	14.61	12.36
0.02	1990	79	73	10	1	11	--	1	19	--	6	1	4	1	2	1	1	--	--	12	8	--	10	50	56	4	12.06	12.76
0.05	900	10	--	--	2	10	2	3	6	--	--	8	4	5	--	5	--	--	12	8	9	--	16	27	30	3	11.11	15.56
0.075	1240	41	32	--	2	9	--	1	--	--	--	12	5	--	1	2	6	--	--	--	10	--	9	46	60	14	10.48	16.13
0.1	1880	27	--	5	5	--	2	2	48	42	--	10	7	12	2	8	1	--	6	4	--	--	19	82	90	18	10.64	19.31
																										Average of treatments	11.78	15.22

TABLE 16: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Nostoc commune* Vaucher ex Born. *et* Flah. in *Allium cepa* L. root tips after 2 hour treatment

Treatment	Total No. of Cells	Prophase				Metaphase					Anaphase							Telophase & Cytokinesis						Interphase			Mitotic Index (%)	Abnormality (%)
		Normal	Bizarre form of nucleus	Differential condensation	Unequal grouping of chromatin	Normal	Lollypop metaphase	Scattering and grouping of chromosomes	Giant cell with C-metaphase	Double metaphase in a binucleate cell	Normal	Early movement of chromosomes	Giant cell with a bridge	Disturbed anaphase	Shift in MTOC & unequal separation	Misorientation of chromosomes	Stellate anaphase in a giant cell	Normal telophase	Stellate telophase in a giant cell	Diagonal sticky telophase	Condensed diagonal telophase	Normal cytokinesis	Hyper condensation during cytokinesis	Bizarre form of nucleus	Club shaped nucleus	Giant cell with nucleus at the periphery		
Control	1010	60	--	--	--	45	--	--	--	--	37	--	--	--	--	--	--	33	--	--	--	28	--	--	--	--	20.1	--
0.01	1090	100	10	6	2	10	2	--	9	1	--	1	--	6	--	1	--	--	--	--	4	--	8	53	57	--	14.68	14.68
0.02	1390	90	37	3	2	10	--	4	16	--	--	10	--	--	--	--	--	--	--	8	--	--	--	102	27	1	12.95	15.11
0.05	1200	18	25	17	8	--	--	3	27	--	--	2	1	3	--	1	1	10	--	1	11	--	10	50	38	2	11.5	16.67
0.075	1080	--	36	1	3	--	--	7	21	2	10	6	2	1	1	12	--	--	8	--	--	--	--	64	36	--	10.19	18.52
0.1	1290	8	51	--	1	--	1	8	19	2	--	2	2	8	1	15	5	--	1	1	1	--	12	89	21	8	10.7	19.22
																										Average of treatments	12	16.84

TABLE 17: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Nostoc spongiaeforme* C. Agardh ex Born. *et* Flah. in *Allium cepa* L. root tips after ½ hour treatment

Treatment	Total No. of Cells	Prophase				Metaphase				Anaphase				Telophase & Cytokinesis						Interphase			Mitotic Index (%)	Abnormality (%)
		Normal	Condensed chromatin	Scattering of chromosomes	Bizarre form of nucleus	Normal	Ball metaphase	Giant cell with misoriented chromosomes	Displaced chromosomes	Normal	Sticky diagonal anaphase	Disturbed spindle organization at one pole	Scattered movement of chromosomes	Normal telophase	Diagonal sticky telophase	Abnormal clumping & grouping of chromosomes	Equatorial separation	Normal cytokinesis	Fragmentation of one daughter nucleus	Binucleate cell	Elongated nucleus with a micro nucleus	Giant cell with nucleus at the periphery		
Control	780	53	--	--	--	44	--	--	--	36	--	--	--	25	--	--	--	12	--	--	--	--	21.79	--
0.01	1490	160	34	--	19	--	8	12	--	--	2	3	7	10	9	4	4	--	5	61	20	9	18.59	13.22
0.02	820	90	9	5	6	--	12	14	4	--	--	--	--	--	--	--	--	--	--	59	11	10	17.07	15.85
0.05	1290	80	--	6	14	--	--	24	16	--	2	2	4	--	--	5	1	--	6	85	39	6	12.4	16.28
0.075	1780	130	2	8	--	--	15	25	--	--	13	5	--	--	--	1	--	--	1	98	54	12	11.24	13.15
0.1	1050	40	29	--	1	10	--	12	15	--	3	--	--	--	--	--	--	--	--	117	3	--	10.48	17.14
Average of treatments																							13.96	15.13

TABLE 18: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Nostoc spongiaeforme* C. Agardh ex Born. *et* Flah. in *Allium cepa* L. root tips after 1 hour treatment

Treatment	Total No. of Cells	Prophase				Metaphase				Anaphase				Telophase & Cytokinesis						Interphase			Mitotic Index (%)	Abnormality (%)
		Normal	Condensed chromatin	Scattering of chromosomes	Bizarre form of nucleus	Normal	Ball metaphase	Giant cell with misoriented chromosomes	Displaced chromosomes	Normal	Sticky diagonal anaphase with a broken bridge	Disturbed spindle organization at one pole	Scattered movement of chromosomes	Normal telophase	Diagonal sticky telophase	Abnormal clumping & grouping of chromosomes	Equatorial separation	Normal cytokinesis	Fragmentation of one daughter nucleus	Binucleate cell	Elongated nucleus with a micro nucleus	Giant cell with nucleus at the periphery		
Control	760	49	--	--	--	41	--	--	--	32	--	--	--	27	--	--	--	11	--	--	--	--	21.05	--
0.01	1010	60	18	4	18	--	10	6	4	--	3	2	4	--	--	1	--	--	--	35	22	3	12.87	12.87
0.02	1280	70	21	1	8	--	11	6	3	--	1	8	1	--	2	4	2	--	2	44	50	6	10.94	13.28
0.05	1390	50	31	7	32	--	9	4	7	--	--	2	6	--	1	1	--	--	--	37	60	3	10.79	14.39
0.075	1050	40	24	1	5	--	4	--	26	--	--	--	--	--	--	--	--	--	--	73	45	2	9.52	17.14
0.1	1210	43	37	3	10	--	--	--	18	--	--	--	1	--	--	--	1	--	--	107	39	3	9.33	18.1
Average of treatments																							10.69	15.16

46:W

TABLE 19: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Nostoc spongiaeforme* C. Agardh ex Born. *et* Flah. in *Allium cepa* L. root tips after 1½ hour treatment

Treatment	Total No. of Cells	Prophase				Metaphase				Anaphase				Telophase & Cytokinesis						Interphase			Mitotic Index (%)	Abnormality (%)
		Normal	Condensed chromatin	Scattering of chromosomes	Bizarre form of nucleus	Normal	Ball metaphase	Giant cell with misoriented chromosomes	Displaced chromosomes	Normal	Sticky diagonal anaphase	Disturbed spindle organization at one pole	Scattered movement of chromosomes	Normal telophase	Diagonal sticky telophase	Abnormal clumping & grouping of chromosomes	Equatorial separation	Normal cytokinesis	Fragmentation of one daughter nucleus	Binucleate cell	Elongated nucleus with a micro nucleus	Giant cell with nucleus at the periphery		
Control	980	76	--	--	--	62	--	--	--	34	--	--	--	28	--	--	--	20	--	--	--	--	22.45	--
0.01	1024	68	45	5	8	--	12	2	1	--	7	1	2	--	5	--	--	--	--	35	18	10	15.23	14.75
0.02	1230	69	18	7	9	--	8	1	1	--	6	1	3	--	9	10	7	--	4	76	45	9	12.44	17.4
0.05	1260	60	22	5	13	--	17	2	6	--	5	2	3	--	8	--	2	--	5	80	45	5	11.9	17.46
0.075	1300	30	31	3	46	--	4	7	9	--	8	1	1	--	1	1	6	--	2	82	51	11	11.54	20.31
0.1	1212	43	62	--	18	--	--	--	--	--	--	--	--	--	--	--	--	--	--	94	67	16	10.15	21.2
																			Average of treatments				12.25	18.22

TABLE 20: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Nostoc spongiaeforme* C. Agardh ex Born. *et* Flah. in *Allium cepa* L. root tips after 2 hour treatment

Treatment	Total No. of Cells	Prophase				Metaphase				Anaphase				Telophase & Cytokinesis						Interphase			Mitotic Index (%)	Abnormality (%)
		Normal	Condensed chromatin	Scattering of chromosomes	Bizarre form of nucleus	Normal	Ball metaphase	Giant cell with misoriented chromosomes	Displaced chromosomes	Normal	Sticky diagonal anaphase	Disturbed spindle organization at one pole	Scattered movement of chromosomes	Normal telophase	Diagonal sticky telophase	Abnormal clumping & grouping of chromosomes	Equatorial separation	Normal cytokinesis	Fragmentation of one daughter nucleus	Binucleate cell	Elongated nucleus with a micro nucleus	Giant cell with nucleus at the periphery		
Control	640	48	--	--	--	30	--	--	--	41	--	--	--	27	--	--	--	4	--	--	--	--	23.44	--
0.01	1260	72	23	2	3	--	25	5	10	--	18	10	2	--	10	--	--	--	--	27	31	22	14.44	15.1
0.02	1560	50	32	8	--	--	26	4	19	--	20	12	8	--	22	8	4	--	9	39	42	21	14.23	19.29
0.05	1117	60	21	7	2	--	39	--	1	--	--	--	9	--	14	--	2	--	--	48	50	27	13.88	19.7
0.075	1180	63	28	1	1	--	27	--	3	--	--	--	--	10	12	--	--	--	--	53	57	29	12.29	17.88
0.1	1470	--	67	3	40	--	54	2	4	--	--	--	10	--	--	--	--	--	--	61	38	12	12.24	19.8
Average of treatments																							13.42	18.35

TABLE 21: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Oscillatoria obscura* Brühl *et* Biswas in *Allium cepa* L. root tips after ½ hour treatment

Treatment	Total No. of Cells	Prophase					Metaphase							Anaphase							Telophase & Cytokinesis						Interphase						Mitotic Index (%)	Abnormality (%)
		Normal	Condensed chromatin	Nuclear disintegration	Pulverized prophase	Bizarre prophase	Normal	Sticky metaphase	Pulverized metaphase	Chromosome gaps & fragments	Stratified metaphase	Bizarre metaphase	Polyploid cell	Normal	Sticky anaphase with bridges	Ball anaphase	Disturbed anaphase with laggards	Stathmoanaphase	Diagonal pulverized anaphase	Unequal movement of chromosomes	Normal telophase	Bizarre telophase	Stellate telophase	Abnormal clumping and grouping of chromosomes	Normal cytokinesis	Stickiness during cytokinesis	Dissolution of chromatin	Nuclei showing hyperchromasia	Nuclear pulverization	Nuclear diminution	Nuclear fragmentation	Micronuclei		
Control	779	53	--	--	--	--	35	--	--	--	--	--	--	30	--	--	--	--	--	--	30	--	--	--	12	--	--	--	--	--	--	20.54	--
0.01	1370	45	12	7	8	23	5	11	9	2	12	17	3	--	--	--	--	10	9	11	--	--	12	--	--	--	4	--	18	1	--	79	14.31	18.10
0.02	1240	65	7	8	5	--	5	12	11	--	--	14	--	--	6	1	4	4	2	--	8	1	1	1	2	20	1	9	39	--	--	81	14.27	18.31
0.05	1280	60	23	--	2	15	--	1	1	1	2	1	4	10	4	11	--	2	3	1	--	15	10	--	--	20		8	54	3	--	57	14.45	18.52
0.075	1410	55	19	1	3	27	5	3	6	--	1	18	2	--	15	10	9	11	5	8	--	--	1	--	--	1	1	--	61	--	1	59	14.18	18.58
0.1	1180	40	45	--	--	31	10	--	--	--	--	4	--	10	--	6	--	14	--	1	--	--	--	--	--	--	--	--	53	1	1	68	13.64	18.98
Average of treatments																																	14.17	18.5

TABLE 22: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Oscillatoria obscura* Brühl *et* Biswas in *Allium cepa* L. root tips after 1 hour treatment

Treatment	Total No. of Cells	Prophase					Metaphase							Anaphase							Telophase & Cytokinesis						Interphase						Mitotic Index (%)	Abnormality (%)
		Normal	Condensed chromatin	Nuclear disintegration	Pulverized prophase	Bizarre prophase	Normal	Sticky metaphase	Pulverized metaphase	Chromosome gaps & fragments	Stratified metaphase	Bizarre metaphase	Polyploid cell	Normal	Sticky anaphase with bridges	Ball anaphase	Disturbed anaphase with laggards	Stathmoanaphase	Diagonal pulverized anaphase	Unequal movement of chromosomes	Normal telophase	Bizarre telophase	Stellate telophase	Abnormal clumping and grouping of chromosomes	Normal cytokinesis	Stickiness during cytokinesis	Dissolution of chromatin	Nuclei showing hyperchromasia	Nuclear pulverization	Nuclear diminution	Nuclear fragmentation	Micronuclei		
Control	790	52	--	--	--	--	43	--	--	--	--	--	--	39	--	--	--	--	--	--	18	--	--	--	15	--	--	--	--	--	--	--	21.14	--
0.01	1290	91	7	3	8	12	4	2	--	--	4	4	6	5	--	7	1	8	1	--	--	9	8	--	--	40	44	4	50	1	--	21	17.05	18.6
0.02	1234	112	6	12	--	21	4	--	3	2	1	5	--	4	--	10	--	--	--	--	--	--	--	--	--	30	47	10	52	1	--	37	17.02	19.21
0.05	1010	60	36	14	3	27	--	6	--	1	--	--	3	--	--	--	--	--	--	--	--	--	--	--	--	22	30	11	--	--	2	45	17.03	19.8
0.075	1037	90	18	11	9	14	--	10	1	--	--	9	--	--	2	--	1	--	1	1	--	1	1	1	--	2	12	3	--	3	3	111	16.59	20.64
0.1	1006	82	19	3	14	11	--	2	--	1	--	--	--	--	2	1	2	2	2	1	--	--	--	1	--	--	35	--	14	8	--	96	14.21	21.27
Average of treatments																																	16.38	19.9

TABLE 23: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Oscillatoria obscura* Brühl *et* Biswas in *Allium cepa* L. root tips after 1½ hour treatment

Treatment	Total No. of Cells	Prophase					Metaphase							Anaphase							Telophase & Cytokinesis						Interphase						Mitotic Index (%)	Abnormality (%)
		Normal	Condensed chromatin	Nuclear disintegration	Pulverized prophase	Bizarre prophase	Normal	Sticky metaphase	Pulverized metaphase	Chromosome gaps & fragments	Stratified metaphase	Bizarre metaphase	Polyploid cell	Normal	Sticky anaphase with bridges	Ball anaphase	Disturbed anaphase with laggards	*Stathmoanaphase*	Diagonal pulverized anaphase	Unequal movement of chromosomes	Normal telophase	Bizarre telophase	Stellate telophase	Abnormal clumping and grouping of chromosomes	Normal cytokinesis	Stickiness during cytokinesis	Dissolution of chromatin	Nuclei showing hyperchromasia	Nuclear pulverization	Nuclear diminution	Nuclear fragmentation	Micronuclei		
Control	897	62	--	--	--	--	50	--	--	--	--	--	--	41	--	--	--	--	--	--	37	--	--	--	10	--	--	--	--	--	--	--	22.3	--
0.01	1230	31	22	10	8	20	9	15	5	1	9	11	9	--	3	3	--	1	2	1	8	1	12	2	2	25	50	10	--	1	2	77	17.07	24.39
0.02	1420	30	20	16	--	44	--	29	--	1	--	10	--	--	26	4	2	2	2	4	--	--	20	--	--	30	27	9	--	2		102	16.90	24.65
0.05	1210	40	18	3	37	12	40	16	4	4	4	6	6	--	--	5	--	1	--	4	--	--	--	--	--	--	35	15	3	--	--	127	16.53	24.79
0.075	1370	82	23	5	2	30	11	26	--	4	--	10	--	--	7	--	2	1	2	8	--	3	4	3	--	--	40	20	2	2	--	136	16.27	24.09
0.1	1540	60	20	--	2	88	--	37	3	5	--	5	10	--	9	1	--	--	1	9	10	--	--	--	--	--	45	--	--	5	3	157	16.88	25.97
Average of treatments																																	16.73	24.78

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TABLE 25: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Oscillatoria princeps* Vaucher ex Gomont in *Allium cepa* L. root tips after ½ hour treatment

Treatment	Total No. of Cells	Prophase			Metaphase				Anaphase							Telophase & Cytokinesis					Interphase			Mitotic Index (%)	Abnormality (%)
		Normal	Differential condensation	Non synchronized condensation	Normal	Clumping	Abnormal condensation	Bouquet formation of chromosomes	Normal	Anaphase with a single bridge	Chromosome laggard	Early movement of chromosomes	Delayed movement of chromosomes	Stellate anaphase	Diagonal anaphase	Normal telophase	Bizarre telophase	Diagonal telophase	Normal cytokinesis	Unequal division at cytokinesis	Nuclear lesion	Binucleate cell	Abnormal interphase		
Control	829	61	--	--	38	--	--	--	36	--	--	--	--	--	--	22	--	--	12	--	--	--	--	20.39	--
0.01	920	20	--	--	--	8	1	1	10	6	4	3	2	5	--	10	--	--	--	--	24	10	26	7.61	9.78
0.02	900	21	--	--	10	3	3	4	--	--	--	3	7	5	5	--	--	--	--	--	60	--	10	6.78	11.11
0.05	962	18	4	6	4	4	2	4	--	8	3	--	--	4	2	--	--	--	--	--	55	5	10	6.15	11.12
0.075	881	10	--	--	--	6	7	7	--	3	--	--	7	--	--	10	--	--	--	--	46	9	19	5.68	11.8
0.1	960	20	--	--	2	--	8	2	--	--	--	--	--	--	--	8	6	3	--	1	58	14	22	5.21	11.88
																				Average of treatments				6.29	11.14

TABLE 24: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Oscillatoria obscura* Brühl *et* Biswas in *Allium cepa* L. root tips after 2 hour treatment

Treatment	Total No. of Cells	Prophase					Metaphase							Anaphase							Telophase & Cytokinesis						Interphase						Mitotic Index (%)	Abnormality (%)
		Normal	Condensed chromatin	Nuclear disintegration	Pulverized prophase	Bizarre prophase	Normal	Sticky metaphase	Pulverized metaphase	Chromosome gaps & fragments	Stratified metaphase	Bizarre metaphase	Polyploid cell	Normal	Sticky anaphase with bridges	Ball anaphase	Disturbed anaphase with laggards	Stathmoanaphase	Diagonal pulverized anaphase	Unequal movement of chromosomes	Normal telophase	Bizarre telophase	Stellate telophase	Abnormal clumping and grouping of chromosomes	Normal cytokinesis	Stickiness during cytokinesis	Dissolution of chromatin	Nuclei showing hyperchromasia	Nuclear pulverization	Nuclear diminution	Nuclear fragmentation	Micronuclei		
Control	880	60	--	--	--	--	33	--	--	--	--	--	--	40	--	--	--	--	--	--	30	--	--	--	17	--	--	--	--	--	--	--	20.45	--
0.01	1130	40	18	12	--	20	12	11	7	--	--	8	--	10	16	3	14	13	2	3	--	7	8	--	--	15	25	--	10	2	3	67	19.38	23.36
0.02	1047	47	17	11	12	31	3	12	--	--	1	9	10	--	3	1	6	9	--	--	--	--	10	--	--	20	30	3	11	1	--	96	19.29	27.98
0.05	1140	24	49	6	6	39	6	--	6	1	--	3	11	10	--	7	--	10	--	--	--	--	12	--	--	28	32	--	20	2	2	106	19.12	29.82
0.075	1660	33	79	--	--	51	7	24	5	5	6	16	4	10	--	--	--	--	--	--	--	4	25	2	--	25	30	5	15	3	2	205	17.83	30.48
0.1	1110	26	48	--	--	2	4	10	4	--	--	12	4	--	--	--	--	--	--	--	--	--	15	--	--	25	15	10	--	--	--	275	13.51	37.84
																																Average of treatments	17.83	29.9

TABLE 26: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Oscillatoria princeps* Vaucher ex Gomont in *Allium cepa* L. root tips after 1 hour treatment

Treatment	Total No. of Cells	Prophase			Metaphase				Anaphase							Telophase & Cytokinesis					Interphase			Mitotic Index (%)	Abnormality (%)
		Normal	Differential condensation	Non synchronized condensation	Normal	Clumping	Abnormal condensation	Bouquet formation of chromosomes	Normal	Anaphase with a single bridge	Chromosome laggard	Early movement of chromosomes	Delayed movement of chromosomes	Stellate anaphase	Diagonal anaphase	Normal telophase	Bizarre telophase	Diagonal telophase	Normal cytokinesis	Unequal division at cytokinesis	Nuclear lesion	Binucleate cell	Abnormal interphase		
Control	960	71	--	--	53	--	--	--	39	--	--	--	--	--	--	31	--	--	20	--	--	--	--	22.29	--
0.01	1132	25	--	--	--	17	1	2	15	--	--	7	13	--	--	10	--	--	--	--	62	8	--	7.95	9.72
0.02	1016	30	2	1	12	10	--	--	8	--	--	--	--	4	6	--	--	--	--	--	66	2	12	7.19	10. 14
0.05	1122	16	--	--	--	21	5	4	4	--	--	2	16	--	--	10	1	1	--	--	63	--	7	7.13	10.7
0.075	1127	24	--	--	--	--	6	--	6	--	--	1	9	--	--	--	9	15	--	10	90	1	9	7.1	13.31
0.1	1110	8	--	--	--	--	18	--	--	8	6	11	--	--	5	--	--	--	--	12	71	8	9	6.13	13.33
																							Average of treatments	7.1	11.44

TABLE 27: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Oscillatoria princeps* Vaucher ex Gomont in *Allium cepa* L. root tips after 1½ hour treatment

Treatment	Total No. of Cells	Prophase			Metaphase				Anaphase							Telophase & Cytokinesis					Interphase			Mitotic Index (%)	Abnormality (%)
		Normal	Differential condensation	Non synchronized condensation	Normal	Clumping	Abnormal condensation	Bouquet formation of chromosomes	Normal	Anaphase with a single bridge	Chromosome laggard	Early movement of chromosomes	Delayed movement of chromosomes	Stellate anaphase	Diagonal anaphase	Normal telophase	Bizarre telophase	Diagonal telophase	Normal cytokinesis	Unequal division at cytokinesis	Nuclear lesion	Binucleate cell	Abnormal interphase		
Control	810	58	--	--	42	--	--	--	35	--	--	--	--	--	--	34	--	--	11	--	--	--	--	22.22	--
0.01	1120	29	--	--	15	9	1	--	11	1	--	3	1	3	3	10	--	3	--	7	61	14	25	8.57	11.70
0.02	1140	19	--	--	13	11	--	--	1	6	10	2	8	1	1	--	1	1	--	9	54	21	15	8.16	12.28
0.05	1011	18	--	--	--	45	--	5	2	2	--	1	3	2	2	--	--	--	--	--	59	11	20	7.91	14.84
0.075	1060	20	--	--	--	15	5	--	--	--	--	--	--	--	--	10	4	4	--	12	99		21	6.6	15.09
0.1	1200	10	4	6	--	4	--	6	--	--	--	--	--	--	--	10	9	7	--	14	111	9	20	5.83	15.83
Average of treatments																								7.41	13.95

TABLE 28: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Oscillatoria princeps* Vaucher ex Gomont in *Allium cepa* L. root tips after 2 hour treatment

Treatment	Total No. of Cells	Prophase			Metaphase				Anaphase							Telophase & Cytokinesis					Interphase			Mitotic Index (%)	Abnormality (%)
		Normal	Differential condensation	Non synchronized condensation	Normal	Clumping	Abnormal condensation	Bouquet formation of chromosomes	Normal	Anaphase with a single bridge	Chromosome laggard	Early movement of chromosomes	Delayed movement of chromosomes	Stellate anaphase	Diagonal anaphase	Normal telophase	Bizarre telophase	Diagonal telophase	Normal cytokinesis	Unequal division at cytokinesis	Nuclear lesion	Binucleate cell	Abnormal interphase		
Control	730	40	--	--	40	--	--	--	30	--	--	--	--	--	--	25	--	--	15	--	--	--	--	20.8	--
0.01	1100	24	--	--	--	16	1	3	--	1	2	2	2	12	1	10	6	2	--	2	74	24	32	7.64	16.36
0.02	1011	10	--	--	10	15	2	--	--	6	1	1	1	--	1	10	6	4	--	3	81	33	26	6.92	17.8
0.05	1298	2	--	--	--	--	2	8	--	4	8	6	4	3	5	--	18	20	--	12	92	31	17	7.09	17.72
0.075	1188	10	--	--	--	11	--	--	--	9	7	1	3	2	8	--	6	10	--	13	86	34	22	6.73	17.85
0.1	1023	20	1	3	--	--	--	2	--	--	8	--	--	--	2	--	10	8	--	10	128	2	15	6.26	18.48
Average of treatments																								6.93	17.64

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TABLE 29: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Phormidium tenue* (Menegh.) Gomont in *Allium cepa* L. root tips after ½ hour treatment

Treatment	Total No. of Cells	Prophase				Metaphase							Anaphase					Telophase & Cytokinesis						Interphase				Mitotic Index (%)	Abnormality (%)
		Normal	Condensed chromatin and appendage formation	Bizarre prophase	Dissolution of chromatin	Normal	Ring chromosomes	Hyperstickiness	Convoluted chromosomes	Misoriented C- metaphase	Adhesion of chromosomes	Deproteinized diagonal metaphase	Normal	Equatorial separation of chromosomes	Stathmo anaphase	Pulverized anaphase	Sticky anaphase	Normal telophase	Fragmentation of daughter nuclei	Pulverized chromosomes	Equatorial separation	Normal cytokinesis	Hyper condensation during cytokinesis	Aberrant nucleus	Cytomixis	Blebbing	Giant cell with several lesions		
Control	710	53	--	--	--	43	--	--	--	--	--	--	20	--	--	--	--	20	--	--	--	14	--	--	--	--	--	21.13	--
0.01	1009	47	4	3	3	--	4	1	2	1	1	1	12	--	--	--	--	11	--	--	--	--	--	14	--	9	20	8.92	6.24
0.02	1080	40	--	--	--	14	--	3	--	1	2	1	10	2	7	1	3	6	--	--	--	--	--	7	1	8	34	8.33	6.48
0.05	1017	22	--	--	--	18	--	--	--	--	--	--	17	--	4	1	--	13	1	1	1	--	2	38	2	--	17	7.87	6.59
0.075	1020	34	--	--	--	12	1	5	--	1	6	3	10	--	4	--	--	--	--	--	--	--	--	48	2	10	--	7.45	7.84
0.1	1002	31	--	--	--	11	--	2	--	--	7	1	8	--	--	--	--	7	--	--	--	--	--	63	--	5	2	6.69	7.98
Average of treatments																												7.85	7.03

TABLE 30: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Phormidium tenue* (Menegh.) Gomont in *Allium cepa* L. root tips after 1 hour treatment

Treatment	Total No. of Cells	Prophase				Metaphase							Anaphase					Telophase & Cytokinesis						Interphase				Mitotic Index (%)	Abnormality (%)
		Normal	Condensed chromatin and appendage formation	Bizarre prophase	Dissolution of chromatin	Normal	Ring chromosomes	Hyperstickiness	Convoluted chromosomes	Misoriented C- metaphase	Adhesion of chromosomes	Deproteinized diagonal metaphase	Normal	Equatorial separation of chromosomes	Stathmo anaphase	Pulverized anaphase	Sticky anaphase	Normal telophase	Fragmentation of daughter nuclei	Pulverized chromosomes	Equatorial separation	Normal cytokinesis	Hyper condensation during cytokinesis	Aberrant nucleus	Cytomixis	Blebbing	Giant cell with several lesions		
Control	910	70	--	--	--	51	--	--	--	--	--	--	39	--	--	--	--	32	--	--	--	8	--	--	--	--	--	21.98	--
0.01	1020	20	10	4	--	--	1	1	1	1	2	--	20	6	2	1	1	20	--	--	--	--	--	27	--	3	20	8.82	7.84
0.02	1018	22	2	8	--	12	--	2	--	--	4	--	8	--	8	--	6	6	2	3	3	1	2	32	8	2	--	8.74	8.06
0.05	1011	28	8	--	2	18	--	5	--	--	5	--	8	5	2	2	1	4	--	--	--	--	--	3	11	2	37	8.7	8.21
0.075	1040	38	--	--	--	10	1	1	--	--	6	2	10	2	--	--	8	2	1	4	5	--	--	19	1	--	38	8.65	8.46
0.1	1048	40	--	--	--	--	--	--	--	--	--	--	10	--	--	--	10	20	2	7	--	--	1	36	9	--	34	8.59	8.49
																												Average of treatments	8.7	8.21

TABLE 31: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Phormidium tenue* (Menegh.) Gomont in *Allium cepa* L. root tips after 1 ½ hour treatment

Treatment	Total No. of Cells	Prophase				Metaphase							Anaphase					Telophase & Cytokinesis						Interphase				Mitotic Index (%)	Abnormality (%)
		Normal	Condensed chromatin and appendage formation	Bizarre prophase	Dissolution of chromatin	Normal	Ring chromosomes	Hyperstickiness	Convoluted chromosomes	Misoriented C- metaphase	Adhesion of chromosomes	Deproteinized diagonal metaphase	Normal	Equatorial separation of chromosomes	Stathmo anaphase	Pulverized anaphase	Sticky anaphase	Normal telophase	Fragmentation of daughter nuclei	Pulverized chromosomes	Equatorial separation	Normal cytokinesis	Hyper condensation during cytokinesis	Aberrant nucleus	Cytomixis	Blebbing	Giant cell with several lesions		
Control	780	65	--	--	--	35	--	--	--	--	--	--	33	--	--	--	--	25	--	--	--	12	--	--	--	--	--	21.79	--
0.01	1020	21	16	--	4	--	8	2	1	4	9	1	--	3	7	6	5	--	1	1	1	--	1	52	5	15	5	8.92	14.41
0.02	1042	26	--	--	--	14	10	1	1	12	2	--	--	7	10	5	--	--	--	1	1	--	--	69	8	17	7	8.64	14.49
0.05	1011	28	--	--	--	16	--	16	--	3	4	7	--	--	--	9	1	--	--	--	--	--	--	77	4	18	9	8.31	14.63
0.075	1007	22	9	1	--	--	--	10	--	--	--	--	10	10	2	--	18	--	--	--	--	--	--	75	2	10	11	8.14	14.7
0.1	1014	20	--	8	7	--	--	18	2	4	2	8	--	--	--	--	--	10	--	--	--	--	--	78	--	18	12	7.79	15.48
Average of treatments																												8.36	14.74

TABLE 32: Mitotic index, types and frequency of cellular abnormalities induced by various concentrations of the extract of *Phormidium tenue* (Menegh.) Gomont in *Allium cepa* L. root tips after 2 hour treatment

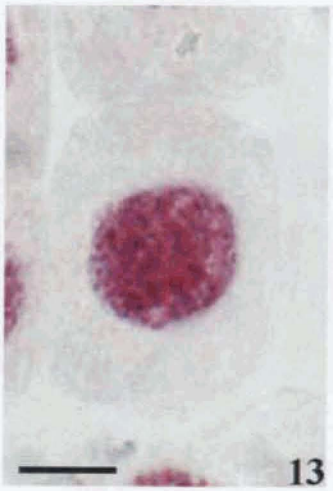
Treatment	Total No. of Cells	Prophase				Metaphase							Anaphase					Telophase & Cytokinesis						Interphase				Mitotic Index (%)	Abnormality (%)
		Normal	Condensed chromatin and appendage formation	Bizarre prophase	Dissolution of chromatin	Normal	Ring chromosomes	Hyperstickiness	Convoluted chromosomes	Misoriented C- metaphase	Adhesion of chromosomes	Deproteinized diagonal metaphase	Normal	Equatorial separation of chromosomes	Stathmo anaphase	Pulverized anaphase	Sticky anaphase	Normal telophase	Fragmentation of daughter nuclei	Pulverized chromosomes	Equatorial separation	Normal cytokinesis	Hyper condensation during cytokinesis	Aberrant nucleus	Cytomixis	Blebbing	Giant cell with several lesions		
Control	758	45	--	--	--	39	--	--	--	--	--	--	36	--	--	--	--	20	--	--	--	16	--	--	--	--	--	20.6	--
0.01	1029	28	--	7	4	13	3	7	2	5	2	1	11	--	--	--	--	10	2	4	1	--	3	27	23	--	20	10.01	10.79
0.02	1006	27	5	5	7	6	--	9	--	--	--	1	--	2	9	11	8	--	--	8	--	--	--	31	3	--	17	9.74	11.53
0.05	1041	31	7	2	1	12	3	3	1	5	2	3	8	--	7	8	--	2	--	--	--	--	--	32	13	--	35	9.13	11.72
0.075	1002	20	--	--	--	11	9	8	--	1	1	1	--	5	10	4	11	--	1	--	--	--	--	76	--	4	--	8.18	13.07
0.1	1034	26	9	1	--	10	--	5	--	--	5	--	9	--	--	--	16	1	--	1	1	--	1	89	--	--	31	8.22	15.38
Average of treatments																												9.06	12.5

PLATE – 4

Figs. 13-18. Normal mitotic stages of *Allium cepa* L.

Fig. 13. Normal Interphase
Fig. 14. Normal Prophase
Fig. 15. Normal Metaphase
Fig. 16. Normal Anaphase
Fig. 17. Normal Telophase
Fig. 18. Normal Cytokinesis

PLATE - 4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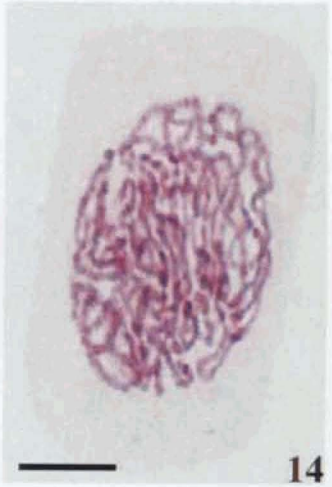
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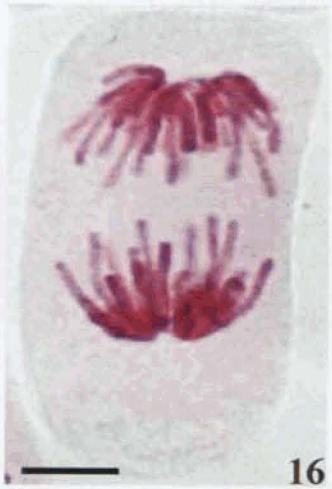
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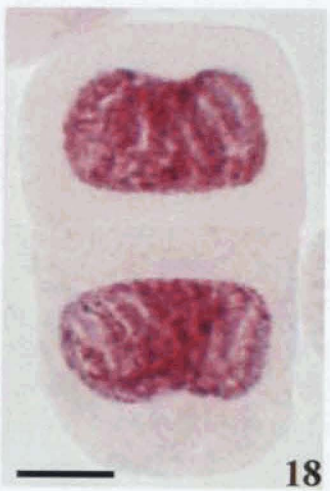
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PLATE – 5

Figs. 19-34. Various cytological aberrations at interphase scored in *Allium cepa* L. root meristem after treatment with cyanobacterial extracts.

Fig. 19. Cytomixis.

Fig. 20. Macro and micronuclei.

Fig. 21. Bridge like connection between macro and micronucleus.

Fig. 22. Cells with one micronucleus each.

Fig. 23. Cell with elongated nucleus and one micronucleus.

Fig. 24. Cell with two micronuclei.

Fig. 25. Binucleate cell.

Fig. 26. Formation of binucleate cells.

Fig. 27. Bizarre binucleate cell.

Fig. 28. Two macronuclei and one micronucleus.

Fig. 29. Two unequal macronuclei and three micronuclei.

Fig. 30. Bizarre trinucleate cell.

Fig. 31. Trinucleate condition.

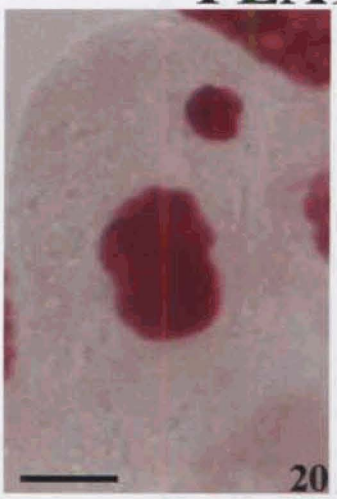
Fig. 32. Tetranucleate cell.

Fig. 33. Condensed chromatin and appendage formation.

Fig. 34. Cells showing nuclear budding.

PLATE - 5


19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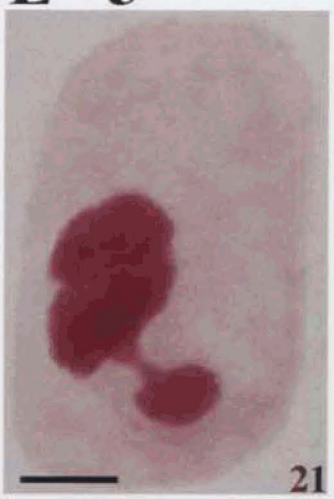
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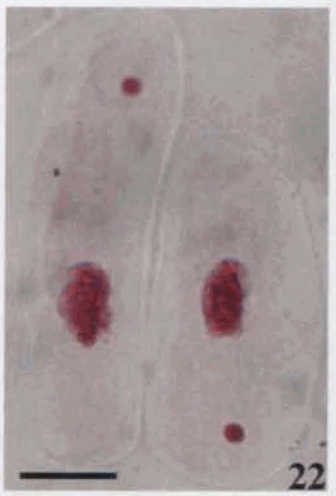
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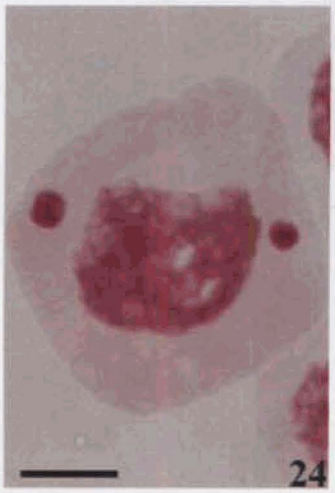
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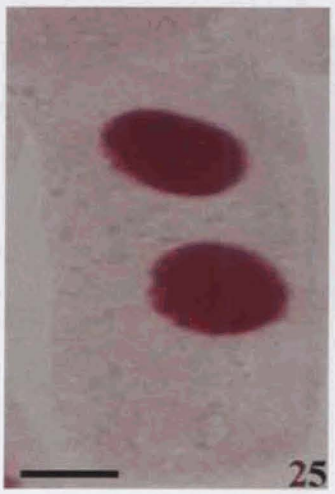
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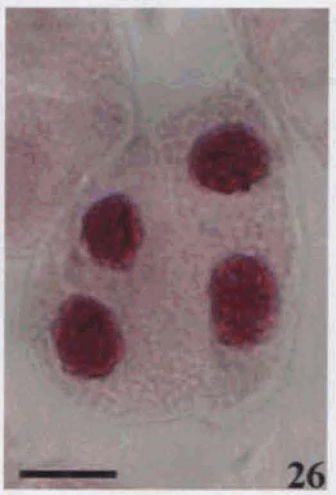
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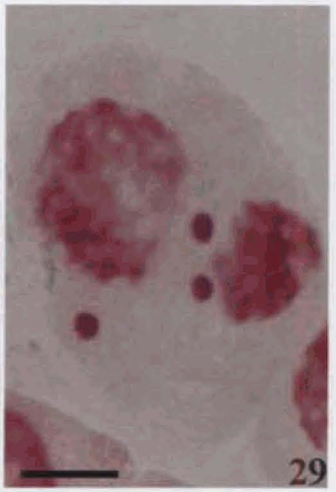
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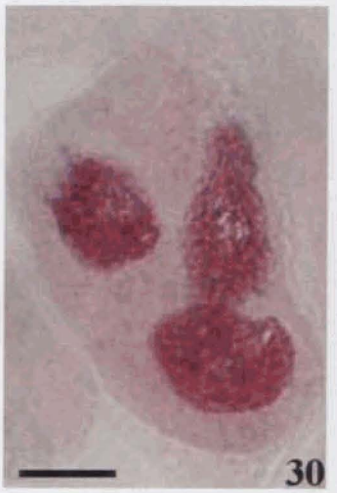
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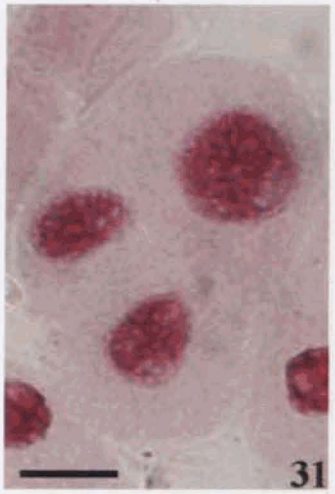
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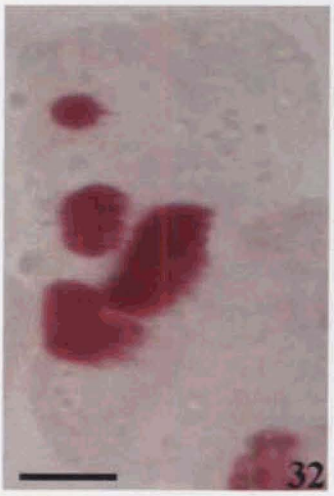
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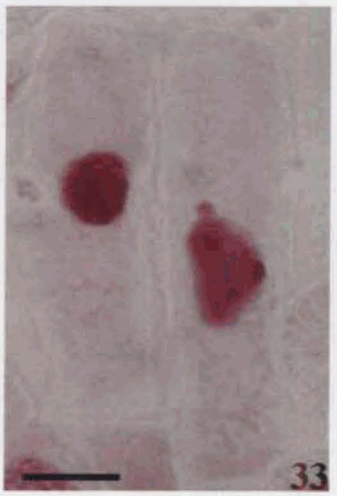
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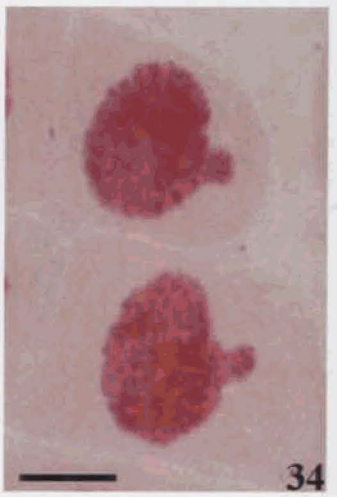
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PLATE – 6

Figs. 35-50. Various cytological aberrations at interphase scored in *Allium cepa* L. root tip meristem after treatment with cyanobacterial extracts.

Fig. 35. Nuclear budding.

Fig. 36. Nuclear diminution.

Fig. 37. Nuclear disintegration.

Fig. 38. Bizarre form of nucleus.

Fig. 39. Blebbing.

Fig. 40. Nuclear extrusion.

Fig. 41. Cell showing nuclear extrusion and another with binucleate cell.

Fig. 42. Nuclear fragmentation.

Fig. 43. Nuclear fragments in elongated cells.

Fig. 44. Elongated cell with nuclear fragments and micronuclei.

Fig. 45. Nuclear lesion.

Fig. 46. Nuclear pulverization.

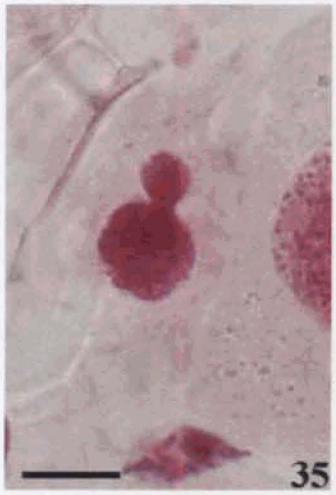
Fig. 47. Nuclear pulverization.

Fig. 48. Cell with nucleus showing hyperchromasia.

Fig. 49. Cell showing dissolution of chromatin.

Fig. 50. Cell with club shaped nucleus.

PLATE - 6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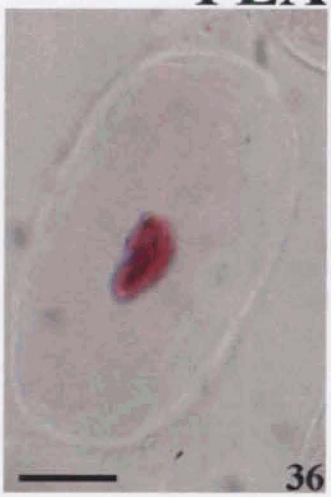
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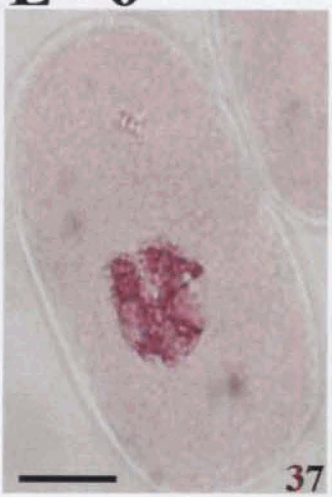
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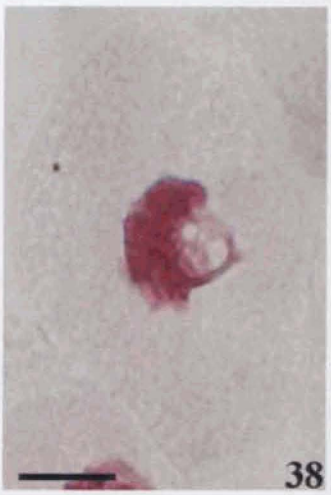
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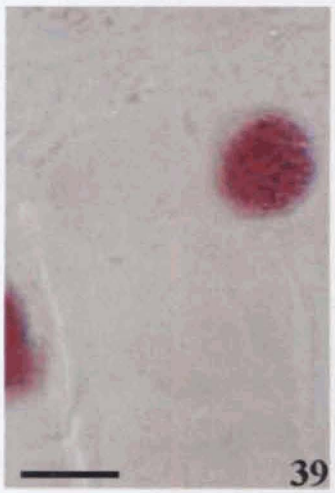
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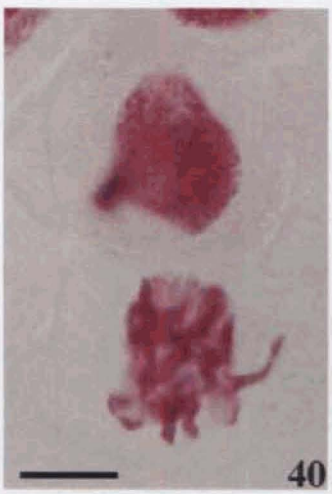
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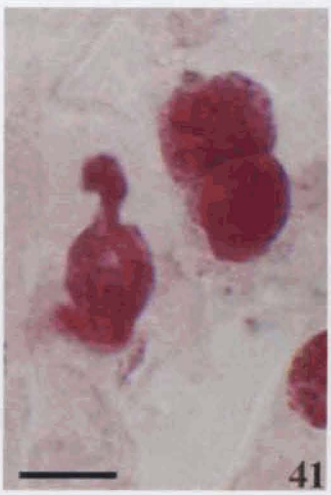
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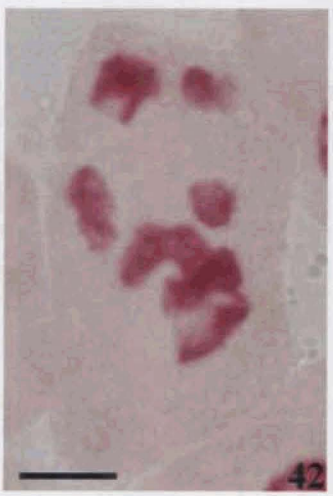
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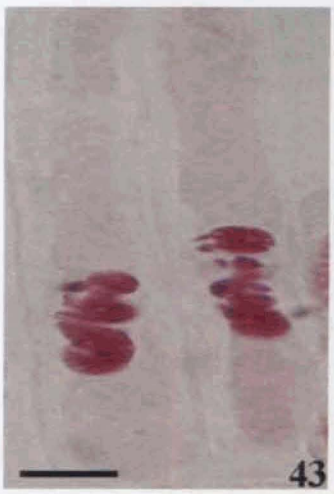
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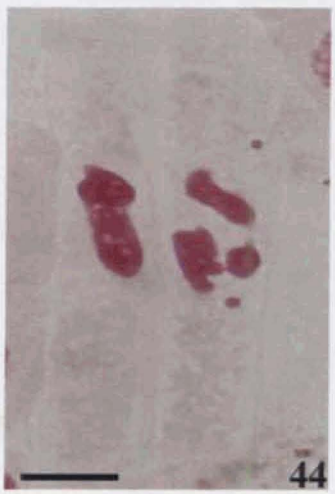
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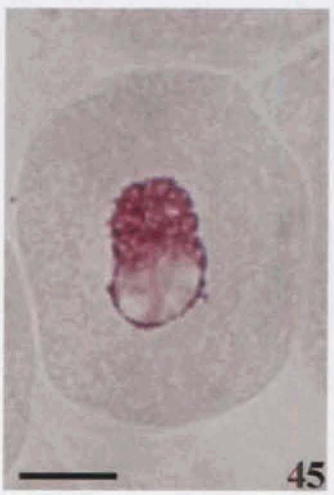
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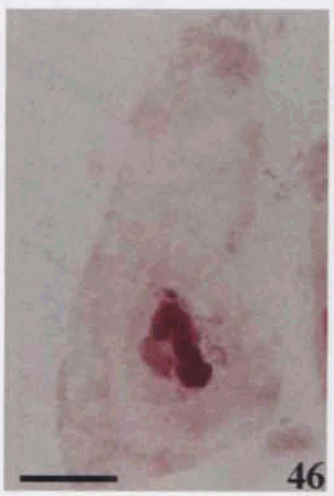
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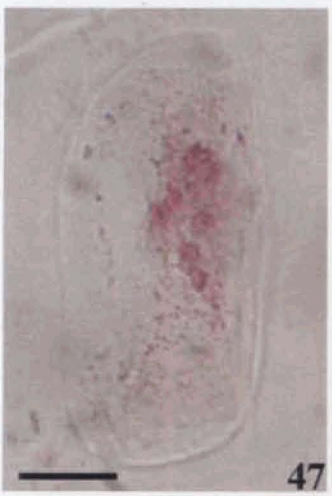
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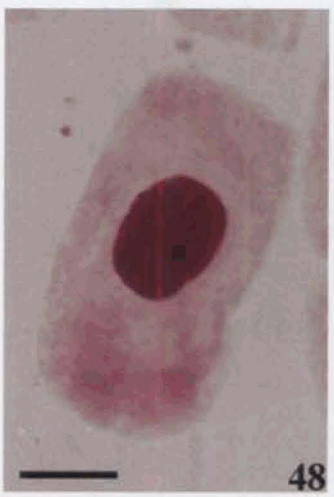
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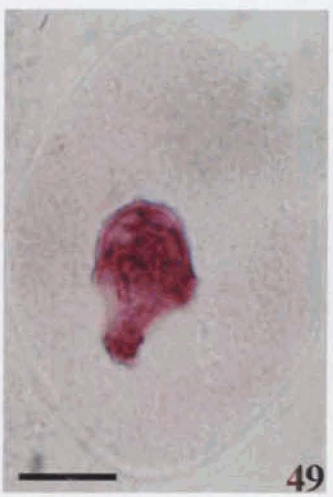
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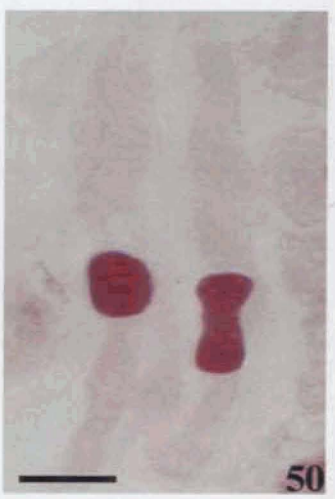
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PLATE – 7

Figs. 51-66. Various cytological aberrations at interphase scored in *Allium cepa* L. root tip meristem after treatment with cyanobacterial extracts.

Fig. 51. Crescent shaped nucleus.

Fig. 52. Crescent shaped nucleus.

Fig. 53. Cell with aberrant nucleus.

Fig. 54. Dissolution of chromatin.

Fig. 55. Bizarre form of nucleus.

Fig. 56. Bizarre form of nucleus.

Fig. 57. Bizarre form of nucleus.

Fig. 58. Bizarre form of nucleus with a micronucleus.

Fig. 59. Heterophasic binucleate cell.

Fig. 60. Diagonal binucleate condition at late prophase.

Fig. 61. Nuclear lesion.

Fig. 62. Nuclear lesion.

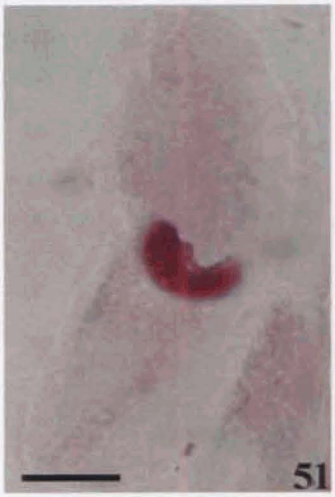
Fig. 63. Multiple nuclear lesions.

Fig. 64. Nuclear erosion.

Fig. 65. Cells with microlesions.

Fig. 66. Condensed chromatin with a lesion.

PLATE - 7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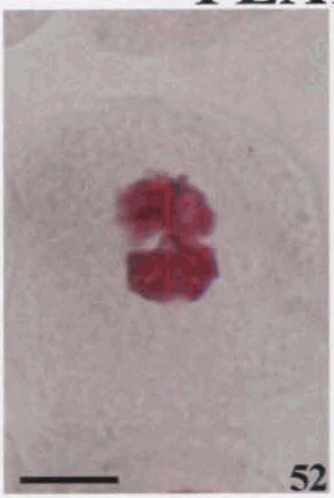
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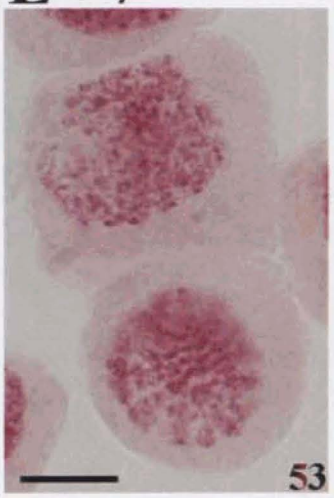
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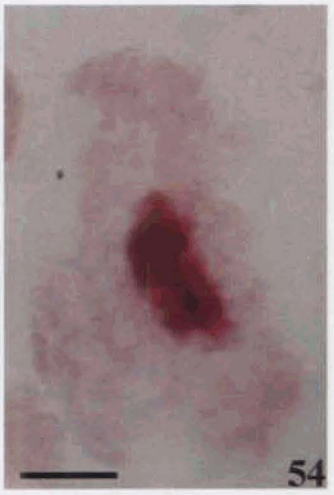
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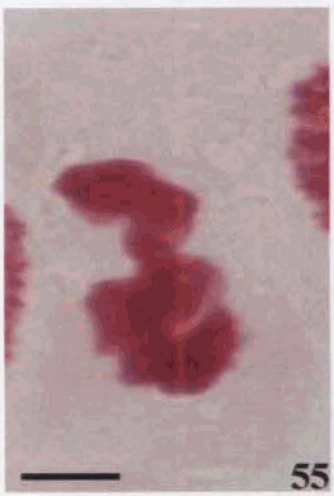
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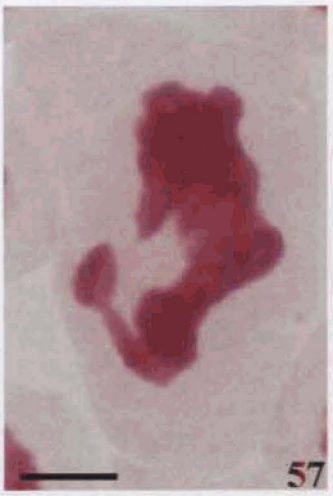
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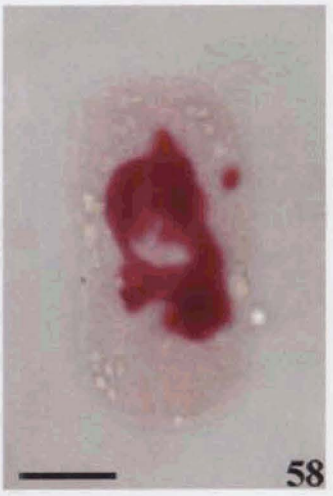
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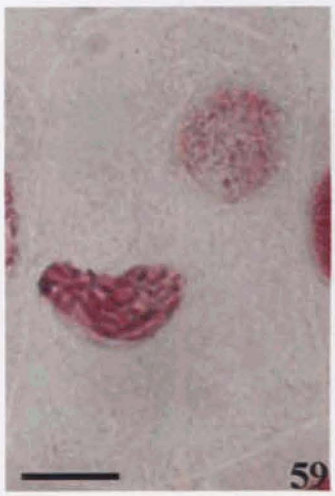
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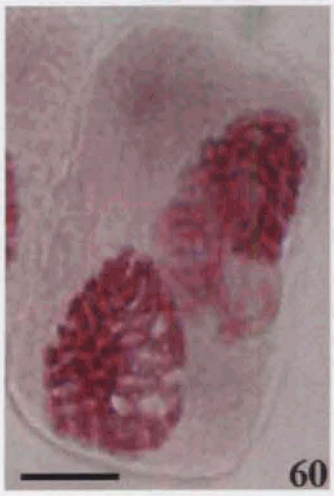
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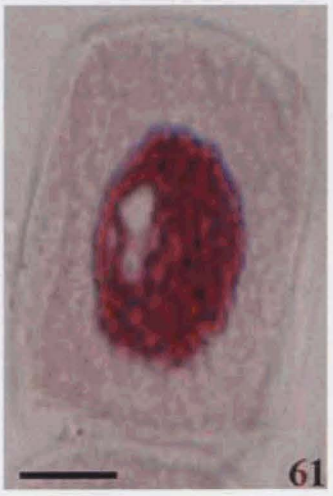
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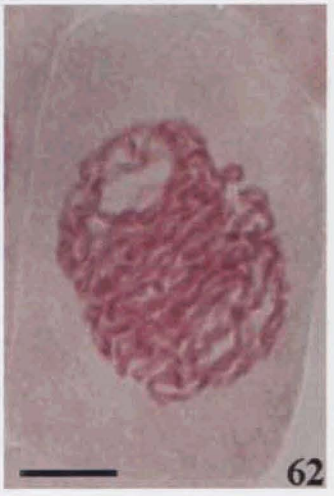
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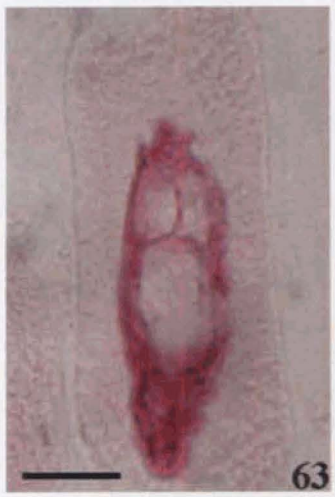
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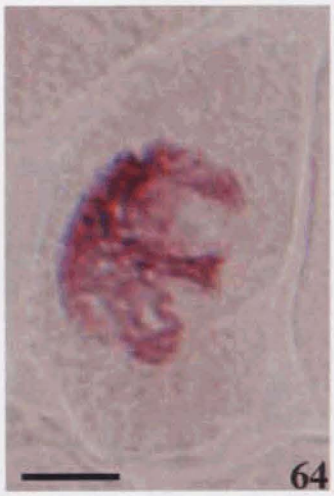
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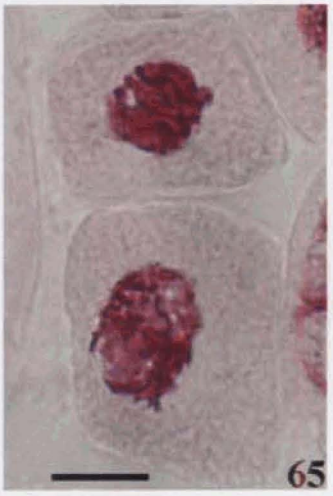
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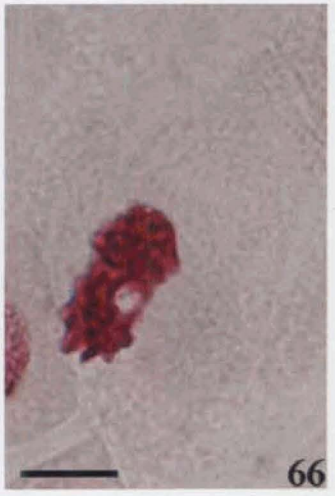
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PLATE – 8

Figs. 67-70. Various cytological aberrations at interphase scored in *Allium cepa L.* root tip meristem after treatment with cyanobacterial extracts.

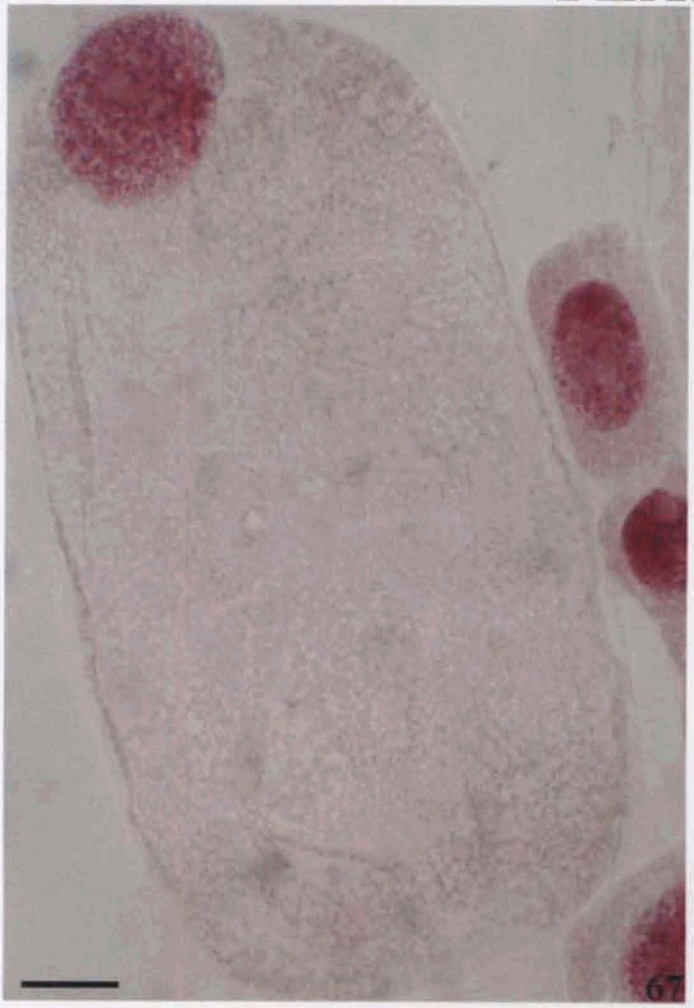
Fig. 67. Giant cell showing nucleus at the periphery.

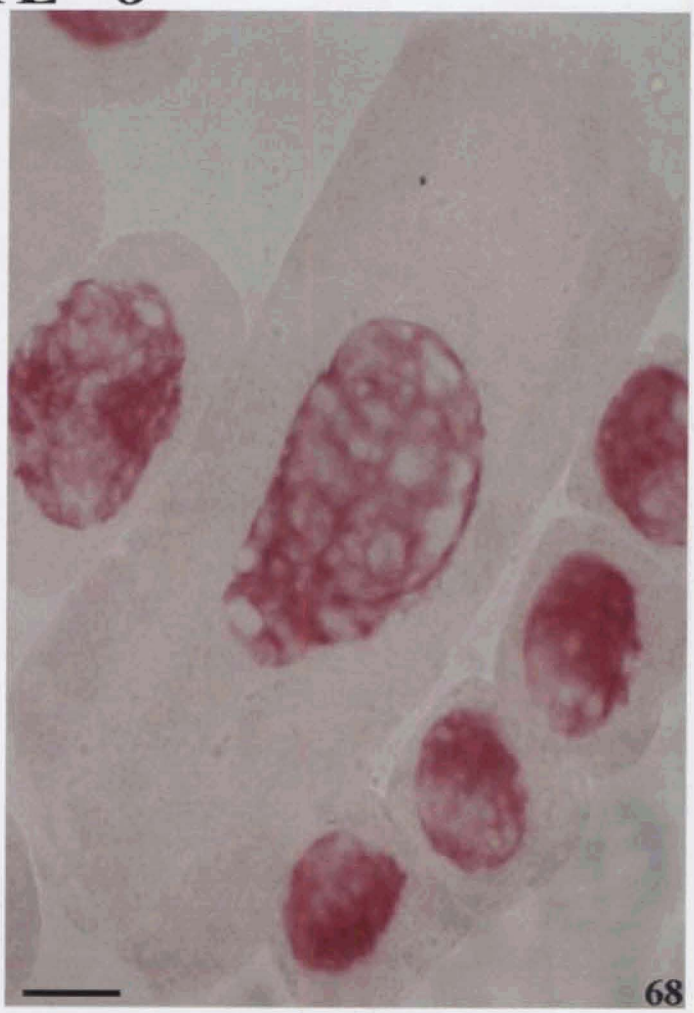
Fig. 68. Giant cell with several lesions in the nucleus.

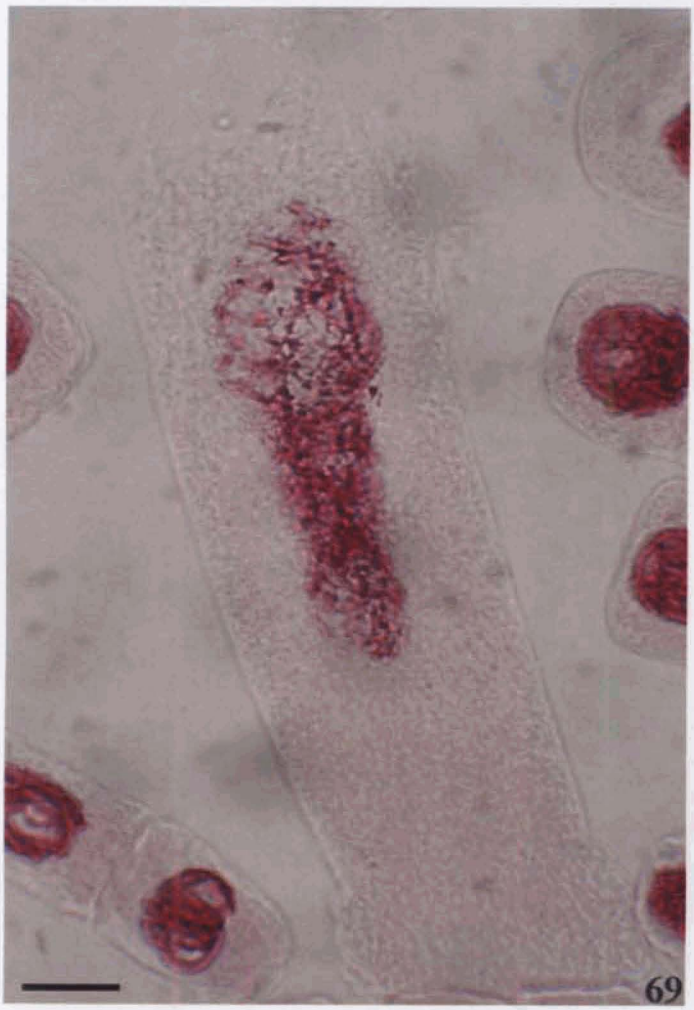
Fig. 69. Giant cell showing pulverized chromatin.

Fig. 70. Giant cell with bizarre nucleus.

PLATE - 8







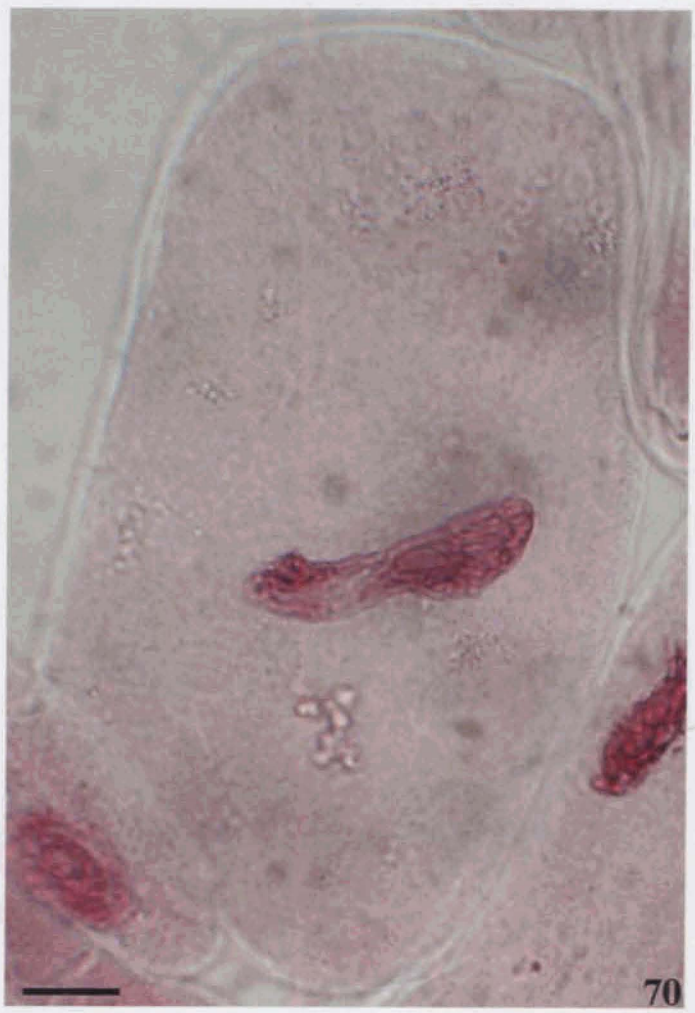


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PLATE – 9

Figs. 71-85. Various cytological aberrations at prophase scored in *Allium cepa* L. root tip meristem after treatment with cyanobacterial extracts.

Fig. 71. Condensed chromatin.

Fig. 72. Contorted chromatin.

Fig. 73. Differential condensation.

Fig. 74. Non-synchronized condensation at prophase.

Fig. 75. Non-synchronized condensation.

Fig. 76. Cells showing dissolution of chromatin.

Fig. 77. Nuclear extrusion.

Fig. 78. Dissolution of chromatin at prophase.

Fig. 79. Pulverized chromatin at prophase.

Fig. 80. Pulverized prophase.

Fig. 81. Abnormal disintegration of nuclear membrane during early prophase.

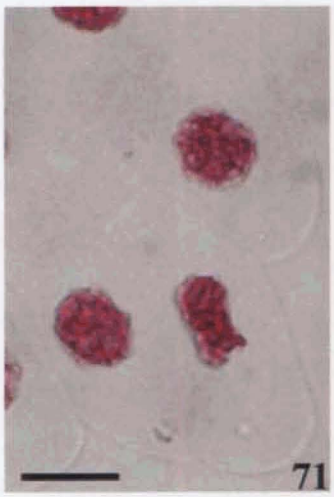
Fig. 82. Abnormal bouquet formation of chromatin.

Fig. 83. Unequal grouping of chromatin at late prophase.

Fig. 84. Bizarre form of nucleus.

Fig. 85. Bizarre form of nucleus.

PLATE - 9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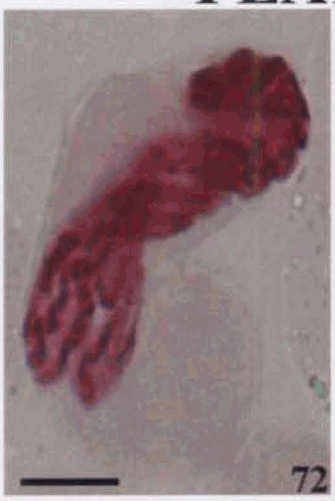
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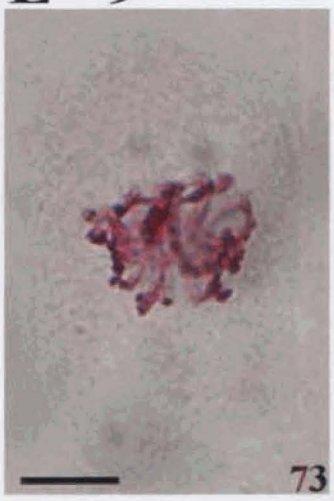
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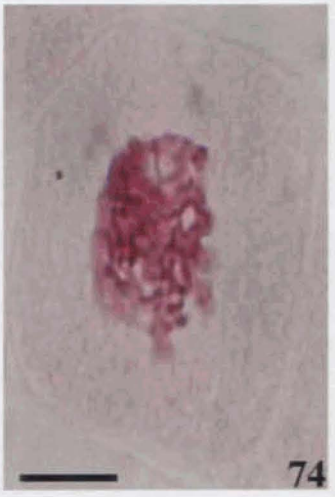
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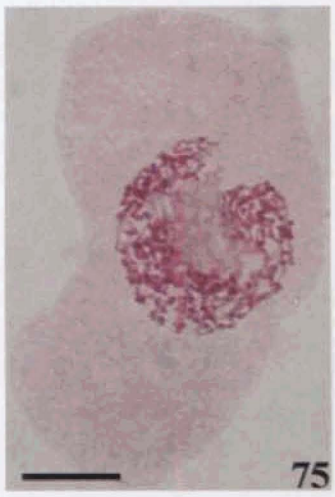
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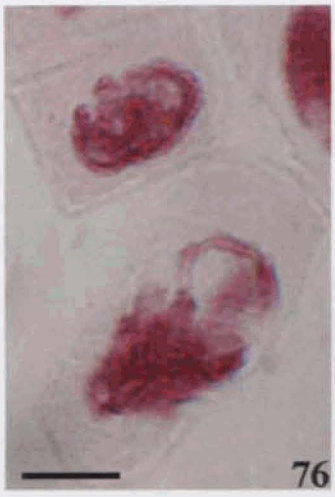
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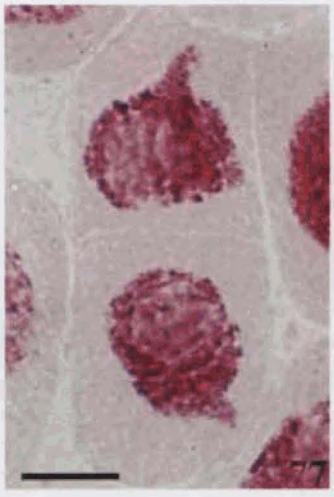
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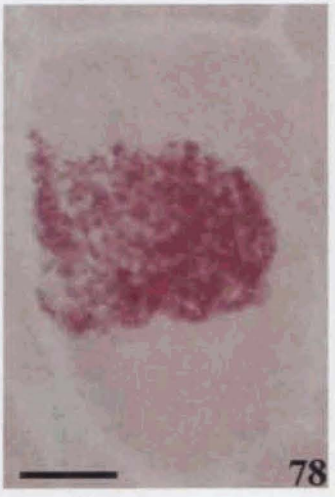
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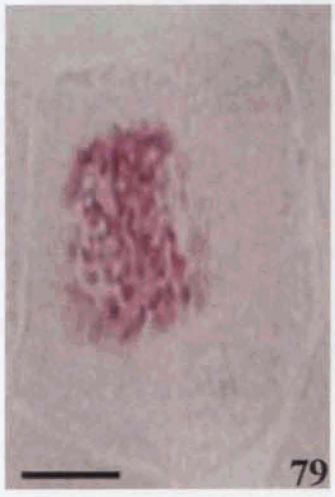
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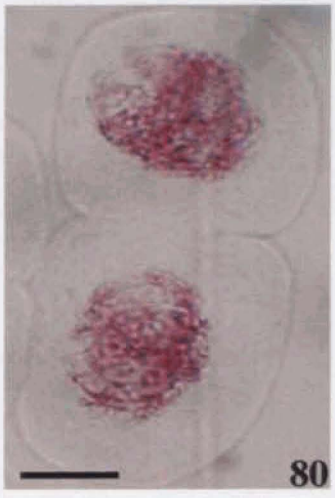
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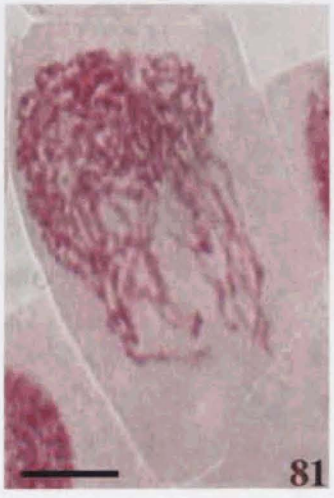
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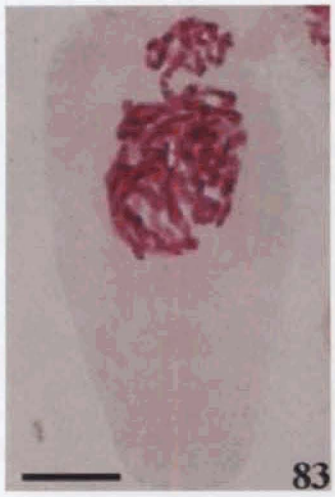
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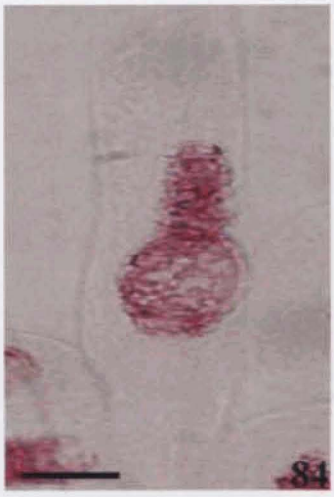
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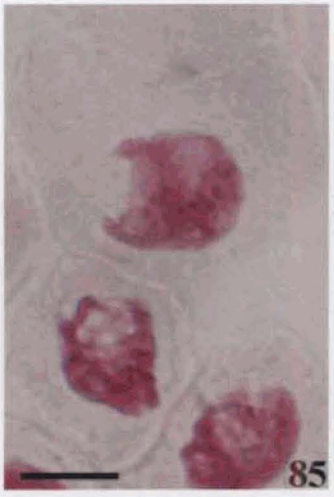
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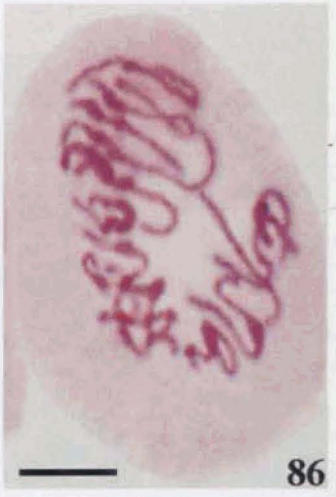
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PLATE – 10

Figs. 86-102. Various cytological aberrations at metaphase scored in *Allium cepa* L. root tip meristem after treatment with cyanobacterial extracts.

Fig. 86. Non - synchronized condensation at early metaphase

Fig. 87. Sticky metaphase.

Fig. 88. Abnormal condensation at metaphase.

Fig. 89. Metaphase clumping.

Fig. 90. Stratified early metaphase.

Fig. 91. Stratified metaphase.

Fig. 92. Scattering of chromosomes at early metaphase.

Fig. 93. Scattering and grouping of chromosomes at metaphase.

Fig. 94. Abnormal bouquet formation of chromosomes at metaphase.

Fig. 95. Lollypop metaphase.

Fig. 96. Stellate metaphase.

Fig. 97. Ring chromosome at metaphase.

Fig. 98. Ring chromosome.

Fig. 99. Ball metaphase.

Fig. 100. Sticky ball metaphase.

Fig. 101. C- metaphase.

Fig. 102. Partial C- metaphase.

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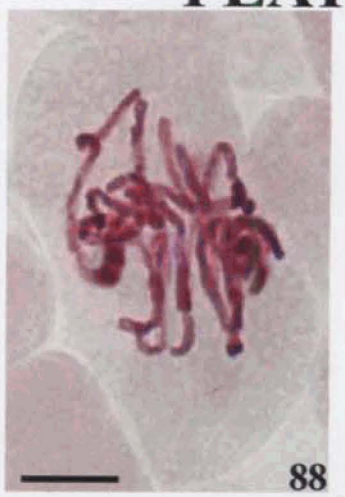
87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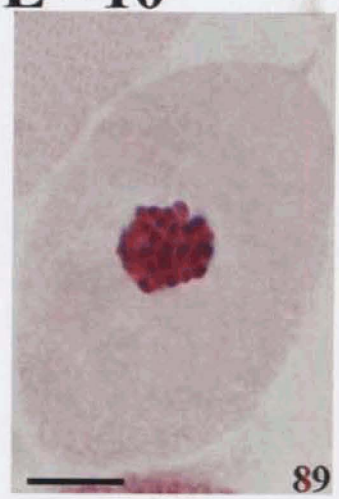
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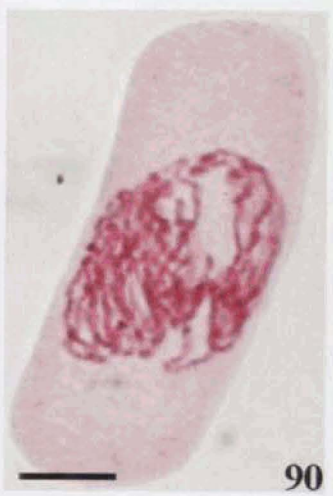
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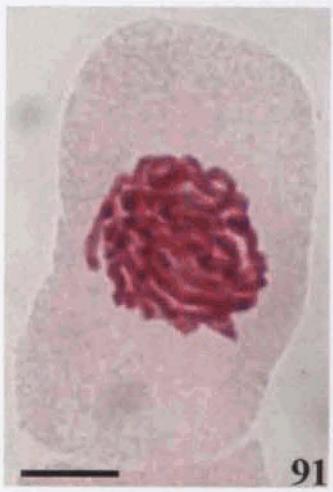
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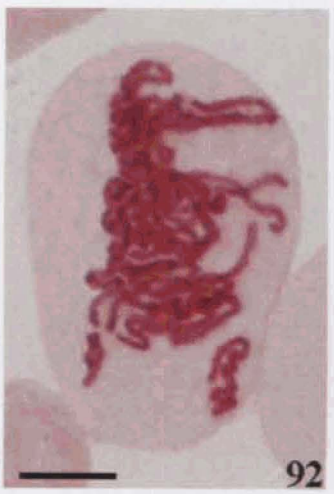
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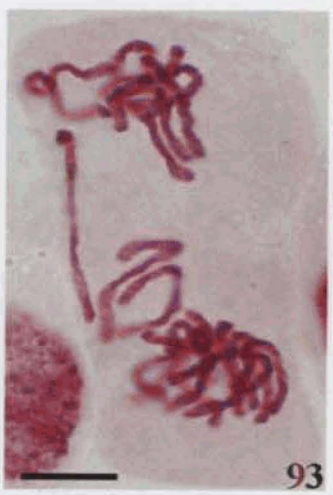
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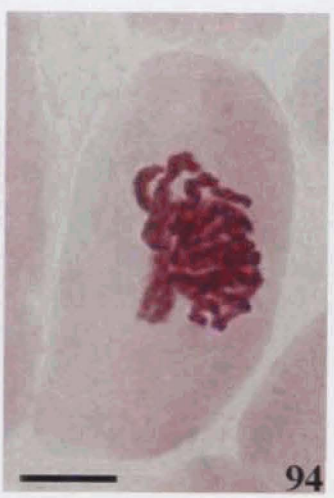
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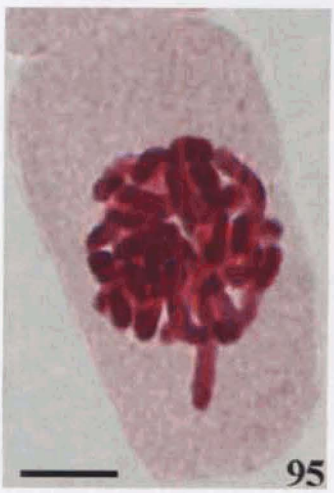
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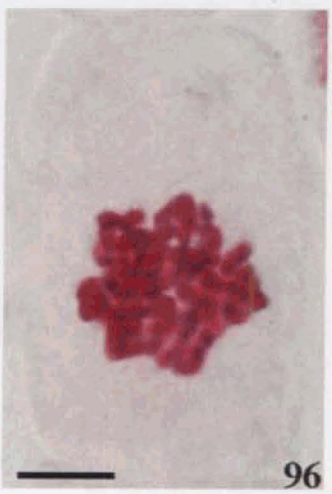
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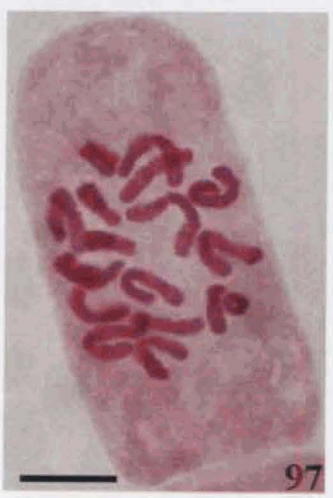
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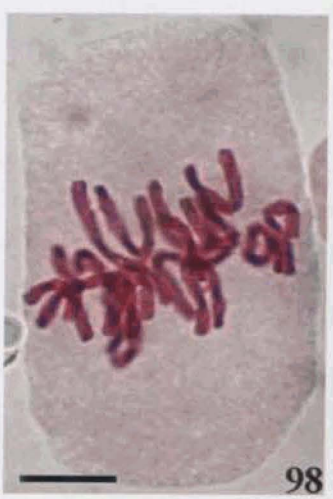
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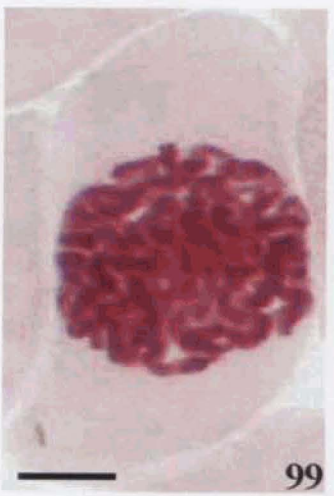
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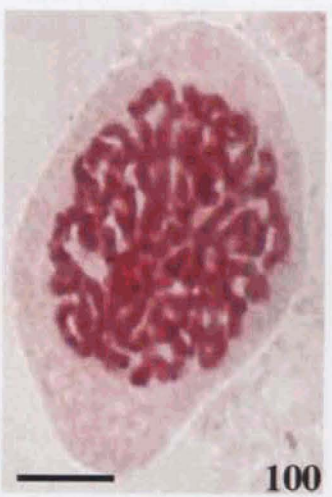
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101


102

PLATE - 11

Figs. 103-118. Various cytological aberrations at metaphase scored in *Allium cepa* L. root tip meristem after treatment with cyanobacterial extracts.

Fig. 103. Sticky metaphase.

Fig. 104. Sticky C- metaphase.

Fig. 105. Adhesion of chromosomes.

Fig. 106. Metaphase clumping.

Fig. 107. Pulverized metaphase.

Fig. 108. Pulverization and differential condensation at metaphase.

Fig. 109. Pulverized C-metaphase.

Fig. 110. Chromosome fragment at metaphase.

Fig. 111. Chromosome fragment at metaphase.

Fig. 112. Chromosome gaps and fragments in a polyploid cell.

Fig. 113. Misoriented C- metaphase.

Fig. 114. Polyploid cell. what is the x number

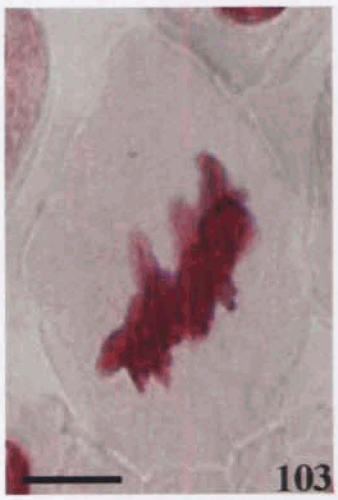
Fig. 115. Chromosome fragment and telosome formation in a polyploid cell.

Fig. 116. Convoluted chromosomes.

Fig. 117. Double metaphase in a binucleate cell.

Fig. 118. Deproteinized diagonal metaphase.

PLATE - 11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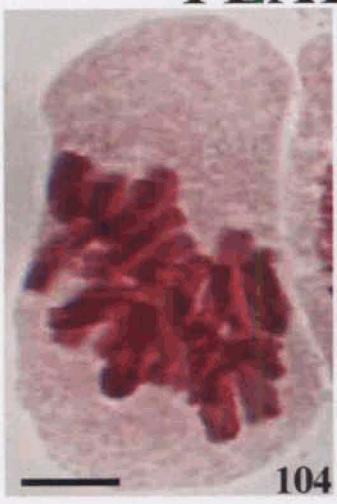
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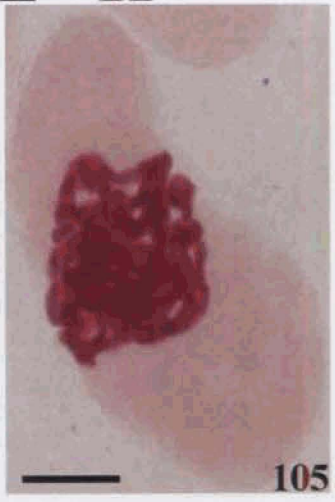
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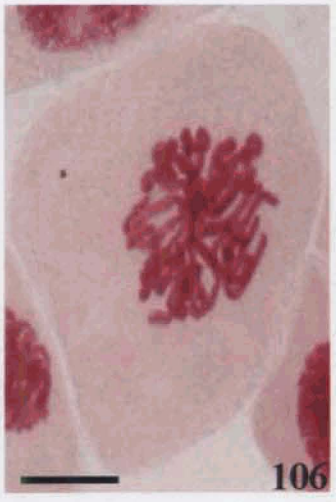
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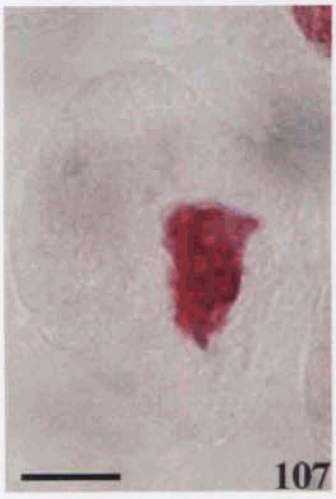
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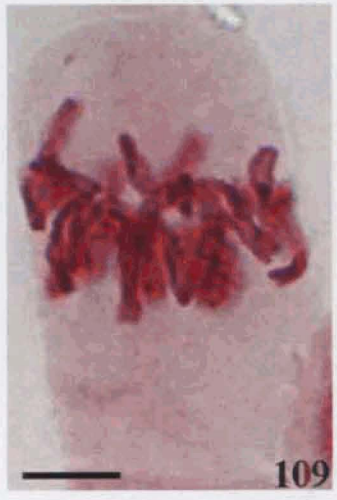
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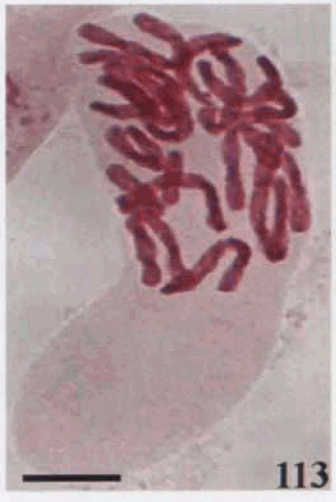
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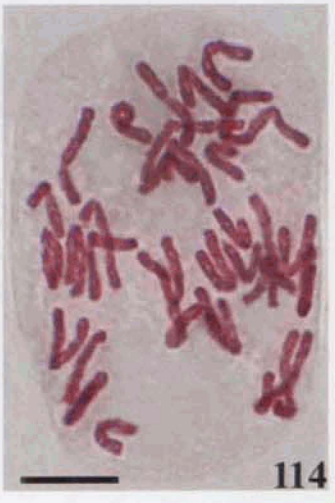
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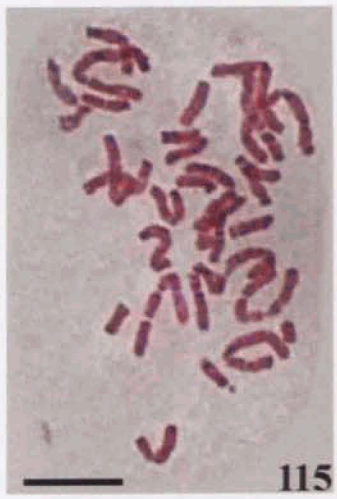
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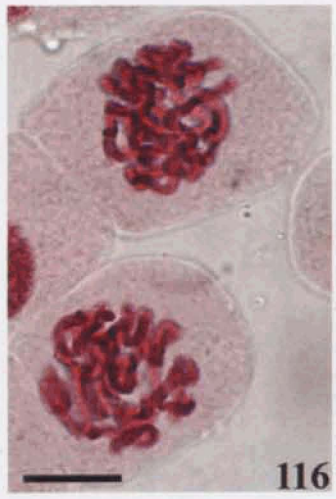
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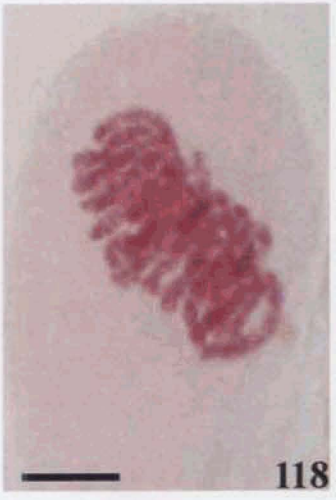
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PLATE - 12

Figs. 119-122. Various cytological aberrations at metaphase scored in *Allium cepa* L. root tip meristem after treatment with cyanobacterial extracts.

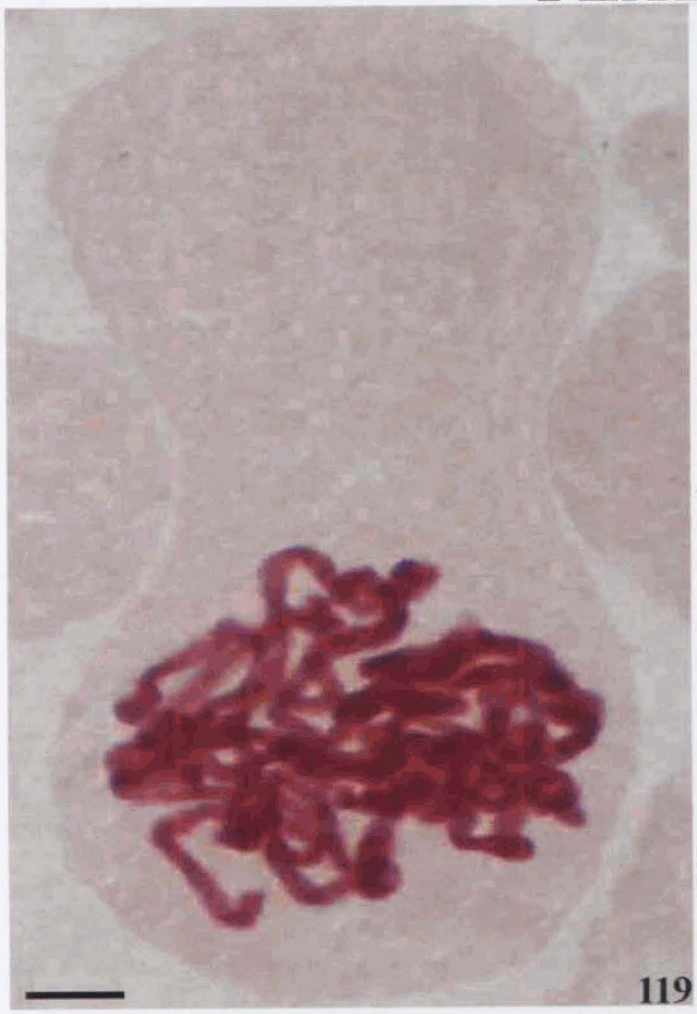
Fig. 119. Misorientation at early metaphase in a giant cell.

Fig. 120. Stratified early metaphase in a giant cell.

Fig. 121. Disturbed metaphase in a hyperploid giant cell.

Fig. 122. Sticky and condensed ball metaphase in a giant cell.

PLATE - 12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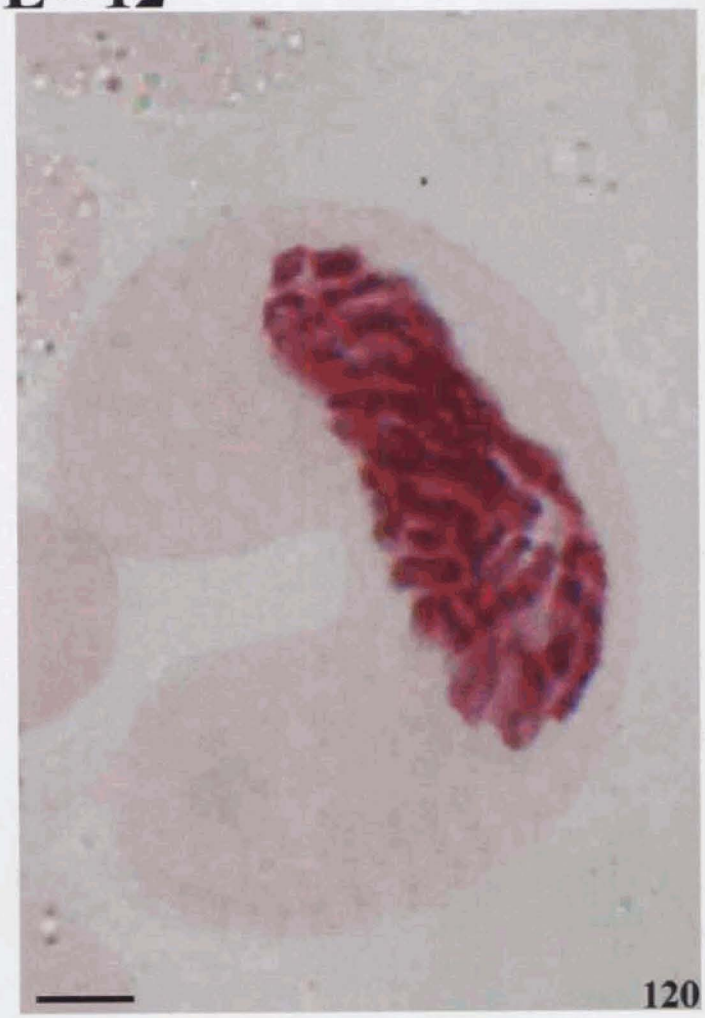
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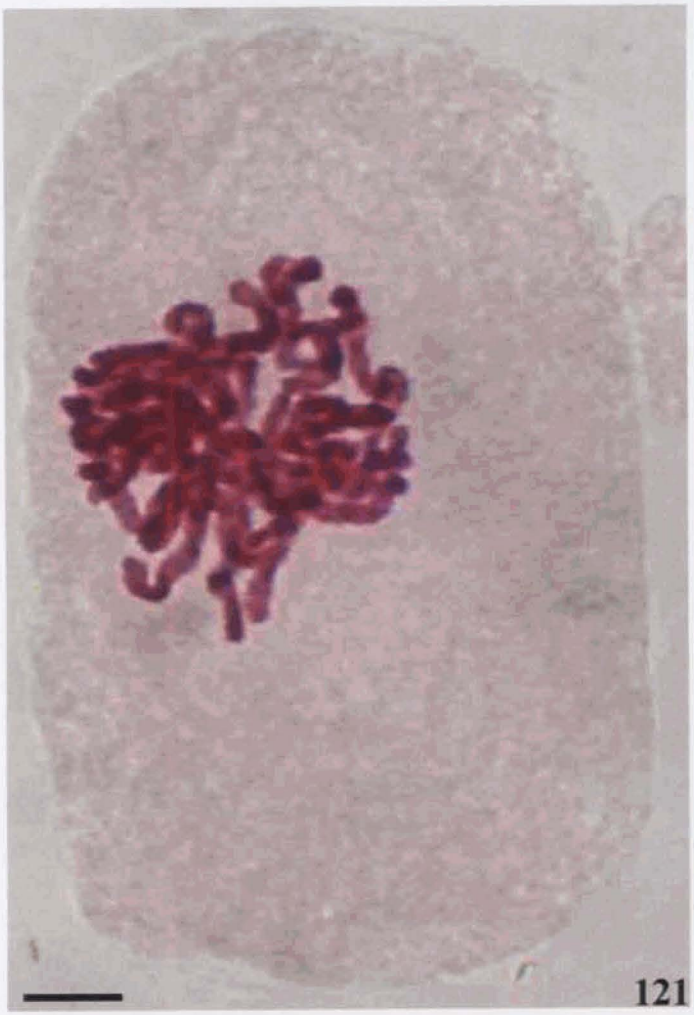
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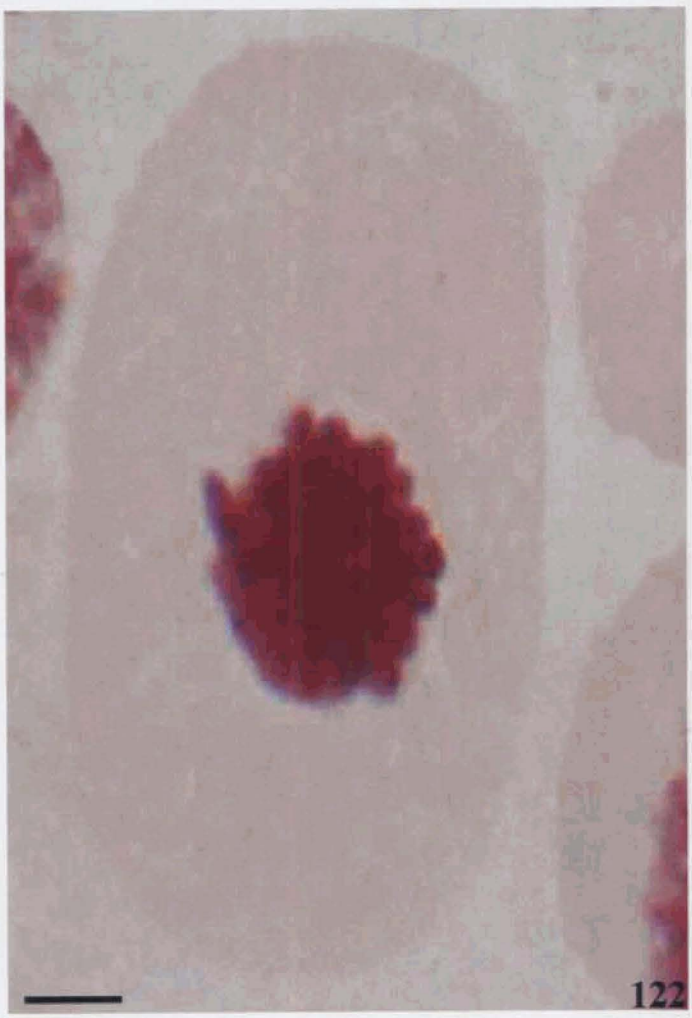
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46. CCC

PLATE - 13

Figs. 123-126. Various cytological aberrations at metaphase scored in *Allium cepa* L. root tip meristem after treatment with cyanobacterial extracts.

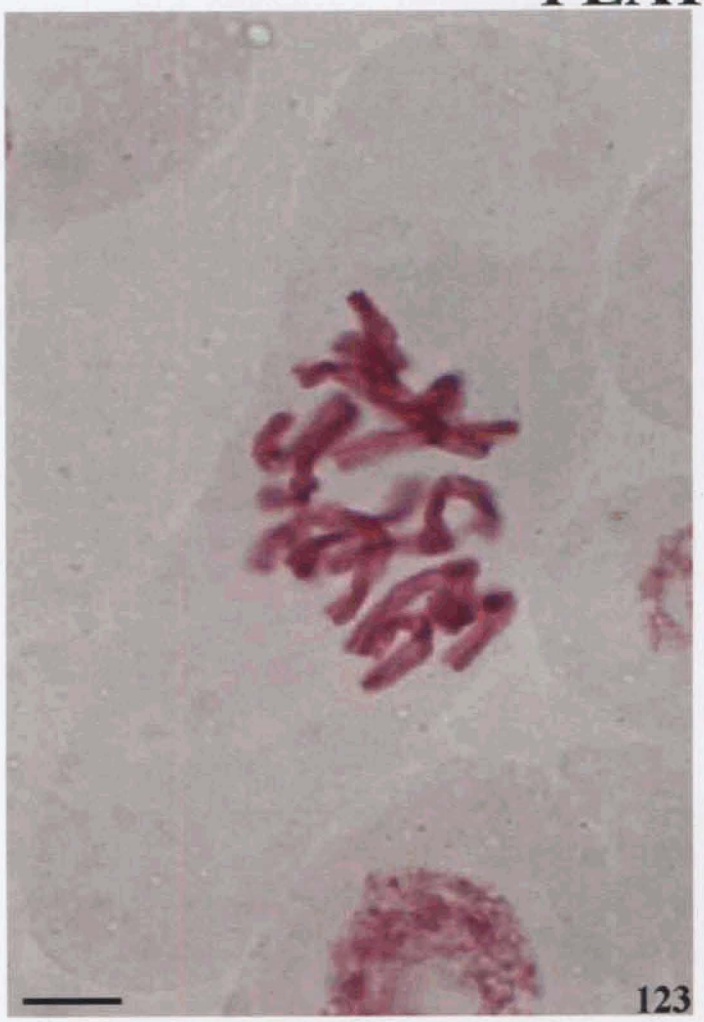
Fig. 123. Diagonal partial C- metaphase in a giant cell.

Fig. 124. Chromosome gaps and several fragments in a giant polyploid cell.

Fig. 125. C-metaphase in a giant cell.

Fig. 126. C-metaphase clumping in a giant cell.

PLATE - 13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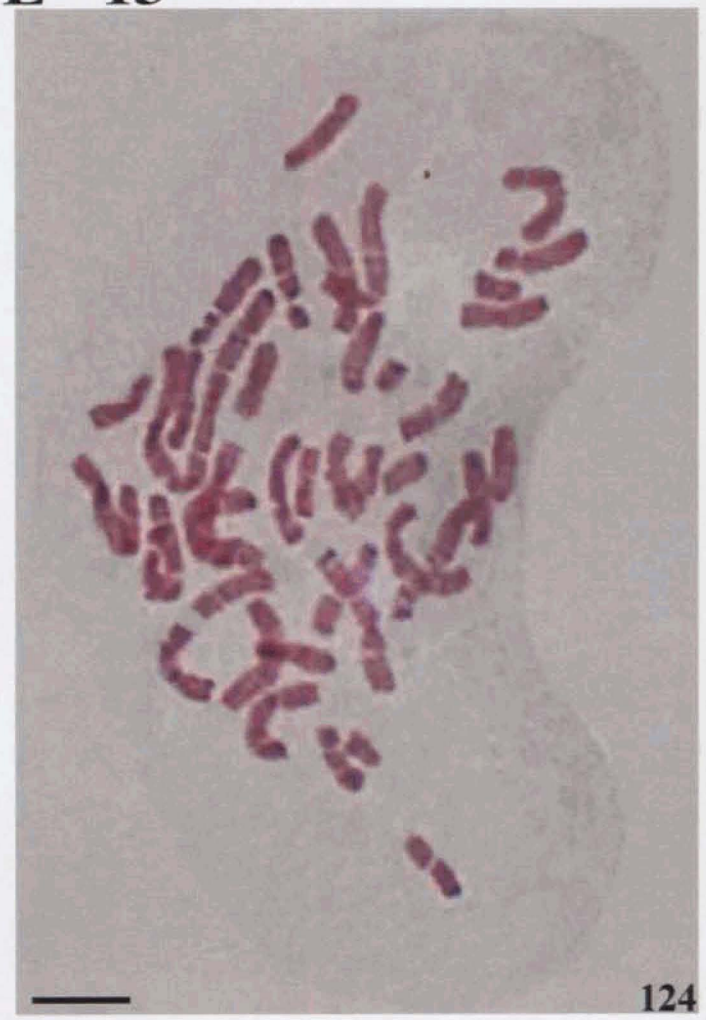
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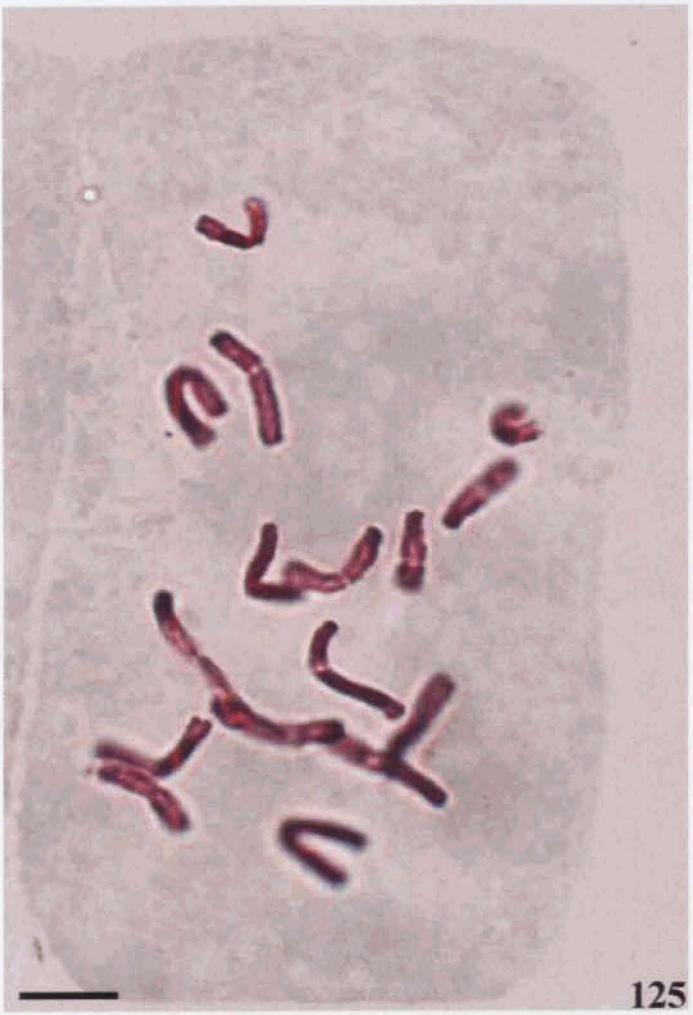
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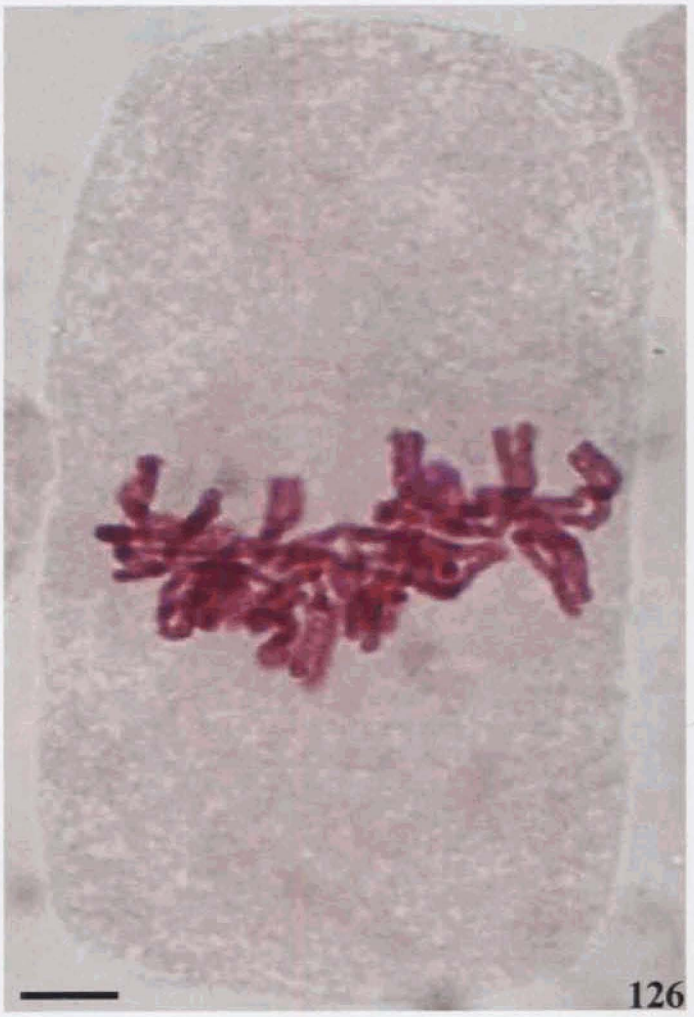
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PLATE - 14

Figs. 127-142. Various cytological aberrations at metaphase (127-134) & anaphase (135-142) scored in *Allium cepa* L. root tip meristem after treatment with cyanobacterial extracts.

Fig. 127. Displaced chromosomes.

Fig. 128. Misoriented chromosomes at metaphase.

Fig. 129. Unequal grouping of chromosome at metaphase.

Fig. 130. Bizarre metaphase.

Fig. 131. Bizarre metaphase.

Fig. 132. Bizarre metaphase.

Fig. 133. Bizarre metaphase.

Fig. 134. Hyperstickiness at metaphase.

Fig. 135. Early ball anaphase.

Fig. 136. Ball anaphase with a laggard.

Fig. 137. Sticky anaphase with a single bridge.

Fig. 138. Anaphase with double bridges.

Fig. 139. Anaphase with triple bridges.

Fig. 140. Sticky multiple bridges.

Fig. 141. Anaphase with five bridges.

Fig. 142. Anaphase with multiple bridges and associations.

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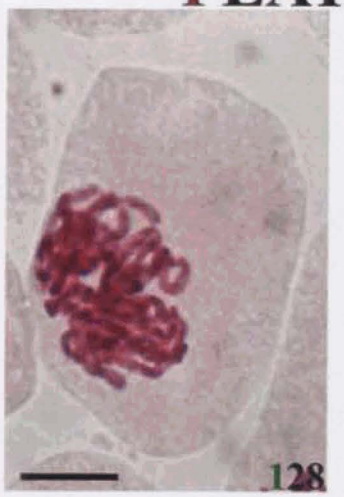
127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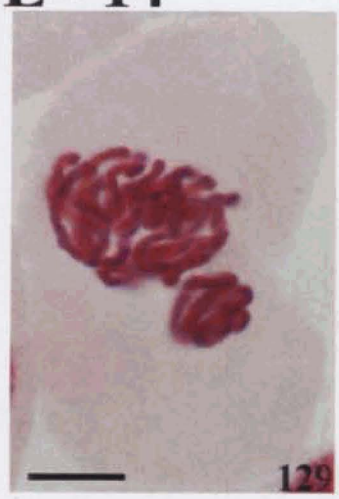
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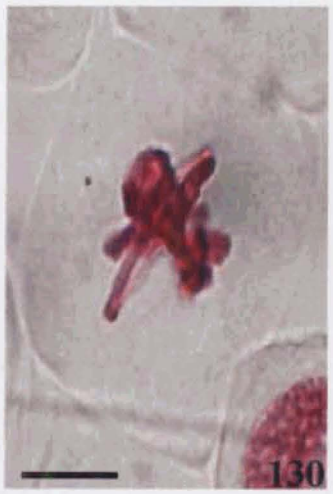
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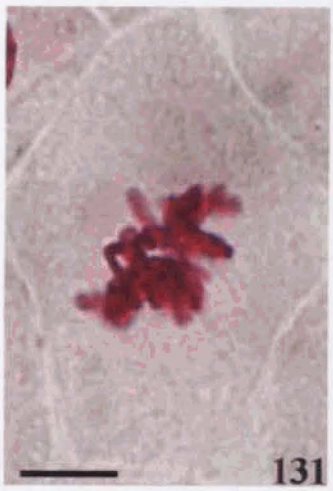
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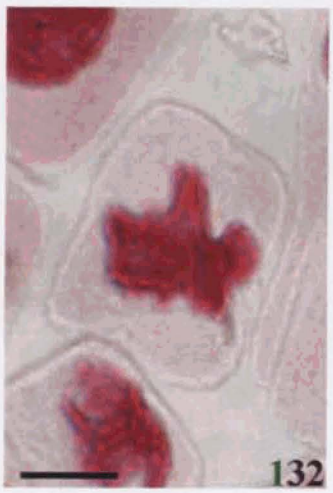
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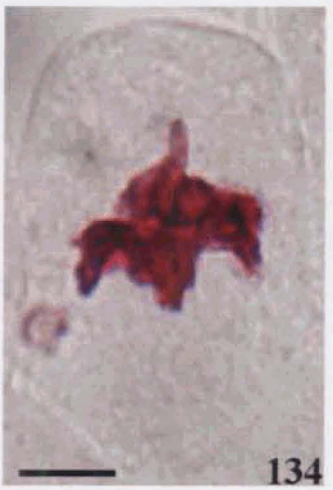
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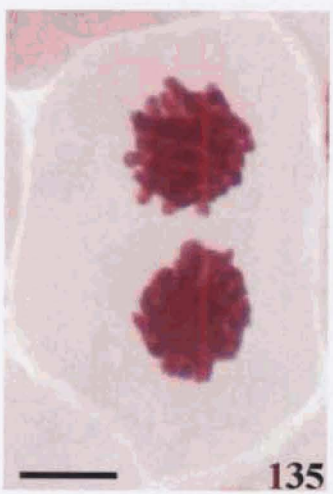
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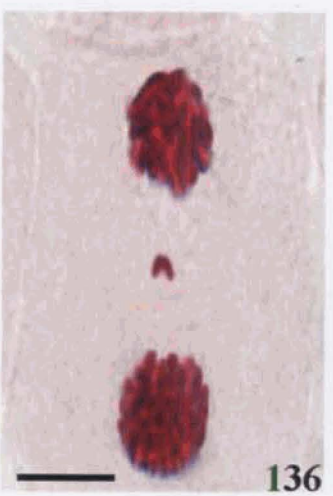
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PLATE - 15

Figs. 143-158. Various cytological aberrations at anaphase scored in *Allium cepa* L. root tip meristem after treatment with cyanobacterial extracts.

Fig. 143. Sticky diagonal anaphase with a bridge.

Fig. 144. Distorted anaphase with a curved bridge.

Fig. 145. Stellate diagonal anaphase with a bridge.

Fig. 146. Stellate anaphase with bridges.

Fig. 147. Stellate early anaphase.

Fig. 148. Stellate late anaphase.

Fig. 149. Chromosome laggard at hemistellate anaphase.

Fig. 150. Stellate sticky chromosomes at anaphase.

Fig. 151. Equatorial separation at anaphase.

Fig. 152. Equatorial separation of chromosomes at anaphase (sticky).

Fig. 153. Equatorial separation with multiple bridges.

Fig. 154. Chromosome laggard.

Fig. 155. Delayed movement of chromosome during anaphase.

Fig. 156. Early movement of chromosomes and fragment at anaphase.

Fig. 157. Displaced chromosome at anaphase.

Fig. 158. Non-synchronized chromosome movement at anaphase.

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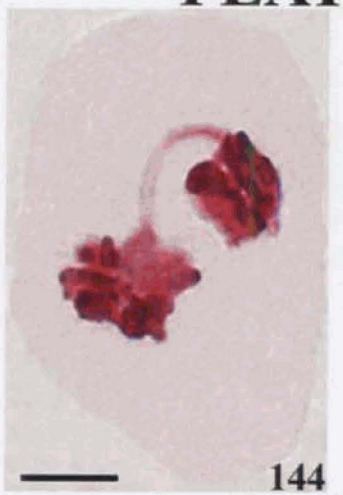
143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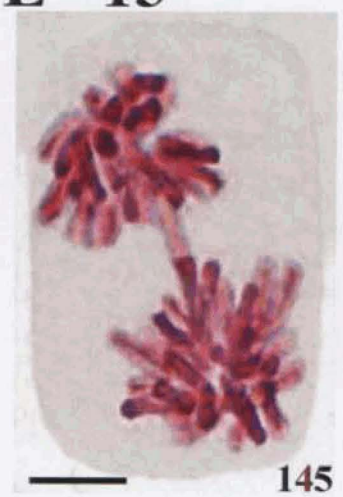
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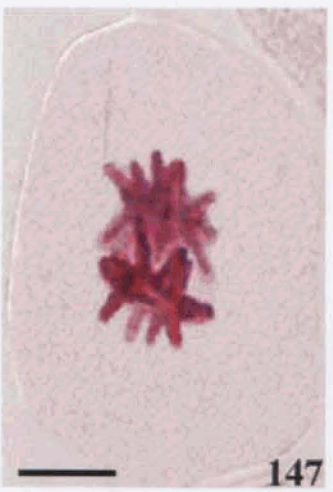
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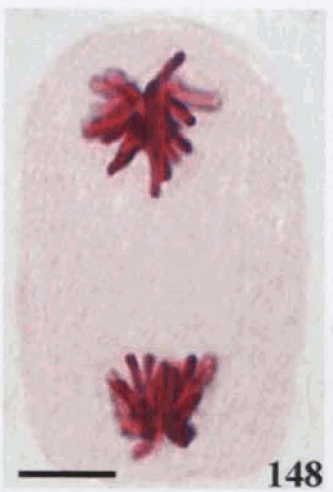
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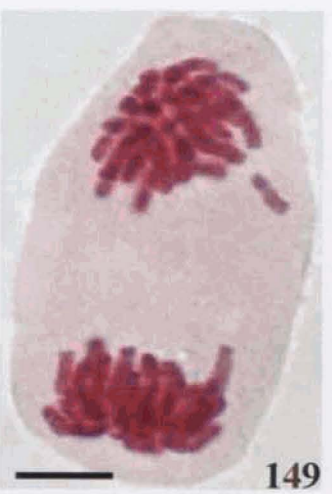
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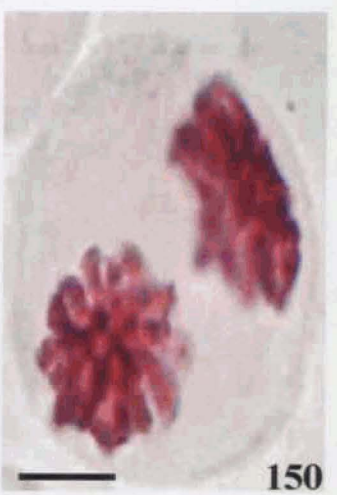
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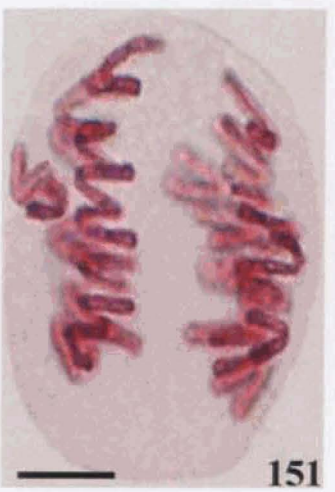
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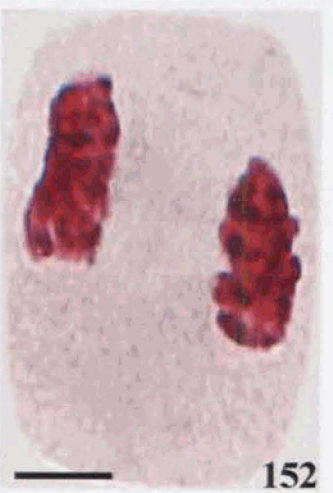
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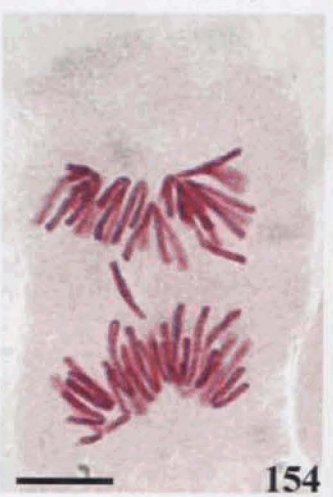
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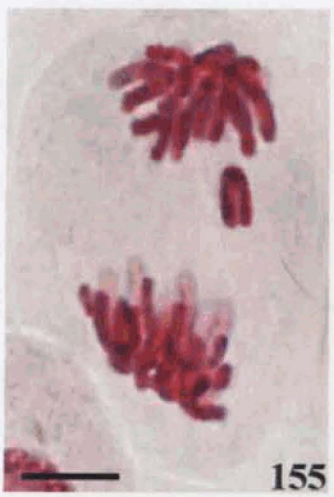
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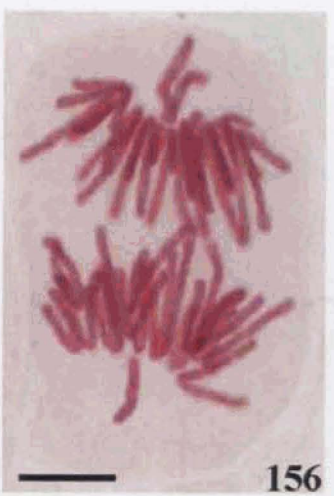
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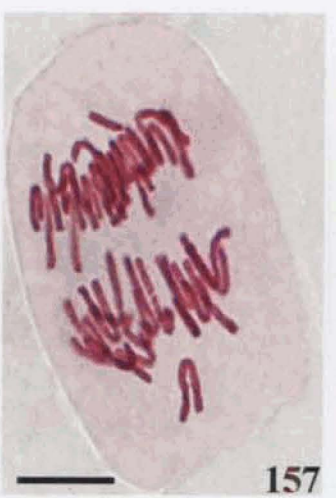
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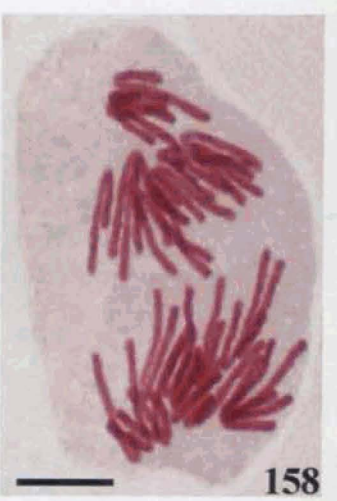
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Figs. 159-162. Various cytological aberrations at anaphase scored in *Allium cepa* L. root tip meristem after treatment with cyanobacterial extracts.

Fig. 159. Early movement of chromosomes at anaphase in a giant cell.

Fig. 160. Giant cell- anaphase with a bridge.

Fig. 161. Chromosome fragment at anaphase in a giant cell.

Fig. 162. Stellate anaphase in a giant cell.

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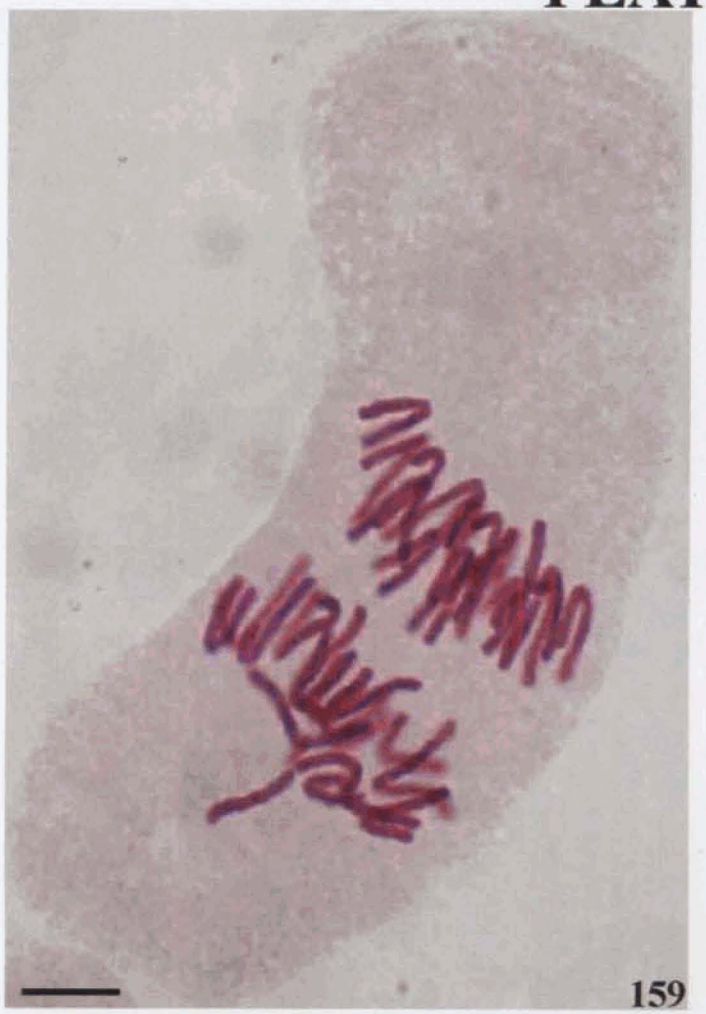
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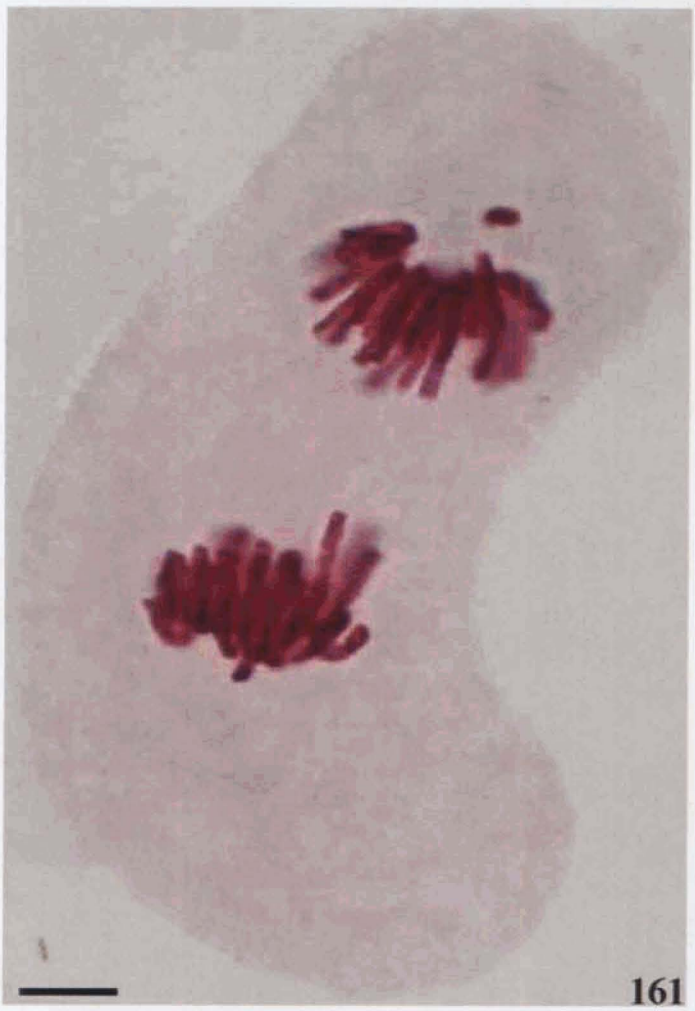
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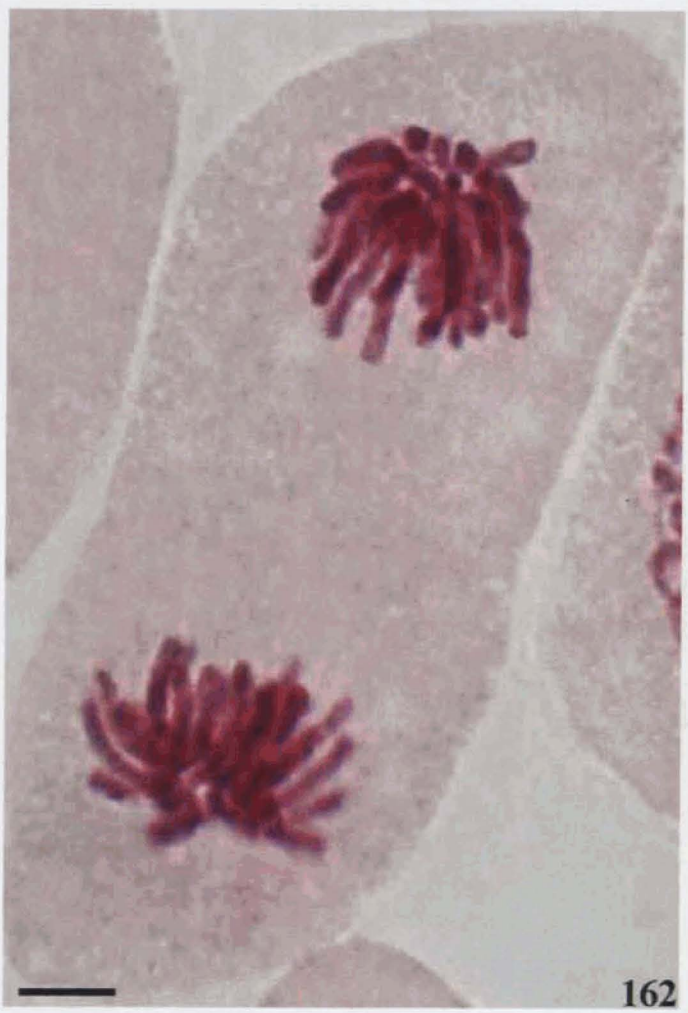
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Figs. 163-166. Various cytological aberrations at anaphase scored in *Allium cepa* L. root tip meristem after treatment with cyanobacterial extracts.

Fig. 163. Hemi- stellate anaphase in a giant cell.

Fig. 164. Misorientation at anaphase in a giant cell.

Fig. 165. Shift in MTOC, unequal separation and fragments in a giant cell.

Fig. 166. Stathmo anaphase in a giant cell.

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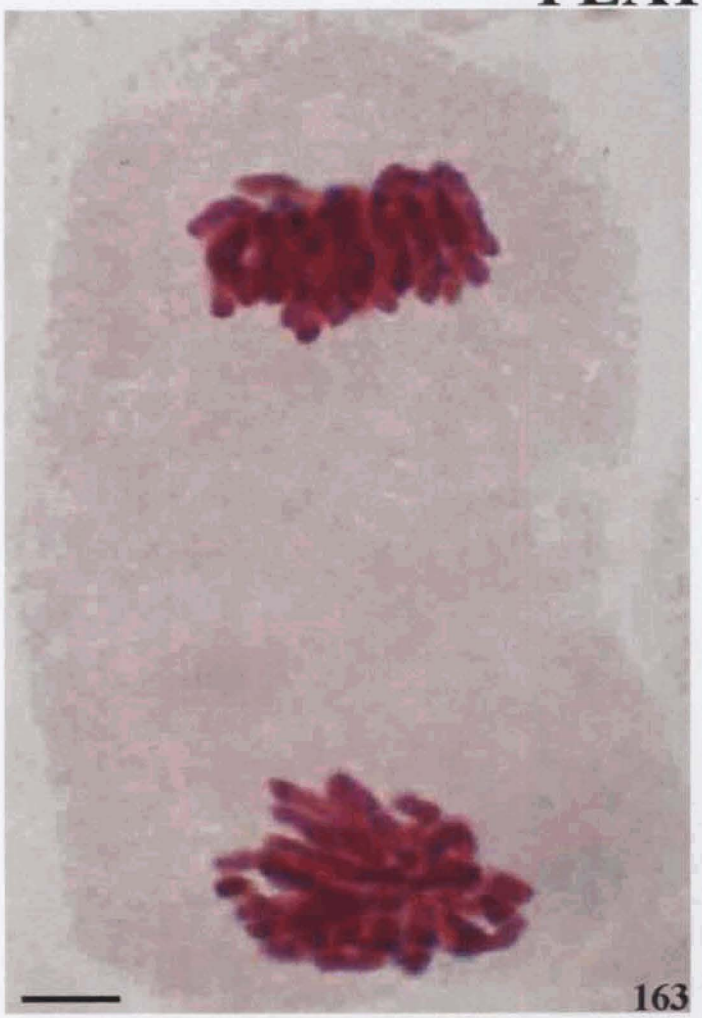
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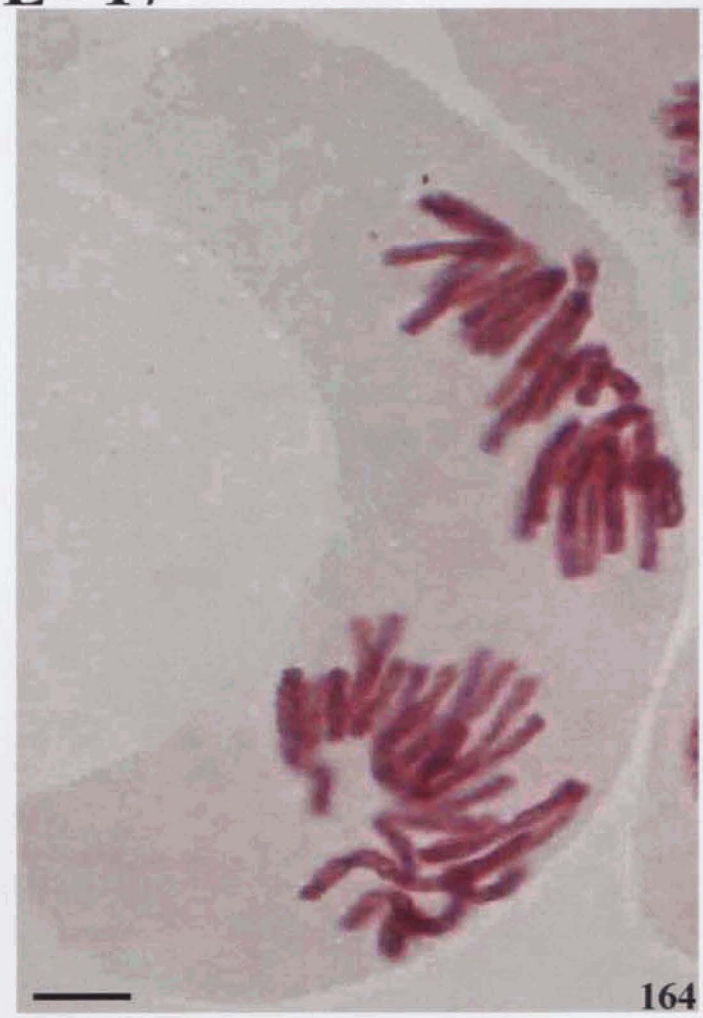
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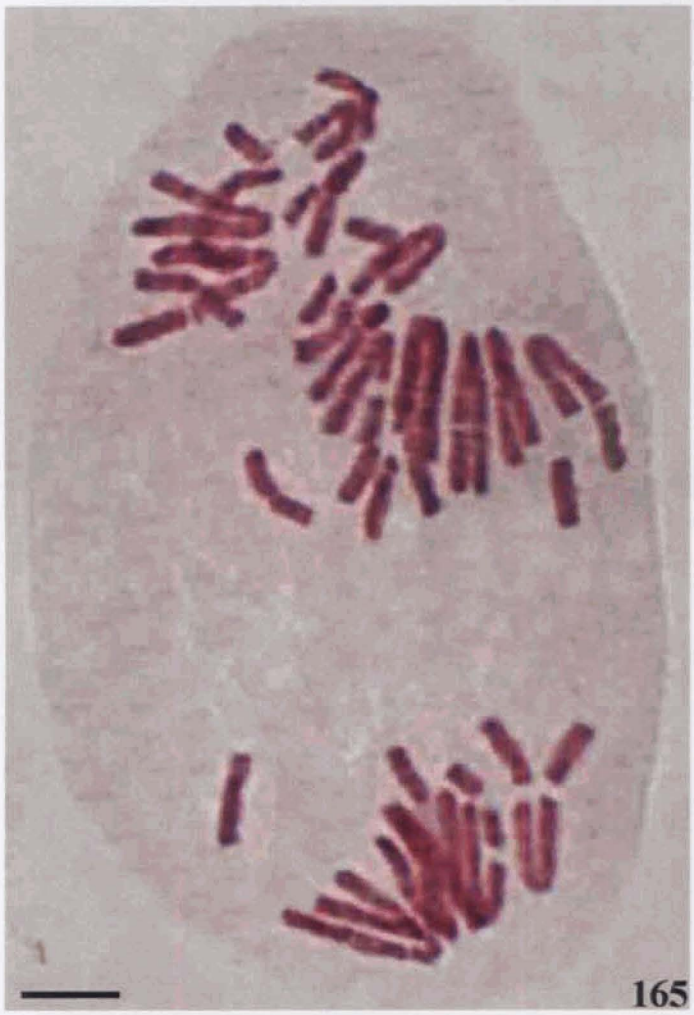
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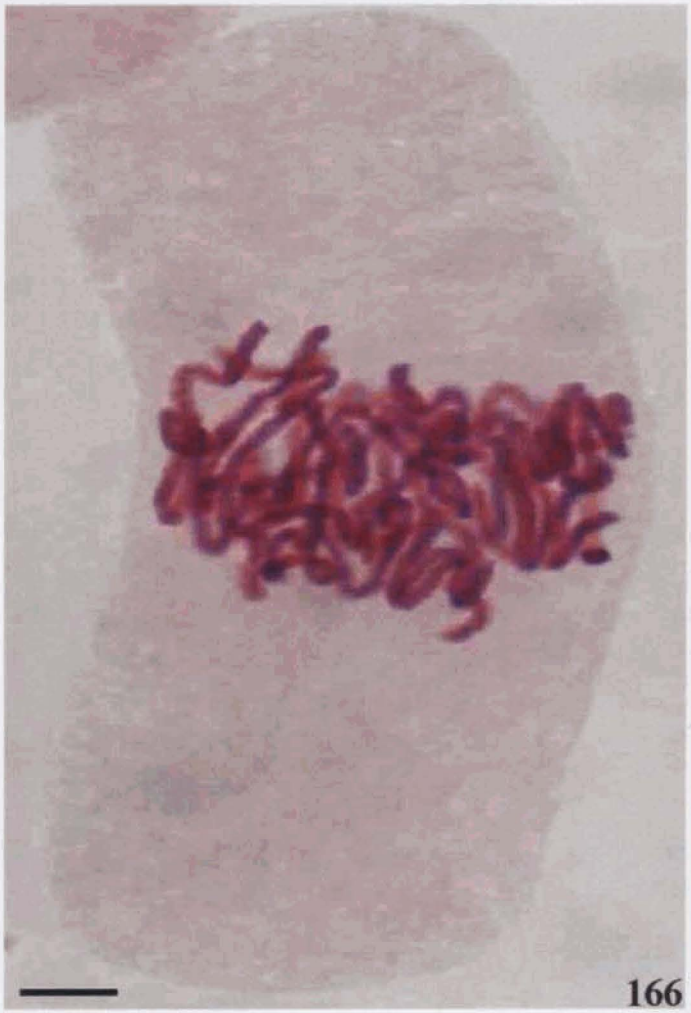
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Figs. 167-182. Various cytological aberrations at anaphase scored in *Allium cepa* L. root tip meristem after treatment with cyanobacterial extracts.

Fig. 167. Chromosome fragment at anaphase.

Fig. 168. Ring chromosome and fragment at anaphase.

Fig. 169. Scattered movement at anaphase.

Fig. 170. Misorientation at anaphase.

Fig. 171. Shift in MTOC during anaphase.

Fig. 172. Hyper condensed chromosomes at anaphase.

Fig. 173. Disturbed anaphase.

Fig. 174. Disturbed early anaphase with laggards.

Fig. 175. Disturbed spindle organization at one pole.

Fig. 176. Disturbed anaphase with bridges.

Fig. 177. Disturbed anaphase and fragments in a polyploid cell.

Fig. 178. Diagonal anaphase.

Fig. 179. Diagonal late anaphase.

Fig. 180. Diagonal anaphase with a fragment.

Fig. 181. Diagonal pulverized anaphase.

Fig. 182. Stathmo anaphase.

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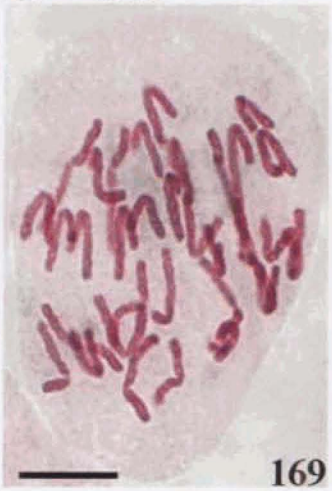
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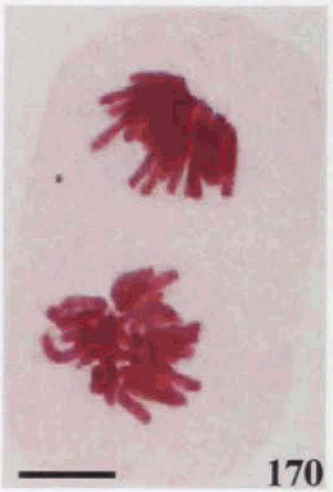
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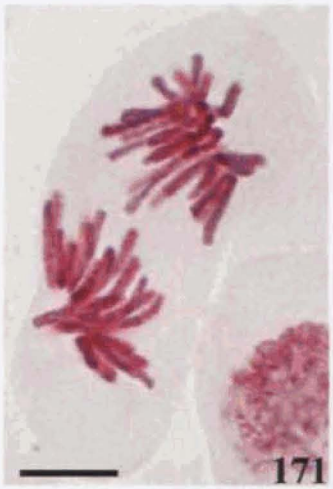
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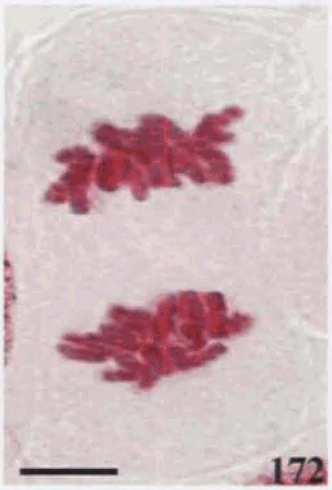
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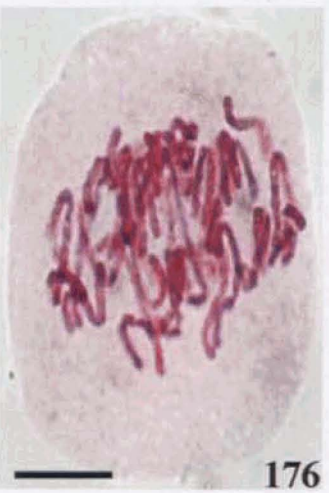
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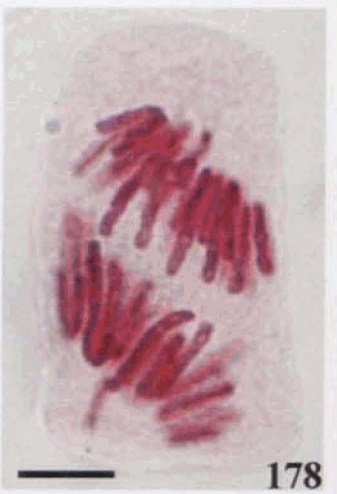
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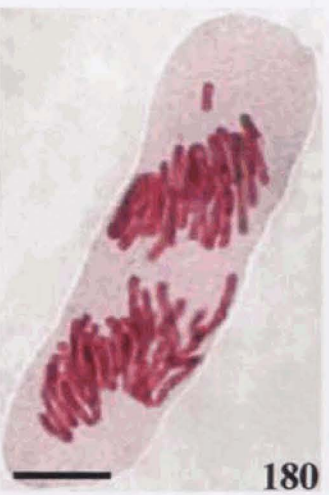
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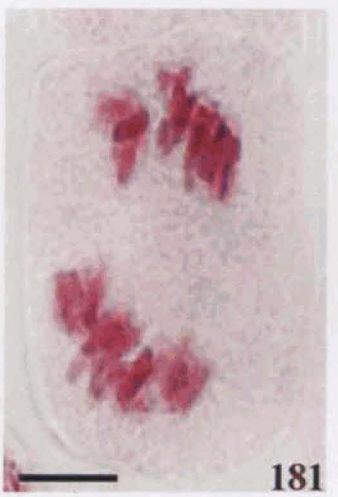
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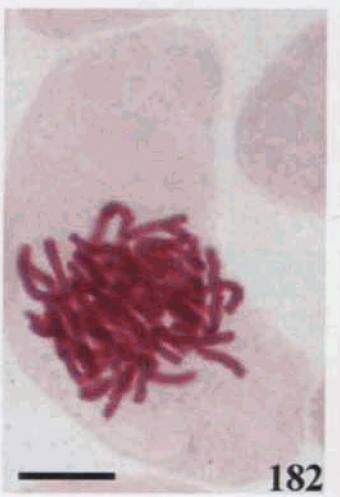
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PLATE - 19

Figs. 183-198. Various cytological aberrations at anaphase scored in *Allium cepa* L. root tip meristem after treatment with cyanobacterial extracts.

Fig. 183. Sticky stathmo anaphase.

Fig. 184. Stathmoanaphase – pulverized.

Fig. 185. Pulverized anaphase - scattered movement.

Fig. 186. Pulverized anaphase with a laggard.

Fig. 187. Pulverized anaphase with multiple bridges.

Fig. 188. Sticky early anaphase.

Fig. 189. Sticky late anaphase.

Fig. 190. Sticky anaphase showing early movement.

Fig. 191. Clumped sticky anaphase.

Fig. 192. Sticky diagonal anaphase.

Fig. 193. Diagonal anaphase showing coagulation/adhesion of chromosomes.

Fig. 194. Deproteinized chromosomes and a laggard.

Fig. 195. Deproteinized chromosomes and abnormal grouping at late anaphase.

Fig. 196. Deproteinized chromosomes showing multipolar movement.

Fig. 197. Bizarre anaphase.

Fig. 198. Bizarre anaphase.

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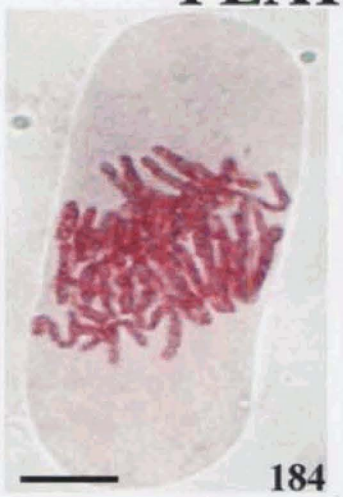
183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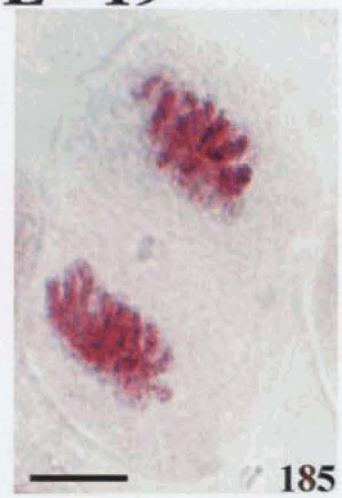
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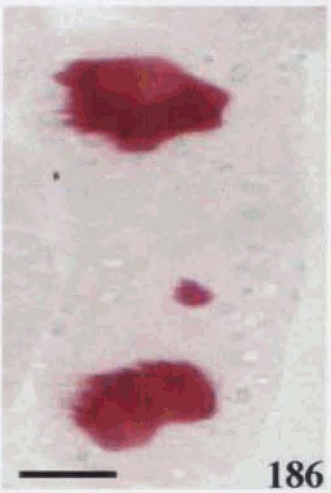
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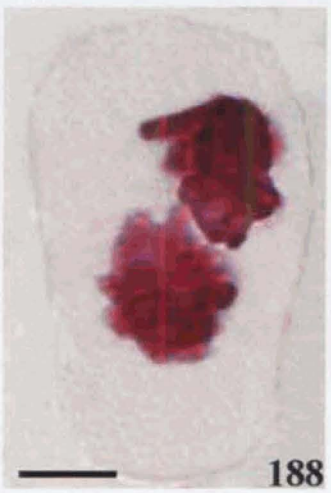
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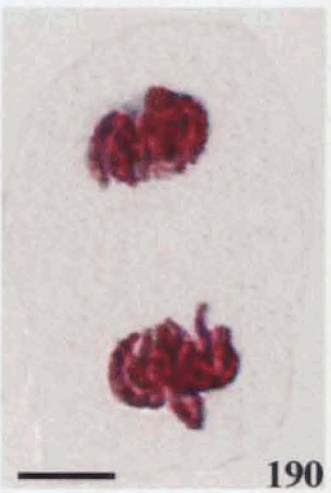
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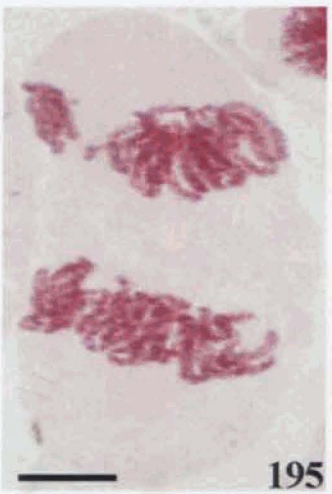
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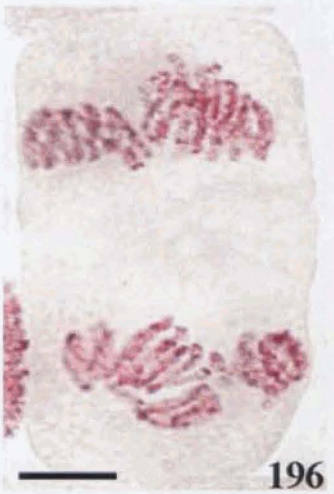
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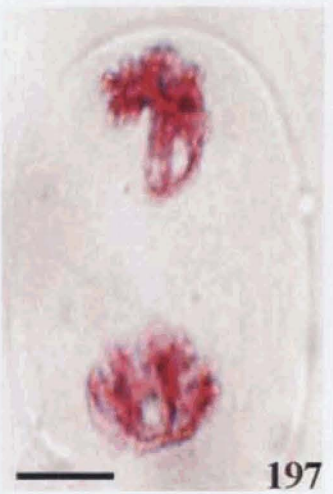
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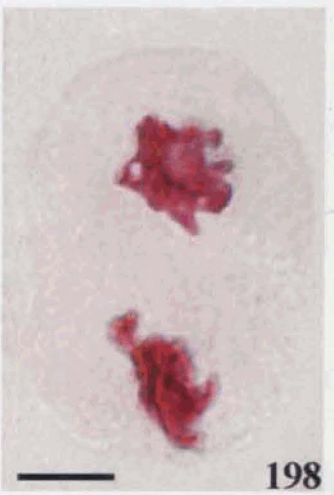
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PLATE - 20

Figs. 199-202. Various cytological aberrations at telophase (199-201) and cytokinesis (202) cored in *Allium cepa* L. root tip meristem after treatment with cyanobacterial extracts.

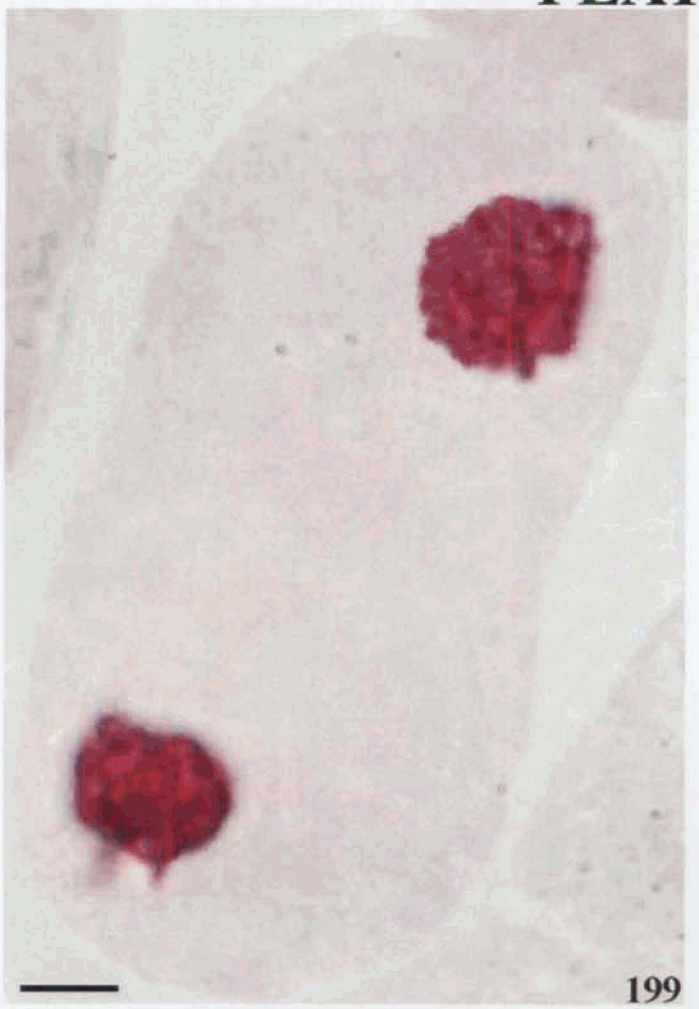
Fig. 199. Diagonal sticky telophase in a giant cell.

Fig. 200. Stellate telophase in a giant cell.

Fig. 201. Hypercondensed chromosomes at telophase in a giant cell.

Fig. 202. Hypercondensed chromosomes during cytokinesis in a giant cell.

PLATE - 20



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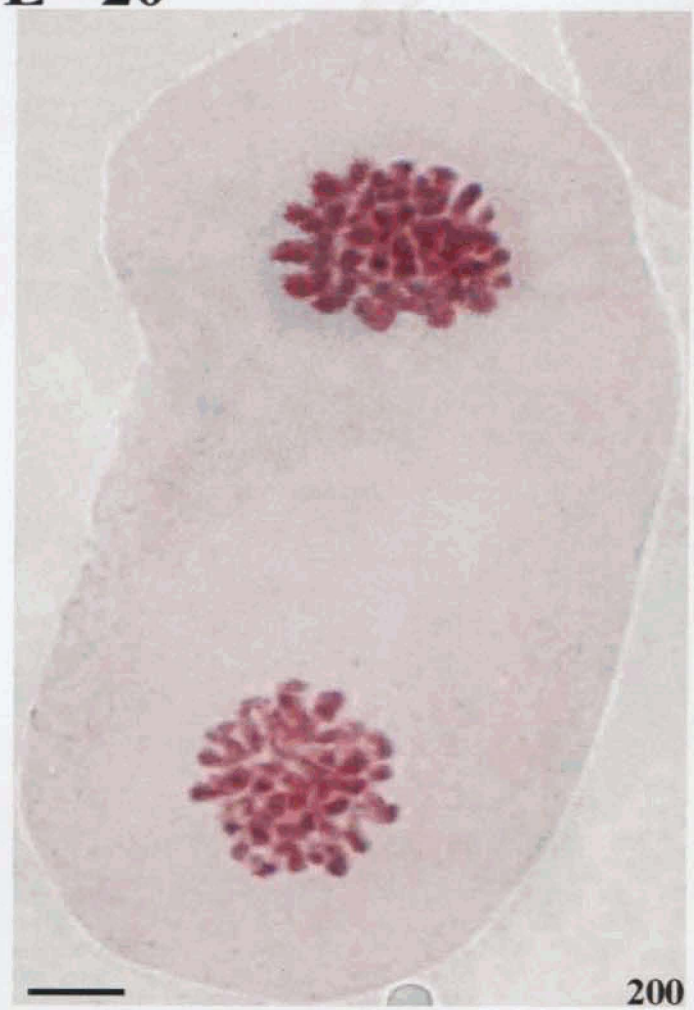

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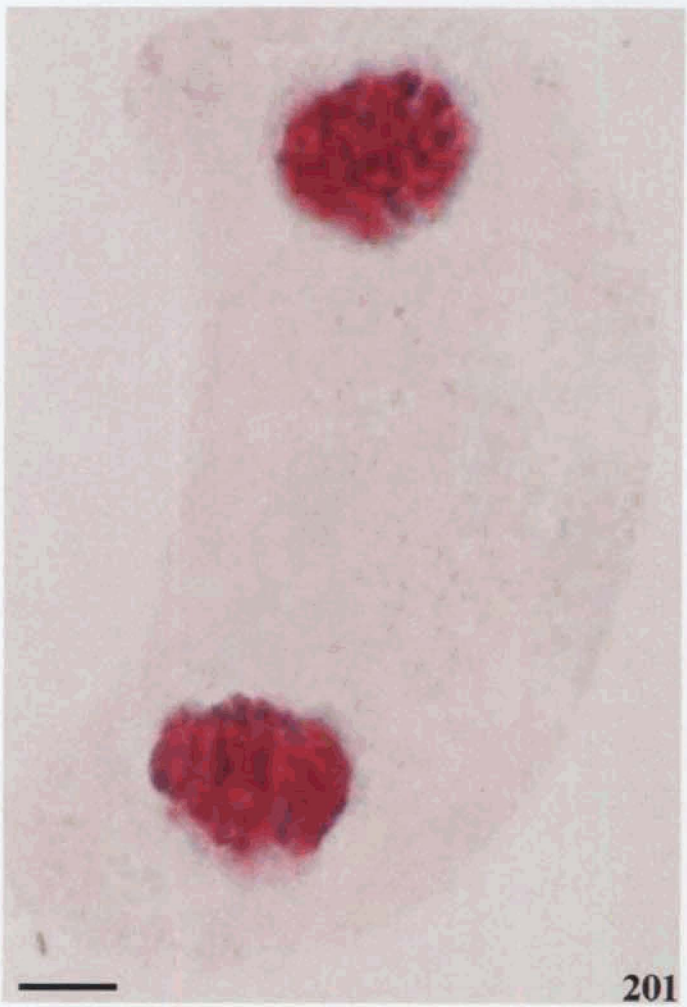

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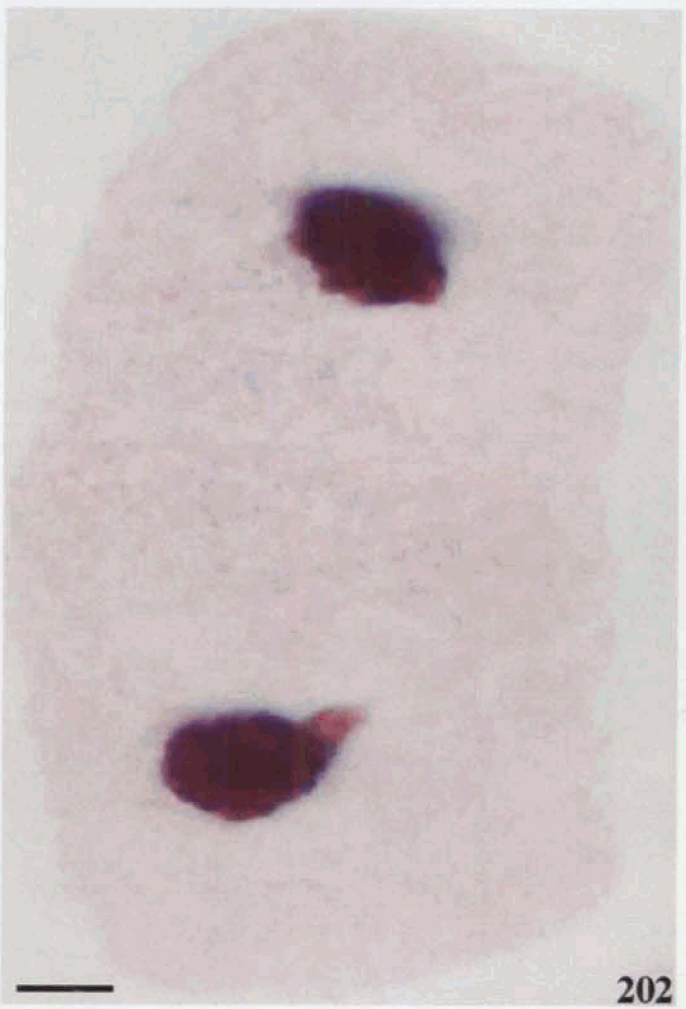

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PLATE - 21

Figs. 203-218. Various cytological aberrations at telophase (203-213) and cytokinesis (214-218) scored in *Allium cepa* L. root tip meristem after treatment with cyanobacterial extracts.

Fig. 203. Diagonal telophase.

Fig. 204. Equatorial separation at telophase.

Fig. 205. Stellate telophase.

Fig. 206. Condensed diagonal telophase.

Fig. 207. Extremely sticky telophase.

Fig. 208. Misorientation and extreme stickiness at telophase.

Fig. 209. Pulverization of chromosomes at late telophase.

Fig. 210. Abnormal grouping of chromosomes at telophase.

Fig. 211. Bizarre telophase.

Fig. 212. Bizarre telophase.

Fig. 213. Bizarre telophase.

Fig. 214. Stickiness during cytokinesis.

Fig. 215. Fragmentation of daughter nucleus during cytokinesis.

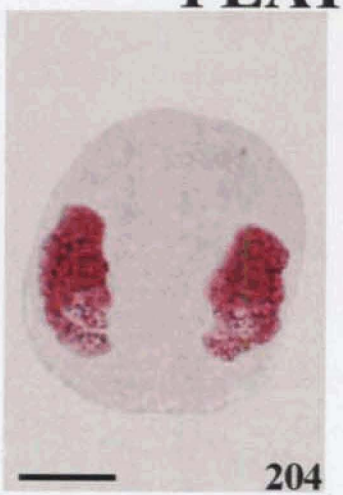
Fig. 216. Unequal division at cytokinesis.

Fig. 217. Macro and micro cells.

Fig. 218. Hyper condensed chromosomes during cytokinesis.

PLATE - 21


203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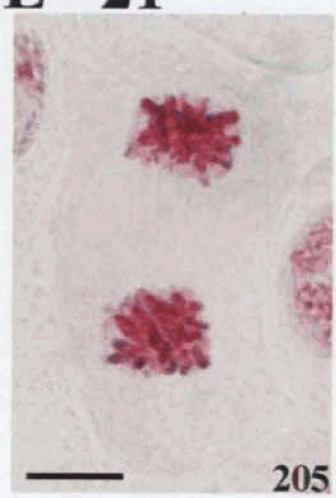
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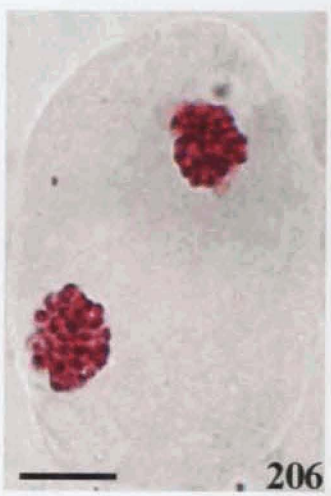
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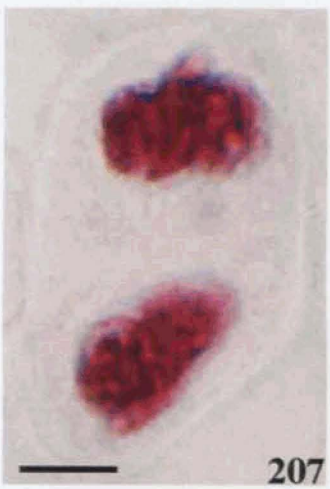
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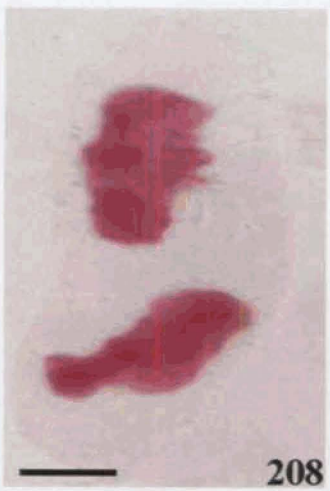
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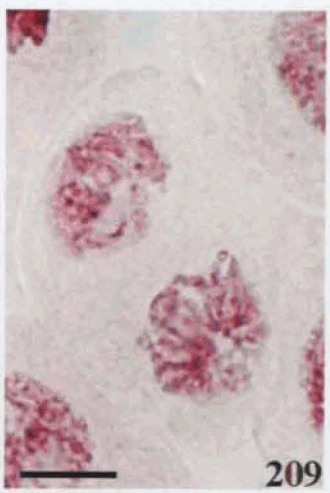
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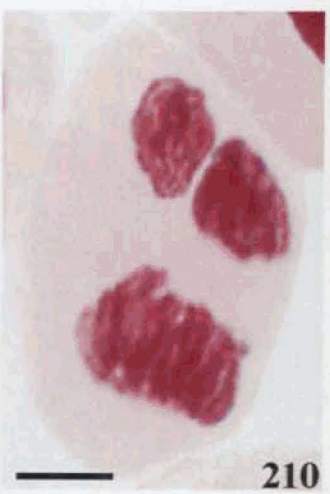
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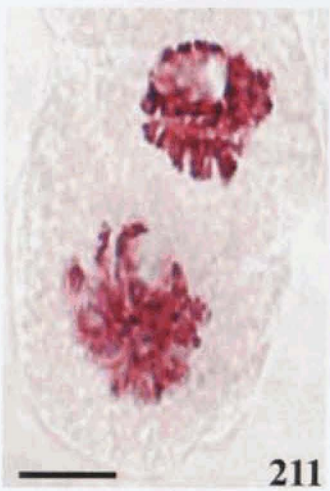
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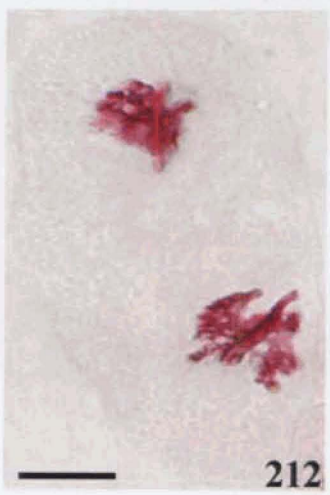
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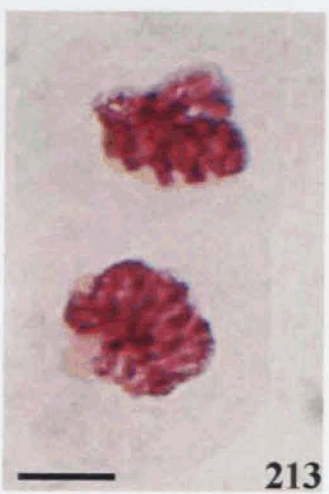
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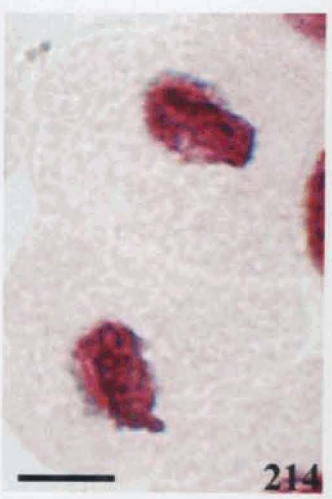
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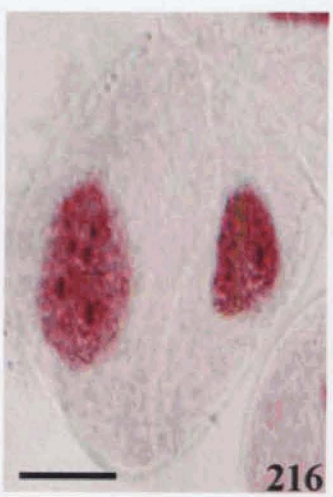
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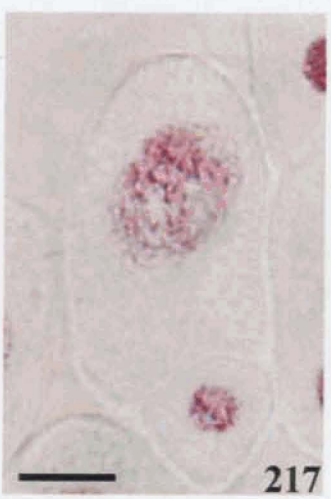
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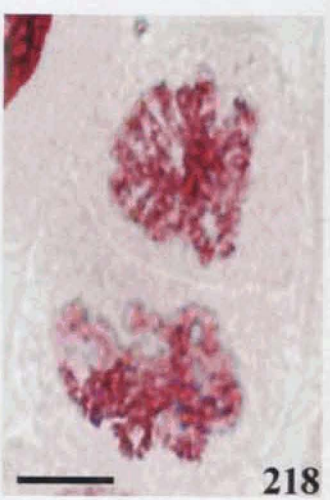
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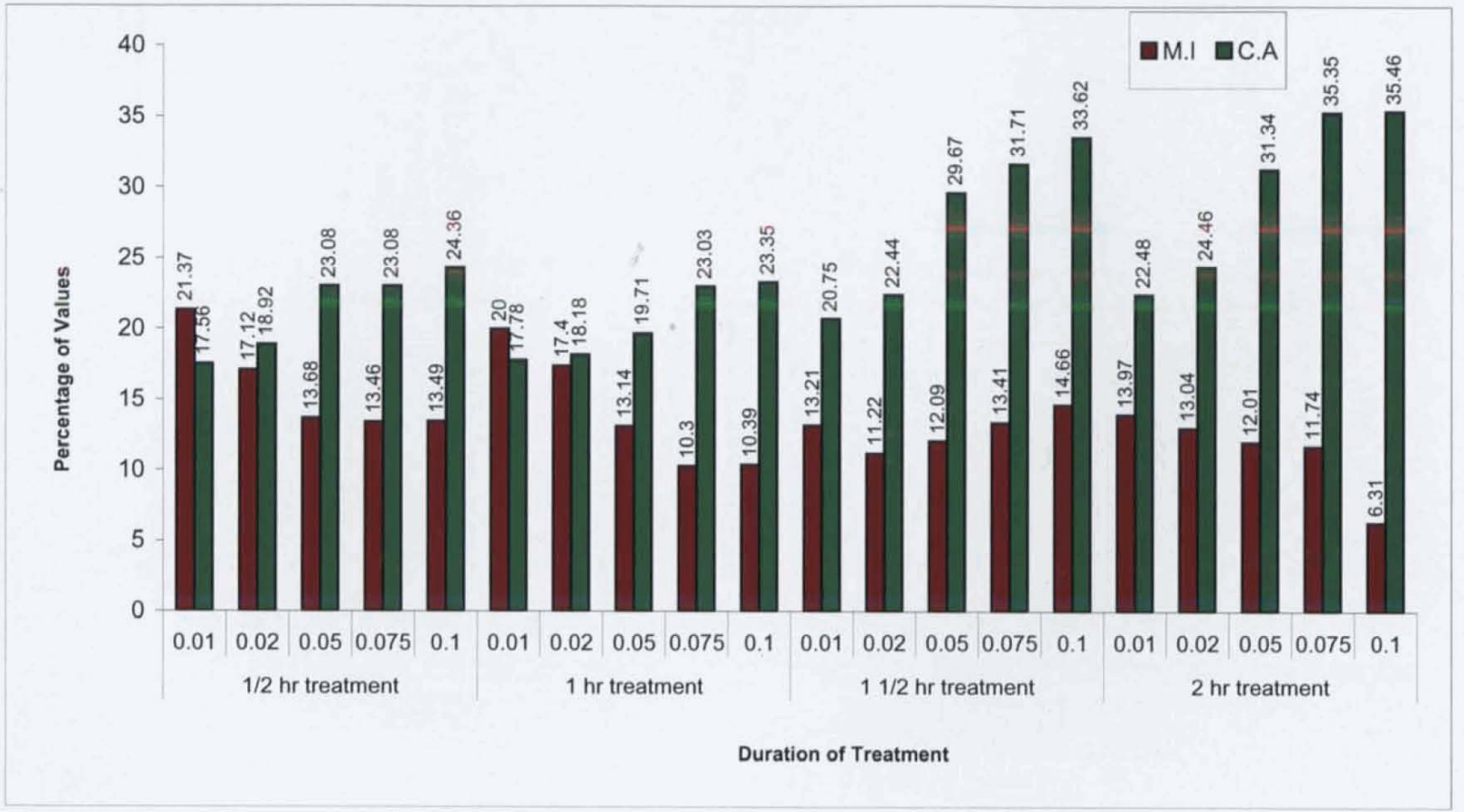
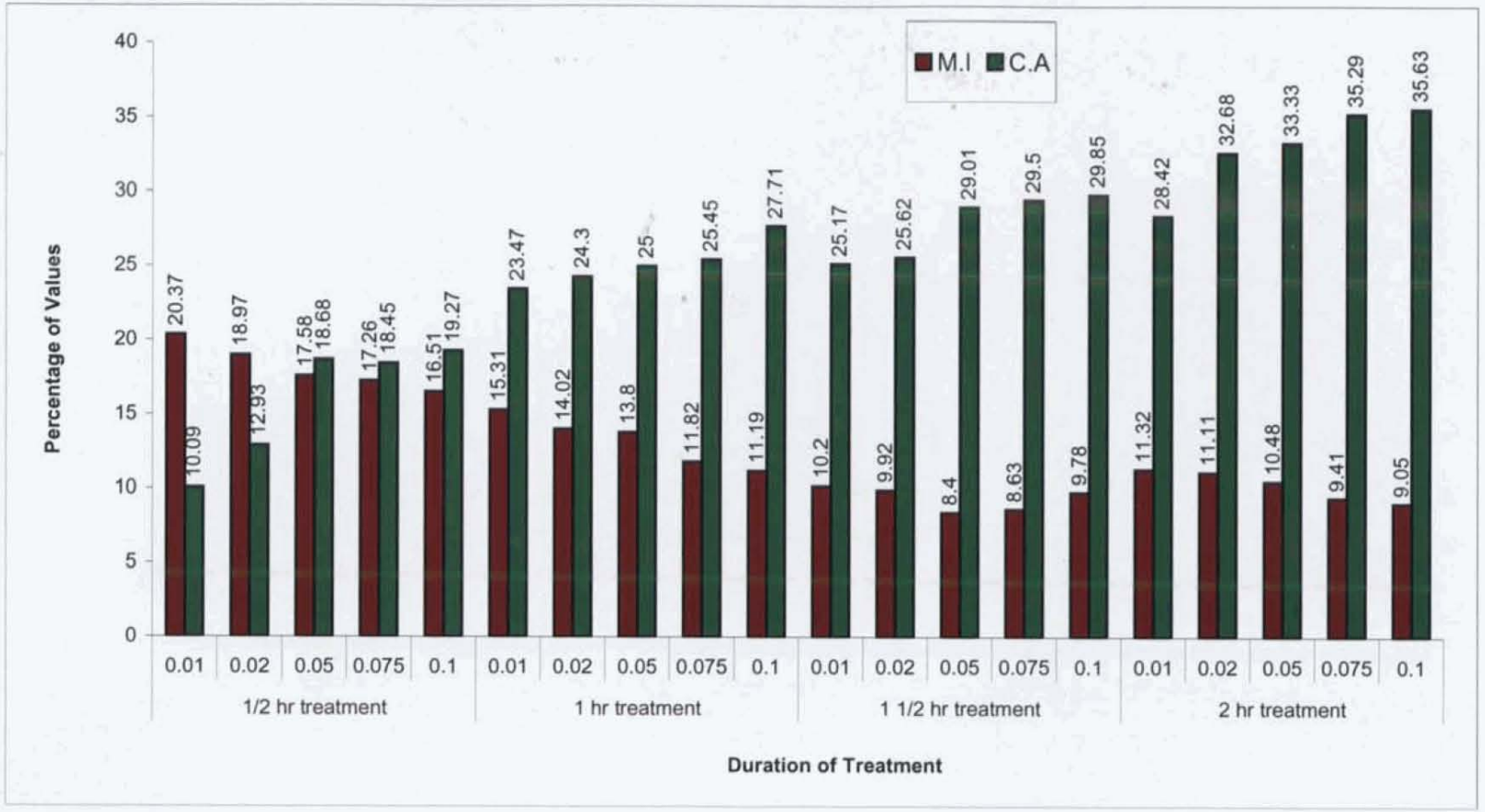
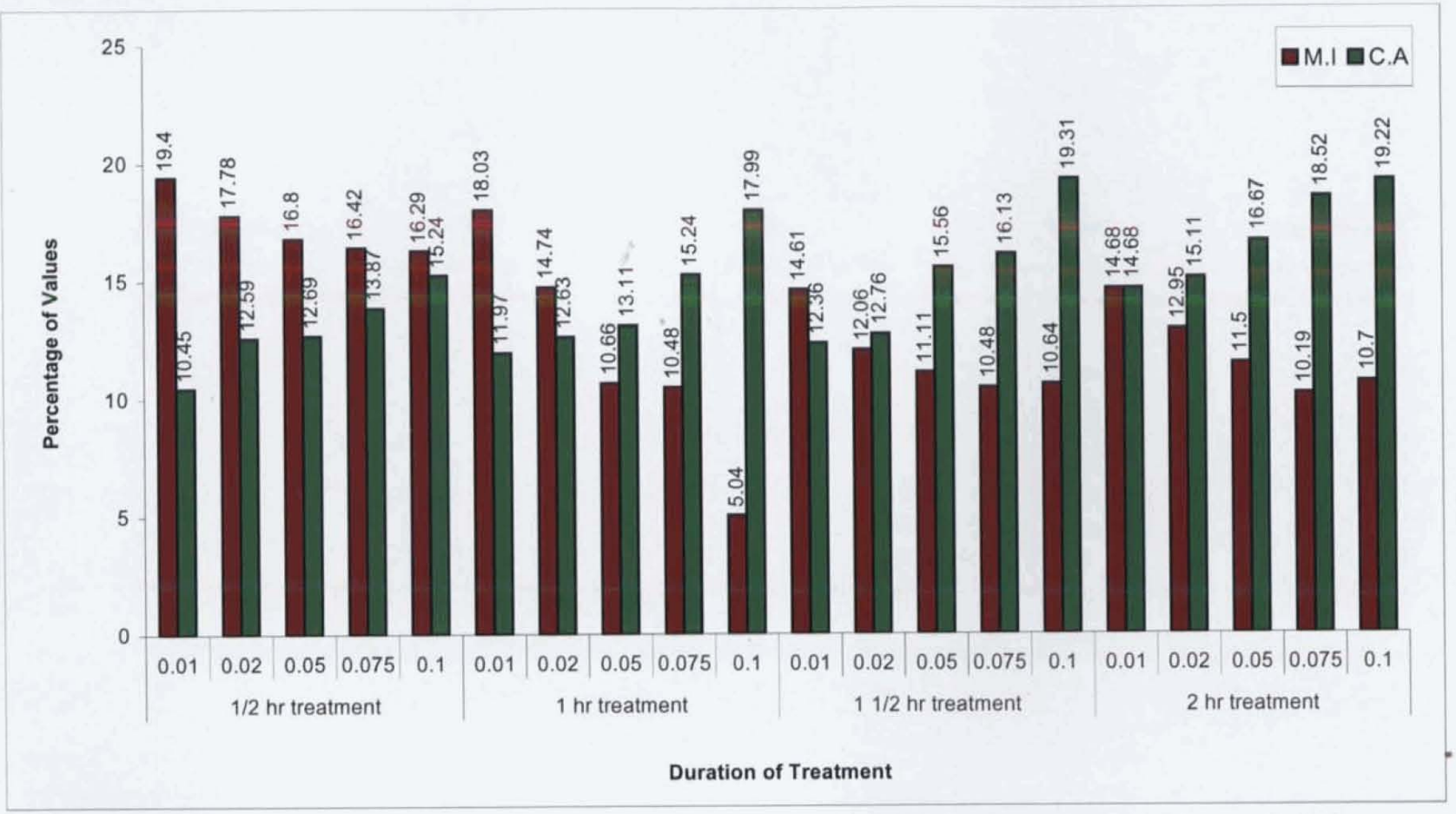
Graph 1: **Comparison of mitotic indices and percentage of cellular abnormalities induced by various concentrations of the extract of *Anabaena circinalis* var. *crassa* Ghose during different treatment durations in *Allium cepa* L. root tip meristem**



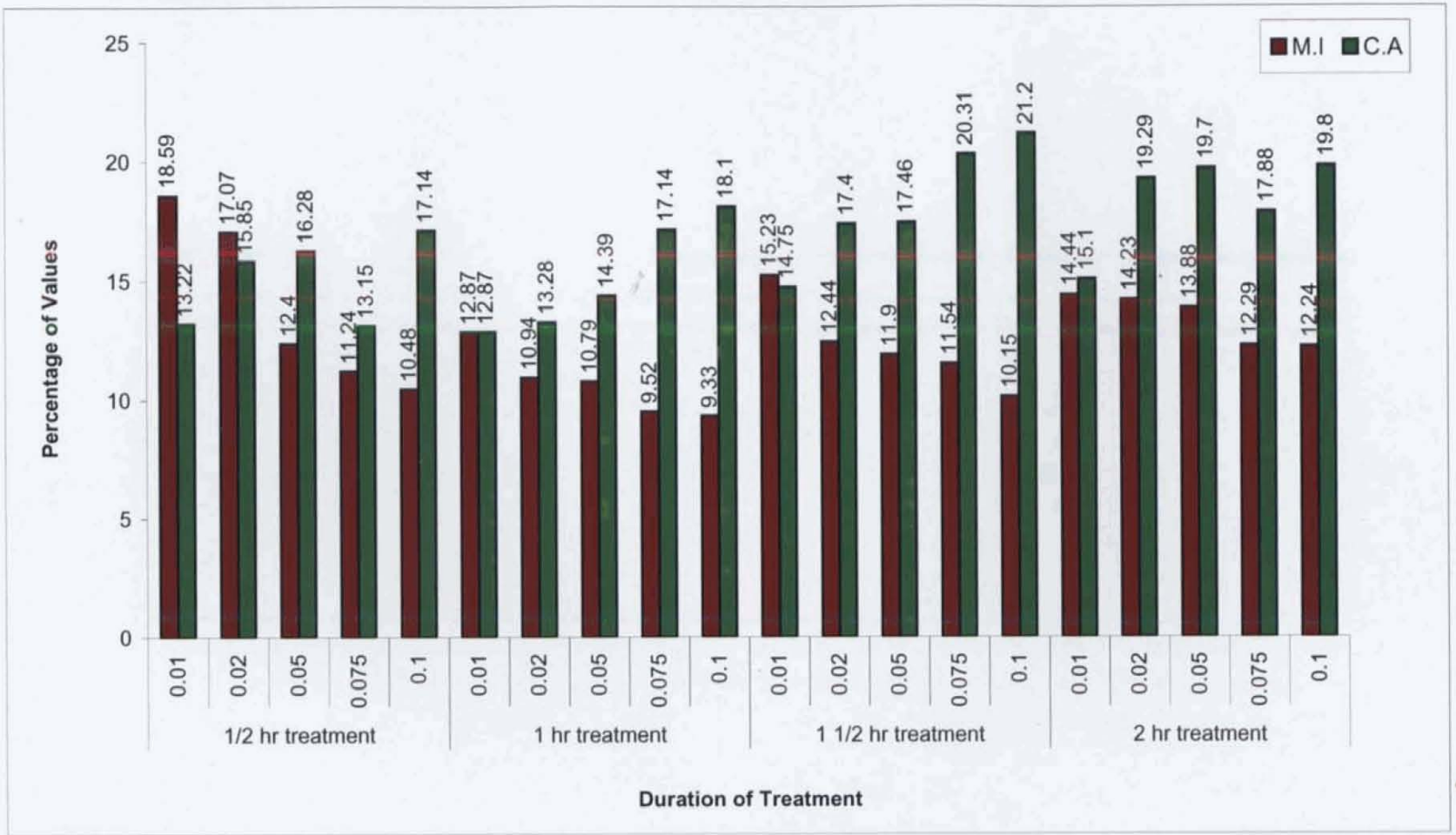
Graph 2: **Comparison of mitotic indices and percentage of cellular abnormalities induced by various concentrations of the extract of *Microcystis aeruginosa* Kütz during different treatment durations in *Allium cepa* L. root tip meristem**



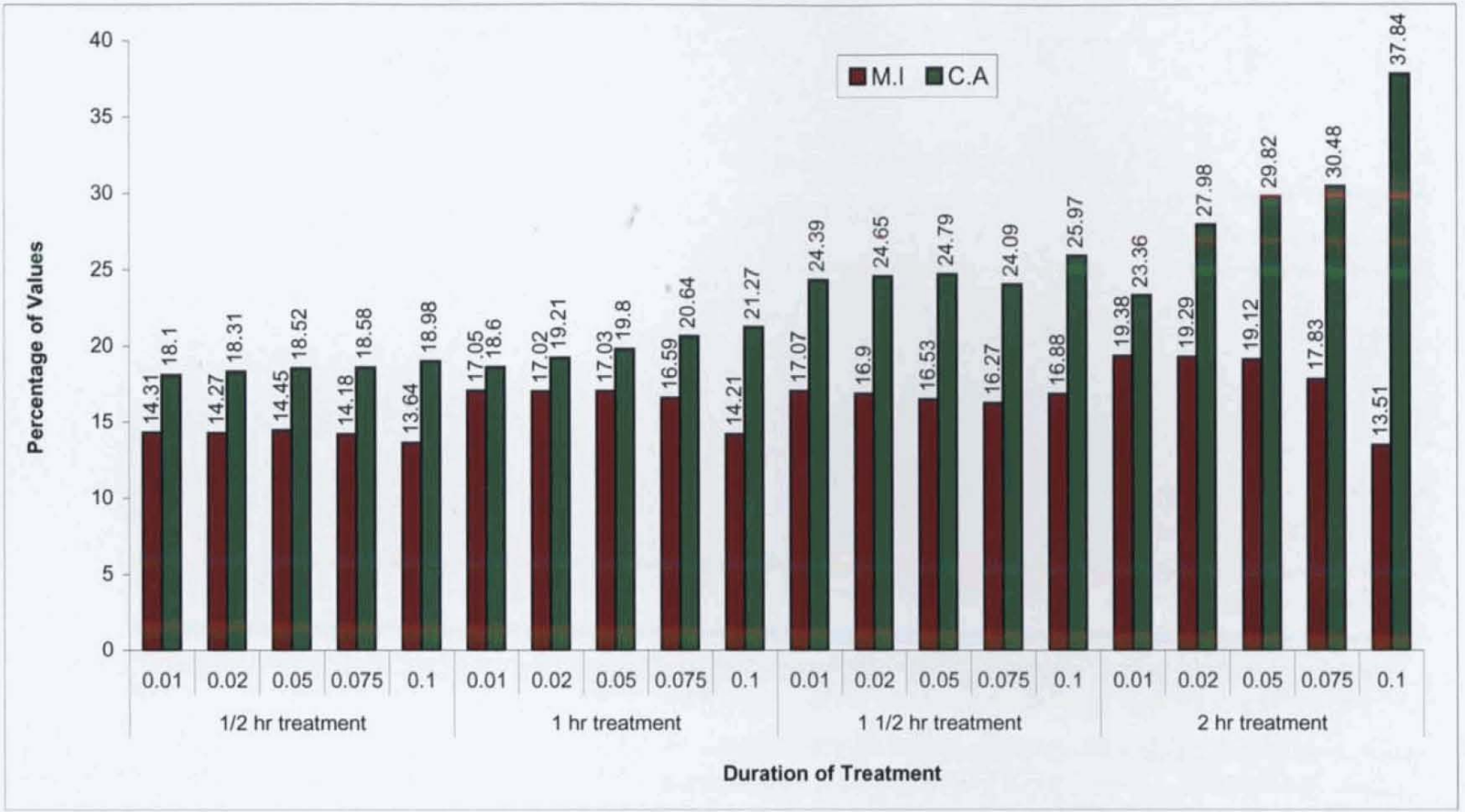
Graph 4: **Comparison of mitotic indices and percentage of cellular abnormalities induced by various concentrations of the extract of *Nostoc commune* Vaucher ex Born. *et* Flah. during different treatment durations in *Allium cepa* L. root tip meristem**



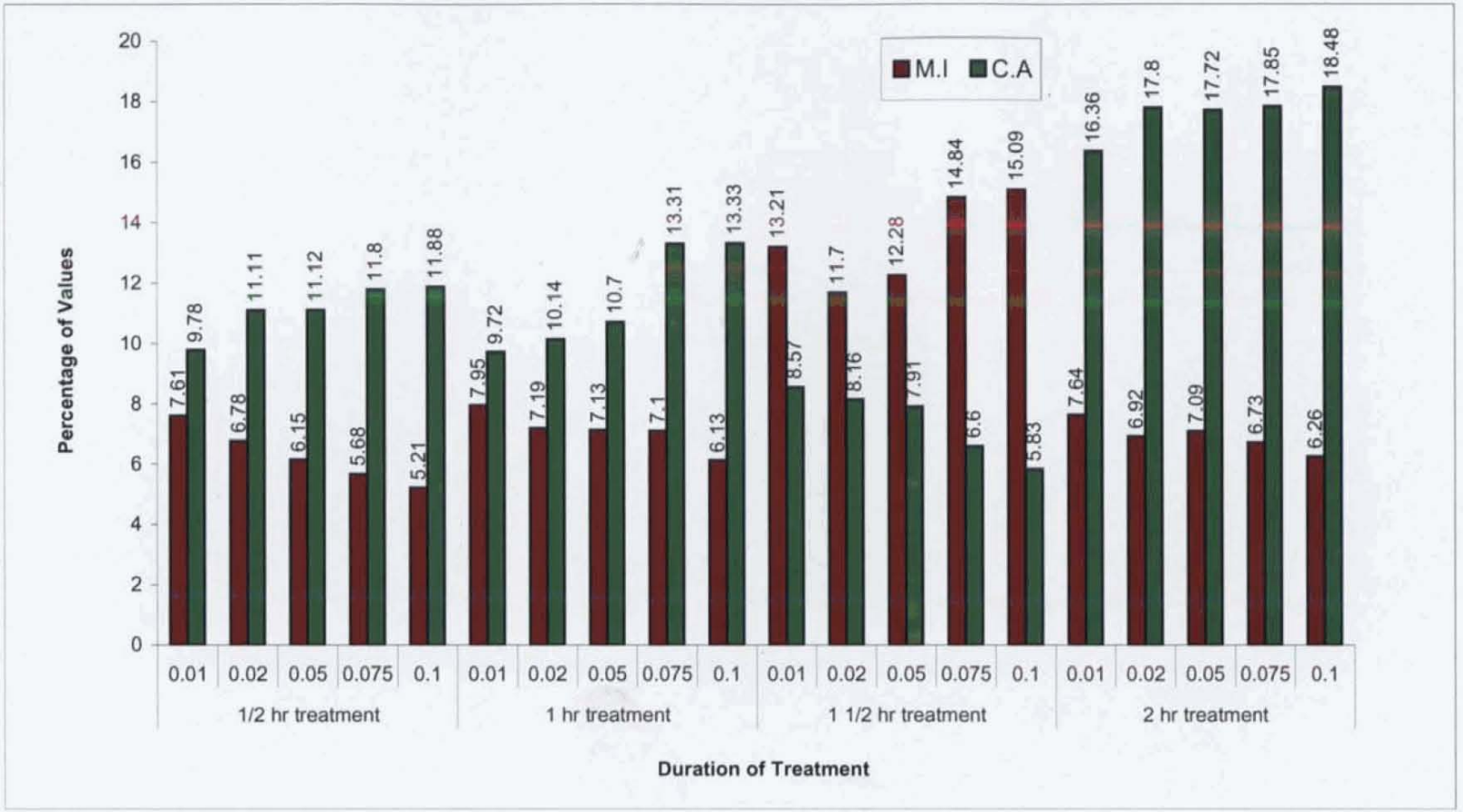
Graph 5: **Comparison of mitotic indices and percentage of cellular abnormalities induced by various concentrations of the extract of *Nostoc spongiaeforme* C. Agardh ex Born. *et* Flah. during different treatment durations in *Allium cepa* L. root tip meristem**



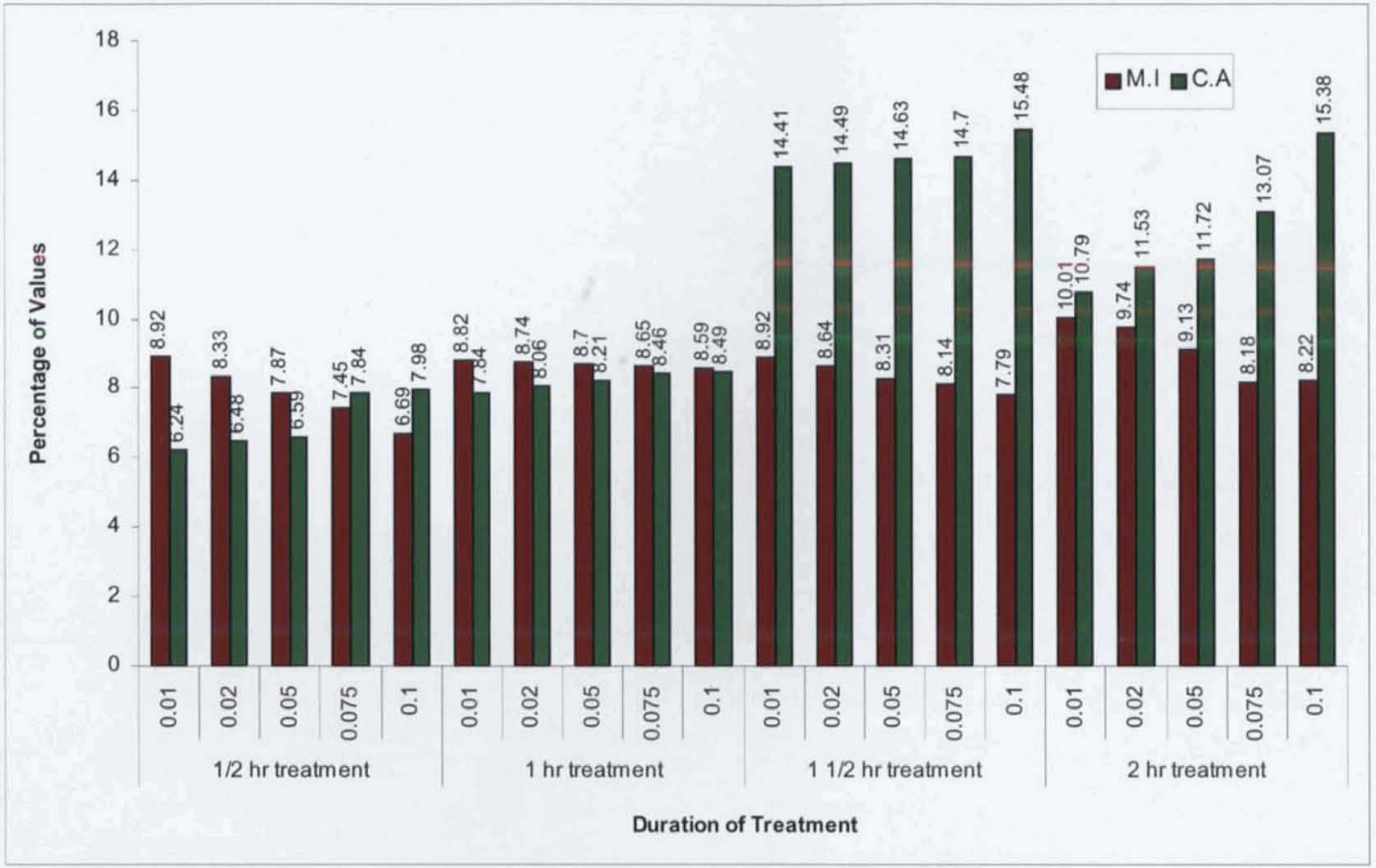
Graph 6: **Comparison of mitotic indices and percentage of cellular abnormalities induced by various concentrations of the extract of *Oscillatoria obscura* Brühl *et* Biswas during different treatment durations in *Allium cepa* L. root tip meristem**



Graph 7: **Comparison of mitotic indices and percentage of cellular abnormalities induced by various concentrations of the extract of *Oscillatoria princeps* Vaucher ex Gomont during different treatment durations in *Allium cepa* L. root tip meristem**



Graph 8: **Comparison of mitotic indices and percentage of cellular abnormalities induced by various concentrations of the extract of *Phormidium tenue* (Menegh.) Gomont during different treatment durations in *Allium cepa* L. root tip meristem**



DISCUSSION

Characterization of cyanobacteria

In the present investigation, eight cyanobacterial taxa were characterized from environmental and cultured samples, which are as follows:

1. *Anabaena circinalis* var. *crassa* Ghose
2. *Microcystis aeruginosa* Kütz.
3. *Nostoc carneum* C. Agardh ex Born. *et* Flah.
4. *N. commune* Vaucher ex Born. *et* Flah.
5. *N. spongiaeforme* C. Agardh ex Born. *et* Flah.
6. *Oscillatoria obscura* Brühl *et* Biswas
7. *O. princeps* Vaucher ex Gomont
8. *Phormidium tenue* (Menegh.) Gomont

The traditional taxonomy of cyanobacteria is based on units, which represent stable morphotypes well recognizable in samples from nature, which occur repeatedly in time and sometimes in very distant localities (in the similar ecological situations). The concept of species as far as cyanobacteria are concerned is a controversial subject (South and Whittick, 1987). Anand (1989) analyzed cyanobacterial taxonomy and commended that the present systems of identification of cyanobacteria at species level are often leading to confusion. Identification of cyanobacteria has proven problematic due to the extensive phenotypic plasticity (Kondo *et al.,* 2000). Phenotypic plasticity is the process by which a single genotype is able to produce different phenotypes when environmental conditions change. *In vitro* culturing of the eight cyanobacterial taxa revealed several interesting features. Strains belonging to the genera *Nostoc* have a profuse growth over agar surface than in liquid media. *Nostoc commune* grow adherent to the surface of the culture vessels and rarely formed submerged or floating colonies. This observation

indicates that the ability of certain strains of cyanobacteria to grow attached to the support or the cyanobacterial epiphytism. The non heterocystic forms viz., *Microcystis aeruginosa, Oscillatoria obscura, O. princeps* and *Phormidium tenue* also have a profuse growth over agar surface than in liquid media. *In vitro* culturing is of utmost importance in the elucidation of plasticity, since they provide the opportunity to work with genetically and environmentally homogeneous populations (Kondo *et al.*, 2000). So the characterization of both natural and *in vitro* samples made in the present study seems to be of great significance.

The identification of cyanobacterial species was made by the conventional methods based on morphological characters. Numerous stable morphotypes (traditional "species") exist in nature, which are ecologically restricted and recognisable one from another. In this study the species were identified only when conspicuous morphological characters were observed. Accordingly only eight species could be recognized. The present study reveals the dominance of eight species belonging to five genera namely *Anabaena, Microcystis*, *Nostoc, Oscillatoria* and *Phormidium* in fresh water blooms present in water reservoirs, fresh water ponds and paddy fields. They belong to two orders viz., Chroococcales and Nostocales. The species were well characterized phenotypically. Morphological characters especially nature of thallus (if colonial - form of colony, size of cells, arrangement of cells), position, size, shape and number of heterocysts, akinetes, spirality, width of trichome *etc.* were considered for taxonomic characterization.

The conventional method to identify species is based on morphological characters. Since cyanobacteria vary in morphological characters especially in thallus nature, position, size, shape and number of heterocysts and akinetes, they can be considered for taxonomic characterization. The diversity at generic level is more easily distinguishable than at species level. The

characters at higher ranks are well demarcated, but as it goes down to the species level the delimitation becomes feeble and rather impractical due to the lack of precise qualitative characters (Umamaheswari, 2005).

A survey of blue-green algae conducted by Anand and Hopper (1987) revealed the identification and description of thirty taxa occurring in rice fields in Kerala State (India). The taxa included unicellular, non-heterocystous filamentous, heterocystous filamentous and heterotrichous forms. The reports of Dominic and Madhusoodanan (1996, 1999) revealed the existence of cyanobacteria in acidic environments. Umamaheswari (2005) reported a wide range of cyanobacterial diversity within the order Nostocales in the rice fields of Kerala. Thus these earlier studies reveal the abundance of cyanobacterial flora that flourishes in the paddy fields and adjacent areas of Calicut University Campus. Instead of the taxonomic revision of cyanobacterial flora, in the present study a special stress has been given to the characterization of cyanobacterial proliferations, also known as blooms of cyanobacteria, which occurred during the months of January to May in the aquatic ecosystems near Calicut University and in a water reservoir at Calicut city.

Cytotoxicity of cyanobacteria

Cyanobacteria are known to produce a wide variety of bioactive substances including antialgal, antifungal, antibiotic and antiviral activities associated with cytotoxic, neurotoxic or hepatotoxic action (Kreitlow *et al.*, 1999; Legrand *et al.*, 2003). In the present investigation cytotoxic assays were conducted with the extracts of the species of the genera, *viz.*, *Anabaena, Microcystis, Nostoc, Oscillatoria* and *Phormidium*. Potential toxin producing genera identified among cyanobacteria are *Anabaena*, *Aphanocapsa, Hapalosiphon, Microcystis, Nostoc* and *Oscillatoria* (Carmichael, 2001). The concept of usage of higher plants as a first-tier assay system for detecting the

effects of chemical mutagens is a potentially viable method. The use of plant tissue (primarily root tips) for studying the induction of chromosomal aberrations is one of the oldest, simplest, most reliable and inexpensive methods available. Chemicals and extracts which cause chromosome aberrations in plant cells are also capable of producing chromosome aberrations in animal and human cells. Moreover, the aberrations are identical in all organisms. Studies have shown that compounds which have a C-mitotic effect on plant cells have the same effect on animal cells. It is recommended that plant systems can be accepted as a first-tier assay system for the detection of possible genetic damage by environmental chemicals (Grant, 1978).

The present study revealed that the treatment of *Allium cepa* root meristem with five different concentrations (0.01%, 0.02%, 0.05%, 0.075% and 0.1%) of crude extracts of eight cyanobacterial species such as *Anabaena circinalis* var. *crassa*, *Microcystis aeruginosa*, *Nostoc carneum*, *N. commune*, N. *spongiaeforme*, *Oscillatoria obscura*, *O. princeps* and *Phormidium tenue* are capable of producing a wide spectrum of abnormalities. However, normal mitotic stages were observed in control treatments (Figs. 13-18). Meristematic cells treated with the eight cyanobacterial extracts revealed a dose- and time-dependent decrease of mitotic indices. The observations of the present study are a clear indication of clastogenic and non-clastogenic property of the cyanobacterial extracts, which is evident from the direct action on the chromosomes and manifestation of spindle abnormalities. The lowering of the mitotic index may be due to inhibition of DNA synthesis at S-phase (Sudhakar *et al.*, 2001).

The abnormalities observed in the present investigation can be categorized into frequently observed clastogenic abnormalities, rarely observed clastogenic abnormalities, frequently observed non - clastogenic

abnormalities and rarely observed non - clastogenic abnormalities, which are listed below.

Frequently Observed Clastogenic Abnormalities

1. Aberrant and Bizarre Nucleus

In the present investigation, aberrant and bizarre nucleus were observed in various treatments (Figs. 27, 30, 38, 50-53, 55-58, 70, 84, 85; Tables 9-32) of *Nostoc carneum*, *N. commune*, N. *spongiaeforme*, and *Oscillatoria obscura*, *O. princeps* and *Phormidium tenue* extracts.

Aberrant nucleus develops in plants as a result of the nuclear instability induced by the extract on *A. cepa* root tip meristem. Nuclear instability is defined as any deviant nuclear behaviour of producing a nucleus (or nuclei) of abnormal structure, karyotype or behaviour (Bennet, 1981).

Bizarre forms of nuclei include nuclei with aberrant shapes with or without non-nucleolar condensations and swollen nuclear envelopes, nuclear envelope rupture in cells that are in an advanced state of disorganization, multinucleate cells or cells which remain joined in unusual configurations closely resembling division profiles or division conformations (Gruber, 1978).

Normal chromosomal arrangement in the interphase nucleus has two main aspects: (1) arrangement of chromosomes with respect to nuclear polarity and to other nuclear components, and (2) arrangement of chromosomes with respect to one another. The latter aspect consists of two main types of spatial relationships; (a) relationships between different members of one chromosomal set, (b) relationships between different chromosomal sets. Genetic control as well as sub cellular mechanisms which are involved in nuclear organization is responsible for the normal orientation of the interphase nucleus. The ordered arrangement of chromosomes in the

nucleus for the regularity of genetic activity and chromosomal behavior is highly essential during interphase, failing which may lead to the development of abnormal interphase nucleus (Avivi and Feldman, 1980).

The cytotoxicants present in the extracts act as potent inhibitor of cell plate formation in dividing plant cells, leading to the formation of bizarre binucleate cell. Previous studies on living cells reveal that the drug always permits the cell plate to arise and grow normally until about 80% complete, but then causes it to break down. Phragmoplast microtubules arise and align in the interzone, golgi vesicles are produced and aggregate in a line that defines the young cell plate, and considerable fusion of these vesicles occurs to form islands of the cell plate material. However, under the influence of the cytotoxic chemical these islands do not fuse to form the enlarged lamellar expanses characteristic of maturing cell plates. Instead, the partially fused material reverts to small vesicles which appear to become resorbed by the cellular membrane systems. Following the cell plate disintegration, the reformed/malformed nuclei move close together and occupy the central region of the cell, thereby forming extravagant binucleate or multinucleate cells (Hepler and Bonsignore, 1990). This may be the probable reason for scoring aberrant and bizarre nucleus in *Allium cepa*, after treatment with the extracts of *Nostoc carneum*, *N. commune*, N. *spongiaeforme*, *Oscillatoria obscura*, *O. princeps* and *Phormidium tenue* in the present investigation.

2. Bizarre Metaphase, - Anaphase and - Telophase

In the present investigation bizarre metaphase, bizarre anaphase and bizarre telophase were observed in various treatments (Figs. 130-133, 197, 198, 211-213; Tables 1-8, 21-28) with extracts of *Anabaena circinalis* var. *crassa*, *Microcystis aeruginosa*, *Oscillatoria obscura* and *O. princeps*.

Factors which bring about drastic changes in the DNA methylation pattern and histone acetylation can alter the architecture of interphase chromosomes leading to the development of bizarre malformations at the subsequent stages of mitosis (Santos *et al.*, 2002). Induced heterochromatinization due to the influence of toxicants in the environment can also lead to the formation of bizarre shapes and aberrant forms of arrangement during the mitotic stages (Hennig, 1999). This may be the probable reason for scoring bizarre metaphase, bizarre anaphase and bizarre telophase in *Allium cepa* after treatment with the extracts of *Anabaena circinalis* var. *crassa*, *Microcystis aeruginosa*, *Oscillatoria obscura* and *O. princeps* in the present investigation.

3. Nuclear Appendage Formation / Nuclear Budding

Formation of nuclear appendages or buds were observed in various treatments (Figs. 33-35; Tables 1-8, 29-32) of extracts of *Anabaena circinalis* var. *crassa*, *Microcystis aeruginosa* and *Phormidium tenue.*

Naturally occurring nuclear budding, apart from the induced bud or appendage formation occurs as a result of the selective entrapment of extrachromosomally amplified DNA by the nucleus and which can probably end in micronucleation during S phase (Shimizu *et al.*, 1998).

Nuclear buds and vesicles resembling protuberances were observed to bud from the outer membrane of the nuclear envelope *in situ* at certain regions of the nuclear envelope. Probably, these nuclear buds arise as a result of the excessive production of nucleic acids and proteins, induced by the cytotoxicants (Hellgren and Morré, 1992). This may be the probable reason for scoring nuclear appendage / nuclear buds in *Allium cepa* after treatment with the toxic cyanobacterial extracts in the present investigation.

4. Nuclear Lesions and Micro Lesions

Lesions were observed in the nucleus at the prophase stage (Figs. 45, 61-63, 65, 66, 68; Tables 9-12, 25-32) in treatments with extracts of *Nostoc carneum*, *Oscillatoria princeps* and *Phormidium tenue*. Previous reports confirmed the occurrence of nuclear lesions, induced by plant derived chemicals in *A. cepa* root meristem (Mercykutty and Stephen, 1980; Sreeranjini and Thoppil, 2001).

Experimental evidences indicate that various mutagenic agents are capable of inducing microlesions in the nucleus of *Arabidopsis* and the aberration seems to be radical-induced leading to cell death (Overmyer *et al.*, 2005). Mitogen-activated protein kinases and various reactive oxygen species radicals are capable of inducing various kinds of cytological aberrations including nuclear microlesions (Pitzschke and Hirt, 2006).

Recent studies have revealed the fact that nuclear lesions are associated with programmed cell death in plants (Pasqualini *et al.*, 2003). So the wide spread occurrence of nuclear lesions in all the cyanobacterial treatments in the present investigation proclaims the acute cytotoxicity of these extracts.

5. Pulverization

Pulverization was observed (Figs. 46, 47, 69, 79, 80, 107-109, 181,184-187, 209; Tables 1-4, 21-24, 29-32) in the treatments with extracts of *Anabaena circinalis* var. *crassa*, *Oscillatoria obscura* and *Phormidium tenue*.

Apart from the induced condition, chromosome pulverization can also be observed in diseased cells (Sakari *et al.*, 1981). Premature chromosome pulverization (PCP) or 'prophasing' may be caused by a number of chemical, physical and biological agents. The morphology of the "pulverized" interphase nucleus will depend on the phase of the cell cycle in which the

interphase cell was in when exposed to a substance present in the cytoplasm of the metaphase cell leading to "prophasing". Prophasing can occur either as a normal cellular phenomenon occurring prematurely or under abnormal induced circumstances (Sandberg, 1978). This may be the probable reason for scoring nuclear pulverization in *Allium cepa* after treatment with the cyanobacterial extracts in the present investigation.

6. Dissolution of Chromatin

Chromatin dissolution at interphase and prophase (Figs. 49, 54, 76, 78; Tables 5-8, 21-24, 29-32) was noticed in treatments with the extracts of *Microcystis aeruginosa*, *Oscillatoria obscura* and *Phormidium tenue*. Dissolution of chromatin was noticed earlier by (Matsumoto, 1988). Studies conducted on the structural changes of pea chromatin due to *in vitro* or *in vivo* treatment with Aluminium showed that chromatin might get condensed and / or aggregated due to the probable sticky nature of the chromatin or may get dissolved. The results suggested that Al absorbed by pea roots is somehow related to alteration of the chromatin structure, *i.e.* its condensation and / or aggregation as well as dissolution. The conclusion reached was that Al toxicity leads to disturbance of the nuclear activity (Matsumoto, 1988). In the present study probably the toxins present in the cyanobacterial extracts may be responsible for the dissolution of chromatin in *Allium cepa*.

7. Abnormal Condensation of Chromatin

Abnormal condensation of chromatin (Figs. 33, 66, 71, 73- 75; Tables 1-32) was noticed in treatments with the extracts of all the eight cyanobacterial species in the present investigation. Abnormal chromatin structure and condensation behavior during the different stages of cell cycle may be expressed as irregular compaction along the chromosome length and chromatin stickiness, which may lead to irregularities in chromosomes at all

stages of cell division (Sosnikhina *et al.*, 2003). This may be the probable reason for scoring abnormal condensation of chromatin in *Allium cepa* after treatment with the cyanobacterial extracts in the present investigation.

8. Abnormal Condensation of Chromosomes

Abnormal condensation of chromosomes was noticed in treaments with the extracts of *Microcystis aeruginosa*, *Nostoc carneum*, *N. commune*, *Oscillatoria princeps* and *Phormidium tenue* (Figs. 86, 88, 108, 122, 172, 201, 202, 206, 218; Tables 5-16, 25-32). Differential condensation of chromosomes may be due to the improper heteropycnosis or due to the quantitative changes in the heterochromatin content present in the cells (Verma, 1988). Abnormal condensation of chromosomes may be due to premature condensation of chromatin during prophase (Sperling and Rao, 1974). In the present study probably the toxins present in the cyanobacterial extracts may be responsible for abnormal condensation of chromosomes in *Allium cepa*.

9. Giant Cells

In the present investigation giant cells were observed in various treatments (Figs. 67-70, 119-126, 159-166, 199-202; Tables 13-20, 29-32) with extracts of *N. commune*, *N. spongiaeforme* and *Phormidium tenue*. Giant cells can be induced either by physical means or by employing chemicals, which are capable of affecting the cell cycle especially in the 'S' phase. Here the cell division is completely arrested and cell expansion seems to be generated and as a result the cells become large giant cells. The frequency of giant cells seems to be increased depending upon the dosage and duration of the treatment with cytotoxic agents (Verma and van Huyste, 1971). More over, it had been experimentally proved that the giant cells are subjected to various kinds of stress and normally they cannot cope with the enormous size

of the protoplasm that they are liable for injury (Menzel, 1988). This may be the probable reason for scoring giant cells in *Allium cepa* after treatment with the cyanobacterial extracts in the present investigation.

Nuclear migration was noticed in the giant cells after treatment with the extracts of *Nostoc commune* and *N. spongiaeforme* during the present investigation. Nuclear migration towards the periphery of the cell is caused by the abnormal spatial arrangement of the participating microtubules. Aberrant protein fibres in the vicinity of the nucleus, disturbs the normal orientation of the nucleus. Disoriented establishment of the cytoskeleton seems to be involved in nuclear migration. It is suggested that interactions between the microtubule organizing center (MTOC), the nuclear envelope and areas of the plasma membrane are functional in the formation, orientation and localization of the nucleus and associated microtubule-microfilament complex (Meindl, 1985). This may be the probable reason for nuclear migration in *Allium cepa* after treatment with the toxic cyanobacterial extracts in the present investigation.

10. Stickiness of Chromosomes

Stickiness of chromosomes were observed in various treatments of (Figs. 87, 100, 103, 104, 122, 134, 137, 140, 143, 150, 152, 183, 188-192, 199, 207, 208, 214; Tables 1-32) all the eight species of cyanobacterial extracts in the present investigation. Stickiness of chromosomes as well as chromatin therefore, is interpreted as entanglement of chromatin fibers between unrelated chromosomes, probably caused by abnormal condensation behaviors that occur either prior to mitosis or during mitotic stages. Presumably, chromatin breaks would occur when sticky chromosomes or chromatin separate. This breakage occurs either during anaphase, telophase or cytokinesis (Mc Gill *et al.*, 1974).

During cytokinesis, the cell cytoplasm of higher plants is partitioned by the construction of a new cell wall inside the cell. It is the spindle, normally, which determines where as well as when partition occurs. All higher plant spindles lack centrosomes. Mitotic and meiotic plant spindles and many animal meiotic spindles are initiated from the chromosomes or after nuclear envelope breakdown (Baskin and Cande, 1990; Waters and Salmon, 1997). The spindles assemble around the mass of chromosomes and initially appear as poorly organized, often multipolar, structures (Yu *et al.*, 1999). The improper functioning of these multipolar structures associated with the daughter chromosomes seems to be responsible for the abnormal stickiness during cytokinesis in the treated cells (Caetano-Pereira and Pagliarini, 2001). This may be the probable reason for stickiness in *Allium cepa* root tip meristemsatic cells after treatment with all the cyanobacterial extracts in the present investigation.

11. Chromosome Fragments

In the present investigation chromosome fragments (Figs. 110-112, 115, 124, 156, 161, 165, 167, 168, 177, 180; Tables 1-8, 21-24) were observed in the treatment with the extracts of *Microcystis aeruginosa*, *Anabaena circinalis* var. *crassa* and *Oscillatoria obscura*. Physical and chemical agents are capable of producing chromatid and chromosome breaks and other nuclear aberrations. These aberrations were produced by the influence of the destructive physical and chemical mutagens, and the secondary structural effects may be perhaps due to spatial mixing of the toxic components of the extract with the nuclear components and consequent interference of the nucleic acid cycle of the nucleus (Newcomer and Wallace, 1949; Lebedeva and Chubykin, 1975).

These chromosome breaks are realized before the prometaphase and by the beginning of the prometaphase the fragments are randomly distributed

within the volume of the nucleus. At the prometaphase most fragments move from the equator to the pole of the cell and thus at the metaphase and anaphase are found to be located outside the equatorial plate. Possibly, the process of development of breaks is also not yet completed by this time, it continues and is completed at the metaphase, partially and also at the anaphase of the mitosis. This may be the probable reason for scoring chromosome fragments both at metaphase and anaphase of mitosis in *Allium cepa*, after treatment with the toxic cyanobacterial extracts in the present investigation.

Experimental studies conducted with environmental mutagens on root tips of *Vicia faba* produces structural chromosome changes especially, chromosome breaks. A tendency of chrosome breaks is that it tends to be concentrated in heterochromatic segments of the chromosomes (Kihlman, 1957).

12. Chromosome Bridges

Chromosome bridge formation (Figs. 21, 137-146, 153, 160, 176, 187; Tables 1-4, 9-28) is a clastogenic abnormality observed in treatments with the extracts of *Anabaena circinalis* var. *crassa, Nostoc carneum, N. commune, N. spongiaeforme, Oscillatoria obscura* and *O. princeps*. Mitotic bridge may arise due to the formation of dicentric chromosomes by breakage and reunion (Raj and Rao, 1972). Double and multiple bridges occur as a result of fusion between broken chromosomes (Wong Young and Woo Young, 1993). Bridge formation arises due to hydration and dehydration process of spindle and chromosomes (Das *et al.,* 1968). Chromosome bridges arise due to the partial impediment of spindle mechanism inducing incomplete cell walls and their irregular positions (Sawamura, 1964). Anaphase bridges in the root tips of *Allium cepa* involving one or more chromosomes is due to stickiness of chromosomes at metaphase stage. This was also observed in the mitosis of

Vicia faba after treatment with organophosphorous insecticides (Amer and Farah, 1983).

Chromosome bridges may be due to chromosomal stickiness and subsequent failure of free anaphase separation or may be due to unequal translocation and inversion of chromosome segments (Najjar and Soliman, 1980). Single, double or triple bridges are due to breaking up of chromosome by proximal chromatid reunion (Grant, 1978) and also due to general stickiness of chromosomes (Abraham and Koshy, 1979). The anaphase bridges may arise due to the effect of chemical in breaking the protein moiety of nucleo-protein backbone (Patnaik *et al.,* 1984). Here the extracts of cyanobacteria may be probably having similar cytotoxic effect.

Rarely Observed Clastogenic Abnormalities

1. Nuclear Diminution

Diminution of the nucleus was observed in (Fig. 36; Tables 21-24) the treatments with the extracts of *Oscillatoria obscura* in the present investigation.

Several different forms of chromatin loss and chromatin diminution were observed: (1) Chromosome elimination (2) Dramatic reduction in heterochromatin containing the satellite DNA and, subsequently, also of many other chromatin moieties, resulting in the formation of small chromosomes or leading to the formation of comparatively minute sized nucleus (3) Appearance of a large number of very small Feulgen positive chromatin particles often arranged in metaphase-like arrays, suggesting that they were effectively distributed during mitosis or the formation of 'miniature nucleus'. These phenomena of selective loss and destruction of chromatin in response to environmental conditions seems to be induced by the mutagens when compared to other forms of chromatin loss, including developmentally

controlled chromatin diminution in certain animals (Deumling and Clermont, 1989). In the present investigation, the extracts of *Oscillatoria obscura* may be probably having similar cytotoxic effect.

2. Nuclear Disintegration

Disintegration of the nucleus was observed in (Figs. 37, 81; Tables 1-8, 21-24) the treatments with the cyanobacterial extracts of *Microcystis aeruginosa*, *Anabaena circinalis* var. *crassa* and *Oscillatoria obscura* in the present investigation. Changes in the viscocity of the protoplasm brought about by stress or after exposure to toxic chemicals may lead to nuclear deformation. The root tip nuclear DNA was found to be deformed or degraded after treatment with the cytotoxicants, followed by the disintegration of the nucleus (Liu *et al.*, 2000). Similar results were also observed in potatoes (*Solanum tuberosum*) and it was found that nuclear damage occurred in the root tip cells, followed by nuclear degradation, which seems to be induced after exposure to the cytotoxicant (Richardson *et al.*, 2001). In the current study, the extracts of *Microcystis aeruginosa*, *Anabaena circinalis* var. *crassa* and *Oscillatoria obscura* may be probably having similar cytotoxic effect.

3. Nuclear Extrusion

Extrusion of chromatin from the nucleus was observed in (Figs. 40, 41, 77; Tables 1-4) the treatments with the extracts of *Anabaena circinalis* var. *crassa* in the present investigation. Nuclear extrusion was discovered much earlier in young *Allium* scales by Wu (1955a, b). Systematic investigation on this phenomenon was carried out with various kinds of microscopic techniques and plant materials to collect more effective evidence to clarify the debate about whether the nuclear extrusion is an artifact or a normal event. Nuclear extrusion occurs either in the growing part of plant abnormally,

induced by toxic substances or in senescent tissue as a normal event. Various kinds of chromosome aberrations have been considered to be closely associated with chromatin extrusion. The nuclear material traverses the nuclear membrane barrier by vigorous contraction and expansion and they may simultaneously extrude out, but often asynchronously migrate from one cell to another. The involvement of cytoplasmic constituents in chromatin extrusion and migration was also detected.

During the process of nuclear extrusion, nucleoli containing vesicles from the nucleus gets leaked into the cytoplasm with the occasional release of the nucleoli and chromatin into the cytoplasmic stream. This phenomenon may develop particularly during stress conditions or in critical phases of development (Martini and Ronchi, 1977). The above mentioned phenomena may be responsible for the occurrence of nuclear extrusion in *A. cepa* after treatment with the cyanobacterial extract.

4. Cytomixis

This aberrant phenomenon was observed in (Fig. 19; Tables. 29-32) the treatments with the extracts of *Phormidium tenue* in the present investigation. Extrusion of chromatin from one cell into the cytoplasm of an adjoining cell is termed as cytomixis. Migration of chromatin has also been reported in somatic cells (Bowes, 1973; George and Geethamma, 1985). The factors responsible for cytomixis are rather ambiguous. Some possible causes attributed to cytomixis are cell response as a consequence of pesticides and antibiotic dosages (Kumar and Sinha, 1991), abnormal genetic behaviour due to treatment with a chemical mutagen (Kumar and Srivastava, 2001; Kumar and Sharma, 2002; Bhat *et al.*, 2006).

Cytomixis in the present investigation may be attributed to abnormal genetic behaviour due to treatment with toxic cyanobacterial extracts. With

the increase in extract concentration, the frequency of cells that showed chromosomal stickiness and cytomixis increased. According to Kaul (1971), some chemicals, which cause stickiness of chromosomes, may be responsible for cytomixis. Failure to find chromosomal stickiness and cytomixis in control plants confirms this view. This may be the probable reason for cytomixis in *Allium cepa*, after treatment with the extracts of *Phormidium tenue* in the present investigation.

5. Nuclear Fragmentation

Fragmentation of the nucleus was observed in (Figs. 42-44, 215; Tables 17-24, 29-32) the treatments with the extracts of *Nostoc spongiaeforme*, *Oscillatoria obscura* and *Phormidium tenue* in the present investigation.

According to Caffaro *et al.* (1982), nuclear fragmentation and related cytological chimeras may be induced as an amitosis event, which probably leads to cell death. Chemical-induced cell death was accompanied by the characteristic features of apoptosis in cells, such as typical changes in nuclear morphology, the fragmentation of the nucleus and DNA fragmentation (De Jong *et al.*, 2000).

The induction of nuclear fragmentation is primarily associated with formation of certain intra-nuclear structures, similar to 'apoptotic bodies' associated with fragmentation of the nuclear DNA into small fragments (Houot *et al.*, 2001). The cytotoxic assays with the cyanobacterial extracts may be probably having similar cytotoxic effect on *A. cepa*.

6. Hyperchromasia

Hyperchromasia was observed in (Fig. 48; Tables 21-24) the treatments with the extracts of *Oscillatoria obscura*.

Hyperchromasia is the most distinguishable state of aberration, where the interphase nucleus takes up intense stain than normal, probably due to abnormal heterochromatinization. Hyperchromasia is an extremely condensed and thereby deeply staining state of interphase nucleus observed during stress induced by the influence of toxic environmental chemicals or during incompatible conditions. Progressive heterochromatinization seems to be responsible for this aberration (Gernand *et al.*, 2005).

Studies conducted by Ning *et al.* (2001) in maize root meristematic tissues that were exposed to cytotoxic reagents, revealed several characteristic patterns of nuclear and chromosomal condensations. DNA cleavage was found to occur before condensation and disorganization of the nucleus, followed by deformation and condensation of metaphase chromosomes, as well as marginalization of chromatin in the interphase nucleus. Finally, nucleoli disappeared and fragmentation of the nucleus occurred. The nuclei shows intense staining pattern, termed as hyperchromasia, due to over condensation and compaction with heterochromatin. This may be the probable reason for hyperchromasia in *Allium cepa* after treatment with the toxic extracts of *Oscillatoria obscura* in the present investigation.

Extremely condensed heterochromatin leading to hyperchromasia had also been observed in cultured tobacco cells after treatment with toxic chemicals (Houot *et al.*, 2001).

7. Nuclear Erosion

Nuclear erosion is observed in the root meristematic cells of *Allium cepa* after treatment with the extract of *Microcystis aeruginosa* (Fig. 64; Tables 5-8). Sharma (1980) attributed chromatin erosion to the partial dissolution of nucleoproteins.

The normal organization of chromatin in the nucleus and chromosome segregation is genetically controlled (Franklin and Cande, 1999). Under the influence of stress or due to the action of environmental mutagens, chromatin degeneration occurs, which may become visible in the nucleus as degeneration / erosion zones (Caetano-Pereira *et al.*, 1999).

8. Contorted Chromatin

Chromatin was found to be contorted or twisted in (Fig. 72; Tables 9-12) the treatments with the extracts of *Nostoc carneum* in the present investigation. Experimental evidences obtained from an angiospermic plant, *Xerophyta villosa* reveals that, nuclei with dense contorted chromatin develop at the time of destruction of the plant by dessication, either natural or induced (Hallam and Luff, 1980).

Certain spindle poisons not only affect the mitotic apparatus, but also cause marked proliferation of the nuclear envelope, increase in nuclear size and contortion. Contorted chromatin is a prominent nuclear anomaly, detected together with cytoplasmic compartmentalization and unusual associations of several organelles after treatment with toxic chemicals (Walne, 1967). In the present investigation treatment of the *Allium cepa* root meristem with the aqueous extracts of the cyanobacterium, *N. carneum* leads to a physiological condition similar to that mentioned above, which was confirmed by the occurrence of contorted chromosomes.

9. Ring Chromosomes

Ring chromosome was noticed in different treatments of *Allium cepa* with the extract of *Phormidium tenue* (Figs. 97, 98, 168; Tables 29-32). Induction of ring chromosomes suggests the possibility of two breaks that occur in the same chromosome. Sax (1940) opined that the two breaks that occur in the same chromosome after the process of rejoining may form a ring

chromosome. Raghuvansi and Singh (1976) attributed the formation of ring chromosomes due to telomeric losses. In the present investigation, the cytotoxic chemicals present in the extract of *P. tenue* act directly on the fragile sites of the chromosomes and lead to the brekage at the terminal regions and the reunion of the raw ends of the chromosomes so as to form a ring.

10. Coagulation / Adhesion of Chromosomes

This abnormality is an after effect of chromosome stickiness, where the chromosomes seems to be adhering to form an intact mass of aberrant chromosome group (Figs. 105, 193; Tables 29-32). It was observed in the present investigation after treatment of *Allium cepa* root meristem with the extract of *Phormidium tenue.* The substrate adhesion molecules (SAM) have been implicated in numerous aspects of cell behavior, including adhesion of chromosomes (Epstein, 1986). In such reunited chromosomes, the chromosome breaks at the point of adhesion (Jennings, 1935). Adhesion of chromosomes suggests that changes have occurred in the viscosity of their constituent materials. It has frequently been assumed that such changes in viscosity are due to depolymerization of deoxyribonucleic acid (Hollaender, 1954). The 'stickiness' of chromosomes is seen in cells that are in division at the time of treatment. The adhesion of chromosomes may lead to failure of complete cell division or at times it may affect divisional stages partially (Duncan, 1977). This may be the probable reason for adhesion / coagulation of chromosomes in *Allium cepa* after treatment with the toxic extracts of *Phormidium tenue* in the present investigation.

11. Chromosome Gaps

Gaps in the chromosomes was observed in the present investigation after treatment of *Allium cepa* root meristem with the extract of *Microcystis*

aeruginosa and *Oscillatoria obscura* (Figs. 112, 124; Tables 5-8, 21-24). Experiments conducted on *Allium cepa, Hordeum vulgare* and *Secale cereale* reveal severe chromosomal aberrations in their root tip cells after exposure to a potent mutagen, LSD. Aberrations occurred in the form of chromatid and isochromatid breaks / gaps with most of these breaks / gaps failing to rejoin. The distribution of chromosome breaks was not uniform over the length of chromosomes, and a majority of the breaks were localized at the centromeric regions. These results are the outcome of the clastogenic activity of the mutagen, acting upon the fragile sites, distributed at random on the chromosomes (Sadasivaiah *et al.*, 1973; Star, 1970). In the present study, the extracts of cyanobacteria may be probably having similar cytotoxic effects. Experimental studies conducted with environmental mutagens on root tips of *Vicia faba* produces structural chromosome changes especially, chromosome breaks. A tendency of chromosome breaks is that it tends to be concentrated in heterochromatic segments of the chromosomes (Kihlman, 1957).

12. Telosome Formation

Telosome formation was observed (Fig. 115; Tables 1-4) in diploid and polyploid cells after treatment of *Allium cepa* root meristem with the extract of *Anabaena circinalis* var. *crassa*. Taz1 protein is a component of telomeric chromatin regulating proper telomere maintenance. Certain mitoclastic substances have been shown to directly bind to the telomeric DNA to form abnormal protein arrays and looped structures termed telosomes or t-loops. The mitoclastic molecules bound to the telomeric regions, initially results in the formation of telosomes, which may sometimes lead to fission or breakage of a telomeric chromosome fragment (Tomaska *et al.*, 2004). In the present work, the extracts of the cyanobacterium, *A. circinalis* var. *crassa* may be probably having similar cytotoxic effects.

Mutation affecting certain genes which control the telomerase pathway in chromosomes may lead to an alternative lengthening of telomeres, probably enhancing the chances for the formation of telosomes, followed by deletion (Iyer *et al.*, 2005).

13. Convoluted Chromosomes

Convoluted chromosomes were observed (Fig. 116; Tables 29-32) in the present investigation as a result of the treatment of *Allium cepa* root meristem with the extract of *Phormidium tenue*.

Genetic instability may arise either by incompatible situations, viz., interspecific hybridizations in nature or occasionally induced instability of genetic material, which leads to several malformations that include convolution and weakness of the chromosomes (Gerstel and Burns, 1967). The shape and molecular structure of chromosomes can deviate from normal, under the influence of abnormal conditions of induction and infection by harmful biological, physical and chemical agents (Diaz and Pavan, 1965). Here the extracts of the Cyanobacterium, *Phormidium tenue* may be probably having similar cytotoxic effects.

14. Deproteinized Chromosomes

Deproteinized chromosomes were observed (Figs. 118, 194-196; Tables 5-8, 29-32) in the present investigation after treatment of *Allium cepa* root meristem with the extracts of *Microcystis aeruginosa* and *Phormidium tenue*. Certain carcinogenic as well as mutagenic chemicals are capable of inducing chromosomal aberrations. The major effect is found when the treatment affects the S phase, which may lead to an array of drastic effects including deproteinization of chromosomes. Other defects include production of chromatid and chromosome-type aberrations — both fragments and exchanges. The aberrations are localized in a non random fashion along the

length of the genome. The control of cell growth was affected by genetic alterations brought about by these chemicals (Vig, 1979).

Cyanide was found to be an apoptosis inducer in stoma guard cells from pea leaf epidermis. The nucleus of the guard cells was found to undergo deproteinization of the chromosomes, before the onset of cell death. The type of cell death-via apoptosis or necrosis-is controlled by the level of toxicity induced by the cytotoxicant (Dzyubinskaya *et al.*, 2006). Programmed cell death or apoptosis was found to be induced by phytotoxins in plants as a result of the ultrastructural changes that take place in the nucleus. These changes include an array of aberrations, which includes deproteinization of the chromosomes as well (Wang *et al.*, 1996). Here the extracts of the Cyanobacteria, *Phormidium tenue* and *Microcystis aeruginosa*, may be probably having similar cytotoxic effects.

Frequently Observed Non-Clastogenic Abnormalities

1. Micro Nuclei

Presence of micronucleus (Figs. 20-24, 28, 29, 44, 58; Tables 1-8, 17-24) was observed in *A. cepa* root meristem during treatment with *Anabaena circinalis* var. *crassa*, *Microcystis aeruginosa*, *Nostoc spongiaeforme* and *Oscillatoria obscura* extracts. Micronucleus may originate from a lagging chromosome of anaphase or from a chromosome fragment (Badr and Ibrahim, 1987). The formation of micronuclei may be due to the action of chemicals present in the extract on the spindle apparatus leading to unequal separation of chromosomes at anaphase. The larger group of daughter chromosomes form a larger nucleus and the smaller group form a micronucleus.

Cytotoxically induced detachment of acentric chromatin or acentric chromosomes in the preceding mitosis are responsible for the formation of

micronuclei in the succeeding interphase. These aberrant acentric fragments in the cytoplasm undergoes localization at G (1) resulting in micronucleation by lamin reorganization at S phase (Tanaka and Shimizu, 2000). Experimental evidences prove that miconulcei arise from acentric chromosomal fragments (Heddle and Carrano, 1977). Micronucleation can also result from abnormally induced nuclear budding (Shimizu *et al.*, 1998). Micronucleus formation, progressive heterochromatinization and DNA fragmentation leads to chromosome elimination in the genome (Gernand *et al.*, 2005).

2. Bi-, Tri-, Tetra- and Multinucleate cells

In the present investigation, bi-, tri-, tetra- and multinucleate cells were observed in the root tip meristem of *A. cepa* after various treatments (Figs. 25-32, 41, 59, 60, 117; Tables 1-4, 9-20, 25-28) with extracts of *Anabaena circinalis* var. *crassa*, *Nostoc carneum*, *N. commune*, N. *spongiaeforme* and *Oscillatoria princeps*. In each multinucleate cell, a complete synchrony of nuclear division was maintained throughout the cell division, and chromosome behavior appeared normal up to the metaphase stage. In most dinucleates, chromosome segregation movement was organized in a common spindle, and the daughter nuclei at the telophase appeared to envelope each other in the newly formed nuclear membrane. Trinucleates were similarly enveloped with separate nuclear membranes and possess unequal number of chromosomes. Segregation of the daughter chromosomes into four groups, each surrounded by its own nuclear membrane during late telophase leads to the formation of tetranucleate configurations. In cells with more than four nuclei, chromosomes separated at random but reaggregated into individual daughter nuclei, with no later cytokinesis (Ito and Maeda, 1974). This may be the probable reason for the occurrence of bi, tri, tetra and multinucleate cells in *Allium cepa* after treatment with the aqueous extracts of *Anabaena*

circinalis var. *crassa*, *Nostoc carneum*, *N. commune*, N. *spongiaeforme* and *Oscillatoria princeps* in the present investigation.

Disturbances in the nuclear and microtubular cycles seem to be associated with the formation of heterophasic bi or multinucleate cells (Alberts *et al.*, 1983). The heterophasic cells exhibited asynchronous nuclei at different stages of mitosis. In heterophasic cells (Fig. 59) displaying prophase and interphase nuclei, the mitotic transition is delayed but is ultimately achieved due to the effect of the advanced nuclei, which induces a premature mitotic entry of the lagging nuclei. In some heterophasic cells, metaphase - anaphase transition did not take place simultaneously in different chromosome groups, signifying that the cells do not exit from the mitotic state after anaphase initiation of the advanced nuclei. Asynchronous pace of mitosis of different chromosome groups was also observed during anaphase and telophase. Multinucleate cells and cells with polyploid nuclei exhibit heterophasic condition probably due to the various abnormal configurations of multiple and complex pre-prophase microtubules (Manandhar *et al.*, 1996). The cytotoxic extracts act on mitotic cells in three different ways. (1) Prophase inhibitor (2) Inhibitor of mitotic spindle formation and orientation, compounds being termed as mitoclastic agents and (3) Inhibitor of cell plate and cell wall formation between daughter nuclei resulting in binucleate and multinucleate cells (Ray and Barman, 1987). In the present study the extracts of the Cyanobacteria may be probably having similar cytotoxic effects.

Chromatin bridge like connection between macro and micronucleus (Fig. 21) was observed in the present investigation after treatment of *Allium cepa* root meristem with the cyanobacterial extract of *Anabaena circinalis* var. *crassa*. This may be due to non-disjunction of a pair of daughter chromosomes and simultaneous unequal separation of daughter chromosomes. The larger group forms the macronucleus and the smaller

group forms the micronucleus. Both these types of nuclei remain attached by the persistent chromatin bridge like connection.

3. Clumping of Chromosomes

Clumping of chormosomes was another most frequent non-clastogenic abnormality observed in the treatments (Figs. 89, 106, 126, 191; Table 5-12, 17-28) with extracts of *Microcystis aeruginosa*, *Nostoc carneum*, N. *spongiaeforme*, *Oscillatoria obscura* and *O. princeps* in the present investigation. According to Pritchard and Court (1968), increase in concentration of the cytotoxicant was found to be hastening the onset of clumping. The clumped metaphase is the full effect of C-mitotic agent (Hadder and Willson, 1958). Here the extracts of the cyanobacteria may be probably having similar cytotoxic effects.

4. Ball Shaped Arrangement of Chromosomes

Ball shaped arrangement of chromosomes were observed in the treatments (Figs. 99, 100, 122, 135, 136; Tables 5-8, 17-24) with extracts of *Microcystis aeruginosa*, *Nostoc spongiaeforme* and *Oscillatoria obscura* in the present investigation. Ball anaphase is the stage in mitosis in which sister chromatids separate into a hollow ball of chromosomes that results from the early cleavage divisions in some aberrant cells (Morgan, 2006). The chromosomes may fail to separate at anaphase, and a single nucleus is restored with a double number of chromosomes. Such under-coiled chromosomes have more coils (Darlington, 1958). Here the extracts of the cyanobacteria may be probably having similar cytotoxic effects.

Ball shaped arrangement of chromosomes at metaphase was observed only rarely. This is a form of C-mitosis with characteristically clumped chromosomes. Ball metaphase is followed by either a complete degeneration of the cell or state similar to interphase (Barber and Callan, 1943).

5. C- Metaphase

Experiments reveal that agents inducing C-mitosis give rise to two distinct effects: stathmokinetic and radiomimetic. Toward the time of reversibility of the first of these effects, the second becomes clearly manifested as bridge formation. The appearance of this pathological form is evidently due to disturbance of cell nucleoprotein metabolism during C-mitosis (Boltovskaya *et al.*, 1979).

C-metaphase is detected in the treatments (Figs. 101, 102, 104, 109, 113, 123, 125, 126; Tables 1-4, 13-16, 29-32) with extracts of *Anabaena circinalis* var. *crassa*, *Nostoc commune* and *Phormidium tenue* in the present investigation. The spindle abnormalities lead to C-metaphase and this sometimes even causes the blockage of metaphase (Nagl, 1970). The inhibition of certain specific proteins, the so called 'spindle –proteins' is cytologically manifested as C-mitosis. Levan (1938) termed scattering of chromosomes by spindle inhibition as C-mitosis or colchicine mitosis. The C-mitosis is the secondary effect of prolonged metaphase (Sharda *et al.*, 1973). The C-metaphase is produced as a result of inhibition of spindle fibre formation (El-Khodary *et al.*, 1989). According to Redei (1998) in the event of C- mitosis, the mitotic anaphase gets blocked by the poisonous effect of the cytotoxic chemical and consequently the cell and its progeny may become polyploid. Here the extracts of the cyanobacteria may be probably having similar cytotoxic effects.

6. Stellate Arrangement of Chromosomes

Stellate arrangement of chromosomes is detected in the treatments (Figs. 96, 145-150, 162, 163, 200, 205; Tables 1-4, 9-16, 21-28) with *Anabaena circinalis* var. *crassa*, *Nostoc carneum*, *N. commune*, *Oscillatoria obscura* and *O. princeps* extracts in the present investigation. Stellate

arrangement of chromosome may be due to the clumping of daughter chromosomes into star like structures near the polar region of the cell. Ostergen *et al.* (1953) have described the occurrence of double star anaphase in higher plants. Amer (1965) considered it as being a fore-step of the complete disturbance of the spindle. Here the extracts of the cyanobacteria may be probably having similar cytotoxic effects.

7. Misorientation of Chromosomes

Misorientation of chromosomes were observed (Figs. 113, 119, 127, 128, 157, 164, 170, 208; Tables 13-20, 29-32) in the treatments with *Nostoc commune, N. spongiforme* and *Phormidium tenue* extracts during this study. It may be due to the disturbed functioning of the spindle apparatus. The disturbance can be due to a distortion of the microtubules and microfilaments of the spindle apparatus (Meske and Hartmann, 1995), a tilt in the equatorial organization of metaphase chromosomes or a change in the direction of the movement of daughter chromosomes during anaphase (Saliem *et al.*, 1981). In the present investigation, the misorientation of chormosomes may be due to the above mentioned effect of the cyanobacterial extracts.

8. Polyploidy

Polyploidy is a major numerical, chromosomal abnormality, observed in the present investigation. It is observed in the treatments (Figs. 114, 115, 121, 124, 177; Tables 1-4, 21-24) of *Anabaena circinalis* var. *crassa* and *Oscillatoria obscura* extracts. Raj and Reddy (1971) as well as Minija *et al.* (1999) attributed polyploidy due to inhibition of spindle mechanism. Here the extracts of cyanobacteria may be probably having similar cytotoxic effects.

The occurrence of hyperploid cells may be attributed to spindle inhibition, lack of anaphase movement or failure of cell plate formation (Nagpal and Grover, 1994). Herichova (1973) observed polyploid cells in

mitosis of barley after application of spindle distructing chemicals. Polyploidy is widely acknowledged as a major mechanism of adaptation and speciation in plants (Ramsey and Schemske, 1998).

9. Diagonal Arrangement of Chromosomes

Diagonal arrangement of chromosomes was observed (Figs. 118, 123, 143, 178-181, 192, 193, 199, 203, 206; Tables 9-32) in the present investigation in the treatments with the extracts of *Nostoc carneum*, *N. commune*, N. *spongiaeforme*, *Oscillatoria obscura*, *O. princeps* and *Phormidium tenue*. According to Das *et al.* (1968) the improper functioning of the spindle apparatus causes the diagonal orientation of chromosomes. This may be due to the slight tilt in the spindle apparatus induced by the failure and improper functioning of the spindle apparatus (Sreeranjini and Thoppil, 2001). Here the extracts of the cyanobacteria may be probably having similar cytotoxic effects.

10. Disturbed Metaphase, -Anaphase and - Telophase

Disturbed metaphase, - anaphase and - telophase was observed (Figs. 121, 144, 173-177; Tables 1-4, 13-24) in the present investigation after treatment with the extracts of *Anabaena circinalis* var. *crassa*, *Nostoc commune*, N. *spongiaeforme* and *Oscillatoria obscura*. It may be due to the loss of activity of microtubules in spindle fibers (Heaps *et al.*, 1982; Saleem *et al.*, 1993). Disturbed metaphase may be the result of disturbances on the spindle apparatus (Shehab *et al.*, 1978) or due to inhibition of spindle formation (Amer and Farah, 1983; El-Khodary *et al.*, 1989). Disturbed metaphase is indicated by the scattering of chromosomes irregularly all over the cell in some cases. In some other cases, chromosome groups show rosette like arrangement at anaphase. This may be due to disturbance of spindle apparatus. Similar reports are available in *V. faba* and *Gossypium barbadense*

after seed soak and root treatment with Sevin (Amer *et al.,* 1971). Multipolar spindles were noted in onion after root treatment with IPC and has attributed to partial suppression of spindle action (Doxey, 1949). The extract is having an action on the spindle, and so can be considered as a stathmokinetic agent (Shehab, 1979). Here the extracts of the cyanobacteria may be probably having similar cytotoxic effects.

11. Chromosome Laggards

Chromosome laggards were observed (Figs. 136, 149, 154, 174, 186, 194; Tables 1-4, 9-12, 21-28) in the present investigation after treatment with the extracts of *Anabaena circinalis* var. *crassa*, *Nostoc carneum*, *Oscillatoria obscura* and *O. princeps*. Barthelmess (1957) and Nagpal and Grover (1994) opined that the lagging of chromosomes might be because of prometaphase movement of chromosomes, accompanied by adhesion of the centromere to the nuclear membrane or to the surrounding surface of the plasma membrane. Lagging of chromosomes can be attributed to delayed terminalization, stickiness of chromosome ends or due to failure of chromosomal movements (Kaur and Grover, 1985). This may be the probable reason for scoring chromosome laggards at anaphase and telophase of mitosis in *Allium cepa*, after treatment with the toxic cyanobacterial extracts in the present investigation.

12. Abnormal Movement of Chromosomes

Abnormal movement of chromosomes was observed (Figs. 155, 156, 158, 159, 190, 196; Tables 9-28) in the present investigation in the treatments with the extracts of *Nostoc carneum*, *N. commune*, *N. spongiaeforme*, *Oscillatoria obscura* and *O. princeps*. Abnormal movement of chromosomes may be due to severe disturbances in the spindle mechanism (Minija *et al.*, 1999).

Multipolar spindles were observed in the present study. The number of poles in a cell depend on the position of the assemblage of RNA and polysachharides, which remain, distributed either in the form of sol or gel (Prasad, 1974). This may be the probable reason for scoring abnormal movement of chromosomes both at metaphase and anaphase of mitosis in *Allium cepa*, after treatment with the toxic cyanobacterial extracts in the present investigation.

The precocious movement of chromosomes might have been caused by the early terminalisation, resulting in the movement of the chromosomes ahead of the rest during anaphase (Kaur and Grover, 1985).

13. Stathmo-Anaphase

Stathmo-anaphase was observed (Figs. 166, 182-184: Tables 21-24, 29-32) in the present investigation after treatments with the extracts of *Oscillatoria obscura* and *Phormidium tenue*. Studies conducted on *Hordeum sativum, Vicia faba* and *Nigella damascena* confirms that stathmo-anaphase is a radiomimetic effect caused by the simultaneous multipolar and spindle poisoning activities induced by the spindle destructing chemicals. Stathmo-anaphase can be considered as stathmo-kinetic as it affects the spindle fibers (Shehab, 1979). In the present investigation, the occurrence of stathmo-anaphase may be due to the above mentioned reasons.

Rarely Observed Non-Clastogenic Abnormalities

1. Blebbing

Blebbing was observed (Fig. 39; Tables 29-32) in the present investigation in the treatments with the extracts of *Phormidium tenue*. Infection with a bacterium, *Pseudomonas avenae* caused cytoplasmic condensation, shrinkage, and plasma membrane blebbing, all of which are

important morphological characteristics of programmed cell death (PCD) in paddy (Che *et al.*, 1999). A very recent experimental assay conducted on tobacco, shows that blebbing is a forerunner of cell death, which was induced by certain toxic chemicals (Surakka *et al.*, 2005; Víteček *et al.*, 2007). So in the present investigation the phenomenon of blebbing induced by some treatments shows the acute toxicity of the respective cyanobacterial extracts.

2. Stratified Metaphase

Stratified metaphase was observed (Figs. 90, 91, 120; Tables 21-24) in the present investigation after treatments with the extracts of *Oscillatoria obscura*. Stratified ultrastructure of chromosomes was found to be the characteristic feature of some chlorophycean and animal chromosomes that have localized centromeres. However, the stratified nature of arrangement of chromosomes appears as an induced aberration in treated cells of test organisms (Godward *et al.*, 1979). Cytotoxic assay conducted on *Rosa chinensis* var. *minima* reveals the chemical induction of polyploidy. Polyploidization was detected together with stratified chromosomal arrangement at metaphase in the meristematic layers of root tip squashes of rooted cuttings (Zlesak *et al.*, 2005). Colchicine treatment on some higher plants also shows the occurrence of stratified metaphase in polyploid tissues as a cytochimera (Mergen and Lester, 1961; Barrett, 1974). So in the present investigation, the occurrence of stratified metaphase may be a cytochimera induced by the extracts of *Oscillatoria obscura.*

3. Scattering of Chromosomes

Chromosome scattering (Figs. 92, 93, 169; Tables 13-20) was observed in *A. cepa* after treatment with extracts of *Nostoc commune* and *N. spongiaeforme.* It could be attributed to the interference of the cyanobacterial extracts with tubulin or polymerization of the microtubular

subunits forming the spindle apparatus (Mathur and Chua, 2000). Chromosomes spread irregularly over the cell due to the disturbance of the spindle apparatus. Such type of chromosome scattering was also reported in *Vicia faba* and *Gossypium barbedence* after seed soak and root treatment with Sevin (Amer *et al.* 1971). In the present study, the scattering of chromosomes observed in *A. cepa* root tip meristem may be due to the above mentioned reason.

4. Bouquet Formation of Chromosomes

Bouquet formation of chromosomes was observed (Figs. 82, 94; Tables 25- 28) in the present investigation in the treatments with the extracts of *Oscillatoria princeps*.

In plants telomeres are known to be specifically associated with many proteins that contribute to their maintenance and to telomere-specific behaviors. Several experimental evidences prove that telomeres are responsible for the coincident initiation of homolog pairing and synapsis during the telomere clustering (bouquet) stage of meiotic prophase (Bass *et al.*, 2000; Carrie *et al.*, 2001).

Bouquet formation is a polarized arrangement of chromosome ends at the periphery while the remaining chromosomes fill the volume of the cell. This is the result of telomeres moving towards the inner side and eventually bunching together at the bouquet site (King *et al.*, 2006). Bouquet formation of chromosomes sometimes occur naturally during zygotene of meiotic prophase at the time of chromosome pairing in some plants and animals. However, abnormal bouquet formation may also occur during somatic cell division (Gillies, 1989). Certain specific telomere-localized proteins may be defective in abnormal bouquet formation in plants. In the present study, the

bouquet formation of chromosomes found in *A. cepa* may be due to the above reason.

5. "Lollypop" Metaphase

Lollypop metaphase was observed (Fig. 95; Tables 13-16) in *Allium cepa* root tip meristem after treatments with the extracts of *Nostoc commune* in the present investigation.

Studies conducted on the mechanism of chromosome segregation in budding yeast, *Saccharomyces cerevisiae* and fission yeast, *S. pombe* reveals the natural existence of lollypop like chromosomal structures in the midst of mitotic spindle apparatus (Page and Snyder, 1993). Experimental evidences from the studies conducted by Adkins *et al.* (2004) also confirms the occurrence of three-dimensional architectural dynamic macromolecular complexes resembling a "lollypop" whose biological functions are intimately linked with their structure and interactions with chromatin-associated proteins (CAPs).

Dynamic motor proteins related to kinesin are required for the normal orientation and structural integrity of spindle fibres during the divisional stages of cell cycle (Nedelec *et al.*, 1997). Spindle poisons are capable of disturbing the structural and functional aspects of the mitotic apparatus leading to the aberrant spatial arrangement of chromosomes at the equatorial plane during metaphase, anaphase and telophase stages (Saunders and Hoyt, 1992). The above mentioned reason might have been responsible for the origin of "Lollypop" metaphase in the present investigation.

6. Abnormal Grouping of Chromosomes

Abnormal grouping of chromosomes was observed (Figs. 83, 129, 195, 210; Tables 13-24) in the present investigation in the treatments with the extracts of *Nostoc commune*, *N. spongiaeforme* and *Oscillatoria obscura*.

Autotetraploid barley plants induced by the use of colchicine revealed various irregularities in chromosome configuration, including unequal grouping. The probable reason of abnormal grouping may be spindle poisoning (Chen *et al.*, 1945; Degraeve and Gilot-Delhalle, 1972). Such induced unequal grouping of chromosomes, manifested as stathmo-kinetic effect may be responsible for the detection of a wide spectrum of euploid and aneuploid variants in *Nicotiana* (Smith, 1943). In the colchicine induced autotetraploid *Physalis pubescens*, meiotic spindle abnormality was observed. It is probable that the colchicine treatment may have disturbed the spindle organiser, thereby causing abnormal spindle behaviour leading to irregular clumping and grouping of chromosomes, multipolar separation of chromosomes, *etc*. The physiological basis for this abnormality (multipolar spindle) may be the abnormal spindle behaviour of the mitotic apparatus induced by the cytotoxicant (Lydia and Raja Rao, 1982). This may be the probable reason for scoring abnormal grouping of chromosomes at mitosis in *Allium cepa*, after treatment with the toxic cyanobacterial extracts in the present investigation.

7. Equatorial Separation of Chromosomes

Equatorial separation of chromosomes was observed (Figs.151-153, 204; Tables 17-20, 29-32) in the present investigation in the treatments with the extracts of *Nostoc spongiaeforme* and *Phormidium tenue*.

Separation of daughter chromosomes parallel to the equator rather than towards the poles is an acute aberrant condition that arises as a result of errors

in the mitotic spindle assembly and dynamics (Ford and Correll, 1992). Abnormal separation of chromosomes during anaphase is an induced phenomenon, rather than a genetic one, depending upon the polarity and plasticity of the mitotic spindles concerned (Brown and Lemmon, 1998). Abnormal orientation of chromosomes at metaphase leading to equatorial separation of chromosomes originates from the aberrant pathways of spindle assembly (Waters and Salmon, 1997). In the present study, equatorial separation of chromosomes in *Allium cepa* may be due to the above reason.

8. Shift in Microtubule Organizing Centres

Shift in microtubule organizing centres was observed (Figs. 165, 171; Tables 13-16) in the present investigation in the treatments with the extracts of *Nostoc commune*. In plants, the absence of organelles such as the centrosome has led to the belief that MTOCs originate on the nuclear envelope and are transported to specific intracellular locations by microtubule motor proteins (Asada and Collings, 1997; Baluska *et al.*, 1997). Alternatively, spontaneous and de novo assembly of such MTOCs may occur in the cell by enhanced microtubule stability (Cyr and Palevitz, 1995). Studies in both animal and plant systems have suggested that stable microtubules form an integral component of microtubule organizing centers or MTOCs (Kimble and Kuriyama, 1992; Vaughn and Harper, 1998). The above mentioned facts may be responsible for the shift in MTOC in the present investigation.

9. Unequal Division and Formation of Macro and Micro cells

Unequal division and formation of macro and micro cells were observed (Figs. 216, 217; Tables 25-28) in the present investigation in the treatments with the extracts of *Oscillatoria princeps*.

In plants mitotic apparatus arise from the microtubule organizing centers (MTOCs) situated in the perinuclear space (Marc, 1997). Alterations brought about by the influence of cytotoxicants may tilt the normal balanced organization of the spindle apparatus, leading to unequal division. The unequal nuclear division, followed by phragmoplast formation may sometimes leads to the formation of macro and microcells (Schmit *et al.*, 1996). Experimental cytological assays conducted on *Zea mays* reveal highly irregular cytokinesis caused by abnormal multiple spindle intervention. Aberrant cytokinesis was found to generate unequal division (Caetano-Pereira and Pagliarini, 2001). In the present investigation, unequal division and formation of macro and micro cells might have occurred as a result of the above mentioned reasons.

The observations of the present study are a clear indication of clastogenic and non-clastogenic property of the cyanobacterial extracts, which is evident from the direct actions on the chromosomes and manifestation of spindle abnormalities. These observations are mainly caused by microcystins present in these toxic cyanobacterial blooms (Rönicke and Chorus, 1999). The lowering of the mitotic index may be due to inhibition of DNA synthesis at S-phase (Sudhakar *et al.*, 2001). It may be due to the slowing of the rate of cell progression through mitosis (Sharma and Sahu, 1977) or due to the arrest of one or more mitotic phases (Kabarity and Mallalah, 1980).

The extract of *Anabaena circinalis* var. *crassa* is able to induce clastogenic and non-clastogenic abnormalities and malformations in the nuclear structure in *Allium cepa* root tip cells. Meristematic cells revealed a dose- and time-dependent decrease of mitotic indices, ranging from 21.37% to 6.31% and increase of percentage of abnormalities from 17.56 to 35.46 (Graph 1; Tables 1-4). These data indicate that the extract of *Anabaena circinalis* var. *crassa* has cytotoxic, mitodepressive and turbogenic effects,

especially at the higher dose. Previous studies shows that several species of *Anabaena* are toxic (Rybicka, 2005; Kobos *et al.*, 2005; Bucka and Wilk-Wozniak, 2005; Kasperoviciene *et al.*, 2005; Surakka *et al.*, 2005), capable of producing biotoxins like microcystins and nodularins (Patterson *et al.*, 1991; Carmichael, 1994, 1997; Harada, 1994; Codd, 1995; Sivonen, 1996; Falconer, 1999; Singh *et al.*, 1999; Chorus *et al.*, 2000; Codd, 2000). Several other cytotoxic compounds have been reported from many species of *Anabaena* (Surakka *et al.*, 2005). The clastogenic and non-clastogenic aberrations in *A. cepa* induced by the aqueous extracts of *Anabaena circinalis* var. *crassa* may be the outcome of the toxic effects of microcystins or non-microcystin compounds present in this cyanobacterium.

Out of the eight cyanobacterial extracts, the extract of *Microcystis aeruginosa* is able to induce maximum clastogenic and non-clastogenic abnormalities and various morphological cell death features were also observed in *Allium cepa* root tip cells. Meristematic cells treated with extract of *Microcystis aeruginosa* revealed a dose- and time - dependent decrease of mitotic indices, ranging from 20.37% to 8.4% (Graph 2; Tables 5-8) and increase of percentage of abnormalities from 10.09 to 35.63. The results indicate that cyanobacterial extract of *Microcystis aeruginosa* induces apoptosis in a dose- and time - dependent manner. These results are similar to those observed by Lankoff *et al.* (2004) on cytotoxicity and apoptotic effects of Microcystin-LR and Anatoxin-a in mouse lymphocytes. Wide spectrum of literature is available regarding the cytotoxicity of microcystic cyanobacterial blooms (Ueno *et al.*, 1998; Romanowska-Duda *et al.*, 2002; Marshall *et al.*, 2005; Rybicka, 2005; Bucka and Wilk-Wozniak, 2005; Kasperovicine *et al.*, 2005; Järvenpää *et al.*, 2007; Jang *et al.*, 2007; [3]http; Ding *et al.*, 1999, 2000). In the present study, the extent of the clastogenic and non-clastogenic aberrations together with the induction of apoptosis in the treated meristematic cells of *A. cepa* may be due to the microsystins and related

compounds present in these cyanobacteria as revealed by earlier reports (Carmichael, 1994, 1997; Harada, 1994; Codd, 1995; Sivonen, 1996; Falconer, 1999; Singh *et al.*, 1999; Chorus *et al.*, 2000; Codd, 2000; Carmichal, 1992a; Fawell *et al.*, 1993; Bishop *et al.*, 1959; Dittman *et al.*, 1997; Nishizawa *et al.*, 1999; Lankoff *et al.*, 2003, 2004; Hooser *et al.*, 1990, 1991).

Meristematic cells of *A. cepa* treated with the extract of *Nostoc carneum*, revealed a dose- and time - dependent decrease of mitotic indices, ranging from 19.57% to 7.07% and increase of percentage of abnormalities from 5.26 to 16.3 (Graph 3; Tables 9-12).

The extract of *N. commune* is able to induce clastogenic and non-clastogenic abnormalities and malformations in the nuclear structure in *Allium cepa* root tip cells and revealed a dose- and time - dependent decrease of mitotic indices, ranging from 19.4% to 5.04% and increase of percentage of abnormalities from 10.45 to 19.31 (Graph 4; Tables 13-16).

The extract of *Nostoc spongiaeforme* was found to induce clastogenic and non-clastogenic abnormalities during both mitotic and non-mitotic stages in *Allium cepa* root tip cells.

Root tip meristematic cells revealed a dose- and time - dependent decrease of mitotic indices, ranging from 18.59% to 9.33% and increase of percentage of abnormalities from 12.87 to 21.2 (Graph 5; Tables 17-20). Several reports are available in literature with regard to the cytotoxicity of many species of *Nostoc* (Piccardi *et al.*, 2000; Surakka *et al.*, 2005). The reported cytotoxicity and other bioactivities may be due to the presence of cytotoxic antibiotics (Patterson *et al.*, 1991) or due to the presence of microcystins or compounds related to microcystins (Carmichael, 1994, 1997; Harada, 1994; Codd, 1995; Sivonen, 1996; Falconer, 1999; Singh *et al.*, 1999;

Chorus *et al.*, 2000; Codd, 2000). In the present investigation the clastogenic and non - clastogenic aberrations and mitotic inhibition caused by the aqueous extracts of *Nostoc carneum, N. commune* and *N. spongiaeforme* may be due to the presence of above mentioned toxic substances in them. The differences observed among the three species of *Nostoc* investigated may be due to the differences in the content and composition of their major chemical components.

The extract of *Oscillatoria obscura* is capable of inducing clastogenic and non - clastogenic abnormalities and malformations in interphase and divisional stages in *Allium cepa* root tip cells. The frequency of aberrations was moderate in various treatments. Mitotic index ranges from 19.38% to 13.51% and frequency of aberrations from 18.10% to 37.84% (Graph 6; Tables 21-24).

The extract of *Oscillatoria princeps* induces clastogenic and non - clastogenic abnormalities during the divisional stages of mitosis and interphase, affecting the nuclear as well as cellular structure in *Allium cepa* root tip meristem. The frequency of aberrations were comparatively low in various treatments. Profound decrease in mitotic index was found to be a characteristic feature of assays with *O. princeps* extracts on *A. cepa* root tip meristem. Mitotic index ranges from 8.57% to 5.21% and frequency of aberrations from 9.72% to 18.48% (Graph 7; Tables 25-28).

The results indicate that cyanobacterial extracts of *Oscillatoria obscura* and *Oscillatoria princeps* shows high mitotic inhibition in a dose- and time-dependent manner. These results are similar to those observed by Chauhan *et al.* (1992) on the effect of growth of some cyanobacteria and a green alga when cultured along with an axenic planktonic *Oscillatoria* sp. Toxicity of waterblooms containing *Oscillatoria* had already been reported by several earlier workers (Patterson *et al.*, 1991; Kobos *et al.*, 2005; Bucka and

Wilk-Wozniak, 2005), probably due to the presence of microcystins (Fastner *et al.*, 1999; Rönicke and Chorus, 1999) and / or microcystin related compounds (Carmichael, 1994, 1997; Harada, 1994; Codd, 1995; Sivonen, 1996; Falconer, 1999; Singh *et al.*, 1999; Chorus *et al.*, 2000; Codd, 2000). In the present study the cytotoxicity and mitotic inhibition exhibited by *Oscillatoria obscura* and *O. princeps* may be due to the presence of the above mentioned chemicals present in these plants. The differences observed among the frequencies of aberrations and mitotic indices owe to their chemical composition.

The enormous spindle abnormalities noticed in the present investigation are in agreement with the studies of Ding *et al.* (2000) on mitotic poisoning effects of microcystic cyanobacterial extracts.

The extract of *Phormidium tenue*, is able to induce clastogenic and non-clastogenic abnormalities and several malformations of nuclei in the nuclear structure in *Allium cepa* root tip cells. The frequency of aberrations were comparatively low in various treatments. Mitotic index ranges from 10.01% to 6.69% and frequency of aberrations from 6.24% to 15.48% (Graph 8; Tables 29-32).

The dose and time - dependent increase of mitotic indices, accumulation of bizarre forms of nuclei during interphase and mitotic stages, with hypercondensed chromosomes, abnormal anaphases with defective chromosome separation and polyploid cells are similar to that observed by Lankoff *et al.* (2003) who examined the influence of microcystin-LR on cell cycle progression, onset of anaphase, segregation of chromosomes by mitotic spindle and apoptosis in Chinese hamster ovary cells.

Blebbing of plasma membrane observed in the present investigation is similar to those observed by Eriksson *et al.* (1989) and Hooser *et al.* (1990,

1991). Blebbing observed after treatment with the extracts of *Phormidium tenue* may be due to the presence of certain toxic chemicals in this cyanobacterium, which in turn induces blebbing, a forerunner of apoptosis or programmed death in the root tip cells of *A. cepa*. Several earlier workers reported the toxicity of blooms of several potentially toxic species of *Phormidium* (Kobos *et al.*, 2005; Surakka *et al.*, 2005), probably due to the presence of microcystins and / or microcystin related compounds present in these species of *Phormidium* (Ballot *et al.*, 2004).

The twelve different categories of clastogenic aberrations that occurred frequently in majority of the treatments and fourteen additional classes of clastogenic abnormalities that occurred rarely in the present investigation may be due to the induced malformations affecting the shape, size, structure and organization of the nuclei (Gruber, 1978; Bennet, 1981; Liu *et al.*, 2000; Richardson *et al.*, 2001) or due to aberrant conditions affecting the structure, size and physiology of the chromosomes / chromatin (Kihlman, 1957; Star, 1970; Sadasivaiah *et al.*, 1973; McGill *et al.*, 1974; Grant, 1994; Patnaik *et al.*, 1984; Epstein, 1986; Matsumoto, 1988; Deumling and Clermont, 1989; Wong Young and Woo Young, 1993), or due to aberrant nucleic acid biosynthesis, metabolism or modifications in the 'S' phase of the cell cycle (Newcomer and Wallace, 1949; Verma and van Huyste, 1971; Lebedeva and Chubykin, 1975; Santos *et al.*, 2002), or due to abnormal genetic behaviour / aberrant genetically controlled mechanism (Gerstel and Burns, 1967; Vig, 1979; Franklin and Cande, 1999; Kumar and Srivastava, 2001; Kumar and Sharma, 2002; Bhat *et al.*, 2006). Many of these aberrations are the precursors / forerunners of apoptosis or programmed cell death (Caffaro *et al.*, 1982; Wang *et al.*, 1996; De Jong *et al.*, 2000; Houot *et al.*, 2001; Dzyubinskaya *et al.*, 2006), pointing towards the acute toxicity of the eight cyanobacterial species investigated.

The thirteen different types of non - clastogenic abnormalities that were encountered frequently in most of the treatments together with the nine additional forms of non-clastogenic abnormalities that were scored rarely in the present investigation may be due to aberrant synthesis and reorganization of spindle protein during G1 /S / G2 phases of the cell cycle (Prasad, 1974; Kimble and Kuriyama, 1992; Cyr and Palevitz, 1995; Marc, 1997; Waters and Salmon, 1997; Brown and Lemmon, 1998; Vaughn and Harper, 1998; Tanaka and Shimizu, 2000) or due to aberrant seggregation and non-synchronous separation mechanism during anaphase (Barthelmess, 1957; Lydia and Raja Rao, 1982; Kaur and Grover, 1985; Ford and Correll, 1992; Page and Snyder, 1993; Nagpal and Grover, 1994; Ramsey and Schemske, 1998) or due to disturbances in the microtubular / microfilament cycles and aberrant cytoskeletal / spindle organization (Doxey, 1949; Heaps *et al.*, 1982; Alberts *et al.*, 1983; El-Khodary *et al.*, 1989; Meske and Hartmann, 1995; Manandhar *et al.*, 1996; Asada and Collings, 1997; Baluska *et al.*, 1997; Nedelec *et al.*, 1997; Mathur and Chua, 2000) or due to stathmokinetic, radio mimetic and spindle poisoning effects (Levan, 1938; Smith, 1943; Chen *et al.*, 1945; Hadder and Wilson, 1958; Mergen and Lester, 1961; Degraeve and Gilot-Delhalle, 1972; Barrett, 1974; Boltovskaya *et al.*, 1979; Shehab, 1979; El-Khodary *et al.*, 1989; Zlesak *et al.*, 2005). Some of these abnormalities were reported to cause complete degeneration of the cells (Barber and Callan, 1943; Amer, 1965; Surakka *et al.*, 2005; Vitecek *et al.*, 2007), thereby confirming the lethal effects of the extracts of the cyanobacteria investigated.

So when looking at these results, it could be concluded that:

(a) Cyanobacteria are ubiquitous in surface waters during the summer season in aquatic ecosystems of Malappuram and Calicut and they are associated with frequent toxic blooms.

(b) Majority of the isolated cyanobacteria can be grown in large scale *in vitro* culture.

(c) *Allium cepa* assay is a reliable assay for the testing of genotoxicity of cyanobacteria. Even the lower dose of cyanobacterial extract induced drastic malformations and altered nuclear ultrastructure in *Allium cepa* root meristem.

(d) Despite the differences in morphology, structure, size, organization and metabolism between the taxa of cyanobacteria investigated, there is a high correlation between the mutagenic effects (both clastogenic and non-clastogenic) induced by them on the test material *A. cepa*.

(e) Cyanobacteria studied contain potentially harmful bioactive compounds which can induce cytotoxic, antimitotic and apoptotic effects on higher organisms including man.

(f) Even though there is no competition for survival, cytotoxic compounds are produced in the cultured condition too. It throws light to the fact that the ability to produce cytotoxic compounds probably may be a genetic character of some cyanobacteria .

(g) The ability to produce cytotoxic compounds probably may be responsible for the evolution and continued success of toxic strains of cyanobacteria over other phytoplanktons.

(h) The cytotoxic compounds present in the cyanobacterial species remain to be characterized, and the mechanisms of cytotoxicity need to be studied further.

The potential for production of cytotoxic compounds by cyanobacteria could have implications on public health. Monitoring the quality of water destined for public supply should also include identification of potentially

toxic cyanobacteria and their population density. Moreover, human diets rich in fish may pose a threat of chronic low level exposure to these toxins.

When unexplained deaths of wild or domestic animals occur in locations with 'blooming' water bodies, the veterinary and health authorities should also consider the possibility of poisoning by cyanobacterial toxins. So water quality should be tested for cyanobacterial presence and cyanotoxins. Hence, by increasing awareness among the veterinary audience, the present investigation may help to save more domestic but also wild animals and perhaps human lives in the future.

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 exracts 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 exracts 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. Subculturing 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%, .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, cytomixis, 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 spongiaeforme* 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.

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