Microcystis Toxic Blooms In Fish Culture Ponds And Their Biological And Chemical Control

Baidyanath Kumar, Ameetha Sinha

Abstract: Cyanobacteria are well recognized for their ability to fix atmospheric nitrogen. This group holds significant as a major natural food source for cultural forms. But, a significant number of them are also known for their nuisance value on account of their ability to produce potentially lethal toxins. The population density of *Microcystis aeruginosa* accounts for bloom like situation in fish culture ponds. The population density of *M. aeruginosa* was found to be maximum in HP, followed by DP and MP. Bloom like situation was recorded during summer and was observed only when its population density was **>** 2.5x10^{4}cells/cm^3. *Ochromonas danica*, a golden brown Chrysophytean alga engulfs and digests *Microcystis aeruginosa* colonies, a situation observed during survey of MP, when water sample was examined microscopically. The population density of *M. aeruginosa* in BRL-III medium inoculated with different concentration of culture suspension of *Ochromonas danica* was studied. In vitro results related to biological control indicated that a population density of 9.9 x 10^4 cells/cm^3 (1.5 ml) to 16.5 x 10^4 cells/cm^3 (2.5 ml) of *O. danica* caused a rapid decline in the population density of *M. aeruginosa* to almost nil only after 6 or 9 days of incubation. Investigations related to growth response of toxic strains of *M. aeruginosa* in BRL-III medium supplemented with different concentrations of Copper sulphate, Potassium permaganate, Quinine, Urea, $KMnO_4$, Ammonia, Simazine, Calcium hypochlorite, Ferric alum and Cupricide indicated that $CuSO_4$, $KMnO_4$ and Quinine were more toxic to *M. aeruginosa* in comparison to urea and ammonia. Copper sulphate and potassium permagnate caused a rapid decline in population density of *M. aeruginosa* to almost nil following 15 days of incubation; the same concentration of quinone brings this effect within 6 days. Quinones was more toxic to *Microcystis aeruginosa* followed by copper sulphate, potassium permagnate, urea and ammonia. Among calcium hypochlorite, ferric alum and cupricide, calcium hypochlorite showed maximum inhibitory effect on the growth of *M. aeruginosa.*

Key words: Biological Control, Chemical control, *Microcystis*, *Ochromonas danica*, Toxic bloom.

1 INTRODUCTION

Cyanobacteria are the most primitive gram negative, oxygenic photo synthesizer prokaryotes and well recognised for their ability to fix atmospheric nitrogen. In aquaculture world, this group holds significant as a major natural food source for cultural forms. But, a significant number of them are also known for their nuisance value on account of their ability to produce potentially lethal toxins. Unfortunately, elaborate investigation on this score has not yet been taken up in context of inland aquaculture scenario. Cyanobacteria are ubiquitous in ponds and occur as permanent blooms in some water bodies. Certain Cyanobacteria give out metabolic products which accumulate in the water and are many a time lethal to the other forms present in the water. Eutrophication of pond water involves enrichment of plant nutrients, especially phosphorus compounds that lead to significant shift in the phytoplanktonic community towards Cyanobacteria. Species of *Microcystis, Anabaena, Aphanizomenon, Oscillatoria* grow profusely under such condition (Lund, 1969; Findenegg, 1973; Meffert, 1975; Hickel, 1975; Burgi, 1977; Bernhardt and Clasen, 1982; Pearl and Ustach, 1982; Skulberg, Codd and Carmichael, 1984; Wetzel, 1983; Brock, 1985; Verma et al., 2002). All of these genera are known to produce potentially lethal toxin (Leeuwangh et al., 1983; Skulberg et al., 1984; Berg and Soli, 1985; Berg et al., 1986; Baidyanath et al, 2006). Toxic Cyanobacteria have been reported from marine brackish water and freshwater habitats throughout the world.

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These algae characteristically form dense unialgal growths often referred to as “blooms” and have been responsible for death of fish, livestock and waterfowl (Gorham et al., 1964; Collins, 1978; Carmichael, 1981). *Microcystis aeruginosa* is the most common bloom forming unicellular cyanobacterium in ponds. Animal poisonings due to toxins produced by this organism has been reported worldwide (Gorham and Carmichael, 1979). A toxic strain of *M. aeruginosa* (NRC – 1) was first isolated by Canadian researchers (Huges et al., 1958). Toxicity of *M. aeruginosa* on Cladixeran arthropod, *Daphnia magna*, air breathing teleost (*Heteropneustes fossilis* and *Clarias batrachus*) and major carps (*Labeo rohita* and *Catla catla*) has been assessed by Baidyanath et al. (2006). It has been found that *M. aeruginosa* strains produce a variety of peptide toxins. A strain of this species, isolated from a toxic bloom of Witbank Dam in the Transvaal, was found to produce four different peptide toxins. All the four purified toxins were cyclic hepta peptides containing β – methyl aspartic acid, glutamic acid and alanine. The other residues in each fraction were variable (Botes et.al., 1982). One of these toxic variants was characterised and designated Cyanoginosin-LA, indicating thereby that it contained the variable amino acids, leucine and alanine. Cyanoginosin-LA has an amino acid composition of erythro β-D-aspartic acid, D-alanine, L-alanine, D-Glutamic acid and L-leucine. An unusual 3-amino-9-methoxy-2, 6, 8-trimethyl-10-Phenyldeca-4, 6-dienoic acid residue (Adda) is also present in addition to these. *Microcystis* toxin is an ‘endotoxin’ i.e., it is not found in the water column until the bloom begins to deteriorate. Henning and Kohl (1981) have shown that the toxic effects produced by *M. aeruginosa* depend on the degree of decomposition or disintegration of the cells. *M. aeruginosa* contains two effective toxic principles, called the fast death factor (FDF) and the slow death factor (SDF). The former is a cyclic peptide called micro cystin and causes rapid death, within four hours of the animals who ingest the alga, whereas the SDF causes death only after a long period. The toxin from *M. aeruginosa* is lethal to a

number of mammalian species and birds, both after intra peritoneal injection or ingestion. Death occurs in 1-3 hr and is apparently due to internal bleeding. Pathological examinations show extensive liver damage (Huges et al., 1958). However, *Microcystis* toxins have relatively hydrophobic properties due to blocking groups and the presence of the unusual aromatic residue Adda. It has been proposed that these compounds may be general membrane disruptions and that liver damage in higher organisms is a consequence of the general detoxification function of this organ (Runnegar et al., 1981) showed that low levels of *Microcystis* toxin caused a relatively rapid deformation of the cell membrane in isolated hepatocytes. Slatkin et al., 1983 reported the occurrence of pulmonary thrombi after lethal injection of *Microcystis* toxins. *Microcystis* toxin is a potent thrombogenic agent which is not counteracted by the usual anticoagulants. The lethal effects of the toxin could be counteracted by hydrocortisone which may be due to the effect of the drug on platelet aggregation. In India, *Microcystis aeruginosa* has been widely recognised as a serious species for causing toxic and permanent bloom. In Mithila (North Bihar) the ponds are gradually leading towards eutrophication due to variable reasons. However, domestic sewage has been identified as critical being one of the chief sources of phosphates. They contain heavy load of surfactants and detergents that significantly alerts the N:P ratio. Mass mortality of fish in this region is a common feature during summer and monsoon months. The phenomenon is locally called 'UJAIH' characterised by unusual surfacing of the fish due to respiration distress, malfunctioning of the chemo receptors, causing loss of balance, muscle fasciculation and subsequent death. Toxic algal blooms have been increasing worldwide. In fresh water bodies, blooms are formed mainly by cyanobacteria that float to the surface and accumulate causing water green in colour (Sellner et al., 2003). Aquatic life is exposed to toxins by eating/ingesting. The toxins can pass through cell membranes including the blood-brain barrier and skin tissues (Kemppainen et al., 1991; Apland et al., 1993). Fish are exposed to toxins by swimming through blooms and ingesting forms of aquatic life that have become contaminated with toxins. They are killed through lack of muscle coordination and paralysis, convulsions and respiratory failure (Kirkpatrick et al., 2004). The methods to control harmful algal blooms have been reviewed by several workers Mehdi Bibak and Seyed Abbas Hosseini (2013), Anderson (2009), Davis (2009), Chorus and Bartram (1999), Lu et al. (2006), Fernanda et al. (2011). The methods to control toxic blooms to minimise the potential adverse effects on the environment and to mitigate economic loss would be advantageous and hence the present investigation has been undertaken to highlights the chemical and biological control of toxic blooms caused by *Microcystis aeruginosa* in fish culture ponds of North Bihar.

2 MATERIALS AND METHODS

2.1 Survey and Sampling:

Three fish culture ponds having past history of recurrent algal blooms were selected for the present investigation viz., Mahaseth pond (MP), Harahi pond (HP) and Dighi pond (DP). Bloom producing Cyanobacteria, *Microcystis* species were collected monthly from June 2012 – May 2013 and the samples were kept in acid washed and sterilized glass vials and immediately preserved in FAA solution for microscopic observations. Toxic bloom producing Cyanobacteria, *Microcystis aeruginosa* were identified following Fritsch (1977) and APHA-AWWA-WPCE, 1980. Population density of *M. aeruginosa* was determined by counting their cells employing haemocytometer and expressed as N x 10^4 cells/cm^3.

2.2 Culture:

Serial dilution technique was employed to isolate *Microcystis aeruginosa* from a mixed population. The sample was homogenized for dislodging the colonies to free cells. The sample was serially diluted to up to 10^{-8} in test tubes containing sterile medium to the point of extinction in the number of cells, so that the last tube contained only a single cell. The last 10^{-8} dilution contained a single cell. One ml of this diluted sample was then poured in molten sterile agar medium in petridish and incubated at 27**±2º** C in presence of 3600 lux light. The colonies appeared following three days of incubation. The number of colonies of *M. aeruginosa* was counted to find out the total number of cells per ml of the original sample using haemocytometer. A single colony which contained genetically homogeneous population of *M. aeruginosa* was transferred to sterilized BRL-III medium as suggested by Baidyanath et al. (2006) and incubated at temperature 25**±2º** C, 100 cc/m aeration and light intensity of 3600 lux for 7 days, with 8 hrs. photoperiod daily. The stock culture of toxin strains of *Microcystis aeruginosa* was maintained in BRL-III medium at 25**±2º** C, 2200 lux light, 8 hrs. photoperiod and 100 cc/m aeration with repeated culture on day 45.

2.3 Biological Control:

Random observation during field survey led us to study the biological control of toxic bloom caused by *Microcystis aeruginosa.* In vitro experiment was conducted with live samples of *Ochromonas danica*, a Chrysophytean alga collected from the Mahaseth pond. Identified strain was cultured in the laboratory in a medium containing glucose-1.09 g; Tryptone-1.09 g; Yeast extract-1.09 g; Liver extract infusion -40.0 ml in 1000 ml distilled water. The suspension was incubated for 21 days at room temperature (25**±2º** C) giving a population density of 6.6 x 10^4 cells/ml. A 35 days old laboratory culture of toxic *M. aeruginosa* with population density of 21.4 x 10^4 cells/cm^3 was taken in five different concentrations viz., 0.5, 1.0, 1.5, 2.0 and 2.5 and *O. danica* culture suspension in 50 ml water was inoculated with 2 ml of toxic *M. aeruginosa* culture. The mixed suspension was incubated for 21 days with count for the population density of *M. aeruginosa* in *O. danica* suspension was recorded periodically on every third day (72 hrs.). A simultaneous record of the Mahaseth pond for population density of *O. danica* in natural conditions conducted between April to September, 2012 was also taken for reference.

2.4 Chemical control of *Microcystis aeruginosa* bloom:

A 35 days old pure culture of toxic strain of *Microcystis aeruginosa* grown in BRL-III medium in which their population density was recorded as 12.5 x 10^4 cells/cm^3 was tested for decline in their population density after supplementing various chemicals in different

concentrations. The effect of different concentrations of copper sulphate, urea, potassium permagnate, quinone, ammonia, simazine (90%), calcium hypochlorite, ferric alum and cupricide on the growth density of *M. aeruginosa* was evaluated. The BRL-III medium was separately supplemented with four different concentrations viz., 0.5, 1.0, 1.5 and 2.0 gm/lit. of copper sulphate, urea, potassium permagnate, quinone, ammonia and five different concentrations of calcium hypochlorite, ferric alum and cupricide (1.0, 1.5, 2.0, 2.5 and 3.0 mg/lit.). The five different concentrations of 90% simazine used were in ppm viz., 5, 10, 15, 20 and 25 ppm. The experiment was carried out in replicates of five. The cultures were incubated at room temperature, 25**±**2**º** C, and decline in the population density of *M. aeruginosa* was recorded at the interval of 3 days up to 21 days. The results of each experiment have been expressed with standard error (SE) and critical difference (CD) at 5% level of significance. The results obtained have been presented in Table 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12**.**

Table 1:
Month-wise record of Population density of *Microcystis aeruginosa* in three fish culture ponds (Population density in N x 10^4 cells/cm^3)

Ponds of Group	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May
Mahaseth Pond (MP)	3.1 x 10^4 SE 0.029 CD ± 0.08	3.5 x 10^4 SE 0.041 CD ± 0.11	5.0 x 10^4 SE 0.089 CD ± 0.25	3.0 x 10^4 SE 0.33 CD ± 0.96	1.5 x 10^4 SE 0.044 CD ± 0.12	...	...	...	1.4 x 10^4 SE 0.15 CD ± 0.43	2.0 x 10^4 SE 0.081 CD ± 0.23	2.3 x 10^4 SE 0.044 CD ± 0.12	3 x 10^4 SE 0.081 CD ± 0.08
Harahi Pond (HP)	36 x 10^4 SE 0.029 CD ± 0.08	14 x 10^4 SE 0.37 CD ± 0.12	40 x 10^4 SE 0.37 CD ± 0.12	35 x 10^4 SE 0.040 CD ± 1.1	10 x 10^4 SE 0.43 CD ± 1.2	0.5 x 10^4 SE 0.12 CD ± 0.35	1 x 10^4 SE 0.08 CD ± 0.23	2 x 10^4 SE 0.083 CD ± 0.23	21 x 10^4 SE 0.422 CD ± 1.2	25 x 10^4 SE 0.36 CD ± 1.05	30 x 10^4 SE 0.81 CD ± 2.3	33 x 10^4 SE 0.47 CD ± 1.3
Dighi Pond (DP)	25 x 10^4 SE 0.029 CD ± 0.08	33 x 10^4 SE 0.036 CD ± 0.12	35 x 10^4 SE 0.24 CD ± 0.70	31 x 10^4 SE 0.47 CD ±1.3	10 x 10^4 SE 0.04 CD ± 0.11	0.4 x 10^4 SE 0.04 CD ± 0.11	1.1 x 10^4 SE 0.04 CD ± 0.11	1.5 x 10^4 SE 0.16 CD ± 0.46	10 x 10^4 SE 0.65 CD ± 1.8	15 x 10^4 SE 0.36 CD ± 1.05	18 x 10^4 SE 0.21 CD ± 0.61	20 x 10^4 SE 0.32 CD ± 0.92

SE = Standard Error; CD = Critical Difference

Table 2: Population density of *M. aeruginosa* in BRL-III medium inoculated with different concentrations of culture suspension of *Ochromonas danica*

(population density in N x 10^4 cells/cm^3)

Initial Population of *M. aeruginosa*	Days of incubation	Concentration of culture suspension of *Ochromonas danica* in ml.				
		0.5 (3.3 x 10^4)	1.0 (6.6 x 10^4)	1.5 (9.9 x 10^4)	2.0 (13.2 x 10^4)	2.5 (16.5 x 10^4)
21.4 x 10^4 cells/cm^3	3	15.4 x 10^4	6.5 x 10^4	2.7 x 10^4	2.0 x 10^4	1.6 x 10^4
	6	6.2 x 10^4	3.7 x 10^4	1.3 x 10^4	1.2 x 10^4	0.6 x 10^4
	9	3.5 x 10^4	1.4 x 10^4	0.4 x 10^4	0.2 x 10^4	...
	12	2.3 x 10^4	0.8 x 10^4	...	...	...
	15	1.6 x 10^4	0.6 x 10^4	...	...	...
	18	0.8 x 10^4	0.5 x 10^4	...	...	...
	21	0.5 x 10^4	0.2 x 10^4	...	...	...

Table 3: Phagotrophic interaction of *O. danica* with *M. aeruginosa* in Mahaseth pond (population density in N x 10^4 cells/cm^3)

Months	Interacting organisms	
	Ochromonas danica	*Microcystis aeruginosa*
April	Nil	30.5 x 10^4
May	3.8 x 10^4	15.6 x 10^4
June	5.2 x 10^4	2.6 x 10^4
July	10.8 x 10^4	Nil
August	8.5 x 10^4	Nil
September	6.7 x 10^4	Nil

Table 4: Population density of *M. aeruginosa* in BRL-III medium amended with Different concentrations of $CuSO_4$ (Decline in population of *M. aeruginosa* in N x 10^4 cells/cm^3)

Population density in BRL-3 medium after stationary phase of growth i.e., after 35 days of incubation	Days of incubation	Amount of $CuSO_4$ in gm/l			
		0.5	1.0	1.5	2.0
2.5 x 10^4 cells/cm^3	3	6.5 x 10^4	4.5 x 10^4	2.6 x 10^4	1.3 x 10^4
	6	2.1 x 10^4	0.8 x 10^4	0.4 x 10^4	0.1 x 10^4
	9	1.5 x 10^4	0.5 x 10^4	...	...
	12	0.7 x 10^4	...	...	...
	15	0.2 x 10^4	...	...	...
	18	...	...	...	...
	21	...	...	...	...

Table 5: Population density of *M. aeruginosa* in BRL-III medium amended with different doses of Urea (population density in N x 10^4 cells/cm^3)

Population density in BRL-3 medium after stationary phase	Days of incubation	Doses of urea in gm/l			
		0.5	1.0	1.5	2.0
17 x 10^4 cells/cm^3	3	9.7 x 10^4	7.5 x 10^4	6.5 x 10^4	4.5 x 10^4
	6	5.6 x 10^4	4.4 x 10^4	3.6 x 10^4	2.6 x 10^4
	9	5.0 x 10^4	3.5 x 10^4	2.1 x 10^4	1.3 x 10^4
	12	4.5 x 10^4	2.4 x 10^4	1.5 x 10^4	0.6 x 10^4
	15	4.1 x 10^4	4.0 x 10^4	0.6 x 10^4	...
	18	4.0 x 10^4	1.4 x 10^4	...	...
	21	3.7 x 10^4	1.0 x 10^4	...	...

Table 6: Population density of *M. aeruginosa* in BRL-III medium amended with differe nt concentrations of $KMnO_4$ (Population density in N x 10^4 cells/cm^3)

Population density in BRL-3 medium	Days of incubation	Concentrations of Quinone in gm/l			
		0.5	1.0	1.5	2.0
12.5×10^4 cells/cm^3	3	6.3×10^4	4.5×10^4	3.7×10^4	2.1×10^4
	6	2.8×10^4	1.4×10^4	1.2×10^4	0.6×10^4
	9	1.7×10^4	0.8×10^4	0.5×10^4	...
	12	0.7×10^4	0.4×10^4	...	...
	15	0.3×10^4	...	...	...
	18	...	...	...	...
	21	...	...	...	...

Table 7: Population density of *M. aeruginosa* in BRL-III medium amended with different concentrations of Quinone (Population densityin N x 10^4 cells/cm^3)

Population density in BRL-3 medium	Days of incubation	Concentrations of Quinone in gm/l			
		0.5	1.0	1.5	2.0
12.5×10^4 cells/cm^3	3	1.3×10^4	0.5×10^4	0.2×10^4	...
	6	0.8×10^4	...	...	...
	9	...	...	...	...
	12	...	...	...	...
	15	...	...	...	...
	18	...	...	...	...
	21	...	...	...	...

Table 8: Population density of *M. aeruginosa* in BRL-III medium amended with different doses of Ammonia (Population density in N x 10^4 cells/cm^3)

Population density in BRL-3 medium	Days of incubation	Doses of urea in gm/l			
		0.5	1.0	1.5	2.0
12.5×10^4 cells/cm^3	3	13.3×10^4	12.2×10^4	1.4×10^4	7.7×10^4
	6	12.6×10^4	11.0×10^4	10.3×10^4	7.0×10^4
	9	11.8×10^4	10.6×10^4	9.5×10^4	6.3×10^4
	12	7.5×10^4	6.5×10^4	4.7×10^4	3.2×10^4
	15	3.2×10^4	2.7×10^4	1.5×10^4	1.7×10^4
	18	2.0×10^4	1.2×10^4	0.6×10^4	...
	21	1.6×10^4	0.6×10^4	...	...

Table 9: Population density of *M. aeruginosa* in BRL-III medium supplemented with different concentrations of Simazine (90%) (Population density in N x 10^4 cells/cm^3)

Initial Population density in BRL-3 medium	Days of incubation	Concentrations in ppm				
		5	10	15	20	25
12.5×10^4 cells/cm^3	3	5.7x 10^4	3.5x 10^4	2.0x 10^4	1.5x 10^4	...
	6	2.3x 10^4	1.7x 10^4	1.3x 10^4	...	...
	9	1.6x 10^4	0.5x 10^4	...	...	...
	12	0.7x 10^4	...	...	...	...
	15	...	...	...	...	...
	18	...	...	...	...	...
	21	...	...	...	...	...

Table 10: Population density of *M. aeruginosa* in BRL-III medium supplemented with different concentrations of Calcium hyhypochlorite (Population density in N x 10^4 cells/cm^3)

Initial population density in BRL-3 medium	Days of incubation	Concentrations in mg/l				
		1.0	1.5	2.0	2.5	3.0
12.5×10^4 cells/cm^3	3	6.5×10^4	5.0×10^4	3.5×10^4	2.0×10^4	0.6×10^4
	6	3.2×10^4	2.5×10^4	1.6×10^4	0.6×10^4	...
	9	2.1×10^4	1.7×10^4	0.5×10^4	...	...
	12	1.2×10^4	0.4×10^4	...	...	...
	15	0.5×10^4	...	...	...	...
	18	...	...	...	...	...
	21	...	...	...	...	...

Table 11: Population density of *M. aeruginosa* in BRL-III medium supplemented with different concentrations of Ferric alum (Population density in N x 10^4 cells/cm^3)

Initial Population density in BRL-3 medium	Days of incubation	Concentrations in mg/l				
		15	30	45	60	75
12.5×10^4 cells/cm^3	3	10.7 x 10^4	7.8×10^4	6.3×10^4	5.6 x 10^4	5.0 x 10^4
	6	9.4 x 10^4	7.8×10^4	4.7×10^4	3.7 x 10^4	3.0×10^4
	9	7.5 x 10^4	5.9×10^4	3.5×10^4	2.5 x 10^4	2.2×10^4
	12	6.3 x 10^4	3.7×10^4	2.6×10^4	1.8 x 10^4	1.6×10^4
	15	4.6 x 10^4	2.5×10^4	0.4×10^4	0.5 x 10^4	0.6×10^4
	18	3.8 x 10^4	1.3×10^4	...	...	...
	21	2.4 x 10^4	0.7×10^4	...	...	...

Table 12: Population density of *M. aeruginosa* in BRL-III medium supplemented with different concentrations of Cupricide (Population density in N x 10^4 cells/cm^3)

Initial Population density in BRL-3 medium	Days of incubation	Concentration in ml/l				
		5	10	15	20	25
12.5 x 10^4 cells/cm^3	3	7.7 x 10^4	5.6 x 10^4	4.6 x 10^4	3.7 x 10^4	2.5 x 10^4
	6	4.7 x 10^4	3.7 x 10^4	2.2 x 10^4	2.0 x 10^4	1.5 x 10^4
	9	3.4 x 10^4	2.6 x 10^4	1.6 x 10^4	1.1 x 10^4	0.7 x 10^4
	12	2.4 x 10^4	1.4 x 10^4	...	...	...
	15	1.3 x 10^4	0.3 x 10^4	...	...	...
	18	0.4 x 10^4	...	...	...	...
	21	...	...	...	...	...

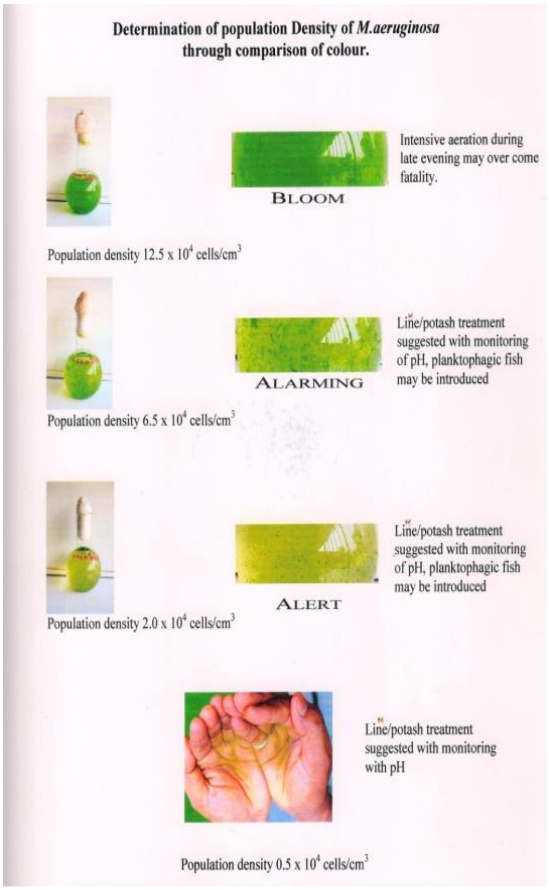

Photo Plate: 1 Relatively normal

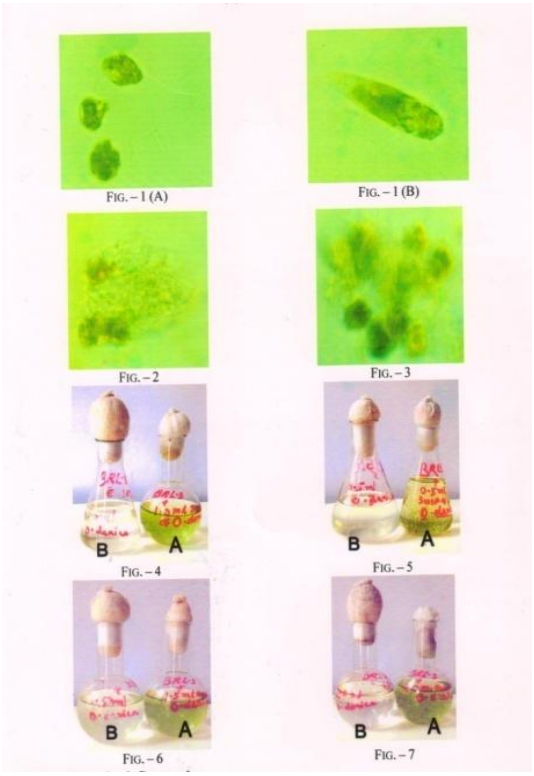

Photo Plate 2:
(Fig: 1 (A),1 (B), 2, 3, 4, 5, 6 and 7)

Fig. 1: (A) Polymorphic forms of *Ochromonas danica.*

Fig. 1: (B) Polymorphic forms of *Ochromonas danica.*
Fig. 2: *Ochromonas danica* around a small (cell less) *microcystis* colony.
Fig. 3: *Ochromonas danica* engulfing cells of *microcystis* colony.
Fig. 4: *Microcystis* colony added with 0.5 ml *O. danica* suspension.

Fig. 5: *Microcystis* colony added with 1.0 ml *O. danica* suspension.
Fig. 6: *Microcystis* colony added with 1.5 ml *O. danica* suspension.
Fig. 7: *Microcystis* colony added with 2.0 ml *O. danica* suspension.
A = Before incubation; B = After incubation with *O. Danica.*

3 RESULTS AND DISCUSSION

The population density of *M. aeruginosa* in three fish culture ponds have been presented in Table 1. It is the population density of this species that accounts for bloom like situation. A sample population count may be taken as a routine practice to predict the forthcoming bloom in the small water bodies. A colour chart has been developed taking colour appearance of *M. aeruginosa* culture with having different strength of population density (Photo plate – 1). Comparision with the colour presented may roughly indicate the population of the *Microcystis aeruginosa* in water sample taken from the pond. The population density of *M. aeruginosa* was maximum in HP followed by DP and MP. In all these ponds bloom-like situation was recorded during summer i.e., from May to September with a population density greater than 2.5 x 10^4 cells/cm^3. During peak season of bloom production i.e., from June to September the average population density of *M. aeruginosa* was in the following sequence:

HP (38×10^4) > DP (31×10^4) > MP (4×10^4)

The luxurious growth of *Microcystis aeruginosa* in all these ponds, which are highly polluted (hypereutrophic) reflected population density much greater than 3 x 10^4 cells/cm^3. This indicate that in the ponds of Mithila bloom-like situation is observed only when it's population density is **>** 2.5 x 10^4 cells/cm^3 as also suggested by Gentile (1971), Verma (1998) and Baidyanath et al. (2006). During the survey of Mahaseth Pond (Darbhanga) one interesting situation led to the investigation of biological control of toxic bloom caused by *Microcystis aeruginosa*. It could be established that *Ochromonas danica*, a golden brown Chrysophytean alga engulfs and digests *M. aeruginosa* colonies, a situation observed during survey of MP, when water sample was microscopically examined. *Ochromonas danica* shows both plant and animal-like characters. The cells appeared spherical, ovoid or elongate. Cell size ranged from 2.5 to 6 µm in length and from 1.75 to 2.75 µm in width. Elongate cells travelled in a straight path with the two flagella directed forwards, while ovoid and spherical forms swimmed in a slower, helical path. On coming in contact with substrata, they rotated in a clockwise direction. The centre of rotations was about a point towards the posterior of the cell. The healthy rotating cells were observed to change shape from elongate to spherical. Each cell contained a chloroplast of pale gold colour, towards the anterior end. An eyespot was present near the flagellar bases. A large vesicle occupied the posterior portion of the cell. Under the light microscopic observation 7 to 13 cells of *Microcystis aeruginosa* were found engulfed within the *O. danica* cells. *O. danica* thus showed mixotrophic nutrition. Mahaseth pond is located in Bakarganj of the town city Laheria Sarai of Darbhanga District. It has a past history of recurrent *Microcystis* blooms. During pond survey it was observed that up to April the pond was heavily infested with *Microcystis aeruginosa* with population density around 30.5 x 10^4 cells/cm^3. In the following month (July, onward), there was a drastic decline in the population of *Microcystis aeruginosa*, causing concern to the further investigations. Periodic samples were brought to laboratory for extensive microscopic examinations. It could be revealed that this Chrysophytean alga, *Ochromonas danica* has fascination for *M. aeruginosa* colonies. A sequential photomicrograph illustrating the event has been presented in fig. 1, 2 and 3 of photoplate 2.

Fig (1): *Ochromonas danica* around a small and eluplied (cell less) *Microcystis* colony.
Fig (2): *Ochromonas danica* engulfing cells of *Microcystis* colony.
Fig (3): Pleomorphic forms of *Ochromonas danica*.

Ochromonas danica is phagotrophic flagellate protist that engulfs live cells of *Microcystis* colony leaving behind the mucilaginous core. Investigations revealed that the fishermen use that pond for regular fish culture operation and had treated the pond with potash @ 1 kg/ha in the month of February 2012, followed by lime treatment @ 1.5 kg/ha before stocking. Fry-fingerlings of *Labeo rohita, Catla-catla, Silver carp* and *Cirrhinas mrigala* were introduced disproportionately. The above field observations encouraged to undertake laboratory investigations to validate the use of *Ochromonas danica* as a biological tool to control the *Microcystis* toxic bloom in freshwater bodies. The population density of *Microcystis aeruginosa* in BRL-III medium inoculated with different concentrations of culture suspension of *Ochromonas danica* was studied and data represented in Table 2. The results indicated that the population density of *M. aeruginosa* declined on increasing the population density of *O. danica*. A population density 3.3 x 10^4 cells/cm^3 of *O. danica* (0.5 ml suspension) caused a decline in the population of *M. aeruginosa* from 21.4 x 10^4 cells/cm^3 to 0.5 x 10^4 cells/cm^3 after 21 days of incubation. Similarly, a population density of 6.6 x 10^4 cells/cm^3 of *O. danica* caused a decline in the population of *M. aeruginosa* to 0.2 x 10^4 cells/ml after 21 days of incubation. A population density of 9.9 x 10^4 cells/cm^3 (1.5 ml) to 16.5 x 10^4 cells/cm^3 (2.5 ml) of *O. danica* caused a rapid decline in the population density of *M. aeruginosa* to almost nil only after 6 or 9 days of incubation. Phagotrophic interaction of *Ochromonas danica* with *Microcystis aeruginosa* in Mahaseth pond has been presented in Table 3. The survey indicated that in the month of April, the population density of *M. aeruginosa* was 30.5 x 10^4 cells/cm^3, and *O. danica* was nil. From May onward, the population density of *M. aeruginosa* showed a rapid decline to almost nil with increasing population of *O. danica*. From the month of July to August the population density of *M. aeruginosa* was almost nil when *O. danica* was present in the range of population density from 6.7 x 10^4 to 10.8 x 10^4 cells/cm^3. The results clearly indicated that *Ochromonas danica* showed a phagotrophic interaction with *Microcystis aeruginosa* and might proved to be an effective biological agent to control toxic bloom caused by *M. aeruginosa.* The present findings gain support from the work of Fernanda et al., (2011) who suggested parasitism as a biological control

agent of dinoflagellate blooms. The biological control processes specifically phagotropism or parasitism thus influence the dynamics of *Microcystis* blooms. Investigations on the growth response of toxic strains of *M. aeruginosa* in BRL-III medium reflect variable susceptibility to different chemicals and their concentrtions also influences the growth rate. Copper sulphate, Potassium permaganate and quinine were more toxic in comparision to urea and ammonia. The population density of *M. aeruginosa* in BRL-III medium supplemented with different concentration of $CuSO_4$ (Table 4), Urea (Table 5), $KmNO_4$ (Table 6), Quinone (Table 7), Ammonia (Table 8), Simazine (Table 9), Calcium hypochlorite (Table 10), Ferric alum (Table 11) and Cupricide (Table 12) has been presented. Perusal of tables (Table 4 – 12) also depict the influence of concentration of these chemicals on controlling population of *M. aeruginosa*. While the copper sulphate and potassium permagnate (0.5 g/l) cause a rapid decline to almost nil following 15 days of incubation, the same concentration of quinone brings the effect within 6 days. Ammonia and urea, however, caused a slow decline and it took about 18 to 21 days to cause 100% mortality. The result clearly indicates that quinone was more toxic to *Microcystis aeruginosa* followed by copper sulphate, potassium permagnate, urea and ammonia. The population density of *M. aeruginosa* in BRL-III medium supplemented with 0.5 ml/l of toxicant of 3 days of incubation was as follows:

Ammonia 13.3×10^4 > Urea 9.7×10^4 > Copper sulphate 6.5×10^4 > Potassium permagnate 6.3×10^4 > Quinone 1.3×10^4

0.5 ml/l of ammonia, data from Table 8 first favoured a slight increase in the population density of *M. aeruginosa* up to 6th day of incubation, but their population showed a gradual decline after 6 days of incubation. In 21 days of incubation, their population density was found minimum (1.6×10^4 cells/cm^3). From the result of Table 9**,** it is evident that the different concentrations of 90% Simazine caused a rapid decline in the population density of *M. aeruginosa* in BRL-III medium supplemented with 5 ppm of 90% Simazine to almost nil after 12 days of incubation. 10, 15 and 20 ppm of Simazine caused similar effect after 9, 6 and 3 days incubation respectively. *M. aeruginosa* could not grow in BRL-III medium supplemented with 25 ppm of 90% Simazine. Among calcium hypochlorite, ferric alum and cupricide, calcium hypochlorite showed maximum inhibitory effect on the growth of *M. aeruginosa* 1.0, 1.5, 2.0, 2.5 and 3.0 ml/l of calcium hypochlorite caused the death of *M. aeruginosa* after 15, 12, 9, 6 and 3 days of incubation respectively which is represented in Table 10. Department of Agriculture, Govt. of Western Australia (2004) has suggested that simazine will continue to kill algae for several days after application. As it is herbicide it will also kill plants. So, treated water should not be used on the garden for at least 14 days. The average half-life of simazine in ponds when it has been applied in 30 days with the actual half-life, is dependent on the level of algae present, the degree of weed infestation and other factors. Although it is regarded as virtually non-toxic to bees, aquatic animals and many mammals, sheep and cattle are susceptible to poisoning by simazine when exposed to dose rates for greater than that of recommended for algal control. The result of Table 9 clearly indicates that 25 ppm of 90% simazine which is toxic to *M. aeruginosa* will not cause any harm to non-target aquatic flora and fauna and will thus prove to be suitable for the control of *Microcystis* bloom. 15 and 30 mg/l of ferric alum, when added to BRI-III medium did not cause death but showed a gradual decline in the population density of *M. aeruginosa*; 45, 60 and 75 mg/l of ferric alum showed a more or less similar trend in growth inhibition of *M. aeruginosa*, but their population density declined to almost nil after 15 days of incubation shown in Table 11. BRI-III medium supplemented with five different concentrations of cupricide viz., 5-25 ml/l also showed a general decline in the population density of *M. aeruginosa* after 18 days of incubation. Death of *M. aeruginosa* after 18 days of incubation and 10 ml/l caused the same after 15 days of incubation. 15, 20 and 25 ml/l of cupricide caused the death of *M. aeruginosa* only after 9 days of incubation as represented in Table 12. Copper sulphate treatment for the control of toxic bloom has been commonly used but this chemical cannot be recommended because it is an acute pesticide and can kill crustaceans, fish and other aquatic life. An effective rate of 12 gm of 70 percent of calcium hypochlorite in 1000 litres of water has been recommended by DAE (2004), but may cause death of fish and crustaceans. *Microcystis* bloom may reappear following chlorine treatment and periodic treatment will therefore be necessary. Ferric alum has been recommended at the rate of 50 ml/l for reducing algal blooms in farm dam, but this chemical removes phosphorus from the water. Cupricide is a complex helated product that kills algae without the toxicity risk as associated with copper sulphate. DAE (2004) has recommended the dose rate which varies from 190 ml to 4.81 per 1000 m^2 depending on the life state of algae being treated. Under field conditions cupricide is effective in controlling a wide spectrum of algae. From the present investigation, it can be safely concluded that 25% of 90% simazine which is virtually non-toxic to aquatic animals sheep, cattle and bees, might be employed in controlling toxic bloom of ponds caused by *Microcystis aeruginosa*. During the summer, shallow hypereutrophic ponds often have dense blue green algal bloom (*Microcystis* bloom) coincident with low concentrations of inorganic nitrogen and elevated concentration of inorganic phosphorus (Barica, 1974; Claesson and Ryding, 1977; Coveney et al., 1977; Olrik, 1981) which is a major plant nutrient stimulating pond eutriphication (Vollen weider, 1968; Schindler, 1977) N is considered growth limiting nutrient in hypereutrophic as well as eutrophic ponds (Gerloff and Skoog, 1957; Claesson and Ryding, 1977; Canfield, 1983). Certain species of cyanobacteria fix atmospheric nitrogen to supplement their nitrogen requirements. However, many cyanobacterial blooms are dominated by species that do not fix nitrogen (Gerloff and Skoog, 1957; Reckhow and Simpson, 1980). Dominance of either blue-green or non-blue green algae in water bodies depend on medium surface concentration of inorganic nitrogen. A threshold level of 0.2 mg/l for blue-green cyanobacterial dominance and 0.8 mg/l for non-blue green algal dominance suggest the adaptability of cyanobacteria to lower Nitrogen levels (Lathrop, 1988).

Total N:P ratios have been gaining wide-spread use as a management tool for nitrogen limiting conditions that favour cyanobacteria (Canfield, 1983; Forsberg et al., 1978; Schindler, 1977; Smith, 1983). Forsberg et al. (1978) reported that ratios <10 (by weight) is N-limiting and >17is P-limiting. Total N:P ratio 8 as high as 29 felicitates cyanobacterial blooms in water bodies (Smith, 1983). The N:P is a key factor regulating the development of *Microcystis* bloom. Therefore the management programs by increasing the N:P ratios will be desirable. To reduce algal biomass in lakes to low or moderate, Schindler (1977) recommended to increase the N:P ratio by decreasing P supplies. Therefore, the toxic algal bloom in hypereutrophic ponds can be controlled by the addition of inorganic nitrogen above a certain threshold concentrations. Barica et.al. (1980) reported the disappearance of Aplanizomenon-flos-aquac by addition of inorganic nitrogen in ponds which causes a shift to small green algae and cryptomonads. It is beyond doubt that nutritional enrichment and subsequent eutrophication is accelarated due to human activities leading to increased productivity of phytoplankton community in the water bodies. Further the landscape changes caused due to various land use patterns also reflect different concentrations of nitrogen and phosphorus in pond water leading to outbreak of toxic algal blooms. Intensive agriculture and increased use of chemicals also triggers significant alterations in the surrounding environment. Consequently, loss in chemical balance of the pond ecosystems lead to rapid proliferation of algae, aquatic weeds and several other non target organisms (Matsumara et.al.,1986) that are important members of food chain and may subsequently lead to human health problem. The pesticides that reach the aquatic ecosystems are concentrated in certain parts or remain in solution for extended periods, adsorbed due to particulate matter and thereby deposited in the sediments. The top micrometers layer of the water column in any natural water body constitutes a preferred zone for hydrophic organic substances. The surface act as a zone of concentration of pesticides in the marine environment. The occurrence of high concentrations of pesticides resides in the surface microlayers but act as a repository and a sink for anthropogenic chemicals (Duce et.al., 1972). The level of pesticide residues in the open water depends on many factors viz., the nature of the drainage basin, extent of flow load of particulate matter, level of productivity and the depth of the water bodies. The size and nature of the water body and the extent of possible dilution influence the level of accumulation of residues by organisms. The residue levels in organisms are greater in closed ponds than that of lakes (Naqvi et.al., 1973). Aquaculture and fisheries play a major role in food production to meet the growing challenges of poverty eradication. Culture of Carps, Cat fishes, Mussels and Pearl spot in wetland ecosystems has gained considerable importance. However, the increased use of agrochemicals and the resultant residual effects in pond ecosystems have become a potential toxicological concern to fishes (Eaton, 1970). When pesticides enter into aquatic ecosystems, the resulting environmental costs can be high. A recent study on pesticides consumption and it's entry into the aquatic system in Kuttanad, Alapuzha District, Kerala, has revealed that the annual consumption of pesticides is 1000 tonnes of which 250 tonnes goes into the aquatic system. The concentration of organo phosphorus compounds has been estimated to be 0.06 – 0.09 ppm and fishes living in such polluted waters in and around Kuttanad bioaccumulates the toxicant at the level of 0.03 ppm in the liver which in turn may result in the destruction of liver enzymes such as acid phosphates. Several such instances have been reported on the toxicity uptake and bioaccumulation of pesticides in fishes (Konar, 1980; Singh and Singh, 1980). Pesticide resistance of fishes is largely based on the toxicity, exposure time, dose rate and degree of persistence in the environment. Exposure of aquatic organisms to a specific pesticide depends on it's biological availability. The bio-availability of pesticides in the aquatic system, in turn, depends on many factors of which the most potent once are biodegradability, adhesion to water sediments, solubility in water medium and the volatility. Bio-concentration and biomagnifications are two important natural phenomena closely associated with pesticide pollution. Bio-concentration is the accumulation of pesticides in tissues of plants and animals at level greater than those in the immediate environment. Bio-magnification refers to the progressive accumulation of pesticides at each trophic level of the food chain. Persistence of pesticide in the aquatic medium is another factor which determines the toxic effect on fishes. Most pesticides contain heavy metals whose discharge in fresh water bodies has resulted in alarming physical, chemical and biological responses including remarkable reduction in the biological activity (Sharma et al., 2000). Heavy metals cause death of fishes by altering haematological parameters, inhibit respiratory enzyme activity and by reducing oxygen consumption (Sornaraj et al., 1995). A variety of chemicals have been tested for controlling the growth of algae in swimming pools or other water bodies. These include copper sulphate, chlorine, potassium permagnate, sodium arsenate and sodium penta chlorophenate. The efficacy of these chemicals is often short-lived and some of them can have adverse side effects. The dosage depends on pH and other characteristics of water to be treated and on the concentration of algae. Chlorophenyl dimethyl urea, Ethionine and 2.5-dichloro-3, 4-ditrothiophene are selectively algicidal to *Anabaena*, *Microcystis* and other cyanobacteria. The response of algae to copper sulphate varies from species to species. Some toxic cyanobacetria viz., *Anabaena*, *Anacystis*, *Aphanizomenon*, *Gomphosphaeria* etc., are highly susceptible to copper sulphate (Palmer, 1980). In algal cultures, copper toxicity is determined not by the total copper concentration present but by the cupric ion activity (Sunda and Lewis, 1978). Mc knight (1981) has shown that the concentration of the free copper ion also determines the toxic response of the phytoplankton in natural water bodies.

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