



# EFFECTS OF FUNGICIDE CM-75 ON GROWTH, BIOCHEMICAL COMPOUNDS AND SOME ENZYMES OF *FISCHERELLA MUSCICOLA* NDUPC001

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## Abstract

*Fischerella muscicola* NDUPC001 was isolated from agricultural fields of Varanasi, India and characterized by morphological as well molecular means. The organism was deposited at NAIMCC (NBAIM), Mau, India (Accession No. NAIMCC-C-00121). Fungicide CM-75 inhibited growth, biochemical composition and some enzymes of *Fischerella muscicola* NDUPC00. LC<sub>50</sub> conc. of fungicide was 6ppm and 3ppm, 6ppm, and 9ppm were concentrations of treatments. All concentrations of CM-75 inhibited the growth of cyanobacteria, and maximum inhibition was observed in 9ppm treatment. Treatment concentrations of fungicide decreased the Chl.-a and carbohydrate content of cyanobacteria with maximum inhibition of 29.29% and 29.9% respectively in 9ppm treatment. Total protein content was slightly increased (2.52%) in 3ppm treatment and decreased in other treatments with maximum inhibition of 19.26% in 9 ppm treatment. Fungicide inhibited the activity of Nitrate reductase and Glutamine synthetase with maximum inhibition of 35.11% and 21.74% respectively in 9ppm. The findings of the experiment suggest that high tolerance level of *Fischerella muscicola* NDUPC001 against fungicide CM-75.

**Key words:** Cyanobacteria, *Fischerella muscicola* NDUPC001 and CM-75

## Introduction

Cyanobacteria are capable of colonizing nearly all habitats on our planet (Whitton & Potts, 2000) and are widely distributed from aquatic to the terrestrial environment. It has been established that cyanobacteria play a significant role in soil genesis and conservation. Cyanobacteria produce humus on the soil surface after their death and decay, and it dissolves certain soil minerals for maintaining a reservoir of the element in a semi available form for higher plants. The pertinent genera in soils include *Anabaena*, *Cylindra spermum*, *Lyngbya*, *Microcoleus*, *Nodularia*, *Nostoc*, *Fischerella*, *Oscillatoria*, *Phormidium*, *Plectonema*, *scytonema*, *Synendra*, and *Tolypothrix*, etc. Role of cyanobacteria in fertility of rice fields are well established. Paddy fields favor the luxuriant growth of cyanobacteria, and most of the biological nitrogen fixation of this ecosystem is done by N<sub>2</sub>-fixing cyanobacteria (Irisarri *et al.*, 2001). Many nitrogen-fixing strains of cyanobacteria have been isolated

and used in biofertilizer consortia in Southeast Asian countries. Pesticides are regularly used in agricultural fields to decrease the loss due to pests. A score of fungi causes disease on plants throughout the world. Damage due to fungal diseases are one of the major problems of agriculture. Crop area of paddy is about 24% with pesticide use of 17.2% (Mamthanayagam and Sharinila, 2004). Use of fungicides increased from 10% (1996) to 21% (2000) amounting 10910 tons (Agnihotri, 2000). Fungicides besides controlling target fungi also have shown an adverse impact on beneficial microbes of soil including cyanobacteria. Fungicide zineb inhibited the growth of *Cylindro spermum* sp. and *Nostoc muscorum* at 1ppm and 5ppm respectively (Venkataraman and Rajyalakshmi, 1971). Soil fungicides nabam and vapan also showed varying degree of toxicity towards rice field cyanobacteria (Venkataraman and Rajyalakshmi, 1972). Captan decreased the growth of *Westiellopsis prolifica*, *Aulosira fertillissima*, *Nostoc* sp., *Tolypothrix tenues*, and *Calothrix* sp. (Gangawane and Saler, 1979a). The growth of *Westiellopsis* sp., *Aulosira* sp., *Calothrix* sp., *Nostoc*

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sp., and *Tolypothrix* sp. was decreased by treatment of Roval, a broad spectrum fungicide (Gangawane and Kulkarni, 1979b). CM-75 is a broad spectrum systemic fungicide. It is used to control leaf spot, rust diseases of groundnut, and blast disease of paddy crop. This fungicide is regularly applied in paddy fields of Varanasi. *Fischerella muscicola* NDUPC001 is one of most abundant cyanobacteria of agricultural fields of Varanasi and report on the effects of CM-75 on this cyanobacteria is lacking. Hence, This experiment was designed to study the effects of fungicide on growth, biochemical composition and some enzymes of *Fischerella muscicola* NDUPC001.

## Materials and Methods

### Cultivation of cyanobacteria and Pesticide Treatment

The cyanobacterium *Fischerella muscicola* NDUPC001 was isolated from agricultural soils of Varanasi, India, characterized by morphological method and confirmed by molecular means (16 rRNA gene, Accession No. JX912574). The strain was deposited at NAIMCC (NBAIM), Mau, India (Accession No. NAIMCC-C-000121). It was grown in nitrogen-free, BG-11 liquid medium (Stanier, 1971) in a culture room maintained at a temperature of  $28 \pm 2^\circ\text{C}$  and illuminated with fluorescent light of  $12 \text{ Wm}^{-2}$ .

Different concentrations of fungicide CM-75 was screened to determine  $\text{EC}_{50}$  which is the concentration of pesticide that reduces the growth of the sample population by 50% in comparison to control in a specified period of exposure. 6 ppm fungicide concentration was  $\text{EC}_{50}$  (Table 1). 3ppm, 6ppm and 9ppm levels of fungicide were decided for treatment and untreated cyanobacterial culture was control (Table 1).

### Growth and Biochemical Analysis

The growth of homogenous cultures were measured turbidometrically at 700nm in spectrophotometer-117 (Systronic). Myers and Kratz (1955) method was followed to measure Chlorophyll-a. Phenol-sulphuric method (Dubois *et al.*, 1956) was followed to measure total carbohydrate. Method of Lowry *et al.*, (1951) was followed to measure total protein content.

### Enzymatic study

Colorimetric methods of Snell and Snell (1949) was followed to measure the activity of nitrate reductase (NR). The activity of nitrate reductase was expressed as  $\mu\text{M NO}_2$  formed  $\text{mg chl}^{-1} \text{ min}^{-1}$ .

Method of Shapiro and Stadtman (1970) was followed to measure the activity of Glutamine synthetase

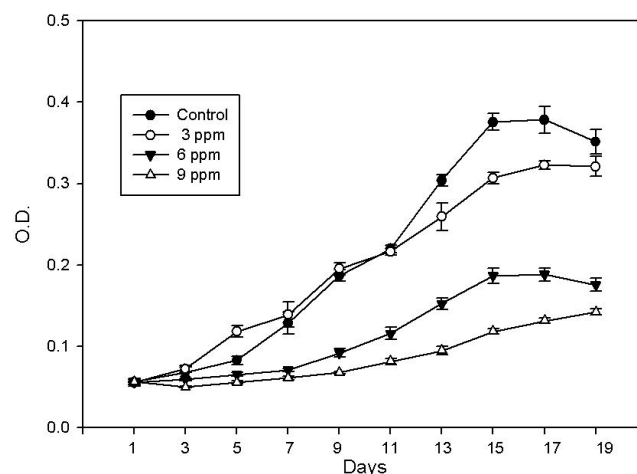
(GS). The activity of Glutamine transferase was expressed as m Moles glutamyl hydroxamate produced  $\text{mg chl}^{-1} \text{ min}^{-1}$ .

## Results and Discussion

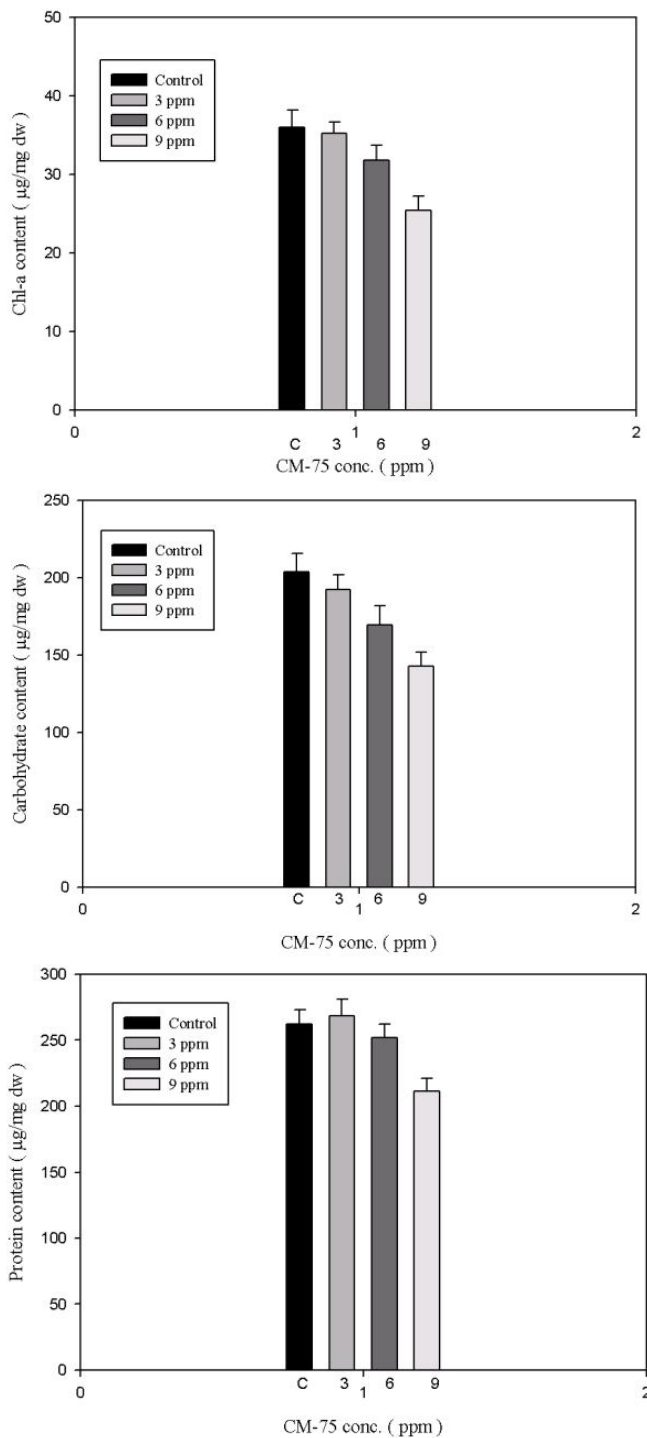
CM-75 is a broad spectrum systemic fungicide and composed of Carbendazim 12+ Mancozeb 63%. It is used to control leaf spot, rust diseases of groundnut, and blast disease of paddy crop. This fungicide is applied regularly in paddy fields of Varanasi. Effects of fungicide on growth, biochemical composition and some enzymes of *Fischerella muscicola* NDUPC001 was studied. Different concentrations of fungicide CM-75 was screened for determination of  $\text{EC}_{50}$  which is the treatment concentration of pesticide that reduces the growth of the sample population by 50% in comparison to control in a specified period of exposure.  $\text{LC}_{50}$  value of fungicide was 6ppm (Table 1). 3ppm, 6ppm and 9ppm concentrations (Table 1) of fungicide were treatment concentrations. Effect of 3ppm, 6ppm and 9 ppm conc. of CM-75 on *Fischerella muscicola* NDUPC001 growth behavior of was studied. All treatment concentrations of fungicide inhibited the growth of cyanobacteria (Fig. 1). Maximum inhibition was observed in 9ppm treatment. Growth was slightly induced in exponential phase in 3ppm but later on, decreased in stationary phase. A similar trend

**Table-1:**  $\text{LC}_{50}$  value of pesticide CM-75

Pesticide	Organism	$\text{LC}_{50}$ (ppm)	Treatment concentrations (ppm)
CM-75	<i>Fischerella muscicola</i> NDUPC001	6	3
			6
			9



**Fig. 1:** Growth behavior of *Fischerella muscicola* NDUPC001 in response to different concentrations of CM-75. Values are mean of triplicate  $\pm$  S.D., bars indicate standard deviation.

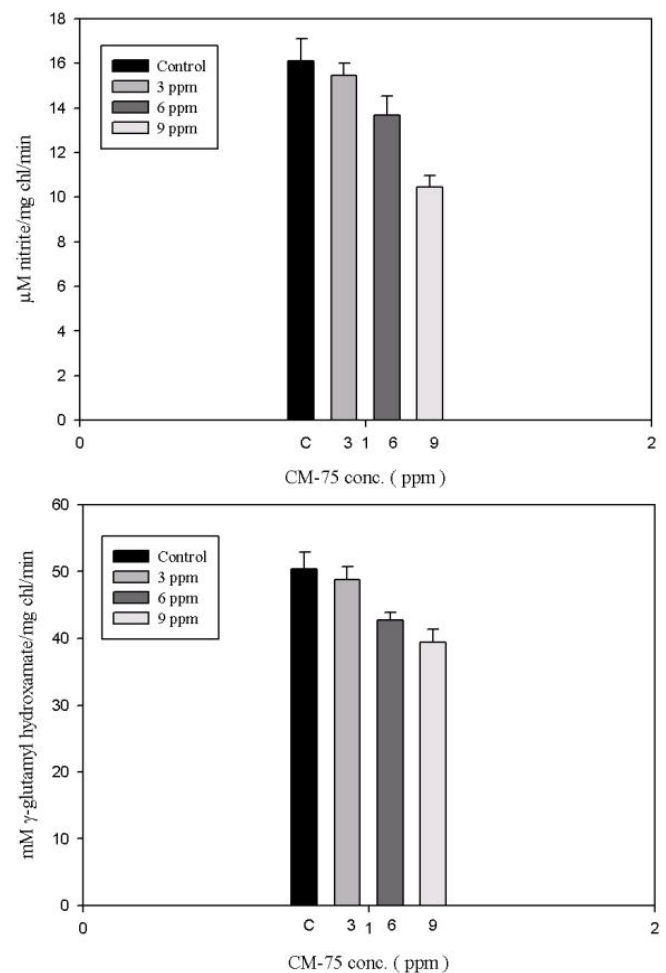


**Fig. 2:** Effect of CM-75 on biochemical composition (Chl-a, Carbohydrate, and Total protein) of *Fischerella muscicola* NDUPC001. Values are mean of triplicate  $\pm$  S.D. bars indicate standard deviation.

of the effect of fungicide on cyanobacteria was observed. Fungicide zineb inhibited the growth of *Cylindro spermum* sp. and *Nostoc muscorum* at 1 ppm and 5 ppm respectively (Venkataraman and Rajyalakshmi, 1971). Soil fungicides nabam and vapan also showed varying degree of toxicity towards rice field cyanobacteria (Venkataraman and

Rajyalakshmi, 1972). Captan decreased the growth of *Westiellopsis prolifica*, *Aulosira fertillissima*, *Nostoc sp.*, *Tolypothrix tenues*, and *Calothrix sp.* (Gangawane and Saler, 1979). The growth of *Westiellopsis sp.*, *Aulosira sp.*, *Calothrix sp.*, *Nostoc sp.*, and *Tolypothrix sp.* was decreased by treatment of Roval, a broad spectrum fungicide (Gangawane and Kulkarni, 1979). Most of the cyanobacteria showed tolerance limit of 5-6 ppm to fungicides but *Fischerella muscicola* NDUPC001 have tolerance limit up to 9 ppm (Fig. 1).

Effect of all treatments on biochemical composition (Chl-a, Total protein, and Carbohydrate) of *Fischerella muscicola* NDUPC001 was studied. All treatment concentrations of fungicide decreased the Chl-a, carbohydrate and protein content of cyanobacteria with maximum inhibition of 29.29%, 29.9%, and 19.26% respectively in 9 ppm treatment (Fig. 2) However, the decrease in biomolecules content was lesser than other reported cyanobacteria. Bavistin decreased the chl-a of



**Fig. 3:** Effects of CM-75 on Nitrate reductase (NR) and Glutamine synthetase (GS) activity of *Fischerella muscicola* NDUPC001. Values are mean of triplicate  $\pm$  S.D. bars indicate standard deviation.

*Tolypothrix scytonemoides* (Rajendran *et al.*, 2006). chlorophyll-a content of *Anabaena fertilissima*, *Aulosira fertilissima* and *Westilopsis prolifica* was decreased by fungicide Tebuconazole (Kumar *et al.*, 2012). Carbohydrate content of cyanobacteria *Anabaena fertilissima*, *Aulosira fertilissima* *Westilopsis prolifica* decreased up to 94 %, 96% and 97% respectively by tebuconazole treatment (Kumar *et al.*, 2012). Bavistin decreased the carbohydrate content of *Tolypothrix scytonemoides* (Rajendran *et al.*, 2006). Total protein content was slightly induced in conc. below LC<sub>50</sub> treatment (Fig. 2) and decreased in the other two treatments. Induction of total protein content in lower conc. of treatment may be due to the formation of stress proteins. Some fungicide like Tebuconazole decreased the total protein content of *Tolypothrix scytonemoides* (Rajendran *et al.*, 2006) and *Anabaena fertilissima*, *Aulosira fertilissima*, and *Westilopsis prolifica* up to 90 %, 95% and 93% respectively (Kumar *et al.*, 2012).

All the treatment concentrations of CM-75 inhibited the Nitrate reductase (NR) and Glutamine synthetase (GS) activity of *Fischerella muscicola* NDUPC001 (Fig. 3). NR and GS activity of cyanobacteria was also decreased by fungicides treatment. NR activity of *Anabaena fertilissima*, *Aulosira fertilissima*, *Westilopsis prolifica* was decreased by 90%, 93% and 93% respectively on fungicide Tebuconazole treatment (Kumar *et al.*, 2012). GS activity of *N. elliposporum* was decreased upto 70% on Bagalol treatment and up to 46% in *W. prolifica* on Mancozeb treatment ( Debnath *et al.*, 2012). Bavistin treatment decreased the GS activity of *Tolypothrix scytonemoides* (Rajendran *et al.*, 2006).

*Fischerella muscicola* NDUPC001 showed high tolerance range to fungicide CM-75 and amount of decrease in growth, biomolecules and enzymes ( NR&GS) was lesser than other fungicides as per available reports. Hence, *Fischerella muscicola* NDUPC001 may be considered as suitable cyanobacterial inocula for agricultural fields of Varanasi.

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