Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 9, Issue, 1(F), pp. 23297-23300, January, 2018

ISSN: 0976-3031

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

EFFECTS OF FUNGICIDE ON GROWTH, BIOCHEMICAL COMPOSITION AND SOME ENZYMES OF *NOSTOC ELLIPSOSPORUM* NDUPC002

Mishra S.K., Jyoti Singh and Dwivedi N*

Department of Botany, U.P. College (Autonomous), Varanasi, India

DOI: http://dx.doi.org/10.24327/ijrsr.2018.0901.1440

ARTICLE INFO

Article History:

Received 15th October, 2017
Received in revised form 21st November, 2017
Accepted 05th December, 2017
Published online 28th January, 2018

Key Words:

Cyanobacteria, *Nostoc ellipsosporum* NDUPC002 and CM-75

ABSTRACT

Effects of fungicide CM-75 on growth, biochemical composition and some enzymes of *Nostoc ellipsosporum* NDUPC002 was studied. The cyanbacterium *Nostoc ellipsosporum* NDUPC002 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-000122). LC_{50} conc. of fungicide was 2ppm. 1ppm, 2ppm, and 4ppm were concentrations of treatments. All concentrations of CM-75 inhibited the growth of cyanobacteria, and maximum inhibition was observed in 4ppm treatment. All treatment concentrations of fungicide decreased the Chl.-a and carbohydrate content of cyanobacteria with maximum inhibition of 23.63% and 19.98% respectively in 4ppm treatment. Total protein content was slightly increased (1.57 %) in 1ppm treatment and decreased in other treatments with maximum inhibition of 15.47 % in 4 ppm treatment. All concentrations of fungicide inhibited the activity of Nitrate reductase and Glutamine synthetase with maximum inhibition of 58.93% and 41.15 % respectively in 4ppm. The findings of experiment suggested that even 1 ppm conc. of CM-75 was inhibiting the growth, biochemical composition and some of the enzymes (NR and GS) of *Nostoc ellipsosporum* NDUPC002.

Copyright © Mishra S.K., Jyoti Singh and Dwivedi N, 2018, this is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Cyanobacteria improve the soil fertility by increasing organic content, water holding capacity, nitrogen content, phosphate solubilization, and secretion of polysaccharides (Tiwari *et al*., 1991; Whitton and Potts, 2000). These properties of cyanobacteria prove it suitable and eco-friendly biofertilizer. Tolerant strains of cyanobacteria to regularly used pesticides and potential to degrade them are desirable qualities for cyanobacterial biofertilizer. Paddy fields favor the luxuriant growth of cyanobacteria and most of the biological nitrogen fixation of this ecosystem is done by N_2-fixing cyanobacteria (Irisarri *et al*., 2001). Many nitrogen-fixing strains of cyanobacteria have been isolated and used in biofertilizer consortia in Southeast Asian countries.

Fungicides, herbicides, and insecticides are common pesticides used in agricultural fields. Pesticides help in improving agricultural productivity. Fungal diseases are most common on plants throughout the world. Substantial amount of fungicides are being poured in fields regularly. Fungicides besides controlling fungi also cause adverse effects on non-target organisms including cyanobacteria. Fungicides Dichlone, Dithane, Bavistin, Blitox, Captafol, Panacide, mercuric chloride, Carbendazim, Thiram, and Fytolan have shown toxic effects on nitrogen-fixing cyanobacteria (Rajendran *et al*., 2006). Fungicide tebuconazole reduced the growth, photosynthetic pigments, carbohydrate, protein and enzymes (Nitrate reductase, Glutamine synthetase, and Succinate dehydrogenase) of *Anabaena fertilissima*, *Aulosira fertilissima* and *Westillopsis prolifica* (Kumar *et al*., 2012).

Fungicide CM-75 is being frequently used in agricultural fields of Varanasi. Hence, this experiment was designed to study effects of CM-75 on growth, biochemical composition and some enzymes of cyanobacteria *Nostoc ellipsosporum* NDUPC002.

MATERIALS AND METHODS

Cultivation of cyanobacteria

The cyanobacterial strain *Nostoc ellipsosporum* NDUPC002 was grown in nitrogen-free, BG-11 liquid medium (Stanier, 1971) in a culture room maintained at a temperature of 28 ± 2^0 C and illuminated with fluorescent light of 12 Wm^{-2}. The strain was isolated from agricultural soils of Varanasi, India, characterized by the morphological method and confirmed by molecular means (16 rRNA gene, Accession No. JX912574).

Corresponding author:* **Dwivedi N
Department of Botany, U.P. College (Autonomous), Varanasi, India

The strain was deposited at NAIMCC (NBAIM), Mau, India (Accession No. NAIMCC-C-000122).

Pesticide Treatment

CM-75 is frequently used the fungicide in rice fields of Varanasi, India. Technically it is composed of Carbendazim (12.25%w/w), Mancozeb (74.12 % w/w), Sodium salt of alkyl aryl sulphonate (2.00%w/w), the Sodium salt of alkyl naphthyl sulphonate (2.000%w/w) and Kaolin (9.63%w/w).

Various concentrations of fungicide CM-75 was screened for determination of EC_{50}. EC_{50} is the concentration of pesticide that reduces the growth of sample population by 50% in comparison to control in a specified period of exposure. 2 ppm fungicide concentration was EC_{50} (Table-1). 1ppm, 2ppm and 4ppm concentrations of fungicide were decided for treatment, and untreated cyanobacterial culture was control (Table-1).

Table 1 LC_{50} value of fungicide CM-75

Pesticide	Organism	LC_{50} (ppm)	Treatment concentrations (ppm)
CM-75	*Nostoc ellipsosporum* NDUPC002	2	1 2 4

Growth and Biochemical Analysis

The growth of homogenous cultures was measured turbidometrically at 700nm in spectrophotometer-117 (Systronic). Chlorophyll-awas measured by the method prescribed by Myers and Kratz (1955). Total carbohydrate was measured by the phenol-sulphuric method (Dubois *et al*., 1956). The total protein content was measured by the method of Lowry *et al*., 1951.

Enzymatic study

The activity of nitrate reductase (NR) in cell suspension was estimated by colorimetric methods of Snell and Snell (1949). Nitrite formed was calculated by the standard graph. The activity of nitrate reductase was expressed as µM NO_2 formed mg chl^{-1} min^{-1}.

Glutamine synthetase (GS) activity was determined by the method of Shapiro and Stadtman (1970). The activity of Glutamine transferase was expressed as mMoles glutamyl hydroxamate produced mg chl^{-1} min^{-1}.

RESULTS

CM-75 is broad spectrum systemic fungicide. It successfully controls leaf spot, rust diseases of groundnut, and blast disease of paddy crop. This fungicide is regularly used in paddy fields of Varanasi. Effects of fungicide on growth, biochemical composition and some enzymes of *Nostoc ellipsosporum* NDUPC002 was studied. LC_{50} value of fungicide was 2ppm (Table-1). 1ppm, 2ppm and 4ppm concentrations (Table- 1) of fungicide were decided to study effects on Growth, Biochemical composition, Nitrate reductase and Glutamine synthetase enzyme of *Nostoc ellipsosporum* NDUPC002.

Effect of 1ppm, 2ppm and 4 ppm conc. of CM-75 on growth behavior of *Nostoc ellipsosporum* NDUPC002 was studied. All concentrations of fungicide inhibited the growth of cyanobacteria (Fig.-1). Maximum inhibition was observed in 4ppm treatment. Growth was slightly induced in exponential phase in 1ppm but later on, decreased in stationary phase. An intermediate amount of growth inhibition was observed in LC_{50} concentration (Fig.-1).

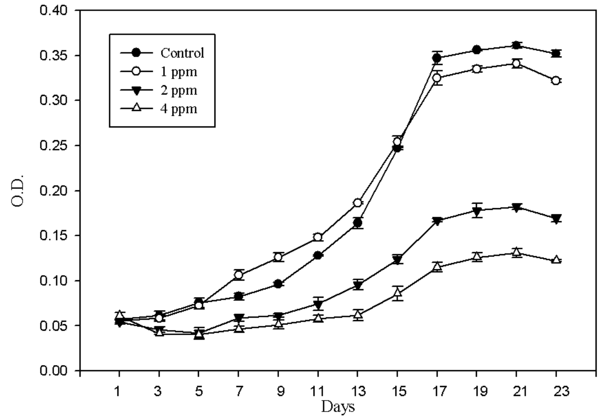

Fig 1 Growth behavior of *Nostoc ellipsosporum* NDUPC002 in response to different concentrations of CM-75. Values are mean of triplicate±S.D., bars indicate standard deviation.

Effect of treatments on biochemical composition of *Nostoc ellipsosporum* NDUPC002 was studied. All treatment concentrations of fungicide decreased the Chl.-a and carbohydrate content of cyanobacteria with maximum inhibition of 23.63% and 19.98% respectively in 4ppm treatment (Fig.-2). Total protein content was slightly increased (1.57 %) in 1ppm treatment and decreased in other treatments with maximum inhibition of 15.47 % in 4 ppm treatment (Fig.-2).

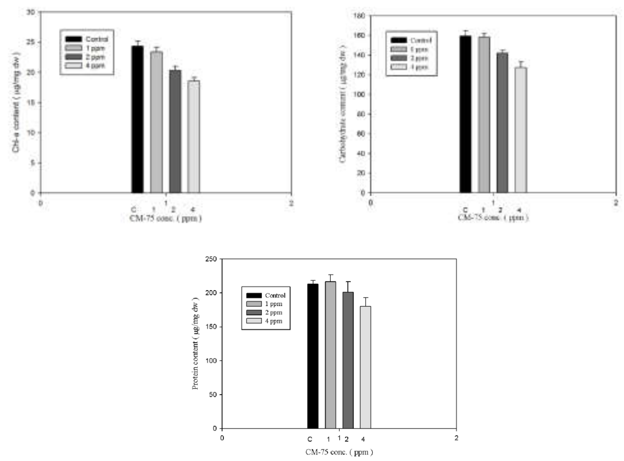

Fig 2 Effect of CM-75 on biochemical composition of *Nostoc ellipsosporum* NDUPC002. Values are mean of triplicate±S.D. bars indicate standard deviation.

Effects of treatments on the activity of Nitrate reductase and Glutamine synthetase was studied. All treatment concentrations of fungicide inhibited the activity of Nitrate reductase and Glutamine synthetase with maximum inhibition of 58.93% and 41.15 % respectively in 4ppm (Fig.-3).

DISCUSSION

According to Food and Agriculture Organization of United Nations (FAOSTAT-Agriculture\\Data, http://apps.fao.org), the world trade of pesticides in 1999 amounted to more than $22billion, of which about 25% was for fungicides.

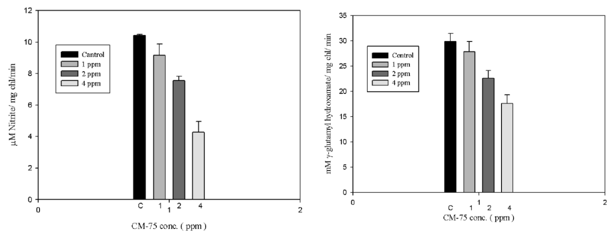

Fig 3 Effects of CM-75 on some enzymes of nitrogen metabolism of *Nostoc ellipsosporum* NDUPC002. Values are mean of triplicate±S.D. bars indicate standard deviation.

Our country is one of the largest consumer of pesticides and highest among south Asian counties (Agnihotri, 2000). Use of fungicides increased from 10% (1996) to 21% (2000) amounting 10910 tons (Agnihotri, 2000). The adverse impact of pesticides on non-target beneficial organisms including cyanobacteria are well established. Fungicides Dichlone, Dithane, Bavistin, Blitox, Captafol, Panacide, mercuric chloride, Carbendazim, Thiram, and Fytolan have shown toxic effects on nitrogen-fixing cyanobacteria (Rajendran *et al*., 2006). Heterocystous cyanobacteria are important contributors to nitrogen economy of agricultural fields. Fungicide CM-75 are frequently used in agricultural fields of Varanasi, India. All the treatments of the CM-75 decreased the growth of cyanobacteria (Fig.-1). Fungicide Tebuconazole decreased the growth and chlorophyll-a content of *Anabaena fertilissima*, *Aulosira fertilissima* and *Westillopsis prolifica* (Kumar *et al*., 2012). Fungicides Bagalol and Mancozeb decreased the growth of four cyanobacteria, i.e., *Nostoc ellipsosporum*, *Scytonema simplex, Tolypothrix tenuis*, and *Westiellopsis prolifica* (Debnath *et al*., 2012). Most of the pesticides inhibit the photosynthetic processes of phototrophs, decrease the biomolecules content and are supposed to be the major reason for decrease in growth of cyanobacterial strain.

Pesticides also cause inhibition of photosynthetic pigments of cyanobacteria. Fungicide CM-75 decreased the chl-a content (Fig.-2) of *Nostoc ellipsosporum* NDUPC002. Fungicide Tebuconazole reduced the chl-a content of *Anabaena fertilissima* and *Westillopsis prolifica* (Kumar *et al*,. 2012). Induction of Active oxygen species (Mostafa and Helling, 2002) and adverse interaction with thylakoids by pesticides inhibit pigment synthesis and accelerate the degradation of pigments. All treatment conc. of fungicide, CM-75 inhibited carbohydrate content of *Nostoc ellipsosporum* NDUPC002. The similar trend was observed with Fungicide Tebuconazole which reduced the carbohydrate content of *Anabaena fertilissima, Aulosira fertilissima Westiellopsis prolifica* up to 94 %, 96% and 97% respectively (Kumar *et al*., 2012). Fungicide Tebuconazole reduced the total protein content of *Anabaena fertilissima, Aulosira fertilissima, and Westiellopsis prolifica* up to 90 %, 95% and 93% respectively (Kumar *et al*., 2012). Fungicide CM-75 slightly induced the total protein content in 1 ppm treatment (Fig.-2) and decreased in other two treatments. Induction of total protein content in 1ppm conc. of treatment may be due to the formation of stress proteins.

All treatment concentrations of fungicide inhibited the activity of Nitrate reductase and Glutamine synthetase with maximum inhibition of 58.93% and 41.15 % respectively in 4ppm (Fig.-3). The similar effect was also observed with Tebuconazole which reduced the NR activity of *Anabaena fertilissima, Aulosira fertilissima Westiellopsis prolifica* by 90%, 93% and 93% respectively (Kumar *et al*., 2012). Tebuconazole reduced the GS activity of *Anabaena fertilissima, Aulosira fertilissima Westiellopsis prolifica* by 59%, 95% and 90% respectively (Kumar *et al*., 2012). Fungicide Bagalol inhibited GS activity by 70% in *N. ellipsosporum*, and Mancozeb inhibited GS activity by 46% in *W. prolifica* (Debnath *et al*., 2012).

Adverse impacts of pesticides on non-target beneficial micro-organisms are well established. *Nostoc ellipsosporum* is one of the common cyanobacteria of agricultural fields of Varanasi. Findings of experiment suggested that even 1 ppm concentration of fungicide CM-75 decreased growth, biochemical composition, the activity of Nitrate reductase (NR) and Glutamine synthetase (GS) of *Nostoc ellipsosporum* NDUPC002.

Acknowledgement

Dr. Nagendra Dwivedi is thankful to the University Grants Commission (UGC), New Delhi for providing financial assistance.

References

Agnihotri, N.P. (2000). Pesticide Consumption in Agriculture in India-an Update. *Pesticide Research Journal*, I2 (1) : 150-155.

Debnath, M., Mandal, N. C. and S. Ray. (2012) Effect of fungicides and insecticides on growth and enzyme activity of four cyanobacteria. *Indian J. Microbiology*, 52 (2): 275-280.

Dubois, K., Gills. K.A. Hemiton, J.K. Rebers, P.A. and Smith, P. 1956. Colorimetric method for determination of sugars and relation substances. *Anal.Chemi*. 28: 350-356.

Irisarri, P., Gonnet, S. and Monza, J. 2001. Cyanobacteria in Uruguayan rice fields; Diversity, nitrogen-fixing ability, and tolerance to herbicides and combined nitrogen, *J. Biotechnol.* 91: 95-103.

Kumar, N., Bora, A., Kumar, R. and Amb, M.K. 2012. Differential effects of agricultural pesticides endosulfan and tebuconazole on photosynthetic pigments, metabolism and assimilating enzymes of three heterotrophic, filamentous cyanobacteria. *J Biol Environ Sci*., 6(16): 67-75.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the folin phenol. Reagent. *J. Biol. Chem*. 193: 265-275.

Mostafa, F.I. and Helling, C.S. 2002. Impact of four pesticides on the growth and metabolic activities of two photosynthetic algae. *J Environ Sci Health*, 37: 417-444.

Myers, J. and W.A. Kratz. 1955. Relation between pigment content and photosynthetic characteristics in blue-green algae. *J. Gen. Physiol*. 39: 11-92.

Rajendran, U.M., Kathirvel, E. and Narayanaswamy, A. 2006. Effects of a fungicide, an insecticide, and a biopesticide on *Tolypothrix scytonemoides*. *Pesticide Biochemistry and Physiology*, 87: 164-171.

Shapiro, B. M., and Stadtman, E.R. 1970. Glutamine synthetase (E .coil). *Meth. Enzymol*. 17: 910-922.

Snell, F.D. and Snell, G.C. 1949. Nitrate by sulphanilamide and N-(1-naphthyl) ethylenediaminehydrochloride. In: Colorimetric Methods of Analyses (3rd Ed.), D. Von Nostrand Company, N.Y., Vol. 2, pp. 804-805.

Stanier, R.Y., Kunisawa, R., Mandel, M. and Cohen–Bazire, G. 1971. Purification and properties of unicellular blue-green algae (order chroococcales)-Bacteriological Reviews. 35: 171-205.

Tiwari, D.N., Kumar, A. and Mishra, A.K. 1991. Use of cyanobacterial diazotrophic technology in rice agriculture. *Appl Biochem Biotechnol* 28/29: 387–396.

Whitton, B.A. and Potts, M. 2000. Introduction to cyanobacteria. In: Whitton, B.A. and Potts, M, (Eds), The Ecology of Cyanobacteria: Their Diversity in Time and Space. Kluwer Academic, Dordretch, Netherlands, 1-11.

How to cite this article:

Mishra S.K., Jyoti Singh and Dwivedi N.2018, Effects of Fungicide on Growth, Biochemical Composition And Some Enzymes of Nostoc Ellipsosporum NDUPC002. *Int J Recent Sci Res.* 9(1), pp. 23297-23300.
DOI: http://dx.doi.org/10.24327/ijrsr.2018.0901.1440

****