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Effects of malathion on growth, biochemical composition and some enzymes of *Nostoc ellipsosporum* NDUPC002

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

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

*Corresponding author:Email: drnagendra.dwivedi@gmail.com

Abstract

Effects of malathion on growth, biochemical composition and some enzymes of *Nostoc ellipsosporum* NDUPC002 was studied. The cyanbacterial strain was isolated from agricultural fields of Varanasi, India. The strain was characterized by morphological as well molecular means. The organism was deposited at NAIMCC (NBAIM), Mau, India (Accession No. NAIMCC-C-000122). 5ppm, 10ppm, and 15ppm were concentrations of malathion treatments, and 10ppm was LC_{50} value. All treatments of malathion inhibited the growth of cyanobacteria, and maximum inhibition was observed in 15ppm treatment. Chl.-a was induced (12.33%) in 5ppm treatment and decreased in other two treatments with the maximum reduction of 15.41% in 15ppm treatment. Total protein and carbohydrate content was slightly induced with maximum induction of 5.72% and 8.08%, respectively in 10ppm treatment. Nitrate reductase (NR) activity was induced in 5ppm (12.47%) and reduced in other two treatments with maximum inhibition of 46.56% in 15ppm treatment. The activity of glutamine synthetase (GS) increased in 5ppm treatment (10.74%) and decreased in other two treatments with maximum inhibition of 32.29% in 15ppm treatment. The finding of experiment suggested that 10 ppm (LC_{50}) and above conc. of malathion was inhibiting the growth, biochemical composition and some of the nitrogen metabolism enzymes (NR and GS) of *Nostoc ellipsosporum* NDUPC002.

Key words: Cyanobacteria, Malathion, *Nostoc ellipsosporum* NDUPC002

Introduction

Cyanobacteria are the prokaryote, Gram-negative and oxygenic phototrophs (Wilmotte, 1994). They have a wide distribution and are ubiquitous in occurrence (Henson et al., 2004) including extreme habitats of the world (Schulz and Scherer, 1999). Role of cyanobacteria in soil fertility is well established. Paddy fields are one of the most favorable natural niches for the growth and proliferation of cyanobacteria (Whitton, 2000) where they play a major role in primary productivity as well as the nitrogen economy of that ecosystem. Cyanobacterial growth and diversity are mainly governed by soil physicochemical properties. They prefer a natural to alkaline p^H for optimum growth. Many cyanobacteria fix nitrogen and comprise one of the largest global suppliers of fixed nitrogen in flooded/irrigated rice fields (Singh,1961; Roger,1996). Many nitrogen-fixing strains of cyanobacteria have been isolated and used in biofertilizer consortia in South east Asian countries. Potentiality of cyanobacteria as biofertilizers, soil conditioners, plant growth regulators and soil health ameliorators has been well recognized (Vaishampayan *et al.,* 2001, Whitton, 2000). Members of the order nostocales and stigonematales are widespread having particular significance in these environments (Desikachary,1959).

The reduction of crop losses is the primary goal of agriculture. Synthetic chemicals have played a fundamental role in suppressing pests and maintaining high crop yields. The world trade of pesticides in 1999 amounted to more than $22billion, of which about 25% was for fungicides, 35% was for herbicides, and 21% was for insecticides (Food and Agriculture Organization of United Nations, FAOSTAT-Agriculture\\Data, http://apps.fao.org). The adverse environmental impact of pesticides has become a significant concern, from the mid-1970s. Persistence of the pesticides in topsoils, leaching to groundwater and their undesired effects on non-target organisms are responsible for environmental impacts. Pesticides affect non-target microorganisms including cyanobacteria. The tolerant cyanobacterial strains to a variety of routinely used agrochemicals are suitable biofertilizers in rice fields. Many experimental findings showed adverse effects of pesticides on heterocystous cyanobacteria (Mahapatra *et al.,* 2003; Galhano *et al*., 2008). Bagalol and Mancozeb (fungicides), Thiodan and phorate (insecticides) inhibited the growth, biochemical composition, nitrogenase and glutamine synthetase activity at EC_{50} concentration (Debnath *et al*., 2012). Insecticide profenofos decreased the growth and biomolecules of *Anabaena* sp. (Chaurasia, 2014). Insecticide Malathion is routinely used in agricultural fields of Varanasi. Hence, this experiment was designed to study effects of malathion on growth, biochemical composition

and some nitrogen metabolism enzymes of one of most abundant cyanobacteria of the region *Nostoc ellipsosporum* NDUPC002.

Materials and Methods

Cultivation of cyanobacteria

Nostoc ellipsosporum NDUPC002 was isolated from agricultural fields of Varanasi, India and characterized by morphological and molecular methods (Singh *et al.,* 2011). 16 rRNA gene of strain was amplified, sequenced and deposited to NCBI with Accession No. JX912574. The strain was deposited at NAIMCC (NBAIM), Mau, India (Accession No. NAIMCC-C-000122). The strain 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}.

Pesticide Treatment

EC_{50} was determined by screening various concentrations of insecticide malathion in logarithmic phase of growth. 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. 10 ppm pesticide concentration was EC_{50} (Table-1). 5ppm, 10ppm and 15ppm concentrations of pesticide treatment were decided, and cyanobacterial culture without any pesticide treatment was control (Table-1).

Table-1: LC_{50} value of pesticide Malathion

Pesticide	Organism	LC_{50} (ppm)	Treatment concentrations (ppm)
.Malathion	*Nostoc ellipsosporum* NDUPC002	10	5
			10
			15

Growth and Biochemical Analysis

The growth of homogenous cultures was measured turbidometrically at 700nm in spectrophotometer-117 (Systronic). Chlorophyll-a was 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). Cyanobacterium *Nostoc ellipsosporum* NDUPC002 was grown in BG-11 liquid nitrogen-free medium (Stanier, 1971) supplemented with 5ppm, 10ppm and 15ppm concentrations of pesticide. The strain was grown at a temperature of 28 ± 2^0 C and illuminated with fluorescent light of 12 Wm^{-2} for six days. Cyanobacterial suspension of all treatments and control was induced by adding 100 µM KNO_3 and after 8 hrs of the induction period cultures were incubated for two hrs. in 100mM KNO_3 then the reaction was terminated by adding 24% TCA. 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 exponentially growing cultures (10.0 ml) of treated and control in triplicates were centrifuged, and the pellets were suspended in 1.0 ml Imidazole buffer then treated with 0.5 ml toluene. shook vigorously and incubated for 20 minutes at 4^0 C. The samples were shaken again to allow complete permeabilization of the cell membrane then centrifuged and discarded the top toluene layer. The 0.5 ml of cell extraction buffer of each was treated with 0.8 ml of reaction mixture. The reaction mixture was incubated at 37^0C for 30.0 minutes then terminated by adding 2.0 ml of stop mixture. The turbid debris in the resultant solution was removed by centrifugation and intensity of coffee color solution was colorimetrically analyzed at 540 nm against the reagent blank prepared by eliminating glutamine and hydroxylamine from the reaction mixture. The activity of Glutamine transferase was expressed as mMoles glutamyl hydroxamate produced mg chl^{-1} min^{-1}.

Results

Malathion is an organophosphate insecticide and regularly used in agricultural fields of Varanasi. Role of cyanobacteria in fertility of agrarian fields is well established. *Nostoc ellipsosporum* is one of most abundant

cyanobacteria of agricultural fields of Varanasi. *Nostoc ellipsosporum* NDUPC002 was isolated from agricultural fields of Varanasi, characterized by morphological and molecular methods (16 rRNA). The strain was deposited at NBAIM, Mau with Accession No. NAIMCC-C-000122.

Different concentrations of malathion were screened to determine LC_{50} value. 10ppm was the LC_{50} value (Table-1). Concentrations of treatment, i.e. 5ppm, 10ppm and 15ppm (Table- 1) were decided to study effects of malathion on Growth, Biochemical composition and some enzymes of *Nostoc ellipsosporum* NDUPC002.

Effect of treatments on growth behavior of *Nostoc ellipsosporum* NDUPC002 was studied. All treatments of malathion inhibited the growth of cyanobacteria (Fig.-1). Maximum inhibition was observed in 15ppm treatment. Growth was slightly induced in exponential phase in 5ppm but later on, decreased in stationary phase. An intermediate amount of growth inhibition was observed in LC_{50} value treatment (Fig.-1).

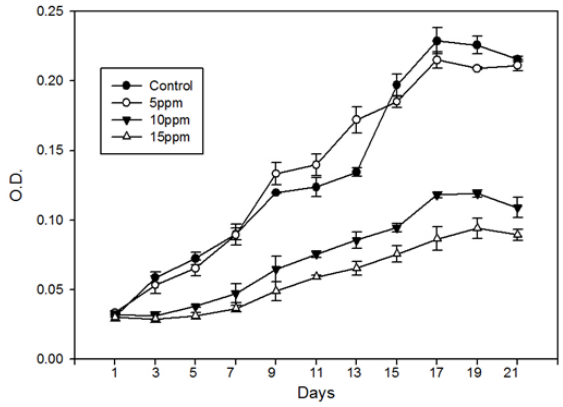


Fig. 1 : Growth behavior of *Nostoc ellipsosporum* NDUPC002 in response to different concentrations of malathion. Values are mean of triplicate±S.D., bars indicate standard deviation.

Effect of all treatments on biochemical composition (Chl-a, Total protein, and Carbohydrate) of *Nostoc ellipsosporum* NDUPC002 was studied. Chl.-a was induced (12.33%) in 5ppm treatment (Fig.-2) and decreased in other two treatments with a maximum reduction of 15.41% in 15ppm treatment (Fig.-2).

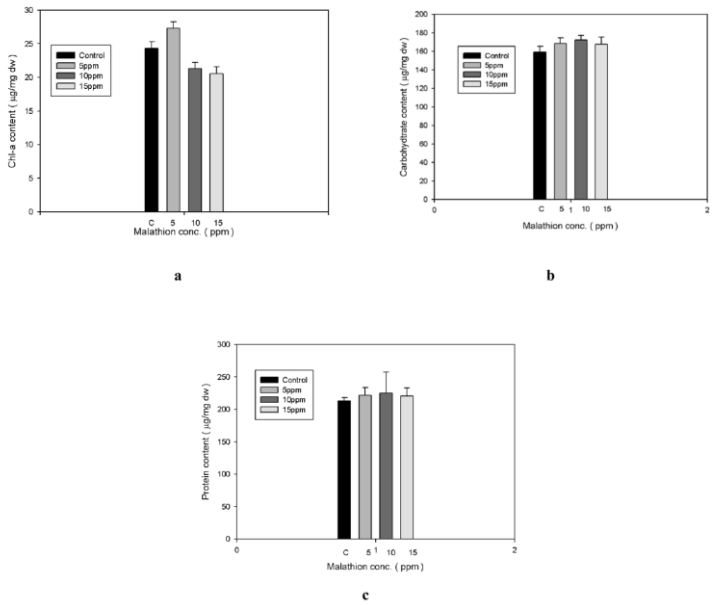
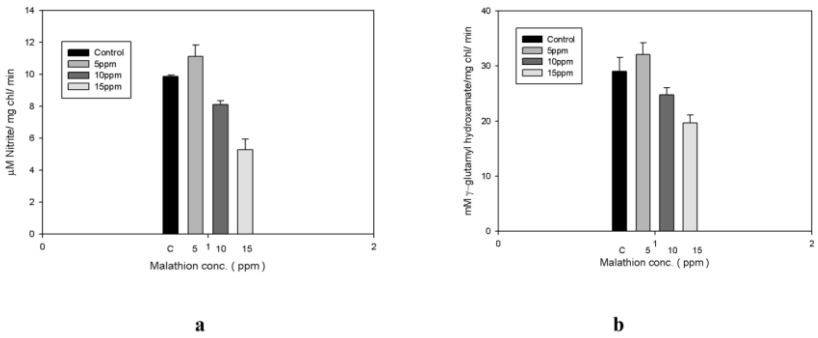


Fig.-2: Effect of malathion on biochemical composition of *Nostoc ellipsosporum* NDUPC002. Values are mean of triplicate±S.D.

Total protein content was increased in all treatments with maximum induction of 5.72% in 10ppm treatment (Fig.-2). Total carbohydrate was also induced in all treatments with maximum induction of 8.08% in 10ppm treatment (Fig.-2).

Effects of treatment on the activity of nitrate reductase and glutamine synthetase were studied. Nitrate reductase activity was induced in 5ppm (12.47%) and reduced in other two treatments with maximum inhibition of 46.56% in 15ppm treatment (Fig-3). A similar pattern of effect was observed on the activity of glutamine synthetase. The activity of glutamine synthetase increased in 5ppm treatment (10.74%) and decreased in other two treatments with maximum inhibition of 32.29% in 15ppm treatment (Fig.-3).



a b

Fig.-3: Effects of malathion on some enzymes of nitrogen metabolism of *Nostoc ellipsosporum* NDUPC002. Values are mean of triplicate±S.D.

Discussion

Pesticides are used in agricultural fields to minimize losses due to pests. Pesticides beside controlling the target pest also have an adverse effect on non-target organisms. Insecticide Malathion is one of common pesticide and regularly used in agricultural fields of Varanasi, India. 5ppm to the 15ppm treatment of malathion decreased the growth of *Nostoc ellipsosporum* NDUPC002 (Fig.-1). Insecticides Thiodan and Phorate reduced the growth up to 50% in the concentration ranging from 0.025 to 0.05 ppm and from 0.40 to 0.80 ppm respectively in all the four cyanobacteria i.e.. *Nostoc ellipsosporum, Scytonema simplex, Tolypothrix tenuis*, and *Westiellopsis prolifica* (Debnath et al., 2012). Insecticide endosulfan inhibited the growth of *Anabaena fertilissima, Aulosira fertilissima, Westiellopsis prolifica* (Kumar *et al*., 2012), *Nostoc calcicola* and *Nostoc muscorum* (Prasad *et al*., 2011). Several other pesticides also cause inhibition in growth of cyanobacteria. Most of the pesticides cause an adverse impact on the photosynthetic process of phototrophs and decreases the biomolecules content; this may be the reason for the decrease in the growth of cyanobacteria. Pesticides also cause decrease in biochemical composition of cyanobacteria. LC_{50} and above conc. of malathion decreased the chl-a content of *Nostoc ellipsosporum* NDUPC002 (Fig.-2). Insecticide endosulfan reduced the chl-a content of *Anabaena fertilissima, Aulosira fertilissima* and *Westiellopsis prolifica* (Kumar *et al*., 2012). Endosulfan also reduced the Carotenoids and Phycobiliprotein content of cyanobacteria (Kumar *et al*., 2012). Mostafa and Helling (2002) suggested insecticide stress induced the formation of Active oxygen species (AOS) which inhibit pigment synthesis and accelerate the degradation of pigments. Some researcher suggested that interaction of pesticide and thylakoids could be the reason for pigment degradation. Lower conc. (up to 5ppm) of endosulfan induced the carbohydrate content of *Nostoc muscorum* and *Anabaena variabilis* (Kumar, 2008). Endosulfan reduced the carbohydrate content of *Anabaena fertilissima, Aulosira fertilissima Westiellopsis prolifica* up to 97 % (Kumar et al., 2012). Malathion induced the carbohydrate content of *Nostoc ellipsosporum* NDUPC002 (Fig.-2) up to 15ppm treatment. Total protein content was slightly induced in all treatments of malathion (Fig.-2). A similar result was observed with lower conc. (7 ppm) of endosulfan treatment (Kumar, 2008) on *Aulosira fertilissima*. This finding suggests that lower conc. of insecticides are stimulating synthesis of stress proteins.

Malathion treatment decreased the NR activity of *Nostoc ellipsosporum* NDUPC002 (Fig.-3). The similar effect was observed by insecticide endosulfan treatment which decreased the NR activity of *Anabaena fertilissima, Aulosira fertilissima* and *Westiellopsis prolifica* by 77%, 90% and 95% respectively (Kumar *et al*., 2012). Glutamine synthetase (GS) leads to the conversion of ammonia to glutamine. Endosulfan decreased the GS activity of *Anabaena fertilissima, Aulosira fertilissima* and *Westiellopsis prolifica* (Kumar *et al.,* 2012). Phorate

inhibited GS activity by 62% in *Tolypothrix tenuis,* and Thiodan inhibited GS activity by 50% in both *Nostoc ellipsosporum* and *Westiellopsis prolifica* (Debnath *et al.,* 2012). Malathion also causes suppression of GS activity of *Nostoc ellipsosporum* NDUPC002 (Fig.-3). Chemical Pesticides are regularly used in agricultural fields to increase production, but these pesticides also have the adverse effect on non-target beneficial micro-organisms. Cyanobacteria are most common inhabitants of soil and ubiquitous in occurrence. Cyanobacteria play important role in fertility of the soil. *Nostoc ellipsosporum* is one of the common cyanobacteria of agricultural fields of Varanasi. Insecticide malathion is commonly used pesticide in agricultural fields of Varanasi. Our finding suggests 10 ppm (LC_{50}) and above conc. of malathion is causing suppression in growth, biochemical composition and some of the enzymes (NR and GS) of *Nostoc ellipsosporum* NDUPC002.

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