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Research Article

BIODEGRADATION AND RAPID REMOVAL OF DIMETHOATE BY CYANOBACTERIA

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ABSTRACT

Mainly pesticide pollution is a serious environmental problem and their remediation is necessary therefore cyanobacteria are used as removal contamination agent. In this study cyanobacteria were used to their growth and utilization of organophosphorus pesticide dimethoate as phosphorus source. These cyanobacteria belong to filamentous heterocystus genera. A sharp decrease in the growth of the cyanobacterial isolate was observed by increasing the concentration of dimethoate. Amongst them *Nostoc* tolerated different concentrations and was recorded as the highest efficient isolate are tolerate of this compound. Moreover, protein content of their cells overtopped the other isolates especially at higher concentrations. The cyanobacterial isolate was further subjected to grow in the absence of phosphorus and presence of dimethoate. Although, the cyanobacterial growth absence phosphorus recorded at very poor level, a massive enhanced growth and phosphorous content of cells were obtained when the dimethoate amended with media. Capabilities of *Nostoc* species to use dimethoate as sole phosphorous is considerably a cheaper and efficient tool for remediation of organophosphorus pesticides from contaminated agriculture soil which is clearly indicated from the above study.

Keywords: Cyanobacteria, Dimethoate, *Nostoc*, Isolate

1. INTRODUCTION

The concept of green revolution has played important role for utilization of variety of pesticides for high yield varieties. The progressive increase of pest problem and demand for agricultural products necessitated the application of agrochemicals and ensure high quality of crop yield [1]. Pesticides are most commonly detected in all aquatic environments [2]. These pesticides are mainly used for agricultural purposes [3]. They enter the aquatic environment via runoff after being sprayed in agricultural fields and can potentially reach groundwater [4]. Pesticides affect environment and health of humans as they are responsible for ground water and marine environmental pollution as well as they pollutes soil and crops.

Pesticide's continuous and excessive use of organophosphorus compounds has led to the contamination of several ecosystems in different parts of the world [5, 6]. Contamination of grains, vegetables and fruits with organophosphorus compounds is also well documented. Use of micro-organisms in detoxification decontamination of organophosphorus compounds is considered a viable and environment friendly approach.

Dimethoate [O, O-dimethyl S-methyl carbamoyl methyl phosphorodithioate] is a systemic organophosphorus insecticide, but is also considered as a carbamate pesticide due to the existence of a carbamate group in its chemical structure. It was patented and introduced in the 1950s by Ameican cyanamid. Dimethoate is considered one of the most important pesticides in killing a wide range of insects, including aphids, thrips, plant hoppers and whiteflies systemically and on contact. Dimethoate while acting as cholinesterase inhibitor, interfering with the activities of this enzyme is responsible for neurotransmission.

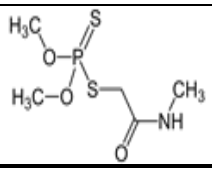
The half-life of dimethoate in soil ranges from 4 to 16 days [7]. Residues of dimethoate and its oxidized analogs have been detected in soil, fruits, vegetables, crops and even cow milk [8]. The physicochemical properties of dimethoate are given in Table 1.

Organophosphorus compounds are totally mineralized by the microorganisms. Most organophosphorus compounds are degraded by microorganisms in the environment as a source of phosphorus or carbon or both [9]. Different pathways of organophosphorus decomposition such as hydrolysis, photolytic oxidation, microbial transformations and other biological processes have been reported [10].

Photoautotrophic microorganisms, such as cyanobacteria, have potential to remove various pollutants, such as dyes

[11] heavy metals [12] and pesticides [13]. Cyanobacteria are one of the oldest life forms and they occupy almost all the possible habitat on the earth. They are a class of autotrophic microorganisms capable of photosynthesis and have a high photosynthetic efficiency (upwards of 10%), significantly better than land plants (~3-4% maximum efficiency) [14, 15].

Table 1: Physico-chemical properties of dimethoate

Chemical Name	Molecular Weight	Formula	Structure	Type	Solubility at 21°C in water(g/l)
O, O-Dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate	229.249g/mol	$C_5H_{12}NO_3PS_2$		Organophosphate insecticide	25 g/l

Majority of them fix atmospheric nitrogen into ammonia and they also mineralize organic phosphorus by producing alkaline phosphatases [16, 17] thus they play an important role in global cycling of carbon nitrogen as well as phosphorus. Cyanobacteria have adapted a molecular mechanism to cope up with Pi limitation by metabolizing organic form of phosphorus present in the environment. Larger fraction ~75% of total dissolve organic phosphorus is contributed by phosphoesters while phosphonate contributes ~25% in marine system [18, 19]. Cyanobacteria produce phosphatases to metabolize phosphoesters whereas phosphonates are metabolized by several pathways but C-P lyase pathway is considered as a major pathway of metabolism. Therefore, this study is conducted to investigate the survival and tolerance of cyanobacteria *Nostoc* with different concentrations of dimethoate, as well as evaluating their efficiency for rapid removing and tolerate this pesticide from contaminated agriculture field.

2. MATERIAL AND METHODS

2.1. Chemicals

All the chemicals used in the media preparation and assay of insecticide were obtained from Hi media, India. The organophosphorus pesticide used in this study is commercially available as dimethoate, was obtained from Bayer company, (35.5% active ingredient). Commercial standards of dimethoate were obtained from Sigma-Aldrich Co., USA.

2.2. Isolation, purification and identification of cyanobacteria

The cyanobacterial isolate (*Nostoc)* was isolated from rice field around Bhopal (23.25°N; 77.41°E) of Madhya Pradesh, India.

Isolation and purification of the organism was performed by standard isolation techniques including spread plate, streak plate method and regular sub-culturing [20]. Pure cultures were maintained in liquid BG11 medium (–N) under 28±1°C temperature and 25-30 µmol photons m^{-2} s^{-1} illumination in 16h light and 8h dark period [21]. The organism was identified following literatures and monograph [22]. The culture vessels were hand-shaken four to five times daily to keep the cultures in homogenous state.

2.3. Dimethoate exposure and uptake experiments

Dimethoate uptake experiments were conducted were in 500ml Erlenmeyer flasks containing 200ml of liquid BG-11 media (-N)supplemented with dimethoate at graded concentrations 1, 10, 20, 30, 40 and 50 ppm. Exponentially growing cultures were inoculated to get initial absorbance 0.5 at 720 nm. To test the cyanobacterial ability using dimethoate as a phosphate source, the BG-11 medium was modified by replacing K_2HPO_4 with dimethoate as a phosphate source. The culture flasks were kept under standard condition during the experiment. Samples were taken after every four-day intervals up to twenty days for the estimation of the growth and protein content in the tested cyanobacterial culture. Protein content of cyanobacterial biomass was determined according to Lowry [23]. Cell growth and total phosphorus content in the cyanobacterial biomass was measured by spectrophotometer at 760 nm [24].

After 20days, 50ml of cyanobacterial cultures was filtrated by centrifugation at 5000 rpm for 20 minutes. The cyanobacterial filtrate was used to determine dimethoate in the culture medium. Then the pellet was disrupted by lysozyme enzyme and extracted three times

with dichloromethane. The solution was centrifuged at 5000 rpm for 10 min. Dichloromethane layer was since then concentrated to final volume of 0.5ml after the discarding of water phase. The supernatant was collected and filtered through a 0.45 μm pore-size filter for analysis in HPLC. An analytical Perkin Elmer HPLC System (USA) was used to analyze the dimethoate. Samples were analyzed by a capillary column phenomenexluna C18 (250×4.6). The mobile phase selected was acetonitrile and water in the ratio 60:40 (v/v) with a flow rate of 1.0 ml/min. The sample volume of 10μl was injected into an oven temperature 30°C, with a UV detector, and detecting wavelength was set at 205 nm. The retention times of dimethoate were identified according to the retention time of the reference standard [25].

2.4. Statistical analysis

Data were presented as mean of replicates from three runs and were analyzed statistically using t test. Statements of significant differences were based on accepting $P \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Isolation, purification and identification of cyanobacterial isolates

The cyanobacterial isolate (*Nostoc*) were isolated from rice field around Bhopal of Madhya Pradesh, India. The pure culture was obtained after primary and secondary screening on BG-11 (-N) media.

The pure isolate was examined for its morphological characterization under the compound microscope (Leica analytical system, Mumbai). These isolate morphologically belonged to heterocystous filamentous with the presence of heterocyst and akinetes that may belong to group *Nostocales*. Microscopic observation of isolate with different characteristics such as cell shape and size, heterocyst, akinetes shapes. thallus gelatinous, colonial, dark blue green in color, colony wide round, bottom dwellers; filaments highly coiled and densely entangled; trichrome 3-4μm broad; cells spherical to barrel shaped, short; heterocyst nearly spherical and 4-7μm broad shown in fig.1.

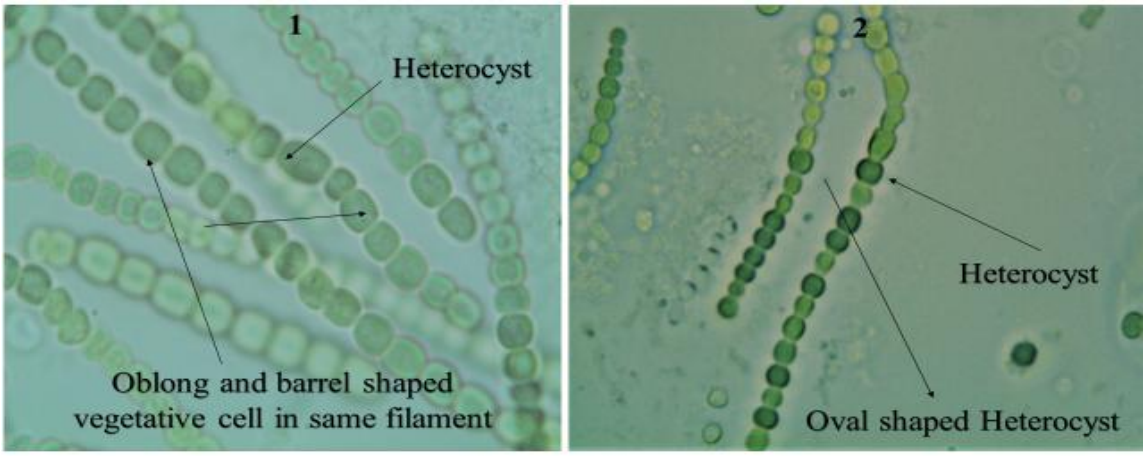


Fig. 1: Microphotographs of morphologically identified genera of cyanobacterial isolate (*Nostoc*) showing heterocyst and vegetative cell under 100X(1) and 40X(2) magnification

3.2. Effect of dimethoate on growth of *Nostoc*

In this study, dimethoate was introduced to BG-11 medium at concentrations of 1, 10, 20, 30, 40 and 50 ppm and its effects on the growth of *Nostoc* were evaluated over 20 days. The typical growth patterns of *Nostoc* in the absence of phosphorous and presence of graded concentration of dimethoate is shown in fig.2. However, with the increase of the concentration of dimethoate, the growth was increase. The growth of *Nostoc* showed the tolerance to higher concentration of dimethoate; its growth maintained to continue under the highest concentration applied. In the absence of phosphorus content in media the cyanobacterial cells can use dimethoate as phosphorous source. A relationship between dimethoate concentration and the cyanobacterial growth was recorded. At concentrations of dimethoate (50 ppm), the maximum growth of *Nostoc* was achieved within 20 days recording an increment of the total growth by respectively. Different cyanobacteria showed different tolerance to the dimethoate; the reason could be due to their surface composition and surface area exposed to the

dimethoate. For instance, cyanobacteria have a thicker layer of extracellular polysaccharide, which may aid the cells to tolerate the toxicity in the environments. The intracellular and extracellular enzymes involved in the degradation of dimethoate are also important in supporting the cells that survive in the stress conditions.

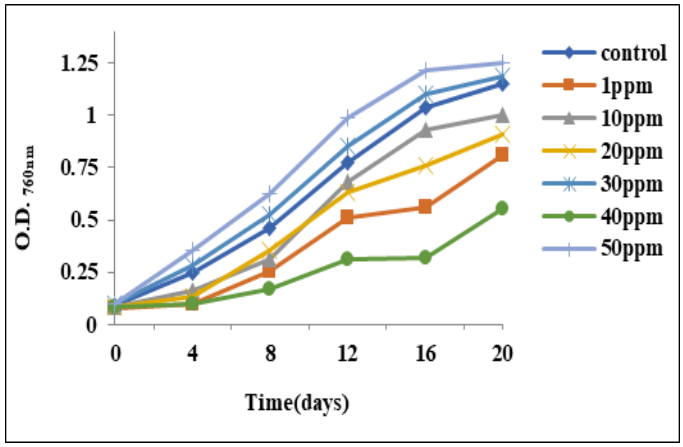


Fig. 2: Effect of different concentration of dimethoate on growth of *Nostoc*

3.3. Effect of dimethoate on protein content of *Nostoc*

Concerning protein content of *Nostoc*, it is evident from fig. 3 that treatment of *Nostoc* with dimethoate, significantly increased protein content at higher concentrations (50 ppm). The organophosphorus pesticide dimethoate stimulates protein content of *Nostoc* [26]. Such a phenomenon may be due to the presence of some enzymes which can hydrolyze this organophosphorus compound and utilize dimethoate as nutrient sources [13].

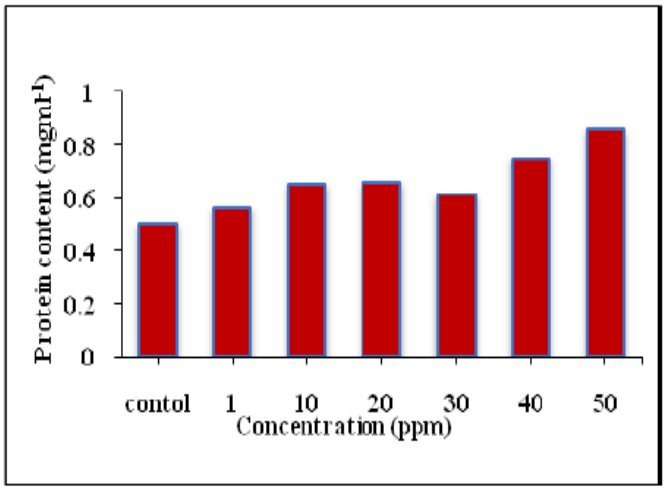


Fig. 3: Effect of different concentration of dimethoate on protein content of *Nostoc*

3.4. Dimethoate removal

Microorganisms may tolerate insecticide either at uptake level or at intracellular level. Dimethoate uptake by *Nostoc* was studied to test whether the organism was able to take up and degrade the insecticide intracellularly. The organism removed 0.040 mg dimethoate in 20 days, its mean 90 % of dimethoate respectively, from medium containing 50 ppm dimethoate. From these results, it appears that dimethoate is initially adsorbed on the surface of the biomass followed by slow intracellular uptake. In the present study, slight decrease in dimethoate was observed when the amount of the insecticide was determined on day 8 in the control flask, which indicated that depletion of dimethoate compound from the medium was biological and is stable under laboratory condition.

3.5. Dimethoate degradation

Six major peaks with the retention time 1.70, 1.83, 2.15, 2.48, 3.11, and 5.51 min were observed in the HPLC chromatogram of cell extracts (fig.4). The peak with retention time 3.11 matched with standard dimethoate, whereas peak with retention time 3.05 corresponded to standard. The presence of dimethoate in all extracts indicated that the organism degraded the insecticide intracellularly as well as extracellularly. Enzyme is found on the surface and also intracellularly which is further showed by them. It is also reported that phosphatases play an important role in the biodegradation of dimethoate. The microbial consortia grew well by utilizing dimethoate as was evident from the increase in optical density (fig.2). The simultaneous loss of dimethoate from the culture was observed by HPLC analyses (fig.4). It was observed that the cyanobacteria could grow in dimethoate-containing medium as the only carbon source. The medium was converted to a milky emulsion as a result of the released of some of the bio surfactants and extracellular enzymes, thus assisting in degrading this compound. On the other hand, cell-free extracts were used to detect enzymes. It is evident that cyanobacteria release exo and end cellular enzymes together with bio surfactants to help degrade dimethoate. Bioaccumulation of dimethoate compound in water is due to its lipophopic nature, which is partially explained. This utilization of the compound is similar to the finding of Deshpande who observed that organochlorine compounds are degraded by emulsification due to their lipophilic nature [27]. Moreover, organophosphorus accumulation rapidly diminishes when they are removed

from the medium, because of their higher bioavailability and metabolization rates [28].

Dimethoate might have metabolized by detoxifying enzymes which is revealed through the study of results. Moreover, the presence of other polar metabolites stimulated the activity of other detoxifying enzymes, such as phosphor triesterases. Microorganisms degrading xenobiotic chemicals have elaborate enzyme systems. Biodegradation of organophosphates involve activities of enzymes phosphatase, esterase, hydrolase and oxygenase. Chemical hydrolysis of twelve common used organophosphorus insecticides is found to be much slower than enzymatic hydrolysis. Therefore, it was concluded that the enzyme in crude extract could degrade the P-S linkage of dimethoate which is different from parathion hydrolases, which attack the P-O bond in gram negative bacterial strains and produced the metabolites of the compound [29].

Membranes permeability thus altered due to the adsorption of this compound on the rich-lipid plasma membranes which is due to the inhibitory effect of dimethoate [30] and diminishing photosynthetic activity 31, 32] as well as increasing reactive oxygen species (ROS) during stress [33]. *Nostoc* was the most tolerant to dimethoate. Kumar *et al.*, studied the tolerance of three cyanobacterial isolates to endosulfan and record the tolerance in the order of *Nostoc*>*Anabaena variabilis*>*Anabaena fertilissima* [34]. Such a may be due to the presence of some enzymes which can hydrolyze this organophosphorus compound and utilize dimethoate as nutrient sources [35]. Therefore, total phosphorus content of algal biomass was estimated in order to confirm their capability to utilize dimethoate as a phosphorus source. The phosphorus content of the cells which grew absence phosphorus and in presence of dimethoate was much higher than that found in cells cultured under the same conditions but without the addition of dimethoate revealing the capability of this species to break down and utilize dimethoate as a sole phosphorus source.

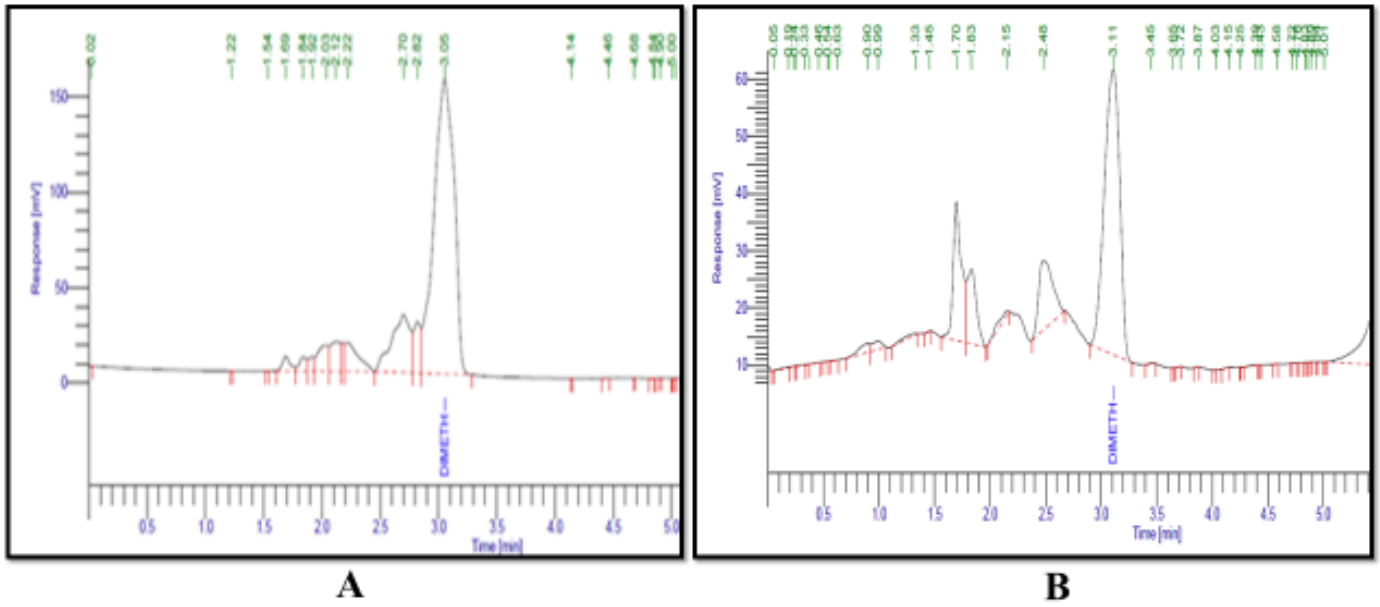


Fig. 4: Quantification of dimethioate by HPLC chromatogram (A) Standard of Dimethioate (B) ***Nostoc***

4. CONCLUSION

The present study is evidence of the ability of *Nostoc* to remove and utilize dimethoate as a source of phosphorus. Overall, the data obtained highlight the efficiency of cyanobacterial isolates to grow under high concentrations of dimethoate with enhancement of biomass and protein content. Moreover, *Nostoc* overtopped the other isolate in removing more than 90% of dimethoate. Hence, work in this regard should continue to characterize enzymatic components responsible for the utilization of dimethoate by of this species in order to evaluate its efficiency for the bioremediation of these environmental pollutants.

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