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Research Article

A study on toxicity of organophosphorous pesticides on cynobacterium *Westiellopsis prolifica* Janet

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Abstract

Effect of two widely used organophosphate pesticide such as Triazophos and Monocrotophos on the growth, biochemical metabolites like protein, carbohydrate, photosynthetic pigment chlorophyll *a* and accessory photosynthetic pigment phycobillin contents and stress induced non protein amino acid proline content in strain of freshwater cyanobacrteium *Westiellopsis prolifica* Janet was studied. The various concentrations of Triazophos and Monocrotophos were exposed to *W. prolifica* the inhibitory concentration (EC 50) was determined. The EC50 value of Triazophos and Monocrotophos were 125 µm and 350 µm respectively for *W. prolifica*. Both the organophosphate insecticides used in the study reduced the protein content of *W. prolifica*. It was interesting to note that organophosphate insecticides inhibit the synthesis of photosynthetic pigments too in the cyanobacteria which was reduced with 51% (Triazophos) and 48% (Monocrotophos) at respective EC50 concentration. The organophosphate pesticide changes the ratio and production of Phycobilin content in *W. prolifica*. The pesticide Triazophos and Monocrotophos inhibited Phycocyanin content 65% and 34%, Allophycocyanin 61 % and 43% Phycoerytherin 45% and 39% respectively. Phycocyanin and Allophycocyanin content of *W. prolific* was severely inhibited by pesticide Triazophos, whereas reduction was comparatively less by Monocrotophos. There was no signification variation noticed in Phycoerytherin content in both stresses imposed in *W. prolifica* similar response was noticed in production of carbohydrate content in Triazophos treated *W. prolifica* the reduction was 73%. The enhanced level Proline synthesis was noted in Triazophos treated *W. prolifica* it has 16 fold increase whereas as only 3 fold increase in Monocrotophos treated culture at respective EC 50 value. Thus reduction in all the biochemical parameters and increased level of proline revealed that Triazophos was more toxic to *W. prolifica* than Monocrotophos.

INTRODUCTION

In modern day agriculture practices, without the application of pesticides has become nearly impossible anywhere in the world. Pesticides are of various types, such as organochlorine, organophosphorus, carbamates, pyrethroids, etc. Among these, Organophosphorus compounds (OPs) are most widely used around the world and have been used as pesticides and chemical warfare agents in agriculture and other fields (Singh and Walker, 2006). As every pesticide used in agricultural practices affects the growth of non target soil microorganisms especially in cyanobacteria (Pipe AE, 1992). Cynaobacteria belongs to a group of ubiquitous photosynthetic prokaryotes has the ability to synthesize Chlorophyll-*a* and carry out an important role in nutrient cycling and maintenance of organic matter in aquatic systems including lakes, river and wetland (Kumar *et al.*, 1998). Kumar (1996) carried out the impact of pesticides on nucleic acids of *Anabaena* sp. 310 and photosynthetic, biochemical and enzymatic investigations of *Anabaena fertilissima* in response to an insecticide-hexa a chloro-hexa hydromethano benzo dioxathiepine –oxide. A great deal of information on toxicological aspects of pesticides on green algae, especially on *Chlorella*, *Scenedesmus* and *Selenastrum* is available (Ma *et al.,* 2007).

However, little information available on the effect of these pesticides on cyanobacteria (Abou-waly *et al.,* 1991; Ma and Chen, 2005) and the susceptibility of cyanobacteria to toxicants such as herbicides, fungicides and heavy metals (Ferrando *et al.,* 1996). Therefore the aim of this work was to establish the differential toxicity effects of the two selected rice field pesticide (Triazophos and Monocrotophos) on growth and survivability potentials on cyanobacteria *W. prolifica*.

MATERIALS AND METHODS

Glass wares, Chemicals and Insecticides

Analytical grade chemicals manufacture by S.D, fine chemicals, E. Merck (India) Ltd. Qualigen and Sigma-Aldrich were used for all experiments. Scrupulously clean borosil glasses wares and double glass-distilled water (pH 6.8 - 7.2) only ware used. Chemicals and reagents were prepared fresh at the time of each experiment. Two organo phosphorus pesticides, Triazophose [o, o-diethyl o-(1-phenyl-1H-1,2,4-4-triazol-3-yl] phosphothioate marketed in the name of Trifos 40 manufactured by Cheminova India Ltd and Monocrotophos [dimethyl (E)-1-methyl-2-methyl carbamoylvinyl phosphate] marked in the name of Phoskill 36% manufactured by United Phosphorus limited were selected for the present investigation in view of their extensive and intensive usage in Indian agriculture for control of pests on crops such as cotton and groundnut.

Cyanobacterial strains and culture conditions

The Cyanobactera *Westiellopsis prolifica* was obtained from center for advanced studies in Botany, University of Madras, Chennai-600025, Tamil Nadu, India. The strains are maintained in BG 11 medium (Rippka *et al.,* 1979). The stock cultures were maintained in BG 11 medium. Culture at the mid-log phase only was used for inoculation. The inoculation was carried out using laminar air flow chamber with HEPA filter. The culture was maintained in growth chamber was under controlled environmental condition. The temperature of the growth chamber maintained at 27 ± 1° C and the RH was maintained between 70 & 80%. A daily photoperiod of 16 hrs was maintained photo-synthetically active radiation (PAR 400-700) was provided by a blank of cool, white fluorescent lamp Philips 40 W.

Imposition of stress condition

Westiellopsis prolifica strains were exposed to various concentration of organophosphate pesticides such as Triazophos (25, 50, 75, 100, 125, 150 μM) and Monocrotophos (100, 200, 300, 400, 500, 600 μM) in BG11 medium on the expontial stage of culture (Figure 1). **Biochemical analysis**

The total protein content was done by Lowry *et al.,* 1951 and the chlorophyll *a* was estimated according to the method of McKinney *et al.,* (1941). The estimation of accessory photosynthetic pigment such as Phycocyanin (PC), Allophycocyanin (APC) and Phycoerytherin (PE) were carried out by Bennett & Bogorad (1973), Proline content (Bates *et al.,* 1973) and Carbohydrate content was determined according to Anthrone method (Morris, 1948).

Figure 1. Organophosphate pesticides treatment of *W. prolifica*

Sensitivity index (%)

The sensitivity index of various parameters of the Cyanobacterium *W. prolifica* to two organophosphate pesticides treatment was determined by applying the formula

$$SI = \frac{(\text{Control} - \text{Pesticide treated})}{\text{Control}} \times 100$$

RESULTS AND DISCUSSION

The growth of *W. prolifica* with different concentration organophosphate insecticide such as Triazophos (25, 50, 75, 100, 125, 150 µm) and Monocrotophos (100, 200, 300, 400, 500, 600 µm) was carried out in controlled environmental conditions .There were no significant effects on growth of the *W. prolifica* at low concentrations. However, at higher concentrations, survival was threatened. The protein content in the algae, which is routinely used as an index of algal growth, was plotted against the various concentrations of pesticides, supplied to the medium. Both the organophosphate insecticides used in the study reduced the protein content of *W. prolifica* (Table 1). However, it was noticed that *W. prolifica* was more susceptible to Triazophos than Monocrotophos, which is evident from the wide difference in their EC 50 (Effect concentration causing 50% inhibition in growth) values of insecticide. While the EC50 value of Triazophos and Monocrotophos were 125 µm and 350 µm respectively for *W. prolifica* the observation that *W. prolifica* has higher susceptibility to Triazophos than Monocrotophos was substantiated when subjected to regression analysis. Triazophos and Monocrotophos showed R^2 = 0.9866 and 0.9972 respectively in *W. prolifica* (Figure 1&2). Further experiment was carried out using the respective EC 50 values of pesticide.

Likewise organophosphate pesticide severely inhibited the synthesis of carbohydrate content. Triazophos and Monocrotophos reduced the carbohydrate 73% and 44% respectively in *W. prolifica*. This result clearly substantiated that Triazophos was more toxic to *W. prolifica* strain than Monocrotophos (Table 1). Kumar *et al*. (2012) reported that pesticide endosulfan reduced the carbohydrate content of *Anabaena fertilissima, Aulosira fertilissima, W. prolifica* up to 97 %.

It was interesting to note that Organophosphate pesticides inhibit the synthesis of photosynthetic pigments too in the cyanobacteria. Varying concentrations of Triazophos and Monocrotophos had a pronounced effect on pigment contents in *W. prolifica*. Chlorophyll *a* was much more affected by toxic pesticides concentrations. Its content was decreased by 51% (Triazophos) and 48% (Monocrotophos) at respective EC 50 concentration (Table 1). Triazophos reduce highest chlorophyll *a* content than Monocrotophos in tested cyanobacteria strain. The result obtained in this study was in agreement with those of previous report. (Mohapatra *et al.*, 2003; Bhunia *et al.*, 1991; Shen *et al.*, 2009) and adverse effects of endosulfan, tebuconazole and malathion pesticides on the photosynthetic pigment content have been reported in *Anabaena fertilisimia*, *Aulosira fertilisimia* and *W. prolifica* (Kumar *et al*., 2012). *Anabaena sphaerica* (Chakraborty *et al*., 2017)

The reduction in chlorophyll content due to different stress may be the result of inhibition of chlorophyll biosynthesis brought about by inhibition of α –aminolevulinic acid dehydrogenase and protochlrophyllide reductaes activity (Sundrum and Soumya, 2011).

The cyanobacteria are unique in possessing certain accessory light harvesting Photosynthetic pigment such as Phycocyanin, Allophycocyanin and Phycoerytherin. The effect of organophosphate pesticide changed the ratio and production of phycobillin content in *W. prolifica*. The pesticides Triazophos and Monocrotophos inhibited the Phycocyanin content 65% and 34%, Allophycocyanin 61 % and 43% Phycoerytherin 45% and 39% respectively. Phycocyanin and Allophycocyanin content of *W. prolifica* was severely inhibited by pesticides Triazophos, whereas reduction was comparatively less by Monocrotophos. There was no signification variation noticed in phycoerytherin content in both stresses imposed in *W. prolifica* (Table 2). Kumar *et al*. (2012); Mohapatra *et al.* (2000) have demonstrated that organophosphate pesticide - thylakoid membrane interaction has been proved to be responsible for reduction in pigment content of *Synechocystis* PCC 6803. These two water-soluble protein pigments were degraded at a faster rate than those of Chlorophyll *a.*

Proline accumulates heavily in several plants under stress, providing the plants protection

Table 1. Protein, Chlorophyll *a* and carbohydrate content (µg ml^{-1}) in *W. prolifica* grown in the presence of respective EC50 levels of organophosphate pesticide (Triazophos and Monocrotophos (µM)) (parenthesis denote percent over control).

S. No	Name of insecticides	Protein (µg ml^{-1})	Chlorophyll *a* (µg ml^{-1})	Carbohydrate (µg ml-1)
1.	Triazophos	17.61±0.5(41)	0.26± 0.03(49)	8.70±0.8 (27)
2.	Monocrotophos	29.60±0.6(54)	0.085± 0.002(52)	47.69±0.5 (56)

Table 2. Phycobillin content (µg m^{-1}) in *Westiellopsis prolifica* grown in the presence of respective EC50 levels of organophosphate pesticide (Triazophos and Monocrotophos µM) (parenthesis denote percent over control).

S. No	Name of insecticides	PC	APC	PE
1	Triazophos	0.013±0.02(45)	0.011±0.03 (39)	0.008±0.001(55)
2	Monocrotophos	0.026±0.003(66)	0.027±0.003 (57)	0.021±0.003(61)

PC-Phycocyanin, **APC** – Allophycocyanin, **PE-** Phycoerytherin

Table 3. Proline content (µgml^{-1}) in *Westiellopsis prolifica* grown in the presence of respective EC50 levels of organophosphate pesticide (Triazophos and Monocrotophos µM) (parenthesis denote fold increase with control)

S. No	Name of insecticides	Proline content (µgml-1)
1	Triazophos	0.44(16 fold)
2	Monocrotophos	0.29 (3 fold)

Table 4. Sensitivity index (SI) for the various parameter analyzed in the Cyanobacterium *W. prolifica* exposed to two organophosphate pesticide

S. No	Parameter	Triazophos	Monocrotophos
1.	Protein	59	46
2.	Carbohydrate	72	43
3.	Chlorophyll *a*	51	47
4.	Phycocyanin	53	33
5.	Allophycocyanin	66	42
6.	Phycoerytherin	55	38
7.	Mean	59.3	41.5

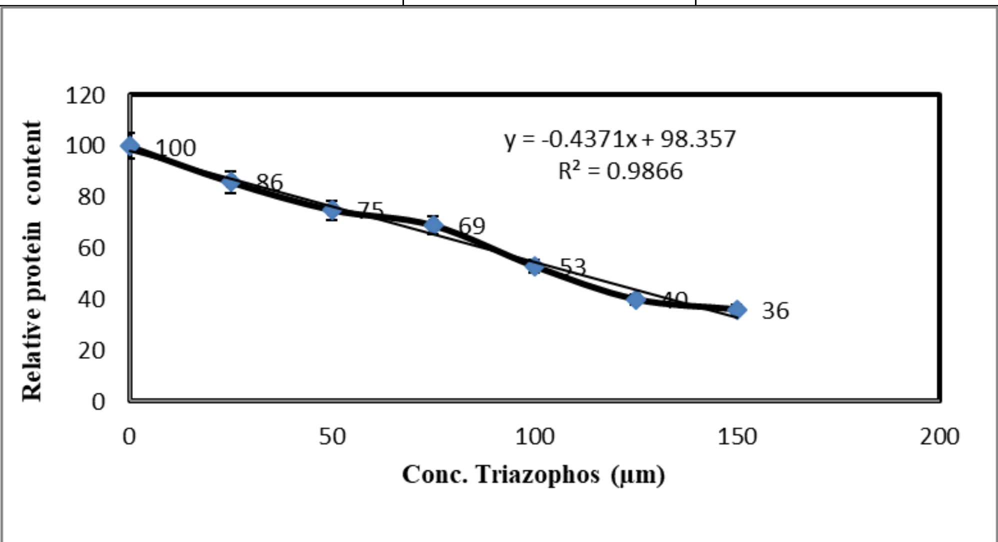

Figure 1. Regression analysis of protein content in *W. prolifica* strains under different Concentrations of Triazophos treatment on 15th day of growth.

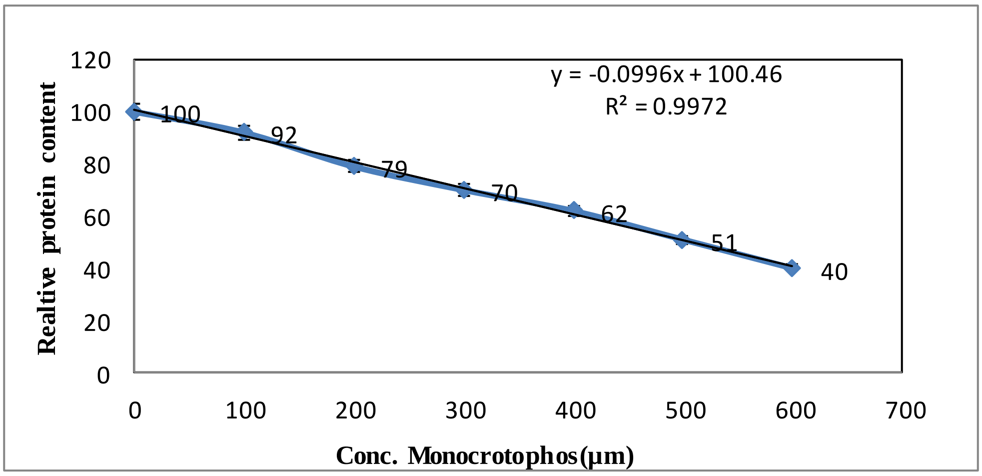

Figure 2. Regression analysis of protein content in *Westiellopsis prolifica* strains under different Concentrations of Monocrotophos treatment on 15th day of growth.

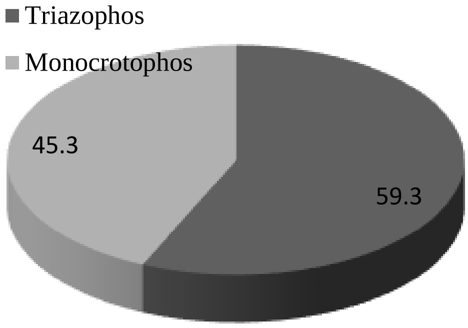

Figure 3. Mean Sensitivity index (SI) for the various parameters analyzed in the Cyanobacterium *Westiellopsis prolifica* exposed to two organophosphate pesticides against damage by ROS.

Proline plays important roles in osmoregulation (Ahmad and Hellebust, 1988; Laliberte and Hellebust, 1989), protection of enzymes (Nikolopoulos and Manetas, 1991; Laliberte and Hellebust,1989; Paleg *et al.*, 1984), stabilization of the machinery of protein synthesis (Kadpal and Rao, 1985) regulation of cytosolic acidity (Venekemp, 1989) and scavenging of free radicals / (Smirnoff and Cumbes, 1989). Increase in the osmoprotectant Proline content was directly proportional to the insecticides concentration. The Proline contents was increased 16 fold in Triazophos treated culture whereas as only 3 fold increase in Monocrotophos treated culture at respective EC 50 value (Table 3).

The sensitivity index compute for the various parameters recorded in the Cyanobacterium *W. prolifica* was under two organophosphate pesticides treatment (Table 4). It is evident that Triazophos was more toxic to *W. prolifica* than Monocrotophos. The mean sensitivity index which was 59.3 for Triazophos and 45.3 for Monocrotophos clearly show that *W. prolifica* more susceptible to Triazophos (Figure 3)

Conclusion

The results obtained from this study indicated that Triazophos more toxic to *Westiellopsis prolifica* than Monocrotophos. The protein, carbohydrate, chlorophyll *a* and phycobillin content were severely inhibited at low concentration of Triazophos, whereas Proline accumulation increased with Triazophos treated *W. prolifica* culture than Monocrotophos treated cell. This result clearly depict that the enhanced level of synthesis of proline content in *W. prolifica* mitigate the adverse effect of organophosphate insecticide stress.

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