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Growth Inhibition Effect of Organophosphate Pesticide, Monocrotophos on Marine Diatoms

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The growth inhibition effect of organophosphate pesticide, Monocrotophos on *Odontella mobiliensis* and *Coscinodiscus centralis* was investigated. Growth of diatoms was reduced on exposure to low concentrations (2 to 8 mg/L) of Monocrotphos (MCP) up to 48 hrs afterwards growth was normalized, but higher concentrations have seized the growth completely. The IC_{50} of MCP was 7.2±0.3 mg/L and 17.7±3.9 mg/L on *O. mobiliensis* and *C. centralis* respectively. The No Observable Effect Concentration (NOEC), Low Observable Effect Concentration (LOEC) and Sub Chronic Values (SChVs) were estimated to be 0.5±0.04, 0.95±0.06 and 0.69±0.03 mg/L on *O. mobiliensis* and 1.05±0.07, 2.13±0.11 and 1.5±0.09 mg/L on *C. centralis*. The results of present study ascertained that the MCP is moderately toxic to diatoms and the safe concentration of MCP for diatoms was determined to be less than 0.5 mg/L.

[Keywords: Diatom, Growth inhibition, Monocrotophos, IC_{50}, SChV]

Introduction

About two million tons of pesticides have been consumed annually in worldwide[1] as pesticide usage continues to increase about 50% include insecticides used widely in agricultural crops. Among the various insecticides, monocrotophos (MCP) is the single largest consumed agrochemical in India[2] and widely used for agricultural crops to control the broad spectrum of pests. Many countries have banned the use of MCP but its use barely restricted in India on vegetables[2]. Of the total pesticides applied, an estimated quantity of 0.1% reaches the target organisms and the remaining 99.9% disperse through air, soil and water, thus resulting pollution of natural ecosystems and in turn affect non-target organisms[3,4]. Thus the assessment of safe concentration for MCP to aquatic environment including marine is essential, using toxicity bioassay endpoints. The toxicity of MCP have reported mostly on birds and its intense toxicity on aquatic invertebrates such as, cladocerans and crustaceans were also reported by few authors[2,4,5,6]. Some previous studies showed the reduction in photosynthesis and growth of diatoms exposed to 2, 4-D, atrazine, metobromuron, triazines[7,8,9] and some other organophosphates[10,11]. Diatoms may serve as effective indicators of pollution caused by organophosphates as reported by previous author[12]. The study on toxicological effects of monocrotophos on phytoplankton particularly in marine diatoms are not reported and therefore, the present study is the first attempt to assess the toxicological effect for the organophosphate pesticide, MCP on two species of centric diatoms from the marine environment.

Materials and Methods

The experimental diatom species *viz.*, *Odontella mobiliensis* and *Coscinodiscus centralis* were isolated from the phytoplankton soup collected from Vellar estuary, Parangipettai and uni-algal strains were treated with mixture of antibiotics penicillin, gentamicin and streptomycin, and maintained in Guillard's F/2 media at 24 ± 2 ºC of room temperature, 30 psu of salinity and 4500 ± 500 Lux at 12:12 light and dark photoperiod. Pure strain of diatoms was optimized for conduct of the growth inhibition experiments under controlled laboratory conditions.

The MCP in 99.9% purity (Sigma-Alrich, Germany) was used for standardization of GC-FPD quantification and the commercial grade

36% v/v solution (Phoskill®) was used as a test chemical in the exposure treatment throughout the experiments.

By following the standard growth inhibition test procedures[13,14], the experiments were performed with 4–5 days aged exponentially grown algal cultures maintained in 250ml conical flasks with the initial cell densities of *O. mobiliensis* at $1.6 \pm 0.14 \times 10^4$ cells mL^{-1} and *C. centralis* at $2.3 \pm 0.52 \times 10^3$ cells mL^{-1}. The range finding tests were conducted for 48 h before definitive test. The stock solution of MCP was prepared in Milli-Q ultra pure water using commercial formulation of MCP (36% v/v). Acute and chronic tests were conducted in triplicate experiments using different test concentrations of MCP (each concentration in duplicate was used in every experiment) for 96 h and 5 days respectively. The concentrations of 2.0, 3.8, 7.2, 13.7 and 26.1 mg L^{-1} and 1.5, 2.9, 5.4, 10.3 and 19.5 mg L^{-1} were exposed to the cultures of *C. centralis* and *O. mobiliensis* respectively. Further, the test concentrations of 0.7, 1.3, 2.5, 4.8 and 9.1 mg L^{-1} were used for exposure treatment on both *C. centralis* and *O. mobiliensis*. The culture flasks were manually shaken 3-4 times every day and maintained at 25 ± 2 ºC of room temperature, 30 psu of salinity and 4500 ± 500 Lux at 12:12 light and dark photoperiod. Cell density was estimated at every 24 h intervals. Growth rate and percentage of growth inhibition were calculated by the equations reported in the OECD guidelines[13].

One hundred µl of culture was made up to 1ml with the addition of lugol's iodine solution. 10 µl of diluted sample was transferred to the meeting point of plus mark in the glass slide. The slide was mounted on the binocular microscope and the cells were counted at 10x (Stage lens) and 10 x (Ocular lens) condition. The results were expressed as cells ml^{-1} of culture.

The growth rate was calculated by using the following formula[13],

$$\mu = In(N_2/N_1)/(t_2-t_1)$$

Where, N_2 and N_1 are the number of diatom cells produced during the time t_1 and t_2.

The percentage of growth inhibition was calculated by using the following formula[13],

$$\% \text{ of growth inhibition} = \mu_{control} - \mu_{concentration}/\mu_{control} * 100$$

To analyze the concentrations of MCP in seawater and in the exposure treated cultures maintained in the conical flasks, the sample was extracted individually using the HPLC grade (Merck, Germany) dichloromethane (DCM) by automatic separating funnel shaker (Recipro Shaker RS-1). Extracted MCP was purified through Florisil-sodium sulphate column and condensed to 1ml under vacuum in a Rotary evaporator (Cyberlab RE 10) at 38 ºC. The analyses of monocrotophos were carried out in GC-FPD (Agilent 6890N). The monocrotophos of 99.9% purity (Sigma-Aldrich) was used as standard. All the toxicity test results were presented based on dissolved concentrations of MCP. The method was standardized in the laboratory and adopted in all the analyses.

The subsurface seawater samples were collected from Parangipettai coast (11.4900° N, 79.7600° E) and the dissolved MCP was extracted from one litre of seawater sample and analysed in GC-FPD. The average background concentration of MCP ranged from 5 to 9 ng/L. The recovery of MCP was found to be 90% measured in the GC.

IC_{50} values were calculated by Probit analysis method[14] whereas, no observable effect concentration (NOEC), low observable effect concentration (LOEC) and chronic values were calculated by Dunnett's method[15]. The data were processed and graphs were plotted using MS-Excel software.

Results

All the observations were made in the exponential growth phase of diatoms in their life cycle. MCP proved to be toxic with IC_{50} values ranged between 7.2 ± 0.3 and 17.7 ± 3.9 and the sub-chronic values between 0.69 ± 0.03 and 1.9 ± 0.09 mg/L on *O. mobiliensis* and *C. centralis* respectively. There was no growth inhibitory effect noticed in the untreated cultures and this has been ascertained from the stock culture by doubling time analysis at every stage of culture.

Table 1. Acute and chronic toxicity values of MCP (Mean ± SD) on *O. mobiliensis* and *C. centralis*

Diatom species	IC_{50}	NOEC	LOEC	SChV
O. mobiliensis	7.2 ± 0.3	0.5 ± 0.04	0.95 ± 0.06	0.69 ± 0.03
C. centralis	17.7 ± 3.9	1.05 ± 0.07	2.13 ± 0.11	1.5 ± 0.09

Increasing concentration of MCP has reduced the growth of *O. mobiliensis* and *C. centralis*. The culture with uniform cell densities was exposed to five different concentrations of MCP. The decrease in the cell density was observed after 24 hrs exposure in all the treated samples. Despite the growth rate of diatoms affected by lower concentrations of MCP at 24 and 48 hrs, it was regularized (as control) during 72 and 96 hrs and attained similar cell density of control. At the higher concentrations of MCP, the growth rate was almost reduced completely and multiplication of cells was observed at 48 and 72 hrs (Fig.1A & B, Fig.2a, Fig.3A & B and Fig.2b). Based on the cell densities at 96 hrs of exposure to five different concentrations of MCP, the percentage of growth inhibitions were calculated and the 96-hrs IC_{50} values were derived by Probit analysis method. The exposure treatment concentration were measured and used for calculation of the median inhibitory concentrations (IC_{50}) and IC_{50} of MCP and were observed to be 7.2 ± 0.3 and 17.7 ± 3.9 mg/L on *O. mobiliensis* and *C. centralis* respectively (Table 1).

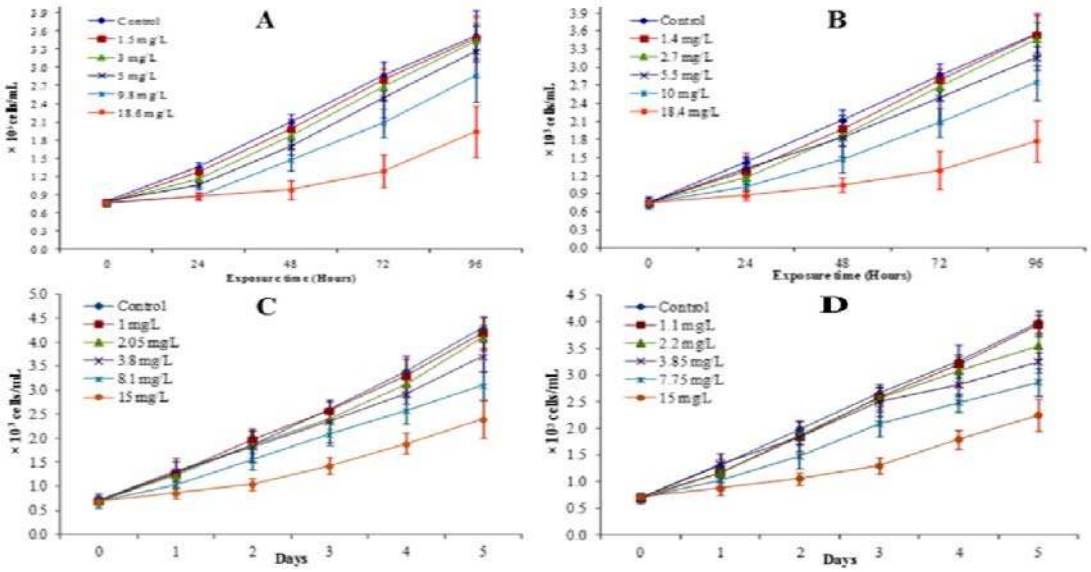

Fig.1. Growth curves of *C.centralis* on MCP exposure. (A) & (B) acute test I & II, (C) & (D) chronic test I & II respectively.

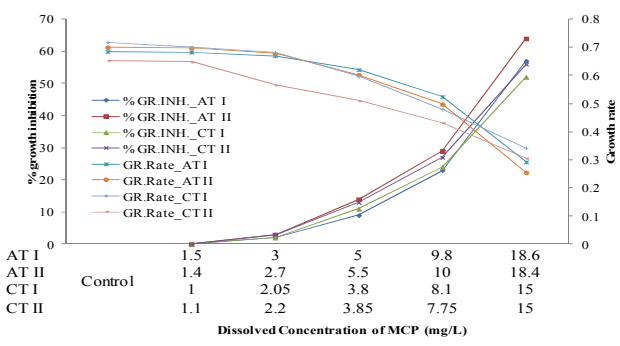

Fig.2b. Concentration-Response curves of acute and chronic toxicity tests on *O. mobiliensis* for MCP (AT-Acute Test; CT-Chronic Test).

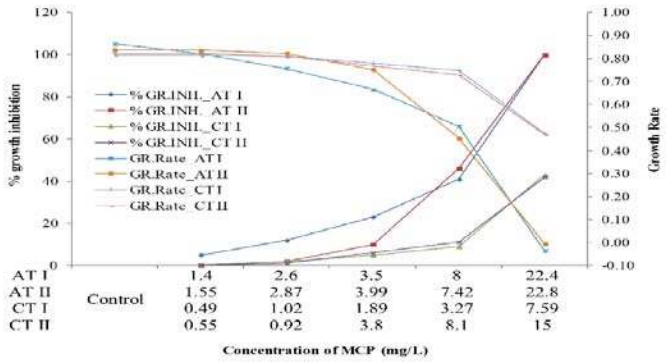

Fig.2a. Concentration-Response curves of acute and chronic toxicity tests on *C. centralis* for MCP (AT-Acute Test; CT-Chronic Test)

The diatom cultures were exposed to lower concentrations of MCP selected based on IC50 value for deriving the No Observable Effect Concentration (NOEC), Low Observable Effect Concentration (LOEC) and sub-chronic values. The growth was not inhibited at the lowest concentrations of MCP (0.5 to 1.1 mg/L) during exposure period of five days. Insignificant decrease in the growth was observed at 1.5 to 2.05 mg/L of MCP (Fig.1C & D, Fig.2a, Fig.3C & D and Fig.2b). The exposure treatment concentrations were also measured and used for calculation of the NOEC, LOEC and Sub Chronic Values (SChVs) which were found to be 0.5 ± 0.04, 0.95 ± 0.06 and 0.69 ± 0.03 mg/L for *O. mobiliensis* and 1.05 ± 0.07, 2.13 ± 0.11 and 1.5 ± 0.09 mg/L for *C. centralis* (Table 1).

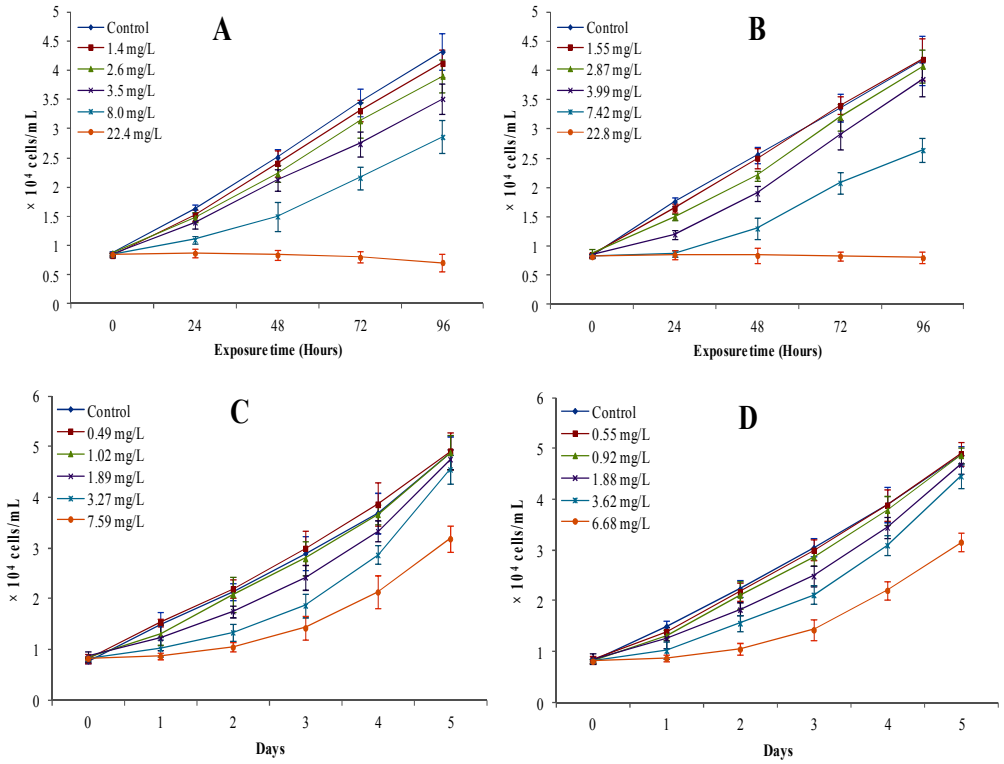

Fig.3. Growth curves of *O.mobiliensis* on MCP exposure. (A) & (B) acute test I & II, (C) & (D) chronic test I & II respectively.

Discussion

The growth inhibition effect of the monocrotophos on two centric diatoms *viz*. *O. mobiliensis* and *C. centralis* were exhibited and 96-h IC_{50} values ranged between 7.2 ± 0.3 and 17.7 ± 3.9 mg/L observed in the present study. Similarly the toxicity effect of organophosphate, Atrazine was reported as 50% reduction in Chl *a* content between 50 and 1000 µg/L on the photosynthetic pigments of four species of marine diatoms and green microalgae[16,17]. During the 72, 48 and 24h of Chl. *a,* EC_{50} of MCP were reported as 6.9, 10. 6 and 11.7 mg/L respectively; Further, 72h and 48h EC_{50} based on carotenoid content of MCP were 8.4 and 11 mg/L reported for *Dicrateria* sp[16]. As far as *Phaeodactylum tricornutum* is concerned, the 72h and 48h Chl *a* EC_{50} of MCP was reported to be 8.9 and 12 mg/L and 72h and 48h of carotenoid EC_{50} of MCP were reported as 10.8 and 14 mg/L[16]. Since the growth of diatoms directly related to photosynthetic pigments, the MCP reduced the concentration of photosynthetic pigment[17] observed in the present study have shown similarity to the work reported by Tang *et al.* on species like *P. tricornutum* and *Dicrateria* sp.[17]. The high reduction in the growth rate during early exposure period (24-72 hrs) and the normalized of growth rate during later exposure period (72-120 hrs) was also observed in the present study. This indicated that the stress caused by MCP and subsequent stable reaction against the stress in the test medium was observed. In lower concentrations, the growth rate was regularized between 48 hrs and 72 hrs. Peterson and Rand *et*

al. evidenced that the tolerance and bioconcentration capacity of microalgae to organophosphorous insecticides[18,19]. Chen and Chen also suggested that the organophosphate compounds are not only act as aquatic pollutants but their accumulation may also cause inhibitory effect to the organisms in the food chains[20]. The significant difference in the nominal and measured concentrations of MCP observed in the present study evidenced the bioaccumulation and/or biodegradation of MCP by diatom cells indicated that the toxic effect is moderate, which is in support of the findings on the blue green alga *Nostoc* and *Tolyptothrix* reported by Mostafa *et al.* due to biodegradation of organophosphate[21]. Even though the mode of toxicity of MCP is the inhibition of acetylcolinesterase, it has also shown the growth inhibition effects on diatoms. The chronic values of MCP was found to be 0.69 ± 0.03 and 1.9 ± 0.09 mg/L on *O. mobiliensis* and *C. centralis* respectively indicated inhibitory effect during the initial period of exposure treatment which demonstrated that it was moderately toxic.

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