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TOXICITY OF CHLORPYRIFOS ON SOME MARINE CYANOBACTERIA SPECIES

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Abstract

Pakistan is an agricultural country and a wide variety of pesticides are used on its cropland. Pesticides pose serious threats to the natural ecosystem. In the present study cyanobacteria (blue green algae) were used to assess the ecotoxicological effect of chlorpyrifos (organophosphate pesticide). Cyanobacteria are the base of the food web providing food resource to consumers in higher trophic level. Cyanobacteria were isolated and purified from water samples collected from Manora channel. Fast growing cultures of cyanobacteria were used to assess the toxicity of test pesticide. The Light and Dark method was used to determine the primary production of the organisms. The acute toxicity of chlorpyrifos was determined by calculating IC_{50} of the test organisms. The IC_{50} was found to be 0.074, 0.013, 0.08 and 0.3 ppm for *Synechocystis aquatilis, Komvophoron minutum, Gloeocapsa crepidinum* and *Gloeocapsa sanguinea* when exposed to chlorpyrifos pesticide. Laboratory experiments with cyanobacteria have demonstrated that organophosphate pesticides are potent inhibitors of photosynthesis.

Introduction

Agriculture is the mainstay of Pakistan economy, cotton being the cash crop so wide varieties of pesticides are used on its cropland. Organophosphate pesticides are largely used against the cotton pests in Pakistan. Pesticides are a major source of pollution in marine environment. Organophosphates are comparatively short lived and are not persistent as mercurial and organochlorine compounds and hence organophosphates are favoured as a replacement for these compounds (Hansen *et al*., 1983; Coats *et al*., 1989).

Cyanobacteria, the photosynthetic prokaryotes, play an important role in the energy transformation to higher trophic levels (Lee *et al*., 2001). Cyanobacteria are a major component in many ecologically significant processes, such as, generation of new nitrogen and carbon production (Bashan *et al*., 1998). Cyanobacterial photosynthesis, growth and heterocysts differentiation is reduced or inhibited by herbicides and pesticides, such as, 2,4-D; atrazine; metsulfuron methyl (Thompson *et al.,* 1993; Berard *et al.,* 1999). In the present study cyanobacteria have been cultured in the laboratory. The fast growing cultures were selected to assess the ecotoxicological effect of organophosphate on these primary producers.

Material and Methods

Collection, isolation & identification of cyanobacteria: Cyanobacteria were isolated and purified from water samples collected from Manora channel using a phytoplankton net (55 µm) towed for ten minutes in the surface water. The cyanobacterial species were isolated through repeated inoculation on solidified ASN III media and in ASN III broth prepared in filtered seawater and incubated under suitable light and temperature conditions. A mixed culture obtained was further isolated by streaking on solid medium as well as through serial dilution (Rippka, 1988). Cyanobacteria, were identified on the basis of their morphological characters (Desikachary, 1959; Anagnostidis & Komarek, 1985, 1988; Komarek & Anagnostidis, 1986, 1989). Chlorpyrifos (organophosphate pesticide) was used to assess the toxicity on four different cyanobacteria species.

Experimental design: The light and dark method (Strickland & Parsons, 1968) was used to determine the rate of photosynthesis. The laboratory grown cultures were maintained in ASN III broth. Known volume of well mixed culture was transferred in the glass BOD bottles in triplicate containing filtered seawater only (control) and different concentration (0.01-1ppm) of the test pesticide prepared in seawater. Two sets of triplicate bottles were used for control and all test concentrations. One set was incubated in light and the other set in the dark at constant salinity (35±1), pH (7.60±1) and temperature 36±1ºC for three hours. At the end of experiment each of the light and dark incubated samples were fixed for the analysis of dissolved oxygen (Wrinkler's method; Hanna C100 multimeter ion specific meter). The gross photosynthesis was calculated both in control and test samples to see the effect of pesticides on photosynthetic ability of cyanobacteria. IC_{50} was calculated using log probit graph and data was statistically analyzed using Minitab v11.

Results

Effects of organophosphate pesticide calculated as IC_{50} for different algal species depicted in Table 1 shows that pesticide have variable effects on the photosynthesis of blue green algae/cyanobacteria. Variability in sensitivity of cyanobacteria was observed according to their response to pesticide tested. The most sensitive specie to chlorpyrifos was *Komvophoron minutum* followed by *Synechocystis aquatilis*, *Gloeocapsa crepidinum* and *Gloeocapsa sanguinea* respectively. Toxicity of pesticide to algal species increased with concentration (Figs. 1-4). The regression equation for *Synechocystis aquatilis* was calculated as y = 62.9-53.6 x, for *Komvophoron minutum* regression equation was calculated as y = 42.1-20.9 x, for *Gloeocapsa crepidinum* it was calculated as y = 56.1 - 26.4 x and for *Gloeocapsa sanguinea* regression equation was calculated as y = 77.1-43.6 x respectively.

Table 1. Effect of chlorpyrifos pesticide on photosynthesis of cyanobacteria. Values are concentration of pesticides at which photosynthesis is reduced by 50% of control values.

S. No.	Cyanobacteria	IC_{50} ppm
		Chlorpyrifos
1.	*Synechocystis aquatilis*	0.074
2.	*Komvophoron minutum*	0.013
3.	*Gloeocapsa crepidinum*	0.08
4.	*Gloeocapsa sanguinea*	0.3

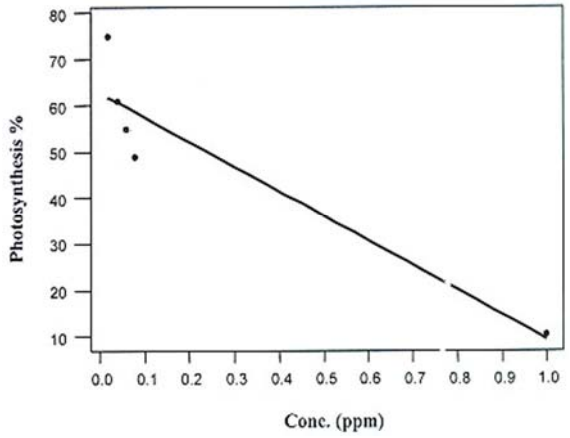

Fig. 1. Effect of chlorpyrifos on photosynthesis of *Synechocystis aquatilis.*

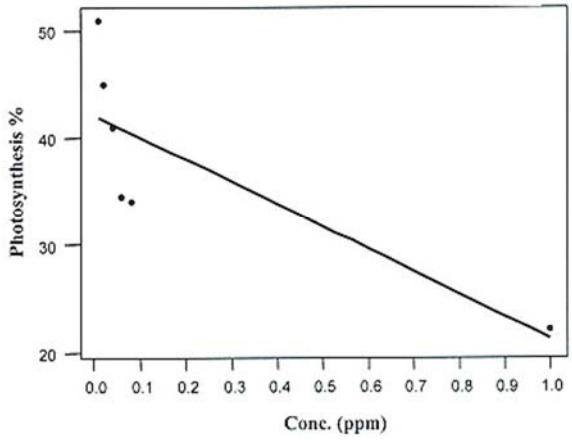

Fig. 2. Effect of chlorpyrifos on photosynthesis of *Komvophoron minutum*.

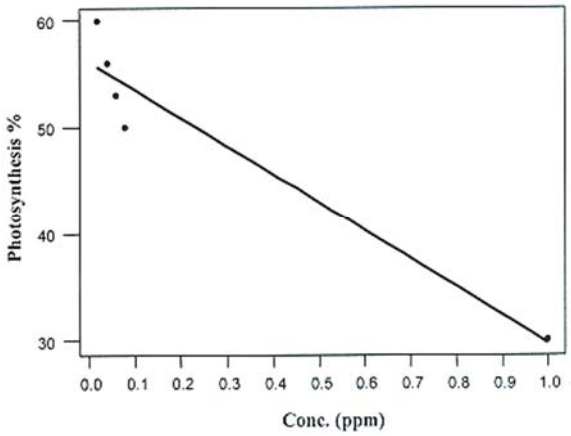

Fig. 3. Effect of chlorpyrifos on photosynthesis of *Gloeocapsa crepidinum*.

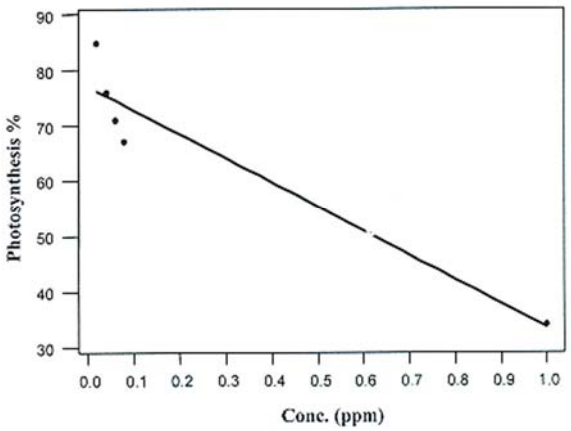

Fig. 4. Effect of chlorpyrifos on photosynthesis of *Gloeocapsa sanguinea.*

Discussion

The toxicity of pesticide to cyanobacteria is generally a function of the concentration of pesticide. Inhibition of photosynthesis in microalgae tested in the present study is in good agreement with previous studies showing inhibitory effect on primary producers exposed to different pesticides (Rajendran *et al.,* 1983; Yee *et al*., 1985; Fahl *et al*., 1995; De Lorenzo *et al*., 2000; Mohapatra *et al*., 2003).

It has been observed in the present study that there is a great variability in species sensitivity to a particular pesticide. Inter-species variation in sensitivity to the organophosphate pesticides has been shown by Kasai *et al.,* (1993) and Peterson *et al*., (1994). Peterson *et al*., (1998) reported toxicity of hexazinone and diquat to green algae (two species), diatoms (two species) and cyanobacteria (five species). The differential sensitivity to herbicide contaminants of different taxonomic groups of plants may have ecological consequences (Peterson *et al*., 1998). Interspecific variations may also be attributed to the chemical nature of pesticides. Number and types of esters present in organophosphates and their stereochemistry regulates pesticide potency, spectrum of activity, and toxicology (Glickman & Casida, 1982; Gray & Soderlund, 1985; Bradbury & Coats, 1989; Reddy &

Rao, 1992). The selective toxic stress by pesticides on certain algae may alter the species composition of a natural phytoplankton community (Wurster, 1968a,b). The imbalance of a flora could favor species normally suppressed by others, thus producing population explosions and dominance of the planktonic community by one or a few species (Korringa, 1968). The annihilation of natural populations of some organisms and increase in noxious groups, as well as an imbalance in the population dynamics can lead to serious problems in the natural ecosystem.

References

Anagnostidis, K. and J. Komarek. 1985. Modern approach to the classification system of cyanophytes: 1- Introduction. *Arch. Hydrobiol. Suppl*.171. *Algol. Stud.,* 38/39: 291-302.

Anagnostidis, K. and J. Komarek. 1988. Modern approach to the classification system of cyanophytes: 3-Oscillatoriales. *Arch. Hydrobiol.*83. *Algol. Stud.,* 50-53: 327-472.

Bashan, Y., M.E. Puente, D.D. Myrold and G. Toledo. 1998. *In vitro* transfer of fixed nitrogen from diazotrophic filamentous cyanobacteria to black mangrove seedlings. *FEMS Microbiol. Ecol.,* 26: 165-170.

Berard, A., C. Leboulanger and T. Pelte. 1999. Tolerance of *Oscillatoria limnetica* Lemmermann to atrazine in natural phytoplankton populations and in pure culture: influence of season and temperature. *Arch. Environ. Contam. Toxicol.,* 37: 472-479.

Bradbury, S.P. and J.R. Coats. 1989. Comparative toxicology of the pyrethroid insecticides. *Rev. Environ. Contam. Toxicol*., 108: 133-177.

Coats, J.R., D.M. Symonik, S.P. Bradbury, S.D. Dyer, L.K. Timson and G.J. Atchison. 1989. Toxicology of synthetic pyrethroids in aquatic organisms: an overview. *Environ. Toxicol. Chem.,* 8: 671-679.

DeLorenzo, M.E., G.I. Scott and P.E. Ross. 2000. Effects of the agricultural pesticides atrazine, deethylatrazine, endosulfan, and chlorpyrifos on an estuarine microbial food web. *J. Appl. Phycol*., 12: 461- 467.

Desikachary, T.V. 1959. *Cyanophyta.* ICAR, Delhi, India, 686 pp.

Fahl, G.M., L. Kreft, R. Altenburger, M. Faust, W. Boedecker and L.H. Grimme. 1995. pH-Dependent sorption, bioconcentration and algal toxicity of sulfonylurea herbicides. *Aquat. Toxicol.,* 31: 175-187.

Glickman, A.H. and J.E. Casida. 1982. Species and structural variations affecting pyrethroid neurotoxicity. *Neurobehav. Toxicol. & Teratol.,* 4: 793-799.

Gray, A.J. and D.M. Soderlund. 1985. Mammalian toxicology of pyrethroids. In: *Progress in Pesticide Biochemistry and Toxicology*: Vol. 5. Insecticides. (Eds.): D.H. Hutson and T.R. Roberts, John Wiley, New York, p. 193-248.

Hansen, D.J., L.R. Goodman, J.C. Moore and P.K. Higdon. 1983. Effects of the synthetic pyrethroids AC 222, 705, permethrin and fenvalerate on sheepshead minnows in early life stage toxicity tests. *Environ. Toxicol. & Chem.*, 2: 251-258.

Kasai, F., N. Takamura and S. Hatakeyama. 1993. Effects of simetryne on growth of various freshwater algal taxa. *Environ. Pollut.,* 79: 77-83.

Komarek, J., K. and Anagnostidis. 1986. Modern approach to the classification system of cyanophytes; 2- Chroococcales. *Arch. Hydrobiol. Suppl.* 73, *Algol. Stud.*, 43: 157-226.

Komarek, J. and K. Anagnostidis. 1989. Modern approach to the classification system of cyanophytes; 4- Nostocales. *Arch. Hydrobiol.* 83. *Algol. Stud.,* 56: 247-345.

Korringa, P. 1968. Biological consequences of marine pollution with special reference to the North Sea fisheries. *Helgoland. wiss. Meeresunters.,* 17: 126-40.

Lee, O.H.K., G.A. Williams and K.D. Hyde. 2001. Diets of *Littoraria ardouiniana* and *L. melanostoma* in Hong Kong. *J. Mar. Biol. Assoc.* U.K., 81: 967-973.

Mohapatra, P.K., S. Patra, P.K. Samantaray and R.C. Mohanty. 2003. Effect of the pyrethroid insecticide cypermethrin on photosynthetic pigments of the cyanobacterium *Anabaena doliolum* Bhar. *Pol. J. Environ. Stud*., 12: 207-212.

Peterson, H.G., C. Boutin, P.A. Martin, K.E. Freemark, N.J. Ruecker and M.J. Moody. 1994. Aquatic phytotoxicity of 23 pesticides applied at expected environmental concentrations. *Bot Rev.,* 60: 182-196.

Peterson, H.G., C. Boutin, K.E. Freemark and P.A. Martin. 1998. Toxicity of hexazinone and diquat to green algae, diatoms, cyanobacteria and duckweed. *Bot. Acta*., 111: 176-190.

Rajendran, N. and V.K. Venugopalan. 1983. Effect of pesticides on phytoplankton production. *Mahasag. Bull. Nat. Inst. Ocean.*, 16: 193-197.

Reddy, M.S. and K.V. Rao. 1992. Toxicity of selected insecticides to the penaeid prawn *Metapenaeus monoceros* (Fabricius). *Bull. Environ Contam. Toxicol*., 48: 622-629.

Rippka, R. 1988. Isolation and purification of cyanobacteria. In: *Methods in Enzymology,* Vol. 167, (Eds.): A.N. Glazer and L. Packer L. Academic Press, San Diego, California. p. 1-7.

Strickland, J.D.H. and T.R. Parsons. 1968. A manual of seawater analysis. *Bull. Fisher. Research Board Canada.*

Thompson, D.G., S.B. Holmes, D. Thomas, L. MacDonald and K.R. Solomon. 1993. Impact of hexazinone and metsulfuron methyl on the phytoplankton community of a mixed- wood/boreal forest lake. *Environ. Toxicol. Chem*., 12: 1695-1707.

Wurster, C.F., Jr. 1968a. DDT threatens ocean life, chemical balance of atmosphere. UST. 2.pp.

Wurster, C.F., Jr. 1968b. DDT reduces photosynthesis by marine phytoplankton. *Science,* N.Y., 159: 1474-1475.

Yee, D., P. Weinberger, D.A. Johnson and C. Dechacin. 1985. *In vitro* effects of the s-triazine herbicide prometryne, on the growth of terrestrial and aquatic microflora. *Arch. Envir. Contam. Toxicol.,* 14: 25-31.

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