Effect of Pesticides on *Vibrio fischeri*

[1]M. ANWAR, [2]F. FABIANA AND [3]A.M. MERICAN
[1]*Department of Chemistry,*
University of Balochistan
Quetta Pakistan
[2]*Instituto Scienze Chimiche,*
Universito de Bologna, Italia
[3]*Department of Chemistry,*
University of Malaya, Malaysia

(Received 16th September, 2002, revised 25th November, 2002)

Summary: A total of 21 pesticides have been tested on a luminous bacteria (*Vibrio fischeri)* and the possible decrease of bacterial luminescence checked. Among pesticides tested, only few are effecting the bacterium in the chemical concentration range upto 200 ppm. The culture of bacterium and methanol (solvent) toxicity is also discussed.

Introduction

The increasing use of pesticides all over the world has generated among the scientific community a growing interest for their environmental monitoring. Almost all pesticides and their metabolites are persistent pollutants and produce residual effects in the environment [1]. The Organo-phosphorous compounds are powerful inhibitors of the enzymes involved in nerve function [2-4]. Likewise Paradoxon, an insecticide has a wide range of biological effects and is very toxic to mammals with a human oral lethal dose of 5mg/kg [5]. The hatching and settling mechanism of the Oyster larvae is greatly influenced by the Organochlorine pesticide [6]. DDT has very obvious effects on the breeding process in birds [7].

In Pakistan the use of pesticide is very common and is increasing very fast. The lack of proper information on toxicity makes human risk assessment and management of a majority of the chemicals impossible. One example of these chemicals is pesticide. By the year 2000 there were 1590 toxic "Active Ingredients" yielded 50,000 different pesticide formulations and none of the 1590

active ingredients have been adequately tested (less than 30%) for health and environmental effects [2]. One reason of less interest is the high cost of their use in conventional animals tests [1]. The 2nd reason is that the multi analyte assays of pesticides in food, agriculture and other commodities not only requires complex and expensive instrumentation like Gas Chromatograph and High Performance Liquid Chromatography but also is taking much longer times [10-12]. While if the tests are with human health involvement, then the case become more serious needing obviously careful observations and monitoring. Contrary to this practice the application of pesticides to lower organism is easy and rapid. Moreover, bacteria are useful for toxicity evaluations because there response to chemical content is significant, Their life cycle is short, their response to environment change is quick and they are readily available and easy to maintain.

Overall pesticide utilization for any purpose gets access to living biota including humans which have been proved to be having fatal consequences [8-9]. The effect of many pesticide have been already tested on a *Vibrio fischeri* and other bacteria's [13-19] but as mentioned earlier, due to large no. of pesticides and many being prepared every day need attention and testing.

The aim of this paper is to present the results of pesticides and its toxicity effects on *Vibrio fischeri*, a photobacterium.

Results and Discussion

The pesticides tested are given in table 1 along with their solubility range in water. Most of the pesticides are lipophilic and so are not easily soluble in water. To make pesticides soluble, Methanol was used as solvent (less toxic for *Vibrio fischeri*) for all the bacterial assays in the final concentration of 1.5%.

Pesticides decreasing bacterial luminescence are tabulated in table 2. The decrease of luminescence is to some extent because of the damage to the cellular wall or some damage is being done to the internal metabolism [2]. The graphs of pesticides which effects bacterial bioluminescence are shown from fig.1 to fig.8.

Table 1. The list of pesticides tested.

S.No.	Name of Pesticide	Mol. Weight	Solu. in water (mg/l)
1	Chloropyriphos	322.54	4.00
2	Molinath	187.30	970.00
3	Carbaryl	201.22	120.00
4	Chlordan	409.78	0.06
5	Mercaptodimether	225.31	10.00
6	Endosulfan	409.93	0.32
7	4,4,DDT	354.49	0.001-4
8	Parathionethyl	291.26	24.00
9	Endrin	380.91	0.23
10	Aldrin	364.91	0.027
11	Dieldrin	380.91	0.186
12	Alphaendosulfan	409.93	0.320
13	Betaendosulfan	409.93	0.320
14	2,4,DDD	320	0.005
15	4,4,DDD (TDE)	320	0.005
16	4,4,DDE	318	0.010
17	2,4,DDE	318	0.010
18	0,P,DDT	354.5	0.001
19	P,P,DDT	354.5	0.001
20	Endrin Aldehyde	380.9	0.23
21	Heptachlorepoxide	373.3	0.056

Table 2. Pesticides list decreasing bacterial luminescence

S.No.	Name	Luminescence
1	Molinath	Decreasing
2	Carbaryl	Decreasing
3	Chlordan	Decreasing
4	Mercaptodimethur	Decreasing
5	4.4.DDT	Decreasing
6	Parathionethyl	Decreasing
7	Endrin	Decreasing
8	4,4,DDD(TDE)	Decreasing

To see whether, if the pesticides are mixed in groups, mixtures are having different toxicity levels, because in the environmental samples chemicals occur as different mixtures rather as pure chemicals. Therefore, the potential synergic and antagonistic interfering of mixture component should always be taken into account. The mixture prepared in groups are given in table 3.

Table 3. Mixtures of pesticides tested.

Group I	Group II	Group III	Group IV
Chloropyriphos	Endosulfan	Dieldrin	4,4,DDE
Molinath	4,4,DDT	Alphaendosulfan	2,4,DDE
Carbaryl	Parathionethyl	Betaendosulfan	0,P,DDT
Chlordan	Endrin	2,4,DDD	P,P,DDT
Mercaptodimether	Aldrin	4,4,DDD (TDE)	Endrin Aldehyde
			Heptachlorepoxide

The results were the same yielding the same net toxicity as was per individual pesticide. Less than two percent difference was noted and was presumed to be human error.

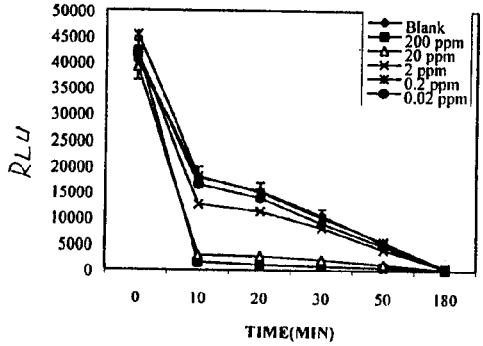

Fig. 1: Mercaptodimether effect on the bacterial bioluminescence.

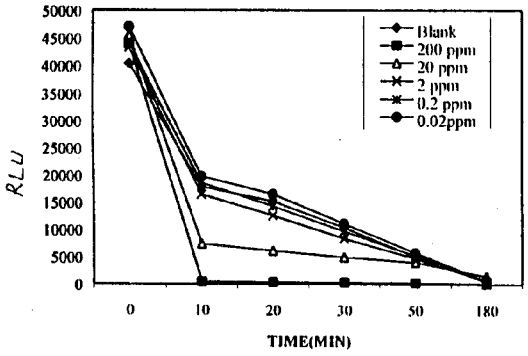

Fig. 2: Chloridane effect on bacterial bioluminescence.

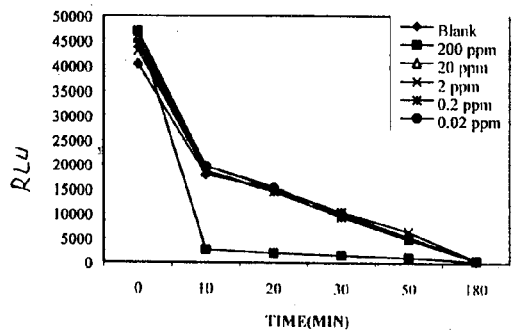

Fig. 3: Molinath effect on bacterial bioluminescence.

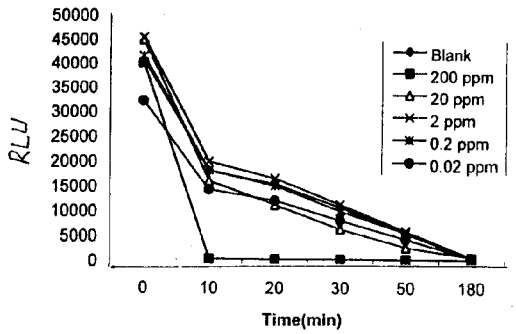

Fig. 4: Endrin effect on bacterial bioluminescence.

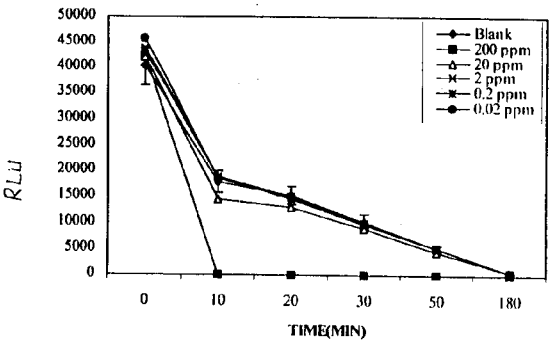

Fig. 5: Parathionethyl effect on bacterial bioluminescence.

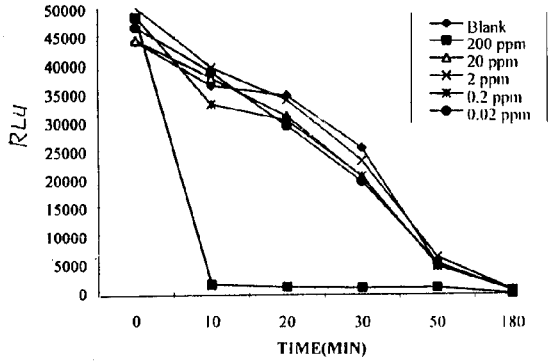

Fig. 6: 4,4,DDT effect on bacterial bioluminescence.

Experimental

Materials

All pesticides were provided by Riedal-en hai of Germany and for double checking by Khurasan Traders Pakistan. Luminometer was Wictor Wallac-1420-002 having multilabel counter from Turku-Finland. *Vibrio fischeri* strains provided by S. Girotti lab. Bologna in freeze dried form and in frozen state (see Acknowledgement). All other reagents were of AnalaR grade.

Methanol effect

Due to solubility problems of pesticides in water, Pure methanol was used throughout the experiment. As the methanol is toxic and many

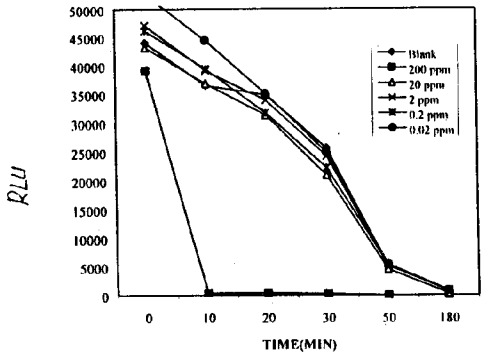

Fig. 7: 4,4,DDD effect on bacterial bioluminescence.

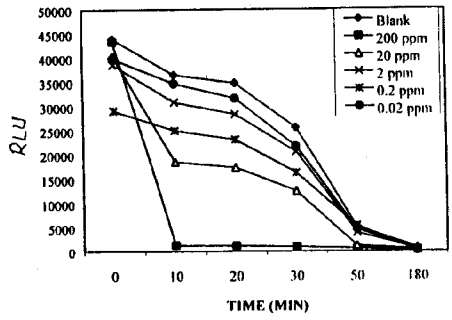

Fig. 8: Carbaryl effect on bacterial bioluminescence.

bacteria's including *Vibrio fischeri* have no tolerance to survive in methanolic environment till its concentration and time for testing the bacteria is kept minimum.

For this reason methanol was used as a solvent from 1% to 15% to dissolve the chemicals and the result was that the maximum of 1.5% was the reasonable concentration where the bacteria is minimumly effected for the period of upto one hour and the chemicals were dissolved very well. If the study is to be carried out for less than ten minutes and long hours then close monitoring and observation is necessary for getting the experimental data for the reasons to be published in detail shortly. Less than 1 % of methanol was found unsuitable as below this concentration the solubility of pesticides was sometimes found as having precipitates and so being unsuitable for analysis.

Bacterial strain preparation

Both freeze dried and frozen bacteria was used through the study carrying each analysis in triplicate. Both strains were constituted in cryoprotective solution (1%NaCl, 12% Lactose, 2% Starch). Culture grown in the petri dishes containing broth (30 gm NaCl, 3 ml glycerol, 5 gm peptone, 3 gm yeast and 12 gms agar/litre) was given a time of 6-10 hours at room temperature (14-18°C). Freezing of bacteria cell causes milder damage to bacterial membranes than freeze drying process [2].

Conclusions

Methanol was chosen as solvent for increasing the solubility of lipophilic compounds (pesticides) As shown in table 2, The toxic effects of pesticides are very obvious, while rest of pesticides given in table 1. are showing no toxicity if the pesticide chemical range is kept as 200 ppm. Above 200 ppm. more analysis and experimental work is under progress and will be reported shortly with the outcome.

Acknowledgement

Many thanks to Prof. Mauro Magnani, of the Biotechnology Interuniversity consortium (CIB), Trieste, Italy for providing the generous funds for the study. I am very thankful to Prof. S. Girotti Instituto Scienze Chimiche, Bologna, Italy for providing me Lab. facilities through out. Thanks also goes to Khurasan traders, Khanozai through which I became able to re-study some of the samples for reconfirmation of the results.

References

1. N.Grossblatt, J.L. Whitterberger, C. J. Bailer, J. Doull, E.A. Pfitzer and A.C-Upton, Natl. Acad-Press, Washington, D.C. (1984).
2. A.Kahru.K.Tomson, T. Pall and I Kulm, *Wat. Sci. Tech.*, **33,** 147 (1996).
3. V. Philip, Ecological cycling, Wiley Inter science NewYork **26,** 607 (1993),
4. J.R. Corbett (Ed.), The Biochemical Mode of Action of Pesticides, Academic Press, New York (1974).
5. J.R Coat (Ed.). Insecticide Mode of Action, Academic Press, NewYork (1982).
6. P.A. Dahm, in R.D, O' Brien and I. Yamamoto (Eds.) Biochemical Toxicology of Insecticides, Academic press, NewYork (1982).
7. G.Russo and S. Calmotti, *Chim. Ind.*, **71,** 52 (1989).
8. D.S. Maclusky; Vbryant and R. Campbell, Marine Biol. Ann. Rev; **24,** 481 (1986).
9. M.R. Reeve, M.A. Walter, K. Darcy and T.

Ikeda, *Bull. Mar. Sci.*, **27**, 105 (1977).
15. D.J. Stom, T.A. Geel A.E. Balayan, G.I. Kuznetsor and S.E. Madvedeva, *Arch. Environ. Contamn. Toxicol.*, **22**, 203 (1992).
16. N. Lofhagon, C. Hartig and W. Babel, *Ecotoxicol. Environ. Safety*, **36,** 269 (1997).
17. D.P. Arfsten, R. Davenport and D-J. Scaeffer; Biomed. *Environ. Sci.*, 7, 101.(1999)
18. D.P. Fini, M. A. Salam, *Environ Toxicol*; **22**, 54, (2000).
19. G.Gellert, *Ecotoxi. Enviroment. Safety.*, **45**, 87 (2001)
10. M. Martin, K. E. Osbom, P. Billing, Mar. Pollu. Bull; **12,** 305 (1981).
11. E. J. Kuenzaer. in proceedings of a Symposium on Radioecology. Ann. Arbor. Michiga (1967) D.S Nelen and F.C. Evans (Eds.) 462673. USA. E. C .Oak. Ridge. Tennesse (Conf. 670503), (1967).
12. A. K. Singh, RJ. Zeiizniker and L. R. Drewes, *J. Chromatogr*, **5**, 324 (1985).
13. M. L. Shih and R.I. Ellin, Anal Lett; **19,** (1986).
14. A. Rode, P. Rouch, E. Ferri and R. Bovorra, *Analytica Chimica Acta*; 294, II (1994).