International Journal of Environmental Planning and Management
Vol. 1, No. 2, 2015, pp. 63-68
http://www.aiscience.org/journal/ijepm

Inhibitory Effect of Some Pesticides on the Freshwater Amoebae *Vahlkampfia Ustina*

Wafaa M. Hikal[1, 2, *], Ahmad Z. Al-Herrawy[2], Ezzat S. El-Daly[3], Shehata E. Elowa[3]

[1]Department of Biology, Faculty of Science, University of Tabuk, Tabuk, Saudi Arabia
[2]Parasitology Laboratory, Water Pollution Research Department, National Research Centre, Dokki, Giza, Egypt
[3]Department of Zoology and Entomology, Faculty of Science, Helwan University, Helwan, Egypt

Abstract

Many countries (including Egypt) still have severe problems in the water quality of their resources with special reference to drinking water. Apart from the poor quality levels detected during monitoring the biological pollutants are considered a direct cause of public health hazards. The inhibitory of 8 pesticides (methomyl, dimethoate, malathion, dicuran, cypermethrin, carbendazim, fenitrothion and butachlor) was estimated using the isolated and purified freshwater amoebae *Vahlkampfia ustina*. Toxicity experiments were carried out using short-term static relative sensitivity toxicity tests. Vahlkampfiaustina was separately exposed to each of the selected 8 chemical substances for 1, 10, 24, 48, 72 and 96 hr. The mean inhibitory effect of pesticides ranged from 0.006790 to 0.008275 mg/l for Vahlkampfia. *Vahlkampfia ustina* could be used as sensitive and convenient bioindicators for evaluating the toxicity of waters polluted with pesticides.

Keywords

Toxicity, Pesticides, Freshwater Amoebae, *Vahlkampfia ustina*

Received: April 11, 2015 / Accepted: April 29, 2015 / Published online: June 5, 2015

1. Introduction

Freshwater ecosystem is one of the environments most seriously affected by pollution. Water pollution may be of biological and/or chemical origin. The chemical contamination may be due to organic or inorganic substances. The organic chemical pollutants include fertilizers in runoff from agricultural fields and seeping of petroleum from tankers (Allen, 1995). The excessive industrial and agricultural activities with inattentive disposal of waste products raised the level of pollution in the aquatic habitats and caused various ecological and biological devastating effects (Chang et al., 1996; Kandeler et al., 2000). The hazards of these pollutants are not only their high toxicity, but also their longevity as most of them persist in the environment, often unchanged for a long period, and can reach human food and drinks (Allen, 1995). Surface water may be polluted by organic pesticides, either directly by application into water and runoff from the agricultural drift and/or indirectly from discharge of industrial wastewater (Allen, 1995). Pesticides are commonly encountered singly and as mixtures in drinking water, rivers, lakes and other aquatic bodies (Allsop et al., 1993). The toxicity of pesticide contaminated effluent depends on the amounts and types of the individual pesticide present. However, even for pure compounds the concentration-toxicity relationships are complex (Faust et al., 1994). The biological indicators of pollution are organisms being used more frequently for monitoring the aquatic contamination. Free-living amoebae (FLA) are unicellular inhabitants of aquatic habitats and

* Corresponding author
E-mail address: wafaahikal@gmail.com (W. M. Hikal)

moist soil. They are common and important organisms of ecological communities within different substrates and biofilms. The role of the gymnamoebae as a most widely distributed protozoan group and their meaning for microbial communities of soil, for example in nutrient cyclisation, is presently still under discussion (Anderson, 2000). The rapid rate of propagation, small size and sensitivity to minor surrounding environmental changes are the characteristics which merit the preliminary use of freshwater amoebae as a biological indicator. So the present work was directed towards the achievement of the following aims: Isolation, identification, purification and maintenance of some predominant strains of freshwater amoebae to be used as test organisms. Also, preliminary assessment of toxic effects of some pesticides to the isolated and purified strains of freshwater amoebae.

2. Materials and Methods

2.1. Preparation of Test Organisms

Freshwater amoebae were used as test organisms in the present work. Freshwater amoebae were collected from Nile river water, purified, identified, and maintained under laboratory conditions (Rogerson and Patterson, 2000).

Samples were collected and concentrated by using the membrane filtration technique. One liter of water was filtered through a nitro-cellulose membrane filter 0.45 µm pore size and 47 mm in diameter (Whatman, WCN type, Cat No. 7141-104) using a stainless steel holder and suction pump. The suction was stopped just before complete dryness of the membrane. After filtration the membrane was inverted face to face on the surface of non-nutrient (NN) agar plate previously seeded with 100 µl Escherichia coli (E. coli) which were used as a source of food for the growth of free-living amoebae. The inoculated plates were wrapped in plastic bags (to avoid dryness) for one week with daily microscopic examination for the presence of any amoebic growth (Hikal, 2005; Hikal et al., 2015)

2.2. Identification of Isolated Freshwater Amoebae

Isolated and purified freshwater amoebae were identified on the bases of both trophozoite and cyst morphology and physiology (Pussered and pons, 1977). After identification of strains of freshwater amoebae, the obtained strains were subcultured on a sterile glucose-salt (GS) agar plates previously seeded with E. coli. After incubation at 30°C for 3-7 days the cultured plates produced enormous numbers of the identified species of freshwater amoebae (Hikal, 2005; Hikal, 2010).

2.3. Preparation of the Pesticides Used

Eight organic compounds in the form of 8 insecticides, acaricides, fungicides and herbicides are already used for the agricultural purposes in Egypt. Methomyl, dimethoate, malathion, dicuran and butachlor were dissolved in distilled water. Cypermethrin was dissolved in chloroform. Carbendazim and fenitrothion were dissolved in acetone and dichloromethane, respectively. Stock solutions of the selected pesticides were prepared on the bases of the concentration of the active ingredient in the raw material. The prepared stock solutions were calculated and adjusted to give a final concentration of the toxicant equivalent to 1 mg/ml (Tomlin, 1994).

2.4. Toxicity Test Procedures

A short-term static relative sensitivity toxicity test was used in the present work according to duration, method of adding test solutions and purpose of the experiments (American Public Health Association, 1998). The isolated test organisms (freshwater amoebae) were separately exposed to duplicate containers of each experimental concentration used. A control sample including amoebae alone was presented with each experiment. From the control amoebae sample, the percentage of positivity (inhibition) was calculated. The collected trophozoites were washed 3 times with distilled water and centrifuged at 500 xg for 5 min. The supernatant was discarded while sediments were resuspended in 2 ml distilled water and counted using Sedgwick Rafter counting cell. Stock solutions of the 8 selected toxicants were separately used for the preparation of desired different concentrations. Three basic preliminary concentrations (1, 0.1 and 0.01 mg/l) were prepared and tested for each toxicant. According to the obtained results from the three preliminary tested concentrations, other ascending (3, 5, 7 and 9 mg/l) or descending (0.007, 0.004 and 0.001 mg/l) concentrations were prepared and used. Test organisms (isolated freshwater amoebae) were exposed to a wide range of concentrations of the test substances, usually in logarithmic ratio such as 1, 0.1 and 0.01 mg/L. This exploratory test allowed the determination of approximate concentration range to be included in the definitive short-term test (Hikal et al., 2015).

2.5. Determination of Median Inhibitory Concentrations (IC_{50})

Amoebae isolate were equally distributed into Petri dishes each containing one of the previously prepared concentrations of each pesticide and incubated at 30ºC for different contact times (1, 10, 24, 48, 72 and 96 h). The mean of three replicates of each pesticide concentration was calculated. A Petri dish containing amoebae only with distilled water was used as a control. After 96 h of exposure,

treated amoebae were examined microscopically to detect pesticide toxicity through loss of movement, rounding and encystations. Inhibition values were estimated as a result of loss of movement, rounding and encystation, but not death of amoebae (Hikal, 2005; Hikal et al., 2015).

2.6. Statistical Analysis

The obtained data were subjected to analysis of variance (ANOVA) according to Snedcor and Cochran (1990). Least significant differences (LSD) were used to compare between the means of treatments according to Waller and Duncan (11969) at probabilities 5% and 1%. Data were statistically analyzed using "MSTATC" computer program V. 2.1 (1985). The IC50 values were calculated by "SPSS" computer program.

3. Results

3.1. Isolation of Free-Living Amoebae from NileRiver Water

Water samples were collected from Nile River at El-Gezera site for the detection and isolation of the predominant species. Freshwater amoebae grown on NN agar media were picked up individually and subcultured on new and fresh NN agar plates for the isolation of pure strains. The morphological and physiological characterization of cultured freshwater amoebae revealed the isolation and purification of Vahlkampfiaustina amoebae.

3.2. Inhibitory Effect of Tested Pesticides on Vahlkampfia Ustina

The toxic effects of 8 different pesticides were tested towards the isolated amoebae strains Vahlkampfiaustina using a wide range of pesticide concentrations (0.001, 0.004, 0.007, 0.01, 0.1 and 1 mg/l) and different contact times (1, 10, 24, 48, 72 and 96 h) (Tables 1; 2). The calculated values of median inhibitory concentrations (IC50) were recorded in Table 3.

Results presented in tables (1; 2) showed a significant toxic effect of tested pesticides (methomyl, dimethoate, malathion, dicuran, cypermethrin, carbendazim, fenitrothion and butachlor) on Vahlkampfiaustina. The highest calculated inhibition percentages (79.66%) were caused by dimethoate while the lowest percentage of inhibition (68.31%) recorded with cypermethrin in Vahlkampfia. Dimethoate was more highly significant than carbendazim> dicuran> butachlor> methomyl> malathion>fenitrothion>cypermethrin

Table 1. Inhibitory effect (%) of tested pesticides on *Vahlkampfia ustina.*

Treatment		*Vahlkampfia ustina*					
Pesticides	Conc. (mg/L)	Contact time (hours)					
		1	10	24	48	72	96
Methomyl	0.001	15	20	35	45	55	60
	0.004	20	35	45	60	65	75
	0.007	30	60	65	80	90	95
	0.01	90	95	100	100	100	100
	0.1	100	100	100	100	100	100
	1	100	100	100	100	100	100
Dimethoate	0.001	10	25	40	60	65	75
	0.004	15	40	55	75	80	95
	0.007	20	65	75	90	95	100
	0.01	90	98	100	100	100	100
	0.1	100	100	100	100	100	100
	1	100	100	100	100	100	100
Malathion	0.001	5	15	25	40	50	70
	0.004	15	30	45	65	75	85
	0.007	25	45	60	85	95	100
	0.01	80	90	95	100	100	100
	0.1	85	97	100	100	100	100
	1	100	100	100	100	100	100
Dicuran	0.001	10	20	30	45	50	60
	0.004	25	30	50	70	85	90
	0.007	35	45	65	80	95	100
	0.01	80	95	100	100	100	100
	0.1	97	100	100	100	100	100
	1	100	100	100	100	100	100

Table 2. Continued the inhibitory effect (%) of tested pesticides on *Vahlkampfia ustina.*

Treatment		***Vahlkampfia ustina***					
Pesticides	**Conc. (mg/L)**	**Contact time (hours)**					
		1	**10**	**24**	**48**	**72**	**96**
Cypermethrin	0.001	3	15	22	30	42	50
	0.004	8	25	30	44	50	68
	0.007	19	35	60	68	72	80
	0.01	70	90	92	98	100	100
	0.1	91	98	99	100	100	100
	1	100	100	100	100	100	100
Carbendazim	0.001	10	30	45	53	60	70
	0.004	23	40	48	68	85	92
	0.007	30	41	70	95	100	100
	0.01	85	93	100	100	100	100
	0.1	98	100	100	100	100	100
	1	100	100	100	100	100	100
Fenitrothion	0.001	3	19	25	33	39	42
	0.004	20	35	39	45	49	60
	0.007	25	40	60	65	72	85
	0.01	78	85	91	98	100	100
	0.1	91	99	100	100	100	100
	1	98	100	100	100	100	100
Butachlor	0.001	11	19	25	40	49	65
	0.004	20	39	50	61	85	90
	0.007	32	40	72	85	92	100
	0.01	80	99	100	100	100	100
	0.1	97	100	100	100	100	100
	1	100	100	100	100	100	100
Mean of main effects:							
Pesticides (A):		Contact time (hours) (B)				Concentration (C)	
Methomyl	75.97	1	54.97			0.001	35.93
Dimethoate	79.66	10	65.56			0.004	50.06
Malathion	74.36	24	73.18			0.007	67.35
Dicuran	76.58	48	80.79			0.01	95.25
Cypermethrin	68.31	72	85.31			0.1	99.00
Carbendazim	78.77	96	89.73			1	99.96
Fenitrothion	69.33						
Butachlor	76.42						
	A	B	C	(AB)	(AC)	(BC)	(ABC)
LSD $_{5\%}$	0.45	0.43	0.39	1.11	1.11	0.96	2.71
LSD $_{1\%}$	0.59	0.56	0.52	1.45	1.45	1.26	3.56

Table 3. Median inhibitory concentrations (1 and 10 hr-IC_{50}) of tested pesticides on *Vahlkampfiaustina.*

Pesticides	**IC $_{50}$ (mg/l)**		
	Vahlkampfia ustina		
	1 hr	**10 hr**	**Mean**
Methomyl	0.007920	0.005940	0.006930
Dimethoate	0.008240	0.005340	0.006790
Malathion	0.008340	0.007020	0.007680
Dicuran	0.007820	0.007010	0.007415
Cypermethrin	0.008920	0.007630	0.008275
Carbendazim	0.007930	0.006950	0.007440
Fenitrothion	0.008310	0.007210	0.007760
Butachlor	0.008080	0.006820	0.007450
	Time(A)	Pest.(B)	(AB)
LSD $_{5\%}$	0.00093	NS	NS
LSD $_{1\%}$	0.00112	NS	NS

4. Discussion

The increasing environmental pollution has led to an over growing concern about the potential effects of these pollutants on human health, directly or indirectly. In recent years, there has been growing concern about the toxic effects of chemical substances in the aquatic environment (Codina et al., 1993). An aquatic toxicity test is a procedure in which the responses of aquatic organisms are used to detect or measure the presence or effect of one or more substances, wastes, or environmental factors, alone or in combination. Toxicity tests are desirable in water quality evaluations because the chemical and physical tests alone aren't sufficient to assess potential effects on the aquatic biota (Grothe et al., 1996). Toxicity tests are classified according to a) duration: short-term, intermediate, and/or long-term, b) method of adding test solutions: static, renewal, or flow-through and c) purpose: effluent quality monitoring, single compound testing, relative toxicity, relative sensitivity, taste or odor, or growth rate (American Public Health Association, 1998).

In the present study short-term static single compound toxicity tests were used to estimate the relative sensitivity of freshwater amoebae Vahlkampfiaustina towards 8 chemical compounds. In the present work, the choice of freshwater amoebae may allow more rapid, less labour-intensive and inexpensive toxicity tests. The prevalence and conspicuousness of naked freshwater amoebae in aquatic ecosystems also make them notable organisms for the assessment of water quality (Lynn and Gilron, 1992). In the present work, the tested 8 pesticides were chosen so as to cover a wide range of chemical groups that were usually used for synthesis and production of pesticides. The mode of actions of these selected pesticides upon target organisms varied from systemic insecticide (methomyl and dimethoate) to non-systemic insecticide (malathion, cypermethrin and fenitrothion), selective herbicide (dicuran and butachlor) and systemic fungicide (carbendazim) (Tomlin, 1994). Moreover, the tested pesticides in the present work were manufactured and consequently applied for usage in agricultural purposes in Egypt. The published data concerning toxicity of these tested pesticides to non-target aquatic micro-organisms, especially protozoa, are scarce. Moreover, the tested pesticides in the present work were manufactured and consequently applied for usage in agricultural purposes in Egypt. The published data concerning toxicity of these tested pesticides to non-target aquatic micro-organisms, especially protozoa, are scarce. Concerning inhibitory of Vahlkamfiaustina in the present study, it was shown that dimethoate was more highly significant than carbendazim>dicuran>butachlor>methomyl>malathion>fenitrothion>cypermethrinIn a study of the effect of pesticides on the populations of bacteria, actinomycetes, fungi and protozoa, Ekundayo (2003) found that agrosan (phenyl mercuric acetate) was the highest toxic one at 5 µg/g soil. It totally eliminated all protozoa, inhibited bacterial density from 4,600,000 to 22 cells/g and reduced the fungal population from 34,000 to 60 cells/g. In general, protozoa and fungi were susceptible to fungicides than bacteria and actinomycetes. On studying the effects of biocides on soil protozoa, Foissner (1997) found that insecticides were usually more toxic than herbicides while fungicides had rather varied effects and most of them didn't influence soil protozoa critically. The effects of organophosphorous insecticide fenitrothion on 12 freshwater algae were studied by Kent and Weinberger (1991). They found that 10 mg/l fenitrothion significantly reduced growth rate in all tested species. In a study conducted by Mohapatra and Mohanty (1992) it was found that the 10-d LC50 for Chlorella vulgaris algae was as high as 51 mg/l dimethoate. In recent years, aquatic toxicity testing has been applied to a variety of different regulatory and scientific purposes, including toxicity testing of municipal and industrial effluents as part of monitoring/permit compliance (Weber, 1993; Lewis et al., 1994; Grothe et al., 1996), the derivation of national and site-specific water quality criteria for individual chemicals (U.S. Environmental Protection Agency, 1994), product safety evaluations (U.S. Environmental Protection Agency, 1985), chemical persistence studies (Weber, 1993), testing sediments and studies included in toxicity reduction evaluation (TRE) programs to identify constituents causing toxicity in effluents (U.S. Environmental Protection Agency, 1991). These diverse applications have broadened the utility of toxicity testing and made more important the judicious interpretation of their results.

In conclusion, freshwater amoebae Vahlkamfiaustina were used as test organisms for the first time.

5. Conclusion

Toxicity tests must be employed with a variety of test organisms to provide data that can be used to indicate toxicant concentrations likely to be harmful to freshwater ecosystems. Further investigations are needed to determine the interactions of different toxicants to each other and in the field.

References

[1] Allen, P. (1995). Accumulation profiles of lead and cadmium in the edible tissue of Oreochromis aurous during acute exposure. J .Fish Biol., 47: 559-597.

[2] Allsop, P.J.,Chisti, Y., Moo-Young, M. and G.R. Sullivan (1993). Dynamics of phenol degradation by Pseudomonas putida. Biotechnol. Bioeng, 41: 572-580.

[3] American Public Health Association (1998). Standard methods for the examination of water and wastewater. 20th ed., APHA, AWWA, WEF, Washington, DC.

[4] Anderson, O. R. (2000). Abundance of terrestrial Gymnamoebae at a Northeastern U.S. site: a four-year study, including the El Nino winter of 1997-1998. J. Eukaryot. Microbiol, 47: 148-155.

[5] Chang, W.F., Hung, T.C. and T.L. Lee (1996). Studies on the heavy metal contents of molluscas in the southwestern coastal waters of Taiwan. J. Mar. Sci., 26: 17-64.

[6] Codina, J. C., Perez-Garcia, A., Romero, P. and A. De Vicente (1993). A comparison of microbial bioassays for the detection of metal toxicity. Arch. Environ. Contam. Toxicol., 25: 250-254.

[7] Ekundayo, E.O. (2003). Effects of common pesticides used in the Niger Delta Basin of Southern Nigeria on soil microbial populations. Environ. Monit. Ass, 89: 35-41.

[8] Faust, M.,Altenburger, R., Boedeker, W. and L.H. Grimme(1994). Algal toxicity of binary combinations of pesticides. Bull. Environ. contam. Toxicol, 53: 134-141.

[9] Foissner, W. (1997). Protozoa as bioindicators in agroecosystem with emphasis on farming practices, biocides, and biodiversity. Agricul. Ecosyst. Environ, 62: 93-103.

[10] Grothe, D. R., Dickson, K. L. and D. K. Reed-Judkins (1996). Whole effluent toxicity testing: an evaluation of methods and prediction of receiving system impacts. SETACPellston workshop on whole effluent toxicity, September 16-25, 1995, Pellston, Mich. SETAC press, Pensacola, Fla.

[11] Hikal,W.M. (2005). Freshwater amoebae as a biological indicator for some environmental chemical pollutants, M. Sc. Thesis, Fac. Sci., Helwan Univ.

[12] Hikal, W. M. (2010). Biochemical and molecular characterization of pathogenic free-living amoeba in the aquatic environment. ph. D. thesis, fac. Sci., Ain Shams Univ., Egypt.

[13] Kandeler, E.,Tscherko,D., Bruce, K. D.,Stemmer,M., Hobbs,P. J., Bardgett,R. D. and W. Amelung (2000): Structure and function of the soil microbial community in microhabitats of a heavy metal polluted. Soil Biol. Fertil. Soils, 32: 390-400.

[14] Kent, R. A. and P. Weinberger (1991).Multibiological level responses of freshwater phytoplankton to pesticides stree. Environ. Toxicol. Chem., 10: 209-216.

[15] Lewis, P. A.,Klemm, D. J.,Lazorchak, J. M., Norberg-King, T. J.,Peltier, W. H. and M. A. Heber(1994). Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, 3rd ed. EPA-6--/4-91-002, environmental monitoring systems lab., U.S. Environmental Protection Agency, Cincinnati, Ohio.

[16] Lynn, D. H. and G. L. Gilron(1992). A brief review of approaches using ciliated protists to assess aquatic ecosystem health. J. Aqua. Ecosyst. Health, 1: 263-270.

[17] Mohapatra, P. K. and R. C. Mohanty (1992). Growth pattern changes of Chlorella vulgaris and Anabaena doliolum due to toxicity of dimethoate and endosulfan. Bull. Environ. Contam. Toxicol., 49: 576-581.

[18] Pussard, M. and R. Pons (1977).Morphologie de la paroikystiqueettaxonomie du genre Acanthamoeba (protozoa, amoebida). Protistol.,TXIII: 557-598.

[19] Rogerson, A. and D. J. Patterson (2000). The naked ramicristate amoebae (Gymnamoebae) in: An illustrated guide to the protozoa, 2nd edition (Eds. Lee J.J., Leedale, G.F. and Bradbury P.). Society of Protozoologists, Lawrence, Kansas. pp. 1023-1053.

[20] Snedcor, G. W. and W.G. Cochran (1990). Statistical Methods, 9th ed. Iowa State University Press, Iowa, USA.

[21] Tomlin, C. (1994). The pesticide manual incorporating the agrochemicals handbook, 10th ed., The Royal Sosciety of Chemistry, Thomas Graham House, Cambridge, UK.

[22] U.S. Environmental Protection Agency (1985). Environmental effects testing guidelines. 40 CFR part 797; Federal Register., 50: 39321.

[23] U.S. Environmental Protection Agency (1991). Technical support document for water quality-based control. EPA-505/2-90-001 (PB91-127415), off. Water, U.S. Environmental Protection Agency, Washington, D.C.

[24] U.S. Environmental Protection Agency (1994). Interim guidance on determination and use of water effect ratios for metals. EPA/823-B-94-001, U.S. EPA, off. Water, Washington, D.C.

[25] Weber, C. I. (1993). Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 4th ed. EPA-600/4-90-027F, environmental monitoring and support lab., U.S. Environmental Protection Agency, Cincinnati; Ohio.

[26] Hikal, W.M., Al-Herrawy, A.Z., El-Daly, E.S. and S.E.Elowa (2015). Assessment of Pesticide Toxicity Using The Freshwater Amoeba Rosculusithacus in vitro. Pyrex Journal of Research in Environmental Studies. 2 (1): 008-012.