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Original Article

Impact of Organic Contamination on Some Aquatic Organisms

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ABSTRACT

Background: Contamination of water systems with organic compounds of agricultural uses pose threats to aquatic organisms. Carbaryl, chlorpyrifos, and diuron were considered as model aquatic pollutants in this study. The main objective of this study was to characterize the toxicity of organic contamination to two different aquatic organisms. **Materials and Methods:** Low concentrations (0.0–60 μmol/L) of carbaryl, diuron and very low concentration (0.0–0.14 μmol/L) of chlorpyrifos and their mixtures were tested against fish and *Daphnia magna*. Percentage of death and immobilization were taken as indicators of toxicity. **Results:** Toxicity results to fish and *D. magna* showed that chlorpyrifos was the most toxic compound (LC_{50} to fish and *D. magna* are 0.08, and 0.001 μmol/L respectively), followed by carbaryl (LC_{50} to fish and *D. magna* are 43.19 and 0.031 μmol/L), while diuron was the least toxic one (LC_{50} values for fish and *D. magna* are 43.48 and 32.11 μmol/L respectively). Mixture toxicity (binary and tertiary mixtures) showed antagonistic effects. Statistical analysis showed a significant difference among mixture toxicities to fish and *D. magma*. **Conclusion:** Fish and *D. magam* were sensitive to low concentrations. These data suggest potent threats to aquatic organisms from organic contamination.

Key words: Carbaryl, chlorpyrifos, diuron, *Daphnia magna*, fish, toxicity, mixture

INTRODUCTION

Contamination of water systems with organic compounds is well known in the world. In this study carbaryl, chlorpyrifos, and diuron were considered as model organic pollutants.

Application of pesticides in agricultural or health sector creates residues in food, water and soil in all Arab countries,[1] health problems have also been reported.[2-4] Pesticides can move from the terrestrial environment into the watershed and may cause ecological problems far from the site of application.[5-7] For instance, a number of nontarget species have been endangered.[8,9]

Many of the studies[10-15] revealed that chlorpyrifos, carbaryl and diuron residues have been detected in rivers with lethal concentration to aquatic life such as fish, shrimp, stoneflies, and frogs.

Moreover, some authors[16-20] mentioned that pesticide residues are usually present as mixtures in aquatic systems. This may enhance the threat to the aquatic organism and referred to as synergistic effects of organic pollutants. In contrast, a reduction of toxic effect may be observed as a result of mixture this effect referred to as antagonistic effect.

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For instance, some studies[21-24] found enhanced toxicity on freshwater fish when carbaryl was mixed with phenthoate or when diazinon was mixed with chlorpyrifos. In contrast, other studies[25-26] revealed reduced toxicity of mixtures to *Daphnia magna* when mixing formetanate and fenamiphos or amphibian populations when mixing chlorpyrifos, and carbaryl.

Fish farming in Gaza become very intensive, in the last years many of farms were established. It has been revealed by the fish breeders in Gaza that many death cases were observed every morning or during changing the water. The farmers mentioned that they used fresh water from agricultural wells in their locations. However, the problem still exists. The authors of this study suggested that the source of water may be contaminated with pesticide residues due to pest control processes, or the fish farms received drift contamination of pesticides during the daily pest control process. Accordingly this study was designed to: (1) Characterize the toxicity of organic pollutants (carbaryl, chlorpyrifos, and diuron) as single, binary and their tertiary mixtures to local fish (*Tilapia nilotica*) and other aquatic organism *D. magma*; (2) evaluate the sensitivity of the tested organisms to the tested pesticides; and (3) to determine the synergism or antagonisms effect of the mixtures.

MATERIALS AND METHODS

Materials

Carbaryl, purity 99.7%, chlorpyrifose, purity 99.2%, and diuron purity 99.4% were purchased from seelze deutschland - Fluka and Riedel-de Haën chemical plant, Germany. More details on physical and chemical properties are shown in Table 1[27], the chemical structures of the tested compounds are shown in Figure 1.

Technical amount equivalent to 60 μmol of carbaryl, 60 μmol of diuron or 3.99 μmol of chlorpyrifos was dissolved separately in 1-2 ml methanol and completed with distilled water to 1 L. The solution of each compound was left close under magnetic stirrer for 3 h to insure complete homogenization and complete solubility of the compounds. Series of gradient concentrations (0, 10, 20, 30, 40, 50, and 60 μmol of carbaryl or diuron, whereas very low concentrations (0, 0.02, 0.05, 0.06, 0.1, and 0.12 μmol) for chlorpyrifos were prepared and tested.

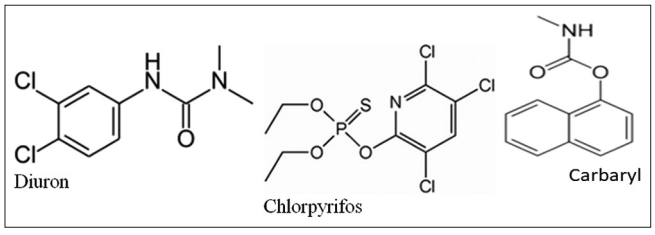


Figure 1: Chemical structure of the tested compounds

Local fish (*T. nilotica*) and *D. magma* were taken as two different aquatic organisms.

Preparation of mixtures

The concentration mentioned above were prepared and mixed together either as even portions or at, 0.75 + 0.25. These mixtures were prepared and tested against fish and *D. magna*.

Breading and acclimatization of *Daphnia magna*

D. magna is a freshwater zooplankton. It belongs to group arthropoda, branchiopoda, and family daphniidae. Daphnia are small about 1–5 mm in size. It feeds on phytoplankton and organic detritus. Sufficient population of *D. magna* was obtained from a storm water collection pond near Wadi Gaza, transformed to a 50 L black plastic pail, fed them with small green algae. After 2 weeks under 16/8 h dark/light cycle under the recommended condition of the OECD static test,[28] the temperature with the range of 22° ±1°C, pH 7.5 ± 0.5, and 16/8 dark-light cycle. The activity of the organisms indicated their acclimatization. These active organisms were used in the toxicity tests.

Breading and acclimatization of fish

Local fish (*T. nilotica*) larvae were purchased from commercial fish supplier (ASSDAI Media city Farm, Khnyonis - Palestine) the average weight of individual fish was 20 ± 1 g and average length of 4 ± 0.5 cm. The fish were held in a 500 L plastic barrel equipped with aeration pump to supply oxygen to the water. The water had the following characteristic: Temperature: 25.00 ± 1°C, pH: 7.5 ± 0.5, de-saline a 16 h light/8 h. Dark photoperiod was maintained. Fish were acclimated for 2 weeks under the laboratory conditions before starting of the experiments. During the acclimatization, the fish were fed on water fleas. During the test 5 fishes were put on glass aquaria (10 cm × 15 cm × 20 cm) half filled with the test solutions that were pre-saturated with oxygen. The dissolved oxygen was tested every 12 h using the dissolved oxygen meter. The temperature also was tested every 12 h to ensure suitable conditions during the test.

Table 1: Selected properties of the tested pesticides

Chemical name	MW	Solubility in water (mg/l)	K_{ow}	K_h	LD_{50} for rat (mg/kg)
Chlorpyrifos	350.6	1.4	4.7	6.76×10^{-1}	135-163
Carbaryl	201.2	120	1.59	NA	500
Diuron	233.1	36.4	2.85	7.04×10^{-6}	3400

K_h = Henry's law constant (atm m^3/mol). NA = Not available, MW = Molecular weight

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Toxicity test on fish and *Daphnia magna*

Two days before toxicity tests, the fish were transferred to a deionized water with no feed. For fish tests, 4 days (96 h) static acute toxicity test[29] was performed to determine the dose-effect curve for carbaryl, chlorpyrifos, and diuron under laboratory conditions. For *D. magna* tests, they were exposed to the concentrations of the tested compound for 48 h under the same conditions of acclimatization as mentioned above. Each test consists of five concentrations of each compound as mentioned above with 2 replicates and 2 control samples, one of them is a positive control which received 50 µl methanol and negative control, which received only deionized water. The fish tests were performed in glass containers 1 l capacity, whereas *D. magna* tests performed in 20 ml glass test tubes.

Calculation of toxicity

The percent death of fish and % immobilization of *D. magma* were taken as indicators of the toxicity of the tested pesticide in single, binary and/or tertiary mixture. Calculation of % death was done using Equation 1 according El-Nahhal *et al*.[30] with a slight modification:

$$\% \text{ Death} = 100 \times (L_c - L_t)/L_c \quad [1]$$

Where L_C and L_t are the number of the live organism in the control and the treated samples respectively. Percent death or immobilization was regressed versus concentration and converted to a log scale to calculate the LC_{50} values; furthermore, the % death was also regressed versus time to calculate LT_{50} (the exposure time required to kill 50% of the tested organisms).

Toxicity of mixtures was calculated based on % death and toxic units available in the solution. According to Sprague and Ramsay,[16] toxic unit was calculated as:

Toxic unit = actual concentration in solution/lethal threshold concentration

Furthermore, the toxic unit was defined as the concentration of a chemical in the toxic mixture divided by its single toxic concentration for the end point measured.[31]

To determine the antagonistic, and/or synergistic effect we calculated the mixture toxicity index (MTI) according to Equation 2.[32,33]

$$\text{MTI} = 1 - (\text{Log M}/\text{Log N}) \quad [2]$$

Where $M = \sum c/EC_{50}$ at 50% effect in the mixture, and n = total number of the compound in the mixture. According to Equation 2, MTI value may have a negative value (antagonistic effect), zero value (no effect) and a positive value (synergistic effect).

Statistical analysis

Average and standard deviation for each treatment were calculated and used for calculation the % death or immobilization for fish and *D. magan* respectively. One-way ANOVA was used to detect a significant difference among treatments.

RESULTS

Influence of concentrations

Toxicity of carbaryl, chlorpyrifos, and diuron to fish and *D. magna* are shown in Figure 2. It is obvious in all cases that % death increased almost linearly in all tested compound as their concentrations increased in the solution. However, for the case of the fish test, it is obvious for carbaryl and diuron that the low observed toxic effect (LOTE) was at 30 mmol/l. Then, a steep increase on % death was observed. For the case of chlorpyrifos, it was not possible to detect the LOTE fish. For *D. magan*, the trend of toxicity is similar, but the magnitude and the shape of toxicity curves are different.

Converting the data in Figure 2 to the corresponding log scale enabled the calculations the LC_{50} values of the test pesticides. The values are presented in Table 2. It is obvious that carbaryl and diuron have nearly equal LC_{50} value for fish, whereas chlorpyrifos has a very low LC_{50} value (0.08 µmol/L). These data indicate that chlorpyrifos is the most toxic one among the tested pesticides. Furthermore, the regression coefficient (R^2) associated with LC_{50} calculations ranged between 0.91 and 0.99 indicating strong positive association statistical analysis of % death obtained by the three tested pesticides against fish does not show any significant difference. *P* values ranged between 0.32 and 0.5. The calculated LC_{50} values of *D. magna* [Table 2], indicates that chlorpyrifos has the lowest value, followed by carbaryl and diuron.

Comparison with the LC_{50} values of fish, one can realize that the compounds were several times higher in the toxicity to *D. magan* than to fish. The correlation factors (R^2) are in the range of 0.96–0.99 indication strong positive association.

Influence of exposure time

Effect of exposure time on the toxicity of carbaryl, chlorpyrifos, and diuron on fish is shown in Figure 3.

Table 2: Essential toxicity parameters on fish and *Daphnia magna*

Compound	Fish				*Daphnia magna*	
	LC_{50} (µmol/L)	R^2	LT_{50} (h)	R^2	LC_{50} (µmol/L)	R^2
Carbaryl	43.19	0.95	60.86	0.96	0.031	0.98
Chlorpyrifos	0.08	0.99	68.04	099	0.001	0.96
Diuron	43.48	0.91	65.01	0.92	32.11	0.99

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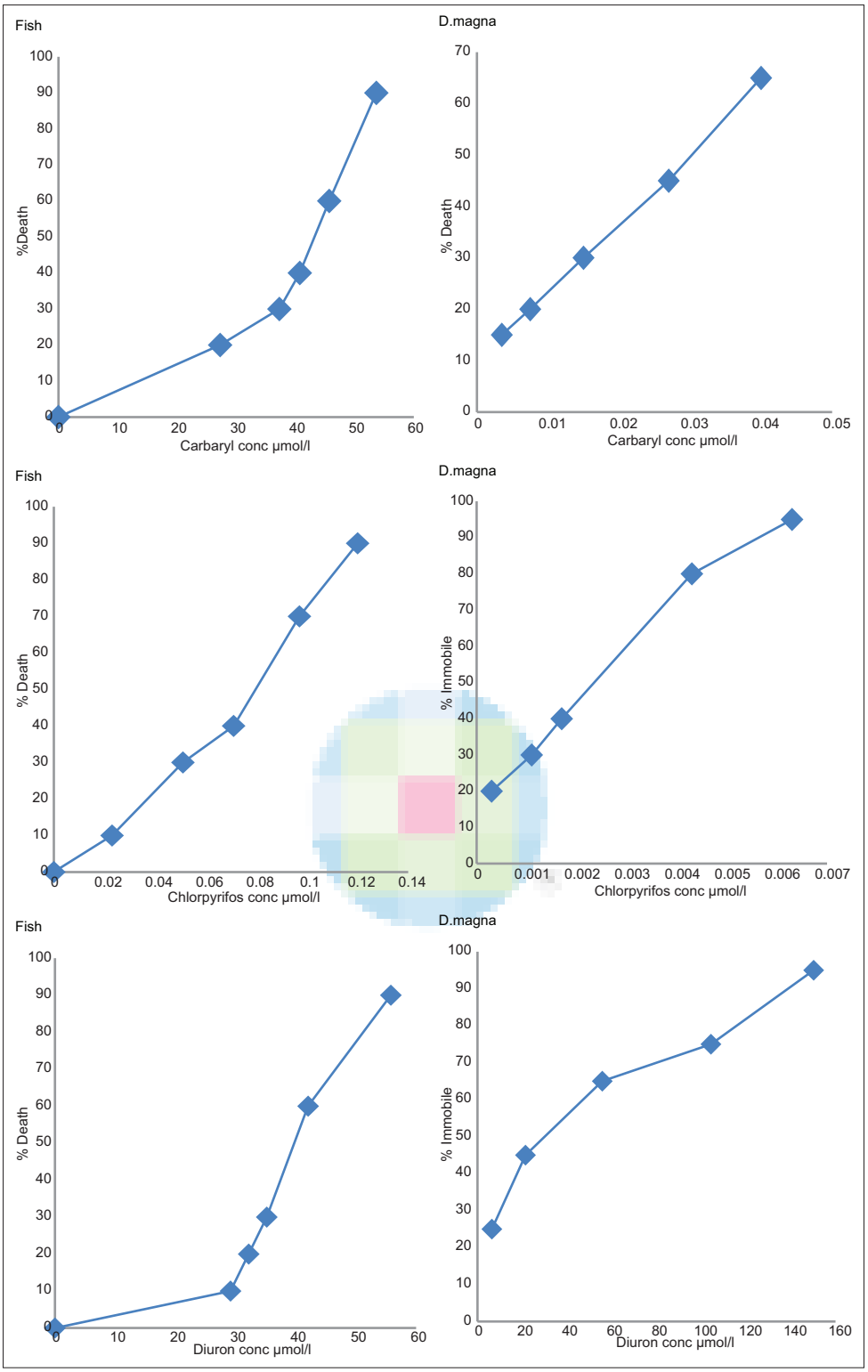


Figure 2: Toxicity of carbaryl, chlorpyrifos and diuron to fish and *Daphnia magna* as individual test, percent death and immobilization were recorded after 96 h and 48 h of exposure for fish and *Daphnia magna* respectively

The effect on *D. magna* was not measured because the toxicity protocol is limited to 48 h which is not suitable to make the same measurements on fish. The data clearly demonstrated the linear relationship between % death of fish and exposure time to carbaryl, chlorpyrifos, and diuron at fixed concentration. It is obvious that the tested compounds have a similar trend of effect. Converting the data in Figure 3 to the corresponding log scale enabled the calculation of LT_{50} values (the exposure time that necessary to kill 50% of the tested organisms).

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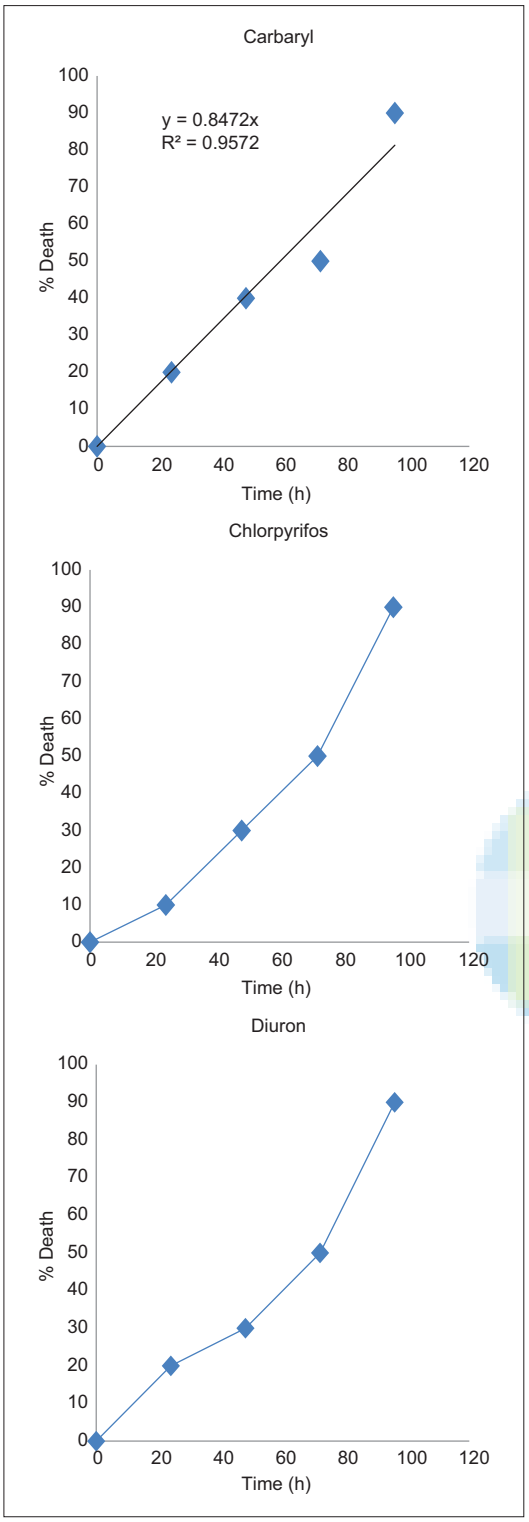


Figure 3: Dynamic effect of carbaryl concentration (53.67 µmol/L), chlorpyrifos concentration (0.12 µmol/L) and diuron concentration (55.8 µmol/L) on fish life

The calculated values are: 60.86, 68.04, and 65.01 h for carbaryl, chlorpyrifos and diuron, respectively. The associate R^2 value exceeded 0.92 for all cases indicating a strong positive association.

Toxicity of mixtures

Toxicity of three binary mixtures to fish and *D. magna* are shown in Figure 4. It can be seen that mixtures are more toxic to *D. magna* than fish. Moreover, the toxicity were high to both cases when the mixture contains carbaryl and chlorpyrifos, as in MX1.

However, when the mixture contains diuron as in MX2 and MX3. A dramatic decrease in the toxicity of fish was observed. Furthermore changing the portion of diuron in the mixtures resulted in a considerable change in the toxicity of the mixtures [Figure 3].

Furthermore, the comparison of LT_{50} values demonstrates significant variations. However, the very close values are due to the variations of K_{ow} and Henry low constant [Table 1].

Toxicity of tertiary

Effects of tertiary mixtures are shown in Figure 5. It is obvious that the effect is more pronounce in *D. magna* tests. This may indicate the antagonistic effect on fish tests.

DISCUSSION

The presented results in Figure 2 clearly demonstrate the potential toxicity of the tested compounds on fish and *D. magna*. This indicates the sensitively and the homogeneity of the tested organisms. Moreover, the data indicate a strong positive association between % death and the tested concentrations. The detectable level of LOTE with fish tests indicate that fish can tolerate some level of toxicity, whereas LOTE level was not possible to detect it with *D. magan* due to high sensitivity. Moreover, above 30 µmol/L of carbaryl and diuron, % death increased rapidly with a steep rise up 85%, whereas a linear increase of % death with chlorpyrifos concentration below 0.15 µmol/L. The explanation of these results is that carbaryl, chlorpyrifos, and diuron are toxic pesticides with different values of LD_{50}.[27] Moreover, these compounds have high K_{ow} values [Table 1] that may enable them to rapidly partitioned from aquatic phase to hydrophobic phase (fish, or *D. magna*), beside the fact that bio-accumulation and distribution become faster. Our explanation is supported by the results of Liu *et al*.[34] who emphasized the value of K_{ow} to explain the bio-accumulation of Amoxicillin to fish. In addition, our toxicity results agree with previous reports[35-37] who found similar results.

In addition, the LC_{50} values of carbaryl and diuron indicating similar toxicity. An interesting outcome of these results is that the high toxicity of diuron to fish. These data are in accord with the presented results in Figure 2.

Furthermore, the low LC_{50} values of the tested compounds to *D. magna* comparing to fish, is probably due to possible

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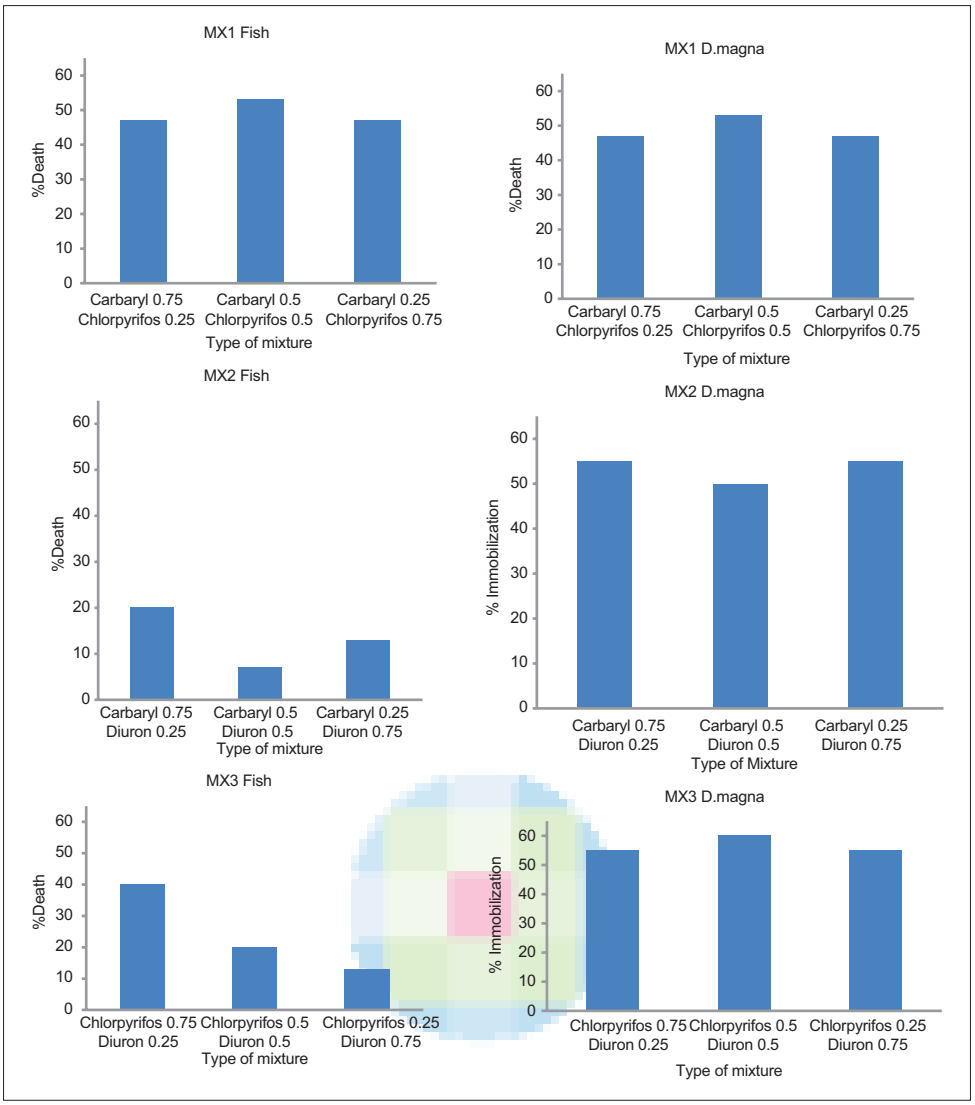


Figure 4: Mixture toxicity of carbaryl and chlorpyrifos (MX1), carbaryl and diuron (MX2) and chlorpyrifos and diuron (MX3) with different combinations of solubility limit in water as binary mixtures on fish and *Daphnia magna*

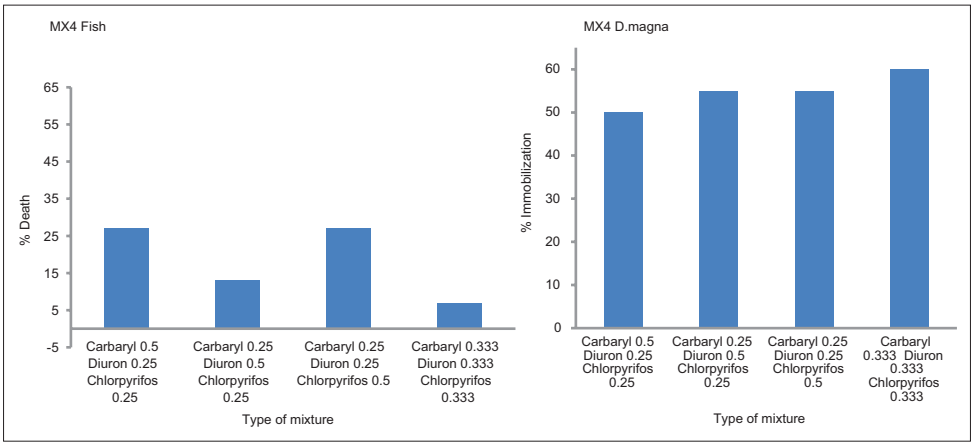


Figure 5: Mixture toxicity of carbaryl, chlorpyrifos and diuron (MX4) with different combinations of solubility limit on water as tertiary mixture on fish and *Daphnia magna*

bio-accumulation of the tested compounds in fish than in *D. magna* due to the fact that fish body is larger than *D. magna* and contains larger fraction of fat bodies. According more bio-accumulation may occur in fish. This

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suggestion is in accord with previous report[38] who found the high potential of bio-accumulation due to chronic exposure to low dose of chlorpyrifos or carbaryl.

Statistical analysis of % death versus exposure time obtained by the three tested pesticides against fish does not show any significant difference. *P* values ranged between 0.43 and 0.46.

The toxicity of binary mixtures is more pronounced in *D. magna* than in fish. This suggests an antagonistic effect on fish and partial synergistic effects on *D. magna*. Moreover, the mixture became more toxic when it contained carbaryl and chlorpyriofs with a different combination. The explanation of these results is that both carbary and chlorpyrifos belong to different chemical groups, but the mode of action of their toxicity is similar to each other. Both molecules had been shown to inhibit acetylcholine esterase,[13] accordingly when they mixed together it is expected to have an additional effect or synergistic effect. In addition mixing carbary with diuron, which has different mode of action reduced % death or % immobilization of the mixture as in M × 2 or M × 3 in fish tests. Our results agree with previous report[19] who found the joint action of carbaryl and phenthoate mixture on fish. Our explanation is also supported by previous work of Sprague[17] who discussed in details the influence of mixture toxicity on different organisms.

In addition, it is also obvious that portion of combination has a role to potentiate the toxicity of mixture, especially when the mixture contained molecules with a different mode of action such as MX2 and MX3. It is obvious in both cases that reducing the fraction of carbaryl or chlorpyrifos in the mixture from 0.75 to 0.25 resulted in a sharp drop in the toxicity of the mixture in fish test, whereas in *D. magan* test, it was not possible to detect the effect due to high sensitivity to the tested concentration.

In addition, although diuron has different mode of action but it is still possible that diuron can potential the toxicity of carbaryl and chlorpyrifos and as shown in MX2 and MX3 in *D. magana,* whereas the effect in fish test is totally different. Our results agree with previous reports[7,10,21,38,39] who found that triazine potentiated the toxicity of chlorpyrifos and diazinon to the midge *Chironomus tentans*.

Furthermore, at similar mode of action as in carbaryl and chlorpyrifos, and even fraction of combination (0.5 + 0.5), each molecule react independently with sensitive site on the nervous system of the fish or *D. magna*. At uneven portion of combination (0.75 + 0.25) the molecules tend to interact to each other and form a larger molecule that geometrically may not fit the interaction with sensitive site in the nervous system of fish or *D. magna* accordingly reduction of % death may be obtained. This explanation agree with El-Nahhal and Safi[40] who found that organic molecules dissolved to each other and form a larger molecule that cannot react with the adsorption site due to steric effect. Our results also agree with Laetz *et al*.[24] who evaluated the mixture toxicity of organophosphate and carbamate pesticides on juvenile *Coho salmon* (Oncorhynchus kisutch) exposed to sublethal concentrations of chlorpyrifos, and carbaryl and observed addition and synergism, with a greater degree of synergism at higher exposure concentrations. Several combinations of organophosphates were lethal at concentrations that were sublethal in single-chemical trials.

An interesting outcome of this comparison is that diuron toxicity is nearly high comparing with data from the previous report.[41]

Evaluation of MTI according to [Equation 2], showed negative values to all mixtures [Table 3] except MX4 with *D. magna*. This indicates the antagonistic effect of the negative values and synergistic effect with the positive value. This agree with the presented results above.

CONCLUSION

This study investigated the toxic effects of organic pollutants (carbaryl, chlorpyrifos, and diuron) on fish and *D. magam* and provides answers to the reasons behind mortality of local fish farming. Moreover, the study ranked chlorpyrifos the most toxic one for fish and *D. magma* with LC_{50} values equal to 0.08 and 0.001 µmol/L respectively. Carbaryl and diuron have similar LC_{50} for fish 43.19 and 43.48 µmol/L respectively. Carbaryl was also very toxic to *D. magma* with an LC_{50} = 0.031 whereas diuron has 32.11 µmol/L.

Time response relationships were nearly similar and LT_{50} values were very close to each other and ranged from 60.86 to 68.04 h indicating similar time of exposure time required to produce the toxic effect. Toxicity evaluation indicates that *D. magma* was more sensitive than fish to organic pollution. Statistical analysis revealed significant differences among mixture toxicities to fish and *D. magama*.

Binary and tertiary mixtures were less toxic to fish than to *D. magma* and did not produce % death more than

Table 3: Values of MTI

Tested mixtures	MTI	
	Fish	*Daphnia magna*
MX1	−3.97±(0.42)	−12.45±(0.49)
MX2	−2.08±(0.44)	−12.09±(0.8)
MX3	−3.64±(0.67)	−9.83±(0.8)
MX4	−1.81±(0.24)	10.06±(0.36)

MTI = Mixture toxicity index

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60% in all cases due to possible antagonistic effects. Application of MTI indicated antagonistic effects of all mixture. Moreover, it is recommended to check water quality before transferring it to fish farms to unsure absence of organic pollutants especially those mentioned in this study.

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Conflicts of interest

There are no conflicts of interest.

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