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Toxicity of Amoxicillin and Erythromycin to Fish and Mosquitoes

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Abstract

This study characterized the toxicity of Amoxicillin (AM), Erythromycin (ER) and Endosulfan (EN) to fish and mosquito larvae obtained from a certified fish farm and from Wadi Gaza. The acute toxicity tests were determined by calculating mortality percentage of fish and mosquitoes through a gradient concentration of the tested compounds. Mortality percentage, exposure time, LC_{50} and LT_{50} were taken as indicators of toxicity. The results showed that AM was the potent compound against fish with LC_{50} value lower that EN. Toxicity to fish is in the following order: AM > EN > ER whereas the toxicity to mosquitoes is in the following order: ER > EN > AM. Fish are more sensitive to AM than mosquitoes whereas mosquitoes are more sensitive to ER than fish. The interesting outcome of the study is the calculated LC_{50} values are far below the concentration found in different water systems. Mixture toxicity of the tested antibiotics indicates antagonistic effect on both fish and mosquitoes.

Key-words: Antibiotics, Fish, Mosquitoes, LC_{50}, Toxicity.

INTRODUCTION

Antibiotics are pharmaceuticals widely used not only for human and veterinary medication but also for livestock and aquaculture growth promotion (Sarmah et al.,2006). After normal application of the antibiotics, 50 to 90% of them and/or their metabolites are eliminated from the body, mainly through urine and feces, which then enter the environment indirectly through sewage treatment plants or directly through fertilizer application to agricultural land (Schlusener & Bester, 2006).

Amoxicillin (AM) and Erythromycin (ER), act by inhibiting the synthesis of bacterial cell wall and/or cross-linkage between the linear peptidoglycan polymer chains that make up a major component of the cell walls of both Gram-positive and Gram-negative bacteria (Mozayani & Raymon, 2004).

Antibiotics may reach the drinking water and cause extreme allergy to some sensitive species including human beings. Moreover, contamination of water and soil systems with low concentrations of antibodies may create bacteria resistant to antibiotics and create public health problems. For instance, Poonia *et al*. (2014) reported that most bacteria isolated from natural sources of water from rural areas of East Sikkim were found to be resistant to commonly used antibiotics including AM and ER. Moreover, Mulamattathil *et al.* (2014) isolated environmental bacteria from surface and drinking water in Mafikeng, South Africa, and found all organisms resistant to AM and ER. In a different study Braschi *et al*. (2013) investigated the persistence and degradation of β-lactam antibiotics in the soil and water environment and found several degradation products in soils at different water potentials indicating resistant species. Distribution and occurrence of antibiotics in the environmental components have also been studied. For instance, Tong *et al*. (2014) studied the occurrence of 19 antibiotics including ER in surface water and groundwater samples collected from Shahu County of Jianghan Plain, central China, in autumn (dry season) and spring (wet season). They reported high concentration of all test antibiotics. Moreover, Liu *et al.* (2014) investigated the distribution, bioconcentration, metabolism, and effects of ER in crucian carp (*Carassius auratus*). They showed that a maximum tissue concentration occurred in the muscle and that the bioconcentration factor (BCF) of 72.2 was lower than the theoretical BCF of 90.4 calculated from the octanol-water coefficient of ER.

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Nevertheless, few studies investigated the toxic effects of antibiotics in the environment. For instance, Oplinger & Wagner (2012) evaluated the effectiveness of antibiotics (ER, streptomycin, & a penicillin-streptomycin mixture) against *F. psychrophilum*. They showed that ER concentrations up to 2,000 mg L^{-1} for 15 min were also ineffective. Moreover, Ji *et al.* (2012) evaluated the toxicity of chlortetracycline, ox tetracycline, sulfamethazine, sulfathiazole, and ER using *Daphnia magna* and fish. They reported considerable effects on the tested organism and revealed the necessity of further long-term investigation. Furthermore, Wang and Gardinali (2012) analyzed different environmental samples and found sulfamethoxazole and ER residues in reclaimed water. In a different study, Gao *et al*. (2012) investigated the occurrence, distribution and bioaccumulation of 22 antibiotics, in the Haihe river, surface water, sediment and fish samples. They found ER and other antibiotics in all surface water samples. Moreover, Carballeira *et al*. (2012) studied the toxicity of AM on fish and found similar sensitivities to all substances tested. In addition, Park *et al.* (2010) detected AM in fish tissue after the 3rd day of spiking in water. Liu *et al.* (2012) studied the toxicity AM and microcystins against algae and found that Spiramycin is more toxic to *M. aeruginosa* than AM according to their EC_{50} values. He *et al*. (2012) investigated the multi-biomarker responses in fish from two typical marine aquaculture regions of South China**.** Tyczkowska *et al*. (2012) found beta-lactam antibiotics in bovine milk using multi-residue analytical method. Li *et al.* (2012) detected antibiotic residues in water samples and correlated it with the detrimental effect on ecological and human health due to bacterial resistance. Liu *et al*. (2011) determined AM and penicillin G, and their major metabolites in bovine milk by ultra-high-performance liquid chromatography in tandem with mass spectrometry. Ebert *et al*. (2011) investigated growth inhibition of fluoroquinolone enrofloxacin and ciprofloxacin on four photoautotrophic aquatic species. Christensen *et al*. (2006) studied the mixture toxicity of aquaculture antibiotics ox tetracycline, oxolinic acid, ER, florfenicol, and flumequine and found synergistic effects when combinations of ER and ox tetracycline were tested on activated sludge microorganisms.

Large quantities of AM and ER are being used in Gaza strip in the human and/or animal health sectors (MOH, 2012). Fish farming breeders claimed high fish mortality after antibiotic application to control fish disease. Moreover, many dead fish of different sizes were found on the coastal area close to Wadi Gaza that is an indispensable part of natural life in Palestine and has an interesting history and rich biodiversity in terms of fauna and flora probability due to contamination of antibiotics and/or pesticides (Abd Rabou, 2005). The presence of those antibiotics or pesticides threating Wadi Gaza's biodiversity. Accordingly, the authors designed this study to investigate the toxicity to fish and mosquitoes using concentrations far below the U.S. official tolerance for AM/ER (10 mg Kg^{-1}) in milk and uncooked edible tissue of cattle (U.S. Code of Federal Regulations, 1991) and the concentrations found in water (Oliveira *et al.,* 2013; Gozlan *et al.,* 2013), wastewater (Novo *et al.,* 2013; Lamm *et al.,* 2009), milk (Liu *et al.,* 2011) and/or fish samples (Smith *et al.,* 2009; Wang *et al.,* 2009). Moreover the authors investigated the toxicity of EN to the tested organism to estimate the relative toxicity of AM and ER.

MATERIALS AND METHODS

Materials

Technical amounts of antibiotics (AM and ER) were purchased from Ramalla Medical Drug Company, Ramalla, Al-Beera, WB, Palestine, whereas EN, a standard toxic substance, was purchased from Sigma, Germany and used in this study to compare the toxicity of AM and ER to fish and mosquitoes. Some physicochemical properties of these compounds are presented in Table1 and their chemical structure in Figure 1. The tested organisms are Juvenile goldfish (*Tilapia nilotica*) and mosquito larvae *(Culex pepans)* were obtained from local certified fish farming breeding sites and from Wadi Gaza. The rationale in selecting two different organisms is that each represents a different position in the food chain in the eco-system and has different sensitivity to environmental pollution (OECD, 1992).

Preparation of stock solution

About 100 mg of ER was dissolved in 25 mL of methanol. One ml methanol was transferred to a one liter volumetric flask and completed with distilled water to the mark and used as stock solution for the necessary dilutions.

A series of concentrations ranging from 0-200 µg L^{-1} was prepared and tested. The same procedure was followed for the case of EN whereas AM was dissolved in 25 mL of acetone because AM does not dissolve in methanol, and the same steps as in ER were repeated for AM.

Breeding and acclimatization of fish and mosquitoes

Acclimatization of fish and mosquitoes was done according to the method previously described (OECD, 1992).

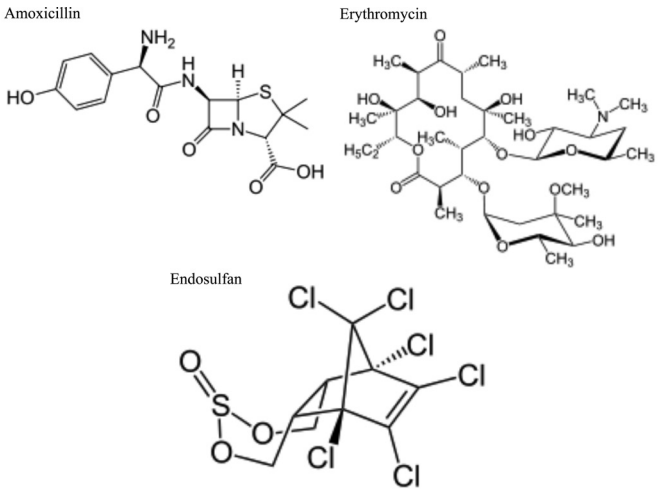

Figure 1. Chemical structure of the tested compounds.

In this procedure the larvae of fish and mosquitoes were bred under laboratory conditions with 12 hours of light followed by 12 hour of dark, for two weeks before starting the toxicity tests. The temperature of the laboratory was controlled at 25±2 °C. The dead fish or mosquitoes were removed from the breeding cage. The average weight of a single fish was 20±1 g and average length was 4± 0.5 cm. The fish were held in a 500 liter plastic barrel equipped with an aeration pump to supply oxygen to the water. The water had the following characteristic: pH: 7.5 ± 0.5, desalinated Fish were fed on daphnia. During the test five fish were put in each glass aquaria (10×15×20cm) half filled with the test solutions that were pre-saturated with oxygen. The dissolved oxygen was tested every 12 hours using the dissolved oxygen meter. The temperature was also tested every 12 hours to ensure suitable conditions during the test. For mosquitoes, acclimatization depends on the development of 1st instars larvae to the 2nd instars.

Toxicity Test on Fish

Two days before testing the fish larvae were put in de-ionized water with no feed for 96 hours , static acute toxicity test (OECD, 1984). In this test, fish were exposed to five gradient concentrations up to the solubility limit on water to determine the concentration response curve for AM, ER and EN on fish under laboratory conditions. The experiments included positive and negative control samples, the positive contained 50 µL of methanol (solvent control) whereas the negative did not include methanol. Mortality was recorded every 24hours for 96hours.

Toxicity test on mosquitoes

The tests were performed in 20 mL capacity glass tubes. Ten mL of desalinated water containing five mosquito larvae were transferred to each glass tube. Appropriate amounts representing gradient concentrations ranging from 0-160 µg L^{-1} were spiked in the test tubes. Each test included five series of concentrations with four replicates each in addition to positive and negative control treatments as mentioned above under the recommended conditions of the OECD (1992). The experiments were kept at a temperature of 22° ± 1° C, pH 7.5±0.5, and 12/12 dark/light photoperiod. The number of dead larvae in each tube was observed and registered every 24 hours for 48 hours.

Calculation of toxicity and statistical analysis

Percent mortality of fish and mosquito larvae were taken as indicators of toxicity of the tested compound in single and/or binary mixture. Calculation of mortality percentage was done using equation 1 according El-Nahhal *et al.* (1998) with a slight modification:

$$Mortality = 100 * \frac{(Lc - Lt)}{Lc} \qquad \text{(Eq.1)}$$

where Lc and Lt are the number of live organisms in the control and the treated samples respectively. Percent mortality was regressed versus concentration and converted to a log scale to calculate the LC_{50} values, furthermore the mortality percentage was also regressed versus time to calculate LT_{50}.

Relative toxicity (RT) was calculated according to equation 2.

$$RT = \frac{LCt_{50}}{LCs_{50}} \qquad \text{(Eq 2)}$$

where LCt_{50} and LCs_{50} are the lethal concentration of the tested and the standard compounds, respectively. A value of RT equal to or less than 1 indicates higher toxicity whereas a value above 1 indicates lower toxicity than the standard toxic substance.

T-test was used to detect variances among treatments. Fish and mosquito tests include three replicates of each concentration in addition to positive and negative control samples. The positive control includes 50µL methanol. Toxicity of mixtures was calculated based on mortality percentage and toxic units available in the solution. According to a previous report (Sprogue & Ramsay, 1965), toxic unit was calculated as: Toxic unit = actual concentration in solution/ lethal threshold concentration.

RESULTS

Single toxicity test on fish

Concentration response relationships of AM, ER and EN on fish and mosquito mortality are shown in Figure 2.

It is obvious that mortality percentage increased rapidly as the concentration of AM, ER, and EN increased in the solution and reached 50 µg L^{-1}. Then a slight increase in mortality percentage above 50 µg L^{-1} was observed in fish whereas in the mosquito tests, considerable in mortality percentage was observed as the concentration increased in the solution up to 160 µg L^{-1}.

Moreover, the results presented in Figure 2 clearly demonstrate that EN at a concentration below 10 µg L^{-1} resulted in less than 10 percent fish mortality. This concentration can be named No-Observed Effects Concentration (NOEC). This point was not detected in AM and ER tests.

Converting the data in Figure 2 to log scales enabled the calculations of LC_{50} values, which are 35.72, 242.7, and 89.32 µg L^{-1} on fish test; the values for mosquito test are 107.6, 60.2, and 63.3 µg L^{-1} for AM, ER and EN respectively. In addition strong positive associations with high correlation coefficients (R^2) were observed (Table 2).

Furthermore, calculating the RT (Eq. 2) indicates a lower value of AM than EN, whereas ER has a higher value than EN on fish. For mosquitoes, the RT values for AM and ER are 1.7 and 0.95 respectively. This indicates the potentially higher toxicity of ER than the other antibiotics. The interesting results are that the RT value of ER, which is nearly half that of AM, indicates greater toxicity of ER to mosquitoes.

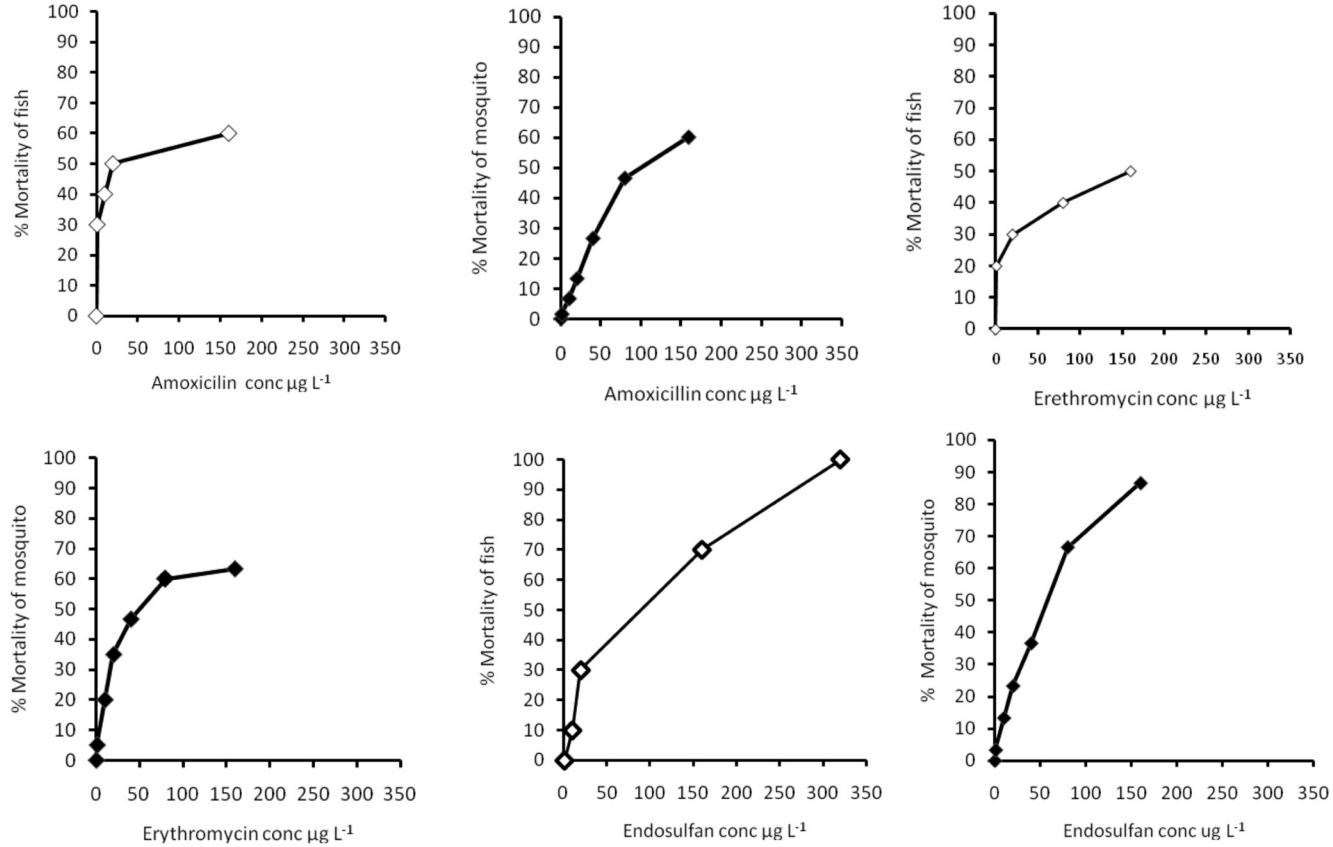

Figure 2. Concentration response relationships of Amoxicillin, Erythromycin and Endosulfan on fish and mosquito mortality. Mortality percentage was recorded after 48 and 96 h for mosquito and fish respectively.

Effect of time on toxicity

Time response relationships of AM, ER, and EN concentrations on fish and mosquitoes are shown in Figure 3. The data clearly demonstrate a progressive relationship between mortality percentage and length of exposure. Linear regression analysis indicated a strong positive association with a high value of R^2.

To compare the toxicity according to length of exposure, we regressed the data in Figure 3 to a linear mode. This enabled the calculation of the length of exposure required to kill 50 percent of fish or mosquitoes at the tested concentration. The calculated values are presented in Table 2. Statistical analysis of mortality percentage versus length of exposure obtained by the three tested compounds against fish show different p-values (0.08-0.14).

Toxicity of mixture

Dose response relationships of AM and ER mixture on fish and mosquito are shown in Figure 4. The mixture showed no mortality rate in fish whereas high mortality rates were observed in mosquitoes.

Moreover, mortality percentage of mosquitoes increased as the concentration of the mixture increased in the solution. However, the maximum mortality percentage value obtained at the highest concentration did exceed 60%. This value is equal to or below the mortality percentage value obtained from mosquitoes in the single toxicity tests (Fig 2).

DISCUSSION

AM and ER (Fig. 1) are antibiotics widely used to treat bacterial infections in the human and veterinary sectors. AM acts by inhibiting the synthesis of bacterial cell wall whereas ER is a macrolide antibiotic and acts as a bacteriostatic that inhibits growth of bacteria. Moreover, EN is an organochlorine insecticide, used in this study as a standard toxic substance to fish and mosquitoes to calculate the relative toxicity of AM and ER. They are solid at room temperature, and have different chemical structures and physical properties.

The results presented in Figure 2 clearly demonstrate the potential toxicity of AM, ER, and EN to fish and mosquitoes. EN, a standard toxic substance, showed high toxicity to both cases. In fact, EN is classified as extremely toxic substance according to WHO (Tomlin, 2000). Our results agree with Echeverría-Sáenz *et al*. (2012) and Bauer *et al*. (2013) who found increased fish mortality due to high levels of insecticides in water.

Moreover, the steep increase in mortality percentage on fish and mosquitoes below 50 µg L^{-1} (Fig. 2) suggests that the majority of the tested population is sensitive to low concentrations of AM and ER, while the remainder are tolerant,

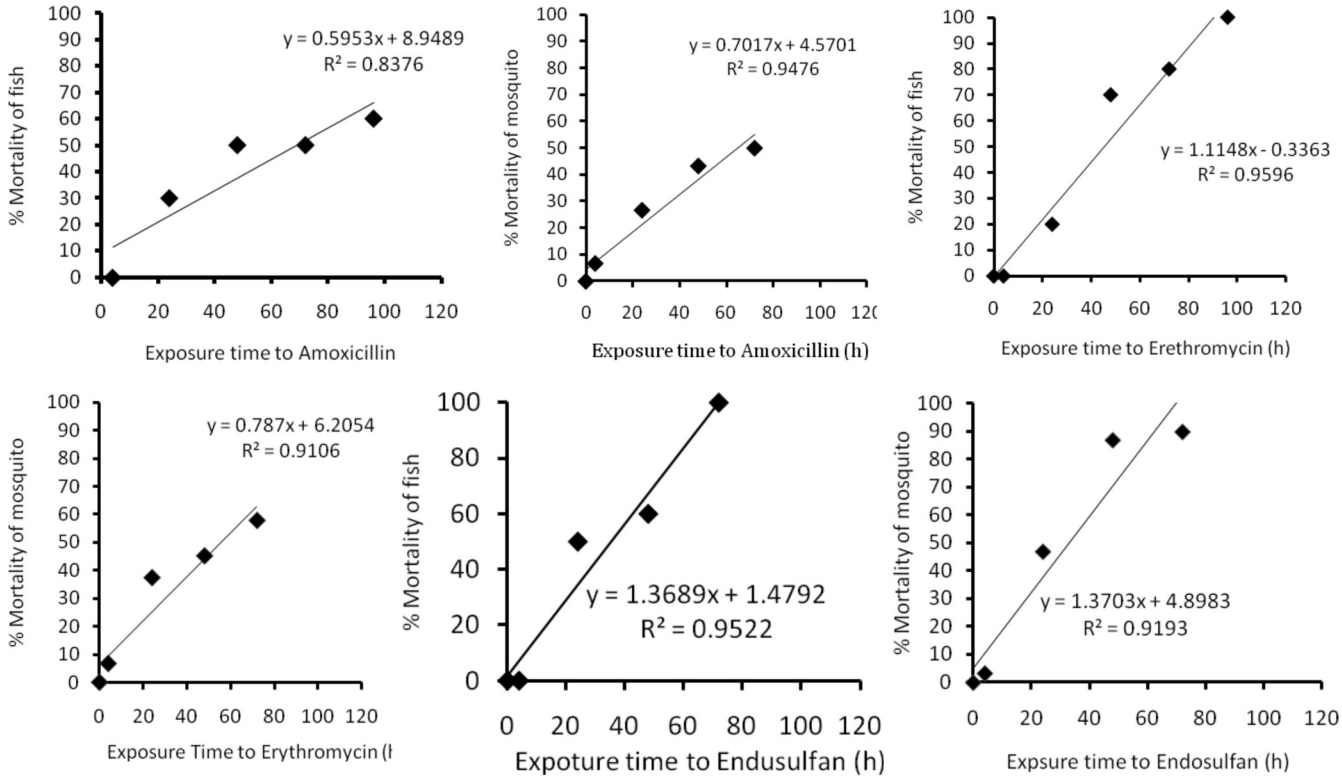

Figure 3 Time response relationships of diluted concentrations ER, AM and EN on fish and mosquito mortality. Mortality percentage was recorded at a certain concentration of each compound (0.08 mg/l) overtime.

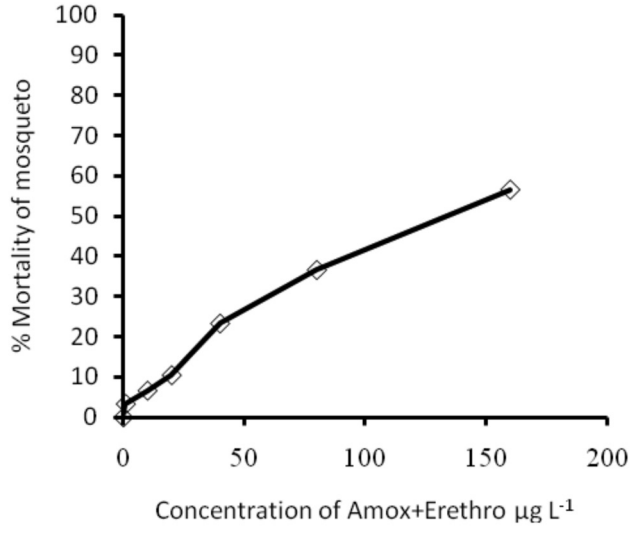

Figure 4. Concentration response relationship of Amoxicillin and Erythromycin Mixture on mosquito larvae. Mortality percentage was recorded after 48 h.

accordingly mortality percentage reached a plateau with the fish and mosquito tests. This tolerance may be attributed to the fact that their exposure to sub-lethal concentrations of AM or ER in the eco-system contributes to the development of a gene resistant to antibiotics. This suggestion is supported by the results of Yi *et al.* (2014) who investigated the prevalence of resistant genes against β-lactams in 119 fish strains and reported that a large number (99.2%) of the present fish strains were resistant to one or more β- lactams including ceftiofur, AM-clavulanic acid, ampicillin, piperacillin and cefpodoxime. More support for our hypothesis can be found in Oliveira *et al*. (2013), who investigated the the sub-lethal effects of oxytetracycline and amoxicillin on zebrafish development and biomarkers and revealed that AM caused premature hatching (48 h-EC_{50}=132.4 mg L^{-1}) whereas oxytetracycline cause delayed hatching of embryos (72 h-EC_{50}=127.6 mg L^{-1}).

In addition, the similarity of the toxicity curves in Figure 2 suggests similar sensitivity to the tested compound. This finding agrees with Carballeira *et al.* (2012), who investigated the toxicity of AM and other antibiotics to embryos of two species of sea urchin (*Paracentrotus lividus* & *Arbacia lixula*) and found that both species showed similar sensitivities to all substances tested.

Furthermore, the toxicity curve of EN to fish and mosquito is nearly similar, these suggest similar responses by the tested organisms. As mentioned above EN has the same mode of action on fish and mosquitoes (Tomlin, 2000).

The calculated LC_{50} values (Table 2) from the experimental results in Figure 2 clearly show the lowest value of AM on fish test. The RT calculation indicates that AM has lower value than EN, regardless of the fact that the apparent toxicity of EN is high and mortality reached 100 percent in both cases.

An interesting finding is the high toxicity of AM to fish, and of ER to mosquitoes. The high values of R^2 indicate

the strong positive association. The toxicity to fish has the following order: AM > EN > ER.

Our results are in agreement with previous reports (Ebert *et al.,* 2011; Liu *et al.,* 2012).

The variations in the toxicity of the tested compounds may be explained by the fact that the tested compounds have different values of K_{ow}. For instance AM has high K_{ow} value which enables it to be partitioned rapidly from the aquatic phase to the hydrophobic phase (fish). This explanation can be supported by the results of Liu *et al.* (2014) who emphasized the importance of K_{ow} on bioaccumulation or biodegradation of antibiotics in fish. Statistical analysis of mortality percentage obtained for the three tested compounds against fish does not show any significant differences between ER and AM. However, comparison with EN showed significant differences. P-values ranged between 0.03-0.000.

Moreover, the increased mortality percentage due to increase in the length of exposure (Fig. 3) can be explained by the relation to K_{ow} and Henry constant of the tested compound (Table 1). The high values of K_{ow} for EN and ER indicate the high potential of movement of these compounds from water to fish. As time increased from four hours to 96 hours, high fraction of ER and EN accumulate in fish tissue and caused mortality. The low K_{ow} value of AM reduces the potential of bioaccumulation in fish tissues, accordingly, the mortality of fish may take a longer time than other cases. This explanation is also supported by the results of Liu *et al.* (2014). The calculated LT_{50} values are presented in Table 2. Furthermore, the shape of the toxicity curve (Fig. 2) is nearly similar which suggests that the tested organisms have similar sensitivity to AM, ER and EN. An important finding of the study is that the calculated LC_{50} values are far below the concentrations detected in several water systems (Oliveira *et al.,* 2013; Gozlan *et al.,* 2013; Novo *et al.,* 2013; Lamm *et al.,* 2009) found in edible parts of fish (Smith *et al*., 2009; Wang *et al.,* 2009), milk (Liu *et al*., 2011), and/or U.S. official tolerance for AM/ER (10 mg Kg^{-1}).

The presented toxicity parameters in Table 2, clearly show that EN has the lowest LT_{50} value (35 h) whereas AM and ER have LT_{50} value nearly 2 and 4 times higher than EN. This is in agreement with K_{ow} values (Table 1). This suggests that EN has a fast knock-down effect whereas AM and ER have a slow knock-down effect on fish or mosquitoes. The high R^2 values of all cases indicate a strong positive association.

Our results agree with Liu *et al.* (2012), who demonstrated the influences of two antibiotics on the eco-systems activity.

Table 1. Physicochemical properties of Amoxicillin ,Erythromycin and Endosulfan.

Properties	AM	ER	EN
MW	365.40	733.93	406.93
Solubility in water mg L^{-1}	3430	1.44	0.32
K_{OW}	0.9	2.8	3.5
Hennery constant	$2.73 * 10^{-19}$	-	1.48
pKa	3.39	8.9	-

Comparing the LT_{50} values of the tested compounds against fish (Table 2), one can realize the highest LT_{50} value of ER. This suggests a slower knock-down effect of ER on fish than AM and EN. Moreover, the LC_{50} values of ER is several times higher than AM and EN (Table 2). This may also lengthen the time required to kill 50% of the tested fish at lower concentrations. Furthermore, EN has the lowest LT_{50} value. The explanation of these results is related to the solubility limit of ER in water which is several times lower than those of AM and EN (Table 1), in addition to the fact that ER has a high K_{ow} value. These data make the partitioning process of ER from water to fish longer than those of AM and EN. These data agree with the results presented in Figure 3.

Statistical analysis of mortality percentage versus length of exposure obtained by the three tested compounds against fish show different p-values (0.08-0.14).

In the case of mosquitoes, the data presented in Table 2 clearly demonstrated that ER has the lowest LC_{50} value among all cases, indicating the highest toxicity on mosquitoes. It is well known that AM and ER have a different mode of action on bacteria. This different mode of action also becomes obvious in fish and mosquito tests. Our results agree with Milam *et al.* (2005). Statistical analysis of mortality percentage versus concentrations shows significant differences among the three tested compounds. P-values ranged between 0.013-0.04. This indicates different responses of mosquitoes to the tested compounds. The explanation of these results is given above, in addition to the fact that the tested compounds have different physicochemical properties (Table 1). Comparing the LC_{50} values (Table 2), the toxicity to fish has the following order: ER > EN > AM. The different LC_{50} values of the tested compounds indicate different sensitivity and response of the tested organism.

The LT_{50} value (Table 2) of AM is the highest value among all tested compounds. This clearly indicates that the response time between mosquitoes and AM is longer than with ER and EN. Furthermore, EN has the lowest LT_{50} value among all tested cases. The explanation of these results is similar to that given above.

Comparison of LT_{50} values of fish and mosquitoes shows that the values are close to each other except for ER, which has LT_{50} value on fish 2.55 times higher than on mosquitoes. The explanation of this variation is that bioaccumulation of the tested compound on fish tissues depends on various factors such as size and, fat content of the subject and K_{ow} of the tested compound. In the presented case, fish larvae is larger than mosquito larvae and has more fat, accordingly ER takes longer to bioaccumulate in fish tissues to cause a lethal effect. This explanation is supported by the results of El-Amrani *et al.* (2012), who found different bioaccumulation modes of pesticides in Zebrafish eleutheroembryos.

The data presented in Figure 4 clearly demonstrated the considerable toxicity against mosquitoes, whereas no mortalitys were reported with fish. This indicates antagonistic effects of the tested antibiotics on fish. Regardless of the

Table 2. LC_{50}, RT, LT_{50}, R^2 and regression equations on fish and mosquito tests.

Parameter	Fish			Mosquito		
	AM	ER	EN	AM	ER	EN
LC_{50} (µg L^{-1})	35.72	242.7	89.32	107.6	60.2	63.3
RT	0.4	2.72	1	1.7	0.95	1
R^2	0.97	0.97	0.92	0.99	0.96	0.99
Re Eq	y=0.14X+1.48	y=0.17X+1.29	y=0.6X+0.55	y=0.76X+0.15	y=0.53X+0.76	y=0.66X+0.51
LT_{50} (h)	69	132	35	63.47	51.76	33.72
R^2	0.84	0.94	0.95	0.99	0.97	0.96
Re Eq	Y=0.6X+8.95	Y=0.38X-0.55	Y=1.4X+1.48	y=0.71X+0.41	y=0.59X+0.23	y=1.20X-0.14

Where LC_{50}, RT, R^2, Re Eq and LT_{50} are leathal concentration, relative toxicity, regression equation and leathal time respectively.

considerable mortality rate with mosquitoes, the presented results confirm the antagonistic effects on mosquitoes. Our results agree with Liu *et al.* (2014) who studied the combined effects of two antibiotics on *Microcystis aeruginosa* and suggested an antagonistic interaction at the median effect level. The explanation of these results is that AM and ER have different modes of action as reported above, besides the fact that the tested concentrations of the molecules were at very low levels. Moreover, the different molecular size (Fig. 1) and weight (Table 1) may limit each molecule to react independently with the sensitive site on the cell wall or nervous system of the fish or mosquitoes. Accordingly, an antagonistic effect was observed on fish and mosquitoes.

Our results agree with Orton *et al.* (2014), who observed complete suppression of dihydrotestosterone effects when chemicals were combined at individual concentrations eliciting 1%, 10% or 20% androgen receptor antagonistic effect. They also reported that the combined androgen receptor antagonistic effects occurred at very low concentrations of individual mixture components.

Furthermore, the molecules of antibiotics in the mixture tests tend to interact with each other and may form a larger molecule which geometrically cannot fit the interaction with sensitive site in the cell wall or the nervous system of fish or mosquitoes. Accordingly a reduction of mortality percentage was obtained. This explanation accords with previous published work (El-Nahhal & Safi, 2004) which found that organic molecules dissolved into each other and formed a larger molecule that cannot react with the adsorption site due to steric effect. However, the mortality percentage obtained in mosquitoes (Fig. 4) clearly demonstrated that mosquito larvae are more sensitive to the tested antibiotics than fish. Moreover, our results agree with a recent report of Berger *et al.* (2014) who reported that exposure to an environmentally relevant mixture of contaminants results in development of abnormalities in the absence of apparent maternal toxicity.

However, the small size of mosquitoes makes them more sensitive to toxic substances. These results agree with a previous report (Kumar *et al.,* 2010).

It is cleare from the results presented that fish and mosquitoes are sensitive to the tested antibiotics, accordingly they will die at low concentrations. Under these conditions, they become easy accessible food to the secondary and tertiary consumers in the eco-system. Accordingly, bioaccumulations of these antibiotics become a serious problem in the eco-system. This hypothesis agrees with El-Amrani *et al.* (2012), who found different bioaccumulation modes of chemicals in Zebrafish eleutheroembryos. Moreover, the biodeterioration of dead fish and mosquitoes may result in a slow release of the antibiotics into the eco-system, accordingly the development of antibiotic-resistant genotypes may occur, and consequently antibiotics may lose their therapeutic effectiveness. Our suggestion is supported by the results of Poonia *et al.* (2014), who isolated antibiotic-resistant bacteria from natural sources of water where antibiotics are commonly used. More support for our suggestion comes from Mulamattathil *et al.* (2014) who isolated environmental bacteria from surface and drinking water that were resistant to antibiotics.

CONCLUSION

This study investigated the single and mixture toxicities of AM, ER, and EN on fish and mosquitoes as non-target organisms of the antibiotic. The study ranked AM the most toxic antibiotic to fish with LC_{50} value equals to 35.72 µg L^{-1}. EN and ER have LC_{50} vales for fish 89.32 and 242.7 µg L^{-1} respectively. The relative toxicity of AM is lower than 1 indicating extreme toxicity, whereas the relative toxicity of ER is 2.6 indicating lower toxicity. ER is more toxic to mosquitoes than AM, LC_{50} values are 60.2 and 107.6 µg L^{-1} respectively. The relative toxicity of ER is 0.95 whereas that of AM is 1.7. Time response relationships (Table 3) are not similar and LT_{50} values ranged from 33.72-63.47 hours, indicating different exposure times are required to produce the toxic effect. Statistical analysis revealed significant differences in toxicities to fish and mosquitoes. Binary mixtures were less toxic to fish and mosquitoes than single toxicity, and did not produce a mortality rate greater than 60% in all cases.

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