

Toxicity of Single and Mixtures of Antibiotics to Cyanobacteria

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Abstract

The present study was designed to investigate the disappearance of cyanobacterial mat from fish breeding lakes and to evaluate the phytotoxic effects of Penicillin, Ciprofloxacin, and Tylosin as single and as mixtures to cyanobacterial mats. Phytotoxic effects were measured as growth inhibition of cyanobacteria using spectrophotometer at 680 nm at low concentrations of antibiotics. Results showed potential phytotoxicity of the tested antibiotics with EC₅₀ values of Penicillin, Ciprofloxacin and Tylosin of 0.13, 0.71, 5.28 mg/l respectively. Relative toxicity indicated that Penicillin and Ciprofloxacin were more toxic to cyanobacterial mats than Diuron (standard toxic material). EC₅₀ values of binary mixtures are 0.077, 0.103, 0.292 TU, for (Penicillin+Tylosin), (Ciprofloxacin+Tylosin) and (Ciprofloxacin+Penicillin) respectively; whereas EC₅₀ of the tertiary mixture is 0.034 TU. Statistical analysis of the results indicated significant differences between the toxic effects of compounds and their mixtures to cyanobacterial mats. Observation of toxicity of over time indicated that cyanobacterial mats were able to overcome the toxic effects after approximately 72 h of exposure time. It can be concluded that antibiotics exert dangerous toxic effects to cyanobacterial mat, an important organism in the eco-system. These results are considered the first of its kind in Palestine.

Keywords: Cyanobacteria; Penicillin; Tylosin; Ciprofloxacin; Toxicity

Introduction

Pharmaceuticals and personal care products including all human and veterinary drugs, are emerging contaminants of concern [1,2]. Among these pharmaceuticals and personal care products are antibiotics [3]. About 50 to 90% of antibiotics and/or their metabolites are eliminated from the body through urine and feces which then enter the environment through sewage treatment plants or organic fertilizers application to agricultural lands [4].

Antibiotics have frequently been detected in wastewater effluents, surface waters, ground waters and sea water [5-8] enhancing the generation of resistant genotypes of bacteria in the environment [9].

Antibiotics are applied to control pathogenic bacteria, but their application creates potential risks to indigenous microorganisms in the eco-system [10]. These non-target microorganisms either provide important ecosystem services, such as nutrient cycling, organic matter mineralization and degradation of pollutants [11], or produce oxygen and carbohydrates [12-13]. Thus, antibiotics may affect both primary producers and decomposers, potentially disrupting ecosystem processes.

Antibiotics may enter the environment via several routes, for instance wastes of pharmaceutical plants, wastes from hospitals, manure from animals, containing antibiotics, is often used as a fertilizer and is spread on fields [14].

Natural antibiotics are rarely completely metabolized in the body [15] whereas synthetically modified antibiotics are less biodegradable, more persistent and hydrophilic [16]. Antibiotics may affect the growth of aquatic organisms including cyanobacterial mats which have important ecological position and essential roles in the nutrient cycling and oxygen production [17].

Cyanobacteria, which are member of free-living micro-organisms of the aquatic ecosystem, can fix atmospheric nitrogen [18] and produce oxygen through photosynthetic activity [19]. They have a long history and are diverse and widespread in marine, freshwater, and terrestrial environments.

Number of studies investigated the toxicity of antibiotics to various organisms. For instance toxicity on: Bacterial growth [11], *Daphnia magna* [20], soil bacteria [21], and algae [22].

Phytotoxicity of amoxicillin at high concentration (50 mg/l) has been reported to *Synechocystis* sp. [23], few studies reported the toxic effect of β -lactam antibiotics and erythromycin, Ciprofloxacin and sulfamethoxazole on algae or cyanobacteria [24-26], the estimated EC₅₀ value against green algae were 1.54-32.25 mg/L. The effects included the reduction in growth rate and inhibition of Photo system II. Furthermore, few reports [27-28] investigated growth-inhibiting and binary joint effects of 12 antibacterial agents on the freshwater green alga *Pseudokirchneriella subcapitata* (Korschikov) and found potentially synergistic effects in binary mixtures of the same class. Fish breeders and aquacultures from Gaza Strip claimed the disappearance of blue green algae and aquatic weeds in fish lakes and aquaculture industry after application of antibiotics to protect fish from bacterial diseases. Accordingly, the authors designed this study to: 1) characterize phytotoxicity of Penicillin G, Tylosin tartrate, and Ciprofloxacin hydrochloride, as individuals and as mixtures to cyanobacterial mats as an aquatic model of vegetation; 2) study the toxico-dynamics of certain concentrations on the toxicity to cyanobacteria.

Materials and Methods

Chemicals

Ciprofloxacin hydrochloride, Penicillin, and Tylosin were purchased from Birzeit-Palestine Pharmaceutical Company. Diuron

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Received January 21, 2015; Accepted February 10, 2015; Published February 14, 2015

Citation: Yasser ELN, Adli A (2015) Toxicity of Single and Mixtures of Antibiotics to Cyanobacteria. J Environ Anal Toxicol 5: 274. doi:10.4172/2161-0525.1000274

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was purchased from Sigma Aldrich, Germany. The chemical structure, generic and IUPAC names are shown in (Figure1).

Collection of cyanobacterial mats

Six cyanobacterial mat samples were collected from the natural area, western part of Wadi Gaza near the Mediterranean Sea where fish farming and aquaculture industry are distributed. Mats collection started early February up to the end of April 2013 following the procedures described previously [29].

Preparation of growth media

Growth media was prepared by collection of natural water samples from Wadi-Gaza, the same places where cyanobacterial mats were naturally growing. The samples were transferred to the laboratory at The Islamic University-Gaza and left for few hours to allow suspended matter to precipitate. Then the supernatant was collected and cleaned up using sand filter developed in this study. The sand filter consisted of a plastic tube of 3 cm diameter and 120 cm long, filled in the bottom with 10 g of glass wall followed of fine sea sand in the range of 630-20 μ m mesh. The sand was washed several times with distilled water until the removal of all salts. This was checked by silver nitrate reaction following the descriptions in a previous report [30]. Then filtered again using microbiological filter paper (MFP), white, grid-marked, cellulose ester, 47 mm diameter and 0.45 μ m micro mesh under vacuum. The filtrate was autoclaved and used as growth media after cooling to room temperature. The properties of growth media were measured to be adapted with the cyanobacterial mats solution collected from the Wadi. The EC and pH were measured during all the experiments and adjusted to the range 3600-5100 μ s/cm, 7.5 -8.7 respectively. The growth media was kept in the refrigerator to be used when it is needed [31].

Preparation of cyanobacterial mats suspension

The collected cyanobacterial mats were incubated in the laboratory at 20-25°C for few hours and shaken carefully using electrical shaker, then the aqueous mixture of cyanobacterial mats was left for half an hour. One hundred ml of the suspension, which contained living cells, was isolated and considered as main suspension of cyanobacterial mats [32]. The EC and pH of the suspension were measured and adapted to properties of growth media [33]. An amount of the main suspension of the mat was diluted with 100 ml growth media to obtain low concentration solution of the mat with EC and pH resembling that of natural environment. The dilution was gently shaken to insure normal distribution of cells by using plates magnetic stirrer- KMC-130 SH for ten minutes [31]. The cyanobacterial mats were diluted to have the following optical density 0.16 ± 0.03 .

Characterization of cyanobacterial mats growth phases

The idea behind this experiment is to characterize the log phase of growth so that the toxicity tests can be designed properly. The cyanobacterial mats, having the following optical density 0.16 ± 0.03 , were allowed to grow under lab conditions, and the optical density was monitored each 4 h up to 120 h using CT-220 spectrophotometer at wavelength of 680 nm [34]. The temperature was maintained at $25 \pm 1^\circ\text{C}$ with 12 h-light: 12 h-dark cycle using 60 watt tungsten lamp. The experiment was performed in three replicates. The population dynamics of cyanobacterial mats was evaluated by plotting OD versus time [35].

Preparation of antibiotics and Diuron stock solutions

Accurate amounts (4 mg) of Tylosin tartrate, Penicillin G procaine and/or Ciprofloxacin were dissolved separately in 1l volumetric flask using distilled water. The experimental tools and solution were free of chlorine. The following concentrations 0, 0.02, 0.2, 0.4, 0.6, 0.8 mg/l, of the antibiotics were prepared and tested whereas Diuron concentrations were in the following range 0, 0.4, 4, 8, 12, 16 mg/l.

Binary mixtures of Penicillin G procaine, Tylosin tartrate, and Ciprofloxacin were prepared by mixing together two antibiotics with the ratio of 1: 1(v/v) whereas tertiary mixtures (Mixture T1) of Penicillin, Tylosin, and Ciprofloxacin were prepared by mixing 0.333: 0.333: 0.333 of each compound together according to the procedure described by Kerkez [32].

Phytotoxicity tests

The following concentrations 0, 0.2, 1, 2, 3, and 4 mg/l of the antibiotics and 0, 0.4, 4, 8, 12, 16 mg/l diuron were prepared and added to round bottom flasks containing 1 ml of cyanobacterial mat and growth media of total volume of 50 ml. The cyanobacterial mats were diluted to match 0.16 ± 0.03 , total optical density of the suspension [36]. The flasks were kept in the laboratory conditions at $25 \pm 1^\circ\text{C}$ and 12 h light/dark cycle to match the natural conditions. The optical densities of the flasks were recorded at 0, 24, 48, 72, 96 h. The recorded data at 24 h were used to estimate the acute toxicity whereas, the recorded data at 72 h was used to estimate the chronic effect. The toxicity was estimated by calculating the growth inhibition. High growth inhibition indicates high toxicity and low growth inhibition indicates low toxicity.

The dynamics of Cyanobacterial mats growth (relative growth) in the control samples evaluated by plotting time versus the $\text{OD}_{\text{max}}/\text{OD}_0$ ratio, where OD_{max} and OD_0 are the optical densities at maximum growth and growth at time zero respectively. According to El-Nahhal et al.[35], % growth inhibition (GI) which represents toxicity was calculated as follows: % (GI)= $100 \times [(\text{ODc}-\text{ODt})/\text{ODc}]$ (1), where ODc and ODt are the optical density of the control and the treated samples, respectively.

Regressing GI versus log antibiotic concentrations, enabled the estimation of EC_{50} values, where EC_{50} is the effective concentration that caused 50% growth inhibition compared with the control in any chosen toxicity endpoint [34].

Toxicity of mixtures

The mixtures mentioned above (Preparation of antibiotics and Diuron stock solutions) were prepared and used as stock solution to make different dilutions. Following the procedure mentioned above, binary and tertiary toxicities were determined.

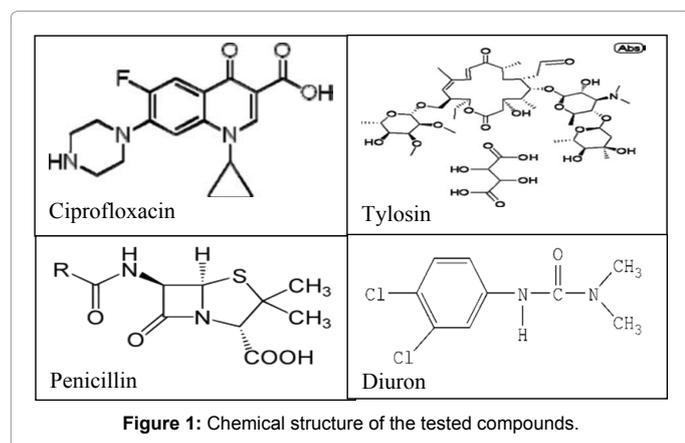


Figure 1: Chemical structure of the tested compounds.

Toxic units (TU_s) in mixtures: According to previous calculation [37], toxic units were calculated as follows:

Toxic units=actual concentration in solution/lethal threshold concentration.

$$\text{Relative toxicity} = EC_{50} \text{ of antibiotics} / EC_{50} \text{ diuron (2)}$$

Mixture toxicity index (MTI): To estimate the synergetic and/or the antagonistic effects of antibiotics mixtures, we calculated the mixture toxicity index (MTI) (Table 1) proposed by Konemann, [38]. $MTI = 1 - (\log M / \log n)$, where $M = \sum C/EC_{50}$ at 50% effect in the mixture, and n =total number of compounds in the mixture.

Based on calculation, MTI value can be a negative (antagonism), zero (no effect) and positive value (synergism).

Statistical analysis

All experiments were performed in three replicates. Averages and standard deviation of the growth inhibition were calculated and fitted to linear regression analysis. The averages of growth inhibition were compared by Tukeys test and P-values were determined to evaluate the significant differences among treatments. One-way ANOVA test which is used to analyze the differences between group means (three or more) was done by using Excel program.

Results

Growth of cyanobacteria

The tested antibiotics are widely used in Gaza strip for human, veterinary medication, and livestock and aquaculture growth promotion. These antibiotics are soluble in water and have a solid state at room temperature. More of the physicochemical properties are shown in Table 1.

Salinity and pH values of the growth media of cyanobacterial mats were $4068 \pm 599 \mu\text{s/cm}$ and 8.04 ± 0.44 respectively. However, pH value of natural growth media ranged between 7.5-8.7 and EC ranged from 3600-5100 $\mu\text{s/cm}$.

Population growth of cyanobacterial mats in the laboratory is demonstrated by optical density in Table 2. Furthermore, regressing the growth data (Table 2) versus time enabled the characterization of 4 growth phases (Figure 2).

Toxicity tests

Single toxicity tests of antibiotics: Toxicity of Penicillin, Tylosin,

Tested compound	MW	Solubility in H ₂ O mg/l	pKa	Log K _{ow}	Classification
Ciprofloxacin	331.3	1.1	5.64 8.87	2.3	Fluoroquinolones
Penicillin G	334.4	22	2.62	1.67	β-lactams
Tylosin	917.1	5000	3.41	13	Macrolides
Diuron	233.1	36.4	na	2.85	Urea

Table 1: Some physico-chemical properties of the tested compounds.

Time (h)	Average optical density ± SD
0	0.090 ± 0.002
24	0.105 ± 0.007
48	0.305 ± 0.022
72	0.305 ± 0.006
96	0.157 ± 0.083

Table 2: Growth of cyanobacterial mats under laboratory conditions.

Ciprofloxacin and Diuron are shown in Figure 3. The toxico-dynamics of exposure time are shown in Figure 4. The presented data showed increased toxicity with increased exposure time to 48 h or 72 h in all cases, and then a decreasing trend at 96 h.

Table 3 illustrates the toxicity parameters and indicates that Penicillin is the most toxic one followed by Ciprofloxacin as shown by the value of EC₅₀. The lowest value of EC₅₀ indicates the highest toxicity. Statistical analysis showed significant difference between the toxicity of diuron and the toxicity of Penicillin, Ciprofloxacin and/or Tylosin; p-value ranged from 0.023-0.0003. Toxicity of Tylosin and Penicillin was also significant p-value equals 0.009.

Toxico-dynamic effect of time: Toxico dynamic effect of Penicillin, Tylosin, Ciprofloxacin and Diuron are shown in Figure 4. It is obvious

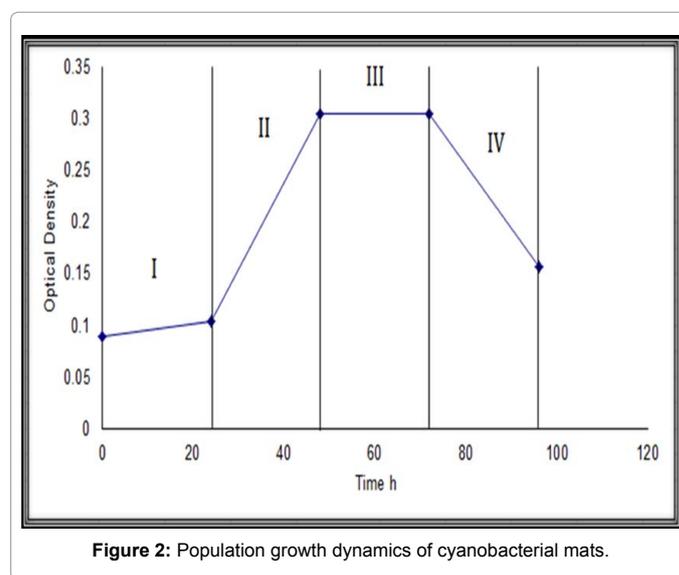


Figure 2: Population growth dynamics of cyanobacterial mats.

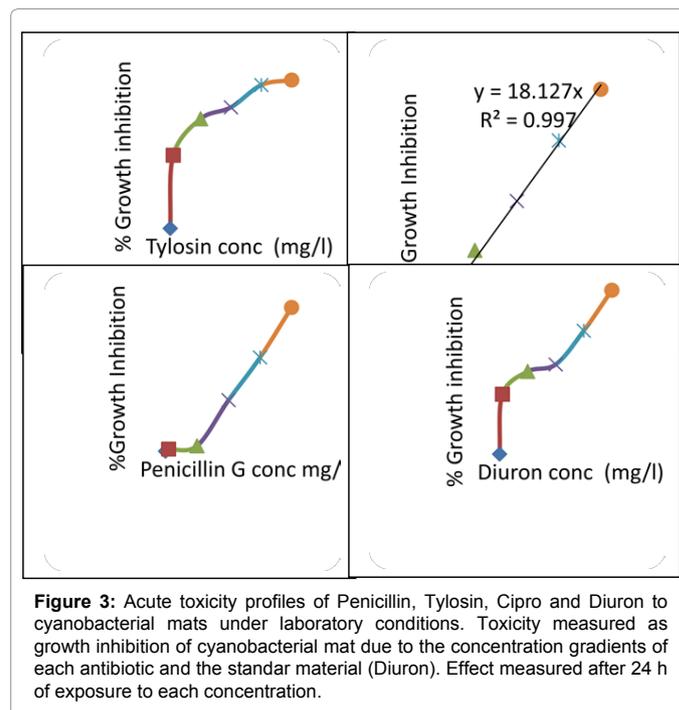


Figure 3: Acute toxicity profiles of Penicillin, Tylosin, Cipro and Diuron to cyanobacterial mats under laboratory conditions. Toxicity measured as growth inhibition of cyanobacterial mat due to the concentration gradients of each antibiotic and the standar material (Diuron). Effect measured after 24 h of exposure to each concentration.

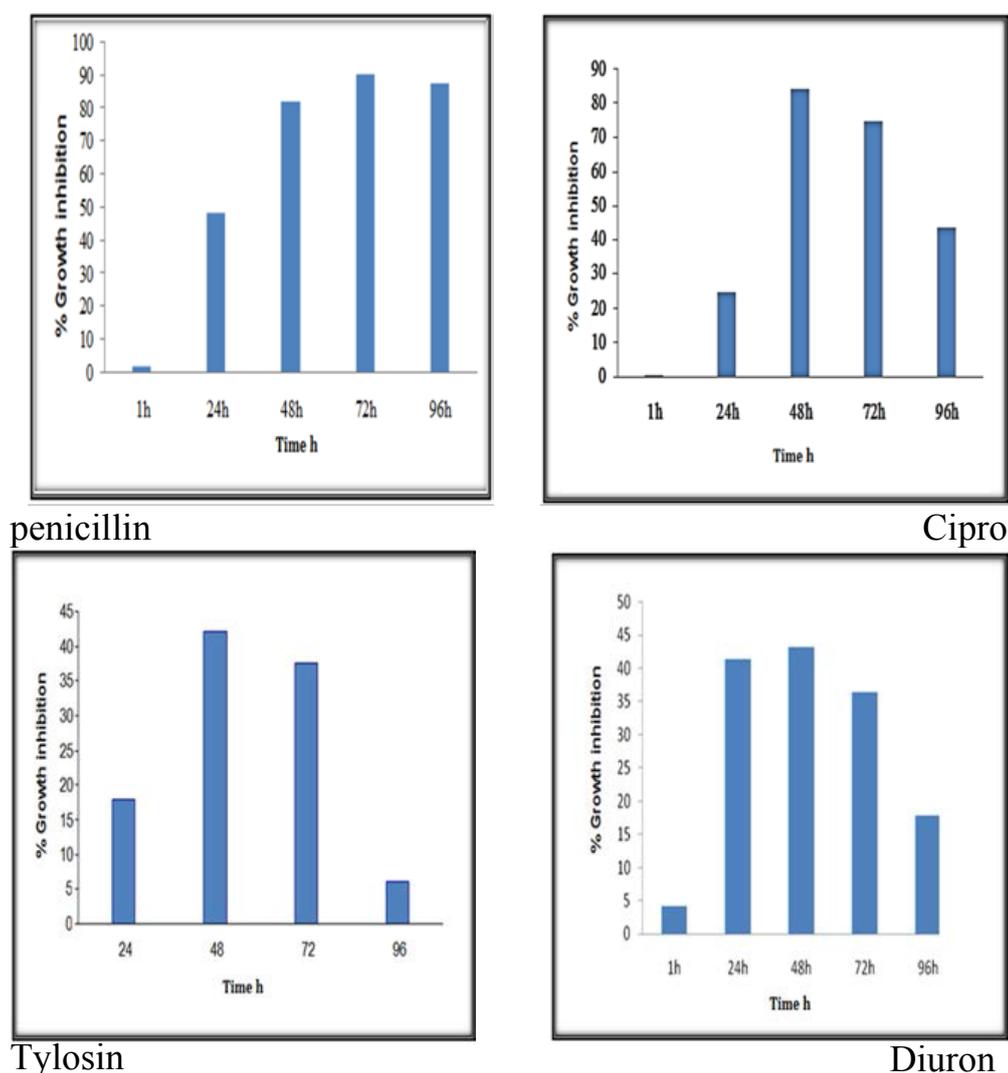


Figure 4: Toxicodynamic effect of penicillin (40 mg/l), Tylosin (20 mg/l), Cipro (0.2 mg/l) and Diuron (4 mg/l) on cyanobacterial mats growth after 96 h.

Compound	EC ₅₀	R ²	Reg. Eq	MTI
Diuron	1.92 mg/l	0.82	Y=0.2302X+1.6336	Nd
Ciprofloxacin	0.71 mg/l	0.976	Y=0.7269X+1.8078	Nd
Tylosin	5.28 mg/l	0.984	Y=0.1906X+1.5612	Nd
Penicillin	0.13 mg/l	0.957	Y=0.0994X+1.7865	Nd
Penicillin 0.5: Tylosin 0.5=B1	0.077 TU	0.917	Y=19.94X+72.217	-3.701
Cipro 0.5: Tylosin 0.5=B2	0.103 TU	0.966	Y=41.612X+91.08	-2.294
Cipro 0.5: penicillin 0.5=B3	0.292 TU	0.83	Y=43.859X+73.46	-0.792
Penicillin 0.33: Tylosin 0.33: Cipro 0.33=T	0.034 TU	0.988	Y=25.08X+86.718	-2.325

Table 3: Toxicity parameters of the tested compounds and Diuron.

that % growth inhibition of Penicillin increased up to 72 h and caused about 90% growth inhibition of cyanobacterial mats. Similar trends were observed for Ciprofloxacin, Tylosin, and Diuron with slight difference in exposure time.

Toxicity of mixtures

The data presented in Figure 5 clearly demonstrate the toxicity of

mixtures to cyanobacterial mats. It is obvious that toxicity of mixtures increased as the concentration of the mixture increased in the solution. Converting the results to the corresponding log scale enabled the calculations of EC₅₀, R² values, regression equation and MTI (Table 3). Statistical analysis showed significant differences among all mixture tests; p-values ranged from 0.0001 to 0.0000. Moreover, no significant difference between B2 and B3, p-value equals to 0.165, was detected.

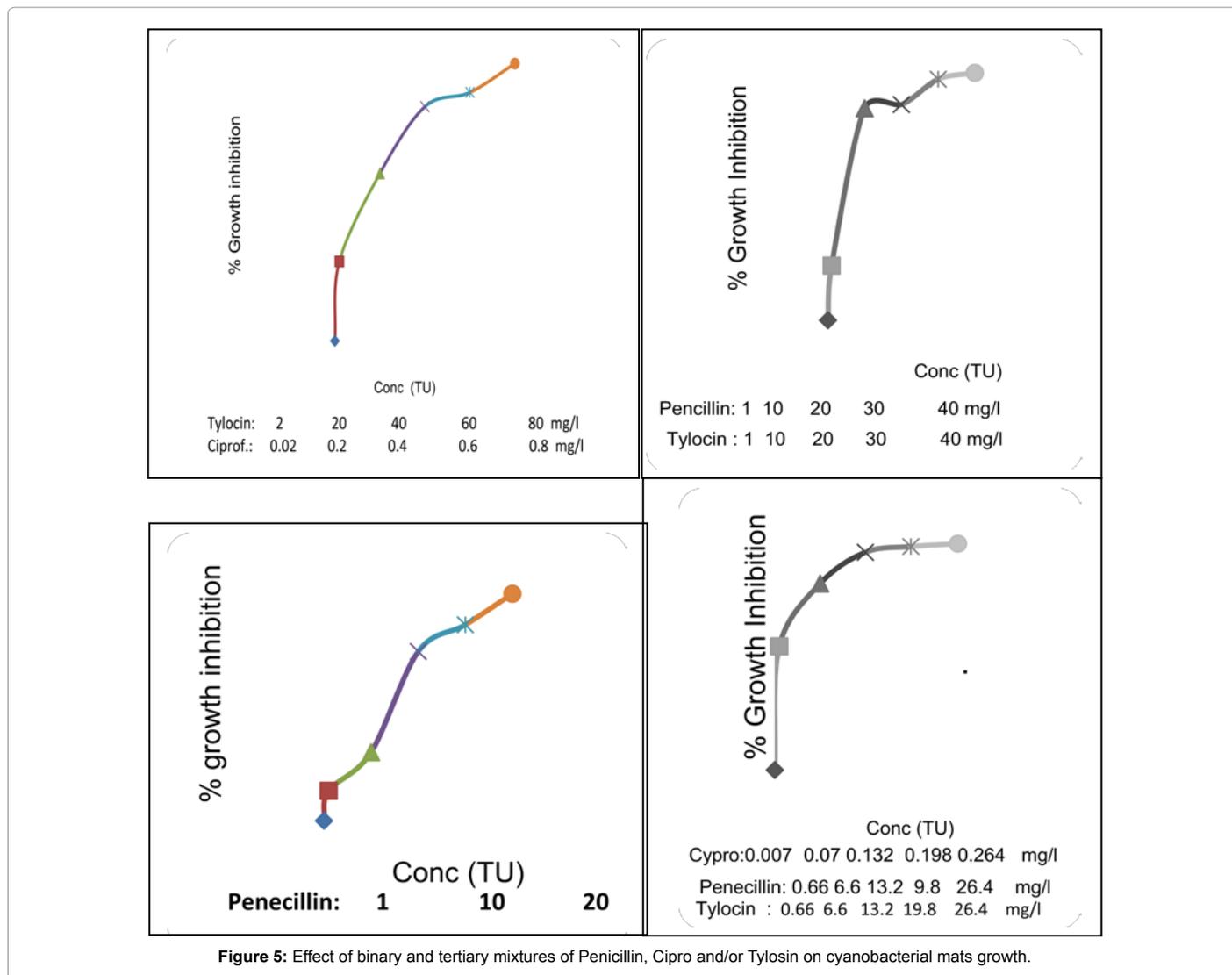


Figure 5: Effect of binary and tertiary mixtures of Penicillin, Cipro and/or Tylosin on cyanobacterial mats growth.

Furthermore, MTI calculations showed negative values. Accordingly, all tests are antagonist effects. However, we can group the MTI values to nearly slightly negative (-0.792) to extremely negative (-3.7013); accordingly, the first one can be categorized to slightly synergistic effect and the one with extreme negative value has the highest antagonistic effect.

Toxico-dynamics of binary and tertiary mixture

Toxicities of mixtures B1 (50% Penicillin and 50% Tylosin), B2 (50% Tylosin and 50% Ciprofloxacin) and B3 (50% Penicillin and 50% Ciprofloxacin) and T (33.3% Penicillin, 33.3% Ciprofloxacin and 33.4% Tylosin) on cyanobacterial mats are shown in Figure 6.

Effect of time on the toxicity of mixtures: Effect of time on toxicity of different combination ratios of antibiotics mixtures on the growth of cyanobacterial mats is shown in Figure 6. Converting the data to the corresponding log scale enabled the calculation of toxicity parameters. However, the effective concentrations for all mixtures are about 0.025 TU that caused different % growth inhibition of cyanobacterial mats. Nevertheless, % growth inhibition reached 40% in case of Mixture B1, and 25% in mixture B2 and 15% in mixture B3. Similar effect was observed in tertiary mixture T1.

Statistical analysis of toxicity of binary and tertiary mixtures:

Statistical analysis of the results indicates significant differences between the effects of mixture B1* B3 and B1* B2 whereas B1* T1 has similar effects regardless of the variations in TU values. Comparing the effects of binary mixture (B) with the tertiary mixture (T1) indicated significant differences in all cases. To evaluate the synergistic and antagonist effect, we calculated the mixture toxicity indices according to equation of Konemann, [38]. MTI for T1 mixture was less than zero which indicates antagonistic effect (Table 3).

Discussion

Growth of cyanobacteria

The data presented in Table 1 clearly indicate the ability of cyanobacterial mats community to grow in different conditions. These values are nearly in accordance with those previously found in Wadi Gaza for other studies [32].

It is obvious (Table 2) that optical density of cyanobacterial mats increased with time indicating normal growth of cyanobacterial mats under laboratory condition. Moreover, calculating the relative growth (Eq. 2) of each experiment (data not shown) and regressing them versus

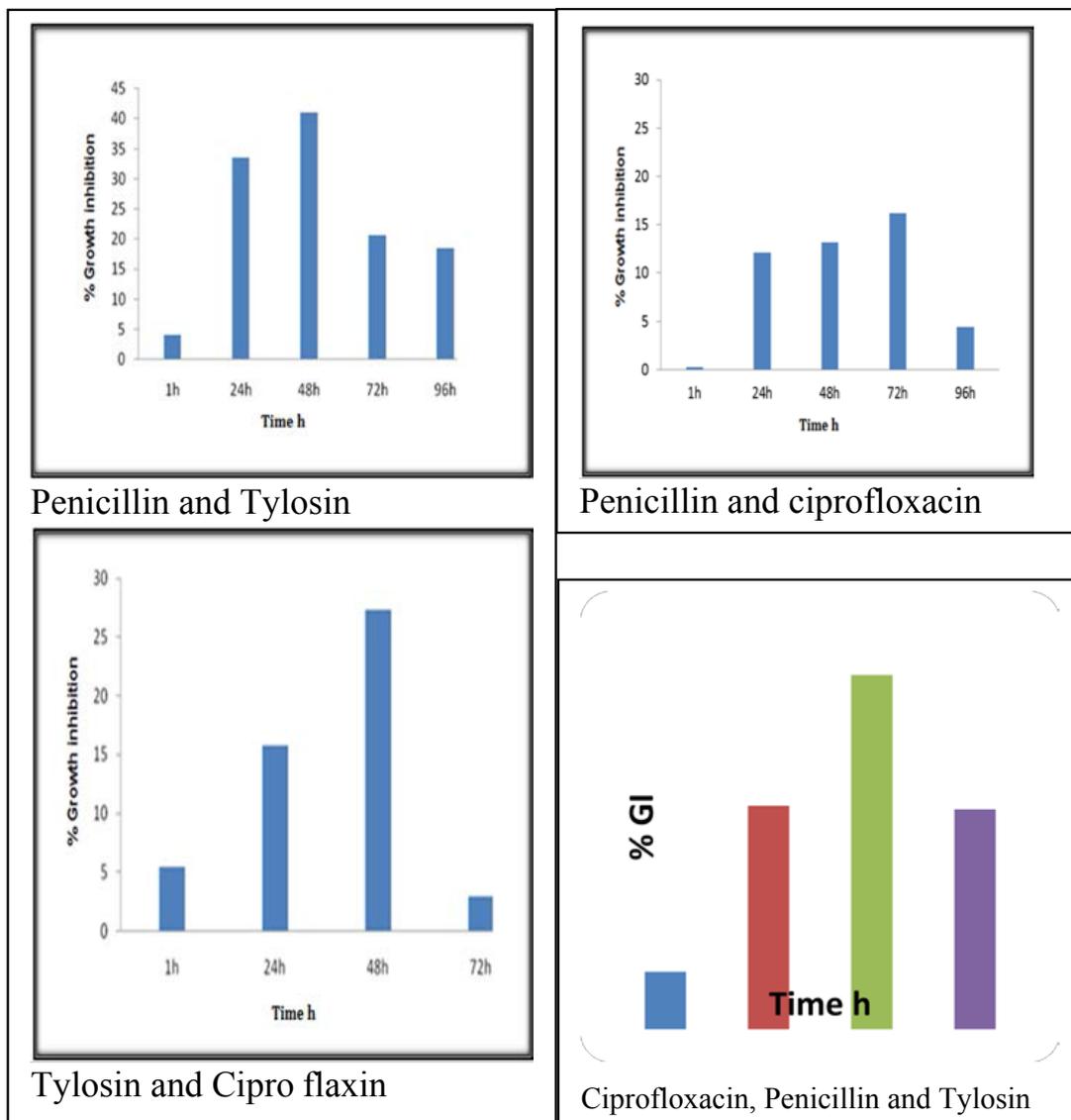


Figure 6: Toxico-dynamics of the binary and tertiary mixtures: B1 (Penicillin+Tylosin), B2 (Tylosin +Ciproflaxin), B3 (Penicillin+Cipro) and T (Penicillin+ciprofloxacin+Tylosin) mixture on the growth of cyanobacterial mats after 96 h. Exposure time measured at concentration equals to 0.025 TU after 72 h.

time indicated linear relationship with an overlapping of standard deviation at the corresponding points of estimation. This indicates adaptation ability of cyanobacterial mats to grow under laboratory conditions at all experiments. Similar growth features were previously reported [32].

The data in Table 2 demonstrated that lag phase of Cyanobacterial mats lasted 22 h of lab conditions followed by log phase which reached the maximum growth at 50 h and stayed at the same level up to 72 h then followed by the decay phase that reached a minimum growth level after 96 h. An interesting outcome of the study is that the optical density of the cyanobacterial mat at the decay phase is still above the optical density at the starting point. This suggests community changes of the cyanobacterial mats. Our results agree with [39] who found community changes of cyanobacterial mats during growth under laboratory conditions. It is obvious (Figure 2) that 4 growth phases of cyanobacterial mat growth are observed.

The presented data in Figure 2 clearly demonstrated the growth phases of cyanobacterial mats during 96 h. The curve can be divided into four phases I, II, III, and IV. Similar growth phases of cyanobacterial mats growth were reported [34]. These growth phases represented, lag phase, log phase, stationary phase, and decay phase [40]. Moreover, Figure 2 shows that log phase was observed during 50 h. Accordingly, our toxicity investigation focused on this period of bacterial growth.

Toxicity tests

The presented data in Figure 3 clearly shows steep increases of growth inhibition of cyanobacterial mats as the concentrations of Penicillin and Ciprofloxacin increased in the solution (Single toxicity tests) then no further increase on growth inhibition was observed. In case of Tylosin and Diuron, gradual increases of growth inhibition were observed as their concentrations increased in the solution.

Converting the data in Figure 3 to the corresponding log scale

enabled the calculation of EC_{50} of each compound along with the regression coefficient (R^2) (Table 3). It can be seen that Penicillin has the lowest EC_{50} (0.13 mg/l) followed by Ciprofloxacin (0.71 mg/l), then Diuron and Tylosin. The low value of EC_{50} indicates high toxicity.

The order of EC_{50} values is 0.13, 0.71, 1.92 and 5.28 mg/l, for Penicillin, Ciprofloxacin, Diuron, and Tylosin respectively. Accordingly, Penicillin is the most toxic compound to cyanobacteria, within 72 h. The explanation of these results is that Penicillin refers to the bactericidal action against sensitive organisms during the stage of active multiplication and due to inhibiting bacterial cell wall synthesis [41]. Moreover, the data in Figure 3 revealed that the tested compounds need longer time to reach the target site in cyanobacterial mats, this is in accord with K_{ow} of the tested compounds (Table 1). However, decreased toxicity after 48 h suggests that tolerant species of cyanobacterial mats adapted themselves to the toxic compound and began to grow under the stress of antibiotic concentrations. This can be attributed to community changes that enabled cyanobacteria to survive under chemical stress. Similar results were observed by Abed et al. [39] when they exposed the cyanobacteria mats to diesel oil. Furthermore, the used cyanobacterial mats in this study were many species with different photoautotrophic activities that enabled them to survive under different pollution conditions [39]. Our results agree with previous reports [32,34] that investigated the growth inhibiting effects of eight antibiotics on freshwater cyanobacteria (*Microcystis aeruginosa*) and green algae (*Selenastrum capricornutum*), and found that *M. aeruginosa* was two orders of magnitude more sensitive than *S. capricornutum* to the tested antibiotics.

Moreover, our results demonstrated that Ciprofloxacin showed strong toxic effect to cyanobacterial mats with EC_{50} (0.71 mg/l). Previous studies reported different EC_{50} values for Ciprofloxacin (2.97 mg/l-20.6 mg/l) against green algae, cyanobacteria, Lemna sp, *Pseudokirchneriella subcapitata* and *Chlorella vulgaris* [34, 42]. These variations in the EC_{50} values for Ciprofloxacin (Table 3) in the present study and that of previous ones are due to different tested organisms, laboratory conditions, and differences in the tested methods used. In contrast, our results indicated that Tylosin (EC_{50} =5.28 mg/l), has moderate toxicity and may pose a potential risk to cyanobacterial mats in aquatic systems, whereas previous studies using green algae *P. subcapitata* growth inhibition, reported lower EC_{50} values 0.95 to 1.38 mg/l [34]. The high EC_{50} value of Tylosin may be due to its instability in acidic and alkaline media [43]. However, Tylosin is a well-known bacteriostatic which inhibits the growth of prokaryotes by binding to the subunit 50S ribosome, and thus preventing the translocation of peptides and interfering with protein synthesis.

Another interesting observation is that Penicillin and Ciprofloxacin are more toxic than Diuron (Table 3), the most toxic herbicide to cyanobacterial mats [33]. This suggests that Penicillin and Ciprofloxacin have specific site of action with cyanobacterial mats beside their regular toxic effects to the bacterial growth. The high correlation coefficient value (R^2) indicates strong positive association between toxicity (%GI) and concentration. Calculating the relative toxicity (RT) of antibiotics (Eq. 2) indicates that Penicillin and Ciprofloxacin have RT value less than 1; whereas Tylosin has RT value higher than 1. This indicates that Penicillin and Ciprofloxacin are more toxic than diuron and pose threats to the aquatic eco-system.

Toxicity of mixtures

The trend of mixture toxicity is similar in all cases, indicating similar response of cyanobacterial mats to the mixture. However converting

the data in Figure 4 to log scale enabled the calculations of EC_{50} of mixture. The toxicity parameters (Table 3) clearly show that mixture B1 (Penicillin and Tylosin) is the most toxic one and has the lowest EC_{50} value (0.077 TU) among all mixtures. The present study suggests that binary mixtures have antagonistic effect (Table 3). Similar results were reported by Yang et al. [44] for Tylosin-triclocarban, triclosan-norfloxacin, and triclocarban-norfloxacin which have slightly antagonistic effect. This may be due to the fact that compounds of a heterogeneous mixture often have different toxic sites and dissimilar modes of action. So concentration addition may not be expected [37]. Another explanation of antagonistic effect could be due to competition for uptake of the same binding sites or suppression of the toxic effect of one drug to the other. In contrast, Chen et al. [27] showed synergistic effect. This is probably due to the fact that we work on cyanobacterial mats which are a community of bacteria.

Toxico-dynamic effects of binary and tertiary mixture on cyanobacterial mats

It is obvious (Figure 5) that there is a steep increase in growth inhibition as toxic units of the mixture increased in the solution up to 0.2. Then no further considerable growth inhibition was observed. Table 3 shows the toxicity parameters of mixtures on cyanobacterial mats and indicates the relationship between the compounds of each mixture. According to Konemann, [38], all tested compounds in binary and tertiary mixtures showed antagonistic effect.

Effect of time on the toxicity of different combination ratios of antibiotics mixtures on cyanobacterial mats are shown in Figure 6. Converting the data to the corresponding log scale enabled the calculation of toxicity parameters. However, the effective concentrations for all mixtures are about 0.025 TU that caused different % growth inhibition of cyanobacterial mats. Nevertheless, % growth inhibition reached 40% in case of Mixture B1, and 25% in mixture B2 and 15% in mixture B3. Similar effect was observed in tertiary mixture T1.

Conclusion

The present study provided answers to the claims of fish breeders and aquacultures and indicated the possible disappearance or destruction of cyanobacterial mats as a model of fish lake vegetation. This effect has considerable effects of oxygen production by cyanobacteria. Consequently, it is recommended to use alternatives to these antibiotics in fish lakes as health promoters or protectives. Moreover, the study provides evidence of the toxic effects of Penicillin, Tylosin, and Ciprofloxacin to cyanobacterial mats. Single toxicity tests demonstrated high potential toxicity against cyanobacterial mats. EC_{50} of individual tests were Penicillin 0.13<Ciprofloxacin 0.71<Tylosin 5.28 mg/l. Relative toxicity values indicate Penicillin, and Ciprofloxacin, are more toxic than Diuron to cyanobacterial mats. The interesting outcome of this study is that Penicillin is the most toxic compound to the cyanobacteria mat followed by Ciprofloxacin whereas Tylosin was the least toxic. The effects of time indicated that cyanobacteria mats were able to overcome the toxic effects after 72 h of exposure time. The mixture B1 was the most toxic one among all showed antagonistic effect. EC_{50} of mixtures are: B1 0.077<B2 0.103<B3 0.292 and T1 0.034 TU/l. The study also demonstrated the sensitive cyanobacterial mats as aquatic microorganisms to antibiotics. It is recommended that hospital wastes should be treated before transferring to the eco-system.

Acknowledgement

Dr. Y. El-Nahhal acknowledges Alexander von Humboldt Stiftung Foundation Fellowship Grant no IV-PAL/1104842 STP, Germany. Special thanks go to Prof Dr G.lagaly at Kiel University, Prof Shourmman Germany.

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