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The Effects of Oxytetracycline and Flumequine on the Rotifers (*Brachionus plicatilis* O.F. MÜLLER)

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Özet: ***Oxytetracycline ve Flumequine'in rotiferler* (Brachionus plicatilis *O.F. MÜLLER*) *üzerindeki etkisi.*** Flumequine ve Oxytetracycline'in farklı dozlarının *Brachionus plicatilis* O.F. MÜLLER üzerindeki etkileri çalışıldı ve antibiyotiklerin farklı dozlarındaki ölüm oranları tespit edildi. Ayrıca rotiferler tarafından absorbe edilen Oxytetracycline miktarı Elisa testi ile saptandı. Her denemede 50 lt'lik polietilen torbalar kullanıldı. 0,0 ppm, 25,0 ppm, 50,0 ppm, 100,0 ppm, 150,0 ppm ve 200,0 ppm'lik antibiyotik dozları torbalara eklendi. Yaklaşık beş saat sonra her gurup 45 µm'lik membran filtreden süzülerek Elisa testiyle analiz edildi ve rotiferler tarafından absorbe edilen antibiyotik miktarı tespit edildi. Yukarıda bahsedilen antibiyotikler arasında balık larvalarının tedavisinde en uygun antibiyotiğin Oxytetracycline olduğu bulundu. Analizlere göre, 25,0 ppm, 50,0 ppm, 100,0 ppm, 150,0 ppm ve 200,0 ppm için absorbe edilen antibiyotik miktarları sırasıyla 3,58 ppb; 1,5 ppb; 3,91 ppb; 2,5 ppb ve 4,46 ppb olarak tespit edildi.

Anahtar kelimeler: *Brachionus plicatilis*, antibiyotik, Oxytetracycline, Flumequine, Elisa testi

Abstract : The effects of different dosages of Flumequine and Oxytetracycline were studied on *Brachionus plicatilis* O.F. MÜLLER and the rates of death for different dosages of antibiotics were determined. In addition, the amounts of the Oxytetracycline absorbed by the rotifers were determined by Elisa test. Polyethylene bags of 50 lt were used in each trial. 0.0 ppm (control group), 25.0 ppm, 50.0 ppm, 100.0 ppm, 150.0 ppm, and 200.0 ppm dosages of antibiotics were added into the bags. Approximately five hours later, each group was filtered through a 45 µm Millipore membrane filter and analysed by Elisa test, being determined the amount of the antibiotic absorbed by the rotifers. It was found that the most suitable antibiotic on the treatment of fish larvae is Oxytetracycline between the antibiotics above. According to the analysis, antibiotic amounts absorbed for 25.0 ppm, 50.0 ppm, 100.0 ppm, 150.0 ppm, and 200.0 ppm were determined as 3,58 ppb; 1,5 ppb; 3,91 ppb; 2,5 ppb; and 4,46 ppb, respectively.

Keywords: *Brachionus plicatilis*, antibiotic, Oxytetracycline, Flumequine, Elisa test

Introduction

Because of its easy digestion, appropriate size to the mouth-opening of larvae, high density stocking capability, ability to swim slowly and appropriate biochemical composition (Reitan et al., 1993 ; Qie et al., 1997), the fact that it remains suspended in the water column and because it is enriched easily by antibiotics and fatty acids (Maragelman et al., 1985), *Brachionus plicatilis*, O.F. MÜLLER is intensively used to cultivate marine fish larvae, having an essential role, especially, in the first feeding.

Depending on continuous development in aquaculture, Artemia and rotifer, which are used as live foods, can be enriched by nutrients (Leger et al., 1986), by fat soluble therapeutics (Mohney et al., 1990 ; Nelis et al., 1991 ; Touraki et al., 1996) and even by water

soluble therapeutics (Duis et al., 1995 ; Touraki et al., 1995). Gatesoupe (1982) and P. Benevante et al. (1988) got successful results in the trial they carried out on turbot larvae (*Scophthalmus maximus*, L.)

In intensive aquaculture mode, more feed come into the rotifer culture, so it is difficult to prevent the occurrence of bacteria in the tanks. Rotifers filter the important part of the bacteria in the culture tank. When the rotifers are given to the larval rearing tanks, larvae will take the bacteria, which come through the rotifers, and the probability of bacterial contamination will increase. The most important losses in marine fish larvae culture occur through bacterial infections, which are generally seen in larval period, and these infections are mostly due to gram negative bacteria (Trust, 1986). The most important reason bacterial diseases occur is that live food makes the pathogen bacteria infect the larval rearing tanks and the infections are cured either by direct antibiotic addition to the culture tank or by the addition of antibiotics to fish feed.

In this study, the problem of how to remove or reduce bacterial contamination of rotifers with the applications of two different antibiotics was researched and the most suitable antibiotic used during the treatments was sought by observing the activity and mortality of the rotifers, as well as antibiotic levels absorbed by the rotifers.

Materials and Method

In this research, two kinds of antibiotics, Flumequine and Oxytetracycline, were applied to the individuals of *B. plicatilis*. Each experiment consisted of six groups that were given various antibiotic dosages: 0,0 ppm (Control), 25,0 ppm, 50,0 ppm, 100,0 ppm, 150,0 ppm and 200,0 ppm. Approximately the same numbers of individual rotifers were put in each experimental group and the rotifers used in the experiment were selected from the rotifers that are suitable for the mouth opening of Sea Bass larvae (250 µm). Experiments were carried with 20 ‰ salinity and 25 °C constant temperature and the environmental temperature was stable by the system of Bain-Mari.

After a certain time, rotifer mass was analysed by Elisa test. In order to obtain the biomass the analysis needs, rotifer cultures were set up as 250-300 individuals/ml in polyethylene plastic bags of 50 lt. The experiments were started after antibiotic and Culture-Selco were added simultaneously into the experiment groups. The activity of rotifers was observed by microscopic observations every ten minutes.

Rotifers were counted by a Sedgwick-Rafter counting-chamber. After the counting operation was finished in each trial, rotifers were filtered through a 45 µm millipore membrane filter and rinsed well to remove the antibiotic residue present in the medium. In the trials, sensitiveness and behaviours of the rotifers were observed for the levels applied, and the absorbed Oxytetracycline levels, made by İzmir Bornova Veterinarian Institute, were found.

Results

Group of Flumequine

In the trial applied Flumequine, Erlenmeyer of 5 lt and 20 ‰ seawater were used. No antibiotic addition was done in control group, and calculated amounts of Flumequine for 25.0, 50.0, 100.0, 150.0 and 200.0 ppm were found to be 125 mg, 250 mg, 500 mg, 750 mg and 1000 mg, respectively.

Numerical changes related to time and the activities of the rotifers were observed in each experiment group with different applications of the antibiotic.

Development of the population density was clarified on the figures below.

As can be seen in Fig.1 (A) and (B), rotifer numbers were about the same in both groups, and they did not indicate much deviation from the initial cell numbers. The activities were very good, except for a few rotifers moved slowly in the group applied 25,0 ppm. The number, which was 188 individuals/ml initially in control group, was 198 ind./ml at the end of the trial. As for in the group applied 25,0 ppm Flumequine, rotifer number was found to be 216 ind./ml initially, being 169 ind./ml at the end of the trial. The rates of mortality in control group and in the group applied 25,0 ppm were found to be 18% and 35%, respectively at the minutes when rotifer numbers were lowest in the trials, i.e., 154 ind./ml at the 255th minute and140 ind./ml at the 185th minute.

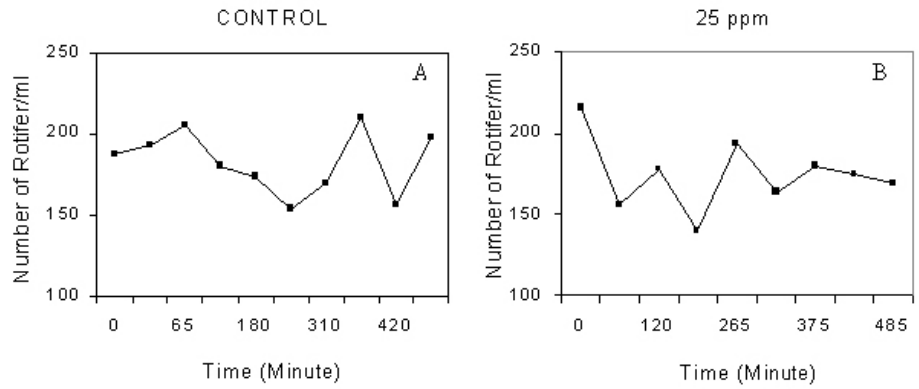

Figure 1. Development of the population (A) in control group (B) in 25,0 ppm Flumequine

In the trial using Flumequine of 50,0 ppm and 100,0 ppm, the initial numbers were 207 ind./ml and 225 ind./ml, respectively. The activities were generally good during the trials, except that a few rotifers moved too slowly in both groups. The lowest numbers of the rotifers were observed to be 142 ind./ml in the group of 50,0 ppm and 137 ind/ml in the group of 100,0 ppm, and the rates of mortality were found to be 31 % and 39 % at these times, respectively [Fig.2 (A) and (B)].

The numbers were 212 ind./ml in the group of 150,0 ppm and 202 ind./ml in the group of 200,0 ppm, and the activities were good at the beginning. However, after minute 155, the activity of the half of the rotifers were slower than the other half in the former group, and at minute 210, the activity of the rotifers became good, except for 10 ind./ml rotifers that made only their rotators move. The lowest number was reached at the 35th minute with 166 ind./ml, and the mortality was found to be 21 % at the moment. In the group with 200,0 ppm Flumequine, in general, most of them could move only the rotators and tails, and the activity started to get better after minute 350. The rotifers reached at their lowest number at min 110 with 88 ind./ml and the mortality was 55 % [Fig.3 (A) and Fig.3 (B)].

Group of Oxytetracycline

In the second trial, 20 ‰ seawater and PE bags of 50 lt were used. The amounts for 25.0, 50.0, 100.0, 150.0 and 200.0 ppm Oxytetracycline being calculated to be 1250 mg, 2500 mg, 5000 mg, 7500 mg and 10000 mg, respectively.

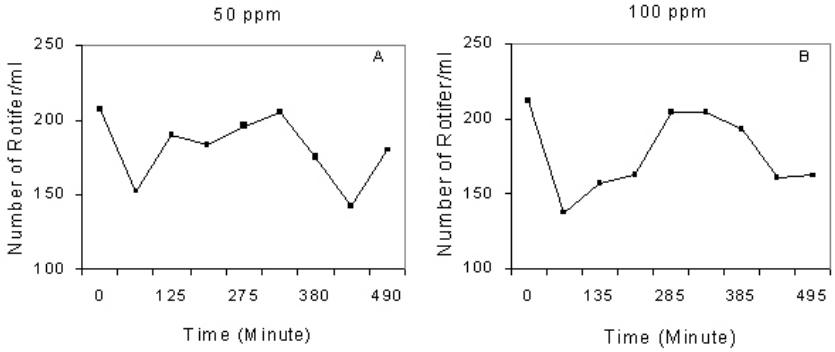

Figure 2. Development of the population (A) in 50,0 ppm (B) in 100,0 ppm Flumequine

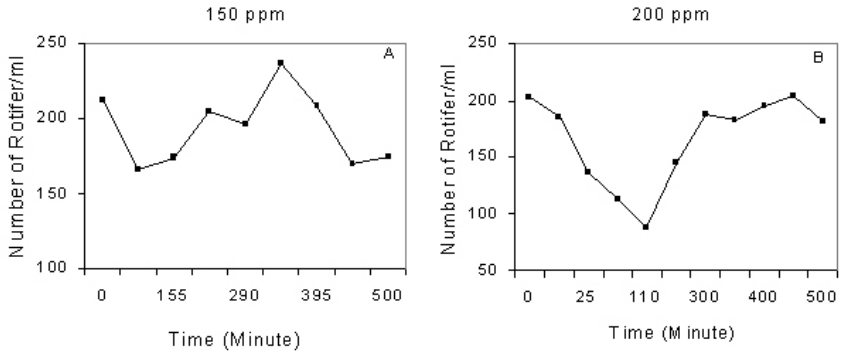

Figure 3. Development of the population (A) in 150,0 ppm (B) in 200,0 ppm Flumequine

In the observations, the number of rotifers and the individuals with eggs were found. After rotifers were put into PE bags, their numbers were determined and the activity of the rotifers and the individuals with eggs were found by microscopic observations every 10 minutes. The values obtained and the individual numbers with eggs in the rotifer cultures are clarified on the figures below.

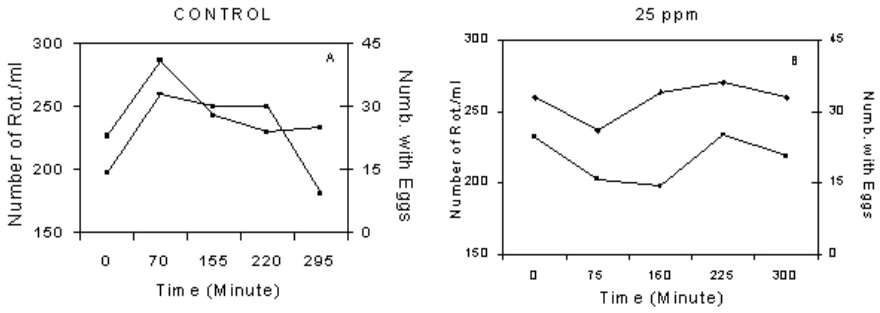

Figure 4. Development of the population and number of the individuals with eggs (A) in control group and, (B) in 25,0 ppm Oxytetracycline. Filled and empty symbols indicates rotifer number and individuals with eggs, respectively.

The initial number of control group was 203 ind./ml, and, in general, the activity was good during the trial. The highest rate of mortality in the group was 11 % at minute 265. In the trial with an application of 25,0 ppm Oxytetracycline, the initial number was 239 ind./ml (34 ind./ml with eggs), and the activity was generally good, except for 4-6 ind./ml dead rotifers and a few rotifers moved slowly at the 160th minute. The rate of mortality was 17 % at minute 160 [Fig.4 (A) and Fig.4 (B)]. The cultures were started with the initial numbers of 207 ind./ml (34 ind./ml with eggs) and 253 ind./ml (33 ind./ml with eggs) in the groups of 50,0 ppm and 100,0 ppm, respectively. There were some dead rotifers in the observation made at minute 230 in the group of 50,0 ppm, i.e., 11 ind./ml, While there were 6 ind./ml dead and 20-25 ind/ml moved slowly at minute 80 in the group of 100,0 ppm. Although there were a few dead individuals in 50,0 ppm, the number reached at 15 ind./ml dead and 64 ind./ml moved slowly at the 235th minute in 100,0 ppm, the rate of dead being 14 % for the group [Fig.5 (A) and (B)].

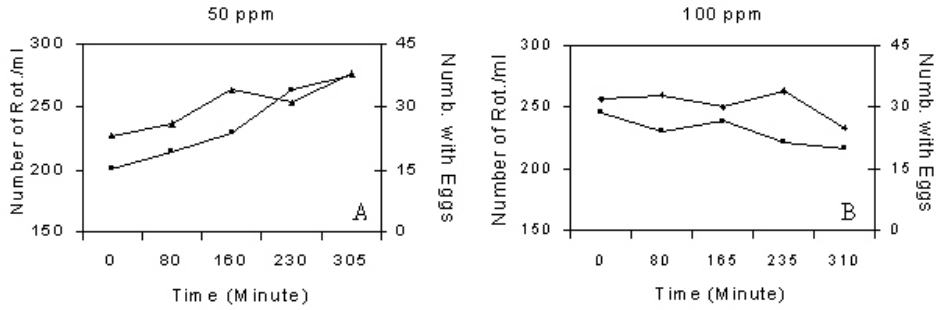

Figure 5. Development of the population and number of the individuals with eggs (A) in 50,0 ppm (B) in 100,0 ppm Oxytetracycline. Filled and empty symbols indicates rotifer number and individuals with eggs, respectively.

The numbers were 225 ind./ml (23 ind./ml with eggs) and 254 ind./ml (27 ind./ml with eggs) initially for the groups of 150,0 and 200,0 ppm, respectively. Numbers of the dead rotifers increased in both groups being related to time, but it increased much faster in 200,0 ppm than 150,0 ppm, i.e., while there were 10 ind./ml dead and 46 ind./ml moved slowly in 150,0 ppm, the number increased to 98 ind./ml dead and 61 ind./ml moved slowly in 200,0 ppm in the ends of the trials. The rates of deaths were 23 and 59 % for 150,0 and 200,0 ppm, respectively [Fig.6 (A) and Fig.6 (B)].

Discussion

In this experiment, the effects of two different antibiotics were researched on the population dynamics and activity of rotifer cultures. It is worth noting that, one of the most important problems in marine larvae culture is larval loss and this loss takes place due to bacterial infections that occurs in the culture tanks. The mass loss of larvae, i.e., the main reason is most likely because of bacterial infections, results in huge economical losses. Different amounts of antibiotics are added to the larval rearing tanks in order to cure the larvae infected by

bacteria. In direct antibiotic addition, the added antibiotics are discharged quickly from the tanks because of water circulation before showing their effect to cure. In addition, the discharged water, e.g., including antibiotic, leads to be negative effect on the environment, so that, this situation causes big damages both on environment and economy (Samuelsen et al., 1997). The best way of preventing this is to give antibiotics to larvae via live food in early periods of them. For this reason, antibiotics are given to *Artemia* sp. or *Brachionus* sp. firstly and it is ensured live food to absorb antibiotic. *Artemia* sp. or *Brachionus* sp. that absorbed a certain dosage of antibiotic is given to larvae, and it is ensured healthy larvae to be protected from bacterial infections or infected larvae to be cured.

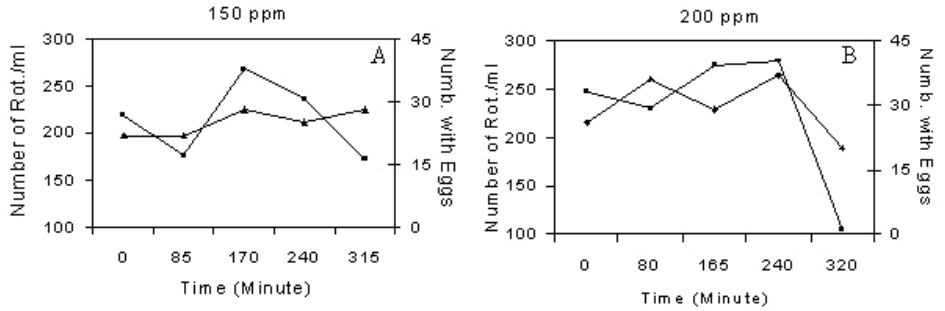

Figure 6. Development of the population and number of the individuals with eggs (A) in 150,0 ppm (B) in 200,0 ppm Oxytetracycline. Filled and empty symbols indicates rotifer number and individuals with eggs, respectively.

Flumequine couldn't be effective sufficiently on the rotifers in the experiment we did. However, Oxytetracycline became more effective than Flumequine because Oxytetracycline dissolved better in the water, consequently, Oxytetracycline should be preferred to Flumequine. In the first trial made with Flumequine, rotifer population reached its lowest between the minutes 35 and 60. But in 200,0 ppm, this numerical lessen in the culture went on and the culture made a peak at the minute 110 with 88 ind./ml. After this numerical lessen occurred, rotifer culture started to increase again by the reproducing of the individuals with eggs. Moreover, because some amount of the antibiotic was absorbed by the rotifers in the culture, a decrease was seen on the levels of antibiotics compared with initial ones, and newly hatched individuals were not affected from the new levels, the number starting to increase. As for in the last trial used Oxytetracycline, the number of individuals with eggs were observed, as well as the number of rotifers in the culture. As expected, especially the levels of 150,0 and 200,0 ppm had a sudden effect on the development of population. At minute 170 in 150,0 ppm, and at the minute 240 in 200 ppm, a quick decrease was seen in the numbers of the cultures. It can be concluded that there is a linear proportion between the development of the population and the individual number with eggs. Very important decrease was not seen among the other groups, but all of them, except that the groups of 25,0 and 50,0 ppm, had a lower numbers than the initial numbers. In this experiment, low levels were not effective on the cultures, but when the dosage is higher, so is the mortality. Especially, the dosages of

100,0 ppm and up significantly prevented the development of the rotifer population.

According to the results of the analysis for the trials made with Oxytetracycline, the levels of the antibiotics absorbed by *B. plicatilis* for 10 g samples are 3,58 ppb, 1,5 ppb, 3,95 ppb, 2,5 ppb and 4,46 ppb for the groups of 25,0 , 50,0 , 100,0 , 150,0 and 200,0 ppm, respectively.

The value absorbed in 50,0 ppm Oxytetracycline was lowest with 1,5 ppb, and in 200,0 ppm it was highest with 4,46 ppb. Although the amount of the Oxytetracycline absorbed in 200,0 ppm was the highest, the highest mortality was also in this group, and the activity was clearly slow. In conclusion, it is not suitable to use these rotifers in the cure of larvae. Both the best activity and the least numerical change were seen in the group of 25,0 ppm Oxytetracycline. Nevertheless, the highest absorption of Oxytetracycline, except for 200,0 ppm, was seen again in this group with 3,58 ppb. To draw a conclusion, *B. plicatilis* applied 25,0 ppm of Oxytetracycline for 5 hours can be used in the cure of larvae.

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