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Alterations of HSP70 gene expression in rainbow trout (*Oncorhyncus mykiss*) exposed to deltamethrin

Muhammed ATAMANALP[1,*], Orhan ERDOĞAN[2]

[1]Department of Aquaculture Engineering, Faculty of Agriculture, Atatürk University, 25240 Erzurum - TURKEY

[2]Department Molecular Biology and Genetic, Faculty of Science, Atatürk University, 25240 Erzurum - TURKEY

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Abstract: The aim of the study was to assess the effects of deltamethrin, a synthetic pyrethroid pesticide and potential toxic pollutant to aquatic ecosystems, on the heat shock protein HSP70 gene expression in rainbow trout. Deltamethrin was applied at 0.3 and 0.6 µg/L for 28 days under laboratory conditions. After the experiment, HSP70 was amplified for 20-40 PCR cycles for the control and deltamethrin-treated fish groups. While the quantitative mRNA level was low in the 40th PCR cycle in the control, it was higher in the fish exposed to deltamethrin. The differences between the treated groups and the control group were statistically significant ($P < 0.01$), but the difference between the treatment groups was not.

Key words: Pesticide, *Oncorhynchus mykiss*, HSP70 gene

Deltamethrin'e maruz bırakılan gökkuşağı alabalıklarında (*Oncorhynchus mykiss*) HSP70 gen ekspresyonu değişimleri

Özet: Bu araştırmada; sucul ekosistemler için potansiyel toksik kirletici olan bir sentetik piretroit, deltamethrin'in gökkuşağı alabalığı HSP70 gen ekspresyonu üzerine etkilerinin belirlenmesi amaçlanmıştır. Laboratuar koşullarında 28 gün süresince 0,3 ve 0,6 µg/L dozlarında deltamethrin uygulandıktan sonra, HSP70 geni 20-40 PCR döngülerinde uygulama grubu ve kontrol grubu balıklar için amplifiye edildi. Kontrol grubunun 40 PCR döngüsünde kantitatif mRNA düzeyi düşük bulunurken deltamethrine maruz bırakılan grupta yüksek çıkmıştır. Uygulama grupları ve kontrol arasındaki farklar istatistikî olarak önemli ($P < 0,01$), uygulama grupları arasındaki farklar ise önemsiz olarak değerlendirilmiştir.

Anahtar sözcükler: Pestisit, *Oncorhynchus mykiss*, HSP 70 geni

Introduction

The increasing use of synthetic pesticides is increasing worldwide pollution risks. Pesticides are toxic and designed to kill unwanted organisms, but when applied on land, they may be washed into the surface water and kill, or at least adversely influence, the life of aquatic organisms.

Pyrethroids are synthetic derivatives of pyrethrins, which are obtained from the flower *Chrysanthemum cinerariaefolium*, commonly used for industrial and

* E-mail: mataman@atauni.edu.tr

agricultural purposes. They have been employed as substitutes for organochlorine, organophosphate, and carbamate insecticides because of their low persistence in the environment and comparatively low mammalian toxicity (1,2). As a result of their beneficial qualities synthetic pyrethroids, such as deltamethrin, have attracted farmers and health departments to use them for pest control (3).

Deltamethrin is a pyrethroid insecticide widely used on crops and in pest-control programs because of its low environmental persistence and toxicity. Deltamethrin was synthesized in 1974, and first marketed in 1977. It has been shown to exert a wide range of effects on non-targeted organisms, including fish (4).

Pyrethroids pose a serious potential hazard to fish because of the use of these compounds in many aquatic larvicidal programs. Pyrethroids are highly toxic to most fish, and deltamethrin is one of the most toxic and widely used. The fish exhibit several symptoms of stress when treated with deltamethrin (5). A high rate of absorption of deltamethrin through the gills also makes fish a vulnerable target of its toxicity (6).

Under laboratory conditions, in water without particulate matter, pyrethroid insecticides have a high toxicity on fish and some aquatic invertebrates. The pyrethroids have very low water solubility/high hydrophobicity, and therefore they are rapidly and strongly adsorbed into particulate material (7).

Deltamethrin is highly toxic to fish and various other aquatic organisms (8). According to the World Health Organization (WHO) the LC_{50} for deltamethrin in fish, after 96 h (acute toxicity), ranges between 0.4 and 2.0 μg L^{-1}. After intoxication with this pyrethroid, fish exhibit symptoms of nervous stimulation, such as hyperexcitability (4,9). The mechanism of deltamethrin's toxicity to fish is the same as that of other pyrethroids containing the cyano-3-phenoxybenzyl groups. These groups block the sodium channels of nerve fibers, thereby lengthening their depolarization phase; moreover, they affect the GABA receptors in the nerve fibers (10).

The rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) is the most common breeding species in Turkey, and has a large market potential. A study of deltamethrin-induced damage and the alterations in rainbow trout could provide useful information on the ecotoxicological consequences of deltamethrin use.

Therefore, the present study was undertaken to experimentally investigate the effects of deltamethrin exposure on the HSP70 gene expression in rainbow trout.

Materials and methods

Fish and pesticide supplementation

The fish material, rainbow trout, was obtained from the Trout Breeding and Research Center of the Fisheries Department of the Faculty of Agriculture at Atatürk University. The fish were 1 year-old, and weighed 130 ± 20 g. Research tanks were designed with a constant water flow of 0.5 L/min/kg. The water temperature was 10.5 ± 0.5 °C, and dissolved oxygen was 8 ± 0.5 ppm during the experiment. The pH and total hardness values were measured and recorded routinely; they were found to be 7.8 and 102 mg as $CaCO_3$, respectively. The fish were fed a commercial pellet diet at a daily ration of 1% of their fresh body mass during the study. After an adaptation period of 21 days, the fish in the treatment groups were exposed to a daily single dose of 0.3 or 0.6 μg/L deltamethrin for 28 days.

At the end of the treatment period, the fish were killed without anesthetic and were sacrificed for tissue samples.

RNA and cDNAs synthesis

The fast-frozen skeletal muscle tissue of each sample was used for purification of total RNA. Total RNA was extracted using TRIzol® Reagent (Invitrogen). First-strand cDNA was synthesized using Super Script III Reverse Transcriptase (Invitrogen) in accordance with the manufacturer's protocol (11).

Quantitative reverse transcription polymerase chain reaction (RT-PCR)

For PCR, ca. 100 ng template, 1× PCR buffer (1.5 mM $MgCl_2$), 200 μM of each dNTP, 0.2 μM gene-specific for both forward (5′-TGCACCTA

GGTTTTCATAGAAT-3′) and reverse (5′-ATG GAGGTGTAGAAGTCGATGC-3′) primers, and 2.5 units of Taq DNA polymerase were mixed in a total reaction volume of 15 µL. Thermal cycling conditions were as follows: initial activation at 94 °C for 3 min, 40 PCR cycles at 94 °C for 30 s, 62.5 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 5 min. PCR products were submitted to electrophoresis on a 1% agarose gel. A distinct band, estimated at ~954 nucleotides, was generated. The same method was performed for the actin gene, using the primers actin F (5′-TGGGGCAGTATGGCTTGTATG-3′) and actin R (5′-CTCTGGCACCCTAATCACCTCT-3′) (12). Amplified products were quantified using ImageJ 1.37c (11).

Results

The rainbow trout were exposed to 2 different doses (0.3 and 0.6 µg/L) of deltamethrin for 28 days after 21 days of adaptation. The HSP70 mRNA was amplified in the 2 treatment groups and the control group as shown in Figure 1. The β-actin gene was used as a positive control for the RNA extraction and PCR method. The control, low dose, and high dose deltamethrin-exposed samples were amplified for 20-40 PCR cycles. The expected HSP70 amplification product was undetectable after 40 PCR cycles with RNA in the control fish, as shown in Figure 1, but was abundant with RNA in the deltamethrin-treated fish at either dose.

The quantitative mRNA levels of HSP70 were plotted for the control and deltamethrin-exposed fish using gel density after 40 PCR cycles (Figure 2). β-actin was used as a positive control. There was a statistically significant difference in the quantity of HSP70 mRNA between the control and deltamethrin-exposed fish groups ($P < 0.01$), but the difference between the groups exposed to different doses was not significant.

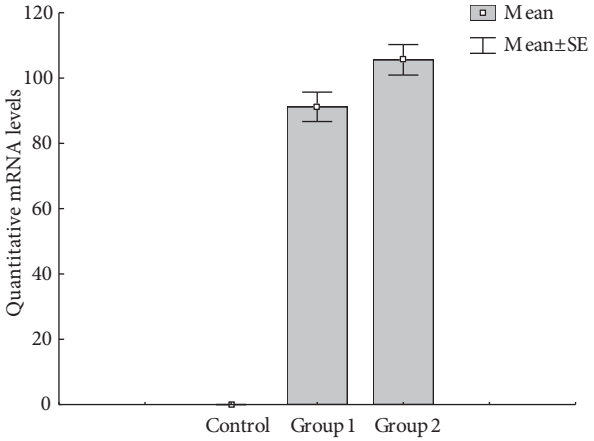

Figure 2. HSP70 quantitative mRNA levels.

Discussion

Under field conditions, the impact of toxic chemicals (insecticides, etc.) is likely to be much less than predicted by laboratory acute or chronic toxicity test data (7). Observing data in field conditions is often difficult and sometimes impossible, and so researchers work with the same chemicals under laboratory conditions to obtain comparable data.

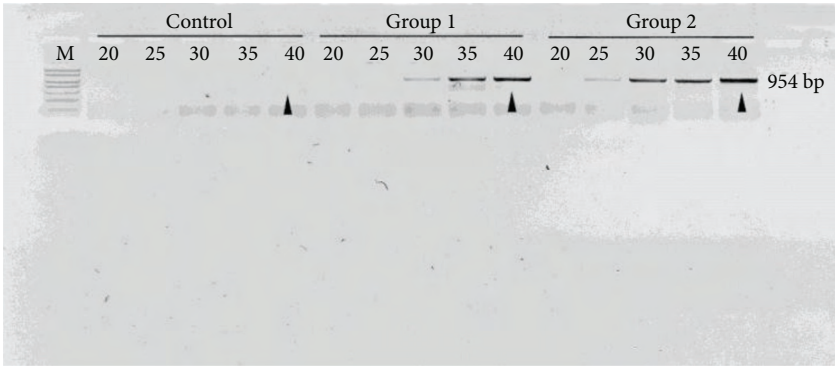

Figure 1. HSP70 gene PCR product agarose gel patterns, control, group 1 (exposed to 0.3 µg/L deltamethrin); group 2 (exposed to 0.6 µg/L deltamethrin).

The present work demonstrates the induction of HSP70 gene expression by deltamethrin. While the quantitative mRNA level was very low in the 40th PCR cycle for the control, it was high in the deltamethrin treatment fish (Figure 1). The quantitative mRNA levels of HSP70 were plotted for the control and deltamethrin exposed fish by using band density in the 40th PCR cycle. β-actin was used as a control. As far as the quantitative mRNA is concerned, the results show that deltamethrin (0.3 or 0.6 µg/L) significantly affects rainbow trout.

A few experiments have dealt with the unfavorable effects of pesticides on fish gene expression, but this is the first one performed with deltamethrin. Erdoğan et al. (11) reported that 1.6 mg/L DDVP (2, 2 Dichlorovinyl Dimethyl Phosphate) increased the HSP70 gene expression in rainbow trout, as we found with deltamethrin. Kumar et al. (13) suggested that deltamethrin affects the overall physiological profile in fish with particular reference to energy metabolism and hematological characteristics. In catfish (*Heteropneustes fossilis*) deltamethrin caused a significant increase in erythrocyte counts, but a small decrease in hemoglobin, mean cell volume, mean cell hemoglobin, and hematocrit (13). Shih and McDonough (14) suggested that the primary effect of pesticides (dichlorvos and other organophosphates) on vertebrate and invertebrate organisms is the inhibition of the enzyme acetylcholinesterase (AChE), which is responsible for terminating the transmission of the nerve impulse. McHenery et al. (15) reported that dichlorvos exposure affected acetylcholinesterase (AChE) activity in mussels (*Mytilus edulis* L.).

Many researchers have reported that pesticides have various unfavorable effects on aquatic organisms (16-22); however, their effect on gene expression remains unclear. We have found that deltamethrin adversely affects gene expression in rainbow trout, and it may also change the organism's gene sequence. This response may vary according to the fish species and other chemicals, and so further studies are needed.

References

1. Aldridge, W.N.: An assessment of the toxicological properties of pyrethroids and their neurotoxicity. Crit. Rev. Toxicol., 1990; 21: 89-104.
2. Parvez, S., Raisuddin, S.: Copper modulates non-enzymatic antioxidants in the freshwater fish *Channa punctata* (Bloch) exposed to deltamethrin. Chemosphere, 2006; 62: 1324-1332.
3. Ural, M.S., Saglam, N.: A study on the acute toxicity of pyrethroid deltamethrin on the fry rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792). Pestic. Biochem. Phys., 2005; 83: 124-131.
4. Pimpão, C.T., Zampronio, A.R. Silva de Assis, H.C.: Effects of deltamethrin on hematological parameters and enzymatic activity in *Ancistrus multispinis* (Pisces, Teleostei). Pestic. Biochem. Phys., 2007; 88: 122-127.
5. Cengiz, E.I.: Gill and kidney histopathology in the freshwater fish *Cyprinus carpio* after acute exposure to deltamethrin. Environ. Toxicol. Phar., 2006; 22: 200-204.
6. Srivastav, A.K., Srivastava, S.K., Srivastav, S.K.: Impact of deltamethrin on serum calcium and inorganic phosphate of freshwater catfish, *Heteropneustes fossilis*. B. Environ. Contam. Tox., 1997; 59: 841-846.
7. Hill, I.R.: Aquatic organisms and pyrethroids. Pestic. Sci., 1989; 27: 429-457.
8. Haya, K.: Toxicity of pyrethroid insecticide to fish. Environ. Toxicol. Chem., 1989; 8: 381-391.
9. Viran, R., Unlü Erkoc, F., Polat, H., Kocak, O.: Investigation of acute toxicity of deltamethrin on guppies (*Poecilia reticulata*). Ecotoxicol. Environ. Saf., 2003; 55: 82-85.
10. Velíšek, J., Jurčíková, J., Dobšíková, R., Svabodová, Z., Piačková, V., Máchová, J., Novotný, L.: Effects of deltamethrin on rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Pharmacol., 2007; 23: 297-301.
11. Erdoğan, O., Atamanalp, M., Şişman, T., Aksakal, E., Alak, G.: Effects of 2,2-dichlorovinyl dimethyl phosphate (DDVP) on HSP70 gene expression in rainbow trout. Isr. J. Aquacult.-Bamid., 2007; 59: 230-234.
12. Ojima, N., Yamashita, M., Watabe, S.: Quantitative mRNA expression profiling of heat-shock protein families in rainbow trout cells. Biochem. Biophys. Res. Commun., 2005; 329: 51-57.
13. Kumar, S., Lata, S., Gopal, K.: Deltamethrin induced physiological changes in freshwater cat fish, *Heteropneustes fossilis*. B. Environ. Contam. Tox., 1999; 62: 254-258.
14. Shih, T.M., McDonough, J.H. Jr.: Neurochemical mechanisms in soman induced seizures. J. Appl. Toxicol., 1997; 17: 255-264.
15. McHenery, J.G., Linley-Adams, G.E., Moore, D.C., Rodger, G.K., Davies, I.M.: Experimental and field studies of effects of dichlorvos exposure on acetylcholinesterase activity in the gills of the mussel, *Mytilus edulis* L. Aquat. Toxicol., 1997; 38: 125-143.

16. Atamanalp, M., Keleş, M.S., Haliloğlu, H.İ., Aras, M.S.: The effects of cypermethrin (a synthetic pyrethroid) on some biochemical parameters (Ca, P, Na and TP) of rainbow trout (*Oncorhynchus mykiss*). Turk. J. Vet. Anim. Sci., 2002; 26: 1157-1160.

17. Atamanalp, M., Yanik, T., Haliloglu, H.I., Aras, M.S.: Alterations in the heamotological parameters of rainbow trout, *Oncorhynchus mykiss*, exposed to Cypermethrin. Isr. J. Aquacult.-Bamid., 2002; 54: 99-103.

18. Atamanalp, M., Yanık, T.: Alterations in hematological parameters of rainbow trout (*Oncorhynchus mykiss*) exposed to mancozeb. Turk. J. Vet. Anim. Sci., 2003; 27: 1213-1217.

19. Reddy, P.M., Bashamohideen M.: Fenvalarate and Cypermethrin induced changes in the hematological parameters of *Cyprinus carpio*. Acta. Hydrochim. Hydrobiol., 1989; 17: 101-107.

20. Santhakumar, M., Balaji, M., Ramudu, K.: Effect of sublethal concentrations of monocrotophos on erythropoietic activity and certain hematological parameters of fish *Anabas testudineus* (Bloch). B. Environ. Contam. Tox., 1999; 63: 379-384.

21. Shakoori, A.R., Iqbal, M.J., Mughal, A.L., Ali, S.S.: Drastic biochemical changes following 48 hours exposure of Chinese grass carp *Ctenopharyngodon idella*, to sublethal doses of mercuric chloride. Proc. 1st Symp. Fish and Fisheries, Pakistan, 1991; 81-98.

22. Shakoori, A.R., Mughal, A.L., Iqbal, M.J.: Effects of sublethal doses of fenvalarate (a synthetic pyrethroid) administered continuously for four weeks on the blood, liver and muscles of a freshwater fish, *Ctenopharyngodon idella.* B. Environ. Contam. Tox., 1996; 57: 487-494.