

## Alterations in Hematological Parameters of Rainbow Trout (*Oncorhynchus mykiss*) Exposed to Mancozeb

Muhammed ATAMANALP, Telat YANIK  
Atatürk University, Faculty of Agriculture, Department of Fisheries, Erzurum - TURKEY

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**Abstract:** Rainbow trout reared in fresh water were exposed to a sublethal concentration of mancozeb (1/2 of  $LC_{50} = 1.1$  mg/l) at 24 h intervals, for 3 weeks in tanks containing 780 l water under natural light conditions with a constant flow ( $1.5$  l  $min^{-1}$ ) of aerated dechlorinated tap water at 9-11 °C and with no recirculation. Approximately 2 cc venous blood was drawn from each subject (after weekly exposure) for the determination of hematological parameters, using heparin as an anticoagulant. Hematological parameters were investigated weekly.

The findings of this study showed that mancozeb had some effect on the hematological parameters of rainbow trout. It was determined that the use of the pesticide caused a slight increase in red blood cell (RBC) numbers and a decrease in hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), packed cell volume (PCV), mean corpuscular volume (MCV) and white blood cell (WBC) levels. Significant decreases ( $P < 0.05$ ) in Hb content and in MCH were observed during exposure to the pesticide. No significant differences were observed in the levels of RBC, MCHC, PCV, MCV and WBC ( $P > 0.05$ ). The RBC/WBC level increased due to the decrease in WBC during the study.

**Key Words:** Mancozeb, hematology, rainbow trout, pesticide

### Mancozeb'e Maruz Bırakılan Gökkuşáđı Alabalıklarının (*Oncorhynchus mykiss*) Hematolojik Parametrelerindeki Deđişimler

**Özet:** Tatlı suda yetiştirilen gökkuşáđı alabalığı mancozeb'in sublethal dozuna (1/2  $LC_{50} = 1,1$  mg/l) 780 l su hacmine sahip, resirkülasyonsuz tanklarda doğal ışıklandırma şartlarında, sabit su akışında (1,5 l dak-1), 9-11 °C'de, havalandırılmış ve klordan arındırılmış şebeke suyunda 24 saat aralıklarla 3 hafta maruz bırakılmıştır. Hematolojik parametrelerin tespiti için antikoagülant olarak heparin kullanılarak balıklardan yaklaşık 2 cc kan alınmıştır. Hematolojik parametreler her hafta incelenmiştir.

Mevcut çalışmanın sonuçları mancozeb'in gökkuşáđı alabalığının hematolojik parametreleri üzerinde bazı etkilere sahip olduğunu göstermiştir. Pestisit kullanımının RBC (eritrosit sayısı)'de hafif bir artışa, Hb (hemoglobin), MCH (eritrosit başına düşen ortalama hemoglobin miktarı), MCHC (eritrosit başına düşen ortalama hemoglobin konsantrasyonu), PCV (hematokrit), MCV (ortalama eritrosit hacmi) ve WBC (lökosit sayısı) seviyelerinde ise düşüşe neden olduğu tespit edilmiştir. Hemoglobin muhtevası ve MCH seviyesinde pestisite maruz bırakılma esnasında önemli derecede düşüş gözlenmiştir ( $P < 0,05$ ). RBC, MCHC, PCV, MCV ve WBC seviyelerinde önemli deđişiklikler belirlenmemiştir ( $P > 0,05$ ). RBC/WBC seviyesi deneme esnasınca WBC'deki düşüşten dolayı artmıştır.

**Anahtar Sözcükler:** Mancozeb, hematoloji, gökkuşáđı alabalığı, pestisit

### Introduction

Synthetic pyrethroids such as azodrin, cypermethrin, deltamethrin, fenvalerate and mancozeb are used to protect many fruit, vegetable, nut and field crops against a wide spectrum of fungal diseases and rust. They are also used for seed treatment of cereal grains, cotton, tomatoes etc., and for the control of undesirable insects in order to increase agricultural production. However,

they have harmful effects on aquatic environments and organisms (1-4).

Mancozeb is not toxic to rats (5,6), honeybees or rabbits (7) at given oral doses of  $LD_{50}$ . However, it is moderately to highly toxic to fish and aquatic organisms (7-10). It has been reported that mancozeb is not mutagenic (11) or is only weakly mutagenic (12), and that is not carcinogenic, although it has caused cancer in

experimental animals exposed to high doses (11,13). Its main target is the thyroid gland and it can be excreted almost completely from the body in 96 h.

Since hematological parameters reflect the poor condition of fish more quickly than other commonly measured parameters, and since they respond quickly to changes in environmental conditions (14), they have been widely used for the description of healthy fish (15), for monitoring stress responses (16,17) and for predicting systematic relationships and the physiological adaptations of animals.

Hematological changes in some fish exposed to various toxicants, i.e. in *Cyprinus carpio* exposed to cypermethrin and fenvalerate (9), in *Tilapia mossambica* exposed to cadmium chloride (18), in *Ctenopharyngodon idella* exposed to fenvalerate (19) and in *Heteropneustes fossilis* exposed to deltamethrin (20), have been investigated. However, there are no data available about the effects of mancozeb on the hematological parameters of rainbow trout.

The objective of the present study was to determine the effects of mancozeb on the hematological parameters of rainbow trout (*Oncorhynchus mykiss*) in order to show the toxic effects of the pesticide.

## Materials and Methods

A group of 20 fish with an average weight of  $170 \pm 14$  g, reared in well water with a constant temperature of  $8.5 \text{ }^\circ\text{C}$  at our farm, located at the Research and Extension Center in Atatürk University, was transferred to the Central Laboratory in the Aquarium Fish Rearing Facility and was exposed to a sublethal dose of 1.1 ppm (equal to  $1/2 \text{ LC}_{50}$ ) of mancozeb at 24 h intervals for 3 weeks in circular fiberglass tanks 780 l in volume (100 cm diameter and 100 cm depth) under natural light conditions with a constant flow ( $1.5 \text{ l min}^{-1}$ ) of aerated dechlorinated tap water at  $9\text{-}11 \text{ }^\circ\text{C}$  and with no recirculation. They were acclimatized for week in April, 2000. The dissolved oxygen and pH levels and total water hardness were 8-9 ppm, 7.8 and 102 mg in  $\text{CaCO}_3$ , respectively. The tanks were aerated with an air pump.

Ten fish were placed into each of 2 tanks, one for testing the mancozeb and the other as the control. At the end of each weekly exposure, 4 fish from the control tank and 4 fish from the treatment tank (2,21) were taken

out and their blood was subjected to hematological analysis (2,3,18,22).

Approximately 2 cc venous blood was drawn from each subject (after weekly exposure) for the determination of hematological parameters, using heparin as an anticoagulant, and for the estimation of the red blood cell (RBC) count (23,24), the total white blood cell (WBC) count (23,24), the hemoglobin (Hb) concentration (17) and the packed cell volume (PCV) (25), whereas the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC) were calculated according to Reddy and Bashamohideen (9).

## Statistical analysis

The results are presented as means  $\pm$  SD. Differences between parameters were analyzed by one-way analysis of variance (ANOVA), and significant means were subjected to a multiple comparison test (Duncan) at the  $\alpha = 0.05$  level (26).

## Results

The blood values of rainbow trout, namely the Hb, MCH, MCHC, MCV, PCV and WBC levels, decreased, whereas the RBC level increased slightly with exposure to the pesticide (Table). No significant changes were observed in the measured variables of the control fish ( $P > 0.05$ ). However, a significant decrease in Hb content ( $F = 5.16$ ) and in MCH ( $F = 3.89$ ) was observed with exposure to mancozeb ( $P < 0.05$ ). No significant differences were observed in the levels of MCV ( $F = 2.40$ ), MCHC ( $F = 1.41$ ), PCV ( $F = 1.45$ ), RBC ( $F = 1.22$ ) and WBC ( $F = 2.09$ ) ( $P > 0.05$ ).

## Discussion

Significant decreases occurred in Hb levels after exposure to a sublethal dose, which may impair oxygen supply to various tissues, thus resulting in a slow metabolic rate and low energy production (21). The significant decrease in the Hb concentration may also be due to either an increase in the rate at which the Hb is destroyed or to a decrease in the rate of Hb synthesis (9). Similarly, in freshwater catfish (*H. fossilis*), the Hb (%) decreased after 30 days exposure to deltamethrin (20).

Table. Changes in the hematological parameters of rainbow trout exposed to mancozeb at a 24 h interval for 3 weeks. Superscripts in a row with different letters represent significant differences ( $P < 0.05$ ). Each value is the mean  $\pm$  SD of 4 individual observations. The values in the parentheses are percentage changes over the control ( $1/2 LC_{50} = 1.1$  ppm).

	1st Week		2nd Week		3rd Week	
	Control	Mancozeb	Control	Mancozeb	Control	Mancozeb
RBC ( $10^6/\text{mm}^3$ )	0.67 $\pm$ 0.05 <sup>a</sup>	0.69 $\pm$ 0.01 <sup>a</sup> (-2.99)	0.68 $\pm$ 0.02 <sup>a</sup>	0.70 $\pm$ 0.01 <sup>a</sup> (-2.94)	0.68 $\pm$ 0.04 <sup>a</sup>	0.72 $\pm$ 0.01 <sup>a</sup> (-5.88)
Hb (g/100 ml)	6.86 $\pm$ 0.54 <sup>a</sup>	4.68 $\pm$ 1.38 <sup>b</sup> (-31.78)	6.82 $\pm$ 0.46 <sup>a</sup>	5.65 $\pm$ 1.04 <sup>ab</sup> (-17.16)	6.89 $\pm$ 0.30 <sup>a</sup>	6.90 $\pm$ 0.64 <sup>a</sup> (-0.15)
PCV (%)	44.75 $\pm$ 3.21 <sup>a</sup>	40.25 $\pm$ 11.65 <sup>a</sup> ( - 10.06)	45.00 $\pm$ 2.71 <sup>a</sup>	38.75 $\pm$ 9.35 <sup>a</sup> ( - 13.89)	44.50 $\pm$ 3.78 <sup>a</sup>	35.25 $\pm$ 3.30 <sup>a</sup> ( - 20.79)
(MCV ( $\mu\text{m}^3$ ))*	663.48 $\pm$ 22.84 <sup>a</sup>	582.18 $\pm$ 164.13 <sup>a</sup> ( - 12.25)	659.46 $\pm$ 34.77 <sup>a</sup>	553.31 $\pm$ 132.73 <sup>a</sup> ( - 16.10)	653.66 $\pm$ 46.54 <sup>a</sup>	488.19 $\pm$ 49.11 <sup>a</sup> ( - 25.31)
MCH (pg)*	102.69 $\pm$ 16.22 <sup>a</sup>	68.01 $\pm$ 20.37 <sup>b</sup> (-33.77)	100.17 $\pm$ 10.31 <sup>a</sup>	80.57 $\pm$ 14.49 <sup>ab</sup> (-19.57)	101.72 $\pm$ 11.16 <sup>a</sup>	95.62 $\pm$ 10.29 <sup>a</sup> (-6.00)
MCHC (g per 100 ml)*	15.46 $\pm$ 2.36 <sup>a</sup>	13.21 $\pm$ 7.38 <sup>a</sup> (-14.55)	15.23 $\pm$ 1.94 <sup>a</sup>	14.83 $\pm$ 2.10 <sup>a</sup> (-2.63)	15.61 $\pm$ 2.06 <sup>a</sup>	19.68 $\pm$ 2.46 <sup>a</sup> (-26.07)
WBC ( $10^4/\text{mm}^3$ )	7.46 $\pm$ 1.06 <sup>a</sup>	6.68 $\pm$ 0.13 <sup>a</sup> ( - 10.46)	7.42 $\pm$ 1.08 <sup>a</sup>	6.51 $\pm$ 0.12 <sup>a</sup> ( - 12.26)	7.47 $\pm$ 1.04 <sup>a</sup>	6.26 $\pm$ 0.12 <sup>a</sup> ( - 16.20)

\*MCV = [PCV (%) / RBC ( $10^6$ )]  $\times$  10  $\mu\text{m}^3$ ; MCH = [Hb (g) / RBC ( $10^6$ )]  $\times$  10 pg; MCHC = [Hb (g) / PCV (%)]  $\times$  100 g per 100 ml

RBC: Red blood corpuscle count; Hb: Hemoglobin; PCV: Packed cell volume; MCV: Mean corpuscular volume; MCHC: Mean corpuscular hemoglobin; WBC: White blood cell count

MCH was significantly decreased in *C. idella* after 48 h exposure to fenvalerate (19). The decreased MCH and MCV levels may be a sign of hypochromic microcytic anemia (2). In contrast to our findings, there were increases in Hb and MCH values in *T. mossambica* exposed to cadmium chloride (18) and *C. idella* exposed to sublethal doses of mercuric chloride (27) and in *O. mykiss* exposed to cypermethrin (22).

Although no significant differences were observed in RBC, it increased slightly during the exposure period. Atamanalp et al. (22) observed a significant increase in the erythrocyte (RBC) count in *O. mykiss* exposed to cypermethrin. Similar findings were also observed in *C. idella* after exposure to fenvalerate (2) and in *H. fossilis* after exposure to deltamethrin (20). On the other hand a decline in RBC was observed in *T. mossambica* after exposure to sumithion and sevin (28), in *C. carpio* after 48 h exposure to cypermethrin (9), and in *A. testudineus* exposed to azodrin (3).

RBC indices such as MCV and MCHC fell during prolonged exposure to mancozeb. The decrease in other

hematological parameters is attributable to reduced MCV (21,29,30). In spite of the increase in the RBC count, a decrease in PCV may show the extent of the shrinking cell size due to insecticide intoxication (21). Decreases in PCV and MCV show that mancozeb treatment may interfere with the normal physiology of RBC. Exposure to danitol and fenvalerate in *C. idella* caused a reduction in the hematocrit (PCV) value (2,21), whereas an increase was observed in *T. mossambica* exposed to mercury chloride (18).

A decrease was reported in the WBC count in the blood of *C. idella* exposed to fenvalerate (2). After insecticide intoxication the RBC/WBC ratio increased. That may be caused by decreased WBC (2). Environmental changes may also cause differences in the physiological and chemical properties of fish blood (31,32).

Our findings showed that mancozeb had some effect on the hematological parameters of rainbow trout. Although the  $LC_{50}$  dose of mancozeb is 2.2 mg/l, it is shown that the  $1/2 LC_{50}$  dose examined in this study also

causes important changes in the hematological properties of rainbow trout. Therefore the use of pesticides in farmlands should be controlled to prevent possible contamination by leaching into the aquatic environments. In this way aquatic organisms could be protected from

these kinds of toxic chemicals. On the other hand, the response to any kind of stress sources such as pesticides may differ in some fish, and therefore further work is needed to determine the responses of different fish species.

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