

# LABORATORY OF ICHTHYLOGY, FISH DISEASES & AQUACULTURE

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## USE OF OREGANO ESSENTIAL OIL FOR THE CONTROL OF PARASITE AND MICROBIAL DISEASES OF MEDITERRANEAN FISH

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## Introduction

- Recent aquaculture growth in Greece , new fish species – increased prevalence of pathogens
- Antiparasitic drugs are limited, no licensed drugs, no MRL's
- Need for low impact substances

**In recent years, especially after the ban on the use of antibiotics in animal feed in the European Union since January 2006, essential oils have emerged as a potential alternative to antibiotics in animal feed.**

# Essential Oils

## What do we really know about essential oils and their antimicrobial properties?

- EOs can comprise more than **sixty** individual components.
- Major components can constitute up to 85% of the EO, whereas other components are present only as a trace.
- The **phenolic components** are responsible for the antibacterial properties of EOs. There is some evidence that **minor components** have a critical part to play in antibacterial activity, possibly by producing **a synergistic effect** between other components. This has been found to be the case for sage, certain species of *Thymus* and *Oregano* (Paster et al., 1995).
- An advantage of essential oils over antibiotics may be that **bacteria do not develop resistance** to essential oils. In addition, some oils actually stimulate immune function.



# *Origanum vulgare* (a member of the Labiatae family)

- Oregano essential oil is produced from the oregano plant through the process of **steam distillation**.
- Oregano essential oil contains the following components:
- **carvacrol** (share 40–70%)
- gamma-terpinene (8–10%)
- p-cymene (5–10%)
- alpha-pinene
- myrcene
- thymol
- flavonoids



# *Origanum vulgare*

## Oregano Oil is Antibacterial and Antimicrobial

According to a recent study published in the *Journal of Agricultural and Food Chemistry*, one of the most promising oregano oil benefits is its high **antimicrobial and antibacterial** activity. In addition, the study found that oregano essential oil has antimicrobial properties which can counteract food borne pathogens, such as *Listeria monocytogenes*.

## Oregano Oil is Anti-Parasitic

Oregano oil has been used to effectively treat internal parasites, including *Blastocystis hominis*, a small parasite that infects the intestinal tracts of humans. A study published in *Phytotherapy Research*.

Also, **antiviral actions** of Origanum oil against RNA and DNA viruses have been reported

**Antioxidant**



# STUDIES PERFORMED

- *Preliminary study: In vitro* activity *V. anguillarum*, *V. alginolyticus* 1995 (Athanassopoulou et al.)
- Myxosporida spp. infections-sea bream, *D. puntazzo* 1997(Athanassopoulou et al.)
- *Ceratomyxa oestroides* infections- sea bass-2008 (Yiagnisis et al)
- *Listonella (Vibrio)sp.* Infections –sea bass-2008(Yiagnisis et al)
- *Aeromonas sombria* infections- carp- 2009(Panopoulos et a)
- *Photobacterium damsella subsp. Piscicida* / Histological lesions / sea bream - 2010(Yiagnisis et al)



***Origanum*** essential oils have exhibited differential degrees of protection against **myxosporean** infections in **gilthead** and **sharp snout sea bream** tested in land-based & field experimental facilities

*Myxobolus* sp. In *D. puntazzo*

*Polysporoplasma sparis* in *Sparus aurata* L

• *Myxidium leei* (*Enteromyxum leei*) in sharp snout sea bream, *Diplodus puntazzo* C, *Sparus aurata* L



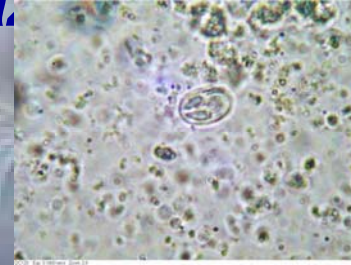
**Athanassopoulou F, Karagouni E, Dotsika E, Ragias V, Tavla J, Christofilloyanis P (2004). Efficacy and toxicity of orally administrated anticoccidial drugs for innovative treatments of *Polysporoplasma sparisi* infection in *Sparus aurata* L., *J.Appl. Ichthyol.* 20,345–354**

**Athanassopoulou F, Karagouni E, Dotsika E, Ragias V, Tavla J, Christofilloyanis P, Vatsos I (2004). Efficacy and toxicity of orally administrated anticoccidial drugs for innovative treatments of *Myxobolus* sp. infection in *Puntazzo puntazzo*, *Dis. Aquat.Org.* 62, 217–226)**

**E. Karagouni,\* , F. Athanassopoulou, A. Lytra, C. Komis, E. Dotsika**  
**Antiparasitic and immunomodulatory effect of innovative treatments against *Myxobolus* sp. infection in *Diplodus puntazzo*. *Veterinary Parasitology* 134, 215-228.**

**Karagouni, E., Athanassopoulou F., P. Tsagozis, E. Ralli, Th. Moustakareas, K. Lytra and E. Dotsika (2005a). The impact of a successful anti-myxosporean treatment on the phagocyte functions of juvenile and adult *Sparus aurata* L. *International Journal of Immunopathology and Pharmacology* 18(1) 121-132**

# Efficacy and toxicity of orally administered anticoccidial drugs for innovative treatments of *Myxobolus* sp. infection in *Puntazzo puntazzo* Dis. Aquat.Org. 62, 217–226



“Athanasopoulou F, Karagouni E, Dotsika E, Ragias V, Tavla J, Christofilloyanis P, Vatsos I (2004).

## EXPERIMENTS

- **In the land based experiment** 25g and 50g fish infected with *P. sparis* were treated with Oreganum essential oils, Toltrazuril with propylene glycol, Amprolium, and a combination of Salinomycin 12%+Amprolium(SA).
- **In the field trial**, 15 and 155g *Sparus aurata* infected with the same parasite were treated with SA, Oreganum essential oils and Fumagillin.

<b>DRUG</b>	<b>ABBREVIATION</b>	<b>COMMERCIAL FORM Composition</b>	<b>TRADE NAME</b>	<b>COMPANY</b>
<b>Fumagillin (dicyclohexylamine)</b>	F (F1-F6)	20mg.g <sup>-1</sup> Powder -oral sol.	Fumidil®	-CEVA
<b>Salinomycin (Salinomycin sodium )</b>	S	Medicated Premix 12%	Salinomycin	<b>Haechst</b>
<b>Amprolium</b>	Amp	Medicated Premix 50%	Amprolium	-Veterin
<b>Oreganum essential oils</b>	R (R1- R2)	5% oil sol.	<b>Orego-Stim ® (Ecodiar Liquid) Ecofarm Hellas</b>	
<b>Toltrazuril</b>	T (T1-T2)	25mg.ml <sup>-1</sup> oral sol	Baycox ®	-Bayer
<b>ESB-3</b>	E3	30% powder	ESB-3	-Ciba-Geigy

## Experimental conditions, protocols, efficacy and resultant mortality of *D. puntazzo* anti - myxosporean drugs. Fish averaged 20g weight – inland experiments

	C	F-1	F-2	S	SA	R-1	R-2	Tp-1*	Tp-2*
<b>Regimen</b>	-	6mg Kg <sup>-1</sup> x6wk	6mg Kg <sup>-1</sup> x 3wk	70g T <sup>-1</sup> x30 d rep 5d	60g T <sup>-1+</sup> 100g T <sup>-1</sup> x30 d	8ml / Kg BW <sup>-1</sup>	12ml 5Kg BW <sup>-1</sup>	600ml To <sup>-1</sup> x2d rep 15d	600ml To <sup>-1</sup> x2d/ 3d-off / 2d-on/ Rep 15d
<b>Sampling interval (d)</b>	weekly	weekly	weekly	weekly	weekly	weekly	weekly	weekly	weekly
<b>Expt duration (d)</b>	70	70	70	70	70	70	70	70	70
<b>Prevalence (%)</b>									
<b>init. Prevalence</b>	15	15	15	15	15	15	15	15	15
<b>final prevalence</b>	33	11	12	14	5	9	8	10	9
<b>Cumulative Mortality(%)</b>	10	1	2	0	1	5	3	3	0

F1, 2: Fumagillin; S: Salinomycin; SA: Salinomycin 12% + Amprolium 50%; R1, 2: Oregano oils; Tp1, 2: Toltrazuril + Propylene glycol; \* : minimal concentration of Propylene glycol to improve taste; To: tone of biomass; rep: repeat whole scheme

## Experimental conditions, protocols, efficacy and resultant mortality of *D.puntazzo* anti - myxosporean drugs. Fish averaged 165g weight – field trials in cages

Drug	C	SA	R	F
Regimen	-	60g To <sup>-1</sup> +100g To <sup>-1</sup> x 30 d	8ml 5kg BW <sup>-1</sup> x30 d	6mg kg BW <sup>-1</sup> x6 wk
Sampling	weekly	weekly	weekly	weekly
Exper Duration (d) Prevalence (%)	73	73	73	73
init. Prevalence	15.6	15.6	15.6	15.6
final prevalence	95.2	9.5	50	50
Cumulative mortality	18.7	10.5	15.5	19.6

SA: Salinomycin 12% + Amprolium 50%; R1, 2: Oregano oils; F1, 2: Fumagillin; To: tone of biomas

**“Efficacy and toxicity of orally administered anti-coccidial drugs for innovative treatments of *Polysporoplasma sparis* Sitja-Bobadilla & Alvarez-Pellitero 1885 infection in *Sparus aurata* L.”**

**F. Athanassopoulou, E. Karagouni, E. Dotsika, V. Ragias, J. Tavla, and P. Christofilloyanis**

**EXPERIMENTS**

- **In the land based experiment** 25g and 50g fish infected with *P. sparis* were treated with Origanum essential oils, Toltrazuril with propylene glycol, Amprolium, and a combination of Salinomycin 12%+Amprolium(SA).
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## Cross tabulation analysis of mortality (%) and prevalence (%) during field treatments for *P. sparis* infections of 155g *Sparus aurata*

Pharmaceutical treatment										
Mortality (%)	Prevalence (%)		C		SA		R		F	
0.8	75.0	<b>C</b>			<b>o</b>	<b>X</b>	<b>o</b>	<b>X</b>	<b>o</b>	<b>o</b>
0.0	5.0	<b>SA</b>					<b>o</b>	<b>X</b>	<b>o</b>	<b>X</b>
0.0	50.0	<b>R</b>							<b>o</b>	<b>o</b>
0.0	66.7	<b>F</b>								

**x=statistically significant** ( $P < 0.05$ ) from the untreated control fish

**o=not statistically significant** from the untreated control fish

C= untreated control fish

**Cross-tabulation analysis of mortality (%) and prevalence (%) during field treatments for *P. sparis* infections of 15g *Sparus aurata***

**Pharmaceutical treatment**

Mortality (%)	Prevalence (%)		C		SA		R		F	
6.7	50.0	C			X	X	O	O	O	O
0.0	10.0	SA					O	X	X	X
2.5	50.0	R							O	X
3.6	45.0	F								

**x=statistically significant ( $P < 0.05$ ) from the untreated control fish**

**o=not statistically significant from the untreated control fish**

**C= untreated control fish**

## Conclusions



- In all experiments, the most effective antiparasitic treatment was found with Amprolium + Salilomycin 12% , however not allowed anymore for animal treatment. Oregano oils second best (reduction up to 50% prevalence)
- No side effects, no toxicity
- All fish treated with Amprolium + Salilomycin 12% and Oregano oils showed a statistically significant secretion of NO-, IL-1 and TNF $\alpha$  compared to treated or untreated groups. In contrast, no differences on lysozyme levels were detected

**EFFECT OF DIETARY OREGANO ESSENTIAL OIL  
SUPPLEMENTATION ON COMBINED INFECTIONS BY  
PATHOGENIC BACTERIA - PARASITES (SEA LICE AND  
COPEPODS) IN EUROPEAN SEA BASS  
*DICENTRARCHUS LABRAX* L.**

Our first study was carried out to examine the effect of dietary of oregano essential oil on combined infection by the isopod sea lice *Ceratothoa oestroides*, and the experimentally administered (orally) pathogenic bacterium *Listonella* (*Vibrio*) *anguillarum*.



- A total of 180 sea bass with mean weigh  $150 \pm 30$  gr.
- Randomly divided into 2 groups with three replicates each
- Naturally infected by the isopod *Ceratothoa oestroides*
- One group was given a commercial diet and served as control while the other was fed with the commercial diet supplemented with oregano essential oil *Origanum vulgare*, 100% pure, at a dose of 1ml essential oil/100 gr. food.



### Experimental infection

100 days after rearing, both groups were experimentally infected by the bath administered bacterium *Listonella (Vibrio) anguillarum* at a level of  $0,6 \cdot 10^7$  CFU/ ml sea water. A negative control of the experiment also was used.



## RESULTS

Results showed that dietary oregano oil at the inclusion level of 0.9-1ml/100gr food has an important antiparasitic-antibacterial effect.

Fifteen days after the experimental infection, supplementation with dietary oregano oil resulted in **34% improvement of the mean survival rate of sea bass treated** and in **50% decrease of parasite presence.**





*The effect of oregano oil on the resistance of carp  
(*Cyprinus carpio* L) experimentally infected with  
*Aeromonas sombria**

*S. Panopoulos, MSc Thesis, University of Thessaly, 2009*

# EXPERIMENTS

- Fish were divided in 4 groups of 30 fish each:
- Group 1 was supplemented with oregano essential oil (Orego-stim with 5% oregano oil) at a dose of 12ml/5g biomass for 6months prior to the experimental infection.
- Group 2 was supplemented with the same amount of oregano oil on the day of the experimental infection with *A. sombria* and for the whole duration of the experiment (224 days, positive control).
- Group 3 was only infected with *A. sombria* and had no oil supplementation. Group 4 was neither supplemented nor infected (negative control).
- Infection with ATCC *A. sombria* was performed by adding 107 CFU/ml of water in a 10L separate tank for 40 min with adequate aeration.
- Four samplings were taken during the experiment for necropsy, microbiological and histopathological examination.
- Mortalities were monitored daily and moribund fish underwent a full laboratory examination.

## RESULTS

- Results showed that group 1 was very tolerant to the *A. sombria* infection in terms of clinical signs, mortality and growth rate.
- Groups 3 and 4 had similar results in terms of total mortality (43%); mortality in group 1 was statistically lower (26,7%,  $P < 0.05$ ).
- Control group 4 had no mortalities and no signs of disease.
- Clinical signs of the infection appeared on 4th day onwards (fish were lethargic, anorexic with erythema on fins and scales) in groups 2 and 3. On the 8th day all fish in groups 1-3 had clinical signs.
- Smaller fish in groups 1-3 were more susceptible (more clinical signs) and had the highest mortality. A four day delay of appearance of clinical signs was shown in group 1 and generally symptoms were less severe. On 10th day fish in groups 2 and 3 did not feed anymore.
- Fish of group 1 had also a statistical better growth rate when compared to all other groups including group 4.

# CONCLUSION

Results showed that dietary oregano oil at the inclusion level of **12ml/5g** biomass has an important antibacterial effect and it also helps the growth rate. Larger fish were less susceptible to microbial challenge.

**EFFECT OF OREGANO ESSENTIAL OIL  
DIETARY SUPPLEMENTATION ON THE  
DEVELOPMENT OF HISTOPATHOLOGICAL  
LESIONS INDUCED BY *Photobacterium*  
*damselae* subsp. *piscicida* IN CULTURED  
(*Sparus aurata* L.)**



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## EXPERIMENT

- **400 unvaccinated sea bream juveniles ( $0.8 \pm 0.2$  SD g)**
- **3 groups , 3 tanks**
- **Group A: Oregano oil in food 10 ml kg<sup>-1</sup>**  
**0l Groups B & C same food without supplementation**
- **45 days experiment, histology samples**
- **30 fish from each tank were transferred to 9 experimental tanks 25L**

## Experimental infection

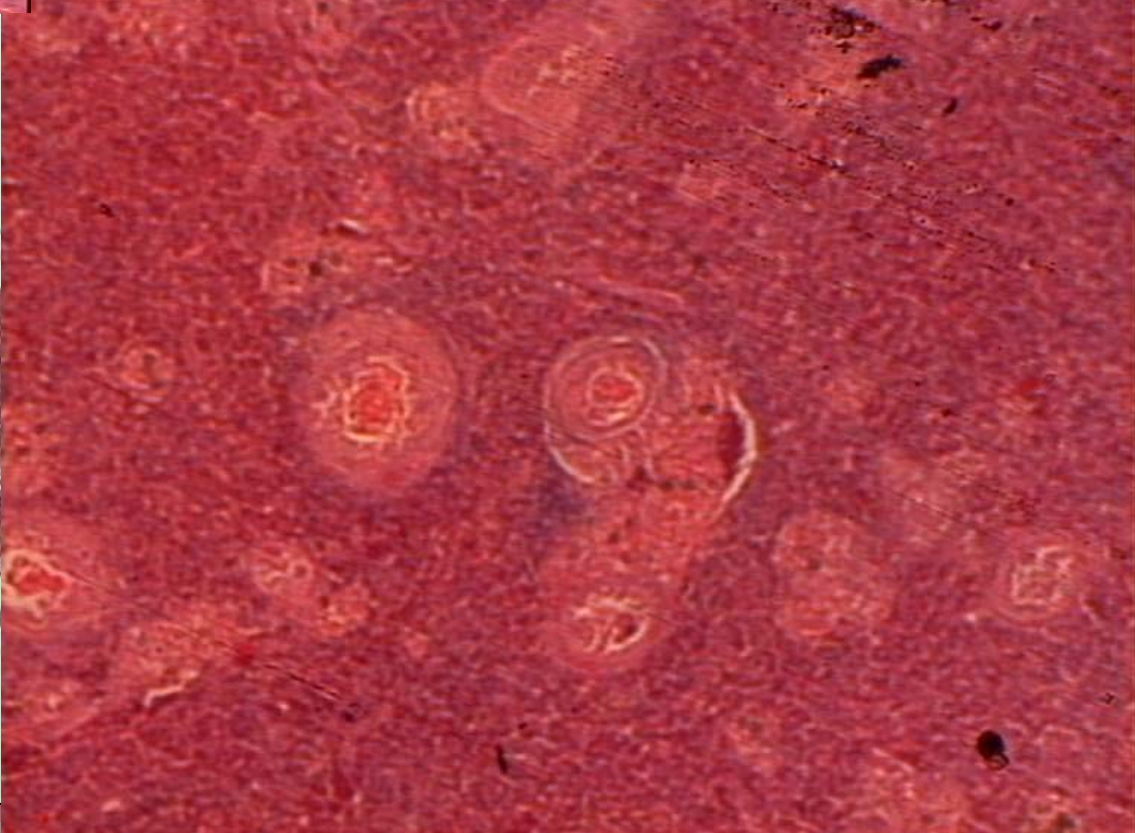
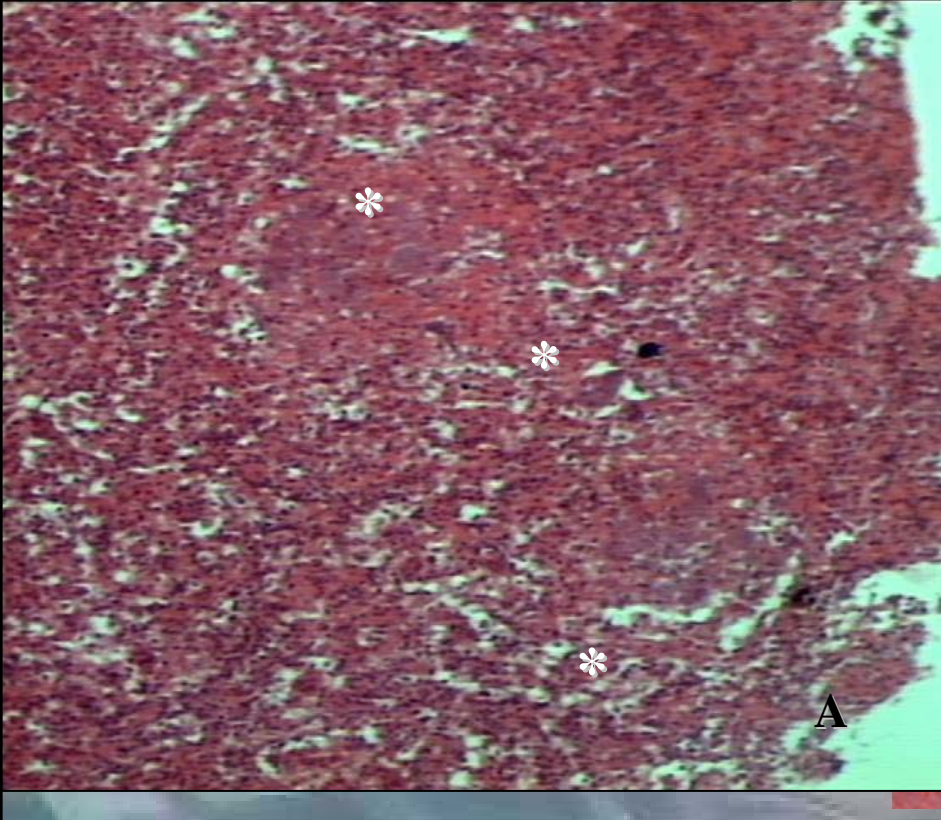
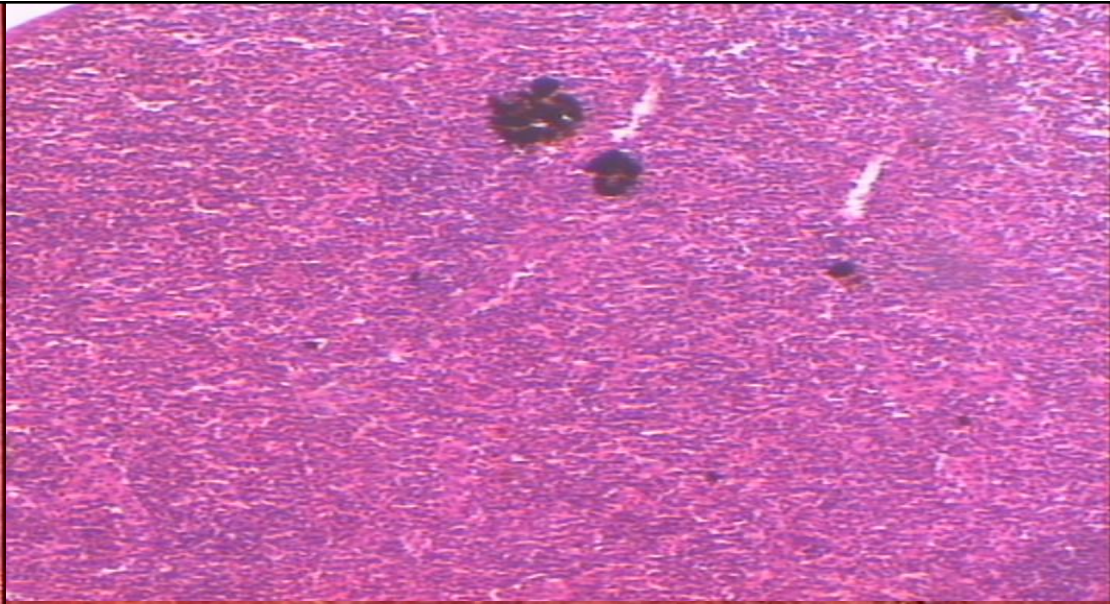
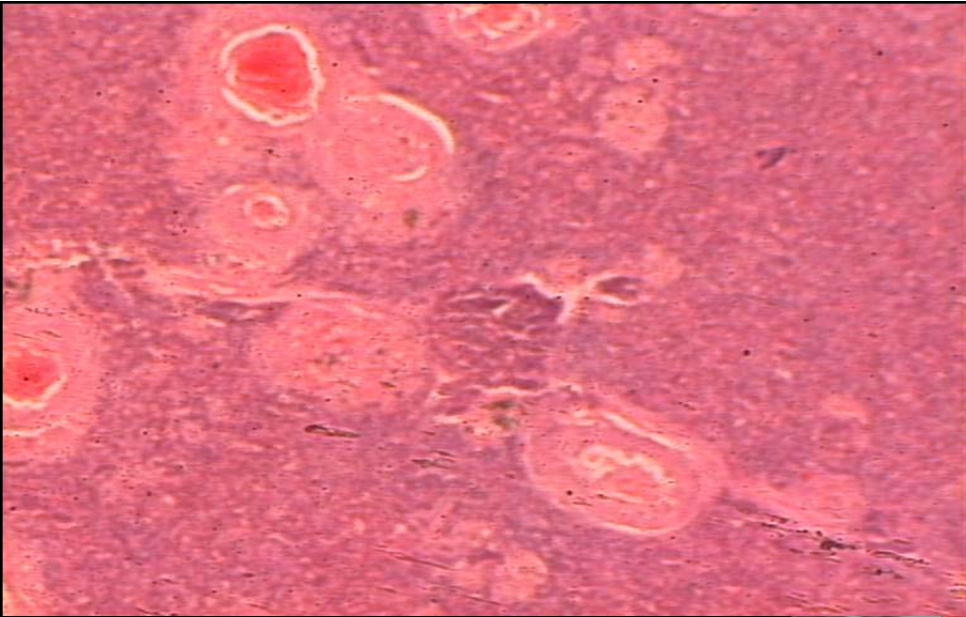


- **$10^7$  CFU *P. damsela* subsp. *piscicida* ml<sup>-1</sup> for 30 ' in tanks A & B**
- **Tank C = negative control**
- **17 days after sampling of spleen for histology (15 fish from each tank)**
- **SPSS General linear mode univariate program analysis**
- **Ct factor was the diet, not ct = % fish and random factor = tanks**

# Table I: % of fish with lesions in spleen in 15 fish sample from each tank

Experimental infection <i>P. damselae</i> subsp. <i>piscicida</i>						No infection		
Diet with oregano oil (Group A)			Diet without oregano oil (Group B)			Diet without oregano oil (Group C)		
Tank 1	Tank 2	Tank 3	Tank 4	Tank 5	Tank 6	Tank 7	Tank 8	Tank 9
40%	46.6%	46.6%	80%*	60%*	66.6%	0%	0%	0%

\*Granulomata formation.



## Results

- Fish fed with oregano oil, mean number of prevalence of histological lesions in spleen was statistically lower ( $44.4 \pm 3.81$  SD) compared to the mean number of fish not fed with oregano oil ( $68.86 \pm 10.19$  SD)
- In fish not fed with oregano oils multiple granulomata were also observed .
- Differences in fish in different tanks with lesions were statistically significant ( $P \leq 0.05$ )

## Conclusions



- **The experimental infection at this dose did not cause any histopathological lesions in sea bream fish**
- **Lesions were necrotic.**
- **The lower number of fish that developed granulomata may show retardation of the infection development due to oregano oil.**



***Thanks  
for your kind attention!***