



**ParaFishControl**

# **Alternative Treatments for *Saprolegnia***

**ParaFishControl Workshop “North European Fish Parasite  
Management Strategies in Aquaculture Farms”**

**Berlin, 9<sup>th</sup> October 2019**

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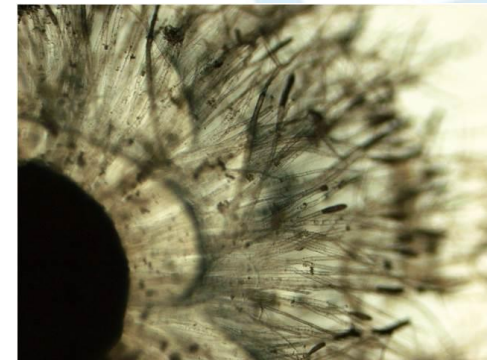
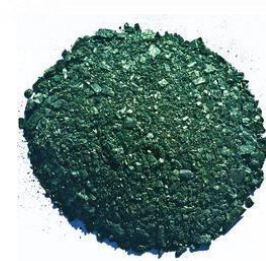
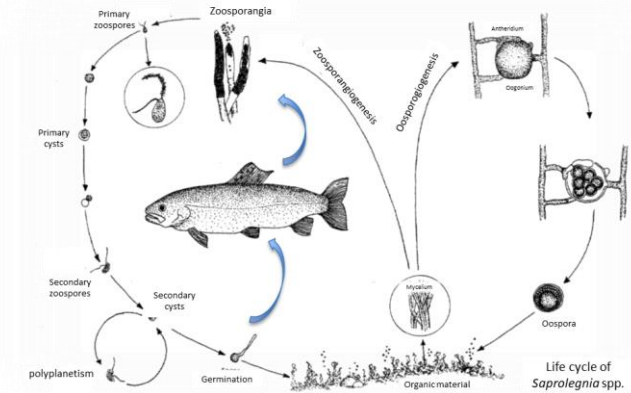
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# 1. Challenge and Impact

## Challenge

- *Saprolegnia* cause high mortality rates in freshwater fish culture
- The lack of alternative treatments with effectiveness comparable to the banned malachite green or other hazardous compounds, urges the identification of new molecules active against this pathogen
- This work focused on an *in vitro* screening aimed at assessing **new and alternative treatment strategies** directly targeting *Saprolegnia*, to replace the use of hazardous chemicals



# 1. Challenge and Impact

## Impact

➤ Selection of treatments that are:

- **environment-friendly**
- **safe for workers**
- **cost-effective**



- Expansion of the chemicals licensed in aquaculture for their use within an integrated parasite management (IPM) strategy
- Increased productivity and sustainability of aquaculture activities



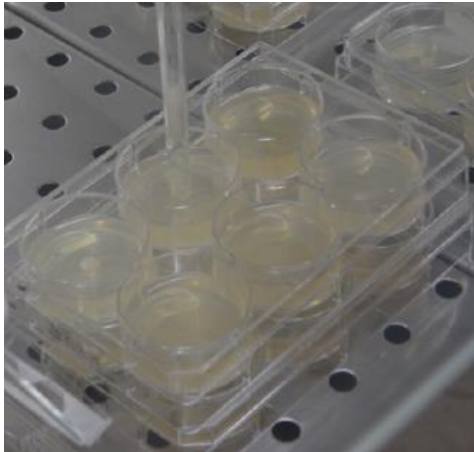
## 2. Our approach and our team

### *In vitro* SCREENING of 62 compounds

#### Methodology

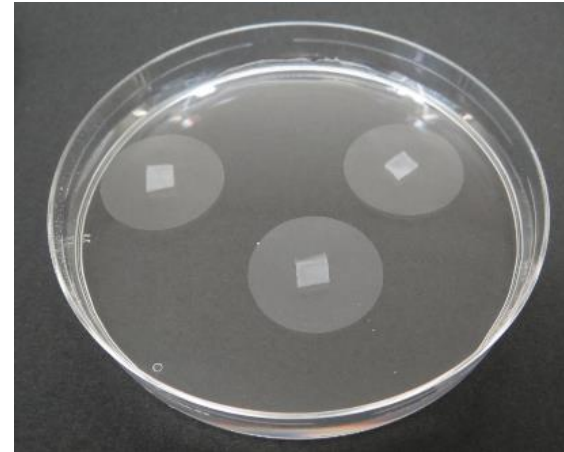
test in agar

(Minimum Inhibitory Concentration)



test in water

(Minimum Lethal Concentration)



#### Team involved in the development

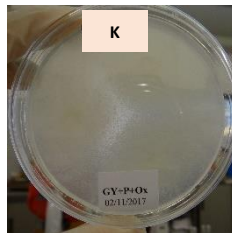
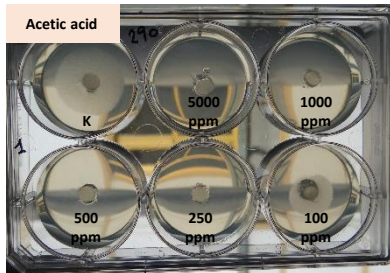
Laboratory of Mycology Staff, Department of Veterinary Medical Sciences,  
University of Bologna

# 3. Our proposed solution (I)

- rapid to implement treatments

**benzoic acid** and **iodoacetic acid** showed the lowest MIC/MLC, respectively;

**acetic acid** and **peracetic acid-based products**, particularly in combination with **hydrogen peroxide**, represent promising candidates for controlling saprolegniosis, due to their effectiveness associated with low environmental impact



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ORIGINAL ARTICLE

WILEY *Journal of Fish Diseases*

## In vitro activity of chemicals and commercial products against *Saprolegnia parasitica* and *Saprolegnia delica* strains

Perla Tedesco <sup>ID</sup> | Maria Letizia Fioravanti | Roberta Galuppi

Compound	MIC			MLC		
	A (ppm)	B (ppm)	C (ppm)	A (ppm)	B (ppm)	C (ppm)
Malachite green	5	5	5	5	5	5
Copper sulphate	250	250	250	1,000	5,000	5,000
Acetic acid	250	250	250	250	500	500
Benzoic acid	100	100	100	250	250	250
Boric acid	1,000	1,000	1,000	a	a	a
Iodoacetic acid	250	250	100	50	50	100
Lactic acid	500	500	5,000	500	500	1,000
Oxalic acid	500	500	1,000	1,000	5,000	5,000
Tartaric acid	500	500	1,000	a	a	a
Sodium percarbonate	a	a	a	a	a	a
Hydrogen peroxide	5,000	5,000	5,000	5,000	5,000	5,000
Actidrox®	5,000	5,000	5,000	500	500	500
Detarox®AP	1,000	1,000	1,000	100	100	100
Virkon™S	1,000	1,000	1,000	1,000	1,000	1,000

Notes. A, *Saprolegnia parasitica* CBS 223.65.

B, *Saprolegnia parasitica* ITT 320/15/20.

C, *Saprolegnia delica* ITT 290/15/15.

<sup>a</sup>Minimum inhibitory concentration or MLC not found at tested concentrations.

# 3. Our proposed solution (II)

- biological compounds

Slower mycelial growth of *S. parasitica* and *S. delica* strains recorded at 24h for all tested compounds at a concentration of 0.1 mM.

**2'4'-Dihydroxychalcone** slowed down mycelial growth at 0.01 mM (2.4 µg/ml).

MICs found for:

**Tomatine** (0.1mM = 99.4 µg/ml)

**Piperine** (0.25 mM = 71.3 µg/ml)

**Plumbagin** (0.25 mM = 47 µg/ml)



Inhibition of aerial mycelium  
(**Camphor**, **Diallyl Sulfide**,  
**Umbelliferone** and **Scclareolide**)



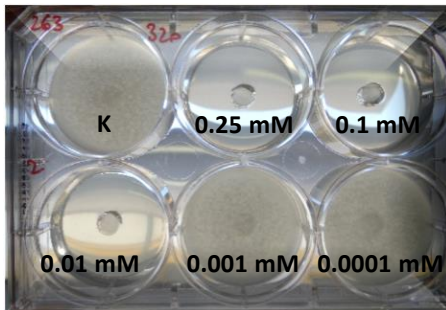
control

treatment

2'4'-DIHYDROXYCHALCONE
7-HYDROXYFLAVONE
CAMPHOR
DIALLYL SULFIDE
ESCULETIN
EUCALYPTOL
PALMATINE CHLORIDE
PIPERINE
PLUMBAGIN
SCLAREOLIDE
UMBELLIFERONE
USNIC ACID
TOMATINE
W42

# 3. Our proposed solution (III)

- array of synthetic and natural compounds



Zinc pyrithione  
and *S. parasitica*  
after 6 days

- MICs determined for 15 out of 35 compounds examined
- antifungals commonly used in human and veterinary medicine included mainly for comparative effectiveness (potential antimicrobial resistance issues)
- most of the compounds considerably slowed down radial mycelial growth and/or inhibited the development of aerial mycelium also after 6 days

COMPOUNDS	MIC mM (µg/ml)	Note about protocol I test	MLC mM (µg/ml)
ZINC PYRITHIONE	0.01 (3.197)		>0.25
CLOTRIMAZOLE	0.1 (34.484)	After 6 days radial mycelial growth was considerably slowed down at 0.01 mM (3.4484 µg/ml)	0.1 (34.484) only for strain 320  0.25 (86.21) only for strain CBS
CICLOPIROXOLAMINE	0.1 (26.836)		>0.25
5-CHLORO-8-HYDROXYQUINOLONE (CLOXYQUIN)	0.1 (17.96)		>0.25
DEQUALINIUM CHLORIDE	0.1 (52.76)		not tested
ECONAZOLE NITRATE	0.1 (44.47)		0.25 (111,175)
SULCONAZOLE NITRATE	0.1 (46.076)		0.25 (115,19)
TRICLOSAN	0.1 (28.954)		0.1 (28,954)
BUTYL 4-HYDROXYBENZOATE (BUTYL PARABEN)	0.25 (48.56)	After 6 days radial mycelial growth was considerably slowed down at 0.1 mM (19.424 µg/ml)	>0.25
BUTOCONAZOLE	0.25 (102.945)  0.1 (41.178) for strain 320	After 6 days, radial mycelial growth was considerably slowed down at 0.1 mM (14.178 µg/ml) for strains CBS and 290	0.25 (102,945)
BRONOPOL	0.25 (49.995)  (only for strain 290)	After 6 days radial mycelial growth was considerably slowed down at 0.25 mM (49.995 µg/ml) for strains CBS and 320	>0.25
CLIMBAZOLE	0.25 (73.19)		>0.25
HEXETIDINE	0.25 (84.905)  (only for CBS strain)	After 6 days radial mycelial growth was considerably slowed down at 0.25 mM (84.905 µg/ml) for strains 290 and 320	0.25 (84,905)
TETRAMETIYLTHIURAM DISULFIDE (THIRAM)	0.25 (60.11)	After 6 days radial mycelial growth was considerably slowed down at 0.1 mM (24.044 µg/ml)	0.25 (60,11)
UNDECYLENIC ACID	0.25 (46.07)		0.25 (46,07)

# 4. Expected benefits for the industry

- Expansion of the chemicals licensed for use in aquaculture. Good candidates:

Acetic and Peracetic acid-based products + hydrogen peroxide



Piperine



2',4'-Dihydroxychalcone



Plumbagin

Zinc pyrithione



- Development of treatments that are **safer for workers** in the aquaculture industry
- Increased sustainability through the use of safe and eco-friendly treatment strategies will help **improve the general public's view** of the industry

## 5. Current status, next steps and conclusions

### Current status:

*In vitro* screening to identify agents of interest is now completed

### Suggested next steps:

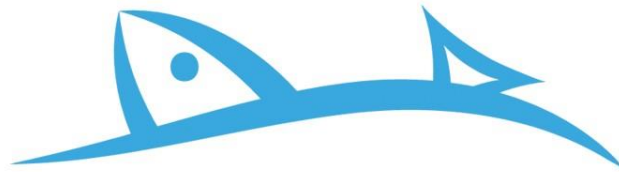
- *In vitro*-reactive agents further tested in *in vivo* trials on the target fish species
- Assessment of dose-response and optimal exposure conditions
- Development of selected compounds from lab to farm level



### Conclusions:

- The selected compounds are environment-friendly, safe for workers and cost-effective
- The development of effective alternative products and their use within an integrated parasite management (IPM) approach will contribute to increase the productivity and sustainability of aquaculture activities

# Thank You



## ParaFishControl

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