ParaFishControl

Alternative Treatments against *Saprolegnia*

ParaFishControl Final Conference: Innovative Strategies to Control Parasites in Aquaculture Farms

11th March 2020

Perla Tedesco, University of Bologna

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 634429. This output reflects the views only of the author(s), and the European Union cannot be held responsible for any use which may be made of the information contained therein.

Table of Contents

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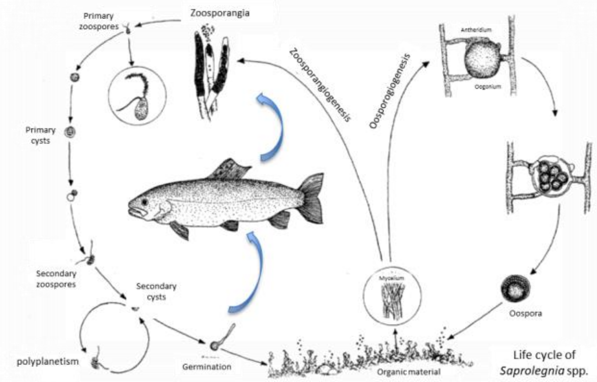
1. Challenge and Impact

Challenge

- *Saprolegnia* infections are among the main parasitic diseases causing economic losses in salmonid aquaculture
- 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
- Our research focused on an *in vitro* screening aimed at assessing **new and alternative treatment strategies** directly targeting *Saprolegnia*, to replace the use of hazardous chemicals

Challenge

- This research will provide preliminary information for the selection of more environment-friendly, safe and cost-effective compounds to be used within an IPMS.



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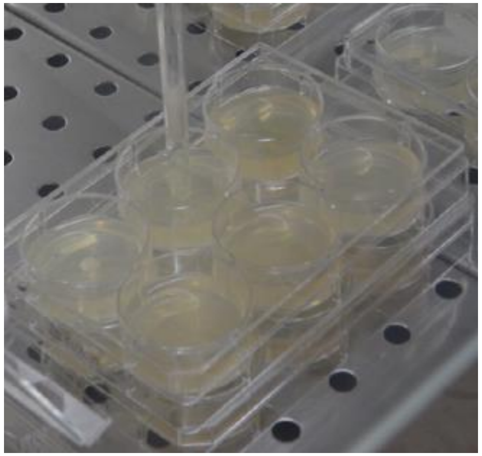
2. Our approach - Our team

In vitro SCREENING of 62 compounds

Methodology

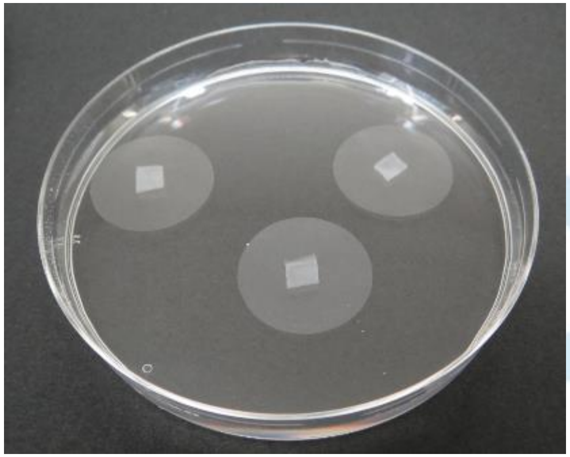
PROTOCOL 1: test in agar

(Minimum Inhibitory Concentration)



PROTOCOL 2: test in water

(Minimum Lethal Concentration)



Team involved in the development

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

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3. Our proposed solution (I)

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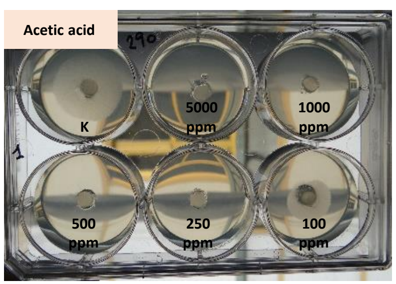
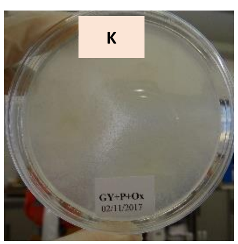
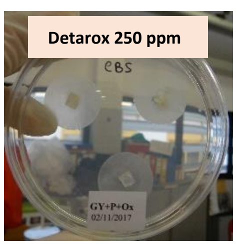
- **rapid to implement treatments**

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 | Maria Letizia Fioravanti | Roberta Galuppi

Compound	MIC A (ppm)	MIC B (ppm)	MIC C (ppm)	MLC A (ppm)	MLC B (ppm)	MLC 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.
[a]Minimum inhibitory concentration or MLC not found at tested concentrations.

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3. Our proposed solution (II)

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- **array of natural compounds**

frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 21 February 2020
doi: 10.3389/fvets.2020.00083

Comparative Therapeutic Effects of Natural Compounds Against *Saprolegnia* spp. (Oomycota) and *Amyloodinium ocellatum* (Dinophyceae)

*Perla Tedesco[1], Paola Beraldo[2], Michela Massimo[2], Maria Letizia Fioravanti[1], Donatella Volpatti[2], Ron Dirks[3] and Roberta Galuppi[1]**

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)

Compounds	MIC mM (µg/ml)
2′,4′-Dihydroxychalcone	>0.1
7-Hydroxyflavone	>0.1
Camphor (1R)	>0.25
Diallyl sulfide	>0.25
Esculetin	>0.1
Eucalyptol	>0.1
Palmatine chloride	>0.1
Piperine	0.25 (71.335)
Plumbagin	0.25 (47.045)
Sclareolide	>0.25
Tomatine	0.1 (99.4) (only for *S. delica*)
Umbelliferone	>0.25
Usnic acid	>0.25

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

control

treatment

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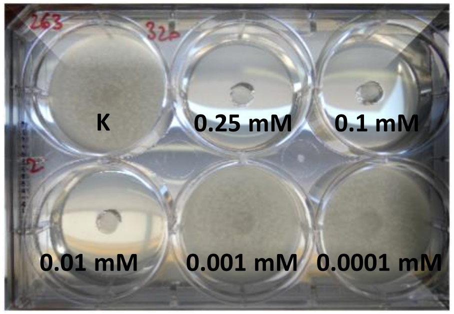
3. Our proposed solution (III)

- **array of synthetic 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)

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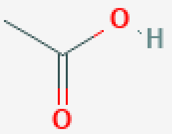
Control compounds

MOLECULE NAME	MIC (mg/L or µl/L)	MLC (mg/L or µl/L)	PRICE (€/mg or €/µl)
MALACHITE GREEN	5	5	banned
FORMALIN	170	not tested	0.000003
COPPER SULFATE	250	1000 (5000 strains 320 and 290)	0,00002
ACETIC ACID	250	250 (500 strains 320 and 290)	0.00001 (Merck)
IODOACETIC ACID	250 (S. parasitica) 100 (S. delica)	50 (S. parasitica) 100 (S. delica)	0.00251 (Merck)
ACTIDROX®	5000	500	0.000024 (Farmec)
DETAROX® AP	1000	100	0.000003 (Perdomini)
BENZOIC ACID	100	250	0.00009 (Merck)
PIPERINE	71.335	not tested	0.010 (Merck)
PLUMBAGIN	47.045	not tested	0.370 (Santa Cruz)
TOMATINE	99.4 (S. delica)	not tested	4.613 (Merck)
2’4’-DIHYDROXYCHALCONE	24.02 (48h, S. parasitica)	not tested	0.255 (Santa Cruz)
ZINC PYRITHIONE	3.197	not determined	0.018 (Merck)
CLOTRIMAZOLE	34.484	34.484 (strain 320); 86.21 (strain CBS)	0.019 (Merck)
CICLOPIROXOLAMINE	26.836	not determined	0.047 (Santa Cruz)
5-CHLORO-8-HYDROXYQUINOLONE (CLOXYQUIN)	17.96	not determined	0.0004 (Merck)
DEQUALINIUM CHLORIDE	52.76	not tested	0.141 (Merck)
ECONAZOLE NITRATE	44.47	111.175	0.009 (Merck)
SULCONAZOLE NITRATE	46.076	115.19	0.070 (Santa Cruz)
TRICLOSAN	28.954	28,954	0.062 (Merck)
BUTYL 4-HYDROXYBENZOATE (BUTYL PARABEN)	48.56	not determined	0.0004 (Merck)
BUTOCONAZOLE	102.945 (41.178 strain 320)	102.945	0.384 (Santa Cruz)
BRONOPOL	49.995 (S. delica)	not determined	0.219 (Merck)
CLIMBAZOLE	73.19	not determined	0.014 (Santa Cruz)
HEXETIDINE	84.905 (strain CBS)	84.905	0.024 (Santa Cruz)
TETRAMETIYLTHIURAM DISULFIDE (THIRAM)	60.11	60.11	0.003 (Merck)
UNDECYLENIC ACID	46.07	46.07	0.030 (Merck)

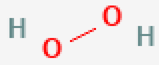
4. Expected benefits for the industry

Good candidates:

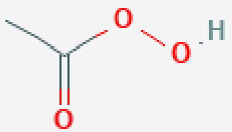
acetic acid



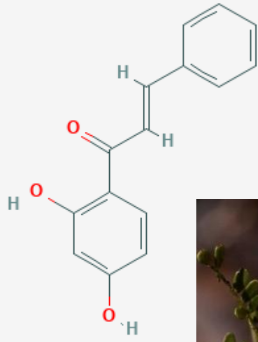
hydrogen peroxide

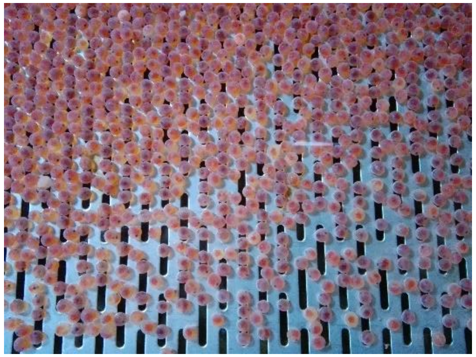


peracetic acid

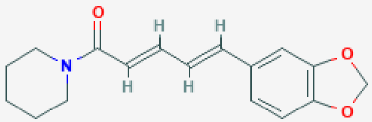


2',4'-Dihydroxychalcone

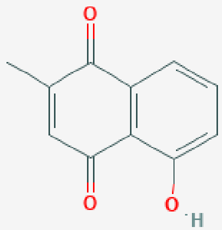




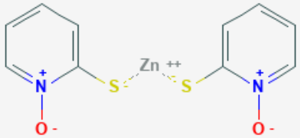
Piperine

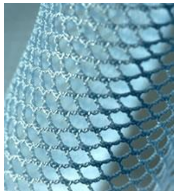


Plumbagin



Zinc pyrithione





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5. Current status, next steps and conclusions

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Current status:

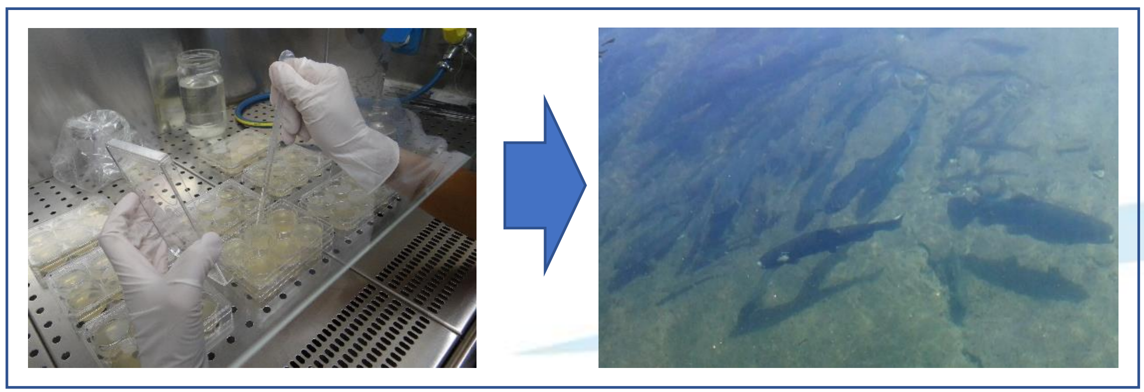
In vitro screening completed

Suggested next steps:

Further investigations needed to define toxicity, persistence and bioaccumulation potential

Development of selected compounds from lab to farm level:

- *In vitro*-reactive agents further tested in *in vivo* trials on the target fish species
- Assessment of dose-response and optimal exposure conditions



Conclusions:

The development of effective alternative products and their use within IPMS will contribute to increase the productivity and sustainability of aquaculture activities.

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Thank You

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Perla Tedesco

perla.tedesco@unibo.it

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