



ParaFishControl

Alternative treatments for *Amyloodinium ocellatum*

ParaFishControl Workshop

“Mediterranean Fish Parasite Management Strategies”

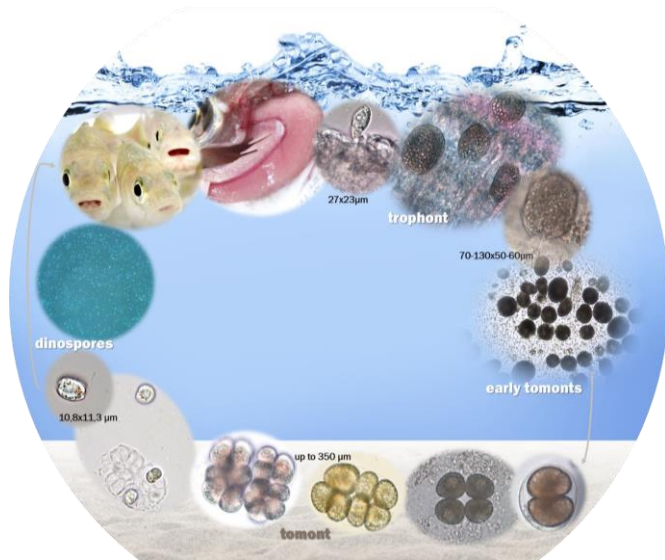
Porto, 10th September 2019

Michela Massimo

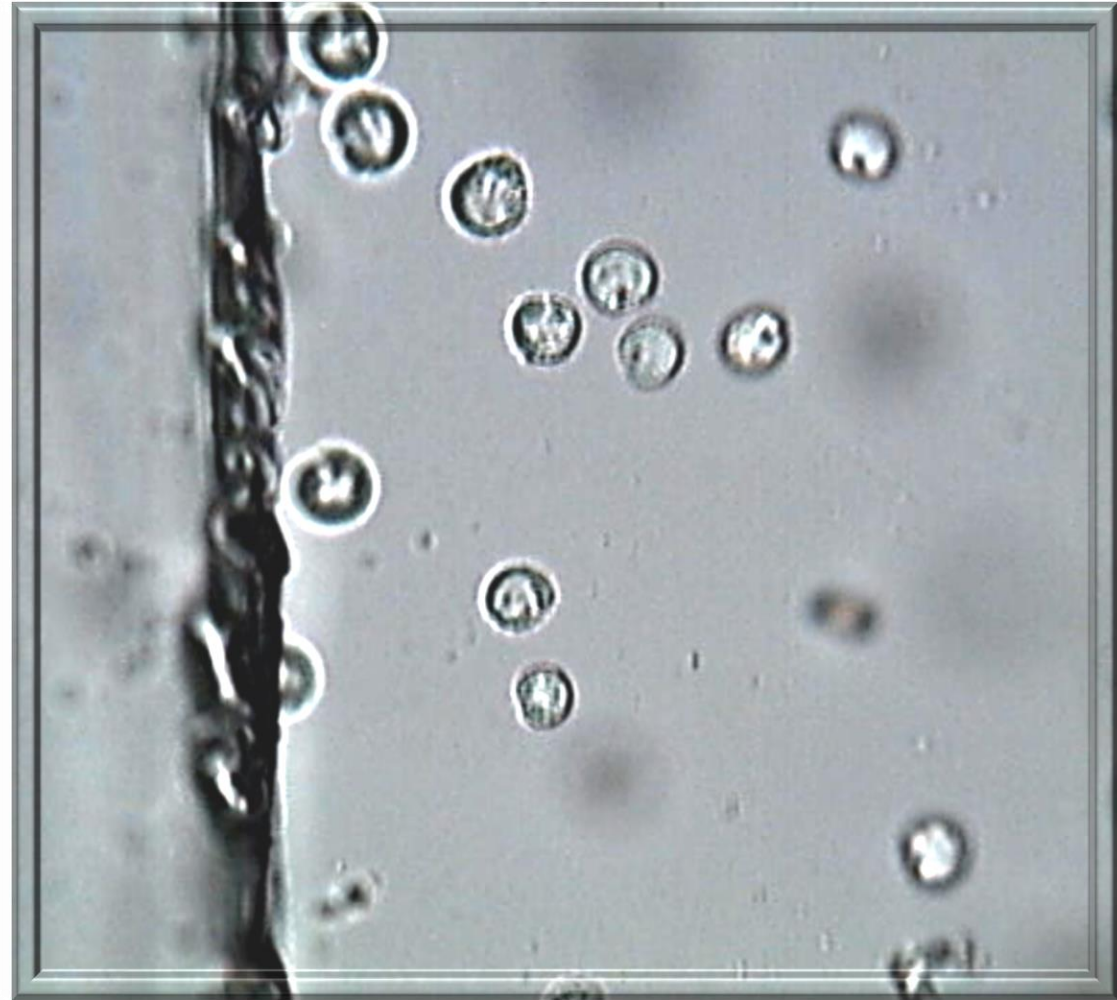
University of Udine



Challenge and Impact



Amyloodinium ocellatum



Our approach and our team

Study to assess the potential *in vitro* antiparasitic effects of 16 plant derived compounds on dinospores of *Amyloodinium ocellatum*

Team involved in the development



Our proposed solution (I)

Table 1. Classification of the 16 active plant compounds provided by Zebra Fish Screens (ZF-Screens)

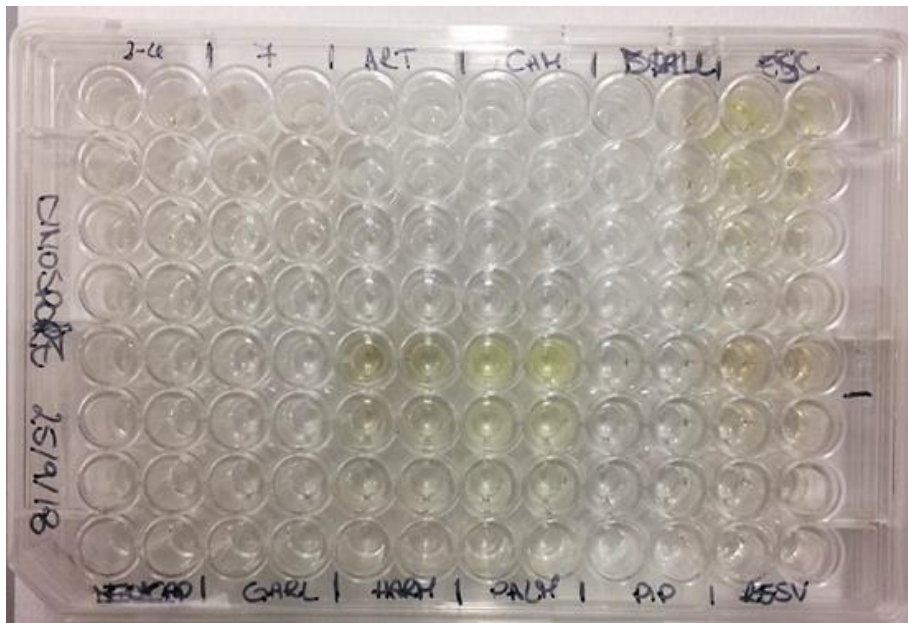
ACTIVE PLANT COMPOUND	CLASSIFICATION	CODE and SUPPLIER
2',4' - Dihydroxychalcone	Flavonoid	Sc – 266263; Santa Cruz Biotech.
7 - Hydroxyflavone	Flavonoid	H4530 – 1G; Sigma-Aldrich
Artemisinin	Sesquiterpene lactone	Sc – 202960A; Santa Cruz Biotech.
Camphor (1R)	Terpenoid	857300 – 5G; Sigma Aldrich
Diallyl sulfide	Organosulfur compound	W204218 – 100G – K; Sigma-Aldrich
Esculetin	Lactone	246573 – 1G; Sigma-Aldrich
Eucalyptol	Monoterpene cyclic ether	C80601 – 100ml; Sigma-Aldrich
Garlicin 80%	Organosulfur compound	317691 – 25G; Sigma-Aldrich
Harmalol hydrochloride dihydrate	Alkaloid	H125 – 1G – A; Sigma-Aldrich
Palmatine chloride	Protoberberine alkaloid	Sc – 205788; Santa Cruz Biotech.
Piperine	Alkaloid	P49007 – 1G; Sigma-Aldrich
Resveratrol	Phytoalexin	R5010 – 100 mg; Sigma-Aldrich
Rosmarinic acid	Caffeic acid ester	536954 – 5G; Sigma-Aldrich
Sclareolide	Sesquiterpene lactone	W379401 – 25G; Sigma-Aldrich
Tomatine	Glycoalkaloid	Sc – 296548; Santa Cruz Biotech.
Umbelliferone	Phenylpropanoid	H24003 – 10G; Sigma-Aldrich

Our proposed solution (II)

Dinospores at a defined concentration of 5.2×10^3 /ml were incubated at RT for 24 hours with serial dilutions (50-0.39 $\mu\text{g}/\text{ml}$) of the 16 compounds

Controls were set as follows:

- positive controls, **chelated copper sulphate** (1 $\mu\text{g}/\text{ml}$) and **formalin** (4 $\mu\text{g}/\text{ml}$)
- negative control (no compounds, only culture medium)



Motility was assessed after 1, 6 and 24 hours of incubation at RT

The experiment was repeated twice, in the 1st attempt dinospores derived from fresh tomonts, in the 2nd study from hibernated ones



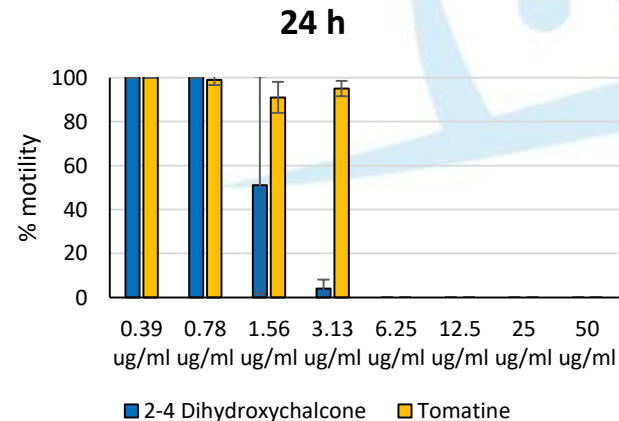
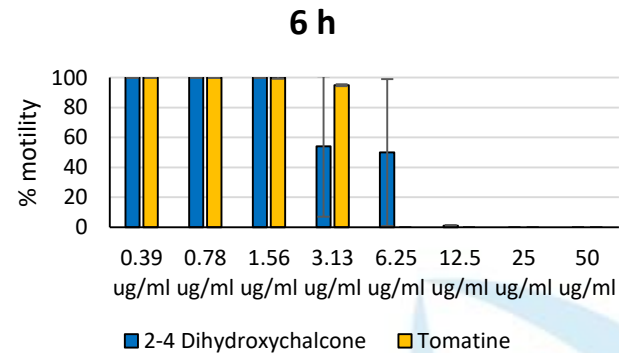
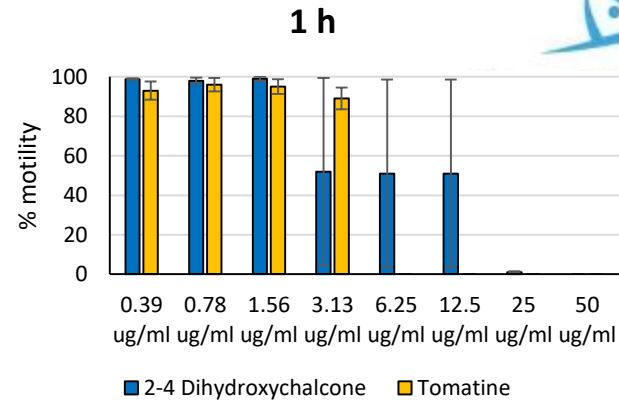
Our proposed solution (III)

Tomatine and 2^l,4^l – dihydroxychalcone reduced the motility of dinospores with a **dose-dependent effect**

Tomatine was the **most effective** by **completely stopping** the motility from the **1st hour** of incubation and till the concentration of **6.25 µg/ml**

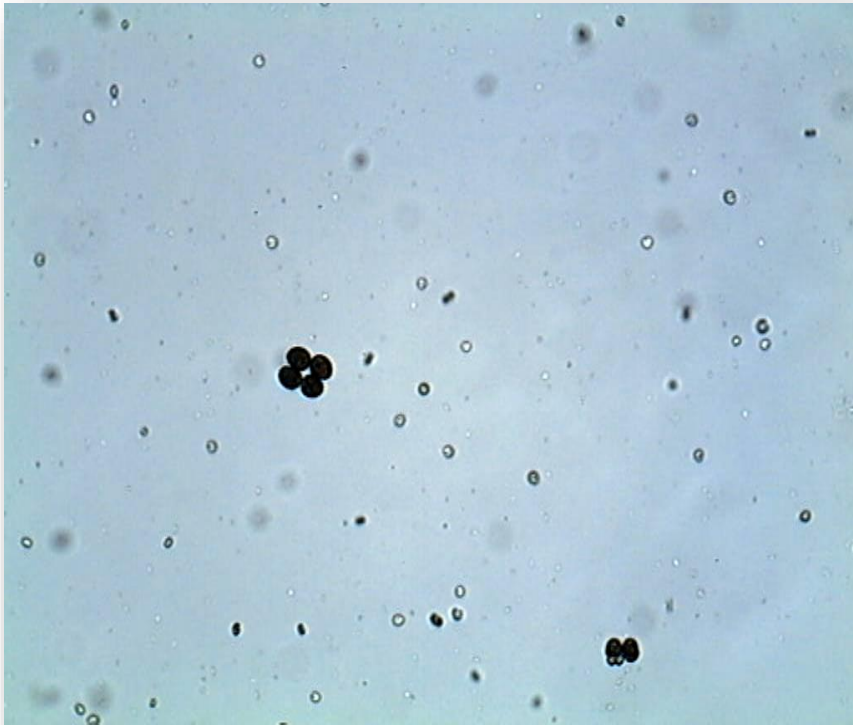
The remaining **14 compounds** did not exert **any relevant action** on dinospore motility

Chelated copper sulphate and **formalin** were effective in inhibiting dinospore motility for the whole duration (**24h**) of the experiment

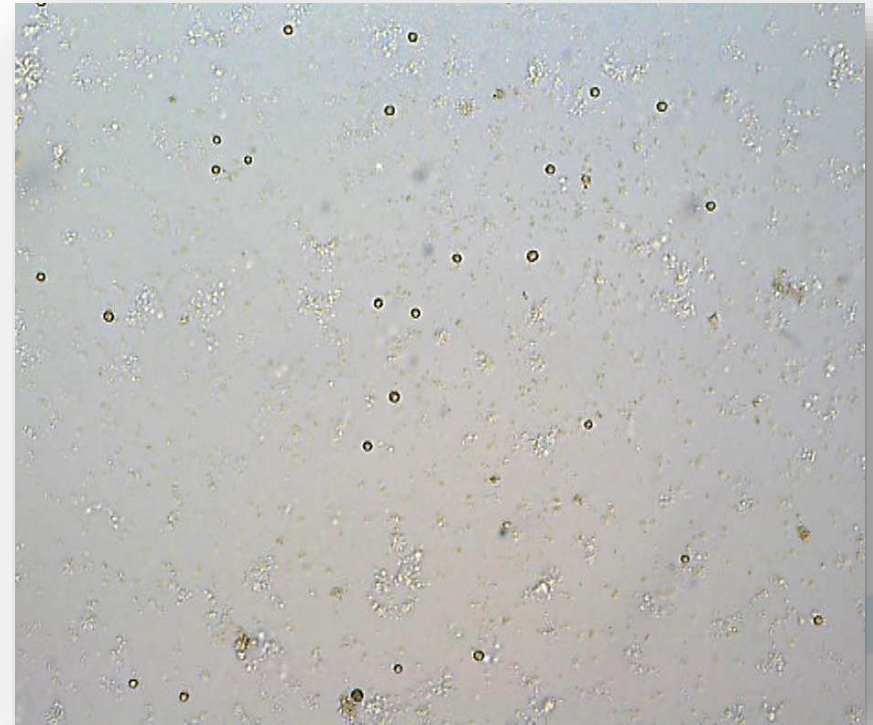


Motility of A. ocellatum dinospores incubated at room temperature with different concentrations (0.39-50 µg/ml) of 2^l-4^l dihydroxychalcone and tomatine. The motility is expressed as percentage of motile dinospores on the total number of dinospores present per well after 1, 6 and 24 hours of incubation. Values were then related to the motility observed in the wells assigned to negative control corresponding to 100%. Data are expressed as mean values ± S.E.

Current status and next steps



Negative control



**Dinospores incubated with 50µg/ml
Tomatine**

Expected benefits for the industry

NOVEL EFFECTIVE TREATMENT

licensed

eco-friendly

cheap

easy to find



Conclusions



UNIUD has interrupted this investigation line to focus on the research of the development of a vaccine

Thank You



ParaFishControl

Dr. Michela MASSIMO

**Università degli Studi di Udine
Dipartimento di Scienze AgroAlimentari, Ambientali e Animali
Sezione di Patologia Veterinaria
Via Sondrio, 2 – Udine - Italy**

michela.massimo@uniud.it

info@parafishcontrol.eu

