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Preliminary tests on in vitro activity of different pure and commercial compounds against Saprolegnia spp.

This is the submitted version (pre peer-review, preprint) of the following publication:

Published Version:
Galuppi R., Tedesco P., Ciardo M., Gustinelli A., Menconi V., Caffara M., et al. (2017). Preliminary tests on in vitro activity of different pure and commercial compounds against Saprolegnia spp.. European Association of Fish Pathologists.

Availability:
This version is available at: https://hdl.handle.net/11585/622818 since: 2019-01-14

Published:
DOI: http://doi.org/

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12 October 2024

ParaFishControl

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PRELIMINARY TESTS ON *IN VITRO* ACTIVITY OF DIFFERENT PURE AND COMMERCIAL COMPOUNDS AGAINST *SAPROLEGNIA* SPP.

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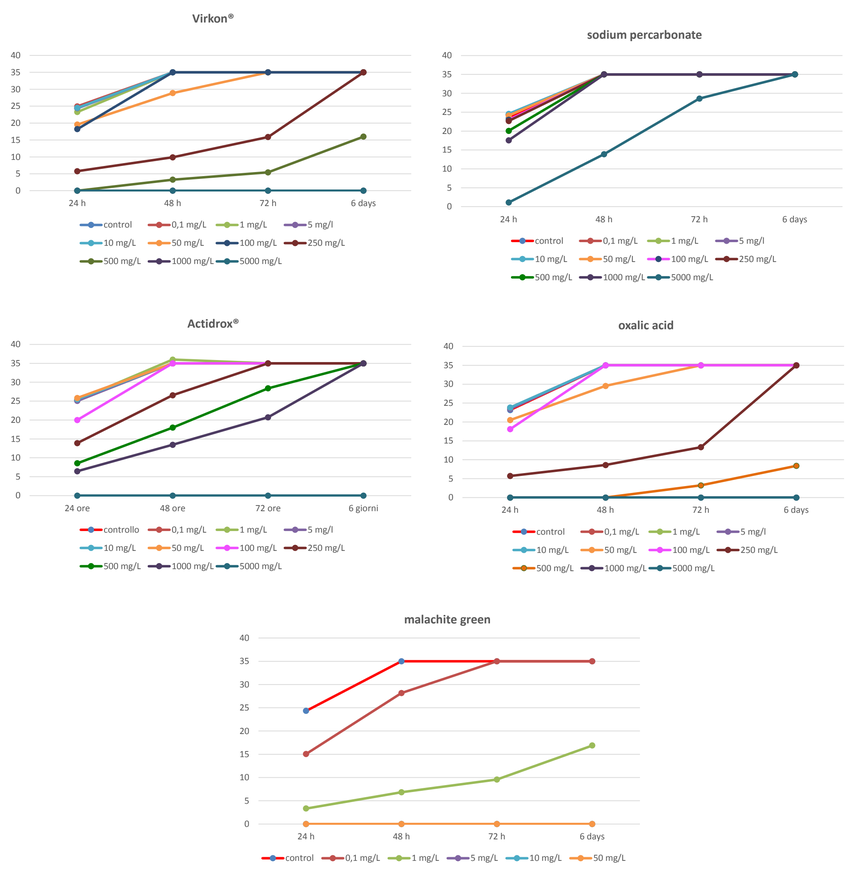
Introduction

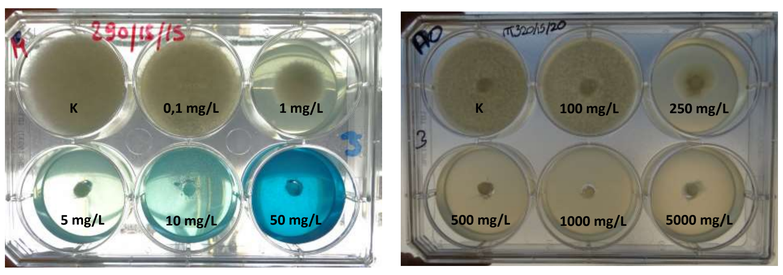
Oomycetes of the genus *Saprolegnia* are responsible for severe economic losses in freshwater aquaculture. Compounds of proven activity against *Saprolegnia* spp. (i.e. malachite green) are potentially hazardous to human health and the environment, and their use is forbidden by European regulations. Therefore, the demand for new treatments pushes towards the selection of more safe and environmentally friendly products.

In the present work, *in vitro* activity of two pure compounds (Oxalic acid; Sodium percarbonate) and two commercial products (Actidrox®, De Marco, Italy; Virkon® S, Dupont) was tested on different strains of *Saprolegnia*. Malachite green was used as reference compound.

protocol I



Average diameters of the colonies with different concentrations of tested compounds



Examples of *Saprolegnia* growth on agar with different concentrations of malachite green (left) and oxalic acid (right) after 24 h of incubation

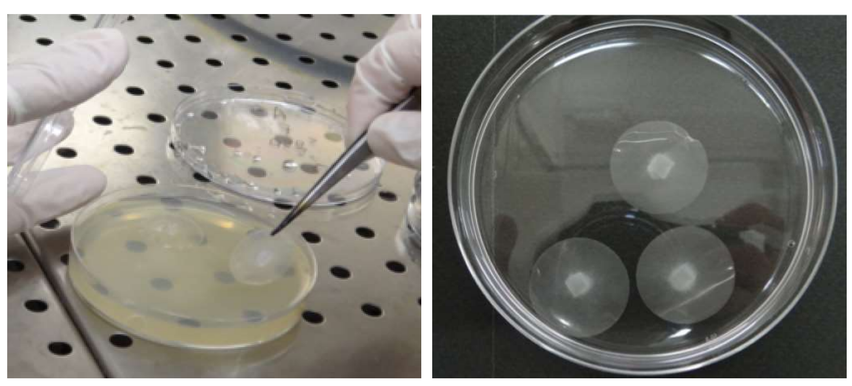
Methodology

Preliminary trials were performed using two protocols available in the literature by Alderman (1982, Journal of Fish Diseases 8:289-298): one screening method in Agar (**protocol I**) aimed at assessing the *minimum inhibitory concentration* (MIC) and one hour bath in aqueous solution of mycelium growing on polycarbonate membrane (**protocol II**), to assess the *minimum lethal concentration* (MLC). Two field strains of *Saprolegnia* spp. isolated from rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) and one reference strain of *S. parasitica* (CBS 223.65 furnished by CSIC-RJB) have been tested in triplicate per each concentration.

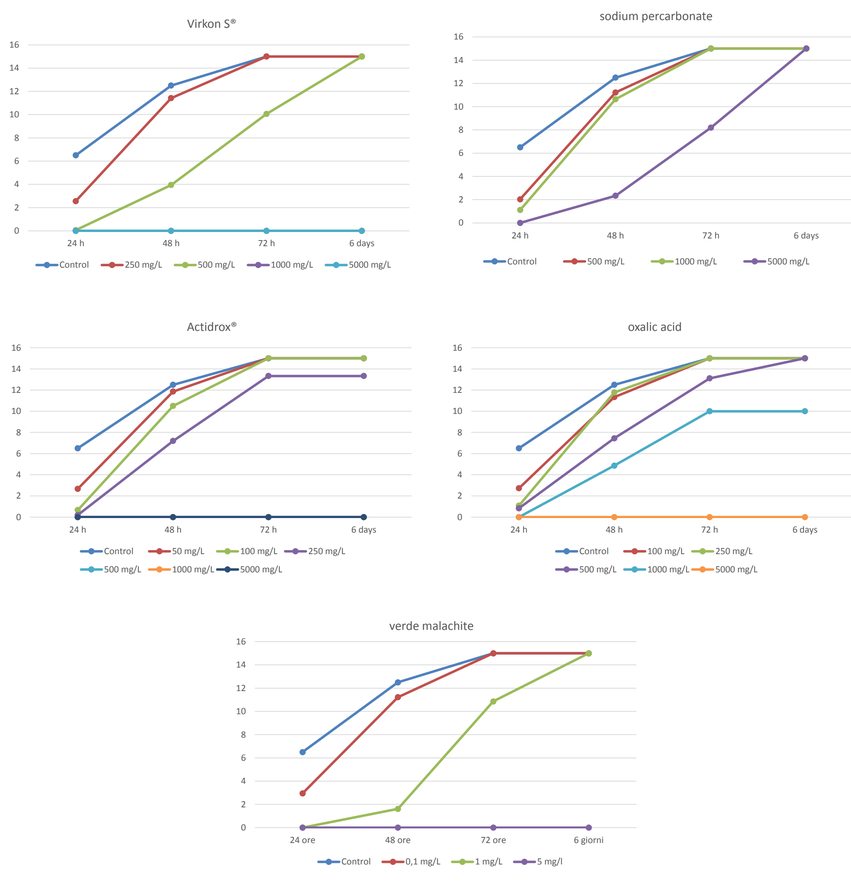
Results

Our results show that oxalic acid and Virkon® are effective in inhibiting the growth of the mycelium, although at concentrations too high to be applied in the field (MIC 1000 mg/L). Actidrox® showed a different activity between the two methods (MIC 5000 mg/L; MLC 500 mg/L), possibly due to its mechanism of action, that requires presence of water. Tested concentrations of Sodium percarbonate were effective only in slowing down the mycelium growth.

	MIC	MLC
compound	**agar**	**water**
malachite green	5 mg/L	5 mg/L
oxalic acid	1000 mg/L	5000 mg/L
		(1000 mg/L for CBS 233.65 strain)
Actidrox®	5000 mg/L	500 mg/L
sodium percarbonate	> 5000 mg/L	> 5000 mg/L
Virkon®	1000 mg/L	1000 mg/L



protocol II



Average radial growth of hyphae beyond the filter area after immersion treatment with different concentrations of tested compounds

Mycelium growth after 6 days at 3 different concentrations of malachite green

Conclusions

Further *in vitro* trials will be necessary, considering a wider range of promising compounds. The combination of the two methods (inoculation in agar and contact in aqueous solution) represents a good investigation approach for screening the activity of different molecules and products against *Saprolegnia* spp.

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.