

Effects of alcoholic extracts of various plants against *Saprolegnia parasitica*

Introduction

Water moulds of the genus *Saprolegnia*, belonging to the oomycetes, include species being among the most important fish and crustacean pathogens. However, they are often neglected due to a lack of study. Before the global prohibition in 2006, the organic dye malachite-green oxalate was used to very effectively control *Saprolegnia* outbreaks. Currently formaldehyde is the remedy of choice

to primarily prevent *Saprolegnia* outbreaks in salmonid eggs or to treat valuable fish like brood stock. Current research on plant derived alternatives is increasing and here we present preliminary results of *in vitro* tests of seven alcoholic plant extracts against *Saprolegnia parasitica* (ATCC 22284).

Material & Methods

Plant material (Table 1) was ground and macerated in 94.5% ethanol for 24 h (1:5, w/v). Afterwards it was filtered through a MN 6151/4 filter and stored dark and dry until used after a maximum of one week. *Saprolegnia parasitica* (ATCC 22284) were propagated on corn meal agar plates supplemented with penicillin (150 mg L⁻¹) and streptomycin (250 mg L⁻¹) via agar plug (ca. 5 x 5 mm) transfer. Agar plugs inoculated with *S. parasitica* were incubated in triplicates for 60 and 120 minutes in the plant extracts (500, 1000 and 2000 ppm), in a negative control (ethanol in the same concentration as present in the extracts) and a positive control (300 ppm formaldehyde, active ingredient) and randomly distributed on fresh corn meal plates. Fungal growth was observed after one and two days and length of longest hyphae measured. Furthermore the amount of hyphae were counted on one side of the agar plug and set in relation to the length of the plug.

Results & Discussion:

None of the tested extracts at the tested concentrations was as good as formalin (positive control) but several showed significant effects when compared to the negative control. The effects on the amount of growing hyphae were more pronounced than the effects on the hyphae length. This was especially the case at 2000 ppm after an incubation time of two hours. Overall curcuma showed the best results at 500 ppm after one hour of incubation. In general, the effects were even more pronounced at the shorter incubation time than after two hours of incubation, which also included the positive control. This points to some kind of adaptation or detoxification mechanism of *Saprolegnia parasitica* when incubated with plant extracts in aqueous solution over a longer period compared to a shorter incubation time.

Variation between replicates of one treatment at a given time and concentration was rather high pointing to variability in homogeneity of extract or varying colonization density of agar plugs and necessitating higher number of replicates. Several studies have used alcoholic and aqueous plant extracts against *Saprolegnia* spp. with varying success (Udomkusomsri et al. 2007, Caruana et al. 2012, Cao et al. 2013) but up to date no replacement being as effective as formalin has been found.



Figure 1: Effects of (from left to right) negative control (500 ppm, 1 h), positive control and curcuma extract (500 ppm, 1 h) on hyphae growth of *S. parasitica*.

Literature:

Udomkusomsri et al., 2007, Kasetsart J. 41, 56-61
 Caruana et al. 2012, Aquaculture 358-359, 146-154
 Cao et al., 2013, J Appl Ichthyol, doi: 10.1111/jai.12316

Table 1: Common and scientific names of applied plants

Common Name	Scientific Name	Extract no.
Cinnamon	<i>Cinnamomum verum</i>	20
Clove	<i>Syzygium aromaticum</i>	21
Curcuma	<i>Curcuma longa</i>	22
Nasturtium	<i>Tropaeolum sp.</i>	23
Savory	<i>Satureja hortensis</i>	24
Black Pepper	<i>Piper nigrum</i>	25
Grapefruit seed	<i>Citrus x aurantium</i>	26

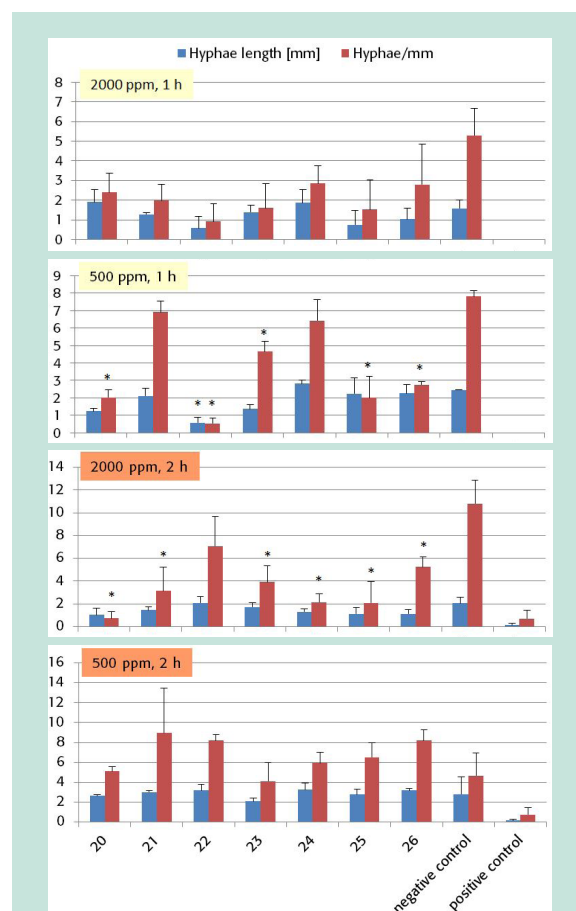


Figure 2: Effect of plant extracts on hyphae growth of *S. parasitica*; values = mean $\pm$ SEM (n = 3), * = significant different from negative control (Dunnett's test)