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In vitro antifungal activity of plant extracts on *Saprolegnia parasitica* from cutaneous lesions of rainbow trout *(Oncorhynchus mykiss)* eggs

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Abstract

Saprolegnia parasitica Coker is the main agent of saprolegniosis, one of the most important causes of economic losses in the rainbow trout farming industry in Iran. In this work, seven essential oils (2 species) and ethanol extracts (5 species) were tested against a strain of *S. parasitica* from cutaneous lesions of *Oncorhynchus mykiss* eggs, using a continuously agitated broth technique that allows both the minimum inhibitory concentration ($MIC_{\geq 50\%}$) and the minimum lethal concentration ($MLC_{\geq 99.9\%}$) to be evaluated. The essential oils of *Thymus daenensis* and *Thymus khuzestanicum* ($MIC_{\geq 50\%}$ = 0.63 µl ml^{-1} and $MLC_{\geq 99.9\%}$ = 22 µl ml^{-1}) and ethanol extracts of *Tanacetum parthenium* and *Mentha longifolia* ($MIC_{\geq 50\%}$ = 31.25 and 62.5 µg ml^{-1} and $MLC_{\geq 99.9\%}$ = 600 and 550 µg ml^{-1}, respectively) showed higher inhibition against *S. parasitica* than the other extracts. In general, our study suggests that natural products derived from some medicinal plants have the potential to be used as health rainbow trout eggs.

Key words: Medicinal plants, minimum inhibitory concentration (MIC), minimum lethal concentration (MLC), *Saprolegnia parasitica*.

Introduction

One of the main types of fungal diseases in farmed trout fish is saprolegniasis, caused by species in the genus *Saprolegnia*. *Saprolegnia parasitica* Coker (Saprolegniaceae) is the causative agent of a disease in aquatic animals, which is responsible for severe damage in all developmental stages of fish [5]. The therapeutic control of such a disease is hard to achieve because the most effective products (e.g. formalin, malachite green and etc.) have been prohibited due to their toxicity and persistence in the environment [5, 7]. It causes considerable economic problems in the fish farming industry [1], infecting both fish and fish eggs [3]. In the past this problem was solved with the extremely effective fungicide (compound which kills fungi) malachite green. The use of malachite green began in 1933 and it was one of the cornerstones used in treatment of fish against a range of parasites [7]. Malachite green is considered carcinogenic, mutagenic and teratogenic [7]. Carcinogenic substances are agents capable of causing cancer [7]. Unfortunately, malachite green is still being used by fish farmers in some of trout farms, Iran. The problems connected with the use of these chemicals stimulate the research for new constituents of natural origin. In a more extensive study of the use of plant extracts against pathogenic fungi, it has been observed that some essential oils are capable of inhibiting the growth or even causing the death of *S. parasitica* at comparatively low concentrations [11]. In the present survey, the activity of some plant extracts against this oomycete was tested in a continuously agitated broth and compared with the activity of formalin in order to identify possible plant extract to be used in the control of the infection.

Materials and Methods

Plant material: The plants studied, including the botanical name and data related to traditional use and plant parts used, are listed in Table 1. The plants were collected from mountain areas of Zagross, Iran, during May-Nov., 2008.

Extract preparation: The leaves of two species of thyme were powdered (200 g) and subjected to hydrodistillation (2000 ml distillated water) for 4 h using a Clevenger-type apparatus according to the method recommended in British Pharmacopoeia [2]. Leaves and flowers of other plants, shade dried and ground into a powder (100 g), macerated in 200 ml of ethanol and filtered, were dried at 35°C under rotary vacuum (Model Zirbus 302®). The extract samples were stored in universal bottles and refrigerated at 4°C prior to use.

Strain tested: The test was carried out on a *S. parasitica* strain isolated from *Oncorhynchus mykiss* eggs and obtained in pure form through serial transplants on GY agar medium, yeast extract 0.25 g and glucose 5 g in 100 ml of deionised water, with the addition of 250 µml^{-1} of penicillin-streptomycin. The purified strain was kept at 18°C on GY agar and transplanted at regular intervals.

Antifungal test: The minimal inhibitory concentration (MIC) and the minimum lethal concentration (MLC) values were determined by serial dilution assay [10, 13], each series inoculated with 1 ml of fungal suspension at a density of 10^5 spore ml^{-1}. Two tubes were used as negative control and positive control (formalin 10 µl ml^{-1})

Table 1. Ethnobotany of Iranian medicinal plant used in this study.

Scientific name	Family	English name	Local name	Parts used	Traditional medicine uses [4, 14]
Thymus daenensis Celak.	Lamiaceae	Denaee thyme	Avishan-e-Denaie	Leaves	Green tea, spice, culinary, cough, anti-bacterial, carminative
Thymus khuzestanicum	Lamiaceae	Khuzestani thyme	Avishan-e-Khuzestani	Leaves	Carminative, cough and antimicrobial
Mentha longifolia (L.) Hudson	Lamiaceae	Mint	Pooneh-e-Koohi	Leaves	Edible as vegetable, flavoring, indigestion, cough
Myrtus communis L.	Myrtaceae	Common myrtle	Mord or morte	Leaves	Skin discords, digestive discords, astringent, good hair condition
Tanacetum parthenium (L.) Schultz-Bip.	Asteraceae	Tansy	Baboneh Kabir	Flower	Headache, migraine, sedative, fat blood, diabetes
Satureja bachtiarica Bunge	Lamiaceae	Bakhtyari savory	Marzeh-e-bakhtyari	Leaves	Edible as vegetable, flavoring, indigestion, cough, anti-bacterial
Hyssopus officinalis L.	Lamiaceae	Hyssop	Zofa	Leaves	Cough, asthma, anti-microbial

Table 2. Effect of ethanol extracts on growth *Saprolegnia parasitica* by serial dilution assay.

Concentration (µg ml^{-1})	Growth inhabitation (%)				
	Mentha longifolia	*Satureja bachtiarica*	*Hyssopus officinalis*	*Tanacetum parthenium*	*Myrtus communis*
1.00	0.28 ± 0.21	9.43 ± 1.51	0.513 ± 0.37	1.56 ± 0.50	1.27 ± 1.38
1.95	22.67 ± 19.07	15.08 ± 4.89	1.583 ± 1.24	14.25 ± 3.65	4.97 ± 3.30
3.91	29.10 ± 16.54	21.56 ± 4.99	4.820 ± 3.81	20.26 ± 1.43	9.50 ± 2.18
7.81	32.52 ± 13.76	30.71 ± 8.74	8.393 ± 6.31	31.32 ± 1.83	11.65 ± 4.70
15.63	35.41 ± 10.12	33.63 ± 7.61	9.203 ± 6.10	42.89 ± 1.99	15.96 ± 4.92
31.25	42.67 ± 14.00	41.99 ± 4.96	19.643 ± 17.59	55.73 ± 3.12	22.62 ± 9.02
62.5	50.37 ± 9.53	47.53 ± 1.67	23.100 ± 17.69	59.55 ± 2.36	25.54 ± 7.92
125	67.32 ± 1.86	51.89 ± 3.48	31.290 ±19.53	65.62 ± 3.93	34.29 ± 8.55
250	77.47 ± 2.91	57.97 ± 5.63	38.35 ±13.75	70.03 ± 3.12	39.81 ± 9.07
500	81.13 ± 1.83	68.9 ± 4.25	40.52 ± 12.48	72.94 ± 5.16	44.91 ± 9.48
Formalin (10 µl ml^{-1})	65.53 ± 5.61	82.84 ± 2.77	81.423 ± 2.72	80.00 ± 3.10	58.12 ± 2.07

while products to be tested were added to the other tubes so as to obtain concentrations of 0.04 to 20 µl ml^{-1} for essential oils (thyme) and 1 to 500 µg ml^{-1} for ethanol extracts. The inoculum was then introduced into each tube under sterile conditions. The tubes so prepared were placed in a thermostat at 18°C on a stirrer and submitted to continuous agitation at 130 rpm throughout the incubation time (7 days) [10]. The growth of microorganisms was observed as turbidity determined by a spectrophotometer at OD_{600} nm. The MIC was defined as the lowest concentration of soluble principle at which percentage of growth inhabitation greater than 50% was observed after incubation. The MLC was defined as the lowest extract concentration at which 99.9% of the fungi have been killed (percentage of growth inhabitation greater than 99.9%). The inhibition demonstrated by the extracts is expressed by the following equation [13]: Inhibition % = $[(OD_c-OD_t)/OD_c]*100$ where OD_c is the OD_{600} for the negative control (containing no extracts) and OD_t is the OD_{600} for the sample treated with the antimicrobial compounds.

Results and Discussion

There were significant differences ($P \leq 0.05$) in the antifungal activity of ethanol extracts and essential oils. Growth inhabitation values of extracts and essential oils on *S. parasitica* are shown in Tables 2 and 3. Among the plants tested, essential oils of *Thymus daenensis* and *Thymus khuzestanicum* and ethanol extract of *Mentha longifolia* showed the best antifungal activity. Also, the ethanol extract of *Tanacetum parthenium* and *Satureja bachtiarica* showed the antifungal activity.

The $MIC_{(\geq 50\% \text{ growth inhabitation})}$ and $MLC_{(\geq 99.9\% \text{ growth inhabitation})}$ for two essential oils of *Thymus* were 0.63 and 22 µl ml^{-1}, respectively. Also, the MIC for ethanol extracts of *Tanacetum parthenium*, *Mentha longifolia* and *Satureja bachtiarica* were 31.25, 62.5 and 125 µg ml^{-1}, respectively. The MLC for extracts of *Tanacetum parthenium*, *Mentha longifolia* and *Satureja bachtiarica* were 600, 550 and 750 µg ml^{-1}, respectively.

In extensive studies of the use of vegetable products against pathogenic fungi, some essential oils and extracts were capable of inhibiting the growth or even causing the death of *S. parasitica* at comparatively low concentrations [6, 9-11]. The essential oils are extremely volatile substances from vegetable sources; the main

Table 3. Effect of essential oils on growth *Saprolegnia parasitica* by serial dilution assay.

Concentration (µl ml^{-1})	Growth inhabitation (%)	
	Thymus daenensis	*Thymus khuzestanicum*
0.04	1.89 ± 1.65	1.40 ± 1.22
0.08	33.37 ± 2.97	9.83 ± 0.90
0.16	39.73 ± 3.06	32.37 ± 17.09
0.31	47.10 ± 5.30	49.40 ± 11.13
0.63	52.03 ± 6.66	54.13 ± 11.49
1.25	55.80 ± 5.56	60.17 ± 8.68
2.5	57.80 ± 5.53	66.27 ± 7.50
5	60.27 ± 5.22	75.80 ± 5.90
10	76.47 ± 1.79	78.63 ± 4.68
20	86.30 ± 1.49	80.07 ± 4.40

components are hydrocarbons; aldehydes, ketones, alcohols, phenols, ethers and esters of terpenic and phenolic origin [12]. In this study, the essential oils of *Thymus daenensis* and *Thymus khuzestanicum* ($MIC_{\geq 50\%}$ = 0.63 µl ml^{-1} and $MLC_{\geq 99.9\%}$ = 22 µl ml^{-1}) and ethanol extract of *Mentha longifolia* showed higher inhibition against *S. parasitica* than the other extracts.

Previous work [6] showed that aqueous extracts of fruits and leaves of *Capsicum fruitescens*, *Capsicum annuum* (Solanaceae) and *Nerium oleandar* (Apocyanceae) inhibited the germination of *Alternaria solani* spores and decreased the mycelial dry weight of *Alternaria solani* and *Saprolegnia.* According to a report [10], the best antifungal activity was shown by the essence of *Cymbopogon flexuosus* (Poaceae), with MIC and MLC 100 ppm, as well as *Cinnamomum verum* (Lauraceae), *Thymus vulgaris* (Lamiaceae) and *Origanum vulgare* (Lamiaceae), that had a fungistatic activity at 100 ppm and a fungicidal activity at 200 ppm, and *Satureja montana* (Lamiaceae) with MIC and MLC at 200 ppm. The essential oils and extracts of some aromatic plants (for example Lamiaceae family) with a high percentage of carvacrol, which was very active, had a higher efficacy against *S. parasitica*, in accord with other papers [8, 10, 11]. Also, Tampieri *et al*. [11] reported that terpenic compounds with an alcoholic function showed a higher fungistatic and fungicidal activity compared with other hydrocarbons.

In general, our study suggests that natural products (essential oil and extract) derived from some of the medicinal and aromatic plants (especially mint family or Lamiaceae) have the potential to be used as health control of rainbow trout eggs against *S. parasitica***.**

References

[1]Bly, J.E., Lawson, L.A., Dale, D.J., Szalai, A.J., Durborow, R.M. and Clem, L.W. 1992. Winter saprolegniosis in channel catfish. Diseases of Aquatic Organisms **13**:155-164.

[2]British Pharmacopoeia 1988. HMSO vol. 2, London, pp. 137–138.

[3]Bruno, D.W. and Wood, B.P. 1999. *Saprolegnia* and other Oomycetes. In Woo, P.T.K. and Bruno, D.W. (eds). Fish Diseases and Disorders. Vol. 3. Viral, Bacterial and Fungal Infections. CABI Publishing, Wallingford, Oxon, United Kingdom, pp. 599-659.

[4]Ghasemi Pirbalouti, A. and Golparvar, A.R. 2007. Evaluation of ethnobotany in the region of Chaharmahal & Bakhtyari, West Central Iran. The Proceeding of Symposium of Medicinal Plants, Georgia, USA, 2007.

[5]Gieseker, C.M., Serfling, S. G. and Reimschuessel, R. 2006. Formalin treatment to reduce mortality associated with *Saprolegnia parasitica* in rainbow trout, *Oncorhynchus mykiss*. Aquaculture **253**:120–129.

[6]Khallil, M. A. 2001. Phytofungitoxic properties in the aqueous extracts of some plants. Pak. J. Biol. Sci. **4**:392-394.

[7]Meyer, F.P and Jorgenson, T.A. 1983. Teratological and other effects of malachite green on development of rainbow trout and rabbits. Transactions of the American Fisheries Society **112**:818-824.

[8]Perrucci, S., Cecchini, S., Pretti, C., Varriale Cognetti, A.M., Macchioni, G., Flamini, G. and Cioni, P.L. 1995. *In vitro* antimycotic activity of some natural products against *Saprolegnia ferax*. Phyto Res. **9**:147–149.

[9]Sharif Rohani, M., Ebrahimzadeh Mosavi, M.H., Khosravi, A.R., Baba, M., Bahonar, A.R., Mirzargar, S.S. and Mehrabi, Y. 2006. Activity of some essential oils of *Geranium herbarum* against fungal species in rainbow trout. Vet. Res. J. (Tehran University) **61**:269-272 (in Persian).

[10]Tampieri, M. P., Galuppi, R., Carelle, M. S., Macchioni, F., Cioni, P. L. and Morelli, I. 2003. Effect of selected essential oils and pure compounds on *Saprolegnia parasitica*. Pharmaceutical Biology **41**(8): 584–591.

[11]Tampieri, M.P., Carelle, M.S. and Galuppi, R. 2001. *In vitro* activity of some essential oils against Saprolegniaceae. Proceedings of 18th International Conference of the World Association for the Advancement of Veterinary Parasitology, Stresa, Italy, 26–30 August 2001, p. 46.

[12]Thompson, D.P. 1989. Fungitoxic activity of essential oil components on food storage fungi. Mycologia **81**:151-153.

[13]Zampini, I. C., Vattuone, M.A. and Isla, M. I. 2005. Antibacterial activity of *Zuccagnia punctata* Cav. ethanolic extracts. J. Ethnopharmacol. **102**:450-456.

[14]Zargari, A. 1989-1992. Medicinal Plants. University Publication, Tehran (in Persian).

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