

DOI 10.24425/pjvs.2021.136805

Short communication

Parasiticidal effects of *Tanacetum vulgare* extract against *Ichthyophthirius multifiliis*

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Abstract

Ichthyophthiriasis, which is caused by *Ichthyophthirius multifiliis* (Ich) infections, has a severe impact on productivity in freshwater aquaculture. These infections were previously treated effectively with malachite green, a compound that is now banned on fish farms due to its carcinogenicity. To find efficacious drugs to control Ich, flowers of tansy *Tanacetum vulgare* were evaluated for their antiprotozoal activity. *Tanacetum vulgare* extract significantly reduced the survival of Ich trophonts and theronts. *In vitro*, the extract killed all trophonts at 3200 mg l⁻¹, terminated tomont reproduction at 50 mg l⁻¹, and caused mortality of all theronts at 100 mg l⁻¹. *T. vulgare* extract may be a new and efficacious drug for the control of Ich.

Key words: fish, *Ichthyophthirius multifiliis*, parasite, *Tanacetum vulgare*, tansy

Introduction

Ichthyophthirius multifiliis (Ich) is an important parasitic ciliate that parasitizes the gills and skin of freshwater fish (Buchmann et al. 2001). Ichthyophthiriasis (white spot disease) causes high mortalities in fish worldwide and leads to heavy economic loss in aquaculture (Buchmann et al. 2001). The life cycle of the parasite has 3 stages: an infective theront, a parasitic trophont, and a reproductive encysted tomont (Buchmann et al. 2001). Although malachite green is effective against Ich, it has been banned from use in food fish because of its carcinogenic and genotoxic effects on humans. Since the ban, the control of ichthyophthiriasis depends largely on the use of therapeutants

that are poorly effective or unsafe for the environment (Lahnsteiner and Weismann 2007). *Tanacetum vulgare* L. (family Asteraceae) is a plant known as tansy. It is native to Europe and Asia and has been used for centuries as a medicinal plant. The aerial parts of this plant are commonly used for many medicinal purposes, including the treatment of migraine, neuralgia and rheumatism, and as an anthelmintic agent and insect repellent (Kumar and Tyagi 2013). Extracts of *T. vulgare* have also exhibited parasiticidal activity against the fish parasite *Spiromucleus vortens* (Puk and Guz 2014). However, the anti-Ich activity of *T. vulgare* extracts has not been reported. The aim of this study was to investigate the parasiticidal effect of *T. vulgare* extract against *I. multifiliis*.

Table 1. Mortality of trophonts and theronts, and Ich reproduction (released theronts) after exposure to different concentrations of the *T. vulgare* extract. The values are means \pm SD of 3 replicates.

	Testing time (h)	Concentration (mg l ⁻¹)								
		0	25	50	100	200	400	800	1600	3200
Mortality (%) of trophonts	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.3 \pm 5.8
	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16.7 \pm 5.8	100 \pm 0.0
	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0 \pm 10.0	100 \pm 0.0
Released theronts	1	+	+	-	-	-	-	-	-	-
	2	+	+	-	-	-	-	-	-	-
	3	+	+	-	-	-	-	-	-	-
	4	+	+	-	-	-	-	-	-	-
Mortality of theronts	1	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+
	3	+	+	+	-	-	-	-	-	-
	4	+	+	+	-	-	-	-	-	-

(+) Live theronts in the test wells, (-) No live theronts in the test wells

Materials and Methods

Flowers of tansy were purchased from the NANGA herb wholesale company (Przemysław Figura, Złotów). 20 g of ground dried plant material were soaked in 100 mL of distilled water for 24 h. The extract was filtered through a filter paper, lyophilized, and stored at -20 °C until use. The anti-Ich assay was performed according to the method described by Fu et al. (2014). *I. multifiliis* was isolated from a common carp (*Cyprinus carpio*) which was heavily infected with mature trophonts. In the anti-trophont experiment, approximately 40 trophonts in 100 μ l of dechlorinated freshwater were placed into each well of a 96-well tissue culture plate. A solution of the extract (100 μ l) was added to each well in triplicate (n=3) to make final concentrations of 25, 50, 100, 200, 400, 800, 1600, 3200 and 0 mg l⁻¹ (negative control). Live and dead trophonts were identified based on their movement; the trophonts were considered dead if no motion of the parasite was observed. The trophonts were counted under a microscope (4x) at 1, 2, 3 and 4 h post treatment. For the Ich reproduction experiment, 10 ml of a solution with 40 trophonts were distributed into Petri dishes and incubated for 6 h until encystment. The parasites were then exposed to the extracts at eight concentrations: 25, 50, 100, 200, 400, 800, 1600, 3200 and 0 mg l⁻¹ (negative control), and maintained at 23°C for 12 h until theronts were released. The release of the theronts was then determined for 4 h. The presence of theronts was marked as positive (+) and their absence as negative (-). For the anti-theront experiment, the trophonts were transferred into glass beakers with 50 ml dechlorinated fresh water and incubated at 23°C for 18 h. After theronts were released,

100 μ l of water containing approximately 200 theronts were placed into each well of 96-well microtiter plates. The theronts were exposed to the extracts at concentrations of 25, 50, 100, 200, 400, 800, 1600, 3200 and 0 mg l⁻¹ (negative control) in triplicate for each concentration. The status of the theronts (alive or dead) in each well was assessed at 1, 2, 3, and 4 h post treatment. The presence of live theronts was marked as positive (+) and their absence as negative (-).

Results and Discussion

This study shows that the water extract of *T. vulgare* flowers can kill *I. multifiliis* trophonts and theronts. It also can stop tomont reproduction. All trophonts were killed after 3 h of exposure to *T. vulgare* extract at a concentration of 3200 mg l⁻¹. At a concentration of 1600 mg l⁻¹ the extract caused 20% mortality of the trophonts within 4 h (Table 1). No theronts were released after the exposure of encysted tomonts to the extracts at a concentration of 50 mg l⁻¹ (Table 1). *T. vulgare* extract killed 100% of *I. multifiliis* theronts at a dose of 100 mg l⁻¹ within 3 h (Table 1). In conclusion, our results have demonstrated that the water extract of *T. vulgare* flowers can kill *I. multifiliis* trophonts and theronts and can stop tomont reproduction. Further studies are needed to evaluate the effect of the *T. vulgare* extract in the control of ichthyophthiriasis in fish farms.

References

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