Vol. 66: 29–32, 2005 | DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org | Published August 9

Effect of Australian tea tree oil on *Gyrodactylus* spp. infection of the three-spined stickleback *Gasterosteus aculeatus*

Dietmar Steverding*, Edward Morgan, Patrick Tkaczynski, Foster Walder, Richard Tinsley

School of Biological Sciences, University of Bristol, Woodland Road, Bristol BS8 1UG, UK

ABSTRACT: *Gyrodactylus* spp. infections of commercially farmed fishes are responsible for significant economic losses. Existing treatments have proved uneconomic, stressful to the fishes, and ecologically damaging. Essential oils are naturally occurring compounds that exhibit a wide range of anti-microbial and anti-fungal activities. This study explored the possibility of using Australian tea tree (*Melaleuca alternifolia*) oil (TTO) to treat *Gyrodactylus* spp. infection on the three-spined stickleback *Gasterosteus aculeatus*. In the presence of 0.01% Tween 80 as an emulsifier, TTO treatments at concentrations between 3 and 30 ppmv (parts per million by volume) lowered the prevalence and significantly reduced the parasite burden of sticklebacks naturally infected with *Gyrodactylus* spp. In addition, Tween 80 alone exhibited parasiticidal activity against *Gyrodactylus* spp. These findings show the potential of TTO in combination with Tween 80 as an effective treatment of *Gyrodactylus* spp. infection of fishes.

KEY WORDS: Australian tea tree oil · Tween 80 · *Gyrodactylus* · Monogenea · Three-spined stickleback

INTRODUCTION

Gyrodactylus spp. are monogenean ectoparasites of 19 orders of teleost fishes (Bakke et al. 2002) and are recognised as a significant threat to commercial fisheries (Scholz 1999) because of their unusual reproduction. They employ a unique mode of viviparous reproduction involving polyembryony and production of a succession of generations *in situ* (Cable & Harris 2002). With very short generation time, *Gyrodactylus* spp. populations can reach epizootic levels very rapidly (Thoney & Hargis 1991, Tinsley 2004). Damage caused by gyrodactylids includes epithelial proliferation and increased mucus production in the gills provoked by their feeding activities and by mechanical action of their attachment apparatus (Cone & Odense 1984). This may eventually lead to host death by asphyxiation due to functional failure of the respiratory epithelium (Schäperclaus 1991). In addition, the epithelial damage predisposes the host to secondary bacterial infection (Heggberget & Johnsen 1982, Cone & Odense 1984).

The main strategy to control and eliminate *Gyrodactylus salaris* from Norwegian rivers involves the killing of entire fish populations with the pesticide rotenone (Johnsen & Jensen 1991) and subsequent re-stocking with parasite-free fishes. This method is not only expensive but also presents environmental and health concerns: rotenone has been suspected of being carcinogenic (Gosalvez 1983) and of causing Parkinson's disease (Sherer et al. 2003). Other treatments include toltrazuril (Schmahl & Mehlhorn 1988), praziquantel (Schmahl & Taraschewski 1987), metals (Soleng et al. 1999, Poléo et al. 2004), hydrogen peroxide (Rach et al. 2000), sodium percarbonate (Buchmann & Kristensson 2003), and formaldehyde (Buchmann & Kristensson 2003). However, with the rise in popularity of 'organic' produce and increasing fastidiousness of the consumer, a natural treatment with no harmful human side-effects is desirable.

*Email: dsteverding@hotmail.com

The oil of the Australian tea tree *Melaleuca alternifolia* has been used as a topical antiseptic for almost 80 yr (Altman 1988). Essential oils have been shown to exhibit parasiticidal activity against nematodes (Hasan 1992, Pandey et al. 2000) and bloodstream forms of *Trypanosoma brucei* (Mikus et al. 2000). As tea tree oil (TTO) is a natural product and therefore potentially 'environmentally friendly', its effect on *Gyrodactylus* spp. infection of three-spined sticklebacks, *Gasterosteus aculeatus*, was investigated.

MATERIALS AND METHODS

Reagents. TTO was obtained from Abbey Essentials, and Tween 80 (cell culture tested) from Sigma.

Experimental fish. We collected three-spined sticklebacks from a wild population in a farmland stream near Bristol in February 2004. Fish captures were random and there was wide variation in fish weight and length. In the laboratory, the fish were kept in transparent plastic tanks in 25 l of original stream water and acclimated at 13°C under a 12:12 h light:dark regime for approximately 19 h prior to experimentation.

Tea tree oil treatment of fish. As TTO is hydrophobic, Tween 80 was employed to increase its solubility in water (Carson et al. 1995). White cylindrical plastic containers were filled with 1 l tap water and 0.22 ml Haloex (for dechlorination), 1 ml 10% Tween 80 (0.01% final concentration), and varying amounts (1 to 30 µl) of TTO corresponding to final concentrations of 1 to 30 ppmv (parts per million by volume) were added. Controls included 0.01% Tween 80 alone in dechlorinated water. A second control consisted only of dechlorinated water. The water was stirred thoroughly and kept at 13°C for approximately 19 h. Subsequently, fish were randomly selected and transferred from the reservoir tank to the containers (2 fish per container) and incubated at 13°C. After 48 h, the fish were killed and examined with a dissecting microscope to determine numbers of *Gyrodactylus* spp. on fins, skin and gills. Although 2 species of *Gyrodactylus* (*G. arcuatus*, *G. gasterostei*) can be found on sticklebacks in the UK (and both occurred in this study population), no attempt was made to differentiate between *Gyrodactylus* species detected on the sticklebacks.

RESULTS

In the presence of 0.01% Tween 80 as solvent, TTO had a negative effect on *Gyrodactylus* spp. infections of three-spined sticklebacks, reducing the overall prevalence from 90 to 50% (Fig. 1). In addition, TTO treatment lowered parasite abundance on sticklebacks, particularly between concentrations of 3 and 30 ppmv (Fig. 2). Mean parasite abundance was reduced from 5.9 worms per host for the Tween 80 control treatment to 1.4 worms per host for the 30 ppmv TTO treatment. Analysis of the data revealed that there was a significant difference between parasite abundance in the different treatments ($H = 11.01$, df = 4, $p = 0.026$, nonparametric Kruskal-Wallis). A series of Mann-Whitney tests showed no significant difference between para-

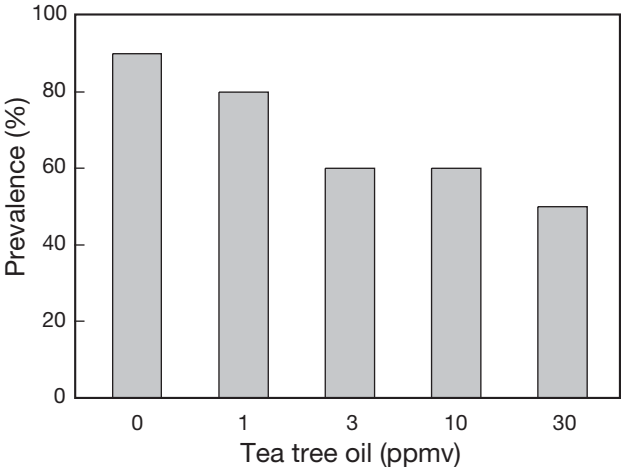

Fig. 1. *Gyrodactylus* spp. Prevalence on sticklebacks *Gasterosteus aculeatus* after tea tree oil (TTO) treatment. Sticklebacks naturally infected with *Gyrodactylus* spp. were treated with different concentrations (parts per million by volume, ppmv) of TTO in the presence of 0.01% Tween 80; control treatment consisted of 0.01% Tween 80 alone. Numbers of parasites per fish were recorded after maintenance at 13°C for 48 h. Each treatment comprised 10 fish from 2 independent captures

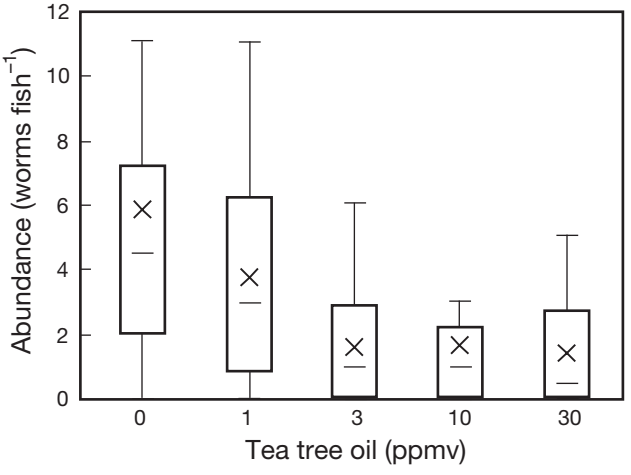

Fig. 2. *Gyrodactylus* spp. Parasite abundance on sticklebacks *Gasterosteus aculeatus* after TTO treatment, showing median (bar), mean (cross), interquartile range containing 50% of values (box) and range (whiskers) for each concentration. Further details as in Fig. 1 legend

site abundance in the control treatment and 1 ppmv TTO treatment (W = 115, p = 0.4697); however, fish treated with 3 to 30 ppmv TTO had significantly fewer parasites than those untreated (control vs. 3 ppmv oil: W = 137.5, p = 0.0139; control vs. 10 ppmv oil: W =135, p = 0.0239; control vs. 30 ppmv oil: W = 136.5, p = 0.0178). Comparison of mean parasite intensities displayed the same trend (data not shown).

Tween 80 alone had an effect on *Gyrodactylus* spp. infection on sticklebacks (Fig. 3). In the absence of Tween 80, mean parasite abundance was 12.6 compared to 5.9 in the presence of 0.01% Tween 80. This difference was significant (W = 132.5, p = 0.0402, Mann-Whitney test). Thus, compared to the control without Tween 80, treatment of fish with 30 ppmv TTO in the presence of 0.01% Tween 80 resulted in a 90% reduction in parasite abundance (from 12.6 to 1.4 worms per host).

No ill effects were detected amongst the fish treated with TTO in Tween 80 at the concentrations employed.

DISCUSSION

This study has shown that TTO has a negative effect on *Gyrodactylus* spp. infection of three-spined sticklebacks. The parasiticidal action of the oil is primarily displayed in the presence of the solubilising agent Tween 80: in the absence of the solvent, TTO has little effect on parasite burdens (data not shown). This is due to the insolubility of TTO in water. Interestingly, Tween 80 alone displayed parasiticidal activity against *Gyrodactylus* spp. worms, although it is a non-toxic surfactant widely used as an emulsifier, dispersant or stabiliser in food, pharmaceutical preparations and cosmetics (National Toxicology Program 1992). However, it has been previously shown that Tween 80 has intrinsic anti-tumour activity (Crispens & Sorenson 1988).

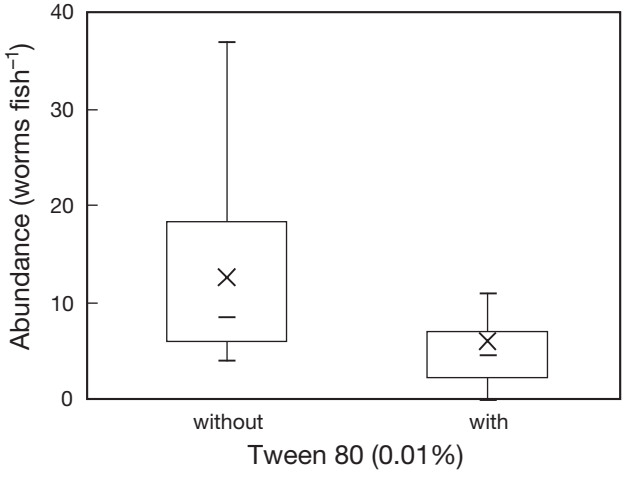

Fig. 3. *Gyrodactylus* spp. Parasite abundance on sticklebacks *Gasterosteus aculeatus* exposed to Tween 80. Sticklebacks naturally infected with *Gyrodactylus* spp. were exposed to 0.01% Tween 80 (with) or water alone (without). Further details as in legends to Figs. 1 & 2

Infections with *Gyrodactylus* spp. are pathogenic to fishes principally at high infection levels (Cone & Odense 1984). This study has shown that, although TTO does not completely remove all *Gyrodactylus* spp. worms within 2 d, it reduces infection levels significantly. It is possible that continuous TTO treatment would maintain an infection level low enough to prevent pathogenicity until the host develops resistance to the parasite. Lindenstrøm & Buchmann (2000) showed that rainbow trout *Oncorhynchus mykiss* acquired resistance against *Gyrodactylus derjavini* 5 wk post infection, exhibiting decreasing parasite infections regardless of high infection pressure. The same general principle (with various timescales) has been demonstrated in other fish–*Gyrodactylus* spp. systems studied experimentally (Harris 1993, Cable et al. 2000).

Gyrodactylus spp. infections in small groups of naturally infected sticklebacks are potentially unstable (Tinsley 2001). *In situ* reproduction could have produced an increase in infection levels during the course of this experiment (above those occurring at capture). Alternatively, some burdens may have declined under the influence of a developing immune response. These effects are likely to have been minor over the 48 h experimental period and would be expected to affect all replicates equally. Whatever the intrinsic capacity for parasite population increase, typically exhibited initially by captive samples of naturally infected sticklebacks (see Tinsley 2001), the significant reduction in infection levels following treatment indicates that TTO is very effective in reversing any potential for increase, and acts directly on the parasite. Whether TTO also has potential effects on parasite reproduction and/or survival of offspring remains to be determined.

The exact mode of action of TTO against helminths is unknown. Investigations into the effect of essential oils as nematicides have suggested that the presence of oxygenated compounds is partially responsible (Adb-Elgawad & Omer 1995). In studies of the efficacy of TTO against bacteria, the oil has been shown to have adhesion-inhibiting activity (Takarada et al. 2004) and to disrupt the permeability of cytoplasmic membranes, leading to loss of chemiosmotic control and cell death (Cox et al. 2000). The latter mode of action specifically involves the lipophilic monoterpen terpinen-4-ol (Carson et al. 2002), the major constituent (30%) of TTO. Terpinen-4-ol is responsible for most of the antimicrobial activities (Carson & Riley 1995) and has been reported to exhibit trypanocidal activity (Mikus et al. 2000).

Epithelial damage caused by *Gyrodactylus* species often promotes secondary infection by micro-organisms that are regarded as the primary cause of fish death (Cone & Odense 1984, Mo 1994). Given the well-known anti-microbial properties of TTO (Carson et al. 1995), it is likely that, in addition to the reduction of *Gyrodactylus* spp. burdens, TTO treatment will aid in sterilising inflammatory wounds. The effect of TTO on other infections was suggested by the reduction of skin ectoparasites such as *Trichodina* species found on sticklebacks in this study (data not shown).

In conclusion, this preliminary study has shown that TTO has potential as a natural, consumer-friendly alternative to current chemotherapy treating *Gyrodactylus* spp. infection of fishes. In addition, this work adds to the growing evidence supporting the use of essential oils as commercial parasiticides.

LITERATURE CITED

Abd-Elgawad MM, Omer EA (1995) Effect of essential oils of some medicinal plants on phytonematodes. Anz Schädlingskd Pflanzenschutz 68:82–84

Altman PM (1988) Australian tea tree oil. Aust J Pharm 69: 276–278

Bakke TA, Harris PD, Cable J (2002) Host specificity dynamics: observations on gyrodactylid monogeneans. Int J Parasitol 32:281–308

Buchmann K, Kristensson RT (2003) Efficacy of sodium percarbonate and formaldehyde bath treatments against *Gyrodactylus derjavini* infestations of rainbow trout. N Am J Aquacult 65:25–27

Cable J, Harris PD (2002) Gyrodactylid developmental biology: historical review, current status and future trends. Int J Parasitol 32:255–280

Cable J, Harris PD, Bakke TA (2000) Population growth of *Gyrodactylus salaris* (Monogenea) on Norwegian and Baltic Atlantic salmon (*Salmo salar*) stocks. Parasitology 121:621–629

Carson CF, Riley TV (1995) Antimicrobial activity of the major components of the essential oil of *Melaleuca alternifolia*. J Appl Bacteriol 78:264–269

Carson CF, Hammer KA, Riley TV (1995) Broth micro-dilution method for determining the susceptibility of *Escherichia coli* and *Staphylococcus aureus* to the essential oil of *Melaleuca alternifolia* (tea tree oil). Microbios 82:181–185

Carson CF, Mee BJ, Riley TV (2002) Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. Antimicrob Agents Chemother 46:1914–1920

Cone DK, Odense PH (1984) Pathology of 5 species of *Gyrodactylus* Nordmann, 1832 (Monogenea). Can J Zool 62: 1084–1088

Cox SD, Mann CM, Markham JL, Bell HC, Gustafson JE, Warmington JR, Wyllie SG (2000) The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). J Appl Microbiol 88:170–175

Crispens CG Jr, Sorenson JR (1988) Treatment of reticulum cell sarcoma in SJF/J mice with Tween 80. Anticancer Res 8:1341–1343

Gosalvez M (1983) Carcinogenesis with the insecticide rotenone. Life Sci 32:808–816

Harris PD (1993) Interactions between reproduction and population biology in gyrodactylid monogeneans—a review. Bull Fr Pêche Piscic 328:47–65

Hasan N (1992) Nematicidal action of essential oils isolated from selected indigenous plants against root-knot nematode, *Meloidogyne incognita*. Acta Bot Indica 20:89–92

Heggberget TG, Johnsen BO (1982) Infestations by *Gyrodactylus* sp. of Atlantic salmon, *Salmo salar* L., in Norwegian rivers. J Fish Biol 21:15–26

Johnsen BO, Jensen AJ (1991) The *Gyrodactylus* story in Norway. Aquaculture 98:289–302

Lindenstrøm T, Buchmann K (2000) Acquired resistance in rainbow trout against *Gyrodactylus derjavini*. J Helminthol 74:155–160

Mikus J, Harkenthal M, Steverding D, Reichling J (2000) *In vitro* effect of essential oils and isolated mono- and sesquiterpenes on *Leishmania major* and *Trypanosoma brucei*. Planta Med 66:366–368

Mo TA (1994) Status of *Gyrodactylus salaris* problems and research in Norway. In: Pike AW, Lewis JW (eds) Parasitic diseases of fish. Samara, Dyfed, p 43–58

National Toxicology Program (1992) NTP toxicology and carcinogenesis studies of polysorbate 80 (CAS No. 9005-65-6) in F344/N rats and B6C3F1 mice (feed studies). Natl Toxicol Program Tech Rep Ser 415:1–255

Pandey R, Kalra A, Tandon S, Mehrotra N, Singh HN, Kumar S (2000) Essential oils as potent source of nematicidal compounds. J Phytopathol 148:501–502

Poléo ABS, Schjolden J, Hansen H, Bakke TA, Mo TA, Rosseland BO, Lydersen E (2004) The effect of various metals on *Gyrodactylus salaris* (Platyhelminthes, Monogenea) infections in Atlantic salmon (*Salmo salar*). Parasitology 128: 169–177

Rach JJ, Gaikowski MP, Ramsay RT (2000) Efficacy of hydrogen peroxide to control parasitic infestations on hatchery-reared fish. J Aquat Anim Health 12:267–273

Schäperclaus W (1991) Fish diseases, 5th edn. Oxonian Press, New Dehli

Schmahl G, Mehlhorn H (1988) Treatment of fish parasites. 4. Effects of sym. triazinone (toltrazuril) on Monogenea. Parasitol Res 75:132–143

Schmahl G, Taraschewski H (1987) Treatment of fish parasites. 2. Effects of praziquantel, niclosamide, levamisole-HCl and metrifonate on Monogenea (*Gyrodactylus aculeati*, *Diplozoon paradoxum*). Parasitol Res 73:341–351

Scholz T (1999) Parasites in cultured and feral fish. Vet Parasitol 84:317–335

Sherer TB, Betarbet R, Testa CM, Seo BB and 6 others (2003) Mechanism of toxicity in rotenone models of Parkinson's disease. J Neurosci 23:10756–10764

Soleng A, Poléo ABS, Alstad NEW, Bakke TA (1999) Aqueous aluminium eliminates *Gyrodactylus salaris* (Platyhelminthes, Monogenea) infections in Atlantic salmon. Parasitology 119:19–25

Takarada K, Kimizuka R, Takahashi N, Honma K, Okuda K, Kato T (2004) A comparison of the antibacterial efficacies of essential oils against oral pathogens. Oral Microbiol Immunol 19:61–64

Thoney DA, Hargis WJ Jr (1991) Monogenea (Platyhelminthes) as hazards for fish in confinement. Annu Rev Fish Dis 1:133–153

Tinsley RC (2001) Population dynamics of *Gyrodactylus* on sticklebacks. In: Halton DW, Behnke JM, Marshall I (eds) Practical exercises in parasitology. Cambridge University Press, Cambridge, p 147–160

Tinsley RC (2004) Platyhelminth parasite reproduction: some general principles derived from monogeneans. Can J Zool 82:270–291

Editorial responsibility: Wolfgang Körting, Hannover, Germany

Submitted: September 27, 2004; Accepted: February 24, 2005
Proofs received from author(s): July 8, 2005