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# Efficacy of ginger-based treatments against infection with *Gyrodactylus turnbulli* in the guppy (*Poecilia reticulata* (Peters))

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### ABSTRACT

Monogenean infections of commercially farmed fishes are responsible for significant economic losses and existing chemical therapeutants, often stressful to the fish, pose associated risks. As part of a recent trend to move towards the use of alternative, plant-based remedies for commonly occurring aquaculture-related diseases, the efficiency of ginger (*Zingiber officinale*) was investigated against the monogenean parasite *Gyrodactylus turnbulli* in the guppy. *In vitro* trials revealed the clear anti-parasitic effects of ginger. Ethanolic and aqueous extracts, prepared from freeze dried ginger, were tested. An increase in extract concentration was associated with reduced time to parasite immobilisation, with ethanolic extract being more efficient; at 75 and 200 ppt aqueous ginger extract parasites died at  $65.6 \pm 2.8$  and  $1.8 \pm 0.2$  min, respectively, whereas at 5 and 40 ppt ethanolic extract parasites died at  $26.1 \pm 0.7$  and  $4.9 \pm 0.3$  min, respectively.

Bathing *G. turnbulli*-infected fish in ethanolic ginger extract (*i.e.* 5 and 7.5 ppt for 90 and 30 min, respectively) significantly reduced infection prevalence and intensity when compared to the water and ethanol controls. The higher concentration (*i.e.* 7.5 ppt) proved as equally effective as Praziquantel, the conventionally used chemical treatment for gyrodactylosis, with the fish appearing to be completely cleared of the infection in both cases. Oral treatments of *G. turnbulli*-infected guppies with diets supplemented with 10 and 20% ginger powder proved to be ineffective in decreasing parasite load. These findings demonstrate that immersion in ginger extract offers an effective, alternative treatment against monogenean infection in fish.

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## 1. Introduction

Ornamental fish production for the aquarium hobbyist trade is a rapidly growing and important sector of the aquaculture industry as a whole (Tlustý, 2002). Statistics reported to FAO (2005) indicate that the world export value

in 1998 of ornamental fishes was US\$174 million and an annual export of around 1 billion ornamental fish has been reported for 2012 (Dykman, 2012). As with food fish, ornamental fish species suffer from parasitic infections which can result in high mortalities and cause significant economic losses, threatening the commercial viability of the industry.

Gyrodactylids, belonging to the family of monogenean flatworms, are commonly occurring ectoparasites of freshwater fish. *Gyrodactylus turnbulli* has been shown to have

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low host-specificity and an ability to infect a wide range of hosts (King and Cable, 2007). Gyrodactylids can be pathogenic to fish; they attach to the epithelium and cause extensive tissue damage using a set of hooks on their attachment organ or opisthaptor, which, in turn, causes an increased production of mucous, disrupting the protective functions of the skin (Harms, 1996) and promoting secondary bacterial and fungal infections (Akmirza, 2013). Their direct life cycle, hyper-viviparity, a combination of sexual and asexual reproduction modes and progenesis (Cable and Harris, 2002; Schelkle et al., 2012) enable parent parasites to produce offspring in as little as 24 h from the day they were born (Scott, 1982). Parasite transmission occurs by direct contact between fish as well by the water (Cable et al., 2002) and a single parasite can initiate an entirely new population (Schelkle et al., 2009). In intensive aquaculture production situations, where stocking densities are high, infestation is more likely to occur and, once present, is very difficult to eradicate. The guppy (*Poecilia reticulata*), a tropical, freshwater fish originating from the Caribbean and Central and South America, is an important cultured ornamental species and, due to the fact that it is a natural host for *G. turnbulli*, has been used extensively to study host–parasite relationship (Harris and Lyles, 1992).

Chemical therapeutants commonly used to treat gyrodactylid infections pose associated problems such as low efficacy, host toxicity and environmental and human health concerns (see review by Schelkle et al., 2009). In addition, antihelminthic-resistant strains of parasites can develop (Schmahl et al., 1989). In recent years a need has been recognised to move away from the currently used chemical treatments in an attempt to find alternative strategies of disease control and new natural treatments which are effective in promoting fish health and are safe for use (Schelkle et al., 2011). A recent review of research into the usage of plant extracts in aquaculture by Reverter et al. (2014) suggests their potential as effective substitutes to conventionally used therapeutants. Ginger (*Zingiber officinale*) is a plant which has been widely used as a medicine in China and India for many years and is considered to have broad-spectrum of prophylactic and therapeutic properties (Ernst and Pittler, 2000). The current study examines its potential as a practical, alternative treatment for *G. turnbulli* infection in guppies. Treatment with different ginger preparations at various concentrations and modes of application was examined and its efficacy was evaluated.

## 2. Materials and methods

### 2.1. Source of animals and parasites

Guppies were obtained from a commercial ornamental fish farm in the Arava, Israel. Upon arrival, fish were anaesthetised using clove oil at a concentration of 250 ppm (Kildea et al., 2004) and confirmed as infected with monogenean parasites using a dissecting microscope (Zeiss Stemi 2000-C, Carl Zeiss, Oberkochen, Germany). The monogenean was identified as *G. turnbulli* based on its morphological attachment structures, using the methods detailed in Paladini et al. (2009). The fish were then

transferred to a 200L tank filled with de-chlorinated tap water (50 mg L<sup>-1</sup> of sodium thiosulphate pentahydrate, and supplied with aeration and submerged biological filters. Fish stocking density was maintained at a high level of c. 2.8 fish L<sup>-1</sup> to promote a high level of infection. Throughout the experiment water temperature was maintained at 25 ± 1 °C and a 12 h light: 12 h dark cycle was maintained. Tanks were siphoned daily and monitored for water quality parameters, including ammonia and nitrites, every 3 days using AquaMerck kits (Merck, Germany). Feeding was done once a day at 2% of the body weight using a commercial guppy feed (Mem Ornamental, BernAqua, Belgium). The experimental protocols were approved by the Ben Gurion University Committee for the Ethical Care and Use of Animals, Ben Gurion University of the Negev, Israel. Authorisation number: IL-79-10-2012.

### 2.2. In vitro effect of ginger extracts

#### 2.2.1. Preparation of ginger extracts

Fresh ginger rhizomes, sourced from a local farm (Tekoa Farms, Israel) were brought to the laboratory, peeled, washed, sliced and freeze-dried before being stored at –80 °C.

Ginger was then ground by a stainless laboratory blender. Two extraction methods were carried out using either double distilled water (DDW) or 75% ethanol. DDW extract preparation: powdered ginger was extracted at a ratio of 1 g per 10 ml DDW in 15 ml falcon tubes. The mixture was vortexed for 2 min, and then centrifuged at 2939 × g (4500 rpm) for 20 min at 4 °C. The supernatant was collected and passed through a filter paper and then through 0.45 and 0.22 μm Millex-GV filter. Finally, the extract (considered as the 100% stock solution) was divided into 1 ml aliquots in 2 ml eppendorf tubes and stored at –20 °C. Ethanol extraction: extraction was similar to the aqueous extraction method with the difference being that, prior to centrifugation, the ginger powder was mixed with 75% ethanol, placed in an oven at 40 °C for 1 h (Mukherjee et al., 2012) and vortexed every 15 min.

#### 2.2.2. In vitro parasite survival

Guppies from the infection tank were randomly chosen, anaesthetised as described above, euthanised by pithing and then examined under a dissecting microscope for the presence of *G. turnbulli*. Fins with parasites were clipped and skin with parasites was scraped off using a cover slip. Scales and fins to which parasites were attached were transferred individually using watchmaker's forceps to wells of a 24-well plate containing 0.5 ml filtered tank water, with various treatments added and mixed by pipetting. Each extract was tested at the following concentrations: aqueous ginger extract at 200, 150, 100, and 75 ppt; ethanolic ginger extract at 40, 20, 10, 7.5, and 5 ppt. For each concentration, 15–30 parasites were examined. As controls, water and 75% ethanol were used. Immediately following exposure to the different treatments, parasites were observed under a dissecting microscope and time to cessation of movement (indicating parasite

immobilisation and death, according to Fridman et al., 2014) was documented.

### 2.3. Bath treatment with ginger extract

#### 2.3.1. Toxicity test

Toxicity trials were performed to determine the concentration of ginger extract which could be used for immersive treatment without compromising the health of the fish. Ten infected guppies were placed in a 1.5 L glass beaker equipped with aeration and containing 1 L of dechlorinated tap water supplemented with ethanolic ginger extract at 2.5, 5 and 7.5 ppt. Control groups of fish were tested under the same condition and included treatments of water and ethanol at the equivalent concentrations to the extract. All tests were carried out in triplicates. Mortality was recorded every hour post-exposure.

#### 2.3.2. *In vivo* bath treatment

*G. turnbulli* infected fish were used in the trial (as determined by examination of 15 fish from the infection tank). The trial was conducted using the same experimental set-up as the toxicity test, using 1 L beakers with 10 fish per beaker, in triplicate, with gentle aeration. Bath treatments of 5 and 7.5 ppt of ethanolic ginger extract for 90 and 30 min, respectively, were applied. For controls, 5 and 7.5 ppt of 75% ethanol and a control of water only were used. For a positive control, fish were treated with Praziquantel at 3 ppm for 24 h. Following immersion period, fish were moved to 1 L beakers containing de-chlorinated tap water for 24 h. Fishes were then anaesthetised as described above and the number of parasites on both sides of the caudal fin was determined by direct examination under a dissecting microscope. In addition, a skin scrape on one side of the fish was performed and the total number of parasites was counted under a light microscope. Prevalence was calculated as the proportion of infected hosts amongst all the hosts examined and intensity was calculated as mean number of parasites per inspected areas of the infected hosts (Bush et al., 1997). There was no mortality in the treated fish and all the fish from each replicate were examined.

### 2.4. In feed treatment

#### 2.4.1. Feed preparation

Dried ginger rhizomes and commercial guppy feed were ground separately using a stainless laboratory blender and then mixed together to obtain 10 and 20% ginger supplemented feed. A paste was prepared by adding 10 ml of DDW per 25 g of food; the mixture was prepared using a mortar and pestle and then spread on a tin foil, and allowed to dry at room temperature for 48 h. Once dry, the food was ground and passed through 500 and 1000  $\mu\text{m}$  laboratory mesh sieves. Feed was stored in sealed specimen collection cups at  $-20^\circ\text{C}$  and used within 1 month. A preliminary palatability trial was performed for 14 days on a separate group of guppies, suggesting that ginger supplementation at 10 and 20% did not affect palatability.

#### 2.4.2. Experimental design

At the start of the trial, a representative group of 25 fish were randomly sampled from the infection tank and examined for the presence of *G. turnbulli* according to the methods described above, revealing mean infection prevalence (%) and intensity of  $86.7 \pm 11.5$  and  $3.5 \pm 2.7$ , respectively. For the feeding trial, guppies from the infection tanks were randomly selected and distributed among sixteen 10 L aquaria at 30 fish per aquarium, i.e. 20 large female guppies (mean weight  $3.2 \pm 0.38$  g) and 10 small males (mean weight  $2.29 \pm 0.27$  g). Each aquarium was equipped with aeration and a submerged biological filter. Water temperature was maintained at  $25 \pm 1^\circ\text{C}$  under a 12 h light:12 h dark cycle.

#### 2.4.3. Feeding trial

Diets, i.e. a commercial feed supplemented with 10 and 20% ginger were administered for a period of 14 days. For a negative control, un-supplemented feed was used, prepared in the same way but without the addition of ginger. For a positive control, on Day 0 fish were bathed in 3 ppm Praziquantel for 24 h, followed by a 100% freshwater exchange and then subsequently fed with control feed for the remainder of the trial. All fish were fed at a rate of 2% of body weight  $\text{day}^{-1}$ , divided into two portions given twice daily (1% each feeding). The feeding dose was re-adjusted weekly according to mortality. A 10% water exchange was carried out daily by siphoning the bottom and water quality parameters, i.e. nitrites, ammonia, oxygen and temperature were monitored every 3 days. When values of ammonia and nitrites exceeded 0.5 and 0.05  $\text{mg L}^{-1}$ , respectively, a larger water exchange was applied. Dead fish were removed daily and freshly dead fish were examined for the presence of *G. turnbulli* to confirm mortality due to infection with the parasite. At the end of the feeding trial 10 large females and 5 small males from each aquarium (total 15 per treatment) were examined for the presence of *G. turnbulli*, according to the method described above, and infection prevalence (%) and intensity were calculated.

### 2.5. Statistical analyses

Statistical analyses were performed using the statistical software SigmaStat version 3.5 (Systat Software, Inc., San José, CA). Results of *in vitro* trials (i.e. time to parasites' immobilisation) were compared using one-way ANOVA followed by Dunn's *post hoc* test. Results of infection intensity from the *in vivo* trials were compared using one-way ANOVA on individual fish followed by Tukey's *post hoc* test. Data, appearing in percentage (infection prevalence and mortality) underwent arcsine transformation before being statistically analysed by one-way ANOVA. Differences were considered statistically significant at  $p < 0.05$ .

## 3. Results

### 3.1. *In vitro* parasite survival

The effect of ginger extract on *G. turnbulli* was apparent soon after exposure; the parasite started to immediately twitch, shortly followed by its detachment from the fin clip

**Table 1**  
*In vitro* effect of differently prepared ginger extracts on the survival of *Gyrodactylus turnbulli*.

Treatment	Conc. (ppt)	N	Time to death (min)	Range of survival (min)
Ginger extract (aqueous)	75	30	65.6 ± 2.8*	25–90
	100	30	83.5 ± 3.7*	20–115
	150	30	6.2 ± 0.5*	2–12
	200	30	1.8 ± 0.2*	1–4
Ginger extract (ethanolic)	5	60	26.1 ± 0.7 <sup>†</sup>	18–45
	7.5	60	18.8 ± 0.6 <sup>†</sup>	12–30
	10	60	14.6 ± 0.3 <sup>†</sup>	9–21
	20	15	9.9 ± 0.3 <sup>†</sup>	8–12
	40	15	4.9 ± 0.3 <sup>†</sup>	4–7
Ethanol control	5	30	645 ± 5.7	600–690
	7.5	30	495 ± 5.3	450–540
	10	15	100 ± 0.2*	47–175
	20	15	67 ± 0.1*	21–100
Water control	0	60	1192.3 ± 8.6	1080–1320
	0	60	11 ± 0*	4–19
Praziquantel	3 ppm	60	306.1 ± 5.7*	240–390

Different symbols indicate significant differences between treatments ( $p < 0.05$ ).

<sup>†</sup> Significant difference between ethanolic ginger extract compared to equivalent concentrations of ethanol control.

\* Significant difference compared to water control.

or scale, after which time movements slowed down and eventually stopped. In the untreated control (water) parasites survived an average of 19.8 h (Table 1). A clear, dose dependent anti-parasitical effect was observed for both extraction methods, however the ethanolic ginger extract showed a greater efficiency; exposure to 75 and 200 ppt aqueous ginger extract resulted in parasite's death after  $65.6 \pm 2.8$  and  $1.8 \pm 0.2$  min, respectively, and exposure to ethanolic ginger extract at 5 and 40 ppt killed the parasite after  $26.1 \pm 0.7$  and  $4.9 \pm 0.3$  min, respectively (Table 1). Praziquantel killed the parasite only after 5.1 h. Exposure to 75% ethanol (control treatment) affected parasites' survival, yet the time to death was significantly greater as compared to the equivalent ginger ethanolic extract at all tested concentrations (e.g.  $26.1 \pm 0.7$  min at 5 ppt of ginger extract as compared to  $654 \pm 5.7$  min at 5 ppt of 75% ethanol). Time to death following exposure to 75% ethanol at the lower concentrations (5 and 7.5 ppt) did not significantly differ from the water control.

### 3.2. Toxicity test

Survival of guppies bathed in ethanolic ginger extract at a concentration of 5 ppt decreased after 2 h to 93.3% and at a concentration of 7.5 ppt after 0.5 h to 90%. Therefore, *in vivo* bath treatment using 5 and 7.5 ppt ginger ethanol extract were chosen for the duration of 90 and 30 min, respectively (Table 2).

### 3.3. Infection rate of *G. turnbulli* following bath treatments

Bath treatment with ethanolic ginger extract at concentrations of 5 and 7.5 ppt resulted in a significant reduction in the prevalence and intensity of *G. turnbulli*, as compared to both the equivalent ethanol and water controls (Table 3). A reduction in both prevalence and intensity was also observed in the ethanol controls as compared to the water control, albeit non-significant. Praziquantel

**Table 2**  
 Survival of guppies (%) following exposure to ginger extract. Mean ± s.e.m. of three replicates with 10 fish per replicate.

Bath treatment	Survival (%)					
	Time of exposure (h)	Concentration (ppt)				
		0	2.5	5	7.5	10
Ginger extract (ethanolic)	0.5	100	100	100	90	73.3 ± 6.7
	1	100	100	96.7	80	63.3 ± 3.3
	2	100	100	93.3	73.3	56.7 ± 3.3
	3	100	100	90	63.3	50
Ethanol control	0.5	100	100	83.3	63.3	50
	1	100	100	100	100	96.7 ± 3.3
	2	100	100	100	100	90 ± 5.7
	3	100	100	100	96.6	86.7 ± 8.8
Water control	0.5	100	100	96.7	100	86.7 ± 3.3
	1	100	100	100	100	100
	2	100	100	100	100	100
	3	100	100	100	100	100
	4	100	100	100	100	100

**Table 3**  
Mean prevalence (%) and mean intensity  $\pm$  s.e.m of *Gyrodactylus turnbulli* infection in guppies following bath treatment ( $n=3$ ; 10 fish per replicate).

Treatment	Conc. (ppt)	Treatment time (min)	Mean prevalence (%)	Mean intensity
Ginger extract (ethanolic)	5	90	0.3 $\pm$ 0.3 <sup>†,*</sup>	0.3 $\pm$ 0.3 <sup>†,*</sup>
	7.5	30	0 <sup>†,*</sup>	0 <sup>†,*</sup>
Ethanol control	5	90	93.3 $\pm$ 0.3	4.9 $\pm$ 0.8
	7.5	30	80 $\pm$ 1.0	7.0 $\pm$ 2.2
Water control	0	90	96.6 $\pm$ 0.3	20.7 $\pm$ 6.8
Praziquantel	3000	1440 (24h)	0 <sup>†,*</sup>	0 <sup>†,*</sup>

Different symbols indicate significant differences between treatments ( $p < 0.05$ ).

<sup>†</sup> Significant difference between ethanolic ginger extract compared to equivalent concentrations of ethanol control.

<sup>\*</sup> Significant difference compared to water control.

**Table 4**  
Effect of ginger supplemented diets on mean prevalence (%) and mean intensity  $\pm$  s.e.m of *Gyrodactylus turnbulli* infection in guppies ( $n=4$ ; 15 fish per replicate).

Treatment	Mean prevalence (%)	Mean intensity
Control	52.5 $\pm$ 8.7 <sup>ab</sup>	3.7 $\pm$ 1 <sup>ab</sup>
Praziquantel <sup>A</sup>	25 $\pm$ 3.2 <sup>b</sup>	1.7 $\pm$ 0.3 <sup>b</sup>
Ginger 10%	75 $\pm$ 7.5 <sup>a</sup>	5.3 $\pm$ 1 <sup>ab</sup>
Ginger 20%	92.5 $\pm$ 3 <sup>a</sup>	6.2 $\pm$ 1.4 <sup>a</sup>

Different superscript letters within columns indicate significant differences between treatments ( $p < 0.05$ ).

<sup>A</sup> Praziquantel at a concentration of 3 ppm was applied as a single 24h bath treatment on Day 0.

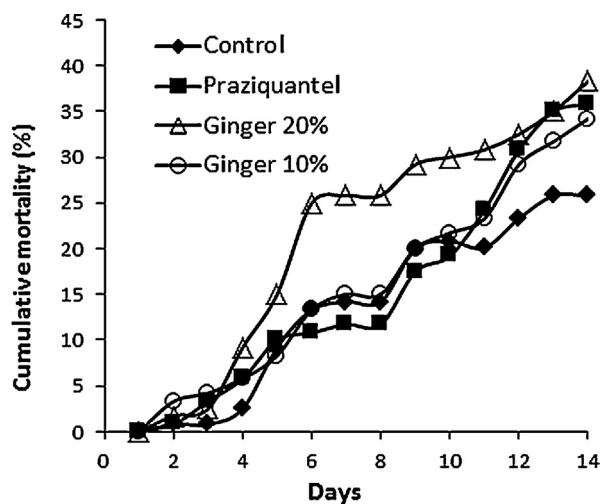
treatment resulted in significant reduction in infection prevalence and intensity as compared to the controls (Table 3).

### 3.4. Infection with *G. turnbulli* following oral treatments

Guppies fed diets supplemented with both 10 and 20% freeze-dried ginger powder at the end of the 14-day feeding trial displayed a higher prevalence (%) and intensity of *G. turnbulli* infection as compared to the control group fed non-supplemented food (Table 4). Treatment with Praziquantel resulted in significant reduction in infection. Mortality did not significantly differ amongst treatment groups ( $p > 0.05$ ) (Fig. 1).

## 4. Discussion

Monogenean infections in fish are a significant constraint for the sustainability and development of the aquaculture sector due to associated losses. Whilst the use of anti-parasitic treatments – either chemotherapeutic or herbal – is currently not regulated in ornamental fishes, the treatment of those destined for human consumption is highly regulated and many previously used anti-parasitic compounds are no longer authorised for use, e.g. malachite green and formalin (European Union Biocide Product Directive 98/8/EC, European Council Regulation 2377/90). Therefore, in the search for natural treatment alternatives, numerous plants have been investigated for their curative properties against monogenean infections in fish (Reverter et al., 2014). Ginger is reported to have anti-oxidant (Kim et al., 2007) and anti-inflammatory (Grzanna et al., 2005) effects in humans and immune modulatory properties in terrestrial animals (Tan and Vanitha, 2004; Zhou et al., 2006; Ali et al., 2008). Similarly, in



**Fig. 1.** Cumulative mortality (%) of guppies treated with ginger supplemented diets for 14 days. An un-supplemented diet was used as a negative control and a single Praziquantel bath (3 ppm) was used as positive control ( $n=4$ ; 25 fish per replicate).

aquatic animals, anti-bacterial and immune-stimulatory properties of ginger have been reported in the Rainbow trout (*Oncorhynchus mykiss*), anti-parasitic properties in the African catfish (*Clarias gariepinus*) (Khalil and Walaa, 2013) and growth enhancement and immune-stimulatory effects in the freshwater prawn (*Macrobrachium rosenbergii*) (El-Desouky et al., 2012). The current study aimed to investigate the efficacy of ginger both as an in-feed and bath treatment against *G. turnbulli*.

In the present study, *in vitro* trials clearly demonstrate the efficacy of ginger against *G. turnbulli*. A clear time and dose dependent effect was seen amongst all treatments which is in agreement with previous studies which examined the anti-helminthic and antibacterial effects of ginger (Malu et al., 2009; Lin et al., 2010). When comparing between the efficacies of the different extracts ethanolic ginger extract was found to be much more effective than the aqueous extract. The lowest concentration of aqueous extract examined, i.e. 75 ppt resulted in around 66 min to parasite death, whereas 40 ppt of the ethanolic extract killed the parasite within 5 min. In general, more lipophilic compounds, which cross the helminth's surface membrane and act within the organism or cause membrane disruption, are extracted by ethanol and methanol as compared to extraction in water (Liu et al., 2010). This could



possibly explain the improved anti-helminthic efficiency of the ethanolic extraction, as compared with aqueous extraction, as seen in the present study. Indeed, previous studies have shown that ethanolic extracts of ginger retain a higher anti-bacterial activity compared to other solvents tested, such as distilled water and chloroform (Malu et al., 2009; Harmalkar and Desai, 2011).

Ginger contains polyphenolic compounds such as eugenol, shogaols, zingerone, gingerdiols, gingerols, etc., which are presumed to be responsible for its therapeutic properties. These include potent anti-oxidant properties (Stoilova et al., 2007; Singh et al., 2008) as well as anti-bacterial, anti-inflammatory, anti-tumour-promoting and anti-angiogenic activities (Kim et al., 2005; Singh et al., 2008). A study by Mukherjee et al. (2012) suggested that extraction of polyphenols from ginger is affected, in order of importance, by the solvent, time and temperature of extraction. Results from the same study showed that optimal extraction was obtained with ethanol, at a concentration of 75%, at 40 °C, for 60 min, conditions which were likewise applied in the current study. Similarly, Liu et al. (2014) showed that the amount of gingerols extracted by 95% ethanol were 4.6-fold higher as compared to water extraction. Therefore, ginger extracts prepared for the present study proved to be effective in killing *G. turnbulli* and efficiency seemed to be influenced by the method of extraction, concentration, and time of exposure.

Bath treatment has been the traditional treatment method of choice against monogenean infection (Schmahl and Mehlhorn, 1988). This method of ginger application was shown to be safe for use and did not cause undesired effects on haematological and biochemical parameters in the sea bass *Dicentrarchus labrax* (Yilmaz and Ergün, 2012). In the current study, ethanolic ginger extract was selected for the *in vivo* trial, based on the results obtained *in vitro*. Indeed such bath treatment completely cleared the infection from the fish. These results are in accordance with the study of Khalil and Walaa (2013), which reported that bathing the North African catfish, (*C. gariepinus*) in ginger from dry powder at a concentration of 20 mg L<sup>-1</sup> resulted in complete detachment and death of the gill monogeneans, *Quadriacanthus* spp. However, in contrast to the results of the current study, Abo-Esa (2008) reported that bathing *C. gariepinus* in ginger was effective at a dose of 20 mg L<sup>-1</sup> against the ectoparasite protozoas *Trichodina* sp. and *Epistylis* sp. but not against gyrodactylids. It is important to note that the ginger used in the study of Abo-Esa (2008) was a patent preparation in a tablet form, thus its content may have differed from the ethanolic extract used in the present study.

In-feed supplementation of ginger offers a practical alternative solution to the use of chemical therapeutants. Indeed the oral route is preferred for administration of therapeutants as it is non-stressful to the fish and is delivered directly to the host, thus less of the material is released to the environment and is consequently wasted. Oral application usually involves less labour, lower costs and a reduction in negative environmental impacts (Dunn et al., 1990). Oral application of Praziquantel for the treatment of monogeneans has proved to be effective, however it is associated with host toxicity, development of resistance

and low palatability (Hirazawa et al., 2004; Williams et al., 2007) therefore, it is not a commonly applied approach. In the present study, feeding of 10 and 20% ginger supplemented diets in was shown to be ineffective in controlling the infection and, in contrast, increased the load of parasites when compared to the control. It is possible that the doses selected in the present study of 10 and 20%, were too high and adversely affected the fish, which were not evident in our monitoring. Chemical compounds that are effective by baths for the treatment of gyrodactylids in fish were shown to not always be effective if given orally (Tojo and Santamarina, 1998). It is possible, therefore, that oral application of ginger in the current study was not effective due to reasons related to unknown physiological processes and metabolism of ginger in the guppies and probably the active ingredient did not reach the site of parasite attachment, i.e. the skin.

## 5. Conclusion

Results obtained from this study have demonstrated that bath treatment with 7.5 and 5 ppt ethanolic ginger extract to be an effective method to control *G. turnbulli* infection in guppies. The findings obtained, adding to the existing knowledge base, can lead to the development of prophylactic and therapeutic treatment protocols and to the development of commercial products for routine use in management of monogenean infections in fish. Further research is needed to characterise the mechanisms of action of the active compounds of ginger which are involved in the prophylactic and anti-helminthic effects against monogenean infections. Further research is needed also to optimise ginger-based preparations for successful in feed treatment. Analysis of the effect of ginger on the guppy's immune response would add important information regarding the potential of ginger in protection and healing from monogenean infections.

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