Acta Aquatica Turcica
E-ISSN: 2651-5474 17(1), 56-63 (2021) DOI:https://doi.org/10.22392/actaquatr.751913

In vitro Antiparasitic Activity of Ginger (*Zingiber officinale*) Bulb and Pomegranate (*Punica granatum*) Peel Against Monogenean Fish Parasite., *Dactylogyrus* sp.

Quyet Phan VAN[1], Bilgenur Harmansa YILMAZ[1*], Hijran Yavuzcan YILDIZ[1]

[1]Ankara University, Faculty of Agriculture, Department of Aquaculture and Fisheries, Ankara, Turkey

* Corresponding author: bilgenurharmansah@gmail.com

Research Article

Received 15 June 2020; Accepted 22 October 2020; Release date 01 March 2021.

How to Cite: Van, Q. P., Yılmaz, B. H., & Yıldız, H. Y. (2021). *In vitro* antiparasitic activity of Ginger (*Zingiber officinale*) Bulb and Pomegranate (*Punica granatum*) peel against Monogenean fish parasite., *Dactylogyrus* sp.. *Acta Aquatica Turcica, 17*(1), 56-63. https://doi.org/10.22392/actaquatr.751913

Abstract

The Monogenean parasite, *Dactylogyrus* sp., is being considered as one of the most dangerous pathogens in freshwater fish with a high infestation in common carp (*Cyprinus carpio*). The treatment of parasites including Monogeneans is an important part of fish health maintenance in the overall cycle of aquaculture. Novel applications of natural plant products to eradicate the parasites have taken high attention in aquaculture since they are reported to have less adverse impacts on the environment and fish in comparison to other chemical treatments. In the present study, *in vitro* antiparasitic effects of the ginger (*Zingiber officinale*) bulb and pomegranate (*Punica granatum*) peel against *Dactylogyrus* sp. were investigated by using *in vitro* tests. Individuals of the parasite were exposed to different concentrations of ginger (10, 50, 100, and 250 mg/ml) and pomegranate peel (50, 100, 250, and 500 mg/ml) for a specified period. *In vitro* cumulative mortality values reached 100% in 5 minutes after exposure to ginger at the concentration of 250 mg/ml in 9 minutes after exposure to 100 mg/ml and 50 mg/ml. Cumulative mortality was 58% in 9 minutes after exposure to ginger at the concentration of 10 mg/ml. In *in vitro* pomegranate tests, cumulative mortality values were assessed 100% in 3 minutes after exposure to pomegranate peel at the concentration of 500 mg/ml and in 6 minutes after exposure to 250 mg/ml and 100 mg/ml. Cumulative mortality was 66% in 6 minutes after exposure to pomegranate peel at the concentration of 50 mg/ml. *In vitro* results revealed that ginger and pomegranate peel solutions had antiparasitic effects on *Dactylogyrus* sp. to some degree, depending on solution concentration and exposure time. The efficacy of the ginger and pomegranate peel solutions against Monogeneans should be confirmed in fish through *in vivo* tests.

Keywords: Antiparasitic activity, *Dactylogyrus* sp., ginger, pomegranate peel, *Cyprinus carpio*.

Zencefil (*Zingiber officinale*) ve Nar Kabuğunun (*Punica granatum*) Monogenean Parazitlerden *Dactylogyrus* sp.'e Karşı Antiparazitik Aktivitesinin *In vitro* Olarak Belirlenmesi

Özet

Monogenean parazitlerden *Dactylogyrus* sp., sazanlarda (*Cyprinus carpio*) yaygın olarak bulunan en tehlikeli patojenlerden biri olarak kabul edilir. Genel olarak, Monogenean parazitlerin tedavisi, su ürünleri yetiştiriciliğinde balık sağlığının muhafazası bakımından önemli bir sorun teşkil eder. Bitkisel ürünlerin paraziter hastalıklarda tedavi edici uygulamaları su ürünleri yetiştiriciliğinde kimyasal tedavilere kıyasla çevre ve balık sağlığı üzerinde daha az olumsuz etkiye göstermesi nedeniyle büyük ilgi görmektedir. Bu çalışmada zencefil (*Zingiber officinale*) ve nar kabuğunun (*Punica granatum*) *Dactylogyrus* sp.'e karşı antiparaziter etkileri incelenmiştir. *Dactylogyrus* sp. parazitleri, farklı sürelerde, farklı konsantrasyonlarda zencefil (10, 50, 100 ve 250 mg/ml) ve nar kabuğu içeren solusyaonlara (50, 100, 250 ve 500 mg/ml) maruz bırakılmıştır. *In vitro* testlerde kümulatif mortalite değerleri, zencefilde 100 mg/ml ve 50 mg/ml'ye maruz bırakıldıktan sonra 9 dakika içinde, 250 mg/ml konsantrasyonuna maruz bırakıldıktan sonra ise 5 dakika içinde % 100'e ulaşmıştır. Kümulatif mortalite oranı, zencefilin 9 dakika süreyle 10 mg/ml konsantrasyonuna maruz bırakılan *Dactylogyrus* sp. için % 58 olarak bulunmuştur. *In vitro* nar kabuğu testlerinde, kümulatif mortalite değerleri 500 mg /ml konsantrasyonuna maruz kaldıktan sonra 3 dakika içinde ve 250 mg/ml ve 100 mg/ml'ye maruz bırakıldıktan 6 dakika içinde %100'e ulaşmıştır. Nar kabuğu solusyonuna 6 dakika süreyle 50 mg/ml konsantrasyonda maruz bırakılan *Dactylogyrus* sp.de kümulatif mortalite oranı % 66 olarak saptanmıştır. *In vitro* testlerle elde edilen sonuçlar, zencefil ve nar kabuğu çözeltilerinin *Dactylogyrus* sp.'e karşı antiparazitik etkisinin zamana ve konsantrasyona bağlı olduğunu göstermiştir. Ancak, balıklarda zencefil ve nar kabuğu çözeltilerinin Monogenean parazitlere karşı antiparazitik etkisi *in vivo* testlerle desteklenmelidir.

Anahtar Kelimeler: Antiparazitik aktivite, *Dactylogyrus* sp., zencefil, nar kabuğu, *Cyprinus carpio*.

INTRODUCTION

Dactylogyrus with more than 900 species is one of the most dangerous sources of parasitic infections of freshwater fish in aquaculture in the world which can cause high morbidity and mortality in many species of freshwater fish. The parasite, *Dactylogyrus* sp., mainly exists on the gills (Jain, 1959) and inhabits the host causing gill hyperplasia, swelling, reduction in surface area for respiration, and excess mucus production, the latter potentially affording the parasite some protection from chemical treatments. There is extensive literature on the increase of occurrence and severeness of the gill *Dactylogyrus* infestations of fish in recent years (Topić et al., 2001; Kır and Özan, 2007). Aggressive compounds and conventional antiparasitics against Monogeneans such as *Dactylogyrus* sp. are applied in aquaculture for removing the parasite from the gills. Formalin is one of the most commonly used chemicals although their use has not been recommended because of their severe side effects (Diggles et al., 1993; Pavanelli et al., 2002). Some other used chemicals against Monogeneans are mebendazole, toltrazuril, praziquantel, and chelidonine (Schmahl and Mehlhorn, 1985; Schmahl et al., 1988; Treves-Brown, 1999; Yao et al., 2011). The risks of chemical use for eliminating the parasites include anthelmintic resistance, risk of residue, environmental contamination, and toxicity to hosts. Thus, the undesired effects of these chemicals have highlighted the need for other novel alternative control methods for protecting fish from parasitic pathogens (Goven et al., 1980; Klinger and Floyd, 2002).

The use of medicinal plants to eradicate the parasites has been tested due to their advantages. Park et al. (2011) and Hao et al. (2012) have mentioned that phytochemicals are a realizable alternative to conventional synthetic pesticides or drugs due to their lower environmental toxicity. The use of phytochemicals as anthelmintic drugs for fish health may help to support sustainable and environmentally acceptable treatment applications. There are previous studies reviewing investigations that have examined the antiparasitic effects of plants on fish parasites (Wink, 2012; Ramudu and Dash, 2013; Reverter et al., 2014; Syahidah et al., 2015; Lieke et al., 2019). In recent years, many researchers have focused on the use of herbal plants against parasitic species in aquaculture (Chitmanat et al., 2005; Puk and Guz, 2014; Williams et al., 2016; Trasvina-Moreno et al., 2017; Yildiz et al., 2019). The various parts of ginger and pomegranate are considered as medicinal plants that can be applied to tackle aquaculture diseases. El-Sayed and El-Saka (2015) have stated that *Z. officinale* has significant anthelmintic activity against *Toxocara canis, Angiostrongylus cantonensis, Dirofilaria immitis, Hymenolepis nana, Schistosoma mansoni, Anisakis simplex* both *in vitro* and *in vivo*, and antiprotozoal activity against *Giardia lamblia, Blastocystis, Trypanosoma brucei* and *Toxoplasma gondii* species. Efficacy of ginger solution against different parasites in fish was studied using both oral and bath treatments (Abo-Esa, 2008; Khalil and Houseiny, 2013; Levy et al., 2015; Fu et al., 2019). In comparison, less literature is observed on the use of pomegranate solution against parasitic infections in fish. To our knowledge, the antiparasitic activity of ginger and pomegranate solutions on the eradication of *Dactylogyrus* sp. was not investigated hitherto. Therefore, in this study, we tested *in vitro* exposure of *Dactylogyrus* sp. to ginger and pomegranate solutions to evaluate their antiparasitic potential.

MATERIALS and METHODS

Fish and parasites

Carp (*Cyprinus carpio*) were obtained from the aquaponic system (co-production of carp and mint (*Mentha* spp)) in Ankara University, Department of Fisheries and Aquaculture. Scrapings from heavily parasitized fish gills were examined under the microscope. The fish length and weight were 11.66 ± 1.15 cm and weight 24.33 ± 4.93 g, respectively. The fish stocking density in fiberglass fish tanks (80x60x50 cm) was 35 kg/m^3. The water in the tanks was at temperature 20-22 °C, dissolved oxygen 5.50- 5.97 mg/L, and pH 6.97-7. Fish were fed with the commercial rainbow trout feed with 45% raw protein at 2% total body weight ratio.

The parasites on the gills were identified as *Dactylogyrus* (Malmberg, 1970; Bruno et al., 2006). Parasite samples on the slides were counted under the microscope for *in vitro* parasite survival tests.

Fish management and experimental protocols (reference number of 2019-7-72) were approved by the Ankara University, Ethics Committee. No fish were killed during the experiments.

Solution preparation

The ginger (*Z. officinale*) and pomegranate (*P. granatum*) were obtained from the local market, Ankara, Turkey. Ginger or pomegranate peel was well crushed and the stock solution of each was prepared by diluting 10 gr of each in 20 ml distilled water. The stock solution was diluted with distilled water to adjust the necessary concentration. The tested ginger concentrations were 10, 50, 100 & 250 mg/ml and pomegranate peel concentrations were 50, 100, 250 & 500 mg/ml.

***In vitro* assay**

During the *in vitro* tests, ginger or pomegranate peel solution was tested by directly pouring on the alive parasite on the slides. The movement and contraction of parasites were continuously observed under the microscope. Ginger solution concentrations of 10, 50, 100 & 250 mg/ml and pomegranate peel solution concentrations of 50, 100, 250 & 500 mg/ml were tested. The parasite behavior was observed and recorded for 10 minutes. Three replicates, each containing 4 parasites, were used per each concentration (Hutson et al., 2018).

Statistics

Statistical analysis was done by using one-way ANOVA. Differences were considered significant at $p < 0.05$.

RESULTS

***In vitro* parasite survival**

In vitro survival of *Dactylogyrus* sp. varied by concentrations of ginger or pomegranate peel solutions and exposure time (F (critical value 2.90) = 7.60) for ginger experiment and (F (critical value 2.68) = 6.72) for pomegranate experiment ($p < 0.05$). The cumulative mortality values of *Dactylogyrus* sp. exposed to ginger and pomegranate peel solutions were shown in Figure 1 and Figure 2, respectively.

Antiparasitic effects of ginger solutions on *Dactylogyrus* sp. were concentration and exposure time-dependent. Cumulative mortality values reached 100% in 5 minutes after exposure to ginger solution of 250 mg/ml, 100% in 9 minutes after exposure to 100 mg/ml and 50 mg/ml ginger solutions. After exposure to a ginger solution of 10 mg/ml concentration for 9 minutes, cumulative mortality was assessed by 58%.

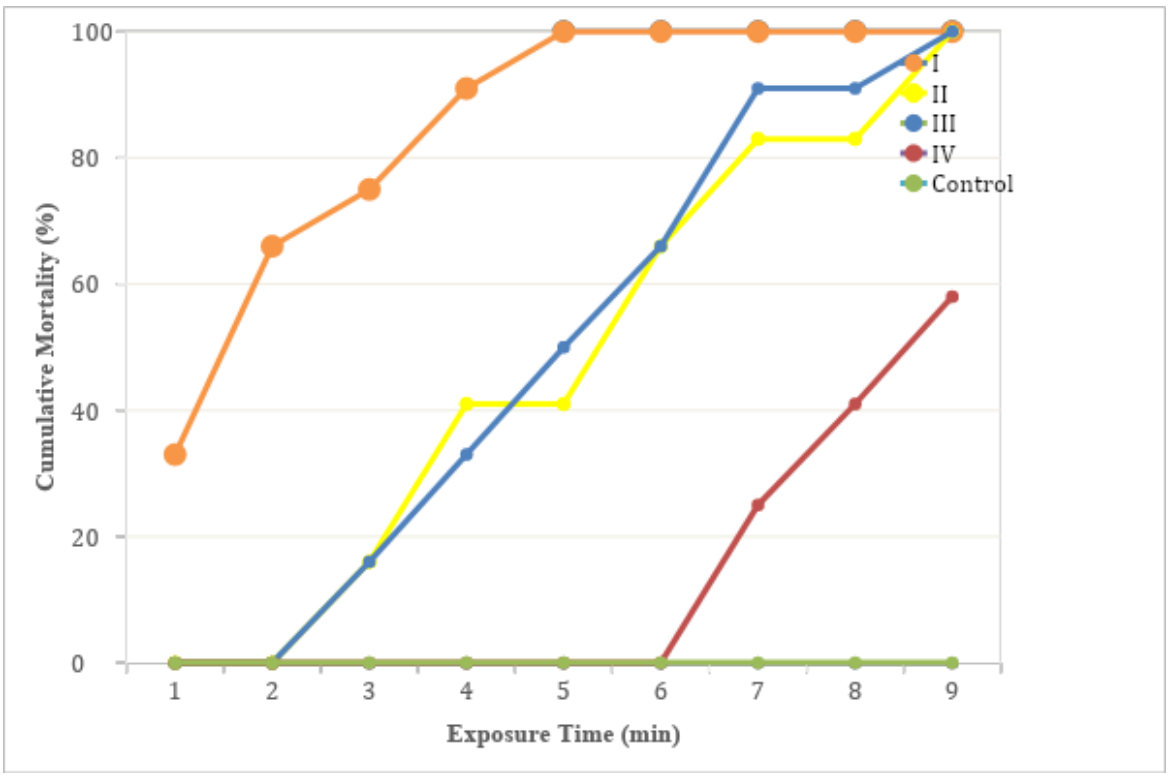


Figure 1. Cumulative mortality values of *Dactylogyrus* sp exposed to ginger (*Z. officinale*) solution at concentrations (mg/mL) of I-250; II-100; III-50; IV-10). Control: no solution exposure.

Antiparasitic effects of pomegranate peel solutions on *Dactylogyrus* sp. were also concentration and exposure time-dependent (Figure 2). Cumulative mortality values reached 100% in 3 minutes after exposure to pomegranate peel solution of 500 mg/ml, 100% in 6 minutes after exposure to 250 mg/ml, and 100 mg/ml pomegranate peel solutions. Cumulative mortality was 66% in 6 minutes after exposure to pomegranate peel at the concentration of 50 mg/ml.

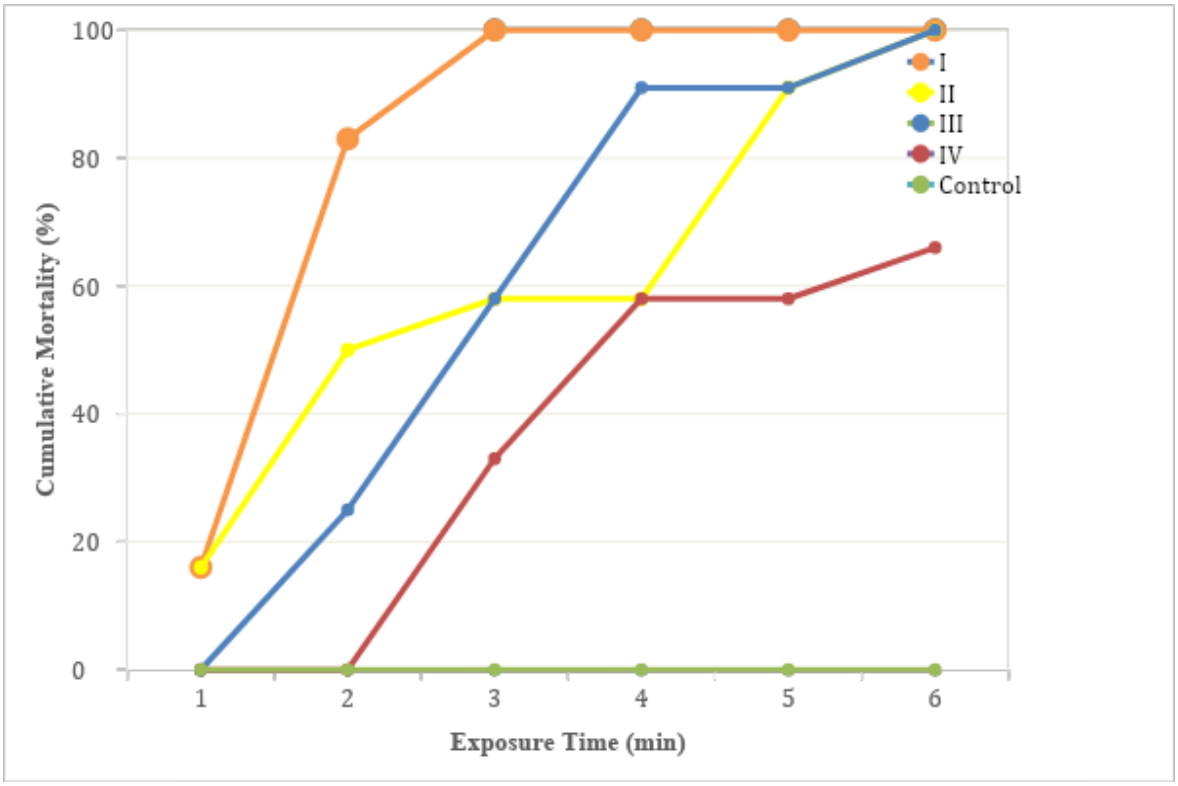
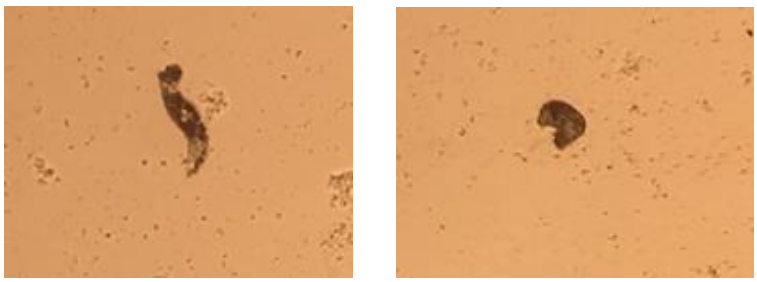


Figure 2. Cumulative mortality of *Dactylogyrus* sp. exposed to pomegranate (*P. granatum*) solution at concentrations (mg/ml) of I-500, II-250, III-100, IV-50. Control: no solution exposure.

The contact of *Dactylogyrus* sp. with the solutions of ginger or pomegranate peel resulted in these in sequence: 1) increase in mobility of the parasite 2) stretching of the parasite 3) contraction of the parasite and 4) shrinking to the smallest size before death (Figure 1).



(a) (b)

Figure 3. Parasite behavior after contact with the ginger (*Z. officinale*) (a) *Dactylogyrus* sp. before exposure (alive); (b) Contraction of *Dactylogyrus* sp. after exposure to ginger solutions

DISCUSSION

Parasitic fish diseases constitute an important problem in aquaculture. It is noted that the sustainability of the aquaculture industry may be compromised by parasitic fish diseases (Soler-Jiménez et al., 2017; Soares et al., 2017a, b). With the development of aquaculture, the number of fish infected by Monogenean parasites is being increased (Topić et al., 2001; Kır and Özan, 2007). Currently, parasitic diseases observed in aquaculture production are generally treated with chemicals such as formalin, acetic acid, potassium permanganate, praziquantel, etc. However, the use of such

substances can lead to problems such as the development of resistant organisms, the accumulation of chemical residues, and damage to the environment and eventually to human beings (Ling et al., 2015; Hashimoto et al., 2016; Soares et al., 2016; Soares et al., 2017a, b). Therefore, the use of chemicals to control fish diseases has been limited in many countries (Ling et al., 2015). Furthermore, it seems that current antiparasitic treatments can be costly. Seeking environmentally friendly, cost-effective, and effective medicines have brought up the use of plant-based substances in fish diseases (Yavuzcan Yildiz and Bekcan, 2020).

In our study, *in vitro* anthelmintic activity of ginger (*Z. officinale*) bulb and pomegranate (*P.granatum*) peel against the Monogenean parasite, *Dactylogyrus* sp., recovered from common carp (*Cyprinus carpio*) were examined. The efficiency of ginger against some Monogeneans has been studied, however, its potential use against *Dactylogyrus* sp. has not been studied before. In the case of pomegranate peel, to our knowledge, no research has been done on its efficiency against Monogenean infections in fish. Our study provides new knowledge on the lethal effects of pomegranate peel on *Dactylogyrus* sp.

During the *in vitro* tests, ginger and pomegranate peel were observed to have a dose-dependent antiparasitic effect against *Dactylogyrus* sp. The time and concentration-dependent activity of ginger against the *Dactylogyrus* sp. (Monogenea) were similar to previous researches on ginger with different parasite species by Trasvina Moreno et al. (2017) and Levy et al. (2015). In our tests with ginger or pomegranate peel, the time to death of *Dactylogyrus* sp. *were* from 1 min to 9 min. In a similar study of Levy et al. (2015) survival period of *Gyrodactylus* sp. (Monogenea) ranged between 1 min and 90 min following exposure to 200, 150, 100, and 75 ppt ginger concentrations. The differences in survival time can be attributed to the differences in the resistance of parasite species against the various antiparasitic compounds. There is no previous research on the anthelmintic effects of ginger on *Dactylogyrus* sp, nevertheless, some other plant extracts have been screened to detect their potential use against *Dactylogyrus* sp. *In vitro* tests conducted by Zoral et al. (2017) using *Rosmarinus officinalis* against *Dactylogyrus minutus* (Monogenea) recovered from *Cyprinus carpio* showed that the survival time ranged from 1 min to 71 min after exposure to the concentrations of 200, 150, and 100 g/l. The findings on the antiparasitic activity of ginger against *Quadriacanthus* sp. (Monogenea) recovered from *Clarias gariepinus (*Khalil and El-Hosseiny 2013) and *Neobenedenia* sp., (Monogenean) (Trasvina Moreno et al. 2017) agree with our results. In the study of Trasvina Moreno et al. (2017), the antiparasitic potential of water-ethanol extracts of garlic *(Allium sativum),* ginger *(Zingiber officinale),* basil *(Ocimum basilicum),* bitter chaparro *(Castela tortuosa),* onion *(Allium cepa),* and papaya *(Carica papaya)* against *Neobenedenia* sp. parasites were examined, reporting that ginger solution had the most toxic effect on the parasites among the all herbal solutions tested. Thus, it is revealed that the antiparasitic potential of ginger against *Dactylogyrus is apparent.*

The peel and seeds of pomegranate (*P. granatum*) have various therapeutic applications such as antibacterial, antifungal, antioxidant, antitumor, antiviral, antimalarial, and antimutagenic effects (Yones et al. 2016). Research has not been found in the literature on pomegranate peel efficiency against Monogenean infections in fish. However, the antiparasitic activity of pomegranate extracts to *Schistosoma mansoni* (Trematoda) has been studied by Fahmy et al. (2009). In their study, the mortality value of the adult worms was observed to reach 100% after 24 h exposure for all tested leaves and peels methanol extract at the concentrations of 100 µg/ml, 300 µg/ml and 500 µg/ml. At the highest concentration of 500 µg/ml, 100% mortality occurred within 10-12 hours. The efficiency depended on the duration of exposure and the concentration, which is in agreement with our test results. The dose-dependent antiparasitic activity of pomegranate extracts against *S.mansoni* was shown by the study of Yones et al. (2016) as well. Abdel-Hafezz (2016) researched rats infected by *Blastocystis* spp. (Protozoan) and the feeding trials with *P. granatum* showed a decrease in the intensity of parasites. In line with our study, they also stated that pomegranate peel can be used as an alternative antiparasitic.

In terms of parasite behavior after contact with the test solutions, typically, *Dactylogyrus* sp. was observed to increase mobility at first, then stretching, contraction, shrinking, and finally stop moving Previous studies on the antiparasitic effect of ginger on different Monogeneans did not indicate an increase in mobility after the parasite contact with the ginger solution. Unlike our observations, it was recorded that relaxation of parasite at first, then contraction, slowing down, and eventually lack of motion of parasite before death (Khalil and El-Hosseiny 2013; Levy et al., 2015; Trasvina Moreno et

al., 2017). The research by Yones et al. (2016) on Trematoda indicated that applying pomegranate peel solution resulted in the light contractions as soon as contact with the related test solution, then motility reduction and paralysis before parasites death, which are similar to the observations in our study concerning parasite response to test solutions. Besides in the study of Yones et al. (2016), the pomegranate was stated as a promising candidate as a new antiparasitic agent.

CONCLUSION

Antiparasitic effects of ginger (*Z. officinale*) bulb and pomegranate (*P. granatum*) peel solutions on *Dactylogyrus* sp. recovered from common carp (*Cyprinus carpio*) were tested in this study. These two plant solutions showed dose and concentration-dependent antiparasitic activity and displayed to have a promising alternative treatment potential against Monogenean infections of fish in aquaculture. However, further evaluations should be done to understand the active constituents available in the solutions eradicating parasites and the mechanism of actions associated with their efficiency in fish. The ginger and pomegranate peel solutions need to be evaluated for their toxicity and side effects on fish, showing the requirement of detailed *in vivo* studies.

REFERENCES

Abdel-Hafeez, E.H., Ahmed, A.K., Abdellatif, M.Z., Kamal, A.M., & Toni, N. D. (2016). The efficacy of pomegranate (*Punica granatum*) peel extract on experimentally infected rats with *Blastocystis* spp. *Journal of Infectious Diseases and Preventive Medicine*, 2-6.

Abo-Esa, J.F.K. (2008). Study on some ectoparasitic diseases of Catfish, *Clarias gariepinus* with their control by ginger, *Zingiber of officiale*. *Mediterranean Aquaculture Journal, 1(*1), 1-9.

Bruno, D.W., Nowak, B., & Elliott, D.G. (2006). Guide to the identification of fish protozoan and metazoan parasites in stained tissue sections. *Diseases of Aquatic Organisms, 70*,1-36.

Chitmanat, C., Tongdonmuan, K., & Nunsong, W. (2005). The use of crude extracts from traditional medicinal plants to eliminate *Trichodina* sp. in tilapia (*Oreochromis niloticus*) fingerlings. *Songklanakarin Journal of Science and Technology, 27*(1), 359-364.

Diggles, B.K., Roubal, F.R., & Lester, R.J.G. (1993). The influence of formalin, benzocaine and hyposalinity on the fecundity and viability of *Polylabroides multispinosus* (Monogenea: Micro cotylidae) parasitic on the gills of *Acanthopagrus australis* (Pisces: Sparidiae). *International Journal for Parasitology, 23,* 877–884.

El-Sayed, N.M., & El-Saka, M.M. (2015). Anti-Parasitic Activity of *Zingiber officinale* (Ginger): A Brief Review. *Aperito Journal of Bacteriology, Virology and Parasitology, 2*(1), 112.

Fahmy, Z.H., El-Shennawy, A.M., El-Komy, W., Ali, E., & Hamid, S.S.A. (2009). Potential Antiparasitic Activity of Pomegranate Extracts Against Shistosomules and Mature Worms of *Schistosoma Mansoni:* in vitro and in vivo Study. *Australian Journal of Basic and Applied Sciences, 3*(4), 4634-4643.

Fu, Y.W., Wang, B., Zhang, Q.Z., Xu, D.H., Liu, Y.M., Hou T.L., & Guo,S.Q. (2019). Efficacy and antiparasitic mechanism of 10-gingerol isolated from ginger *Zingiber officinale* against *Ichthyophthirius multifiliis* in grass carp. *Veterinary Parasitology,265,*74-84.

Goven, B.A., Gilbert, J., & Gratzek, J. (1980). Apparent drug resistance to the organophosphate dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate by monogenetic trematodes. *Journal of Wildlife Diseases, 16*(3), 343–346.

Hao, B., Liu, G.L., Hu, X.G., & Wang, G.X. (2012). Bioassay-guided isolation and identification of active compounds from Semen pharbitidis against *Dactylogyrus intermedius* (Monogenea) in goldfish (*Carassius auratus*). *Veterinary Parasitology, 187*(3-4), 452-458.

Hashimoto, O.G. S., Neto, F. M., Ruiz, M. L., Acchile, M., Chagas, E. C., Chaves, F. C. M., & Martins, M. L. (2016). Essential oils of *Lippia sidoides* and *Mentha piperita* against monogenean parasites and their influence on the hematology of Nile tilapia. *Aquaculture, 450,* 182-186.

Hutson, K. S., Brazenor, A. K., Vaughan, D. B., & Trujillo-González, A. (2018). Monogenean parasite cultures: current techniques and recent advances. *Advances in parasitology, 99,* 61-91.

Jain, S.L. (1959). New Dactylogyrus parasites from the gill filaments of Cyprinid fishes from India. *Zeitschrift für Parasitenkunde, 19*(5), 432-441.

Khalil, A.A., & El-Houseiny, W. (2013). Effect of ginger (*Zingiber officinale*) on *Clarias gariepinus* infested with gill monogenia. *Egyptian Journal of Aquatic Research, 3*(4), 55-61.

Kir, I., & Özan, S.T. (2007). Helminth infections in common carp, *Cyprinus carpio* L., 1758 (Cyprinidae) from Kovada Lake (Turkey). *Türkiye Parazitoloji Dergisi, 31*(3), 232–236.

Klinger, R., & Floyd, R.F. (2002) *Introduction to freshwater fish parasites.* Document CIR716. Institute of Food and Agricultural Science. University of Florida, Gainesville.

Levy, G., Zilberg, D., Paladini, G., & Fridman, S. (2015). Efficacy of ginger-based treatments against infection with *Gyrodactylus turnbulli* in the guppy (*Poecilia reticulata*). *Veterinary Parasitology, 209*(3-4), 235-241.

Lieke, T., Meinelt, T., Hoseinifar, S.H., Pan, B., Straus, D.L., & Steinberg, C.E.W. (2019). Sustainable aquaculture requires environmental-friendly treatment strategies for fish diseases. *Aquaculture*, 1-23.

Ling, F. E. I., Jiang, C., Liu, G., Li, M., & Wang, G. (2015). Anthelmintic efficacy of cinnamaldehyde and cinnamic acid from cortex cinnamon essential oil against *Dactylogyrus intermedius*. *Parasitology, 142*(14), 1744-1750.

Malmberg, G. (1970). The excretory system and the marginal hooks as a basis for the systematics of *Gyrodactylus* (Trematoda, Monogenea). *Arkiv for Zoologi*, 23:235.

Park, H.M., Kim, J., Chang, K.S., Kim, B.S., Yang, Y.J., Kim, G.H., Shin, S.C., & Park, I.K. (2011). Larvicidal activity of myrtaceae essential oils and their components against *Aedes aegypti* acute toxicity on *Daphnia magna* and aqueous residue. *Journal of Medical Entomology, 48*(2), 405-410.

Pavanelli, G.C., Eiras, J.C., & Takemoto, R.M (2002). Doenças de peixes: Profilaxia, diagnóstico e tratamento, EDUEM, Maringá.

Puk, K., & Guz, L. (2014). Effects of medical plant extracts on the growth of the fish parasite Spironucleus vortens. Medycyna Weterynaryjna, 70(3), 165-168.

Ramudu, D.K.R., & Gadadhar. (2013). A Review on Herbal Drugs Against Harmfull Pathogens in Aquaculture. *American Journal of Drug Discovery and Development, 3*(4), 209-219.

Reverter, M., Bontemps, N., Lecchini, D.L, Banaigs, B., & Sasal, P. (2014). Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. *Aquaculture, 433*, 50-61.

Schmahl, G., & Mehlhorn, H. (1985). Treatment of fish parasites. 1. Praziquantel effective against Monogenea (*Dactylogyrus vastator, Dactylogyrus extensus, Diplozoon paradoxum*). *Zeitschrift Für Parasitenkunde, 71*(6), 727–737.

Schmahl, G., Mehlhorn, H., & Haberkorn, A. (1988). Sym. Triazinone (toltrazuril) effective against fish-parasitizing Monogenea. *Parasitology Research, 75*(1), 67–68.

Soares, B.V., Neves, L.R., Ferreira, D.O., Oliveira, M.S.B., Chaves, F.C.M., Chagas, E.C., & Tavares-Dias, M. (2017). Antiparasitic activity, histopathology and physiology of *Colossoma macropomum* (tambaqui) exposed to the essential oil of *Lippia sidoides* (Verbenaceae). *Veterinary Parasitology, 234*, 49-56.

Soares, B.V., Neves, L.R., Oliveira, M.S.B., Chaves, F.C.M., Dias, M.K.R., Chagas, E.C., & Tavares-Dias, M. (2016). Antiparasitic activity of the essential oil of *Lippia alba* on ectoparasites of *Colossoma macropomum* (tambaqui) and its physiological and histopathological effects. *Aquaculture, 452*, 107-114.

Soler-Jiménez, L.C., Paredes-Trujillo, A.I., & Vidal-Martínez, V.M. (2017). Helminth parasites of finfish commercial aquaculture in Latin America. *Journal of Helminthology, 91*(2), 110-136.

Syahidah, A., Saad, C.R., Daud, H.M., & Abdelhadi, Y.M. (2015). Status and potential of herbal applications in aquaculture: A review. *Iranian Journal of Fisheries Sciences, 14*(1), 27-44.

Topić, P.N., Hacmanjek, M., & Teskeredžić, E. (2001). Health status of rudd (*Scardinius erythrophthalmus hesperidicus* H.) in Lake Vrana on the Island of Cres, Croatia. *Journal of Applied Ichthyology, 17*(1), 43-45.

Trasviña-Moreno, A.G., Ascencio, F., Angulo, C., Hutson, K.S., Avilés-Quevedo, A., Inohuye-Rivera, R.B., & Pérez-Urbiola, J.C. (2017). Plant extracts as a natural treatment against the fish ectoparasite *Neobenedenia* sp. (Monogenea: Capsalidae). *Journal of Helminthology, 93*(1), 57-65.

Treves-Brown, K.M. (1999). Availability of medicines for fish. *Fish Veterinary Journal, 4*, 40-55.

Williams, A.R., Soelberg, J., & Jäger, A.K. (2016). Anthelmintic properties of traditional African and Caribbean medicinal plants: identification of extracts with potent activity against *Ascaris suum in vitro. Parasite, 23*, 1-6.

Wink, M. (2012). Medicinal Plants: A Source of Anti-Parasitic Secondary Metabolites. *Molecules, 17*(11), 12771-12791.

Yao, J.Y., Zhou, Z.M., Pay, X.Y., & Hao, G.J. (2011). In vivo anthelmintic activity of chelidonine from *Chelidonium majus* L. against *Dactylogyrus intermedius* in *Carassius auratus*. *Parasitology Research, 109*(5), 1465–1469.

Yavuzcan Yildiz, H., & Bekcan, S. (2020). Control of ectoparasitosis in carp (*Cyprinus carpio*) induced by *Gyrodactylus elegans* (Monogenea) with garlic (*Allium sativum*) and onion (*Allium cepa*) extracts. *Ecocycles*, 6(1), 10-17.

Yildiz, H.Y., Phan, Q.V., Parisi, G., & Dam, M.S. (2019). Anti-parasitic activity of garlic (*Allium sativum*) and onion (*Allium cepa*) juice against crustacean parasite, *Lernantropus kroyeri*, found on European sea bass (*Dicentrarchus labrax*). *Italian Journal of Animal Science, 18*(1), 833–837.

Yones, D.A., Badary, D.M., Sayed, H.M.B., Bayoumi, S.A.H., Khalifa, A.A., & El-Moghazy, A.M. (2016). Comparative Evaluation of Anthelmintic Activity of Edible and Ornamental Pomegranate Ethanolic Extracts against *Schistosoma mansoni*. *BioMed Research International*, 2016(5), 1-15.

Zoral, M. A., Futami, K., Endo, M., Maita, M., & Katagiri, T. (2017). Anthelmintic activity of *Rosmarinus officinalis* against *Dactylogyrus minutus* (Monogenea) infections in *Cyprinus carpio*. *Veterinary Parasitology, 247*, 1-6.