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Fuad Ameen, Khalidah Al-Niaeem, Majid M. Taher & Fatima A. H. Sultan

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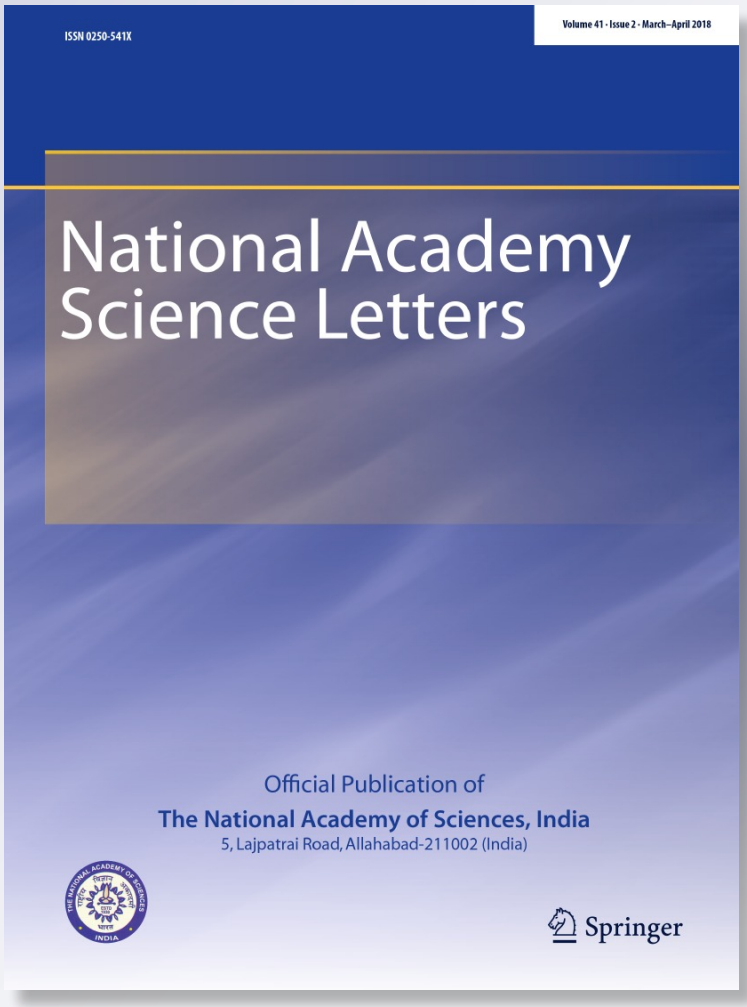

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SHORT COMMUNICATION

Potential of Plant Extracts to Inhibit the *Ichthyophonus* sp. Infection in Blue Tilapia: A Preliminary Study in vitro

Fuad Ameen[1] · Khalidah Al-Niaeem[2] · Majid M. Taher[2] · Fatima A. H. Sultan[2]

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Abstract We studied in vitro the potential of plant extracts to inhibit the infection caused by *Ichthyophonus* sp. in blue tilapia (*Oreochromis aureus*). We inoculated *Ichthyophonus* sp. on agar plates where we placed the filter discs that were prepared by filtering the aqueous extracts (five concentrations) of ginger (*Zingiber officinale*), linseed (*Linum usitatissimum*), henna (*Lawsonia inermis*) and white turmeric (*Curcuma zedoaria*). All plants inhibited the growth of *Ichthyophonus* sp. in the highest concentration (20%); *Z. officinale* appeared to be the most effective, whereas *C. zedoaria* was the least effective. This study suggests that widely available and inexpensive plant extracts might be effective natural agents to prevent the *Ichthyophonus* sp. infection in fish. More research is needed in order to ascertain the efficiency of the plant extracts in vivo as well as to find a proper practice used in aquaculture.

Keywords Aquaculture · Disease · Protist · Parasite · Medicinal plants

Fish production in aquacultures is continuously hampered by infections caused by viruses, bacteria, fungi and fungus-like protists [1]. A genus previously considered to be fungi and recently classified rather as a protist, *Ichthyophonus* sp. [2, 3] affect systemic granulomatous disease, both in wild and cultured fish causing economic losses [1].

✉ Fuad Ameen
fuadameen@ksu.edu.sa; alfouad2004@hotmail.com

[1] Department of Botany and Microbiology, Faculty of Science, King Saud University, Riyadh 11451, Saudi Arabia

[2] Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah, Basrah, Iraq

Ichthyophonus sp. grows in fresh and marine water. Both wild and cultured fish are likely to harbour this disease. The infection is spread by fungal cysts released in the faeces, and by cannibalism of infected fish [4, 5]. The primary route of transmission is through the ingestion of infective spores and the infection spreads under the skin and in muscle tissue [4, 6]. Signs of the infection are species-related and depend on the condition of individual fish. Fish with a mild to moderate infection do not show external signs of the disease [7]. In severe cases, the skin may have a "sandpaper texture" [6]. Some fish may show curvature of the spine. Internally, the organs may be swollen with white to grey–white sores [8].

The use of chemical treatments against parasites in aquacultures is becoming less attractive due to rising environmental and health perspectives. Moreover, many side effects of the chemicals to fish has increased the interest to inhibit diseases by using natural products, like plant extracts [9]. A wide range of medicinal plants, such as herbs, spices and seaweeds seem to act as immunostimulants as reviewed recently [9, 10]. Plants seem to inhibit diseases in aquacultures when used as dietary supplements [11, 12]. Plant extracts might be used also as the bath treatment of infected fish [9, 13].

An infection caused by *Gyrodactylus turnbul*, a monogenean flatworm, was effectively treated with *Zingiber officinale* extract [12]. *Mucuna pruriens* and *Carica papaya* extracts were effective against the protozoan parasite *Ichthyophthirius multifiliis* infection in goldfish [13]. As an emerging area of interest, we, however, do not have enough knowledge about potential plants that could be used against different parasites and *Ichthyophonus* sp. in special.

Our aim was to assess the efficiency of the four plant species, common in Asia, against infection caused by

Ichthyophonus sp. We performed an in vitro laboratory study where we measured the efficiency of the water extracts of the plants to inhibit the growth of *Ichthyophonus* sp. This will increase our knowledge about the possibility to limit and decrease the use of chemical compounds in aquatic environments.

Ichthyophonus sp. infected blue tilapia (*Oreochromis aureus*) were collected from the fishpond of Basrah University, Iraq, during the period from September 2014 to December 2014. Ten blue tilapia from the same pond were investigated, and *Ichthyophonus* sp. was isolated from the abdomen of two fish. Glucose yeast (GY) agar (10% glucose, 0.25% yeast extract, and 1.5% agar, ampicillin and streptomycin sulphate) plates were incoculated and stored at 20 °C. As recommended by Kocan et al. [8]., the identification of *Ichthyophonus sp*. was made by examining the plates under 40× magnification. The species was identified morphologically according to Zadeh et al. [14].

The rhizomes of ginger (*Zingiber officinale*), rhizomes of white turmeric (*Curcuma zedoaria*), leaves of henna (*Lawsonia inermis*) and linseed (*Linum usitatissimum*) were collected from Iraqi local markets, powdered and extracted with ultrapure water. Five different concentrations of plant powder and water (weight/weight) (2, 5, 10, 15, and 20%) were vortexed and centrifuged as described by Levy et al. [15]. The supernatants (20 ml) of the plant extracts were passed through Whatman filter papers (Grade 1; diameter 25 mm). Filter discs of different plant extracts were placed on plates that were recently inoculated with *Ichthyophonus* sp. [16]. Control discs were prepared by filtering ultrapure water. One disc was placed on one plate that were then incubated for 7 days at 20 °C. The inhibition zone diameters were measured to the nearest whole millimeter at the point where a prominent reduction of growth was observed, as described by Espinel-Ingroff and Canton [17]. Three replicates of each plant extract were performed.

We performed a two-way analysis of variance (ANOVA) plant species and concentrations as factors. The differences between the means were determined using Tukey's test. The result was considered significant at $p < 0.05$.

The control discs did not inhibit the growth of *Ichthyophonus* sp. (0 mm inhibition zone). Two-way ANOVA indicated a significant interaction between the plant extract concentration and plant species. One-way-ANOVA indicated a significant difference in all concentrations studied ($p < 0.05$). In each concentration, *Z. officinale* extract had the significantly (Tukey) highest inhibition zone and thus it inhibited the growth of *Ichthyophonus* sp. markedly in all concentrations (Fig. 1). The lowest concentrations (2%) of both *Z. officinale* and *L. inermis* exhibited an inhibitory effect, the inhibition zones being 12.5 and 4.2 mm,

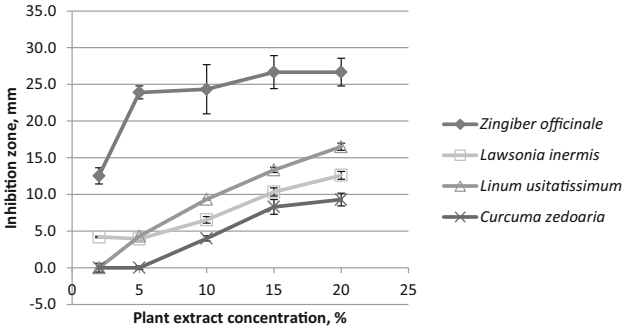

Fig. 1 Inhibitory effects (inhibition zone, mm) of the four plant species extracted with water in five different concentrations on the growth of *Ichthyophonus* sp.

respectively. *C. zedoaria* and *L.usitatissimum* had no inhibitory effect at the lowest concentration. The highest concentration (20%) of each of the species inhibited the *Ichthyophonus* sp. growth. The inhibition zones from the highest to lowest were 26.7, 16.5, 12.6, and 9.3 mm for *Z. officinale*, *L. usitatissimum*, *L. inermis* and *C. zedoaria*, respectively.

The *Ichthyophonus* disease is a major problem in aquaculture because it reduces fish production and thus causes economic losses [1]. Our results showed that the in vitro growth of *Ichthyophonus* sp. was inhibited by the aqueous extracts of the rhizomes of ginger (*Zingiber officinale*), linseed (*Linum usitatissimum*), leaves of henna (*Lawsonia inermis*) and rhizomes of white turmeric (*Curcuma zedoaria*). These plants are used traditionally as medicinal herbs or as healthy, functional food. Thus, they have a potential to be used to prevent the *Ichthyophonus* disease.

Antibacterial and antifungal properties of different plants have been used in Chinese medicine for a long time. Recently, many studies have been published in order to study the actual functional mechanisms as well as the exact chemical composition of the plants. Herbal compounds, such as phenolics, polyphenols, alkaloids, quinones, terpenoids, lectines and polypeptides, have been shown to be effective alternatives to synthetic compounds against pathogens [18]. A variety of bacterial and fungal species have been inhibited by the essential oil constituents extracted from the rhizomes of *Z. officinale* [18, 19]. The extracts of the rhizomes of several *Curcuma* species, among them *C. zedoaria*, have been shown to act as antimicrobial and anti-inflammatory agents [20, 21]. The medicinal value of *C. zedoaria* is based on its essential oil constituents, terpens and other aromatic compounds [20, 22]. *L. inermis* has been shown to contain several bioactive molecules and its pharmacological studies have indicated antimicrobial, anti-inflammatory and antiparasitic actions [23, 24]. *Linum usitatissimum* is a natural health

product and functional food, mainly because of its healthy oil composition, whereas its other compounds have received less attention [25]. Antimicrobial activity of *L. usitatissimum* was observed in a study of Palla et al. [26], whereas no or only low antimicrobial activity was observed by Abuelgasim et al. [27].. We observed the inhibitory effect of *L. usitatissimum* on *Ichthyophonus* sp. growth to be on about the same level as the effect of *C. zedoaria* and *L. inermis*, while the effect of *Z. officinale* was the most pronounced.

In conclusion, the aqueous extracts of ginger, white turmeric, henna and linseed were able to inhibit the growth of *Ichthyophonus* sp. in vitro, ginger being the most effective. The use of widely available and inexpensive natural plant extracts would be superior to the continuous usage of artificial chemicals from the aspect of the side effects to fish. Especially, when water would be used as the solvent. Water, however, extracts lower amounts of effective compounds than other solvents [28, 29] and the efficiency of the extracts should be tested in vivo with infected fish. Moreover, natural compounds derived from plants probably accumulate less in the flesh of fish and in the environment because natural compounds in general tend to be more easily biodegradable than synthetic chemicals. Natural plant compounds are less likely to induce resistance in parasites due to the high diversity of affecting molecules [9]. More research is needed in order to ascertain the efficiency of the plant extracts in vivo as well as to find a proper practice used in aquaculture.

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