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The efficacy of four common anthelmintic drugs and traditional Chinese medicinal plant extracts to control *Dactylogyrus vastator* (Monogenea)

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ABSTRACT

Disease caused by *Dactylogyrus vastator* has resulted in considerable economic damage in aquaculture. To control this parasite effectively, the anthelmintic properties of several extracts obtained from *Euphorbia fischeriana* and four common anthelmintic drugs (trichlorfon, praziquantel, 40% phoxim and mebendazole) against adults and eggs of *D. vastator* were assessed *in vitro* and *in vivo*. Trichlorfon (0.5, 1 and 1.5 mg/L), praziquantel (10 and 20 mg/L), 40% phoxim (0.1, 0.2 and 0.4 mg/L), mebendazole (0.02 and 0.04 mg/L) and the ethyl acetate extract of *E. fischeriana* (10 and 20 mg/L) were effective to kill the parasites *in vitro*, while the other extracts (petroleum ether, n-butanol and distilled water) of *E. fischeriana* had no significant effects. Praziquantel effectively killed adults with the efficacy of 80.3% at 20 mg/L and inhibited egg hatching, while trichlorfon and the ethyl acetate extract of *E. fischeriana* effectively eliminated adult parasites with the efficacy of 87.3% and 80.1% at 2.5 mg/L and 14 mg/L, but had no effect on egg viability. 40% phoxim was highly effective in suppressing egg hatching, but mebendazole was not significantly effective on either adults or eggs. Overall, this study found that 40% phoxim and praziquantel could prevent horizontal infection *via* eggs in aquaculture facilities and that trichlorfon, praziquantel and the ethyl acetate extract of *E. fischeriana* could be effective against adult parasites. However, the effective dose of the ethyl acetate extract of *E. fischeriana* was very close to the toxic dose, a factor that likely limits its practical application in aquaculture.

1. Introduction

Aquaculture was an important economic activity in China that had grown rapidly over recent decades. However, deterioration in water quality combined with heavy losses from a variety of fish diseases had proved a major obstacle to the further development of fish aquaculture industries. In particular, diseases caused by monogenean parasites have resulted in considerable economic damage to the aquaculture industry (Bondad-Reantaso et al., 2005; Buchmann et al., 1995). For example, *Dactylogyrus vastator* Nybelin, 1924, a common monogenean parasite found on the gills of cyprinid, has caused serious economic damage to aquaculture industries worldwide (Galli et al., 2005; Ogawa and Egusa, 1979). Monogeneans exhibit a direct, single-host life cycle, and are thus able to multiply rapidly in high-density aquaculture environments (Klinger and Floyd, 2002). Fish infected with monogenean parasites can suffer serious disorders including gill inflammation, excessive mucous secretions, and accelerated respiration (Reed et al., 2009).

To date the treatment and prevention of monogenean infections had involved the use of various anthelmintic parasiticides, including formalin (Diggles et al., 1993; Sharp et al., 2004), trichlorfon (Buchmann et al., 1987), praziquantel (Schmahl and Mehlhorn, 1985) and mebendazole (Katharios et al., 2006). However, the extended and frequent use of these drugs at incorrect doses had led to the evolution of drug resistance, which had limited their anthelmintic efficacy. For example, Buchmann et al. (1992) revealed that extended exposure to subtherapeutic doses of mebendazole resulted in drug resistant parasite populations, demonstrating that the administration of accurate therapeutic doses is critical for effective control. This finding had since stimulated the re-screening of common drugs and extensive research to identify and develop alternative control strategies. In particular, this research has focused on the use of traditional medicinal plants as an alternative control strategy (Wang et al., 2008). Medicinal plants are considered to be more environmentally friendly and biodegradable than chemical anthelmintic agents (Rahuman et al., 2008).

Euphorbia fischeriana (Steud: Euphorbiaceae) is a perennial herbaceous plant whose dried roots ('*lang-du*') are used in Chinese traditional medicine. Chemical analyses have revealed that *E. fischeriana* roots contain a variety of diterpenoids (Ma et al., 1997; Wang et al., 2006), and

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the plant has been used effectively in the treatment of human cancer, ascites and edema (Su et al., 2007). To date, however, the anthelmintic properties of this plant against fish parasites in aquaculture had not been investigated.

In this study, therefore, we investigated the effect of the four anthelmintic drugs and the *E. fischeriana* extracts against *D. vastator* adults both *in vitro* and *in vivo*. In addition, we also assessed the effects of the five effective compounds on *D. vastator* eggs *in vitro*. Through these trials we aimed to determine the optimal doses of each of these drugs and the anthelmintic compound derived from *E. fischeriana* required for effective control of *D. vastator*.

2. Materials and methods

2.1. Preparation of goldfish, parasites and drugs

Goldfish, *Carassius auratus*, naturally infected with *Dactylogyrus* spp. were obtained from a farmer and reared in an aquarium for 3 days. On the fourth day, goldfish were killed, and *D. vastator* adults were collected from the gills using fine needles. Then eggs were collected after being laid by adults, placed in an incubator and observed daily. When egg hatching began, the oncomiracidia were removed with a pipette, and introduced into the container with healthy goldfish in dechlorinated tap water. The fish infected with *D. vastator* were obtained. Other experimental goldfish, weighing 2.9 ± 0.4 g were obtained from a commercial fish farm. To ensure that goldfish are healthy, every 5 days, 10 goldfish were randomly selected for examination of parasite infection on the gills under stereoscope for a month. Fish were chosen for experiment when no parasites were observed. Then these healthy goldfish were placed in an aquarium with goldfish infected with *D. vastator* which were reared at a ratio of 80%. After 15 days, 10 goldfish from the aquarium were randomly selected and examined for *D. vastator* infection. Fish were selected for experiments when the infection prevalence reached 100%, and the mean number of the parasites per fish was between 30 and 50.

All synthetic drugs (trichlorfon, praziquantel, mebendazole and 40% phoxim) were obtained from Wuhan Chopper Fishery Bio-Tech Co., Ltd., China. Dried *E. fischeriana* roots were purchased from a local drug store. The extracts of *E. fischeriana* used in our experiments were obtained from Hubei University of Chinese Medicine, Wuhan, China. The basic extraction process was as follows: the dried *E. fischeriana* roots were washed thoroughly, air-dried and finally oven-dried at 45 °C for 48 h. The dried plant material was then crushed and reduced to fine powder using a strainer (30–40 mesh), which was then freeze-dried at −54 °C to ensure complete removal of water. Powdered dry samples of the plant material were extracted with 80% ethanol for 48 h for complete extraction; a process that was repeated three times. To obtain solidified crude extracts, the powdered extracts were filtered and concentrated under reduced pressure in a vacuum rotary evaporator (R-201, Shanghai Shenshen) until all the solvents had evaporated. These crude extracts of *E. fischeriana* were then re-extracted with ethyl acetate, petroleum ether, n-butanol and distilled water in successive stages. The extracts were concentrated, and the solvents evaporated to obtain solidified extracts. All solid extracts were dissolved in acetonum to obtain a 5% (sample/solution) stock solution from which dilutions of the desired experimental concentrations were obtained (see Fig. 1).

2.2. The anthelmintic efficacy of the experimental compounds against adult *D. vastator in vitro*

All the compounds and concentrations were listed in Fig. 1. Active adult parasites were removed from infected fish using fine needles. Twenty active parasites were then manually transferred into one well of a 24-well culture plate containing 2 mL of the specific treatment solution and incubated at 22 °C. All experimental assays were replicated twice and compared with a control treatment (administered with 2 mL of aerated tap water). Parasites were observed every 2 h. The survival of parasites, including worm response and egg laying was recorded. Death was defined as either a lack of movement or presence of obvious autolysis (Reimschuessel et al., 2011). We recorded the time taken to kill 100% of the parasites and hypothesized that a treatment was effective if 100% parasite mortality was achieved within 24 h.

2.3. The anthelmintic efficacy of the experimental compounds against adult *D. vastator in vivo*

Those compounds that exhibited anthelmintic properties *in vitro* (trichlorfon, phoxim, mebendazole, praziquantel and the ethyl acetate extract of *E. fischeriana*, see Section 3.1) were further tested for their anthelmintic efficacy against adult *D. vastator in vivo*. *In vivo* tests were performed on 10 infected goldfish placed in glass tanks containing 20 L aerated tap water at 20–22 °C and each chemical was dissolved to the previously designated concentrations. Three replicates were used for all chemical treatments and further three tanks were used as control groups (no chemicals added). No food was added and fish mortalities were recorded during the treatment period. Forty-eight hours

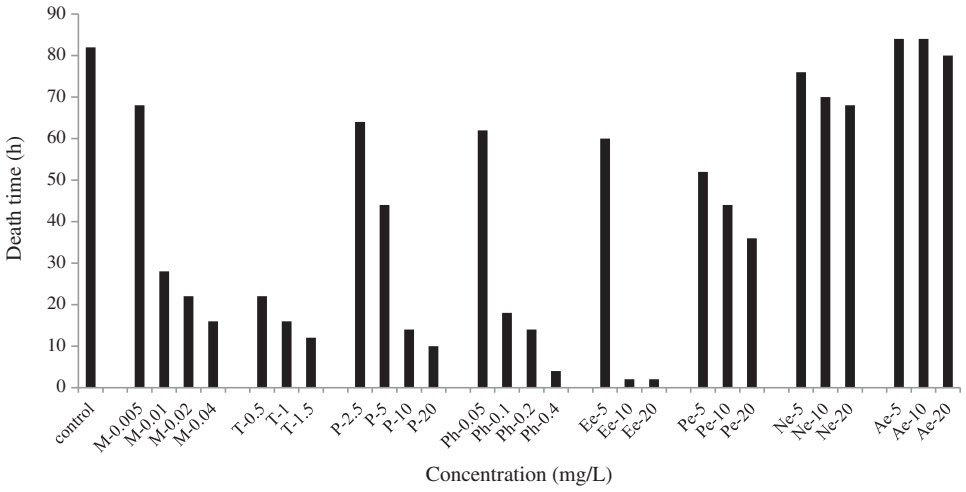

Fig. 1. The time taken to kill all adult *Dactylogyrus vastator* parasites exposed *in vitro* to the various chemical compounds for 48 h (100% death time). M, mebendazole; T, trichlorfon; P, praziquantel; Ph, phoxim; Ee, ethyl acetate extract of *Euphorbia fischeriana*; Pe, petroleum ether extract of *Euphorbia fischeriana*; Ne, n-butanol extract of *Euphorbia fischeriana*; Ae, aqueous extract of *Euphorbia fischeriana*. Treatment concentrations (mg/L) were listed after the compound abbreviation.