Short Communication

The use of crude extracts from traditional medicinal plants to eliminate *Trichodina* sp. in tilapia (*Oreochromis niloticus*) fingerlings

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Abstract

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The treatment for ectoparasitic diseases in freshwater fish with formalin seems at present to be ineffective. For this reason it is evidently a useless cost. In addition, formalin possibly leaves toxic residues in fish flesh and in the environment which are eventually harmful to consumers. The alternative way to solve this problem is to use traditional medicinal plants instead. The purpose of this research is to determine the possibility of using garlic (*Allium sativum*) and Indian almond (*Terminalia catappa*) as optional chemicals to treat fish ectoparasites, *Trichodina* sp. The results showed that crude extracts of either garlic or Indian almond at 800 mg/l significantly ($P < 0.05$) eliminated *Trichodina* sp. infections in tilapia (average weight 3.62±0.06 g each). To evaluate the acute toxicity of these products to the host fish, groups of 20 tilapia (same size as above) were exposed to 3 concentrations of each product for 96 h. Mortality was then determined. The 2 h LC_{50} for tilapia exposed to crude extract of garlic was 2,259.44 mg/L while the 16 h LC_{50} for tilapia exposed to Indian almond extract was 46,665.94 mg/L. This information is the beneficial and fundamental knowledge to develop guidelines to reduce the use of chemicals and antibiotics in freshwater fish culture businesses. The research is underway to determine the long-term effect of Indian almond and garlic to tilapia, if any.

Key words : *Trichodina*, parasite, tilapia, medicinal plant, garlic, Indian almond

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การใช้สารสกัดหยาบจากพืชสมุนไพรในการกำจัดเห็บระฆัง (*Trichodina* sp.) ในลูกปลานิล (*Oreochromis* niloticus)

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ปัจจุบันการใช้ฟอร์มาลินในการรักษาโรคสัตว์น้ำที่เกิดจากปรสิตภายนอกดูเหมือนว่าจะไม่ได้ผลดีเท่าที่ควร นอกจากนั้นฟอร์มาลินอาจก่อให้เกิดการตกค้างในเนื้อปลาและอาจก่อให้เกิดผลทางลบแก่สิ่งแวดล้อม ซึ่งอาจทำให้เกิดอันตรายต่อผู้บริโภคได้ การใช้สมุนไพรเพื่อทดแทนการใช้สารเคมีซึ่งเป็นพิษน่าจะเป็นทางเลือกหนึ่งในการแก้ปัญหาโรคระบาดปลาที่เกิดจากปรสิตภายนอกได้ งานวิจัยนี้มีจุดประสงค์เพื่อทดสอบความเป็นไปได้ในการใช้กระเทียมและใบหูกวางเพื่อกำจัดเห็บระฆัง ผลการทดลองชี้ให้เห็นว่า สารสกัดหยาบจากกระเทียมหรือใบหูกวางอย่างใดอย่างหนึ่งในความเข้มข้นที่ 800 มก/ลิตร สามารถใช้กำจัดเห็บระฆังในลูกปลานิล (น้ำหนักเฉลี่ยตัวละ 3.62 ± 0.06 กรัม) ได้ หลังจากนั้นได้ทดสอบความเป็นพิษเฉียบพลันของสารสกัดดังกล่าวต่อลูกปลา ใช้ลูกปลานิลขนาดเดียวกันกับการทดลองข้างต้น โดยใช้ลูกปลากลุ่มละ 20 ตัว เติมสารสกัดในความเข้มข้นที่ต่างกัน 3 ระดับ สังเกตผลนาน 96 ชั่วโมง พบว่า ค่าความเป็นพิษเฉียบพลันของสารสกัดกระเทียมที่ทำให้ลูกปลานิลตายครึ่งหนึ่งภายในเวลา 2 ชั่วโมง มีค่าเท่ากับ 2,259.44 มก/ลิตร ส่วนค่าความเป็นพิษเฉียบพลันของสารสกัดใบหูกวางที่ทำให้ลูกปลานิลตายครึ่งหนึ่งภายในเวลา 16 ชั่วโมง มีค่าเท่ากับ 46,665.94 มก/ลิตร ข้อมูลพื้นฐานที่ได้นี้จะเป็นประโยชน์ต่อการพัฒนาแนวทางในการลดการใช้สารเคมีและยาปฏิชีวนะในการทำธุรกิจการเพาะเลี้ยงปลาน้ำจืด งานวิจัยต่อเนื่องที่กำลังดำเนินการอยู่คือการตรวจสอบผลข้างเคียงระยะยาวของการใช้กระเทียมและใบหูกวางที่มีต่อปลานิล

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Tilapia (*Oreochromis niloticus)* is one of many economical freshwater fish that are cultured worldwide. In 2003 the increased export of tilapia brought in almost 146 million Thai baht. Unfortunately, the parasitic outbreak acts as an important limiting factor for aquaculture businesses. The especially heavy infection of *Trichodina sp*. in small fish has caused gigantic financial losses. Infected fish are lethargic, generate excessive mucus, and become off-feed eventually which results in considerable deaths. Pinkate *et al*. (2003) reported that every single tilapia raised by farmers in Chiang Mai, Thailand has a *Trichodina* infection. This problem was significantly related to the high organic matter in water. Trichodiniasis used to be effectively controlled with formalin, but it now appears insufficient to control this parasitic infection (Madsen *et al*., 2000b). This leads to increased needless cost and possibly causes undesired toxic residues in fish flesh and in the environment (Jung *et al*. 2001).

Food safety is a great concern worldwide so the producers need to generate clean food products. There is a fast growing interest in screening antiparasitic substances from plants to replace chemical and antibiotic alternatives. Two such plants are garlic and Indian almond. Garlic is one of the edible plants that have had a strong interest to scientists, thus their attention to plants for medicinal use. A wide range of microorganisms including bacteria, fungi, protozoa, and viruses have been shown to be sensitive to crushed garlic preparations. Ankri and Mirelman (1999) reported that Allicin, one of the active ingredients in garlic, was found to exhibit antiparasitic activity, including some major intestinal protozoan parasites such as *Entomoeba histolytica* and *Giardia lamblia*.

Madsen *et al*. (2000b) reported that raw and squeezed garlic (*Allium sativum*) at 200 mg/l had potential to treat trichodiniasis in eel. Indian almond, *Terminalia catappa,* is another plant that is promising to prevent fish diseases. It is commonly used as an herbal drug in Taiwan (Liu *et al*. 1996). It has been locally claimed to be a wound healing substance for Siamese fighting fish hurt after matches in Thailand as well. Burapadaja (1997) showed the crude ethanol extract from the leaves of Indian almond was active against *Staphylococcus aureus* with a minimum inhibitory concentration of 512 µg/ml. In addition, the crude extracts of *T. catappa* had *in vitro* antifungal properties against *Pythium ultimum*, *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Aspergillus fumigatus* (Goun *et al*. 2003). Nonaka *et al*. (1990) showed that the punicalin from the leaves of Indian almond (*Terminalia catappa*) inhibited HIV replication in infected H9 lymphocytes with little cytotoxicity. However, there is no report on applying Indian almond to treat fish ectoparasites. Using the crude extract from either garlic or Indian almond is one of the new challenging methods for trichodiniasis treatment.

The purpose of this present research was to (1) determine the efficacy of garlic and Indian almond as chemical alternatives to control trichodiniasis epizootic in tilapia fingerlings, and (2) to evaluate the toxicity of these alternative parasiticides to tilapia. The benefits would cut down expense and get rid of the toxic residue problem in food products.

Materials and Methods

1. Experimental fish

Tilapia fingerlings (average weight 3.62 ± 0.06 g) derived from a local farm in Chiang Mai, Thailand, were kept in 50x50x100-cm^3 aquaria with high density for 20 days to induce trichodiniasis. A commercial pellet feed was given once a day until satiation. After that, fish were randomly selected to check for *Trichodina* sp. infection before experiment initiation. Sample mucus was scraped from the tilapia body and the two pieces of gill arches were cut for ectoparasite examination. The overall *Trichodina* sp. was counted under a microscope and then the total number was recorded.

2. Anti-parasitic test

The completely randomized design was used in this study. Thirty parasitic-infected tilapias were randomly introduced into glass aquaria with 20 L water in each replication unit. The water was continuously aerated. A series of dosages of garlic and Indian almond extracts were used to determine the best concentration to treat this ectoparasite infection before the experiment started. In several pretests of the garlic and Indian almond preparations 800 ppm seemed to work best in the treatment of ectoparasites. For this reason, the experiment was divided into 3 treatments with three replications per treatment. The trial was conducted to compare the effectiveness of crude extract garlic and Indian almond by exposure of the tilapia to either 800 ppm of crude extract garlic or 800 ppm of Indian almond. A control group was not treated with any chemical. The pH and dissolved oxygen of water were determined before and after treatments. Two days later, groups of 5 tilapias were randomly selected for examination. Mucus was scraped from total surface of skin and two gill arches were removed from experimental tilapia. The number of *Trichodina* sp. from mucus and gills was subsequently counted under a microscope. Experimental fish were then randomly checked for parasite infections in the next two and four weeks.

3. Toxicity of natural substances to tilapia

The acute toxicity of garlic and Indian almond was examined in tilapia fingerlings. The experiment was also used the completely randomized design. Groups of twenty tilapias were exposed to different concentrations of the substances without any water exchange for 96 h. Mortality and abnormal reactions were recorded during exposure. The control group remained in water. The LC_{50} values were calculated as following Reed and Muench (1983) formula;

$$LC_{50} = \text{antilog } \{A + [(B/C)D]\}$$

Where A = log concentration below 50% mortality
B = 50 – mortality below 50%
C = mortality above 50% - mortality below 50%
D = log concentration above 50% - log concentration below 50%

Results and Discussion

Either garlic or Indian almond at 800 ppm was able to remove all *Trichodina* sp. from tilapia after 2-day treatment (Table 1). All treatments were significantly different from control groups. Both garlic and Indian almond are more economical than formalin and, for this reason, the cost of treatments would be reduced. However, *Trichodina* sp. became re-apparent after two weeks. As both Indian almond and garlic contain high organic matter, they are possibly able to cause water deterioration. It is suggested to exchange the water a day after treatment. This trial, however, was conducted with continuous aeration so we did not see the reduction of dissolved oxygen. Although the crude substance generated slightly acidic water as shown in Table 2, at this range it is not going to affect the fish growth.

The acute toxicity of garlic and Indian almond to tilapia fingerling was determined. Fish showed similar behavior when initially exposed to these extracts. Their respiration was increased referring to quicker opercular movement. They swam in a random direction all over the tanks. The surviving fish returned to normal behavior around 12 h after treatment. The 2 h LC_{50} of crude extract garlic for tilapia was 2,259.44 mg/L while the 16 h LC_{50} of Indian almond for tilapia was 46,665.94 mg/L. It was found that a heavy suspension of solids adhered to the gills. This might be an additional cause of fish deaths due to a blockage of oxygen. However, the working concentration for *Trichodina* treatment is much less than the concentration that causes fish deaths. Based on this

Table 1. Parasiticidal activity of garlic and Indian almond on *Trichodina* sp. infection in tilapia.

Treatment	Mean *Trichodina* sp. number		
	2-day post treatment	14-day post treatment	30-day post treatment
Control (water)	49.53±19.53[a]	45.90±28.81[a]	127.33±82.91[a]
800 ppm. garlic	0[b]	1.40±1.54[b]	1.73±1.58[b]
800 ppm. Indian Almond	0[b]	0.20±0.33[b]	0.47±0.55[b]

[a] **Overall, 15 specimens were collected from randomly selected tilapia bodies per treatment and two pieces of gill arches per fish were counted under a microscope and then the total number was recorded.**
[b] **The table shows the mean *Trichodina* sp. number after exposure to crude extract plants for 2, 14, and 30 days. The data following by the different letters are significantly different (P < 0.05).**

Table 2. Water parameters before and after garlic and Indian almond treatment

Treatment	pH		D.O. (ppm)		Temperature (°C)	
	before	after	before	after	before	after
Control (water)	7.72±0.04	7.72±0.05	4.28±0.06	4.20±0.07	26.0±0	26.0±0
garlic (800 ppm)	7.77±0.03	7.65±0.04	4.42±0.09	4.37±0.09	26.0±0	26.0±0
Indian Almond (800 ppm)	7.76±0.07	7.38±0.07	3.72±0.39	4.02±0.37	26.0±0	26.0±0

outcome, either garlic or Indian almond could be developed for safer ectoparasitic treatment.

As it is extremely desirable to reduce the use of hazardous therapeutics for trichodiniasis control, there is a great potential of using garlic and Indian almond for this parasitic treatment. All *Trichodina* sp. were killed two days after treated with either 800 ppm garlic or Indian almond ($P < 0.01$). In this study, we could not use the same amount of garlic extract as shown in Madsen *et al*. (2000b) to eradicate *Trichodina* infection. The possible explanation might be due to the variation of raw material. This is one of the drawbacks of crude extract plant application. Referring to this evidence, the active ingredient for this treatment is needed to identify and find out the effective dosage before commercial application. Additionally, it is difficult to eradicate all *Trichodina* infection from the system. We found some *Trichodina* re-occurred after two week of treatment. Trichodiniasis is primarily a problem of overstocking and poor water management; for this reason, the proper stocking density and water quality management is strongly required to relieve this problem. Madsen *et al*. (2000a) suggested that the infection pressure from trichodiniasis in farms with a relatively high load of organic matter (> 15-20 mg/l) may be relieved by reducing the content of organic dry matter in the processed water.

Some other medicinal plants have been used as antibiotic and chemical alternatives. For example, Dugenci *et al*. (2003) has shown that rainbow trout fed with diets containing aqueous extracts of mistletoe (*Viscum album*), nettle (*Urtica dioica*), and ginger (*Zingiber officinale*) exhibited significant non-specific immune responses. Since all medicinal plants are able to stimulate only non-specific immune responses, vaccines might be a better way to prevent the deadly diseases. These plants could be used as vaccine adjuvants though in order to create more effective vaccines.

The acute toxicity response of garlic to tilapia fingerlings was much higher than that of Indian almond. The 2 h LC_{50} for tilapia exposed to crude extract garlic was 2,259.44 mg/L while the 16 h LC_{50} for tilapia exposed to Indian almond was 46,665.94 mg/L. Both garlic and Indian almond had low acute toxicity to tilapia fingerlings at the working concentration to treat trichodiniasis.

In conclusion, garlic and Indian almond can be used as an alternatives to chemicals to treat *Trichodina* sp. infections in tilapia fingerlings in laboratory trials. Further studies, including the chronic effect on growth, survival rate, and reproduction need to be investigated. The mechanism of these plants on the non-specific immune responses must be conducted before acceptance for field treatment.

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