Antiparasitic activity of cloves (*Syzygium aromaticum*) against *Trichodina* sp. parasites in white snapper (*Lates calcarifer*)

[1]Inem Ode, [2]Tekat D. Cahyono, [1]Jahra Wasahua

[1] Water Resource Management Study Program, Faculty of Fisheries and Marine Sciences, Darussalam Ambon University, Indonesia; [2] Forestry Study Program, Faculty of Agriculture, Darussalam Ambon University, Indonesia. Corresponding author: I. Ode, inem_ode@yahoo.com

Abstract. *Trichodina* sp. is one type of parasite that causes trichodiniasis (itchy disease) in fish. Control of *Trichodina* sp. in fish farming has been using chemicals. This study aimed to evaluate the potential of cloves (*Syzygium aromaticum*) as an antiparasitic for *Trichodina* in white snapper (*Lates calcarifer*). In vitro antiparasitic activity test used 4 treatments with boiled clove water at concentrations of 50, 70, 100 and 130 ppm and 2 control treatments, namely positive control using 5 ppm formalin and negative control using sterile seawater. In vivo antiparasitic test used 4 treatments, namely 3 treatments with boiled clove water at concentrations of 70, 100, 130 ppm and 1 control treatment without boiled clove water. The results of the in vitro antiparasitic test showed that the mortality of *Trichodina* sp. increased significantly ($P<0.05$) in the treatments with boiled clove water compared to the negative control, and was not significant between the treatments at concentrations of 100, 130 ppm and the positive control. The results of the in vivo test showed that the treatment of boiled clove water was able to reduce the average intensity of *Trichodina* sp. in white snapper. The antiparasitic efficacy value was not significantly different ($P<0.05$) between the concentrations of boiled clove water treatments. The results of this study can be the basis for the utilization of cloves as an alternative antiparasitic for fish diseases caused by parasite *Trichodina* sp.

Key Words: aquaculture, cloves, ectoparasite, *Lates calcarifer*, *Trichodina* sp.

Introduction. The success indicators in aquaculture are the achievement of rapid fish growth and high survival rates, thus increasing production value (Ode et al 2023a). One of the important aquaculture commodities is white snapper (*Lates calcarifer*), which is currently cultivated in all coastal waters of Indonesia. The advantages of white snapper include rapid growth, high economic value, and high tolerance to environmental changes. The main constraint in marine fish culture is fish mortality due to disease attacks. Fish diseases cause stunting, longer rearing periods, high feed conversion, low stocking density, and mortality, which can lead to decreased production and economic losses (Ode 2014). *Trichodina* sp. is one type of parasite that causes trichodiniasis (itchy disease) in fish. This parasite is one of the sources of disease in white snapper aquaculture, both at the seed and grow-out stages. The control of *Trichodina* sp. in fish has been done using chemicals such as Methylene blue, Malachite green, formalin, and povidone-iodine (Betadine) (Agustina et al 2019). The continuous use of chemicals with inappropriate doses can cause the accumulation of antibiotic residues in fish meat, which potentially threatens consumer health. In addition, the use of chemicals for fish treatment can also worsen water quality and pollute the environment (Manage 2018; Soares et al 2017).

The application of antibiotics in aquaculture leads to the proliferation of antibiotic-resistant microorganisms. As much as 20-30% of antibiotics are found in the fish's body, and the remaining 70-80% are transmitted to the environment (Tolga et al 2019). To reduce the use of antibiotics and the impacts caused, new breakthroughs are needed in fish disease control using materials that are safe for fish, consumers, and the environment. One alternative that can be used is natural materials derived from plants.

Cloves have the potential to be used as an antiparasitic as they contain phytochemicals that have antimicrobial and anti-inflammatory activities (Batiha et al 2020), gastrointestinal activity against infectious agents and as anthelmintics (Santika et al 2021). This can be the basis for the utilization of cloves as an antiparasitic material. This study aimed to evaluate the potential of cloves as an antiparasitic for *Trichodina* in *L. calcarifer.*

Material and Method

Clove collection and preparation. Dried cloves were obtained from the Mardika Market, Ambon City, Maluku Province, Indonesia. Dried cloves were boiled with sea water in a ratio of 1:6 (100 g cloves: 600 mL sea water). For 5 minutes, cooled, then filtered and put into a sterile container as initial stock to be used in the next stage.

In vitro antiparasitic activity test. The treatments used were 4 concentrations of boiled clove water (50, 70, 100 and 130 ppm) obtained by diluting boiled clove water from the initial stock and 2 control treatments, namely positive control using 5 ppm formalin and negative control using sterile seawater. *Trichodina* parasites were collected from white snapper with a total length of 6-7 cm in the nursery tank of the Ambon Marine Aquaculture Center (BPBLA), by scraping the mucus on the surface of the body and gills of the fish. A total of 15 *Trichodina* were exposed to each treatment for 1 hour. *Trichodina* were observed under a microscope to assess their motility. Mortality was recorded did not move and did not respond when touched with a needle.

In vivo test of antiparasitic activity. The study was conducted in June-July 2024 at the nursery room of BPBLA, Maluku, Indonesia. The study was conducted, in the rainy season, when *Trichodina* parasites were found with high intensity in white snapper nurseries. The test fish used were white snapper with a total length of 6-7 cm. The study used four treatments, namely 3 concentrations of boiled clove water (70, 100, 130 ppm) and 1 control treatment without boiled clove water. Each treatment was performed with 3 replications. The recipient used was a 70 L plastic container with 12 units. To obtain the concentration of boiled clove water according to the treatment, the dilution formula (C1) (V1) = (C2) (V2) was used, where: V1 is the volume of stock solution needed to make the new solution, C1 is the concentration of stock solution, V2 is the final volume of new solution, C2 is the final concentration of new solution. The stocking density of each container is 12 fish. Before this stage begins, a toxicity test of the concentration that will be used as a treatment is first carried out, and the results show that there is no mortality in the test fish. The test fish are adapted for 3 days, then 9 fish are taken from each treatment to calculate the amount of *Trichodina* in the mucus on the surface of the body and gills. After that, the water in the container is reduced according to the required treatment concentration. After 1 hour of immersion treatment, 9 fish were taken randomly from each treatment to calculate the number of *Trichodina* in the mucus on the body surface and gills.

The intensity of *Trichodina* sp. in test fish was calculated using the formula of Reyes-mero et al (2024):

$$\text{Intensity} = \sum P/N$$

Where:
∑ P - the total number of *Trichodina* parasites;
N - the number of samples of infected fish specimens.

The antiparasitic efficacy was calculated using formula based on Ikefuty et al (2015):

$$AE = 100-[(D\times100)/A]$$

Where:
AE - the antiparasitic efficacy (%);
A - the *Trichodina* average before treatment;
D - the *Trichodina* average after treatment.

Statistical analysis. The data were analyzed qualitatively and quantitatively. Statistical analysis using an analysis of variance was applied to identify the comparative treatment effect, followed by Duncan's continuous test at 5% confidence level using the Statistical Program Software System (SPSS) version 24.

Results and Discussion

Trichodina sp. mortality. Mortality of *Trichodina* sp. increased significantly (P<0.05) in boiled clove water treatment compared to negative control, and was not significant at concentrations of 100, 130 ppm compared to positive control. Mortality of *Trichodina* sp. began to occur after 10 minutes in all clove treatments. In the positive control, 100% *Trichodina* mortality occurred in the first 10 minutes of exposure, while in the negative control, no mortality occurred during the 1st hour of exposure (Figure 1). *Trichodina* sp. is able to survive for more than two days without a host (Simbolon et al 2017).

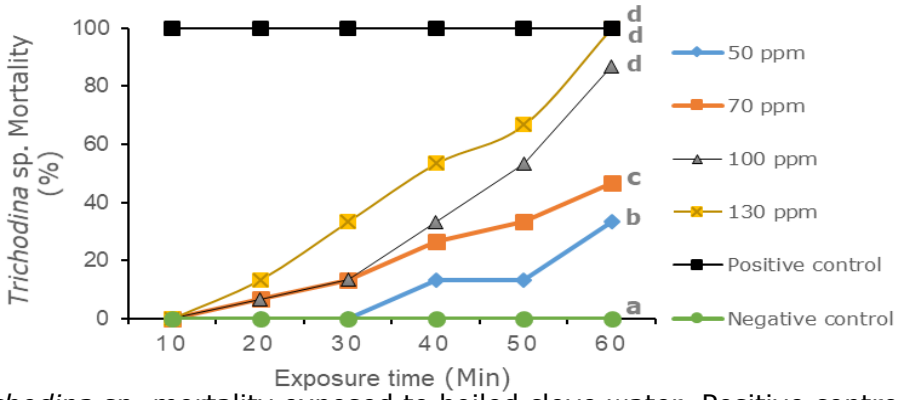

Figure 1. *Trichodina* sp. mortality exposed to boiled clove water. Positive control formalin 5%; Negative control sterile sea water.

The high mortality of *Trichodina* sp. exposed to boiled clove water compared to negative controls shows a good inhibitory action. This is thought to be caused by the active compounds in cloves. The major (64.07%) compound in cloves is eugenol (Phenol, 2-methoxy 4-(2-propenyl) (Ode et al 2023b). According to Ulanowska & Olas (2021), eugenol compounds in cloves can cause cell membrane disruption and cell swelling, cell membrane hyperpolarization and increased membrane permeability, and ultimately leakage of intracellular cell components. Shang et al (2021) stated that eugenol can reduce ATP levels, which results in impaired mitochondrial function and causes cell death in the parasite *Psoroptes cuniculi*. In the field of parasitology, the antiparasitic activity of eugenol has been tested and reported to affect the morphology and growth of parasites such as *Trypanosoma cruzi*, *Giardia lamblia*, *Leishmania donovani*, and *Trichinella spiralis* (El Ghannam et al 2023). *Trichodina* spp. is disc-shaped with a body diameter of 64-88 µm (Khallaf et al 2020). Changes in *Trichodina* morphology after being treated cloves are possibly related to cell membrane damage due to clove bioactive compounds (Figure 2).

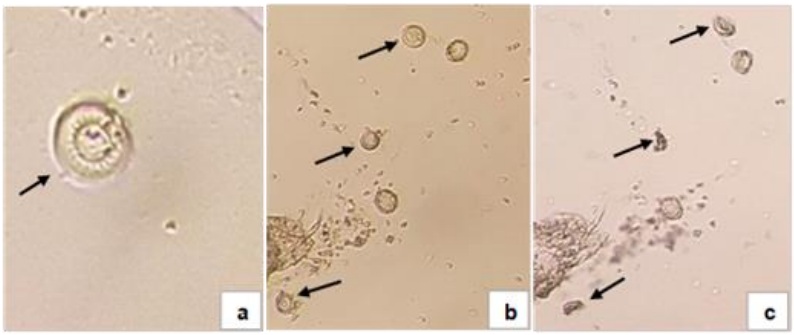

Figure 2. *Trichodina* sp. (a); before exposure (alive) (b); after exposure boiled clove water (death) (c).

Mean intensity of Tricodina sp. The pathogenicity of *Trichodina* is characterized by high intensity values. According to Khallaf et al (2020), *Trichodina*'s pathogenesis is linked to its ability to infect the host. When *Trichodina* firmly attaches to its host, the aboral limiting membrane creates a suction-like motion on the epithelial cell surface, potentially damaging the epithelial cells and irritating the fish tissue. *Trichodina* possesses a concave adhesive disc that enables strong attachment to the host's surface. The adhesive disc is encircled by the aboral limiting membrane (Nimah et al 2022). In this study, the administration of boiled clove water reduced the average intensity value of *Trichodina* sp. (Figure 3). This is likely due to the bioactive compounds in boiled clove water disrupting the aboral limiting membrane of *Trichodina*, thereby impairing its ability to infect fish.

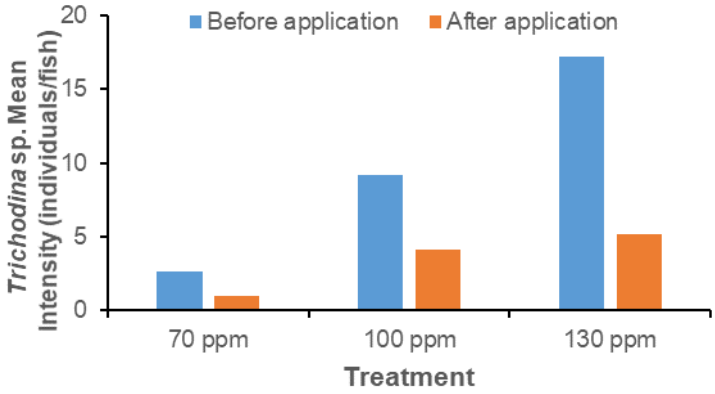

Figure 3. *Trichodina* sp. mean intensity before and after application boiled clove water.

In addition, cell membrane disruption can also affect the reproductive ability of *Trichodina*. Rokhmani et al (2020), *Trichodina* reproduces through binary fission where the new individuals produced will attach directly to the same host or look for new hosts in the water column. High *Trichodina* infection is due to its rapid reproductive ability and active and rapidly spreading movement. The decrease in the average intensity of *Trichodina* after treatment with boiled clove water is thought to be caused by cell membrane disruption that affects *Trichodina* sp. cell division, thus affecting the level of *Trichodina* infection. Research by Vercellini et al (2023) found that 20 mg L^{-1} clove treatment reduced the average abundance of *Diaphorocleidus* sp. parasites.

Antiparasitic efficacy. The antiparasitic efficacy value was not significantly different ($P<0.05$) between all concentrations of clove boiled water treatments. The antiparasitic efficacy value in this study ranged from 62.77-73.49% (Figure 4). Research by Ikefuty et al (2015) found an efficacy of 62.8 to 64.1% for the Teflubenzuron against *Trichodina*, at a concentration of 30-50 mg L^{-1} and for an exposure time of 1 hour. The use of chloramine-t at a concentration of 20 mg L^{-1} significantly reduced *Trichodina* sp. parasitism with an efficacy of 50.27% and 53.23% (Bentes et al 2022).

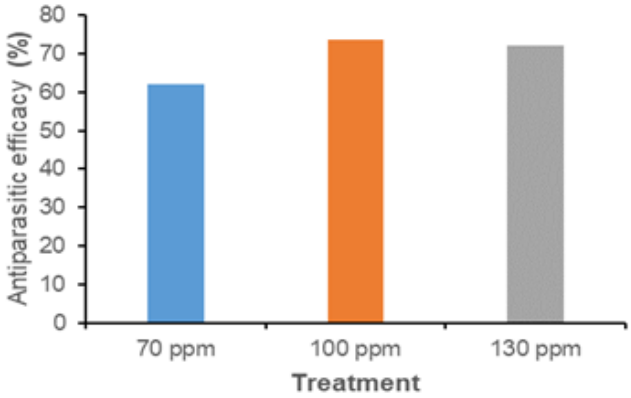

Figure 4. Antiparasitic efficacy.

Conclusions. Clove boiled water treatment can increase *Trichodina* sp. mortality in vitro and it can reduce *Trichodina* intensity in tilapia and white snapper with antiparasitic efficacy values ranging from 62.09 to 73.49%. The results of this study can be the basis for the use of cloves as an alternative antiparasitic for fish diseases caused by *Trichodina* sp. parasites.

Acknowledgements. This research was supported by the DRTPM No. SP DIPA-023.17.1.690523/2024 from the Ministry of Education, Culture, Research and Technology, of Republic Indonesia. The authors are very grateful to the Higher Education Service Institution (LLDIKTI) Region XII, the Research and Community Service Institution (LPPM) Darussalam Ambon University, Laboratory of aquatic organism health in the BPBL Ambon for facility support. We would like to thank Ms. Rara Samin and Risma Ely for their precious help to this study.

Conflict of interest. The authors declare that there is no conflict of interest.

References

Agustina S., Tasruddin, Layoo N., 2019 The use of concentration of piper betle leaf extracts in commercial feed to parasites intensity of *Trichodina* sp. that attacked tilapia (*Oreochromis niloticus*). Journal of Physics: Conference Series 1179-012182:1-6.

Batiha G. E., Alkazmi L. M., Wasef L. G., Beshbishy A. M., Nadwa E. H., Rashwan E. K., 2020 *Syzygium aromaticum* (Myrtaceae): traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. Biomolecules 10(2):202-216.

Bentes C., da Cruz M., Jerônimo G., Coimbra F., Gonçalves L., 2022 Chloramine-T application for *Trichodina* sp. in *Arapaima gigas* juveniles: acute toxicity, histopathology, efficacy, and physiological effects. Veterinary Parasitology 303:109667.

El Ghannam M., Dar Y., El Mehlawy M. H., Mokhtar F. A., Bakr L., 2023 Eugenol; effective anthelmintic compound against foodborne parasite *Trichinella spiralis* muscle larvae and adult. Pathogens 12(1):127-132.

Ikefuti C., Carraschi S., Barbuio R., da Cruz C., Pádua S., Onaka E., Paiva M., 2015 Teflubenzuron as a tool for control of *Trichodinids* in freshwater fish: acute toxicity and *in vivo* efficacy. Experimental Parasitology 154:108-112.

Khallaf M., El-Bahrawy A., Awad A., Elkhatam A., 2020 Prevalence and histopathological studies of *Trichodina* spp. infecting *Oreochromis niloticus* in Behera Governorate, Egypt. Journal of Current Veterinary Research 2:1-7.

Nimah A., Galuh A., Plumeriastuti H., Koesdarto S., Budiarto, 2022 Identification of ectoparasites in pearl catfish (*Clarias gariepinus*) with one and three month age in maclele cultivation, Tuban District, Tuban Regency. Journal of Parasite Science 6(2):60-66.

Manage P. M., 2018 Heavy use of antibiotics in aquaculture: emerging human and animal health problems – A review. Sri Lanka Journal of Aquatic Science 23(1):13-27.

Ode I., 2014 [Ectoparasites on cultured fish in the waters of Ambon Bay]. Jurnal Agribisnis Perikanan 7(1):66-72. [In Indonesian].

Ode I., Sukenda, Widanarni, Dinamella W., Munti Y., Mia S., 2023a Effect of clove powder *Syzygium aromaticum* supplementation on growth and health status of cantang grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂) in floating net cage. Jurnal Perikanan Universitas Gadja Mada 25(1):1-7.

Ode I., Sukenda, Widanarni, Dinamella W., Munti Y., Mia S., 2023b The antibacterial activity of clove *Syzygium aromaticum* extract and its effects on the survival rate of hybrid grouper *Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂ infected with *Vibrio alginolyticus*. Jurnal Akuakultur Indonesia 22(1):1-11.

Reyes-mero B., Qiuntana Y. C., Canul R. R., Gisbet E., Pineros A. M., 2024 The effect of parasitization by *Trichodinid* ciliates on the mortality of cultured pacific fat sleeper larvae (*Dormitator latifrons*). Animals 14(20):3037.
Rokhmani, Darsono, Riwidiharso E., 2020 Diversity of *Trichodina* spp. on the larvae of freshwater fish in Banyumas and surrounding areas of Central Java. IOP Conference Series: Earth and Environmental Science 593:012039.
Santika A., Irkham W., Soedarmanto I., Joko P., 2021 In vitro anthelmintic activity of clove-leaf extract (*Syzygium aromaticum*) against *Ascaridia galli*. Livestock Research for Rural Development 33(7):1-5.
Shang X., Dai L., Yang C., Xiao G., Liu Y., Miao X., Zhang J., 2021 A value-added application of eugenol as acaricidal agent: The mechanism of action and the safety evaluation. Journal of Advanced Research 34:149-158.
Simbolon D., Gultom T., Harahap F., 2017 [Identification of ecto-parasite on the larvae of tilapia (*Oreochromis niloticus*) in Balai Benih Ikan Kabupaten Samosir]. Prosiding Seminar Nasional III Biologi dan Pembelajarannya. Universitas Negeri Medan 1:118-126. [In Indonesian].
Soares B. V., Neves L. R., Ferreira D. O., Oliveira S. B., Chaves F. C., Chagas E. C., Gonçalves R., 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.
Tolga B., Hakan C., İsmail S., Sevket T., 2019 Antibiotic application in fish farm and environmental problems. Turkish Journal of Engineering 3(20):60-67.
Vercellini M., Garcia I., Rearte R., Vargas M. S., Montes M., 2023 Eugenol, menthol, and benzocaine as anesthetic and antiparasitic treatments for *Cheirodon interruptus* (Ostariophysi: Characidae). Aquaculture International 12:1-13.
Ulanowska M., Olas B., 2021 Biological properties and prospects for the application of eugenol—A review. International Journal of Molecular Science 22(7):3671.

Received: 08 November 2024. Accepted: 03 December 2024. Published online: 15 December 2024.
Authors:
Inem Ode, Water Resource Management Study program, Faculty of Fisheries and Marine Sciences, Darussalam Ambon University, Indonesia, e-mail: inem_ode@yahoo.com
Tekat Dwi Cahyono, Forestry study program, Faculty of Agriculture, Darussalam Ambon University, Indonesia, e-mail: tekat@unidar.ac.id
Jahra Wasahua, Water Resource Management Study program, Faculty of Fisheries and Marine Sciences, Darussalam Ambon University, Indonesia, e-mail: jahwasahua_83@yahoo.co.id

How to cite this article:
Ode I., Cahyono T. D., Wasahua J., 2024 Antiparasitic activity of cloves (*Syzygium aromaticum*) against *Trichodina* sp. parasites in white snapper (*Lates calcarifer*). AACL Bioflux 17(6):2899-2904.