Effectiveness of Various Plant Crude Extract on Controlling Parasitic Ciliates (*Colpoda sp*.) Affecting Fishes

ABSTRACT

Aquaculture plays a crucial role in the national economy of many countries. Ciliate protozoans are recognized as economically significant parasites in fish aquaculture. Colpodean ciliates are particularly abundant and are commonly found in terrestrial settings such as soils, leaf litter, and mosses. Diseases are recognized as significant impediments to sustainable animal production, leading to substantial economic losses, particularly in the aquaculture sector. Traditional medicinal plants offer a promising alternative for controlling various diseases in aquaculture. This study examined the efficacy of several plant extracts, including tobacco, neem paste, rue seeds, thyme seeds, and pepper seeds, in managing ciliates. Among the tested plant extracts, Colpoda cultures treated with rue seeds demonstrated the highest effectiveness.

Keywords: Aquaculture; freshwater; diseases; pathogens; medicinal plants.

1. INTRODUCTION

The global expansion of finfish aquaculture in both freshwater and marine settings continues unabated (FAO, 2020), with well-documented potential for significant growth in marine environments (Gentry et al., 2018). A major challenge for all aquaculture species, whether traditional or innovative, lies in the variety of pathogens linked to each new fish type. Fish hosts in their natural habitats harbour a range of specific and non-specific parasites (both specialists and generalists) (Woo et al., 2020). This situation poses risks to both fish health and welfare, as well as the economic viability of aquaculture operations (Shinn et al., 2015). Diseases are acknowledged as a primary obstacle to sustainable animal farming, often resulting in considerable economic setbacks, particularly in aquaculture. While various chemotherapeutic agents have traditionally been employed for the treatment and prevention of diseases in cultivated fish, their use is cautioned against because improper and prolonged use of antibiotics may lead to the development of antibiotic-resistant bacteria (Seyfried et al., 2010). Furthermore, environmental pollution and the accumulation of toxic substances in fish, along with residual antibiotics in the muscles of commercially sold fish, could adversely affect human health (Cabello et al., 2006; Romero et al., 2012). As a result, researchers are increasingly focused on harnessing natural products, such as herbs, to create alternative dietary supplements that support fish growth, health, and immune systems, as these options tend to be cost-effective, safe, efficient, easily prepared, and biodegradable (Citarasu et al., 2010; Mohapatra et al., 2013).

During the last years, the search for new and natural treatments to mitigate the side effects of chemicals used in aquaculture included bioactive chemicals from plants (Wang et al., 2010). Plants are a rich source of bioactive compounds like alkaloids and glycosides, and they might be an alternative source of natural parasitic control (Rahuman et al., 2011). Medicinal plants have been reported as appetite stimulation, antimicrobial, immunostimulant, anti-inflammatory, biopesticides and anti-parasitic properties and their use in traditional medicine has been known for thousands of years around the world (Mehlhorn et al., 2014). Traditional medicinal plants are the most promising alternative source to control various diseases in aquaculture. Because it contains an active compound such as tannins, alkaloids, terpenoids, saponins, phenolics, steroids and flavonoids that possess various biological activities (Chakraborty et al., 2011). These active compounds have characteristics of

antibacterial, antiparasitic, antifungal and antiviral activities that prevent various fish diseases. Besides, their use could reduce the costs of treatment and be more environmentally friendly as they tend to be more biodegradable than synthetic molecules and they are less likely to produce drug resistance in parasites due to the high diversity of plant extract molecules (Olusola et al., 2013).

Neem leaf extract (*Azadirachtaindica* A. Juss.) has been shown to effectively manage approximately 127 pests and functions as a fungicide, bactericide, nematicide, and molluscicide (Kardinan et al., 2002). Research by (Majeed et al., 2018) indicated that neem seed extract is the most toxic among various plant extracts. Additionally, tobacco extract has been reported to be effective in controlling insect pests (Susi et al., 2023). Maitham(2019) investigated *Pegnum harmala*, revealing its antimicrobial properties and noting that the smoke produced from its seeds has antiseptic effects. Ciliates, a highly specialized group of microbial eukaryotes, can be found in almost every environment on Earth's surface where water is available to support their survival (Bai et al., 2020; Agatha et al., 2021). The class Colpodea(Small et al., 1981) represents a significant structural component of soil protozoa, and these ciliates have been documented in diverse terrestrial habitats, ranging from arid deserts to permanently saturated wetlands and bogs (Vdacny et al., 2019). Ciliate protozoans are recognized as economically significant parasites in fish aquaculture (Bastos et al., 2017). with Colpodean ciliates being particularly prevalent in terrestrial ecosystems such as soils, leaf litter, and mosses. Reyes (2024) studied the effect of parasitization by Trichodinid Ciliates on the mortality of cultured Pacific fat sleeper larvae (*Dormitatorlatifrons*). However, Colpoda ciliates have received limited attention in research, as their impacts on humans and domestic animals are minimal (Shen et al., 1999). The aquaculture industry needed much more study on alternative medication to control parasites.

The present study highlights the need to control the growth of Colpoda, a ciliate harmful to fish, and tests various plant extracts for their effectiveness in managing this ciliate. The control of these ciliates through plant extracts has proved to be an efficient method and thus can be used in the aquaculture industries. The cost effectiveness is a major advantage in the usage of plant extracts. Growth of fishes without parasitic attack through commonly available plant extracts is an essential advantage for the fishermen community.

2. MATERIALS AND METHODS

Culture media preparation: Approximately 5 grams of paddy straw were cut into pieces roughly 3 centimeters in length and placed into a 250 ml conical flask. Then, 200 ml of water was added to the flask, which was sealed with cotton and left overnight to allow for the growth of ciliates. Upon examination of the culture media under a microscope, some colpoda were observed. After 12 hours, the culture media was again inspected microscopically, revealing that the ciliates had grown to a minimal size and were relatively few in number. The medium was left for another night to promote better growth. Upon subsequent observation under the microscope, an increased number of colpoda was noted. These colpoda were then subjected to various compounds to evaluate the efficacy of these substances against their growth.

Preparation of various plant - Water Extracts: For the current study, 1 gram each of tobacco powder, neem paste, rue seed powder, thyme seed powder, and pepper seed powder were used to evaluate the efficacy of the plant extracts. The seeds were individually soaked in 10 ml of water for 2 hours to create the water extracts. After this period, four 100 ml conical flasks were labelled 1, 2, 3, and 4. Each flask contained 40 ml of culture media. To flask 1, 1 ml of the corresponding plant extract was added; to flask 2, 2 ml; to flask 3, 5 ml; while flask 4 served as the control. The experiment was conducted in triplicate, and observations were made using a microscope after 24 hours.

Serial dilutions of various plant water extracts: A 1 ml solution of plant water extract from Rue and Thyme seeds is mixed with 9 ml of distilled water in test tube A. From test tube A, 1 ml is transferred to test tube B, which contains 9 ml of distilled water. This process of serial dilution is continued through test tubes C, D, and E, with the dilutions labeled as 10^1,10^2,10^3,10^4 and 10^5 respectively. For each dilution, four 100 ml conical flasks are prepared and labeled 1, 2, 3, and 4. Each flask contains 40 ml of culture media. To flask 1, 1 ml of the diluted plant water extract from test tube A is added; to flask 2, 2 ml; to flask 3, 5 ml; and flask 4 serves as a control, containing 1 ml of distilled water. Observations are recorded after 24 hours. This procedure is repeated for the various serial dilutions from test tubes B, C, D, and E.

Preparation of various plant alcohol Extracts: For the current study, 1 gram each of tobacco,

neem paste, rue seeds, thyme seeds, and pepper seeds were utilized to evaluate the efficacy of various plant extracts. The seeds were extracted separately in 10 ml of alcohol and allowed to sit for 2 hours to obtain the alcohol extract. Following this period, three petri dishes were prepared and labelled A, B, and C. In dish A, 1 ml of the respective plant extracts was added, 2 ml in dish B, and 5 ml in dish C. The dishes were then placed in sunlight for a few minutes to allow the alcohol to evaporate. After drying, 0.01 g of sterile talc was incorporated and mixed into each petri dish to absorb the residues. Four conical flasks of 100 ml were used, labelled 1, 2, 3, and 4. Each flask contained 40 ml of culture media, with the contents of dish A combined with flask 1, dish B with flask 2, and dish C with flask 3; flask 4 served as the control. Observations were made under a microscope after 24 hours. Additionally, serial dilutions of the alcohol extracts were performed using thyme seeds.

pH3 Extract: One gram of rue seed grains was ground into a fine powder and dissolved in 10 mL of water at pH 3, allowing it to steep for 2 hours to create a pH3 extract. After this period, four 100 mL conical flasks were prepared and labeled 1, 2, 3, and 4. Each flask was filled with 40 mL of culture media; to flask 1, 1 mL of rue seed extract was added, to flask 2, 2 mL of extract, and to flask 3, 5 mL of extract was added. Flask 4 served as a control. Observations were made under a microscope after 24 hours.

3. RESULTS AND DISCUSSION

Colpods sp. were cultured in the required media. These colpoda were subjected to treatment with different plant extracts to assess the effectiveness of these compounds in inhibiting their growth. Observations were made for each treatment, utilizing both water and alcohol extracts, to evaluate the survival rates of the *Colpods sp*.

Treatment with tobacco: Colpoda cultured in media were subjected to treatment with both water and alcohol extracts of tobacco, and their survival was monitored after 24 hours. The ciliates remained alive in all concentrations of the water extract; however, they exhibited no movement at the 2 ml concentration, and 100% mortality was recorded at the 5 ml concentration of the alcohol extract. Tobacco has long been regarded as an anti-social compound, and powdered tobacco was introduced to the Colpoda culture. The water extract proved ineffective, while the alcohol extract demonstrated significant efficacy against Colpoda mortality. Notably, even the lowest concentration of tobacco alcohol extract (2 ml) was effective in killing Colpoda. These findings align with the research conducted by (Fatemeh et al., 2016), which indicated that tobacco alcohol extract had a pronounced effect on adult nematodes. Tobacco leaves contain nicotine, a ganglion stimulant, which may be responsible for the anthelmintic activity observed (Bowman et al., 1980). The immobility of adult *M. marshalli* induced by tobacco extracts was found to be both time and dose dependent, with the alcohol extract at a concentration of 75 mg/ml proving to be the most effective against adult worms.

Treatment with Neem: Colpods cultured in media were treated with both water and alcohol extracts of Neem and monitored after 24 hours. The ciliates remained alive in all concentrations of both extracts, exhibiting normal movement. Therefore, Neem appears to be ineffective against Colpods. Both the water and alcohol extracts of Neem indicated 0% mortality in the cultured Colpoda. Contrary to previous studies on *Azadirachtaindica*, the anticipated efficacy was not demonstrated in this instance with Colpoda.

Treatment with Rue seeds: Colpods cultivated in culture media were treated with water, alcohol, and pH3 extracts of rue seeds, and observations were made after 24 hours. Ciliate mortality was observed across all concentrations of the water extract. Serial dilutions of the water extract from rue seeds demonstrated its effectiveness. At a concentration of 10^5, the lowest level of extract, the ciliates showed survival in 1 ml and 2 ml, exhibiting slow movement in the 5 ml concentration. All other dilution concentrations resulted in 100% mortality. In all concentrations of the alcohol extract, the ciliates were found deceased, whereas they remained alive when treated with the pH3 extract. Overall, the water and alcohol extracts of rue seeds proved to be more effective than the pH3 extract. Thus, the study indicated that even at their lowest concentrations, rue seeds exhibited significant efficacy against colpods. Hashemi (2011) reported that ethanol extracts of common rue (*Rutagraveolens*) displayed antifungal properties and inhibited the growth of *Saprolegnia sp*. Rue seeds contain β-carbolines such as harmine, harmalol, and harman, with harmine being the primary hallucinogenic alkaloid. Additionally, the seeds contain a red pigment utilized for dyeing wool and carpets, as well as for culinary purposes and traditional medicine, where they are valued as aphrodisiacs, as noted by (Nazim

et al., 2018). According to Hamid (2004), harmaline, the final product of extraction, serves as the key effective antinociceptive agent in extracts of *Peganum harmala* alkaloids.

Treatment with Thyme seeds: Colpods cultured in media were treated with thyme seed extracts, both in water and alcohol and monitored after 24 hours. The Colpods survived at a 1 ml concentration of the water extract, but complete mortality was observed at 2 ml and 5 ml concentrations. In contrast, none of the Colpods survived when exposed to 1 ml, 2 ml, and 5 ml of the alcohol extracts. Serial dilutions of the alcohol extracts revealed differing survival rates, with no Colpods surviving at a dilution of 10^1. However, cyst formation was noted at a dilution of 10^2 in the 5 ml concentration. At dilutions of 10^3 and 10^4, the Colpods remained unaffected by the thyme extracts. The findings suggest that thyme seeds possess effective plant compounds against Colpoda, with both the water and alcohol extracts demonstrating greater efficacy in killing the Colpods. Similar antibacterial properties of thyme seeds were also reported by (Sienkiewicz et al., 2011).

Treatment with pepper seeds: Colpods cultivated in culture media were exposed to water and alcohol extracts from pepper seeds, with observations made after 24 hours. Mortality was detected in both the 2ml and 5ml concentrations of the water extract, while the colpods in the 1ml concentration survived. In the case of the alcohol extract, the ciliates in the 5ml concentration were found to be lethal, but those in the 1ml and 2ml concentrations remained alive. The colpods were unable to survive at the highest concentrations of both the water and alcohol extracts.

4. CONCLUSION

The present study on the parasite Colpoda utilizing various plant extracts provided valuable insights into effective control products. Among all the plant extracts tested, Rue seeds demonstrated the highest efficacy. The use of plant-derived compounds as alternative treatments for parasites in aquaculture has shown minimal or no negative impact on the environment, as their residues are typically biodegradable in water. In contrast to traditional chemotherapeutics, administering plant-derived compounds to fish is linked to few or no adverse effects. However, the persistence of these compounds in the environment and their potential side effects on human health have received limited attention. Therefore, further research is needed to assess the actual environmental impact of these plant-derived compounds and their effects on human health.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)
Please write this section
Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of this manuscript. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology.

Details of the AI usage are given below:

1.
2.
3.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Agatha, S., Ganser, M. H., &Santoferrara, L. F. (2021). The importance of type species and their correct identification: A key example from tintinnid ciliates (*Alveolata, Ciliophora, Spirotricha*). *The Journal of Eukaryotic Microbiology*, *68*.

Amid Reza, M., & Monsef, M. (2004). Antinociceptive effects of *Peganum harmala* L. alkaloid extract on mouse formalin test. *Journal of Pharmaceutical Science*, *7*, 65–69.

Bai, Y., Wang, R., Song, W., Suzuki, T., & Hu, X. Z. (2020). Redescription of five *Tintinnine* ciliates (*Alveolata: Ciliophora: Oligotrichea*) from coastal waters of Qingdao, China. *Marine Life Science and Technology*, *2*, 209–221.

Bastos Gomes, G., Jerry, D. R., Miller, T. L., & Hutson, K. S. (2017). Current status of parasitic ciliates *Chilodonella* spp. (*Phyllopharyngea: Chilodonellidae*) in freshwater fish aquaculture. *Journal of Fish Diseases*, *40*(5), 703–715.

Bowman, W. C., & Rand, M. J. (1980). *Textbook of Pharmacology* (pp. 29–42). Blackwell Scientific Publications.

Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. *Environmental Microbiology*, *8*, 1137–1144.

Chakraborty, S. B., &Hancz, C. (2011). Application of phytochemicals as immunostimulant, antipathogenic, and antistress agents in finfish culture. *Review in Aquaculture*, *3*, 103–119.

Citarasu, T. (2010). Herbal biomedicines: A new opportunity for aquaculture industry. *Aquaculture International*, *18*, 403–414.

FAO. (2020). *The State of World Fisheries and Aquaculture (SOFIA)*. United Nations Organisation for Food and Agriculture. Rome, Italy.

Gentry, R. R., Froehlich, H. E., Grimm, D., Kareiva, P., Parke, M., Rust, M., Gaines, S. D., & Halpern, B. S. (2017). Mapping the global potential for marine aquaculture. *Nature Ecology and Evolution*, *1*(8), 220–232.

Hashemi Karouei, S. M., Sadeghpour Haji, M., &Gholampour Azizi, I. (2011). Isolation of *Saprolegnia* and the influence of root ethanolic extract of *Rutagraveolens* on *Saprolegnia* spp. growth. In *International Conference on Biotechnology and Environment Management (IPCBEE)* (pp. 19–23).

Kardinan, A. (2002). *PestisidaNabatiRamuan dan Aplikasi* (4th ed.). PenebarSwadaya.

Maitham, M., &Abdulridha, A. (2019). Phytochemical and antibacterial activity of the *Pegnum harmala* seeds and its alkaloids. *Plant Archive*, *19*, 1439–1444.

Majeed, M. Z., Nawaz, M. I., Khan, R. R., & Farooq, C. S. (2018). Tropical and Subtropical Agroecosystems, *21*(3), 421–430.

Mehlhorn, H., Wu, Z., & Ye, B. (Eds.). (2014). *Treatment of Human Parasitosis in Traditional Chinese Medicine* (1st ed.). Springer-Verlag.

Mohapatra, S., Chakraborty, T., Kumar, V., DeBoeck, G., &Mohanta, K. N. (2013). Aquaculture and stress management: A review of probiotic intervention: Stress management by probiotics administration. *Journal of Animal Physiology and Animal Nutrition*, *97*, 405–430.

Nazim, A., & Mamedov. (2018). Pharmacological studies of Syrian rue (*Peganum harmala* L., Zygophyllaceae). *International Journal of Secondary Metabolite*, *5*, 292–302.

Nouri, F. (2016). In vitro anthelminitic effect of tobacco extract on parasitic nematode, *Marshallagiamarshalli*. *Journal of Parasitic Diseases*, *40*, 643–647.

Olusola, S. E., Emikpe, B. O., &Olaifa, F. E. (2013). The potentials of medicinal plant extracts as bio-antimicrobial in aquaculture. *International Journal of Medicine and Aromatic Plants*, *3*, 404–412.

Rahuman, A. A. (2011). Efficacies of medicinal plant extracts against blood-sucking parasites. In H. Mehlhorn (Ed.), *Nature Helps…How Plants and Other Organisms Contribute to Solve Health Problems* (pp. 19–53). Springer-Verlag.

Reyes-Mero, B. M., Cruz-Quintana, Y., Rodríguez-Canul, R., Gisbert, E., & Santana-Piñeros, A. M. (2024). The effect of parasitization by *Trichodinid* ciliates on the mortality of cultured Pacific fat sleeper larvae (*Dormitatorlatifrons*). *Animals*, *14*(20), 3037. https://doi.org/10.3390/ani14203037

Romero Ormazábal, J. M., Feijoó, C. G., & Navarrete Wallace, P. A. (2012). Antibiotics in aquaculture-use, abuse and alternatives. In E. D. Carvalho, J. S. David, & R. J. Silva (Eds.), *Health and Environment in Aquaculture* (pp. 159).

Seyfried, E. E., Newton, R. J., Rubert, K. F., Pedersen, J. A., & McMahon, K. D. (2010). Occurrence of tetracycline resistance genes in aquaculture facilities with varying use of oxytetracycline. *Microbiological Ecology*, *59*, 799–807.

Shen, Y. (1999). *Protozoology* (1st ed.). Science Press.

Shinn, A. P., Pratoomyot, J., Bron, J. E., Paladini, G., Brooker, E. E., & Brooker, A. J. (2015). Economic costs of protistan and metazoan parasites to global mariculture. *Parasitology*, *142*, 196–270.

Sienkiewicz, M. (2011). Antibacterial activity of thyme and lavender essential oil. *Medicinal Chemistry*, *7*, 20–31.

Small, E. B., & Lynn, D. H. (1981). A new macrosystem for the phylum *Ciliophora*Doflein, 1901. *BioSystems*, *14*, 387–401.

Vdacny, P., &Foissner, W. (2019). Re-analysis of the 18S rRNA gene phylogeny of the ciliate class *Colpodea*. *European Journal of Protistology*, *67*, 89–105.

Wang, K. Y., Yao, L., Du, Y. H., Xie, J. B., Huang, J. L., & Yin, Z. Q. (2011). Anthelmintic activity of the crude extracts, fractions, and osthole from *Radix angelicaepubescentis* against *Dactylogyrus intermedius* in goldfish

(*Carassius auratus*) in vivo. *Parasitology Research*, *108*, 195–200.

Woo, P. T. K., Leong, A. L., & Buchmann, K. (Eds.). (2020). *Climate change and infectious fish diseases (CCIFD)* sections I, II, III. CAB International.

Wuryantini, S., Endarto, O., Wicaksono, R. C., Istianto, M., Hussain, Z., &Triasih, U. (2023). Bioinsecticide activity of neem oil and tobacco extract mixture against citrus aphids and green scale on citrus. *E3S Web of Conferences*, *432*.