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**PLANT EXTRACT AS ALTERNATIVE TREATMENT FOR NILE TILAPIA
FINGERLINGS INFESTED WITH *Trichodina* spp.**

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ABSTRACT

This study was conducted in order to evaluate the potential of different plant extracts (guava *Psidiumguajava*, annatto *Bixaorellana*, red sage *Lantana camara* and jambolan *Syzygiumcumini*) against *Trichodinaspp.* of Nile tilapia fingerlings. Initial parasite abundance and prevalence was compared after two-day treatment using a commercial anti-parasitic drug and plant extracts (50 mg/L). Percent prevalences of *Trichodina* spp. in the gills, skin and fins remained very high (93.30 to 100.00%) after two days treatment. Extracts from annatto (128.1±97.8), red sage (178.4±209.5), guava (52.3±75.9) and jambolan (56.1±71.7), and the commercial anti-parasitic drug (163.7±101.5) were significantly effective in reducing the abundance of *Trichodina* spp. in the gills of Nile tilapia fingerlings as compared to the initial abundance (215.2±190.1). Except jambolan (26.9±34.8), plant extracts from guava (9.3±10.1), annatto (16.2±12.3) and red sage (16.8±21.4) were also found effective in decreasing the initial abundance (25.7±21.9) of skin and fins *Trichodina* spp. Commercial anti-parasitic drug was not successful in controlling the parasite in the skin and fins of tilapia fingerlings.

Keywords: Nile tilapia, plant extracts, *Trichodina* spp., abundance, prevalence

INTRODUCTION

Tilapias (*Oreochromis* spp.) are hailed as the culture species of the 21st century [1]. In the Philippines, tilapia is considered as one of the major fishery products [1]. It has been reported that the Philippine tilapia production from aquaculture had an average of 2,256,000 MT [2].

Parasitic infestation of cultured fishes in tropical and subtropical countries represents a serious problem for aquaculture [3]. Heavy infestation of these parasites cause reduced growth rate and mortality if not treated properly, thus results in great economic loss [4]. Occurrence of fish kills can be related to changes in parasite density and community composition [5]. Tissue damage is relative to the rate of parasite infestation [5].

Although Nile tilapia (*O. niloticus*) has been reported to have a relatively higher resistance to parasitic diseases than other common cultured freshwater fish, especially at optimum temperatures, the fingerlings and young fishes are most susceptible to infestations under stressful conditions [6]. The most common parasites recorded in tilapias are species of *Trichodina* [7].

Trichodina species are considered pathogenic to both wild and cultured fishes. This parasite is identifiable by its saucer

shape, presence of hair-like projections in its body and rapid gliding movement [8]. This protozoan parasite has been reported to cause serious mortality in wild and farmed reared tilapia in both hatcheries and rearing facilities [9].

Parasite-infested fishes may show injuries on the skin and gills, and these injuries may become sites for secondary infection commonly caused by bacteria, fungi or protozoa [10]. In order to address parasitic problems in intensive culture system, several devices such as clarifier and biofilter are used [11].

Most of the medicinal plants locally available have secondary metabolites which include alkaloids, phytosteroids, saponins, triterpenoids, proteins, tannins and flavonoids responsible for their anti-parasitic activity [12]. The therapeutic preparations from these plants are cheap and cost effective [13].

The general objective of this study was to evaluate the potential of different plant extracts against *Trichodina* spp. of Nile tilapia fingerlings.

MATERIALS AND METHODS

Collection of Samples

Young leaves of guava (*Psidium guajava*), annatto (*Bixa orellana*),

red sage (*Lantana camara*) and jambolan (*Syzygium cumini*) were collected in Science City of Muñoz, Nueva Ecija. Meanwhile, Nile tilapia fingerlings infested with *Trichodina* spp. were obtained from a private hatchery located in the same area.

Preparation of Ethanolic Extract

Leaves of the plant samples were sun-dried for 1 hour and air dried for 2 to 3 weeks depending on the moisture content of the plant samples. The dried leaves were cut into smaller pieces, powdered and stored in plastic containers until ready for use. Twenty-five grams (25 g) of the powdered leaves were soaked in 70 mL ethanol and 30 mL distilled water for 48 hours at room temperature to give a concentration of 833 mg/mL. The resulting mixture was filtered using filter paper and the filtrate was concentrated in a water bath. The resulting residue was diluted in distilled water to give the desired doses of 25, 50, 100, 200 and 300 mg/mL [14].

Bioassay

The toxicity of the plant extracts at various dosages (25, 50, 100, 200 and 300 mg/L) was evaluated first. Plastic containers were filled with 3.5 L of tap water. The plant extract was added at a ratio of 1 mL extract: 1 L tap water. Each container was stocked with 35 pre-conditioned tilapia fingerlings

(size 24). Fish mortality was recorded after 48 hours. Plant extracts of higher concentration with less than 25% mortality were used in the anti-parasitic experiment.

Anti-Parasitic Experiment

Fifty pieces of naturally-infected tilapia fingerlings (size 24) were stocked in 6 L capacity circular plastic containers [15]. The study employed positive and negative control, C (+) = commercial anti-parasitic drug (Trichlorfon as active ingredient) and C (-) = tap water only. Plant extracts served as the treatments. The concentration of the plant extracts was based on the bioassay result. Each control and treatment was replicated thrice. The fish was fed with fry mash following the recommended feeding guide for tilapia fingerlings of the Freshwater Aquaculture Center -Central Luzon State University (CF-CLSU).

Data Gathered

Initial counting of *Trichodina* spp. in tilapia fingerlings was done one day before the anti-parasitic experiment. The final counting was executed two days after the anti-parasitic experiment. Smears of gills, skin and fins were made and examined under the light microscope for the presence of *Trichodina* spp. The parasite was identified using the keys of Kabata (1985). Abundance and prevalence (%) of *Trichodina* spp. was

estimated following the formulae proposed by Margolis *et al.* (1982) as follows [16]:

$$\text{Abundance of parasite} = \frac{\text{number of parasite counted}}{\text{number of fish host examined}}$$

$$\text{Prevalence of parasite} = \frac{\text{number of fish host with parasite}}{\text{number of fish host examined}} \times 100$$

Statistical Analysis

Significant differences in the abundance of *Trichodina* spp. during initial count and after the two-day treatment were analyzed using One-Way Analysis of Variance.

RESULTS AND DISCUSSION

Bioassay

In general, percent mortality of fingerlings increases with increasing extract concentration. Tilapia fingerlings can only tolerate the 25 and 50 mg/L extract concentrations. Higher extract concentrations (100, 200 and 300 mg/L) already resulted to 98 to 100% mortalities (Table 1). Concentration of 50 mg/L was used for the anti-parasitic experiment.

Anti-Parasitic Experiment

Although, the prevalence of *Trichodina* spp. in the gills, skin and fins remained very high (93.30 to 100.00%) after two days treatment, it was evident that the four plant extracts had potentials in reducing the abundance of the parasite. Abundance of *Trichodina* spp. in the gills of untreated

tilapia fingerlings (C-) remained high and was still comparable with the initial parasite count. The effectiveness of commercial anti-parasitic drug (C+) in reducing the abundance of *Trichodina* spp. in the gills was comparable to annatto (T2) and red sage (T3). Extracts from guava (T1) and jambolan (T4) were considered to be the most effective against gills *Trichodina* spp. because it recorded the lowest parasite abundances. Meanwhile, except jambolan (T4), plant extracts from guava (T1), annatto (T2) and red sage (T3) were also found effective in controlling skin and fins *Trichodina* spp. Commercial anti-parasitic drug (C+) was not successful in reducing the parasite in the skin and fins of tilapia fingerlings (Table 2).

Natural molecules present in plants are important in medicine not only in their pharmaceutical or chemotherapeutic effects but also in their role as a base molecule for the production of synthetic drugs [17]. Terpenes, phenolic compounds and nitrogen containing compounds are the three major secondary metabolites present in plants [18].

Terpenes are found to be toxic to herbivores and probably to parasites. Phenolic compound such as tannins and flavonoids present in guava possess toxin which are found to be anti-microbial, insecticidal and anti-estrogenic [18]. Some flavonoids in guava can kill many bacterial strains, reduce important viral enzymes, like reverse transcriptase and protease, and kill some pathogenic protozoans [19]. Saponin which is also an active compound in guava and annatto can cause intercalation in the cell membranes of parasite due to its hydrophobic action resulting to formation of pores. Nitrogen containing compounds include alkaloids, cyanogenic glycosides, glucosinolates and non-protein amino acids. Alkaloids are present in acacia and jambolan, and this active compound act as stimulant, anesthetic and analgesic at lower doses but poisoning agents at high doses. Alkaloids also show nematocidal activity on migrating larvae in host's tissues [20]. Cyanogenic glycoside found in jambolan releases hydrogen cyanide while glucosinolates release volatile toxins which are toxic to animals [18]. Essential oils or some of their constituents are effective against a large variety of organisms including bacteria,

fungi, viruses, protozoa as well as metazoan parasites [21]. Condensed tannins which are present in acacia are found to be effective in reduction of worm burden, hatching of eggs and fecal age count [22, 23]. The active component of red sage is called latadenes. Extracts from the leaves of red sage were studied for its anti-helminthic activity against *Pheretima posthuma* [24].

Phytochemicals in plants are found to be effective against parasites because it hampered the parasite's energy metabolism. Also, the central targets of these phytochemicals are the DNA (intercalation, alkylation), membrane integrity, microtubules and neuronal signal transduction of the parasites. Phytochemicals from plants also interfere the energy generation of parasites by uncoupling oxidative phosphorylation, consequently leading to depletion of parasite energy (ATP) [25].

Plants contain anti-oxidant that improves physiological condition of fish which may result to stronger immunity. Consumption of such bioactive plants had the beneficial effects on the host physiology and the ability to maintain homeostasis under parasitic challenge [26].

Table 1: Mean mortality (%) of tilapia fingerlings exposed to ethanolic leaf extract at various concentrations (T1 = guava, T2 = annatto, T3 = red sage, T4 = jambolan; A = 25 mg/L, B = 50 mg/L, C = 100 mg/L, D = 200 mg/L, E = 300 mg/L).

Treatment	Mean Mortality	Treatment	Mean Mortality
T1A	3.81±4.36	T3A	10.48±11.55
T1B	16.19±4.36	T3B	24.76±8.73
T1C	100.00±0.00	T3C	98.10±3.30
T1D	100.00±0.00	T3D	100.00±0.00
T1E	100.00±0.00	T3E	100.00±0.00
T2A	11.43±11.43	T4A	8.57±4.95
T2B	22.86±10.30	T4B	14.29±2.86
T2C	100.00±0.00	T4C	100.00±0.00
T2D	100.00±0.00	T4D	100.00±0.00
T2E	100.00±0.00	T4E	100.00±0.00

Table 2: Comparison of mean abundance (parasite/fish) and prevalence (%) of *Trichodinaspp.* in the gills, skin and fins of Nile tilapia fingerlings after two days treatment in commercial anti-parasitic drug (Trichlorfoan) and various plant extracts (C+ = commercial anti-parasitic drug; C- = tap water; T1 = guava; T2 = annatto; T3 = red sage; T4 = jambolan).

	Gills		Skin and Fins	
	Abundance	Prevalence	Abundance	Prevalence
Initial Count	215.2±190.1 ^a	100.0	25.7±21.9 ^a	98.9±1.7
Positive Control (C+)	163.7±101.5 ^b	100.0	33.2±30.2 ^a	100.0±0.0
Negative Control (C-)	202.8±134.0 ^a	100.0	23.7±15.5 ^a	100.0±0.0
T1	52.3±75.9 ^c	93.3	9.3±10.1 ^c	100.0±0.0
T2	128.1±97.8 ^b	100.0	16.2±12.3 ^b	100.0±0.0
T3	178.4±209.5 ^b	100.0	16.8±21.4 ^b	93.3±11.5
T4	56.1±71.7 ^c	100.0	26.9±34.8 ^a	93.3±11.5

CONCLUSION

Nowadays, multiple anti-parasitic commercial drugs have been developed due to indiscriminate number of parasites that causes problems and losses in aquaculture [27]. However, these commercial drugs are costly and sometimes cause adverse effects in fish.

Results from this study revealed that extracts from annatto, red sage, guava and jambolan were effective in reducing the abundance of *Trichodina* spp. in the gills of tilapia fingerlings. *Trichodina* spp. count in skin and fins was also controlled upon using

guava, annatto and red sage extracts. Commercial anti-parasitic drug was not successful in decreasing skin and fins *Trichodina* spp.

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