Tridax procumbens EXTRACT AGAINST *Argulus spp.* (FISH LICE) IN *Cyprinus carpio* (KOI FISH)

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Abstract*—The study aimed to develop an alternative antiparasitic treatment from Tridax procumbens (coatbutton) which may cause no damage to both the fish population and the environment against the fish lice (Argulus spp.) on Koi fish (Cyprinu carpio). The researchers conducted the Toxicity test and found out that the different concentrations of Tridax procumbens extract (100%, 75% and 50%) does not affect the color, mucus production and swimming behavior of Koi fishes. No mortality occurred thus, Tridax procumbens extract is not lethal to the Koi fishes. In vitro test showed high mortality of Argulus spp. Analysis showed that there is no significant difference between the effects of Tridax procumbens extract and the positive control (100% Neguvon solution) on Argulus spp. These only imply that using the different concentrations of Tridax procumbens extract as an antiparasite to Argulus spp. is as effective as the positive control (100% Neguvon solution) and it does not cause damage to the koi fish.

***Indexed Terms*—Argulus spp., Coatbutton, Koi fish, Tridax Procumbens, Tridax procumbens extract**

I. INTRODUCTION

The popularity of Japanese Koi fish has greatly affected the Philippine aquaculture industry. It motivates many Filipinos to be involved in the ornamental fish culture business (Kyodo, 2008). According to Dela Vega of the Bureau of Fish and Aquatic Resources, Philippines which is a tropical country, is a thriving site for breeding ornamental fish like goldfish, koi and guppies. However, there are some factors that affect the success of the propagation of Koi fish in the Philippines. One of this is the infestation of *Argulus spp.*, which causes disease outbreaks and mortalities in aquaculture resulting in serious economic losses (Zafar I., Rabia M. & Ramsha S., 2013).

One of the most common parasitic crustacean that infest a wide range of fish species including Koi fishes is *Argulus spp*. Argulus are ectoparasites of ornamental and food fish that have become wide spread due to anthropogenic introduction of their host (Walker, 2011). In severe infestation, the host displays hemorrhagic and edematous body and fins resulting to dermatitis, anemia, and lethargic movement. In addition, Argulus act as a vector for other fish disease such as Spring viremia of carp and carp pox (cyprinid herpesvirus 1), conjoined with Aeromonas (bacteria) and Saprolegnia (water mold) (Mayer et. al, 2012).

Drugs available for treatment of Argulus infestation are organophosphate pesticide, diflubenzuron, luferunon, emamectin benzoate (in-feed product), and potassium permanganate which are said to be limited for general use since its effectiveness are overcome by the risk that it causes to the fishes (Steckler, 2012). Some suggest the use of salts and formalin for the control of *Argulus spp.* but increasing the salt concentration does not appear to be effective given that it irritates the skin of the fish causing them to flash and rub against objects creating wounds to its body. It is vital to find new and alternative treatments to manage the Argulus infestation considering this issue.

Grundy (2010) and Ikewuchi (2009) stated that plants have bioactive compounds. Bioactive compounds are mainly secondary plants metabolites eliciting pharmacological or toxicological effects in man and animals such as alkaloids, flavonoids, saponin, tannins and terpenes. *Tridax procumbens* (coatbuttons), belong to the family of Compositae has been used as traditional medicine by the native population of India. The different parts of the plant have been used because

it is proven effective in the treatment of ailments such as bronchial catarrh, dysentery, malaria, stomachache, diarrhea, high blood, bruises and wounds and prevention of falling of hair. It also possesses antiseptic, insecticidal, anti-parasitic and hepatoprotective properties and has depressant properties on respiratory system that can prevent the organism from having respiratory related diseases.

Mundada and Shivhare (2010) revealed in *Tridax procumbens'* phytochemical screening of the leaf extracts had revealed the presence of alkaloids, carotenoids, flavonoids, saponins and tannins that may have the potential to kill Argulus spp. To control Argulus spp., this study sought to propose an alternative treatment using *Tridax procumbens* (coatbuttons), which may not harm the fish population, handlers, or environment.

Statement of the Problem

This study aims to control *Argulus spp.* (fish lice) in Koi fish using *Tridax procumbens* (coatbutton) extract.

This study answers the following sub-problems:

1. What are the effects of different concentration of *Tridax procumbens* extract on the Koi fish in terms of the following:
a) Physical qualities
i. Color
ii. mucus production
b) Swimming behavior
2. Is there any significant difference in the effects of different concentration of *Tridax procumbens* extract on the Koi fish in terms of the following:
a) Physical Properties
i. color
ii. mucus production
b) Swimming behavior
3. What is the mortality of Koi fish in different concentrations of *Tridax procumbens*?
4. What is the mortality of Argulus spp. in different concentrations of *Tridax procumbens*?
5. Is there any significant difference in the mortality of Argulus spp. in different concentration of *Tridax procumbens*?

Significance of the study

Greater than 90% of the world's aquaculture is being contributed by Asia such as the Philippines together with the different countries. Fish industry has greatly influenced the Philippines since it is known as a country that cultivates and propagates fishes.

Fish production does not only serve as a source for food, but it also serves as an income for many. In 2010, Philippines ranked 5th among top fish producing countries in the world. Aside from being known as site for fish production Philippines, it was also considered as a good propagating site for ornamental fishes such as Koi fish.

In line with the success in the propagation of fish in different countries, its aquaculture industry has also been overwhelmed with its share of diseases and problems caused by viruses, bacteria, fungi, parasites and other undiagnosed and emerging pathogens. Disease is now a primary constraint to the culture of many aquatic species impeding both economic and social development in many countries (Bondad R. et. al., 2005).

Philippines is not an exception to this wide problem in aquaculture industry in fact According to Zafar I., Rabia M. and Ramsha S.(2013), Argulus spp. were reported to be the cause of high mortality in reared tilapias, mullets, eels, as well as Koi fishes. In response to this problem, some chemically produced treatments are offered such as organophosphate pesticide, diflubenzuron or luferunon, emamectin benzoate, and potassium permanganate, which effectively eradicate the said parasite, but the use of these treatments entails some harmful side effects that can harm the environment, holder, as well as the fish. Therefore, the researchers came out with the aim of the study which is to develop an alternative control agent from *Tridax procumbens* (coatbuttons) which may cause no damage to both the fish population and the environment to control argulosis.

The study also accentuates the importance of plants in treating pathogenic and parasitic diseases in fishes and offers another usage of *Tridax procumbens* aside from its usual usage. Exploring the potential antiparastic activity of this plant against harmful pathogen and

parasite that cause disease will be a big help to the whole aquaculture industry.

This study also aims to enlighten the farmers about the destructive effect of *Argulus spp.* outbreak as well as the harmful side effect of the use of chemically produced treatments to treat argulosis in fishes as well as the environment. Thus, farmers can maximize the use of their available resources such as the weed *Tridax procumbens* instead of buying commercialized drugs, hence lowering the expenses of the farmers.

In addition, the study enhanced the researchers' writing skills, as well as developing their laboratory skills. In addition, it will give the researchers the opportunity to improve their fish handling skills.

Scope and Limitations of the Study

The study mainly focused on the extraction of the biological active component of *Tridax procumbens* such as alkaloids, flavonoids, saponin, tannins and terpenes that can be used to control the fish ectoparasite, *Argulus spp.* The study mainly treated the Argulus in vitro. The experiment used the leaf extract of *Tridax procumbens* to test it against the Argulus sp. through the solvent extraction of the leaves and defatting and subjecting the crude extract obtained to a reflux process that will give to the pure extract of *Tridax procumbens* leaves. The solvent used was ethyl acetate. The determined concentrations of the leaf extract used in the experiment are 100%, 75% and 50%. 100% Neguvon was used as the positive control to kill the parasite.

Koi (*Cyprinus carpio*), 4-5 inches in length were used as a subject for the toxicity test. The study also involved the computation of the lethal concentration of the leaf extract of *Tridax procumbens* to determine the concentration that is lethal to the Koi fish. The experiment had lasted for four weeks. The study was conducted at Philippine Normal University.

II. METHODOLOGY

The researcher used experimental research, the attempt to maintain control over all factors that may affect the result of an experiment. In doing this, the researchers attempt to determine what may occur. In this type of research, the researchers manipulated the independent variable to determine its effect on the dependent variable. The independent variable of this research was the different concentration of the crude extract of *Tridax procumbens*. The dependent variables were the mortality of the *Argulus* and Koi fish in different concentration of *Tridax procumbens* extract. The extraneous variables were the following: number of *Argulus* and Koi fish in each set-up, amount of different concentration of crude extract of *Tridax procumbens* in each set up, amount of water, amount of Neguvon (100%), size of container, and days of toxicity test.

The design used in the study is the complete randomized design specifically treatment by subjects or repeated measure design where in the researchers used different treatments that was assigned to the experimental specimens completely by chance. The study was divided into two parts, the toxicity test and the in vitro test (experimental test).

In toxicity test, five fishes were selected randomly and put in each section of each aquarium and treated with the assigned treatment solution. The fishes were exposed to three different concentrations of the crude extract of the *Tridax procumbens* leaves (coatbuttons) which are 100%, 75%, and 50% and the negative control (water). The toxicity test lasted for four days in which every twelve hours observation and monitoring were conducted. After four days, each concentration of *Tridax procumbens* extract were evaluated for its toxicity to Koi fish the lethal concentration of the *Tridax procumbens* solution were computed as well as the mortality of the Koi fish were. In the in vitro test, fifty fish lice (Argulus) were selected randomly and were placed in each separated clear plastic cups with water and were treated with the assigned treatment solution. The fish lice (Argulus) were exposed to three different concentrations of the crude extract of the *Tridax procumbens* leaves (coatbuttons) which are 100%, 75%, 50%, and in the commercialized anti fish lice which is the positive (100% Neguvon) control as well as to the negative control (water). The in vitro test lasted for two days in which every six hours observation and monitoring were conducted. After two days, the mortality of the Argulus in different concentration of *Tridax* procumbens solution was evaluated and the

antiparasitic efficacies of *Tridax procumbens* extract were evaluated.

A. Materials and Methods of the Study

Collection and Preparation of Tridax Procumbens extract

Before the preparation of Tridax procumbens extract, all the needed materials that were used in the study were sterilized. The beakers, graduated cylinders, airtight bottles, funnel, stirring rod and other materials used in the reflux set-up were washed with running water to remove the dirt and prevent contamination. The crude extraction was carried out using the method of Abubakar et. al. (2012) with some modifications. *Tridax procumbens* leaves were collected from Molino Road in Bacoor, Cavite. The collected leaves were put in a clean plastic bag and were weighed using a weighing scale for its wet weight which is one kilogram (1 kg). The leaves were then washed with running tap water and dried under shade at room temperature until all the moisture of the leaves were gone. Dried leaves were weighed for its dry weight which is two hundred fifty grams (250 g). The dried leaves sample were homogenized to fine powder using an electric blender and were stored in clean airtight bottles until required for use. Fifty grams (50g) of dried powdered samples of *Tridax procumbens* were mixed with 500 ml of ethyl acetate, macerated and stored for 24 hours at room temperature with mild shaking. Solutions were filtered using filter paper then the filtrate was subjected to rotary evaporator for the crude extraction. The obtained extracts were transferred into airtight bottle for storage at refrigerated temperature until required for reflux.

Preparation of different concentration of Tridax procumbens extract

The extract from rotary evaporator was then mixed with little amount of ethyl acetate and small amount of hexane. The hexane, which dissolves the fat content where the biological active compound is attached to, was used to separate the biological active compounds from unwanted compounds of the extract. After mixing hexane with the extract, it was stored overnight. This is to give time for the hexane solution to separate from the unwanted compounds. After one day storage, the upper layer (unwanted compound) and lower layer (hexane solution) were separated with the use of the separatory funnel. The hexane solution was then subjected to reflux to defat the solution and to separate the crude extract from hexane and ethyl acetate. The solution was allowed to boil for one hour while monitoring its temperature not to exceed the boiling point of hexane. After one hour of reflux, the solution was then allowed to stand for 10 minutes or so until the solution cools. The solution was then put in a separatory funnel allowing the solution to separate into upper layer (hexane (fat content) ethyl acetate mixture) and the lower layer (crude extract). The collected lower layer was stored in an airtight bottle and the remaining the extract was subjected again in another round of reflux.

After which the crude extract was then diluted in different amount of distilled water to obtain the different concentration of *Tridax procumbens* extract (100%, 75%. 50%). The 100% concentration was obtained by getting 100 ml of the crude extract. The 75% concentration was obtained through the dilution of 75 ml crude extract to 25 ml of distilled water. Lastly, the 50% concentration was obtained through the dilution of 50 ml crude extract to 50 ml of distilled water. The formulated concentrations of Tridax procumbens crude extracts were stored in clean airtight bottles.

B. Toxicity test

Preparation of Experimental set up

The experimental set up was placed on tables at the back of CED Ayala, Taft Ave. wing. Four (4) aquariums of ten (10) gallon capacity, filter sponges, plastic tubes, compressor, two (2) scoop nets, and four (4) pails were purchased from Cartimar, Pasay city. One stirring rod, graduated cylinder, and beaker were borrowed from Science Resource Center in Philippine Normal University. These materials were used in acclimatizing the Koi fishes (*Cyprinus carpio*). These materials were sterilized first by washing with diluted chlorine solution, prepared by the dilution of four (4) grams of chlorine in twenty-five (25) liters of water, and after which was washed with clean water. These were air dried for one day to prevent contamination.

Each of the aquaria was properly labeled with letters A, B, C, and D according to the crude extract concentration (100%, 75%, and 50%) and 0ml/L (control set up) assign to it. A (100% *Tridax procumbens* extract), B (75% *Tridax procumbens* extract), C (50% % *Tridax procumbens* extract) and D

(negative control) was labeled to aquarium 1, 2, 3 and 4 respectively.

The water used was first stocked in pails for two days to separate its chlorine content to avoid unnecessary death of Koi fishes. Each aquarium was filled with eight (8) gallons of tap water (non-chlorinated) from Philippine Normal University. Each of the aquaria filled with water (non-chlorinated tap water) was pre-aerated for one hour to a day for full oxygen saturation before the Koi fishes was transferred to each of the aquaria. Fine net cover or glass cover was used to cover the top of the aquarium to prevent the experimental set-up from contamination.

Collection and Acclimatization of Koi fishes

A total of twenty (20) disease-free Koi fishes (*Cyprinus carpio*) with the size of 4-5 inches in length and was collected from National Fisheries Biological Center (BFAR-NFRDI); Butong Taal, Batangas. They were packed in thick plastic bags with enough water and oxygen accompanied with some drops of methylene blue to lessen the stress during transport. Once the Koi fishes had arrived to its destination, they were first acclimatized with the water temperature in the holding tank before it is stocked to water filled holding tank which are fully pre- aerated with oxygen to full saturation within moderate temperature until required for testing.

Once the Koi fishes are settled, the holding tank was covered with fine net or glass cover to prevent contamination during the acclimatization period. The fishes were fed with fish pellets according to their feeding time from (BFAR-NFRDI) Butong, Taal Batangas. When death occurs, the dead fish will be quickly removed, separated from healthy fishes, and transferred to plastic bags for proper disposal.

Toxicity test of Koi fishes (Cyprinus carpio)

Proper handling was observed through the experiment to minimize the stress subjected to the fishes that are being tested. After a day of rest of the Koi fishes, the different concentrations of the plant extract (100%, 75% and 50%) was added to the different testing aquarium (A, B, and C). Then with the use of a scoop net a total of five disease-free healthy Koi fishes was randomly selected and transferred from the holding tank to the different testing aquarium (A, B, C, and D), each aquarium was divided into five (5) spaces, each fish was placed in each of these divisions. Each aquarium was then subjected to the different concentrations of the plant extract (100%, 75% and 50%) respectively. Once the Koi fishes were settled, each testing aquarium was covered with fine net or glass cover to prevent contamination of the Koi fishes all throughout the toxicity test.

Fifteen (15) drops of each of the crude extract (100% crude extract, 75 % crude extract and 50 % crude extract) was given every 12 hours to each testing aquaria a total of 30 drops each testing day.

Monitoring and analysis of data collected

Toxicity test of Koi fishes was allotted four (4) days (12, 24, 36, 48, 60, 72, 84 and 96 hours). Each aquarium was properly monitored, and data was properly collected in every 12 hours of observation. Observable changes in Koi fishes during the test, like in its physical characteristics such as color of scales and production of mucus; swimming behavior; and other outcomes, was properly monitored and recorded every 12 hours. The researchers observed the effects of different concentration of *Tridax procumbens* extract on the Koi fish by doing the following: a. Physical characteristics: a.1. Change of the color of scales-noting the change of the Koi fishes scale color from bright to dull through taking pictures of each of the Koi fishes, and a.2. Mucus production-holding of Koi fishes properly and rubbing its body with the free hand to see if the fish had produce excessive mucus than the usual or no excessive mucus production at all, b. Swimming behavior- observing the way the Koi fishes swim, the Koi fish is said to be poisoned if it demonstrate top swimming (swims near the oxygen supply aerator sponge filter) and flashing or its usual swimming behavior, and.

In addition to this, the fish mortality, from the hour of stocking to every 12 hours within four days, was properly monitored and recorded. Dead fishes were removed immediately from the test aquaria. Death was assumed when the fish is immobile and show no response when it is being touch with glass rod or its body is floating lifelessly. Dead fishes were immediately transferred to a plastic bag for proper disposal. The collected data was analyzed.

Statistical analysis of data

The data collected was analyzed to determine the lethal concentration of the concentration *of Tridax procumbens* to the test specimen by plotting the concentrations (100%, 75% and 50%) of the test plant extract against fish mortality within 24, 48, 72 and 96 hours after their exposure to the different treatments. One way ANOVA was used to determine the effects of different concentration of *Tridax procumbens* extract on the Koi fish in terms of the following: Physical properties, color, and mucus production and Swimming behavior.

The study used one way ANOVA because of single treatment was used in this research. The lethal concentration was determined by plotting concentration of the test plant against fish mortality within 12, 24, 36, 48, 60, 72, 84 and 96 hours after exposure to the treatment. One-way analysis of variance single factor was used for the computation of the mortality of the Koi fish. It used only one factor, extract of *Tridax procumbens* leaves, having four treatments with 100%, 75%, 50% and 0% (no treatment).

C. In vitro test

Preparation of experimental set up

The experimental set up was placed on tables situated at the back of CED Ayala, Taft Ave. wing. Stirring rods, beaker and graduated cylinder were borrowed from Science Resource Center in Philippine Normal University. Other materials such as fifty (50) small clear plastic cups, compressor, plastic tubes, one (1) scoop nets, one (1) big plastic jar, forceps, spoons or droppers and one (1) pail was used in acclimatizing the Argulus spp. (Fish lice). These materials were purchased from Cartimar, Pasay city and SM Manila. All the materials were sterilized by washing with diluted chlorine solution, prepared by diluting four (4) grams of chlorine in twenty five (25) liters of water, after which was washed with clean water. These were air dried for one day to prevent contamination.

Each of the plastic cups were properly labeled with letters A, B, C, D and E according to the crude extract concentration (100%, 75% and 50%) and positive (100 % Neguvon) and negative (water) control set up assign to it. A (100% crude extract concentration), B (75% crude extract concentration), C (50% crude extract concentration), D (negative control) and E (positive control: 100% Neguvon) was labeled to plastic cups 1 to 5 respectively, which is replicated 10 times giving a total of 50 plastic cups.

The water that was used in the in vitro test was first stocked in a pail for two days to let the traces of chlorine content separate and to avoid unnecessary death of fish lice using only the upper tap water in the container. Each plastic cups were filled with twenty (20) ml of tap water (non-chlorinated) from Philippine Normal University. Each of the fish lice was then transferred to its respective plastic cups, filled with water (non-chlorinated tap water), randomly. Fine net cover was used to cover the top of the plastic jars to prevent the water from contamination.

Collection and Acclimatization of Fish lice (Argulus spp.)

Infected fishes with Argulosis, juvenile to adult stage, was purchased together with the twenty (20) Koi fishes in National Fisheries Biological Center (BFAR-NFRDI) Butong Taal Batangas, in Bioresearch in Sucat, Paranaque City and Cartimar, Pasay city. The fishes were placed in thick plastic bags with enough water and oxygen. Once the fishes had arrived at its destination, they were stocked in holding tanks filled with water (non-chlorinated) fully pre-aerated with oxygen to full saturation with moderate temperature for 30 minutes until required for the selection of fish lice for testing.

Selection of fish lice was done by scrapping the fish lice from the infected fishes with the use of forceps or bare hands. This was done by carefully scooping the infected fishes from the holding tanks transferring them to a basin with water (non-chlorinated tap water) supplied with oxygen through the aerator. The selection of fish lice from infected fishes was done by properly holding each fish to its head and body. Fishes was held with great care, not too tight to lessen the stress imposed to the infected fish. Spotted fish lice from the fins, beside the gills and body of the fish were scraped out of the fish using bare hands or by carefully picking it with the use of forceps. Fish lice was transferred to a container letting it to settle down for thirty (30) minutes before transferring it to testing cups for in vitro test. The container was covered with fine net preventing the contamination during the

acclimatization period. When mortality occurred, the fish lice were quickly removed and transferred to plastic bags for proper disposal. Infected fishes were returned to the holding tank and were treated with antiparasitic drug and were taken home for proper care.

In vitro test of Argulus spp.

After thirty (30) minutes, the prepared 100%, 75%, 50% crude extract concentrations and positive (100% Neguvon) control was added to plastic cups A, B, C, D and E respectively. Then, a total of fifty (50) actively moving fish lice was randomly selected with the use of a dropper or spoon and transferred from the container to the different testing plastic cups, one fish louse was assigned to each plastic cups. Each of the plastic cups contained twenty (20) ml of water (non-chlorinated) and each was assigned with the different concentrations of the plant extract (100%, 75% and 50%) and negative and positive control (water and 100% Neguvon) respectively. Each testing plastic cups was covered with plastic cover to prevent contamination of the fish lice all throughout the in vitro test. Fifteen (15) drops of each of the extract (100% crude extract, 75 % crude extract and 50 % crude extract) was given to each respective testing cup.

Monitoring and analysis of data collected

In vitro test of fish lice was allotted two days (6, 12, 18, 24, 30, 36, 42 and 48 hrs.) in which each plastic cups was properly monitored. Observable changes of the fish lice during the test and other outcomes were properly recorded. In addition to this, fish lice mortality starting from the hour it was transferred to every six hours for two (2) days was properly monitored and recorded. Death was assumed when the fish lice were immobile and shows no response, for five minutes, when being touch with glass rod. Dead fish lice were removed immediately from test cups and transferred to a plastic bag for proper disposal. The collected data was analyzed.

Experimental containers, beakers, aerators with filter sponges, scoop nets, basins, forceps, spoon or dropper, pail and other materials used in toxicity test and in vitro test was washed with soap and clean water and taken home.

Statistical analysis of data

The data collected was analyzed to determine if the concentrations of *Tridax procumbens* extract is as effective in killing Argulus sp. as that of the positive control (100% Neguvon) by plotting the concentrations (100%, 75% and 50%) of the test plant extract against parasite mortality within 24 and 48 hours after their exposure to the different treatments. One Way Analysis of variance was used, since the study uses only one factor which is the extract of *Tridax procumbens* leaves, having four treatments with 25%, 50%, 100% and 0% (no treatment), to compute the mortality of the Argulus spp.

III. RESULTS AND DISCUSSION

1. Effects of Tridax procumbens extract on the Koi fish

a. Physical properties

i. Color change

There was no recorded change in color or 0% color change after 96 hours of exposure of Koi fishes in the different concentrations of *Tridax procumbens* extract (100%, 75%, and 50%) as well as in the control set-up. In summation, the different concentrations of *Tridax procumbens* extract (100%,75%, and 50%) have no effect on the color of the Koi fishes.

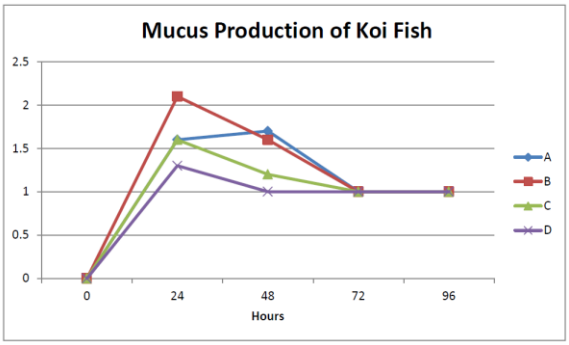
ii. Mucus Production

Table 4.1 Summary of Mucus production of Koi fish

Treatment	HOURS				TOTAL MEAN	QUALITATIVE MEANING
	24	48	72	96		
A	1.6	1.7	1	1	1.325	Medium Mucus Production
B	2.1	1.6	1	1	1.425	Medium Mucus Production
C	1.6	1.2	1	1	1.2	Medium Mucus Production
D	1.3	1	1	1	1.075	Medium Mucus Production

Legend:
Mean
0 - 1 - No Mucus Production
1.1 - 2 - Medium Mucus Production } Normal
2.1 - 3 - Excessive Mucus Production → Poisoned

Figure 4.1 Graph of the mucus production of Koi fishes throughout 24, 48, 72, and 96 hours of exposure to different concentrations of *Tridax procumbens* extract (100%, 75% and 50%).



Legend:
Concentration
Treatment A- 100% *Tridax procumbens* extract
Treatment B- 75% *Tridax procumbens* extract
Treatment C- 50% *Tridax procumbens* extract
Treatment D- Negative control

Table 4.1 shows the summary of the mucus production of Koi fishes (*Cyprinus carpio*) after 24, 48, 72 and 96 hours of exposure to water with different concentration of test plant (*Tridax procumbens*) and negative control, which is water. Mucus is an important barrier in fish because it provides the substrate in which antibacterial mechanism may act. Before observation, the fishes showed no mucus production when it is being placed in assigned aquarium.

After 24 and 48 hours of the exposure to the different concentration of test plant (*Tridax procumbens*) and negative control, there were 1 to 2.1 recorded mean mucus production in different concentration of test plant (*Tridax procumbens*) and in negative control. After 72 and 96 hours of exposure to the different concentration of test plant (*Tridax procumbens*) and negative control there was a decreased mucus production of Koi fishes. The graph (Figure 4.1) shows the said observation.

There was a recorded medium mucus production, which is considered normal, after 24 and 48, and 72 of exposure of Koi fishes in the Treatment A and C (100%, 75%, and 50%) of *Tridax procumbens* as well as in the negative control except in treatment B which showed excessive mucus production in the first 24 hours. While after 96 hours, there was no mucus production recorded of Koi fishes in all treatments and in negative control (water). The production of mucus is significantly decreased because the effects of the extract doesn't impose stress in the fishes (Tort, L., Balasch, S., Mackenzie, S., 2003)

The total mucus production of Koi fishes after 96 hours of exposure to concentration A (100% *Tridax procumbens* extract) was 1.325 while for concentration B was 1.425 and C (75% and 50% *Tridax procumbens* extract) was 1.2 and in negative control was 1.075. These only show that Koi fishes subjected to treatment B had produce more mucus than the test specimen did subjected to treatment A, C and D which are considered under normal mucus production. Thus, the mucus production of the Koi fishes was not affected by the different concentrations of *Tridax procumbens* extracts (100%, 75%, and 50%).

b. Swimming Behavior

Table 4.2 Summary of Swimming behavior of Koi fish

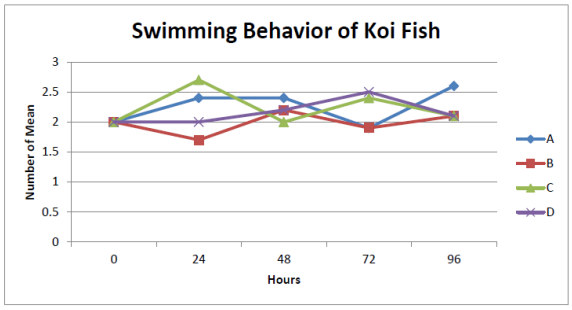
TREATMENT	HOURS				TOTAL MEAN	QUALITATIVE MEANING
	24	48	72	96		
A	2.4	2.4	1.9	2.6	2.325	Slow Moving
B	1.7	2.2	1.9	2.1	1.975	Moderately Moving
C	2.7	2.0	2.4	2.1	2.3	Slow Moving
D	2.0	2.2	2.5	2.1	2.2	Slow Moving

Legend:

Mean

0 – 1 – Fast → Abnormal swimming behavior
1.1 – 2 Moderate } Normal swimming behavior
2.1 – 3 – Slow }

Figure 4.2 Graph of the swimming behavior of Koi fishes throughout 24, 48, 72, and 96 hours of exposure to different concentrations of *Tridax procumbens* extract (100%, 75% and 50%).



Legend:
Concentration
Treatment A- 100% Tridax procumbens extract
Treatment B- 75% Tridax procumbens extract
Treatment C- 50% Tridax procumbens extract

Treatment D- Negative control

Table 4.2 shows the swimming behavior of Koi fishes (*Cyprinus carpio*) after 24, 48, 72 and 96 hours of exposure to water with different concentration of test plant (*Tridax procumbens*) and negative control which is water. As Yosha (2004) had stated, an observable indicator that a fish is poisoned is through its swimming behavior. When the fish is observed to demonstrate top swimming wherein the fish stays near the aerator trying to gulp oxygen from above, and bottom swimming wherein the fish swims on one side, upside down, or inability of the fish to swim properly. Another abnormal swimming behavior demonstrated by poisoned fish is flashing. Flashing is swimming against objects, jumping out of water showing its underside and abnormally fast swimming.

After 24, 48, 72 and 96 hours of the Toxicity test, the Koi fishes expose to the different concentration of *Tridax procumbens* (100%, 75% and 50%) and negative control (water) displayed moderate to slow or resting swimming behavior which is likewise the observable behavior of the Koi fishes before the Toxicity test which is observed to be under the normal swimming behavior. Thus, there were no recorded abnormal swimming behaviors of Koi fishes after 96 hours of exposure to the different concentrations of coatbutton extracts. The graph (Figure 4. 2) above shows the said observation.

The total mean swimming behavior of Koi fishes after 96 hours of exposure to treatment A (100% *Tridax procumbens* extract) is 2.325 while for treatment B (75% *Tridax procumbens* extract) is 1.975, in treatment C (50% *Tridax procumbens* extract) there was a 2.3 while in the negative control there was 2.2 swimming behavior. The following mean only shows which test specimens are more active than the others indicating that Koi fishes under treatment B are the most active followed by those in treatment A, C, and D. To summarize, the exposure to the different concentration of *Tridax procumbens* (100%, 75% and 50%) and negative control which was water has no effect on the swimming behavior of Koi fishes which were under the normal range of swimming behavior since none of the Koi fishes demonstrated any abnormal swimming behavior.

2. Is there any significant difference in the effects of different concentration of Tridax procumbens extracts (100%, 75% and 50%) on Koi fish in terms of the following: a.) physical qualities i) color and ii) mucus production, and its b.) swimming behavior.

Table 4.3 Summary of One-Way ANOVA on the Different Mean Physical qualities of the Koi fish

	Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Mucus Production	Between Groups	1.201875	3	0.400625	7.311787	0.004784	3.490295
	Within Groups	0.6575	12	0.054792			
	Total	1.859375	15				
Swimming Behavior	Between Groups	0.005	3	0.001667	0.017316	0.996723	3.490295
	Within Groups	1.155	12	0.09625			
	Total	1.16	15				

As shown in the table 4.3 presented above is an Analysis of Variance (ANOVA) which shows the results of the comparison among the means of mucus production and swimming behavior of Koi fishes after the 96-hour exposure to the different concentration of *Tridax procumbens* extract (100%, 75%, and 50%). The Null Hypothesis (H_o) is accepted in terms of the physical quality such as the color and swimming behavior of Koi fishes. This is due to that there is no significant difference among the computed means of the physical quality which is color and in the swimming behavior of Koi fishes and since the F-value is equal to 0 and is less than the F crit (Critical value) which is equal to 3.490295. These only imply that the mean effects to the color and swimming behavior of Koi fishes of the different concentrations of Tridax procumbens extract (100%, 75%, and 50%) have no difference from each other. These only mean that the different concentrations of *Tridax procumbens* extract (100%, 75%, and 50%) and the negative control has no effect on the color and swimming behavior of Koi fishes. While in mucus production, the Null Hypothesis (H_o) is rejected in terms of the physical quality which is the mucus production of Koi fishes. This is due to that there is a significant difference among the computed means of the mucus production of Koi fishes and since the F-value is equal to 7.311787 and is greater than the F crit (Critical value) which is equal to 3.490295. This only implies that the mean effects to the mucus production of Koi fishes of the different concentrations of *Tridax*

procumbens extract (100%, 75%, and 50%) have differences from each other. It only means that among the different concentrations of *Tridax procumbens* extract (100%, 75%, and 50%) that had been used or applied to the Koi fishes have different effects on the mucus production of the Koi fishes.

3. What is the mortality of Koi fish in different concentrations of Tridax procumbens?

After 24, 48, 72 and 96 hours of the Toxicity test, the recorded mortality was 0 in all treatment of the coatbuttons as well as the negative control. The recorded mortality was 0 in all concentrations of *Tridax procumbens* after 96 hours of exposure of Koi fishes in the different concentrations of *Tridax procumbens* extract (100%, 75%, and 50%) as well as in the control set-up. In summation, the different concentrations of *Tridax procumbens* extract (100%, 75%, and 50%) were not lethal to the Koi fishes.

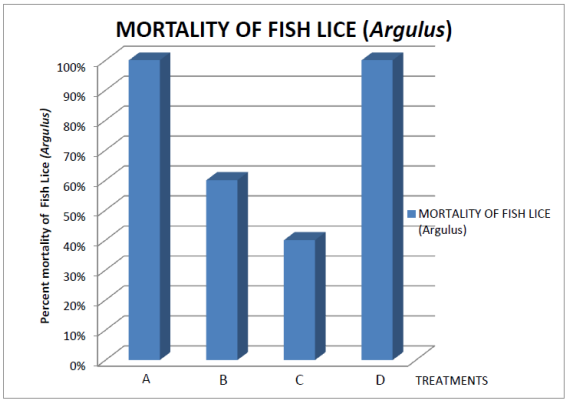
4. What is the mortality of Argulus spp. in different concentrations of Tridax procumbens?

Table 4.4 Summary of the Mortality of Fish Lice (*Argulus spp.*)

Treatment	DAY		TOTAL	Mortality (%)
	1	2		
A	10	0	10	100%
B	3	3	6	60%
C	3	1	4	40%
D	10	0	10	100%

Table 4.4 shows summary of the mortality of fish lice (*Argulus spp.)* after 24 and 48 hours of exposure to water with the different concentration of *Tridax procumbens* extract and water.

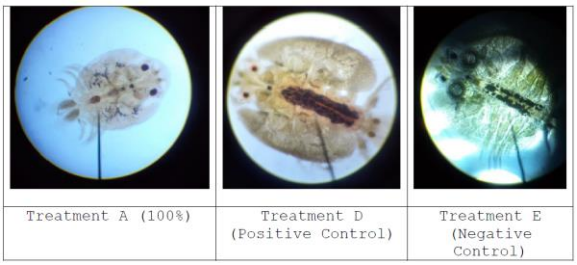
Figure 4.3 Graph of the mortality of fish lice on 48 hours of exposure to different concentrations *of Tridax procumbens* extract (100%, 75% and 50%)



Legend:
Concentrations
Treatment A – 100% *Tridax procumbens* extract
Treatment B – 75% *Tridax procumbens* extract
Treatment C – 50% *Tridax procumbens* extract
Treatment D – Positive control

Table 4.4 shows the summary of the mortality of Fish lice (*Argulus spp.)* after 24 and 48 hours of exposure to water with different concentrations of *Tridax procumbens* extract. After 24 hours of In vitro test, the recorded mortality was 10 in each of the treatment A and D (100% *Tridax procumbens* extract and positive control) while there was 3 in each of the treatment B and C (75% and 50% *Tridax procumbens* extract). Furthermore, there was an increase in the mortality of fish lice after 48 hours of exposure in Treatment B with an addition of 3 deaths and 1 in Treatment C. The graph (Figure 4.3) above illustrated the said observation. The mortality that had occurred was due to the action of the bioactive compounds of *Tridax procumbens* extract concentrations (100%, 75% and 50%) which was found out to have antiviral, insecticidal, and parasitical properties. Some of these bioactive compounds are alkaloids, flavonoids, saponin, tannins and terpenes. Alkaloids were generally considered as a poisonous deterrent to animals and insects since it is also responsible for both the short-term and long-term toxicity of some herbs and had antimicrobial and anthelminthic activity. Flavonoids were found to have the following abilities such as enzyme inhibitor, antimicrobial, and cytotoxic antitumor activity. Saponins were used as insecticidal, pesticides and molluscicides while Tannins was said to have greater potential to cause toxicity. Tannins which inhibit the growth of many fungi, yeast, bacteria, and viruses. There are some observable indicators which show that the fish lice are already dead such as being immobile and showing no response after being touched by forceps. The changing of color is also noted, which in the 100% concentration of *Tridax procumbens* extract the fish lice suckers turns red and from transparent carapace it becomes pinkish in color while in the positive control the suckers also turn red and also its abdominal part compared to the negative control (Plate 1).

Plate 1: Shows the images of the fish lice (*Argulus spp*.) after 24 hours of exposure to 100% concentration of Tridax procumbens extract (left), positive control (middle) and negative control or water (right).



Treatment A (100%)	Treatment D (Positive Control)	Treatment E (Negative Control)

The total percentage mortality of Fish lice after 48 hours of exposure to treatment A (100*% Tridax procumbens* extract) was 10 or 100%, treatment B (75% *Tridax procumbens* extract) was 6 or 60%, treatment C (50% *Tridax procumbens* extract) was 4 or 40% and treatment D (positive control) was 10 or 100%.

5. Is there any significant difference in the mortality of Argulus spp. in different concentration of Tridax procumbens?

Table 4.5 Anova of the Mean Mortality of Fish Lice(*Argulus*)

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	60.5	1	60.5	6.6	0.042389	5.98737 8
Within Groups	55	6	9.16666 7			
Total	115.5	7				

As shown in table 4.5 presented above is the Analysis of Variance (ANOVA), which shows the results of the comparison among the mortality of Argulus spp. after the 48-hour exposure to the different concentration of *Tridax procumbens* extract (100%, 75%, and 50%). The Null Hypothesis (H0) is rejected, due to that there is a significant difference among the mortality of Argulus spp. and since the F-value is equal to 6.6 and is greater than the F crit (Critical value) which is equal to 5.987378. It only implies that the effects of the different concentrations of *Tridax procumbens* extract (100%, 75%, and 50%) have differences from each other. This only means that among the different concentrations of *Tridax procumbens* extract (100%, 75%, and 50%) that had been used in the in vitro test have different effects on Argulus spp. with the 100% concentration of *Tridax procumbens* extract being the most effective antiparasitic treatment among the different concentrations of *Tridax procumbens* extract (100%, 75%, and 50%) which is somewhat as effective as that of the positive control (100% Neguvon) in killing the parasite, fish lice (*Argulus spp*.).

IV. FINDINGS

The results and statistical analysis acquired were summarized according to the order in which the sub-problems are stated on this study. The different results of the study undertaken are the following:

The physical qualities such as color and mucus production and the swimming behavior of Koi fishes were not affected when the testing specimens (Koi fishes) were exposed to the different concentration of *Tridax procumbens* extract (100%, 75% and 50%). There was no recorded color change or 0% color change after 96 hours of exposure of Koi fishes in the different concentrations of *Tridax procumbens* extract (100%, 75%, and 50%) as well as in the control set-up. In mucus production, there was a recorded medium mucus production all throughout the 24 to 96 hours exposure of Koi fishes in the A, B, and C treatment (100%, 75%, and 50%) of *Tridax procumbens* as well as in the negative control with a trend of decreasing mucus production from medium to no mucus produced at all, these mucus productions are considered just normal. In the swimming behavior, after 96 hours of the Toxicity test, the Koi fishes exposed to the different concentration of *Tridax procumbens* (100%, 75% and 50%) and in negative control (water) had displayed moderate to slow (resting) swimming behavior which was under the normal swimming behavior. Furthermore, the determined lethal concentration 50 of *Tridax procumbens* (coatbuttons) was 0% thus it is not lethal to the Koi fishes.

There is no significant difference in the physical qualities in terms of its color and the swimming behavior of Koi fishes subjected to the different concentrations of *Tridax procumbens* extract from Koi fishes subjected to the negative control which is water after the Toxicity test except for the in-mucus production which has a significant difference. Since the color, mucus production and the swimming behavior of the Koi fishes were not affected after the 96 hours exposure of the Test specimen to the different concentration of *Tridax procumbens* extracts (100%, 75% and 50%) thus the different concentration of

Tridax procumbens extracts (100%, 75% and 50%) was not lethal to Koi fishes. The summary of the mortality of Koi fishes after 96 hours of exposure to all the different concentration of *Tridax procumbens* extracts (100%, 75% and 50%) was 0 which is the same with the control set-up (water).

The summary of the mortality of *Argulus spp.* after 48 hours of exposure to the different concentration of *Tridax procumbens* extracts (100%, 75% and 50%) were 100% *Argulus sp.* in treatment A (100% *Tridax procumbens* extracts), 60% *Argulus sp.* in treatment B (75% *Tridax procumbens* extracts), and 40% *Argulus sp.* in treatment C (50% *Tridax procumbens* extracts) and lastly 100% *Argulus sp.* in treatment D (positive control: 100% Neguvon)

The different concentrations of *Tridax procumbens* extract (100%, 75% and 50%) effects of the different concentrations of *Tridax procumbens* extract (100%, 75%, and 50%) have differences from each other. This only means that among the different concentrations of *Tridax procumbens* extract (100%, 75%, and 50%) that had been used in the in vitro test have different effects on Argulus spp. with the 100% concentration of *Tridax procumbens* extract being the most effective antiparasitic treatment among the different concentrations of *Tridax procumbens* extract (100%, 75%, and 50%) which is somewhat as effective as that of the positive control (100% Neguvon) in killing the parasite, fish lice (*Argulus spp.).*

CONCLUSION

After 96-hours of exposure of Koi fishes to the different concentrations of *Tridax procumbens* leaf extract, it was observed that the test specimen (Koi fishes) was not affected in terms of its physical qualities such as color, mucus production, and its swimming behavior.

Comparing the physical quality which is color and the swimming behavior of Koi fishes subjected to the different concentrations of *Tridax procumbens* (100%, 75% and 50%) to the Koi fishes exposed to the negative control, which is water, it shows that there is no significant difference between the two. But in the mucus production of Koi fishes it has a significant difference. It only implies that using the different concentrations of *Tridax procumbens* (100%, 75% and 50%), as an antiparasite against *Argulus spp.,* to Koi fishes would not affect the fishes.

In line with this, the mortality of Koi fish was recorded, after the 96-hours of exposure of test specimen to the different leaf extract of *Tridax procumbens*, 0 mortality occurred. This led to the conclusion that all the concentrations of *Tridax procumbens* extract were considered not lethal to Koi fishes in lined with the determined Lethal Concentration 50 (LC50) of the test specimen (Koi fishes).

Measuring the mortality of Argulus spp. after 48 hours of exposure to the different concentration of *Tridax procumbens* extracts (100%, 75% and 50%), high mortality was observed. It led to the conclusion that all the concentrations of the test plant (*Tridax procumbens*) were considered lethal to the *Argulus spp.*

Comparing the mortality of *Argulus spp.* subjected to the different concentrations of *Tridax procumbens* (100%, 75% and 50%) and the positive control (100% Neguvon); it showed that there is a significant difference between the mention extract concentrations. It only implies that the effects of the different concentrations of *Tridax procumbens* extract (100%, 75%, and 50%) have differences from each other. With the 100% concentration of *Tridax procumbens* extract being the most effective antiparasitic treatment among the different concentrations of *Tridax procumbens* extract (100%, 75%, and 50%) which is somewhat as effective as that of the positive control (100% Neguvon) in killing the parasite, fish lice (Argulus spp.). It only implies that using the concentration A of *Tridax procumbens* (100%) as an antiparasite to *Argulus spp.* is as effective as the positive control (100% Neguvon).

With these conclusions, the researchers are now able to conclude for the main problem of this study. The main problem of this study was to determine the effectiveness of the antiparasitic activity of *Tridax procumbens* (coatbutton) extract against the fish lice (Argulus spp.) in Koi fish. In comparing

Tridax procumbens (coatbutton) extract (100%, 75%, and 50%) with that of the positive control (Neguvon),Tridax procumbens (coatbutton) extract is as effective as Neguvon (100%) in controlling the Fish lice (Argulus spp.). In addition, *Tridax procumbens* extract is not lethal to test specimen (Koi fish).

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