

# Study of the Potentials of *Jatropha Curcas* Extracts in Control of Some Endoparasitic Diseases of *Clarias gariepinus* in Bakajeba Reservoir, Niger State

<sup>1</sup>Omole. O. O, <sup>2</sup>Gimba, U.N, and <sup>1</sup>Kolo, M.A

<sup>1</sup>Department of MSEP, National Mathematical Centre Kaduna-Lokoja Road Sheda Kwali Abuja. P.M.B 118 Abuja

<sup>2</sup>Department of Biological Sciences, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria

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## ABSTRACT

The potency of *Jatropha curcas* crude extracts against helminth parasites of *Clarias gariepinus*; *Procamallanus* sp. and *Wenyonia* sp. with less toxicological effect on host fingerlings were investigated. Average Survival Time and Percentage Reduction in Survival Time relative to saline solution for the intestinal parasites exposed to the extracts at the least interval; 0.1 LC50 g/L host fingerling, showed trend in susceptibility *Jatropha curcas* shown a very high potency against helminth parasites. The effects of plant extracts on parasites and the sensitive life stage of their fish host is dose-dependent and to avoid over dosage, the need for determination of suitable concentration is of great importance.

**Keywords:** *Jatropha curcas* extracts, Reduction in Survival Time, Intestinal parasites, Relative Toxicity Factors, *Clarias gariepinus*

## INTRODUCTION

Freshwater fish serve as definitive and intermediate host in the life cycle of many species of protozoan, metazoan and crustacean parasites (Kumar *et al.*, 2014). Fish parasites are one of the most serious threats to fish health. Fish parasites have long been a source of concern since they can cause a variety of diseases in fish. These diseases can also weaken the host's immune system, making them more susceptible to secondary infections (Eyo *et al.*, 2014). These diseases cause nutritional devaluation of fish and fish loss as a result of their impacts. Parasites also cause fish to lose their ability to swim, grow at a slower rate, and die more frequently (Kumar & Singh 2014).

A parasite is an organism that receives nutrients from another species by living on or within a portion of that species. Ecto-parasites or Endo-parasites could be present. The protozoan and helminth parasites are said to be the most common types of fish parasites in Nigeria (Martinez-Herrera *et al.*, 2010).

Endoparasitic organisms that produce higher mortalities can be found in both wild and domestic fish. According to reports, roughly 50 to 90 percent of freshwater fishes host at least one endoparasite (Abobatta, 2016). Under current conditions, parasite losses are at high concentrations, and if allowed unchecked, they might reach catastrophic proportions (Ingle *et al.*, 2017). Fish's regular growth is hampered by endoparasites if the fish is heavily contaminated, it lives in the fish. Endoparasites have a direct impact on fish survival by reducing their condition (fish size), altering their behavior, and making them more susceptible to infection (Widiyastuti and Sutardi, 2016), but it also reduced their swimming abilities, slowed their growth rate, and increased the number of deaths.

To eliminate the problem, Understanding the mode of transmission and potential intermediary hosts is critical (Tomar *et al.*, 2014). In comparison to ponds, hatcheries, and captivity, In the wild, parasitism is the most

common and diversified. Infection in fish is caused by environmental stress and overcrowding (Diabaté *et al.*, 2014). Fish farming has been a major commercial industry in Pakistan over the last two decades. Pathogens, including parasites, pose a serious challenge to the intensification and expansion of fish culture, since they are one of the leading causes of chronic mortalities and poor growth, impacting fish productivity and marketability. Fish parasites can be spread by food, alien species introduced into aquatic ecosystems, and the handling and processing of sick fish. Endoparasitic helminths cause infections in fish are more common as pollution levels rise (Ishag and Osman, 2020). If fish are fed Infectious life stages are found in living foods that convey the infective stages of vectors, they will become parasitized (Widiyastuti and Sutardi, 2016). Variations in the aquatic environment, whether natural or artificial, can disrupt equilibrium of parasites on the host and cause sickness or mortality in fish. Fish parasites harm fish physiologically, reproductively, and physically (Tomar *et al.*, 2014).

## MATERIALS AND METHOD

### Study Area

Lapai is a Local Government Area adjoining the Federal Capital Territory in Niger State. Its base camp is located at 9°03'00"N 6°34'00"E near the town of Lapai, on the A124 throughway in the west of the area. According to 2006 figures, it has a zone of 3,051 km<sup>2</sup> and a population of 110,127 people. The territory is roughly coterminous with the Emirate of Lapai. The investigation areas are arranged in Lapai LGA, Niger State which is situated between scope 120 25' N to 120 40' N and Longitude 80 35' E to 45' East of the Greenwich Meridian, that is moderately at the focal point of Niger State. It has a territory of about 50km<sup>2</sup> and is comprised of 10 wards. The atmosphere of the territory is the tropical dry and wet atmosphere. The normal yearly precipitation is 884.4mm as recorded at the Lapai Secretariat Niger State and the overwhelming time of precipitation is from June to September and August, which is the wettest month of the year, receives over 40% of the annual precipitation. Despite a relatively mild time between November and February, the temperature system is consistently warm to hot. The average annual temperature ranges between 220°C in the coolest months (December or January) to 310°C in the hottest months (July or August) (April or May). The average yearly potential disappearance, transpiration, daylight, and relative stickiness are 1,772mm, 8.5 hours per day, and half of each day separately. Sudan savanna is the common vegetation of the region, and to be sure, it covers the majority of the Niger district.



Figure 1: Map of Lapai Showing the Study Locations.

Source: Cartographic Department in Lapai Local Government Secretariat.

## Test Animals

One thousand (1000) specimens of life *Clarias gariepinus* (Fingerlings) which were 6 weeks old were bought from Bakajeba reservoir and acclimatized to the laboratory condition in a well aerated dechlorinated tap water for a period of ten days. Test animals were of similar size, weight and age. They were categorized into treated and control groups (20 animals in each group in two replicates).

## Collection of Plants

Plant with medicinal potential were carefully selected and bought from Gwagwalada town early in the morning for fresh specimen. The leafy ones were rinsed in a running tap water and blotted with filter paper, spread over newspaper for air drying under shade 2-3 weeks.

## Extraction of Phytochemicals

The succulent and woody plant parts were pulverized using mortar and pestle while the dried leaves were blended with a grinder after complete dryness. A known quantity of leaf powder of each plant and other plant parts was put in a 250 ml conical flask and added with 200 ml of Methanol (95%). The Methanol-leaf powder mixtures were kept at room temperature for 72 hours and rapidly stirred using glass rod every 8 hours. After 72 hours, the extract of each plant was filtered through Whatman No. 1 filter paper to exclude the plant tissue debris. The filtrate was concentrated under reduced pressure using rotary evaporator. Concentrated extracts were transferred in a beaker and dried at 40.5°C in a water bath. Crude extract, obtains from each plant parts, was transferred to screw cap bottles, labelled and store under refrigerator (4°C) condition till use. Their yields and other physical properties were noted and recorded.

## Bioassay Technique

The test media was made by adding 1.0 litre of fresh water to the glass containers. The plant extracts were then added to the assay using different calibrations depends on the activeness of the phytochemical, after a range test was carried out. Acute testing lasted for 96 hours and this includes the controls. Fish was added to test chambers within 30 minutes of addition of test material to dilution water. 20 fingerlings of *Clarias gariepinus* in each group, a minimum of ten fish per replicate and two replicate per test concentration to provide statistical baseline. Construction materials and equipments for testing followed standard procedure. Gentle aeration of test vessels was used in the static-renewal. A minimum of five test concentrations was employed. Mortality observations were recorded at 6, 24, 48, 72 and 96 hour, additional observations were made every 24 hours until termination. Constant conditions were maintained throughout the test period. The test fishes were fed with commercial fish pellets (coppen zeigler) 1mm, twice daily. However, the fishes were not fed for 24 hrs before test initiation. In addition to these, abnormal behavior was recorded, such as, erratic swimming, loss of reflex, increased excitability, lethargy, and changes in appearance or physiology such as discoloration, excessive mucous production, hyperventilation, opaque eyes, curved spine, or hemorrhaging. They were taken to be dead if they show no body movement even when probed with a pointed glass rod and they also appear whitish.

## Toxicity Factor

Relative Toxicity factor (RTF) for the plant extract at 96 hours exposure to 0.1 LC50, 1.0 LC50, and 1.5 LC50 concentrations were estimated using the formulae below;

$$\text{Relative Toxicity factor (RTF)} = \frac{\text{The highest 96h LC50 value}}{96\text{h LC50 value for each plant extract}}$$

## Determination of Average Survival Time for the parasites

Intestinal parasites were extracted from sixty (60) wild adult each of *Parachanna obscura* (African Snakehead) weighing 41g to 65g and length 17.2 cm to 20.0 cm and *Clarias gariepinus* (African Cat fish) weighing 60g to 90g and length 16.0 cm to 18.0 cm were collected in Bakajeba Reservoir, 4° 00 and 4° 15E; trations of each plant extract was estimated using low observed concentration level i.e. 0.1 LC50 for 96 hour - host fry determined from the acute toxicity for *Procamallanus longus*, a cestode parasite and, *Wenyonia* sp a nematode of *Clarias gariepinus* in saline solution (0.0085g/ml, NaCl) were estimated. Percentage Reduction in Survival Time (%RST) in each plant extract was estimated using the formulae below;

$$\%RST = 100\% - \frac{(\text{AST in each plant extract})}{\text{AST in Saline solution}} \times 100$$

*Clarias gariepinus* caught from the wild after two day laboratory acclimatization of the parasites in direct exposure to the sub-lethal concentration.

## Statistical Analysis

Mortality rate was analysed using probit analysis. This was done by converting concentration to logarithm and percentage response which was converted to probit (Regression Analysis). Number of organism exposed in each assay was also taken into consideration. The probit values were then plotted against logarithm of concentration in order to determine the lethal concentration (LC50). Statistical analysis was executed using the Sigma Stat software (SPSS), IBM Statistic Package.

## RESULTS

Crude plant extracts from *Jatropha curcas* were obtained fresh were highly soluble in water with pH range of 5.50 – 6.13, Total Dissolved Solid; 581 – 887 mg/L, Temperature of 26.4 – 29.00 C, *Clarias gariepinus*. Lethal Concentrations (LC50) of plant crude extracts on fingerlings of *Clarias gariepinus*.

There were decreases in lethal concentrations with exposure time, from 24h LC50 to 96h LC50 in the plant extract. Relative Toxicity Factors (RTF) of Plant Extracts at increasing toxicities on fingerlings of *Clarias gariepinus* (96h LC50 Toxicity) using Probit.

Relative toxicities of the plant crude extracts on fingerlings of *Clarias gariepinus* a 96 hour exposure, using probit analysis showed decreased in RTFs.

It is important to have suitable dosing to obtain desired effects of *Jatropha curcas* on parasites of fishes at safe concentration. Extracts appears to be more toxic than some others at higher concentrations, this is not true at lower concentration.

## Percentage Reduction in Survival Time of Intestinal parasites of *Clarias gariepinus*

The average survival time of intestinal parasites; *Procamallanus* sp, a cestode and, *Wenyonia* sp a nematode in saline solution (0.0085g/ml NaCl) were 40.00±0.69 and 42.50±0.96 hr respectively with statistical significance at 0.01 level.

Average survival time and percentage reduction in survival time relative to saline solution of the intestinal parasites exposed to *Jatropha curcas* shown a high potency against helminth parasites.

## Histopathological Examination

Tissue specimens from the skin, gills and pseudobronchial organs of the infested catfish were taken.



Specimens were fixed immediately in 10% buffered neutral formalin, dehydrated and embedded in paraffin wax. Paraffin blocks were sectioned at 4-5  $\mu\text{m}$  thickness and stained with Hematoxylin & Eosin (H&E) and examined under light microscope (Leica) using  $\times 200$  and  $\times 400$  magnification power according to Bancroft and Gamble

### Parasitological Examination

Microscopic smears taken from gills, skin and fins of examined *C. gariiepinus* revealed the presence of some endoparasitic parasites in Bakajeba. *Dactylogyrus* sp. have the highest rate of the infestation (25.8%) followed by *Gyrodactylus* sp. (17.8%), *Trichodina* sp. (10.8%) then *Chilodenella* sp. (6.8%). Adult worms isolated from the gills of infested catfish; were flat and elliptical in shape. Their anterior end (prohaptor) was divided into four cephalic lobed heads, with sticky and adhesive organs (cephalic glands), in addition to four black eye spots. The posterior end, appeared a dome shape and composed of one pair of connecting bars (V-shaped) and seven pairs of small marginal booklets. The intestinal limbs were connected, the ovary located in front to the testes. Such adult worms are related to the phylum Platyhelminthes, class Trematoda, order Mongenea family Dactylogyridae and genus *Dactylogyrus claridii*. However, the adult worms isolated from the skin of infested catfish; were flat and elliptical in shape and provided with a pair of too long and strong anchors in the opisthaptor and 7 pairs of small strong hook lets used for fixation firmly on the external body surface of its host. Such adult worms are related to the phylum Platyhelminthes, class Trematoda, order Mongenea, family Gyrodactylidae and genus *Gyrodactylus claridii*. Regarding monogenean trematodes (*Gyrodactylus* sp. and *Dactylogyrus* sp.), they were morphologically and parasitologically described and were nearly similar to the descriptions given by Ishag and Osman (2020). Microscopic smears taken from skin and gills of examined fish, showed a peritrichus ciliated protozoan. A denticulate ring of hollow conical structures was found with flat lateral projections. The centrifugal projections of denticles were semicircular. The macronucleus was large horseshoe shaped with a round micronucleus. Such ciliated protozoans were identified as *Trichodina heterodontata* (Tomar *et al.*, 2014). Another protozoan appeared as large, flattened, ovoid or heart shaped ciliates with bands of cilia along the long axis of organisms. A single oval to round macronucleus as well as round micronucleus, were easily seen. Such ciliated protozoan was identified as *Chilodonella* sp. The ciliated protozoans (*Trichodina* sp. and *Chilodenella* sp. were morphologically and parasitologically identified and were nearly similar to the descriptions given by Martinez-Herrera *et al.* (2010).

### Incidences of Fish Endoparasitic Parasites among Different Seasons

Parasitological examination of 400 *C. gariiepinus* revealed the presence of different endoparasitic parasites in 244 positive infested cases (61%) with a seasonal prevalence of 15.5% in autumn and winter and 15% in spring and summer season. A higher infection rate of *Dactylogyrus claridii* was observed during spring and summer (27%) for both, followed by winter (26%) then autumn (23%), while, a higher infestation rate of *Gyrodactylus claridii* was observed during autumn and winter (19%) for both, followed by spring (18%) then summer (15%). On the other hand, a higher infection rate of *Trichodina* sp. was observed during autumn and summer (12%) for both, followed by spring (10%) then winter (9%).

### Histopathological Examination:

Histopathological alterations of the skin of the infested *C. gariiepinus* revealed presence of the parasitic cysts in the underlying degenerated muscle tissues A, B, C and D) together with necrosis of some muscle fibers While, the gills showed congestion and hemorrhages as well as proliferation of the cartilaginous tissues inside the gill filament tissues with degenerated lamellae. The induced gill damage by protozoan parasites, may be due to the feeding activity, attachment, fixation and locomotion and caused a massive destruction of the respiratory epithelial cells; these agree with that reported by Ingle *et al.* (2017) where the histopathological examination of tilapias for trichodina affections showed hemorrhage, congestion. Also, similar to that recorded by (Widiyastuti & Sutardi, 2016). That mentioned that the most common response of the gill to damage by protozoan parasites is hyperplasia and hypertrophy of epithelial cells.

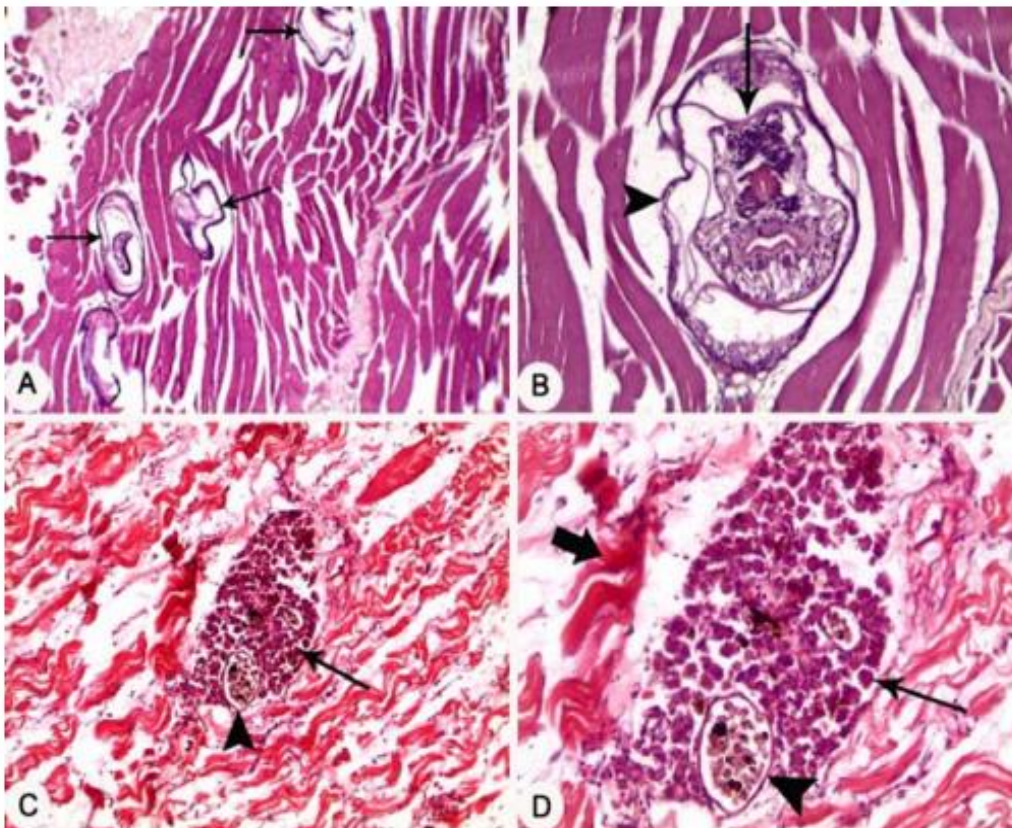


Fig: 1 Muscles of African catfish (*Clarias gariepinus*). A & B) showing parasitic cyst in muscle tissues without any inflammatory reaction. Arrow indicating the parasite and arrowhead indicating the cyst capsule. C) showing parasitic cysts in degenerated muscular tissues. Arrow indicating the parasites in ruptured cyst; and the arrowhead indicating unruptured parasitic cyst. D). showing parasitic cysts in degenerated muscular tissues. Thin arrow indicating the parasites in ruptured cyst; the arrowhead indicating unruptured parasitic cyst; and the thick arrow indicating necrosis in muscle fibers. H&E stain. A X100; B X200; C X100 & D X200

### Efficacy of Jatropha Bath on the Endoparasites Infesting *C. Gariepinus*:

The results of *Jatropha* efficacy on the infested *C. gariepinus* with mixed infestation of *Trichodina*, *Epistylis* and *Gyrodactylus* spp. indicated that long exposure of infested fish for three days (long bath) to jatropha at concentration 10 mg/L was insufficient to eradicate the protozoa and monogenea while jatropha concentration of 20 mg/L was sufficient to eradicate the protozoa (*Trichodina* and *Epistylis* spp.) but cannot eradicate the monogenean (*Gyrodactylus* spp.)

Overdose of jatropha caused ulceration and erosion in the skin and fins of catfish.

The estimation of water parameters (Dissolved oxygen, pH and total ammonia) pre and during treatment with jatropha revealed that the water quality during treatment was better than pre-treatment especially the total ammonia which decreased in value in comparing with pre-treatment.

## DISCUSSION

Parasitic diseases of fish seem to be one of the major problems confronting fish culturists. Application of aquatic medicine in treatment and prevention of diseases in fisheries and aquaculture is also gaining great momentum in recent years. The organism is classified at a given time as having responded after treatment. Quantal response is employed to obtain quantitative results because the percentage responding in randomly chosen group of organisms in general increases with concentration. In this study, they were decreased in lethal concentrations with increase in exposure time, from LC50 of 24 h exposure to LC50 of 96 h exposure in all the plant extracts. It is important to have suitable dosing to obtain desired effects on parasites of the fishes at safe

concentration. One extract appears to be more toxic than the other at higher concentrations, this is not true at lower concentration. A number of studies have demonstrated the chemopreventive activity of garlic by using different garlic preparations including fresh garlic extract, aged garlic, garlic oil and a number of organosulfur compounds derived from garlic (Abobatta *et al.*, 2019). Most recent efforts have been made in identifying potential activities on different plant species. However, identifying the active molecules responsible for the observed bioactivities would allow optimization of extraction procedure, estimate appropriate dosage and understand mechanism of actions (Reverter *et al.*, 2014). Enriched diets with plant extracts will have beneficial effects on fish health and enhance the immune system and hence play importance role in preventing disease outbreak (Abobatta *et al.*, 2016).

## CONCLUSION

The parasites showed susceptibility to the extracts as their host fingerlings. The beneficial properties and efficacy of plant extracts on health of fish depend on the active ingredients, method of extraction and the extract concentration. Besides the effects of plant extracts on parasites and their fish host is dose-dependent and there is a potential for overdosing, so determining suitable concentration is of great importance.

From the current study, the African catfish (*Clarias gariepinus*) which is one of the most economically important fish species for successful aquaculture, is suffering from highly obvious problems due to parasitic infestation. The highest prevalence of the endoparasitic parasitic infestation was (15.5%) in both autumn and winter, and (15%) in spring and summer season. Many endoparasitic parasites could be isolated. *Dactylogyrus* sp., is the most dominant isolated endoparasitic parasite (25.8%) with highest infestation rate in spring and summer, followed by *Gyrodactylus* sp., (17.8%) with highest infestation rate in autumn and winter, *Trichodina* sp., (10.8%) with highest infestation rate in autumn and summer, then *Chilodenella* sp., (6.8%) with highest infestation rate in autumn and winter.

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