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In vivo antiparasitic effects of an African's traditional plant *Ocimum gratissimum* (Linneaus, 1758) on fish louse *Argulus spp*. infesting the Nile tilapia males *Oreochromis niloticus* (Linneaus, 1758) in fish farming

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Abstract

The crude extract of the plant *Ocimum gratissimum* was used to control the parasites *Argulus spp.* infestation on Nile tilapia *Oreochromis niloticus* by *in vitro* and *in vivo* tests, which permitted to determine plant's efficacy, LC_{50} values, and the delay of antiparasitic effect. For that, the randomized design were performed into petri dishes using the crude extract of the African's plant *O. gratissimum* against the parasites *Argulus spp*. for *in vitro* test toxicity, and into raceways for *in vitro* and *in vivo* tests of this plant against *O. niloticus*. The crude extract of the plant *O. gratissimum* had toxic effect to the parasite *Argulus spp.* with a LC_{50} value of 197.66 mg / L with 95 % confidence interval of 194.65 – 200.01 mg / L. For the Nile tilapia *O. niloticus*, the LC_{50} value was 1271.22 mg / L with 95 % confidence interval of 1251.20 – 1291.20 mg / L. The 36 hours 100 % efficacy of the crude extract of *O. gratissimum* was obtained at the concentration of 800 mg / L. The plant, *O. gratissimum,* had anti-parasitic effects after bath treatments, which can prevent for 3 months against high infestation of Nile tilapia by parasite *Argulus spp.*.

Keywords: *Oreochromis niloticus*, *Argulus spp.*, treatment, *Ocimum gratissimum*.

Introduction

This paper aims to show the anti-parasitic effects of the crude extract of an African plant called *Ocimum gratissimum* (Linneaus, 1758) by *in vitro* tests on the fish louse *Argulus spp.*, to determine its real efficacy against these parasites by *in vivo* test, and to determine its action time or effect delay on Nile tilapia males *Oreochromis niloticus* (Linneaus, 1758) from fish farming.

Argulose is one of the significant ectoparasite diseases of bred fish (Saurav et *al.*, 2012). This disease is caused by a crustacean ectoparasite of the genus *Argulus* belonging to the order Arguloida from arthropods' phylum. These ectoparasites have been described as economically important pathogens of fish in temperate and tropical regions (Walker et *al.*, 2004). Fishes infested by these parasites shown behavioral abnormality, which includes irritations, discolorations, lethargy and anorexia. The mode of infestation results in puncturing the host's skin, injecting a cytolytic toxin through pre-oral proboscis and feeding on blood, besides mucus and epithelial cells (Lamarre & Cochran, 1992). This disease can lead to 50-60 % of fish mortality. In Ivory Coast, fish farming of Nile tilapia, *Oreochromis niloticus*, located near lagoons are usually exposed to attacks of *Argulus spp*. since a decade. This disease causes important losses of bred fishes, which can reach 40 to 60 % of mortality. For the treatment of this disease, like others ectoparasites diseases, fish farmers apply various recommended bath treatments substances as formalin, hydrogen peroxide, dichlorvos, deltamethrin-C oestroides, azamethiphos, chloramine-T, cypermethrin, levamisole, diflubenzuron, ivermectin, (McAndrew *et al.*, 1998; Powell and Speare, 1996; Pike and Wadswath, 1999; Toovey and Lyndon, 2000). Despite the useful of these pharmaceuticals by fish farmers, *Argulus* species persist in different fish farming exposed to these parasites. It is the case in Ivory Coast's lagoons fish farming. Some time ago, the use of chemical drugs such as these indicated above were restricted because of mechanisms of bioaccumulation and residual formation, which were respectively induced in the environment and in the fish meat. To avoid these phenomena, alternative methods of parasites control may be explored. In recent year, many traditional medicine plants have been used to control bacterial and parasitic infections in humans and non-aquatic animals (Ijah and Oyebanji, 2003). However, the use of traditional medicine plants for the treatment of parasitic diseases in fish has rarely been reported (Pandey *et al.*, 2012), but the use of medicinal plant extracts as an effective alternative to antibiotic

and pesticides is well documented. Phytotherapy is shown as an important field to combat diseases problems in aqua-farm as well as in ornamental practices due to its efficacy, cost-effectiveness and eco-friendly (Saurav et *al*., 2012). In this logic, some fish farmers of Côte d'Ivoire traditionally use certain plants such as leaves of *Citrus limon* and *Nicotiana tabacum* as anti-parasitic remedies. Some others traditional plants like *Phyllanthus amarus* (Coulibaly et *al*., 2011) and *Ocimum gratissimum* (Orwa *et al*., 2009) are used against human's infections and recognized for their multi-effective powers against many diseases.

1- Material and methods

1- 1- Fish

Fishes used for this experimental purpose were mature males of Nile tilapia, *Oreochromis niloticus,* weighting 100 ± 3 g. The stock was derived from private fish farming in Bingerville village called "Adjin" (5°24'54" N - 3°52'42" W, Côte d'Ivoire). Tilapias were put in quarantine for 15 days. Fishes were supplied with well-aerated freshwater using compressed air system. They were put at a stocking density of 4 fishes by square meter according to Lacroix (2004).

1-2- Crude extraction of the traditional medicine plan

Leaves of African traditional plant *Ocimum gratissimum* were collected in the natural park and air-dried in darkness for 4 weeks. The air-dried and finely ground sample of this plant was used in accordance with Guédé *et al*. (1993). A 500 g dry weight sample of *Ocimum gratissimum* powder was added to 5 L of distilled water for 24 hours of maceration at room temperature. After this time, the solution was successively filtrated through a cotton and Watt-man paper. The obtained filtrate was put in an evaporator (Blender) at 60°C during 24 hours to have a dry powder. Fifty (50) gram of this powder were then put to 1 L of distilled water to obtain a stock solution, which was used to carry out serial concentrations for experiment. The stock solution of traditional plant *Ocimum gratissimum* was sterilized at 121°C for 15 min according to Chehregani *et al*. (2007).

1-3- *In vitro* toxicity effect of plant *Ocimum gratissimum*

1-3-1- Effect on parasite *Argulus sp*.

The *in vitro* test was performed into six (6) Petri dishes containing each 20 ml of serial concentrations (0, 100, 200, 400, 800 and 1.600 mg / L) of crude extracts of the plant *Ocimum gratissimum*. The Petri dish containing 0 mg / L of the plant was used as control. *Argulus* species were previously picked with the help of plastic forceps on heavily infected fishes and put into the plastic plate. Species of parasite were identified as *Argulus sp*. according to the African's Argulidae identification key proposed by Rushton-Mellor (1994). Ten (10) actively moving parasites were selected with the help of a small hairbrush and put into each Petri dish. At every 10 min, the number of killed parasites was counted. Parasitic death was considered when the organism did not exhibit any movement after 5 min of observation and after a slight touch with a feather forceps. The experiment was conducted in triplicate. The LC_{50} value, corresponding to the lethal concentration, which caused 50 % of exposed organisms dead, was calculated tanks to the Probit Analysis method in accordance with Beamish *et al*. (2009).

1-3-2- Effect on the Nile tilapia *Oreochromis niloticus*

Five (5) groups composed of ten (10) males of Nile tilapia each were formed in a completely randomized design. One (1) group was used as control and the four (4) others as assays. Fishes were exposed to serial concentrations of plant substance without any water exchange for 48 hours. The solution of assays concentrations had started by the LC_{50} which had killed *Argulus sp in vitro* test. Different concentrations following a geometric reason 2 (Coulibaly *et al*., 2011) were 0 mg / L as control and 100 mg / L, 200 mg / L, 400 mg / L, 800 mg / L and 1600 mg / L as assays. Mortality and abnormal reaction of fishes were recorded during exposure. The experiment was triplicate. The LC_{50} value was also calculated as following Beamish *et al*. (2009) proposed method.

1-4- Efficacy of the plant *Ocimum gratissimum* on infected fishes

The *in vivo* test was performed in a completely randomized design in accordance with Wang *et al*. (2009) with a slight modification. Six (6) experimental groups were formed with tanks (1.5 x 1.5 x 0.9 m). One group, taken as control, was not treated with the plant extract. The others (6) were

constituted to following geometric reason 2 concentrations of 100, 200, 400, 800 and 1600 mg / L. Each tank was filled with 250 L of water with 36 hours of aeration and had received 10 highly Argulus-infected fishes. *In vivo* evaluation of *Ocimum gratissimum* crude extract effects was performed by bath treatments. The Parasite mortality was recorded after each 6 h during this exposure. In the same time, observations were carried out on fishes behaviors during the bath treatment. The efficacy of each concentration was confirmed by the comparison of the average number of surviving parasites or parasites intensity in each assay group with those of control group. The experiment was also triplicate. The anti-parasitic efficacy of each concentration was calculated using the following equation (Wang *et al*., 2009):

$AE = ((I_c - I_a) / I_c) \times 100$ % with:

AE: Anti-parasitic Efficacy

I_c: Intensity of surviving *Argulus sp* in the control

I_a: Intensity of surviving *Argulus sp* in the assay.

1-5- Delay of plant's anti-parasitic effect on treated fishes

After bath treatments, treated fishes with the best effectiveness concentration were transferred to normal water and were followed until the reappearance of moderate intensity (15-20) of *Argulus sp* on treated fish. Fishes of control group were manually freed of *Argulus sp* and no treated by the plant. This process had permitted to determine the time of *Ocimum gratissimum* anti-parasitic activity on treated fishes (Chitmanat *et al*., 2004). The experiment was triplicate. According to the time, the prevalence and the mean intensity of *Argulus sp* reappearance into control and assay group was compared. These epidemiological indexes were calculated according to Bush *et al*. (1997). The prevalence, which is the percentage of the sampled fishes, infected by the parasite *Argulus spp*., and the mean intensity of infection, which is the mean number of parasites by infected fish, were calculated according to these formula:

1) $P = Nb.i.f / T.sf \times 100$ % with:

P: Prevalence

Nb.i.f : Number of infected fishes

T.s.f: Total of sampled fishes

2) I = T.p / Nb.i.f with:

I: Mean intensity of infection

T.p: Total of parasites

2-8- Statistical analysis

The LC_{50} value and the percentage of crude extract efficacy were estimated at 95 % confidence in level with upper confidence limit and lower confidence limit (Beamish *et al.*, 2009) by Probit Analysis method. The prevalence and the mean intensity values appeared in the mechanic and plant treatments groups were compared with Tukey HSD test. The software Statistica 10.0 was used for different analysis.

2- Results

2-1- *In vitro* toxicity effect of African traditional plant *Ocimum gratissimum*

Lethal concentrations of the crude extract of the traditional plant *Ocimum gratissimum* in which fifty percent of exposed organisms die (LC_{50}) are represented by Probit analysis in figure 1. In this figure, the Probit analysis representing the lethal concentration of the tropical plant *Ocimum gratissimum* for the parasite *Argulus spp*. was above those of the Nile tilapia *Oreochromis niloticus*. The LC_{50} value of plant extract was 197.66 mg / L for the parasite *Argulus sp* with 95 % confidence interval of 194.65 – 200.01 mg / L. For the Nile tilapia *Oreochromis niloticus*, the LC_{50} value was 1271.22 mg / L with 95 % confidence interval of 1251.20 – 1291.20 mg / L.

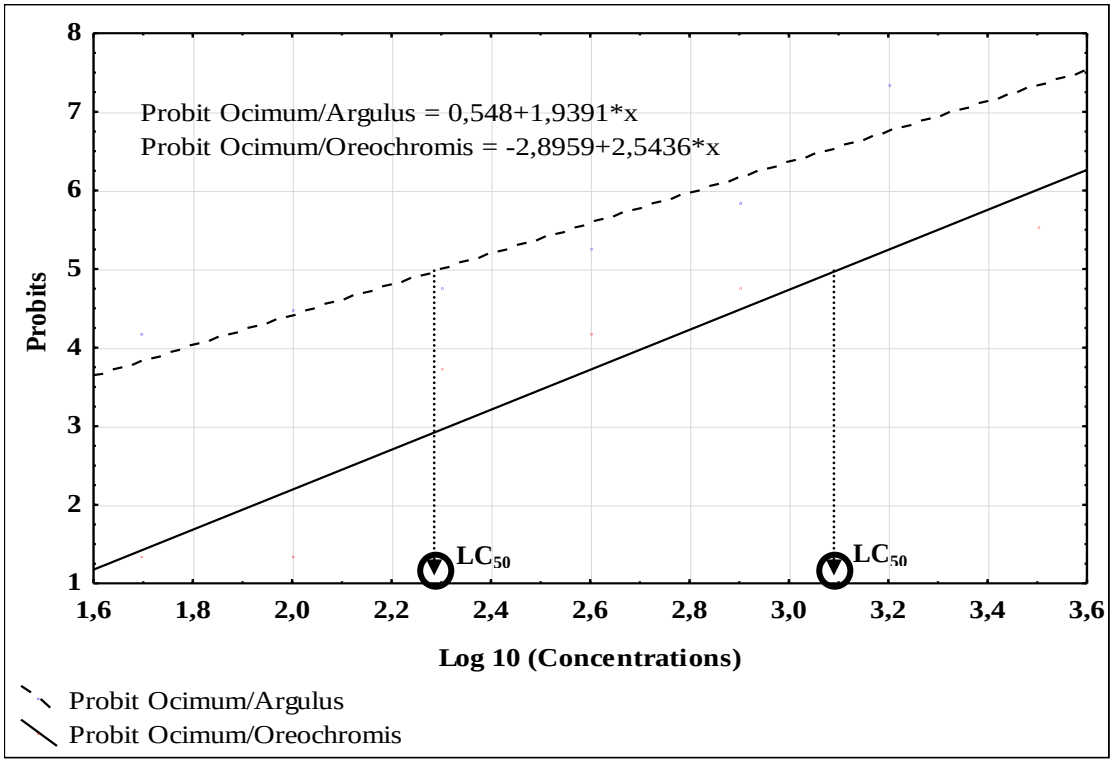

Figure 1: Probit analysis of *Ocimum gratissimum* LC_{50} determination for the parasites *Argulus spp*. and the Nile tilapia *Oreochromis niloticus*

2-2- Efficacy of plant *Ocimum gratissimum* on infected fishes

2-2-1- Anti-parasitic efficacy of the crude extract

The bath treatments carried out on natural moderate infested fishes with different concentrations of the crude extract of the plant *Ocimum gratissimum* resulted in a significant reduction in the *Argulus* species burden of Nile tilapia males *Oreochromis niloticus*. Efficacy rates had varied from 11.3 to 100 % in trials concentrations according to the time and concentrations of exposure. The first 50 % efficacy appeared was obtained in the concentration of 400 mg / L between 12 hours and 18 hours of exposition. The 30 hours 100 % in vivo efficacy of the crude extract of African traditional plant *Ocimum gratissimum* was found at the concentration of 800 mg / L (Table 1).

Table 1: *in vivo* efficacy test of the plant *Ocimum gratissimum* against the parasites *Argulus spp*.

Concentrations	*In vivo* efficacy of *Ocimum gratissimum* (%)					
	6 h	12 h	18 h	24 h	30 h	36 h
Control	-	-	-	-	-	-
50 mg / L	11.3	23.3	26.9	29.25	31.00	33.50
100 mg / L	13.5	25.8	27.6	31.00	34.1	38.3
200 mg / L	26.3	37.4	39.00	41.5	44.6	47.9
400 mg / L	33.8	46.4	51.6	59.7	66.2	87.4
800 mg / L	42.00	49.7	67.8	866.2	100.00	-

2-2-2- Delay of plant's anti-parasitic effect on treated fishes

The table 2 indicates the prevalence and the average intensity (numbers of parasite by fish) of *Argulus spp* reappeared on post-treated Nile tilapias during certain time. A significant difference ($p < 0.05$) was observed between prevalence and average intensity values of *Argulus spp.* reappearance on fishes of control and assay groups during the first three months of observation. No significant difference ($p > 0.05$) was observed between these values of both groups. During this study, the prevalence of *Argulus spp* had varied from 18.70 to 42.2 % and from 0.00 to 41.30 % respectively in control and assay groups. The average intensity values varied from 14.4 ± 1.26 to 29.3 ± 1.49 parasites per re-infected fish into control and from 0 to 27.4 ± 3.46 parasites per re-infected fish into assay group. At the first three month, the epidemiological index values were weak into the group treated with the crude extract of the plant *Ocimum gratissimum* at 800 mg / L.

Table 2: Delay of *Ocimum gratissimum* efficacy after plant and manual treatments

Groups	First month		Second month		Third month		Fourth month	
	Prevalence (%)	Intensity	Prevalence (%)	Intensity	Prevalence (%)	Intensity	Prevalence (%)	Intensity
Control	18.70	14.4^{a} ± 1.26	24.64	21.36^{c} ± 1.03	31.7	24.1^{e} ± 1.37	42.2	29.3^{g} ± 1.49
Essays (800 mg / l)	0.00	0^{b}	9.7	2^{d} ± 0.67	13.7	12.1^{f} ± 1.91	41.3	27.4^{g} ± 3.46

The values of the same parameter on a same column having in exponent identic alphabetic letters do not have significant difference ($p \geq 0.05$). These having different alphabetic letters have significant difference ($p \leq 0.05$)

3- Discussion

The plant *Ocimum gratissimum* is known for its relaxant effect on isolated intestinal smooth muscle (Madeira *et al*., 2002), antinociceptive properties (Aziba *et al*., 1999; Rabelo *et al*., 2003), Hypoglycaemic property (Aguiyi *et al*., 2000), antibacterial activity (Orafidiya *et al*., 2000), antihelminthic effect (Njoku & Asuzu, 1998), and antifungal activity, but there were not studies on its effects on crustacean (copepods) infecting the fishes. In this study, the observed anti-parasitic effects of crude extract of traditional plant *O. gratissimum in vitro* tests may be due to the effect of one or several of its mean components such as eugenol, thimol, and geraniol, which were tested to be efficacy against pathogens (Vieira *et al*., 2001). The same effects of *O. gratissimum* were discovered for others traditional plants like *Radix sp*, *Fructus sp*, *Caulis sp*, *Semen aesculi*, *Semen pharbitidis* and *Azadirachta indica* against *Argulus* species (Schmutterer, 2002; Chitmanat et al., 2004; Liu *et al*., 2010; Omima, 2010). The *in vitro* weak value of LC_{50} (197.66 mg / L) of the African's traditional plant *Ocimum gratissimum* against parasite *Argulus spp* in comparing to the LC_{50} value (1271.22 mg / L) of the same plant against Nile tilapia *Oreochromis niloticus*, indicates us that this plant has not a toxicity risk to Nile tilapias males in the conditions of this experiment. Saurav *et al*. (2012) showed similar finding, which ensure the safety of hosts exposed to crude extracts of the plant *Azadirachta indica*. The *in vivo* test 30 hours 100 % efficacy of the traditional plant *Ocimum gratissimum* against parasite *Argulus sp* indicated in this study has been also shown for certain plants (*Terminalia catappa*, *Allium Sativum* and *Artemisia vulgaris*). These plants had 100 % efficacy against some fish's ecto-parasites (Chitmanat *et al*., 2004; Noor El Deen and Mohamed, 2009). The difference between *in vitro* and *in vivo* efficacies of the crude extract of the plant *Ocimum gratissimum* could be explained the fact that ecto-parasites *Argulus sp* are vulnerable to *in vitro* treatment because they have been detached to their host and directly exposed to the plant extract. In this situation (*In vitro* test), exposed parasites have not place to hide themselves from dangers. However, *in vivo* situation, exposed parasites are attached to their hosts and can be protected by scales, operculum and gills of hosts. These results are similar to those of Kirby (1996), who had remarked that the lack of correlation between the Lc_{50}

value *in vitro* and *in vivo* bath treatments was verified for all areas of substances' anti-biotic effect researches.

The significant difference ($p \leq 0.05$) between prevalence and mean intensity values of reappearance of parasites after manual treatments, on control and bath treatment, on assay groups during the experiment would show that crude extract of *Ocimum gratissimum* possesses the anti-parasitic effects, which continued to fight against parasites. Chitmanat *et al*. (2004) who, tested crude extracts of two traditional plants to eliminate the ciliate parasites *Trichodina sp* on tilapia fingerlings *Oreochromis niloticus*, had find similar results. Therefore, the moderate intensity of 15 - 20 *Argulus spp* per infected fish, beyond which fishes mortality could note (Saurav et *al*., 2012), was observed after 90 days on fishes treated with the crude extract of *Ocimum gratissimum*. In control group with the manual treated fishes, this value appeared after 30 days. This difference would signify that the crude extract of African traditional plant *Ocimum gratissimum* have an anti-parasitic effect for a certain time.

4- Conclusion

The crude extract of the African traditional plant *Ocimum gratissimum* had toxic effect to the parasite *Argulus sp* with a LC_{50} value of 197.66 mg / L with 95 % confidence interval of 194.65 – 200.01 mg / L. For the Nile tilapia *Oreochromis niloticus*, the LC_{50} value was 1271.22 mg / L with 95 % confidence interval of 1251.20 – 1291.20 mg / L.

The 36 hours 100 % efficacy of the crude extract of African traditional plant *Ocimum gratissimum* was at the concentration of 800 mg / L. The plant, *Ocimum gratissimum,* had anti-parasitic effects after bath treatments, which prevent for 3 months against high infection of Nile tilapia by parasite *Argulus sp*.

The traditional plant *Ocimum gratissimum* can be used as an alternative to chemicals to treat *Argulus* species infection on males of Nile tilapia *Oreochromis niloticus*. However, further studies, including chronic effects on reproduction, meat cells quality, mechanism of non-specific immune responses of fishes and the determination of the active ingredient need to be investigated before acceptance for field treatment in aquaculture.

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