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## Treatment of zooplankton with neem oil in the laboratory setting

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### Abstract

An experiment investigating the effects of varying neem oil doses on zooplankton treatment was carried out from march 15 and April 30, 2024 at the fisheries and aquaculture research and application farm integrated kanhé-moyo of Baham (FRAPAIK). its objectives were to contribute to the improvement of specific production zooplankton groups through treatment with different doses of neem oil (*azadirachta indica*). An experimental device including a T0 control only fertilized with chicken manure, as well as five treatments TN1, TN2, TN3, TN4 and TN5 reated respectively with neem oil at the dose of 1; 0.1; 0.5; 1.5; 2ml /l of water. Each treatment was applied in completely randomized triplicates in 18 transparent breakable aquariums with a capacity of 36 liters. These aquariums are inseminated with phytoplankton three day later pre-fertilized with chicken droppings, and 72 hours later, then with zooplankton at a density of 11ind/l of water with 8 rotifers; and 3 cladocerans. Water quality (physico-chemistry, zooplankton) was analyzed daily. After 45 days of observations, the following results were recorded: The values of the physicochemical parameters of the water (dissolved oxygen,transparency, pH, temperature (did not vary significantly ( $p<0.05$ ) between treatments. Density, biomass, daily productivity and intrinsic rate biomass increase were significantly different ( $p<0.05$ ) between the treatments, the highest values of density (2914ind/l) and intrinsic rate biomass increase (911) was recorded in the T0 and lower in batches (1033ind/l) TN3, TN4 and TN5 respectively. As for the biomass and the daily productivity (2128 ( $\mu$ g) and 592( $\mu$ g)) the highest values were obtained in TN3 and the lowest (295 ( $\mu$ g), 311) respectively in batches T0 and TN4.

The use of neem oil at a dose of 0.5ml/l is recommended for mass production of cladocerans.

**Keywords:** Cladoceran, rotifer, neem oil, density, fertilization

### Introduction

Fish, whether from the fishing or aquaculture sector, plays a crucial role in improving household food and nutrition security in developing countries development; it contributes to the diversification of livelihoods and to the income generation (Béné and al., 2015; Lazard, 2017) [5, 16]. Annual aquaculture production in 2023 in Cameroon, to increase by 3% (FAO, 2024) between 2022 and 2023, thus increasing from 145621 tonnes to 150086 tonnes. But, despite the efforts made by producers, the sectorAquaculture is still struggling to take off. Its development faces various challenges.constraints including limited production of fry, marked by a supply insufficient food in the larval stages (Arimoro, 2006, Pouomogne, 2013) [4, 22]. The mass production of juveniles of prized species such as African catfish or Common carp remains limited. They require live food (zooplankton) adequate, of good quality and quantity; which aquaculturists can only provide with many difficulties (Piasecki and al., 2004; Jha and al., 2006) [21, 15]. The limited availability of Artemia (saltwater zooplankton), due in particular to the high cost and supply difficile (Agadjhouédé and al., 2010) [31] does not solve the problem. Zooplankton freshwater remains to this day the basic and unparalleled food for needs of fish larvae (Schlumberger and al., 2002) [27]. It is not only producible but also accessible locally in fish farms. In this sense, several works with organic fertilization have already been carried out (Agadjiouédé and al., 2011, Efole and al., 2017; Nana and al., 2018, Songmo and al., 2018, Pouomogne and al., 2024) [9, 19, 28]. Excluding the whole of This previous work led to a multi-group

production which not only interacts among themselves through predation and competition, but also with fish of the same way depending on its stage of development. The success of aquaculture production would be more assured with a single-group production of zooplankton groups each adapted to the developmental stage of the target fish. Looking into this question, several researchers have conducted trials aimed at selective production of zooplankton (Agadjhouédé and al., 2011, Pouomogne and al., 2022) <sup>[23]</sup> this with stem cells. This strain nevertheless remains unavailable to local producers. It is therefore necessary to evaluate techniques aimed at directing the production of zooplankton groups in a mass production. To our knowledge, no work has been done in this direction. This investigation therefore aims to evaluate the use of other compounds in the selective production of zooplankton groups. The oil extracted from neem seeds contains triterpenoid, compounds such as azadirachtin, gedunin, nimbin and nimbidin which have several properties including antibacterial and antifungal properties (Makeri *et al.*, 2007; Valarmathy *et al.*, 2010) <sup>[18]</sup>. The general objective of this study is to contribute to improving the specific production of zooplankton intended for feeding fish larvae through the use of different doses of aqueous extracts (neem oil) locally available natural products. More specifically, it is a question of evaluating the effect of neem (*Azadirachta indica*) on density, biomass, daily productivity and rate intrinsic specific increase of zooplankton (rotifers and cladocerans) in the environment control.

## Materials and Methods

**Study period and area:** The study was conducted from March

15 to April 30, 2024 at the Ferme de Research and Application in Integrated Fisheries and Aquaculture of Kanké-Moyo (FRAPAIK) in Baham located in the West region of Cameroon, Hauts Plateaux Department, Baham district on the southeastern border of Baho'o village (5°17'54"-5°17'57"North latitude and 10°22'20"-10°22'31" East longitude). The average altitude is 1700 m (PCD, 2015). The climate is of the high altitude Cameronian type with an alternation of two seasons: a rainy season that runs from mid-March to mid-November and a dry season from mid-November to mid-March. The average annual temperature is 23 °C with a range of 2 °C and an annual average precipitation varying between 1500 and 2000 mm (PCD, 2015). Experimental design and conduct of the test: The test was carried out in triplicate in 18 transparent breakable aquariums (0.33x0.33x0.33m<sup>3</sup>) labelled by treatment and arranged randomly on a wooden support placed in a laboratory at the farm north side of the window, with continuous exposure to sunlight between 6:30 a.m. and 6 p.m. These aquariums are filled with 30 liters of water (20 liters of borehole water and 10 liters of pond water filtered under a 50 µm plankton sieve to seed the medium inphytoplankton). Three days later the aquariums were then fertilized with sieved dry poultry droppings at a rate of 600 g/m<sup>3</sup> and then 3 days (72 hours) later, the environment was seed with 12 zooplankton/liter of water (9 rotifers, 3 cladocerans). Every two weeks, a third of the droppings (200 g/m<sup>3</sup>) was introduced into the environment to maintain the fertilization (Songmo and al., 2018) <sup>[28]</sup>. The physicochemical characteristics of the water during the test are summarized in table 1.

**Table 1:** Physicochemical characteristics of the water during the test

Caractéristiques	T0	TN1	TN2	TN3	TN4	TN5
Ph	6.96±0.38 <sup>a</sup>	6.97±0.39 <sup>b</sup>	6.96±0.41 <sup>b</sup>	6.95±0.42 <sup>b</sup>	6.96±0.38 <sup>b</sup>	6.96±0.41 <sup>b</sup>
T°	22.38±0.24 <sup>a</sup>	21.48±0.56 <sup>b</sup>	21.78±0.7 <sup>b</sup>	21.38±0.51 <sup>b</sup>	21.98±0.66 <sup>b</sup>	21.38±0.56 <sup>b</sup>
Trans	26.76±2 <sup>a</sup>	35±0.42 <sup>b</sup>	35±0.42 <sup>b</sup>	35±0.42 <sup>b</sup>	35±0 <sup>b</sup>	35±0.42 <sup>b</sup>
O <sub>2</sub>	3.73±0.74 <sup>a</sup>	6±0.5 <sup>b</sup>	6±0.5 <sup>b</sup>	6±0.5 <sup>b</sup>	6.3±0.67 <sup>b</sup>	6.3±0.67 <sup>b</sup>

a, b means with identical letters for the same characteristic is not significantly ( $P>0.05$ ).

Data collected and parameters studied: pH (UI), temperature (°C), oxygen dissolved (mg/l) and transparency (cm) were taken daily in situ respectively with a HANNA Hi 9813-5 brand multi-parameter, a brand oximeter MILWAUKEE and a suitable 30cm long Secchi disc. Every day between 6am and 8am, after homogenization of each aquarium and collection of physicochemical data, a 2 liter sample of water from each aquarium of each treatment was taken and filtered using a 40µm mesh plankton sieve of the AFNOR type. Then a sub-sample of 25ml of the rotifer concentrate was recovered, fixed by adding 5% formalin in the proportions 25% formalin in 75% of the sample volume (Nguetsop and al., 2009) <sup>[20]</sup> and stored in 100 ml bottles for quantitative analyses (Legendre and Watt, 1972) <sup>[17]</sup> and qualitative in the farm laboratory. The identification of zooplankton was done by promoting the determination keys and works of Zébazé (2000) <sup>[30]</sup> and Fernando (2002) <sup>[12]</sup>. The identification, counting and photography of different species of rotifers were made using the optical microscope brand DN-107T, No.000350 with USB interface. Renewed observation of petri dishes for counting groups of zooplankton in each sample was made until at least 100 individuals were obtained (Frontier, 1972). At the end of this inventory, the densities (D), the biomass, the daily production (P) and the intrinsic rate of

increase in biomass of zooplankton (Kr) were calculated for each treatment from the following formulas:

### Density

$$D = \frac{n}{v1} \times \frac{V2}{V3}$$

n: number of individuals counted; V1 volume of the sub-sample taken; V2: volume of the concentrated sample; V3: total volume of filtered water.

### Zooplankton biomass(µg)

Biomass (B) corresponding to the average individual dry mass of zooplankton in micrometer was calculated by the method developed by Gras and Saint-Jean in 1981; Legendre and al., 1987 <sup>[17]</sup>. Namely rotifers 0.19 (µg) PS for *B. calyciflorus* for dry weight (PS) (µgPS/l).

$$B=D \times P,$$

D=density; P=individual weight of zooplankton

**Daily production (P)**

$$P = (N_T - N_0) / T$$

$N_t$  = final number per ml of filtrate,  $N_0$  = initial number per ml of filtrate;  $t$  = duration of colonization of zooplankton species (in days).

**Intrinsic rate of increase in zooplankton biomass (Kr)**

It was calculated from the following formula:

$$K_r = \ln B_{mT} - \ln B_{mi} / t$$

$b_{mT}$  = final biomass/l of filtrate;  $b_{mi}$  = biomass/l of filtrate;  $t$  = colonization time of zooplankton species (in days).

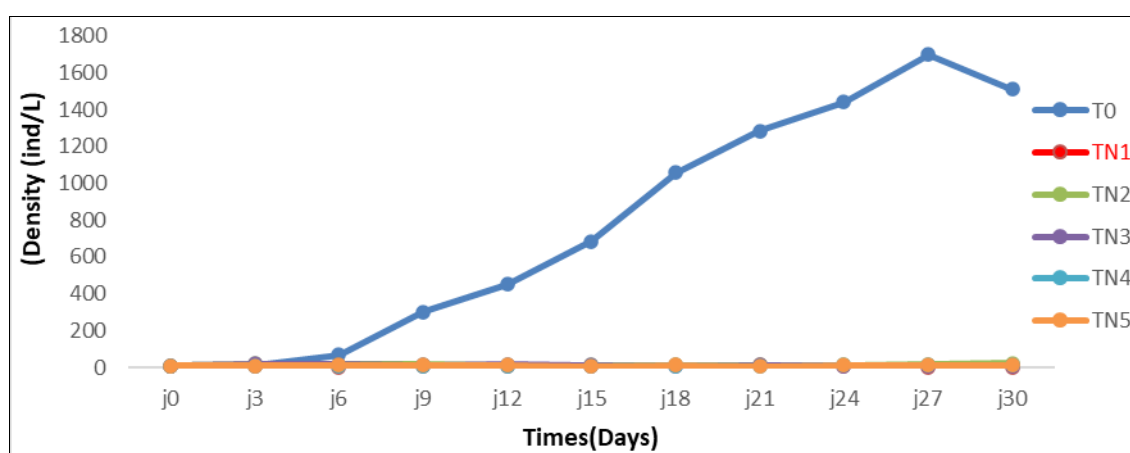
**Statistical analysis of the results**

The calculations of the various parameters were first submitted to the software Excel 2010, which also allowed us

to do descriptive statistics. All the data was subjected to one-way analysis of variance. Whenever there were significant differences between treatments, the means were subjected to the Duncan test which allowed to separate the means. These statistical analyses were carried out using the software SPSS version 21.0.

**Results****1.1 Effect of neem oil dose on zooplankton group density**

Zooplankton density as a function of neem oil dose in the medium is illustrated by Figure 1. It appears that with the exception of batch T0 (only fertilized) without oil neem all other lots have evolved in a comparable manner whatever the period chosen. Zooplankton density to evolve very weakly and constantly in the batches containing neem oil compared to the batch fertilized only (T0) which increased exponentially throughout the test.



T0: Treatment only fertilized; TN1; TN2; TN3; TN4; TN5: batches treated respectively with 1ml; 0.1ml; 0.5ml; 1.5ml; 2ml of neem oil.

**Fig 1:** Evolution of zooplankton density as a function of neem oil dose.

The effect of neem oil dose on zooplankton group density is summarized in table 1 it is clear from this Table that the dose

of neem oil influenced significantly ( $p < 0.05$ ) the production regardless of the zooplankton group.

**Table 1:** Zooplankton density (ind/l) as a function of neem oil dose.

Caractéristiques	Groupes zooplanktoniques	Treatments					
		T0	T1	T2	T3	T4	T5
Density (ind/l)	Rotifères	2865.00±26.06 <sup>d</sup>	1300.00±200.00 <sup>e</sup>	1533.33±152.75 <sup>c</sup>	833.33±152.75 <sup>b</sup>	533.33±57.74 <sup>a</sup>	550.00±229.13 <sup>a</sup>
	Cladocera	49.67±5.51 <sup>a</sup>	383.33±189.30 <sup>b</sup>	520.67±152.7 <sup>c</sup>	550.00±229.1 <sup>d</sup>	500.00±100.00 <sup>e</sup>	500.00±100.00 <sup>e</sup>
	Total	2914.67±3157 <sup>a</sup>	1683.63±389.3 <sup>c</sup>	2200±305.5 <sup>b</sup>	1383.33±381.88 <sup>c</sup>	1033.33±157.74 <sup>c</sup>	1050±329.13 <sup>d</sup>

a,b,c,d means with identical letters on the same lines are not significantly ( $P > 0.05$ ) different.

T0: Treatment only fertilized; TN1; TN2; TN3; TN4; TN5: batches treated respectively with 1ml; 0.1ml; 0.5ml; 1.5ml; 2ml of neem oil.

**1.2 Effect of neem oil dose on the biomass of zooplankton groups**

The effect of neem oil dose on the biomass of zooplankton

groups is summarized in table 2. it is clear from this table that the dose of neem oil influenced significantly ( $p < 0.05$ ) the production regardless of the zooplankton group.

**Table2:** Zooplankton biomass (µg) as a function of neem oil dose. Zooplankton density (ind/l) as a function of neem oil dose.

Caractéristiques	Groupes zooplanktoniques	Treatments					
		T0	T1	T2	T3	T4	T5
Biomass (µg)	Rotifères	200.55±1.82 <sup>d</sup>	91.00±14.00 <sup>c</sup>	107.33±10.69 <sup>c</sup>	58.33±10.69 <sup>b</sup>	37.33±4.04 <sup>a</sup>	38.50±16.04 <sup>a</sup>
	Cladocera	94.90±81.43 <sup>a</sup>	1053±500.10 <sup>b</sup>	1800±400.43 <sup>c</sup>	2070.4±412.43 <sup>d</sup>	1350±270.00 <sup>e</sup>	1350±270.00 <sup>e</sup>
	Total	295.45±83.25	1144±514.10	1907.33±411.39 <sup>c</sup>	212.33±409.3	1033.33±157.74 <sup>c</sup>	1388.5±267 <sup>d</sup>

a,b,c,d means with identical letters on the same lines are not significantly ( $P > 0.05$ ) different.

T0: Treatment only fertilized; TN1; TN2; TN3; TN4; TN5: batches treated respectively with 1ml; 0.1ml; 0.5ml; 1.5ml; 2ml of neem oil.

### 1.3 Effect of neem oil dose on daily zooplankton productivity

The effect of neem oil dose on daily zooplankton productivity

is summarized in table 3. it is clear from this table that the dose of neem oil influenced significantly ( $p < 0.05$ ) the daily productivity regardless of the group zooplankton.

**Table 3:** Zooplankton daily productivity as a function of neem oil dose

Caractéristiques	Groups zooplanktoniques	Treatments						
		T0	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	P
daily productivity	Roti-fers	122.14 ± 1.24 <sup>d</sup>	47.62 ± 9.52 <sup>c</sup>	58.73 ± 7.27 <sup>c</sup>	25.40 ± 7.27 <sup>b</sup>	11.11 ± 2.75 <sup>a</sup>	11.90 ± 10.91 <sup>a</sup>	0.001
	Cladocera	259.85 ± 213.60 <sup>a</sup>	183.33 ± 189.30 <sup>a</sup>	466.67 ± 152.75 <sup>c</sup>	566.67 ± 152.75 <sup>b</sup>	300.00 ± 100.00 <sup>c</sup>	300.00 ± 100.00 <sup>c</sup>	0.001
	Total	381.99	230.95	525.4	592.07	311.11	311.9	0.001

a, b, c, d means with identical letters on the same lines are not significantly ( $P > 0.05$ ) different.

T0: Treatment only fertilized; TN1; TN2; TN3; TN4; TN5: batches treated respectively with 1ml; 0.1ml; 0.5ml; 1.5ml; 2ml of neem oil.

### 1.4 Effect of neem oil dose on intrinsic rate biomass increase of zooplankton groups

The effect of neem oil dose on intrinsic rate biomass increase

is summarized in table 4. it is clear from this table that the dose of neem oil influenced significantly ( $p < 0.05$ ) the daily productivity regardless of the group zooplankton.

**Table 4:** Zooplankton intrinsic rate biomass increase as a function of neem oil dose

Caractéristiques	Groups zooplanktoniques	Treatments						
		T0	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	P
intrinsic rate biomass increase	Rotiferes	219.33 ± 187.90 <sup>a</sup>	0.13 ± 0.01 <sup>b</sup>	0.13 ± 0.01 <sup>b</sup>	0.11 ± 0.01 <sup>b</sup>	0.08 ± 0.01 <sup>b</sup>	0.08 ± 0.2 <sup>b</sup>	0.001
	Cladocera	691.77 ± 593.6 <sup>a</sup>	0.24 ± 0.02 <sup>b</sup>	0.27 ± 0.01 <sup>b</sup>	0.28 ± 0.01 <sup>b</sup>	0.25 ± 0.01 <sup>b</sup>	0.25 ± 0.01 <sup>b</sup>	0.093
	Total	911.1 <sup>a</sup>	0.37 <sup>b</sup>	0.4 <sup>b</sup>	0.39 <sup>b</sup>	0.33 <sup>b</sup>	0.33 <sup>b</sup>	0.094

a, b, c, d means with identical letters on the same lines are not significantly ( $P > 0.05$ ) different.

T0: Treatment only fertilized; TN1; TN2; TN3; TN4; TN5: batches treated respectively with 1ml; 0.1ml; 0.5ml; 1.5ml; 2ml of neem oil.

## Discussion

During this work the dose of neem oil influenced the density and biomass of the zooplankton groups.

The values of the density of zooplankton groups varied between 1050 and 2914 (ind/l), although that the highest value 2914 (ind/l) is found in the batch (control) receiving only the fertilization. The values obtained in this work are comparable to those (1581-3592 (ind/l) of Tonfack *et al.* (2018) [25] or those (148.16-3045.53 (ind/l) of Agadjihouede *et al.* (2010) [31] obtained by zooplankton production in fertilized ponds respectively to different doses of chicken manure and different types of fertilizers. They remain however superior to those (133-863 (ind/l) obtained by Akodogbo *et al.* (2014) [3] by Conclusion and application of results fertilization at different doses of pig manure. The difference between these results would be due to the fact that neem oil would have a destructive effect on certain microorganisms in particular zooplankton. This corroborates the work of Mamadou Faye 2010 who demonstrated that neem oil is known for its insecticidal properties.

Biomass ranged from 295-2128.3 (µg), with the highest value in the batches treated with neem oil at dose TN3 (2.5 ml) and the smallest in the only fertilized batches. These results are higher than those (63.80-423.33(µg)) reported by Akodogbo *et al.* (2014) [3] in a zooplankton production fertilized with different doses of pig manure. But comparable to 426-1592(µg) obtained by Tonfack *et al.* in 2018 [25]. These results could be explained by a difference in weight between the zooplankton groups present in the culture media (0.07µg/ind for rotifers and up to 2.7µg/ind for cladocerans (Dabbadie, 1996) [7]. Indeed, media containing neem oil showed strong concentrations of cladocerans. This suggests that the dose of neem oil would have a much higher destructive action on rotifers than on cladocerans.

Daily productivity values ranged from 311 to 592 (ind/l). These values are higher than those (20-41 ind/l) of Agadjihouede *et al.* (2010) [31] and comparable to those (262-597(ind/l) of Tonfack *et al.* (2018) [25]. This difference would

be linked to zooplankton groups present in the environment. Indeed, in our environment there is no presence of copepods which are competitors and predators of other zooplankton groups of cladocerans and rotifers.

Although the dose of neem oil had no effect on the intrinsic rate biomass increase, its values (0.115 to 0.135) remained lower than those (0.86 and 0.99) of Tonfack *et al.* (2018) [25] or those (0.42 to 0.66) reported by (Gras *et al.* 1981, Clement 1990). These compounds act as insect repellents and/or insecticides, limiting the presence or development of pests on the host plant. Makeri *et al.*, 2007 [18] found, in trials conducted in the locality of Bankim, that the aqueous extract of the leaves of Azadirachta indica and Lantana camara can control 90% of *S. frugiperda* caterpillars. Makeri *et al.*, 2007 [18] describes that neem oil contains a quantity of limonoids (C-seco-Limonoids), composed of several principles' active ingredients (azadirachtin, salanin, nimbin and 6-desacetylnimbin), which interact perfectly together for a more effective fight when the dose is high.

## Conclusion

The dose of neem oil had a significant effect ( $p < 0.05$ ) regardless of the chosen production characteristic. The incorporation of neem oil tended to decrease the values of the production characteristics of rotifers unlike those of cladocerans. The use of neem oil at a dose of 0.5ml/l is recommended for the mass production of cladocerans

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