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Acute toxicity of monocalm 400sl (*monocrotophos)* and profenalm 720ec (*profenofos)* on *Oreochromis niloticus* (Linnaeus, 1758)

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ABSTRACT
Objective: This study aimed at assessing acute toxicity of Monocalm 400SL (monocrotophos) and Profenalm 720EC (profenofos)**,** two organophosphorous pesticides widely used in the Far North region of Cameroon, to fight against insects and mites parasitizing cotton, rice, maize, sorghum, beans and potatoes.
Methodology and results: A 48 h static acute toxicity test was carried out, to determine the LC_{50} of commercial formulations of monocrotophos (Monocalm 400SL) and profenofos (Profenalm 720EC) on the freshwater *Oreochromis niloticus* and its behaviours, during the exposition. The fishes were exposed to increasing concentrations of the two pesticides, in a non-renewed medium. The numbers of dead fishes were recorded, after 48 h and the behaviours observed, during the exposition. The 48h-LC_{50} values, estimated by probit analysis, were of 20.42 and 0.046 mgL^{-1}, for monocrotophos and profenofos, respectively. Lowest Observed Effect Concentration (LOEC) means were of 10 and 0.02 mgL^{-1}, for monocrotophos and profenofos, respectively. Elsewhere, fish behaviours such as slow opercular movements, loss of balance, increased surfacing activities, loss of pigmentation; increased aggression and erratic swimming were observed, during exposition to the pesticides.
Conclusion and application of results: Monocrotophos was found to be slightly toxic and profenophos highly toxic to *Oreochromis niloticus.* Water contamination with these products can represent a great threat for fish and aquatic invertebrates. So it is recommended to apply these pesticides with caution.
Key words: Acute toxicity, LC_{50}, monocrotophos, profenofos, *Oreochromis niloticus.*

INTRODUCTION

In order to reduce food shortage, according to the objectives of the Millennium Development Goals, most African countries have opted for revolutionary agricultural policies (Mayet, 2009) which require a varied and important range of pesticides. In the savannah zone of Central Africa (Cameroon, Chad and Central African Republic), insecticides alone represent 48 % of pesticides used (Sougnabe *et al.*, 2009). In the region of the Far North of Cameroon, cotton farmers use a lot of insecticides due to insects and dust mites attacking cotton and to boost their production (Roberts, 1987; Dülmler 1993; Mamadou *et al.*, 2001). The Monocalm 400SL and Profenalm 720EC are two organophosphorous pesticides widely used in the Far North region of Cameroon, to fight against insects and mites parasitizing cotton, rice, maize, sorghum, beans, and potatoes (Sougnabe *et al*., 2009). Monocalm 400SL and

Profenalm 720EC, have monocrotophos and profenofos, as active substances, respectively (PAN-UK, 1997). These substances, off-farm plots, may become toxic to non-target organisms. In the cotton regions of Paraguay, monocrotophos has been identified as a cause of paralysis in children. It was found that the paralysis appeared after two to three weeks of exposure (Dinham, 1993). In agricultural areas, huge amounts of pesticides can be drained by storm water and discharged into nearby rivers plantations, jeopardizing, not only the survival of exposed organisms, but also the quality of these waters (Marchand, 1989; Monkiedje et *al.,* 2004; Babut et *al.*, 2006). Tests performed in laboratory microcosm, had previously shown different levels of tolerance of aquatic organisms to pesticides exposure (Monod, 2001). Tilapia (*Oreochromis niloticus*) is a useful model for ecotoxicological studies (Jos et *al.*, 2005). Its level of antioxidant enzymes may be used as a biomarker of exposure to pollutants in water (Prieto *et al.*, 2007). This fish can spawn in all types of water, withstand extreme water temperatures and low dissolved oxygen amount (Assiah, 1996). Determining degrees of tolerance, including lethal concentrations of Monocalm 400SL and Profenalm 720EC towards *O. niloticus*, may serve as a warning signal for other less robust bodies. In Cameroon and, specifically in the Far North region, tilapia occupies an important place in aquaculture production and it is bred in ponds and diversion dam. With the increased use of pesticides in agriculture in warmer regions of Cameroon, water pollution by organophosphorous as Monocalm 400SL and Profenalm 720EC may represent a serious hazard to aquatic animal communities. Few studies reveal toxic effects of monocrotophos or profenofos on *Oreochromis niloticus* (Phommakone, 2004; Muthukumaravel *et al.,* 2013). Moreover, most existing studies use separate active ingredients, or aquatic media measurements highlight that it is a mixture of two or more compounds that exerts toxic effects (Babut *et al.*, 2001). The overall objective of this study was to evaluate the acute toxicity of Monocalm 400SL (Monocrotophos) and Profenalm 720EC (Profenofos) to tilapia (*Oreochromis niloticus*) . To do so, No Observed Effect Concentration (NOEC), Lowest Observed Effect Concentration (LOEC), 48h- LC_{50} (Lethal Concentration for 50 % of tested individuals, after 48h) of Profenalm 720EC and Monocalm 400SL towards tilapia were evaluated.

MATERIAL AND METHODS

Fish acclimatization: The study was conducted in laboratory microcosms in the Center of Inland Fisheries of Maga (Far North Cameroon). The freshwater *Oreochromis niloticus* fingerlings, with a mean length of 6.41 ± 0.87 cm and mean weight of 8.01± 0.52 g, were obtained from the Center of Inland Fisheries of Maga (Far North Cameroon). Fingerlings were transferred to a 1 m^3 plastic aquarium filled with 0.25 m^3 of a local drilling water and acclimated to laboratory conditions for 4 days, prior to experimentation. They were fed, once a day, with rice ground bran, supplied by the Center of Inland Fisheries and the medium was change daily. The fry feeding was stopped 24 hours before the test (OCDE, 1992).

Acute toxicity tests: Toxicity tests were conducted in accordance with the static test model of APHA (1998) and USEPA (1996) protocol. Preliminary tests were conducted, to find out the range of concentrations to be used in the bioassays (OCDE, 1992). Stock solutions of monochrotophos and profenophos were prepared by diluting their respective commercial formulations (obtained from Maroua market) Monocalm 400SL (400g/l of monochrotophos, ALM International/Agrochem, France) and Profenalm 720EC (720g/l of profenophos, ALM International/Agrochem, France) with boring water. The nominal test concentrations were of 0, 10, 16, 24, 32 and 40 mg/l, for monocrotophos and of 0, 0.02, 0.04, 0.06, 0.08 and 0.1 mg/l, for profenophos. In both cases, each concentration was tested in triplicate and 10 specimens of fish were placed in an 18-litres transparent plastic aquarium, filled with 12 litres of test solution. Small mesh net was used to cover the aquariums, to prevent the specimens jumping out of the test solutions. The behaviour of the fries was observed every 3 hours and death was recorded after 24 and 48 hours. The dead fishes were promptly removed and preserved in a healthy medium for observations. An essay with the binary mixture Monocalm 400SL-Profenalm 720EC was also, run, to evaluate the potential effects of the mixture of monocrotophos and profenofos on fish. Some physico-chemical parameters of the experimental drilling water were measured. Temperature was taken with an Exteck EC 400 model digital apparatus. Levels of TDS (Total Dissolved Solids) and electrical conductivity were measured, using a Wagtech model, 3937-40 series digital conductivimeter. Turbidity was measured using a digital Wagtech model, 3999-76 series digital turbidimeter. The pH was measured, using a Wagtech model, 3916-55 series digital pH-meter. Free and total Chlorine levels were measured by calorimetry with a Wagtech model, series 1024-C3 el photometer, dissolved oxygen was measured with an Exteck DO 400 model digital oxygenmeter and total hardness was measured, according to APHA (1998).

Data Analysis: The LC_{50} was calculated according to the method of Litchfield and Wilcoxon (1949). Per cent mortality was calculated and the values obtained were transferred into probit scale. Regression lines of probit against logarithmic transformation of concentrations were made, using the Microsoft Office Excel 2007 software. 95%-Confidential limits (upper and lower) of the CL_{50}, with chi square test were calculated, according to the method of Litchfield and Wilcoxon (1949) too.

RESULTS AND DISCUSSION

Experimental water quality: The physico-chemical parameters of the drilling water are shown in Table 1. These parameters show that the water is of an enough good quality, as the OCDE (2007) recommends the use of a water with a total hardness between 10 and 250 mgL^{-1} of $CaCO_3$, a pH between 6.0 and 8.5 and a residual chlorine < 10 μgL^{-1}. The used waters have physico-chemical characteristics tolerable by *O. niloticus* and several other species of fish. Moreover, the absence of deaths in the control aquaria, during testing, shows that the experimental water supplies the demands of the fingerlings for their survival and that our tests can be validated.

Table 1: Physico-chemical parameters of the drilling experimental water.

Parameters	Values
Temperature (°C)	28 ± 2
pH	7.70 ± 0,1
Turbidity (NTU)	0.22 ± 0.02
Electrical conductivity (µS/cm)	408 ± 50
TDS (ppm)	202 ± 28
Free chlorine ($mg.L^{-1}$)	0.08 ± 0.01
Dissolved oxygen ($mg.L^{-1}$)	6.05 ± 0,5
Total hardness ($mg.L^{-1}$ of $CaCO_3$)	225 ± 2,5
Total chlorine ($mg.L^{-1}$)	0.22 ± 0.03

Observation of fish behaviours: Once in contact with the test solutions, the following malfunctions due to poisoning were observed: agitation and attempt to jump out of aquariums, erratic swimming, abnormal posture, imbalance in posture (fishes swim rotated, diving to the bottom of the tank before resurfacing again), morbidity (fishes fall on their sides at the bottom of the aquarium and move only their covers and caudal fins). Dead fishes sank to the bottom of the aquarium and did not perform any movement. Swelling and redness of the lids, a light gray colour and stretching or display of constitutive thorns of all the fins were, also observed. Signs of poisoning, namely the imbalance, aggressiveness, progressive loss of movement, spines erection and depigmentation may be explained by the fact that the toxic effects of Monocalm and Profenalm are based on the interruption of transmission of nerve impulses. This disruption would be by inhibition of cholinesterase activity (acetylcholinesterase and non-specific esterase) in the central nervous system and at the neuromuscular junctions (Hill, 2002). When the organophosphate binds to the cholinesterase, the connection is stable and prevents cholinesterase from deactivating the acetylcholine neurotransmitter. It is followed by an accumulation of acetylcholine and overstimulation of the somatic nervous system, which would be reflected in the signs of poisoning observed (Hudson *et al.*, 1984, Ecobichon, 1996; cited in Hill, 2002). These symptoms are similar to those described by Santhakumar and Balaji (2000) and Muthumaravel *et al.* (2013) who, when exposing, respectively, freshwater fishes *Allabas testudineus* and *Labeo rohita to* monocrotophos, observed behaviors such as slow opercular movements, loss of balance, increased surfacing activities, strong mucus secretion, increased aggression and erratic swimming. The observed death, occurred when the inhibition of cholinesterase in the brain reached 85 and 92 %, respectively, in male and female fish *Aphanius iberus* (Varó *et al*., 2008).

Assessment of acute toxicity: According to the dose-response curves, after 48h of exposition of *Oreochromis niloticus to* monocrotophos (Figure 1) and to profenalm (Figure 2), it appears that the LOEC is 10 mgL^{-1}, for the first pesticide and 0.02 mgL^{-1}, for the second. The results obtained, do not give the exact values of the NOEC, however, indicate that it is in the concentrations range [0-10[mgL^{-1}, for monocrotophos and [0-0, 02[mgL^{-1}, for profenalm. The LOEC as the NOEC of profenalm are 500 times smaller than that of monocrotophos. The curves have the appearance of a sigmoid and indicate that mortality rate of exposed fishes increased, as the concentration of pesticides increased.

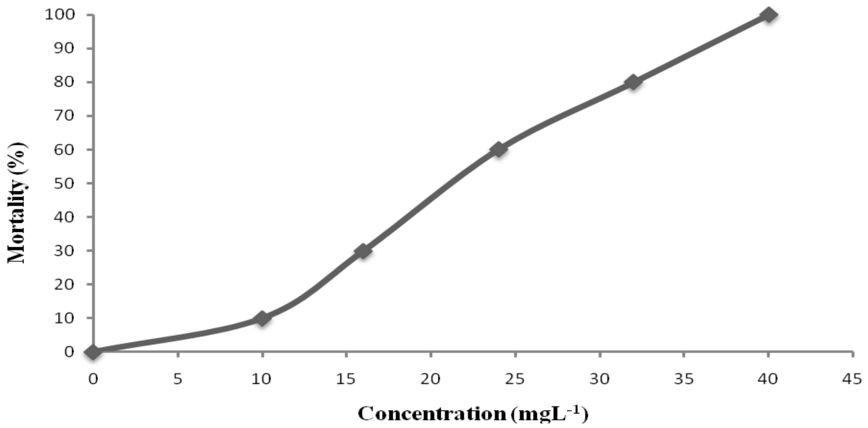

Figure 1: Dose-response curve, after 48h of exposition of *Oreochromis niloticus to* monocrotophos.

Based on the log concentration-probit regression lines (Figures 3 and 4), 48h-LC_{50} were found to be 20.42 mgL^{-1}, with 95%-confidential limits of 15.59-26.75, for monocrotophos and 0.046 mgL^{-1}, with 95%-confidential limits of 0.026-0.079, for profenalm (Table 2).

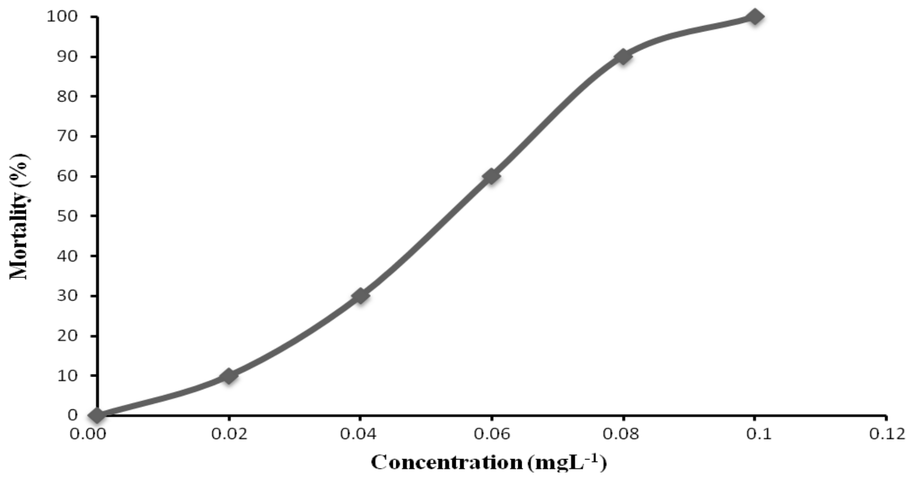

Figure 2: Dose-response curve, after 48h of exposition of *Oreochromis niloticus to* profenophos.

These results show that profenalm is more toxic to *Oreochromis niloticus* than monocalm. Monocrotophos, Monocalm 400SL active substance, is known to be of low toxicity to fish (Meister, 1992; Tomlin, 1994; RECORD, 2007). Previous studies on the toxicity of monocrotophos on fish indicate a 48h-LC_{50} of 7 mgL^{-1} and of 23 mgL^{-1}, respectively, on *Oncorhynchus mykiss* and *Lepomis macrochirus* (bluegill). (Kidd and James, 1991; Tomlin, 1994). Cruz (2002) found a 48h-LC_{50} of monocrotophos equal to 400 mgL^{-1}, for *Astyanax altiparanae* (Lambari).

These findings, compared with those obtained in this work; show that *A. altiparanae* and *L. macrochirus* are more resistant to monocrotophos than *O. niloticus,* which appears to be less sensitive than *O. mykiss*. Cruz (2002) had also conducted a study on acute toxicity of monocrotophos *to O. niloticus* and had found a 48h-LC_{50} of 20 mgL^{-1}. This value is noticeably close to that obtained in the course of our work.

Table 2: 48h-LC_{50} values of Monocalm and Profenalm to *Oreochromis niloticus*.

Pesticide	48h-LC_{50} (mgL^{-1})	95%-Confidential limits		Calculated χ^2	Table χ^2 value
		Lower	Upper		
Monocalm	20.42	15.59	26.75	0.154	7.815
Profenalm	0.046	0.026	0.079	5.288	7.815

The high toxicity of Profenalm, compared to that of Monocalm, may be explained by its ability to be absorbed by the body and to form a more stable complex with acetylcholine esterase (Elizabeth, 2008). According to RECORD (2007) table of pesticides toxicity on fish and aquatic invertebrates classification, Profenalm is very toxic, whereas Monocalm is slightly toxic to fish. Phommakone (2004), studying the effect of profenofos on Nile tilapia, had found a 48h-LC_{50} of 0.046 $mg.L^{-1}$, indicating, that the fish species they used, was more sensitive to profenofos than the one used in this work. Values of 96h-LC_{50} of 0.08 mgL^{-1}, 0.09 mgL^{-1} and 0.3 mgL^{-1} were determined, respectively, on *Oncorhynchus mykiss*, crucian carp and *Lepomis macrochirus* (Tomlin, 1994). We obtained a value of 48h-LC_{50} of 0.045 mg L^{-1} for *O. niloticus*, indicating that this fish would be more sensitive to profenofos than *O. mykiss*, crucian carp and *L. macrochirus*.

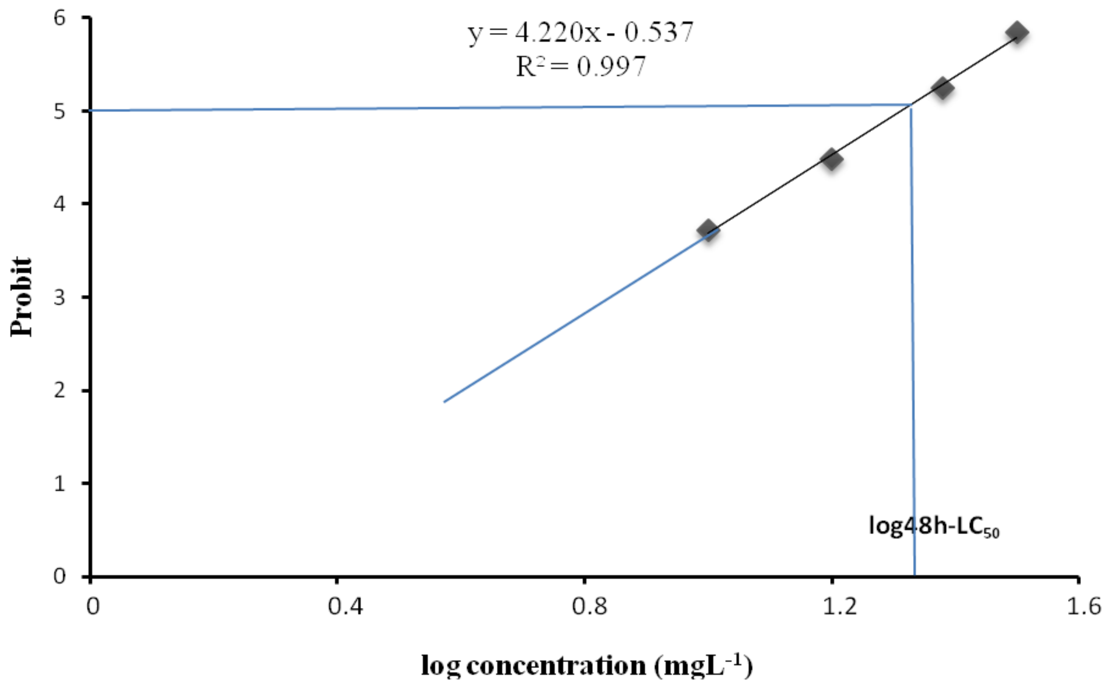

Figure 3: Regression line log concentration-Probit, after 48h of exposition of *Oreochromis niloticus to* monocrotophos.

Monocrotophos-profenofos mixture is characteristic of a mixture of two products of the same family having the same biological targets and different toxicity. A mortality rate of 20% was observed at the mixture concentrations of 16 mg L^{-1} + 0.04 mg L^{-1}, near to those of the respective 48h-LC_{50} of both pesticides. These results may be explained by the fact that toxicities additivity tendency may be reduced for a mixture of two products, if each is at the half of its LC_{50} (Warne and Hawker 1995). Studies on mixtures of plant protection substances showed other combined synergy effects, antagonism or no interaction (Bianchi et *al.*, 1994; Bianchi-Santamaria *et al.*, 1997).

The lack of mortality observed at the other concentrations test might indicate a lack of toxicity because these two products, at these concentrations, had interacted and their toxicity had been neutralized.

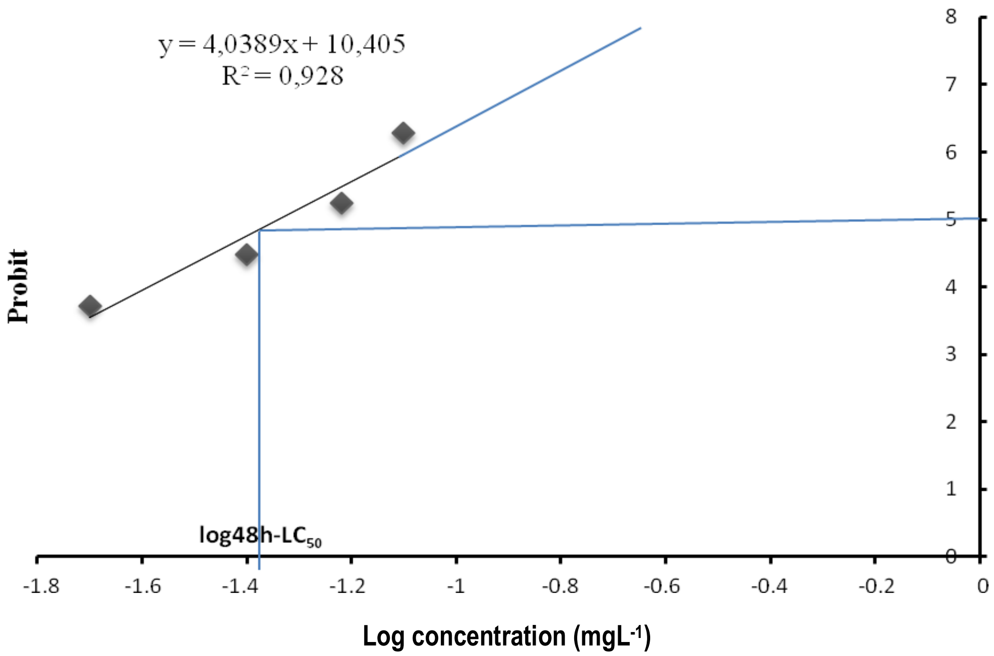

Figure 4: Regression line log concentration-Probit, after 48h of exposition of *Oreochromis niloticus to* profenophos.

CONCLUSION

Several mechanisms contribute to the transfer of Monocalm 400 SL and Profenalm 720 EC in water stream. It was, therefore, necessary to make an ecotoxicity study of these two products. At the end of the acute toxicity assessments of the two organophosphates on tilapia, performed in microcosm, the 48h-LC_{50} amounted to 20.42 mg L^{-1} and 0.046 mgL^{-1} , for Monocalm and Profenalm, respectively . According to the classification table of the toxicity of substances on fish and aquatic invertebrates, monocrotophos has low toxicity and profenophos is highly toxic to *Oreochromis niloticus*. Elsewhere, fish behaviors such as slow opercular movements, loss of balance, increased surfacing activities, loss of pigmentation; increased aggression and erratic swimming were observed, during exposition to the pesticides. Water contamination with these products can represent a great threat for fish and aquatic invertebrates. So it is recommended to apply these pesticides with caution. Following this study, chronic toxicity tests to understand the nature of injuries and the enzymatic mechanisms involved, during the exposition and genotoxicity tests to investigate the ability of the two products to generate DNA damage are needed.

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