BERKELEY PUBLICATION AND RESEARCH INTERNATIONAL

09.30.2020

Journal of Med., Biological and Pharm. Sci. Vol. 13 No. 3

EFFECTS OF AGRO-COMET PESTICIDE ON THE SURVIVAL OF AFRICAN CAT FISH (*Clarias gariepinus,* BURCHELL 1822) JUVENILES IN CROSS RIVER STATE, NIGERIA

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ABSTRACT

Pesticides have been in use by farmers for long and most often sprayed on farmlands that are near water bodies without recourse to the physiological effect on aquatic life especially fishes. Moreover, this can create and/or result in biomagnifications or bioaccumulation. The aim of this study was to investigate the sub-lethal effect of agro comet in fish, (Clarias gariepinus sub-adults). The study was carried out in the Department of Zoology and Environmental Biology, University of Calabar for 96 hours after acclimatizing the fish to laboratory conditions for two weeks. Ten (10) sub-adults of Clarias gariepinus were used for each aquarium, exposed to five different concentrations of the agro-comet and a control group. The experiment was carried-out in triplicate. The experimental fishes were exposed to 0, 300, 500, 2000, 3500 and 4000ppm of the test chemical. The mean length and

Introduction:

A pest is any organism that causes economic damage or injury to man, his livestock or crops in at least one or two life stages of its lifecycle (Bridget, 2012). It has been known to cause widespread crop destruction and wholesome death of domestic animals and epidemics of insect-borne human diseases. Pest is any organism that is noxious, destructive or troublesome, which includes a tremendous variety of organisms that interfere directly or indirectly with humans or their social and economic means of livelihood (Ubong and Akuro, 2001). Pesticide was defined by the Food and Agricultural Organization (FAO, 2002) as a substance or mixture of substances with the intention of preventing, destroying, or

ISSN: 1211-4401

weight of the fish used for this study were 29.87 ± 1.863cm and 148.00 ± 25.733g respectively. The mortality data of Clarias gariepinus sub-adults exposed to the chemical were concentration-exposure-duration dependent. The 96 hours lc_{50} value with 95% confidence limit of Clarias gariepinus sub-adult exposed to agro-comet chemical was 1800ppm ± 1.353, and was significant with a determination coefficient (r^2=0.89) at $p<0.05$.

***Keywords**: pesticides, concentration, Clarias gariepinus, biomagnification, sub-lethal, Agro-comet.*

Controlling pest, which includes vectors of human and/or animal disease. These include substances used to regulate plant growth, as desiccant, as defoliant, for thinning fruit or to prevent premature fall of fruits. Pesticides are also used as substances that are applied to crops before or after harvesting and for protection of the commodity from spoilage during transportation and storage.

Pests and pesticide

Pesticides are made up of organic micro pollutants that have ecological impacts in a broad range. Pesticides have different categories which also have different effects on living organisms. The most important pathway that causes most ecological impact is water runoff contaminated by pesticide. There are two important mechanisms through which pesticides become toxic and harmful. These include bio-concentration and biomagnifications (Dorothy and Richard, 2011). Many of these effects have consequences on food chain, usually unnoticed by mere observations, but chronic or not lethal (Ubong and Akuro, 2001)]. It has been estimated that, of the pesticides applied mostly only less than 0.1 per cent reaches target pest, the remaining 99.9 percent become environmental pollutant that end up in air, nearby vegetation, soil or water (Panigrahi *et al*., 2014).

Pollution

Pollution refers to the introduction into the natural environment of contaminants that can causes adverse change in the environment. This change can affect plants, animals and convenience of the ecosystem functions. According to OECD 2014, pollution is the production and/or "introduction by man directly or indirectly of substances or energy into the environment resulting into deleterious effects of such nature as to endanger human health, harm other living resources in the ecosystem and impairs or interferes with amenities or other legitimate uses of the environment". Pesticides are made up of organic micro pollutants that have ecological impacts in a broad range.

Agro-comet

Agro-Comet 72WP is a chemical pesticide used in the control of fungus diseases in cocoa, potato, cashew and coffee. According to (Bruck *et al*., 1980), Agro Comet is a copper and metalaxyl-based fungicide which is of short term solutions for the black-pod disease of cocoa (*Phytophthora*pod rot), and generally most reliable and popular with farmers because of their quick, effective action. Agro-Comet 72WP has been approved by the Cocoa Research Institute of Ghana (CRIG). Ridomil plus (12% metalaxyl and 60% copper-1-oxide) and Agro Comet are among the fungicides used in Nigeria to control cocoa *Phytophthora* pod rot (Opoku *et al*., 2007).

African catfish (*Clarias gariepinus* Burchell, 1822) [Clariidae]

This catfish is indigenous to northern parts of the continent of Africa, which has been translocated into many river systems outside its natural range. Man primarily predates on African catfish because of its nutritious value. This is due to their characteristics which include their ability to tolerate poor water quality conditions, adaptability to overcrowding, wide distribution, give extremely high yields, high standard response to artificial feed, fast rate of growth, resistance to disease, good taste, ease of artificial breeding and high market quality(Nahar *et al*., 2000).

Statement of the problem

A pesticide is supposed to be lethal to the target pest organism and not to non-target organisms like man. Unfortunately, this is not so, hence the apparent controversial use and abuse of pesticides. There has been uncontrollable use of the chemicals, which is triggered by the fact that some people think "if little is good, a lot will be better". This has completely disrupted human and other living organisms. Poisoning by agricultural pesticides is a serious public health problem especially in the developing world, killing about 250,000 to 370,000 people every year (Dawson *et al*., 2010). According to Opoku *et al*., 2007, most application of pesticides to crops are done during planting and growing (raining) seasons where the torrential tropical rainfall can readily wash leach pesticides into nearby streams and wells that local residents use for drinking, cooking, bathing and laundry purposes.

However, most effective and widely used pesticides against the black pod disease of cocoa are those of copper and metalaxyl-based fungicides. These fungicides are equally used in all cocoa farms in Ikom LGA, Cross River State. Since most of the cocoa farms are close to streams and rivers that most people use for other purposes, it is therefore easy to imagine how waterways could be contaminated in all areas where pesticides are used.

MATERIALS AND METHODS

The study area

This study was carried out in the Post Graduate Laboratory of the Department of Zoology and Environmental Biology, University of Calabar, Calabar, Cross River State, Nigeria.

Collection and transportation of samples

A hundred and eighty (180) live sub-adults of *Clarias gariepinus* with mean length and weight of 29.87 ± 1.863cm and 148.00 ± 25.733g were bought from the University of Calabar fish farm and transported in a plastic bucket containing water to the Department of Zoology and Environmental Biology Postgraduate laboratory, University of Calabar for the study.

Acclimatization of samples

In the Department, the fishes were allowed to acclimatize for 2 weeks, so as to get used to their new environment. During this period, they were fed twice daily 4% of their body weight using Coppen. The water was changed every day, so as to maintain adequate water quality during the acclimation according to (Adetola *et al*., 2011). Feeding stopped 2 days to the commencement of the trials.

Preparation of test chemicals

A stock solution of the test chemical was prepared according to(OECD, 1977). 6g of Agro-comet 72WP was dissolved in 1000mls of water. From the stock solution, serial dilutions of 300ppm, 500ppm, 2000ppm, 3500ppm and 4000ppm were made as exposure concentrations.

Preliminary toxicity test

A range-finding test was first carried out, in order to reveal the actual concentration that is capable of causing mortality.

Test procedures

A 96 hour static bioassay was conducted in the departmental laboratory to determine the sub-lethal effect of Agro-comet 72WP on sub-adults of African catfish (*Clarias gariepinus*). The Pesticide was obtained from Okey Agro-chemicals Ltd., Ikom market, Cross River State, Nigeria. The experimental fish were distributed randomly into 18 transparent rectangular plastic aquaria. A total of 180 sub-adults *Clarias gariepinus* were used. Ten (10) fish samples were stocked in each aquarium, with each group exposed to 300ppm, 500ppm, 2000ppm, 3500ppm and 4000ppm of test chemical in triplicates. There was also a control group where no chemical was introduced. The mortality and behavioural responses were monitored after 24, 48, 72 and 96 hour. Scoop net was used to remove dead fishes that may contaminate the test medium and were preserved in 10% formalin for histological processing. The length and weight of the samples were randomly measured using measuring board in centimetres and electronic sensitive weighing balance in grams before the commencement of the practical.

Toxicity observations

Mortality.

Mortality were monitored and recorded for a 24, 48, 72 and 96 hours. The inability of any of the fish to respond to external stimuli will formally be used as an index of death. The 96hour LC_{50} toxicity for each concentration was determined as a total summary of percentage mortality data using the method of (Hoque *et al*., 1993).

Statistical analysis

The data obtained were subjected to statistical analyses (descriptive and analytical). The 96hr LC_{50} (mean lethal concentration) computed and determined using probit analysis (Andem *et al*., 2017). Analysis of variance (ANOVA) was used to test the significant differences between the different culture water groups contaminated with different concentrations of chemical. Probit analysis was carried out on the mortality to determine the concentration that will kill 50% of the test fish (LC_{50}). All statistical analyses were carried out using predictive analytical software (PASW) version 20.

RESULTS

Percentage mortality and survival of *Clarias gariepinus* sub-adults exposed to different concentrations of Agro-comet 72WP

The summary of the survival, percentage survival, mortality and percentage mortality of *Clarias gariepinus* exposed to different treatment concentrations of Agro-comet chemical is shown in Table 1. The initial mean length and weight were 29.87 ± 1.863cm and 148.00 ± 25.733g respectively. *Clarias gariepinus* sub-adults exposed to concentration of 0, 300, 500, 2000, 3500 and 4000ppm of test chemical had a percentage survival of 100%, 70%, 60%, 40%, 20% and 10% respectively, with percentage mortality of 0%, 30%, 40%, 60%, 80% and 90% respectively (Table 1).

Table 1. Survival and Mortality profile of *C. gariepinus* sub-adults exposed to different conc. of Agro-comet 72WP for 96 hrs.

Toxicant conc. (ppm)	Survival	% survival	Mortality	% mortality
0 (Control)	10	100	0	0
300	7	70	3	30
500	6	60	4	40

2000	4	40	6	60
3500	2	20	8	80
4000	1	10	9	90

Probit transformation at 96 hours

The summary of the probit transformation mortality data for *Clarias gariepinus* that were exposed to the different concentrations of Agro-comet is shown in Table 2. The mortality data were dependent on the concentration, and showed signs of stress and erratic behaviour and gasping for air when exposed to the different concentrations of Agro-comet, due to respiratory impairment.

The regression equation for the probit transformation of the sub-adults of *Clarias gariepinus* exposed to different concentrations was $y = 0.0184X + 18.449$ (Table 3). A log concentration probit regression analysis was significant at $P<0.05$, yielding a determination coefficient ($r^2 = 0.89$), chi-square value of 0.911 (Table 4), and a 96 hours LC_{50} with 95% confidence limit of 1800ppm $\pm$ 1.353 (Figure 1). The lower and upper limit of the LC_{50} value for sub-adults of the *Clarias gariepinus* exposed to the different concentrations of agro-comet was 0.515 and 2.191 respectively (Table 5).

TABLE 2. Probit Transformation of mortality data of sub-adults of *Clarias gariepinus* exposed to different concentrations of Agro-comet 72WP at 96 hours

Conc. (ppm)	Log conc. (x)	N	R	P	M_R	Y	R_P	P
0	0.000	10	0	0.00	0	0.000	0.000	0.000
300	2.477	10	3	0.30	30	2.806	0.194	0.281
500	2.699	10	4	0.40	40	3.893	0.107	0.389
2000	3.301	10	6	0.60	60	7.031	-1.031	0.703
3500	3.544	10	8	0.80	80	8.057	-0.057	0.806
4000	3.602	10	9	0.90	90	8.266	0.734	0.827

n = Number of fish tested at each concentration, r = Number of fish responding, p = Response rate ($\frac{r}{n}$), M_R = Mortality rate, Y = Expected probit from visual regression line, Rp = Residual probit, P = Probability.

TABLE 3. Results of regression analysis of 96hours Log concentration – probit relationship of sub-adults of *Clarias gariepinus* that were exposed to different concentrations of Agro-comet 72WP

Conc. (Log unit)	Response rate, p	Equation	Coefficient of determination, r^2	Significant level, α
0	0.00			
300	0.30			
500	0.40	y = 0.0184x+18.449	0.89	0.05 (sig)
2000	0.60			
3500	0.80			
4000	0.90			

TABLE 4. Chi-square test of sub-adults of *Clarias gariepinus* exposed to different concentrations of Agro-comet 72WP

	Chi-square	df[a]	Sig.
Probit pearson Goodness-of-fit	0.911	3	0.899[a]

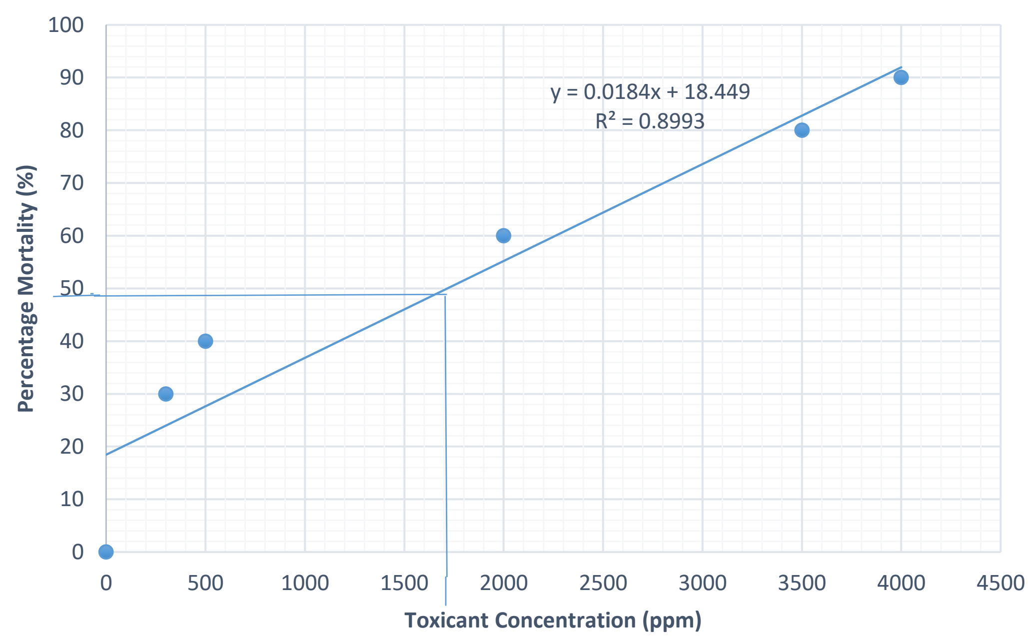

Fig.1: A probit graph of sub-adults African catfish (*Clarias gariepinus*) exposed to different concentrations of Agro-comet 72WP

TABLE 5. LC_{50} with 95% confidence limits of sub-adults of *Clarias gariepinus* exposed to different concentrations of Agro-comet 72WP

LC50 with ± 95% CL	Confidence Limits	
1800ppm ± 1.353	Lower	Upper
	0.515	2.191

DISCUSSION

Agro-comet 72WP effluents through handling carelessly, spilling accidentally and indiscriminate and over-use, end up in the aquatic systems thereby causing harm on populations of fish and other forms of life in aquatic environments.

The waters are used by humans for many purposes like cooking, washing, bathing and even drinking. Animals also drink the waters. The toxicity of Agro-comet 72 WP on Sub-adults of African catfish increased with increasing concentration and exposure time and this corroborated with the findings of (Moshen *et al.,* 2012). Frequency of the pesticides in agricultural wastewaters in high concentrations exposes fishes and other aquatic organisms to their toxic effects. This has been reported by (Mustapha *et al.,* 2014), (Andem *et al*., 2017) and (Akerblom, 2004).

There was no mortality observed in all the control tests, while varying degrees of mortality were observed in the concentrations. This is a clear indication that the effect of pesticide (Agro-comet 72WP) could be regarded as possible cause of death of the test organisms. The toxicity of agro-comet in this study was concentration and exposure dependent, with the mortality increasing with increasing concentration. The highest mortality was found at the highest concentration, suggesting dose-dependent survival and concentration graded lethality. This observation was in conformity with the findings of (Adetola *et al*., 2011) and (Andem *et al*., 2017). Mortality of the sub-adults of *Clarias gariepinus* was observed and this confirms the fact that synthetic pesticides cause mortality of aquatic organisms and deterioration of the aquatic ecosystem. The 96 hours LC_{50} of the sub-adults of the *Clarias gariepinus* that were exposed to different concentrations of Agro-comet at 95% confidence limit was

1800ppm for this study, which did not tally with the findings report of (Adetola *et al*., 2011) when studying the acute toxic effect of Endosulfan on fingerlings of African catfish, and (Andem *et al*., 2017) when studying the toxicological and Histopathological Changes in *Clarias gariepinus* Fingerlings exposed to synthetic pyrethroid Pesticide (Cypermethrin). This discrepancy in the LC_{50} value of fish exposed to the different toxicants could be due to the difference in fish species, duration of exposure, difference in elimination, age of fish and metabolic degradation from the body. The higher LC_{50} value of *Clarias gariepinus* sub-adult when exposed to agro-comet is as a result of the fact that the sub-adult of *Clarias gariepinus* is older and as such tougher and takes a higher concentration of the chemical for its toxicity to be observed.

CONCLUSION

Acute toxicity test using species of fish under laboratory conditions help us to assess the possible danger or risks on other animal and plant species in the natural environment. For the purpose of regulations, it also aids in determining possible criteria for water quality and for use to correlate acute toxicity testing of other animal species for the purpose of comparison. It was observed that short-term exposure to Agro-comet 72WP resulted in mortality of fish. This indicates that Agro-comet 72WP is very toxic to *Clarias gariepinus*, and therefore, the use of Agro-comet 72WP fungicide on or near aquatic environments should be discouraged.

RECOMMENDATIONS

1. Biological methods of pest control should be used instead of the chemical method because of its toxicity to aquatic lives
2. Pest resistant crop species should be encouraged to curtail the use of pesticides.
3. Further studies using other chemicals should be carried out to investigate if there is a pesticides which can be more favourable to the aquatic environment

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