ISJ 14: 140-148, 2017 ISSN 1824-307X

RESEARCH REPORT

Lethal and sub-lethal effects of cypermethrin and glyphosate on the freshwater's copepod, *Acanthocyclops robustus*

AM Houssou[1,2], EJ Daguégué[2], E Montchowui[1,2]

[1]*Laboratory of Research in Aquaculture and Aquatics Biology and Ecology, School of Aquaculture of Vallée, National University of Agriculture, Porto-Novo, BP 43 Kétou, Republic of Bénin*
[2]*Laboratory of Hydrobiology and Aquaculture/Faculty of Agricultural Sciences / University of Abomey-Calavi, BP 526 Cotonou, Republic of Bénin*

Accepted April 18, 2017

Abstract

The study aims to evaluate the acute and chronic toxicity of cypermethrin and glyphosate to a freshwater's copepod, *Arcanthocyclops robustus*. The acute sensibility was assessed by estimating lethal concentrations. Then the chronic exposure allowed to assess the effects of low concentrations (0.2489 ppb and 0.4978 ppb respectively 10 % and 20 % of LC_{50} at 48 h of cypermethrin and 1.3 ppm and 2.6 ppm respectively for glyphosate) on the species. The estimated lethal concentrations at 1%, 50 % and 99 % were 2.353 ppb, 4.755 ppb and 9.610 ppb in 24 h, respectively 0.567 ppb, 2.489 ppb and 10.929 ppb in 48 h for cypermethrin. Regarding glyphosate, the lethal concentrations 1 %, 50 % and 99% were 5 ppm, 19 ppm and 73 ppm in 24 h, respectively 8 ppm, 13 ppm and 21 ppm in 48-h. hatching was affected by 20 % LC_{50} of cypermethrin; only 20 % of females have hatched their eggs against 60 % in the control treatment. Females and nauplii survival was affected by both pesticides. *A. robustus* is then more sensitive to cypermethrin compared to glyphosate. Low concentration like 0.4978 ppb of cypermethrin could affect it population and then all the ecosystem biodiversity.

Key Words: *Acanthocyclops robustus*; chronic sensibility; cypermethrin; glyphosate; lethal dose

Introduction

The use of chemical pesticides has greatly expanded in Africa, as everywhere in the world, to fight against both the endemic diseases vectors and crops pests (Lévèque and Paugy, 2006). It results chronic contamination of all ecosystem (aquatic, terrestrial or atmospheric) (Druart, 2011). Only 0.1 % of sprayed pesticides reach their target, the rest is distributed in ecosystems and contaminate the land, water and air. The atmospheric fraction also finally regains, like atmospheric fallout, soil and surface waters during rainfall (Haraguchi *et al*., 1994). Soil leaching and soil-water dispersion of pesticides therefore lead to contamination of aquatic environments (Kao, 2002; Lalancette, 2012). It results various effects, on ecosystem balance and lead to the extinction of some species (Adégbidi, 2000; Fadoegnon and Midingoyi, 2006; Pesce *et al*., 2008; Houssou *et al*., 2015, 2016).

Corresponding author:
Arsène Mathieu Houssou
Laboratory of Research in Aquaculture and Aquatics Biology and Ecology
School of Aquaculture of Vallée
National University of Agriculture
BP 43 Kétou, Republic of Bénin
E-mail: arsnehous@yahoo.fr

In Bénin republic, aquatic ecosystems are contaminated with agricultural pesticides especially in the cotton basin (Agbohessi *et al*., 2011), in the Ouémé River (Yèhouénou *et al*., 2006a and b). Even, cypermethrin and glyphosate are two of the most used pesticides in food crops production in Bénin (preliminary personal investigation: unpublished). The increasing of food crops production in the Ouémé River basin causes a consequent increase of the use of cypermethrin and glyphosate, then the increasing of contamination of the ecosystem.

Freshwater's copepods are an important part of the planktonic biodiversity. They are part of the first organisms that may present a quickly observable responses facing chemical pollution. Due to numerous factors affecting the responses of these organisms to pollutant, specie in two different environments can present wide range of responses to the same substance. Then, to efficiently monitor the status of ecosystems, it is important to have biological models for different environment and know their responses facing different type of pollutants. *Acanthocyclops robustus* is one of the most abundant copepod species in the Ouémé River basin (Houssou *et al*., unpublished). Nowadays, there is no data on the sensitivity of plankton species to the different pollutants in Bénin aquatic environments. The aim of this study is to evaluate the response of *A. robustus* exposed to cypermethrin and glyphosate, used in food crops production in Bénin.

Material and Methods

Test organisms: culture and rearing

A plankton sample was taken in Ouémé River at Bonou (6°54'30.7"N and 2°26'58.0"E) by using plankton net of 50 µm mesh size. This sample was rushed to the laboratory in ambient conditions in absence of fixative. It was then cultured for 2 weeks in the presence of organic fertilizer (chicken droppings) at 0.6 g/L (Agadjihouèdé *et al*., 2011). The species were then identified under photonic microscope and ovigerous females of *A. robustus* were isolated. A total of 10 females have been placed in a culture of phytoplankton (*Scenedesmus* spp.) and rotifers (*Brachionus plicatilis*). After hatching, the nauplii were kept in the culture until adulthood. The culture solution was renewed every two weeks. At the time of this study, culture has gone through four generations.

Chemicals

Cypermethrin is a high active synthetic pyrethroid insecticide. It has light yellow appearance with acidity (as H_2SO_4) of 0.3 % (w/w) maximum. It toxicity classification is II (Moderatly hazardous). Cypermethrin is lowly soluble in water (4 to 10 µg/L). In this study, cypermethrin is obtained in it supplied formulation (Cyperforce®) containing Cypermethrin 10 % EC (emulsifiable concentrate). Glyphosate is a non-selective systemic herbicide. It is a weak organic acid. Its molecular weight is 169.07 and its solubility in water of 12 g/L at 25 °C. Glyphosate is used in this study as supplied formulation (Kumark® (480 g/L). Different concentrations of both pesticides were dissolved in water to have the needed concentrations of active ingredient. The structural formula of cypermethrin and glyphosate are following (see below).

Acute toxicity test

Test design and handling

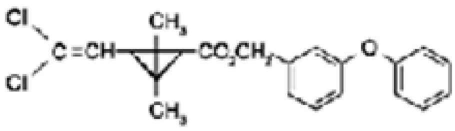
The experimental design for each pesticide is composed of twenty-eight (28) glass cup (petri box) (Six concentrations and one control with four replications each) and seven (07) pillboxes. A pillbox containing the test solution of each treatment was used for the measurement of physico-chemical parameters. Seven adults of *A. robustus* were placed in each glass cup immediately after distribution of the test solutions (28 individuals per treatment and a total of 168 per test). An initial test was performed to determine the concentration range to be used in the definitive test (data not presented). The definitive nominal concentrations used are: 0 (control), 1, 2, 4, 6, 8 and 10 ppb for cypermethrin and 0 (control), 2, 4, 8, 12, 16 and 20 ppm for glyphosate. Each test lasted 48 h. The test solutions were not renewed and the copepods were not fed (USEPA, 2002). The loss of active ingredients concentration in the 48-h was considered insignificant, view cypermethrin is non-volatile (vapor pressure = 3.1×10^{-9} mmHg at 20 °C; Henry's constant = 4.21×10^{-7} atm.m^3/mol) (SAgE pesticide, 2015). Also its half-life time in the water is 14 days. Glyphosate also is non-volatile (vapor pressure = 1.84×10^{-7} mmHg at 45 °C; Henry's constant = 4.08x10-19 atm.m^3/mol).

The photoperiod was 16/8-h (light/darkness). Temperature (26.49 ± 0.25° C), pH (7.11 ± 0.11) and dissolved oxygen (3.91 ± 0.19 mg/L) were measured daily in the different treatments. The individual's mobility and mortality were monitored at 1-h , 24-h and 48-h of exposure. Copepod was declared dead with lack of movement after a mild stimulus.

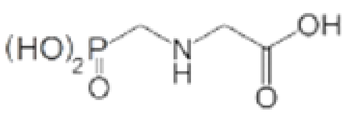
Chronic tests

Test design and handling

Hatching, survival of females and nauplii of *A. robustus* were studied in this part of the study. A total of 30 glass cups (petri box) of 50 ml each (three treatments with 10 replications each) was used for each pesticide. A pillbox (120 ml) was provided for each treatment allowing the measurement of physico-chemical parameters. Two sub-lethal concentrations (10 % and 20 % LC_{50} in 48 h) were tested in addition to the zero-concentration (control). After distribution of the test solutions in the experimental design, one ovigerous female of *A. robustus* was immediately placed in each cup. The feeding was ad-libitum with concentrated freshwater's rotifer (*B. plicatilis*) and phytoplanktons. The test solution was renewed in all tests every 96-h. Cypermethrin and glyphosate being non-volatile with half-life time in water more than 96-h, the test solution renewal time made insignificant the loss of active mater. The test lasted 10 days and the hatching was controlled daily. Female and nauplii mortality have also been monitored daily. The photoperiod was 16/8-h (light/darkness). The temperature (24.73 ± 0.06°C), pH (6.67 ± 0.06) and dissolved oxygen (3.57 ± 0.12 mg/L) were measured daily in the different treatments.



Cypermethrin



Glyphosate

Table 1 Cumulative mortality of *Acanthocyclops robustus* (n = 28 each concentration) exposed to cypermethrin and glyphosate

Glyphosate			Cypermethrin		
Concentration (ppm)	**No of mortality**		**Concentration (ppb)**	**No of mortality**	
	24-h	48-h		24-h	48-h
0	4	4	**0**	2	2
2	3	5	**1**	5	9
4	5	6	**2**	10	13
8	7	8	**4**	12	17
12	11	14	**6**	20	28
16	15	23	**8**	28	28
20	14	28	**10**	28	28
Chi-Square	15.076	14.203		23.385	17.624
df	22	22		22	22

Data analysis

The copepod survival values were used to estimate the lethal concentrations ($LC_{1-10-20-30-40-50-60-70-80-90-99}$) at 24-h and 48-h. Lethal concentrations were calculated using probit method in the computing program PoloPlus v.1.0. The chi-square test was used to assess difference in response other exposure concentration. The hatching rate, the death rate of nauplii and females were calculated of each treatment. The One way analysis of variance (ANOVA) was used to assess variability of data other treatment (Statistica v.7 Software was used).

Results

Acute toxicity

Cumulative numbers of dead individual of *A. robustus* to increase concentration of cypermethrin and glyphosate during the exposure time (24 and 48-h) are presented in Table 1. There is significant increased number of dead individual with increasing concentration for both pesticides. Respectively two deaths were recorded in the control treatment (zero concentration) at 24-h and 48-h for cypermethrin exposure and four in the two periods for glyphosate exposure. In cypermethrin exposure, there was 100 % mortality from 8 ppb to 10 ppb (24-h) and from 6 ppb to 10 ppb (48-h). Respective to the glyphosate exposure, a maximum of 53.57% (24-h) and 100% (48-h) was respectively observed at 16 ppm and 20 ppm. The estimated lethal concentrations of cypermethrin and glyphosate to 1 %, 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 % and 99 % of *A. robustus* population are respectively presented in Tables 2 and 3. In case of cypermethrin, the median lethal concentration (LC_{50}) at 24-h (4.755 ppb) was high than that of 48-h (2.489 ppb). Regarding glyphosate, the LC_{50} was 19 ppm (24-h) and 13 ppm (48-h).

Sub-lethal effects

The percentage of females that hatched their eggs according to different treatments is presented in Table 4 respectively for cypermethrin and glyphosate. Hatching was very low in females exposed to 20 % LC_{50} of cypermethrin. Only 20 % of females have hatched their eggs against 60 % in the control and 10 % LC_{50} cypermethrin treatment. In the case of glyphosate contamination, the maximum hatching rate was 70 % in the control treatment. The lowest hatching was observed in the 20 % LC_{50} treatment.

Table 2 Lethal concentration ($LC_{1\ to\ 99}$) and 95 % confidence limits of cypermethrin to *Acanthocyclops robustus*

Point	**Concentration (ppb)**			
	24h		48h	
	Doses	95%CL		95%CL
LC_1	2.353	0.667 - 3.314	0.567	0.156 - 1.001
LC_{10}	3.228	1.853 - 3.964	1.102	0.466 - 1.632
LC_{20}	3.687	1.932- 4.464	1.457	0.734 - 2.015
LC_{30}	4.058	2.415 - 4.778	1.783	1.015 - 2.355
LC_{40}	4.405	2.912 - 5.080	2.119	1.332 - 2.705
LC_{50}	4.755	3.451 - 5.409	2.489	1.706 - 3.099
LC_{60}	5.134	4.048 - 5.817	2.924	2.160 - 3.59
LC_{70}	5.573	4.705 - 6.417	3.474	2.726 - 4.290
LC_{80}	6.134	5.389 - 7.495	4.251	3.454 - 5.472
LC_{90}	7.006	6.145 - 9.839	5.623	4.526 - 8.126
LC_{99}	9.610	7.745 - 20.352	10.929	7.698 - 23.230
Chi-square	10.96	Degrees of freedom: 2		*p* = 0.004

Table 3 Lethal concentrations ($LC_{1\ to\ 99}$) and corresponding 95 % confidence limits of glyphosate to *Acanthocyclops robustus*

Point	Concentration (ppm)			
	24h		48h	
	Doses	95%CL	Doses	95%CL
LC_1	5	1 - 8	8	5 - 10
LC_{10}	9	2 - 12	10	7 - 12
LC_{20}	12	6 - 15	11	8 - 12
LC_{30}	14	9 - 18	12	9 - 13
LC_{40}	16	12 - 22	12	10 - 14
LC_{50}	19	14 - 28	13	11 - 14
LC_{60}	22	17 - 39	14	12 - 15
LC_{70}	26	20 - 55	14	13 - 16
LC_{80}	31	23 - 86	15	14 - 17
LC_{90}	40	27 - 162	17	15 - 20
LC_{99}	73	40 - 751	21	18 - 28
Chi-square	28.08	Degrees of freedom: 2	p = 0.000	

Regarding the females which hatched their eggs, 100 % had died within 10 days of exposure to 20 % LC_{50} of cypermethrin (Table 4). Only 50 % of females had died under 10 % LC_{50} while in the control 33,33 % mortality was observed. Respective to the females which have not hatched their eggs, 100 % mortality was observed with 20 % LC_{50} of cypermethrin against 75 % and 50 % respectively for 10 % LC_{50} and the control. In case of glyphosate exposure (Table 4) 57.14 % of females which hatched their eggs was died in control treatment. In 10 %LC_{50} and 20 %LC_{50} treatment, 66.67 % and 80 % of died was observed respectively. For those which didn't hatch their eggs in glyphosate exposure, 100 % was died under 10 % LC_{50} while 66.67 % and 60 % was noted respectively in control and 20 % LC_{50} treatment.

The cumulative mortality of hatched nauplii during the study showed increase sensitivity to the exposure dose of cypermethrin (Fig. 1). The dose of 20 % LC_{50} in 48-h (0.4978 ppb) was more toxic to the nauplii of *A. robustus* during the 10 days of exposure. A high mortality rate was also observed in the absence of contaminants (control). Respective to glyphosate (Fig. 2), 10 % LC_{50} and 20 % LC_{50} induced more mortality in nauplii.

The average number of live nauplii obtained per female after 10 days of exposure is presented on Figures 3 and 4 respectively for cypermethrin and glyphosate. In cypermethrin exposure, the lowest average number of nauplii per female was obtained with 20 % LC_{50} (9.5 ± 3.5 nauplii/female). This mean number is significantly different from those obtained in the other two treatments (ANOVA 1, Tukey test, $p < 0.05$). The averages of 21.67 ± 5.8 nauplii/female and 24.17 ± 10.96 nauplii/female were observed in the control and 10 % LC_{50} respectively. For glyphosate, no significant difference was observed between treatments. 21.5 ± 7.6 nauplii/female was the highest survival nauplii obtained in glyphosate exposure after 10 days.

Table 4 Hatching rate and mortality of female of *Acanthocyclops robustus* under sub-lethal concentrations of cypermethrin and glyphosate.

		Control	10%LC_{50}	20%LC_{50}
Hatching (%)	**Cyperméthrin**	60	60	20
	Glyphosate	70	60	50
Mortality of females after hatching (%)	**Cyperméthrin**	33,33	50	100
	Glyphosate	57,14	66,67	80
Mortality of females without hatching (%)	**Cyperméthrin**	50	75	100
	Glyphosate	66,67	100	60

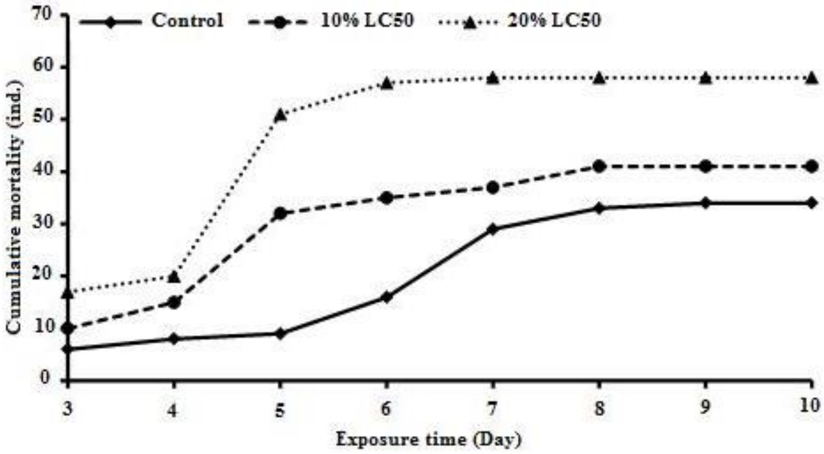


Fig. 1 Cumulative mortality of hatched nauplii other cypermethrin treatments

Discussion

The immobilization follow by mortality of *A. robustus* is higher when the exposure time is long and the concentration of cypermethrin or glyphosate is high. The increasing doses have therefore lead dysfunction of the nervous system causing paralysis. Cypermethrin's action has focused on interference with the functioning of the sodium channel in the central nervous system, stimulating the nervous discharges repeatedly, causing paralysis (SAgE pesticide, 2015). Christensen et al. (2005) reported that cypermethrin at concentrations greater than 0.1 ppb causes immobilization of crustacean zooplankton in general and in *Daphnia magna* particularly after respectively 6-h, 24-h, and in 48-h exposure.

Lethal doses determined showed that *A. robustus* is very sensitive to cypermethrin (Cyperforce®). Kuldeep *et al.* (2014) reported that cypermethrin is highly toxic to aquatic invertebrates. This high toxicity is observed on *A. robustus* with median lethal concentrations (LC_{50}) 24-h and 48-h respectively 4.755 ppb and 2.489 ppb. In case of glyphosate, 19 ppm and 13 ppm was LC_{50} to *A. robustus* at 24-h and 48-h exposure respectively. These results showed that cypermethrin is more toxic to *A. robustus* than glyphosate, as Golombieski *et al.* (2008) reported that insecticides are more toxic to aquatic organisms than other pesticides. Chris (2009) also reported that glyphosate is less toxic to fish than other pesticide as: methidathion, beta-cyfluthrin, endosulfan et carbendazine. Summarizing these observations, the sensitivity of aquatic species to a pollutant, is affected by both interspecific differences and the type of pollutant (see Tables 5 and 6). Takahashi *et al.* (2006) have also showed a large difference in sensitivity between life stages of specie exposed to same pollutant. They demonstrated that adult copepods are higher resistant to Diazinon and Carbaryl than nauplii. In the present paper, this aspect was not evaluated for *A. robustus* facing cypermethrin and glyphosate. But the high acute resistance observed by Takahashi *et al.* (2006) in adult copepods to Diazinon and Carbaryl, confirmed the interspecific and pollutant difference effect in sensitivity of aquatic invertebrates.

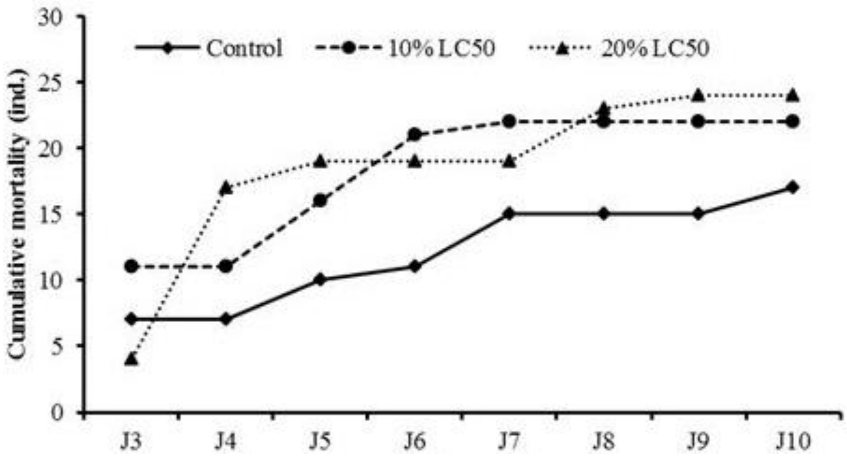


Fig. 2. Cumulative mortality of hatched nauplii other glyphosate treatments.

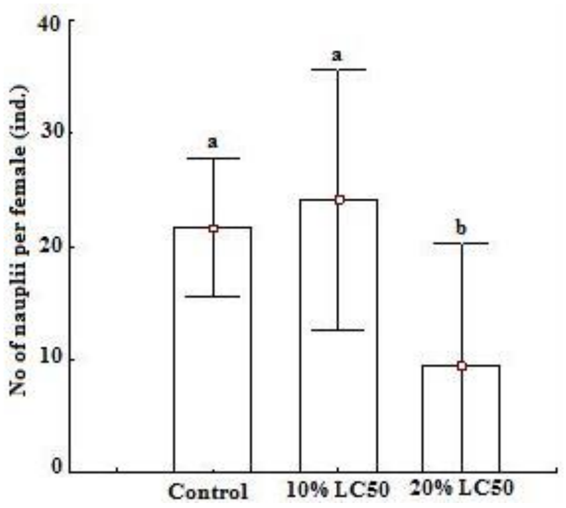


Fig. 3. Mean number of nauplii obtained per female of *Acanthocyclops robustus* after 10 days of exposure to different doses of cypermethrin. Plots having different letter are significantly different (Turkey, $p < 0.05$).

In aquatic ecosystems where shores are strongly used for agricultural production (as Oueme river), concentrations higher than LC_{50} estimated for *A. robustus* (especially for cypermethrin) can easily be exceed in water. It would result a strong change in its population and zooplankton community in general (Relyea, 2005). According to Sarkar *et al*. (2005), the 96-h LC_{50} of aquatic invertebrates to cypermethrin are generally between 0.01 and 5 ppb. The observations of this study at 24-h and 48-h of exposure fit into this range and confirm the high sensitivity of the species.

Regarding the glyphosate, the lethal dose observed is largely high than that of Roundup® (glyphosate) to the callanoïda *Phyllodiaptomus annae* (1.06 ppm) (Ashoka-Deepananda *et al*., 2011). The difference may be due to the difference in used formulation of glyphosate (Kumark® in the present study) also in the genetic difference between the two species of copepod (table 6). However, the department of agriculture of UAS has reported that lethal doses of glyphosate Roundup® to aquatic invertebrates varied between 4 and 37 ppm (Ashoka-Deepananda *et al*., 2011).

The chronic effects tests showed that chronic exposure of *A. robustus* at sub-lethal doses of cypermethrin and glyphosate affects reproduction and survivorship of nauplii. In fact, chronic exposure to both pesticides caused mortality in ovigerous females before and after hatching (reproduction). Hatching is strongly affected and survivorship of nauplii is also affected. The results confirm those of Hanazato (2001) which showed that pesticides can affect population dynamics of freshwater zooplankton by reducing their survival rates, by affecting the eggs hatching and reducing their richness and specific diversity. Lina *et al*. (2003) also showed zooplankton population reduction in the presence of cypermethrin.

Ratushnyak *et al*. (2005) showed that cypermethrin at concentrations above 0.002 ppb to 0.2 ppb does not affect the survival of freshwater's invertebrates after 21 days of exposure. Lutnicka *et al*. (2014) also showed that a concentration of 0.02 ppb of cypermethrin has no observable toxic effect on reproduction and growth of freshwater organisms. This justified the fact that a concentration of 0.2489 ppb (10% LC_{50}, 48-h), had no significant effect neither on reproduction neither on the survival of *A. robustus* after 10 days of exposure. Significant effects were then obtained with a concentration of 0.4978 ppb (20 % LC_{50}, 48-h) after 10 days of exposure. Contrary to Ratushnyak *et al*. (2005), Wendt-Rasch *et al*. (2003) have previously reported that cypermethrin causes a reduction in zooplankton population at concentrations greater than 0.13 ppb during 11 days of exposure. Also Lina *et al*. (2003) showed that copepods facing concentrations of cypermethrin greater than 0.03 ppb, presented firstly ovigerous females mortality and secondly an increase egg hatching rate among survivors. This demonstrates the variation of species responses to a single pollutant whether the environments are different.

After 21 days of exposure of *Daphnia magna* to different concentration (0.26 and 0.38 ppm) of glyphosate, no effect was observed on neonates' survivorship and growth, but the reproduction has affected with increasing concentration (Maycock *et al*., 2010). On freshwater copepods, concentration

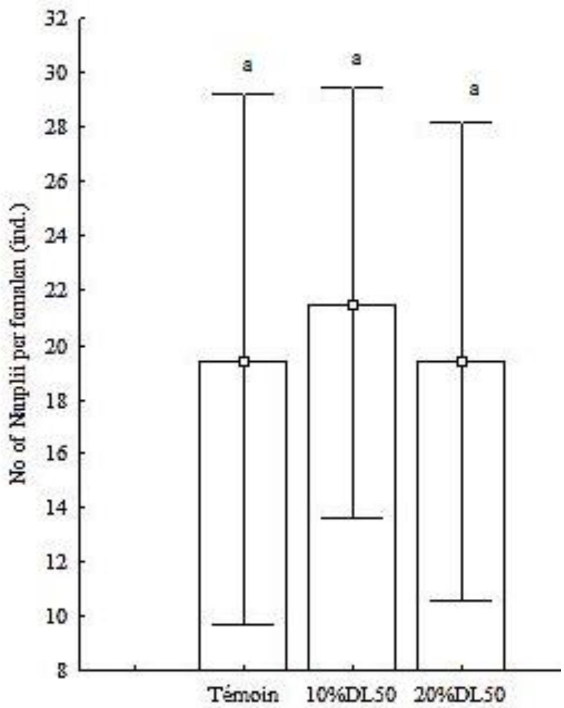


Fig. 4 Mean number of nauplii obtained per female of *Acanthocyclops robustus* after 10 days of exposure to different doses of glyphosate. Plots having different letter are significantly different (Turkey, $p < 0.05$).

Table 5 Comparative toxicity of cypermethrin in different formulation to different planktonic crustacean species

Species	Cypermethrin formulation	Acute toxicity		Chronic effects		References
		Exposure time	Toxicity LC_{50} (ppb)	Concentration (ppb)	Effects	
Ceriodaphnia dubia **(cladoceran)**	Pestanal®	48-h	0.23	0,0978	Affected growth of neonate	Shen *et al.* (2012)
Daphnia magna **(cladoceran)**	Pestanal®	48-h	0.72			Ashauer *et al.* (2011)
Daphnia magna **(cladoceran)**	Cypermethrin (CAS no.52315-07-8)			≥0.1	Significant reduction of the swimming ability.	Christensen *et al.* (2005)
Daphnia schoedleri **(cladoceran)**	Pestanal®	48-h	0,6	0.0005, 0.0054 and 0.054	All population parameters were significantly reduced. Reduction in reproduction is observed	Martínez-Jerónimo *et al.* (2013)
Diaptomus forbesi **(copepod)**	Pestanal®	48-h	0.03			Saha and Kaviraj (2008)
Acartia clause **(copepod)**	Pestanal®	48-h	2.67			Willis and Ling (2004)
Pseudocalanus elongates **(copepod)**		48-h	>5			
Acanthocyclops robustus **(copepod)**	Cyperforce®	48-h	2.49	0.25	Reduced egg hatching in ovigerous females Reduced nauplii survivorship	Present study

greater than 0.01 ppm shouted reduce eggs hatchability (Maycock *et al.*, 2010). In the present study, *A. robustus* exposed to chronic doses 1.3 ppm and 2.6 ppm (respectively 10% and 20% of LC_{50} in 48-h) of glyphosate has presented any significant effects on hatching rate. Significant effect on female survival after eggs hatching at 10% LC50 exposure (may be due to physical treatments). It is then observed a high resistance of *A. robustus* to sub-lethal doses of glyphosate.

Conclusion

A. robustus, a copepod species present in Ouémé River ecosystem, appeared very sensitive to cypermethrin. The median lethal concentration (LC_{50}) is 4.755 ppb in 24 h and 2.489 ppb respectively in 48-h. This specie was less sensitive to glyphosate with respective LC_{50} of 19 ppm and 13 ppm. Low concentrations rang of 0.2489 ppb and 0.4978 ppb of cypermethrin affected it reproduction by reducing egg hatching. The survival of ovigerous females and nauplii in the early development stages was also affected. Regarding glyphosate, exposure to sub-lethal dose as 2.6 ppm also reduced survival factors of the species. Low levels of cypermethrin in the aquatic environment can therefore significantly affect aquatic biodiversity. Glyphosate in fact is less toxic for aquatic invertebrates.

Table 6 Comparative toxicity of glyphosate in different formulation to different planktonic crustacean species

Species	Glyphosate formulation	Acute toxicity		Chronic effects		References
		Exposure time	Toxicity LC_{50} (ppm)	Concentration (ppm)	Effects	
Daphnia magna **(cladoceran)**	Glyphosate IPA	48-h	31	4.05	Significant reduction in survival Significant reduction of body size Significant decrease of fecundity	Cuhra *et al.* (2013)
Simocephalus vetulus **(cladoceran)**	Eskobat®	48-h	21.5	≥6.4	Age at first reproduction was delayed 2 to 4 days number of neonates/females was significantly reduced	Reno *et al.* (2014)
Notodiaptomus conifer **(Copepod)**	Eskobat®	48-h	95.2	80 ≥160	Significant delayed of the sexual maturity *N. conifer* could not reach the adult stage	Reno *et al.* (2014)
Phyllodiaptomus annae **(Copepod)**	Roundup®	48-h	1.06			Ashoka-Deepananda *et al.* (2011)
Acanthocyclops robustus **(copepod)**	Kumark® (480 g/L)	48-h	13	2.6	Reduced nauplii survivorship	Present study

Acknowledgement

We are grateful to the West Africa Agricultural Productivity Program (WAAPP) which founded this research. We thank all people who have contributed on any way to the effectiveness of this study. We also thank any reviewer who contributed to the scientific level of the paper.

References

Adégbidi A. Etude des Filières des Intrants Agricoles au Bénin: Engrais minéraux, produits phytosanitaires, semences, matériels et équipements agricoles, fertilisants organiques, (Tome 3). Presse de la FSA: Cotonou, 2000.

Agadjihouèdé H, Bonou CA, Montchowui E, Lalèyè PH. Recherche de la dose optimale de fiente de volaille pour la production spécifique de zooplancton à des fins piscicoles. Cahiers Agricultures 20: 247-260. 2011.

Agbohessi TP, Imorou Toko I, Yabi AJ, Dassoundo-Assogba JFC, Kestemont P. Caractérisation des pesticides chimiques utilisés en production cotonnière et impact sur les indicateurs économiques dans la Commune de Banikoara au nord du Bénin. Int. J. Biol. Chem. Sci. 5: 1828-1841. 2011.

Ashauer R, Hintermeister A, Potthoff E, Escher BI. Acute toxicity of organic chemicals to *Gammarus pulex* correlates with sensitivity of *Daphnia magna* across most modes of action. Aquat. Toxicol. 103: 38-45. 2011.

Ashoka-Deepananda KHM, Gajamange D, De Silva WAJP, Wegiriya HCE. Acute toxicity of a glyphosate herbicide, Roundup®, to two freshwater crustaceans. J.Natn.Sci.Foundation Sri Lanka 39 (2): 169-173, 2011.

Chris LS. Ecotoxicological endpoints for Fish and other aquatic organisms. Chemical Review Work Order CR294-1, 2009.

Christensen BT, Lauridsen TL, Ravnh W, Baylwy M. A comparison of feeding efficiency and swimming ability of *Daphnia magna* exposed to cypermethrin. Aquat. Toxicol. 73: 210-220, 2005.

Cuhra M, Traavik T, Bøhn T. Clone-and age-dependent toxicity of a glyphosate commercial formulation and its active ingredient in *Daphnia magna*. Ecotoxicology 22: 251-262, 2013.

Druart C. Effets des pesticides de la vigne sur le cycle biologique de l'escargot dans divers contextes d'exposition. Thèse de Doctorat, spécialité: Environmental Sciences. Université de Franche-Comté, 327p, 2011.

Fadoegnon B, Midingoyi S. Produire du coton de meilleure qualité. Référentiel technico-économique de la production agricole, ProCGRN-INRAB, 2006.

Golombieski JI, Marchesan E, Baumart JS, Reimche GB, Júnior CR, Storck L, et al. Cladocers, copepods and rotifers in rice-fish culture handled with metsulfuron-methyl and azimsulfuron herbicides and carbofuran insecticide. Cienc Rural 38: 2097-2102, 2008.

Hanazato T. Pesticide effects on freshwater zooplankton: an ecological perspective. Environ Pollut. 112: 1-10, 2001.

Haraguchi K, Kitamura T, Yamashuta T, Kido A. Simultaneous determination of traces pesticides in urban air. Atmosphere Environ. 28: 1319-1325, 1994.

Houssou AM, Agadjihouédé H, Montchowui E, Bonou CA, Lalèyè P. Structure and seasonal dynamics of phytoplankton and zooplankton in Lake Azili, small Lake of the pond of River Ouémé, Benin. Int. J. Aquat. Biol. 3: 161-171, 2015.

Houssou AM, Yaovi R, Ahouansou Montcho S, Bonou CA, Montchowui E. Diversity and seasonal variation of zooplankton of Lake Hlan, Republic of Bénin (West Africa). J. Appl. Biosci. 102: 9723-9737, 2016.

Kao C, Vernet G, Le Filleul JM, Nedelec Y, Carluer N. Elaboration d'une méthode de typologie des fossés d'assainissement agricole et de leur comportement potentiel vis-à-vis des produits phytosanitaires. Ingénieries-EAT. 49-65, 2002.

Kuldeep KS, Arti S, Hiteshi J, Neha S, Amandeep K. Toxic effects (by LC_{50} analysis) of cypermethrin on a Cyclopoid copepod, *Mesocyclops leuckarti* (Claus, 1857) Int. J. Recent Sci. Res. 5: 215-217, 2014.

Lalancette A. Méthodes de lutte à la contamination des eaux de surface en montérégie par les pesticides agricoles. Mémoire maîtrise, Université de Sherbrooke, 122p., 2012.

Lévèque C, Paugy D. Les poissons des eaux continentales africaines : Diversité, écologie, utilisation par l'homme IRD Éditions, 573p, 2006.

Lina WR, Ursula FJ, Per W, Kirsten C. Effects of the pyrethroid insecticide cypermethrin on a freshwater community studied under field conditions. II. Direct and indirect effects on the species composition. Aquat. Toxicol. 63: 373-389, 2003.

Lutnicka H, Fochtman P, Bojarski B, Ludwikowska A, Formicki G. The influence of low concentration of cypermethrin and deltamethrin on phyto- and zooplankton of surface waters. Folia Biol. (Kraków) 62: 251-257, 2014.

Martínez-Jerónimo F, Arzate-Cárdenas M, Ortiz-Butrón R. Linking sub-individual and population level toxicity effects in *Daphnia schoedleri* (Cladocera: Anomopoda) exposed to sublethal concentrations of the pesticide α-cypermethrin. Ecotoxicology, 22(6): 985-995. 2013.

Maycock D, Crane M, Atkinson C, Johnson I. Water Framework Directive United Kingdom Technical Advisory Group. SNIFFER 25 Greenside Place Edinburgh EH1 3AA Scotland, 2010.

Pesce S, Fajon C, Bardot C, Bonnemoy F, Portelli C, Bohatier J. Longitudinal changes in microbial planktonic communities of french river in relation to pesticide and nutients input. Aquat. Toxicol. 86: 352-360, 2008.

Ratushnyak AA, Andreeva MG, Trushin MV. Influence of the pyrethroid insecticides in ultralow doses on the freshwater invertebrates. Fres. Environ. Bull. 14: 823-834, 2005.

Relyea RA. The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. Ecol. Appl. 15: 618-627, 2005.

Reno U, Gutierrez MF, Regaldo L, Gagneten AM. The Impact of Eskobat, a Glyphosate Formulation, on the Freshwater Plankton Community. Water Environ. Res. 86: 2294-2300. 2014.

SAgE pesticide. Caractéristiques des produits commerciaux. Available at www.sagepesticides.qc.ca/ Recherche/RechercheProduits.aspx (accessed le 25 juillet 2015).

Saha S, Kaviraj A. Acute toxicity of synthetic pyrethroid cypermethrin to some freshwater organisms. Bull. Environ. Contam. Toxicol. 80: 49-52. 2008.

Sarkar S, Justus J, Fuller T, Kelley C, Garson J, Mayfield M. Effectiveness of environmental surrogates for the selection of conservation area networks. Cons. Biol. 19: 815-825, 2005.

Shen MF, Kumar A, Ding SY, Grocke S. Comparative study on the toxicity of pyrethroids, α-cypermethrin and deltamethrin to *Ceriodaphnia dubia*. Ecotox. Environmental Safe. 78: 9-13, 2012.

Takahashi H, Chang KH, Hanazato T. Acute Toxicity of the Insecticide Diazinon and Carbaryl to Calanoid and Cyclopoid Copepoda (*Eodiaptomus*, *Mesocyclops* and *Thermocyclops*) in Different Life Stages. Japanese J, Environ. Toxicol. 9: 133-139, 2006.

United States Environmental Protection Agency (USEPA). Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 5a ed (p. 266). NW. Washington, DC: US Environmental Protection Agency, Office of Water (4303T). 20460. EPA-821-R-02-012, 2002.

Wendt-Rasch L, Friberg-Jensen U, Woin P, Christo-Ffersen K. Effects of the pyrethroid insecticide cypermethrin on a freshwater community studied under field conditions. II. Direct and indirect effects on the species composition. Aquat. Toxicol. 63: 373-389, 2003.

Willis KJ, Ling N. Toxicity of the aquaculture pesticide cypermethrin to planktonic marine copepods. Aquaculture Res. 35: 263-270. 2004.

Yèhouénou APE, Boko M, Cornelis AM, Gestel V, Ahissou H, Lalèyè P, *et al*. Contamination of fish by organochlorine pesticide residues in the Ouémé River catchment in the Republic of Bénin. Environ. Int. 32: 594-599, 2006a.

Yèhouénou APE, Boko M, Cornelis AM, Gestel V, Ahissou H, Lalèyè P, et al. Organochlorine and organophosphorous pesticide residues in the Ouémé River catchment in the Republic of Bénin. Environ. Int. 32: 616-623, 2006b.