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ORIGINAL RESEARCH

Exposure to agricultural pesticides impairs growth, feed utilization and energy budget in African Catfish *Clarias gariepinus* (Burchell, 1822) fingerlings

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Abstract The African catfish *Clarias gariepinus* is a widespread species in the Beninese cotton basin. In this study, the impacts of exposure to agricultural pesticides endosulfan [Thionex 350 EC (emulsifiable concentrate)] and Tihan 175 O-TEQ (oil toxicity equivalent) on growth, feed utilization and energy budget of *C. gariepinus* were investigated. Fingerlings (1.58 ± 0.02 g) were exposed to borehole water (control), 0.23 ppb (environmental concentration), 440 ppb (Lethal Concentration 50 %/20, LC50/20) and 880 ppb (LC50/10) of Tihan; and to 11 ppb (LC50/20), 22 ppb (LC50/10) and 29.40 ppb (environmental concentration) of Thionex for 28 days. Fish biomass was assessed weekly and fish samples were taken from different aquaria to determine the specific growth rate (effect on growth), feed efficiency rate and protein efficiency ratio (impact on feed utilization), and the biochemical composition of fish (impact on the energy budget). The results showed that endosulfan environmental concentration induced 100 % of mortality in catfish fingerlings while mortality rate was comparable between control fish and Tihan-treated fish over the 28-day period ($p > 0.05$). In contrast to survival, the two pesticide types tested induced a marked decrease in growth only during the first 2 weeks of exposure ($p < 0.05$). The negative impact of endosulfan on growth was associated to a lower feed utilization and protein efficiency compared with control fish or those exposed to Tihan ($p < 0.05$). The energy reserves were more rapidly exhausted in fish exposed to endosulfan to meet energy demand generated by this chemical stressor.

Keywords African catfish · Endosulfan and Tihan · Growth parameters · Energy budget

Introduction

The growing use of pesticides in agriculture significantly contributes to environmental pollution. It is currently estimated that the contribution of pesticides to pollution is comparable to emissions from industrial sources (Velisek et al. 2012). Only 0.1 % of the sprayed pesticides reaches the target pests, the rest being distributed into the ecosystems where it contaminates the land, water and air (Primentel and Levitain 1986). All these pollutants finally reach aquatic ecosystems (Gillium 2007; Chao et al. 2009). The biocides contaminate fish by

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integumentary, gill road or through food (Mackay and Fraser 2000; Wang et al. 2005; Kan and Meijer 2007; Singh and Singh 2007). Even at non-lethal doses, these products are susceptible to disrupt the nervous system, liver device, hormonal regulation, reproduction, embryonic development and growth of fish (Barse et al. 2007; Singh and Singh 2007, 2008; Palma et al. 2008, 2009a, b).

As in other countries of the West African sub-region, the cotton culture in Benin is associated to the use of huge quantities of pesticides. Recommended pesticides are not necessarily those used. Agbohessi et al. (2011) reported that endosulfan is the pesticide used the most extensively (75 %) in the Benin cotton basin through Thionex 350 EC (51 %) and Cotofan 350 EC (24 %). The use of endosulfan has been banned in Benin since 2007 due to various nuisances on the environment and several cases of human poisoning detection by the Ministry of Health (Mbaye 2008). Endosulfan was replaced by Tihan 175 O-TEQ, a mixture of flubendiamide (100 g/L) and spirotetramat (75 g/L) which represents only 1.70 % of the pesticides applied in cotton culture (Agbohessi et al. 2011). During the cotton season 2011–2012, Beninese authorities decided to subsidize Tihan to accelerate its adoption by the cotton producers, resulting in the coexistence of endosulfan and active ingredients of Tihan in aquatic ecosystems.

Endosulfan (6,7,8,9,10,10-hexachloro1,5,5a,6,9,9a-hexahydro-6,9-methano-2,3,4-benzodio xathiepine-3-oxide, CAS No. 115-29-7) is an organochlorine pesticide considered among the most hazardous with respect to environmental pollution, since it is highly persistent, non-biodegradable and capable of bio-magnification as its moves up along the food chain (Pandey et al. 2006). Endosulfan has been reported highly toxic to fish and shellfish (Kamrin 1997). Its exposure, even at sub-lethal doses, can cause behavioral and biochemical changes in fish, and hence death (Shafiq-ur- 2006). Several authors have demonstrated that endosulfan causes reduction in the growth performance and survival of different tropical fish species (Petri et al. 2006; Sarma et al. 2009; Beyger et al. 2012).

Tihan 175 O-TEQ belongs to a new generation of insecticides that are considered as more environmental friendly than the previous generations of biocide chemicals. Flubendiamide, N2-[1,1-dimethyl-2-(methyl-sulfonyl)ethyl]-3-iodo-N1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl) ethyl] phenyl]-1,2-benzene dicarboxamide, belongs to phthalic acid diamide group (Das and Mukherjee 2011). Chronic exposure of rodents to flubendiamide has shown its negative impact on growth (Justus et al. 2007). Females have generally a higher sensitivity to flubendiamide than males (APVMA 2009). Toxicity studies on fathead minnow *Pimephales promelas* have indicated a $NOEC\text{-}_{21\ day}$ of 60 μg/L (Australian Pesticides and Veterinary Medicines Authority 2009). Spirotetramat (cis-4-(ethoxycarbonyloxy)-8-methoxy-3-(2, 5-xylyl) -1-azaspiro [4.5] dec-3-en-2-one) belongs to the chemical class of ketoenols (Mohapatra et al. 2012). In the same way, an assessment of the toxicity of this compound on fathead minnow has reported adverse effects on growth, with a $NOEC_{33\ days} = 1.16$ mg/L (PRD 2008).

To our knowledge, there were no published papers on the chronic effects of Tihan on any tropical fish species. The present work aimed to determine, under experimental conditions, the chronic effects of the key pesticides used in Benin, i.e. endosulfan through Thionex 350 EC and Tihan, on growth of African catfish *Clarias gariepinus* fingerlings. This species is one of the major economical fish species in the Benin cotton basin (Lalèye et al. 2004).

Materials and methods

Chemicals

Tihan 175 O-TEQ and Thionex 350 EC were purchased from the "*Société de Distribution des Intrants (SDI)*" (Benin). Tihan is a milky-white liquid and Thionex a light yellow-colored liquid, both stored at ambient temperature. Water used for the preparation of test solutions was analyzed for quality (nitrate 24.17 ± 0.01 mg/L, nitrite 0.03 ± 0.01 mg/L, total hardness 81.0 ± 0.3 mg/L). Tihan and Thionex stock solutions were dissolved in water without a carrier solvent. A stock solution is generally manufactured and the test solution is obtained by dilution of this stock solution according to the desired final solution. All working stock solutions were made immediately prior the tests.

Biological material

African catfish fingerlings were bought from Royal Fish Benin S.A., where they were produced artificially from parents reared in recirculating water conditions. Fish were acclimated during 12 days in plastic tanks (120 L) at the stocking density of one fish L^{-1} according to OECD guideline 203, at the Research Unit in Aquaculture and Aquatic Ecotoxicology, University of Parakou, Benin. They were fed twice daily at 4 % of their biomass still 24 h before the beginning of the test with a commercial dry feed (Coppens pellets of 2 mm, 45 % crude proteins, 12 % crude fat, The Netherlands).

Experimental design and handling

The experiment was conducted according to OECD guidelines 215 with some modifications. Twenty-one aquaria (volume = 50 L) were used for seven conditions with three replicates per treatment. Few minutes after the preparation of experiment solution, 20 fingerlings (1.58 ± 0.02 g), i.e. fish in their exponential growth phase were carefully counted, weighed and placed into each tank at the stocking density of 1 g of fish/L. Fish were fed at apparent satiation five times daily at 5 % of their biomass with a commercial dry feed Coppens pellets of 2 mm (the Netherlands) during the experiment. According to the manufacturer's data, the feed contained 45 % crude proteins, 12 % crude fat and 9.5 % ash. The experiments were conducted in semi-static systems with renewal of 75 % of the aquarium solution each 48 h after checking of water quality parameters by standard methods. The experiments lasted 28 days (4 weeks). The food that was not served by aquarium was weighed at the end of each feeding day. Uneaten food and feces were siphoned upon renewal of experiment solutions. Temperature (27.2 ± 0.6 °C), pH (7.1 ± 0.2) and dissolved oxygen (5.6 ± 0.7 mg/L) were measured daily in each aquarium. During the experiment, the photoperiod was maintained at LD 12:12.

The control and the pesticide treatments were run simultaneously (day 0, D0). The nominal concentrations tested were as follows:

- C = control, borehole water,
- Tihan: TA-E = 0.23 ppb (environmental concentration), TA-1 = $LC50_{-96h}/20$ = 440 ppb and TA-2 = $LC50_{-96h}/10$ = 880 ppb,
- Thionex: TE-E = 29.4 ppb (environmental concentration), TE-1 = $LC50_{-96h}/20$ = 11 ppb and TE-2 = $LC50_{-96h}/10$ = 22 ppb.

$LC50_{96h}$ is the concentration of each pollutant that kills 50 % of the fish within 96 h. $LC50_{Tihan}$ = 8.79 ppm and $LC50_{Thionex}$ = 0.22 ppm (Agbohessi et al. 2013). Environmental dose of endosulfan represents the average concentrations obtained after dosing water at various sites of the Beninese cotton basin watercourses. Note that TE-E is greater than TE-1 and TE-2.

As mentioned previously (Agbohessi et al. 2013), nominal concentrations were not confirmed by chemical analyses. Precise measurement of the actual concentrations was considered to be of minor importance in these series of increasing concentrations. In addition, the half-lives of flubendiamide (20.2 days; Australian Pesticides and Veterinary Medicines Authority 2009) and spirotetramat (8.6–47.6 days; PRD 2008; U.S. EPA 2008) in neutral and acidic environments are greater than the water-renewal times applied in the present experiment. Furthermore, the active components are not highly volatile (vapor pressures: flubendiamide $<10^{-4}$ Pa; spirotetramat 5.6×10^{-9} Pa). Endosulfan is stable at ambient temperature (CCME 2010). Although its vapor pressure (0.83 mPa at 20 °C) indicates that it is semi-volatile (Tomlin 2000), its half-life of 4–7 days in natural water is greater than the water-renewal time used here. We therefore did not expect a significant quantity of these compounds to be lost by volatilization during the study.

Growth and feed utilization data collection

The fish were individually weighed at the beginning of the experiment, after 7 days (week 1), 14 days (week 2), 21 days (week 3) and at the termination (week 4) of the experiment. The biomass was always determined by weighing all fish from each aquarium. The data were used to calculate:

- Mortality rate: 100. (fish dead). Total fish^{-1}
- Average weight: (P, g) = B.n^{-1}
- Specific growth rate: (SGR, % d^{-1}) = 100(ln Bw_2 − ln Bw_1). Δt^{-1}
- Feed efficiency (FE): =TFS (FB − IB)$^{-1}$
- Protein efficiency ratio: PER = (FB – IB). FPS^{-1}

where B is biomass, n total number, $Bw_{1,2}$ initial and final body weight (g); Δt, duration of the experiment (days); IB and FB, initial and final stock biomass (g), respectively; TFS, total feed served (g); FPS, feed protein served.

At the onset of the experiment, a sample of 10 fish (initial sample) was collected. Every 7 days until the end of the experiment, 9 fish per concentration (3 fish/aquaria) were sampled and stored at −20 °C. At the end of the experiment, fish were separately mashed and homogenized. A portion of each sample of homogenate was frozen and brought back to Belgium for analysis of protein and fat content and the remaining material was used in Benin for the determination of glycogen content to evaluate energy reserves.

Available energy reserves

Whole-body protein and lipid contents were measured in the laboratory of the Research Unit in Environmental and Evolutionary Biology, Namur, Belgium by Kjeldahl and Soxlet methods respectively. Whole-body glycogen content was determined with the Anthrone reagent by spectrophotometry at a wavelength of 620 nm (Roe and Dailey 1966) in laboratory of Regional Institute of Industrial Engineering Biotechnology and Applied Sciences in Cotonou (Benin). Glycogen concentrations were calculated by means of a standard curve of glycogen. Changes in body composition were expressed as changes in energy budget per day and were calculated using the formula:

$$A_x = \frac{(T_x - T_{x-1})(Y_x - Y_{x-1})/2 + (T_x - T_{x-1})(Y_{x-1} - Yo)}{T_x}$$

with T_x being the time x and Y_x the composition (glycogen, lipid, protein) at time x. This approach allows the quantification of changes in energy budget between different exposure regimes and periods. When calculating energetic values, an enthalpy of combustion of 17 kJ/g for glycogen, 39.5 kJ/g for lipids, and 24 kJ/g for proteins was used (Jobling 1994). Using these values, the different energy sources may be summed up to give the changes in whole-body energy budget.

Statistical treatment

The experimental unit is the aquarium. The results were expressed as mean ± standard deviation of the mean. For each sampling time point and each parameter studied (mortality, P, SGR, FE, PER, total glycogen budget, total protein budget, total lipid budget, etc.), differences between means were evaluated by a two-way analysis of variance (ANOVA II) with pesticide and dose as factors. Whenever significant differences were revealed, Duncan's multiple range tests were applied. The statistical calculations were performed using Statistica Software® (StatSoft,Tulsa, OK, USA). P value of 0.05 or less was considered significant.

Results

Mortality rate

All fish (100 %) exposed to the TE-E dose (29.4 ppb) died from D0 to D12 ($p < 0.05$) (Fig. 1). Fish exposed to low endosulfan doses TE-2 and TE-1 showed different mortality rates when compared with control fish at week 1 and week 2 for TE-2 and week 3 for TE-1. But, whatever the dose, fish exposed to Tihan showed mortality rates similar to those of control fish.

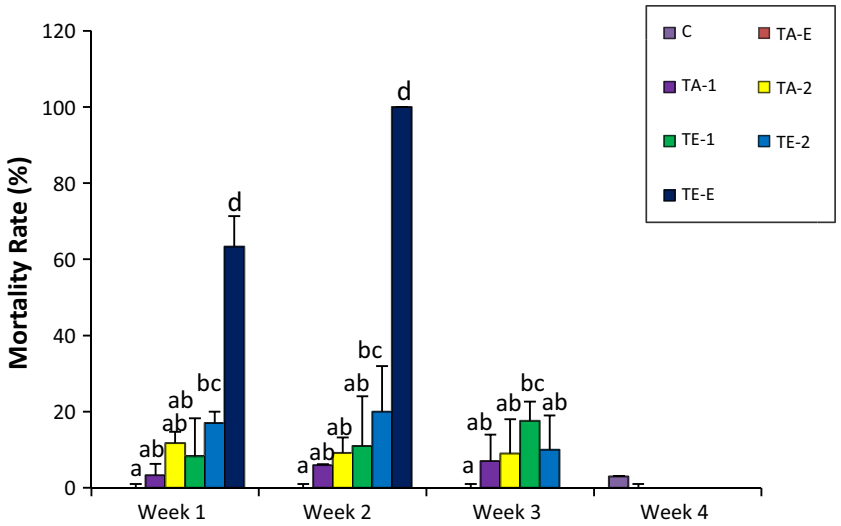

Fig. 1 Impact of increasing doses of endosulfan (TE-E = 29.4 ppb, TE-1 = 11 ppb, TE-2 = 22 ppb) and Tihan (TA-E = 0.23 ppb, TA-1 = 440 ppb, TA-2 = 880 ppb) on mortality rates of fingerlings of African catfish. Data are given as mean ± SD; $n = 3$. In each sampling time point values having different letters are significantly different at $p < 0.05$

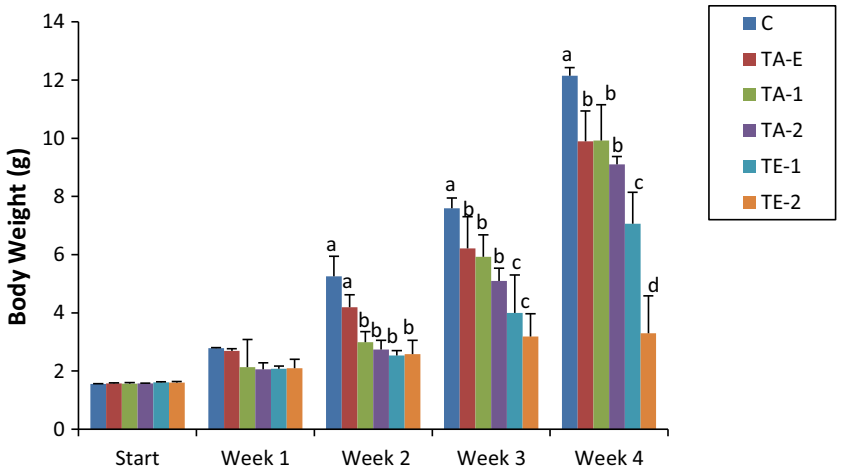

Fig. 2 Impact of increasing doses of endosulfan (TE-1 = 11 ppb, TE-2 = 22 ppb) and Tihan (TA-E = 0.23 ppb, TA-1 = 440 ppb, TA-2 = 880 ppb) on body weight of fingerlings of African catfish. Data are given as mean ± SD; $n = 3$. In each sampling time point values having different letters are significantly different at $p < 0.05$

Fish growth and feed efficiency

The tested pesticides were found to significantly affect the growth of fingerlings (Figs. 2, 3). The specific growth rates of control fish ranged from 5.25 to 9.10 %/d. During the first 2 weeks, except for fish exposed to the lowest dose of Tihan (TA-E), fish from other treatments displayed SGR significantly lower than in control groups ($p < 0.05$). From the 2nd week of exposure, SGR of TE-1-exposed fish increased to further decrease toward the termination of the test. However, SGR of TE-2 exposed fish did not change at the end of the 3rd week but fell toward the end of the test. SGR of the other Tihan treatments was high, almost until the end of the 4th week. At the end of the 3rd week, SGR of fish in treatments TA-1 and TA-2 even exceeded that of the control groups ($p < 0.05$). At the end of the test, Tihan mean body weight of fish belonging to the different Tihan treatments was similar ($p > 0.05$) and significantly higher than those of the Thionex treatments ($p < 0.05$). Mean weight of control fish was significantly higher than in all other treatments ($p < 0.05$).

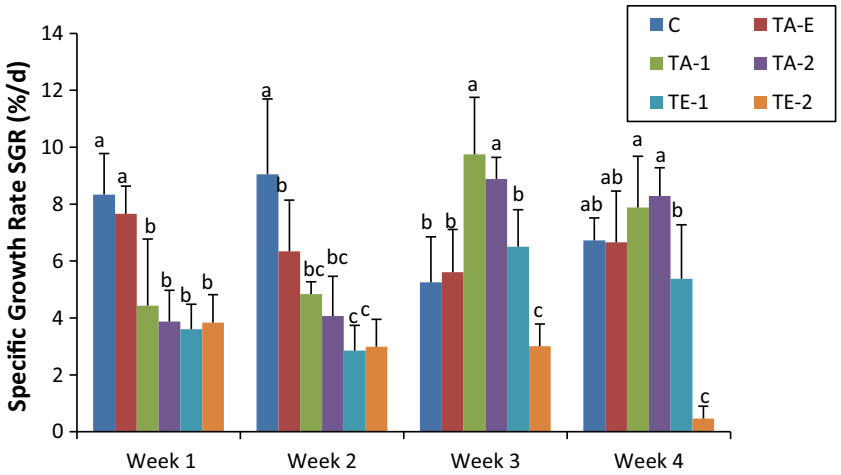

Fig. 3 Impact of increasing doses of endosulfan (TE-1 = 11 ppb, TE-2 = 22 ppb) and Tihan (TA-E = 0.23 ppb, TA-1 = 440 ppb, TA-2 = 880 ppb) on specific growth rate of fingerlings of African catfish. Data are given as mean ± SD; $n = 3$. In each sampling time point values having different letters are significantly different at $p < 0.05$

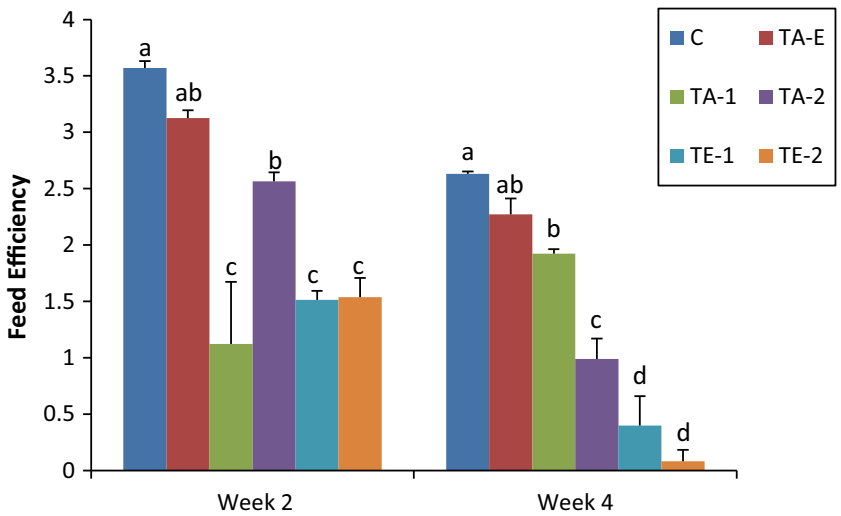

Fig. 4 Impact of increasing doses of endosulfan (TE-1 = 11 ppb, TE-2 = 22 ppb) and Tihan (TA-E = 0.23 ppb, TA-1 = 440 ppb, TA-2 = 880 ppb) on Feed Efficiency of fingerlings of African catfish. Data are given as mean ± SD; $n = 3$. In each sampling time point values having different letters are significantly different at $p < 0.05$

Food efficiencies FE (Fig. 4) of the control fish ranged from 2.63 to 3.57 and were significantly greater than that of all treatments during the test. During the first 2 weeks, FE values of fish exposed to Thionex remained statistically similar to that of TA-1 fish ($p > 0.05$). But in the last 2 weeks, FE values of fish exposed to Thionex became significantly lower than those of Tihan ($p < 0.05$). Throughout the test, the protein efficiency ratio (Fig. 5) of fish exposed to the two doses of Thionex remained lower than that of all fish belonging to the Tihan treatments ($p < 0.05$). PER of fish in the Tihan treatments was also lower ($p < 0.05$) than that of controls (7.0–8.2).

Changes in energy budget

At the beginning of the experiment, the average whole-body energy content of the fish based on the sum of glycogen, lipid and protein energy content was 3676 J/g dry weight (DW). This whole-body energy consisted of 19.4 % glycogen, 29.0 % lipids and 51.6 % protein (DW). The glycogen content of the exposed fish was

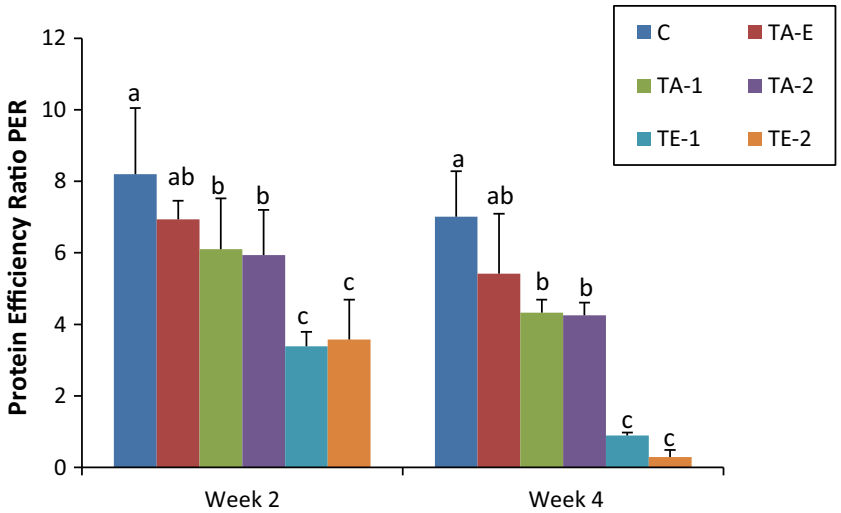

Fig. 5 Impact of increasing doses of endosulfan (TE-1 = 11 ppb, TE-2 = 22 ppb) and Tihan (TA-E = 0.23 ppb, TA-1 = 440 ppb, TA-2 = 880 ppb) on PER of fingerlings of African catfish. Data are given as mean ± SD; $n = 3$. In each sampling time point values having different letters are significantly different at $p < 0.05$

significantly low compared to the control, from the start of the experiment until the end, except in TA-E where the difference with the control was significant from the 3rd week only. There was an increase in glycogen content from week 3 in all Tihan treatments (TA-E, TA-1 and TA-2) as well as in the lowest concentration of endosulfan (TE-1) (Fig. 6a). A significant effect of pesticide exposure on the total lipid content of fish was observed in the different treatments (Fig. 7a). Fish exposed to Thionex contained much less lipids than those exposed to the different doses of Tihan from the 3rd week and a trend of difference was also observed at the end of the experiment between TE-1 and TE-2. The difference between Tihan and Thionex treatments was also observed in the total protein content of fish (Fig. 8a). However, protein levels edged up early in all treatments, and from the 3rd week, this increase was greater in fish from all Tihan treatments and TE-1 compared to the control. During this time it fell in fish TE-2 at the end of the experiment compared to control and other treatments.

The effects of Tihan and Thionex on the absolute values of energy content are rather confused. However, if the changes in energy content are integrated over time the resulting energy budget provides much clearer results. The glycogen budget dropped after 1-week exposure to all pesticide treatments (Fig. 6b). Its level in TA-E treatment fish was low compared to control while, in other treatments, it remained deficit throughout the duration of the test. The effects of pesticide exposure on the glycogen budget of fish were already highly significant for TA-1, TA-2, TE-1 and TE-2 after 1-week experiment. In all treatments, the lipid budget increased from the start to the end of the experiment. However, from the 3rd week onwards a gap which widened between Tihan treatments and those of Thionex, in week 1, was confirmed (Fig. 7b). Still reporting that the lipid budget of TA-E and control that were statistically significantly different at the beginning of the test have become similar from week 3. The pattern of protein budget was, however, completely different (Fig. 8b). During the first 3 weeks a significant difference can be noted between Tihan treatments and those of Thionex, and between TA-1 and TA-2 and control. TA-E and control remained similar throughout the test. At the end of the test, the protein budget of fish exposed to Tihan was similar to that of control, but significantly different from that exposed to Thionex. The protein budget of TE-1 was statistically higher than that of TE-2 at the end of the test. The changes in individual energy budgets for glycogen, lipids and proteins were combined to determine the effects of pesticide exposure on changes in the whole-body energy budget (Fig. 9b). After one week of exposure, whole-body energy of fish exposed to both pesticides (except TA-E) was significantly lower than that in fish from the control groups. Fish exposed to Thionex displayed the lowest energy budget, with negative values most of the time.

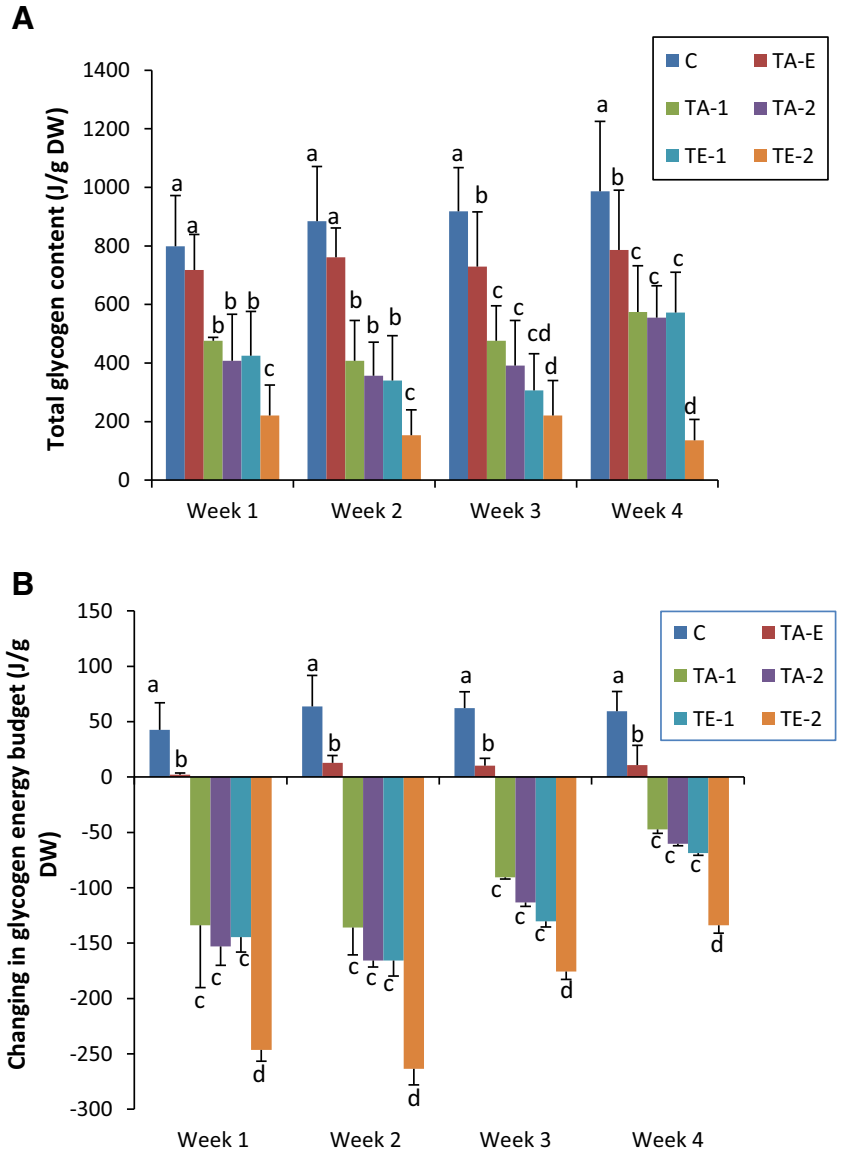

Fig. 6 Impact of increasing doses of endosulfan (TE-1 = 11 ppb, TE-2 = 22 ppb) and Tihan (TA-E = 0.23 ppb, TA-1 = 440 ppb, TA-2 = 880 ppb) on **a** total glycogen content and **b** changes in glycogen energy budget of fingerlings of African catfish. Data are given as mean ± SD; $n = 3$. In each sampling time point values having different letters are significantly different at $p < 0.05$

Discussion

The purpose of this investigation was to determine under experimental conditions the effects of chronic doses of key agricultural pesticides used in Benin cotton fields (Tihan 175 O-TEQ and endosulfan through Thionex 350 EC) on African catfish survival, growth, feed efficiency and change in whole-body energy budget.

Mortality

The doses tested in the experiment were usually sub-lethal and induced slight mortalities, except in the group submitted to an environmental dose of endosulfan (100 % of mortality after 12 days). The dose of 29.4 ppb of Thionex, corresponding to 10.29 ppb of endosulfan, is the average concentration of endosulfan assayed during the rainy season in several sites of the Alibori River, which crosses the main area of cotton production in northern Benin. Although this dose is much lower than the $LC50_{96h}$ determined by Agbohessi et al. (2013) and

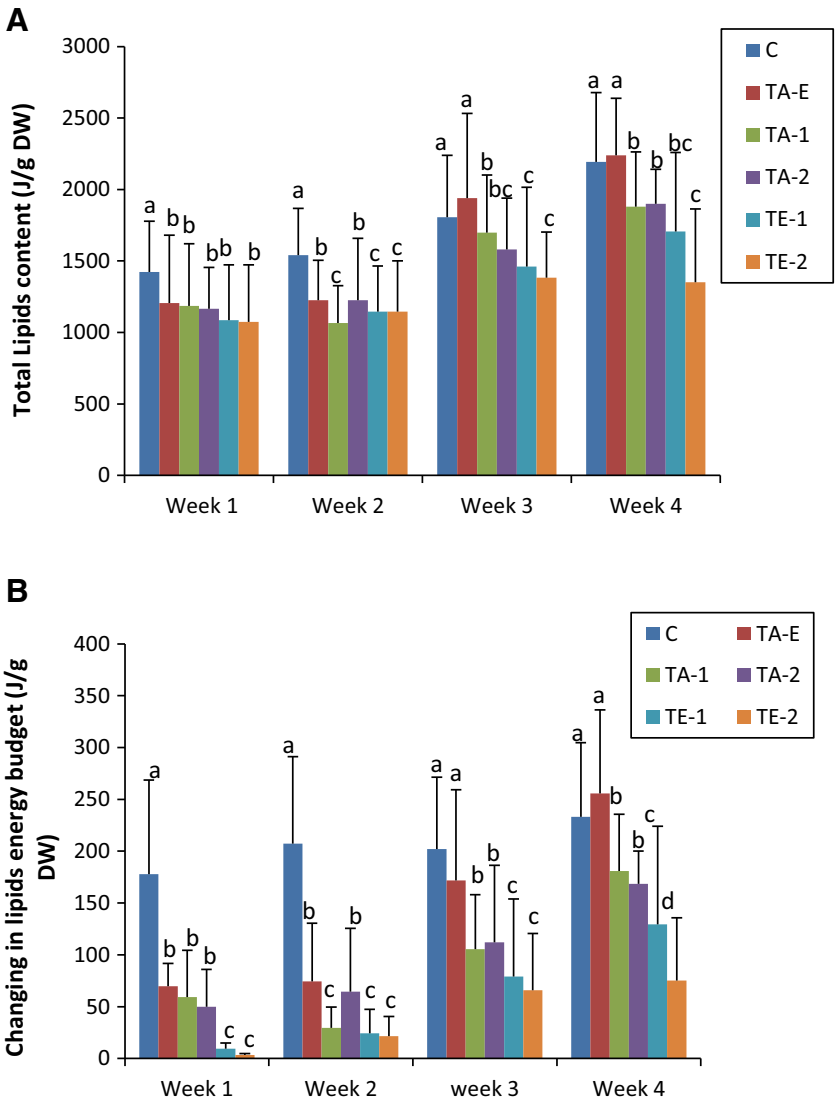

Fig. 7 Impact of increasing doses of endosulfan (TE-1 = 11 ppb, TE-2 = 22 ppb) and Tihan (TA-E = 0.23 ppb, TA-1 = 440 ppb, TA-2 = 880 ppb) on **a** total lipids content and **b** changes in lipids energy budget of fingerlings of African catfish. Data are given as mean ± SD; $n = 3$. In each sampling time point values having different letters are significantly different at $p < 0.05$

Yekeen and Fawole (2011) for African catfish (0.077 and 0.052 ppm, respectively), it is much higher than the value of 0.77 ppb reported by Ezemonye and Ikpesu (2011). The death of all fish after 12 days supports the hypothesis that endosulfan is among the main causes of recurrent death of many fish often seen floating at the surface or along the banks of several rivers in the cotton basin during the rainy season. Fish mortalities observed in the other groups of Thionex (TE-1 and TE-2) did not differ significantly from those recorded in the Tihan treatments, all these doses being much lower than the $LC50_{96h}$ determined previously for African catfish juveniles (Agbohessi et al. 2013). Fish exposed to pesticides displayed abnormal behaviors. In Tihan treatments TA-1 and TA-2 some fish were restless and moved quickly in all directions while uncoordinated swimming and loss of equilibrium were noted a few minutes before death in fish exposed to Thionex. Ezemonye and Ikpesu (2011), Yekeen and Fawole (2011) and Agbohessi et al. (2013) also reported these behavioral abnormalities in juveniles of African catfish exposed to endosulfan. Such swimming behaviors were also observed after exposure to endosulfan by Carter (1971), Munawar (1975) and Manoharan and Subbiah (1982) in spotted snakehead *Channa punctatus*, Channel catfish *Ictalurus punctatus* and *Barbus*

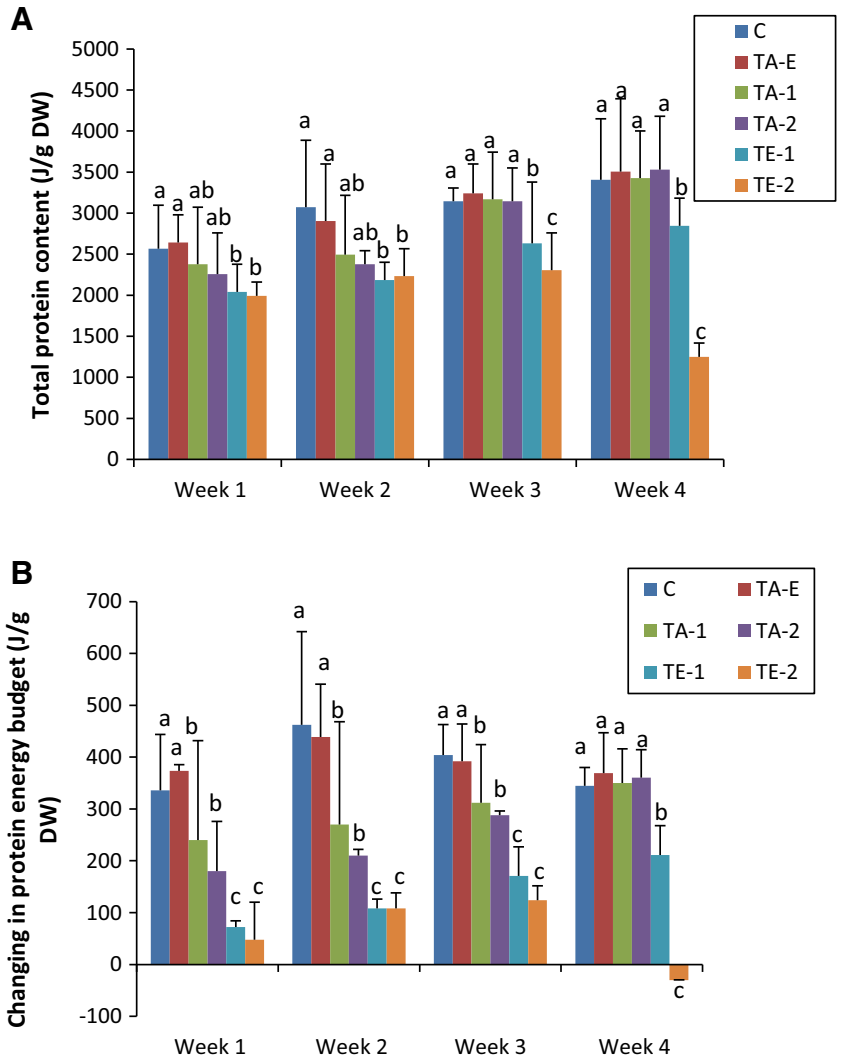

Fig. 8 Impact of increasing doses of endosulfan (TE-1 = 11 ppb, TE-2 = 22 ppb) and Tihan (TA-E = 0.23 ppb, TA-1 = 440 ppb, TA-2 = 880 ppb) on **a** total protein content and **b** changes in protein energy budget of fingerlings of African catfish. Data are given as mean ± SD; $n = 3$. In each sampling time point values having different letters are significantly different at $p < 0.05$

stigma, respectively. These two pesticides tested may probably have negative effects on the brain of African catfish.

Growth and feed efficiency

Growth of African catfish fingerlings was significantly affected by exposure to pesticides, whatever the type of pesticide and tested dose. Similar observation had been made by McCarthy and Fuiman (2008) in red drum *Sciaenops ocellatus* exposed to concentrations of 40 and 80 µg/L of atrazine or 1–10 µg/L of malathion. Weight reduction was also found in Australian catfish *Tandanus tandanus* exposed to 2 or 10 µg/L of chlorpyrifos (Huynh and Nugegoda 2012), in Nile tilapia *Oreochromis niloticus* exposed to 5–20 mg/L of dimethoate or 0.5, 1.0 and 2.0 mg/L of malathion (Sweilum 2006), in *Barbus stigma* exposed to a concentration of 3 µg/L of endosulfan (Manoharan and Subbiah 1982) and in spotted snakehead contaminated with diazinon (Cong et al. 2009). A study of Hanson et al. (2007) showed the adverse effects of lindane, pentachlorophenol and propoxur on the growth of Nile tilapia, African catfish and Bagrid catfish *Chrysitchtys nigrodigitatus*. On the contrary a concentration of 10 ng/L of β-endosulfan did not affect the growth of

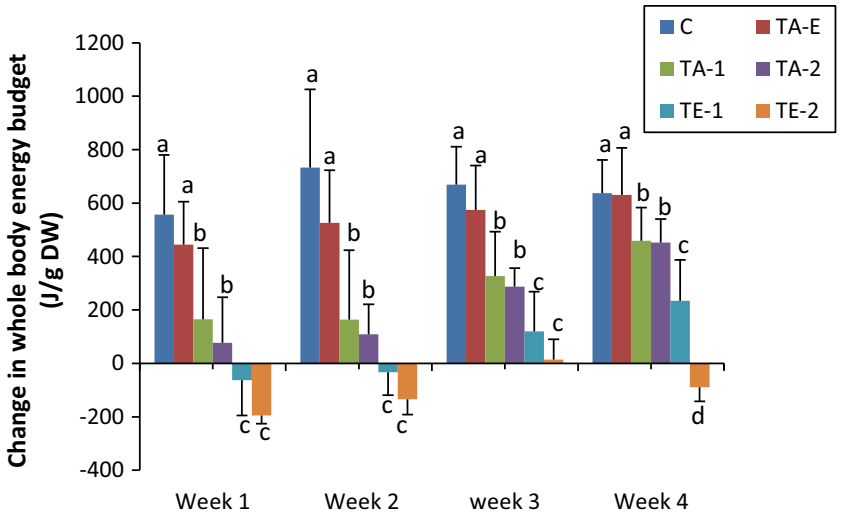

Fig. 9 Impact of increasing doses of endosulfan (TE-1 = 11 ppb, TE-2 = 22 ppb) and Tihan (TA-E = 0.23 ppb, TA-1 = 440 ppb, TA-2 = 880 ppb) on whole-body energy budget of fingerlings of African catfish. Data are given as mean ± SD; $n = 3$. In each sampling time point values having different letters are significantly different at $p < 0.05$

zebrafish (Han et al. 2011). Several factors can explain the delay observed in growth of fish exposed to the pesticides tested, such as the difference of feed intake (Wang et al. 1998; Qian et al. 2002; Lal et al. 2013) or the difference in the food metabolism (Qian et al. 2002). A reduction of feed intake was indeed noted in fish exposed to the different pesticides (data not shown). After week 4, fish from all treatments, and particularly those exposed to Thionex, displayed a significantly lower feed efficiency than that of control. Similarly the protein efficiency ratio was lower in exposed fish and particularly in those submitted to Thionex, indicating that these fish used less efficiently the dietary proteins. The delay of growth was also due to this weak use of dietary proteins. Lal et al. (2013) found a significant decline in plasma levels of GH and IGF-I in malathion-exposed Asian catfish *Heteropneutes fossilis* and showed that this decline was related to reductions in fish growth, also due to low food intake and influence of the pesticide on metabolization of feed into somatic growth. The weak growth of contaminated fish could also be the result of inhibition of acetyl-cholinesterase as reported by other authors such as Jarvinen and Tanner (1982), Cleveland and Hamilton (1983), Nagel et al. (1991) and Huynh and Nugegoda (2012). Other important factor explaining the delay in growth could be the transformation into energy of a portion of nutrients from digestion of food consumed to cope with chemical stress that constitutes the exposure to agricultural pesticides.

The specific growth rate of fish exposed to Tihan remained lower than the one of control fish during the first 2 weeks, but increased significantly from the 3rd week, exceeding significantly the SGR of control. This is probably due to compensatory growth as previously reported by McCarthy and Fuiman (2008) in red drum.

Changes in energy budgets

Significant effects of pesticide exposure on the glycogen budget of African catfish were observed from the 1st week of exposure until the end of the experiment, suggesting that glycogen reserves were used quickly after chemical exposure to cope with energy demand. According to Kharat et al. (2009), glycogen depletion is due to the increase of glycogenolysis induced by an elevation of the activities of phosphorylase, succinate and pyruvate dehydrogenase leading to anaerobic metabolism during anoxic stress conditions caused by toxicants. Vijayavel et al. (2006) studied the effects of naphthalene on carbohydrate metabolism of crab *Scylla tranquebarica*. They found depletion in carbohydrate level and suggested that naphthalene can induce hypoxic conditions, which result in the extra expenditure of carbohydrate metabolism. The decrease in glycogen content of the organisms exposed to pollutants was observed by several authors (Sarojini et al. 1990; Mane and Kulkarni 1999; Mulet et al. 2007; Moorthikumar and Muthulingam 2011; Tendulkar and Kulkarni 2012). The difference in glycogen energy budget observed between TA-E, TA-1 and TA-2 on the one hand and between TE-1 and TE-2 on the other hand shows that the amount of glycogen depleted was dose dependent. These

observations were also made by Heath (1987), Schramm et al. (1998) and Smolders et al. (2003). After 14 days, the depletion of the glycogen budget in the TA-1, TA-2 and TE-1 decreased significantly. As suggested above, this might be explained by the compensatory growth of fish during the last 2 weeks. Compensatory growth is a phenomenon that is usually observed after starvation of fish, and in our case, it might be an indication that fish in TA-1, TA-2 and TE-1 were recovering from an initial shock that impacted mainly the food intake or assimilation while fish of the TE-2 groups were still forced to starvation. This phenomenon of compensatory growth was also revealed by Smolders et al. (2003) in zebrafish exposed to sewage effluents.

The impact of pesticides on lipid content of exposed fish was also visible from the 1st week onwards. The reduction of fat content was particularly marked in fish exposed to TE-2. The differences between treatments suggest that lipid depletion was also dose dependent. The depletion of lipids after pollutant exposure has been documented in different fish species chronically exposed to pollutants (Palackova et al. 1994; Sancho et al. 1998; Handy et al. 1999; Smolders et al. 2003). Loss of lipids may be due to lipid synthesis inhibition and mobilization of the stored lipids, either through β-oxidation or through a gradual unsaturation of lipid molecules as suggested by Jha (1991).

Protein levels were significantly different in fish exposed to pesticides (except TA-E) compared to control. Even though protein is a prominent source of energy in fish, stress preferably causes depletion of glycogen and lipid reserves instead of proteins (Mckee and Knowles 1986; Heath 1987; Giesy and Graney 1989). A significant increase in protein content was observed in the 3rd and 4th week of exposure to the different pesticide concentrations except TE-2 in week 4. Such increase in protein content after pollutant exposure has also been observed by other authors. Racotta and Hernandez-Herrera (2000) found a significantly higher protein content in hemolymph of white shrimp *Penaeus vannamei* exposed to 1.07 mmol/L of ammonia-N than in control group. Brumley et al. (1995) reported a 1.5-fold increase in liver protein content when injecting sand flathead with 400 mg/kg Aroclor-1254 and De Coen and Janssen (1997) found significant increases in protein reserves in *Daphnia magna* when exposing them to different concentrations of cadmium, tributyltin, linear alkyl sulfonic acid, lindane and 2,4-dichlorophenoxy acetic acid (2,4-D). It appears that low to intermediate levels of pollution trigger increased protein synthesis (e.g. for detoxification processes and other defense mechanisms) when other sources of readily available energy like glycogen and lipids are still sufficiently present. In our study we noted a decrease of protein levels after 4 weeks of exposure to the highest dose of Thionex while the highest dose of Tihan did not lead to such decline of protein level. As mentioned by Somnath (1991) and Mulet et al. (2007), a decrease of protein levels means hydrolysis and oxidation through tricarboxylic acid cycles to meet the increased demand for energy caused by the chemical stress.

Conclusion

This study clearly showed that endosulfan and Tihan slow the growth of African catfish and that the impact of endosulfan is more intense than the one of Tihan. The growth decline induced by these agricultural pesticides was due inter alia to the poor feed utilization, protein efficiency and the use of energy to cope with the chemical stress caused by these pesticides. All fish exposed to the environmental concentration of endosulfan died before D12 but environmental concentration of Tihan did not have much impact on the growth of exposed fish.

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