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Effects of chlorpyrifos ethyl on acetylcholinesterase activity in climbing perch cultured in rice fields in the Mekong Delta, Vietnam

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ABSTRACT

Climbing perch is commonly harvested in rice fields and associated wetlands in the Mekong Delta. Despite its importance in providing food and income to local households, there is little information how this fish species is affected by the high use of pesticides in rice farming. Organophosphate insecticides, such as chlorpyrifos ethyl, which are highly toxic to aquatic organisms, are commonly used in the Mekong Delta. This study shows that the brain acetylcholinesterase (AChE) activity in climbing perch fingerlings cultured in rice fields, was significantly inhibited by a single application of chlorpyrifos ethyl, at doses commonly applied by rice farmers (0.32–0.64 kg/ha). The water concentration of chlorpyrifos ethyl decreased below the detection level within 3 days, but the inhibition of brain AChE activity remained for more than 12 days. In addition, the chlorpyrifos ethyl treatments had a significant impact on the survival and growth rates of climbing perch fingerlings, which were proportional to the exposure levels. The results indicate that the high use of pesticides among rice farmers in the Mekong Delta could have a negative impact on aquatic organisms and fish yields, with implications for the aquatic biodiversity, local people's livelihoods and the aquaculture industry in the Mekong Delta.

1. Introduction

Climbing perch (*Anabas testudineus*, Bloch, 1792) is one of the five most commonly harvested native fish species in rice fields in the Mekong Delta, and plays an important role for many households in terms of additional sources of income and nutrition (Klemick and Lichtenberg, 2008). However an overuse of pesticides in intensive rice farming constitutes a potential threat to climbing perch and other aquatic organisms. Although there is no official statistics on climbing perch populations, there is a decreasing trend in wild fish yields from agro-ecosystems in the Mekong Delta (Edwards et al., 1997).

Organophosphates (OPs), which are commonly used insecticides in the delta, are often highly toxic to aquatic animals. Chlorpyrifos ethyl (CPF) is frequently used to control a variety of insects (Pope et al., 2005). The recommended application rate is 0.6–0.8 L/ha of the commercial product (Vitashield 40EC), while a recent study indicated that farmers in the Mekong Delta commonly apply much higher concentrations (RCRD, 2014).

CPF is a well known acetylcholinesterase (AChE) inhibitor (Taylor and Brown, 1999), and in this study, brain AChE activity was used as a bio-marker to assess sub-lethal effects on climbing perch fingerlings exposed to CPF sprayed on rice fields. This method was chosen because it is well known and easy to apply, with comparatively low analytical costs. It can be applied on a range of different organisms and provides ecological relevant endpoints (Nunes, 2011). As pointed out by Chuiko et al. (2003), the AChE sensitivity varies among different fish species, and in this study climbing perch was selected as a test organism, to gain more information on the AChE sensitivity in this native species under tropical field conditions.

Thus, the objective of this study was to investigate how CPF, applied at concentration used in rice farming, affect the brain AChE activity in climbing perch fingerlings from rice fields in the Mekong Delta. Survival and growth rates were also measured to assess the effects of CPF on climbing perch fingerlings, and to evaluate if these were influenced by the AChE inhibition levels.

2. Materials and methods

2.1. Fish

Climbing perch fingerlings (3–4 g) were bought from a hatchery in Co Do district, Can Tho city, Viet Nam. The fingerlings were

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produced by a brood-stock caught from an area with no use of pesticides in the Mekong Delta, to ensure that the fingerlings were not affected by any previous exposure of pesticides to the brood-stock. The fingerlings were reared in 20 cages ($1 \times 1 \times 2$ m) placed in a pond, which used the same water sources as the experimental rice fields, for approximately 3 weeks. The fingerlings were fed pelleted feed (40% protein) daily at approximately 3–5% of the total fish fresh weight. The feeding stopped 1 day before the experiment was initiated.

2.2. Insecticide

Vitashield 40EC, an organophosphate insecticide (containing 40% chlorpyrifos ethyl [O,O-diethyl O-(3,5,6-trichloro-2-pyrinidyl)-phosphorothioate; common name, chlorpyrifos)] was purchased from Thanh Son Hoa Nong Company (Binh Chanh district, HCM city, Vietnam).

2.3. Experimental design

The experiment was carried out in 9 rice fields (approximately 1000 m^2/field) in Thoi Lai district, Can Tho city, Viet Nam. In each rice field, a canal (50 m long, 1.2 m wide and 0.5 m deep) was constructed along one side of the rice field. The experiment included a control and two treatments with Vitashield 40EC at a concentration of 0.8 L Vitashield 40EC/ha (R), corresponding to the highest recommended dose by the manufacture, and 1.6 L Vitashield 40EC/ha ($2R$), corresponding to the dose used by many farmers. Each treatment had three replicates. The experimental plots were bordered by 40 cm high and 40 cm wide mud dykes, covered by plastic sheets, which almost eliminated any water exchange between the different plots. A local rice variety (IR50404) was sown at a density of 15 kg seed per 1000 m^2. This variety is usually harvested 100 days after sowing.

Thirty-eight days after sowing, six cages ($1 \times 1 \times 2$ m), including three cages for the AChE assay and three cages for the growth and survival measurements, were placed along the central axis of each rice field. At the same time, six cages were evenly distributed along the adjacent canal. Water depths were approximately 20 cm in the rice fields and 80 cm in the canals. The cages were left for one day to allow suspended solids to settle from the water. Sixty fingerlings (average weight of 5.17–5.49 g/fish) were placed in each cage for the AChE assay and growth measurements. The fingerlings were fed commercial feed (40% protein) daily at approximately 5% of the total fish weight. Seven days after placing the fingerlings in the cages, the test fields were sprayed with Vitashield 40 EC.

Water temperature and dissolved oxygen (DO) were measured in the morning (7:00–8:00 am) and in the afternoon (14:00–15:00 pm) every second day throughout the experiment, using an Orion 830A DO meter (Thermo, Beverly, MA, USA). pH was measured only in the morning using a Portamess 911 pH meter (Knick, Berlin, Germany).

Water samples (1 L) were collected in brown glass bottles from each of the cages one hour before and one hour after spraying the rice fields with CPF, and 1, 3, 5, 7 and 12 days after spraying. The water samples were collected inside the fish cages, which were placed in the rice areas and canals. In each cage, one litre of water was sampled. The samples were kept on ice and brought to the laboratory, where they were frozen at −20 °C until analysis. The chemical analysis was conducted at the accredited laboratory, Sac Ky Hai Dang Scientific Services Joint Stock Company (EDC-HD), Vietnam, using the gas chromatography method described by Parfitt (2000).

Fish were sampled for AChE analyses at the same occasion as the collection of water samples, as described above. At each sampling time, two fishes were removed randomly from each cage. The fish were kept on ice during the transportation to the laboratory, where they were immediately processed for AChE activity measurements.

Every 15 days, 10 fingerlings were randomly sampled from each of the "growth" cages to measure the wet weight and length for growth rate calculations. After measurement, the sampled fish were stocked back into the same cages. The survival rates were measured by the end of the experiment.

2.4. Acetylcholinesterase assay

The brain was dissected out on ice and placed in a pre-weighted Eppendorf tube on ice before measurement of the brain weight. Each brain was then homogenized on ice in 6 ml of 0.1 M phosphate buffer (pH 7.4, prepared by mixing mono and dibasic sodium hydrogen phosphate) using a glass homogenizer. The homogenates were transferred into 10 ml glass tubes and centrifuged at $2000 \times g$ at 4 °C for 20 min (Centrifuge 4k15; Sigma, Osterode am Harz, Germany). 1.5 ml of the supernatant was removed to an Eppendorf tube and kept on ice for AChE analysis within 12 h. The AChE activity was determined according to the method described by Ellman et al. (1961). All measurements were performed in an air-conditioned room at 25 °C. For each measurement, a cuvette was prepared containing 2.65 ml of the 0.1 M phosphate buffer (pH 7.4), 100 μl of 3 mM 5.5 dithio-bis (2-nitrobenzoic acid) (DTNB; Sigma Aldrich Chemie, Steinheim, Germany). Immediately before measurements, 50 μl of 10 mM acetylthiocholine iodide (Sigma Aldrich Chemie) and 200 μl of the supernatant were added, and the solution was mixed well. Blanks were prepared with 200 μl of buffer instead of supernatant. Two blanks were used for each sample measurement. The AChE enzyme activity was detected using an ultraviolet/visible spectrophotometer (model UV2 2000E; ATI Unicam, Cambridge, UK) for 10 cycles (3 min and 18 s) with auto interval (22 s) at a wavelength of 412 nm, when the increase in absorbance with time was linear. The results of these measurements were expressed as a rate (absorbance per min), from which the AChE activity was calculated.

2.5. Data analysis

The data were checked for normality and variance of homogeneity prior to the statistical analysis. The Chi-squared test was applied for data that did not meet normality and the variance of homogeneity requirements. The data were analyzed with one-way ANOVA and Dunnett's post-hoc test for multiple comparisons. SPSS for windows (Ver 17.0; SPSS, Chicago, IL, USA) was used to analyze the data.

3. Results

The temperature, dissolved oxygen and pH were similar among treatments. Temperature varied from 30 °C to 32.8 °C. Dissolved oxygen level ranged from 3.5 mg/L to 4.8 mg/L and the pH ranged from 7.2 to 7.4.

No chlorpyrifos ethyl (CPF) was detected in the water before the start of the experiment (Table 1). The highest water concentration of CPF (4.23 μg/L) was found in the rice fields receiving the highest dose. The lower dose resulted in an almost 50% lower concentration of CPF in the water of the rice fields (2.32 μg/L). The concentrations in the canals were in general lower than the concentrations in the rice fields (Table 1).

The CPF concentrations in water decreased quickly, and after one day only some 20–30% of the original concentrations in the