Assessing the nuclear level impacts upon exposure to Bispyribac–sodium and Carbosulfan in Poecilia reticulata and Aplocheilus parvus

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Abstract Conventional chemical controlling, due to harmful effects on the environment and animal health, is less appreciated and discouraged today. The impacts to exposed organisms are multiple, but changes at nuclear level can result in long-term impacts to exposed populations. Such chemical exposure can also negatively impact fish that are intentionally introduced to aquatic systems for mosquito vector control. Hence, two types of fish, guppy (Poecilia reticulata) and dwarf panchax (Aplocheilus parvus) used for mosquito control, were tested to the sensitivity to insecticide, Carbosulfan, and weedicide, Bispyribac–sodium in the present study. Lethal average concentration (LC₅₀) was measured for both chemicals. Gills and liver of moribund and survived fish were stained with Heamotoxylin and Eocin to determine the histological changes. Level of cell necrosis was calculated through terminal deoxynucleotidyl transferase-mediated d’UTP nick end labeling (TUNEL) method. The 96h acute LC₅₀ value of Bispyribac–sodium to A. parvus and P. reticulata were 1.280 mg L⁻¹ and 2.370 mg L⁻¹ respectively. The 96h acute LC₅₀ value of Carbosulfan to A. parvus and P. reticulata were 0.315 mg L⁻¹ and 0.028 mg L⁻¹ respectively. Lamellar fusion, filament and lamellar epithelium proliferation, curling of secondary lamellae, hypoplasia and necrosis in gills were observed in treated fish. The percentage of damaged nuclei in the liver of treated A. parvus to Bispyribac–sodium indicated a significantly higher number of damaged nuclei in all treatments except in the lowest concentration (0.025–0.075 mg L⁻¹) compared to control (P<0.005) . P. reticulata which were exposed to Carbosulfan resulted in a significantly higher percentage of damaged nuclei in all treatments (1.10–1.85 mg L⁻¹) compared to control group (P<0.005). Results highlighted potential nuclear level impacts due to exposure to Bispyribac–sodium and Carbosulfan. In addition to determining LC₅₀ for chemcials, studies should also focus on surviving fish and their genetic make up.

Keywords: Aplocheilus parvus, Bispyribac–sodium, Carbosulfan, LC₅₀, Poecilia reticulata, TUNEL Staining

INTRODUCTION

Scientists have recently cautioned that more than the number of species that are killed, the individuals that survive after an exposure to chemicals can pose threats to the species’ future, as survivors can potentially transfer muted/damaged genetic material to next generations (Malins and Gunselman 1994; Ayas et al. 2007, Simoniello et al. 2009; Ribeiro et al. 2013). Further, genotoxic compounds represent major ecological challenges, which may lead to unusual disorders that could be transmitted to the next generations (Haldsrud and Krokje 2009). The increase of chemical releases into water bodies has created series of deleterious effects for aquatic organisms, besides direct and indirect hazards to human health (Simoniello et al. 2009). Certain pollutants may not cause acute effects but in the long run, they may decrease the life span (Nehls and Segner 2001). Hence, just as the subsequent development of resistance (Humphries 2013) through genetical modifications, attention is also required on damages to cell nuclei of exposed species.

As a result of above problems, exploring alternative, environmentally sounds methods to avoid introduction of chemicals is promoted (Kumar and Hwang 2006; Mattews 2011). One such option
is the use of biological control of vectors. For an example, larvivorous fish like *Gambusia affinis* (Ghosh et al. 2011), *Poecilia reticulata* (Kusumawathie et al. 2006) and *Gambusia holbrooki* (Willems et al. 2005), have been introduced globally, to control mosquito-borne diseases. However, the effective use of any biological control agent depends on the hardiness of the introduced species (Chandra et al. 2008). In tropics, aquatic systems, both lentic and lotic, are increasingly polluted (Li et al. 2009). Therefore, in addition to screening for consumption of targeted vector organism, they should also be tested for the tolerance and survival under agricultural and industrial pollutant loadings (Chhonkar et al. 2000).

In Sri Lanka, exotic guppy (*P. reticulata*) has been widely recommended and released as a biological control agent (Kusumawathie et al. 2008). Further, genus *Aplocheilus* (Family: Aplocheilidae) also has been successfully tested and used (Wickramasinghe and Costa 1986; Chandra et al. 2008). Compared to *P. reticulata* which is exotic to Asia, Family Aplocheilidae is native. In Sri Lanka, *Aplocheilus parvus* is a commonly found native species and has been documented as a native candidate for biological control of mosquito larvae. Both species are, however, exposed to pesticide and weedicide loadings during the farming season due to haphazard use of chemicals and release of effluent water. Therefore, a study was conducted to test the sensitivity of native *A. parvus* and exotic *P. reticulata* to a widely used insecticide, Marshal 20® (Carbosulfan) and a weedicide, Nominee® (Bispyribac–sodium) with the aim of calculating 50% Lethal Average Concentration (LC50), histological changes in gills and the level of cell necrosis in liver.

**MATERIALS AND METHODS**

**Determining the sensitivity of *A. parvus* and *P. reticulata* to some selected chemicals used in paddy culture**

About 300 *A. parvus* and *P. reticulata* (150 males and 150 females) were captured by hand nets from a perennial reservoir and a manmade canal during May 2014. Fish were acclimated to laboratory conditions in glass aquaria filled with aged tap water for 2 weeks. Stock solutions of Carbosulfan and Bispyribac–sodium were made by diluting commercial formulations of the chemicals with distilled water to obtain the required concentrations. In the case of Bispyribac–sodium, the same amount of surfactant (a mixture of Nonyl phenol polyethylene glycol ether and Poly ethylene glycol) was used as recommended by the manufacture in order to dissolve the chemical.

**Preparation of aquaria**

Aquaria were prepared applying the standard procedures and range finding tests (EPA 1996) were carried out for each chemical for both the species using two replicates with five fish each.

**Definitive test–96 hours acute toxicity test**

In this study, 96 hours static system was followed throughout the experiment. Five concentrations of test solutions were used in the definitive test with each concentration replicated in thrice with control aquaria with 5 males and 5 females each. Feeding was terminated 48 hours prior to the initiation of the experiment. The aeration was stopped only during dosing period. Mortality, temperature, DO and pH of each aquarium was recorded hourly. Percentage mortalities under each concentration at 96 hours were log transformed and were analyzed using probit analysis to determine the LC50.

A dilution series of 0.025, 0.0375, 0.05, 0.065, 0.075 mg L$^{-1}$ was used for Bispyribac–sodium for *A. parvus*, while 2.00, 2.25, 2.50, 2.75, 3.00 mg L$^{-1}$ was used for *P. reticulata* for the same chemical. In the case of Carbosulfan 0.25, 0.30, 0.35 mg L$^{-1}$ and 1.10, 1.25, 1.40, 1.75, 1.85 mg L$^{-1}$ dilution series was used for *A. parvus* and *P. reticulata* respectively.

Live or moribund fish were fixed in 10% buffered formalin separately as the death occurred in different treatments. The staining adopted in the current study is Hematoxylin and Eosin method (Meyers 2000). In addition, TUNEL staining was used to reveal cellular level damages (Gavrieli et al. 1992; Zhu et al. 2002; Qu et al. 2006). Hundred cells from liver were selected randomly from each stained slide and number of cells showing cell necrosis was calculated under Stereo Zoom Microscope (x100). Between 1500–2500 cells from 5 replicate slides were observed for each treatment. Percentage of cells indicating some form of cell damage was calculated for each agro chemical for each
Results indicated a significantly higher number of damaged nuclei in cells in all treatments compared to control group (ANOVA, df = 5, 54, P < 0.005) (Fig. 3).

DISCUSSION

The current study highlighted the damages that have occurred to nuclei in the liver of the treated fish compared with the control. The terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) method, based on direct, specific, labeling of DNA breaks is considered highly specific for the detection or early apoptosis stages, when apoptotic cells are not yet morphologically recognizable (Gavrieli et al. 1992; Negoescu et al. 1998). Among all the organisms, fish are particularly vulnerable and heavily exposed to pollution because they cannot escape from the detrimental effects of pollutants (Yarsan and Yipel 2013). Similar liver apoptosis was also observed in Atlantic bluefin tuna (Thunnus thynnus) found in the northern Adriatic Sea exposed to pollutants (Corriero et al. 2013). At present, organisms are exposed to a complex mixture of contaminants as toxic metals, organochlorides compounds, polycyclic aromatic hydrocarbons (PAHs) and sewage. Thus, depending on the dynamics of hydrographical and geomorphological conditions, the bioavailability can increase, providing more opportunities to contact with those pollutants (Haldsrud and Krokje 2009; Kousar and Javed 2015).

Histological findings from this study reiterated the susceptibility of gills and liver to tested chemicals. The large surface area of the secondary epithelium makes the gill a well-known target organ in fish and it is the first organ to react to environment pollutants (Bernet et al. 1999; Dalzell and Macfarlane 1999). The gill alterations including lamellar fusion, filament and lamellar epithelium proliferations, curling of secondary lamellae, hypoplasia and necrosis (Figs. 1a–g).

TUNEL staining

Results indicated a significantly higher number of damaged nuclei in the liver in all treatments except in the lowest concentration (1.1 mg L⁻¹) compared to control for A. parvus treated with Bispyribac – sodium. (ANOVA, df = 5, 54, P < 0.005) (Fig. 2 and Fig. 4).

Similar results were obtained for P. reticulata which were exposed to Carbofuran, with a significantly higher percentage of damaged nuclei in cells in all treatments compared to control group (ANOVA, df = 5, 54, P < 0.005) (Fig. 3).
The calculated 96 h acute LC$_{50}$ value of Bispyribac–sodium for *A. parvus* was lower than for *P. reticulata* which indicated the more sensitive nature of *A. parvus* compared to *P. reticulata* for the weedicide. According to the material data sheet of Bispyribac–sodium, the LC$_{50}$ value of rainbow trout (*Oncorhynchus mykiss*), bluegill sunfish (*Lepomis macrochirus*) and sheep head minnow (*Cyprinodon variegatus variegatus*) are >100 mg L$^{-1}$. These values are hundred times greater than the values.

![Figure 1](image_url)  
*Figure 1* Gills of *P. reticulata* stained with Heamotoxyin and Eocin. a: control treatment; b-g exposed to Bispyribac–sodium and Carbosulfan. Figures show lamellar fusion (w); filament and lamellar epithelium proliferations (z); curling of secondary lamellae (y); hypoplasia (v) and necrosis (u) (10x10 magnification).
Figure 2 The percentage of damaged nuclei of the liver tissues of *A. parvus* exposed to Bispyribac–sodium. Means with different letters are significantly different (Tukey’s Test, *P*<0.005).

Figure 3 The percentage of damaged nuclei of the liver tissues of *P. reticulata* exposed to Carbosulfan. Means with different letters are significantly different (Tukey’s Test, *P*<0.005).
Figure 4 Damaged nuclei of the liver tissues of *A. parvus* exposed to Bispyribac–sodium. x: control; y and z: treatments (1.25 and 1.75 mg L$^{-1}$ respectively). Letter “a” marked in plates depicts the damaged nuclei.

obtained from the current experiment which emphasize the need to test the tolerance for a wider range of species than relying on studies done elsewhere for non-related species.

Bispyribac–sodium is applied with a surfactant and the current results are for the combined toxicity of both. The combined effects and toxicity by itself has also been tested by others. For instance, Wan et al. (1989) found that in a commercial formulation of glyphosate, the added surfactant was more toxic than the active constituent, glyphosate. Further, laboratory studies have also shown that glyphosate alone has a low toxicity, while the surfactant can be highly toxic to a variety of taxa including amphibians (Mann and Bidwell 1999; Lajmanovich et al. 2003).

The 96h LC$_{50}$ for *P. reticulata* for Carbasulfan (0.028 mg L$^{-1}$) was lower than the values reported by Boran et al. (2007) for the same species (0.122 mg L$^{-1}$). However, in the current study 96h LC$_{50}$ for *A. parvus* was much higher (0.315 mg L$^{-1}$) than previously reported. For example, the 96h LC$_{50}$ calculated for the Carbosulfan for *Channa punctatus* was 0.268 mg L$^{-1}$ (Nwani et al. 2010), 0.231 mg L$^{-1}$ for *Oncorhincus mykiss* (Boran et al. 2007) and 1.2 mg L$^{-1}$ for *Labeo rohita* (Nagaraju and Rathnamma 2014), 0.042 mg L$^{-1}$ for rainbow trout (Yi et al. 2006) and 0.015 mg L$^{-1}$ for bluegill sunfish (Boran et al. 2007). Therefore, this study also affirms the fact LC$_{50}$ of this pesticides varies from species to species and for the same species under the influence of number of factors including size, time of exposure (Nagaraju et al. 2011), hardiness of the test species and water quality parameters (Nwani et al. 2010). Overall, both the species selected in the current study exhibited their sensitivity towards tested toxicants and the results indicated the likely
interferences of agro chemicals with future biological control programs in Sri Lanka.

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