

RESEARCH ARTICLE

Cocktail effect of Profenophos and Abamectin on tadpoles of Asian Common Toad (*Duttaphrynus melanostictus*)

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Abstract: Farmers mix two or more pesticides as mixing saves the spraying time and the labour cost and also with the idea that it increases the efficiency. The cocktail effect of two agrochemicals, profenophos and abamectin, was tested on the tadpoles of the Asian common toad, *Duttaphrynus melanostictus* under laboratory conditions. First, the 48 hr lethal concentrations (LC_{50}) was determined. Then, a chronic exposure to a series of ecologically relevant concentrations (profenophos: 0.125, 0.25, 0.5, 1.0, 2.0 ppm and abamectin: 0.01, 0.02, 0.03, 0.04, 0.05 ppm) and a mixture of these were carried out. A control was set up using de-chlorinated tap water. Survival, growth, behaviour and development of malformations were observed until metamorphosis. The LC_{50} value of profenophos and abamectin were 3.78 ppm and 0.12 ppm, respectively. Both pesticides caused lethal and sub lethal effects at low concentrations while abamectin was more toxic. In the chronic exposure to field level had greatly reduced the survival of tadpoles in profenophos (Chi square test, $\chi^2 = 133.8$, $p = 0.001$), abamectin ($\chi^2 = 105.5$, $p = 0.001$) and the cocktail ($\chi^2 = 137.5$, $p > 0.001$) when compared with the control. All the exposures caused a reduction in the growth parameters with a significant reduction in snout-vent length and body mass in 15 days post-hatch tadpoles (one way ANOVA, $p = 0.001$). Moreover, exposed tadpoles took a longer time to metamorphose (40-42 days), developed malformations and showed behavioural abnormalities like altered feeding and swimming behaviours. Profenophos induced scoliosis and kyphosis, abamectin caused oedema while the cocktail exposed tadpoles developed both scoliosis and oedema. The effects of the cocktail exposure on survival, growth and development of malformations were higher than the lone effects. Although pesticide cocktail may have potential benefits in pest resistance management, what chemicals to be mixed have to be studied before recommending the mixing of chemicals to farmers to minimize the harmful effects on the environment.

Keywords: Amphibians, Insecticides, Profenophos, Abamectin, Survival, Malformations.

INTRODUCTION

Amphibians are a fundamental component of many ecosystems playing a pivotal role as both predators and prey species (Beebee, 1995; Gardner, 2001). In the past few

decades, amphibians have experienced many threats leading to population declines and species extinctions throughout the world (Blaustein *et al.*, 2003). Researchers have identified various reasons for amphibian decline: the major factor being the habitat destruction and alteration (Alford and Richards, 1999; Blaustein *et al.*, 2011). Other causes such as climate change (Pounds *et al.*, 1999; Kiesecker *et al.*, 2001; Carey and Alexander, 2003), increased ultraviolet (UV)- B radiation, chemical contaminants (Hayes *et al.*, 2002; Blaustein *et al.*, 2003), infectious diseases (Daszak *et al.*, 2003) have also been listed as significant contributors.

Chemical contaminants are common in nature and many have studied their effect on natural communities (Relyea and Hoverman, 2006). In aquatic systems, pesticides are a common contaminant. Pesticides are extensively used especially to control agricultural pests because it is the most viable and easiest method. Most of them are poorly degradable and accumulate in aquatic habitats contaminating the environment and affecting the associated fauna when the residues leach into aquatic habitats (Relyea, 2004). Due to the aquatic larval stage, greater permeability of the skin and the presence of gills during the larval stage, amphibians are more susceptible to chemical contaminants compared to other aquatic organisms (Blaustein *et al.*, 2003; Mann *et al.*, 2009). Pesticides can affect amphibians directly or indirectly and can cause lethal or sub lethal effects (Bridges and Semlitsch, 2000). Sublethal effects include decreased growth, developmental and behavioural abnormalities, decreased reproductive success and weakened immune system (Hayes *et al.*, 2006; Sparling *et al.*, 2010). Wei *et al.* (2014) observed that the amphibian diversity has reduced in agricultural areas where usage of agrochemicals is high compared to nearby non-agricultural areas and some species have become extinct in those areas. Beebee and Griffiths (2005) highlight the connection between amphibian decline and pesticides. Early studies mainly focus on individual chemicals. While it is important to understand the principal mechanisms of different classes of pesticides and their effects on organisms, in nature, habitats are exposed to mixtures of pesticides (Le-Noir *et al.*, 1999) and therefore later studies focus more on the effects of mixtures of pesticides on aquatic organisms

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including amphibians (Hayes *et al.*, 2006; Relyea and Jones, 2009). Since many adult amphibians are insectivorous, they experience bioaccumulation of pesticides in their bodies (Fagotti *et al.*, 2005; Smalling *et al.*, 2015). Some studies point out that mixtures of pesticides with different modes of actions do not enhance the effects on anurans in mesocosms (Boone and Bridges, 1999). Others report that mixtures with different modes of actions enhance the effects on anurans than the corresponding pesticides alone (Hayes *et al.*, 2006; Relyea and Jones, 2009).

Despite the use of toxic pesticides becoming more widespread, they remaining poorly regulated and are commonly misused especially in developing countries where working conditions are often poor and the educational level of the farmer population is low. Misuse of pesticide is common among farmers in Sri Lanka too (Jeyratnam, 1985; Jeganathan and Rajakaruna, 2014). Jeyaratnam in 1985 pointed out that although farmers in Sri Lanka have knowledge about simple precautionary measures necessary to prevent the hazards arising from pesticide usage; they are unable to put this knowledge into practice. Farmers mix two or more pesticides before application as mixing saves the spraying time and the labour cost and also, they believe that mixing different types of pesticides increased the efficiency of pesticide solution and so ensure effective control of the target pest (Jeganathan and Rajakaruna, 2014). Although, there are no specific instructions on the pesticide label, studies show that some pesticides have reduced efficacy when mixed with other chemicals (Sibanda *et al.*, 2000). At the same time, some studies show that mixtures of pesticides help to restore the resistance of previously widely used chemicals to which high resistance has been developed by the pests showing that toxicities of the resistant chemical are enhanced by using a combination (Ahamed *et al.*, 2009; Abbas *et al.*, 2013). The present study determined lone and combined effect of two common agrochemicals: profenophos and abamectin on the Asian common toad, *Duttaphrynus melanostictus* under laboratory conditions.

MATERIALS AND METHODS

Test animal

The Asian common toad *Duttaphrynus melanostictus* (Anura: Bufonidae) is a widely distributed species across Asia. In Sri Lanka, it is found all over the country and occurs up to elevations of about 1700 m (Manamendra-Arachchi and Pethiyagoda, 2006). It is more abundant in human altered habitats such as agricultural landscapes and urban settings (Bandara *et al.*, 2012). *Duttaphrynus melanostictus* is distinguished from all the other Sri Lankan *Duttaphrynus* species because of the absence of parietal ridges and the presence of two longitudinal rows of large warts which distributed all length of inter parotid area. Snout-vent length of mature males is 50.3-90.0 mm and of gravid females it is 70.0-95.0 mm (Manamendra-Arachchi and Pethiyagoda, 2006). Asian common toad is listed as a "Least Concern (LC)" species in the Red list of the World Conservation Union (IUCN, 2012).

Egg strands of *D. melanostictus* were collected from two locations, a pond near Science Education Resource

Centre, in the Peradeniya University Park (7° 15' N and 80° 35' E) and a home garden pond in Pilimathalawa (7° 16' N and 80° 34' E) in Kandy District in Sri Lanka. These egg strands were brought to the laboratory and were placed in a glass tank containing de-chlorinated tap water. Emergent tadpoles were initially fed with Anchor® milk powder by placing in a small container in tanks containing tadpoles. After about five days, they were given boiled lettuce.

Test chemicals

Profenophos (0-[4-bromo-2-chlorophenyl] 0-ethyl S-propylphosphorothioate) is an organophosphate insecticide. Sri Lankan farmers use it to control pests in crops like cabbage, chilli, tomato, mustard, paddy, etc. It is marketed under the trade names Baurcron®, Polycron® and Calcron®. Usually, the dilution is 20 mL/10 L of water. A dosage of 640-800 mL ha⁻¹ and 640-800 mL ha⁻¹ is recommended for early and late growth stages.

Abamectin is a mixture of avermectins containing >80% avermectin B1a and <20% avermectin B1b (Tisler *et al.*, 2006). It is traded in market as Mightee®, Vertimac® and Mitsu®. In Sri Lanka, it is used to control pests in chilli, potato, beans, beet root, brinjal, cucumber, paddy, etc. It is highly effective against a broad spectrum of common pests in agriculture and used as an acaricide and insecticide. The dilution is usually 6 mg/10 L of water. A dose of 190-240 mL ha⁻¹ and 300-360 mL ha⁻¹ is recommended for early and late growth stages respectively.

Commercially, available abamectin (Abamectin 18 g/L EC) and profenophos (Profenophos 500 g/L EC) were used in the experiment. Profenophos and abamectin are common insecticides among those mixed and sprayed by the vegetable farmers (Jeganathan and Rajakaruna, 2014; Personal communication with farmers). First, the tadpoles of *D. melanostictus* were exposed to lethal concentrations of the two chemicals and the cocktail to determine the LC₅₀ (concentration at which 50% of tadpoles die) and then to ecologically relevant field concentrations.

Acute exposure to determine LC₅₀ values

Five days post-hatch tadpoles (Gosner stage 25-26; Gosner, 1960) were exposed to a concentration series of the two pesticides to determine the LC₅₀ for 48 hr. Commercially available form of two pesticides was dissolved in de-chlorinated tap the water to obtain a dilution series of 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 ppm of profenophos and 0.08, 0.09, 0.10, 0.11, 0.12, and 0.13 ppm of abamectin, after a preliminary range finding exposure. Tadpoles from three egg clutches were used to determine LC₅₀ (one clutch in the first trial and pooled eggs of two clutches in the second trial). Twenty tadpoles were selected randomly and placed in a glass tank (measuring 15 × 15 × 25 cm) containing 2 L of the test solution. Mortality at 48 hr after exposure was recorded.

Table 1: Concentrations of the two insecticides used in the chronic exposure to five days post-hatch tadpoles of *Duttaphrynus melanostictus*.

Pesticide	Concentration Series (ppm)				
Profenophos	0.125	0.250	0.500	1.000	2.000
Abamectin	0.010	0.020	0.030	0.040	0.050
Cocktail (Profenophos+ Abamectin)	0.125+0.01	0.25+0.02	0.5+0.03	1.0+0.04	2.0+0.05

Chronic exposure to pesticides until metamorphosis

Five days post-hatch tadpoles were exposed to a series of ecologically relevant concentrations of the two pesticides and a mixture of it. Information about field concentrations of profenophos was obtained from the Pesticide Registrar's Office, Peradeniya, Sri Lanka. Since the information about field concentration of abamectin was not available it was estimated using available data. Concentration series of two pesticides were prepared as in Table 1.

Exposure was carried out using glass tanks (measuring 15 × 15 × 25 cm) containing 2 L of the respective solution. Twenty tadpoles (five days post-hatch tadpoles at Gosner stage 25-26) were put into each tank and they were exposed to relevant pesticide concentrations and raised until metamorphosis. A control was set up using de-chlorinated tap water. Tadpoles were initially fed with Anchor® milk powder followed by boiled lettuce. The medium in the tanks were renewed once a week until metamorphosis. The medium was maintained under room temperature and constant pH. The experiment was repeated twice with one egg clutch in the first trial and two pooled egg clutches in the second trial. Mortality of tadpoles was recorded daily. The snout-vent length and the body mass were recorded at 15 and 30 days after exposure and at metamorphosis. The snout-vent length was measured to the nearest 0.01 cm using an electronic Vernier caliper and the body mass was weighed to the nearest 0.001g using an analytical balance. Time required for the fore limb emergence of half the number of tadpoles (TE_{50}) in each tank was recorded. Tadpoles and metamorphs were carefully examined for any malformations which if present were characterized using the "Field guide to malformations of frogs and toads" (Meteyer, 2000). Malformations observed in tadpoles and metamorphose were photographed and the behaviour of tadpoles was video graphed. Feeding and swimming behaviour of tadpoles exposed to pesticides were observed and it was compared with the tadpoles the control set up.

Statistical analyses

Results of acute and chronic exposure to pesticides were analyzed using MINITAB version 16.0. The LC_{50} values of the pesticides were calculated using Probit Analysis. Individual effect of concentration of pesticides on the survival of metamorphosed tadpoles was analysed using a chi square test. The effect of each pesticide on growth parameters (snout-vent length, body mass and TE_{50}) was analyzed using a one way ANOVA.

RESULTS

LC_{50} values for acute exposure of pesticides

A total of 480 tadpoles were exposed to the two chemicals. The lethal concentrations (LC_{50}) at 48 hrs for *D. melanostictus* for profenophos and abamectin were 3.78 ppm and 0.12 ppm, respectively.

Exposure to pesticides at field concentrations

Survival of tadpoles

The percentage survival of unexposed tadpoles at 15 days, 30 days post-hatch and at metamorphosis was 100.0%, 97.5% and 90.0%, respectively. Pesticide exposed tadpoles had a lower survival compared to the control (Table 2). Percentage survival decreased with increasing concentration of pesticides as well as with increasing exposure duration (Figure 1). There was a significant reduction in survival of *D. melanostictus* at metamorphosis with the increasing concentration of pesticides when compared to the control (Chi square test, profenophos, $\chi^2 = 133.8$, $p = 0.001$; abamectin, $\chi^2 = 105.5$, $p = 0.001$) with a more profound effect in the tadpoles exposed to the insecticide cocktail (cocktail, $\chi^2 = 137.5$, $p = 0.001$; Table 2). The mortality of tadpoles in the highest concentration of the two pesticides and the cocktail was 100%. Moreover there was a significant reduction in survival in the lone exposures compared with the cocktail (Chi square test, profenophos vs cocktail $\chi^2 = 205.4$, $p = 0.001$; abamectin vs cocktail, $\chi^2 = 117.1$, $p = 0.001$).

Tadpole growth

Tadpoles exposed to higher concentrations of pesticides were smaller in size with lower SVL and body mass. The mean SVL and body mass of 15 days post hatch tadpoles in the control was 0.736±0.058 cm and 0.050±0.003 g, respectively. There was a significant reduction in SVL of 15 days post-hatch tadpoles with increasing concentration of both pesticides and cocktail (one way ANOVA, profenophos, $F_{5,213} = 254.3$, $p < 0.001$; abamectin, $F_{5,186} = 88.7$, $p = 0.001$; cocktail, $F_{5,151} = 114.8$, $p = 0.001$). Moreover, a significant reduction in weight of 15 days post-hatch tadpoles with increasing concentration of pesticides was also observed (one way ANOVA, profenophos, $F_{5,213} = 163.4$, $p = 0.001$; abamectin, $F_{5,186} = 57.7$, $p = 0.001$; cocktail, $F_{5,151} = 65.7$, $p = 0.001$). Snout vent length and body weight of cocktail exposed tadpoles (15 days post-

Table 2: Percentage survival of *D. melanostictus* tadpoles at 15 days post-hatch, 30 days post-hatch and at metamorphosis exposed to two pesticides and a cocktail at different concentrations.

Pesticide	Concentration (ppm)	Percentage survival %		
		0-15 days	16-30 days	Metamorphosis
Profenophos	0.125	100.0	97.5	77.5
	0.250	100.0	77.5	70.0
	0.500	95.0	67.5	52.5
	1.000	87.5	42.5	12.5
	2.000	67.5	27.5	0.0
Abamectin	0.010	100.0	85.0	60.0
	0.020	87.5	70.0	35.0
	0.030	82.5	67.5	52.5
	0.040	62.5	27.5	12.5
	0.050	50.0	10.0	0.0
Cocktail	0.125+0.010	90.0	70.0	27.5
	0.250+0.020	65.0	50.0	17.5
	0.500+0.030	65.0	50.0	10.0
	1.000+0.040	52.5	30.0	7.5
	2.000+0.050	22.5	5.0	0.0
Control	0	100.0	97.5	90.0

hatch) were smaller than the profenophos and abamectin exposed tadpoles (Table 3).

The mean TE_{50} of unexposed tadpoles was 30 days. A lengthening of TE_{50} was observed in tadpoles that were exposed to pesticides (Figure 2). Moreover, the average TE_{50} increased with increasing concentration of the two pesticides and cocktail. Tadpoles exposed to profenophos took a longer time to develop forelimb (33.5 days) compared to that of with the control group (one way ANOVA, $p < 0.05$). A similar observation was made for the cocktail (41.4 days; one way ANOVA, $p < 0.05$).

Malformations of tadpoles

Tadpoles in some exposures developed malformation but none of those in the control group had any malformations. The percentage of tadpoles having malformations increased with the increase in the chemical concentration (Figure 3). Three main types of malformations were observed: scoliosis (curvature of the spine in the lateral plane), kyphosis (curvature of the spine in the dorso-ventral plane) and edema (Figure 4). Profenophos exposed tadpoles showed only scoliosis and kyphosis whereas abamectin exposed once showed only edema. These fluid filled swellings caused shifting the centre of gravity of tadpoles, and resulted in twisting of the body axis and altering their swimming behaviour. They were unable to balance the body during swimming and they tend to swim upside down. All the tadpoles with edema died due to rupturing of the swelling. In the cocktail exposure there were tadpoles with both scoliosis and edema, though kyphosis was not observed. The downward pattern of malformations observed in high concentration tanks were due to high mortality of tadpoles at those concentrations. Most of the malformations were

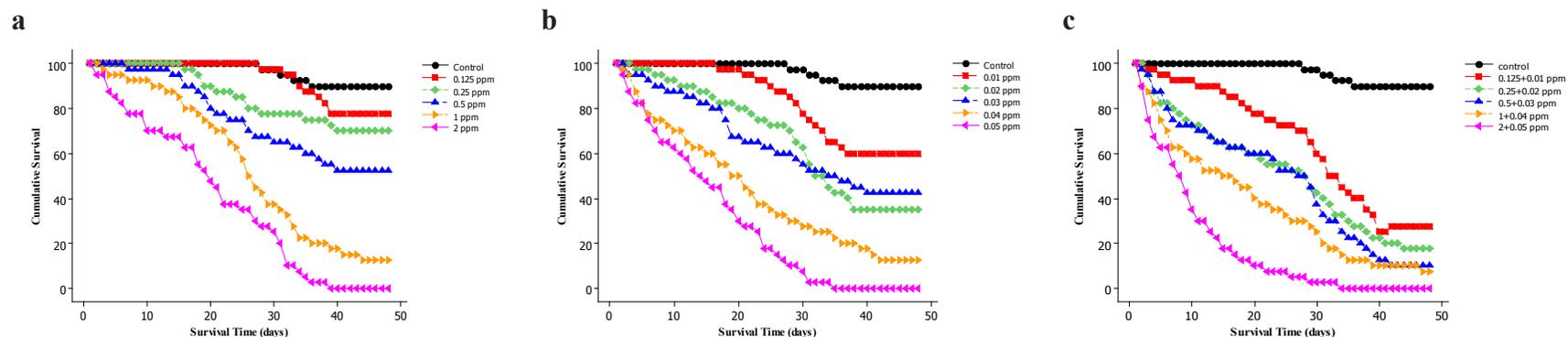


Figure 1: Cumulative survival of tadpoles of *D. Melanostictus* exposed to ecologically relevant concentration series of a) profenophos b) abamectin and c) cocktail.

Table 3: Mean snout vent length and mean body weight of *D. melanostictus* at 15 days post-hatch, 30 days post-hatch and at metamorphosis exposed to two pesticides and a mixture of it at different concentrations.

Pesticide	Concentration (ppm)	Mean snout vent length (cm)			Mean body weight (g)		
		15 days	30 days	Metamorphosis	15 days	30 days	Metamorphosis
Profenophos	0.125	0.624±0.056	0.678±0.047	0.692±0.044	0.044±0.006	0.050±0.007	0.044±0.006
	0.250	0.629±0.063	0.725±0.053	0.685±0.055	0.042±0.008	0.059±0.008	0.043±0.008
	0.500	0.600±0.070	0.636±0.081	0.672±0.038	0.039±0.007	0.039±0.012	0.038±0.007
	1.000	0.381±0.042	0.475±0.069	0.623±0.022	0.081±0.004	0.029±0.006	0.035±0.004
	2.000	0.342±0.031	0.470±0.098	*	0.016±0.005	0.027±0.007	*
Abamectin	0.010	0.688±0.074	0.674±0.062	0.690±0.041	0.050±0.009	0.045±0.009	0.039±0.004
	0.020	0.622±0.114	0.650±0.060	0.663±0.054	0.042±0.014	0.042±0.006	0.046±0.008
	0.030	0.521±0.053	0.646±0.056	0.712±0.040	0.033±0.007	0.039±0.008	0.047±0.006
	0.040	0.442±0.074	0.546±0.028	0.651±0.007	0.024±0.007	0.030±0.007	0.038±0.001
	0.050	0.399±0.079	0.563±0.007	*	0.021±0.008	0.034±0.001	*
Cocktail	0.125+0.010	0.534±0.046	0.644±0.080	0.644±0.080	0.032±0.007	0.041±0.008	0.041±0.008
	0.250+0.020	0.548±0.042	0.669±0.033	0.669±0.033	0.036±0.006	0.046±0.003	0.046±0.003
	0.500+0.030	0.499±0.063	0.585±0.061	0.585±0.061	0.029±0.008	0.041±0.007	0.041±0.007
	1.000+0.040	0.454±0.094	0.556±0.067	0.556±0.067	0.025±0.009	0.032±0.004	0.032±0.004
	2.000+0.050	0.337±0.026	0.432±0.028	*	0.015±0.003	0.025±0.004	*
Control	0	0.736±0.058	0.746±0.050	0.734±0.051	0.050±0.003	0.050±0.007	0.041±0.005

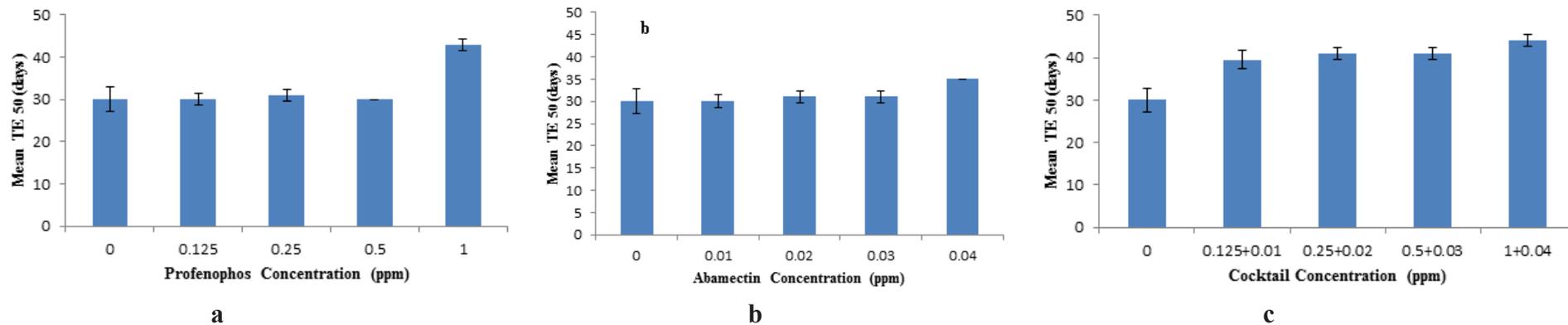


Figure 2: Mean TE₅₀ (time required for forelimb emergence of half the number of exposed tadpoles) values of *D. melanostictus* tadpoles exposed to different concentrations of a) profenophos b) abamectin and c) cocktail.

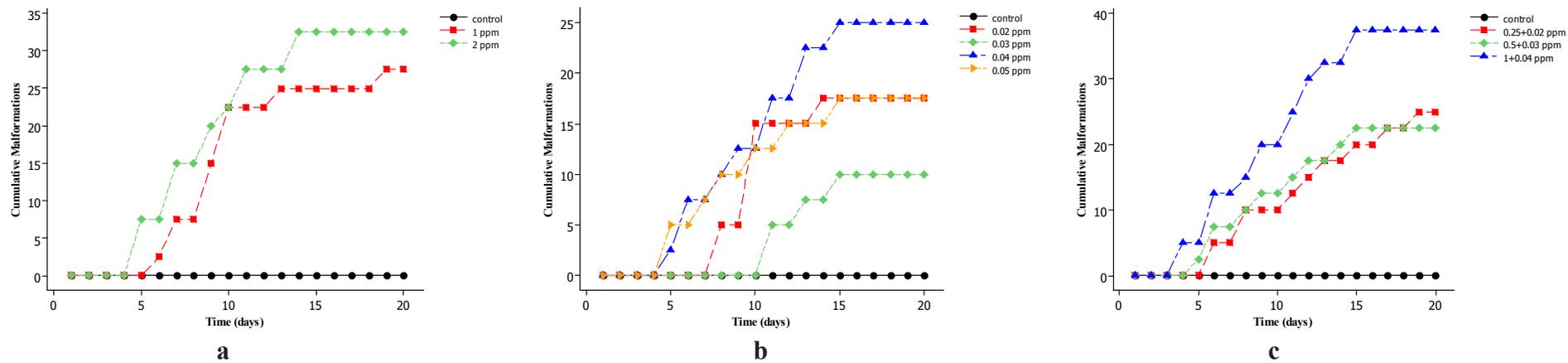


Figure 3: Cumulative malformations of *D. melanostictus* tadpoles and metamorphs exposed to ecologically relevant concentration series of a) profenophos b) abamectin and c) cocktail.



Figure 4: Malformations of *D. Melanostictus* exposed to profenophos, abamectin and cocktail A) a tadpole with scoliosis (abnormal curvature of the spine in lateral plane B) a tadpole with kyphosis (abnormal curvature of the spine in dorso-ventral plane C & D) tadpoles with scoliosis at tail region at Gosner stage 43 E) a tadpoles with edema in one side of the body F) a tadpole with edema in both sides of the body at Gosner stage 42.

observed in 5-15 days post-hatch tadpoles.

Feeding and swimming behaviour

Tadpoles exposed to pesticides had an altered feeding and swimming behaviour. Feeding efficiency of tadpoles in exposed groups was lower compared to tadpoles in the control group. Tadpoles in control and low concentrations of pesticides were more active compared to those in high pesticide concentrations who were lethargic and showed limited movements. Moreover, they showed unusual swimming behaviours and those with oedema found it difficult to balance during swimming.

DISCUSSION

The acute exposure shows that both chemicals have low LC_{50} values of which the value for abamectin was much lower compared to profenophos. This indicates that the acute toxicity of abamectin is higher than that of profenophos. Profenophos and abamectin have been classified as toxic and moderately toxic pesticides by World Health Organization (WHO). The LC_{50} values reported for other organisms including frogs are comparable with the values reported for *D. melanostictus*. Li *et al.* in 2010 reported 24 and 48 hr LC_{50} of profenophos to tadpoles of a Chinese native amphibian, *Rana spinose* as 1.59 ppm and

1.14 ppm, respectively. The 96 hr LC_{50} value for rainbow trout (*Oncorhynchus mykiss*) is 3.2 ppb (Jencic *et al.*, 2006).

At ecologically relevant concentrations, survival of tadpoles was greatly affected by the exposure to both profenophos and abamectin. The field concentration of profenophos is 1.00 ppm (Pesticide Registrar Office, Peradeniya) at which only 12.5% tadpoles survived to metamorphose and none survived in the highest lone exposures in both chemicals as well as the cocktail. Lone effect of the two chemicals was highly toxic to the tadpoles while the cocktail showed even higher toxicity. A study conducted to determine the effects of combinations of malathion and cypermethrin on tadpoles of Indian cricket frog (*Fegervary alimnocharis*) suggest that cypermethrin is more toxic than malathion and combinations of higher concentrations of cypermethrin with malathion are more deleterious to the survivability of tadpoles (Nataraj and Crishnamurthy, 2012).

Ahamed *et al.* (2009) showed that chlorpyrifos, profenophos and fipronil can be used in mixtures to restore the resistance to cypermethrin and deltamethrin in *Spodoptera litura*, a lepidopteran pest worldwide infesting over 112 host plants. Moreover, the effectiveness of the pesticide cocktails against resistance management of the

house fly, *Musca domestica* to some insecticides have been studied by Abbas *et al.* (2013). They have shown that the toxicities of cypermethrin, bifenthrin, and deltamethrin in the resistant population of house flies are enhanced by the combination with chlorpyrifos, profenophos, emamectin and fipronil. Mixing of two pesticides and applying to the field is a common practice in Sri Lanka (personal communication with farmers, 15 March 2015). A recent survey carried out by Jeganathan and Rajakaruna (2014) show that 32.5% of the vegetable farmers in Sri Lanka, always mix two or more pesticides before applying as the farmers believe that mixing different types of pesticides increased the efficiency of chemical and therefore have a better more effective control of the target. The farmers have further stated that mixing save spraying time and labour costs (Jeganathan and Rajakaruna, 2014). In Sri Lanka, there are no specific instructions in the label on the pesticide bottle not to mix any chemicals or the Agricultural Instructors who visit the farms regularly do not advise the farmers on the negative effects of cocktailing chemicals and spraying (personal communications with the farmers, 15 March 2015). It is important to study which pesticide combinations are effective in resistance management before using as mixing enhanced the harmful effect on the non-target organisms.

The survival of tadpoles in the lone exposures of the two pesticides was different, abamectin being more toxic which can be attributed to the disparity in the activity of the two chemicals. Profenophos is an organophosphorous insecticide and is an acetylcholinesterase inhibitor while the primary target of abamectin (avermectin B1a and avermectin B1b) is the nervous system which also acts as an endocrine disruptor. However, the mechanism of action of abamectin has not been fully elucidated yet.

Pesticide exposed tadpoles took longer time to metamorphose and were smaller in size. Many studies have shown the lengthening of the tadpole period when exposed to pesticides but the size at metamorphosis varies depending on the chemical and amphibian species. Hayes *et al.* (2006) reported that with exposure to pesticide mixtures, larvae take longer to metamorphosis, but do not obtain a size advantage. However, *Polypedates cruciger* and *D. melanostictus* exposed to chlorpyrifos, dimethoate, glyphosate, propanil take a longer time to metamorphose (Jayawardena *et al.*, 2010; 2011). *Polypedates cruciger* shows a reduction in growth parameters at metamorphosis (Jayawardena *et al.*, 2010) while *D. melanostictus* shows an increase in growth parameters (Jayawardena *et al.*, 2011). For amphibians, growth period and size at metamorphosis are important factors as it influences the fecundity, juvenile dispersal, adult fitness and survivability (Sparling *et al.*, 2010).

The types of malformations caused by the two pesticides were different. Tadpoles exposed to profenophos developed malformations such as scoliosis and kyphosis in the spine whereas those exposed to abamectin developed oedema. In the cocktail exposure, the tadpoles developed kyphosis, scoliosis and oedema. Similar malformations were observed in *D. melanostictus* exposed to chlorpyrifos,

dimethoate, glyphosate, propanil (Jayawardena *et al.*, 2011). Malformations are likely to increase the vulnerability of the tadpoles or the frog to predation (Cohen, 2001). Although this study did not examine the gonads of the adult frogs, feminisation has been observed in *P. cruciger* that was exposed to ecologically relevant concentrations of profenophos under laboratory conditions (Jeganathan and Rajakaruna, 2016). These authors have observed development of oocytes in the histological preparations of the cross sections of male testes.

CONCLUSION

The results show that the exposure to the commercial grade of two pesticides, profenophos and abamectin, reduced the survival and growth, lengthened the time taken for metamorphosis and induced malformations in tadpoles of *D. melanostictus* under laboratory conditions. The cocktail effect of the two pesticides was more profound than the lone effect. Being an agricultural based country, the usage of pesticides in Sri Lanka is very high. Misuse and overuse of pesticides is very common in Sri Lanka (Jeganathan and Rajakaruna, 2014). Farmers often ignore technical recommendations and use their own experience during usage of pesticides to fields leading to an indiscriminate use of pesticides (Wilson and Tiddsell, 2001). They use stronger concentrations of pesticides with increased frequency of application (Selvarajah and Thiruchelvan, 2007). Studies have shown mixing of pesticides may have potential benefits in resistance management (Das, 2014). It is important to study what chemicals are to be mixed and when to be mixed rather than mixing them randomly. The results of the study highlight the importance of educating the farmers that mixing of chemicals may lead to harmful effects in the environment.

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