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Ecotoxicological Effects of Imidacloprid and Lambda-Cyhalothrin (Insecticide) on Tadpoles of the African Common Toad, Amietophrynus Regularis (Reuss, 1833) (Amphibia: Bufonidae)

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Abstract

Agriculture is the main activity carried out in Cameroon and pesticide use is believed to be a common rule for its success. Most of the farms are situated close to water bodies, thus constituting a potential risk to non-target aquatic organisms. Declines of amphibian populations have been a worldwide issue of concern for the scientific community during the last several decades. The aim of this study was to assess the effects of an insecticide used by local farmers in Buea on amphibians. Parastar 40WP® which is constituted of imidacloprid + lambda-cyhalothrin a commonly used insecticide was applied on tadpoles of the African common toad Amietophrynus regularis (Reuss, 1833) in a static renewal experiment. The acute toxicity test was carried out after a range finding test from which seven test concentrations were selected. These concentrations were 0.0025 mg/l, 0.005 mg/l, 0.01 mg/l, 0.05 mg/l, 0.1 mg/l, 0.5 mg/l and 0.2 mg/l. Signs of toxicity such as hyperactive symptoms, loss of balance, motionlessness and death were recorded. A varying degree of mortality (dose-dependent) was noticed during the test. On the contrary, no such toxicity signs and mortality occurred in the control, indicating that they were caused by the test substance. The 24h LC50 was 3.66mg/l, which is less than the recommended application dose (125 mg/l). Products constituted of imidacloprid and lambda-cyhalothrin should therefore be handled with care and far from water bodies because of their potential to cause harm to non-target aquatic biota.

Keywords:

Acute;
Amphibians;
Insecticide;
Tadpoles;
Dose-dependence.

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1- Introduction

Agriculture is the major industry in Cameroon because of economic factors [8]. In the South West Region, agriculture is carried out by small scale farmers and two main companies: the Cameroon Development Corporation (CDC) and Del Monte to supply the local market and neighbouring countries [14]. This is one of the few activities from where potentially harmful chemicals are intentionally released into the environment [11]. The use of pesticides is a common rule in agriculture; almost all the farmers apply pesticides to protect their crops [12], even banned pesticides [12, 13]. Pesticides are substances or mixtures of substances intended to repel, destroy or mitigate pests that destroy plants or act as vectors for human and animal diseases [4]. The economic crisis after 1989 brought about the liberalization of the pesticide sector in Cameroon [8]. Therefore, the importation, distribution and use of pesticides are done in conditions that are far from ideal [9]. In many poor countries, pesticide use is synonymous to improper use [15] Farmers don't follow recommended doses, use pesticide in combination, lack protective gears, and throw pesticide remains and containers in water or the bush [12, 13]. These practices favour the accumulation of pollutants on the environment pollutants accumulation into the environment [9].

Agriculture is most often carried out near water bodies for irrigation purposes and the presence of a stream is sometimes the criterion to create a plantation [12]. Hence there is a possible risk of harm for non-target organisms, as pesticides can find their way into aquatic ecosystems through spray drift and run-offs. The impact of pesticides on water quality is associated with active ingredients in the pesticide formulations, contaminants that exist as impurities in the

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active ingredients, additives that are mixed with the active ingredients and degradates that are formed during chemical, microbial or photochemical degradation of the active ingredient [11]. Pesticides act in water through trophic cascade, affecting a wide range of organisms and causing tadpoles to have less food and grow more slowly [6]. Effects at the organism or ecological level are usually considered to be an early warning indicator of potential human health impacts [11]. In a study carried out in the Fako Division in the South-West Region of Cameroon, up to 14 post-application symptoms were recorded in farmers that used pesticides in farms, as well as cases of pesticides intoxications where men significantly suffered more than women [12].

Existing risk assessment procedures for pesticide regulation are not protecting amphibians [18]. Declines of amphibian populations have been a worldwide issue of concern for the scientific community during the last several decades [19]. Indeed, amphibians may be at greater risk of toxic effects of pesticides than other aquatic vertebrates because their preferred breeding habitats are often shallow lentic or ephemeral water bodies [3]. Amphibians play a key role in food chains: tadpoles feed on algae and debris (plants and animals) found in water. After the metamorphosis, they serve as food for small mammals and birds, and are predators for insects, spiders, molluscs and other small animals. As such they contribute to the biomagnification of pollutants in aquatic systems. The ecological effects of pesticides on living organisms vary because different categories of pollutants have different effects. Pesticides accumulate in living tissues (bioaccumulation) and cause lethal (death) or sub-lethal (histopathology, carcinogenesis, reduced reproduction, immune-suppression, endocrine disruption, teratogenesis, intergenerational) effects [11].

Worldwide, there is limited data pertaining to the effects of pesticides on amphibians [3]. In Cameroon, studies linking pesticides to amphibian community structure are very rare or completely absent. Imidacloprid and lambda-cyhalothrin are frequently used by farmers in different pesticide formulations in the South-West Region of Cameroon. In a previous study, it has been reported that 19.7% of farmers in the Fako Division used this combination of insecticides [12]. The use of these pyrethroid insecticides by market gardeners has also been reported in Balessing, a locality in the West Region of Cameroon [13]. Imidacloprid is a neonicotinoid insecticide which is highly soluble in water and non-toxic to fish [17], but in a laboratory work, this pesticide caused an increase in the mortality of fish following the duration of exposure [16]. It has a low potential of bioaccumulation and is highly toxic to birds and honey bees. However, it is moderately toxic to mammals and earthworms but non-toxic to fish [17]. Acting as sodium channel modulator, lambda-cyhalothrin is a pyrethroid insecticide with a low aqueous solubility. It is highly toxic to mammals and is a known irritant. It is also highly toxic to fish, aquatic invertebrates and honey bees, but only moderately toxic to earthworms [17]. In order to contribute to ecotoxicological studies in Cameroon, this study aimed at assessing the acute effects of imidacloprid + lambda-cyhalothrin (Parastar 40WP®) on tadpoles of the African common toad, Amietophrynus regularis (Reuss, 1833) (Amphibia: Bufonidae).

2- Material and Methods

2-1- Test Substance

Parastar 40WP® (insecticide) is sold in 50g sachets in powder form and this content is recommended to be diluted in 16l of water before application in tomato farms. This 50g of powder contains 2g of active ingredients in the following proportions: imidacloprid 20g/kg + lambda-cyhalothrin 20g/kg. With a molar mass of 255.66 g/mol and a chemical formula of $C_9H_{10}ClN_5O_2$, imidacloprid ((*E*)-1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine) is a chloronicotine insecticide used to control sucking and soil insects [17]. Lambda-cyhalothrin((*R*)-a-cyano-3-phenoxybenzyl(1*S*)-cis-3-[(*Z*)-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate and (*S*)-a-cyano-3-phenoxybenzyl (1*R*)-cis-3-[(*Z*)-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate) has a molar mass of 449.85g/mol and the chemical formula is $C_{23}H_{19}ClF_3NO_3$. It is a synthetic pyrethroid insecticide having a low aqueous solubility. It is relatively volatile and non-mobile. Based on its chemical properties, there is a low risk of it leaching to ground water [17].

2-2- Sample Collection and Acclimatization

The species of tadpoles that was collected was the *A. regularis* about 150 tadpoles were collected and taken to the laboratory. They were allowed to stand at room temperature (around 25°C) for 48hrs. An aerator was used to supply enough oxygen to the tadpoles during the acclimatization period at room temperature.

2-3- Preparation of Test Concentration

Dechlorinated tap water was used for our dilutions, following the titration formula: $c_iv_i=c_fv_f$ (c= concentration, v= volume, i= initial, f= final). A sachet of Parastar 40WP was diluted in 1l of water to constitute a mother solution at 2g/l, from which test concentrations were prepared.

2-4- Range Finding Test

A range finding test with concentrations ranging from 0.00025mg/l to 1875 mg/l was carried out. This test enabled us to choose seven test concentrations for the definitive test. The test concentrations values were at interval between those that indicated “no observable effect concentration” where no death was recorded) and those that indicated LC_{100}

(which is the concentration at which 100% of the tadpoles were killed.

2-5-Acute Toxicity Test

The design followed the static renewal [7]. One control and seven test concentrations were used in order to determine the 24hrs LC_{50} value. Each concentration and the control were tested in three replicates. The seven tests concentrations for the definitive test were as follows: 0.0025mg/l, 0.005mg/l, 0.01mg/l, 0.05 mg/l, 0.1 mg/l, 0.5 mg/l and 0.2 mg/l. The behaviour of the tadpoles was noted as the experiments were set. These experiments were labelled in ascending order based on concentrations.

The experiments were allowed to stand in the laboratory for 24h and the number of dead tadpoles was recorded. They were considered dead when they turned upside down and sank to the bottom of the container, or when they failed to show any sign of movement after their ailed was touched with a glass rod [1].

2-6-Behavioural Observations

Tadpoles' behaviour was monitored in situ through the observation of the swimming activity in the control and test containers.

2-7-Data Analysis

The susceptibility of tadpoles to pesticides was determined using the Probit method of analysis for LC_{50} at 24hrs. The safe concentration was obtained by multiplying the median lethal concentration by a factor of 0.1 [1].

3- Results

3-1-Behavioral Response

When introduced into tests containers, abnormal reactions were recorded and these reactions increased with insecticide concentration. Tadpoles swam actively, jumped and were trying to escape from the test containers, while those in the control swam normally.

3-2-Acute Effects

No mortality was recorded in the control group (water), meaning that tadpole mortality in the test concentrations was due to the presence of the chemical. Tadpole mortality exposed to Parastar 40WP has a dose-dependent relationship, with a positive and significant correlation ($R^2 = 0.86$) as shown in Figure 1. The LC_{50} from the graph was 3.66mg/l.

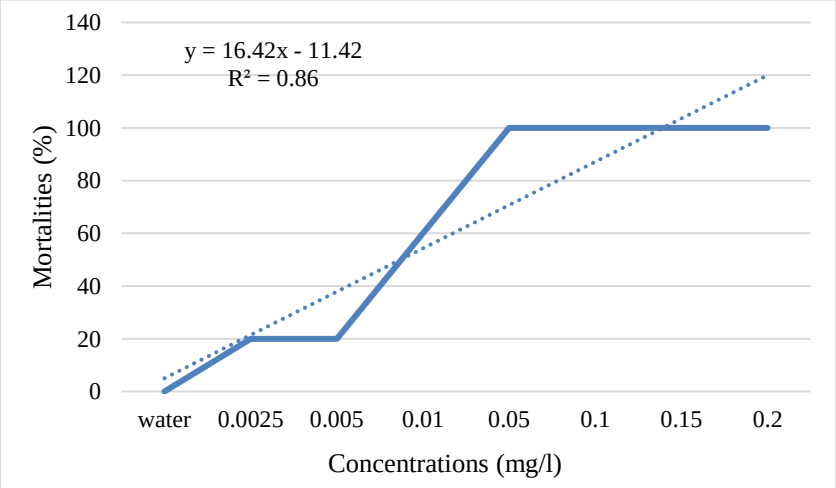

Figure 1. Tadpoles mortalities

The safe concentration for Parastar 40WP (imidacloprid 20 g/kg + lambda-cyhalothrin 20g/kg), was then derived as 0.37mg/l (Table 1).

Table 1. LC50 and safe concentration for Parastar 40 WP (insecticide)

Variable	LC_{50}	Safe Concentration	Recommended Dose
Value (mg/l)	3.66	0.37	125

4- Discussion

Amphibians' populations have been declining in an alarming rate over the past 25 years [5]. There is limited literature on the effects of imidaclopride+lambda-cyhalothrine (Parastar 40WP) on amphibians. Information on the effects of other pesticides on amphibians exists, but the end point is very controversial. This study evaluated the acute and chronic effects of Parastar insecticide to A. regularis tadpoles. Parastar is used by farmers in Buea for tomatoes cultivation. Tadpoles exposed to different concentrations of Parastar pesticide undoubtedly experienced stress due to irritations and neurotoxic effects. The increase erratic movements exhibited by the tadpoles in test solutions may be an attempt to be relieved from such stress environment. Negative effects of pesticides on amphibians are probable because the skin is highly permeable to allow gas, water and electrolyte exchange with the environment [18].

The LC50 value after 24h was 3.66 mg/l in a static renewal experiment. These findings corroborate those of a study done on the effects of glyphosate (herbicide) in four different formulations on the amphibian Rhinella arenarum, where the 24h LC50 ranged from 2.42 to 77.52 mg/l. [20]. However, this value is relatively small as compared to the 24h LC50 value of 5.37mg/l with Chlorantraniloprole (insecticide), 4.90mg/l with Flubendiamide-abamectin (insecticide) and 4.68mg/l with Penoxsulam (herbicide) on tadpoles in a static renewal experiment with Hoplobatrachus chinensis (Chinese tiger frog) [10]. A. regularis tadpoles seemed to be a little bit more sensitive to pollutants. This susceptibility depends on factors such as age and size. The onset of metamorphosis may cause tadpoles to be more vulnerable to toxic effects. The tadpole developmental stage may be more relevant to determining toxicity than tadpole age. In a study carried out in Costa Rica, it was found that chlorothalonil (fungicide) is highly toxic to native amphibian species and that low concentrations can cause biochemical responses related to phase II of biotransformation and effects on development [19].

No mortality was reported in the control test in this study, while varying degrees of mortality were reported in the tests concentrations. This is a clear indication that the effects of the Parastar 40WP could be regarded as possible cause of death of test organisms. The results clearly indicates that variation in the doses of this pesticides varied greatly in their effects on survival of A. regularis .The highest mortality was found at the highest concentration, suggesting dose dependent survival and concentration graded lethality. The LC50 value found in the work is lower than the recommended applied dose, confirming the risk of using this compound near water bodies. In a study done in Germany on juvenile European common frogs (Rana temporaria), it was realized that acute mortality due to pyraclostrobin (fungicide) ranged from 100% after one hour to 40% after seven days at the recommended label rate of currently registered pesticide products [18].

The change in animal behaviour after exposure to toxicants may be related to consequent alteration in physiological process. Certain signs of toxicity such as hyperactive symptoms at the beginning then loss of balance, followed by motionlessness and finally death were observed in the behaviour of the African common toad. Similar behavioural effects in response to laboratory exposure to Endosulfan, Diazinon, atrazine in larvae amphibians have been reported [1]. These behavioural effects are not surprising as most of these pesticides are neurotoxins. It is also known that behavioural changes of these types increase the chance of amphibian predation.

5- Conclusion

At the end of this work that aimed at assessing the impact of Parastar 40 WP on tadpoles of the common toad, it can be concluded that very toxic to amphibians because of the impact on mortality and behavioural changes. The high mortality recorded for Parastar 40WP may be mainly due to the active ingredients imidachloprid and lambda-cyhalothrin that might have accumulated in the system of the tadpoles. Intentional or accidental release of this compound into water bodies and other wetlands can be a great threat to amphibians and probably other aquatic organisms. Parastar 40WP (insecticide) should be handled with great care since it is very toxic and farmers should follow the recommended dose. The tadpole bioassay carried out in this study can be of tremendous use in ecological monitoring programmes for Cameroonian water bodies and wetlands.

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