



Behavioral changes in *Anabas testudineus* exposed to sub-lethal concentration of Nuvan

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Abstract

The organophosphorus insecticide, Nuvan is widely used in agricultural field and private industrial premises to control pests, easily accumulates in aquatic ecosystems and exerts toxic effects on aquatic animals. The study aimed to evaluate the acute toxicity (LC_{50}) of Nuvan on *Anabas testudineus*. *Anabas testudineus* were exposed to different concentrations of nuvan ranging from 0.00025 ppm to 2.048 ppm with parallel untreated control. At the doses of 0.0005 ppm, 0.001 ppm, 0.002 ppm, and 0.004 ppm 40%, 50%, 70%, and 90% mortality of *Anabas testudineus* were observed within 96 hrs, respectively, whereas, 100% mortality was observed at doses above 0.008 ppm. Based on probit analysis, the LC_{50} value of diazinon for 96 hrs of exposure on *Anabas testudineus* was found as 0.001 ppm. During the exposure trial several behavioral alterations including restlessness, sudden and quick movements, loss of equilibrium, increased opercular activities and paralysis were observed in the fish. The present study demonstrated that Nuvan is highly toxic especially to *Anabas testudineus* and thus, indiscriminate use of this insecticide in the agriculture and industrial sectors should be strictly controlled.

Key words: Nuvan, *Anabas testudineus*

Introduction

In India, Nuvan is widely used to control a variety of sucking and leaf eating insects of rice, fruit trees, sugarcane, corn, tobacco, potatoes and other horticultural plants. It is assumed that a certain percent (about 25%) of the used Nuvan is easily washed into surface waters and then enters into ground water. Generally Nuvan degrades rapidly but under conditions of low temperature, low moisture, high alkalinity and lack of microbiological degraders, it may remain biologically active in soils for six months or longer (Hamm and Hinton, 2000). Because of indiscriminate use, Nuvan may severely affect the normal physiology, biology, early development, and sometimes may cause sudden death of fishes and other aquatic organisms (Burkepille *et al.*, 2000; Rashid *et al.*, 2012). However, farmers use Nuvan and such type of other pesticides without considering their immediate and residual impacts on fish and other aquatic organisms. Unfortunately, the production of indigenous fish species especially SIS (small Indigenous species) in natural water bodies decreases very sharply due to various manmade and natural interventions and a number of fish species is going to vulnerable to endangered to critically endangered situation for the last two

decades.

The freshwater fish *Anabas testudineus* is one of the widely distributed. This fish is also available as endemic in India, Pakistan, Myanmar and Far-East (Nazrul, 2004). It has a special preference in the diet of people of India because of its special taste, low fat and high protein content (Islam and Joadder, 2005). Generally this species use shallow inland waters adjacent to the crop cultivation fields as breeding, feeding and nursing ground (Nazrul, 2004). Contamination of the water of those habitats by the huge amount of insecticides or pesticides applied for crop cultivation is considered as one of the crucial causes for decreasing the abundance of *Anabas testudineus*. Toxic effects of these pesticides vary with the degree of exposure and the type and concentration.

As Nuvan is one of the most frequently used pesticides, therefore, its effects on various physiological aspects of different freshwater species have been studied so far (Rahman *et al.*, 2002; Dutta and Meijer, 2003; Ahmed, 2011; Shamoushaki *et al.*, 2012; Rashid *et al.*, 2012; Rouf and Arain, 2013). However, very limited work has been done effects of Nuvan on fishes and there is no report about the effect of Nuvan on *Anabas testudineus*. To protect this species from being extinct through better management it is important to determine the effects of Nuvan on *Anabas testudineus*. Therefore, the present experiment was undertaken to know the toxicity effects, median lethal concentration (LC₅₀) of Nuvan and behavioural changes of *Anabas testudineus* were also identified.

Materials and Methods

To identify the effect of Nuvan on *Anabas testudineus*, A total of 200 *Anabas testudineus* were collected from fishermen stocked in the cisterns. Fish were reared in the cisterns and fed with trash fish 2 times daily. The average length and weight of the fish were 10.30±0.360 cm and 16.455±0.346 g, respectively.

To determine the acute toxicity (LC₅₀) on *Anabas testudineus*, fourteen concentrations (0.00025, 0.0005, 0.001, 0.002, 0.004, 0.008, 0.016, 0.032, 0.064, 0.128, 0.256, 0.512, 1.024, and 2.048 ppm) of nuvan were used. Nine glass aquaria of size 60×30×30 cm³ with water holding capacity of 50 L were used. Desired concentration of Nuvan test solution was prepared by serial dilution of Nuvan.

After acclimatization in the cisterns ten (10) *Anabas testudineus* with the average length of 24.80 cm and average weight of 141 g were released in each aquarium containing different concentrations (treatment) of Nuvan for different times with three replications for each treatment against a control. All tests were done at room temperature. The behavioural and other external changes in the body of *Anabas testudineus* were observed during the exposure periods. Mortality was recorded at 0.5, 1, 3, 6, 12, 24, 36, 48, 60, 72, 84, and 96 hrs of exposure time and dead fishes were removed immediately. The LC₅₀ value for *Anabas testudineus* was calculated for 96 hrs.

Results and Discussion

At 0.512, 1.024, and 2.048 ppm of Nuvan all the fish died within 12 hrs of exposure. Similarly, at 0.016, 0.032, 0.064 ppm and 0.002, 0.004, 0.006, 0.008 ppm of Nuvan all the fish died within 36 hrs and 72 hrs of exposure, respectively. Therefore, the lower concentrations from the acute toxicity test viz. 0.001, 0.0005, and 0.00025 ppm were used to identify the LC₅₀ value of Nuvan for *Anabas testudineus*. The effects of different concentrations and exposure time of Nuvan on *G. giuris* are presented in Table 1. Mortality of all (100%) *Anabas testudineus* was observed at the 0.512 ppm and above doses within 12 hrs exposure period and from 0.008 to 0.256 ppm within 48 hrs of exposure period. At the doses of 0.004, 0.002, 0.001, 0.0005, and 0.00025 ppm of Nuvan, about 90%, 70%, 50%, 40%, and 10% mortality of *Anabas testudineus* were observed within 96 hrs, respectively. All of *Anabas testudineus* were found alive in control treatment. Hence, based on probit analysis the LC₅₀ value of Nuvan was found to be 0.001 ppm for *Anabas testudineus* for 96 hrs of exposure.

During the study period, the behavioural changes and reactions of *Anabas testudineus* were observed to different concentrations of the Nuvan . The first reactions of treated fish were observed within five minutes at the concentrations of 2.048 ppm. Several abnormal behaviours such as restlessness, sudden quick and rolling movement, swimming to the back (at higher doses) were observed. Finally, the affected fish became very weak, settled at the bottom and died. The number of dead *Anabas testudineus* increased with the increasing concentration of Nuvan . The most important effect of the toxicant was observed with lack of equilibrium and spiral swimming pattern of fish. The colour of the fishes also became pale progressively with higher doses at the end of 96 hrs of exposure time. On the other hand, shiny colour and normal behaviour of fishes were observed in the control group.

Table 1. Cumulative mortality of *Anabas testudineus* at nuvan treatments during the experimental period

Concentration (ppm)	Cumulative Mortality (%)											
	30min	1h	3h	6h	12h	24h	36h	48h	60h	72h	84h	96h
0.00025	-	-	-	-	-	-	-	-	-	10	10	10
0.0005	-	-	-	-	-	-	-	10	20	30	40	40
0.001	-	-	-	-	-	-	-	10	30	40	40	50
0.002	-	-	-	-	-	10	30	40	50	50	60	70
0.004	-	-	-	-	10	20	40	50	50	70	80	90
0.008	-	-	-	30	50	80	90	100				
0.016	-	-	-	20	50	80	90	100				
0.032	-	-	-	30	40	80	80	100				
0.064	-	-	-	50	70	80	80	100				
0.128	-	-	30	60	70	80	100					
0.256	-	30	40	80	90	100						
0.512	20	30	60	80	100							
1.024	40	60	70	80	100							
2.048	50	70	80	100								
Control	-	-	-	-	-	-	-	-	-	-	-	-

To determine the LC₅₀ of Nuvan on *Anabas testudineus* 14 different doses were used. The highest and most rapid mortality was observed in fish exposed to the highest concentration of Nuvan tested. The LC₅₀ value of *Anabas testudineus* for 96 hrs was found to be as low as 0.001 ppm. compared to *G. giuris*. On the other hand, Hossain and Halder (1997) found that the median lethal concentration (LC₅₀) of Talstar 2.5 EC on *Labeo rohita* fry was 0.0014 ppm for 48 hrs which is very much similar to the findings of the present study (LC₅₀ of Nuvan on *Anabas testudineus* although the exposure time was less for *Labeo rohita*. Observing of similar LC₅₀ values for two small fish i.e., *Labeo rohita* fry and *Anabas testudineus* suggested that SIS fish species and/or large fish species in their early stages are very vulnerable to the exposure of Nuvan compared to larger ones. Dutta and Meijer (2003) reported that the age of fish, size, and duration of exposure affect the toxicity potential of Nuvan in various fish species and the degree of its sensitivity varied even among the fish of the same genus and family. Rahman *et al.* (2002) also reported that the LC₅₀ value for 96 hrs of exposure to Nuvan for the small fish *Barbodes gonionotus* to be 2.72 ppm which is much higher than the present findings. Phylogenetic difference between the species might be the reason for large variation in the LC₅₀ value. The difference in the toxicity of Nuvan may be attributed to differences in susceptibility and tolerance regarding absorption, biotransformation, and excretion of Nuvan .

Hence, Nuvan is considered a severely toxic substance to *Anabas testudineus* as this fish is very sensitive to the presence of any kind of toxic chemicals. Rahman *et al.* (2002) reported LC₅₀ values of Nuvan for the air breathing fishes, *Anabas testudineus* and *Channa* potential toxic effects of pesticide exposure. In the present study, several abnormal behaviours such as restlessness, loss of equilibrium, increased opercular activities, surface to bottom movement, sudden quick movement, and resting at the bottom were observed. From these studies a similarity was observed in the LC₅₀ values obtained for different larger sized species and these values are significantly higher important effect of Nuvan that was found in the present study may be the alterations of the nervous and brain systems, which was obvious with lack of equilibrium and spiral swimming pattern of *Anabas testudineus* to that found by Shamoushaki *et al.* (2012) on *Rutilus frisii kutum* due to the effect of diazinon.

Conclusion

The result of the present study revealed that Nuvan is toxic to fish. The recorded 96 hrs LC₅₀ value for *Anabas testudineus* is very lower which indicate that *Anabas testudineus* is quite susceptible to Nuvan and its mortality increases proportionally at higher doses. Therefore, strict biosecurity should be taken into consideration when Nuvan is used by the farmers in agriculture fields particularly surrounding aquatic environment.

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