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Acute toxicity of a Biopesticide Spinosad to benthic Oligochaete worm, *Branchiura sowerbyi* and the fry of Common Carp, *Cyprinus carpio.*

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ABSTRACT

Toxicity of a Biopesticide Spinosad to benthic Oligochaete worm, *Branchiura sowerbyi* and the fry of common carp, *Cyprinus carpio* along with their behavioural changes were evaluated in the present study. The 24, 48, 72 and 96 h LC_{50} values of Spinosad to *Branchiura sowerbyi* were 21.19, 12.40, 8.76 and 6.14 mg/l and to the fry of *Cyprinus carpio* were 5.03, 3.20, 2.16, 1.77 mg/l respectively. The mortality rate of *B. sowerbyi* and *C. carpio* showed significant variation ($p<0.05$) with the increasing concentrations at all exposure times. On the other hand, the mortality rate of *B. sowerbyi* and *C. carpio* also varied significantly ($p<0.05$) at all the doses with increasing exposure times (24, 48 and 72 and 96h). Excess mucous secretion, decreasing movements and decreasing clumping tendency were recorded in *B. sowerbyi* at higher concentrations. The excess mucous secretion with increasing concentration of toxicant and exposure time was recorded in the exposed fish. The treated fish showed faster movement and wide opening of mouth and gills at higher concentrations at 24 and 48h of exposure. At 72 and 96h of exposure the fish became sluggish and the movement was gradually reduced with increasing concentration. Similarly, the mouth and gill openings were reduced and were almost absent at the higher concentrations after 72h of exposure. The opercular movement of fish was increased significantly ($p<0.05$) at 24 and 48h but decreased significantly ($p<0.05$) at 72 and 96h.

Keywords: Spinosad, LC_{50}, *Branchiura sowerbyi*, *Cyprinus carpio*, behavioural change, opercular movement.

INTRODUCTION

The pesticides and some of their active ingredients are highly toxic to non-target organisms and the natural ecosystem (Delaplane, 2000). These pesticides come to the adjacent water bodies as agricultural runoff and affect non-target species such as fish which have great economic importance to man (Adhikari *et al*., 2004). At present the new generation biopesticides instead of synthetic pesticides are used in the agricultural field to minimize the toxicity to the non-target organisms. Among these new pesticides, Spinosad (Dow Elanco, Indianapolis, IN, USA) (Thomson *et al*., 2000) is common. It is a mixture of spinosyns A and D which are the fermentation products of the soil bacteria (*Saccharopolyspora spinosa*, Actinomycetes) and is an effective agent of pest control especially for control of Lepidopteran pest (Wanner *et al*., 2000; Brickle *et al*., 2001; Crouse *et al*., 2001). The Spinosad is neurotoxic in nature which acts as contact and stomach poison (Salgado *et al*., 1998).

Spinosad is generally more toxic in nature to pest than other beneficial species (Elzen, 2001), but some studies indicate that Spinosad exhibits toxicity to beneficial species also (Consoli *et al*., 2001). Among aquatic organisms, fish are very susceptible to environmental contamination of water (Naveed *et al*., 2011). Furthermore, fish prefers to consume the benthic oligochaete worm *B. sowerbyi* (Hossain *et al*. 2006). This prey and predator relationship between tubificid worm and fish can accelerate the biomagnifications process of toxicant from lower to higher trophic levels (Dhara *et al*., 2014). As a considerable part of the world food comes from fish, it is necessary to secure the health of fish from pesticide pollution (Tripathi *et al*, 2002). The reports on the potential harmful effect of Spinosad on fish and other aquatic organisms are scanty (Borth *et al*. 1996; www.pesticideinfo.org, Journal: Environmental Fate and Effect division, U.S. EPA. Washington DC). Therefore, the present study was undertaken to evaluate the acute toxicity of commonly used biopesticide Spinosad on benthic Oligochaete worm (*Branchiura sowerbyi*) and fry of Common carp (*Cyprinus carpio*) along with their behavioural changes.

METHODOLOGY

The benthic Oligochaete worm, *Branchiura sowerbyi* (Class: Oligochaeta, Family: Tubificidae; mean length 2.05 ± 0.50 cm; mean weight 2.02 ± 1.20 mg) and the fry of common carp, *Cyprinus carpio* (Order: Cypriniformes and family: Cyprinidae; mean length 4.30± 0.65 cm, mean weight 2.50 ±0.48 gm.) were used as test organisms in the bioassay. The worms were collected from local market and the fish were collected from nearby commercial fish farm. They were acclimatized gradually to the test water for 3 days in the laboratory before experiment. The commercial grade of Spinosad (45% SC) was used as test chemical. Static replacement bioassay was conducted in laboratory. Acute toxicity tests for oligochaete worm were conducted in 500 ml Borosil glass beakers containing 250 ml water. For fish,15l glass aquarium each containing 10l of un-chlorinated tap water was taken to determine the acute toxicity of Spinosad following the methods of earlier workers (American Public Health Association, 2012; Mukherjee and Saha 2013; Sarkar *et al*, 2016). Un-chlorinated tap water (temperature 24.5 ± 0.12 ^{0}C, pH 7.8 ± 0.23, free CO_2 10.9 ± 0.42 mg/l, DO 5.48 ± 0.34 mg/l, alkalinity 169 ± 11.23 mg/l as $CaCO_3$, hardness 115 ± 4.30 mg/l as $CaCO_3$) was used as diluent medium during the study. The fish were not fed for 24h before and during the bioassay.

Each test was accompanied by four replicates with sufficient control. Each replicate was provided with ten organisms. Initially rough range finding experiments were conducted to determine range of doses at which the mortality of worm and fish may occur. Finally, the selected test concentrations of Spinosad were used to *Branchiura sowerbyi* and *Cyprinus carpio* to determine the LC_{50} values at 24, 48, 72 and 96h of exposure. During the experiment the dead organisms were removed quickly to avoid any microbial decomposition causing depletion of dissolved oxygen. The dead organisms were recorded at every 24h.The 10% of the test water was replaced by freshly prepared test water at every 24h to maintain a fixed concentration. The mean opercular movement (number of movement/min/fish) of *Cyprinus carpio* was noted at every 24h during the experiment to evaluate the effects of Spinosad on respiratory rate of fish. The behavioural changes (clumping tendency, irregular movements, excess mucous secretion, and suffocation) of the exposed worms and fish were also recorded during the experiment (Mukherjee and Saha 2013).

Mean mortality of *Branchiura sowerbyi* and *Cyprinus carpio* after 24, 48, 72 and 96h of experiment were used to calculate the LC_{50} values (with 95% confidence limit) through a statistical software, Probit program version 1.5 (US EPA 1999). The values of percent mortality of both organisms and opercular movements of fish were subjected to analysis of variance (ANOVA) using R-software (R Development Core Team, 2011) succeeded by Duncan's Multiple Range Test (DMRT) to find out the significant difference within the mean values at various concentrations of Spinosad at 24, 48, 72 and 96h of exposure.

RESULTS AND DISCUSSION

The 24, 48, 72 and 96 h LC_{50} values of Spinosad to *Branchiura sowerbyi* were 21.19, 12.40, 8.76 and 6.14 mg/l and to the fry of *Cyprinus carpio* were 5.03, 3.20, 2.16, 1.77 mg/l respectively (Table 1). The mortality rate of *B. sowerbyi and C. carpio* showed significant variation ($p<0.05$) with the increasing concentrations at all exposure times (Table 2 and 3). On the other hand, the mortality rate of *B. sowerbyi and C. carpio* also varied significantly ($p<0.05$) with increasing exposure times (24, 48 and 72 and 96h) at all the doses (Table 2 and 3).

Excess mucous secretion, decreasing movements and reduced clumping tendency were recorded in *B. sowerbyi* at higher concentrations (Table 4). The excess mucous secretion with increasing concentration of toxicant and exposure time was recorded in the exposed fish (Table 5). The treated fish showed faster movement and wide opening of mouth and gills at higher concentrations at 24 and 48h of exposure (Table 5). At 72 and 96h of exposure the fish became sluggish and the movement was gradually reduced with increasing concentration. Similarly, the mouth and gill openings were reduced and were almost absent at the higher concentrations after 72h of exposure. The opercular movement of fish was increased significantly ($p<0.05$) at 24 and 48h but decreased significantly ($p<0.05$) at 72 and 96h (Table 6).

Table 1: LC_{50} values (with 95% confidence limits) of **Spinosad** to the *Branchiura sowerbyi* and *Cyprinus carpio* at different times of exposure (24, 48, 72 and 96h)

Test organisms	Concentration (mg/l)			
	24h	*48h*	*72h*	*96h*
Branchiura sowerbyi	**21.19** (17.37-25.49)	**12.40** (7.87-17.51)	**8.76** (5.99-11.5)	**6.14** (3.98-8.22)
Cyprinus carpio	**5.03** (4.37-5.88)	**3.20** (2.48-3.99)	**2.16** (1.58-2.74)	**1.77** (1.30-2.24)

Table 2: Mean values (±SD) of % mortality of *Branchiura sowerbyi* exposed to different concentrations of Spinosad at different times of exposure (24, 48, 72 and 96h). Mean values within columns indicated by different superscript letters (a-h) and mean values within rows indicated by different superscript letters (m-p) are significantly different (DMRT at 5% level)

Dose (mg/l)	% mortality of worm at different times of exposure (h)			
	24h	***48h***	***72h***	***96h***
3.2	00^{am}±0.00	20^{an}±0.50	20^{an}±0.43	30^{ao}±0.50
3.6	00^{am}±0.00	30^{bn}±0.43	30^{abn}±0.43	40^{bo}±0.43
7.2	10^{bm}±0.00	40^{bcn}±0.83	40^{bcn}±0.43	50^{co}±0.43
10.8	20^{cm}±0.43	40^{bcn}±0.43	50^{cdo}±0.43	60^{dp}±0.50
14.4	30^{dm}±0.50	40^{bcm}±0.83	60^{den}±0.43	70^{en}±0.50
18.0	30^{dm}±0.50	50^{cn}±0.43	60^{deo}±0.43	80^{fp}±0.00
21.6	40^{em}±0.43	50^{cn}±0.43	70^{efo}±0.83	90^{gp}±0.43
27.0	50^{fm}±1.19	50^{cm}±0.83	80^{fn}±2.17	100^{hn}±0.43
30.6	70^{gm}±0.00	70^{dm}±0.43	100^{gn}±0.50	100^{hn}±0.00
36.0	70^{gm}±0.71	80^{en}±0.43	100^{go}±0.00	100^{ho}±0.00
36.2	90^{hm}±0.43	100^{fn}±0.43	100^{gn}±0.00	100^{hn}±0.00

Table 3: Mean values (± SD) of % **mortality** of ***Cyprinus carpio*** exposed to different concentrations of **Spinosad** at different times of exposure (24, 48, 72 and 96h). Mean values within columns indicated by different superscript letters (a-h) and mean values within rows indicated by different superscript letters (m-p) are significantly different (DMRT at 5% level)

Dose (mg/l)	% mortality of fish (*Cyprinus carpio*) at different times of exposure (h)			
	24h	***48h***	***72h***	***96h***
0.72	00^{am}± 0.00	10^{an}± 0.50	20^{ao}± 0.50	20^{ao}± 0.43
0.9	00^{am}± 0.00	20^{bn}± 0.43	30^{bo}± 0.71	40^{bp}± 0.50
1.8	10^{bm}± 0.50	30^{cm} ± 0.43	50^{co}± 0.50	50^{co}± 0.43
2.7	10^{bm}± 0.00	30^{cn} ± 0.43	50^{co}± 0.83	60^{dp}± 0.83
3.15	20^{cm}± 0.50	30^{cn}± 0.83	50^{co}± 0.5	60^{dp}± 0.50
3.6	20^{cm}± 0.43	40^{dn} ± 0.43	60do± 1.22	70^{ep}± 0.83
4.5	30^{dm}± 0.50	60^{en} ± 0.50	70^{eo}± 0.71	80^{fp}± 0.50
5.4	40^{em}± 0.50	60^{en}± 0.43	70^{eo}± 0.71	80^{fp}± 0.83
5.85	50^{fm}± 0.43	70^{fn}± 0.83	90^{fo}± 0.43	90^{go}± 0.43
6.0	50^{fm}± 0.71	80^{gn}± 0.50	100^{go}±0.50	100^{ho}±0.00
6.2	70^{gm}± 0.50	80^{gn}± 0.83	100^{go}±0.43	100^{ho}±0.00
6.4	80^{hm}± 0.50	90^{hn}± 0.43	100^{go}±0.00	100^{ho}±0.00

Table 4: Behavioural responses of ***Branchiura sowerbyi*** (CT= Clumping Tendency, M=Movement, MS= Mucous Secretion, –: absent, +: mild, ++: moderate, +++: high, X= not recorded due to death) exposed to different concentrations of **Spinosad** at different times of exposure

Dose (mg/l)	**Behavioural responses of worm at different times of exposure**											
	24h			***48h***			***72h***			***96h***		
	CT	M	MS	CT	M	MS	CT	M	MS	CT	M	MS
0.0	+++	++	-	+++	++	-	+++	++	-	+++	++	-
3.2	+++	++	-	+++	++	-	+++	++	-	+++	++	-
3.6	+++	++	-	+++	++	-	+++	++	-	++	+	-
7.2	+++	++	-	+++	++	-	++	+	-	+	+	+
10.8	+++	++	-	++	+	-	++	+	-	+	-	+
14.4	++	++	-	++	+	-	+	+	+	+	-	+
18.0	++	+	-	++	+	-	+	-	+	-	-	+
21.6	++	+	-	+	+	+	+	-	+	-	-	+
27.0	++	+	-	+	+	+	-	-	+	-	-	+
30.6	+	+	+	+	+	+	-	-	+	X	X	X
36.0	+	+	+	-	-	+	-	-	+	X	X	X
36.2	+	+	+	-	-	+	X	X	X	X	X	X

Table 5: Behavioural responses of *Cyprinus carpio* (MS=Mucous Secretion, WOMG=Wide Opening of Mouth & Gills, M= Movement, –: absent, +: mild, ++: moderate, +++: high, X= not recorded due to death) exposed to different concentrations of **Spinosad** at different times of exposure

Dose (mg/l)	**Behavioural responses of fish at different times of exposure**											
	24h			**48h**			**72h**			**96h**		
	MS	WOMG	M	MS	WOMG	M	MS	WOMG	M	MS	WOMG	M
0.0	-	+	+	-	+	+	-	+	+	-	+	+
0.72	-	+	+	-	+	+	-	+	+	-	+	+
0.9	-	+	+	-	++	+	-	++	++	-	++	++
1.8	-	++	++	-	+++	++	-	++	++	-	++	++
2.7	-	++	++	-	+++	++	-	++	++	+	+	+
3.15	-	++	++	-	+++	++	-	++	++	+	+	+
3.6	-	++	+++	-	+++	+++	+	++	++	+	-	+
4.5	-	+++	+++	+	++	+++	+	+	+++	++	-	-
5.4	+	+++	+++	+	++	+++	+	+	++	++	-	-
5.85	+	++	+++	+	+	++	+	+	++	++	-	-
6.0	+	++	+++	+	+	++	+	-	+	X	X	X
6.2	+	++	+++	+	+	++	++	-	+	X	X	X
6.4	+	++	+++	+	+	++	++	-	+	X	X	X

Table 6: Mean values (±SD) of **opercular movement**/minute of ***Cyprinus carpio*** exposed to different concentrations of **Spinosad** at different times of exposure (24, 48, 72 and 96h). Mean values within columns indicated by different superscript letters (a-i) and mean values within rows indicated by different superscript letters (m-p) are significantly different (DMRT at 5% level)

Dose mg/l	Opercular movement of fish at different times of exposure/minute			
	24h	***48h***	***72h***	***96h***
0.0	86^{cp}±1.0	72^{bm}±0.83	82^{fo}±0.43	77^{gn}±0.71
0.45	90^{do}±0.83	82^{cn}±0.83	75^{em}±0.71	82^{hn}±0.71
0.72	72^{bn}±0.83	69^{am}±1.29	75^{eo}±0.83	70^{fm}±1.0
0.9	60^{am}±0.71	72^{bo}±1.09	82^{fp}±0.71	66^{en}±0.83
1.8	72^{bn}±0.71	90^{eo}±0.43	60^{cm}±0.71	61^{cm}±0.71
2.7	86^{co}±1.0	86^{do}±0.83	66^{dn}±1.29	64^{dm}±0.71
3.15	95^{eo}±1.22	90^{en}±1.48	61^{cm}±0.71	61^{cm}±1.0
3.6	100^{fo}±0.71	100^{fo}±0.83	60^{cm}±0.43	63^{dn}±0.43
4.5	105^{gn}±0.83	110^{go}±1.29	58^{bm}±0.83	57^{bm}±0.71
5.4	116^{ho}±1.29	112^{hn}±1.58	57^{abm}±0.83	57^{bm}±0.43
5.85	120^{in}±0.83	120^{in}±0.83	56^{am}±0.83	55^{am}±0.43

In the present study the LC_{50} values indicate that *Cyprinus carpio* is more susceptible to Spinosad than *Branchiura sowerbyi*. The 96h LC_{50} value of Spinosad to fish (1.77 mg/l) also indicates that the fry of *Cyprinus carpio* is more sensitive to Spinosad than Carp (5 mg/l), juvenile Dagger blade grass Shrimp (*Palaemonetes pugio*) (9.76 mg/l), Sheepshead minnow (*Cyprinodon variegatus*) (7.9 mg/l), Bluegill (*Lepomis macrochirus*) (5.9 mg/l), and Rainbow trout (*Oncorhynchus mykiss*) (30 mg/l) (Borth *et al*., 1996; www.pesticideinfo.org, Journal: Environmental Fate and Effect division, U.S. EPA. Washington DC).

The different abnormal behaviours (table 4 and 5) like excess mucous secretion, erratic movements and suffocation observed in the present study were probably due to the enzymatic as well as ionic alteration in tissues and blood (Larsson *et al*. 1981). The clumping tendency of worm varies inversely with increasing pesticide concentrations and exposure times. The excessive mucous secretion with loss of balance and movement was observed both in worm and fish at higher pesticide concentrations at 72 and 96h of exposure. This was probably an early indication of their avoidance reaction from the pesticide. The toxicity of Spinosad to worm was probably due to the formation of mucous-toxicant complex which precipitates over the body of worm and blocks the exchange of O_2 and CO_2 (Whitley, 1967). The excess mucous secretion over the body of treated fish was probably due to the dysfunction of pituitary gland (Pandey *et al*. 1990). The changes in behaviour of the exposed fish were probably due to acclimatization by a compensatory mechanism to obtain energy and to escape from stress caused due to toxicity (Joshi 2011).

CONCLUSION

The present findings may provide additional data on the toxicity of Spinosad which will help in the proper management activities for natural water resources in respect to the input of this Biopesticide from the agricultural field.

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