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Toxicological studies on the effect of herbicide (atrazine) on the fecundity and moulting of a tropical soil isopod- *Cylisticus convexus*

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Abstract
Soil isopods plays critical role to increase soil fertility. Indiscriminate use of Herbicides on agricultural purposes resulted deleterious effects on the biology and species diversity of soil arthropods. The effect of sublethal concentration of a herbicide atrazine, were treated to find out the fecundity and moulting of a soil isopod (Cylisticus convexus). The LC 50 and LC 100 values of Cylisticus convexus were calculated. The safe and sublethal concentrations of atrazine were very low indicating high vulnerability of these isopods to agrochemicals. The safe level concentration of atrazine was 0.2897ppm. The moulting intervals of Cylisticus convexus showed variation between herbicide treated and normal Cylisticus convexus. Herbicide treated C. convexus showed drastic reduction in fecundity and also their moulting intervals were prolonged.

Keywords: atrazine, isopod, *Cylisticus convexus*, herbicide, LC 50, LC 100

Introduction
Soil is contaminated by different xenobiotics arising from sectors like agriculture, industry, transport etc. Modern agricultural practices, such as, use of heavy machinery for tillage operation, chemical fertilizers and pesticides have led to severe impacts on the soil ecosystem. A major portion of these chemicals may persist in the soil and affect non-target and ecologically important organisms. Among these impacts the reduction in soil biodiversity and degradation of soil quality are often viewed as major threats for the future (Solbrig, 1991) [13]. Land use change and agricultural intensification generate severe habitat degradation or destruction for soil biota (Decaens *et al.*, 2006) [3]. A multi level approach in toxicity testing with terrestrial isopods has previously been successfully used to identify the hazard of different pesticides and nanomaterials (Stanek *et.al,.2006;* Drobne *et.al,*. 2009) [15], Due to their important ecological role as decomposers of organic material, terrestrial isopods are widely accepted as test organisms in terrestrial ecotoxicology and ecophysiology (Drobne,1997; Lokke and Vangestel,1998; Lapanje *et al,*.2007). The objective of the present study is to assess the toxicity of Atrazine ($C_8H_{14}ClN_5$) on the fecundity and moulting of a tropical soil isopod-*Cylisticus convexus*.

Materials and Methods
Collection and rearing of Cylisticus convexus
Cylisticus convexus were collected from the study area. They were transferred into culture chambers of 5×3.5 cm with a mixture of Plaster of paris and activated charcoal in the ratio 5:1 as base (Sinder, 1973). Baker's yeast were provided as food for hatching organism along with water soaked decaying leaf bits. Mature animals were separated and reared in subculture bottles. They were acclimatized in the laboratory condition for about 20 days prior to experiment.

Preparation of Stock Solution of Atrazine
1000 ppm stock solution of Atrazine was prepared by dissolving required quantity of chemicals in one liter of distilled water (APHA, 2012) [1]. From this stock solution various concentration like 1, 1.5, 2, 2.5, 3 and 3.5 ppm Atrazine were prepared for bioassay.

Bio assay
Experiments conducted in laboratory using cultured animals. Ten replicates of *C. convexus* and a control were tested for each concentration of this agro chemicals. The mortality was recorded at 12, 24, 48, 72 & 96 hours intervals. Bioassay lethal concentrations LC 100 and LC 50 were calculated using probit analysis of Finney (Finney, 1980); Safe concentrations were determined by the method suggested by Hart *et al*., (1945) [8]. Total fecundity was calculated by counting the number of eggs in total oviposition and compared the normal group fecundity with atrazine treated groups. Moulting interval of normal and agrochemical treated groups were also recorded. A pair of subadults of ten replicates were exposed to sub lethal concentration for 15 days and the fecundity was observed, compared the fecundity with normal group

Result and Discussions

Mortality

Table 1: Percentage mortality of *Cylisticus convexus* treated with herbicide, Atrazine

	Percentage mortality				
Con: in ppm	12 hr	24 hr	48hr	72 hr	96 hr
1	6.9	16.9	27.8	32.8	40.6
1.5	12.8	18.9	31.8	44.9	42.6
2	22.8	28.7	37.6	48.6	56.5
2.5	24.7	45.9	44.8	56.8	86.9
3	42.7	53.8	66.9	82.8	96.2
3.5	44.8	60.7	74.8	94.8	100

Cylisticus convexus treated with atrazine were found that at 1 ppm, the mortality started. The mortality was recorded at 12, 24, 48, 72 and 96 hours intervals. 27,31, 37, 44, 66 and 74 percentage mortality were recorded for atrazine concentration of 1,1.5,2,2.5,3 and 3.5 ppm at 48 hours intervals. 32, 44, 48, 56, 82 and 94 percentage mortality was found at 72 hours intervals. 40, 42, 56, 86, 96, and 100 percentage mortality was recorded for 1,1.5,2,2.5,3 and 3.5 ppm concentration of atrazine at 96 hours intervals (Table 1). The result showed that the mortality rate of *Cylisticus convexus* increases with increase in concentration of herbicide, atrazine.

Table 2: Lethal Concentration 50(LC 50) and Lethal Concentration 100(LC 100) values of atrazine for *Cylisticus convexus*

Atrazine	12 hr	24 hr	48hr	72 hr	96 hr
LC 50	3.6887	2.8697	2.3770	1.8654	1.5683
LC 100	6.6587	5.3447	4.7120	3.7654	3.2333

Lethal concentration 50 of Herbicide, atrazine treated *Cylisticus convexus* were 3.6887 ppm at 12 hours, 2.8697 ppm at 24 hours, 2.3770 ppm at 48 hours, 1.8654 ppm at 72 hours and 1.5683 ppm at 96 hours. LC 100 values of atrazine treated *C.convexus* were calculated to be 6.6587, 5.3447, 4.7120, 3.7654 and 3.2333 ppm at 12, 24, 48, 72 and 96 hours intervals (Table 2). The safe level concentration of Herbicide, atrazine was 0.2897 ppm and its sublethal concentration found to be 0.4020 ppm. (Table 3).

Table 3: Safe level and sublethal concentrations of Atrazine for *Cylisticus convexus*

Herbicide	S	SF (ppm)	Sublethal (ppm)
Atrazine	1.27	0.2897	0.4020

Fecundity

Table 4: Fecundity of normal *Cylisticus convexus*

Replicates	Oviposition 1	Oviposition 2	Oviposition 3	Oviposition 4	Oviposition 5	Mean
1	25	27	23	18	16	21.8
2	23	20	25	21	18	21.4
3	23	25	21	16	13	19.6
4	25	22	19	17	12	19
5	21	22	19	16	14	18.4
6	23	21	22	17	12	19
7	24	26	25	21	17	22.6
8	22	25	23	19	14	20.6
9	25	24	20	19	14	20.4
10	24	26	24	18	13	21

The maximum fecundity of normal *C.convexus* was seen in oviposition 2 in each groups. The maximum fecundity was observed in the group 1 & 7, 22 eggs per broods and minimum was recorded in group 5, 18 eggs per broods (Table 4).

Table 5: Fecundity of *Cylisticus convexus* after treatment with sublethal concentration of Atrazine

Replicates	Oviposition 1	Oviposition 2	Oviposition 3	Oviposition 4	Oviposition 5	Mean
1	8	9	6	7	8	7.6
2	5	6	7	7	6	6.2
3	9	10	6	6	9	8

4	8	10	11	8	7	8.8
5	8	7	6	8	7	7.2
6	11	11	8	9	6	9
7	9	11	11	9	8	9.6
8	10	10	8	10	9	9.4
9	9	12	8	6	8	8.6
10	11	9	11	8	8	9.4

The average number of eggs laid by *C.convexus* after the treatment of sublethal concentration of atrazine was observed between a minimum of 6.2 eggs/broods and maximum of 9.6 eggs /broods. After the treatment of atrazine the number of eggs produced by females were less. This means that the herbicide affect drastically on isopods (Table 5).

Moulting Interval

Table 6: Normal moulting interval of *Cylisticus convexus*

Stage	Sample 1 (Days)	Sample 2 (Days)	Sample 3 (Days)	Sample 4 (Days)	Sample 5 (Days)	Sample 6 (Days)	Sample 7 (Days)	Sample 8 (Days)	Sample 9 (Days)	Sample 10 (Days)	Group mean (Days)
Rest stage	5.3	4.5	4.5	5.3	4	5.3	5.5	4.7	4.8	5	4.8
1st moult	13.26	14.23	12.25	13.3	14.25	14.3	12	13	13.65	13	13.324
2nd moult	17	18	18	18.2	18	17.5	18.5	17	18.23	17	17.743
3rd moult	20.56	21	21.5	22	21	22.54	21.26	21.32	20.45	20	21.163
4th moult	30.23	29.25	30.35	30	31	29.3	31	29.5	30	30.26	30.089
5th moult	37.12	38.15	38.56	37	38	37	38.25	37.13	38.42	37.5	37.713

Normal *Cylisticus convexus* showed 5 moults, the first moult started after 5 days of hatching, second moult after 13 days of hatching, third moult after 20 days of hatching, fourth moult after 30 days of hatching and fifth moult after 37 days of hatching (Table 6).

Table 7: Moulting interval of *Cylisticus convexus* after treatment with Atrazine

Stage	Sample 1(Days)	Sample 2(Days)	Sample 3(Days)	Sample 4(Days)	Sample 5(Days)	Sample 6(Days)	Sample 7(Days)	Sample 8(Days)	Sample 9(Days)	Sample 10(Days)	Group mean(Days)
Rest stage	6	6.7	6.5	7	6.8	6.94	7.46	6	7	7.28	6.768
1st moult	18.23	17.25	18	17	17.5	18.43	16.5	17.32	16.9	17.8	17.493
2nd moult	27.23	28	28.54	28	27.5	28.5	27.4	28.63	27.56	28.4	27.976
3rd moult	33.12	33.6	32.16	33	33.5	32.86	32.56	33.25	33.38	32.5	32.993
4th moult	42.56	41.45	41.26	42.22	42	40.36	42.15	41	42.5	40.5	41.6
5th moult	52.3	50.23	51.48	50.2	50.5	51.13	49.64	52.11	50.2	52.36	51.015

After the treatment with the sub lethal concentration of atrazine, the moulting intervals of *C.convexus* became prolonged. First moult only being after 17 days, second moult on 27 days, third moult on 32 days, fourth moult on 41 days and fifth moult on 51 days of hatching (Table 7).

Conclusion

Indiscriminate use of toxic agrochemicals has led to the contamination of soil as well as water resources and subsequent loss of population of many useful terrestrial and aquatic organisms. Another important consequence of the use of agrochemicals is the bioaccumulation of these toxins by the soil invertebrates (Sun *et al*., 2005) [14] and the rebyaffecting their physiology (Drobne *et al*., 2008) [4]. The soil dwelling organisms do a responsible function in the ecosystem by organic matter breakdown, nutrient cycling and soil structure stability. Agrochemicals have long been used in agriculture to control pests and diseases in crops and thereby increasing agricultural production. However most of them are toxic to non-target species and may cause negative impacts on beneficial soil macro invertebrates. In the present study it is clear that the extensive application of herbicide affects the reproductive capacity of *Cylisticus convexus.* The body of *C.convexus* is covered with thin cuticle so that it can easily absorb the toxic herbicide through their body surface. The study shows a drastic reduction in the fecundity of herbicide treated *Cylisticus convexus.* Moulting interval of this species were prolonged due to the effect of atrazine. The LC 50 and LC 100 values obtained in the present study underlined the toxicity of this

chemical to soil organism like *C.convexus.* Also the residual remains of this agrochemical in the soil pose a threat to the habitat of soil isopods.

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