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Effect of herbicides on *Cyphoderus javanus* (Hexapoda: Collembola) under laboratory conditions

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Abstract
Cyphoderus javanus was used to evaluate toxicity of two herbicide formulations Pretilachlor (50 EC) and Pendimethalin (30 EC) under laboratory conditions. LC_{50} and LT_{50} value of Pretilachlor on *Cyphoderus javanus* could not be established at Recommended Agricultural Dose (RAD) of the chemical during an exposure period of 24 hrs. Twenty four hours LC_{50} value of Pendimethalin was less than its corresponding RAD and LT_{50} value was 7 hours. Significant reduction in hatching success was noted with the application of the herbicide formulation of Pendimethalin at all sublethal doses except 1/10th of LC_{50} in comparison to control. Juveniles of *Cyphoderus javanus* exposed to 1/6th, 1/8th and 1/10th of LC_{50} for Pendimethalin survived and exhibited increased moulting frequency in comparison to control. Test specimens required significantly less time to attain sexual maturity exposed to Pendimethalin in comparison to control.

Keywords: *Collembola, hatching success, herbicides, LC_{50}, moulting*

1. Introduction
Several works have been done to find out the impact of herbicides that are frequently used in agro-farming on non-target soil organisms like collembolans. However, little data is available for the tropical conditions. Notable contributions among them are Bhattacharya T *et al. and* Bhattacharya T *et al. and* Joy VC *et al. and* Bandyopadhyaya I and Park EK *et al. and* Amorim MJ *et al. and* Frampton GK *et al. and* Lins VS [3, 4, 15, 17, 2, 18, 1, 12, 16]. There are evidences that collembolans are more susceptible to pesticides than other soil arthropods. In the present study, the effects of two selected herbicide formulations were evaluated on *Cyphoderus javanus* (Collembola: Hexapoda) under laboratory conditions. Herbicide formulations tested were Pretilachlor and Pendimethalin which were easily available in local markets and commonly used by farmers. Main purpose of present study was to evaluate the comparative toxicity of the two herbicide formulations on *Cyphoderus javanus* and their potential to damage soil ecosystems.

2. Materials and Methods
2.1 Period of study
The period of study was premonsoon and postmonsoon season of 2012 and 2013.

2.2 Collection and Extraction of Test Specimens
C. javanus were collected from the nearby soil having good amount of organic matter around Midnapore town (West Bengal, India) that has never been used for agriculture purpose and free from herbicide treatment.
Extraction was done using "Tullgreen apparatus". Polythene rearing jars (6.5 cm diameter and 7.5 cm height) were used to maintain stock culture on soil medium (sterilization by sun-dry technique). Bakers yeast was given to the animals as food. Culture jars were kept inside B.O.D incubator at 28±0.5 °C. Moisture of the soil was maintained by adding distilled water from time to time.

2.3 Experimental Apparatus
Inert polythene containers measuring 2.0 cm diameter and 3.0 cm height were used as treatment vials.

2.4 Experiment Medium and its Properties

Natural grassland soil (sandy loam) that has never been used for any agricultural purpose was used as the test soil. The physiological parameters of test soil (organic carbon, pH and water holding capacity) were determined following the methods of [19] and [13].

2.7 Herbicides used

Commercially available herbicide formulations were used in the present investigation. Technical information of the herbicide formulations used in the present study are given in Table 1.

Table 1: Herbicide formulations used

a	Trade Name	Formulation	Manufacturer	RAD* (g a.i/ha)
Pretilachlor	Prince	50EC	Krishi Rasayan Exports Pvt. Ltd. Samba, Jammu & Kashmir	500
Pendimethalin	Kristop	30EC	Krishi Rasayan Exports Pvt. Ltd. Samba, Jammu & Kashmir	1250

* Recommended Agricultural Dose

2.5 Treatments

Different dilutions of the herbicide formulations based on their recommended agricultural doses as provided by the manufacturer (viz. RAD, ½ x RAD, ¼ x RAD, $^1/_6$ x RAD, $^1/_8$ x RAD and $^1/_{10}$ x RAD) were used to determine the LC_{50} of the selected herbicide formulations on *C. javanus*. For determination of LT_{50}, recommended agricultural doses of the herbicide formulations were applied. To study the effects of the sub-lethal doses of the herbicide formulations on hatching success, moulting and maturity of the test organisms, doses based on their respective LC_{50} values (i.e. ½ x LC_{50}, ¼ x LC_{50}, $^1/_6$ x LC_{50}, $^1/_8$ x LC_{50} and $^1/_{10}$ x LC_{50}) were applied. Appropriate amount (0.65 ml dilution of herbicide formulations in 2 g of soil) of herbicide formulations were applied in treatment vials 15 minutes before the incorporation of test animals for smooth spreading of herbicide formulations. Ten matured test animals of the same age group and size were introduced in each replicate. No food was provided during 24 h exposure. Vials were kept inside B.O.D incubator at 28±0.5 °C. Three replicate vials and control sets were maintained at each level of experiment. In case of determination of LC_{50}, mortality was assessed after 24 h period. Observations were made every hour following the methods of [6] for the determination of LT_{50}. Specimens who showed no apparent sign of life when touched with a needle were considered dead.
For the investigation of life cycle parameters exposed to sublethal doses, adult specimens in experimental vials (maintained at control set) were incorporated and reared. Adults were removed after laying of eggs and then sublethal doses of the herbicide formulations were applied to record the effects of the herbicides on hatching success, moulting and maturity of the test organisms. During the experimental period bakers yeast was given as food after hatching of the eggs.

2.6 Statistical Analysis

Mortality data from the 24 h exposure were analysed by probit analysis using EPA Probit Analysis Programme, version 1.5 (US EPA, 2006) for the determination of LC_{50}. The data on percentage on percentage of hatching success for each herbicide formulation was analysed by single factor ANOVA followed by least significance difference (LSD) test to establish significant variation between treatments at 5% of probability. Data of frequency of moulting and duration for sexual maturity were analysed by *'f'* test.

3. Results

Physiochemical properties of the test soil are shown in Table 2.

Table 2: Physiochemical properties of test soil

Physiochemical Properties	Value
Organic carbon	1.5%
pH	6.2
Water holding capacity	34%

Mortality was not found in the control sets. Test animals in the control sets showed normal activities. In respect of 24 h LC_{50} value (Table 3), Pretilachlor with maximum 30% mortality at RAD during 24 hrs exposure period did not achieve LC_{50} value and was found less toxic than Pendimethalin (LC_{50}-581.0 g a.i./ha) and was not used for further study of life cycle parameter at sub-lethal (below LC_{50}) doses. There was significant difference between LC_{50} value and with the RAD of herbicide formulations of Pendimethalin (p< 0.05). LT_{50} value of the herbicide formulations at respective RAD (Figure 1) showed that Pretilachlor did not attain the LT_{50} during 24 hrs of exposure period showing significant heterogeneity with Pendimethalin (p< 0.05) having LT_{50} of 7 hours.

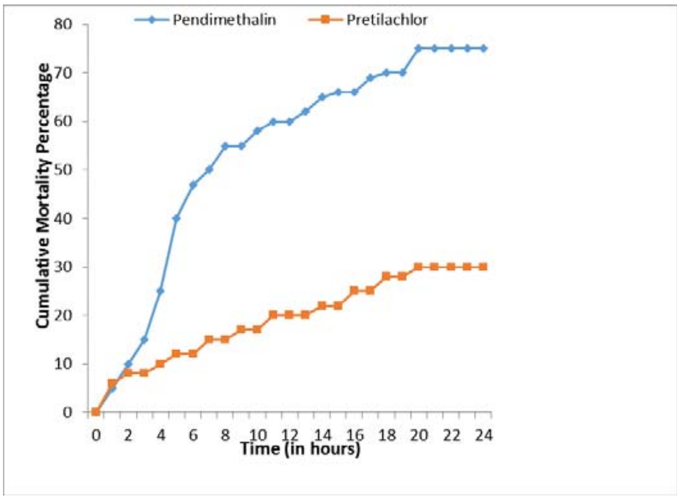

Fig 1: Mean lethal time of *Cyphoderus javanus* at RAD doses of two selected herbicides Pendimethalin and Pretilachlor

Table 3: LC_{50} values of two selected herbicides on *Cyphoderus javanus*

Herbicide	24 h LC_{50} (g a.i/ha)	95% confidence limit
Pendimethalin	581	465-782
Pretilachlor	Not established	

Mean value of the life span of *C. javanus* in the control was found to be 53±1.36 days. A significant reduction of 7%, 13% and 17% of life span were noticed in $^1/_{10}$of LC_{50}, $^1/_8$ of LC_{50} and $^1/_6$ of LC_{50} respectively. Mean value of eggs laid/female specimen and hatching success were 25±2.56 and 80.0±1.0% respectively in the control. In the lowest applied dose ($^1/_{10}$ x LC_{50}) hatching success attained almost the same value as control for the herbicide formulation of Pendimethalin (Table 4). The values showed 81.3% reduction in hatching success for the highest applied dose (½ x LC_{50}) for Pendimethalin.

Table 4: Hatching success percentage of *Cyphoderus javanus* at control and in different sublethal doses of Pendimethalin.

Treatment	Dose (g a.i/ha)	Hatching success (%)*
0	0.00	80.00±1.00^a
$^1/_{10}$ x LC_{50}	58.1	77.00±1.50^a
$^1/_8$ x LC_{50}	72.6	63.25±1.25^b
$^1/_6$ x LC_{50}	96.8	43.00±1.00^c
¼ x LC_{50}	145.2	20.50±1.50^d
½ x LC_{50}	290.5	15.25±1.05^e

* 1. Values are expressed as mean ± S.D.
2. n= 30 eggs per vial, mean value of 3 vials were considered
3. Different superscript letters in the same column denote significant difference at p < 0.05

It was observed that after hatching, juveniles produced exuvia in every 5.0±2.5 days in control where as in treated vials significant increase of exuvia production observed. The insects did not survive in ¼ x $LC_{50}$and ½ x LC_{50}. About 30%, 25% and 6% increased of exuvia production observed in $^1/_6$ x LC_{50}, $^1/_8$ x LC_{50} and $^1/_{10}$ x LC_{50} respectively per week. They attained sexual maturity (egg laying) after 8th moulting (40 days). In experimental sets, juveniles did not survive the doses of ¼ of LC_{50} and ½ of LC_{50} in Pendimethalin. The sublethal doses at which juvenile survived to produce exuvia did so after every 4 days and attained sexual maturity after 7th moulting in 30 days. There was significant difference in duration of the instar stages and sexual maturity between test and control sets ($p<0.05$) of the test specimens in the herbicide formulation.

4. Discussion

Several researchers have observed the negative impact of herbicides on collembolan species [14, 17, 5]. The findings of present investigation on two selected herbicides formulations, Pretilachlor and Pendimethalin also exhibited their toxic effect on *C. javanus*. Pretilachlor was found toxic to the test species while Pendimethalin (LC_{50} 581 g a.i/ha) was found highly toxic with respective to their respective RAD (500 and 1250 g a.i/ha, respectively) at $p<0.05$. Similar toxicity was also found in case of the LT_{50} value where Pretilachlor did not attain LT_{50} in 24 hrs exposure and Pendimethalin attained LT_{50} in 7 hrs. [12] Noticed the sensivity of collembolan against different herbicides. Crouau Y *et al.* [8] investigated the sensitivity of growth and reproduction of four xenobiotics of *Folsomia candida* and found the negative effect of these chemicals on several life cycle parameters of *Folsomia* sp. [20] noticed negative role of herbicide dinoseb on survival, growth and reproduction of *F. candida*. Chakravorty PP *et al.* [7] Reported reduction of hatching success on collembolan *Cyphoderus* species exposed to sublethal doses of insecticides under laboratory conditions. Haque A *et al.* [11] also found reduction of hatching success on collembolan *Xenylla welchi* exposed to sublethal doses of herbicides Pendimethalin and Pretilachlor. Significant decrease in hatching success due to exposure to these herbicides on *C. javanus* was also recorded in the present investigation.

Eijsackers H *et al.* [10] reported shorter life span, decreased egg laying and increased frequency of moulting due to impact of herbicide 2, 4, -5T on collembola. Chakravorty PP *et al.* [7] and Haque A *et al.* [11] reported shorter life span, increased frequency of moulting and early maturity on *Cyphoderus* species (collembola) exposed to different insecticides and herbicides respectively. Similar results were found in the present investigation where significant variation was observed between control and test data ($p< 0.05$) with respect to moulting frequency and time to attain sexual maturity of *C. javanus* when exposed to sublethal doses of Pendimethalin.

Thus it can be concluded from the present study that based on LC_{50} and LT_{50} values of the herbicides formulations on *C. Javanus* Pretilachlor was found less toxic than Pendimethalin and at sublethal doses only Pendimethalin adversely affected the life cycle of *C. javanus* indicating its potential role in damaging the soil ecosystem.

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