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Effect of Two Herbicides on *Xenylla Welchi* (Hexapoda:Collembola) Under Laboratory Conditions

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Abstract *Xenylla welchi* was used to evaluate toxicity of two herbicide formulations, pretilachlor (50 EC) and pendimethalin (30 EC) under laboratory conditions. Twenty four hours LC_{50} value of pretilachlor and pendimethalin formulations on *Xenylla welchi* were 72.7 and 190.0 g a.i/ha respectively which were less than their corresponding recommended agricultural doses. Again pretilachlor attained fastest LT_{50} (110 min) followed by pendimethalin (140 min). Significant reductions in hatching success were noted with the application of both the herbicide formulations at all doses excepting $1/_8$ and $^1/_{10}$th of LC_{50} (9.1, 7.3 and 23.8, 19.0 g a.i/ha for pretilachlor and pendimethalin, respectively). Hatching success of the test specimens recorded 44.1 and 63.3% reduction from control for the highest applied dose (½ of LC_{50}) of pretilachlor and pendimethalin, respectively. Juveniles of *Xenylla welchi* exposed to $^1/_6$, $^1/_8$ and $^1/_{10}$th LC_{50} for pretilachlor (12.1, 9.1, 7.3 g a.i/ha) and $^1/_8$ and $^1/_{10}$th LC_{50} for pendimethalin (23.8, 19.0 g ai/ha) survived and exhibited increased moulting frequency (7 moultings in 28 days in both the herbicide treatments) in comparison to control (8 moulting in 42 days). Test specimens required 26.0 ± 1.2 and 28.1 ± 2.1 days to attain sexual maturity exposed to pretilachlor and pendamethalin respectively which was significantly less than control (42 ± 2.6 days).

Keywords Collembola · Herbicides · LC_{50} · Hatching success · Moulting

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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 by Bhattacharya and Joy (1980a, b), Joy and Bhattacharya (1981), Mitra et al. (1983), Bandyopadhya (1995), Park and Lees (2005), Amorim et al. (2005), Frampton et al. (2006) and Lins et al. (2007). 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 *Xenylla welchi* (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 the present study was to evaluate the comparative toxicity of the two herbicide formulations on *Xenylla welchi* and their potential to damage soil ecosystem.

Materials and Methods

Xenylla welchi 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.

Inert polythene container measuring 2.0 cm diameter and 3.0 cm height were used as treatment vials. Natural

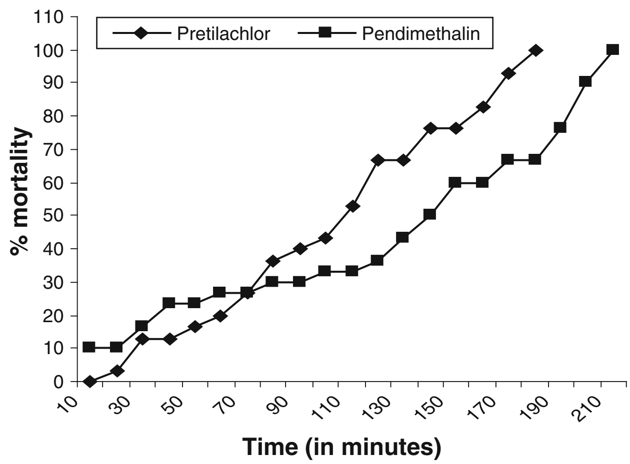

Fig. 1 LT_{50} value of pretilachlor and pendimethalin on *Xenylla welchi*

grassland soil (sandy loam) that has never been used for any agricultural purpose was used as the test soil. The physicochemical parameters of test soil (organic carbon, pH and water holding capacity) were determined following the methods of Piper (1942) and Jackson (1962).

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

Different dilutions of the herbicide formulations based on their recommended agricultural doses as provided by the manufacturer (viz. RAD, ½ × RAD, ¼ × RAD, $^1/_6$ × RAD, $^1/_8$ × RAD and $^1/_{10}$ × RAD) were used to determine the LC_{50} of the selected herbicide formulations on *Xenylla welchi*. For determination of $LT_{50,}$ recommended agricultural doses of the herbicide formulations were applied. To study the effect of 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 × LC_{50}, ¼ × LC_{50}, $^1/_6$ × LC_{50}, $^1/_8$ × LC_{50}, $^1/_{10}$ × 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 min before the incorporation of test animals for smooth spreading of herbicide formulations. Ten no matured test animals of the same age group and size were introduced in each replicate. No food was provided during the 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 Chakravorty (1990) for the determination of LT_{50}. Specimens who showed no apparent sign of life even when touched with a needle were considered dead.

For the investigation of life cycle parameters exposed to sub lethal doses, adult specimens in experimental vials (maintained as control set) were incorporated and reared. Adults were removed after laying of eggs and then sub-lethal 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.

Mortality data from the 24 h exposure were analyzed by probit analysis using EPA Probit analysis programme, version 1.5 (US EPA, 2006) for the determination of LC_{50}. The data on percentage of hatching success for each herbicide formulation was analyzed for 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 analyzed by '*f*' test.

Results and Discussions

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

Table 2 Physiochemical properties of test soil

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

Table 1 Herbicide formulations used

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

* Recommended agricultural dose

Table 3 LC_{50} values of two selected herbicide formulations on *Xenylla welchi*

Herbicide	24 h LC_{50} (g a.i/ha)	95% confidence limit
Pretilachlor	072.7	057.0–087.9
Pendimethalin	190.0	159.0–223.0

Table 4 Hatching success (%)

Treatment	Dose (g a.i/ha)		Hatching success (%)*	
	Pretilachlor	Pendimethalin	Pretilachlor	Pendimethalin
0	0.00	0.00	90.6 ± 1.3^{a}	90.6 ± 1.3^{a}
$^{1}/_{10} \times LC_{50}$	7.30	19.0	88.3 ± 2.8^{a}	87.0 ± 5.0^{a}
$^{1}/_{8} \times LC_{50}$	9.10	23.8	86.6 ± 2.8^{b}	66.6 ± 2.8^{b}
$^{1}/_{6} \times LC_{50}$	12.1	31.7	83.3 ± 2.8^{b}	58.3 ± 2.8^{c}
$^{1}/_{4} \times LC_{50}$	18.2	47.5	68.3 ± 2.8^{c}	45.0 ± 5.0^{d}
$^{1}/_{2} \times LC_{50}$	36.4	95.0	50.6 ± 1.1^{d}	32.6 ± 2.5^{e}

* Values are expressed as mean ± S.D, n = 30 eggs per vial, mean value of 3 vials were considered; different superscripts in the same column denote significant difference at $p < 0.05$

LC_{50} value of 72.7 g a.i/ha was found more toxic than pendimethalin (LC_{50}–190.0 g a.i/ha). There was significant difference between respective LC_{50} values with their RAD and also between LC_{50} values of two herbicide formulations ($p < 0.05$). LT_{50} value of the herbicide formulations at respective RAD (Fig. 1) showed that pretilachlor attained the fastest LT_{50} (110 min) showing significant heterogeneity with pendimethalin ($p < 0.05$).

Mean value of the life span of *Xenylla welchi* in the control was found to be 120 ± 6.21 days. Mean value of eggs laid/female specimen and hatching success were146 ± 10.98 and 90.6 ± 1.3, respectively in the control. In the lowest applied dose ($^{1}/_{10} \times LC_{50}$) and also in the next higher dose ($^{1}/_{8} \times LC_{50}$), hatching success attained almost the same value as control for both the herbicide formulations tested (Table 4). The values showed 44.1 and 63.3% reduction in hatching success for the highest applied dose (½ × LC_{50}) for pretilachlor and pendimethalin, respectively.

It was observed that after hatching, juveniles produced exuvia in every 5.5 ± 0.5 days in control and attained sexual maturity (egg laying) after 8th moulting (42 days). In experimental sets, juveniles did not survive in the doses of ¼ of LC_{50} and ½ of LC_{50} in case of Pretilachlor and $^{1}/_{6}$ of LC_{50}, ¼ of LC_{50} and ½ of LC_{50} in case of pendimethalin. The sub lethal doses at which juvenile survived to produce exuvia did so after every 4 days and attained sexual maturity after 7th moulting in 28 days. There were significant difference in duration of the instar stages and sexual maturity between test and control sets ($p < 0.05$) of the test specimens in case of both the herbicide formulations.

Several researchers have observed the negative impact of herbicides on collembolan species (Joy and Bhattacharya 1977; Mitra et al. 1983; Brooks et al. 2005). The findings of present investigation on two selected herbicides formulations, pretilachlor and pendimethalin also exhibited their toxic effect on *Xenylla welchi*. Pretilachlor with LC_{50} 72.7 g a.i/ha was found more toxic than pendimethalin (LC_{50} 190 g ai/ha) with respect 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 attained fastest LT_{50} in 110 min and pendimethalin attained LT_{50} in 140 min. The values exhibited heterogenicity at $p < 0.05$. Frampton et al. (2006) noticed the sensitivity of collembolan against different herbicides. Crouau and Moia (2006) investigated the sensitivity of growth and reproduction of four xenobiotics on *Folsomia candida* and found negative effect of these chemicals on several life cycle parameters of *Folsomia sp.* Staempfli et al. (2007) noticed negative role of herbicide dinoseb on survival, growth and reproduction of *F. candida*. Significant decrease in hatching success due to exposure to these herbicides on *Xenylla welchi* was also recorded in the present investigation.

Eijsackers (2009) reported shorter life span, decreased number of egg laying and increased frequency of moulting due to impact of herbicide 2, 4, 5-T on collembola. 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 *X. welchi* when exposed to sub-lethal doses of pretilachlor and pendimethalin.

Thus it can be concluded from the present study that based on the LC_{50} and LT_{50} values of the herbicide formulations on *Xenylla welchi*, pretilachlor was found more toxic than pendimethalin and at sub-lethal doses both the herbicides adversely affected the life cycle of *Xenylla welchi* indicating their potential role in damaging the soil ecosystem.

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