Toxicological Studies on a Soil Micro-Arthropod: An Indication on the Potential Threat in Soil Humus Formation

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Abstract: ***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. The four agrochemicals which are toxic in nature are tested for their toxicity to Phylosciajavanensis at different concentrations. The results of the experiments revealed that all the agrochemicals tested viz., indofil, 2, 4-D, furudan and sevin, are highly toxic to the test animal even at low concentrations. The values LC-50 and LC-100 values obtained in the present study underlined the toxicity of these chemicals to soil organisms like Phyloscia. Also the residual remains of these agrochemicals in the soil pose a threat to the habitat of soil isopods.***

Keywords: *Phyloscia*, soil isopod, agrochemical, toxicity, LC-50

1. Introduction

Isopods group of organisms play a dominant role to increase soil fertility. Indiscriminate use of pesticides,herbicide and fungicide in virtue of agricultural purposes has deleterious effects on the biology of soil arthropods.The chemical treatments that eliminate the group of microorganism undoubtedly create a partial biological vacuum in soil. Many pesticides, fungicide and herbicides can kill more than just their intended targets, namely necessary microorganism in the soil. Once in the soil, they can kill the microorganism living in the soil that breaks down organic material and aid in plant growth. It can take years before microorganism can once again live in soil that has had toxic chemicals applied to it.

Pesticides are the worst enemy of many of the soil organisms on this planet. Pesticides widely affect the life of aquatic fauna which are manifested as change in physiology, biochemistry and activity levels of many enzymes. In isopods, digestive gland epithelial thickness is related to contaminated food (Odendaal and Reinecke, 2004). Drobne*et.al*, (2008) confirmed the occurrence of epithelial thinning as a result of stress and that reduced feeding rate coincides with reduced epithelial thickness. The action of pesticide may bring external or internal damage to many parts of the organism.

Only recently a few studies on toxicity to soil invertebrates were published, including earthworms, springtails and enchytraeids (Wislocki*et.al*,. 1989; Sun *et.al*,. 2005; Jensen *et.al*,. 2007; Kolar*et.al*,. 2008). However, its effects on terrestrial isopods are not well known (Kolar*et.al*, 2008). Terrestrial isopods are abundant in different ecosystems and habitats, and have an important ecological role as macro decomposers. These animals have been recognized as useful for the characterisation of chemical toxicity (Hornung*et.al*,. 1998; Walker *et.al*,. 2001), because they are easy to sample, handle and culture, and large enough to perform a variety of sub-organism studies. 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).Due to their important ecological role as decomposers of organic material, terrestrial isopods are widely accepted as test organisms in terrestrial ecotoxicology and ecophysiology (Lapanje*et al*., 2007).

Pesticide can serve farmers money by preventing crop losses to insect and other pests. Pesticide can be classified by target organism, chemical structure, and physical state. Pesticide can also be classified as inorganic, synthetic, and biological.Like modern insecticides, herbicides were first applied on a large scale basis shortly after world war –II. Generally herbicide falls into two groups depending upon their mode of action which include Mounron and Simazin, interfere with photosynthesis and thus cause the plant to die from lack of energy. The second group is typed by the commonly used 2, 4-D (2, 4-Dicholoro phenoxy acetic acid).

A preliminary survey in the study area revealed that agrochemicals of common use in the study area are 2,4-D, indofil, carbofuran and sevin.The study assess the toxicity of commonly used agrochemicals, 2, 4 D, carbofuran, indofil and sevin on soil isopod, *Phylosciajavanensis* (Rich).

2. Materials and Methods

1. Collection and rearing of mother culture

Phylosciajavanensis (Rich) were collected from the study area. They were transferred to large culture chambers of which plaster of paris and activated animal charcoal in the ratio 5:2 as base. They were acclimatized in the laboratory condition for about 20 days prior to experiment. Decayed leaf bits soaked in water were given as food.

2. Collection and rearing for subculture

(a) Preparation of subculture medium

Large plastic containers were used as culture bottles; sterile plastic bottles were kept in oven for 24 hours at 40°C to desterilize the bottle. Plaster of Paris and activated animal charcoal in the ratio 5:2 mixed in distilled water were kept in this chambers for setting (Plate 2.a.) This was used as a base and these bottles were fixed with water for through setting for a period of 24 hours.

(b) Collection from soil and extraction

Soil samples were collected from different locations of Chengannurthalukof Alleppey district, Kerala and the work was carried out during December, 2014. The soil samples were loaded to series of Berlese funnel for the extraction of organisms. 40 W bulbs were illuminated above each funnel for a period of 24 hours to extract the micro arthropod of the soil into a beaker containing thin layer of water. The collected live*Phyloscia* were transferred to culture bottle to acclimatise in the laboratory condition. Cleaned decayed leaves and wood pieces were given to them as food.

3. Preparation of Stock Solution of Agrochemicals

1000 ppm stock solution of Indofil (Mancozeb), 2,4-D (Hedonal), Furdan (Carbofuran) and Sevin (Carbaryl) was prepared by dissolving required quantity of chemicals in one litre of distilled water(APHA, 2005) . From this stock solution different concentration of fungicide and herbicide were prepared for bioassay.

4. Bioassay

Various concentration like 1,3,5,7,9 and 11 ppm Indofil, 2,4 D, Carbofuran and Sevin were prepared from stock solution. A group of 150 mature isopods in three replicates and a control were tested for each concentration of these agro chemicals. Leaves were dosed with pesticide as described by Staneket.*al*,. (2006). The food (Decaying leaves) was soaked in respective concentration of pesticides for a period of 24 hours and was given to experimental animals and leaves soaked in distilled water was given to control animals. The mortality was recorded at 24,48,72& 96 hours interval and the percentage mortality at different hours were calculated.

5. Toxicity Analysis

From the data of bioassay, lethal concentration -100 (LC-100) lethal concentration - 50 (LC-50) and safe level concentration were calculated. LC-50 values was calculated by probit analysis (Finney, 1980) safe concentration were determined by the method suggested by Hart *et. al.*, (1945).

6. Determination of Lethal Concentrations:LC-100, LC-50 and Safe Level

Lethal concentration-100 (LC-100) Lethal Concentration-50 (LC-50) was calculated usingprobit analysis by Finney. Sub-lethal concentration was calculated following the method described by Loomis and Sternberg, 1968.One third (1/3) of the lethal concentration -50 was taken as sub lethal concentration.

3. Results

1. Mortality

(a) With 2, 4-D

Percentage mortality of *Phylosciajavenensis* at different hours 2, 4-D is given in table 1. The rise in mortality was gradual as the concentration of the pesticide increases. 30, 40, 40, 50, 60, and 80% mortality was recorded for 1, 3, 5, 7, 9, and 11 ppm concentration of 2, 4-D respectively at 48 hours. 30, 40, 40, 70, 80, and 80% mortality was recorded for 1, 3, 5, 7, 9 and 11 ppm concentration of 2, 4-D at 72 hours. 40, 50, 60, 70, 80, and 90% mortality was recorded for 1, 3, 5, 7, 9, and 11 ppm concentration of 2, 4-D at 96 hours.

Table 1: Percentage Mortality of *Phyloscia*Exposed To Different Concentration of 2, 4-D

Concentration (ppm)	Mortality (%)			Control Mortality %
	48 hours	*72 hours*	*96 hours*	
1	30.21	30.31	40.28	0
3	40.33	40.31	50.21	0
5	40.34	40.30	60.21	0
7	50.21	70.21	70.21	5
9	60.21	80.11	80.01	0
11	80.81	80.32	90.02	0

(b)With Indofil

Here also there was a gradual rise in the mortality as the fungicide concentration increases 50% mortality was observed for 1 ppm concentration of fungicide at 48 hours. Cent percent mortality was observed for 11 ppm indofil at 96 hours. 80, 90, and 100% was the mortality rate when *Phyloscia* were exposed to 11 ppm fungicide at 48 hours, 72 and 96 hours respectively (Table 2).

Table 2: Percentage Mortality of *Phyloscia*Exposed to Different Concentration of Indofil

Concentration (ppm)	Mortality (%)			Control Mortality (%)
	48 Hours	72 Hours	96 Hours	
1	50.01	50.01	50.21	0
3	60.08	60.02	60.22	0
5	60.21	60.01	70.34	0
7	70.31	70.88	70.28	0
9	70.21	80.31	80.28	0
11	80.01	90.30	100	0

(c) With Carbofuran

The rise in mortality was gradual as the concentration of the pesticide increases. 10,20,30,40,and 60% mortality was recorded for 1,3,5,7,9 and 11 ppm concentrations of furadan (carbofuran) respectively at 48 hours. 20,30,40,50 and 80% mortality was recorded for 1,3,5,7,9 and 11ppm concentrations of carbofuran at 72 hours 50,60,70 and 90% mortality was recorded for 1,3,5,7,9 and 11 ppm concentrations of carbofuran at 96 hours (Table 3)

Table 3: Percentage Mortality of *Phyloscia* Exposed to Different Concentration of Carbofuran

Concentration (ppm)	*Mortality (%)*			Control Mortality (%)
	48 Hours	*72 Hours*	*96 Hours*	
1	10.21	20.22	50.31	0
3	20.23	30.31	50.21	0
5	30.21	40.21	50.08	0
7	30.31	50.21	60.01	0
9	40.33	50.33	70.03	0
11	60.21	80.31	90.04	0

(d) With Sevin

Here also there was a gradual rise in mortality as the pesticide concentration increases. 10,30,40,50 and 100% mortality was recorded for 1, 3, 5, 7, 9, and 11 ppm concentrations of seven respectively at 48 hour. 30, 40, 50, 70, and 100% mortality was recorded for 1, 3, 5, 7, 9 and 11 ppm concentrations of sevin at 72 hour. 50, 60, 70, 100% mortality was recorded for 1, 3, 5, 7, 9 and 11 ppm concentrations of sevin at 96 hour (Table 4.)

Table 4: Percentage Mortality of *Phyloscia*Exposed to Different Concentration of Sevin

Concentration (ppm)	*Mortality (%)*			*Control Mortality (%)*
	48 Hours	*72 Hours*	*96 Hours*	
1	10.00	30.33	50.09	0
3	30.01	40.34	60.03	0
5	30.08	50.33	60.07	0
7	40.21	50.01	70.01	0
9	50.23	70.08	100	0
11	100	100	100	0

2. Lethal concentration 100 (LC-100) and lethal concentration 50 (LC-50)

(a) 2, 4-D.

Lethal concentration 100 of 2, 4-D was found to be 16.000 ppm (48 hours) and 10.7041 ppm (72 hours), 13.000 ppm (96 hours). The LC-50 values were calculated to be 6.000 ppm (48 hours) and 4.9391 ppm (72 hours) and 3.000 ppm (96 hours). The LC-100 values of 2, 4-D is quite high for *Phylosciajavanensis* (Table 5).

(b) Indofil.

The LC- 100 value of Indofil was 18.0925 (48 hours), 13.8922 (72 hours) and 11.9210 (96 hours). The LC-50 value was found to be 0.8175 (48 hours), 1.4322 (72 hours) and 1.471 (96 hours).

(c) Carbofuran

Lethal concentration-100 of carbofuran was found to be 20.281 ppm (48 hour) 15.5659 ppm (72 hour) and 14.0646 ppm (96 hour). The LC-50 values were calculated to be 9.831 ppm (48 hour) 7.1259 ppm (72 hour) and 30.5446 ppm (96 hour) respectively.

(d) Sevin

The LC 100 value of sevin was 12.228 ppm (48 hour) 12.0835 ppm (72 hour) and 10.34 45 ppm (96 hour). The LC-50 values were calculated to be 6.733 ppm (96 hours) respectively.

Table 5: LC 100 and LC 50 (in ppm) of different agrochemicals at different hours

Chemicals	*LC -100*			*L C - 50*		
	48 Hours	*72 Hours*	*96 Hours*	*48 Hours*	*72 Hours*	*96 Hours*
2,4-D	16.000	10.7041	13.000	6.000	4.9391	3.000
Indofil	18.0925	13.8922	11.9210	0.8175	1.4322	1.471
Carbofuran	20.281	15.5659	14.0646	9.831	7.1259	3.5446
Sevin	12.228	12.0835	10.3445	6.733	5.0635	2.1995

3. Safe concentration and sublethal concentration

The safe level concentration of 2, 4-D was found to be 0.6099 ppm and that of indofil was found to be 1.3545 ppm. The sublethal concentration was found to be 0.3 ppm for 2, 4-D and 0.1471 ppm for indofil. The concentration of 0.5587 ppm cabofuran was found to be safe for *Phylosciajavanensis* (safe concentration). The concentration of 0.35446 ppm furadan was found to be sub-lethal to this animal. Similarly 0.3732 ppm sevin is the safe concentration and 0.21995 ppm sevin is the sub lethal level for *Phylosciajavanensis*(Table 6, Figure 6).

Table 6: Safe concentration and sublethal concentration of agrochemicals tested

Chemicals	*Sublethal Concentration (ppm)*	*Safe Concentration (ppm)*
2,4-D	0.3	0.6099
Indofil	0.1471	1.3540
Carbofuran	0.35446	0.5587
Sevin	0.21995	0.3732

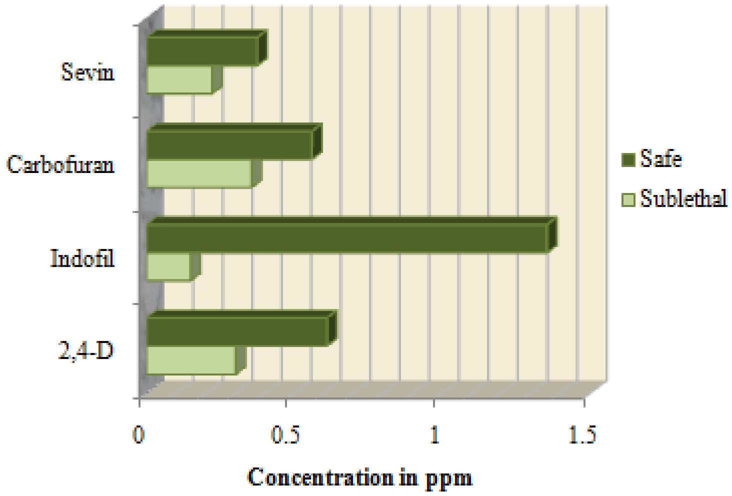

Figure 6: Safe concentration and sublethal concentration of agrochemicals tested

4. Discussion

Indiscriminate use of insecticides, pesticides and other 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. There are reports of reduction in number of earthworms, collembolans, mites and other organisms in soil consequent to the regular application of these toxic agrochemicals (Andrew and Sanil, 1991; Pall and Chatterjee, 1986). Another important consequence of the use of agrochemicals is the bioaccumulation of these toxins by the soil invertebrates (Sun *et.al*,2005; Diao*et.al*, 2007) and thereby affecting their physiology (Drobne*et.al*, 2008).

The percentage mortality of *Phyloscia* rises with the increasing concentration of all the agrochemicals tested and with increasing time of exposure. 11 ppm concentration at 96 hour of all these pesticides tested showed 90-100 percent mortality of the organism. But at low concentrations of 1-3 ppm and the same exposure time of 96 hour, the mortality is 40-60 percent only. This means that these agrochemicals and its residual remains in the habitat of *Phyloscia*have a long term impact in the sustenance of the species.

The LC-50 value of furudan (3.5446) is the highest as compared with other agrochemicals tested followed by 2,4-D (3.000), sevin (2.1995) and indofil (11.921) at 96 hours. Also the LC-100 values showed a similar trend being the highest is furudan (14.0646) followed by 2,4-D (13.000), indofil (11.921) and sevin (10.3445). The results showed that furudan is highly toxic to *Phyloscia* even at low concentration and sevin is the least toxic out of the tested agrochemicals. The probit analysis results revealed that indofil is safe even upto 1.354 ppm but the other chemicals tested are safe at less than 0.6 ppm only.

The present study of the toxicity of different commonly used agrochemicals on soil isopod *Phylosciajavanensis* is in conformity with the earlier research works in this field. The isopod seems to be highly prone to the action of the agrochemicals studied and indiscriminate use of these pesticides will drastically decrease the population density and diversity of soil isopods and thereby reduces the soil fertility.

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