A Study on the Effect of two Agrochemicals on the Fecundity of a Soil Collembolan Cryptopygus Thermophilus

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Abstract

Agrochemicals play a significant role in increasing the productivity of crops but it has very serious effect on the fauna and flora of the soil ecosystem. Soil micro arthropods especially Collembolans help in increasing the soil fertility by decomposing the litter. Collembola is a group that function as a bio- indicator of the soil condition. The normal fecundity of Cryptopygus thermophilus and the effect of a herbicide Glyphosate and the fungicide Indofil on the fecundity was studied. The studies revealed that both the agrochemicals has a profound effect in reducing the fecundity of Cryptopygus thermophilus, but the effect of Glyphosate is high when compared to Indofil.

Key words: Collembola, Fecundity, Agrochemicals, Glyphosate, Indofil

1. Introduction

The ever growing global population leading to the enormous demand in food supply is one of the biggest challenges in agriculture. Agrochemicals have been an indispensable evil of modern industrial agriculture. Pesticides are those substances which are used to destroy, control, repel or attract pest in order to minimize their detrimental effect. The United Nations Environmental Programme (UNEP, 1979) has ranked pesticide resistance as one of the top four environmental problems of the world. In modern agriculture practice the demand for pesticides is frequently increasing and this may lead to the use of new compounds in agriculture. In order to increase crop production varieties of herbicides, insecticides, fungicides, nematicides and other chemical fertilizers are used in high quantities. Even as pesticides have become an important tool for global food security, their undesirable effect is alarmingly increasing in agricultural sector.

Some of these agrochemicals are persistent, toxic, bio accumulating and biomagnifying in animal tissue as well as humans. Most of the agrochemicals used in the agriculture are non-specific and kill the useful organism too. This may affect the trophic structure of the ecosystem. The indiscriminate use of these chemicals may lead to environmental problems and ecological imbalance and consequently affect the flora and fauna and sometimes it may extend up to human beings. The extensive use of agrochemicals in the environment may pose toxic to Human beings. Human beings are exposed to these harmful chemicals through different pathway such as inhalation, ingestion, and dermal contact.

Agrochemicals can contaminate soil, water, turf, and other vegetation. Insecticides are used to kill insects or weeds while they may be toxic to other host organisms including birds, fish, beneficial insects, and non-target plants. Insecticides are generally the most acutely toxic class of pesticides, but herbicides can also cause risks to non-target organisms. Pesticides are hydrophobic, persistent, bio accumulate and are strongly bound to soil. Agrochemicals can reach surface water through runoff and leaching from treated plants and soil. Due to persistent nature, pesticides may bio accumulate and bio magnify in organisms of food chain. Though these chemicals are found as common contaminants in soil, air and water, they may act on some non-target organisms. They may drift or volatilize from the treated area and contaminate air, soil, and non-target plants. They can harm plants and animals ranging from beneficial soil micro organisms and insects to non-target plants, fish, birds, and other wildlife. Some herbicides can also be toxic to fish.

Indiscriminate use of these chemical substances decreases the decomposition process by affecting the micro fauna and flora of the soil (Darlong & Alfred, 1982). The decomposition process is initiated by soil micro arthropods, which includes Collembolans proturans, thysanurans, diplopodans, myriapodans etc. Collembola are among the most abundant soil arthropods and play an important role in decomposer food webs (Butcher *et al.*1971); (Peterson & Luxton 1982); (Peterson 2002). The effects of Collembola on decomposition processes and nutrient mineralisation may depend on species composition and dominant structure of collembolan communities, since different species of collembola have different feeding preferences (Mebes & Filser, 1998); (Chahartaghi *et al.,* 2005).

Cryptopygus thermophilus is a major collembolan playing important role in increasing the soil fertility by decomposing the litter. The present investigation is to study the effect of two commonly used agrochemical namely Glyphosate- a herbicide and Indofil – a fungicide on the fecundity of *Cryptopygus thermophilus*.

2. Materials and Methods

2.1. Bioassay studies

The agrochemicals were selected for the study is Glyphosate 41 EC-a herbicide and Indofil 45 EC- a Fungicide. 5, 7,10,12,15 and 17 ppm Glyphosate was prepared by dissolving appropriate quantity of herbicide in 1000 ml distilled water. 10, 12.14,18,20, and 22 ppm Indofil was prepared by dissolving appropriate quantity of fungicide in 1000 ml distilled water (APHA, 2012)

A group of 50 adult individuals were exposed to each concentration of Glyphosate and Indofil in different culture chambers. The decaying leaves washed in water and soaked in respective quantity of agrochemicals for 24 hours were given as food for the experimental group. A control was also maintained without treating the agrochemical. Mortality was recorded at every 12, 24, 48, 72, and 96 hours.

2.2. Analysis

Probit analysis (Finney 1972) was used for the calculation of lethal concentration 50 (LC50) lethal concentration100 (LC100), Sub lethal concentration and safe concentration of each agrochemical.

2.3. Normal Fecundity studies

Five sub adult female individuals were separated in separate culture chamber and were given decaying jack leaves as food. Five adult males were also introduced in each chamber. Fecundity was recorded in each oviposition by carefully separating eggs from the culture chamber using a fine brush. The number of eggs in each oviposition was counted.

2.4. Fecundity studies after treating with Agrochemicals

Five replicates were maintained for the agrochemical treated fecundity studies. Individuals of Five replicates were given jack leaves soaked in sub lethal concentration of Indofil and another five replicates were given food treated with sub lethal concentration of Glyphosate. Number of eggs in each oviposition was counted and removed using a fine brush.Two Way ANOVA was conducted to find out any difference between number of eggs in different replicates and also in different oviposition for normal and agrochemical treated individuals.

3. Results

3.1. Normal Fecundity

Nine ovipositions were observed for the entire five replica studied. The number of eggs were between 58 and 64 in first oviposition, 60 and 68 in second oviposition, between 64and 72 in third oviposition, between 72 and 78 in fourth oviposition, between 76 and 84 in fifth oviposition, between 70 and 78 in sixth oviposition, between 64 and 74 in seventh oviposition, between 60 and 66 in eighth oviposition and between 58 and 65 in ninth oviposition.

Table 1. Fecundity of *Cryptopygus thermophilus* under normal conditions

Replica	Ovi position									Mean
	1	2	3	4	5	6	7	8	9	
1	60	65	70	78	80	76	74	66	65	70.44
2	64	68	72	78	82	74	68	66	64	70.61
3	62	64	70	77	80	75	69	64	65	69.55
4	58	60	64	72	76	70	64	60	58	64.44
5	62	66	70	76	84	78	68	66	62	70.22

Two way Anova revealed that there is significant difference in the number of eggs in each replicate during different oviposition (P 1.79×10^{-9} $P < 0.05$). There is also significant variation in the number of eggs during different ovipositions as revealed by P value (P 1.02×10^{-19} $P < 0.05$).

Table 2: Two way ANOVA showing Fecundity of *Cryptopygus thermophilus* between different oviposition.

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between group	246.5778	4	61.64444	25.08988	1.79E-09	2.668437
Between Oviposition	1765.6	8	220.7	89.82702	1.02E-19	2.244396
Error	78.62222	32	2.456944			
Total	2090.8	44				

3.2. Fecundity of *Cryptopygus thermophilus* treated with Sublethal Concentration of indofil.

Indofil treated individuals also showed nine oviposition, but the number of eggs in each oviposition was less when compared to normal. In the first oviposition the number of eggs ranged to 34 and 38 in different replicates. In second oviposition it was 36 to 40, 34 to 45 in the third oviposition, 40 to 48 in the fourth oviposition, 42 to 50 in the fifth oviposition,40 to 45 in the sixth oviposition,41to 44 in the seventh oviposition,35 to 42 in eighth and 35 to 40 in ninth oviposition. The average number of eggs in replica one was 43.55 in each ovipoition,42.22 in replica two,38.44 in replica three , 38.11 in replica four and 39.77 in replica five.

Table 3: Fecundity of *Cryptopygus thermophilus* after treatment with sub lethal Concentration of Indofil

	Ovi position									
Replica	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Mean**
1	38	40	45	48	50	45	44	42	40	43.55
2	36	38	44	46	45	42	42	40	38	41.22
3	34	36	38	42	42	40	40	38	36	38.44
4	35	36	34	40	43	42	43	35	35	38.11
5	35	37	38	43	45	43	41	39	37	39.77

Two way ANOVA showed that there is significant differences in the number of eggs between different ovipositions ($P = 1.66 \times 10^{-11}$ $P < 0.05$) and between different replicates

($P = 1.71 \times 10^{-08}$ $P < 0.05$).

Table 4: Two way ANOVA showing Fecundity of sublethal concentrations of Indofil treated *Cryptopygus thermophilus*

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between group	179.3333	4	44.83333	20.65259	1.71E-08	2.668437
Between Oviposition	420.9778	8	52.62222	24.24056	1.66E-11	2.244396
Error	69.46667	32	2.170833			
Total	669.7778	44				

3.3. Fecundity of *Cryptopygus thermophilus* treated with Sublethal Concentration of Glyphosate

When *Croyptopygus thermophilus* was exposed to the sublethal concentration of glyophosate there is a drastic reduction in number of eggs laid in each oviposition. The numberof ovipositions still remain as 9. The number of eggs produced in the first oviposition was between 15 to 19 in different replicates, between17 to 21 in the second oviposition, 17 to 20 in the third, 17 to 21 in the forth, 17 to 22 in fifth, 15 to 21 in sixth, 17 to 21 seventh, 16 to 21 in the eighth, 17 to 21 in the ninth oviposition. The mean number of eggs laid in each oviposition was found to be 19.33 in the first replicates, 19.44 in the second, 19.55 in the third, 17.22 in the fourth, and 17.55 in the fifth replicate.

Table 5: Fecundity of *Cryptopygus thermophilus* after treatment with sub lethal Concentration of Glyphosate.

Replica	**Ovi position**									**Mean**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	
1	18	20	19	21	20	19	21	17	19	19.33
2	18	21	17	19	21	21	18	19	21	19.44
3	19	18	20	17	22	20	19	21	20	19.55
4	15	17	19	17	19	15	17	19	17	17.22
5	17	19	18	18	17	16	18	16	19	17.55

Two way ANOVA showed a great significant difference in the number of eggs laid between different replicates (P=0.00127, P<0.05) and there is no significant differences between number of eggs laid in each oviposition (P=0.036724, P>0.05)

Table 6: Two way ANOVA showing Fecundity of sublethal concentrations of Glyphosate treated *Cryptopygus thermophilus*

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Group	46.35556	4	11.58889	5.790423	0.00127	2.668437
Between oviposition	18.17778	8	2.272222	1.135323	0.36724	2.244396
Error	64.04444	32	2.001389			
Total	128.5778	44				

3.4. Bioassay studies

Glyphosate 41 EC

5ppm,7ppm,10ppm,12ppm,15ppm,17ppm,glyphosate were tested for different groups of 50 individuals till 96 hours. 22.98 % mortality was observed at 96 hours for 5ppm, 32.84% for 7ppm, 48.96% for 10ppm, 62.97% for 12ppm, 78.93% for 15ppm, and 96.66%for 17ppm.Here also mortality was gradual as the increase in the concentration of herbicide and length of exposure.

Table 7: Percentage mortality of *Cryptopygus thermophilus* at different hours of Glyphosate25 EC

Concentration	12hour	24hour	48hour	72hour	96hour
5ppm	2.46	8.96	12.86	16.98	22.98
7ppm	6.99	14.85	16.86	26.87	32.84
10ppm	15.78	28.22	34.78	40.56	48.96
12ppm	32.89	36.88	44.87	56.87	62.97
15ppm	40.77	48.87	59.88	74.86	78.93
17ppm	48.14	59.97	68.99	82.87	96.66

3.5. Indofil 45 EC

10ppm, 12ppm, 14ppm, 18ppm, 20ppm and 22ppm indofil were tested for different groups of 50 individuals till 96 hours. There was no mortality till 9ppm indofil was tested. At 96 hrs 20.44% mortality was observed for 10ppm, 30.77% for 12 ppm, 46.94% for 14 ppm, 56.74% for 18 ppm and 70.68% for 20ppm and 94.89% for 22ppm respectively.

Table 8: Percentage mortality of *Cryptopygus thermophilus* at different hours of Indofil.

Concentration	12hour	24hour	48hour	72hour	96hour
10ppm	8.88	10.54	12.66	18.94	20.44
12ppm	12.44	16.89	22.87	26.99	30.77
14ppm	16.87	24.87	28.76	34.98	46.94
18ppm	26.88	30.88	36.87	44.88	56.74
20ppm	36.88	48.46	58.97	68.96	70.68
22ppm	56.87	64.87	78.88	86.33	94.89

3.6. LC50, LC 100, safe and sublethal concentrations

The LC100 value for indofil was found to be 23.9436ppm at 96 hours, 13.7960ppm and 18.4023 ppm for glyphosate. The LC50 of Indofil was found to be 15.4186ppm at 96 hours and 10.5973ppm for glyphosate.

Table 9: LC 100 of different agrochemicals on *Cryptopygus thermophilus*

Name of agrochemical	12Hr	24Hr	48Hr	72Hr	96 Hr
Indofil	34.2869	30.9497	27.0093	25.3491	23.9436
Glyphosate	32.2373	28.5657	24.5041	20.4335	18.4023

Table 10: LC 50 of different agrochemicals on *Cryptopygus thermophilus*

Name of agrochemical	12Hr	24Hr	48Hr	72Hr	96 Hr
Indofil	21.8519	21.8347	19.8593	18.5541	15.4186
Glyphosate	17.8023	15.2307	13.0791	10.8285	10.5973

The safe concentration at 96 hours was found to be 1.9737ppm for glyphosate and 3.5745ppm for indofil. Sub lethal concentration at 96 hours was found to be 2.645ppm for glyphosate and 3.86ppm for indofil.

Table 11: Safe and Sub lethal Concentration of Glyophosate and Indofil on *Cryptopygus thermophilus*

Name of agrochemical	Safe Concentration (96Hr) ppm	Sublethal Concentration (96Hr) ppm
Glyophosate	1.9737	2.645
Indofil	3.5745	3.86

4. Discussion

The detritus food chain in the top and sub pedological layers were totally disturbed through the indiscriminate use of compounds having ethyl, methyl,and thionate groups. Such long persistent chemicals had a cumulative adverse effect on the fragile fauna and flora of the detritivorous food chain that lead to the disequilibrium of the pedological community and population as a whole. Of the different varieties of agro chemicals organochlorine, organophosphates and carbamides were mostly used in the home gardens, agroecosystems and agro forestry ecosystems of different countries. The soil ecosystem lost its natural capacity of rejuvenation and recycling of organic nutrients. Humus formation was disrupted leading to less production of humic acid, in turn increase in alkalinity of the soil. .

The microarthropods including collembolans, proturans, thysanurans, diplopodans, myriapodans, accari, diplurans, orthopterans and coleopterans from the bulk of the soil microarthropod community, those were indiscriminately massacred by the heavy application of fungicides containing thionate compounds. Along with this destruction there occurs prodigality of production in the number of undesirable species of bacteria. This added new dimensions to the existing imbalance and chaos.

The very thin cuticle of most of the microarthropods especially collembolans persuade the influx of methyl, ethyl and thionate groups of fungicides and herbicides in to the haemolymphs of these vulnerable organisms. The partial or complete elimination of some of these groups from the detritivorous food chain leads to the disruption of energy flow, nutrient cycling and biomass accumulation.

Cryptopygus thermophilus is a small vulnerable collembolan with a thin cuticle covering on the outer surface of the body. Most of these agrochemicals diffused into the integumentary system of these animals. The percolation of thionates, salicylic, methyl, ethyl and other such functional groups found in these organic compounds slowly diffuses in to the haemolymph. The haemolymph has a remarkable ability to detoxify most of the toxicants entering the body. But these chemicals may affect the physiology of some of the vital systems of these animals. The reproductive system is the most vulnerable organ system of any animal. Intoxication and intrusion of toxicants into the system may lead to the disruption of vital functions, total disturbance of the reproductive hormones thereby reducing the fecundity. In the present experiment also the result is in accordance with the finding of earlier works like (Sanal kumar & Nair, 1999); (Veltcamp, 2012); (Hussain & Zahira, 2010).

Among the agrochemicals tested Glyphosates had great effect on the egg production by the ovary followed by the fungicide Indofil. The methyl compounds of the herbicides had much impact on the fecundity of this animal. Exposure of sub lethal concentration of these chemicals leads to drastic reduction in the fecundity. Direct heavy doze application of these chemicals into the soil ecosystem may have much detrimental effect on the very existence of these tiny arthropods.

Collembolans generally show a recovery from the adverse impact of soil pollutants, but the present experiment in the laboratory condition showed less chance of recovery even after 9^{th} oviposition. This indicates the rejuvenation of soil ecosystem after a heavy doze application of these chemicals may take decades to come back to the virgin condition. So it is recommended that these agrochemicals are to be applied only in permissible concentrations approved by the agricultural scientists. The application of agrochemicals in concentrations above the recommended level may lead to irreparable damages to soil microbiota and soil ecosystem at large.

5. Summary and Conclusion

Nine ovipositions were observed for the *Cryptopygus thermophilus* in the five replicas studied and the mean number of eggs was between 64.44 and 70.61. The agrochemical treated individuals also showed nine ovipositions but the number of eggs in each oviposition was less when compared to normal. The LC 100 value for indofil was found to be 23.9436ppm and for Glyphosate was 18.4023ppm at 96 hours. The safe concentration at 96 hours was found to be 1.9737ppm for glyphosate and 3.5745ppm for indophil. Sub lethal concentration at 96 hours was found to be 2.645ppm for glyphosate and 3.86ppm for indofil. Glyphosate treated organisms showed less egg production because the methyl compound in the herbicide had effect on egg formation and maturation.

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