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Influence of agrochemicals on the reproductive biology of *Bilobella braunerae* Dhervang 1981, (Collembola: Neanuridae) in lab conditions

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Abstract
Agrochemicals play a significant role in increasing the productivity of crops but it has a very serious effect on the fauna and flora of the soil ecosystem. Soil collembolans play a decisive role in boosing soil fertility. Unsystematic use of agrochemicals for agricultural purposes resulted in harmful effects on the biology and species diversity of soil microarthropods. Collembola is a group that functions as a bio-indicator of the soil condition. Laboratory toxicity tests were carried out, to evaluate the effects of agrochemicals- Acrinathrin (pyrethroid pesticide), Mancozeb (Fungicide) and Altrazine (Herbicide) on fecundity and moulting intervals of an edaphic collembolan Bilobella braunerae (Collembola) grown in the sub lethal concentration of agrochemicals. The LC 50 values of agrochemicals for Bilobella braunerae were calculated. The safe and sub-lethal concentrations of agrochemicals were very low, indicating the high vulnerability of these collembolans to agrochemicals. The safe level concentration of Acrinathrin (pyrethroid pesticide), Mancozeb (Fungicide) and Altrazine (Herbicide) were 1.25ppm, 0.1850ppm, 0.0355ppm respectively. The collembola feeding on leaves of jackfruit containing sub lethal concentration agrochemicals exhibited trends of increased days in moulting and decreased fecundity rates. The moulting intervals of B.braunerae showed variation between the agrochemical treated and control group. The moulting intervals were prolonged after treatment. The inhibition of growth, moulting and fecundity was seen above the sub-lethal concentration. Both moulting and fecundity rates were significantly affected by the agrochemicals when presented as food to the collembola. The untreated control sets recorded growth, moulting fecundity for B. braunerae, but chronic toxicity of the insecticides on adults confined to the treated food resulted into very low fecundity and prolonged moulting intervals. Even short duration exposure to agrochemicals treated food for 24 or 72 hours, was found to delay the egg-laying and decrease the fecundity of the species. It is concluded that population responses and reproductive sensitivity in non-target soil microarthropods are potential eco- toxicological parameters for detecting agrochemical pollution in soil and for ecological health assessment. The present studies revealed that agrochemicals has a profound effect in reducing the fecundity of B. braunerae, but the toxic effect of Altrazine (Herbicide) is high when compared to Acrinathrin, Mancozeb.

Keywords: *Bilobella braunerae*, Acrinathrin, Mancozeb, Altrazine, sub lethal concentration, moulting intervals, fecundity, soil microarthropods

Introduction
Unsystematic application of pesticides can disturb the ecological equilibrium through addition of toxic residues in the environment. In a developing country like India agricultural fields represent the greatest arena of pesticide pollution because of direct application to soil for uptake by roots or against soil-borne pests. Excluding a few groups that are pests or parasites the greater part of the soil fauna constitute an essential component of the detritus food chain. Any agricultural practice, including the use of insecticides, which interferes with the composition of the decomposer community, shifts the component population's equilibrium and this may result in reduced organic decomposition which could affect soil fertility (Broadbent & Tomlin, 1980). Microarthropods, for example, play a key role in maintaining soil fertility and microbial propagation during feeding and in mixing the organic and mineral components of soil. Therefore, a comparative assessment of the direct toxicity and residual ill effects of pesticides on ecologically important nontarget organisms is very significant. Collembola, or springtails (Insecta: class Entognatha), represent one of the most abundant arthropod groups in soil (Hale, 1967) [8]. They contribute to the decomposition of organic matter, mineralization of nutrients, as well as distribution and control of soil microflora (Butcher etal., 1971) [5]. The density of Acarina and Collembola, suffered a statistically significant and constant decline in the aldrin 30 EC (0.25%)- and endosulfan 35 EC (0.33%)-treated soil of wheat fields (Joy & Chakravorty, 1991). An increase in altrazine dose from 1.46 mg active ingredient /g to 2.33 mg a.i /g, decreasedegg production and increased the duration length of instars in Entomobrya musatica (Collembola) (Al-Assiuty & Khalil, 1996). Even short duration exposure to heptachlor and endosulfan treated soil for 24 or 72 hours only was found to delay the egg-laying and decrease the fecundity of both the species,

Cyphoderus javanus (Collembola) and Archegozetes longisetosus (Acari) (Joy etal., 2005) [13]. Application of chlorpyrifos reduced collembolan density to a greater extent than dimethoate; the effect of the combined application on total collembolan numbers was similar to that of chlorpyrifos (Endlweber etal., 2006). The growth regulator (IGR) pesticides on soil microarthropod *Cyphoderus javanus* under lab conditions (Saha & Joy, 2014) [17] showed negative effects on its life history.

Acrinathrin is a pyrethroid used as an insecticide and an acaricide derived from hexafluoro-2-propanol. It is used to control the mites on wine grapes, table grapes, pepper and ornamentals. A high risk to non-target arthropods in the in-field and off-field area was indicated from the available laboratory studies. Extensive laboratory studies showed that after 111 – 112 days of ageing of agrochemicals in the field, there is probability for re-establishmentof collembolans. The consequence of acrinathrin 75 g/L EW was found to be persistent to *Folsomia candida.*

Materials and methods

Collection and rearing of *Bilobella braunerae*

The live specimens for the present study were collected largely from soil and litter of decaying leaves in Konni forest division in Pathanamthitta District using a soil augar. The organisms extracted from the above soil sample were transferred by a camel brush into plastic containers of 7x 3 cm size, containing a base of plaster of paris – charcoal. These organisms in plastic container were maintained as stock colonies. These animals in culture were fed with fruit of jackfruit tree (*Artocarpus heterophyllus*). Thus *Bilobella braunerae* were acclimatized to the laboratory condition for about three months and by this time, three to four generations were readily complete. For experimental purposes, freshly laid eggs from stock culture were transferred to smaller plastic culture container of 5x 3.5cms with perforated plastic lids. These culture containers were provided with substratum of about 1 cm in thickness, consisting of a mixture of plaster of paris and animal charcoal in 5:1 ratio (Snider etal., 1969). These animals in culture were fed with decaying leaves of jackfruit tree. To keep the plaster of paris base saturated fresh distilled water was sprinkled every day. The saturation was judged by the speed with which the water added to the base was absorbed.

Observations were made on the oviposition, fecundity, duration of instars, number of instars, and moulting of *Bilobella braunerae* species at room temperature. Experiments were conducted by transferring pairs from stock culture. Daily observations were made and eggs laid by the pairs of individuals were counted and removed under Labomed Stereozoom trinocular microscope.

Preparation of Stock Solution of agrochemicals

1000 ppm stock solution of agrochemicals Acrianthrin 2%EC, Mancozeb 75%WP, Altrazine 50% WP was prepared by dissolving required quantity of chemicals in one liter of distilled water. From this stock solution seven concentrations (in ppm) plus a control were prepared.

Bioassay

Experiments conducted in laboratory using cultured animals. Five replicates of ***B.*** *braunerae* were tested for each concentration of the agro chemicals. 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 with the agrochemical. 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, 1964); Safe concentrations and sublethal concentrations were determined by the method suggested by (Hart, 1945). For fecundity studies fourty, one day old juveniles were separated and cultured in separate culture bottle.

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.

Fecundity studies after treating with Agrochemicals

For fecundity studies five replicates of *Bilobella braunerae* were maintained for each agrochemical. Individuals of were given jack leaves soaked in sub lethal concentration of acrianthrin, mancozeb and altrazine. 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

Results and Discussion

Bioassay studies

Acrinathrin 2% EC

0.5 ppm,1 ppm,2 ppm,5 ppm,8 ppm,12 ppm,15ppm acrinathrin were tested for different groups of 70 individuals till 96 hours. 7.05 % mortality was observed at 96 hours for 0.5ppm, 15.34 % for 1ppm,35.90% for 2 ppm, 49.03

% for 5 ppm, 57.73for 8 ppm, and 89.09 for 12 ppm and 100% mortality for 15 ppm Table (1). There was gradual increase in mortality with concentration and length of exposure of pesticide.

Table 1: Percentage Mortality of *B. braunerae* at different hours of Acrinathrin 2% EC

Conc. In ppm	12hrs	24hrs	48hrs	72hrs	96hrs
0.5	0	2.5	3.09	4.80	7.05
1	5.01	7.23	8.09	12.11	15.34
2	12.34	15	21.45	28.46	35.90
5	18.12	19.43	28.79	38.63	49.03
8	25.52	35.13	39.23	48.67	57.73
12	42.33	58.78	69.78	78.09	89.09
15	46.34	65.45	78.09	89.46	100

Mancozeb 75% WP

0.4ppm, 0.6ppm, 0.7ppm, 0.8ppm, 1ppm and 2ppm and 2.5ppm Mancozeb were tested for different groups of 70 individuals till 96 hours. There was no mortality till 0.3 ppm concentration of Mancozeb. At 96 hrs 23.68% mortality was observed for 0.4ppm, 28.58% for 0.6ppm, 35.90% for 0.7 ppm, 49.24% for 0.8 ppm and58.75% for 1ppm, 84.08% for 2ppm and 100% for 2.5ppm respectively Table (2).

Table 2: Percentage Mortality of *B. braunerae* at different hours of, Mancozeb 75% WP

Conc. in ppm	12hrs	24hrs	48hrs	72hrs	96hrs
0.4	2.1	6.02	11.02	19.80	23.68
0.6	8.23	12.13	18.40	22.56	28.58
0.7	14.09	25.45	29.21	31.37	35.90
0.8	22.21	29.01	37.34	45.75	49.24
1	25.34	35.06	39.56	48.60	58.75
2	37.67	45.50	49.45	66.78	84.08
2.5	45.09	58.70	72.90	89.01	100

Altrazine 50%WP

0.05ppm, 0.1ppm,0.2ppm .0.3ppm,0.4ppm,0.6ppm and 0.7ppm Altrazine were tested for different groups of 70 individuals till 96 hours. There was no mortality till 0.04% ppm concentration of Altrazine. At 96 hrs 21.22% mortality was observed for 0.05ppm, 36.16% for 0.1ppm, 41.25% for 0.2 ppm, 52.73% for 0.3 ppm, 72.34 % for 0.4ppm, 89.04% for 0.6 ppm and 100% for 0.7ppm respectively Table (3).

Table 3: Percentage Mortality of *B. braunerae* at different hours of, altrazine50%WP

Conc. in ppm	12hrs	24hrs	48hrs	72hrs	96hrs
0.05	3.08	7.70	14.90	16.09	21.22
0.1	8.45	14.67	25.46	28.54	36.16
0.2	15.34	18.02	28.79	38.92	41.25
0.3	18.56	19.43	35.23	48.98	52.73
0.4	29.48	37.56	39.12	54.79	72.34
0.6	38.57	47.09	59.23	77.09	89.04
0.7	40.78	57.01	68.05	98.01	100

LC50, LC 100, safe and sublethal concentrations of Agrochemicals

The LC100 value at 96 hours for acrinathrin was found to be 14.07 ppm, 2.368 ppm for mancozeb and0.685 ppm for altrazine. The LC50 at 96 hours of acrinathrin was found to be 6.11 ppm and 1.0238 ppm for mancozeb and for altrazine was found to be 0.265

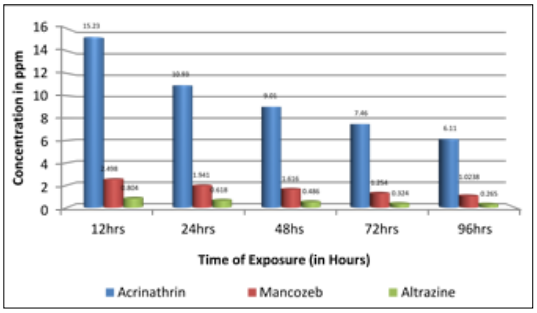

Fig 1: LC 50 of agrochemicals at different hours of exposure on *B. braunerae*

Safe and Sub lethal Concentration of agrochemicals on *B. braunerae*

The safe level concentration at 96 hours was found to be 1.25ppm for acrinathrin,0.185ppm for mancozeb and0.0355 altrazine. Sub lethal concentration at 96 hours was found to be 1.527ppm for acrinathrin, 0.255ppm for mancozeb and 0.0663ppm altrazine.

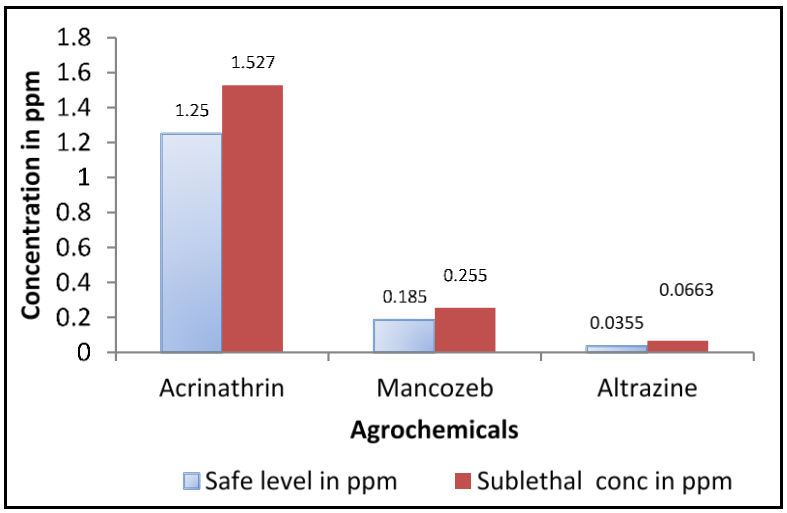

Fig 2: Safe and Sub lethal Concentration of agrochemicals on *B. braunerae*

Normal fecundity studies

Six ovipositions were observed for the entire five replica studied. Each oviposition continued for 36-48 hrs for each replicate. The average number of eggs laid was between 80 and 89 in first oviposition, 94 and 98 in second oviposition, between 75 and 78 in third oviposition, between 62 and 69 in fourth oviposition, between 54 to 65 in fifth oviposition, between 50 and 58 in sixth oviposition. The eggs were laid under the fragments of food provided or on the side wall of culture chamber. The eggs were generally seen attached together with a sticky substance to form cluster of eggs. During oviposition the female lowers the posterior end of the abdomen and depress the head. The eggs are laid one by one and heaped together with the help of ovipositor on the ventral side of body below the female genetilia. Eggs are laid in clusters which contain 6-17 eggs. Generally each oviposition was preceded by a moult but rarely every adult moult was strictly followed by an oviposition. The female *B. braunerae* oviposits 5 to 6 times with a mean of 5.5 oviposition at 29± 1 ºC. The maximum fecundity of normal *B. braunerae* was seen in oviposition 2 in each replicate Table (1.4). The maximum fecundity was observed in the replicate 2, with 73.166 eggs per brood and minimum was recorded in replicate 1, with 71.667 eggs per brood (Table 4). The two-way ANOVA conducted showed that there was no significant difference in number of eggs between replicates (P>0.05) but there was significant difference between different oviposition (P<0.05).

Table 4: Fecundity of *B. braunerae* under normal conditions

Replicates	Oviposition 1	Oviposition 2	Oviposition 3	Oviposition 4	Oviposition 5	Oviposition 6	Mean
1	80	94	76	65	65	50	71.667
2	87	95	78	66	55	58	73.166
3	86	95	75	68	58	51	72.167
4	89	98	77	69	54	54	73.5
5	82	95	78	62	60	56	72.17

Fecundity of *B. braunerae* treated with Sublethal Concentration of acrinathrin.

Acrinathrin treated individuals also showed six 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 45and 56 in different replicates. In second oviposition it was 64 to 78, 52 to 55 in the third oviposition, 41 to 45 in the fourth oviposition, 41 to 52 in the fifth oviposition,41 to 45 in the sixth oviposition. The average number of eggs in replica one was 53.666 in each oviposition, 52.166 in replica two, 48.5in replica three, 50.66 in replica four and 50 in replica five. The mean number of eggs laid by *B. braunerae* after the treatment with sublethal concentration of acrinathrin was found to be 48.5 to 53.67 compared to normal fecundity which was 71 to 73 eggs. So the fecundity decreased by 22-20 eggs after treatment. The reduction of eggs in each oviposition was also clearly noticed. The maximum fecundity of normal *B. braunerae* was seen in oviposition 2 in each replicate. The maximum fecundity was observed in the replicate1, with 53.67eggs per brood and minimum was recorded in replicate 4, containing 48.5 eggs per brood (Table 5). The two-way ANOVA showed that there was no significant difference in number of eggs laid between replicates ((P= 0.219349, P>0.05) but there was significant difference between different ovipositions (P= 1.87E-09, (P<0.05).

Table 5: Fecundity of *B. braunerae after* treatment with sublethal concentration of acrinathrin (pesticide)

Replicates	Oviposition 1	Oviposition 2	Oviposition 3	Oviposition 4	Oviposition 5	Oviposition 6	Mean
1	55	78	55	41	52	41	53.666
2	56	76	54	42	41	44	52.166
3	45	65	52	45	42	42	48.5
4	56	64	54	44	45	41	50.666
5	46	69	54	44	42	45	50

Fecundity of *B. braunerae* treated with Sublethal Concentration of Mancozeb (fungicide)

When *B. braunerae* was exposed to the sublethal concentration of mancozeb (fungicide) there was further reduction in number of eggs laid in each oviposition. The number of ovipositions still remains as 6. The number of eggs produced in the first oviposition was between 41 to 46 in different replicates, 51 to 55 in the second oviposition, 32 to 39 in the third, 22 to 25 in the fourth, 24 to 32 in fifth, 20 to 28 in sixth .The mean number of eggs laid in each oviposition was found to be 32.667 in the first replicates, 34.333 in the second, 34.5 in the third, 36.667 in the fourth, and 35.83in the fifth replicate.. The maximum fecundity was observed in the replicate 4, 36.67eggs per brood and minimum was recorded in replicate 1, 32.67 eggs per brood Table (6). The two-way ANOVA showed that there was no significant difference in number of eggs laid between replicates (P= 0.065979, P>0.05) but there was significant difference between different ovipositions (P= 1.18E-14, P<0.05). The mean number of eggs laid by *B. braunerae* after the treatment with sublethal concentration of Mancozeb (fungicide) was found to be between 32.67 to 36.67compared to normal fecundity which was between 71 to 73 eggs. There was reduction in fecundity by 39-37 eggs after treatment.

Table 6: Fecundity of *B. braunerae after* treatment with sublethal concentration of Mancozeb (fungicide)'

Replicates	Oviposition 1	Oviposition 2	Oviposition 3	Oviposition 4	Oviposition 5	Oviposition 6	Mean
1	44	54	32	22	24	20	32.667
2	42	51	35	25	32	21	34.333
3	41	52	39	24	31	20	34.5
4	45	55	38	22	32	28	36.667
5	46	53	38	22	32	24	35.83

Fecundity of *B. braunerae* treated with Sublethal Concentration of altrazine(herbicide)

When *B. braunerae* was exposed to the sublethal concentration of altrazine (herbicide) there is a drastic reduction in number of eggs laid in six ovipositions. The number of eggs produced in the first oviposition was between 35 to 39 in different replicates, between 40 to 42 in the second oviposition, 15 to 22 in the third, in the fourth 12 to 14 and 8 to 11 in fifth and sixth. The mean number of eggs laid in each oviposition was found to be 22 in the first replicates, 21 in the second, 20.83 in the third, 21.33 in the fourth and 20.67 in the fifth replicate. The maximum fecundity of normal *B. braunerae* was seen in oviposition 2 in each replicate. The maximum fecundity was observed in the replicate 1, 22 eggs per brood and minimum was recorded in replicate 5, 20.67 eggs per broods Table (7). The two-way ANOVA showed that there was no significant difference in number of eggs laid between replicates (P= 0.685082, P>0.05) but there was significant difference between different ovipositions (P= 1.72E-18, P<0.05). The mean number of eggs laid by *B. braunerae* after the treatment with sublethal concentration of altrazine (herbicide) was found to be between 20.67to 22 compared to normal fecundity which was between 71 to 73 eggs. There was drastic change in fecundity of 50-51 eggs after treatment with altrazine. The number of eggs produced by females after the treatment of Altrazine was less, it's revealed that the herbicide affect drastically on collembolans The one way ANOVA conducted showed significant difference between fecundity in normal and agrochemical treated *Bilobella braunerae* (F=1362.817, F crit=3.238872; P= 1.78E-19, P<0.05)

Table 7: Fecundity of *B. braunerae* treated with Sublethal Concentration of Altrazine (herbicide)

Replicates	Oviposition 1	Oviposition 2	Oviposition 3	Oviposition 4	Oviposition 5	Oviposition 6	MEA N
1	35	41	22	12	11	11	22
2	35	40	20	14	9	8	21
3	36	40	20	12	8	9	20.83
4	39	42	15	13	10	9	21.33
5	36	40	16	13	11	8	20.67

Table 8: Normal moulting interval of *B. braunerae*

Stages	Group 1 Days	Group 2 Days	Group 3 Days	Group 4 Days	Group (mean days)
Rest	4.5	4.6	4.5	4.5	4.525
1st moult	10.5	11.4	10.3	10.4	10.65
2nd moult	15	14	17	16	15.5

3rd moult	20	23	22	20	21.25
4th moult	26	28	27	25	26.5
Adult	Egg laid	Egg laid	Egg laid	Egg laid	

Normal *B. braunerae* showed 4 moults before laying eggs, the first moult started after 11 days of hatching, second moult after 15 days of hatching, third moult after 21 days of hatching, fourth moult after 26 days of hatching. (Table 8). The female *B. braunerae* attained sexual maturity after 4th moult and 5th instar individual began to lay eggs under laboratory condition both in normal and some of the treated groups.

Table 9: Moulting interval of *B. braunerae* after treatment with sub lethal concentration of acrinathrin (pyrithroid pesticide)

Stages	Group 1 days	Group 2 days	Group 3 days	Group 4 days	Group (mean days)
Rest	6.8	8.6	7.9	8.9	8.05
1st moult	15.9	16.8	16.9	17.6	16.8
2nd moult	18	19	19	21	19.25
3rd moult	24	27	25	23	24.75
4th moult	37	34	34	35	35
Adult	Egg laid	Egg laid	Egg not laid	Egg laid	

After the treatment with the sub lethal concentration of acrinathrin, moulting intervals becomes prolonged. First moult only begins after 17 days, second moult roughly by 19 days, third moult on 25 days, fourth moult on 35 days (Table 9).

Table 10: Moulting interval of *B. braunerae* after treatment with sub lethal concentration of Mancozeb (fungicide)

Stages	Group 1 Days	Group 2 Days	Group 3 Days	Group 4 Days	Group (mean days)
Rest	10	11	12	13	11.5
1st moult	20.6	21.2	24.3	22.1	22.05
2nd moult	22.6	24.5	25.4	24.3	24.2
3rd moult	33.5	33	33	35	33.63
4th moult	42	42	45	42	42.75
Adult	Egg laid	Egg laid	Egg laid	Egg laid	

Table 11: Moulting interval of *B. braunerae* after treatment with sub lethal concentration of Altrazine (herbicide)

Stages	Group 1 Days	Group 2 Days	Group 3 Days	Group 4 Days	Group (mean days)
Rest	15	14	17	12	14.5
1st moult	22	25	24	27	24.5
2nd moult	34	35	39	34	35.5
3rd moult	50	53	49	48	50
4th moult	67	68	61	59	63.75
Adult	Egg not laid	Egg laid	Egg laid	Egg laid	

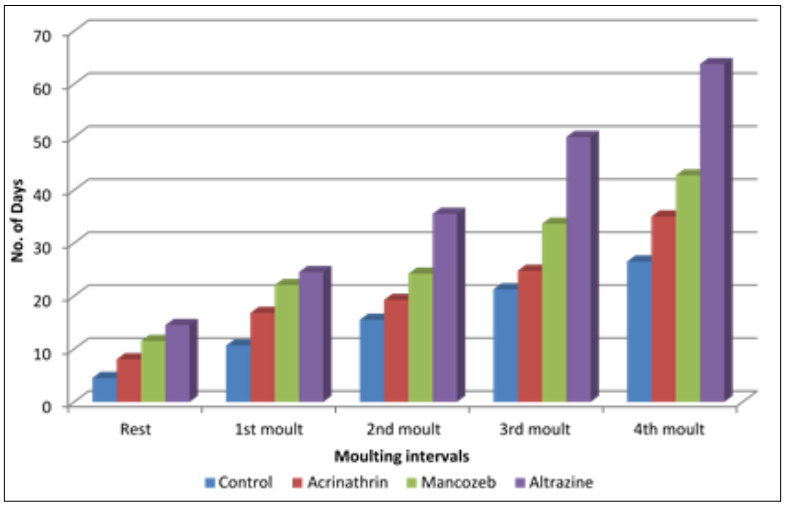

Fig 3: Moulting interval of *B. braunerae* in control and treated groups

After the treatment with the sub lethal concentration of mancozeb, the days taken for hatching of egg delayed compared to control group, moulting intervals also becomes prolonged. Eggs in control group hatch in 5days but in mancozeb treated group hatching of egg takes place after 11-13 days First moult is observed after 22 days, second moult roughly by 24 days, third moult on 33 days, fourth moult on 42 days (Table 10).
After the treatment with the sub lethal concentration of altrazine, the days taken for hatching of egg delayed compared to control group, and accordingly moulting intervals also changed. Eggs in control group hatch in 5days but in altrazine treated grouph, egg hatches after 14-17 days First moult is observed after 24 days, second moult roughly by 35 days, third moult was quite delayed and was seen in one group after 50 days (Table 11).

Discussion

The detritus food chain in the top and sub pedological layers were totally disturbed through the indiscriminate use of compounds having fluoride, chlorinated, ethylene 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. *B.braunerae* 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) [20]; (Hussain & Zahira, 2010). Among the agrochemicals tested altrazine had great effect on the egg production by the ovary followed by the fungicide mancozeb. 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 6th 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.

Soil collembolans ar*e* soil-dwelling, wingless, unpigmented insect that is widely distributed around the globe, plays an important role in soil ecosystems. Because of abundant distribution and ease of culture and short reproductive cycle, the springtail is one of the most extensively used animals in terrestrial ecotoxicology. Collembola contribute to the breakdown of soil organic matter and to mineralisation of nutrients (Verhoef & Brussaard, 1990) [21]. There is, therefore, reason to be concerned with the potential toxic effects of different toxicants on these organisms. They are exposed to toxins via the epidermis, ventral tube (water uptake), or gut via food; however, it is not clear which uptake routes are the most important (Pedersen *et al*., 1997) [14]. The moulting processes temperature dependent, the rate showing a linear relation with temperature. Below 3-5° C no moulting or growth occurs, the animals do not feed (Joosse, 1971) [10] and mostly rest in aggregations (Joosse & Groen, 1970) [11]. In Orchesella cincta (L.) (Collembola) males moulted more frequently than females in in cadmium and zinc treated groups (Posthuma *et al*., 1992) [16]. In general, metals are much less toxic when added to the food of *F. candida* than when incorporated into soil in standard tests. It is suggested that Collembola have a greater tolerance of metals in the diet since they avoid contaminated food, and are able to excrete assimilated metals at moulting via exfoliation of the midgut epithelium where the elements are retained as part of a storage–detoxification system

The Nonylphenol stimulates fecundity but not population growth rate of Folsomia candida (Widarto *et al*., 2007) [22]. The present study was designed to explore the relation-ships between individual- and population-level responses of a terrestrial invertebrate to agrochemicals. The LC50 at 96 hours of acrinathrin was found to be 6.11 ppm and 1.0238 ppm for mancozeb and for altrazine was found to be 0.265. This shows among the

agrochemicals altrazine is highly toxic to *B. braunerae.* These low values indicate pesticide is highly determintal to soil collembolans like *B.braunerae.* There was no significant difference between moulting intervals in *B. braunerae* but significant difference was observed in oviposition between normal and groups treated with sub lethal concentration of agrochemicals. (Badejo & Van Straalen, 1992) indicated that soil temperature accounts for a higher percentage of variation in springtail number. The pesticide treatments showed that a negative relationship exists between pesticide concentration and the density of the test species (Al-Assiuty & Khalil, 1996). Agrochemical treated food at certain doses caused a marked effect on both female fecundity and the length of instar duration. These two vital functions probably affect the population peaks. Similar field observations on soil microarthropod populations have been reported earlier by (Popovici, 1977) [15] and (Edwards, 1979). (Badejo & Van Straalen, 1992) confirmed that the lethal dose in a laboratory experiment greatly exceeds that in a field experiment. This implies a difference in uptake efficiency of agrochemicals, which occurred only during feeding and during body surface contact with the contaminated food for a long time. The agrochemicals dose taken via ingestion is frequently subjected to metabolic processes. The very thin cuticle of most of the microarthropods especially collembolans persuade the influx of methyl, ethyl and thionate groups of pesticides,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 biomassaccumulation. The haemolymph 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 there by reducing the fecundity. 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 6th oviposition. This indicates the rebuilding of soil ecosystem after a heavy doze application of these chemicals may take decades to come back to the virgin condition. So it is suggested 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.

Summary and Conclusion

Six ovipositions were observed for the *B. braunerae* in the five replicas studied and the mean number of eggs was between 71.6 and 73.5. The agrochemical treated individuals also showed six ovipositions but the number of eggs in each oviposition was less when compared to normal. The safe level concentration at 96 hours was found to be 1.25ppm for acrinathrin,0.185ppm for mancozeb and 0.0355 altrazine. Sub lethal concentration at 96 hours was found to be 1.527ppm for acrinathrin, 0.255ppm for mancozeb and 0.0663ppm altrazine. Among the three agrochemicals studied maximum ill effect was shown by altrazine. Altrazine has low LC 50 among the three agrochemicals that means high toxicity than acrinathrin and mancozeb. Altrazine treated organisms showed less egg production because the methyl compound in the herbicide had effect on egg formation and maturation. From the study revealed that extensive application of agrochemicals adversely affect the soil microarthropod *B. braunerae,* which will indirectly affecting the soil fertility. Soil collembolans body is covered with thin cuticle which can absorb the agrochemicals easily through their body surface. Among the agrochemicals tested altrazine had great effect on the egg production by the ovary followed by the fungicide mancozeb followed by acrinathrin. 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. In agrochemical treated groups prolonged moulting intervals were observed compared to control group which clearly suggest that protein synthesis for ecdysis was affected. Because of delayed protein synthesis moulting period has prolonged. As protein synthesis is affected it clearly suggests that agrochemicals do affect the DNA and RNA of the collembolan species. Direct heavy doze application of these chemicals into the soil ecosystem may have much detrimental effect on the very existence of these tiny arthropods. Herbicide treated *B. braunerae* showed drastic reduction in fecundity and also prolonged moulting intervals compared to pesticide and fungicide. Indiscriminate agrochemical applications kills both pests and natural enemies concurrently and results in the revival of non-target insects, and the misuse and overuse of agrochemicals can harm farmers, consumers, and the environment too.

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