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IMPACT OF PESTICIDE ACRINATHRIN ON *Bilobella braunerae* DHERVANG (COLLEMBOLA: NEANURIDAE) IN LABORATORY EXPERIMENTS

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration of both authors. Author NT designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Author MGSK managed the analyses of the study and gave the necessary guidance. Both authors read and approved the final manuscript.

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ABSTRACT

Laboratory toxicity tests were carried out, to evaluate the effects of acrinathrin on fecundity and moulting intervals of *Bilobella braunerae* Dhervang (Collembola) grown in sub lethal concentration is 1.2612 ppm of acrinathrin pesticide. The Collembola feeding on leaves of jackfruit containing Acrinathrin pesticide exhibited trends of increased days in moulting and decreased fecundity rates up to 1.2612 ppm An inhibition of growth and fecundity was seen above the sublethal concentration. Both moulting and fecundity rates were significantly affected by the pesticide presented as food to the collembola. The untreated control sets recorded high fecundity for *B. braunerae*, but chronic toxicity of the insecticides on adults confined to the treated food resulted into very low fecundity and high moulting rates. Even short duration exposure to acrinathrin treated food for 24 or 72 hours only 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 pesticide pollution in soil and for ecological health assessment.

Keywords: *Bilobella braunerae*; acrinathrin pesticide; moulting; fecundity; soil microarthropods.

1. 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-

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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 [1]. 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 [2]. They contribute to the decomposition of organic matter, mineralization of nutrients, as well as distribution and control of soil microflora [3]. The density of major groups of microarthropods, namely Acarina and Collembola, suffered a statistically significant and persistent decline in the aldrin 30 EC (0.25%)- and endosulfan 35 EC (0.33%)-treated soil of wheat fields [4]. As the atrazine herbicide dose was increased to 1.46 mg a.i. g−1and at the dose 2.33 mg a.i. g−1, a marked negative effect on egg production and the duration length of instars was noticed in *Entomobrya musatica* (Collembola) [5]. 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) [6]. 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 [7]. The likely ill effects of insect growth regulator (IGR) pesticides on ecologically significant soil microarthropod fauna was evaluated using life history parameters in *Cyphoderus javanus* under microcosm conditions [8].

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. Extended laboratory studies showed that there is potential for recolonisation of the in-field area after 111 – 112 days of ageing of residues. Effect of acrinathrin 75 g/L EW was found to be chronic to *Folsomia candida.* A high risk to soil dwelling arthropods was reported on the basis of the available data [9].

2. MATERIALS AND METHODS

The live specimens for the present study were collected largely from soil and litter of decaying leaves in Ranni forest division and rubber plantation of Seethathode village in Pathanamthitta District .Sufficient soil samples of 5X5 cm^2 area were randomly collected from different altitudinal places of study site using a soil augar. The soil samples were collected in a polythene bag, tied using rubber band, properly marked and taken to the laboratory. Live specimens of *Bilobella braunerae* were extracted from the above sample into bits of moist decaying leaves using a battery of Berlese Tullgren funnels, 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 integrifolia*). Thus *B. 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 3.5 cms 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 et al. 1969). These animals in culture were fed with decaying fruit 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, incubation period of eggs, duration of instars, number of instars, moulting and longevity of *B. 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. Acrinathrin pesticide was used to test the toxicity on *B. braunerae*. Six sublethal concentrations plus a control were used: 0, 1, 2, 5, 8, 12 and 15 ppm concentration of pesticide were prepared. Adult individuals were kept in separate culture chambers and given food soaked in respective concentrations of pesticide. Three replicates were used for testing each concentration of pesticide. Mortality was recorded at every 12, 24, 48, 72 and 96 hours interval. From these data LC 50, LC 100, sublethal and safe concentrations were calculated using LC 50 software.

For fecundity studies eighty, one day old juveniles were separated and cultured in separate culture

bottle. Forty were given food soaked in sublethal concentration of pesticide and rest was given normal food. Both control and treated organisms were again divided into four groups each containing five pairs. Moulting intervals (in days) and the fecundity in each oviposition of normal and pesticide treated organisms were counted.

2.1 Statistical Analysis

One way ANOVA was used to test difference in moulting intervals of *B. braunerae* in different groups and also between control and treated. Two way Analysis of variance was also done to test difference in fecundity between control and treated groups. Effects were considered significant when p≤0:05.

3. RESULTS AND DISCUSSION

3.1 Reproduction

B. braunerae 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. 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°C. The fecundity was observed to be maximum with an average number of 441 eggs in six ovipositions. The average number of eggs per individual, pooled over the six broods (Fig. 1) showed that acrinathrin pesticide significantly affected egg production in the springtails.

The female *B. braunerae* attained sexual maturity after 4^{th} moult and 5^{th} instar individual began to lay eggs under laboratory condition both in normal (Table 1.1) and treated groups (Table 1.2). The one way ANOVA showed no significant difference in moulting intervals between control and treated group (F=0.742952, F crit=5.317655; P.>0.05). *B. braunerae* released their first exuviae (cuticle) at the age of 10–11 days. Sublethal concentration of acrinathrin pesticide increased the time for first moult to 16-17 days (Fig. 2). After the first exuviae, *B. braunerae* cast off its cuticle every 6–7 days, and pesticide did not affect this molting period. Thus, during the 74-day experiment, 12 moultings were recorded on average.

Springtails laid eggs for the first time at an age of 25–26 days (Table 1.1), and then produced eggs every 5–6 days, during the 74-day experiment. Whereas in *B. braunerae* treated with sublethal concentration of acrinathrin pesticide there was a delay in laying eggs and eggs were laid at an age of 34-37 days (Table 1.2). Total of six ovipositions was analyzed from each of five females per treatment. The early broods contained an average of 80–89 eggs per individual in oviposition 1, brood size increased in second oviposition reaching approximately 96 eggs per brood and there was a decrease in eggs laid with each oviposition and by sixth oviposition the only 50-58 eggs were laid (Table 1.3). In pesticide treated group a decrease in fecundity was observed in all six ovipositions with an average of 45-56 eggs in oviposition 1 and increase in brood size in second oviposition to average of 70 eggs and then there was decrease in number of eggs in each oviposition and approximately 42 eggs laid in sixth oviposition (Table 1.4). There was drastic reduction of 15-20 eggs when compared to normal fecundity. Two way ANOVA showed no significant difference in fecundity of *B. braunerae* between different replicates but significant difference between different oviposition in both control and treated groups groups. The one way ANOVA conducted showed significant difference between fecundity in control and pesticide treated *B. braunerae* (F=510.151,F crit=5.317655; P<0.05).

3.2 Bioassay

At 1 ppm concentration of pesticide 5%, 7%, 8%, 12% and 15% mortality was observed. At 96 hours 15%, 35%, 49%, 57% 89% and 100% mortality was recorded for 1, 2, 5, 8, 12 and 15 ppm concentration of pesticide (Table 1.5). Lethal concentration -100 was found to be 19.7126 ppm for 48 hours, 16.687 ppm for 72 hrs and 14.392 for 96 hrs. Lethal concentration -50 was found to be 9.3176 ppm for 48 hrs, 7.3876 ppm for 72 hrs and 5.8942 ppm for 96 hrs (Table 1.6). LC -50 value at near to 5 ppm at 96 hours shows that acrinathrin is highly toxic to *B. braunerae.* The value of safe concentration was found to be 1.1117 ppm and sublethal concentration was found to be 1.2612 ppm. These low values indicate pesticide is highly determintal to soil collembolans like *B. braunerae.*

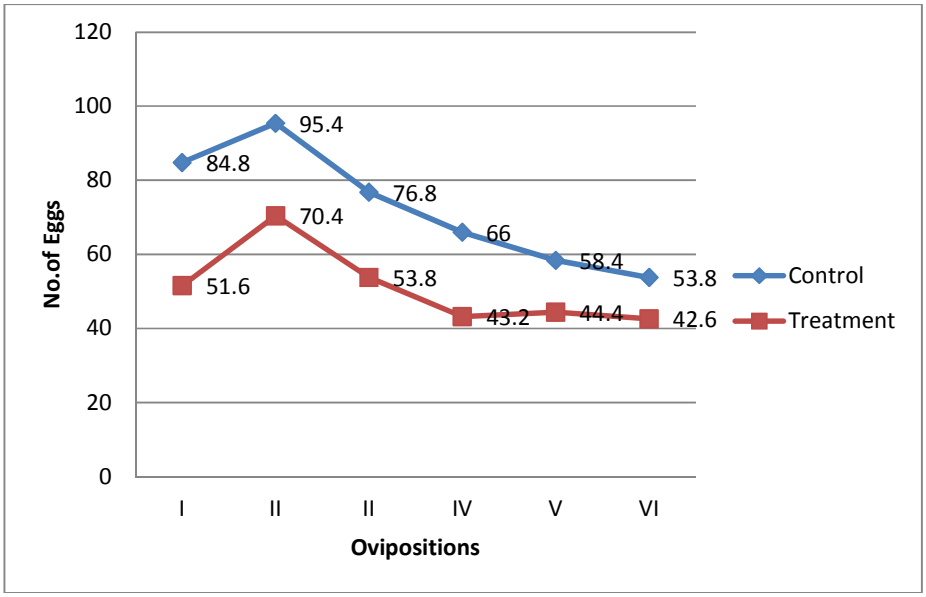

Fig. 1. Mean number of eggs in each oviposition of *B. braunerae* in control and treated groups

Table 1.1. Normal moulting interval of *B. braunerae* (average days)

Stages	Group 1	Group 2	Group 3	Group 4	Mean±Se
Rest	4.5	4.6	4.5	4.5	4.525±0.025
1st moult	10.5	11.4	10.3	10.4	10.65±0.253
2nd moult	15	14	17	16	15.5±0.646
3rd moult	20	23	22	20	21.25±0.75
4th moult	26	28	27	25	26.5±0.645
Adult	Egg laid	Egg laid	Egg laid	Egg laid	

Table 1.2. Moulting interval (in average days) of *B. braunerae* treated with sub lethal concentration of acrinathrin (pyrithroid pesticide)

Stages	Group 1	Group 2	Group 3	Group 4	Mean±Se
Rest	6.8	8.6	7.9	8.9	8.05±0.466
1st moult	15.9	16.8	16.9	17.6	16.8±0.349
2nd moult	18	19	19	21	19.25±0.629
3rd moult	24	27	25	23	24.75±0.854
4th moult	37	34	34	35	35±0.707
Adult	Egg laid	Egg laid	Egg not laid	Egg laid	

Table 1.3. Normal fecundity of female *B. braunerae*

Replicates	Oviposition 1	Oviposition 2	Oviposition 3	Oviposition 4	Oviposition 5	Oviposition 6
1	80	94	76	65	65	50
2	87	95	78	66	55	58
3	86	95	75	68	58	51
4	89	98	77	69	54	54
5	82	95	78	62	60	56
Mean	84.8	95.4	76.8	66	58.4	53.8

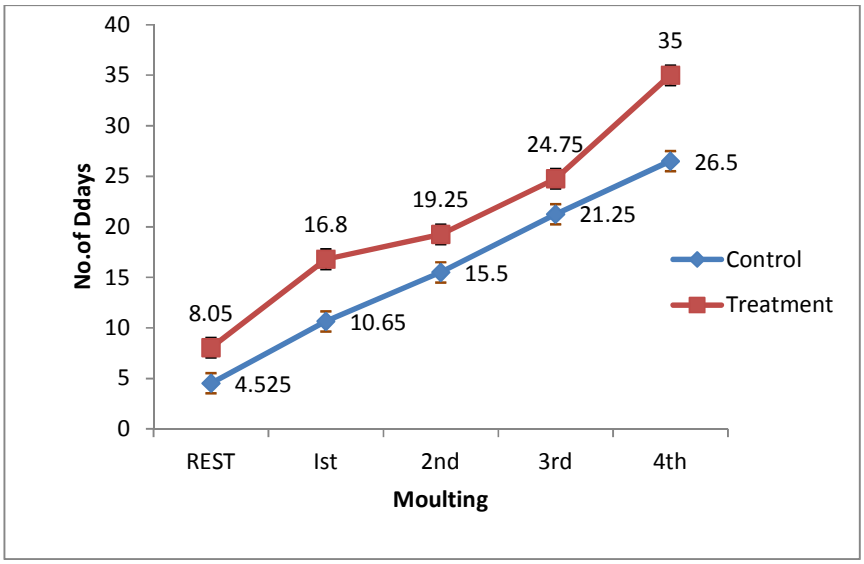

Fig. 2. Mean number of days for moulting of *B. braunerae* in control and treated groups

Table 1.4. Fecundity of female *B. braunerae* treated with sublethal concentration of acrinathrin

Replicates	Oviposition 1	Oviposition 2	Oviposition 3	Oviposition 4	Oviposition 5	Oviposition 6
1	55	78	55	41	52	41
2	56	76	54	42	41	44
3	45	65	52	45	42	42
4	56	64	54	44	45	41
5	46	69	54	44	42	45
Mean	51.6	70.4	53.8	43.2	44.4	42.6

Table 1.5. Percentage mortality of *B. braunerae at* different concentration of acrinathrin at different hours

Concentration in PPM	12 hrs	24 hrs	48hrs	72 hrs	96 hrs
1	5	7	8	12	15
2	12	15	21	28	35
5	18	19	28	38	49
8	25	35	39	48	57
12	42	58	69	78	89
15	46	65	78	89	100

Table 1.6. LC50 and LC -100 values of acrinathrin pesticide on *B. braunerae*

	12HR	24HR	48HR	72HR	96HR
LC50	15.6733	11.0510	9.3176	7.3876	5.8942
LC 100	32.4583	22.5810	19.7126	16.6876	14.3992

4. CONCLUSION

Soil collembolans are 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 [10]. There is, therefore, reason to be concerned with the potential toxic effects of different toxicants on these organisms. Several researchers have observed the negative impact of agrochemicals on collembolan species. The findings of present investigation on (pyrithroid pesticide) formulation, acrinathrin also exhibited toxic effect on *B. braunerae*. 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 [11] and mostly rest in aggregations [12]. The Nonylphenol stimulates fecundity but not population growth rate of Folsomia candida [13]. Pretilachlor and Pendimethalin exhibited toxic effect on *Cyphoderus javanus* [14]. Eijsackers, H [15] reported shorter life span, decreased egg laying and increased frequency of moulting due to impact of herbicide 2, 4, -5T on collembola. Haque, A et al. [16] reported shorter life span, increased frequency of moulting and early maturity on Cyphoderus species (collembola) exposed to different insecticides and herbicides respectively. Badejo MA et al. [17] 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 [18]. The present study was designed to explore the relation-ships between individual- and population-level responses of a terrestrial invertebrate to a toxicant. LC -50 value at near to 5 ppm at 96 hours shows that Acrinathrin 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 treated groups with sub lethal concentration of acrinathrin (pyrithroid pesticide) .Effect of pesticide was significant as it has decreased the fecundity of *B. braunerae* Acrinathrin 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 [19] and [20]. Badejo MA et al. [17] confirmed that the lethal dose in a laboratory experiment greatly exceeds that in a field experiment. This implies a difference in uptake efficiency of Acrinathrin, which occurred only during feeding and during body surface contact with the contaminated food for a long time. The Acrinathrin dose taken via ingestion is frequently subjected to metabolic processes. Consequently, a reduction of toxicant action of Acrinathrin could be detected and the mode of action of Acrinathrin may become more efficient on the outer body surface of such delicate animals.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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