

Assessing the toxic effects of seven insecticides on soil arthropods in the rice crop ecosystem

Comment [L1]: Toxicity of selected insecticides to soil arthropods of rice ecosystem

Abstract

~~It is known that s~~Soil ~~oi~~ fauna performs a critical role in the biological turnover and nutrient release from plant residue. The presence of pollutants in the soil leads to disruptions that cause both qualitative and quantitative changes in the fauna, ultimately impacting soil functioning. Globally, rice (*Oryza sativa* L.) is one of the most important food crop, ~~particularly in Asia,~~ ~~where it serves as and~~ a staple food ~~crop~~ for billions of people. The present study was undertaken to evaluate the impact of ~~seven~~ insecticides viz., ~~chlorantraniliprole, fipronil, cartap hydrochloride, flubendamide, indoxacarb, fipronil+imidacloprid and fipronil+indoxacarb~~ on the collembolan population, a key ~~component member~~ of soil arthropod ~~communities,~~ ~~within in~~ rice ~~cropping system~~ecosystem. The experiment was carried out at the Crop Research Centre, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, during the *kharif* season of 2022. . The study revealed significant reductions in collembolan populations across all ~~treated groups~~treatments, with the most substantial effects observed in fipronil+imidacloprid and fipronil+indoxacarb ~~combination treatments~~. The current findings highlight the potential adverse impacts of insecticides on soil health, emphasizing the need of careful selection of pest management strategies to preserve ~~soil~~biodiversity ~~of soil arthropods~~ and maintain ecological balance.

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Introduction

Rice (*Oryza sativa* L.) stands as the world's most important crop cultivated across 117 countries ~~and serving as the and is a~~ staple food ~~source~~ for more than 3.5 billion people worldwide, which is nearly half of the global population. It is especially important in Asia, where it ~~serves is considered as~~ as the primary source of calories and nutrition for the majority of the population (FAO, 2004). ~~Globally,~~ India ~~ranks stands~~ first ~~globally~~ in ~~rice~~ cultivation area, covering 44.6 million hectares, and is the second-largest producer, with 137 million metric tons produced in 2023-24, accounting for 26% of the world's rice production (USDA, 2023-24).

Many species of arthropods inhabit rice fields, ~~although most of them are not and only a~~ ~~few are~~ truly noxious to the crops. For instance, ~~some about~~ 500 species of insects and spiders

may appear in a rice field in a particular season, ~~with only a few of them are causing~~ potential threat to the crop. In addition to the terrestrial arthropods, various ~~other~~ arthropods are also found in ~~the~~ soil that significantly contribute to the soil fauna ~~diversity~~. ~~The Soil-soil~~ fauna is typically categorized based on their size into microfauna (e.g., protozoa, nematodes), mesofauna (e.g., mites, springtails), and macrofauna (e.g., earthworms, beetles). Each group contributes to the overall functioning and biodiversity of the soil ecosystem.

In terms of species richness, arthropods account for up to 20% of the soil fauna and constitute a significant portion of the meso- and macro- fauna populations within the soil environment. Soil-dwelling arthropods carry out several important functions within the soil. Nutrients in the soil are mineralized by arthropods through microbial consumption, thereby completing the process of nutrient recycling and the ~~composition of soil mainly consists of is~~ ~~enriched with the arthropod's excreta and the dead remains of the soil dwelling arthropods~~. (Sagi and Hawlena, 2021). Collembola are the most abundant arthropods in soil and play a significant role as decomposer in food webs (Petersen, 2002).

The introduction of high-yielding varieties led to a substantial increase in the use of commercial inputs and agrochemicals. While these inputs have contributed to higher crop yields and protection against pests, ~~but~~ their indiscriminate use has ~~indirectly contributed to reduced~~ ~~reduction in~~ soil fertility. Soils have become repositories for various chemical inputs, ~~including especially~~ insecticides (Bhuyan *et al.*, 1993), ~~which are that are considered~~ beneficial for ~~enhanced yields and better crop protection crop production~~ but ~~have pose~~ negative ~~impacts consequences that include including~~ toxic residues in food, water, air, and soil, pest resurgence, resistance and adverse effects on non-target organisms.

The extent of these hazards is directly co-related with the persistence of the insecticides. The longer they remain in the soil, the higher the risk of affecting soil fauna and contaminating the environment, ultimately leading to disruptions in soil health. When insecticides are applied, they often ~~mix or~~ accumulate in the top ~~layers at around a depth of~~ 10–15 cm of soil, the layer with the highest microbial and faunal activity (Blasco and Pico, 2009). This creates conditions for interactions between insecticides and soil fauna, impacting soil functions (Cortet *et al.*, 1999).

Soil micro arthropods are frequently used as bio-indicators of soil quality (Cortet *et al.*, 2002). Since pesticides are not entirely species-specific, they can cause both lethal and sublethal effects

on non-target species, which may vary depending on developmental stages (Adamski *et al.*, 2007; Charmillot *et al.*, 2001). Collembola are one of the non-target organisms that gets for pesticides still they are often exposed to them pesticides that are applied for crop protection (De Santo *et al.*, 2018). Biodiversity studies are needed need for effective sampling, particularly for highly diverse terrestrial groups like arthropods (Colwell and Coddington, 1994). Therefore the present study was conducted to understand and conclude how agrochemicals, particularly insecticides, impact soil arthropods in rice crop ecosystem.

Materials and methods

The Experiment experiment was conducted in the Crop Research Centre, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, located at 29°-01'-33.60"-N latitude and 79°-28'-16.19"-E longitude during *kharif* season of 2022 in rice cropping system. Rice (variety cv. HKR-47) saplings were sowed was sown in plots measuring 5m × 5m, at a spacing of 20×10cm between rows and leaving 20 cm between plants to plant.

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The Seven insecticides under evaluation viz. chlorantraniliprole 20 SC at 150 ml/ha, fipronil 5 SC at 1000 ml/ha, cartap hydrochloride 50 SP at 1000g/ha, flubendamide 480 SC at 50 ml/ha, indoxacarb 14.5 SC at 500 ml/ha, fipronil 15 SC + imidacloprid 5 SC at 500 ml/ha, and fipronil 15 SC + indoxacarb 5 SC at 1000 ml/ha were applied at recommended dose in foliar application during at 25-30 days after transplanting. Besides the above insecticides an untreated control was maintained. Three replications were maintained.

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Comment [L4]: Any replications? include if at all present.

The diversity indexes of **Shannon (H)** and **Simpson diversity index (1-D)**, as well as the evenness index of **Pielou (J)**, were used to assess insect diversity within the ecosystem? In accordance with **Magurran (2004)**, the unequal distribution of abundance between species allowed the use of the dominance index of **Berger-Parker (1/d)** to express the proportion of individuals accounted for, by the most abundant species in each site. The species richness index of was used to highlight the most species-rich site.

1. Shannon Diversity Index (H):

The Shannon diversity index measures the entropy (uncertainty or diversity) within a dataset. It's calculated as:

$$H = - \sum (p_i \cdot \ln p_i)$$

Where p_i is the proportion of the i -th species. Higher values indicate greater diversity.

2. Simpson Diversity Index (1-D):

Simpson's index measures the probability that two individuals randomly selected from a sample will belong to the same species. It's calculated [by using the following formula \(reference\)](#):

$$D = \sum p_i^2$$

Where p_i is the proportion of the i -th species. However, $1-D$ (Simpson's Index of Diversity) is often used, where higher values indicate greater diversity.

3. Pielou's Evenness Index (J):

Pielou's evenness index measures how evenly individuals are distributed across the species in a community. It's calculated [by using the following formula \(reference\)](#)as:

$$J = \frac{H}{\ln S}$$

Where H is the Shannon diversity index and S is the total number of species. Values range from 0 to 1, with 1 indicating perfect evenness.

4. Berger-Parker Index (1/d):

The Berger-Parker index gives the proportional dominance of the most abundant species. It's calculated [by using the following formula \(reference\)](#)as:

$$d = \frac{N_{max}}{N}$$

N_{max} is the number of individuals of the most abundant species, and N is the total number of individuals. The inverse, $1/d$, is used to express diversity, where higher values indicate greater diversity. All these indices are commonly used in ecological community studies (Magurran, 2004).

To study the arthropod abundance in the experimental site, observations were taken [pretreatment prior to applying insecticides \(pre-treatment\)](#) - and at 15, 30, and 75 days after insecticide application [and labeled accordingly](#) (Rajagopal, Kumar, & Gowda, 1990). The samples were then moved to the laboratory where the Tullgren Funnel was installed. Extraction methods were designed to suit behavior and body structures of the organisms (Hopkin, 1997;

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Wallwork, 1970). The soil micro arthropods were collected and put into containers with 70% alcohol within 48h of extraction and examined under stereo microscope for proper identification. ~~All the experiments were replicated thrice.~~ The collected data was subjected to statistical analysis using Duncan's multiple range test (DMRT) to compare the specific difference of mean with the help of SPSS (26) software.

Results and Discussion

The soil under investigation hosts a diverse assemblage of arthropods, predominantly from the class Insecta, which represents 83.54% of the total population. Within this collection, Collembola (springtails) ~~are were~~ the most abundant, comprising 42.57% of the total soil arthropod collected from the field. ~~Following Collembola, Followed by this,~~ Isoptera (termites) constituted 29.32%, and Hymenoptera (ants) ~~make made~~ up 7.23%. Other insect orders include Coleoptera (beetles) at 3.21% and Dermaptera (earwigs) at 1.21%. The class Arachnida, ~~accounting accounted~~ for 7.23% of the total, ~~and is was~~ predominantly represented by Oribatida (mites) at 5.62%, ~~with and~~ Araneae (spiders) contributing 1.61%. Diplopoda (millipedes) represented 4.83%, Chilopoda (centipedes) 2.01%, and Annelida (earthworms) 2.41% ~~were the other members of the Phylum Arthropoda~~. This distribution highlights a rich and varied soil ecosystem, with a clear dominance of insect species, particularly springtails, which play a crucial role in the soil environment.

~~The present study evaluated the impact of seven different insecticidal treatments on the collembolan population soil at different time intervals: 15, 30, and 75 days after spraying (DAS). This analysis helps understand the implications of insecticide use on soil fauna. The insecticides tested included chlorantraniliprole 20 SC, fipronil 5 SC, cartap hydrochloride 50 SP, flubendamide 480 SC, indoxacarb 14.5 SC, Fipronil 15 + imidacloprid 5 SC, fipronil 15 and Indoxacarb 5% SC. An untreated control was also maintained for comparative analysis.~~

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Results indicate significant reductions in collembolan populations across all treated groups compared to the control. The most pronounced reductions were observed in the combination treatments of fipronil 15% + Imidacloprid 5% SC and fipronil 15% + indoxacarb 5% SC, with mean populations of 2.71 and 2.75 individuals per 500g of soil, respectively. These treatments showed per cent reduction of 51.74% and 50.98% over ~~control~~. Conversely, chlorantraniliprole 20 SC and flubendamide 480 SC showed relatively lower reductions, with

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mean populations of 4.06 and 3.95 individuals/ 500g of soil and percentage reductions of 27.56% and 29.62%, respectively. The control group exhibited a stable population of approximately 5.61 individuals/ 500g of soil throughout the study period, emphasizing the natural resilience of the collembolan population in untreated soil.

Comment [L8]: 3+ in treatment and 5+ in control groups cannot be considered a significant difference.

The results suggest that the combination insecticides, particularly those containing fipronil, have a significant detrimental effect on the collembolan population. This could be attributed to the synergistic effects of the combined active ingredients, leading to relatively higher ~~contribution-toxicity in reducing and subsequent reduction in~~ soil-dwelling arthropods. These findings align with previous studies that have highlighted the broad-spectrum activity of fipronil-based insecticides and their impact on non-target soil organisms (Kim *et al.*, 2014; Oliver *et al.*, 2016). The substantial reduction in collembolan populations raises concerns about the potential long-term ecological consequences, given the crucial role of collembolans in soil health and nutrient cycling (Hopkin, 1997). The differential impact observed across the various insecticides underscores the need for careful consideration when selecting pest control strategies in agricultural ecosystems.

Comment [L9]: ?? fipronil belongs to phenyl pyrazole group. Do you mean this group is toxic?

Although, ~~the~~ effective pest management is essential, the potential adverse effects on beneficial soil organisms must be weighed to maintain ecological balance. Further research is warranted to explore the recovery dynamics of collembolan populations post-insecticide application and to investigate alternative pest management practices that mitigate harm to soil biodiversity (Pisa *et al.*, 2015).

Table 1. Abundance of soil arthropods found in the experimental location

Class	Order	Individuals	% of Total Count
Insecta	Coleoptera (beetles)	8	3.21%
	Collembola (Springtail)	106	42.57%
	Hymenoptera (Ant)	18	7.23%
	Isoptera (Termite)	73	29.32%
	Dermaptera (Earwigs)	3	1.21%
Total Insecta	Total	208	83.54%
Arachnida	Oribatida (Mite)	14	5.62%
	Araneae (Spider)	4	1.61%
Diplopoda	Millipedes	12	4.83%
Chilopoda	Centipedes	5	2.01%
Annelida	Earthworms	6	2.41%

Grand Total		249	100%
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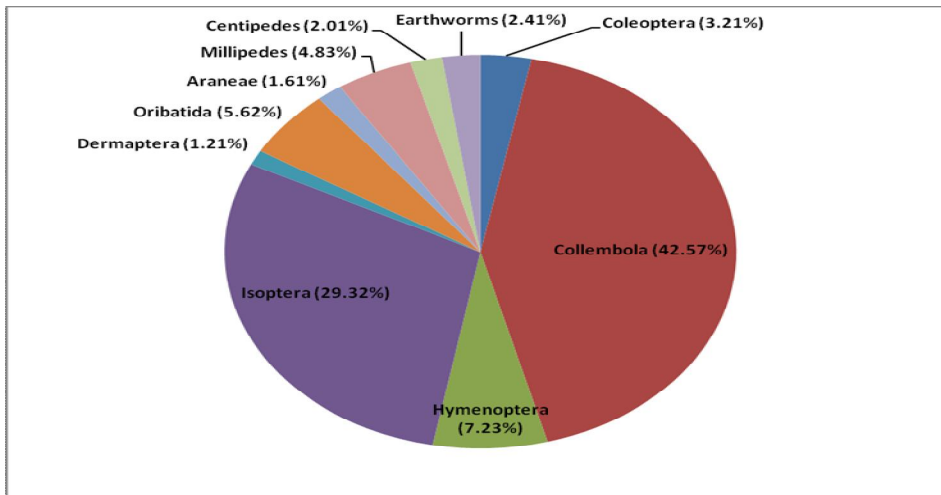


Fig 1. Abundance of soil arthropods found in the experimental location

Table 2. Species diversity and species richness indices calculated for insects associated

Diversity Index	Value
Shannon Diversity Index (H)	1.498
Simpson Diversity Index (1-D)	0.723
Pielou's Evenness Index (J)	0.651
Berger-Parker Index (1/d)	2.35

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Table 3. Effect of insecticides on collembolan population at different interval

Treatment No.	Pre Treatment	15DAS	30DAS	75DAS	Mean	% ROC
T1*	4.41±0.03 ^b	3.18±0.03 ^f	3.94±0.08 ^g	4.71±0.03 ^g	4.06	27.56
T2	4.25±0.10 ^{ab}	2.16±0.04 ^c	2.45±0.06 ^c	2.93±0.07 ^c	2.92	47.90
T3	4.12±0.07 ^a	2.46±0.06 ^d	3.12±0.05 ^e	3.67±0.09 ^e	3.34	40.37
T4	5.21±0.11 ^c	2.88±0.07 ^e	3.52±0.06 ^f	4.17±0.10 ^f	3.95	29.62
T5	4.28±0.06 ^{ab}	2.29±0.05 ^c	2.78±0.04 ^d	3.38±0.07 ^d	3.18	43.22
T6	5.63±0.06 ^d	1.38±0.01 ^a	1.64±0.02 ^a	2.17±0.03 ^a	2.71	51.74
T7	5.21±0.09 ^c	1.53±0.06 ^b	1.89±0.02 ^e	2.36±0.02 ^b	2.75	50.98
T8	5.55±0.01 ^d	5.63±0.06 ^g	5.68±0.13 ^h	5.56±0.01 ^h	5.61	27.56

Treatment No.	Pre Treatment	15DAS	30DAS	75DAS	Mean	% ROC
SEm(±)	0.06	0.04	0.07	0.06		
CD 5%	0.19	0.12	0.20	0.20		
CV	13.00	50.16	41.26	32.16		

*T₁= Chlorantraniliprole 20 SC, T₂= Fipronil % 5 SC, T₃= Cartap Hydrochloride 50 SP (T3), T₄= Flubendamide 480 SC (T4), T₅= Indoxacarb 14.5% SC (T5), T₆= Fipronil 15%+ Imidacloprid 5% SC, T₇= Fipronil 15%+Indoxcarb 5 SC, T₈= Control

**Numbers followed by same letters are statistically at par (otherwise significantly different at $p < 0.05$)

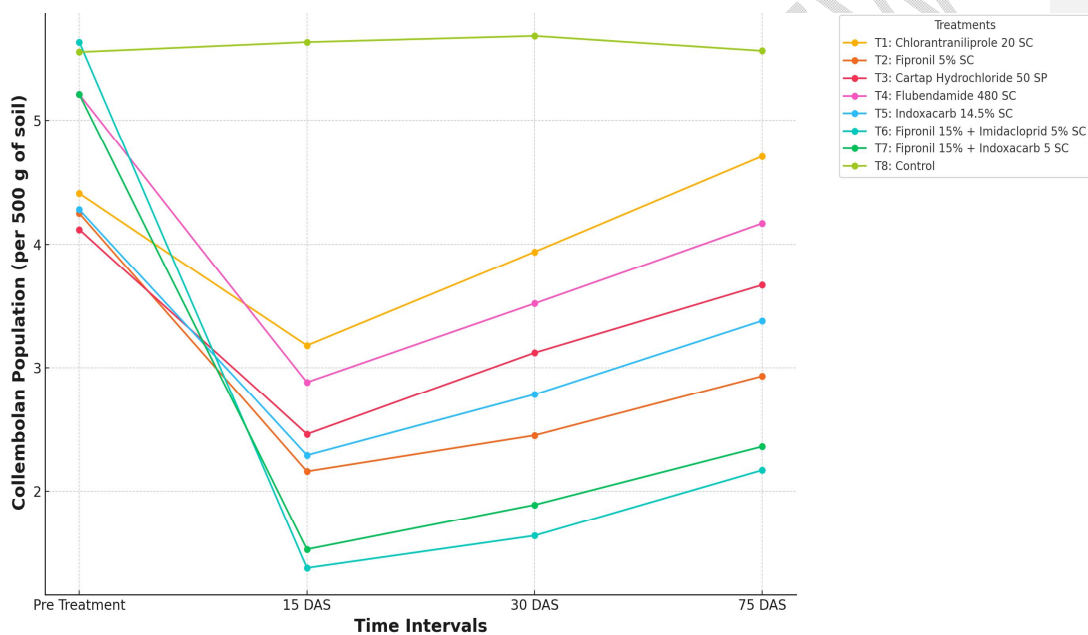


Fig 2. Effect of insecticides on collembolan population at different intervals

Conclusion:

This study demonstrates that the application of various insecticides, particularly those containing fipronil in combination with Imidacloprid or Indoxacarb, significantly reduces the collembolan population in rice cropping systems. The reduction in collembolan numbers, which play a vital role in soil health and nutrient cycling, raises concerns about the long-term ecological consequences of these insecticides. The results underscore the importance of considering the broader ecological impacts when selecting insect pest control strategies. Further research is necessary to investigate the recovery of collembolan populations post-insecticide

application and to explore alternative pest management practices that minimize harm to beneficial soil organisms. These findings contribute to the understanding of how agrochemicals affect soil biodiversity and highlight the need for sustainable agricultural practices.

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