

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

Abstract

It is known that soil 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 crops, particularly in Asia, where it serves as a staple food for billions of people. The present study was undertaken to evaluate the impact of seven different insecticides viz., chlorantraniliprole, fipronil, cartap hydrochloride, flubendamide, indoxacarb, fipronil+imidacloprid and fipronil+indoxacarb on the collembolan population, a key component of soil arthropods, within specifically in rice cropping systems. 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, with the most substantial effects observed in fipronil+imidacloprid and fipronil+indoxacarb. 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 and maintain ecological balance.

Introduction

Rice (*Oryza sativa* L.) stands as the world's most important crop cultivated across 117 countries and serving as the 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 as the primary source of calories and nutrition for the majority of the population (FAO, 2004). India ranks first globally in terms of area of 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 total rice production (USDA, 2023-24).

Many species of arthropods inhabit rice fields, although most of them are not truly noxious to the crops. For instance, some 500 species of insects and spiders may appear in a rice

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field in a particular season, ~~of which~~ only few ~~of them~~ are potential threat to the crop. ~~In addition to the terrestrial arthropods, various other arthropods are also found in soil that significantly contribute to the soil fauna.~~ 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.

Comment [NT2]: Cite reference.

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, viz., mineralization of soil nutrients~~ through microbial consumption, thereby completing the process of nutrient recycling and the composition of soil mainly consists of arthropod's excreta (**Sagi and Hawlena, 2021**). Collembola are the most abundant arthropods in soil and play a significant role as decomposer in food webs (**Petersen, 2002**).

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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 reduced soil fertility. Soils have become repositories for various chemical inputs, including insecticides (**Bhuyan et al., 1993**), which are beneficial for crop ~~production-protection~~ but have negative impacts that include toxic residues in food, water, air, and soil, pest resurgence, resistance and adverse effects on non-target organisms.

Comment [NT3]: Cite more references that are latest from last 5 years

The extent of these hazards is directly ~~eo-related~~ ~~correlated~~ with the persistence ~~as well as the relative toxicity~~ 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 10–15 cm ~~layer~~ of soil, ~~the layer with~~ ~~having~~ the highest microbial and faunal activity (**Blasco and Pico, 2009**). This ~~creates~~ ~~enhances the conditions for~~ interactions between insecticide ~~residues~~ and soil fauna, impacting soil ~~health and~~ functions (**Cortet et al., 1999**).

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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). There are very few collembolans known to damage crop plants hence, most of them are considered beneficial in terms of improving soil structure and other properties. These non-target Collembola collembolans are non target organisms for pesticides still they are often exposed to their lethal effects of pesticides (De Santo et al., 2018). Biodiversity studies need effective sampling, particularly for highly diverse terrestrial groups like arthropods (Colwell and Coddington, 1994). Therefore, present study was conducted to understand and conclude how agrochemicals, particularly insecticides, impact soil arthropods in rice crop ecosystem.

Comment [NT6]: Add few examples of collembolans having pest status along with latest references. i.e., *Sminthurus viridis*

Materials and methods

The Experiment was conducted in at 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 HKR-47) saplings were sowed in plots measuring 5m × 5m at a spacing of 20 × 10cm between rows and 20 cm between plants to plant. All the experiments were replicated thrice.

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The insecticides under evaluation viz., chlorantraniliprole 20 SC at 150 ml/ha, fipronil 5 SC at 1000 ml/ha, cartap hydrochloride 50 SP at 1000 g/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 by foliar application during at 25-30 days after transplanting.

Comment [NT8]: How many experiments were conducted? Avoid use of generalized terminologies.

The diversity indices 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 soil arthropods. In accordance with Further Magurran (2004), the unequal distribution of abundance between individuals of different species was computed using allowed the use of the dominance index of Berger-Parker (1/d) to express the proportion of individuals accounted for by of the most abundant species in each sampling site (Magurran, 2004). The species richness index of was used to highlight the most species-rich site.

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Comment [NT9]: The sentence does not express its meaning properly. Reform the sentence.

1. Shannon Diversity Index (H):

The Shannon diversity index measures the entropy (uncertainty or diversity) within a dataset. It is 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 whether the randomly selected~~ two individuals ~~randomly selected~~ from a sample ~~will would~~ belong to the same species. ~~It's It is~~ calculated as:

$$D = \sum p_i^2$$

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 It is~~ calculated as:

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

Where, H is the Shannon's 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 It is~~ calculated 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 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

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the Tullgren Funnel was installed. Extraction methods were designed to suit behaviors and body structures of the organisms (Hopkin, 1997; 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 three. The collected data was/were subjected to statistical analysis using Duncan's Multiple Range Test (DMRT) to compare the specific difference of/among means with the help of SPSS (26) software.

Comment [NT11]: Explain all extraction methods you utilized in detail with respect to each different soil arthropod.

Comment [NT12]: Acknowledge taxonomist, if assisted.

Comment [NT13]: How many experiments were conducted? Avoid use of generalized terminologies.

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 the most abundant, comprising 42.57% of the total soil arthropod collected from field. Following collembola, isoptera (termites) constitute 29.32%, and hymenoptera (ants) make up 7.23%. Other insect orders include coleoptera (beetles) at 3.21% and dermaptera (earwigs) at 1.21%. The class arachnida, accounting for 7.23% of the total, is predominantly represented by oribatida (mites) at 5.62%, with araneae (spiders) contributing 1.61%. Diplopoda (millipedes) represent 4.83%, chilopoda (centipedes) 2.01%, and annelida (earthworms) 2.41%. 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.

Comment [NT14]: Use scientific names of each collected/ observed species of arthropod, if identified scientifically by taxonomist. This seems very general observations and reduces the quality of the work. Try to be specific (for example, beetles. There are lot of soil inhabiting beetles. Which ones were in your study?)

Comment [NT15]: The work is finished. Use past tense only. Recast the text accordingly.

Comment [NT16]: Do not put facts. Just mention and describe your output and results in R & D section.

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Results indicated significant reductions in collembolan populations across all treated groups compared to the control. The most pronounced reductions were/was 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 the highest per cent reduction of 51.74% and 50.98% over control.

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Conversely, chlorantraniliprole 20 SC and flubendamide 480 SC showed relatively lower reductions, with 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 mean population of ~~approximately~~ 5.61 individuals/ 500g of soil throughout the study period, emphasizing the natural resilience of the collembolan population in untreated soil.

The results suggested 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 higher contribution in reducing 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 harmful impact observed ~~across the~~ due to various insecticides underscores ~~the need for~~ careful consideration ~~when for selecting~~ selection of pest control strategies in agricultural ecosystems.

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

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

<u>Class</u>	<u>Order</u>	<u>Individuals</u>	<u>% of Total Count</u> <u>Relative Abundance (%)</u>
Insecta	Coleoptera (beetles)	8	3.21%
	Collembola (Springtail)	106	42.57%
	Hymenoptera (Ant)	18	7.23%
	Isoptera (Termite)	73	29.32%
	Dermaptera (Earwigs)	3	1.20%
Total Insecta		208	83.5453%
Arachnida	Oribatida (Mite)	14	5.62%
	Araneae (Spider)	4	1.61%

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Diplopoda	Millipedes	12	4.8382%
Chilopoda	Centipedes	5	2.01%
Annelida	Earthworms	6	2.41%
Grand Total		249	100%

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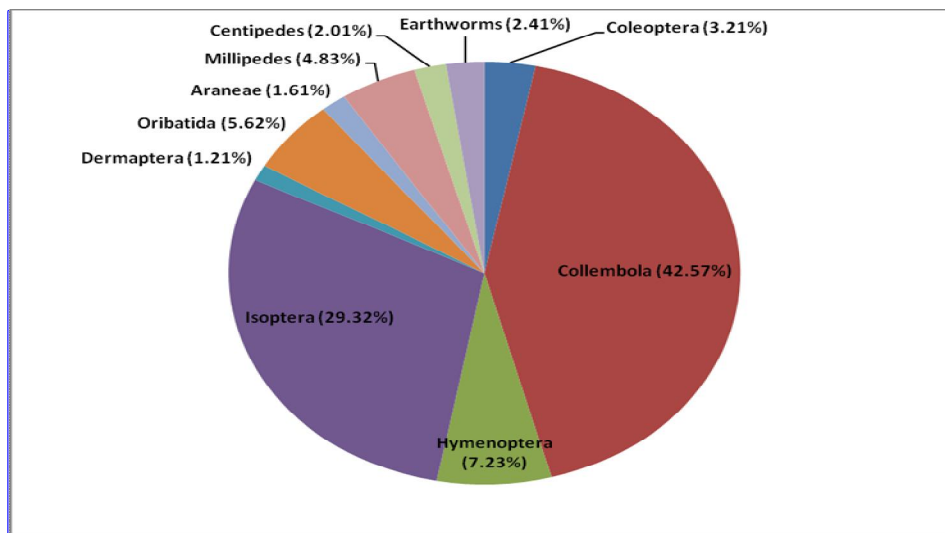


Fig 1. Relative Abundance of soil arthropods found in the experimental location

Comment [NT20]: Figure is just replication of the Table data.

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

Comment [NT21]: Merge the data of Table 2 within Table 1

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

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

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Treatment No.	Pre Treatment	15DAS	30DAS	75DAS	Mean	% ROC
T3	4.12±0.07 ^a	2.46±0.06 ^d	3.12±0.05 ^c	3.67±0.09 ^e	3.34	40.37
T4	5.21±0.110 ^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
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		

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*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%+Indoxacarb 5 SC, T₈= Control
 **Numbers followed by same letters are statistically at par (otherwise significantly different at $p < 0.05$)

Comment [NT22]: Figure is repetition of the data mentioned in the table.

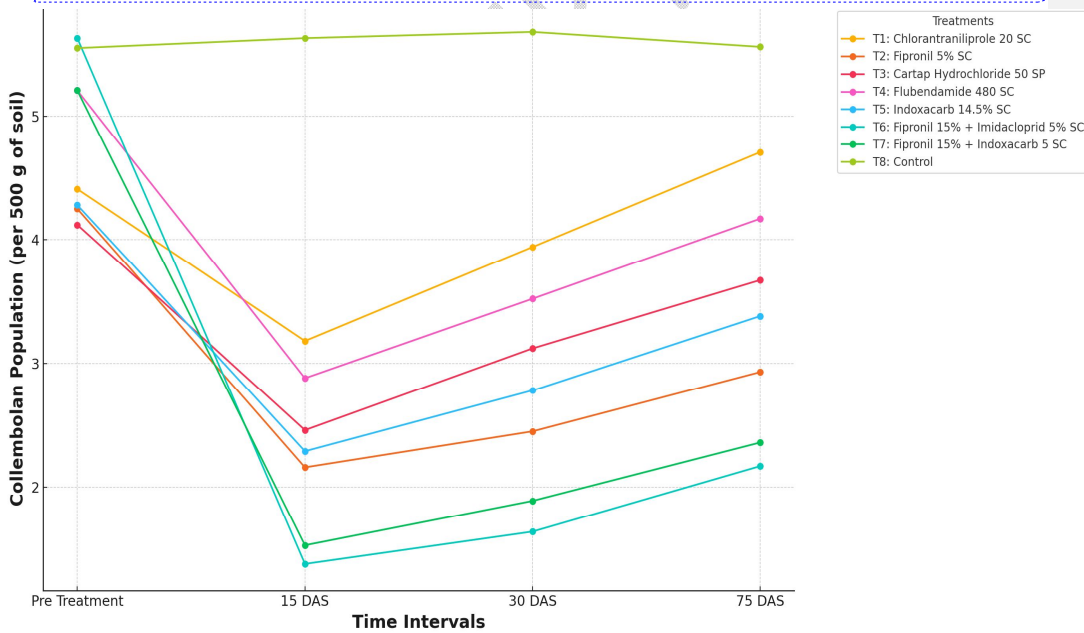


Fig 2. Effect of insecticides on collembolean population at different intervals

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.

Comment [NT23]: Recast the conclusion. Draw significant conclusion from the experiment. Recast whole text. Major reforms required.

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