

Culturable phylloplane microbiota and beneficial insects population dynamics in rice under drone spraying of pesticides

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Received: 10 May 2025

Revised: 02 September 2025

Accepted: 05 January 2026

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Abstract

Aim: To evaluate and compare the effects of drone and conventional power sprayer-based pesticide applications on the culturable phylloplane microbiota and beneficial insects' population in rice (*Oryza sativa*), focusing on key microbial groups and beneficial insects and their ecological implications.

Methodology: Phylloplane microflora isolated from leaf samples were collected pre- and post-application of treatments by Dickinson leaf washing method and analysed. Beneficial insects like spiders and coccinellids (lady bird beetles) population were recorded at pre- and post-application of treatments by visual counts.

Results: Drone-based pesticide applications reduced the total bacterial and *Pseudomonas* populations, whereas actinobacteria remained stable and fungal populations varied across treatments. Some fungi, like *Trichoderma* spp., increased in drone-treated plots. Gram-positive bacteria showed resilience post-application whereas Gram-negative bacteria exhibited variable responses. Coccinellid populations were not significantly affected, however, spider populations varied notably, with higher counts in treatments T₁ (chlorantraniliprole) and T₂ (tetraniliprole) and lower counts in T₃ and T₄ treatments, respectively.

Interpretation: Drone spraying induced distinct shifts in microbial communities and significant variations in spider populations were observed among the treatments both after the first and second spray applications, highlighting the need for further research on its long-term ecological effects and implications for sustainable agriculture.

Key words: Drone spraying, Microbial ecology, Natural enemies, Phylloplane microflora, Spiders, Sustainable agriculture

Impact of drone spraying of pesticides on phylloplane microbiota and beneficial insects

Study Design



Drone spraying

Conventional spraying

Sampling:
Pre & Post-Application (7 & 14 DAS)

Observations:

- Microbial populations
- Beneficial insects

Results

Phylloplane Microflora

Total bacterial	-ve
<i>Pseudomonas</i> spp.	-ve
Actinomycetes	stable
<i>Trichoderma</i> spp.	+ve
G-ve bacteria	Variable


Coccinellids
No significant change


Spiders
↑ in T₁ & T₂
↓ in T₃ & T₄

Interpretation



Drone spraying alters
Microbial and Beneficial insects'
population dynamics

Ecological implications
and need for further
studies in sustainable
pest and disease
management

Introduction

The increasing reliance on chemical pesticides in modern agriculture has undeniably contributed to enhanced crop protection and yield stability. However, such interventions often come at an ecological cost, particularly affecting non-target organisms that are crucial for the sustainability of agroecosystems. Two such components, phylloplane microbiota and beneficial insects, play integral roles in disease suppression and natural pest regulation in crop ecosystems like rice (*Oryza sativa* L.), one of the world's most important staple foods. The rice phyllosphere is an oligotrophic and dynamic environment, influenced by both abiotic (relative humidity, temperature, light, nitrogen deposition) and biotic factors (plant exudates and secondary metabolites). Epiphytic microorganisms have evolved several adaptation mechanisms to survive these ever-changing conditions (Bringel and Couee, 2015; Etemadi et al., 2018). Pesticide elicits a distinct shift in rare phyllosphere microbiota, with minor or no effect on the core microbiota. Generally, treated plants show increased microbial diversity compared to untreated samples, but this rise in diversity may be detrimental to function (Cernava et al., 2019a). Beneficial bacteria, intrinsically associated with plants, are among the most sensitive responders to pesticide application (Chen et al., 2021).

Pesticide-driven biodiversity increase is often linked to replacement effects, where niches vacated by pathogens are occupied by microbial competitors, along with community expansion of taxa possessing stress-response systems (Xu et al., 2020). Evidence from several crops demonstrates such microbial shifts. For example, in tea leaves, lime sulfur pesticide signatures included *Brevundimonas*, *Arsenophonus*, and *Candidatus* Portiera, species are known to tolerate harsh conditions (Cernava et al., 2019a). In tobacco, the broad-spectrum fungicide N-(3,5-dichlorophenyl) succinimide has been found to increase Gamma proteobacteria relative to Alpha proteobacteria. Genes related to stress responses, biofilm formation and efflux pumps has elevated following pesticide treatments (Yu et al., 2023). Similar patterns have been observed in rice, where the phyllosphere is predominantly colonized by Proteobacteria, with Alpha proteobacteria comprising up to 33.8% of the community, and pesticide application influencing both the core and rare microbiome (Chen et al., 2021; Varghese et al., 2025; Feng et al., 2023).

Phospholipid Fatty Acid (PLFA) analysis and molecular fingerprinting have reported significant but transient pesticide effects on epiphytic microbial communities (Zhang et al., 2009; Gu et al., 2010). Later sequencing studies revealed microbial resilience (Perazzolli et al., 2014; Ottesen et al., 2015). However, fungicide applications in maize and soybean have reduced Tremellomycetes yeasts, including the family Bulleribasidiaceae, which harbors core microbiome members (Noel et al., 2022). Despite their ecological significance, commercial pesticides are rarely evaluated for their effects on non-pathogenic phylloplane microflora, with their selectivity remaining largely speculative (Huang et al., 2023).

Among the beneficial insects, coccinellid (lady bird beetles) and spiders stand out as important contributors to natural biological control systems. Coccinellids are voracious predators of various agricultural pests, including aphid, thrips and mite, while spiders act as general predators, preying on various insect pests in agricultural fields. These beneficial faunae play a vital role in regulating pest populations, reducing reliance on chemical pesticides, and maintaining ecological balance in agricultural ecosystems. The rapid adoption of drone-based pesticide application introduces novel deposition patterns, as drones generate finer droplets than conventional methods. In response to this technological advancement, the Government of India, as of April 2023, introduced standard operating protocols for safe application of pesticides using drones. Although drone spraying offers increased precision and efficiency, its potential impact on phylloplane microflora and natural enemies has not been studied. This is of particular concern, because natural microbial populations and natural enemies exert antagonistic effects on foliar pathogens and insect pests, thereby contributing to biological control (Biondi et al., 2012).

Given the frequent use of pesticides in rice cultivation, it is important to understand how drone-based applications affect these microorganisms, which play a vital role in disease suppression and plant health. Literature on the impact of pesticide spraying on phylloplane microflora is limited. In this context, the present study compares drone and manual spraying by assessing culturable microbial populations and natural enemies in rice, highlighting drone spraying's impact on phylloplane microbes and biological control.

Materials and Methods

The present investigation was carried out during *kharif* (Vanakalam), 2022 at the Institute of Rice Research, Agricultural Research Institute, Rajendranagar, Hyderabad, using the variety Samba Mahsuri (BPT 5204), with each plot measuring 360 m² and a total treatment area of 1080 m². To draw a valid and standard conclusions on the impact on phylloplane microflora, requires a series of sample collection over a period. For phylloplane microflora analysis, leaf samples were collected before spraying, at 7 and 14 days after spraying. Similarly, beneficial insects' population (spiders and coccinellids) were recorded pre- and post-application (7 and 14 DAS) of treatments.

Spraying equipment: As depicted in Fig. 1, the UAV model employed for aerial spraying was battery-powered AGRICOPTER AG 365. It operated on two 22,000 mAh Li-Po batteries, providing a flight endurance of approximately 15 min when fully loaded. The drone had a tank capacity of 10 l and was operated at a flight speed of 3.6 m sec⁻¹. Equipped with four hydraulic nozzles, symmetrically positioned on each side of the fuselage, the UAV achieved precise flight control through ground radar guidance and the expertise of a trained drone operator. The flight altitude was maintained 2.5 m above the crop canopy, while the effective spray swath covered a width of 3.5 m. The spray



Fig. 1: Agricopter Ag365.

system used XR 11002 VP nozzles (manufactured at M/s. Teejet Technologies India Pvt. Ltd., Bengaluru), which were of the extended-range flat fan type with a spray angle of 110°.

Treatment details: The present study comprised 13 treatments, including two insecticides (chlorantraniliprole 18.5% SC and tetraniliprole 18.18% w/w SC) and two fungicides (picoxystrobin 7.5%+tricyclazole 22.5% SC and tebuconazole 50% + trifloxystrobin 25% WG). These were applied individually using a drone (T1–T4), in different insecticide–fungicide combinations using both drone (T5–T8) and power sprayer (T9–T12), and compared against an untreated control (T13). The required amount of water was taken for each treatment and recommended dosage of insecticide or fungicide alone or in combination was added, mixed well and then sprayed uniformly for three replications. The recommended pesticide dose (g or ml a.i. ha^{-1}) was kept constant for both drone and power sprayer applications. The only difference was in the spray fluid volume used for dilution. For drone application, the required pesticide dose was diluted in 40 l of water per hectare, whereas for power sprayer application, the same pesticide dose was diluted in 375 l of water per hectare. At the time of first spray, the initial GPS mapping of treatments and replications for autonomous drone spraying was done and the same maps were then utilized for second application, ensuring consistency in treatment application within the field.

Isolation of phylloplane microflora: Leaf washing method was carried out following Dickinson *et al.* (1975). Fresh leaves were collected before spraying and at 7 and 14 DAS from 10 randomly selected points per treatment and pooled to form a composite sample. From this, three subsamples were taken as technical replicates. Ten gram of leaf tissue was washed in sterile distilled water on a rotary shaker, and the washings were serially diluted up to 10^{-7} . Dilutions of 10^{-3} – 10^{-4} were used for fungi and actinomycetes, and 10^{-5} – 10^{-6} for bacteria using the pour-plate method. Microbes were isolated on nutrient agar, *Pseudomonas* agar, Kenknight & Munier's medium and PDA for bacteria,

Pseudomonas, actinomycetes, and fungi, respectively. Plates were incubated under standard conditions appropriate for each group. Colonies were counted as CFU g^{-1} ; fungi were purified by single-spore or hyphal-tip isolation, while bacterial and actinomycete cultures were recorded only as population counts.

Assessment of microbial cultures: The number of bacterial colonies developed on the plates after incubation period of 24–48 hrs were counted on a digital colony counter (M/s. Labtronics, Haryana) and the number of colonies per gram of sample were computed (Atlas *et al.*, 1978). The number of fungal colonies developed on the plates after an incubation period of 4 days were referred to as colony-forming units (CFU), and the number of CFU per gram of sample was computed (Das *et al.*, 2003). Gram staining was performed on nutrient agar culture plates for 10 randomly selected colonies for each treatment (Lacy and Lukezic, 2004). The fungal isolates were identified up to genus level following the standard morphological criteria, including colony morphology, conidiophore architecture, and conidial characteristics. For *Aspergillus*, species-level identification was conducted based on distinct diagnostic features such as conidiophore morphology, conidial form, and colony pigmentation following the keys provided by Samson *et al.* (2014). The per cent occurrence of fungus was calculated (Dong *et al.*, 2014).

Beneficial fauna sampling: Standard sampling methods were used to record the populations of coccinellid and spiders at each treatment and at different time intervals. Ten hills were randomly selected for each treatment, and the population of coccinellid and spiders were recorded by visual counts both before (pre-count) and after the application of pesticide mixtures at 7 and 14 days after spraying (DAS). The per cent occurrence of beneficial insects was calculated by the following formula:

$$\text{Beneficial fauna (\%)} = \frac{\text{Per cent No of beneficial insects in treatment}}{\text{No of beneficial insects in untreated control}} \times 100$$

Statistical analysis: The experimental data on beneficial insect populations were statistically analyzed in RCBD as per Gomez and Gomez (1984). Significant differences between treatments were calculated by ANOVA and Duncan's multiple range test at a significance level of 95% with OPSTAT software package. Treatment means were compared with DMRT at 5% probability level. For microbial data, statistical analysis was not performed as the replicated plates exhibited high dispersion due to spatial heterogeneity across large treatment area.

Results and Discussion

The perusal of data (Table 1) showed that different pesticide treatments had varying effects on the proliferation of bacterial populations on the phylloplane of rice. Total bacterial counts ranged widely before spraying and declined at 7 DAS, with partial recovery by 14 DAS. *Pseudomonas* populations showed similar fluctuations across sampling days. *Pseudomonas* spp. serve as an important plant growth-promoting and biocontrol roles, these population shifts indicate complex interactions between pesticide residues and beneficial phyllosphere communities (Arya and Harel, 2019). Drone-applied treatments (T_5 - T_8) consistently recorded lower mean bacterial populations than power-sprayer treatments (T_9 - T_{12}). The mean total bacterial counts were 122.69×10^5 CFU g^{-1} under drone spraying compared with 257×10^5 CFU g^{-1} under power spraying; *Pseudomonas* counts showed similar trend (114.13; 170×10^5 CFU g^{-1}). These differences may reflect higher formulation concentrations in drone applications and variations in pesticide mode of action. Similar pesticide-induced alterations of phylloplane microbiota have been reported in other crops, including significant shifts in bacterial and fungal communities following iprodione application

(Katsoula et al., 2020). Although pesticides can reduce microbial abundance, such changes do not inherently indicate negative impacts on plant health. Rather, they highlight the sensitivity of phylloplane communities to application method and chemistry, underscoring the need for further work linking microbial shifts with functional outcomes.

Actinomycetes play a key role in biological control and nutrient cycling, while phylloplane fungi include both beneficial and pathogenic groups. Actinomycete and fungal populations varied among treatments (Table 2). The highest mean actinomycete counts were recorded in T_2 (138×10^4 CFU g^{-1}), followed by T_9 and T_{11} , with populations remaining relatively stable across sampling days. The fungal counts were maximum in T_{13} (21×10^4 CFU g^{-1}), whereas T_{11} and T_9 treatments showed comparatively lower fungal populations. Comparison of application methods revealed minimal differences for actinomycetes, drone treatments (T_5 - T_8) averaged (119.5×10^4 CFU g^{-1}), similar to power-sprayer treatments (118.1×10^4 CFU g^{-1}). Fungal populations were slightly lower under power spraying (10.75×10^4 CFU g^{-1}) than drone spraying (13.5×10^4 CFU g^{-1}), though overall variation remained modest. These results align with previous studies that have reported limited effects of pesticides on actinobacterial and fungal phyllosphere communities, despite treatment-level differences (Dai et al., 2024; Chaudhary et al., 2025).

Different pesticide treatments have influenced the abundance of Gram-positive and Gram-negative bacteria (Table 3). One day before spraying, the initial bacterial community composition of rice phylloplane was relatively consistent. At 7 DAS, the Gram-positive bacterial group showed a slight increase in abundance, ranging between 5 to 7 colonies across different

Table 1: Effect of pesticides applied using a drone and power sprayer on phylloplane bacterial population

Treatments	Total bacteria (CFU $\times 10^5$) g^{-1}				<i>Pseudomonas</i> (CFU $\times 10^5$) g^{-1}			
	Sampling days							
	1 DBS	7 DAS	14 DAS	Mean	1 DBS	7 DAS	14 DAS	Mean
T_1	304	132	66	99.0	109	101	78	89.5
T_2	316	142	226	184.0	136	202	61	131.5
T_3	266	114	417	265.5	114	86	66	76.0
T_4	168	112	91	101.5	86	171	90	130.5
T_5	179	61	127	94.0	152	182	90	136.0
T_6	96	74	101	87.5	192	72	33	52.5
T_7	123	93	175	134.0	235	110	168	139.0
T_8	95	147	203	175.0	168	155	103	129.0
T_9	431	102	71	86.5	347	146	129	137.5
T_{10}	88	221	437	329.0	545	188	155	171.5
T_{11}	386	240	403	321.5	277	104	301	202.5
T_{12}	509	248	334	291.0	274	186	151	168.5
T_{13}	129	184	270	227.0	258	267	289	278.0
Average	237.69	143.85	224.69	-	222.54	151.54	131.85	-

DBS = Day Before Spraying, DAS = Days After Spraying

Table 2: Effect of pesticides applied using a drone and power sprayer on phylloplane actinomycetes and fungi

Treatments	Actinomycetes (CFU x 10 ⁴) g ⁻¹				Fungi (CFU x 10 ⁴) g ⁻¹			
	Sampling days							
	1 DBS	7 DAS	14 DAS	Mean	1 DBS	7 DAS	14 DAS	Mean
T ₁	134	113	144	128.5	17	14	18	16
T ₂	126	122	154	138.0	12	10	16	13
T ₃	112	94	132	113.0	14	12	18	15
T ₄	116	108	124	116.0	18	16	20	18
T ₅	115	120	134	127.0	20	16	18	17
T ₆	110	92	117	104.5	12	10	16	13
T ₇	87	68	123	95.5	9	12	16	14
T ₈	132	106	148	127.0	22	14	18	16
T ₉	117	124	146	135.0	12	10	14	12
T ₁₀	62	54	87	70.5	15	8	14	11
T ₁₁	116	62	194	128.0	10	6	8	7
T ₁₂	86	92	148	120.0	10	11	13	12
T ₁₃	96	128	189	158.5	12	18	24	21
Average	108.38	98.69	141.54	-	14.08	12.08	16.38	-

DBS = Day Before Spraying, DAS = Days After Spraying

Table 3: Effect of pesticides applied using a drone and power sprayer on Gram-positive and Gram-negative bacterial populations

Treatments	1 DBS		7 DAS		14 DAS	
	G +ve	G -ve	G +ve	G -ve	G +ve	G -ve
T ₁	4	6	6	4	8	2
T ₂	5	5	7	3	6	4
T ₃	6	4	5	5	7	3
T ₄	4	6	7	3	6	4
T ₅	7	3	6	4	7	3
T ₆	5	5	6	4	9	1
T ₇	6	4	7	3	7	3
T ₈	4	6	6	4	10	0
T ₉	5	5	5	5	7	3
T ₁₀	4	6	6	4	8	2
T ₁₁	6	4	5	5	7	3
T ₁₂	5	5	6	4	6	4
T ₁₃	4	6	5	5	6	4
Average	5.00	5.00	5.92	4.08	7.23	2.77

DBS = Day Before Spraying, DAS = Days After Spraying

treatments, with an average of 5.92 colonies. This increase may be due to various factors, such as nutrient availability and the release of organic compounds from pesticide degradation (Rui *et al.*, 2025). The higher average abundance suggests that, the Gram-positive bacterial group has a higher growth rate or tolerance to the applied pesticides compared to the Gram-negative group. The observed variations may be influenced by specific pesticide formulations and their mode of action, as well as interactions with other microorganisms or with the host plant. By 14 DAS, the Gram-positive bacteria showed a further rise (6–10

colonies; mean: 7.23), supporting evidence of their resilience and competitive advantage under pesticide stress (Biswas *et al.*, 2021).

The reduced abundance of Gram-negative bacteria likely reflects their greater sensitivity to pesticide exposure or reduced competitiveness in the post-treatment phylloplane. Overall, pesticide applications significantly influenced the relative proportions of Gram-positive and Gram-negative groups. Knief *et al.* (2012) reported a strong dominance of methylotrophic bacteria, particularly *Methylobacterium*, in the rice phyllosphere,

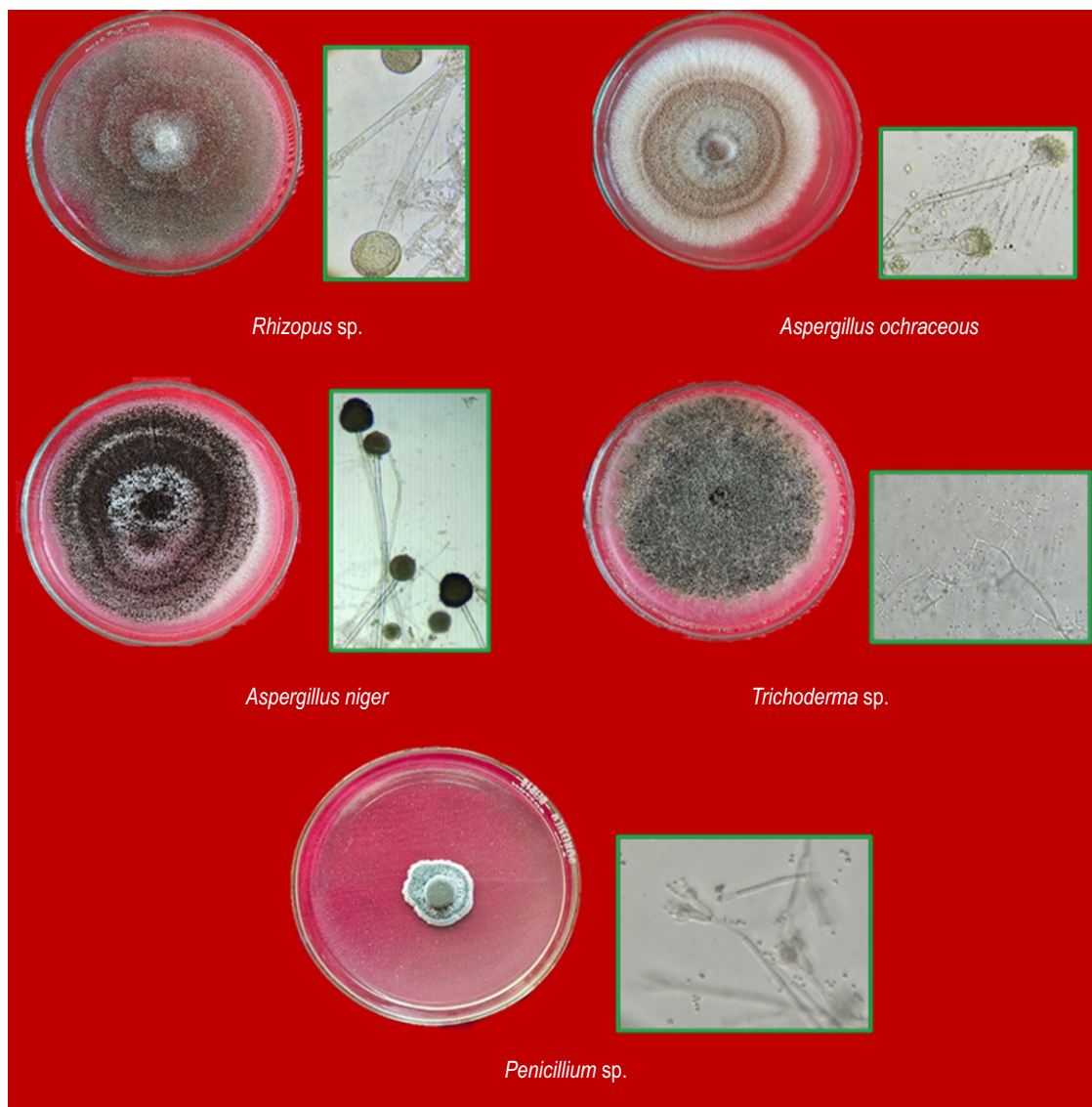


Fig. 2: Pure cultures of major fungi isolated from phylloplane samples and photographs of fungal morphology observed at 400X magnification.

supporting our observation that Gram-positive bacteria were generally more abundant. Nonetheless, the microbial responses to pesticides can vary with crop type and environmental conditions, underscoring the context-dependent nature of phylloplane community shifts. The predominant fungal genera detected in the rice phylloplane included *Rhizopus* spp., *Aspergillus ochraceous*, *Aspergillus niger*, *Trichoderma* spp., and *Penicillium* spp. (Fig. 2; Table 4). *Rhizopus* spp. were consistently present across all treatments, with a mean occurrence of 16.12%. *Aspergillus ochraceous* and *A. niger* also appeared frequently, showing mean occurrence of 17.95 and 15.75%, respectively.

Trichoderma spp. exhibited moderate overall abundance (16.39%), with noticeably higher occurrence under T_6 on specific

sampling days. *Penicillium* spp. were present in all treatments but at lower mean abundance (10.71%), with temporal fluctuations across sampling days. Pesticide applications clearly influenced the composition and abundance of phylloplane fungi, as reflected by treatment- and time-dependent variations in these genera. Similar pesticide-driven shifts in fungal communities have been reported in other crops, such as tebuconazole-induced reductions in key fungal OTUs in cucumber (Wu *et al.*, 2023), rice (Danso Ofori *et al.*, 2024) and in citrus (Ozdemir and Erkilic, 2018). The elevated *Trichoderma* presence under T_6 may indicate either a direct growth advantage or reduced competition following this treatment. Overall, these results highlight the sensitivity of phylloplane fungal communities to pesticide regimes and their potential ecological consequences.

Table 4: Effect of pesticides alone or in combinations applied using a drone and power sprayer on the distribution of predominant genera of fungi in the phylloplane of rice ecosystem

Treatments	Sampling days														
	<i>Rhizopus</i> spp.			<i>Aspergillus ochraceous</i>			<i>Aspergillus niger</i>			<i>Trichoderma</i> spp.			<i>Penicillium</i> spp.		
	1DBS	7 DAS	14 DAS	1DBS	7 DAS	14 DAS	1DBS	7 DAS	14 DAS	1DBS	7 DAS	14 DAS	1DBS	7 DAS	14 DAS
T ₁	22.22*	18.18	0.00	22.22	27.27	0.00	22.22	27.27	0.00	22.22	18.18	33.33	11.11	9.09	66.67
T ₂	0.00	33.33	0.00	50.00	33.33	0.00	25.00	33.33	0.00	0.00	0.00	0.00	25.00	0.00	0.00
T ₃	50.00	0.00	28.57	50.00	25.00	28.57	0.00	75.00	28.57	0.00	0.00	0.00	0.00	0.00	14.29
T ₄	20.00	25.00	0.00	40.00	25.00	0.00	0.00	25.00	0.00	40.00	25.00	100.00	0.00	0.00	0.00
T ₅	28.57	25.00	25.00	28.57	25.00	25.00	0.00	25.00	25.00	14.29	12.50	25.00	28.57	12.50	0.00
T ₆	0.00	0.00	28.57	0.00	0.00	28.57	0.00	0.00	28.57	0.00	0.00	14.29	0.00	100.00	0.00
T ₇	50.00	33.33	25.00	33.33	33.33	25.00	0.00	33.33	25.00	0.00	0.00	0.00	16.67	0.00	25.00
T ₈	0.00	16.67	0.00	25.00	16.67	0.00	25.00	16.67	0.00	0.00	33.33	0.00	50.00	16.67	0.00
T ₉	33.33	0.00	33.33	33.33	50.00	33.33	33.33	50.00	33.33	0.00	0.00	0.00	0.00	0.00	0.00
T ₁₀	0.00	25.00	0.00	0.00	25.00	50.00	0.00	25.00	50.00	0.00	0.00	0.00	0.00	25.00	0.00
T ₁₁	28.57	0.00	33.33	28.57	60.00	0.00	28.57	0.00	0.00	0.00	20.00	33.33	14.29	20.00	33.33
T ₁₂	25.00	0.00	0.00	25.00	50.00	0.00	25.00	50.00	0.00	0.00	0.00	0.00	25.00	0.00	0.00
T ₁₃	23.53	20.00	35.71	23.53	20.00	42.86	29.41	30.00	14.29	11.76	20.00	7.14	11.76	10.00	0.00
Mean	21.63	15.12	16.12	27.66	30.05	17.95	14.50	30.05	15.75	6.79	9.92	16.39	14.03	14.87	10.71

*% Occurrence of fungi, DBS = Day Before Spraying, DAS = Days After Spraying.

Table 5: Effect of different treatments on the incidence of Coccinellids

Treatments	Coccinellids per 10 hills									
	1 st Spray					2 nd Spray				
	1DBS	7 DAS	14 DAS	Mean	1DBS	7 DAS	14 DAS	BH	Mean	
T ₁	7.00	2.33	5.00	3.67	8.33	6.67	12.67	14.67	11.33	
T ₂	5.00	3.67	5.00	4.33	8.33	7.33	11.00	14.00	10.78	
T ₃	4.00	2.33	5.00	3.67	8.67	4.67	9.33	13.67	9.22	
T ₄	4.33	2.00	4.00	3.00	6.00	8.00	11.33	13.00	10.78	
T ₅	6.33	6.00	7.00	6.50	9.67	8.33	8.33	12.67	9.78	
T ₆	5.67	3.33	6.67	5.00	8.67	7.00	10.67	10.00	9.22	
T ₇	4.00	3.00	4.33	3.67	6.33	5.33	6.00	9.00	6.78	
T ₈	4.00	5.00	6.00	5.50	6.67	5.67	7.33	10.33	7.78	
T ₉	4.67	7.67	9.33	8.50	11.00	7.67	6.33	8.33	7.44	
T ₁₀	5.33	4.67	7.00	5.83	9.33	6.67	10.33	13.00	10.00	
T ₁₁	9.33	7.67	7.00	7.33	9.00	5.33	9.00	10.00	8.11	
T ₁₂	7.33	5.67	7.33	6.50	9.00	8.67	10.67	13.00	10.78	
T ₁₃	7.00	8.33	9.33	8.83	9.33	14.67	13.00	17.00	14.89	
SE(m)±	-	-	-	-	-	-	-	-	-	
CD (at 5%)	NS	NS	NS	-	NS	NS	NS	NS	-	
CV (%)	-	-	-	-	-	-	-	-	-	

DBS = Days Before Spraying, DAS = Days After Spraying, B.H= Before Harvest, mean = Mean of two observations after spraying

The incidence of coccinellids was monitored at three time points: 1 day before spraying, 7 days after spraying, and 14 days after spraying for the 1st and 2nd spray. The number of coccinellid per 10 hills was recorded for each treatment and time point. The findings, demonstrate the coccinellid populations observed across different treatments and time intervals (Table 5). The mean

number of coccinellid per 10 hills ranged from 3.67 to 8.83, and no significant differences in coccinellid populations were observed after the first or second spray applications. These findings are consistent with those of previous studies, demonstrating that certain pesticide mixtures have a minimal impact on coccinellid population (Biondi *et al.*, 2012).

Table 6: Effect of different treatments on the incidence of Spiders

Treatments	Spiders per 10 hills								
	1 st Spray				2 nd Spray				
	1 DBS	7 DAS	14 DAS	Mean	1 DBS	7 DAS	14 DAS	BH	Mean
T ₁	12.33	3.67 ^e	5.00	4.33	13.67	10.67 ^b	12.33 ^b	15.67	12.89
T ₂	12.33	7.00 ^{bc}	8.33	7.67	12.67	7.33 ^b	11.00 ^p	14.67	11.00
T ₃	8.67	6.33 ^{cd}	9.00	7.67	10.00	5.67 ^b	11.33 ^b	16.00	11.00
T ₄	8.67	6.33 ^{cd}	6.33	6.33	12.33	5.00 ^b	9.00 ^b	12.00	8.67
T ₅	10.00	8.00 ^{abc}	11.00	9.50	11.67	9.00 ^b	10.33 ^b	13.00	10.78
T ₆	10.33	8.33 ^{bc}	10.67	9.50	8.33	8.00 ^b	12.33 ^b	15.67	12.00
T ₇	11.00	5.00 ^{cd}	12.33	8.67	12.00	6.33 ^b	9.00 ^b	10.67	8.67
T ₈	9.33	6.33 ^{cd}	11.00	8.67	12.67	7.33 ^b	8.33 ^b	12.00	9.22
T ₉	17.00	13.33 ^{bc}	15.00	14.17	13.33	6.67 ^b	7.67 ^b	10.00	8.11
T ₁₀	14.33	14.67 ^{bc}	17.00	15.83	15.00	9.33 ^b	10.67 ^b	16.00	12.00
T ₁₁	10.67	12.00 ^{bc}	15.00	13.50	15.67	8.33 ^b	10.00 ^b	13.00	10.44
T ₁₂	11.67	11.67 ^{bc}	15.33	13.50	15.67	10.00 ^b	11.67 ^p	15.00	12.22
T ₁₃	12.00	13.33 ^a	15.67	14.50	18.00	19.00 ^a	23.00 ^a	27.33	23.11
SE(m)±	-	1.46	-	-	-	1.73	1.65	-	-
CD (at 5%)	NS	4.32	NS	-	NS	5.10	4.86	NS	-
CV (%)	-	10.82	-	-	-	9.20	12.05	-	-

DBS = Days Before Spraying, DAS = Days After Spraying, B.H= Before Harvest, mean = Mean of two observations after spraying. Alphabets represent the DMRT analysis

Spiders are recognized as important predators of agroecosystems, and contribute to the biological control of pests. Understanding the potential effects of pesticide treatment on spider population is crucial for maintaining ecological balance in agricultural systems. The perusal of data in Table 6 represent the spider population observed for each treatment at different time intervals. The mean number of spider per 10 hills was 4.33 to 15.83 at all treatments and time points. Significant variations in spider population were observed among the treatments both after first and second spray. Treatments T₁₁, chlorantraniliprole and T₂, tetraniliprole exhibited relatively higher spider populations, with mean values ranging from 11.00 to 15.83 spiders per 10 hills. Conversely, T₃ (picoxystrobin + tricyclazole) and T₄ (tebuconazole + trifloxystrobin) treatments resulted in lower spider populations, with mean values ranging from 7.33 to 8.67 spider per 10 hills. The results of this study indicate that the treatments had varying effects on spider population on rice plants. These findings corroborate with previous studies reporting the potential negative impact of certain pesticides on spiders (Biondi *et al.*, 2012).

The observed reduction in spider populations can be attributed to direct toxic effects or alterations in prey availability, vegetation structure, and microhabitats (Main *et al.*, 2018). Treatments exhibiting lower coccinellid and spider populations should be used with caution in integrated pest management programs because, spiders play a significant role in biological control. Alternative strategies that minimise the negative impact on spider populations while effectively controlling pests should be explored.

In conclusion, this study is among the first to assess the effects of drone-based pesticide applications on the culturable phylloplane microflora and beneficial insect populations in rice. While providing preliminary insights, it is important to note that only the culturable microbiota, representing about 0.5% of the total community, was studied, and microbial dynamics are also influenced by environmental factors such as rainfall, leaf wetness, and temperature. Further research, particularly using metagenomic approaches, is needed to fully understand the impact of drone spraying on the complete phylloplane microbiota and to standardize evaluation methodologies, thereby guiding sustainable pest management strategies.

Acknowledgments

Authors duly thank the authorities of Professor Jayashankar Telangana Agricultural University (PJTU) and Institute of Rice Research (IRR), ARI, Rajendranagar, Hyderabad for providing necessary infrastructural facilities and financial support to conduct this research work. We also extend our heartfelt thanks to Marut Dronetech Pvt. Ltd., Hyderabad, and their technical team for their valuable assistance in arranging and operating the drone used in this study.

Authors' contribution: S. Ragiman, K.B. Talluri, N.R.G. Varma and B.V. Sagar: Conceptualized and designed the experiment; S. Ragiman: Performed the experiment; Analysed the data and prepared manuscript. All authors revised the manuscript and gave suggestions to improve the manuscript.

Funding: The funding for this study was generously provided by Professor Jayashankar Telangana Agricultural University (PJTU), Hyderabad, and the Institute of Rice Research (IRR), Agricultural Research Station (ARI), Rajendranagar, Hyderabad.

Research content: The research content of manuscript is original and has not been published elsewhere.

Ethical approval: Not applicable.

Conflict of interest: All authors declare that there is no conflict of interest.

Data availability: All the data generated or analysed during this study are included in this manuscript.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology*.

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