Unveiling the Ecological Ramifications of Aerial Pesticide Application by Drones on Soil Microbiota in Rice

Abstract

The ecological consequences of aerial pesticide application by drones on soil microbiota in rice fields were investigated in this study. The quantitative and qualitative effects of different pesticide treatments, both applied *via* drones and taiwan sprayer, were examined on soil bacteria, actinomycetes, and fungi. The average population of total bacteria and pseudomonas in the rhizosphere soil tended to be slightly higher in the drone-sprayed treatments compared to the taiwan sprayer treatments. It is evident that the drone spraying treatments resulted in higher average populations of actinomycetes and fungi (124.75 CFU × 10^5 g^{-1} soil and 21.12 CFU × 10^4 g^{-1} soil, respectively) compared to the taiwan sprayer treatments with average populations of 127.75 CFU × 10^5 g^{-1} soil for actinomycetes and 22.5 CFU ×10^4 g^{-1} soil for fungi. Qualitative assessment of microbial groups revealed that, the abundance of G -ve bacterial groups are higher when compared to G +ve bacterial groups in rhizospheric soil before harvest of the crop. The distribution of fungal genera varied due to pesticide applications. The mean per cent occurrence of *Curvularia* spp., *Penicillium* spp., and *Trichoderma* spp. was slightly higher in the drone-sprayed treatments (9.85%, 8.51%, and 8.33%) compared to the taiwan-sprayed treatments (2.48%, 2.24%, and 2.00%). However, the mean per cent occurrence of *Aspergillus* species (*A. ochraceous*, *A. niger,* and *A. flavus*) was relatively higher in the taiwan sprayer treatments (9.14%, 12.81%, and 4.09%) when compared to the drone-sprayed treatments (3.75%, 2.31%, and 0.83%). Overall, this study underscores the need for further research to comprehensively understand the implications of different pesticide application methods on soil microbial communities and their potential impact on soil fertility and ecosystem functioning over time.

Keywords: Aerial pesticide application, Soil microbial communities, Ecological consequences, Soil health.

1. INTRODUCTION

As the agricultural sector embraces new technologies like drone spraying for pesticide application, concerns regarding its potential impact on the environment, soil, water bodies, and natural ecosystems have arisen. In this context, the Govt. of India has given general and crop specific standard operating protocols for safe application of pesticides using drones during April, 2023. One of the key assumptions is that, drone spraying contains the higher pesticide concentration in each droplet compared to manual spraying methods as the droplets are fine to

very fine in drone spraying, as opposed to medium to coarse droplets in taiwan sprayers, raises questions about the reach and effect of pesticides on soil microbiota. Recognizing the significance of these assumptions, the present study focused on investigating the impact of pesticide application through both drone and taiwan sprayers on soil microbiota. By examining the potential effects on these essential ecological components, the research aimed to provide valuable insights into the environmental implications of adopting drone spraying technology in agriculture.

As this technology is still in its infancy stage, it is essential to address these concerns and generate scientifically sound data to facilitate informed decision-making. The study aimed to nullify any uncertainties and shed light on the actual effects of drone spraying on phylloplane and soil microbiota. Understanding the implications of this emerging technology is of paramount importance, especially in the context of India's agricultural production facing labour scarcity and dwindling natural resources. Any challenges or issues arising from the adoption of drone spraying could significantly impact agricultural productivity and sustainability, making rigorous research is imperative for shaping the future of this technology in the farmer fields. Pesticides can potentially be altering the soil bacterial populations, serving as indicators of their toxicity and environmental impacts. The previous studies on pesticides such as tebuconazole and carbendazim have indicated that higher concentrations of these substances can adversely affect soil microbial activity (Onwana *et al*., 2020). Therefore, understanding the effects of pesticide application using drones on soil microbiota is crucial for maintaining the generative capacities of agroecosystems and ensuring sustainable agricultural practices. The present investigation was to study the impact of pesticides application using drone and manual spraying (taiwan spryer) on soil microbiota. To achieve this, microbial analysis was conducted on samples collected from the field experiment before and after spraying of treatments, with a specific emphasis on the effects of drone spraying on phylloplane and soil microbiota. This analysis allowed for a comprehensive evaluation of the microbial composition and diversity present on phylloplane as well as in the soil.

In the present study, the experiment was conducted as pre-liminary study on effect of pesticides application using drone *via a vis* taiwan sprayer on soil microbiota. In order to draw the valid and standard conclusion on impact of drone sparing of pesticides on soil microbiota requires a series of sample collection over a period of time. The very limited literature is available on impact of drone spraying on phylloplane and soil microbiota. After thorough scrutiny of literature pertaining to impact of pesticide spraying on soil microbiota, it is deduced that, first of its kind of attempt has been made to study the impact of pesticide applied alone and in combination using drone on microbial population in soil.

2. MATERIAL AND METHODS:

The present investigation to study the impact of drone spraying of pesticides on soil microbiota was carried out at Institute of Rice Research, Agricultural Research Institute, Rajendranagar, Hyderabad, which is situated at an altitude of 542.6 m above the MSL on 18'50^0 North latitude and 77'.53^0 East longitude during *kharif (Vanakalam),* 2022. The samples of the soil were collected from all the treatment plots before and after spraying and were then subjected to laboratory-based microbial analysis to evaluate the composition and abundance of microorganisms.

Variety: Samba Mahsuri (BPT 5204), Net area of each plot per treatment / replication: 360 m^2, Total plot area per treatment: 1650 m^2.

2.1 Spraying Equipment

As shown in Fig 1, the model of UAV (drone) used in this aerial spraying was AGRICOPTER AG 365 and it was powered by two 22,000 mAh Li-Po batteries and has 15 min. endurance with full tank. The optimum flight speed was 3.6 m s^{-1}, flight height was 2.5 m and effective spraying swath width was 3.5 m. The nozzle tip used for drone sprayer was XR 11002 VP (M/s. Teejet Technologies India Pvt. Ltd., Bengaluru) an extended range flat fan type with spray angle of 110^0 and operatable at a spray pressure of 20-30 PSI (Fig 2).

2.2 Treatment Details

The current study comprised of 13 treatments (Table 1) wherein two insecticides *viz.*, chlorantraniliprole 18.5% SC and tetraniliprole 200% SC and two fungicides *viz.*, picoxystrobin 7.5% SC + tricyclazole 22.5% SC and tebuconazole 50% WG + trifloxystrobin 25% WG were applied alone with drone and applied in combination (both with drone and taiwan sprayer) and their bio-efficacy was compared with untreated control. 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 it was sprayed uniformly for three replications. The recommended pesticide dosage (g/ml *a.i.* ha^{-1}) for both drone spraying and taiwan sprayer was same. However, there was a difference in the spray fluid volume applied in the two methods. For drone spraying, a spray volume of 40 L/ha and for taiwan sprayer, a spray volume of 375 L/ha was utilized. 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 the second application, ensuring consistency in treatment application within the field.

Table 1 Treatment details of field experiment conducted during *kharif*, 2022 at IRR, ARI, Rajendranagar

Trt. No.	Treatment particulars	Spraying equipment
T1	Chlorantraniliprole 18.5% SC @ 3.75 ml/l	Drone

T2	Tetraniliprole 200 SC @ 6.25 ml/l	Drone
T3	Picoxystrobin 7.5% + Tricyclazole 22.5% SC @ 25 ml/l	Drone
T4	Tebuconazole 50% + Trifloxystrobin 25% WG @ 5 g/l	Drone
T5	Chlorantraniliprole 18.5% SC @ 3.75 ml/l + (Picoxystrobin 7.5% + Tricyclazole 22.5% SC) @ 25 ml/l	Drone
T6	Chlorantraniliprole 18.5% SC @ 3.75 ml l^{-1}+ (Tebuconazole 50% + Trifloxystrobin 25% WG) @ 5 g/l	Drone
T7	Tetraniliprole 200 SC @ 6.25 ml/l+ (Picoxystrobin 7.5% + Tricyclazole 22.5% SC) @ 25 ml/l	Drone
T8	Tetraniliprole 200 SC @ 6.25 ml/l+ (Tebuconazole 50% + Trifloxystrobin 25% WG) @ 5 g/l	Drone
T9	Chlorantraniliprole 18.5% SC @ 0.4 ml/l + (Picoxystrobin 7.5% + Tricyclazole 22.5% SC) @ 2.66 ml/l	Taiwan sprayer
T10	Chlorantraniliprole 18.5% SC @ 0.4 ml/l + (Tebuconazole 50% + Trifloxystrobin 25% WG) @ 0.53 g/l	Taiwan sprayer
T11	Tetraniliprole 200 SC @ 0.6 ml/l + (Picoxystrobin 7.5% + Tricyclazole 22.5% SC) @ 2.66 ml/l	Taiwan sprayer
T12	Tetraniliprole 200 SC @ 0.6 ml/l + (Tebuconazole 50% + Trifloxystrobin 25% WG) @ 0.53 g/l	Taiwan sprayer
T13	Untreated Control	_

To prevent the potential issue of drift and contamination between the treatments, a buffer zone of 5 m was maintained between adjacent treatments / replication. Each replication consisted of a minimum plot size of 360 m² in order to ensure adequate coverage during the drone-based pesticide application in rice. The crop specific standard operating procedures (SOPs) for the application of pesticides with drone was released by the Ministry of Agriculture and Farmers Welfare, Govt. of India and standard operating protocols for drone-based pesticide application in rice developed by (Varma *et al*., 2022) were followed in the present investigation. While operating the drones in the field for conducting the experiment, the weather conditions such as wind speed was measured using an Anemometer (Lutron, AM 4201), while a hand-held hygrometer (HTC, 288 CTH) was used to record temperature and relative humidity.

Table 2 Pesticide usage and application dates

Application date	Pesticide	Applied rate (*a.i.*/ha)	Label claim	Spray equipment
14.09.2022 (67 DAT)	Chlorantraniliprole 18.5% SC	60 ml	Stem borer & Leaf folder	Drone + Taiwan sprayer
	Tetraniliprole 200 SC	100 ml	Stem borer & Leaf folder	
	Picoxystrobin 7.5% + Tricyclazole 22.5% SC	400 ml	Blast & Sheath blight	
	Tebuconazole 50% +Trifloxystrobin 25% WG	80 g	Blast, Sheath blight & GD	
17.10.2022 (105 DAT)	Chlorantraniliprole 18.5% SC	60 ml	Stem borer & Leaf folder	
	Tetraniliprole 200 SC	100 ml	Stem borer & Leaf folder	
	Picoxystrobin 7.5% + Tricyclazole 22.5% SC	400 ml	Blast & Sheath blight	
	Tebuconazole 50% +Trifloxystrobin 25% WG	80 g	Blast, Sheath blight & GD	

2.3 Sample collection

For soil microbiota analysis, three rhizospheric soil samples per each treatment were randomly collected *i.e.,* before spraying, one month after spraying and before harvest.

2.4 Isolation of soil microbiota

Soil samples were collected before spraying, one month after spraying and before harvest at 3 different places from each treatment and they were mixed thoroughly to make a composite sample. From the composite sample 10 g of finely pulverized, air-dried soil was made for serial dilution and plate count method by Aneja (2003) for the isolation of fungi, bacteria, and actinomycetes from rhizosphere soil. Dilutions of 10^{-3} and 10^{-4} were used for isolation of fungi and actinomycetes, while dilutions of 10^{-5} and 10^{-6} were used for isolation of bacterial colonies. Then pour plate method was followed for microbial isolation where, 1 ml aliquots were transferred into 3 petri plates for each dilution for maintaining 3 replications and added 15 ml cooled nutrient agar, pseudomonas agar, ken knight's medium and potato dextrose agar medium for isolation of bacteria, pseudomonas, actinomycetes and fungi respectively. Upon solidification of the media, plates were incubated in an inverted position at 25 ± 2°C for 3-4 days for fungi, 28 ± 2°C for 24-48 hours for bacteria / Pseudomonas, 28 ± 2°C for 10-15 days for isolation actinomycetes. After completion of incubation, the number of similar colonies was counted and sub-cultured to obtain pure cultures. The pure cultures of fungal colonies were obtained by single spore and single hyphal tip method whereas, only population counts were taken into consideration for enumeration of bacteria, pseudomonas and actinomycetes population.

2.5 Quantitative assessment of microbial cultures

2.5.1 Bacteria

The number of bacterial colonies developed in the plates after the incubation period of 24-48 hours were counted on digital colony counter (M/s. Labtronics, Haryana) and number of colonies per gram of sample were computed (Atlas *et al*., 1978) by using following formula:

$$\text{Bacteria / g of sample} = \frac{\text{Number of colonies/ plate} \times \text{dilution factor}}{\text{Dry weight of sample taken}}$$

2.5.2 Fungi

The number of fungal colonies developed in the plates after the incubation period of 4 days were referred as colony forming units (CFU) and number of CFU per gram of sample were computed (Das *et al*., 2003) by using following formula:

$$\text{Fungi / g of sample} = \frac{\text{Number of colonies/ plate} \times \text{dilution factor}}{\text{Dry weight of sample taken}}$$

2.6 Qualitative assessment of microbial cultures

2.6.1 Bacteria

For qualitative analysis of bacteria, the gram staining was performed on nutrient agar culture plates for 10 randomly selected colonies for each treatment (Lacy and Lukezic, 2004).

In the study focused on bacterial qualitative analysis, the isolation and enumeration of Pseudomonas and Actinomycetes were conducted using specific growth media, namely Pseudomonas agar and Ken Knight's medium in addition to the gram staining.

2.6.2 Fungi

Qualitative analysis in terms of per cent occurrence of a fungus was calculated (Dong *et al.*, 2014) using the following formula:

$$\text{Per cent occurrence of a fungus} = \frac{\text{The number of colonies of a particular fungus in 3 replicate plates}}{\text{Total number of all fungi in 3 replicate plates}} \times 100$$

2.7 Statistical analysis

The experimental data on various characters recorded throughout the course of investigation were statistically analyzed in RCBD as per Gomez and Gomez (1984). Significant differences between treatments were calculated using analysis of variance (ANOVA) and Duncan's test (DMRT) at a significance level of 95% with OPSTAT software package. Wherever statistical significance was observed, critical difference (CD) at 0.05 level of probability was worked out for comparison. Non- significant comparison was indicated as NS.

3. RESULTS AND DISCUSSION:

3.1 Quantitative assessment of soil microbiota

3.1.1 Bacteria

The present study aimed to investigate the impact of pesticide application alone and their combination on proliferation of bacterial populations in the rhizosphere soil of rice. The treatment particulars and the number of total bacteria (CFU x 10^5) and pseudomonas (CFU x 10^5) per gram of soil at different sampling days were presented in Table 3. The results shown that, the application of different pesticides had varying effects on the bacterial population in the rhizosphere soil of rice (Fig 3). Among the treatments, T3 (picoxystrobin + tricyclazole) resulted in a significant decline in the number of total bacteria at one day before spraying (1 DBS) and one month after spraying (1 MAS). However, the total bacterial count was increased at before harvest. Similar patterns were observed for pseudomonas populations. This suggests that (picoxystrobin + tricyclazole) initially had a negative impact on bacterial populations, but the populations recovered due course of time. The treatment T4 (tebuconazole + trifloxystrobin) exhibited a different trend. It led to a higher total bacterial count compared to the control group throughout the sampling period. Similarly, pseudomonas populations were also higher in T4

treatment (tebuconazole + trifloxystrobin). Upon analysing the data, it was observed that, the bacterial population varied across treatments and sampling days. The treatments sprayed using drones (T5, T6, T7, and T8) showed comparable results to those sprayed with the taiwan sprayer (T9, T10, T11, and T12) in terms of their impact on bacterial populations. The mean number of total bacteria and pseudomonas in the rhizosphere soil tended to be slightly higher in the drone-sprayed treatments compared to the taiwan sprayer treatments. However, the differences between the two methods were not enough to establish a clear superiority of one over the other.

The results of this study align with previous research conducted by Rahman *et al.* (2021), who found that, the overuse of chemical fertilizers and pesticides negatively affects the populations of nitrifying bacteria, denitrifying bacteria, and anamox bacteria in paddy soils. They also reported that, heavy metals can influence nitrification rates. Similarly, Endo *et al.* (1982) reported that, the populations of microorganisms were decreased with the application of cartap hydrochloride at a high concentration. This finding is consistent with our study, where the application of certain insecticides led to a decline in bacterial populations. The results showed that different pesticides had varying effects on bacterial populations irrespective of the spraying equipment, with some pesticides leading to a decline, while others stimulated bacterial growth. These findings are consistent with previous studies, highlighting the complex interactions between pesticides and soil microorganisms.

3.1.2 Actinomycetes and Fungi

The present study aimed to investigate the effect of different pesticide application alone and its combination either using drone and taiwan sprayer on the proliferation of actinomycetes and fungi in the rhizosphere soil of rice presented in Table 4. The number of actinomycetes and fungi (Fig 3) was quantified at different sampling days, including 1 day before spraying (DBS), 1 month after spraying (MAS), and before harvest (BH). Among the treatments, T1 (139.0 CFU $\times 10^5$ g^{-1} soil) applied using drone showed a relatively higher average population of actinomycetes compared to the untreated control. Similarly, T2 (143.0 CFU $\times 10^5$ g^{-1} soil) using drone also resulted in a significant increase in actinomycetes population. The treatments T10 (27.0 CFU $\times 10^4$ g^{-1}) and T11 (24.5 CFU $\times 10^4$ g^{-1}) exhibited a higher number of fungal population. In contrast, other treatments showed the lower fungal populations compared to the untreated control. The results showed that both drone and taiwan sprayer application had varying impacts on bacterial populations. It is evident that the drone spraying treatments (T5, T6, T7, and T8) generally resulted in higher average populations of actinomycetes and fungi (124.75 CFU $\times$ 10^5 g^{-1} soil and 21.12 CFU $\times$ 10^4 g^{-1} soil, respectively) compared to the taiwan sprayer treatments (T9, T10, T11, and T12) with average populations of 127.75 CFU $\times$ 10^5 g^{-1} soil for actinomycetes and 22.5 CFU $\times 10^4$ g^{-1} soil for fungi. However, it is crucial to note that, the

differences in population numbers were not substantial. Both application methods seem to have relatively similar effects on the microbial populations in the rhizosphere soil of rice. The variations in bacterial populations observed could be attributed to factors other than the application method, such as the specific pesticide formulations used in each treatment and the environmental conditions during application and sampling. In conclusion, there are slight differences in the microbial populations between drone and taiwan sprayer treatments, it is difficult to draw definitive conclusions solely based on this study. Further studies and a comprehensive analysis are needed for better understanding of the specific factors contributing to the observed variations in actinomycetes and fungal populations in the rhizosphere soil of rice under different pesticide application methods. These findings can be valuable for optimizing pesticide application practices and understanding their impact on soil microbial communities.

The findings of our study align with Das *et al.* (2003), who observed that an increase in the population of bacteria, actinomycetes, and fungi in rhizosphere soil after the application of insecticides. The results of present study also support the findings of Roman *et al.* (2021) and Onwana *et al.* (2020) regarding the negative effects of triazole fungicides on soil microbiota. Roman *et al.* (2021) who reported that, decrease in soil microbial populations and enzyme activities due to application of triazole fungicides in rice.

3.2 Qualitative assessment of soil microbiota

3.2.1 Bacteria

The effect of pesticide application alone or in combination applied using drone and taiwan sprayer on the abundance and composition of G +ve and G -ve bacterial groups in the rhizosphere soil of rice (Table 5) revealed that, the abundance of G +ve and G -ve bacterial groups in soil varied after the pesticide application at 1 month after spraying. The abundance of G -ve bacterial groups are higher when compared to G +ve bacterial groups in rhizospheric soil before harvest of the crop. It is worth noting information on pesticide combinations, such as chlorantraniliprole with picoxystrobin + tricyclazole has exhibited mixed effects on both the G +ve and G -ve bacterial groups. The results obtained in the present study also suggests that, the interactions between different pesticides may have complex consequences on soil microbial communities. Further, the findings of Bacmaga *et al.* (2022) supports this notion, as they have demonstrated that, the tebuconazole application stimulated the organotrophic bacteria and fungi, indicating potential shifts in microbial community composition. Overall, the results highlight the importance of considering the effects of pesticide application alone either in combination applied using drone and taiwan sprayer on soil microbial communities, particularly G +ve and G -ve bacterial groups. The observed changes in bacterial abundance and composition may have implications for soil fertility, nutrient cycling, and overall ecosystem functioning. Future

research should be further investigating the long-term effects of pesticide exposure on soil microbial communities by collecting the sample over a period of time.

3.2.2 Fungi

The distribution of fungal genera (Fig 4a, 4b), including *Curvularia* spp., *Penicillium* spp., *Trichoderma* spp., *Aspergillus ochraceous, Aspergillus niger,* and *Aspergillus flavus*, was assessed at different sampling days, including 1 day before spraying (1DBS), 1 month after spraying (1MAS), and at before harvest (BH). The results of present study (Table 6) indicated that, the mean per cent occurrence of fungal genera along different sampling days are as follows: *Curvularia* spp. (8.33%), *Penicillium* spp. (5.38%), *Trichoderma* spp. (3.75%), *Aspergillus ochraceous* (54.72%), *Aspergillus niger* (9.14%), and *Aspergillus flavus* (4.09%). The above findings are suggesting that, pesticide application has influenced the relative abundance of fungal genera in the rhizosphere soil. The mean per cent occurrence of *Curvularia* spp., *Penicillium* spp., and *Trichoderma* spp. was slightly higher in the drone-sprayed treatments (9.85%, 8.51%, and 8.33%, respectively) compared to the taiwan-sprayed treatments (2.48%, 2.24%, and 2.00%, respectively). However, the mean per cent occurrence of *Aspergillus* species (*A. ochraceous, A. niger,* and *A. flavus*) was relatively higher in the taiwan sprayer treatments (9.14%, 12.81%, and 4.09%, respectively) when compared to the drone-sprayed treatments (3.75%, 2.31%, and 0.83%, respectively). It is important to note that, this is a simplified analysis based solely on the predominant genera of fungi in the rhizosphere soil. Nonetheless, these initial findings could guide further research on better understanding of the effects of drone and taiwan spraying treatments on both fungal and bacterial populations in rice cultivation.

Table 3 Effect of pesticides applied alone or in combination using drone and taiwan sprayer on the proliferation of bacterial population in the rhizosphere soil of rice ecosystem

Trt. No.	Treatment particulars	Number of total bacteria (CFU x 10^5) g^{-1} soil				Number of pseudomonas (CFU x 10^5) g^{-1} soil			
		Sampling days				Sampling days			
		1 DBS	1 MAS	BH	Mean	1 DBS	1 MAS	BH	Mean
T1	Chlorantraniliprole 18.5% SC @ 3.75 ml l^{-1}	131	184	267	225.5	69	185	331	258.0
T2	Tetraniliprole 200 SC @ 6.25 ml l^{-1}	154	218	225	221.5	82	164	308	236.0
T3	Picoxystrobin 7.5% + Tricyclazole 22.5% SC @ 25 ml l^{-1}	428	423	207	315.0	119	233	318	275.5
T4	Tebuconazole 50% + Trifloxystrobin 25% WG @ 5 g l^{-1}	443	476	392	434.0	145	216	335	275.5
T5	Chlorantraniliprole 18.5% SC @ 3.75 ml l^{-1} + Picoxystrobin 7.5% + Tricyclazole 22.5% SC @ 25 ml l^{-1}	134	191	268	229.5	268	233	254	243.5
T6	Chlorantraniliprole 18.5% SC @ 3.75 ml l^{-1} + Tebuconazole 50% + Trifloxystrobin 25% WG @ 5 g l^{-1}	186	69	248	158.5	122	289	319	304.0
T7	Tetraniliprole 200 SC @ 6.25 ml l^{-1} + Picoxystrobin 7.5% + Tricyclazole 22.5% SC@ 25 ml l^{-1}	202	293	321	307.0	201	216	281	248.5
T8	Tetraniliprole 200 SC @ 6.25 ml l^{-1} + Tebuconazole 50% + Trifloxystrobin 25% WG @ 5 g l^{-1}	66	354	427	390.5	193	231	296	263.5
T9	Chlorantraniliprole 18.5% SC @ 0.4 ml l^{-1} + Picoxystrobin 7.5% + Tricyclazole 22.5% SC @ 2.66 ml l^{-1}	200	475	371	423.0	144	223	246	234.5
T10	Chlorantraniliprole 18.5% SC @ 0.4 ml l^{-1} + Tebuconazole 50% + Trifloxystrobin 25% WG @ 0.53 g l^{-1}	350	441	364	402.5	261	282	252	267.0
T11	Tetraniliprole 200 SC @ 0.6 ml l^{-1} + Picoxystrobin 7.5% + Tricyclazole 22.5% SC @ 2.66 ml l^{-1}	652	230	428	329.0	168	226	273	249.5
T12	Tetraniliprole 200 SC @ 0.6 ml l^{-1} + Tebuconazole 50% + Trifloxystrobin 25% WG @ 0.53 g l^{-1}	356	494	452	473.0	166	243	288	265.5
T13	Untreated Control	252	230	248	239.0	223	251	314	282.5
	Average	**273.38**	**313.69**	**324.46**	**-**	**166.23**	**230.15**	**293.46**	**-**

***DBS** = Day Before Spraying, **MAS** = Month After Spraying, **BH** = Before Harvest.*

Table 4 Effect of pesticides applied alone or in combination using drone and taiwan sprayer on the proliferation of actinomycetes and fungi in the rhizosphere soil of rice ecosystem

Trt. No.	Treatment particulars	Number of actinomycetes (CFU x 10^5) g^{-1} soil				Number of fungi (CFU x 10^4) g^{-1} soil			
		Sampling days				Sampling days			
		1 DBS	1 MAS	BH	Mean	1 DBS	1 MAS	BH	Mean
T1	Chlorantraniliprole 18.5% SC @ 3.75 ml l^{-1}	123	134	144	139.0	10	17	23	20.0
T2	Tetraniliprole 200 SC @ 6.25 ml l^{-1}	136	132	154	143.0	14	16	24	20.0
T3	Picoxystrobin 7.5% + Tricyclazole 22.5% SC @ 25 ml l^{-1}	94	82	102	92.0	12	17	18	17.5
T4	Tebuconazole 50% + Trifloxystrobin 25% WG @ 5 g l^{-1}	115	120	132	126.0	8	12	14	13.0
T5	Chlorantraniliprole 18.5% SC @ 3.75 ml l^{-1} + Picoxystrobin 7.5% + Tricyclazole 22.5% SC @ 25 ml l^{-1}	108	116	132	124.0	17	18	21	19.5
T6	Chlorantraniliprole 18.5% SC @ 3.75 ml l^{-1} + Tebuconazole 50% + Trifloxystrobin 25% WG @ 5 g l^{-1}	92	110	117	113.5	7	13	27	20.0
T7	Tetraniliprole 200 SC @ 6.25 ml l^{-1} + Picoxystrobin 7.5% + Tricyclazole 22.5% SC@ 25 ml l^{-1}	126	118	137	127.5	15	18	22	20.0
T8	Tetraniliprole 200 SC @ 6.25 ml l^{-1} + Tebuconazole 50% + Trifloxystrobin 25% WG @ 5 g l^{-1}	60	70	110	90.0	11	15	24	19.5
T9	Chlorantraniliprole 18.5% SC @ 0.4 ml l^{-1} + Picoxystrobin 7.5% + Tricyclazole 22.5% SC @ 2.66 ml l^{-1}	117	124	156	140.0	9	14	15	14.5
T10	Chlorantraniliprole 18.5% SC @ 0.4 ml l^{-1} + Tebuconazole 50% + Trifloxystrobin 25% WG @ 0.53 g l^{-1}	126	132	184	158.0	14	20	34	27.0
T11	Tetraniliprole 200 SC @ 0.6 ml l^{-1} + Picoxystrobin 7.5% + Tricyclazole 22.5% SC @ 2.66 ml l^{-1}	122	134	156	145.0	12	20	29	24.5
T12	Tetraniliprole 200 SC @ 0.6 ml l^{-1} + Tebuconazole 50% + Trifloxystrobin 25% WG @ 0.53 g l^{-1}	87	68	112	90.0	18	18	19	18.5
T13	Untreated Control	132	144	168	156.0	21	22	24	23.0
Average		**110.62**	**114.15**	**138.77**	**-**	**12.92**	**16.92**	**22.62**	**-**

***DBS** = Day Before Spraying, **MAS** = Month After Spraying, **BH** = Before Harvest.*

Table 5 Effect of pesticides applied alone or in combination using drone and taiwan sprayer on G + ve and G -ve bacterial groups in rhizosphere soil of rice

Trt. No.	Treatment particulars	Effect of different treatments on gram +ve and gram -ve groups					
		1 DBS		1 MAS		BH	
		G +ve	G -ve	G +ve	G -ve	G +ve	G -ve
T1	Chlorantraniliprole 18.5% SC @ 3.75 ml l^{-1}	4	6	3	7	3	7
T2	Tetraniliprole 200 SC @ 6.25 ml l^{-1}	5	5	4	6	3	7
T3	Picoxystrobin 7.5% + Tricyclazole 22.5% SC @ 25 ml l^{-1}	4	6	4	6	4	6
T4	Tebuconazole 50% + Trifloxystrobin 25% WG @ 5 g l^{-1}	3	7	4	6	5	5
T5	Chlorantraniliprole 18.5% SC @ 3.75 ml l^{-1} + Picoxystrobin 7.5% + Tricyclazole 22.5% SC @ 25 ml l^{-1}	5	5	3	7	4	6
T6	Chlorantraniliprole 18.5% SC @ 3.75 ml l^{-1} + Tebuconazole 50% + Trifloxystrobin 25% WG @ 5 g l^{-1}	9	1	3	7	2	8
T7	Tetraniliprole 200 SC @ 6.25 ml l^{-1} + Picoxystrobin 7.5% + Tricyclazole 22.5% SC@ 25 ml l^{-1}	5	5	5	5	3	7
T8	Tetraniliprole 200 SC @ 6.25 ml l^{-1} + Tebuconazole 50% + Trifloxystrobin 25% WG @ 5 g l^{-1}	3	7	2	8	3	7
T9	Chlorantraniliprole 18.5% SC @ 0.4 ml l^{-1} + Picoxystrobin 7.5% + Tricyclazole 22.5% SC @ 2.66 ml l^{-1}	6	4	4	6	3	7
T10	Chlorantraniliprole 18.5% SC @ 0.4 ml l^{-1} + Tebuconazole 50% + Trifloxystrobin 25% WG @ 0.53 g l^{-1}	4	6	4	6	2	8
T11	Tetraniliprole 200 SC @ 0.6 ml l^{-1} + Picoxystrobin 7.5% + Tricyclazole 22.5% SC @ 2.66 ml l^{-1}	7	3	4	6	3	7
T12	Tetraniliprole 200 SC @ 0.6 ml l^{-1} + Tebuconazole 50% + Trifloxystrobin 25% WG @ 0.53 g l^{-1}	5	5	3	7	4	6
T13	Untreated Control	7	3	4	6	4	6
Average		**5.15**	**4.85**	**3.62**	**6.38**	**3.31**	**6.69**

***DBS** = Day Before Spraying, **MAS** = Month After Spraying, **BH** = Before Harvest.*

Table 6 Effect of pesticides applied alone or in combination using drone and taiwan sprayer on distribution of predominant genera of fungi in the rhizosphere soil of rice

Trt. No.	***Curvularia*** spp.			***Penicillium*** spp.			***Trichoderma*** spp.			***A. ochraceous***			***A. niger***			***A. flavus***		
	Sampling days			**Sampling days**			**Sampling days**			**Sampling days**			**Sampling days**			**Sampling days**		
	1DBS	**1MAS**	**BH**	**1DBS**	**1 MAS**	**BH**	**1DBS**	**1MAS**	**BH**	**1DBS**	**1MAS**	**BH**	**1DBS**	**1MAS**	**BH**	**1DBS**	**1MAS**	**BH**
T1	16.67*	16.67	20.00	11.11	25.00	0.00	5.56	0.00	10.00	61.11	50.00	50.00	0.00	0.00	0.00	5.56	8.33	20.00
T2	9.52	0.00	0.00	4.76	10.00	0.00	0.00	0.00	0.00	80.95	90.00	0.00	0.00	0.00	0.00	4.76	0.00	0.00
T3	18.18	21.43	20.00	27.27	21.43	13.33	0.00	0.00	6.67	54.55	57.14	53.33	0.00	0.00	0.00	0.00	0.00	6.67
T4	6.67	0.00	20.00	13.33	20.00	0.00	0.00	0.00	10.00	66.67	60.00	60.00	13.33	20.00	10.00	0.00	0.00	0.00
T5	8.33	9.09	16.67	16.67	18.18	8.33	0.00	0.00	0.00	50.00	54.55	66.67	8.33	9.09	8.33	16.67	9.09	0.00
T6	0.00	10.00	7.69	0.00	0.00	15.38	0.00	10.00	7.69	0.00	60.00	61.54	0.00	0.00	7.69	0.00	20.00	0.00
T7	15.38	18.18	9.09	15.38	18.18	9.09	0.00	0.00	9.09	61.54	63.64	63.64	0.00	0.00	0.00	7.69	0.00	9.09
T8	5.26	0.00	0.00	5.26	10.00	0.00	5.26	10.00	0.00	73.68	70.00	0.00	0.00	0.00	0.00	10.53	10.00	0.00
T9	0.00	0.00	0.00	11.11	20.00	14.29	0.00	0.00	0.00	88.89	80.00	85.71	0.00	0.00	0.00	0.00	0.00	0.00
T10	0.00	10.00	9.52	0.00	10.00	9.52	0.00	10.00	0.00	0.00	60.00	80.95	0.00	0.00	0.00	0.00	10.00	0.00
T11	26.32	18.18	0.00	10.53	18.18	0.00	0.00	0.00	5.26	47.37	54.55	94.74	10.53	0.00	0.00	5.26	9.09	0.00
T12	8.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	50.00	50.00	0.00	0.00	0.00	0.00	41.67	50.00	0.00
T13	13.33	7.14	5.26	13.33	0.00	0.00	0.00	0.00	0.00	46.67	42.86	94.74	0.00	0.00	0.00	26.67	50.00	17.39
Mean	9.85	8.51	8.33	9.90	13.15	5.38	0.83	2.31	3.75	52.42	60.98	54.72	2.48	2.24	2.00	9.14	12.81	4.09

% Occurrence of the fungi, **DBS = Day Before Spraying, **MAS** = Month After Spraying, **BH** = Before Harvest.*

4. CONCLUSION:

The results revealed diverse responses in bacterial populations, with certain pesticides leading to decline while others spurred growth. Comparatively, drone-sprayed treatments exhibited slightly higher average populations of actinomycetes and fungi than taiwan-sprayed ones, though differences were not substantial. The qualitative assessment highlighted shifts in G +ve and G -ve bacterial groups, indicating potential complexities due to pesticide interactions. Similarly, fungal genera distribution showed variations, influenced by both pesticide application and other factors. Our findings underline the need for further research to comprehensively understand the implications of different pesticide application methods on soil microbial communities and their potential impact on soil fertility and ecosystem functioning over time. The complex and nuanced responses observed highlight the importance of considering various factors beyond just the method of application. These findings can guide further research to better understand the long-term implications of pesticide exposure on soil microbial communities. Investigating microbial dynamics over extended periods and under different environmental conditions will offer deeper insights into the resilience and shifts within these communities.

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Fig 1 AGRICOPTER AG365

Fig 2 XR 11002 VP Nozzle

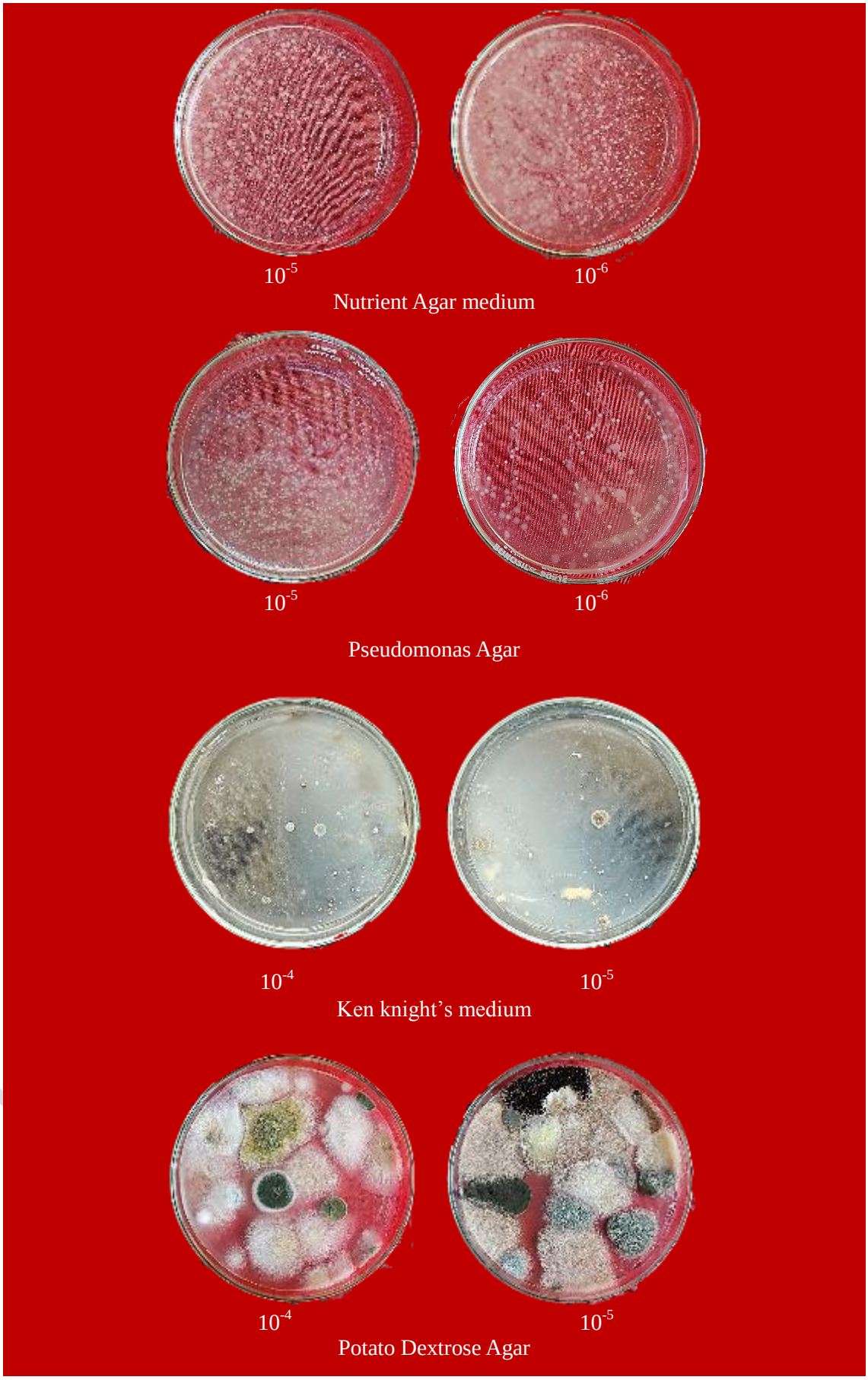

Fig 3 Variation in microbiota from rhizosphere soil samples on different media

Fig 4a Pure cultures of major fungi isolated from rhizosphere soil samples & photographs of fungal morphology observed at 40X magnification

Fig 4b Pure cultures of major fungi isolated from rhizosphere soil samples & photographs of fungal morphology observed at 40X magnification