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Comparison of tebuconazole and trifloxystrobin residue levels on paddy grains and paddy straw harvested from paddy fields applied with knapsack sprayer, motorised knapsack sprayer or drone application

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Abstract

A study on comparison of pesticide residue concentrations on paddy grain and paddy straw that were treated using three application methods, namely knapsack sprayer, motorised knapsack sprayer and drone sprayer, was conducted in a paddy field. Nativo (containing active ingredient of tebuconazole and trifloxystrobin) was applied once on a paddy field at 21 days before harvest. On the harvesting day, paddy grain and paddy straw were sampled from the plots that were treated with the three respective three application methods. Tebuconazole was detected in paddy grains (<0.01 - 0.371 mg/kg) and paddy straw (<0.01 - 0.171 mg/kg) at concentrations near to Limit of Quantification (0.01 mg/kg). The concentration ranges of trifloxystrobin in paddy grain and paddy straw were <0.01 – 0.025 mg/kg and <0.01 – 0.011 mg/kg, respectively. In ascending order, residue concentrations from plot treated with drone was the lowest, followed by motorised knapsack sprayer and knapsack sprayer. Spray drift factor may have contributed to the observed magnitude of residues in the treatment plots. Detected residues were within Maximum Residue Limits of tebuconazole and trifloxystrobin on rice. Except for tebuconazole concentration in paddy grain, drone application did not cause concentrations that were significantly differ from the conventional application method.

Keywords: *pesticide residues, paddy field, knapsack sprayer, motorised knapsack sprayer, drone*

Introduction

In line with technological advancement, Unmanned Aerial Vehicle (UAV) or drone technology has been used in seed sowing, irrigation, pesticide and fertiliser spraying, crop monitoring and mapping and remote sensing (Talaviya et al. 2020). In addition, drone technology can also be linked with Internet of Things and Artificial Intelligence (AI) as technology evolves (Subeesh & Mehta 2021). Drone technology is applied in the area of pesticide spraying and crop monitoring for precision agriculture (Hafeez et al. 2022). Pesticide application by UAV or drone has become the latest trend. In major paddy granary areas in Peninsular Malaysia, drone technology has been used widely as vehicle to spray pesticides. The acquisition cost of drone for agricultural use was high when the drone was introduced. However, reduction in drone cost over time enable a wider spread adoption of drone technology in agriculture sector (Kulbacki et al. 2018). Beside cost reduction in drone price, Drone has been adopted widely as a preferred method to apply pesticides because of its enormous advantages in terms of cost effectiveness and in addition to the comparable efficacy of pest and disease control by drone application. In terms of efficacy in controlling *Spodoptera exigua* on cabbage fields in South Korea, insecticide spraying by drone was comparable with conventional insecticide such as broadcast sprayer, knapsack sprayer, etc. (Park et al. 2019). Drone is viewed as able to spray agrochemicals faster as compared to other application methods (Rani *et al.* 2019).

UAV for pesticide application has been reported in East Asian countries in China (Matthews 2019; Wachenheim et al. 2021), Taiwan (Guo et al. 2019), Japan and South Korea (Xiongkui et al. 2017). Use of UAVs can help in reducing these deaths and other health problems (Nivas et

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al. 2020). Robotics and automatic spraying technologies like variable rate sprayers, UAV sprayers, and electrostatic sprayers has gained more attention to enhance. These advanced spraying technologies not only reduces the labour cost but also effective in environmental protection (Ahmad et al. 2021). Furthermore, occupational health perspective, worker is much less exposed to pesticide risk.

Despite its numerous advantages, concerns have been raised in drift exposure from drone application, especially to residences near to paddy fields. In terms of pesticide residue originated from drone application, not much of information was available. In theory, as long as application rate is similar regardless of application method, residues in crop should not differ significantly.

Even though drone application is very beneficial in terms of addressing lack of labour resource and reducing occupational hazard exposure during pesticide applications, its frequent and ubiquitous use may warrant further studies on other possible impacts in other aspects such as efficacy in pest and disease control, pesticide residue levels, possibility of significant spray drift to contaminate area nearby, etc. The current study compared pesticide residues levels on paddy grain and paddy straw from plots applied with three pesticide application methods including drone.

In Malaysia, Nativo (active ingredients: tebuconazole and trifloxystrobin) is used in paddy field for control of *Helminthos porium oryzae* (brown spot disease), *Rhizoctonia solani* (sheath blight) and *Pyricularia oryzae* (rice blast disease). Trifloxystrobin mode of action is classified under mode of action Code C according to FRAC. Trifloxystrobin disrupts respiration by acting on target site of complex III: cytochrome bc1 (ubiquinol oxidase) Qo site (cyt b gene). Tebuconazole mode of action is classified under Code G, with its mode of action that interrupts sterol biosynthesis in membranes.

The aim of the study was to determine if there was significant difference in terms of pesticide residue levels on paddy grain and paddy straw from for paddy fields treated separately with three application methods namely, knapsack sprayer, motorised knapsack sprayer, and drone.

Materials and method

Residue field trials

The field trial was conducted at paddy plots (variety MR 269) in MARDI Seberang Perai within May-August 2019. A total of four treatment plots (*Table 1*) with dimensions of at least 10 m × 15 m for each treatment plot were established as shown in *Figure 1*. The four treatment plots were Treatment 1 (control plot, not sprayed with Nativo), Treatment 2 (sprayed Nativo with knapsack sprayer), Treatment 3 (sprayed Nativo with motorized knapsack sprayer) and Treatment 4 (sprayed Nativo with drone). Nativo was applied at 21 days before harvest. Nativo was applied at the rate of 150 g/ha based on the spray volume of 250 L/ha for Treatment 2 and Treatment 3. The spray volume of Treatment 4 was 18 L/ha with the same application rate of 150 g/ha. Other herbicides, insecticides and fungicides that do not contain tebuconazole and trifloxystrobin were applied as maintenance pesticides to protect paddy crops from pests and diseases. The maintenance pesticides were applied using motorised knapsack sprayer.

Table 1. Treatment types according to Nativo application methods

Treatment type	Nativo application method
Treatment 1 (T1)	Control – No Nativo application
Treatment 2 (T2)	Nativo applied with knapsack sprayer
Treatment 3 (T3)	Nativo applied with motorised knapsack sprayer
Treatment 4 (T4)	Nativo applied with drone

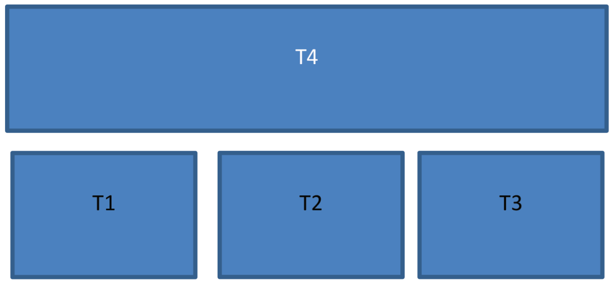

Figure 1. Treatment plots layout

At the harvest date (110 days after sown), paddy grain and paddy straw samples were taken from each treatment plot at 1 kg and 500 g, respectively. A total of three replicates of field crop samples (paddy grain and paddy straw) were collected from each of the four treatments plots. Samples were taken randomly in the middle of the treatment plots. Samples were not taken near the bordering area of the treatment plot (10 m from bordering bund) where drift from nearby plots was likely to occur. Samples were sent to the analytical laboratory located at the headquarters of Malaysian Agricultural Research and Development Institutes (MARDI) at Serdang, Selangor for analysis of tebuconazole and trifloxystrobin residues.

Paddy sample processing

Grinder was used to convert paddy grain samples were into fine particle form. The grinded paddy grain was transferred into small container for further sampling of analytical portion. Paddy straw were cut into shorter pieces (about 2 cm in length) and all the pieces were macerated in Blixer® 5 Food Processor (brand: Robot Coupe) into smaller particles. The homogenised paddy straw was transferred into small container for further sampling of analytical portion.

Residue extraction

An amount of 10 g of grinded paddy grain (20 g of homogenised paddy straw) was weighed into 50 ml centrifuge tube (250-mL bottle for homogenized paddy straw). A volume of 10 ml (35 ml for paddy straw) of distilled water was added into the tube and the tube was sonicated (sonicator model B-92H of Branson brand) for 30 minutes. Then a volume of 20 ml (45 ml for paddy straw) acetonitrile containing 0.1% formic acid was added into the tube, followed by 6 g of magnesium sulphate and 1.5 g of natrium acecate. The tube containing paddy grain and solvents was shaken vigorously for 1 minute and centrifuged (centrifuge model Sigma 3-16 KL of Sartorius brand) at 4000 rpm for 5 minutes. The 250 mL containing 20 g paddy straw and solvents was stirred with ultraturax at high speed setting for 3 minutes. Then a volume of 5 ml of extract (paddy grain or paddy straw) was transferred into 15 ml centrifuge tube. The extract was added with 0.5 g Primary Secondary Amines (PSA) powder, vortexed for 1 minute and followed by centrifugation at 2000 rpm for 2 minutes. The extract was filtered using syringe filter that was attached with 0.22 µm nylon syringe filter. The filtered extract was diluted 5 times with acetonitrile (1% formic acid). The filtered extract was transferred into 2 mL vial for residue analysis using Liquid Chromatoraph Mass Spectrometry equipped with triple quadrupoles detector.

Residue analysis

Agilent 1290 Infinity UHPLC (Ultra High Pressure Liquid Chromatograph) and AB Sciex QTrap 5500 mass spectrometer were used in analysis of tebuconazole and trifloxystrobin. Residues of trifloxystrobin and its metabolite, trifloxystrobin acid were analysed using HPLC-MS/MS in paddy straw, bran, brown rice and soil (Chen et al. 2014). The column used in the UHPLC was Synergi 4µ Fusion-RP 80A (4 µm pore size, 50 mm length, 2 mm outer diameter). Temperature of column was maintained at 30°C. Injection volume was set at 5 µL. The mobile phases and gradient mode of Liquid Chromatograph was shown in *Table 2*.

Table 2. LCMS gradient setting for analysis of trifloxystrobin and trifloxystrobin acid

Time (min)	%A	%B	Flow (mL/min)
0.0	90.0	10.0	0.4
3.0	5.0	95.0	0.4
4.0	5.0	95.0	0.4
4.1	90.0	10.0	0.4
6.0	90.0	10.0	0.4

A: 0.1% formic acid in water
B: 0.1% formic acid in HPLC-grade methanol

Statistical analysis

Residue data from the treatments were analysed using The SAS System (version 9.4) in which, Duncan multiple range test was used to determine significance ($p < 0.05$) of mean of treatments.

Results and discussion

The Limit of Quantification (LOQ) of the analytical method for quantification of tebuconazole, trifloxystrobin was 0.01 mg/kg, respectively. In method validation in the both laboratories, mean recoveries were observed within 70 – 120% for tebuconazole and trifloxystrobin.

Results of residue analysis of field samples are shown in *Table 4* (tebuconazole) and *Table 5* (trifloxystrobin). No residue of trifloxystrobin was detected at or above the Limit of Quantification in samples from T1 (untreated). However, tebuconazole was detected in paddy grains (<0.01 - 0.371 mg/kg) and paddy straw (<0.01 - 0.171 mg/kg) at concentrations near to Limit of Quantification (0.01 mg/kg). The presence of tebuconazole in paddy grains and paddy straw from T1 (untreated) indicated that there was probably a spray drift cross-contamination during applications from nearby plots (T2, T3 & T4). In a study by Kim et al. (2020), pesticide residues were detected in surrounding crops that were situated next to the main plot that was treated with UAV. Based on the results obtained, there was a potential of spray drift resulted from the drone use during the trial which could have contaminated the samples from the untreated plots.

The concentration ranges of trifloxystrobin in paddy grain and paddy straw were <0.01 – 0.025 mg/kg and <0.01 – 0.011 mg/kg, respectively. The concentration ranges of trifloxystrobin in paddy grain and paddy straw were <0.01 – 0.025 mg/kg and <0.01 – 0.011 mg/kg, respectively. Overall, tebuconazole and trifloxystrobin concentrations in paddy straw were observed to be lower than its concentrations in paddy grain.

The highest detected concentrations of tebuconazole and trifloxystrobin in paddy grains were 0.371 mg/kg and 0.025 mg/kg, respectively. These highest values were still within national Maximum Residue Limits of tebuconazole and trifloxystrobin for milled rice are 0.15 mg/kg and 5 mg/kg, respectively. This finding indicates that spraying pesticides with drone on paddy field would result in pesticide concentrations in rice that are within MRLs.

In terms of mean concentrations, pesticide concentrations were highest in paddy samples from T4 (knapsack sprayer) followed by T3 (motorised power sprayer) and T2 (drone). In SAS analysis, there were no significant differences ($p < 0.05$) in treatment using drone and treatment using knapsack sprayer for all pesticide-crop combination with the exception of tebuconazole-paddy grain. No significant difference was observed in treatments from motorised knapsack sprayer and knapsack sprayer.

Table 3. Tebuconazole concentration in paddy samples

Sample	Concentration (mg/kg)			Mean*	Standard deviation
	R1	R2	R3		
Paddy grain					
T1 (untreated)	0.012	<0.01	<0.01	0.011[c]	0.009
T2 (drone)	0.202	0.119	0.185	0.169[b]	0.044
T3 (motorised knapsack sprayer)	0.231	0.206	0.292	0.243[ab]	0.044
T4 (knapsack sprayer)	0.214	0.371	0.336	0.307[a]	0.083
Paddy straw					
T1 (untreated)	<0.01	<0.01	0.012	0.011[b]	0.005
T2 (drone)	0.098	0.032	0.171	0.100[a]	0.069
T3 (motorised knapsack sprayer)	0.051	0.032	0.056	0.046[ab]	0.013
T4 (knapsack sprayer)	0.025	0.046	0.027	0.033[ab]	0.012

* P value <0.05
<0.01 – Less than limit of quantification of 0.01 mg/kg

Table 4. Trifloxystrobin concentration in paddy samples

Sample	Concentration (mg/kg)			Mean*	Standard deviation
	R1	R2	R3		
Paddy grain					
T1 (untreated)	<0.01	<0.01	<0.01	<0.01[c]	0
T2 (drone)	0.012	0.013	0.010	0.012[a]	0.002
T8 (motorised knapsack sprayer)	0.019	0.010	0.019	0.016[b]	0.005
T9 (knacksack sprayer)	0.015	0.025	0.021	0.020[b]	0.005
Paddy straw					
T1 (untreated)	<0.01	<0.01	<0.01	<0.01[c]	0
T2 (drone)	0.010	0.010	0.011	0.011[b]	0.001
T8 (motorised knapsack sprayer)	0.010	0.010	0.010	0.010[ab]	0
T9 (knapsack sprayer)	0.010	0.010	0.010	0.010[a]	0

* P value <0.05
<0.01 – Less than limit of quantification of 0.01 mg/kg

The observed significant difference in tebuconazole-paddy grain between drone and knapsack sprayer could be due higher range of detected concentrations. In trifloxystrobin-paddy grain combination, no significant difference between drone and knapsack sprayer was observed. This discrepancy may be due to the magnitude of concentration ranges as the authors hypothesize that if higher range of concentrations were observed for trifloxystrobin-paddy grain combination, there would be significant difference between the two treatments. The observed higher ranges of tebuconazole concentrations than trifloxystrobin concentrations were expected as the percentage of tebuconazole (50%) is higher than trifloxystrobin (25%) in Nativo formulation. The authors suggest future studies should explore applying pesticides with higher application rate that would theoretically yields higher pesticide concentration ranges.

The authors hypothesise that spray coverage from knapsack sprayer was more focus on crop area and spray drift was less severe as compared to the other two application methods. In drone application, because of longer distance (about 1 – 2 m) between the drone nozzle and the paddy crop as the drone needs to hover at certain elevation, the spray drift could be significantly enhanced in situation when wind occur. One of the major problems encountered during drone application was drift (Caner 2017). Overall, faster wind speeds, finer droplet sizes, and a heavier initial payload were associated with more drift on average (Grant et al. 2022).

This could explain relatively lower residue of tebuconazole and trifloxystrobin in paddy grain from T2 (drone). Spray drift cross over to neighbouring plot tends to occur during application around the perimeter of the plot as the spray path propels by the motorised pump (T3) could reach further distance as compared to knapsack sprayer (T4). This could explain the relatively higher tebuconazole and trifloxystrobin residues in paddy grain from T4 plot.

In a comparison study between UAV and aerial spray on commercially grown alfalfa grass by Li et al. (2021), chlorantraniliprole residue in alfalfa grass applied with drone application was comparable and consistent with chlorantraniliprole residue in alfalfa grass applied with airplane application.

Conclusion

With the exception of tebuconazole in paddy grains, there was no significant difference in terms of pesticide residues concentrations in paddy grains and paddy straw from plots treated by using knapsack sprayer, motorized knapsack sprayer and drone. Hence, in terms of pesticide residue concentrations, adoption of drone in place of conventional spraying method should result in pesticide residue concentrations that are not significantly different from concentrations that would be expected from conventional spraying methods. Application of pesticides using drone technology can be considered as a new technological tool in agriculture modernisation and more studies of its use on pesticide residues concentrations by using other pesticides or on other crops should be explored so that pesticide intake from food crops that have been applied by drone can be thoroughly assessed and evaluated.

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