Phytopathology Research

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Evaluation of adjuvants for reducing the risk of phytotoxicity in low-volume spray of propiconazole

Lijie Teng[1,4], Tuqiang Gao[1], Anyu Gu[2], Qizhen Zhang[1], Maolin Hu[3*], Jianjun Hao[4], Xiaolin Li[2*] and Pengfei Liu[1*]

Abstract

Unmanned aerial vehicles (UAVs) have been increasingly employed for fungicide applications in plant disease control. However, due to weight limitations, the fungicides sprayed through UAVs must be in low volumes with high concentrations in many instances, which may result in potential phytotoxicity. Here we evaluate the safety of low-volume spray of chemicals on rice plants. The plants were sprayed with propiconazole emulsifiable concentrate (EC) at 250 g/L mixed with various adjuvants and applied at a low volume, which contained the fungicide at concentrations equivalent to or higher than that used in UAV application. The spray adjuvants included YS-20, Biaopu adjuvant, TriTek, Yipinsongzhi, AgriSolv-C100, and Hongyuyan. Potential phytotoxicity on rice plants was examined based on surface tension and crop growth. Additives suitable for a low-volume spray of propiconazole were also assessed on three rice varieties for phytotoxicity. The results showed that after 72 h of fungicide application at 2, 4, and 8 times the recommended dose of 7500 μg/mL for UAV spray, rice leaves exhibited abnormal growth, and the dry weight of rice significantly decreased 21 days after application. Phytotoxicity was evaluated on three rice varieties 5 days after spraying propiconazole EC at 2 × recommended dose with one of the spray adjuvants. The addition of 1% YS-20, Biaopu adjuvant, TriTek, and Yipinsongzhi significantly augmented the phytotoxicity. However, both AgriSolv-C100 and Hongyuyan significantly reduced the comprehensive index of phytotoxicity and, therefore, could be used for UAV applications.

Keywords Unmanned aerial vehicle (UAV), Surface tension, Crop safety, Water-sensitive card

Background

Fungicides are a common strategy to control plant diseases. For example, propiconazole belonging to triazoles is effective in controlling diseases of rice crops, such as blast, sheath blight, and false smut (http://www.icama.org.cn/, as of December 9, 2022). As rice is China's primary food crop and the country is the largest producer of rice (Food and Agriculture Organization of the United Nations Crop Production 2020), disease control is of utmost importance. Triazoles inhibit the activity of the cytochrome P450 monooxygenase lanosterol 14α-demethylase, which affects the biosynthesis of ergosterol in fungi (Berg et al. 1988; Zhang et al. 2020; Chen et al. 2022). These fungicides also affect the synthesis

*Correspondence:
Maolin Hu
maolin522612@126.com
Xiaolin Li
lixiaolin@yaas.org.cn
Pengfei Liu
pengfeiliu@cau.edu.cn
[1] College of Plant Protection, China Agricultural University, Beijing 100193, China
[2] Yunnan Institute of Grain Crops, Kunming 650201, China
[3] Shenzhen Agricultural Technology Promotion Center, Shenzhen 518000, China
[4] School of Food and Agriculture, University of Maine, Orono, ME 04469, USA

of gibberellin in plants (Izumi et al. 1985; Fletcher et al. 1999; Yang et al. 2016; Cao et al. 2020).

To improve the efficiency of fungicides and deliver precise disease control, aerial applications via unmanned aerial vehicles (UAVs) have been increasingly used for chemical sprays (Ministry of Agriculture and Rural Affairs of the People's Republic of China, 2014). This approach offers several advantages, such as high efficiency, adaptability, and reduced water and chemical usage (Huang et al. 2009; Xue et al. 2016; Lan et al. 2019; Wang et al. 2022). However, there are some concerns related to UAV applications, such as low-volume spray ranging from 5 to 30 L/hm^2 (Yuan et al. 2018), which requires low-volume and high-pressure settings. This generates a large amount of fine mist droplets, leading to pesticide drift and environmental pollution. Additionally, high concentrations of fungicides can cause phytotoxicity and microbial toxicity (Yang et al. 2014). It is known that incorrect application of triazole fungicides can result in phytotoxicity in rice, such as inhibiting rice plant growth and defective heading. Therefore, it is crucial to consider crop safety issues when using triazole fungicides for plant disease control via UAVs. Most plant leaves are constructed with a surface covered with cuticles, which are composed of cuticle wax (von Wettstein-Knowles 1993; Xu et al. 2010). These substances increase surface tension and leaf skin hardness, thereby protecting plants from pathogen infection. However, such a structure makes the droplets of applied fungicides hard to adhere to leaf surfaces. To overcome this obstacle, adjuvants can be used with fungicides to break the surface tension, enhance the deposition and attachment, and so on. When using a UAV for pesticide application, adding an appropriate spray adjuvant can improve physicochemical properties, enhance stability, reduce leaf surface tension, reduce droplet drift and evaporation, improve the deposition of pesticides, enhance the spread, and reduce chemical losses (Dong et al. 2015; Meng et al. 2018; Wang et al. 2022). This significantly improves the spraying effect and application quality of UAV-based applications.

Some adjuvants for aerial pesticide applications, such as wettable adjuvants and penetrating adjuvants, are available on the market. However, it is unclear whether these adjuvants can alleviate the potential phytotoxicity of pesticides on crops. This study aims to identify suitable adjuvants for improving the efficacy of low-volume spray of propiconazole and reducing phytotoxicity in controlling rice diseases with fungicides.

Results

Spray quality under UAV conditions

The spray card designed to detect water sensitivity turned blue upon contact with water droplets (Fig. 1a). The droplets had the highest level of uniformity at the middle and lower parts than at the upper part of the plant (Table 1), and the droplet point density was 62.50 points/cm^2, 24.65 points/cm^2, and 10.48 points/cm^2, for middle, lower, and top parts, respectively.

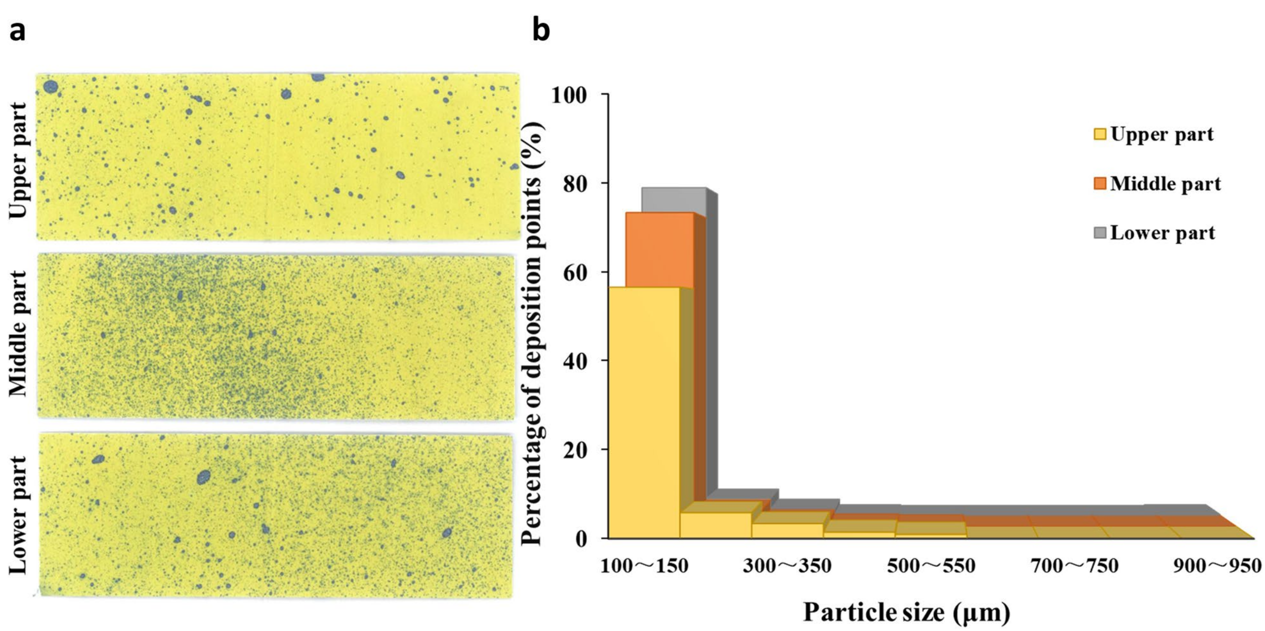

Fig. 1 Patterns of droplet distribution on **a** water-sensitive papers and **b** droplet size spectra at different heights of 40 cm (upper part), 55 cm (middle part), and 68 cm (lower part)

Table 1 Physical properties of chemical-suspension droplets on water-sensitive spray cards placed at 40, 55, and 68 cm from a sprayer, which referred to the upper, middle, and lower parts of rice plants

Position	Number of countable droplets	Density (dots/cm^2)
Upper part	207	10.48
Middle part	1235	62.50
Lower part	487	24.65
Average	643	32.54

Standard density was ≥ 20 dots/cm^2 based on the spray volume of the pesticide

While the density at the middle and lower parts met the standard, it did not meet the standard at the upper part. Despite this, the total average point density of droplets at different positions was 32.54 points/cm^2, which met the standard (Table 1). More than 50% of the droplets had particle sizes of 100–150 μm, which were fine droplets (Fig. 1b). Based on these findings, the experimental spray method met the quality requirements of UAV application and was utilized for the subsequent studies.

Safety of propiconazole on rice under UAV spray concentrations

After 72 h of applying propiconazole, the rice leaves displayed varying degrees of chlorosis, yellowing, curling, and frangibility at all concentrations of the fungicide (1×F, 2×F, 4×F, and 8×F) (Fig. 2a). The level of phytotoxicity was positively correlated with the fungicide concentrations. However, no obvious symptoms were found at concentrations of 1×S. After 21 days of propiconazole application, the leaves treated with the low-volume and high concentration of propiconazole showed severe symptoms, such as withering, chlorosis, and whitish, and a few plants from the 4×F and 8×F treatment groups even killed as a result of severe symptoms.

Plant height, root length, fresh weight, and dry weight of rice were determined 21 days after treatment with propiconazole at concentrations of 1×F, 2×F, 4×F, and 8×F. The results showed a decrease in plant biomass as the concentration of propiconazole increased (Fig. 2b, c).

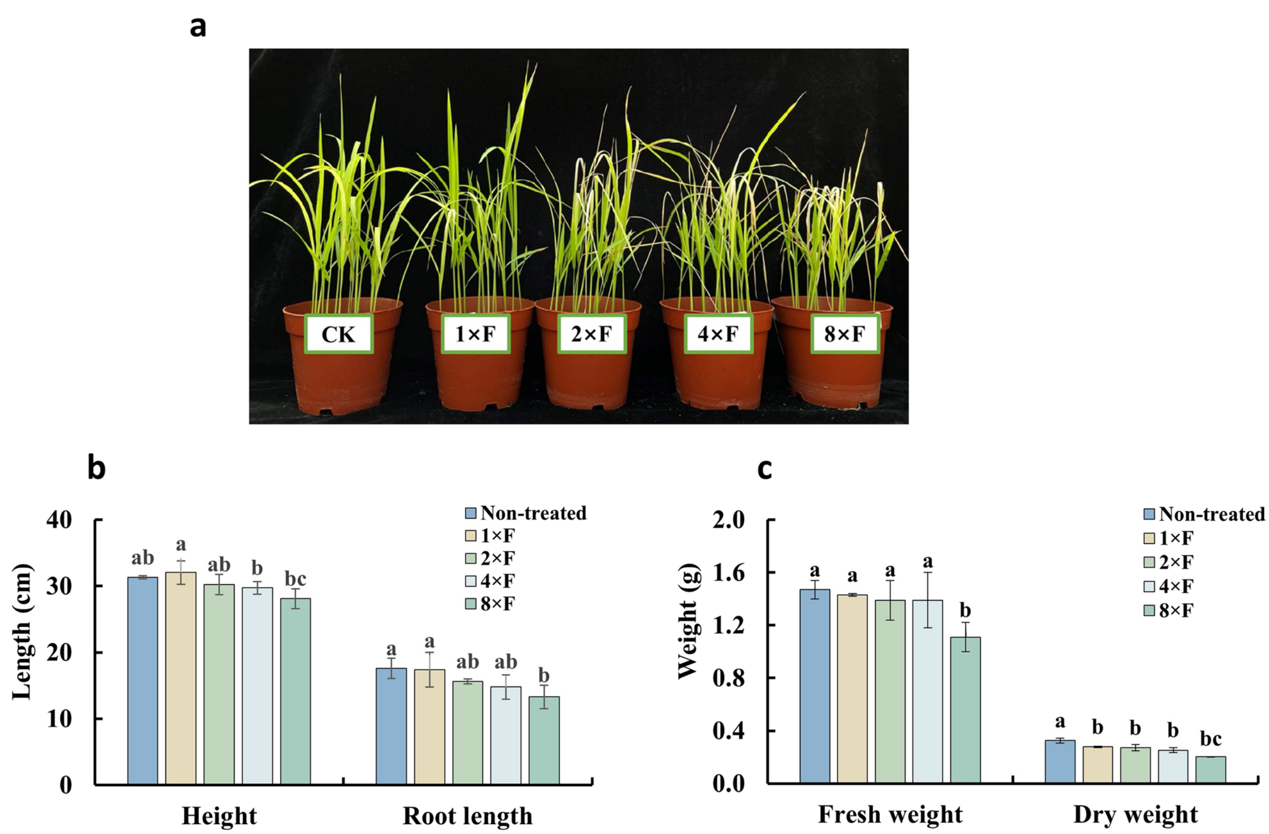

Fig. 2 Effects of propiconazole concentrations on **a** rice seedling leaf color 3 days after application, **b** length, and **c** weight 21 days after application. CK was a non-treated control. F = 7500 μg/mL. Different letters within the same column indicate significant differences ($p < 0.05$)

While there was no significant difference in plant height, root length, and fresh weight between the 1×F, 2×F, and 4×F treatment groups and control group, the dry weight of plants was significantly decreased in the 1 × F, 2×F, 4×F, and 8×F treatment groups compared to the control (Fig. 2b, c).

Effects of adjuvants on surface tension of propiconazole and rice growth

The surface tension of the control group was 30.44 mN/m, which was very low compared to 72 mN/m of water, indicating that the commercial product propiconazole at 250 g/L demonstrated superior performance for target surface wetting and chemical spreading (Table 2). The addition of one of the six spray adjuvants resulted in a reduction of surface tension in the propiconazole suspension. TriTek, YS-20, and Hongyuyan had a significant effect in reducing surface tension.

Test adjuvants were sprayed onto rice plants at a 1% aqueous suspension. After 21 days, the addition of 6 adjuvants had no significant impacts on the growth of rice plants, and no phytotoxicity was observed (Fig. 3a–c). Therefore, these six adjuvants are safe for use on rice and can be applied in conjunction with other fungicides.

Alleviative effects of adjuvants on propiconazole-caused phytotoxicity

Six spray adjuvants were examined when they were added at 1% to propiconazole suspension at 2×F to determine the alleviation effect of adjuvants on propiconazole phytotoxicity. The canopy of rice treated with Yipinsongzhi, YS-20, TriTek, and Biaopu adjuvant showed noticeable phytotoxicity, such as yellowing, whitish, or dry leaves. However, the addition of AgriSolv-C100 and Hongyuyan did not increase phytotoxicity and even showed some alleviation (Fig. 4a).

The comprehensive index of phytotoxicity (CIP) increased from 7.94% to 41.61% in the control group from 2 and 5 days after application. Propiconazole suspensions added with either YS-20, Biaopu adjuvant, TriTek, or Yipinsongzhi all increased CIP (Fig. 4b). CIP was 82.00% and 89.64%, respectively, after 2 days and 5 days of treatment with TriTek. The phytotoxicity of the treatments with AgriSolv-C100 and Hongyuyan added after spraying for 2 days was not significantly different from the control group. The indices were 7.98% and 10.33%, respectively, indicating that it has the function of alleviating the phytotoxicity of propiconazole in rice plants.

The 2×F propiconazole mixed with 1% adjuvant did not affect rice height but reduced both the fresh and dry weights to some extent. Because Hongyuyan and AgriSolv-C100 had the least negative effect on rice, they were selected to carry out the greenhouse safety test on three rice varieties (Fig. 3d–f).

Hongyuyan and AgriSolv-C100s were added at 1% to 1×F, 2×F, and 4×F propiconazole suspensions. After 21 days of application on rice cultivar Jingliangyou 1125, Tenuo 2072, and Liangyou H108, propiconazole at all three concentrations had negative effects on the dry weight of Jingliangyou 1125 seedlings, while the addition of the other two adjuvants caused phytotoxicity (Fig. 5d). The height, root length, fresh weight, and dry weight of 'Liangyou H108' were affected by propiconazole at 4×F and the addition of the two additives at 1×F affected dry weight of rice seedlings, indicating that they were safe to crops (Fig. 5a–d). For Tenuo 2072, under the treatment of three concentrations of propiconazole, the addition of the two adjuvants did not affect plant growth (Fig. 5a–d). This indicates that the application of propiconazole at a higher concentration was safe on Tenuo 2072 when applied via a drone.

Table 2 Effect of adjuvants on the surface tension of propiconazole suspension

Adjuvants	Surface tension (mN/m)
No adjuvants added	30.44 ± 0.58 a
Biaopu adjuvant	30.40 ± 0.60 a
AgriSolv-C100	29.94 ± 0.43 ab
Yipinsongzhi	29.31 ± 1.04 abc
TriTek	29.10 ± 1.08 bc
YS-20	28.56 ± 0.68 cd
Hongyuyan	27.33 ± 0.33 d

Different letters within the same column indicate significant differences ($p < 0.05$)

Discussion

Since UAVs are equipped with low-volume spray, the concentration of pesticides can be much higher than the manufacturer's recommendations. To achieve the same level of fungicide delivery to plants as tractor-driven sprays, a smaller volume is needed. However, there is a risk of crop damage if the droplets are not evenly distributed for unforeseen reasons. Triazole fungicides can affect plant growth by disrupting gibberellin biosynthesis (Izumi et al. 1985; Fletcher et al. 1999; Yang et al. 2016), and higher concentrations can cause phytotoxicity by overstimulating plant growth. Therefore, when applying propiconazole via UAVs, there is a risk of phytotoxicity.

Propiconazole at higher concentrations has been found to cause various symptoms of phytotoxicity on rice leaves. When propiconazole EC was applied at the recommended dose of 7500 μg/mL or higher, it caused

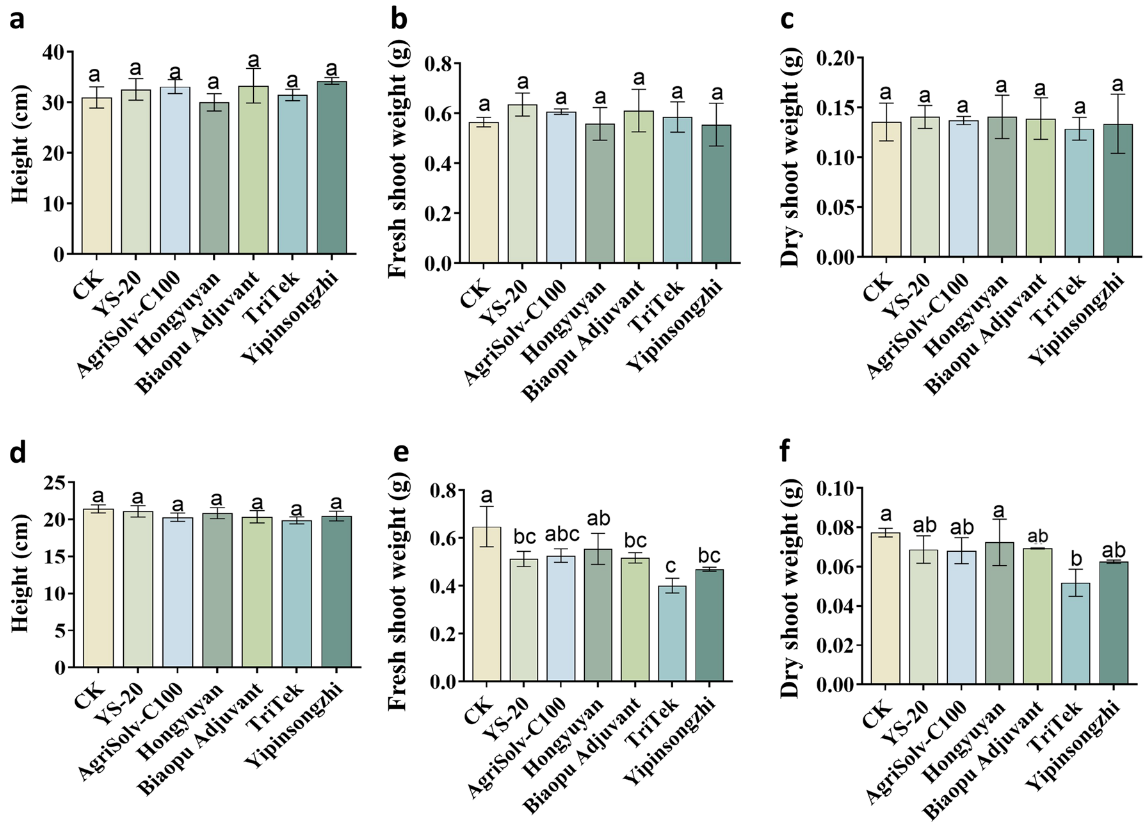

Fig. 3 Effects of 1% adjuvants on **a** and **b** seedling height, **b** and **e** fresh shoot weight, and **c** and **f** dry shoot weight **f** of rice 'Jingliangyou 1125' seedlings, compared to the non-adjuvant control (CK). Rice plants were applied with either an adjuvant only **a** to **c** or the adjuvant mixed with 15,000 μg/mL propiconazole **d** to **f**. Different letters in the same column indicate significant differences ($p < 0.05$)

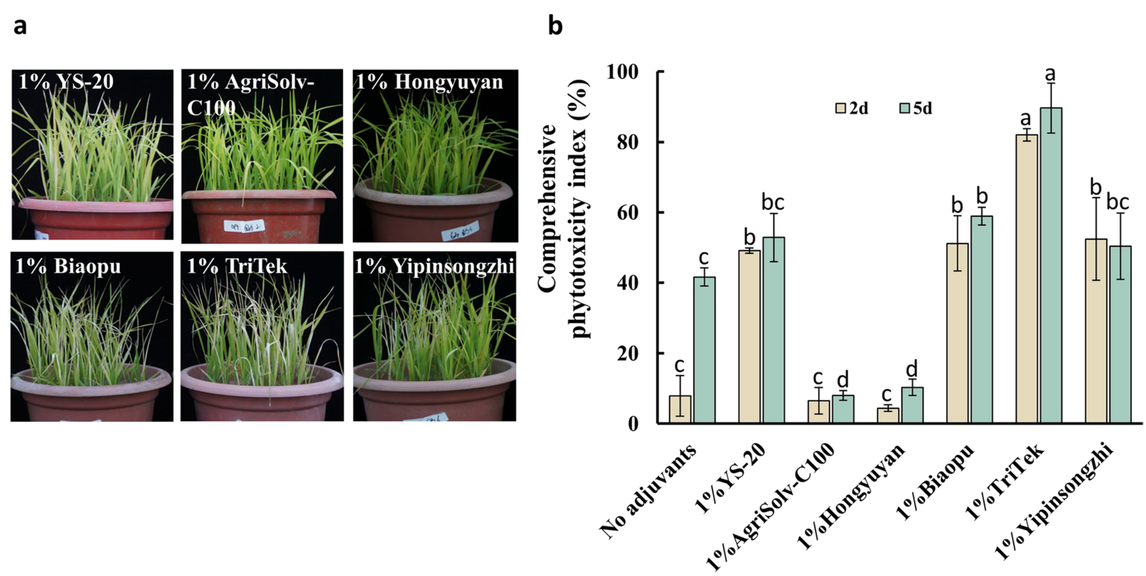

Fig. 4 Phytotoxicity of propiconazole mixed with 1% adjuvants sprayed on rice leaves. **a** Performance of rice leaves 3 days after receiving low-volume spray of propiconazole and **b** phytotoxicity index on 2 days and 5 days. Treatments included 2×F propiconazole mixed either 1% AgriSolv-C100, 1% Hongyuyan, 1% Yipinsongzhi, 1% YS-20, 1% Biaopu Adjuvant, or 1% TriTek

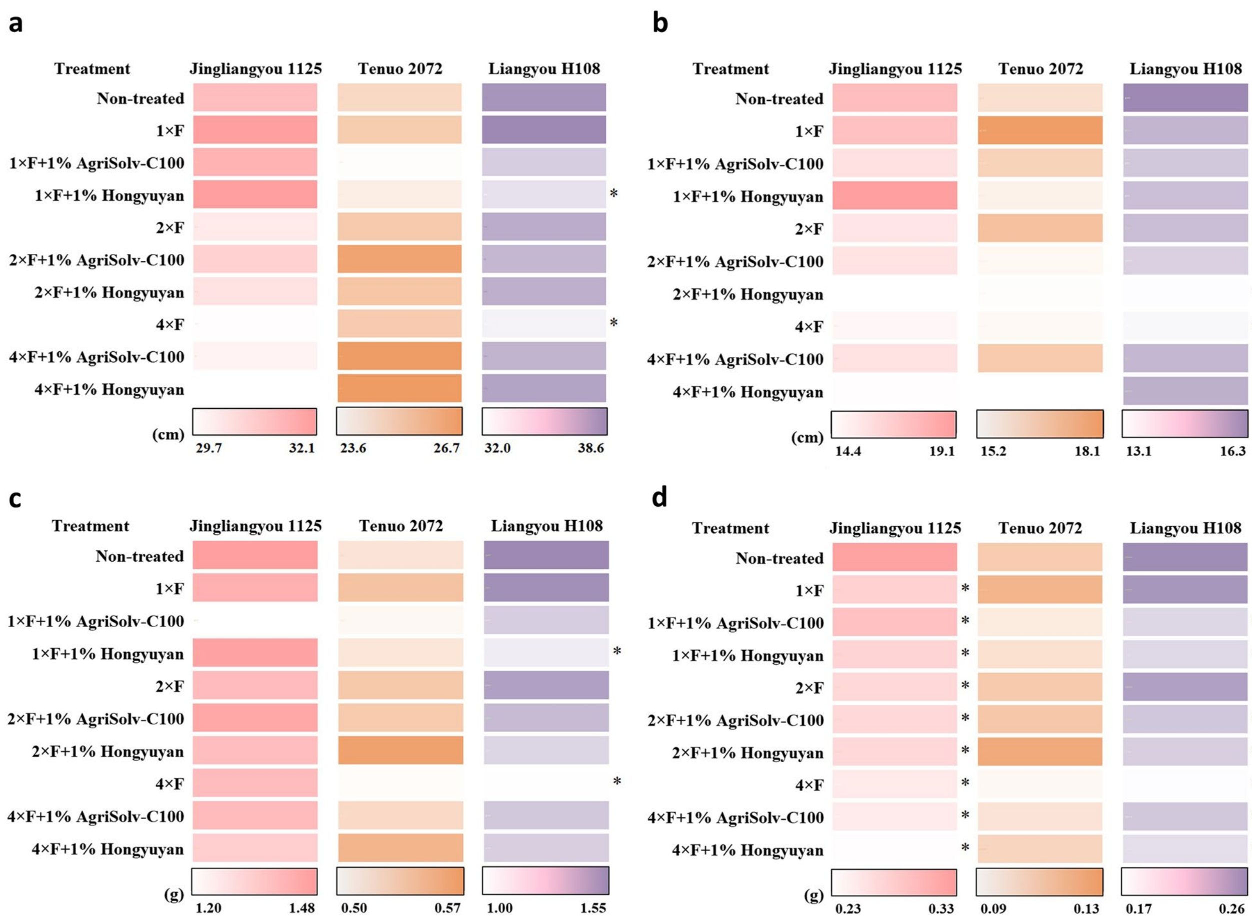

Fig. 5 Effects of propiconazole with AgriSolv-C100 or Hongyuyan on the **a** seedling height, **b** seedling root, **c** fresh weight, and **d** dry weight of three different rice varieties. * indicates significant differences within the same column ($p < 0.05$)

phytotoxicity, although the symptoms were different compared to other reports (Percich 1989).

In order to minimize the risk of phytotoxicity when using UAVs for crop disease control, we investigated the use of adjuvants in combination with propiconazole. The addition of some spray additives can improve the permeability of the suspension and increase the wetting area of the pesticide. Our results were consistent with previous reports (Appah et al. 2020; Chen et al. 2020; Wang et al. 2022).

It is unknown whether different additives will affect crop safety when spraying pesticides. Studies have shown that the addition of non-ionic surfactants (NIS) and an organosilicon (OS) at appropriate concentration can increase the phytotoxicity of coumarin and p-vanillin to *Eleusine indica* (Chuah et al. 2013). Additionally, the use of adjuvants with flumioxazin has been found to increase its phytotoxicity to sunflower (Jursík et al. 2013). However, either AY904-1, AY904-2, AY904-3, or AY904-4 added into 200 g/L chlorfenam does not cause toxicity to corn (Zhang et al. 2018). In this study, we observed that the addition of AgriSolv-C100 or Hongyuyan at the same concentration of propiconazole reduced phytotoxicity, while the use of four other additives increased it. Therefore, the selection of additives is crucial for safe and effective pesticide use.

In this study, none of the six adjuvants caused phytotoxicity on rice when they were solely applied; therefore, they were safe to use on rice. However, varying levels of phytotoxicity were observed when they were co-applied with propiconazole under the UAV application conditions. The addition of TriTek, YS-20, Biaopu adjuvant, and Yipinsongzhi increased the CIP value to different levels, presumably due to the penetrant and oil contained in the adjuvants, which helped promote the absorption of propiconazole. On the other hand, the addition of AgriSolv-C100 and Hongyuyan greatly remediated phytotoxicity when used with propiconazole. Hongyuyan was found to be the most effective, possibly because it had the lowest surface tension among the six adjuvants. Spray additives can reduce the surface tension of pesticide solutions, which is conducive to their wetting and spreading performance on the surface of plant leaves (Gimenes et al. 2013; Gitsopoulos et al. 2014; Prado et al. 2016). Therefore, we proposed that this addition would be beneficial for reducing the phytotoxicity of propiconazole on the surface of rice leaves due to local high concentrations. In our results, although there was no difference in surface tension between AgriSolv-C100 and the non-adjuvant control group, it was effective in alleviating phytotoxicity. This could be attributed to citric acid, an important component of AgriSolv-C100, which provides substrates for some metabolic pathways, promotes photosynthesis, and cellular respiration of plants, and participates in the regulation of plant physiological metabolism (Tahjib-Ul-Arif et al. 2021). Citric acid can also improve plant resistance to stress and endow plants with a tolerance to abiotic stress (Tahjib-Ul-Arif et al. 2021). These effects may have helped the plant improve its ability to alleviate the phytotoxicity caused by propiconazole. It is possible that the polyols contained in both adjuvants may have also contributed to this activity, but further research is required to confirm this.

The sensitivity of rice to propiconazole varied depending on the varieties. This result is consistent with a previous report (Percich 1989). Therefore, it is important to consider the rice varieties, types of adjuvants, and fungicides used when spraying via UAVs. Among the three varieties examined, only rice cultivar Tenuo 2072 did not show significant phytotoxicity in any category of rice growth under propiconazole at various concentrations, making it suitable for UAV applications.

Conclusions

The application of pesticides by using UAVs requires very high concentrations of active ingredients within the tank mix. This, however, can elevate the risk of crop damage. In this study, we have confirmed that propiconazole EC causes toxicity to rice when applied at low-volume and high concentrations. The level of phytotoxicity is related to the concentration of the fungicide, the rice varieties used, and the specific types of tank mixing additives employed. Notably, the adjuvants AgriSolv-C100 and Hongyuyan significantly mitigate the toxicity to rice due to propiconazole. This remediation of phytotoxicity is likely attributed to improved wetting and spreading properties of spray droplets, which prevents localized fungicide accumulation on the leaf surface. This research offers valuable insights to ensure the safe and effective use of UAVs in fungicide applications.

Methods

Plants and chemicals

Rice Jingliangyou 1125, Indica rice, and Guishendao 2021214 were collected from Guangxi, China, in 2021. Tenuo 2072 indica, Medium indica, and Yushengdao 2002002 were provided by Xinyang Academy of Agricultural Sciences, Xinyang, Henan, China. Liangyou H108, Medium Indica, and Minshen Rice 20190008 were provided by Nanping Institute of Agricultural Sciences, Nanping, Fujian, China.

Chemicals included 250 g/L propiconazole EC (ADAMA, Huaian, Jiangsu), Yipinsongzhi (40% methylated vegetable oil and 30% oleoresin-based vegetable oil, Shenzhen Yuyan Intelligent Technology Service Co., Ltd., Shenzhen, Guangdong), Hongyuyan (30% glycerol polyols, 30% polycondensates, and 10% fatty alcohol ethoxylates, Shenzhen Yuyan Intelligent Technology Service Co. Ltd., Shenzhen, Guangdong), Biaopu adjuvant (70% methyl oleate, 5% rapid penetrant T, 20% mineral oil, and 5% non-ionic emulsifier, Anyang Quanfeng Biotechnology Co., Ltd., Anyang, Henan), Ys-20 (45% methyl oleate, 5% rapid penetrant T, 40% mineral oil, 5% non-ionic emulsifier, and 5% dispersant, Anyang Quanfeng Biotechnology Co., Ltd., Anyang, Henan), AgriSolv-C100 (30% tetrahydrofurfuryl alcohol, 2% corn oil, 0.5% sodium lauryl sulfate, 0.2% citric acid, 67.3% other inert ingredients, Sino US joint Venture Hebei Daosheng Bairui, Co. Ltd., Shijiazhuang, Hebei), and TriTek (80% mineral oil, Benson Biotechnology Co. Ltd., Huizhou, Guangdong).

Fungicide preparation

The maximal concentration of the label-recommended dose of propiconazole was used as a baseline (1×), and concentrations of 1×, 2×, and 4× were applied, referring to the literature method (Ministry of Agriculture of the PRC, 2010). A treatment without fungicide application was used as a control (CK). To control sheath blight on rice, the recommended dosage of the commercial 250 g/L propiconazole EC is 450 to 900 mL/hm^2; therefore, we selected the highest active ingredient dosage of 225 g/hm^2 and diluted it with water to create standard spray liquid (1 × S = 300 μg/mL) at 750 L/hm^2 for conventional spray and a low-volume spray dose for flight defense (1 × F = 7500 μg/mL) at 30 L/hm^2 for low-volume spray. Therefore, the concentrations of propiconazole for phytotoxicity conventional spray and low-volume spray simulating UAV included 1 × S (300 μg/mL), 1 × F, 2 × F, 4 × F, and 8 × F, where F = 7500 μg/mL.

Simulated UAV spray conditions and spray quality

The experiment was conducted in a greenhouse located at China Agricultural University, Beijing, China, during the night when there was no wind. Rice seeds were sown in plastic pots containing potting soil. When the rice plant reached the three-leaf stage, a small watering can was used to mimic the low-volume spray of a UAV. This was placed 40 cm from the plant canopy. Water-sensitive spray cards (Syngenta Systems Co., Wheaton, USA) were placed at three different positions (heights) to collect the droplets. These positions included the upper (top of the canopy, 40 cm from the spray can), middle (55 cm from the can), and lower (68 cm from the can) parts of the rice plant. The droplet deposition on the sprayed card was photographed. The image was analyzed using the iDAS system (National Research Center of Intelligent Equipment for Agriculture, Beijing, China). The spray quality was examined based on the spectrum of deposition amount point density standard, which was set at ≥ 20 dots/cm^2, and the degree of conformity between the test spray and standard references, as per the Quality Indexes of Agricultural Aviation Operation-Part 1 Spraying Operation MH/T 1002.1-2016 from the Civil Aviation Administration of China in 2016.

Potential phytotoxicity of propiconazole on rice under UAV spray conditions

When the rice plant reached the three-leaf stage, propiconazole was sprayed at concentrations of 1 × F, 2 × F, 4 × F, and 8 × F. Plants were frequently examined for phytotoxicity. After 21 days of spray application, plant height, root length, fresh weight, and dry weight of the plants were determined. Subsequently, the plant samples were dried in an oven (model 101-3A, Tianjin Taisi Instrument Co., Ltd, China) at 70 °C for 3 days.

Surface tension of rice leaves applied with propiconazole and adjuvants

The propiconazole suspension (7500 μg/mL [1 × F]) was supplemented with either Yipinsongzhi, Hongyuyan, Biaopinnongye, AgriSolv-C100, TriTek, or YS-20 at a concentration of 1%, while a control consisting of the propiconazole suspension without any adjuvant was also prepared. Surface tension was measured using the JK99B automatic tensiometer (resolution < 0.05 m/Nm, Powereach, Shanghai, China), and the value was recorded by the Wilhelmy plate method (Wilhelmy 1863; Liu et al. 2021).

Alleviative effects of adjuvants on propiconazole-caused phytotoxicity

To determine the effects of adjuvants on rice growth, various test adjuvants were sprayed onto rice plants at a 1% aqueous suspension. Seedling leaves of rice cultivar Jingliangyou 1125 with similar growth conditions were collected at the three-leaf stage. One percent of Yipinsongzhi, Hongyuyan, Biaopu adjuvant, AgriSolv-C100, and TriTek were uniformly sprayed on the collected leaves. After 21 days of spray, plant height, fresh weight, and dry weight were measured. One of the adjuvants (1%) was added to 2 × F propiconazole. Propiconazole at 2 × F without an adjuvant was used as a control. These prepared chemicals were sprayed onto the leaves of the rice cultivar Jingliangyou 1125. The level of phytotoxicity was determined on the second and fifth days after the spray. Each treatment was replicated three times with 30 plants per replication.

One of the adjuvants was mixed with propiconazole at 1 × F, 2 × F, and 4 × F; the final concentration of all adjuvants was 1%. The treatment without adjuvants was used as a control. Plant height, root length, fresh weight, and dry weight of the plants were measured 21 days after spray. Phytotoxicity index = ∑ (number of toxified plants × level of toxicity)/(total number of control plants × number of the highest toxicity level) × 100.

Abbreviations

CIP	The comprehensive index of phytotoxicity
EC	Mulsifiable concentrate
NIS	Non-ionic surfactants
UAVs	Unmanned aerial vehicles

Acknowledgements
Not applicable.

Author contributions
LJT designed the experiment, developed the protocol, employed the experiment, collected and analyzed the data, and prepared the manuscript. TQG performed the data validation and analyses. AYG performed data curation analysis and visualization. QZZ employed the experiment. MLH used software applications and data analysis. JJH employed the manuscript development and revision. XLL employed the experimental design. PFL investigated and revised methods, overall project management, and manuscript revision.

Funding
This work was funded by Yunnan Province Major Science and Technology Special Plan (202302AE090003).

Availability of data and materials
The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
Not applicable.

Received: 21 April 2023 Accepted: 8 November 2023
Published online: 12 December 2023

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