agronomy

MDPI

Article

Seed Coating with Triflumezopyrim Induces the Rice Plant's Defense and Inhibits the Brown Planthopper's Feeding Behavior

Chanyuan Xi [1,†], Sheraz Ahmad [1,†], Julong Yu [2,†], Jieyu Zhang [1], Yu Chen [1], Guo Zhang [2], Haowen Zhu [1], Linquan Ge [1,*], Xiangyang Yu [3,*] and Zhaolin Shu [2]

1 School of Horticulture and Plant Protection, Yangzhou University, Yangzhou 225009, China; xichauanyuan@163.com (C.X.); dh20010@yzu.edu.cn (S.A.); zjy1151692891@126.com (J.Z.); 771594350@yzu.edu.cn (Y.C.); 1697267220@yzu.edu.cn (H.Z.)
2 Institute of Agricultural Sciences in Hilly Area of Jiangsu Province, Jurong 212400, China; yujulong@126.com (J.Y.); guo871015@126.com (G.Z.); shuzl2005@163.com (Z.S.)
3 Institute of Plant Protection, Jiangsu Academy of Agricultural Science, Nanjing 210014, China
* Correspondence: lqge@yzu.edu.cn (L.G.); yuxy@jaas.ac.cn (X.Y.)
† These authors contributed equally to this work.

Abstract: Triflumezopyrim (TFM), a novel mesoionic insecticide, has been effective in controlling sucking insect pests such as the brown planthopper (BPH). However, the mechanism of TFM as a seed coating agent in paddy fields remains unclear. Here, we investigate the seed germination rates (>80%) and seedling emergence rates (>85%) of rice treated with TFM at 0, 22.5, 45.0, 67.5, and 90.0 g a.i. ha^{-1} with no significant effect on germination rates. In addition, the low TFM residue concentration (0.04 mg. kg^{-1}) is maintained in the rice stem. Meanwhile, the TFM seeds' treatments lead to increased oxalic acid, flavonoids, total phenol, callose contents, and elevated C/N ratio in rice plants at 60 and 90 days after sowing (DAS). The electrical penetration graph (EPG) results indicate that TFM as a seed coating treatment prolongs the non-probing period and inhibits phloem sap ingestion at 90 DAS. Furthermore, the mechanically transplanted rice treated with TFM provides long-term prevention against the BPH infestation. This study demonstrates that seeds treated with TFM play a vital role in controlling the BPH population up to >90%. These results provide a novel valuable control strategy for BPH in the rice fields.

Keywords: trifluemzopyrim; *Nilaparvata lugens*; feeding behavior; rice resistance

Citation: Xi, C.; Ahmad, S.; Yu, J.; Zhang, J.; Chen, Y.; Zhang, G.; Zhu, H.; Ge, L.; Yu, X.; Shu, Z. Seed Coating with Triflumezopyrim Induces the Rice Plant's Defense and Inhibits the Brown Planthopper's Feeding Behavior. *Agronomy* **2022**, *12*, 1202. https://doi.org/10.3390/agronomy12051202

Academic Editors: Zhiqiang Kong and Ran Wang

Received: 31 March 2022
Accepted: 12 May 2022
Published: 17 May 2022

1. Introduction

The brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), is a serious rice pest inflicting damage on a massive scale across Asia [1]. The BPH nymphs and adults cause direct damage by feeding on phloem sap from tillering to milking stages of rice and, in the process transmitting viral pathogens such as rice ragged stunt virus (RRSV) and rice grassy stunt virus (RGSV) [2,3]. Insecticides have become an integral part of agriculture protection, such as in forming land and stored grain and in eradicating infectious diseases transmitting pests [4]. However, overuse of chemical insecticides has consequences. For example, it sharpens planthopper resistance [5], induces resurgence [6], and threatens natural enemies [7]. Thus, new insecticides are urgently needed to control the BPH infestation effectively and keep natural predators intact [6].

Thanks to scientific advances, several recently developed insecticides have shown noticeable results in controlling the pest population; whereas, there are some consequences to the natural predators, such as *Cyrtorhinus lividipennis*. Moreover, the previously developed pyrifluquinazon and pymetrozine inhibit insect feeding by disturbing their behavior and causing starvation [8,9]. Although, numerous studies have revealed that organophosphate

insecticides triazophos and deltamethrin are pyrethroid insecticides that influence BPH reproductive biology and result in reduced fecundity [10,11]. However, insecticides might play a role by altering the nutrients, such as the number of free amino acids, sucrose concentration, changes in the secondary metabolism, physiological-biochemical processes, growth, and development, which indirectly induce pest resurgence [12]. Although the insecticide's combined effects have a role in affecting the locomotor pest's host-finding tendency, feeding behavior ultimately reduces fecundity [13,14].

Triflumezopyrim (TFM), the recently developed insecticide by DuPont Crop protection USA, is a new class of insecticides categorized as mesoionics [15]. The TFM has high efficacy in controlling several planthopper and leafhopper species. Particularly, the TFM has shown enhanced control in both susceptible and resistant strains of BPH that have developed tolerance to imidacloprid [15]. The TFM mainly acts by inhibiting the orthosteric site of the nicotinic acetylcholine receptor (nAChR), deviating from the action of neonicotinoids and other receptor agonists, which stimulates the receptor, leading to over-excitation of the insect nervous system [16]. Furthermore, the TFM and related mesoionic insecticides, having native and expressed nAChRs, compete for the same binding site as imidacloprid. Further, several other studies have unfolded the role of TFM as a BPH population controlling agent in China [17], India [18], and Malaysia [19]. The TFM showed an effective response and high efficiency to rice planthoppers with no toxicity to non-target arthropods. For example, *Atypena formosana* predatory spiders and *Cyrtorhinus lividipennis* feed on BPH eggs and adults in rice paddy fields [20,21].

In response to insect pests, the secondary metabolites (SM) accumulate at an increased level and act as signaling molecules to upregulate the defense responsive genes, which play a vital role in adaptation and defense processes [22]. For example, a study by Liu et al. (2010) reported that the activation of GABA shunt and shikimate-mediated secondary metabolisms was vital for rice plants to resist BPH infestation [23]. Previously, we reported that the free amino acids level, secondary metabolites, and sucrose contents of rice have a crucial role in inducing BPH fecundity [18]. For instance, the TFM 10% coated seeds at 60 DAS substantially lengthened the BPH non-probing period and prevented the phloem intake with a reduced egg-laying capacity [17]. However, it is necessary to understand the underlying mechanism of physiological and biochemical processes induced by TFM post-seed dressing. Additionally, the insectoids influence the biochemistry of several immune regulators, such as oxalic acid and phenolic compounds, that possibly provoke resistance against BPH [24,25]. In the current study, we investigate physiological and biochemical processes, such as secondary metabolism, oxalic acid, flavonoids, total phenol, callose contents, elevated Carbon/nitrogen (C/N) ratio, and residual toxicity in rice plants exposed to TFM via seed dressing in a field-based study. Our study contributes to adapting and applying mesoionic insecticide in future rice production programs.

2. Materials and Methods

2.1. Insects Rearing, Seed Treatment, and Insecticide Application

The colonies of BPH were fed on seedlings of rice plants in the lab chamber at 26 ± 2 °C, with 70 ± 10% relative humidity and 16 Light:8 Dark photoperiods at Yangzhou University. The original colonies of BPH were initially obtained from China National Rice Research Institute, Hangzhou. Seeds of rice Nanjing 9108 were purchased from the Zhenjiang Institute of Agricultural Sciences in the Hilly Area of Jiangsu Province (China). Ten percent TFM SC was obtained from DuPont Agricultural Chemicals Ltd., Shanghai, China. The Nanjing 9108 rice variety (japonica rice) was used for all experiments. The rice Nanjing 9108 variety that is not resistant to BPH is usually planted in Jiangsu Province, China. One kilogram of rice seeds were moved into a plastic mesh bag and kept soaked in water. After 48 h, the seeds were dried (dark treatment) at 25 ± 2 °C for about 24 h.

Subsequently, 1 kg of seeds treated by TFM was transferred to a ready plastic bag (L 60 cm × W 40 cm); then, 10% TFM SC was added according to the following concentrations: 0 g, 3.75 g (22.5 g a.i. ha^{-1}), 7.5 g (45.0 g a.i. ha^{-1}), 11.25 g (67.5 g a.i. ha^{-1}), and 15 g

(90.0 g a.i. ha^{-1}), respectively. The seeds were mixed in the sealed plastic mesh bag for 10 min and then dried at 25 ± 2 °C under a dark treatment. Furthermore, the treated and control seeds were evenly sowed on plastic transplanting trays (60 cm × 30 cm × 3.5 cm) on 16 May 2020, covered with a thin layer of soil to keep the soil moist, and then placed in the climate chamber (25 ± 2 °C with a 16 L: 8 D photoperiod). A small amount of water was poured daily to keep it moist. When the seedlings were at the four-leaf stage after sowing for 25 days, they were mechanically transplanted to paddy fields; 60 and 90 days after sowing, seedlings were at tillering and booting stages, respectively, waiting for further use. The paddy field's location was 119.3111° E, 31.9646° N, consisting of 7500 hectares of rice paddies at the Agricultural Science and Technology Research Center in Zhenjiang Institute of Agricultural Sciences in the hilly area of Julong, Jiangsu Province, China.

2.2. Determination of Germination and Seedling Emergence Rates

Germination tests of rice seeds were a condition in which the day (16 h) was at 22 °C and the night (8 h) was at 18 °C, and the relative humidity was 65 ± 10%. One hundred seeds of randomly selected treated and control were sowed petri dish (D 20 cm) with three layers of soaking filter paper; 5 mL of tap water was in a petri dish. To each petri dish, 3 mL of tap water was added to moisten the filter paper. According to germination determination, a seed with a visible radicle longer than 1mm was identified as germination following the standards [26,27]. Each treatment contained about three hundred rice seeds. The germinated seeds were recorded seven days later, and the germination rate was calculated [28,29]. Each treatment and control have three independent replicates.

$$\text{Germination rate } (100\%) = \frac{\text{Number of germinating seeds (treated)}}{\text{Number of germinating seeds (control)}} \times 100\% \quad (1)$$

One hundred seeds of randomly selected treated and control were evenly sowed on plastic transplanting trays (60 cm × 30 cm × 3.5 cm), covered with a thin layer of earth to keep the soil moist, placed indoors and darkened for 5 days, and then placed outdoors; a small amount of water was poured daily to keep the soil moist. The number of seedlings that emerged was recorded after 15 days, and the seedlings' emergence rate was calculated [27]. At least three biologically independent replicates were conducted for each treated and control group, including the experiments below; more than three replicates were specially noted.

$$\text{Seedling emergence rate } (100\%) = \frac{\text{Number of seedling emergence (treated)}}{\text{Number of seedling emergence (control)}} \quad (2)$$

2.3. LC-MS/MS Method for the Determination of TFM in the Rice Plant Stem

According to the previous study, the extraction methods of TFM in the rice plant stem were followed [30]. A pulverizer (Model: RS-FS150, Royalstar, Hefei, China) was used to crush the rice stem samples dried at 50 ± 2 °C for 14 h. Experimental samples used in each treatment and period were randomly sampled from the mechanically transplanted field. Crushed experimental samples (0.5 g) were collected in a centrifuge tube (50 mL) containing 60% acetonitrile (20 mL) and then kept in a metal bath at 50 °C for 30 min, centrifuged at 6000 rpm for 5 min. The supernatant (1 mL) dissolved with 60% acetonitrile was dried under a gentle nitrogen stream; then, the extract was filtered into a clean sample vial using a PVDF syringe filter (13 mm × 0.22 μm) for further UPLC-MS analysis. The standard TFM was obtained from the Jiangsu Academy of Agricultural Sciences (Jiangsu Agro-product Quality Test Center, Nanjing, China). Two published articles referred to the method of determining TFM residue in rice stems [31]. The UPLC analysis was performed through Agilent 1290 UPLC system, including a diode array detector, a binary pump, a column oven, and an automatic sampler. The oven temperature was adjusted to 40 °C, and 1μL experimental sample was added to the Agilent Eclipse Plus chromatographic columns C_{18} with its dimension parameter 2.1 mm × 50 mm (i.d.), 1.7 μm. The flow rate

was 0.2 mL/min, in which the mobile phase component included two solvents, 0.1% formic acid solution and acetonitrile. The procedure elution gradient (0.1% formic acid solution was marked solvent AS) was as follows: 0–1 min, 95% AS; 1–4.5 min, 95–10% AS; 4.5–6 min, 10–95% AS; 6–6.1 min, 95% AS, 6.1–10 min ended. Next, the fragment ion m/z parameters were set to 399.0/121.0 and 399.0/287.9, using positive ion multiple reaction monitoring modes to analyze TFM, setting the collision energy at 80 V declustering potential at 25 V. Mass spectra were conducted by an AB Sciex 4500 (ESI-MI) assembled with an ESI source. Data achievement and system control were performed by MutiQuant 3.0.2 software (AB Sciex Company, Framingham, MA, USA). According to the published article, we followed the procedure for obtaining the best analyte response, and they are as follows: curtain gas was at 35 psi, ion spray voltage was at 5500 V, source temperature was at 450 °C, and both ion source gas I and ion source gas II were at 40 psi. We turned the interface heater on while setting the collision gas on medium. Furthermore, the ESI was processed with positive ion mode in multiple reactions for monitoring.

2.4. Determination of Oxalic Acid, Flavonoids, Phenolic Compounds, and Callose Content

To reveal the correlation effect between secondary metabolites of rice plants and rice resistance against BPH, oxalic acid, flavonoids, phenolics, and callose contents were determined at 60 and 90 DAS in rice plants. We used the trichloride titanium method to determine the oxalic acid content of rice plant stem mediated by TFM seed coating treatment. Stems (1 g) of tillering rice plants were obtained and weighed 60 and 90 DAS, in which the interception part of stems was between 1 and 15 cm above the soil surface (primary BPH feeding site); then, they were washed and clean with ultrapure-water and placed into a clean flask of 50 mL. Decolorized supernatants by activated carbon were removed from the solution and centrifuged for 5 min, at 6000 rpm, which was repeated three times; then, trichloride titanium was added to experimental samples, and absorbance was measured, referring to the published article [32].

The determination of flavonoid content in moderate rice stems was sheared and dried at 80 °C and homogenized in liquid nitrogen [17,33]. Next, 1 g of homogenates component samples were transferred to a clean centrifugal tube (15 mL), 60% ethanol was added (10 mL), and they were sonicated for 30 min under 350 W. After that, centrifugation of samples took place for 5 min, 10,000× *g*, at 25 °C; this was repeated three times, collecting supernatants. The supernatants were combined into a clean 25 mL volumetric flask, and volume was brought up to 25mL with 60% ethanol; then, 1 mL standards of which was transferred to a new clean volumetric flask of 25 mL, adding 1mL of 5% $NaNO_2$ and 1 mL of 5% $NaNO_2$. The mixture was incubated for 5 min, 1mL of NaOH (1M) was added, and it was adjusted to 25mL using 30% ethanol; then, the mixed sample stood for 15 min, and the value of A_{415} was determined. Flavonoid contents determination was done with a standard curve. Further, each treatment was repeated four times, with four independent biological replicates.

The phenolic content tests were done according to the Folin–Ciocalteu experimental method with a minor modification. First, the stems of tillering rice were sheared and collected, homogenized, adding liquid nitrogen, and blended using tungsten carbide beads and methanol [17]. After that, the suspension was homogenized for 5 min at 30 Hz; 200 µL Folin–Ciocalteu reagent (10% *v*/*v*) and 700 mM of Na_2CO_3 were added and incubated for 2 h, of which 200 µL samples were transferred to a 96-well microplate, measuring the value of A_{765}. Phenols content was calculated according to a standard curve achieved with gallic acid as standard, in which milligrams of gallic acid equivalent per g dry weight was the unit of measurement.

The rice plant's stem callose content was tested referring to a previously published study [34]. The rice stems of 0.5 g were sheared and collected in a centrifuge tube (10 mL) with 98% (*v*/*v*) ethanol (1 mL), standing for 1 h. The excess ethanol was removed by suctioning, transferring rice plant stem samples to a clean centrifuge tube (5 mL) containing 1 mL NaOH (1 M), incubating for 15 min at 80 °C, and then cooling it naturally to room

temperature (25 ± 2 °C), removing particulates by centrifugation. The content of callose was determined with stepwise and aniline blue methods. A Perkin Elmer LS-5B Fluorimeter (Wellesley, MA, USA) was used to obtain the value of absorbance.

2.5. Quantification of Sucrose

The rice plant stem's free amino acids content was measured with ninhydrin according to what was previously described at 60 d and 90 after sowing [12,13]. The spectrophotometer (722 series) was used to measure the values of A_{570} (Analytical Instrument Company, Shanghai, China), and a standard curve with glutamic acid was achieved. Three biological replicates were taken for treated and control. After sowing, a modified anthrone method was used to measure the sucrose content at 60 and 90 days. Furthermore, 20 mg rice plant stem samples were soaked in liquid nitrogen so that they would be crushed and the previously described extraction and quantity methods were used [35].

2.6. Feeding Behavior of BPH Female Adults

The feeding behavior of BPH was recorded by electrical penetration graph (EPG) at 26 ± 2 °C and relative humidity of 80 ± 10% under the condition treated with continuous light. EPG data recording the feeding behavior of BPH female adults was performed with a Giga-8 DC EPG amplifier (Wageningen Agricultural University, The Netherlands) at 90 d after sowing under the condition that the BPH population was abundant in the paddy field [8]. The BPH female feeding on untreated plants and the pre-EPG test recording were performed for 4 and 8 h. Four different effective doses of TFM (22.5, 45.0, 67.5, and 90.0 g a.i. ha^{-1}) were used for the experimental treatments. Firstly, BPH female adults needed to be treated with a soaked piece of cotton for 2 h and 2 DAS. Then, these BPH were settled on the rice plant stems (90 DAS) and treated with the same condition to conduct EPG recording experiments. In the EPG experiment, the dorsal thorax of a BPH female adult was connected by a gold wire with a size 10 cm length ×20 µm diameter, and the other end was attached to the amplifier referred to in a published article [35]. The plant electrode, a copper wire size 10 cm in length × 2 mm diameter, was embedded in the soil. Then, BPH female adults attached to the gold wire were moved to rice plant stems. The amplifier gain was adjusted to 50×, and the voltage was set at −5, and +5 V. EPG signals were performed by PROBE 3.0 software developed by Wageningen Agricultural University. Fifteen independent recordings were recorded and collected for four TFM concentrations, respectively. "The EPG patterns contain seven waveforms as follows NP, non-penetration; N1, initial penetration; N2, salivation and stylet movement; N3, extracellular activity adjacent to phloem; N4-a, intracellular activity in phloem; N4-b, phloem ingestion; and N5, activity in the xylem region" (Figure S1) [36,37].

2.7. Field Surveys and Control Efficiency Analysis

Seedlings derived from seeds treated with TFM at 0, 22.5, 45.0, 67.5, and 90.0 g a.i. ha^{-1} rates were transplanted to the experimental field at the sixth-leaf stage following the Hummel et al. (2014) method [27]. A ridge was applied to prevent water loss between adjacent sections. The surveys were conducted from 61 days to 130 days after sowing from 16 July to 23 September 2020 and surveyed every 7 days. Each treatment contained five plots, and twenty plots/hills were investigated, in which 100 hills in total were counted in our experimental paddy field for each period. Furthermore, for each treatment, five biological independent replicates were conducted to ensure efficiency.

$$\text{Control efficiency } (100\%) = \frac{\text{Number of rice planthoppers (control)} - \text{Number of rice planthoppers (treated)}}{\text{Number of rice planthoppers (control)}} \times 100\% \quad (3)$$

2.8. Statistical Analyses

Firstly, the data were tested for equal variance and normality according to the Bartlett test before analyzing variance (ANOVA). Furthermore, one-way ANOVA was applied, followed by Turkey's multiple comparison tests for most of the figures, in which only

Figure 6A,D were analyzed using two-way ANOVA. Additionally, multiple comparisons of the means were analyzed using the Fisher-protected least significant difference. Furthermore, the values were expressed as means ± SEM, and the *p*-value ($p < 0.05$) represented a significant difference. Statistical analysis was performed using the DPS data processing system [38]. Statistical analysis data are shown (Table S1).

3. Results

3.1. Effects of TFM as Seed Coating Treatment on Germination and Seedling Emergence Rates

To explore the effects of TFM as seed coating treatment on germination and seedling emergence rates, four different TFM concentrations were used compared with the uncoated seeds. The experimental results revealed no significant reduction in germination rate at 7 days after the seed was coated with TFM (Figure 1A). Similarly, no significant reduction in seedling emergence was recorded between the coated and uncoated TFM at 15 days (Figure 1B). This study indicated that seeds coated with TFM did not influence seed germination and seedling emergence rates.

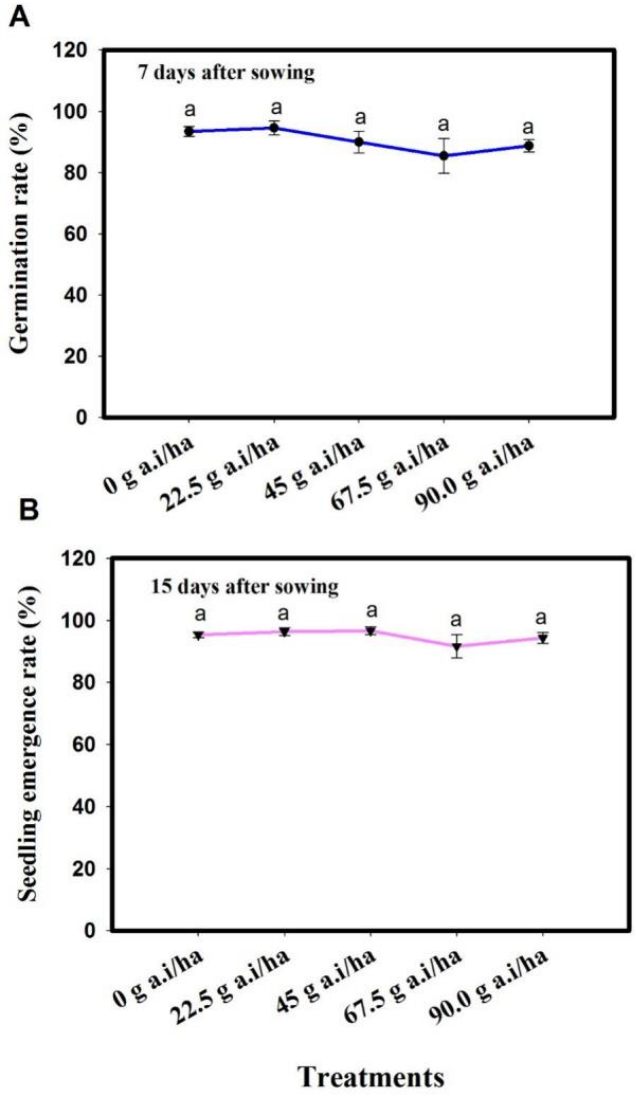

Figure 1. Effects of germination and seedling emergence rates mediated by seeds coated with TFM. (**A**) Germination of treatment seeds coated by TFM and control group. (**B**) The emergence of seedlings derived from the seeds coated with TFM and the control group. Means with the same letter represent no significant difference (Fisher's test, $p < 0.05$).

3.2. TFM Residual Dynamic of Rice Plant Stem Grown via Seed Coated with TFM

Our results indicated the TFM residue contents significantly reduced between 61 and 130 days after sowing (DAS) in the TFM coated treatment (four concentrations: 22.5, 45.0, 67.5, and 90.0 g a.i. ha^{-1}) in 2020 (Figure 2A–D). From 61 to 82 DAS, the TFM residue contents in TFM treatment (four concentrations) significantly decreased (Figure 2A–D); however, from 90 to 130 days, the values of TFM (four concentrations) residue contents showed no significant difference. The TFM residue content remained comparatively stable by 0.04 mg. kg^{-1} at 82 DAS (Figure 2).

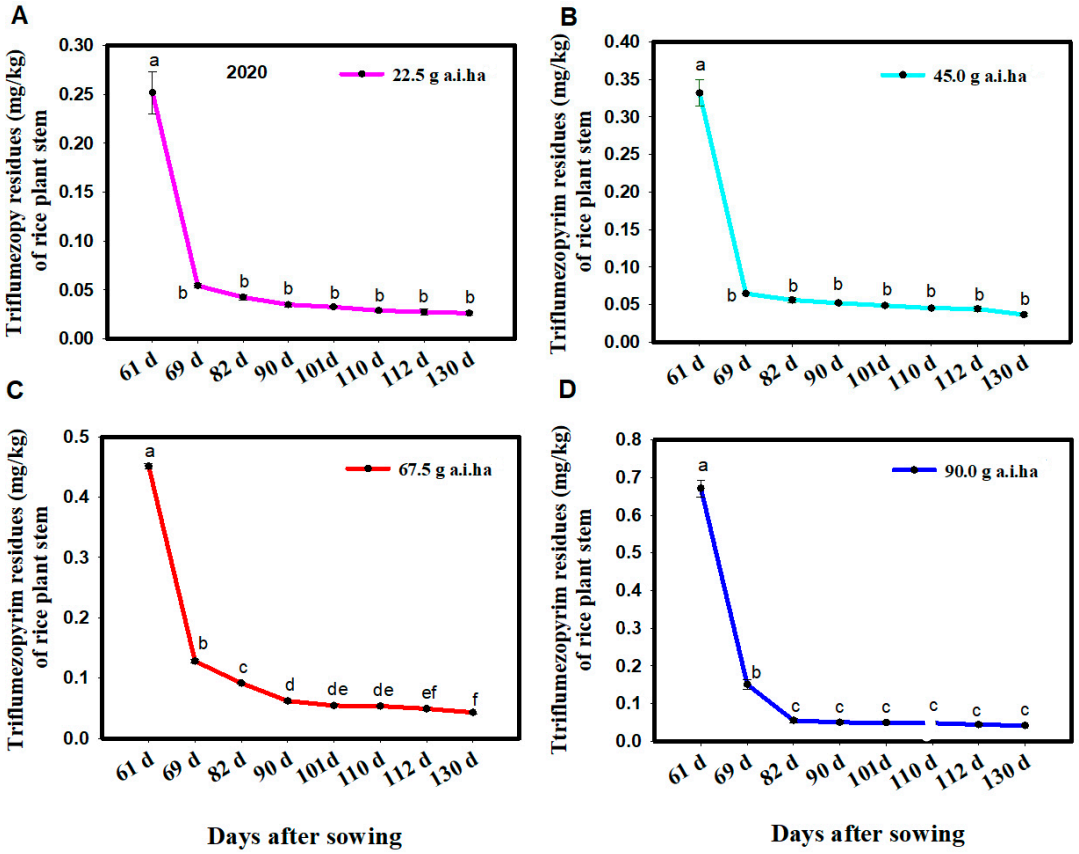

Figure 2. The TFM residue contents of rice plant stem of four different TFM concentrations seed-coating treatments from 61 days after sowing to 130 days after sowing. (**A–D**) represent the residue content of rice stem of four different TFM concentration seed-coating treatments; values are shown as means ±SEM (N = 3). Means with the same letter represent no significant difference (Fisher's test, $p < 0.05$).

3.3. TFM as Seed Coating Treatment Affects Rice Plant's Resistance and Nutrition

Our data showed that oxalic acid production was 26% and 37%, 51% and 75%, 158 and 174%, 163%, and 185% higher in comparison to untreated control when seeds were coated with 22.5, 45.0, 67.5, and 90.0 g a.i. ha^{-1} TFM at 60 and 90 DAS, respectively (Figure 3A). Flavonoids were 58% and 82%, 170% and 175%, 289%, and 256%, and 271% and 264% higher than the untreated control when seeds were coated with 22.5, 45.0, 67.5, and DAS, respectively (Figure 3B). Phenol's content was 89% and 29%, 166% and 128%, 259% and 221%, and 262% and 213% higher than the untreated control when seeds were coated with 22.5, 45.0, 67.5, and 90.0 g a.i. ha^{-1} TFM at 60 and 90 DAS, respectively (Figure 3C). Callose production was 60% and 61%, 129% and 131%, 213% and 204%, and 245% and 231% higher than untreated control when seeds were coated with 22.5, 45.0, 67.5, and 90.0 g a.i. ha^{-1} TFM at 60 and 90 DAS, respectively (Figure 3D). However, the mean contents of oxalic acid (Figure 3A), flavonoids (Figure 3B), phenols (Figure 3C), and callose (Figure 3D) showed no

significant difference between 60 and 90 DAS. The mean contents of oxalic acid, flavonoids, phenols, and callose significantly differed among four concentrations (Figure 3A–D). No interaction between days after sowing and concentrations (Figure 3A–D) was observed. These results demonstrated that secondary metabolites of the rice plant played a key role in enhancing the rice resistance against BPH.

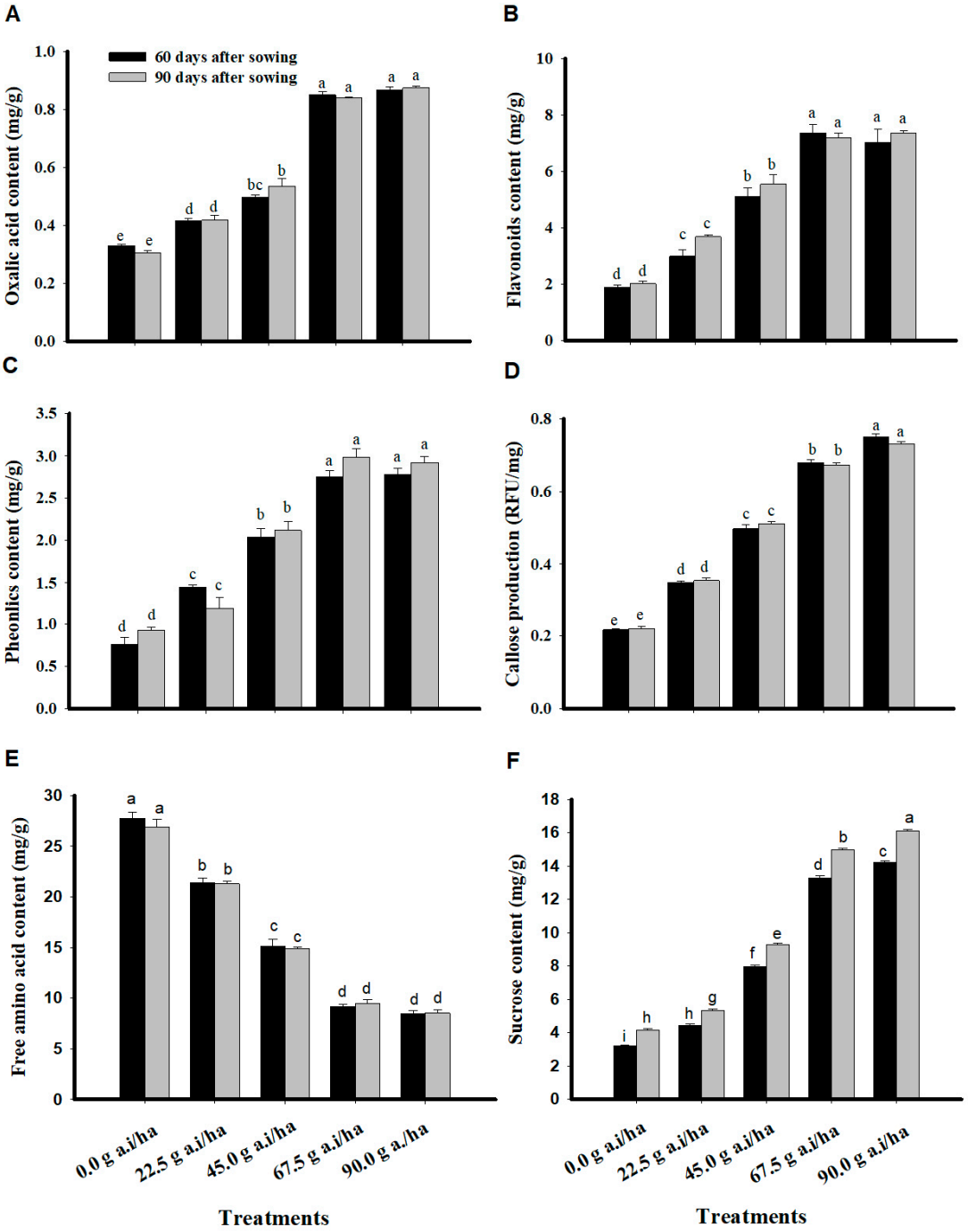

Figure 3. Dynamics of secondary metabolites and nutrition of rice plants (their seeds coated with TFM) at 60 days and 90 days after sowing. Black columns represent the values at 60 days, and gray columns represent the values at 90 days. (**A**–**F**) represent the change contents of oxalic acid, flavonoids, phenolics, callose, free amino acid, and sucrose, respectively. Each treatment contains three biological replicates. Different lowercase letters mean significant differences. Means with the same letter indicate no significant differences (Fisher's test, $p < 0.05$).

Seeds coated with TFM resulted in a reduced free amino acid level and elevated sucrose contents at 60 and 90 DAS in rice plants (Figure 3E, F). Free amino acid content was 22.9% and 20.9%, 45.6% and 44.7%, 66.8% and 64.8%, and 69.4% and 68.3% lower than untreated control (Figure 3E), respectively. Sucrose content was 37.3% and 28.5%, 148% and 123%, 314% and 260%, and 342% and 287% higher than untreated control (Figure 3F), respectively. The mean content of free amino acid in rice plants was not significantly different between 60 and 90 DAS, except for the mean sucrose content. The mean contents of free amino and sucrose in the rice plant differed significantly among four different concentrations. No interactions between days after sowing and concentration were observed.

3.4. TFM as Seed Coating Treatment Affects BPH Female Feeding Behavior

To explore whether seeds coated with TFM affect the BPH female feeding behavior, an electrical penetration graph (EPG) was used to record the complete process of BPH feeding behavior (Figure S1). The recording period on plants was 8 h monitored by EPG and TFM as seed coating treatment at 90 DAS. Here, all the experimental insects were BPH female adults.

The EPG data showed that the np waveform duration was 84.7 ± 4.5 min in the control (untreated TFM) group. The total duration of the np waveform derived from seeds treated with TFM (four concentrations: 22.5, 45.0, 67.5, and 90 g a.i. ha^{-1}) was 66%, 126%, 188%, and 198% higher than the untreated control (Figure 4A). Nevertheless, the number of np occurrences showed no significant differences among treatments and the control group (Figure 5A). These data indicated the np waveform was prolonged when seeds were coated with TFM and had no effect on a number of np rates. The N1 waveform occurrence number with TFM treatments (four concentrations above) was significantly reduced compared with the control group; the relatively reduced percentage was 11%, 37%, 62%, and 61%, respectively (Figure 5B). These results indicated that the number of BPH female adults piercing rice plant tissue was significantly reduced in TFM coating treatment. The total duration of N2 waveforms (Figure 4B) was lower than the untreated control (21%, 40%, 56%, and 66%), and the number of N2 occurrences (Figure 5C) was also lower than the untreated control (25%, 51%, 53%, 55%), respectively. The N3 waveforms' entire duration and the number of occurrences was 14% and 29%, 28% and 45%, 43% and 57%, 45%, and 51% lower on rice treated with 22.5, 45.0, 67.5, and 90.0 g a.i. ha^{-1} (Figures 4C and 5D) as compared to the untreated control. These results showed that the moving route of the BPH stylet tip from the extracellular location to the phloem of the rice plants was prevented. The total duration of the N4-a waveform was 26%, 48%, 68%, and 69% lower than the untreated control (Figure 4D), and the number of N4-a occurrences was 37%, 47%, and 46% lower on rice treated with TFM (four concentrations above) (Figure 5E), respectively. The entire duration of the N4-b waveform was 21%, 44%, 72%, and 73% lower than the untreated control (Figure 4E), and the number of N4-b occurrences was 30%, 48%, and 52% lower on the rice plant treated TFM (four concentrations above) (Figure 5F), respectively. The EPG data of N4-a and N4-b indicated that seeds coated with TFM significantly suppressed phloem sap ingestion for BPH female adults. No notable differences were recorded in the N5 waveform's entire duration (Figure 4F) and the number of N5 occurrences (Figure 5G) between the control and the seed coated with the TFM rice plant.

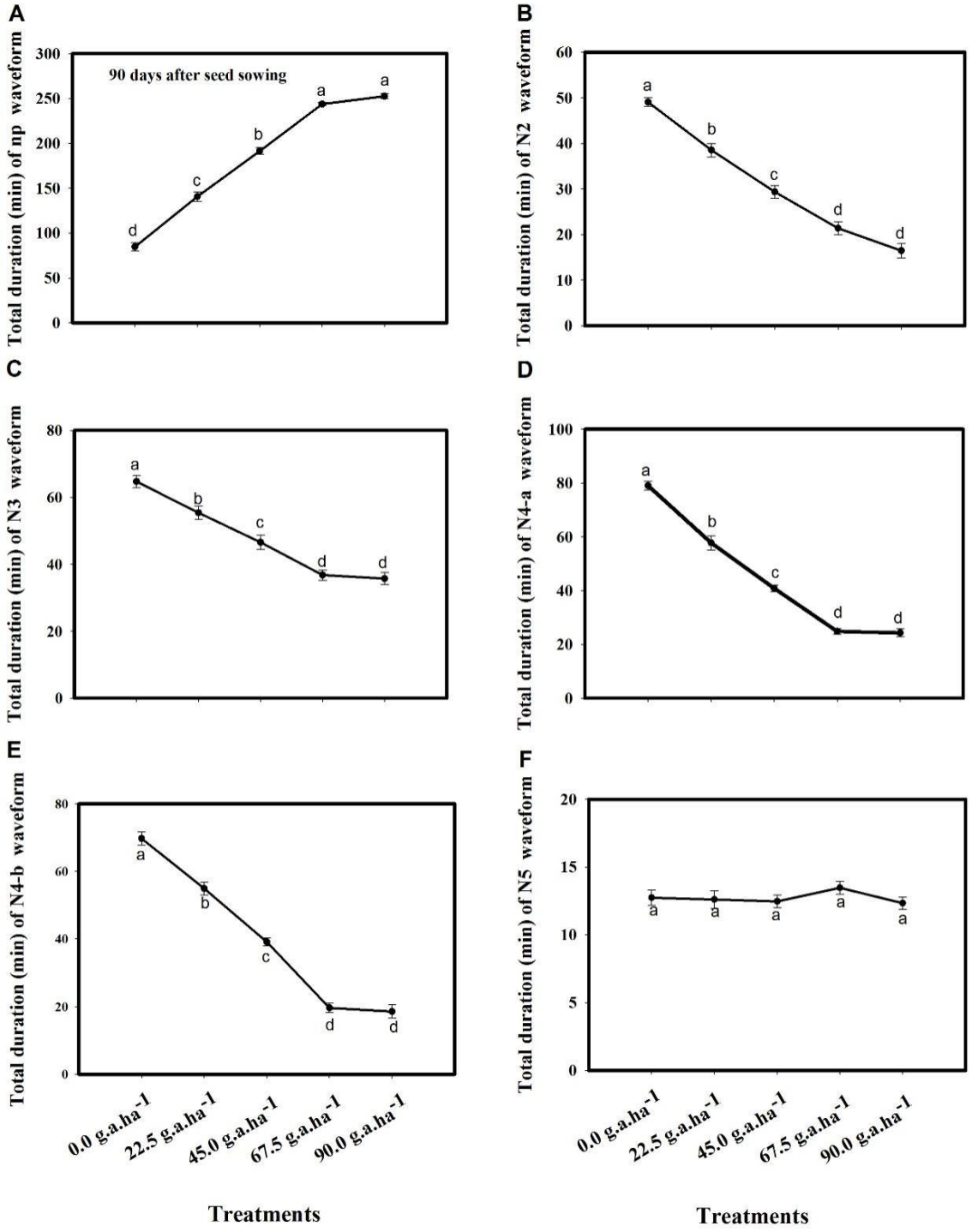

Figure 4. Duration of electrical penetration graph (EPG) waveforms associated with BPH female adults sucking rice seedlings derived from seeds coated with TFM (0, 22.5, 45, 67.5, and 90 g a.i. ha^{-1}) 90 days after sowing. (**A**–**F**) represent the total duration of waveforms NP, N2, N3, N4-a, N4-b, and N5, respectively. Fifteen independent biological replicates were conducted for the EPG experiment. Different lowercase letters mean significant differences. Means with the same letter indicate no significant differences (Fisher's test, $p < 0.05$).

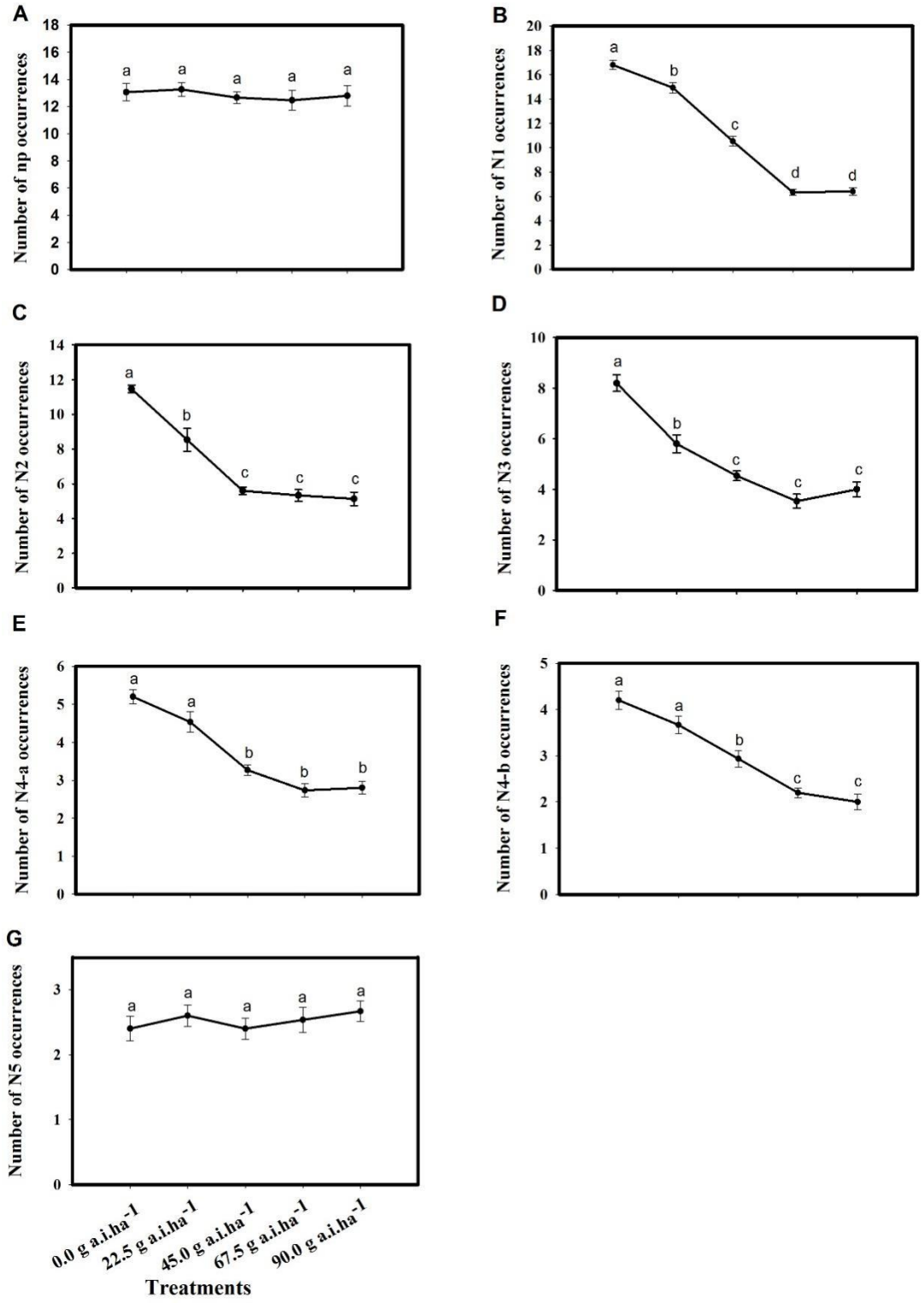

Figure 5. Number occurrences of electrical penetration graph (EPG) waveforms associated with BPH female adults sucking rice seedlings derived from seeds coated with TFM (0, 22.5, 45, 67.5, and 90 g a.i. ha^{-1}) 90 days after sowing. (**A**–**G**) represent the number of occurrences of waveforms NP, N1, N2, N3, N4-a, N4-b, and N5, respectively. Fifteen independent biological replicates were conducted for the EPG experiment. Different lowercase letters mean significant differences. Means with the same letter indicate no significant differences (Fisher's test, $p < 0.05$).

3.5. TFM as Seed Coating Treatment Affects the BPH Population in the Mechanically Transplanted Rice Fields

To ensure the impacts of TFM as seed coating treatments on the BPH population in mechanically transplanted rice in paddy fields, the number of BPH per 100 hills of rice was counted from 61 to 130 DAS from 16 July to 23 September 2020 (Figure 6). The results showed that the rice planthoppers per 100 hills were approximately 460~3155 rice planthoppers from 61 to 130 DAS in the untreated control rice paddy fields and 30~100 rice planthoppers in the seed coated with TFM (four concentrations in Section 3.4) rice field. Seeds coated with TFM led to excellent control efficiency, beyond 92% compared to the untreated control in the mechanically transplanted rice in fields, except for a low dose of 22.5 g a.i. ha^{-1} at 110 DAS (Table 1) from 61 days to 130 days.

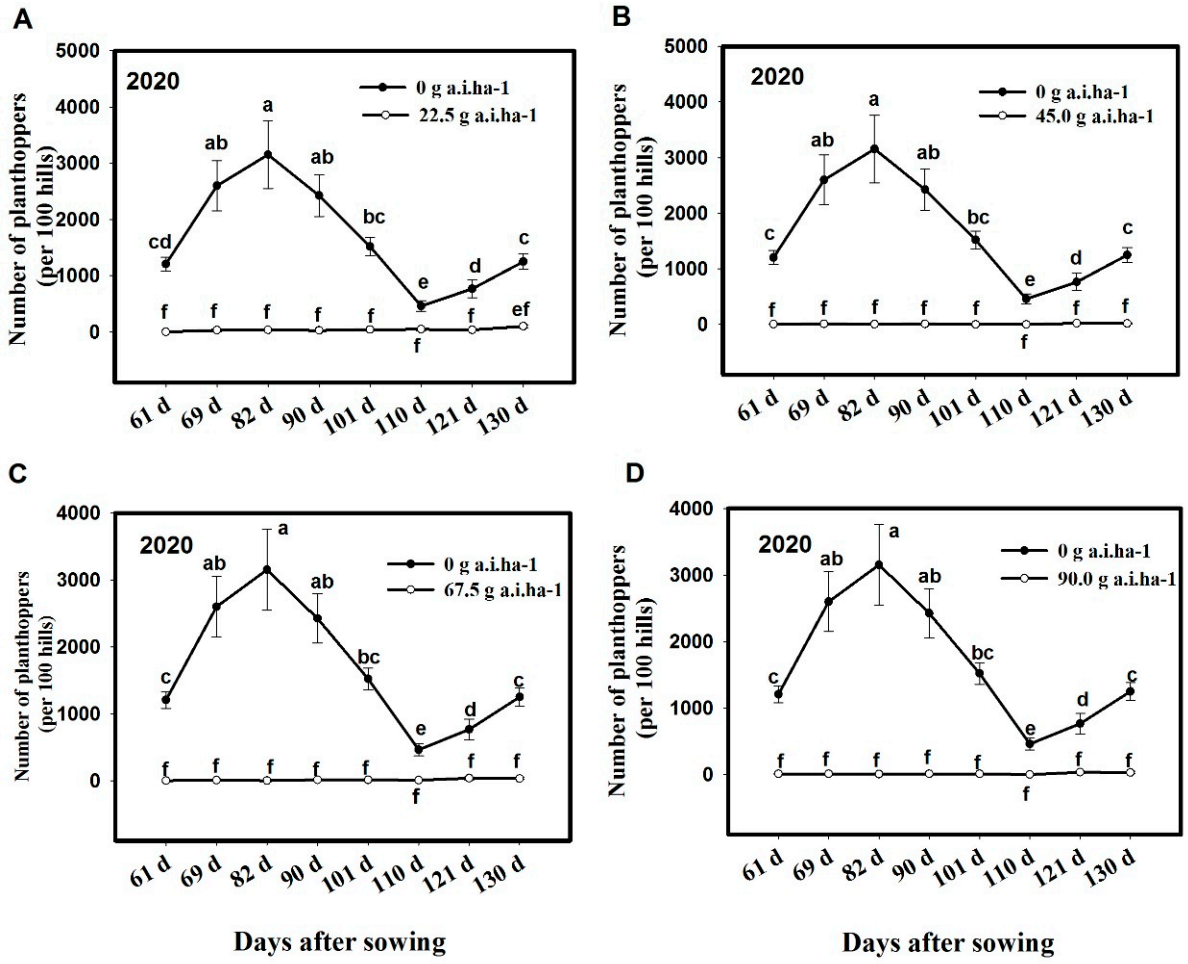

Figure 6. The number of rice planthoppers per 100 hills of rice. (**A**–**D**) represent the dynamic changes in the number of rice planthoppers, respectively. A sketch map was used to compare the four index curves obtained. Five independent biological replicates were conducted for each treatment and control. Means with the same letter represent no significant difference (Fisher's test, $p < 0.05$).

Table 1. The control efficiency of seeds coated with TFM on the number of rice planthoppers in mechanically transplanted rice plants in the rice paddy.

TFM Concentrations (g a.i. ha^{-1})	Control Efficiency (%)							
	Days After Sowing							
	61 d	69 d	82 d	90 d	101 d	110 d	121 d	130 d
22.5	100 ± 0.0a	99.2 ± 0.6a	98.2 ± 1.2a	99.0 ± 0.5a	97.2 ± 0.6b	76.2 ± 1.7b	94.2 ± 2.2a	92.1 ± 1.9b
45	99.6 ± 0.5a	99.4 ± 0.6a	99.8 ± 0.2a	99.6 ± 0.3a	100 ± 0.0a	100 ± 0.0a	94.3 ± 3.7a	98.5 ± 0.7a
67.5	100 ± 0.0a	99.9 ± 0.1a	100 ± 0.0a	99.6 ± 0.3a	99.2 ± 0.8ab	98.9 ± 1.1a	93.4 ± 5.3a	97.7 ± 1.1ab
90	99.2 ± 0.5a	99.4 ± 0.4a	99.8 ± 0.2a	99.6 ± 0.3a	99.4 ± 0.4a	100 ± 0.0a	96.5 ± 1.8a	97.1 ± 2.1ab

Notes: Five independent biological replicates were conducted for each treatment. Different lowercase letters mean significant differences. Means with the same letter indicate no significant differences (Fisher's test, $p < 0$).

4. Discussion

BPH infestation causes huge economic damage to rice farmers across Asia. To control BPH infestation, farmers use insecticides. However, overuse of insecticide causes environmental damage and insecticide resistance in BPH and disturbs the population of natural predators [29]. Furthermore, the insecticide's side effects threaten natural enemies, stimulate pest reproductive parameters, and cause insecticide-induced susceptibility of rice to BPH [38]. Additionally, the application of insecticides causes physiological changes in the rice plant that later affect the overall physiology of BPH [13]. However, the latest insecticide TFM at 22.5, 45.0, 67.5, and 90.0 g a.i. ha^{-1} concentration was used in rice paddy fields, and it enhanced rice protection and reduced the BPH population by approximately 90% [27]. Similarly, another indoor study showed that the TFM seed coating treatment improved rice plant resistance and influenced BPH feeding behavior with a reduced egg-laying capacity at 60 days after sowing (DAS) [17]. In comparison, our study showed that TFM 10% coated seeds maintained the same low-level residual concentration in the rice plant stem at 69–130 DAS, enhanced the BPH-associated resistance and reduced the free amino acids, and elevated the C/N ratio. Based on our obtained results (Table 1), we can recommend the TFM 10% at the rate of 22.5~45.0 g a.i. hm^{-1} to reduce the BPH damage up to 90%.

Imidacloprid or thiamethoxam insecticide seed coating with a low concentration can inhibit seed germination in oil palm and onion [39,40]. Imidacloprid as a seed dressing reduced rice plant seedlings' shoot/root ratio and showed enhanced anti-aphid characteristics. These studies indicate that some neonicotinoid insecticides might not be suitable for seed coating. However, our study showed that seed germination and seedling emergence rates were not influenced by TFM treatments (Figure 1). This suggests that the TFM as a seed coating is a safe option and could be promoted in rice cultivation.

It has been found that the insecticide seed coating treatment enhances the plant's defence against pests via induction in secondary metabolites [41]. The BPH feeds on rice phloem, which affects the chemistry of several immune regulators, such as oxalic acid and phenolic compounds that possibly provoke resistance against BPH [42].

Oxalic acid is widely distributed in plants, animals, bacteria, and fungi; several studies have demonstrated that oxalic acid plays essential roles in plants, such as being a defense against herbivory [24]. Further, oxalic acid is an effective inhibitor in aliphatic organic acids, disturbing BPH sucking activity [43]. Previous studies showed increased oxalic acid contents with TFM seed coating at 45.0, 67.5, and 90.0 g a.i. ha^{-1} at 60 DAS indoors [16]. However, the present study showed the oxalic acid elevated level of 22.5, 45.0, 67.5, and 90.0 g a.i. ha^{-1} at 60-90 DAS in rice, cultivated in paddy fields. We infer that oxalic acid is a key factor affecting insect feeding behavior by hindering extracellular activities adjoining the phloem (N3) and intracellular activities in the phloem (N4-a).

A more resistant approach is the biochemical defensive pathways induction in plants through salicylic acid, primarily induced by microbial pathogens, and Jasmonic acid, primarily induced by insects [44]. Furthermore, the signaling molecules activate gene regulatory proteins in the induction process, leading to defensive molecule synthesis. For

instance, the methyl jasmonate activates the ORCA3 transcription factor from *Catharanthus roseus*, which contributes to the production of terpenoid indole alkaloids [45]. Recently, in *Arabidopsis thaliana*, the Anthocyanin Pigment 1 (PAP1) gene was produced, an MYB transcription factor that activates the phenylpropanoid biosynthetic pathway [46]. Constituent overexpression of the PAP1 gene results in purple pigmentation in most plants [46]. "Because the phenylpropanoid compounds increased by PAP1 overexpression are associated with insect resistance" [47]. In line with that, a recent study observed increased flavonoid contents with TFM seed treatment compared with the untreated control, resulting in a significantly inhibited BPH feeding behavior [17].

Phenolics are secondary metabolites synthesized and accumulated in the sub-epidermal layers of plant tissues exposed to stress and pathogen attack [48]. The phenolics play the primary role in the plant's defense against various biotic and abiotic stresses, such as antecedent, antioxidant, antiapoptosis, anti-aging, anticarcinogen, anti-inflammation, antiatherosclerosis, inhibition of angiogenesis, and cell proliferation activity [49]. For instance, in the Strawberry *Fragaria vesca L.*, the spider mites *Tetranychus urticae* showed a repulsion effect on the plants with an elevated level of catechol-based phenolics [50]. Rani and Pratyusha (2014) reported that the feeding behavior and growth development of *Achaea Janata* and *Spodoptera litura* were significantly interfered with by phenolic compounds in castor beans; phenolic resulted in induced defense [51]. The phenolic compounds were shown to be the feeding deterrents to BPH in rice plants and positively correlated with rice plant resistance [52]. In addition, most of the resistant rice landraces were observed with an increased percentage of phenolic contents, which were corroborated by our findings.

Callose, a polysaccharide, accumulated around the edge of each sieve, functioning as a resistance or defense of plants toward pests [53]. The cell wall containing callose appositions, known as papillae, are highly effective barriers induced at attacking sites in the relatively early stages of pathogen invasion, thus preventing BPH from phloem sucking [54]. Maag et al. [55] stated that increased levels of secondary metabolites, such as callose and their deposition, trigger the host plant defence system, which plays a dual role in deterring pathogens and attracting natural enemies. Alternatively, the flavonoids and phenolics may be responsible for the poor feeding rate and prolonged non-probing phase, which may be due to the presence of antifeedants or lack of phagostimulant. The callose accumulation may form a physical barrier, thereby prolonging the BPH probing time and enhancing rice resistance. He et al. [8] found that pymetrozine treated rice plants might be distasteful to BPH, observed by a drastic increase in the NP period of electropenetrography (EPG) decreased phloem ingestion. The TFM works by desensitizing the nicotinic acetylcholine receptor [19]. However, pymetrozine interferes with neuroregulation and triggers feeding termination for prick-suck insects, resulting in starvation [8].

Furthermore, several studies have reported that the insects' feeding behavior is affected by insecticides, such as the green peach aphid *Myzus persicae* [56], and tobacco whitefly (*Bemisia tabaci*) [57], and rice planthoppers (*N. lugens*) [8]. This suggests that both internal and external changes of the host plant are responsible for changing feeding behavior. The precise molecular mechanism affecting insect feeding behavior mediated by secondary metabolism and their underlying mechanism remains unclear and needs further study.

Insects require nitrogen to maintain basic physiology, growth, and development, primarily obtained from free amino acids [28,58]. Furthermore, nitrogen nutrition regulation directly affects insects' feeding behavior by modulating their feeding rate, feeding amount, feeding time, and feeding site. Although, the TFM influences the elevated C/N ratio in rice plants or decreases free amino acids, which reduces BPH feeding and, ultimately fecundity [17].

In comparison, our results showed that TFM coated seed reduced free amino acid concentration and high sucrose content than untreated controls of rice stem in paddy fields. Furthermore, we demonstrated that the elevated C/N ratio in rice plants might reduce BPH feeding, leading to a significantly decreased BPH population. In addition, Rashid et al. (2016) [26] found that rice volatiles impedes BPH feeding and choosing oviposition site

preference. Besides nitrogen (N), other elements, such as phosphorus (P), potassium (K), ATP, and nucleic acid synthesis, are required by the herbivores for normal physiological activities; growth and development might be altered by TFM, which requires further study. The primary nutrients play a crucial role in nymph survival and egg hatching of BPH. The synergistic relationship between nutrients and the higher feeding rate of BPH, possibly owing to ready-made succulence in leaf sheath using N content, may affect rice plant biochemistry and play an essential role in resistance [57]. Further study is needed on the effect of volatiles produced by TFM treated with rice plants on BPH feeding behavior.

The TFM sublethal concentration (LC_{30}) has no obvious effects on BPH egg hatchability, the longevity of the F0 and (F1) generations, and fecundity [58]. Our findings (Figure 2) showed that the TFM residues in the rice stem remained at a relative level of 0.04 $mg.kg^{-1}$ at 82 DAS; the BPH began to occur in large numbers in the paddy field. This suggests that the lower sublethal dose has no effects on BPH fecundity. Using the rice-stem drip method, the TFM LC50 value is 0.064 mg/L against the BPH third instar nymphs susceptible strain [30,59]. Our field survey data indicated that a smaller rice planthopper population was observed in the seed coated with 22.5, 45.0, 67.5, and 90 g a.i. ha^{-1} TFM concentrations in the paddy field compared with the untreated control (Figure 6A–D) at 61 to 131 DAS, which showed high control efficiency (Table 1). However, the BPH populations of control and TFM treated groups (concentrations 22.5, 45.0, 67.5, and 90 g a.i. ha^{-1}) have no significant differences at 110 days to 121 days less than the base number of the BPH population in the paddy field. We infer that the rice planthopper population is inhibited due to high TFM residual toxicity and elevated C/N ratio. Furthermore, the enhanced secondary metabolites, including oxalic acid, flavonoids, phenolics, and callose, have an elevated C/N ratio in the earlier stage of rice growth at 61 and 82 DAS.

The TFM has been demonstrated to be harmless on BPH predatory spiders, such as *Pirata subpiraticus*, *Pardosa pseudoannulata*, *Ummeliata insecticeps*, *Theridion octomaculatum*, and *Hylyphantes graminicola* [20]. A population of these spiders will help to avoid expanding BPH populations in the middle and later stages of rice plant growth at 82–130 DAS. Along with the enhanced insect resistance, there was a C/N ratio of rice in paddy fields. The underlying mechanism of how TFM coated seed treatment effectively controls rice planthoppers needs to be further studied.

5. Conclusions

Seed coating with TFM showed a low residue concentration in the middle and later phases of rice plants, in line with enhanced resistance substances associated with BPH, such as oxalic acid, flavonoids, total phenol, callose contents, and elevated levels of C/N ratio in the rice plant stem at 60 and 90 DAS. Furthermore, the electrical penetration graph (EPG) data revealed that seed coating with TFM treatments extended the non-probing period of BPH and inhibited the duration of phloem sap ingestion at 90 DAS. In addition, the field survey data showed that seed coating with TFM exhibited a long duration controlling efficiency against rice planthoppers, particularly BPH in mechanically transplanted rice paddy fields. Taken together, we infer that TFM is a seed coating treatment with effective control efficiency and long duration to prevent rice planthoppers population infestation on rice plants due to enhanced resistance substances and elevated C/N ratio combined with high TFM residual toxicity in the early stage of rice plants. It then enhanced resistance substances, elevated the C/N ratio, and increased the abundance of natural enemies in the middle and later stages of rice plants, resulting in reduced using times of TFM for rice planthoppers occurrences. Meanwhile, seed coating with the low dose TFM (22.5~45.0 g a.i. hm^{-1}) effectively controlled rice planthoppers in paddy fields, which did not exceed the TFM spaying dosage in the paddy field. TFM demonstrated reasonable control of BPH when used as a seed coating, thus introducing a new approach for effectively controlling BPH in rice paddy fields.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12051202/s1, Table S1: Significance statistics of figures. Figure S1: represents the EPG experiment.

Author Contributions: L.G. and Z.S. designed the experiment. L.G., C.X., X.Y. wrote the main manuscript text and J.Y., Y.C., G.Z., J.Z., S.A. and H.Z. performed data analysis and prepared all figures. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Natural Science Foundation of China (32072415), the Agricultural Science and Technology Independent Innovation Project of Jiangsu Province (CX (20) 3123) and Yangzhou University Interdisciplinary Research Foundation for Agronomy Discipline of Targeted Support (yzuxk202005).

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

References

1. Hu, G.; Lu, F.; Zhai, B.-P.; Lu, M.-H.; Liu, W.-C.; Zhu, F.; Wu, X.-W.; Chen, G.-H.; Zhang, X.-X. Outbreaks of the brown planthopper *Nilaparvata lugens* (Stål) in the Yangtze River Delta: Immigration or local reproduction? *PLoS ONE* **2014**, *9*, e88973. [CrossRef] [PubMed]
2. Du, B.; Zhang, W.; Liu, B.; Hu, J.; Wei, Z.; Shi, Z.; He, R.; Zhu, L.; Chen, R.; Han, B.; et al. Identification and characterization of Bph14, a gene conferring resistance to brown planthopper in rice. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 22163–22168. [CrossRef] [PubMed]
3. Cabauatan, P.Q.; Cabunagan, R.C.; Choi, I.R. Rice viruses transmitted by the brown planthopper Nilaparvata lugens Stål. In *Planthoppers: New Threats to the Sustainability of Intensive Rice Production Systems in Asia*; International Rice Research Institute: Los Baños, Philippines, 2009; pp. 357–368.
4. Fahad, S.; Nie, L.; Hussain, S.; Khan, F.; Khan, F.A.; Saud, S.; Muhammad, H.; Li, L.; Liu, X.; Tabassum, A. Rice pest management and biological control. In *Sustainable Agriculture Reviews*; Springer: Berlin, Germany, 2015; pp. 85–106.
5. Wang, Y.; Chen, J.; Zhu, Y.C.; Ma, C.; Huang, Y.; Shen, J. Susceptibility to neonicotinoids and risk of resistance development in the brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae). *Pest. Manag. Sci.* **2008**, *64*, 1278–1284. [CrossRef] [PubMed]
6. Zhang, Y.X.; Zhu, Z.F.; Lu, X.L.; Li, X.; Ge, L.Q.; Fang, J.C.; Wu, J.C. Effects of two pesticides, TZP and JGM, on reproduction of three planthopper species, *Nilaparvata lugens* Stål, *Sogatella furcifera* Horvath, and *Laodelphax striatella* Fallén. *Pestic. Biochem. Physiol.* **2014**, *115*, 53–57. [CrossRef]
7. Zhang, X.; Xu, Q.; Lu, W.; Liu, F. Sublethal effects of four synthetic insecticides on the generalist predator *Cyrtorhinus lividipennis*. *J. Pest. Sci.* **2015**, *88*, 383–392. [CrossRef]
8. He, Y.; Chen, L.; Chen, J.; Zhang, J.; Chen, L.; Shen, J.; Zhu, Y.C. Electrical penetration graph evidence that pymetrozine toxicity to the rice brown planthopper is by inhibition of phloem feeding. *Pest. Manag. Sci.* **2010**, *67*, 483–491. [CrossRef]
9. Kang, M.A.; Seo, M.J.; Hwang, I.C.; Jang, C.; Park, H.J. Insecticidal activity and feeding behavior of the green peach aphid, *Myzus persicae*, after treatment with nano types of pyrifluquinazon. *J. Asia-Pacif. Entomol.* **2012**, *15*, 533–541. [CrossRef]
10. Ge, L.Q.; Wu, J.C.; Zhao, K.F.; Chen, Y.; Yang, G.Q. Induction of Nlvg and suppression of *Nljhe* gene expression in *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) adult females and males exposed to two insecticides. *Pestic. Biochem. Physiol.* **2010**, *98*, 269–278. [CrossRef]
11. Zhu, Z.F.; Cheng, J.; Lu, X.L.; Li, X.; Ge, L.Q.; Fang, J.C.; Wu, J.C. Comparisons of topical and spray applications of two pesticides, triazophos and jinggangmycin, on the protein content in the ovaries and fat bodies of the brown planthopper *Nilaparvata lugens* Stål (Hemiptera: Delphacidae). *Pestic. Biochem. Physiol.* **2014**, *114*, 97–101. [CrossRef]
12. Wu, J.C.; Xu, J.X.; Yuan, S.Z.; Liu, J.L.; Jiang, Y.H.; Xu, J.F. Pesticide-induced susceptibility of rice to brown planthopper *Nilaparvata lugens*. *Entomol. Exp. Appl.* **2001**, *100*, 119–126. [CrossRef]
13. Haynes, K.F. Sublethal effects of neurotoxic insecticides on insect behavior. *Annu. Rev. Entomol.* **1988**, *33*, 149–168. [CrossRef] [PubMed]
14. Ge, L.-Q.; Wang, L.-P.; Zhao, K.-F.; Wu, J.-C.; Huang, L.-J. Mating pair combinations of insecticide-treated male and female *Nilaparvata lugens* Stål (Hemiptera: Delphacidae) planthoppers influence protein content in the male accessory glands (MAGs) and vitellin content in both fat bodies and ovaries of adult females. *Pestic. Biochem. Physiol.* **2010**, *98*, 279–288. [CrossRef]
15. Cordova, D.; Benner, E.A.; Schroeder, M.E.; Holyoke Jr., C.W.; Zhang, W.; Pahutski, T.F.; Leighty, R.M.; Vincent, D.R.; Hamm, J.C. Mode of action of triflumezopyrim: A novel mesoionic insecticide which inhibits the nicotinic acetylcholine receptor. *Insect Biochem. Mol. Biol.* **2016**, *74*, 32–41. [CrossRef] [PubMed]
16. Divekar, P.A.; Narayana, S.; Divekar, B.A.; Kumar, R.; Gadratagi, B.G.; Ray, A.; Singh, A.K.; Rani, V.; Singh, V.; Singh, A.K.; et al. Plant secondary metabolites as defense tools against herbivores for sustainable crop protection. *Int. J. Mol. Sci.* **2022**, *23*, 2690. [CrossRef] [PubMed]

17. Holyoke Jr., C.W.; Cordova, D.; Zhang, W.; Barry, J.D.; Leighty, R.M.; Dietrich, R.F.; Rauh, J.J.; Pahutski Jr., T.F.; Lahm, G.P.; Tong, M.-H.T.; et al. Mesoionic insecticides: A novel class of insecticides that modulate nicotinic acetylcholine receptors. *Pest Manag.* **2017**, *73*, 796–806. [CrossRef]
18. Liu, C.; Hao, F.; Hu, J.; Zhang, W.; Wan, L.; Zhu, L.; Tang, H.; He, G. Revealing different systems responses to brown planthopper infestation for pest susceptible and resistant rice plants with the combined metabonomic and gene-expression analysis. *J. Proteome Res.* **2010**, *9*, 6774–6785. [CrossRef]
19. Wu, Q.; Zhang, G.; Chen, Y.; Yu, J.L.; Zhou, Y.K.; Shu, Z.L.; Ge, L.Q. Seed dressing with triflumezopyrim controls brown planthopper populations by inhibiting feeding behavior, fecundity and enhancing rice plant resistance. *Pest. Manag. Sci.* **2021**, *77*, 2870–2886. [CrossRef]
20. Guruprasad, G.S.; Pramesh, D.; Reddy, B.G.M.; Mahantashivayogayya, K.; Ibrahim, M.; Pampapathy, G. Triflumezopyrim (DPX-RAB55) A novel promising insecticide insecticide for the management of planthoppers in paddy. *J. Exp. Zool. India* **2016**, *19*, 955–961.
21. Holyoke, C.W.; Zhang, W.; Pahutski, T.F.; Lahm, G.P.; Tong, M.-H.T.; Cordova, D.; Schroeder, M.E.; Benner, E.A.; Rauh, J.J.; Dietrich, R.F.; et al. Triflumezopyrim: Discovery and Optimization of a Mesoionic Insecticide for Rice. In *Discovery and Synthesis of Crop Protection Products*; ACS Symposium Series: Wachingtonm, DC, USA, 2015; pp. 365–378.
22. Zhu, J.; Li, Y.; Jiang, H.; Liu, C.; Lu, W.; Dai, W.; Xu, J.; Liu, F. Selective toxicity of the mesoionic insecticide, triflumezopyrim, to rice planthoppers and beneficial arthropods. *Ecotoxicology* **2018**, *27*, 411–419. [CrossRef]
23. Preetha, G.; Stanley, J.; Suresh, S.; Samiyappan. Risk assessment of insecticides used in rice on miridbug, *Cyrtorhinus lividipennis* Reuter, the important predator of brown planthopper, *Nilaparvata lugens* (Stål). *Chemosphere* **2010**, *80*, 498–503. [CrossRef]
24. Usha Rani, P.; Jyothsna, Y. Biochemical and enzymatic changes in rice plants as a mechanism of defense. *Acta Physiol. Plant.* **2010**, *32*, 695–701. [CrossRef]
25. Nuyttens, D.; Devarrewaere, W.; Verboven, P.; Foque, D. Pesticide-laden dust emission and drift from treated seeds during seed drilling: A review. *Pest. Manage. Sci.* **2013**, *69*, 564–575. [CrossRef] [PubMed]
26. Rashid, M.M.; Jahan, M.; Islam, K.S. Impact of nitrogen, phosphorus and potassium on brown planthopper and tolerance of its host rice plants. *Rice Sci.* **2016**, *23*, 119–131. [CrossRef]
27. Lank, S.K.; Ottea, J.A.; Davis, J.A.; Hernandez, A.B.; Stouta, M.J. Systemic effects of thiamethoxam and chlorantraniliprole seed treatments on adult *Lissorhoptrus oryzophilus* (*Coleoptera: Curculionidae*) in rice. *Pest Manag. Sci.* **2013**, *69*, 250–256. [CrossRef]
28. Xu, P.; Shu, R.; Gong, P.; Li, W.; Wan, H.; Li, J. Sublethal and transgenerational effects of triflumezopyrim on the biological traits of the brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae). *Crop Protect.* **2019**, *117*, 63–68. [CrossRef]
29. Hummel, N.A.; Mészáros, A.; Ring, D.R.; Beuzelin, J.M.; Stout, M.J. Evaluation of seed treatment insecticides for management of the rice water weevil, *Lissorhoptrus oryzophilus* Kuschel (*Coleoptera: Curculionidae*), in commercial rice fields in Louisiana. *Crop. Protect.* **2014**, *65*, 37–42. [CrossRef]
30. Zhang, Y.C.; Feng, Z.R.; Zhang, S.; Pei, X.G.; Zeng, B.; Zheng, C.; Gao, C.F.; Yu, X.Y. Baseline determination, susceptibility monitoring and risk assessment to triflumezopyrim in *Nilaparvata lugens* (Stal). *Pestic. Biochem. Physiol.* **2020**, *167*, 104608. [CrossRef]
31. Gorim, L.; Asch, F. Effects of composition and share of seed coatings on the mobilization efficiency of cereal seeds during germination. *J. Agron. Crop Sci.* **2012**, *198*, 81–91. [CrossRef]
32. Yu, J.; Zhang, G.; Miao, K.; Zhao, L.; Yang, H.; Fang, J.; Guo, H.; Zhuang, Y.; Yao, K.; Shu, Z. Control efficiency of *Cnaphalocrocis medinalis* by rice seed treatment with chlorantraniliprole and its safety evaluation. *Chin. J. Pestic. Sci.* **2019**, *21*, 300–308. [CrossRef]
33. Peng, J.H.; Liao, L.P.; Nie, S.Q.; Liang, J.; Fu, Q.M.; Wu, D.X.; Xu, W.J. Analysis of triflumezopyrim residues in rice, soil and field water. *Agrochemicals* **2018**, *57*, 50–53. [CrossRef]
34. Fan, T.; Chen, X.; Xu, Z.; Liu, L.; Shen, D.; Dong, S.; Zhang, Q. Uptake and translocation of triflumezopyrim in rice plants. *J. Agric. Food Chem.* **2020**, *68*, 7086–7092. [CrossRef] [PubMed]
35. Shen, Y.; Jin, L.; Xiao, P.; Lu, Y.; Bao, J. Total phenolics, flavonoids, antioxidant capacity in rice grain and their relations to grain color, size and weight. *J. Cereal Sci.* **2009**, *49*, 106–111. [CrossRef]
36. Jones, D.; Blancaflor, E.; Kochian, L.; Gilroy, S.J. Spatial coordination of aluminium uptake, production of reactive oxygen species, callose production and wall rigidification in maize roots. *Plant Cell Environ.* **2006**, *29*, 1309–1318. [CrossRef] [PubMed]
37. Xu, H.; He, X.; Zheng, X.; Yang, Y.; Tian, J.; Lu, Z. Southern rice black-streaked dwarf virus (SRBSDV) directly affects the feeding and reproduction behavior of its vector, *Sogatella furcifera* (Horváth) (Hemiptera: Delphacidae). *Virol. J.* **2014**, *11*, 1–6. [CrossRef]
38. Zhang, G.; Yu, J.; Shu, Z.; Fang, J.; Wu, J.; Yao, K. Control effects on rice planthopper and safety evaluation of natural enemies by seed dressing with 10% triflumezopyrim SC. *J. South. Agric.* **2019**, *50*, 2695–2702. [CrossRef]
39. Jahn, G.C.; Litsinger, J.A.; Chen, Y.; Barrion, A.T. *15 Integrated Pest Management of Rice: Ecological Concepts*; Cabi: Wallingford, UK, 2006.
40. Cheng, Y.; Shi, Z.-P.; Jiang, L.-B.; Ge, L.-Q.; Wu, J.-C.; Jahn, G.C. Possible connection between imidacloprid-induced changes in rice gene transcription profiles and susceptibility to the brown plant hopper Nilaparvata lugens Stål (*Hemiptera: Delphacidae*). *Pestic. Biochem. Physiol.* **2012**, *102*, 213–219. [CrossRef]
41. Chanprasert, W.; Myint, T.; Srikul, S.; Wongsri, O.J.J.o.O.P.R. Effects of neonicotinoid and method of breaking dormancy on seed germination and seedling vigour of oil palm (Elaeis guineensis Jacq.). **2012**, *24*, 227–234.

42. Taylor, A.G.; Eckenrode, C.J.; Straub, R.W. Seed coating technologies and treatments for onion: Challenges and progress. *Hortscience A Publ. Am. Soc. Hortic. Sci.* **2001**, *36*. [CrossRef]
43. Ghaffar, M.B.A.; Pritchard, J.; Ford-Lloyd, B. Brown planthopper (*N. Lugens* Stål) feeding behaviour on rice germplasm as an indicator of resistance. *PLoS ONE* **2011**, *6*, e22137. [CrossRef]
44. Rani, P.U.; Pratyusha, S. Role of castor plant phenolics on performance of its two herbivores and their impact on egg para-sitoid behaviour. *Biol. Control.* **2014**, *59*, 513–524. [CrossRef]
45. Luczynski, A.; Isman, M.B.; Raworth, D.A. Strawberry foliar phenolics and their relationship to development of the twospotted spider mite. *J. Econ. Entomol.* **1990**, *2*, 557–563. [CrossRef]
46. Korth, K.L.; Doege, S.J.; Park, S.-H.; Goggin, F.L.; Wang, Q.; Gomez, S.K.; Liu, G.; Jia, L.; Nakata, P.A. Medicago truncatula mutants demonstrate the role of plant calcium oxalate crystals as an effective defense against chewing insects. *Plant Physiol.* **2006**, *141*, 188–195. [CrossRef] [PubMed]
47. Yoshihara, T.; Sogawa, K.; Pathak, M.D.; Juliano, B.O.; Sakamura, S. Oxalic acid as a sucking inhibitor of the brown planthopper in rice (Delphacidae, Homoptera). *Entomol. Exp. Appl.* **1980**, *27*, 149–155. [CrossRef]
48. Maleck, K.; Dietrich, R.A. Defense on multiple fronts: How do plants cope with diverse enemies? *Trends Plant Sci.* **1999**, *4*, 215–219. [CrossRef]
49. Van der Fits, L.; Memelink, J.J. ORCA3, a jasmonate-responsive transcriptional regulator of plant primary and secondary metabolism. *Science* **2000**, *289*, 295–297. [CrossRef]
50. Borevitz, J.O.; Xia, Y.; Blount, J.; Dixon, R.A.; Lamb, C. Activation tagging identifies a conserved MYB regulator of phenyl-propanoid biosynthesis. *Plant Cell* **2000**, *12*, 2383–2393. [CrossRef]
51. Johnson, E.T.; Dowd, P.F. Differentially enhanced insect resistance, at a cost, in *Arabidopsis thaliana* constitutively expressing a transcription factor of defensive metabolites. *J. Agric. Food Chem.* **2004**, *52*, 5135–5138. [CrossRef]
52. Cheynier, V.; Comte, G.; Davies, K.M.; Lattanzio, V.; Martens, S.J. Plant phenolics: Recent advances on their biosynthesis, genetics, and ecophysiology. *Plant Physiol. Biochem.* **2013**, *72*, 1–20. [CrossRef]
53. Luna, E.; Pastor, V.; Robert, J.; Flors, V.; Mauch-Mani, B.; Ton, J. Callose deposition: A multifaceted plant defense response. *Mol. Plant Microbe Interact.* **2011**, *24*, 183–193. [CrossRef]
54. Hao, P.; Liu, C.; Wang, Y.; Chen, R.; Tang, M.; Du, B.; Zhu, L.; He, G. Herbivore-induced callose deposition on the sieve plates of rice: An important mechanism for host resistance. *Plant Physiol.* **2008**, *146*, 1810–1820. [CrossRef]
55. Maag, D.; Erb, M.; Köllner, T.G.; Gershenzon, J. Defensive weapons and defense signals in plants: Some metabolites serve both roles. *Bioessays* **2015**, *37*, 167–174. [CrossRef] [PubMed]
56. Nisbet, A.J.; Woodford, J.A.T.; Strang, R.H.C. The effects of azadirachtin on the acquisition and inoculation of potato leafroll virus by *Myzus persicae*. *Crop Protect.* **1996**, *15*, 9–14. [CrossRef]
57. Polston, J.E.; Sherwood, T. Pymetrozine interferes with transmission of tomato yellow leaf curl virus by the whitefly *Bemisia tabaci*. *Phytoparasitica* **2003**, *31*, 490–498. [CrossRef]
58. Karley, A.J.; Douglas, A.E.; Parker, W.E. Amino acid composition and nutritional quality of potato leaf phloem sap for aphids. *J. Exp. Biol.* **2002**, *205*, 3009–3018. [CrossRef]
59. Lu, J.; Li, J.; Ju, H.; Liu, X.; Erb, M.; Wang, X.; Lou, Y. Contrasting Effects of Ethylene Biosynthesis on Induced Plant Resistance against a Chewing and a Piercing-Sucking Herbivore in Rice. *Mol. Plant* **2014**, *7*, 1670–1682. [CrossRef]