Research Article

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Seed dressing with triflumezopyrim controls brown planthopper populations by inhibiting feeding behavior, fecundity and enhancing rice plant resistance

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Abstract

BACKGROUND: Triflumezopyrim (TFM), a novel mesoionic insecticide, has high efficiency at a low dosage, and is mainly used to control hopper species. A previous study demonstrated that seed dressing with TFM effectively controlled rice planthopper populations in mechanically transplanted rice fields; however, mode of action for control was unclear.

RESULTS: The study shows that seed dressing with TFM resulted in elevated levels of oxalic acid, flavonoids, phenolic substances, callose and other compounds associated with *Nilaparvata lugens* resistance in rice plants, and low TFM residue content in rice plant stem and grain. Host choice behavioral experiments showed that *N. lugens* females prefer feeding on untreated rice plants. Electrical penetration graph (EPG) data showed that seed dressing with TFM at medium and high doses significantly prolonged the non-probing period and inhibited phloem ingestion in *N. lugens* females. These changes led to a significant decrease in female secretion of honeydew, expression of genes encoding vitellogenin and juvenile hormone acid methyltransferase, body weight and longevity, and significantly influenced several physiological parameters resulting in impaired oocyte growth, fecundity and population. Field survey data showed that seed dressing with TFM was efficacious and relatively durable in protecting rice plants from infestation by planthoppers.

CONCLUSION: This study revealed that seed dressing with TFM enhances rice plant resistance to *N. lugens* by limiting phloem ingestion and increasing the *N. lugens* non-probing period; this leads to reduced fecundity of females and lowers *N. lugens* numbers in the field.

Supporting information may be found in the online version of this article.

Keywords: *Nilaparvata lugens*; triflumezopyrim (TFM); electrical penetration graph (EPG); feeding behavior; resistance

1 INTRODUCTION

The brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), is a common pest of rice in Asia.[1] *N. lugens* causes damage in rice directly by phloem-feeding and indirectly by transmitting viral pathogens.[2] Pesticides are commonly used for control of *N. lugens*[3]; however, widespread use of chemical pesticides often leads to insecticide resistance and the elimination of natural enemies.[4,5] Thus, the development of new insecticides with high efficacy for resistant planthopper populations and high selectivity to preserve natural enemies is urgently needed.

Triflumezopyrim (TFM), a novel mesoionic insecticide developed by DuPont Crop Protection, functions by desensitizing nicotinic acetylcholine receptors (nAChRs).[6] TFM has been registered in China for two years.[7] TFM has been used to control both leafhopper and planthopper populations.[8,9] Rice growers in Asia are beginning to use TFM to manage brown planthoppers, and TFM has been shown to be efficacious in controlling *N. lugens* in both laboratory and field trials. Furthermore, TFM has been shown to

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be long-lasting and not injurious to natural enemies.[10,11] Application of 10.6% TFM SC at 25 g or 35 g a.i. ha^{-1} and 237 ml ha^{-1} has been shown to be effective in controlling field populations of *N. lugens* and *Sogatella furcifera* (Hoverth).[12,13] Low (75 ml hm^{-2}), medium (150 ml hm^{-2}), and high (225 ml hm^{-2}) doses of 10% TFM SC showed a high level of insecticidal activity against *N. lugens* compared 25% pymetrozine SC (300 ml hm^{-2}) and thiamethoxam WDG (90 g hm^{-2}).[11] Seed dressing with 10% TFM SC at 22.5, 45.0 and 67.5 g a.i. ha^{-1} was found to be effective in controlling planthoppers in directly seeded and mechanically transplanted rice.[14] However, the mode of action of TFM when used as a seed dressing for rice planthopper control is unclear.

At sublethal concentrations, some insecticides stimulate population growth and reproduction in *N. lugens* by altering the physiology and gene expression, including changes in soluble proteins, hormone metabolism, and vitellogenin (*Vg*) expression in females.[15–18] Furthermore, pesticides can indirectly impact insects by inducing changes in host plant physiology and development[19,20] or by disturbing *N. lugens* feeding behavior.[21,22] Although TFM shows excellent promise in controlling *N. lugens* populations in the rice plant field,[14] it is important to understand the underlying mechanisms of control to avoid unwanted nontarget effects by TFM seed coating agent.

Here, we examine the physiological and biochemical processes altered in *N. lugens* populations exposed to TFM via seed dressing. These results will help understand TFM toxicity to *N. lugens* and may ultimately lead to new control strategies.

2 MATERIALS AND METHODS

2.1 Insects, pesticide application, seed treatment and instruments

Colonies of *N. lugens* were originally collected from the China National Rice Research Institute (Hangzhou, China). *N. lugens* colonies were reared on seedlings of rice plants cv. Nanjing 9108 in the laboratory at 26 ± 2°C, 80 ± 10% relative humidity (RH) and a 16:8 h light/dark photoperiod.

Triflumezopyrim (10%) SC was purchased from DuPont Agricultural Chemicals Ltd. We used rice variety Nanjing9108 (japonica rice) for all experiments. This variety is commonly planted in Jiangsu Province, China and is not resistant to *N. lugens* (Jiangsu Academy of Agricultural Sciences). Rice seeds (1 kg) were weighed, transferred to a mesh bag, soaked in water for 48 h, and dried in darkness at room temperature for 24 h. Seeds (1 kg per treatment) were then transferred to a plastic bag (40 × 20 cm), and 10% TFM SC was added at 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}). Plastic bags were sealed, gently mixed for 5 min, and then dried in darkness at room temperature for 24 h.[14] Treated and control seeds were sown outdoors in cement tanks (length 200 cm × width 100 cm × high 80 cm) under natural conditions in mid-May every year (21–26°C, 50–90% RH). Seedlings were transplanted at the sixth-leaf stage (30 days after sowing) and grown to the tillering stage (60 days after sowing) under natural conditions for use in experiments.

An Agilent 1290 series UPLC system (Agilent Co.) equipped with an ABSciex 4500 (ESI-MS) Triple Quadrupole LC/MS system (AB Sciex Co), a Lyoquest-55 freeze-dryer (Telstar), XP105DR electronic balance (Sartorus Scientific Instruments Co.), AWL-020L-P ultrapure water machine (Aquapro International Comp), desktop high-speed centrifuge and H2050R medical centrifuge (Hunan Xiangyi Instrument Equipment Co. Ltd) were used. C_{18} (Chemical Reagent Co., Ltd), acetonitrile (chromatographic purity; Tedia Comp), and formic acid (≥98%; Merck & Co) were also used.

2.2 Determination of oxalic acid, flavonoids, phenolic compounds, and callose content

The oxalic acid content of rice plant stems via TFM seed coating agent was measured by the trichloride titanium method with minor modifications. Stems (1 g) (from 1–15 cm above the soil layer, the primary *N. lugens* feeding site) of tillering rice plants (60 days after sowing) (untreated control and seeds treated with TFM) were weighed, washed with 10 ml of ultrapure water, and placed in a 50-ml flask. Supernatants were decolorized with activated carbon, which was then removed from the solution by centrifugation.[23] The decolorization step was repeated, and trichloride titanium was then added, and absorbance measured as described previously.[23] Treatments and controls consisted of three independent biological replicates.

The flavonoid content of rice plant stems was determined as described previously with minor modifications. Rice stems (60 days after sowing) were collected,[24] dried at 80°C, and homogenized after adding liquid nitrogen. The resulting homogenates (1 g) were transferred to 15-ml polypropylene conical tubes, 10 ml of 60% ethanol was added, and homogenates were sonicated (30 min at 350 W). Samples were then subjected to centrifugation (10 000 *g* for 5 min at room temperature), supernatants were collected and the process was repeated three more times. Supernatants were then combined in a 25-ml volumetric flask, and the volume was adjusted to 25 ml with 60% ethanol. Aliquots (1 ml) of diluted extracts or standards were transferred to another 25-ml volumetric flask, and 5% $NaNO_2$ (1 ml) and 10% $AlCl_3{\cdot}6H_2O$ (1 ml) were added. The mixture was incubated for 5 min, 1 mol L^{-1} NaOH (1 ml) was added, and the volume was adjusted to 25 ml with 30% ethanol. The solution was mixed, allowed to stand for 15 min, and A_{415} was determined. Flavonoid content was determined with a rutin standard curve. Treatments and controls consisted of four independent biological replicates.

The phenolic content was determined with the Folin–Ciocalteu method with minor changes. Stems (100 mg) of tillering rice (60 days after sowing) were collected, frozen in liquid nitrogen, homogenized, and mixed with tungsten carbide beads in methanol as described previously.[25] The suspension was homogenized (5 min, 30 Hz), tungsten carbide beads were removed, and samples were processed and incubated for 2 h with 200 µl 10% (v/v) Folin–Ciocalteu reagent and 700 mmol L^{-1} Na_2CO_3. Samples (200 µl) were transferred to a 96-well microplate, and A_{765} was measured for each well. A standard curve was obtained using gallic acid standards. Phenols content was calculated as milligrams of gallic acid equivalent (mg GAE) per g dry weight. Treatments and controls contained three independent biological replicates.

The callose content of rice plant stem was determined as described previously.[26] Rice plant stems (0.5 g) were collected 60 days after sowing and transferred to 10 ml tubes containing 1 ml of 98% ethanol (v/v) for 1 h. Excess ethanol was removed by suction, and stems were homogenized in liquid nitrogen and transferred to 5 ml tubes containing 1 mol L^{-1} NaOH (1 ml); samples were then incubated at 80°C for 15 min, allowed to cool to room temperature, and centrifuged to remove particulates. Callose content was determined with aniline blue and the stepwise protocol. Absorbance was read using a Perkin Elmer LS-5B Fluorimeter. Treatments and controls contained three independent biological replicates.

2.3 Determination of free amino acid and sucrose content

Free amino acids in rice plant stems (60 days after sowing) from the same treatments were determined using ninhydrin as described previously.[20,27] A_{570} values were measured with a 722 series spectrophotometer (Analytical Instrument Co.), and a standard curve was obtained with glutamic acid. Treatments and controls each had three independent biological replicates.

The sucrose content of rice plant stems (60 days after sowing) was estimated using a modified anthrone method. Rice stems (20 mg) were macerated in liquid nitrogen, and sucrose was extracted and quantified as described previously.[28] Sucrose quantification was calculated three times using independent samples for each treatment and untreated control.

2.4 Feeding behavior of *N. lugens* female adults

EPG experiments were performed at 26 ± 2°C and 80 ± 10% RH with continuous light. EPG data for *N. lugens* female adults feeding on the same treatments of tilling rice plants (60 days after sowing) in Faraday cages were recorded for 8 h using a Giga-8 DC EPG amplifier (Wageningen Agricultural University) as described previously.[21] To determine suitable EPG recording duration, a preliminary EPG test was conducted by testing *N. lugens* females feeding on untreated plants for 4 and 8 h (Figure 4A). The experimental treatments included the four effective doses of TFM (22.5, 45.0, 67.5, and 90.0 g a.i. ha^{-1}) and a water control. Before experiments, brachypterous *N. lugens* females at 2 days after emergence (2 DAE) were provided with water delivered via a saturated piece of cotton for 2 h. They were then allowed to settle on of rice plant stems (60 days after sowing) under the same treatments for EPG recordings. One end of a gold wire (20 μm diameter × 10 cm length) was attached to the dorsal thorax of female adult *N. lugens* and the other end was connected to the amplifier as described previously.[29] A copper wire (2 mm diameter × 10 cm length) was used as the plant electrode and was placed in the soil. *N. lugens* females attached to the gold wire were transferred to rice stems. The amplifier gain was set at 50×, and the voltage was adjusted to −5, and + 5 V. PROBE v. 3.0 software (Wageningen Agricultural University) was used to analyze EPG signals. Fifteen independent recordings were collected for each TFM concentration and used for data analysis. The EPG patterns were assigned to one of the following seven waveforms described previously[30]: 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).

2.5 *N. lugens* female adult honeydew production and host choice

Excretion of honeydew by *N. lugens* female adults at 2 DAE was measured using Parafilm® sachets.[31,32] The same treatments for rice plants (60 days after sowing, tillering stage) were grouped in a randomized complete block and maintained at 25–28°C with a 12:12 h light/dark photoperiod. *N. lugens* nymphs were reared on rice plants at the tillering stage (60 days after sowing); female adults were recovered at 2 DAE, starved for 2 h, and transferred into individual Parafilm sachets attached to rice stems in the same treatments. After a 48-h feeding period, females were removed from sachets as described previously.[31] Sachets with honeydew were weighed (W1), the honeydew was removed and the sachets were reweighed (W0). The quantity of honeydew was calculated as the difference between the two weights (W1 − W0). Treatments and controls each consisted of 15 independent replicates.

Methods for evaluating *N. lugens* host choice were similar to those described by Lu *et al*. with minor modification.[33] Rice seedlings derived from TFM-treated seeds and the water control were planted in plastic pots (20 × 45 cm) with four hills per pot and three rice plants per hill and grown to the tillering stage (60 days after sowing). One replicate consisted of five pots (four treatments and control); these were randomly placed in a greenhouse maintained at 26 ± 2°C, 80% RH and 16:8 h light/dark photoperiod. A 10-cm dish containing 50 *N. lugens* female adults at 2 DAE was placed in the middle of the five pots. Numbers of *N. lugens* inhabiting each plant were counted at 24 h after release. Each treatment and control consisted of five independent biological replicates.

2.6 Quantitative real-time PCR

RNA was isolated from five *N. lugens* adult females as described previously.[17] cDNA was synthesized in a 20-μl reaction volume at 37°C using oligo(dT) primers and random hexamers. A quantitative real-time polymerase reaction (qPCR) was conducted in 96-well plates with the SYBR Premix™ Taq Kit (Takara) and a 96 CFX Touch PCR system (Bio-Rad). Each reaction contained cDNA template (1 μl), SYBR master mix (5 μl), primers (0.4 μl/per primer at 10 μmol), and double-distilled water (3.2 μl). The qPCR program was as follows: 95°C for 30 s, 35 cycles of 95°C for 5 s, 60°C for 15 s and 72°C for 30 s. Expression was normalized with *β-actin* (GenBank accession no. EU179850). The $2^{-\Delta\Delta ct}$ method was used to obtain relative mRNA expression levels.[34] The primers utilized for qPCR are listed in Table S1.

2.7 Determination of total proteins, juvenile hormone III and ecdysteroids, and body weight

Third-instar nymphs were collected and transferred into glass jars (12 × 6 cm, 20 nymphs/jar) containing the same treatments of tillering rice stems (15 cm long) in an environmental chamber (26 ± 2°C, 80% RH, 16:8 h light/dark photoperiod) (Model: RXZ500, Ningbo Jiangnan Instrument Co. Ltd). We observed the fifth instar every 12 h; when the fifth instar nymphs emerged, a newly emerged female and a newly emerged male were transferred to new glass jar containing the same treatments of tilling rice stems (a mated group). We collected mated brachypterous *N. lugens* female at 2 DAE. Total proteins in the ovaries and fat bodies of mated brachypterous *N. lugens* females feeding on TFM-treated rice plants (n = 50, each treatment) and control rice plants (n = 50) at 2 DAE were extracted as described previously.[17] The Bradford method was used to determine protein concentrations as described previously.[35] Titers of juvenile hormone III (JH III) and ecdysteroid in adult females at 2 DAE were measured by high-performance liquid chromatography mass spectrometry (HPLC/MS) as described previously.[17] We determined the fresh body weight using 15 mated females at 2 DAE. The insects were placed in pre-weighed centrifuge tubes and then weighed using a Mettler-Toledo electronic balance (Ec 100 mode: 1/1000 *g* sensitivity). Each treatment and control were replicated three times.

2.8 LC–MS/MS method for the determination of TFM in the rice stem and grain

The extraction procedure for TFM in the rice stem or grain referred to Peng *et al*.[36] The rice plant stem samples or grains (randomly collected samples in the mechanically transplanted field for each treatment and each period) were dried at 50°C for 12 h and then

crushed by using a pulverizer (Model: RS-FS150, Royalstar). A 0.5 g sample of drying rice plant stem or grain was collected in a 50-ml centrifuge tube, and 20 ml of 60% acetonitrile was added. The centrifuge tube was placed in a 50°C constant temperature bath for 30 min, then centrifuged for 5 min at 6000 rpm (10,000 xg). One milliliter of supernatant was taken, dried under a gentle nitrogen stream and dissolved in 60% acetonitrile, the extract was then filtered through a polyvinylidene difluoride (PVDF) syringe filter (13 mm × 0.22 μm) into a sample vial for Ultra High Performance Liquid Chromatography-Mass Spectrum (UPLC-MS) analysis. Each treatment and control were three independent biological replicates.

Standard triflumezopyrim was provided by Jiangsu Agro-product Quality Test Center (Jiangsu Academy of Agricultural Sciences). Determination of TFM in rice stem or grain followed the methods of Peng *et al*. and Fan *et al*.[36,37] UPLC analysis was performed with an Agilent 1290 UPLC system equipped with a binary pump, automatic sampler, column oven, and diode array detector. A 1-μl aliquot of the sample solution was injected into an Agilent Eclipse Plus chromatographic column C_{18} (2.1 × 50 mm i.d., 1.7 μm). The temperature of the column in the oven was maintained at 40°C. Solvent A (0.1% formic acid solution) and solvent B (acetonitrile) were used as the mobile phase at a flow rate of 0.2 ml min^{-1}. The elution gradient was: 0–1 min, 95% A; 1–4.5 min, 95% to 10% A; 4.5–6 min, 10% A; 6–6.1 min, 10% to 95% A; 6.1–10 min, 95% A. TFM was analyzed in positive ion multiple reaction monitoring modes. The fragment ion *m/z* values were 399.0/121.0 and 399.0/287.9. The declustering potential and collision energies were set at 80 and 25 V, respectively. Mass spectra were recorded on an AB Sciex 4500 (ESI-MI) equipped with an electrospray ionization (ESI) source. System control and data acquisition were controlled using MutiQuant 3.0.2 software (AB Sciex Co.). The best analyte response was achieved under the following conditions: curtain gas, 35 psi; ion spray voltage, 5500 V; source temperature, 450°C; ion source gas (1), 40 psi; ion source gas (2), 40 psi; interface heater, on; and collision gas, medium. ESI was operated in positive ion mode in multiple reaction monitoring (MRM).

2.9 Effects of seed dressing with four TFM concentrations on *N. lugens* female adults' reproductive parameters

Brachypterous newly emerged females and the newly emerged males were collected as described in Section 2.7. Fifteen copulating pairs were analyzed for the preoviposition and oviposition periods, fecundity, body weight, and longevity in *N. lugens* female adults. The same rice stem treatments (60 days after sowing) were replaced daily during preoviposition and at 2-day intervals until female death. Eggs deposited on rice stems were counted under light microscopy, and the fecundity of 15 mated pairs was determined by calculating the mean number of eggs laid. Subsets of mated females ($n = 10$) were dissected for immunofluorescent microscopy.

2.10 Western blot analysis

Proteins were isolated from ovaries and fat bodies, separated using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), and blotted to PVDF membranes as described previously.[17] After a blocking step, membranes were incubated with anti-Vg antiserum (1:5000) (provided by Haijun Xu, Zhejiang University); antiserum to β-actin (1:5000) (Cell Signaling) was used as a loading control. Membranes were then washed, incubated with goat anti-rabbit IgG secondary antibodies (1:8000), and treated with chemiluminescent substrates as described previously.[17] Mean gray values of detected proteins were normalized to β-actin with ImageJ (https://imagej.nih.gov/ij/).

2.11 Immunofluorescence microscopy

Ovaries from mated *N. lugens* females were removed at 2 DAE and washed three times in cold phosphate-buffered saline (PBS; pH 7.4). The fixation and incubation process with anti-Vg and Alexa Fluor 488-labeled goat anti-rabbit secondary antibody (Beyotime) followed established protocols. Nuclei were visualized by staining with 100 nM 4′,6-diamidino-2-phenylindole (DAPI; Beyotime) as described previously.[17] Fluorescence images of Vg (green) and DAPI (blue) were detected at 405 and 488 nm, respectively, using a Zeiss LSM 780 confocal microscope (Carl Zeiss MicroImaging). Sequential scanning mode was used to capture images at a resolution of 1024 × 1024 pixels; more intense fluorescence indicated a higher level of gene expression.

2.12 Population growth

Five groups of brown planthoppers were established to monitor population growth and included *N. lugens* fed on rice subjected to seed dressing with 0 (untreated control), 22.5, 45.0, 67.5, and 90.0 g a.i. ha^{-1} TFM. Five replicates were organized in a randomized complete block. The newly emerged, mated female adults described in Section 2.7 were released on tillering (60 days after sowing) (control or TFM-treated) and enclosed in nylon cages. Groups were inspected daily, and third-instar nymphs were counted and transferred to new plants using established protocols. Nymphs were inspected on alternate days until adults emerged, and *N. lugens* numbers were logged until the females died. Hatch rates, the ratio of adults/adults + unhatched eggs, and population growth index (PGI) were determined using established protocols.[17,23]

2.13 Field surveys

Seedlings at the sixth-leaf stage were mechanically transplanted to the field. A 500 m^2 area was used for the five treatments (see Section 2.10)[14] and contained five replicates each. A ridge separated sections of the paddy to prevent water flow between sections. Three surveys were conducted as follows: 10 July to 26 September 2017 (56 to 133 days after sowing), 11 July to 26 September 2018 (56 to 133 days after sowing), and 3 July to 25 September 2019 (48 to 124 days after sowing), surveyed every 7 days. Five plots and 20 hills/plots were surveyed for each treatment, resulting in a total of 100 hills counted in mechanically transplanted fields for each period. Treatments and controls consisted of five independent biological replicates.

2.14 Statistical analyses

Before performing an analysis of variance (ANOVA), normality and homogeneity of variance were verified based on the Bartlett test. One-way ANOVA followed by Tukey's multiple comparison tests in all figures except for a two-way ANOVA in Figure 9. Multiple comparisons of the means were analyzed using the Fisher-protected least significant difference (PLSD). Data points were considered significant at $P < 0.05$, and values were expressed as means ± SEM. All statistical analysis was conducted manipulating the DPS data processing system developed by Tang and Feng.[38]

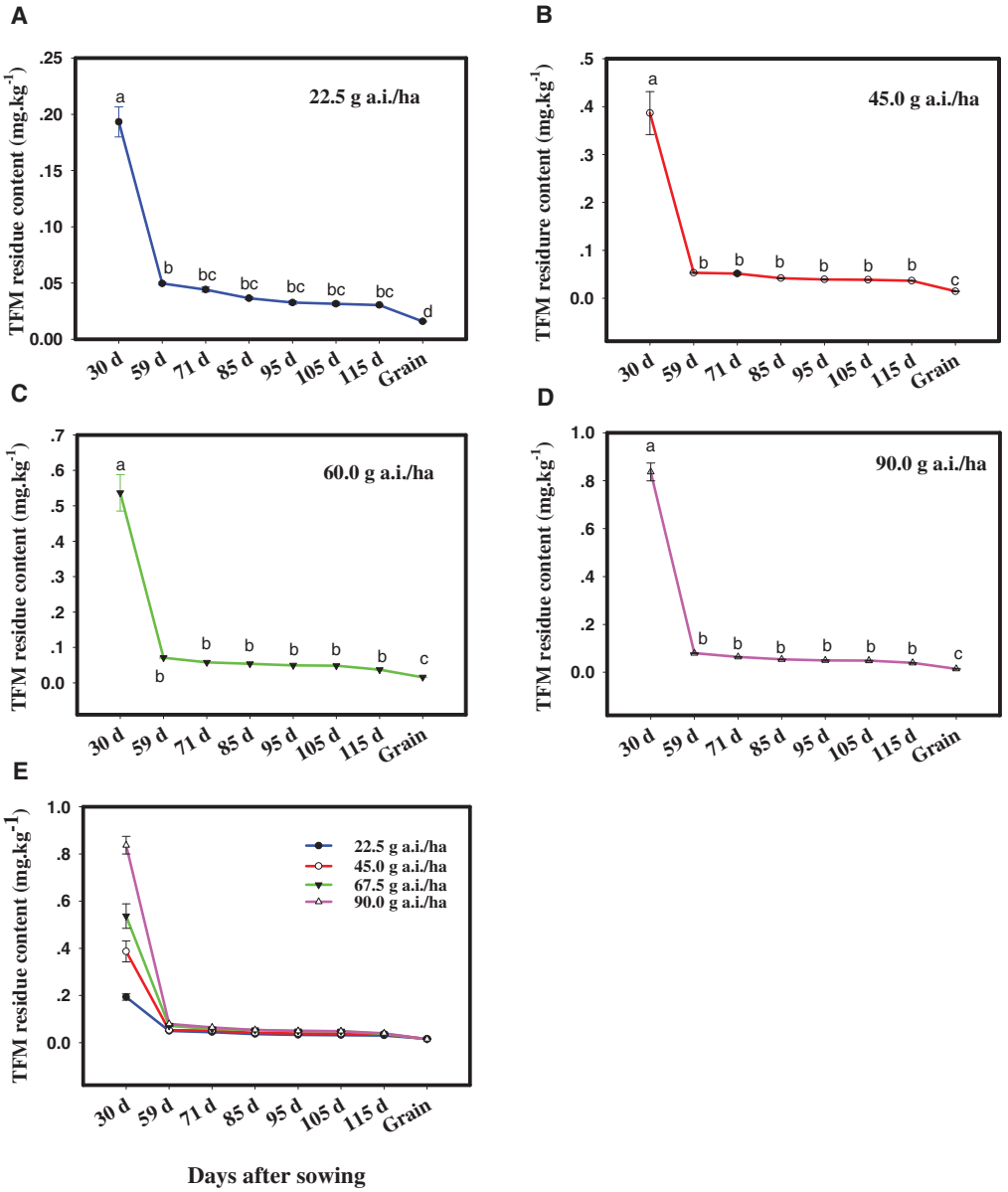

FIGURE 1. Triflumezopyrim (TFM) residue contents of rice plant stems and grain via seed coated with four TFM concentrations from 30 to 115 days after sowing. (A–E) TFM residue contents of rice plant stems and grain via seed coated with four TFM concentrations. Values are means ± SEM ($N = 3$). Means with the same letter are not significantly different (Tukey test, $P < 0.05$).

3 RESULTS

3.1 The TFM residue content of rice plant stems via seed coated with TFM

The TFM residue contents of rice plant stems grown from seed coated with 22.5, 45.0, 67.5, and 90.0 g a.i. ha^{-1} TFM were significantly decreased from 30 to 115 days after seed sowing ($F = 137.0$, df = 7, 23, $P = 0.0001$ for 22.5 g a.i. ha^{-1}, Figure lA; $F = 60.2$, df = 7, 23, $P = 0.0001$ for 45.0 g a.i. ha^{-1}, Figure 1B; $F = 57.1$, df = 7,23, $P = 0.0001$ for 67.5 g a.i. ha^{-1}, Figure 1C; $F = 435.7$, df = 7,23, $P = 0.0001$ for 90.0 g a.i. ha^{-1}, Figure 1D). The TFM residue contents of rice plant stems grown from seed coated with four TFM concentrations showed a significant difference from 30 to 59 days after sowing (Figure 1E). However, there was no significant difference from 71 to 115 days after sowing (Figure 1E) and the level remained relatively stable (approximately 0.04 mg.kg^{-1}). The TFM residue contents of grain from seed coated with four TFM concentrations also remained relatively stable (approximately 0.015 mg kg^{-1}) (Figure 1). This study indicated that the TFM residue content of grain from seed coated with TFM was remarkably safe, and far below the standard residue content (1.0 mg kg^{-1}).

3.2 Seed dressing with TFM affects rice resistance and nutrition

Seed dressing with TFM resulted in significant increases in secondary metabolites associated with resistance, including oxalic acid, flavonoids, phenols, and callose. Oxalic acid production was 69%, 89%, 198%, and 267% higher than the untreated control when rice seeds were treated with 22.5, 45, 67.5, and 90 g a.i. ha^{-1} TFM, respectively ($F = 38.3$, df = 4,14, $P = 0.0001$; Figure 2A). Flavonoids were 63%, 155%, 265%, and 434% higher in rice plants from seed treated with 22.5, 45, 67.5, and 90 g a.i. ha^{-1} TFM,

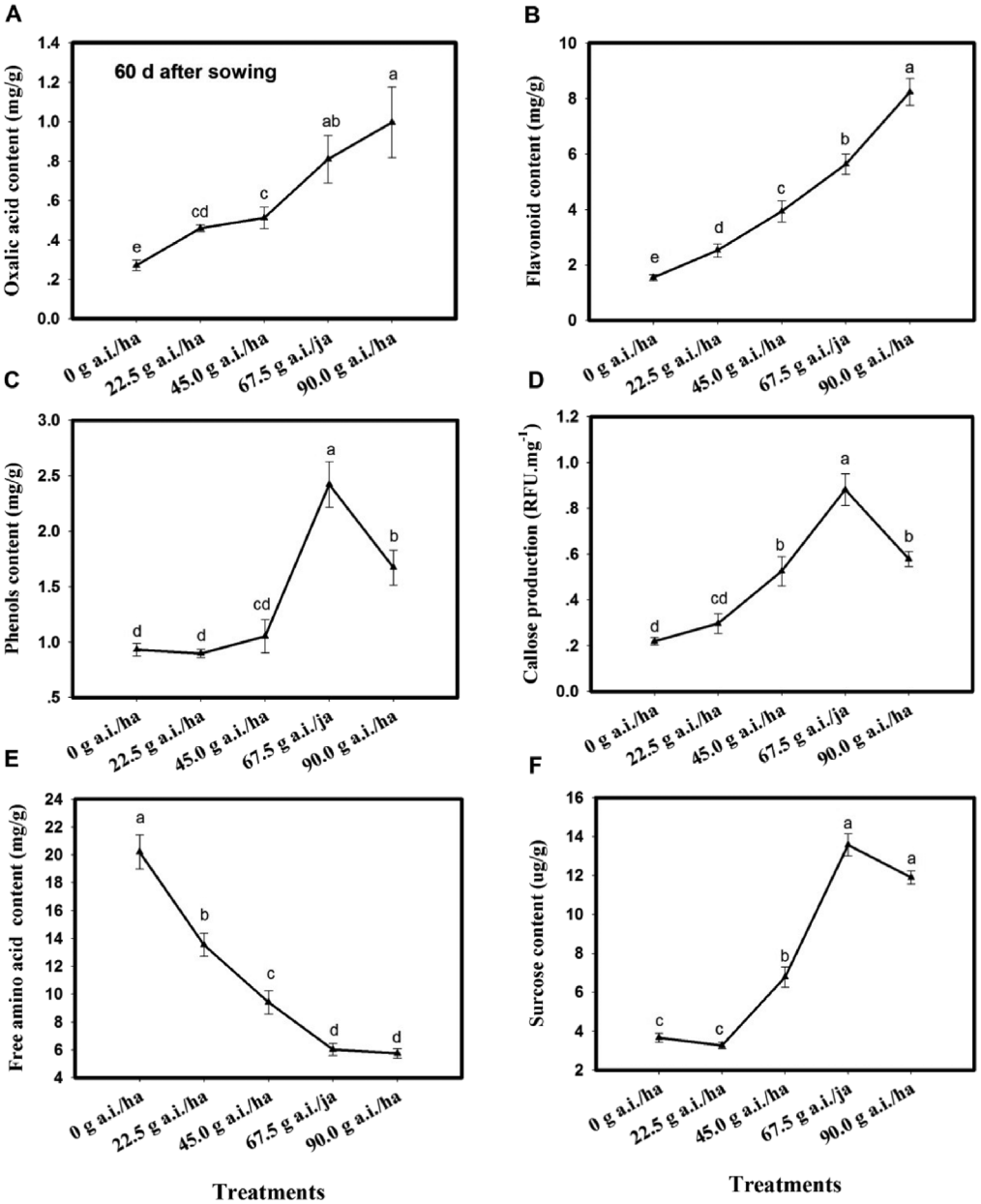

FIGURE 2. Measurement of secondary metabolites, free amino acids, and sucrose in rice plants at tillering (60 days after sowing seed). Prior to planting, seeds were treated with 0 (untreated control), 22.5, 45, 67.5, or 90 g a.i. ha^{-1} triflumezopyrim (TFM). (A–D) Quantity of (A) oxalic acid, (B) flavonoids, (C) phenols and (D) callose, in response to the five TFM concentrations. (E,F) Free amino acid (E) and sucrose (F) content. Data are from three independent biological replicates and are presented as means ± SEM. Data points labeled with different lowercase letters are statistically significant at $P < 0.05$ (Tukey's test).

respectively ($F = 60.5$, df = 4,14, $P = 0.0001$; Figure 2B). Phenols content was increased by 159% and 79% in response to seed dressing at 67.5, and 90 g a.i. ha^{-1} TFM, respectively ($F = 22.7$, df = 4,14, $P = 0.0001$; Figure 2C). Callose production was 139%, 301%, and 164% higher when seeds were treated with 45, 67.5, and 90 g a.i. ha^{-1} TFM, respectively ($F = 28.0$, df = 4,14, $P = 0.0001$; Figure 2D). Free amino acid levels were reduced in response to TFM and were decreased by 33%, 53%, 70%, and 71% when seeds were treated with 22.5, 45, 67.5, and 90 g a.i. ha^{-1} TFM, respectively ($F = 56.9$, df = 4,14, $P = 0.0001$; Figure 2E). Sucrose content was elevated in response to TFM and was 85%, 271%, and 225% higher than the control at 45, 67.5, and 90 g a.i. ha^{-1} TFM, respectively ($F = 143.2$, df = 4, 14, $P = 0.0001$; Figure 2F). This study demonstrated that seed dressing with TFM enhanced rice plant resistance and changed its nutritional value.

3.3 Seed dressing with TFM affects *N. lugens* host choice and honeydew excretion

The effect of TFM-treated rice seed on host choice selectivity was evaluated for *N. lugens* female adults. *N. lugens* females preferred to feed on untreated rice; the numbers of adults colonizing plants grown from rice seeds treated with 45.0, 67.5, and 90.0 g a.i. ha^{-1} TFM were 76%, 97%, and 94% lower than the untreated control at 24 h post-release ($F = 83.5$, df = 4, 24, $P = 0.0001$; Figure 3A). Furthermore, honeydew secretion was 23% and 19% lower in

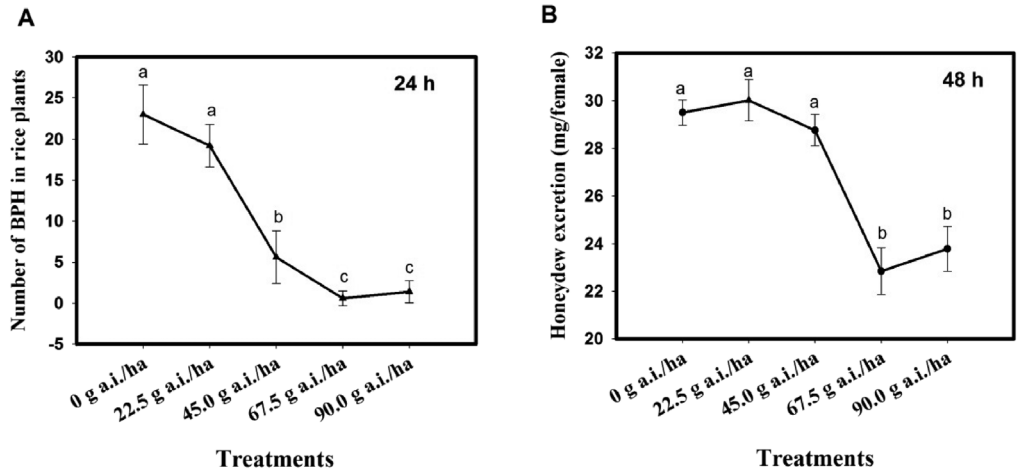

FIGURE 3. Seed dressing with triflumezopyrim (TFM) affects host choice and honeydew excretion in *Nilaparvata lugens* females. Prior to planting, seeds were treated with 0 (untreated control), 22.5, 45, 67.5, or 90 g a.i. ha^{-1} TFM. (A) Numbers of *N. lugens* inhabiting rice ($n = 50$, $N = 5$) at 24 h post-release. (B) Amount of honeydew excreted ($N = 15$, 15 biological replicates) at 48 h. The data are from independent biological replicates and are presented as means ± SEM. Different lowercase letters indicate significant difference at $P < 0.05$ (Tukey's test).

N. lugens female adults when rice was pre-treated with 67.5 and 90.0 g a.i. ha^{-1} TFM, respectively ($F = 17.3$, df = 4, 99, $P = 0.0001$; Figure 3B).

3.4 Seed dressing with TFM affects feeding behavior of *N. lugens* females

The feeding behaviors of *N. lugens*, including non-penetration, stylet penetration of the rice epidermis, sap ingestion in the phloem, and water ingestion in xylem during feeding, were estimated using the NP, N1, N2, N3, N4, and N5 parameters, respectively. Multiple observations of *N. lugens* waveforms on control rice revealed similar EPG patterns at 4 and 8 h recording; however, waveform total duration was higher monitored for an 8-h period (Figure 4A). Based on this observation, we chose to monitor EPG data for an 8-h recording period on plants derived from control and TFM-coated seed.

During the 8-h recording period, the duration of the np waveform for female adults was 40.1 ± 3.3 min on the control rice plant; by contrast, the duration of the np waveform for female adults was 56%, 80%, and 74% higher than the untreated control for rice plant seeds treated with 45.0, 67.5, and 90 g a.i. ha^{-1} TFM ($F = 254.0$, df = 4, 74, $P = 0.0001$; Figure 4B). However, there were no significant differences in the number of np occurrences among treatments and controls ($F = 1.63$, df = 4, 74, $P = 0.096$; Figure 5A). This result demonstrated that seed dressing with TFM significantly prolonged the np waveform but did not influence the number of np occurrences.

Occurrences for the N1 waveform were 44% and 20% lower than the control for rice seeds treated with 67.5 and 90.0 g a.i. ha^{-1} TFM, respectively ($F = 35.3$, df = 4, 74, $P = 0.0001$; Figure 5B). This result demonstrated that seed dressing with TFM significantly decreased the number of *N. lugens* female adults piercing plant tissue. The total duration of N2 waveforms ($F = 0.595$, df = 4,74, $P = 0.6672$; Figure 4C) and number of N2 occurrences ($F = 2.0$, df = 4, 74, $P = 0.1075$, Figure 5C) were not significantly different for *N. lugens* females feeding on control versus treated rice. The duration of N3 waveforms for *N. lugens* females was 12%, 50%, and 28% lower on rice treated with 45.0, 67.5, and 90.0 g a.i. ha^{-1} TFM, respectively, ($F = 42.9$, df = 4,74, $P = 0.0001$; Figure 4D) compared with the untreated control. There was no significant difference in the number of N3 occurrences ($F = 0.54$, df = 4, 74, $P = 0.7092$; Figure 5D) on control versus treated rice. This study showed that seed dressing with TFM prevented the stylet tip of *N. lugens* female adults moving from an extracellular location to the rice plant phloem.

Duration of the N4-a waveform, which indicates intracellular activity in the phloem region, was 23%, 67%, and 55% lower on rice treated with 45.0, 67.5, and 90.0 g a.i. ha^{-1} TFM, respectively, compared with the untreated control ($F = 217.7$, df = 4,74, $P = 0.0001$; Figure 4E). The number of N4-a occurrences in *N. lugens* females was 29%, 61%, and 37% lower than the control when rice seed was treated with 45.0, 67.5, and 90.0 g a.i. ha^{-1} TFM, respectively ($F = 82.4$, df = 4,74, $P = 0.0001$; Figure 5E). Duration of the N4-b waveform, which represents phloem sap ingestion, was 25%, 76%, and 56% lower than the control when rice seed was treated with 45.0, 67.5, and 90.0 g a.i. ha^{-1} TFM, respectively ($F = 128.9$, df = 4,74, $P = 0.0001$; Figure 4F). The number of N4-b occurrences in *N. lugens* females was 22%, 57%, and 39% lower than the control when rice seeds were treated with 45.0, 67.5, and 90.0 g a.i. ha^{-1} TFM, respectively ($F = 24.5$, df = 4,74, $P = 0.0001$; Figure 5F). This result demonstrated that seed dressing with TFM significantly inhibited phloem sap ingestion by *N. lugens* female adults. Duration of N5 waveforms ($F = 0.175$, df = 4,74, $P = 0.9508$; Figure 4G) and number of occurrences ($F = 0.394$, df = 4, 74, $P = 0.8122$, Figure 5G) were not significantly different between control and TFM-treated rice.

3.5 Seed dressing with TFM affects the total protein, hormone titers, and body weight in *N. lugens* females

Total protein content in fat bodies was 38%, 71%, and 66% lower in *N. lugens* females feeding on rice plants derived from seed treated with 45.0, 67.5, and 90.0 g a.i. ha^{-1} TFM, respectively, compared with untreated controls ($F = 18.1$, df = 4,14, $P = 0.0001$; Figure 6A). Similarly, the amount of the total protein in *N. lugens* ovaries was 21%, 44%, and 39% lower for females feeding on rice plants derived from seed treated with 45.0, 67.5, and 90.0 g a.i. ha^{-1} TFM, respectively, compared with the control ($F = 67.3$, df = 4,14, $P = 0.0001$; Figure 6B). The same three TFM treatments (45.0, 67.5, and 90.0 g a.i. ha^{-1}) also resulted in reduced JH III and ecdysteroid titers, and body weight (67.5 and 90.0 g a.i. ha^{-1}); JH III

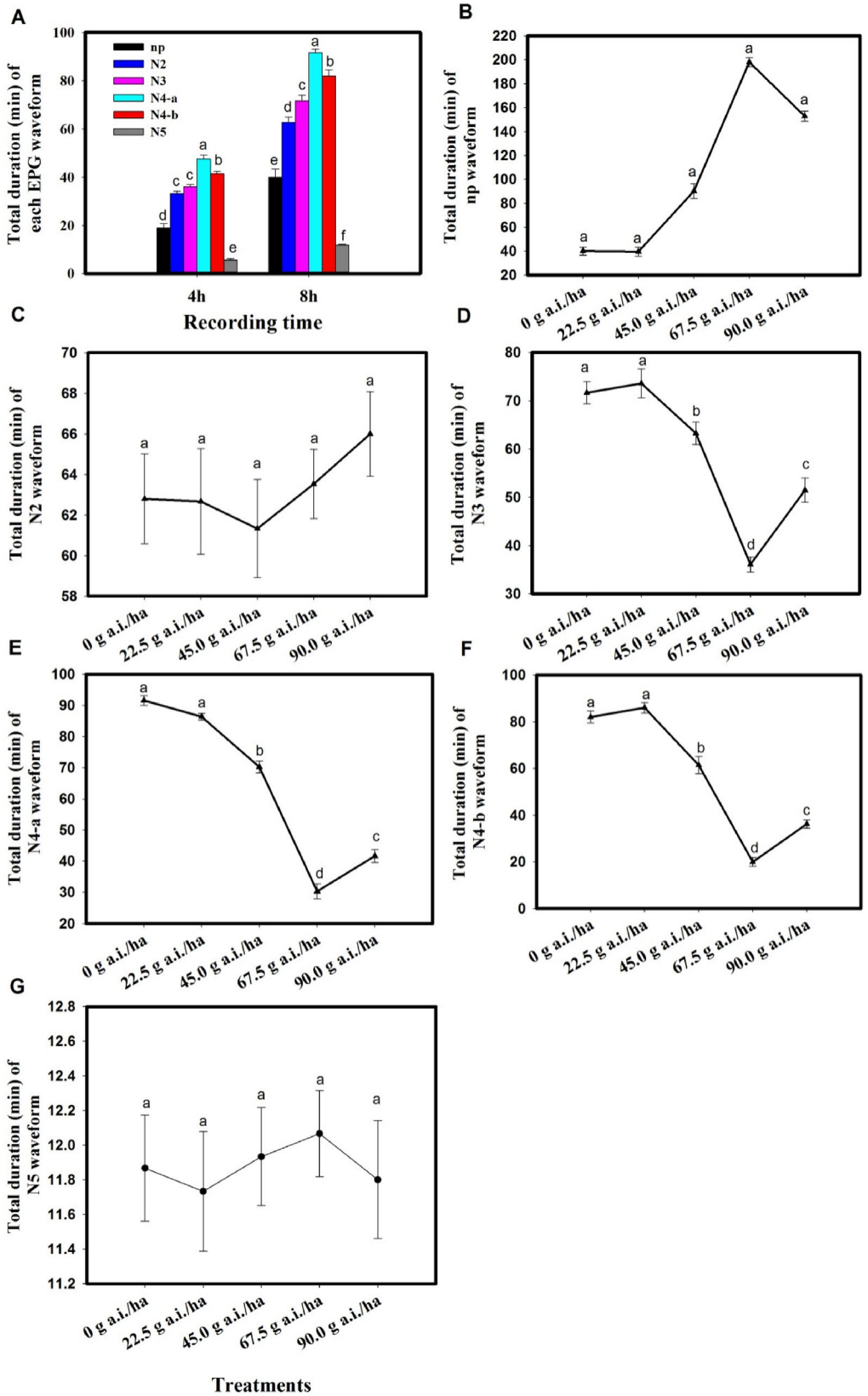

FIGURE 4. Duration (min) of each electrical penetration graph (EPG) waveform produced by *Nilaparvata lugens* feeding on rice plants obtained after seed dressing with triflumezopyrim (TFM) at 0, 22.5, 45, 67.5, or 90 g a.i. ha^{-1}. (A) Duration of each EPG waveform produced by *N. lugens* during a 4- and 8-h recording period on an untreated control rice plant (60 days after sowing). (B–F, H) Total duration of the waveforms np (B), N2 (C), N3 (D), N4-a (E), N4-b (F), and N5 (H). (G) Total duration of both N4 waveforms. The data are from three independent biological replicates and are presented as means ± SEM. Data points labeled with different lowercase letters denote a significant difference at $P < 0.05$ (Tukey's *t* test).

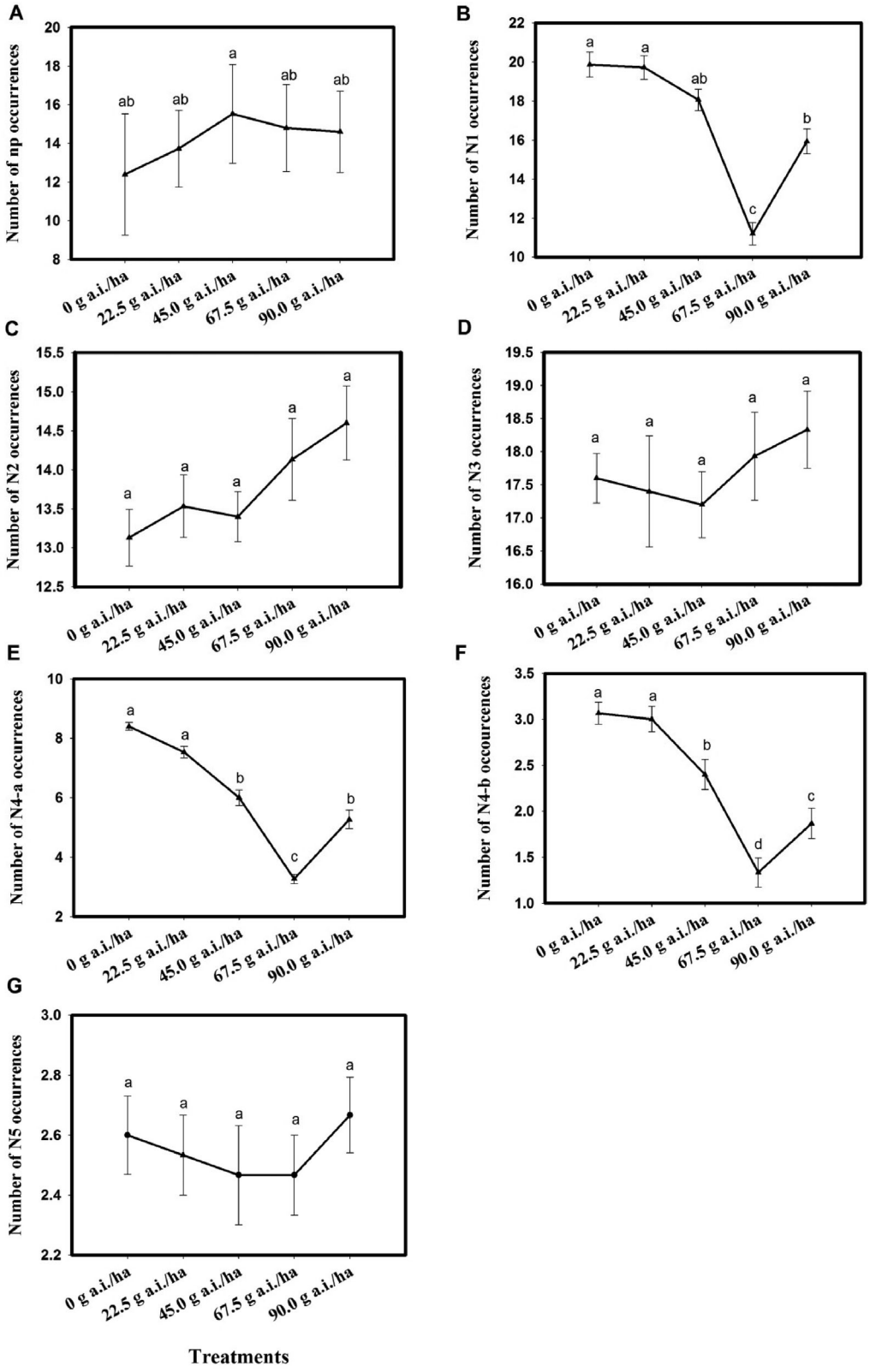

FIGURE 5. Number of occurrences of electrical penetration graph (EPG) waveforms produced by *Nilaparvata lugens* feeding on rice plants obtained after seed dressing with triflumezopyrim (TFM) at 0, 22.5, 45, 67.5, or 90 g a.i. ha^{-1}. (A–G) Mean number of occurrences for np (A), N1 (B), N2 (C), N3 (D), N4-a (E), N4-b (F), and N5 (G) waveforms. The data are from three independent biological replicates and are presented as means ± SEM. Data points labeled with different lowercase letters represent a significant difference at $P < 0.05$ (Tukey's test).

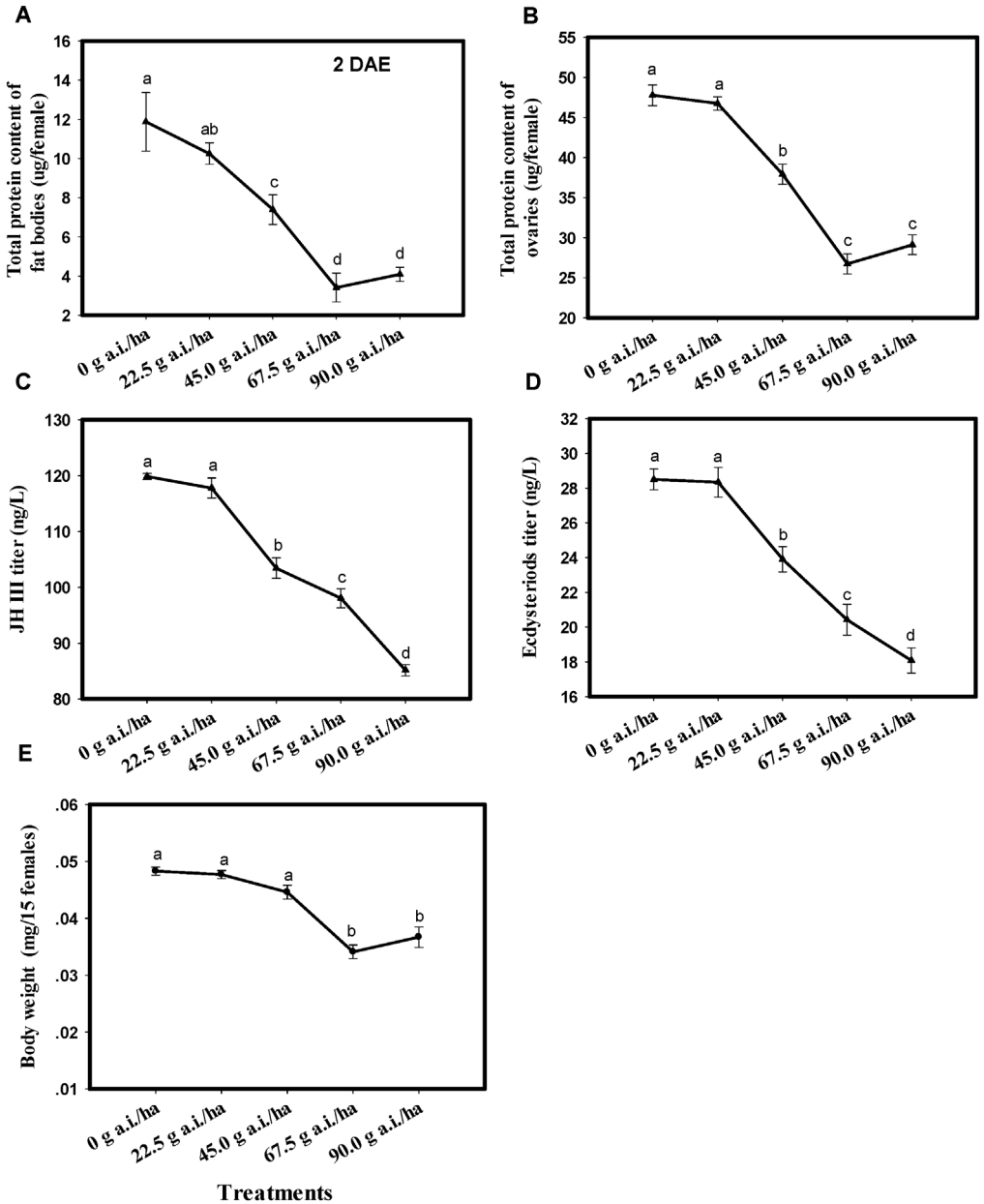

FIGURE 6. Effect of seed dressing with triflumezopyrim (TFM) on hormone titers, the total protein content of fat bodies and ovaries, and body weight in *Nilaparvata lugens* adult females. (A,B) Mean soluble protein content in fat bodies and ovaries. (C,D) Mean titers of JH III and ecdysteroids, respectively. (E) Mean body weight. Data are from three replicates and are presented as means ± SEM. Data points with different lowercase letters are significantly different at $P < 0.05$ (Tukey's test).

was down by 14%, 18%, and 29% ($F = 97.2$, df = 4, 29, $P = 0.0001$; Figure 6C), ecdysteroid down by 16%, 28%, and 37% ($F = 37.0$, df = 4, 29, $P = 0.0001$; Figure 6D), and body weight down by 29% and 24% ($F = 29.5$, df = 4, 14, $P = 0.001$; Figure 6E).

3.6 Seed dressing with TFM affects Vg synthesis, hormone metabolism, and Vg uptake by oocytes

Vg expression was down by 62% and 46% in *N. lugens* females feeding on plants derived from seed treated with 67.5 and 90.0 g a.i. ha^{-1} TFM, respectively, compared with untreated controls ($F = 11.1$, df = 4, 14, $P = 0.0001$; Figure 7A). Similarly, *JHAMT* expression in *N. lugens* females was downregulated by 23%, 54%, and 37% at 45, 67.5, and 90.0 g a.i. ha^{-1} TFM, respectively ($F = 17.2$, df = 4,14, $P = 0.0002$; Figure 7C). However, TFM treatments had no effect on expression of *VgR* ($F = 0.37$, df = 4, 14, $P = 0.8247$; Figure 7B) or *Met* ($F = 1.2$, df = 4, 14, $P = 0.3686$; Figure 7D) at 2 DAE. Western blot analysis showed that Vg protein abundance in *N. lugens* female adults was 55% and 41% lower at 67.5 and 90.0 g a.i. ha^{-1} TFM, respectively, compared with untreated control plants (Figure 7E). These studies indicated that *N. lugens* females feeding on rice plants derived from seed treated with high doses of TFM showed impaired Vg protein synthesis and hormone metabolism. Immunohistochemical staining showed that Vg uptake in oocytes was inhibited when *N. lugens* females fed on rice plants derived from seeds treated with high doses of TFM (Figure 8H,K,N).

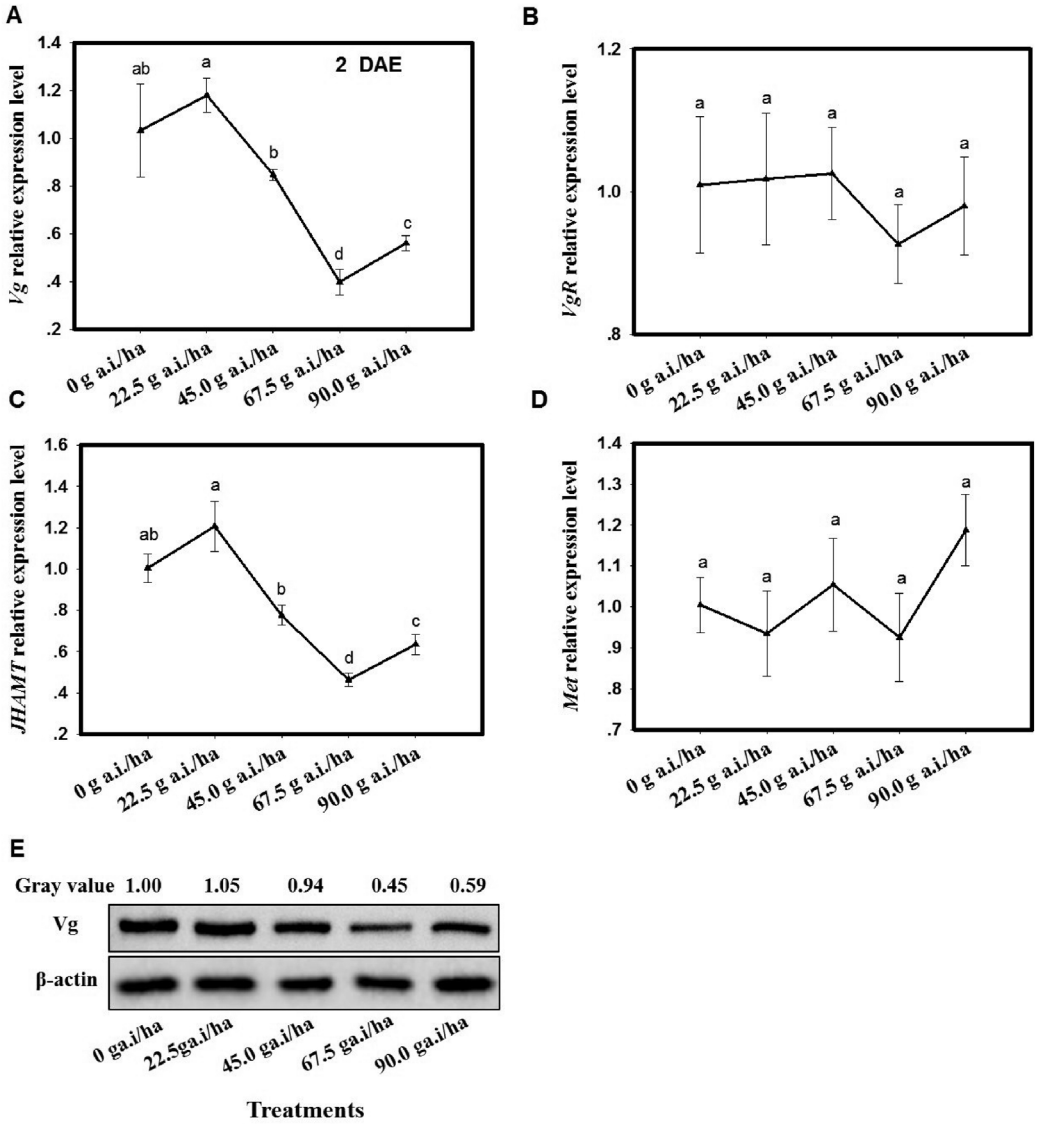

FIGURE 7. Seed dressing with triflumezopyrim (TFM) impacts Vg protein synthesis and hormone metabolism in *Nilaparvata lugens* females. (A,B) *Vg* and *VgR* expression; (C,D) *JHAMT* and *Met* expression. (E) Vg production using anti-Vg antisera, where anti-*β*-actin was used as a loading control. Gene expression and Vg production were measured by quantitative polymerase chain reaction and western blot analysis, respectively, at 2 days after emergence (DAE) in *N. lugens* feeding on plants derived from TFM-treated rice and the untreated control. Relative mRNA levels were determined by comparison with standard curves and normalized with *β-actin*. Anti-*β*-actin was used as a loading control in western blots, and the relative gray values normalized to *β*-actin are shown above corresponding bands. Data are from three independent biological replicates and are presented as the means ± SEM. Data points with different lowercase letters are significantly different at $P < 0.05$ (Tukey's test).

3.7 Seed dressing with TFM affects reproductive parameters in *N. lugens* females

When *N. lugens* adult females fed on rice seedlings derived from seed treated with high doses of TFM, the number of laid eggs, oviposition period, and longevity showed significant decreases (Figure 9). The numbers of laid eggs were decreased by 68% and 61% ($F = 19.7$, df = 4,74, $P = 0.0001$; Figure 9A), the oviposition period decreased by 62% and 53% ($F = 12.5$, df = 4, 74, $P = 0.0001$; Figure 9C), longevity by 63% and 51% ($F = 11.0$, df = 4, 74, $P = 0.0001$; Figure 9D), compared with untreated controls, respectively. There was no difference in the preoviposition period for *N. lugens* females feeding on TFM-treated rice seedlings and the untreated control ($F = 1.2$, df = 4,74, $P = 0.3138$; Figure 9B).

3.8 Seed dressing with TFM affects the number of offspring, hatching rate, and PGI

When *N. lugens* females fed on rice derived from seed treated with medium (45.0 g a.i. ha^{-1}) and high doses of TFM (67.5 and 90.0 g a.i. ha^{-1}), offspring numbers, hatching rates, and PGI decreased (Table 1). For example, numbers of offspring were down by 22%, 88%, and 83% ($F = 291.9$, df = 4, 74, $P = 0.0001$) and PGI decreased by 22%, 88%, and 83% ($F = 291.9$, df = 4,74, $P = 0.0001$) for seed were treated with 45.0, 67.5, and 90.0 g a.i. ha^{-1} TFM, compared with untreated controls, respectively (Table 1). Hatching rates decreased by 61% and 51% ($F = 32.3$, df = 4,74, $P = 0.0001$) when seeds were treated at 67.5 and 90.0 g a.i. ha^{-1} TFM, compared with untreated controls, respectively. There were no significant differences in sex ratios for TFM-treated seed versus untreated control (Table 1).

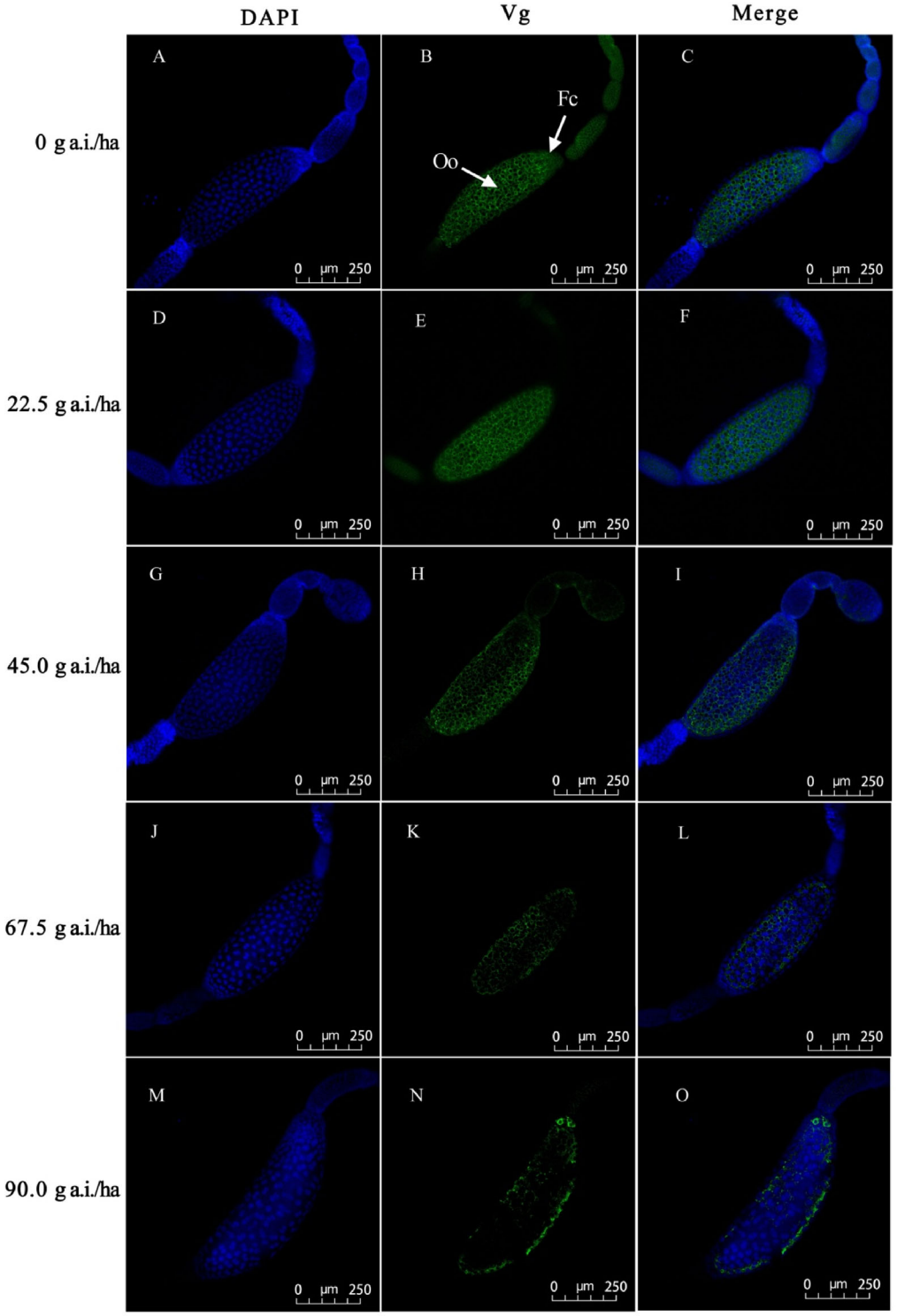

FIGURE 8. Immunofluorescent visualization of failed Vg protein uptake in *Nilaparvata lugens* females at 2 days after emergence (DAE). Ovaries were prepared as described in Section 2.9. (A), (D), (G), (J) and (M) DAPI-stained nuclei (blue) in *N. lugens* ovaries; the latter were derived from rice seeds treated with 0, 22.5, 45, 67.5, or 90 g a.i. ha^{-1} triflumezopyrim (TFM), respectively. (B), (E), (H), (K), and (N) show Vg uptake (green) in *N. lugens* ovaries; rice seedlings were obtained from seeds were treated with 0, 22.5, 45, 67.5, or 90 g a.i. ha^{-1} TFM, respectively. (C), (F), (I), and (L) show merged images. Fc, follicular cell; Oo, oocyte. Scale bars, 250 μm.

3.9 Seed dressing with TFM impacts rice planthopper populations in mechanically transplanted rice fields

To further evaluate the effects of seed dressing with TFM on rice planthopper populations in mechanically transplanted rice plants, we investigated the number of *N. lugens* per 100 hills of rice from July to September in 2017, 2018, and 2019 (Figure 10). In 2017, the number of rice planthoppers per 100 hills was down 30–96%, 43–97%, 54–96%, and 78–95% for 22.5, 45, 67.5, and 90 g a.i. ha^{-1} TFM, respectively, compared with the untreated control from 10 July to 25 September (56 to 133 days after sowing)

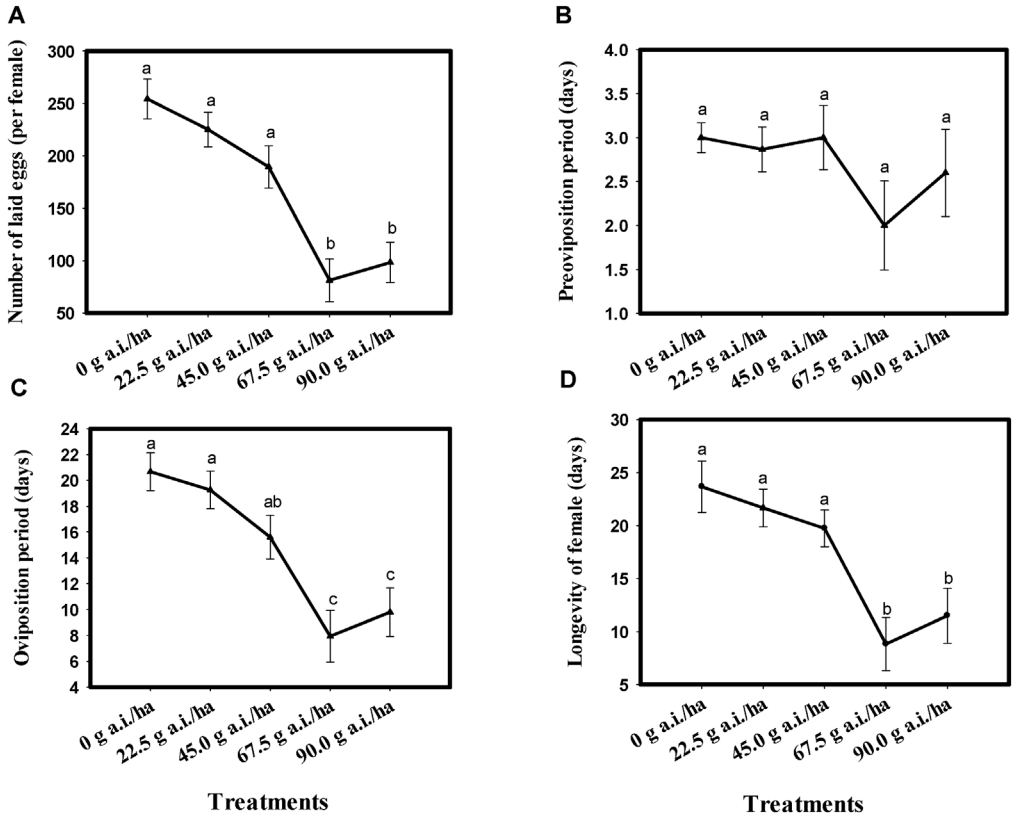

FIGURE 9. Seed dressing with triflumezopyrim (TFM) alters reproductive parameters, longevity and body weight of *Nilaparvata lugens* females. (A) Number of eggs laid. (B) Preoviposition period. (C) Oviposition period. (D) Longevity. Data were acquired from 15 independent replicates and are presented as means ± SEM. Data points labeled with different lowercase letters indicates significance at $P < 0.05$ (Tukey's test).

(Figure 10A) ($F = 234.2$, df = 4, 299, $P = 0.0001$ for TFM concentrations; $F = 42.7$, df = 11, $P = 0.0001$ for survey days). In 2018, *N. lugens* numbers were down 38–97%, 31–100%, 44–100%, and 34–100% for 22.5, 45, 67.5, and 90 g a.i. ha^{-1} TFM, respectively, compared with the untreated control from 11 July to 26 September (56 to 133 days after sowing) (Figure 10B) ($F = 380.7$, df = 4, 299, $P = 0.0001$ for TFM concentrations; $F = 19.2$, df = 11 299, $P = 0.0001$ for survey days). Similarly, *N. lugens* numbers in 2019 were down 21–92%, 40–97%, 60–97%, and 62–97% for 22.5, 45, 67.5, and 90 g a.i. ha^{-1} TFM, respectively, compared with the control from 3 July to 25 September (48 to 124 days after sowing) (Figure 10C) ($F = 387.5$, df = 4299, $P = 0.0001$ for TFM concentrations; $F = 21.8$, df = 11, 299, $P = 0.0001$ for survey days). The data showed that seed dressing with TFM is a highly effective control for *N. lugens* and has a relatively long duration.

4 DISCUSSION

Earlier studies showed that insecticides altered biochemical and physiological processes in rice, thus impacting *N. lugens* feeding and survival.[19,20,39,40] In a previous report, rice planthopper populations were effectively controlled in mechanically transplanted rice fields by treating seeds with 22.5, 45.0, 67.5, and 90.0 g a.i. ha^{-1} TFM. At 112 days after sowing, *N. lugens* control exceeded 90%[14]; however, the mechanisms underlying the control were unclear. We found that seed dressing with TFM enhanced rice resistance, decreased free amino acids, and increased sucrose content. These changes resulted in aberrant *N. lugens* feeding behavior, decreased body weight and longevity in females, lower numbers of offspring, a reduced hatching rate, and lower PGI. Furthermore, seed dressing with TFM was an effective control and was relatively durable in mechanically transplanted fields.

Oxalic acid is most active in inhibiting feeding when delivered as a pinch of salt or as free acid. It is a potent inhibitor of *N. lugens* sucking activity.[41,42] Seed dressing with TFM at 45.0, 67.5, and 90.0 g a.i. ha^{-1} resulted in increased oxalic acid content in rice (Figure 2A). In *Cicer arietinum* (chickpea), elevated amounts of oxalic acid resulted in reduced oviposition, decreased larval survival, and reduced body weight of *Helicoverpa armigera*.[43] Wu *et al.* reported that rice treatment with the insecticide bisultap and the fungicide jinggangmycin resulted in significantly lower oxalic acid content and a concomitant decrease in rice resistance to *N. lugens*.[23] Furthermore, oxalic acid content decreased when rice was treated with the insecticides buprofezin and imidacloprid, which led to a resurgence in populations of *Tryporyza incertulas*.[44] Flavonoids are secondary metabolites that interfere with insect feeding and result in toxicosis of insects.[45,46] In the present study, treatment of seeds with TFM led to increased flavonoid concentrations compared with the untreated control (Figure 2B). In a previous study, flavonoids extracted from the stems of resistant rice inhibited *N. lugens* feeding, and high flavonoid content was associated with *N. lugens* resistance[47]; these results support our findings with TFM-treated seed and flavonoid content. Plant phenolics play an essential role in herbivore defense and are known to limit insect damage by functioning as toxins, deterrents, and antifeedants.[48,49] In the present study, seed dressing with TFM increased the phenolic content of rice plants (Figure 2C). In

Table 1. Effects of triflumezopyrim-treated seed on the number of *Nilaparvata lugens* offspring, hatching rate, sex rate and population growth index

Treatment (g a.i. ha^{-1})*	Number of offspring†	Hatching rate‡	Sex rate‡	PGI§
0	761.1 ± 45.5a	0.85 ± 0.04a	1.85 ± 0.09a	190.2 ± 11.4a
22.5	707.1 ± 57.8a	0.83 ± 0.04a	1.91 ± 0.09a	176.8 ± 14.4a
45.0	594.7 ± 92.5b	0.76 ± 0.05a	1.85 ± 0.11a	148.7 ± 12.8b
67.5	91.3 ± 68.3c	0.33 ± 0.06b	1.82 ± 0.91a	22.8 ± 4.4c
90.0	128.7 ± 89.8c	0.42 ± 0.07b	1.83 ± 0.86a	32.2 ± 5.8c

*Treatments included four different triflumezopyrim concentrations and the untreated control.
† Values labeled with different letters indicate a significant difference at $P < 0.05$ by Tukey's test.
‡ Hatching rates and gender ratios were calculated as described by Ge *et al.*[17].
§ The population growth index (PGI) was expressed as described by Ge *et al.*[17].

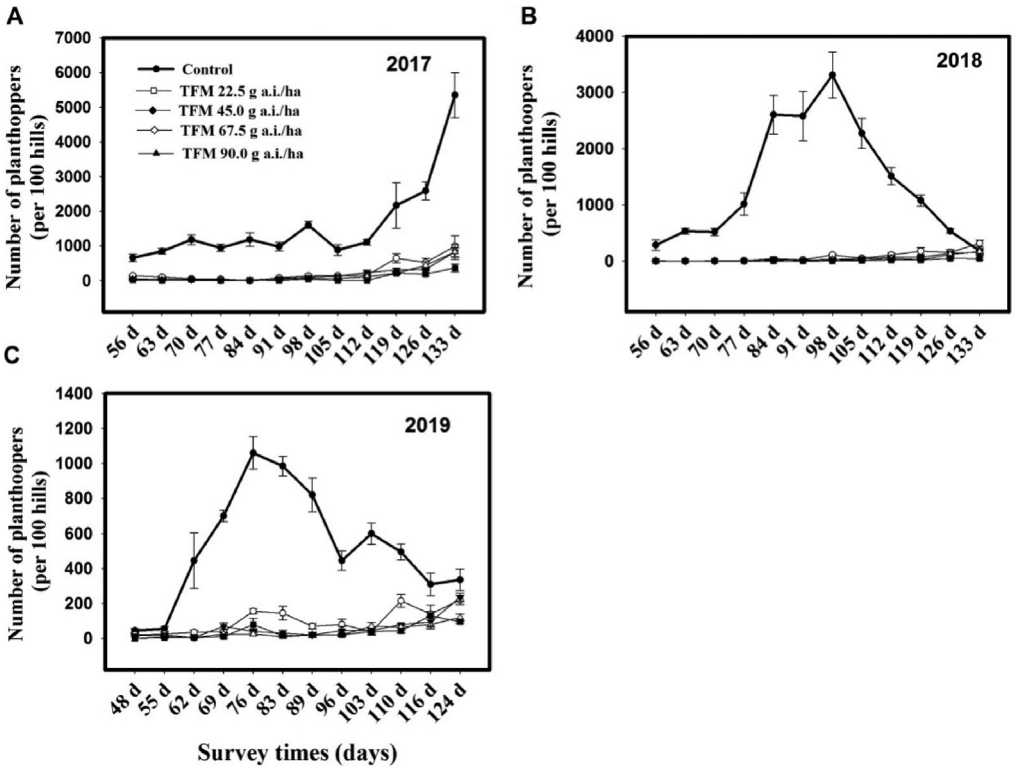

FIGURE 10. Seed dressing with triflumezopyrim (TFM) impacts rice planthopper numbers in mechanically transplanted rice fields. (A–C) Mean number of rice planthopper per 100 hills in 2017 (A), 2018 (B), and 2019 (C). Data were obtained from five replicates and are presented as means ± SEM. Data points labeled with different lowercase letters indicate significance at $P < 0.05$ (Fisher's test).

a related study, total phenolics in resistant rice were significantly higher than in susceptible varieties, and the majority of larvae either died or developed more slowly.[50] Similarly, phenolic compounds in castor bean significantly impacted feeding and growth of *Spodoptera litura* and *Achaea janata* and functioned in induced defense.[51] Callose is a polysaccharide found in the cell wall of higher plants and is often associated with resistance or defense.[52] In the present study, rice plants derived from TFM-treated seed exhibited significantly increased callose production compared with the untreated control (Figure 2D). In the context of *N. lugens* resistance, previous studies have demonstrated that callose deposition inhibits *N. lugens* feeding.[53,54]

Nutrients such as sucrose stimulate *N. lugens* feeding, and amino acids in the host plant may serve as a nitrogen source for the planthopper.[55] Moreover, resistant rice cultivars showed lower amino acid and sucrose content compared with susceptible varieties.[56,57] Our results show that seed treatment with TFM decreased free amino acid and increased sucrose content in rice plants (Figure 2E,F). Nymphs feeding on rice with a high nitrogen content were shown to have a greater body mass, lay more eggs as adults and live longer.[58] It is also noteworthy that total soluble sugar content in highly resistant rice varieties was lower than in susceptible varieties.[57] An increase in free amino acids or a decrease in the C/N ratio stimulates *N. lugens* feeding. In the current study, seed dressing with TFM resulted in a significant increase in the C/N ratio in rice plants, which may contribute to reduced *N. lugens* feeding. Select amino acids may also function to reduce *N. lugens* probing and increase honeydew excretion.[2] We show that *N. lugens* adults preferred to feed on untreated rice compared with rice derived from TFM-treated seed, and honeydew excretion was reduced on

the latter (Figure 3A,B); these results suggest that the decrease in free amino acids might be involved in the reduction of *N. lugens* feeding. Rice volatiles, such as 2-heptanol, 2-heptanone, and linalool, significantly inhibited *N. lugens* feeding and oviposition preference, resulting in significantly reduced honeydew excretion and enemy attraction.[59] However, whether rice plants from seeds dressed with TFM affect *N. lugens* feeding behavior by releasing volatiles needs further study.

Sap-sucking insects are categorized as xylem, phloem, or mesophyll cell feeders, and *N. lugens* is a phloem-feeder.[60] The N4-a and N4-b EPG waveforms indicate intracellular activity in the phloem and phloem sap ingestion, respectively. In this study, we monitored both N4 waveforms in *N. lugens* fed on untreated and TFM-treated rice. The total duration of N4 waveforms (Figure 4E,F) and the number of occurrences of N4-a (Figure 5E) and N4-b (Figure 5F) were significantly lower on rice derived from TFM-treated seed compared with the untreated control. Inhibition of *N. lugens* activity in the phloem was dose-dependent, and there was complete suppression of phloem activity at the highest TFM concentration (90 g a.i. ha^{-1}). EPG data also indicated that seed dressing with TFM prolonged the non-probing phase (Figure 4B). However, TFM had no significant impact on stylet movement (Figures 4C and 5C) or xylem sap ingestion (Figures 4H and 5G).

In a related study, pymetrozine treatment significantly prolonged the non-probing phase and decreased the phloem ingestion by *N. lugens*.[21] Pymetrozine impacts neuroregulation and causes cessation of feeding in sucking insects, which results in starvation.[61] TFM inhibits the nicotinic acetylcholine receptor (nAChR) orthosteric binding site,[6] which functions as the primary receptor for rapid excitatory neurotransmission.[62] Previous studies indicated that pesticides might interfere with feeding behavior in vectors such as aphids (*Myzus persicae*),[63] whiteflies (*Bemisia tabaci*),[64] and rice planthoppers (*N. lugens*).[22] Insect feeding is modulated by complex mechanisms that respond to internal and external signals.[65] The present results demonstrated that seed dressing with TFM significantly increased rice resistance by interfering with *N. lugens* feeding behavior. Accordingly, we speculated that behavioral changes in *N. lugens* caused by the TFM seed-coating agent may be due to disturbed neurotransmission or may be the outcome of an insect's innate response to alterations in insecticide-treated host plants, such as secondary metabolism product and nutritional substance.

Multiple hormonal signals are involved in ovary development in insects.[66] Total protein content in fat bodies and ovaries (Figure 6A,B), JH III and ecdysteroid titers (Figure 6C,D), *Vg* and *JHAMT* expression (Figure 7A,C), and Vg protein levels (Figure 7E) were significantly lower in *N. lugens* feeding on rice plants derived from TFM-treated seed versus the untreated control. Research has indicated that fecundity in female insects is modulated by the production of Vg and vitellin; furthermore, JH also controls Vg synthesis in many insects.[43,67,68] JH regulates ovary development via gonadotropin, modulates the invertebrate endocrine system, and stimulates Vg production.[69–71] Prior studies have also demonstrated that *Vg* upregulation promotes Vg protein synthesis in the fat bodies of *N. lugens* adult females, and stimulates fecundity of *N. lugens* adult females.[16,72] However, recent studies also indicated TFM treatment increased *Vg*, *VgR*, and *EcR* expression level and contents in F_4 female adults in *Sogatella furcifera*, compared with the F_0 generation, promoting growth and reproduction in *S. furcifera*.[73] When seeds were pretreated with TFM, *N. lugens* females had lower JH III titers (Figure 6C), which resulted in reduced Vg levels (Figure 7E), body weight (Figure 6E), and lower fecundity (Figure 9A) compared with females feeding on untreated controls. JH has a role in vitellogenesis within oocytes and is known to induce patency in follicular cells[74–76]; moreover, fertility is positively associated with ovariole numbers in oocytes.[77] Our results show that seed dressing with TFM altered fecundity by interfering with the development of both oocytes and ovarioles (Figure 8). Furthermore, seed dressing with TFM reduced the longevity of *N. lugens* females (Figure 9D) and reduced hatching rates, lowering numbers of offspring and decreasing PGI (Table 1). Ultimately, pretreatment of seed with TFM led to decreased numbers of rice planthoppers in mechanically transplanted rice fields compared with controls (Figure 10). Field survey data indicated that seed dressing with TFM helped reduce the use of chemical pesticides to control the *N. lugens* population in the rice field. The residue content of TFM in treated rice plant stems remained relatively stable (about 0.04 mg kg^{-1}) 71 days after sowing (Figure 1). A large number of rice planthoppers occurred in the rice field after 71 days after sowing. Zhang *et al*. reported that the median lethal concentration (LC_{50}) of TFM is 0.15 mg L^{-1} (0.120–0.181) using seedling-dip or 0.064 mg L^{-1} (0.051–0.082) against the susceptible strain of *N. lugens* (third-instar nymph) using rice-stem drip.[78] Zhu *et al*. also reported that the LC_{50} of TFM is 0.22 mg L^{-1} (0.18–0.26) against the susceptible strain of *N. lugens* (newly emerged female adult) using rice-seedling dip.[22] Accordingly, we inferred that seed dressing with TFM effectively controls *N. lugens* populations, and this may be due to the interaction between the residue toxicity and a secondary metabolism product in plants grown from TFM-coated seed.

5 CONCLUSION

This study was the first to report that seed dressing with TFM effectively controls *N. lugens* by enhancing rice plant resistance mechanisms, decreasing free amino acids, and increasing sucrose contents. These changes resulted in an increase in the non-probing period, interfered with phloem ingestion, disturbed *N. lugens* feeding behavior, inhibited Vg uptake in oocytes and reduced fecundity. In mechanically transplanted rice plant fields, TFM exhibited reasonable control of *N. lugens* when used as a seed dressing, thus presenting a new approach for efficiently controlling *N. lugens* in rice fields.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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