Environmental Science and Pollution Research (2022) 29:30206–30216
https://doi.org/10.1007/s11356-022-18551-1

RESEARCH ARTICLE

Residue dynamics and bio-efficacy of triflumezopyrim against *Nilaparvata lugens* and non-targeted effect on natural enemies in a rice ecosystem

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Received: 16 September 2021 / Accepted: 3 January 2022 / Published online: 8 January 2022

Abstract

Triflumezopyrim (TMP), a mesoionic insecticide, is commonly used for controlling planthoppers in rice. However, the relationship between the TMP residue and toxicity against brown planthoppers (BPHs) has not been studied in detail. We are reporting the dissipation of TMP from rice plant and soil under field conditions. The median lethal dose and median lethal concentration were 0.036 ng per insect and 0.525 mg L^{-1}, respectively. TMP at recommended dose (25 g a.i. ha^{-1}) recorded 1.25 live BPH per hill as against 25.5 per hill in control at 14 days after treatment. TMP was considered to be harmless to the natural enemies, namely, *Cyrtorhinus lividipennis* and *Lycosa pseudoannulata* in the rice ecosystem. The residue of TMP from rice plant and soil was estimated using the QuEChERS method using three different doses (12.5, 25, and 50 g a.i. ha^{-1}). The limit of quantitation (LOQ) of TMP in plant and soil was 5 μg kg^{-1} and 1 μg kg^{-1}, respectively. The maximum content of TMP in soil was less than 1% that of plant content on day 1. The dissipation pattern of TMP both from plant and soil was better explained by the first-order double-exponential decay model (FODED) as compared to the first-order kinetic model. Overall, the half-lives of TMP were ranged from 2.21 to 3.02 days in plant tissues and 3.78 to 4.79 days in soil as per the FODED model. Based on the persistence and toxicity of TMP, we could conclude that TMP will be effective against BPH up to 7–10 days after application. Triflumezopyrim with reasonable persistence and high efficacy could be recommended as an alternate pesticide in BPH management in rice.

Keywords LD_{50} · LC_{50} · Bio-efficacy · Dissipation · Persistent toxicity · Natural enemies

Introduction

Rice planthoppers (Hemiptera: Delphacidae), namely, brown planthopper (BPH) (*Nilaparvata lugens* (Stål)), small brown planthopper (SBPH) (*Laodelphax striatella* (Fallén)), and white-backed planthopper (WBPH) (*Sogatella furcifera* (Horvath)), are the most economically important sucking pests of rice in Asia (Zhang et al. 2015a, b). Planthoppers suck the sap from rice stems to damage the crop by producing symptoms of "hopper burn." These hoppers transmit different viral pathogens, namely, rice grassy stunt virus, rice stripe virus, ragged stunt viruses, rice black streak dwarf virus, etc. (Zhu et al. 2018). Outbreaks of rice planthoppers are common in various Asian countries and cause severe crop losses. BPH can cause up to 60% crop losses in rice (Li et al. 2015). To manage the regular outbreak of planthoppers, farmers go for routine application of synthetic chemicals. Pesticides may be the last resort in integrated pest management, but it is most convenient, efficient, and cost-effective for farmers to manage planthoppers (Endo and Tsurumachi 2001). Pymetrozine, flonicamid, imidacloprid, etc., are the main insecticides against rice planthoppers in India.

Responsible Editor: Gangrong Shi

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The efficacy of these existing molecules is questioned due to the development of insecticide resistance (Yang et al. 2016; Wu et al. 2018). The pest resurgence, the development of insecticide resistance, and the destruction of natural enemies are major problems aroused from the mishandling of chemical pesticides (Matsumura et al. 2008; Wang et al. 2008a, b; Preetha et al. 2010; Zhang et al. 2015a, b). Hence, there is an urgent requisite of new molecules in India as well as around the globe with improved efficacy to manage the devastation caused by BPH.

Triflumezopyrim (TMP) is a mesoionic insecticide (2,4-dioxo-1-(pyrimidin-5-ylmethyl)-3-[3-(trifluoromethyl) phenyl]-3,4-dihydro-2H-pyrido[1,2-a]pyrimidin-1-ium-3-ide), which acts on nicotinic acetylcholine receptor (nAChR). It is grouped under 4E in the Insecticide Resistance Action Committee mode of action classification (IRAC 2021). The neonicotinoid group insecticides block the nicotinic acetylcholine receptor, which might have negative effects on natural enemies, pollinators, poultry, and mammals (Ihara et al. 2017). TMP acts on a different mode of action to kill the pests from existing neonicotinoids. The inhibitory effect on the nAChR upon application of TMP leads to lethargic poisoning among planthoppers as compared to acute excitatory symptoms on neonicotinoid application (Cordova et al. 2016). TMP could be absorbed and translocated easily in rice plant (Fan et al. 2020). TMP application modulates the behavior of BPH and WBPH by prolonging the non-penetration duration and decreasing the phloem sap ingestion duration (Jun et al. 2020). TMP can be recommended in cotton, rice, corn, and soybean crops against planthoppers, leafhoppers, etc. (Yang 2016). A few reports suggest that TMP is more effective against planthoppers than imidacloprid (Guruprasad et al. 2016; Zhu et al. 2018). A sub-lethal concentration of TMP retards the generational growth and reproduction of small brown planthoppers (Zhang et al. 2020).

The non-target toxicity to honey bees and natural enemies is important for any pesticide. TMP falls under the broad IRAC group of neonicotinoids (group 4). Neonicotinoid concentrations in agricultural nectar and pollen can reach levels that influence bee colony reproduction (Whitehorn et al. 2012). Since rice is a self-pollinated crop (Matsui and Kagata 2003), wind and insect pollination is of little significance (Jackson 2008), and TMP application may not pose a direct risk to honey bees and other pollinators. Moreover, the oral LD_{50} of TMP against honey bees is 0.39 mg kg^{-1} (Casida 2018). TMP is generally harmless to natural enemies, namely, *Anagrus nilaparvatae*, *Cyrtorhinus lividipennis*, and spiders (*Pirata subpiraticus*, *Hylyphantes graminicola*, *Pardosa pseudoannulata*, *Ummeliata insecticeps*) but slightly harmful to *Theridion octamaculatum* (Zhu et al. 2018). TMP had no negative effect on red fire ants (*Solenopsis invicta*) (Li et al. 2019).

TMP was recommended by the Central Insecticide Board & Registration Committee in 2018 for its application and use in India to control brown planthoppers and white-backed planthoppers. Similarly, it is registered in the USA, Japan, South Korea, and China to manage the rice hoppers. A wide range of maximum residue limit (MRL) values are fixed by different regulatory agencies. The MRL value of 0.01 mg/kg is fixed by the Japan Food Chemical Research Foundation. The Environmental Protection Agency (EPA) establishes tolerances for the residues of TMP in rice grains and rice hulls of 0.4 ppm and 1.0 ppm, respectively (EPA, USA https://www.federalregister.gov/documents/2017/10/16/2017-22356/triflumezopyrim-pesticide-tolerances, 2017). But, there are limited reports of susceptibility of BPH against TMP and its non-target toxicity in the varying sub-tropical field conditions of India. As India is a major rice producing as well as consuming country of the world, any new molecule that comes in to the market should be of importance to understand the residue dynamics in the Indian rice ecosystem.

The use of pesticides delivers irrefutable advantages for food and nutrition, but it contaminates the environment and may cause risk to human health (Bhanti and Taneja 2007; Zhang et al. 2010). Therefore, it is vital to analyze the dissipation of pesticides to know its persistence as well as effectiveness (Yi and Lu 2006). The non-judicious and extensive use of pesticides contaminates the cropping areas, non-cropping areas, and the ground water (Jiries et al. 2002; Yu and Zhou 2005). Triflumezopyrim is relatively soluble in water (230 mg L^{-1}) and has a moderate octanol–water partition coefficient (log K_{OW}: 1.24). This may cause non-point pollution through leaching and surface run-off. The behavior of residues and their kinetics in the leaf tissues of rice and the manner in which the residues change their aspects in the soil and other matrices of the rice ecosystem is yet to be explored. In a study, TMP residue in rice grain was less than 0.015 mg kg^{-1}, when TMP was used as a seed treatment (Wu et al. 2021). Despite that, residue dynamics of TMP upon foliar application has not been studied extensively in different environmental matrices. The residue of triflumezopyrim from rice plant and soil was extracted using the QuEChERS method and estimated by the LCMSMS instrument (Fan et al. 2020).

Taking these factors into consideration, the experiment was designed to assess the toxicity of TMP against BPH in controlled as well as in field conditions. The non-target toxicity and the residue dynamics of TMP were also assessed. The residue concentration present in rice plants will also help to understand the residue dynamics in the environment and the risk of use involved, as related to the insecticide toxicity.

Materials and method

Instruments and chemicals

The certified reference material of triflumezopyrim (TMP) was purchased from OMC Chemicals, Delhi, India. TMP 10.6% SC (Pexalon™, powered by PYRAXALT™, DuPont, USA) was purchased from a local vendor. Instruments, namely, the liquid chromatography mass spectrometer (LC–MS/MS) (Qsight LX50 (LC) Qsight™ 110 (MS), PerkinElmer, USA), centrifuge (Heraeus Megafuse 16R, Thermo Scientific, Germany), vortex (Vortexer, Heat Throw Scientific, China), nitrogen evaporator (Nitrovap-1LV, Parker Hanifinn, USA, and Nitrovap, Athena technologies, India), homogenizer (T25 digital Ultra-Turrax®, IKA, Germany), pH and EC meter (PCSTestr™35, Eutech Instruments, Oakton, Singapore), etc., were used in the experiment. LCMS grade solvents from J.T. Baker, India, were procured. All other chemicals and solvents were of analytical or highest grade and procured from Merck, India.

Laboratory bioassays

Insects

BPH adults (4 ± 1 day old) and nymphs (3 ± 1 day old) used in the study were originally collected from the field of ICAR-NRRI, Cuttack, Odisha. The insects were reared a minimum of three generations on susceptible rice seedlings (variety: TN1, *Indica* type) at 28 ± 2 °C, 75 ± 5% relative humidity, and a 14:10-h light:dark photoperiod before use.

Toxicity bioassays

The toxicity of TMP to BPH was assessed using topical application method, and lethal dose (LD_{50}) was calculated (Fukuda and Nagata 1969). The doses required to cause 10–90% mortality of planthopper adults were tested as proposed by Wang et al. (2008a, b). TMP standard was dissolved in acetone and diluted to have eight different doses, and acetone was used as the control. A 0.2-µl droplet of each dose (equivalent to 0.001, 0.005, 0.01, 0.02, 0.1, 0.2, 0.4, 0.6 ng/insect) was applied topically onto the dorsal thorax of an anesthetized macropterous adult BPH female (4 ± 1 day old) using Hamilton's repeating syringe (PBS600-1 dispenser with 1700 Series Syringe, Nevada, USA). A set of 10 adults was considered to be one replicate, and each dose had five replicates. Three plastic cups of 9 cm in diameter and 11 cm in height were used for this experiment (Fig. S1). A hole was made at the base of the first cup to insert three rice seedlings (7–10 days old). This cup along with rice seedlings was put into the second cup which was filled with enough water to dip the root of the rice seedlings. In between two cups, a support was given to keep the system stable. The third cup was used to cover the first cup with seedlings and small punctures were made for ventilation. After treatment, the insects were released into these cups and were maintained at 28 ± 2 °C, 75 ± 5% relative humidity, and a 14:10-h light:dark photoperiod. The number of dead adults was counted at 24 h. The lethal dose was estimated by Probit regression analysis using EPA Probit Analysis Program (version 1.5) (Londingkene et al. 2016).

Toxicity of TMP against BPH was also analyzed following the IRAC susceptibility test (IRAC Susceptibility Test 05) with minor modifications (IRAC; http://www.irac-online.org/content/uploads/Method_005_v4.1.pdf. Visited February 12, 2020) and lethal concentration (LC_{50}) was calculated. Ten rice seeds were sown in plastic cups that contain alluvial soil. Rice seedlings with four leaves were used for bioassay. To cover the soil surface of the plastic cups, cooled agar powder solution (37 °C) was poured on the soil surface. Rice seedlings (upside down) were dipped into nine concentrations of insecticide solution (7, 5, 3, 2, 1, 0.5, 0.25, 0.1, 0.01 mg L^{-1}) for 30 s and kept at room temperature (approximate 15 min) until the seedling dried. Ten third instar nymphs were transferred onto the rice seedling and the cup was covered with a transparent plastic tube with a plastic mesh above. A test was replicated five times. There was a control treatment where water was applied. Dead BPHs were counted after 24, 48, and 72 h after treatment (HAT). The mortality data were analyzed by Probit regression equation using EPA Probit Analysis Program (version 1.5).

Experimental site and design

The field experiment was done at the experimental field of ICAR-National Rice Research Institute, Cuttack, India (20° 45′ N latitude, 85° 93′ E longitude and 36 m altitude) during the rice crop season of August to December 2020. The weather parameters are listed in Fig. S2. The field soil was sandy clay loam texture (alluvial soil) and AericEndoaquept Type. The pH, electrical conductivity (EC), and organic carbon content of soil were 6.6 ± 0.2, 0.29 ± 0.05 ds min^{-1}, and 0.61 ± 0.02%, respectively. The experiment was conducted in randomized block design with 4 replications. The plot size for each replication was 9 m × 3 m and separated with an isolation bund. Rice seedling was planted in 20-cm row spacing and 15-cm plant to plant spacing. Agronomic practices were followed uniformly across the different treatments. Fertilizers were applied at the recommended dose ($N:P_2O_5:K_2O$::80:40:40 kg ha^{-1}), half dose of N and full dose of P and K were applied as basal application, and the remaining dose of N was applied in 2 equal splits, one at the tillering stage and another at the panicle initiation stage. Three doses

of TMP 10.6 SC, namely, TMP at the recommended dose (T100, 25 g a.i. ha^{-1}), half of the recommended dose (T50, 12.5 g a.i. ha^{-1}), and double the recommended dose (T200, 50 g a.i. ha^{-1}), were applied 45 days after transplanting (DAT), i.e., the mid-tillering stage. Insecticide applications were carried out using a high-volume knapsack sprayer fitted with a hollow cone nozzle. The spray volume was 500 L of water per hectare. Along with the three pesticide treatments, an untreated control was kept, where water was sprayed.

Field efficacy of TMP and its effect on natural enemies

Pre-count of BPH was taken just before spraying. Pest count was taken 3, 7, and 14 days after the spraying of TMP. Three rice plants were tagged in each plot for pest count. Natural enemy population, green mirid bugs (*Cyrtorrhinus lividipennis*), and wolf spiders (*Lycosa pseudoannulata*) were counted from 10 tagged rice plants in each plot. The reductions in populations of natural enemies were calculated. Based on these data, the treatments were classified according to the International Organization for Biological and Integrated Control (IOBC) classes of toxicity (Boller et al. 2005).

Pesticide extraction and clean up

Plant

The representative samples of soil and plant were collected at 0 (2 h after spraying), 1, 3, 5, 7, 10, 15, and 30 days after application of TMP and freshly collected samples were used for TMP extraction (Pandey et al. 2020). Rice plants were cut at 5 cm from the base and the whole plant (100 g) from each treatment was finely cut, macerated in a household mixture grinder. For each sample, the grinder was cleaned and cooled in an ice bucket before grinding. Macerated plant tissue (2 g) leaves were taken in a 50-mL centrifuge tube and homogenized by adding 5 mL water and 10 mL acetonitrile (Wu et al. 2021). Then, 1 g of NaCl and 4 g of $MgSO_4$ were added in each tube, vortexed for 5 min, and centrifuged at 5000 rpm for 5 min. An aliquot of 2 mL was transferred in an Eppendorf tube containing 300 mg of anhydrous magnesium sulfate and 50 mg of silicated PSA. Ten milligrams of activated charcoal was added to remove chlorophyll pigments. Afterwards, it was vortexed for 1 min and then centrifuged at 3000 rpm for 5 min. Finally, samples were filtered through 0.22-µ PTFE filter for analysis.

Soil

TMP from soil samples was extracted as per the modified QuEChERS method (Pandey et al. 2020). Soil samples (500 g) were well collected from 0 to 15 cm depth and mixed properly, and a representative sub-sample of 10 g was taken in a 50-mL centrifuge tube and 10 mL of acetonitrile was added. One gram of NaCl and 4 g of $MgSO_4$ were added in each tube, vortexed for 5 min, and centrifuged at 5000 rpm for 5 min. An aliquot of 2 mL was transferred to a 15-mL centrifuge tube containing 300 mg of anhydrous $MgSO_4$ and 50 mg of silicate PSA. The samples were vortexed for 1 min and centrifuged at 5000 rpm for 5 min. Later, the samples were filtered through a 0.22-µ PTFE filter for analysis.

Instrument parameters

Ultra-performance liquid chromatography system with an autosampler, a binary pump, and a vacuum degasser was used for residue analysis. A C18 column (100 mm × 2.1 mm, 3 µm particle size) was used to separate the target compound. The mobile phase was in gradient mode with two solvent systems (A) water (containing 0.1% formic acid and 5 mM ammonium formate) and (B) methanol (containing 0.1% formic acid and 5 mM ammonium formate). The flow rate was 0.4 ml min^{-1} throughout the run time. The optimized gradient elution program was started with 90% A, and it was decreased to 30% A within 5 min of injection followed by it being decreased to 5% A within 14 min of injection and held for 2.5 min, subsequently increased to 90% A within 17.50 min injection, and held for another 2.10 min. The column was finally re-equilibrated with 90% A and held for 2 min before the next run. The injection volume was 7 µL and the column oven temperature was 45 °C.

Mass spectrometric detection was carried out using Qsight™ 110 fitted with an electrospray ion source (ESI) operating in positive ionization and multiple reaction monitoring (MRM) mode. Identification and quantification of analyte were achieved by measuring mass transitions with *m*/*z* 399.10/278.10 and *m*/*z* 399.10/306.10 as quantifier and qualifier ions, respectively. ESI parameters were capillary voltage of 5500 V, hot surface-induced desolvation (HSID) temperature was 320 °C, and nebulizer gas temperature was 250 °C. The entrance voltages (EV) were 49 and 51 V and collision energies (CE) were 39 and 28 V for quantitative transition and confirmation transition, respectively. The retention time of TMP was 4.7 ± 0.1 min.

Method validation

SANTE guidelines (SANTE/12682/2019) (SANTE 2019) were followed for method validation of TMP. The residues of TMP were quantified by LC–MS/MS. Linearity, matrix effects (ME), limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision were estimated. To evaluate linearity, solvent-matched and matrix-matched calibration standards of 6 different concentrations (1–200 µg L^{-1}) of TMP were constructed. Matrix effect was calculated

as ME % = (Slope of matrix-matched calibration curve/slope of solvent calibration curve – 1) × 100. The matrix-matched calibration standard curve was used for further calculation. LOD is the lowest concentration of target analyte in different samples resulting to a signal-to-noise (S/N) ratio of 3:1. LOQ is the lowest concentration of target compound in the samples that could be quantified with acceptable precision and accuracy with an S/N ratio of 10:1. A pesticide recovery study (accuracy) was carried out at LOQ levels (1 ng g^{-1} for soil and 5 ng g^{-1} for plant) as per SANTE guidelines (SANTE 2019). The precision was estimated by measuring % relative standard deviation (% RSD) of the six replicates at LOQ levels in soil and plant.

Statistical analysis

LD_{50} and LC_{50} values, 90% confidence limits, χ^2, and regression equations were calculated by the Probit method using EPA Probit Analysis Program (version 1.5) to determine the toxicity of triflumezopyrim to BPH (Bliss 1935). The toxicity data of triflumezopyrim against BPH and natural enemies were square root transformed to satisfy assumptions of normality prior to being analyzed. One-way ANOVA followed by Tukey's honesty significant difference (HSD) test was done to find out the effect of different doses against BPH and natural enemies using SAS (http://stat.iasri.res.in/sscnarsportal/main.do). Mean survival data of BPH on triflumezopyrim treatment were fitted in regression equation using SAS (http://stat.iasri.res.in/sscnarsportal/main.do). The model parameters were determined.

The residue data was fitted in both linear (first-order kinetic model, FOK) and non-linear models (first-order double-exponential decay model, FODED) (Sarmah and Close 2009). The FODED model was considered assuming that the pesticide in solution phase material dissipates faster than the pesticide in sorbed phase.

First-order kinetic model (FOK):

$$X = X1Exp(-K1t) \quad (1)$$

where X is the pesticide concentration (μg kg^{-1}) at time t (d) after application, $X1$ is the initial concentration (μg kg^{-1}), and $K1$ is the first-order rate constant (d^{-1}).

First-order double-exponential decay model (FODED):

$$X = X1Exp(-K1t) + X2Exp(-K2t) \quad (2)$$

where $X1$ and $X2$ are constants representing pesticide concentrations initially distributed between two phases. $K1$ and $K2$ are dissipation rate constants in these two phases, respectively.

Parameters for the FODED model were obtained from the best-fit models using Microsoft Excel Solver (Microsoft, 2019) using a generalized reduced gradient (GRG2) non-linear optimization code. The FOK model was fitted using the log-transformed residue data in Microsoft Excel. The best-fit parameters were obtained by minimizing the sum of squares of the residuals (SSRes) between measured and fitted values. Apart from regression coefficient, root mean square error (RMSE) and coefficient of residual mass (CRM) were calculated to find out the best-fit model as per the previous literature (Sarmah and Close 2009). The values of RMSE and CRM in different models close to "zero" indicate good prediction by the model with respect to observed values. Estimation of 50% (DT50) dissipation times of initial applied concentration for triflumezopyrim was also calculated as per the previous literature (Sarmah and Close 2009).

Results and discussion

Bio-efficacy of TMP against BPH

TMP was effective against BPH as observed in the topical application method. The LD_{50} was calculated from the dose-mortality response curve and it was 0.036 ng TMP per insect, at 24 h after application (Table 1). The dose-mortality response curve was fit to determine the lethal doses as the calculated χ^2 value was less than that of the table value. The concentration-mortality curve was constructed for BPH

Table 1 Susceptibility of brown planthopper to triflumezopyrim

	Number of insect tested	Dose (ng per insect)/ concentration (mg L^{-1})	95% confidence limits		Slope	Standard error	χ^2 calculated	df	$p > \chi^2$
			Lower	Upper					
Lethal dose (LD_{50})	400	0.036	0.023	0.054	0.734	0.078	8.900	7	0.259
Lethal concentration (LC_{50})									
24 HAT	270	0.525	0.296	0.872	0.728	0.105	3.190	8	0.921
48 HAT	270	0.131	0.057	0.237	0.708	0.111	5.522	7	0.597
72 HAT	270	0.024	0.004	0.060	0.613	0.122	0.659	6	0.995

HAT hours after treatment

against TMP at 24, 48, and 72 h after treatment (HAT) and LC_{50} were 0.525, 0.131, and 0.024 mg L^{-1}, respectively (Table 1). The 95% confidence limits, χ^2 values, and regression slopes are listed in Table 1. The efficacy study suggests TMP at low concentration will be able to protect rice plants from BPH incidence.

The LD_{50} of TMP against BPH is low as compared to other insecticides reported earlier. For example, the LD_{50} of imidacloprid was 0.120 ng/insect against susceptible laboratory strains and 8.740 ng/insect against resistant laboratory strains of BPH (Zewen et al. 2003). This implies that TMP can provide a higher mortality of BPH at a very low dosage in the field. Similarly, LC_{50} of TMP was 0.525 mg L^{-1} indicating that TMP is highly effective against BPH. In a previous study in China, LC_{50} of TMP against planthoppers in rice dipping treatment was 0.535 mg L^{-1} 24 h after application (Fan et al. 2020).

TMP can be an alternative to selective insecticides (neonicotinoids, avermectins, pymetrozine, and buprofenzin) to manage the BPH population. These pesticides were unable to reduce planthopper density in Vietnam and it was reported that BPH had developed resistance against them (Matsukawa-Nakata et al. 2019). It was also found that emergence of insecticide resistance in BPH against eight insecticides (imidacloprid, dinotefuran, nitenpyram, pymetrozine, buprofenzin, etofenfox, fenobucarb, and fipronil) (Khoa et al. 2018). Imidacloprid resistance was found in BPH which had resulted in a gradual decrease of efficacy in rice planthopper management (Matsumura et al. 2008; Wang et al. 2008a, b). For example, the resistance ratios (RR) for neonicotinoid insecticides, viz., imidacloprid, thiamethoxam, and clothianidin, were 35.1, 10.8, and 4.9 respectively, against BPH in Godavari Delta of Andhra Pradesh (Lakshmi et al. 2010). In another study in southern Karnataka, India, the resistance ratios varied greatly among the BPH populations, viz., imidacloprid (as high as 13.50 RR), clothianidin (as high as 4.86 RR), dinotefuran (as high as 2.22 RR), acephate (as high as 5.32 RR), thiamethoxam (as high as 2.19 RR), and buprofezin (as high as 5.43 RR) (Basanth et al. 2013). Field populations of BPH had shown resistance to acephate, thiamethoxam, and buprofezin (maximum *RR*: 20.92, 14.99, and 18.09, respectively) in South India (Malathi et al. 2017). The development of insecticide resistance is due to the extensive and non-judicious use of these insecticides for suppressing BPH. This has resulted in frequent control failures in the field. Thus, our study exemplifies the use of TMP as a substitute of these insecticides that have reported cases of insecticide resistance.

Field efficacy of TMP against BPH

The survival pre-count data of BPH before treatment was statistically non-significant indicating a homogeneous population of BPH in the field and the population was above the economic threshold level (5–10 nymphs or adults/hill). It was ranged from 30.5 to 32.75 per hill (Table 2). The mean survival of BPH in TMP-treated plots was ranged from 2.25 to 6.25, 1.25 to 4.00, and 0.25 to 2.50 per hill on 3, 7, and 14 days after spraying (DAS), respectively. The effect of different concentrations of triflumezopyrim against BPH was plotted in linear regression (Fig. S3). The regression parameters are presented in Table 2. The regression model is well fitted (3 days after spray: F: 25.83, R^2: 0.648, p: < 0.0002; 7 days after spray: F: 21.96, R^2:0.611, p: < 0.0003; 14 days after spray: F: 22.97, R^2: 0.621, p: < 0.0003). The results suggested that all the treatments proved to be significantly superior over control.

The results revealed that TMP is an efficient insecticide for the management of planthoppers in the rice field. Earlier, TMP at 25 g a.i ha^{-1} or TMP 10.6% SC at 237 mL ha^{-1} was used to manage the planthopper populations in the field (Guruprasad et al. 2016; Suri and Makkar 2018; Kumar et al. 2020). TMP 10% SC (225 mL ha^{-1}) showed a high level of insecticidal activity against BPH as compared to 25% pymetrozine SC (300 mL ha^{-1}) and thiamethoxam WDG (90 g ha^{-1}) (Zhang et al. 2019). Mesoionic insecticides like TMP inhibit the orthosteric binding site of the nAChR and the inhibitory action of TMP is rapid and prolonged in nature (Cordova et al. 2016). Being a different mode of action, it requires low concentration to be effective against a number of pests. Rice planthoppers mainly attack on leaf sheath, and pesticides with good translocation properties only will be effective to manage these pests. Triflumezopyrim under foliar treatment distributes itself in different parts of the plants (Fan et al. 2020). In the same literature, it was suggested to apply triflumezopyrim on target sites to avoid loss. In another study, triflumezopyrim as seed treatment disturbed the non-probing period and feeding behavior of BPH and fecundity was also reduced (Wu et al. 2021).

Effect on natural enemies

The mean population of green mirid bugs per hill was ranged from 7.00 to 7.75 before TMP treatment (Table 3). The mean population was 6.00–7.25, 6.00–6.75, and 5.25–5.75 per hill on 3, 7, and 14 days, respectively, after TMP treatment. The mean population of spiders per 10 hills was ranged from 6.50 to 8.00 before TMP application whereas it ranged from 6.25 to 7.50, 6.00 to 7.25, and 5.25 to 6.25 per 10 hills on 3, 7, and 14 days after TMP application, respectively (Table 3.). The population of green mirid bugs and wolf spiders was found to be homogeneous before and after treatment as there was no significant difference among the treatments. Hence, TMP is considered to be harmless to green mirid bugs and wolf spiders.

Table 2 Mean survival data of brown planthopper on triflumezopyrim treatment in field condition

Treatment	Brown planthopper population (insect per hill)			
	Pre-count	3 DAS	7 DAS	14 DAS
T50	31.75 ± 3.40 (5.72)	6.25 ± 1.25 (2.69)[b]	4.00 ± 1.41 (2.22)[b]	2.50 ± 1.29 (1.85)[b]
T100	32.75 ± 0.57 (5.81)	4.00 ± 1.15 (2.22)[ab]	2.50 ± 0.57 (1.87)[ab]	1.25 ± 1.00 (1.47)[ab]
T200	30.50 ± 2.88 (5.61)	2.25 ± 0.95 (1.79)[a]	1.25 ± 0.50 (1.49)[a]	0.25 ± 0.50 (1.10)[a]
Control	31.50 ± 1.29 (5.70)	34.25 ± 2.21 (5.93)[c]	33.00 ± 1.82 (5.83)[c]	25.50 ± 1.29 (5.15)[c]
p-value	0.7088	< .0001	< .0001	< .0001
CV (%)	4.16	7.30	5.99	8.88
Tukey HSD at 5%	NS	0.509	0.377	0.469
Regression parameters of toxicity of triflumezopyrim against brown planthopper				
F value	0.35	25.83	21.96	22.97
Error DF	14	14	14	14
Root MSE	0.22	1.06	1.19	1.11
R^2	0.024	0.648	0.611	0.621
Adjusted R^2	−0.045	0.623	0.583	0.594
Intercept	5.71 ± 0.09	4.68 ± 0.41	4.41 ± 0.46	3.84 ± 0.43
Slope	−0.002 ± 0.003	−0.072 ± 0.014	−0.075 ± 0.016	−0.072 ± 0.015
p-value	0.5642	0.0002	0.0003	0.0003

Figures in parentheses are square root-transformed values. Means with the same letters are not significantly different using Tukey's honest significant difference

DAS days after spraying

Table 3 Effect of triflumezopyrim on mean survival of Mirid Bugs and Spiders in rice field

Treatment	Mirid bugs (number per hill)				Spiders (number per 10 hills)			
	Pre-count	3 DAS	7 DAS	14 DAS	Pre-count	3 DAS	7 DAS	14 DAS
T50	7.25 ± 0.50 (2.87)	6.00 ± 0.50 (2.63)	6.25 ± 0.95 (2.69)	5.50 ± 0.57 (2.87)	8.00 ± 0.81 (3.00)	6.75 ± 0.50 (2.78)	6.50 ± 0.57 (2.74)	5.75 ± 0.95 (2.59)
T100	7.75 ± 0.50 (2.96)	7.25 ± 0.50 (2.87)	6.00 ± 0.81 (2.64)	5.25 ± 0.50 (2.96)	6.75 ± 1.89 (2.77)	6.75 ± 0.50 (2.77)	6.25 ± 0.50 (2.69)	5.25 ± 0.50 (2.50)
T200	7.00 ± 0.00 (2.83)	6.25 ± 0.50 (2.69)	6.75 ± 0.50 (2.78)	5.75 ± 0.95 (2.83)	6.50 ± 1.29 (2.73)	7.00 ± 0.00 (2.83)	6.00 ± 0.81 (2.64)	5.50 ± 0.57 (2.55)
Control	7.50 ± 0.57 (2.91)	7.75 ± 0.50 (2.96)	7.50 ± 1.29 (2.91)	6.25 ± 0.50 (2.91)	7.75 ± 0.50 (2.96)	7.50 ± 0.57 (2.91)	7.25 ± 0.50 (2.87)	6.25 ± 0.50 (2.69)
p-value	0.2427	0.1134	0.1880	0.2427	0.3379	0.1610	0.1186	0.1650
CV (%)	2.97	6.76	6.09	2.97	8.26	2.97	4.48	4.35
Tukey HSD at 5%	NS	NS	NS	NS	NS	NS	NS	NS

Figures in parentheses are square root-transformed values

DAS days after spraying, *T50* triflumezopyrim at half of the recommended dose (12.5 g a.i. ha^{-1}), *T100* triflumezopyrim at the recommended dose (25 g a.i. ha^{-1}), *T200* triflumezopyrim at double the recommended dose (50 g a.i. ha^{-1})

TMP is a safe insecticide and the population of the beneficial arthropods is not affected by its contact exposure. Our findings are in agreement with an earlier research (Zhu et al. 2018). They reported that there was only 10% mortality of mirid bugs on TMP exposure for 48 h in a laboratory study. TMP did not have any negative activity on the fitness of *Solenopsis invicta* at an exposure level of 0.5 µg mL^{-1} (Li et al. 2019). Pesticide toxicity against natural enemies should be tested under the field conditions and could be classified in three categories, namely "N, harmless or slightly harmful" (0–50% reduction of population); "M, moderately harmful" (51–75% reduction of population), and "T, harmful" (75% reduction of population), respectively, as per the IOBC (Boller et al. 2005). In this study, the percent reduction of

green mirid bugs and wolf spiders was less than 20%. So, all the three doses could be considered harmless or slightly harmful according to IOBC classes.

Method validation

The regression coefficient for the solvent match curve was 0.999 and that of the matrix match curve was 0.989 and 0.998 for plant and soil, respectively, for 1–200 µg L^{-1} (Table S1). The matrix effect for plant and soil was 17% and 12%, respectively. The limit of quantification (LOQ) of TMP from plant and soil was 5 µg kg^{-1} and 1 µg kg^{-1}, respectively. The recovery percentages for plant and soil at LOQ were 78 ± 6% and 84 ± 7%, respectively. The RSDs of recovery of TMP from plant and soil were in acceptable ranges, i.e., within 20% as per SANTE guidelines (SANTE 2019). Representative chromatograms are presented in Fig. S4.

Dissipation kinetics of TMP in rice ecosystem

The initial deposits of TMP in T50, T100, and T200 treatments were 4.59, 7.38, and 10.29 mg kg^{-1}, respectively, and the residue was declined to 0.01, 0.02, and 0.04 mg kg^{-1}, respectively, on day 30 (Fig. 1). The initial deposits of TMP in soil were 10.53, 12.18, and 15.65 µg kg^{-1} in T50, T100, and T200, respectively, and then it was increased to 23.10, 28.98, and 61.60 µg kg^{-1}, respectively, on day 1. The deposits of TMP in soil in T50, T100, and T200 treatments were declined to 1.39, 2.15, and 5.42 µg kg^{-1} 30 days after application (Fig. 1).

The residue kinetics of TMP in plant and soil was fitted in two models: first-order kinetics (FOK) and first-order double-exponential decay model (FODED). The model parameters including rate constant, coefficient of determination, sum of squares of the residuals (SSres), root mean square error (RMSE), coefficient of residual mass (CRM), and residual half-life of TMP in different treatments are listed in Table 4. Based on the above parameters, the loss of TMP from plant can be well described by the FODED model. The RMSE, CRM, and SSRes were less in the FODED model as compared to the FOK model. The regression coefficient was 0.99 in the FODED model in all three doses. The residual half-life of TMP in plant was 2.21, 2.69, and 3.02 days for T50, T100, and T200, respectively. Similarly, the loss of TMP in soil was fitted well in the FODED model with the regression coefficient value of 0.95 in all three doses. The calculated residual half-life was 3.78, 4.03, and 4 for T50, T100, and T200, respectively.

The residues of TMP were higher in plant than those of the soil in all days of sampling. The maximum residue of TMP in soil was observed on day 1 and it was less than 1% that of plant content. Rice plant serves as the target matrix of the insecticide. Our findings corroborate with Fan et al. (2020) who reported that TMP is absorbed and translocated

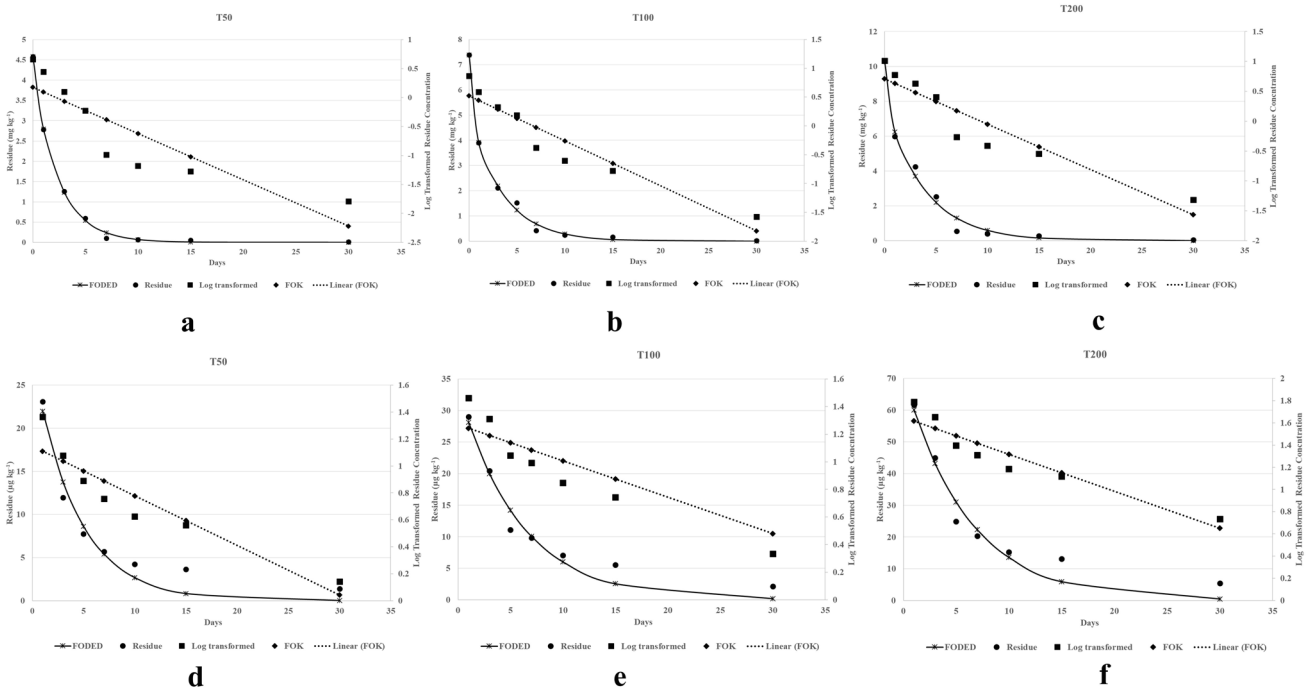

Fig. 1 Residue dynamics of triflumezopyrim (observed and model values) from rice leaf tissues and soil. Linear (first-order kinetic model, FOK) and non-linear models (first-order double-exponential decay model, FODED) were used. **A** Plant_T50, **B** Plant_T100, **C** Plant_T200, **D** Soil_T50, **E** Soil_T100, **F** Soil_T200

Table 4 Model (linear and non-linear) parameters of triflumezopyrim dissipation from plant and soil

First-order kinetic model (FOK)						
Variables	Plant			Soil		
	T50	T100	T200	T50	T100	T200
X_1 (μg kg^{-1})	0.18	0.52	0.71	1.15	1.27	1.65
K_1 (day^{-1})	0.08	0.08	0.076	0.04	0.03	0.03
SSRes	1.31	0.47	0.55	0.12	0.14	0.14
R^2	0.77	0.89	0.88	0.87	0.91	0.88
RMSE	75.96	141.58	747.19	17.16	14.90	10.93
CRM	$2.2*10^{-4}$	$1.36*10^{-3}$	$7*10^{-3}$	$5.3*10^{-4}$	$4*10^{-2}$	$1.4*10^{-2}$
DT_{50} (days)	8.67	8.87	9.14	18.83	19.3	20.81
First-order double-exponential decay model (FODED)						
Variables	Plant			Soil		
	T50	T100	T200	T50	T100	T200
X_1 (μg kg^{-1})	0.36	2.16	2.19	2.19	2.19	2.19
K_1 (day^{-1})	12.71	12.71	12.72	12.71	12.72	12.72
X_2 (μg kg^{-1})	4.22	5.22	8.09	27.77	33.42	70.88
K_2 (day^{-1})	0.41	0.29	0.26	0.23	0.17	0.16
SSRes	0.02	0.18	1.11	17.54	24.25	124.96
R^2	0.99	0.99	0.99	0.95	0.95	0.95
RMSE	4.65	7.58	12.28	17.94	14.32	14.89
CRM	$7*10^{-4}$	$2*10^{-3}$	$8.2*10^{-3}$	$8.2*10^{-2}$	$4.7*10^{-2}$	$5*10^{-2}$
DT_{50} (days)	2.21	2.69	3.02	3.78	4.03	4.79

X_1 is the initial concentration, and K_1 is the first-order rate constant in the FOK model; X_1 and X_2 are constants representing initial pesticide concentrations in two phases with dissipation rate constants of K_1 and K_2 in the FODED model

SSRes sum of squares of the residuals, R^2 regression coefficient, *RMSE* root mean square error, *CRM* coefficient of residual mass, DT_{50} residual half-life, *T50* triflumezopyrim at half of the recommended dose (12.5 g a.i. ha^{-1}), *T100* triflumezopyrim at the recommended dose (25 g a.i. ha^{-1}), *T200* triflumezopyrim at double the recommended dose (50 g a.i. ha^{-1})

within rice plant. There is very little chance of translocation of TMP from plant to soil. An increase in concentration of TMP in soil at day 1 may be due to the dislodgement of loosely adsorbed surface residues on plant due to wind or dew at night. TMP can be safe to soil environment as it was detected at very low concentration in rhizosphere soil.

TMP followed a fast degradation initially and then later a slow degradation as described in the FODED model. At the initial phase, loosely held TMP in plant sap might undergo a combination of physical, photochemical, and volatilization processes at a faster rate. In the second phase, pesticide may be adsorbed within the plant compartment or conjugate with different metabolites which makes it unavailable for degradation. In the second phase, dissipation may occur due to the combination of biotic, abiotic, and enzymatic hydrolysis (Sarmah and Close 2009). Previously, the dissipation of thiamethoxam from soil was reported to be biphasic with very fast dissipation during the initial period followed by slower loss in soil (Gupta et al. 2008). In another study, the dissipation of gibberellic acid (GA3), 6-benzylaminopurine, forchlorfenuron, and ethephon in grape berries was non-linear two-compartment first + first-order kinetics and the dissipation was faster at the initial phase, and it was slowed down with the passage of time (Ugare et al. 2013). There was a 4–fivefold of difference in residual half-life between the two models. There is no universal regression coefficient to choose the best model but regression coefficient < 0.7 in the FOK model should be avoided as per the European regulators (European Commission 1995). The FOK model gives greater effect to later sampling times on the result of the linear regression due to the log transformation (Wolt et al. 2001). In this study, we may conclude the FODED model was best to describe the TMP loss.

The loss of pesticide from plant was 98, 94, and 94% in T50, T100, and T200 treatments, respectively, within 7 days after pesticide application. Similarly, there was 75, 66, and 66% loss of TMP in T50, T100, and T200 treatments, respectively, within 7 days after pesticide application. The TMP concentration on day 7 was 0.42 mg kg^{-1} and the LC_{50} was 0.53 mg L^{-1}; hence, it can be related that TMP will be effective up to 7 days. Furthermore, if there is another wave of planthopper incidence, it may not be effective as the concentration of the TMP in the

plant sap will not be enough to provide protection against BPH. Hence, the persistent toxicity of TMP may last for 7–10 days in the plant sap.

Conclusion

The LD_{50} of TMP was 0.036 ng per insect and the LC_{50} was 0.525 a.i. mg L^{-1} at 24 h after treatment. The field efficacy data demonstrated that TMP could serve as an excellent substitute of other insecticides recommended against BPH. It can also act as an alternate insecticide for insecticide rotation technique in insecticide resistance management program. TMP can be considered as harmless to the biocontrol agents of the BPH. Acetonitrile extraction-based residue analysis method to quantify TMP residues in rice plant and soil is reported. The half-lives of TMP was 2.69 days in the rice plant and which may be sufficient to manage the BPH attack. This work will be thus useful in the safe, judicious, and efficient utilization of TMP to manage BPH incidence in the rice-growing belts of India.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11356-022-18551-1.

Acknowledgements The authors duly acknowledge the technical and financial support provided by the Director, ICAR-National Rice Research Institute, Cuttack, under ICAR-NRRI/In-house Project 3.5/2020-2025 scheme (fund received by Dr. Totan Adak). The authors sincerely thank Mr. Arnab Malik, Technical Assistant, and Mr. Rakesh Kumar Nayak, Graduate Assistant, EAP-261, ICAR-NRRI, Cuttack, for their technical help.

Author contribution TA and HPM formulated the idea of this experiment; RM and SSP conducted the experiments; BG, GGPP, and TA analyzed the data; RM, BG, GGPP, AM, HPM, NKB, PCR, and TA wrote and edited the manuscript.

Funding The research was funded by ICAR-NRRI/In-house Project 3.5/2020–2025 scheme (fund received by Dr. Totan Adak).

Data availability All data generated or analyzed during this study are included in this article. Furthermore, the datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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