J Bangladesh Agril Univ 19(2): 192–197, 2021 https://doi.org/10.5455/JBAU.72976

ISSN 1810-3030 (Print) 2408-8684 (Online)

Journal of Bangladesh Agricultural University

Journal home page: http://baures.bau.edu.bd/jbau

Toxicity Evaluation of Different Chemical Pesticides against *Riptortus pedestris* (Hemiptera: Alydidae) Under Laboratory Condition in Bangladesh

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ARTICLE INFO

Article history
Received: 13 Apr 2021
Accepted: 06 Jun 2021
Published: 30 Jun 2021

Keywords
Acute toxicity, Riptortus *pedestris*, Topical application, Residual exposure,

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ABSTRACT

The bean bug, *Riptortus pedestris* Fabricius (Hemiptera: Alydidae) is a polyphagous seed-sucking major pest of leguminous crops distributed worldwide. Effective management of this notorious insect pest is a major concern to growers. In this study, the efficacy of seven pesticides was assayed at field recommended doses against *R. pedestris* by topical application, exposure to residue and oral ingestion in laboratory condition. Our results showed that cypermethrin and chlorpyrifos + cypermethrin exhibited high toxicity causing 100% mortality within 4 hours after treatment both by topical and oral ingestion method. Profenofos + cypermethrin showed 100% mortality by topical application and oral ingestion within 16 and 12 hours after treatment. On the other hand, chlorpyrifos + cypermethrin and cypermethrin caused 100% mortality within 8 hours after treatment by residue on substrate. Therefore, for the integrated pest management program, cypermethrin (1.0 ml/L), chlorpyrifos + cypermethrin (1.0 ml/L), and profenofos + cypermethrin (2.0 ml/L) can be used for chemical control of *R. pedestris*. Further experimentation is needed to check their efficacy in field condition.

Introduction

The bean bug, *Riptortus pedestris* Fabricius (Hemiptera: Alydidae) is a seed-sucking major pest infesting many crops in Asian countries (Osakabe and Honda, 2002; Kang *et al*., 2003; Lee *et al.,* 2004) including Japan, China, Korea, Taiwan, Thailand, Indonesia, India and Bangladesh (Kikuhara, 2005; Alim and Hossain, 2018; Arifunnahar *et al*., 2019). It feeds on wide range of host plants such as soybean (*Glycine max* L.), sesame (*Sesamum indicum* L.), rice (*Oryza sativa* L.), apple (*Malus domestica* Borkh.), sweet persimmon (*Diospyros spp*) (Kim *et al.,* 1992; Son *et al*., 2000; Wada *et al*., 2006) and causes serious damage (60-70%) to the leguminous crops and the fruits trees (Kono, 1989; Chung *et al.,* 1995; Kang *et al*., 2003). Both nymphs and adults of *R. pedestris* form a stylet-sheath to produce watery saliva that contains digestive enzymes and the damages caused by this infestation lead to significant reductions in yield, seed quality, and germination rate (Daugherty *et al.,* 1964; Todd and Turnipseed, 1974; McPherson *et al.,* 1979).

There are a few management strategies have been introduced against this notorious pest throughout the world. The strong flight capacity of *R. pedestris* helps it to avoid insecticide treated areas making chemical control ineffective (Son *et al.,* 2000; Wada *et al.,* 2006). In addition, chemical pesticides not only increase production cost, but result in reduction of natural enemy populations, develop pest resurgence and other environmental problems (Liu *et al.,* 2014). Cultural control methods such as delayed planting and use of resistant varieties have been suggested for *R. pedestris* management (Wada *et al*., 2006). Biological control using egg parasitoids such as *Gryon japonicum* Ashmead (Hymenoptera: Scelionidae), *Gryon nigricorne* Dodd (Hymenoptera: Scelionidae), *Ooencyrtus nezarae* Ishii (Hymenoptera: Encyrtidae) have been used successfully for the control of *R. pedestris* in augmentation or introduction programs (Orr, 1988; Alim and Lim, 2011).
Even though the use of synthetic chemical insecticides has adverse effects, it is a widely used management tactic against *R. pedestris* (Kim *et al.,* 1992; Lee *et al.*,

Cite This Article
Arifunnahar, M., Khatun, M.M., Hossain, M.A., Alim, M.A. 2021. Toxicity Evaluation of Different Chemical Pesticides against *Riptortus pedestris* (Hemiptera: Alydidae) Under Laboratory Condition in Bangladesh. *Journal of Bangladesh Agricultural University*, 19(2): 192–197. https://doi.org/10.5455/JBAU.72976

2004; Choi *et al*., 2005; Wada *et al*., 2006). Additionally, chemical pesticides remain a major strategy in integrated pest management (IPM) systems against the insect due to its various advantages such as easiness in use and handling, quick action and reliability (Endo and Tsurumachi, 2001). Major advantages of chemical pesticides include quick action, easy use, convenience, cost-effectiveness, and reliability.

The legume growers of Bangladesh face *R. pedestris* as a new insect problem in their field (Arifunnahar *et al.,* 2019). Most farmers are adopted to use different kinds of broad-spectrum synthetic insecticides for the control of insect pests including the *R. pedestris* (Alim and Lim, 2014). However, not all of them may not be suitable for the control of *R. pedestris*. There is no previous research work has been done and published about the efficacy of chemical insecticides against *R. pedestris* in Bangladesh context, but reported from East Asian countries like Korea, Japan and China and used chloronicotinile, synthetic pyrethroids, organophosphorus, neonicotinoid, lothianidin and phenylpyrazole (Bae *et al.,* 2008; Takeuchi and Endo, 2012). So, there is a gap to identify the suitable insecticides against *R. pedestris* in Bangladesh context. So, to ensure sustainable plant protection and environmentally safe for the beneficial insects, need to identify the appropriate pesticides for controlling *R. pedestris.* Therefore, in this study the efficacy of seven selected chemical pesticides were evaluated against *R. pedestris* in laboratory condition.

Materials and Methods

Collection and mass rearing of insects

Adults of *R. pedestris* were collected from mungbean (BARI Mug 5) fields in Hajee Mohammad Danesh Science and Technology University (HSTU) campus and reared in the laboratory of the department of Entomology, HSTU during 2019. The mass culture of *R. pedestris* was maintained in the laboratory according to the methods described by Alim and Lim (2010). The nymphs and adults were kept in separate acryl cages (40L × 40W × 40H cm) with windows in three lateral sides covered with meshed (0.50 mm) screens for ventilation at ambient temperature (31.62°C ± 0.16), relative humidity (79.25% ± 1.60) and natural photoperiod (L:D, 13:11 h). Temperature and relative humidity were measured by the Hydro-thermometer (HTC-1), (Brand Name: Ace Instruments; Manufacturer: HTC instruments, China). Ascorbic acid dissolved in water and dried soybean seeds were provided to adult *R. pedestris* as food. The nymphs were transferred into another glass cage with windows in three lateral sides covered with meshed screens for ventilation. Mungbean plants with cotyledonous leaves provided for nymphs as food sources. Four pieces of gauze cloth were placed on upper and bottom corners inside the adult cages as oviposition substrates. Water-soaked cotton and soybean seeds were changed twice a week. The cage was cleaned two times in a week. Eggs were collected from the acryl cages and new gauzes were placed in acryl cages daily and kept in another cage for the use in experiment. Adults (7 days old) were collected for the purpose of experiments.

Lethal effect of pesticides

Six insecticides and one herbicide were collected from local market of Dinajpur district and tested in this experiment. Each pesticide was diluted with 250 ml of distilled water to make field recommended concentration (Table 1).

Topical application

Single adult of female *R. pedestris* (7 days old) was placed in a centrifuge tube (50 ml cap.) and sprayed with each pesticide using a hand sprayer (300 ml cap.). Each pesticide was diluted to make field recommended concentration (Table 1) and sprayed one stroke (0.62ml spray materials) for an individual in a centrifuge tube. The adult insect was then immediately moved into a new Petri dish (Plastic) (5.5 D×1.3 H cm) individually and kept at ambient condition without food sources. Mortality of the individuals was recorded every four hours interval. These procedures were repeated using 30 insects for each pesticide. Distilled water was used as control in this study.

Residue on substrate

The bottoms Petri dishes (Plastic) (5.5 D×1.3 H cm) were sprayed with recommended doses of each pesticide (Table 1) and dried for 1 hour. One adult of female *R. pedestris* (7 days old) then placed in each Petri dish (Plastic) with pesticide residue and kept at ambient condition without food source. Mortality of *R. pedestris* was recorded every four hours interval. Total 30 adults were replicated for each pesticide including control.

Oral ingestion

Same aged adults of female *R. pedestris* were starved for 12 hours and then placed individually in the Petri dishes (Plastic) with a contaminated diet which was prepared by mixing with 5 ml of honey, 9.95 ml of distilled water and 0.05 ml of each pesticide separately (Alim and Lim, 2014). The Petri dishes (Plastic) were kept at ambient condition. Mortalities were recorded every four hours interval. A total 30 adults were tested for each pesticide including control. Fresh honey was used as a control.

Table 1. List of pesticides with group name, chemical name, trade name, field recommended dose and manufacturer's name

Type	Group/Class name	Active ingredient name (Chemical name)	Trade name	Field recommended dose	Manufacturer's name
Insecticide	Synthetic pyrethroid	Cypermethrin	Ripcord 10 EC	1.0 ml/L	BASF Bangladesh Limited
	Organophosphate+ synthetic pyrethroid	Chlorpyrifos + cypermethrin	AC Mix 55 EC	1.0 ml/L	ACI Formulations Limited
	Organophosphate+ synthetic pyrethroid	Profenofos + cypermethrin	Shobicron 425 EC	2.0 ml/L	Syngenta Bangladesh Limited
	Neonicotinoids	Imidacloprid	Tiddo 20 SL	0.50 ml/L	ACI Formulations Limited
	Neonicotinoids	Thiamethoxam	Actara 25 G	0.20 g/L	Syngenta Bangladesh Limited
	Avermectin	Abamectin	Lakad 1.8 EC	1.20 ml/L	Intefa
Herbicide	Sulfonyl urea	Pyrazosulfuran Ethyl	Lubada 10 WP	150 g/ ha	Intefa

Statistical analyses

Lethal effects, Chi-square and LT_{50} values were obtained by probit analysis using PoloPlus (Robertson *et al*., 2007). Significant differences among the treatments in each assay were recorded when confidence intervals (CI) were 95%. Data of spraying methods were compared among the treatments by a normal approximation of the chi-square test on pooled data (Zar, 2010) and using two factors ANOVA in SPSS program (version 32.0). Toxicity Index (TI) was calculated by LT_{50} of control divided by LT_{50} of each treatment.

Results and Discussion

Efficacy of pesticides by topical application

The efficacy of the tested pesticides in terms of survival rate is presented in Figure 1, 2, and 3. Our results showed that all the tested insecticides were found effective although they varied individually. Among the pesticides assayed, cypermethrin and chlorpyrifos + cypermethrin showed the highest mortality on *R. pedestris* (Table 2 and Fig. 1). Cypermethrin and chlorpyrifos + cypermethrin caused 100% mortality within 4 hours after treatment (HAT). In this experiment, *R. pedestris* was found highly susceptible (100%) to profenofos + cypermethrin. Thiamethoxam was proved as effective to *R. pedestris* within 28 HAT. Profenofos+cypermethrin showed the lowest LT_{50} (4.30) and control was the highest LT_{50} (51.32) (Table 2). On the other hand, cypermethrin and chlorpyrifos+cypermethrin showed the highest Toxicity Index (TI) (51.32) and lowest was in control treatment (1.00) (Table 2).Similar result was obtained by Alim and Lim (2014) with 24 HAT against *Ooencyrtus nezarae* Ishii (Hymenoptera: Encyrtidae). *Riptortus pedestris* was less susceptible to imidacloprid. Bae *et al*. (2008) revealed that clothianidin, deltamethirn, ethofenprox, fenitrothion, fipronil and phenthoate showed highest susceptibility for *R. clavatus* and that stink bugs can be effectively controlled by spraying insecticides in soybean field in Korea. Imidacloprid 0.005% treated plants recorded significantly lower counts of *R. pedestris* until 15 days after treatment (Bharathimeena and Sudharma, 2009). Abamectin was found less effective against *R. pedestris*. This insect was also less susceptible to herbicide, pyrazosulfuran ethyl remained up to 48 hours after treatment.

Efficacy of pesticides by residue on substrate

Cypermethrin showed the highest toxic effect on *R. pedestris* and killed 100% insects within 4 hours after application (Table 2), while chlorpyrifos + cypermethrin showed the highest toxic effect on *R. pedestris* and killed 100% insects within 8 hours after treatment (Table 2 and Fig. 2). *R. pedestris* was found highly susceptible to profenofos + cypermethrin (Fig. 2). Imidacloprid was proved as effective to *R. pedestris* within 28 hours after treatment (Fig. 2). *R. pedestris* was less susceptible to thiamethoxam. Profenofos+cypermethrin showed the lowest LT_{50} (4.78) and control was the highest LT_{50} (59.81) (Table 2). On the other hand, cypermethrin and chlorpyrifos+cypermethrin showed the highest Toxicity Index (TI) (59.81) and lowest was in control treatment (1.00) (Table 2). Lee *et al*. (2015) showed that dinotefuran WP, etofenprox WP, chlorpyrifos WP, cabaryl WP, chlothianidin SC, and bifenthrin WG has residual effects and were effective on both male and female of stink bugs (*Halyomorpha halys* and *Plautia stali*), while chlorpyrifos and bifenthrin were showed higher residual toxicity on both male and female of *Halyomorpha halys* in laboratory condition. Bifenthrin have a high residual effect on *Plautia stali* until 5 days after treatment and chlorpyrifos showed higher residual toxicity in the laboratory (Lee *et al*., 2015). Chlorpyrifos and bifenthrin showed higher residual toxicity in the laboratory against different stink bug populations (Lee *et al*., 2015). *R. pedestris* was less susceptible to herbicide, pyrazosulfuran ethyl and abamectin remained up to 48 hours after treatment.

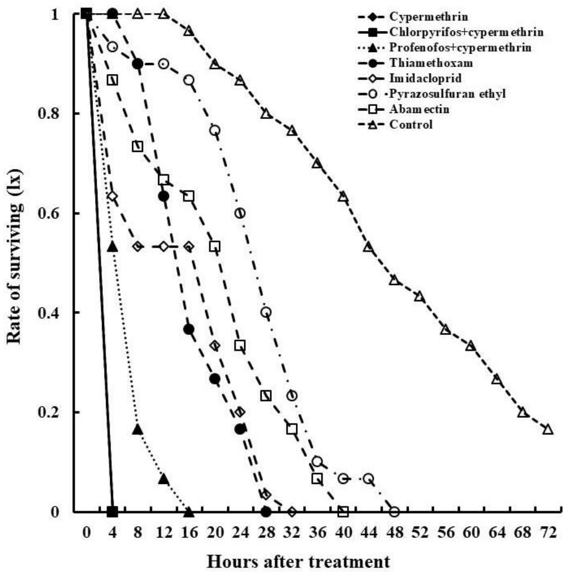

Figure 1. Survival rate of adult female *R. pedestris* exposed to seven different pesticides at recommended doses by topical application

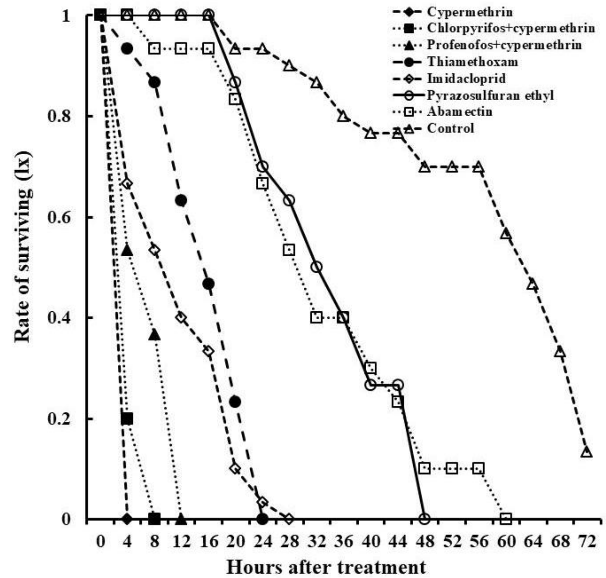

Figure 2. Survival rate of adult female *R. pedestris* exposed to seven different pesticides at recommended doses by residue on substrate

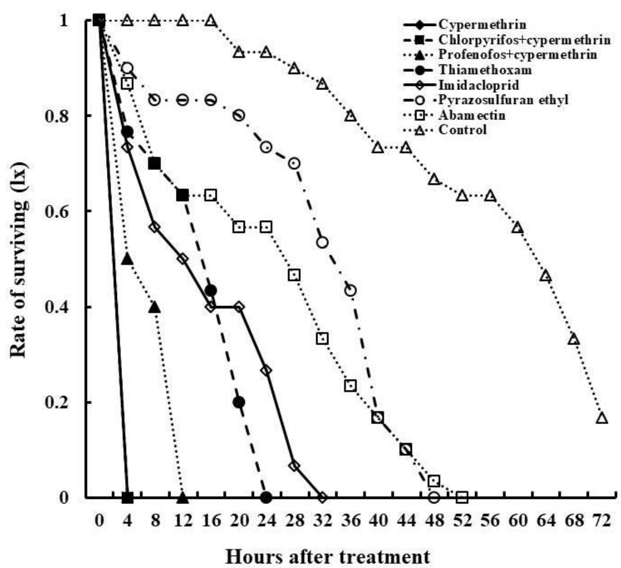

Figure 3. Survival rate of adult female *R. pedestris* exposed to seven pesticides at recommended doses by oral ingestion

Efficacy of pesticides by oral ingestion

Cypermethrin and chlorpyrifos + cypermethrin were most effective to *R. pedestris* and caused 100% mortality within 4 hours after exposure with contaminated food (Table 2 and Fig. 3). In oral ingestion, profenofos + cypermethrin showed 100% mortality within 12 hours after treatment (Fig. 3). Thiamethoxam and imidacloprid were proved as effective to *R. pedestris* within 32 hours after treatment (Fig. 3). Profenofos+cypermethrin showed the lowest LT_{50} (4.71) and control was the highest LT_{50} (58.37) (Table 2).

On the other hand, cypermethrin and chlorpyrifos + cypermethrin showed the highest Toxicity Index (TI) (58.37) and lowest was in control treatment (1.00) (Table 2). *R. pedestris* showed highest toxicity ratio for fenitrothion, etofenprox, silabuofen and fenthion (Takeuchi and Endo, 2012). The herbicide, pyrazosulfuran ethyl and abamectin were less effective to *R. pedestris* in oral ingestion than topical and residual substrate and adults were remained up to 72 hours after treatment. Results of the statistical analysis are summarized in Table 2.

Table 2. Statistical comparison of lethal effects of seven pesticides on female *R. pedestris* (n=30)

Assayed by	Pesticides	LT_{50} (hrs.)	95% CI (lower limit, upper limit)	Slop (±) SE	χ^2(df)	TI
Topical application	[a]Cypermethrin	-	-	-	-	51.32
	[b]Chlorpyrifos+cypermethrin	-	-	-	-	51.32
	Profenofos+cypermethrin	4.30 c	3.21-5.26	6.97 ± 0.38	1.265 (10)	11.93
	Thiamethoxam	14.09 b	12.72-15.37	6.64 ± 0.59	6.009 (10)	3.64
	Imidacloprid	14.23 b	10.57-17.52	4.26 ± 0.35	29.75 (10)	3.60
	Pyrazosulfuran ethyl	22.13 b	12.50-33.46	3.76 ± 0.35	26.00 (10)	2.31
	Abamectin	14.90 b	11.19-18.44	3.64 ± 0.29	25.86 (10)	3.44
	Control	51.32 a	49.17-53.60	6.92 ± .388	3.28 (16)	1.00
Residual application	[a]Cypermethrin	-	-	-	-	59.81
	[b]Chlorpyrifos+cypermethrin	-	-	-	-	59.81
	Profenofos+cypermethrin	4.78 c	3.52-5.84	4.08 ± 0.63	10.44 (10)	12.51
	Thiamethoxam	12.75 c	9.83-15.47	5.06 ±0.48	31.76 (10)	4.69
	Imidacloprid	7.715 c	5.48-9.72	3.10 ± 0.32	17.29 (10)	7.75
	Pyrazosulfuran Ethyl	31.39 b	29.48-33.40	6.55 ± 0.70	8.71 (10)	1.90
	Abamectin	28.70 b	25.39-32.04	4.45 ± 0.38	23.08 (10)	2.08
	Control	59.81 a	54.76-67.1 2	4.19 ± 0.46	1.978 (16)	1.00
Oral ingestion	[a]Cypermethrin	-	-	-	-	58.37
	[b]Chlorpyrifos+cypermethrin	-	-	-	-	58.37
	Profenofos+cypermethrin	4.71 d	3.18-5.94	3.89 ± 0.60	13.48 (10)	12.39
	Thiamethoxam	10.26 c	7.07-13.21	3.63 ±0.34	34.02 (10)	5.69
	Imidacloprid	9.94 c	6.46-13.46	2.78 ± 0.28	27.28 (10)	5.87
	Pyrazosulfuran Ethyl	26.89 b	18.55-42.82	2.66 ± 0.29	55.30 (10)	2.17
	Abamectin	17.33 b	18.97-21.49	2.64 ± 0.24	34.97 (10)	3.36
	Control	58.37 a	53.94-64.47	4.17 ± 0.45	10.37 (16)	1.00

Means followed by different letters of column significantly different (Robertson *et al*., 2007); [a]Cypermethrin and [b]chlorpyrifos+cypermethrin killed all the *R. pedestris* within 4 hours after treatment and Profenofos+cypermethrin killed all the *R. pedestris* within 16 hours after treatment in topical application; [a]Cypermethrin killed all the *R. pedestris* within 4 hours after treatment and [b]Chlorpyrifos+cypermethrin killed all the *R. pedestris* within 8 hours after treatment in residue on substrate method; [a]Cypermethrin and [b]chlorpyrifos+cypermethrin killed all the *R. pedestris* within 4 hours after treatment and *R. pedestris* were provided with uncontaminated honey solution and survived the 72 hours experimental period in oral ingestion method; TI = Toxicity index

Comparative performance of different methods of bioassay

A (topical vs residual vs oral method) × (seven pesticides with control) between subjects ANOVA was conducted to study the difference between topical, residual and oral application methods as a function of the pesticides mortality (Table 3). There was not significant different in the pesticides applying methods, $F (2, 408) = 0.001$, $P = 0.999$, such that topical (Mean = 1.63, SD= 4.10), residual (Mean =1.61, SD= 3.81), oral (Mean =1.61, SD= 4.01) had not significant different after 72 hours of treatment. The main effect of pesticides was not also significant, $F (7, 408) = 0.026$, $P = 1.00$ in the same interval time (Table 3). However, the interaction effect was not also significant, $F (14,408) = 0.002$, $P = 1.00$ (Table 3). Our results showed that all the tested pesticides were found effective although those were applied using three different method.

Table 3. Comparative performance of three different pesticides applying methods against *R. pedestris*

Pesticides	Method		
	Topical	Residual	Oral
Cypermethrin	100.00±1.66	100.00±1.66	100.00±1.66
Chlorpyrifos + cypermethrin	100.0±1.66	100.00±1.35	100.00±1.66
Profenofos + cypermethrin	100.00±0.95	100.00±0.83	100.00±0.87
Imidacloprid	100.00±0.71	100.00±0.67	96.66±0.59
Thiamethoxam	100.00±0.56	100.00±0.57	96.66±0.59
Abamectin	100.00±0.43	90.00±0.43	100.00±0.38
Pyrazosulfuran Ethyl	100.00±0.48	100.00±0.57	100.00±0.46
Control	100.00±0.20	86.66±0.42	83.33±0.35

Value in each column that are not significant differences from the Tukey test (α = 0.05).

Conclusion

In this study, we had tested six commonly used insecticides and one herbicide against *R. pedestris* by three different methods, i.e., topical application, residual exposure and oral ingestion. The results showed that cypermethrin, chlorpyrifos + cypermethrin, profenofos + cypermethrin were highly toxic to *R. pedestris* in laboratory assay of topical application, exposure to residue and oral ingestion. Therefore, cypermethrin, chlorpyrifos+cypermethrin, profenofos+cypermethrin, imidacloprid and thiamethoxam were suggested to use the control of *R. pedestris* in Bangladesh.

Acknowledgements

The authors acknowledge the Institute of Research and Training (IRT), Hajee Mohammad Danesh Science and Technology University, Bangladesh for financial support.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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