Journal of Agriculture and Veterinary Science
ISSN: 2959-1198 (Print), 2959-1201 (Online)

Article History
Received: July 19, 2023
Accepted: August 24, 2023
Published: August 31, 2023

Research Article

Unleashing the Power of Post-Emergence Herbicides: Eradicating Red Sprangletop (*Leptochloa chinensis* L.) Invaders in Rice Nurseries with a Fiery Precision

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Abstract

Red/Chinese sprangletop (*Leptochloa chinensis* L.) is a serious grass weed in rice paddies. Red sprangletop (*Leptochloa chinensis* L.), has become a problematic terminal weed in rice-growing areas of Punjab, Pakistan. This study aimed to evaluate the efficacy of three combinations of synthetic herbicides: Lepto kill (Penoxulam+Fenoxaprop-P-Ethyl) @ 1000 mL hc^{-1}, Gorgan (Cyhalofope butyl+Bispyribac Sodium+ Fenoxaprop-P-Ethyl) @ 1000 mL hc^{-1}, and Ricer (Penoxulam+Cyhalofope butyl) @ 2500 mL hc^{-1}, when applied as leaf sprays in rice nurseries, compared to a control (No treatment applied). The results showed that Gorgan exhibited highly significant ($P<0.001$) effectiveness in controlling this invasive weed, with mortalities of 23.33%, 42.21%, and 79.76% recorded after 7, 14, and 21 days of spraying (DAS) in 2020. Similarly, significant ($P<0.001$) mortalities were observed with Gorgan (23.33%, 32.21%, and 72.76%) and Leptokill (12.57%, 26.17%, and 59.43%) during the same period in 2021. In contrast, Ricer showed relatively lower mortality rates (12.57%, 29.19%, and 57.49% in 2020; 19.32%, 24.34%, and 66.56% in 2021). The coefficient of determination (R^2) for mortality indicated a positive correlation for Leptokill, Gorgan, and Ricer, with values of 0.83, 0.96, and 0.95 in 2020, and 0.94, 0.88, and 0.83 in 2021, respectively. These results suggest that Gorgan is highly effective in controlling this invasive weed species in rice fields.

Keywords: *Gorgan; Invassive; Leptokill; Red sprangletop; Ricer.*

Introduction

China, one of the largest rice planting and production countries globally, contributes around 20% to the world's rice planting area. The significant growth in China's rice grain

https://doi.org/10.55627/Javs.02.2.0351

production serves as a crucial safeguard for ensuring food security. Nevertheless, weeds cover a substantial portion of China's rice planting area, accounting for approximately 15 million hectares or 45%. Consequently, an estimated 10 million tons of rice, equivalent to roughly 15% of the total production, are lost each year. The absence of weed control typically leads to a reduction in paddy rice yield ranging from 5% to 15%, escalating to 15% to 30% in severe instances. Consequently, chemical weed control plays a vital role in the modernization of global agriculture (Fang *et al.*, 2020; Liu *et al.*, 2021).

In the Indian sub-continent, including Pakistan, rice (*Oryza sativa* L.) is a highly significant staple food and a favorite diet of the majority population. However, the presence of different weed species poses a major limitation to achieving maximum yield and serves as alternative hosts for insects and various pathogens (Tao and Hu, 2009). The most effective tools for weed control are herbicides, which have the ability to kill 90 to 99% of weed flora (Délye *et al.*, 2013; Mallory-Smith and Retzinger, 2003; Wakabayashi and Böger, 2002). However, the development of herbicide-resistant weed species has become a significant concern. Therefore, there is a need for herbicides with novel action mechanisms to combat the development of resistance in these weeds. Unfortunately, the introduction of herbicides with advanced action mechanisms in the agrochemical market is still limited.

Weeds pose a major obstacle to maximizing rice production, with grass species like barnyardgrass (*Echinochloa crus-galli* L. Beauv), red sprangletop (*Leptochloa chinensis* L. Nees), and grass-like fimbry (*Fimbristylis miliacea* L. Vahl) being particularly prevalent and formidable competitors in rice fields. Approximately 50 weed species are discovered in rice fields worldwide, leading to significant declines in productivity. Notably, the Asian sprangletop (*Leptochloa chinensis* L.) and barnyard grass (*Echinochloa crus-galli*) swiftly thrive, particularly in areas where rice is directly sown. Meanwhile, Jungle rice (*Echinochloa colona* L.) flourishes vigorously in direct-seeded rice fields and is predominantly observed in both directly seeded and transplanted rice cultivation (Allard *et al.*, 2005; Caton *et al.*, 2004). Each of these species, when allowed to compete with rice throughout the growing season, can significantly diminish rice yields by 50-70% (Chin, 2001; Labrada *et al.*, 1994; Xuan *et al.*, 2006). Red sprangletop (Leptochloa chinensis L.) is one of the most widespread and competitive grass species in rice fields. It establishes itself rapidly in rice nurseries, where it competes with young rice plants for essential resources such as sunlight, water, and nutrients. The dense growth of red sprangletop can severely hamper the growth and development of rice seedlings, leading to substantial yield reductions. According to a study by Wang *et al.* (2019), uncontrolled red sprangletop competition can result in yield losses of up to 50-70% during the rice-growing season.

Among different weed species of rice crop, *Leptochloa chinensis* (red sprangletop) is a highly tufted grass that typically acts as an annual invasive summer weed in paddy fields and other rainy/tropical areas of Pakistan. It primarily reproduces through seeds (Chuah *et al.*, 2014). The invasiveness of this perennial grass-weed species is attributed to its abundant production of seeds, which exhibit high tolerance to harsh environments and are easily dispersible. *Leptochloa chinensis* reproduces through narrow ovate panicle-

https://doi.org/10.55627/Javs.02.2.0351

like structures, ranging in length from 10 to 60 cm. These structures contain sub-sessile spikelet with 3-7 reddish or yellowish green florets (Chin, 2001). The length of this weed typically reaches up to 1 meter, and it possesses a branching-base originated geniculate, erect, or slender culm. The leaves of *Leptochloa chinensis* have a length of 15-20 cm and appear linear and smooth with a thin, acute, and flat morphology. The upper side of the leaves is rough and membranous, while the ligule measures 1-2 mm in length *L. chinensis*, commonly known as red sprangletop (Chuah *et al*., 2014). While it is capable of vegetative propagation (Benvenuti *et al*., 2004), its primary mode of reproduction is sexual. However, there is limited available data regarding its germination ecology, including temperature and light requirements, as well as its mechanisms of tolerance to hypoxic and flooded environments.

In Penang, Malaysia, the highly invasive weed species *Leptochloa chinensis* has been historically reported in paddy fields(Sunyob *et al*., 2012). To control *L. chinensis*, different chemical herbicides, such as benthiocarb, fenoxaprop-p-ethyl, molinate, quinclorac, and propanil, have been applied. a mixture of sethoxydim and cyhalofope in light concentration has also been found to be effective in controlling *L. chinensis* with minimal harm to rice plants (Sahid *et al*., 2011). However, the excessive use of these herbicides has led to better control of this weed since the early 1990s, but repeated application of the same formulations has shown less effectiveness and has caused herbicide resistance in these weeds (Azmi, 2002). Similarly, *L. chinensis* has shown resistance to propanil, quizalofop-p-ethyl, fenoxaprop-p-ethyl, and profoxydim (Sahid *et al*., 2011).

Integrated weed management stands as the optimal choice for weed control, with cultural weed control serving as a crucial element within it. By manipulating various strategies for managing weeds, crop competitiveness against weeds for both above and below ground resources can be bolstered. Limited research exists regarding the use of new chemistry mixture herbicides against *L. chinensis*. To address this research gap, the present study aimed to evaluate the efficacy of various herbicide mixtures, namely Penoxulam + Fenoxaprop-P-Ethyl, Cyhalofop-butyl + Bispyribac-sodium + Fenoxaprop-P-Ethyl, and Penoxulam + Cyhalofop-butyl, against *L. chinensis* under field conditions in Gujrat, Punjab, Pakistan.

Methodology

Experimental Design and Site Selection for Assessing Herbicide Effectiveness on Leptochloa chinensis in Rice Nursery Fields

The study site was selected based on a comprehensive survey of rice nursery fields in District Gujrat, Pakistan, with the aim of identifying hot-spot areas infested with *Leptochloa chinensis* (red sprangletop). These hot-spot sites were chosen to assess the effectiveness of three herbicides in controlling the spread of this invasive weed species. The comparison was made with control areas within the experimental setup.

One specific hot-spot area of *Leptochloa chinensis* was identified in the rice nursery of Basmati Super (B-Sup) variety located in Surakh Pur Tehsil, District Gujrat. The nursery was sown in the last week of May for two consecutive years, 2020 and 2021. The seeds used for sowing were treated with thiophenate methyl at a rate of 2.5g per kilogram of seed, with an application rate of 12.5 kg hc^{-1}. Table 1 presents the post-emergence

https://doi.org/10.55627/Javs.02.2.0351

herbicides selected for the current study, which were evaluated for their efficacy against *Leptochloa chinensis*.

Experimental Setup and Herbicide Application

The experiment was conducted using a randomized complete block design (RCBD) on 0.5-hectare plots. Each experimental block was separated by walk-path lines, and the experiment was replicated three times for each treatment.

The herbicide mixtures were prepared and applied using a spray method under appropriate moist conditions. A regular manual sprayer machine was used, and the water volume was maintained at 247 liters per hectare (L hc-1). The T-Jet nozzle with a droplet size of 225 μm was employed, producing a fine spray mist at a pressure of 2 pounds per square inch (PSI). The herbicides were applied 22 days after nursery sowing, specifically at the 4-6 leaf stage of *L. chinensis*.

Prior to the herbicide application, water was drained from the field. After the herbicides were applied, the field was re-irrigated within the next 24 hours. This irrigation practice was implemented to obtain results that align with the recommendations provided by the herbicide manufacturers.

Table 1. Comprehensive Compilation of Herbicides Employed for Controlling Red Sprangletop (*Leptochloa chinensis* L.).

Sr. No.	Chemical Name	Trade Name	Chemical Family	Firm/Companies name	Rate of application
1	Penoxulam + Fenoxaprop-P- Ethyl	Lepotokill 10 % OD	Triazolopyrimidine Sulfonamide + Aryloxyphenoxy propionate	Comega Life Sciences (Pvt.) Limited	1000 $mLha^{-1}$
2	Cyhalofope butyl+ Bispyribac Sodium+ Fenoxaprop-P-Ethyl	Gorgan 16 % OD	Aryloxyphenoxy propionate+ Pyrimidinlthio-benzoate	Imperial Crop Sciences (Pvt.) Limited	1000 $mLha^{-1}$ + 250 $mLha^{-1}$
3	Penoxulam + Cyhalofope butyl	Ricer 60 % OD	Triazolopyrimidine Sulfonamide + Aryloxyphenoxy propionate	Syngenta Pakistan (Pvt.) Limited	1000 $mLha^{-1}$

OD=Oil Dispersion.

Pest Scouting and Population Counting Methodology

The pest scouting method employed for data collection in each treatment at five distinct locations was the random square feet ring approach. Prior to the application of herbicide spray, the population of Red Sprangletop (*Leptochloa chinensis* L.) was recorded from each treatment-block. Subsequently, the experimental field was visited on days 7, 14, and 21 following the spray application, and the populations (number of weeds in the scouting method) of *L. chinensis* in various blocks were quantified.

Evaluation of Model Fitness and Performance for Herbicides: Coefficient of Determination (R2) and Root Mean Square Error (RMSE) Analysis

In the study, the relationship between the model fitness for herbicides and the comparison of the coefficient of determination (R2) values with root mean square error (RMSE) was examined. The coefficient of determination R2 and RMSE were utilized as

https://doi.org/10.55627/Javs.02.2.0351

indicators of the standard deviation of the residuals, which represent the prediction errors. Residuals provide a measure of how far the values deviate from the regression lines that the data points were fitted to. This measure also informs us about the level of data clustering around the line of best fitness and model validity (Hossain *et al.*, 2017). To assess the performance of the model in predicting herbicides, RMSE was used. In the experiment with 'n' observations, RMSE was calculated using the following equation (Equation 1) as described by (Debaeke *et al.*, 1997).

$$\text{RMSE} = \sqrt{\frac{1}{n} \times \sum_{j=1}^{n} ((Yield_{mes} - Yield_{sim})^2}$$

Statistical Analysis of Data using ANOVA and Duncan's Multiple Range Test

The data was subjected to rigorous statistical analysis using ANOVA (Analysis of Variance). The significance level was set at $P > 0.05$, indicating a threshold above which results were considered statistically significant. To perform the statistical analyses, SPSS statistics version 25.0 was utilized, ensuring accuracy and reliability in the interpretation of the results.

Duncan's Multiple Range Test, a widely accepted post-hoc test, was employed to compare the means and identify significant differences among the treatments. This test provides valuable insights into the variations observed in the dataset, allowing for a comprehensive understanding of the experimental outcomes.

By employing these rigorous statistical methods, we ensured robust analysis and accurate interpretation of the data, thus enhancing the credibility and reliability of our findings.

Results and Discussion

Efficacy of Herbicides in Controlling Mortality of *Leptochloa chinensis*

The impact of herbicides on the mortality of *Leptochloa chinensis* is depicted in Table 2. Gorgan demonstrated highly significant efficacy in controlling this weed, resulting in mortality rates of 23.33%, 42.21%, and 79.76% after 7, 14, and 21 days, respectively. Similarly, Leptokill displayed notable effectiveness, with mortality rates of 19.32%, 24.34%, and 66.56% during the same time periods. Conversely, Ricer exhibited the lowest mortality rates, reaching 12.57%, 26.17%, and 59.43% in 2020. Comparable trends in mortality were observed with mixed herbicides in 2021. During the year 2021, Gorgan exhibited high mortality rates of 23.33%, 32.21%, and 72.76%, followed by Leptokill with rates of 12.57%, 26.17%, and 59.43%. In contrast, Ricer displayed relatively lower mortality rates of 12.57%, 29.19%, and 57.49% in 2020, and 19.32%, 24.34%, and 66.56% in 2021, respectively. Supporting visual evidence is presented in Figure 1.

Validation Performance of Herbicide Treatment on Mortality Response of *L. chinensis*

The mortality response of *Leptochloa chinensis* to herbicide treatment was evaluated and the results are summarized in Table 3. The validation performance of the model indicated strong correlation, with R^2 values of 0.83, 0.96, and 0.95 for Gorgan, Leptokill, and Ricer, respectively, in the year 2020. Notably, these herbicides demonstrated superior performance compared to root mean square error (RMSE), displaying excellent curve fitting and accurately representing the obtained data.

https://doi.org/10.55627/Javs.02.2.0351

Figure 1. Efficacy of Gorgan (Cyhalofope butyl+ Bispyribac Sodium+ Fenoxaprop-P-Ethyl) after 22 days of application, red sprangletop is killed by the application of Gorgan. Arrows indicates the red sprangletop.

Table 2. Effect of Various Herbicides on Mortality of Red Sprangletop (*Leptochloa chinensis*).

Sr. No.	Herbicide Treatment	Mortality % DAS (2020)			Mortality % DAS (2021)		
		7	14	21	7	14	21
1	Penoxulam+ Fenoxaprop-P-Ethyl	19.32±2.3[b]	24.34±2.1[b]	66.56±3.2[b]	12.57±2.3[c]	29.19±2.9[b]	57.49±2.4[c]
2	Cyhalofope butyl+ Bispyribac Sodium+ Fenoxaprop-P-Ethyl	23.33±1.8[a]	42.21±1.2[a]	79.76±2.7[a]	23.33±1.9[a]	32.21±1.2[a]	72.76±2.7[a]
3	Penoxulam+ Cyhalofope butyl	12.57±2.3[c]	26.17±2.9[b]	59.43±2.4[c]	19.32±2.3[b]	24.34±2.1[b]	66.56±3.2[b]
4	Control	0±0[d]	0±0[c]	0±0[d]	0±0[d]	0±0[c]	0±0[d]

DAS = days after spraying; Coefficient of Variance; mean + SD (Standard Deviation).

Control=No application of weedicides for the control. 0 indicates the no mortality of the red sprangletop.

Table 3. Regression Models Assessing the Impact of Various Herbicides on the Mortality of Red Sprangletop (*Leptochloa chinensis*) in 2020 and 2021.

Sr. No.	Chemical Name	2020			2021		
		Regression model	R^2	RMSE	Regression model	R^2	RMSE
1	Penoxulam + Fenoxaprop-P- Ethyl	y=23.62x-10.5	0.83	8.77	y=23.43x-14.13	0.94	4.63
2	Cyhalofope butyl+ Bispyribac Sodium+ Fenoxaprop-P-Ethyl	y=28.215x-7.99	0.96	4.40	y=24.715x-6.66	0.88	7.47
3	Penoxulam + Cyhalofope butyl	y=23.43x-14.13	0.95	4.63	y=23.62x-10.5	0.83	8.77

Whereas; R^2 = coefficient of determination; RMSE = root mean square error

Red sprangletop (*Leptochloa chinensis*) has emerged as a predominant weed species in paddy fields across various countries. Efforts to eradicate this weed have involved the

https://doi.org/10.55627/Javs.02.2.0351

use of both pre-emergence and post-emergence herbicides. Our findings align with previous studies, confirming their observations. Singh *et al.* (2018) conducted a study where they identified Pendimethalin at a rate of 1000 g/ha (5.0 m-2) as the most effective pre-emergence herbicidal treatment for *L. chinensis*. They also found that Fenoxaprop-p-ethyl at a rate of 67g ha^{-1} and Chlorimuron ethyl + Metsulfuron methyl at a rate of 4g ha^{-1} exhibited considerable control. Notably, Fenoxaprop at 67g ha^{-1} effectively controlled *L. chinensis* that had shown resistance to Bispyribac-sodium at a rate of 25g ha^{-1}. Additionally, Singh et al. (2004) reported similar findings regarding the efficacy of Fenoxaprop against *L. chinensis*, further corroborating our own results.

The efficacy of herbicide treatments on reducing the biomass of *L. chinensis*, a problematic weed, was investigated in this study. Results demonstrated that Penoxulam at a rate of 25 g active ingredient (a.i.) ha^{-1}, Bispyribac-sodium at 25g a.i. ha^{-1}, and Azimsulfuron at 20g a.i. ha^{-1} exhibited significant reductions in *L. chinensis* biomass by 70%, 73%, and 72% respectively, in comparison to the control group (Mahajan and Chauhan, 2013).

Building upon these findings, our research supports the efficacy of Gorgan, a combination of Cyhalofope butyl, Bispyribac Sodium, and Fenoxaprop-p-Ethyl, which resulted in a notable mortality rate of 79.76% for *L. chinensis*. This outcome further validates the potential of Bispyribac sodium as an effective herbicide, as it has demonstrated high control efficiency against broad-leaved weed *Lindernia crustacea* in previous studies (Prameela *et al.*, 2014). *L. chinensis*, a weed species widely known for its resistance to Quinclorac and Bentazone, has exhibited vulnerability to Pretilachlor, Fenoxaprop, and Molinate (Ampong-Nyarko and De Datta, 1991). However, there have been conflicting reports regarding the efficacy of propanil against L. chinensis. While it has been listed as ineffective, Pane and Mansor (1994) reported propanil as an effective control for *L. chinensis*. In Thailand, a commercially prepared formulation of Pretilachlor + safener demonstrated efficient control of *L. chinensis* (Ooi and Chong, 1988). Another study by Vongsaroj and Price (1987) highlighted fluazifop-butyl as an effective control option. Moreover, the application of Fenoxaprop-p-ethyl, under both flooded and dry conditions, proved effective in controlling *L. chinensis* and resulted in yield increases ranging from 10% to 100% in the presence of varying degrees of weed infestation.

Chauhan and Abugho (2012) demonstrated that a combination of Penoxulam and Cyhalofope, applied during the early stages of weed growth (specifically, the four-leaf stage), effectively suppressed 89-100% of *L. chinensis* biomass. The effectiveness of various post-emergence herbicides, including penoxsulam + cyhalofop, fenoxaprop + ethoxy sulfuron (combined), and bispyribac-sodium (alone), on four types of weeds: *E. colona*, *Digitaria ciliaris*, *Leptochloa chinensis*, and *E. crus-galli*. These herbicides were applied at the four, six, and eight-leaf stages. Fenoxaprop + ethoxy sulfuron achieved over 97% weed control for all weed species studied. Additionally, early application of post-emergence herbicides yielded better weed control compared to late application. Fenoxaprop + ethoxy sulfuron effectively controlled *Digitaria ciliaris* and *Leptochloa chinensis*, penoxsulam + cyhalofop controlled *Leptochloa chinensis*, and bispyribac-sodium effectively controlled *E. colona*. However, high weed density led to significant reductions

https://doi.org/10.55627/Javs.02.2.0351

in tiller production and grain yield in rice (Ranjit and Suwanketnikom, 2005). Applying bensulfuron, bispyribac-sodium, and cyhalofop-butyl during the early growth stage, followed by Bentazon/2-methyl-4-chlorophenoxyacetic acid (MCPA) during the mid-growth stage, effectively controlled weeds and increased rice productivity (Anwar et al., 2012). Various herbicides such as ethoxy sulfuron, cyhalofop-butyl, chlorimuron, metsulfuron, bispyribac-sodium, and penoxsulam demonstrated effective control of different weed types in dry-seeded rice (Mann et al., 2007; Mahajan et al., 2009). Hussain et al., (2008) reported that bispyribac-sodium and ethoxy sulfuron showed high efficiency with 90% and 87% weed control, respectively, in rice. They also found that applying bispyribac-sodium followed by ethoxy sulfuron resulted in the highest paddy yield and net benefits, while the lowest yields were recorded in the weedy check. These finding underscores the significance of utilizing post-emergence herbicides in intensive crop production to combat herbicide-resistant weeds (Khaliq *et al.*, 2012). In line with these results, Rahman *et al.* (2014) reported that the application of Cyhalofop-butyl at a rate of 0.64g m^{-2} resulted in visual mortality rates of 78%, 75%, 72%, and 70% after 1, 2, 3, and 4 weeks of treatment, respectively. These findings are consistent with the outcomes of our current study.

Weed resistance to herbicides is often overlooked compared to resistance to insecticides or fungicides (Zein et al., 2010). Many believe that resistance is solely caused by specific active ingredients, but it actually stems from agricultural systems that heavily rely on herbicides for weed control. In rice farming, herbicides targeting acetyl co-enzyme A carboxylase (ACCase) inhibitors, acetolactate synthase (ALS), thiocarbamates, synthetic auxins, and amides are commonly used, leading to a significant problem of herbicide resistance in many regions (Valverde and Itoh, 2001). Limited data exists on the efficacy of new chemistry mixture herbicides, prompting the need for further research. In light of this, our study aimed to evaluate the effectiveness of three mixture herbicides in controlling *Leptochloa chinensis* in rice nurseries. The results of our investigation revealed that Gorgan emerged as the most effective post-emergence herbicide for suppressing the growth of *L. chinensis*, solidifying its position as a valuable tool in managing this invasive species. To mitigate this issue, it is crucial to regularly monitor and detect herbicide resistance, understand its mechanisms, and implement appropriate management strategies to preserve the effectiveness of herbicides in weed control.

Conclusion

Weeds pose a significant threat to agriculture by competing with crops for resources and growing rapidly. Their resilience and adaptability make them difficult to control, particularly in rice fields where they cause yield reduction and increased production costs. Chemical weed control is commonly used due to its effectiveness and efficiency, but a single herbicide is not sufficient to tackle the diverse weed communities present in the field. Plant density plays a role in weed growth, with high density leading to intra-plant competition and low density providing space for weed growth. Integrated weed management, combining various strategies, can enhance crop competitiveness. The invasive weed species, red sprangletop, poses a significant challenge in rice-

https://doi.org/10.55627/Javs.02.2.0351

growing regions of Punjab, Pakistan. In our study, we examined the effectiveness of different combinations of chemical herbicides in combating red sprangletop. Our findings revealed that Gorgan exhibited remarkable efficacy, surpassing Leptokill and Ricer in 2020 and 2021, respectively. These results suggest that this particular formulation of chemical herbicides holds great promise for effectively managing red sprangletop in rice. Monitoring and addressing herbicide resistance is crucial, and future research should focus on developing effective herbicides and integrated management strategies to ensure optimal crop yield and discourage weed resistance.

Conflict of Interest

The authors have not declared any conflict of interest.

Authors Contributions

MA, MA, ARS, S, TH and ZN conceived the experiment; MA and AS conducted the experiment; MA, FA, MFA and MH analyzed the data**;** MA wrote the manuscript.

Acknowledgment

The author extends heartfelt gratitude to all the contributors who dedicated their time and assistance throughout the experiment. Special appreciation goes to Professor Ji Mingshan for their invaluable supervision, conceptual input, and financial support. The final manuscript was thoroughly reviewed and approved by all authors.

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