

# Tolerance of sugarcane cultivars to Mahanarva fimbriolata

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**ABSTRACT**: Mahanarva fimbriolata (Stål) (Hemiptera: Cercopidae), the sugarcane spittlebug, is one of the most important pests of this crop in Brazil. The magnitude of its damage depends on the pest population, the plants size when they are infested and the cultivar tolerance. The knowledge about those parameters allows the elaboration of the spittlebug management matrix, in which small plants and less tolerant varieties fields are prioritized for sampling and controlling in relation to developed plants and tolerant varieties fields. Therefore, this study aimed to evaluate the response of sugarcane genotypes to M. fimbriolata, so that they can be correctly allocated in the management matrix. Two experiments were carried out under laboratory conditions using a randomized block design with treatments in a factorial arrangement of  $2 \times 11$  (experiment 1) and  $2 \times 21$  (experiment 2), with six (experiment 1) or five replicates (experiment 2). The first factor included two levels of infestation (infested and noninfested plants with spittlebugs), while the second consisted of the cultivars. Cultivars IACSP01-5503, CTC 9004 and RB925211 were considered tolerant to spittlebug, they did not show significant reductions in aboveground biomass, despite showing symptoms of pest attack, as leaves yellowing, while CV6654 and IACSP01-3127 were the least tolerant cultivars, showing the highest reduction in aboveground biomass. due to M. fimbriolata infestations caused 30.9 % of reduction of aboveground biomass. Key words: spittlebug spintlebug, spintlebug spintlebug and control words.

#### Tolerância de cultivares de cana-de-açúcar a Mahanarva fimbriolata

**RESUMO:** A cigarrinha das raízes, Mahanarva fimbriolata (Stål) (Hemiptera: Cercopidae), é uma das mais importantes pragas da cana-deaçúcar no Brasil. A grandeza de seus danos depende da população, do tamanho da planta ao sofrer o ataque e da tolerância da cultivar. O conhecimento sobre esses parâmetros permite a elaboração da matriz de manejo de cigarrinha das raízes, na qual canaviais de plantas pouco desenvolvidas e cultivares não tolerantes são priorizados para amostragem e controle em relação a canaviais de plantas mais desenvolvidas e variedades tolerantes. Portanto, o objetivo do presente trabalho foi avaliar a reação de cultivares de cana-de-açúcar à M. fimbriolata, para que elas possam ser adequadamente locadas na matriz de manejo. Dois experimentos foram conducidos em condições de laboratório, usando o delineamento de blocos casualizados com seis (experimento 1) ou cinco (experimento 2) repetições e tratamentos em arranjo fatorial 2×11 (experimento 1) e 2×21 (experimento 2). O primeiro fator foi representado pelos dois níveis de infestação de cigarrinha (infestado e não infestado), enquanto o segundo, pelas cultivares em estudo. As cultivares IACSP01-5503, CTC 9004 e RB925211 foram consideradas tolerantes a M. fimbriolata, visto que a praga não causou redução significativa da massa verde da parte aérea, apesar de ter provocado alguns sintomas de ataque, como amarelecimento de folhas, enquanto CV6654 e IACSP01-3127 foram as cultivares menos tolerantes, pois mostraram as mais altas reduções na massa verde da parte aérea das plantas. Assim, CV6654 e IACSP01-3127 deveriam ser priorizadas na amostragem e controle em relação às demais. Na média, a cigarrinha das raízes provocou 30,9 % de redução na massa verde da parte aérea das plantas. **Palavras-chave**: cigarrinha das raízes, resistência de plantas, Saccharum.

## **INTRODUCTION**

The spittlebug *Mahanarva fimbriolata* (Stål) (Hemiptera: Cercopidae) is one of the most significant sugarcane pests in Brazil. In addition to a marked reduction of stalk yield, high populations of this pest result in alterations in the quality of the sugarcane by reducing stalk sugar content and

increasing fiber content. Industrial processes are also harmed because the damaged stalks reduce milling capacity. Since the stalks are often cracked, deteriorated and contaminated, contaminants make sugar recovery difficult and inhibit fermentation (DINARDO-MIRANDA, 2018).

The magnitude of the damage caused by the sipttlebug depends on the pest population, the

Received 07.07.20 Approved 07.07.21 Returned by the author 09.23.21 CR-2020-0632.R2 Editors: Leandro Souza da Silva plants size when they are infested (DINARDO-MIRANDA et al., 1999; 2001) and the cultivar tolerance (GARCIA et al., 2011; DINARDO-MIRANDA et al., 1999; 2014; 2016; 2018).

The knowledge about the influence of cultivar tolerance and the plants size on damage caused by spittlebug allowed the construction of the spittlebug management matrix, in which small plants and less tolerant varieties fields are prioritized for sampling and controlling in relation to developed plants and tolerant varieties fields (DINARDO-MIRANDA, 2018).

Thus, it is relevant for farmers knowing the cultivars reaction in relation to spittlebug. Since the cultivars studied in the aforementioned works are no longer cultivated or have even been released for planting in commercial areas, the objective of this study was to evaluate the tolerance of new genotypes and of various commercial sugarcane genotypes to *M. fimbriolata* attack.

## MATERIALS AND METHODS

Two conducted experiments were between January 2018 and March 2019, under laboratory conditions (room at 26  $\pm$  1 °C; 70  $\pm$ 10% RH; 12-h photoperiod) in Ribeirão Preto -SP, Brazil. In experiment 1, the following cultivars were evaluated: IACSP95-5094, IACSP95-6007, IACSP97-4039, IACSP01-3127, IACSP01-5503, IACSP04-7060, IACCTC05-2562, IACCTC05-8069, IACCTC07-8008, IACCTC07-8044 and SP81-3250, while in experiment 2, the cultivars studied were: CTC7, CTC14, CTC15, CTC17, CTC20Bt, CTC9002, CTC9003, CTC9004, CTC9005, CV6654, RB928064, CV7870, RB925211, RB965902, RB975201, RB975952, RB985476, RB988082, SP83-2847 and SP83-5073. The cultivar SP81-3250 was included due to its known susceptibility to the pest (DINARDO-MIRANDA et al., 2001) and the others due to be among the most planted cultivars in Central-South Region of Brazil or to be recently released to commercial planting (LANDELL & BRAGA JR., 2016).

To conduct the experiments, pots (5-liter) were filled with a mixture of agricultural substrate and clay soil (1:1) and a slow-release fertilizer (14-14-14, NPK; 200 g/25 kg soil + substrate). In each one, one bud of a given cultivar was planted and the pots were kept in a greenhouse for sugarcane growth. Approximately 90 days later, when the roots were well developed, the pots were taken to the laboratory for testing (DINARDO-MIRANDA et al., 2016). The

spittlebug populations used in both experiments had been reared on IACSP85-5000 sugarcane cultivar, according to a technique described by GARCIA et al. (2007), in the laboratory of Sugarcane Center of the Agronomic Institute (Instituto Agronômico - IAC).

To evaluate cultivar tolerance to nymph infestation, on January 23, 2018 (experiment 1) and on January 9, 2019 (experiment 2), 12 plants from each cultivar in experiment 1 and 10 plants of each cultivar in experiment 2 were uprooted, exposing great part of their roots, and placed on plastic dishes in the laboratory room for testing. Six plants from each cultivar from experiment 1 and five plants from each cultivar from experiment 2 were infested with spittlebug nymphs, while others six plants (experiment 1) or five plants (experiment 2) were not infested and were used as a control group. Each infested plant received six newly hatched nymphs, which were carefully transferred to the roots. Since there were not enough nymphs to infest the plants at once, the plants from each repetition were infested on consecutive days. Thus, the experiments were carried out using a randomized blocks design with six replicates in experiment 1. Due to availability of M. fimbriolata nymphs, experiment 2 was carried out with five replicates. Treatments were arranged in a factorial design  $(11 \times 2 \text{ in experiment } 1 \text{ and } 21 \times 2$ in experiment 2) with one of the factors representing the cultivars (11 or 21) and the other representing the presence or absence of spittlebug infestation (2). To prevent the escape of emerged adults from infested plants and to keep the uninfested plants in the same conditions, all plants were kept under a nylon-screen cages (approximately 1-mm mesh size). Every week, infested plants were inspected and, if needed, newly hatched nymphs were transferred to their roots, so that each plant remained infested with six nymphs during the experiment period. Thirty days after the first infestation, the damage caused by spittlebug nymphs to each plant was assessed by using a damage score based on a 1-to-5 visual scale, and adpted from CARDONA et al. (1999) (1 = no detectable damage; 2 = plants with mild symptoms, with slightly yellow leaves; 3 =plant with moderate symptoms, with several yellow leaves, some of them necrotic; 4 =plant with severe symptoms, with many yellow and necrotic leaves; 5 = dead plant). After that, the leaves and stalks of all plants were cut at soil level and weighed to obtain the aboveground biomass weight.

The analysis of variance was performed considering the effect of cultivar, infestation and cultivar  $\times$  infestation interaction, since the treatments were arranged in a factorial design. With the aboveground biomass weight data obtained on infested and non-infested plant, the percent reduction of aboveground biomass, caused by spittlebug infestations in each cultivar, was calculated. These data were transformed by the arcsine of the square root of (x/100) and the analysis of variance was performed considering a randomized-block design with six or five replicates. The means were compared using the Tukey test at 5 % significance.

#### **RESULTS AND DISCUSSION**

In both experiments, a significant difference between infested and non-infested plants was registered in relation to the symptoms caused by spittlebug, expressed in scores, revealing that the pest affected the development of all cultivars. On average, in trail 1 infested plots presented a score  $3.6 \pm 0.2$ , while non-infested plants  $1.0 \pm 0.1$ . In experiment 2, the scores were  $3.1 \pm 0.3$  and  $1.0 \pm 0.1$  in infested and non-infested plants, respectively (Tables 1 and 2).

The first spittlebug attack symptom on all cultivars plants was a slight yellowing of the leaves. In several cultivars, the yellowing evolved to severe chlorosis, necrosis of the leaves and, in some cultivars as IACSP97-4039, IACSP01-3127 and IAC04-7060, in experiment 1 and CV 6654, in experiment 2, to plant death. Several researchers have described that the spittlebug attack were followed by reductions in chlorophyll content, noticeable by the leaf chlorosis (BOINA et al., 2005; DIAZ-MONTANO et al., 2007; LÓPEZ et al., 2009). In *Brachyaria ruziziensis*, RESENDE et al. (2012) registered that the reduction

in chlorophyll leaves, with consequent reduction in photosynthetic activity, was caused by toxic saliva injected by *M. spectabilis* adults into leaves. However, DINARDO-MIRANDA (2008) state that nymphs feeding on the sugarcane roots also inject toxic saliva, that reduces leaf chlorophyll content and, consequently, the photosynthetic rate, with a decrease in plant production. These facts were observed in the present work and by several authors, including DINARDO-MIRANDA et al. (2014, 2016; 2018), MELO et al. (2018) and VALVERDE et al. (2018), in experiments involving several sugarcane varieties.

In this study, just cultivars IACSP01-5503 in experiment 1 and CTC 9004 and RB925211 in experiment 2 did not show significant difference in relation to aboveground biomass between infested and non-infested plants, suggesting that these cultivars have some degree of tolerance to M. fimbriolata. However, since even those three cultivars showed symptom of spittlebug attack, it is expected that under a longer period of infestation or a higher infestation, they could have great loss of aboveground biomass. The other cultivars showed a significant difference in relation to aboveground biomass between infested and non-infested plants, being considered non-tolerant to M. fimbriolata. On average, M. fimbriolata-infested plants showed loss of 30.9 % of aboveground biomass compared with uninfested plants, in both experiments (Tables 1 and 2).

In addition to IACSP01-5503, CTC 9004 and RB925211, included in this study, other cultivars, such as IACSP94-2094, IACSP96-7569

 Table 1 - Symptom scores and aboveground biomass weigh (g) (mean ± standart error) for each cultivar, both infested and non-infested with Mahanarva fimbriolata. Ribeirão Preto/SP, January 2018. Experiment 1.

Cultivar	Symptom score		Aboveground biomass weight (g)	
	infested	non-infested	infested	non-infested
IACSP95-5094	$2.7\pm0.3$ a	$1.0\pm0.1 \text{ b}$	$91.8\pm3.8~a$	$109.5\pm3.2~b$
IACSP95-6007	$3.5\pm0.4\;a$	$1.0\pm0.1\ b$	$69.5\pm6.0~a$	$112.2\pm6.7~b$
IACSP97-4039	$4.3 \pm 0.3 \ a$	$1.0\pm0.1\;b$	$41.8 \pm 4.3$ a	$61.7\pm6.5~b$
IACSP01-3127	$4.8\pm0.2~a$	$1.0\pm0.1 \text{ b}$	$30.4 \pm 5.1 \text{ a}$	$83.5\pm8.6\ b$
IACSP01-5503	$2.8\pm0.2$ a	$1.0\pm0.1\;b$	$91.3 \pm 7.4$ a	$106.0 \pm 6.9$ a
IACSP04-7060	$4.3\pm0.3\ a$	$1.0\pm0.1\ b$	$72.1\pm8.5~a$	$100.9\pm6.7~b$
IACCTC05-2562	$2.7\pm0.3~a$	$1.0\pm0.1\ b$	$100.2 \pm 6.9 \text{ a}$	$129.1\pm3.9~b$
IACCTC05-8069	$4.0\pm0.4~\mathrm{a}$	$1.0\pm0.1 \text{ b}$	$63.1 \pm 6.0 \text{ a}$	$126.4\pm4.6\ b$
IACCTC07-8008	$2.8\pm0.3~a$	$1.0\pm0.1\ b$	$101.6 \pm 2.5 \text{ a}$	$127.3\pm9.6~b$
IACCTC07-8044	$3.3\pm0.2$ a	$1.0\pm0.1 \ b$	$88.2\pm7.0~a$	$116.9\pm5.0\ b$
SP81-3250	$4.7\pm0.2$ a	$1.0\pm0.1 \text{ b}$	35.3 ± 3.1 a	$62.4\pm6.1~b$
Mean	$3.6\pm0.2\ a$	$1.0\pm0.1\ b$	$71.3\pm6.5~a$	$103.3\pm6.5~\text{b}$

Means within the same cultivar and same parameter followed by the same latter are not significantly different (Tukey test,  $P \le 0.5$ ).

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Cultivar	Symptom score		Aboveground biomass weight (g)	
	infested	non-infested	infested	non-infested
CTC 7	$3.0\pm0.4$ a	$1.0\pm0.1\;b$	$55.6\pm6.8~a$	$76.7\pm6.9\ b$
CTC 14	$2.6 \pm 0.3$ a	$1.0\pm0.1\ b$	$68.2\pm3.2~a$	$84.7\pm5.3\ b$
CTC 15	$3.2\pm0.2$ a	$1.0\pm0.1\ b$	$57.7\pm4.5~a$	$73.8\pm6.4\ b$
CTC 17	$3.6 \pm 0.2 \ a$	$1.0\pm0.1\;b$	$48.1\pm5.2\;a$	$70.6\pm5.2\;b$
CTC 20Bt	$2.2\pm0.2$ a	$1.0\pm0.1\;b$	$70.2\pm4.8~a$	$101.1 \pm 12.7 \text{ b}$
CTC 9002	$3.2\pm0.4$ a	$1.0\pm0.1\ b$	$65.6\pm6.9~a$	$90.9\pm1.3\ b$
CTC 9003	$3.0\pm0.4$ a	$1.0\pm0.1~\mathrm{b}$	$32.1 \pm 7.7 \text{ a}$	$54.7\pm6.9~b$
CTC 9004	$2.0\pm0.1$ a	$1.0\pm0.1~\mathrm{b}$	$59.1\pm3.2$ a	$71.5 \pm 4.2 \text{ a}$
CTC 9005	$3.4\pm0.4$ a	$1.0\pm0.1~\mathrm{b}$	$25.5\pm2.9~a$	$42.7\pm2.0\ b$
CV 6654	$5.0\pm0.1$ a	$1.0\pm0.1\ b$	$2.4\pm2.6\;a$	$30.9\pm4.6\;b$
CV 7870	$2.8\pm0.3$ a	$1.0\pm0.1~\mathrm{b}$	$78.9\pm5.5~a$	$113.6\pm10.1b$
RB925211	$3.0\pm0.4$ a	$1.0\pm0.1\ b$	$59.4\pm3.3~a$	$72.8\pm4.5~a$
RB928064	$2.8\pm0.2$ a	$1.0\pm0.1~\mathrm{b}$	$35.3 \pm 4.8 \text{ a}$	$58.7\pm4.5\ b$
RB965902	$3.0\pm0.5~a$	$1.0\pm0.1~\mathrm{b}$	$43.0 \pm 6.1 \text{ a}$	$59.0\pm3.5~b$
RB975201	$3.2\pm0.5~a$	$1.0\pm0.1~\mathrm{b}$	$34.3 \pm 4.3 \text{ a}$	$60.2\pm4.1~\text{b}$
RB975952	$3.0\pm0.3$ a	$1.0\pm0.1~\mathrm{b}$	$47.5 \pm 4.3 \text{ a}$	$67.5\pm3.9~b$
RB985476	$2.8\pm0.2$ a	$1.0\pm0.1~\mathrm{b}$	$50.5\pm5.2$ a	$69.2\pm6.7~b$
RB988082	$3.4\pm0.3$ a	$1.0 \pm 0.1$ b	$40.7\pm8.0~a$	$60.7\pm4.5~b$
SP81-3250	$3.0\pm0.3$ a	$1.0\pm0.1\ b$	$22.2\pm1.9~\mathrm{a}$	$39.6\pm4.9\ b$
SP83-2847	$3.4\pm0.5~a$	$1.0\pm0.1~\mathrm{b}$	$46.4 \pm 11.0$ a	$65.3 \pm 11.2 \text{ b}$
SP83-5073	$3.0\pm0.3$ a	$1.0\pm0.1~\mathrm{b}$	$45.1 \pm 2.0$ a	$60.7\pm5.3~b$
Mean	$3.1\pm0.3$ a	$1.0\pm0.1\;b$	$47.0 \pm 4.1 \text{ a}$	$69.2\pm6.6~b$

 Table 2 - Symptom scores and aboveground biomass weigh (g) (mean ± standart error) for each cultivar, both infested and non-infested with Mahanarva fimbriolata. Ribeirão Preto/SP, January 2019. Experiment 2.

Means within the same cultivar and same parameter followed by the same latter are not significantly different (Tukey test supported by Anova,  $P \le 0.5$ ).

and IACSP96-7586, were also considered tolerant to *M. fimbriolata* (DINARDO-MIRANDA et al., 2014). The reasons why some sugarcane cultivars are more tolerant than others to *M. fimbriolata* are unknown.

Tolerant cultivars play an important role in pest-management programs (SMITH, 2005). Since pest populations are kept at high levels when tolerant cultivars are cultivated, it is less likely the development of pest-insect biotypes that can break host resistance in this condition, compared to situations in which cultivars that show antibiosis or antixenosis resistance are cultivated. Nevertheless, several researchers concur that there is a significant risk when tolerant cultivars are planted across large and continuous areas, because the populations can grow so much that they break the tolerance of cultivars and cause severe crop damage (CARDONA et al., 2004; LAPOINTE et al., 1992). According to DINARDO-MIRANDA et al. (2014), the use of tolerant cultivars, without any degree of antibiosis and antixenosis, in spittlebug management in sugarcane involves a certain risk in Brazil, because this crop is cultivated in large continuous areas. Therefore, the eventual cultivation in large areas of cultivars IACSP01-5503, CTC 9004 or RB925211 demands attention because, although the varieties showed a certain tolerance, they presented some symptoms of pest attack, such as leaves yellowing, suggesting that, under more severe pest infestation or subjected to a longer pest infestation period, the tolerance can be broken and other pest control measures can be necessary.

The decrease in aboveground biomass varied from  $13.6 \pm 6.6$  %, in IACSP01-5503 to 60.3  $\pm$  7.6 % in IACSP01-3127 in experiment 1, while in experiment 2, it varied from  $17.2 \pm 3.1$  % in CTC 9004 to 91.5  $\pm$  5,7 % in CV 6654 in experiment 2 (Table 3). DINARDO-MIRANDA et al. (2014; 2016; 2018) also found different tolerance degrees among sugarcane cultivars, some of them could be considered tolerant, as IACSP96-7569, for example. In SP81-3250, the susceptibility standard, the decrease

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Experiment 1		Experiment 2		
Cultivar	Reduction (%)	Cultivar	Reduction (%)	
IACSP01-3127	$60.3 \pm 7.6$ a	CV 6654	$91.5 \pm 5.7 \text{ a}$	
SP81-3250	$45.3\pm8.0\ ab$	CTC 9003	$43.3\pm6.0\ b$	
IACSP95-6007	$37.8 \pm 3.0 \text{ ab}$	RB975201	$41.2 \pm 5.5 \ bc$	
IACSP97-4039	$33.1\pm8.3$ ab	SP81-3250	$41.0 \pm 4.1 \text{ bc}$	
IAC04-7060	$29.4 \pm 7.1 \text{ ab}$	CTC 9005	$40.8\pm3.7~\text{bc}$	
IACCTC07-8044	$24.2\pm5.6\ b$	RB928064	$39.6\pm2.6$ bc	
IACCTC05-2562	$22.4\pm4.8\ b$	RB988082	$37,7 \pm 7.5 \text{ bc}$	
IACCTC05-8069	$19.7\pm7.3~b$	CTC 17	$31.9 \pm 4.9 \text{ bc}$	
IACCTC07-8008	$17.3 \pm 2.2 \text{ b}$	CTC 20Bt	$31.5 \pm 4.1 \text{ bc}$	
IACSP95-5094	$16.1\pm1.5\ b$	RB975952	$30.1 \pm 3.2 bc$	
IACSP01-5503	$13.6\pm6.6$ b	CV 7877	$29.0\pm8.5~bc$	
		RB965902	$28.8\pm6.0\ bc$	
		SP83-2847	$28.4 \pm 4.9 \text{ bc}$	
		CTC 9002	$28.4 \pm 3.8$ bc	
		CTC 7	$26.8 \pm 2.2 \text{ bc}$	
		RB985476	$25.1 \pm 3.6$ bc	
		SP83-5073	$24.5 \pm 2.8 \text{ bc}$	
		CTC 15	$20.7 \pm 3.4 \text{ bc}$	
		CTC 14	$19.3\pm6.0\ bc$	
		RB925211	$18.2\pm4.1\ c$	
		CTC 9004	$17.2 \pm 3.1 \text{ c}$	
Mean	$30.9\pm4.2$	Mean	$30.9\pm3.2$	

Table 3 - Reduction (%) in aboveground biomass weight (mean ± standart error) due to Mahanarva fimbriolata infestation on each cultivar, in experiment 1 and 2. Ribeirão Preto/SP, January 2018 and January 2019.

Means within the same cultivar and same parameter followed by the same latter are not significantly different (Tukey test,  $P \le 0.5$ ).

in aboveground biomass due to *M. fimbriolata* attack was  $43.5 \pm 8.0$  % in experiment 1 and  $41.0 \pm 4.1$  % in trail 2, intermediate values to those observed in experiments conducted by DINARDO-MIRANDA et al. (2014; 2016; 2018), in similar conditions, in which the authors registered values ranging from  $30.3 \pm 5.6$  % to  $53.9 \pm 9.7$  %. Considering the decrease in aboveground biomass due to *M. fimbriolata* attack, IACSP01-3127 (experiment 1) and CV 6654 (experiment 2) should be considered more susceptible to spittlebugs than SP81-3250 (Table 3).

Although almost all tested cultivars are non-tolerant to *M. fimbriolata*, the differences on the degree of susceptibility may help to establish the management schedule, mainly sampling and adoption of control measures. Since the nymphs emerge in field from diapause eggs in the beginning of the rainy season (spring and summer), the spittlebug population grow up at the same time in all cultivars. Thus, to elaborate the spittlebug management matrix, the knowledge about the cultivar's rection in relation to spittlebug is relevant, since that small plants and less tolerant varieties fields are prioritized for sampling and controlling in relation to developed plants and tolerant varieties fields (DINARDO-MIRANDA, 2018). Therefore, those cultivars less tolerant, that present greater productivity reduction due to the spittlebug attack, such as CV6654 and IACSP01-3127, should be prioritized in sampling and control over those more tolerant, that present less damage, such as IACSP01-5503, IACSP95-5094, IACCTC07-8008, CTC9004 and RB925211.

## **CONCLUSION**

The cultivars IACSP01-5503, CTC 9004 and RB925211 are tolerant to *M. fimbriolata*.

# DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## **AUTHORS' CONTRIBUTIONS**

Conceptualization and Data acquisition: LLDM, JVF, HDSS and IDM. Design of methodology and data analysis: LLDM, JVF and HDS. LLDM and IDM prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

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