

## *In-vitro* and *in-vivo* evaluation of Inorganic Salts for the Management of Wheat Stem Rust (*Pucciniagraminis f.sp. tritici*)

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### ABSTRACT

**Purpose :** The development of new resistant pathogen races is increasing the concern of food safety and the environments are driving new search for alternative disease management strategies. This study was conducted to evaluate the effect of inorganic salts on wheat stem rust under *in-vitro* and *in-vivo* conditions.

**Research Method :** Inorganic salts were evaluated on their effect on the percentage uredospore germination, pustule number and pustule length of *Pucciniagraminis f.sp. tritici* at different concentrations, *in-vitro* and *in-vivo*. All the data were subjected to analysis of variance using SAS version 9.1.3 software. The least significant difference at 5% level of significance was used to separate treatment means.

**Findings :** There was a significant ( $P < 0.05$ ) difference among inorganic salts and their concentrations on the germination of *Pgturedospores*, *in-vitro*. Potassium carbonate completely inhibited the germination of *Pgturedospores* (100%) at all concentrations (i.e. 10, 15, and 20 gl<sup>-1</sup>) followed by potassium bicarbonate, sodium bicarbonate and ammonium bicarbonate with inhibitions of 99.7, 97.33 and 97%, respectively. The lowest inhibition of spore germination was recorded from potassium dihydrogen phosphate (6.7%) and potassium chloride (9.3%) at 5 gl<sup>-1</sup>. The *in-vivo* study showed that six inorganic salts significantly ( $P < 0.05$ ) reduced the number of pustules and pustule lengths on susceptible wheat cultivar 'McNair'.

**Originality / Value :** The present study identified potassium carbonate and potassium bicarbonate as the best inorganic salts to manage wheat stem rust.

**Keywords:** Inorganic salts, *In-vitro*, *In-vivo*, *Pucciniagraminis f.sp. tritici*, Wheat stem rust

### INTRODUCTION

Wheat (*Triticum*spp.) is one of the most important cereal crops in the world in terms of production and nutritional value. In Ethiopia, it is an important staple food crop. It is the preferred diets of Ethiopians, providing about 15% of the caloric intake for over 90 million population (FAO, 2015), placing it second after maize and slightly ahead of teff, sorghum and enset, which contribute 10-12% each (Minot *et al.*, 2015). The area under wheat production is estimated to be around 1.7 million hectares (CSA, 2018), which makes the country the largest wheat producer (4.64 million tons) in Sub-Saharan Africa. However, productivity of wheat in Ethiopia is very low. The low productivity is attributed to several factors including biotic (diseases, insect pests and weeds), abiotic (moisture, soil fertility, etc) and lack or poor adoption of new

agricultural technologies (Zegeye *et al.*, 2001).

Among those biotic factors, stem rust caused by the fungus *Pucciniagraminis f.sp. tritici* Eriks. and E. Henn, has been the most devastating wheat production problem. The disease is mostly severe at the later stage of crop development and is mainly a disease of vegetative and the reproductive portion of the host (Roelfs, 1985).

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Under favorable environmental conditions, stem rust can cause yield losses of up to 100% in susceptible wheat varieties (Leonard and Szabo, 2005; Roelfs, 1985). In Ethiopia, yield losses due to stem rust have been reported to be in the range of 61-100% depending on the susceptibility of the variety and environmental conditions (Bechere *et al.*, 2000).

Management practices such as host resistance, use of chemicals and cultural methods are implemented to control stem rust in Ethiopia. The use of synthetic pesticides remains the most widely used disease control measure against plant pathogens, even though they have shown major drawbacks such as their lack of long-term efficacy due to the development of resistance by plant pathogens (Avis, 2007). In addition, Kibria *et al.*, (2010) and Komarek *et al.*, (2010) reported that the regular use of fungicides can potentially pose a risk to the environment, particularly if residual effect in the soil migrate off site and enter waterways. For instance, concerns have been raised over the long term use of copper-based fungicides, which can result in the accumulation of copper in the soil. This in turn can have adverse effects on soil organisms and pose potential risks to the long term fertility of the soil (Komarek *et al.*, 2010). Therefore, there is a need for finding alternative management options which are environmentally friendly, accessible and affordable to local farmers in the control of the disease. Several inorganic salts have been tested worldwide as alternative method to manage plant diseases on a wide range of crops. Foliar spray of sodium or potassium bicarbonates led to decreased powdery mildew (*Leveillulataurica*) severity in pepper plants (Fallik *et al.*, 1997). Furthermore, septoria leaf blotch (*Septoria tritici*) of winter wheat was significantly reduced by foliar application of potassium chloride (Mann, 2004). The use of inorganic salts for disease management has advantages over application of synthetic fungicides because it is less costly, and non-poisonous to humans and the environment (Reuveni *et al.*, 1995). Therefore, the present study was designed to evaluate the effect of inorganic salts on wheat stem rust (*Pucciniagraminis* f.sp. *tritici*) under *in-vitro* and *in-vivo* conditions.

## MATERIALS AND METHODS

The present study consisted of two independent experiments i.e. *in-vitro* and *in-vivo* evaluation of inorganic salts on uredospore germination of *Pgt*, and infection of wheat by *Pgt* under greenhouse conditions, respectively. The experiments were conducted at Ambo Agricultural Research Center.

### *Effect of inorganic salts on uredospore germination of Pgt, in-vitro*

The inhibitory effect of inorganic salts on uredospore germination of *Pucciniagraminis* f.sp. *tritici* was evaluated using eight inorganic salts namely: potassium bicarbonate ( $\text{KHCO}_3$ ), sodium bicarbonate ( $\text{NaHCO}_3$ ), ammonium bicarbonate ( $\text{NH}_4\text{HCO}_3$ ), potassium carbonate ( $\text{K}_2\text{CO}_3$ ), dipotassium hydrogen phosphate ( $\text{K}_2\text{HPO}_4$ ), potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ), sodium chloride ( $\text{NaCl}$ ) and potassium chloride ( $\text{KCl}$ ) in *in-vitro* condition. A solution of each inorganic salt at concentrations of 5, 10, 15 and 20  $\text{g l}^{-1}$  was prepared. Five (5) milliliter of each solution was added on to a water agar medium in Petri dishes, and mixed gently to dispense uniformly. *Pgt* uredospore suspension adjusted at 100 spores  $\text{ml}^{-1}$  was inoculated to the water agar medium having the various concentrations of inorganic salts. Water agar medium without any inorganic salts served as a control. All Petri dishes were later incubated in a chamber adjusted at  $20 \pm 2$  °C and 95-100% RH for 48 hrs. The experiment was set in a completely randomized design with three replications. The experiment was repeated following the same procedure.

### *Foliar spray of inorganic salt on wheat stem rust infection*

The effect of inorganic salt foliar sprays on wheat stem rust infection was evaluated using the eight inorganic salts mentioned above. The experiment was conducted using the universally susceptible wheat variety 'McNair'. Five seeds of the variety 'McNair' were grown in 8 cm diameter pots in the greenhouse. The inorganic salt solutions were applied at 5, 10, 15 and 20  $\text{g l}^{-1}$  concentrations on the third leaves (21 days after planting) of each plant. Each 'McNair'

plant received 30 ml of the salt solution which was sprayed to cover the whole leaves ensuring both upper and lower surface of the leaves. Plants were kept at ambient temperature for one hour for the solution on the leaves to dry. An hour later, plants were inoculated with *Pgt* uredospore suspension adjusted at  $1 \times 10^5$  spores  $\text{ml}^{-1}$ . The spore suspension was applied using the spore inoculators 'Vacuubrand' (Wertheim, Germany). Inoculated plants were placed in a dew chamber at  $18^\circ\text{C}$  for 24 hrs in the dark and  $20^\circ\text{C}$  in the light without the polyethylene cover. Water treated plants were used as control. Disease infection was assessed seven days after the application of inorganic salt solutions and inoculum of *Pgt*. The experiment was laid out in a completely randomized design with three replications and the experiment was repeated.

### Data collection

The number of germinated and non-germinated uredospores on each agar medium was counted using compound microscope after 48 hrs of inoculation. The percentage of germinated spores was calculated using the following formula.

$$\text{Percentage of Uredospore germination} = \frac{\text{Number of germinated Uredospores}}{\text{Total number of inoculated Uredospores}} \times 100$$

Additional disease data, such as the number of pustules and pustule lengths, from the *in-vivo* experiment were collected from seedlings. The number of pustules was counted at the middle of single leaves of randomly selected five plants. The shape of pustules was elliptical, hence the pustule length was measured by taking the length of five pustules from each plant per pot using graduated ruler. The average pustule length of the five measurements was used for the final analysis.

### Data analysis

All the data, percent of uredospore germination, number of pustules and pustule length, were subjected to analysis of variance (ANOVA) using SAS version 9.1.3 software (SAS, 2003). The least significant difference (LSD) at 5% level of significance was used to separate treatment means.

## RESULTS AND DISCUSSION

### *The effects of inorganic salts on uredospore germination*

There was a significant difference ( $P < 0.05$ ) among inorganic salts and their concentrations on the inhibition of uredospore germination of *Puccinia graminis* sp. *tritici* under *in-vitro* condition. Potassium carbonate showed the highest inhibition rate (100%) of uredospore germination at all concentrations (Table 01 and Figure 01). Potassium bicarbonate was the second best with 99.7% inhibition rate, followed by sodium bicarbonate and ammonium bicarbonate with 97.3 and 97% at concentrations of  $20 \text{ gl}^{-1}$  respectively. Potassium carbonate at all concentrations, sodium bicarbonate at 15 and  $20 \text{ gl}^{-1}$ , ammonium bicarbonate at 10, 15 and  $20 \text{ gl}^{-1}$ , potassium bicarbonate at 10, 15 and  $20 \text{ gl}^{-1}$ , dipotassiumbiposphate at  $20 \text{ gl}^{-1}$  inhibited spore germination by 95-100%. The two compounds that were ineffective were potassium dihydrogen phosphate and sodium chloridewith spore inhibition rates ranging from 6.7- 8.3 and 14.7-37.3%, respectively (Table 01).

Several studies showed that inorganic salts affect the germination of several plant pathogens. According to Turkkan *et al.*, (2017) the germination of spores of *Botrytis cinerea* was completely inhibited by the use of potassium carbonate at 25 and 50 mM and potassium bicarbonate at 50 and 75 mM. The previous studies have also shown that carbonate and bicarbonate salts including ammonium, sodium and potassium to have inhibitory effects on the mycelial growth of many fungal species (Droby *et al.*, 2003; Latifa *et al.*, 2011; Palou *et al.*, 2001; Youssef *et al.*, 2012). Similarly, Bombelli and Wright (2006) found that potassium bicarbonate reduced the mycelial growth of *B.cinereato* some extent (65%) at 0.5%; and had complete inhibitory effect at 1% and higher concentrations.

**Table 01:** The effect of inorganic salts on *in-vitro* germination of uredospore of *Pgt*

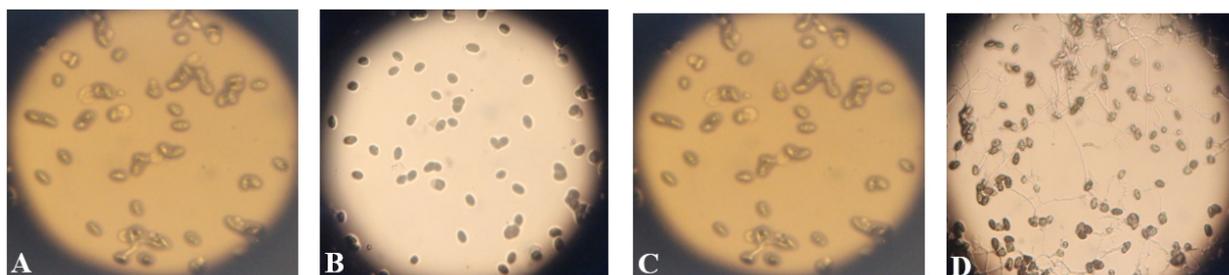
Inorganic salt	Concentration (gl <sup>-1</sup> )	Germination (%)
NaHCO <sub>3</sub>	5	51.7 <sup>g</sup>
	10	12.0 <sup>ih</sup>
	15	4.3 <sup>j</sup>
	20	2.7 <sup>j</sup>
NH <sub>4</sub> HCO <sub>3</sub>	5	48.3 <sup>g</sup>
	10	3.7 <sup>j</sup>
	15	3.3 <sup>j</sup>
	20	3.0 <sup>j</sup>
KCl	5	90.7 <sup>bc</sup>
	10	76.7 <sup>e</sup>
	15	68.3 <sup>f</sup>
	20	16.7 <sup>ih</sup>
NaCl	5	85.3 <sup>dc</sup>
	10	81.0 <sup>de</sup>
	15	67.0 <sup>f</sup>
	20	62.7 <sup>f</sup>
KHCO <sub>3</sub>	5	17.7 <sup>h</sup>
	10	3.0 <sup>j</sup>
	15	1.0 <sup>j</sup>
	20	0.3 <sup>j</sup>
KH <sub>2</sub> PO <sub>4</sub>	5	93.3 <sup>ba</sup>
	10	90.0 <sup>bc</sup>
	15	86.7 <sup>dc</sup>
	20	81.7 <sup>de</sup>
K <sub>2</sub> HPO <sub>4</sub>	5	88.0 <sup>bc</sup>
	10	63.3 <sup>f</sup>
	15	11.0 <sup>i</sup>
	20	2.3 <sup>j</sup>
K <sub>2</sub> CO <sub>3</sub>	5	0.3 <sup>j</sup>
	10	0.0 <sup>j</sup>
	15	0.0 <sup>j</sup>
	20	0.0 <sup>j</sup>
Control	0	97.3 <sup>a</sup>
LSD (5%)		6.21
CV (%)		9.58

Means within a column followed by the same letter are not significantly different, CV=Coefficient of Variation, LSD = Least Significance Difference

### ***The effects of inorganic salt foliar sprays on wheat stem rust***

There was a significant ( $P < 0.05$ ) difference among inorganic salts and their concentrations in reducing pustule length and pustule number of *Pgt* on the susceptible host 'McNair' (Table 02). The lowest number of pustules and pustule lengths were recorded from plants treated with potassium carbonate and potassium bicarbonate (Figure 02). Plants treated with potassium

carbonate at concentrations of 10-20 gl<sup>-1</sup> had the lowest mean pustule count of 5.3-8.0 followed by potassium bicarbonate and sodium bicarbonate at concentration of 10-20 gl<sup>-1</sup> with mean pustule counts of 6.7-8.5 and 7.7-10 per single leaf, respectively. The highest number of pustules and pustule lengths were recorded from plants treated with potassium dihydrogen phosphate and sodium chloride at 5 gl<sup>-1</sup>, respectively.

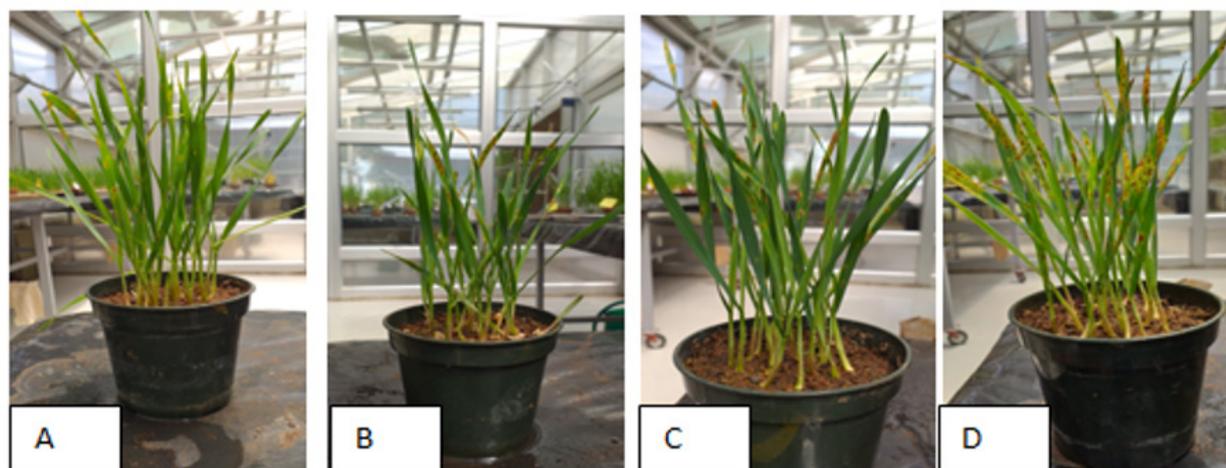


**Figure 01:** Inhibitory effect of inorganic salts on the germination of *Pgt* uredospores under *in-vitro* condition. A)  $\text{KHCO}_3$  at  $15\text{gl}^{-1}$  B)  $\text{K}_2\text{CO}_3$  at  $15\text{gl}^{-1}$  C)  $\text{NaHCO}_3$  at  $15\text{gl}^{-1}$  D) Water (control)

**Table 02:** The effect of inorganic salts on the infection of *Pgt* under greenhouse condition

Inorganic salt	Concentration ( $\text{gl}^{-1}$ )	Number of pustule/leaf	Pustule length (mm)
$\text{NaHCO}_3$	5	10.0 <sup>mn</sup>	2.7 <sup>ba</sup>
	10	9.0 <sup>pno</sup>	2.0 <sup>de</sup>
	15	8.3 <sup>pqo</sup>	1.3 <sup>gghi</sup>
	20	7.7 <sup>pqr</sup>	1.1 <sup>ji</sup>
$\text{NH}_4\text{HCO}_3$	5	12.0 <sup>kij</sup>	2.2 <sup>dc</sup>
	10	11.5 <sup>kl</sup>	1.7 <sup>gef</sup>
	15	10.7 <sup>ml</sup>	1.2 <sup>jhi</sup>
	20	9.7 <sup>mno</sup>	1.0 <sup>j</sup>
KCl	5	14.5 <sup>ghefd</sup>	2.7 <sup>ba</sup>
	10	13.8 <sup>ghi</sup>	2.6 <sup>b</sup>
	15	13.2 <sup>hij</sup>	2.0 <sup>de</sup>
	20	12.5 <sup>kij</sup>	1.3 <sup>gghi</sup>
NaCl	5	15.5 <sup>cebd</sup>	2.8 <sup>ba</sup>
	10	14.5 <sup>ghefd</sup>	2.5 <sup>bc</sup>
	15	15.0 <sup>ghf</sup>	2.0 <sup>de</sup>
	20	13.3 <sup>hij</sup>	1.7 <sup>gef</sup>
$\text{KHCO}_3$	5	8.5 <sup>po</sup>	2.0 <sup>de</sup>
	10	7.7 <sup>pqr</sup>	1.4 <sup>ghi</sup>
	15	7.0 <sup>sqr</sup>	1.0 <sup>j</sup>
	20	6.7 <sup>str</sup>	1.0 <sup>j</sup>
$\text{KH}_2\text{PO}_4$	5	16.8 <sup>b</sup>	2.0 <sup>de</sup>
	10	16.0 <sup>cb</sup>	2.0 <sup>de</sup>
	15	15.3 <sup>cefd</sup>	1.4 <sup>ghi</sup>
	20	14.5 <sup>ghefd</sup>	1.4 <sup>ghi</sup>
$\text{K}_2\text{HPO}_4$	5	15.7 <sup>cbd</sup>	2.0 <sup>de</sup>
	10	14.8 <sup>geefd</sup>	2.0 <sup>de</sup>
	15	14.2 <sup>ghf</sup>	2.0 <sup>de</sup>
	20	13.8 <sup>ghi</sup>	1.8 <sup>def</sup>
$\text{K}_2\text{CO}_3$	5	8.0 <sup>pqr</sup>	1.5 <sup>ghf</sup>
	10	7.0 <sup>sqr</sup>	1.0 <sup>j</sup>
	15	6.2 <sup>st</sup>	1.0 <sup>j</sup>
	20	5.3 <sup>t</sup>	1.0 <sup>j</sup>
Control	0	18.3 <sup>a</sup>	3.0 <sup>a</sup>
LSD (5%)		1.37	0.34
CV (%)		7.17	11.82

Means within a column followed by the same letter are not significantly different, CV= Coefficient of Variation, LSD = Least Significance Difference



**Figure 02:** Effect of inorganic salts on the spore development of *Pgt* under greenhouse condition. A)  $K_2CO_3$  at  $15\text{ g l}^{-1}$  B)  $NaHCO_3$  at  $15\text{ g l}^{-1}$  C)  $KHCO_3$  at  $15\text{ g l}^{-1}$  D) Water (Control)

A previous study by Caldwell *et al.*, (2013) showed that the use of potassium bicarbonate resulted in good control of black rot and *Phomopsis* grapes and leaf spot on strawberry. Powdery mildew, caused by *Sphaerotheca pannosavar. rosae*, and black spot, caused by *Diplocarpon rosae*, were significantly controlled by weekly sprays of sodium bicarbonate on *Rosa* species (Horst *et al.*, 1992). Arslan *et al.*, (2006) showed that potassium bicarbonate, sodium bicarbonate and ammonium bicarbonate, concentrations ranging from  $0.47\text{--}12\text{ g l}^{-1}$  completely inhibited spore germination and germ tube elongation of the uredospores of bean rust (*Uromyces appendiculatus*) and wheat leaf rust (*Puccinia triticina*) under *in-vitro* and pot experiments in controlled environments indicating that spray applications of potassium bicarbonate ( $6\text{ g l}^{-1}$ ), sodium bicarbonate ( $10.1\text{ g l}^{-1}$ ) and ammonium bicarbonate ( $9.4\text{ g l}^{-1}$ ) to 10-day-old wheat plants of the highly susceptible Gonen cultivar reduced the number of *P. triticina* pustules on the leaves by 82, 92 and 90%, respectively, relative to controls. In the present study potassium chloride was not effective in controlling wheat stem rust disease but Mann *et al.*, (2004) showed that potassium chloride was effective in reducing the severity of septoria leaf blotch on winter wheat. Prabhu *et al.*, (2007) noted that the effect of potassium on disease can be classified as “increased”, “decreased” and having “no effect” or “variable effect”. The variable effects of potassium on

disease could be affected by the amount and source of the element, plant and pathogen species.

The present study showed that potassium carbonate and potassium bicarbonate as the best inorganic salts in controlling stem rust development on the susceptible wheat cultivar ‘McNair’ by suppressing the germination of uredospores and limiting the development of pustules. Various authors have proposed different mechanisms in which potassium improves the resistance of plants against pathogens. For example, Feng & Zeng (2006) and Machinandiarena *et al.*, (2012) stated that potassium increases the resistance of plants to stresses and supports the natural defense of plants against pathogens. These have been linked to processes such as pH elevation on the leaf surface (Ziv and Zitter, 1992), and collapse of fungal cell walls due to  $K^+$  imbalance or dehydration of fungal spores (HDC, 2005).

It is also important to mention that ammonium bicarbonate, potassium carbonate and potassium bicarbonate had a slight burning effect on the leaf margins of wheat at the highest concentration ( $20\text{ g l}^{-1}$ ). This was an indicator that at higher concentrations inorganic salts may have phototoxic effects on wheat leaves. The present observation concurs with previous studies by various authors (Ilhan *et al.*, 2006; Karabulut *et al.*, 2005) about the phytotoxic effects of inorganic salts on several plant species. Hence, there is a need to be cautious

when recommending inorganic salts for the management of plant diseases.

## CONCLUSION

Stem rust caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*) is a serious wheat disease that causes high yield reduction in wheat. Their emergence of a new virulent race in eastern African regions and the subsequent detection of its variants have rendered important commercial varieties become susceptible. The use of fungicide is not safe to the environment and applicators. Hence, inorganic salts may overcome the challenges associated with fungicides. The present study showed that germination of uredospores of *Pgt* was strongly affected by application of potassium carbonate and potassium bicarbonate, *in-vitro*. Potassium carbonate completely inhibited (100%) the germination of the fungal spores at 10-20 g<sup>l</sup><sup>-1</sup> concentrations, whereas potassium bicarbonate reduced germination by 99% at 15g<sup>l</sup><sup>-1</sup> and 100% at 20g<sup>l</sup><sup>-1</sup> concentrations. Similarly, the number of pustules and pustule lengths were significantly reduced by these two inorganic salts when tested *in-vivo*, in a

greenhouse environment. This is an indication that the two inorganic salts, potassium carbonate and potassium bicarbonate, have the potential to manage wheat stem rust. However, further investigations on the rate, frequency and time of application under field environment is required before making definitive conclusions.

## Data Availability Statement

The datasets generated and analyzed during the current study are not publicly available. They are available from the corresponding author on reasonable request.

## Conflict of Interests

The authors had not declared any conflict of interests.

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