

PLANT SCIENCE TODAY ISSN 2348-1900 (online) Vol 11(sp4): 01–10 <https://doi.org/10.14719/pst.5208>

RESEARCH ARTICLE

Influence of biogenic silica seed coating on the biochemical parameters of sorghum (var. k12) seeds stored in different containers under ambient conditions

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ARTICLE HISTORY

Received: 22 September 2024 Accepted: 31 October 2024 Available online Version 1.0 : 05 December 2024

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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CITE THIS ARTICLE

Krishnaarivanandhan A, Alex Albert V, Sujatha K, Kannan P, Arunachalam P. Influence of biogenic silica seed coating on the biochemical parameters of sorghum (var. k12) seeds stored in different containers under ambient conditions. Plant Science Today.2024;11(sp4):01-10. <https:/doi.org/10.14719/pst.5208>

Abstract

This research aimed to investigate the biochemical properties of Biogenic silica-coated sorghum seeds stored in various containers at ambient temperatures. Seeds often lose viability and vigour due to suboptimal storage conditions. The packaging material and storage conditions influence a seed's durability and long-term viability. Applying a protective substance to the seeds before storage can help preserve their quality over time. In this study, pre-storage seed coating with Biogenic silica effectively prevented seed degradation, thereby maintaining seed quality throughout storage. The results revealed that, after six months of storage period, seed coated with Biogenic silica with carbon $@$ 5 ml kg⁻¹ exhibited the lowest electrolytic leakage compared to the control. The natural antioxidants in silica, which accumulate in the epidermal layers of seed cell walls, serve as a physical and mechanical barrier, effectively safeguarding the seeds from deterioration. Among the storage containers used, seeds packed in super grain bags performed better than those stored in cloth bags. The findings demonstrated that coating seeds with biogenic silica containing carbon at 5 ml $kg⁻¹$ and storing them in super grain bags preserved seed viability, as indicated by decreased electrical conductivity, lower sugar levels, reduced lipid peroxidation and stable biochemical parameters during the storage time frame.

Keywords

biogenic silica; seed coating; cloth bag; super grain bag; seed biochemical parameters

Introduction

Sorghum (*Sorghum bicolor* L. Moench) is an adaptable cereal crop that plays a vital role in global agriculture. It is also considered a camel crop for its drought resistance and ability to thrive in arid, poor soil conditions, making it a vital crop in regions with limited water (1, 2). It ranks as one of the world's most important cereal crops, following wheat, rice and maize in global importance (3). Sorghum is a fundamental food source in Africa, India and China, with India and the United States being its leading producers globally. For millions in semi-arid areas, it is a crucial source of energy, protein, vitamins and essential minerals, making it a vital nutritional staple for some of the world's most impoverished populations (4).

Proper seed storage until the next sowing season is crucial for sorghum seed production. Seeds of every crop degrade in their vigour and viability at a certain rate during storage, either by intrinsic aging or due to the effects of adverse environmental factors. Reducing seed vigour reduces seed quality, germination rates, yields and increases susceptibility to environmental stress (5). The conditions of seed storage and the materials utilized can influence the duration of seed viability. Coating seeds before storing can help preserve them for future use. It protects seeds by managing moisture levels, which helps ensure uniform germination. They also provide a physical barrier against pests, pathogens and environmental stressors. Furthermore, these coatings can supply vital nutrients, growth regulators, or pesticides directly to the seed, aiding in its initial development. Some coatings are designed for the controlled release of these substances, which optimizes germination and growth, ultimately improving seed survival and overall performance (6, 7). Seed coating treatment before storage also removes the risk of seed loss and enhances the quality of seeds during storage (8, 9).

Applications of silica may be found in a variety of fields and technology. It serves as a filter in beverage processing, a filler in paint and coating, and a binding agent in rubber and plastic (10). Silicon has demonstrated a beneficial effect on seeds when applied as a coating. The application of silicon seed coating may promote seed germination and seedling growth by bolstering antioxidant defense and improving iron nutrition (11). Silica, produced from plant origins, has been identified to have better benefits than silica obtained from synthetic or mineral sources. Biogenic silica coatings, with their natural composition and high porosity, provide better protection against stress than synthetic silica. Derived from sources like rice husk ash, they enhance antioxidant activity, neutralize reactive oxygen species (ROS) and help preserve seed quality during storage (12, 13). Bio-silica from rice husk is economical to produce as compared to the manufacturing cost of production from synthetic silica sources, mainly because of the cheap rice husk (14) and the cost of manufacturing could be less expensive if the product was utilized in an established manner (15). It uses environmentfriendly methods to extract silica from rice husks. Rice husk ash (RHA) is often a more efficient and cost-effective feedstock compared to the direct extraction of silica from the husk (16).

Thus, the quality of seeds during storage depends on proper storage containers. Containers regulate temperature, humidity and moisture levels. These eventually ensure that integrity in the seed is secured. Proper storage minimizes biological activity and guards against environmental influences that may further shorten safe storage periods. Optimal conditions and appropriate packaging materials provide effective seed germination rates and viability while minimizing the dangers of insect and disease infestations, hence decelerating the aging process. Given this context, the present study was designed to investigate the effectiveness of Biogenic silica seed coating and storage containers under ambient conditions in maintaining the biochemical parameters of sorghum seeds over a six-month storage period.

Materials and Methods

Various extraction methods are used to produce Biogenic silica from agricultural waste, including Electric/Muffle Furnace (poor yield, low efficiency) (17), Inclined Step-Grate Furnace (high labour requirement) (18), Cyclone Furnace (high investment cost) (19), Rotary Kiln (not yet developed for higher capacity) (20) and Alkaline Extraction (efficient, simple method with good yield quality) (21). Based on comparing these methods, alkaline extraction is the most efficient for silica extraction due to its lower energy consumption, simplicity and environmental friendliness, without the need for sophisticated infrastructure or costly reagents.

Preparation of Biogenic silica from rice husk ash

Biogenic silica is prepared by alkaline extraction followed by the acid neutralization method (21). This is an efficient and simple process for extracting silica from rice husks.

Preparation of Biogenic Silica with Carbon

Rice husk ash (RHA) is added to the 1N NaOH solution in the 250 ml Erlenmeyer flask and boiled for 1h with constant stirring to dissolve silica and form a sodium silicate solution. The formed sodium silicate solution obtained is black due to the presence of carbon. Carbon alters the colour of silica by absorbing light, resulting in deeper colours such as grey or black. Residual carbon in silica affects its natural white colour, with more carbon having a more significant darkening effect (22). From this, the Biogenic silica with carbon was prepared by titrating the sodium silicate solution with 1 N hydrochloric acid (HCl) solution for 18 h at 7.0 pH with constant stirring. Biogenic silica with carbon **(Fig. 1)** in gel form was obtained after the stirring period.

Fig 1. Biogenic silica with carbon

Preparation of Biogenic silica without carbon

Rice husk ash (RHA) is added to the 1N NaOH solution in the 250 ml Erlenmeyer flask and boiled for 1h with constant stirring to dissolve silica and form a sodium silicate solution. To prepare Biogenic silica without carbon, sodium silicate solution was filtered through ashless filter paper and the residue (carbon) was removed. The further solution was titrated with 1N hydrochloric acid (HCl) solution for 18 h at 7.0 pH with constant stirring. Biogenic silica without carbon **(Fig. 2)** in gel form obtained after the stirring period.

Fig 2. Biogenic silica without carbon

Seed Coating

Sorghum seeds were coated with Biogenic silica coating materials and shade-dried for 24 h to retain their original moisture content. To assess the storability of Biogenic silica coated seeds (with and without carbon treatments), the coated seeds were stored in a cloth bag **(Fig. 3)** and super grain bag **(Fig. 4)** under ambient conditions (mean temperature 26 \pm 1°C and RH 70 \pm 2%) for six months in the Department of Seed Science and Technology, Agricultural College and Research Institute, Madurai. The stored seeds were evaluated monthly to assess seed biochemical parameters.

Fig 3. Cloth bag

Fig 4. Supergrain bag

Containers:

 C_1 - Cloth bag

C² - Super grain bag

Seed treatments:

T0- Without seed treatment (Control)

T1- Seeds Coated with 5 ml/kg of Biogenic Silica with Carbon

T2- Seeds Coated with 5 ml/kg of Biogenic Silica without Carbon

Using a lower concentration, such as 5 ml/kg (millilitre per kilogram), for seed coating is preferred for its costeffectiveness. This reduces treatment costs while maintaining protection. It also minimizes environmental impact by lowering leaching into soil and water. Additionally, lower concentrations support seed health, avoiding negative effects on germination and allowing for more uniform application. This approach also helps in meeting regulatory limits on chemical use in seed treatments (23, 24).

The seeds were then used to measure the following biochemical parameters: electrical conductivity (25), dehydrogenase activity (26), lipid peroxidation (27), catalase activity (28), peroxidase activity (29) and leachate sugars (30) were recorded on both controls as well as treated seeds. The output from various experiments was analysed with the help of the software AGRES (31). Then the critical difference was obtained at 5 per cent (%).

Results

Electrical conductivity (dSm-¹)

Electrical conductivity significantly differed among the seed coating, containers and storage periods and also significantly differed in between their interactions of coating treatments, containers and storage periods. Electrical conductivity was expressed in Deci Siemens per metre (dSm-¹). Biogenic silica-coated seeds showed lower electrical conductivity than the control seeds. T_1 (Biogenic silica with carbon) contains carbon, which increases the mechanical strength and endurance of the silica coating, providing superior seed protection. Carbon also increases enzyme activity, essential for preserving seed viability and vigour during storage. Also, biogenic silica coatings restrict electrical conductivity by creating a barrier that prevents ion leaching, maintains seed integrity and slows cellular disintegration. They control moisture to avoid excessive water absorption and leakage and offer antimicrobial protection to reduce tissue deterioration and ion release (32-34). The electrical conductivity was less in the super grain bag (0.38 dSm-¹) compared to the cloth bag recorded (0.41 dSm⁻¹). Among treatments, T_1 (0.26 dSm⁻¹) was the most effective, showing minimal electrical conductivity compared to the control $T_0 (0.59 \text{ dSm}^{-1})$ at the end of the storage period of 6 months, irrespective of containers and treatments **(Table 1).** In the interaction of seed coating and storage period, T_1 seeds stored in super grain bag have minimal EC (0.45 dSm⁻¹) (T_1P_6) after the storage of 6 months while higher EC (0.76 dS m⁻¹) (T₀P₆) was recorded in the control.

Table 1. Influence of seed coating, storage container and storage period on Electrical Conductivity in Sorghum var. K 12

		Containers (C) and storage periods in months (P)															Grand mean
Т	Cloth bag (C_1)								Super grain bag (C_2)								
	P ₀	P ₁	P ₂	P_3	P ₄	P ₅	P_6	Mean	P ₀	P ₁	P ₂	P_3	P ₄	P ₅	P_6	Mean	
T,	0.39	0.46	0.54	0.63	0.68	0.72	0.77	0.60	0.39	0.42	0.51	0.58	0.65	0.72	0.76	0.58	0.59
T_{1}	0.08	0.13	0.17	0.25	0.33	0.41	0.49	0.27	0.08	0.11	0.14	0.21	0.30	0.37	0.45	0.24	0.26
T ₂	0.15	0.21	0.29	0.36	0.44	0.52	0.58	0.36	0.15	0.18	0.25	0.32	0.39	0.47	0.55	0.33	0.35
Mean	0.21	0.27	0.33	0.41	0.48	0.57	0.60	0.41	0.21	0.24	0.30	0.37	0.45	0.52	0.59	0.38	0.40
Grand		P ₀	P ₁		P ₂ 0.32 P		P_3 0.39 т		P ₄ 0.47 $C \times P$		P ₅ 0.55 $C \times T$		P_6 0.60				
Mean		0.21	0.26														
				C									$P \times T$	$C \times P \times T$			
S. Ed			0.0020			0.0038		0.0025		0.0054			0.0035		0.0067		0.0094
$CD (P=0.05)$			$0.0041**$		$0.0077**$		$0.0050**$		$0.0108**$			$0.0071**$		$0.0133**$ $0.01882**$			

**- Significant at 5% level; NS- Non-Significant; C- Containers; P- Storage period in months; T- Treatments

Dehydrogenase activity (Optical Density value) T0 - Control T1 - Biogenic silica with carbon 5 ml/kg T2 - Biogenic silica without carbon 5 ml/kg

Lipid peroxidation (Optical Density value)

Dehydrogenase activity differed significantly among the seed treatments, containers, and storage periods but was non-significant among the interactions. As the storage period increased, the dehydrogenase activity decreased. The rate of decline was less in T_1 -treated seeds (0.418) compared to control (0.220). Among the containers, super grain bags (0.348) registered the highest dehydrogenase activity compared to cloth bags (0.337). At 6 months of storage, the effect was higher in super grain bags (0.274) than in cloth bags (0.257). Initially, dehydrogenase enzyme activity was 0.406, which gradually declined to 0.266 after 6 months of storage, regardless of containers and treatments. In the interaction between treatment and storage period, after 6 months of storage, the highest dehydrogenase activity was observed in $C_2T_1P_6$ (0.354), while the lowest was recorded in the control, $C_2T_0P_6$ (0.148) (Table 2).

Lipid peroxidation significantly varied with the treatments and was directly proportional to the storage period. Under ambient conditions, among the Biogenic silica coating formulation, seeds coated with treatments, T_1 @ 5 ml kg⁻¹ recorded the lowest lipid peroxidation value (0.888) and control seeds recorded the highest lipid peroxidation value (1.314), irrespective of containers and storage periods. Among the containers, seeds stored in super grain bags had lower lipid peroxidation (1.031) than those in cloth bags (1.049) at the end of the storage period **(Table 3).**

Table 2. Influence of seed coating, storage container and storage period on Dehydrogenase in Sorghum var. K 12

**- Significant at 5% level; NS- Non-Significant; C- Containers; P- Storage period in months; T- Treatments

Table 3. Influence of seed coating, storage container and storage period on Lipid Peroxidation in Sorghum var. K 12

Containers (C) and storage periods in months (P)

**- Significant at 5% level; NS- Non-Significant; C- Containers; P- Storage period in months; T- Treatments

Catalase activity (µg g-¹) T0 - Control T1 - Biogenic silica with carbon 5 ml/kg T2 - Biogenic silica without carbon 5 ml/kg

Catalase activity significantly differed among the seed treatments, containers, and storage period, with nonsignificant differences in interactions. Catalase activity was expressed in micrograms per gram (μ g g⁻¹). Catalase enzyme activity was maximum at the initial storage period(P_0) (811), and it slowly declined to (696) at the final storage period(P_6) after 6 months, irrespective of containers and treatments. Among the seed treatments, the control group (T0) exhibited the lowest catalase activity (623), while the treatment group (T1) demonstrated the highest catalase activity (834). Between containers, the super grain bag (C_2) recorded maximum catalase activity (759) and the lowest catalase activity was observed in cloth bag C_1 (750) and showed very minimum variations **(Table 4)**.

Peroxidase (µg g-¹)

A significant difference was observed in peroxidase activity with Biogenic silica seed coating treatments and storage period. Peroxidase activity was expressed in micrograms per gram (μ g g⁻¹). Initially, peroxidase activity was 0.535 μ g g⁻¹, which decreased to 0.426 μ g g⁻¹ after 6 months, regardless of seed coatings **(Table 5).** Among the different treatments, T_1 had the highest activity of peroxidase (0.559 μ g g⁻¹), followed by T₂(0.522 μ g g⁻¹) and control (T₀) $(0.367 \,\mu g \, g^{-1})$ recorded lower activity of peroxidase. A similar trend was observed in dehydrogenase and catalase activity. Seeds in super grain bags recorded the highest peroxidase activity (0.487 μ g g⁻¹) than those in cloth bags (0.478 μ g g⁻¹).

Table 4. Influence of seed coating, storage container and storage period on Catalase in Sorghum var. K 12

**- Significant at 5% level; NS- Non-Significant; C- Containers; P- Storage period in months; T- Treatments

T0 – Control T1 - Biogenic silica with carbon 5 ml/kg T2 - Biogenic silica without carbon 5 ml/kg

Containers (C) and storage periods in months (P)

**- Significant at 5% level; NS- Non-Significant; C- Containers; P- Storage period in months; T- Treatments

T0 - Control T1 - Biogenic silica with carbon 5 ml/kg T2 - Biogenic silica without carbon 5 ml/kg

Leachate sugars (µg g-¹)

The leachate sugars showed a rising trend during all storage periods. It was expressed in micrograms per gram (μ g g⁻¹). Increasing leachate sugars during seed storage is often an indicator of seed deterioration. This event is crucial as it indicates the disintegration of cellular membranes and the release of cellular constituents, thereby jeopardizing seed viability and vigor. High levels of leachate sugars suggest that the seeds undergo stress or damage, leading to reduced germination rates and overall seed quality (35, 36). Among the containers, minimum leachate sugars were observed in the super grain bag (0.41 μ g g⁻¹) compared to the cloth bag (0.45 μ g g⁻¹). T₁ showed the lowest leachate

sugar value (0.36 μ g g⁻¹), while the control had the highest $(0.54 \mu g g^{-1})$ irrespective of containers and treatments. Initially, leachate sugars were below P_0 (0.17 μ g g⁻¹) and progressively grew to P_6 (0.72 μ g g⁻¹) over a storage duration of 6 months. **(Fig. 5).** Among the different treatments, T_1 had minimum leachate free sugars (0.36 μ g g⁻¹) followed by $T_2(0.40 \mu g g^{-1})$ while the control (T₀) recorded the highest leachate free sugars. The free sugars exuded from seeds during storage are mostly free monosaccharides (glucose and fructose) and disaccharides (sucrose). Leachate contains free sugars due to the lysis of the cellular membrane, which results in the escape of these soluble sugars from inside the cells (37).

Fig 5. Influence of seed coating, storage container and storage period on Leachate free sugars (μg g⁻¹) in Sorghum var. K 12

Discussion

Seed storage is a critical component of the seed industry, as seeds are hygroscopic and absorb moisture from their storage environment (38). Seed cells consist of lipoprotein layers that absorb water while storing seeds. This process leads to lipid peroxidation, accelerating cellular decay and, ultimately, seed death (39). Several key physicochemical factors influence seed vigour and viability during storage, including initial seed quality, chemical and physical composition, moisture content, temperature, storage structure, gaseous exchange and type of packaging material.

Biogenic silica derived from rice husk ash is eco-friendly, bio-friendly, less expensive and utilizes agricultural waste. It possesses high porosity and bioavailability to enhance seed germination, nutrient uptake and moisture retention (40, 41). However, its supply is altered by the processing method. On the other hand, Synthetic silica ensures consistent quality but is more expensive and less sustainable than biogenic silica. This gives biogenic silica an advantage in agriculture, as it is more cost-effective and environmentally friendly. Regardless of the type of containers used, the seed biochemical parameters are better in seeds coated with Biogenic silica with carbon (T_1) . Biogenic silica coating ensures the nutrients are not degraded and washed away, ensuring healthy seedling growth post-germination. It prevents erosion by acting as a protective coat against environmental stressors that reinforce the cell walls of seeds to maintain structural integrity and minimize cellular degradation. It also has antioxidant effects, neutralizing oxidative stress while preserving seed quality (42-44).

Seed leachate's electrical conductivity is a good indicator of seed deterioration. The changed permeability of the plasma membrane during storage is the principal indicator of aging, resulting from the degradation of lipoproteins (45). The electrical conductivity of seed leachate of seed increased over the storage period irrespective of containers. The positive effect of Biogenic silica-coated seeds is likely to be attributed to its protective coating, avoiding direct contact of seeds with oxygen and hence, protecting through providing maximum integrity to the cell walls, seed deterioration reduces to the optimum level. In the presence of calcium and pectin ions, a cellulose membrane-Si layer is formed; this layer may potentially support sustainability because silica precipitates on the walls of vascular tissues and the epidermis, where the deposited silica plays an important role as a physicomechanical barrier (46, 47). Since infiltration of moisture and vapor in the container was not allowed, the seed in the super grain bag was decaying at a very slow rate. The electrical conductivity of the seed leachate is directly proportional to membrane integrity (48). Increasing the storage period would cause membrane damage and might be reflected in increasing seed EC; lower EC was observed for seeds packed in super grain bag C_2 than those packed in cloth bag C_1 while those were stored at ambient conditions. Similar results were observed in other studies (49, 50).

Dehydrogenase enzyme activity is a stable metabolic marker for assessing seed vigour (51), and it correlates positively with seed viability and vigour. For both treated seeds, dehydrogenase activity was higher in seeds stored in a super grain bag (C_2) compared to those stored in a cloth bag (C_1) . This is likely because super grain bags limit oxygen, creating an anaerobic environment. Such an environment can influence enzyme activity, with some enzymes becoming more active in anaerobic conditions (52-54).

Regarding storage durations and containers, the T_1 treatment showed higher catalase and peroxidase activity levels than other treatments. One of the most crucial enzymes for determining seed quality is peroxidase, which acts as a shield to prevent peroxide formation and promote the conversion of hydrogen peroxide to oxygen and water (55). The addition of antioxidant-rich silica was able to increase the activity of antioxidant enzymes SOD and CAT, decrease the activities of superoxide anion (O2-) and hydrogen peroxide (H_2O_2) (56), increase the activity of the enzyme's protease and α-amylase (57) and slow down the process of seed deterioration with higher peroxidase enzyme activity.

The seed viability and vigour were impacted by the leaching of sugar and lipid peroxidation (58). Loss and decline of seed vigour were directly correlated with variations in sugar content and lipid peroxidation, which increased with longer storage periods (59, 60). Lipid peroxidation, a measure of the response to free radicals, was reduced in the storage of coated seeds in a super grain bag (C_2) compared to the seeds in a cloth bag (C_1) . These damages can be healed through the application of Biogenic silica treatments. Biogenic silica reduces lipid peroxidation and increases enzyme activity during seed storage. This is accomplished by strengthening plants antioxidant defense systems, which aid in reducing oxidative stress and preserving seed quality. Furthermore, silicon treatment improves metabolism and ion stability, which adds to the seeds resistance and longevity during storage (61-63). Apart from protecting the cell membrane from structural and functional deterioration caused by environmental stressors (64), silicon also contributes to increased lipid stability in cell membranes by reducing the number of organic molecules such as phenols and proteins (65). It can strip off the toxic compounds that result from this storage process and protect through its antioxidant activities that may inhibit the action of free radicals and lipid peroxidation products (66, 67). Natural antioxidants in biogenic silica are assumed to inherently protect this from oxidative stress due to the extreme environmental conditions (56). Silica has also been shown to present multiple radical scavenging activities (68).

Seeds coated with Biogenic silica containing carbon and stored in super grain bags-maintained enzyme activity better than those stored in cloth bags at ambient conditions. This may be because denaturation of protein bands accelerates DNA degradation and antioxidants in silica form a layer over the seed cell wall that is not exposed to direct moisture. This maintains the viability and vitality of the seeds throughout the storage period. (69, 70).

Conclusion

These findings suggest that Biogenic silica coatings can be a viable, eco-friendly solution for improving seed storage longevity and quality. The current study showed that sorghum seeds coated with Biogenic silica with carbon $T_1 @$ 5 ml /kg kept in a super grain bag (C_2) were effective in preserving key seed biochemical properties such as electrical conductivity, dehydrogenase, catalase, peroxidase, leachate sugars and lipid peroxidation throughout the storage period of six months under ambient condition. It is due to the presence of natural antioxidants in silica, which coats the epidermal layers of seed cell walls and serves as a physico-mechanical barrier, effectively safeguarding against seed deterioration. Biogenic silica coatings provide considerable agricultural advantages, including improved seed germination, nutrient retention, and protection against environmental stresses. These coatings keep seeds viable during storage by lowering lipid peroxidation and increasing antioxidant enzyme activity. They also promote sustainable farming by reducing the demand for chemical inputs and increasing drought resilience. Future studies might look into integrating biogenic silica with other bio stimulants and applying nanotechnology to increase its potency.

Acknowledgements

The authors gratefully acknowledge Tamil Nadu Agricultural University, Coimbatore, for providing the platform to publish this article. Our heartful thanks to the Department of Seed Science and Technology, Agricultural College and Research Institute, Madurai, for facilitating the work.

Authors' contributions

KA and AAV conceived the idea and wrote the manuscript. AAV gave the idea, and KA designed the diagrams and tables. SK revised the manuscript. AAV, SK, KP and AP finalized the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used Grammarly to improve the language and readability. After using this tool/service, the authors reviewed and edited the content as needed and took full responsibility for the publication's content.

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