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Enhancing Vigor and Viability of Deteriorated True Shallot Seed by Matriconditioning Using Biofertilizer and Washed Rice Water

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Abstract. *True Shallot Seed (TSS) is a healthy, cost-effective, and high-yielding alternative planting material instead of shallot bulbs. However, TSS has a short shelf life due to deterioration during storage. The viability and vigor of deteriorated seeds can be enhanced by Plant Growth Promoting Rhizobacteria (PGPR). Washed rice water containing macro- and micronutrients also supports metabolic processes and improves the accumulation of seed nutrients during germination. Therefore, this study aimed to investigate the role of matriconditioning with a mixture of PGPR and washed rice water for enhancing shallot seed vigor and viability. In addition, the physiological quality of shallot seeds in the study had decreased their moisture and germination by 9.09% and 52.25%, respectively. The treatment of Matriconditioning consisted of five levels: Control (M0), Matriconditioning without PGPR (M1), Matriconditioning plus PGPR RhizomaX (M2), Matriconditioning plus PGPR BenprimA (M3), and Matriconditioning plus PGPR FloraOne (M4). Washed rice water consisted of three levels: control (A0), 50% concentration (A1), and 100% concentration (A2). Observed variables were germination rate, maximum growth potential, relative growth rate, uniformity of growth, and vigor index. Data were analyzed by ANOVA, and the Duncan Multiple Range Test was applied for significant results at the 5% level. The results showed that matriconditioning using charcoal and PGPR RhizomaX (M2) could enhance the vigor and viability of deteriorated shallot seeds. The application of washed rice water for five days during germination, especially at a concentration of 100% (A2), also significantly improved the vigor and viability of deteriorated shallot seeds.*
Keywords: *Allium cepa L., germination, seed degeneration, seed priming.*

Type of the Paper: Regular Article.

1. Introduction

Shallot is widely used in daily Indonesian consumption, both for health and culinary purposes. According to Shahrajabian et al. [1], shallot is rich in bioactive compounds, such as flavonoids, organosulfur, and phenolic acids. The compounds contained in shallot have antioxidant and anti-inflammatory properties [2]. These compounds also have the potential to inhibit the proliferation of tumor cells, induce apoptosis, and suppress angiogenesis [3]. Furthermore, shallot can reduce the risk of chronic diseases, such as cardiovascular, diabetes, and specific types of cancer [4]. A previous study showed that shallot had the potential as an antimicrobial agent and immunomodulator [5].

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The selection of planting material is an important stage for successful shallot cultivation. Shallot bulbs are the most widely used source of cultivation seed among farmers in Indonesia but are prone to seed-borne diseases [6]. A previous study attributed high seed pathogenicity to the unregulated sale of shallot bulbs by farmers and growers, leading to high mutational variations [7]. Therefore, ensuring high-quality seed as planting material is important to prevent additional challenges in shallot cultivation [8].

True Shallot Seed (TSS) has the potential to replace shallot bulbs as a high-quality seed source [9]. Saputri et al. [6] reported significantly lower disease incidence in TSS compared to shallot bulbs. TSS also has lower mutational variations, causing more uniform growth and yield of shallot plants [7]. Atman [10] also reported that the use of TSS reduced seed procurement costs by up to 66.7% and increased harvest yields to 30-34 tons/ha. Therefore, TSS provides an alternative for obtaining quality planting materials in shallot cultivation.

The use of TSS is still uncommon among farmers due to the need for seedling establishment through sowing. A previous study reported that seedling establishment in TSS fails often with low germination rates [7] caused by deterioration during storage. TSS has a relatively short shelf life in tropical regions, less than a year [11]. Rahayu et al. [12] reported the Pancasona variety can be stored for up to 6 months at room temperature. Meanwhile, Yulyatin and Haryati [11] reported that TSS of the Bima variety can only be stored at room temperature for a maximum of 3 months, and an extension beyond this duration will lead to decreasing quality.

Advancing technology is important to provide TSS that maintains high-quality standards after storage. Several strategies can be implemented to achieve high-quality TSS seed, including optimizing production processes [13,14], storage under optimal conditions [12], enhancing quality through pre-planting treatments [15], and adjusting nursery environments [16]. Invigoration is a pre-planting treatment which is a particularly accessible method for farmers to improve seed quality after storage [17].

Matriconditioning (seed priming) is an effective invigoration method for various seed types to improve deterioration [18]. This method can be combined with bioagents, growth regulators, fertilizers, and pesticides [19]. Plant Growth Promoting Rhizobacteria (PGPR) is a commonly used bioagent in seed priming [20]. The use of PGPR in seed priming is reported to enhance viability and vigor of deteriorated seed [20–22]. Additionally, nutrient supplementation can further improve the quality of deteriorated seed by supporting metabolic processes and enhancing nutrient accumulation during germination [23].

Washed rice water is an organic material rich in starch, protein, and various macronutrients and micronutrients [24]. Utami and Hariyanto [25] reported that organic content, such as carbohydrates, minerals, vitamins, and amino acids enhanced seed germination and stimulated development. Abdelkader et al. [26] also reported that the exogenous addition of amino acids (glutamine, proline, and tryptophan) improved the germination ability of shallot seed. The use of washed rice water as a nutritional source to enhance seed vigor and viability remains uncommon. Therefore, this study aimed to investigate the role of matriconditioning with a mixture of PGPR and rice washing water in enhancing shallot seed vigor and viability.

2. Materials and methods

2.1. Study Site and Materials

This study had been conducted from January 16 to April 8, 2023, in a greenhouse and Seed Technology Laboratory, Faculty of Agriculture, University of Jember. The material used was shallot seed of Lokananta variety, which had substandard quality according to the Minister of Agriculture standards for sowing seed class [27]. Shallot seed has a moisture content of 9.09% and a germination rate of 52.25%. Other materials used include PGPR-biofertilizer RhizomaX (contains PGPR *Rhizobium* sp. 10^{10} cfu/ml, *Bacillus polymixa* 10^{10} cfu/ml, and *Pseudomonas flourescens* 10^{10} cfu/ml), PGPR-biofertilizer BenprimA (contains PGPR *Bacillus polymixa* 10^{7} cfu/ml, dan *Pseudomonas flourescens* 10^{7} cfu/ml), and PGPR-biofertilizer FloraOne (contains PGPR *Rhizobium* sp. $3{,}4\text{x}10^{10}$ cfu/ml, *Pseudomonas flourescens* $9{,}3\text{x}10^{10}$ cfu/ml, *Azospirilum* sp. $7{,}3\text{x}10^{8}$, *Aspergillus niger* $3{,}4\text{x}10^{7}$, and *Tricoderma harzianum* $1{,}3\text{x}10^{7}$).

2.2. Experimental Design

A split-plot design was adopted with a factorial complete randomized design (CRD) with two factors. The first was matriconditioning treatment with a mixture of PGPR biofertilizer, consisting of 5 levels, namely Control (M0), Matriconditioning using rice husk charcoal (M1), Matriconditioning using rice husk charcoal plus PGPR biofertilizer RhizomaX (M2), Matriconditioning using rice husk charcoal plus PGPR biofertilizer BenprimA (M3), and Matriconditioning using rice husk charcoal plus PGPR biofertilizer FloraOne (M4). The second factor was the application of washed rice water, with 3 levels, namely Control (0% concentration) (A0), 50% concentration (A1), and 100% concentration (A2). Each experiment was repeated 4 times, with each replication containing 100 shallot seeds.

2.3. Seed Treatment

The concentrations of PGPR biofertilizer solutions were 10 g/liter (BenprimA), 10 g/liter (RhizomaX), and 15 ml/liter (FloraOne). A total of 500 g rice was washed with 500 ml well water 4 times, resulting in a stock solution with a concentration of 100%. The stock solution was diluted with well water to make a 50% concentration of washed rice water.

Matriconditioning media were prepared with a mixture of shallot seed, rice husk charcoal, distilled water (control), or PGPR biofertilizer (according to the study treatments). This procedure also included 3 g, 50 g, 20 ml, and 20 ml of washed rice water, which were applied during germination from day 1 - 5, after planting, with a total of 40 ml used.

2.4. Seed Testing and Observational Variables

The evaluation of vigor and viability of shallot seed had been conducted using the sand test method and incubated in a greenhouse with a daily temperature range of 32-40°C. The testing was carried out on 400 seed samples with 4 replications [28]. The observed variables for vigor and viability were based on previous studies [27,29]. These variables included a) germination rate (%), representing the percentage of normal seedlings after 12 days of planting, b) maximum growth potential (%), including both normal and abnormal seedlings after 12 days of planting, c) relative growth rate (%), showing the percentage of normal seedlings in 24 hours during the 12-day period, d) uniformity of growth (%), representing the percentage of normal seedlings after 7 days of planting, and e) vigor index (%), showing the percentage of normal seedlings at the first count, 6 days after planting.

2.5. Data Analysis

Data analysis was carried out using Analysis of Variance (ANOVA), followed by Duncan's Multiple Range Test (Duncan's test) for significant results at the 5% level. Data analysis was conducted using IBM SPSS Statistics 25.0 software.

3. Results and Discussion

3.1. ANOVA Result

The interaction between matriconditioning and washed rice water application significantly affected the relative growth rate. Matriconditioning significantly affected germination, growth rate, vigor index, uniformity, and maximum growth potential. The application of washed rice water significantly affected germination rate, relative growth rate, vigor index, and uniformity of growth, as shown in Table 1.

Table 1. Results of ANOVA on the effects of matriconditioning and washed rice water application on vigor and viability of TSS seed.

Observational Variables	F-Value			CV
	M	A	M*A	
Germination rate	5.0449**	3.9677*	2.1165ns	11%
Maximum growth potential	8.8836**	6.7960**	2.4535*	14%
Relative growth rate	6.2932**	4.5280*	2.1241ns	16%
Uniformity of growth	6.0337**	3.4084*	2.0823ns	12%
Vigor index	4.8535**	1.5727ns	1.1873ns	11%
F 5%	2.895	3.316	2.266	
F 1%	4.500	5.390	3.173	

Notes: * = significant at the 5% level, ** = significant at the 1% level, ns = not significant, M = Matriconditioning, A = Washed rice water; M*A = Interaction of matriconditioning and washed rice water application; CV = Coefficient of variation

3.2. The Effect of Matriconditioning with PGPR on Vigor and Viability of Shallot Seed

The treatment of control (M0) showed the lowest vigor and viability compared to other matriconditioning treatments (Table 2). Seed deterioration causes oxidative stress, leading to damage in proteins, lipids, and deoxyribonucleic acid [30]. This results disrupt hormone activity and seed nutrient mobilization, as reported by a previous study [31]. Seed deterioration was further increased when germination occurred under stressed conditions [32]. Matriconditioning is a priming method that uses a substrate medium to stimulate metabolism and regulate oxygen circulation, optimizing seed germination [19]. Chomontowski et al. [33] reported that solid-matrix priming (matriconditioning) enhanced seed strength and growth rate of *Beta vulgaris* L. Fig. 1 shows the impact of matriconditioning with PGPR on vigor and viability of shallot seed.

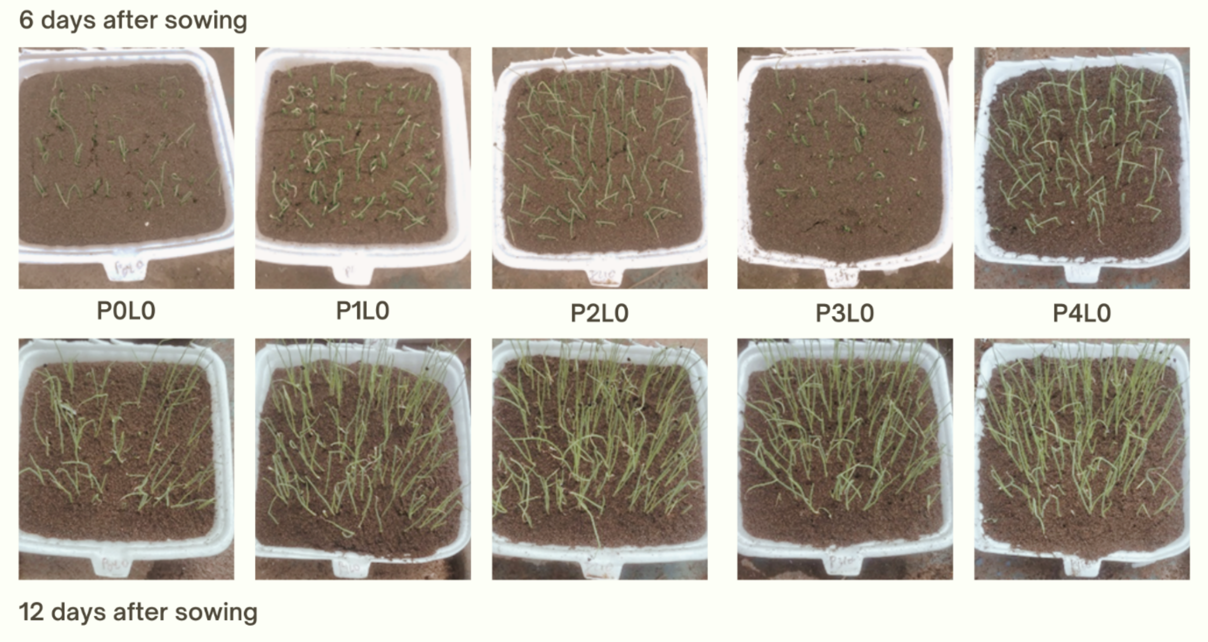

Fig. 1. Shallot seedlings treated with matriconditioning were observed on the 6th and 12th days after sowing.

Table 2. Effect of matriconditioning treatment on vigor and viability of shallot seed

Matriconditioning	Germination Rate (%)	Relative Growth Rate (%.etmal^{-1})	Vigor Index (%)	Uniformity Of Growth (%)	Maximum Growth Potential (%)
M0	56.75±10.89 d	9.61±2.26 c	38.33±3.19 c	54.00±3.48 c	68.58±2.06 c
M1	67.92±6.90 bc	12.40±2.00 b	46.42±3.72 b	65.33±1.96 bc	75.42±2.76 bc
M2	78.17±8.70 a	15.66±2.31 a	61.33±5.21 a	76.08±3.83 a	88.42±4.09 a
M3	64.58±11.62 c	12.12±2.89 b	45.42±8.89 b	63.67±8.83 b	73.33±6.67 bc
M4	72.25±9.09 ab	15.26±2.37 a	57.42±1.65 a	72.00±4.25 ab	80.67±2.24 b

Notes: Data is presented as mean ± standard deviation (n = 12). Values followed by the same letter show no significant difference based on Duncan's analysis at a significance level of 5%

Matriconditioning treatment with a mixture of PGPR biofertilizer Rhizomax (M2) showed the best vigor and viability results compared to other matriconditioning treatments. Hydro-priming can enhance seed respiration, but the effect is relatively low [34]. Seed priming with exogenous phytohormones can further improve seed vigor and viability [35]. Furthermore, PGPR activity includes the production of phytohormones, such as auxins, gibberellins, cytokinins, and abscisic acid, which enhance seed vigor and viability [20]. PGPR biofertilizer of Rhizomax contains a more complex PGPR content compared to FloraOne. According to a previous study, PGPR bacteria function optimally when operating as a consortium for phytohormone production [36]. However, FloraOne biofertilizer has a more comprehensive PGPR content compared to Rhizomax biofertilizer, resulting in lower vigor and viability of shallot seed. This result was attributed to the presence of *Trichoderma hazarnum* fungi in FloraOne biofertilizer. The use of *Trichoderma* as a seed priming material has been reported to decrease seed germination ability in several horticultural commodities [37].

3.3. The effect of washed rice water treatment on vigor and viability of shallot seed

Treatment A2 resulted in better vigor and viability compared to other washed rice water treatments. The lowest vigor and viability of shallot seed were shown by treatment A1 (50% washed rice water) but were not significantly different from the control (Table 3). Furthermore, the impact of washed rice water on vigor and viability of shallot seed was visualization in Fig. 2. A previous study reported that deteriorated seed often experience a loss of stored nutrients, such as starch, protein, and lipids [38]. Washed rice water contains various organic substances, such as starch, protein, lipids, amylose, and vitamin E [39], as well as various macro and micronutrients [24]. The presence of macro and micronutrients in washed rice water has the potential to enhance the germination ability of deteriorated seed. The priming of seed in inorganic salt solutions, such as NaCl, KCl, KNO_3, K_3PO_4, KH_2PO_4, $MgSO_4$, and $CaCl_2$ has been reported to induce seed metabolism during germination. Similarly, the priming of seed with macro and micronutrient ion solutions allows for the adjustment to osmotic pressure, enhances enzyme activity, promotes embryo enlargement, and increases metabolism [23].

Table 3. Effect of washed rice water treatment on vigor and viability of shallot seed

Washed Rice Water	Germination Rate (%)	Relative Growth Rate (%.etmal^{-1})	Vigor Index (%)	Uniformity Of Growth (%)	Maximum Growth Potential (%)
A0	66.2±14.08 b	12.9±3.64 b	48.8±11.17 b	64.4±14.02 b	76.7±9.29 a
A1	65.75±9.71 b	12.0±2.73 b	46.7±8.08 b	64.2±10.24 b	75.3±5.72 a
A2	71.85±10.75 a	14.1±2.98 a	53.9±8.59 a	70.1±11.37 a	80.0±7.37 a

Notes: Data is presented as mean ± standard deviation (n = 10). Values followed by the same letter show no significant difference based on Duncan's analysis at a significance level of 5%

The application of washed rice water at a concentration of 100% could significantly improve vigor and viability of shallot seed. However, a concentration of 50% was less effective in enhancing vigor and viability of shallot seed. The mechanism behind the improvement was more clearly influenced by the presence of macro and micronutrients. Although washed rice water was reported to contain a lot of starch [39], no study has reported that adding exogenous starch can enhance seed germination ability. Furthermore, exogenous glucose in seed, when present in sufficiently high concentrations, can induce abscisic acid hormone activity, leading to dormancy

[40,41]. The addition of exogenous glucose at low concentrations (0.1 – 0.5 mM) can increase seed germination ability under saline stress conditions [42].

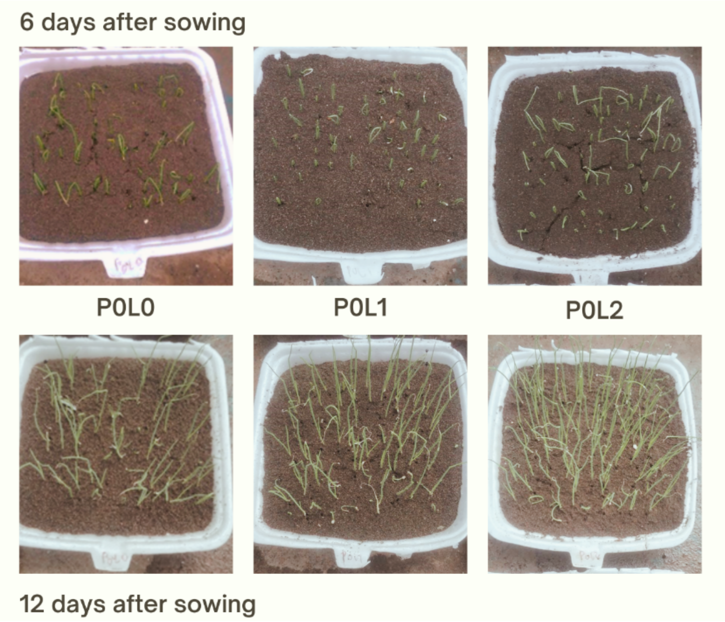

Fig. 2. Shallot seedlings treated with washed rice water were observed on the 6th and 12th days after sowing.

Table 4. Effect of the interaction between matriconditioning with PGPR and washed rice water treatment on vigor and viability of shallot seed

Matriconditioning	Washed Rice Water	Germination Rate (%)	Relative Growth Rate (%.etmal^{-1})	Vigor Index (%)	Uniformity Of Growth (%)	Maximum Growth Potential (%)
	A0	55.25±12.79a	9.52±1.94gh	37.75±12.97a	52.75±13.16a	67.25±12.17a
M0	A1	53.25±6.180a	8.46±0.95h	34.75±7.41a	50.50±7.23a	67.00±6.75a
	A2	61.75±10.69a	10.85±2.78fg	42.5±13.96a	58.75±13.05a	71.5±10.36a
	A0	68.25±7.50a	12.77±1.78cdef	50.50±9.40a	65.75±7.08a	78.75±10.85a
M1	A1	65.00±4.53a	11.48±1.67efg	41.50±9.75a	62.75±3.34a	72.00±5.79a
	A2	70.50±7.12a	12.97±2.17cdef	47.25±9.22a	67.5±6.34a	75.50±8.41a
	A0	76.00±6.75a	15.66±1.77ab	61.75±4.43a	73.25±5.63a	88.75±4.97a
M2	A1	75.50±10.23a	14.06±2.25bcd	54.75±9.64a	73.50±10.62a	83.25±10.23a
	A2	83.00±6.44a	17.25±1.66a	67.50±7.33a	81.50±5.02a	93.25±3.77a
	A0	53.50±9.63a	9.53±2.070gh	34.25±10.72a	52.25±8.14a	64.75±8.64a
M3	A1	65.75±3.63a	12.02±2.11def	46.00±12.94a	65±3.81.00a	74.25±6.26a
	A2	74.50±8.73a	14.81±1.55abc	56.00±8.98a	73.75±8.07a	81.00±10.98a
	A0	78.00±10.63a	17.03±2.06a	59.75±8.73a	78.00±10.63a	83.75±9.55a
M4	A1	69.25±5.54a	13.93±1.84bcde	56.25±8.02a	69.25±5.54a	79.75±6.65a
	A2	69.50±7.40a	14.8±2.030abc	56.25±9.91a	68.75±8.04a	78.50±7.26a

Notes: Data is presented as mean ± standard deviation (n = 4). Values followed by the same letter show no significant difference based on Duncan's analysis at a significance level of 5%

3.4. Effect of the interaction between matriconditioning with PGPR and washed rice water treatment on vigor and viability of shallot seed

The control treatment M0A0 showed the lowest relative growth rate compared to others (Table 4). The interaction between matriconditioning and washed rice water treatment caused varied vigor and viability of shallot seed. Treatment M3A0 showed the lowest results in germination rate, vigor index, uniformity, and maximum growth potential, but was not significantly different from the control treatment (M0A0). However, interactions between M3A1 and M3A2 produced better vigor and viability of shallot seed compared to M0A1 and M0A2.

The interaction between matriconditioning with a mixture of PGPR biofertilizer and washed rice water showed different responses. M3A2 exhibited the best vigor and viability compared to M3A0 and M3A1. However, M4A0 showed the best results in vigor and viability of shallot seed compared to M4A1 and M4A2. The combination of washed rice water and PGPR biofertilizer enables the formation of a complex bacterial consortium that could influence the activity of rhizobacteria as plant growth-promoting agents through mechanisms, such as nitrogen fixation, phytohormone production, phosphorus, and potassium solubilization [43]. Non-fermented washed rice water contains several strains of rhizobacteria from the groups *Pantoea*, *Klebsiella*, *Bacillus*, *Enterobacter*, and *Stenotrophomonas* [24]. Furthermore, the complexity of PGPR bacterial population in matriconditioning treatment with a mixture of PGPR biofertilizer and washed rice water may lead to competition among rhizobacteria for nutrients, space, and environmental factors. According to Dhungana and Itoh [44], the majority of rhizobacteria have the ability to produce IAA (indole-3-acetic acid), while others play a role in degrading IAA in plants.

4. Conclusions

In conclusion, both matriconditioning and washed rice water treatments, particularly when applied individually, improved the vigor and viability of deteriorated shallot seeds. The best outcome was observed with matriconditioning using rice husk media combined with the PGPR biofertilizer Rhizomax. Additionally, treating the seeds with 100% concentration of washed rice water for 5 days also significantly enhanced shallot seed vigor and viability.

Data Availability Statement

Data will be shared upon request by the readers.

CRediT Authorship Contribution Statement

M.W.S: Conceptualization, Data curation, Investigation, Methodology, Project administration, Writing – original draft. V.K.S.: Validation, Writing – review & editing, Project administration. I.F.: Methodology, Validation, Writing – review & editing, administration. SL: Methodology, Projectadministration, Resources, Validation, Visualization, Writing – review & editing. T.C.C: Validation, Writing – review & editing, Validation. A.W.: Validation, Writing – review & editing, Visualization. S.H.: Supervision, Funding acquisition, Validation, review & editing, Project administration, Software, Validation, Visualization, Writing original draft.

Declaration of Competing Interest

The authors declare no known competing financial interests or personal relationships that could have influenced this study.

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