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Original Research Article

Acquisition of Vermiwash from *Eudrilus eugeniae* and Its Influence on the Post-Germination Growth of Mustard Greens

Oanh N Huynh[1,2]*, Tien T T Le[1,2], Thu TA Dao[1,2], Phuc T Vo[1,2]

[1]*Department of Biotechnology, Faculty of Chemical Engineering, Ho Chi Minh City University of Technology (HCMUT), 268 Ly Thuong Kiet Street, Ho Chi Minh City, Vietnam*
[2]*Vietnam National University Ho Chi Minh City, Linh Trung Ward, Thu Duc City, Ho Chi Minh City, Vietnam*

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ABSTRACT

Vermiwash is a nutrient-rich liquid extract derived from vermicompost, containing essential minerals, enzymes, and plant growth regulators that support plant development. This study aimed to extract vermiwash from *Eudrilus eugeniae* reared in a substrate enriched with cow manure. The extraction process involved passing water at 15°C (Vermiwash A) and 30°C (Vermiwash B) through layers of sand and gravel to obtain the final vermiwash product. The study further evaluated the effects of these vermiwash on the post-germination growth of mustard green seedlings. On day 10, the mass of Vermiwash A obtained was 318.33 ± 7.64 g, and for Vermiwash B, it was 316.67 ± 11.55 g. By day 25, Vermiwash A exhibited a higher increase in protein content (0.61±0.02 mg/mL) compared to Vermiwash B (0.54±0.02 mg/mL). Vermiwash A also had a higher amino acid content (5.82±0.45 µg/mL) and reducing sugar content (0.58±0.02 mg/mL) than Vermiwash B (4.77±0.86 µg/mL and 0.40±0.03 mg/mL, respectively). Foliar application of vermiwash improved seedling growth compared to both auxin (a plant growth hormone) and the control group. Vermiwash A at a 50% concentration was more effective than Vermiwash B at the same concentration. Seedlings treated with Vermiwash A and NPK fertilizer (on days 7 and 15) showed the best growth, with 7 leaves and a length of 3.18 cm after 21 days. In conclusion, Vermiwash A is more effective than Vermiwash B in enhancing plant growth. These findings suggest that Vermiwash serves as a highly efficient liquid fertilizer, offering a sustainable and eco-friendly alternative for improving crop productivity.

Keywords: Vermiwash, Earthworms, Coconut coir, Mustard greens.

Introduction

The excessive use of synthetic fertilizers and agricultural chemicals has negatively impacted soil health and crop productivity. Therefore, natural fertilizers offer a promising solution to this issue. *Eudrilus eugeniae*, commonly known as the African nightcrawler, is highly valued for its rich nutritional content. Its digestive system processes organic waste, producing nitrogen-rich vermicompost fertilizer that enhances soil quality and crop performance.[1] Vermiwash, a nutrient-rich liquid fertilizer, is derived from the coelomic fluid released during vermicomposting. It is collected as leachate during the decomposition of organic matter decomposes. The nutrient content of vermiwash is highly impressive. It contains a wide range of essential compounds, including amino acids, proteins, vitamins, enzymes, and key nutrients such as nitrogen (N), potassium (K), magnesium (Mg), calcium (Ca), iron (Fe), along with plant growth hormones that support overall plant development. According to Tiwari and Singh, a combination of foliar-applied vermiwash and vermicompost significantly enhanced tomato plant growth.[2]

*Corresponding author. Email: ngocoanh_cnsh@hcmut.edu.vn;
Tel: (084) 904421602

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Similarly, Gajjela and Chatterjee reported that vermiwash application in bitter melon (*Momordica charantia* L.) resulted in increased fruit length (10.64 cm), number of fruits per plant (1.45%), fruit weight (5.36%), total soluble solids (10.45%), ascorbic acid content (8.71%), and protein levels (3.57%). These findings highlight the potential of vermiwash as a sustainable, eco-friendly biofertilizer that enhances both plant health and agricultural productivity.[3]
Mustard greens (*Brassica juncea L.*) are widely consumed in Vietnam and are valued for their high content of essential minerals and vitamins, making them a nutritious addition to the diet and beneficial for overall health. In recent years, organic farming has gained significant popularity as it produces clean and safe agricultural products with minimal heavy metal residues. Phu Lam Organic Fertilizer increased the yield of mustard greens to 31.43 tons/ha.[4] In the aeroponic system, the growth of mustard greens was significantly improved by the organic solution derived from fish by-products, resulting in a yield of 3.55 kg/m².[5]
However, research on the effects of liquid organic fertilizers on mustard greens remains limited. Therefore, this study aimed to evaluate the acquisition of vermiwash from *Eudrilus eugeniae* and assess its influence on the post-germination growth stage of mustard greens.

Materials and Methods

Materials and chemicals
African earthworms were supplied by Phu Thanh Organic Agriculture Company Limited. RADO 57 mustard green seeds were provided by Rang Dong Co., Ltd. Lavamix cow dung and NPK fertilizer were provided by Duc Thuan Trading and Service Company Limited and Binh Dien Fertilizer Joint Stock Company, Vietnam.
Chemicals used in this study included Sodium Potassium Tartrate ($C_4H_4O_6KNa \cdot 4H_2O$, Xilong Scientific, China), Citric Acid (99.5%, Xilong Scientific, China), Methanol (99.8%, Xilong Scientific, China),

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Ascorbic Acid (99.7%, Xilong Scientific, China), Ninhydrin (JHD Chemical, China), Sodium Phosphate ($NaH_2PO_4 \cdot 2H_2O$, Xilong Scientific, China), Glucose ($C_6H_{12}O_6 \cdot H_2O$, JHD Chemical, China), Egg Albumin Powder (protein ≥ 78%, HiMedia, India), L-Leucine (HiMedia, India), and DPPH (2,2-Diphenyl-1-picrylhydrazyl, 95%, Alfa Aesar, USA).

Designing a vermiwash collection model at home

The vermiwash collection model was adapted from an industrial design utilizing manure and organic waste.[6] The filtration system begins with an initial 3 cm layer of gravel, followed by a 2 cm layer of sand, ensuring efficient filtration. Water flows continuously through these layers until the filtrate becomes clear and free of sand-colored impurities, stabilizing the filtration process. Before proceeding to the next layer, water is continuously allowed to flow through to stabilize the filtration process until the filtrate becomes clear and free of sand-colored impurities. Next, a 5 cm layer of compost, mixed with 30 grams of earthworms, is evenly distributed to allow the earthworms to acclimate and burrow into the compost. A top layer of coconut coir is then added to act as a protective cover, minimizing water evaporation within the system. The model maintains a stable substrate moisture level of 70% and is placed in an area shielded from direct sunlight and unintended animal interference (Figure 1).

The substrate humidity was maintained at 70%, and the model was placed in an area not exposed to direct light. To maintain the substrate's moisture, 100 g of water was added to the tank daily, and the temperature was measured before and after watering. Vermiwash solution was collected periodically on days 5, 10, 15, 20, and 25, and stored at 4°C after collection.

Vermiwash solution was collected and stored in bottles every five days. Vermiwash A was obtained at a water temperature of 15°C, while Vermiwash B was collected at a water temperature of 30°C. In addition, a control tank was maintained under similar conditions to the experimental tanks, but without the presence of any worms, serving as the baseline for comparison.

Determination of some properties of vermiwash solution

Vermiwash was centrifuged (Hettich EBA 21, Merk) at 4000 rpm for 10 minutes to remove residue, and then evaluated according to the following criteria: pH value (Hi 8424, Hanna, USA), protein content, amino acid content, reducing sugar content, the total number of immobilized microorganisms nitrogen and phosphorus-degrading microorganisms. Particularly, protein content was determined using the Biuret method, amino acid content using the Ninhydrin method,[6] and reducing sugar content using the DNS method.[7] These methods were performed using a CT-2200 spectrophotometer (Taiwan).

The optimal vermiwash sample was selected and subsequently analyzed for total nitrogen-fixing and insoluble phosphorus-metabolizing microorganisms at QUATEST 3 (Quality Assurance and Testing Center 3, 49 Pasteur, Nguyen Thai Binh Ward, District 1, Ho Chi Minh City, Vietnam).

Influence of vermiwash on the post-germination growth stage of Mustard Greens

The seeds were soaked in warm water for 4 hours and then incubated in a damp towel for 24 hours to stimulate germination. Afterwards, the seeds were sown in a mixture of coco peat and soil. One week post-germination, the seedlings were fertilized with NPK fertilizer at a rate of 1 g per pot. Ten days later, the survey solutions (Table 1) were sprayed onto the leaves of each plant every three days for the following nine days, using 100 mL of solution per pot. The survival rate, number of leaves, leaf length, plant height, root length, leaf color, and stem characteristics were recorded after 21 days.

Statistical analysis

The results of the experiments were analyzed using one-way ANOVA in Statgraphics 10. Probability values of $p < 0.05$ were considered statistically significant.

Results and Discussion

Vermiwash Solution Collection Process

The survey was conducted using the model depicted in Figure 1, and the vermiwash collection process was investigated and analyzed.

Sensory characteristics of vermiwash

On day 5, the vermiwash was obtained from all three models: Vermiwash A (water at 15°C), Vermiwash B (water at 30°C) and the control without earthworms, resulting in a cloudy, cockroach-colored solution. However, from day 10 onwards, the filtration system stabilized, and the vermiwash solution in all three models became clear and cockroach-colored.

Table 1: Survey conduct arrangements

Treatments	NPK fertilizer (Day 7 and 15)	Type of solution (Sprayed on Day 10, 13, 16, 19)
Water – NPK	+	-
Cow manure solution 50%* –NPK	+	Cow manure solution 50%
NAA 10 µg/mL – NPK	+	NAA 10 µg/mL
Vermiwash A 50% **- NPK	+	Vermiwash A 50%
Vermiwash B 50% **- NPK	+	Vermiwash B 50%
Vermiwash A 50%**	-	Vermiwash A 50%
Vermiwash B 50%**	-	Vermiwash B 50%

**: Cow manure solution 50%*: 50 mL of cow manure solution diluted with 50 mL of tap water*
***: 50 mL of vermiwash diluted with 50 mL of tap water*

Notably, the fluid obtained from the control model began to develop a foul odor starting on day 10, which was not present in Vermiwash A and Vermiwash B. These findings demonstrate that Vermiwash A and Vermiwash B are superior to the control model in both odor control and solution clarity.

Volume evaluation

According to the survey conducted (Figure 2), a statistically significant difference (**P** < 0.05) in the volume of vermiwash was observed between Models A and B. While the mass changes over time were relatively similar for both models, the highest mass recorded on day 10 was 318.33 ± 7.64 g for Vermiwash A and 316.67 ± 11.55 g for Vermiwash B. The earthworms in the substrate were able to dig and move freely, which enhanced the water-holding capacity of the substrate. As a result, the volume of vermiwash gradually decreased on days 15, 20, and 25.

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Compositional analysis of vermiwash solution

Protein Content

During the production of Vermiwash A and Vermiwash B, protein content increased from day 5 to day 15 (Figure 3), a trend attributed to the growth in both the size and number of earthworms, which led to higher protein levels in the mucus. However, by day 20, the protein content decreased due to the mineralization process of microorganisms in vermicompost. On day 25, there was a subsequent increase in protein content, which reached its highest concentration with Vermiwash A and Vermiwash B at 0.61±0.02 mg/mL and 0.54±0.02 mg/mL, respectively.

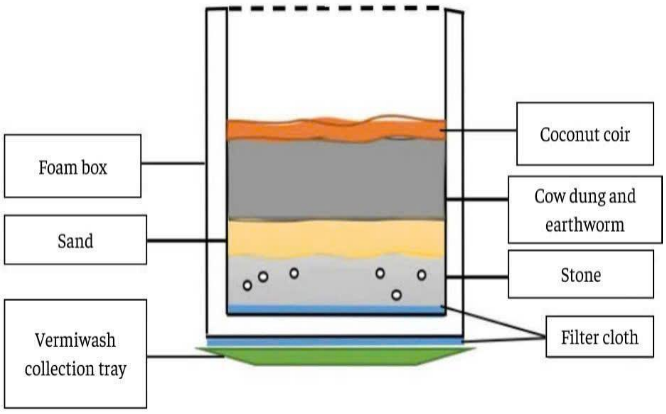

Figure 1: Vermiwash collection design model

Enzymes, which are proteins acting as biological catalysts, play a crucial role in accelerating chemical reactions and facilitating essential biological processes. According to Sahoo, the presence of enzymes in vermiwash fluid was investigated.[8] The hydrolytic enzymes break down complex compounds into simpler units, such as amino acids and monomeric sugars, making it easier for plants to absorb vital nutrients.

Amino acid content

The study revealed that Vermiwash A and B remained stable over time and outperformed the control (Figure 4). The amino acid content changed slightly on days 5, 10, 15, and 20; however, there was no significant difference (P>0.05). On day 25, both Vermiwash A and Vermiwash B reached their highest amino acid levels, at 5.82 ± 0.45 µg/mL and 4.77 ± 0.86 µg/mL, respectively. Furthermore, a study by Sundaravadivelan (2024) found that the nutritional composition of vermiwash can be significantly influenced by the type of composting materials used.[9] Additionally, the earthworms burrow and move, they secrete substantial amounts of mucus, which plays a crucial role in increasing the amino acid content of vermiwash.

Reducing sugar content

The reducing sugar content in both Vermiwash A and B exhibited an increasing trend, peaking on day 25 (Figure 5). Vermiwash A recorded a reducing sugar concentration of 0.58 ± 0.02 mg/mL, while Vermiwash B had a slightly lower concentration of 0.40±0.03 mg/mL. The substrate was a mixture of soil, coconut fiber, and cow dung, which had fully decomposed into humus. Earthworms played a crucial role in this process by digesting organic matter, particularly cellulose-rich materials, and excreting nutrient-rich waste. Their intestinal tract contains cellulase enzymes, which actively hydrolyze cellulose present in the organic mixture. This enzymatic breakdown releases monomeric sugars, enriching vermiwash with essential nutrients. These sugars serve as precursors for the synthesis of various bioactive compounds. As reported in another study, vermiwash is rich in monomeric sugars, which serve as precursors for the formation of diverse compounds and valuable nutrients for plants.[8]

Based on these findings, Vermiwash A demonstrated greater effectiveness than Vermiwash B, making it the preferred choice for further analysis at QUATEST 3. The analysis revealed a nitrogen-fixing microorganism density of 2.8 × 10^7 CFU/g and an insoluble phosphorus-metabolizing microorganism density of 2.0 × 10^6 CFU/g. According to another study, vermiwash also contains a beneficial microbial system that enhances soil quality and protects plants.[10] These attributes further underscore its value as a sustainable solution for improving soil health and crop productivity.

Effect of vermiwash on post-germination growth of mustard greens

Effect of NPK fertilizer on the growth of mustard greens plants

After 10 days of growth, the plants treated with NPK fertilizer had more leaves and longer leaf lengths than untreated ones. The leaf length in the plants that received NPK fertilizer reached 1.42 ± 0.24 cm, 1.77 times higher than in the untreated plants. Although there was no significant difference in root length and plant height between the two groups, more rootlets appeared in the plants treated with NPK fertilizer.

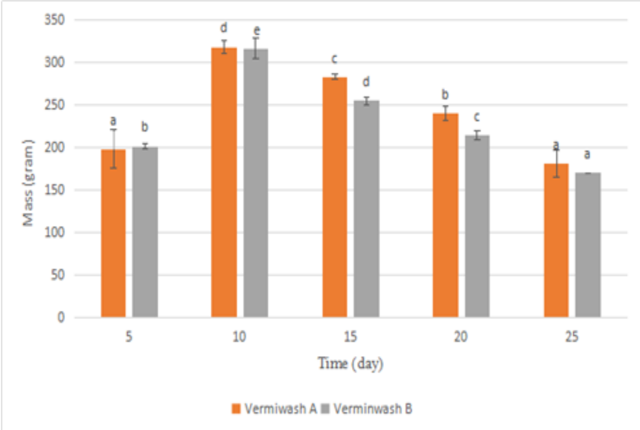

Figure 2: The volume evaluation of vermiwash, collected over a period of time

Note: a, b, c, d, e: Statistically significant differences according to the same-colored column in the one-way ANOVA analysis for each type of vermiwash.

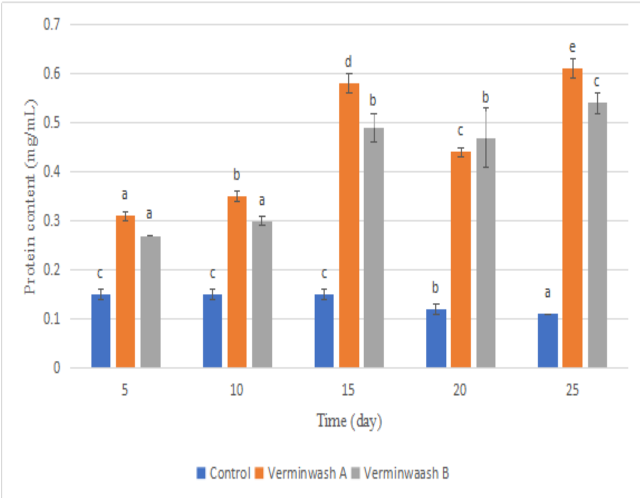

Figure 3: The protein content in vermiwash, collected over a period of time

Note: a, b, c, d, e: Statistically significant differences according to the same-colored column in the one-way ANOVA analysis for each type of vermiwash.

Effect of vermiwash on the growth of mustard greens plants

According to the data in Table 2, treatments involving vermiwash and water-NPK resulted in a survival rate exceeding 90%, with the highest survival observed in the Vermiwash A 50% - NPK treatment (93.33%). Plant height and number of leaves were also at the highest in vermiwash treatments

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Table 2: Effect of vermiwash on the growth of mustard greens after 21 days

Treatments	Survival rate (%)	Leaves height (cm)	Number of leaves	Leaf length (cm)	Root length (cm)
Water – NPK (ĐC-1)	90	6.98 ± 0.08^{b}	4.60 ±0.55^{b}	1.72 ± 0.19^{ab}	1.82 ± 0.20^{a}
Cow dung solution 50% – NPK (ĐC-2)	60	6.46 ± 0.25^{a}	4.20 ±0.45^{ab}	1.54 ± 0.05^{a}	2.30 ± 0.31^{ab}
NAA 10 µg/mL – NPK	20	6.04 ± 0.63^{a}	3.80 ± 0.45^{a}	1.84 ± 0.11^{b}	2.08 ± 0.26^{a}
Vermiwash A 50%	90	7.90 ± 0.47^{c}	6.40 ± 0.55^{cd}	2.28 ± 0.16^{cd}	2.96 ± 0.46^{c}
Vermiwash B 50%	92	7.56 ± 0.38^{c}	6.20 ± 0.45^{c}	2.18 ± 0.13^{c}	2.84 ± 0.32^{c}
Vermiwash A 50% - NPK	**93.33**	7.80 ± 0.33^{c}	**7.00 ± 0.71^{d}**	**3.18 ± 0.15^{e}**	2.70 ± 0.70^{c}
Vermiwash B 50% - NPK	92	7.92 ± 0.19^{c}	6.4 ± 0.55^{cd}	2.42 ± 0.19^{d}	3.06 ± 0.26^{c}

Different letters in the same column represent signifiant differences with p-values < 0.05.

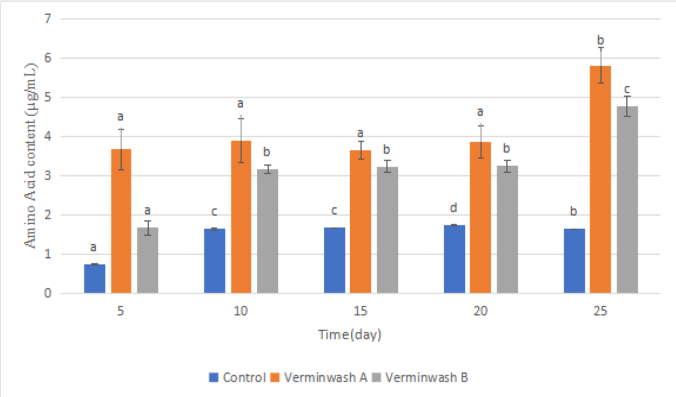

Figure 4: The amino acid content in vermiwash, collected over a period of time
Note: a, b, c, d, e: Statistically significant differences according to the same-colored column in the one-way ANOVA analysis for each type of vermiwash.

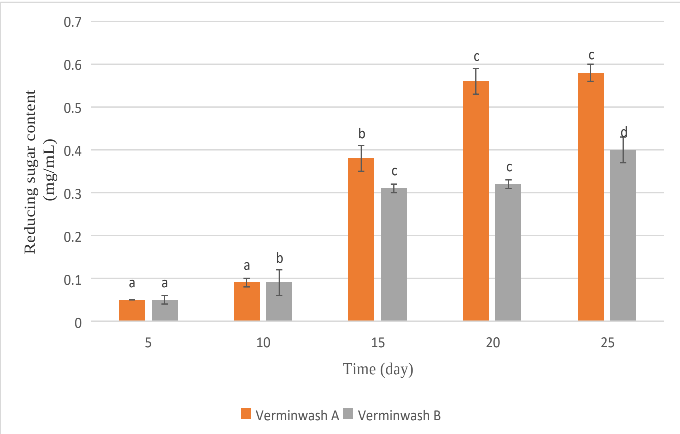

Figure 5: The reducing sugar content in vermiwash, collected over a period of time
Note: a, b, c, d, e: Statistically significant differences according to the same-colored column in the one-way ANOVA analysis for each type of vermiwash

Additionally, the Vermiwash A 50% - NPK treatment had the highest count in the number of leaves (7 leaves) among all the vermiwash treatments. The plants treated with vermiwash showed an increase in leaf size, and the highest leaf length was observed in the Vermiwash A 50% - NPK treatment (3.18 cm), which was approximately twice as long as the control treatment. Additionally, foliar application of vermiwash significantly increased root length compared to control plants. Vermiwash showed a positive effect on the growth of roots and the number of rootlets. The plants treated with vermiwash mostly had green, large leaves and many young leaves. The roots also adhered well to the substrate and produced many rootlets.
Vermiwash effectively improved the germination of rice and mustard seeds, showing the highest germination index (GI) and minimal gibberellic acid (GA) concentration.[11] Using a 100% vermiwash it increased the germination rate and yield of papaya.[12]
The coelomic fluid (CF) of *Eudrilus eugeniae* earthworms which were obtained through the cold stress method was evaluated for its effects on various growth parameters in *Oryza sativa* L. subsp. *indica*. These parameters included seed germination, shoot length, root length, seedling length, and vigor index. Gibberellic acid served as a control for comparison with CF. Moreover, seed germination rates and root length showed greater improvement in CF-treated seedlings. While other growth parameters also exhibited enhancement with CF treatment compared to the control, their effects were either slightly lower or comparable to those induced by GA.[13] The results above indicate that vermiwash can promote the growth of mustard greens, particularly in their leaves and roots. This is due to the nutritional components found in vermiwash, such as vitamins, amino acids, proteins, enzymes, plant growth hormones (including auxin, cytokinin, gibberellin, and humic acid), as well as soluble nutrients (including N, P, K, Na, Mg, etc.). Additionally, experiments showed that Vermiwash A was more effective than Vermiwash B in enhancing the growth of mustard greens. Vermiwash A contains higher levels of protein, amino acids, and reducing sugars compared to Vermiwash B. This may explain why plants treated with Vermiwash A showed a significant increase in the number of leaves and leaf length compared to those treated with Vermiwash B. According to Singh, vermicompost and vermiwash are used as green pesticides to support sustainable crop production.[14]

Conclusion

The results of this study demonstrate that the significant impact of irrigation water temperature on the vermiwash collection process from earthworms. Specifically, at 15°C, the mild stress induced by the lower temperature appears to stimulate earthworms to secrete higher levels of bioactive compounds, including proteins, amino acids, and reducing sugars. Vermiwash collected under these conditions is not only nutrient-rich but also demonstrates enhanced effectiveness in promoting plant growth. These results emphasize the potential of vermiwash as a sustainable and eco-friendly organic fertilizer that is both easy to produce and highly beneficial for agriculture. By optimizing collection conditions, vermiwash can serve as a valuable resource for improving soil health and fostering sustainable farming practices in the future.

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Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The work presented in this article is original, and any liability for claims related to the content of this article will be borne by the authors.

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