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Thidiazuron and Coconut Water Effects on In Vitro Shoot Multiplication of Red Potato (*Solanum tuberosum* L.)

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Abstract:
Red potato has higher yield potential than the Granola variety, but its cultivation is limited by the high cost and poor quality of seed tubers. This study aimed to determine the effects of thidiazuron (TDZ), coconut water, and their interaction on in vitro shoot multiplication of red potato. The experiment was conducted at Politeknik Negeri Jember from June to September 2025 using a factorial completely randomized design with TDZ (1.5, 2.5, and 3.5 mg/L) and coconut water (50, 100, and 150 mL/L) as factors. Time to shoot emergence, number of shoots, shoot height, number of leaves, root length, and number of roots were analyzed by ANOVA and Duncan's multiple range test at 5%. The TDZ × coconut water interaction significantly affected shoot height; 1.5 mg/L TDZ with 150 mL/L coconut water gave the tallest shoots (3.13 cm), statistically similar to 1.5 mg/L TDZ with 100 mL/L (2.66 cm). Coconut water increased the number of shoots, with 150 and 100 mL/L producing 3.04 and 2.78 shoots, respectively. TDZ at 1.5 mg/L produced the most leaves (10.43). Shoot emergence and rooting were not affected. TDZ at 1.5 mg/L combined with 100–150 mL/L coconut water is recommended for red potato shoot multiplication.

Keywords:
coconut water, cytokinin, micropropagation, Solanum tuberosum, thidiazuron

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INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the world's most important food crops, and the availability of healthy seed tubers is the single most important input for its cultivation. Because potato is propagated vegetatively, seed tubers degenerate quickly as viruses and other pathogens accumulate over successive generations, so seed must be replaced regularly (Buckseth et al., 2022). In many tropical and low-income regions, poor seed quality is a major cause of the large gap between potential and actual yields (Forbes et al., 2020). In Indonesia, red potatoes are a cultivar group with high economic value and greater production potential than the widely grown Granola variety, and consumer demand for them is increasing. However, its expansion is constrained by the high cost of seed tubers and the limited availability of high-quality planting material.

Micropropagation offers a practical way to overcome this constraint. It can produce large numbers of uniform, pathogen-free plantlets in a short time and independently of the growing season (Cardoso et al., 2018). In modern seed potato systems, micropropagated plantlets and microtubers provide the nuclear stock from which minitubers and later seed generations are produced (Buckseth et al., 2022). The efficiency of this system largely depends on the shoot multiplication stage, as the number of shoots, nodes, and leaves produced per explant determines how many cuttings are

Volume 02 Number 02
November 2026
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available for the next subculture. The multiplication rate is strongly influenced by the composition of the culture medium, particularly the type and concentration of plant growth regulators (Phillips and Garda, 2019; Pasternak and Steinmacher, 2024).

Cytokinins are the main growth regulators used to stimulate shoot multiplication because they promote cell division and the outgrowth of axillary buds. Cytokinin signals are perceived by membrane-localized receptors and transduced to transcription factors that regulate shoot development (Kieber and Schaller, 2018). Thidiazuron (TDZ) is a phenylurea-type compound with strong cytokinin-like activity that is widely used to induce regeneration in recalcitrant species (Deepa et al., 2018; Erland et al., 2020). TDZ induces de novo shoot organogenesis at low concentrations and somatic embryogenesis at higher concentrations, and it also alters primary metabolism, sugar uptake, and stress metabolism in the treated tissues (Erland et al., 2020). Its high potency, however, means that the effective range is narrow. At higher concentrations, hyperhydricity, abnormal shoot morphology, and rooting problems have been reported (Zhang et al., 2019). In potato, TDZ has been used successfully for microtuber production at concentrations as low as 0.1 mg/L (Yagiz et al., 2020), and, in combination with BAP, it enhanced microtuber formation in the Spunta cultivar (Soliman et al., 2022). In other species, the best shoot multiplication was obtained with relatively low TDZ concentrations, for example, 0.45 ppm in Kaempferia rotunda (Murgayanti et al., 2020), while high cytokinin levels can limit the subsequent growth of plantlets (Murgayanti et al., 2023).

Natural organic supplements are another way to improve in vitro growth at low cost. Coconut water, the liquid endosperm of Cocos nucifera, contains sugars, amino acids, vitamins, minerals, and phytohormones, and it has been used successfully in the tissue culture of many field crops, ornamentals, and medicinal plants (Mu et al., 2024). In potato, coconut water has been evaluated as a medium additive for improving shoot growth of in vitro explants (Winarto et al., 2021). Because coconut water supplies both nutrients and cytokinin-like activity, its effect may depend on the level of synthetic cytokinin already present in the medium. Combining a potent synthetic cytokinin such as TDZ with a natural supplement could therefore produce additive, synergistic, or even antagonistic effects, depending on the concentrations used.

Optimizing a multiplication medium is not simply a matter of maximizing shoot number. A useful medium must also produce shoots that are long enough to be cut into nodal segments, have sufficient leaves to support growth after subculture, and remain free of abnormalities such as hyperhydricity, which is a recurring challenge in commercial micropropagation (Abdalla et al., 2022). These traits are governed by the balance between growth regulators in the medium, because the relative levels of cytokinin and auxin determine whether cells divide, elongate, or differentiate into new organs (Long et al., 2022). Medium components other than growth regulators, such as the type of carbohydrate, also interact with cytokinins in determining multiplication responses (Murgayanti et al., 2021). Evaluating several growth variables together is therefore necessary to identify a medium that supports both shoot proliferation and shoot quality.

Previous work on potato multiplication in Indonesia has focused mainly on BAP, kinetin, and 2-iP. Munggarani et al. (2018) reported that 1.5 mg/L 2-iP significantly increased the number of leaves, nodes, and branches of potato mericlone shoots. In contrast, information on the combined use of TDZ and coconut water for red potato is not available. This study therefore aimed to determine the effects of TDZ concentration, coconut water concentration, and their interaction on the in vitro shoot multiplication of red potato, and to identify the most effective combination for shoot growth, leaf formation, and rooting.

Volume 02 Number 02
November 2026
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METHOD

The research was conducted at the Plant Tissue Culture Laboratory, Politeknik Negeri Jember, East Java, Indonesia, from June to September 2025. The experiment was arranged in a factorial completely randomized design (CRD) with two factors. The first factor was TDZ concentration (T), with three levels: T1 = 1.5 mg/L, T2 = 2.5 mg/L, and T3 = 3.5 mg/L. The second factor was coconut water concentration (A), with three levels: A1 = 50 mL/L, A2 = 100 mL/L, and A3 = 150 mL/L. The combination of the two factors produced nine treatments (Table 1). The TDZ range was selected based on previous studies, while the coconut water range covered concentrations commonly added to tissue culture media as an organic supplement. Each treatment was replicated three times, and each replicate consisted of culture bottles with explant(s) per bottle. Treatments were assigned to experimental units at random, and culture bottles were placed randomly on the culture racks to minimize positional effects.

Table 1. Treatment combinations of TDZ and coconut water concentrations

TDZ concentration	A1 (50 mL/L)	A2 (100 mL/L)	A3 (150 mL/L)
T1 (1.5 mg/L)	T1A1	T1A2	T1A3
T2 (2.5 mg/L)	T2A1	T2A2	T2A3
T3 (3.5 mg/L)	T3A1	T3A2	T3A3

Note: A = coconut water concentration. All treatments used the same MS basal medium.

The data were analyzed according to the linear model of a two-factor CRD: $Y_{ijk} = \mu + T_i + A_j + (TA)_{ij} + \varepsilon_{ijk}$, where Y_{ijk} is the observed value of the *k*-th replicate receiving the *i*-th TDZ concentration and the *j*-th coconut water concentration, μ is the general mean, T_i is the effect of the *i*-th TDZ concentration, A_j is the effect of the *j*-th coconut water concentration, $(TA)_{ij}$ is the interaction effect, and ε_{ijk} is the random error.

In vitro plantlets of red potato (*Solanum tuberosum* L.) cultivar obtained from the source of plantlets were used as the source of explants. Plantlets were maintained on hormone-free MS medium and subcultured for 2 weeks prior to the experiment to obtain a uniform stock. Explants consisted of single-node cuttings approximately 1 cm long, each bearing one axillary bud. Only healthy, green explants of similar size were used to reduce variation unrelated to the treatments.

Coconut water was collected from young green coconuts, approximately 5 months old, filtered through filter paper / sterile cloth to remove debris, and used fresh on the day of medium preparation. The basal medium was Murashige and Skoog (MS) medium supplemented with 30 g/L sucrose and solidified with 7–8 g/L agar. TDZ was added from a stock solution, and coconut water was added by volume according to the treatment combinations in Table 1, with coconut water replacing an equivalent volume of distilled water to ensure a final volume of 1 L. The pH of the medium was adjusted to 5.8 before sterilization in an autoclave at 121 °C for 15 minutes, and the media were dispensed into sterile culture bottles.

All inoculation work was carried out under aseptic conditions in a laminar airflow cabinet using sterilized instruments. Explants were placed upright on the medium, with the basal end slightly inserted into the agar. Cultures were incubated in the culture room at 25 ± 2 °C under a 16-hour light and 8-hour dark photoperiod provided by white fluorescent/LED lamps. Cultures were inspected regularly, and contaminated bottles were excluded from the analysis. Explants were labeled individually and observed every 5 days to track shoot emergence and the development of each explant throughout the culture period. Contamination, browning, and any abnormal growth such as hyperhydricity were also noted during observation.

Volume 02 Number 02
November 2026
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Six variables were recorded (Table 2). Time to shoot emergence was recorded as the number of days after planting (DAP) until the first new shoot became visible. The number of shoots was counted as the number of new shoots formed per explant. Shoot height was measured in centimeters from the base of the shoot to the shoot apex. The number of leaves was counted as the number of fully opened leaves per explant. Root length was measured from the base of the explant to the tip of the longest root, and the number of roots was counted per explant. Shoot and root variables were recorded at the end of the observation period, two weeks after planting.

Table 2. Observed variables, units, and methods of observation

Variable	Unit	Method of observation	Time
Time to shoot emergence	DAP	Days from planting until the first new shoot was visible	Daily
Number of shoots	shoots/explant	Number of new shoots per explant	End
Shoot height	cm	From shoot base to shoot apex	End
Number of leaves	leaves/explant	Number of fully opened leaves	End
Root length	cm	From explant base to the tip of the longest root	End
Number of roots	roots/explant	Number of roots per explant	End

Note: DAP = days after planting; End = end of the observation period.

Data were subjected to a two-way analysis of variance (ANOVA) in a factorial CRD to test the main effects of TDZ and coconut water, as well as their interaction. The calculated F values were compared with tabulated F values at the 5% and 1% significance levels. When a factor or the interaction was significant, means were separated using Duncan's multiple range test (DMRT) at the 5% level. For a significant interaction, all nine treatment combinations were compared; for a significant main effect without interaction, the levels of that factor were compared across the other factor's levels. Means followed by the same letter were considered not significantly different. Before analysis, the normality of the residuals and the homogeneity of variances were assessed, and count data were transformed if necessary. Data were analyzed using statistical software.

RESULTS

The ANOVA results for all observed variables are summarized in Table 3. The TDZ × coconut water interaction significantly affected shoot height. Coconut water concentration significantly affected the number of shoots and shoot height, while TDZ concentration significantly affected the number of leaves. Neither factor nor their interaction significantly affected the time to shoot emergence, root length, or number of roots.

Table 3. Recapitulation of ANOVA results (F values) for the observed variables of red potato shoots

No.	Variable	TDZ (T)	Coconut water (A)	T × A
1.	Time to shoot emergence	0.22 ns	1.59 ns	0.45 ns
2.	Number of shoots	1.83 ns	3.84 *	1.01 ns
3.	Shoot height	1.91 ns	4.20 *	3.92 *
4.	Number of leaves	4.05 *	1.38 ns	0.46 ns
5.	Root length	1.71 ns	0.93 ns	0.61 ns
6.	Number of roots	0.28 ns	0.56 ns	0.34 ns

Note: Values are calculated F values. ** = highly significant ($p < 0.01$); * = significant ($p < 0.05$); ns = not significant.

Volume 02 Number 02
November 2026
e-ISSN: 3124-8179
p-ISSN: 3124-7865

Number of Shoots

Coconut water had a significant effect on the number of shoots (F = 3.84), whereas TDZ and the interaction were not significant. The number of shoots increased with coconut water concentration, from 2.13 shoots per explant at 50 mL/L to 2.78 at 100 mL/L and 3.04 at 150 mL/L (Table 4). The 150 mL/L treatment differed significantly from 50 mL/L but not from 100 mL/L, and the 100 mL/L treatment did not differ from either of the other two levels.

Table 4. Effect of coconut water concentration on the number of shoots of red potato

Coconut water concentration	Number of shoots	DMRT 5% value
A1 (50 mL/L)	2.13 b	0.74
A2 (100 mL/L)	2.78 ab	0.71
A3 (150 mL/L)	3.04 a	–

Note: Means followed by the same letter are not significantly different according to DMRT at the 5% level.

Shoot Height

Shoot height was significantly affected by the interaction between TDZ and coconut water (F = 3.92) and by coconut water alone (F = 4.20). The tallest shoots were produced by 1.5 mg/L TDZ combined with 150 mL/L coconut water (T1A3; 3.13 cm), which was not significantly different from 1.5 mg/L TDZ with 100 mL/L coconut water (T1A2; 2.66 cm). The shortest shoots were obtained with 1.5 mg/L TDZ and 50 mL/L coconut water (T1A1; 1.47 cm) (Table 5). The response of shoot height to coconut water depended on the TDZ level. At 1.5 mg/L TDZ, shoot height more than doubled as coconut water increased from 50 to 150 mL/L. At 2.5 mg/L TDZ, the tallest shoots occurred at 100 mL/L (2.54 cm) and declined at 150 mL/L (1.77 cm). At 3.5 mg/L TDZ, shoot height varied within a narrow range (1.66–2.23 cm) and was lowest at 150 mL/L.

Table 5. Interaction effect of TDZ and coconut water concentrations on shoot height (cm) of red potato

TDZ concentration	A1 (50 mL/L)	A2 (100 mL/L)	A3 (150 mL/L)
T1 (1.5 mg/L)	1.47 e	2.66 ab	3.13 a
T2 (2.5 mg/L)	1.73 cde	2.54 b	1.77 cde
T3 (3.5 mg/L)	2.16 bcd	2.23 bc	1.66 de

Note: Means followed by the same letter are not significantly different according to DMRT at the 5% level (DMRT values 0.50–0.57).

Number of Leaves

TDZ concentration had a significant effect on the number of leaves (F = 4.05), whereas coconut water and the interaction were not significant. The number of leaves decreased as TDZ concentration increased (Table 6). The lowest TDZ concentration (1.5 mg/L) produced the highest number of leaves (10.43), which was not significantly different from 2.5 mg/L (9.67) but was significantly higher than 3.5 mg/L (7.00).

Table 6. Effect of TDZ concentration on the number of leaves of red potato

TDZ concentration	Number of leaves	DMRT 5% value
T1 (1.5 mg/L)	10.43 a	–
T2 (2.5 mg/L)	9.67 ab	2.66
T3 (3.5 mg/L)	7.00 b	2.79

Note: Means followed by the same letter are not significantly different according to DMRT at the 5% level.

Volume 02 Number 02
November 2026
e-ISSN: 3124-8179
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Time to Shoot Emergence and Root Growth

Time to shoot emergence, root length, and number of roots were not significantly affected by TDZ, coconut water, or their interaction (Table 3). All treatments therefore produced similar emergence and rooting responses within the tested concentration ranges. A summary of the best treatments for each variable is presented in Table 7.

Table 7. Summary of significant factors and best treatments for each observed variable

Variable	Significant factor	Best treatment	Mean of best treatment
Time to shoot emergence	None	–	–
Number of shoots	Coconut water	A3 (150 mL/L)	3.04 shoots (A2: 2.78, not different)
Shoot height	T × A; coconut water	T1A3 (1.5 mg/L + 150 mL/L)	3.13 cm (T1A2: 2.66 cm, not different)
Number of leaves	TDZ	T1 (1.5 mg/L)	10.43 leaves (T2: 9.67, not different)
Root length	None	–	–
Number of roots	None	–	–

Note: Comparisons are based on DMRT at the 5% level.

DISCUSSION

This study shows that TDZ and coconut water influenced different components of red potato shoot multiplication. Coconut water mainly increased the number of shoots, TDZ mainly affected leaf formation, and the two factors interacted to determine shoot height. The best overall performance was achieved with the lowest TDZ concentration (1.5 mg/L) combined with a moderate-to-high level of coconut water (100–150 mL/L). In contrast, increasing TDZ above 1.5 mg/L did not improve any variable and tended to reduce leaf number and shoot elongation. These results suggest that the tested TDZ concentrations were at or above the optimal range for red potatoes, while coconut water served as a beneficial supplement.

The positive effect of coconut water on shoot number can be explained by its composition. Coconut water contains sugars, amino acids, vitamins, minerals, and phytohormones, including compounds with cytokinin-like activity (Mu et al., 2024). These components provide additional energy and nitrogen for cell division and may promote the release of axillary buds from dormancy. The number of shoots increased steadily from 50 to 150 mL/L, and the 100 and 150 mL/L treatments were statistically similar. This indicates that 100 mL/L already provides most of the benefit, while higher concentrations give only a small additional gain. The absence of a TDZ effect on shoot number may indicate that the lowest TDZ level (1.5 mg/L) was already sufficient to saturate the cytokinin response for bud activation, so that higher concentrations did not increase shoot formation further. A similar lack of response to added cytokinin, including TDZ, on shoot number has been reported in strawberry multiplication (Raisya et al., 2020).

The significant interaction for shoot height is the most informative result of this study. At 1.5 mg/L TDZ, shoot height increased markedly with coconut water, from 1.47 cm at 50 mL/L to 3.13 cm at 150 mL/L. At 2.5 mg/L TDZ, the benefit of coconut water disappeared at the highest level, and at 3.5 mg/L TDZ, shoots remained short regardless of the coconut water concentration. This pattern suggests that the total cytokinin-like activity of the medium, supplied by both TDZ and coconut water, is what determines shoot elongation. When TDZ is low, coconut water supplies nutrients and mild hormonal stimulation that support elongation. When TDZ is high, the additional cytokinin-like activity

Volume 02 Number 02
November 2026
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from coconut water may push the culture beyond the optimum, favoring cell division and bud formation over cell elongation. Strong cytokinin activity is known to suppress shoot elongation, and high TDZ levels have been associated with hyperhydricity and abnormal shoot morphology (Zhang et al., 2019). High cytokinin levels were also reported to limit plantlet growth in Curcuma zedoaria, thereby requiring gibberellin to optimize growth (Murgayanti et al., 2023).

The decrease in the number of leaves with increasing TDZ concentration supports the same interpretation. TDZ is considerably more potent than adenine-type cytokinins, and its effects persist because it is metabolized slowly in plant tissues. Erland et al. (2020) proposed that TDZ accumulates in tissues in an oligomeric form that acts as a reservoir, slowly releasing the active compound, and that it redirects primary and stress metabolism. Such prolonged activity can disturb the normal development of leaves and internodes, especially at high concentrations. In potato, very low TDZ concentrations are sufficient for some purposes; for example, 0.1 mg/L TDZ was effective for microtuber production (Yagiz et al., 2020). The highest leaf number in this study (10.43 leaves at 1.5 mg/L TDZ) is consistent with reports that relatively low TDZ concentrations give the best multiplication responses in other species (Murgayanti et al., 2020). It is also comparable to the effect of 1.5 mg/L 2-iP, which increased the number of leaves, nodes, and branches of potato mericlone shoots (Munggarani et al., 2018). For red potato, therefore, TDZ concentrations below 1.5 mg/L should be tested in future studies, as they may produce equal or better results.

Time to shoot emergence was not affected by the treatments. Shoot emergence from a single-node or shoot-tip explant depends largely on the pre-existing axillary bud, which is already organized and can grow out rapidly once the explant is placed on a nutrient medium. Under these conditions, the timing of bud outgrowth is controlled mainly by the physiological state of the explant and by endogenous hormones rather than by the concentration of exogenous regulators (Ikeuchi et al., 2019; Pasternak and Steinmacher, 2024). Because all explants were taken from uniform stock material, they probably had a similar endogenous status, which would explain the uniform emergence time.

Root length and number of roots were also unaffected. Potato roots readily in vitro, and hormone-free MS medium is widely used for large-scale potato micropropagation (Buckseth et al., 2022). The endogenous auxin produced by growing shoots is generally sufficient to induce adventitious roots in potato, so exogenous cytokinin and coconut water had little additional effect. At the same time, cytokinins usually antagonize root formation, and TDZ in particular has been associated with rooting problems (Zhang et al., 2019). The absence of a negative effect on rooting at the tested concentrations is therefore a positive finding, because it means that shoots multiplied on TDZ-containing media can still form roots without a separate rooting stage. This simplifies the propagation protocol and reduces cost.

From a practical standpoint, the combination of 1.5 mg/L TDZ and 100 mL/L coconut water is an efficient choice for red potato multiplication. Although the tallest shoots were obtained with 150 mL/L coconut water, the 100 mL/L treatment was statistically equivalent for both shoot height and number of shoots, and it reduces the amount of coconut water required per liter of medium. Using the lowest effective TDZ concentration also lowers the risk of abnormal growth and reduces the cost of the synthetic regulator. Coconut water is inexpensive and widely available in Indonesia, making this combination attractive to local seed producers. Because micropropagation provides the nuclear stock for minituber production (Buckseth et al., 2022), even moderate improvements in multiplication rate can significantly increase the number of healthy plantlets available for seed potato production.

The results also illustrate why multiple growth variables should be evaluated together when optimizing a multiplication medium. If only the number of shoots had been considered, 150 mL/L

Volume 02 Number 02
November 2026
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coconut water would have been selected regardless of TDZ concentration. However, the interaction for shoot height shows that this coconut water level is beneficial only when combined with the lowest TDZ concentration. Similarly, high TDZ did not increase shoot number but reduced leaf number, thereby lowering the number of usable nodal cuttings in the next subculture. These responses align with the general principle that the balance among growth regulators, rather than the absolute level of any one, determines whether cells divide, elongate, or differentiate (Long et al., 2022). A balanced medium that supports moderate shoot proliferation together with good elongation and leaf development is therefore more valuable in practice than a medium that maximizes only one trait, especially because excessive cytokinin can also increase the risk of hyperhydricity and other abnormalities (Abdalla et al., 2022).

This study has several limitations. First, the tested TDZ concentrations (1.5–3.5 mg/L) were relatively high for potato, and a TDZ-free control and lower concentrations were not included, so the true optimum may lie below 1.5 mg/L. Second, coconut water is a natural product whose composition varies with the coconut's age and variety (Mu et al., 2024), potentially limiting the reproducibility of results. Third, the stability of the multiplication rate over several subcultures and the genetic fidelity of the regenerated plants were not evaluated. Future studies should include a TDZ-free control, TDZ concentrations between 0.1 and 1.5 mg/L, standardized coconut water from fruits of known age, evaluation over several subcultures, and acclimatization of the plantlets to assess their performance under ex vitro conditions.

CONCLUSION

TDZ and coconut water affected different aspects of in vitro shoot multiplication of red potato. Their interaction significantly affected shoot height, with 1.5 mg/L TDZ combined with 150 mL/L coconut water producing the tallest shoots (3.13 cm), statistically similar to 1.5 mg/L TDZ with 100 mL/L coconut water (2.66 cm). Coconut water increased the number of shoots, with 150 and 100 mL/L producing 3.04 and 2.78 shoots per explant, respectively, while 1.5 mg/L TDZ produced the highest number of leaves (10.43). Time to shoot emergence, root length, and number of roots were not affected by the treatments. Therefore, 1.5 mg/L TDZ combined with 100 mL/L coconut water is recommended as an efficient medium for in vitro shoot multiplication of red potato.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Volume 02 Number 02
November 2026
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