

Early Growth Response of *Capsicum frutescens* L. (Ori 202 Variety) under Humic Acid Application in Cocopeat-Based Growing Media

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ABSTRACT

Responses to humic acid (HA) are strongly influenced by plant genotype and cultivation conditions; however, information regarding HA application rates for bird's eye chili (*Capsicum frutescens* L. cv. Ori 202) under container-based cultivation remains limited. This study evaluated the growth response of bird's eye chili to HA applied at five concentrations in a completely randomized design consisting of H0 = 0, H1 = 2.5 mL L⁻¹, H2 = 5 mL L⁻¹, H3 = 7.5 mL L⁻¹, and H4 = 10 mL L⁻¹. Applications began seven days after planting and were repeated at seven-day intervals. Vegetative performance was assessed using biomass-related parameters, including plant height, leaf number, stem diameter, root length, root and shoot biomass, fresh and dry weights, and root-to-shoot ratio. Data were analyzed using analysis of variance followed by Duncan's Multiple Range Test at $\alpha = 0.05$. HA application significantly enhanced vegetative growth and biomass accumulation compared with the control treatment ($p < 0.05$). The H1 treatment (2.5 mL L⁻¹) produced the highest total dry weight (1.94 g) and total fresh weight (17.82 g), indicating superior early biomass accumulation. Meanwhile, H2 (5 mL L⁻¹) resulted in the greatest root length (13.43 cm). In contrast, the highest concentration (H4) did not provide growth benefits and performed similarly to or lower than the control. Under the experimental conditions, HA at 2.5 mL L⁻¹ was the most effective treatment for promoting biomass accumulation in *C. frutescens* cv. Ori 202, although further validation under cultivation environments is still required to confirm its applicability.

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INTRODUCTION

Bird's eye chili (*Capsicum frutescens* L.) is an economically important horticultural crop in Indonesia due to its high market demand and widespread culinary use. In 2024, total chili pepper production in Indonesia reached 15,687,569.77 quintals, reflecting the substantial economic importance of chili cultivation in the national horticultural sector (BPS Indonesia, 2024) with average consumption increased 1.995% from 2019 to 2023 (Pusdatin, 2024). However, productivity is often constrained by declining soil fertility and inefficient fertilizer utilization in intensive cultivation systems (Jeksen & Sari, 2022). Continued reliance on synthetic fertilizers may degrade soil quality and undermine long-term agricultural sustainability (Krasilnikov et al., 2022). Therefore, environmentally friendly cultivation approaches that improve nutrient-use efficiency and support sustainable crop production are increasingly needed. One potential approach is the use of biostimulants such as humic acid (HA), which may enhance plant growth and improve nutrient availability in the growing medium. Despite the increasing interest in HA application in horticultural crops, information regarding the growth response of bird's eye chili, particularly the Ori 202 variety, to different HA application rates under controlled cultivation conditions remains limited.

The early vegetative stage of bird's eye chili is a critical phase that strongly influences subsequent plant establishment and productivity. Optimal vegetative growth is associated with improved root development, greater photosynthetic capacity, and enhanced tolerance to environmental stress (Blunk et al., 2019). However, inadequate nutrient availability and low fertilization efficiency in cultivation systems may limit early plant growth and biomass accumulation. Therefore, strategies that improve nutrient uptake and support sustainable plant development are increasingly important in bird's eye chili cultivation. The use of suitable growing media and environmentally friendly inputs has been reported to influence plant performance and growth responses (Sari & Fantashe, 2015; Tanari et al., 2023). In this context, humic acid has attracted attention as a potential biostimulant that enhances nutrient availability and promotes vegetative growth.

In practical cultivation settings, bird's eye chili production continues to face various constraints, particularly declining soil fertility and reduced fertilization efficiency. Long-term and excessive application of inorganic fertilizers can degrade soil physical, chemical, and biological properties, ultimately impairing plant growth (Bhatt et al., 2019). These challenges highlight the

need for more environmentally friendly and sustainable cultivation approaches (Simanihuruk, 2011; Tyasmoro, 2023).

One promising approach to address these limitations is the application of humic acid (HA), an organic-based biostimulant known to improve soil conditions and enhance nutrient uptake efficiency. HA may increase cation exchange capacity, improve nutrient availability, and stimulate microbial activity in the growing medium, thereby supporting better plant growth and development (Ranesa & Tejawulan, 2024). In addition to its effects on soil properties, HA has also been associated with enhanced root development and improved nutrient absorption efficiency. Therefore, HA represents a potential environmentally friendly input for supporting sustainable bird's eye chili cultivation systems.

Humic acid, one of the major fractions of organic matter, is widely recognized for its important role in supporting plant growth and development. It can stimulate root growth, enhance the uptake of macro- and micronutrients, and modulate plant growth regulators such as auxins and cytokinins (Nuraini & Zahro, 2020). Previous studies have reported that humic acid application can improve vegetative growth and biomass accumulation in several horticultural crops, including maize (Trimansyah et al., 2024) and mung bean (Hermawan et al., 2025). However, information regarding the appropriate humic acid application rate for bird's eye chili, particularly the Ori 202 variety during the early vegetative stage, remains limited. In addition, the effectiveness of soil-drenching humic acid application under controlled growing-medium conditions has not been clearly established. Therefore, evaluating the growth response of bird's eye chili to different humic acid concentrations is necessary to identify the most effective treatment under the present experimental conditions.

However, plant responses to humic acid application are strongly influenced by dosage, plant species, and the properties of the growing medium. The application of improperly calibrated doses may lead to suboptimal plant performance or even adverse effects on growth (Manukoto et al., 2024). Determining the optimum dose of humic acid is therefore a key aspect in its effective use for *C. frutescens* cultivation.

To date, information regarding the appropriate humic acid application rate for the growth of bird's eye chili (*Capsicum frutescens* L.) var. Ori 202 remains limited, particularly during the early vegetative stage. Therefore, this study aimed to evaluate the growth response of *C. frutescens* var. Ori 202 to different concentrations of humic acid and to identify the most effective concentration

for promoting vegetative growth and biomass accumulation under the present experimental conditions.

RESEARCH METHODS

Time and Place of the Research

The experiment was conducted at the GRC Garden and the Biology Laboratory, University of Bengkulu, from January to February 2026 under controlled cultivation conditions. During the experimental period, the average environmental temperature ranged from approximately 27–32°C, with relative humidity between 70–85%, representing typical lowland tropical conditions in Bengkulu, Indonesia. Plants were irrigated regularly to maintain adequate moisture throughout the experiment. The cultivation conditions followed standard chili pepper-growing practices in tropical environments (Berke et al., 2021).

The growing medium consisted of cocopeat and compost mixed at a ratio of 2:1 (v/v). Cocopeat was selected due to its high water-holding capacity and good aeration properties, whereas compost served as an organic nutrient source to support early vegetative growth. Before planting, the medium was homogenized thoroughly to ensure uniformity among treatments. The medium was maintained under similar moisture conditions throughout the experimental period for all experimental units. The use of cocopeat-based media followed commonly applied horticultural substrate practices for *Capsicum* cultivation.

Materials and Tools

The materials used in this study consisted of bird's eye chili seeds of the Ori 202 variety (*Capsicum frutescens* L.), commercial humic acid, planting media, and polybags used as planting containers. The humic acid used in this experiment was a commercial liquid product obtained from Subur Green. According to the manufacturer's specification, the formulation contained 90% humic acid and 10.5% organic C, along with 0.2% P and 0.1% K, as well as several micronutrients including 20.5 ppm Fe, 11 ppm Mn, 6.5 ppm Co, 15 ppm Cu, 14 ppm Zn, and 125 ppm B. Additional physicochemical characteristics such as pH and K₂O equivalent content were not specified by the manufacturer. These components may contribute to enhanced nutrient availability and improved plant growth performance. The planting media were prepared uniformly before treatment application.

The instruments employed included a ruler for measuring plant height and root length, a caliper for measuring stem diameter, an analytical balance for measuring fresh and dry biomass, and a drying oven for determining plant dry weight.

Experimental Design

This study employed a Completely Randomized Design (CRD) consisting of five humic acid concentration treatments: H0 (0 mL L⁻¹) as the control, H1 (2.5 mL L⁻¹), H2 (5 mL L⁻¹), H3 (7.5 mL L⁻¹), and H4 (10 mL L⁻¹). Each treatment was replicated three times, resulting in 15 experimental units. Each experimental unit consisted of a single plant grown in a polybag containing 3 kg of growing medium.

The experimental units were arranged randomly within the cultivation area to minimize positional bias and reduce potential microclimatic variation among treatments. In addition, the positions of the polybags were rotated periodically during the experimental period to maintain relatively uniform environmental exposure among experimental units.

Humic Acid Application

Humic acid was applied using the soil-drenching method: the product was dissolved in water to the designated treatment concentrations, and the solution was applied directly to the planting medium. Each plant received 20 mL of solution per application to ensure uniform exposure across experimental units. Applications were initiated 7 days after transplanting and repeated at 7-day intervals until the end of the observation period, resulting in a total of 5 applications during the experiment.

The term “planting” in this study refers to the transplanting of 14-day-old bird’s eye chili seedlings into polybags containing the prepared growing medium. Standardization of application volume and timing was maintained throughout the study to minimize variability unrelated to treatment effects.

Observation Variables

Vegetative growth variables included plant height, leaf number, stem diameter, and root length. Plant height was measured from the base of the stem to the highest growing point using a ruler. Leaf number was determined by counting fully expanded leaves and expressed as the total number of leaves. Stem diameter was measured using a caliper at approximately 1 cm above the surface of the growing medium and recorded in millimeters. Root length was measured after harvest by

determining the length of the longest root from the stem base to the root tip and expressed in centimeters.

Plant biomass variables included the fresh and dry weights of shoots, roots, and total plants. Fresh weight was measured immediately after harvest by separating the root and shoot portions and weighing them using an analytical balance, with results expressed in grams. The dry weight was determined by oven-drying plant samples at 70 °C until a constant weight was achieved. Constant weight was defined as two consecutive weighings showing a difference of less than 0.01 g after additional drying intervals.

Data Analysis

The observational data were analyzed using analysis of variance (ANOVA) to evaluate the effects of different humic acid concentrations on plant growth parameters. For variables measured repeatedly over time on the same experimental units, repeated measures ANOVA was applied to account for within-subject correlations. Duncan's Multiple Range Test (DMRT) was used for mean comparison at a 5% significance level ($\alpha = 0.05$) whenever significant differences were detected. In cases where the ANOVA assumptions were not satisfied, data transformation was applied before analysis. All statistical analyses were conducted using IBM SPSS Statistics 20.

RESEARCH RESULT

Plant height increased progressively from week 0 to week 3 (Figure 1A), rising from approximately 10.16 cm at week 0 to about 22.30 cm at week 3. A marked increase was observed particularly after week 1, with plant height reaching approximately 17.36 cm at week 2. Significant differences among observation times were indicated by different significance letters shown in the figure. Under different humic acid treatments (Figure 1D), plant height responses varied among treatments. Treatment H1 produced the highest mean plant height (20.24 cm), followed by H2 (17.57 cm) and H3 (approximately 15.83 cm), whereas the control treatment (H0) showed the lowest value (10.38 cm). Treatment H4 showed an intermediate response of approximately 13.26 cm. However, several treatments were not significantly different from one another, as indicated by shared significance letters in the figure.

The number of leaves also increased progressively with plant age (Figure 1B), from approximately 6.67 leaves at week 0 to around 8.60 leaves at week 3. Significant differences were detected among most observation periods. However, week 2 and week 3 did not differ significantly, suggesting a slower rate of leaf production toward the end of the observation period.

Across humic acid treatments (Figure 1E), leaf number was highest in treatments H1, H2, and H3 and these treatments were not significantly different from one another. In contrast, the control treatment (H0) produced the lowest leaf number (6.08 leaves), while H4 showed an intermediate response of approximately 6.92 leaves.

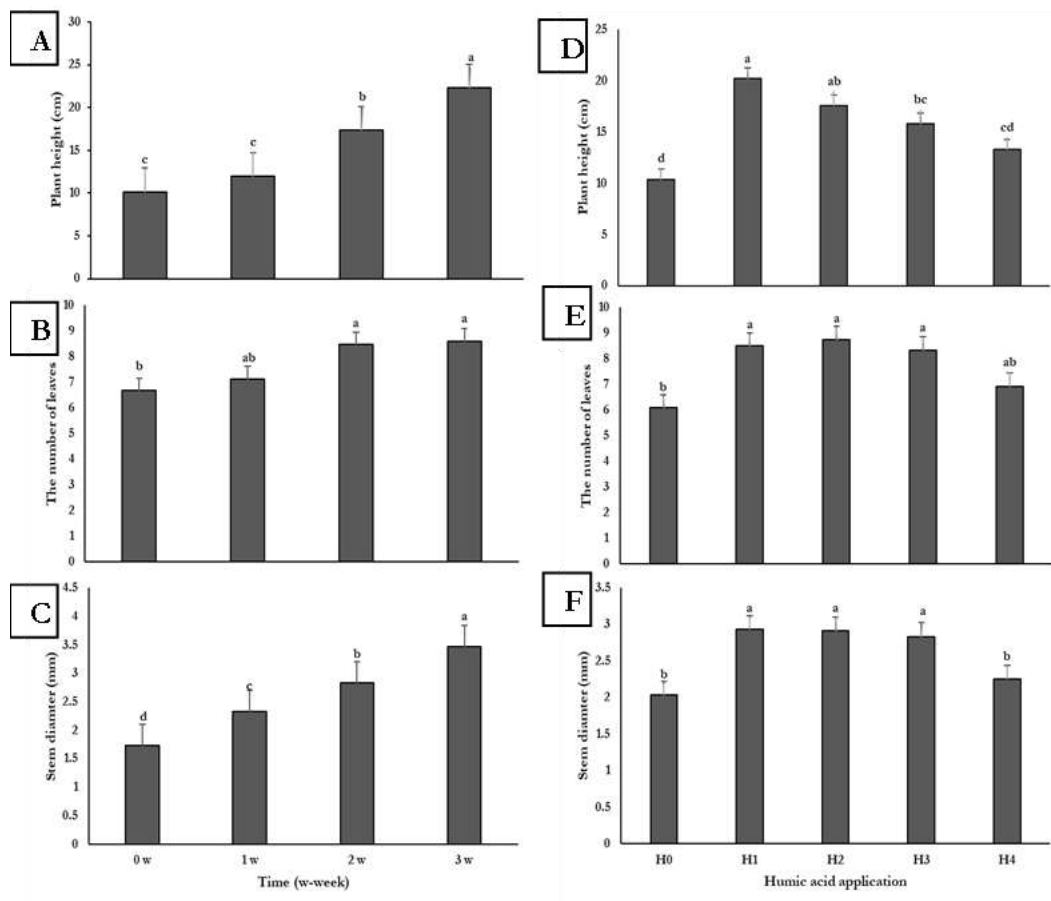


Figure 1. Growth response of *Capsicum frutescens* L. (Ori 202 variety) over time and under different humic acid applications. (A) Plant height at weeks 0–3. (B) Number of leaves at weeks 0–3. (C) Stem diameter at weeks 0–3. (D) Plant height under different humic acid treatments (H0–H4). (E) Number of leaves under different humic acid treatments. (F) Stem diameter under different humic acid treatments. Data are presented as mean $\pm$ SD ($n = 3$). Different letters above the bars indicate significant differences according to DMRT at $\alpha = 0.05$. In panels A–C, letters indicate comparisons among observation weeks, whereas in panels D–F, letters indicate comparisons among humic acid treatments.

Stem diameter increased throughout the observation period (Figure 1C). The smallest stem diameter was recorded at week 0, while the largest was at week 3. Each weekly measurement differed significantly based on the grouping letters. Differences in stem diameter were also observed among humic acid treatments (Figure 1F). Treatments H1, H2, and H3 produced the

largest stem diameters, with no statistical difference among them. The lowest stem diameter was recorded in H0, while H4 produced intermediate values.

The fresh and dry biomass components of *Capsicum frutescens* L. (Ori 202 variety) varied among humic acid treatments (Table 1). Root length ranged from 9.10 cm to 13.43 cm. Although treatment H2 showed the highest numerical root-length value, no significant differences were observed among the research treatments, as indicated by the identical statistical grouping letters. Root fresh weight ranged from 0.51 g to 2.46 g, with H1 showing the highest mean value, whereas H4 and the control treatment (H0) produced the lowest values. Shoot fresh weight exhibited a similar trend, with H1 producing the highest mean value (15.36 g), followed by H2, while H0 and H4 showed comparatively lower values. Total fresh weight ranged from 2.37 g to 17.82 g, with H1 producing the greatest biomass accumulation overall, although some treatments sharing similar statistical groupings were not significantly different according to DMRT.

Root dry weight values were relatively low across treatments, ranging from 0.05 g to 0.29 g, with H1 showing the highest mean value. Shoot dry weight ranged from 0.31 g to 1.65 g, and the highest mean value was also observed in H1. Total dry weight varied between 0.40 g and 1.94 g, with H1 producing the greatest total dry biomass accumulation, whereas H4 and the control treatment (H0) showed comparatively lower values. The root-to-shoot (R/S) ratio ranged from 0.10 to 0.39. Although H3 exhibited the highest numerical R/S ratio, no significant differences among treatments were detected by DMRT analysis.

Table 1. Fresh and dry weight of plants with varied humic acid treatment

Num	Treatments	Root length (cm)	RFW (g)	SFW (g)	TFW (g)	RDW (g)	SDW (g)	TDW (g)	R/S Ratio
1	H0 (0 ml L ⁻¹)	9.10 ^a	0.53 ^c	1.84 ^c	2.37 ^c	0.09 ^b	0.31 ^b	0.40 ^b	0.31 ^a
2	H1 (2.5 ml L ⁻¹)	12.93 ^a	2.46 ^a	15.36 ^a	17.82 ^a	0.29 ^a	1.65 ^a	1.94 ^a	0.28 ^a
3	H2 (5 ml L ⁻¹)	13.43 ^a	1.52 ^b	10.58 ^{ab}	12.10 ^{ab}	0.24 ^a	1.36 ^b	1.60 ^{ab}	0.19 ^a
4	H3 (7.5 ml L ⁻¹)	12.13 ^a	1.06 ^{bc}	7.46 ^{bc}	8.51 ^{bc}	0.26 ^a	1.00 ^{ab}	1.26 ^{ab}	0.39 ^a
5	H4 (10 ml L ⁻¹)	9.33 ^a	0.51 ^c	3.10 ^c	3.62 ^c	0.05 ^b	0.46 ^{ab}	0.52 ^b	0.10 ^a

Note: Values are presented as mean $\pm$ SD (n = 3). Means followed by the same letters within a column are not significantly different at $P \leq 0.05$ according to DMRT. RFW = root fresh weight; SFW = shoot fresh weight; TFW = total fresh weight; RDW = root dry weight; SDW = shoot dry weight; TDW = total dry weight.

DISCUSSION

Humic acid application markedly influenced the vegetative growth of *Capsicum frutescens* L. (Ori 202), as reflected by increases in plant height, leaf number, stem diameter, and biomass

accumulation across the observation period (Figure 1A–F). Among the treatments, H1 (2.5 mL L⁻¹) produced the greatest biomass accumulation, increasing total fresh weight and total dry weight by approximately 652% and 385%, respectively, compared with the control treatment. These findings indicate that low-dose humic acid application was effective in promoting early vegetative growth under the present experimental conditions.

The observed responses are consistent with the known role of humic substances (HS) as plant biostimulants that enhance nutrient uptake and growth-related physiological processes (Ampong et al., 2022; Rathor et al., 2023). Mechanistically, HS/HA exhibit auxin-like activity and stimulate plasma membrane H⁺-ATPase activity in roots, thereby promoting cell-wall loosening, cell elongation, and lateral root formation through hormone and redox signaling interactions (Canellas et al., 2002; de Souza et al., 2025). Recent evidence also suggests that HA may regulate root development through RBOH/ROS/H⁺-ATPase/TOR signaling pathways and stimulate fine-root formation, thereby improving nutrient acquisition efficiency (Zandonadi et al., 2025). In addition, the biological activity of HS is strongly influenced by molecular characteristics such as functional groups, hydrophobicity, and molecular size, which may contribute to the dose-dependent growth responses observed in this study (Nardi et al., 2021).

Table 1 shows that treatment H1 generally produced among the highest fresh (RFW, SFW, TFW) and dry (RDW, SDW, TDW) biomass values, whereas the control treatment (H0) and the highest concentration treatment (H4) tended to exhibit lower biomass accumulation. However, several biomass variables showed overlapping statistical groupings between H1 and H2/H3, indicating that these treatments were not significantly different for all parameters according to DMRT analysis. These findings are consistent with meta-analytical evidence showing that humic substances (HS) commonly enhance shoot and root biomass, although the magnitude of the response is often dose-dependent and influenced by HS source and application rate (Rose et al., 2014). At the crop level, humic acid may improve yield and nitrogen-use efficiency under specific environmental and dosage conditions, which aligns with the relatively better performance of low-to-moderate HA concentrations observed in the present study compared with the highest concentration treatment (H4) (Ma et al., 2024).

Treatment H2 showed the numerically greatest root length; however, differences among treatments were not statistically significant according to DMRT analysis. In contrast, whole-plant biomass accumulation (TFW and TDW) tended to be greater in H1, suggesting that lower humic

acid concentrations were generally associated with more favorable vegetative growth responses under the present experimental conditions. Previous studies have indicated that improvements in root architecture do not always correspond directly to maximum whole-plant biomass accumulation because carbon assimilation and biomass partitioning are influenced by both humic substance characteristics and application rates (Nardi et al., 2021).

The root-to-shoot (R/S) ratio showed some numerical variation among treatments, with H3 presenting the highest mean value; however, no significant differences were detected among treatments. Therefore, interpretation of biomass allocation patterns should be made cautiously, although the observed trend may suggest variation in biomass partitioning responses under different humic acid concentrations (Jindo et al., 2020).

Previous studies in *Capsicum* spp. have demonstrated that humic-based biostimulants may enhance plant growth and productivity, although responses vary depending on species, cultivar, application method, and developmental stage. For example, foliar application of humic acid at intermediate concentrations increased plant height, stem diameter, and yield in chili pepper, while humic-based biostimulants improved yield and quality-related traits in *Capsicum* species under greenhouse and field conditions (Jan et al., 2020; Mezeyová et al., 2024). In line with these reports, the present study further highlights the potential of humic acid application in *C. frutescens* var. Ori 202 under early vegetative growth conditions.

In addition to growth promotion, humic acid fertilization has also been associated with improvements in postharvest structural integrity and metabolite composition in sweet pepper (Zamljen et al., 2025). Furthermore, integrated nutrient management combining HA with NPK fertilizers has been reported to improve chlorophyll content, sugar accumulation, and soil fertility indicators in chili cultivation, although treatment effectiveness depended on the HA:NPK ratio applied (Ichwan et al., 2022). These findings collectively support the context-dependent nature of humic acid responses in *Capsicum* cultivation systems.

Under the present experimental conditions, H1 (2.5 mL L⁻¹) generally produced among the highest fresh and dry biomass values, particularly for TFW and TDW, indicating that low-to-moderate humic acid concentrations were more favorable for early vegetative growth of *Capsicum frutescens* L. var. Ori 202 than the highest concentration treatment (H4). These findings are consistent with previous reports indicating that humic substances often exhibit non-linear dose responses, in which moderate application rates may promote plant growth more effectively than

excessive concentrations (Rose et al., 2014; Ma et al., 2024). However, overlapping DMRT statistical groupings among several treatments indicate that some biomass responses were not significantly different across all humic acid concentrations. Therefore, the present findings should be interpreted cautiously and considered specific to the experimental conditions used in this study.

CONCLUSION

Humic acid application influenced the early vegetative growth and biomass accumulation of *Capsicum frutescens* L. var. Ori 202 under the present experimental conditions. Among the evaluated treatments, H1 (2.5 mL L⁻¹) generally produced the highest fresh and dry biomass values, particularly for total fresh weight and total dry weight, indicating that low-to-moderate humic acid concentrations were more favorable than the highest concentration treatment (H4). Although H2 showed the highest numerical root-length value, differences in root length among treatments were not statistically significant. The findings suggest that humic acid at 2.5 mL L⁻¹ may be considered for supporting early vegetative establishment and biomass accumulation of *C. frutescens* var. Ori 202 under conditions similar to those used in this study. However, further validation using larger sample sizes, additional growing conditions, and longer cultivation periods is required before broader agronomic recommendations can be made.

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CONFLICTS OF INTEREST

The authors declare no competing interests and confirm that there are no financial or non-financial conflicts of interest related to the publication of this work.

KREDIT AUTHORSHIP CONTRIBUTION STATEMENT

E. V. Andesmora: Conceptualization, Methodology, Formal analysis, Writing – Original Draft, Writing – Review & Editing, Supervision, Project administration. **R. Aprianto:** Methodology, Formal analysis, Writing – Review & Editing. **W. Syahmi:** Resources; Visualization; Writing – Review & Editing. **R. J. Putra:** Validation; Visualization; Resources. **D. Amaliya:** Investigation, Validation, Writing – Review & Editing.

DECLARATION OF THE USE OF AI

During the preparation of this work, the authors used ChatGPT to enhance the clarity of the writing. After using this tool, the authors carefully reviewed and edited the content as needed and took full responsibility for the final published content.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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