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RESEARCH ARTICLE

Effect of different molecular weight and concentrations of chitosan on the phenology of tomato in greenhouse conditions

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ABSTRACT

The use of synthetic fertilizers is among the most expensive and pollutant supplies in agriculture. An alternative to reduce their use is through complementary products such as biostimulants like chitosan. Biostimulants represent a sustainable alternative to chemicals as they contain or help generate beneficial substances for plant growth. The present study evaluated the effect of different molecular weights of chitosan (low, medium, and high) and different doses (120, 210, and 300 mg) on the growth of tomato (*Solanum lycopersicum* L.) plants in greenhouse conditions. The evaluated variables were plant dry weight, foliar dry weight, root volume and root dry weight, plant height, stem diameter, and analysis of foliar nutrients. Results showed a positive effect of chitosan supplementation on plant growth and nutrient uptake, showing that both dose and molecular weight influenced growth and nutrient absorption. Treatment with low molecular weight chitosan at a dose of 300 mg increased root dry weight by 107% and plant height by 35% compared to the control, on the other hand, while high molecular weight chitosan applied at 210 mg, increasing plant dry weight by 73% and foliage dry weight by 76% compared to water control. Total plant weight is a good measure of overall plant growth and nutrient absorption. It was concluded that high molecular weight chitosan represents a good biostimulant alternative to chemical fertilizers such as urea for tomato plant cultures.

Key words: Biostimulant, chitosan, molecular weight, *Solanum lycopersicum*, tomato plants.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the most important vegetable crop in the world due to its great nutritional value and production volume, of which a high percentage is directed for human consumption. Tomato production is constantly increasing due to high population demand, reaching 192 million tons in 2024 (FAO, 2025). Growing this crop requires large amounts of agrochemicals, most of which have negative effects on the environment and human health (Sharma and Singhvi, 2017).

In Sinaloa, the annual amount of fertilizer applied to tomato crops ranges from 300 to 350 kg ha^{-1} N (INIFAP, 2017), whereas the N plant uptake is approximately 50% (Wang et al., 2025). The nutrients not taken up by plants have a negative impact on the environment, causing damage to soil and water ecosystems as well as to human health. One of the alternatives used to reduce synthetic fertilization is the application of biostimulants, which improves plant metabolism and enhances nutrient uptake (Halpern et al., 2015). Unlike fertilizers and biofertilizers, biostimulants do not directly supply nutrients; rather, they are substances or microorganisms that regulate physiological processes in plants (Yakhin et al., 2017; Rouphael and Colla, 2020; Quille et al., 2025).

Chitosan is a biopolymer obtained from chitin, which is the second most abundant natural polysaccharide in the world, found in the exoskeletons of invertebrates such as insects and crustaceans including crabs, lobsters and shrimp, the latter being the most common source used for its extraction. Chitin is subjected to a deacetylation process to obtain chitosan, which is characterized by its biodegradability and biocompatibility (Kozma et al., 2022). These properties make chitosan a biopolymer with various applications in the food and medical industries, in water treatment, and as a plant growth promoter, among others (Sreelakshmi et al., 2024). As a result, chitosan has gained increasing interest as a sustainable agricultural biostimulant due to its ability to promote plant growth, improve crop productivity, and enhance physiological performance (Khaengkhan et al., 2024). In addition, it can help plants tolerate abiotic stresses, such as drought and high temperatures, which are becoming increasingly frequent challenges due to climate change (Hidangmayum et al., 2019). Chitosan is a cationic linear polysaccharide composed of D-glucosamine and *N*-acetyl-D-glucosamine units linked by β-1,4-glycosidic bonds (Chandrasekaran and Paramasivan, 2024), and it can be classified according to its molecular weight as high (HMW), medium (MMW), and low molecular weight (LMW) (Ardean et al., 2021).

It has been demonstrated that chitosan acts as an activator of metabolic pathways in tomato, inducing the production of hormones such as indole-3-acetic acid, salicylic acid and jasmonic acid, which are related to plant growth and defense (De Tender et al., 2020). Chitosan has different effects on crops depending on its molecular weight due to changes in its solubility, viscosity and capacity to interact with plant tissues. Therefore, low molecular weight chitosan has a better ability to interact with soil and plant tissues and is the most recommended as a growth promoter and in plant defense (Román-Doval et al., 2023). Due to these differences, plants respond to chitosan as a biostimulant depending on its molecular weight and concentration. Nonetheless, limited information is available on optimal doses and administration regime, which limits its use as a sustainable alternative to the extensive use of synthetic fertilizers. Hence, the present study aimed to evaluate the effect of three molecular weight (low, medium and high) and three doses of chitosan on growth of tomato plants to provide information on its agricultural use.

MATERIAL AND METHODS

This research was carried out under greenhouse conditions from October to December 2024, at Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR) Unidad Sinaloa (25°32'48.0" N, 108°28'54.0" W) located in Guasave, Sinaloa, Northwest Mexico. Commercial chitosan (Tokyo Chemical Industry [TCI], Tokyo, Japan) of three different molecular weights (low, medium and high) was used (Table 1).

Distilled water with 0.05% acetic acid was used to dissolve the three types of chitosan, solutions were stirred for 2 h in a magnetic stirrer, and potassium hydroxide (KOH) was used to adjust the pH to 5.5-6.0.

Table 1. Molecular weight characteristics of the commercial chitosan used in this study.

Molecular weight	Specifications
Low	5-20 mPa·s^{-1}, 0.5% in 0.5% acetic acid at 20 °C
Medium	20-100 mPa·s^{-1}, 0.5% in 0.5% acetic acid at 20 °C
High	200-600 mPa·s^{-1}, 0.5% in 0.5% acetic acid at 20 °C

Experimental design

A greenhouse experiment was conducted, where the experimental unit consisted of a 3 L pot with a 1:1 peat moss-soil substrate and two tomato (*Solanum lycopersicum* L.) plants per pot. Nine treatments were established (Table 2), each with four replicates in a completely randomized design, using the saladette tomato 'SV8579TE' (Bayer, Mexico). The treatments consisted of chitosan at three different molecular weights and three different doses (120, 210, and 300 mg), which were divided into three applications at 10, 25, and 40 d after sowing. The chitosan treatments were applied through irrigation at the corresponding concentration (40, 70, or 100 mg) per application for each molecular weight. A control with four replicates was included, consisting of the same medium used to prepare the chitosan solutions, but without chitosan.

Table 2. Description of chitosan treatments. LMW: Low molecular-weight; MMW: medium molecular-weight; HMW: high molecular-weight; LD: low dose; MD: medium dose; HD: high dose.

Treatment	Molecular weight	Total applied dose
	$mPa \cdot s^{-1}$	mg
LWM LD	5-20	120
LMW MD	5-20	210
LMW HD	5-20	300
MWM LD	20-100	120
MWM MD	20-100	210
MWM HD	20-100	300
HMW LD	200-600	120
HMW MD	200-600	210
HMW HD	200-600	300
Control	Control	0

Physicochemical analyses of water and soil

A physicochemical analysis of the soil and water used in the experiment was conducted in accordance to the criteria established in the Official Mexican Standard (NOM-021-RECNAT-2000), published in the Official Gazette of the Federation. Soil pH was determined using the AS-02 method, and the percentage of organic matter was determined from organic C content following the method described by Walkley and Black (1934). Electrical conductivity was determined using the saturation extract method according to AS-07 of NOM-021-RECNAT-2000. Available P was analyzed following the method described by Olsen et al. (1954), corresponding to AS-10 of NOM-021-RECNAT-2000. Exchangeable cations (K^+, Ca^{2+}, Mg^{2+}, and Na^+) were determined using ammonium acetate extraction according to AS-12 of NOM-021-RECNAT-2000, and total N was determined by the Kjeldahl method following NOM-021-RECNAT-2000.

Evaluation of plant growth parameters

Plant growth parameters were evaluated, including plant, foliar and root dry weight. Plants were cut at the base of the stem and roots were washed with water to remove the substrate. Samples were dried in an oven (Felisa, Feligneo, S.A. de C.V. Mexico) for 72 h at 70 °C. Plant height (cm) was measured using a graduated ruler and stem diameter (mm) with a vernier caliper. Once the foliage was dried, it was weighed using a digital balance and then ground using an electric mill to perform foliar nutrient analysis. The methodology described by Alcántar and Sandoval (1999) was followed, performing a wet digestion using a mixture of sulfuric and perchloric acids. Nitrogen concentration was determined by the Kjeldahl method; total P was measured using a UV spectrophotometer (Genesys 20, Rochester, New York, USA) by the yellow vanadate-molybdate method; K was quantified using a flame photometer (PFP-7, Ansonia, Connecticut, USA) by flame emission photometry; and Ca, Mg, Fe, Mn, Zn, and Cu ions were determined using an atomic absorption spectrophotometer (Varian 50B, Santa Clara, California, USA).

Statistical analysis

Data were analyzed for normality using the Shapiro-Wilk test and for homogeneity of variances using Levene’s test using R (R Foundation for Statistical Computing, Vienna, Austria). Once the assumptions of normality and homoscedasticity were met, an ANOVA was performed. Differences among treatments were examined using Tukey’s test with a significance level of $\alpha = 0.05$ using the Statistical Analysis System (SAS Institute, Cary, North Carolina, USA).

RESULTS

All chitosan treatments showed significant differences compared to the control, indicating increased plant growth (Figure 1). Differences were also observed among the three chitosan molecular weights and the three evaluated doses. A trend was observed in low and medium molecular-weight (LMW and MMW) chitosan, in which higher concentration resulted in greater growth. In contrast, treatment with high molecular weight

(HMW) chitosan, displayed greater plant growth at the low concentration. The highest plant dry weight (13.2 g) occurred with the treatment of HMW chitosan at the lowest dose, representing a 73% increase compared to the control, which showed the lowest value.

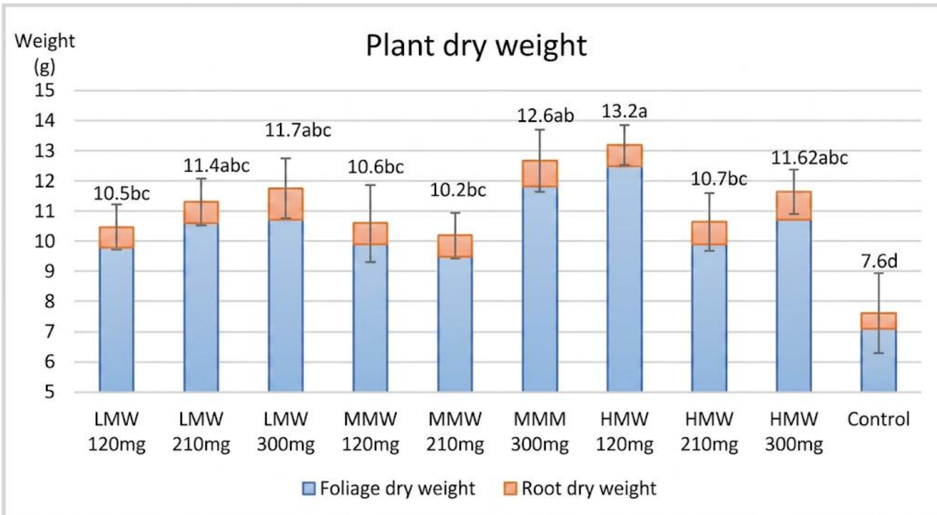

Figure 1. Comparison of mean total tomato plant dry weight at different chitosan concentrations and molecular weights. LMW: Low molecular-weight (5-20 mPa s^{-1}); MMW: medium molecular-weight (20-100 mPa s^{-1}); HMW: high molecular-weight (200-600 mPa s^{-1}). Means with the same letter do not show a significant difference, according to Tukey ($\alpha \leq 0.05$).

Foliar and root dry weight

In foliar dry weight, the results showed the same trend as in tomato plants dry weight. All treatments with chitosan significantly increased foliar dry weight compared to the control, with values ranging from 7.1 g (control) to 12.5 g (HMW low dose) (Figure 2).

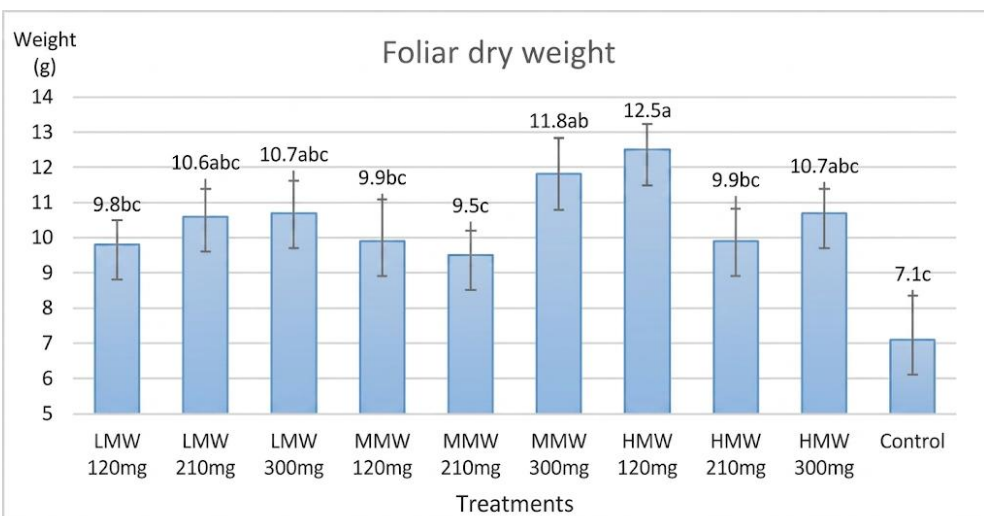

Figure 2. Comparison of mean tomato foliar dry weight at different chitosan concentrations and molecular weights. LMW: Low molecular-weight (5-20 mPa s^{-1}); MMW: medium molecular-weight (20-100 mPa s^{-1}); HMW: high molecular-weight (200-600 mPa s^{-1}). Means with the same letter do not show a significant difference, according to Tukey ($\alpha \leq 0.05$).

In root dry weight, all treatments with chitosan had significantly higher values than the control (Figure 3). A trend was observed in which increasing chitosan concentration, regardless of molecular weight, increased root dry weight. The LMW chitosan applied at the highest dose showed the highest root dry weight, without significant differences to MMW and HMW chitosan at the same concentrations.

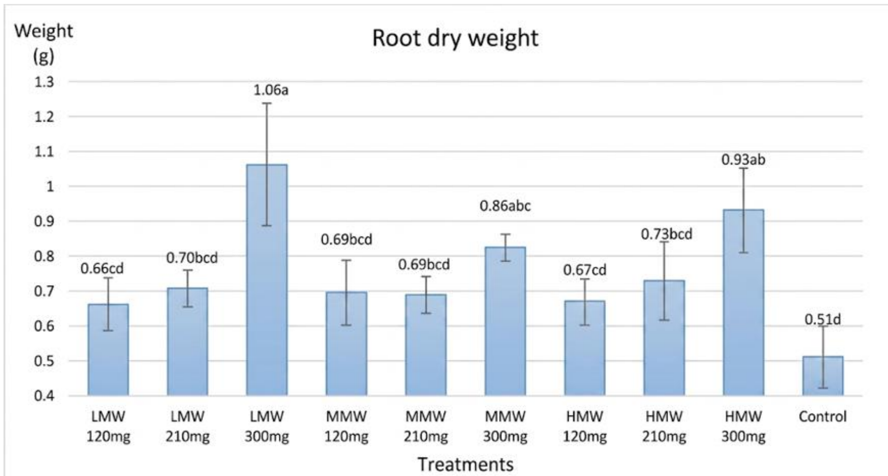

Figure 3. Comparison of tomato root dry weight at different chitosan concentrations and molecular weights. LMW: Low molecular-weight (5-20 mPa s^{-1}); MMW: medium molecular-weight (20-100 mPa s^{-1}); HMW: high molecular-weight (200-600 mPa s^{-1}). Means with the same letter do not show a significant difference, according to Tukey ($\alpha \leq 0.05$).

Plant height and stem diameter

The effect of chitosan on plant height showed an increase between 21% and 35% compared to the control (Figure 4), with differences observed at the same concentration among different molecular weights (Figure 5). The MMW chitosan applied at the highest dose showed the greatest height, although significant difference occurred only with the control but not with other chitosan treatments. Regarding stem diameter, all treatments showed a significant increase compared to the control (Figure 4).

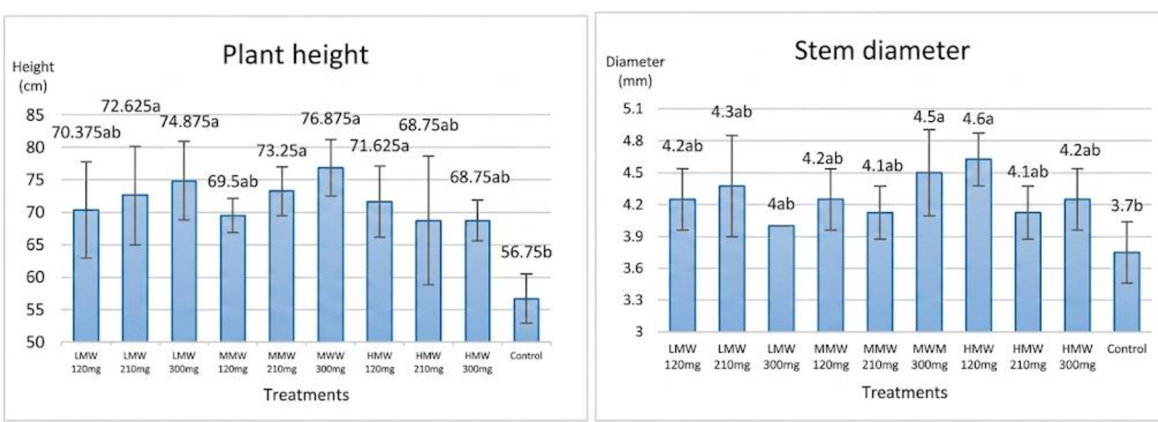

Figure 4. Comparison of mean tomato plant height and stem diameter at different chitosan concentrations and molecular weights. LMW: Low molecular-weight (5-20 mPa s^{-1}); MMW: medium molecular-weight (20-100 mPa s^{-1}); HMW: high molecular-weight (200-600 mPa s^{-1}). Means with the same letter do not show a significant difference, according to Tukey ($\alpha \leq 0.05$).

Figure 5. Comparison of control (water) tomato plant growth to different chitosan treatments. High molecular weight (HMW) (200-600 mPa s^{-1}) chitosan at 300 mg had the biggest growth, but HMW, medium (MMW) and low (LMW) molecular weight chitosan at concentration of 210 mg also showed bigger growth than control.

Nutrient concentration of the foliage

Nutrient analysis showed that chitosan application resulted in increased nutrient uptake, with all treatments presenting higher foliar nutrient concentrations compared to the control (Table 3). The results showed variations in nutrient concentration among the different chitosan molecular weights and concentration treatments. The LMW chitosan showed higher N concentration at all three doses compared to control, with the highest N value observed in the LMW treatment at the highest dose, whereas HMW chitosan at the highest dose showed the highest concentrations of P and K.

Table 3. Nutrient content in the foliage of tomato plants subjected to different chitosan concentrations and molecular weights. LMW: Low molecular-weight (5-20 mPa s^{-1}); MMW: medium molecular-weight (20-100 mPa s^{-1}); HMW: high molecular-weight (200-600 mPa s^{-1}). Means with the same letter do not show a significant difference, according to Tukey (α ≤ 0.05).

Treatments	Nutrients				
	N	P	K	Ca	Mg
	%				
LMW 120 mg	4.17^{ab}	0.40^{ab}	1.65^{bc}	2.12^{a}	1.12^{a}
LMW 210 mg	3.73^{abc}	0.37^{bc}	1.65^{bc}	1.80^{a}	0.94^{a}
LMW 300 mg	4.37^{a}	0.40^{ab}	1.73^{abc}	2.18^{a}	1.04^{a}
MMW 120 mg	3.53^{bc}	0.38^{ab}	1.65^{abc}	1.99^{a}	1.05^{a}
MMW 210 mg	3.51^{c}	0.39^{ab}	1.77^{ab}	2.03^{a}	1.12^{a}
MMW 300 mg	3.55^{bc}	0.38^{abc}	1.67^{abc}	2.01^{a}	1.09^{a}
HMW 120 mg	3.46^{c}	0.39^{abc}	1.55^{cd}	2.17^{a}	0.92^{a}
HMW 210 mg	3.75^{ab}	0.40^{ab}	1.77^{ab}	1.83^{a}	0.83^{a}
HMW 300 mg	3.64^{bc}	0.42^{a}	1.85^{a}	1.79^{a}	1.04^{a}
Control	3.08^{c}	0.39^{c}	1.44^{d}	2.03^{a}	1.10^{a}

DISCUSSION

Chitosan is a sustainable alternative in crop production, as it promotes plant growth and nutrient uptake; however, as a biopolymer, its properties interact differently depending on the length of its chains (Román-Doval et al., 2023). Therefore, it is important to evaluate different chitosan molecular weights to understand their differences when applied through irrigation at different doses in tomato plants.

The increase in plant dry weight following the application of chitosan to the soil is consistent with the findings of Sukanya and Hewavitharana (2023), who applied chitosan via foliar spray at different growth stages and achieved increases of up to 120% compared to the control group, which received no biostimulants. This increase in biomass may be associated with greater production and allocation of photoassimilates, resulting from increased photosynthetic activity and improved C assimilation in the plant (Simkin et al., 2019), these results agree with those reported by Khaengkhan et al. (2024), who observed that chitosan promoted plant growth and improved physiological responses, supporting its use as a sustainable agricultural biostimulant.

The increase in plant height is consistent with the findings of Reyes-Pérez et al. (2020), who applied chitosan to tomato seedlings and observed 20% increase 45 d after planting. This effect may be due to chitosan's ability to induce the synthesis of phytohormones such as auxins and salicylic acid, which are directly related to cell elongation and plant growth. Plant height is a parameter that reflects vegetative growth and plant vigor.

Root development is essential for overall plant performance, as a larger root system increases soil exploration, enhancing the uptake of water and nutrients (Lynch, 2019). A trend was observed in which increasing the applied dose of chitosan increased root development, regardless of the molecular weight applied, although with variations among them. This effect could be attributed to what was reported by De Tender et al. (2020), who indicated that chitosan has the capacity to induce physiological responses in tomato plants, including the stimulation of auxin synthesis, particularly indole-3-acetic acid, which is related to root formation and elongation. Therefore, increasing the applied concentration promotes auxin synthesis and enhances root growth.

The better root development observed with low molecular weight (LMW) chitosan could be due to its higher solubility in water and its ability to infiltrate plant tissues, promoting the activation of hormonal pathways such as auxins and abscisic acid, which favor cell division and root system growth. This is consistent with the results of Sánchez et al. (2021), who reported that LMW chitosan promoted greater root growth than high molecular weight (HMW) chitosan.

Nutrient uptake is a key factor determining plant productivity and physiological status (Nieves-Cordones et al., 2020). Chanratana et al. (2019) reported that chitosan application enhances macronutrient uptake in tomato, increasing N by up to 28%, P by 14%, K by 6% and Ca by 10%, which agrees with the increase in nutrient absorption observed in this study. For Ca and Mg concentrations, nonsignificant differences were found among treatments, possibly because irrigation water contained sufficient concentrations of these nutrients.

Although some studies report that LMW chitosan produces greater effects due to its higher solubility, lower viscosity and greater mobility in plant tissues, allowing a faster plant response (El Hadrami et al., 2010; Malerba and Cerana, 2016; Román-Doval et al., 2023), in this study HMW chitosan showed a greater effect on plant growth in terms of plant dry weight, possibly due to its higher viscosity and stability (Sharp, 2013).

CONCLUSIONS

The application of chitosan at different molecular weights and doses resulted in greater tomato plant development compared to the control, showing its positive effect on tomato, which was dependent on molecular weight and concentration. Using higher doses of chitosan did not necessarily produce a greater response, as its effect depends on its physicochemical properties. High molecular weight chitosan stood out for promoting plant development and when applied through irrigation, its greater stability and persistence may have enhanced its biostimulant effect through interactions in the soil and rhizosphere. From a practical standpoint, the use of high-molecular-weight chitosan offers an advantage over medium- and low-molecular-weight chitosan due to its greater availability and lower production cost, in addition to being a viable alternative for reducing the amount of synthetic fertilizers used, which facilitates its implementation in agricultural production systems.

Author contribution

Conceptualization: A.D.A.B., D.A.C.A., C.M.E.B., J.A.G.L., B.E.L.V. Methodology: A.D.A.B., D.A.C.A., J.A.G.L., B.E.L.V. Analysis: D.A.C.A., J.A.G.L., B.E.L.V. Investigation: A.D.A.B., D.A.C.A., J.A.G.L., B.E.L.V., C.M.E.B. Resources: A.D.A.B., C.M.E.B. Writing-original draft: D.A.C.A. Writing-review & editing: D.A.C.A., A.D.A.B., C.M.E.B. Visualization: A.D.A.B., D.A.C.A., C.M.E.B., J.A.G.L., B.E.L.V. Supervision: A.D.A.B., C.M.E.B. Project administration: A.D.A.B., C.M.E.B. Funding acquisition: A.D.A.B., C.M.E.B. All co-authors reviewed the final version and approved the manuscript before submission.

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