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Influence of exogenously applied k-carrageenan at various concentrations on plant growth, phytochemical content, macronutrients, and essential oils of *Ocimum basilicum*

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Food safety and security are now among the most urgent problems to be resolved as the world's population continues to grow. Intensive agriculture is required to meet the demands of a growing population and guarantee greater agricultural yield. Chemical pesticides and fertilizers are an essential part of intensive farming. Their extensive use accelerates the depletion of other important and minor nutrients, resulting in poor soil fertility and nutritional imbalance. There are serious health and environmental hazards associated with several of these hazardous agricultural chemicals. In context, for the first time, this study represents an innovative experiment exploring the impact of exogenously applied k-carrageenan on plant growth, physiological parameters, phytochemical content, macronutrients, and essential oil percentage in Ocimum basilicum plants. The investigation assessed the effect of varying k-carrageenan levels; 0.30, 0.60, 0.90, and 1.20 mM versus untreated control. The findings revealed that all k-carrageenan treatments significantly enhanced growth indicators compared to the control. The phytochemical analysis demonstrated that foliar application of k-carrageenan, particularly at 1.20 mM, significantly enhanced total chlorophyll, chlorophyll a, chlorophyll b, and total carbohydrate and essential oil percentage compared to the untreated control. O. basilicum essential oils show rich, nuanced flavors with higher levels of Methyl cinnamate, Camphor, transmethyl cinnamate, Eucalyptol, Linalool, and β -Caryophyllene among treatments. Treatment effects were also observed in the macroelements content of Nitrogen (N), phosphorus (P), and potassium (K). k-carrageenan-induced alterations were noted in the contents of essential oil compounds. These results suggest that k-carrageenan can be a growth-promoting agent and significantly enhance essential oil yield, particularly in O. basilicum plants.

Keywords Chlorophyll, Essential oils, K-carrageenan, Macroelements, Ocimum basilicum

Ocimum basilicum, or basil, is a member of the Lamiaceae family, which also includes many other significant plants like lavender, rosemary, and mentha^{1,2}. Compounds extracted from the plant have been employed in medicine, either in their natural form or after chemical modification³. *O. basilicum* exhibits extensive ethnomedicinal applications and its essential oil has demonstrated antiviral, larvicidal, antinociceptive, and antimicrobial properties^{4–8}. Moreover, its essential oil is highly valued in the food industry for its flavoring and aromatic qualities, as well as its unique scent in perfumery^{9,8,10}.

As a rich source of aromatic compounds, essential oil induces various biological processes, indicating its potential as a nematocidal, antibacterial, antifungal, antioxidant, and insect-repellent^{3,10}. O. basilicum accessions

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have been categorized into different chemotypes based on their oil composition and geographical origin. The European type is characterized by methyl chavicol and linalool as the primary oil components, while most tropical chemotypes contain methyl cinnamate. Reunion *O. basilicum*, conversely, has a high concentration of methyl chavicol, while chemotypes in North Africa, Russia, Eastern Europe, and parts of Asia are rich in eugenol^{11,12}.

In modern horticulture crop production, emphasis is placed on product quality, safety, and yield improvement. Enhancing the production of bioactive ingredients is particularly crucial in medicinal herbs, given the diverse biological functions of secondary metabolites⁶.

Carrageenans are sulfated linear polysaccharides isolated from various marine red algae¹³. These ingredients consist of a linear chain of D-galactose remains connected by glycosidic bonds replaced by ester sulphonic groups present in every two repeating galactose units. Carrageenans are categorized into three types based on sulfate groups that are present in each disaccharide unit: kappa (k)-carrageenan has 1, iota (k)-carrageenan has 2, and lambda (k)-carrageenan has 3 sulfate groups^{14,15}.

Enzymes such as pyruvate dehydrogenase, glutamate dehydrogenase, inosine mono-phosphate dehydrogenase, isocitrate dehydrogenase, and glucose 6-phosphate dehydrogenase demonstrate increased activity in the presence of rubisco¹⁶. Furthermore, carrageenan serves as an elicitor to activate defense mechanisms against plant infections¹⁶. It primarily promotes plant development by controlling various metabolic pathways. Such as photosynthesis, cell division, purine and pyrimidine biosynthesis, and sulfur and nitrogen assimilation. Additionally, they trigger plant defensive responses against Abiotic stress like drought, salinity, and temperature^{17–22} by modulating several defense pathways, including ethylene, jasmonate, and salicylate signaling pathways^{15,23,17}. Treatments with carrageenan have been shown to significantly increase plant height, trunk diameter, chlorophyll content, and net photosynthesis in various plant species^{24,25,16}.

The present study aimed to determine the optimal treatment for enhancing plant development and phytochemical production as well as improving the quality and quantity of *O. basilicum* essential oils. While previous research has focused on increasing secondary metabolites in *O. basilicum*, this study is the first to investigate the influence of k-carrageenan on *O. basilicum* growth, phytochemical content, macronutrients, and essential oil. The research shows that k-carrageenan significantly improves *O. basilicum* growth parameters, nutrient content, and essential oil yield. It enhances plant health and productivity, particularly in culinary and medicinal contexts. The study also reveals that higher concentrations of k-carrageenan, a novel growth enhancer, offers innovative agricultural practices, enhancing crop yields and quality. It contributes to sustainable agriculture by improving plant growth and nutrient uptake, reducing chemical fertilizer use, and enhancing soil health. The study on k-carrageenan application in *O. basilicum* has identified gaps in its long-term effects, molecular understanding of its effects on plant growth and nutrient uptake, its broad applicability in agriculture, and its environmental impact, necessitating further research to understand its potential ecological consequences.

Materials and methods

Plant material and growing conditions

This study was carried out at the Ornamental and Medicinal Plants Farm, Department of Horticulture, Ain Shams University, Egypt. Uniform and healthy *O. basilicum* transplants, one-month-old and 15 cm in height, were sourced from a nursery in Giza, Egypt. The experimental design was a completely randomized design with 3 replicates. All pots (75 pots) were handed out by five k-carrageenan applications × 5 pots × 3 replicates. Each transplant was placed in a pot (0.35-m diameter) filled with a mixture of peat moss and sand (1:1) during the first week of March 2023. Irrigation was conducted regularly, 2 to 3 times per week. Fertilization was carried out using approximately half-strength Hoagland's nutrition solution, applied once every ten days. After one month of cultivation, all experimental pots (75 pots) were located into five groups in the third week of April to receive exogenous application of k-carrageenan (Sigma Aldrich, St. Louis, MO, USA) at concentrations of zero (distilled water as a control), 0.30, 0.60, 0.90 and 1.20 mM. In the third week of May, the plants were harvested to examine their growth characteristics and plant chemical components.

Measured parameters

When the plants achieved the best vegetative growth stage (at 75 days after planting) two pots from each replication were collected to assess the growth parameters. Shoot fresh weight of the whole plant (leaves and stem, g) was evaluated after sampling, while shoot dry weight (g) was identified by drying the samples in an airforced ventilation oven (Binder FP056-230 V, Germany) at 105 °C. To determine the total content of nitrogen, phosphorus, and potassium, the plant samples were oven-dried at 70 °C and then wet-digested using a mix of H_2SO_4 and H_2O_2 as outlined by Cottenie et al.²⁶. Total N content was estimated by the micro-Kjeldahl method utilizing 40% NaOH and 5% boric acid following AOAC²⁷. Total K and P were estimated by employing ICP Mass Spectrometry (ICP-MS, China)²⁸. Total carbohydrates were determined as g/100 g dry weight of herb as outlined by AOAC (2005)²⁹. Chlorophyll-a and Chlorophyll-b (mg/g FW) were determined following Sumanta et al.³⁰.

Steam distillation was utilized to separate the volatile oil from the dried vegetative parts of the *O. basilicum* plant, including the leaves and stem (both treated and untreated plants), employing a Clevenger glass apparatus. One hundred grams of the dried whole *O. basilicum* plant (leaves and stem) was separated in the steam distillation apparatus for 3 h. The *O. basilicum* oil was separated from the water and stored in dark glass bottles at 4 °C until the active ingredients were isolated and analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) (GCMS 2400, USA). The oil percentage was calculated as grams of oil per 100 g of dry whole *O. basilicum* plant. After evaporation, the separated oil residue was thawed into 3 mL of ethyl acetate, and then 1 mL was transferred to a GC vial for GC/MS analysis. GC-MS was utilized to analyze the various components of *O. basilicum* volatile oil, including those present in modest quantities, as well as the main components of *O. basilicum* essential oil.

Identification of components was based on a comparison of their mass spectra and retention times with those of authentic compounds, computer matching the WILEY and NIST libraries, and comparison of the fragmentation pattern of the mass spectral data with those reported in the literature. The analysis was conducted using a GC system (Agilent Technologies 7890 A) coupled with a mass-selective detector (MSD, Agilent 7000) and equipped with a polar Agilent HP-5ms (5%-phenyl methyl polysiloxane) capillary column (30 m × 0.25 mm i.d. and 0.25 µm film thickness). Helium was used as the carrier gas with a linear velocity of 1 mL/min. The injector and detector temperatures were set at 200 °C and 250 °C, in the same order, with a sample volume injected with 1 μ L. The MS operating parameters were as follows: ionization potential 70 eV, interface temperature 250 °C, and acquisition mass range 50–800³¹.

Statistical analysis

Statistical analyses were applied utilizing R software version 4.1.1 (https://www.r-project.org/). The Tukey's range test at a significance level of $P \le 0.05$ was calculated to assess the significant differences among the studied treatments. The heatmap was generated using the Rcolor-Brewer package within the R software environment³².

Results

Growth parameters

The impact of k-carrageenan on *O. basilicum* growth parameters is depicted in Fig. 1. The data suggest that the application of exogenous k-carrageenan led to an increase in all growth parameters (plant height, stem diameter, branch number, fresh weight, and dry weight) compared to untreated control plants. The lowest values of plant growth parameters were observed in the untreated control group, while the highest values were recorded in plants treated with 1.20 mM of k-carrageenan, with plant height reaching 83.73 cm, stem diameter measuring 2.35 cm, branch number totaling 40.20, fresh weight amounting to 363.07 g, and dry weight reaching 115.0 g compared to the other concentrations tested.

Chlorophyll A, B, total chlorophyll, and total carbohydrates

The results of chlorophyll a, b, total chlorophyll, and total carbohydrates are presented in Fig. 2. The application of k-carrageenan led to a significant enhancement in chlorophyll a, b, and total chlorophyll. The lowest values of 24.20, 21.95, and 46.15 were observed under untreated control conditions, respectively. Conversely, the data revealed that treatment with 1.20 mM k-carrageenan recorded the maximum values of 46.89, 44.09, and 90.98 for chlorophyll a, b, and total chlorophyll, respectively. Total carbohydrates were reduced in nontreated plants, while they increased and reached the highest value of 9.57 with the highest level of k-carrageenan.

Content of macroelements

The N, P, and K content in *O. basilicum* was influenced by k-carrageenan, as depicted in Fig. 3. Overall, the application of k-carrageenan led to a significant increase in the levels of N, P, and K. Moreover, escalating the concentration of k-carrageenan from 0.30 to 1.20 mM resulted in a significant rise in the percentages of N, P, and K. The highest percentages of N, P, and K were noticed with the application of 1.20 mM k-carrageenan (3.45, 0.56, and 2.52%, respectively), compared to all other concentrations and untreated control.

Oil percentage

The results illustrated in Fig. 4 showed that the oil percentage was at its minimum under untreated control. However, the oil percentage reached its maximum (0.39%) at a high level of k-carrageenan 1.20 mM followed by 0.90 and 0.60 mM.

Association among applied k-carrageenan concentrations and evaluated parameters

Exploring the association between applied treatments and the parameters studied is pivotal for providing valuable insights. Utilizing the heatmap and hierarchical clustering, studied parameters including plant growth, phytochemical content, macronutrients, and essential oil percentage of *O. basilicum* plants, grouped the applied treatments into distinct clusters (Fig. 5). It was observed that the applied k-carrageenan with a concentration of 1.20 mM, followed by 0.90, 0.60, and 0.30 mM, demonstrated favorable performance across all evaluated parameters. Conversely, the untreated control exhibited unfavorable performance (Fig. 5).

Impact of k-carrageenan on O. basilicum essential oils

The application of k-carrageenan influenced the content of essential oils in *O. basilicum* plants. Table 1 presents the results of GC-MS analysis of essential oils. Following cultivation under various concentrations of k-carrageenan, 24 chemical compounds were identified in *O. basilicum*. These compounds were detected across different k-carrageenan concentrations, whereas the lowest number of essential oil compounds (19) was observed in the untreated plants with k-carrageenan (Fig. 6). Additionally, the data revealed a significant and noticeable increase in the percentage of essential oil in treated plants with k-carrageenan at all concentrations (0.30, 0.60, 0.90, and 1.20 mM) compared to untreated control. k-carrageenan treatments led to elevated values of essential oil components compared to the control. Compounds such as α -Pinene, Camphene, β -Pinene, D-Limonene, Eucalyptol, β -Ocimene, γ -Terpinene, Linalool, (+)-Camphor, Terpinen-4-ol, α -Terpineol, trans-Methyl cinnamate, Methyl cinnamate, β -Caryophyllene, β -Copaene, Bicyclogermacrene, δ -Guaiene, γ -Cadinene, and Tau-Cadinol acetate were detected in plants both with and without k-carrageenan treatments. Sabinene hydrate and Eugenol were detected in plants treated with 1.20 mM of k-carrageenan. Humulene was recorded only in the treated plants with k-carrageenan (Table 1).



Fig. 1. Influence of k-carrageenan treatment on plant height (**A**), Stem diameter (**B**), Number of branches (**C**), Fresh weight (**D**), and Dry weight (**E**) of *O. basilicum*. The bars represent standard deviation (SD) values, and different letters indicate significant differences, as determined by Tukey's Studentized Range (HSD) Test (p < 0.05).

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Discussion

This study reported for the first time the usage of k-carrageenan to promote the biosynthesis of active ingredient substances in *O. basilicum*. *O. basilicum* plants treated with k-carrageenan exhibited a significant increase in all growth parameters; plant height, number of branches, stem diameter, and fresh and dry weight, compared to untreated plants. Overall, the application of 1.20 mM k-carrageenan resulted in substantial improvements across all growth parameters compared to the untreated control group by 1.5, 2.35, 1.48, 1.73, and 1.64-folds increases for plant height, stem diameter, total branch number, fresh weight, and dry weight, respectively. All applied k-carrageenan concentrations resulted in a significant enhancement in chlorophyll a, b, total chlorophyll, and total carbohydrate content in *O. basilicum* plants compared to untreated plants by 1.94, 2.0, 1.97, and 2.5, respectively. In addition, N, P, and K were noticed to increase by 3.45, 0.56, and 2.52% with 1.46, 2.8, and 1.94-folds, respectively compared to control plants. This suggests that k-carrageenan acts as an effective biostimulant, promoting better growth, structural integrity, and biomass accumulation in *O. basilicum* plants.



Fig. 2. Influence of k-carrageenan treatment on chlorophyll a (**A**), Chlorophyll b (**B**), Chlorophyll a (**C**), Total chlorophyll (**D**), and Total carbohydrate (**E**) of *O. basilicum*. The bars represent standard deviation (SD) values, and different letters indicate significant differences, as determined by Tukey's Studentized Range (HSD) Test (p < 0.05).



Fig. 3. Influence of k-carrageenan treatment on content of Nitrogen (A), Phosphorus (B), and Potassium (C) of *O. basilicum*. The bars represent standard deviation (SD) values, and different letters indicate significant differences, as determined by Tukey's Studentized Range (HSD) Test (p < 0.05).



Fig. 4. Influence of k-carrageenan treatment on yield of *O. basilicum* essential oil (%) in the dried vegetative parts (leaves and stem). The bars represent standard deviation (SD) values, and different letters indicate significant differences, as determined by Tukey's Studentized Range (HSD) Test (p < 0.05).

The untreated control group consistently showed the lowest values across all parameters, highlighting the beneficial effects of k-carrageenan treatment. This may be attributed to the high sulfur content in k-carrageenan. Sulfur plays a crucial function in plant metabolism as it is required for the synthesis of plant proteins, vitamins, phytochelatins, thiamine, phenolic compounds, flavonoids, coenzyme A, glutathione, amino acids, and enzymes as well as its necessity for chlorophyll synthesis, which enhances the photosynthesis process, leading to increased carbohydrate accumulation^{33–36}. Sulfur also stimulates nodulation in legumes and aids in the development and activation of specific enzymes and vitamins, ultimately contributing to increased plant growth and productivity. Since sulfur is water-soluble, it is readily absorbed by plant leaves from k-carrageenan extract, which is soluble³⁴. This finding aligns with previous studies by Naeem et al.³⁷, who noticed that spraying k-carrageenan increased productivity in Catharanthus roseus and significantly increased its performance. Also, the enhancement in leaf area resulting from k-carrageenan treatment enhanced solar harvesting, carbon dioxide (CO₂) consumption, and chlorophyll content, thereby improving photosynthesis rates and leading to the accumulation of plant dry matter. The k-carrageenan application significantly boosted nitrogen, phosphorus, and potassium levels in O. basilicum plants. Increasing the concentration from 0.30 to 1.20 mM led to higher macroelements percentage, with the highest values at 1.20 mM being 3.45% nitrogen, 0.56% phosphorus, and 2.52% potassium, exceeding all other concentrations and the untreated control. El-Beltagi et al.³⁸ reported that the use of k-carrageenan at concentrations of 400, 600, and 800 ppm resulted in a substantial increase in all growth parameters, including plant height, number of branches, and stem diameter. Furthermore, Mousavi et al.³⁹ found that treatments with 1 g/L k-carrageenan increased O. basilicum shoot length and leaf area. This treatment stimulated O. basilicum growth by enhancing shoot length and leaf area, as well as increasing phenylalanine ammonia-lyase activity, phenolic ingredients, lignin concentrations, and antioxidant activity. Consequently, k-carrageenan can be considered to activate the phenylpropanoid pathway due to its high sulfur content, which is a type of linear sulfated polysaccharide⁴⁰. Sulfur interacts with nearly every major macronutrient, secondary nutrient, and micronutrient, altering nutrient uptake and usage, which can impact crop growth and production. Sulfur and nitrogen are strongly associated, with sulfur increasing the efficiency of plant nitrogen absorption. Sulfur's involvement in nitrate conversion to amino acids also links it to nitrogen. Crops with high nitrogen requirements usually have high sulfur requirements³³. Sulfur and phosphorus are both essential nutrients absorbed by plants from the soil in their anionic state, and plants require equivalent amounts of these components⁴¹. The role of sulfur and potassium in increasing agricultural output and increasing crop quality is well-established^{42,43}, with increased potassium absorption resulting in more blooms in plants. The oil percentage in O. basilicum was lowest in the untreated control and reached a maximum of 0.39% with 1.20 mM k-carrageenan, with 1.56- a 1.56-fold increase compared to the control treatment. The quantity and quality of essential oils components in plants can be affected by various environmental factors and nutrition. For instance, it has been reported that the essential oil of O. basilicum plants changes with nutrition⁴⁴. k-carrageenan is an important biostimulant that



Fig. 5. Heatmap for the evaluated parameters of *O. basilicum* plants treated with different concentrations of k-carrageenan. Parameters. Red color and blue color reveal high and low values for the corresponding parameters, respectively.

affects essential oil biosynthesis, with enhanced essential oils content 45,46 . The use of k-carrageenan significantly increased the essential oil output and the content of the key ingredients of the essential oils compared to the control observed in the fennel study⁴⁵. Essential oils of O. basilicum exhibited nuanced and rich flavor notes, with higher levels of Methyl cinnamate, representing between 45.24 and 49.9% in the oil among treatments. Intermediate proportions of Camphor (10.63 to 11.56%), trans-methyl cinnamate (7.4 to 7.92%), Eucalyptol (7.16 to 7.89%), Linalool (4.32 to 6.18%), and β -Caryophyllene (3.34 to 3.63%) were also observed among all treatments. The presence of Methyl cinnamate as the main component in our species is consistent with previous research on different O. basilicum species^{1,47}. Camphor was detected in this study at the highest level under 0.30 mM k-carrageenan, as reported in previous studies^{1,48}. Some compounds were not found in the untreated plants but appeared under k-carrageenan treatment. Sabinene and Terpinolene were recorded in plants treated with 0.60 mM k-carrageenan compared to other treatments. Additionally, Sabinene⁴⁹ and Terpinolene⁵⁰ have been reported in O. basilicum. k-carrageenan at 0.90 mM enhanced more compounds compared to the control, such as Tran sabinene hydrate⁵¹ and Eugenol⁵². Furthermore, Humulene was observed in k-carrageenan-treated plants⁵³. The essential oil percentage increased with k-carrageenan application due to a significant increase in dry and fresh matter yields. A similar effect was found in Mungbam⁵⁴ and peachy⁵⁵, as well as an increase in essential oils in fennel⁴⁵.

Conclusions

The investigation into the effects of exogenously applied k-carrageenan on *O. basilicum* has yielded significant insights into its potential as a growth-promoting agent. The study demonstrates that varying concentrations of k-carrageenan can enhance key growth parameters, improve phytochemical content, and increase essential oil yield in basil plants. These findings underscore the importance of k-carrageenan not only as a biostimulant that can lead to higher agricultural productivity but also as a sustainable alternative to synthetic fertilizers. The research highlights the potential economic benefits for farmers, particularly in the context of increasing market demand for high-quality and organic herbs. Additionally, it opens avenues for further exploration into the mechanisms by which k-carrageenan influences plant physiology, nutrient uptake, and overall health. Addressing existing research gaps, such as the long-term effects and broader applicability of k-carrageenan across different crops, will be essential for developing comprehensive agricultural practices that are both effective and sustainable. In conclusion, this study contributes valuable knowledge to the field of horticulture and agricultural science, advocating for the integration of natural biostimulants like k-carrageenan in modern farming practices.

			K-carrageenan concentrations				
Peak	RT	Component name	Untreated control	0.30 mM	0.60 mM	0.90 mM	1.20 mM
1	6.334	α-Pinene	0.53	0.59	0.64	0.66	0.65
2	6.698	Camphene	0.85	0.95	1.07	1.06	1.04
3	7.298	Sabinene				0.40	
4	7.391	β-Pinene	0.68	0.82	0.83	0.83	0.79
5	8.730	D-Limonene	1.55	1.73	1.83	1.81	1.79
6	8.817	Eucalyptol	7.44	7.8	7.89	7.8	7.16
7	9.233	β-Ocimene	0.53	0.59	0.54	0.65	0.58
8	9.545	γ-Terpinene	0.67	0.66	0.7	0.76	0.71
9	9.811	Trans-Sabinene hydrate					0.53
10	10.371	Terpinolene				0.47	
11	10.683	Linalool	4.84	4.32	4.84	5.33	6.18
12	11.988	(+)-Camphor	11.28	10.95	11.54	11.56	10.63
13	12.900	Terpinen-4-ol	2.58	2.43	2.56	2.78	2.69
14	13.270	α-Terpineol	0.65	0.69	0.69	0.73	0.70
15	16.394	Trans-Methyl cinnamate	7.77	7.92	7.77	7.4	7.50
16	17.781	Eugenol					0.67
17	18.491	Methyl cinnamate	49.9	48.36	46.76	46.27	45.24
18	19.432	β-Caryophyllene	3.38	3.44	3.63	3.38	3.34
19	20.290	Humulene		0.68	0.74	0.66	0.69
20	20.969	β-Copaene	2.26	2.38	2.4	2.41	2.84
21	21.344	Bicyclogermacrene	1.08	1.15	1.18	1.24	1.56
22	21.558	δ-Guaiene	0.88	0.96	0.97	0.88	1.08
23	21.754	γ-Cadinene	0.77	0.83	0.83	0.73	0.87
24	24.728	Tau-Cadinol acetate	2.35	2.76	2.58	2.2	2.74

Table 1. Influence of k-carrageenan treatments on oil components in O. basilicum plants.

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As the agricultural sector continues to face challenges related to food security and environmental sustainability, research of this nature is crucial for fostering innovative solutions that benefit both farmers and the ecosystem.





Data availability

Data sets generated during the current study are available from the corresponding author upon reasonable request.

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Competing interests

The authors declare no competing interests.

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Additional information

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