

Growth Parameters and Oxidative Stress Indicators in Lemon Balm Grown Under Light-Emitting Diodes and Greenhouse Lighting

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ABSTRACT

Recent advancements in LED technology have created new opportunities for cultivating medicinal plants in controlled environments. This study compares the growth of two lemon balm genotypes under four different LED light treatments with standard greenhouse lighting conditions. The photoperiod was set at 18 hours of light and 6 hours of darkness, with a photosynthetic photon flux density (PPFD) of approximately 300 $\mu\text{mol}/\text{m}^2/\text{s}$ and an average temperature of 25 to 35 °C. The experiments were conducted using a completely randomized design, consisting of five treatments of light quality and three replications. The primary variables assessed included shoot fresh weight, shoot dry weight, chlorophyll content, carotenoid levels, and the concentration of oxidative stress marker metabolites, specifically hydrogen peroxide (H_2O_2) and malondialdehyde (MDA). The results showed that red+blue LED lighting increased shoot fresh weight, shoot dry weight, chlorophyll, and carotenoids while minimizing the levels of H_2O_2 and MDA, indicating reduced oxidative stress. These findings demonstrate that red+blue LED lighting is optimal for promoting growth and antioxidant capacity, thereby enhancing the quality of lemon balm plantlets compared to those grown under greenhouse conditions.

Keywords: LEDs, Markers of oxidative stress, *Melissa officinalis*, Pigments

Abbreviations

Car: carotenoids, Chl: Chlorophyll, H_2O_2 : Hydrogen Peroxide, LED: Light emitting diodes, MDA: Malondialdehyde, PPFD: Photosynthetic Photon Flux Density, ROS: Reactive Oxygen Species, SDW: shoot dry weight, SFW: shoot fresh weight.

INTRODUCTION

Plant-derived antioxidants are crucial for human health, offering protection against various diseases. In plants, these antioxidants mitigate reactive oxygen species (ROS) created under stress by converting harmful compounds, such as hydrogen peroxide and lipid hydroperoxides, into benign molecules like water and lipid hydroxides [1-3].

Light quality significantly influences the biosynthesis of these secondary metabolites [4]. Previously, controlled cultivation was limited by light conditions, high-pressure sodium lamps, and fluorescent lights, which lacked stability and efficiency for promoting photosynthesis, for example [5]. Light emitting diode (LED) technology provides the advantage of customizable wavelengths and intensities, closely resembling natural light. This capability significantly enhances plant growth and metabolism [6].

Plants primarily absorb red and blue light with ~90% efficiency. Red light promotes photosynthetic apparatus development, starch accumulation, and chloroplast activity [7], while blue light enhances chlorophyll-binding proteins and photosynthetic activity [8]. Combining these wavelengths improves photosynthesis and biomass production [9,10].

Among medicinal plants, *Melissa officinalis* (lemon balm), a perennial herb of the Lamiaceae family, has been valued for its anti-inflammatory, antiviral, and antibacterial properties [11, 12]. This study investigates the impacts of red+blue, red, blue, and white LEDs versus greenhouse lighting on growth parameters, pigment composition, and ROS production in *M. officinalis*.

MATERIAL AND METHODS

Plant Materials and Treatments

Lemon balm rhizomes are procured from agricultural plots located in the provinces of Isfahan and Ilam, Iran. Initially, homogenous plantlets were cultivated within a regulated greenhouse environment, maintaining temperatures between 25 and 35 °C and adhering to a 16/8 hours light/dark regimen. One month later of cultivation, plantlets displaying 3–4 leaves were subsequently transferred to incubators containing LEDs (ASM51 apparatus, Arvin Tajhiz Espadana Co., Iran), which were calibrated to sustain a 16/8 photoperiod at approximately 300 $\mu\text{mol}/\text{m}^2/\text{s}$ of photosynthetic photon flux density (PPFD). The experimental treatments included white LED illumination

(380–760 nm), red LED illumination (650 nm), blue LED illumination (460 nm), and a mixture of red and blue LED illumination at a ratio of 70:30. The experiments were conducted as a mixed analysis in a completely randomized design with three replications.

Plant Growth Measurements

The weights of both fresh and desiccated shoots, as well as the number of leaves, were carefully measured. The dry weights were determined following a standardized procedure, wherein the samples were oven-dried at 65 °C for 48 hours.

Chlorophyll and Carotenoid Analysis

Chlorophyll (Chl) and carotenoids (car) were extracted from leaf samples weighing 0.1 g utilizing 80% acetone at a temperature of 4 °C, and their concentrations were assessed via the methodologies established by Arnon [13] and Lichtenthaler [14], respectively. The samples underwent centrifugation at 5000 ×g for 3 minutes at 4 °C. The resultant supernatant was utilized to measure the absorbance of chl a, chl b, and car, as determined by a spectrophotometer (JENWAY 6300), at the specific wavelengths of 663, 645, and 470 nm.

Hydrogen Peroxide (H₂O₂) and Malondialdehyde (MDA) Measurements

H₂O₂ content was assessed following Alexieva *et al.* [15], using a reaction mixture of TCA, K₂HPO₄, and KI, with absorbance measured at 390 nm. MDA levels were determined via a thiobarbituric acid assay, with fluorometric detection at 532 nm [16].

Statistical Analysis

Statistical analysis of the data was executed employing ANOVA in conjunction with the SAS software (ver. 8.0). The means were contrasted utilizing LSD tests at a 95% confidence level.

RESULTS

Impression of Illumination of LED on Growth Parameters

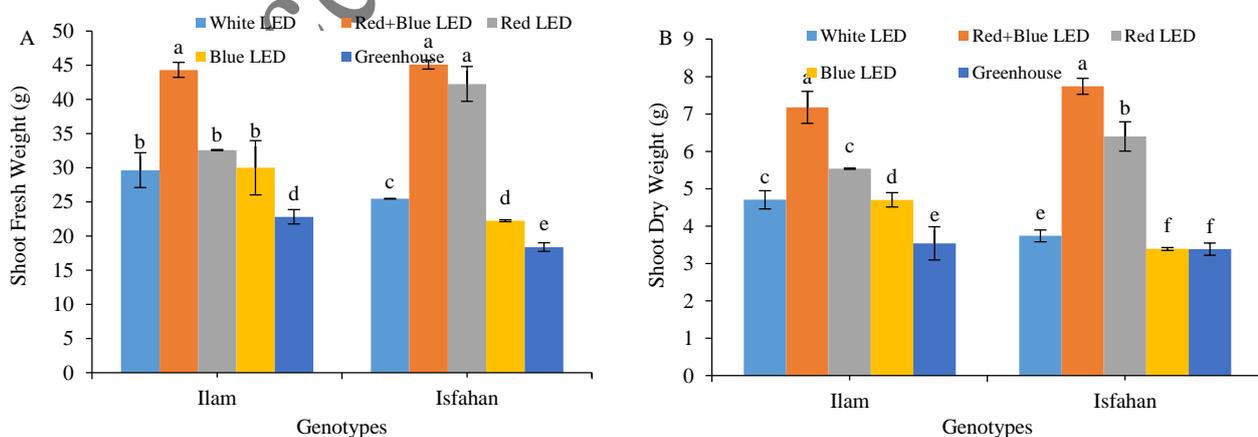
The statistical evaluation of variance concerning shoot fresh weight (SFW), shoot dry weight (SDW), and leaf count is delineated in Table 1. Both illumination sources and plant genotypes had a statistically significant impact on all growth parameters evaluated. Plantlets cultivated under LED illumination exhibited superior SFW and SDW as well as leaf counts in comparison to those grown under conventional greenhouse lighting.

Table 1 The statistical evaluation of variance (mean squares) of light, genotype, and their interactions on SFW, SDW, leaf number, Chl a, Chl b, total Chl, Chl a/b, carotenoid, H₂O₂ and MDA.

Variable	Df	SFW	SDW	Leaf number	Chl a	Chl b	Total Chl	Chla/b	carotenoid	H ₂ O ₂	MDA
Genotype	1	487.14 **	34.32 ns	25317.07 **	0.06 ns	0.57 ns	0.31 **	0.22 **	0.00011 *	1741144.9 **	22239.85 **
Light	4	596.70 **	174.99 **	75676.36 **	0.08 ns	2.49 **	4.76 **	0.35 **	0.00008 **	1625177.6 **	9031.88 **
Genotype × Light	4	50.97 **	5.96 **	22707.42 **	0.62 **	2.14 **	3.22 **	0.07 **	0.00044 **	1344443.3 **	6392.34 **
Error	20										
CV (%)		5.08	15.40	7.20	20.30	18.49	7.08	14.29	5.53	0.71	10.61

* and ** denote statistically significant differences at $P \leq 0.05$ and 0.01 respectively, while ns signifies no statistical significance.

As illustrated in Fig. 1A, the Ilam genotype displayed the most substantial fresh weight under the mixture of red and blue illumination (red+blue), while the genotype of Isfahan demonstrated comparably favorable outcomes under both the mixture of red and blue illumination and red LED conditions. In the case of the Ilam genotype, the employment of the mixture of red and blue LED illumination resulted in a fresh weight augmentation of up to 51% relative to greenhouse lighting, whereas the Isfahan genotype experienced increases of 2.45-fold and 2.29-fold respectively under mixture of red and blue illumination and red LED bulbs. Similarly, shoot dry weight attained its apex under the mixture of red and blue illumination and red LED bulbs for both genotypes, with increases of 62% and 60% for Ilam and 50% for Isfahan when contrasted with greenhouse conditions (Fig. 1B). The leaf count was maximized under mixture of red and blue illumination, with red LED bulbs following closely, for both genotypes (Fig. 1C).



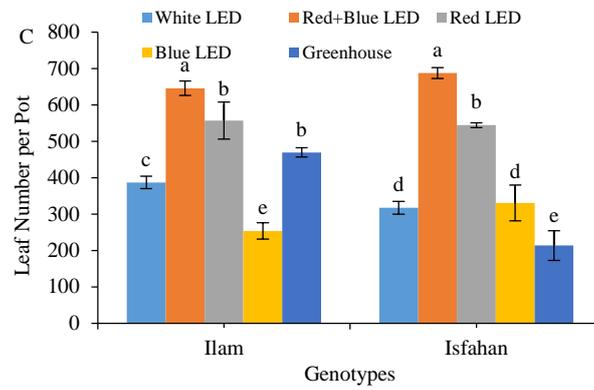


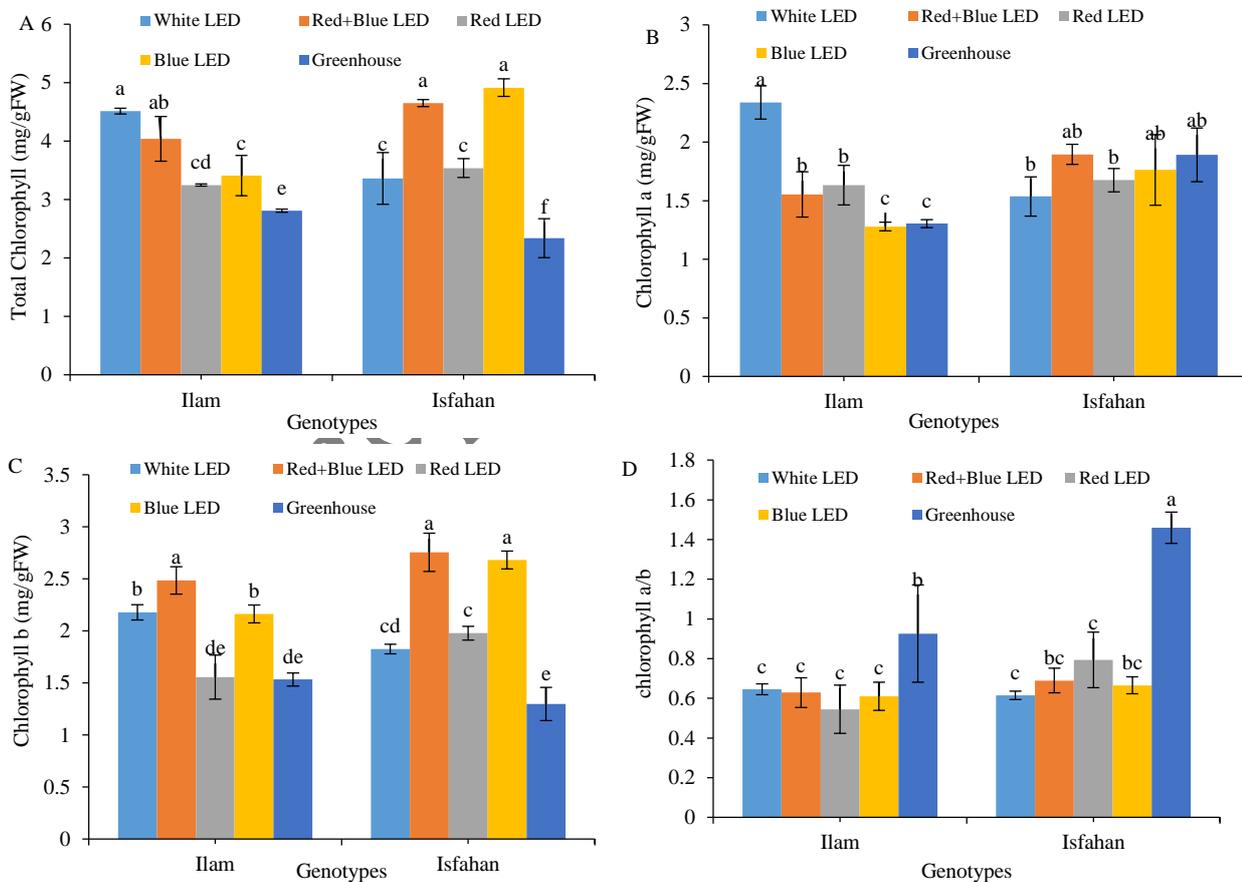
Fig. 1 A-C. Growth parameters in lemon balm's of two genotypes cultivated under five distinct illuminations. Data represent mean values \pm SE. Error bars represent the standard error. Differentiated letters signify significant differences ($P \leq 0.01$).

Impression of Illumination of LED on Chlorophyll and Carotenoid Accumulation

Genotype had a significant effect on total chlorophyll content, the chlorophyll a/b ratio, and carotenoids at 0.01 and 0.05 probability levels, respectively. However, chl a and chl b were not significantly affected by genotype. The light treatment significantly influenced all pigments except chlorophyll a, which showed no significant differences.

Within the Ilam genotype, plantlets nurtured under white LEDs exhibited the highest concentration of chl a, whereas the Isfahan genotype revealed no statistically significant differences in chl a levels across treatments and the control. Regarding chl b, the Ilam genotype recorded the peak levels under the mixture of red and blue LEDs, followed by white and blue LEDs. Conversely, the Isfahan genotype attained the greatest chl b concentration under the mixture of red and blue LEDs and blue LEDs. The total chlorophyll accumulation was at its zenith under white LEDs in the Ilam genotype, followed by the mixture of red and blue LEDs; in contrast, the Isfahan genotype achieved the highest total chlorophyll levels under the mixture of red and blue LEDs and blue LEDs.

The chlorophyll a/b ratio declined significantly under LED treatments in both genotypes, with the highest ratios observed under greenhouse lighting. Carotenoid levels were elevated in the mixture of red and blue LEDs and red LEDs in comparison to greenhouse conditions in the genotype of Ilam, with red+blue LEDs producing the highest carotenoid accumulation in the Isfahan genotype.



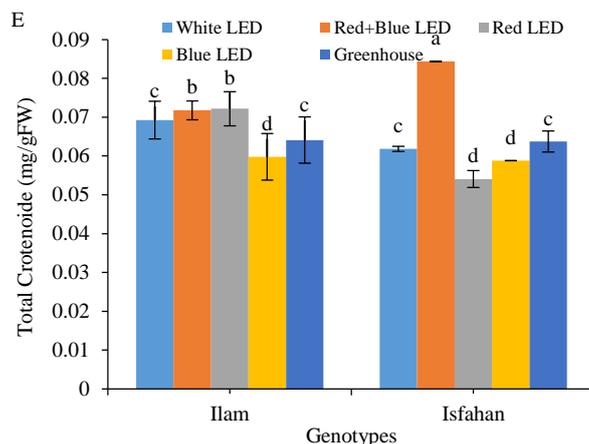


Fig. 2 A-E. Pigments in the lemon balm^s of two genotypes cultivated under five distinct illuminations. Data represent mean values \pm SE. Error bars represent the standard error. Differentiated letters signify significant differences ($P \leq 0.01$).

Impression of Illumination of LED on Hydrogen Peroxide

Variance analysis revealed that both genotype and light treatments significantly influenced H_2O_2 levels at a 0.01 probability level (Table 1). In the Ilam genotype, greenhouse lighting and red LEDs resulted in higher H_2O_2 production compared to other treatments. For the Isfahan genotype, the highest H_2O_2 levels were observed under white LEDs. In two genotypes, the mixture of red and blue illuminations created the lowest H_2O_2 levels (Fig. 3).

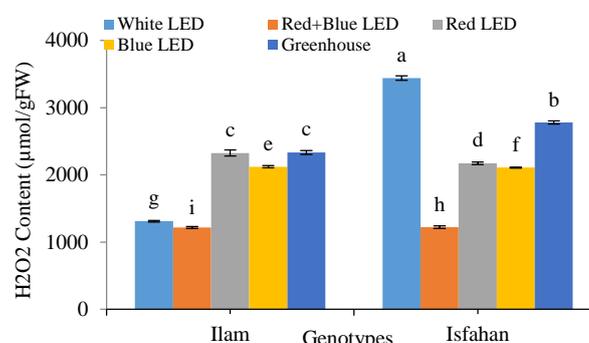


Fig. 3 H_2O_2 content in the lemon balm^s of two genotypes cultivated under five distinct illuminations. Data represent mean values \pm SE. Error bars represent the standard error. Differentiated letters signify significant differences ($P \leq 0.01$).

Impression of Illumination of LED on Malondialdehyde

The interaction between genotype and light treatment significantly affected MDA levels at a 0.01 probability level. In the Ilam genotype, the lowest MDA levels and membrane damage were observed under the mixture of red and blue illuminations, while the zenith levels occurred under red LED bulbs. Conversely, in the genotype of Isfahan, both the mixture of red and blue illuminations and red LED bulbs resulted in the lowest MDA levels, with the highest levels observed under white LED treatment (Fig. 4).

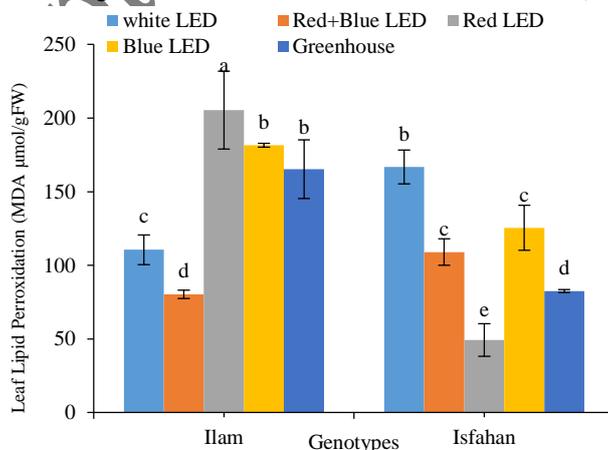


Fig. 4 MDA content in the lemon balm^s of two genotypes cultivated under five distinct illuminations. Data represent mean values \pm SE. Error bars represent the standard error. Differentiated letters signify significant differences ($P \leq 0.01$).

DISCUSSION

Growth Parameters under LEDs

The findings of the present research (Table 1) revealed that different light sources significantly affected all growth parameters in lemon balm plants. SFW, SDW, and leaf counts were higher under LED illumination sources compared to greenhouse light, with the mixture of red and blue illuminations producing the apex values for these parameters in both genotypes. Plant growth and product quality are affected by various environmental parameters, like light quality. In particular, Light quality affects plant growth and morphology. For instance, exposure to red LEDs alone can lead to elongated growth and reduced biomass in lettuce, while blue LED light promotes leaf expansion, increased leaf area, and biomass production [17].

It has been established that plants cannot fully develop using just red light; there needs to be at least a small percentage of blue light, even as low as 10% [18]. According to Kim *et al.* [5], under the mixture of red and blue LED, and red and blue light, the increase in SFW and SDW, chlorophyll accumulation, and leaf area reached the maximum, with a threefold rise in comparison with treatments with blue and far-red light. The LED with red spectrum also corresponds to the absorption peaks of chlorophyll a and b in the chloroplast, while blue light creates effects complementary to that, even as red light contributes more to photosynthesis [19].

Liu *et al.* [20], in a similar way, observed that pepper plants produced less biomass when cultivated under red LEDs exclusively, as opposed to a combination of UV-A and blue LEDs. White LEDs, which comprise a blend of low-intensity red, blue, and other wavelengths, are less effective for photosynthesis and can hinder plant growth and development compared to the use of red+blue LEDs. Multiple studies focusing on lettuce cultivation under LED lighting [5, 21] have consistently revealed that a mixture of red and blue illumination leads to greater fresh weight and overall plant growth compared to other lighting treatments, including fluorescent light.

The enhanced growth observed under LEDs may be attributed to the high intensity of photons they emit [22]. For instance, *Ocimum gratissimum* showed increased leaf production under intense light, although leaf area decreased [23]. Blue and red light wavelengths also regulate stomatal aperture, influencing water content in plant tissues and thereby affecting plant size and height [22].

Pigments under LEDs

This study showed that red+blue LEDs were the most effective light sources for increasing pigment levels, followed by white and blue LEDs. Light quality significantly impacts pigment synthesis. For example, Chen *et al.* [24] observed that a combination of red and blue light positively influenced the total chlorophyll content in rice seedlings. Similarly, Dong *et al.* [25] stated that red light enhanced total chlorophyll in wheat leaves, although adding blue light decreased chlorophyll content. These findings were similar to Shuai *et al.* [26], who observed the highest chlorophyll content in grape leaves under red light.

Studies from the Wisconsin and Kennedy Space Center projects showed the necessity of complementing red LEDs with blue light for optimal chlorophyll synthesis. For instance, wheat seedlings under red LEDs at 500 $\mu\text{mol}/\text{m}^2/\text{s}$ exhibited defective chlorophyll development, which improved when supplemented with blue light (30–100 $\mu\text{mol}/\text{m}^2/\text{s}$) [27].

In this research, red+blue LEDs significantly increased total chlorophyll content in both genotypes. Choi *et al.* [28] said that strawberry plants cultivated under red+blue illumination had 40% more total chlorophyll than those grown in greenhouses. Similarly, Amoozgar *et al.* [29] concluded that plantlets cared with red+blue LED lamps had the highest levels of chl a, chl b, and carotenoid.

Red+blue LEDs also promoted carotenoid accumulation in the present research. Carotenoids, crucial for photosynthesis and chloroplast development, are regulated by light quality and intensity [30]. Studies have shown that blue LEDs, in particular, enhance chlorophyll and carotenoid synthesis in lettuce and other plants [31, 21].

H₂O₂ under LEDs

In the present research, red and white LEDs, as well as greenhouse light, had the most stimulating effect on H₂O₂ production in lemon balm genotypes. These light sources likely induced oxidative stress, leading to increased H₂O₂ levels. ROS for example hydroxyl radicals, superoxide, and H₂O₂ can attack cell membranes, causing lipid peroxidation and cellular damage [32].

Gupta and Sahoo *et al.* [33] stated that red LEDs induced the highest H₂O₂ levels, followed by blue LEDs, during early branching differentiation stages. Red LEDs, associated with oxidative stress, result in high H₂O₂ accumulation and lipid peroxidation. Similarly, Wang *et al.* [34] found that short wavelengths, including red and blue light, caused oxidative stress and get bigger ROS creation in *Houttuynia cordata*.

MDA under LEDs

This study revealed differences in MDA production between genotypes under various light treatments. In the genotype of Ilam, red and blue LED lamps, alone, induced the greatest MDA levels, while white LEDs had the most meaningful impact in the other genotype. These treatments caused significant damage to cell membrane lipids.

MDA, a marker of lipid peroxidation and oxidative stress, increases under high-intensity light [35]. Ilieva *et al.* [36] proved that red LEDs induced the highest MDA levels, while blue LEDs caused moderate oxidative stress. The oxidative damage from red light is linked to high H₂O₂ accumulation and lipid peroxidation, while blue light's effects are milder [33].

CONCLUSION

Energy consumption and output are key metrics for evaluating artificial illumination sources in controlled conditions. The findings of this study suggest that the mixture of red and blue LED lamps is the most effective illuminating source for promoting growth, biomass, and physiological, and biochemical parameters in lemon balm plants. The performance of these LEDs not only enhances the nutritional quality of plants but also promotes their overall health by providing essential antioxidant protection.

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Conflict of Interests

The authors declare no Conflict of interest.

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