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Blue light inhibits gray mold infection by inducing disease resistance in cherry tomato

Abstract: Induced resistance is considered as a sustainable strategy to control postharvest decay of fruits, while light emitting diodes (LEDs) as a green physical technology are of more and more interest in postharvest fruit preservation field. In this study, we evaluated for the first time the resistance inducing ability of LED irradiation with different light wavelengths and photoperiods for cherry tomatoes (*Solanum lycopersicum* L. 'Qianxi'). Results indicated the exposure to 40 W m^{-2} of four light wavelengths for 3 d decreased *B. cinerea* lesion diameter on harvested cherry tomatoes, notably the best effect in blue light (470 nm). Meanwhile, the mechanism of blue light-induced disease resistance is the enhancement of defense-enzyme activity and the expression of defense-related genes. Moreover, results revealed that blue light enhanced vitamin C content and the firmness of the fruit exocarp, suggesting the potential usage of blue light in the postharvest preservation of cherry tomatoes.

Keywords: Blue light emitting diodes, Cherry tomato fruit, Disease resistance, Defense enzymes, Fruit quality

1. Introduction

Due to their high moisture content and thin exocarp (epidermis), cherry tomatoes (*Solanum lycopersicum* var. *cerasiforme*) are vulnerable to postharvest decay caused by a number of necrotrophic fungal pathogens, including *Botrytis cinerea*, *Rhizopus stolonifera*, and *Alternaria alternata* (Carrieri et al., 2015). Among these pathogens, *B. cinerea,* the causal agent of gray mold, is a significant problem and results in substantial reductions in postharvest quality and marketability. At present, the use of synthetic fungicides is the main method used to prevent postharvest fungal infections (Malandrakis et al., 2013). However, synthetic fungicides pose an environmental and health risk and are being more tightly regulated or banned for postharvest use by government agencies (Jin et al., 2019). Therefore, finding safe and environmentally friendly preservation techniques to replace chemical fungicides is a critical need.

Host disease resistance can be induced by a variety of physical, chemical, or biological stimuli, and the induced resistance can provide long-lasting and broad-spectrum antimicrobial defense (Petriacq et al., 2018). Light-emitting diodes (LEDs) can provide monochromatic light of different colors in a range of narrow bandwidth emission wavelengths (480 nm-730 nm) (D'Souza et al., 2015), that has been used to inactivate putrefying fungi which induces postharvest fruit decay. For example, several studies have demonstrated the direct inhibitory effect of purple and blue light on spore germination and fungal growth (Hui et al., 2017; Ghate et al., 2021; Chong et al., 2022). Meanwhile, the exposure to red and blue light has been shown to stimulate disease resistance of fruits. For the red light, previous studies have reported that it can enhance the resistance of cucumber to *Sphaerotheca fuliginea*, as well as the resistance of broad bean to *Alternaria tenuissima* and tomato to *Botrytis cinerea* (Rahman et al., 2003; Wang et al., 2010; Hui et al., 2017). While for the blue one, studies of fruit disease resistance induced by light have been just limited to citrus (Liao et al., 2013; Lafuente et al., 2020; Du et al., 2023). In addition, most studies on LED light-induced disease resistance have evaluated the effect with

43 a single wavelength, however, different fruits may be more sensitive to light parameters such
44 as different wavelength (color), intensity, photoperiod, etc, especially for the induction of
45 disease resistance. Hence, the optimum LED light parameters to induce postharvest disease
46 resistance need to be experimentally determined, thus achieving better economic loss reduction.

47 Therefore, the objective of the present study was to determine the LED irradiation
48 parameters inducing disease resistance in cherry tomato fruit against *Botrytis cinerea* and to
49 provide insight into the underlying mechanisms involved. Fruit quality is a major determinant
50 of consumer acceptance, thus, the effect of blue light on cherry tomato fruit quality was also
51 evaluated.

52 **2. Materials and methods**

53 **2.1 Fruit and pathogens**

54 Fully ripened, 'Qian xi' cherry tomatoes of uniform size and without any indications of injury,
55 were harvested from a farm in Qingdao city, Shandong Province, China. The harvested
56 tomatoes were transported to the Laboratory of Ocean University of China in under two hours.
57 The fruit were then surface disinfected by soaking them in an aqueous solution of 0.2 % (v/v)
58 commercial sodium hypochlorite for 2 min, allowed to air dry, and then divided into treatment
59 groups.

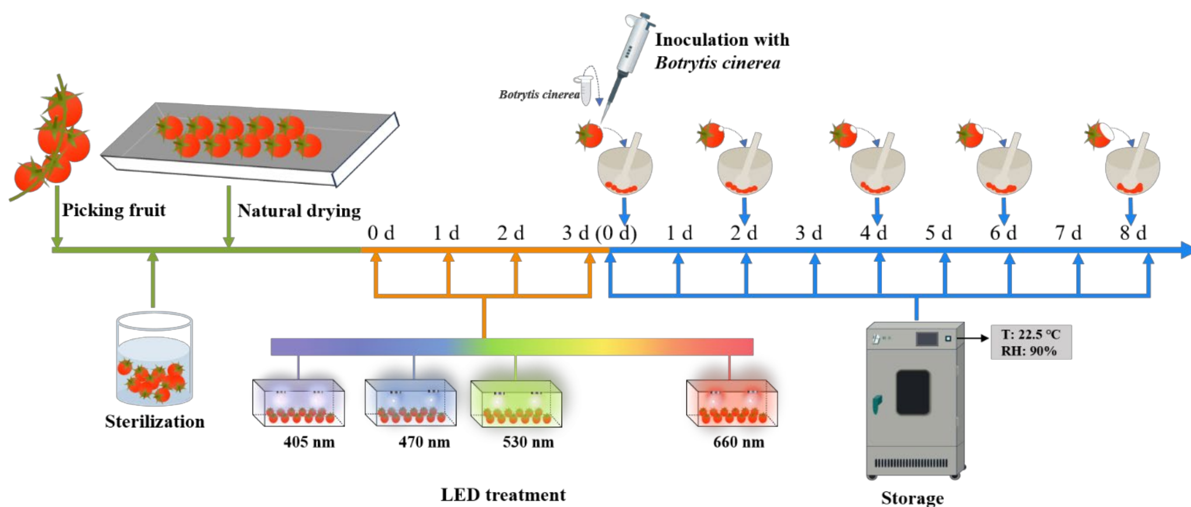
60 *Botrytis cinerea* (B05.10) was obtained from the Institute of Botany, Chinese Academy of
61 Sciences. *B. cinerea* was grown on potato dextrose agar at 22 °C and 90 % humidity for two
62 weeks, at which time the cultures began to sporulate. The concentration of the spore in sterile
63 water was adjusted to 1.0×10^4 CFU mL^{-1} with the aid of a hemocytometer.

64 **2.2 LED treatments**

65 A light panel containing 16 small LEDs (CREE LIGHTING, Washington, United States) that
66 could be adjusted to emit purple (405 nm), blue (470 nm), green (530 nm), or red (660 nm)
67 light at an intensity of 40 W m^{-2}. Two different photoperiods were used: 12 h light/12 h darkness

68 (12 DL) or 24 h constant light (24 LL), with constant darkness (DD) serving as a control. The
69 experimental flowchart used in the study is presented Fig. 1.

70 Cherry tomato fruit were exposed to four wavelengths light under two different
71 photoperiods for 3 d, after which a sterile perforator was used to make a 3 mm × 3 mm (depth
72 and diameter) wound on the equator of each fruit. Then, 10 μL of a 1.0×10^4 spore suspension
73 of gray mold was injected into each fruit wound. The inoculated wounds were allowed to air
74 dry for 2 h after which the fruit were placed in the constant temperature incubator set at 22.5 °C
75 and 90 % relative humidity. Fruit were considered infected when mycelia were visible in the
76 wound, and the incidence of gray mold infection was recorded at 2, 3 and 4 d, the lesion
77 diameter was measured beginning on the fourth day after inoculation using a digital caliper.
78 Lesion diameter was measured in two opposing directions an averaged. Each treatment included
79 three replicates of 15 fruit in each replicate, and the experiment was conducted three times.



80

81 **Figure 1.** Flow chart of the experimental design used in the present study.

82 **2.3 Sample collection**

83 Fifteen fruit were randomly selected from each treatment group (including the control) at 0, 2,
84 4, 6, 8 d post inoculation for biochemical analysis. Samples were also collected at 0, 12, 24, 48,
85 96, 144, and 192 h to analyze gene expression. Visibly infected areas of a fruit were removed,

86 and the surrounding fruit tissues were collected, pulverized into a powder in liquid nitrogen,
87 and stored at -80 °C for subsequent analysis.

88 **2.4 CHI and GLU activity**

89 One gram of sample was extracted with 5 mL acetate buffer (100 mM, pH 5.2) containing 1
90 mM ethylene diamine tetraacetic acid (EDTA), 5 mM β-mercaptoethanol (β-ME), and 8 % (w/v)
91 polyvinylpyrrolidone (PVP). The mixture was centrifuged at 12000 × g for 20 min at 4 °C and
92 the supernatant was collected and used to measure chitinase (CHI) and β-1,3-glucanase (GLU)
93 activity.

94 CHI activity was assessed using a commercial assay kit (NJBI, Nanjing, China) according
95 to the manufacturer's instructions. One unit (U) of CHI activity was defined as the amount of
96 enzyme needed to reduce chitin per hour to produce 1 mg of N-acetylglucosamine. Absorbance
97 was measured at 585 nm and expressed as U per mg of protein.

98 GLU activity was determined according to the method of Li et al. (2019). Briefly, 0.1 mL
99 of supernatant was added to 0.1 mL 0.4 % fucoidan solution, 1.8 mL of distilled water and 1.5
100 mL of 3,5-dinitrosalicylic acid reagent. The absorbance was measured at 540 nm after mixing.
101 One unit of GLU activity was defined as the amount of enzyme needed to break down fucoidan
102 per second to produce 1×10^{-9} mol of glucose. Results are expressed as U per mg of protein.

103 **2.5 O^{2-} generation and H_2O_2 content**

104 Superoxide anion (O^{2-}) was extracted from 1 g of sample using 3 mL phosphate buffer (50
105 mM, pH 7.8) containing 0.3 % Triton X-100, 2 % (w/v) PVP, and 1 mM EDTA, then analysis
106 as described by Li et al. (2019). The rate of O^{2-} production was expressed as mmol kg^{-1} min^{-1}
107 of fruit. Hydrogen peroxide (H_2O_2) was extracted from 1 g of sample using 3 mL phosphate
108 buffer (100 mM, pH 7.0). H_2O_2 content was measured using a commercial assay kit (NJBI,
109 Nanjing, China) according to the manufacturer's instructions and expressed as mol kg^{-1} of fruit.

110 **2.6 GPX, APX, SOD, POD, and CAT activity**

111 Glutathione peroxidase (GPX) activity was measured as described by Li et al. (2014), and
112 one unit was defined as the amount of enzyme per milligram of protein to catalyze 1 nmol
113 glutathione oxidation per minute. Absorbance was measured at 412 nm and expressed as U per
114 mg of protein.

115 Ascorbic acid peroxidase (APX) activity was measured according to the method of Tang
116 et al. (2020), and one unit was defined as the decrease of 0.01 in absorbance at 290 nm per
117 minute and expressed as U per mg of protein.

118 Superoxide dismutase (SOD) activity was measured according to Zhang et al. (2021), and
119 one unit was defined as the amount of enzyme needed to inhibit 50 % of the photochemical
120 reduction of NBT at 560 nm and expressed as U per mg of protein.

121 Peroxidase (POD) activity was measured as described Zhao et al. (2009), and one unit was
122 defined as an increase in absorbance of 0.01 per minute at 460 nm and expressed as U per mg
123 of protein.

124 A total of 3 mL phosphate buffer (50 mM, pH 7.5) containing 5 mM DTT and 5 % (w/v)
125 PVP was used to extract catalase (CAT) from 1 g of sample. CAT activity was measured using
126 a commercial assay kit (NJBI, Nanjing, China). One unit of CAT was defined as the
127 decomposition of 1 μM H_2O_2 per milligram of protein per second. Absorbance was measured
128 at 405 nm and expressed as U per mg of protein.

129 Determination of proteins in the supernatant was performed according to the Bradford
130 (1976).

131 **2.7 PAL activity and secondary metabolites**

132 A total of 3 mL borate buffer (100 mM, pH 8.8) containing 4 % (w/v) PVP, 5 mM β-ME, and 2
133 mM EDTA was used to extract phenylalanine ammonia-lyase (PAL) from 1 g of sample. The
134 reaction mixture was kept at 37 °C for 60 min, followed by the addition of 0.1 mL 6 M
135 hydrochloric acid solution to terminate the reaction. One unit of PAL activity was defined as an

136 increase in absorbance of 0.01 per hour at 290 nm and expressed as U per mg of protein.

137 Total phenolics and flavonoids were measured from 2 g of sample using 25 mL 1 % (v/v)
138 hydrochloric acid-methanol, then analysis as described by Yu et al. (2021). The total phenolics
139 and flavonoids content were expressed as mg kg^{-1} of fruit.

140 Lignin content was expressed as mg kg^{-1} of fruit. A total of 1 g of sample was rinsed 5 x
141 with 95 % ethanol, and then dried at 60 °C. A total of 1 mL 25 % bromoacetyl glacial acetic
142 acid solution was then added to the dried precipitate. The tube containing the reaction mixture
143 was placed in a 70 °C water bath for 30 min, after which the reaction was terminated by the
144 addition of 1 mL 2 M NaOH. Subsequently, 0.1 mL 7.5 M hydroxylamine hydrochloride and 3
145 mL glacial acetic acid were added to the assay solution and absorbance was measured at 280
146 nm.

147 **2.8 Assay of fruit quality**

148 Fresh fruit were irradiated with blue light for three days without subsequent wounding and
149 inoculation with *B. cinerea* spores, fruit quality parameters were then measured. Total soluble
150 solids (TSS), titratable acid (TA), firmness, and vitamin C were measured in 15 randomly
151 selected fruit at two-day intervals.

152 Fifteen fruit were homogenized in a juicer (JYL-C020E, Joyoung, China), and 5 g fruit
153 puree were squeezed and filtered to obtain a filtrate that was then used to determine TSS content
154 utilizing a handheld refractometer (P216950, Atago, Japan). Distilled water was added to 10 g
155 fruit puree and was allowed to obtain a volume of 100 mL for TA determination, and the results
156 are expressed as a percentage of malic acid.

157 Fruit skin firmness was measured using a texture meter (TMS-Touch, FTC, United States)
158 equipped with a needle-tipped probe. The penetration distance was 5 mm, the starting force was
159 0.1 N, and the experimental test speed was 120 mm min^{-1}. Results are reported in Newtons (N).
160 Fluorescence spectrophotometry was used to assess the content of vitamin C, the content was

161 expressed as mg kg^{-1} of fruit.

162 **2.9 RNA extraction and Reverse-transcription-quantitative PCR (RT-qPCR)**

163 Total RNA was extracted from 0.15 g of previously, frozen, and powdered tomato tissue from
164 the different treatment group and control samples using an RNAprep Pure Total RNA Extraction
165 Kit for Polysaccharide-Polyphenol Plants (DP441) (TIANGEN, China). The RNA (900 ng) was
166 reversely transcribed into cDNA using the HiScript II 1st Strand cDNA kit (Vazyme, Nanjing,
167 China). RT-qPCR was performed on a Bioer StepOneTM Real-time PCR system (Bioer,
168 Hangzhou, China) using ChamQ Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China).
169 The thermocycler was programmed to run at 95 °C for 3 min, followed by 40 cycles of 95 °C
170 for 10 s and 60 °C for 30 s.

171 Melting curve analysis was used to evaluate the suitability of each reaction and primer set
172 for each gene expression assay. The expression of each target gene *(LeCHI*, *LeGLU*, *LePAL*,
173 *LeSOD*, *LePOD* and *LeCAT*) was normalized to that of the reference gene *Actin* and the $2^{-\Delta\Delta CT}$
174 method was used to calculate relative expression. The gene primer pairs used for the analysis
175 of each of the indicated genes are listed in Supplementary Materials Table 1.

176 **2.10 Statistical analysis**

177 The results represent the mean ± SD. The data were statistically analyzed using SPSS 27 (SPSS
178 Inc, Chicago, IL, USA). Duncan's multiple range test was employed following analysis of
179 variance (ANOVA) to ascertain the presence of significant differences (p<0.05) between
180 treatment group means. An independent samples t-test was utilized to determine whether there
181 was a significant difference (p<0.05) between two groups, when just two groups were compared.

182 **3. Results**

183 **3.1 Optimization of LED-induced disease resistance**

184 Cherry tomatoes were exposed to different wavelengths of LED light using two different
185 photoperiods, prior to inoculation with *B. cinerea*. Results indicated that disease incidence and

186 lesion diameter increased as storage time increased. The 12 DL photoperiod did not decrease
187 disease incidence, relative to the dark-treated control, regardless of the wavelength used.
188 However, disease incidence did decrease in cherry tomatoes exposed to a 24 LL photoperiod of
189 LED irradiation (Fig. 2A). Therefore, a 24 LL photoperiod was used to induce resistance to
190 gray mold in cherry tomato fruit in subsequent experiments.

191 Constant 24 h irradiation of cherry tomatoes with either blue or green light significantly
192 decreased the incidence of gray mold in cherry tomato fruit inoculated with *B. cinerea*. Disease
193 incidence in blue and green irradiated fruit was approximately 17 % and 12 % lower,
194 respectively, then it was in the control at 2 d post inoculation (Fig. 2A). No appreciable
195 differences in disease incidence between the other light wavelengths (colors) and the control
196 group were observed at that time. Notably, the blue light treatment reduced disease incidence
197 to a significantly greater extent than the other color LEDs as storage time increased. Lesion
198 diameter was also significantly smaller than it was in the control and other color LED treatment
199 groups across all storage days (Fig. 2B). In this regard, lesion diameter in cherry tomatoes
200 exposed to blue light treatment was approximately 43 % smaller after 8 d of storage than it was
201 in fruit in the dark-treated control. In brief, constant blue light was the best of all the color LEDs
202 in preventing gray mold incidence and lesion development in cherry tomatoes. In following
203 experiments, 3 d blue light irradiation was conducted prior to inoculation with *B. cinerea* to
204 further investigate the mechanism of resistance enhancement.

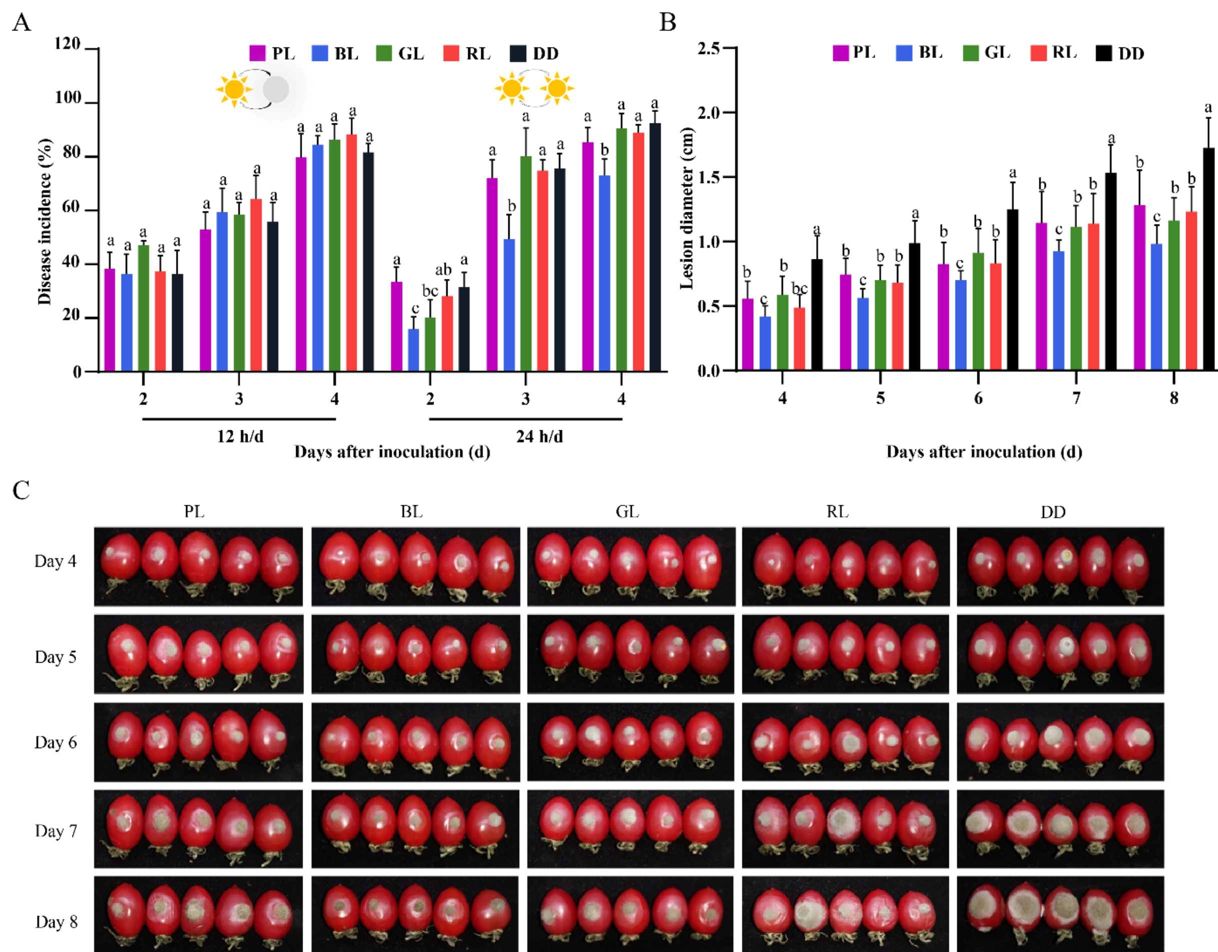
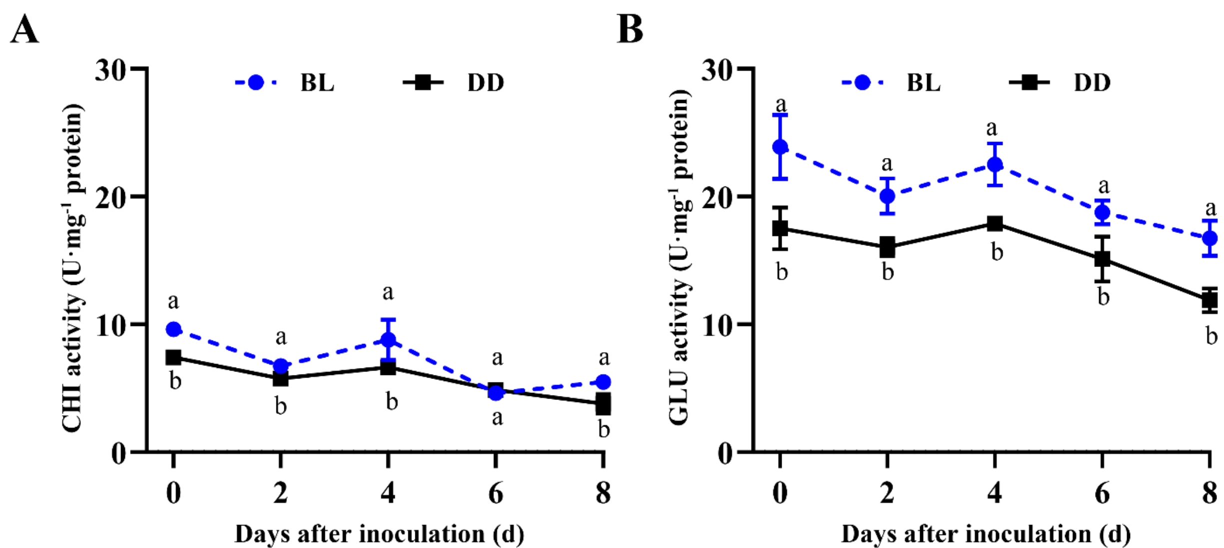


Figure 2. Induction of disease resistance in cherry tomatoes against *B. cinerea* by different color LED irradiation. (A) Cherry tomatoes were subjected to a 12 h dark/12 h light (12 DL) or constant 24 light (24 LL) LED exposure at different wavelengths (colors) for 3 d and then inoculated with *B. cinerea.* Disease incidence was measured over 4 d. (B) Lesion diameter in cherry tomatoes exposed to constant (24 LL) LED irradiation of different wavelengths (colors) for 3 d and then wound inoculated with *B. cinerea.* Lesion diameter was measured starting at 4 d post-inoculation. (C) Representative photographs of cherry tomatoes exposed to constant (24 LL) LED irradiation of different wavelengths (colors) for 3 d and then wound inoculated with *B. cinerea* and then placed in storage. Different letters indicate a significant difference between treatment groups at the specified time point according to Duncan's test (p<0.05). Abbreviations: DD: constant darkness; 12 DL: 12 h light/12 h dark; 24 LL: 24 h constant light; PL: purple light (405 nm); BL: blue light (470 nm); GL: green light (530 nm); RL: red light (660 nm).

218 **3.2 Effect of blue LED treatment on CHI and GLU activity**

219 CHI and GLU are significant defense-related enzymes in cherry tomatoes. As shown in Fig. 3,
220 CHI and GLU activity in both the blue light (BL) and dark (DD) treatment groups exhibited a
221 similar pattern of expression over the course of 8 d of storage. Notably, however, CHI and GLU
222 activity in the blue LED treatment group of cherry tomatoes was generally (CHI) or consistently
223 (GLU) significantly higher than it was in the dark-treated treatment group.



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225 **Figure 3.** Effect of blue LED irradiation (constant for 3 d) CHI and GLU activity in cherry
226 tomato fruit during 8 d of storage. Fruit were exposed to blue LED light for 3 d and then
227 inoculated with *B. cinerea* and placed in storage. (A) CHI activity and (B) GLU activity.
228 Different letters above the data points on each day of storage indicate a significant difference
229 between blue LED and dark treatment groups according to the independent samples t-test
230 (p<0.05).

231 **3.3 Effect of blue LED irradiation on O^{2-} production, H_2O_2 content, GPX, APX, SOD,**
232 **POD, and CAT activity**

233 Exogenous exciters induce a ROS burst in fruit tissues, which produce H_2O_2 as a signaling
234 molecule in the defense response of fruits to activate a disease resistance response cascade. In
235 the present study, we monitored ROS levels by assessing the rate of O^{2-} production and the

236 H_2O_2 accumulation in cherry tomatoes that were subjected to a dark (DD) or blue light (BL)
237 treatment for 3 d prior to inoculation with *B. cinerea*. As shown in Fig. 4A, results indicated
238 that O^{2-} production in cherry tomato samples exposed to blue light was significantly lower than
239 in the dark treatment group. The greatest difference in the rate of O^{2-} production between the
240 BL and DD treatment groups was observed on the 6th d of storage. Notably, O^{2-} production in
241 BL treatment group of cherry tomatoes was 37.5 % lower than it was in the dark-treated (DD)
242 control group on the 6th d of storage. H_2O_2 content decreased and then increased during storage
243 in both treatment groups. H_2O_2 content in the BL treatment group was significantly greater than
244 it was in the control (DD) treatment group during the first 2 d of storage, especially on day 0
245 when the H_2O_2 content in BL treatment group was 12.4 % greater than that in the DD treatment
246 group. After 2 d of storage, subsequently, however H_2O_2 content in the BL treatment was
247 significantly lower or the same as it was in the dark-treated control treatment group.

248 On the other side, GPX and APX from the fruit antioxidant defense systems are essential
249 antioxidant enzymes which can reduce the oxidative injury by excessive levels of ROS. Results
250 indicated that cherry tomato fruit treated with blue light exhibited significantly higher levels of
251 GPX and APX activity than dark-treated samples throughout storage (Fig 4B and 4C). This was
252 especially true for APX activity where APX activity was 270.3 % greater in BL treatment group
253 than in the DD treatment group on day 6 of storage.

254 SOD activity increased and then decreased during the storage period in both treatment
255 groups (Fig. 4D). SOD activity in BL treatment group was significantly higher than it was in
256 the DD treatment group throughout storage (except on day 4), especially on the 8th d of storage.
257 POD activity increased in both treatment groups over the course of storage (Fig. 4E). Notably,
258 however, POD activity in the BL treatment group was significantly higher than in the DD
259 treatment group throughout storage. POD activity in the BL group was 31 % and 36 % higher
260 than in the DD group on day 2 and day 6 of storage, respectively. A gradual increasing trend in

CAT activity throughout the storage period was observed in both treatment groups, however, blue light increased CAT activity only on day 8 (Fig. 4F).

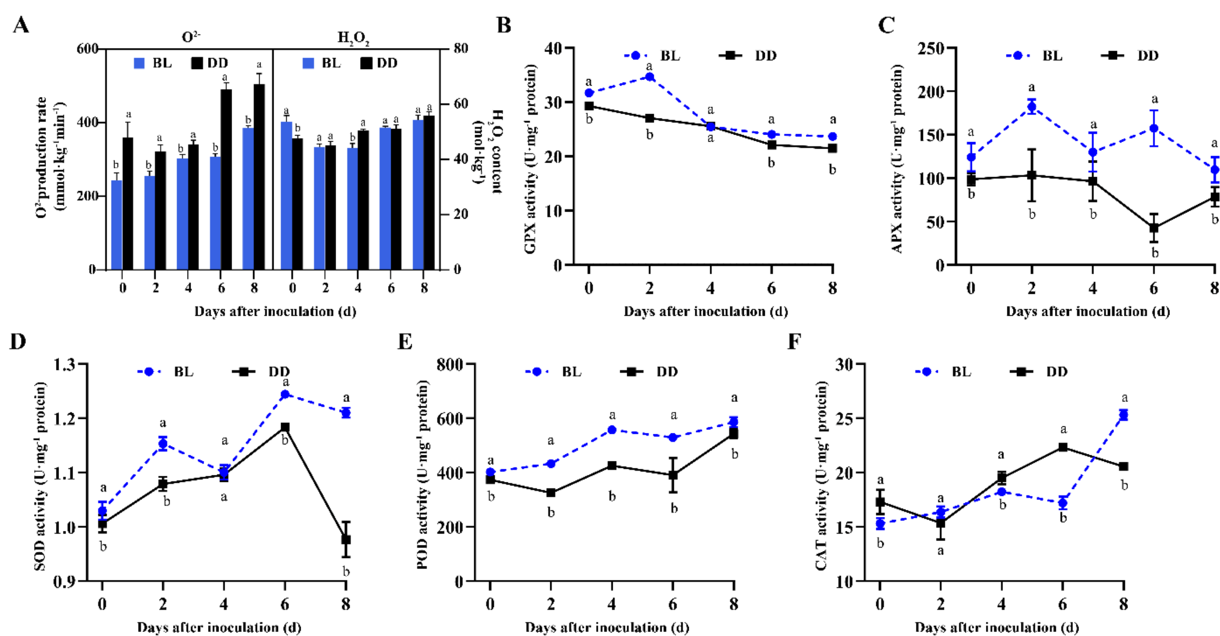
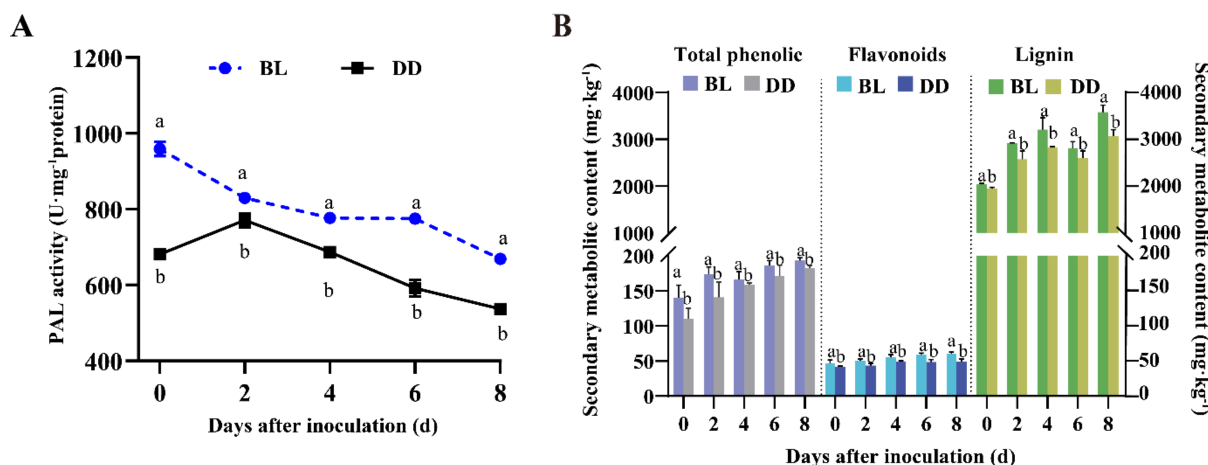


Figure 4. Effect of blue LED irradiation on ROS levels and antioxidant enzyme activity in cherry tomato fruit. Fruit were exposed to constant blue LED irradiation or kept in the dark for 3 d prior to being inoculated with *B. cinerea*, after which they were placed in storage. (A) O^{2-} production and H_2O_2 content; (B) GPX activity; (C) APX activity; (D) SOD activity; (E) POD activity; (F) CAT activity. Different letters above the bars or data points indicate a significant difference between treatment groups at that timepoint of storage as determined by an independent samples t-test ($p<0.05$).

3.4 Effect of blue LED irradiation on the phenylpropanoid pathway

PAL regulates the synthesis of secondary metabolites (such as phenolic, flavonoids, and lignin) in the phenylpropanoid pathway that significantly contributes disease resistance. Results of our study indicated that cherry tomatoes exposed to blue LED irradiance exhibited significantly higher levels of PAL activity than dark-treated fruit throughout storage (Fig. 5A). Notably, PAL activity in the BL treatment group was on average 41 % higher than it was in the DD treatment group throughout storage. The level of lignin, flavonoids, and total phenolics in cherry tomatoes

278 exhibited an overall increasing trend throughout 8 d of storage (Fig. 5B). However, the level of
279 these compounds was always higher in the BL treatment group than the DD treatment group,
280 exhibiting an average 27 %, 24 %, and 17 % greater level of phenolics, flavonoids, and lignin,
281 respectively, than the levels in the dark-treated (DD) control samples (Fig. 5B).



282

283 **Figure 5.** Effect of blue LED irradiation on the phenylpropanoid pathway in cherry tomato fruit.
284 (A) PAL activity; (B) total phenolic, flavonoid and lignin content. Different letters above the
285 bars and data points indicate a significant difference between treatment groups on the
286 designated storage day as determined by an independent samples t-test (p<0.05).

287 **3.5 RT-qPCR analysis**

288 RT-qPCR was used to determine the relative expression of six genes encoding defense-related
289 enzymes in wounds of cherry tomato fruit. Results indicated that *LeCHI* and *LeGLU* expression
290 were upregulated from 12 to 144 h in samples exposed to blue LED irradiation, relative to dark-
291 treated samples (Fig. 6A). The relative expression of *LePAL* in blue light-treated samples
292 increased 10.0 x compared to the dark-treated control at 0 h after inoculation (Fig. 6A). The
293 relative expression of *LeSOD*, *LePOD* and *LeCAT* increased 1.3, 17.9 and 1.8 x, respectively,
294 compared to their expression in dark-treated control samples at 24 h (Fig. 6A). Based on data
295 obtained in this study, we developed a model of the response of cherry tomatoes to constant
296 blue light irradiation in relation to enhanced disease resistance response (Fig. 6B).

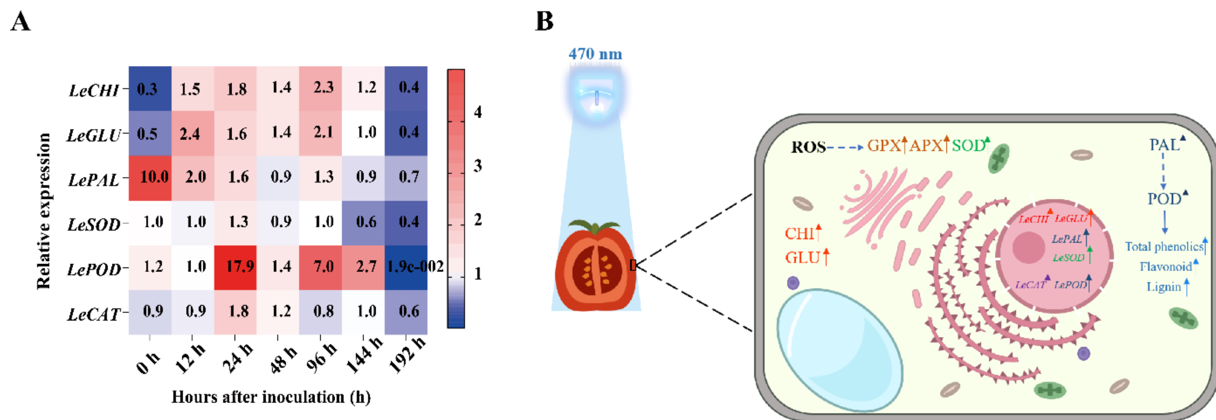


Figure 6. Effect of blue LED irradiation on the relative expression of genes encoding defense-related enzymes in cherry tomato fruit. (A) RT-qPCR of the relative expression of genes encoding defense-related enzymes in blue LED irradiated samples relative to dark-treated control samples; (B) A model of the response of cherry tomatoes to blue light irradiation in relation to the induction of disease resistance.

3.6 Effect of blue LED irradiation on fruit quality parameters

Data on fruit quality parameters are presented in Supplementary Materials Table 2. No discernible differences in TA were observed between the BL and DD treatment groups during storage. After four days of storage, the concentration of TSS increased in cherry tomato fruit exposed to blue LED light and was considerably greater than TSS levels in dark-treated control fruit. Blue light irradiation enhanced peel firmness, relative to the control, and enhanced vitamin C content during the first four days of storage.

4. Discussion

LEDs with different wavelengths have been used for postharvest preservation of decay and to delay senescence, thus, preventing a loss in economic value. However, little research has been conducted on determining if blue light induces a disease resistance response in fruits and vegetables against fungal pathogens, and the studies that have been conducted have been mainly focused on citrus. Previous study reported that red light increased the resistance of tomato leaves to *B. cinerea* (Hui et al., 2017), however, fruit, stems, and leaves of the same plant may

317 react differently to different wavelengths of light and/or possess different levels of sensitivity
318 to a given wavelength. Therefore, in the current study, we investigated the effect of four
319 different light wavelengths (colors) provided by LED irradiation on the resistance of cherry
320 tomato fruit to *B. cinerea*.

321 Results indicated that constant LED irradiation of cherry tomato fruit with blue light, with
322 a peak of 470 nm, at 40 W m^{-2} for three consecutive days had a greater ability than other
323 wavelengths (colors) to control postharvest gray mold (*B. cinerea*) decay in inoculated cherry
324 tomato fruit (Fig. 2). Interestingly, we found that red light, green light, and purple light can all,
325 to varying degrees, reduce lesion diameter in cherry tomato fruit resulting from gray mold
326 infection (Fig. 2B and C). This phenomenon may be potentially attributed to plant
327 photoreceptors. Different photoreceptor types, such as LOV Kelch protein 2 (LKP2),
328 photopigments (PHY), cryptochromes (CRY), and phototropin (PHOT), as well as zeitlupe
329 (ZTL), and UV-B resistance locus 8 (UVR8), are used by plants to detect the wavelength and
330 intensity of light (Loi et al., 2020). They may induce an abiotic or biotic defense response due
331 to their sensitivity to specific light wavelengths and level of irradiance, a perception that is then
332 transmitted to evoke a plant response (Paik & Huq, 2019). We speculate that the four
333 wavelengths and irradiation intensity of light used in our study were perceived in the fruit and
334 stimulated a host defense cascade. In this regard, blue light was the most effective and long-
335 lasting one, thus, its mechanism needs to be further explored. We also found that 3 d of
336 continuous irradiation was superior to 12 h of intermittent irradiation in regard to reducing
337 disease incidence (Fig. 2A). We suggest that this is due to the light-induced alterations being
338 dissipated once fruit is placed in the dark, as would occur in a 12 h light/12 h dark cycle.
339 Alternatively, inducing the changes in cherry tomato fruit that make it more disease resistant
340 may simply require a level of light saturation that is only provided by constant irradiation. Our
341 data confirm the findings of Lafuente et al. (2020), who reported that blue light irradiation

342 altered the physiology and metabolism of fruit (compared to control samples preserved in the
343 dark), however, most of the alterations did not persist after the fruit was placed in the dark.

344 Several studies have indicated that the activation of highly coordinated defense-related
345 enzymes, including CHI, GLU, PAL, and POD, may be underly the induction of disease
346 resistance in fruits (Cao et al., 2013; Jiao et al., 2018). CHI and GLU are two pathogenesis-
347 related proteins with enzymatic properties that facilitate the degradation of fungal cell walls,
348 which inhibits fungal growth and sporulation, thus, increasing the ability of fruits to prevent
349 pathogen ingress (Souza et al., 2017). Our study indicated that CHI and GLU activity were
350 significantly higher in blue LED irradiated cherry tomatoes than dark-treated, control tomatoes
351 during storage. Furthermore, RT-qPCR data indicated that blue light upregulated the expression
352 of *LeCHI* and *LeGLU* in cherry tomato fruit beginning 12 h after the end of light exposure. PAL
353 is a crucial rate-limiting enzyme in the phenylpropanoid pathway that supplies precursors for
354 the downstream production of secondary metabolites, such as lignin and other phenolic
355 compounds, that strengthen plant cell walls to prevent pathogen penetration (Yadav et al., 2020).
356 POD can be triggered in plants after an infection has been established and is directly involved
357 in stimulating lignin production and other processes that strengthen cell walls (van Loon et al.,
358 2006). Results of the present study indicated that blue LED irradiation increased the PAL and
359 POD activity, as well as the expression of *LePAL* and *LePOD* encoding these enzymes. The
360 relationship between blue LED irradiation and the phenylpropanoid pathway was demonstrated
361 by the increase in the concentration types of secondary metabolites, including total phenolics,
362 flavonoids, and lignin, by the blue light treatment. These findings are in agreement with Du et
363 al. (2023), who reported that blue light enhanced CHI, GLU, PAL, and POD activity in citrus,
364 which enhanced resistance to the fungal pathogen, *Geotrichum citri-aurantii*. Collectively, our
365 data indicated that blue LED irradiation of cherry tomatoes induced resistance to gray mold in
366 cherry tomato fruit by enhancing defense enzyme activity and the expression of their encoding

367 genes.

368 H_2O_2 serves as a pivotal signaling molecule within fruit tissues, mediating responses to
369 various environmental stresses. The H_2O_2 content in cherry tomato fruit inoculated with *B.*
370 *cinerea* increased by 12.4 % under blue LED irradiation, which may have enhanced the defense
371 response of cherry tomato fruit. Additionally, GPX, APX, SOD, and POD activity in cherry
372 tomato increased to varying degrees under blue LED irradiation, relative to dark-treated control
373 fruit. Consequently, a lower level of ROS was observed, which was consistent with the decrease
374 in superoxide anion production and H_2O_2 content that was recorded. CAT is an essential
375 antioxidant enzyme in fruits, that functions in the decomposition of H_2O_2, mitigating its
376 cytotoxic effects. Although a reduction in H_2O_2 levels was observed in response to blue LED
377 irradiation, a corresponding increase in CAT activity was not observed. Similar findings have
378 also been reported, these results are similar to the study by Hui et al. (2017), in which red light
379 was found to increase CAT activity in tomato leaves but blue light did not. These data suggested
380 that the decrease in the concentration of H_2O_2 may be a consequence of increased activity of
381 the overall antioxidant system, rather than just an increase in CAT activity. Notably, the blue
382 light treatment significantly upregulated genes encoding the defense-related enzymes *LeSOD*,
383 *LePOD*, and *LeCAT* after 24 h of storage based on the RT-qPCR analysis (Fig. 6). In summary,
384 blue LED irradiation reduced ROS levels and potential oxidative damage by increasing the
385 overall activity of all the components of the ROS scavenging system, both enzymatic and non-
386 enzymatic, in cherry tomato, resulting in an enhancement of resistance to *B. cinerea*.

387 Our study also examined the effect of blue LED irradiation on several indicators of
388 postharvest quality in cherry tomatoes. This aspect is essential as fruit quality determines
389 customer acceptability (Tarangini et al., 2022). Our results indicated that blue LED irradiation
390 increased TSS and vitamin C content but had no discernible effect on TA. In contrast, Du et al.
391 (2023) reported that blue light decreased TA but had no appreciable effects on TSS or vitamin

392 C content in citrus. This may possibly be attributed to the thin peel in cherry tomato fruit,
393 relative to citrus fruit, which would enable the blue light to penetrate more readily into the
394 internal tissues of cherry tomato, increasing TSS and vitamin C content. Peel firmness was also
395 greater in the blue light treatment group than in the control group, which in agreement with our
396 observation that blue light promoted PAL and POD activity in the phenylpropanoid pathway
397 and elevated the concentration of lignin. In a preliminary study, we determined the effect of
398 blue LED irradiation on the content of pectin and cellulose in the cell walls of cherry tomato
399 peel (unpublished). The results of the preliminary study indicated that blue light inhibited the
400 degradation of acid-soluble pectin in the early stages of storage but had no significant effect on
401 cellulose content. Therefore, the enhancement in the firmness of cherry tomato peel may be
402 mainly due to an increase in lignin content. Thus, blue light may improve fruit disease resistance
403 by delaying senescence and maintaining the strength of cell walls in the peel.

404 **5. Conclusions**

405 This study compared the resistance of cherry tomato to *B. cinerea* during postharvest storage
406 under different wavelengths and photoperiods, and proved that blue LED irradiation had the
407 best effect. The specific mechanism can be divided into two aspects, one is that the blue light
408 strengthens the resistance of cherry tomato to gray mold by enhancing the activities of defense-
409 related enzymes, defense-related proteins, antioxidant enzymes activity and the PAL. On the
410 other side, PAL enhances the firmness of peel tissues by using secondary metabolites of
411 phenylpropanoid pathway (especially the lignin produced plays a major regulatory role),
412 making the fruit more resistant to the ingress of pathogens, such as *B. cinerea*. Therefore, the
413 application of blue LEDs in the control of post-harvest fruit decay is a meaningful and
414 promising technology.

415 **Credit authorship contribution statement**

416 Jiayi Sun: Writing - original draft, Data curation, Investigation.

Xinhui Tan: Methodology, Visualization.

Maurizio Battino: Writing - review & editing.

Xianghong Meng: Visualization.

Fang Zhang: Conceptualization, Investigation, Funding acquisition.

Conflicts of interest

The authors declared no competing financial interest.

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Statement

During the preparation of this work the author used [Quillbot] to embellish language. After using this tool, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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