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Comparison of toxicological effects of oxybenzone, avobenzone, octocrylene, and octinoxate sunscreen ingredients on cucumber plants (*Cucumis sativus* L.)

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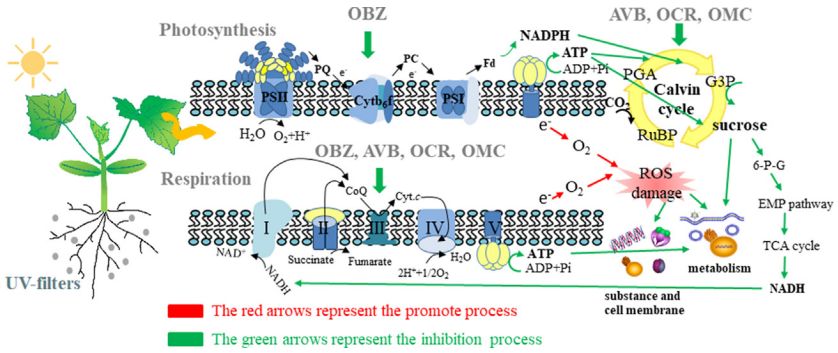
HIGHLIGHTS

- All of the UV-filters inhibited photosynthesis and respiration of cucumber plant.
- The OBZ instantly inhibited PET, while the others inhibited Calvin-Benson cycle.
- The damage caused by over-production of ROS is thought as the secondary damage.

GRAPHICAL ABSTRACT



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ABSTRACT

Oxybenzone (OBZ), avobenzone (AVB), octocrylene (OCR) and octinoxate (OMC) are ultraviolet (UV) filters commonly added to chemical sunscreens. These UV filters are known to widely contaminate the environment through a variety of anthropogenic sources, including sewage discharge. However, systematic studies of the damage caused by these four UV filters and their toxicopathological differences in a variety of plant species are lacking. In this study, we demonstrated that irrigation with water containing these four UV filters could significantly inhibit the aboveground growth of cucumber plant. All of the UV filters decreased photosynthesis through nonstomatal factors but via different inhibitory mechanisms. Only OBZ inhibited photosynthesis by directly inhibiting photosynthetic electron transport, while the other three (AVB, OCR, and OMC) inhibited photosynthesis by inhibiting the Calvin-Benson cycle. Additionally, these four UV filters also decreased plant respiration under long-term treatment. Photosynthesis and respiration inhibition led to the over production of reactive oxygen species (ROS) and the formation of lipid peroxidation damage products, which further damaged the structure and function of plant cells, causing secondary pathologies and potentially leading to reduced crop yields. The study also demonstrated that these four UV filters caused different degrees of phototoxic damage to cucumber plants. On the basis of comprehensive evaluation, we speculated that the order of the four UV filters in terms of plant damage was OBZ > AVB > OMC > OCR. Because of the severe damaging effects of these UV filters on

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plant growth, the application of contaminated biosolids/reclaimed water in agriculture reduces agricultural production and may damage ecosystems. The results of this study can advance recognition of the hazards associated with environmental and agricultural pollution via UV filters and encourage consumers and the industry to limit or reduce the application of cosmetics and over-the-counter drugs containing these substances.

1. Introduction

Oxybenzone (OBZ, CAS:131-57-7), avobenzone (AVB, CAS:70356-09-1), octocrylene (OCR, CAS:6197-30-4), and octinoxate (OMC, CAS:5466-77-3) are the most widely used ultraviolet (UV) filters in sunscreens and cosmetics; they are "high-production-volume chemicals" (HPVCs),with >1000 metric tons produced per year in the European Union (Rastogi, 2002). Because UV filters in sunscreens can reduce damage caused by UV (UVB and UVA) rays (Marrot et al., 2005; Moyal and Fourtanier, 2008), they are widely used in pharmaceutical personal care products (PPCPs) and food product packaging. Recently, recognition of the pollution of aquatic environments by these substances has increased dramatically, in part due to the wide spread use of sunscreen and an increase in coastal tourism. These UV filters are known to contaminate oceans, lakes, rivers, and groundwater through human activities via either direct release into surface water (from the skin during swimming and bathing) or indirect release into surface water through sewage treatment plants (via water used for bathing or washing textiles) (Balmer et al., 2005). To date, four major UV filters have been detected in aquatic systems in various areas (Bachelot et al., 2012; Farré et al., 2008; Li et al., 2007; Magi et al., 2013; Zwiener et al., 2007). Several studies have documented the difficulty in removing these UV filters using traditional sewage treatment methods (Schneider and Lim, 2018). The continuous contamination of aquatic systems by UV filters has directed worldwide attention towards environmental pollution and the associated damage to ecosystems (Sieratowicz et al., 2011).Previous studies of the toxicity and damaging effect of UV filters on flora and fauna focused on OBZ. Most studies have indicated that OBZ is a potential estrogen analog that can affect the embryonic development and fertilization of mammals (Blüthgen et al., 2012), change their endocrine or reproductive endpoint (Coronado et al., 2008), change their mammary gland morphology and function (Laplante et al., 2018), and even change the epigenetic state of their neurons, leading to severe neuron apoptosis (Wnuk et al., 2018). In addition to animal damage, OBZ can also damage aquatic phytoplankton (Sieratowiczet al. 2011, Zhong et al., 2019c), decrease algal pigment content (Mao et al., 2017), lead to coral bleaching and morbidity (Downs et al., 2016), and cause oxidative stress in plants (Chen et al., 2018). Due to its harmful effects on animals and plants, OBZ has been banned in some areas (e.g., Hawaii, Bonaire, Aruba, Palau, U.S. Virgin Islands, and the city of Key West).

Previous toxicological studies on UV filters focused predominantly on animals, including humans (Dinardo and Downs, 2017). For example, OBZ, AVB, OCR and OMC in sunscreen can be readily absorbed by human skin, greatly accumulate in human plasma and urine and contaminate cord blood and milk in humans and dolphins (Hany and Nagel, 1995; Janjua et al., 2008; Gago-Ferrero et al., 2013; Molins-Delgado et al., 2018; Matta et al., 2019).Several other UV filters have been demonstrated to induce mutagenic, endocrine and developmental toxicities in various animal species (Schlumpf et al., 2004a, 2004b; Paredes et al., 2014; Zhang et al., 2016; Park et al., 2017).

Studies on the environmental contamination and organismal/environmental fate of these UV filters have mainly focused on chemical migration, transformation and removal from aqueous solutions (Buchberger, 2010; Caliman and Gavrilescu, 2010; Trebše et al., 2016) and their light stability in PPCPs (Kawakami and Gaspar, 2015). Wastewater influent and effluent contamination is an issue of concern for environmental engineering because of the considerable inefficiency of current wastewater treatment systems, which results in significant environmental contamination (Ekpeghere et al., 2016; O'Malley et al., 2019). The concentration of UV filters in water ranges from dozens of $ng.L^{-1}$ to thousands of $ng.L^{-1}$ (Bratkovics and Sapozhnikova, 2011; Bratkovics et al., 2015; Downs et al., 2016), which is likely to have toxic effects on animals and plants.

The use of sewage biosolids and irrigation of crops with treated and untreated wastewater are becoming common practices worldwide (Frank, 1998; Gray et al., 2017; Ghirardini and Verlicchi, 2019). Chemical transfer from biosolids/wastewater (such as antibiotics, pharmaceuticals, steroids, heavy metal ions, estrogen-like endocrine-disrupting compounds, and some chemicals in PPCPs, including galaxolide, tonalide, and triclosan) to agricultural crops has only recently been recognized (Clarke and Smith, 2011; Engler and Lemley, 2013; Cabrera-Peralta and Pena-Alvarez, 2017; 40 CFR Part 503, USEPA, 1993, 1994). Furthermore, river and lake source waters used for irrigation can also be polluted by UV filters, further aggravating pollution of agricultural resources (Chen et al., 2011; Wu et al., 2018; Kleywegt et al., 2019). Currently, there have been numerous studies and reports on the concentration of UV filters in water, the concentration of OBZ is 17–5429 $ng \cdot L^{-1}$ in worldwide surface water (Tsui et al., 2014), 10–60 $ng \cdot L^{-1}$ in rivers and lakes and 700–7700 $ng \cdot L^{-1}$ in waste water (Balmeret al. 2005; Ekpeghere et al., 2016); that of OCR is 950–3060 $ng \cdot L^{-1}$ in influent water and 410–1490 $ng \cdot L^{-1}$ in effluent water (Ramos et al., 2016); and that of OMC is 500–19,000 $ng \cdot L^{-1}$ in untreated water (Balmer 2005). Besides, there are researchers who have showed that the UV-filters exist in sludge, one of the main use of sewage sludge is as fertilizer in agriculture, the large quantities of UV filters in sludge (for example, OBZ: 16–2100 $ng \cdot g^{-1}$ DW in treated sludge; OCR: 303–8000 $ng \cdot g^{-1}$ DW in raw sludge and 138–41,610 $ng \cdot g^{-1}$ DW in treated sludge (Ramos et al., 2016) will bring about a contaminating pathway to soils, animals and even humans. In addition, it has been demonstrated that these substances also exist outside the source of the polluted areas, which indicates that the flow of water can disperse these biological solids beyond the initial deposition range and they last for quite long time (Tsui et al., 2014). Due to the lipophilic characteristics of these UV-filters (Sieratowicz et al., 2011; Jentzsch et al., 2019), it's easy for them to accumulate in the sludge and organisms (Sieratowicz et al., 2011; Ramos et al., 2016) and it is hard to remove them from the retreated water (Blüthgenet al. 2014; Ekpeghere et al., 2016). Even though, at present, the observed concentrations are relative lower in the soil, the biological enrichment function of plants and organisms will accumulate these UV filters in the plants, and with the increase use of the personal care products containing these UV filters, the contamination of the soils will be exacerbated. Risk assessments of the danger of these practices are lacking, with very little consideration of how PPCPs chemicals, especially UV filters, can adversely impact crop yield or the chemical quality of agricultural products in relation to safety for human consumption (Kinney et al., 2006; Kinney et al., 2012; Careghini et al., 2015).

This study focused on the toxicity of four UV-filter chemicals commonly used in sunscreen (OBZ, AVB, OMC, and OCR) to a global crop vegetable, cucumber (*Cucumis sativus* L.). Our primary goal was to determine the toxicopathological reactions of photosynthesis, oxidative phosphorylation, membrane structures, active oxygen metabolism, and photosensitivity to these four UV filters. A secondary objective was to distinguish the pathologies of the four chemicals in order to

help ascertain forensic differences in the four chemicals (e.g., does one chemical reduce the light reaction versus dark reaction of photosynthesis?). Another goal of this study was to increase awareness of the potential threat that contaminated biosolids and irrigation waters can pose to agriculture, hopefully with the consequences of not only better assessing and monitoring these agricultural applications to mitigate reductions in crop yield but also better managing the threat that these chemicals can pose to public health through exposure via food consumption.

2. Materials and methods

2.1. Plant materials

A widely cultivated cucumber (*Cucumis sativus* L.) cultivar, "Jinyou-35", was used in this study. All experiments were performed in Tai'an, China. Cucumber seedlings were cultured in plastic pots (7 cm × 7 cm) with culture medium (peat: perlite: horticultural vermiculite = 3:1:1) outside under natural sunlight (the maximum photosynthetic photon flux density (PPFD) of sunlight at noon was approximately 1600 μmol.$m^{-2}.s^{-1}$). One-half-concentration Hoagland nutrient solution (purchased from Sigma Aldrich Company) (Hewitt 1966) and water were provided during the research period. The cultivation conditions were the same as those described by Zhong et al. (2019b).

2.2. Preparation of suspensions of the 4 kinds of UV filters

The four UV filters were purchased from Sigma Aldrich Company. Due to the water insolubility of OBZ, AVB and OMC, they exist as solid particles in aqueous solution rather than forming soluble molecular solutions; therefore, we were unable to use molar concentrations to express their contents in aqueous solution. The four UV filters were first dissolved in small amounts of alcohol to form solutions with equal molarities. These solutions were used to prepare suspensions with the same molar weight, either with 1/2 Hoagland nutrient solution or reaction agents, for use in the experiment. The control group consisted of 1/2 Hoagland nutrient solution or reaction agents with the same amount of alcohol. Although the suspensions of UV filters could not be expressed in molar concentrations, all the suspensions of the four UV filters used in the same treatment were prepared with the same molarity, and we used "same-molar-weight suspensions" (μM.L^{-1}) in this study. The 1 μM.L^{-1} suspensions were 0.228 mg.L^{-1} OBZ, 0.31 mg.L^{-1} AVB, 0.361 mg.L^{-1} OCR, and 0.29 mg.L^{-1} OMC suspensions. The 10 μM.L^{-1}, 50 μM.L^{-1}, 100 μM.L^{-1}, and 200 μM.L^{-1} suspensions refer to the 1 μM.L^{-1} suspension multiplied by 10, 50, 100 and 200, respectively.

2.3. Treatment of the plant material

The cucumber seedlings were treated with the 4 kinds of UV filters, beginning when the first cotyledon fully unfolded. The seedlings were irrigated with 1/2-concentration Hoagland nutrient solution containing the different UV filters with the same molar weight (50 μM.L^{-1}) once a day in the afternoon. The seedlings were irrigated every day with fresh solutions containing the UV filters; a volume twice the maximum water content of the soil medium in the pots was applied (Al-Khaliel, 2010; Duke et al., 1986; Oster et al., 1984), aiming to reduce the UV filters accumulated the day before and to ensure that the UV-filter contents did not fluctuate excessively throughout the treatment period. Although we could not rule out the possibility of UV filter absorption by soil particles, we aimed to simulate natural irrigation conditions under which the UV filters may be absorbed by soil particles. Our objective was to explore the damaging effect of these 4 UV filters under such irrigation conditions. After treatment for five days, seedling growth (plant height, stem diameter, and leaf area) and photosynthetic gas exchange were measured. In a photoinhibition experiment, the cucumber seedlings, after being treated for five days, were placed under natural conditions at noon (12:00–14:00) for 2 h. The environmental conditions were as follows: maximum light intensity (PPFD) of 1600 μmol.$m^{-2}.s^{-1}$, ambient temperature of approximately 33–35 °C, and relative humidity of approximately 55%–61%. After this five-day incubation period, chlorophyll fluorescence quenching and the chlorophyll fluorescence transient were measured.

In an experiment on the influence of UV filters on seedling leaf photosynthesis and respiration, the first fully expanded leaves of the control were cut into discs with a 0.19 cm^2 area, and all the discs were placed into tubes with same-molar-weight suspensions (100 μM.L^{-1}) of the 4 UV filters, slightly vacuumed with a syringe and then soaked in the UV-filter suspensions for 1 h. The discs were then removed to measure photosynthetic and respiration rates. Leaf discs treated with water were used as the controls.

To quantify ROS, the first fully expanded leaves of the control were cut into discs with a 0.785 cm^2 area, and the discs were placed into tubes with the different concentration suspensions (1 μM·L^{-1}, 10 μM·L^{-1}, and 100 μM·L^{-1}) of the four kinds of UV filters, slightly vacuumed with a syringe and then soaked in the suspensions for approximately 1 h. The discs were then placed under 1000 μmol·m^{-2}·s^{-1} light for 2 h. Finally, the O_2^-, H_2O_2 and malondialdehyde (MDA) contents were measured.

To explore the phototoxic effects of the four kinds of UV filters, the first expanded leaves of the control were cut into discs with a 0.19 cm^2 area, and all the discs were placed into tubes with same-concentration suspensions (100 μM·L^{-1}) of the four UV filters, slightly vacuumed with a syringe and then soaked in the suspensions for 1 h. Half of the discs were treated under 1000 μmol·m^{-2}·s^{-1} light, and the other half were placed in the dark. After 2 h, the discs were removed, and the chlorophyll fluorescence and chlorophyll content were measured.

2.4. Measurement of plant growth

Plant height, stem diameter and leaf area were measured after treatment with the UV filters for 5 days. Main stem height was measured as plant height. Stem diameter 1 cm above the base was measured using a micrometer. Leaf area was measured using a CI-202 leaf area meter (CID Inc., USA). The chlorophyll content was measured according to Porra (2002) with an ultraviolet spectrophotometer (UV-1601, Shimadzu, Japan).

2.5. Measurement of gas exchange

Gas exchange measurements were performed using a CIRAS-3 photosynthesis system (PP Systems, USA) under an ambient CO_2 concentration (400 μmol.mol^{-1}), a saturated light intensity (1000 μmol.$m^{-2}.s^{-1}$), room temperature (25 °C) and a 60% relative humidity, which were controlled by a CIRAS-3 automatic control device. All the measurements were performed at 08:00–11:00 on sunny days. The P_n-C_i response curves were constructed according to Zhong et al. (2019b). Carboxylation efficiency (*CE*) was calculated based on the initial slope of the P_n-C_i response curve. The maximal RuBP regeneration rate was calculated as the maximal P_n under a saturated CO_2 concentration and saturated light intensity (Farquhar et al. 1980).

2.6. Measurement of the chlorophyll fluorescence transient

The chlorophyll fluorescence transients (OJIP curves) were measured using a Handy-PEA fluorimeter (Hansatech, UK) according to Strasser et al. (2000).

2.7. Measurement of the photosynthetic and respiration rates of leaf discs

Leaf discs treated with the UV filters were used to measure photosynthetic and respiration rates by an Oxytherm oxygen electrode

(Hansatech, UK). An O_2 saturation curve was established with ambient oxygen-saturated water, and a zero-O_2 curve was established by using $Na_2S_2O_3$ to deplete oxygen in the water. Five leaf discs (0.19 cm^2), 1.8 mL of deionized water and 0.2 mL of 500 mM $NaHCO_3$ were placed into the reaction cup. The respiration rates were recorded after the O_2 uptake became stable in the dark, and the photosynthetic rate was recorded after the O_2 release rate became stable under a 1000 μmol. $m^{-2}.s^{-1}$ light intensity.

2.8. Measurement of O_2^-, H_2O_2 and MDA contents

The O_2^- content was detected using the nitroblue tetrazolium (NBT) staining method according to Kawai-Yamada et al. (2004). The leaf discs treated with UV filters under light were placed into tubes with 0.5 $mg.mL^{-1}$ NBT dye mixture for 4 h, and then the leaf discs were taken out and placed into tubes with 20 mL of destaining solution (lactic acid: glycerinum: absolute ethyl alcohol = 1:1:4). Then, the tubes were placed into a boiling water bath for over 20 min until the leaf discs were completely decolored.

The H_2O_2 content was detected using DAB staining according to Thordal et al. (1997). The leaf discs treated with UV filters under light were placed into tubes with 1 $mg.mL^{-1}$ DAB for over 24 h, and then the leaf discs were taken out and placed into tubes with 20 mL of absolute ethyl alcohol. Then, the tubes were heated in a boiling water bath until the leaf discs were completely decolored.

The MDA content was measured according to Heath and Packer (1968). After treatment with UV filters, 0.1 g of leaf discs was ground with 2 mL of precooled 50 mM phosphate buffer (pH = 7.8) using a mortar and pestle, the pellet was centrifuged at 4000 r for 10 min, and 1 mL of liquid supernatant was added to the tube with 2 mL of 0.6% TBA solution. Then, the tubes were placed into a boiling water bath for 15 min. After cooling, the solution was centrifuged at 4000 r for 10 min, and the MDA content was determined by using the colorimetric method at wavelengths of 532 nm, 450 nm and 600 nm.

The optical density (OD) of the colored leaf discs was analyzed with Image J software (National Institutes of Health, NIH, US). The OD value of leaves treated with 100 $\mu M.L^{-1}$ OBZ was taken as 100%, while the value of those treated with the other UV filters was expressed as a percentage of the value observed for the leaves treated with 100 $\mu M.L^{-1}$ OBZ.

2.9. PET assay

Chloroplast thylakoid membrane extraction was performed according to Zhong et al. (2019a), and photosynthetic electron transport (PET) activities were measured using an Oxytherm oxygen electrode system (Hansatech, UK). The activity of the whole linear electron transport chain was detected as O_2 absorption with the addition of 0.1 mM methylviologen (MV) (Brandle et al., 1977). PET was measured according to Zhong et al. (2019a). The instantaneous inhibition of PET was measured by adding 10 μL of different concentrations of the 4 kinds of UV filters (in alcohol solutions) to the reaction cup during the process of measuring PET activity, ensuring that the final molar weights of these UV filters in the system were the same (10 $\mu M \cdot L^{-1}$, 100 $\mu M \cdot L^{-1}$, and 200 $\mu M \cdot L^{-1}$).The O_2 uptake rate of the control was determined by adding 10 μL of alcohol to the reaction system. The differences in O_2 uptake rate between the treatments before and after adding the four kinds of UV filters were determined to reflect the inhibition degree.

2.10. Measurement of fluorescence quenching

After high-temperature and strong-light treatments, the seedlings were dark adapted for 30 min, and ΦPSII and qP were determined by using an FMS-2 pulse-modulated fluorimeter (Hansatech, UK) according to Zhang et al. (2011). Steady-state fluorescence (Fs) was recorded by illumination under approximately 800 μmol $m^{-2} \cdot s^{-1}$ actinic light, and then a 0.8 s pulse of saturating light of approximately 8000 μmol $m^{-2} \cdot s^{-1}$ was applied to measure the maximum fluorescence under light (F_m'). The actinic light was then switched off, and the minimum fluorescence under light (F_o') was determined after illumination with far-red light for 3 s. When measuring the initiation of actual photochemical efficiency (ΦPSII), ΦPSII was recorded every 30 s, and the total determination time was 630 s.

The following parameters were calculated in accordance with Maxwell and Johnson (2000):

(1) Actual quantum yield of PSII photochemistry: $\Phi PSII = 1 - F_s/F_m' = qP \times F_v'/F_m'$

(2) Linear electron transport rate (ETR) = ΦPSII×PPFD×0.5 × 0.83.

(3) Openness degree of PSII reaction centers: $qP = (F_m' - F_s)/(F_m' - F_o')$

2.11. Statistical analysis

Data were tested for normality (Shapiro-Wilk test) and equal variances. All variances between all the treatments were homogenous, and a Fisher one-way analysis of variance (ANOVA) was applied to the data. To facilitate comparisons between the control and UV-filter treatments, the figure legends represent the results of Dunnett's post hoc tests.

3. Results

3.1. The effects of irrigation with four kinds of UV filters in same-concentration nutrient suspensions on plant growth

After irrigation with four UV filters at the same concentration (50 $\mu M \cdot L^{-1}$) for 5 days, the plant growth of cucumber was significantly inhibited compared with that in the control group (Fig. 1).

3.2. The effects of irrigation with four kinds of UV filters in same-molar-weight nutrient suspensions on the photosynthesis and respiration of plants

After irrigation with 4 UV filters with the same molar weight (50 $\mu M \cdot L^{-1}$) for 5 days, the photosynthesis and respiration of cucumber seedlings were obviously inhibited (Fig. 2 A and B). The g_s of leaves treated with UV filters showed a significant downward trend compared with that in the control group, but the C_i was not significantly different from that in the control group (Fig. 2 C and D). There were also significant differences in the P_n-C_i curve between the different UV-filter treatments (Fig. 2 E), and the *CE* and maximum RuBP regeneration rate of leaves were significantly lower than those in the control group, indicating that all the UV filters inhibited photosynthetic activity via nonstomatal factors (Fig. 2 F and G).

3.3. Effects of 4 kinds of UV filters on the photochemical efficiency of cucumber leaves under light

After 2 h of exposure to natural conditions(light intensity of 1600 $\mu mol.m^{-2}.s^{-1}$, temperature of 34.7 °C, and relative humidity of 61%) at noon (12:00–2:00) in summer, the plants treated with OBZ, AVB and OMC showed obvious photoinhibition under high-light stress after irrigation for 5 days, which indicated that some of the UV filters caused photoinhibition of cucumber plants. When the plants were moved from total darkness to a normal light intensity, their photosynthetic initiation process changed significantly (Fig. 3A), and the time required for the photochemical efficiency (ΦPSII) to reach its maximum, the ETR when the maximum photochemical efficiency was reached, and the openness degree of the PSII reaction centers were significantly lower under the UV filter treatments than in the control group (Fig. 3 B, C and D).

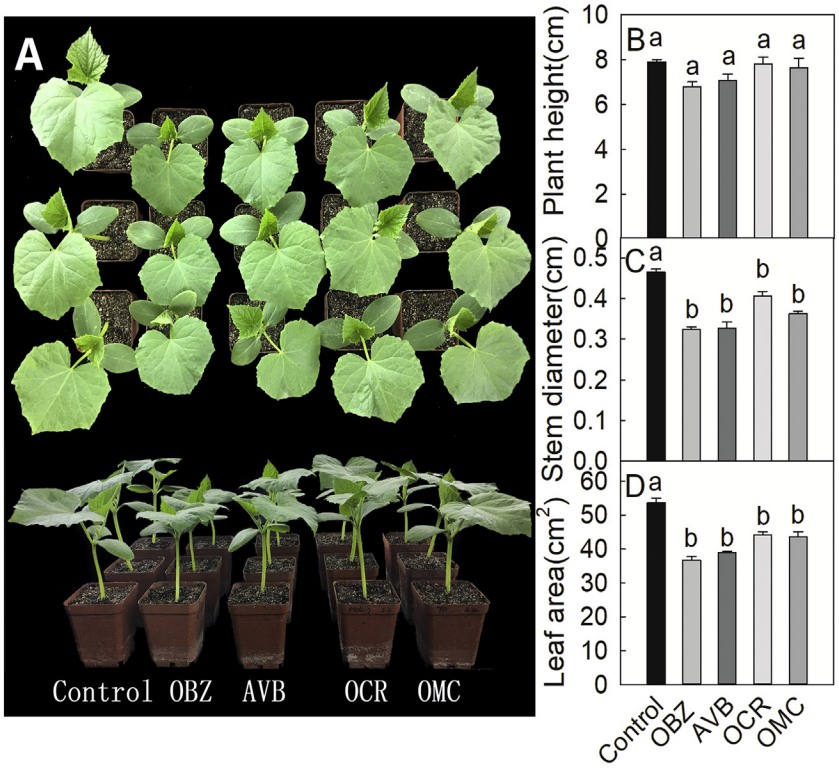

Fig. 1. After irrigation with 4 UV filters with the same molar weight (50 μM.L^{-1}) for 5 days, the effect of UV filters on plant growth (A), plant height (B), stem diameter (C) and leaf area (D). Different letters above bars represent significant differences between the treatment and control groups. In this and all other figures, the 1 μM·L^{-1} suspensions were 0.228 mg·L^{-1} OBZ, 0.31 mg·L^{-1} AVB, 0.361 mg·L^{-1} OCR, and 0.29 mg·L^{-1} OMC suspensions, and the 10 μM·L^{-1}, 50 μM·L^{-1}, 100 μM·L^{-1}, and 200 μM·L^{-1} suspensions refer to the 1 μM·L^{-1} suspension multiplied by 10, 50, 100 and 200, respectively. Data were tested for normality (Shapiro-Wilk test) and equal variances. Variances between all the treatments were homogenous, and a Fisher one-way ANOVA was applied to the data. To facilitate comparisons between the control and UV-filter treatments, the figure legends represent the results of Dunnett's posthoc tests (panel A: $P < .05$ level, $F = 2.829$; panel B: $P < .05$ level, $F = 36.414$; panel C: $P < .05$ level, $F = 37.802$; panel D: $P < .05$ level, $F = 2.983$; panel D: $P < .05$ level, $F = 2.829$; panel E: $P < .05$ level, $F = 3.820$). Error bars represent standard errors.

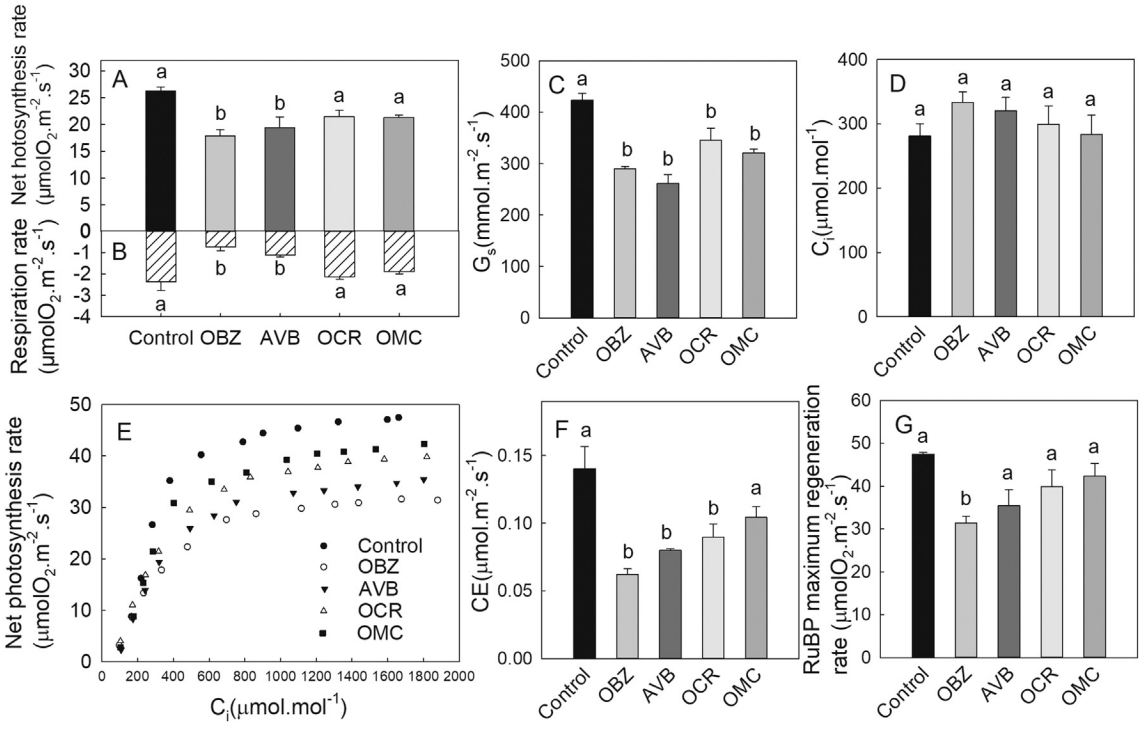

Fig. 2. After irrigation with 4 UV filters with the same molar weight (50 μM·L^{-1}) for 5 days, the effect of the UV filters on the net photosynthetic rate (A), the respiration rate (B), leaf stomatal conductance (C), intercellular CO_2 concentration (D), the P_n-C_i response curve (E), CE (F) and the maximum RuBP regeneration rate (G).Different letters above bars represent significant differences between the treatment and control groups. Data were tested for normality (Shapiro-Wilk test) and equal variances. Variances between all the treatments were homogenous, and a Fisher one-way ANOVA was applied to the data. To facilitate comparisons between the control and UV-filter treatments, the figure legends represent the results of Dunnett's posthoc tests (panel A: $P < .05$ level, $F = 6.466$; panel B: $P < .05$ level, $F = 6.956$;panel C: $P < .05$ level, $F = 12.070$; panel D: $P < .05$ level, $F = 0.943$; panel F: $P < .05$ level, $F = 6.626$; panel G: $P < .05$ level, $F = 3.227$). Error bars represent standard errors.

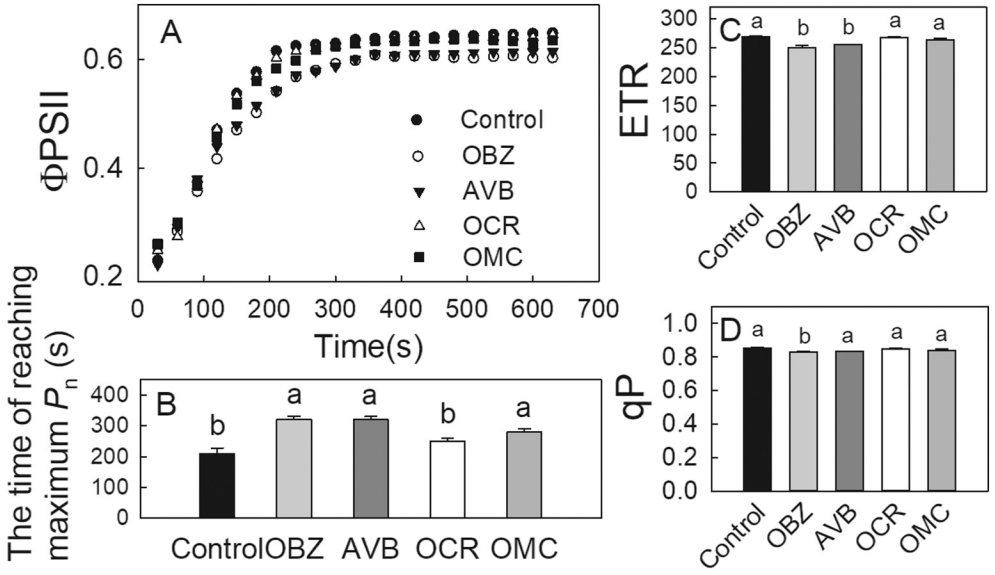

Fig. 3. After irrigation with 4 UV filters with the same molar weight (50 μM·L^{-1}) for 5 days, the effect of the 4 UV-filter treatments on the initiation of actual photochemical efficiency (ΦPSII) (A), the time required for ΦPSII to reach its maximum value (B), the linear ETR (C) and the photochemical quenching (qP) (D)in leaves of cucumber seedlings at noon under outdoor conditions(light intensity of 1600 μmol.m^{-2}.s^{-1}, temperature of 34.7 °C, and relative humidity of 61%). Different letters above bars represent significant differences between the treatment and control groups. Data were tested for normality (Shapiro-Wilk test) and equal variances. Variances between all the treatments were homogenous, and a Fisher one-way ANOVA was applied to the data. To facilitate comparisons between the control and UV-filter treatments, the figure legends represent the results of Dunnett's posthoc tests (panel B: $P < .05$ level, $F = 15.929$; panel C: $P < .05$ level, $F = 13.999$; panel D: $P < .05$ level, $F = 3.907$). Error bars represent standard errors.

3.4. Effects of 4 kinds of UV filters on the chlorophyll fluorescence transient curve of leaves

After treatment under natural summer noon conditions for 2 h (12:00–2:00), the chlorophyll fluorescence transient curves (OJIP curves) of the plants treated with the four UV filters showed obvious differences (Fig. 4 A).The J points of the plants treated with UV filters were significantly higher than those of the control group, which indicated that the UV filters restricted PET from Q_A downstream.

3.5. Effects of UV-filter treatments on the active oxygen content in leaves

The results showed that all four kinds of UV-filter treatments increased the level of ROS, including the O_2^- and H_2O_2contents as well as the MDA content in leaves, which indicated that all the UV filters caused significant membrane lipid peroxidation. For a given UV-filter content, the leaves treated with OBZ exhibited the highest ROS content and the highest membrane lipid peroxidation (Figs. 5, 6, and 7).

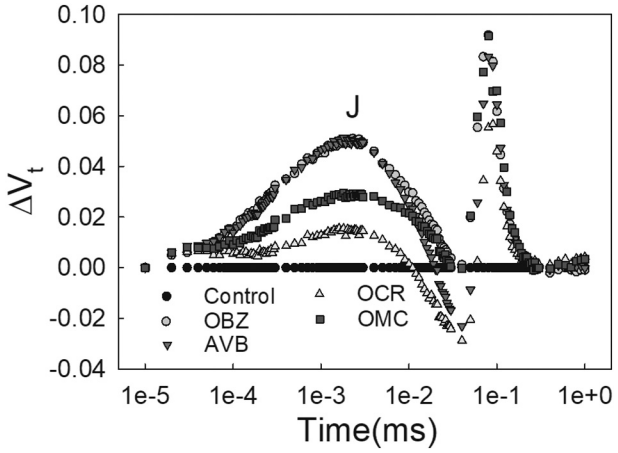

Fig. 4. After irrigation with 4 UV filters with the same molar weight (50 μM·L^{-1}) for 5 days, the effect of the 4 UV-filter treatments on the ΔVt curve of leaves of cucumber seedlings at noon under outdoor conditions. $V_t = (F_j\text{-}F_o)/(F_m\text{-}F_o)$, $\Delta V_t = V_{t(\text{UV-filter treatment})}\text{-}V_{t(\text{control})}$. The values of the points in each curve are the averages of five independent measurements.

3.6. The direct effects of short-term treatment with 4 kinds of UV filters on the photosynthesis and respiration of leaves

The respiration and photosynthetic rates of leaves treated with OBZ for a short period of time were significantly inhibited. Although the photosynthetic rates of leaves treated with the other three UV filters were significantly decreased, there were no significant differences in respiration rate between the three UV filter (AVB, OCR, and OMC) treatments and the control group (Fig. 8).

3.7. Instantaneous inhibition of the PET of cucumber chloroplasts by 4 kinds of UV filters

The results showed that only OBZ instantaneously inhibited the PET activity of chloroplasts, and the degree of PET inhibition increased with increasing OBZ content. However, various AVB, OCR and OMC contents were unable to instantaneously inhibit PET (Fig. 9).

3.8. The phototoxic effects of the 4 UV filters on cucumber leaves

the OBZ significantly increased the J point in the OJIP curve of the leaves under dark conditions compared with that observed in the control, while there were no significant differences in the J point between the control and the other three UV-filter treatments (Fig. 10 A), indicating that OBZ can directly inhibit PET from Q_A downstream without causing photooxidation-induced secondary damage, while the other three UV filters could not. After treatment under light for 2 h, the J point (V_J) of the leaves treated with OBZ markedly increased to the same height as the P point, and the J points of leaves treated with the other three UV filters also increased obviously. The order of the UV filters in terms of the increase in the J point was OBZ > AVB > OMC ≥ OCR. (Fig. 10 A, B, E, and F). The decreases in PSII maximum photochemical

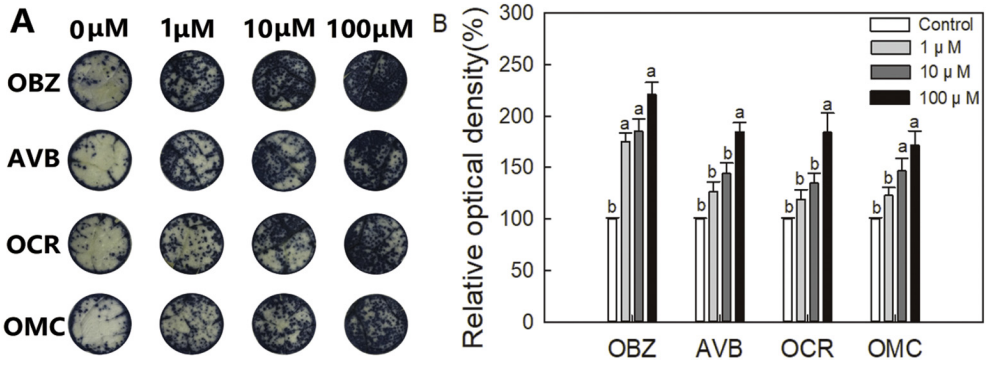

Fig. 5. After treatment with different levels of 4 UV filters under 1000 μmol·m^{-2}·s^{-1} light for 2 h, the effects of the 4 UV-filter treatments on the O_2^- content in leaf discs (A, B). The OD of leaves is used to quantitatively express the ROS content in leaves. The OD value of the control leaves was taken as 100%, while that of the leaves treated with the other UV filters was expressed as a percentage of that in the control group. Different letters above bars represent significant differences between the treatment and control groups. Data were tested for normality (Shapiro-Wilk test) and equal variances. Variances between all the treatments were homogenous, and a Fisher one-way ANOVA was applied to the data. To facilitate comparisons between the control and UV-filter treatments, the figure legends represent the results of Dunnett's posthoc tests (panel B: OBZ: $P < .05$ level, $F = 138.068$; AVB: $P < .05$ level, $F = 33.498$; OCR: $P < .05$ level, $F = 20.625$; OMC: $P < .05$ level, $F = 23.664$). Error bars represent standard errors.

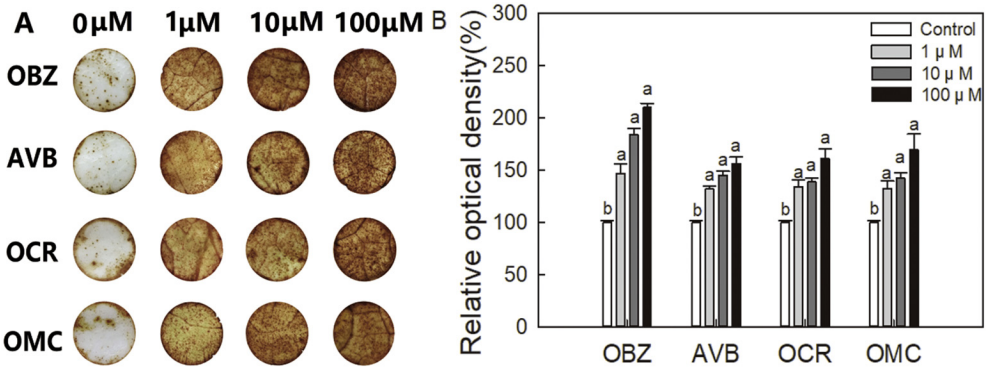

Fig. 6. After treatment with different levels of 4 UV filters under 1000 μmol·m^{-2}·s^{-1} light for 2 h, the effects of the 4 UV-filter treatments on the H_2O_2 content in leaf discs (A,B). The OD of leaves is used to quantitatively express the ROS content in leaves. The OD value of the control leaves was taken as 100%, while that of the leaves treated with the other UV filters was expressed as a percentage of that in the control group. Different letters above bars represent significant differences between the treatment and control groups. Data were tested for normality (Shapiro-Wilk test) and equal variances. Variances between all the treatments were homogenous, and a Fisher one-way ANOVA was applied to the data. To facilitate comparisons between the control and UV-filter treatments, the figure legends represent the results of Dunnett's posthoc tests (panel B: OBZ: $P < .05$ level, $F = 1.271$; AVB: $P < .05$ level, $F = 38.464$; OCR: $P < .05$ level, $F = 14.959$; OMC: $P < .05$ level, $F = 16.325$). Error bars represent standard errors.

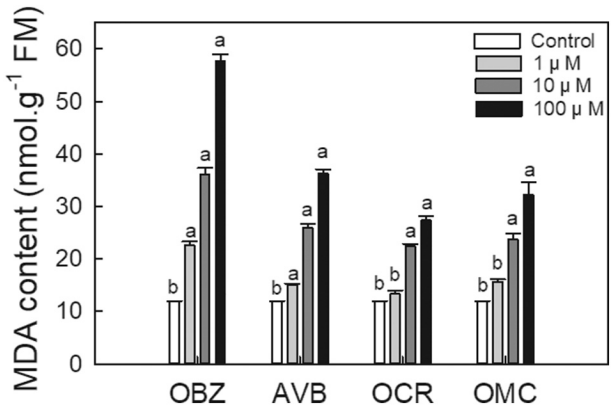

Fig. 7. After treatment with different levels of 4 UV filters under 1000 μmol·m^{-2}·s^{-1} light for 2 h, the effects of the 4 UV-filter treatments on the MDA content in leaf discs. Different letters above bars represent significant differences between the treatment and control groups. Data were tested for normality (Shapiro-Wilk test) and equal variances. Variances between all the treatments were homogenous, and a Fisher one-way ANOVA was applied to the data. To facilitate comparisons between the control and UV-filter treatments, the figure legends represent the results of Dunnett's posthoc tests (OBZ: $P < .05$ level, $F = 290.159$; AVB: $P < .05$ level, $F = 258.568$; OCR: $P < .05$ level, $F = 115.085$; OMC: $P < .05$ level, $F = 30.845$). Error bars represent standard errors.

efficiency (F_v/F_m) in response to all four UV-filter treatments were more severe under light than in the dark, which revealed that the function of PSII was severely damaged by the UV filters under light. The comprehensive performance indexes for the photochemical reaction (PI_{CSO}) under the four UV-filter treatments were significant different from that in the control, and this difference was larger under light conditions than under dark conditions (Fig. 10 G and H). There were no significant differences in leaf chlorophyll content between the four UV-filter treatments and the control group in the dark, while the chlorophyll content of leaves significantly decreased after OBZ treatment under light (Fig. 10 I and J). These results indicated that the four UV filters had different degrees of phototoxic effects, and the possible order of the UV filters in terms of their phototoxic effects was OBZ > AVB > OMC > OCR.

4. Discussion

Our study demonstrated the four UV filters used in chemical sunscreens, namely, OBZ, AVB, OCR and OMC, can significantly inhibit aboveground growth, photosynthesis and respiration of the cucumber plant when irrigated with water containing these four UV filters. The inhibition of light and dark reactions of photosynthesis led to the over accumulation of ROS, further inhibiting plant growth and resulting in chlorophyll bleaching. We also observed that the possible order of the four UV filters in terms of plant damage was OBZ > AVB > OMC > OCR.

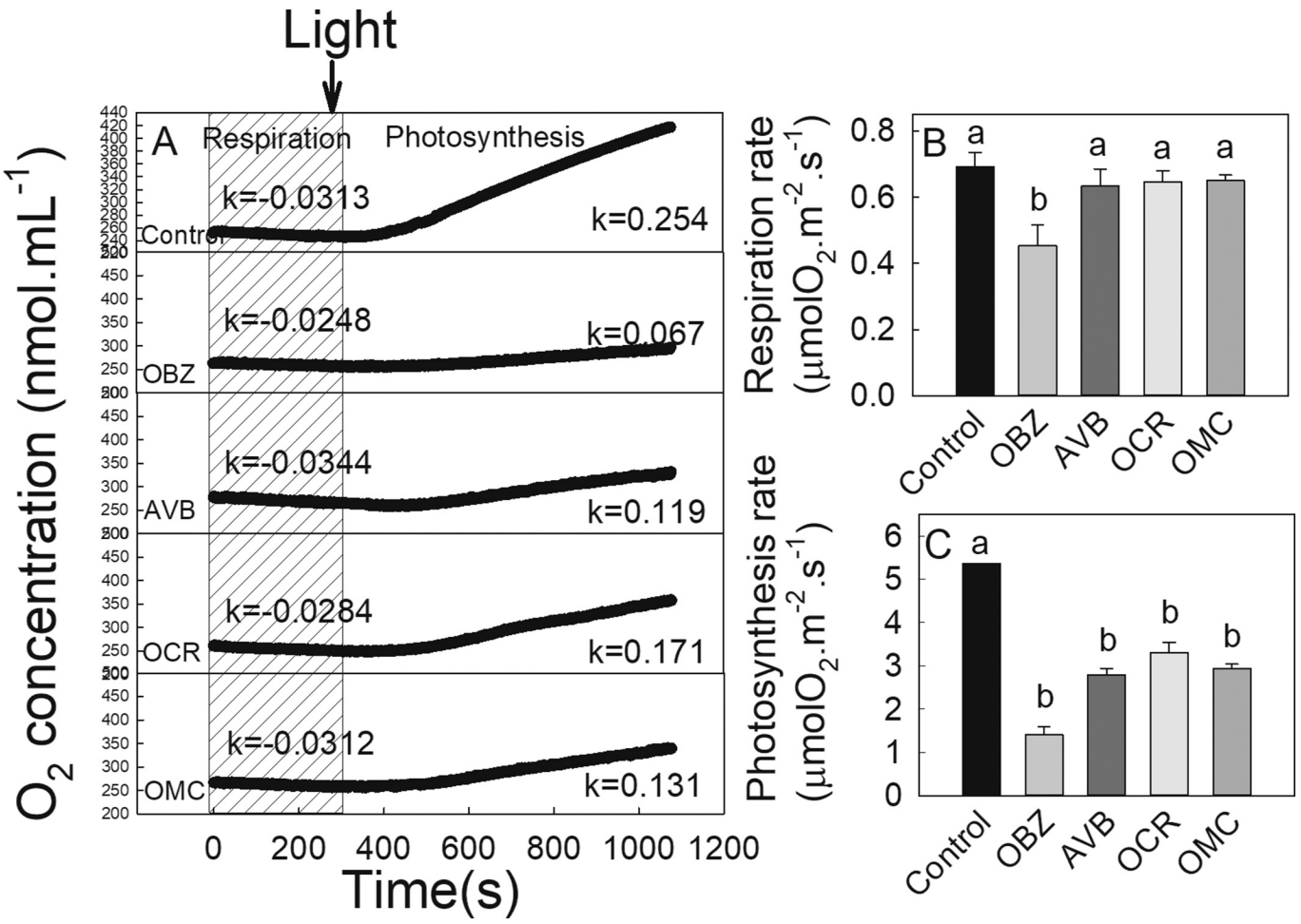

Fig. 8. The direct effects of the four UV filters (100 μM·L^{-1}) on the progress of photosynthesis and respiration, respiration rate (B) and photosynthesis rate (C) of the detached leaf discs. Different letters above bars represent significant differences between the treatment and control groups. Data were tested for normality (Shapiro-Wilk test) and equal variances. Variances between all the treatments were homogenous, and a Fisher one-way ANOVA was applied to the data. To facilitate comparisons between the control and UV-filter treatments, the figure legends represent the results of Dunnett's posthoc tests (panel B: $P < .05$ level, $F = 4.247$; panel C: $P < .05$ level, $F = 77.906$). Error bars represent standard errors.

Previous studies have shown that solid particles released by PPCPs into the natural environment and retained in soil can be absorbed by crops through their root systems (Wu et al., 2010). Our results also demonstrated that even though all four UV filters are not water soluble, the solid particles of these filters present in the soil can significantly affect photosynthesis and respiration in the aboveground parts and inhibiting plant growth (Figs.1, 2, and 8). These 4 UV filters have been widely released into aquatic environments and agricultural irrigation water sources through human activities (Bachelot et al., 2012; Farré et al., 2008; Li et al., 2007). The concentrations of these UV filters found in nature in some regions are close to or even higher than those used in this experiment. For example, Bratkovics et al. found that the concentrations of UV filters in seawater along coastal South Carolina were > 3700 ng·$L^{-1}$for OCR and ~2200 ng·L^{-1} for OBZ (Bratkovics et al., 2015), and the concentrations of OBZ, AVB, OCR and OMC in seawater samples collected from Folly Beach, South Carolina, in the summer of 2010 ranged from 10 to 2013 ng·L^{-1} (Bratkovics and Sapozhnikova, 2011). Downs et al. (2016) found that in some regions of Hawaii, the OBZ content reached 1.395 mg·L^{-1}. The OMC content in untreated water was found to be 500–19,000 ng·L^{-1} in Switzerland (Balmer 2005). If appropriate measures are not taken in a timely manner, the ecological environment of agricultural areas and aquatic systems will be threatened.

To explore the mechanisms by which these 4 UV filters harm plants, we investigated two of the most important life processes in plants: photosynthesis and respiration. Although the stomatal conductance of leaves decreased significantly after treatment with UV filters, the intercellular CO_2 concentration was not significantly different from that of the control group (Fig. 2 B and C), which indicated that the restriction of the photosynthetic rate by the UV filters was caused by the inhibition of mesophyll factors of photosynthesis defined by Farquhar and Sharkey's criterion (Farquhar and Sharkey, 1982). Mesophyll factors mainly include the light reaction (PET) in the chloroplast thylakoid membrane and the dark reaction (Calvin-Benson cycle) in the chloroplast stroma. Our study revealed that only OBZ instantaneously inhibited the PET process of plants, while none of the other three filters had this effect (Fig. 9). Thus, only OBZ can directly inhibit photosynthesis by directly inhibiting the PET of the light reaction, while the other 3 UV filters inhibit photosynthesis by inhibiting the Calvin-Benson cycle (Figs. 2, 3, and 8). In addition to direct inhibition of photosynthesis, all four UV filters inhibited photosynthesis by secondary damage resulting from the overproduction of ROS (Figs. 5, 6, and 7).

Although the gas exchange results showed that the UV filters could limit aboveground plant photosynthesis and respiration through root treatment (Fig. 2), the effects of the UV-filter irrigation treatments on whole plants may include the effect of UV filters on the plant root system, the blocking of root moisture absorption by UV filters, and damage to photosynthetic, respiratory and membrane systems caused by ROS produced after long-term treatment. To explore the direct effects of these 4 UV filters on the photosynthesis and respiration of cucumber

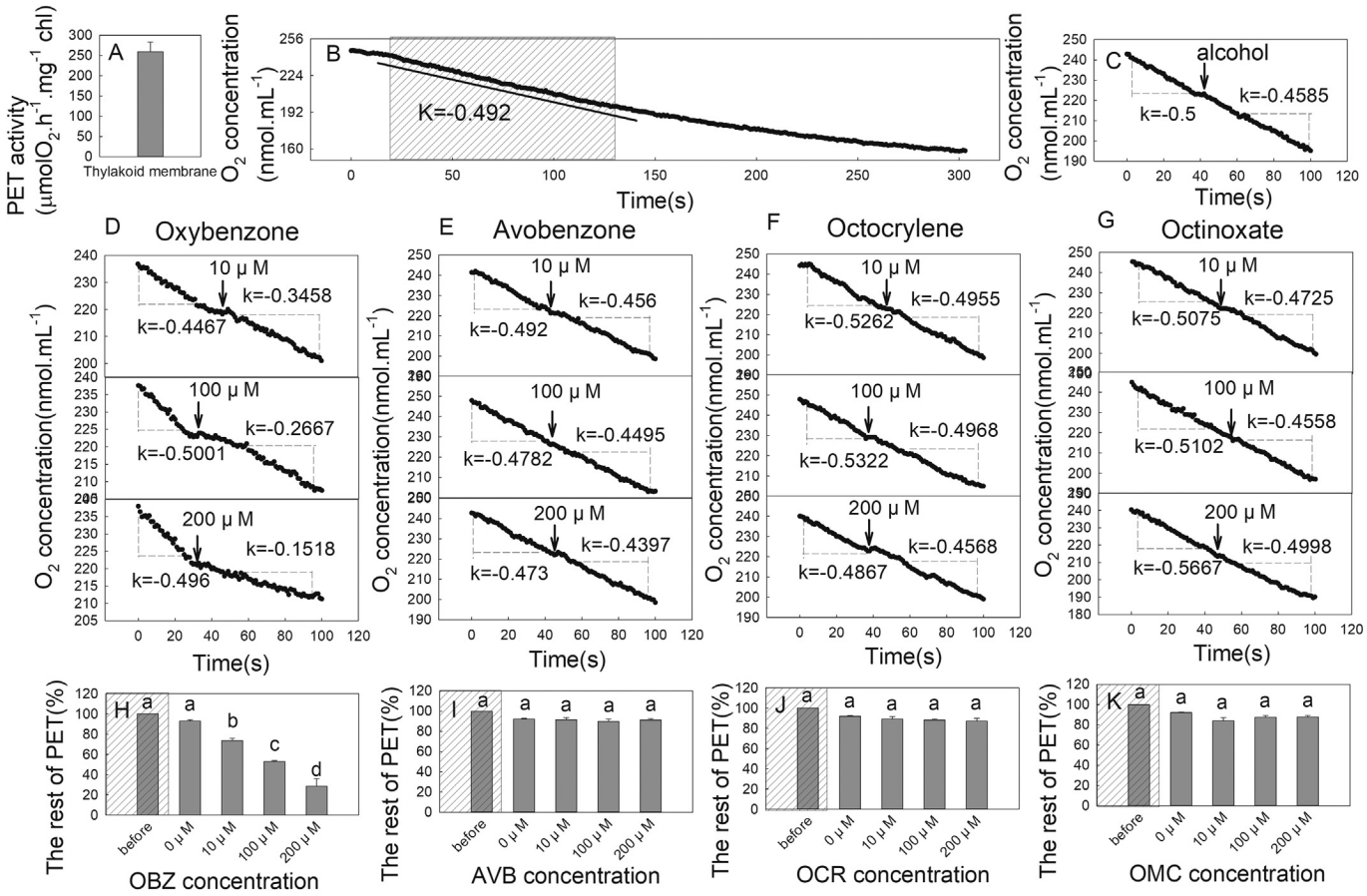

Fig. 9. The instantaneous effects of the four UV filters on PET in cucumber chloroplasts. A: The PET activity per unit chlorophyll in the control group. B: The PET activity measured over five minutes beginning with the first measurement of PET (adding chloroplasts and electron transport receptors to the reaction cup); the straight-line slope segment (the phase in which the PET activity remained unchanged) was used in the next instantaneous inhibition test. C: The change in PET in the control group (caused by the addition of 10 μL of alcohol). D: The changes in PET after adding OBZ with different molar weights. E: The changes in PET after adding AVB with different molar weights. F: The changes in PET after adding OCR with different molar weights. G: The changes in PET after adding OMC with different molar weights. H: The arrest of PET activity after adding OBZ with different molar weights. I: The arrest of PET activity after adding AVB with different molar weights. J: The arrest of PET activity after adding OCR with different molar weights. K: The arrest of PET activity after adding OMC with different molar weights. Different letters above bars represent significant differences between the treatment and control groups. Data were tested for normality (Shapiro-Wilk test) and equal variances. Variances between all the treatments were homogenous, and a Fisher one-way ANOVA was applied to the data. To facilitate comparisons among treatments, figure legends represent results of Holm-Sidak posthoc tests, which compare each treatment to all others (panel H: $P < .05$ level, $F = 51.4$; panel I: $P < .05$ level, $F = 0.350$; panel J: $P < .05$ level, $F = 1.102$; panel K: $P < .05$ level, $F = 3.372$). Error bars represent standard errors.

leaves, normally growing cucumber leaves were immersed in suspensions containing the UV filters for 2 h in the dark, which eliminated light stress and allowed an assessment of the direct effects of these UV filters on the photosynthesis of cucumber leaves in the short term. The results showed that short-term treatment with all 4 kinds of UV filters significantly inhibited the photosynthetic rate (Fig. 8), indicating that although AVB, OCR and OMC cannot directly inhibit PET, they likely inhibit the photosynthetic process by inhibiting the Calvin-Benson cycle.

Under natural summer noon conditions, photoinhibition usually happens because of the high temperatures and strong light (Powles, 1984). The results for the seedlings treated with the 4 kinds of UV filters under natural conditions (light intensity of 1600 $\mu mol \cdot m^{-2} \cdot s^{-1}$, temperature of 34.7 °C, and relative humidity of 61%) at noon (12:00–14:00) in summer for 2 h showed that the UV filters exacerbated the photoinhibition of leaves (Fig. 3). When plants were moved from darkness to light, their photosynthetic rates did not peak immediately, and slower photosynthetic induction took place. Previous studies showed that the inhibition of photosynthetic induction was mainly due to the delay of activation of photosynthetic carbon assimilation enzymes, the accumulation of photosynthetic carbon assimilation intermediates and the closing of stomata in leaves, all of which require some time, and that the speed of photosynthetic induction reflects the state of the photosynthetic apparatus (Edwards and Walker, 1983; Prinsley and Leegood, 1986). Yentsch and Lee (1966) reported that the plant cannot maintain high activities of dark enzymes under darkness or low light intensities and that dark reaction activity is quickly saturated by a number of limitations of activating enzymes during the initiation of photosynthesis when plants are transferred from dark to light. Therefore, it is reasonable to assume that the longer photosynthetic induction time under the UV-filter treatments than in the control was due to the inhibition of enzyme activity related to photosynthetic dark reactions. The change in ΦPSII not only reflects the change in light energy conversion efficiency but also reflects the change in the PET rate. The fact that ΦPSII was significantly lower in the UV-filter treatments than in the control group implies that due to the limitation of dark-reaction enzyme activity, feed-back inhibition of PET happened, which reduced linear electron transport and caused the closure of PSII reaction centers.

Our research demonstrated that only OBZ directly inhibited the respiration of plant leaves (Fig. 6). Our previous study demonstrated that OBZ inhibited the respiration of cucumber plants by directly interfering with the oxidative phosphorylation ETR (Zhong et al., 2019a). However, in this study, we did not observe direct inhibition

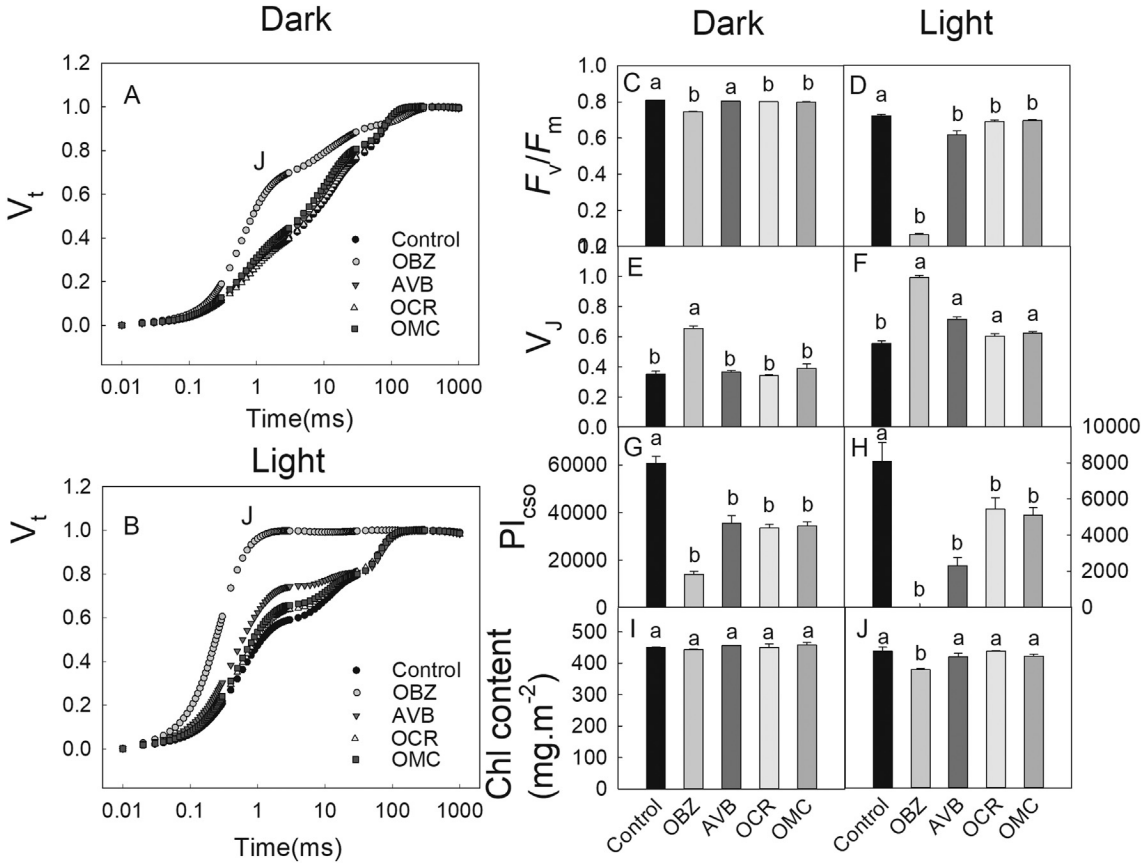

Fig. 10. After treatment with different levels of 4 UV filters under 1000 μmol·m^{-2}·s^{-1} light for 2 h, the effects of the 4 UV-filter treatments on the normalized chlorophyll fluorescence transient (V_t) curve in the dark (A) or under light (B); F_v/F_m in the dark (C) or under light (D); J point of the OJIP curve (V_J) in the dark (E) or under light (F); PI_{CSO} in the dark (G) or under light (H) and chlorophyll content in the dark (I) or under light (J) of the detached leaves. Different letters above bars represent significant differences between the treatment and control groups. Data were tested for normality (Shapiro-Wilk test) and equal variances. Variances between all the treatments were homogenous, and a Fisher one-way ANOVA was applied to the data. To facilitate comparisons between the control and UV-filter treatments, the figure legends represent the results of Dunnett's posthoc tests (panel C: $P < .05$ level, $F = 132.688$; panel D: $P < .05$ level, $F = 1584.733$; panel E: $P < .05$ level, $F = 99.396$; panel F: $P < .05$ level, $F = 41.112$; panel G: $P < .05$ level, $F = 40.5706$; panel H: $P < .05$ level, $F = 32.066$; panel I: $P < .05$ level, $F = 2.066$; panel J: $P < .05$ level, $F = 5.567$). Error bars represent standard errors.

of respiration of cucumber leaves by the other three UV filters, indicating that the other three UV filters do not have a blocking effect on the respiratory ETR of cucumber leaves. The other three UV filters can significantly inhibit plant photosynthesis, and the carbohydrates produced by plants through photosynthesis are the substrate for respiration (Ekblad and Högberg, 2001). The inhibition of photosynthesis by the UV filters significantly reduces the substrates available for respiration, which will inevitably inhibit respiration after longer-term treatment by the other 3 UV filters. In addition, significant inhibition of photosynthesis will increase excess excitation energy and cause overproduction of ROS. The fact that the O_2^-, H_2O_2 and MDA contents all increased with increasing UV-filter content verifies that oxidative stress occurred in the plant leaves. Over accumulation of ROS can damage the membrane structure of mitochondria (Richter and Schweizer, 1997) and inhibit the activity of the enzymes associated with respiration (Kirkinezos and Moraes, 2001). Furthermore, oxygen-evolving complex (OEC) structure is also damaged by ROS (Henmi et al., 2004), which limits O_2 production and further inhibits respiration. Furthermore, the lack of ATP and NADPH production caused by the inhibition of photosynthesis will result in an imbalance of energy metabolism in plants (Kramer and Evans, 2010), leading to a lack of reduced form of nicotinamide-adenine dinucleotid (NADH), which is necessary for respiration. Therefore, it is reasonable to assume that the inhibition of respiration by the other three UV filters (AVB, OCR, and OMC) after a longer treatment period (such as 5 days of treatment in this study or longer) is associated with the inhibition of photosynthesis or other processes.

ROS, which cause severe injury to plants, not only inhibit the respiration of cells but also cause extensive damage to cellular structure and DNA (Blokhina et al., 2003; Richter and Schweizer, 1997). When plants are exposed to light, the suppression of photosynthesis and respiration will result in excessive excitation energy, causing overproduction of ROS and in turn affecting the whole metabolism of the plant, blocking electron transport, and slightly bleaching leaves. Therefore, all 4 UV filters are phototoxic, and their damage to plants is more obvious under light than in the dark (Fig. 10). Under natural light conditions in the field, plants subjected to light stress at noon will experience stronger oxidative stress than under other conditions, which will likely damage agricultural production.

However, the actual concentrations of the filters in the soil and the plant tissue under the given irrigation have not been analyzed in this study, and the pathway that these UV filters enter the plant and their subsequent transfer in plant tissues has not been explored either. These questions are worth studying in future researches. In addition, However, the mechanisms by which OBZ inhibits PET in plants and by which AVB, OCR and OMC specifically inhibit the dark reaction of photosynthesis, whether the degree of damage caused by the 4 UV filters to lower plants such as algae is the same as that to higher plants, and whether the inhibitory mechanisms of the 4 filters are the same across crops still require more research for clarification.

5. Conclusion

The active ingredients in sunscreen (OBZ, AVB, OCR and OMC) can be absorbed by plants through the root system, thus inhibiting their normal photosynthesis, respiration and growth. The possible order of these UV filters in terms of their toxicological effects on cucumber plants is OBZ > AVB > OMC > OCR. The inhibition of photosynthesis by OBZ occurs mainly through the direct inhibition of PET, while that by AVB, OCR and OMC occurs mainly through inhibition of the dark reaction (Calvin-Benson cycle). The inhibition of photosynthesis reduces the substrate available for respiration and leads to excess excitation energy accumulation, which leads to the over production of ROS and inhibits the respiration of plants, thereby aggravating the inhibition of photosynthesis, further damaging the structure and function of cells, and in turn leading to serious inhibition of plant growth and development. The results of this study will enhance recognition of the harmful effects of UV filters on plants and will be significant for the protection of aquatic and terrestrial ecosystems and agricultural production.

Author statement

Xin Zhong, Qingming Li and Huiyuan Gao designed the whole research plan; Xin Zhong performed most of the experiments and wrote the article with Huiyuan Gao and Craig A. Downs; Craig A. Downs also did all the statistical analysis of the data. Yuting Li, Zishan Zhang and Binbin Liu assisted Xin Zhong to do part of the experiments, and provided technical supports for the whole experiment. Qingming Li and Huiyuan Gao supervised and completed the writing. Qingming Li agrees to serve as the author responsible for contact and ensures communication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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