Bulletin of Environmental Contamination and Toxicology (2018) 100:849–855
https://doi.org/10.1007/s00128-018-2331-7

Ameliorating Effects of Leaf Water Extract of Three Aromatic Plant Species on Ozone-Polluted Snap Bean (*Phaseolus vulgaris* L. 'Jiangjunyoudou')

Y. J. Chen[1] · M. X. Wen[1] · J. X. Sui[1] · Y. Q. Yan[1] · W. Yuan[1] · L. Hong[1] · L. Zhang[1]

Received: 5 December 2017 / Accepted: 21 March 2018 / Published online: 23 March 2018

Abstract
Ozone (O_3) is one of the major pollutants in near-surface air. In order to protect sensitive plants from O_3 pollution, many kinds of protectants including synthetic ones, were assessed in previous studies. Although they have certain protective effects, some of them are not environment-friendly. In the present study, leaf water extracts of aromatic plants [*Plectranthus hadiensis* var. *tomentosus* (PHT), *Pelargonium hortorum* (PHB), *Tagetes patula* (TP)] were compared for mitigating the damages caused by O_3 (150 ppb for 3 days, 8 h day^{-1}) on snap bean (*Phaseolus vulgaris* 'Jiangjunyoudou'). Our results showed that O_3 fumigation impaired plasma membrane, decreased chlorophyll content, increased contents of malondialdehyde and superoxide anion, inhibited photosynthesis, and caused visible injury. Leaf water extracts of PHT, PHB or TP ameliorated the negative effects of O_3. Among them, extract of PHT showed the greatest potential to alleviate the O_3-caused injury, followed by PHB and TP.

Keywords Antioxidant enzyme · Gas exchange · Leaf water extract · Ozone injury · *Phaseolus vulgaris*

Ozone (O_3) has become one of the major pollutants in near-surface air (Paoletti and Cudlin 2012). With the acceleration of industrialization and urbanization, tropospheric O_3 has risen about 0.5%–2% annually in the twenty-first century (Feng et al. 2015). In most areas of China, the average O_3 concentration is more than 50–60 ppb per day for 8 h (from 9:00 to 17:00) from late spring to autumn harvest period (Wang and Mauzerall 2004). It has been well reported that elevated O_3 causes serious harm to human, animal and plant species (Feng et al. 2014; Izuta 2017).

As a gaseous strong oxidizer, O_3 enters the leaf tissue through stomata and dissolves into the water solutions in the apoplast (Esposito et al. 2009; Li et al. 2017), and then is transformed into reactive oxygen species (ROS) (Dizengremel et al. 2009; Frei et al. 2010; Cotrozzi et al. 2016). The ROS attacks the cell membrane, induces membrane lipid peroxidation (Li et al. 2017), increases the levels of malondialdehyde, decreases cell membrane stability and ultimately leads to programmed cell death (Zhang et al. 2017b; Dumont et al. 2017). Furthermore, it can inhibit plant net assimilation, change assimilates distribution, slow down plant growth, and reduce crop yield (Fiscus et al. 2005; Singh and Agrawal 2017).

In order to alleviate O_3-caused damage on sensitive plants, a range of protectants have been evaluated, including antioxidants (Zhang et al. 2017a), anti-senescence agents (Navakoudis et al. 2003), growth regulators (Paoletti et al. 2011), flavonoids and polyamines (Didyk and Blum 2011), fungicides (Hassan et al. 2007). Many studies have found that the application of ethylene diurea (EDU) is highly effective on decreasing O_3-caused damage (Manning et al. 2011; Agathokleous 2017; Tiwari 2017). However, synthetic protectants may contaminate environment and agricultural products, and may also cause dose-dependent toxicity of natural vegetation (Didyk and Blum 2011). Moreover, some of these substances are expensive because they are hard to manufacture. It has been reported that leaf water extract from aromatic plants such as French marigold (*Tagetes patula*) and basil (*Ocimum basilicum*) can alleviate O_3 damage to some extent (Blum and Didyk 2006, 2007). We postulated that other aromatic plant species, whose leaf water extract

✉ L. Zhang
caszhanglu@hotmail.com

[1] College of Horticulture and Landscape Architecture, Northeast Agricultural University, Changjiang Road 600, Harbin 150030, China

usually contain flavonoids, could also mitigate O_3 damage. So far, the information about this area is still very limited.

The objectives of this study therefore were: (1) to compare mitigating effects of leaf water extract from three aromatic plant species on O_3-caused injury, (2) to reveal physiological mechanisms related to the different mitigating effect. This study can provide the basis for screening environmental friendly and low-cost reagent resistance to O_3 pollution.

Materials and Methods

Seeds of snap beans (*Phaseolus vulgaris* L. 'Jiangjunyoudou') were bought from Harbin Academy of Agricultural Science, Harbin, CN. On 10 July 2016, the seeds were sown in plastic pots (10 cm in diameter) filled with a mixture of vermiculite and peat (1:2, v:v). Seedlings were cultivated in a greenhouse at the Horticultural Experiment Station (HES) of Northeast Agricultural University (NEAU, 45°74′15″N, 126°73′14″E), Harbin, CN. To avoid water deficiency, the seedlings were irrigated every day to field capacity throughout the experiment.

Leaf water extract of *Tagetes patula* L. (TP), *Pelargonium hortorum* Bailey (PHB), and *Plectranthus hadiensis* var. *tomentosus* (PHT) were prepared according to Huang et al. (2015). Fresh fully expanded and healthy leaves of plants in each species were sampled (25 g). The samples were cut into small pieces, then put into flask filled with 125 mL of distilled water (CK) for 48 h in a dark room. After filtration, a mother liquid (200 g L^{-1}) was obtained and stored in a refrigerator at 4°C.

Six open-top chambers (OTCs, 1 m in diameter and 1 m in height) were constructed with transparent polymethyl methacrylate plates for treatments. On 23 July 2016, 20 potted seedlings were placed in each OTC for 5 days' adaptation [the air was charcoal-filtered (CF) and O_3 concentration was <5 ppb] at the HES of NEAU. During the experiment, the daily average max/min relative humidity and temperature in the OTCs were 63/27% and 29/18°C, respectively. The daytime average photosynthetic photon flux density (PPFD) inside the OTCs was about 850 µmol (photon) m^{-2} s^{-1}. After adaptation every five plants in each OTC were sprayed with diluted leaf water extract (20 g L^{-1}, 10 mL for each plant) of TP, PHB, PHT, or CK (10 mL for each plant), respectively. After 12 h three OTCs were ventilated with O_3, where concentration was maintained at 150 ± 20 ppb for 3 days (from 8:30 to 16:30, 8 h day^{-1}), and the other three chambers were treated with CF air. Every day, when the fumigation was finished, the plants were sprayed again until the end of the experiment (16:30, on 31 July 2016). Ozone was produced by an O_3 generator (CF-KG1, Beijing Sumsun EP Hi-Tech, Beijing, CN). In order to maintain the target value, O_3 concentrations at the plant height were monitored using an O_3 analyzer (Model 202, 2B Technologies Inc., Boulder, Colorado, USA).

After 3 days of exposure, two plants of each spraying treatment from each of six OTCs (O_3 and CF air, $n=3$) were randomly selected for observation. Visible injuries on the first pair of fully expanded leaves in each plant were observed and recorded by three surveyors independently using a 5% and 1% step scale when visible injury was above and below 5%, respectively (Paoletti et al. 2009). Then the values were averaged as visible injury of the plant.

Instantaneous gas exchange was measured on the first pair of fully expanded leaves from 8:00 to 11:30 on the first day after O_3 exposure using a portable photosynthesis system (Li-6400, Li-Cor, Lincoln, NE, USA). Two plants of each spraying treatment from each of six OTCs were randomly selected. The light intensity and the air temperature inside the leaf chamber were set to 1200 µmol (photon) m^{-2} s^{-1} and 35°C, respectively. Ambient CO_2 concentration (410 ± 5 µmol mol^{-1}) was used as reference. Net photosynthetic rate (Pn), stomatal conductance (gs), transpiration rate (E), and the ratio of intercellular/ambient CO_2 concentration (Ci/Ca) were calculated.

The first pair of fully expanded leaves of snap bean were sampled and cut into tiny pieces, then mixed for the following measurements.

Fresh leaf sample (0.2 g) was put into test tubes containing 20 mL of CK at 25°C. After 24 h, electrolyte leakage (EL) was measured using a conductivity meter (Delta 326, Mettler-Toledo, Switzerland) as EL_1. Then tubes were maintained in boiling water bath (100°C) for 20 min. After cooling to 25°C, EL was measured again as EL_2. The relative conductivity was calculated according to Liu et al. (2016) as: $EL_1/EL_2 \times 100\%$.

Fresh leaf sample (0.2 g) was ground and homogenized with trichloroacetic acid (10%). The homogenates were centrifuged at 4000×*g* for 10 min. The supernatants (1 mL) were mixed with 5 mL of thiobarbituric acid (0.6%) and then maintained in boiling water bath for 15 min. After cooling, the mixture was centrifuged at 4000×*g* for 10 min. The absorbance of supernatants was measured using a UV–Visible spectrophotometer (T6 New Century, CN) at 450, 532, and 600 nm. The malondialdehyde (MDA) content was calculated according to Hao et al. (2004) as: $\left[6.45 \times \left(OD_{532} - OD_{600}\right) - 0.56 \times OD_{450}\right]/0.2$.

Leaf pigment content was measured according to Sui et al. (2017). Fresh leaf sample (0.3 g) was ground with quartz sand, calcium carbonate powder, and ethanol (95%, v/v) in a dark room, then was filtered to 25 mL brown volumetric flask. The final constant volume was kept at 25 mL by ethanol. Absorbance was measured using a UV–Visible spectrophotometer (T6 New Century, CN) at 649 and 665 nm. Contents of chlorophyll (Chl) *a*, Chl *b*, and total Chl were calculated.

Fresh leaf sample (0.5 g) was homogenized in ice-cold mortar using 2 mL of 50 mmol L^{-1} phosphate extraction buffer (PBS) (pH 7.8). The residue was rinsed three times with 1 mL of PBS. Then the homogenate was centrifuged at 12,000×*g* for 15 min at 4°C. After that the supernatant was used to determine enzyme activities of superoxide dismutase (SOD), peroxidases (POD), catalase (CAT) and the content of superoxide anion ($O_2^{\bullet-}$). Activity of SOD was based on the methods of Syeed et al. (2011). One unit of SOD activity (U) was defined as the amount of enzyme that inhibited 50% of nitro blue tetrazolium (NBT) photoreduction and the absorbance was read at 560 nm. SOD activity was expressed as U g^{-1} fresh weight (FW). POD activity was determined according to the method described by Wang et al. (2014) with minor modifications. The enzyme extract (0.1 mL) was mixed with 100 mL PBS (50 mmol L^{-1}, pH 6.0) containing 56 µL guaiacol and 38 µL 30% H_2O_2, and the increase in absorbance at 470 nm was monitored for 4 min. Unit of POD activity expressed the amount of enzyme oxidizing 1 µmol (guaiacol) g^{-1} min^{-1} FW. CAT activity was estimated based on the method reported by Diaz-Vivancos et al. (2008), which measures the decline of H_2O_2 at the maximum absorption at 240 nm. One unit was defined as the amount of enzyme catalyzing the decomposition of 1 µmol (H_2O_2) g^{-1} min^{-1} FW. The content of $O_2^{\bullet-}$ was determined according to the method described by Tian et al. (2003). The extract (1 mL) was mixed with 1 mL hydroxylamine hydrochloride (10 mmol L^{-1}), then water bathed (25°C) for 20 min. Absorbance was determined at 530 nm.

The experimental design had three blocks, each block contained one CF air and one O_3 chamber, each chamber had four kinds of spraying treatment, and each treatment contained five replicate plants. All data were analyzed using SPSS (v.12, SPSS, Chicago, IL, USA). Normal distribution was checked by Kolmogorov–Smirnov test. Two-way analysis of variance (ANOVA) was used to identify the effect of O_3 treatment (Treatment), leaf water extract (Extract), and their interactions (ns not significant, $*p \le 0.05$, $**p \le 0.01$, $***p \le 0.001$). Post-hoc Duncan's test was used to compare means of each parameter among different treatments. Relative change of each parameter was expressed as percentage difference between O_3-exposed (O_3) plants and CF air plants, $(O_3 - CF)/CF \times 100\%$.

Results and Discussion

Acute O_3 fumigation induced interveinal chlorotic and necrotic lesions on the adaxial surface of snap bean leaves (Fig. 1). This symptom is consistent with other studies on snap bean, although the cultivars for test are different (Guidi et al. 2009; Paoletti et al. 2014). This result means that 'Jiangjunyoudou' is also a sensitive cultivar. The average

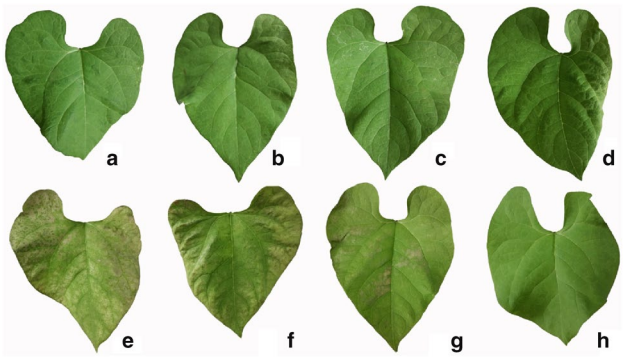

Fig. 1 Leaf visible symptoms of *P. vulgaris* L. 'Jiangjunyoudou' under charcoal-filtered air (CF, 0 ppb) or elevated ozone (O_3, 150 ppb). Plants sprayed with CK (**a**), leaf extract of TP (**b**), of PHB (**c**), or of PHT (**d**) in CF air; plants sprayed with CK (**e**), leaf extract of TP (**f**), of PHB (**g**), or of PHT (**h**) under O_3

Table 1 Visible injury on the first fully expanded leaves in plants of *P. vulgaris* L. 'Jiangjunyoudou' sprayed with CK, leaves extract of TP, of PHB, of PHT under elevated ozone (O_3, 150 ppb) and charcoal-filtered air (CF, 0 ppb)

Treatment	CF visible injury (%)	O_3 visible injury (%)
CK	0	77.17 ± 2.24a
TP	0	52.83 ± 1.08b
PHB	0	24.17 ± 1.54c
PHT	0	5.33 ± 0.80d

Data were shown as means ± S.E. (n = 6). Values with different letters indicate significant differences between treatments (Duncan's test, $p \le 0.05$)

percentage of visible injured surface over the first pair of fully expanded leaves was significantly lower after spraying leaf extract compared with CK (Table 1). The most effective extract was that of PHT (− 93.09% of injury relative to CK), followed by PHB (− 68.68%) and TP (− 31.53%).

Significant interactions between O_3 treatment and extract spraying were found in the Pn, gs, E, and Ci/Ca of snap bean (Fig. 2). Compared with plants in CF air Pn, gs and E were significantly decreased, while Ci/Ca increased under O_3 fumigation. The significant inhibition of Pn and the slight increase of Ci/Ca induced by O_3 fumigation demonstrated that the decline of Pn was mainly caused by non-stomatal factors, probably biochemical processes. These results are in accordance with previous researches (Zhang et al. 2010; Sui et al. 2017). Leaf extract spraying reduced the O_3-caused loss of Pn. The relative loss of Pn was the lowest in PHT (− 27.20%), followed by PHB (− 45.91%), TP (− 69.11%), and CK (− 76.10%). The trend of Pn under O_3 fumigation was in accord with that of visible injury.

Ozone fumigation significantly decreased the contents of Chl *a*, Chl *b* and total Chl (Fig. 3).This result is consistent

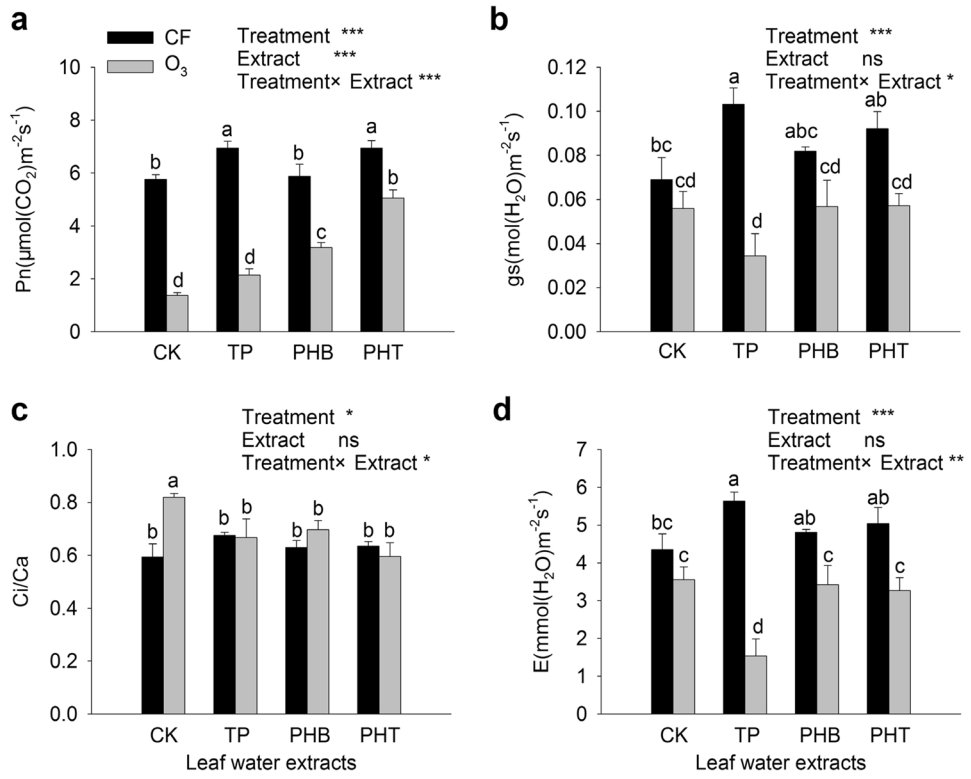

Fig. 2 Net photosynthesis (Pn, **a**), stomatal conductance (gs, **b**), the ratio of intercellular/ambient CO_2 concentration (C_i/C_a, **c**), and transpiration rate (E, **d**) of *P. vulgaris* L. 'Jiangjunyoudou' sprayed with CK, leaf extract of TP, of PHB, or of PHT exposed to charcoal-filtered air (CF, 0 ppb) or elevated ozone (O_3, 150 ppb) for 3 days (8 h per day). Each value is mean of six replicates, error bars represent + SE. Different letters show significant differences among bars (Duncan's test, $p \leq 0.05$). Results of two-way ANOVA are shown in the inset

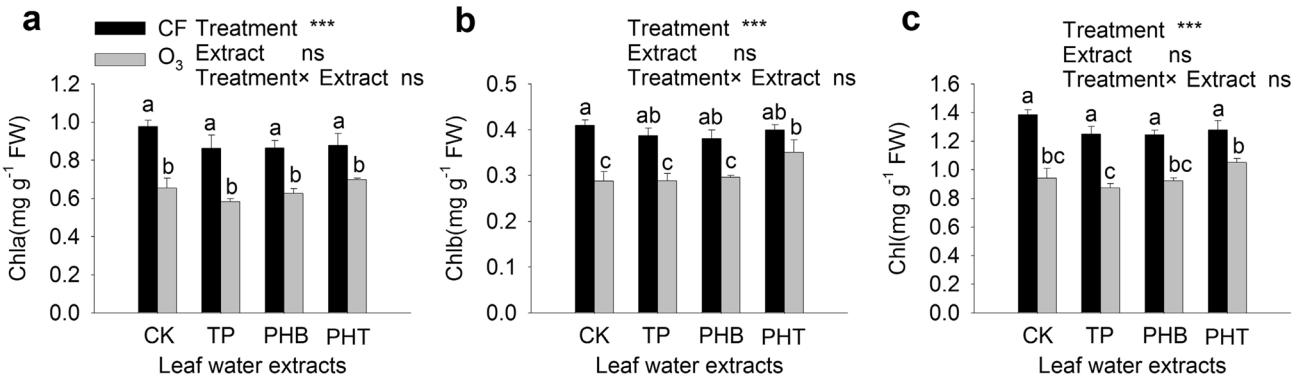

Fig. 3 Contents of chlorophyll (Chl) *a* (**a**), Chl *b* (**b**), total Chl (**c**) of *P. vulgaris* L. 'Jiangjunyoudou' sprayed with CK, leaf extract of TP, of PHB, or of PHT exposed to charcoal-filtered air (CF, 0 ppb) or elevated ozone (O_3, 150 ppb) for 3 days (8 h per day). FW was the FW of the sample. Each value is mean of six replicates, error bars represent + SE. Different letters show significant differences among bars (Duncan's test, $p \leq 0.05$). Results of two-way ANOVA are shown in the inset

with other studies (Bagard et al. 2015; Zhang et al. 2017b). The decrease of Chl probably partially caused the reduction of Pn. Leaf extract spraying had no significant effect on the O_3-caused decrease of Chl. These results indicated that the mitigating effect of leaf extract on the loss of Pn was not due to the change of Chl.

Significant interaction between O_3 treatment and extract spraying was observed in EL and MDA (Fig. 4). Ozone significantly increased EL and MDA content. The significant increases of EL and MDA content were indicative of plasma membrane damage caused by O_3 fumigation (Liu et al. 2015). The relative increase of EL and MDA was the lowest in PHT (0.07% and 4.03%, respectively), followed by PHB (3.31% and 13.03%, respectively), TP (5.72% and 21.46%, respectively) and CK (10.23% and 21.09%, respectively). This result indicated that extract of PHT could significantly protect the plasma membrane from the ROS attack. Responses of EL and MDA to O_3 had a similar pattern to that of visible injury. These results

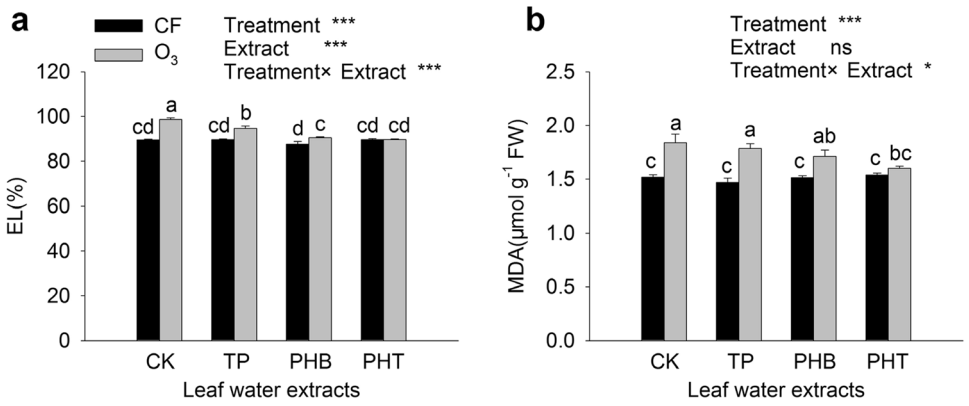

Fig. 4 Electrolyte leakage (EL, **a**) and malondialdehyde contents (MDA, **b**) of *P. vulgaris* L. 'Jiangjunyoudou' sprayed with CK, leaf extract of TP, of PHB, or of PHT under charcoal-filtered air (CF, 0 ppb) and elevated ozone (O_3, 150 ppb) for 3 days (8 h per day). FW is the fresh weight of the sample. Each value is mean of six replicates, error bars represent + SE. Different letters show significant differences among bars (Duncan's test, $p \leq 0.05$). Results of two-way ANOVA are shown in the inset

confirmed that the observed visible injuries were due to, at least in part, cell membrane damage.

The interactions between O_3 treatment and extract spraying on $O_2^{\bullet-}$, SOD, POD and CAT were significant (Fig. 5). Ozone fumigation significantly increased $O_2^{\bullet-}$ contents relative to plants in CF. Leaf extract spraying inhibited the increases of $O_2^{\bullet-}$ contents. The relative increase of $O_2^{\bullet-}$ in the PHT-sprayed plants was the lowest (4.03%), followed by PHB-sprayed plants (15.61%), TP-sprayed plants (20.71%), and CK (33.63%). SOD, POD and CAT can cooperate with each other to remove ROS such as $O_2^{\bullet-}$ produced by plant under stress (Pan et al. 2006). As the first line of defense, SOD is the key to remove $O_2^{\bullet-}$ in cells and convert $O_2^{\bullet-}$ into H_2O_2, which can be scavenged by POD and CAT (Liu et al. 2015; Xu et al. 2015). It has been observed that O_3 fumigation increased the SOD activity (Zhang et al. 2010), while in the current study the activity of SOD was decreased by elevated O_3. This result might be due to increased concentrations of other antioxidants, which had higher ability to scavenge superoxide radicals (Daripa et al. 2016). It has

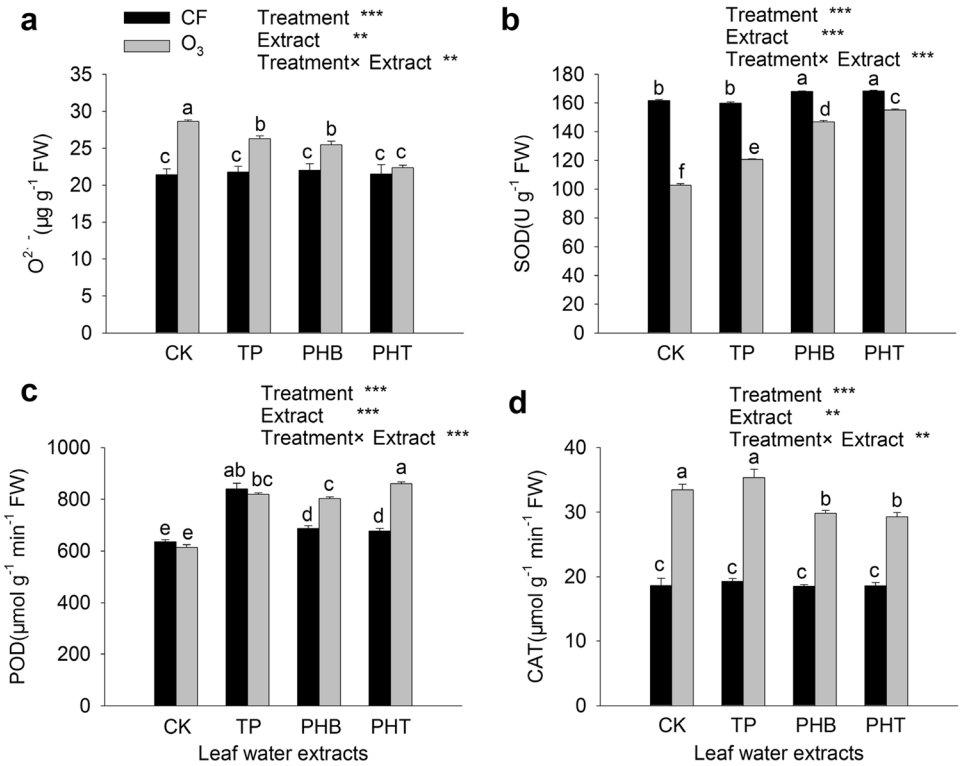

Fig. 5 Superoxide anion content ($O_2^{\bullet-}$, **a**) and SOD (**b**), POD (**c**), CAT (**d**) activities of *P. vulgaris* L. 'Jiangjunyoudou' sprayed with CK, leaf extract of TP, of PHB, or of PHT under charcoal-filtered air (CF, 0 ppb) and elevated ozone (O_3, 150 ppb) for 3 days (8 h per day). FW is the fresh weight of the sample. Each value is mean of six replicates, error bars represent + SE. Different letters show significant differences among bars (Duncan's test, $p \leq 0.05$). Results of two-way ANOVA are shown in the inset

been reported that the leaves of PHT and TP contain lots of flavonoids and phenolic compounds (Blum and Didyk 2006, 2007; Mothana et al. 2010; Li et al. 2014; Rijo et al. 2014; Daripa et al. 2016), which probably played key roles in scavenging the $O_2^{\bullet-}$. Leaf water extract of TP has been proved to alleviate O_3 damage (Blum and Didyk 2006, 2007; Daripa et al. 2016), which is in accordance with our results. PHT and PHB extracts had higher ability to scavenge the $O_2^{\bullet-}$ than TP suggesting their higher contents or activities of antioxidants. Furthermore, in plants treated with PHT extract the relative decrease of SOD activity was lower than that in the other extract treatments after O_3 fumigation. This result suggested that the PHT extract could maintain the SOD activity to a certain degree. This may be another reason why the plant sprayed with PHT extract had lower content of $O_2^{\bullet-}$ than other extract treatments. Under O_3 fumigation, POD activity significantly increased in PHT-sprayed and PHB-sprayed plants, but did not change in TP-sprayed plants and CK. Compared with plants in CF air, the activity of CAT was significantly increased by O_3 fumigation. The relative increases of CAT activity in PHT-sprayed and PHB-sprayed plants were lower than those in TP-sprayed plants and CK. These results showed that POD played more important roles in scavenging ROS in PHT-sprayed and PHB-sprayed plants after $O_2^{\bullet-}$ was converted to H_2O_2. In TP-sprayed or CK plants, H_2O_2 was scavenged depending on the increase of CAT.

In summary, an acute O_3 fumigation caused significant visible foliar injury, impaired the plasma membrane, reduced the content of Chl, increased the content of MDA and $O_2^{\bullet-}$, thus decreased photosynthesis. Spraying with extract of PHT, PHB or TP ameliorated the negative effects of O_3. PHT proved the best ability to mitigate the damages caused by O_3, followed by PHB and TP.

Funding This study was financially supported by National Natural Science Foundation of China (31401895) and University Nursing Program for Young Scholar with Creative Talents in Heilongjiang Province (UNPYSCT-2017023).

References

Agathokleous E (2017) Perspectives for elucidating the ethylenediurea (EDU) mode of action for protection against O_3 phytotoxicity. Ecotoxicol Environ Saf 142:530–537

Bagard M, Jolivet Y, Hasenfratz-Sauder MP et al (2015) Ozone exposure and flux-based response functions for photosynthetic traits in wheat, maize and poplar. Environ Pollut 206:411–420

Blum O, Didyk N (2007) Study of ambient ozone phytotoxicity in Ukraine and ozone protective effect of some antioxidants. J Hazard Mater 149:598–602

Blum O, Didyk N (2006) Ambient ozone phytodetection with sensitive clover (*Trifolium subterraneum* L. cv. Geraldton) in Ukraine. In: Arapis G et al (ed) Ecological risk assessment and multiple stressors. Ecotoxicology. Springer, Dordrecht, pp 279–289

Cotrozzi L, Remorini D, Pellegrini E et al (2016) Variations in physiological and biochemical traits of oak seedlings grown under drought and ozone stress. Physiol Plant 157:69–84

Daripa A, Bhatia A, Ojha S et al (2016) Chemical and natural plant extract in ameliorating negative impact of tropospheric ozone on wheat crop: a case study in a part of semiarid north west india. Aerosol Air Qual Res 16:1742–1756

Díaz-Vivancos P, Clemente-Moreno MJ, Rubio M et al (2008) Alteration in the chloroplastic metabolism leads to ROS accumulation in pea plants in response to plum pox virus. J Exp Bot 59:2147–2160

Didyk NP, Blum OB (2011) Natural antioxidants of plant origin against ozone damage of sensitive crops. Acta Physiol Plant 33:25–34

Dizengremel P, Thiec DL, Hasenfratz-Sauder MP et al (2009) Metabolic-dependent changes in plant cell redox power after ozone exposure. Plant Biol 11:35–42

Dumont J, Keskisaari S, Keinänen M et al (2017) Ozone affects ascorbate and glutathione biosynthesis as well as amino acid contents in three Euramerican poplar genotypes. Tree Physiol 34:253–266

Esposito MP, Ferreira ML, Sant'Anna SMR et al (2009) Relationship between leaf antioxidants and ozone injury in *Nicotiana tabacum* 'Bel-W3' under environmental conditions in São Paulo, SE—Brazil. Atmos Environ 43:619–623

Feng Z, Sun J, Wan W et al (2014) Evidence of widespread ozone induced visible injury on plants in Beijing, China. Environ Pollut 193:296–301

Feng Z, Hu E, Wang X et al (2015) Ground-level O_3 pollution and its impacts on food crops in China: a review. Environ Pollut 199:42–48

Fiscus EL, Booker FL, Burkey KO (2005) Crop responses to ozone: uptake, modes of action, carbon assimilation and partitioning. Plant Cell Environ 28:997–1011

Frei M, Tanaka JP, Chen CP et al (2010) Mechanisms of ozone tolerance in rice: characterization of two QTLs affecting leaf bronzing by gene expression profiling and biochemical analyses. J Exp Bot 61:1405–1417

Guidi L, Degl'Innocenti E, Martinelli F, Piras M (2009) Ozone effects on carbon metabolism in sensitive and insensitive *phaseolus* cultivars. Environ Exp Bot 66:117–125

Hao Z, Chang J, Xu Z (2004) Plant physiology experiment. Harbin Inst Technol Press, Harbin, pp 106–108 (**Chinese**)

Hassan IA, Bell JNB, Marshall FM (2007) Effects of air filtration on Egyptian clover (*Trifolium alexandrium* L. cv. Messkawy) grown in open top chambers in a rural site in Egypt. Res J Biol Sci 2:395–402

Huang YM, Zhang YX, Liu QL et al (2015) Research on allelopathy of aqueous extract from *Tagetes patula* to four garden plants. Acta Pratacult Sin 24:150–158 (**Chinese**)

Izuta T (2017) Air pollution impacts on plants in East Asia. Springer, Tokyo, p. 321

Li AN, Li S, Li HB et al (2014) Total phenolic contents and antioxidant capacities of 51 edible and wild flowers. J Funct Foods 6:319–330

Li S, Harley PC, Niinemets Ü (2017) Ozone-induced foliar damage and release of stress volatiles is highly dependent on stomatal openness and priming by low-level ozone exposure in *Phaseolus vulgaris*. Plant Cell Environ 40:1984–2003

Liu X, Sui L, Huang Y et al (2015) Physiological and visible injury responses in different growth stages of winter wheat to ozone stress and the protection of spermidine. Atmos Pollut Res 6:596–604

Liu L, Li H, Zeng H et al (2016) Exogenous jasmonic acid and cytokinin antagonistically regulate rice flag leaf senescence by mediating chlorophyll degradation, membrane deterioration, and senescence-associated genes expression. J Plant Growth Regul 35:366–376

Manning WJ, Paoletti E, Sandermann H Jr, Ernst D (2011) Ethylenediurea (EDU): a research tool for assessment and verification of

the effects of ground level ozone on plants under natural conditions. Environ Pollut 159:3283–3293
Mothana RAA, Abdo SAA, Hasson S et al (2010) Antimicrobial, antioxidant and cytotoxic activities and phytochemical screening of some yemeni medicinal plants. Evid Based Complement Alternat Med 7:323–330
Navakoudis E, Lütz C, Langebartels C et al (2003) Ozone impact on the photosynthetic apparatus and the protective role of polyamines. Biochim Biophys Acta 1621:160–169
Pan Y, Wu LJ, Yu ZL (2006) Effect of salt and drought stress on antioxidant enzymes activities and SOD isoenzymes of liquorice (*Glycyrrhiza uralensis Fisch*). Plant Growth Regul 49:157–165
Paoletti E, Cudlin P (2012) Ozone, climate change and forests. Environ Pollut 169:249
Paoletti E, Ferrara AM, Calatayud V et al (2009) Deciduous shrubs for ozone bioindication: *Hibiscus syriacus* as an example. Environ Pollut 157:865–870
Paoletti E, Manning WJ, Ferrara AM, Tagliaferro F (2011) Soil drench of ethylenediurea (EDU) protects sensitive trees from ozone injury. iforest 4:66–68
Paoletti E, Castagna A, Ederli L et al (2014) Gene expression in snap-beans exposed to ozone and protected by ethylenediurea. Environ Pollut 193:1–5
Rijo P, Matias D, Fernandes AS et al (2014) Antimicrobial plant extracts encapsulated into polymeric beads for potential application on the skin. Polymers 6:479–490
Singh AA, Agrawal SB (2017) Tropospheric ozone pollution in India: effects on crop yield and product quality. Environ Sci Pollut Res 24:4367–4382
Sui JX, Wen MX, Jia LL et al (2017) Effects of elevated ozone on polka dot plant (*Hypoestes phyllostachya*) with variegated leaves. Bull Environ Contam Toxicol 99:445–451
Syeed S, Anjum NA, Nazar R et al (2011) Salicylic acid-mediated changes in photosynthesis, nutrients content and antioxidant metabolism in two mustard (*Brassica juncea* L.) cultivars differing in salt tolerance. Acta Physiol Plant 33:877–886
Tian M, Gu Q, Zhu M (2003) The involvement of hydrogen peroxide and antioxidant enzymes in the process of shoot organogenesis of strawberry callus. Plant Sci 165:701–707
Tiwari S (2017) Ethylenediurea as a potential tool in evaluating ozone phytotoxicity: a review study on physiological, biochemical and morphological responses of plants. Environ Sci Pollut Res 24:14019–14039
Wang X, Mauzerall DL (2004) Characterizing distributions of surface ozone and its impact on grain production in China, Japan and South Korea: 1990 and 2020. Atmos Environ 38:4383–4402
Wang J, Zeng Q, Zhu J et al (2014) Apoplastic antioxidant enzyme responses to chronic free-air ozone exposure in two different ozone-sensitive wheat cultivars. Plant Physiol Bioch 82:183–193
Xu S, He X, Chen W et al (2015) Differential sensitivity of four urban tree species to elevated O_3. Urban For Urban Gree 14:1166–1173
Zhang L, Xu H, Yang JC et al (2010) Photosynthetic characteristics of diploid honeysuckle (*Lonicera japonica* Thunb.) and its autotetraploid cultivar subjected to elevated ozone exposure. Photosynthetica 48:87–95
Zhang L, Jia LL, Sui JX et al (2017a) Ameliorating effects of three kinds of antioxidants to ozone-polluted painted nettle (*Coleus blumei*, benth.). Photosynthetica. https://doi.org/10.1007/s11099-017-0693-4
Zhang L, Xiao S, Chen YJ et al (2017b) Ozone sensitivity of four Pakchoi cultivars with different leaf colors: physiological and biochemical mechanisms. Photosynthetica 55:478–490