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Interactive effects and competitive shift between *Triticum aestivum* L. (wheat) and *Chenopodium album* L. (fat-hen) under ambient and elevated ozone[☆]

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ABSTRACT

The aim of this study was to evaluate the impact of ambient and elevated O₃ (ambient+20 ppb) under the competition between a crop plant *Triticum aestivum* L.cv. HD 2967 and a weed, *Chenopodium album* L. (fat-hen) grown singly and in mix-culture (1:1) using open-top chambers. The competition posed a relatively lesser effect on the growth of fat-hen as compared to the wheat under ambient O₃ at both the sampling time, however, the effects of stress factors (competition and O₃) were more pronounced at the reproductive stage on both the plants. Fat-hen possess a stronger antioxidative potential against elevated O₃ (eO₃), irrespective of competition, making it more resistant against the existing stress factors. Significant stimulation in the activities of CAT, POX, GR and SOD in fat-hen and non-enzyme antioxidants (AsA, thiols, and total phenolics) might have helped the plants to pose a superior ROS scavenging potential under competition + O₃. Strong stimulation of flavonols (kaempferol and quercetin) and phenolic acid (*p*-coumaric acid and ferulic acid) in fat-hen not only helped the plants to withstand the oxidative damage under eO₃ but also might have influenced the allelopathic interaction (competition + O₃). Yield loss in wheat was observed to be larger under competition + O₃ (33.1%) followed by O₃ (20.5%) than only under competition (16.3%). The study suggests stringent weed management strategies to be established recognizing the existing threat from O₃ to the productivity of a staple crop-wheat.

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1. Introduction

Since the last 50 years of rapid industrial development, emission of fossil fuel exhaust has increased across the globe (Zou et al., 2016), with Tropical Asia being recognised for its favourable meteorological conditions conducive for high photochemical activity (David et al., 2019). Tropospheric region of the atmosphere dominated by such photochemical reactions concomitant with increased pollutant emissions from anthropogenic sources generally give rise to high ambient surface-level ozone (O₃) concentration (Simpson et al., 2014). The growing problem of O₃ pollutant has gained much limelight due to its high phytotoxic nature (Guarin et al., 2019) and thereby increasing vulnerability of agricultural

sectors with subsequent economic loss (Hu et al., 2020; Shao et al., 2020; Feng et al., 2019; Mills et al., 2018).

The deleterious effects of alarming O₃ pollution hovering over Indo-Gangetic Plain (IGP) has been assessed based on the data from satellite images (Kumari et al., 2018) and different atmospheric models (Sharma et al., 2020). Recently, a modelling study conducted by Sharma et al. (2019) estimated an O₃ mediated relative yield loss of about 21% for wheat and 6% for rice in India. More than 50% of the countrywide crop production losses accounted for rice and wheat under existing high O₃ concentration predominated in the IGP region, commonly contemplated as agriculture dominated regions of the Northern India (Sharma et al., 2019). Studies have projected the future rise of O₃ concentration (2045–2054) with much higher increment over IGP which may endanger the food security of the nation until effective mitigation measures are being implemented to tackle the emission of its precursors.

Besides the menace of O₃ pollution in agriculture, the inter-specific competition also delineates the struggle of the crop

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plants for its healthy survival under a competitive environment. The invasive characteristics of weed coupled with its poly-phenotypic nature and large genetic diversity generally provide them advantages for their luxuriant growth over crop plants, especially under the changing pattern of climate conditions (Clements et al., 2014). Oerke (2006) reported ~34% annual yield loss for some major crop species due to weed interference.

Chenopodium album L. (fat-hen) is a vigorously growing invasive weed with almost all winter sown crops of subtropical and tropical regions. It is a weedy annual often used as a vegetable crop in Northern India, therefore, often allowed growing along with the wheat and other winter grown crops. Some of the conventional field and pot trial studies have already reported the deleterious effects of fat-hen on the wheat yield (Oad et al., 2007). A field trial experiment by Anjum and Bajwa (2010) reported 50–60% of biomass loss in the wheat cultivars Inq1ab-91 and Punjab-96 due to competition from fat-hen.

An important factor regulating the aggressiveness of weed species is their potential allelopathic interference, which was realized later in the field of agro-ecosystem management. The study of Majeed et al. (2012) concluded that the allelopathic activity of fat-hen which corresponded to the presence of a high concentration of alkaloidal and phenolic compounds in their leaves were assumed to reach the soil and thereby affecting the wheat plants by altering its absorption capacity for nutrients and other physiological functions. Since limited information is available regarding the mechanism underlying the effects of integrated O₃ pollution on the interaction between crop and weed species, the present study mainly focuses to examine (i) Is there any metabolic adjustment/shift in the crop-weed interaction under ambient and eO₃, based on their morphological and physiological alteration? (ii) To find the change in economic yield under individual and combined stresses of competition and eO₃. Furthermore, we hypothesized a shift in the production of allelochemicals (phenolic compounds) under eO₃ in both wheat and fat-hen, which might protect the wheat/fat-hen to some extent against the combined effect of competition and O₃ stress.

2. Material and methods

2.1. Experimental site, test plants and field preparation

The experiment area was located at the Banaras Hindu University, Varanasi (25° 81' N and 83° 1' E) (Botanical Garden, Department of Botany). Experiments were carried out from mid-November 2018 to mid-April 2019. Popular wheat (*Triticum aestivum* L.) cultivar HD 2967 of Northern India was used as a test plant. Seeds of wheat and fat-hen were hand sown inside the open-top chambers (OTCs) maintaining a distance of 10 cm between the plants (wheat-wheat/wheat-fat-hen) and both the plants species under mix-culture conditions were maintained till wheat's maturity stage. Doses and methods of application of fertilizer along with other agronomic practices were similar as described in the study of Ghosh et al. (2020a).

2.2. Ozone treatment

The experiments were performed in OTCs adopting the design of Bell and Ashmore (1986). Experimental setup comprised of six treatments for each test plants: (i) monoculture of wheat/(SAW)/fat-hen (SAC) under ambient O₃; (ii) monoculture of wheat (SEW)/fat-hen (SEC) under eO₃; and (iii) mix-culture wheat MAW/fat-hen (MAC) under ambient O₃ and (iv) mix-culture wheat (MEW)/fat-hen (MEC) under eO₃. The wheat/fat-hen ratios were achieved as 20:20 under mix-culture condition, 50:0 under wheat

monoculture and 40:0 under monoculture of fat-hen, respectively. For each treatment, three chambers were installed for replication, thus making use of 18 OTCs in total. Blowers of high-speed were installed as described by Ghosh et al. (2020b) along with O₃ generators since 14 days after germination till the wheat plants reach its maturity. Daily 4 h O₃ exposure was provided to the test plants from 11:00 a.m. to 3:00 p.m. of local standard time.

2.3. AOT40 calculation and air quality monitoring

Ozone monitoring for continuous 8 h (9:00 to 17:00 h) was carried out with the help of O₃ analyzers (Model APOA 370, HORIBA Ltd., Kyoto, Japan) all through the growth period of the wheat plants (Ghosh et al., 2020). Accumulated O₃ beyond a threshold limit of 40 ppb or AOT 40 was calculated for the present study with the help of the formula of Mauzerall and Wang (2001).

2.4. Plant sampling and analysis

Plant sampling was done from each treatment at 40 and 80 days after germination (DAG) for various analyses. Randomly three plants were selected from each chamber, hence constituting a replication of nine for each treatment (n = 9). Soil monoliths (11 × 11 × 20 cm) were dug for the collection of intact roots parts. Root length (RL), plant height (PH), and leaf area (LA) per plant were evaluated as growth parameters. Leaf area was estimated using a portable leaf area meter (Model LI-3000, LI-COR, Inc. USA). To carry out the analysis for biomass (TB), plant parts were separated and dried in oven to acquire constant weight. Calculation of root shoot ratio (RSR) was taken as a ratio of root biomass to shoot biomass.

2.5. Gas exchange parameters

Leaf gas exchange and chlorophyll fluorescence parameters were measured at different sampling times (40 and 80 DAG) using Ciras-3 Portable Photosynthesis System, US. Recording of measurements was done as already described by Ghosh et al. (2020a). Three replicates were taken for measurements on a random basis from each OTC, thereby making a total of nine measurements for each treatment for each plant species (n = 9). To calculate the O₃ flux, stomatal conductance was measured at an hourly interval after every 10 days in different treatments (9:00 to 16:00 h of local time). The stomatal uptake of O₃ was calculated as given by Yadav et al. (2019).

2.6. Biochemical parameters

Fully expanded third leaves of wheat and fat-hen from the canopy top was selected randomly from three plants from each of the chamber of different treatment at 40 DAG, while flag leaves of wheat and second branched leaves from canopy top for fat-hen were sampled at 80 DAG. Three samples were selected randomly from each of the OTC per treatment (n = 9). Processes of enzyme extraction (superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), and ascorbate peroxidase (APX) and their respective spectrophotometric assay has been elaborately described by Singh et al. (2014). The standardized protocols of Schaedle and Bassham (1977) and Bradford (1976) were followed for the estimation of glutathione reductase (GR) activity and protein quantification, respectively.

Details of the extraction procedure for the assay of superoxide production (*O₂), hydrogen peroxide (H₂O₂) content, and total phenol content (TP) have been provided in the study conducted by Singh et al. (2014). The estimation of malondialdehyde (MDA), a

marker for lipid peroxidation, and total flavonoids (TF) (in roots and leaves) have been elaborated in the study of [Fatima et al. \(2018a\)](#). The standardized protocols of [Babbs and Gale \(1987\)](#) and [Keller and Schwager \(1977\)](#) were followed for the estimation of hydroxyl radical content (*OH) and ascorbic acid, respectively. The protocol of [Sedlak and Lindsay \(1968\)](#) has been followed for the assay of total thiols content.

Detailing of estimation procedures for free radical scavenging activities {superoxide radical (SSA), hydrogen peroxide (HSA) and hydroxyl radical scavenging activity (HOSA)}, chlorophyll *a* and *b* and carotenoid contents estimation is elaborated in [Takshak and Agrawal \(2015, 2014\)](#), respectively.

Extraction procedures of quercetin and kaempferol have been performed following the method of [Choudhary and Agrawal \(2014\)](#). The extraction procedure for ferulic acid and *p*-coumaric acid was similar to that of quercetin and kaempferol. The assay was carried out on an isocratic HPLC (Waters, USA) with a photodiode array detector (Waters, 2998), HPLC pump (Waters, 515), and reverse-phase C18 column (Nova-pak). Methanol: (0.8%) formic acid dissolved in sonicated double distilled water (6:4) has been used as the mobile phase with 1 ml/min flow rate, 20 μ l injection volume and 35 °C as column temperature. The wavelength used for detection fixed at 326 nm.

2.7. Soil parameters

Description of the collection of soil samples and further processing at different growth stages of the plants have been mentioned in the study by [Fatima et al. \(2018b\)](#). For soil collection, plants were uprooted with intact cores of the soil (6 × 6 × 15 cm³). The adhered soil particles to the roots of the respective plant species were collected for the analyses. The protocol of [Tabatabai \(1994\)](#) was followed for the assay of amylase. Microbial biomass was estimated following the methodology of [Pandey et al. \(2015\)](#). Activities of β -glucosidase and polyphenol oxidase (PPO) were analyzed by using the procedures of [Allison and Jastrow \(2006\)](#), whereas the methodology of [Kandeler and Gerber \(1988\)](#) was followed to measure the activity of urease.

2.8. Yield attributes

At 135 DAG, final harvesting of wheat was performed and yield was assessed as the weight of grains/m².

2.9. Statistical analysis

Before performing the statistical analysis, the whole data set was checked for the normality and homogeneity based on Shapiro Wilk and Levene's test. Each OTC chamber was considered as a statistical unit to carry out the statistical analysis. Data of plant growth, gas exchange, chlorophyll fluorescence and biochemical parameters along with soil parameters were subjected to one-way ANOVA, to examine the effects of different treatments. Tukey's post hoc test was also performed to illustrate the significant differences between each treatment for each growth stage denoted by lower-case alphabets. Moreover, a three-way ANOVA was also carried out on the similar data set to find the individual and combined effects of the respective factors (O₃, Competition (C), sampling time (T)).

Partial least square-structural equation modelling (PLS-SEM) found to be an adequate statistical tool to study the causal relationships between different variables in ecological studies ([Hair et al., 1998](#)). It is worth identifying the path through which eO₃ and weed competition individually, and their interaction could possibly affect the wheat yield. Accordingly, O₃, C, and their interaction (competition + O₃), along with 'biochemical alteration', 'soil

health', 'plant growth', 'disruption of physiological mechanisms' were considered as exogenous latent variables and yield as target endogenous latent variable in the present established models ([Fig. 5](#)). The present model has been analyzed by SmartPLS 3.2.9. Detail assessment and validation of the PLS model have been provided in the supplementary material (S 2.10).

3. Results

3.1. Ozone monitoring, ozone flux and meteorological parameters

A heat map of daily average O₃ concentration has been provided in [Fig. 1A](#). The average 8 h (9:00 to 17:00 h) O₃ concentration was 46.6 ± 0.7 ppb in the ambient condition during the entire experimental period conducted from November 2018 to March 2019 ([Fig. 1B](#)) with AOT 40 value to be 8.3 ppm h. Minimum mean 8 h O₃ concentration was observed in the month of December (41.7 ppb) and maximum in the month of March (55 ppb). The lowest (maximum) temperature was recorded in the month of December (24.5 °C) while, highest during March (31.4 °C), concomitant with low relative humidity (68.4%) which was conducive for the formation of high concentration of O₃ ([Fig. 1B](#)). However, the mean O₃ concentration in the chambers with eO₃ fumigation was 58.3 ± 0.8 ppb, ranging from 37.6 ppb to 76 ppb. The AOT40 recorded in the O₃ fumigated chambers were 18 ppm during the wheat growing season (mid–November to March).

O₃ flux was observed to be higher in fat-hen compared to wheat under control and stressed conditions ([Fig. 1C](#)). An increase of O₃ flux was observed significant under both SEW/SEC and MEW/MEC.

3.2. Plants growth

Ozone negatively affected wheat's growth: 18% and 15% reductions were observed for root length (RL) and plant height (PH) at 40 DAG while, reductions of 21.8 and 22% were detected for the same at 80 DAG, respectively, as compared to their respective controls (SAW) (SF1). During the vegetative period (40 DAG), the susceptibility of fat-hen to O₃ was observed to be at the higher side as compared to the wheat, with respect to the percent reductions of the growth parameters under SEC. Ozone had significant effects on weed growth, showing ~19, 18, 26 and 23% reductions for RL, PH, leaf area (LA) and total biomass (TB), respectively, at 40 DAG, as compared to SAC, however, such effects on weeds have not been intensified under competition + O₃. Significant reductions of root shoot ratio (RSR) in monoculture of fat-hen (22.6%) under eO₃ were observed only at 40 DAG, while such reductions were not significant at 80 DAG (SF1).

Drastic reductions in the growth of wheat were noticed under competition (both ambient and eO₃). The effects of competition + O₃ were more adverse for wheat's LA and RL followed by TB at 80 DAG. Based on such observations, it is inferred that the growth of the wheat was affected more strongly by competition than that of fat-hen under eO₃. Under O₃ × C, PH, LA, and TB showed significant variations in both the test plants (ST1 and 2).

3.3. Gas exchange parameters

Ozone induced significant reductions in assimilation rate (A) for both the test plants whereas it was insignificant for fat-hen at 80 DAG under SEC ([Fig. 2](#)). Wheat showed reductions in An under competition at 40 and 80DAG, whereas, a significant reduction was observed only at 80 DAG in fat-hen under similar conditions as compared to their respective controls. Stomatal conductance (g_s) and water use efficiency (WUE) tended to reduce under O₃

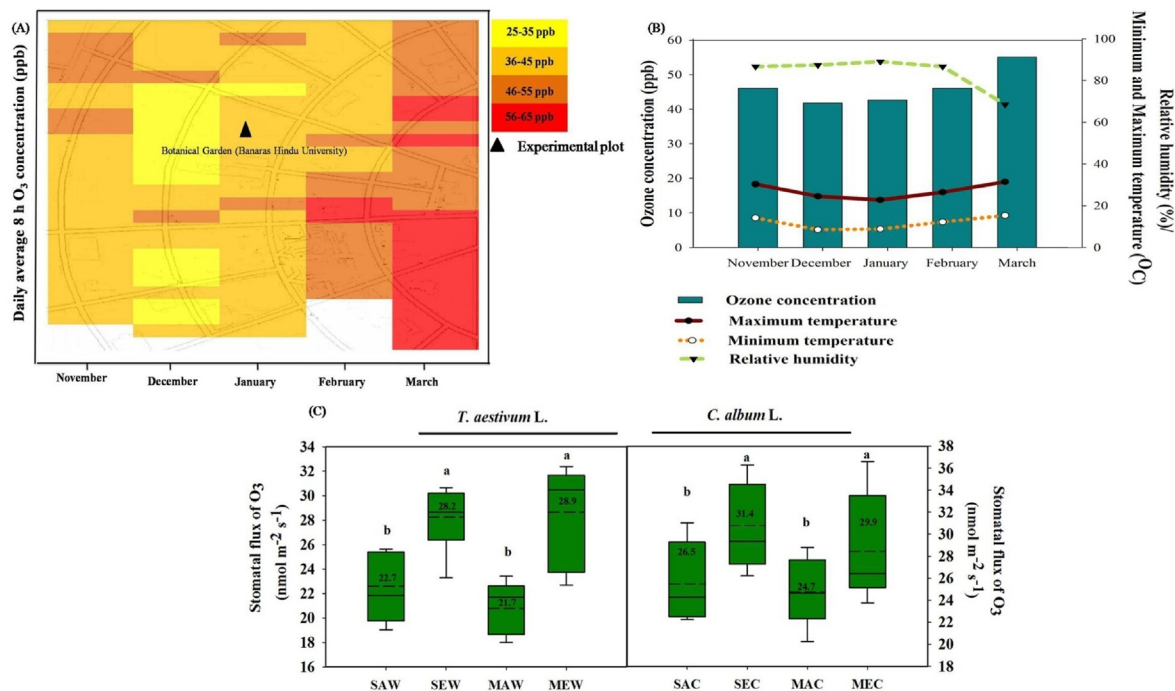


Fig. 1. (A) Heat map showing the daily average O₃ concentration for the study period for the year 2018–2019 with a background highlighting the map of experimental site (Botanical garden, Banaras Hindu University) (B) Illustration showing monthly variation of minimum and maximum temperature (°C) and relative humidity (%) with O₃ concentration recorded during the experimental period at the study site for the year 2018–2019 (C) Box plot showing the variations in stomatal flux of O₃ in *T. aestivum* and *C. album* during the study period. The box is divided by a line represented as median, the area of the box above the median depicts 75th percentile and the area below the median depicts 25th percentile, respectively. The top whiskers represents 95th and bottom whisker represents 5th percentile, respectively. The dotted lines represent the mean value.

exposure irrespective of competition and it was of higher magnitude in wheat, whereas competitive environment did not significantly alter the g_s at 40 DAG in both the plants. However, significant downregulation of these parameters were observed under competition + O₃ in both test plants. Increments in intercellular CO₂ (C_i) (SF2) and transpiration rate (E) have been observed in both plants under eO₃ and inter-specific competition, individually and their interaction, with more percent increments in E has been observed in wheat. Fat-hen displayed insignificant changes in C_i at 40 DAG under competitive environment, irrespective of O₃ concentration. As per F ratio values, the effect of O₃ on A and g_s was stronger than the competition in both wheat and weed (ST1 and 2). Under O₃ × C, all the gas exchange parameters displayed significant variations in both the plants.

3.4. Chlorophyll fluorescence

SF3 exhibiting that dark-adapted leaves subjected to the individual and combined treatment of eO₃ and competition showed negative effects on Chlorophyll *a* fluorescence parameters in both the plant species. Results have been discussed in details in the supplementary sections (S4.4).

3.5. Photosynthetic pigments and non-enzymatic antioxidants

Ozone and competition tended to decrease carotenoids (Car) and total chlorophyll (TC) at 40 DAG in wheat, consistent with its reduction of photosynthetic rate (Fig. 3). Such an impact was more pronounced under competition + O₃, depicting higher reduction (27.6 and 16%, respectively) under MEW. On the contrary, Car content increased in the leaves of fat-hen at the later growth stage under eO₃, irrespective of inter-specific competition. The ratio of Chl *a/b* also reduced under the individual treatment of O₃ and

competition. Significant increments of Chl *a/b* ratio were observed by 30% under individual treatment of eO₃ and by 15% under O₃+competition in fat-hen at 40 DAG.

Ascorbic acid (AsA) in the leaves of wheat and fat-hen sharply increased under eO₃ by 14 and 21% at 40 DAG, respectively, as compared to their respective controls (Fig. 3). Similarly, up-regulation of total thiols was significant under O₃ fumigation and inter-specific competition in both the plants. Even, higher increase was detected under competition + O₃ in wheat at 40 DAG by 26% and in fat-hen by 28%, as compared to their respective controls.

3.6. Lipid peroxidation, reactive oxygen species and their scavenging activities

O₃ significantly induced malondialdehyde (MDA) formation in wheat's leaves irrespective of competition, with maximum increment under competition + O₃ (36.5%, 40 DAG and 46%, 80 DAG). In contrast, lipid peroxidation (LPO) tended to be low in fat-hen under SEW (12 and 17.4%), MAC (4.6 and 24.8%) and their interaction MEC (13.8% at 40 DAG and 32% at 80 DAG) as compared to SAC, respectively (ST3).

Amongst reactive oxygen species (ROS), O₃ fumigated leaves displayed a significant increase of hydrogen peroxide radicals (H₂O₂) and hydroxyl radical (*OH⁻) content in wheat at both the growth stages, the maximum increment being recorded for H₂O₂ content at 80 DAG (24.5%; P < 0.05) followed by *OH⁻ content at 40 DAG (21%; P < 0.05) under SEW. However, high H₂O₂ scavenging activity (HSA) under eO₃ might have checked the content of H₂O₂ to some extent in wheat at 40 DAG (ST3). Significant increments of superoxide radical (*O₂⁻) scavenging activities (SSA) might have resulted in a non-significant increase of *O₂⁻ production in wheat at both the sampling stages due to eO₃ exposure. Contrary to this, increments of ROS (H₂O₂ and *O₂⁻) in fat-hen under O₃ fumigation was

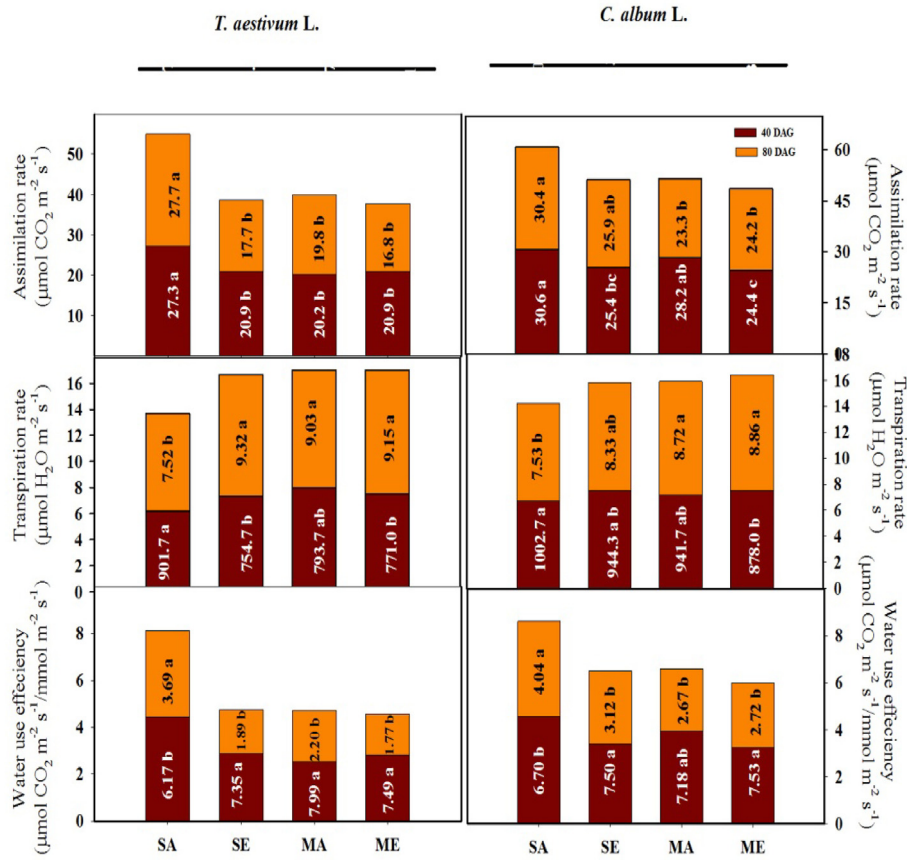


Fig. 2. Various gas exchange parameters of *T. aestivum* cv.HD 2967 and *C. album* grown under different treatments at 40 and 80 DAG (Mean). Bars showing different lowercase letters indicate significant differences between the treatments according to Tukey's test at $p < 0.05$; $n = 9$.

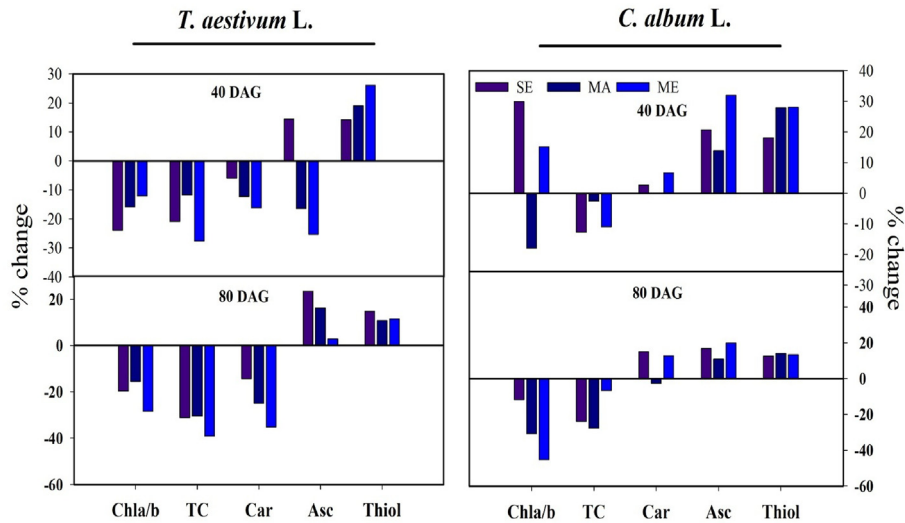


Fig. 3. Graph showing percentage changes for photosynthetic pigments and non-enzymatic antioxidants under different treatments at 40 and 80 DAG. Chla/b chlorophyll a/b; TC total chlorophyll; Car carotenoids; Asc ascorbic acid; DAG days after germination.

observed to be significant only at 80 DAG due to high HSA and SSA. However, under competition + O₃, both wheat and fat-hen displayed higher ROS contents; although with higher scavenging activities exhibited by fat-hen, indicating its higher potentiality in coping up the oxidative stress.

3.7. Antioxidative enzymes

Exposure with eO₃ stimulated the activities of APX (1.5 times) and SOD (1.1 times) at 40 DAG in wheat (SF4). On the other hand, all the measured enzymatic activities involved in H₂O₂ scavenging (APX, CAT, POX and GR) showed sharp increments at 40 DAG in fat-

hen under eO₃. Besides, induction of SOD activities in weed at 40 DAG (1.3 times) might have checked the production of *O₂⁻ radicals against O₃ stress.

In fat-hen, significant inductions of GR activities under competition were observed at both 40 and 80 DAG and only CAT activity at 80 DAG under SEC. A powerful increase of CAT, POX, GR and SOD activities (~1.5 times) in fat-hen were found under competition + O₃ at 40 DAG as compared to SAW (SF4). Increment of POX activity under O₃ at 80 DAG has been reported in the leaves of wheat plants, irrespective of competition (SF4).

3.8. Phenols and flavonoids

Ozone stimulated the pool of total phenolic (TP) and total flavonoids (TF) in the leaves and roots of both the plants; higher percentage increments being observed in the roots of fat-hen (Fig. 4). O₃ induced stimulation of TF in wheat root remained insignificant at 40 DAG while was significant at 80 DAG. Although sharp increments of TF in wheat leaves were observed at both the growth stages, it was much higher in the foliage and roots of fat-hen. However, the roots of fat-hen did not exhibit any significant change in flavonoid profiles under mix-culture, but sharp increment was observed under SEC (40% at 40 DAG and 24.4% at 80 DAG), as compared to SAC.

Ozone induced significant increments of ferulic acid (FA) and *p*-coumaric acid (CA) only in the leaves of wheat, independent of competition at both the stages, while the wheat roots showed much lesser increments as compared to the leaves (SF5). FA sharply increased by 22.6% and 20.8% in wheat leaves and by 11% and 19.5% in roots under SEW and MEW at 40 DAG, as compared to SAW. Ozone sharply increased FA and CA contents both in the leaves and roots of fat-hen, higher increments were observed under competition + O₃ at both the stages (except FA (roots) at 80 DAG).

Quercetin displayed significant increments under eO₃ in wheat leaves and roots at 40 DAG, while the increase of the flavonols was significant in fat-hen (kaempferol at 40DAG while, quercetin at 80DAG) (SF6). However, the production of these flavonols remained unchanged in fat-hen (leaves and roots) under competition while prominent increments were observed in the roots and leaves of fat-hen at both sampling times under competition + O₃. Competition did not affect the profiles of these flavonols in different wheat parts, irrespective of O₃ concentration, except at 80 DAG where wheat roots showed increment of kaempferol under ambient O₃.

3.9. Soil enzymes and microbial biomass

The individual and combined exposure of stressors negatively affected the parameters of the rhizospheric regions of both the

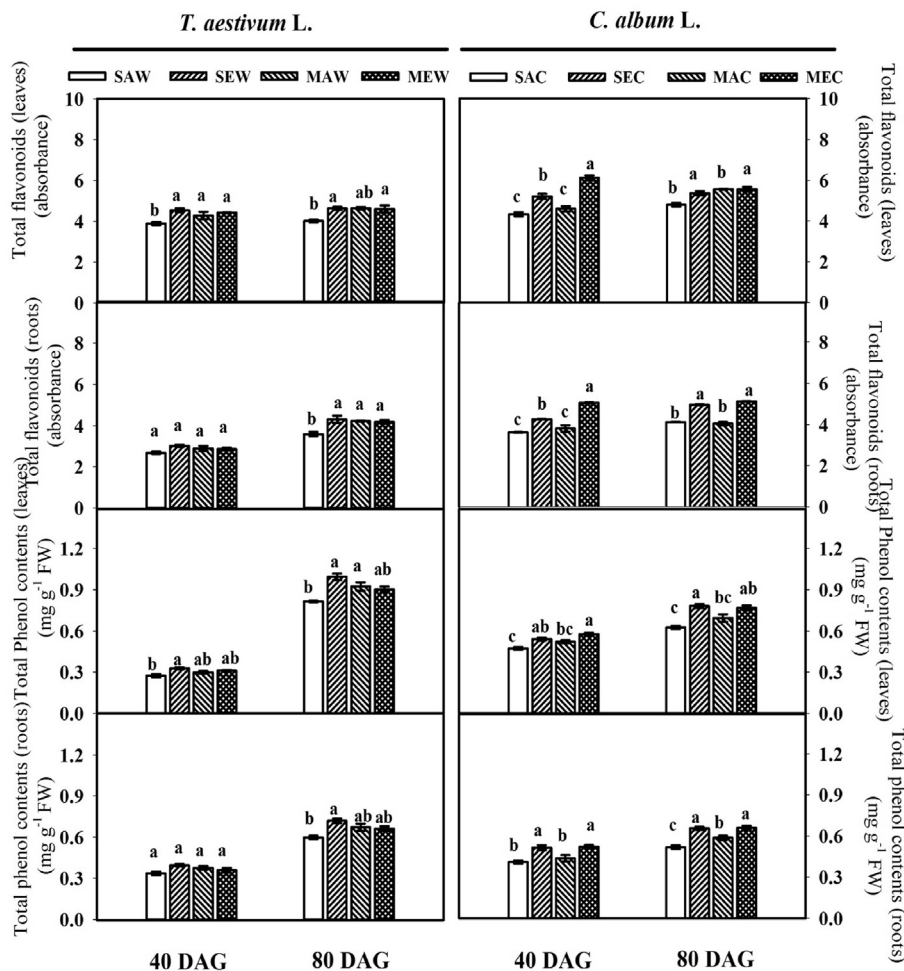


Fig. 4. Parameters showing variations in the total phenolic and total flavonoids contents in roots and leaves of the *T. aestivum* cv.HD 2967 and *C. album* grown under different treatments at 40 and 80 DAG (Mean \pm SE). Bars showing different lowercase letters indicate significant differences between the treatments according to Tukey's test at $p < 0.05$; $n = 9$.

plants. Detail result has been mentioned in the supplementary (S3.10 and ST4).

3.10. Grain yield

Grain yield of wheat was observed to be significantly affected due to eO₃, weed competition and their interaction. Reduction of grain yield was arranged in the descending order MEW (166 ± 2.5) > SEW (197 ± 1.0) > MAW (208 ± 1.7) > SAW (248 ± 2.3).

3.11. PLS-SEM

The illustrations of the PLS-SEM have been provided in Fig. 5 for O₃, competition, and O₃+competition, respectively. Fig. 5 shows that under eO₃, 'biochemical alteration/BA' has been observed as the most influential factors regulating the "Yield" of the wheat via O₃ with a negative association (-0.68). Under SEW, R² for the yield was 0.88, indicating that "Yield" is largely explained by its latent variables through significant direct and indirect paths. Besides, the direct influences of O₃ have been observed to be highest on biochemical parameters (0.94) showing positive relationships followed by 'disruption of physiological mechanism/DPM' (0.70) and 'plant growth/PG' (0.58). On the other hand, under competitive environment the indirect paths highly influenced the 'yield' was through 'PG' which showed positive associations whereas; 'BA' displayed a negative association in influencing the wheat yield. Besides, the influence of competition on 'BA' (+), 'DPM' (+), and 'PG' (-) was equally high, unlike that observed under O₃. However, under MEW, the 'yield' was highly influenced via 'soil health/SH' followed by BA and PG. 'DPM' in wheat was the most influenced latent variable by O₃+competition, which was positively associated via a direct path from the stress factor. Direct path from O₃+competition displayed highest negative β -coefficient (-0.98) followed by O₃ (-0.96) and competition (-0.90), indicating a much higher negative influence under the interaction of both the stresses, contributing to wheat yield loss in the present study.

4. Discussion

Phytotoxicity induced under high O₃ concentration is a well-known fact and is evidenced worldwide, on different crop plants (Feng et al., 2019; Hayes et al., 2019). However, very few studies have evaluated the impact of the interaction of O₃ and weed competition to date. Wheat and fat-hen displayed differential responses under eO₃ and such effects varied under competition.

4.1. Which metabolic adjustments are induced under individual and combined effects of inter-specific competition/O₃ exposure?

The results from the present experiment with each plant reflected some important factors controlling their sensitivity to eO₃: stomatal control regulating the O₃ flux, biochemical defence capacity of the plants and leaf area (LA). Availability of photosynthates determines the tolerant capacity of plants to overcome oxidative stress under the presence of different stressors (Calzadilla et al., 2019). Reduction in LA in O₃ fumigated plants might have negatively affected the production of photosynthates, also noticed in plants under competitive environment (MAW/MAC). Such suppression of assimilation rate under eO₃ and weed competition might have jeopardized the biomass of the wheat, translating to yield reduction.

Although, stomatal conductance (g_s) determines the stomatal uptake of O₃ in the plants, this does not necessarily correlate with the enhancement of O₃ induced damages (Xu et al., 2019). Tissue response to such oxidative stress includes an array of processes

involving defence and repair mechanism and also depends on the alteration of carbon allocation patterns. For example, relatively, lower reduction of g_s and higher O₃ flux has been observed in fat-hen under individual exposure of eO₃ and competition as compared to that of wheat, resulting in higher O₃ uptake by fat-hen. However, the process of O₃ uptake is generally simultaneous with the uptake of CO₂ (Panek and Goldstein, 2001) which might have helped fat-hen in maintaining higher assimilates to cope up with the oxidative stress under the individual presence of both the stressors.

Our study displayed significant increments of intercellular CO₂ (C_i) in wheat at both the growth stages, which might be due to the inhibition of the carbon assimilation process (Wang et al., 2019). Percent increment of transpiration rate in wheat was observed to be higher as compared to fat-hen, reflecting strong stomatal control in weed foliage under stress conditions. As expected, fat-hen displayed a better WUE as compared to that of wheat. Chlorophyll fluorescence kinetics is considered as an important non-invasive approach to govern the plant photosynthetic performance (Kalaji et al., 2016). Reduction of F_v/F_m under O₃, especially at 80 DAG, indicated the damage caused to photosystem II of wheat cultivar which might be due to photoinhibition effect. Such data was corroborated well with the results of Yadav et al. (2020). However, such effects seemed to get intensified under competition + O₃ at 80 DAG in wheat. This is confirmed by the reductions of non-photochemical and photochemical quenching in the wheat under different stress conditions, while fat-hen reflected a better non-radiative dissipation capacity of excess energy under stress condition. The efficacy of this mechanism regulating the photoinhibition was confirmed by the destruction of membrane integrity in wheat leaves under the individual and combined effect of both the stresses.

Carotenoids, a light-harvesting complex, is known to take part in oxidative stress resistance mechanism and serve to prevent photo oxidation of chlorophyll (Gill and Tuteja, 2010; Havaux et al., 2005). Hence, such loss of carotenoids and chlorophyll together can produce a decline in the absorbing capacity of light harvesting complex, and also affect the plant capacity of dissipation of excess energy in form of heat under stress conditions, as observed in the wheat. On contrary, the increment of carotenoids content concomitant with chlorophyll a/b in the fat-hen might have offered protection against higher O₃ flux under eO₃.

Reactive oxygen species (ROS) are toxic by-products of plant physiological metabolism, generally produced under stress conditions (Noctor et al., 2018), but also plays a pivotal function in signalling reactions when present in low concentration (Pellegrini et al., 2019). Our study displayed a species-specific pattern of ROS accumulation which was higher in wheat under both eO₃ and competition as compared to fat-hen. Moreover, H₂O₂ and *O₂⁻ production was observed to be higher under competition rather than under eO₃, whereas, a reverse pattern is observed for *OH⁻ radical content in both the plants. Furthermore, induction of oxidative burst under the presence of both the stressors were found to be higher in wheat at 80 DAG which could be correlated to the membrane injury as demonstrated by the significant increase of MDA content.

A most familiar mechanism to scavenge excessive ROS production is through the enzymatic defence pathway. Our study displayed comparatively strong O₃ induced up-regulation of APX and SOD at an early growth stage in wheat, resulting into significant scavenging of H₂O₂ and *O₂⁻ radicals. Although, activities of APX (both the stages) and GR (80 DAG) in wheat were induced under competition, but such stimulation could not cope up with the existing H₂O₂ concentration, resulting in its higher accumulation. On the contrary, activities of CAT, SOD, POX and GR increased sharply under each stress factor in fat-hen which resulted in better

protection and lower oxidative stress as compared to wheat. Our results are in agreement with the work of Li et al. (2013), where an antioxidatively stronger defense mechanism was exhibited by flaxweed under eO₃ as compared to winter wheat.

Non-enzymatic antioxidants such as ascorbic acid (AsA), thiols, flavonoids and phenols also played a crucial role in the avoidance of oxidative damage under stress conditions. Our result exhibited that oxidative stress induced by O₃ and competition (singly and combination) modified the pool of ascorbate in both plant species, confirming the role of AsA in the first line of defence against O₃ (Fatima et al., 2018); however, competition alone and combined treatment with O₃ reduced the ascorbate pool at 40DAG but negligible rise at 80DAG in the wheat has been observed. On the contrary, competition (singly) did not interfere in regulating the ascorbate pool in fat-hen. However, in terms of ROS content and membrane damage in wheat, it seems that such stimulation of AsA was not enough to cope up with the adverse effect of O₃.

4.2. Can allelopathic interaction be triggered as a response to the high concentration of O₃?

Concentrations of phenolic compounds are often believed to get altered under the presence of stresses and allocation of assimilates plays a vital role in such adaptation (Tang et al., 1995). Though, O₃ fumigation resulted in a high accumulation of phenolic content in the wheat leaves and roots at both the growth stages, the plants could not maintain high phenol concentration under the presence of combined stressors. On the contrary, maximum phenolic content was observed in the roots of fat-hen at 40DAG under the combined stressors followed by O₃ and competition. Ozone raised the concentration of ferulic acid (FA) and *p*-coumaric acid (CA) sharply only in wheat leaves, irrespective of competition whereas, a slight increment has been exhibited by the wheat roots. Dos Santos et al. (2008) reported that high FA in the plant cells might alter the glucose metabolism, through its utilisation in the up-regulation of defence mechanism and resulting in reducing the carbon flux towards plant growth. Increments of FA and CA in the roots of fat-hen under competition + O₃ allowed us to expect a transport of phenolic compounds to the soil, which might have attributed to induction of some indirect effect on the wheat root length (RL) via allelopathic interaction. Klein and Blum (1990) observed that the RL of cucumber decreased under the presence of well known allelochemical FA, negatively affecting the WUE and nutrients uptake of the plants. Li et al. (2010) reported the inhibition of nutrients uptake of plants in the presence of such phenolic allelochemicals, thereby resulting in limitation of essential nutrients for plants' physiological processes. Likewise, the role of CA in the root length inhibition has also been observed in lettuce.

Flavonoids are generally released through the roots in the rhizospheric zone and might involved in plant/plant interaction (Weston and Mathesius, 2013). Under such interactive studies, allelopathic compounds targeting the root of neighbouring plants via its free movement in soil (Majeed et al., 2012) which may alter the physiological function of less competitive species growing together. Strong stimulation of the flavonoid in fat-hen not only helped the plants to withstand the oxidative damage under eO₃ but also might have influenced the allelopathic interaction (competition + O₃) and enhanced the growth-inhibitory effect on wheat root system (Kacienė et al., 2019), thus increasing competitive pressure of the neighbouring plants favouring root growth inhibition of wheat (Kato-Noguchi et al., 2013). Similarly, the rise of kaempferol and quercetin in different parts of fat-hen offered resistance against O₃ stress, competition and their interaction. However, to validate such assumption of allelopathic interaction in crop-weed studies, the observation related to root exudates must

be considered for the same.

Soil enzymatic activities are well-known factor to be used as a quality indicator of soil. Previous studies manifested the importance of root exudates to drive a shift in microbial community composition in soil rhizosphere (Broeckling et al., 2008) and furthermore, the species-specific root chemical profiles may result in a distinctive rhizospheric microbial environment (Micallef et al., 2009). Our findings do not directly include the study related to root exudates but distinct root phenolic profiles observed under different stress conditions of studied plant species might be taken into account to consider the shift in the microbial environment which directly influenced the soil carbon and nutrient cycling (Zwetsloot et al., 2018). Higher stimulation of phenolic compounds in the roots of fat-hen under the combination of competition and eO₃ (compared to wheat) might have caused inhibition of the organic matter decomposition as a result of the formation of phenolic-enzyme complex, thereby reducing soil microbial decomposition and growth suppression (Stalheim et al., 2009). Although, the phenolic compounds were observed to be higher in the roots of fat-hen, contrary to our expectation, reductions of soil enzymatic activities and microbial biomass were more pronounced in the rhizosphere of wheat rather than that of fat-hen under competitive environment and eO₃ in combination, which might help us to assume the lower microbial degradation of these phenolic acids in the wheat's rhizosphere, resulting in a build-up of phenol compound to phytotoxic level in the soil (Blum, 1998).

Ozone induced stimulation of kaempferol and quercetin in wheat has been reported by Fatima et al. (2018a). Kaempferol and quercetin is well-known iron-chelating agents (Cesco et al., 2010) due to their high cation exchange capacity. Up-regulation in the profile of these flavonoids in the root of fat-hen in under competition + O₃, might have caused chelation of important metal ions for microbial growth, thereby reducing the plants' uptake of nutrients (Soobrattee et al., 2005).

Soil enzymatic activities of amylase and β -glucosidase reduced significantly under eO₃, competition and their combination, with higher reduction of amylase activity observed in wheat under competition + O₃ at both the sampling times. Suppression of these enzymatic activities clearly stipulated the reduction of microbial activities which possibly might be due to the alteration of the nutrient pool resulting from the low litter input/decomposition (Fatima et al., 2018). However, the increment of microbial biomass carbon under mix-culture condition in the rhizosphere of fat-hen can be correlated to the slight increment of amylase activity, an enzyme governing the labile carbon pool in the soil.

Unlike the result of Fatima et al. (2018b), the activity of polyphenol oxidase, catalyzes the breakdown of phenolic compounds in the soil, was suppressed in the rhizosphere of both the plants under different stress conditions; however the reductions were higher in the wheat under competition, irrespective of the O₃ concentration which might have resulted in higher accumulation of phenolic compounds in the rhizosphere of wheat. Likewise, the activity of urease, involved in nitrogen cycling, reduced significantly in both the plants under competitive environment, irrespective of O₃ concentration. Such results have supported the shift in the production of allelochemicals under eO₃ concentration in both the test plants. Although, such shift did not protect the wheat against stress conditions and triggered allelopathic interaction. Strong stimulation of allelochemicals in fat-hen, however, helped them to withstand the oxidative damage against O₃ stress, thereby supporting the hypothesis proposed in the study.

4.3. Estimation of damage in wheat with respect to yield loss

Numerous studies revealed O₃ induced oxidative damage

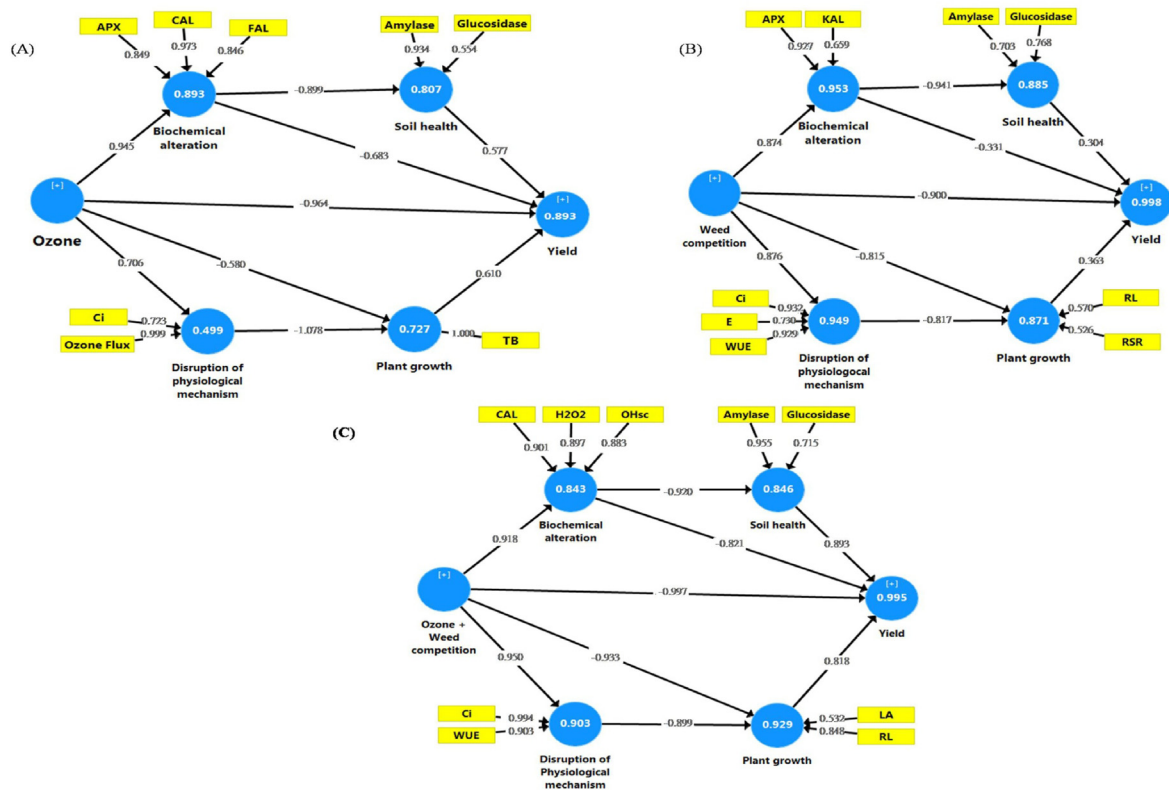


Fig. 5. Result of PLS-SEM showing path analysis under (A) eO₃ (SEW) (B) weed competition (MAW) (C) O₃+ weed competition (MEW). APX ascorbate peroxidase; CAL coumaric acid (leaf); FAL ferulic acid (leaf); Ci intercellular CO₂; TB total biomass; KAL kaempferol (leaf); E transpiration rate; WUE water use efficiency; RL root length; RSR root shoot ratio; H2O2 hydrogen peroxide radical content; OHsc hydroxyl radical scavenging activity; LA leaf area.

resulted in limitation of growth and productivity of various crops (Brewster et al., 2019; Feng et al., 2019; Pandey et al., 2018). Our study demonstrated that the O₃ fumigation reduced the TB and grain yield of the wheat cultivar. Such yield reduction may be explained with the help of the path analysis by PLS-SEM models (Fig. 5). Under O₃ SEW, 'biochemical alteration'/'BA' represented by APX, *p*-coumaric acid (leaf)/CAL and ferulic acid (leaf)/FAL, contributed maximally to determine "Yield" via indirect path ($\beta = -0.683$). The result was an expected one supporting the concept of 'trade-off', suggesting prioritization towards the implementation of defence imposed substantial allocation of resources which might have affected the wheat yield. Furthermore, the yield was also affected by competition. Under MAW, 'plant growth'/'PG' showed substantial influence in governing 'Yield' of HD 2967. However, PG is further influenced by 'disruption of physiological mechanism'/'DPM', displaying a negative relationship between the two. The model depicted that competition disrupted the physiological mechanism via WUE, C_i and E and 'PG' through RL and RSR, which ultimately translated to yield loss for HD 2967. Our study also reinforces the results of Li et al. (2016) which demonstrated yield losses in wheat due to inter-specific competition by flaxweed. Furthermore, an aggravated effect of yield loss has been observed under combined stress factors, suggesting eO₃ causes a shift in the competitiveness of wheat. Under MEW, 'soil health'/'SH' is influenced by the 'BA' which in turn was determined by H₂O₂ content, hydroxyl radical scavenging activity (HOSA) and CAL in wheat itself. The positive association between 'SH' and "Yield" revealed that if SH deteriorates, the yield will be negatively affected which holds true for our study.

5. Conclusions

The study displayed differential responses by wheat and fat-hen (weed) under ambient and eO₃ in mono and mix-culture conditions with respect to the assessed parameters. Although higher g_s along with consecutive higher O₃ flux has been observed in fat-hen under individual stress as compared to that of wheat, fat-hen maintained higher assimilation capacity to cope up with the oxidative stresses, translating to higher biomass. Under stress conditions, fat-hen performed better in terms of WUE and fluorescence kinetics along with strong antioxidative protection resulting in superior scavenging activities compared to wheat. Our study showed complete agreement with Li et al. (2013) documenting the better adaptable capability of flaxweed (weed) as compared to winter wheat (crop) owing to their stronger antioxidative system, rendering to their highly competitive nature. The competitiveness of fat-hen increased towards wheat, especially under the concomitant elevation of O₃ concentration. All-inclusive, the yield loss of wheat was evidenced maximally under MEW followed by SEW and MAW, suggesting an additive interaction under co-occurrence of both the stress factors. Therefore, the intentional growing of fat-hen by the farmers is not recommended in the wheat field in return of small gain at the cost of large economic losses in wheat crop, especially in the Tropical regions where O₃ concentration generally remains high.

Declaration of competing interest

The authors declare that they have no conflict of interest.

CRediT authorship contribution statement

Annesha Ghosh: Data curation, Writing - original draft. **Bhanu Pandey:** Formal analysis, Writing - review & editing. **Madhoolika Agrawal:** Visualization, Investigation. **Shashi Bhusan Agrawal:** Conceptualization, Methodology, Visualization, Investigation, Writing - review & editing, Supervision.

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Appendix A. Supplementary data

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