Evaluation of changes in lipid peroxidation, ROS production, surface structures, secondary metabolites and yield of linseed (Linum usitatissimum L.) under individual and combined stress of ultraviolet-B and ozone using open top chambers

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The individual and interactive effects of supplemental UV-B (sUV-B) (ambient + 7.2 kJ m$^{-2}$ d$^{-1}$) and elevated O$_3$ (ambient + 10 ppb) were evaluated under field conditions using open top chambers on two cultivars, Padmini and T-397 of linseed (Linum usitatissimum L.). Mean monthly surface level of O$_3$ concentrations varied from 27.7 ppb to 59.0 ppb during the experimental period. Both UV-B and O$_3$ induced the production of ROS (H$_2$O$_2$ and O$_2$.-) resulting in significant damage of membranes due to lipid peroxidation and electrolyte leakage. Synthesis of secondary metabolites (flavonoids, anthocyanin, lignin and wax) was also enhanced in all the treatments, whereas biomass and yield were reduced. Alterations in frequency of stomata and wax distribution were also observed through scanning electron microscopy (SEM). Cultivar Padmini was found to be more sensitive because of higher damage of membrane vis-a-vis reduction in biomass and seed yield. However, concentrations of flavonoids, anthocyanin, lignin and wax were higher in T-397, suggesting its relative resistance against applied stress. Combined exposure of sUV-B and O$_3$ was less harmful, as compared to their individual treatment. Among the three treatments, O$_3$ was found to be more detrimental for overall growth and sUV-B for economic yield.

**Keywords**: sUV-B, O$_3$, Linum usitatissimum L., Reactive oxygen species, Secondary metabolite, Growth, Yield.

During past decades, ozone in stratosphere and troposphere has become an important global environmental issue. Anthropogenically produced halon compounds (CFCs, HCFCs and CH$_3$Br, etc.) are continuously depleting the stratospheric O$_3$ layer, resulting in increase of UV-B radiation on the Earth’s surface. Global average total column O$_3$ has declined by 3.5% from pre 1980s concentration$^1$. According to European O$_3$ Research Coordinating Unit (EORCU) O$_3$ layer is depleting by approximately 0.6% per year$^2$. With every 1% depletion of O$_3$ layer about 2% more UV-B reaches to the Earth’s surface. With the full implementation of Montreal Protocol, the annual average total O$_3$ was projected to return to 1960 level around the middle of the century, however, in tropics, below 1960s level will remain throughout the 21$^{st}$ century$^3$. Therefore, urgent attention needs be given to monitor the harmful effects of increasing concentration of UV-B radiation on tropical plants.

Along with depletion in stratosphere, O$_3$ is increasing in troposphere due to rapid urbanization, industrialization and economic growth. In troposphere, O$_3$ is photochemically produced from natural and anthropogenic precursors, mainly methane (CH$_4$), volatile organic carbon (VOCs) and nitrogen oxides (NOx). Background concentration of O$_3$ has raised from < 10 ppb i.e. the pre-industrial level to a day time concentration exceeding 40 ppb in many parts of Northern hemisphere$^4$. Global photochemical models further predict that under current legislation emission scenarios, parts of Asia will experience significant increase in O$_3$ concentrations by 2030$^5$. Intrinsically, there is a requisite to assess the detrimental effects of these simultaneously existing stresses like UV-B and O$_3$ on plants.

UV-B and O$_3$ are known to induce leaf senescence, affect photosynthesis, growth and productivity of the plants. Phytotoxic nature of both UV-B and O$_3$ is...
mostly because of their capability to generate reactive oxygen species (ROS). Ozone gets access inside plants mainly via open stomata and mesophyll air space and degrades in the apoplast forming various ROS (H$_2$O$_2$, O$_2^-$, OH etc) which induces oxidation of lipids, proteins and DNA and activate programmed cell death pathway. Likewise, O$_3$ and UV-B also induce formation of ROS, membrane disruption and generation of oxidative stress possibly via UV-B activated membrane localized NADH-oxidase. Plants adapt various strategies to limit the UV-B and O$_3$ induced damage, such as induction of polyamine metabolism, phenolics and lignin biosynthesis, rendering leaf surface changes (wax deposition), defense related proteins as well as antioxidants (enzymatic and non-enzymatic).

Before entry in the leaf tissue both UV-B and O$_3$ react with foliar surface and modify its configuration like change in composition and ornamentation of wax, altered structure of cuticle of epidermal layer and change in size and frequency of stomata. Previous studies have reported change in size and frequency of stomata and in UV-B exposure. Oilseed crops are major crops which yield oils of both edible and industrial purposes. India is the fourth largest oilseed producing country in the world, next to USA, China and Brazil. In India, linseed (Linum usitatissimum L.) is fourth most important oilseed crop after mustard, sesame and groundnut which yield oil of both edible and industrial purpose.

The present study has been designed to assess: i) effects of elevated levels of sUV-B and O$_3$ singly and in combination on production of ROS and secondary metabolites, lignin and wax synthesis, leaf surface changes, biomass and yield of linseed (Linum usitatissimum L.), and ii) to find out, if any adaptive mechanism operating under simultaneous exposure of sUV-B and O$_3$ to alleviate their individual impact.

**Plant material**

Two high yielding and locally grown cultivars of north-east region of India (Padmini and T-397) of linseed (Linum usitatissimum L.), an oil yielding crop were selected as test plant for the present study. The seeds were provided from the Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

**Experimental setup**

Twenty-four open top chambers (OTCs) of 1.5 m diameter and 1.8 m height were installed at the experimental site, following the design of Bell and Ashmore. Soil of each OTC was provided with recommended dose of NPK (80: 40: 30 kg ha$^{-1}$) and plots were irrigated at regular interval to maintain the uniform soil moisture. Twenty four OTCs were divided into four treatments as: Control (C) i.e. non-filtered chambers (NFCs), NFC with supplemental UV-B (sUV-B), NFC supplied with elevated ozone (O$_3$) (ambient + 10 ppb) and NFC with combination of sUV-B and elevated O$_3$ (sUV-B + 10 ppb O$_3$). Six NFCs (three for each cultivar) were artificially supplied with sUV-B by Q-Panel 313, 40 W fluorescent lamps (Q-Panel Inc. Cleveland, OH, USA) held in mobile and adjustable iron frames over each planted row.

In open plots, UV-B tubes were covered with 0.13 mm polyester filter (to absorb radiation below 320 nm), however, in UV-B treatment plots, they were covered with 0.13 mm cellulose diacetate filter (to absorb radiation below 280 nm). Distance between the top canopies of the plants and lamps were maintained at 45 ± 2 cm by adjusting the frames to provide sUV-B of 7.2 KJ m$^{-2}$ d$^{-1}$ (unweighted) that mimics 20% reduction in stratospheric O$_3$ at Varanasi during clear sky conditions. UV-B was measured under lamp at plant apices with UV intensity meter (UV P Inc. San Gabriel, (A), USA) and the readings were converted to UV-B$_{BE}$ values by comparing with spectropower meter (Sciencent, Boulder, USA).

Plants were irrigated for 3 h d$^{-1}$ in the middle of the photoperiod. Twelve OTCs were provided with O$_3$ generators with their delivery ends connected to the distribution system, so that air coming from blowers became diluted and circulated the O$_3$ gas into the chamber with three air changes min$^{-1}$. All twelve OTCs were equipped with facility to expose plants with 10 ppb O$_3$ for 3 h d$^{-1}$ during mid day (11:30 a.m. - 2:30 p.m.) and out of which six of them were provided with treatment having combination of both sUV-B and elevated O$_3$. 

**Materials and Methods**

**Experimental site**

The field experiment was conducted at the Botanical garden of Banaras Hindu University, Varanasi (25° 14’ N latitude, 82°03’E and 76.19 m above mean sea level) between the months of December 2007 to March 2008 in the Rabi growing season. Mean monthly temperature ranged from minimum 9.8 to 28.5°C. Total sunshine hour ranged from 3.6 to 9.8 h d$^{-1}$ and relative humidity ranged from 78.9% to 41.2%. Total mean rainfall of study period was 14.2 mm.
Ozone monitoring

Eight hourly O$_3$ monitoring was done during the growth period of the plants with a UV-absorption ambient O$_3$ monitor (Model APOA 370, HORIBA Ltd, Japan).

Plant sampling and analysis

**LPO, solute leakage, H$_2$O$_2$ content and superoxide radical production rate**

Three plants were randomly sampled from each chamber for each treatment and their leaf tissues were used for analysis. Membrane damage was measured in the form of malondialdehyde (MDA) content and solute leakage. ROS was estimated as O$_2^•−$ production rate and H$_2$O$_2$ content.

**Flavonoids and anthocyanin content**

In fresh leaf tissues, non-photosynthetic pigments flavonoid and anthocyanin were estimated.

**Lignin and wax estimation**

Fully expanded uppermost leaf of the canopy was used for epicuticular wax estimation by earlier reported choloform method. Lignin content was measured from dry leaf samples by thioglycolate method.

**Leaf surface view morphology (Scanning electron microscopy, SEM)**

Leaf specimens were prepared for SEM using the protocol adapted from standard procedures. The fresh leaf samples (of 5 mm$^2$ from a similar middle portion) of six leaves from each treatment were dissected and immediately fixed in a solution of 2% glutaraldehyde in a 0.1 M sodium phosphate buffer (pH 7.0) for 12 h at room temperature. The specimens were washed three times in 25 mM sodium phosphate buffer (pH 6.8) overnight at 4°C and then dehydrated to absolute ethanol using 15-min series steps of 25%, 50%, 75%, 95% and 100% ethanol and then stored at -20°C until required. At the time of examination, the specimens were rinsed, post-fixed in 2% osmium tetroxide, critical point dried and sputter coated with gold palladium before being mounted on aluminium stubs. The specimens were viewed and photographed by a scanning electron micro-scope (LEO Stereoscan 360 SEM (LEO Electron Microscopy Inc., Thornwood, NY, USA).

Biomass accumulation and yield

Plants were sampled randomly in triplicates from each chamber and open plot at 100 days after germination (DAG) by carefully digging the monolith of 10 × 10 × 15 cm$^3$ containing intact root system. Plants were cleaned under running tap water after keeping it in a 2 mm pore size sieve. For biomass estimation, component wise plant parts were separated and dried in oven at 80°C till the constant weight achieved. Dry weight of each component part was measured separately and biomass was expressed in terms of g plant$^{−1}$. Seed yield was measured at the time of final harvest during the end of March by harvesting ten plants each from different treatments.

Statistical analysis

Data of lipid peroxidation (LPO), solute leakage, free radicals, anthocynain, flavonoids and biomass were subjected to three-way ANOVA test to assess the significance of changes due to different treatments (T), age (A) and cultivars (Cv). Duncan’s multiple range tests were performed as post-hoc on parameters subjected to one-way ANOVA test. Data of wax, lignin content, biomass and yield were analyzed through two-way ANOVA for assessing the significance of changes due to treatments and cultivars. Correlations between LPO, solute leakage and superoxide radical production rate of both the cultivars were performed using linear regression analysis. All the statistical tests were performed using SPSS software (SPSS, Inc, version 16.0).

Results

**O$_3$ monitoring**

During growth period of linseed mean monthly ambient O$_3$ concentration varied from minimum 27.7 ppb in December’07 to maximum 59.0 ppb in March’08. Mean O$_3$ concentrations in January and February were recorded to be 46.3 ppb and 48.6 ppb, respectively (Table 1).

<table>
<thead>
<tr>
<th>Month/year</th>
<th>O$_3$ concentration (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>December’07</td>
<td>27.7</td>
</tr>
<tr>
<td>January’08</td>
<td>46.3</td>
</tr>
<tr>
<td>February’08</td>
<td>48.6</td>
</tr>
<tr>
<td>March’08</td>
<td>59.0</td>
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</table>

**LPO, solute leakage, H$_2$O$_2$ content and superoxide radical production rate**

Both LPO and solute leakage showed increase in all the three treatments in both the test cultivars (Fig. 1). Ozone-treated plants showed maximum
increment of 66.3% in LPO, followed by 49% in sUV-B and 44.2% in sUV-B + O₃ treated plants of cultivar Padmini at 100 DAG. Similar trend was observed in T-397 with 57.2%, 49.9% and 37.2% increase under O₃, sUV-B and sUV-B + O₃ treatments, respectively in LPO at 50 DAG. Solute leakage also showed the same trend, but increment was maximum at initial age of sampling i.e. 50 DAG (Fig. 1).

H₂O₂ and O₂⁻ production rate also followed similar trend of increment in both the cultivars. Padmini showed an increase of 61.7% in O₃ treated plants for H₂O₂ content, followed by 44.8% in sUV-B and 35% in sUV-B + O₃ treated plants at 100 DAG, however, T-397 showed maximum increase at 50 DAG (Fig. 1). Superoxide radical production rate was 70.6%, 55.9% and 39.7% in Padmini and 65.1%, 57.3% and 39% in O₃, sUV-B and sUV-B + O₃ treated plants, respectively of T-397 at 50 DAG (Fig. 1). Three-way ANOVA test showed significant response of A (p<0.001), Cv (p<0.001) and T (p<0.001), individually as well as in their interaction on LPO and solute leakage, but their interaction showed insignificant difference for H₂O₂ and superoxide radical production rate (Table 2).

Figure 2 depicts the relationship among LPO, solute leakage and superoxide radical production rate of both the cultivars. Cultivar T-397 showed highly significant relationship of LPO with solute leakage ($r^2 = 0.99$) and between LPO and superoxide radical production rate ($r^2 = 0.98$).

### Flavonoids and anthocyanin

Both flavonoids and anthocyanin showed significant increment under all the treatments for both the cultivars. Flavonoids showed an increment of 47.2, 41.3 and 29.7% in sUV-B, sUV-B + O₃ and O₃-treated plants of Padmini, however, increments of 48.9, 40.5 and 32.8% were observed in T-397 at 100 DAG (Fig. 3). Similar trend was also reported for anthocyanin showing maximum increase of 59.4, 45.6

![Fig. 1—Variations in LPO, solute leakage and free radicals of sUV-B, O₃ and sUV-B + O₃ exposed two cultivars of linseed (Mean ± SE) [Bars with different letters in each group show significant differences at p< 0.05]](image)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Age (A)</th>
<th>Cultivar (Cv)</th>
<th>Treatment (T)</th>
<th>A × Cv</th>
<th>A × T</th>
<th>Cv × T</th>
<th>A × Cv × T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid peroxidation</td>
<td>0.003***</td>
<td>337.9***</td>
<td>0.001***</td>
<td>92.14***</td>
<td>89.64***</td>
<td>9.19***</td>
<td>11.3***</td>
</tr>
<tr>
<td>Solute leakage</td>
<td>0.0028***</td>
<td>188.2***</td>
<td>0.002***</td>
<td>10.36**</td>
<td>4.77**</td>
<td>6.71***</td>
<td></td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>291.7***</td>
<td>24.2***</td>
<td>163.2***</td>
<td>6.94*</td>
<td>0.71*</td>
<td>0.79*</td>
<td></td>
</tr>
<tr>
<td>Superoxide radical production rate</td>
<td>566.3***</td>
<td>12.9***</td>
<td>187.8***</td>
<td>6.35*</td>
<td>0.73*</td>
<td>0.78*</td>
<td>0.39*</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>0.001***</td>
<td>19.5***</td>
<td>173.4***</td>
<td>3.7***</td>
<td>33.67***</td>
<td>0.16*</td>
<td>1.2*</td>
</tr>
<tr>
<td>Anthocyanin</td>
<td>0.002***</td>
<td>5.63*</td>
<td>97.7***</td>
<td>1.12*</td>
<td>39.8***</td>
<td>0.21*</td>
<td>0.12*</td>
</tr>
</tbody>
</table>

Level of significance: *Significant at P<0.05, ** Significant at P<0.01, ***Significant at P<0.001, ns, non-significant
and 34.4% in sUV-B, sUV-B + O₃ and O₃-treated plants of T-397 at final age of sampling (Fig. 3). Results of three-way ANOVA showed significant response of A (p<0.001), Cv (p<0.001), T (p<0.001), A×T (p<0.001), however, their interactive effects were insignificant (Table 2).

**Lignin and wax content**

Lignin and epicuticular wax contents increased significantly in all the three treatments. Lignin content was maximum in sUV-B (55.2%) treated plants, followed by sUV-B + O₃ (43.4%) and minimum in O₃ (14.1%) treated plants of T-397 (Fig. 4). Similarly, increment of epicuticular wax content was maximum in T-397, as compared to Padmini. Two-way ANOVA test showed significant difference due to individual response of Cv and T (p<0.001) (Table 3).

**Leaf surface level changes**

SEM of abaxial surface of healthy leaf showed intact stomata and even distribution of wax on subsidiary and guard cells (Fig. 5). Wax crystallites were less prominent under sUV-B, O₃ and sUV-B + O₃ exposure and amorphous layers were more evident. Stomata were also embedded by surrounding cells and appeared as sunken in stressed leaves. Combined
exposure was less affective as compared to individual treatment of sUV-B and O₃. Total number of stomata observed was 19, 23, 11 and 12 in C, sUV-B, O₃ and sUV-B + O₃ (in 10.9 x 10⁻⁴ µm² area).

Biomass and yield
Total biomass showed maximum reduction of 54.7%, 40.6% and 27.4% under O₃, sUV-B and sUV-B + O₃ treatments, respectively in Padmini (Fig. 6). Maximum reduction of 56.9% was recorded in seed yield of sUV-B exposed plants of Padmini, followed by 42.5% in O₃ and 27.6% in sUV-B + O₃ (Fig. 5). Two-way ANOVA test showed significant variation in biomass due to Cv, T and Cv × T, while seed yield was significant only for T and Cv × T (Table 3).

Discussion
Study site of present study was a sub urban site, where O₃ precursors are transported from nearby urban and industrial area. During study period, mean monthly ambient O₃ concentration varied from minimum 27.7 ppb in December to maximum 59.0 ppb in March. High O₃ concentration in March might be due to high temperature and availability of high solar radiation. Variation in mean O₃ concentration from 30.3 to 46.6 ppb is also reported earlier at the same site²². Another study has also reported that different cities of India, including Varanasi is experiencing more than 40 ppb O₃ concentrations, with northern part of India facing higher concentrations of O₃ than southern area²³. Some other reports from Asian countries have also shown high concentrations of O₃ like 13.8-74.2 ppb in China²⁴ and 72 ppb in Pakistan²⁵. Likewise, significant declining trend of total O₃ column is also reported in Northern part of India²⁶.

About 40% part of Northern India, encompassing the highly fertile Indo-Gangetic plain is experiencing the 1.5% depletion per decade, which can have serious impact on agricultural produce and food security²⁷. Indo-Gangetic basin has significant load of sulphate aerosols and dust particles, which react with atmospheric gases and play an important role in TOC decline in these areas²⁸. During the winter season, thickness of O₃ layer was depleted up to 243.4 DU at Varanasi site for the period 1979-1993²⁸.

Exposure of sUV-B, O₃ and sUV-B + O₃ stimulated the production of ROS (H₂O₂, O₂⁻) in linseed. Cultivar Padmini showed maximum production of ROS, as compared to T-397. Earlier studies also reported an increase in production of H₂O₂ and O₂⁻ in citrus plants after O₃ exposure²⁹ and in cowpea plants after UV-B exposure³⁰. Both H₂O₂ and O₂⁻ are capable of oxidizing membrane constituents and at the same time also act as signaling molecule to upregulate the activity of antioxidants and production of defense-related metabolites⁶⁻⁸.

In present study, LPO and H₂O₂ was maximum at 100 DAG and solute leakage and O₂⁻ production rate was highest at 50 DAG in Padmini, while responses of all the parameters were maximum in T-397 at 50 DAG. These findings clearly suggested that initial increase in levels of LPO and ROS at 50 DAG might have aggravated the membrane leakage, but at later stage, it was compensated and thus indicating the induction of antioxidant defense system at a faster
rate in T-397. Increments in electrical conductivity and MDA content are also reported in O₃-exposed wheat leaves. Similarly, an increase in electrolyte conductivity after UV-B exposure is observed in earlier study, suggesting the possible effect of UV-B in inhibiting K⁺ATPase and peroxidation of lipids which decreases membrane integrity and increases electrolyte conductivity.

Plants manifest various strategies for tolerance and avoidance against UV-B radiation, such as synthesis of UV screening compounds, increase in thickness of leaf surface, synthesis of epicuticular wax and trichomes, etc. A number of experiments have been performed to estimate UV-B induced flavonoids production and the enzymes of phenyl propanoid pathway. Ozone is also known to increase the activity of enzymes of phenylpropanoid pathway namely, phenylalanin ammonia lyase (PAL), chalcone synthase (CHS) and chalcone synthase isomerase (CHI), which results in synthesis of flavonoids, lignin and various other phytoalexins. In the present study, plants exposed to sUV-B, O₃, sUV-B + O₃ stimulated the synthesis of flavonoids and anthocyanin, however, the increment was more in sUV-B, followed by sUV-B + O₃ and minimum in O₃. Role of flavonoids in reducing the penetration of UV-B and diminishing the effect of ROS by scavenging them has been reported by various workers. An increase in production of transcripts of PAL, CHS and CHI is also reported in O₃ sensitive Phaseolus vulgaris plants. Anthocyanin is a water soluble pigment derived from flavonoids via “Shikimic acid pathway” and plays pivotal role in epidermal screening.

The outermost layer of leaf with cuticle and epicuticular wax content protect the leaf surface against pollution and is defined as “the first line of defense”. Present study observed significant increments in wax and lignin contents, when exposed to sUV-B and O₃. Lignin and wax synthesis showed maximum induction in sUV-B treatment, followed by sUV-B + O₃ and O₃ and cultivar T-397 was more responsive as compared to Padmini. Previous studies have also suggested that UV-B induces wax synthesis, and especially crystalline wax which is very effective in reducing UV-B penetration to mesophyll cells. Another study has reported more induction of wax tubes along with increased thickness of epidermal layer after UV-B exposure in cotton leaves. Ozone-induced lignin biosynthesis has not been well-characterized, however, in poplar, O₃-dependent increase in lignin content is reported.

Scanning electron micrograph showed alterations in epidermal layer, wax crystallites and number of stomata under sUV-B, O₃ and sUV-B + O₃ exposure. Stomata appeared as embedded with surrounding cells and looked sunken in appearance. Number of stomata was increased under sUV-B exposure, however, O₃ and combined exposure showed a reduction in its number. An increase in number of stomata area after UV-B exposure is also reported in Taxus chinensis however, size of individual stomata is reduced. Similarly, alteration in structure of stomata and wax content of mastic plant has been observed after O₃ exposure.

Reduction in biomass accumulation is generally used as a reliable indication of plant sensitivity, because it represents the cumulative effect of all the altered physiological and biochemical functions. Cultivar Padmini showed maximum reduction in biomass after O₃ exposure, followed by sUV-B and sUV-B + O₃. Significant reduction of 25.8% is reported in total biomass of Amaranthus plants due to UV-B treatment. Similarly, O₃ has also been reported to reduce biomass of clover cultivar Vardan by 46.2%. Under combined exposure of UV-B and O₃, 20.6% reduction in biomass of wheat plants is recorded, however, the response is significant under individual exposure.

In the present study, there was a reduction in seed yield, but loss was maximum in sUV-B treated plants, followed by O₃ and sUV-B + O₃, suggesting that UV-B was more effective in flower suppression or delay of flowering, ovule abortion and lowering the final fruit set. The reduction in yield has also been reported in a wide range of crops with ambient and elevated dose of UV-B and O₃ exposure.

The high levels of flavonoids, lignin and epicuticular wax on leaf surface have been reported to reduce the epidermal transmittance of UV-B and thus may be helpful in providing protection against the harmful effects. Results of the present study were in agreement with earlier studies that high level of flavonoids, anthocyanin, lignin and epicuticular wax on leaves might provide some protection to plants by acting as barrier for penetration of harmful UV-B and thus reducing the magnitude of damage under sUV-B + O₃ treatments. Similar response has been reported in spruce plants, where both the stresses lower their harmful effect in combination.

**Conclusions**

The present study demonstrated that supplemental dose of UV-B (+7.2 kJ m⁻² d⁻¹) and O₃ (ambient +
10 ppb) induced the production of ROS, altered membrane integrity, as well as secondary metabolism and finally the yield of linseed under their individual and combined exposures. LPO and ROS production were highest in cultivar Padmini, which also showed maximum reduction in biomass and seed yield. However, T-397 was less affected in terms of membrane damage, biomass and yield. Secondary metabolites (flavonoids, antocyanin, lignin and wax) were also induced in high concentrations in T-397, indicating its higher resistance towards the applied stress(s). Combined exposure of uUV-B and O₃ was less effective, as compared to their individual treatment.

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