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Evaluation of physiological, growth and yield responses of a tropical oil crop (*Brassica campestris* L. var. Kranti) under ambient ozone pollution at varying NPK levels

Poonam Singh, Madhoolika Agrawal*, Shashi Bhushan Agrawal

Laboratory of Air Pollution and Global Climate Change, Ecology Research Circle, Department of Botany, Banaras Hindu University, Varanasi 221005, India

NPK level above recommended alleviates the adverse effects of ambient ozone on a tropical mustard cultivar.

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ABSTRACT

A field study was conducted to evaluate the impact of ambient ozone on mustard (*Brassica campestris* L. var. Kranti) plants grown under recommended and 1.5 times recommended NPK doses at a rural site of India using filtered (FCs) and non-filtered open top chambers (NFCs). Ambient mean O_3 concentration varied from 41.65 to 54.2 ppb during the experiment. Plants growing in FCs showed higher photosynthetic rate at both NPK levels, but higher stomatal conductance only at recommended NPK. There were improvements in growth parameters and biomass of plants in FCs as compared to NFCs at both NPK levels with higher increments at 1.5 times recommended. Seed yield and harvest index decreased significantly only at recommended NPK in NFCs. Seed quality in terms of nutrients, protein and oil contents reduced in NFCs at recommended NPK. The application of 1.5 times recommended NPK provided protection against yield loss due to ambient O_3.

1. Introduction

The rapid deterioration of the air quality has been recognized as a significant threat to food production throughout the world. The National Crop Loss Assessment Programme (NCLAN) was initiated in USA using open top chambers at different regional sites following a standardized protocol on relationship to assess yield losses due to air pollution especially O_3. Based on the NCLAN crop response studies, Legge et al. (1993) reported a crop growth threshold for O_3 as 35 ppb. In late 1980s, yield reductions due to O_3 were calculated to be about 5% of national production in USA and the economic benefit by reducing O_3 concentrations to 40% was calculated to be about U.S.$ 3 billion annually (Heck et al., 1988). On a similar approach, Commission of European Communities (CEC) started a large-scale experimental programme named European Open Top Chamber Programme (EOTCP) involving many countries to find out the response of a range of species in rural areas, where O_3 is the main pollutant. These experiments have demonstrated that O_3 has significantly reduced the growth and yield of crops in many parts of Europe (Schenone and Lorenzini, 1992; Jager et al., 1994; Adaros et al., 1991; Fuhrer et al., 1989). Yield losses of 20% in sensitive crops were reported from Mediterranean sites of Europe, where higher mean O_3 concentrations were recorded (Schenone et al., 1992).

Implications of air pollutant effects on crop plants are also reported from many developing countries, which are rapidly industrializing like China (Wang and Mauzerall, 2004; Wang et al., 2007, 2008), India (Agrawal et al., 2003; Tiwari et al., 2006; Rai et al., 2007) and Pakistan (Wahid, 2006a,b). Evidences are, however, limited and based on a wide range of experimental approaches ranging from transect studies along pollution gradients, use of chemical protectants and air filteration studies on selected staple and vegetable crops of Asia. Wahid et al. (1995) observed that mean O_3 concentrations of 35.6 ppb caused reductions in total grain weight plant^{-1} by 42% for Basmati-385 and 37% for IRRI-6 varieties of rice in unfiltered air chambers (UFA) as compared to those grown in charcoal filtered air chambers (FA) in Lahore, Pakistan. Wahid (2006a) reported that unfiltered air (UFA) caused grain yield reductions of 43% for Pasban-90, 39% for Punjab-91 and 18% for Inquilab-91 varieties of wheat as compared to those grown in charcoal filtered air (FA). Rai et al. (2007) reported reductions of 27.1% in photosynthetic rate, 18.8% in test weight, 8.4%

Abbreviations: OTCs, open top chambers; NFCs, non-filtered chambers; FCs, filtered chambers; OPs, open plots; ppb, parts per billion; Ps, photosynthetic rate; Cs, stomatal conductance; F_o, initial fluorescence; F_m, maximum fluorescence; F_v, variable fluorescence; PAR, photosynthetically active radiation; DAG, days after germination; RGR, relative growth rate; NAR, net assimilation rate; LAR, leaf area ratio; SLW, specific leaf weight; RSR, root shoot ratio; HI, harvest index.

* Corresponding author. Tel.: +91 542 2368156; fax: +91 542 2368174.
E-mail address: madhoo58@yahoo.com (M. Agrawal).

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in harvest index and 20.7% in yield of wheat var. HUW 234 grown in NFCs as compared to those in FCs.

Impact of ambient air pollution especially O_3 has largely been studied on major staple crops and vegetables throughout the world. However, information on response of mustard plants under field conditions at ambient and elevated concentrations of O_3 are scanty despite its most important contribution in oil production. Adverse impact of O_3 on seed yield and quality and seedling vigour of two oil seed rape (*Brassica napus* L.) varieties observed in controlled environment chambers suggested greater implications of effects on seed quality and quantity under field conditions (Bosac et al., 1994, 1998). Ollerenshaw et al. (1999) found reductions in seed yield and quality (crude protein and oil contents) of a sensitive variety of *B. napus* cv. Eurol at ambient and elevated O_3 concentrations using OTCs. Reductions in stomatal conductance and net photosynthesis rate, vegetative growth and number of reproductive sites on the terminal raceme and oil and protein contents of seeds of *Brassica campestris* cv. Wisconsin Fast Plants were reported at 10-d exposure of 70 ppb O_3 for 7 h d^{-1} in controlled environment exposure chambers (Black et al., 2007). Recently Wang et al., (2008) have shown that more reductions in biomass production and pod and seed yield of *B. napus* cv. Huyou 19 were caused by a complete diurnal O_3 exposure regime than a steady state single O_3 showing the same mean O_3 concentration.

Responses of plants to air pollutants vary with the supply of mineral nutrients and with specific nutrient element also (Ormrod and Adedipe, 1974). Soybean (*Glycine max* L. cv. JS-72-44) plants grown at low fertility levels have been found to be more sensitive to SO_2 (Verma and Agrawal, 1996). Soil with high available phosphorus increased the photosynthetic rate of *Pinus pinaster* by 17% (Rousseau and Reid, 1990). Adverse impacts of ambient air pollutants on growth and biomass accumulation of *Beta vulgaris* L. were minimized at high fertility levels under pot experiments (Singh et al., 2005).

The air quality of most of the Indian cities is increasingly deteriorated due to rapid industrialization with poor emission control and unorganized urbanization with growing motor traffic. India is the third largest mustard seed producer in the world. Mustard is the most important oil seed crop accounting approximately 25% of total oil seed production in the country for domestic consumption. Its production has increased substantially from 0.903 million tonnes in 1950–1951 to 6.69 million tonnes in 2006–2007.

The present study was undertaken to quantify the changes in morphological and physiological characteristics, biomass accumulation and allocation, yield attributes and quality of seeds of mustard (*B. campestris* L. var. Kranti) grown under natural field conditions at a rural site experiencing elevated levels of O_3, using open top chambers. Plants were grown at recommended and 1.5 times recommended NPK levels to examine the possibility of alleviating the adverse effects of ambient O_3 at higher fertility levels.

2. Material and methods

2.1. Experimental site and design

The study was conducted at a rural site of Varanasi located in the eastern Gangetic plains of India at 25°14′N latitude, 82°03′E longitude and 76.19 m above sea level. The experiment was carried out between the months of November 2006 and March 2007. This period of the year showed mean maximum temperature variations from 24.2 to 28.7 °C and mean minimum temperature from 7.84 to 13.72 °C. The mean maximum relative humidity varied from 79 to 84.5% and mean minimum from 37.5 to 54.5%. The total rainfall during the entire experimental period was 105.2 mm. The sunshine hours varied from 3.6 to 10.2 h.

Twelve open top chambers were installed at the experimental site during early November 2006. Open top chambers of 1.5 m in diameter and 1.8 m height were fabricated following the design of Bell and Ashmore (1986). A detailed description of the design of the OTCs is given by Tiwari et al., (2006). Each of the chambers was attached to high speed blower to subject three air changes per min around the inner perimeter of the chamber. Six chambers were ventilated with non-filtered air (NFCs) and another six with air that passed through activated charcoal filter (FCs) to remove pollutants from the atmosphere. All the chambers were provided with the prefilters made up of non-woven polyester to remove the dust or dirt entrained in the air flow. Six open plots (OPs) were also established to study the chamber effects on crops.

2.2. Climatology and O_3 monitoring

Climatic parameters (temperature, humidity and light) were assessed within and outside OTCs. Measurement of microclimatic conditions showed that mean temperature within the chambers was 0.1–0.2 °C higher as compared to OPs. Light intensity was 5% less inside the chambers as compared to the open plots. Relative humidity inside the chambers was 2–4% higher than observed for OPs.

Twelve hourly air monitoring for O_3 was conducted from germination to harvesting of the plants. Air samples were drawn through Teflon tube (0.35 cm diameter) at canopy height from different chambers between 8:00 and 20:00 h. The sampling tube was moved up as the plants grew. O_3 concentration was monitored using UV absorption photometric O_3 analyzer (Model 400A, API, Inc., USA).

2.3. Plant material and growth conditions

Mustard (*B. campestris* L. var. Kranti) was selected as an experimental plant because it is widely grown in the North-eastern plain zones of India. This variety is derived from mutant of varuna and has a life span of 125 days. It is used both as timely or late sown variety and is resistant to diseases.

The field was prepared by ploughing upto 20 cm depth. There were two treatments of NPK, recommended and 1.5 times recommended given as urea, single super phosphate and muriate of potash. Recommended dose of NPK was 80, 40 and 40 kg ha^{-1}, whereas 1.5 times recommended was 120, 60 and 60 kg ha^{-1}. Half dose of N and full doses of P and K were given as basal dressing and another half dose of N was given as top dressing. Seeds were hand sown in rows during mid-November and after one week of germination, plants were thinned to one plant every 15 cm. Field was irrigated time to time to maintain the soil moisture uniformity in chambers as well as open plots. There were three replicates of each treatments, i.e. NFCs with recommended, NFCs with 1.5 times recommended, FCs with recommended, FCs with 1.5 times recommended, OPs with recommended and OPs with 1.5 times recommended NPK.

2.4. Plants sampling and analysis

2.4.1. Physiological parameters

Photosynthetic rate (Ps) and stomatal conductance (Cs) were quantified using portable photosynthesis system (Model LI-6200, LI-COR, USA). The measurements were made on the third fully expanded mature leaf from the top of each plant on cloud free days between 09:30 and 10:30 h local time at 40 and 60 days after germination (DAG) on three randomly selected plants in each chamber. During the measurements, photosynthetically active radiation (PAR) ranged between 1100 and 1200 μmol m^{-2} s^{-1}. The system was calibrated using a known CO_2 source of 509 ppm concentration.

Chlorophyll fluorescence was determined between 10:30 and 11:30 h using portable plant efficiency analyzer (Model, MK2, 9414, Hansatech Instrument Ltd., UK) on the same leaves where Ps was measured. Leaf clips for dark adaptation were placed on the adaxial side of the leaves for 10 min before measurement at excitation irradiance of 2000 μmol m^{-2} s^{-1}. Minimum fluorescence (F_o) and maximum fluorescence (F_m) were measured from which variable fluorescence ($F_v = F_m - F_o$) and ratio of variable and maximum fluorescence (F_v/F_m) were calculated.

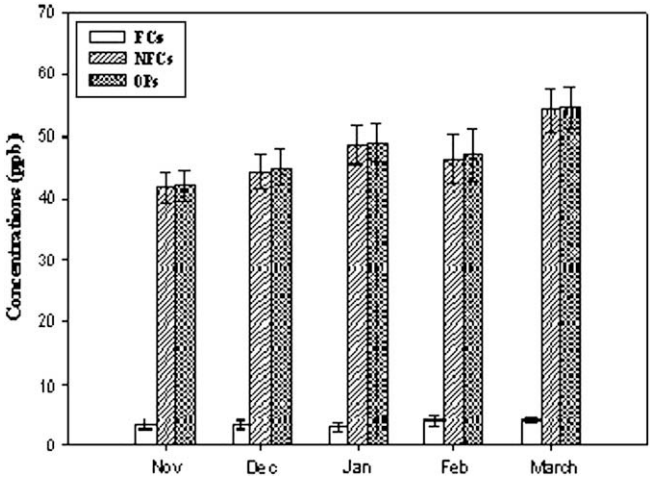

Fig. 1. Mean concentrations (ppb) of ozone at the experimental site (12-h daily mean). Values are mean ± 1 SE.

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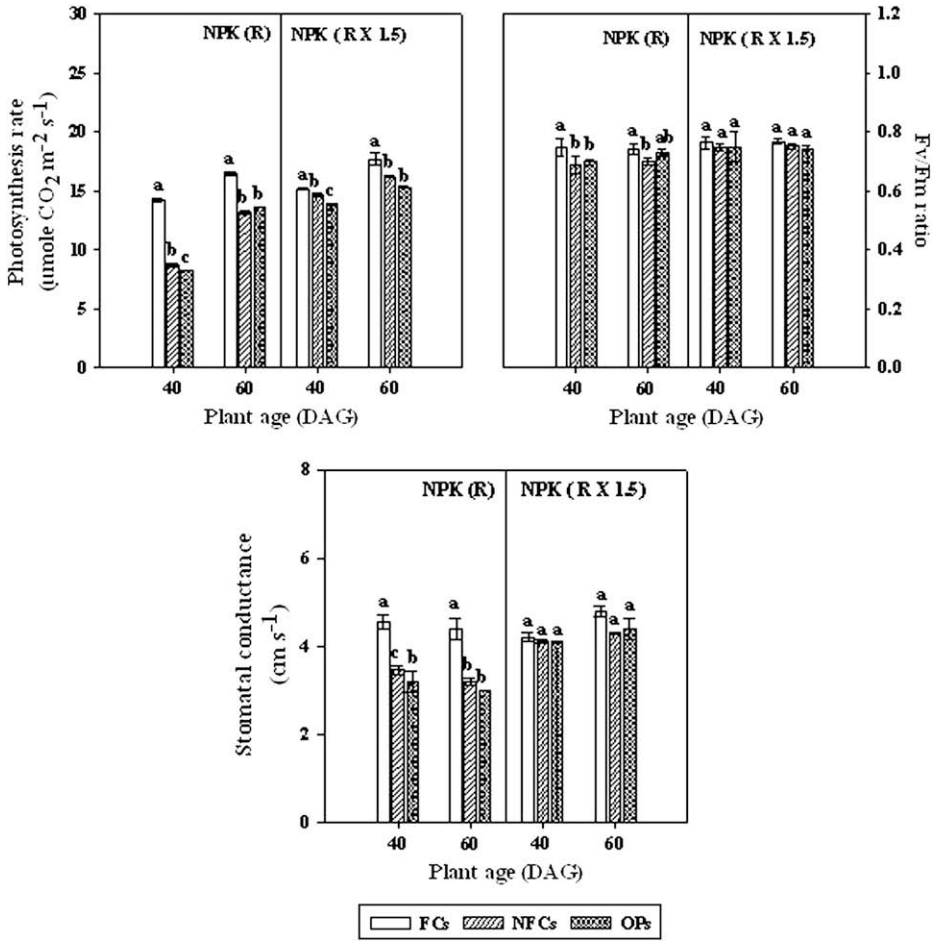

Fig. 2. Photosynthesis rate, stomatal conductance and F_v/F_m ratio in mustard plants grown in filtered chambers (FCs), non-filtered chambers (NFCs) and open plots (OPs) at different levels of NPK. Bars represent mean ± SE. Bars followed by different letter are significantly different from each other at $p < 0.05$.

2.4.2. Morphological parameters and biomass

For biomass and growth determinations, three random samples were taken from each chamber and open plots at 40 and 60 DAG by carefully digging monoliths ($10 \times 10 \times 20$ cm^3) containing intact roots. These were thoroughly washed by placing on a sieve of 1 mm diameter under running tap water to remove soil particles adhering to the roots. Shoot and root lengths, leaf area, number of leaves and pods plant^{-1} were estimated. Leaf area was measured using portable leaf area meter (Model LI-3000, LI-COR, Inc., USA). For biomass determination component wise plant parts were separated and oven dried at 80 °C till constant weight was achieved. For understanding the biomass allocation pattern growth indices relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), specific leaf weight (SLW), root shoot ratio (RSR) were calculated on dry weight data by following the formulae modified by Hunt (1982).

2.4.3. Yield parameters

Yield parameters were assessed at the time of final harvest during the end of March by harvesting five plants from different treatments. Number and weight of pods plant^{-1}, number of seeds pod^{-1}, number and weight of seeds plant^{-1}, weight of above ground parts and weight of 1000 seeds (test weight) were calculated. Harvest index (HI) was calculated as the ratio of weight of seeds and total above ground biomass plant^{-1}.

2.4.4. Seed quality characteristics

Seed protein content was determined by the method of Lowry et al. (1951). The oil content of seeds was determined by using the Soxhlet extraction method (Chopra and Kanwar, 1991). For nutrient analysis, oven dried samples of seeds were ground in a stainless steel grinder and passed through a 2 mm sieve. For determination of Ca, Mg, K, P and Zn, 0.1 g powdered sample was digested in a mixture of $HClO_4$, HNO_3 and H_2SO_4 (5:1:1) by following the method of Allen et al. (1986). The digested samples were filtered through Whatmann No. 42 filter paper and volume was maintained to 25 ml with distilled water. Concentrations of Ca, K, Mg and Zn in filtrate were determined with the help of Atomic Absorption Spectrophotometer (Model 2380, Perkin Elmer, USA) and of P by following the method given by Jackson (1958). Total nitrogen was quantified by micro-kjeldahal technique through Gerhardt Automatic N Analyzer (Model KB8S, Germany).

Table 1
Yield parameters of mustard plants grown in filtered chambers (FCs), non-filtered chambers (NFCs) and open plots (OPs) at different levels of NPK (mean ± SE).

Parameters	Recommended NPK			Recommended ($R \times 1.5$)		
	FCs	NFCs	OPs	FCs	NFCs	OPs
No. of pods plant^{-1}	496.60^a ± 3.82	488.80^a ± 5.37	492.40^a ± 5.30	498.60^a ± 4.46	492.20^a ± 4.43	495.60^a ± 4.12
Pod weight (g plant^{-1})	44.96^a ± 1.09	43.36^a ± 0.42	43.16^a ± 0.51	46.28^a ± 0.69	44.96^a ± 0.49	44.40^a ± 0.45
No. of seeds plant^{-1}	6066.60^a ± 468	4892.0^b ± 353	4952.0^b ± 531	5973.0^a ± 310	5923.80^a ± 453	5910.42^a ± 426
No. of seeds pod^{-1}	12.20^a ± 0.86	8.60^b ± 0.70	8.0^b ± 0.86	12.0^a ± 0.70	11.40^a ± 0.86	11.0^a ± 0.86
Seed weight (g plant^{-1})	75.80^a ± 1.41	63.38^b ± 4.93	64.30^b ± 1.24	78.40^a ± 4.95	77.40^a ± 3.33	76.04^a ± 3.73
Test weight (g)	3.56^a ± 0.2	3.48^a ± 0.2	3.44^a ± 0.2	4.40^a ± 0.29	4.16^a ± 0.29	3.72^a ± 0.24
Above ground biomass (g plant^{-1})	193.12^a ± 1.15	179.66^b ± 5.5	183.18^b ± 1.97	197.32^a ± 5.5	195.0^a ± 2.73	194.06^a ± 2.79
Harvest index (g g^{-1})	0.394^a ± 0.008	0.352^b ± 0.001	0.350^b ± 0.003	0.396^a ± 0.001	0.396^a ± 0.001	0.390^a ± 0.006

Values followed by different letters are significantly different ($p < 0.05$).

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Table 2
Seed quality characteristics of mustard plants grown in filtered chambers (FCs), non-filtered chambers (NFCs) and open plots (OPs) at different levels of NPK (mean ± 1 SE).

Parameters	Recommended NPK			Recommended ($R \times 1.5$)		
	FCs	NFCs	OPs	FCs	NFCs	OPs
Calcium ($mg\,g^{-1}$)	$44.78^{a} \pm 0.02$	$37.05^{b} \pm 0.03$	$36.67^{b} \pm 0.02$	$51.80^{a} \pm 0.03$	$51.50^{a} \pm 0.02$	$50.81^{a} \pm 0.02$
Magnesium ($mg\,g^{-1}$)	$20.09^{a} \pm 0.02$	$14.70^{b} \pm 0.13$	$14.12^{b} \pm 0.11$	$27.62^{a} \pm 0.04$	$27.56^{a} \pm 0.02$	$27.50^{a} \pm 0.03$
Potassium ($mg\,g^{-1}$)	$8.80^{a} \pm 0.03$	$6.04^{b} \pm 0.02$	$6.00^{b} \pm 0.03$	$9.90^{a} \pm 0.02$	$8.5^{a} \pm 0.03$	$8.10^{a} \pm 0.02$
Zinc ($\mu g\,g^{-1}$)	$40.00^{a} \pm 0.28$	$26.08^{b} \pm 0.36$	$26.00^{b} \pm 0.32$	$40.00^{a} \pm 0.28$	$40.00^{a} \pm 0.28$	$39.8^{a} \pm 0.25$
Phosphorus ($mg\,g^{-1}$)	$0.28^{a} \pm 0.002$	$0.24^{a} \pm 0.003$	$0.24^{a} \pm 0.002$	$0.32^{a} \pm 0.001$	$0.32^{a} \pm 0.002$	$0.33^{a} \pm 0.002$
Nitrogen ($mg\,g^{-1}$)	$8.55^{a} \pm 0.02$	$7.76^{b} \pm 0.03$	$7.83^{b} \pm 0.02$	$9.76^{a} \pm 0.03$	$9.38^{a} \pm 0.02$	$9.36^{a} \pm 0.01$
Protein ($mg\,g^{-1}$)	$95.60^{a} \pm 0.23$	$83.60^{b} \pm 0.71$	$78.50^{c} \pm 0.75$	$96.50^{a} \pm 0.75$	$95.40^{a} \pm 0.92$	$93.0^{a} \pm 1.3$
Oil ($g\,100\,g^{-1}$)	$25.09^{a} \pm 0.22$	$22.0^{b} \pm 0.20$	$21.8^{b} \pm 0.22$	$27.27^{a} \pm 0.21$	$26.0^{a} \pm 0.12$	$25.89^{a} \pm 0.15$

Values followed by different letters are significantly different ($p < 0.05$).

Table 3
Results of three-way ANOVA test showing *F*-values and level of significance for selected physiological and growth characteristics of mustard plants grown at different NPK levels in FCs, NFCs and OPs.

Parameters	Age	NPK level	Treatment	Age × NPK level	Age × treatment	NPK level × treatment	Age × NPK level × treatment
Root length	97.73^{***}	10.3^{**}	20.1^{***}	27.3^{***}	2.0^{NS}	2.7^{NS}	0.1^{NS}
Shoot length	3061.2^{***}	186.9^{***}	69.5^{***}	242.7^{***}	11.8^{***}	7.6^{**}	4.9^{*}
No. of leaves	0.04^{NS}	11.5^{**}	1.9^{NS}	21.1^{***}	0.2^{NS}	0.04^{NS}	1.2^{NS}
Leaf area	12.6^{**}	0.1^{NS}	0.4^{NS}	0.4^{NS}	0.1^{NS}	0.04^{NS}	0.1^{NS}
No. of pods	1131.4^{***}	37.6^{***}	1.1^{NS}	37.6^{***}	1.1^{NS}	0.3^{NS}	0.3^{NS}
No. of inflorescence	180.1^{***}	22.6^{***}	1.3^{NS}	22.6^{***}	1.3^{NS}	0.1^{NS}	0.1^{NS}
F_v/F_m ratio	0.1^{NS}	5.7^{*}	1.7^{NS}	0.1^{NS}	0.09^{NS}	0.5^{NS}	0.2^{NS}
Photosynthetic rate	743.7^{***}	827.0^{***}	333.5^{***}	107.7^{***}	7.6^{**}	93.1^{***}	35.6^{***}
Stomatal conductance	1.3^{NS}	81.7^{***}	46.7^{***}	13.1^{***}	0.8^{NS}	20.6^{***}	0.4^{NS}
Root biomass	276.8^{***}	2.1^{NS}	14.4^{***}	0.003^{NS}	9.0^{***}	0.1^{NS}	7.4^{**}
Shoot biomass	2399.0^{***}	179.1^{***}	48.1^{***}	44.0^{***}	16.0^{***}	1.9^{NS}	5.8^{**}
Leaf biomass	5.0^{*}	51.1^{***}	9.3^{***}	3.7^{NS}	0.8^{NS}	1.9^{NS}	0.6^{NS}
Pod biomass	723.0^{***}	6.4^{*}	4.1^{*}	6.4^{*}	4.1^{*}	3.6^{*}	3.6^{*}

Level of significance: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; NS = not significant.

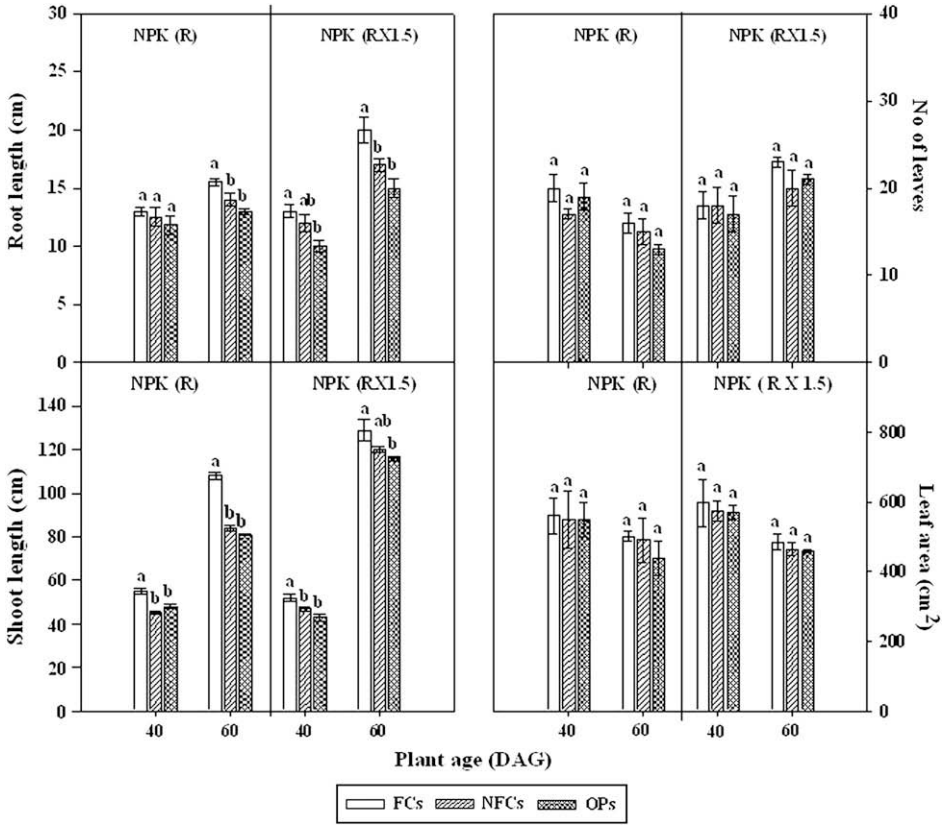

Fig. 3. Morphological parameters of mustard plants grown in filtered chambers (FCs), non-filtered chambers (NFCs) and open plots (OPs) at different levels of NPK. Bars represent mean ± SE. Bars followed by different letter are significantly different from each other at $p < 0.05$.

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2.5. Statistical analysis

Data of the physiological and growth characteristics were subjected to three-way analysis of variance (ANOVA) tests to examine the individuals and combined effects of age, NPK level and treatment. Data of yield parameters were analyzed through two-way ANOVA tests for assessing the significance of changes due to treatment and NPK level. Duncan's multiple range tests were performed as post hoc on parameters subjected to various ANOVA tests. All the statistical tests were performed using SPSS software (SPSS Inc., version 10.0).

3. Results

3.1. Ozone concentration

At the experimental site, 12 hourly mean concentrations of O_3 varied from 41.65 ppb during November 2006 to 54.2 ppb during March 2007 (Fig. 1). Concentration of O_3 was 92.6% higher in NFCs as compared to FCs. The concentration of O_3 in NFCs and OPs was more or less similar.

3.2. Physiological parameters

Significantly higher photosynthetic rate (Ps) was observed for plants growing in FCs in comparison to those in NFCs and OPs at all the ages of observations (Fig. 2). Ps decreased by 39 and 20% at recommended NPK and 3.3 and 8.5% at 1.5 times recommended NPK in NFCs as compared to FCs, respectively, at 40 and 60 DAG. Stomatal conductance was significantly more in FCs as compared to those of NFCs and OPs at recommended NPK, but not significantly different at 1.5 times recommended NPK (Fig. 2). F_v/F_m ratio showed significantly lower values in plants of NFCs and OPs grown at recommended NPK as compared to FCs (Fig. 2).

Three-way ANOVA tests showed significant variations in photosynthetic rate due to all the individual factors and their interactions, but stomatal conductance varied significantly due to NPK level, treatment, age × NPK level and NPK level × treatment (Table 3). F_v/F_m ratio only showed significant variation due to NPK level.

3.3. Morphological parameters

Root length was significantly higher in FCs as compared to those in NFCs and OPs at recommended NPK at 60 DAG, but was not significantly different at 40 DAG (Fig. 3). Root length at 1.5 times recommended NPK reduced significantly in NFCs at both the ages of observations. Shoot length significantly decreased by 18.2 and 22.2% at recommended and 9.6 and 7.0% at 1.5 times recommended NPK in NFCs as compared to FCs, respectively, at 40 and 60 DAG (Fig. 3). Number of leaves and inflorescence and leaf area did not vary significantly between FCs, NFCs and OPs at any of NPK levels and ages of observations (Figs. 3 and 4). Number of pods plant^{-1} decreased at recommended NPK in NFCs and OPs than those in FCs (Fig. 4).

A three-way ANOVA test showed that variations in shoot length were significant due to all the individual factors and their interactions (Table 3). Root length, however, varied significantly due to all the individual factors and age × NPK level (Table 3). Leaf area showed variations with age, whereas number of leaves varied significantly with NPK level and age × NPK level. Number of pods and inflorescence showed significant effects of age, NPK level and their interaction (Table 3).

3.4. Biomass accumulation and allocation

Root biomass did not vary significantly between FCs, NFCs and OPs at recommended NPK at both the ages of observations, but reduced significantly at 60 DAG at 1.5 times recommended (Fig. 5).

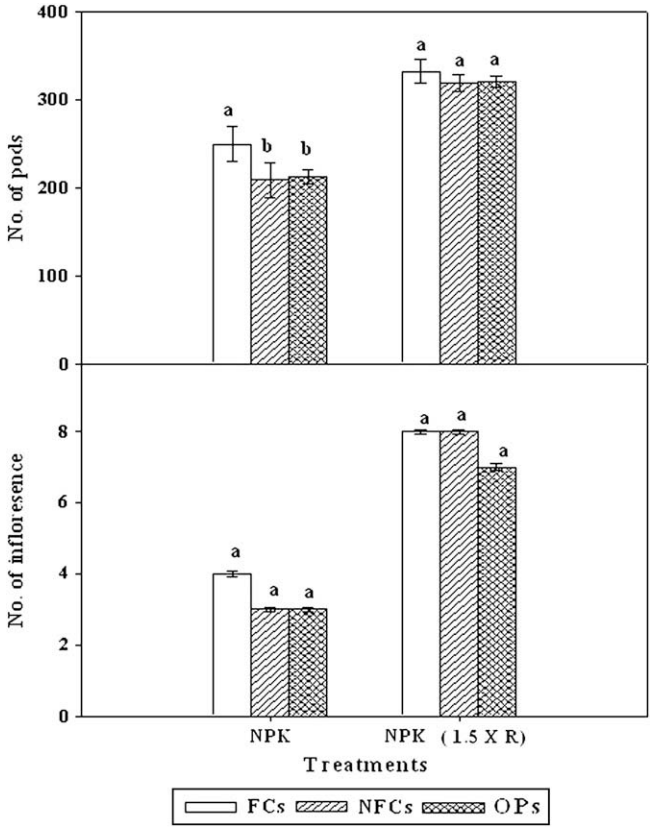

Fig. 4. Numbers of pods and inflorescence plant^{-1} in mustard plants grown in filtered chambers (FCs), non-filtered chambers (NFCs) and open plots (OPs) at 60 DAG at different levels of NPK. Bar represents mean ± SE. Bars followed by different letter are significantly different from each other at $p < 0.05$.

Shoot biomass was significantly lower in plants of NFCs and OPs at recommended NPK at both the ages of observations (Fig. 5). Shoot biomass at 1.5 times recommended NPK did not vary between treatments at 40 DAG, but decreased significantly at 60 DAG in NFCs and OPs (Fig. 5). Leaf biomass was significantly more in FCs as compared to those of NFCs and OPs at recommended NPK at 40 DAG, but no significant difference was observed at 60 DAG. Leaf and pod biomass did not vary significantly between treatments at 1.5 times recommended NPK (Fig. 5). Pod biomass was significantly more in FCs as compared to those of NFCs and OPs at recommended NPK.

Three-way ANOVA tests revealed that all the biomass components varied significantly due to individual factors, age, NPK level and treatment except root biomass, which did not vary significantly due to NPK level (Table 3). Significant effects of age × NPK level on shoot and pod biomass, age × treatment on root, shoot and pod biomass, NPK level × treatment on pod biomass and age × NPK level × treatment on root, shoot and pod biomass were recorded (Table 3).

Relative growth rate (RGR) and net assimilation rate (NAR) were significantly lower in plants of NFCs and OPs at recommended NPK at 40 DAG, but no significant difference was observed at 60 DAG. A contrasting trend was observed for RGR and NAR at different treatments at 1.5 times recommended NPK (Fig. 6) except NAR at 60 DAG. Leaf area ratio (LAR) did not vary significantly between treatments at both the NPK levels and ages of observations (Fig. 6). Specific leaf weight (SLW) was significantly lower in plants grown in NFCs and OPs at recommended NPK at 40 DAG. But at 1.5 times recommended NPK, variations in SLW were not significant (Fig. 7). Root shoot ratio (RSR) did not vary significantly between

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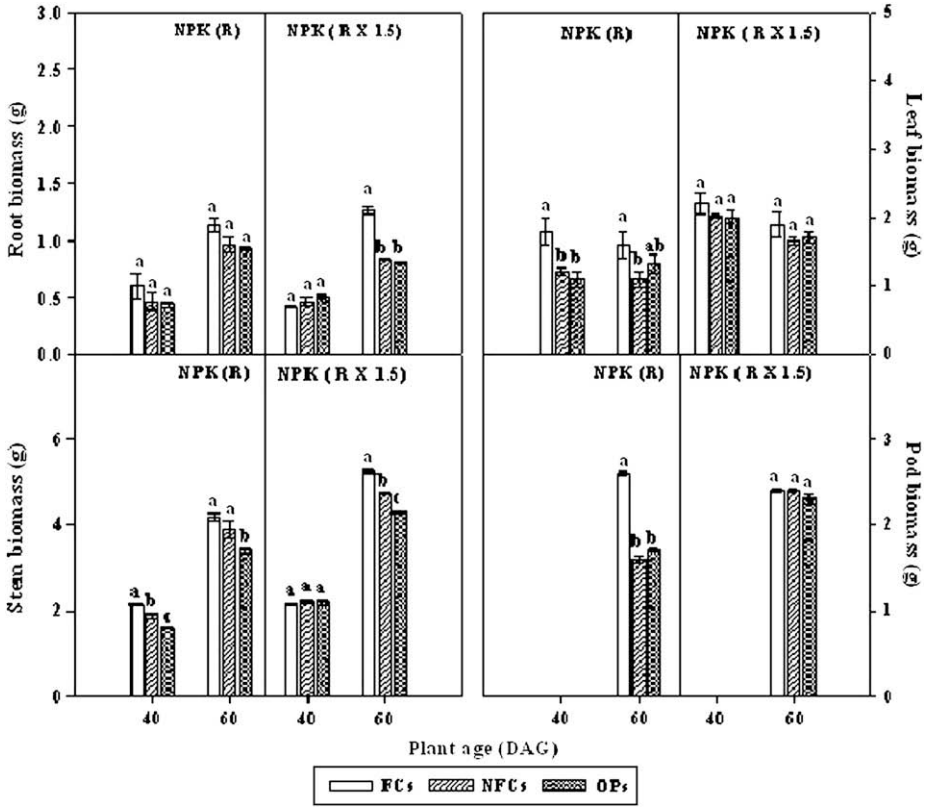

Fig. 5. Root, stem, leaf and pod biomass (g plant^{-1}) of mustard plants grown in filtered chambers (FCs), non-filtered chambers (NFCs) and open plots (OPs) at different levels of NPK. Bars represent mean ± SE. Bars followed by different letter are significantly different from each other at $p < 0.05$.

treatments at recommended NPK at both the ages of observations, but declined significantly at 60 DAG at 1.5 times recommended.

3.5. Yield parameters

Number of pods and pod weight plant^{-1} did not vary significantly between treatments at both the NPK levels. Number of seeds plant^{-1} and pod^{-1} decreased by 19.4 and 29.5%, respectively, at recommended NPK in plants growing in NFCs as compared to FCs, but did not vary significantly at 1.5 times recommended NPK (Table 1). Yield and HI decreased by 16.4 and 10.7%, respectively, at recommended NPK in NFCs as compared to FCs, but did not vary significantly at 1.5 times recommended NPK (Table 1). Above ground biomass at the time of harvest significantly reduced at recommended NPK by 7.0 and 5.1%, respectively, in NFCs and OPs as compared to FCs, but did not vary significantly at 1.5 times recommended NPK (Table 1). Test weight did not vary between FCs, NFCs and OPs at both NPK doses (Table 1).

Two-way ANOVA test showed that variations in yield (seed weight plant^{-1}), above ground biomass and harvest index were significant due to NPK level (Table 4). Variation in number of seeds pod^{-1} was significant due to all the individual factors and their interaction (Table 4). Other parameters, however, did not show significant variations.

3.6. Seed quality characteristics

The oil content was significantly lower by 12.3 and 13.1%, respectively, in plants grown in NFCs and OPs as compared to FCs at recommended NPK (Table 2). No significant difference in oil content was observed between treatments at 1.5 times recommended NPK. The concentrations of protein, N, P, Ca, Mg, K and Zn in seeds were lower in plants grown in NFCs as compared to FCs at recommended NPK, but no significant changes were observed at 1.5 times recommended NPK (Table 2). The percent reductions at recommended NPK were 12.5 for protein, 9 for N, 14 for P, 17 for Ca, 26.8 for Mg, 31 for K, and 34.8 for Zn, respectively, in seeds of plants grown in NFCs as compared to FCs.

4. Discussion

The experiment performed in open top chambers clearly suggests that ambient O_3 levels at experimental rural area of Varanasi exhibited potential risk for yield loss of mustard plants at recommended NPK level. Monthly mean O_3 concentrations varied from 41.65 to 54.2 ppb during the growth period of the test plant. Mean O_3 concentrations in suburban area of Varanasi varied from 35.33 to 43.74 ppb during December 2002 to March 2003 (Tiwari et al., 2006) and 36.4 to 48 ppb during December 2005 to March 2006 (Rai et al., 2007). Wahid et al. (2006a,b) reported mean O_3 concentrations of 72 and 71 ppb at a rural site in Lahore, Pakistan during 2003–2004 and 2004–2005, respectively. Higher O_3 concentrations at rural than suburban/urban areas were earlier reported by Agrawal et al. (2003) and Hassan et al. (1995) in dry tropical areas of India and Egypt, respectively. O_3 monitoring data showed lower concentrations during the early stage of plant growth (vegetative; December to January) as compared to reproductive stage (February to March). During the present study, charcoal filters efficiently scrubbed O_3 from ambient air. Filteration efficiencies of charcoal filters for O_3 were found to be 92% and 90.4% in OTC experiments conducted by Wahid (2006a) and Rai et al. (2007), respectively, during the same season. Microclimatic conditions did not vary considerably between the chambers and OPs during the study period, which may be ascribed to the cool dry season of the year when mustard is grown. Rai et al. (2007) also observed similar variations in microclimatic conditions during same months of 2005–2006.

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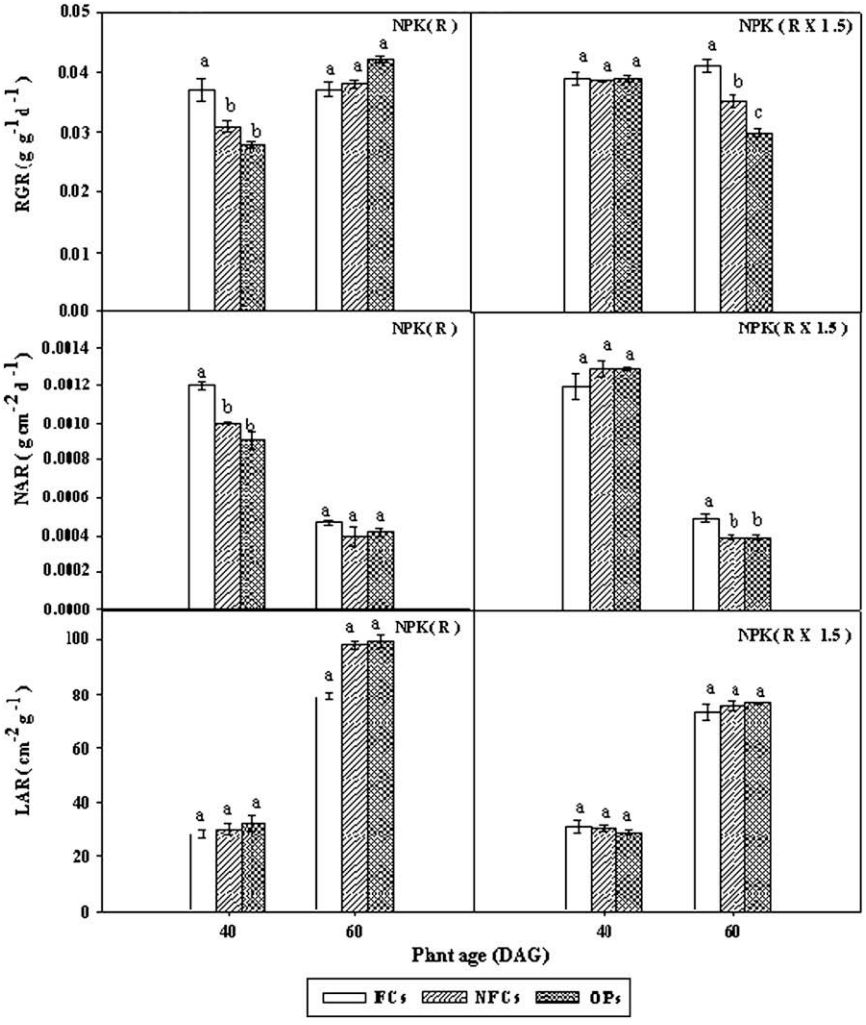

Fig. 6. Relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR) of mustard plants grown in filtered chambers (FCs), non-filtered chambers (NFCs) and open plots (OPs) at different levels of NPK. Bars represent mean ± SE. Bars followed by different letter are significantly different from each other at $p < 0.05$.

Plants growing in FCs showed significant increments in Ps and Cs as compared to those growing in NFCs and OPs at both the treatments of NPK. Reduction in Ps under O_3 stress is correlated with reduced activity and levels of Rubisco (Clark et al., 1996). Wahid (2006a) also observed significant reductions in Ps and Cs of three varieties of wheat grown in UFA and AA than those in FA. Rai et al. (2007) recorded 27 and 20% decline in Ps and Cs, respectively, of wheat cv. HUW 234 grown in NFCs as compared to FCs. O_3 generally causes closure of stomata, which may consequently reduce Ps (Calatayud and Barreno, 2004). O_3 concentration of 45 ppb for 8 h daily for 4 weeks during anthesis period has been shown to reduce Ps of flag leaves of wheat cv. Satu by 40% (Ojenpera et al., 1998). Pell et al. (1992) suggested that decline of photosynthesis can be a result of the direct effect of O_3 on stomata, but oxyradicals generated by O_3 can also alter photosynthetic electron transport and enzymatic activities.

In the present study, F_v/F_m ratio was lower in plants grown in NFCs and OPs as compared to FCs, but the difference was only significant at recommended NPK. Slight decline in photosynthetic efficiency (F_v/F_m ratio) indicates chronic photoinhibition. Reductions in Ps and Cs of plants grown in NFCs and OPs were several fold lower at 1.5 times recommended than recommended NPK. Maheshwari et al. (1993) observed that N application helps to increase photosynthesis by increasing RuBP carboxylase activity. Avdeeva and Andreeva (1973) also reported that under double recommended NPK, plants showed better result than recommended NPK due to nitrogen, which helps in photosynthesis by increasing RuBP carboxylase activity. In the present study at 1.5 times recommended NPK, plants may have maintained higher Rubisco content and activity even under O_3 stress condition and hence showed lower magnitude of reduction in Ps as compared to recommended NPK.

Plants grown at 60 DAG at 1.5 times recommended NPK dose showed higher root and shoot lengths as compared to those grown at recommended NPK. Higher nutrient availability is, however, known to reduce root length in plants (Levin et al., 1989). Root and shoot lengths were found to reduce in wheat cv. HUW 234 at ambient air pollutant concentrations at a suburban area of Varanasi (Rai et al., 2007). No significant changes in leaf area and number of leaves between treatments at both the ages of observations under both NPK levels suggest that plants are able to keep the photosynthate for its repair and hence maintained the leaf formation and expansion. Production of more number of leaves with smaller area is, however, reported in polluted environment (Pandey and Agrawal, 1994). Agrawal et al. (2003) showed lower magnitude of reductions in plant height and leaf area in mustard plants grown in pots at different sites experiencing variable pollutant concentrations around Varanasi as compared to wheat, mung bean and palak.

Stem, leaf and pod biomass reduced significantly in plants grown in NFCs and OPs at recommended NPK at both the ages of

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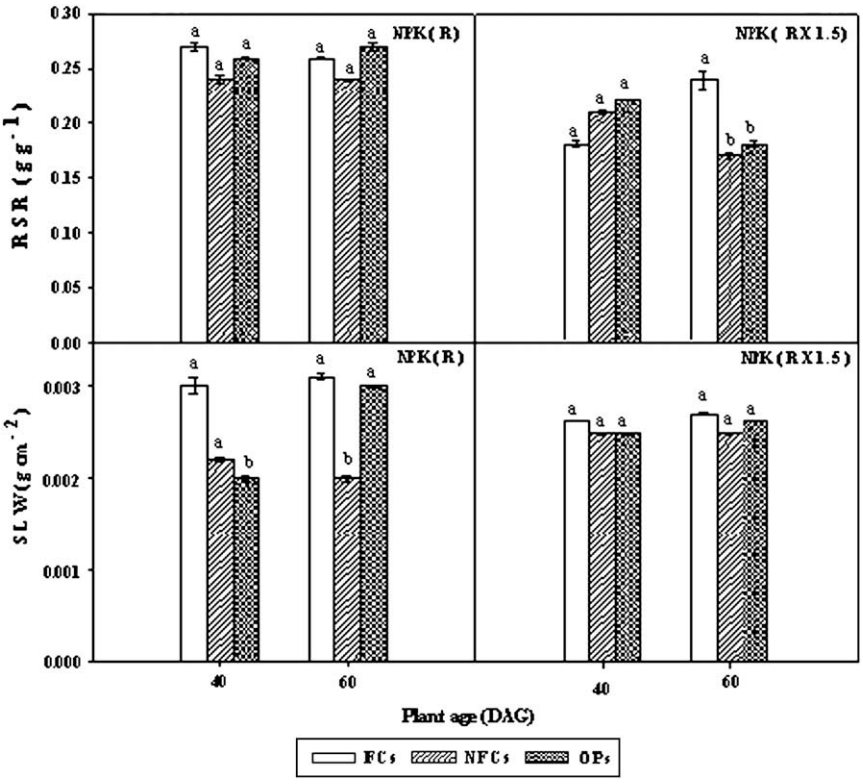

Fig. 7. Root shoot ratio (RSR) and specific leaf weight (SLW) of mustard plants grown in FCs, NFCs and OPs at different levels of NPK. Bars represent mean ± SE. Bars followed by different letter are significantly different from each other at $p < 0.05$.

observations, whereas at 1.5 times recommended NPK, leaf and pod biomass did not vary significantly between NFCs and FCs. This suggests that at higher NPK level, more biomass was retained in leaf and also partitioned more in pod under stress with simultaneous decline in root and stem biomass at 60 DAG. At recommended NPK, however, there was no significant reduction in root biomass at both the ages of observations.

The reduction in RGR at 40 DAG at recommended NPK clearly showed a stress on production efficiency of plants when grown in NFCs and OPs. But the same did not vary at 60 DAG, which suggests that plants might have adapted to ambient O_3 levels and hence able to keep the physiological function at a normal state. RGR depends upon NAR and LAR. NAR represents an increase in plant dry weight per unit assimilatory surface per unit time. NAR showed a trend similar to RGR whereas LAR did not vary significantly between treatments at both the NPK levels. This suggests that RGR was more influenced by NAR as compared to LAR. In the present study, at 1.5 times NPK, photosynthesis decreased significantly in plants of NFCs as compared to FCs at 60 DAG, but NAR declined which suggests acceleration of respiration in these plants. Ozone is reported to reduce Ps but increase respiration rate, which may lead to overall decrease in cumulative carbon gain (Weber et al., 1993).

Ozone is known to affect the source-sink balance leading to more retention of biomass in shoot, thus reducing the root shoot ratio. But in the present study, such response is only found in plants grown at 1.5 times recommended NPK at 60 DAG. This suggests that these plants afforded greater protection to the assimilatory surface against O_3 stress by allocating more resources in the above ground portion. Wahid et al. (1995) found significant reduction in RSR of two different rice cultivars grown in FCs than NFCs. The study suggests that the photosynthate allocation priority not only change with ambient O_3, but also due to difference in NPK levels. SLW, which represents dry weight accumulation per unit leaf area reduced significantly in NFCs and OPs at recommended NPK as compared to 1.5 times recommended NPK. Higher SLW shows more thickness of leaf which is suggested to be a response to avoid the entry of O_3 in the leaf interior (Postiglione et al., 2000). During the vegetative stage of plant growth, leaf expansion is more important and hence the plants at recommended dose showed greater sensitivity, but at later stage the difference in SLW become insignificant.

Yield (seed weight plant^{-1}) reduced significantly by 16.4% in NFCs and 15.2% in OPs at recommended NPK, whereas no significant yield reductions were found at 1.5 times recommended NPK (Table 1). The reduction in seed weight was due to reductions in number of seeds plant^{-1} as well as number of seeds pod^{-1} (Table 1). Number of pods plant^{-1}, however, did not differ significantly between the treatments at any of the NPK levels (Table 1). These parameters did not differ significantly between FCs, NFCs and OPs at 1.5 times recommended NPK. Under O_3 stress decrease in storage of assimilates in leaves had reduced the photosynthate supply to the grains of wheat due to decrease of sucrose and fructan contents of the internodes leading to weight reductions (Kuhbauch

Table 4
Results of two-way ANOVA test showing *F*-values and level of significance for selected yield parameters of mustard plants grown at different NPK levels in FCs, NFCs and OPs.

Parameters	NPK level	Treatment	NPK level × treatment
No. of pods plant^{-1}	0.6^{NS}	1.4^{NS}	0.01^{NS}
Pod weight	2.6^{NS}	1.7^{NS}	0.01^{NS}
No. of seeds plant^{-1}	0.2^{NS}	2.1^{NS}	1.0^{NS}
No. of seeds pod^{-1}	16.0^{***}	11.6^{***}	4.9^{*}
Seed weight	12.2^{**}	2.8^{NS}	1.6^{NS}
Test weight	3.3^{NS}	0.2^{NS}	1.3^{NS}
Above ground biomass	11.3^{**}	2.6^{NS}	1.1^{NS}
Harvest index	7.8^{**}	2.2^{NS}	1.7^{NS}

Level of significance: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; NS = not significant.

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and Thome, 1989). Ollerenshaw et al. (1999) have reported 14% reduction in seed yield of *B. napus* cv. Eurol grown at elevated O_3 concentrations of 77–80 ppb about 49 days at two intervals as compared to those grown in ambient air experiencing O_3 concentrations of 30–31 ppb. Bosac et al. (1998) have reported that 6 h exposure of inflorescence of *B. napus* cv. Libravo to 100 ppb O_3 as compared to filtered air in controlled environment chambers reduced the number of pods plant^{-1} by 18%, number of seeds pod^{-1} by 17% and seed dry weight plant^{-1} by 44%. However, no significant effects of exposure on these parameters were observed in cv. Tapidor. Black et al. (2007) also recorded significant reductions in total number of seeds pod^{-1} and total number of seeds plant^{-1} of *B. campestris* cv. Wisconsin Fast plants upon exposure of 70 ppb O_3 for 7 h d^{-1} for 10 d during 4–13 d after sowing. The mature seed weight plant^{-1} was not significantly affected at above exposure although the value was 20% lower in O_3 treated plants as compared to those exposed in filtered air (Black et al., 2007). The difference in response of *B. campestris* may be due to short-term exposure of plants during their vegetative growth in the study of Black et al. (2007), whereas in the present study the ambient O_3 concentrations exceeded several times above 70 ppb during the seed setting stage of mustard during February. Rai et al. (2007) recorded reductions of 20.7% in yield and 14.5% in number of grains plant^{-1} of wheat cv. HUW 234 at mean concentration of 40.1 ppb O_3 in NFCs as compared to those in FCs.

No significant difference in number of pods in plants grown in FCs and NFCs suggests that number of fertile sites is not changed in plants at ambient O_3 concentrations. Bosac et al. (1994) have also reported that fertile sites in 100 ppb O_3 exposed inflorescence of *B. napus* cv. Tapidor were comparable to control. Wang et al., (2008), however, reported significant decline in number of pods at primary and secondary branches upon exposure in OTCs at diurnal mean concentration of 100 ppb O_3. The supply of 1.5 times recommended NPK, however, protected the mustard plants against yield loss due to ambient O_3. This response was also accompanied by higher weight of seeds and number of seeds plant^{-1} at 1.5 times recommended NPK as compared to recommended NPK. Photosynthate translocation priorities favoured the leaf growth and biomass accumulation during vegetative phase and translocation to pod during reproductive phase at 1.5 times recommended NPK, which helped the plants showing higher HI than at recommended NPK under ambient O_3 concentration. Rai et al. (2007) recorded significant reduction in HI of wheat cv. HUW 234 grown in NFCs as compared to FCs at recommended NPK. Test weight although did not differ significantly between NFCs, OPs and FCs at any of ages of observations was significantly higher at 1.5 times recommended NPK.

Seed quality parameters showed varying levels of changes in response to ambient O_3 and NPK levels. In general oil, protein and nutrient (Ca, Mg, K, P, Zn) contents significantly decreased in plants grown in NFCs and OPs as compared to those in FCs at recommended NPK (Table 2). At 1.5 times recommended NPK, these parameters did not differ significantly between treatments (Table 2). This clearly shows that higher NPK dose not only maintained a higher yield under ambient O_3 concentrations, but also maintained the quality characteristics in the plants. Bosac et al. (1998) have reported reductions of 55% in oil and 40% in protein content of *B. napus* cv. Libravo following 6 h exposure of inflorescence to 100 ppb O_3 as compared to filtered air in controlled environment chambers. In contrast, these parameters did not differ significantly in cv. Tapidor. Wahid (2006a), however, did not find any change in protein content of wheat seeds grown in NFCs as compared to FCs. Ollerenshaw et al. (1999) reported 5% reduction in crude protein and oil content of seeds of *B. napus* cv. Eurol exposed at elevated O_3 concentrations of 77–80 ppb as compared to ambient concentrations of 30–31 ppb.

5. Conclusions

The present field study conducted at a rural site of India clearly provides evidence of high O_3 levels, potentially capable of influencing vegetative and reproductive development of a major oil producing species at recommended levels of NPK. Ambient O_3 levels have induced detrimental effects on photosynthesis, gaseous exchange, vegetative growth, yield component and seed quality of plants. Mustard oil is the commercial product and the seed protein is also important for various mustard meals. The reduction in these two important characteristics suggests a greater threat to the quality maintenance of these plants under elevated O_3 concentrations. The experimental cultivar of mustard seems to be sensitive under current levels of ambient O_3. The inconsistency observed between the responses reported under controlled environment chambers than the present study clearly suggests the appropriateness of field research for the response evaluation. The study further suggests that adequate assimilate partitioning during the reproductive phase played a major role in sustaining quantity and quality of yield under 1.5 times recommended NPK. A higher nutrient amendment may be required to sustain yield in areas having high ambient O_3 concentrations.

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