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Interactive effect of ambient and elevated levels of tropospheric ozone, nutrition and PGPR on growth and yield of chickpea (*Cicer arietinum*)

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ABSTRACT

An experiment was conducted during 2020 and 2021 at ICAR-Indian Agricultural Research Institute, New Delhi, to study the interactive effect of nutrient and PGPR (Plant growth promoting rhizobacteria) on growth and yield of chickpea (*Cicer aeritinum* L.) cv. Pusa 3043 under ambient and elevated levels of ozone in FAOE (Free air ozone enrichment). A loss of 15–16% in dry matter production was observed under elevated ozone (EO_3) when compared to ambient ozone (AO_3) conditions. Seed treatment with PGPR was found to compensate for this loss as an increase in seed yield 18–19% was observed over untreated seeds. In terms of seed yield, the PGPR treatments with 75% nitrogen showed significant improvement compared to the recommended dose of fertilizer (RDF) application. A yield increase of 12% and 14% was observed with PGPR like RPAN8 (*Anabena laxa*) and An-Rh (*Anabena torulosa* with *Mesorhizobium ciceri*) (chickpea rhizobium) respectively, suggesting an advantage of an–Rh over RPAN8 under EO_3. A combination of both PGPRs outperformed the individual PGPR, showing a 19% yield increase over RDF. This proves the potential of seed treatment with PGPR (RPAN8 and An-Rh) in ameliorating tropospheric ozone-induced stress in chickpea plants.

Keywords: Chickpea, Growth, Nitrogen, Ozone, PGPR, Yield

Tropospheric ozone (O_3) is a critical air pollutant in India generated by photochemical reactions of volatile organic compounds with nitrogen oxides, which harms vegetation and human health (Sharma *et al*. 2022). Background O_3 concentration in the troposphere has increased by 36% since pre-industrial times and the current rate of increase is 0.5–2.5% per year (Wang *et al*. 2022). In Asia, vast agricultural areas of India and China are under severe O_3 pollution (Gao *et al*. 2020). Irrespective of O_3 precursor emissions, climate change is also expected to increase the O_3 level by 4% in the northern part of India (Pommier *et al*. 2018). O_3 enters leaves via stomata and rapidly transforms to produce reactive oxygen species (ROS), which damage plant tissues and alter their physiological and biochemical properties such as decreased photosynthesis, stomatal conductance, leaf injury, lower plant yield leads to production losses (Ashrafuzzaman *et al*. 2017). Indo-Gangetic Plains (IGPs) are India's most fertile and productive agricultural areas which also faces the highest amount of yield losses for most crop species under present and future O_3 concentrations, grain yield loss ranging between 7–13% under ambient O_3 concentration (~30ppb) in the northern region of India (Mukherjee *et al*. 2021). In Asia, when ambient ozone levels varied from 35–75 ppb during crop growth, legumes and pulse crops being more susceptible to ozone stress lost 10–66% of their yield (Emberson *et al*. 2018). Among the legumes, India ranks first in chickpea (*Cicer arietinum* L.) production accounts for 70% of global production although its vulnerability to biotic and abiotic stressors has also increased resulting in yield fluctuations (Muehlbauer and Sarker 2017). There are very few studies which have quantified the impact of ozone stress on chickpea. Studies have shown the beneficial role of PGPR as a biofertilizer and in alleviating abiotic stresses like drought, salinity and metal toxicity in plants (Emberson 2020). However, elevated ozone (EO_3) levels induce abiotic stress in plants, no study has been reported where PGPR was used for reducing ozone stress in legume crops like chickpea. The present work aims to elucidate the amelioration of ozone stress through nutrients and PGPR on the growth and yield of chickpeas.

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MATERIALS AND METHODS

A pot experiment was conducted in the Free Air Ozone

Enrichment facility (FAOE) at the experimental farm of ICAR-Indian Agricultural Research Institute, New Delhi (28º35'N and 77º12'E, 228.16 m amsl) during the winter (*rabi*) season of 2020 and 2021. The soil for pot experiment was silty clay loam with (*p*H of 7.6) electrical conductivity of 0.47 dS/m, organic carbon of 0.51% and the available N, P, K contents 212.8 kg/ha, 24.2 kg/ha, 255.2 kg/ha respectively. Duration of elevated ozone exposure was 7 h/day (except rainy days) from 9.30 AM to 4.30 PM. The average ambient O_3 concentration was 30 ± 10 ppb and the elevated O_3 concentration in FAOE was about 60 ± 10 ppb (using O_3 generator) throughout the crop growth period. The treatments comprised two ozone exposure (Ambient-AO_3; Elevated-EO_3) levels along with a soil application of 3 different nitrogen doses and 2 strains of PGPR (seed treatment) were tested using factorial completely randomized design (FCRD) with three replications. Chickpea cultivar Pusa 3043 was used for the present study. Experiment consisted of 8 treatment combinations, viz. T_1, Absolute control; T_2, 100% RDF (Recommended Dose of Fertilizers); T_3, 75% RDN + RPAN8 (*Anabena laxa*); T_4, 50% RDN + RPAN8; T_5, 75% RDN + An-Rh (*Anabena torulosa* with *Mesorhizobium ciceri*) (chickpea rhizobium); T_6, 50% RDN + An-Rh; T_7, 75% RDN + RPAN8 + An-Rh; T_8, 50% RDN + RPAN8 + An-Rh. Recommended dose of NPK for chickpea crop was 20:40:20 kg/ha. Two PGPR used in the current study were obtained from the Division of Microbiology, ICAR-Indian Agricultural Research Institute, New Delhi. First cyanobacterial culture, RPAN8 (*Anabena laxa*) terrestrial nitrogen fixing cyanobacterium fixes N upto 20–30 kg/ha used in agriculture for biofertilizer and biopesticide development. The second PGPR is a cyanobacterial biofilm, An-Rh *Anabena torulosa* with *Mesorhizobium ciceri* (chickpea rhizobium) reported for tolerance to abiotic stress and promotion of plant growth used for seed treatment. For microbial treatment, seeds were coated with carrier-based formulation of the microbial consortia @50 g/kg seed using 3% sucrose solution as adhesive agent. The coated seeds were shade-dried and sown immediately in pots. For observations, five plants per treatment were randomly selected and tagged. After harvest, plant height and number of secondary branches were measured at harvest stage. Dry and fresh weight were determined, and the cumulative addition of all plant parts gave the total biomass as g/plant. Yield parameters like number of pods/plants, number of seeds/pods, pod weight, seed weight and test weight of seeds (100 seed weight) were also recorded. Harvest index of the crop was calculated with the formula given below.

$$\text{Harvest index} = (\text{Seed yield/Total biological yield}) \times 100$$

ANOVA (Analysis of variance) was done for factorial completely randomized design (FCRD) to check whether the treatment differences were statistically significant using OPSTAT software. Multivariate analysis was performed using the R software package.

RESULTS AND DISCUSSION

Growth parameters: The plant height exhibited significant differences among the treatments in both seasons (Fig. 1). The maximum plant height was reflected in T_7 (75% N + RPAN8 + An-Rh) under ambient ozone (AO_3) treatment in both first year and second year as 48.13 cm and 48.84 cm, respectively. Decline in plant height was observed under EO_3 treatments (29.69 cm and 30.09 cm in T_1) compared to ambient ozone levels. The number of secondary branches determines the total number of leaves and hence the total photosynthetic area. Under AO_3 and EO_3, the number of secondary branches (Fig. 1) showed a significant difference. The higher number of secondary branches/plant (10.65 and 11.85) resulted under AO_3 PGPR treatment (T_7) at flowering stage. There was a 7.2 to 8% decrease in secondary branches production under EO_3 as compared to AO_3 in both years of the experiment. However, the compensation effect of PGPR over EO_3 was about 7.9–9%. This was because PGPRs provided favourable condition in the rhizosphere for plant growth and yield. The dry matter production (biomass g/pot) was significantly lowered by 16% during 2020–21 and 15% during 2021–22 in the EO_3 treatments compared to AO_3 conditions (Table 1). An increase of dry matter accumulation was found in the range of 19% during 2020–21 and 18% during 2021–22 in PGPR treated seeds over untreated seeds.

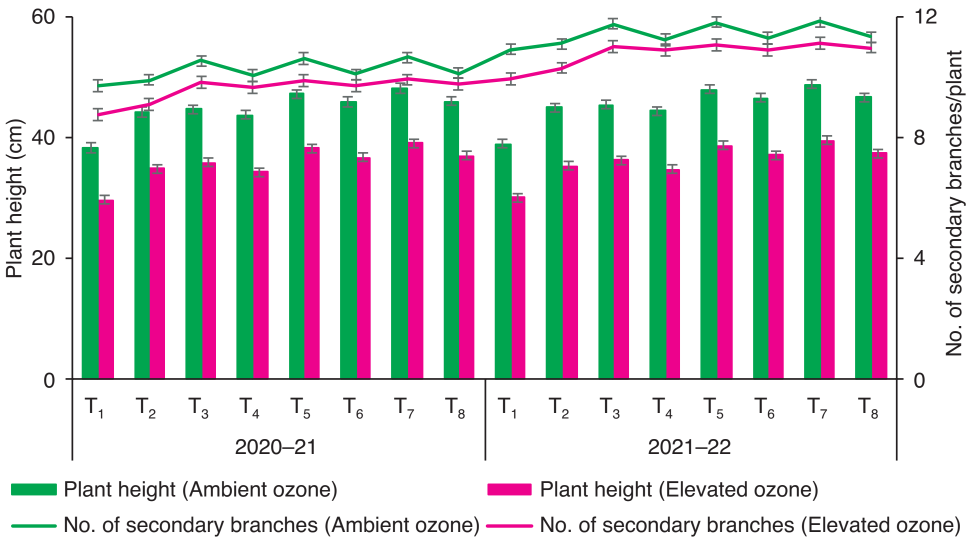

Fig. 1 Interactive effect of tropospheric ozone, nutrient and PGPR on plant height and number of secondary branches of chickpea under ambient and elevated levels. Treatment details are given under Materials and Methods.

Table 1 Interactive effect of ambient and elevated O_3 and PGPR on total biomass (g/pot) of chickpea

Treatment	2020–21		2021–22	
	AO_3	EO_3	AO_3	EO_3
T_1	41.47 ± 1.03^{a}	34.1 ± 0.5^{g}	43.06 ± 1.06^{a}	35.69 ± 0.76^{g}
T_2	44.58 ± 0.86^{d}	37.61 ± 0.24^{f}	46.17 ± 1.06^{d}	39.2 ± 0.33^{f}
T_3	49.75 ± 0.16^{b}	38.67 ± 0.51^{f}	51.34 ± 0.11^{b}	40.26 ± 0.42^{f}
T_4	44.08 ± 0.28^{d}	38.06 ± 0.81^{f}	45.67 ± 1.09^{d}	39.65 ± 0.31^{f}
T_5	51.24 ± 0.38^{ab}	38.98 ± 0.79^{f}	52.83 ± 0.74^{ab}	40.57 ± 0.61^{f}
T_6	43.96 ± 0.44^{d}	38.39 ± 0.58^{f}	45.55 ± 0.69^{d}	39.98 ± 0.77^{f}
T_7	53.12 ± 0.69^{a}	39.14 ± 0.71^{f}	54.71 ± 0.91^{a}	40.73 ± 0.04^{f}
T_8	47.61 ± 0.99^{c}	38.51 ± 0.7^{f}	49.2 ± 0.92^{c}	40.1 ± 0.69^{f}
Mean	46.97	37.93	48.56	39.52
Factors	C.D.	SE(m)	C.D.	SE(m)
AO_3	0.67	0.23	0.74	0.25
EO_3	1.34	0.46	1.49	0.51
(AO_3 + EO_3) × (Fertilizer and PGPR)	1.89	0.65	2.11	0.73

± Standard error; AO_3, Ambient ozone; EO_3, Elevated ozone. Values followed by the same letters within columns are not significantly different at $P\leq 0.05$.

Treatment details are given under Materials and Methods.

Table 2 Interactive effect of ambient and elevated O_3 and PGPR on number of pods/plant in chickpea

Treatment	2020–21		2021–22	
	AO_3	EO_3	AO_3	EO_3
T_1	31 ± 0.54^{ij}	26 ± 0.49^{k}	34.28 ± 0.17^{h}	29.28 ± 0.22^{j}
T_2	36 ± 0.49^{ef}	32 ± 0.78^{hi}	39.28 ± 0.25^{f}	35.28 ± 0.28^{h}
T_3	38.5 ± 0.32^{cd}	37.25 ± 0.43^{de}	41.78 ± 0.74^{de}	40.53 ± 0.36^{ef}
T_4	33.5 ± 0.75^{gh}	29.5 ± 0.61^{j}	36.78 ± 0.11^{g}	32.78 ± 0.07^{i}
T_5	40 ± 0.44^{c}	39 ± 0.53^{c}	43.28 ± 0.23^{c}	42.28 ± 0.44^{cd}
T_6	34.6 ± 0.67^{fg}	31.3 ± 0.77^{i}	37.88 ± 0.97^{g}	34.58 ± 0.34^{h}
T_7	50 ± 0.21^{a}	49 ± 0.26^{a}	53.28 ± 0.19^{a}	52.28 ± 0.54^{a}
T_8	46 ± 0.84^{b}	45.5 ± 0.05^{b}	49.28 ± 0.51^{b}	48.78 ± 0.48^{b}
Mean	38.69	36.19	41.98	39.47
Factors	C.D.	SE(m)	C.D.	SE(m)
AO_3	0.56	0.19	0.44	0.15
EO_3	1.13	0.39	0.89	0.30
(AO_3 + EO_3) × (Fertilizer and PGPR)	1.60	0.55	1.26	0.43

±, Standard error; AO_3, Ambient ozone; EO_3, Elevated ozone. Values followed by same letters with in columns are not significantly different at $P\leq 0.05$.

Treatment details are given under Materials and Methods.

There is evidence in the scientific literature that EO_3 affects important metabolic processes leading to the reduction in growth and total biomass production (Hoshika *et al*. 2013), in O_3-sensitive species/cultivars. A recent study on common bean with PGPR reported that enhancing the phytohormones results in enhanced root growth, shoot growth, yield, and biochemical activity alleviation of toxicity in plants under adverse conditions (Kumar *et al*. 2020).

Yield attributes: The yield contributing characters, viz. number of pods/plant, pod weight/plant, number of seeds/pod, seed weight, 100 seed weight and harvest index was negatively influenced by the EO_3 levels. The results of number of pods/plant (Table 2) and pod weight/plant indicate that the T_7 (75% RDN + RPAN8 + An-Rh) recorded the highest range under AO_3. Among the treatment effects of number of pods/plant, the EO_3 showed a significant decrease in the range of 11% (2020–21) and 10.1% (2021–22).

A significant increasing per cent difference of PGPR treated seeds over non-treated seeds was observed between 8.3–12% in AO_3 and 25–22.2% in EO_3 condition. The results of pod weight/plant exhibited a range of (16.24–26.80 g) at AO_3 and (13.16–22.30 g) at EO_3 conditions. However, at harvest stage, the difference in the pod weight/plant was found to be non-significant. Reduced photosynthetic activity during stress may affect assimilate translocation and carbon fixation, resulting in fewer pods, poor seed set, and lowered sink activity in chickpeas (Nadeem *et al*. 2019). Increased photosynthates availability by microbes might have been utilized for repair and detoxification processes against EO_3 stress (Backer *et al*. 2018), and hence yield increase significantly higher. The reduction in growth characteristics is directly proportional to yield attributes.

Significantly higher number of seeds/pod (Fig. 2) and seed yield (Table 3) was observed for seed treated with PGPR in AO_3 treatments, while EO_3 treatment exhibited reduced number of seeds and seed weight than ambient ozone levels. Treatment T_7 (75% N+RPAN8+An-Rh) recorded the highest no. of seeds/pod range of 1.8–2.1 and 23.96–25.19 g of seed weight in AO_3 levels. But, the number of seeds/pod decreased by 11% (2020–21) and 10% (2021–22) from ambient to EO_3 condition in untreated seed treatment however, T_7 (75% N+RPAN8+An-Rh) has shown 18–21% increase compared to EO_3 condition. The exposure of EO_3 caused decrease in seed weight as compared to ambient condition whereas 19.5–21.1% increase was observed in seed weight from T_7-EO_3 treatment. The interactions among O_3 levels, fertilizer and PGPR treatments were found to be

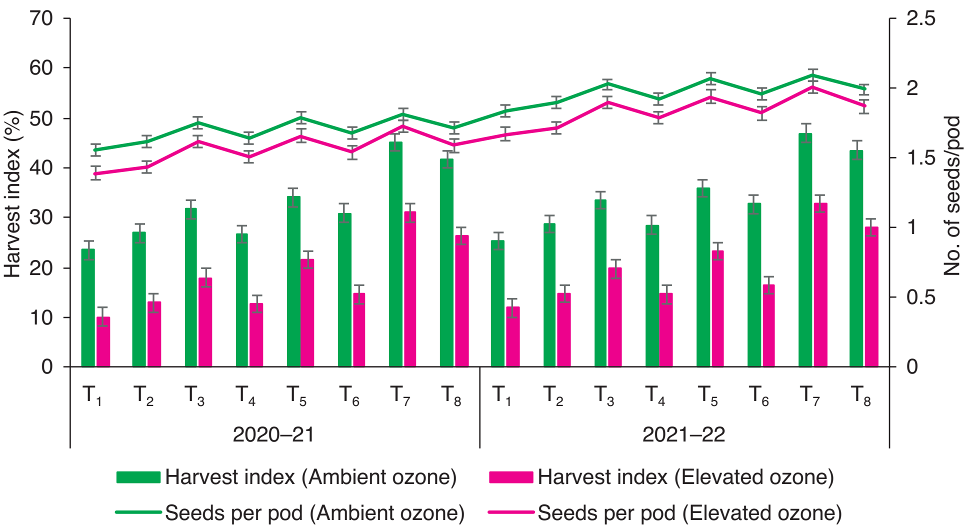

Fig. 2 Interactive effect of tropospheric ozone, nutrient and PGPR on number of seeds per pod and harvest index of chickpea under ambient and elevated levels.
Treatment details are given under Materials and Methods.

non-significant. Moreover, the results indicate that the PGPR had a considerable influence in ameliorating O_3 induced damage to crops.

Table 3 Interactive effect of ambient and elevated O_3 and PGPR on seed yield per plant in chickpea

Treatment	2020–21		2021–22	
	AO_3	EO_3	AO_3	EO_3
T_1	9.74 ± 0.17^g	3.43 ± 0.07^i	10.97 ± 0.24^g	4.66 ± 0.07^i
T_2	11.98 ± 0.05^f	4.83 ± 0.02^k	13.21 ± 0.15^f	6.06 ± 0.14^k
T_3	15.79 ± 0.35^d	6.93 ± 0.08^i	17.02 ± 0.21^d	8.16 ± 0.14^i
T_4	11.78 ± 0.17^f	4.85 ± 0.12^k	13.01 ± 0.28^f	6.08 ± 0.02^k
T_5	17.46 ± 0.2^c	8.38 ± 0.06^h	18.69 ± 0.27^c	9.61 ± 0.05^h
T_6	13.6 ± 0.26^e	5.63 ± 0.03^j	14.83 ± 0.14^e	6.86 ± 0.09^j
T_7	23.96 ± 0.04^a	12.15 ± 0.29^f	25.19 ± 0.04^a	13.38 ± 0.09^f
T_8	19.85 ± 0.15^b	10.13 ± 0.07^g	21.08 ± 0.41^b	11.36 ± 0.06^g
Mean	15.52	7.04	16.75	8.27
Factors	C.D.	SE(m)	C.D.	SE(m)
AO_3	0.16	0.05	0.18	0.06
EO_3	0.33	0.11	0.37	0.12
$(AO_3 + EO_3)$ × (Fertilizer and PGPR)	0.47	0.16	0.52	0.18

±, Standard Error; AO_3, Ambient ozone; EO_3, Elevated ozone. Values followed by same letters with in columns are not significantly different at $P \leq 0.05$.

Treatment details are given under Materials and Methods.

During EO_3 exposure, the advancement in pod maturity by 8 and 10 days compared to AO_3 were reported (Bhatia *et al*. 2021) and this decrease in the duration of reproductive period may have resulted in reduced seed weight and yield in our experiment. Pollen tube growth rate may decrease under stress, affecting pod and seed formation (Kaloki *et al*. 2019). Kumar *et al*. (2020) showed role of stress tolerant PGPRs on growth and yield, this study showed that combined effects of 32 stress tolerant PGPR strains (8 Rhizobium, 8 Pseudomonas, 8 potassium releasing bacteria, 8 zinc solubilizing bacteria) gives highest yield as compared to control condition. The 100-seed weight ranged from 21.01–22.0 g under AO_3 while in EO_3 it ranged from 20.91–21.76 g. The harvest index (HI) (Fig. 2) in both seasons has shown significant difference between the treatments. Reduction recorded in HI was more in EO_3 than AO_3 suggesting more unfilled seeds/plant in former than latter (Singh *et al*. 2012). The HI reduction showed less dry matter partitioned into seeds under ambient air pollution stress. The presence of tropospheric O_3 is known to hinder the synthesis of carbohydrates and the movement of assimilates from the source to seeds. Similar results of lower yield were obtained by (Bhatia *et al*. 2021) in chickpea, 15.4% in mungbean (Chaudhary and Agrawal 2015), turnip (Sethupathi *et al*. 2018), 37.15% rice (Kumar *et al*. 2021) and cauliflower (Kovilpillai *et al*. 2023).

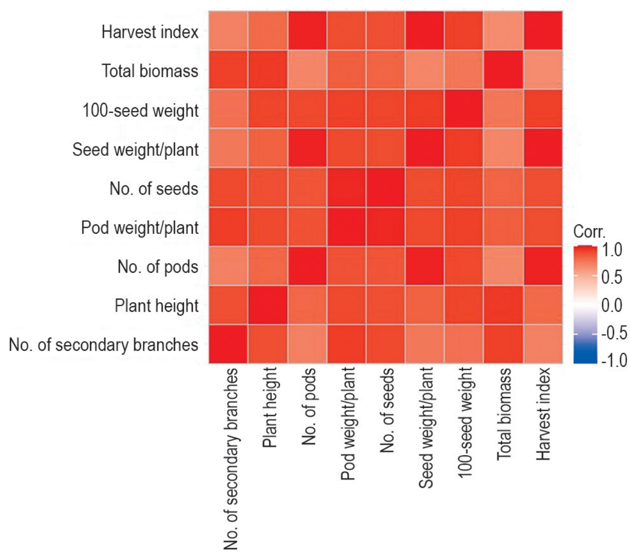

Fig. 3 Multivariate analysis correlation between growth and yield parameters of chickpea.

PGPR application escalated the number of pods/plant, number of seeds/plant and seed yield by 50, 63 and 37%, respectively in chickpea (Laranjeira *et al*. 2022). The PGPR inoculation could mitigate MDA accumulation caused by EO_3, and enhance the chlorophyll content, leading to increased rice productivity (Autarmat *et al*. 2023). The growth and yield parameters were positively correlated with the yield of chickpea (Fig. 3).

The findings of the present study showed that when compared to the recommended fertilizer dose, the application of 75% N + RPAN8 and 75% N + An-Rh increased seed yield by 12% and 14%, respectively. Whilst, the combination of both PGPR (75% N + RPAN8 + An-Rh) increased yield by 19%. This finding indicates that An-Rh exhibited greater efficacy than RPAN8 in mitigating the impact of ozone stress, whereas the synergistic effect of both of these PGPR exceeded the sum of their contributions. Along with nutrients, use of PGPR can be a sustainable as well as cost effective solution to overcome the deleterious effects of rising tropospheric O_3. The evidence on the amelioration effects of nutrient and PGPR over O_3 was limited and its physiological role needs to be documented in future research with a focus on studying the tropospheric ozone's impacts on different crops and identifying the best PGPR to nullify the tropospheric ozone's impact for ensuring sustainable agriculture.

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